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4-Hydroxy-3-(naphthalen-1-ylmethyl)thiophen-2(5H)-one as inhibitors of tyrosyl-tRNA synthase: Synthesis, molecular docking and antibacterial evaluation

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HIGHLIGHTS

- A series of 4-hydroxy-3-(naphthalen-1-ylmethyl)thiophen-2(5H)-ones were first synthesized.
- Compound **29** is the most potent agent against *Staphylococcus aureus* ATCC 25923 with MIC_{50} value of 0.21 μg/mL.
- Their biological activities are also evaluated for tyrosyl-tRNA synthase inhibitory activity.

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ABSTRACT

A series of novel 4-hydroxy-3-(naphthalen-1-ylmethyl)thiophen-2(5H)-ones as tyrosyl-tRNA synthetase inhibitors were synthesized. Of these compounds, 4-(naphthalen-1-ylmethyl)-5-oxo-2,5-dihydrothiophen-3-yl-2-(4-hydroxyphenyl)acetate (**29**) was the most potent. The binding model and structure–activity relationship indicate that replacement of phenyl acetate in the side chain of **29** with a substituent containing more hydrophilic groups would be more suitable for further modification. Antibacterial assay revealed that the synthetic compounds are effective against growth of Gram-positive organisms, and **29** is the most potent agent against *Staphylococcus aureus* ATCC 25923 with MIC_{50} value of 0.21 μg/mL.

1. Introduction

The emergence of resistance to clinically used drugs has promoted development of novel antimicrobial agents which selectively inhibit novel bacterial targets [1–3]. In this regard, aminoacyl-tRNA synthetases (aaRSs) have attracted considerable interest in antibacterial drug discovery [4–6]. It is well known that aaRSs are the enzymes that catalyze the transfer of amino acids to their cognate tRNAs [7,8] and their catalytic activities determine the genetic code. Consequently, once these enzymes are inhibited, cell growth is inhibited due to protein biosynthesis being halted. This concept is proven by the success of the broad-spectrum antibacterial drug mupirocin, which targets bacterial isoleucyl-tRNA synthetases (IleRS) [9]. Additionally, AN-2690 (Fig. 1), an inhibitor of leucyl-tRNA synthetases, is currently in clinical development for the topical treatment of onychomycosis and icofungipen. These enzymes, therefore, are attractive targets for antibacterial agents [10–12].

Thiophenone framework is a part of many natural and synthetic compounds, which possess useful biological activities such as antitubercular and antibacterial activity [13–19]. Xiao et al. have shown that 3-aryl-4-alkylaminofuran-2(5H)-ones are potent inhibitors against tyrosyl-tRNA synthetase (TyrRS) [20,21], one of the aminoacyl-tRNA synthetases (aaRSs). Structure–activity relationships of the two classes of compounds disclosed that bioisosteric replacement of NH group (a) with an O (b) was more favorable for activity (Fig. 1). Recently, Benneche et al. reported 5-(chloromethylene)-and 5-(bromoalkylidene)thiophen-2(5H)-ones showing better antibacterial activity than furanones [22]. Therefore, derivatives of thiophenone have been receiving significant attention, which encourages us to synthesize and evaluate the antibacterial activity of a series of substituted thiophenones. To learn more about the structure-antibacterial activity relationship of thiophenones, we herein report the synthesis of 4-hydroxy-3-(naphthalen-1-ylmethyl) thiophen-2(5H)-ones as

