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Research paper

# Synthesis and evaluation of new tyrosyl-tRNA synthetase inhibitors as antibacterial agents based on a *N*2-(arylacetyl)glycinanilide scaffold

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ABSTRACT

Tyrosyl-tRNA synthetase (TyrRS), an essential enzyme in bacterial protein biosynthesis, is an attractive therapeutic target for finding novel antibacterial agents, and a series of *N*2-(arylacetyl)glycinanilides has been herein synthesized and identified as TyrRS inhibitors. These efforts yielded several compounds, with $IC_{50}$ in the low micromolar range against TyrRS from *Staphylococcus aureus*. Out of the obtained compounds, **3ap** is the most active and exhibits excellent activity against both Gram-positive (*S. aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains. In comparison with the parent scaffold 3-arylfuran-2(5*H*)-one, *N*2-(arylacetyl)glycinanilide significantly improved the potency against Gram-negative bacterial strains, indicating that this scaffold offers a significant potential for developing new antibacterial drugs.



## 1. Introduction

The aminoacyl-tRNA synthetases (aaRS) belong to a group of enzymes, which play a central role in the correct translation of genetic information into proteins [1]. Each aaRS catalyzes a specific amino acid to attach the cognate tRNA molecule in the presence of ATP, yielding an aminoacyl-tRNA [2]. Therefore, inhibition of any enzyme in the cell will lead to protein synthesis inhibition and cell growth arrest. Furthermore, the topology of the ATP binding domain and the functions of the human aaRSs differ from those of bacteria, indicating that the binding site for ATP may provide an opportunity to selectively inhibit aaRSs in bacteria [3,4]. These enzymes are therefore considered as attractive targets for antibacterial agents [5,6]. Most aaRS inhibitors bind to the ATP binding site are analogs of ATP, or aminoacyl-adenylate intermediates, such as Microcin C, SB-236996 and Leu-AMS [4,7–9].

Structure-based drug design is now widely applied in most stages of the drug development process, from initial hit identification to lead optimization. Our recent efforts have focused on the development of novel antimicrobial agents, and we applied this approach to find 3-arylfuran-2(5*H*)-ones to target tyrosyl-tRNA synthetase (TyrRS). Since 2011, several new chemical entities have been determined as potent antibacterial agents in our group [6,10–14]. Many analogs of compound **1** (Scheme 1) showed good inhibitory activity against TyrRS and good potency against intact bacterial cells [6]. Compound **1** could be therefore considered to have a potential for the development of antibacterials devoid of cross-resistance with present drugs. To learn more about the structure-antibacterial activity relationships and to develop compounds as potential antibacterial agents, we undertook an additional design for improving the present scaffold (**1**) and for screening active compounds in animal infection models. Specifically, the furan-2(5*H*)-one moiety of **1** was bioisosterically replaced with an acetoxyacetyl group to give a scaffold (**2**). The ester functional group in **2** was subsequently substituted with an amide group to obtain **3** for acquiring more stability in physiological conditions (Scheme 1). On one hand, the acetylglycinamide moiety as a pharmacophore is found in a large quantities of natural and synthetic compounds, which possess useful biological activities

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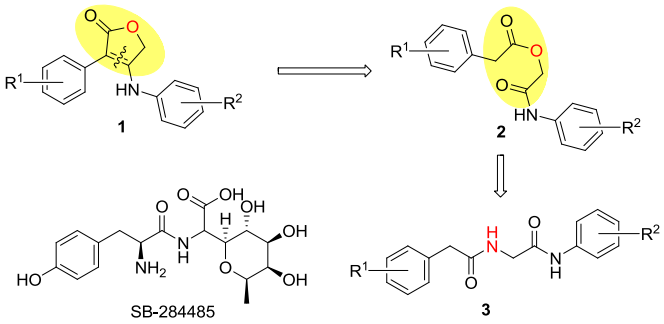



**Scheme 1.** Design of the *N*2-(arylacetyl)glycinanilide (**3**).

including antibacterial, antifungal, antitumorigenic and anti-Hepatitis C virus replication activities [15–19]. On the other hand, in terms of the energy-minimized conformation and geometrical dimension, scaffold **3** shows very similarity to the well known TyrRS inhibitor, SB-284485 (Scheme 1), which indicates that analogs of **3** will display potent inhibition against TyrRS. Based on this conception, a series of *N*2-(arylacetyl)glycinanilides (**3aa**-**3be**) were synthesized and evaluated as TyrRS inhibitors. The results showed that some of these synthesized compounds exhibited excellent antibacterial activities.

## 2. Results and discussion

### 2.1. Chemistry

Thirty-one *N*2-(arylacetyl)glycinanilides were designed and synthesized by the route outlined in Scheme 2. Briefly, substituted phenylacetic acids **4a**–**l** were subjected to condensation with methyl glycinate hydrochloride in the presence of TBTU to yield the corresponding phenylacetamides **5a**–**l**, which were then further amidated in toluene with an appropriated aniline to give the desired products **3aa**–**bg** (Scheme 2).

### 2.2. Inhibitory activities of N2-(arylacetyl)glycinanilides against tyrosyl-tRNA synthetase from Staphylococcus aureus

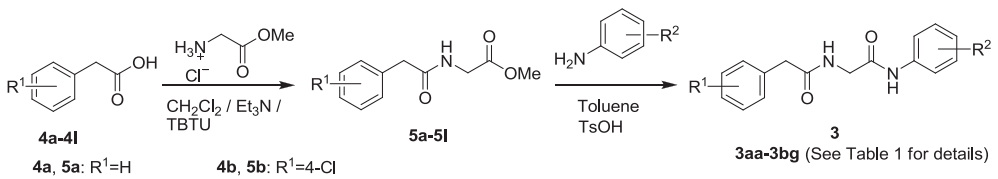
In the process of discovering anti-infection agents, we prepared and evaluated a series of 3-arylfuran-2(5*H*)-one derivatives as tyrosyl-tRNA synthetase (TyrRS) inhibitors. The chain analogs, *N*2-(arylacetyl)glycinanilides (**3aa**–**bg**), were synthesized for a further understanding of structure–activity relationships (SAR), and all were evaluated for the inhibitory activity against TyrRS. As shown in Table 1, the compound with 4-nitro substituent (**3ad**) in the anilino moiety (Scheme 1) affords a 22-fold improvement over the unsubstituted analog (**3ai**), while 4-bromo, 4-methyl or 4-methoxy group significantly reduces the activity, and the 4-chloro analog preserves about equal activity. Modification of the 2- or 3-position of this phenyl ring seems less fruitful, and further modification on the phenylacetyl moiety likewise seems less rewarding. Only the 3-chloro derivative **3ap** ($IC_{50}$ = 0.70 ± 0.03 μM) showed a slight increase in activity, being the most active among all synthesized compounds. Generally speaking, our efforts of modification furnished several good TyrRS inhibitors with submicromolar potency, suggesting an opportunity of this scaffold for further exploration.

