

Green Synthesis and Urease Inhibitory Activity of Spiro-Pyrimidinethiones/Spiro-Pyrimidinones-Barbituric Acid Derivatives

Ghodsi Mohammadi Ziarani^{a*}, Shima Asadi^a, Sakineh Faramarzi^a and Massoud Amanlou^b

^aResearch Laboratory of Pharmaceutical, Alzahra University, Tehran, Iran. ^bDrug Design and Development Research Center and Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Abstract

Sulfonic acid functionalized SBA-15 (SBA-Pr-SO₃H) with pore size 6 nm as an efficient heterogeneous nanoporous solid acid catalyst exhibited good catalytic activity in the Biginelli-like reaction in the synthesis of spiroheterobicyclic rings with good yield and good recyclability. Spiro-pyrimidinethiones/spiro-pyrimidinones-barbituric acid derivatives were synthesized in a simple and efficient method using the one-pot three-component reaction of a cyclic 1,3-dicarbonyl compounds (barbituric acid), an aromatic aldehyde and urea or thiourea in the presence of nanoporous silica SBA-Pr-SO₃H under solvent free conditions. Urease inhibitory activity of spiro compounds were tested against Jack bean urease using Berthelot alkaline phenol-hypochlorite method. Five of 13 compounds were inhibitor and two of them were enzyme activators. Analysis of the docking results showed that, in most of the spiro molecules, one of the carbonyl groups is coordinated with both nickel atoms, while the other one is involved in the formation of hydrogen bonds with important active-site residues. The effect of inserting two methyl groups on N atoms of barbiturate ring, S substituted, *ortho*, *meta* and *para* substituted compounds were investigated too.

Keywords: Biginelli like reaction; Spiro heterobicyclic rings; SBA-Pr-SO₃H; Multicomponent reaction (MCR); Urease inhibitory; Barbituric acid.

Introduction

Many hundreds of chemical compounds across the world are tested daily to find new treatments for different kinds of diseases including bacterial infections and especially gastrointestinal disorders. Among these compounds, optically active dihydropyrimidine (DHPM) derivatives have shown a broad spectrum of biological and pharmacological activities (1-5). The medicinal importance of DHPMs such as: antibacterial, antiviral, antitumor, anti-inflammatory and

calcium channel blockers activities have been identified (6, 7).

The fast and low-cost synthetic methodologies of novel pharmacologically active compounds are the bottleneck of each drug development. Among these methods, the Biginelli reaction is one of the most applicable multicomponent reactions for the preparation of multi-functionalized 3,4-dihydropyrimidin-2(1*H*)-ones and similar compounds (8-13). Literature survey revealed that, not only the common open-chain β -dicarbonyl compounds were used in novel Biginelli-like scaffold synthesis but also cyclic β -diketones (14), β -ketolactones (15), cyclic β -diesters (16) or β -diamides (16, 17), benzocyclic ketones and

* Corresponding author:

E-mail: gmziarani@hotmail.com

α -keto acids were utilized (18).

Urease which is distributed in a variety of organisms is a key enzyme in final step of nitrogen metabolism that catalyzes hydrolysis of urea to ammonia (19). *Helicobacter pylori* plays an important role in gastro duodenal diseases such as gastritis, gastric and duodenal ulcers, and even gastric cancer (20, 21) as well as some other various extraintestinal pathologies. Up to now, many compounds have been proposed as urease inhibitors (22), but some of them are prevented from using *in-vivo* because of their toxicity, poor pharmacokinetic and side effects. On the other hand, natural based compounds are appropriate substitutions for existing inhibitors. In this regard, some isoflavones and deoxybenzoin showed urease inhibitory effect (23). Barbiturate derivatives exhibited broad variety of biological effects such as inhibition of MMP-3 (24), MetAP-1 (25), mushroom tyrosinase (26) and they have also antibacterial (27) and sedative (28) properties.

Previously, our efforts to find urease inhibitors resulted in some derivatives with barbiturate based scaffold (29, 30). Besides, other researchers have shown that compounds possessing barbiturate scaffold inhibit urease apart from their biological significance (31, 32). Moreover, *in vivo* studies have confirmed the possible efficacy of barbituric acid on urea splitting activity of gastrointestinal contents of chicks (33). In the present study, thirteen compounds were synthesized and their effect against urease have evaluated.

As part of our ongoing program for developing environmentally friendly synthetic methods (30, 34-36), sulfonic acid functionalized ordered mesoporous silica was evaluated as nano acid catalyst (37-39) which mediates Biginelli reaction (Scheme 1).

Herein we want to report a simple, rapid, one-pot SBA-Pr-SO₃H mediated synthesis of thirteen spiropyrimidinethiones/spiropyrimidinones-barbituric acids derivatives and their urease inhibitory activities.

Experimental

Material and methods

GC-Mass analysis was measured using the GC-Mass model: 5973 network mass selective

detector, GC 6890 Agilent. IR spectra were recorded from KBr disk on the FT-IR Bruker Tensor 27. Melting points were measured using the capillary tube method with an electro thermal 9200 apparatus. The ¹H-NMR (250 MHz) spectra were recorded on a Bruker DPX, 250 MHz. N₂ adsorption and desorption isotherms were measured at -196 °C with a Japan Belsorb II system after the samples were vacuum dried at 150 °C overnight.

