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ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 2246–2251
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Amines and oximes derived from deoxybenzoins as *Helicobacter pylori* urease inhibitors

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Received 19 December 2007; received in revised form 23 May 2008; accepted 2 June 2008
Available online 11 June 2008

Abstract

Twenty amines and oximes from deoxybenzoins were prepared and evaluated for their inhibitory activity against *Helicobacter pylori* urease. Among these compounds, high inhibitory activities were observed in amines and oximes, especially amines **1b** ($IC_{50} = 0.011$ mM) and **6b** ($IC_{50} = 0.047$ mM) exhibited good in vitro activities, and were comparable to acetohydroxamic acid (AHA). The hydroxyl groups on deoxybenzoin skeleton may be responsible for the inhibitory activity and coordinate with the nickel (active site) on enzyme. A direct interaction may exist between the OH group of hydroxylamines or NH group of amines and His α323 of *H. pylori* urease, which is on the flap of the enzyme active site.

Keywords: Amines; Oximes; Deoxybenzoin derivatives; *H. polyri* urease; Structure–activity relationships

1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5) is widely distributed in a variety of bacteria such as *Helicobacter pylori* and *Proteus mirabilis*. *H. pylori* is a Gram-negative microaerophilic bacterium that infects up to 50% of the world's human population [1]. Investigations revealed that the bacterium could cause many gastroduodenal diseases such as gastritis, gastric and duodenal ulcers, and even gastric cancer [2,3], as well as some other various extraintestinal pathologies. Structural studies of the enzymes from *Klebsiella aerogenes*, *Bacillus pasteurii*, and *H. pylori* have revealed a dinuclear Ni active site with a carbamylated lysine residue that bridges the deeply buried metal atoms [4,5]. One nickel ion (Ni-1) binds and activates urea, with one resonance structure stabilized by a nearby carboxylate, while the second (Ni-2) binds a hydroxide. The latter molecule is activated for attack on the urea carbon by a protein residue acting as a general base. The tetrahedral intermediate collapses, eliminating ammonia with the help of an active site thiol, which was proposed to act as a general acid [6]. Many urease inhibitors have been described in the past decades, as fluorofamide, hydroxyureas, and acetohydroxamic acids [4], but part of them were prevented from using in vivo because of their toxicity or instability. Hence, there are unmet medical needs for novel and efficacious urease inhibitors with low toxicity.

As the most abundant natural products, isoflavones had been demonstrated potent biological activity such as anticarcinogenic [7], anti-inflammatory [8], antiviral [9] and antiprotozoals [10]. As important derivatives of isoflavones, deoxybenzoins also exhibited their broad variety of biological activities including antimicrobials [11] and urease inhibitors with low toxicity [12]. Many compounds with amine or oxime group indicated potent biological activities and low toxicity [13,14], some of them have been used as clinical medical agents. We previously investigated the inhibitory activity against *H. pylori* urease of some deoxybenzoins and found that the carbonyl group might be detrimental to the inhibitory activity [12]. To find the more potent urease inhibitors for further structure–activity relationship research of deoxybenzoins, we focused on the carbonyl group modification and inhibitory activity against *H. polyri* urease of deoxybenzoin. In this paper, 20 amines and oximes were designed from

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doi:10.1016/j.ejmech.2008.06.001

deoxybenzoins for their urease inhibitory activities against *H. pylori* urease for the first time. The structure–activity relationships have also been discussed upon the evaluation results.

2. Results and discussion

2.1. Synthesis

A series of amines and oximes were designed and synthesized from deoxybenzoins for urease inhibitors by the routes outlined in Scheme 1. Ten deoxybenzoins **1–10** were first synthesized by the method in our previous paper [12]. Our interest in this area was to design and synthesize amines and oximes for SAR studies in *H. pylori* urease inhibitory activity. Thus, the treatment of selected deoxybenzoins **1–10** with hydroxylamine hydrochloride in pyridine afforded the oximes **1a–10a**. An oxime has *Z*- and *E*-configuration isomers in theory, but the two pseudo isomers are configurationally unstable and transformed each other quickly at room temperature. Then **1a–10a** were submitted to the Zn/HCOOH (aq) reductive conditions [15], leading to the corresponding amines **1b–10b** with moderate to high overall yields. All compounds (Fig. 1) were fully characterized by spectroscopic method and elemental analysis. All the amines and oximes **1a–10a** and **1b–10b** were synthesized for the first time.

