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Research Article

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2-Substituted-5-(5-nitro-furan-2-yl)-1,3,4-thiadiazoles: Anti-*Helicobacter pylori* activity evaluation and docking study

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ABSTRACT

Helicobacter pylori causes gastric and gastroduodenal ulcer disease and chronic infection of H. pylori is attributed to adenocarcinoma. 2-Substituted-5-(nitro-aryl)-1,3,4-thiadiazoles are being extensive evaluated against H. pylori isolates. In this study, a series of 2-Substituted-5-(5-nitro-furan-2-yl)-1,3,4-thiadiazoles selected and evaluated for anti-Helicobacter pylori activity. Molecular docking simulation on H. pylori urease active site improves showed potential interaction between selected ligands and H. pylori urease. In this study in vitro anti-Helicobacter pylori assay was evaluated for some selected 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole containing compounds. Docking simulation for designed compound was performed to explain mechanism of interactions. It was found that most compounds exhibited significant anti-Helicobacter pylori activity more potent than the standard drug metronidazole. In vitro anti-Helicobacter pylori activity was performed that 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-propylacetamide was the most potent compound among all tested compounds against clinical isolates of H. pylori. Development of new 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol derivatives containing thio-linker (N-(alkyl) acetamides) as potentially potent anti-Helicobacter pylori agents is greatly suggested.

Keywords: *Helicobacter pylori*, 1,3,4-thiadiazole, gastric ulcer

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INTRODUCTION

Gastric and duodenal ulcers are the most common ulcer of an area of the gastrointestinal tract and are extremely painful. *Helicobacter pylori* is a Gram-negative, microaerophilic bacterium which was identified in 1982. Goodwin found that *H. pylori* was present in patients with gastric ulcers and chronic gastritis [1,2]. This time it became clear for scientists that *Helicobacter pylori* is one of the most common cause of duodenal and gastric ulcer and chronic infection of *H. pylori* is attributed to gastric cancer, the second-highest cause of cancer death and the fourth-most common type of cancer, duodenal cancer and some other complicated malignancies [3,4].

The first-line therapy of gastric ulcer is a one-week triple therapy consisting of antibiotics amoxicillin or metronidazole and clarithromycin and the proton pump inhibitors such as pantoprazole, rabeprazole or esomeprazole. However variations of the triple therapy have been developed over the past years, such as adding a bismuth colloid to change the triple therapy to quadruple therapy. The most important treatment failure in triple or quadruple therapy regimens is the contribution of antibiotic-resistant strains of *H. pylori* in these diseases [5,6]. Therefore, finding new agents with highly selective antibacterial activity against *H. pylori*, but without risk of other untoward effects and resistance have become necessary.

In this concept, wide range of heterocyclic compounds with different attachments as anti-*H. pylori* agents is well documented. We have reported some 1,3,4-thiadiazole derivatives containing nitrofuran, nitrothiophene and nitroimidazole heterocycles with moderate to strong anti-*Helicobacter pylori* activity [7-14]. The structure-activity relationships of these compounds indicate that anti-*H. pylori* activity dramatically impacted by the pendent group on 1,3,4-thiadiazole and type of nitroaryl moiety. Among them 5-(nitrofuran-2-yl)-1,3,4-thiadiazoles demonstrated the strong inhibitory potential against the resistant strain, see figure 1. In continuation of our investigations to introduce the new compounds with *H. pylori* inhibitory activity, here in we described the *in vitro H. pylori* inhibition assay for some selected 5-(nitrofuran-2-yl)-1,3,4-thiadiazole containing compounds followed by molecular docking simulation to show the potentially mechanism of anti-*H. pylori* activity [15,16].

EXPERIMENTAL SECTION

Marvin sketch was used for drawing, displaying and characterizing chemical structures, Marvin 6.0.3, 2013, ChemAxon (http://www.chemaxon.com). Physicochemical properties of selected compounds are presented in Table 1.

