

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of El Oued

Faculty of Nature and Life Sciences

Department of Cellular and Molecular Biology



كلية علوم الطبيعة والحياة
Faculty of Nature and Life Sciences

MASTER THESIS

In partial fulfilment of the requirements for the degree of Master

Major: Biological Sciences

Speciality: Toxicology

THEME

**Ferrocenyl Acetylaniline Derivatives as Potential
Modulators of Thyroid Receptors: Synthesis, Char-
acterisation and Computational Insights**

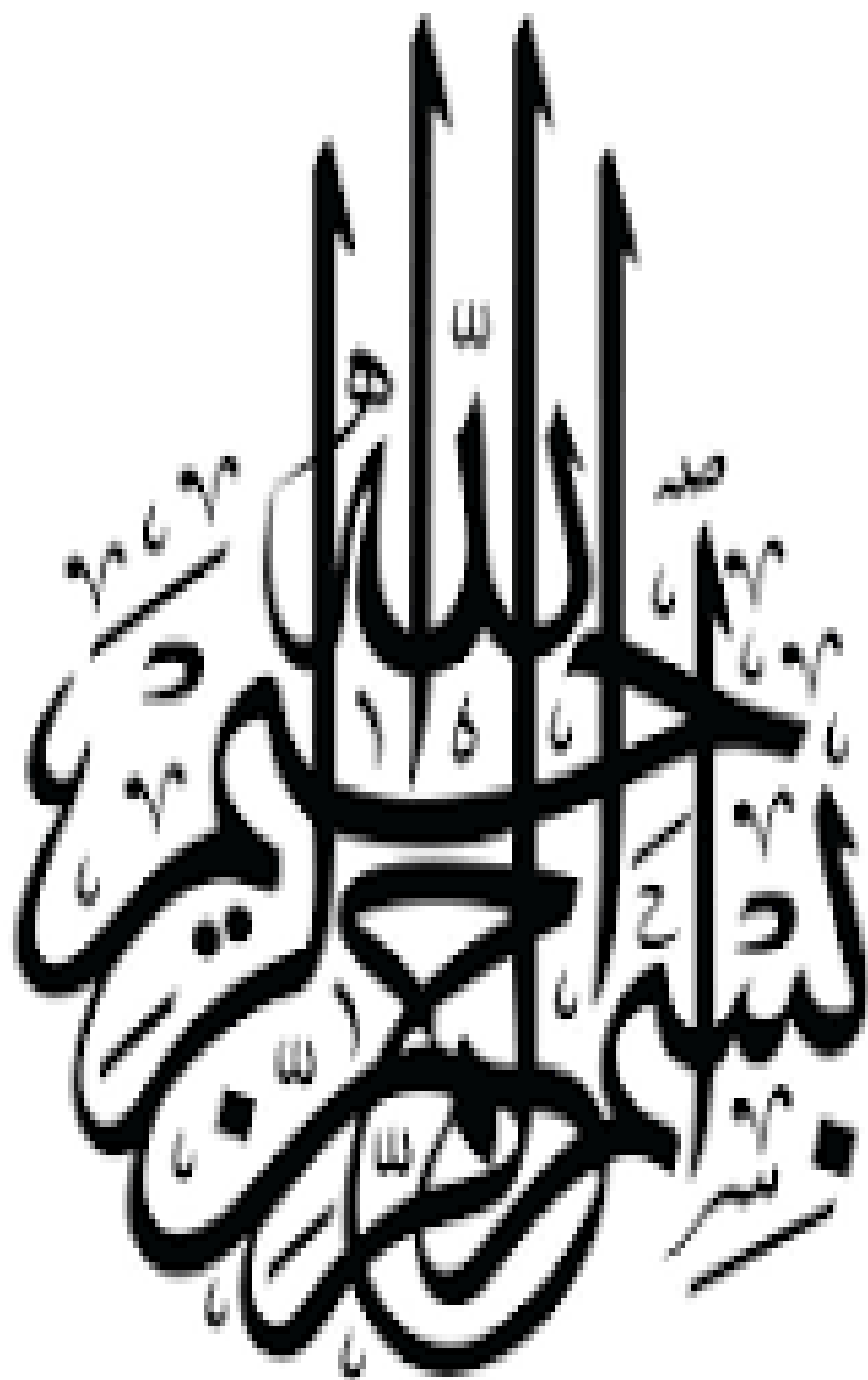
Presented by:

➤ **Fatima El Zahra DOUIS** ➤ **Mohammed SADOON**

Jury Members:

- | | | | |
|-------|------------------|-----|------------|
| • Dr. | Touati Said | Dr | President |
| • Dr. | Ghania Ahmed | Dr | Examiner |
| • Dr. | Elhafnaoui LANEZ | MCA | Supervisor |

Academic Year: 2025 – 2026



DEDICATE

*In the name of Allah, the Most Gracious, the Most Merciful
All praise is due to Allah, whose grace makes all good things possible. I ask Him to bless this work and make it sincerely for His sake.*

*My dear grandmother... **Bechiri Khadidja**, may Allah have mercy on her*

I miss you in every achievement, as if my joy is incomplete without you. You were my safety, and you remain a prayer that never leaves my heart.

*My dear grandfather... **Mustafa Bachir Hiba**, may Allah have mercy on him*

Your memory is with me in every success. I ask Allah to have mercy on you and grant you Paradise.

*My dear father... **Reda Douis***

My first support and my light in every path, I dedicate this work to you as a sign of love

May Allah protect you and grant you a long life.

*My dear mother **Hiba Toufaha***

My light and my support, to you I dedicate this work with all my love.

May Allah protect you and grant you a long life.



*My dear siblings: **Abd El CHafi, Aymen, Wafaa, CHourouk, Ahmed Yassine***

Those souls who are like me, your presence by my side means a lot.

*To my uncles and aunts: **Khaled, Naima, Saida, Faiza**
May you always remain my support; your presence in my life is a blessing.*

*To my childhood friends: **Amina, Walid, Soumia, Chaimaa, Bouchra, Mohamed El Eid, Bachir**
Thank you for always being a beautiful part of my life.*

*To my little friends: **Khadidja, Kholoud, Hodaiifa, Maimouna, Hala, Imrane, Johaina, Sara**
With you, my joy is complete.*

*To my special friend: **Intissar** you have always been and remain the most beautiful coincidence in my life.*

DOUIS Fatima El Zahra



*{And their final supplication will be, "Praise be to Allah, Lord of
the Worlds."}*

*Praise be to Allah, who mends broken hearts and rewards the
patient, even if the wait is long.*

*I dedicate this achievement and effort to my dear father; may
God have mercy on him.*

*To my dear mother, who inspires me with passion and determi-
nation; she is my paradise and my first destination. May God re-
ward her abundantly.*

*And to those whom God has blessed with my presence, to the
source of my strength, my solid ground, and the strong wall of my
heart: my brothers and sisters*

Sadoun Mohammed



ACKNOWLEDGMENTS

All praise is due to Allah, truly deserving of praise, for the completion of His favor and grace. I thank Him for all His blessings, both manifest and hidden. To You belongs praise befitting the majesty of Your countenance and the immensity of Your dominion. Peace and blessings be upon the best of all creation, our Prophet Muhammad, may the best of prayers and purest peace be upon him, for enabling us to reach the end of this long-awaited stage and for facilitating our affairs.

We extend our deepest gratitude and appreciation to Professor LANEZ Elhafnaoui, the supervisor of this study, who never hesitated to provide sound guidance and a helping hand. To him, we offer our sincere thanks and the highest expressions of appreciation and respect. May Allah reward him abundantly on our behalf.

We also extend our thanks and appreciation to those who carry the most sacred message in life, to those who paved the way for us on the path of knowledge and learning, to all our esteemed professors:



for all they have given us throughout our academic journey. We express our sincere gratitude to the honorable Director and all members of the administrative and technical staff who oversee the management of the Vocational Training and Apprenticeship Center.

And to my dear family, who have been a true support throughout my academic journey, may Allah protect them. We extend our gratitude to the discussion committee, whose guidance and evaluation of this study were invaluable.

In conclusion, we pray to God Almighty that this work be accepted for His sake and that it may be a beneficial contribution, however small, to the field of research.



Abstract

This thesis reports the synthesis, physicochemical characterisation and comprehensive *in silico* evaluation of a series of ferrocenyl acetylaniline derivatives (FcMe2Ac, FcMe3Ac and FcMe4Ac), with the aim of elucidating the relationship between molecular structure, electronic properties and interaction behaviour towards thyroid-related molecular targets.

The target compounds were successfully synthesised via a nucleophilic substitution strategy and isolated in moderate to good yields ranging from **48% to 80%**. Structural elucidation was achieved using complementary spectroscopic techniques, including FT-IR, UV–Visible spectroscopy and nuclear magnetic resonance (^1H , ^{13}C and 2D NMR). FT-IR spectra exhibited characteristic absorption bands corresponding to N–H stretching ($\sim 3290\text{--}3445\text{ cm}^{-1}$) and carbonyl groups ($\sim 1647\text{--}1651\text{ cm}^{-1}$), while UV–Visible analysis revealed absorption maxima in the range of **259–432 nm**, associated with $\pi\rightarrow\pi^*$ and d–d transitions. NMR data confirmed the expected structural features, including signals assigned to the ferrocenyl moiety ($\sim 4.1\text{--}4.3\text{ ppm}$) and aromatic protons ($\sim 6.6\text{--}7.8\text{ ppm}$).

Electrochemical studies using cyclic voltammetry revealed a quasi-reversible Fe (II)/Fe (III) redox system, with formal potentials of **43.9, 51.0 and 57.5 mV** for FcMe2Ac, FcMe3Ac and FcMe4Ac, respectively. Peak-to-peak separations ($\Delta E_p = 87.8\text{--}115\text{ mV}$) and current ratios close to unity confirmed quasi-reversible behaviour. Diffusion coefficients ranged from **1.11×10^{-7} to $5.74 \times 10^{-7}\text{ cm}^2\cdot\text{s}^{-1}$** , while heterogeneous electron-transfer rate constants (k_s) varied between **0.14×10^{-3} and $0.62 \times 10^{-3}\text{ cm}\cdot\text{s}^{-1}$** , reflecting the influence of substitution on electron-transfer kinetics.

Density functional theory calculations indicated HOMO energies between **-5.83 and -5.68 eV** and HOMO–LUMO gaps ranging from **3.99 to 4.29 eV**, suggesting moderate chemical stability combined with controlled reactivity. Global reactivity descriptors revealed hardness values between **1.995 and 2.143 eV** and electrophilicity indices up to **3.519 eV**, while dipole moments increased from **3.82 to 5.12 D**, indicating enhanced molecular polarity across the series.

Molecular docking studies against thyroid-related targets, including the thyroid hormone receptor β and the thyroid-stimulating hormone receptor, revealed favourable binding affinities ranging from **-7.34 to $-8.58\text{ kcal}\cdot\text{mol}^{-1}$** , with FcMe4Ac exhibiting the strongest interaction. The binding mode analysis highlighted the importance of hydrophobic contacts, hydrogen bonding and π -interactions in stabilising the ligand–receptor complexes.

Molecular dynamics simulations (100 ns) further confirmed the stability of the FcMe4Ac–receptor complexes, with RMSD values stabilising within **0.14–0.24 nm** and reduced residue fluctuations, particularly in the thyroid-stimulating hormone receptor system, indicating enhanced structural rigidity and persistent interaction networks.

ADMET predictions demonstrated favourable pharmacokinetic properties, including high gastrointestinal absorption, LogP values ranging from **3.18 to 3.52**, and absence of predicted mutagenic or hepatotoxic effects. The lack of blood–brain barrier permeability further supports their suitability for peripheral endocrine targets.

Overall, the results demonstrate that structural modification of ferrocenyl derivatives significantly influences their electronic properties, electrochemical behaviour and interaction profiles with thyroid-related targets. Among the studied compounds, **FcMe4Ac emerges as the most promising candidate**, combining optimal electronic characteristics, strong binding affinity, high dynamic stability and favourable pharmacokinetic properties, making it a potential scaffold for further development in thyroid-related therapeutic research.

Resume

Cette thèse présentée la synthèse, la caractérisation physico-chimique et l'évaluation in silico complète d'une série de dérivés de ferrocénylacétylaniline (FcMe₂Ac, FcMe₃Ac et FcMe₄Ac), dans le but d'élucider la relation entre la structure moléculaire, les propriétés électroniques et les interactions avec des cibles moléculaires liées à la thyroïde.

Les composés cibles ont été synthétisés avec succès par une stratégie de substitution nucléophile et isolés avec des rendements modérés à bons, compris entre 48 % et 80 %. L'élucidation structurale a été réalisée à l'aide de techniques spectroscopiques complémentaires, notamment la spectroscopie FT-IR, la spectroscopie UV-visible et la résonance magnétique nucléaire (¹H, ¹³C et RMN 2D). Les spectres FT-IR ont présenté des bandes d'absorption caractéristiques correspondant à l'élongation N–H (~3290–3445 cm⁻¹) et aux groupements carbonyle (~1647–1651 cm⁻¹), tandis que l'analyse UV-Visible a révélé des maxima d'absorption dans la gamme 259–432 nm, associés aux transitions $\pi \rightarrow \pi^*$ et d–d. Les données RMN ont confirmé les caractéristiques structurales attendues, notamment les signaux attribués au fragment ferrocényle (~4,1–4,3 ppm) et aux protons aromatiques (~6,6–7,8 ppm).

Les études électrochimiques par voltampérométrie cyclique ont révélé un système redox Fe (II)/Fe (III) quasi-réversible, avec des potentiels formels de 43,9, 51,0 et 57,5 mV pour FcMe₂Ac, FcMe₃Ac et FcMe₄Ac, respectivement. Les séparations pic à pic (ΔE_p = 87,8–115 mV) et les rapports de courant proches de l'unité ont confirmé un comportement quasi-réversible. Les coefficients de diffusion variaient de $1,11 \times 10^{-7}$ à $5,74 \times 10^{-7}$ cm²·s⁻¹, tandis que les constantes de vitesse de transfert d'électrons hétérogènes (k_s) variaient entre $0,14 \times 10^{-3}$ et $0,62 \times 10^{-3}$ cm·s⁻¹, reflétant l'influence de la substitution sur la cinétique de transfert d'électrons.

Les calculs de la théorie de la fonctionnelle de la densité ont indiqué des énergies HOMO comprises entre -5,83 et -5,68 eV et des écarts HOMO-LUMO compris entre 3,99 et 4,29 eV, suggérant une stabilité chimique modérée associée à une réactivité contrôlée. Les descripteurs de réactivité globale ont révélé des valeurs de dureté comprises entre 1,995 et 2,143 eV et des indices d'électrophilie atteignant 3,519 eV, tandis que les moments dipolaires augmentaient de 3,82 à 5,12 D, indiquant une polarité moléculaire accrue au sein de la série.

Des études de docking moléculaire ciblant des récepteurs liés à la thyroïde, notamment le récepteur β de l'hormone thyroïdienne et le récepteur de la TSH, ont révélé des affinités de liaison favorables comprises entre -7,34 et -8,58 kcal·mol⁻¹, le FcMe₄Ac présentant l'interaction la plus forte.

L'analyse du mode de liaison a mis en évidence l'importance des contacts hydrophobes, des liaisons hydrogène et des interactions π dans la stabilisation des complexes ligand-récepteur.

Les simulations de dynamique moléculaire (100 ns) ont confirmé la stabilité des complexes FcMe4Ac-récepteur, avec des valeurs RMSD stabilisées entre 0,14 et 0,24 nm et une réduction des fluctuations résiduelles, notamment au niveau du récepteur de la TSH, indiquant une rigidité structurale accrue et des réseaux d'interaction persistants.

Les prédictions ADMET ont démontré des propriétés pharmacocinétiques favorables, incluant une absorption gastro-intestinale élevée, des valeurs de LogP comprises entre 3,18 et 3,52, et l'absence d'effets mutagènes ou hépatotoxiques prédits. L'absence de perméabilité à la barrière hémato-encéphalique renforce leur intérêt pour les cibles endocriniennes périphériques.

En résumé, les résultats démontrent que la modification structurale des dérivés ferrocényliques influence significativement leurs propriétés électroniques, leur comportement électrochimique et leurs profils d'interaction avec les cibles thyroïdiennes. Parmi les composés étudiés, le FcMe4Ac se distingue comme le candidat le plus prometteur, combinant des caractéristiques électroniques optimales, une forte affinité de liaison, une grande stabilité dynamique et des propriétés pharmacocinétiques favorables, ce qui en fait une structure potentielle pour le développement ultérieur de recherches thérapeutiques liées à la thyroïde.

ملخص

تتناول هذه الأطروحة عملية التخليق والخصائص الفيزيائية والكيميائية والتقييم الشامل باستخدام الحاسوب لسلسلة من مشتقات الفيروسينيل أسيتيل أنيلين (FcMe_2Ac) و FcMe_3Ac و FcMe_4Ac ، بهدف توضيح العلاقة بين التركيب الجزيئي والخصائص الإلكترونية وسلوك التفاعل تجاه الأهداف الجزيئية المتعلقة بالغدة الدرقية.

تم بنجاح تخليق المركبات المستهدفة باستخدام استراتيجية الاستبدال النيوكليوفيلي، وعُزلت بنسب إنتاج تتراوح بين المتوسطة والجيدة، من 48% إلى 80%. وتم تحديد البنية باستخدام تقنيات طيفية متكاملة، بما في ذلك مطيافية الأشعة تحت الحمراء بتحويل فورييه (FT-IR)، ومطيافية الأشعة فوق البنفسجية والمرئية، والرنين المغناطيسي النووي (^1H ، ^{13}C ، و $^2\text{D NMR}$). أظهرت أطياف FT-IR نطاقات امتصاص مميزة تتوافق مع تمدد الرابطة N-H (حوالي 3290–3445 سم $^{-1}$) ومجموعات الكربونيل (حوالي 1647–1651 سم $^{-1}$)، بينما كشف تحليل الأشعة فوق البنفسجية والمرئية عن قيم امتصاص قصوى في نطاق 259–432 نانومتر، مرتبطة بانتقالات $\pi \rightarrow \pi^*$ و d-d. أكدت بيانات الرنين النووي المغناطيسي السمات الهيكلية المتوقعة، بما في ذلك الإشارات المنسوبة إلى جزء الفيروسينيل ($\sim 4.1\text{--}4.3$ جزء في المليون) والبروتونات العطرية ($\sim 6.6\text{--}7.8$ جزء في المليون).

كشفت الدراسات الكهروكيميائية باستخدام الفولتامترية الدورية عن نظام أكسدة-اختزال شبه عكسي للحديد الثنائي/الثلاثي، بجهود رسمية تبلغ 43.9 و 51.0 و 57.5 ملي فولت لكل من FcMe_2Ac و FcMe_3Ac و FcMe_4Ac على التوالي. وأكدت فروق الجهد بين الذروتين ($\Delta E_p = 87.8\text{--}115$ ملي فولت) ونسب التيار القريبة من الواحد صحة السلوك شبه العكسي. تراوحت معاملات الانتشار بين 1.11×10^{-7} و 5.74×10^{-7} سم ^{2} /ثانية.

10^{-7} سم² ث⁻¹، بينما تراوحت ثوابت معدل نقل الإلكترون غير المتجانس (k_s) بين 0.14×10^{-3} و 0.62

$\times 10^{-3}$ سم² ث⁻¹، مما يعكس تأثير الاستبدال على حركية نقل الإلكترون.

وأشارت حسابات نظرية الكثافة الوظيفية إلى طاقات HOMO بين -5.83 و -5.68 إلكترون فولت، وفجوات HOMO-LUMO تتراوح بين 3.99 و 4.29 إلكترون فولت، مما يوحي باستقرار كيميائي معتدل مقترن بتفاعلية مضبوطة. أظهرت مؤشرات التفاعل العالمية قيم صلابة تتراوح بين 1.995 و 2.143 إلكترون فولت، ومؤشرات محبة للإلكترونات تصل إلى 3.519 إلكترون فولت، بينما زادت عزوم ثنائي القطب من 3.82 إلى 5.12 ديبياي، مما يشير إلى زيادة قطبية الجزيئات عبر السلسلة.

أظهرت دراسات الالتحام الجزيئي مع أهداف متعلقة بالغدة الدرقية، بما في ذلك مستقبل هرمون الغدة الدرقية بيتا ومستقبل الهرمون المنبه للغدة الدرقية، تقاربًا ارتباطيًا جيدًا يتراوح بين -7.34 و -8.58 كيلو كالوري/مول، حيث أظهر $FcMe4Ac$ أقوى تفاعل. وأبرز تحليل نمط الارتباط أهمية التفاعلات الكارهة للماء، والروابط الهيدروجينية، وتفاعلات باي في تثبيت معقدات الرابطة-المستقبل.

أكدت محاكاة الديناميكا الجزيئية (100 نانو ثانية) استقرار معقدات $FcMe4Ac$ المستقبل، حيث استقرت قيم RMSD ضمن نطاق 0.14 – 0.24 نانومتر، مع انخفاض تقلبات البقايا، لا سيما في نظام مستقبل هرمون تحفيز الغدة الدرقية، مما يشير إلى صلابة هيكلية معززة وشبكات تفاعل مستمرة.

أظهرت تنبؤات ADMET خصائص حركية دوائية مواتية، بما في ذلك امتصاص عالٍ في الجهاز الهضمي، وقيم LogP تتراوح بين 3.18 و 3.52 ، وغياب التأثيرات المطفرة أو السامة للكبد المتوقعة. كما يدعم عدم نفاذية الحاجز الدموي الدماغي ملاءمتها للأهداف الهرمونية الطرفية.

بشكل عام، تُظهر النتائج أن التعديل الهيكلي لمشتقات الفيروسسينيل يؤثر بشكل كبير على خصائصها الإلكترونية وسلوكها الكهروكيميائي وملامح تفاعلها مع الأهداف المتعلقة بالغدة الدرقية. من بين المركبات المدروسة، يبرز مركب FcMe₄Ac كأكثر المرشحين الواعدين، إذ يجمع بين الخصائص الإلكترونية المثلى، وقوة الارتباط العالية، والاستقرار الديناميكي العالي، والخصائص الحركية الدوائية المواتية، مما يجعله أساساً محتملاً لمزيد من التطوير في البحوث العلاجية المتعلقة بالغدة الدرقية.

Contents

<i>DEDICATE</i>	I
<i>ACKNOWLEDGMENTS</i>	IV
Abstract	VI
LIST OF TABLES.....	XIX
LIST OF FIGURES.....	XX
ABBREVIATIONS	XXIII
General Introduction.	XXVIII
PART I: Bibliographic Study	1
Chapter 01: Ferrocene Derivatives: Structure, Reactivity and Applications	2
1. Introduction	3
2. Structural and Electronic Properties of Ferrocene	4
2.1 Ferrocene: Chemical Definition and Overview	4
2.2 Structural Description of Ferrocene	5
2.3 Fundamental Electronic Characteristics	7
3. Synthesis and Functionalization of Ferrocenyl Derivatives.....	8
3.1 Ferrocene Synthesis.....	8
3.1.1 The Chemical process of synthesizing ferrocene.....	8
3.2 Synthesizing some ferrocene Derivatives.....	9
3.2.1 N1-Ferrocenylmethylcytosine Synthesis	10
3.2.2 N1-Ferrocenylmethylthymine Synthesis.....	10
3.2.3 N6, N9-diferrocenylmethyladenine Synthesis	11
3.2.4 Metal-Catalyzed Synthesis of Ferrocenyl Heterocycles	11
4. Electrochemical Behaviour of Ferrocene Compounds.....	13
4.1 Fundamental Redox Characteristics and Kinetic Reversibility	13

4.2	Molecular Orbital Contributions and Structural Stability	13
4.3	Influence of Substituents on Redox Potentials	13
4.4	Interfacial Electrochemical Behavior at Liquid-Liquid Boundaries	14
4.5	Role as a Redox Standard.....	14
5	Biological and Pharmacological Relevance of Ferrocene Derivatives	15
5.1	Biological Properties of Ferrocene Derivatives.....	15
5.2	Pharmacological Applications of Ferrocene Derivatives	16
5.2.1	Anticancer Activity	16
5.2.2	Antioxidant Activity.....	16
5.2.3	Antimicrobial Activity	16
5.2.4	Antimalarial and Anti-parasitic Activity.....	17
	CONCLUSION	18
	Chapter 02: Computational Approaches in Molecular Modelling and Drug Design.....	19
1.	Introduction	20
2.	Density Functional Theory (DFT) and Electronic Descriptors	21
3.	Molecular Docking and Ligand–Protein Interactions	21
3.1	Molecular docking.....	21
3.2	The basic theory of molecular docking	22
3.3	Types of Docking.....	23
3.4	Approaches of Molecular Docking.....	24
3.4.1	Simulation Approach.....	24
3.4.2	Shape Complementarity Approach.....	25
3.5	APPLICATIONS OF MOLECULAR DOCKING.....	26
3.5.1	Lead optimization.....	26
3.5.2	Hit Identifications.....	27

3.5.3	Drug-DNA Interactions Studies	27
3.6	Molecular docking software	27
4.	Molecular Dynamics Simulation.....	28
4.1	Theoretical Background	28
4.2	Research Aim and Objectives.....	28
4.3	Methodological Approach	29
4.3.1	Model Selection:	29
4.3.2	Energy Minimization, Heating and Equilibration:	29
4.3.3	Production Run and Analysis	31
4.4	Scientific Applications.....	31
5.	ADMET Prediction and Drug-Likeness Evaluation	35
5.1	Definition of ADMET and Its Importance.....	35
5.2	Drug-Likeness Evaluation	35
5.3	Role of Computational Prediction in Drug Discovery	36
5.4	Examples of Tools and Models.....	37
5.4.1	QSAR/QSPR Models	37
5.4.2	Machine Learning Approaches.....	37
5.4.3	Common Computational Tools	37
	CONCLUSION	38
	PART II: EXPERIMENTAL	39
	Chapter 01: Materials & Methods.....	40
1.	General Introduction	41
2.	Chemicals and Reagents.....	42
3.	Instrumentation and Experimental Conditions.....	42
3.1.	Spectroscopic Analysis	42

3.1.1.	FT-IR Spectroscopy.....	42
3.1.2.	UV–Visible Spectroscopy	43
3.2.	Nuclear Magnetic Resonance (NMR).....	43
3.2.1.	¹ H NMR Analysis.....	43
3.2.2.	¹³ C NMR Analysis.....	43
3.2.3.	Two-Dimensional NMR (HSQC/HMBC).....	44
3.3.	Electrochemical Measurements (Cyclic Voltammetry)	44
4.	Synthesis of Ferrocenyl Acetylaniline Derivatives	44
4.1.	General Synthetic Procedure.....	45
4.2.	Synthesis of Individual Compounds	46
4.2.1.	N-[(ferrocenyl)methyl]-2-acetylaniline (FcMe2Ac).....	46
4.2.2.	N-[(ferrocenyl)methyl]-3-acetylaniline (FcMe3Ac).....	46
4.2.3.	N-[(ferrocenyl)methyl]-4-acetylaniline (FcMe4Ac).....	47
5.	Computational Methodology.....	47
5.1.	Density Functional Theory (DFT) Calculations	47
5.2.	Molecular Docking Studies.....	48
5.2.1.	Protein Preparation.....	48
5.2.2.	Ligand Preparation	49
5.2.3.	Docking Protocol and Validation	49
5.3.	Molecular Dynamics (MD) Simulations.....	50
5.3.1.	System Setup and Solvation.....	50
5.3.2.	Energy Minimisation and Equilibration.....	51
5.3.3.	Production Run Parameters.....	52
5.4.	MM-PBSA Binding Free Energy Calculations.....	53
5.5.	ADMET Prediction.....	53

Chapter 02: Results & Discussion.....	54
1. General Introduction	55
2. Synthesis of Ferrocenyl Acetylaniline Derivatives	55
3. FT-IR Spectral Analysis	58
4. UV–Visible Spectral Analysis.....	60
5. NMR Spectral Analysis.....	62
5.1. ¹ H NMR Analysis.....	63
5.2. ¹³ C NMR Analysis.....	64
5.3. Two-Dimensional NMR Analysis (HSQC and HMBC).....	65
6. Cyclic Voltammetry Characterization	68
6.1. Redox Properties and Voltammetric Response	68
6.2. Scan Rate Dependence and Mass Transport Regime.....	70
6.3. Diffusion Coefficients and Transport Properties	71
6.4. Electron Transfer Kinetics	72
7. Density Functional Theory (DFT) Analysis.....	74
7.1. Frontier Molecular Orbitals and Electronic Structure	74
7.2. Global Reactivity Descriptors and Thermodynamic Parameters.....	75
7.3. Molecular Electrostatic Potential (MEP) Analysis	77
7.4. Electron Localisation and Non-Covalent Interaction Analysis.....	78
8. Molecular Docking Study	81
8.1. Docking Protocol Validation.....	81
8.2. Binding Affinity and Energetic Analysis	82
8.3. Binding Mode and Interaction Analysis	82
8.4. Structure–Interaction Relationship	84
9. Molecular Dynamics Simulation.....	84

9.1.	System Stability: RMSD Analysis	85
9.2.	Residue Flexibility: RMSF Analysis	86
9.3.	Interaction Profile Analysis.....	86
10.	ADMET Prediction and Pharmacokinetic Evaluation	87
10.1.	Drug-Likeness and Physicochemical Properties.....	88
10.2.	Absorption and Distribution Properties	88
10.3.	Toxicological Profile.....	88
	General Conclusion.....	90
	References: Bibliographic Study.....	93
	References: EXPERIMENTAL.....	102

LIST OF TABLES

N°	Titled	Page
01	Molecular Docking Approaches. Simulation Approach	26.27
02	Yields and physical properties of the synthesised ferrocenyl acetylaniline derivatives.	58
03	Characteristic FT-IR absorption bands (cm^{-1}) and their assignments for the synthesised compounds.	60
04	UV-visible absorption maxima (λ_{max}) of the synthesised ferrocenyl acetylaniline derivatives.	62
05	Diffusion coefficients and linear regression parameters obtained from Randles-Ševčík analysis	73
06	Nicholson kinetic parameters (Ψ , ΔE_p) and heterogeneous electron-transfer rate constants (k_s).	74
07	Frontier molecular orbital energies (eV) of the synthesised compounds.	76
08	Global reactivity descriptors and thermodynamic parameters of the synthesised derivatives at 298.15 K.	77
09	Molecular docking results of ferrocenyl acetylaniline derivatives against thyroid-related targets.	82
10	Predicted physicochemical properties and drug-likeness parameters of ferrocenyl derivatives	88
11	Predicted absorption and distribution properties of the studied compounds.	88
12	Predicted toxicity profiles of ferrocenyl acetylaniline derivatives.	88

LIST OF FIGURES

N°	Titled	Page
01	Ferrocene structure	05
02	the chemical structure depiction of ferrocene (2Dimensional Structure)	05
03	Wrong (a) and corrected (b) structures of ferrocene	06
04	A model of the ferrocene molecule with staggered (D5d) and eclipsed (D5h) symmetries.	07
05	Molecular Orbital Diagram of Ferrocene	08
06	Chemical formula of N1-ferrocenylmethylcytosine	11
07	Chemical formula of N1-ferrocenylmethylthymine	11
08	Chemical formula of N6, N9-diferrocenylmethyladenine	12
09	Synthesis of 4-ferrocenyl-2-phenylquinoline derivatives	13
10	Synthesis of 2,5-bis(2-ferrocenyl-6-methylpyridin-4-yl) thiophene	13
11	Synthesis of ferrocene-functional thiophene derivative	13
12	shows the first proposed “lock-and-key model “and “induced fit model”	23
13	Molecular docking process	24
14	The protein-DNA docking	24
15	Molecular docking software classification	28
16	Synthetic route for the preparation of N-[(ferrocenyl)methyl] acetylaniline derivatives (FcMe2Ac, FcMe3Ac and FcMe4Ac)	46
17	Three-dimensional structures of the target proteins used in docking studies: (a) xanthine oxidase (PDB ID: 1FIQ) and (b) NADPH oxidase (PDB ID: 8WEJ).	49
18	Optimised molecular structures of ferrocenyl derivatives used as ligands: FcMe2Ac (a), FcMe3Ac (b) and FcMe4Ac (c).	50
19	Molecular docking workflow illustrating protein and ligand preparation, grid generation, docking execution and interaction analysis	51
20	Solvated protein–ligand complex showing the explicit water box used for molecular dynamics simulations	52

21	Molecular dynamics simulation workflow including system preparation, energy minimisation, equilibration and production phases	53
22	Proposed reaction mechanism for the formation of N-[(ferrocenyl)methyl] acetylaniline derivatives via nucleophilic substitution	57
23	Chemical structures of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac (ortho), (b) FcMe3Ac (meta), and (c) FcMe4Ac (para)	58
24	FT-IR spectra of the synthesised ferrocenyl acetylaniline derivatives	60
25	UV–visible absorption spectra of the synthesised ferrocenyl acetylaniline derivatives	62
26	¹ H NMR spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac	64
27	¹³ C NMR spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac	65
28	HSQC spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac	66.67
29	HMBC spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac	67.68
30	Cyclic voltammograms of Fc, FcMe2Ac, FcMe3Ac and FcMe4Ac recorded in DMSO containing 0.1 M Bu ₄ NBF ₄ at a scan rate of 100 mV·s ⁻¹ (referenced to Fc ⁺ /Fc).	70
31	Cyclic voltammograms of Fc, FcMe2Ac, FcMe3Ac and FcMe4Ac at different scan rates (100–500 mV·s ⁻¹).	71
32	Linear variation of anodic peak current (<i>i</i> _{pa}) as a function of the square root of scan rate (<i>v</i> ^{1/2}). 72	72
33	Frontier molecular orbitals of ferrocenyl acetylaniline derivatives: HOMO and LUMO surfaces.	75
34	Molecular electrostatic potential (MEP) maps of ferrocenyl acetylaniline derivatives showing charge distribution	78
35	Electron localisation (ELF and LOL) analyses of FcMeAc	79.80
36	Non-covalent interaction (RDG/NCI) analyses of FcMeAc	80

37	Superposition of the co-crystallised ligand and the redocked pose within the active site (validation of docking protocol)	81
38	Three-dimensional binding mode of FcMe4Ac within the binding pocket of the thyroid receptor	83
39	RMSD profiles of receptor and receptor–FcMe4Ac complexes during 100 ns simulation: (A) thyroid hormone receptor β and (B) thyroid-stimulating hormone receptor systems.	85
40	RMSF profiles of receptor residues in complex with FcMe4Ac: (A) TR β and (B) TSHR systems	86
41	. Interaction fraction analysis showing hydrogen bonds, hydrophobic interactions and water bridges: (A) TR β and (B) TSHR complexes	87

ABBREVIATIONS

η^5 : denotes those five atoms of the cyclopentadienyl ring are coordinated to the metal center

C_5H_5 : cyclopentadienyl ring

Fe: iron

NMR: Nuclear Magnetic Resonance

D₅: point group symmetry

DMSO: dimethyl sulfoxide

HCl: hydrogen chloride

THF: tetrahydrofuran

FcCy: N1-ferrocenylmethylcytosine

FcTh: N1-ferrocenylmethylthymine

(Fc)₂Ad: N6, N9-diferrocenylmethyladenine

Ce (OTf)₃: cerium (III) trifluoromethanesulfonate

Y(OTf)₃: yttrium (III) trifluoromethanesulfonate

Sc (OTf)₃: scandium (III) trifluoromethanesulfonate

Yb (OTf)₃: ytterbium (III) trifluoromethanesulfonate

CeCl₃: cerium (III) chloride

Cu(I): copper(I)

I_{pa}: anodic peak current

I_{pc}: cathodic peak current

ΔE_p : peak-to-peak potential difference

mV: millivolt

E_{1/2}: half-wave potential

V: volt

SCE: saturated calomel electrode

NHE: normal hydrogen electrode

Fc: ferrocene

Fc⁺: ferrocenium ion

Fe²⁺: iron (II) ion

Cp: cyclopentadienyl

HOMO: highest occupied molecular orbital

LUMO: lowest unoccupied molecular orbital
HIV: human immunodeficiency virus
ROS: reactive oxygen species
TDZ: thiadiazol
P. falciparum: Plasmodium falciparum
P. berghei: Plasmodium berghei
DFT: Density Functional Theory
DNA: deoxyribonucleic acid
GOLD: Genetic Optimization for Ligand Docking
ICM: Internal Coordinate Mechanics
VMD: Visual Molecular Dynamics
MMTK: Molecular Mechanics Toolkit
pdb: Protein Data Bank file format
psf: protein structure file
PDB: Protein Data Bank
CA: alpha carbon
ID: identifier
T: temperature
BFGS: Broyden–Fletcher–Goldfarb–Shanno
MD: molecular dynamics
NVT: constant number of particles, volume, and temperature
NPT: constant number of particles, pressure, and temperature
ANM: Anisotropic Network Model
ENM: Elastic Network Model
PCA: Principal Component Analysis
SBDD: structure-based drug design
LBDD: ligand-based drug design
ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity
Da: Dalton
LogP: octanol–water partition coefficient
QSAR: Quantitative Structure–Activity Relationship
QSPR: Quantitative Structure–Property Relationship

MM-PBSA: Molecular Mechanics Poisson–Boltzmann Surface Area
FT-IR: Fourier Transform Infrared spectroscopy
FT-IR: Fourier Transform Infrared
KBr: potassium bromide
UV: ultraviolet
¹H NMR: proton nuclear magnetic resonance
MHz: megahertz
ppm: parts per million
TMS: tetramethylsilane
¹³C NMR: carbon-13 nuclear magnetic resonance
HSQC: heteronuclear single quantum coherence
HMBC: heteronuclear multiple bond correlation
SAS: Société par Actions Simplifiée
Hg/Hg₂Cl₂: mercury/mercury(I) chloride electrode
MEP: molecular electrostatic potential
ELF: electron localisation function
LOL: localised orbital locator
RDG: reduced density gradient
ADT: AutoDock Tools
PDBQT: Protein Data Bank, charge, and atom type format
CHARMM36: Chemistry at Harvard Macromolecular Mechanics 36
CGenFF: CHARMM General Force Field
TIP3P: Transferable Intermolecular Potential with 3 Points
NVT: constant number of particles, volume, and temperature
NPT: constant number of particles, pressure, and temperature
ps: picosecond
atm: atmosphere
ns: nanosecond
fs: femtosecond
PME: Particle Mesh Ewald
LINCS: Linear Constraint Solver
RMSD: root mean square deviation

RMSF: root mean square fluctuation

Rg: radius of gyration

SASA: solvent accessible surface area

g_mmpbsa: GROMACS Molecular Mechanics Poisson–Boltzmann Surface Area

ΔG_{bind} : binding free energy

LD₅₀: median lethal dose

2D NMR: two-dimensional nuclear magnetic resonance

MM-PBSA: Molecular Mechanics Poisson–Boltzmann Surface Area

SN2: bimolecular nucleophilic substitution

¹JCH: one-bond proton–carbon coupling constant

Bu₄NBF₄: tetrabutylammonium tetrafluoroborate

²JCH: two-bond proton–carbon coupling constant

³JCH: three-bond proton–carbon coupling constant

v: scan rate

R²: coefficient of determination

A (area): electrode surface area

D: diffusion coefficient

k_s: standard heterogeneous electron-transfer rate constant

n: number of electrons transferred

F: Faraday constant

C·mol⁻¹: coulomb per mole

V·s⁻¹: volt per second

R: gas constant

J·mol⁻¹·K⁻¹: joule per mole per kelvin

T: absolute temperature

K: kelvin

Ψ: Nicholson kinetic parameter

ΔE_p : peak-to-peak potential separation

NCI: non-covalent interaction

LEU : leucine

ILE : isoleucine

VAL : valine

PHE: phenylalanine

TYR: tyrosine

ASN: asparagine

GLU: glutamic acid

TR β : thyroid hormone receptor beta

TSHR: thyroid-stimulating hormone receptor

BBB: blood–brain barrier

General Introduction

Ferrocene and its derivatives are widely studied in organometallic chemistry because of their well-defined structure, stability, and accessible reactivity. These features make them useful not only for fundamental studies but also for the development of functional compounds. In particular, the possibility of modifying the cyclopentadienyl rings allows the preparation of a wide range of derivatives with different chemical and physical properties. In this context, Chapter 1 presents the main aspects related to ferrocene derivatives. It begins with their structural and electronic properties, which are essential for understanding their behaviors. It then considers common methods of synthesis and functionalization, followed by a description of their electrochemical properties. Finally, some biological and pharmacological aspects are discussed, highlighting the interest of these compounds in applied fields.