2.2. Biological activity

Twenty corresponding compounds **1a–10a** and **1b–10b** were evaluated against *H. polyri* urease. Deoxybenzoins **1–10** were also investigated for structure–activity relationships. Percent inhibition at a 400 μg/mL concentration of tested compounds was initially determined, and the results were reported in Table 1. Most of the amines and oximes showed good inhibitory activities, especially the amines **1b** ($IC_{50} = 0.011$ mM) and **6b** ($IC_{50} = 0.047$ mM) exhibited potent in vitro activities, which were comparable to acetohydroxamic acid (AHA), a reversible urease inhibitor (Table 2). The comparison of the inhibitory activities of amines and oximes with corresponding deoxybenzoins indicated that amines (**1b–10b**) and oximes (**1a–10a**) demonstrated better inhibitory activities than the corresponding deoxybenzoins **1–10**. The result revealed that changing the carbonyl group of deoxybenzoins for hydroxylamine (**1a–10a**) or amido group (**1b–10b**) significantly increased their activities, which implied that the carbonyl group was clearly detrimental to the inhibitory activity. These results implied that a direct interaction might exist between the OH group of hydroxylamines or NH group of amide and His α323 of *H. pylori* urease, which was on the flap of the enzyme active site [16].

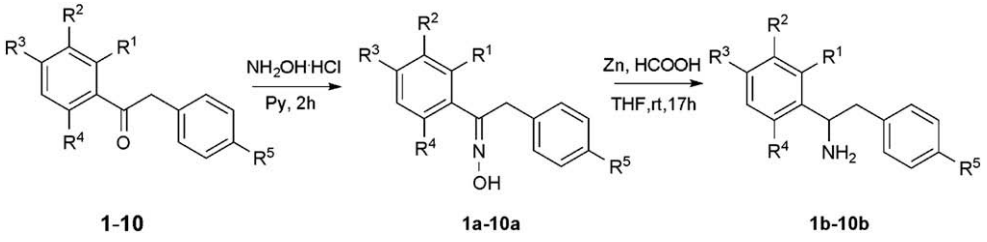
In amines **1b–10b**, compounds **1b–4b**, **6b**, **8b** and **9b** exhibited good inhibitory activities, however, replacing one or all of the hydroxyl groups on the phenol or catechol skeleton by methoxy or nitro group produced a loss in activity (**5b**, **7b** and **10b**). So the hydroxyl groups on deoxybenzoin skeleton may be responsible for the inhibitory activity and coordinate with the nickel (active site) on enzyme. Compounds **3b–5b** with resorcinol skeleton showed weaker inhibitory activity than **1b** and **2b**, possibly because of the long distance between the two hydroxyl groups at 1 and 3 positions on benzene ring counted for less interaction with the nickel atoms of the enzyme. The same comparison was also exhibited in oxime compounds **1a–10a**.

H. pylori-infection leads to different clinical and pathological outcomes in humans, including chronic gastritis, peptic ulcer disease and gastric neoplasia. Up to now, complete eradiation of *H. pylori* is still the most effective therapy, which prompts people to make great efforts to find more and new anti-*H. pylori* natural or synthetical agents. Structure studies of the enzymes from *H. pylori* have revealed a dinuclear Ni active site with a carbamylated lysine residue that bridged the deeply buried metal atoms [4]. It was reported that some urease inhibitors depressed *H. pylori* urease activities by interacting with the sulfhydryl groups, especially the Cystein-592 residue, which is a key residue in the active sites [17]. In the amines and oximes compounds **1a–10a** and **1b–10b**, the interaction of deoxybenzoins with sulfhydryl group may be related to their inhibitory influence on urease, and the hydroxylamine and amide group may be responsible for the interaction.

As important derivatives of natural product isoflavones, deoxybenzoins exhibited their broad variety of biological activities with low toxicity, and the amines **1b** and **6b** based on the deoxybenzoins exhibited good inhibitory activities against *H. polyri*. This result attracted our interest in further structure modification of compounds **1b** and **6b** as lead compounds for new urease inhibitors or anti-*H. polyri* agents.

3. Conclusions

In conclusion, 20 amines and oximes from deoxybenzoins were prepared and evaluated for their inhibitory activity



Scheme 1. Synthetic routes of amine and oximes from deoxybenzoins.

