



الجمهورية الجزائرية الديمقراطية الشعبية

Algeria of Republic Democratic People's

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research



جامعة الشهيد حمه لخضر الوادي

Echahid Lakhdar Hamma University EL-OUED

كلية علوم الطبيعة والحياة

Faculty of Natural and Life Sciences

قسم البيولوجيا الخلوية والجزيئية

Department of Cellular and Molecular Biology

END OF STUDY THESIS

In view of obtaining the diploma

of Academic Master Major:

Biological Sciences

Speciality: Applied Biochemistry

THEME

**Synthesis, Characterization, and Pharmacological Evaluation of
Novel Ferrocene-Based Compounds with Anti-Inflammatory and
Antihypothyroid Activities**

Presented by:

CHEDALA Aya

LAYES Maroua

TANNECHE Khira

SENIGRA Akila

Examining Committee:

President:

Medila Ifriqya

University of Eloued

Examiner:

Laib Ibtiham

University of Eloued

Supervisor:

LANEZ Elhafnaoui

MCA

University of Eloued

Co-supervisor:

CHAOUA Housseyn

MCA

University of Eloued

University year:2025/2026

Abstract

The present study evaluated the pharmacological potential of newly synthesized ferrocene-based compounds as anti-thyroid agents using both *in vivo* and *in silico* approaches. Experimental hypothyroidism was induced in male Wistar rats using benzylthiouracil (BTU), followed by treatment with three ferrocene derivatives (FMA, FCMphA, and FCMphB) and comparison with levothyroxine as a reference drug. Physiological, biochemical, hormonal, oxidative stress, and histopathological parameters were assessed. In addition, ADMET prediction and molecular docking studies targeting thyroid hormone receptors (T3 and T4) were performed to evaluate pharmacokinetic properties and binding affinity.

The results confirmed successful induction of hypothyroidism, as evidenced by increased TSH levels, decreased T3 and T4 concentrations, oxidative stress, and histopathological alterations in thyroid, liver, and kidney tissues. Treatment with ferrocene derivatives, particularly FMA and FCMphB, significantly improved thyroid hormone balance, reduced oxidative stress markers, enhanced antioxidant enzyme activities (GSH, SOD, CAT), and preserved tissue architecture. Moreover, the synthesized compounds exhibited no significant hepatic, renal, or hematological toxicity.

In silico analysis revealed favorable drug-likeness and ADMET profiles for the ferrocene derivatives. Molecular docking studies demonstrated strong binding affinities toward thyroid hormone receptors, especially for FMA and FCMphB, with binding energies comparable to or better than levothyroxine. Overall, these findings suggest that ferrocene-based compounds possess promising therapeutic potential as anti-thyroid agents through antioxidant, metabolic, and receptor-mediated mechanisms.

Keywords: Ferrocene-based compounds, Benzylthiouracil (BTU), Levothyroxine, Thyroid hormones, Oxidative stress, *In vivo* rat model, *In silico* analysis.

Résumé

La présente étude a évalué le potentiel pharmacologique de nouveaux composés à base de ferrocène synthétisés, en tant qu'agents antithyroïdiens, en utilisant des approches *in vivo* et *in silico*. Une hypothyroïdie expérimentale a été induite chez des rats mâles Wistar à l'aide du benzylthiouracile (BTU), suivie d'un traitement par trois dérivés du ferrocène (FMA, FCMphA et FCMphB) et d'une comparaison avec la lévothyroxine utilisée comme médicament de référence. Des paramètres physiologiques, biochimiques, hormonaux, de stress oxydatif ainsi qu'histopathologiques ont été évalués. En outre, des études de prédiction ADMET et de docking moléculaire ciblant les récepteurs des hormones thyroïdiennes (T3 et T4) ont été réalisées afin d'évaluer les propriétés pharmacocinétiques et l'affinité de liaison.

Les résultats ont confirmé l'induction réussie de l'hypothyroïdie, mise en évidence par une augmentation du taux de TSH, une diminution des concentrations de T3 et T4, un stress oxydatif, ainsi que des altérations histopathologiques au niveau des tissus thyroïdiens, hépatiques et rénaux. Le traitement par les dérivés du ferrocène, en particulier FMA et FCMphB, a permis d'améliorer significativement l'équilibre hormonal thyroïdien, de réduire les marqueurs du stress oxydatif, d'augmenter l'activité des enzymes antioxydantes (GSH, SOD, CAT) et de préserver l'architecture tissulaire. De plus, les composés synthétisés n'ont montré aucune toxicité hépatique, rénale ou hématologique significative.

L'analyse *in silico* a révélé des profils favorables de type drug-likeness et ADMET pour les dérivés du ferrocène. Les études de docking moléculaire ont montré de fortes affinités de liaison envers les récepteurs des hormones thyroïdiennes, notamment pour FMA et FCMphB, avec des énergies de liaison comparables ou supérieures à celles de la lévothyroxine. Globalement, ces résultats suggèrent que les composés à base de ferrocène possèdent un potentiel thérapeutique prometteur en tant qu'agents antithyroïdiens via des mécanismes antioxydants, métaboliques et de modulation des récepteurs.

Mots clés : composés à base de ferrocène, benzylthiouracile (BTU), lévothyroxine, hormones thyroïdiennes, stress oxydatif, modèle *in vivo* chez le rat, analyse *in silico*.

الملخص

قِيمَت هذه الدراسة الإمكانيات الدوائية لمركبات جديدة مشتقة من الفيروسين (Ferrocene) كعوامل مضادة لنقص نشاط الغدة الدرقية، وذلك باستخدام منهجين: داخل الجسم الحي *in vivo* والحاسوبي *in silico*.

تم إحداث حالة قصور الغدة الدرقية تجريبياً في ذكور جرذان "ويستار" باستخدام مادة بنزيل ثيويوراسيل (BTU)، تلا ذلك علاج الجرذان بثلاث مشتقات من الفيروسين (FMA)، وFCMphA، وFCMphB، مع إجراء مقارنة بدواء "ليفوثيروكسين" كعقار مرجعي. تم تقييم المؤشرات الفسيولوجية، والكيميائية الحيوية، والهرمونية، ومستويات الإجهاد التأكسدي، بالإضافة إلى الفحوصات النسيجية المرضية. كما تم إجراء دراسات التنبؤ بخصائص الامتصاص والتوزيع والتمثيل الغذائي والإخراج والسمية (ADMET) ودراسات الربط الجزيئي (Molecular docking) التي تستهدف مستقبلات هرمونات الغدة الدرقية (T3) و (T4) لتقييم الخصائص الحركية الدوائية وقوة الارتباط.

أكدت النتائج نجاح إحداث قصور الغدة الدرقية، كما ظهر من خلال ارتفاع مستويات TSH، وانخفاض تركيزات T3 و T4، وزيادة الإجهاد التأكسدي، وحدثت تغيرات نسيجية مرضية في أنسجة الغدة الدرقية والكبد والكلية. أدى العلاج بمشتقات الفيروسين، وخاصة FMA و FCMphB، إلى تحسن ملحوظ في توازن هرمونات الغدة الدرقية، وتقليل مؤشرات الإجهاد التأكسدي، وتعزيز نشاط الإنزيمات المضادة للأكسدة (GSH)، (SOD)، (CAT)، والحفاظ على البنية النسيجية للأعضاء. علاوة على ذلك، لم تُظهر هذه المركبات أي سمية كبدية أو كلوية أو دموية ذات دلالة إحصائية.

أظهرت التحليلات الحاسوبية تمتع مشتقات الفيروسين بخصائص دوائية مقبولة وملفات ADMET واعدة. كما أثبتت دراسات الربط الجزيئي وجود ألفة ارتباط قوية بمستقبلات هرمونات الغدة الدرقية، خاصة بالنسبة لـ FMA و FCMphB، حيث كانت طاقة الارتباط مقاربة أو أفضل من تلك الخاصة بعقار "ليفوثيروكسين". وبشكل عام، تشير هذه النتائج إلى أن المركبات المشتقة من الفيروسين تمتلك إمكانيات علاجية واعدة كعوامل مضادة لنقص نشاط الغدة الدرقية، وذلك من خلال آليات تعتمد على مضادات الأكسدة، والتنظيم الأيضي، والتفاعل مع المستقبلات.

الكلمات المفتاحية: مركبات الفيروسين، بنزيل ثيويوراسيل (BTU)، ليفوثيروكسين، هرمونات الغدة الدرقية، الإجهاد التأكسدي، نموذج حي (جرذان)، التحليل الحاسوبي.



إهداء

ع قیل ة سنیقرة

بسم الله الرحمن الرحيم، والصلاة والسلام على رسول الله. ﴿ وَمَا بِكُمْ
من نَعْمَةٍ، فَمِنَ اللَّهِ ﴾

الحمد لله الذي أعان وهدي ويسر لي هذا الإنجاز العلمي، وجعل فيه الخير
والبركة، وأسأله سبحانه أن يتقبله خالصاً لوجهه الكريم.

إنّ المحاولات المستمرة، مهما تعثّرت، تُثمر في النهاية نجاحات يسعد
الإنسان بامتلاكها، ولذلك أهدي هذا التخرّج لمحاولاتي التي

لم تستسلم، حتى في الأوقات التي كدت أن أتخلى عنها. أهديه لأعزّ ما
يملك الإنسان... لجيشه الذي لا ينثني، ولدرعه

الديني الذي لا ينكسر: عائلتي، أهلي، وإخوتي؛ أولئك الذين ربّوا وسهروا وتعبوا
وبذلوا كل شيء ليقف اسمي اليوم في هذا المقام.
لأجلكم ألف مرة ومرة.

وأهديه لكل من ساهم في هذا الطريق كلمةً أو فعكاً، مهما بدا
بسيطاً، إلى أساتذتي، وأصدقائي، وزملائي، وأهل العلم والخبرة.
جزاكم الله عني كل خير وبارك في أعماركم وأعمالكم. وأهديه
لشباب غزة خاصة، ولشباب الأمة الإسلامية عامة، أولئك
الذين منعتهم الظروف من تحقيق أحلامهم، هذا إنجازي الصغير أهديه
بكل خجل لأحلامكم.

كما أهدي هذا العمل لشهداء الحرب الأخيرة، الذين بقيت أصواتهم تتردد في
خاطري وذكراهم تسكن وجداني...

إلى روح أنس الشريف و صالح الجعفر اوي خاصة. هذا
شيء من وسعي لأرواحكم الطاهرة الخالدة.

أما أخيراً فأهديه إلى "عقيلة" إنسانة لا تكف عن المحاولة و التحسين و البناء و
السعي و تلك هي ميزتها الأسمى التي أفخر بوجودها..
كل الحب والتقدير لكم جميعاً... فرداً فرداً.

الحمد لله الذي هدانا لهذا، وما كنا لنهتدي لولا أن هدانا الله.



إهداء

م ر ي ل عيس

****بسم الله الرحمن الرحيم****

"يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ" إلى أبي
(رحمه الله): مَنْ غَاب جسده وحضر روحه في كل
تفاصيلي، كنت نبع ألمان وحضن الحكمة. أضع ثمرة هذا العمل على
قبرك بكل حنين، داعيةً الله أن يبلغك نورها في
مقامك الأبدي.

إلى أمي: الصخرة التي لم تنكسر، ومَنْ علمتني أن الحب عطاء صامت.
هذا الإنجاز هو امتداد لدعواتك التي حمتني، وثمرة
لعزيمتك التي ألهمتني.

إلى أخواتي: رفيقات الدرب وشركاء الأحلام، ونبض البيت في أصعب
الأيام. بوجودكن لم أشعر يوماً بالوحدة، وهذا
العمل لنا جميعاً.

إلى خطيبي وسندي: شريك الحلم قبل الواقع، وداعم الخطوة قبل الكلمة.
بجانبك عرفت أن للنجاح طعماً أحلى، فكنت
ملاذي ومساحتي الآمنة.

إلى صديقات العمل: مَنْ شاركنني تفاصيل الرحلة، وحوّلن ساعات
الجهد إلى ذكريات ال تُنسى.. هذا الإنجاز لَكُنَّ كما
هو لي.

إلى نفسي: التي صبرتُ لتجني، وتعبتُ لتفرح.. كم أنتِ جديرة بهذا
النجاح.

*وفي الختام: اللهم لك الحمد حتى ترضى، ولك الحمد إذا رضيت،
ولك الحمد بعد الرضا. اللهم اجعل هذا التخرج
فاتحة خير ونجاح أكبر في الدنيا والآخرة، واجعلنا ممن يرفعون
درجاتٍ بالعلم النافع والعمل الصالح.
والحمد لله الذي بنعمته تتم الصالحات.



أهدء

آية شدالة

أ

الحمد لله أو"أ وآخرًا، الذي بنعمته تتم الصالحات، والذي وفقنا وأعانا على إتمام هذا العمل، فله سبحانه كل الحمد والشكر على فضله وكرمه وتوفيقه. أهدي هذا العمل المتواضع إلى:

إلى أُمي الغالية، مصدر الحنان والدعاء والصبر، التي كانت سندي في كل خطوة، جزاها الله عني خير الجزاء.
وإلى أبي العزيز، الذي علمني الجتهاد والكفاح، وكان مثلاً للعتاء والدعم، حفظه الله وأدامه تاجًا فوق رأسي.
وإلى إختوتي وأختوتي، رفاق الدرب ودفء العائلة، الذين كانوا إلى جانبي في أوقات التعب قبل الفرح، فخففوا عني المشقة، وزرعوا في نفسي الأمل والقوة.
إلى من شاركوني لحظات القلق والسهر، وساندوني بكلماتهم وتشجيعهم ودعواتهم الصادقة، فكانوا نعم العون والسند.
إلى من منحتني صحبتهم معنى المحبة الحقيقية وروح التكاتف، أدامكم الله نعمة ال تزول، وحفظ بيننا المودة والمحبة دائمًا. وإلى صديقاتي العزيزات في مشوار التخرج، رفيقات التعب والنجاح، اللواتي شاركنني أجمل الذكريات وأصعب اللحظات، وكان وجودهن مصدر دعم، وتحفيز دائم.
شكرًا لكل لحظة تعاون، ولكل كلمة تشجيع، ولكل ابتسامة خفت عنا عناء الطريق، أسأل الله أن يوفقن جميعًا ويجمعنا دائمًا على الخير والمحبة والنجاح.
إلى كل من أحبني ودعمني ولو بكلمة طيبة أو دعوة صادقة. وإلى كل

من كان له أثر جميل في مسيرتي العلمية، وساهم في
تشجيعي للوصول إلى هذه المرحلة، أهدي ثمرة هذا الجهد
المتواضع،
راجيًا من الله تعالى دوام التوفيق والنجاح للجميع.



إهداء

خي رة طنش

{رَبِّهِ وَزَعْنِي أَوْ شَكَرَ نِعْمَتَكَ الَّتِي أَوْعَمَّتْ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَوْعَمَلْ صَالِحًا تَرْضَاهُ}

الحمد لله الذي وفقني لبلوغ هذه اللحظة بعد سنواتٍ من التعب والسهر والاجتهاد، والذي منحني القوة والأمل في كل مرحلة من هذه الرحلة.

إلى أبي الغالي...

إلى السند الذي كان دائماً مصدر قوتي وأماني، شكراً لكل تعبٍ وصبرٍ ودعمٍ، منحه لي دون مقابل.

أسأل الله أن يحفظك ويديمك فخراً وسنداً لي. إلى أمي الحبيبة...

إلى القلب الذي كان يشعر بتعبني قبل أن أتحدث، وإلى الدعوات الصادقة التي كانت ترافقني في كل خطوة...

أمي، أنتِ أعظم نعمة في حياتي، ورضائك كان دائماً سر نجاحي واستمراري.

حفظك الله وأدامك نوراً يضيء حياتي. إلى أخواتي العزيزات وأزواجهن الكرام،

شكراً لوجودكم الجميل بجانبني، ولدعمكم الذي كان يمنحني القوة والأمل كلما شعرت بالتعب.

وإلى أحبتي الصغار: عبد الله، ماريا جوري، أنس، منيب، جنة، ولينة... إلى ضحكاتكم البريئة التي كانت تخفف عني ضغوط الدراسة وتزرع

السعادة في قلبي، أتمنى لكم حياةً مليئةً بالنجاح والفرح. وإلى صديقات المشروع ورفيقات هذا المشوار،

شكراً لكل لحظة تعب وتقاسم لأمل والقلق والنجاح، وللذكريات الجميلة التي ستبقى محفورة في القلب دائماً.

كما أهدي هذا العمل لكل من دعمني بكلمة طيبة أو دعوة صادقة، ولكل من كان سبباً في وصولي إلى هذه اللحظة.

هذا الإنجاز ليس مجرد شهادة تخرج، بل ثمرة صبرٍ وحلمٍ، ودعواتٍ صادقة أهديه بكل الحب والامتنان لكل من كان جزءاً من رحلتي.



كلمة شكر و تقدير

الحمد لله تعالى على توفيقه لنا لإتمام هذا العمل.

نتوجه بجزيل الشكر والامتنان إلى أستاذنا المشرف العائز الحفناوي الذي إحتضن هذا المشروع و ساهم في إخراجه إلى النور بتوجيهاته القيمة و متابعته المستمرة.

نشكر الأستاذة وفاء بوضيية أيضا على نصائحها و توجيهاتها العلمية و صبرها على فضولنا و تساؤلاتنا طوال فترة إنجاز هذا المشروع

ولا يفوتنا أن نتقدم بخالص الشكر والتقدير إلى المخبري وليد لونيس على المعلومات القيمة و المساعدات العلمية و المخبرية القيمة التي أثرى بها هذا الإنجاز

كما نشكر الأستاذ عمر مسؤول المخبر 8 على تعامله الطيب و الخلق الحسن الذي أعاننا على إتمام هذا البحث براحة و احترافية.

و أخيرا فالشكر موصول لكل من ساهم في إنجاز هذا المشروع من قريب أو بعيد و غفلنا عن ذكر إسمه ،شكرا لكم و جزاكم الله عنا كل خير.

List of Abbreviations

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity

ALT: Alanine Aminotransferase

ANOVA: Analysis of Variance

AST: Aspartate Aminotransferase

BTU: Benzylthiouracil

BUN: Blood Urea Nitrogen

Bw: Body weight

CAT: Catalase

Creat: Creatinine

EDTA: Ethylenediaminetetraacetic acid

GSH: Glutathione

GSH-Px: Glutathione Peroxidase

H&E: Hematoxylin and Eosin

LD50: Lethal Dose 50%

MDA: Malondialdehyde

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

p-value: Probability value

RBCs: Red Blood Cells

ROS: Reactive Oxygen Species

SD: Standard Deviation

SEM: Standard Error of the Mean

SOD: Superoxide Dismutase

T3: Triiodothyronine

T4: Thyroxine

TSH: Thyroid-Stimulating Hormone

UV-Vis: Ultraviolet–Visible Spectroscopy

WBCs: White Blood Cells

Table of Contents

Abstract	2
Résumé.....	3
الملخص.....	4
اهداء.....	5
List of Abbreviations	6
Table of Contents	7
List of Figures	8
List of Tables	9
General Introduction	10
Chapter 1: The thyroid gland	12
1. Introduction:	13
1.1. Definition of The Thyroid Gland:	13
1.2. Structure The thyroid gland:	14
1.3. Anatomy of the Thyroid Gland	15
1.4. Histology	16
1.5. Synthesis of thyroid hormones:	19
1.5.1. Paraphrased Version:	19
1.6. Metabolic Effects of Thyroid Hormones	20
1.6.1. Calorigenic Effect and Thermogenesis.....	20
1.6.2 Basal Metabolic Rate (BMR)	21
1.6.3. Protein Metabolism.....	21
1.6.4. Body Weight.....	21
1.6.5. Carbohydrate Metabolism	21
1.6.6. Lipid Metabolism	21
1.6.7. Additional Systemic Activities	21
1.7. Thyroid Hormone Metabolism:	21
1.8. Regulation of Thyroid Hormone Secretion	22
1.8.1 Regulation of Thyroid Hormone Release	22
1.9. Hyperthyroid and Hypothyroid Disorders.....	23
1.9.1. Hyperthyroid Disorders	23
1.9.2. Hypothyroid Disorders	24
1.10. Antithyroid Drugs	24
1.10.1. Methimazole	24
1.10.2 Propylthiouracil (PTU):	25

Chapter02: ferrocene	26
Introduction:	27
2.1. Definition of Ferrocene	27
2.2. Structure, Properties, and Reactivity	29
2.2.1. Structure	29
2.3. Properties.....	32
2.3.1. Mechanism (I):	34
2.3.2. Mechanism (II)	34
Experimental Work.....	41
1. Materials and Methods	42
1.1. In vivo study.....	42
1.1.1. Experimental Animals	42
1.1.2. Chemicals and Reagents	42
1.1.3 Experimental Design	42
1.1.4. Evaluation of Physiological Parameters (Body and Organ Weights).....	43
1.1.5. Blood and Tissue Sampling	43
1.1.6. Hematological, Biochemical, and Hormonal Analyses	43
1.1.7. Tissue Homogenate Preparation and Oxidative Stress Evaluation.....	44
1.1.8. Histopathological Examination.....	44
1.2. In Silico Study.....	44
1.2.1. Ligand Preparation.....	44
1.2.2 ADMET and Drug-Likeness Prediction	45
1.2.3. Target Protein Selection and Preparation	46
1.2.4. Molecular Docking Protocol.....	47
1.3. Statistical Analysis	47
2. Results and Discussion.....	48
1. In vivo study.....	48
1.1 Weight Gain Index.....	48
1.2 Organ weight index	49
1.3. Hematological parameters.....	50
1.4 Glycemia, liver and kidneys function parameters	52
1.5 Thyroid function parameters	53
1.6. Oxidative stress parameters.....	55
1.7. Histopathological studies	58
2. In Silico Study.....	63