At the same time, the study of such systems increasingly relies on computational methods. Chapter 2 introduces several approaches used in molecular modelling and drug design. Density functional theory and electronic descriptors are used to analyze molecular properties at the electronic level. Molecular docking provides information on possible ligand–protein interactions, while molecular dynamics simulations allow the study of the stability and flexibility of these systems over time. In addition, ADMET prediction and drug-likeness evaluation offer preliminary insight into the behaviors of molecules in a biological context.

Together, these chapters provide a general overview that combines experimental knowledge of ferrocene derivatives with computational tools commonly used to interpret and predict their properties.

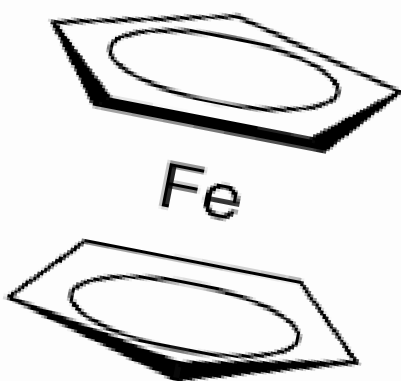
PART I:

Bibliographic Study



Chapter 01:

Ferrocene Derivatives: Structure, Reactivity and Applications



1. Introduction

In 1951, Thomas J. Kealy and Peter L. Pauson at Duquesne University reported the reaction of cyclopentadienyl magnesium bromide with ferric chloride. (Kealy & Pauson, 1951)

with the aim of preparing fulvalene via a Grignard reagent. Instead, they obtained a yellow–orange powder containing iron and two cyclopentadienyl ligands. (Miller et al, 1952) Although the initial reports already noted its high and unexpected stability, the correct structure was soon afterward independently proposed by Geoffrey Wilkinson (Wilkinson et al., 1952) and Ernst Otto Fischer. (Fischer & Pfab, 1952).

Owing to the similarity of its reactivity to that of benzene, the name ferrocene was coined for this compound by Robert Burns Woodward. (Woodward et al., 1952) The term “sandwich compound” is now widely used to describe this structure and a broader class of related compounds.

The discovery of ferrocene and the elucidation of its remarkable structure are widely considered to mark the beginning of modern organometallic chemistry. In recent years, bioorganometallic chemistry has emerged as a rapidly developing field linking classical organometallic chemistry with biology, medicine, and molecular biotechnology. (Jaouen et al., 1993) The stability of the ferrocenyl group in aqueous and aerobic media, the accessibility of a wide range of derivatives, and its favorable electrochemical properties have made ferrocene and its derivatives highly attractive for biological applications and for conjugation with biomolecules. (Van Staveren & Metzler-Nolte, 2004).

In this chapter, the structural and electronic properties of ferrocene, its synthesis and functionalization, its electrochemical behaviors, and its biological and pharmacological relevance will be discussed.

2. Structural and Electronic Properties of Ferrocene

2.1 Ferrocene: Chemical Definition and Overview

Bis-cyclopentadienyl iron or ferrocene is an organometallic compound with the formula $(\eta^5\text{C}_5\text{H}_5)_2\text{Fe}$, in this formula η^5 indicates that the five atoms of the C_5H_5 rings are coordinating to the iron cation, it is the prototypical metallocene, a type of organometallic chemical compounds consisting of two cyclopentadienyl rings bound on opposite sides of a central of a metal cation, such organometallic compounds are also known as "Sandwich Compound ". (Kealy & Pauson, 1951) Figure 01 shows the ferrocene structure, Figure 02 shows the chemical structure depiction of ferrocene (2Dimensional Structure).

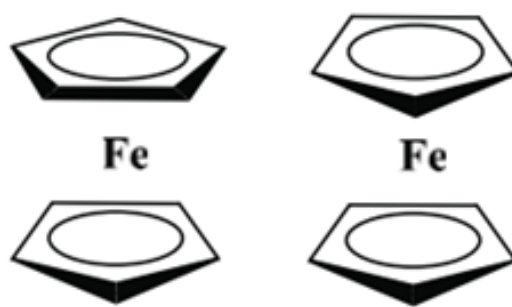


Figure 01: Ferrocene structure.

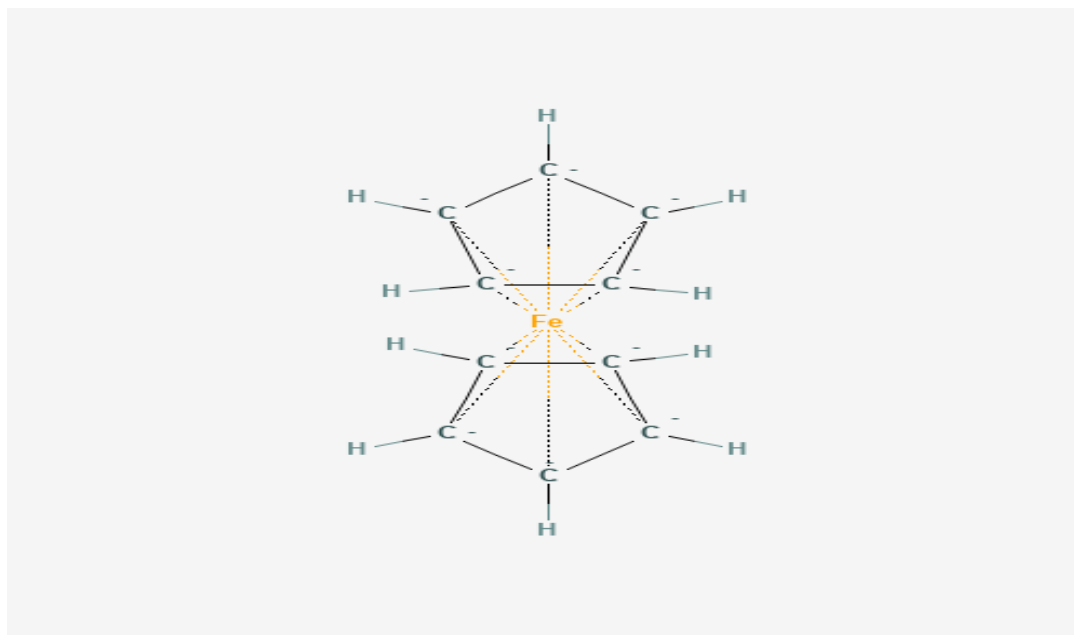


Figure 02: the chemical structure depiction of ferrocene (2Dimensional Structure) [National Center for Biotechnology Information, 2026](#)

2.2 Structural Description of Ferrocene

Ferrocene's molecular structure was determined by Woodward and Wilkinson of Imperial College London through an understanding of its chemical properties, which are analogous to those exhibited by aromatic compounds like benzene. (Wilkinson et al., 1952) Eiland and coworkers confirmed the ferrocene structure using two complementary techniques. First, X-ray crystallography established the precise arrangement in the solid state. Then, NMR spectroscopy provided additional structural confirmation and information consistent with the expected symmetry and bonding. (Eiland & Pepinsky, 1952) .

In the solid phase, the two cyclopentadienyl rings exist in several phases in which the rings are co-parallel but in different orientations, but in the gas phase, the two cyclopentadienyl rings are eclipsed, Figure 03. This is due to the low barrier to rotation at ambient temperature, which is so low that there is even rotation in the solid state after crystallization. Figure 03a presents the incorrect molecular structure of ferrocene proposed by Pauson and Kealy, Figure 03b represents the corrected structure proposed by Woodward and Wilkinson. The two parallel cyclopentadienyl rings of ferrocene can be orientated in the eclipsed conformation (D_{5h} symmetry) or staggered conformation (D_{5d} symmetry). It has been settled. (Gryaznova et al., 2010).

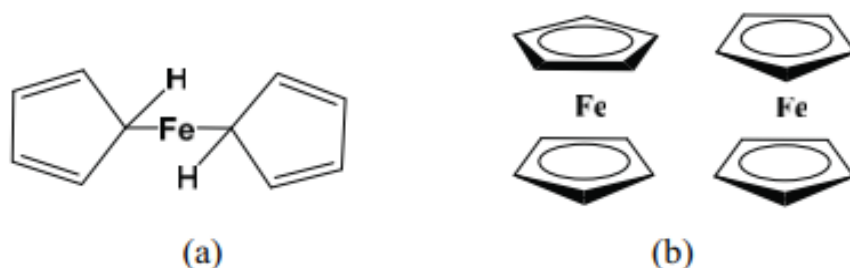


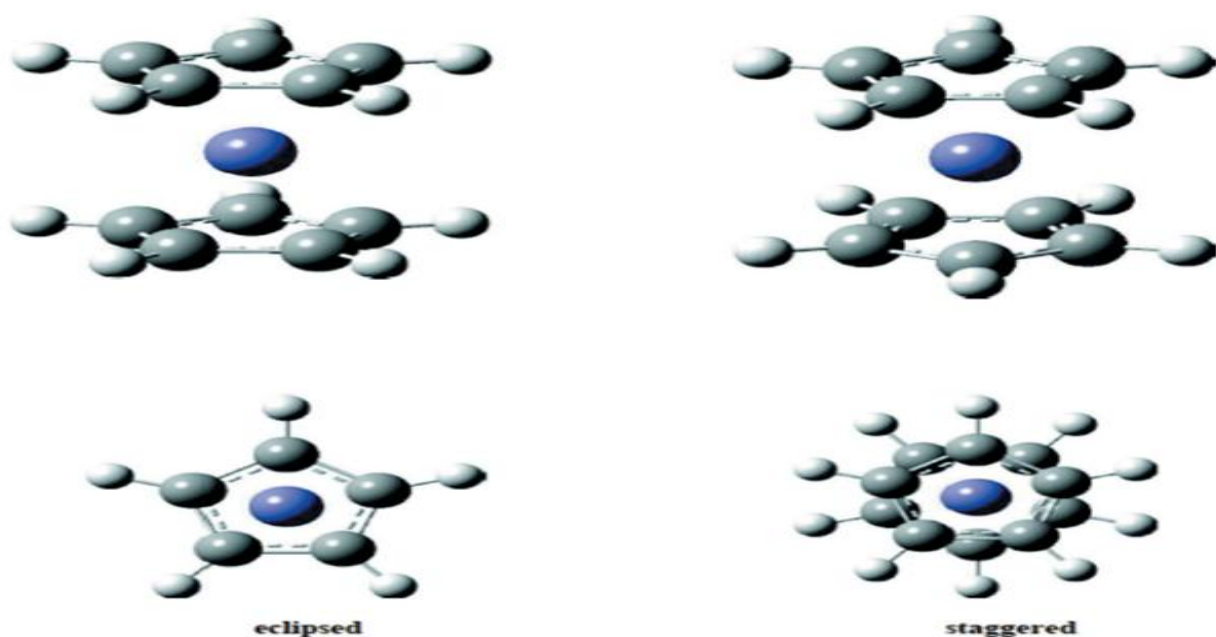
Figure 03. Wrong (a) and corrected (b) structures of ferrocene

that a staggered ferrocene structure of D_{5d} point group symmetry dominates experiments in the condensed phase. (Fischer & Pfab, 1952; Eiland & Pepinsky, 1952; Dunitz & Orgel, 1953; Dunitz et al., 1956). whereas the eclipsed structure of D_{5h} point group symmetry is found in the gas phase. (Bohn & Haaland, 1966; Haaland et al., 1974) The eclipsed conformation was also observed at 90 K in the solid (Seiler & Dunitz, 1982). The eclipsed conformation was also observed at 90 K in the solid and at room temperature in solutions. (Chantler et al., 2012) As most of the theoretical studies of ferrocene refer to gas phase or solutions, the eclipsed (D_{5h}) structure of

ferrocene has been more extensively studied than the D_{5d} conformer, this being due to the small difference in energy of the two conformations and the low energy barrier for the rotation about the Fe-C₅H₅ axis which is equal approximately to 4 KJmol⁻¹. (Cotton & Wilkinson, 1988; Mohammadi et al., 2012).

Properties of the staggered and eclipsed conformers of ferrocene are not markedly different due to the shape of the conformers. Although the electronic state symmetries of the eclipsed conformation D_{5h} differs from the staggered conformation D_{5d}, in the upcoming presentation of the electronic structure of ferrocene the conformation D_{5d} will be used as it simplifies the symmetry matching of the cyclopentadienyl rings molecular orbitals and the iron atomic orbitals.

Figure 04. A model of the ferrocene molecule with staggered (D_{5d}) and eclipsed (D_{5h}) symmetries. (Chantler et al., 2012).



2.3 Fundamental Electronic Characteristics

Although the electronic state symmetries of the eclipsed conformation D_{5h} differs from the staggered conformation D_{5d} , in the upcoming presentation of the electronic structure of Ferrocene the conformation D_{5d} will be used as it simplifies the symmetry matching of the cyclopentadienyl rings molecular orbitals and the iron atomic orbitals. Each carbon atom of the two cyclopentadiene anions in the ferrocene chemical structure has a p-orbital perpendicular to the ring, these p-orbitals can be combined to produce ten molecular orbitals of varying energies. If D_{5d} symmetry is considered, so that there is a center of symmetry in the ferrocene molecule through the iron atom, there will be center-symmetric (g) and anti-symmetric (u) combinations filled by the 12 electrons in the two cyclopentadienyl rings. The molecular orbitals of the two cyclopentadiene anions can be considered together to produce eleven symmetry adapted linear combinations, nine of which form bonding and antibonding combinations with the central iron cation's 3d, 4s and 4p orbitals. Each of these above ten molecular orbital of the two-cyclopentadienyl anion combines to give ten ligand molecular orbitals in three energy levels. In this diagram ten electrons are contributed by the two cyclopentadiene molecules, whereas the iron contributes eight electrons, making it an eighteen-electron system. These eighteen electrons are accommodated in the low-lying molecular orbitals, excluding the antibonding ones. Stability is attributed to the aromatic character of the cyclopentadienyls. (Lanez, 2019)

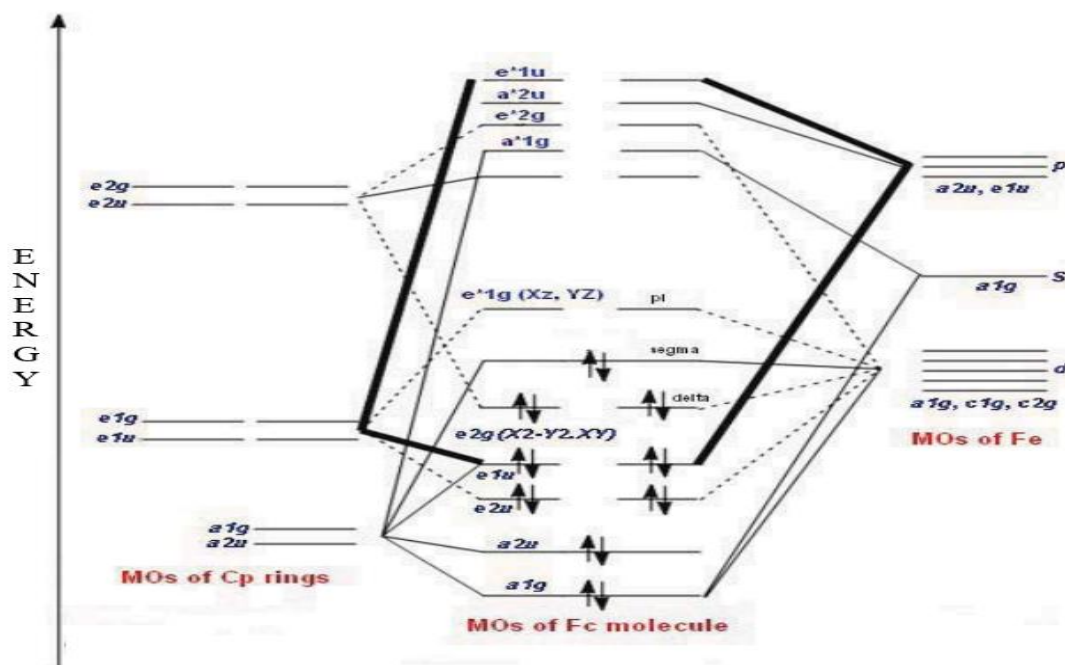


Figure 05 Molecular Orbital Diagram of Ferrocene

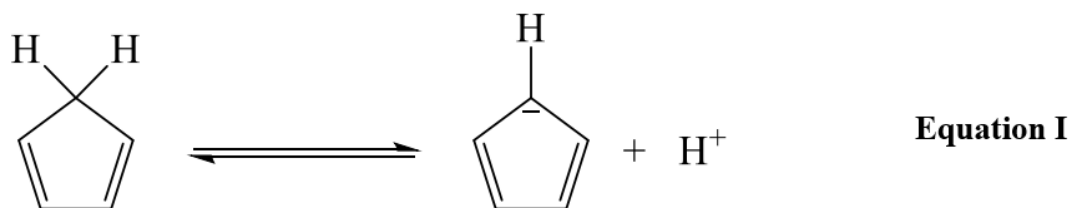
3. Synthesis and Functionalization of Ferrocenyl Derivatives

3.1 Ferrocene Synthesis

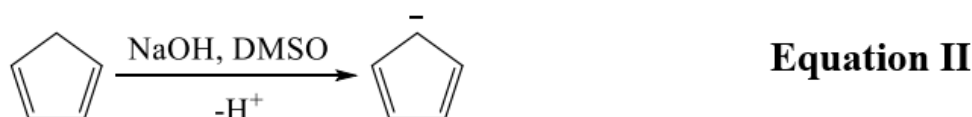
Ferrocene synthesis represents a foundational transformation in organometallic chemistry, marking the formation of a highly stable sandwich complex. Its preparation is widely studied due to its simplicity and historical importance. In this section, we will discuss how ferrocene is synthesized chemically and some of its derivatives.

3.1.1 The Chemical process of synthesizing ferrocene

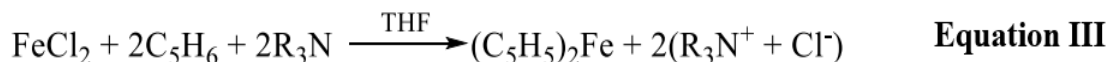
Cyclopentadiene ($\text{pK}_a \approx 20$) is significantly more acidic than alkanes, whose acidity is much lower—for example, methane has a pK_a of about 60. (Equation I)



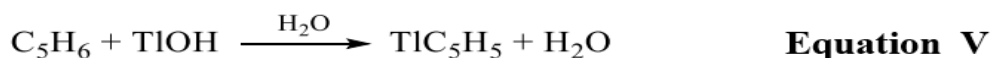
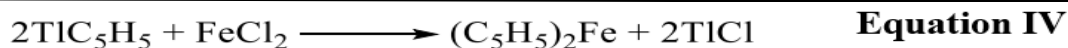
This level of acidity allows cyclopentadiene to react with sodium hydroxide in DMSO, forming the cyclopentadienyl anion. DMSO, being a polar aprotic solvent, increases the basicity of the hydroxide ion by effectively solvating the Na^+ counterion. (Equation II)



A sufficiently strong base like diethylamine can deprotonate cyclopentadiene to form the cyclopentadienyl anion. In a related method, cyclopentadiene is combined directly with ferrous chloride and diethylamine; in this case, the amine (or a diamine) acts as an HCl scavenger, promoting the generation of the cyclopentadienyl ion. (Equation III) ([Wilkinson, 1956](#))



Cyclopentadienylthallium can also be used as a starting material for the preparation of ferrocene as shown in Equation IV and Equation V. ([Leigh, 1964](#))



The Grignard reagent derived from cyclopentadiene—an easily obtained intermediate—was employed by Kealy and Pauson. (Kealy & Pauson, 1951) in their historic synthesis of ferrocene (Equation VI). This method was developed by Pauson in 1955. (Pauson, 1955)

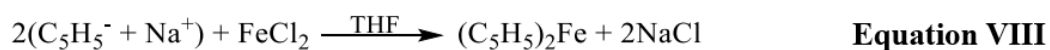
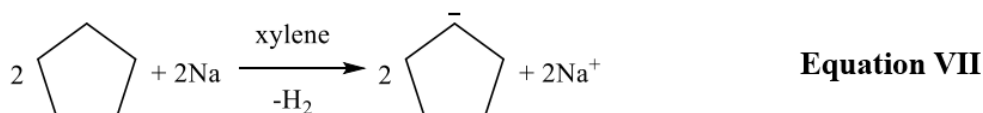


This method succeeds because ferric chloride is soluble in organic solvents. If the ferrous halide remains insoluble in the solvent, no reaction takes place; however, chelation can "solubilize" the ferrous ion, resulting in high yields of ferrocene. The Grignard reaction also requires an excess of the reagent.

Several other synthetic routes to ferrocene have been proposed. The compound can be obtained by directly reacting cyclopentadiene with metallic iron (Miller et al., 1952), by reacting ferrous chloride with sodium cyclopentadienyl in an ammonia solvent, and by treating cyclopentadiene with the ferrous complex of acetylaceton-dipyridine (Wilkinson et al., 1954).

A method for preparing ferrocene and analogous compounds was developed by drawing on the work of several research teams. (Wilkinson & Birmingham, 1954; Pauson & Wilkinson, 1954).

The approach essentially involves the deprotonation of cyclopentadiene through a redox reaction with sodium in xylene (Equation VII), followed by treatment of the resulting cyclopentadienyl salt with ferrous chloride in THF (Equation VIII).



3.2 Synthesizing some ferrocene Derivatives

Ferrocene derivatives constitute an important class of organometallic compounds. Currently, ferrocene is considered a widely applicable organometallic scaffold for the synthesis of various functional derivatives. (Larik et al., 2016). Representative examples of ferrocene derivative synthesis are provided below.

3.2.1 N1-Ferrocenylmethylcytosine Synthesis

The synthesis of the derivative N1-ferrocenylmethylcytosine (FcCy), shown in [Figure 06](#), was carried out using the well-known quaternary salt trimethylferrocenylmethylammonium iodide and the cytosine nitrogenous base in boiling water. The molar ratio cytosine/quaternary salt was 2/1.3, and the reaction time was six hours. The obtained product was washed with water and recrystallized from dimethylformamide to yield FcCy in 65%.

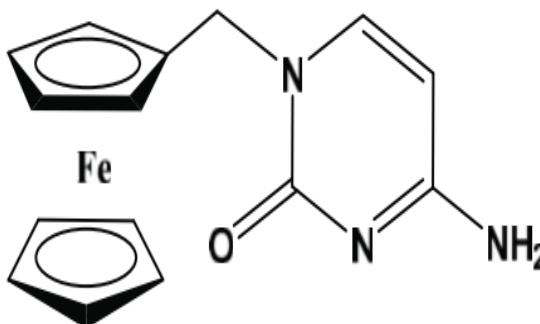


Figure 06 Chemical formula of N1-ferrocenylmethylcytosine

3.2.2 N1-Ferrocenylmethylthymine Synthesis

The synthesis of the derivative N1-ferrocenylmethylthymine (FcTh), shown in [Figure 07](#), was achieved using the well-known quaternary salt trimethylferrocenylmethylammonium iodide and the thymine nitrogenous base in boiling water. The molar ratio cytosine/quaternary salt was 2/1.3, and the reaction time was six hours. The final product was washed with water and purified by column chromatography on alumina, eluting with a 9/1 mixture of chloroform/methanol. The main fraction, upon evaporation, yielded FcTh in 25%.

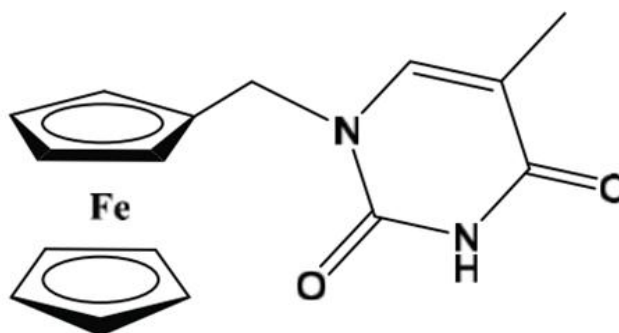


Figure 07 Chemical formula of N1-ferrocenylmethylthymine

3.2.3 N6, N9-diferrocenylmethyladenine Synthesis

The synthesis of the derivative N6, N9-diferrocenylmethyladenine ((Fc)₂Ad), shown in [Figure 08](#), was achieved using the well-known quaternary salt trimethylferrocenylmethyl-ammonium iodide and the adenine nitrogenous base in boiling water. The molar ratio cytosine/quaternary salt was 2/1.3, and the reaction time was six hours. The formed precipitate was washed with water and chromatographed on neutral alumina using chloroform as the eluent. The first fraction was collected and evaporated to produce a yellow precipitate, which was recrystallized from a mixture of acetonitrile/methanol to yield (Fc)₂Ad in 10%. ([Houlton et al., 1999](#)).

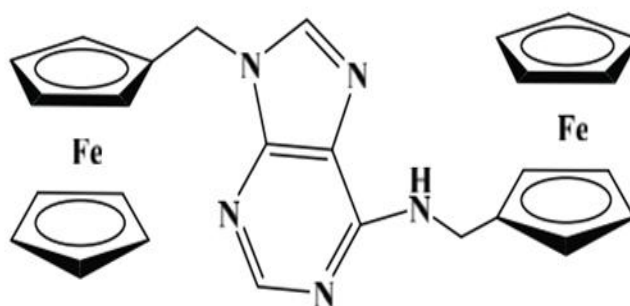


Figure 08 chemical formula of N6, N9-diferrocenylmethyladenine

3.2.4 Metal-Catalyzed Synthesis of Ferrocenyl Heterocycles

The Ce (OTf)₃-catalyzed one-pot three-component ‘domino’ cyclization/aromatization reaction ([Figure 09](#)) of ferrocenylacetylene 1 with aniline 2 and substituted benzaldehydes 3 under an air atmosphere to afford 4-ferrocenyl-2-phenylquinoline derivatives 4 in good yields. ([Chen et al., 2013](#)). A series of quinoline derivatives were readily prepared from easily available starting materials. Various catalysts such as Y(OTf)₃, Sc (OTf)₃, and Yb (OTf)₃ were employed, but the most effective catalyst was Ce (OTf)₃, affording the product in 75% yield. Other metal triflates and CeCl₃ gave only moderate yields. The synthesis of thiophenyl-substituted 2-ferrocenylpyridine derivatives via palladium-catalyzed Stille coupling reaction of 5 with 2,5-bis(tributylstannyl)thiophene to afford 2,5-bis(2-ferrocenyl-6-methylpyridin-4-yl) thiophene 6 was reported ([Figure 10](#)). ([Podolan et al., 2015](#)).

The synthesized compound 6 was used as a ligand in organometallic chemistry and catalysis. An expeditious method for the synthesis of the ferrocene-functional thiophene derivative (ThiFc) compound 9 via 1,3-dipolar cycloaddition between azides 7 and ethynylferrocene 8 ([Figure 12](#)) through a Cu(I)-catalyzed ‘click’ reaction was reported by Karagollu et al. The triazole thus produced was used in ion-sensing applications and also served as a chemical probe for phosphate-

containing organic molecules, such as phospholipids. (Karagollu et al., 2014) Azide-functional Merrifield resin was used to scavenge excess ethynyl ferrocene upon completion of the reaction. Cu(I)- catalyzed click chemistry is therefore a versatile and effective strategy, even in heterogeneous reaction systems. (Larik et al., 2016).

Figure 09: Synthesis of 4-ferrocenyl-2-phenylquinoline derivatives

Figure 10: Synthesis of 2,5-bis(2-ferrocenyl-6-methylpyridin-4-yl) thiophene

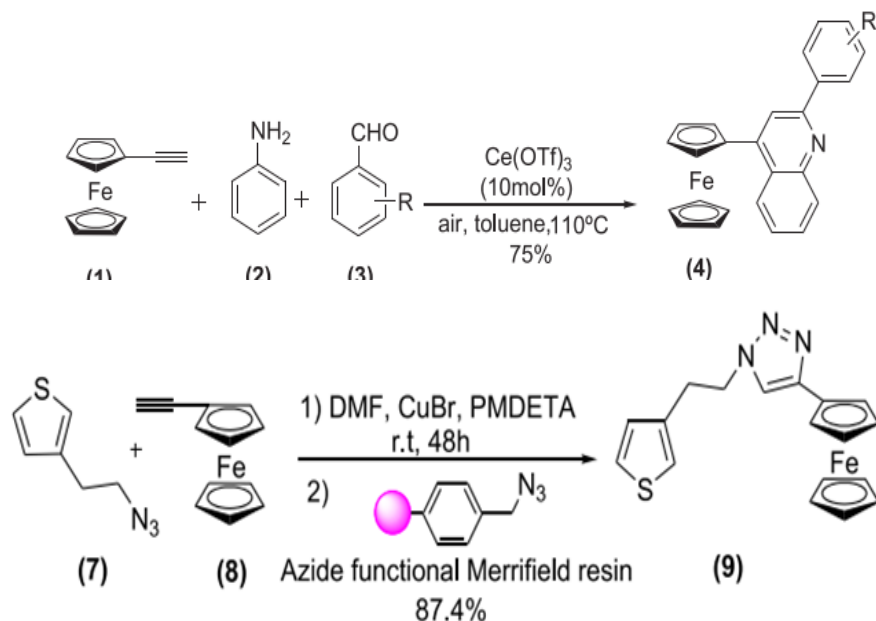
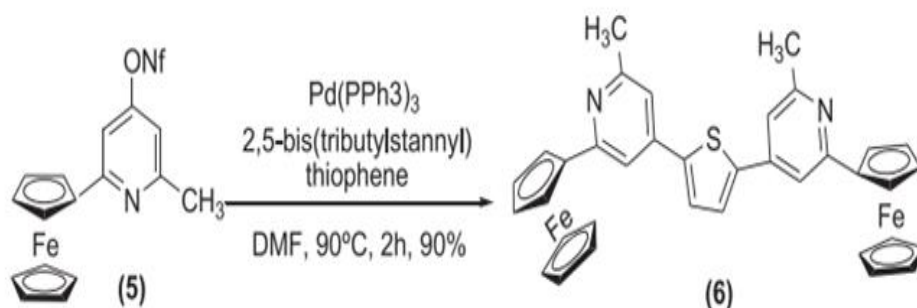


Figure11: Synthesis of ferrocene-functional thiophene derivative



4. Electrochemical Behaviour of Ferrocene Compounds

4.1 Fundamental Redox Characteristics and Kinetic Reversibility

Ferrocene exhibits complex electrochemical behavior characterized by a stable, and reversible Faradaic process, fast charge-transfer kinetic, and a current density ratio of oxidation to reduction that remains nearly unity ($I_{pa}/I_{pc} \approx 1$). The peak-to-peak difference, ΔE_p is very close to ideal value (59 mV for 1e⁻ transferred redox-process). The first polarographic investigation of the ferrocene/ferrocenium couple determined a half-wave potential ($E_{1/2}$) of 0.31 V vs SCE (0.56 V vs NHE) in ethanol/water 9:1. This one-electron Fc/Fc^+ redox change is chemically and thermally stable, making ferrocene an iconic probe for unravelling electrochemical charge-transfer dynamics at electrode interfaces. The self-exchange reaction is fast and kinetically uncomplicated, and its rate is notably solvent-independent. In cyclic voltammetry profiles at 25°C, the heterogeneous electron transfer is considered electrochemically reversible because the electron transfer coefficient exceeds the mass transport coefficient.

