

Original Research Article

Synergistic anti-*Helicobacter pylori* effect of licorice extract and metronidazole-2-thiouracil derivatives: *In vitro* and *in silico* considerations

Mahmood J Jawad¹, Ali H Ijam¹, Jalal H Mohammed², Fatima Razzaq Mohammed³, Samir M Awad^{1,4}, Mohamed F El-Shehry⁵, Ahmed A El-Rashedy^{6,7*}

¹Pharmacy Department, Al Zahrawi College University, Karbala, ²Pharmacy Department, University of Karbala, Karbala, ³Clinical Laboratory Sciences department, Faculty of Pharmacy, Alzahraa University for Women, Karbala, Iraq, ⁴Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Helwan, ⁵Pesticides Chemistry Department, National Research Centre, Dokki, Giza 12622, ⁶Chemistry of Natural and Microbial Products, National Research Centre, Dokki, Giza, ⁷Department Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Sadat City, Menoufia 32897, Egypt

*For correspondence: Email: ahmedelrashedy45@gmail.com

Sent for review: 27 April 2025

Revised accepted: 2 October 2025

Abstract

Purpose: To assess the metronidazole-resistant *Helicobacter pylori* properties of licorice extract and new metronidazole derivatives from 2-thiouracil moiety using *in vitro* and *in silico* models.

Methods: *In vitro* anti-*Helicobacter pylori* activity of licorice extract and new compounds 4a, 4b, and 7 were evaluated against clinical isolates of *Helicobacter pylori* using the disk diffusion method with concentrations of 8, 16, and 32 µg/disk for each sample, using metronidazole as the standard. The docking studies were carried out using Auto Dock 4.2 on *Helicobacter pylori* urease enzyme with protein data bank (PDB) ID code 1E9Z as *Helicobacter pylori* inhibitor.

Results: All synthesized compounds and licorice extract demonstrated significantly greater inhibitory activity against metronidazole-resistant *H. pylori* compared to metronidazole alone ($p < 0.01$ at 32 µg/disk). Compound 4a exhibited the highest potency, with a mean zone of inhibition of 28.5 ± 1.2 mm at 32 µg/disk, outperforming both metronidazole (12.3 ± 0.8 mm) and other derivatives. A synergistic combination of compound 4a and licorice extract resulted in a ~35 % increase in zone of inhibition compared to 4a alone. Docking studies confirmed that compound 4a had the strongest binding affinity to the urease active site (calculated binding energy: -9.8 kcal/mol), forming stable hydrogen bonds with key residues, which correlates with its superior efficacy.

Conclusion: Compound 4a is promising lead compound with efficacy against resistant *H. pylori*. Furthermore, licorice extract acts as a potent synergistic agent, significantly enhancing the activity of 4a. Compound 4a-licorice combination represents a viable and novel therapeutic strategy to overcome metronidazole-resistant *H. pylori* infection and warrants further *in vivo* investigation.

Keywords: Licorice, Metronidazole, 2-Thiouracil, Anti-*Helicobacter pylori* agents

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Over half of the world's population is afflicted with the *Helicobacter pylori* (*H. pylori*) infection,

which causes stomach ulcers in humans, particularly during childhood. Since the bacterial infection has no symptoms in its early stages, the majority of people are unaware of it [1].

Moreover, there are numerous treatment protocols, the most well-known of which is the triple therapy, which consists of downoprazol, metronidazole, and clarithromycin. One of the inconvenient conditions that affects the lining of the digestive system, particularly the duodenum and stomach, is peptic ulcer. In severe stages, symptoms include bleeding, epigastric pain, and a burning or gnawing stomach. It could be a stomach ulcer, which accounts for about 20 % of instances, or a duodenal ulcer, which accounts for nearly 80% of cases. Up to 70 % of people have peptic ulcers without any symptoms [1].

Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and ibuprofen, which block prostaglandins from repairing tissue damage, are one of the many possible causes of peptic ulcers. In addition, stomach cancer, Zollinger-Elison syndrome, Crohn's disease, chemotherapy, radiation therapy, ischemia, or extreme physiological stress may be the reason [2]. Infections with *Helicobacter pylori*, which can cause overgrowth and alter the natural balance of microorganisms lining the stomach, are another important cause. This triggers an inflammatory reaction. Antibiotics like amoxicillin, clarithromycin, metronidazole, and doxycycline are among the drugs used in this situation [3]. Even though metronidazole is widely used in treatment, resistance to this drug has emerged; therefore, new derivatives must be created to solve this problem.