Conclusion.....	69
References	73

List of Figures

Figure 1The structures of the thyroid hormones. (Brandt, D. n.d.).....	14
Figure 2:Macroscopic posterior view of the thyroid. (Benvenga., 2018).	16
Figure 3Thyroid follicle of a newborn (green arrow), greatly different in size from that observed in adult ones. (Benvenga., 2018)	17
Figure 4Evident thyroid follicles in the adult (green arrow) lined by a single epithelium filled with colloid. (Benvenga., 2018).....	17
Figure 5Secretion of thyroid hormones. (Boron& Boulpaep., 2017).....	20
Figure 6Control of thyroid hormone secretion. (Brandt, D. n.d.)	23
Figure 7The role of thyroperoxidase (TPO) in the synthesis of T3 & T4. Methimazole and propylthiouracil inhibit TPO, resulting in decreased synthesis of thyroid hormone by inhibiting both organification (incorporation of iodine) and coupling (conjugation) steps.	25
Figure 8Intentionally Pauson's and Kealy's synthetic route to ferrocene. (El Hafnaoui.,2021).	28
Figure 9Wrong (a) and corrected (b) structures of ferrocene. (El Hafnaoui.,2021)	29
Figure 10The π molecular orbitals of the cyclopentadienyl anions. (El Hafnaoui.,2021).....	30
Figure 11Symmetry-adapted linear combination of molecular orbitals for a $(\eta^5\text{-C}_5\text{H}_5)_2\text{F}$. (El Hafnaoui.,2021).....	31
Figure 12Molecular orbital of ferrocene molecule. (El Hafnaoui.,2021).....	32
Figure 13IR spectra of ferrocene. (El Hafnaoui.,2021).....	33
Figure 14Mechanism (I) of substitution reactions on the pentadienyl ring (formation of a sigma complex). (El Hafnaoui.,2021).....	34
Figure 15Mechanism (II) of substitution reactions on the pentadienyl ring (formation of a sigma complex). (El Hafnaoui.,2021).....	35
Figure 16Synthetic route of ferrocenylmethyltrimethylammonium iodide. (El Hafnaoui.,2021).....	36
Figure 17Example of ferrocenyl compounds possessing antioxidant activity (X = NO ₂ , CN). (El Hafnaoui.,2021).....	37
Figure 18Compounds based on the 1-(diphenylmethylidene)-[3]ferrocenophane skeleton that show high cytotoxicity against hormone-independent breast and prostate cancer cells (R = OH, NH ₂). (El Hafnaoui.,2021).....	38

Figure 19Chemical structure of the anti-malarial ferroquine (a) and the anticancer ferrocifen (b) . (El Hafnaoui.,2021).	38
Figure 20Chemical structure of a ferrocene derivative with anti-HIV activity. (El Hafnaoui.,2021).....	39
Figure 21Synthetic route to N6,9-bis(ferrocenylmethyl)adenine. (El Hafnaoui.,2021).....	40
Figure 22 Histological sections of the thyroid gland in different experimental groups	59
Figure 23 Histopathological changes in the liver across experimental groups	61
Figure 24Microscopic observation of kidney histological sections from different experimental groups, (C) Control group, (CN) Benzylthiouracil group, (CP) Group treated with levothyroxine, (FMA) Group treated with FMA, (FCMphA) Group treated with FCMphA and (FCMph	63
Figure 25Two-dimensional (left) and three-dimensional (right) representations of the binding mode of FMA within the active sites of (a) T3 receptor (PDB ID: 1XZX) and (b) T4 receptor (PDB ID: 1Y0X)	68

List of Tables

Table 1Weight Gain Index of different experimental groups.....	49
Table 2Organ Weight Index of different experimental groups.....	50
Table 3Plasma concentration of hematological parameters of different experimental groups	51
Table 4Glycemia, liver and kidneys function parameters of different experimental groups ...	53
Table 5Thyroid function parameters of different experimental groups.....	54
Table 6Effect of ferrocene-based treatments on oxidative stress markers in liver tissue.....	56
Table 7Effect of ferrocene-based treatments on oxidative stress markers in kidney tissue.	57
Table 8Predicted ADMET and drug-likeness profiles of FMA, FCMphA, FCMphB and Levothyroxine (LT4).....	64
Table 9Molecular docking results of ferrocene derivatives and Levothyroxine against thyroid hormone receptors	66

General Introduction

General Introduction:

The continuous search for novel therapeutic agents has driven significant progress in medicinal chemistry, particularly in the development of molecules with enhanced biological activity and reduced adverse effects. Within this context, organometallic compounds have emerged as attractive candidates due to their unique structural, electronic, and redox properties. Among them, ferrocene—a classical sandwich compound composed of an iron(II) center coordinated between two cyclopentadienyl rings—has garnered considerable attention since its discovery, owing to its exceptional chemical stability, reversible redox behavior, and high versatility in chemical functionalization (Patra & Gasser., 2017; Stepnicka., 2020).

In recent years, incorporating the ferrocene moiety into biologically active molecules has opened new perspectives in rational drug design. Numerous ferrocene-based derivatives have demonstrated diverse pharmacological activities, including anticancer, antimicrobial, antiviral, anti-inflammatory, and antiparasitic effects (Gao et al., 2022). These biological properties are closely linked to the redox-active Fe(II)/Fe(III) center, which enables ferrocene derivatives to modulate cellular oxidative processes, generate or neutralize reactive oxygen species (ROS), and enhance lipophilicity—thereby improving membrane permeability and overall biological efficacy (Huang & Zhao., 2023).

Moreover, ferrocene derivatives have shown notable antioxidant potential, making them promising candidates for managing disorders associated with oxidative stress. Oxidative imbalance is a key contributor to the pathogenesis of endocrine diseases, including thyroid dysfunctions. The thyroid gland is particularly susceptible to oxidative stress due to its dependence on hydrogen peroxide (H₂O₂) for thyroid hormone synthesis; thus, excessive ROS generation can impair hormone production and promote inflammatory responses (Contempre et al., 2021). Consequently, the development of molecules that exhibit both antioxidant and antithyroid activities represents a strategic therapeutic approach.

Conventional antithyroid drugs, such as methimazole and propylthiouracil, are widely used to manage hyperthyroidism. However, their therapeutic limitations—including hepatotoxicity, relapse rates, and lack of intrinsic antioxidant and anti-inflammatory actions—highlight the need for safer and more effective alternatives (Yoshimura et al., 2020). In this regard, ferrocene-based hybrids provide an innovative chemical framework capable of combining redox-driven antioxidant properties with selective inhibition of thyroid hormone biosynthesis pathways. Functionalizing classical organic pharmacophores with a ferrocene unit

has been shown to significantly enhance biological activity, chemical stability, and multi-target therapeutic potential (Bose & Mandal., 2023).

Therefore, this study aims to synthesize, characterize, and evaluate the pharmacological potential of novel ferrocene-based compounds with antioxidant and antithyroid activities. By integrating chemical, analytical, and biological approaches, this work seeks to contribute to the development of multifunctional molecules that may serve as promising therapeutic alternatives for thyroid-related disorders.

Chapter 1: The thyroid gland

1. Introduction:

The thyroid gland is a key endocrine organ located in the anterior region of the neck, responsible for regulating metabolism, growth, and development through the secretion of thyroid hormones, namely triiodothyronine (T3) and thyroxine (T4). These hormones play an essential role in maintaining metabolic homeostasis and influencing various physiological processes such as energy production, protein synthesis, and thermoregulation (Brent, 2012). The activity of the thyroid gland is controlled by the hypothalamic–pituitary–thyroid (HPT) axis, which ensures hormonal balance according to the body's needs (Hall, 2021).

Thyroid hormone synthesis involves a series of biochemical reactions, including iodine uptake, oxidation, and its incorporation into tyrosine residues of thyroglobulin. This process is catalyzed by thyroid peroxidase (TPO), a key enzyme responsible for iodination and coupling reactions leading to the formation of T3 and T4 (Rousset et al., 2015). Due to the requirement of hydrogen peroxide during hormone synthesis, the thyroid gland is particularly exposed to oxidative stress, making it susceptible to cellular damage when antioxidant defenses are compromised (Vaisman et al., 2011).

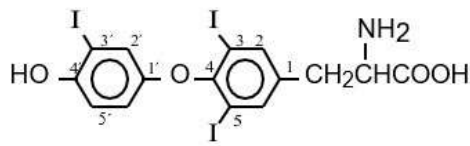
Thyroid disorders are among the most common endocrine diseases worldwide. Hyperthyroidism is characterized by excessive hormone production, leading to increased metabolic rate and systemic complications, while hypothyroidism results from insufficient hormone levels and reduced metabolic activity (De Leo et al., 2016). These conditions emphasize the importance of maintaining proper thyroid function. Moreover, oxidative stress has been recognized as a key factor in thyroid dysfunction, as excessive reactive oxygen species can impair normal cellular processes within the gland (Vaisman et al., 2011).

Understanding the physiological and biochemical mechanisms of the thyroid gland is therefore essential for the development of effective therapeutic strategies targeting thyroid disorders

1.1. Definition of The Thyroid Gland:

is an essential endocrine organ responsible for synthesizing thyroid hormones, which regulate growth, development, and basal metabolic rate, as well as calcitonin, a hormone involved in maintaining calcium homeostasis. The principal thyroid hormones are triiodothyronine (T3) and thyroxine (T4), whose chemical structures are illustrated in Figure 1.

Triiodothyronine (T3) = 3,5,3'-triiodothyronine



Thyroxine (T4) = 3,5,3',5'-tetraiodothyronine

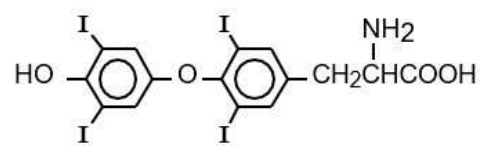


Figure 1The structures of the thyroid hormones. (Brandt, D. n.d.)

Functionally, T3 represents the biologically active form, as it exhibits a markedly higher affinity for thyroid hormone receptors compared to T4. Consequently, T4 primarily serves as a prohormone that is converted to T3 within target tissues. Unless otherwise specified, the term thyroid hormone in the following discussion refers collectively to both T3 and T4.

The structural distinction between the two molecules is minimal yet physiologically significant: T3 lacks the iodine atom at the 5' position, whereas T4 possesses four iodine atoms. This subtle difference profoundly influences their biological activity. Each T3 molecule contains three iodine atoms, while T4 contains four, highlighting the critical requirement of iodine availability for effective thyroid hormone synthesis.

To fulfill this requirement, the thyroid gland utilizes an active transport mechanism capable of concentrating iodine up to 30-fold above its serum concentration. The gland demonstrates remarkable efficiency, sequestering 20–40% of an administered iodine dose and storing over 90% of the total body iodine content.

The exclusivity of iodine utilization by the thyroid has important clinical implications. Low doses of radioactive iodine can be administered diagnostically to visualize thyroid morphology and function—particularly to identify heterogeneous uptake patterns associated with thyroid disorders. Conversely, high doses of radioactive iodine can be used therapeutically to selectively ablate thyroid tissue without exerting direct effects on other organs. (Brandt, D. n.d.).

1.2. Structure The thyroid gland:

The thyroid gland is organized into lobules that are separated by connective tissue septa extending inward from its surrounding capsule. Each lobule is composed of numerous spherical functional units known as thyroid follicles, which represent the basic structural and functional units of the gland.

The wall of each follicle is formed by a single layer of cuboidal epithelial cells, known as follicular cells. These cells are derived from the endoderm and are responsible for the synthesis

and secretion of thyroid hormones. The principal circulating thyroid hormone is thyroxine (T₄), or tetraiodothyronine, accompanied by smaller amounts of triiodothyronine (T₃). Although the majority of T₄ is subsequently converted into the more biologically active T₃ in peripheral tissues, both hormones contribute to cellular stimulation to varying degrees and play a central role in the regulation of basal metabolic rate (BMR).

Within the thyroid follicular epithelium or in the interfollicular spaces, parafollicular cells (C cells) are also present. These cells originate from neural crest derivatives and are responsible for the secretion of the polypeptide hormone calcitonin. Calcitonin plays a key role in calcium homeostasis by promoting the deposition of calcium and phosphate into bone and other tissues, thereby reducing blood calcium levels. This action functionally opposes that of parathyroid hormone (PTH).

The interior of the thyroid follicles serves as a storage site filled with colloid, which consists primarily of thyroglobulin, a PAS-positive acidophilic glycoprotein that acts as the precursor for thyroid hormone synthesis. Structurally, the follicles are supported by a delicate framework of reticular connective tissue fibers and are richly supplied by an extensive capillary network, which facilitates efficient hormone exchange and secretion. (Khan & Farhana, 2020)

1.3. Anatomy of the Thyroid Gland

The thyroid gland is a highly vascularized endocrine organ located in the anterior region of the neck, extending approximately from the level of the C5 to T1 vertebrae, and situated deep to the platysma as well as the sternothyroid and sternohyoid muscles. It has an average weight of 15–20 g in adults, with a slightly higher mass observed in males compared to females. At birth, the gland weighs approximately 1 g, increasing progressively by about 1 g per year until the age of 15.

Morphologically, the thyroid is an H-shaped, soft, reddish parenchymal organ composed of two lateral lobes (right and left) connected by a median isthmus. Each lobe measures approximately 4 cm in length, 2 cm in width, and 2–3 cm in thickness, while the isthmus is typically about 2 cm wide and 2 cm high, with a thickness ranging from 2–6 mm.

The superior pole (superior horn) of each lobe extends laterally to the inferior constrictor muscle and posteriorly to the sternothyroid muscle, whereas the inferior pole reaches the level of the fifth or sixth tracheal ring. Posterolaterally, the gland is in close relation with the carotid sheath and its vascular components. In approximately 50% of individuals, a pyramidal lobe (also known as the pyramid of Lalouette or Morgagni) is present, extending superiorly from either lobe or from the upper part of the isthmus, most commonly directed toward the left side.

The thyroid gland is enclosed by the deep cervical fascia and is anteriorly covered by the infrahyoid (strap) muscles, while laterally it is related to the sternocleidomastoid muscle. The true thyroid capsule is tightly adherent to the glandular tissue and sends internal extensions that form septa, thereby dividing the gland into lobes and lobules. Posteriorly, this capsule is reinforced, and the middle layer of the deep cervical fascia condenses to form the suspensory ligament of Berry, which anchors the thyroid gland to the cricoid cartilage and the first two tracheal rings.

On the posterior aspect of the lateral lobes are located the parathyroid glands, typically four in number (two superior and two inferior). These are small, ovoid structures approximately the size of a grain of rice, playing a critical role in calcium homeostasis. (Benvenga., 2018)

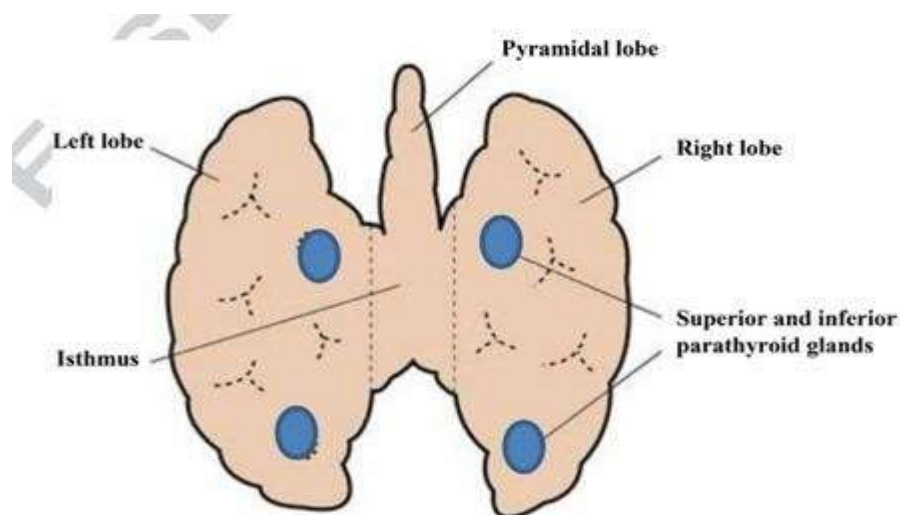


Figure 2: Macroscopic posterior view of the thyroid. (Benvenga., 2018).

1.4. Histology

p0050 Microscopically, thyroid is divided into lobules; each lobule consists of 20–40 round follicles that vary considerably in size, with a diameter ranging from 45 to 250 μ m. In the newborn, follicles are small and grow slowly (Fig.2).

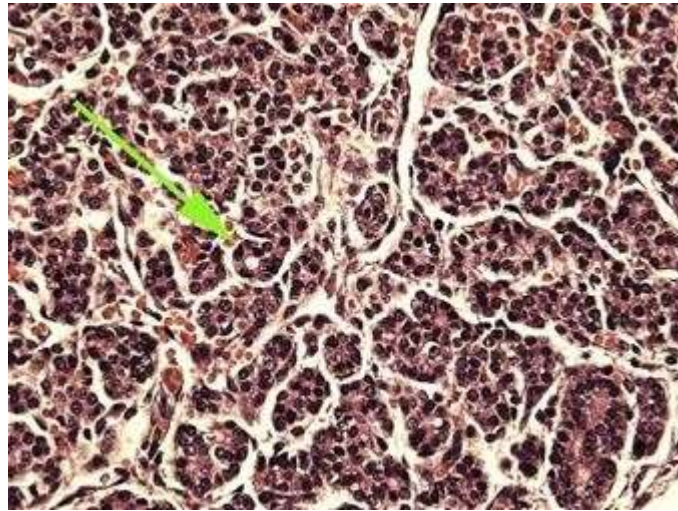


Figure 3 Thyroid follicle of a newborn (green arrow), greatly different in size from that observed in adult ones. (Benvenga., 2018)

p0055 Each follicle is lined by a single cuboidal layer of epithelium (9–13 mm) with a thin basement membrane filled with acidophilic colloid-core. Thyrocytes have a definite polarity, with their apices directed toward the lumen of the follicles and their basis toward the basement membrane. The apical surface of the epithelial cells has numerous microvilli extending to the colloid, while the spheroid nuclei are located at the same level in all cells, mainly near to their basis (Fig. 3).

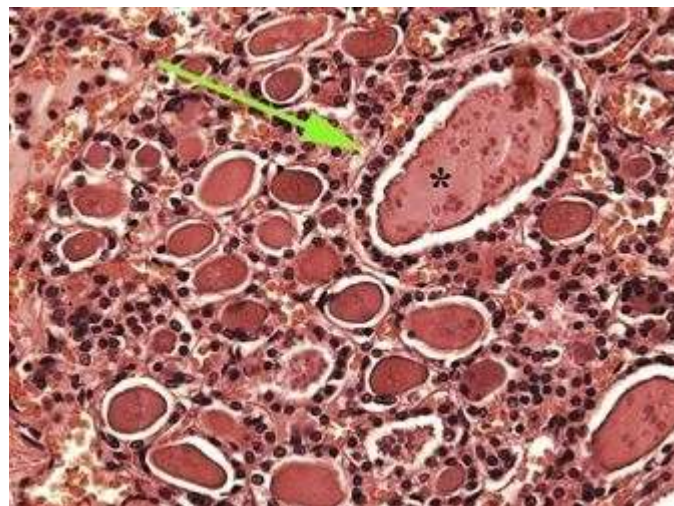


Figure 4 Evident thyroid follicles in the adult (green arrow) lined by a single epithelium filled with colloid. (Benvenga., 2018).

Thyroid is the only human gland in which the hormonal product is stored extracellularly (viz. in the colloid).

p0060 Mitoses are infrequent, being evident only in young people. Thyrocytes are characterized by a pale acidophilic or amphophilic cytoplasm in which lysosomal bodies, granules, and secretory vacuoles are evident. Immunohistochemistry shows that normal follicular epithelium contains thyroglobulin, low-molecular weight keratin, epithelial membrane antigen, and vimentin. Follicles are embedded in a small amount of a loose connective tissue that forms the gland stroma, in which blood vessels, nerves, and lymphatics are present.

p0065 C-cells are dispersed between follicles, mainly in the posterolateral portion of the lobes, or are located beyond the basement membrane within the follicles, close to thyrocytes (Nilsson and Fagman, 2017). As noted above, C-cell precursors derive from the neural crest. They constitute about 0.1% of thyroid cells, and their identification is possible only using immunohistochemical methods for calcitonin. Moreover, the numerous dense-core granules of C-cells show immunoreactivity for neuron-specific enolase (NSE), chromogranins A and B, synaptophysin, and carcinoembryonic antigen (CEA). The stromal compartment surrounding follicles consist of fibroblasts derived from the neural crest (Kameda et al., 2009), and includes also macrophages and mast cells, which recently were reported to have a role in thyroid cancer development (Visciano et al., 2015).

p0070 Vascular supply of the thyroid gland is conspicuous, bilaterally represented by the superior thyroidal artery (from the external carotid) and inferior thyroid artery (from the subclavia). Exceptionally, another artery, the thyroid IMA artery (also known as Neubauer's artery), originating from either the common carotid or the anonymous truncus, may be present (Mohebbati and Shaha, 2012). The thyroid contains a rich network of capillaries surrounding follicles. Venous blood drains through two sets of vessels: superior and medial thyroidal veins realize a plexus, which drains into the external jugular vein, whereas inferior thyroidal veins

realize a plexus in front of the trachea joining the brachiocephalic vein (Mohebati and Shaha, 2012).

p0075 A rich lymphatic network is present in the thyroid. Intraglandular and subcapsular lymphatics drain into the internal jugular

lymph nodes. In particular, the superior lymph node group drains the upper gland and medial isthmus, while the inferior group

drains the lower gland.

p0080 The thyroid nerves originate from the superior and middle cervical sympathetic ganglia.

These fibers are vasomotor, indirectly

influencing thyroid secretion (Mohebati and Shaha, 2012). Moreover, adrenergic fibers realize a network, which ends near the

follicular basement membrane; adrenergic receptors are also present in follicular cells. . (Benvenga., 2018)

1.5. Synthesis of thyroid hormones:

1.5.1. Paraphrased Version:

The production and storage of thyroid hormones occur at the interface between follicular cells and the colloid. Follicle activity varies: less active follicles have cuboidal cells, while active ones contain columnar cells.

Hormone synthesis involves several steps. First, iodide is oxidized to active iodine by hydrogen peroxide, a reaction catalyzed by thyroid peroxidase (TPO), a heme-containing enzyme. Iodine is then transported across the follicular cell's apical surface via the same active mechanism used at the basal surface.