4.2 Molecular Orbital Contributions and Structural Stability

The exceptional reversibility of ferrocene is closely related to its 18-electron configuration and its sandwich structure. The oxidation process involves removal of an electron from a molecular orbital that is essentially non-bonding and has predominant d_{xz} character.

Because this orbital is non-bonding, the overall geometry of the molecule is preserved upon one-electron oxidation, with only a slight elongation of the Fe–C bond distances. In addition, the iron center is located between the cyclopentadienyl rings, which provides effective shielding from solvent interactions and contributes to the stability of the redox process across different media.

The covalent interaction between the sp^2 -hybridized carbon atoms of the cyclopentadienyl ligands and the d orbitals of Fe^{2+} results in a robust molecular framework that exhibits high chemical stability, including resistance to strongly acidic and basic conditions.

4.3 Influence of Substituents on Redox Potentials

The redox potential of the ferrocene system is highly sensitive to chemical functionalization on the cyclopentadienyl (Cp) rings, though such modifications generally not affect electrochemical reversibility. It is observed that substituting ferrocene with an electron-withdrawing group increases the oxidation potential, whereas electron-donating group decreases.

For instance, introducing simple electron-donating units like methyl or tert-butyl groups gradually lowers the potential, with decamethylferrocene (*Me10Fc*) showing a shift of approximately 0.5 V toward negative values compared to pristine ferrocene.

Conversely, highly conjugated alkynyl-ferrocene and -biferrocene derivatives exhibit potentials shifted to the more positive side due to the electron-withdrawing effect of ethynylarene substituents. Additionally, the oxidation state of the ferrocene unit can modulate the acidity of attached groups; for ferrocenecarboxylic acid, the positive charge of the oxidized Fc^+ form enhances substantially the acidity of the carboxylic group. (Roy & Mougel, 2022; Fabbrizzi, 2020).

4.4 Interfacial Electrochemical Behavior at Liquid-Liquid Boundaries

Electrochemical investigations at oil/water interfaces reveal distinct potential shifts compared to bulk aqueous media. Research indicates an approximately 0.07 V shift when the ferrocene oxidation was conducted at the oil/water interface compared with the direct oxidation in the conventional aqueous phase. This potential shift is directly influenced by the partitioning of the redox species in both solvents. The ionic strength of the aqueous phase is the most determining factor in these interfacial studies, as a higher aqueous phase ionic strength dramatically decreases the ferrocene diffusion from the oil to the aqueous phase. Steady-state polarization curves recorded as Tafel plots show that reaction mechanisms and reaction geometry are different when the electrode is placed at the interface versus directly in the aqueous phase, which is attributed to a loss of a degree of freedom when the ferrocene is bound in the oil phase. (Silva et al., 2016)

4.5 Role as a Redox Standard

Due to its well-defined and reproducible redox behavior, ferrocene is widely used as an internal redox standard in non-aqueous electrochemistry. It is recommended for this purpose because it helps to minimize uncertainties associated with liquid junction potentials.

It is commonly employed for the calibration of potential stats and reference electrodes, and for the estimation of HOMO and LUMO energy levels in organic, organometallic, and coordination compounds. (Roy & Mougel, 2022; Fabbrizzi, 2020).

5. Biological and Pharmacological Relevance of Ferrocene Derivatives

Ferrocene is an organometallic compound consisting of a transition metal (iron) sandwiched between two cyclopentadienyl rings. Due to its structural characteristics, ferrocene and its derivatives have been studied extensively, especially in medicinal chemistry. In particular, several ferrocenyl compounds have demonstrated significant biological activities, including kinase inhibition, which may contribute to the modulation of enzymatic pathways involved in various diseases. (Sanoosook et al., 2023)

Organometallic chemistry and biochemistry have been merged into a new field known as bioorganometallic chemistry, which focuses on the synthesis of organometallic compounds and their biological and medical effects. In this context, ferrocene has attracted growing interest due to its stability and redox properties, and several promising biological and medicinal applications have been reported. (Fouda et al., 2007)

5.1 Biological Properties of Ferrocene Derivatives

The incorporation of ferrocene moieties into bioactive compounds often enhances their biological activity. (Larik et al., 2016) This is partly because replacing a benzene core with a ferrocene unit significantly alters molecular properties, including solubility, hydrophobicity, and lipophilicity. (Top et al., 1996; Ferreira et al., 2006).

Consequently, ferrocene derivatives are associated with a diverse array of biological activities, such as anti-HIV, antitumor, antimalarial, antioxidant, antimicrobial. (Top et al., 2003; Yu et al., 2008). The redox behavior of these organometallic compounds is a defining feature of their cellular interactions, as ferrocene has the ability to undergo facile iron oxidation and act as a mediator for protein redox reactions.

A primary mechanism through which ferrocene derivatives exert biological effects, particularly cytotoxicity, is the generation of oxidative stress. (Larik et al., 2016). Derivatives with low oxidation potentials have the ability to catalyze the production of reactive oxygenated species (ROS) under physiological conditions. (Neuse, 2005) This generation of reactive oxygenated species can oxidatively modify essential cellular components, such as DNA, and disturb the overall redox balance within the cell. (Huang et al., 2005)

Additionally, the production of these species may interfere with redox-related cellular signaling pathways. (Sai Krishna et al., 2005)

5.2 Pharmacological Applications of Ferrocene Derivatives

5.2.1 Anticancer Activity

In the last decade, a revolution in cancer treatment has been enacted by organometallic chemists. (Frantz et al., 2004; Yu et al., 2008) Many ferrocene derivatives show good results as antitumor agents, and some of them are now in clinical trials (Getty et al., 2005).

In breast cancer, ferrocifens were the first molecules shown to be active against both hormone-dependent and hormone-independent breast cancer cells. (Welipitiya et al., 1996). Ferrocenyl-tagged 1,4-dihydropyrimidines and N-(ferrocenylmethyl)fluorobenzenecarboxamide derivatives have also shown significant in vitro cytotoxic activity against breast cancer cell lines. (Davis et al., 2005)

In lung cancer, a series of N-(ferrocenyl) naphthoyl and benzoyl dipeptide esters have been evaluated against lung cancer cell lines (e.g., H1299). Some of these compounds are more cytotoxic in vitro than clinically employed drugs such as carboplatin and cisplatin. (Qiu et al., 2009)

5.2.2 Antioxidant Activity

Furthermore, ferrocene derivatives act as effective antioxidants. Research on derivatives containing amine, amide, and hydrazine groups indicates that those with hydrazine groups exhibit free radical scavenging activity close to that of the standard antioxidant ascorbic acid. (Parveen et al., 2016; Khelef & Lanez, 2015).

5.2.3 Antimicrobial Activity

Ferrocene derivatives have also demonstrated antimicrobial activity against a variety of pathogens, including bacteria, fungi, and parasites. (Yu et al., 2008; Top et al., 2003).

Ferrocenylvinyl-flavones exhibit both cytotoxic and antibacterial activities. (Kowalski et al., 2013) Ferrocenylthioureas have been identified as new leads for antimicrobial agents, showing potency against Gram-positive and Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli*, as well as fungal strains like *Saccharomyces cerevisiae* and *Candida albicans*. (Yavuz & Yildirim, 20 Top et al., 2003)

Ferrocenyl-1,3,4-thiadiazole (TDZ) derivatives have also been synthesized for their antitubercular properties, although they often face challenges regarding solubility. (Quintana et al., 2015; Zapata et al., 2010).

5.2.4 Antimalarial and Anti-parasitic Activity

The development of ferrocene-based antimalarial agents has attracted significant attention due to the increasing resistance of parasites like *P. falciparum* to traditional drugs. Ferroquine is a prominent example, demonstrating greater effectiveness than chloroquine in inhibiting *P. falciparum* in vitro and *P. berghei* in vivo, showing 22-fold higher activity than chloroquine in mice models. [\(Larik et al., 2016\)](#)

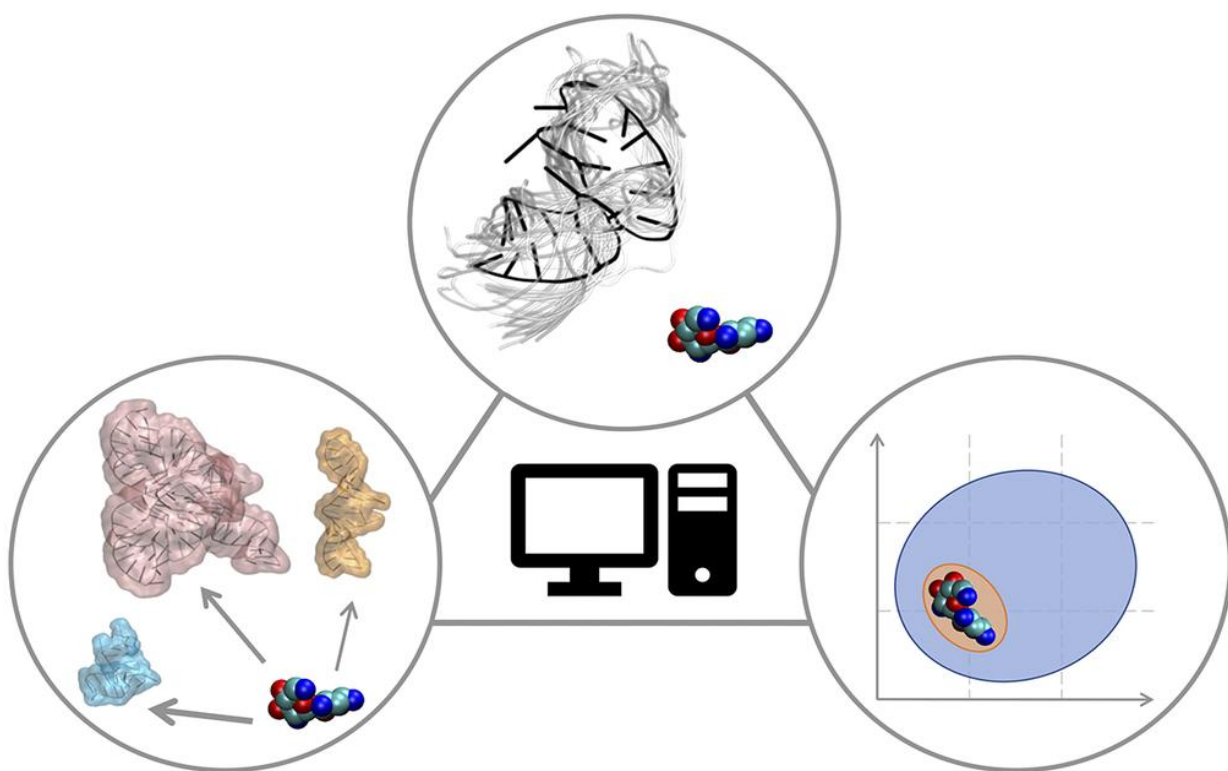
CONCLUSION

In conclusion, ferrocene and its derivatives represent an important class of organometallic compounds characterized by their structural stability and well-defined electronic properties. The availability of different synthetic approaches and the possibility of functionalisation allow access to a wide range of ferrocenyl compounds with diverse characteristics. In addition, their electrochemical behaviour and stability under various conditions contribute to their continued interest in both fundamental and applied research. These features also explain the growing attention given to ferrocene derivatives in biological and pharmacological contexts.

Based on these considerations, the study of such compounds can be complemented by computational approaches, which provide additional insight into their structural and physicochemical properties.

Chapter 02:

Computational Approaches in Molecular Modelling and Drug Design



1. Introduction

In each step of the drug discovery and development, computational methods provide valuable and interesting resources. To explore the design and development of therapeutic agents, a computer-aided drug design approach came into existence that uses various chemical, molecular, and quantum strategies for designing a potential drug candidate. (Veselovsky & Ivanov, 2003)

Structure activity relationships are the basis of many computer-aided drug design strategies as it provides the information about the biological activity. computer-aided drug design's principal aspects are to design and improve the biological activity of a drug, and also to understand the biological mechanism of action at the molecular level. (Guha, 2013)

Three major steps in the drug development processes are as follows: (1) to identify specific bioactive compounds and their molecular targets or hits, (2) testing of the compound using wet-lab experiments (preclinical, clinical I, II, and III trial), and (3) registration of drug compound to facilitate the marketing and therapeutic use (Sliwoski et al., 2014)

The objective of computer-aided drug design approaches is to screen, optimize, and evaluate the activity of compounds against a drug target. (Singh & Dwivedi, 2019)

This virtual screening is intended to reduce the time, labor, and cost associated with drug development. The use of computational approaches, such as homology modeling, cavity prediction, big data mining and analysis, Structure activity relationships, and virtual screening, made significant improvements in the process of drug discovery. (Yu & Mackerell, 2017; Chan et al., 2019)

The purpose of this chapter is to introduce computer-aided drug design in a general way, covering key techniques and their applications, and future directions in the drug discovery process.

2. Density Functional Theory (DFT) and Electronic Descriptors

Density functional theory is an essential building block for modern theoretical physics, chemistry, and engineering, especially those concerning electronic properties. At its core, density functional theory replaces the complex many-body wavefunction with the electron density as the fundamental variable, dramatically simplifying the quantum mechanical description of interacting electrons. Through decades of development, various program packages for first-principles electronic structure calculation are now available, such as the Vienna Ab initio Simulation Package, Quantum ESPRESSO, and Gaussian. Their sophisticated interfaces allow users to apply density functional theory to actual systems—ranging from molecules and solids to surfaces and interfaces—even without knowing the underlying theory.

It is hence becoming more and more important to recall the fundamentals of how density functional theory enables accurate calculations. Key among these are the Hohenberg-Kohn theorems, which establish the one-to-one mapping between the external potential and the ground-state electron density, and the Kohn-Sham formulation, which maps the interacting system onto a fictitious non-interacting system, with all many-body effects captured by an exchange-correlation functional. The accuracy of density functional theory in practice hinges on approximations for this functional, such as the local density approximation, generalized gradient approximations, or hybrid functionals.

This article attempts to provide such knowledge with a minimal overview of density functional theory—its basic foundations, relations to observable electronic and nuclear dynamical properties (for example, through forces and stress tensors for structure relaxation or molecular dynamics), and some of its cutting-edge applications, including the prediction of catalytic activity, band gaps in semiconductors, and properties of novel two-dimensional materials. (Nomura & Akashi, 2024)

3. Molecular Docking and Ligand–Protein Interactions

3.1 Molecular docking

Molecular docking is a kind of computational modeling of the complexes, which is formed from the interaction of two or more molecules. It predicts the three-dimensional structure of adducts, based upon binding properties of participating ligand and target molecules. Molecular docking generates different possible candidate structures, which are ranked and grouped together using scoring function in the software of molecular docking tool. Docking simulations predict optimized

docked conformer based upon total energy of the system. Various computational aspects of molecular docking with respect to its approaches and types.

3.2 The basic theory of molecular docking

Molecular docking is to simulate the optimal conformation according to the complementarity and pre-organization, which could predict and obtain the binding affinity and interactive mode between ligand and receptor. (Morris & Lim-Wilby, 2008) **Figure 12A** shows the first proposed “lock-and-key model”. (Morrison et al., 2006)

which refers to the rigid docking of receptors and ligands to find the correct orientation for the “key” to open up the “lock”. This model emphasizes the importance of geometric complementarity

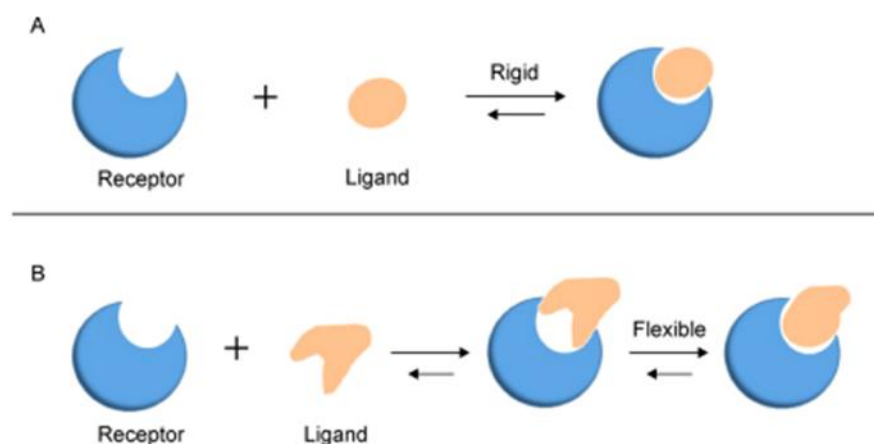


Figure 12A shows the first proposed “lock-and-key model” and “induced fit model”

However, the real docking process is so flexible that receptors and ligands have to change their conformation to fit each other well. Thus, we develop “induced fit model” (**Figure 12B**). (Koshland, 2010)

Based on geometric complementarity, the energy complementarity and pre-organization guarantee that receptors and ligands would obtain the most stable structure in such a manner that minimizes the free energy. (Audie & Scarlata, 2007)

As shown in **Figure 13**, the molecular docking software can help us to find the optimal conformation and orientation according to complementarity and pre-organization with specific algorithm, followed by applying a scoring function to predict the binding affinity and analyze the interactive mode. **Figure 14** shows the protein-DNA docking with AutoDock Vina. (Trott & Olson, 2010) displayed in PyMOL. (DeLano, 2002)

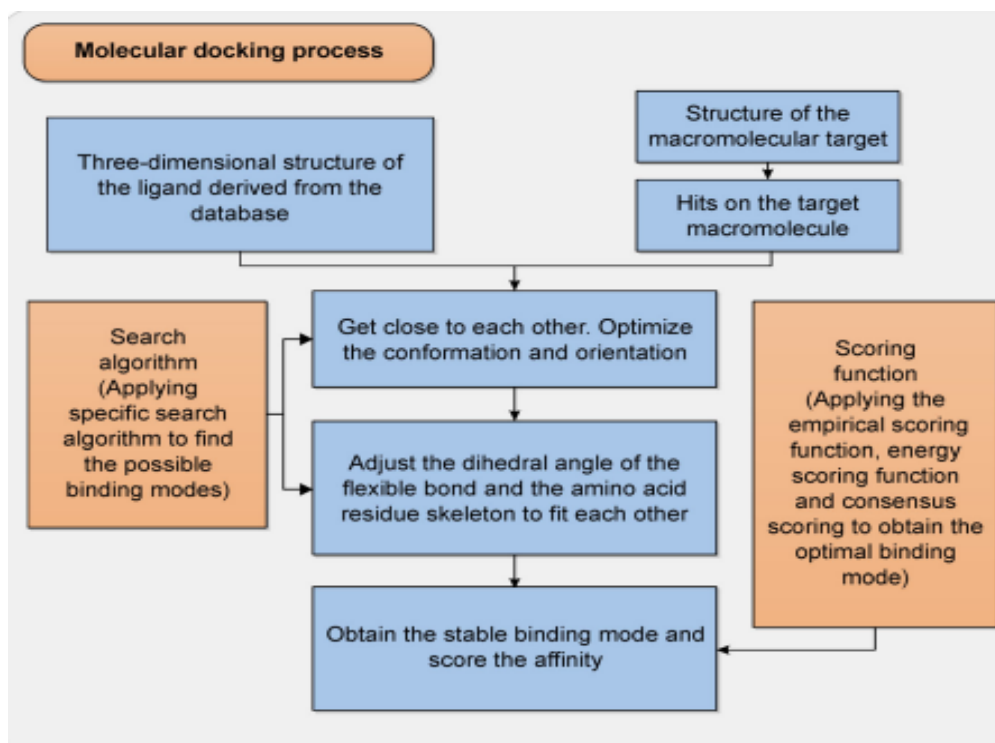


Figure 13 Molecular docking process.

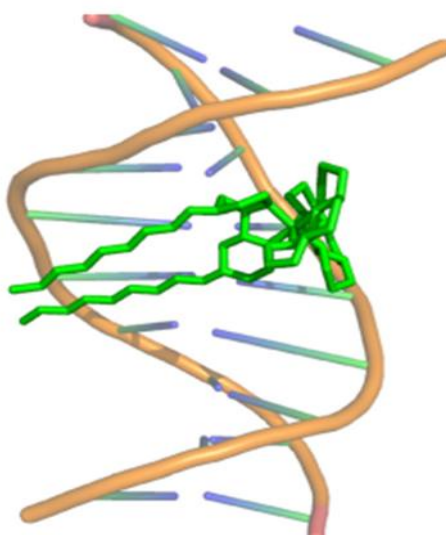


Figure 14 The protein-DNA docking.

3.3 Types of Docking

Comprehensively utilized docking tools employ search algorithms such as genetic algorithm, fragment-based algorithms, Monte Carlo algorithms and molecular dynamics algorithms. Besides this, there are some tools such as DOCK, GOLD, FlexX and ICM, which are mainly used for high

throughput docking simulations. There are various kinds of molecular docking procedures involving either ligand/target flexible or rigid based upon the objectives of docking simulations. (Guedes et al., 2014; Shoichet et al., 2002; Gschwend et al., 1996).

- I. **Flexible ligand docking**, which incorporates target as rigid molecule. This is the most commonly used in docking.
- II. **Rigid body docking**, where both the target and ligand molecules are kept as rigid molecules.
- III. **Flexible docking** that involves both interacting molecules as flexible.

3.4 Approaches of Molecular Docking

For performing molecular docking, primarily two types of approaches are used. One of the approaches employs computer simulations, in which energy profiling is estimated for ligand target docked conformer. Whereas, second approach utilizes a technique that calculates surfaces complementarity between ligand and target. (Mukesh & Rakesh, 2011)

Both approaches are described below; in brief and their main properties are also summarized in (Table 1).

3.4.1 Simulation Approach

In this approach, ligand and target molecules are separated by some physical distance and then, ligand is allowed to bind into groove/pocket of target molecule after a “definite time of moves” in its conformational space. The moves involve variations to the ligand structure either internally (torsional angle rotations) or externally (rigid body transformations such as rotations and translations). Every move in the conformational limits of ligand generates energy, which is calculated as “Total Energy of the System”. This approach is more advantageous over shape complementarity one as it is more compatible to accept ligand flexibility in to molecular modeling tool. An additional benefit of this approach is that, it is more real to assess the molecular recognition between ligand and target molecule. However, molecular docking using this approach takes longer duration to appraise optimal docked conformer, since large energy landscapes need to be calculated for each pose. Nevertheless, fast optimization method and grid-based tools have appreciably revolutionized this drawback to make computer simulation approach more user-friendly. (Mukesh & Rakesh, 2011; Lamb & Jorgensen, 1997).

3.4.2 Shape Complementarity Approach

This approach employs ligand and target as a set of surface structural features that facilitate their molecular docking. In order to achieve molecular docking, molecular surface of target is elucidated with respect to its solvent-accessible surface area, whereas; ligand's molecular surface is described in terms of matching surface illustration. The complementarity between two molecular surfaces is evaluated based on shape matching illustration, which assist in searching the complementary groove/pocket for ligand docking on target molecular surface. In particular, for protein target molecules, hydrophobicity is also estimated employing number of turns in the main-chain atoms. Shape complementarity approach is rather quick and robust, which involves the rapid scanning of numerous thousands of ligands in a few seconds to find out the possible binding properties of ligand on target molecular surface. (Mukesh & Rakesh, 2011; Lamb & Jorgensen, 1997).

Table 1: Molecular Docking Approaches. Simulation Approach

Simulation Approach Shape	Complementarity Approach
In this approach, interaction energy as per ligand-receptor pair are calculated	This approach implies the estimation of complementarity between ligand and receptor surface
To achieve the best docked conformer of ligand and receptor, ligand is allowed to fit into receptor's groove based upon minimum energy consideration	To attain the docked conformer via this approach, solvent accessible topographic features of ligand and receptor in terms of matching surface is described. This is followed by the estimation of shape complementarity between interacting molecules for finding out optimal groove/pocket for ligand binding on its target.
Every move of ligand into receptor's pocket for best fitting generates an energy as Total Energy of System, which is compared to find out best docked conformer with minimum energy	This method consists of surface representation of receptor and ligand (i.e., surface construction and smoothing), features/curvature calculation followed by docking and scoring contingent to geometric complementary criteria.

This approach is more compatible to accept lig- and flexibility in molecular modeling tool, which facilitates real assessment of molecular perception and interaction between ligand and receptor molecules	Shape complementarity approach allows both types of docking; flexible docking and rigid docking. In case of flexible or soft docking conformational changes may take place among bound and free interacting molecules. This is accompanied with the penetration and overlapping of both interacting molecules on each other. However, rigid docking does not let spatial alteration into shape of interacting molecules during molecular modeling
Performing the molecular modeling, through this approach, requires much longer time as large energy profiling needs to be estimated. However, grid-based tools and fast optimization methods have significantly transfigured this downside	This method encompasses the rapid scanning of large number of ligands for the binding on its target in a few seconds and hence, provides quick and robust outcomes

3.5 APPLICATIONS OF MOLECULAR DOCKING

Molecular docking is the need of today's research. It can demonstrate the feasibility of any task, if it carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction investigations between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are described below

3.5.1 Lead optimization

Molecular docking can predict an optimized orientation of small molecule or ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. Knowledge gained from such type of investigations may be employed to develop more potent, selective and efficient analogs (Shoichet et al., 2002; Gschwend et al., 1996)

3.5.2 Hit Identifications

Molecular docking in combination with scoring function can be used to screen huge databases for finding out potent drug candidates in silico, which can target the molecule of interest ([Ferreira et al., 2015](#))

3.5.3 Drug-DNA Interactions Studies

In recent times, majority of regimens and approaches, leading to cancer treatment, involve the utilization of chemotherapy. Despite inexorably significant role of chemotherapy in cancer cure and control, cytotoxic mechanisms of several chemotherapeutic agents are not well characterized. Many of these anticancer chemotherapeutic drugs possess nucleic acid and auxiliary processes as their main cellular target. Keeping this in view, researchers are constantly putting their efforts to elucidate the underlying anticancer mechanism of drugs at molecular level by investigating the interaction mode between nucleic acid and drugs ([Agarwal et al., 2014](#); [Agarwal et al., 2015](#))

Here, molecular docking plays a significant role in the preliminary prediction of drug's binding properties to nucleic acid. The information gathered from the outcome of such investigations is helpful in the establishment of a correlation between drug's molecular structure and its cytotoxicity. Furthermore, this knowledge would be instrumental in the detection of those structural modifications in a drug that could result in sequence/structure specific binding to their target (nucleic acid). This comprehension can be exploited in the rational designing and synthesis of new drugs, possessing better efficacy and reduced side effects, since; non-specific binding restricts drug dose and regularity in cancer treatment ([Holt et al., 2008](#); [Gschwend et al., 1996](#))

3.6 Molecular docking software

Figure 15 lists the main three types of software for molecular docking. Flexible-rigid docking has been widely used. However, since flexible docking is usually more accurate, the relevant researches have become the hot studying spot in recent years, and lists the widely used molecular docking software and its algorithms, evaluation methods, features and application areas.

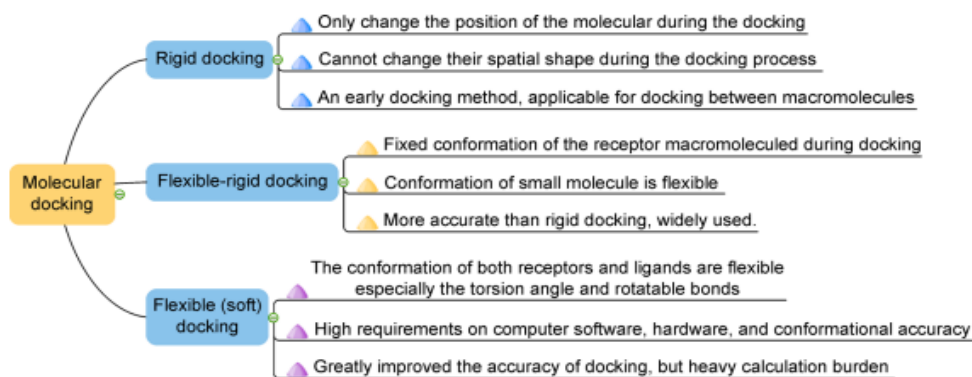


Figure 15. Molecular docking software classification.

4. Molecular Dynamics Simulation

4.1 Theoretical Background

Computer simulation for the study of the dynamic behavior of molecules to understand the enigma behind the complexity of the biological world is a demanding task. It necessitates the need of optimally developed models capable of mimicking the cellular environment, physical forces that can simulate the laws of physics and thermodynamics, and provide dynamicity to the model and heavy computations keeping in view the temporal aspect of the technique. Today, tools have been developed for molecular modelling, energy calculations, algorithms to simulate the chemical aspect of the real systems, docking-scoring techniques, etc., thereby making the whole technique robust. To make the simulation realistic, the structure is placed in a "bath" of thousands of water molecules. Let us generate a fundamental idea about this incredibly amazing technology-enhanced technique.

4.2 Research Aim and Objectives

The goal of Molecular Dynamic Simulation is to predict the behavior of atoms in a biological system and how they move as a time-dependent function thereby providing the ultimate details concerning the atoms based on algorithms of physics that govern the interatomic interactions (Hollingsworth & Dror, 2018).

Through this, we hope to understand the properties of molecules with respect to their structure and their conduct under different conditions. It serves as an important suffix to the lab experiments thus saving time, cost, and labor of the scientists and bridges the gap between the latest technological advancements in the modern scientific community and the conventional experimental scientists. It aims at lowering the amount of guesswork and fittings traditional scientists

make and helps them get an idea about the simulations that are difficult or impossible in the laboratory. We should always keep in mind that it is possible that one might not necessarily have a flawlessly realistic molecular model but the model should be able to portray the essential properties of physics and chemistry and also follow the concerned laws of mathematics along with possessing the correct biological attributes and that should be enough.

4.3 Methodological Approach

There are a number of software available for performing the molecular dynamic simulation of bio-molecules like GROMACS, Open Babel, VMD, UCSF Chimera, etc. We can select a software of our choice and perform the task but always remember that different software uses different Force Fields. MD Simulations are performed in three main steps which further consist of smaller steps.

- I. Model Selection
- II. Energy Minimization. Heating and Equilibration
- III. Production Run and Analysis

If we talk about Chimera, Molecular Dynamics Simulation can be thought of as a link to minimization and molecular dynamics routines provided by Molecular Mechanics Toolkit (MMTK) which is incorporated with it. Standard residues are assigned amber parameters while non-standard residues are assigned parameters using Chimera's Antechamber module.

4.3.1 Model Selection:

A model system of interest should be chosen. Most of the time complete models are available for use, but in case complete models are unavailable, the missing segments are secured and the protonation states conditioned. All atoms of interest should be considered and included in this step because models not included here will be ignored. The prepared molecule should be read in pdb and psf file.

Obtaining Files- Simulations generally start with a crystal structure from the Protein Data Bank, in the standard PDB file format. The information about atoms of use to us are the atom names (N, C, CA), Residue name and ID, Occupancy, Coordinates, Beta factor or Temperature Factor, and Segment ID.

4.3.2 Energy Minimization, Heating and Equilibration:

This step includes the equilibration of the observed structure with the force field used($T=0$) by solving Newton's equations of motion. We also decide the number of equilibration steps

(default 5000). Then the system is heated by rescaling the velocities and its stability ensured until the properties of the system no longer change with time and the system reaches a particular temperature. Preparing the System for Energy Minimization- The energy of the system can be found using the force field calculations. The conformation of the system can be changed to locate lower energy conformations through the process called minimization. There are different minimization algorithms available, like

- steepest descent (slowly converging – used for highly restrained systems)
- conjugate gradient (efficient, uses intelligent choices of search direction – used for large systems)
- Broyden-Fletcher-Goldfarb-Shanno (BFGS) (quasi-newton variable metric method)
- Newton-Raphson (calculates both slopes of energy and rate of change)

Periodic Boundary conditions should be used whenever a solvent box is added. If periodic boundary conditions are used, cut-off distance should not exceed half of the smallest box dimension. Fixed Atoms help specify whether one needs to freeze some atoms in a position during the calculations. Such atoms to be frozen in place are highlighted by selection, but one must always remember that all atoms in the desired model will be included in the energy calculations, whether they are fixed or not.

Translation Remover aids in subtracting out a global translational motion during MD, and also decides which steps, by default the first, third, fifth, etc., through the end.

Rotation Remover aids in subtracting out a global rotational motion during MD, and also decides which steps, by default the first, third, fifth, etc., through the end.

Topology files: In them, the atom types are assigned, for the identification of different elements and different molecular orbital environments. Also, charges are assigned to each atom, and connectivity between them is established.

Parameter files contain force constants requisite to describe the bond energy, nonbonded interactions (van der Waals and electrostatic), angle energy, torsion energy, etc. and also the suggested parameters for setting up the energy calculations.

Solvation is an important step because most biological processes occur in aqueous solutions and solvation effects play a decisive role in the determination of molecular conformation, electronic properties, binding energies, etc. ([Rosenthal et al., 1994](#))

There are two methods of model solvation- explicit treatment and implicit treatment. In explicit treatment, solvent molecules are added to the molecular system whereas, in implicit treatment, the solvent is modelled as a continuum dielectric.

4.3.3 Production Run and Analysis

The model is then simulated under desired conditions of NVT, NPT, etc. Finally, a production run is performed for a relevant period of time to get the output trajectories. The ‘include production phase’ helps us decide whether to include the production MD in a phase and if so, how many steps should be included. We also need to mention the time steps at which we write the trajectory files which are further analyzed to obtain the desired properties of interest ([Kaboli et al., 2018](#))

The steady development potential computational sampling methods now let us carry out the simulation process on a time scale of seconds to microsecond and even millisecond. Here it should be significantly noted that in simulation, these millisecond scales are believed to be very large that contradict the in vitro experiment owing to the fact that in computer simulation, coordinates are generated at the femtosecond level. Moving from femtoseconds to milliseconds scale gives a large ensemble of conformations that have the potential to reveal many underlying biological mysteries.

4.4 Scientific Applications

MD simulations have a wide range of applications not only in the field of biological science but in any field one can imagine ranging from physics, chemistry, biology to climatology and meteorology, video games to film industries. Let us focus on the applications of MD Simulations in biological complexes.

Determination of Structures and Movements of Biomolecules: As already mentioned, we now know that the most common application of MD Simulation in biomolecules is to study, analyze and mimic the flexibility, movements and interactions of and among the different proteins. Structures determined by experimental studies by X-Ray Crystallography or NMR studies reveal only an average approximation of what the real thing could be, but with the usage of computational simulation techniques, one could make an even more precise approximation of the types of structural fluctuations the molecules undergo. By just examining a simulation of these structures, one can quantify the movements of various regions of the molecule at equilibrium and the types of structural fluctuations that occur ([Miao et al., 2001](#)) .

Such simulations also have the capacity to show the dynamic behavioral properties of water molecules and salt ions, the effects of which are often critical for the proper functioning of protein and also for ligand binding.

Assessment of accuracy and Refinement of modelled structures This method can also be used to assess the accuracy of already modelled structures or even to refine the structures built using molecular modelling techniques or experimentally in the lab. For example, it is frequently seen that experimentally determined X-ray crystal structures are refined by a computational MD simulated annealing protocol and fits the model to the experimental data even more precisely while simultaneously maintaining a physically stable structure ([Terwilliger et al., 2012](#)) .

One advantage of this approach is that this approach has been shown to control model errors that are otherwise present. Let us consider another example. It is possible that a membrane protein suffers from artifacts due to the absence of a lipid bilayer or crystal structure suffers from such errors as a result of the crystal lattice packing but owing to the lucidity of the near accuracy of the simulated structures, it is now possible to correct such artifacts by performing a simulation inappropriate solvation environment as per the requirements of the structures one is working with. Though MD simulations are extremely useful in the refinement of existing homology models, quite a number of attempts to do this have been unsuccessful ([Mirjalili & Feig, 2013](#)) .

MD simulations have also been used to retrieve ensembles of conformations, against a single structure, from NMR data ([Lindorff-Larsen et al., 2005](#)).

In each of these cases, the molecular mechanic's force field is augmented by terms that have to be taken from experimental data, which results in lower energy for structures (or structural ensembles) that agree better with.