Modified metronidazole derivatives have been created using AutodockVina software and their efficacy has been increased by adding either uracil or 2-thiouracil in this study. However, traditional antibiotics are not recommended for long-term use; instead, alternative phytotherapy, which is the use of plant-derived medicinal products for therapeutic purposes, is recommended as a complementary approach for *Helicobacter pylori* (*H. pylori*) infections due to the antimicrobial, anti-inflammatory, and gastroprotective properties of certain medicinal plants [4]. While conventional treatment typically involves antibiotics (like clarithromycin and amoxicillin) and proton pump inhibitors (PPIs), antibiotic resistance and side effects have led to interest in phytotherapeutic alternatives. The term "licorice" refers to the rhizomes and roots of glycyrrhizin species (*Glycyrrhiza glabra*), which have historically been utilized for therapeutic purposes as anti-*H. pylori* medications, six glycyrrhizin and its metabolites-glycyrrhizin, 18 β -glycyrrhetic acid, glabridin, licochalcone A, licoricidin, and glycycomarin-are used [5,6]. Furthermore, licorice root prevents bacteria from

adhering to cell membranes and functions as a healing agent.

Adding licorice to the clarithromycin-based triple therapy regimen enhances the eradication of *H. pylori*, which is used as an anti-ulcer medication in the treatment of peptic ulcers [7] active constituents of licorice extract that have anti-*H. pylori* activity includes glabridin and glabrene (*Glycyrrhiza glabra*), licochalcone A (*G. inflata*), licoricidin and licoisoflavone B (*G. uralensis*), in addition to 3-arylcoumarin, pterocarpan, isoflavan, gancaonols A-C, vestitol, licoricone, 1-methoxyphaseollidin, gancaanol, glycerin, formononetin, glyasperin D, and others [8,9].

EXPERIMENTAL

Plant preparation

The aqueous extract of licorice roots (*Glycyrrhiza glabra*) was prepared by grinding well-dried plant roots, and the resulting powdered sample was then soaked in water; the mixture was subsequently placed on a rotary shaker. The extract was purified by filtering twice through the Whatman No. 1 filter paper. After purification, each extract was diluted to the required volume for each concentration [10].

Preparation of alcohol extract by Soxhlet method

A muslin cloth bag containing 100 grams of powdered licorice root was added to the Soxhlet extractor's body. 500 milliliters of absolute ethanol were then added as a solvent. The isomantle was used to heat the solvent under reflux, causing it to evaporate and condense into the reservoir holding the plant extract. The procedure lasted for six hours. After gathering the extract, the ethanol was evaporated at 40 °C, leaving behind 2 - 3 mL of extract, which was then stored in a porcelain bowl until all the ethanol had evaporated. The extract was refrigerated until subsequent biological investigation [11].

Procedures

1-(2-Hydroxyethyl)-5-methyl-2-nitro-1*H*-imidazole (3) [12], and 1-(2-chloroethyl)-5-methyl-2-nitro-1*H*-imidazole (6) [13] were used.

General procedure for synthesis of compounds (4a and b)

A mixture of 2-(2-methyl-5-amino-1*H*-imidazole-1-yl) ethanol **2** (0.7 g, 0.005 mole) and 2-thiouracil-and/or uracil-5-sulfonyl chloride (0.005

mole) and pyridine (0.4 mL, 0.005 mole) in 50 mL absolute ethanol was refluxed for 12 hours, then cooled and neutralized by pouring into acidified ice/water, filtered off, dried and recrystallized from Dimethylformamide (DMF)/water.

***N*-(1-(2-Hydroxyethyl)-2-methyl-1*H*-imidazol-5-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-sulfonamide (4a):**

Yield: 75 %; mp: 257 - 259 °C; IR (KBr cm⁻¹): 3359 (OH), 3239 (NH), 3165 (CH aromatic), 1688 (C=O), 1652 (C=N), 1383, 1137 (SO₂), 1271 (C=S). ¹H NMR (DMSO-*d*₆), δ : 2.0₂ (s, 3H, CH₃), 3.31, 3.94 (2t, 4H, 2CH₂), 4.31 (br.s, 1H, OH, D₂O exchangeable), 7.69, 7.87 (m, 2H, aromatic H), 9.13, 9.84, 11.28 (3s, 3H, 3NH's, D₂O exchangeable), ¹³C NMR (DMSO-*d*₆): δ 17.23, 55.25, 64.13, 120.85, 126.26, 124.4, 126.2, 127.91, 128.18, 128.84, 128.93, 130.16, 152.25, 157.32, 166.22, 173.79. MS: *m/z* (%), 331.04 (M⁺, 100 %), Elemental Anal calculated for C₁₀H₁₃N₅O₄S₂: C, 36.25 %; H, 3.95 %; N, 21.13 %. Found: C, 36.32 %; H, 3.92 %; N, 21.43 %.