Biological assay

Patients and bacterial strains

Strains of *H. pylori* were isolated from 160 dyspeptic patients who were referred to Endoscopy Unit, Shariati Hospital, Tehran, Iran. Patients involved 82 women with the age range of 16-75 years old (mean age: 43 years) and 78 men with the age range of 21- 90 years old (mean age: 48 years). Patients' diseases and frequencies'-based on endoscopic diagnosis-were gastritis (124, 77.5 %), ulcers (32, 20%) and cancer (4, 2.5%: 2 esophageal cancers and 2 gastric cancers).

Antral biopsies with positive rapid urease tests were transported to the microbiology lab in semisolid (0.1% agar) normal saline. Plates were incubated at 37°C under microaerobic conditions (CO_2 incubator; Heraeus, Germany). Cultures were checked out after 3-5 days for observation of pinpoint (1-2 mm) glistening colonies. Subsequent primary selective isolation, *H. pylori* bacteria cells were identified according to colony morphology, Gram negative stain and spiral microscopic appearance as well as positive activities of urease, oxidase, and catalase and negative activities of nitrate and hydrogen sulfide.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed using disk diffusion method (DDM). Recruited antibiotics included metronidazole, tetracycline. One hundred and ten strains were recruited in the first step of the susceptibility tests. The frequency of bacterial resistance to metronidazole and tetracycline appeared to be remarkably higher than our prior studies. Therefore, in the second step in order to increase the accuracy of the metronidazole resistance rate, an added 50 strains were employed for susceptibility testing with metronidazole (32, 16, 8, and 4 µg/mL). These 50 strains were also examined with 2, 1, and 0.5 µg/mL of tetracycline. Susceptibility tests were done again twice for the strains which exhibited resistance to metronidazole or tetracycline. Bacterial suspensions with the turbidity of Mac-Farland standard No.2 (equivalent to 6×108 cell/mL) were prepared in normal saline. Non-selective blood agar plates were surface inoculated with 100 µL of each bacterial suspension. Bent glass rods were utilized for the even spreading of bacterial suspensions. Plates were let to be dried at room temperature for about 10 minutes. Sterile blank disks were placed on the surface of incubated plates. A 10 µL volume of each antibiotic dilution was introduced into a blank disk.

Control plates included those growth positive bacterial cultures with blank disks impregnated with 10 µL of the antibiotic solutions. Plates were incubated as mentioned before and examined after 3-5 days. The inhibition zone diameters (IZDs) were recorded. *H. pylori* isolates with IZDs of ≥ 20 mm for metronidazole were considered susceptible. All tests were performed in triplicate and the antibacterial activities were expressed as the mean of inhibition diameters (mm) produced by title compounds in four concentrations (100, 50, 25 and 12.5 µg/mL) and the antibacterial activity assays have done against three strains that showed resistant to metronidazole and were obtained from clinic. IZDs of ≤ 10mm for title compounds have been designated resistant.

Anti-*Helicobacter pylori* activity assay

Growth inhibition was performed by the filter paper disk diffusion method on selective Brucella agar with 7% defibrinated horse blood under microaerophilic conditions at 37 ◦C. The samples were evaluated for their anti-Helicobacter activity, dissolved in dimethylsulfoxide (DMSO). All compounds were assayed against metronidazole-resistant H. pylori strains at 3 concentrations (100, 50, and 25 and 12.5 µg/disk); the surfaces of the *Brucella* blood agar plates were inoculated with 100 µL of bacterial suspensions. Blank standard disks (6 mm in diameter) were deposited on the plates and impregnated with 10 µL of different dilutions of test compounds. Following incubation for 3-5 days at 37 ◦ C, the inhibition zone around each disk (average diameter), if any, was recorded. The control disks received 10 µL of DMSO. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the tested compounds and the 11-15 mm; and little or no response, zone diameter ≤ 10 mm.

Molecular modeling study

Docking studies for selected compound **1l** was performed using Autodock Vina (ver. 1.1.1) [17]. The crystal structure of *Helicobacter pylori* urease in complex with acetohydroxamic acid (PDB ID: 1E9Z, resolution [Å]: 3) was retrieved from protein data bank [18]. Crystal structure was cleaned from Co-crystallized ligand and water molecules and the protein was converted to pdbqt format using Autodock Tools (1.5.4). 2D Structures of ligands converted to 3D by Openbabel (ver. 2.3.1) [19]. The docking parameters were set on vina docking parameters as follow: center_x = 127.227; center_y = 125.695; center_z = 86.661; size_x = 40; size_y = 40; size_z =40;. The other parameters were left as default for the program.