Flexibility of Molecules The flexibility of biomolecules directly modulates its association with the neighboring atoms, molecules and ions, and thus plays an active role in cellular function. We have already studied that the molecular dynamic system gives us clear insights into the dynamic evolution of any system, it can also be seen as reflecting its flexibility to an extent. Techniques such as Anisotropic Network Model (ANM), Elastic Network Model (ENM), Principal Component Analysis (PCA) among few others have recently been developed which have the ability to extrapolate the prime contributing motions of the system under study ([Bakan & Bahar, 2009](#)).

Another interestingly important application of MD simulation is to ascertain the mechanism in which a biomolecular system will respond to perturbation. Say, for example, someone changes the molecular environment of the protein like the salt concentration or lipid composition, or adds

a ligand where there was originally no ligand present or replaces a bound ligand by a different ligand or changes the amino acid residues present in a particular protein by mutating them or by changing the protonation state of the amino acid (Fribourg et al., 2011; Peng et al., 2018).

In all the cases mentioned above, simulations help one in getting a thorough understanding of the system under study. One thing to be kept in mind while performing such simulations is that one should perform it several times by using both perturbed and unperturbed systems to get clear insights into the consistent differences in the results and thus ascertain one's results.

Analysis of results of MD simulation of different systems helps one to answer such questions about the role of structure, flexibility and the interactions among different biomolecules that are experimentally very difficult to address. Since simulations can take place at the scale of femtoseconds, it is possible for us to observe such biological processes which occur in a jiffy, like the order in which the substructures form during protein folding (Lindorff-Larsen et al., 2011; Snow et al., 2002).

One can also perform a thorough study of processes like ligand binding, protein folding, conformational changes, membrane transport, etc. They also help us understand the factors controlling ligand binding and dissociation kinetics, the process of assembly of disordered proteins to form fibrils (Dedmon et al., 2005; Nguyen & Hall, 2004).

Simulations may capture an entire process in one go, or they may capture it in parts which can then be used to reconstruct the entire process (Bernardi et al., 2015; Schwantes et al., 2014).

Modelling of Drug Receptor Interactions – Experimental studies help us determine the 3D structures of most ligands (drug molecules) which interact with the receptors but the structures of the receptors are still unknown. The interactions among receptors and ligands are the focal step in most biological processes like pharmacological actions of drugs, regulatory mechanisms, toxic effects of particular chemicals, etc. It is significantly important to know the structure of both the receptor and ligands before carrying out further simulations by making modifications for drug design. In recent times, structure-based drug design (SBDD) and ligand-based drug design (LBDD) in computational forms have become important components of modern drug discovery.

SBDD can be carried out with the available 3D structure of the target. Free-energy calculation methods in SBDD are used to calculate the absolute or relative binding free energy. The relative binding free energy methods, also known as alchemical calculations, use MD simulations to first sample and then compute binding free energy differences between structurally similar ligands. This is immensely important from the point of view of drug discovery. In short, simulations are used to

determine the location for the molecule to bind to its receptor, and how it changes the binding strength and affinity of molecules that bind elsewhere. This information along with other geometrical, physical, chemical and thermodynamic properties are used to alter the structure as many times as possible so as to design a drug which fulfils one's needs. Once this computational task is done, the experimental scientists take over and after its testing and approval, clinical trials take place and if it passes the clinical trial, the drug is ready to be launched in markets.

Gives Insights into Molecular Interactions on a Temporal Scale Molecular Dynamics Simulation generates pictures of atomic-level details of the dynamic evolution of the biomolecular system. This property clubbed with temporal scale for MD Simulations, enables us to predict different possible cellular interactions and behaviors based on which modifications in existing structures can be made and seen and is immensely helpful in studying the properties of these samples which can be used as potent drugs in the market.

Docking is the process by which two or more molecular structures orient themselves in such a way that they bind to each other to form a stable complex. MD simulation is widely used to study the protein-ligand, protein-protein, DNA-protein, and DNA-ligand interactions. Researchers in general are always excited to study the effect of new kinds of molecular interactions. For this reason, docking is performed first and then MD simulation is done so that one can know the effect of interactions on a temporal scale. Docking software like Glide, GOLD, AutoDock, etc., make use of different algorithms to calculate a docking score based on different parameters like surface of contact, electrostatics, etc. (Kroemer, 2007), and a good score is considered as one which has a good binding affinity. It can be further studied by in-vitro, in-vivo, pre-clinical, and clinical trials. As each of these processes is time taking and expensive, the docked structures can help filter out structures thermodynamics and structural analyses over the trajectories obtained from molecular dynamics simulation and the streamlined results can be further studied in the labs or in vivo.

Protein Folding Protein folding is one of the most exciting topics in biology. Though the three-dimensional structure of protein has been studied pretty well but the mechanism of protein-folding is still not accurately known. Molecular dynamics simulation is being used to study the folding mechanism using computational power. For example, the MD simulation of a sub-domain of villin protein was done which gave significant insights and thus a glimpse of hope towards the proper understanding of protein folding mechanism (Duan & Kollman, 1998).

To understand how Mutation affects Interactions – To understand and know the effects of a particular amino-acid in binding with its counterpart, the residue is mutated and the difference in

the simulated trajectories both before and after the process is studied well. Refinements and modifications in the ligand can also be done so as to improve its affinity and get clear insights into the structural and interaction properties of the particular ligand (Karaa, 2015).

5. ADMET Prediction and Drug-Likeness Evaluation

5.1 Definition of ADMET and Its Importance

ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity, and it represents the key pharmacokinetic and safety properties that determine the fate of a drug within a biological system. Absorption describes how a compound enters systemic circulation, while distribution refers to its dispersion throughout tissues and organs. Metabolism involves the biochemical transformation of the compound, mainly by liver enzymes such as cytochrome P450, and excretion defines how the compound and its metabolites are eliminated from the body. Toxicity evaluates the potential harmful effects a compound may exert on biological systems.

These parameters are crucial in drug discovery because they directly influence a compound's bioavailability, efficacy, and safety profile. A molecule with poor ADMET properties is unlikely to become a successful drug, even if it shows strong biological activity. In fact, a significant proportion of drug candidates fail during clinical trials due to inadequate pharmacokinetics or unexpected toxicity. Therefore, early evaluation of ADMET properties is essential to minimize late-stage failures and reduce development costs (Gadaleta et al., 2024)

5.2 Drug-Likeness Evaluation

Drug-likeness is a key concept in early drug discovery that refers to the similarity of a chemical compound to known orally active drugs. It is used as a filtering step to identify molecules with a higher probability of becoming successful drug candidates before expensive biological testing.

A widely accepted framework for evaluating drug-likeness is Lipinski's Rule of Five, introduced by Lipinski and co-workers. This rule is based on statistical analysis of physicochemical properties of orally active drugs and proposes that poor absorption or permeation is more likely when a compound has more than one of the following characteristics:

- Molecular weight greater than 500 Da
- LogP (octanol–water partition coefficient) greater than 5
- More than 5 hydrogen bond donors (OH and NH groups)

- More than 10 hydrogen bond acceptors (O and N atoms)

These parameters are not absolute rules but empirical guidelines, meaning that successful drugs may still violate one or more of them. However, compounds that significantly deviate from these ranges often show reduced oral bioavailability.

Lipinski's study demonstrated that most orally active drugs share a relatively narrow range of physicochemical properties, highlighting the importance of balancing hydrophilicity and lipophilicity to achieve adequate absorption and membrane permeability. As a result, drug-likeness evaluation is now routinely used in combination with ADMET prediction to filter large chemical libraries and prioritize promising candidates for further development. ([Lipinski et al., 1997](#)).

5.3 Role of Computational Prediction in Drug Discovery

Computational (in silico) methods have become indispensable tools for predicting ADMET properties at the early stages of drug discovery. These approaches rely on the relationship between chemical structure and biological behavior, allowing researchers to estimate pharmacokinetic and toxicological properties without performing laboratory experiments.

Using computational models, researchers can:

- Rapidly screen large chemical libraries
- Predict oral bioavailability and permeability
- Identify potential toxicity risks such as mutagenicity or hepatotoxicity
- Optimize lead compounds before synthesis

This significantly accelerates the drug development process and reduces the dependence on costly and time-consuming in vitro and in vivo experiments. Moreover, computational ADMET prediction supports ethical considerations by limiting animal testing. As highlighted in recent studies, these methods are now an integral part of modern medicinal chemistry and rational drug design ([Gadaleta et al., 2024](#)).

5.4 Examples of Tools and Models

A variety of computational models and software tools have been developed to predict ADMET properties with increasing accuracy:

5.4.1 QSAR/QSPR Models

Quantitative Structure–Activity Relationship (QSAR) and Quantitative Structure–Property Relationship (QSPR) models use mathematical correlations between molecular descriptors (such as polarity, molecular weight, and electronic properties) and experimentally measured ADMET parameters. These models are widely used for predicting absorption, solubility, and toxicity.

5.4.2 Machine Learning Approaches

Machine learning techniques, including neural networks and random forest algorithms, are trained on large experimental datasets to improve prediction accuracy. These models are particularly effective for complex endpoints such as metabolism and toxicity.

5.4.3 Common Computational Tools

Several widely used platforms for ADMET prediction include:

SwissADME → evaluates drug-likeness, lipophilicity, and pharmacokinetics

pkCSM → predicts toxicity and pharmacokinetic parameters using graph-based signatures

ADMETlab → provides a comprehensive ADMET profile including metabolism and excretion

These tools allow researchers to make informed decisions about compound selection and optimization, improving the efficiency and success rate of drug discovery pipelines ([Gadaleta et al., 2024](#)).

CONCLUSION

In this chapter, the main computational methods used in molecular modelling and drug design have been presented. Each method focuses on a different aspect. Density functional theory and electronic descriptors are used to study the electronic structure and reactivity of molecules. Molecular docking helps to estimate how a ligand can interact with a biological target, while molecular dynamics simulations are used to follow the stability and flexibility of these interactions over time. ADMET prediction and drug-likeness evaluation provide initial information about the pharmacokinetic properties of the compounds.

These methods do not replace experimental work, but they help to guide it and reduce the number of compounds to be tested. When used together, they give a more complete view, although the results must be interpreted carefully. In general, computational approaches are useful tools in the early stages of drug development

PART II:

EXPERIMENTAL



Chapter 01:

Materials & Methods



1. General Introduction

This chapter presents the experimental procedures and computational methodologies employed for the synthesis, structural characterisation, and *in silico* investigation of the ferrocenyl acetylaniline derivatives. The adopted approach integrates synthetic organic chemistry, advanced spectroscopic and electrochemical techniques, and state-of-the-art computational modelling in order to achieve a comprehensive understanding of the structural and electronic properties of the studied compounds, as well as their potential biological relevance.

The synthesis of the target derivatives was carried out at the Laboratory of Valorisation and Technology of Saharian Resources (VTRS), Faculty of Technology, University of El Oued, under controlled experimental conditions. The same laboratory hosted all computational investigations, including density functional theory (DFT) calculations, molecular docking, molecular dynamics simulations and MM-PBSA analyses, thereby ensuring a coherent and consistent interpretation of both experimental and theoretical data.

Structural characterisation was performed in collaboration with the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques (CRAPC), University of Ouargla, using complementary analytical techniques including FT-IR, UV–visible spectroscopy, and one- and two-dimensional NMR experiments. In addition, cyclic voltammetry was employed to investigate the electrochemical behaviour of the ferrocenyl core, which plays a crucial role in its redox properties.

From a biological perspective, the *in-silico* investigation was directed towards thyroid-related molecular targets, particularly those involved in hormonal regulation and endocrine function. Molecular docking and molecular dynamics simulations were performed to explore ligand–protein interactions and evaluate the stability of the resulting complexes.

Furthermore, *in silico* pharmacokinetic and toxicity properties (ADMET) were predicted using validated computational tools in order to assess the drug-likeness, bioavailability and safety profile of the synthesised compounds. This additional analysis provides a preliminary evaluation of their potential suitability as bioactive agents.

Overall, this multidisciplinary strategy ensures the reliability, reproducibility and scientific robustness of the obtained results, while establishing a solid foundation for the interpretation of the physicochemical behaviour and biological relevance of the ferrocenyl derivatives.

2. Chemicals and Reagents

All chemicals and solvents used in this study were of analytical grade and were employed without further purification unless otherwise stated. The selection of reagents was based on their suitability for organometallic synthesis and compatibility with subsequent spectroscopic and electrochemical analyses.

Ferrocene (Fc, 99%) and orthophosphoric acid (85%) were obtained from Alfa Aesar. The o-, m-, and p-aminoacetophenone isomers (99%), as well as tetrabutylammonium tetrafluoroborate (Bu_4NBF_4 , electrochemical grade, 99%), used as supporting electrolyte in electrochemical measurements, were purchased from Sigma-Aldrich. Iodomethane and anhydrous magnesium sulfate (97%) were supplied by Acros Organics. Acetic acid (99–100%) and ethanol (95%) were obtained from Biochem Chemopharma.

The ferrocenylmethyl-based quaternary ammonium intermediate was prepared according to previously reported procedures and used without further modification (Khand et al., 1989). All aqueous solutions were prepared using double-distilled water, and solvents used for spectroscopic and electrochemical measurements were of appropriate purity to ensure reproducibility.

Stock solutions were freshly prepared when required and handled under standard laboratory conditions to minimise contamination and degradation.

3. Instrumentation and Experimental Conditions

A combination of spectroscopic and electrochemical techniques was employed to confirm the structural integrity and to investigate the physicochemical properties of the synthesised ferrocenyl acetylaniline derivatives. All measurements were carried out under controlled experimental conditions using calibrated instruments to ensure accuracy, reproducibility and reliability of the obtained data.

3.1. Spectroscopic Analysis

3.1.1. FT-IR Spectroscopy

Fourier-transform infrared (FT-IR) spectra were recorded using a Shimadzu 8000S spectrophotometer over the wavenumber range of $4000\text{--}400\text{ cm}^{-1}$. The samples were prepared in the solid state using KBr pellets.

This technique was primarily used to identify the characteristic functional groups present in the synthesised compounds, including N–H stretching vibrations, carbonyl (C=O) groups, and aromatic C–H bonds. The observed absorption bands allowed confirmation of the successful formation of the ferrocenyl acetylaniline framework (Lanez et al., 2020).

3.1.2. UV–Visible Spectroscopy

UV–visible absorption spectra were obtained using a Shimadzu UV-1800 spectrophotometer in the wavelength range of 200–900 nm. Measurements were performed in dimethyl sulfoxide (DMSO) using quartz cuvettes with a path length of 1 cm.

This analysis enabled the investigation of electronic transitions associated with both the ferrocenyl moiety and the conjugated aromatic system. In particular, π – π^* and n – π^* transitions, as well as metal-centred d–d transitions, were identified, providing insight into the electronic structure of the compounds (Lanez et al., 2020).

3.2. Nuclear Magnetic Resonance (NMR)

3.2.1. ^1H NMR Analysis

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker ARX-500 spectrometer operating at 400 MHz the samples were dissolved in deuterated dimethyl sulfoxide (DMSO- d_6), and chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard.

This technique was used to identify the different proton environments corresponding to the ferrocenyl unit, aromatic protons, methylene bridge ($-\text{CH}_2-$), and acetyl group ($-\text{COCH}_3$), thus confirming the structural features of the synthesised derivatives (Lanez et al., 2023).

3.2.2. ^{13}C NMR Analysis

Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were acquired under similar conditions using the same spectrometer. The spectra provided detailed information on the carbon framework of the compounds, including the identification of carbonyl carbons, aromatic carbons, cyclopentadienyl rings, and methylene carbons.

This analysis was essential for confirming the molecular structure and verifying the successful functionalisation of the ferrocenyl core (Benamara et al., 2020).

3.2.3. Two-Dimensional NMR (HSQC/HMBC)

Two-dimensional NMR experiments, including heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC), were performed to establish proton–carbon connectivity.

HSQC spectra enabled the identification of direct one-bond correlations between protons and their attached carbon atoms, while HMBC provided long-range (two- and three-bond) correlations. These complementary techniques allowed unambiguous assignment of the molecular structures and confirmation of the substitution pattern on the aromatic ring (Lanez et al., 2019).

3.3. Electrochemical Measurements (Cyclic Voltammetry)

Electrochemical characterisation was carried out using a PGZ301 potentiostat/galvanostat (Radiometer Analytical SAS, France) in dimethyl sulfoxide (DMSO) under an inert nitrogen atmosphere at 298 K.

A conventional three-electrode system was employed, consisting of a glassy carbon working electrode (surface area 0.013 cm²), a platinum wire counter electrode (0.05 cm²), and a saturated Hg/Hg₂Cl₂ reference electrode.

Tetrabutylammonium tetrafluoroborate (Bu₄NBF₄, 0.1 M) was used as the supporting electrolyte. Prior to each measurement, the solution was purged with nitrogen to remove dissolved oxygen and ensure stable electrochemical conditions.

Cyclic voltammetry measurements were performed within an appropriate potential window to investigate the reversible Fe (II)/Fe (III) redox behaviour of the ferrocenyl derivatives. The obtained electrochemical parameters, including anodic and cathodic peak currents and potentials, provided valuable insight into the electron transfer properties of the compounds (Lanez et al., 2020).

4. Synthesis of Ferrocenyl Acetylaniline Derivatives

This section describes the synthetic strategy employed for the preparation of the target ferrocenyl acetylaniline derivatives. The adopted methodology is based on a nucleophilic substitution reaction involving a ferrocenylmethyl quaternary ammonium intermediate, enabling efficient formation of the C–N bond under relatively mild and environmentally favourable conditions. This approach ensures good selectivity and reproducibility for the preparation of the three Regio isomeric compounds.

The overall synthetic pathway leading to the formation of the target compounds is presented in Figure 16, which illustrates the formation of the ferrocenylmethyl intermediate followed by its reaction with the corresponding aminoacetophenone isomers.

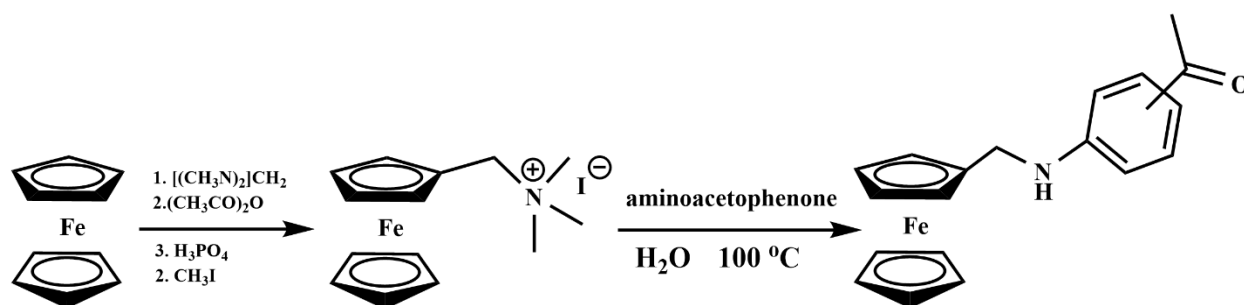


Figure 16. Synthetic route for the preparation of N-[(ferrocenyl)methyl] acetylaniline derivatives (FcMe2Ac, FcMe3Ac and FcMe4Ac).

4.1. General Synthetic Procedure

The synthesis of N-[(ferrocenyl)methyl] acetylaniline derivatives was achieved through a nucleophilic substitution reaction between trimethylferrocenylmethylammonium iodide and the corresponding aminoacetophenone isomers (ortho, meta and para), as illustrated in Figure 16.

In a typical procedure, the appropriate aminoacetophenone (3.84 mmol) was added gradually to a well-stirred aqueous solution of trimethylferrocenylmethylammonium iodide (2.56 mmol) in distilled water (60 mL). The reaction mixture was refluxed for 4 hours under continuous stirring to ensure complete conversion.

Upon completion of the reaction, the mixture was allowed to cool to room temperature, leading to the formation of a yellow precipitate. The crude product was isolated by vacuum filtration and thoroughly washed with distilled water to remove residual inorganic salts and unreacted species.

The obtained solid was purified by recrystallisation from a 90% ethanol/water mixture. In the case of the meta-substituted derivative, additional purification by column chromatography (chloroform/methanol, 99:1 v/v) was performed to achieve higher purity.

This synthetic methodology presents several advantages, including the use of aqueous medium, operational simplicity, and avoidance of harsh reaction conditions, making it suitable for reproducible preparation of ferrocenyl derivatives (Lanez et al., 2019).

4.2. Synthesis of Individual Compounds

4.2.1. N-[(ferrocenyl)methyl]-2-acetylaniline (FcMe2Ac)

Yield: 650 mg (77%).

Appearance: Yellow crystalline solid.

Melting point: 148–150 °C.

¹H NMR (500 MHz, DMSO-d₆): δ 2.60 (3H, m, CH₃), 3.40 (2H, t, α-C₅H₄), 4.05 (2H, t, β-C₅H₄), 4.15 (5H, s, C₅H₅), 4.30 (2H, d, CH₂N), 6.62 (1H, m, Ar-H), 6.84 (1H, d, Ar-H), 7.40 (1H, m, Ar-H), 7.85 (1H, d, Ar-H), 9.01 (1H, s, NH).

¹³C NMR (500 MHz, DMSO-d₆): δ 29 (CH₃), 42 (CH₂N), 67, 68 (C₅H₄), 69 (C₅H₅), 87 (ipso-C), 112, 116, 118, 134, 138, 150 (aromatic carbons), 210 (C=O).

FT-IR (KBr, ν, cm⁻¹): 3426, 3291 (N–H), 3082 (Ar–C–H), 1647 (C=O), 1566, 1516 (C=C), 1454, 1416, 1146, 1003, 617.

UV–Vis (DMSO): λ_{max} = 259 nm (π–π*), 380 nm (d–d).

Cyclic voltammetry (DMSO, Fc⁺/Fc): I_{pa} = 29.13 μA, I_{pc} = –28.52 μA, E_{pa} = 2.17 mV, E_{pc} = –85.60 mV, ΔE = 87.8 mV, E° = 43.9 mV, I_{pa}/I_{pc} = 1.02.

4.2.2. N-[(ferrocenyl)methyl]-3-acetylaniline (FcMe3Ac)

Yield: 480 mg (48%).

Appearance: Yellow–orange crystalline solid.

Melting point: 138–140 °C.

¹H NMR (500 MHz, DMSO-d₆): δ 2.45 (3H, s, CH₃), 3.42 (2H, t, α-C₅H₄), 4.08 (2H, t, β-C₅H₄), 4.18 (5H, s, C₅H₅), 4.32 (2H, d, CH₂N), 6.70–7.20 (3H, m, Ar-H), 7.65 (1H, d, Ar-H), 8.95 (1H, s, NH).

¹³C NMR (500 MHz, DMSO-d₆): δ 28 (CH₃), 43 (CH₂N), 66, 68 (C₅H₄), 69 (C₅H₅), 86 (ipso-C), 114, 118, 125, 131, 136, 152 (aromatic carbons), 205 (C=O).

FT-IR (KBr, ν, cm⁻¹): 3432, 3338 (N–H), 2925, 1650 (C=O), 1582, 1510, 1450, 1365, 1170, 820.

UV–Vis (DMSO): λ_{max} = 268 nm, 402 nm.

Cyclic voltammetry (DMSO, Fc⁺/Fc): I_{pa} = 52.4 μA, I_{pc} = –51.9 μA, E_{pa} = 10 mV, E_{pc} = –92 mV, ΔE = 102 mV, E° = 51.0 mV, I_{pa}/I_{pc} = 1.01.

4.2.3. N-[(ferrocenyl)methyl]-4-acetylaniline (FcMe4Ac)

Yield: 690 mg (80%).

Appearance: Deep yellow crystalline solid.

Melting point: 162–164 °C.

^1H NMR (500 MHz, DMSO- d_6): δ 2.35 (3H, m, CH_3), 3.41 (2H, t, $\alpha\text{-C}_5\text{H}_4$), 4.10 (2H, t, $\beta\text{-C}_5\text{H}_4$), 4.20 (5H, s, C_5H_5), 4.31 (2H, d, CH_2N), 6.65 (2H, d, Ar-H), 6.78 (1H, t, NH), 7.76 (2H, d, Ar-H).

^{13}C NMR (500 MHz, DMSO- d_6): δ 28 (CH_3), 43 (CH_2N), 66 (C_5H_4), 68 (C_5H_5), 87 (ipso-C), 112, 125, 132, 154 (aromatic carbons), 194 ($\text{C}=\text{O}$).

FT-IR (KBr, ν , cm^{-1}): 3445, 3345 (N-H), 2932, 1651 ($\text{C}=\text{O}$), 1589, 1562, 1369, 1281, 1173, 822.

UV-Vis (DMSO): $\lambda_{\text{max}} = 432$ nm.

Cyclic voltammetry (DMSO, Fc^+/Fc): $I_{\text{pa}} = 71.07$ μA , $I_{\text{pc}} = -71.66$ μA , $E_{\text{pa}} = 16$ mV, $E_{\text{pc}} = -99$ mV, $\Delta E = 115$ mV, $E^\circ = 57.5$ mV, $I_{\text{pa}}/I_{\text{pc}} = 0.99$.

5. Computational Methodology

Computational investigations were conducted to provide a comprehensive understanding of the electronic properties and molecular interactions of the synthesised ferrocenyl acetylaniline derivatives. The adopted *in silico* strategy integrates density functional theory (DFT) calculations, molecular docking, molecular dynamics (MD) simulations and MM-PBSA binding free energy analysis, complemented by ADMET predictions to evaluate pharmacokinetic and toxicity profiles.

This combined approach enables correlation between molecular structure, electronic behaviour and biological relevance, particularly in relation to thyroid-related targets involved in endocrine regulation.

5.1. Density Functional Theory (DFT) Calculations

Density functional theory calculations were performed using Gaussian 16 (Frisch et al., 2009) to investigate the electronic structure and reactivity of FcMe2Ac, FcMe3Ac and FcMe4Ac.

All molecular geometries were optimised at the B3LYP level of theory using the 6-311++G (d, p) basis set for C, H, N and O atoms, while the Stuttgart–Dresden (SDD) effective core potential was applied for the iron centre (Andersson & Uvdal, 2005). Frequency calculations were carried out at the same level to confirm that the optimised geometries correspond to true minima, characterised by the absence of imaginary frequencies.

The optimised structures were used to determine frontier molecular orbitals (HOMO and LUMO), energy gaps, electronegativity, chemical hardness, softness and electrophilicity index.

Molecular electrostatic potential (MEP), electron localisation function (ELF), localised orbital locator (LOL), and reduced density gradient (RDG) analyses were also performed to characterise charge distribution and weak intramolecular interactions. Visualisation and analysis were carried out using GaussView and Multiwfn (Lu & Chen, 2012).

5.2. Molecular Docking Studies

Molecular docking simulations were performed to investigate the binding affinity and interaction mechanisms of the synthesised compounds with thyroid-related targets, namely the thyroid hormone receptor β (PDB ID: 1XZX) and the thyroid-stimulating hormone receptor (PDB ID: 7FIY).

5.2.1. Protein Preparation

The three-dimensional structures of the target proteins used in this study are presented in Figure 17, illustrating the structural organisation of the thyroid hormone receptor and the thyroid-stimulating hormone receptor prior to preparation.

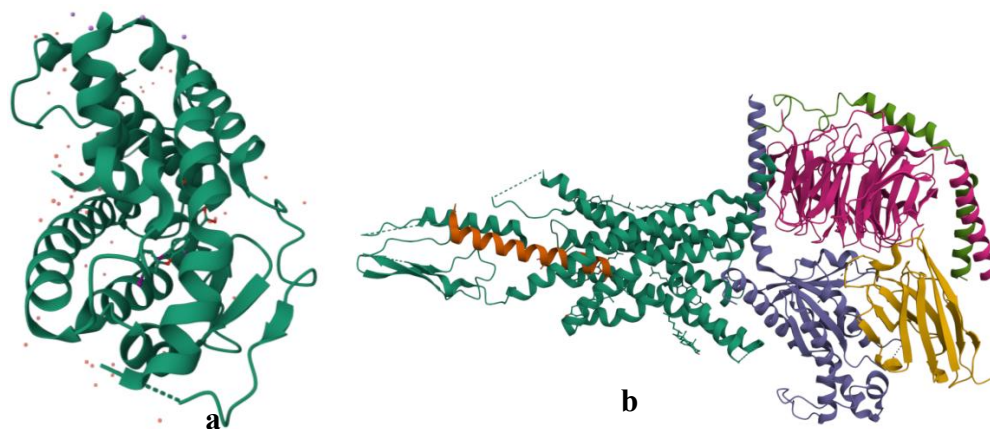


Figure 17. Three-dimensional structures of the target proteins used in docking studies: (a) xanthine oxidase (PDB ID: 1FIQ) and (b) NADPH oxidase (PDB ID: 8WEJ).

The crystallographic structures were retrieved from the Protein Data Bank (<https://www.rcsb.org/>) (Rose et al., 2017), and prepared using AutoDock Tools (ADT 1.5.7) (Steffen et al., 2010). All crystallographic water molecules, co-crystallised ligands and non-essential heteroatoms were removed to prevent interference with ligand binding and to ensure accurate representation of the active site.

Polar hydrogen atoms were added, and Kollman charges were assigned. The prepared proteins were then converted into PDBQT format.

5.2.2. Ligand Preparation

The optimised molecular structures of the ferrocenyl derivatives used as ligands are shown in [Figure 18](#), highlighting their geometry prior to docking calculations.

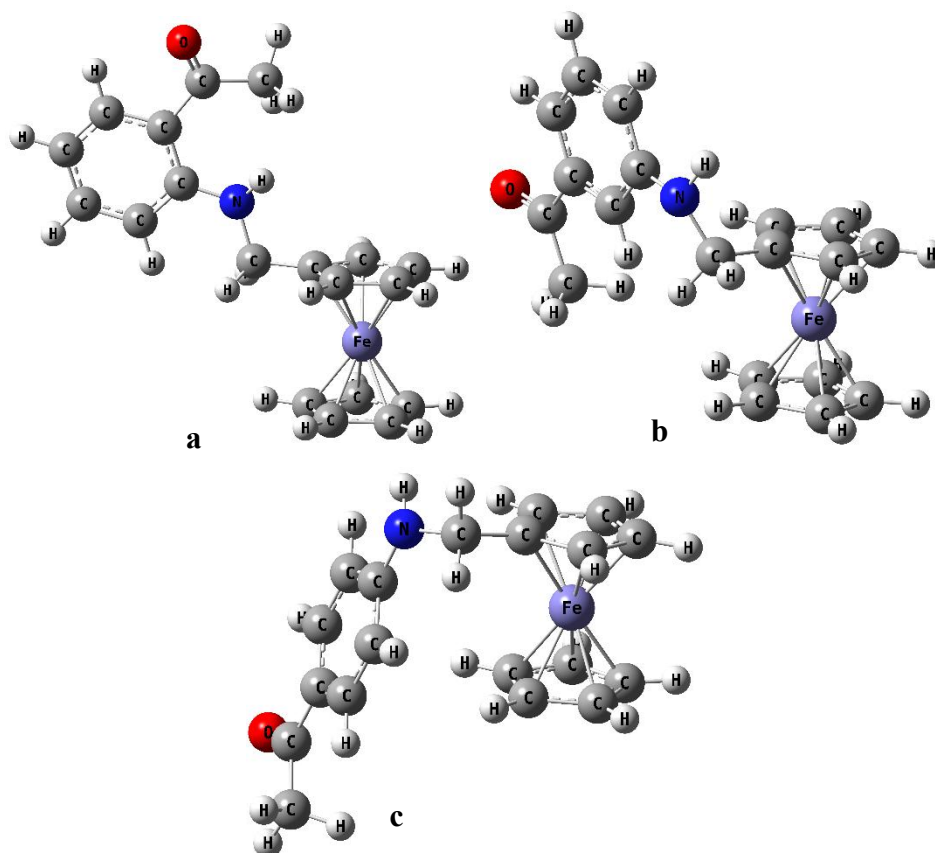


Figure 18. Optimised molecular structures of ferrocenyl derivatives used as ligands: FcMe2Ac (a), FcMe3Ac (b) and FcMe4Ac (c).

The ligands were prepared using AutoDock Tools by assigning Gasteiger charges and defining rotatable bonds. The structures were then saved in PDBQT format.

5.2.3. Docking Protocol and Validation

The overall docking workflow adopted in this study is illustrated in [Figure 19](#), showing the sequence of protein preparation, ligand preparation, grid generation, docking and interaction analysis.

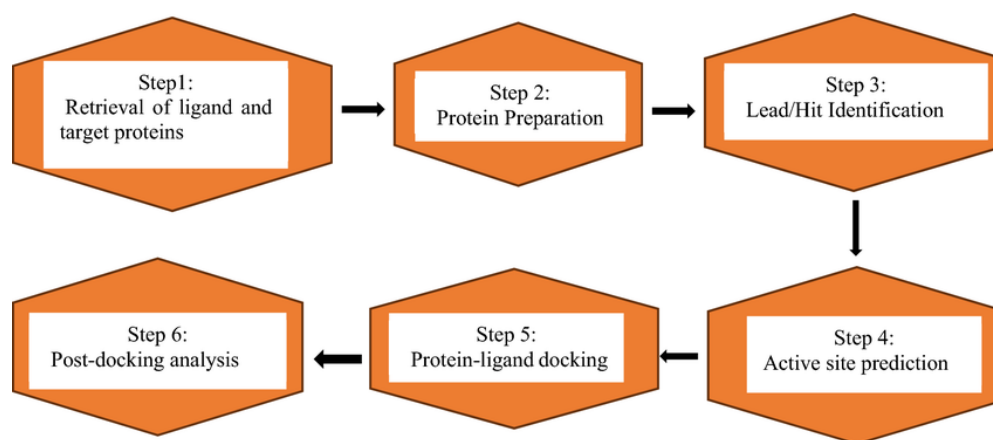


Figure 19. Molecular docking workflow illustrating protein and ligand preparation, grid generation, docking execution and interaction analysis.

Docking calculations were carried out using AutoDock 4.2 with the Lamarckian genetic algorithm. A grid box of $60 \times 60 \times 60$ Å with a spacing of 0.375 Å was centred on the coordinates of the co-crystallised ligand within the active site.

For each system, 100 independent docking runs were performed. The most stable conformation was selected based on the lowest binding energy and cluster population.

Validation of the docking protocol was performed by redocking the native ligand into the active site, in order to confirm the robustness and reliability of the docking procedure (Lanez et al., 2024).

5.3. Molecular Dynamics (MD) Simulations

Molecular dynamics simulations were performed to evaluate the stability and dynamic behaviour of the selected protein–ligand complexes using GROMACS (Lanez et al., 2023).

5.3.1. System Setup and Solvation

The solvation of the protein–ligand complex within an explicit water box is presented in Figure 20, which illustrates the simulation environment prior to energy minimization.

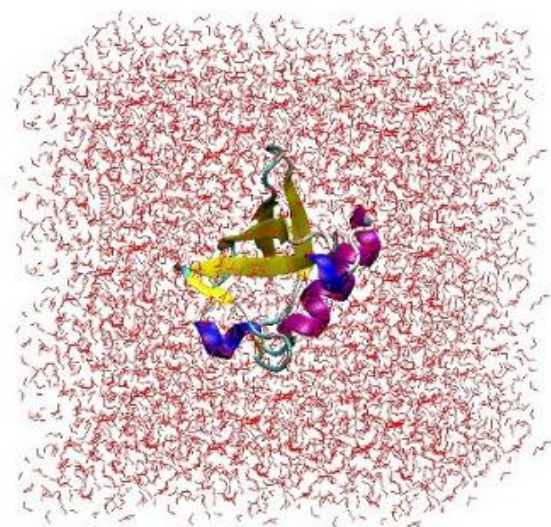


Figure 20. Solvated protein–ligand complex showing the explicit water box used for molecular dynamics simulations.

The selected complexes were parameterised using the CHARMM36 force field ([Huang & MacKerell, 2013](#)), and ligand parameters were generated using the CGenFF protocol ([Vanommeslaeghe & MacKerell, 2012](#)). Each system was placed in a cubic simulation box with a minimum distance of 10 Å from the protein surface to the box boundary and solvated using the TIP3P water model. Counter ions were added to neutralise the system.