Preparation of catalyst

The preparation, fictionalization and reusability of the nanoporous SBA-15 were studied based on our previous report (36). The modified SBA-15-Pr-SO₃H was used as nanoporous solid acid catalyst in the following reaction.

General procedure for the preparation of spiroheterobicyclic rings

The SBA-Pr-SO₃H (0.02 g) was activated in vacuum at 100 °C, and then was cooled to room temperature. In the next step, barbituric acid (0.65 g, 5 mmol), benzaldehyde (1.65 g, 15 mmol) and urea (0.3 g, 5 mmol) were added to the catalyst in a reaction vessel (Scheme 1). The reaction mixture was heated in oil bath at 150 °C for 20 min. After completion of the reaction (indicated by TLC), the catalyst was filtered and the filtrate was cooled to afford the pure solid product. The solid was recrystallized from ethanol, acetonitrile or methanol to afford pure spiro-(2-oxo-4,6-diphenylhexahydro-pyrimidine-5,5'-barbituric acid). The spectroscopic and analytical data for selected compounds are presented in the following part. The catalyst was washed subsequently with acetonitrile, diluted acid solution, distilled water and acetone, and after drying under vacuum, it can be used for several times without significant loss of activity.

Similarly, the corresponding spiroheterocycles 4b-m were prepared in high yields by the treatment of other substituted aldehydes, barbituric acids or substituted barbituric acids, and urea or thiourea. The results were summarized in the Table 2.

*Spiro-[2-thio-4,6-di-(3-methylphenyl)hexahydropyrimidine-5,5'-barbituric acid]*4g
M.p. 236–240 °C. IR (KBr) cm⁻¹: 3367, 3251

(NH), 2969, 2792, 1715 (CO) 1556 and 1609 (NH Bar bending). ¹H NMR (250 MHz, CDCl₃): δ_H, 2.25 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 5.5 (s, 2H, 2CH), 6.95–7.19 (m, 8H, Ar), 7.85 (s, 2H, NH), 11.02 and 11.36 (2s, 2H, NH) ppm. MS (EI): m/z: 408 (M⁺), 215 (100), 368, 353, 338, 327, 313, 293, 285, 276, 264, 255, 247, 230, 186, 172, 162.

Spiro-[2-oxo-4,6-di-(2-methylphenyl)hexahydropyrimidine-5,5'-barbituric acid]4h

M.p. 214–216 °C. IR (KBr) cm⁻¹: 3330, 3193 (NH), 2846, 3061 and 1715 (CO). ¹H NMR (250 MHz, CDCl₃): δ_H, 2.25 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 5.1 (s, 2H, 2CH), 7.1–7.50 (m, 8H, Ar), 8.40 (s, 2H, NH), 11.17 and 11.40 (2s, 2H, NH) ppm. MS (EI): m/z: 392 (M⁺), 215 (100), 368, 361, 353, 339, 327, 313, 299, 285, 276, 265, 257, 239, 230, 172, 142.

Spiro-[2-thio-4,6-di-(2-methylphenyl)hexahydropyrimidine-5,5'-barbituric acid]4i

M.p. 224–226 °C. IR (KBr) cm⁻¹: 3400, 3193 (NH), 2962, 2860 and 1715 (CO). ¹H NMR (250 MHz, CDCl₃): δ_H, 2.25 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 5.45 (s, 2H, 2CH), 7.16–7.57 (m, 8H, Ar), 8.39 (s, 2H, NH), 11.16 and 11.40 (2s, 2H, NH) ppm. MS (EI): m/z: 408 (M⁺), 328 (100), 272, 300, 285, 215, 386, 368, 365, 341.

Docking approach

Autodock 4.2 (40), which uses a stochastic search based algorithm, was used for the docking study. AutoDockTools 1.5.4 (ADT) (41) was used to prepare receptor and compounds structures for docking studies by following steps: merge nonpolar hydrogens, adding gasteiger and kollman charges for each compound and enzyme respectively as well as set up rotatable bonds. To evaluate the interaction of each compound in enzyme active site, grid maps and an electrostatic map of each point were calculated.

Grid calculation was performed by Autogrid 4.2 (40). The parameters were defined 0.375 nm for grid spacing, and each grid map consisted of 40 × 40 × 40 Å points around the active site. Average coordinates of the two Ni²⁺ ions in the α chain of H. pylori urease was set as the center of the grid. A Lamarckian genetic algorithm (LGA) was used which consisted of 250 runs.

The initial population considers 150 structures, and the maximum number of energy evaluations sets as 2.5 × 10⁷. The other parameters were set as default values. The final structures were clustered and ranked according to the lowest docking energy.

Computational resources

The computational studies were carried out on a computer cluster comprising four sets of HP Proliant ML370-G5 tower servers equipped with two quad-core Intel Xeon E5355 processors (2.66 GHz) and 4 GB of RAM, running a Linux platform (SUSE 10.2).