### 2.3. Antibacterial activity

Encouraged by the results of TyrRS inhibitory assays, all synthetic compounds were evaluated for their antimicrobial activities against two Gram-positive bacterial strains (*S. aureus* ATCC 6538, *S. aureus* ATCC 25923) and three Gram-negative bacterial strains (*Escherichia coli* ATCC 8739, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 9027), and the results are presented in Table 2. In general, the antibacterial potency is totally dependent on the inhibitory activity against TyrRS, and the obtained compounds showed more sensitivity to Gram-positive bacterial strains than Gram-negative bacterial strains. Four compounds (**3ad**, **3ap**, **3as** and **3ax**) were found to exhibit good activity against both Gram-positive and Gram-negative bacterial strains with MIC values lower than 20 μM, and the most active enzyme inhibitor **3ap** also showed the highest potency of antibacterial activity with MIC of 10.1 μM against *P. aeruginosa* ATCC 9027 and 1.84 μM against *S. aureus* ATCC 6538, showing a compared potency to that of the positive control kanamycin and a 3.9-fold more potency than the marketed drug penicillin G, respectively.

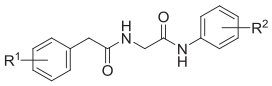
### 2.4. Molecular docking

To interpret the enzyme inhibitory activities at the molecular level, we performed molecular docking studies. The most potent inhibitor **3ap** was docked into the active site of TyrRS based on the binding model of SB-239629-TyrRS complex structure (1jij.pdb) [21], where SB-239629 is a typical TyrRS inhibitor. The docking model obtained in this study indicates that **3ap** docks favorably into the active site of TyrRS (Figs. 1 and 2). Upon analyzing the docking studies, the binding affinity could be associated to hydrogen-bonding as well as hydrophobic interactions (DS = −8.63 kcal/mol). As shown in Fig. 2, 4-nitrophenyl moiety is of primary importance for its hydrogen-bonding interactions network formed between nitro group and two amino-acid residues Tyr-170 and Gln-174. Specifically, $NH_2$ of Gln-174 as the donor coordinates nitro group in a quadridentate fashion with H···O bond length in the range of 1.847–3.364 Å. By contrast, a relatively weak contribution to the binding affinity is observed between OH of Tyr-170 and nitro group, which establishes a hydrogen-bond with longer distance (3.918 Å). These findings indicated that the removal of nitro group at position 4 (**3ai**, DS = −5.87 kcal/mol) or replacement with a weak




**Scheme 2.** Preparation of compounds **3aa**–**be**.

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

| Compound | Structure | | | | cLog P[b] | $IC_{50}$ (μM) | DS (kcal/mol) |
|---|---|---|---|---|---|---|---|
| |  | | | | | | |
| | $R^1$ | EP [20] | $R^2$ | EP [20] | | | |
| **3aa** | H | 2.10 | 4-Br | 2.96 | 3.04 | 50.6 ± 4.1 | −4.72 |
| **3ab** | H | 2.10 | 4-OMe | 2.86[a] | 1.99 | 82.0 ± 5.0 | −4.24 |
| **3ac** | H | 2.10 | 3-$NO_2$ | 3.31[a] | 1.99 | 47.8 ± 4.3 | −5.07 |
| **3ad** | H | 2.10 | 4-$NO_2$ | 3.31[a] | 1.85 | 0.87 ± 0.05 | −8.35 |
| **3ae** | H | 2.10 | 3,5-Dimethoxyl | 2.86[a] | 2.01 | 162.1 ± 8.1 | −3.23 |
| **3af** | H | 2.10 | 3,5-Difluoro | 3.16 | 2.55 | 13.1 ± 0.9 | −6.51 |
| **3ag** | H | 2.10 | 4-Cl | 3.16 | 2.88 | 16.6 ± 0.8 | −6.19 |
| **3ah** | H | 2.10 | 3,5-Dichloro | 3.16 | 3.69 | 132.8 ± 13.5 | −3.61 |
| **3ai** | H | 2.10 | H | 2.10 | 1.91 | 19.3 ± 2.0 | −5.87 |
| **3aj** | H | 2.10 | 2-Cl | 3.16 | 2.03 | 205 ± 27 | −2.72 |
| **3ak** | H | 2.10 | 3-Cl | 3.16 | 2.88 | 144.0 ± 11.5 | −3.54 |
| **3al** | 4-Cl | 3.16 | 3,5-Dichloro | 3.16 | 4.40 | 254 ± 23 | −2.49 |
| **3am** | 4-Cl | 3.16 | 4-OMe | 2.86[a] | 2.70 | 70.1 ± 4.9 | −4.42 |
| **3an** | 3-Cl | 3.16 | 4-OMe | 2.86[a] | 2.70 | 66.5 ± 4.7 | −4.52 |
| **3ao** | 3-Cl | 3.16 | 4-Br | 2.96 | 3.75 | 8.36 ± 0.74 | −6.62 |
| **3ap** | 3-Cl | 3.16 | 4-$NO_2$ | 3.31[a] | 2.56 | 0.70 ± 0.03 | −8.63 |
| **3aq** | 2-Cl | 3.16 | 4-OMe | 2.86[a] | 2.70 | 189.3 ± 15.2 | −2.95 |
| **3ar** | 2-Cl | 3.16 | 4-Br | 2.96 | 3.75 | 77.8 ± 7.7 | −4.32 |
| **3as** | 2-Cl | 3.16 | 4-$NO_2$ | 3.31[a] | 2.56 | 2.52 ± 0.15 | −7.72 |
| **3at** | 3-Br | 2.96 | 4-Br | 2.96 | 3.90 | 58.4 ± 3.1 | −4.63 |
| **3au** | 4-F | 3.98 | 4-OMe | 2.86[a] | 2.13 | 112.4 ± 12.4 | −3.83 |
| **3av** | 4-F | 3.98 | 4-$NO_2$ | 3.31[a] | 2.00 | 5.25 ± 0.26 | −7.49 |
| **3aw** | 3-F | 3.98 | 4-OMe | 2.86[a] | 2.13 | 92.7 ± 8.4 | −4.06 |
| **3ax** | 3-F | 3.98 | 4-$NO_2$ | 3.31[a] | 2.00 | 1.14 ± 0.06 | −8.02 |
| **3ay** | 3,4-OMe | 2.86[a] | 4-OMe | 2.86[a] | 1.65 | 556 ± 56 | −1.30 |
| **3az** | 3,4-OMe | 2.86[a] | 4-$NO_2$ | 3.31[a] | −1.60 | 88.5 ± 6.2 | −4.17 |
| **3ba** | 4-OH | 2.82[a] | 4-OMe | 2.86[a] | 1.32 | 319 ± 26 | −1.95 |
| **3bb** | 4-OH | 2.82[a] | 4-$NO_2$ | 3.31[a] | 1.19 | 10.2 ± 0.6 | −6.57 |
| **3bc** | 3-OH | 2.82[a] | 4-OMe | 2.86[a] | 1.32 | 279 ± 25 | −2.09 |
| **3bd** | 3-OH | 2.82[a] | 4-$NO_2$ | 3.31[a] | 1.19 | 6.41 ± 0.26 | −7.08 |
| **3be** | 2-OH | 2.82[a] | 4-OMe | 2.86[a] | 1.27 | 478 ± 57 | −1.72 |
| **3bf** | 3-Cl | 3.16 | 4-Me | 2.29[a] | 3.13 | 21.4 ± 2.3 | −5.74 |
| **3bg** | 4-Me | 2.29[a] | 4-$NO_2$ | 3.31[a] | −0.76 | 44.6 ± 3.7 | −4.95 |

