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ORIGINAL ARTICLE

SYNTHESIS, CHARACTERIZATION, AND INITIAL PHARMACOLOGICAL ASSESSMENT OF NEW NAPROXEN WITH 1,3,4-THIADIAZOL-2-AMINE DERIVATIVES

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ABSTRACT

Background: Phosphorous oxychloride (POCl3) was employed as a catalyst in this particular research to facilitate the reaction between naproxen and thiosemicarbazide. The main objective of this study was to synthesize four distinct compounds, namely naproxen with 1,3,4-thiadiazol-2-amine moiety derivatives followed by N-alkylation, denoted as Gh5, Gh6, Gh7, and Gh8. This study aimed to synthesize and characterize novel naproxen derivatives and evaluate their anti-inflammatory efficacy. A molecular docking analysis was conducted on the newly synthesized compounds against the binding pocket of receptor for COX-2 to predict their potential activity.

Materials & Methods: The synthesis of the compounds involved the reaction of naproxen and thiosemicarbazide in the presence of POCl3. The resulting products, Gh5, Gh6, Gh7, and Gh8, were subjected to FT-IR and 1H NMR spectroscopy for comprehensive characterization. Additionally, the anti-inflammatory efficacy of the compounds was assessed in vivo using paw edema tests in Albino rats induced by egg whites. The evaluation involved comparing the effectiveness of the synthesized compounds with naproxen as the reference substance and DMSO serving as a control and solvent.

Results: The results of the anti-inflammatory effectiveness assessment revealed that the formulated compounds exhibited noteworthy properties in alleviating induced paw edema in Albino rats, demonstrating efficacy comparable to that of the reference compound. The experimental findings suggested that the synthesized compounds might even exhibit superior anti-inflammatory effects compared to the reference compound. The molecular docking analysis provided insights into the potential interactions within the COX-2 binding pocket, supporting the observed biological activity.

Conclusion: The synthesized naproxen derivatives, Gh5, Gh6, Gh7, and Gh8, exhibited significant anti-inflammatory properties, potentially surpassing the effectiveness of naproxen. These findings highlight the promising potential of these compounds for further exploration in anti-inflammatory research.

KEY WORDS: Nonsteroidal anti-inflammatory drugs; Heterocyclic compounds; 1,3,4-thiadiazole; Naproxen; thiosemicarbazide.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a popular class of medications across the world.[1] Extensive research has provided significant evidence of their effectiveness in providing pain relief, reducing inflammation, and lowering fever.[2] Furthermore, it has been proposed that they may protect against a variety of major disorders, including cancer and cardiovascular disease.[3,4] However, past research has shown that NSAID usage has deleterious effects on a variety of systems, including the neurological, hepatic, gastrointestinal, cardiovascular, and renal.[5-8]

After receiving FDA approval, naproxen gained popularity as a nonsteroidal anti-inflammatory drug (NSAID) in the latter part of the 20th century. Bicyclic propionic acid is the source of S-naproxen, a reversibly and non-selectively blocked cyclooxygenase with about a five-fold stronger preference for COX1. This compound has analgesic and anti-inflammatory

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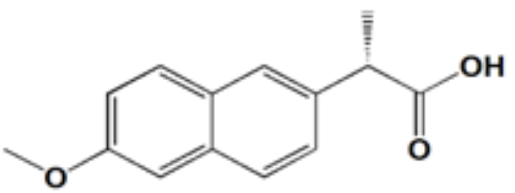
effects. This particular selectivity pattern contributes to the development of gastrointestinal toxicity by diminishing the stomach mucosa's ability to produce physiologically active prostaglandins. Additionally, owing to its acidic nature, naproxen's free carboxyl group has the potential to instigate localized erosion of the stomach lining. Consequently, naproxen's effectiveness as an NSAID lies in its inhibition of cyclooxygenases, thereby impeding the synthesis of prostaglandins responsible for pain and inflammation.[9-11]

Using alternative physiologically productive chemical motifs to mask this functional group might help develop novel compounds that are less ulcer-toxic and more effective anti-inflammatory drugs. Because of this structural change, naproxen may be less effective at inhibiting COX-1 while still being effective at inhibiting COX-2.[12,13]



(*S*)-naproxen

The development of novel and more efficient pharmaceuticals, insecticides, and pesticides is a crucial focus for researchers, who should utilize natural models for inspiration in this endeavor.[14,15] Heterocycles represent a class of drugs that exhibit biological activities akin to those found in natural compounds. Within heterocyclic compounds, there is a substitution of one or more carbon atoms in the ring structure with a different element.[15,16] Researchers can leverage the unique properties of heterocycles to create innovative solutions for various health and environmental challenges, drawing on the rich diversity of natural substances for inspiration in their work. By exploring the structural and functional characteristics of heterocyclic compounds, researchers can unlock new possibilities for the development of safer and more effective medications, insecticides, and pesticides.[16-18]