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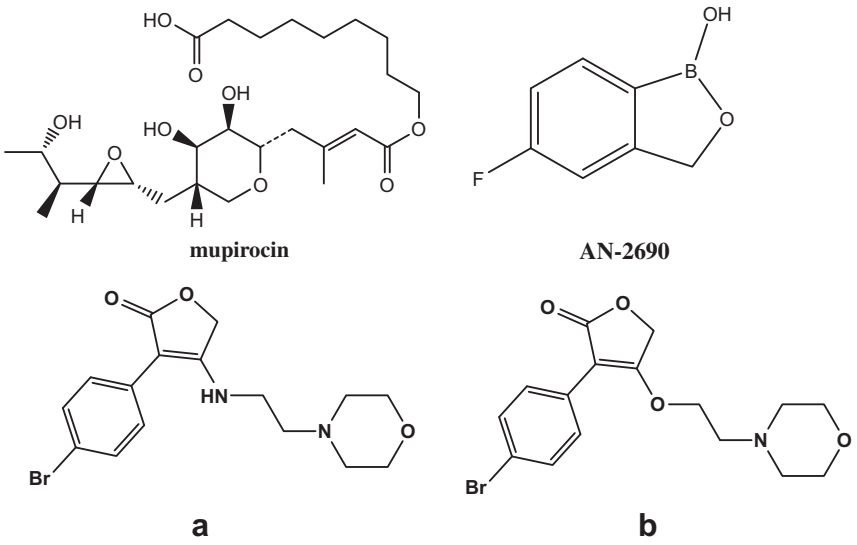


Fig. 1. Structures of some compounds.

TyrRS inhibitors and antibacterial agents. The results show that some of the synthesized compounds exhibit excellent antibacterial activities.

2. Experimental

2.1. Preparation of the TyrRS and enzyme assay

Staphylococcus aureus TyrRS was over-expressed in *Escherichia coli* and purified to near homogeneity (~98% as judged by SDS–PAGE) using standard purification procedures [7]. TyrRS activity was measured by aminoacylation using modifications to previously described methods [8]. The assays were performed at 37 °C in a mixture containing (final concentrations) 100 mM Tris/Cl pH 7.9, 50 mM KC1, 16 mM $MgCl_2$, 5 mM ATP, 3 mM DTT, 4 mg/mL *E. coli* MRE600 tRNA (Roche) and 10 μM L-tyrosine (0.3 μM L-[ring-3,5-3H] tyrosine (PerkinElmer, Specific activity: 1.48–2.22 TBq/mmol), 10 μM carrier). TyrRS (0.2 nM) was preincubated with a range of inhibitor concentrations for 10 min at room temperature followed by the addition of pre-warmed mixture at 37 °C. After specific intervals, the reaction was terminated by adding aliquots of the reaction mix into ice-cold 7% trichloroacetic acid and harvesting onto 0.45 mm hydrophilic Durapore filters (Millipore Multiscreen 96-well plates) and counted by liquid scintillation. The rate of reaction in the experiments was linear with respect to protein and time with less than 50% total tRNA acylation. IC_{50}s correspond to the concentration at which half of the enzyme activity is inhibited by the compound. The results are presented in Table 1.

2.2. Antimicrobial activity

The antibacterial activities of the synthesized compounds was tested against two Gram-positive bacterial strains (*Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, kanamycin as positive control) and two Gram-negative bacterial strains (*Pseudomonas aeruginosa* ATCC13525, *E. coli* ATCC 35218, *penicillin G* as positive control) using LB medium. The MIC_{50}s of the test compounds were determined by a colorimetric method using the dye MTT. A stock solution of the synthesized compound (1000 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (50% (v/v) of DMSO in PBS). A specified quantity of the medium containing the test compound was poured into 96-well plates. Suspension of the microorganism was prepared to contain approximate 10^5 cfu/mL and applied to 96-well plates with serially diluted compounds to be tested and incubated at 37 °C for 24 h. The optical density (OD) was measured with a microplate reader at 550 nm. The observed MIC_{50}s were presented in Table 2.

2.3. Protocol of docking study

Automated docking studies were carried out using Discovery Studio (version 3.1) as implemented through the graphical user interface DS-CDocker protocol.

The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2010)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger–Hückel charges of ligands were assigned. The crystal structures of TyrRS (PDB code: 1jij.) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins.