EP: electronegativity parameter.
DS: docking score.
[a] Data are from the Ref. [20].
[b] Data are calculated by using ChemDraw 11.0.

hydrogen bond acceptor results in a reduced binding affinity (**3aa**, DS = −4.72 kcal/mol; **3ab**, DS = −4.24 kcal/mol and **3ag**, DS = −6.19 kcal/mol), and is consistent with those observed in SAR study.

Acetylglycinamide moiety, the linkage between two benzene rings, also furnishes a considerable contribution to the binding affinity, which packs in a hydrophilic cleft, forming three strong hydrogen-bonding interactions together with a relatively weak one. The aniline moiety by using NH group bonds with the residue His-50, and the distance between the NH group and the nitrogen atom of the imidazole ring in His-50 is 3.624 Å. With the respect to aminoaceto group, the carbonyl group forms a typical hydrogen bond with the NH group of Gly-193 (O···H, 2.031 Å), while the NH group of aminoaceto moiety has no significant interactions to be considered as a contribution for binding. The carbonyl group of the phenylaceto moiety as an acceptor establishes two hydrogen bonds with His-50 and His-47, which confirmed that this carbonyl group is also crucial for binding.

The 3-chlorophenyl group of **3ap** drops completely into a hydrophobic pocket built by Leu-14, Leu-52, Pro-53, Ile-221, Leu-223, Val-224, Phe-232 and Phe-217, indicating a detriment of a hydrophilic substituent on this ring. Therefore, the substitution of a hydroxyl group (**3ba**–**be**, DS from −7.08 to −1.72 kcal/mol) for a halogen atom (**3al**–**ax**, DS from −8.63 to −2.49 kcal/mol) resulted in a considerable decrease in potency. This docking model also revealed that change of 3-Cl to 4-Cl may cause a steric hindrance to the residue Val-224, leading reduction of binding affinity. Similarly, introduction of 3,4-dimethoxyl group (**3ay**, DS = −1.30 kcal/mol and **3az**, DS = −4.17 kcal/mol) may cause steric hindrances to both Leu-52 and Val-224, resulting dramatic decrease in potency. For a definite conclusion, modification at the 3-position of phenylaceto moiety with a hydrophobic group having suitable steric hindrance would improve the antibacterial activity.

## 3. Conclusions

Given the global health threat posed by multiantibiotic resistant bacteria, there is considerable interest in the discovery of antibacterial agents with novel skeleton or/and antibacterial mechanism. Derivatives of *N*2-(arylacetyl)glycinanilide, chain analogs of 3-arylfuran-2(5*H*)-ones, were determined as TyrRS inhibitors, showing promise as antibacterial agents with novel mechanism in terms of clinical drugs. The bioactivity assays demonstrated that some *N*2-(arylacetyl)glycinanilides showed good potency against both extracted enzyme and intact cells. In comparison with the parent scaffold 3-arylfuran-2(5*H*)-one, *N*2-(arylacetyl)glycinanilide not only maintained the potency against Gram-positive bacterial strains, but also significantly improved the potency against Gram-