Thiadiazole stands out as the most widespread and significant heterocyclic moiety within the realm of organic chemistry. This particular moiety has a distinct five-membered ring structure that includes one sulfur atom and two nitrogen atoms, thereby solidifying its classification as a heterocyclic moiety composed of five members. Serving as a fundamental structural framework, thiadiazole acts as a pivotal building block for a diverse array of naturally occurring compounds and materials that exhibit substantial potential in the field of medicine. Noteworthy examples encompass pharmaceutical agents like anti-leishmanial, anti-HIV, antihypertensive, and antibacterial drugs, all of which owe their biological activities to the unique properties conferred by thiadiazole.[19-21]

Thiosemicarbazide holds a significant place.The chemical characteristics of thiosemicarbazide closely resemble those of its semicarbazide counterpart, albeit with a more intricate nature attributed to the thione group's enhanced chemical adaptability compared to the keto group. This increased chemical flexibility of thiosemicarbazide contributes to its broader range of behaviors and potential applications in medicinal chemistry.[22,23]

MATERIALS AND METHODS

Pioneer Co. pharmaceutical companies and Hyper-chem Company (China) provided naproxen. Commercial sources provided the solvents and other chemicals needed for the synthesis, including ethyl acetate, ethanol, hexane, toluene, thionyl chloride, methanol, POCl3, and thiosemicarbazide.

To determine the melting points in open capillary tubes, the Stuart SMP3 melting point equipment was utilized unmodified. The University of Baghdad's College of Pharmacy used Japanese Shimadzu Thin-film technology and an FT-IR spectrophotometer (u, cm-1) to obtain FT-IR spectra. 1H-NMR analysis was conducted at Mashhad University of Medical Sciences utilizing a Bruker ultra shield model operating at 300 MHz, with DMSO serving as the solvent. Chemical changes were expressed in terms of (ppm).

Chemical synthesis:

Synthesis of 5-(1-(6-methoxynaphthalen-2-yl) ethyl)-1, 3, 4-thiadiazole-2-amine. Compound (1).[1,15,24]

The synthesis of 5-(1-(6-methoxynaphthalen-2-yl) ethyl)-1, 3, 4-thiadiazole-2-amine involved the reaction of Naproxen, thiosemicarbazide, and phosphorous oxychloride (POCl3) to produce the desired compound.1 Initially, a solution was prepared by combining 2gm (8.68mml) of naproxen with 0.74gm (8.68mmol) of thiosemicarbazide in a 250 ml round-bottomed flask. This flask was then cooled in an ice bath, and phosphorous oxychloride (4ml) was added slowly to the chilled mixture while stirring continuously. The stirring was carried out at room temperature for 15 minutes before gradually increasing the temperature to 75°C-80°C. The mixture transitioned into a clear, yellow solution, indicating the progression of the reaction. The reflux was maintained for (3-4) hours. Heating was then ceased to bring the reaction back to room temperature. Subsequently, 250 ml of ice water was introduced to the mixture with vigorous stirring. The temperature was raised to 80°C, and stirring was continued for an additional 3 hours. Upon cooling, filtration was performed to isolate the precipitate, which was then neutralized with an aqueous solution containing 10%

KOH. The resulting product was filtered, washed with water multiple times, and allowed to dry for recrystallization using an aqueous ethanol solution.

5-(1-(6-methoxynaphthalen-2-yl) ethyl)-1, 3, 4-thiadiazole-2-amine. Compound (1)

The result was a yellow powder with an M.P. of 160–163 °C and a 90% yield.IR, (υ cm-1): 3283, 3255 symmetric and asymmetric stretching vibration of (NH2), 3105 Aromatic (C-H) stretching vibration, 2974, 2935 (C-H) asymmetrical stretching vibration of –CH3 group, 2839 (C-H) symmetrical stretching vibration of –CH3 group, 1604 stretching vibration of (C=N), 1604, 1485 Stretching vibration of (C=C) skeleton, 1454 (C-H) in-plane bending vibration of –CH3 group, 1373 (C-H) in-plane bending vibration of –CH3 group, 1261 (C-N) stretching vibration, 1215 (C-O-C) stretching vibration of ether, 856 and 813 Out-of-plane bending vibration of β substituted naphthalene.