	R^1	R^2	R^3	R^4	R^5
1a, 1b	OH	OH	OH	H	OH
2a, 2b	OH	OH	OH	H	F
3a, 3b	OH	H	OH	H	F
4a, 4b	OH	H	OH	H	OCH_3
5a, 5b	OH	H	OH	H	NO_2
6a, 6b	H	OH	OH	H	OH
7a, 7b	OCH_3	OCH_3	OCH_3	H	OCH_3
8a, 8b	OH	OCH_3	OH	OH	OH
9a, 9b	OH	H	OH	OH	OH
10a, 10b	OH	H	OCH_3	OCH_3	OCH_3

Fig. 1. Chemical structures of compounds **1a**–**10a** and **1b**–**10b**.

against *H. polyri* urease. Among these compounds, amines **1b** ($IC_{50} = 0.011$ mM) and **6b** ($IC_{50} = 0.047$ mM) exhibited good in vitro activities, and comparable to acetohydroxamic acid (AHA). The results revealed that changing the carbonyl group of deoxybenzoins for hydroxylamine or amide group significantly increases the biological activity of deoxybenzoins, and the hydroxyl groups on deoxybenzoin skeleton may be responsible for the inhibitory activity and coordinate with the nickel (active site) on enzyme. A direct interaction may exist between the OH group of hydroxylamines or NH group of amines and His α323 of *H. pylori* urease, which is on the flap of the enzyme active site.

4. Experimental part

4.1. General

The solvents used were purchased from Shanghai Chemical Reagent Company (Shanghai, China), and were used as received without purification. Reactions and the resulted products were monitored by thin-layer chromatography (TLC), and were run on the silica gel coated aluminum sheets (silica gel 60 GF_{254}, E. Merck, Germany) and visualized in UV light (254 nm). Sonication was performed in a Kunshan KQ 500E ultrasonic cleaner (Jiangsu, China) with irradiation delivered at 40 kHz and 500 W. All the NMR spectra were recorded on a Bruker DRX 500 model spectrometer in DMSO-d_6. Chemical shifts (δ) for ^{1}H NMR spectra were reported in parts per million to residual solvent protons. Melting points were measured on a Boetius micro melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer.

Table 1
Percent inhibition of compounds **1a**–**10a** and **1b**–**10b** against *H. pylori* urease at the concentration of 400 μg/mL

Compound	Percent inhibition (%)	Compound	Percent inhibition (%)	Compound	Percent inhibition (%)
1	51	**1a**	73	**1b**	97.3
2	44	**2a**	55	**2b**	80
3	16	**3a**	23	**3b**	48
4	14	**4a**	36	**4b**	73
5	–[b]	**5a**	–	**5b**	–
6	39	**6a**	62	**6b**	92.5
7	–	**7a**	–	**7b**	11
8	17	**8a**	38	**8b**	62
9	–	**9a**	13	**9b**	28
10	–	**10a**	15	**10b**	–
AHA[a]	94.3				

[a] Used as a positive control.
[b] These compounds were found as inactive.

Table 2
Inhibition of *H. pylori* urease by compounds **1b** and **6b**

Compound	IC_{50} (mM)
1b	0.011 ± 0.0012
6b	0.047 ± 0.005
AHA	0.017 ± 0.002

4.2. Compounds

*General procedure of preparation of oximes **1a–10a** and amines **1b–10b***. A solution of deoxybenzoins **1**–**10** [12] (0.90 mmol) and hydroxylamine hydrochloride (5.4 mmol) in dry pyridine (18 mL) was refluxed for 2–2.5 h. The reaction mixture was allowed to cool to room temperature and evaporated to dryness in vacuo, and the residue was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The organic extract was washed with water (2 × 10 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts dried over anhydrous sodium sulfate and evaporated in vacuo gave the corresponding oximes **1a**–**10a**. The resulting oximes **1a**–**10a** were dissolved in the smallest amount of THF, 70% aqueous formic acid (17 mL) was added, and zinc powder (26.7 mmol) was added portion wise over 30 min after cooling the solution with an ice bath. The reaction mixture was stirred for 17–19 h at room temperature, filtered on sand, and washed with EtOAc. The filtrate was neutralized with a concentrated ammonia solution to pH 8 and then extracted with EtOAc (3 × 30 mL). The organic phase was washed with water (50 mL), dried over anhydrous sodium sulfate, and evaporated in vacuo to give a residue that was purified by flash chromatography on silica gel using 10–40% EtOAc/CH_2Cl_2 or 2–5% MeOH/CH_2Cl_2 as the eluent gave the amines **1b**–**10b**.