**Table 2**
Inhibitory activity (MIC) of the synthetic compounds against microbes.

| Compound | MIC (μM) | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| **3aa** | 113.2 | >160 | >160 | >160 | >160 |
| **3ab** | >160 | >160 | >160 | >160 | >160 |
| **3ac** | 103.2 | 156.2 | >160 | >160 | >160 |
| **3ad** | 2.81 | 3.67 | 11.2 | 15.8 | 18.2 |
| **3ae** | >160 | >160 | >160 | >160 | >160 |
| **3af** | 40.5 | 47.7 | 120.1 | >160 | 148.3 |
| **3ag** | 49.7 | 69.2 | 123.2 | 158.9 | >160 |
| **3ah** | >160 | >160 | >160 | >160 | >160 |
| **3ai** | 52.7 | 73.5 | 133.9 | >160 | >160 |
| **3aj** | >160 | >160 | >160 | >160 | >160 |
| **3ak** | >160 | >160 | >160 | >160 | >160 |
| **3al** | >160 | >160 | >160 | >160 | >160 |
| **3am** | >160 | >160 | >160 | >160 | >160 |
| **3an** | 148.2 | 148.2 | >160 | >160 | >160 |
| **3ao** | 20.9 | 24.9 | 62.4 | 83.4 | 96.1 |
| **3ap** | 1.84 | 2.36 | 6.80 | 9.50 | 10.1 |
| **3aq** | >160 | >160 | >160 | >160 | >160 |
| **3ar** | 69.7 | 103.4 | >160 | >160 | >160 |
| **3as** | 6.51 | 8.41 | 13.5 | 19.7 | 34.0 |
| **3at** | 97.2 | >160 | >160 | >160 | >160 |
| **3au** | >160 | >160 | >160 | >160 | >160 |
| **3av** | 14.1 | 17.0 | 42.3 | 75.8 | 65.6 |
| **3aw** | >160 | >160 | >160 | >160 | >160 |
| **3ax** | 3.14 | 4.11 | 12.3 | 19.6 | 18.2 |
| **3ay** | >160 | >160 | >160 | >160 | >160 |
| **3az** | >160 | >160 | >160 | >160 | >160 |
| **3ba** | >160 | >160 | >160 | >160 | >160 |
| **3bb** | 28.6 | 34.1 | 86.9 | 143.8 | 134.0 |
| **3bc** | >160 | >160 | >160 | >160 | >160 |
| **3bd** | 15.2 | 18.4 | 47.1 | 89.4 | 72.0 |
| **3be** | >160 | >160 | >160 | >160 | >160 |
| **3bf** | 64.9 | 91.1 | 155.4 | >160 | >160 |
| **3bg** | 80.7 | 132.1 | >160 | >160 | >160 |
| Kanamycin | _ | _ | 1.26 | 4.35 | 7.16 |
| Penicillin G | 7.13 | 9.28 | _ | _ | _ |

Note: A, *S. aureus* ATCC 6538; B, *S. aureus* ATCC 25923; C, *E. coli* ATCC 8739; D, *E. coli* ATCC 35218; E, *P. aeruginosa* ATCC 9027.

negative bacterial strains. Compound **3ap** was identified as the most potent antibacterial agent with 3-fold more potency than penicillin G against *S. aureus* ATCC 6538 ($MIC_{50}$ = 0.52 μM) and compared potency to kanamycin against *P. aeruginosa* ATCC 9027 ($MIC_{50}$ = 3.37 μM), which deserves further investigation. Enzyme assays and molecular studies revealed that 4-nitro group of aniline moiety and a hydrophobic group at 3-position with a suitable steric hindrance of phenylaceto moiety are quite important for improving activity. Consequently, the *N*2-(arylacetyl)glycinanilide scaffold offers a significant potential for the discovery of a new class of antibacterial drugs, and a further chemical pharmacomodulation is undergoing.

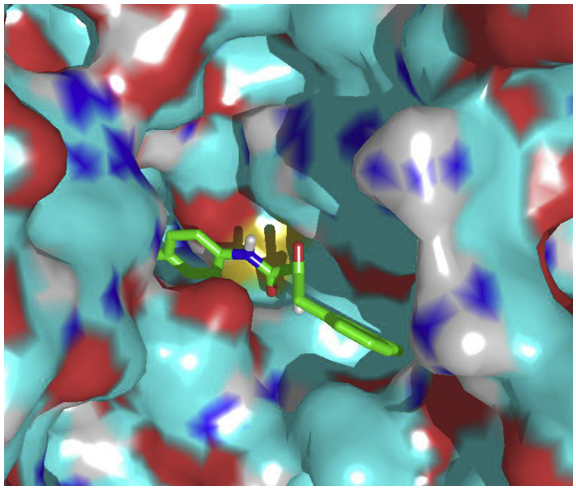


**Fig. 1.** Binding mode of compound **3ap** with TyrRS. The enzyme is shown as surface; while **3ap** docked structures are shown as sticks.

## 4. Experiments

### 4.1. Preparation of the tyrosyl-tRNA synthetase and enzyme assay

*S. aureus* tyrosyl-tRNA synthetase was over-expressed in *E. coli* and purified to near homogeneity (~98% as judged by SDS-PAGE) using standard purification procedures [22]. Tyrosyl-tRNA synthetase activity was measured by aminoacylation using modifications to previously described methods [23]. 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-$^3$H] tyrosine (PerkinElmer, Specific activity: 1.48–2.22 TBq/mmol), 10 μM carrier). Tyrosyl-tRNA synthetase (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. All compounds were tested in triplicate, and the results were presented in Table 1.

### 4.2. Antimicrobial activity

The antibacterial activities of the synthesized compounds was tested against two Gram-positive bacterial strains (*S. aureus* ATCC 6538, *S. aureus* ATCC 25923, penicillin G as positive control) and three Gram-negative bacterial strains (*E. coli* ATCC 8739, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 9027, kanamycin as positive control) using LB medium. The MICs of the test compounds were determined by a colorimetric method using the dye MTT [24,25]. 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. Fifty μL of PBS containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h, and 100 μL of 10% sodium dodecyl sulfate containing 5% isopropanol and 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm. The observed MICs were presented in Table 2.

### 4.3. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based

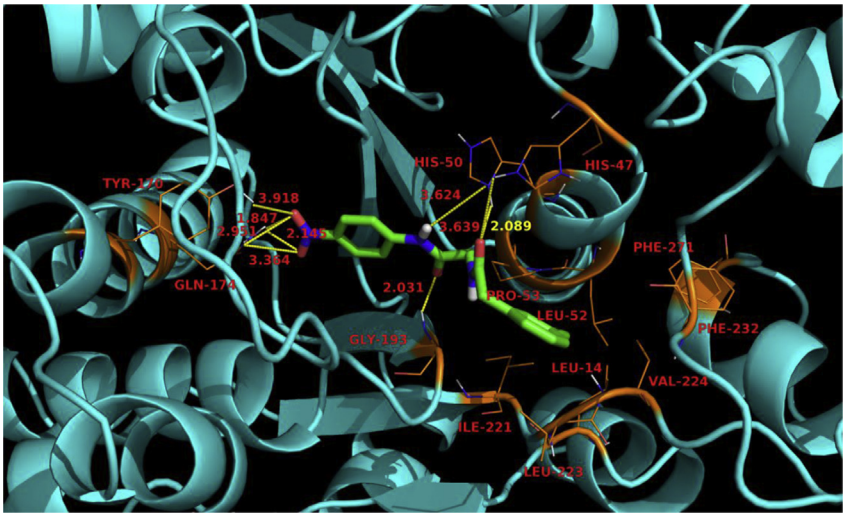



**Fig. 2.** Binding mode of compound **3ap** with TyrRS. For clarity, only interacting residues were labeled. Hydrogen bonding interactions are shown in dash. The figure was made using PyMol.

on the macromolecular target using the AMBER force field. A grid box of 46 × 58 × 48 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The genetic algorithm with local search (GA-LS) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 7.5 × $10^6$ energy evaluations, and a maximum number of 2.7 × $10^4$ generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

### 4.4. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A) and Sinopharm Chemical Reagent Co., Ltd (China). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck). The quantity of silica gel used was 30–70 times the weight charged on the column. Then, the eluates were monitored using TLC. Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Agilent 6400 mass spectrometer, and $^1$H NMR spectra were recorded on a Bruker AV 300, 400 or 500 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm ($\delta$). Elemental analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values.