Synthesis of 1, 3, 4-thiadiazole-2-amine derivatives (Gh5-8).[1,25]

In a 250 ml round flask, we dissolve 0.25 g, equivalent to 0.96 mmol, of Compound (1), in 25 ml of acetone. Subsequently, we introduce either 0.18 gm, corresponding to 1.2 mmol, of potassium carbonate or 7 drops, equating to 1.2 mmol, of triethylamine into the flask. The solution is then subjected to continuous stirring. Gradually, we introduce 1.06 mmol of each of the substituted groups separately. These groups include 0.14 ml of 3-bromobenzyl chloride (for compound Gh5), 0.33 gm of 4-bromophenacyl bromide (for compound Gh6), 0.25 gm of 3-methoxybenzyl chloride (for compound Gh7), and 0.15 ml of 3-chlorobenzyl chloride (for compound Gh8). The next step involves a reflux reaction for a duration of 7 hours. Subsequent to this reaction, cooling is initiated, after which the solvent evaporates to produce a powdered material. After the reaction mixture has reached room temperature, distilled water is added to neutralize it. Three separate 50 ml volumes of ethyl acetate are used for the extraction of the final product. The combined organic layer is then washed with 50 cc of brine, dried over 20 g of anhydrous sodium sulfate, and filtered. A crude product was created after the solvent evaporated. This product can be refined even more by recrystallization using 70% ethanol.

(N-(3-bromobenzyl)-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine (compound Gh5)

A satisfactory powdery material possessing a delicate nutty scent was acquired with a yield percentage of 71%. The substance exhibited a melting point range of 120-123 °C. Upon analysis, the infrared spectrum presented a variety of peaks, such as 3259 cm^{-1} corresponding to the stretching vibration of secondary amine (NH), 3059 cm^{-1} related to aromatic (C-H) stretching vibration, 2974 cm^{-1} and 2935 cm^{-1} associated with the asymmetrical stretching vibration of the –CH3 group, 2839 cm^{-1} denoting the symmetrical stretching vibration of the –CH3 group, 1604 cm^{-1} signifying the stretching vibration of (C=N), and 1604 cm^{-1} along with 1570 cm^{-1} for the stretching vibration of (C=C) skeleton. Additionally, the spectrum displayed peaks at 1454 cm^{-1} for the in-plane bending vibration of the –CH3 group, 1373 cm^{-1} representing the in-plane bending vibration of the –CH3 group, 1261 cm^{-1} indicating the stretching vibration of (C-N), 1215 cm^{-1} for the stretching vibration of ether (C-O-C), and 856 cm^{-1} as well as 813 cm^{-1} for the out-of-plane bending vibration of β-substituted naphthalene. Moreover, the proton NMR spectrum depicted peaks at 1.28 ppm (d, 3H, CH3-), 3.05 ppm (S, 3H, CH3-O), 3.94 ppm (S, 2H, CH2 –N), 4.49 ppm (q, 1H, CH), 5.58 ppm (S, 1H, NH), and a multiplet spanning from 7.10 to 7.83 ppm representing 10 aromatic hydrogens.

1-(4-bromophenyl)-2-((5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-yl)amino)ethan-1-one (compound Gh6)

A dark yellow powder with a yield percentage of 78% and M.P. of 105–108 °C.IR, (υ cm-1): 3267 secondary amine (NH) stretching vibration, 3105 aromatic (C-H) stretching vibration, 2974, 2931 (C-H) asymmetrical stretching vibration of –CH3,CH2 groups, 2839 (C-H) symmetrical stretching vibration of –CH3 group, 1697 (C=O) ketone stretching vibration, 1604 (C=N) stretching vibration, 1604, 1485 stretching vibration of (C=C) skeleton, 1454 (C-H) in-plane bending vibration of –CH3 group, 1373 (C-H) in-plane bending vibration of –CH3 group, 1261 (C-N) stretching vibration, 1215 (C-O-C) stretching vibration of ether, and 852 and 813 Out-of-plane bending vibration of β substituted naphthalene.H NMR: 4.49 ppm (q, 1H, CH), 5.26 ppm (S, 1H,NH), 7.14-7.83 ppm (m,10H, aromatic H), 3.89 ppm (S, 2H, CH2 –N), 1.21 ppm (d, 3H, CH3-), 3.10 ppm (S, 3H, CH3-O).

N-(3-methoxybenzyl)-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine(compound Gh7)

A white powder with a yield percentage of 74%, M.P. (217–220 °C).IR, (ω cm^{-1}): 3255: secondary amine (NH) stretching vibration; Among the others are 3105: aromatic (C-H) stretching vibration; 2974, 2931 (C-H) asymmetrical stretching vibration of –CH3, CH2 groups; 2839 (C-H) symmetrical stretching vibration of –CH3 group; 1604: stretching vibration of (C=N); 1604, 1512: stretching vibration of (C=C) skeleton; 1454: C-H in-plane bending vibration of –CH3 group; 1373: C-H in-plane bending vibration of –CH3 group; 1261 (C-N) stretching vibration; 1215 (C-O-C) stretching vibration of ether; and 856 and 813 Out-of-plane bending vibration of β substituted naphthalene are among the others.H

NMR data: 3.07 ppm (S,6H, (CH3-O)2), 3.74 ppm (S,2H, CH2 –N), 4.76 ppm (q,1H, CH), 5.41 ppm (S,1H, NH), 6.85-7.82 ppm (m,10H, aromatic H).

