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Developing potential *Helicobacter pylori* urease inhibitors from novel oxoindoline derivatives: Synthesis, biological evaluation and *in silico* study

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ABSTRACT

By recruiting the important moiety from Shikonin, a series of novel oxoindoline derivatives **S1–S20** have been synthesized for inhibiting *H. pylori* urease. The most potent compound **S18** displayed better activity (IC_{50} = 0.71 μM; MIC = 0.48 μM) than the positive controls AHA (IC_{50} = 17.2 μM) and Metronidazole (MIC = 31.3 μM). With low cytotoxicity, it showed considerable potential for further development. Docking simulation revealed the possible binding pattern of this series. 3D QSAR model was built to discuss SAR and give useful hints for future modification.

Urease is a common enzyme in bacteria, fungi, higher plant and invertebrates.[1–3] It mainly catalyzes the hydrolysis reaction from urea to ammonia and carbon dioxide, therefore assists microorganisms to utilize urea as indispensable nitrogen source.[4] Despite the roles in botany and microbiology, urease is also significant in biochemical and medicinal researches.[5,6] Recruiting the functions of urease, *Helicobacter pylori* (*H. Pylori*) may cause the abnormal rise of ammonia concentration and subsequent increase in pH, which aids *H. Pylori* itself to endure acidity in the stomach during colonization.[7] Since *H. Pylori* is a major culprit for gastricism and corresponding diseases, urease inferred important effect in *H. Pylori*-inducing pathogenesis of gastric and peptic ulcer.[8] Moreover, the development of infection stones, pyelonephritis, hepatic coma are also convinced to be directly affected by *H. Pylori* urease.[9–11] Inhibitory agents and deuterogenic therapies targeting on urease have supplied available approaches for treatment of *H. Pylori* caused diseases.[8,12] Although hydroxamic acids[13,14] or phosphoramidates[15,16] are relative potent inhibitors of urease at present, teratogenicity and unstability stops them from further medicinal use[17,18]. Since daidzein was found to be weakly inhibitory against *H. pylori* urease, exploration of more potent and low toxic inhibitors from natural products has become a hotspot.[19,20]

Inspired by the previous research on screening *H. pylori* urease from flavonoids[21], we attempted to perform a transformation of flavonoid with oxoindoline due to their similarities in both structure and function[22,23]. The benzoyl hydrazone or phenyl hydrazine groups were selected as candidates to raise new possibilities after comparing available pharmacophores for the target[24–28]. The most attractive moiety was recruited from shikonin, a natural product with multi functions including *H. Pylori* inhibition.[29] Shikonin mainly consists of a flavonoid-like backbone and a typical 3-methylbut-2-enyl moiety. Since we conducted the transformation of flavonoid, we intended to use the same moiety for probable analogy. The new series were designed accordingly and the prepositive molecular docking evaluation implied preferable binding situations. The subsequent synthesis, biological evaluation and structure-activity relationship (SAR) discussion were performed in this work.

After wiping off the generated compounds who failed in virtual screening and synthetic feasibility, oxoindolin derivatives **S1–S20** were synthesized. All of them were reported for the first time. The general synthesis method and the structures were organized in Scheme 1. They were prepared in two steps. First, isatin was stirred in DMF at 50 °C and 3,3-dimethylallyl bromide was then added with NaH, yielding 1-(3-methylbut-2-en-1-yl)indoline-2,3-dione (**B**). Second, different hydrazine derivatives participated the condensation reaction which led to the corresponding target compounds **S1–S20**. Refined compounds were achieved through recrystallisation, giving satisfactory analytical and spectroscopic data.

All the synthesized compounds **S1–S20** were evaluated for their antimicrobial and urease inhibitory activities. The results were expressed as MIC (minimum inhibitory concentration) and IC_{50} (the half maximal inhibitory concentration of *H. pylori* urease mediated ammonia production) with Hammet Sigma values of mono substitutes,

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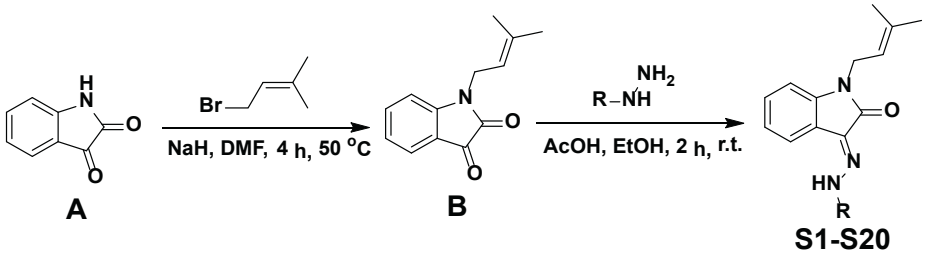

Scheme 1. General synthesis of compounds **S1–S20** and their structures.

Table 1
Antimicrobial and urease inhibitory activities of **S1–S20**.