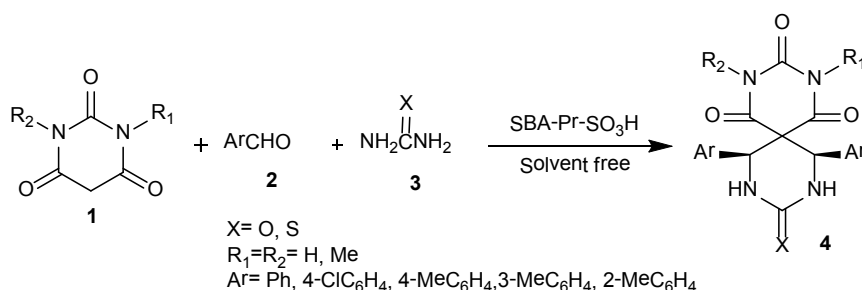
Reliability of the docking protocol

The reliability of the applied docking protocol was determined by re-docking crystallized compound into the active site of the H. pylori urease. If the software could predict binding modes of ligand with receptor, it can be used for further studies. To test this, a ligand is taken out of the X-ray structure of its protein–ligand complex and re-docked into enzyme. After comparison of predicted position of ligand in urease active site, the resulted RMSD was 1.42 Å. This protocol was then similarly applied for all synthesized compounds (29).

Urease inhibition assay

The biological evaluations were performed by the indophenol method. This is based on the release of ammonia (NH₃), which reacts with hypochlorite (OCl⁻) to form monochloramine (42). Then the resulted product reacts with phenol to form blue-colored indophenols whose absorbance is measured at 625 nm.

Briefly, 10 μL of stock enzyme solution (2 mg/mL) was incubated with 140 μL of urea and 5 μL of inhibitor (test compounds) at final concentrations of 1–1.7 mM in phosphate buffer solution (pH 7.6, 100 mM) for 15 min at 37 °C. The released ammonia was estimated using equal volume of solution A (containing 5.0 g phenol and 25 mg of sodium nitro prusside) and 500 μL of solution B [containing 2.5 g sodium hydroxide and 4.2 mL of sodium hypochlorite (5% chlorine) in 500 mL of distilled water] at 37 °C for 30 min, and the absorbance was measured at 625 nm against the control sample.



Scheme 1. Synthesis of spiro-pyrimidinethiones/spiro-pyrimidinones-barbituric acid derivatives 4a-m.

Results and Discussion

This solvent-free one-pot method involves the reaction of barbituric acid **1**, benzaldehyde **2** and urea **3** in the presence of nanoporous acid catalyst of SBA-Pr-SO₃H to afford novel heterobicyclic compounds **4a** in good yields (Scheme 1).

Different solvents in the presence of SBA-Pr-SO₃H as an efficient and recyclable catalyst were screened in the model of reaction. As can be seen in Table 1, in solvent free condition, the desired product, Spiro-(2-oxo-4,6-diphenylhexahydropyrimidine-5,5'-barbituric acid) **4a**, was obtained in good yield (Entry 5) and short reaction time (15 min). Our investigations revealed that the best results were obtained in solvent free condition by heating in oil bath at 150 °C, therefore these conditions were selected as the optimized one.

The proposed mechanism of product **4** is shown in Scheme 2 as a representative model and the stereochemistry of aryl rings is assigned *cis* (43).

In regard to library construction and evaluation of the substrate scope of this reaction, different barbituric acids, urea or thiourea and aromatic aldehydes were employed under similar circumstances (Table 2). Distinguished utilities

of this method are operational simplicity, good yields, and an easy workup protocol without using any chromatographic methods.

In order to find the best catalyst for the formation of the spiro-fused compounds, we compared the reactions in the presence of various protic liquid or solid acids and Lewis acids such as: 1) In acetic acid/microwave (46), 2) using Iodine/microwave (45), 3) using CoCl₂/microwave (47), 4) in HCl (49), 5) in acetic acid (44) and NiCl₂+KI (48) as indicated in Table 3. The results showed that the best yields were obtained in the presence of SBA-Pr-SO₃H which could acts as nanoreactor.

Preparation and characterization of catalyst

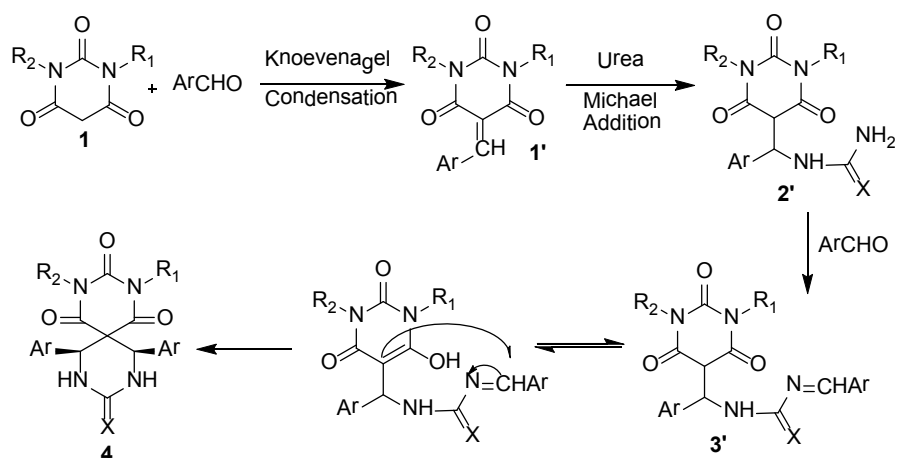
The SBA-15 silica was functionalized with (3-mercaptopropyl)trimethoxysilane (MPTS) and then, the thiol groups were oxidized to sulfonic acid by hydrogen peroxide as shown in Figure 1. Analyzing of the catalyst surface was performed by various methods such as TGA, BET, SEM, TEM and CHN methods which was mentioned in our previous work (30).