(N-(3-chlorobenzyl)-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine (compound Gh8)

A light yellow powder with a yield percentage of 68%, M.P. (160–163 °C).IR, (u cm-1): 3263 secondary amine (NH) stretching vibration, 3120 aromatic (C-H) stretching vibration, 2974, 2931 (C-H) asymmetrical stretching vibration of –CH3, CH2 groups, 2839 (C–H) symmetrical stretching vibration of –CH3 group, 1604 stretching vibration of (C=N), 1604, 1504 stretching vibration of (C=C) skeleton, 1454 (C–H) in-plane bending vibration of –CH3 group, 1373 (C–H) in-plane bending vibration of –CH3 group, 1261 (C-N) stretching vibration, 1215 (C-O-C) stretching vibration of ether, and 852 and 813 Out-of-plane bending vibration of β substituted naphthalene.H NMR: d, 3H, CH3-; 3.06 ppm; S, 3H, CH3-O; 3.87 ppm; S, 2H, CH2 –N; 4.77 ppm; q, 1H, CH; 5.46 ppm; S, 1H, NH; 7.17-7.82 ppm (m,10H, aromatic H).

Molecular docking study

In the process of selecting the molecular targets, it is crucial to examine the potential functions of the recently created compounds Gh5-8 and compare them thoroughly with various other ligands. The goal of this comparative research is to pinpoint the pharmacophoric characteristics of the drugs that enable them to bind to target site important amino acid residues in an efficient manner. Using information from the protein data bank at https://www.rcsb.org/ resulted in a thorough decision-making process. To choose the appropriate location. The compounds were extensively tested against numerous binding sites to assess their interactions, with the results playing a key role in identifying suitable proteins for further docking studies. Once a specific protein was chosen as the target site, a series of procedures were carried out to understand how the tested compounds bind at a molecular level within the receptor's binding pocket for COX-2. The docking simulation was performed using Glide, an advanced software program developed by Schrödinger (version 2023). The ligand co-crystallized with the crystal protein provided the binding sites employed in the analysis (PDB codes: 3q7d). The complex's water molecules were first eliminated, and any crystallographic flaws and unfilled valence atoms were then fixed utilizing the software tool's clean protein functions, utility, and protein report.[26,27]

Anti-inflammatory activity.[28-29]

The anti-inflammatory properties of the resultant compounds, Gh5, Gh6, Gh7, and Gh8, were assessed in vivo using the egg-white induced paw edema method, which measures the reduction in paw swelling. Subsequently, these findings were contrasted with the anti-inflammatory efficacy of naproxen (utilized as a standard) and DMSO (employed as a control) for further analysis and comparison. The analysis comprised determining how the produced chemicals compared to well-known anti-inflammatory drugs such as naproxen and DMSO in terms of their effect on inflammation in a living organism, specifically with regard to the alleviation of paw edema.

The methods for anti-inflammatory study

The target compounds' anti-inflammatory properties were investigated at the University of Karbala's College of Pharmacy in Karbala, Iraq.

The weight of albino rats was estimated to be arranged (170 – 215) grams, with careful measurements taken for accuracy. Each group of rats consisted of six individuals, carefully categorized based on sex for research purposes. All animals were accommodated in the facility's specialized animal housing units, ensuring a controlled environment for the study. The rats were placed in identical locations within the facility, following strict protocols to maintain standardized conditions conducive the experiment. Furthermore, the rats were provided with a balanced diet of commercial chow and had unrestricted access to clean water throughout the duration of the study.

- Group 1: six rats were employed as a control group in the experiment to ensure accurate comparisons and were administered intraperitoneal injections of dimethyl sulfoxide (DMSO), a common solvent utilized in various research studies.
- Group 2: As reference subjects, six rats were given a dosage of naproxen dissolved in DMSO at a concentration of 10 mg/kg.
- Groups 3-6: The rats were injected with specific compounds from each of the four groups (Gh5, Gh6, Gh7, and Gh8). DMSO has been dissolved.

Thirty minutes later, the rats received the drugs or their carriers (IPE), and 0.1 ml of undiluted egg white was injected into the plantar side of their hind feet to cause inflammation. Using a Vernier caliper, the width of the paw was measured seven times after the medication injection: 0. 30 60, 120, 180, 240, and 300 minutes.