Code	MIC (μM) *H. pylori*	IC_{50} (μM) urease	Hammet Sigma	Code	MIC (μM) *H. pylori*	IC_{50} (μM) urease	Hammet Sigma
S1	171 ± 14.3	129 ± 10.1	0.00	**S11**	72.5 ± 6.71	38.1 ± 2.53	+0.37
S2	> 200	154 ± 12.3	+0.06	**S12**	50.5 ± 4.28	27.6 ± 1.98	+0.39
S3	185 ± 16.3	143 ± 11.7	+0.23	**S13**	15.2 ± 1.29	8.21 ± 0.65	+0.12
S4	137 ± 10.8	83.9 ± 7.32	+0.23	**S14**	108 ± 9.36	65.8 ± 5.31	+0.43
S5	58.1 ± 5.23	29.9 ± 2.15	−0.17	**S15**	19.5 ± 1.67	11.7 ± 0.95	–
S6	10.3 ± 0.98	6.99 ± 0.53	−0.27	**S16**	6.75 ± 0.52	4.38 ± 0.30	–
S7	27.5 ± 2.16	14.9 ± 1.19	−0.15	**S17**	1.96 ± 0.16	1.35 ± 0.10	–
S8	30.8 ± 2.87	19.5 ± 1.56	−0.20	**S18**	0.48 ± 0.04	0.71 ± 0.06	–
S9	83.9 ± 7.37	44.1 ± 3.87	+0.54	**S19**	3.94 ± 0.33	3.82 ± 0.29	–
S10	41.8 ± 3.86	22.7 ± 1.76	–	**S20**	> 200	> 300	0.00
Metro	31.3 ± 2.85	–	–	AHA	–	17.2 ± 0.14	–

Metro: Metronidazole; AHA: Acetohydroxamic acid.

presented in Table 1. Though slightly affected by a relative high concentration of DMSO (0.2%), most of the compounds showed potential potency. Meanwhile, the antimicrobial effect indicated correlation with the urease inhibition.

Most of this series inferred potency against *H. pylori* urease. The most potent compound **S18** displayed better activity (IC_{50} = 0.71 μM; MIC = 0.48 μM) than the positive controls AHA (IC_{50} = 17.2 μM) and Metronidazole (MIC = 31.3 μM). This kind of potency was also far beyond that of shikonin. Compendious Structure-Activity Relationship (SAR) discussion was then conducted. First, for simple substitutes at *para*-position, electron-donating groups indicated better potency, with the order –OMe (IC_{50} = 6.99 μM) > –Me (IC_{50} = 29.9 μM) > –CF_3 (IC_{50} = 44.1 μM) > –Br (IC_{50} = 83.9 μM) > –H (IC_{50} = 129 μM) > –Cl (IC_{50} = 143 μM) > –F (IC_{50} = 154 μM). 4-Trifluoromethyl seemed to be an exception with the most electron-withdrawing nature but moderate potency. A possible explanation for this point might be the steric effect. Other relatively bulky groups such as isopropyl (IC_{50} = 14.9 μM), tertiary butyl (IC_{50} = 19.5 μM) or phenoxyl (IC_{50} = 22.7 μM) also showed favorable potency but could not bring further improvement to the top choice. Second, for *meta*-position, simple electron-donating group also seemed preferable, with the order -OMe (IC_{50} = 8.21 μM) > –Br (IC_{50} = 27.6 μM) > –Cl (IC_{50} = 38.1 μM) > –CF_3 (IC_{50} = 65.8 μM). Third, for electron-donating group such as –OMe, *para*-substitute (IC_{50} = 6.99 μM) seemed better than a *meta*- (IC_{50} = 8.21 μM) or *ortho*-one (IC_{50} = 11.7 μM). Finally, we came to multi substitutes. Limited by the virtual performance and practical synthetic difficulties, this kind of compounds in our series were relatively few. The acquired compounds generally obeyed the aforementioned preference of electron-donating group and the top ones all belonged to this category. An extra hint was that the carbonyl could not be omitted as **S1** was slightly better than **S20**. Thus, the future orientation might be involving more electron-donating groups as multi substitutes. The data were visualized as maps and a more brief SAR analysis was displayed in the 3D QSAR part below.

Moreover, the top 3 (**S18**, **S17**, **S19**) and bottom 3 (**S20**, **S2**, **S3**) compounds were set as representatives to evaluate the cytotoxicity of this series on HEK293T (human embryonic kidney cell line) and LO2 (human embryonic liver cell line) cells. Seen in Table 2, all of them suggested low toxic.

For *in silico* study, the ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) simulation[30–31] for **S1–S20** was initially shown in Fig. 1. The parameters of AlogP (the partition coefficient of drug in octanol/aqueous solution calculated by ACD/PhysChem Suite Software) and PSA_2D (the fast calculated polar surface area from the 2D structure) were used to predict the Absorption (human intestinal absorption) and BBB (blood-brain barrier penetration) including 95% and 99% confidence ellipses as referenced[32]. All the compounds suggested good potential in druggability.

Molecular docking was performed for preliminary screening and visualization the possible binding model. All twenty compounds were docked into the active site of *H. pylori* urease (PDB code: 4HI0). Seen in Fig. 2, the CDOCKER Interaction Energy (interaction energy between the ligand and the receptor) agreed with the *H. pylori* urease inhibitory trend for all compounds. This result hinted the consistency of the SAR and molecular docking. The 3D maps of the most potent compound **S18** within 4HI0 were depicted in Fig. 3, showing both the detailed surrounding situation and the laconic binding site.

Seen in Fig. 3, **S18** formed two hydrogen bonds with GLY13 (O…H–N: 2.36 Å, 125.566°) and THR15 (O…H–N: 2.41 Å, 143.143°), indicating the introduction of hydrazide moiety was favorable. The π–cation interaction (distance: 4.34 Å) between LYS146 and oxoindoline maintained the basic binding pattern of this series. The substitutes on the benzoyl ring did not show key interactions with residues of the binding site on F Chain of urease, but they interacted with the E Chain residues. The amino group on the side chains of LYS127 and LYS131 on E Chain might push electron-donating groups of this series to introduce