4.2.1. 2-(4-Hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl) ethanone oxime (*1a*)

Yellow solid, m.p. 151–153 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 4.11 (dd, $J = 3.5$, 9 Hz, 2H), 6.27 (d, $J = 9$ Hz, 1H), 6.82 (d, $J = 8$ Hz, 2H), 6.87 (d, $J = 9$ Hz, 1H), 7.15 (d, $J = 8$ Hz, 2H), 8.91 (s, 1H), 9.64 (s, 1H), 10.32 (s, 1H), 12.66 (s, 1H), 12.98 (s, 1H). ESI-MS: 276.6 ($C_{14}H_{13}NO_5$

$[M+H]^+$). Anal. Calcd for $C_{14}H_{13}NO_5$: C 61.09, H 4.76, N 5.09%; found: C 61.15, H 4.73, N 5.16%.

*4.2.2. 4-(1-Amino-2-(4-hydroxyphenyl)ethyl)benzene-1,2,3-triol (**1b**)*

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.81 (br s, 2H), 2.98 (dd, $J=3.5$, 9 Hz, 2H), 4.07 (d, $J=10.5$ Hz, 1H), 6.39 (d, $J=9$ Hz, 1H), 6.65 (d, $J=8$ Hz, 2H), 7.02 (d, $J=9$ Hz, 1H), 7.17 (d, $J=8$ Hz, 2H), 8.71 (s, 1H), 9.45 (s, 1H), 10.11 (s, 1H), 12.45 (s, 1H). ESI-MS: 262.1 ($C_{14}H_{15}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{15}NO_4$: C 64.36, H 5.79, N 5.36%; found: C 64.39, H5.73, N 5.41%.

*4.2.3. 2-(4-Fluorophenyl)-1-(2,3,4-trihydroxyphenyl) ethanone oxime (**2a**)*

Yellow solid, m.p. 172–175 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 3.93 (dd, $J=3.5$, 9 Hz, 2H), 6.37 (d, $J=9$ Hz, 1H), 6.92 (d, $J=8$ Hz, 2H), 7.15 (d, $J=8$ Hz, 2H), 7.34 (d, $J=9$ Hz, 1H), 8.84 (s, 1H), 10.21 (s, 1H), 12.56 (s, 1H), 12.92 (s, 1H). ESI-MS: 278.2 ($C_{14}H_{12}FNO_4$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}FNO_4$: C 60.65, H 4.36, F 6.85, N 5.05%; found: C 60.56, H 4.25, F 6.77, N 5.11%.

*4.2.4. 4-(1-Amino-2-(4-fluorophenyl)ethyl)benzene-1,2,3-triol (**2b**)*

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.83 (br s, 2H), 2.85 (dd, $J=3.5$, 9 Hz, 2H), 4.12 (d, $J=10.5$ Hz, 1H), 6.36 (d, $J=9$ Hz, 1H), 6.92 (d, $J=8$ Hz, 2H), 7.17 (d, $J=8$ Hz, 2H), 7.23 (d, $J=9$ Hz, 1H), 8.92 (s, 1H), 10.11 (s, 1H), 12.21 (s, 1H). ESI-MS: 264.1 ($C_{14}H_{14}FNO_3$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{14}FNO_3$: C 63.87, H 5.36, F 7.22, N 5.32%; found: C 63.83, H 5.31, F 7.27, N 5.38%.

*4.2.5. 1-(2,4-Dihydroxyphenyl)-2-(4-fluorophenyl) ethanone oxime (**3a**)*

Yellow solid, m.p. 187–189 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 4.13 (dd, $J=3.5$, 9 Hz, 2H), 6.24 (s, 1H), 6.39 (d, $J=9$ Hz, 1H), 6.90 (d, $J=8$ Hz, 2H), 7.08 (d, $J=9$ Hz, 2H), 7.88 (d, $J=9$ Hz, 1H), 9.29 (s, 1H), 10.65 (s, 1H), 12.64 (s, 1H). ESI-MS: 262.1 ($C_{14}H_{12}FNO_3$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}FNO_3$: C 64.36, H 4.63, F 7.27, N 5.36%; found: C 64.19, H 4.77, F 7.33, N 5.41%.