Finally, the conformation for the best free energy of binding was selected for analyzing the interactions between the enzyme and selected inhibitor. 3D models of ligand-receptor interactions generated by using the Autodock Tools (1.5.4) [20].

RESULTS AND DISCUSSION

Selected compounds **1a-p** and their physicochemical properties are presented in Table 1. **1a-p** has been synthesized from starting material, 2-nitro-5-[(bis-acetyloxy)methyl]furan according to scheme 1 [21].

Anti-*Helicobacter pylori* activity assay

The *in vitro* anti-*Helicobacter pylori* activities of **1a-p** were performed by paper disk diffusion bioassay against three metronidazole resistant *H. pylori* isolates. The averages of IZD of compounds in three isolates at four different doses (100, 50, 25 and 12.5 µg/disk) are summarized in Table 2. The anti-*H. pylori* activity could be divided to strong, moderate, weak, and little responses with zone diameters value of > 20 mm, 16-20 mm, 11-15 mm, and < 10 mm respectively. As it shown in Table 2, almost compounds showed strong anti-*Helicobacter pylori* activity however their potency are greatly affected by acetamide moiety substitutions. Based on acetamide moieties, tested compounds can divide to two sub groups: phenyl acetamide and non-phenyl acetamide derivatives. The obtained averages of IZD of compounds revealed the following structure-activity relationship for 2-Substituted-5-(5-nitro-furan-2-yl)-1,3,4-thiadiazoles:

Investigation of different substitutes into N-(phenyl)acetamide derivatives

The results indicated that introduction of different substitutes into the *N*-(phenyl) acetamide derivatives alters the anti-*H. pylori* of compounds. The phenyl acetamide derivative **1a**, exhibited strong inhibitory potential at 100-25 µg/ml (IZD = 36-21 mm) Substitution of nitro group into *meta*-position of phenyl acetamide moiety resulted in enhanced potency of compounds against different *H. pylori* resistant strain. This promising compound **1g** demonstrated strong inhibitory potential at different evaluated concentrations (IZD = 33-23 mm at 100-12.5 µg/ml). In contrast to nitro substitution, introduction of chlorine substitute into *meta*-position of phenyl acetamide moiety (compound **1c** with IZD = 35-18 mm at 100-12.5 µg/ml) didn’t significantly alter the anti-*H. pylori* potential of *N*-(phenyl) acetamide derivative. Moreover substitution of chlorine moiety into *ortho* and *para* positions of phenyl acetamide, diminished the growth inhibitory activity of *N*-(phenyl) acetamide derivatives **1b** and **1d** (IZD = 13 and 11 mm at 100µg/ml, respectively).

Substitution of other moieties such as methoxy and trifluromethxy groups into *para* position of phenyl acetamide side chain resulted in decreased inhibitory potential of these derivatives to some extent (compounds **1e** and **1f** demonstrated weak anti-*H. pylori* activity at 50 µg/ml).

Replacement of N-(heteroaromatic) acetamide moieties

Substitution of *N*-(5-methylisoxazole-3-yl) carboxamide moiety resulted in the compound (**1j**) with strong anti-*H. pylori* activity at 100-50 µg/ml (IZD = 35-20 mm). This compound demonstrated moderate to weak activity at 25 and 12.5 µg/ml (IZD = 17 and 12 mm, respectively).

The *N*-(benzo[d]thiazol-2-yl) acetamide derivative **1k** is almost inactive against resistant strains of *H. pylori* isolates (IZD = 10.5 at 50 µg/ml).