Determination of Dose*:* While the naproxen dosage was given in mg/kg, the dosages of the synthesized targeted compounds (Table1) were estimated following the basic procedure outlined below. **The dose of the target compound / its molecular weight = the dose of the reference / its molecular weight.**[28]

Table (1): The final compounds, DMSO, and naproxen molecular weights and dosages

Compound sample	Molecular weight	Dose mg/ kg
Naproxen(reference)	230.259	10
DMSO(control)	------	2ml
Gh5	454.39	19.73
Gh6	482. 4	20.95
Gh7	405.52	17.61
Gh8	409.93	17.80

Statistical analysis[13,30,31]

The data gathered for this study was analyzed with the Statistical Package for the Social Sciences (SPSS version 26). The information was displayed in the relevant tables and graphs as the mean and standard deviation. When necessary, post hoc analysis and two-way ANOVA were performed to examine differences between and within groups as well as any potential associations between the study's associated variables, when the p-value was equal to or less than 0.05 (P-value $\leq$ 0.05).

Evaluation of anti-inflammatory activity for final synthesized compounds(Gh5, Gh6, Gh7, and Gh8):

An experimental model involving the induction of paw edema using egg white was employed to assess the potential anti-inflammatory properties exhibited by the newly synthesized compounds (Gh5, Gh6, Gh7, and Gh8), where pure egg white is injected subcutaneously into the hind paw of rats on the plantar side for evaluation purposes, resulting in inflammation that is characterized by increased exudation of tissue water and plasma proteins, extravasation of neutrophils, and extravasation of plasma. The metabolism of arachidonic acid is the root cause of all these actions.[32] This in vivo technology has a number of benefits over other approaches, including speedy evaluation since inflammation is detected early and quickly, high paw sensitivity for inflammation, no anesthetic requirement, cost, and usability.[33,34]

RESULTS AND DISCUSSION

Chemistry

Scheme 1 depicts the synthetic methodologies employed in the fabrication of the ultimate chemical targets (Gh5, Gh6, Gh7, and Gh8). These targets were obtained by the reaction of the parent nucleus (naproxen) and thiosemicarbazide with POCl3, which acted as a catalyst. The potent oxidation effect of this agent leads to the conversion of the carbonyl group of naproxen into an acid chloride. When NH2, particularly thiosemicarbazide, is present, the ring closes to generate a 1,3,4-thiadazole-2-amine moiety, resulting in the production of compound (1), namely, 5-(1-(6-methoxynaphthalen-2-yl) ethyl)-1, 3, 4-thiadiazole-2-amine. Then, by a reflux reaction in a basic media, one of the two hydrogen atoms in the 1,3,4-thiadiazole ring is replaced with an alkyl halide group in an alkylation reaction of NH2. Following this series of reactions, the intended final compounds Gh5, Gh6, Gh7, and Gh8 are formed.

The unique asymmetrical and symmetrical stretching vibration of (NH2) at (3283,3255) cm^{-1}, which corresponds to the principal amine N–H in the FTIR spectrum data of the produced compounds, made it easier to identify Naproxen 1,3,4-thiadazole-2-amine (compound 1). The wide band linked to the carboxylic acid (O–H) group of naproxen at 3159 cm-1 vanished along with this. Two different bands, indicative of ring formation, were identified upon the synthesis of the 1,3,4-thiadiazole-2-amine moiety: the (C=N) stretching band at 1604 cm-1 and the (C=C) skeleton stretching band at 1485 cm^{-1}.

The last stage of the process was the disappearing of bands connected to the main amine in the thiadiazole ring at (3283) cm^{-1}, which indicated that the synthesis of the final compounds (Gh5, Gh6, Gh7, and Gh8) had been completed. This spectral pattern held true for all of the completed compounds. Interestingly, compound (Gh6) showed the appearance of a strong (C=O) ketone band at 1697 cm^{-1}, which provided more insight into the structure and chemical makeup of the produced product.

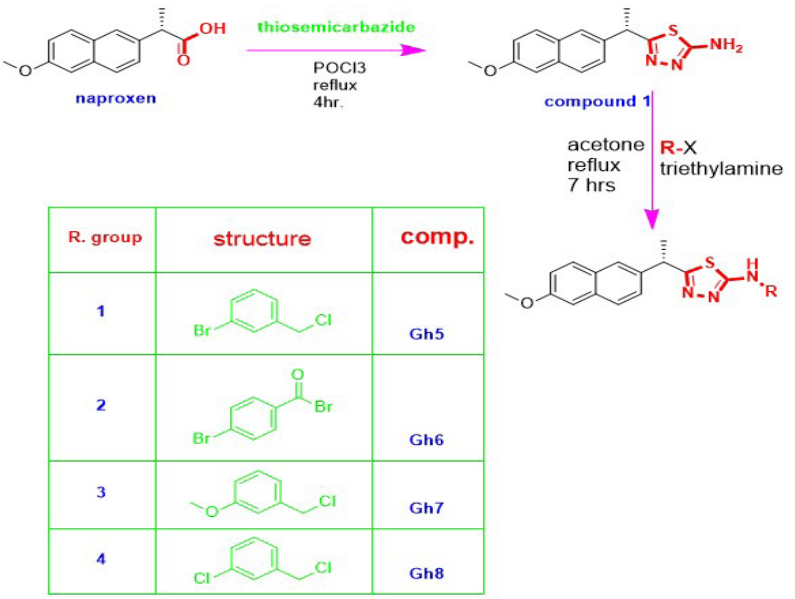
The ^{1}HNMR spectra supported the indicated derivative structure. Compound (Gh5) had a quartet peak (4.49) ppm generated by the (C-H) of (CH-CH3), a singlet peak (3.15) ppm induced by the protons (OCH_3), and a doublet peak for (CH3) of (CH3-CH) at (1.28) ppm. The (C-H2) protons of the (CH2-N) generated a singlet peak at 3.94 ppm. At 5.58 ppm, the (H) proton of the (H-N) generated another singlet signal. Finally, several (10H) aromatic peaks were seen at 7.10–7.83) ppm.