***N*-(1-(2-Hydroxyethyl)-2-methyl-1*H*-imidazol-5-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (4b):**

Yield: 73 %; mp: 272 - 274 °C; IR (KBr cm⁻¹): 3487 (OH), 3340 (NH), 3255 (NH), 2975 (CH aliphatic), 1681, 1650 (2C=O), 1654 (C=N), 1380, 1135 (SO₂). ¹H NMR (DMSO-*d*₆), δ : 2.15 (s, 3H, CH₃), 3.92, 4.15 (2t, 4H, 2CH₂), 3.44 (s, 1H, OH, D₂O exchangeable), 7.83, 7.98 (2s, 2H, aromatic H), 9.68, 10.01, 11.30 (3s, 3H, NH's, D₂O exchangeable), ¹³C NMR (DMSO-*d*₆): δ 19.30, 54.51, 63.35, 120.72, 126.36, 130.07, 130.16, 133.41, 149.72, 152.00, 161.51, 166.20. MS: *m/z* (%), 315.06 (M⁺, 100 %), Elemental Anal calculated for C₁₀H₁₃N₅O₅S: C, 38.09 %; H, 4.16 %; N, 22.21 %. Found: C, 38.15 %; H, 4.21 %; N, 22.31 %.

2-(2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethylthio) pyrimidine-4-(3*H*)-one (7)

A mixture of 2-thiouracil (**6**; 1.02 g, 0.001 mol), compound (**5**; 0.52 g, 0.001 mol), and sodium hydroxide (0.5 g) in absolute ethanol (50 mL) was refluxed for eight hours, then cooled and neutralized by pouring into acidified ice/water. The produced solid was filtered off, washed several times with water, dried under suction, and crystallized from DMF/water. Yield: 69 %; mp: 281-283 °C; IR (KBr cm⁻¹): 3378 (NH), 2919 (CH aliphatic), 1681 (C=O), 1653 (C=N), 1350, 1550 (NO₂), ¹H NMR (DMSO-*d*₆), δ : 2.27 (s, 3H, CH₃), 3.01, 3.41 (2t, 4H, 2CH₂), 7.94 - 7.96, 8.02

(m, 3H, aromatic H), 11.78 (s, 1H, NH, D₂O exchangeable), ¹³C NMR (DMSO-*d*₆): δ 19.93, 32.26, 46.98, 109.46, 125.68, 128.97, 130.16, 146.33, 152.23, 158.25, 167.34. MS: *m/z* (%), 281.29 (M⁺, 100%), Elemental Anal calculated for C₁₀H₁₁N₅O₃S: C, 42.70 %; H, 3.94 %; N, 24.90 %. Found: C, 42.75 %; H, 3.89 %; N, 24.83 %.

Instrumental analysis

Melting point was determined using an Electrothermal IA 9100 apparatus (Shimadzu, Japan). Infrared (IR) spectra were recorded on a Perkin-Elmer 1650 spectrophotometer (USA), while ¹H NMR spectra (300 MHz) were obtained on a Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) using dimethyl sulfoxide (DMSO-*d*₆) as the solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units (ppm). Mass spectra and microanalyses (C, H, N) were performed using a Vario Elementary apparatus (Shimadzu, Japan). The progress of all reactions was monitored by thin-layer chromatography (TLC) on silica gel 60 plates (Merck) using a chloroform-methanol (3:1 v/v) mobile phase. Compounds were visualized under ultraviolet (UV) light (254 nm) or by exposure to iodine vapour.