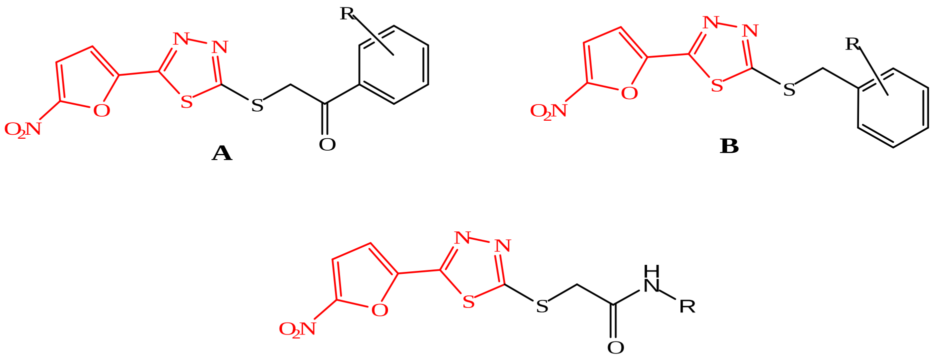
Assessment of N-alkyl acetamide derivatives

Substitution of different *N*-(alkyl) acetamide moieties into thio linker side chain enhanced the anti-*H. pylori* potential of resulted derivatives in most cases.

The most promising compound **1l,** containing *N*-(propyl)acetamide pendant, demonstrated strong inhibitory potency at all evaluated concentrations (IZD = 59-33 mm at 100-12.5 µg/ml). Introduction of different phenyl substitutes into *N*-(alkyl) acetamide pendant decreases the potency of compound to some extent; e.g. compound **1p** containing *N*-(3,4-dimethoxy phenethyl)acetamide substitute (IZD = 43-20 mm at 100-12.5 µg/ml), were slightly less potent than *N*-(propyl)acetamide derivative **1l.** Nevertheless, **1l** is the most strong inhibitor of bacterial growth at all concentrations (IZD > 30) in this study.

The *N*-benzyl acetamide derivative **1m** demonstrated superior anti-*H. pylori* activity than its *N*-phenyl acetamide counterpart **1a** to some extent**.** Compound **1m** demonstrated strong inhibitory activity at 100-25µg/ml (IZD = 44-22 mm) and almost moderate activity at 12.5µg/ml (IZD = 19 mm).

Substitution of heteroaromatic moieties into *N*-alkyl acetamide side chain was also investigated. Attachment of furan-2-yl group through the methylene bridge into *N*-acetamide side chain (compound **1o**) retained the inhibitory potency of compound. Compound **1o** strongly inhibited the *H. pylori* isolates at 100-25 µg/ml (IZD = 45-21 mm).

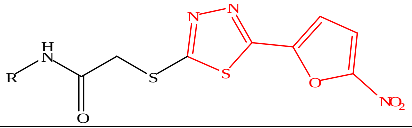
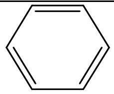
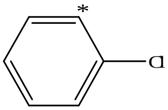
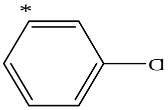
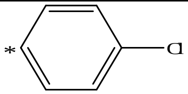
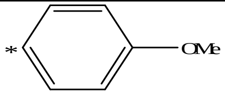
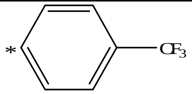
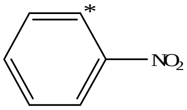
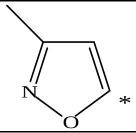
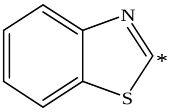
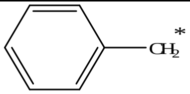
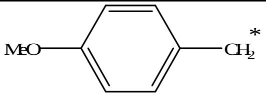
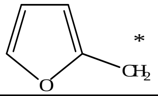
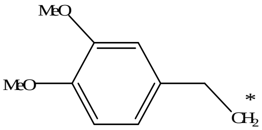