All prepared products (**4**) were tested against Jack bean urease and showed the range of inhibition potency, depending on the structural feature (Table 4). Some of synthesized compounds have shown inhibition potency of sub-micromolar

Table 1. The optimization of reaction conditions in the synthesis of compound **4a**.

No.	Solvent	Time (min)	Yield ^a (%)
1	EtOH	30	71
2	EtOH/H ₂ O	35	81
3	H ₂ O	40	57
4	CH ₃ CN	60	80
5	-	15	85

^aIsolated yields.


Table 2. Synthesis of spiropyrimidinethiones/spiropyrimidinones-barbituric acids derivatives.

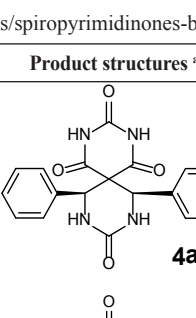
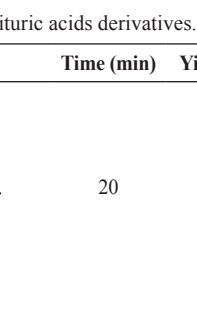
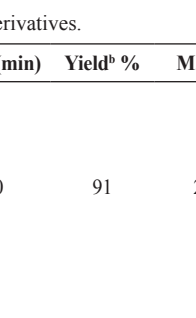
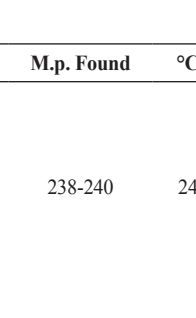
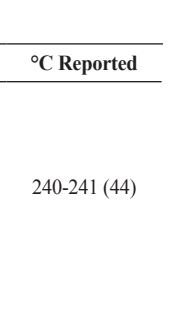
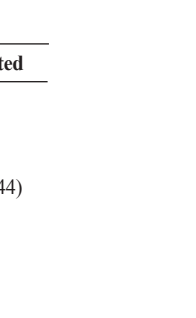
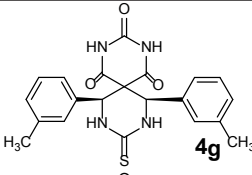
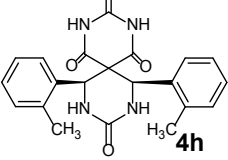
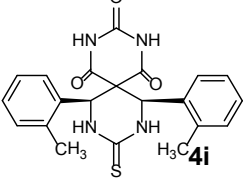
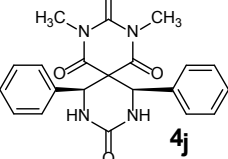
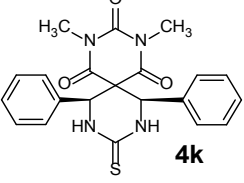
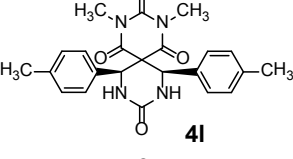
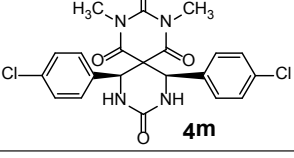
Entry	Ar	R ₁ ,R ₂	X	Product structures ^a	Time (min)	Yield ^b %	M.p. Found	°C Reported
1	Ph	H,H	O		20	91	238-240	240-241 (44)
2	Ph	H,H	S		25	70	245-246	245-247 (45)
3	4-ClC ₆ H ₄	H,H	O		25	80	289-290	289-290 (46)
4	4-MeC ₆ H ₄	H,H	O		15	85	250-252	255-256 (47)
5	4-MeC ₆ H ₄	H,H	S		20	85	240-242	240-242 (48)
6	3-MeC ₆ H ₄	H,H	O		15	92	244-246	246-248 (44)

Table 2. Continue.

7	3-MeC ₆ H ₄	H,H	S		25	77	236-240	New
8	2-MeC ₆ H ₄	H,H	O		20	70	214-216	New
9	2-MeC ₆ H ₄	H,H	S		20	75	220-226	New
10	Ph	CH ₃ ,CH ₃	O		15	78	227-229	230 (46)
11	Ph	CH ₃ ,CH ₃	S		20	79	240-242	242-244 (45)
12	4-MeC ₆ H ₄	CH ₃ ,CH ₃	O		15	78	229-230	227-229 (46)
13	4-ClC ₆ H ₄	CH ₃ ,CH ₃	O		20	80	269-270	271-273 (46)

^a All the compounds were characterized by IR, NMR, MS, and Mp.