5.3.2. Energy Minimisation and Equilibration

The general molecular dynamics simulation protocol, including minimisation, equilibration and production stages, is summarised in [Figure 21](#).

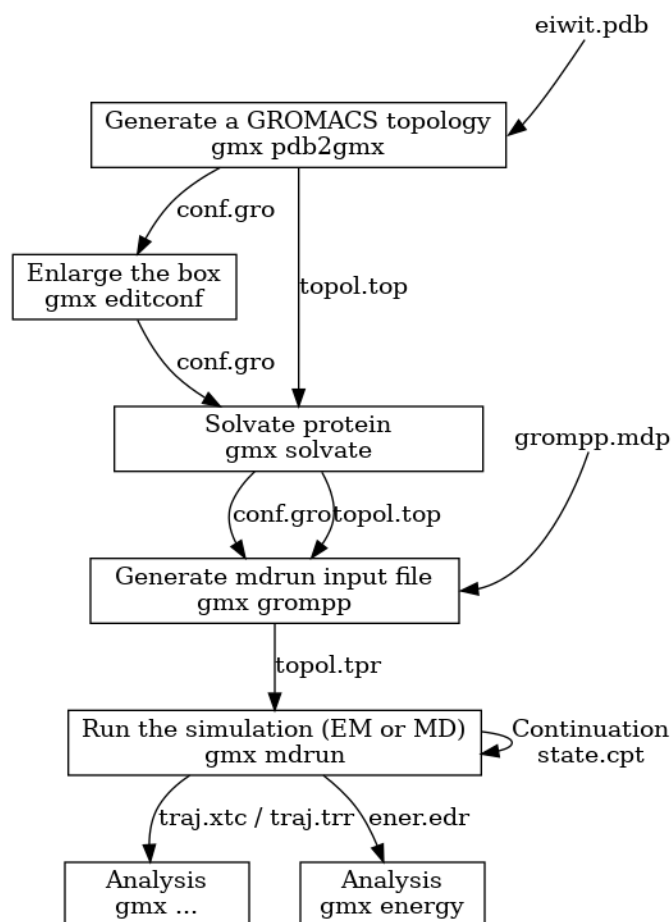


Figure 21. Molecular dynamics simulation workflow including system preparation, energy minimisation, equilibration and production phases.

Energy minimisation was performed using the steepest descent algorithm. The system was then equilibrated under NVT conditions (300 K, 500 ps) followed by NPT conditions (1 atm, 500 ps) using the V-rescale thermostat and Parrinello–Rahman barostat.

5.3.3. Production Run Parameters

Production simulations were conducted for 100 ns with a time step of 2 fs. Periodic boundary conditions were applied in all directions, and long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method. Covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm.

Trajectory analyses were carried out using GROMACS built-in tools, including RMSD, RMSF, radius of gyration (Rg), and solvent surface accessibility area (SASA).

5.4. MM-PBSA Binding Free Energy Calculations

Binding free energy calculations were performed using the g_mmpbsa tool ([Kumari et al., 2014](#)). Snapshots extracted from the last 20 ns of equilibrated trajectories were used.

The total binding free energy (ΔG_{bind}) was estimated as the sum of van der Waals, electrostatic, polar solvation and non-polar solvation contributions.

5.5. ADMET Prediction

ADMET predictions were performed using ADMETlab 2.0 ([Dong et al., 2018](#)) and ProTox-II ([Banerjee et al., 2018](#)) to evaluate pharmacokinetic and toxicity profiles of the synthesised compounds.

Parameters assessed included gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 interactions, plasma protein binding, hepatotoxicity and acute toxicity (LD_{50}).

Chapter 02:

Results & Discussion



1. General Introduction

This chapter presents and discusses the experimental and computational results obtained for the synthesised ferrocenyl acetylaniline derivatives. The analysis aims to establish clear relationships between molecular structure, electronic properties and their potential biological relevance through a comprehensive interpretation of both experimental data and *in silico* findings.

The discussion begins with the evaluation of the synthetic outcomes, including reaction efficiency and substituent effects, followed by detailed structural characterisation using FT-IR, UV–visible spectroscopy, nuclear magnetic resonance (^1H , ^{13}C and 2D NMR) and cyclic voltammetry. These techniques provide complementary information allowing confirmation of the molecular structures and insight into the electronic properties of the ferrocenyl core.

Subsequently, computational investigations are presented to support and extend the experimental observations. Density functional theory calculations are used to analyse electronic descriptors and charge distribution, while molecular docking and molecular dynamics simulations provide insight into the interaction of the compounds with thyroid-related molecular targets. Finally, MM-PBSA and ADMET analyses are discussed to evaluate binding stability and pharmacokinetic potential.

2. Synthesis of Ferrocenyl Acetylaniline Derivatives

The ferrocenyl acetylaniline derivatives (FcMe2Ac, FcMe3Ac and FcMe4Ac) were successfully synthesised via a nucleophilic substitution reaction involving a ferrocenylmethyl quaternary ammonium intermediate and the corresponding aminoacetophenone isomers. The adopted synthetic approach proved to be efficient and reproducible, yielding the desired compounds as yellow to orange crystalline solids under relatively mild experimental conditions.

The reaction mechanism is illustrated in [Figure 22](#), which highlights the key steps involved in the formation of the C–N bond through nucleophilic substitution at the ferrocenylmethyl position.

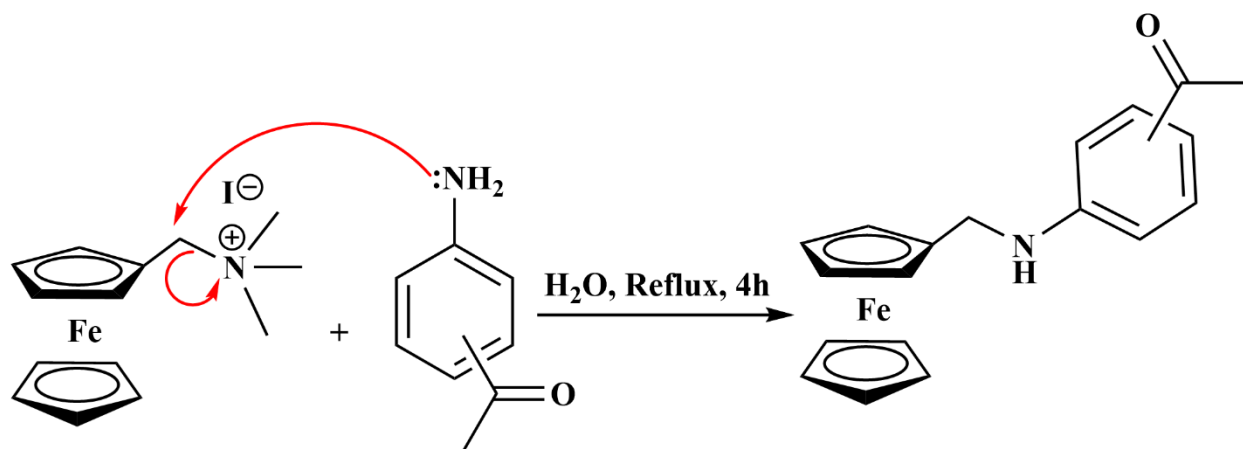


Figure 22. Proposed reaction mechanism for the formation of N-[(ferrocenyl)methyl] acetylaniline derivatives via nucleophilic substitution.

As shown in [Figure 22](#), the reaction proceeds via an S_N2-type mechanism in which the lone pair of electrons on the amino group attacks the electrophilic methylene carbon attached to the quaternary ammonium moiety ([Ouennoughi et al., 2017](#)). This results in the displacement of trimethylamine as a leaving group and formation of the desired ferrocenylmethylaniline linkage ([Ouennoughi et al., 2017](#)).

The efficiency of this transformation can be attributed to several factors. First, the ferrocenyl unit acts as an electron-donating group, which stabilises the transition state and enhances the electrophilicity of the methylene carbon ([Chebbah et al., n.d.](#)). Secondly, the quaternary ammonium group constitutes an excellent leaving group, facilitating bond cleavage under aqueous conditions. Such behaviour is well documented in the literature for ferrocenylmethyl derivatives, where nucleophilic substitution reactions proceed readily due to the combined electronic and structural features of the ferrocenyl system ([Hanane et al., 2023](#); [Adaika et al., 2025](#)).

The chemical structures of the synthesised compounds are presented in [Figure 23](#), highlighting the positional variation of the acetyl substituent on the aromatic ring.

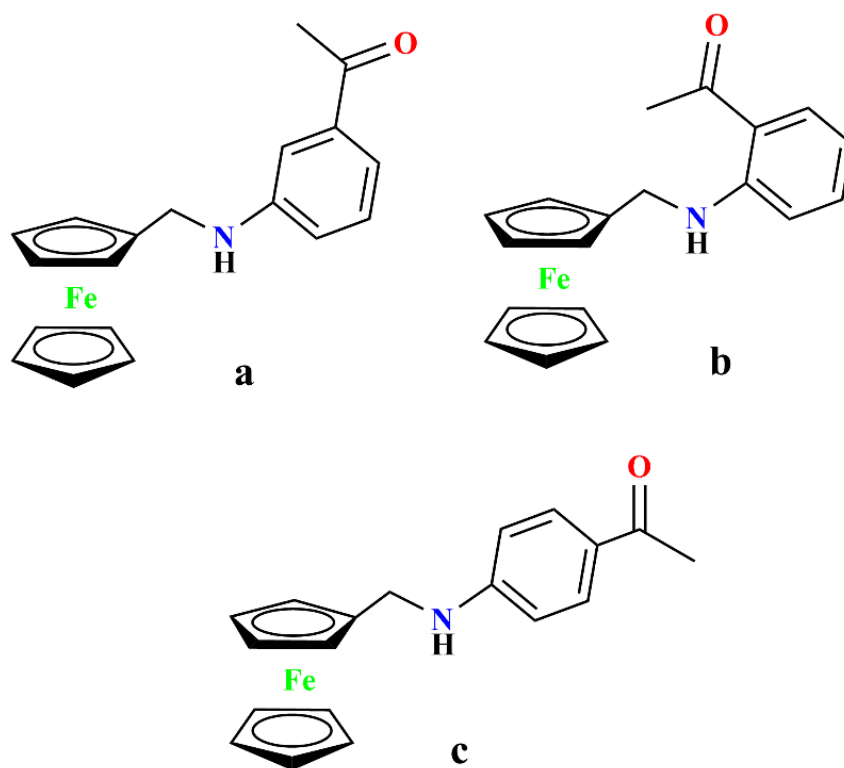


Figure 23. Chemical structures of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac (ortho), (b) FcMe3Ac (meta), and (c) FcMe4Ac (para).

As illustrated in [Figure 23](#), the three derivatives differ only by the position of the acetyl group (ortho, meta or para), which is expected to influence both steric accessibility and electronic distribution. These factors play a crucial role in determining the reactivity of the amino group during the substitution process ([Lanez et al., 2025](#)).

To better evaluate the synthetic efficiency, the yields and physical characteristics of the obtained compounds are summarised in [Table 2](#).

Table 2. Yields and physical properties of the synthesised ferrocenyl acetylaniline derivatives.

Compound	Substituent Position	Yield (%)	Appearance	Melting Point (°C)
FcMe2Ac	Ortho	77	Yellow crystalline solid	148–150
FcMe3Ac	Meta	48	Yellow–orange solid	138–140
FcMe4Ac	Para	80	Deep yellow solid	162–164

As shown in **Table 2**, the reaction yields follow the order: FcMe4Ac > FcMe2Ac > FcMe3Ac

This trend can be rationalised by considering both steric and electronic effects, which are known to govern nucleophilic substitution reactions in aromatic amines. The para-substituted derivative (FcMe4Ac) exhibited the highest yield (80%), reflecting minimal steric hindrance and favourable resonance interaction between the acetyl group and the amino functionality. This electronic delocalisation enhances the nucleophilicity of the amine, thereby facilitating the substitution process (Lanez et al., 2025).

In contrast, the meta derivative (FcMe3Ac) showed a significantly lower yield (48%), which can be attributed to the absence of resonance interaction between the substituents. In the meta position, the acetyl group exerts mainly an inductive electron-withdrawing effect, reducing the electron density on the amino group and consequently its nucleophilicity.

The ortho derivative (FcMe2Ac) presented intermediate behaviour (77%), where steric hindrance between adjacent substituents partially limits the accessibility of the nucleophilic site, despite favourable electronic effects.

These observations are in good agreement with previously reported studies on substituted aniline derivatives, where both steric congestion and electronic factors significantly influence reactivity. Furthermore, the results confirm that the adopted synthetic strategy is robust and well adapted for the preparation of ferrocenyl derivatives with controlled structural variation (Benine et al., 2025).

3. FT-IR Spectral Analysis

The structural features of the synthesised ferrocenyl acetylaniline derivatives were first examined by Fourier-transform infrared spectroscopy. The recorded FT-IR spectra for FcMe2Ac, FcMe3Ac and FcMe4Ac are presented in Figure 24, while the main absorption bands and their assignments are summarised in Table 3.

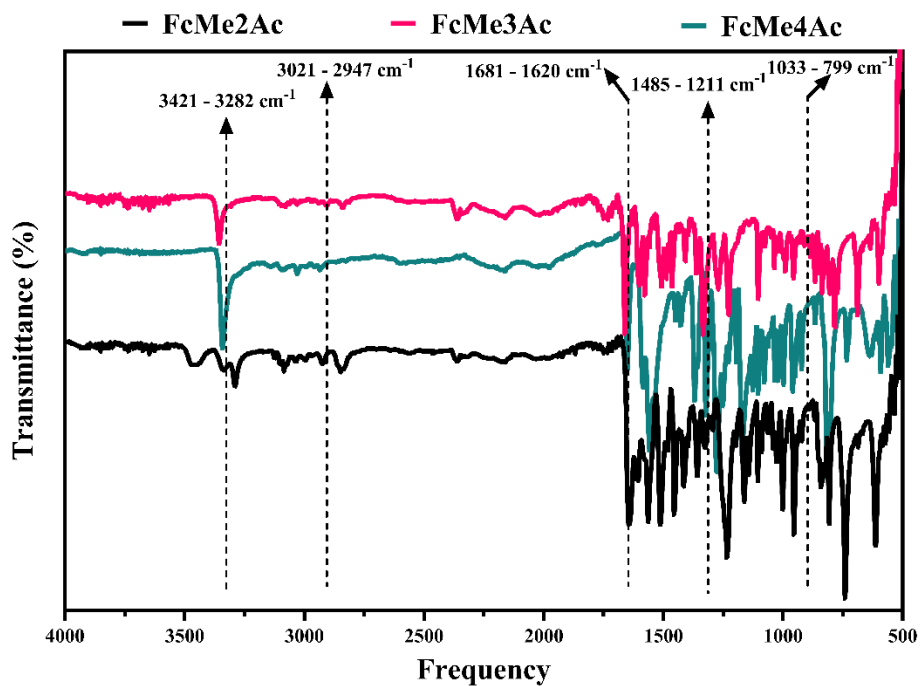


Figure 24. FT-IR spectra of the synthesised ferrocenyl acetylaniline derivatives.

Table 3. Characteristic FT-IR absorption bands (cm^{-1}) and their assignments for the synthesised compounds.

Assignment	FcMe2Ac (cm^{-1})	FcMe3Ac (cm^{-1})	FcMe4Ac (cm^{-1})	Description
N-H stretching	3426, 3291	3432, 3338	3445, 3345	Secondary amine vibration
Ar-C-H stretching	3082	—	—	Aromatic C-H
C=O stretching	1647	1650	1651	Acetyl carbonyl group
C=C (aromatic)	1566, 1516	1582, 1510	1589, 1562	Aromatic ring vibrations
C-N stretching	1146	1170	1173	Amine linkage
C-H bending	1454, 1416	1450, 1365	1369	Methyl/aromatic bending
Out-of-plane C-H	1003, 617	820	822	Aromatic substitution pattern

As shown in [Figure 24](#), all three compounds exhibit similar spectral profiles, confirming the preservation of the ferrocenyl acetylaniline framework across the series, with only minor variations attributable to substituent position ([Tlili et al., 2024](#)).

The presence of broad absorption bands in the region $3445\text{--}3291\text{ cm}^{-1}$ is characteristic of N–H stretching vibrations, confirming the formation of the secondary amine linkage resulting from the nucleophilic substitution reaction. The slight variation in band positions among the three derivatives reflects differences in hydrogen bonding interactions and local electronic environments ([Tlili et al., 2025](#)).

A strong absorption band observed around $1651\text{--}1647\text{ cm}^{-1}$ corresponds to the stretching vibration of the carbonyl (C=O) group of the acetyl moiety. The consistency of this band across all compounds confirms the preservation of the acetyl functionality during the synthetic process.

The aromatic C=C stretching vibrations appearing in the region $1589\text{--}1510\text{ cm}^{-1}$ further support the presence of the substituted benzene ring. Additionally, the bands observed in the range $1173\text{--}1146\text{ cm}^{-1}$ can be attributed to C–N stretching vibrations, providing direct evidence for the successful formation of the C–N bond between the ferrocenylmethyl group and the aniline derivative.

The out-of-plane C–H bending vibrations observed between 820 and 617 cm^{-1} are particularly informative, as they reflect the substitution pattern on the aromatic ring. Differences in these bands among the ortho, meta and para derivatives further confirm the positional variation of the acetyl group.

Overall, the FT-IR analysis confirms the successful synthesis of the target ferrocenyl acetylaniline derivatives, with all characteristic functional groups clearly identified. The obtained spectral data are in good agreement with previously reported values for structurally related ferrocenyl and aniline-based compounds, further supporting the structural assignment ([Khamouli et al., 2022](#)).

4. UV–Visible Spectral Analysis

The electronic absorption properties of the synthesised ferrocenyl acetylaniline derivatives were investigated by UV–visible spectroscopy in DMSO. The recorded spectra are presented in [Figure 25](#), while the corresponding absorption maxima are summarised in [Table 4](#).

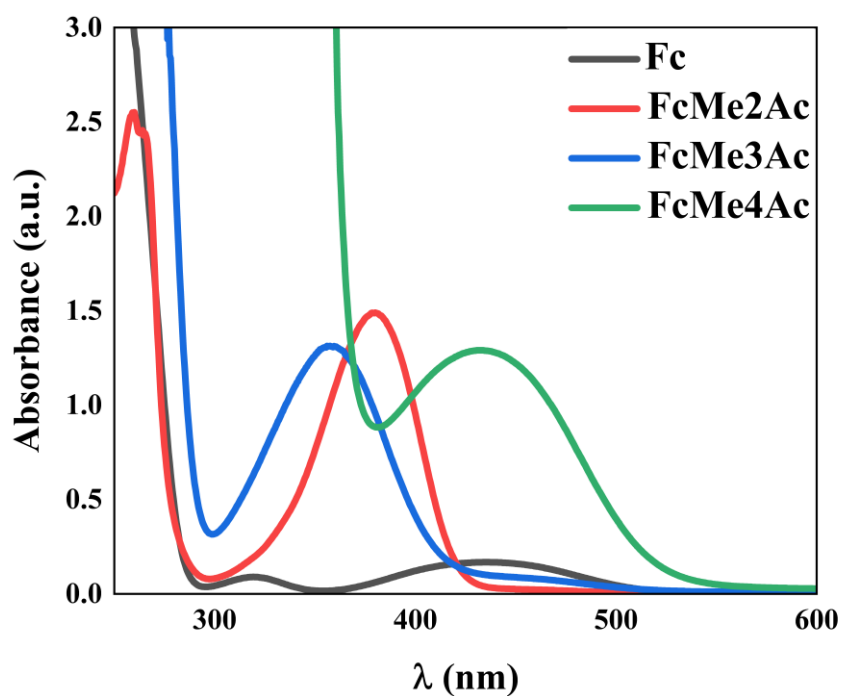


Figure 25. UV–visible absorption spectra of the synthesised ferrocenyl acetylaniline derivatives.

Table 4. UV–visible absorption maxima (λ_{max}) of the synthesised ferrocenyl acetylaniline derivatives.

Compound	λ_{max} (nm)	Assignment
FcMe2Ac	259	$\pi \rightarrow \pi^*$ (aromatic system)
	380	d–d (Fe^{2+} centre)
FcMe3Ac	268	$\pi \rightarrow \pi^*$
	402	d–d transition
FcMe4Ac	432	d–d / charge transfer

As shown in [Figure 25](#), all compounds exhibit characteristic absorption bands associated with both the aromatic system and the ferrocenyl moiety. The presence of intense absorption bands in the ultraviolet region (259–268 nm) is attributed to $\pi \rightarrow \pi^*$ transitions within the conjugated aromatic system. These transitions arise from electron excitation within the benzene ring and are commonly observed in substituted aniline derivatives ([Zouaghi et al., 2025](#)).

In the visible region, the absorption bands observed between 380 and 432 nm are associated with transitions involving the ferrocenyl iron centre. These bands can be attributed to metal-centred d–d transitions and, in some cases, ligand-to-metal charge transfer (LMCT), reflecting the interaction between the iron atom and the surrounding π -electron system.

A notable bathochromic shift is observed from FcMe2Ac (380 nm) to FcMe4Ac (432 nm), indicating an increase in conjugation and electronic delocalisation in the para-substituted derivative. This behaviour can be explained by the more effective resonance interaction between the acetyl group and the aromatic system in the para position, which enhances electron delocalisation and lowers the energy gap between molecular orbitals ([Zegheb et al., 2021](#)).

In contrast, the meta derivative (FcMe3Ac) exhibits intermediate behaviour, as the meta position does not allow efficient resonance interaction between substituents, leading to less pronounced electronic delocalisation. The ortho derivative (FcMe2Ac), although capable of resonance interaction, is affected by steric hindrance, which may slightly distort planarity and reduce conjugation efficiency.

These observations are consistent with previously reported studies on ferrocenyl and substituted aromatic systems, where substituent position significantly influences electronic transitions and absorption behaviour.

Overall, the UV–visible analysis confirms the presence of the ferrocenyl chromophore and highlights the impact of substituent position on the electronic structure of the compounds, which is directly related to their redox behaviour and electronic properties ([Ben Amor et al., 2025](#)).

5. NMR Spectral Analysis

The structural elucidation of the synthesised ferrocenyl acetylaniline derivatives was comprehensively achieved using ^1H NMR, ^{13}C NMR and two-dimensional NMR techniques. In order to ensure clarity and facilitate interpretation, the spectra are presented separately for each compound, allowing direct correlation between spectral features and molecular structure.

5.1. ^1H NMR Analysis

The ^1H NMR spectra of the three derivatives are presented in Figure 26.

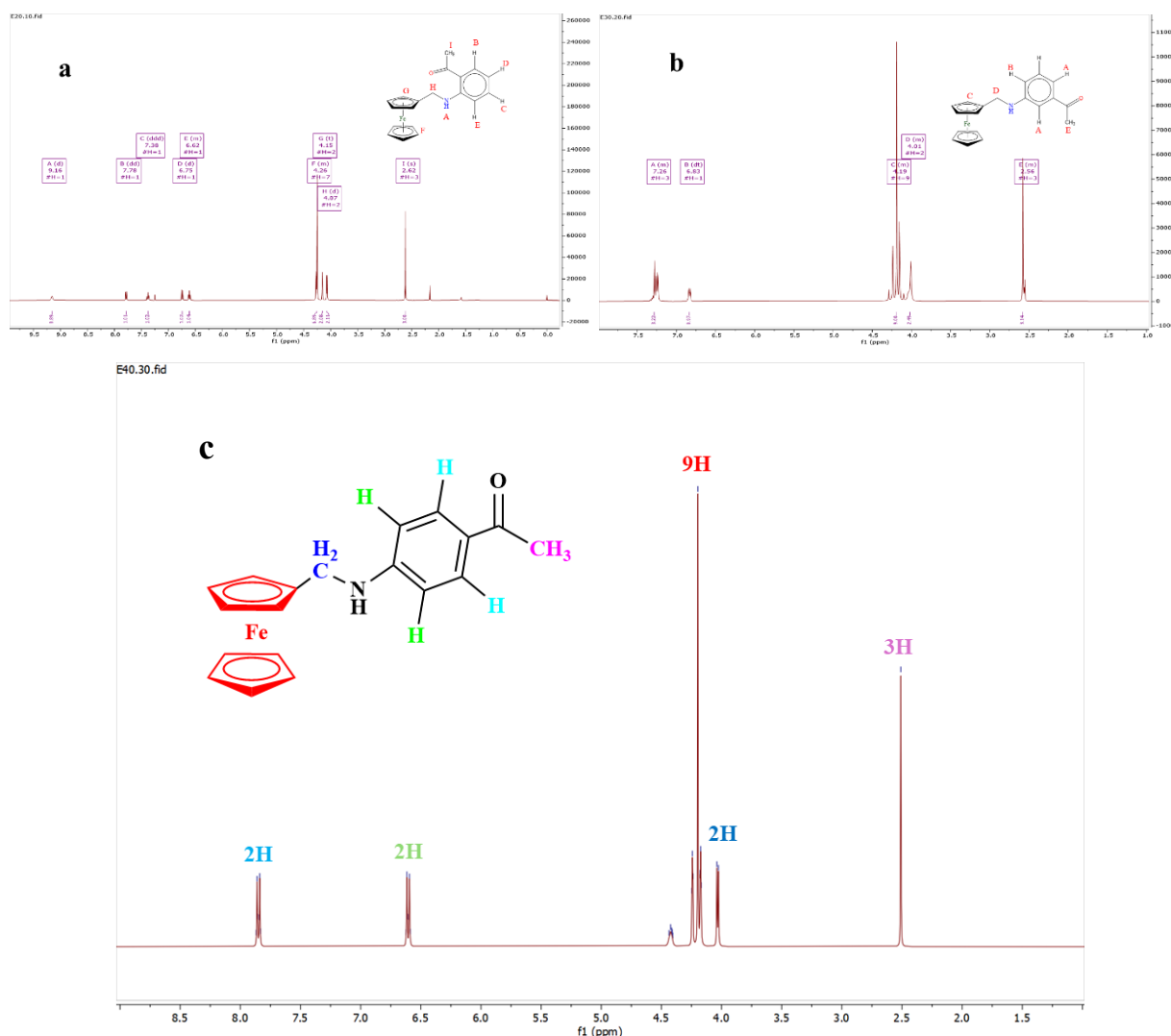


Figure 26. ^1H NMR spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe₂Ac, (b) FcMe₃Ac, and (c) FcMe₄Ac.

As shown in Figure 26, all compounds exhibit characteristic proton signals confirming the successful formation of the target structures.

The methyl group of the acetyl moiety ($-\text{COCH}_3$) appears as a singlet in the region 2.35–2.60 ppm. The methylene bridge ($-\text{CH}_2-\text{N}-$), which links the ferrocenyl unit to the aromatic ring, is observed as a doublet around 4.30 ppm, confirming the formation of the C–N bond (Rebiai et al., 2014).

The aromatic protons are observed in the region 6.6–7.8 ppm, with distinct patterns depending on the substitution position. The ortho, meta and para isomers exhibit clearly different splitting behaviours, allowing unambiguous differentiation between the three compounds.

The NH proton appears as a downfield signal ($\approx 8.9\text{--}9.0$ ppm) in FcMe2Ac and FcMe3Ac, indicating possible hydrogen bonding interactions. In FcMe4Ac, a slight up field shift is observed, which may be attributed to differences in electronic delocalisation in the para-substituted system (Ben Amor et al., 2025).

The ^{13}C NMR spectra of the synthesised compounds are shown in [Figure 27](#).

Figure 2. ^{13}C NMR spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac.

As illustrated in Figure 27, the spectra provide clear evidence for the carbon framework of the compounds.

The carbonyl carbon ($\text{C}=\text{O}$) is observed in the downfield region between 194 and 210 ppm, confirming the presence of the acetyl group. The variation in chemical shift among the three derivatives reflects differences in electronic environment due to substituent position.

The methylene carbon ($\text{CH}_2\text{-N}$) appears consistently around 42–43 ppm, confirming the linkage between the ferrocenyl moiety and the aromatic system.

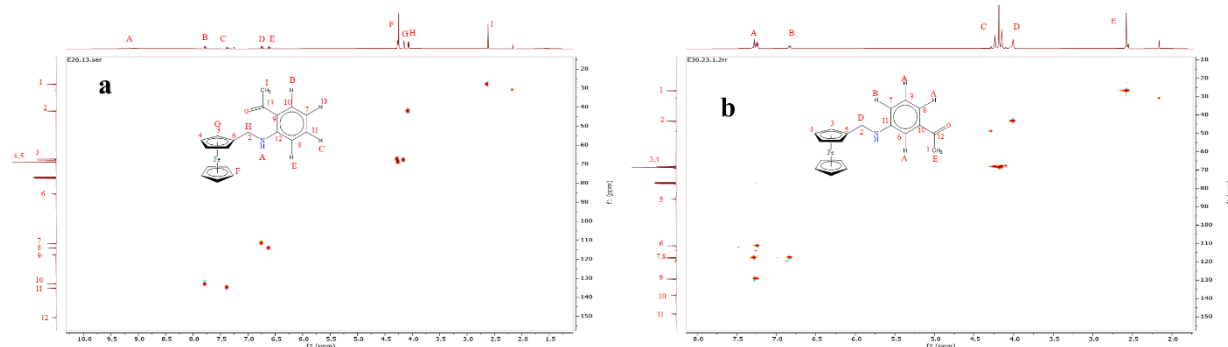
The ferrocenyl carbons are detected in the range 66–69 ppm, corresponding to the cyclopentadienyl rings. The ipso carbon bonded to the substituent appears slightly downfield due to its distinct electronic environment.

Aromatic carbons are distributed between 112 and 154 ppm, with slight variations depending on substitution pattern, which is consistent with substituted benzene derivatives (Khamouli et al., 2019).

5.3. Two-Dimensional NMR Analysis (HSQC and HMBC)

Two-dimensional NMR spectroscopy was employed to achieve unambiguous structural assignment and to confirm the connectivity within the synthesised ferrocenyl acetylaniline derivatives. The HSQC and HMBC spectra are presented separately to allow a clear interpretation of both direct and long-range correlations.

The HSQC spectra of FcMe2Ac, FcMe3Ac and FcMe4Ac are shown in Figure 28, while the corresponding HMBC spectra are presented in Figure 29.



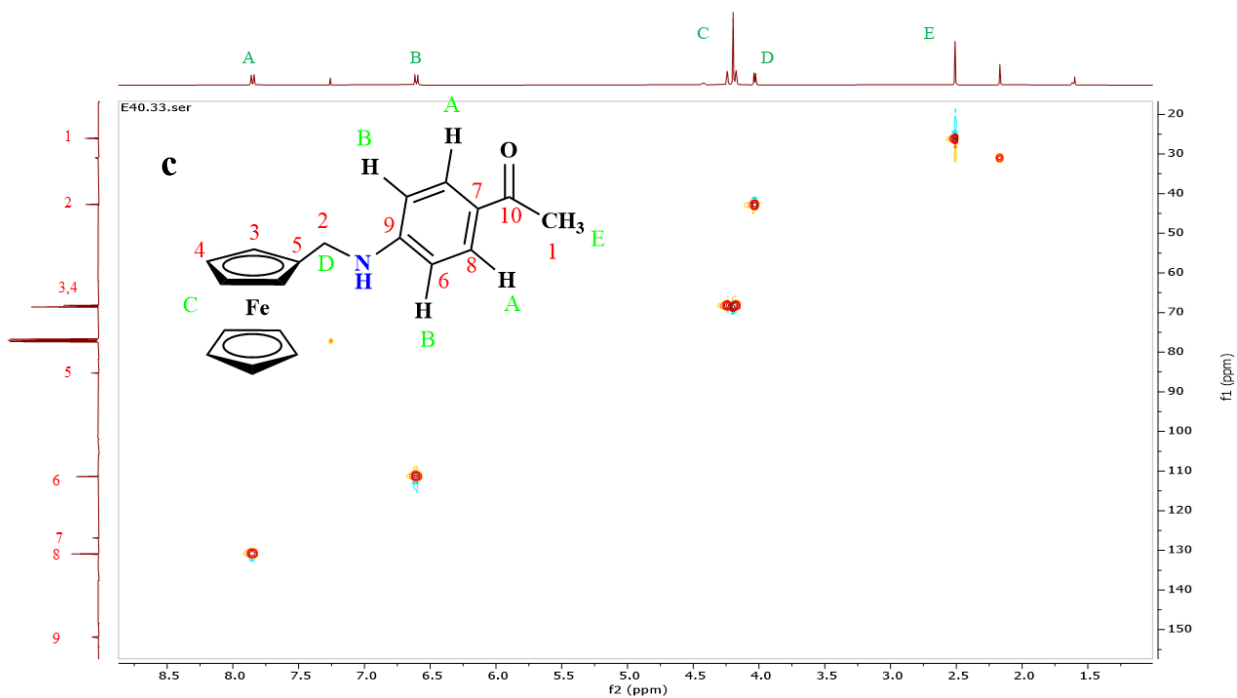
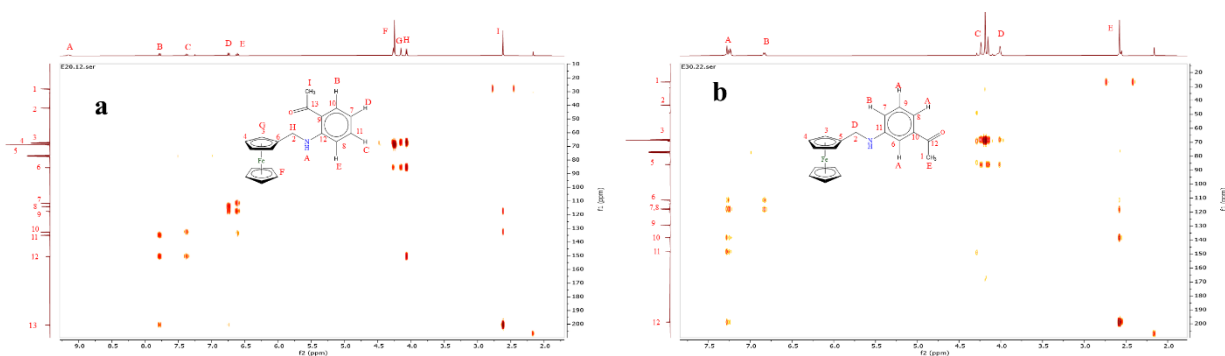


Figure 3. HSQC spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac.



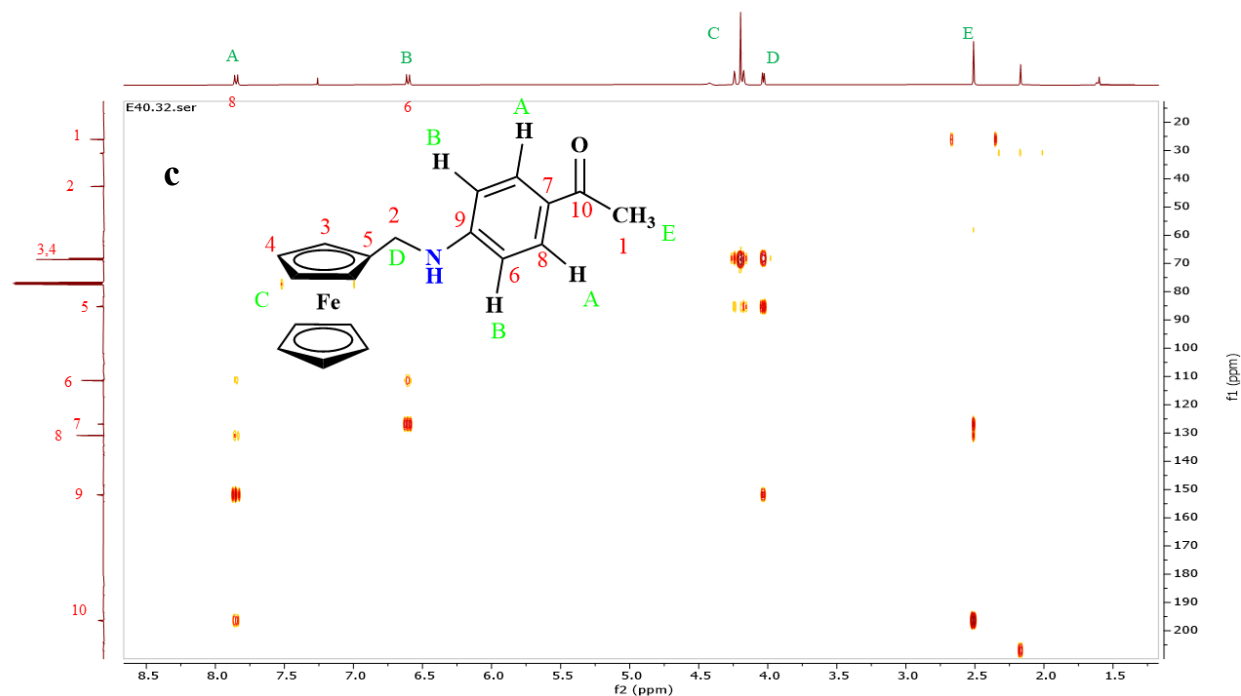


Figure 29. HMBC spectra of the synthesised ferrocenyl acetylaniline derivatives: (a) FcMe2Ac, (b) FcMe3Ac, and (c) FcMe4Ac.