A **B**

1a-p

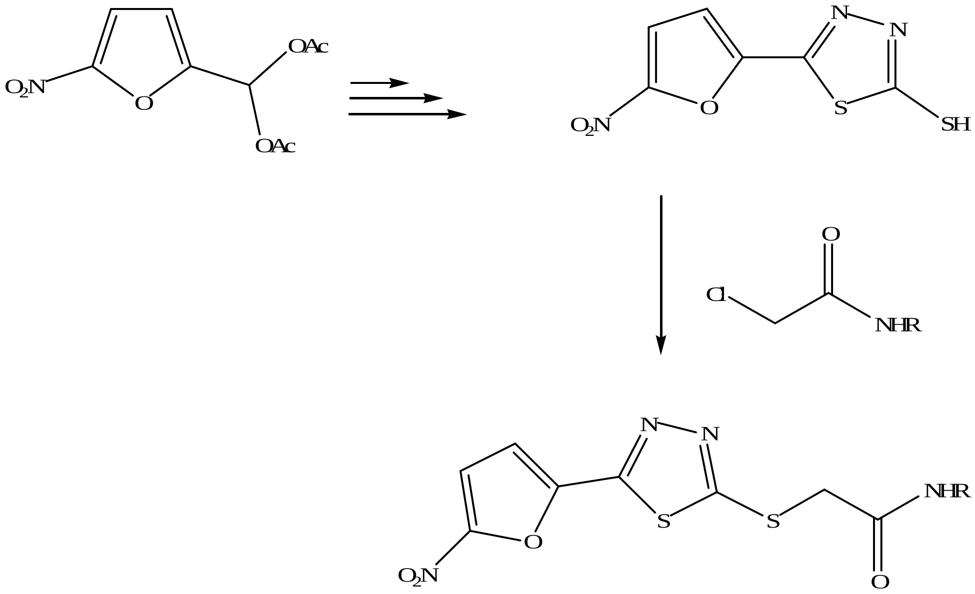
Figure 1. General structures of 2-Nitrofuran-1,3,4-thiadiazole containing anti-*H. pylori* agents (A, B), designed compounds 1a-p

Table 1. Physicochemical properties of selected compounds

Code		**Physicochemical parameters**				
	R	**MW**	**Log P**	***H*-bond donor(s)**	***H*-bond acceptor(s)**	**Rotatable bond(s)**
1a		362	2.78	1	4	6
1b		396	3.38	1	4	6
1c		396	3.38	1	4	6
1d		396	3.38	1	4	6
1e		392	2.62	1	5	8
1f		430	3.65	1	7	10
1g		407	2.72	1	6	7
1j		366	1.36	1	5	6
1k		419	3.60	1	4	6
1l	$-C_3H_7$	328	1.64	1	4	8
1m		376	2.48	1	4	6
1n		406	2.33	1	5	8
1o		366	1.54	1	5	6
1p		450	2.46	1	6	10

link position

Log P values were calculated by Marvin Sketch$^{®}$



Scheme 1. Synthesis of selected compounds 1a-p [21]

Table 2. Average of inhibition zone diameters of compounds "1a-p" at different doses against three metronidazole resistant *Helicobacter pylori* isolates

Code	**Average of Inhibition Zone Diameters** (range, mm)*			
	100 µg/disk	**50** µg/disk	**25** µg/disk	**12.5** µg/disk
1a	18.7 (17-20)	21.3 (18-24)	28.3 (20-35)	35.7 (32-40)
1b	ND	ND	ND	13 -
1c	17.7 (16-19)	19.7 (19-20)	25.3 (25-26)	35.3 (33-38)
1d	ND	ND	ND	11 (10-12)
1e	ND	ND	10 -	22 -
1f	- -	10 -	10.5 (10-11)	19 (18-20)
1g	19.3 (18-20)	23 (20-25)	27.3 (25-30)	32.7 (29-36)
1j	12.3 (10-15)	17 (15-20)	20 (17-23)	35.3 (33-38)
1k	ND	ND	10.5 (10-11)	16.3 (15-18)
1l	33 (30-39)	38.3 (33-44)	47.7 (45-50)	59.3 (58-60)
1m	19 (18-20)	22 (20-25)	31.3 (25-39)	44.3 (40-48)
1n	13 (10-16)	17.3 (15-19)	18.7 (15-21)	26.7 (25-30)
1o	16 (15-18)	21 (18-25)	31.3 (25-35)	44.7 (40-50)
1p	19.7 (19-20)	24 (23-25)	27.7 (25-30)	42.7 (40-44)
Metronidazole	16 (15-18)	19 (18-25)	27.3 (25-35)	34.7 (40-50)