#### 4.4.1. General procedure for preparation of compounds ***5***

To a solution of a substituted phenylacetic acid (**4a**–**l**) in dichloromethane at room temperature, were successively added 3 equivalents of triethylamine, 1.2 equivalents of methyl glycinate hydrochloride and 1.1 equivalents of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU). The reaction mixture was well stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude material was taken up in ethyl acetate. The organic phase was washed with saturated aqueous solutions of sodium bicarbonate and sodium chloride. The organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by chromatography using ethyl acetate/petroleum as eluents (3:1 to 6:1), the pure product (**5a**–**l**) was therefore obtained.

#### 4.4.2. General procedure for preparation of compounds ***3aa–bg***

A mixture of a selected compound **5a**–**l** (0.5 mmol), an appropriately substituted aniline (0.6 mmol) and *p*-toluene sulphonic acid (TsOH, 3.4 mg, 0.02 mmol) was heated to 90 °C for 10 min. Five milliliters toluene was then added and refluxed for 3–7 h. After toluene was removed under reduced pressure, the residue was purified by column chromatography on silica gel, eluting with EtOAc/petroleum ether.

*4.4.2.1. N-(4-Bromophenyl)-N2-(phenylacetyl)glycinamide (**3aa**).* Colorless crystal, 57%, mp 228–230 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.55 (s, 2H); 3.95 (d, $J$ = 5.2 Hz, 2H); 7.16–7.31 (m, 6H); 7.39–7.50 (m, 4H); 9.60 (s, 1H); EIMS $m/z$ 346 ($M^+$). Anal. Calcd for $C_{16}H_{15}BrN_2O_2$: C, 55.35; H, 4.35; Br, 23.01; N, 8.07; found: C, 55.44; H, 4.35; Br, 22.98; N, 8.08.

*4.4.2.2. N-(4-Methoxyphenyl)-N2-(phenylacetyl)glycinamide (**3ab**).* Colorless crystal, 49%, mp 187–188 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.51 (s, 2H); 3.71 (s, 2H); 3.87 (d, $J$ = 5.6 Hz, 2H); 6.88 (d, $J$ = 8.8 Hz, 2H); 7.20–7.31 (m, 5H); 7.49 (d, $J$ = 8.8 Hz, 2H); 8.41 (s, 1H); 9.89 (s, 1H); EIMS $m/z$ 298 ($M^+$). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39; found: C, 68.36; H, 6.07; N, 9.40.

*4.4.2.3. N-(3-Nitrophenyl)-N2-(phenylacetyl)glycinamide (**3ac**).* Colorless crystal, 52%, mp 192–193 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.62 (s, 2H); 4.03 (s, 2H); 7.23–7.33 (m, 5H); 7.47 (t, $J$ = 7.8 Hz, 1H); 7.86–7.94 (m, 3H); 8.58 (s, 1H); 10.23 (s, 1H); EIMS $m/z$ 313 ($M^+$). Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41; found: C, 61.41; H, 4.83; N, 13.40.

*4.4.2.4. N-(4-Nitrophenyl)-N2-(phenylacetyl)glycinamide (**3ad**).* Light yellow crystal, 58%, mp 227–229 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.52 (s, 2H); 3.96 (d, $J$ = 5.6 Hz, 2H); 7.22–7.30 (m, 5H); 7.83 (d, $J$ = 9.1 Hz, 2H); 8.23 (d, $J$ = 9.0 Hz, 2H); 8.50 (bs, 1H); 10.66

(s, 1H); EIMS *m/z* 313 ($M^+$). Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41; found: C, 61.39; H, 4.82; N, 13.40.

*4.4.2.5. N-(3,5-Dimethoxyphenyl)-N2-(phenylacetyl)glycinamide (**3ae**).* White powder, 71%, mp 162–164 °C, $^1$H NMR (300 MHz, $CDCl_3$): 3.69 (s, 2H); 3.79 (s, 6H); 4.03 (d, $J$ = 5.5 Hz, 2H); 6.26 (s, 2H); 6.72 (s, 2H); 7.32–7.40 (m, 5H); 8.16 (bs, 1H); EIMS *m/z* 328 ($M^+$). Anal. Calcd for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53; found: C, 65.77; H, 6.15; N, 8.54.

*4.4.2.6. N-(3,5-Difluorophenyl)-N2-(phenylacetyl)glycinamide (**3af**).* White powder, 79%, mp 201–203 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.52 (s, 2H); 3.90 (d, $J$ = 5.6 Hz, 2H); 6.89–6.94 (m, 1H); 7.22–7.30 (m, 7H); 8.46 (bs, 1H); 10.40 (s, 1H); EIMS *m/z* 304 ($M^+$). Anal. Calcd for $C_{16}H_{14}F_2N_2O_2$: C, 63.15; H, 4.64; F, 12.49; N, 9.21; found: C, 63.06; H, 4.64; F, 12.50; N, 9.22.

*4.4.2.7. N-(4-Chlorophenyl)-N2-(phenylacetyl)glycinamide (**3ag**).* White powder, 82%, mp 220–222 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.55 (s, 2H); 3.95 (d, $J$ = 5.2 Hz, 2H); 7.14–7.27 (m, 6H); 7.43–7.45 (m, 4H); 9.56 (s, 1H); EIMS *m/z* 302 ($M^+$). Anal. Calcd for $C_{16}H_{15}ClN_2O_2$: C, 63.47; H, 4.99; Cl, 11.71; N, 9.25; found: C, 63.53; H, 4.99; Cl, 11.70; N, 9.24.