As illustrated in Figure 28, the HSQC spectra provide direct one-bond correlations between proton and carbon nuclei (^1JCH), allowing precise assignment of all protonated carbon atoms. The methylene protons ($-\text{CH}_2-\text{N}-$), observed around 4.30 ppm in the ^1H NMR spectra, show clear cross-peaks with their corresponding carbon signals at approximately 42–43 ppm, confirming the presence of the ferrocenylmethyl linkage.

Similarly, the signals corresponding to the cyclopentadienyl rings exhibit well-defined correlations. The unsubstituted Cp ring (C_5H_5) shows a single correlation pattern consistent with its symmetry, whereas the substituted Cp ring (C_5H_4) displays multiple correlations reflecting the loss of equivalence upon substitution. These observations are characteristic of monosubstituted ferrocenyl systems and further confirm the structural integrity of the organometallic core.

The aromatic proton signals also show distinct HSQC correlations with their corresponding carbon atoms in the region 112–140 ppm, enabling clear assignment of the benzene ring protons. The differences observed between the ortho, meta and para derivatives further support the positional variation of the acetyl substituent (Mouada et al., 2024; Lanez & Henni, 2015).

In contrast, the HMBC spectra ([Figure 29](#)) provide long-range correlations (^2JCH and ^3JCH), which are essential for confirming the connectivity between different fragments of the molecule. In particular, strong correlations are observed between the methylene protons ($-\text{CH}_2-\text{N}-$) and the aromatic carbons, confirming the formation of the C–N bond linking the ferrocenyl unit to the aniline moiety.

Additional long-range correlations between aromatic protons and the carbonyl carbon ($\text{C}=\text{O}$) are observed, allowing precise localisation of the acetyl group on the aromatic ring. These correlations are especially important for distinguishing between the Regio isomers, as the HMBC patterns differ significantly depending on the substitution position (ortho, meta or para).

Furthermore, correlations involving the ferrocenyl carbons provide additional confirmation of the substitution pattern on the cyclopentadienyl ring. The combined HSQC and HMBC data therefore establish a complete mapping of proton–carbon connectivity within the molecules ([Bekkar et al., 2025](#)).

Overall, the two-dimensional NMR analysis provides definitive structural confirmation of the synthesised ferrocenyl acetylaniline derivatives. The observed correlation patterns are fully consistent with the proposed structures and are in excellent agreement with literature reports on related ferrocenyl compounds, where HSQC and HMBC techniques are commonly used to validate substitution patterns and molecular connectivity.

6. Cyclic Voltammetry Characterization

6.1. Redox Properties and Voltammetric Response

The electrochemical behaviour of FcMe_2Ac , FcMe_3Ac and FcMe_4Ac was investigated by cyclic voltammetry in DMSO (1 mM) containing 0.1 M Bu_4NBF_4 as supporting electrolyte, using $\text{Hg}/\text{Hg}_2\text{Cl}_2$ as the reference electrode. The measurements were performed within a potential window of -0.60 to $+0.60$ V at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$. Ferrocene (Fc/Fc^+) was employed as an internal reference ($E^\circ = 0.00 \text{ V}$).

The corresponding cyclic voltammograms are presented in [Figure 30](#), where the electrochemical responses of the substituted derivatives are compared with that of ferrocene.

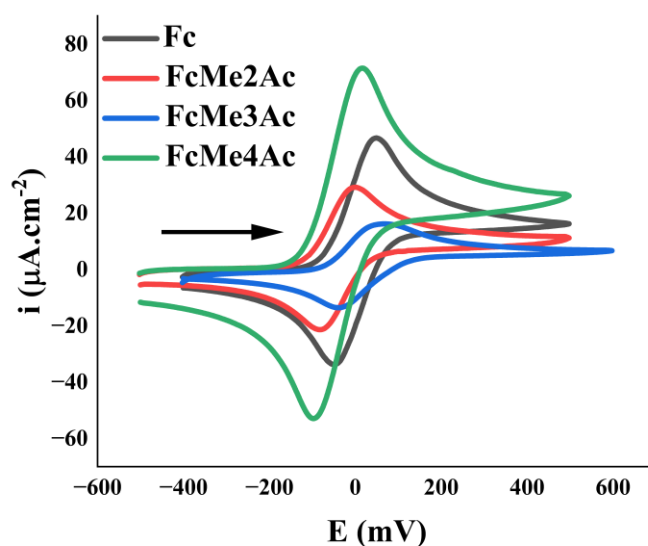


Figure 30. Cyclic voltammograms of Fc, FcMe2Ac, FcMe3Ac and FcMe4Ac recorded in DMSO containing 0.1 M Bu₄NBF₄ at a scan rate of 100 mV·s⁻¹ (referenced to Fc⁺/Fc).

As observed in Figure 30, all compounds exhibit a single, well-defined redox couple corresponding to the Fe (II)/Fe (III) transition of the ferrocenyl centre. This confirms that the electro-active ferrocene unit retains its characteristic reversible behaviour after functionalisation (Lanez et al., n.d.).

A progressive anodic shift of the formal potential (E°) is observed upon substitution: FcMe2Ac (43.9 mV) < FcMe3Ac (51.0 mV) < FcMe4Ac (57.5 mV). This shift indicates a modulation of the electronic environment of the ferrocenyl core by the acetylaniline substituent. The increase in oxidation potential suggests a relative stabilisation of the Fe (II) state and/or a decreased electron density at the metal centre. Such behaviour is commonly reported in substituted ferrocenes, where electron-withdrawing groups induce anodic shifts in redox potentials (Nacer & Lanez, 2013).

The anodic-to-cathodic peak current ratios ($I_{pa}/I_{pc} \approx 1$) confirm that the redox process is chemically reversible. However, the peak-to-peak separation ($\Delta E_p = 87.8\text{--}115$ mV) exceeds the theoretical value for a fully reversible one-electron transfer (59 mV), indicating a quasi-reversible system. This deviation may arise from kinetic limitations of electron transfer, uncompensated resistance in the medium, or structural reorganisation associated with oxidation (Laraoui et al., 2023).

6.2. Scan Rate Dependence and Mass Transport Regime

The influence of scan rate on the electrochemical response was examined in the range 100–500 $\text{mV}\cdot\text{s}^{-1}$. The resulting voltammograms are shown in Figure 31, while the dependence of anodic peak current on the square root of scan rate is presented in Figure 32.

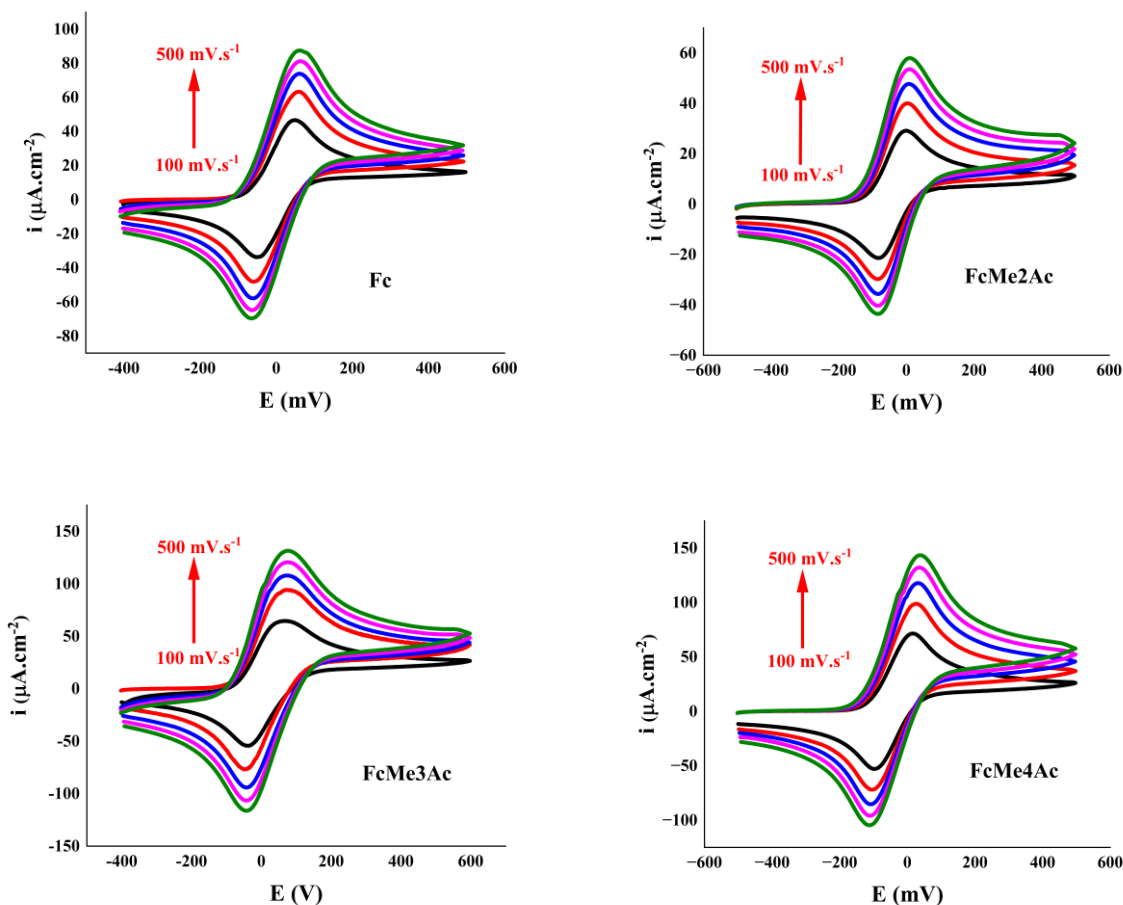


Figure 31. Cyclic voltammograms of Fc, FcMe2Ac, FcMe3Ac and FcMe4Ac at different scan rates (100–500 $\text{mV}\cdot\text{s}^{-1}$).

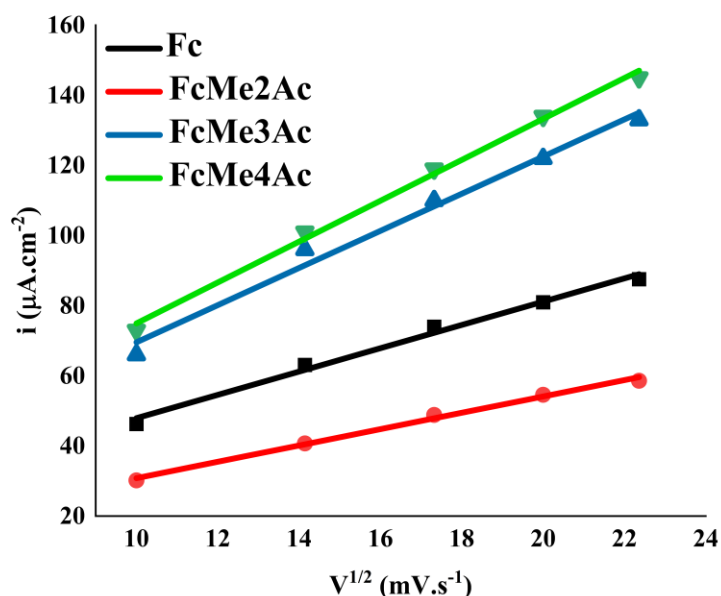


Figure 32. Linear variation of anodic peak current (i_{pa}) as a function of the square root of scan rate ($v^{1/2}$).

As illustrated in [Figure 31](#), increasing the scan rate results in a proportional increase in both anodic and cathodic peak currents, without significant distortion of peak shape. This behaviour indicates that the redox process is not governed by adsorption phenomena [\[149\]](#).

A linear relationship between anodic peak current (i_{pa}) and $v^{1/2}$ is clearly observed in [Figure 32](#) for all compounds. The corresponding linear regression equations ([Table 5](#)) exhibit high correlation coefficients ($R^2 = 0.980\text{--}0.994$), confirming excellent linearity ([Lanez & Henni, 2025](#)).

The high R^2 values demonstrate that the experimental data closely follow the Randles–Ševčík model, thereby confirming that the electron transfer process is diffusion-controlled. Slight deviations from ideal linearity, particularly for FcMe3Ac ($R^2 = 0.980$), may reflect minor contributions from kinetic limitations or increased solution resistance ([Khelef & Lanez, 2015](#)).

6.3. Diffusion Coefficients and Transport Properties

The diffusion coefficients of the electroactive species were determined using the Randles–Ševčík [Equation 1](#) for a reversible system, which relates the peak current to the square root of the scan rate:

$$ip_a = 2.69 \times 10^5 \left(\sqrt{n} \right)^3 SC \sqrt{D} \sqrt{v} \quad \text{Equation 1}$$

where i_p is the peak current (A), n is the number of electrons transferred, A is the electrode surface area (cm^2), C is the concentration of the electroactive species ($\text{mol} \cdot \text{cm}^{-3}$), D is the diffusion coefficient ($\text{cm}^2 \cdot \text{s}^{-1}$), and v is the scan rate ($\text{V} \cdot \text{s}^{-1}$).

The diffusion coefficients (D) were calculated using the Randles–Ševčík equation, and the results are presented in [Table 5](#).

Table 5. Diffusion coefficients and linear regression parameters obtained from Randles–Ševčík analysis.

Compound	Equation	R ²	D × 10 ⁻⁷ (cm ² ·s ⁻¹)
Fc	y = 3.321x + 14.727	0.987	6.952
FcMe2Ac	y = 2.328x + 7.539	0.993	1.108
FcMe3Ac	y = 5.298x + 16.578	0.980	5.738
FcMe4Ac	y = 5.831x + 16.638	0.994	2.255

As shown in [Table 5](#), the diffusion coefficients of the substituted derivatives are significantly lower than that of ferrocene. This decrease can be attributed to several physicochemical factors:

- Increased molecular volume, leading to reduced translational mobility
- Enhanced solute–solvent interactions in polar DMSO medium
- Greater structural complexity and conformational flexibility

FcMe2Ac exhibits the lowest diffusion coefficient ($1.11 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$), suggesting stronger intermolecular interactions and possibly a more hindered diffusion pathway. In contrast, FcMe3Ac retains a relatively higher diffusion coefficient, which may be associated with a more favourable molecular geometry and reduced steric constraints ([Lanez & Hemmami, 2016](#); [Benyza et al., 2022](#)).

6.4. Electron Transfer Kinetics

The heterogeneous electron-transfer rate constants were estimated using Nicholson’s method, which relates the peak-to-peak separation to the kinetic parameter Ψ and the standard rate constant as described in [Equation 2](#):

$$\psi = \frac{K_s}{\sqrt{\pi D \frac{nFv}{RT}}} \quad \text{Equation 2}$$

where k_s is the standard heterogeneous electron-transfer rate constant ($\text{cm} \cdot \text{s}^{-1}$), D is the diffusion coefficient ($\text{cm}^2 \cdot \text{s}^{-1}$), n is the number of electrons transferred, F is the Faraday constant ($96,500 \text{ C} \cdot \text{mol}^{-1}$), v is the scan rate ($\text{V} \cdot \text{s}^{-1}$), R is the gas constant ($8.314 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), and T is the absolute temperature (K).

The heterogeneous electron-transfer rate constants (k_s) were estimated using Nicholson's method, and the results are summarised in [Table 6](#).

Table 6. Nicholson kinetic parameters (Ψ , ΔE_p) and heterogeneous electron-transfer rate constants (k_s).

Compound	Ψ	ΔE_p (mV)	$k_s \times 10^3$ ($\text{cm} \cdot \text{s}^{-1}$)
Fc	1.51	73	2.88
FcMe2Ac	0.323	124	0.62
FcMe3Ac	0.074	263	0.14
FcMe4Ac	0.100	223	0.19

The obtained kinetic parameters clearly indicate a decrease in electron-transfer rates upon substitution. The order: $\text{Fc} > \text{FcMe2Ac} > \text{FcMe4Ac} > \text{FcMe3Ac}$, reflects the increasing complexity of the molecular structure and its impact on electron-transfer dynamics ([Lanez et al., 2024](#)).

The relatively high k_s value for ferrocene confirms its well-known fast and reversible electron-transfer behaviour. In contrast, the substituted derivatives exhibit slower kinetics, which can be explained by:

- Increased **steric hindrance**, limiting effective interaction with the electrode surface
- Higher **reorganisation energy** associated with structural changes during oxidation
- Reduced **electronic coupling** between the ferrocenyl centre and the electrode

The particularly low k_s value for FcMe3Ac suggests that the meta substitution pattern is least favourable for efficient electron transfer, likely due to suboptimal electronic communication within the molecule ([Filote et al., 2022](#)).

7. Density Functional Theory (DFT) Analysis

The electronic structure and intrinsic reactivity of the synthesised ferrocenyl acetylaniline derivatives were investigated using density functional theory (DFT). These calculations provide a quantitative and molecular-level interpretation of electronic distribution, orbital interactions and physicochemical properties, allowing a deeper understanding of the behaviour of the compounds beyond experimental characterisation (Boutarfaia et al., 2019).

7.1. Frontier Molecular Orbitals and Electronic Structure

The frontier molecular orbitals (HOMO and LUMO) constitute fundamental descriptors of molecular reactivity, as they govern electron donation and acceptance processes and influence molecular interaction behaviour. The calculated orbital energies for FcMe2Ac, FcMe3Ac and FcMe4Ac are presented in Table 7, while the corresponding orbital distributions are illustrated in Figure 33.

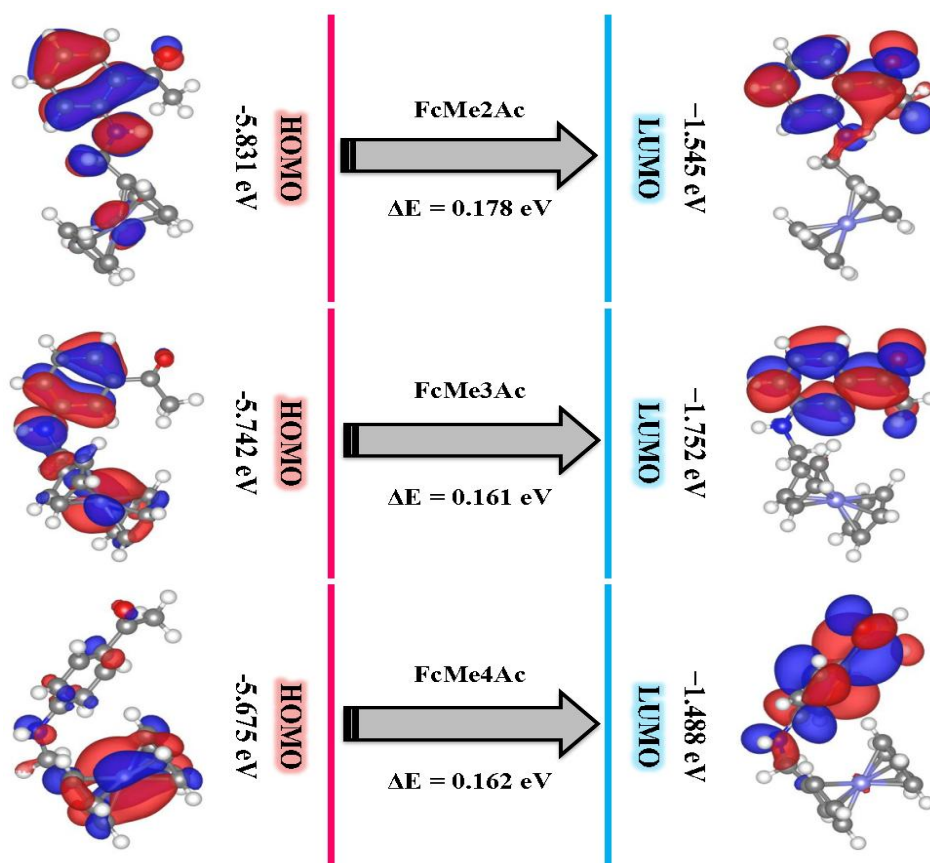


Figure 33. Frontier molecular orbitals of ferrocenyl acetylaniline derivatives: HOMO and LUMO surfaces.

Table 7. Frontier molecular orbital energies (eV) of the synthesised compounds.

Compound	EHOMO (eV)	ELUMO (eV)	ΔE (eV)
FcMe2Ac	−5.8306	−1.5453	4.285
FcMe3Ac	−5.7421	−1.7521	3.990
FcMe4Ac	−5.6752	−1.4882	4.187

As shown in [Table 7](#), a gradual increase in HOMO energy is observed from FcMe2Ac to FcMe4Ac. This trend indicates a progressive enhancement of electron-donor character with increasing substitution, which can be attributed to the influence of the acetylaniline moiety on the electronic density of the ferrocenyl system.

The HOMO–LUMO energy gap (ΔE) ranges from 3.990 to 4.285 eV, suggesting a balance between chemical stability and electronic flexibility. Notably, FcMe3Ac exhibits the smallest energy gap (3.990 eV), indicating relatively higher softness and enhanced reactivity compared to the other derivatives. In contrast, FcMe2Ac presents the largest gap, reflecting slightly higher kinetic stability ([Yahiaoui et al., 2023](#)).

The spatial distribution of the orbitals ([Figure 33](#)) reveals a clear electronic separation within the molecule. The HOMO is predominantly localised on the ferrocenyl unit and partially on the amine bridge, highlighting the role of the iron centre and cyclopentadienyl rings as electron-rich regions. Conversely, the LUMO is mainly concentrated on the aromatic ring and the carbonyl group, identifying these regions as electron-accepting sites.

This spatial separation of frontier orbitals indicates an intramolecular charge-transfer character, where electron density can be transferred from the ferrocenyl core towards the substituted aromatic system. Such behaviour is commonly observed in donor–acceptor systems and is strongly influenced by substituent position and electronic effects, which may play a role in ligand–target interactions ([Kedadra et al., 2022](#)).

7.2. Global Reactivity Descriptors and Thermodynamic Parameters

To further quantify the reactivity and stability of the compounds, global reactivity descriptors derived from frontier orbital energies were calculated. The results are summarised in [Table 8](#).

Table 8. Global reactivity descriptors and thermodynamic parameters of the synthesised derivatives at 298.15 K.

Parameter	FcMe2Ac	FcMe3Ac	FcMe4Ac
Hardness, η (eV)	2.143	1.995	2.094
Electronegativity, χ (eV)	3.688	3.747	3.582
Chemical potential, μ (eV)	-3.688	-3.747	-3.582
Softness, S (eV ⁻¹)	0.233	0.251	0.239
Electrophilicity, ω (eV)	3.174	3.519	3.064
Nucleophilicity, N	2.829	2.918	2.985
Dipole moment (D)	3.82	4.46	5.12
Polarizability (Å ³)	38.5	42.7	47.3
Enthalpy (kJ·mol ⁻¹)	-1245.6	-1388.2	-1531.4
Entropy (J·mol ⁻¹ ·K ⁻¹)	412.5	448.9	486.7
Heat capacity (J·mol ⁻¹ ·K ⁻¹)	236.4	258.7	281.3

The calculated hardness (η) values confirm that all compounds exhibit moderate chemical stability, consistent with their HOMO–LUMO gaps. Among them, FcMe3Ac shows the lowest hardness (1.995 eV) and highest softness (0.251 eV⁻¹), reinforcing its relatively higher reactivity.

The electrophilicity index (ω) follows the order: FcMe3Ac > FcMe2Ac > FcMe4Ac, indicating that FcMe3Ac has a stronger tendency to accept electrons, whereas FcMe4Ac displays higher nucleophilicity. This dual behaviour highlights the subtle balance between electron-donating and electron-accepting capacities within the series, which is relevant for molecular recognition processes (Meraghni et al., 2023).

The negative chemical potential values for all compounds indicate thermodynamic stability and a spontaneous tendency for charge redistribution. Additionally, the progressive increase in dipole moment from FcMe2Ac to FcMe4Ac reflects an increase in molecular polarity, which is directly related to the asymmetry and electronic distribution within the molecule.

A similar increasing trend is observed for polarizability, suggesting that the electronic cloud becomes more easily deformable with increasing substitution. This behaviour is associated with enhanced intermolecular interaction potential and increased responsiveness to external electric fields.

Thermodynamic parameters, including entropy and heat capacity, also increase across the series, indicating greater molecular flexibility and a higher number of accessible vibrational states. This can be attributed to the increasing structural complexity and conformational freedom of the substituted derivatives (Khennoufa et al., 2021).

7.3. Molecular Electrostatic Potential (MEP) Analysis

The molecular electrostatic potential maps provide valuable insight into charge distribution and reactive sites. The calculated MEP surfaces are presented in Figure 34.

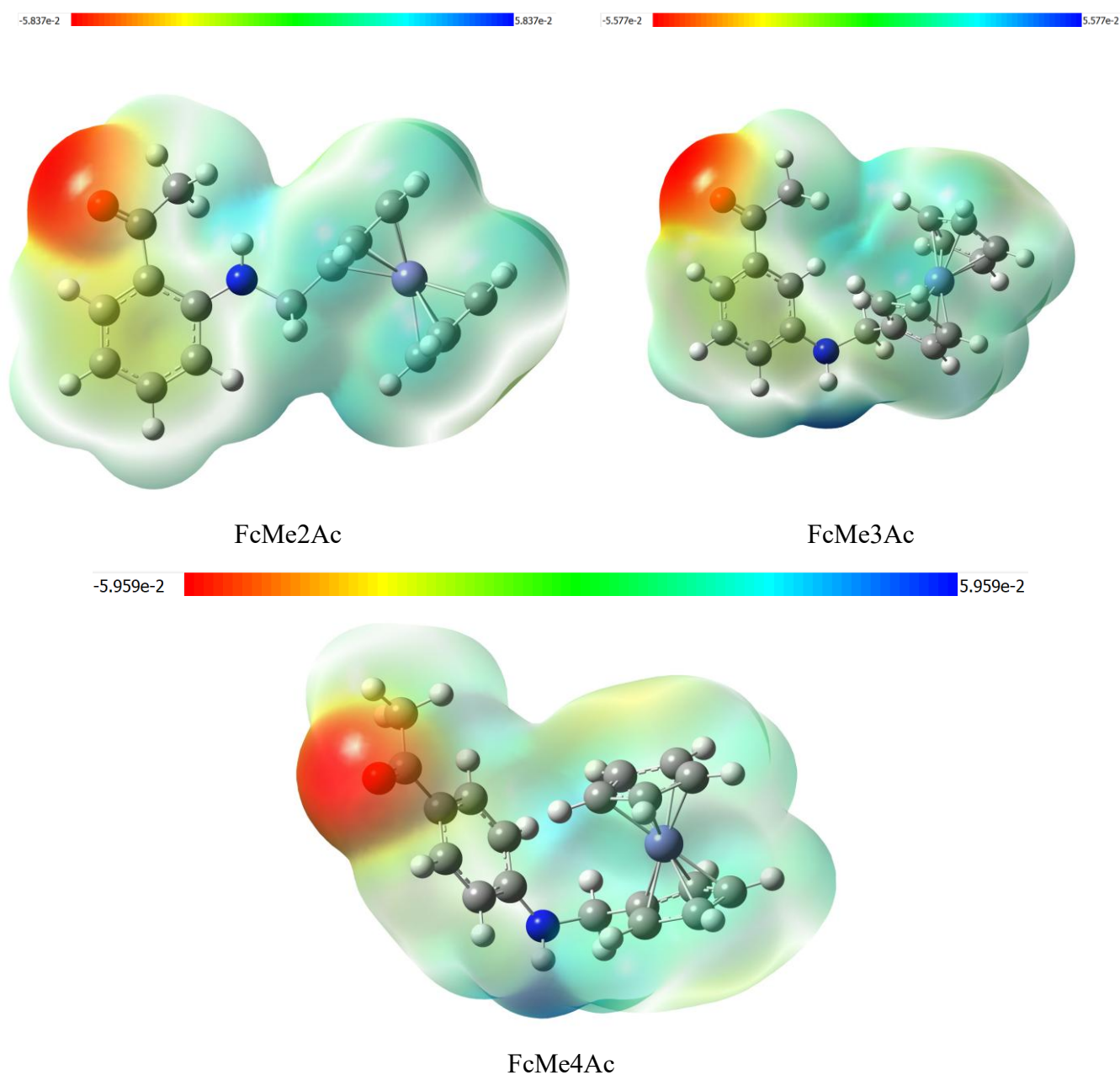


Figure 34. Molecular electrostatic potential (MEP) maps of ferrocenyl acetylaniline derivatives showing charge distribution.

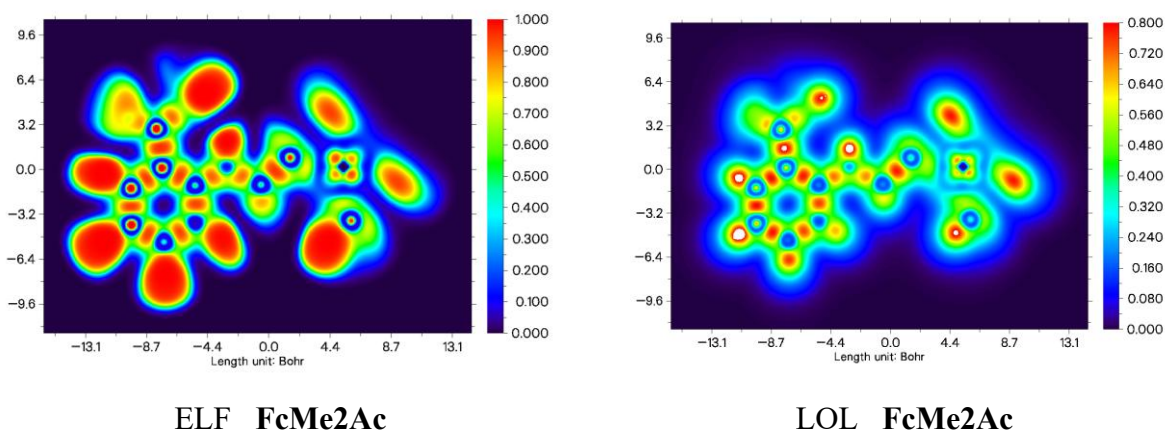
The MEP surfaces reveal that the most negative electrostatic potential regions are localised around the carbonyl oxygen atoms, indicating high electron density and strong nucleophilic character. These regions are therefore expected to be preferential sites for electrophilic interactions or hydrogen bonding (Zegheb et al., 2018).

In contrast, positive electrostatic potential regions are mainly distributed over hydrogen atoms and partially over the ferrocenyl unit, reflecting electron-deficient zones.

The similarity of electrostatic patterns across the three derivatives suggests that substitution does not drastically alter the overall charge distribution, although subtle differences in intensity and localisation are observed, particularly in the para derivative, which exhibits more delocalised electronic distribution (Lanez et al., 2024).

7.4. Electron Localisation and Non-Covalent Interaction Analysis

To further investigate electronic behaviour, ELF, LOL and RDG analyses were performed. Representative maps are presented in Figure 35 and 36.



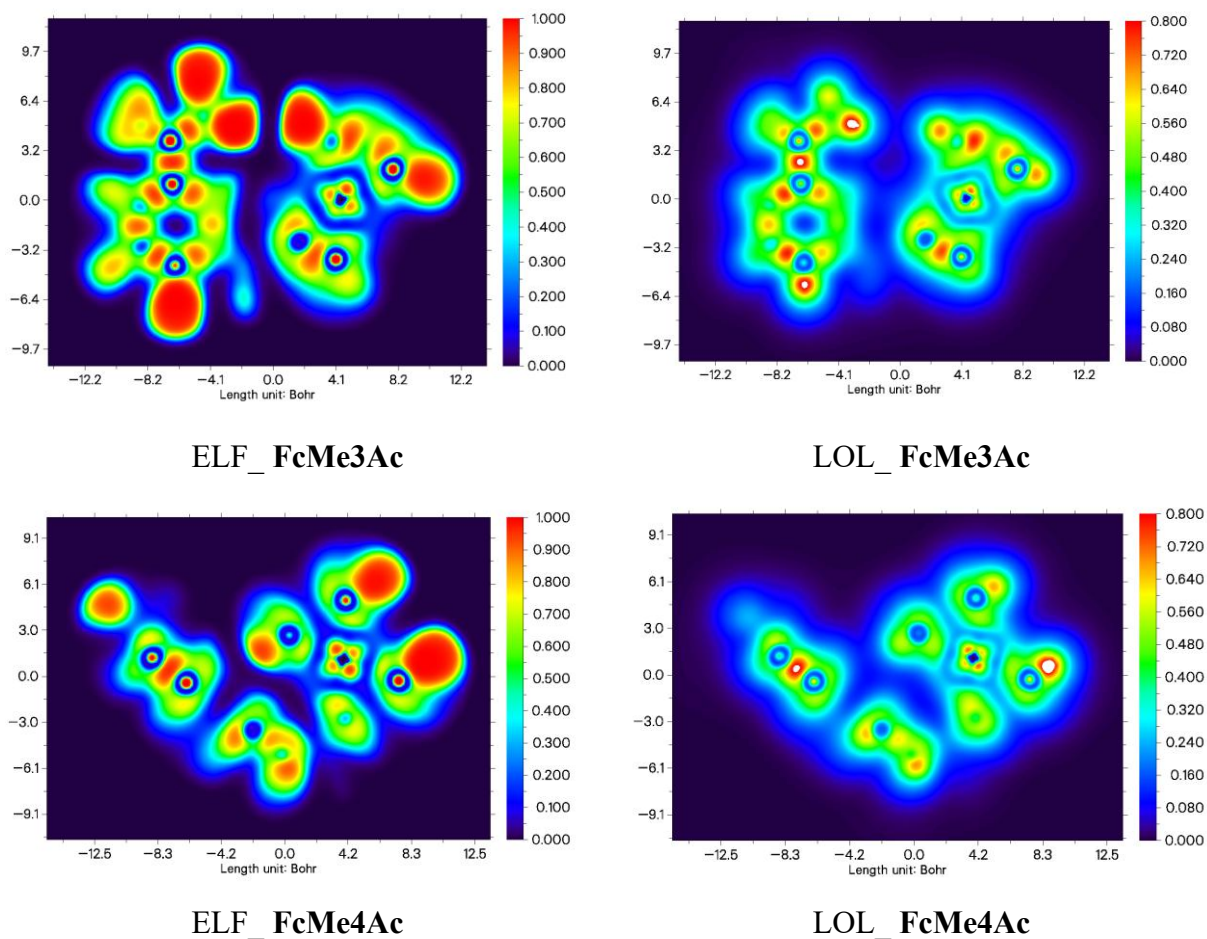
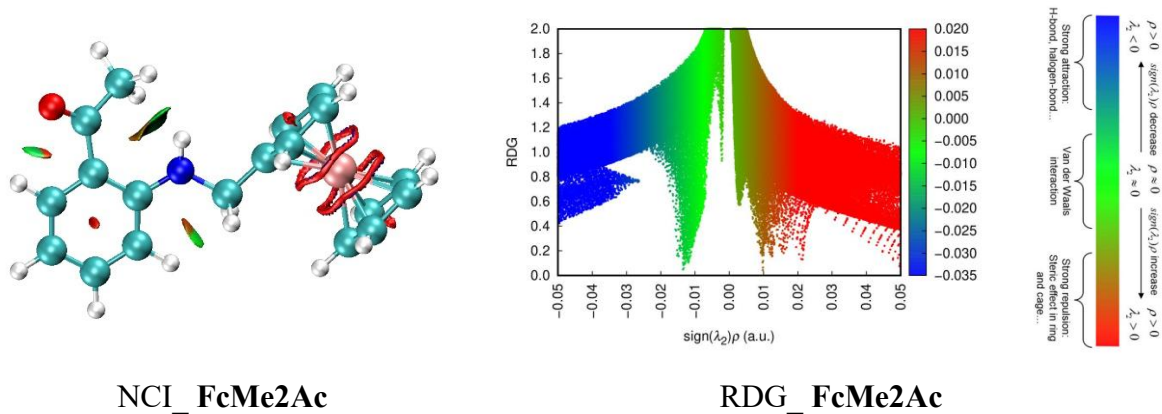


Figure 35. Electron localisation (ELF and LOL) analyses of FcMeAc.