*4.2.6. 4-(1-Amino-2-(4-fluorophenyl)ethyl)benzene-1,3-diol (**3b**)*

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.84 (br s, 2H), 2.78 (dd, $J=3.5$, 9 Hz, 2H), 4.03 (d, $J=10.5$ Hz, 1H), 6.24 (d, $J=9$ Hz, 1H), 6.42 (d, $J=9$ Hz, 1H), 6.87 (d, $J=8$ Hz, 2H), 7.11 (d, $J=9$ Hz, 2H), 7.93 (d, $J=9$ Hz, 1H), 10.54 (s, 1H), 12.11 (s, 1H). ESI-MS: 248.2 ($C_{14}H_{14}FNO_2$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{14}FNO_2$: C 68.00, H 5.71, F 7.68, N 5.66%; found: C 68.09, H 5.77, F 7.63, N 5.61%.

*4.2.7. 1-(2,4-Dihydroxyphenyl)-2-(4-methoxyphenyl) ethanone oxime (**4a**)*

Yellow solid, m.p. 193–194 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 3.76 (s, 3H), 4.08 (dd, $J=3.5$, 9 Hz, 2H), 6.28 (d, $J=9$ Hz, 1H), 6.42 (dd, $J=2$, 9 Hz, 1H), 6.87 (d, $J=9$ Hz, 2H), 7.21 (d, $J=9$ Hz, 2H), 7.87 (d, $J=9$ Hz, 1H), 10.45 (s, 1H), 12.21 (s, 1H), 12.56 (s, 1H). ESI-MS: 274.1 ($C_{15}H_{15}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}NO_4$: C 65.92, H 5.53, N 5.13%; found: C 65.97, H 5.45, N 5.21%.

*4.2.8. 4-(1-Amino-2-(4-methoxyphenyl)ethyl)benzene-1,3-diol (**4b**)*

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.81 (br s, 2H), 2.98 (dd, $J=3.5$, 9 Hz, 2H), 3.76 (s, 3H), 4.11 (d, $J=10.5$ Hz, 1H), 6.26 (d, $J=9$ Hz, 1H), 6.42 (dd, $J=2$, 9 Hz, 1H), 6.87 (d, $J=9$ Hz, 2H), 7.17 (d, $J=9$ Hz, 2H), 7.76 (d, $J=9$ Hz, 1H), 10.54 (s, 1H), 12.11 (s, 1H). ESI-MS: 258.4 ($C_{15}H_{17}NO_3$ $[M-H]^+$). Anal. Calcd for $C_{15}H_{17}NO_3$: C 69.48, H 6.61, N 5.40%; found: C 69.41, H 6.65, N 5.31%.

*4.2.9. 1-(2,4-Dihydroxyphenyl)-2-(4-nitrophenyl) ethanone oxime (**5a**)*

Yellow solid, m.p. 167–169 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 4.11 (dd, $J=3.5$, 9 Hz, 2H), 6.26 (s, 1H), 6.42 (d, $J=9$ Hz, 1H), 7.64 (d, $J=8$ Hz, 2H), 7.94 (d, $J=9$ Hz, 1H), 9.19 (d, $J=8$ Hz, 2H), 10.65 (s, 1H), 12.25 (s, 1H), 12.64 (s, 1H). ESI-MS: 289.1 ($C_{14}H_{12}N_2O_5$ $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}N_2O_5$: C 58.33, H 4.20, N 9.72%; found: C 58.25, H 4.23, N 9.61%.

*4.2.10. 4-(1-Amino-2-(4-nitrophenyl)ethyl)benzene-1, 3-diol (**5b**)*

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.82 (br s, 2H), 2.98 (dd, $J=3.5$, 9 Hz, 2H), 4.03 (d, $J=10.5$ Hz, 1H), 6.28 (d, $J=9$ Hz, 1H), 6.42 (d, $J=9$ Hz, 1H), 7.56 (d, $J=8$ Hz, 2H), 7.91 (d, $J=9$ Hz, 1H), 9.08 (d, $J=8$ Hz, 2H), 10.76 (s, 1H), 12.21 (s, 1H). ESI-MS: 275.1 ($C_{14}H_{14}N_2O_4$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{14}N_2O_4$: C 61.31, H 5.14, N 10.21%; found: C 61.38, H 5.11, N 10.26%.