*4.4.2.8. N-(3,5-Dichlorophenyl)-N2-(phenylacetyl)glycinamide (**3ah**).* White powder, 58%, mp 293–295 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.51 (s, 2H); 3.90 (d, $J$ = 5.6 Hz, 2H); 7.22–7.30 (m, 6H); 7.65 (s, 2H); 8.47 (bs, 1H); 10.35 (s, 1H); EIMS *m/z* 336 ($M^+$). Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_2$: C, 56.99; H, 4.18; Cl, 21.03; N, 8.31; found: C, 56.91; H, 4.19; Cl, 21.05; N, 8.30.

*4.4.2.9. N-Phenyl-N2-(phenylacetyl)glycinamide (**3ai**).* White powder, 49%, mp 215–217 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.53 (s, 2H); 3.92 (d, $J$ = 5.5 Hz, 2H); 6.94–7.12 (m, 5H); 7.20–7.31 (m, 5H); 8.51 (bs, 1H); 10.31 (s, 1H); EIMS *m/z* 268 ($M^+$). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44; found: C, 71.51; H, 6.02; N, 10.46.

*4.4.2.10. N-(2-Chlorophenyl)-N2-(phenylacetyl)glycinamide (**3aj**).* White powder, 86%, mp 156–158 °C, $^1$H NMR (300 MHz, $CDCl_3$): 3.72 (s, 2H); 4.11 (d, $J$ = 5.1 Hz, 2H); 6.16 (s, 1H); 7.08 (t, $J$ = 7.5 Hz, 1H); 7.28–7.41 (m, 7H); 8.28 (double s, 2H); EIMS *m/z* 302 ($M^+$). Anal. Calcd for $C_{16}H_{15}ClN_2O_2$: C, 63.47; H, 4.99; Cl, 11.71; N, 9.25; found: C, 63.39; H, 5.00; Cl, 11.72; N, 9.25.

*4.4.2.11. N-(3-Chlorophenyl)-N2-(phenylacetyl)glycinamide (**3ak**).* White powder, 81%, mp 177–179 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.51 (s, 2H); 3.90 (d, $J$ = 5.6 Hz, 2H); 7.11 (d, $J$ = 7.8 Hz, 1H); 7.23–7.36 (m, 6H); 7.43 (d, $J$ = 8.1 Hz, 1H); 7.79 (s, 1H); 8.44 (bs, 1H); 10.20 (s, 1H); EIMS *m/z* 302 ($M^+$). Anal. Calcd for $C_{16}H_{15}ClN_2O_2$: C, 63.47; H, 4.99; Cl, 11.71; N, 9.25; found: C, 63.52; H, 4.98; Cl, 11.70; N, 9.24.

*4.4.2.12. N2-[(4-Chlorophenyl)acetyl]-N-(3,5-dichlorophenyl)glycinamide (**3al**).* Colorless crystal, 47%, mp 190–192 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.52 (s, 2H); 3.90 (d, $J$ = 5.8 Hz, 2H); 7.28 (t, $J$ = 1.9 Hz, 1H); 7.31 (d, $J$ = 8.5 Hz, 2H); 7.37 (d, $J$ = 8.4 Hz, 2H); 7.64 (d, $J$ = 1.9 Hz, 2H); 8.47 (t, $J$ = 5.7 Hz, 1H); 10.34 (s, 1H); EIMS *m/z* 370 ($M^+$). Anal. Calcd for $C_{16}H_{13}Cl_3N_2O_2$: C, 51.71; H, 3.53; Cl, 28.62; N, 7.54; found: C, 51.65; H, 3.53; Cl, 28.64; N, 7.55.

*4.4.2.13. N2-[(4-Chlorophenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3am**).* Colorless crystal, 55%, mp 172–174 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.52 (s, 2H); 3.72 (s, 3H); 3.86 (d, $J$ = 5.8 Hz, 2H); 6.88 (d, $J$ = 9.0 Hz, 2H); 7.31 (d, $J$ = 8.5 Hz, 2H); 7.36 (d, $J$ = 8.5 Hz, 2H); 7.47 (d, $J$ = 9.0 Hz, 2H); 8.39 (t, $J$ = 5.6 Hz, 1H); 9.83 (s, 1H); EIMS *m/z* 332 ($M^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; Cl, 10.65; N, 8.42; found: C, 61.42; H, 5.14; Cl, 10.64; N, 8.41.

*4.4.2.14. N2-[(3-Chlorophenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3an**).* White powder, 49%, mp 170–172 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.55 (s, 2H); 3.72 (s, 3H); 3.88 (d, $J$ = 5.8 Hz, 2H); 6.90 (d, $J$ = 9.0 Hz, 2H); 7.25–7.39 (m, 4H); 7.49 (d, $J$ = 9.0 Hz, 2H); 8.47 (t, $J$ = 5.5 Hz, 1H); 9.88 (s, 1H); EIMS *m/z* 332 ($M^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; Cl, 10.65; N, 8.42; found: C, 61.22; H, 5.15; Cl, 10.68; N, 8.43.

*4.4.2.15. N-(4-Bromophenyl)-N2-[(3-chlorophenyl)acetyl]glycinamide (**3ao**).* White powder, 72%, mp 182–183 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.55 (s, 2H); 3.91 (d, $J$ = 5.8 Hz, 2H); 7.24–7.38 (m, 4H); 7.50 (d, $J$ = 8.9 Hz, 2H); 7.57 (d, $J$ = 8.9 Hz, 2H); 8.51 (t, $J$ = 5.7 Hz, 1H); 10.17 (s, 1H); EIMS *m/z* 380 ($M^+$). Anal. Calcd for $C_{16}H_{14}BrClN_2O_2$: C, 50.35; H, 3.70; Br, 20.94; Cl, 9.29; N, 7.34; found: C, 50.44; H, 3.70; Br, 20.89; Cl, 9.28; N, 7.35.

*4.4.2.16. N2-[(3-Chlorophenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3ap**).* White powder, 48%, mp 226–228 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.56 (s, 2H); 3.98 (d, $J$ = 5.6 Hz, 2H); 7.25–7.39 (m, 4H); 7.84 (d, $J$ = 8.8 Hz, 2H); 8.24 (d, $J$ = 8.7 Hz, 2H); 8.57 (t, $J$ = 5.2 Hz, 1H); 10.67 (s, 1H); EIMS *m/z* 347 ($M^+$). Anal. Calcd for $C_{16}H_{14}ClN_3O_4$: C, 55.26; H, 4.06; Cl, 10.19; N, 12.08; found: C, 55.32; H, 4.05; Cl, 10.16; N, 12.05.