Evaluation of biological activities

Bacterial isolates in a culture condition

Samples of *H. pylori* isolates from stomach biopsies were obtained from the Internal Medicine Department of Hawamdia Hospital. Primary isolation was performed on selective blood agar base no. 2 (Oxoid, Basingstoke, Hants, UK) supplemented with horse blood 5 % (v/v) and one SelectaTab tablet 500 mg (Mast Diagnostic, Merseyside, UK). Following primary selective isolation, *H. pylori* cells were identified according to colony morphology, Gram staining, microaerophilic growth at 37 °C, oxidase+, catalase+, urease+, nitrate-, H₂S-, and hippurate hydrolysis. Growth of *H. pylori* was maintained at 37 °C for 3 - 5 days in an atmosphere of 5 % O₂, 15 % CO₂, and 80 % N₂ in an anaerobic chamber (Hirayama, Tokyo, Japan). Bacterial strains were stored at -70 °C in brain heart infusion broth (BHIB; Difco, East Molesey, UK) containing 10 % (v/v) fetal calf serum (FCS) and 15 % (v/v) glycerol. Frozen clinical isolates were thawed and inoculated on Mueller-Hinton agar (MHA) plates (Oxoid) supplemented with 10 % horse blood and incubated under microaerophilic conditions. Given the importance of inoculum homogeneity, cellular viability was controlled

microscopically by morphological observation with Gram staining to check the proportions of coccoid cells in cultures. Cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Suspensions were prepared in sterile distilled water to an opacity of 2 McFarland standards (10^7 - 10^8 CFU/mL) [14].

Bacterial growth inhibition assay (disk diffusion method)

Under microaerophilic conditions at 37 °C, the filter paper disk diffusion method was used with Mueller-Hinton agar containing 7 % defibrinated horse blood [15,16]. The samples were tested using different amounts of the synthesized compounds. A microsyringe was used to apply 40 µL of methanol to each sample, which was dissolved, and the paper disks had a diameter of 6 mm. The disks were inoculated with *H. pylori* on agar surfaces and allowed to dry in a fume hood for three days at 37 °C.

If there existed an inhibition zone surrounding each disk, it was identified. The anti-*H. Helicobacter pylori* activity was measured as the mean of the inhibition diameters (mm) that the target compounds and licorice produced. All tests were performed in triplicate, and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the title compound.

Molecular modeling

Using AutoDockVina, computer-guided docking studies were performed—the outcome of biological screening led to the selection of just one ligand. On the RCSB Protein Data Bank website (<https://www.rcsb.org/>), the three-dimensional (3D) sequence for the *Helicobacter pylori* Urease Complex (PDB ID: 1E9Z) was obtained [17,18]. The ChemOffice application (ChemDraw 16.0) was utilized to sketch the structures of the synthesized compounds, ensuring appropriate 2D orientation [19]. ChemBio3D was used to minimize each molecule's energy, and the resulting data was fed into AutoDockVina to perform the docking simulation. Using CASTp analysis and the MOE tool's binding site finder, the binding sites for each protein structure were predicted [20]. The target protein file was prepared by leaving the associated residue with protein using Auto preparation of target protein file AutoDock 4.2 (MGLTools 1.5.6); the protein preparation was carried out using the reported standard protocol by removing the co-crystallized ligand, water molecules, and cofactors. The grid box for the

docking simulations was configured using the graphical user interface software. The grid was positioned to encircle the macromolecule's region of interest. The ligand-protein optimal docked conformation was found using the docking algorithm included with AutoDockVina v.1.2.0. For every ligand, a maximum of nine conformers were taken into consideration throughout the docking procedure. After that, interactions with binding pocket residues were investigated, and the final docking postures were visually inspected using BIOVIA Discovery Studio. The best-scoring poses that fit into the binding pocket and demonstrated beneficial ligand-enzyme interactions were chosen [21].

RESULTS

Chemistry

Initially, by heating metronidazole (**1**) with Fe/HCl, the nitro group may be converted into an amino group, yielding 2-(2-methyl-5-amino-1*H*-imidazole-1-yl) ethanol (**2**). Target sulfonamide derivatives (**4a**) and (**4b**), respectively, were obtained by further condensing the latter (**2**) with 2-thiouracil and/or uracil-5-sulfonyl chloride derivative (**3**) in the presence of anhydrous pyridine as an acid binder. Additionally, thionyl chloride chlorinated metronidazole to produce chloroethyl derivative (**5**), which then interacted with 2-thiouracil (**6**) in basic media to produce S-alkylated thiopyrimidine derivative (**7**) as shown in Figure 1.

