

## Anti-*Helicobacter pylori* activity and Structure-Activity Relationship study of 2-Alkylthio-5-(nitroaryl)-1,3,4-thiadiazole Derivatives

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### Abstract

Nitro-containing heteroaromatic derivatives structurally related to nitroimidazole (Metronidazole) are being extensively evaluated against *Helicobacter pylori* isolates. On the other hand, 1,3,4-thiadiazole derivatives have also demonstrated promising antibacterial potential. In present study, we evaluated anti-*H. pylori* activity of novel hybrid molecules bearing nitroaryl and 1,3,4-thiadiazole moieties.

Anti-*H. pylori* activity of novel 5-(5-nitroaryl)-1,3,4-thiadiazole derivatives bearing different bulky alkylthio side chains at C-2 position of thiadiazole ring, were assessed against three different metronidazole resistant *H. pylori* isolates by paper disk diffusion method.

Most of the compounds demonstrated moderate to strong inhibitory response especially at 25 µg/disk. The structure-activity relationship study of the compounds demonstrated that introduction of different alkylthio moieties at C-2 position of thiadiazole ring alter the inhibitory activity which is mainly dependent on the type of C-5 attached nitroheterocyclic ring. The promising compound of this scaffold, bearing 1-methyl-5-nitroimidazole moiety at C-5 and  $\alpha$ -methylbenzylthio side chain at C-2 position of thiadiazole ring, showed strong inhibitory response against metronidazole resistant *H. pylori* isolates at 12.5 µg/disk (the inhibition zone diameter at all evaluated concentrations (12.5-100 µg/disk) is > 50 mm).

Novel 5-(5-nitroaryl)-1,3,4-thiadiazole scaffold bearing different C-2 attached thio-pendant moieties with promising anti-*H. pylori* potential were identified. Among different nitroheterocycles, 5-nitrofurane and 5-nitroimidazole moieties were preferable for the substitution at C-5 position of 1,3,4-thiadiazole ring. Introduction of different alkylthio side chains at C-2 position of central ring alter the inhibitory activity which is mainly dependent on the type of C-5 attached nitroheterocyclic ring.

**Keywords:** 1,3,4-thiadiazole; anti-*Helicobacter pylori* activity; Nitroheterocycle.

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## Introduction

*Helicobacter pylori* (*H. pylori*), an invading gram negative microaerophilic bacterium causes common chronic gastric infection known as the major cause of gastric and gastroduodenal ulcer disease (1-2). In addition, chronic infection of *H. pylori* is attributed to adenocarcinoma and low grade B-cell mucosa-associated lymphoid tissue lymphoma of stomach and therefore; it is the first bacterium known as definite carcinogen by the World Health Organization's International Agency for Research on Cancer (3-5). The treatment of *H. pylori* is complicated and includes combination of proton-pump inhibitor (PPI) agents with two or more different antibiotics such as amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin for at least 7 days (2, 6). Although eradication of infection results in ulcer healing and reduces the risk of gastric cancer (7), however; emerging resistance to current antibiotics is encountered as a major concern in the treatment of *H. pylori* infection (8). From this, different factors such as poor patient compliance, common side effects of combination therapy and also significant cost of multi-drug therapy, limits the eradication of infection and therefore reveals the urgent need to search novel candidates for the eradication or prevention of infection (7-8).

Recent studies reported some novel natural sources (9-13) and synthetic scaffolds (14-15) as the therapeutic agents with significant inhibitory activity against *H. pylori* isolates. Among the different chemical structures, nitro-containing heteroaromatic derivatives (such as nitrofurans and nitrothiophen derivatives) that are structurally related to nitroimidazole family (Metronidazole) are being extensively evaluated against *Helicobacter pylori* isolates (16). In this regard, the current nitroheterocyclic antibiotics, tinidazole and furazolidone have demonstrated promising anti-*H. pylori* properties to be used in place of metronidazole (17). The results of different studies indicate that the possible mechanism of antimicrobial activity of 5-nitroheterocycles involves the reduction of the 5-nitro group with the formation of reactive intermediates that can interact to various cell

macromolecules. Therefore; the nitroheterocycle moiety is essential for the antimicrobial activity of these derivatives (18).