^b Isolated yields.

through urease. Possibly, the significant enhanced in inhibition of urease potency could be due to stabilization through an additional barbiturate moiety. In this regard, hydrophilic groups interact with the side chains of Lys, and Asp residues surrounding Ni²⁺ ions active site, providing their role in enhancing the potency (50).

In most of the derivatives, the oxygen contain derivatives were found to be more potent than the sulphur derivatives which can be explained by their ability to bridge two nickel ions (51). This effect is more apparent in 4a compare to 4b with 48% and 2.8% of inhibition respectively. Moreover, obtained percent of inhibition of

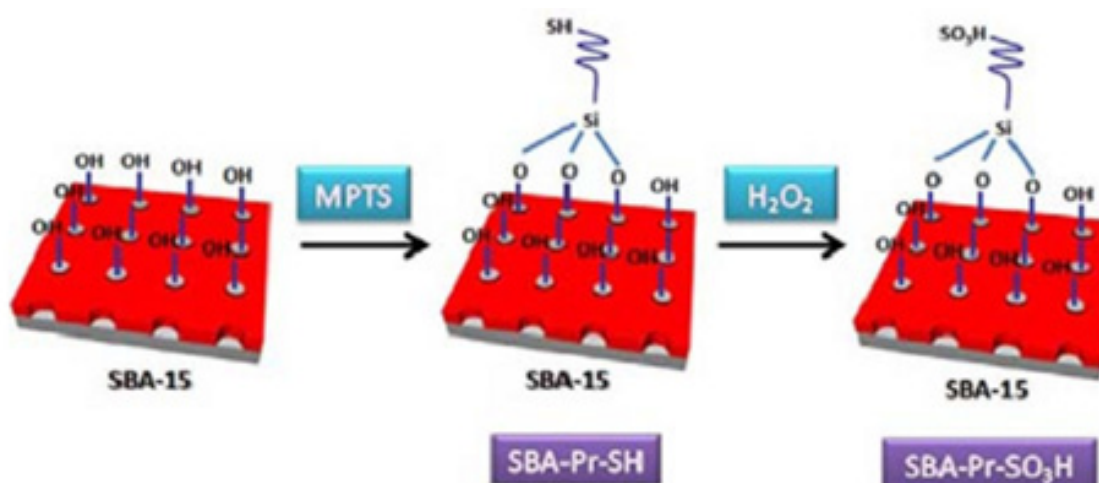


Figure 1. Schematic illustration for the preparation of SBA-Pr-SO₃H

compounds 4h and 4i, 59% and 11% improves this effect. Interestingly, in tested compounds 4j and 4k activate enzyme by different potencies, which means that these two compounds facilitated urease hydrolysis. Indeed, inserting 2 methyl groups on N atoms of barbiturate ring in 4j, result in totally different effect compared to 4a which may cause to construct more hydrophobic interactions in hydrophobic urease binding pocket (52), however in S substituted compound 4k decrease in activity may due to steric hindrance for S to complex with Ni ions. Indeed, hydrophobic interactions of methyl groups in 4k make longer distance between S and Ni to interact.

For better understanding of nature and position of substitute, other derivatives were synthesized. According to the obtained results, introduction of electron donating group of methyl in *ortho* position of phenyl ring resulted in increase in activity to 59 % (compare compounds 4h and

4a). Herein, this effect could be explained by better compound stabilization in binding pocket by hydrophobic interactions (53). In compound 4d with *para* methyl substitutes, lowest steric hindrance resulted in 51% inhibition. For investigation of halogen effects in *para* position, corresponded derivatives have been synthesized, but in experienced concentration, none of them showed significant inhibition through urease.

By contrast, irreversible inhibitors showed the time-dependent manner (54). In this series, compounds 4h and 4k accounted as irreversible one that their inhibition varied in 0.5 h and 3 h. Therefore, obtained results motivate our interest in further structural modifications of 4b as a lead compound which provides new template structures for urease in subsequent researches. From all, decline in inhibitor flexibility considerably owing to the rigid aromatic character result in better inhibition which are included in this series and account them as inhibitors for urease.

Table 3. The efficiency comparison of various catalysts for the synthesis of 4.

Entry	Catalyst	Solvent	Condition	Time	Yield	Year	Ref.
1	CH ₃ COOH	-	MW	4 min	70-83	2004	(46)
2	Iodine	-	MW	4 min	52-94	2010	(45)
3	CoCl ₂	-	MW	4 min	80-88	2004	(47)
4	-	Acetic acid	Reflux	60 min	57-94	1989	(44)
5	HCl	Ethanol	Heating	24 h	39-90	2003	(49)
6	NiCl ₂ + KI	-	Heating	6 h	80-90	2010	(48)
7	SBA-Pr-SO ₃ H	-	Heating	5-30 min	75-92		This work
8	-	-	Heating	60 min	70		This work



Figure 2. Docking structures of inhibitor (4h, yellow) and activator (4j, pink) of spiro compounds in urease enzyme active site.