On the other hand, there are numerous ways to extract licorice root for use as a phytotherapy. The most widely used ways are Soxhlet and cold maceration, which have strong antimicrobial properties, particularly against *Streptococcus mutans* (*S. mutans*) and *H. pylori* [22]. The Soxhlet approach, which is more effective than the traditional old ways, was the primary focus of this study. Glycyrrhizic acid and its glucoside, glycyrrhizin, are among the several phytochemical elements found in the alcoholic extract that have demonstrated good anti-*H. pylori* activity, along with coumarin, flavonoids, isoflavonoids, pterocarpenes, saponins, and stilbenes.

Anti-Helicobacter pylori activity

In addition to regular metronidazole, target-synthesized derivatives and licorice extract were tested and assessed as anti-*H. pylori* drugs by comparing the inhibition zone diameter obtained by paper disk diffusion bioassay.

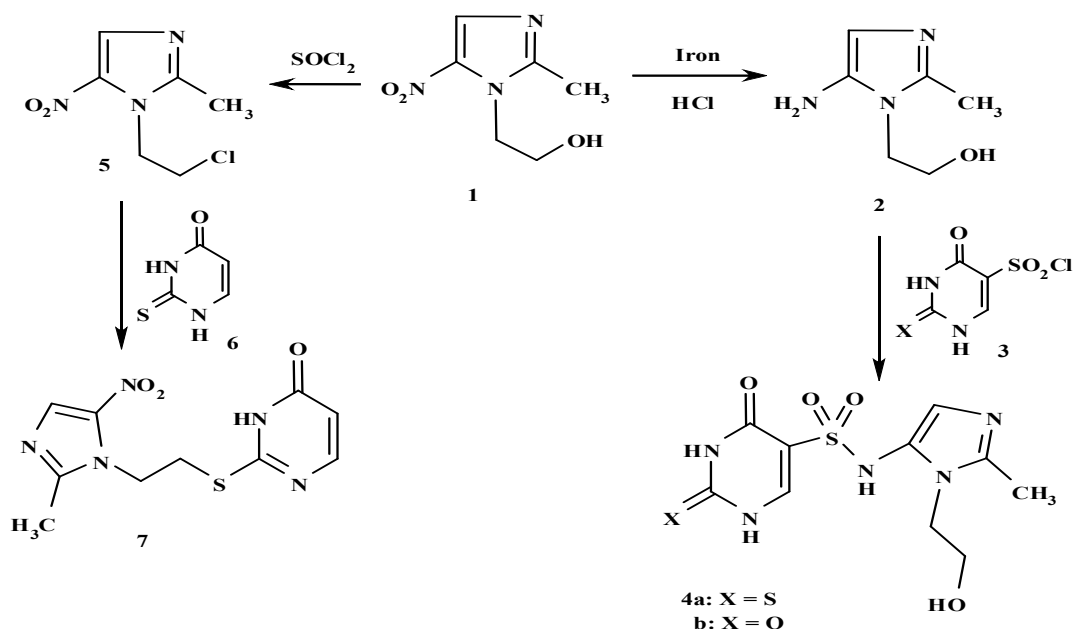


Figure 1: Synthesis of compounds (4), (5), and (7)

On standard disks (6 mm in diameter), various quantities of each sample were submitted and then placed on Muller-Hinton agar plates that had already been inoculated with bacterial suspension. The inhibitory zone was identified following a 4 - 5-day incubation period at 37 °C. All tests were performed in triplicate, and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the title compounds against metronidazole-sensitive and metronidazole-resistant *H. pylori* strains at three concentrations (8, 16, and 32 µg/disk); the results are presented in the Table. The mean inhibition diameter (mm) was used to express the antibacterial activity. The division of the task was as follows: Zone of inhibition > 20 mm: strong response; zone diameter 16 - 20 mm: moderate response; zone diameter < 16 mm: weak response (Table 1).

The paper disk diffusion bioassay determined the anti-*Helicobacter pylori* activity. All tests were performed in triplicate, and the antibacterial activity was expressed as the mean of inhibition zone diameters (mm) produced by the title compounds.

Molecular modeling study

To learn more about the molecular interactions between compound (4a) and the target receptor, molecular docking was carried out. There is no co-crystallized ligand for *Helicobacter pylori* Urease crystal structures (PDB code: 1E9Z) [23], so the MOE site finder module was utilized for active pocket prediction (Figure 2). In metronidazole, the nitro group is essential for activity.