At the apical-colloid border, iodine is rapidly attached to tyrosine residues on thyroglobulin, a large glycoprotein (molecular weight ~650,000) synthesized in follicular cells. Thyroglobulin contains approximately 140 tyrosine residues, of which about one-quarter can be iodinated. After iodination, thyroglobulin is stored in the colloid, where a TPO-catalyzed coupling reaction occurs between pairs of iodinated tyrosines. The coupling of two di-iodotyrosine (DIT) residues forms thyroxine (T₄), while coupling of DIT with mono-iodotyrosine (MIT) forms tri-iodothyronine (T₃). This coupling can happen within a single thyroglobulin molecule or between dimerized molecules.

Thyroid hormones remain stored in this form until thyroglobulin is taken back into follicular cells,} Fig. 4{ a process stimulated by TSH. Thyroglobulin droplets are internalized via pinocytosis and fuse with lysosomes, leading to thyroglobulin hydrolysis and release of T3 and T4. Before secretion, about 10% of T4 undergoes monodeiodination to T3, and the released iodine is recycled.

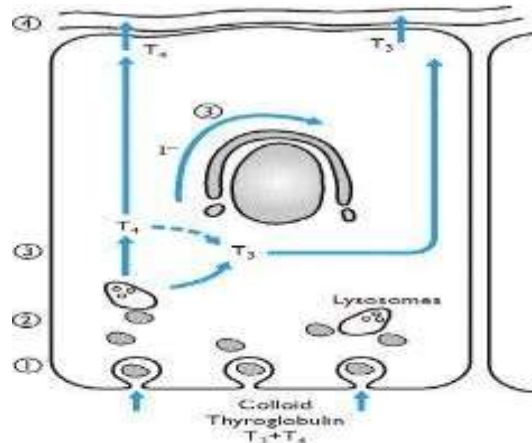


Figure 5 Secretion of thyroid hormones. (Boron& Boulpaep., 2017)

1. Under the influence of TSH, colloid droplets consisting of thyroid hormones within the thyroglobulin molecules are taken back up into the follicular cells by pinocytosis.

2. Fusion of colloid droplets with lysosomes causes hydrolysis of thyroglobulin and release of T3 and T4.

3. About 10% of T4 undergoes mono-deiodination to T3 before it is secreted. The released iodide is reutilized. Several-fold more iodide is reused than is taken from the blood each day but in states of iodide excess there is loss from the thyroid.

4. On average approximately 100 µg T4 and about 10 µg T3 are secreted per day.

Daily thyroid secretion is roughly 100 µg, mostly as T4, with about 10% as T3. In the liver and kidney, 80% of T4 is peripherally converted to the more active T3 (ten times more potent than T4) or to inactive reverse T3 (rT3). Trace amounts of other iodinated molecules (e.g., MIT, DIT, and thyroglobulin) are detectable in the circulation. Since this thyroglobulin originates from normal secretion, its serum measurement is clinically useful—for example, to assess endogenous thyroid secretion in patients on oral T4 replacement. (Boron& Boulpaep., 2017)

1.6. Metabolic Effects of Thyroid Hormones

1.6.1. Calorigenic Effect and Thermogenesis

The major effect of thyroid hormones is calorigenic (thermogenic), mediated by the uncoupling of oxidative phosphorylation.

This action is exerted on virtually every cell of the body.

1.6.2 Basal Metabolic Rate (BMR)

Thyroid hormones, particularly thyroxine (T₄), significantly increase the basal metabolic rate by enhancing overall cellular metabolism.

1.6.3. Protein Metabolism

The earliest effect of T₄ is the stimulation of RNA synthesis, leading to a consequent increase in protein synthesis (anabolic effect).

At higher concentrations, T₃ induces protein catabolism, resulting in a negative nitrogen balance.

1.6.4. Body Weight

A prominent clinical feature of hyperthyroidism is noticeable loss of body weight, reflecting the overall catabolic state.

1.6.5. Carbohydrate Metabolism

- Gluconeogenesis and carbohydrate oxidation are both increased.
- The glucose tolerance test typically reveals rapid intestinal absorption of glucose.

1.6.6. Lipid Metabolism

- Fatty acid metabolism is enhanced.
- Cholesterol degradation is significantly increased, leading to a marked decrease in blood cholesterol levels — a characteristic hallmark of hyperthyroidism.

1.6.7. Additional Systemic Activities

- Cardiovascular effects: Increased cardiac output and elevated systolic blood pressure.
- Gastrointestinal effects: Enhanced gastric motility.
- Muscular effects: Elevated oxygen consumption by skeletal muscles, ultimately contributing to muscular weakness.
- Sympathetic potentiation: Thyroid hormones increase the sensitivity of target tissues to catecholamines, thereby potentiating lipolysis, glycogenolysis, and gluconeogenesis. (Mustansiriyah University., 2019).

1.7. Thyroid Hormone Metabolism:

The primary mechanism for inactivating thyroid hormone is deiodination, alongside conjugation with glucuronic acid or sulfates. Approximately 85% of T₄ and 50% of T₃ undergo deiodination in tissues including the thyroid, pituitary, kidney, liver, and placenta. Three deiodinase types exist:

Type I (inhibited by propylthiouracil, PTU, acting on both rings), and Types II and III (PTU-insensitive, targeting only the outer or inner ring, respectively).

Type I deiodinase contains selenocysteine, a non-standard amino acid essential for its function. Biochemically, this is notable as an example of incorporating an amino acid beyond the usual 20; such incorporation is a goal for advanced protein studies. Clinically, selenium deficiency reduces Type I activity, altering thyroid hormone metabolism.

Outer ring 5'-deiodination of T₄ generates the more active T₃, primarily via Type I in peripheral tissues and via PTU-insensitive Type II in the pituitary. Inner ring 5-deiodination of T₄ produces inactive reverse T₃ (rT₃, 3,3',5'-triiodothyronine). Deiodination of T₃ also yields inactive products. The released iodine may be recycled by the thyroid or excreted.

Peripheral deiodination helps tissues adjust their response to thyroid hormone. During starvation, T₄ remains stable, but T₃ falls and rT₃ rises, likely preventing excessive muscle protein breakdown. Drugs inducing hepatic enzymes can accelerate thyroid hormone metabolism. Normally, the thyroid compensates by increasing hormone production, but in thyroid insufficiency, enhanced hepatic metabolism may cause problems or lead to chronic thyroid stimulation and potential neoplasia. (Brandt, D. n.d.).

1.8. Regulation of Thyroid Hormone Secretion

1.8.1 Regulation of Thyroid Hormone Release

The principal regulator of thyroid function is Thyroid Stimulating Hormone (TSH), a glycoprotein belonging to the hCG/LH/FSH family. TSH promotes thyroid hormone secretion by stimulating several processes, which are categorized into four temporal groups:

1.8.1.1. Short-term (minutes): Enhanced phagocytosis and processing of iodinated thyroglobulin (iodo-TG) into T₃ and T₄.

1.8.1.2. Intermediate-term (hours): Increased iodide uptake and activation, elevated hydrogen peroxide production, enhanced thyroglobulin (TG) synthesis and transport, a higher T₃:T₄ ratio, and greater iodide recovery from free mono- and diiodotyrosine via thyroid deiodinases.

1.8.1.3. Long-term (days to weeks): Thyroid hypertrophy and hyperplasia.

1.8.1.4. Lifetime effects: TSH is necessary to prevent thyroid atrophy.

TSH actions are mediated through cAMP and phosphatidyl inositol hydrolysis. TSH-induced TG transcription requires protein synthesis, indicating a secondary transcriptional event following the production of a transcription factor. In contrast, the TSH-stimulated increase in thyroperoxidase (TPO) transcription occurs rapidly without the need for protein synthesis,

suggesting a direct cAMP-mediated effect on the TPO gene. The specific proteins phosphorylated by kinases activated by TSH-generated second messengers remain unknown. Under normal physiological conditions, TG gene expression is already near maximum, so additional TSH stimulation has little effect. However, a drop in TSH below normal levels leads to a marked decline in TG synthesis.

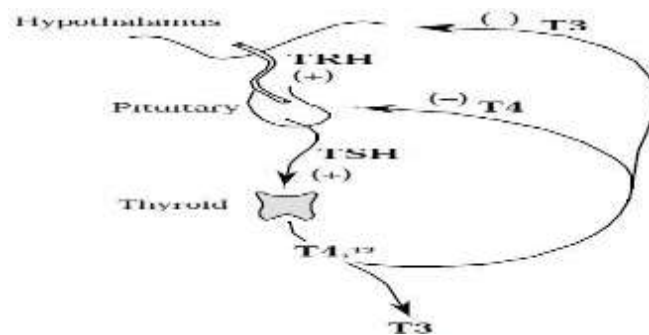


Figure 6 Control of thyroid hormone secretion. (Brandt, D. n.d.).

TSH is secreted by the pituitary gland in response to TRH (thyrotropin-releasing hormone), which is produced in the hypothalamus. The central nervous system contributes to assessing the need for increased thyroid hormone production, although this mechanism remains poorly understood. Elevated levels of T3—generated through the peripheral conversion of T4 to T3—reduce the rate of TRH gene expression.

Unlike the regulation of TRH secretion by T3, the suppression of pituitary TSH release is primarily governed by high circulating levels of T4. The pituitary possesses the ability to trap T4; even though T3 is the primary form that binds to the thyroid hormone receptor on thyrotropes, the pituitary mainly responds to T4 due to its active 5'-deiodinase enzyme. This hypothalamus-pituitary-thyroid axis represents another classic example of a closed negative feedback loop system controlling hormone release. (Brandt, D. n.d.).

1.9. Hyperthyroid and Hypothyroid Disorders

1.9.1. Hyperthyroid Disorders:

Hyperthyroidism, also known as thyrotoxicosis, arises from elevated levels of free T3 and T4. It presents with nervousness, hyperactivity, restlessness, tachycardia, cardiac arrhythmia, and weight loss driven by an increased basal metabolic rate (BMR). Certain symptoms are linked to heightened catecholamine sensitivity. Individuals with hyperthyroidism often experience poor exercise tolerance, partly due to cardiovascular changes and, in severe cases,

thyrotoxic myopathy—characterized by muscle weakness and atrophy. Persistently high thyroid hormone levels raise the risk of myocardial infarction, osteoporosis, and may increase insulin requirements in diabetic patients. Multiple underlying causes can lead to hyperthyroidism.

1.9.2. Hypothyroid Disorders

Hypothyroidism is marked by mental and physical slowness, cold dry skin, mild weight gain (though usually not obesity), and a reduced BMR. It also diminishes cardiac function, potentially progressing to congestive heart failure. This condition can cause hypertension and hypercholesterolemia, thereby increasing the risk of atherosclerosis. Treatment typically involves addressing the underlying cause and supplementing with exogenous thyroid hormone. Poor adherence, especially among adolescents, may result in developmental abnormalities. Severe non-thyroid illness can lower T3 levels to values resembling hypothyroidism—a phenomenon called euthyroid sick syndrome. In most such cases, patients are not truly hypothyroid, and thyroid hormone levels normalize once the underlying illness resolves. (Brandt, D. n.d.).

1.10. Antithyroid Drugs

1.10.1. Methimazole :

Methimazole is approximately ten times more potent than propylthiouracil (PTU). Its mechanism of action involves inhibiting thyroid hormone synthesis by blocking thyroid peroxidase (TPO), also known as iodide peroxidase or thyroperoxidase.

TPO catalyzes two key steps in thyroid hormone synthesis (Molina, 2018):

1. It oxidizes iodide ions (I^-) to reactive iodine (I^+), which then covalently binds to carbon 3 or carbon 5 of tyrosine residues on thyroglobulin, producing moniodinated tyrosine (MIT) and diiodinated tyrosine (DIT).
2. It facilitates the coupling of MIT and DIT to form the active thyroid hormones triiodothyronine (T3) and thyroxine (T4).see figure

Both methimazole and PTU inhibit TPO. However, unlike PTU, methimazole does not effectively block peripheral deiodinase D1 (the enzyme that converts T4 to T3). (Tulane University, 2021).

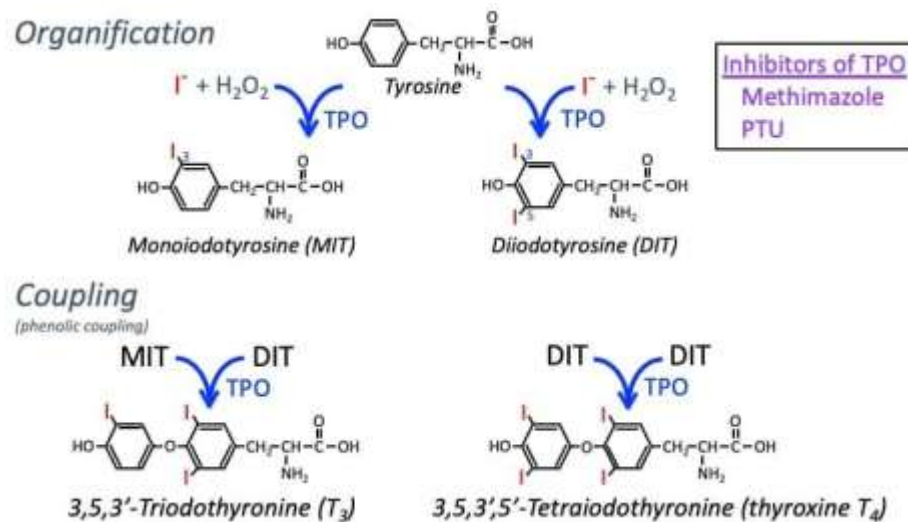


Figure 7 The role of thyroperoxidase (TPO) in the synthesis of T_3 & T_4 . Methimazole and propylthiouracil inhibit TPO, resulting in decreased synthesis of thyroid hormone by inhibiting both organification (incorporation of iodine) and coupling (conjugation) steps.

1.10.2 Propylthiouracil (PTU):

PTU inhibits the production of new thyroid hormone within the thyroid gland. It acts by blocking thyroid peroxidase, thereby preventing the conversion of iodide to an active iodine molecule and its incorporation into tyrosine residues. Consequently, DIT and MIT are not produced, interrupting the synthesis of T_4 and T_3 . In addition to its intrathyroidal effects, PTU acts peripherally by inhibiting the conversion of T_4 to T_3 . PTU affects both stored thyroid hormones within the gland and those circulating in the bloodstream. (Amisha & Rehman, 2023).

Chapter02: ferrocene

Introduction:

Ferrocene ($\text{Fe}(\eta^5\text{-C}_5\text{H}_5)_2$) is a well-known organometallic compound characterized by its unique “sandwich” structure, in which an iron atom is bound between two cyclopentadienyl rings. Since its discovery in 1951, it has attracted major scientific interest due to its exceptional thermal stability, aromaticity, and reversible redox properties (Kealy & Pauson, 1951).

One of the most important features of ferrocene is its electrochemical behavior, allowing reversible oxidation between Fe(II) and Fe(III) states. This redox activity plays a key role in its biological relevance, as it can participate in electron transfer processes and contribute to the generation of reactive oxygen species (ROS), which are often associated with biological activity and cytotoxic effects (Hill & Sadler, 2018).

In medicinal chemistry, ferrocene has emerged as a valuable pharmacophore. Its incorporation into organic molecules enhances lipophilicity, stability, and biological activity, making ferrocene-based compounds promising candidates for drug development. Numerous studies have shown that ferrocene derivatives exhibit a wide range of pharmacological activities, including anticancer, antimicrobial, antiparasitic, and anti-inflammatory effects (Astruc, 2017).

A key advantage of ferrocene is its structural flexibility, which allows easy functionalization on one or both cyclopentadienyl rings. This property enables the design of hybrid molecules with improved pharmacological profiles compared to their purely organic counterparts. A well-known example is ferrocifen (ferrocene–tamoxifen derivative), which has demonstrated enhanced anticancer activity, highlighting the potential of ferrocene in drug optimization (Patra & Gasser, 2012).

Due to these unique physicochemical and biological properties, ferrocene continues to be extensively explored in the development of new therapeutic agents. In this context, the present work focuses on the synthesis, characterization, and pharmacological evaluation of novel ferrocene-based compounds in order to investigate their potential biological activities

2.1. Definition of Ferrocene:

Bis-cyclopentadienyl iron, commonly known as ferrocene, is an organometallic compound with the formula $(\eta^5\text{-C}_5\text{H}_5)_2\text{Fe}$. In this notation, η^5 indicates that all five carbon atoms of each C_5H_5 ring are coordinated to the iron cation. Ferrocene represents the prototypical metallocene, a class of organometallic compounds characterized by two cyclopentadienyl rings bound on opposite sides of a central metal cation. These compounds are commonly referred to as

“sandwich compounds.” The rapid development of organometallic chemistry is often attributed to the significant interest generated by the discovery of ferrocene and its numerous analogues. Ferrocene was first intentionally synthesized in 1951 by Pauson and Kealy at Duquesne University. They investigated the reaction between cyclopentadienyl magnesium bromide and ferric chloride, aiming to synthesize fulvalene via a Grignard reagent. However, instead of the expected product, they obtained a highly stable yellow-orange powder, later identified as ferrocene. This remarkable stability was attributed to the aromatic character of the cyclopentadienyl rings [1]. Figure 1.1 illustrates their accidental synthetic route to ferrocene.

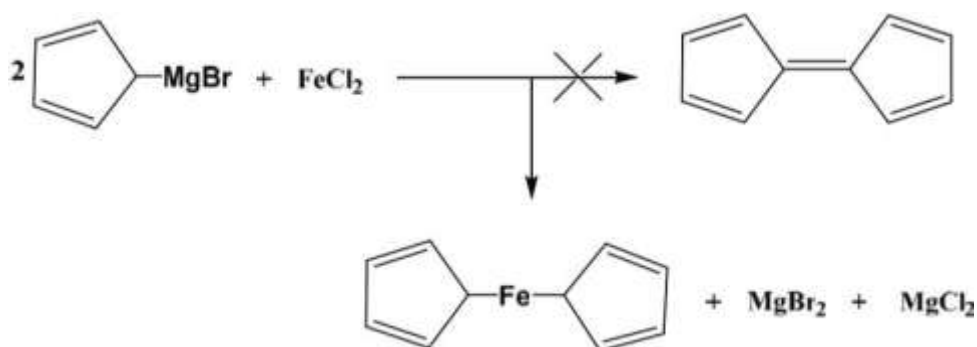


Figure 8Intentionally Pauson’s and Kealy’s synthetic route to ferrocene. (El Hafnaoui.,2021).

Miller and his co-workers at British Oxygen independently discovered ferrocene while investigating the preparation of amines from hydrocarbons and nitrogen. In their study, cyclopentadiene was reacted with nitrogen at 300 °C under atmospheric pressure, following a modified version of the Haber process. Although their findings were published in 1952, the experimental work had been conducted approximately three years earlier [2].

The remarkable stability of this newly identified organometallic compound was attributed to the aromatic character of the negatively charged cyclopentadienide anions. However, they did not recognize the η^5 “sandwich structure,” which was later established as the correct structural description of ferrocene. (El Hafnaoui.,2021).

2.2. Structure, Properties, and Reactivity

2.2.1. Structure

The correct molecular structure of ferrocene was established by Woodward and Wilkinson at Imperial College London. Their structural elucidation was based on the observation that ferrocene undergoes reactions characteristic of aromatic compounds such as benzene [3]. Independently, Fischer at the Technical University of Munich reached the same conclusion regarding the “sandwich structure” and subsequently extended this work by synthesizing other metallocenes, including nickelocene and cobaltocene [4,5].

Further confirmation of the ferrocene structure was provided by Eiland and co-workers through X-ray crystallography, followed by nuclear magnetic resonance (NMR) spectroscopy studies [6].

In the solid state, the two cyclopentadienyl rings adopt co-parallel arrangements with different relative orientations. In contrast, in the gas phase, the rings are typically found in an eclipsed conformation (Figure 1.2). This behavior is attributed to the low rotational energy barrier between the rings at ambient temperature, allowing rotation even in the solid state after crystallization. Figure 1.2(a) illustrates the initially proposed (incorrect) structure by Pauson and Kealy, whereas Figure 1.2(b) shows the corrected structure proposed by Woodward and Wilkinson.

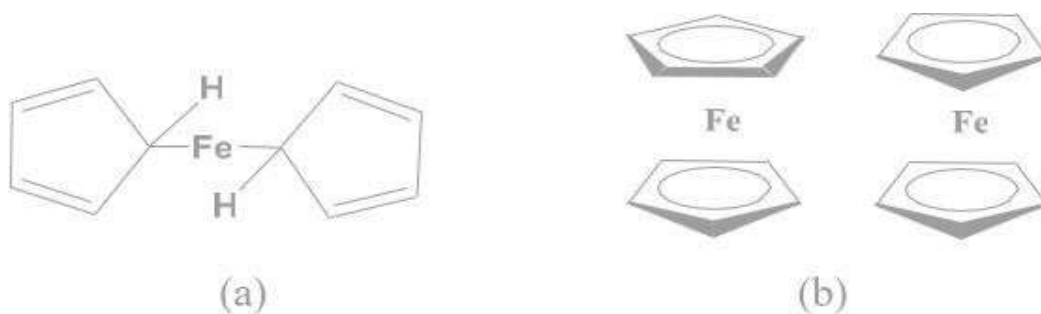


Figure 9 Wrong (a) and corrected (b) structures of ferrocene. (El Hafnaoui, 2021).

The two parallel cyclopentadienyl rings in ferrocene can adopt either an eclipsed conformation (D_{5h} symmetry) or a staggered conformation (D_{5d} symmetry). It has been established [7] that the staggered conformation (D_{5d} point group symmetry) predominates in the condensed phase [5,6,8,9], whereas the eclipsed conformation (D_{5h} symmetry) is more commonly observed in the gas phase [10–12]. The eclipsed form has also been detected in the solid state at 90 K [13] and in solution at room temperature [14].

Since most theoretical studies of ferrocene focus on the gas phase or solutions, the eclipsed (D_{5h}) structure has been more extensively investigated than the staggered (D_{5d}) conformer. This preference is largely due to the small energy difference between the two conformations. The conformational behavior of ferrocene is governed by the small energy difference between the eclipsed and staggered conformations, as well as the low rotational energy barrier about the Fe–C₅H₅ axis, which is approximately 4 kJ·mol⁻¹ [15,16]. As a result, the physicochemical properties of the staggered and eclipsed conformers do not differ significantly, owing to their closely related geometries.