On the other hand, the antibacterial property of 1,3,4-thiadiazole derivatives against both Gram(+) and Gram(-) bacteria has been well established (19-21). We have previously demonstrated the anti-*Helicobacter pylori* properties of different 5-(5-nitroaryl)-1,3,4-thiadiazoles with different substitutes at C-2 position of thiadiazole ring. The structure-activity relationship study of these compounds indicates that the anti-*H. pylori* property of these scaffolds is highly dependent on the type of C-2 substitute. Different C-2 attached moieties to 1,3,4-thiadiazole derivatives such as piperazine derivatives and different sulfur containing side chains were evaluated (22-24). In continuation of our research program on 2,5-disubstituted-1,3,4-thiadiazole scaffold (20-25), we have previously reported novel 2-substituted-thio-1,3,4-thiadiazoles containing different 5-nitroaryl substitutes on C-5 position of thiadiazole ring with potent antilishmanial properties (26). These compounds could also be served as potent scaffold against *H. pylori* infection: (Figure 1)

1- The nitroaryl ring (A) comprises 5-nitrofurans (which is derived from the nitroaromatic part of furazolidone), 5-nitrothiophen and 1-methyl-5-nitroimidazole substitutes (that are isosterically replaced instead of nitroaromatic part of furazolidone and metronidazole; respectively).

2- The 1,3,4-thiadiazole ring (B) could partly mimic the methyleneamino-oxazolidinone fragment of furazolidone and enhances the antibacterial and particularly anti-*H. pylori* properties of scaffold.

3- Substituted-thio side chain (C) of these novel derivatives could serve as a new part against the resistant strains of *H. pylori* and affects the potency of these derivatives against the resistant isolates of this organism.

We have previously reported the synthetic pathway of designed compounds "1-13". The chemical structure of these compounds is demonstrated in Table 1. The structure of these compounds was confirmed by elemental analysis, <sup>1</sup>H NMR and IR spectra (26).

According to our ongoing research program on 1,3,4-thiadiazole derivatives as potent antiparasitic scaffold (20-26), herein, we report the anti-*H. pylori* activity of 2-alkylthio-5-(nitroaryl)-1,3,4-thiadiazole derivatives.

## Experimental

### *Bacterial isolates and culture conditions*

Clinical *H. pylori* isolates from gastric biopsy specimens were obtained from Shariati Hospital (Tehran, Iran). Primary isolation was performed on a selective blood agar base (Oxoid, Basingstoke, UK) supplemented with horse blood (5%, v/v) and 1 Selectatab tablet (500 mg; Mast Diagnostics, Merseyside, UK). Following primary selective isolation, *H. pylori* bacteria cells were identified according to colony morphology, Gram staining, microaerophilic growth at 37 °C, oxidase +, catalase +, urease +, nitrate -, H<sub>2</sub>S, and hippurate hydrolysis. Growth of *H. pylori* was maintained at 37 °C for 3-5 days in an atmosphere of 5% O<sub>2</sub>, 15% CO<sub>2</sub>, and 80% N<sub>2</sub> in an anaerobic chamber (Hirayama, Tokyo, Japan). To maintain a moist atmosphere, a moist paper towel was placed in the chamber. 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. In order to control the proportions of coccoid cells in the cultures, cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Bacterial growth was taken from the plates and resuspended in sterile saline. The inoculum was prepared to contain 5 × 10<sup>7</sup> CFU/mL by adjusting the turbidity of the suspension to match the McFarland No. 2 standard.

### *Bacterial growth inhibition assay (disk diffusion method)*

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.

The one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons was used to analyze the data. A value of p < 0.05 was considered as the significance level between the groups.