*4.4.2.17. N2-[(2-Chlorophenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3aq**).* White powder, 76%, mp 160–161 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.69 (s, 2H); 3.72 (s, 3H); 3.90 (d, $J$ = 5.6 Hz, 2H); 6.89 (d, $J$ = 9.0 Hz, 2H); 7.25–7.33 (m, 2H); 7.41–7.44 (m, 2H); 7.49 (d, $J$ = 8.8 Hz, 2H); 8.41 (t, $J$ = 5.6 Hz, 1H); 9.86 (s, 1H); EIMS *m/z* 332 ($M^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; Cl, 10.65; N, 8.42; found: C, 61.42; H, 5.15; Cl, 10.62; N, 8.40.

*4.4.2.18. N-(4-Bromophenyl)-N2-[(2-chlorophenyl)acetyl]glycinamide (**3ar**).* White powder, 73%, mp 184–185 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.69 (s, 2H); 3.93 (d, $J$ = 5.7 Hz, 2H); 7.27–7.32 (m, 2H); 7.40–7.44 (m, 2H); 7.50 (d, $J$ = 8.9 Hz, 2H); 7.57 (d, $J$ = 8.8 Hz, 2H); 8.45 (t, $J$ = 5.8 Hz, 1H); 10.16 (s, 1H); EIMS *m/z* 380 ($M^+$). Anal. Calcd for $C_{16}H_{14}BrClN_2O_2$: C, 50.35; H, 3.70; Br, 20.94; Cl, 9.29; N, 7.34; found: C, 50.29; H, 3.70; Br, 20.96; Cl, 9.30; N, 7.35.

*4.4.2.19. N2-[(2-Chlorophenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3as**).* White powder, 52%, mp 228–230 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.70 (s, 2H); 4.00 (d, $J$ = 5.6 Hz, 2H); 7.29–7.33 (m, 2H); 7.41–7.43 (m, 2H); 7.84 (d, $J$ = 8.7 Hz, 2H); 8.25 (d, $J$ = 8.8 Hz, 2H); 8.52 (t, $J$ = 5.6 Hz, 1H); 10.66 (s, 1H); EIMS *m/z* 347 ($M^+$). Anal. Calcd for $C_{16}H_{14}ClN_3O_4$: C, 55.26; H, 4.06; Cl, 10.19; N, 12.08; found: C, 55.20; H, 4.06; Cl, 10.20; N, 12.10.

*4.4.2.20. N-(4-Bromophenyl)-N2-[(3-bromophenyl)acetyl]glycinamide (**3at**).* White powder, 58%, mp 182–184 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.55 (s, 2H); 3.92 (d, $J$ = 5.5 Hz, 2H); 7.27–7.30 (m, 2H); 7.42–7.45 (m, 1H); 7.49 (d, $J$ = 8.9 Hz, 2H); 7.50–7.53 (m, 1H); 7.57 (d, $J$ = 8.8 Hz, 2H); 8.51 (t, $J$ = 5.3 Hz, 1H); 10.17 (s, 1H); EIMS *m/z* 424 ($M^+$). Anal. Calcd for $C_{16}H_{14}Br_2N_2O_2$: C, 45.10; H, 3.31; Br, 37.50; N, 6.57; found: C, 45.17; H, 3.31; Br, 37.47; N, 6.576.

*4.4.2.21. N2-[(4-Fluorophenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3au**).* White powder, 66%, mp 192–194 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.51 (s, 2H); 3.72 (s, 3H); 3.87 (d, $J$ = 5.7 Hz,

2H); 6.89 (d, $J$ = 9.0 Hz, 2H); 7.13 (t, $J$ = 8.9 Hz, 2H); 7.33 (dd, $J$ = 8.4 Hz, $J$ = 5.8 Hz, 2H); 7.49 (d, $J$ = 9.0 Hz, 2H); 8.41 (t, $J$ = 5.6 Hz, 1H); 9.87 (s, 1H); EIMS $m/z$ 316 ($M^+$). Anal. Calcd for $C_{17}H_{17}FN_2O_3$: C, 64.55; H, 5.42; F, 6.01; N, 8.86; found: C, 64.48; H, 5.43; F, 6.03; N, 8.88.

*4.4.2.22. N2-[(4-Fluorophenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3av**).* White powder, 70%, mp 246–248 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.53 (s, 2H); 3.98 (d, $J$ = 5.7 Hz, 2H); 7.14 (t, $J$ = 8.9 Hz, 2H); 7.33 (dd, $J$ = 8.4 Hz, $J$ = 5.8 Hz, 2H); 7.84 (d, $J$ = 9.2 Hz, 2H); 8.25 (d, $J$ = 9.2 Hz, 2H); 8.52 (t, $J$ = 5.6 Hz, 1H); 10.67 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 40.42; 43.44; 115.37 (d, $J$ = 20.9 Hz); 119.18; 125.55; 131.38 (d, $J$ = 7.9 Hz); 132.81 (d, $J$ = 3.2 Hz); 142.63; 145.51; 161.50 (d, $J$ = 240.3 Hz); 169.29; 171.15; EIMS $m/z$ 331 ($M^+$). Anal. Calcd for $C_{16}H_{14}FN_3O_4$: C, 58.01; H, 4.26; F, 5.73; N, 12.68; found: C, 58.06; H, 4.25; F, 5.72; N, 12.66.

*4.4.2.23. N2-[(3-Fluorophenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3aw**).* White powder, 79%, mp 153–155 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.56 (s, 2H); 3.72 (s, 3H); 3.89 (d, $J$ = 5.7 Hz, 2H); 6.89 (d, $J$ = 9.0 Hz, 2H); 7.07 (dt, $J$ = 8.6 Hz, $J$ = 2.1 Hz, 1H); 7.15 (t, $J$ = 8.2 Hz, 2H); 7.32–7.38 (m, 1H); 7.49 (d, $J$ = 9.0 Hz, 2H); 9.88 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 42.03; 43.10; 55.59; 113.63 (d, $J$ = 20.6 Hz); 114.33; 116.31 (d, $J$ = 21.3 Hz); 121.10; 125.75 (d, $J$ = 2.7 Hz); 130.47 (d, $J$ = 8.3 Hz); 132.46; 139.53 (d, $J$ = 7.9 Hz); 155.64; 162.49 (d, $J$ = 241.4 Hz); 167.59; 170.53; EIMS $m/z$ 316 ($M^+$). Anal. Calcd for $C_{17}H_{17}FN_2O_3$: C, 64.55; H, 5.42; F, 6.01; N, 8.86; found: C, 64.49; H, 5.42; F, 6.03; N, 8.87.