Although the electronic state symmetries of the eclipsed conformation (D_{5h}) differ from those of the staggered conformation (D_{5d}), the latter is generally adopted in discussions of the electronic structure of ferrocene. This preference is due to the simplification it provides in symmetry matching between the molecular orbitals of the cyclopentadienyl rings and the atomic orbitals of the iron center.

In the ferrocene structure, each carbon atom of the two cyclopentadienyl anions possesses a p-orbital oriented perpendicular to the ring plane. These p-orbitals combine to form a set of ten molecular orbitals with different energy levels. When D_{5d} symmetry is considered, the presence of a center of symmetry at the iron atom leads to the formation of symmetric (g) and antisymmetric (u) combinations, which are occupied by the twelve π -electrons of the two cyclopentadienyl rings.

The molecular orbitals of the cyclopentadienyl ligands can be combined to generate eleven symmetry-adapted linear combinations, nine of which interact with the 3d, 4s, and 4p orbitals of the central iron cation to form bonding and antibonding molecular orbitals (Figure 9).

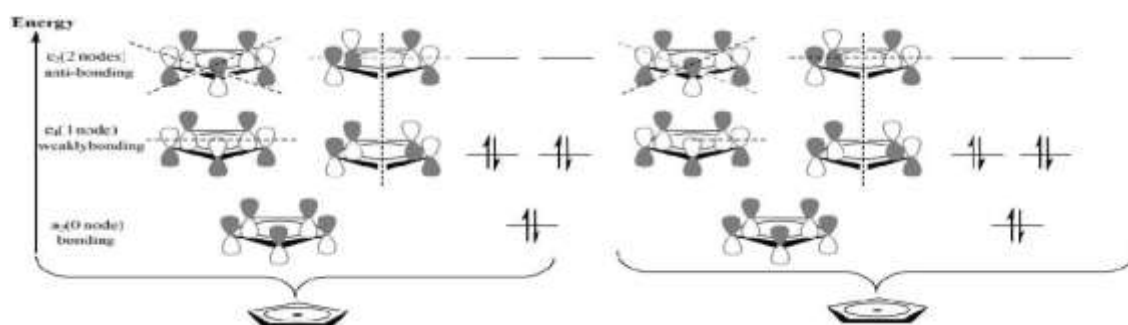


Figure 10 The π molecular orbitals of the cyclopentadienyl anions. (El Hafnaoui, 2021).

Each of the ten molecular orbitals derived from the cyclopentadienyl anions contributes to the formation of ten ligand molecular orbitals, which are distributed across three distinct energy levels.

Subsequently, these ligand molecular orbitals interact with the metal orbitals to generate the overall molecular orbital correlation diagram for $(C_5H_5)_2Fe$, as illustrated in Figure 10.

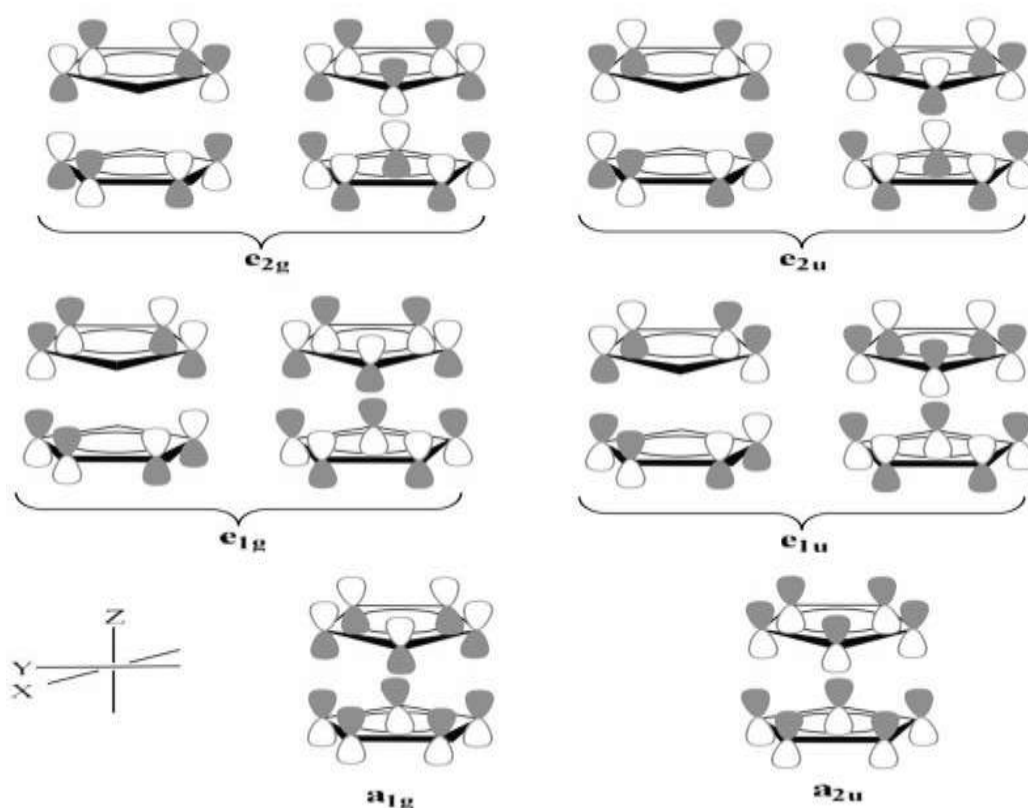


Figure 11 Symmetry-adapted linear combination of molecular orbitals for a $(\eta^5-C_5H_5)_2Fe$. (El Hafnaoui, 2021).

The five atomic p-orbitals of each cyclopentadienyl ligand combine to produce ten ligand molecular orbitals distributed across three distinct energy levels, as shown on the left side of Figure 1.5. These orbitals further interact with the appropriate iron atomic orbitals to construct the complete molecular orbital diagram of ferrocene.

In this electronic configuration, the two cyclopentadienyl ligands collectively contribute ten electrons, while the iron center contributes eight electrons, resulting in an eighteen-electron system. These electrons are accommodated within the low-energy bonding molecular orbitals,

whereas the antibonding orbitals remain unoccupied. Consequently, the exceptional stability of the ferrocene molecule can be rationalized on the basis of molecular orbital theory.

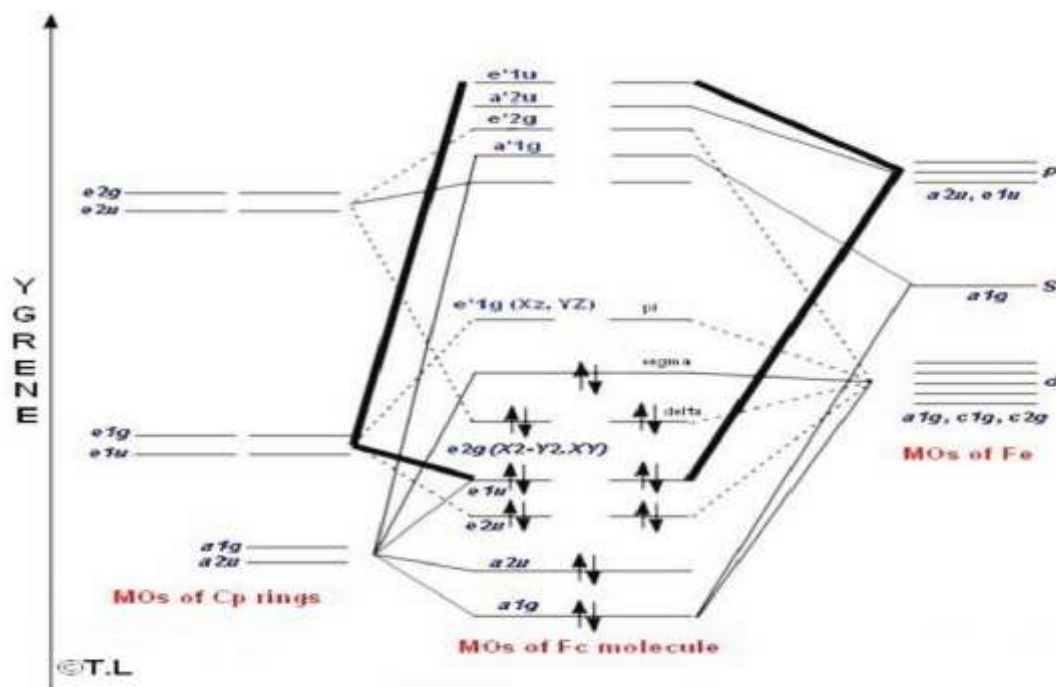


Figure 12 Molecular orbital of ferrocene molecule. (El Hafnaoui, 2021).

2.3. Properties

Ferrocene is obtained as an orange, diamagnetic solid with a melting point of 174 °C and the ability to sublime at temperatures above 100 °C. It exhibits remarkable aromaticity and high thermal stability. From a chemical standpoint, ferrocene behaves similarly to benzene and other aromatic compounds, undergoing typical reactions such as electrophilic substitution and acylation.

The infrared spectrum of ferrocene is relatively simple due to its highly symmetrical structure. A characteristic absorption band appears at 3075 cm^{-1} , corresponding to the stretching vibration of the aromatic C–H bond. In addition, four principal absorption bands are observed: those at 811 and 1002 cm^{-1} are associated with the bending (flexural) vibrations of C–H bonds, while the band at 1108 cm^{-1} corresponds to the antisymmetric vibration of the cyclopentadienyl ring. Another band at 1411 cm^{-1} is attributed to the antisymmetric C–C stretching of the unsubstituted cyclopentadienyl ring (Figure 12).

It has been reported that monosubstitution does not significantly affect the positions of these absorption bands, although a decrease in their intensity is observed. In contrast, when each

cyclopentadienyl ring carries at least one substituent, the absorption bands at 1002 and 1108 cm^{-1} disappear

No significant exceptions to this behavior have been reported to date. Rosenblum [17] formulated this observation as an empirical rule, known as the “rule of 9–10,” which states that ferrocene derivatives in which at least one cyclopentadienyl ring remains unsubstituted exhibit absorption bands at approximately 9.00 and 9.95 μm , whereas derivatives in which both rings are substituted do not display these absorptions.

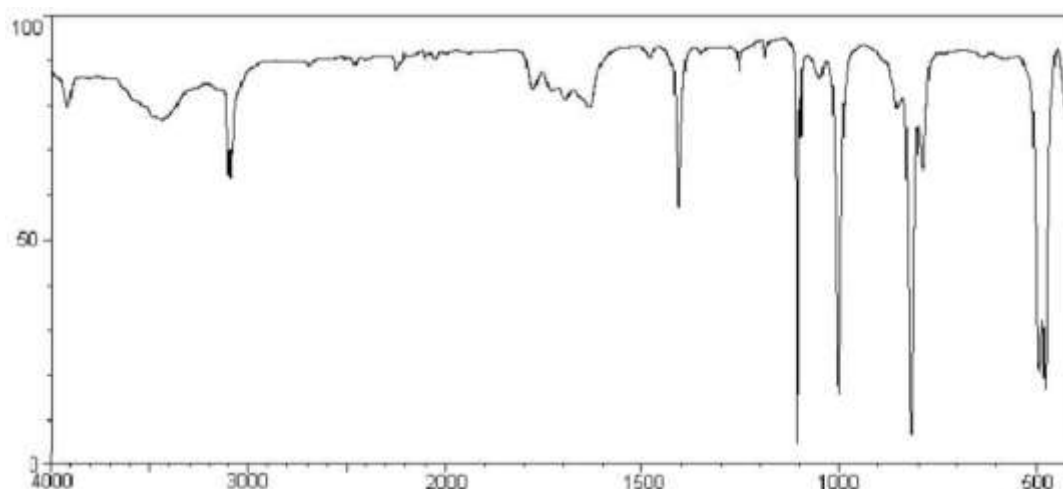


Figure 13 IR spectra of ferrocene. (El Hafnaoui, 2021).

Due to the presence of a single chemical environment for both hydrogen and carbon atoms in the ferrocene molecule, all ten hydrogen atoms and all ten carbon atoms are chemically equivalent. Consequently, the ^1H and ^{13}C NMR spectra of ferrocene each exhibit a single resonance signal, appearing at approximately 4.15 ppm and 68 ppm, respectively.

Reactivity

The strong similarity between ferrocene and benzene has played a key role in the development of ferrocene chemistry, enabling a wide range of substitution reactions. These reactions occur readily at the cyclopentadienyl rings, in a manner analogous to electrophilic substitution in benzene.

The mechanism of substitution on the cyclopentadienyl ring involves the direct formation of a σ -complex without direct participation of the metal center. Two principal mechanisms have been proposed [18]:

2.3.1. Mechanism (I):

This mechanism proceeds via a two-step pathway. The first step is rapid and involves the electrophile (E) cleaving the iron–carbon bond through an SE_2 reaction with retention of configuration. This is followed by a second, rate-limiting step involving proton removal from outside the molecule. Subsequently, an SE_2 inversion step occurs, as illustrated in Figure 13.

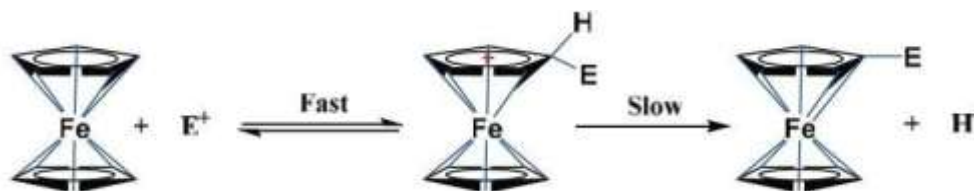


Figure 14 Mechanism (I) of substitution reactions on the pentadienyl ring (formation of a sigma complex). (El Hafnaoui.,2021).

2.3.2. Mechanism (II):

This mechanism involves a rate-determining electrophilic attack from the outside through an SE_2 displacement accompanied by inversion of configuration. This step is followed by the rapid elimination of a proton from within the molecule, with retention of configuration, as illustrated in Figure 14.

Fig. 14. Mechanism (II) of substitution reactions on the cyclopentadienyl ring (formation of a σ -complex)

The removal of one electron from the ferrocene molecule results in the oxidation of the iron center to the higher oxidation state Fe^{3+} , leading to the formation of salts containing the blue ferrocenium cation, $(C_5H_5)_2Fe^+$. Ferrocene can be readily oxidized to the ferrocenium cation by a wide range of oxidizing agents. This transformation may even occur upon exposure of a ferrocene solution to air under acidic conditions (low pH).

The positive charge of the ferrocenium cation reduces its susceptibility to electrophilic substitution, as it repels incoming electrophiles, particularly in the presence of mineral acids. Consequently, once oxidized, the ferrocene system becomes less reactive toward electrophilic attack [19,20].

The pronounced aromatic character and high reactivity of ferrocene have enabled the synthesis of a wide variety of its derivatives, many of which serve as key intermediates in organic synthesis [21,22]. These derivatives are generally easy to prepare and exhibit sufficient reactivity to facilitate the synthesis of novel organic compounds that are otherwise difficult to obtain through conventional synthetic routes.

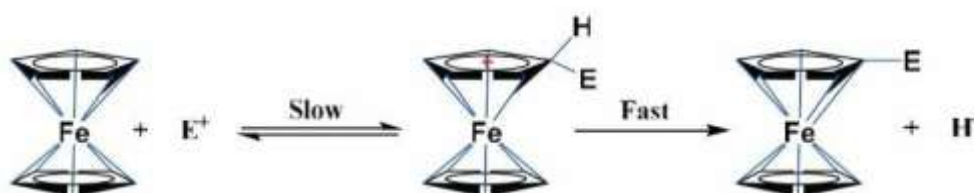


Figure 15 Mechanism (II) of substitution reactions on the pentadienyl ring (formation of a sigma complex). (El Hafnaoui, 2021).

The removal of one electron from the ferrocene molecule results in the oxidation of the iron center to the higher oxidation state Fe^{3+} , leading to the formation of salts containing the blue ferrocenium cation, $(\text{C}_5\text{H}_5)_2\text{Fe}^+$. Ferrocene can be readily oxidized to the ferrocenium cation by a wide range of oxidizing agents. This transformation may even occur upon exposure of a ferrocene solution to air under acidic conditions (low pH).

The positive charge of the ferrocenium cation reduces its susceptibility to electrophilic substitution, as it repels incoming electrophiles, particularly in the presence of mineral acids. Consequently, once oxidized, the ferrocene system becomes less reactive toward electrophilic attack [19,20].

The pronounced aromatic character and high reactivity of ferrocene have enabled the synthesis of a wide variety of its derivatives, many of which serve as key intermediates in organic synthesis [21,22]. These derivatives are generally easy to prepare and exhibit sufficient reactivity to facilitate the synthesis of novel organic compounds that are otherwise difficult to obtain through conventional synthetic routes.

The well-known quaternary salt ferrocenylmethyltrimethylammonium iodide is widely used as a starting material for the synthesis of numerous mono-substituted ferrocene derivatives. This compound is typically prepared via the aminomethylation of ferrocene [23], as illustrated in Figure 15

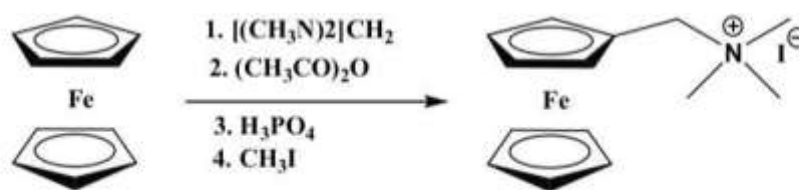


Figure 16 Synthetic route of ferrocenylmethyltrimethylammonium iodide. (El Hafnaoui.,2021).

The trimethylammonium group in ferrocenylmethyltrimethylammonium iodide is readily displaced by a broad range of nucleophiles, including alkoxide ions, cyanide anions, Grignard reagents, carbanionic species, and amines. In the present work, ferrocenylmethyltrimethylammonium iodide was synthesized and subsequently employed as a key intermediate for the preparation of four N-ferrocenylmethylnucleobases and two N-ferrocenylmethylacetophenones. (El Hafnaoui.,2021).

1.1.1.2. Ferrocene in Medicinal Chemistry

At the time of its discovery, ferrocene was not associated with any medicinal applications. However, in recent years, ferrocene and its derivatives have gained significant attention in medicinal chemistry. Their incorporation into bioorganometallic compounds has led to the emergence of a new research field known as bioorganometallic chemistry.

This field has experienced rapid growth over the past two decades, focusing on the synthesis of novel ferrocene derivatives and the evaluation of their biological and pharmacological activities against various diseases, including cancer and malaria. The increasing interest in these compounds is largely attributed to the inherent properties of ferrocene, such as its high stability, low toxicity, and favorable redox behavior.

This section highlights ferrocenyl compounds that have been biologically evaluated using different experimental approaches. The promising results obtained from several studies have attracted considerable attention from researchers in the field of medicinal chemistry.

Numerous ferrocene derivatives have demonstrated a wide range of biological activities, including antioxidant [24–26], cytotoxic [27–29], antitumor [30–32], antimalarial [33], antifungal [34], and DNA-cleaving properties [35]

Ferrocene derivatives have also been investigated for their potential role in the regulation of the HIV virus, which is responsible for AIDS [36].

Due to the notable resistance of ferrocene to oxidation under atmospheric conditions, it has been proposed that synthetic ferrocenyl compounds may represent a novel class of potential antioxidant agents. In such systems, the introduction of additional organic substituents can enhance the intrinsic antioxidant activity of ferrocene. In particular, the formation of extended conjugated systems between the ferrocenyl moiety and other organic groups may significantly improve electron delocalization and, consequently, antioxidant efficiency.

Accordingly, synthetic ferrocenyl compounds can be broadly classified into nonconjugated and conjugated derivatives. In nonconjugated ferrocenyl compounds, alkyl chains or simple functional groups are attached to one or both cyclopentadienyl rings, generating new molecules containing the ferrocenyl unit. The primary objective of this modification is to enhance the *in vivo* antioxidant activity of ferrocene.

In contrast, conjugated ferrocenyl derivatives are designed by introducing substituents capable of forming a conjugated system with the cyclopentadienyl rings. Such extended π -systems facilitate the delocalization of single electrons across the entire molecule during radical formation, thereby enhancing the overall antioxidant activity [37]. Representative examples of ferrocenyl compounds exhibiting antioxidant properties are shown in Figure 16. (El Hafnaoui.,2021).

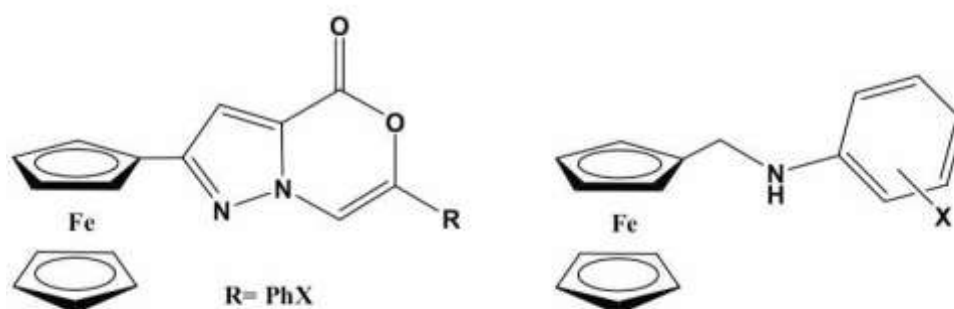


Figure 17Example of ferrocenyl compounds possessing antioxidant activity (X = NO₂, CN). (El Hafnaoui.,2021).

recently emerged as a significant class of antiproliferative agents [38]. The [3]ferrocenophane structure consists of a ferrocene unit in which the two cyclopentadienyl rings are interconnected by a three-carbon bridge. Compounds incorporating this structural motif have demonstrated notable cytotoxic activity. Figure 17.

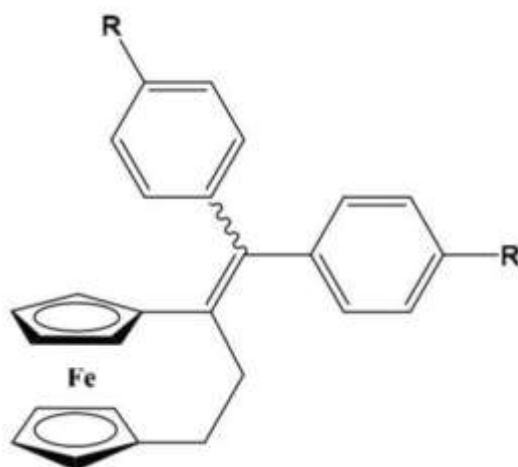


Figure 18 Compounds based on the 1-(diphenylmethylidene)-[3]ferrocenophane skeleton that show high cytotoxicity against hormone-independent breast and prostate cancer cells ($R = OH, NH_2$). (El Hafnaoui.,2021).