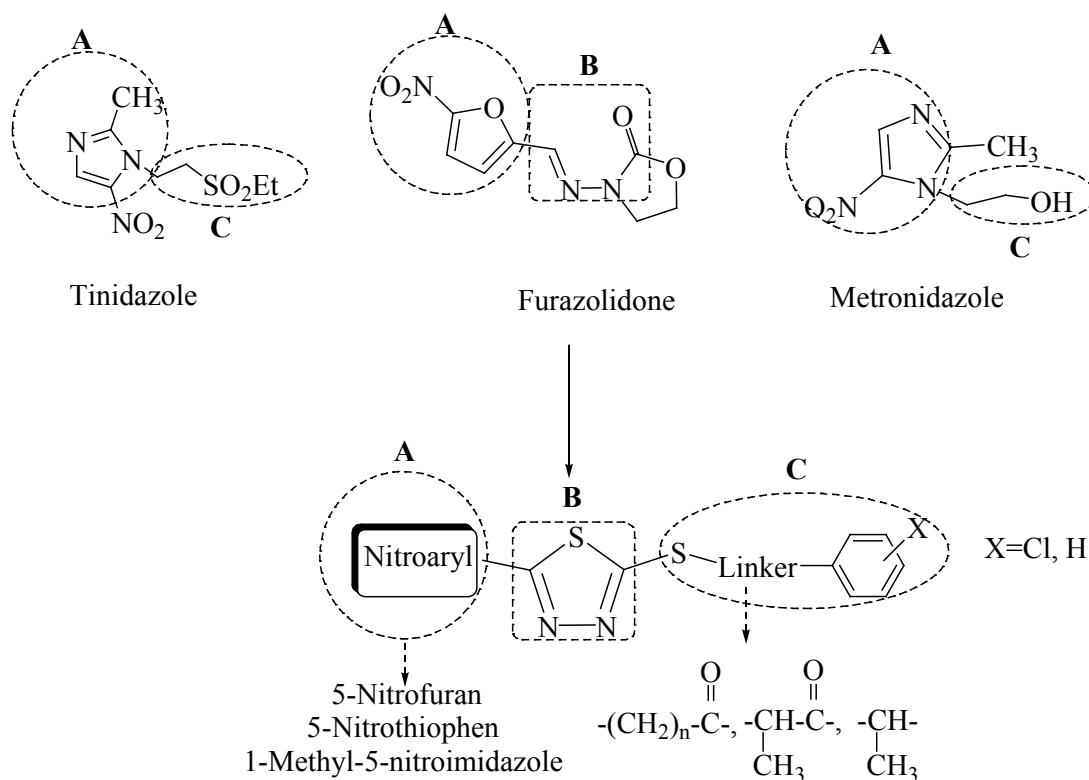
## Result and Discussion

The anti-*Helicobacter pylori* activity of novel 1,3,4-thiadiazole derivatives “1-13” was determined by paper disk diffusion bioassay against three metronidazole resistant *H. pylori* isolates. The average of inhibition zone diameters of compounds in three isolates at different doses (100, 50, 25 and 12.5 µg/disk) is summarized in Table 1. The anti-*H. pylori* activity was classified as follows: strong response, zones range diameter > 20mm; moderate response, zone diameter 16-20 mm; weak response, zone diameter 11-15 mm; and little or no response, zone diameter < 10 mm (22).

### *Structure activity relationship*

#### *Assessment of 5-nitroaryl substitute*

The inhibition zone diameter (IZD) of test compounds indicates that most of the compounds exhibited strong anti-*H. pylori* at concentration of 100-25 µg/disk against



**Figure 1.** Chemical structure of 2-alkylthio-5-(nitroaryl)-1,3,4-thiadiazoles (compounds 1-13) derived from the structure of current anti-*H. pylori* nitroheterocyclic agents (tinidazole, furazolidone and metronidazole).

metronidazole resistant strains. All the test compounds could be classified into three groups according to the 5-nitroaryl substitute: 5-nitrofuran “1-6”, 5-nitrothiophen “7-10” and 1-methyl-5-nitroimidazole (nitroimidazole) “11-13” derivatives. The results demonstrated that compound 13, containing 1-methyl-5-nitroimidazole substitute at C-5 position and  $\alpha$ -methylbenzylthio-pendant group at C-2 position of the 1,3,4-thiadiazole ring, is the promising compound of these series with the most potent anti-*H. pylori* activity (the inhibition zone diameter of this compound at all evaluated concentrations (12.5-100  $\mu$ g/disk) is > 50 mm). In general; comparison of the inhibition zone diameter of compounds at different doses revealed that most of the nitroimidazole and nitrofuran derivatives demonstrated strong anti-*H. pylori* response at 25  $\mu$ g/disk (IZD > 20mm); while the nitrothiophen derivatives (compounds “7-10”) showed moderate (IZD:16-20 mm) to weak (IZD:11-15 mm) response at 25  $\mu$ g/disk.