Table 1: Zone of inhibition (mm) of all tested samples with concentrations of 8,16, and 32 in µg/disk for each sample to study synergistic activity against metronidazole-sensitive and metronidazole-resistant *H. pylori* strains

Compound no.	Inhibition zone against the metronidazole-sensitive strain (mm)			Inhibition zone against metronidazole-resistant strain (mm)		
	Dose (µg/disk)			Dose (µg/disk)		
	8	16	32	8	16	32
4a	30	26	20	24	22	20
4b	28	24	20	21	20	20
7	21	20	20	20	19	18
Licorice	20	20	19	20	20	19
Licorice + 4a	35	29	26	27	25	23
Licorice + 4b	29	26	21	22	21	21
Licorice + 7	21	20	20	20	20	18
Metronidazole	25	21	19	13	12	10

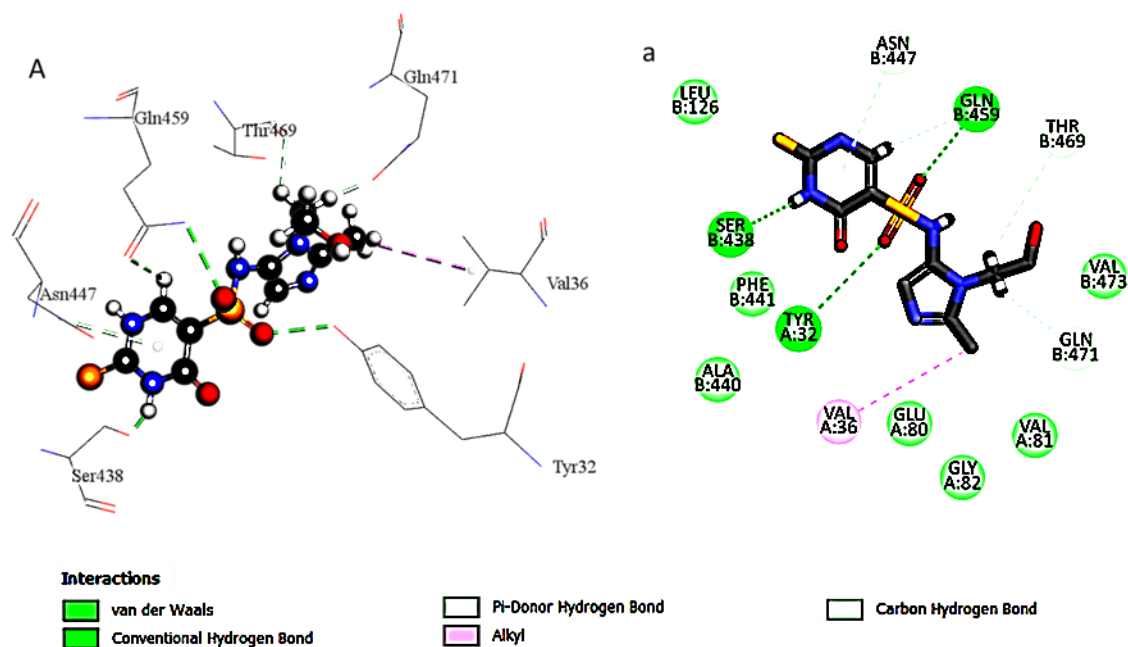


Figure 2: Molecular visualization of compound (**4a**) at the catalytic binding sites of *Helicobacter pylori* Urease Complex receptor

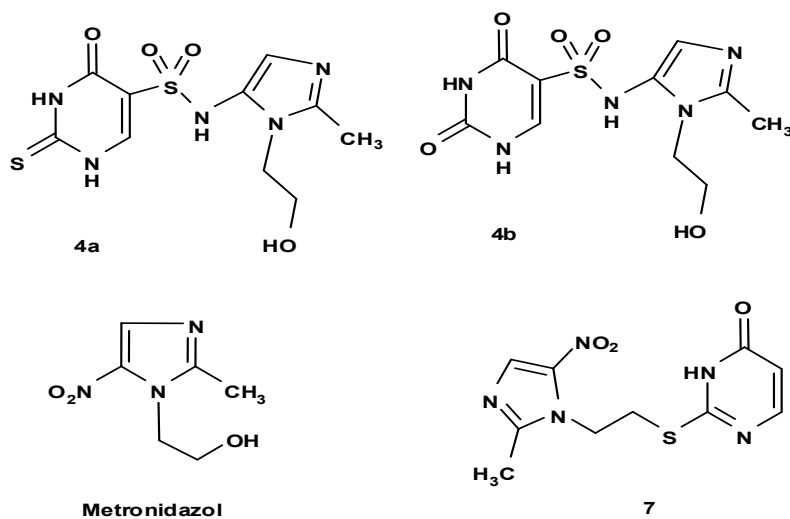


Figure 3: A potent 2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-sulfonamide (**4a**)