The anti-Helicobacter pylori activity was determined by the paper disc diffusion bioassay,* ***ND* *means not determined.*

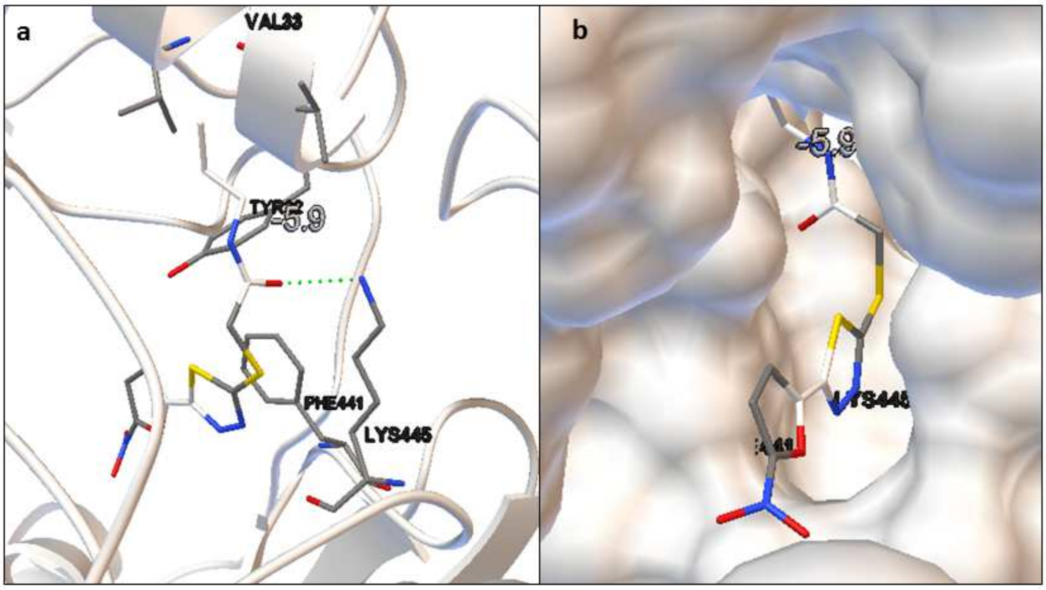


Figure 2. Ligand receptor interaction, a) hydrogen bond is showed in green dotted line, b) coordination of 1l in active site pocket of urease enzyme of *H. pylori*

Molecular modeling study

In order to understand the binding mode of active compounds in the active site pocket of urease, docking study was performed using Autodock Vina. To attain this aim, the potent compounds, **1l** was docked into target enzyme. Docking strongly suggested that the hydrogen bond between carbonyl group on acetamide side chain and hydrogen of amine group on lysine 445 in enzyme B residue is effective in activity of biologically active synthesized compounds. According to Figure 2, **1l** fit in the pocket of urease enzyme completely, and coordination of ligand in pocket make a π-π interaction between thiadiazole moiety and phenyl side chain oh phenylalanine 241 in enzyme B residue.

Synthesized 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-*N*-substituted acetamides derivatives were found as effective anti- *H. pylori* agents against three different resistant strains of *Helicobacter pylori* isolates. The structure-activity relationship study revealed that the anti-*H. pylori* activity of synthesized derivatives was considerably dependent on substituted moiety into acetamide thio-linker side chain. Most of synthesized derivatives demonstrated strong activity against isolated strains at 100-25 µg/ml. The *N*-(alkyl) acetamide derivatives demonstrated superior inhibitory potential than the *N*-(aryl) acetamides. The most promising derivative **1l**, demonstrated strong anti-*H. pylori* potential at all evaluated concentrations (IZD>30). Compound **1l** could be considered as a suitable anti-*H. pylori* candidate especially against metronidazole resistant strains. Therefor; development of new 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol derivatives containing thio-linker (*N*-(alkyl) acetamides) would be of great importance for better understanding of the structure-activity relationship of this scaffold.

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