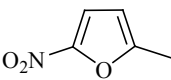
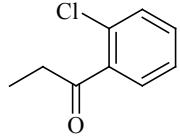
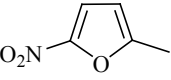
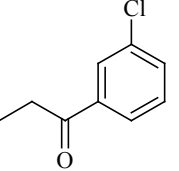
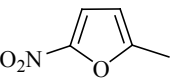
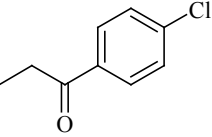
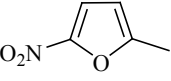
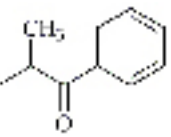
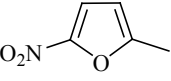
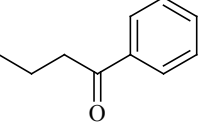

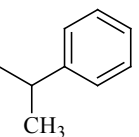
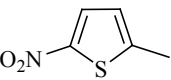
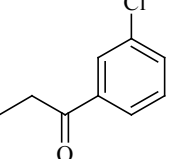
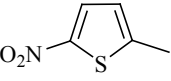
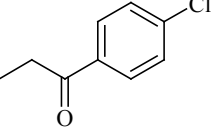
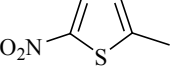
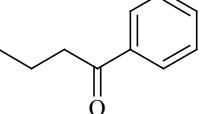
For instance, the average of inhibition zone diameter of 5-nitroimidazole derivatives containing  $\alpha$ -methylbenzylthio-pendant substitute at C-2 position of central ring (compounds 6 and 13) at 25  $\mu$ g/disk was 24 mm and > 50mm respectively. While the 5-nitrothiophen counterpart (compound 10) demonstrated weak response at this dose (IZD: 12 mm) [F(6, 10) = 8.640,  $p = 0.042$  ( $p < 0.05$ )]. Therefore; it seems that the preferable nitroaryl substitution at C-5 position of 1,3,4-thiadiazole ring is as follows.

5-nitroimidazole > 5-nitrofuran > 5-nitrothiophen

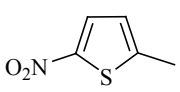
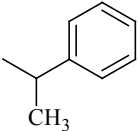
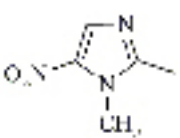
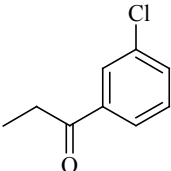
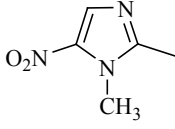
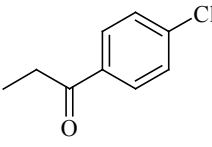
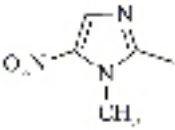
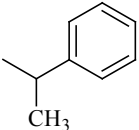
#### Assessment of 2-alkylthio side chain

Different C-2 attached substituted-thio side chains were evaluated in 5-nitroaryl-1,3,4-thiadiazole derivatives against metronidazole resistant *H. pylori* isolates (Table 1). Regio-isomeric chlorine substitution and  $\alpha$ -methylphenacylthio-pendant group were investigated. Comparison of different

**Table 1.** Average of inhibition zone diameters of compounds "1-13" at different doses against three metronidazole resistant *H. pylori* isolates.

Compound	Nitroaryl	R	Average of Inhibition Zone Diameters (range, mm)*			
			100 µg/disk	50 µg/disk	25 µg/disk	12.5 µg/disk
1			34 (32-36)	30 (29-32)	29 (27-30)	27 (25-28)
2			25 (23-27)	23 (19-25)	21 18-23	19 (15-22)
3			29 (25-32)	24 (20-27)	22 (20-24)	16 (12-20)
4			18 (16-20)	12 (12-12)	10 (9-10)	10 (10-10)
5			29 (27-32)	25 (20-30)	22 (18-28)	20 (16-25)
6			31 (28-35)	28 (25-30)	24 (20-28)	22 (18-26)
7			31 (25-35)	24 (22-25)	20 (19-22)	18 (15-20)
8			24 (20-27)	20 (17-23)	17 (15-20)	14 (10-18)
9			22 (20-24)	19 (16-21)	15 (13-20)	12 (9-15)

Contiue Table 1.

10			15 (14-19)	14 (12-15)	12 (10-14)	11 (10-12)
11			26 (25-27)	22 (20-23)	20 (18-22)	11 (10-12)
12			33 (32-35)	29 (25-33)	25 (20-30)	23 (21-24)
13			>50	>50	>50	>50
Metronidazole			26 (24-31)	24 (20-31)	18 (14-21)	12 (8-15)

\* The anti-*Helicobacter pylori* activity was determined by the paper disc diffusion bioassay.

derivatives reveals that the inhibitory effect of alkylthio side chain is partly dependent on the type of nitroheterocyclic moiety. Different regio-isomer of chlorine in phenacylthio side chain demonstrated strong inhibitory response especially in 5-nitrothiophene (compounds 1-3) and 5-nitroimidazole derivatives (compounds 11-12) at concentration range of 100-25 µg/disk. Among the nitrothiophene derivatives, meta-chlorine substituted phenacyl thio (compound 7) demonstrated strong inhibitory response at the concentration range of 100-25 µg/disk.