2.4. Chemistry

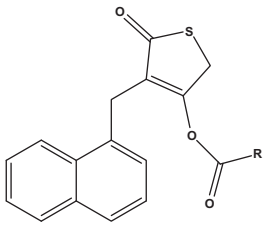
All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^1H NMR spectra were collected on a Bruker DPX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

3. Results and discussion

3.1. Chemistry

Thirty 4-hydroxy-3-(naphthalen-1-ylmethyl) thiophen-2(5H)-ones (**5**–**34**) were designed and synthesized by the routes outlined

Table 1
In vitro inhibitory activity data of the synthesized compounds against *S. aureus* TyrRS.



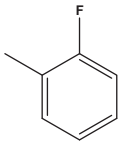
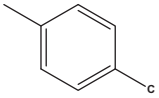
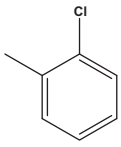
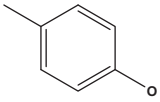
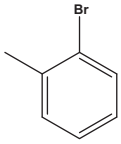
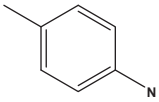
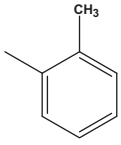
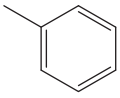
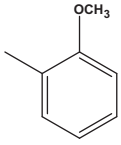
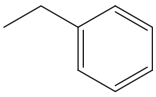
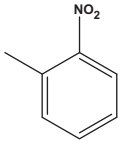
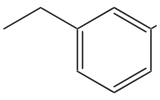
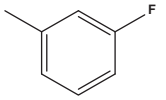
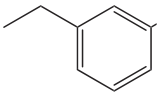
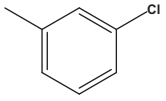
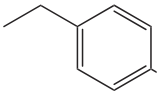
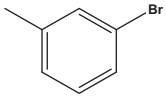
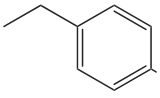
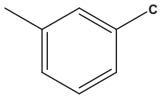
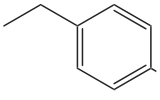
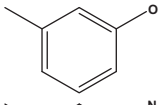
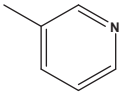
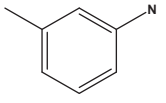
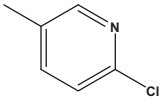
Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
5		>100	**20**		50.08
6		>100	**21**		44.76
7		>100	**22**		48.94
8		88.62	**23**		43.32
9		92.10	**24**		8.30
10		>100	**25**		18.98
11		82.14	**26**		15.86
12		86.13	**27**		11.10
13		83.13	**28**		13.79
14		69.00	**29**		0.25
15		72.11	**30**		>100
16		71.09	**31**		>100

Table 1 (*continued*)

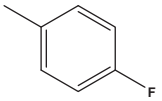
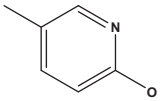
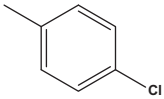
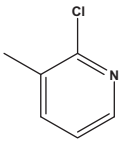
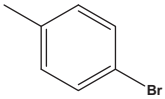
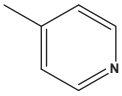
Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
17		65.14	**32**		>100
18		55.23	**33**		>100
19		51.17	**34**		>100

Table 2
Inhibitory activity of the synthetic compounds against microbes.

Compound	MIC_{50} (μg/mL)			
	A	B	C	D
24	23.50	10.08	>50	>50
25	>50	33.12	>50	>50
26	>50	35.78	>50	>50
27	42.12	21.99	40.0	>50
28	29.81	33.12	>50	>50
29	3.66	0.21	>50	>50
Kanamycin	0.48	1.20	3.93	>50
Penicillin	–	–	–	2.70