*4.2.11. 1-(3,4-Dihydroxyphenyl)-2-(4-hydroxyphenyl) ethanone oxime (**6a**)*

Brown solid, m.p. 231–233 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 4.08 (dd, $J=3.5$, 9 Hz, 2H), 6.67 (d, $J=8$ Hz, 2H), 6.80 (d, $J=1$ Hz, 1H), 7.02 (d, $J=8$ Hz, 2H), 7.37 (s, 1H), 7.46 (d, $J=8$ Hz, 1H), 9.22 (s, 1H), 9.35 (s, 1H), 10.02 (s, 1H), 11.23 (s, 1H). ESI-MS: 260.1 ($C_{14}H_{13}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{13}NO_4$: C 64.86, H 5.05, N 5.40%; found: C 64.78, H 5.11, N 5.46%.

*4.2.12. 4-(1-Amino-2-(4-hydroxyphenyl)ethyl)benzene-1, 2-diol (**6b**)*

Yellow oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.85 (br s, 2H), 3.02 (dd, $J=3.5$, 9 Hz, 2H), 4.03 (d, $J=10.5$ Hz, 1H), 6.56 (d, $J=8$ Hz, 2H), 6.73 (d, $J=1$ Hz, 1H), 7.05 (d, $J=8$ Hz, 2H), 7.39 (s, 1H), 7.48 (d, $J=8$ Hz, 1H), 9.22

(s, 1H), 9.35 (s, 1H), 9.97 (s, 1H). ESI-MS: 246.1 ($C_{14}H_{15}NO_3$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{15}NO_3$: C 68.56, H 6.16, N 5.71%; found: C 68.58, H 6.11, N 5.76%.

4.2.13. 2-(4-Methoxyphenyl)-1-(2,3,4-trimethoxyphenyl) ethanone oxime (**7a**)

Yellow oil, ^{1}H NMR (500 MHz, DMSO-d_6): 3.71 (s, 3H), 3.76 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.12 (dd, $J = 3.5$, 9 Hz, 2H), 6.78 (d, $J = 8$ Hz, 2H), 6.89 (d, $J = 9$ Hz, 1H), 7.11 (d, $J = 8$ Hz, 2H), 7.36 (d, $J = 9$ Hz, 1H). ESI-MS: 332.1 ($C_{18}H_{21}NO_5$ $[M+H]^+$). Anal. Calcd for $C_{18}H_{21}NO_5$: C 65.24, H 6.39, N 4.23%; found: C 65.31, H 6.32, N 4.28%.

4.2.14. 2-(4-Methoxyphenyl)-1-(2,3,4-trimethoxyphenyl) ethanamine (**7b**)

Yellow oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.81 (br s, 2H), 2.84 (dd, $J = 3.5$, 9 Hz, 2H), 3.71 (s, 3H), 3.76 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.11 (d, $J = 10.5$ Hz, 1H), 6.86 (d, $J = 8$ Hz, 2H), 6.89 (d, $J = 9$ Hz, 1H), 7.13 (d, $J = 8$ Hz, 2H), 7.38 (d, $J = 9$ Hz, 1H). ESI-MS: 318.1 ($C_{18}H_{23}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{18}H_{23}NO_4$: C 68.12, H 7.30, N 4.41%; found: C 68.17, H 7.37, N 4.48%.

4.2.15. 2-(4-Hydroxyphenyl)-1-(2,4,6-trihydroxy-3-methoxy phenyl)ethanone oxime (**8a**)

Yellow solid, m.p. 212–213 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 3.65 (s, 3H), 4.08 (dd, $J = 3.5$, 9 Hz, 2H), 6.02 (s, 1H), 6.65 (d, $J = 8$ Hz, 2H), 7.01 (d, $J = 8$ Hz, 2H), 9.22 (s, 1H), 10.35 (s, 1H), 11.42 (s, 1H), 12.53 (s, 1H), 12.98 (s, 1H). ESI-MS: 306.1 ($C_{15}H_{15}NO_6$ $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}NO_6$: C 59.01, H 4.95, N 4.59%; found: C 59.07, H 4.97, N 4.51%.