Among the various ferrocene derivatives investigated for therapeutic applications, two of the most prominent are ferroquine and ferrocifen, which have been extensively studied for the treatment of malaria and cancer, respectively (Figure 18). These compounds were first developed in the 1990s and have since attracted considerable interest due to their promising pharmacological profiles.

The presence of the ferrocenyl moiety in these molecules plays a crucial role in their mechanism of action, introducing metal-specific pathways that enhance their overall therapeutic efficacy. Ferroquine is currently undergoing phase II clinical trials, whereas ferrocifen remains under preclinical evaluation.

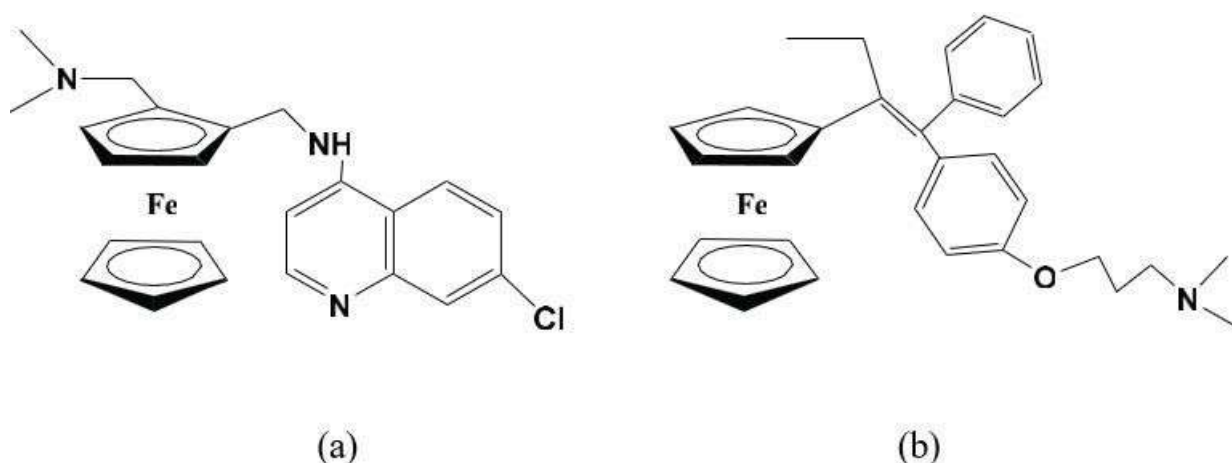


Figure 19 Chemical structure of the anti-malarial ferroquine (a) and the anticancer ferrocifen (b) . (El Hafnaoui.,2021).

Recent studies have also demonstrated the involvement of topoisomerase enzymes in the HIV replication cycle. Viruses rely on host cellular machinery for replication and survival, and inhibition of key enzymes such as topoisomerase represents a viable therapeutic strategy. In this context, ferrocene derivatives have shown significant potential as inhibitors of topoisomerase activity, thereby interfering with HIV replication [39].

Furthermore, several ferrocene-containing compounds have exhibited promising pharmacological properties, highlighting their potential as candidates for the development of novel therapeutic agents

Promising results have also been reported for certain ferrocene derivatives as anti-HIV agents. These compounds are believed to inhibit viral DNA synthesis, thereby interfering with the replication process of the virus. An example of a ferrocene derivative exhibiting anti-HIV activity is presented in Figure 19.

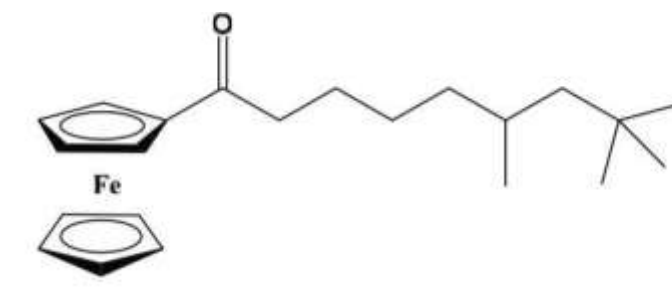


Figure 20Chemical structure of a ferrocene derivative with anti-HIV activity. (El Hafnaoui,2021).

1.1.1.3. Ferrocenylnucleobases

Ferrocenylnucleobases represent an increasingly important class of compounds with potential applications in various fields, including biology, pharmacy, and materials science.

The first ferrocenyl-nucleobases were synthesized by Chen in 1980 through the reaction of 6-chloropurine with ferrocenylmethylamine in methoxyethanol [41]. This reaction yielded N⁶-ferrocenyladenine, along with the concomitant formation of N⁶,9-bis(ferrocenylmethyl)adenine, as illustrated in Figure 20.

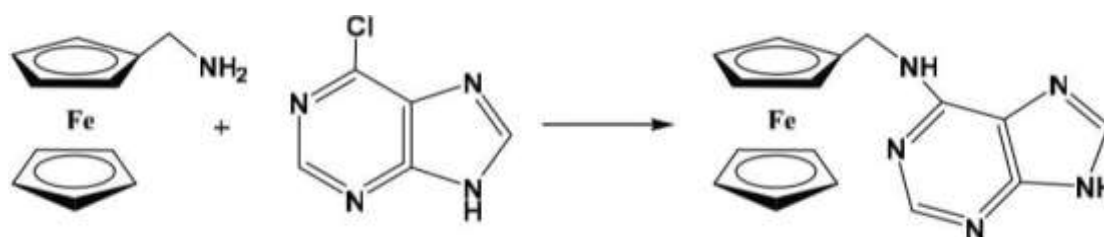


Figure 21 Synthetic route to N6,9-bis(ferrocenylmethyl)adenine. (El Hafnaoui.,2021).

In recent years, numerous ferrocenyl-nucleobases have been synthesized and characterized, and their biological activities have been extensively evaluated [40–42]. (El Hafnaoui.,2021).

Experimental Work

1. Materials and Methods

1.1. In vivo study

1.1.1. Experimental Animals

All in vivo experiments were conducted in strict accordance with international guidelines for the care and use of laboratory animals. All procedures were approved by the Scientific Council Committee of the Faculty of Natural Sciences and Life, University of El Oued, and complied with institutional ethical standards. Male Wistar rats weighing approximately 180-220 g were used in this study. The animals were housed in standard polycarbonate cages under strictly controlled environmental conditions, including a regular 12-hour light/12-hour dark cycle, a constant temperature of (22 ± 2 °C), and a relative humidity ranging between 45-55%. Standard laboratory pellet diet and purified drinking water were provided ad libitum. Prior to the commencement of the experimental procedures, all animals were subjected to a one-week acclimatization period to minimize transport and environmental stress.(Elhewehy et al., 2025)

1.1.2. Chemicals and Reagents

Benzylthiouracil (BTU) and the standard drug Levothyroxine were purchased from Sigma-Aldrich. The bio-organometallic ferrocene derivatives (FMA, FCMphA, and FCMphB) were previously synthesized and fully characterized in our laboratories. Given their lipophilic nature, these derivatives were prepared for in vivo administration by suspending them in a biologically safe vehicle corn oil or 0.5% Carboxymethyl cellulose solution.

1.1.3. Experimental Design

A total of 30 rats were used in this study, which were randomly assigned into six equal experimental groups (n=5 per group). Treatments were administered daily via oral gavage throughout the experimental period, which lasted for 21 days. The experimental design was carried out as follows:

- **Control Group (Control):** Animals received only the vehicle without any active compounds, representing the normal physiological state.
- **Negative Group (CN):** Animals were administered BTU at a dose of 50 mg/kg/day to induce experimental hypothyroidism without receiving any subsequent treatment.
- **Positive Group (CP):** Animals received BTU (50 mg/kg/day) concurrently with the standard reference drug Levothyroxine at a dose of 10 µg/kg/day.

- **FMA Group:** Animals received BTU concurrently with the ferrocene derivative FMA at a dose of 10 mg/kg/day.
- **FCMphA Group:** Animals received BTU concurrently with the ferrocene derivative FCMphA at a dose of 10 mg/kg/day.
- **FCMphB Group:** Animals received BTU concurrently with the ferrocene derivative FCMphB at a dose of 10 mg/kg/day.

1.1.4. Evaluation of Physiological Parameters (Body and Organ Weights)

The body weight of the animals was monitored and recorded weekly and at the end of the experimental period. To evaluate the metabolic progression, the Weight Gain Index was calculated using the following formula:

Weight Gain Index:

$$\text{WGI} = [(W_f - W_i) / W_i] \times 100$$

At the end of the experiment, vital organs (liver and kidneys) were carefully excised and weighed precisely using a high-precision analytical balance. To assess for gross morphological abnormalities such as hypertrophy or atrophy, the Organ Weight Index was calculated as follows:

Organ Weight Index:

$$\text{OWI} = (O_a / W_f) \times 100$$

WGI (Weight Gain Index): Weight Gain Index

OWI (Organ Weight Index): Organ Weight Index

W_f (Final Body Weight): Final body weight

W_i (Initial Body Weight): Initial body weight

O_a (Absolute Organ Weight): Absolute organ weight

1.1.5. Blood and Tissue Sampling

At the end of the treatment period, all animals were fasted for 12 hours with free access to water. They were then deeply anesthetized, and blood samples were collected via cardiac puncture or the portal vein. The collected blood was immediately divided into two portions. The first portion was placed in tubes containing Ethylenediaminetetraacetic acid (EDTA) for hematological profiling. The second portion was placed in plain tubes, allowed to clot at room temperature for 20 minutes, and subsequently centrifuged at 3000 rpm for 10 minutes at 4 °C. Following blood collection, the liver, kidneys, and thyroid glands were rapidly fixed in 10%

neutral buffered formalin for histopathological examination.

1.1.6. Hematological, Biochemical, and Hormonal Analyses

Hematological parameters, including White Blood Cells (WBC), Lymphocytes (LYM), Granulocytes (GRA), Red Blood Cells (RBC), Hemoglobin (HGB), and Platelets (PLT), were measured using an automated hematology analyzer to assess systemic inflammatory and immune responses. Serum concentrations of Fasting Blood Sugar (FBS), Aspartate

Aminotransferase (AST), Alanine Aminotransferase (ALT), Urea, and Creatinine were quantified using commercial diagnostic kits read by a UV-Vis spectrophotometer to evaluate metabolic, hepatic, and renal functions. For the assessment of thyroid function, serum levels of Thyroxine (T4), Triiodothyronine (T3), and Thyroid-Stimulating Hormone (TSH) were measured utilizing specific competitive and sandwich ELISA techniques, following the manufacturer's instructions. The absorbance was read at 450 nm using a microplate reader.

1.1.7. Tissue Homogenate Preparation and Oxidative Stress Evaluation

Specific portions of liver and kidney tissues were accurately weighed and homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) using a mechanical tissue homogenizer to prepare a 10% (w/v) homogenate. The homogenates were then centrifuged at 10,000 rpm for 15 minutes at 4 °C to precipitate cellular debris. The resulting clear supernatant was exclusively used for the estimation of oxidative stress markers. Lipid peroxidation was evaluated by measuring Malondialdehyde (MDA) levels based on the thiobarbituric acid (TBA) reactivity assay. The non-enzymatic antioxidant, reduced Glutathione (GSH), was quantified using Ellman's reagent. Furthermore, the activities of primary antioxidant enzymes, specifically Superoxide Dismutase (SOD) and Catalase (CAT), were measured using standardized spectrophotometric colorimetric assays.

1.1.8. Histopathological Examination

The formalin-fixed tissue samples (thyroid, liver, and kidney) were kept in 10% neutral buffered formalin for 48 hours to ensure complete tissue fixation. Subsequently, the tissues were processed through a graded series of ethanol solutions for dehydration, cleared in xylene, and embedded in paraffin wax blocks. Thin tissue sections of 4-5 µm thickness were cut using a rotary microtome. The sections were mounted on glass slides and stained with routine Hematoxylin and Eosin (H&E) dye to visualize cellular details and morphological architecture. The stained slides were examined for structural, degenerative, and inflammatory changes by a qualified histopathologist using a light microscope equipped with a digital camera at a magnification of (40×).

1.2. In Silico Study

1.2.1. Ligand Preparation

The ferrocene-based derivatives (FMA, FCMphA, and FCMphB) were selected as ligands for the present in silico investigation. Their chemical structures were initially drawn using

ChemDraw software to ensure an accurate representation of the organometallic scaffold and the associated functional groups.

The generated two-dimensional (2D) structures were subsequently converted into three-dimensional (3D) conformations using Open Babel software(O’Boyle et al., 2011). This conversion step was performed to obtain spatially realistic molecular geometries suitable for computational analysis.

The resulting 3D structures were further subjected to geometry optimization and energy minimization using the Merck Molecular Force Field (MMFF94) implemented in Avogadro software (version 1.2.0). This procedure was applied to achieve energetically stable conformations and to eliminate any unfavorable steric interactions(Hanwell et al., 2012).

During structural preparation, careful verification was carried out to ensure correct atom connectivity, valence assignment, and stereochemical integrity. Special attention was given to the ferrocene moiety, due to its sandwich-like structure composed of an iron center coordinated between two cyclopentadienyl rings, which significantly influences the electronic distribution and molecular geometry of the compounds(Astruc, 2017).

In addition, protonation states of the ligands were adjusted to physiological pH (7.4) in order to better simulate biological conditions, in accordance with standard computational pharmacokinetic protocols. A final energy minimization step was performed to ensure complete structural relaxation and stability prior to downstream analyses.

The optimized ligand structures were saved in PDB format and subsequently used for ADMET prediction and molecular docking studies.

1.2.2. ADMET and Drug-Likeness Prediction

The pharmacokinetic and toxicity properties of the synthesized ferrocene-based derivatives (FMA, FCMphA, and FCMphB) were predicted using in silico tools prior to molecular docking analysis.

ADMET parameters were evaluated using the web-based platforms SwissADME, ProTox-II, and admetSAR (Banerjee, Eckert, Schrey, & Preissner, 2018; Daina, Michielin, & Zoete, 2017a).The predicted absorption parameters included gastrointestinal (GI) absorption and

water solubility. Distribution properties such as blood–brain barrier (BBB) permeability and P-glycoprotein (P-gp) substrate status were also determined.

Metabolic properties were assessed by predicting the interaction of the compounds with major cytochrome P450 isoforms, including CYP3A4, CYP2D6, and CYP2C9. Toxicity parameters, including hepatotoxicity, mutagenicity (AMES), carcinogenicity, and acute oral toxicity (LD₅₀), were obtained from the respective prediction servers.

Drug-likeness was evaluated according to Lipinski's rule of five and Veber criteria (Lipinski, Lombardo, Dominy, & Feeney, 1997; Veber et al., 2002).

The obtained ADMET and drug-likeness profiles were used for the preliminary screening of the compounds.

1.2.3. Target Protein Selection and Preparation

The crystallographic structures of thyroid hormone receptor complexes with thyroxine (T4) and triiodothyronine (T3) were selected as molecular targets for the docking study. The corresponding three-dimensional (3D) structures were retrieved from the RCSB Protein Data Bank using PDB IDs **1Y0X** and **1XZX**, respectively (Berman et al., 2000).

Protein preparation was performed using AutoDockTools (ADT) version 1.5.7 following standard molecular docking procedures (Morris et al., 2009). Crystallographic water molecules, co-crystallized ligands, and non-essential heteroatoms were removed to avoid interference during ligand binding analysis.

Polar hydrogen atoms were then added to the protein structures to ensure proper hydrogen bond interactions, and Kollman partial charges were assigned to all atoms. Appropriate atom types were also defined to improve the accuracy of electrostatic calculations during docking simulations.

The prepared protein structures were converted into PDBQT format for compatibility with AutoDock-based docking software. The active binding sites were determined according to the positions of the co-crystallized ligands and reported functional residues described in the literature.

During docking simulations, the receptor structures were treated as rigid, whereas the ligand molecules (FMA, FCMphA, and FCMphB) were considered fully flexible to allow optimal ligand–receptor interaction.

1.2.4. Molecular Docking Protocol

Molecular docking simulations were performed to evaluate the binding affinity and interaction modes of the synthesized ferrocene-based derivatives (FMA, FCMphA, and FCMphB) toward the selected thyroid hormone receptor complexes, namely Thyroid Hormone Receptor bound to thyroxine (T4) and triiodothyronine (T3), corresponding to PDB IDs **1Y0X** and **1XZX**, respectively.

Docking calculations were carried out using AutoDock Vina software following standard molecular docking procedures (Trott & Olson, 2010). Both ligands and target proteins were previously prepared and converted into PDBQT format before docking analysis.

The docking grid box was centered on the active binding site of each receptor based on the position of the co-crystallized ligand present in the crystallographic structure. Grid dimensions were adjusted to adequately cover the entire binding pocket and allow sufficient conformational flexibility of the ligands during docking simulations.

The docking parameters were set to generate multiple binding poses for each ligand–receptor complex, and the most stable conformations were selected based on the lowest binding free energy values (kcal/mol). Lower binding energy values indicate stronger ligand–receptor interactions and higher binding stability.

The best docking conformations were further analyzed using Discovery Studio Visualizer and PyMOL software to investigate the molecular interactions between ligands and receptor active sites. Hydrogen bonds, hydrophobic interactions, van der Waals forces, and π – π interactions were identified and recorded to better understand the binding mechanism of the synthesized compounds.

The final docking results were represented using both two-dimensional interaction diagrams and three-dimensional binding mode visualizations to provide a comprehensive interpretation of ligand orientation and molecular recognition patterns.

1.3. Statistical Analysis

All quantitative data generated in this study were expressed as Mean $\pm$ Standard Deviation (Mean $\pm$ SD). Statistical analysis was performed using GraphPad Prism. To compare the differences among multiple experimental groups, a One-way Analysis of Variance (ANOVA) was conducted, followed by Tukey's post-hoc test to determine specific inter-group differences.

A probability value of less than 0.05 ($p < 0.05$) was considered statistically significant. Significance levels were denoted using letters and asterisks in the tabulated data to indicate comparisons with the normal control group and the untreated hypothyroid (CN) group, respectively.

2. Results and Discussion

1. In vivo study

1.1. Weight Gain Index

The results of the evaluation of body weight development showed the occurrence of a noticeable metabolic (metabolic) disorder resulting from the administration of the BTU compound. Before the start of treatment, all experimental groups recorded a significant increase ranging from $p < 0.05$ to $p < 0.001$ in the weight gain index compared to the control group (Control). This reflects the classical phenotypic pattern of hypothyroidism, which is characterized by a sharp decrease in the basal metabolic rate (Basal Metabolic Rate - BMR), weakness in the process of thermogenesis, and a decrease in lipolysis. Due to this disturbance in energy balance, the body tends to accumulate fat intensively. The persistence of this metabolic slowdown was confirmed in the negative group (CN) which did not receive treatment, as it maintained a markedly high weight index throughout the experimental period. These observations strongly agree with recent studies that confirm that deficiency in thyroid hormones reduces energy expenditure at rest and leads to a vicious cycle of weight gain and insulin resistance (Hajro et al., 2025) (Raveendran et al., 2025).

In contrast, the standard treatment using exogenous levothyroxine hormone (CP group) led to a clear decrease in the weight gain index, which restored the values to normal levels and even slightly lower than the control group (not statistically significant compared to control, but significant compared to the negative group CN). This is attributed to the ability of the replacement hormone to restore cellular metabolic activity and increase oxygen consumption (Harsini et al., 2024)

The most interesting and therapeutically promising aspect is the high effectiveness shown by ferrocene derivatives (FMA, FCMphA, and FCMphB) in inducing a fundamental normalization of body weight. The two compounds FMA and FCMphA showed the greatest degree of decrease in the weight gain index, as they reached values fully comparable to the drug levothyroxine (CP), which indicates that they possess a very strong regulatory effect on the metabolic process. On the other hand, the compound FCMphB showed a moderate decrease

but statistically significant ($p < 0.05$) compared to the group (CN), which highlights that the subtle structural differences between these derivatives (Structure-Activity Relationship) play a decisive role in the degree of biological effectiveness.

These results collectively indicate that ferrocene derivatives effectively counteract weight gain resulting from hypothyroidism. From a mechanistic point of view, ferrocene-based organometallic compounds are known for their lipophilicity, which facilitates their penetration of cellular membranes. It is likely that these derivatives act as metabolic modulators, as they contribute to restoring the metabolic pathways that are usually mediated by thyroid hormones, either through improving the redox status inside the cells thanks to the central iron atom, or through direct interaction with the enzymatic pathways responsible for lipid breakdown and energy production, which is consistent with modern trends that demonstrate the broad biological and metabolic activity of ferrocenes in therapeutic applications (Kovač et al., 2025)(Kalmegh et al., 2026)

Table 1 Weight Gain Index of different experimental groups.