According to docking studies, in most of the examined compounds, one of the carbonyl groups interacts tightly with both nickel atoms, and the other is involved in the hydrogen bonds with active site residues (Figure 2).

Conclusions

In conclusion, an efficient, one-pot strategy for the synthesis of spiropyrimidinones-barbituric acids derivatives with urease inhibitory/stimulatory activity using SBA-Pr-SO₃H as a nano catalyst was reported for the

first time. Urease inhibitory activity of spiro compounds was tested against Jack bean urease using Berthelot alkaline phenol–hypochlorite method. For the first time, it was found that five of 13 compounds were inhibitor and two of them were activator.

Acknowledgments

We gratefully acknowledge for financial support from the Research Council of Alzahra University and Tehran University of Medical Sciences.

Table 4. Urease inhibitory activity of spiro-pyrimidinethiones/spiro-pyrimidinones-barbituric acids derivatives.

Product	Water solubility	Concentration (mM)	Inhibition (%)
4a	+	1.7	48 ± 0.026
4b	+	1.0	2.8 ± 0.046
4c	-	1.0	-
4d	-	1.7	51 ± 0.052
4e	-	1.0	-
4f	-	1.0	-
4g	-	1.0	-
4h	+	1.7	59 ± 0.019
4i	+	1.0	11 ± 0.042
4j	+	1.7	- 42 ± 0.061
4k	-	1.0	-13 ± 0.039
4l	-	1.0	-
4m	-	1.0	-
Std	+	0.38	90 ± 0.23

References

- (1) Kappe CO. Biologically active dihydropyrimidones of the Biginelli-type a literature survey. *Chem. Eur. J.* (2000) 35: 1043-1052.
- (2) Kappe CO. The generation of dihydropyrimidine libraries utilizing biginelli multicomponent chemistry. *QSAR Comb. Sci.* (2003) 22: 630-645.
- (3) Lewandowski K, Murer P, Svec F and Fréchet JMJ. A combinatorial approach to recognition of chirality: preparation of highly enantioselective aryl-dihydropyrimidine selectors for chiral HPLC. *J. Comb. Chem.* (1998) 1: 105-112.
- (4) Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL and Mitchison TJ. Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* (1999) 286: 971-974.
- (5) Rovnyak GC, Kimball SD, Beyer B, Cucinotta G, DiMarco JD, Gougoutas J, Hedberg A, Malley M and McCarthy JP. Calcium entry blockers and activators: conformational and structural determinants of dihydropyrimidine calcium channel modulators. *J. Med. Chem.* (1995) 38: 119-129.
- (6) Atwal KS, Swanson BN, Unger SE, Floyd DM, Moreland S, Hedberg A and O'Reilly BC. Dihydropyrimidine calcium channel blockers. 3. 3-Carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidinecarboxylic acid esters as orally effective antihypertensive agents. *J. Med. Chem.* (1991) 34: 806-811.
- (7) Sawant R and Sarode V. Synthesis, Spectral characterization and analgesic activity of 2-methylthio-1, 4-dihydropyrimidines. *Iran. J. Pharm. Res.* (2011) 10: 733-739.
- (8) Biginelli P. Aldehyde-urea derivatives of aceto- and oxaloacetic acids. *Gazz. Chim. Ital.* (1893) 23: 360.
- (9) Burke MD and Schreiber SL. A planning strategy for diversity-oriented synthesis. *Angew. Chem. Int. Ed.* (2004) 43: 46-58.
- (10) Guillena G, Ramón DJ and Yus M. Organocatalytic enantioselective multicomponent reactions (OEMCRs). *Tetrahedron: Asymmetry* (2007) 18: 693-700.
- (11) Kappe CO. A reexamination of the mechanism of the biginelli dihydropyrimidine synthesis. Support for an N-acyliminium ion intermediate. *J. Org. Chem.* (1997) 62: 7201-7204.
- (12) Foroughifar N, Mobinikhaledi A and Fathinejad Jirandehi H. Microwave assisted synthesis of some pyrimidine derivatives using polyphosphate ester (ppe) in ceramic bath. *Phosphorus, Sulfur Silicon Relat. Elem.* (2003) 178: 1241-1246.