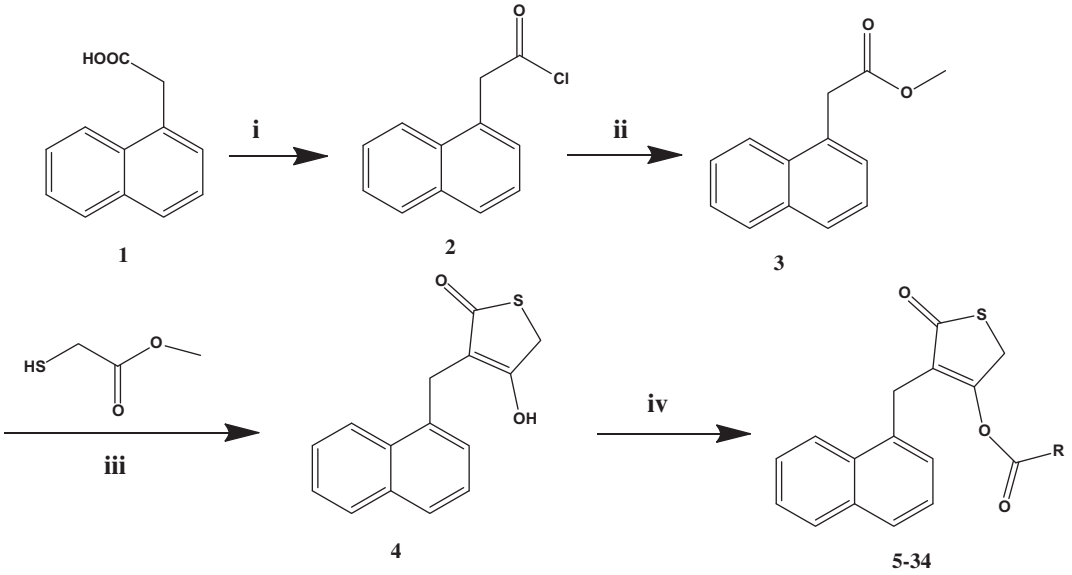
Note: (A) *B. subtilis* ATCC 6633; (B) *S. aureus* ATCC 25923; (C) *P. aeruginosa* ATCC13525; (D) *E. coli* ATCC 35218.

in Scheme 1. In brief, compound **2** was formed in the presence of thionyl chloride. Ester **3** was prepared using concentrated sulfuric acid in methanol. The key compound thiophenone (**4**) was obtained by alkalization of **3** with appropriate methyl thioglycolate in THF in the presence of *t*-BuOK. The target compounds (**5**–**34**) were obtained by treatment with different benzoic acids in the presence of EDC.HCl and HoBt in anhydrous dichloromethane. All 4-hydroxy-3-(naphthalen-1-ylmethyl) thiophen-2(5H)-ones are reported here for the first time and were fully characterized by spectroscopic methods and elemental analysis (see Supporting Information).

3.2. Inhibitory activities of 4-hydroxy-3-(naphthalene-1-ylmethyl) thiophen-2(5H)-ones against TyrRS from S. aureus

All the synthesized compounds (**5**–**34**) were tested for inhibitory activity against TyrRS from *S. aureus*. The IC_{50}s of these compounds are presented in Table 1. Compounds **5**–**23** were prepared to study the utility of benzoic acid side chains and all of the compounds showed poor activity, indicating that separation of benzene moiety and thiophenone fragment with an carbon atom spacer led to compounds (**24**–**29**) being more potent. Therefore, this position seems to be more suitable for further modification with a substituent containing more aliphatic chains.

We then turned our attention toward exploring the SAR profile of the phenylacetic acid group (**24**–**29**). The compound **29** with a hydroxyl group at the *para* position of the benzene-ring fragment



Scheme 1. General synthesis of compounds **5**–**34**. Reagents and conditions: (i) Thionyl chloride, reflux, 1 h; (ii) Methanol, reflux, 3 h; (iii) Tetrahydrofuran, Potassium tert-butoxide, reflux, overnight; (iv) Dichloromethane, EDC.HCl, HoBt. rt, 3–10 h.

exerted the highest inhibitory activity against *S. aureus* TyrRS with an IC_{50} of 0.25 μg/mL. Replacement of 4-hydroxyl group with *chloro* or bromo group resulted in 100-fold loss of activity (**27**–**28**). In the case of compounds (**30**–**34**) with pyridine-ring substituents, introduction of electron-donating group (methoxy) and electron-withdrawing group (*chloro*) led to complete loss in activity, indicating that these substituents may cause a steric clash with the protein.