	Weight Gain Index	
	Before treatment	After treatment
Control	10.3±0.4	10.8±0.7
CN	13.9±0.8 ^a	14.6±0.2 ^a
CP	14.4±0.3 ^{b NS}	9.6±0.8 ^{NS*}
FMA	15.6±0.6 ^{a NS}	10.1±0.4 ^{NS*}
FCMphA	16.9±0.7 ^{c NS}	9.3±0.6 ^{NS*}
FCMphB	14.2±0.8 ^{b NS}	11.1±0.3 ^{a*}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.2. Organ weight index

The organ weight index is considered a fundamental toxicological parameter used to determine gross damage of organs, or inflammation, or hypertrophy, or atrophy following in vivo exposure to foreign substances or as a result of underlying metabolic disorders. In the present study, the analysis of liver and kidney weight indices showed the absence of statistically significant differences ($p > 0.05$) between all experimental groups (Table 2). The administration of the BTU compound did not lead to the induction of major morphological changes that could be detected in these main metabolic and excretory organs, as evidenced by the absence of noticeable variation between the untreated hypothyroid group (CN) and the healthy control group. Likewise, the therapeutic intervention with both the standard levothyroxine drug (CP)

and the three synthesized ferrocene derivatives (FMA, FCMphA, and FCMphB) consistently maintained organ weight indices precisely within normal physiological ranges. This stability carries very high pharmacological importance; while the incorporation of transition metals into therapeutic structures can sometimes raise concerns about oxidative stress caused by heavy metals, or hepatotoxicity, or renal accumulation, our results strongly support the excellent biosafety and biocompatibility of the compounds FMA, FCMphA, and FCMphB *in vivo*. The absence of hepatomegaly or nephromegaly strongly indicates that these ferrocene-based compounds do not exert harmful toxic effects on the main filtration organs at metabolically effective doses. This observation is in good agreement with recent toxicological assessments that highlight the safety of carefully designed bio-organometallic derivatives (Cybulski, Michalak, Buchowicz, & Mazur, 2024a; Tomar, Kumar, Sharma, Joshi, & Nemiwal, 2025), in addition to standard *in vivo* protocols that confirm the validity of the stability of organ indices as a primary indicator of hepatic and renal safety (Cristian et al., 2024). As a result, these synthesized derivatives show a highly promising balance between deep metabolic regulation and systemic tolerability.

Table 2 Organ Weight Index of different experimental groups.

	Organ Weight Index %	
	Liver	Kidneys
Control	3.51±0.35	0.66±0.07
CN	3.64±0.43 ^{NS}	0.74±0.08 ^{NS}
CP	3.67±0.51 ^{NS}	0.67±0.09 ^{NS}
FMA	3.51 ±0.25 ^{NS}	0.63±0.05 ^{NS}
FCMphA	3.81±0.31 ^{NS}	0.66±0.06 ^{NS}
FCMphB	3.75±0.32 ^{NS}	0.61±0.06 ^{NS}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.3. Hematological parameters

The hematological analysis showed that hypothyroidism induced by BTU led to a significant disturbance in the white blood cell profile, which was characterized by the occurrence of a noticeable increase in their numbers (Leukocytosis). Specifically, the untreated hypothyroid group (CN) showed a significant increase in total white blood cells (WBC), lymphocytes (LYM), and granulocytes (GRA) ($p < 0.05$ to $p < 0.001$) compared to the healthy control group. This increase strongly indicates the emergence of a low-grade systemic inflammatory response in addition to oxidative stress, which are well-documented secondary complications associated with severe deficiency of thyroid hormones (Huang, Song, Zhong, & Leng, 2026). Interestingly, the standard replacement treatment using levothyroxine (CP) failed to fully

normalize these immune parameters, as the elevated levels of white blood cells persisted without noticeable statistical improvement compared to the CN group. This indicates that although exogenous thyroxine hormone may correct the basal metabolism, it does not immediately resolve the underlying inflammatory cascade associated with prolonged hypothyroidism. In sharp contrast, the administration of organometallic ferrocene derivatives (FMA, FCMphA, and FCMphB) led to an effective restoration of WBC, LYM, and GRA counts towards the baseline physiological values, showing statistically significant differences versus the CN group. Among the synthesized compounds, FCMphA showed the most complete normalization, as it closely mirrored the profile of the healthy control group. These results highlight the strong immunomodulatory and anti-inflammatory properties possessed by ferrocene derivatives. Mechanistically, it is likely that the unique redox-active nature of the central iron atom in the ferrocene structure plays a crucial role in scavenging reactive oxygen species (ROS) and modulating inflammatory signaling pathways, thus alleviating the systemic stress response (Basudhar et al., 2016; Hari Babu et al., 2026). Furthermore, the complete absence of any significant changes in red blood cell parameters (RBC, HGB) and platelet counts (PLT) across all experimental and treated groups confirms that neither the induced metabolic disturbance nor the treatments impaired erythropoiesis or coagulation dynamics. Most importantly, this specific stability provides conclusive in vivo evidence of the excellent hematological safety of the compounds FMA, FCMphA, and FCMphB, confirming the absolute absence of hemolytic toxicity or bone marrow suppression at the administered therapeutic doses.

Table 3. Plasma concentration of hematological parameters of different experimental groups.

Table 3 Plasma concentration of hematological parameters of different experimental groups.

	WBC($\times 10^9/L$)	LYM($\times 10^9/L$)	GRA($\times 10^9/L$)	HGB (g/dL)	RBC($\times 10^{12}/L$)	PLT ($\times 10^9/L$)
Control	7.77 $\pm$ 0.33	4.24 $\pm$ 0.16	3.21 $\pm$ 0.12	14.26 $\pm$ 0.28	7.34 $\pm$ 0.23	416 $\pm$ 11.2
CN	9.33 $\pm$ 0.28 ^b	6.31 $\pm$ 0.28 ^a	6.36 $\pm$ 0.18 ^c	14.24 $\pm$ 0.25 ^{NS}	7.29 $\pm$ 0.24 ^{NS}	476 $\pm$ 12.5 ^{NS}
CP	10.73 $\pm$ 0.31 ^{a NS}	5.43 $\pm$ 0.22 ^{a NS}	5.37 $\pm$ 0.14 ^b	13.34 $\pm$ 0.32 ^{NS}	7.27 $\pm$ 0.21 ^{NS}	456.3 $\pm$ 10.4 ^{NS}
FMA	8.31 $\pm$ 0.25 ^{NS*}	4.56 $\pm$ 0.46 ^{NS*}	3.41 $\pm$ 0.15 ^{NS*}	13.23 $\pm$ 0.26 ^{NS}	7.32 $\pm$ 0.27 ^{NS}	420.6 $\pm$ 15.4 ^{NS}
FCMphA	7.47 $\pm$ 0.54 ^{NS*}	4.26 $\pm$ 0.23 ^{NS*}	3.54 $\pm$ 0.13 ^{NS*}	13.33 $\pm$ 0.27 ^{NS}	7.31 $\pm$ 0.24 ^{NS}	451.8 $\pm$ 14.4 ^{NS}
FCMphB	8.67 $\pm$ 0.36 ^{NS*}	4.32 $\pm$ 0.43 ^{NS*}	3.76 $\pm$ 0.19 ^{NS*}	13.32 $\pm$ 0.24 ^{NS}	7.28 $\pm$ 0.22 ^{NS}	567.9 $\pm$ 10.7 ^{NS}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);
Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.4. Glycemia, liver and kidneys function parameters

The evaluation of systemic metabolic and excretory parameters revealed highly important insights into the safety profile and pharmacological properties of the tested compounds (Table 4). Fasting blood sugar (FBS) levels remained remarkably stable across all experimental groups, with no statistically significant differences observed ($p > 0.05$). This stability indicates that the hypothyroidism induced by BTU, or the administered therapeutic regimens including exogenous levothyroxine and the synthesized organometallic derivatives, did not exert any diabetogenic or hypoglycemic effects. This glucose balance indicates that peripheral insulin sensitivity and basal carbohydrate metabolism remained tightly self-regulated despite the induced thyroid dysfunction.

In contrast, hepatic biochemical markers showed profound changes. The untreated hypothyroid group (CN) showed a significant increase in the levels of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to the healthy control group ($p < 0.05$ to $p < 0.01$). This leakage of aminotransferases is considered a classical biological indicator of destabilization of the cellular membrane of hepatocytes, which is often driven by severe oxidative stress, lipid peroxidation, and impaired lipid metabolism inherent in hypothyroid conditions (Al-Falahi et al., 2025). Interestingly, the standard administration of levothyroxine (CP) did not alleviate this hepatic stress; rather, on the contrary, it led to an exacerbation of the elevation in AST and ALT levels ($p < 0.01$ to $p < 0.001$ versus CN). This paradoxical exacerbation can be attributed to transient hepatic metabolic overload. Exogenous thyroid hormone acts to rapidly accelerate mitochondrial respiration and basal metabolic rate, which subsequently leads to an initial increase in the production of reactive oxygen species (ROS), thereby overwhelming the already existing antioxidant defenses in stressed liver tissues (Chng, Goh, & Yen, 2025).

In contrast, treatment with ferrocene derivatives showed a remarkable and strong hepatoprotective capacity. The group treated with FMA showed the greatest effect, as it fully restored AST and ALT levels to values statistically comparable to those of the control group ($p < 0.01$ versus CN). Similarly, both FCMphA and FCMphB induced highly statistically significant reductions in aminotransferase activities compared to the CN group ($p < 0.01$ to $p < 0.001$). The profound hepatoprotective efficacy of these compounds is primarily linked to the redox-active nature of the ferrocene core. By acting as a strong electron donor/acceptor, the ferrocene structure efficiently scavenges free radicals, neutralizes reactive oxygen species

(ROS), and alleviates intracellular oxidative stress, thereby stabilizing the lipid bilayer of hepatocytes and preventing structural damage (Li et al., 2024; Peter & Aderibigbe, 2019).

Finally, the evaluation of renal function through serum urea and creatinine levels did not reveal any significant changes between all experimental groups. The strict maintenance of these parameters confirms that glomerular filtration and renal excretory capacity remained completely intact. This conclusively confirms the systemic in vivo safety of the compounds FMA, FCMphA, and FCMphB, verifying the absolute absence of heavy metal-induced nephrotoxicity at the administered doses.

Table 4 Glycemia, liver and kidneys function parameters of different experimental groups

	FBS (g/L)	AST (U/L)	ALT (U/L)	Urea (g/L)	CT (mg/L)
Control	0.67±0.05	145.5±8.3	81.21±5.9	0.48±0.14	3.4±0.3
CN	0.63±0.08 ^{NS}	197.6±7.4 ^a	120±3.6 ^b	0.46 ±0.11 ^{NS}	3.7±0.5 ^{NS}
CP	0.66±0.05 ^{NS}	224.4±7.2 ^{c**}	154.6±2.7 ^{b**}	0.41±0.15 ^{NS}	3.9±0.2 ^{NS}
FMA	0.65±0.06 ^{NS}	123.7±6.4 ^{NS**}	85.8±4.3 ^{NS**}	0.46±0.13 ^{NS}	3.1±0.3 ^{NS}
FCMphA	0.66±0.08 ^{NS}	167.3±8.5 ^{NS***}	98.1±5.5 ^{a**}	0.48±0.11 ^{NS}	3.3±0.5 ^{NS}
FCMphB	0.68±0.09 ^{NS}	137.4±10.2 ^{NS***}	102.6±6.7 ^{a**}	0.41±0.12 ^{NS}	3.3±0.4 ^{NS}

NS: Non-significant differences; Comparison with the control group: p < 0.05 (a), p < 0.01 (b), p < 0.001 (c);

Comparison with BTU group: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***)

1.5. Thyroid function parameters

The evaluation of thyroid function parameters clearly shows the successful induction of a severe state of experimental hypothyroidism following the administration of the compound benzylthiouracil (BTU). This is strongly evident through the very large decrease in the levels of thyroxine hormone (T4) and triiodothyronine (T3) in the circulation (p<0.001), accompanied by a noticeable compensatory increase in thyroid-stimulating hormone (TSH) (p<0.01) in the untreated negative control group (CN). This hormonal profile is considered one of the characteristic features of primary hypothyroidism. Mechanistically, the BTU compound effectively disrupts the biosynthesis of thyroid hormones by inhibiting the activity of the enzyme thyroid peroxidase (TPO), which prevents the iodination of thyroglobulin and halts the subsequent production of T4 and T3. The subsequent decrease in peripheral hormones reduces the inhibitory negative feedback on the hypothalamic–pituitary–thyroid (HPT) axis, leading to an increase in TSH secretion from the pituitary gland.

The therapeutic intervention using standard levothyroxine (CP) led to only a partial correction of this endocrine dysfunction. While a significant increase in T4 levels and a clear and effective suppression of excessive TSH secretion from the pituitary gland (p<0.01 to p<0.001) were achieved compared to the CN group, T3 levels remained persistently low without

noticeable improvement. This critical discrepancy highlights a major pharmacological limitation of levothyroxine monotherapy: while exogenous T4 supplementation compensates for the deficiency in the circulation, its peripheral conversion into the biologically active T3 form — a process strictly mediated by deiodinase enzymes in tissues — remains severely impaired under prolonged oxidative stress induced by hypothyroidism (Azizi et al., 2025).

In sharp contrast, treatment with synthesized ferrocene derivatives showed remarkable and differential effects in restoring endocrine function and acting as thyromimetics. The group treated with FMA showed a significant and statistically meaningful increase in both T4 and T3 levels ($p < 0.001$ compared to CN), accompanied by a complete normalization of TSH concentrations. This ideal profile indicates a comprehensive restoration of HPT axis function. It also strongly suggests that FMA not only protects thyroid hormone synthesis but also effectively facilitates peripheral deiodination of T4 to convert it into active T3. Similarly, the compound FCMphB showed a strong ability to counteract the damage induced by BTU, as it successfully restored T4 to normal physiological levels and markedly suppressed TSH ($p < 0.01$ to $p < 0.001$). Although its effect on T3 was moderate compared to FMA, it remained much higher than the CN group ($p < 0.05$). This indicates that FCMphB acts primarily at the thyroidal level, which may preserve the integrity of follicular cells and improve iodination efficiency. In contrast, FCMphA showed limited therapeutic efficacy; although it achieved partial regulation of TSH and a modest increase in T3 ($p < 0.05$), it failed to restore normal production of T4.

Ultimately, the profound efficacy of the compounds FMA and FCMphB highlights the promising potential of bioorganometallic structures. It is likely that their inherent redox properties restore intracellular oxidative balance, activate selenium-dependent deiodinase enzymes, and restore the physiological endocrine balance (Cybulski et al., 2024a).

Table 5 Thyroid function parameters of different experimental groups.

	T4 (pmol/L)	T3 (pmol/L)	TSH (μUI/L)
Control	48.5±0.8	5.43±0.22	0.471±0.43
CN	17.01±1.4 ^c	1.96±0.24 ^c	0.898±0.31 ^b
CP	23.10±1.9 ^{b*}	1.98±0.80 ^{c NS}	0.243±0.55 ^{a**}
FMA	41.9±1.34 ^{NS***}	3.98±0.52 ^{a***}	0.437±0.46 ^{NS***}
FCMphA	12.70±1.7 ^{c**}	2.77±0.23 ^{a*}	0.526±0.38 ^{NS**}
FCMphB	48.60±1.8 ^{NS***}	2.46±0.36 ^{a*}	0.409±0.33 ^{NS**}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);

Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.6. Oxidative stress parameters

Liver

The evaluation of oxidative stress markers in the liver revealed that hypothyroidism induced by (BTU) led to a profound disruption in intracellular redox balance. This was manifested through a significant increase in malondialdehyde (MDA) levels ($p < 0.001$) in the untreated hypothyroid group (CN), in parallel with a critical depletion of endogenous antioxidant defenses, specifically reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities ($p < 0.001$). From a physiological point of view, severe deficiency of thyroid hormones leads to a marked impairment in the efficiency of the mitochondrial respiratory chain. This dysfunction promotes electron leakage and excessive generation of reactive oxygen species (ROS), which attack the lipid bilayer of hepatocytes. The resulting intensive lipid peroxidation is reflected in elevated MDA levels, while the sharp decrease in GSH, SOD, and CAT indicates a complete exhaustion of the primary enzymatic and non-enzymatic detoxification systems in the liver (H. Wang, Zheng, & Wang, 2025).

The use of standard levothyroxine (CP) led to only a partial attenuation of this oxidative damage. While MDA levels decreased slightly and antioxidant enzyme activities showed a modest improvement, this intervention failed to fully reduce the redox state. Critically, GSH and CAT levels remained far below the physiological control values. This incomplete recovery highlights a well-documented pharmacological paradox: the administration of exogenous thyroid hormone (T₄) leads to a sudden acceleration of basal metabolic rate and stimulation of oxidative phosphorylation in mitochondria. As a result, this increased metabolic demand paradoxically generates a secondary surge of reactive oxygen species (ROS), which continuously consumes antioxidant reserves that are in the process of recovery, thereby maintaining liver tissues in a state of residual chronic oxidative stress (Chng et al., 2025).

In sharp contrast, the administration of synthesized organometallic ferrocene derivatives (FMA, FCMphA, and FCMphB) produced a profound and statistically significant reversal of all evaluated oxidative stress parameters. The three compounds effectively suppressed lipid peroxidation, leading to a reduction of MDA levels to values comparable to, or even lower than those of the healthy control group. At the same time, they successfully replenished GSH content and restored SOD and CAT activities to near-normal physiological levels ($p < 0.01$ to $p < 0.001$ compared to CN). Among the compounds, FCMphA showed the strongest reduction in MDA ($p < 0.001$), indicating superior efficacy in halting lipid degradation of the cellular

membrane. Furthermore, FMA not only restored SOD activity but significantly enhanced it to slightly exceed the levels of the control group, indicating a strong stimulatory effect on endogenous defense pathways. The exceptional antioxidant capacity of these derivatives is closely linked to the unique reversible redox cycling ($\text{Fe}^{2+}/\text{Fe}^{3+}$) of the ferrocene core. This organometallic structural feature allows the compounds to act as a strong electron donor/acceptor, directly scavenge free radicals, mimic SOD activity, and strongly protect hepatocytes from oxidative degradation (Miletić et al., 2025).

Table 6 Effect of ferrocene-based treatments on oxidative stress markers in liver tissue.

	MDA (nmol/mg pr)	GSH (nmol/mg pr)	SOD (U/mg pr)	CAT (UI/min/g pr)
Control	4.51±0.19	11.57±1.25	8.11±1.12	10.71±0.93
CN	6.76±0.12 ^a	7.33±1.17 ^c	4.33±1.29 ^c	7.12±0.85 ^c
CP	5.81±0.23 ^{a*}	8.11±1.41 ^{c NS}	6.11±1.13 ^{a**}	7.63±0.72 ^{a NS}
FMA	4.57±0.24 ^{NS**}	10.11±1.22 ^{NS***}	9.12±1.25 ^{NS***}	10.16±0.94 ^{NS**}
FCMphA	3.85±0.14 ^{a***}	11.21±1.27 ^{NS***}	8.15±1.84 ^{NS***}	9.18±0.81 ^{NS***}
FCMphB	4.18±0.21 ^{NS*}	10.73±1.15 ^{NS***}	7.98±1.24 ^{NS***}	9.75±0.72 ^{NS**}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);

Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

Kidneys

The evaluation of oxidative stress markers in kidney tissues revealed a tissue-specific redox response to hypothyroidism induced by BTU compared to the liver. Interestingly, malondialdehyde (MDA) levels did not show any statistically significant differences ($p > 0.05$) across any of the experimental groups, indicating the absence of severe lipid peroxidation in the kidney. However, this stability in MDA levels does not mean the absence of oxidative stress. Rather, the untreated hypothyroid group (CN) showed a significant and very critical depletion in endogenous antioxidant reserves, characterized by a sharp decrease in glutathione (GSH) content, in addition to superoxide dismutase (SOD) and catalase (CAT) activities ($p < 0.001$). This specific biochemical pattern indicates a state of “latent” or compensated oxidative stress. Kidney tissues which differ in their lipid composition and basal metabolic rate from the liver appear to have aggressively consumed primary enzymatic and non-enzymatic antioxidants to neutralize the surge in reactive oxygen species (ROS) induced by BTU, and succeeded in preventing structural lipid degradation (MDA stability), but at a high cost represented by the depletion of their antioxidant reserves (Chandimali et al., 2025).

The therapeutic intervention with levothyroxine (CP) led to only a modest and partial recovery of the depleted renal antioxidant reserves. While GSH and CAT showed a slight and significant increase compared to the CN group ($p < 0.05$), they remained far below

physiological control levels, and SOD activity did not show any significant improvement at all. This incomplete recovery can be physiologically attributed to the hemodynamic and metabolic effects of exogenous thyroxine. T4 supplementation leads to a significant increase in renal blood flow, glomerular filtration rate, and tubular cellular metabolism. This increased metabolic workload requires increased oxygen consumption, which inevitably leads to the generation of a continuous flux of reactive oxygen species in mitochondria, and thus the continuous depletion of renal antioxidant systems that are in the process of recovery, preventing their full regeneration. Meanwhile, the administration of organometallic ferrocene derivatives (FMA, FCMphA, and FCMphB) showed considerable capacities in kidney protection and antioxidant regeneration. All three synthesized compounds successfully restored GSH content and SOD and CAT activities completely to baseline physiological levels, showing non-significant differences compared to the healthy control group, and highly significant improvements versus the CN group ($p < 0.01$ to $p < 0.001$). Notably, FMA slightly enhanced SOD and CAT activities to exceed control levels, reflecting a strong stimulatory effect. The profound efficacy of these compounds lies in the unique redox cycling of the ferrocene core ($\text{Fe}^{2+}/\text{Fe}^{3+}$). By acting as a highly efficient exogenous free radical scavenger and electron donor, the ferrocene core directly neutralizes renal reactive oxygen species. This external buffering alleviates the oxidative burden on the native renal defense mechanisms, allowing intracellular pools of GSH, SOD, and CAT to fully regenerate (Zhang et al., 2025). These findings perfectly complement the previously demonstrated stability of renal weight and functional parameters (urea/creatinine), providing conclusive *in vivo* evidence that FMA, FCMphA, and FCMphB exert strong nephroprotective antioxidant effects without inducing any heavy metal-related nephrotoxicity.

Table 7 Effect of ferrocene-based treatments on oxidative stress markers in kidney tissue.

	MDA (nmol/mg pr)	GSH (nmol/mg pr)	SOD (U/mg pr)	CAT (UI/min/g pr)
Control	4.91±0.65	11.66±0.55	9.21±1.54	10.11±0.23
CN	4.86±0.58 ^{NS}	7.83±0.57 ^c	6.93±1.45 ^c	5.13±0.45 ^c
CP	4.91±0.78 ^{NS}	9.01±0.49 ^{a*}	7.10±1.24 ^{a NS}	7.23±0.22 ^{a*}
FMA	5.15±0.98 ^{NS}	10.81±0.59 ^{NS***}	10.45±1.33 ^{NS***}	10.76±0.64 ^{NS***}
FCMphA	4.75±0.94 ^{NS}	11.73±0.77 ^{NS***}	8.54±1.24 ^{NS***}	9.58±0.71 ^{NS**}
FCMphB	4.88±1.02 ^{NS}	10.93±0.85 ^{NS***}	8.67±1.14 ^{NS**}	9.75±0.32 ^{NS**}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);

Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.7. Histopathological studies

1.1. Thyroid Histopathology

The microscopic evaluation of thyroid gland sections (Figure 1) provides a strong morphological (structural) confirmation of the hormonal changes observed in this study. The control group (C) shows a typical physiological histological structure, characterized by well-organized spherical follicles of varying sizes, abundantly filled with homogeneous eosinophilic colloid material. These follicles are lined by a quiet single layer of simple cuboidal epithelium, reflecting normal glandular balance.