Compound (Gh6) featured a singlet peak at 3.10 ppm brought on by the (3) protons of the (OCH_3) group, and a quartet peak at (4.49) ppm produced by the (C-H) of (CH-CH3), and a doublet peak for (CH3) of (CH3-CH) at (1.21) ppm. The (C-H2) protons of the (CH2-N) generated a singlet peak at 3.89 ppm. At 5.26 ppm, the (H) proton of the (H-N) generated another singlet signal. Finally, several (10H) aromatic peaks were seen at 7.14–7.83) ppm.

Compound (Gh7) had a quartet peak (4.76) ppm resulting from the (C-H) of (CH-CH3), a singlet peak (3.17) resulting from the (6) protons of two (OCH_3) groups, and a doublet peak for (CH3) of (CH3-CH) at (1.21) ppm. The (C-H2) protons of the (CH2-N) generated a singlet peak at 3.74 ppm. At 5.41 ppm, the (H) proton of the (H-N) generated another singlet signal. Finally, several (10H) aromatic peaks were seen at 6.85–7.82 ppm.

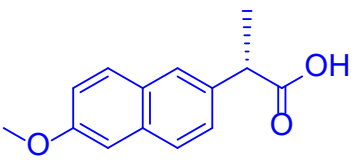
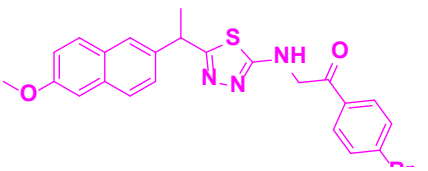
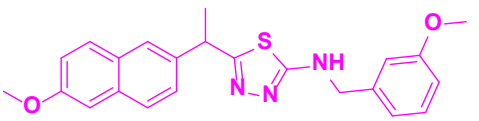
Ultimately, Compound (Gh8) displayed a quartet peak

(4.77) ppm resulting from the (C-H) of (CH-CH3), a singlet peak (3.16) ppm resulting from the protons (OCH_3), and a doublet peak for (CH3) of (CH3-CH) at (1.21) ppm. The (C-H2) protons of the (CH2-N) generated a singlet peak at 3.87 ppm. At 5.46 ppm, the (H) proton of the (H-N) generated another singlet signal. Finally, several (10H) aromatic peaks were seen at 7.17–7.82 ppm.



Scheme 1. Synthesis of 1, 3, 4-thiadiazole-2-amine derivatives compounds.

Table (2): The docking scores (ΔG, kcal/mole) for the naproxen and final compounds with the COX 2 enzyme binding site are listed in PDB ID: 3q7d.

Comp.	structure	Docking score in (kcal/mole)
Naproxen		-8.707
Gh5		-8.024
Gh6		-3.653
Gh7		-8.49
Gh8		-7.335

Molecular docking results:

The grid-based CDOCKER methodology was utilized to conduct the molecular docking approach, where ligands are inserted into receptor binding sites using a CHARMM-based molecular dynamics (MD) approach. Throughout the refining process, it is common for the ligands to exhibit flexibility while the receptor remains rigid in structure. This method typically involves each chemical having three potential interactions with the protein being studied, providing a detailed insight into the molecular interactions at play. In order to further analyze and compare the results, the docking scores, known as CDOCKER interaction energy, were recorded for the most optimal positions within the active site - specifically, the binding site of the COX 2 enzyme. These findings were compiled and presented in Table (2) for easy reference and examination by researchers and stakeholders in the field.

In addition to the illustrations in figures (1) and (2) depicting a two-dimensional view of some examples of tested compounds (naproxen and Gh7) docked in the binding site of the COX 2 enzyme.