- (13) Zendejdel M, Mobinikhaledi A and Asgari A. Zeolite an efficient catalyst for the Biginelli condensation reaction. *J. Inclusion Phenom. Macroscopic Chem.* (2008) 60: 353-357.
- (14) Ananda Kumar K, Kasthuraiah M, Suresh Reddy C and Devendranath Reddy C. Mn(OAc)₃·2H₂O-mediated three-component, one-pot, condensation reaction: an efficient synthesis of 4-aryl-substituted 3,4-dihydropyrimidin-2-ones. *Tetrahedron Lett.* (2001) 42: 7873-7875.
- (15) Ranu BC, Hajra A and Jana U. Indium(III) chloride-catalyzed one-pot synthesis of dihydropyrimidinones by a three-component coupling of 1,3-dicarbonyl compounds, aldehydes, and urea: an improved procedure for the biginelli reaction. *J. Org. Chem.* (2000) 65: 6270-6272.
- (16) Rosi Reddy K, Venkateshwar Reddy C, Mahesh M, Raju PVK and Narayana Reddy VV. New environmentally friendly solvent free synthesis of dihydropyrimidinones catalysed by N-butyl-N,N-dimethyl- α -phenylethylammonium bromide. *Tetrahedron Lett.* (2003) 44: 8173-8175.
- (17) Bose DS, Fatima L and Mereyala HB. Green chemistry approaches to the synthesis of 5-alkoxycarbonyl-4-aryl-3,4-dihydropyrimidin-2(1H)-ones by a three-component coupling of one-pot condensation reaction: Comparison of ethanol, water, and solvent-free conditions. *J. Org. Chem.* (2002) 68: 587-590.
- (18) Dondoni A and Massi A. Parallel synthesis of dihydropyrimidinones using Yb(III)-resin and polymer-supported scavengers under solvent-free conditions. A green chemistry approach to the Biginelli reaction. *Tetrahedron Lett.* (2001) 42: 7975-7978.
- (19) Mobley HLT and Hausinger RP. Microbial ureases: significance, regulation, and molecular characterization. *Microbiol. Rev.* (1989) 53: 85-108.
- (20) Katsura Y, Nishino S, Ohno M, Sakane K, Matsumoto Y, Morinaga C, Ishikawa H and Takasugi H. Anti-Helicobacter pylori Agents. 3. 2-[(Arylalkyl)guanidino]-4-furylthiazoles. *J. Med. Chem.* (1999) 42: 2920-2926.
- (21) Tripathi G, Singh S and Nath G. Formulation and *in-vitro* evaluation of pH-sensitive oil entrapped polymeric blend amoxicillin beads for the eradication of helicobacter pylori. *Iran. J. Pharm. Res.* (2012) 11: 447-455.
- (22) Mahmood Biglar, Khadijeh Soltani, Farzaneh Nabati, Roya Bazl, Faraz Mojab and Massoud Amanlou. A preliminary investigation of the jack-bean urease inhibition by randomly selected traditional used herbal medicine. *Iran. J. Pharm. Res.* (2012) 11: 831-837.
- (23) HQ Li, ZP Xiao, Y Luo, T Yan, PC Lv and HL Zhu. Amines and oximes derived from deoxybenzoin as Helicobacter pylori urease inhibitors. *Eur. J. Med. Chem.* (2009) 44: 2246-2251.
- (24) Kim SH, Pudzianowski AT and Leavitt KJ. Structure-based design of potent and selective inhibitors of collagenase-3 (MMP-13). *Bioorg. Med. Chem. Lett.* (2005) 15: 1101-1106.
- (25) Halder MK, Scott MD and Sule N. Synthesis of barbiturate-based methionine aminopeptidase-1 inhibitors. *Bioorg. Med. Chem. Lett.* (2008) 18: 2373-2376.
- (26) Yan Q, Cao R, Yi W, Chen Z, Wen H, Ma L and Song H. Inhibitory effect of 5-benzylidene barbiturate derivatives on mushroom tyrosinase and their antibacterial activities. *Eur. J. Med. Chem.* (2009) 44: 4235-4243.
- (27) Tomas T, Zidar N, Mueller-Premru M, Kikelj D and Peterlin Mas L. Synthesis and antibacterial activity of 5-ylidenethiazolidin-4-ones and 5-benzylidene-