4.2.16. 2-(1-Amino-2-(4-hydroxyphenyl)ethyl)-4-methoxy benzene-1,3,5-triol (**8b**)

Yellow oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.83 (br s, 2H), 2.84 (dd, $J = 3.5$, 9 Hz, 2H), 3.65 (s, 3H), 4.11 (d, $J = 10.5$ Hz, 1H), 6.02 (s, 1H), 6.65 (d, $J = 8$ Hz, 2H), 7.01 (d, $J = 8$ Hz, 2H), 9.22 (s, 1H), 10.35 (s, 1H), 11.42 (s, 1H), 12.53 (s, 1H). ESI-MS: 292.1 ($C_{15}H_{17}NO_5$ $[M+H]^+$). Anal. Calcd for $C_{15}H_{17}NO_5$: C 61.85, H 5.88, N 4.81%; found: C 61.81, H 5.91, N 4.85%.

4.2.17. 2-(4-Hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl) ethanone oxime (**9a**)

White solid, m.p. 184–186 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 4.08 (dd, $J = 3.5$, 9 Hz, 2H), 5.87 (s, 1H), 6.67 (d, $J = 8$ Hz, 2H), 7.01 (d, $J = 8$ Hz, 2H), 9.15 (s, 1H), 10.34 (s, 1H), 12.11 (s, 2H), 12.98 (s, 1H). ESI-MS: 276.1 ($C_{14}H_{13}NO_5$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{13}NO_5$: C 61.09, H 4.76, N 5.09%; found: C 61.14, H 4.79, N 5.11%.

4.2.18. 2-(1-Amino-2-(4-hydroxyphenyl)ethyl) benzene-1,3,5-triol (**9b**)

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.81 (br s, 2H), 2.95 (dd, $J = 3.5$, 9 Hz, 2H), 4.11 (d, $J = 10.5$ Hz, 1H), 6.92 (s, 1H), 6.68 (d, $J = 8$ Hz, 2H), 7.08 (d, $J = 8$ Hz, 2H), 9.22 (s, 1H), 10.35 (s, 1H), 12.23 (s, 2H). ESI-MS: 262.1 ($C_{14}H_{15}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{14}H_{15}NO_4$: C 64.36, H 5.79, N 5.36%; found: C 64.32, H 5.72, N 5.33%.

4.2.19. 1-(2-Hydroxy-4,6-dimethoxyphenyl)-2-(4-methoxy phenyl)ethanone oxime (**10a**)

White powder, m.p. 115–117 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 3.39 (s, 3H), 3.65 (s, 3H), 3.68 (s, 3H), 3.89 (s, 2H), 5.95 (d, $J = 18$ Hz, 2H), 6.70 (d, $J = 8$ Hz, 2H), 7.00 (d, $J = 9$ Hz, 2H), 9.74 (br, 1H), 10.92 (s, 1H). ESI-MS: 318.1 ($C_{17}H_{19}NO_5$ $[M+H]^+$). Anal. Calcd for $C_{17}H_{19}NO_5$: C 64.34, H 6.03, N 4.41%; found: C 64.28, H 6.08, N 4.35%.

4.2.20. 2-(1-Amino-2-(4-methoxyphenyl)ethyl)-3,5-dimeth oxyphenol (**10b**)

Colorless oil, ^{1}H NMR (500 MHz, DMSO-d_6): 1.81 (br s, 2H), 2.82 (dd, $J = 3.5$, 9 Hz, 2H), 3.41 (s, 3H), 3.67 (s, 3H), 3.78 (s, 3H), 5.94 (d, $J = 18$ Hz, 2H), 6.78 (d, $J = 8$ Hz, 2H), 7.04 (d, $J = 9$ Hz, 2H), 9.71 (s, 1H). ESI-MS: 304.1 ($C_{17}H_{21}NO_4$ $[M+H]^+$). Anal. Calcd for $C_{17}H_{21}NO_4$: C 67.31, H 6.98, N 4.62%; found: C 67.34, H 6.93, N 4.65%.

4.3. Bacteria

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as previously described [3].

4.4. Preparation of *H. pylori* urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000*g*, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80 °C. *H. pylori* was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000*g*, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham–Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.5. Measurement of urease activity

The assay mixture, containing 25 μL (4U) of *H. pylori* urease and 25 μL of the test compound, was preincubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [18].

Acknowledgments

The work was financed by grants (Projects 30772627) from National Natural Science Foundation of China.

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