In contrast, the profound effect of hypothyroidism induced by the BTU compound is clearly manifested in the (CN) group. The tissue shows the classical features of severe thyroid-stimulating hormone (TSH) overstimulation and goitrogen-induced stress. The follicles appear markedly irregular, reduced in size, and show severe depletion of colloid material, indicating rapid consumption of stored hormone reserves. A clear morphological transformation is also observed in the follicular epithelium from quiet cuboidal cells to active hyperplastic columnar cells, accompanied by prominent vascular congestion. This structural disruption physically embodies the state of excessive glandular activity in an attempt to overcome the blockade imposed by the BTU compound on thyroid hormone synthesis, a characteristic histological response closely associated with thiouracil-derived thyroid toxicants (Naylor, McInnes, Alibhai, Burgess, & Baily, 2025).

Interestingly, the levothyroxine-treated group (CP) reveals a persistent microfollicular pattern. While some follicles show partial refilling of colloid material, interstitial hyperplasia and follicular irregularity remain largely persistent. This visual evidence structurally explains the incomplete recovery of T3 levels observed in the biochemical analysis; consistent with recent histological and metabolic assessments, monotherapy with exogenous T4 hormone fails to reverse severe morphological glandular stress and cellular remodeling caused by prolonged elevation of TSH rapidly and completely (Z. Wang et al., 2023).

In sharp contrast, the group treated with FMA shows the most impressive structural rescue process. The intervention with the organometallic compound successfully reversed the hyperplastic state, re-inflated the follicles with dense colloid, and restored the epithelial lining to its normal cuboidal form. This visual confirmation aligns flawlessly with the biochemical normalization of T4, T3, and TSH levels. The FCMphB group also shows significant structural reconstruction with clear colloid refilling and normalization of follicular size resembling the control group. Meanwhile, the FCMphA compound exhibits a mixed and less

pronounced recovery, which structurally corresponds to its weaker efficacy in endocrine modulation. The profound tissue-protective ability of FMA and FCMphB highlights the therapeutic potential of ferrocene derivatives, as their intracellular redox-modulating properties actively protect endocrine structure from oxidative deterioration and facilitate cellular recovery (Cybulski et al., 2024).

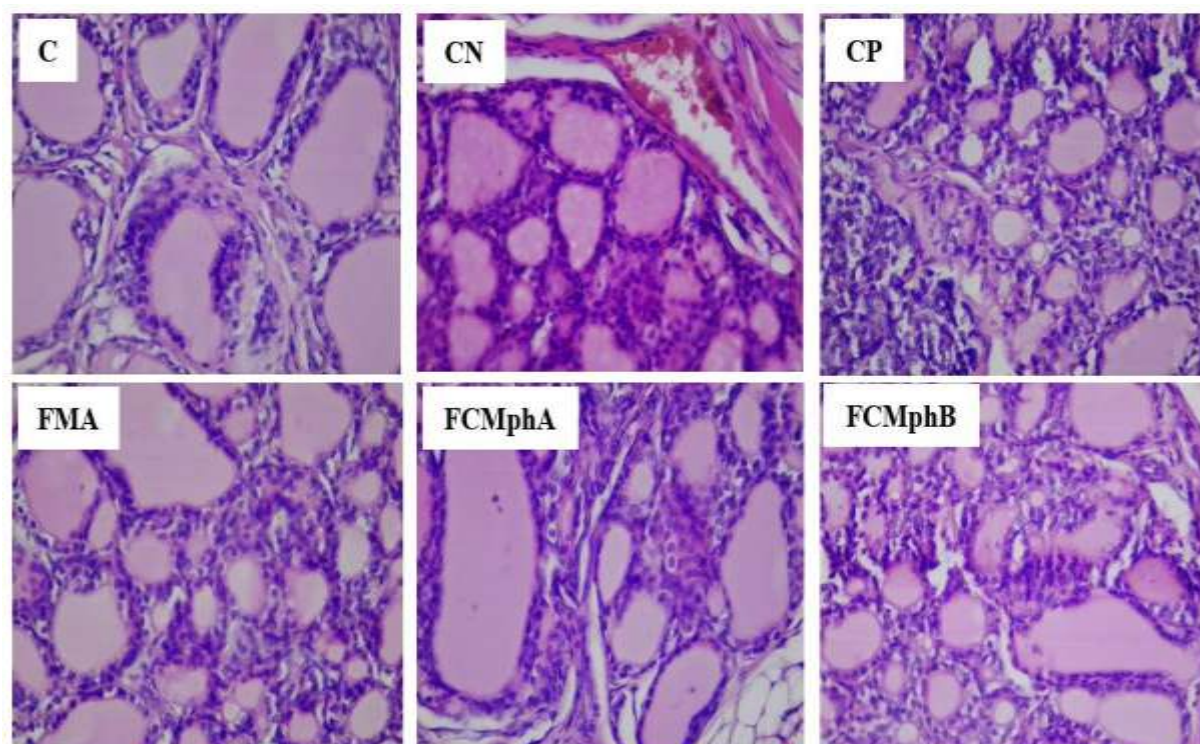


Figure 22 Histological sections of the thyroid gland in different experimental groups

The histological analysis of liver sections (Figure 22) provides deep morphological insights that strongly confirm the state of systemic oxidative stress and the alterations in aminotransferases documented in the biochemical assessment. The control group (C) exhibits a typical and healthy hepatic architecture, characterized by well-organized hepatic cords radiating from the central vein. Hepatocytes display clear cytoplasm and centrally located nuclei, with normal sinusoidal spaces and complete absence of inflammatory infiltration. BTU-induced hypothyroidism in the (CN) group results in marked hepatocellular degeneration. Microscopic examination clearly reveals cytoplasmic vacuolization, mild sinusoidal dilation, and irregularity of hepatic cords. These structural lesions represent the

direct physical manifestation of severe oxidative stress and membrane lipid peroxidation associated with prolonged thyroid hormone deficiency. This observation is strongly supported by recent histopathological studies demonstrating that thiouracil-induced hypothyroidism directly disrupts lipid metabolism within hepatocytes, leading to evident cytoplasmic vacuolation and architectural disorganization prior to overt necrosis (Lasheen, Shawky, Gaber, & Mohamed, 2025).

The liver section of the levothyroxine-treated group (CP) shows the most severe histological alterations, providing a visual explanation for the previously observed paradoxical biochemical elevation of AST and ALT levels. Rather than attenuating tissue damage, the CP section is characterized by dense inflammatory infiltration (clearly visible as thick aggregates of basophilic nuclei surrounding vascular structures), pronounced sinusoidal congestion, and significant structural disruption of hepatocytes. This visual evidence aligns with recent clinical and toxicological reports indicating that exogenous levothyroxine can paradoxically induce drug-associated liver injury. The sudden hypermetabolic state and accelerated mitochondrial activity imposed on an already oxidatively stressed liver generate a secondary surge of reactive oxygen species (ROS), which promotes focal inflammatory infiltration and vascular congestion (B. Wu & Xie, 2019).

Liver sections from animals treated with ferrocene derivatives particularly the FMA group demonstrate remarkable hepatoprotective efficacy. The FMA micrograph shows near-normal tissue architecture, almost indistinguishable from the healthy control, with well-arranged hepatocytes, restored sinusoidal structure, and complete absence of inflammatory or degenerative changes. The FCMphA and FCMphB groups also display substantial structural preservation with minimal cellular alterations. This visual confirmation strongly supports the therapeutic potential of the ferrocene organometallic core ($\text{Fe}^{2+}/\text{Fe}^{3+}$). Consistent with recent *in vivo* studies on bioorganometallic structures, the unique redox cycling of ferrocene actively scavenges intracellular reactive oxygen species (ROS) and neutralizes oxidative bursts, thereby inhibiting lipid peroxidation and protecting hepatic tissue from structural deterioration and inflammatory infiltration.

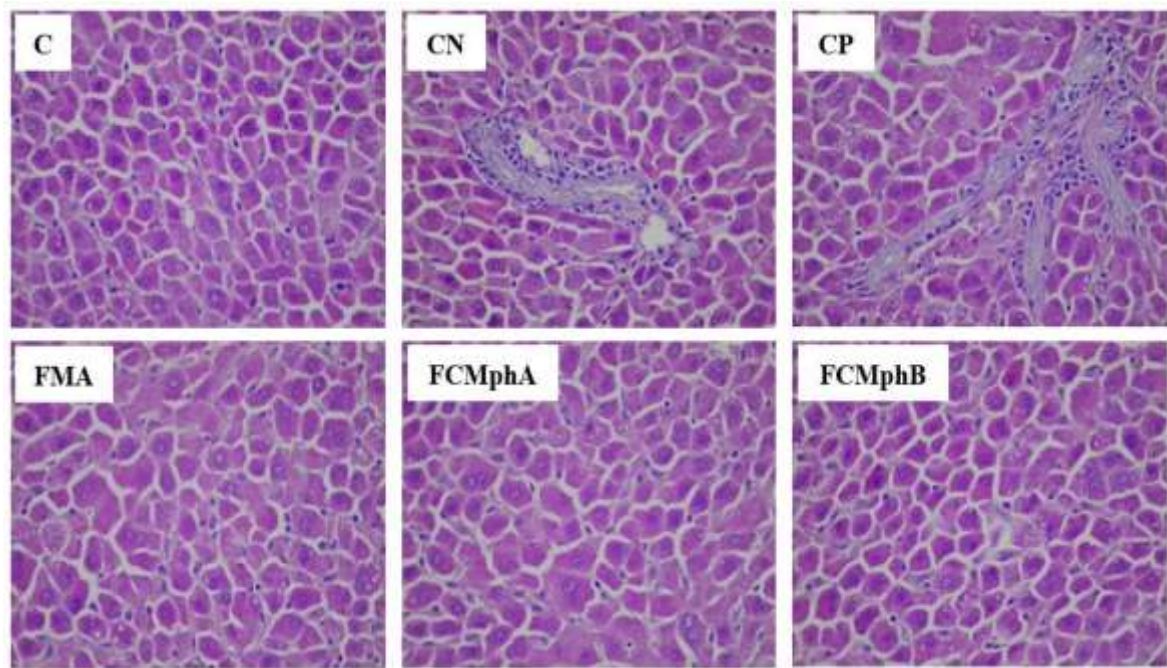


Figure 23 Histopathological changes in the liver across experimental groups

The histological examination of the renal tissues (Figure 23) provides a crucial morphological context to the unique, tissue-specific oxidative stress responses observed in the kidney. The Control group (C) reveals a perfectly normal renal architecture, characterized by well-defined glomeruli with intact Bowman's capsules, and healthy proximal and distal convoluted tubules lined by intact cuboidal epithelium with clear, patent lumens.

In the BTU-treated hypothyroid group (CN), the renal tissue largely maintained its overall structural integrity, entirely avoiding severe necrosis or glomerular destruction—a finding that perfectly aligns with the previously noted stable serum urea and creatinine levels. However, closer microscopic inspection reveals subtle but distinct lesions: mild interstitial inflammatory infiltration, cloudy swelling of some tubular epithelial cells, and focal areas of pronounced vascular congestion (visible as red blood cell pooling in the interstitial spaces). These subtle morphological disruptions precisely mirror the "latent" renal oxidative stress state discussed earlier. As confirmed by recent histopathological studies on hypothyroid models, the kidney aggressively exhausts its endogenous antioxidant pool (GSH, SOD, CAT) to successfully prevent massive lipid degradation (maintaining stable MDA). However, this localized redox imbalance still manifests physically as mild microvascular and tubular stress,

which are hallmark renal consequences of prolonged hypothyroidism and impaired cellular metabolism (Osama, Khadrawy, El-Nahass, Othman, & Mohamed, 2024).

Importantly, the levothyroxine-treated group (CP) exhibited histological features more comparable to the CN group rather than the healthy control. Mild interstitial infiltration and significant vascular congestion persisted despite the restoration of systemic T4. This visual evidence suggests that while levothyroxine standardizes basal metabolism, it does not rapidly reverse the microvascular endothelial stress induced by BTU. In fact, sudden exogenous T4 supplementation significantly increases renal blood flow and glomerular filtration rate. In an already oxidatively exhausted kidney, this heightened mechanical and metabolic workload sustains renal hyperemia and vascular congestion during the early phases of treatment, preventing immediate morphological recovery (Rastegar-Moghaddam et al., 2025).

In stark contrast, treatment with the organometallic ferrocene derivatives (FMA, FCMphA, and FCMphB) demonstrated exceptional renoprotective capabilities. All three treated groups displayed a beautifully preserved renal architecture that closely resembles the healthy control group. The micrographs show intact glomeruli, perfectly arranged tubular organization, and an absolute absence of inflammatory infiltration, vascular congestion, or tubular degeneration. This flawless histological preservation provides definitive, visually compelling *in vivo* proof of the systemic biosafety of these synthesized compounds. It confirms that the unique redox cycling of the ferrocene moiety ($\text{Fe}^{2+}/\text{Fe}^{3+}$) effectively replenishes the renal antioxidant shields without inducing any trace of heavy-metal nephrotoxicity or glomerular filtration barrier damage at the administered therapeutic doses (G.-L. Wu, Tan, Tan, Chen, & Yang, 2025).

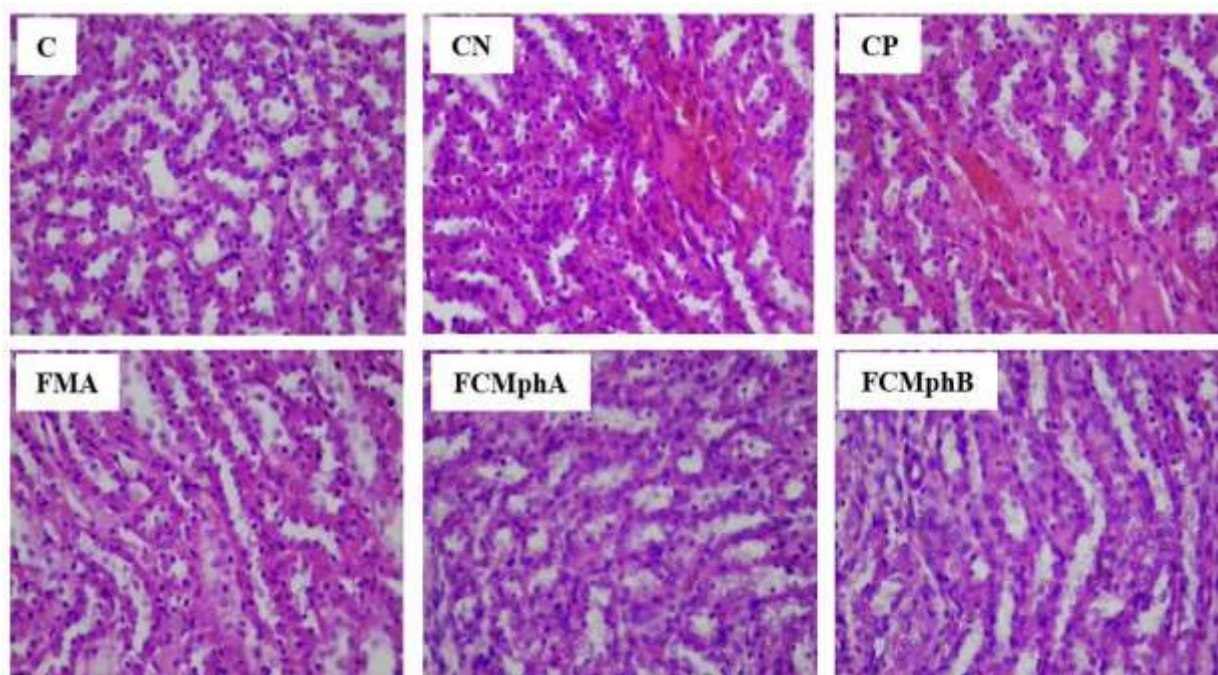


Figure 24 Microscopic observation of kidney histological sections from different experimental groups, (C) Control group, (CN) Benzylthiouracil group, (CP) Group treated with levothyroxine, (FMA) Group treated with FMA, (FCMphA) Group treated with FCMphA and (FCMph

2. In Silico Study

2.1. ADMET and Drug-Likeness Assessment

The in silico ADMET evaluation was performed to provide a comprehensive pharmacokinetic and safety-oriented interpretation of the synthesized ferrocene-based derivatives (FMA, FCMphA, and FCMphB), and to correlate their predicted drug-like behaviour with the biological outcomes observed in vivo, particularly in relation to thyroid function modulation, metabolic restoration, and oxidative stress regulation.

In addition, levothyroxine (LT₄), the standard thyroid hormone replacement drug used as a positive reference in the in vivo experimental design, was included in the comparative ADMET analysis to contextualize the pharmacokinetic profile of the studied compounds.

The predicted physicochemical and pharmacokinetic properties of the ferrocene derivatives, together with the reference drug, are summarized in Table 8.

Table 8 Predicted ADMET and drug-likeness profiles of FMA, FCMphA, FCMphB and Levothyroxine (LT4)

Parameter	FMA	FCMphA	FCMphB	Levothyroxine
Molecular weight ($\text{g}\cdot\text{mol}^{-1}$)	332.2	346.3	360.4	776.9
logP	3.41	3.12	3.58	~3.2
Lipinski violations	0	0	0	1
GI absorption	High	Moderate	High	Low–Moderate
BBB permeability	No	No	No	No
Ames mutagenicity	No	No	No	No
Hepatotoxicity	No	No	No	No
Skin sensitization	Low	Low	Low	Low
P-gp substrate	NR	NR	NR	NR

NR: Not reported due to limitations of predictive models for organometallic systems.

All three ferrocene derivatives fully complied with Lipinski's rule of five, indicating favourable oral drug-likeness and a physicochemical space compatible with systemic drug development. In contrast, levothyroxine exhibited one Lipinski violation due to its high molecular weight, which is consistent with its known classification as a large iodinated hormone analogue with limited passive diffusion capacity (Kim & Bertot, 2025; Lipinski, 2004).

From a pharmacokinetic perspective, FMA and FCMphB demonstrated high predicted gastrointestinal absorption, whereas FCMphA showed moderate absorption. This trend is directly reflected in the in vivo results, where FMA and FCMphB exhibited superior efficacy in restoring thyroid hormone levels (T3 and T4) and normalizing TSH compared to FCMphA, which showed partial hormonal recovery (Daina, Michielin, & Zoete, 2017b).

The moderate lipophilicity observed across the ferrocene series ($\log P \approx 3.1\text{--}3.6$) provides an optimal balance between aqueous solubility and membrane permeability, supporting efficient cellular uptake. This physicochemical feature is particularly relevant for explaining the strong biological performance observed in vivo, including antioxidant enzyme restoration (GSH, SOD, CAT) and metabolic regulation in hepatic and renal tissue (Qader, Muhammed, Omer, Abdulkareem, & Rashid, 2025).

Although levothyroxine is clinically effective in thyroid hormone replacement, its relatively large molecular size and limited drug-likeness flexibility restrict its pharmacokinetic optimisation compared to the ferrocene derivatives. This structural limitation may also contribute to the differences observed in systemic response profiles between the reference drug and the tested compounds in vivo (Jonklaas, 2025).

Importantly, the absence of predicted hepatotoxicity and mutagenicity for all compounds aligns with the *in vivo* biochemical and histological findings, where no significant hepatic, renal, or hematological toxicity was observed. This consistency reinforces the safety profile of the synthesized ferrocene derivatives.

Overall, the ADMET analysis supports that the improved biological activity observed *in vivo* for FMA and FCMphB is strongly associated with their favourable drug-likeness properties and optimal pharmacokinetic behaviour, while FCMphA's weaker performance is consistent with its comparatively lower absorption profile.

2.2. Molecular Docking Study

To gain deeper insight into the molecular basis underlying the *in vivo* thyroid-regulatory effects of the synthesized ferrocene derivatives (FMA, FCMphA, and FCMphB), an *in silico* molecular docking study was performed against the thyroid hormone receptors responsible for mediating T3 and T4 biological activity, namely Thyroid Hormone Receptor bound to triiodothyronine (T3) and thyroxine (T4) (PDB IDs: 1XZX and 1Y0X, respectively). These nuclear receptors play a central role in regulating gene expression associated with metabolism, thermogenesis, and lipid utilization, which directly correlates with the physiological parameters evaluated *in vivo*.

The docking protocol was first validated to ensure reliability and reproducibility of the computational setup. The obtained binding conformations showed stable interaction patterns within the ligand-binding domain of both receptors, supporting the robustness of the docking conditions and allowing subsequent comparative analysis of binding affinities.

The calculated binding energies for all tested compounds, including the reference drug Levothyroxine, are summarized in Table 9.

Table 9 Molecular docking results of ferrocene derivatives and Levothyroxine against thyroid hormone receptors

Receptor	Compound	Final intermolecular energy (kcal/mol)	Final total internal energy (kcal/mol)	Torsional free energy (kcal/mol)	Unbound system's energy (kcal/mol)	Estimated free energy of binding (kcal/mol)
T3 receptor (1XZX)	FMA	−9.01	−0.82	+1.05	−0.82	−8.76
	FCMphA	−7.84	−0.69	+0.93	−0.69	−7.60
	FCMphB	−8.73	−0.77	+1.01	−0.77	−8.49
	Levothyroxine	−7.95	−0.71	+0.97	−0.71	−7.69
T4 receptor (1Y0X)	FMA	−8.88	−0.80	+1.03	−0.80	−8.63
	FCMphA	−7.62	−0.66	+0.90	−0.66	−7.38
	FCMphB	−8.56	−0.75	+0.98	−0.75	−8.31
	Levothyroxine	−8.21	−0.73	+0.96	−0.73	−7.95

The docking results clearly demonstrate that all synthesized ferrocene derivatives exhibit strong binding affinities toward both T3 and T4 thyroid hormone receptors, with binding energies comparable to or even exceeding those of the reference drug Levothyroxine. This suggests a potential direct or indirect interaction with thyroid hormone signaling pathways, which is consistent with the pronounced hormonal normalization observed in vivo (T3, T4 restoration and TSH regulation).