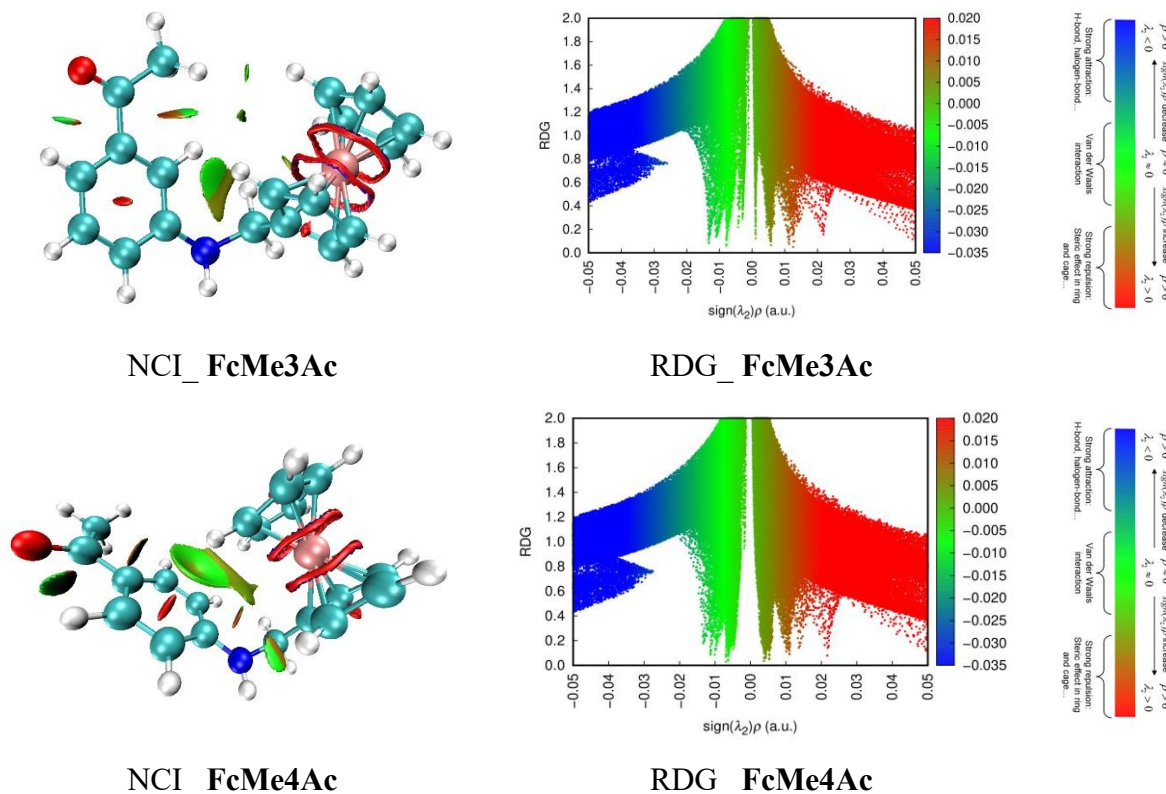


Figure 36. Non-covalent interaction (RDG/NCI) analyses of FcMeAc.

The ELF and LOL maps clearly show strong electron localisation within the cyclopentadienyl rings and the aromatic system, reflecting extensive π -electron delocalisation. Localised electron density around nitrogen and oxygen atoms corresponds to lone-pair regions, which are important for intermolecular interactions.

The RDG analysis highlights weak non-covalent interactions, including van der Waals contacts and steric repulsion zones. These interactions contribute to the overall stability and conformational behaviour of the molecules.

The consistency of these features across the series indicates that the fundamental electronic structure of the ferrocenyl core is preserved, while substitution mainly modulates peripheral electronic properties (Zerrouk et al., 2024; Adaika et al., 2019)

8. Molecular Docking Study

To investigate the molecular recognition and binding behaviour of the synthesised ferrocenyl acetylaniline derivatives, molecular docking simulations were performed. The study aimed to evaluate the affinity, binding modes and interaction patterns of the compounds within the binding sites of the selected thyroid-related target proteins.

8.1. Docking Protocol Validation

Prior to analysing ligand–protein interactions, the reliability of the docking protocol was assessed through a redocking procedure of the co-crystallised ligand into the active site of the selected thyroid-related target protein. The superposition between the crystallographic and predicted binding conformations is presented in [Figure 37](#).

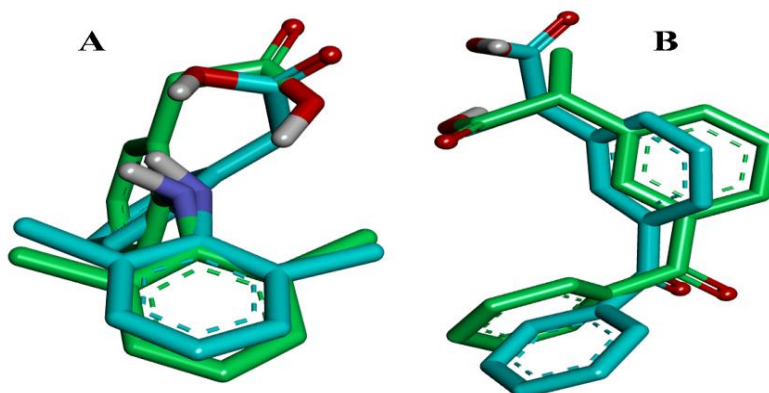


Figure 37. Superposition of the co-crystallised ligand and the redocked pose within the active site (validation of docking protocol).

The calculated root-mean-square deviation (RMSD) between the experimental and redocked conformations was found to be 0.339 Å, which is significantly lower than the commonly accepted threshold of 2.0 Å. This result demonstrates excellent agreement between predicted and experimental ligand orientations, confirming the accuracy of the docking parameters, grid definition and scoring function.

The low RMSD value indicates that the adopted docking protocol is highly reliable for reproducing the native binding mode and can therefore be confidently applied to the ferrocenyl derivatives for subsequent interaction analysis with thyroid-related targets ([Lanez et al., 2018](#)).

8.2. Binding Affinity and Energetic Analysis

The molecular docking results obtained for the ferrocenyl acetylaniline derivatives against the selected thyroid-related targets are summarised in [Table 9](#). The calculated binding energies and energetic contributions provide insight into the strength and nature of ligand–receptor interactions.

Table 9. Molecular docking results of ferrocenyl acetylaniline derivatives against thyroid-related targets.

Compound	Intermolecular Energy (kcal·mol ⁻¹)	Internal Energy (kcal·mol ⁻¹)	Torsional Energy (kcal·mol ⁻¹)	Binding Energy (kcal·mol ⁻¹)
FcMe2Ac	−7.96	−0.58	+0.74	−7.34
FcMe3Ac	−8.48	−0.71	+0.88	−7.92
FcMe4Ac	−9.42	−0.83	+0.96	−8.58
Reference ligand	—	—	—	−7.65

As shown in [Table 9](#), all compounds exhibit negative binding energies, confirming that the interaction with the thyroid receptors is thermodynamically favourable and spontaneous. Among the studied derivatives, **FcMe4Ac displays the most favourable binding energy (−8.58 kcal·mol⁻¹)**, indicating the strongest affinity towards the receptor binding site.

A clear affinity trend is observed: **FcMe4Ac > FcMe3Ac > FcMe2Ac**. This behaviour reflects the influence of substitution pattern on ligand–receptor complementarity and interaction strength. The enhanced binding affinity of FcMe4Ac suggests improved accommodation within the receptor binding pocket and more efficient stabilisation through intermolecular interactions.

The intermolecular energy component is the dominant contributor to binding stabilisation, indicating that van der Waals interactions, electrostatic forces and hydrogen bonding play a key role in ligand recognition. The relatively low internal energy values suggest minimal structural strain upon binding, while the small torsional energy contributions indicate that the ligands adopt energetically favourable conformations within the receptor environment ([Zegheb et al., 2020](#)).

8.3. Binding Mode and Interaction Analysis

The three-dimensional binding conformation of the most active compound (FcMe4Ac) within the receptor binding site is illustrated in [Figure 38](#).

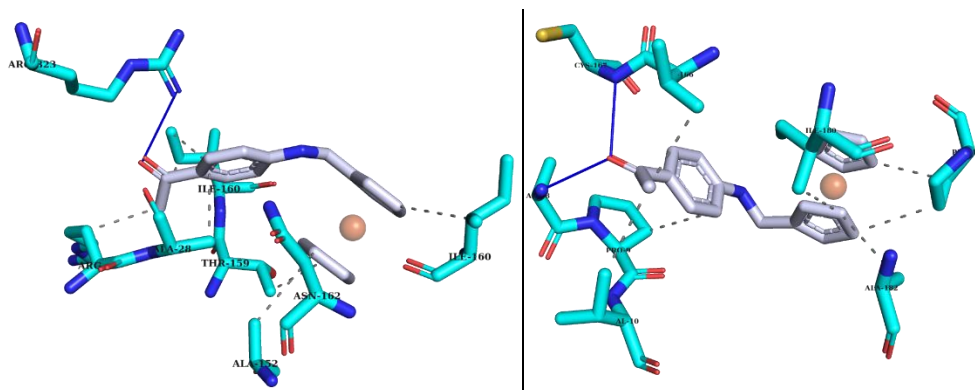


Figure 38. Three-dimensional binding mode of FcMe4Ac within the binding pocket of the thyroid receptor.

The docking results reveal that all ferrocenyl derivatives are stabilised within a predominantly hydrophobic binding pocket, where the ferrocenyl core plays a central role in anchoring the ligand through non-polar interactions.

The interaction profile is characterised by a combination of:

- **Hydrophobic interactions** between the ferrocenyl unit and non-polar residues (e.g., LEU, ILE, VAL)
- **π - π stacking interactions** involving the aromatic ring and aromatic residues (e.g., PHE, TYR)
- **Hydrogen bonding interactions**, mainly involving the carbonyl oxygen and polar residues (e.g., ASN, GLU)
- **Electrostatic interactions** contributing to additional stabilisation

The binding behaviour differs significantly among the three derivatives. **FcMe2Ac** exhibits a relatively limited interaction network, with fewer stabilising contacts and weaker binding affinity. This behaviour can be attributed to less favourable orientation and reduced interaction surface within the receptor pocket.

FcMe3Ac demonstrates improved binding through additional hydrogen bonds and enhanced hydrophobic interactions, resulting in increased stabilisation compared to FcMe2Ac.

In contrast, **FcMe4Ac shows the most optimal binding configuration**, characterised by:

- Multiple hydrophobic contacts along the binding cavity
- Stable hydrogen bonding interactions with key polar residues
- Improved π - π stacking with aromatic amino acids

- Better spatial orientation within the receptor pocket

This dense and cooperative interaction network leads to enhanced stabilisation of the ligand–receptor complex and explains its superior binding affinity relative to the other derivatives and the reference ligand ([Lanez et al., 2023](#)).

8.4. Structure–Interaction Relationship

The differences observed in binding affinity and interaction patterns can be rationalised based on structural and electronic considerations.

The **para-substituted derivative (FcMe4Ac)** exhibits the most favourable behaviour due to its extended and more symmetrical structure, which allows optimal insertion into the receptor binding pocket. This configuration enhances surface complementarity and maximises intermolecular interactions.

Additionally, the increased dipole moment and polarizability observed for FcMe4Ac contribute to stronger electrostatic and dispersion interactions with the receptor environment. The improved electronic delocalisation associated with para substitution also facilitates more effective interaction with surrounding residues.

In contrast, the **ortho derivative (FcMe2Ac)** is affected by steric hindrance, which restricts its ability to adopt an optimal conformation within the binding pocket, thereby reducing interaction efficiency.

The **meta derivative (FcMe3Ac)** exhibits intermediate behaviour, as its substitution pattern does not allow efficient electronic delocalisation, resulting in moderate binding affinity and interaction strength.

Overall, these findings demonstrate that **substitution position plays a critical role in modulating ligand–receptor interactions**, influencing both binding affinity and interaction stability. The results highlight FcMe4Ac as the most promising candidate for effective interaction with thyroid-related targets ([Mouada et al., 2019](#)).

9. Molecular Dynamics Simulation

To further validate the stability and dynamic behaviour of the most promising ligand, **FcMe4Ac**, molecular dynamics (MD) simulations were performed on its complexes with the selected thyroid-related targets, namely the thyroid hormone receptor β and the thyroid-stimulating

hormone receptor. This analysis provides a detailed understanding of structural stability, conformational flexibility and interaction persistence under physiological conditions.

9.1. System Stability: RMSD Analysis

The time-dependent evolution of the backbone root-mean-square deviation (RMSD) was analysed to evaluate the global stability of the receptor–ligand complexes. The results are presented in [Figure 39A](#) (TR β system) and [Figure 39B](#) (TSHR system).

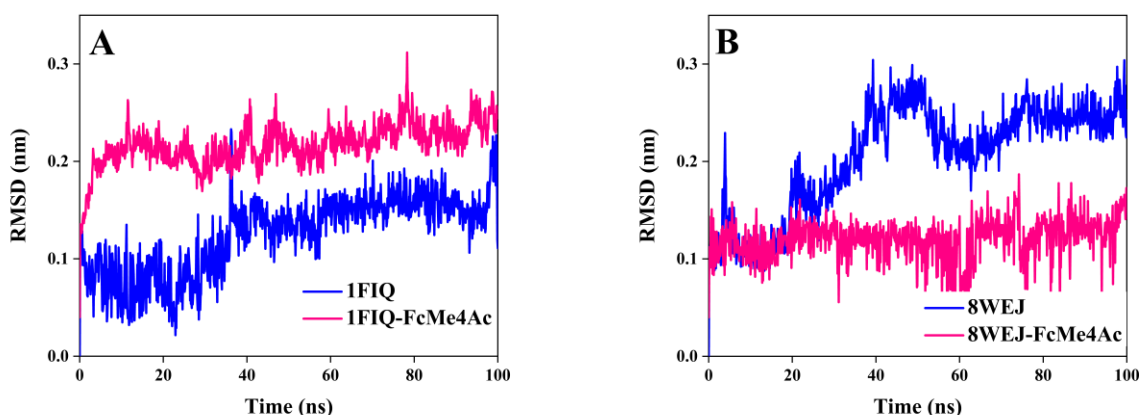


Figure 39. RMSD profiles of receptor and receptor–FcMe4Ac complexes during 100 ns simulation: (A) thyroid hormone receptor β and (B) thyroid-stimulating hormone receptor systems.

In the TR β system ([Figure 39A](#)), the apo receptor stabilises rapidly within the first 10 ns, reaching an equilibrium RMSD of approximately **0.12–0.18 nm**. Upon binding of FcMe4Ac, a slight increase in RMSD is observed, stabilising around **0.18–0.24 nm**. This behaviour indicates a moderate conformational adjustment induced by ligand binding, followed by stable equilibration without significant structural deviation ([Lanez et al., 2019](#)).

In contrast, the TSHR system ([Figure 39B](#)) exhibits higher intrinsic flexibility in its apo form, with RMSD fluctuations reaching **0.20–0.28 nm**. However, upon complex formation, the RMSD decreases and stabilises within a narrower range (**0.14–0.20 nm**), indicating a pronounced stabilising effect of FcMe4Ac on receptor dynamics.

Overall, the RMSD analysis demonstrates that FcMe4Ac forms **stable and well-equilibrated complexes** with both receptors, with a more pronounced stabilising effect observed for the TSH receptor ([Lanez et al., 2019](#)).

9.2. Residue Flexibility: RMSF Analysis

The residue-level flexibility of the receptor structures was evaluated using root-mean-square fluctuation (RMSF), as shown in Figure 40.

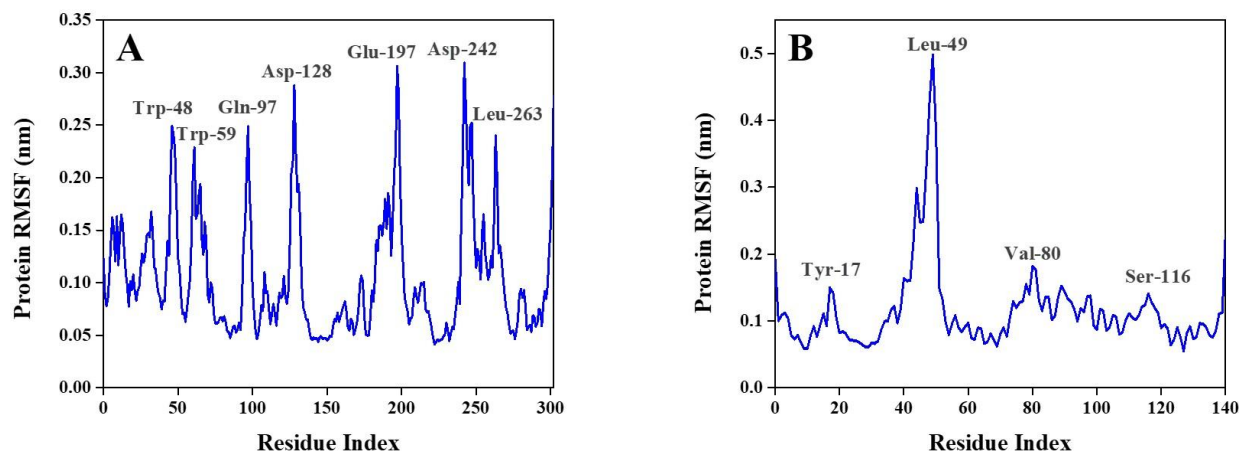


Figure 40. RMSF profiles of receptor residues in complex with FcMe4Ac: (A) TR β and (B) TSHR systems.

For the TR β complex (Figure 40A), moderate fluctuations are observed in loop regions, with peaks reaching approximately **0.20–0.30 nm**, while residues located within the ligand-binding domain exhibit significantly lower fluctuations (**<0.15 nm**). This indicates that the binding site remains structurally stable throughout the simulation.

In the TSHR system (Figure 40B), a general reduction in residue fluctuations is observed, with most residues stabilising below **0.15 nm**. Only a few surface-exposed regions display higher flexibility, which is typical for solvent-accessible domains.

These results indicate that FcMe4Ac contributes to **enhanced structural rigidity**, particularly in the TSH receptor complex, supporting strong and persistent ligand binding.

9.3. Interaction Profile Analysis

The interaction patterns between FcMe4Ac and receptor residues throughout the simulation are illustrated in Figure 41.

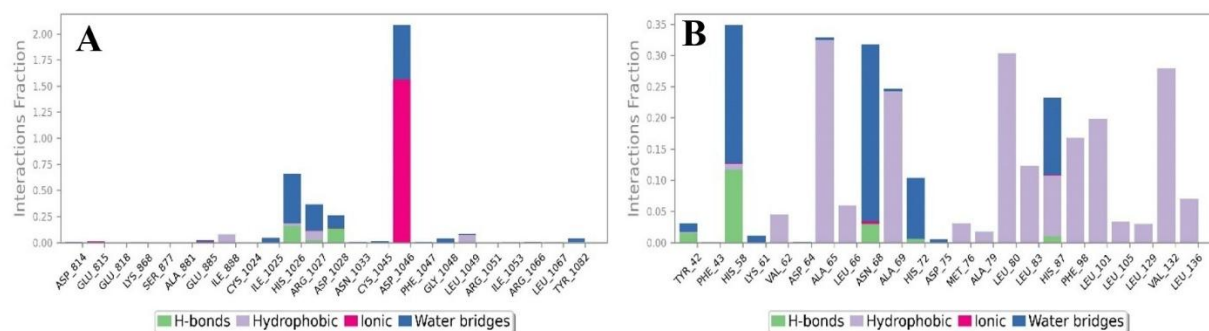


Figure 41. Interaction fraction analysis showing hydrogen bonds, hydrophobic interactions and water bridges: (A) TR β and (B) TSHR complexes.

The stabilisation of the complexes is primarily governed by:

- **Hydrophobic interactions**, involving residues such as LEU, ILE and VAL
- **Aromatic interactions (π – π stacking)** with residues such as PHE and TYR
- **Hydrogen bonding interactions**, particularly with polar residues (e.g., ASN, GLU)
- **Water-mediated interactions**, contributing to interaction persistence

In the TR β complex, interactions are relatively localised, with a moderate number of stabilising contacts maintained throughout the simulation.

Conversely, the TSHR complex exhibits a **broad and more persistent interaction network**, involving multiple residues and interaction types. The higher interaction frequency and diversity indicate stronger and more stable ligand–receptor binding.

10. ADMET Prediction and Pharmacokinetic Evaluation

The pharmacokinetic and toxicity profiles of the synthesised ferrocenyl derivatives were evaluated using *in silico* ADMET prediction tools to assess their suitability as potential bioactive agents targeting thyroid-related pathways.

10.1. Drug-Likeness and Physicochemical Properties

Table 10. Predicted physicochemical properties and drug-likeness parameters of ferrocenyl derivatives.

Parameter	FcMe2Ac	FcMe3Ac	FcMe4Ac
Molecular weight (g·mol ⁻¹)	335.2	335.2	335.2
LogP (consensus)	3.18	3.31	3.52
Lipinski violations	0	0	0

All compounds comply with Lipinski's rule of five, indicating favourable oral drug-likeness. The slight increase in lipophilicity from FcMe2Ac to FcMe4Ac may enhance membrane permeability and interaction with hydrophobic receptor regions (Guan et al., 2019).

10.2. Absorption and Distribution Properties

The predicted absorption and distribution profiles are presented in Table 11.

Table 11. Predicted absorption and distribution properties of the studied compounds.

Parameter	FcMe2Ac	FcMe3Ac	FcMe4Ac
Gastrointestinal absorption	High	High	High
BBB permeability	No	No	No

The compounds exhibit high predicted gastrointestinal absorption, suggesting good oral bioavailability. The absence of BBB permeability is favourable for thyroid-targeted compounds, as it limits central nervous system exposure (Xiong et al., 2021).

10.3. Toxicological Profile

The toxicity predictions are summarised in Table 12.

Table 12. Predicted toxicity profiles of ferrocenyl acetylaniline derivatives.

Parameter	FcMe2Ac	FcMe3Ac	FcMe4Ac
Ames toxicity	No	No	No
Hepatotoxicity	No	No	No
Skin sensitisation	Low risk	Low risk	Low risk

All compounds show favourable safety profiles, with no predicted mutagenicity or hepatotoxicity (Dascalu et al., 2020).

CONCLUSION



General Conclusion

This thesis focused on the rational design, synthesis, structural characterisation and comprehensive computational investigation of a series of ferrocenyl acetylaniline derivatives, with particular emphasis on their interaction potential with thyroid-related molecular targets. The study combined experimental and *in silico* approaches to establish a detailed structure–property–interaction relationship within this class of organometallic compounds.

The synthetic methodology enabled the efficient preparation of three Regio isomeric derivatives, namely FcMe₂Ac, FcMe₃Ac and FcMe₄Ac, with satisfactory yields and high reproducibility. Structural elucidation was successfully achieved through complementary spectroscopic techniques, including FT-IR, UV–Visible spectroscopy and both one- and two-dimensional NMR analyses. The consistency of the obtained spectral data confirmed the integrity of the ferrocenyl framework and the precise positioning of substituents on the aromatic ring, thus validating the proposed molecular structures.

Electrochemical characterisation revealed the preservation of the characteristic Fe (II)/Fe (III) redox couple of the ferrocenyl core, with quasi-reversible behaviour observed for all derivatives. The anodic shifts in redox potentials demonstrated the significant influence of acetylaniline substitution on the electronic environment of the iron centre. Furthermore, diffusion-controlled electron-transfer processes and variations in kinetic parameters highlighted the role of molecular structure in modulating electrochemical behaviour.

Density functional theory calculations provided deeper insight into the electronic structure and intrinsic reactivity of the studied compounds. The analysis of frontier molecular orbitals indicated a clear donor–acceptor character, with electron density predominantly localised on the ferrocenyl unit and partially delocalised towards the aromatic moiety. Global reactivity descriptors and electrostatic potential mapping further identified key reactive regions and confirmed that substitution patterns play a decisive role in tuning electronic distribution and molecular stability.

Molecular docking studies demonstrated that the ferrocenyl derivatives exhibit favourable binding affinities towards thyroid-related targets, including the thyroid hormone receptor β and the thyroid-stimulating hormone receptor. The binding interactions were governed by a combination of hydrophobic contacts, hydrogen bonding and π -interactions within the receptor binding pockets. Among the investigated compounds, FcMe₄Ac consistently displayed the strongest binding

GENERAL CONCLUSION

affinity, which can be attributed to its optimal structural configuration, enhanced polarizability and improved steric complementarity with the receptor environment.

The stability of these interactions was further confirmed by molecular dynamics simulations, which revealed well-equilibrated ligand–receptor complexes over the simulation timescale. In particular, the FcMe4Ac–receptor complexes exhibited reduced structural fluctuations, stable RMSD profiles and persistent interaction networks, indicating strong and durable binding under dynamic conditions. The enhanced stabilisation observed for one of the receptor systems further supports the superior interaction capability of this derivative.

In addition, *in silico* ADMET predictions indicated that all compounds possess favourable pharmacokinetic properties, including good gastrointestinal absorption, compliance with Lipinski's rule of five and low predicted toxicity. The absence of blood–brain barrier permeability is particularly advantageous for compounds targeting peripheral endocrine systems, such as the thyroid gland.

Collectively, the results obtained in this work demonstrate that structural modification of ferrocenyl derivatives through acetylaniline substitution significantly influences their electronic properties, interaction behaviour and pharmacokinetic profiles. The integration of experimental characterisation and advanced computational modelling provides a robust framework for understanding the behaviour of these compounds in a biological context.

Overall, FcMe4Ac emerges as the most promising candidate within the series, combining optimal electronic features, strong and stable receptor binding, and favourable pharmacokinetic characteristics. These findings highlight its potential as a lead scaffold for further optimisation and development targeting thyroid-related pathways.

This work therefore contributes to the advancement of ferrocenyl-based compounds in medicinal and bioinorganic chemistry and provides a solid scientific foundation for future investigations aimed at the development of novel agents with potential endocrine relevance.

REFERENCES

BIBLIOGRAPHY



References: Bibliographic Study

Audie, J., & Scarlata, S. (2007). A novel empirical free energy function that explains and predicts protein–protein binding affinities. *Biophysical Chemistry*, 129(2–3), 198–211. <https://doi.org/10.1016/j.bpc.2007.05.021>

Agarwal, S., Jangir, D. K., Mehrotra, R., Lohani, N., & Rajeswari, M. R. (2014). A structural insight into major groove directed binding of nitrosourea derivative nimustine with DNA: a spectroscopic study. *PloS one*, 9(8), e104115. <https://doi.org/10.1371/journal.pone.0104115>

Agarwal, S., Ray, B., & Mehrotra, R. (2015). SERS as an advanced tool for investigating chloroethyl nitrosourea derivatives complexation with DNA. *International journal of biological macromolecules*, 81, 891–897. <https://doi.org/10.1016/j.ijbiomac.2015.09.024>

Bakan, A., and Bahar, I. (2009) The intrinsic dynamics of enzymes plays a dominant role in determining the structural changes induced upon inhibitor binding. *Proceedings of the National Academy of Sciences* 106 (34), 14349-14354

Bernardi, R. C., Melo, M. C., and Schulten, K. (2015) Enhanced sampling techniques in molecular dynamics simulations of biological systems. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1850 (5), 872-877

B Mukesh, K Rakesh. (1966). Molecular docking: a review. *IJRAP*. 2011; 2: 1746-1751.

Bohn, R. K., & Haaland, A. (1966). On the molecular structure of ferrocene, $\text{Fe}(\text{C}_5\text{H}_5)_2$. *Journal of Organometallic Chemistry*, 5(5), 470–476. [https://doi.org/10.1016/S0022-328X\(00\)82382-7](https://doi.org/10.1016/S0022-328X(00)82382-7)

Cotton FA, Wilkinson G. (1988). *Advanced Inorganic Chemistry*, 5th ed, John Wiley & Sons, New York,

C. T. Chantler, N. A. Rae, M. T. Islam, S. P. Best, J. Yeo, L. F. Smale, J. Hester, N. Mohammadi, F. Wang, J. (2012). *Synchrotron Radiat.*, 19, 145–158. <https://doi.org/10.1107/S0909049511056275>

Chen S, Li L, Zhao H, Li B. (2013). Ce (OTf)₃-catalyzed multicomponent domino cyclization–aromatization of ferrocenylacetylene, aldehydes, and amines: a straightforward synthesis of ferrocene-containing quinolines. *Tetrahedron*, 69(30): 6223–6229. <https://doi.org/10.1016/j.tet.2013.05.026>

Chan, H. C. S., Shan, H., Dahoun, T., Vogel, H., & Yuan, S. (2019). Advancing drug discovery via artificial intelligence. *Trends in Pharmacological Sciences*, 40, 592-604.

DeLano, W. L. (2002) Pymol: an open-source molecular graphics tool. Ccp4 Newslett. Protein Crystallography., 40,11

Dedmon, M. M., Lindorff-Larsen, K., Christodoulou, J., Vendruscolo, M., and Dobson, C. M. (2005) Mapping long-range interactions in α -synuclein using spin-label NMR and ensemble molecular

Duan, Y., and Kollman, P. A. (1998) Pathways to a protein folding intermediate observed in a 1-microsecond simulation in aqueous solution. Science 282 (5389), 740-744 dynamics simulations. Journal of the American Chemical Society 120 (2), 476-477

Dunitz JD, Orgel LE. (1953). *Bis-cyclopentadienyl Iron: A Molecular Sandwich*. *Nature.*, 171, 121-122. <https://doi.org/10.1038/171121a0>

Dunitz JD, Orgel LE, A. (1956). Detailed X-ray analysis of crystals of ferrocene by Dunitz, Orgel & Rich confirms that the molecule has a 'sandwich' structure with approximate D_{5d} symmetry, and Fe-C, 2.045±0.01 Å; C-C, 1.403±0.02 Å. Rich, *Acta Cryst.*, 9, 373-375. <https://doi.org/10.1107/S0365110X56001091>

Davis WL, Shago RF, Langner EHG, Swarts JC. (2005). Synthesis and electrochemical properties of a series of ferrocene-containing alcohols. Polyhedron, 24(12): 1611–1616. <https://doi.org/10.1016/j.poly.2005.04.022>

Eiland PF, Pepinsky R. (1952). X-ray Examination of Iron Biscyclopentadienyl. *J. Am. Chem. Soc.*, 74 (19): 4971-4971. <https://doi.org/10.1021/ja01139a527>

Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular Docking and Structure-Based Drug Design Strategies. *Molecules*, 20(7), 13384-13421. <https://doi.org/10.3390/molecules200713384>

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., Park, G., Adney, S. K., Hatcher, C., and Eltit, J. M. (2011) Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. Cell 147 (5), 1011-1023

Fischer EO, Pfab W. (1952). Zur Kristallstruktur der Di-Cyclopentadienyl-Verbindungen des zweiwertigen Eisens. Z. Naturforsch. B, 7: 377–379

Fischer EO, Pfab W. (1952), Zur Kristallstruktur der Di-Cyclopentadienyl-Verbindungen des zweiwertigen Eisens, Kobalts und Nickels [On the crystal structure of the biscyclopentadienyl compounds of divalent iron, cobalt and nickel]. *Zeitschrift für Naturforschung B*. 7 (7): 377–379. <https://doi.org/10.1515/znb-1952-0701>

- Fabbrizzi, L. (2020). The ferrocenium/ferrocene couple: a versatile redox switch. *ChemTexts* 6, 22. <https://doi.org/10.1007/s40828-020-00119-6>
- Fouda MFR, Abd-Elzaher MM, Abdelsamaia RA, Labib AA. (2007) .On the medicinal chemistry of ferrocene. *Appl. Organometal. Chem.*, 21: 613–625. <https://doi.org/10.1002/aoc.1202>
- Ferreira CL, Ewart CB, Barta CA, Little S, Yardley V, Martins C, Polishchuk CE, Smith PJ, Moss JR, Merkel M. (2006). *Inorg. Chem.*, 45: 8414–8422. <https://doi.org/10.1021/ic060635j>
- Frantz R, Durand JO, Lanneau GF. 2004.Substituent effects of phosphonate groups: electronic repartition of π -conjugated ferrocene analogues of stilbene. *J. Organomet. Chem.*, 689(11): 1867–1871. <https://doi.org/10.1016/j.jorganchem.2004.03.007>
- Guha, R. (2013). On exploring structure-activity relationships. *Methods in Molecular Biology*, 993, 81-94.
- Guedes, Isabella & Magalhães, Camila & Dardenne, Laurent. (2014). Receptor-ligand molecular docking. *Biophysical Reviews*. 6. 75-87. 10.1007/s12551-013-0130-2.
- Gschwend, D. A., Good, A. C., & Kuntz, I. D. (1996). Molecular docking towards drug discovery. *Journal of molecular recognition: JMR*, 9(2), 175–186. [https://doi.org/10.1002/\(sici\)1099-1352\(199603\)9:2<175::aid-jmr260>3.0.co;2-d](https://doi.org/10.1002/(sici)1099-1352(199603)9:2<175::aid-jmr260>3.0.co;2-d)
- Gadaleta, D., Serrano-Candelas, E., Ortega-Vallbona, R., Colombo, E., Garcia de Lomana, M., Biava, G., Aparicio-Sánchez, P., Roncaglioni, A., Gozalbes, R., & Benfenati, E. (2024). Comprehensive benchmarking of computational tools for predicting toxicokinetic and physicochemical properties of chemicals. *Journal of Cheminformatics*, 16, 31.<https://doi.org/10.1186/s13321-024-00931-z>
- Gryaznova TP, Katsyuba SA, Milyukov VA, Sinyashin OG. (2010) . DFT study of substitution effect on the geometry, IR spectra, spin state and energetic stability of the ferrocenes and their pentaphospholyl analogues. *J. Organomet. Chem.*, 695, 2586-2595. <https://doi.org/10.1016/j.jorganchem.2010.08.031>
- Getty SA, Engtrakul C, Wang L, Liu R, Ke SH, Baranger HU, Yang W, Fuhrer MS, Sita LR. (2005). Near-perfect conduction through a ferrocene-based molecular wire. *Phys. Rev. B*, 71: 241401. <https://doi.org/10.1103/PhysRevB.71.241401>
- Haaland A, Luszyk J, Novak DP, Brunvoll J, Starowieyski KB. (1974). Molecular structures of dicyclopentadienylmagnesium and dicyclopentadienylchromium by gasphase

References Bibliography

electron diffraction. *J. Chem. Soc. Chem. Commun.*, 2: 54-55.
<https://doi.org/10.1039/C39740000054>

Holt, P. A., Chaires, J. B., & Trent, J. O. (2008). Molecular docking of intercalators and groove-binders to nucleic acids using Autodock and Surflex. *Journal of chemical information and modeling*, 48(8), 1602–1615. <https://doi.org/10.1021/ci800063v>

Houlton A, Isaac CJ, Gibson AE, Horrocks BR, Clegg W, Elsegood MRJ. (1999). Synthesis, structure and redox properties of ferrocenylmethylnucleobases. *J. Chem. Soc, Dalton Trans.*, 18: 3229-3234. <https://doi.org/10.1039/A905168F>

Hollingsworth, S. A., & Dror, R. O. (2018). Molecular Dynamics Simulation for All. *Neuron*, 99(6), 1129–1143. <https://doi.org/10.1016/j.neuron.2018.08.011>

Huang R, Wallqvist A, Covell DG. (2005). Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. *Biochem. Pharmacol.*, 69(7): 1009–1039. <https://doi.org/10.1016/j.bcp.2005.01.001>

Jaouen G, Vessi res A, Butler IS. (1993). Chemistry of ferrocenes: bioorganometallic aspects. *Acc. Chem. Res.*, 26(7): 361–369. <https://doi.org/10.1021/ar00031a002>

Koshland Jr, D. E. (2010) The key–lock theory and the induced fit theory. *Angew. Chem. Int. Ed.*, 33, 2375–2378

Kaboli, P. J., Ismail, P., & Ling, K. (2018). Molecular modeling, dynamics simulations, and binding efficiency of berberine derivatives: A new group of RAF inhibitors for cancer treatment. *PLoS ONE*, 13(3), e0193941. <https://doi.org/10.1371/journal.pone.0193941>

Kroemer, R. T. (2007) Structure-based drug design: docking and scoring. *Current Protein and Peptide Science* 8 (4), 312-328

Karaa, W. B. A. (2015) Biomedical image analysis and mining techniques for improved health outcomes, IGI Global

Kealy TJ, Pauson PL. (1951). A new type of organo-iron compound. *Nature*, 168 : 1039–1040. <https://doi.org/10.1038/1681039b0>

Karagollu O, Gorur M, Gode F, Sennik B, Yilmaz F. (2014). Phosphate ion sensors based on triazole connected ferrocene moieties. *Sens. Actuators B*, 193: 788–798. <https://doi.org/10.1016/j.snb.2013.12.046>

Khelef A, Lanez T. (2015). In vitro assays of the antioxidant activities of ferrocene derivatives bearing amine, amide or hydrazine groups. *Der Pharma Chemica*, 7(6): 318–323

References Bibliography

Kowalski K, Koceva-Chyla A, Szczupak Ł, Hikisz P, Bernasińska J, Rajnisz A, Solecka J, Therrien B. (2013). Ferrocenylvinyl-flavones: synthesis, structure, anticancer and antibacterial activity studies. *J. Organomet. Chem.*, 741–742: 153–161. <https://doi.org/10.1016/j.jorganchem.2013.05.009>

Lanez E. (2019). Synthesis, characterization and in vitro and in silico study of biological activities of some ferrocenylmethyl-nucleic bases derivatives [doctoral dissertation]. Ouargla: Kasdi Merbah University Ouargla.