3.3. Antibacterial activity

Compounds with $IC_{50} < 20$ μM against *S. aureus* TyrRS were tested against representative Gram-positive organisms (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923) and Gram-negative organisms (*P. aeruginosa* ATCC13525 and *E. coli* ATCC 35218), and the results are presented in Table 2. The tested compounds showed good activity against Gram-positive organisms, especially against *S. aureus*, while all of them were inactive against Gram-negative bacterium, *P .aeruginosa* and *E. coli*. Compound **29** displayed levels of inhibition against *S. aureus* compared to Kanamycin. From comparing the data in Tables 1 and 2, a positive correlation between the IC_{50} values in the enzyme assay and the MIC_{50} values against whole cell bacteria was found. This suggests that the antibacterial activity of thiophenone, as that of 4-hydroxy-3-(naphthalen-1-ylmethyl) thiophen-2(5H)-ones, may be due to their inhibition against TyrRS.

3.4. Molecular docking

With the aim to explore the structural determinants responsible for the activity of these new inhibitors of TyrRS, molecular docking of the most potent inhibitor **29** into SB-239629 binding site of TyrRS was performed on the binding model based on the TyrRS complex structure (1jij.pdb). The binding model of compound **29** and TyrRS is depicted in Fig. 2 and the enzyme surface model is showed in Fig. 3, which revealed that the molecular is well filled in the active pocket.

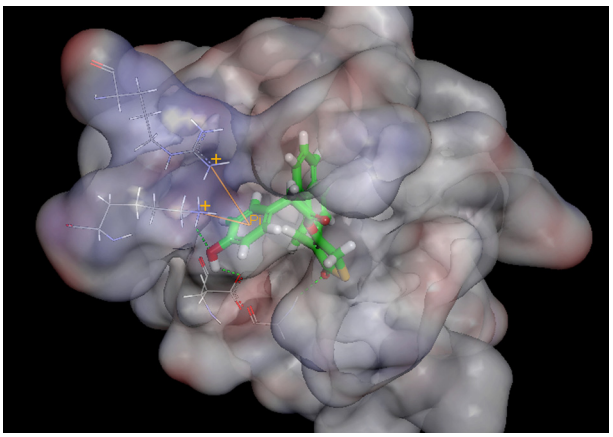


Fig. 3. The 3D model structure of compound **29** binding model with TyrRS.

Several hydrogen-bonding interactions together with some hydrophobic interactions anchoring **29** to the active site tightly may explain its excellent inhibitory activity. In brief, the benzene-ring system in the side chain of **29** occupies the SB-239629 piperidyl-binding pocket, and is oriented towards the entrance cavity (Fig. 3) surrounded by Ala39, Leu70, Gln174, Val191, Gly192 and Ile200. The O atom of thiophenone-ring as a hydrogen bond acceptor is hydrogen-bonded to the backbone nitrogen of Gly193 with O. . .H bond. (Fig. 2). Obviously, the benzene-ring system is located at a hydrophilic pocket of the active site. Thus, replacement with a substituent containing stronger hydrophilic