Among the nitrothiophene derivatives, ortho-chlorine substituted phenacylthio and  $\alpha$ -methylbenzylthio-side chain (compounds 1 and 6; respectively), demonstrated the strong inhibitory potential especially at 12.5 µg/disk against the resistant strain. In addition,  $\alpha$ -methyl branching of phenacylthio-side chain (compound 4) diminishes the inhibitory response; the corresponding inhibition zone diameter at 50-12.5 µg/disk was 10-12 mm [F(1,4) = 9.16, p = 0.014 (p < 0.05)].

The best inhibitory response in 5-nitroimidazole series could also be achieved by the introduction of  $\alpha$ -methylbenzylthio-side

chain; compound 13 with inhibition zone diameter of > 50 at all the tested concentrations. While introduction of this side chain in 5-nitrothiophene derivatives, generates compound with weak anti-*H. pylori* activity at all concentrations (compound 10).

## Conclusion

In conclusion, we have identified novel 2- $\alpha$ Alkylthio-5-(nitroaryl)-1,3,4-thiadiazole derivatives with promising anti-*H. pylori* potential. Among different nitroaryls, 5-nitrothiophene and 5-nitroimidazole moieties are preferable for substitution at C-5 position of 1,3,4-thiadiazole ring. Introduction of different substituted-thio side chains at C-2 position of central ring alter the inhibitory activity which is mainly dependent on the type of C-5 attached nitroheterocyclic ring.