However, compounds (**4a**) and (**4b**) lack this group, which a sulfonamide group replaces to retain activity (Figure 3).

DISCUSSION

To increase metronidazole's efficacy against *H. pylori*, three derivatives of the medication that contain a 2-thiouracil moiety have been developed. By causing bacterial death through DNA chain breaks and degradation, metronidazole itself inhibits the growth of *H. pylori*. Uracil and thiouracil correlate to the metronidazole structure to obtain active compounds acting by these dual mechanisms

because *H. pylori* need uridine and uracil as precursors for *de novo* synthesis of pyrimidine pathways required for DNA synthesis [24,25]. As a result, uracil and thiouracil derivatives could act as false metabolites (antimetabolites) and inhibit the DNA synthesis of bacteria. After obtaining a strong chemical (**4a**), licorice and it were used together as a binary treatment for *H. pylori* infection [26].

All newly synthesized compounds containing metronidazole and pyrimidine moieties are more potent than metronidazole and also licorice extract. Compound (**4a**) activity was augmented by its combination with licorice extract. The inhibition zone diameters indicate that compound

4a, followed by compounds 4b and 7, are superior in inhibiting the growth of *H. pylori* (inhibition zone diameter ≥ 14 mm). The averages of the inhibition zone diameters indicate that substitution of the thiouracil sulfonamide moiety attached to the imidazole nucleus modulates the anti-*H. pylori* activity. Substitution of thiouracil group with uracil group resulted in compound 4b with moderate anti-*H. pylori* activity. Furthermore, compound 7, having a pyrimidine group, was found to be the lowest active compound tested. The docked (4a) compound *Helicobacter pylori* Urease complex established a reliable hydrogen bonding interaction with Tyr 32, Ser438, and Gln 459. Finally, a pi-alky interaction was established between Val 36 and compound (4a) with a docking score of -11.878 Kcal/mol.

CONCLUSION

Three compounds (4a), (4b), and (7) were newly synthesized bearing metronidazole and 2-thiouracil nuclei in an attempt to augment metronidazole activity against *Helicobacter pylori*. Compound (4a) showed potent activity, especially when combined with licorice extract. The concept that compound (7) could be helpful as a *Helicobacter pylori* inhibitor is supported by the valuable information that the molecular docking studies have provided regarding how these compounds bind to target proteins in *Helicobacter pylori* urease receptors.

DECLARATIONS

Acknowledgement/Funding

We are grateful to members of National Research Center in Dokki, Egypt, for performing spectral analyses.

Ethical approval

Not required.

Use of Artificial Intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. Figueiredo C, Machado JC, Yamaoka Y. Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* 2005; 10: 14-20. DOI: 10.1111/j.1523-5378.2005.00339.x
2. Reshetnyak VI, Burmistrov AI, Maev IV. *Helicobacter pylori*: commensal, symbiont or pathogen? *World J Gastroenterol* 2021; 27(7): 545. DOI: 10.3748/wjg.v27.i7.545
3. Nyssen OP, Martínez B, Mégraud F, Savarino V, Fallone CA, Bazzoli F, Gisbert JP. Sequential versus standard triple therapy for first-line *Helicobacter pylori* eradication: an update. *Antibiotics* 2024; 13(2): 136. DOI: 10.3390/antibiotics13020136
4. Maghrabia AE, Elshebrawy HA, Mahros MA, Elgazzar MM, Sallam KI. Clarithromycin-and metronidazole-resistant *Helicobacter pylori* isolated from raw and ready-to-eat meat in Mansoura, Egypt. *Int J Food Microbiol* 2023; 387: 110052. DOI: 10.1016/j.ijfoodmicro.2022.110052
5. Khan SF, Shetty B, Fazal I, Khan AM, Mir FM, Moothedath M, Reshma VJ, Muhamood M. Licorice as a herbal extract in periodontal therapy. *Drug Target Insights* 2023; 17: 70. DOI: 10.33393/dti.2023.2583
6. Li G, Nikolic D, Van Breemen RB. Identification and chemical standardization of licorice raw materials and dietary supplements using UHPLC-MS/MS. *J Agric Food Chem* 2016; 64(42): 8062-8070. DOI: 10.1021/acs.jafc.6b02954
7. Sathianarayanan S, Ammanath AV, Biswas R, Anita B, Sukumaran S, Venkidasamy BA. New approach against *Helicobacter pylori* using plants and their constituents: a review study. *Microb Pathogenesis* 2022; 168: 105594. DOI: 10.1016/j.micpath.2022.105594
8. Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T. Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sci* 2002; 71(12): 1449-1463. DOI: 10.1016/s0024-3205(02)01864-7
9. Malek JM, Ghazvini K. In vitro susceptibility of *Helicobacter pylori* to licorice extract. *Iranian J Pharma Res* 2007; 6(1): 69-72. DOI: 10.22037/ijpr.2007.701
10. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. Trieste, Italy: International Centre for Science and High Technology 2008; 21-54.