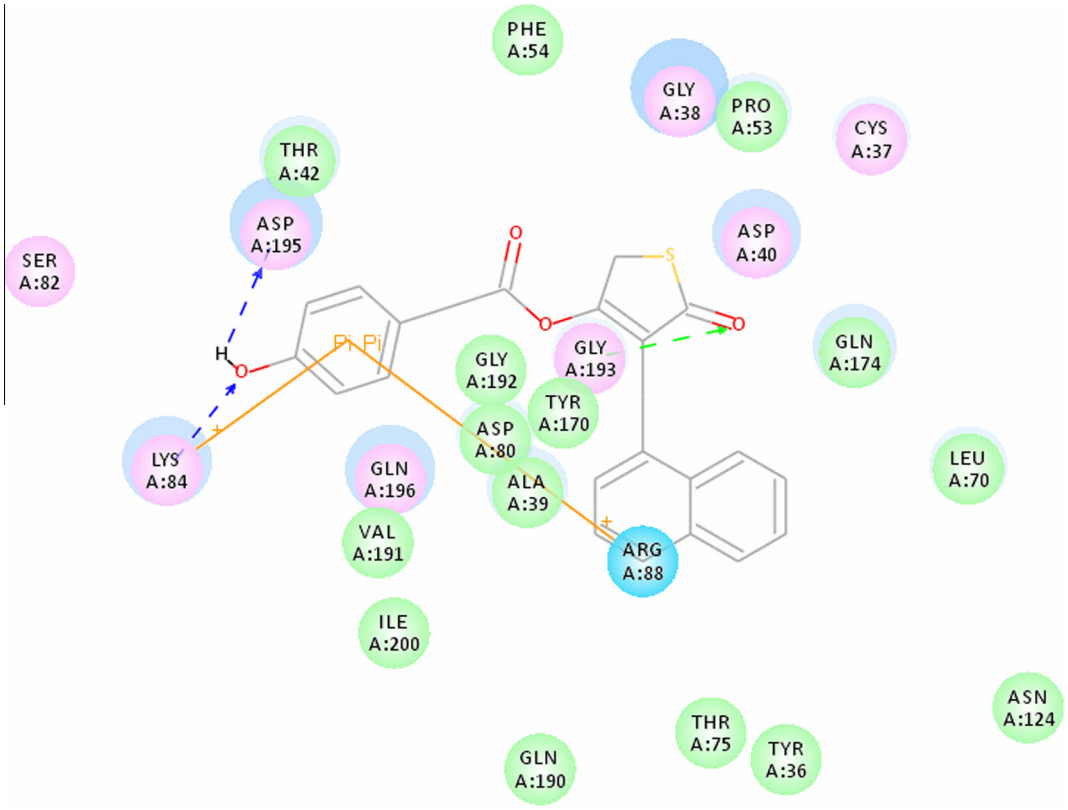


Fig. 2. Ligand interaction diagram of compound **29** with TyrRS using Discovery Studio program with the essential amino acid residues at the binding site are tagged in circles. The purple circles show the amino acids which participate in hydrogen bonding, electrostatic or polar interactions and the green circles show the amino acids which participate in the Van der Waals interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group should increase the potency, and further investigations on the optimization of **29** as leading compounds are being carried out in our laboratory.

The 4-hydroxy-3-(naphthalen-1-ylmethyl)-thiophenone moiety is located at the bottom of the active site cavity (Fig. 3), making some π-cation interactions with Lys84 and Arg88 side chains. In addition to these interactions, the hydroxyl group forms one O–H...O and one O...H–N hydrogen bonds with Lys84 and Asp195 residue, respectively (Fig. 2).

Our modeling results reveal that hydrophilic group substituted at the side chain are important to the interactions of the protein–ligand complex and are crucial to the potency of TyrRS inhibitory activity. Therefore, removing the hydrophilic group or substituting a hydrophobic group can obviously reduce the enzyme inhibitory activity.

4. Conclusion

In summary, we have synthesized thirty novel 4-hydroxy-3-(naphthalen-1-ylmethyl) thiophen-2(5H)-ones derivatives. Several of the target compounds showed good inhibitory activity against TyrRS from *S. aureus*, with **29** being the most active (IC_{50} = 0.25 μM). Antibacterial assay revealed that the synthetic compounds are effective against growth of Gram-positive organisms (especially against *S. aureus*), while all are inactive against Gram-negative organisms. Out of the tested compounds, **29** is the most potent against *S. aureus* ATCC 25923 with MIC_{50} value (0.21 μg/mL) lower than that of the positive control (1.20 μg/mL). Molecular docking studies showed **29** well fits the active site making various close contacts with the active site residues, and disclosed that substitution in the side chain with a hydrophilic group is more suitable for further modification.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2013.10.032.

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