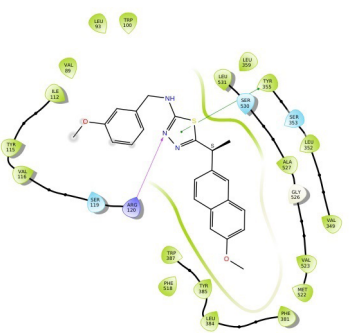


Figure (2): Two-dimensional view of compound Gh7 docked in the COX 2 enzyme binding site.

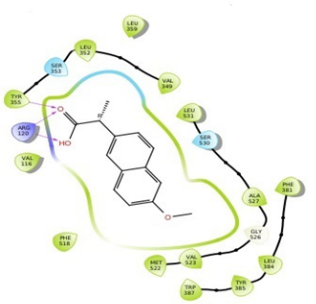


Figure (1): Two-dimensional view of naproxen docked in the COX 2 enzyme binding site.

The results of the molecular docking analysis for the compounds being studied showed a strong similarity to the binding outcomes of naproxen with the COX2 enzyme. Initially, naproxen had a docking score of (-8.7) kcal/mole, indicating a high binding affinity, supported by important hydrogen bond formations with TYR355 and ARG120 involving the carbonyl group, and with ARG120 involving the hydroxyl group, as shown in Figure (1). These specific interactions are crucial for stabilizing the ligand in the enzyme's active site, enhancing its inhibitory effect.

Another example illustrated in Figure (2) concerns compound Gh7, where hydrogen bonds were formed through the nitrogen atom in the thiadiazole ring with ARG120, and the thiadiazole ring interacted with TYR355 via pi-pi stacking interactions. The docking score for this compound was (-8.49) kcal/mol. In essence, the ability of these compounds to establish strong and lasting interactions with key residues within the active site significantly impacts their binding strengths. Typically, higher binding affinities are achieved with more hydrogen bonding and pi-pi stacking interactions, a trend observed for all tested compounds except compound Gh6, which displayed a notably low docking score of (-3.653) kcal/mole.

The Anti-Inflammatory Activity

An egg white-induced paw edema model was used to test the anti-inflammatory effects of the final synthesized compounds (Gh5, Gh6, Gh7, and Gh8). In this experimental setting, rats get subcutaneous injections of undiluted egg whites into the plantar side of their hind foot. This causes an inflammatory response that is characterized by an increase in tissue water content, exudation of plasma proteins, and extravasations of neutrophils and plasma. These inflammatory manifestations are primarily driven by the metabolism of arachidonic acid within the biological system. The utilization of this in vivo technique offers several advantages over traditional approaches, such as prompt assessment facilitated by the early and rapid detection of inflammation, heightened paw sensitivity to inflammatory stimuli, absence of anesthesia requirement, cost-effectiveness, and user-friendly application.[8,27,30,31]

Table: (3): Time's influence on the thickness of paw edema in various groups.

Group	Mean ± S.T.D
R	6.856±1.382
C	7.143±1.384a,b
Gh5	6.708 ±1.229a,c,d
Gh6	6.780±1.070b,e
Gh7	7.086±1.296c,e
Ch8	7.111±1.563d

[a] There is Significant difference between C, Gh5 (P-value = 0.0006) < 0.05

[b] There is Significant difference between C, Gh6 (P-value = 0.0179) < 0.05

[c] There is Significant difference between Gh5, Gh7 (P- value= 0.0032) < 0.05

[d] There is Significant difference between Gh5, Gh8 (P- value = 0.0038) < 0.05

[e] There is Significant difference between Gh6, Gh7 (P- value = 0.0253) < 0.05.