- 4,6-pyrimidinediones. *Eur. J. Med. Chem.* (2010) 45: 1667-1672.
- (28) Kliethermes CL, Metten P and Belknap JK. Selection for pentobarbital withdrawal severity: correlated differences in withdrawal from other sedative drugs. *Brain Res.* (2004) 1009: 17-25.
- (29) Azizian H, Nabati F, Sharifi A, Siavoshi F, Mahdavi M and Amanlou M. Large-scale virtual screening for the identification of new *Helicobacter pylori* urease inhibitor scaffolds. *J. Mol. Model.* (2012) 18: 2917-2927.
- (30) Mohammadi Ziarani G., S. Faramarzi, S. Asadi, A. Badiiei, R. Bazl and M. Amanlou. Three-component synthesis of pyrano[2,3-d]-pyrimidine dione derivatives facilitated by sulfonic acid nanoporous silica (SBA-Pr-SO₃H) and their docking and urease inhibitory activity. *Daru* (2013) 21: 3-16.
- (31) Khan KM, Ali M, Wadood A, Ul-Haq Z, Zia-Ullah KM, Lodhi MA, Perveen S, Choudhary MI and Volter W. Molecular modeling-based antioxidant arylidene barbiturates as urease inhibitors. *J. Mol. Graph. Model.* (2011) 30: 153-156.
- (32) Rauf A, Ahmed F, Qureshi AM, Aziz-ur-Rehman KA, Qadir MI, Choudhary MI, Chohan ZH, Youssoufid M and Haddad TB. Synthesis and urease inhibition studies of barbituric and thiobarbituric acid derived sulphonamides. *J. Chin. Chem. Soc.* (2011) 58: 1-10.
- (33) Harbers LH, Alvares AP, Jacobsen AI and Visek WJ. In growth of chicks fed diets supplemented with chlortetracycline or barbituric acid. *Fed. Prec. Abstr. Federation Amer. Soc. Exp. Biol.* (1962) 20814-3998: 386.
- (34) Mohammadi Ziarani G, Badiiei A and Haddadpour M. Application of sulfonic acid functionalized nanoporous silica (SBA-Pr-SO₃H) for one-pot synthesis of quinoxaline derivatives. *Int. J. Chem.* (2011) 3: 87-94.
- (35) Mohammadi Ziarani G., Badiiei A, Aslani Z and Lashgari N. Application of sulfonic acid functionalized nanoporous silica (SBA-Pr-SO₃H) in the green one-pot synthesis of triazoloquinazolinones and benzimidazoquinazolinones. *Arabian J. Chem.* (2015) 8: 54-61.
- (36) Mohammadi Ziarani G., Badiiei A and Haddadpour M. One-pot synthesis of polyhydroquinolines catalyzed by sulfonic acid functionalized SBA-15 as a new nanoporous acid catalyst under solvent free conditions. *Iran. J. Chem. Chem. Eng.* (2010) 29: 1-10.
- (37) Das B, Venkateswarlu K, Holla H and Krishnaiah M. Sulfonic acid functionalized silica: A remarkably efficient heterogeneous reusable catalyst for α -monobromination of carbonyl compounds using N-bromosuccinimide. *J. Mol. Catal.* (2006) 253: 107-111.
- (38) Onaka M, Hashimoto N, Kitabata Y and Yamasaki R. Aluminum-rich mesoporous aluminosilicate (Al-HMS) as a solid acid catalyst for the Diels-Alder reaction of acrylates with 1,3-dienes. *Appl. Catal., A* (2003) 241: 307-317.
- (39) Van Rhijn WM, De Vos DE, Sels BF and Bossaert WD. Sulfonic acid functionalised ordered mesoporous materials as catalysts for condensation and esterification reactions. *Chem. Commun.* (1998) 317-318.
- (40) Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* (1998) 19: 1639-1662.
- (41) Sanner M. Python: a programming language for software integration and development. *J. Mol. Graph. Model.* (1999) 17: 57-61.
- (42) Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* (1967) 39: 971-974.
- (43) Byk G, Gottlieb H, Herscovici J and Mirkin F. New regioselective multicomponent reaction: one pot synthesis of spiro heterobicyclic aliphatic rings. *J. Comb. Chem.* (2002) 2: 732-735.
- (44) Mokrosz JL, Paluchowska MH, Szneler E and Drozd B. The Effect of Aryl Substituents in Some Spiro[2-oxo-4, 6-bis (aryl)hexahydropyrimidine-5,5'-barbituric acids]. *Archiv der Pharmazie* (1989) 322: 231-235.
- (45) Prajapati D, Bhuyan D and Gohain MHW. Green chemistry approaches to the regioselective synthesis of spiro heterobicyclic rings using iodine as a new and efficient catalyst under solvent-free conditions. *Mol. Divers* (2010) 15: 257-261.
- (46) Shaabani A and bazgir A. Microwave-assisted efficient synthesis of spiro-fused heterocycles under solvent-free conditions. *Tetrahedron Lett.* (2004) 45: 2575-2577.
- (47) Saini A, Kumar S and Sandhu JS. Nickel mediated biginelli-like three component coupling reaction: a solvent-free microwave assisted synthesis of spiro-fused heterocycles. *Ind. J. Chem. B* (2004) 43: 2482-2484.
- (48) Gupta P, Gupta S, Sachar A, Kour D, Singh J and RL S. One pot synthesis of spiro pyrimidinethiones/spiro pyrimidinones, quinazolinethiones/quinazolinones, and pyrimidopyrimidines. *J. Heterocyclic Chem.* (2010) 47: 324-333.
- (49) Abelman MM, Smith SC and James DR. Cyclic ketones and substituted α -keto acids as alternative substrates for novel Biginelli-like scaffold syntheses. *Tetrahedron Lett.* (2003) 44: 4559-4562.
- (50) Amtul Z, Rasheed M, Choudhary MI, Rosanna S, Khan KM and Rahman A. Kinetics of novel competitive inhibitors of urease enzymes by a focused library of oxadiazoles/thiadiazoles and triazoles. *Biochem. Biophys. Res. Commun.* (2004) 319: 1053-1063.
- (51) Domínguez MaJ, Sanmartín C, Font Ma, Palop JA, San Francisco S, Urrutia O, Houdusse F and García-Mina JM. Design, synthesis, and biological evaluation of phosphoramidate derivatives as urease inhibitors. *J. Agric. Food Chem.* (2008) 56: 3721-3731.
- (52) Tanaka T, Kawase M and Tani S. Urease inhibitory activity of simple α,β -unsaturated ketones. *Life Sci.* (2003) 73: 2985-2990.
- (53) Mamiya G, Takishima K, Masakuni M, Kayumi T, Ogawa K and Sekita T. Complete amino acid sequence of jack bean urease. *Proc. Japan Acad. B* (1985) 61: 395-398.
- (54) Dixon M and Webb E. Enzyme inhibition and activation. *Enzymes* (1979) 3: 126-136.