## Acknowledgment

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## Reference

- (1) Gisbert JP, Pajares R and Pajares JM. Evolution of *Helicobacter pylori* therapy from ameta-analytical perspective. *Helicobacter* (2007) 12 Suppl : 50-8.
- (2) Gisbert JP and Pajares JM. Treatment of *Helicobacter pylori* infection: The past and the future. *Eur. J. Intern. Med.* (2010) 21: 357-359.
- (3) Bayerdorfer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S and Stolte M. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. *Lancet* (1995) 345: 1591-1594.
- (4) Logan RP. *Helicobacter pylori* and gastric cancer. *Lancet* (1994). 344: 1078-1079.
- (5) Petersen AM and Krogfelt KA. *Helicobacter pylori*: an invading microorganism? A review. *FEMS Immunol. Med. Microbiol.* (2003) 36: 117-126.
- (6) Candelli M; Nista EC; Carloni E; Pignataro G; Zocco MA; Cazzato A; Di Campli C; Fini L; Gasbarrini G and Gasbarrini A. Treatment of *H. pylori* infection: a review. *Curr. Med. Chem.* (2005) 12: 375-384.
- (7) Sepulveda AR. *Helicobacter pylori* and gastric malignancie. *Helicobacter* (2002) 7: 37-42.
- (8) Sherif M, Mohran Z, Fathy H, Rockabrand DM, Rozmajzl PJ and Frenck RW, Universal high-level primary metronidazole resistance in *Helicobacter pylori* isolated from children in Egypt. *J. Clin. Microbiol.* (2004) 42: 4832-4834.
- (9) Ndip RN, Malange Tarkang AE, Mbullah SM, Luma HN, Malongue A, Ndip LM, Nyongbela K , Wirmum C and Efange SM. *In-vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon. *J. Ethnopharmacol.* (2007) 114: 452-457.
- (10) Hajimahmoodi M, Shams-Ardakani M, Saniee P, Siavoshi F, Mehrabani M, Hosseinzadeh H, Foroumadi P, Safavi M, Khanavi M, Akbarzadeh T, Shafiee A and Foroumadi A. *In-vitro* antibacterial activity of some Iranian medicinal plant extracts against *Helicobacter pylori*. *Natural Product Research* (2011) 25: 1059-1066.
- (11) Galan MV, Kishan AA and Silverman AL. Oral broccoli sprouts for the treatment of *Helicobacter pylori* infection: a preliminary report. *Dig. Dis. Sci.* (2004) 49: 1088-1090.
- (12) Nariman F, Eftekhari F, Habibi Z and Falsafi T. Anti-*Helicobacter pylori* activities of six Iranian plants. *Helicobacter* (2004) 9: 146-151.
- (13) Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H and Hirai Y. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol. Immunol.* (2004) 48: 251-61.
- (14) Chimenti F, Bizzarri B, Bolasco A, Secci D, Chimenti P, Granese A, Carradori S, Rivanera D, Zicari A, Scaltrito MM and Sisto F. Synthesis, selective anti-*Helicobacter pylori* activity, and cytotoxicity of novel N-substituted-2-oxo-2H-1-benzopyran-3-carboxamides. *Bioorg. Med. Chem. Lett.* (2010) 20: 4922-4926.
- (15) Phillips OA and Matowe WC. Antibacterial agents (oxazolidinones, quinolones, macrolides and new agents): patent highlights June to December 2000. *Curr. Opin. Investig. Drugs* (2001) 2: 745-751.
- (16) Nair MD and Negarajan K. Nitroimidazoles as Chemotherapeutic Agents. *Prog. Drug. Res.* (1983) 27: 163-257.
- (17) Williamson JS. *Helicobacter pylori*: Current chemotherapy and new targets for drug design. *Curr. Pharm. Des.* (2001) 7: 355-392.
- (18) Edwards DI. Nitroimidazoles drugs. Action and resistance mechanisms. I. Mechanism of action. *J. Antibiot. Chemother.* (1993) 31: 9-20.
- (19) Amir M and Kumar H and Javed SA. Condensed bridgehead nitrogen heterocyclic system: synthesis and pharmacological activities of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid. *Eur. J. Med. Chem.* (2008) 43: 2056-2066.
- (20) Foroumadi A, Sakhteman A, Sharifzadeh Z, Mohammadhosseini N, Hemmatinejad B, Moshafi MH, Vosooghi M, Amini M and Shafiee A. Synthesis, antituberculosis activity and QSAR study of some novel 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole derivatives. *Daru* (2007) 15: 218-226.
- (21) Padmavathi V, Reddy GS, Padmaja A, Kondaiah P and Shazia A, Synthesis, Antimicrobial and Cytotoxic Activities of 1,3,4-Oxadiazoles, 1,3,4-Thiadiazoles and 1,2,4-Triazoles. *Eur. J. Med. Chem.* (2009) 44: 2106-2112.
- (22) Moshafi MH, Sorkhi M, Emami S, Nakhjiri M, Yahya-Meymandi A, Negahbani AS, Siavoshi F, Omrani M, Alipour E, Vosooghi M, Shafiee A and Foroumadi A. 5-Nitroimidazole-based 1,3,4-Thiadiazoles: Heterocyclic Analogs of Metronidazole as Anti-*Helicobacter pylori* Agents. *Arch. Pharm. Chem. Life Sci.* (2011) 11: 178-183.
- (23) Fallah Tafti A, Akbarzadeh T, Saniee P, Siavoshi F, Shafiee A and Foroumadi A. Synthesis and anti-*Helicobacter pylori* activity of (4-nitro-1-imidazolylmethyl)-1,2,4-triazoles, 1,3,4-thiadiazoles, and 1,3,4-oxadiazoles. *Turk. J. Chem.* (2011) 35: 307-316.
- (24) Mohammadhosseini N, Asadpour A, Letafati B, Vosooghi M, Siavoshi F, Shafiee A and Foroumadi A. Synthesis and *in-vitro* anti-*Helicobacter pylori* activity of 2-(substituted benzylthio)-5-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives. *Turk. J. Chem.* (2009) 33: 471-478.
- (25) Tahghighi A, Rezazade Marznaki F, Kobarfard F, Dastmalchi S, Shahbazi Mojarrad J, Razmi S, Kabudanian Ardestani S, Emami S, Shafiee A and Foroumadi A. Synthesis and antileishmanial activity of novel 5-(5-nitrofuranyl)-1,3,4-thiadiazoles with piperazinyl-linked benzamidine substituents. *Eur. J. Med. Chem.* (2011) 46: 2602-2608.
- (26) Alipour E, Emami S, Yahya-Meymandi A, Nakhjiri M, Johari F, Ardestani SK, Poorrajab F, Hosseini M, Shafiee A and Foroumadi A. Synthesis and antileishmanial activity of 5-(5-nitroaryl)-2-substituted-thio-1,3,4-thiadiazoles. *J. Enzyme Inhib. Med. Chem.* (2011) 26: 123-128.