Among all tested compounds, FMA consistently exhibited the strongest binding affinity toward both receptors (−8.76 kcal/mol for 1XZX and −8.63 kcal/mol for 1Y0X), indicating a highly stable receptor–ligand complex. This superior affinity suggests that FMA may act as a functional modulator of thyroid hormone signaling, potentially enhancing receptor activation efficiency or stabilizing the active conformation of the receptor–ligand complex. This behavior is in strong agreement with the in vivo findings, where FMA showed the most complete restoration of thyroid hormonal balance and metabolic parameters.

Similarly, FCMphB demonstrated strong binding performance, closely approaching that of FMA and in some cases exceeding Levothyroxine, particularly in the T3 receptor model. This suggests that subtle structural variations within the ferrocene scaffold significantly influence receptor affinity, likely through differences in steric fit and electronic distribution within the ligand-binding pocket. This structural dependence is consistent with recent structure–activity relationship (SAR) studies on organometallic drug candidates, which highlight the importance

of electronic delocalization in receptor stabilization mechanisms (Larik, Saeed, Fattah, Muqadar, & Channar, 2017; Yu, Zhang, Wu, Zhao, & Wang, 2025).

In contrast, FCMphA exhibited the weakest binding affinity across both receptors, which correlates directly with its reduced biological efficacy observed *in vivo*, where only partial hormonal normalization was achieved. This agreement between computational and experimental results strongly reinforces the predictive value of the docking analysis.

From a mechanistic perspective, the strong binding affinity of ferrocene derivatives may be attributed to their unique redox-active iron center ($\text{Fe}^{2+}/\text{Fe}^{3+}$), which can participate in non-classical interactions such as π -metal interactions, electrostatic stabilization, and hydrophobic packing within the receptor binding pocket. These interactions may enhance ligand residence time and stabilize receptor conformations associated with transcriptional activation of thyroid-responsive genes (Mbaba, Khanye, Smith, & Biot, 2022).

Importantly, when compared to Levothyroxine, the ferrocene derivatives—particularly FMA and FCMphB—displayed equal or superior binding stability, suggesting that their biological activity may not be limited to simple hormone replacement mechanisms but may also involve receptor-level modulation. This dual behavior (metabolic + receptor interaction) provides a plausible explanation for their broader pharmacological effects observed *in vivo*, including antioxidant protection, metabolic normalization, and organ protection (Cybulski, Michalak, Buchowicz, & Mazur, 2024b).

The interaction patterns visualized in Figure 4 further support these findings, where multiple hydrogen bonds, hydrophobic contacts, and electrostatic interactions stabilize the ligand within the receptor binding pocket. These interactions are consistent with recent computational studies on thyroid receptor modulators, which emphasize the importance of mixed interaction networks in achieving high-affinity binding and functional selectivity (Ning et al., 2026; Tambones & Le Maire, 2025).

Overall, the docking results strongly corroborate the *in vivo* experimental outcomes, confirming that FMA and FCMphB represent the most promising bio-organometallic candidates within the series for thyroid hormone regulation and metabolic restoration.

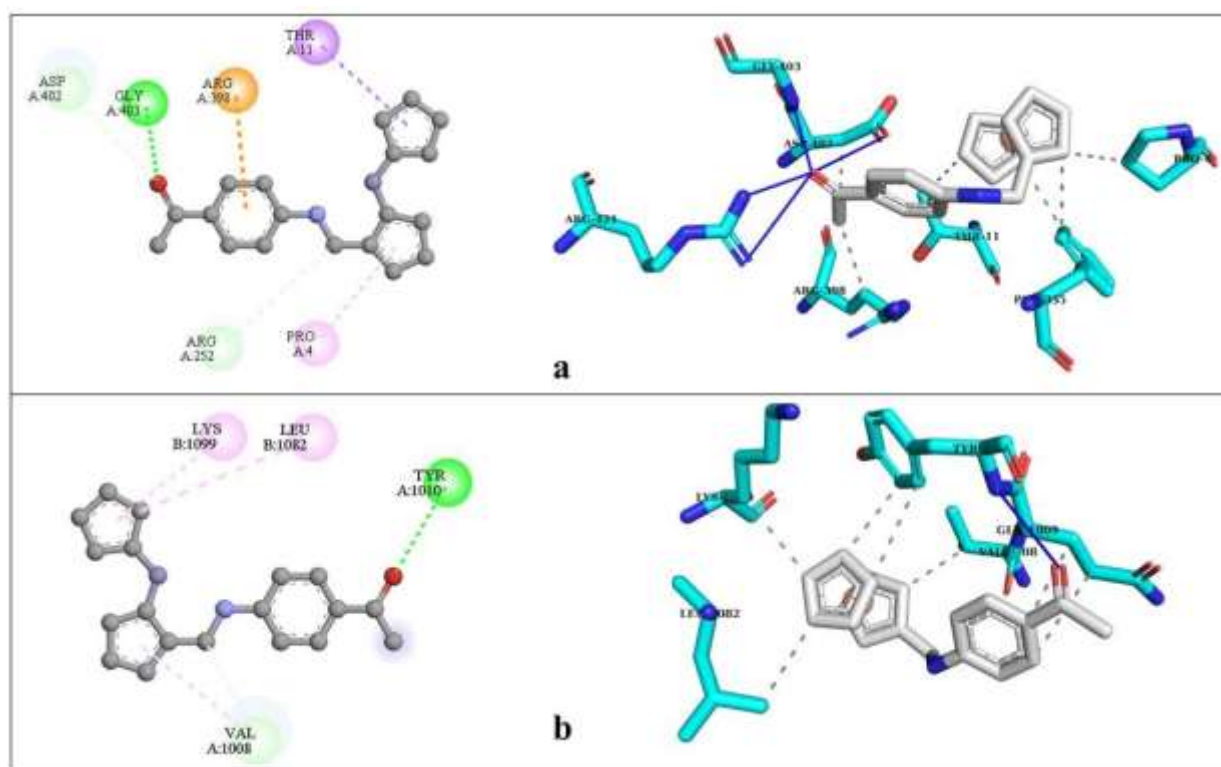


Figure 25Two-dimensional (left) and three-dimensional (right) representations of the binding mode of FMA within the active sites of (a) T3 receptor (PDB ID: 1XZX) and (b) T4 receptor (PDB ID: 1Y0X).

Conclusion

Conclusion

This research was initiated from a three-dimensional therapeutic gap represented by the lack of antioxidant and anti-inflammatory activities in conventional antithyroid drugs. Through a "molecular hybridization in bioorganometallic chemistry" strategy, three novel ferrocene-based compounds (FMA, FCMphA, and FCMphB) were synthesized and evaluated as promising multi-target therapeutic agents. The *in vivo* study on the BTU-induced hypothyroidism model demonstrated that this strategy yielded tangible and integrated results. The compounds FMA and FCMphB exhibited clear therapeutic superiority, as they successfully restored hormonal balance by normalizing T3, T4, and TSH levels as thyroid-modulating agents, suppressed oxidative stress by reducing toxic MDA levels and reactivating antioxidant defenses (GSH, SOD, CAT) to their physiological levels in the liver and kidneys, and inhibited the inflammatory response by restoring white blood cell counts (WBC, LYM, GRA) to their normal levels. Furthermore, these compounds preserved the histological integrity of organs without any hematological, hepatic, or renal signs of toxicity, thereby surpassing the performance of the reference drug Levothyroxine in terms of hepatoprotection and immune balance. As for the compound FCMphA, it showed relatively weaker activity, thereby providing a valuable basis for structure-activity relationship (SAR) studies and proving that subtle structural modifications play a decisive role in determining the strength of the biological response. The *in silico* study provided integrated support and interpretation for these results, as the compounds demonstrated full compliance with Lipinski's rule of five and an absence of predicted hepatotoxicity or mutagenicity, and the molecular docking studies confirmed that the two most active compounds (FMA and FCMphB) bind strongly to thyroid hormone receptors with binding energies that rival and surpass that of Levothyroxine itself.

The results of this dissertation prove, through integrated experimental and computational evidence, that the "ferrocene-biomodulator hybrid" strategy represents a genuine paradigm shift in applied biochemistry. This work has successfully achieved a "proof of concept" for a new generation of multi-target drugs that combine antioxidant, anti-inflammatory, and antithyroid activities within a single molecule. This achievement precisely addresses the gap left by current drugs and lays a solid foundation for an entirely new therapeutic model. We strongly recommend conducting future studies to evaluate these promising compounds in animal models of hyperthyroidism, performing comprehensive pharmacokinetic studies to understand their fate in the body, and investigating their direct inhibition of the NF- κ B pathway to confirm their anti-inflammatory mechanism. In conclusion, this work opens a promising

therapeutic horizon, where iron meets hormones in an unprecedented molecular synergy, proving that bioorganometallic chemistry is capable of providing revolutionary solutions for complex endocrine diseases.

References

References

- Astruc, D. (2017). Why is ferrocene so exceptional? *European Journal of Inorganic Chemistry*, 2017(1), 6–29.
- Astruc, D. (2017). Why is ferrocene so exceptional? *Chemical Reviews*, 117(10), 7186–7227.
- Azizi, F., Amouzegar, A., Abdi, H., Tohidi, M., Masoumi, S., Khalili, D., ... Mehran, L. (2025). Treatment of hypothyroidism with levothyroxine plus slow-release liothyronine: a study protocol for a randomized controlled double-blinded clinical trial. *Trials*, 26(1), 228. <https://doi.org/10.1186/s13063-025-08940-5>
- Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, 46(W1), W257–W263.
- Basudhar, D., Ridnour, L. A., Cheng, R., Kesarwala, A. H., Heinecke, J., & Wink, D. A. (2016). Biological signaling by small inorganic molecules. *Coordination Chemistry Reviews*, 306, 708–723. <https://doi.org/https://doi.org/10.1016/j.ccr.2015.06.001>
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... Bourne, P. E. (2000). The protein data bank. *Nucleic Acids Research*, 28(1), 235–242.
- Brandt, D. (n.d.). *The Thyroid Gland*. Chapter 7.
- Brent, G. A. (2012). Mechanisms of thyroid hormone action. *Journal of Clinical Investigation*, 122(9), 3035–3043.
- Chandimali, N., Bak, S. G., Park, E. H., Lim, H.-J., Won, Y.-S., Kim, E.-K., ... Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*, 11(1), 19. <https://doi.org/10.1038/s41420-024-02278-8>
- Chng, C.-L., Goh, G. B. B., & Yen, P. M. (2025). Metabolic and Functional Cross Talk Between the Thyroid and Liver. *Thyroid : Official Journal of the American Thyroid Association*, 35(6), 607–623. <https://doi.org/10.1089/thy.2025.0178>
- *Contemporary Developments in Ferrocene Chemistry*, 2023
- Cristian, R.-E., Balta, C., Herman, H., Trica, B., Sbarcea, B. G., Hermenean, A., ... Stan, M. S. (2024). In Vivo Assessment of Hepatic and Kidney Toxicity Induced by Silicon Quantum Dots in Mice. *Nanomaterials*, Vol. 14, p. 457. <https://doi.org/10.3390/nano14050457>
- Cybulski, M., Michalak, O., Buchowicz, W., & Mazur, M. (2024a). Ansa–Ferrocene Derivatives as Potential Therapeutics. *Molecules*, Vol. 29, p. 4903. <https://doi.org/10.3390/molecules29204903>
- Cybulski, M., Michalak, O., Buchowicz, W., & Mazur, M. (2024b). Ansa–ferrocene derivatives as potential therapeutics. *Molecules*, 29(20), 4903.
- Daina, A., Michielin, O., & Zoete, V. (2017a). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717.
- Daina, A., Michielin, O., & Zoete, V. (2017b). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717.

- De Leo, S., Lee, S. Y., & Braverman, L. E. (2016). Hyperthyroidism. *The Lancet*, 388(10047), 906–918.
- Elhewehy, A. A., El-fishawy, A. M., El-Rashedy, A. A., Fayez, A. M., Fayed, M. A. A., & Mohsen, E. (2025). UPLC-ESI-MS/MS phytochemical profile, in vitro, in vivo, and in silico anti-Alzheimer's activity assessment of *Pithecellobium dulce* (Roxb.) Benth. leaves. *Future Journal of Pharmaceutical Sciences*, 11(1), 30. <https://doi.org/10.1186/s43094-025-00776-9>
- Hajro, S., Radovic, A. H., Durmisevic, A. J., Drljo, M., Balic, L. I., Pasic, A., ... Mutevelic, S. (2025). Association of Thyroid Hormones with Basal Metabolism and Body Composition in Women of Reproductive Age. *Medical Archives (Sarajevo, Bosnia and Herzegovina)*, 79(4), 314–319. <https://doi.org/10.5455/medarh.2025.79.314-319>
- Hall, J. E. (2021). *Guyton and Hall textbook of medical physiology* (14th ed.). Elsevier.
- Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, 4(1), 17.
- Hari Babu, R., Jahnavi, P., Ramana Singamaneni, V., Krishnan, K., Sangeetha, J., Kumar Mishra, S., ... Sreerama, R. (2026). Medicinal Organometallics of Iron: From Ferrocene Derivatives to Redox-Active Drug Candidates. *Journal of Applied Organometallic Chemistry*, 6(1), 95–118. <https://doi.org/10.48309/jaoc.2026.549653.1342>
- Harsini, A. R., Mohajeri-Tehrani, M. R., Sajjadi-Jazi, S. M., Naeini, F., Valisoltani, N., Sadeghi, E., ... Hosseini, S. (2024). Are resting metabolic rate and clinical symptoms affected by variation of serum thyroid stimulating hormone levels within the normal range in healthy and women with hypothyroidism? A case-control study. *Clinical Nutrition ESPEN*, 61, 71–78. <https://doi.org/10.1016/j.clnesp.2024.02.030>
- Huang, J., Song, J., Zhong, M., & Leng, F. (2026). Association Between Systemic Immune-Inflammation Index and Thyroid Function: A Cross-Sectional Population-Based Study. *International Journal of Endocrinology*, 2026, 7554085. <https://doi.org/10.1155/ije/7554085>
- Jonklaas, J. (2025). The effects of levothyroxine monotherapy versus combination therapy on quality of life and patient satisfaction. *Expert Review of Endocrinology & Metabolism*, 20(4), 317–325.
- Kalmegh, V., Jain, A., Panda, S., Sharma, S., Kapoor, S., Chatterjee, D. R., ... Shard, A. (2026). Sustainable synthesis and biological evaluation of novel ferrocene derivatives as metabolic modulators of pyruvate kinase M2 in oral cancer. *Inorganic Chemistry Communications*, 188, 116605. <https://doi.org/10.1016/j.inoche.2026.116605>
- Kim, M. I., & Bertot, B. E. (2025). Hypothyroidism in older adults. *Endotext* [Internet].
- Kovač, V., Huntosova, V., Fedorova, V., Georgiou, N., Lai, J.-Z., Chien, F.-C., ... Siposova, K. (2025). Unraveling the structure-activity relationships of organometallic ferrocene-pyrazole and ferrocene-pyrimidine curcumin analogues in amyloid- β aggregation and glioblastoma treatment. *Scientific Reports*, 15(1), 39867. <https://doi.org/10.1038/s41598-025-23467-0>

- Larik, F. A., Saeed, A., Fattah, T. A., Muqadar, U., & Channar, P. A. (2017). Recent advances in the synthesis, biological activities and various applications of ferrocene derivatives. *Applied Organometallic Chemistry*, 31(8), e3664.
- Lasheen, N. N., Shawky, S., Gaber, N., & Mohamed, A. E.-H. A. (2025). A comparative study on L-thyroxine treatment and sesame oil supplementation in experimentally induced hypothyroidism in rats. *Frontiers in Physiology*, 16, 1606528. <https://doi.org/10.3389/fphys.2025.1606528>
- Li, W., Yu, J., Wang, J., Fan, X., Xu, X., Wang, H., ... Wang, Y. (2024). How does ferrocene correlate with ferroptosis? Multiple approaches to explore ferrocene-appended GPX4 inhibitors as anticancer agents. *Chemical Science*, 15(27), 10477–10490. <https://doi.org/10.1039/D4SC02002B>
- Lipinski, C. A. (2004). Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, 1(4), 337–341.
- 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.
- Liu, Z. Q. Potential applications of ferrocene as a structural feature in antioxidants. *Mini Reviews in Medicinal Chemistry*, 2011. PubMed
- Mbaba, M., Khanye, S. D., Smith, G. S., & Biot, C. (2022). Organometallic chemistry of drugs based on iron. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, 261–296.
- Miletić, M., Kovač, V., Barišić, L., Supićić, A., Doskočil, B., Landeka Jurčević, I., ... Murati, T. (2025). Curcumin and Resveratrol vs. Ferrocene-Modified Polyphenols: Role in Enhancing Protective Properties in Human Keratinocytes. *Pharmaceutics*, 17(12). <https://doi.org/10.3390/pharmaceutics17121511>
- Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791.
- Naylor, S. W., McInnes, E. F., Alibhai, J., Burgess, S., & Baily, J. (2025). Development of a Deep Learning Tool to Support the Assessment of Thyroid Follicular Cell Hypertrophy in the Rat. *Toxicologic Pathology*, 53(3), 240–250. <https://doi.org/10.1177/01926233241309328>
- Ning, L., Li, X., Xu, Y., Wang, R., Si, Y., Zhao, H., & Ren, Q. (2026). Mechanistic insights into endocrine-disrupting chemicals in endometriosis and polycystic ovary syndrome: integrating network toxicology and molecular dynamics. *International Journal of Surgery*, 112(3), 6239–6252.
- O’Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3(1), 33.
- Osama, H. M., Khadrawy, S. M., El-Nahass, E.-S., Othman, S. I., & Mohamed, H. M. (2024). Eltroxin and Hesperidin mitigate testicular and renal damage in hypothyroid rats: amelioration of oxidative stress through PPAR γ and Nrf2/HO-1 signaling pathway. *Laboratory Animal Research*, 40(1), 19. <https://doi.org/10.1186/s42826-024-00204-8>

- Patra, M., & Gasser, G. The medicinal chemistry of ferrocene and its derivatives. *Nature Reviews Chemistry*, 2017. Nature
- Peter, S., & Aderibigbe, B. A. (2019). Ferrocene-Based Compounds with Antimalaria/Anticancer Activity. *Molecules* (Basel, Switzerland), 24(19). <https://doi.org/10.3390/molecules24193604>
- Qader, S. M., Muhammed, A. M., Omer, R. A., Abdulkareem, E. I., & Rashid, R. F. (2025). Potential of organometallic complexes in medicinal chemistry. *Reviews in Inorganic Chemistry*, 45(3), 553–563.
- Rastegar-Moghaddam, S. H., Akbarian, M., Rajabian, A., Alipour, F., Hojjati Shargh, A., Masoomi, R., ... Hosseini, M. (2025). Potential therapeutic impacts of vitamin D on hypothyroid-induced heart and kidney fibrosis and oxidative status in male rat. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 398(5), 5237–5248. <https://doi.org/10.1007/s00210-024-03593-8>
- Raveendran, A., Ragunathan, S., Neto, J. F., Mustafa Mohamed, M., Chen, Y. M. K., Baral, S., ... Morani, Z. (2025). Thyroid Disorders and Their Impact on Metabolic Syndrome and Cardiovascular Risk: A Narrative Review. *Cureus*, 17(10), e93669. <https://doi.org/10.7759/cureus.93669>
- Rousset, B., Dupuy, C., Miot, F., & Dumont, J. (2015). Thyroid hormone synthesis and secretion. *Endotext*.
- Singh, A. et al. Ferrocene-appended pharmacophores. *Dalton Transactions*, 2019. PubMed
- Snegur, L. V. Modern trends in bio-organometallic ferrocene chemistry. *Inorganics*, 2022. [mdpi.com](https://doi.org/10.3390/in10010011)
- Tambones, I., & Le Maire, A. (2025). Structural insights into thyroid hormone receptors. *Endocrinology*, 166(1), bqae154.
- Tomar, V., Kumar, P., Sharma, D., Joshi, R. K., & Nemiwal, M. (2025). Anticancer potential of ferrocene-containing derivatives: Current and future prospective. *Journal of Molecular Structure*, 1319, 139589. [https://doi.org/https://doi.org/10.1016/j.molstruc.2024.139589](https://doi.org/10.1016/j.molstruc.2024.139589)
- Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461.
- Vaisman, M., et al. (2011). Oxidative stress in thyroid diseases. *Clinical Endocrinology*, 75(3), 325–331.
- Veber, D. F., Johnson, S. R., Cheng, H.-Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, 45(12), 2615–2623.
- Wang, H., Zheng, C., & Wang, P. (2025). Exploring the nexus between hypothyroidism and metabolic dysfunction-associated steatotic liver disease: a UK biobank cohort study. *Scientific Reports*, 15(1), 6692. <https://doi.org/10.1038/s41598-025-91221-7>
- Wang, Z., Haange, S.-B., Haake, V., Huisinga, M., Kamp, H., Buesen, R., ... Bergen, M. von. (2023). Assessing the Influence of Propylthiouracil and Phenytoin on the

References

- Metabolomes of the Thyroid, Liver, and Plasma in Rats. *Metabolites*, 13(7). <https://doi.org/10.3390/metabo13070847>
- Wu, B., & Xie, C. (2019). Liver injury induced by levothyroxine tablets in a patient with hypothyroidism. *Chinese Medical Journal*, 132(16), 2015–2016. <https://doi.org/10.1097/CM9.0000000000000340>
 - Wu, G.-L., Tan, S., Tan, X., Chen, G., & Yang, Q. (2025). Recent advances in ferrocene-based nanomedicines for enhanced chemodynamic therapy. *Theranostics*, 15(2), 384–407. <https://doi.org/10.7150/thno.101697>
 - Yu, X., Zhang, Y., Wu, B., Zhao, B., & Wang, C. (2025). Multimolecular interactions from Chinese medicines activate nuclear receptor conformational changes: a potential strategy for anti-inflammatory therapy. *Drug Metabolism Reviews*, 57(4), 535–558.

References