Lamb, M. L., & Jorgensen, W. L. (1997). Computational approaches to molecular recognition. *Current Opinion in Chemical Biology*, 1(4), 449–457. [https://doi.org/10.1016/s1367-5931\(97\)80038-5](https://doi.org/10.1016/s1367-5931(97)80038-5)

Lindorff-Larsen, K., Best, R. B., DePristo, M. A., Dobson, C. M., and Vendruscolo, M. (2005) Simultaneous determination of protein structure and dynamics. *Nature* 433 (7022), 128-132

Lindorff-Larsen, K., Piana, S., Dror, R. O., and Shaw, D. E. (2011) How fast-folding proteins fold. *Science* 334 (6055), 517-520

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1–3), 3–25. [https://doi.org/10.1016/S0169-409X\(96\)00423-1](https://doi.org/10.1016/S0169-409X(96)00423-1)

Leigh, T., (1964) . Ferrocene derivatives containing tertiary alkyl groups. Synthesis by the Friedel– Crafts and other methods. *Journal of the Chemical Society*, p. 3294-3302.

Larik FA, Saeed A, Fattah TA, Muqadar U, Channar PA. (2016) . Recent advances in the synthesis, biological activities and various applications of ferrocene derivatives. *Appl Organomet Chem* <https://doi.org/10.1002/aoc.3664>

Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. *Methods in Molecular Biology*, 443, 365–382. https://doi.org/10.1007/978-1-59745-177-2_19

Morrison, J. L., Breitling, R., Higham, D. J., & Gilbert, D. R. (2006). A lock-and-key model for protein–protein interactions. *Bioinformatics*, 22(16), 2012–2019. <https://doi.org/10.1093/bioinformatics/btl338>

Miao, J., Hodgson, K. O., and Sayre, D. (2001) An approach to three-dimensional structures of biomolecules by using single-molecule diffraction images. *Proceedings of the National Academy of Sciences* 98 (12), 6641-6645

References Bibliography

Mirjalili, V., and Feig, M. (2013) Protein structure refinement through structure selection and averaging from molecular dynamics ensembles. *Journal of chemical theory and computation* 9 (2), 1294-1303

Miller SA, Tebboth JA, Tremaine JF. (1952). Dicyclopentadienyliron. *J. Chem. Soc.:* 632–635. <https://doi.org/10.1039/JR9520000632>

Mohammadi N, Ganesan A, Chantler CT, Wang F. (2012). Differentiation of ferrocene D-5d and D-5h conformers using IR spectroscopy. *J Organomet Chem*, 713: 51-59. <https://doi.org/10.1016/j.jorganchem.2012.04.009>

Miller, S., J. A. Tebboth, and JF Tremaine. (1952) *J Chem Soc*, 632: p. 127-128.

Nomura, Y., & Akashi, R. (2024). Density functional theory☆. In T. Chakraborty (Ed.), *Encyclopedia of condensed matter physics* (2nd ed., pp. 867–878). Academic Press.

<https://doi.org/10.1016/B978-0-323-90800-9.00148-7>

Nguyen, H. D., and Hall, C. K. (2004) Molecular dynamics simulations of spontaneous fibril formation by random-coil peptides. *Proceedings of the National Academy of Sciences* 101 (46), 16180-16185

National Center for Biotechnology Information (2026). PubChem Compound Summary for CID 517796, CID 517796. Retrieved April 24, 2026 from <https://pubchem.ncbi.nlm.nih.gov/compound/517796>

Neuse EW. (2005). Macromolecular ferrocene compounds as cancer drug models. *J. Inorg. Organomet. Polym. Mater.*, 15(1): 3–31. <https://doi.org/10.1007/s10904-004-2371-9>

Pauson, P. (1955), Ferrocene and related compounds. *Quarterly Reviews, Chemical Society*, 9(4): p. 391-414.

Pauson, P. and G. Wilkinson, (1954). Bis-indenyl Derivatives of Iron and Cobalt. *Journal of the American Chemical Society*, 76(7): p. 2024-2026.

Peng, Y., McCorvy, J. D., Harpsøe, K., Lansu, K., Yuan, S., Popov, P., Qu, L., Pu, M., Che, T., and Nikolajsen, L. F. (2018) 5-HT_{2C} receptor structures reveal the structural basis of GPCR polypharmacology. *Cell* 172 (4), 719-730. e714

Podolan G, Hettmanczyk L, Hommes P, Sarkar B, Reissig HU. (2015). Synthesis and (Spectro)electrochemistry of new ferrocenyl-substituted pyridine derivatives. *Eur. J. Org. Chem.*, 7317–7323. <https://doi.org/10.1002/ejoc.201501163>

References Bibliography

Parveen H, Mukhtar S, Azam A. (2016). Novel ferrocenyl linked pyrazoline analogs as potent antiamoebic agents. *J. Heterocycl. Chem.*, 53(2): 473–479. <https://doi.org/10.1002/jhet.2427>

Qiu X, Zhao H, Lan M. (2009). Novel ferrocenyl nitroxides: synthesis, structures, electrochemistry and antioxidative activity. *J. Organomet. Chem.*, 694(24): 3958–3964. <https://doi.org/10.1016/j.jorganchem.2009.08.012>

Quintana C, Klahn AH, Artigas V, Fuentealba M, Biot C, Halloum I, Kremer L, Arancibia R. (2015). Cyrhetrenyl and ferrocenyl 1,3,4-thiadiazole derivatives: synthesis, characterization, crystal structures and in vitro antitubercular activity. *Inorg. Chem. Commun.*, 55: 48–50. <https://doi.org/10.1016/j.inoche.2015.03.008>

Rosenthal, S. J., Jimenez, R., Fleming, G. R., Kumar, P., and Maroncelli, M. (1994) Solvation dynamics in methanol: Experimental and molecular dynamics simulation studies. *Journal of Molecular Liquids* 60 (1-3), 25-56

Roy, G., & Mougél, V. (2022). *Ferrocene as an iconic redox marker: From solution chemistry to molecular electronic devices*. *Coordination Chemistry Reviews*, 469, 214666. <https://doi.org/10.1016/j.ccr.2022.214666>

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W., Jr. (2014). Computational methods in drug discovery. *Pharmacological Reviews*, 66, 334-395.

Singh, D. B., & Dwivedi, S. (2019). Computational screening and ADMET-based study for targeting Plasmodium S-adenosyl-L-homocysteine hydrolase: Top scoring inhibitors. *Network Modeling and Analysis in Health Informatics and Bioinformatics*, 8, 4.

Shoichet BK, McGovern SL, Wei B, Irwin JJ. (2002). Lead discovery using molecular docking. *Curr Opin Chem Biol.* Aug;6(4):439-46. doi: 10.1016/s1367-5931(02)00339-3. PMID: 12133718.

Snow, C. D., Nguyen, H., Pande, V. S., and Gruebele, M. (2002) Absolute comparison of simulated and experimental protein-folding dynamics. *Nature* 420 (6911), 102-106

Schwantes, C. R., McGibbon, R. T., and Pande, V. S. (2014) Perspective: Markov models for long-timescale biomolecular dynamics. *The Journal of chemical physics* 141 (9), 09B201_201

Seiler P, Dunitz JD. (1982). Low-temperature crystallization of orthorhombic ferrocene: structure analysis at 98 K. *Acta Crystallogr. Sect. B.*, 38: 1741-1745. <https://doi.org/10.1107/S0567740882007080>

References Bibliography

Silva, R. M. P., Silva, G. D., Coutinho-Neto, M. D., & Suffredini, H. B. (2016). *Ferrocene in oil/water interfaces: An electrochemical approach*. *Electrochimica Acta*, 212, 195–200. <https://doi.org/10.1016/j.electacta.2016.06.138>

Sansook S, Phumivanichakit K, Hilae A. (2023). Ferrocene and its derivatives for medical applications. *J. Life Sci. Agric. Technol.*, 2(1): 33–43. <https://li04.tcithaijo.org/index.php/psj/article/view/1154>

Sai Krishna AD, Panda G, Kondapi AK. (2005). Mechanism of action of ferrocene derivatives on the catalytic activity of topoisomerase II α and β : distinct mode of action of two derivatives. *Arch. Biochem. Biophys.*, 438(2): 206–216. <https://doi.org/10.1016/j.abb.2005.04.014>

Top S, Tang J, Vessieres A, Carrez C, Provot C, Jaouen G. (1996). *Chem. Commun.*, 955–956. <https://doi.org/10.1039/cc9960000955>

Trott, O. and Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*, 31, 455–461

Terwilliger, T. C., Read, R. J., Adams, P. D., Brunger, A. T., Afonine, P. V., GrosseKunstleve, R. W., and Hung, L.-W. (2012) Improved crystallographic models through iterated local density-guided model deformation and reciprocal-space refinement. *Acta Crystallographica Section D: Biological Crystallography* 68 (7), 861-870

Top S, Vessi res A, Leclercq G, Quivy J, Tang J, Vaissermann J, Huch  M, Jaouen G. (2003). Synthesis, biochemical properties and molecular modelling studies of organometallic specific estrogen receptor modulators (SERMs), the ferrocifens and hydroxyferrocifens: evidence for an antiproliferative effect of hydroxyferrocifens on both hormone-dependent and hormone-independent breast cancer cell lines. *Chem. Eur. J.*, 9: 5223–5236. <https://doi.org/10.1002/chem.200305024>

Veselovsky, A. V., & Ivanov, A. S. (2003). Strategy of computer-aided drug design. *Current Drug Targets Infectious Disorders*, 3, 33-40.

van Staveren DR, Metzler-Nolte N. (2004). Bioorganometallic chemistry of ferrocene. *Chem. Rev.*, 104: 5931–5986. <https://doi.org/10.1021/cr0101510>

Wilkinson G, Rosenblum M, Whiting MC, Woodward RB. (1952). The structure of iron bis-cyclopentadienyl. *J. Am. Chem. Soc.*, 74(8): 2125–2126. <https://doi.org/10.1021/ja01128a527>

Woodward RB, Rosenblum M, Whiting MC. (1952). The structure of iron bis-cyclopentadienyl (ferrocene). *J. Am. Chem. Soc.*, 74(13): 3458–3459. <https://doi.org/10.1021/ja01133a543>

References Bibliography

Wilkinson G, Rosenblum M, Whiting MC, Woodward RB. (1952). The structure of iron biscyclopentadienyl. *J. Am. Chem. Soc.*, 74 (8): 2125-2126.

<https://doi.org/10.1021/ja01128a527>

Wilkinson, G., *Org Syn* 1956. 36: p. 31-34.

Wilkinson, G., P. Pauson, and F. Cotton, (1954). Bis-cyclopentadienyl compounds of nickel and cobalt. *Journal of the American chemical society*, 76(7): p. 1970-1974.

Wilkinson, G. and J. Birmingham. (1954)., Bis-cyclopentadienyl compounds of Ti, Zr, V, Nb and Ta. *Journal of the American Chemical Society*, 76(17): p. 4281-4284.

Welipitiya D, Green A, Woods JP, Dowben PA, Robertson BW, Byun D, Zhang J. (1996). Ultraviolet and electron radiation induced fragmentation of adsorbed ferrocene. *J. Appl. Phys.*, 79(12): 8730–8734. <https://doi.org/10.1063/1.362474>

Yu, W., & Mackerell, A. D., Jr (2017). Computer-aided drug design methods. *Methods in Molecular Biology*, 1520, 85-106

Yu H, Wang L, Huo J, Ding J, Tan Q. (2008) Synthesis and curing behavior of a novel ferrocene-based epoxy compound. *J. Appl. Polym. Sci.*, 110 : 1594–1601. <https://doi.org/10.1002/app.28584>

Yavuz S, Yildirim H. (2013), Ferrocene derivatives carrying urea, thiourea, and sulfonamide moieties: synthesis and evaluation of antibacterial and antifungal activities. *J. Chem.*: 149693. <https://doi.org/10.1155/2013/149693>

Zapata F, Caballero A, Molina P, Tarraga A. (2010). A ferrocene-quinoxaline derivative as a highly selective probe for colorimetric and redox sensing of toxic mercury (II) cations. *Sensors*, 10(12): 11311–11321. <https://doi.org/10.3390/s101211311>

References: EXPERIMENTAL

- A. Adaika, Y. Bekkar, S. Youmbai, L. Bourougaa, E. Lanez, M.L. Ben Amor, K. Nesba, T. Lanez, L. Bechki, Synthesis, (2025). Antioxidant, and Antidiabetic Potential of Ferrocenylmethyl-nucleobase Compounds: In Vitro, In Silico Molecular Docking, DFT Calculation, and Molecular Dynamic Simulations, Appl. Organomet. Chem. 39 e7988.
- A. Rebiai, T. Lanez, M.L. Belfar, (2014). Total polyphenol contents, radical scavenging and cyclic voltammetry of algerian propolis, Int. J. Pharm. Pharm. Sci. 6 395–400.
- A. Khelef, T. Lanez, (2015). In vitro assays of the antioxidant activities of ferrocene derivatives bearing amine, amide or hydrazine groups, 7 318–323.
- A. Boutarfaia, L. Bechki, T. Lanez, E. Lanez, M. Kadri, (2019). Synthesis, Antioxidant Activity, and Determination of Binding Parameters of Meso-Tetra-4-Actophenyl-Porphyrin and its Palladium (II) Complex with Superoxide Anion Radicals, Curr. Bioact. Compd. 16 1063–1071. <https://doi.org/10.2174/1573407215666191017105239>.
- A. Yahiaoui, N. Benzya, A. Messai, T. Lanez, E. Lanez, (2023). Voltametric and molecular docking investigations of ferrocenylmethylaniline and its N-acetylated derivative interacting with DNA, Journal of Electrochemical Science and Engineering. <https://doi.org/10.5599/jese.2061>.
- A. Kedadra, T. Lanez, E. Lanez, H. Hemmami, M. Henni, (2022). Synthesis and antioxidant activity of six novel N-ferrocenylmethyl-N-(nitrophenyl) and-N-(cyanophenyl)-acetamides: Cyclic voltammetry and molecular docking studies, Journal of Electrochemical Science and Engineering 12 293–304. <https://doi.org/10.5599/jese.1162>
- A. Khennoufa, L. Bechki, T. Lanez, E. Lanez, N. Zegheb, (2021). Spectrophotometric, voltammetric and molecular docking studies of binding interaction of N-ferrocenylmethylnitroanilines with bovine serum albumin, J. Mol. Struct. 1224 129052. <https://doi.org/10.1016/j.molstruc.2020.129052>.
- A. Adaika, A. Adaika, T. Lanez, E. Lanez, (2019). in Vitro and in Silico Evaluation of Anticancer Activity of N, N-Dimethylaminomethylferrocene, Journal of Fundamental and Applied Sciences 11 748–768. <https://jfas.info/index.php/JFAS/article/view/289>.
- C. Filote, E. Lanez, V.I. Popa, T. Lanez, I. Volf, (2022). Characterization and Bioactivity of Polysaccharides Separated through a (Sequential) Biorefinery Process from Fucus spiralis Brown Macroalgae, Polymers (Basel). 14 4106. <https://doi.org/10.3390/polym14194106>.

- C. Benine, D.A. Boutlelis, L.A. Touhami, E. Lanez, D.G. Amara, G. Rim, N. Hanen, W. Zahnit, M. Messaoudi, (2025). Green synthesis, characterization, antioxidant, interaction with DNA/BSA, and investigation of cytotoxicity against MCF-7 cancer cells of zinc oxide nanoparticles using Hammada scoparia (Pomel) Iljin extract, *Int. J. Biol. Macromol.* 295 139709.
- C. Steffen, K. Thomas, U. Huniar, A. Hellweg, O. Rubner, A. Schroer, (2010). AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility, *J. Comput. Chem.* 31 2967–2970.
- D. Dascalu, D. Larisa Roman, M. Filip, A. Ciorsac, V. Ostafe, A. Isvoran. (2020), Solubility and Admet Profiles of Short Oligomers of Lactic Acid, *ADMET DMPK* 8 425–436. <https://doi.org/10.5599/admet.801>.
- E. Lanez, L. Bechki, T. Lanez, (2020). Antioxidant Activities, Binding Parameters, and Electrochemical Behavior of Superoxide Anion Radicals Towards 1-Ferrocenylmethylthymine and 1-Ferrocenylmethylcytosine, *Curr. Phys. Chem.* 10 10–22.
- E. Lanez, L. Bechki, T. Lanez, (2020). Ferrocenylmethylnucleobases: Synthesis, dft calculations, electrochemical and spectroscopic characterisation, *Chemistry (Easton)*. 14 146–153.
- E. Lanez, M. Saidi, T. Lanez, (2023). Assessment of antioxidant and DPPH free radical scavenging activity of 1,2-dithiole-3-thione derivatives by using cyclic voltammetry, spectroscopic, and molecular docking studies, *Journal of Sulfur Chemistry* 44 542–558. <https://doi.org/10.1080/17415993.2023.2200142>.
- E. Lanez, L. Bechki, T. Lanez, Antioxidant Activities, (2019). Binding Parameters, and Electrochemical Behavior of Superoxide Anion Radicals Towards 1-Ferrocenylmethylthymine and 1-Ferrocenylmethylcytosine, *Curr. Phys. Chem.* 10 10–22. <https://doi.org/10.2174/1877946809666190424143752>.
- E. Lanez, L. Bechki, T. Lanez, (2020). Ferrocenylmethylnucleobases: Synthesis, dft calculations, electrochemical and spectroscopic characterisation, *Chemistry and Chemical Technology* 14 146–153. <https://doi.org/10.23939/chcht14.02.146>.
- E. Lanez, L. Bechki, T. Lanez, (2019). N6, 9-bis (ferrocenylmethyl) adenine: synthesis, cyclic voltammetric, spectroscopic characterization, and DFT calculations, *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry* 20 509–519.

- E. Lanez, N. Zegheb, T. Lanez, (2023). In silico ADME, toxicological analysis, molecular docking studies and Molecular dynamics simulation of Afzelin with potential antibacterial effects against *Staphylococcus aureus*, *Turkish Computational and Theoretical Chemistry* 7 10–16. <https://doi.org/10.33435/TCANDTC.1196422>.
- E. LANEZ, D. BENKHELIFA, F.Z. CHERIET, K. GORI, C. SLIMANI, S. KEBACHE, R. OUAKEOUAK, O. ABDELLAOUI, Y. DJOUGHI, M.L. BENAMOR, (2025) Theoretical Investigation of Mitomycin Derivatives as Dual Inhibitors of HER2 and ER- α : Prospects for Novel Breast Cancer Treatment Strategies, *Algerian Journal of Biosciences* 6 1–20.
- E. Lanez, Y. Bekkar, L. Bourougaa, M.L. Benamor, R. Bouraoui, O. Boudebia, A. Adaika, K. Nesba, H. Chaoua, L. Bechki, (2025). Antidiabetic potential of mentha piperita essential oil: GC-MS profiling, in vitro, in vivo and in silico analyses, *J. Mol. Struct.* 144239.
- E. Lanez, L. Zerrouk, L. Bechki, T. Lanez, A. Adaika, N. Zegheb, K. Nesba, (n.d.). Synthesis, molecular docking, drug-likeness analysis, and ADMET prediction of nickel and zinc tetraphenyl-porphyrin complexes as possible antioxidant agents, *Journal of the Chinese Chemical Society*.
- E. Lanez, T. Lanez, N. Zegheb, (2023). In silico ADMET, toxicological analysis, molecular docking studies and Molecular dynamics simulation of Afzelin with potential antibacterial effects against *Staphylococcus aureus*, *Turkish Computational and Theoretical Chemistry* 7 10–16.
- E. Lanez, L. Bechki, T. Lanez, (2019). Computational molecular docking, voltammetric and spectroscopic DNA interaction studies of 9N-(Ferrocenylmethyl) adenine, *Chemistry and Chemical Technology* 13 11–17. <https://doi.org/10.23939/chcht13.01.011>.
- E. Lanez, A. Kedadra, T. Lanez, A. Adaika, N. Zegheb, In vitro antioxidant activity, QSAR, in silico toxicity prediction, molecular docking studies, and molecular dynamics of a series of N-ferrocenylmethylanilines as potent antioxidant agents, *J. Organomet. Chem.* (2024) 123284.
- G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, A. Lu, (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties, *Nucleic Acids Res.* 49 W5–W14.

- H. Mouada, T. Lanez, E. Lanez, (2019). Investigations of the binding parametres of the interaction of N'-ferrocenylmethyl-N'-phenylaceto-and propionohydrazide with DNA, *Journal of Fundamental and Applied Sciences* 11 875–882.
- H. Laraoui, E. Lanez, N. Zegheb, A. Adaika, T. Lanez, M. Benkhaled, (2023) Anti-Diabetic Activity of Flavonol Glucosides from *Fumana montana* Pomel: In vitro Analysis, In Silico Docking, ADMET Prediction, and Molecular Dynamics Simulations, *ChemistrySelect* 8 e202204512. <https://doi.org/10.1002/slct.202204512>.
- H. Mouada, T. Lanez, I. Zafar, (2024). ROS scavenging, DNA binding and NADPH oxidase inhibition potential of N'-Ferrocenylmethyl-N'-phenylpropionohydrazide using cyclic voltammetry and molecular docking, *J. Organomet. Chem.* 1007 123026.
- H. Benamara, T. Lanez, E. Lanez, (2020). Bsa-binding studies of 2-and 4-ferrocenylbenzonitrile: Voltammetric, spectroscopic and molecular docking investigations, *Journal of Electrochemical Science and Engineering* 10 335–346. <https://doi.org/10.5599/jese.861>.
- I.U. Khand, T. Lanez, P.L. Pauson, (1989). Ferrocene derivatives. Part 24. Synthesis of dihydro-2-pyrindines and dihydro-3 H-2-cyclopent [c] azepines by photolysis of their cyclopentadienyliron derivatives, *J. Chem. Soc. Perkin 1* 2075–2078.
- J. Huang, A.D. Mackerell, (2013). CHARMM36 all-atom additive protein force field: Validation based on comparison to NMR data, *J. Comput. Chem.* 34 2135–2145. <https://doi.org/10.1002/jcc.23354>.
- J. Dong, N.N. Wang, Z.J. Yao, L. Zhang, Y. Cheng, D. Ouyang, A.P. Lu, D.S. Cao, (2018). Admetlab: A platform for systematic ADMET evaluation based on a comprehensively collected ADMET database, *J. Cheminform.* 10 1–11. <https://doi.org/10.1186/s13321-018-0283-x>
- K. Vanommeslaeghe, A.D. MacKerell, (2012). Automation of the CHARMM general force field (CGenFF) I: Bond perception and atom typing, *J. Chem. Inf. Model.* 52 3144–3154. <https://doi.org/10.1021/ci300363c>.
- L. ZERROUK, L. BECHKI, E. LANEZ, T. LANEZ, (2024). Synthesis, characterization, cyclic voltammetry, and molecular docking studies of the antioxidant activities of superoxide anion radicals towards meso-tetramethophenyl-porphyrin and meso-tetrabiphenyl-porphyrin, *Not. Sci. Biol.* 16 11823. <https://doi.org/10.55779/nsb16211823>.

- L. Guan, H. Yang, Y. Cai, L. Sun, P. Di, W. Li, G. Liu, Y. Tang, (2019). ADMET-score-a comprehensive scoring function for evaluation of chemical drug-likeness, *Medchemcomm* 10 148–157. <https://doi.org/10.1039/C8MD00472B>.
- M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, Gaussian 09. Wallingford, CT: Gaussian, 2009.
- M.P. Andersson, P. Uvdal, (2005). New scale factors for harmonic vibrational frequencies using the B3LYP density functional method with the triple- ζ basis set 6-311+ G (d, p), *J. Phys. Chem. A* 109 2937–2941.
- M. Chebbah, E. Lanez, S. Bouacida, H. Athmani, M.L. Ben Amor, R. Hafidi, A. Bouchemma, Synthesis, Characterization, Structural Analysis, Hirshfeld Surface Studies, and Molecular Docking of 1, 3, 5-Tris (P-Bromophenyl)-1, 3, 5-Triazacyclohexane, Hirshfeld Surface Studies, and Molecular Docking Of 1 (n.d.).
- M. Hanane, T. Lanez, I. Zafar, M.S. Afghan, N. Zegheb, (2023). N'-Ferrocenylmethyl-N'-phenylbenzohydrazide as a potential DNA binding compound: a combined experimental and computational study, *J. Coord. Chem.* 76 1984–1998. <https://doi.org/10.1080/00958972.2023.2275247>.
- M.L. Tlili, I. Laib, K. Salemi, I. Chetehouna, I. BenMoussa, E. Lanez, (2024). Phytochemical Analysis and Evaluation of the Antioxidant, Anti-Inflammatory, Hemolytic, and Antibacterial Effects of *Astragalus gombo* (L.) Leaves., *Acta Chim. Slov.* 71.
- M.L. Tlili, F. Alia, E. Bahri, T. Beyat, K. Redouan, E. Lanez, (2025). In vivo evaluations of the antidiabetic and antioxidant effects of *Olea europaea* L. (Chemlali variety) leaves, *Acta Periodica Technologica* 3.
- M.L.B.E.N. AMOR, E. LANEZ, Y. Bekkar, A. ADAIKA, T. LANEZ, K. NESBA, L. BECHKI, (2025). Exploring the Interactions of Ferrocenylmethylnucleobase Derivatives with BSA and HHb: Insights from Electrochemical, Spectroscopic, ADMET, in Silico Docking, and MD Simulations, *ChemistrySelect* 10 e202404678.
- M.L. Ben Amor, E. Lanez, Y. Bekkar, A. Adaika, T. Lanez, K. Nesba, L. Bechki, (2025). EVALUATION OF FERROCENYLMETHYLNUCLEOBASES DERIVATIVES INTERACTING WITH DNA: INSIGHTS FROM ELECTROCHEMICAL, SPECTROSCOPIC, DFT CALCULATION, MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATIONS., *Studia Universitatis Babes-Bolyai, Chemia* 70.

- M. Meraghni, T. Lanez, E. Lanez, L. Bechki, A. Kennoufa, (2023). Experimental and theoretical study on corrosion inhibition of mild steel by meso-tetraphenyl-porphyrin derivatives in acid solution, *Journal of Electrochemical Science and Engineering* 13 217–229. <https://doi.org/10.5599/jese.1400>.
- N. Zouaghi, E. Lanez, M.L. Benamor, S.N. Nacer, T. Lanez, Y. Bekkar, H. Haddad, K. Al-Abdallat, M. Zihlif, Y. Al-Abdallat, (2025). Targeting Alzheimer's Disease via Phytochemicals from *Artemisia campestris* L.: GC/MS Analysis with an Integrated Computational Approach, *Food Biosci.* 108128.
- N. Zegheb, C. Boubekri, T. Lanez, E. Lanez, T.T. Küçükkılınç, E. Öz, A. Khennoufa, S. Khamouli, S. Belaidi, (2021). In Vitro and In Silico Determination of Some N-ferrocenylmethylaniline Derivatives as Anti-Proliferative Agents Against MCF-7 Human Breast Cancer Cell Lines, *Anticancer Agents Med. Chem.* 22 1426–1437. <https://doi.org/10.2174/1871520621666210624141712>.
- N. Benyza, F. Allouche, S.W. Dammak, E. Lanez, T. Lanez, (2022). Chemical Reactivity, Topological Analysis, and Second-Order Nonlinear Optical Responses of M3O@ Al12N12: A Quantum Chemical Study, *Russian Journal of Physical Chemistry A* 96 2909–2920.
- N. ZEGHEB, L. Elhafnaoui, T. Lanez, (2020). N-ferrocenylmethyl-derivatives as spike glycoprotein inhibitors of sars-cov-2 using in silico approaches.
- P.W. Rose, A. Prlić, A. Altunkaya, C. Bi, A.R. Bradley, C.H. Christie, L. Di Costanzo, J.M. Duarte, S. Dutta, Z. Feng, R.K. Green, D.S. Goodsell, B. Hudson, T. Kalro, R. Lowe, E. Peisach, C. Randle, A.S. Rose, C. Shao, Y.P. Tao, Y. Valasatava, M. Voigt, J.D. Westbrook, J. Woo, H. Yang, J.Y. Young, C. Zardecki, H.M. Berman, S.K. Burley, (2017). The RCSB protein data bank: Integrative view of protein, gene and 3D structural information, *Nucleic Acids Res.* 45 D271–D281.
- P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals, *Nucleic Acids Res.* 46 W257–W263. <https://doi.org/10.1093/nar/gky318>.
- R. Kumari, R. Kumar, A. Lynn, (2014). G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations, *J. Chem. Inf. Model.* 54 1951–1962. <https://doi.org/10.1021/ci500020m>

- S. Khamouli, S. Belaidi, M. Ouassaf, T. Lanez, S. Belaaouad, S. Chtita, (2022). Multi-combined 3D-QSAR, docking molecular and ADMET prediction of 5-azaindazole derivatives as LRRK2 tyrosine kinase inhibitors, *J. Biomol. Struct. Dyn.* 40 1285–1298.
- S. Khamouli, S. Belaidi, T. Lanez, (2019). Molecular doking and ADMET studies of amino-pyrimidine derivaties as mycobacterium tuberculosis Ser/Thr protein kinases B inhibitors, *Journal of Fundamental and Applied Sciences* 11 914–939.
- S.N. Nacer, T. Lanez, (2013). Electrochemical properties of N'-ferrocenylmethyl-N'-phenylbenzohydrazide in aqueous and organic mediums, *International Letters of Chemistry, Physics and Astronomy* 5 76–85.
- T. Lu, F. Chen, (2012). Multiwfn: A multifunctional wavefunction analyzer, *J. Comput. Chem.* 33 580–592.
- T. Lanez, M. Lanez, R. Lanez, E. Lanez, B. Talbi-Lanez, (2024). Computational Screening of Novel Nitroimidazole Candidates: Targeting Key Enzymes of Oral Anaerobes for Anti-Parasitic Potential, *Curr. Drug Discov. Technol.*
- T. Lanez, M. Henni, H. Hemmami, (2015). Development of cyclic voltammetric method for the study of the interaction of antioxidant standards with superoxide anion radicals case of α -tocopherol, *Scientific Study and Research: Chemistry and Chemical Engineering, Biotechnology, Food Industry* 16 161–168.
- T. Lanez, M. Henni, (2025). Dual Anti-Inflammatory and Antidiabetic Potential of Ferrocenylmethylcyanophenyl Derivatives: Integrated In Vitro and In Silico Evaluation, *J. Organomet. Chem.* 123956.
- T. Zaiz, T. Lanez, (2015). Corrosion inhibition of carbon steel XC70 in H₂SO₄ solution by ferrocene derivative 3-(ferrocenylmethylamine) benzonitrile, *Journal of Fundamental and Applied Sciences* 4 182. <https://doi.org/10.4314/jfas.v4i2.8>.
- T. Lanez, H. Hemmami, (2016). Antioxidant Activities of N-ferrocenylmethyl-2- and -3-nitroaniline and Determination of their Binding Parameters with Superoxide Anion Radicals, *Curr. Pharm. Anal.* 13 110–116. <https://doi.org/10.2174/1573412912666160831145524>.
- T. Lanez, M. Feizi-Dehnyebi, E. Lanez, (2024). Assessment of the electrostatic binding of ferrocenylmethyl-nitroaniline derivatives to DNA: A combined experimental and theoretical study, *J. Mol. Struct.* 138386.

References Bibliography

- T. Lanez, H. Benaicha, E. Lanez, M. Saidi, (2018). Electrochemical, spectroscopic and molecular docking studies of 4-methyl-5-((phenylimino)methyl)-3H- and 5-(4-fluorophenyl)-3H-1,2-dithiole-3-thione interacting with DNA, *Journal of Sulfur Chemistry* 39 76–88. <https://doi.org/10.1080/17415993.2017.1391811>.
- Y. Bekkar, E. Lanez, T. Lanez, L. Bourougaa, A. Adaika, Z. Saada, A. Benine, (2025). Antidiabetic Potential of Synthesized Ferrocenylmethylaniline Derivatives: Insights From In Vitro Studies, Molecular Docking, ADMET, DFT Calculations, and Molecular Dynamics Simulation, *Biotechnol. Appl. Biochem.*
- Y. Bekkar, E. Lanez, T. Lanez, L. Bourougaa, A. Adaika, A. Benine, Z. Saada, (2025). Combined In Vitro and In Silico analysis of ferrocenylmethylaniline derivatives: Antibacterial potential, DFT calculations, and molecular dynamics insights, *J. Organomet. Chem.* 123618.
- Y. Ouennoughi, H.E. Karce, D. Aggoun, T. Lanez, R. Ruiz-Rosas, B. Bouzerafa, A. Ourari, E. Morallon, (2017). A novel ferrocenic copper (II) complex Salen-like, derived from 5-chloromethyl-2-hydroxyacetophenone and N-ferrocenmethylaniline: Design, spectral approach and solvent effect towards electrochemical behavior of Fc^+/Fc redox couple, *J. Organomet. Chem.* 848 344–351.