11. Guetat S. TocVit: optimization of tocopherols extraction through conventional and emerging techniques, and bioactive characterization of the obtained extracts. *Food Sci Technol* 2015; 52(6): 3242-3253.
12. Mustafa FY, Al-Dabbagh AK, Mohammed FM. Synthesis of new metronidazole derivatives with suspected antimicrobial activity. *Iraqi J Pharm* 2008; 7(1): 34-41.
13. Atia AJ. Synthesis and antibacterial activities of new metronidazole and imidazole derivatives. *Molecules* 2009; 14(7): 2431-246. DOI: 10.3390/molecules14072431
14. Mohammadhosseini N, ASADIPOR A, Letafat B, Vosooghi M, Siavoshi F, Shafiee A, Foroumadi A. Synthesis and in vitro anti-*Helicobacter pylori* activity of 2-(substituted benzylthio)-5-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives. *Turkish J Chem* 2009; 33(4): 471-478.
15. Sankeshwari RM, Ankola AV, Bhat K, Hullatti K. Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against *Streptococcus mutans*? *J Scientific Soc* 2018; 45(2): 67-71.
16. National Committee for Clinical Laboratory Standards 2004. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A6. 6th ed. National Committee for Clinical Laboratory Standards, Villanova, A, USA.
17. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Computational Chem* 2010; 31(2): 455-461. DOI: 10.1002/jcc.21334
18. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nature Structural Biol* 2001; 8(6): 505-509. DOI: 10.1038/88563
19. Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Feng Z, Gilliland GL, Iype L, Jain S, Fagan P. The protein data bank. *Acta Crystallographica section D: Biol Crystallograph* 2002; 58(6): 899-907. DOI: 10.1107/s0907444902003451
20. Halford B. Reflections on CHEMDRAW. *Chemical & Engineering News*. 2014; 92(33): 26-27.
21. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Res* 2018; 46(W1): W363-7. DOI: 10.1093/nar/gky473
22. Colvin DM. A review on comparison of the extraction methods used in licorice root: Their principle, strengths and limitations. *Med Aromat Plants* 2018; 7(6): 323.
23. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nature structural Biol* 2001; 8(6): 505-509. DOI: 10.1038/88563
24. Sun L, Liu M, Gong Y, Zhai K, Lv F, He L, Xue X, Liu X, Wang H, Fan D, You Y. Rapid antimicrobial susceptibility test of *Helicobacter pylori* to metronidazole via single-cell Raman spectrometry. *Helicobacter* 2024; 29(5): e13136. DOI: 10.1111/hel.13136
25. Segura AM, Gutiérrez O, Otero W, Ángel A, Genta RM, Graham DY. Furazolidone, amoxycillin, and bismuth triple therapy for *Helicobacter pylori* infection. *Alimentary Pharmacol Therapeut* 1997; 11(3): 529-532. DOI: 10.1046/j.1365-2036.1997.00172.x
26. Kim SY, Choi DJ, Chung JW. Antibiotic treatment for *Helicobacter pylori*: Is the end coming? *World J Gastrointestinal Pharmacol Therapeut* 2015; 6(4): 183. Doi: 10.4292/wjgpt.v6.i4.183