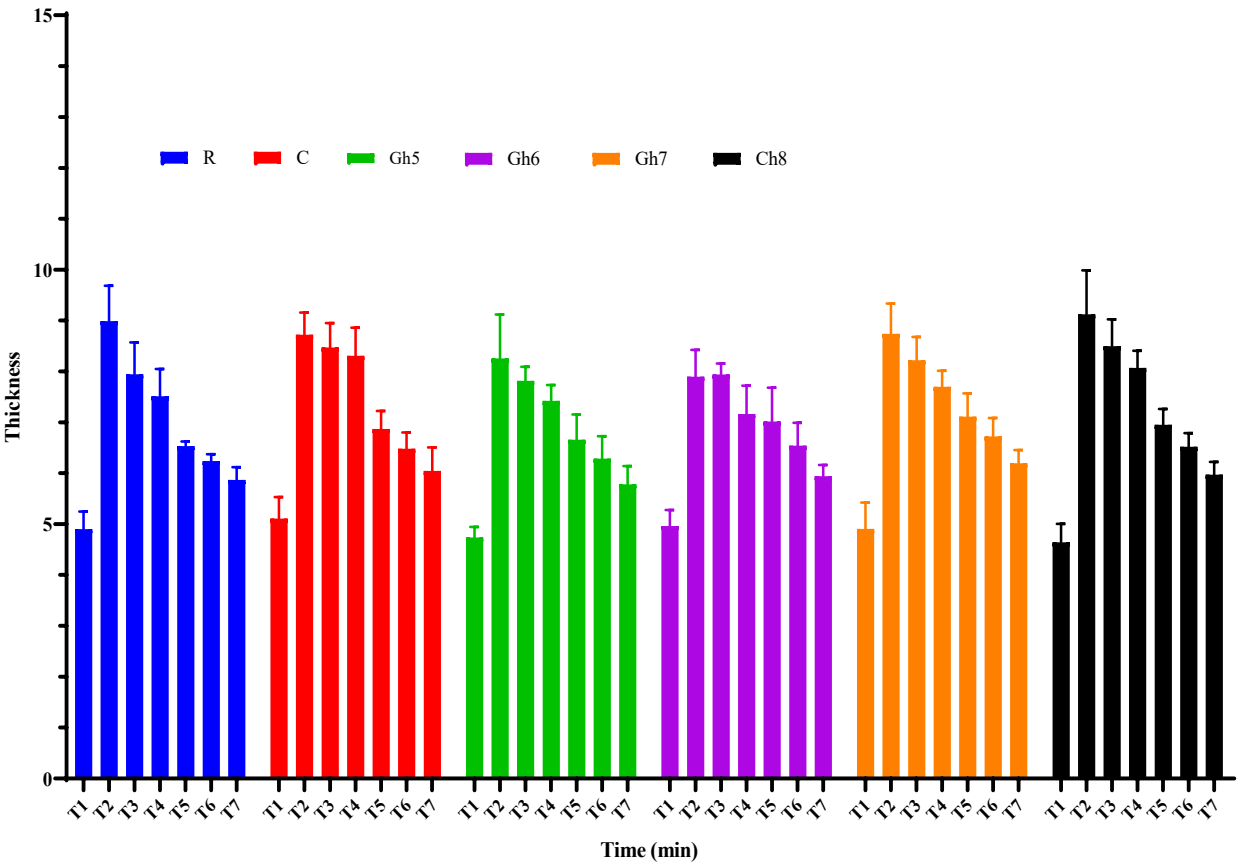


Figure (3): Naproxen, dimethyl sulfoxide (DMSO), and the chemicals Gh5, Gh6, Gh7, and Gh8 on egg whites caused paw edema in rats. The data are presented as percentages and means ± SEM.

The investigation into the paw edema caused by egg whites in rats was conducted concerning naproxen, dimethyl sulfoxide (DMSO), and the chemicals Gh5, Gh6, Gh7, and Gh8, as illustrated in Figure (1). The results are presented as mean ± SEM along with percentages, offering a comprehensive view of the outcomes. In the comparative analysis, the standard drug, naproxen, was juxtaposed with the control group treated with DMSO, as well as the experimental compounds Gh5, Gh6, Gh7, and Gh8, as delineated in Table 3. Notably, the findings revealed the highest level of inhibition among the substances assessed. Interestingly, statistical analysis indicated significant differences among these compounds at various time points as well as between control versus Gh5 and Gh6 where p-value were less than (0.05%) and equal to (0.0006, 0.0179) respectively.

Also the same case recording among each following compounds through practical experience where there is Significant difference between:

1. Gh5, Gh7 (P- value= 0.0032) < 0.05
2. Gh5, Gh8 (P- value = 0.0038) < 0.05
3. Gh6, Gh7 (P- value = 0.0253) < 0.05.

In fact, post administration the final target compounds to the Albino rats the efficacy of each synthesized molecule in reducing the induced edema was found to be nearly equivalent to that of the reference compound, naproxen, particularly for compounds Gh5-7. The mean anti-inflammatory activity values were calculated at 6.708 mm for Gh5, 6.780mm for Gh6, and 7.086mm for Gh7, whereas naproxen exhibited a value of 6.856mm.

CONCLUSION

The synthesized naproxen derivatives, Gh5, Gh6, Gh7, and Gh8, exhibited significant anti-inflammatory properties, potentially surpassing the effectiveness of naproxen. These findings highlight the promising potential of these compounds for further exploration in anti-inflammatory research. Naproxen is a nonsteroidal anti-inflammatory medication, and its anti-inflammatory qualities are preserved through the production of new molecules. Rats with paw edema generated by egg whites were used in in vivo anti-inflammatory investigations, and compounds (Gh5-6) at time T4 (120min) and compound (Gh5) at time T7 (300min) frequently outperformed them.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	GAM, ZAD
Acquisition, Analysis or Interpretation of Data:	GAM, ZAD
Manuscript Writing & Approval:	GAM, ZAD

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.