*4.4.2.24. N2-[(3-Fluorophenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3ax**).* White powder, 56%, mp 232–233 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.57 (s, 2H); 3.99 (d, $J$ = 5.7 Hz, 2H); 7.07 (dt, $J$ = 8.7 Hz, $J$ = 2.4 Hz, 1H); 7.13–7.16 (m, 2H); 7.32–7.38 (m, 1H); 7.84 (d, $J$ = 9.2 Hz, 2H); 8.24 (d, $J$ = 9.2 Hz, 2H); 8.57 (t, $J$ = 5.6 Hz, 2H); 10.68 (s, 1H); EIMS $m/z$ 331 ($M^+$). Anal. Calcd for $C_{16}H_{14}FN_3O_4$: C, 58.01; H, 4.26; F, 5.73; N, 12.68; found: C, 58.06; H, 4.26; F, 5.74; N, 12.70.

*4.4.2.25. N2-[(3,4-Dimethoxyphenyl)acetyl]-N-(4-methoxyphenyl) glycinamide (**3ay**).* White powder, 72%, mp 167–168 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.44 (s, 2H); 3.72 (s, 3H); 3.73 (s, 3H); 3.75 (s, 3H); 3.87 (d, $J$ = 5.3 Hz, 2H); 6.80 (d, $J$ = 8.2 Hz, 1H); 6.86–6.90 (m, 3H); 6.95 (s, 1H); 7.49 (d, $J$ = 7.2 Hz, 2H); 8.32 (bs, 1H); 9.86 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 42.15; 43.10; 55.59; 55.82; 55.97; 112.15; 113.39; 114.33; 121.03; 121.52; 129.12; 132.50; 147.90; 148.91; 155.62; 167.71; 171.34; EIMS $m/z$ 358 ($M^+$). Anal. Calcd for $C_{19}H_{22}N_2O_5$: C, 63.67; H, 6.19; N, 7.82; found: C, 63.67; H, 6.19; N, 7.82.

*4.4.2.26. N2-[(3,4-Dimethoxyphenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3az**).* White powder, 75%, mp 224–226 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.45 (s, 2H); 3.73 (s, 3H); 3.75 (s, 3H); 3.97 (d, $J$ = 5.8 Hz, 2H); 6.81 (dd, $J$ = 8.2 Hz, $J$ = 1.8 Hz, 1H); 6.88 (d, $J$ = 8.2 Hz, 1H); 6.94 (d, $J$ = 1.7 Hz, 1H); 7.83 (d, $J$ = 9.2 Hz, 2H); 8.24 (d, $J$ = 9.2 Hz, 2H); 8.42 (t, $J$ = 5.8 Hz, 1H); 10.66 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 42.03; 42.46; 55.84; 55.98; 112.16; 113.37; 119.16; 121.51; 125.56; 128.99; 142.63; 145.54; 147.91; 148.91; 169.39; 171.51; EIMS $m/z$ 373 ($M^+$). Anal. Calcd for $C_{18}H_{19}N_3O_6$: C, 57.90; H, 5.13; N, 11.25; found: C, 57.85; H, 5.14; N, 11.27.

*4.4.2.27. N2-[(4-Hydroxyphenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3ba**).* White powder, 53%, mp 216–220 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.37 (s, 2H); 3.72 (s, 3H); 3.85 (d, $J$ = 5.8 Hz, 2H); 6.68 (d, $J$ = 8.4 Hz, 2H); 6.89 (d, $J$ = 9.0 Hz, 2H); 7.08 (d, $J$ = 8.4 Hz, 2H); 7.48 (d, $J$ = 9.0 Hz, 2H); 8.27 (t, $J$ = 5.6 Hz, 1H); 9.34 (bs, 1H); 9.86 (s, 1H); EIMS $m/z$ 314 ($M^+$). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91; found: C, 64.91; H, 5.78; N, 8.92.

*4.4.2.28. N2-[(4-Hydroxyphenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3bb**).* White powder, 51%, mp 240–242 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.39 (s, 2H); 3.95 (d, $J$ = 5.8 Hz, 2H); 6.69 (d, $J$ = 8.4 Hz, 2H); 7.08 (d, $J$ = 8.4 Hz, 2H); 7.83 (d, $J$ = 9.3 Hz, 2H); 8.24 (d, $J$ = 9.2 Hz, 2H); 8.38 (t, $J$ = 5.7 Hz, 1H); 9.25 (s, 1H); 10.65 (s, 1H); EIMS $m/z$ 329 ($M^+$). Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76; found: C, 58.32; H, 4.59; N, 12.79.

*4.4.2.29. N2-[(3-Hydroxyphenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3bc**).* White powder, 69%, mp 156–157 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.42 (s, 2H); 3.72 (s, 3H); 3.87 (d, $J$ = 5.7 Hz, 2H); 6.62 (d, $J$ = 7.5 Hz, 1H); 6.71 (d, $J$ = 6.4 Hz, 1H); 6.72 (s, 1H); 6.89 (d, $J$ = 8.9 Hz, 2H); 7.08 (t, $J$ = 8.0 Hz, 1H); 7.49 (d, $J$ = 8.9 Hz, 2H); 8.33 (t, $J$ = 5.4 Hz, 1H); 9.32 (s, 1H); 9.85 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 42.58; 43.10; 55.59; 113.79; 114.32; 116.52; 120.23; 121.07; 129.55; 132.47; 137.96; 155.63; 157.65; 167.64; 171.02; EIMS $m/z$ 314 ($M^+$). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91; found: C, 65.01; H, 5.76; N, 8.89.

*4.4.2.30. N2-[(3-Hydroxyphenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3bd**).* White powder, 60%, mp 197–198 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.43 (s, 2H); 3.97 (d, $J$ = 5.7 Hz, 2H); 6.63 (d, $J$ = 7.8 Hz, 1H); 6.71 (d, $J$ = 6.4 Hz, 1H); 6.72 (s, 1H); 7.09 (t, $J$ = 8.0 Hz, 1H); 7.84 (d, $J$ = 9.2 Hz, 2H); 8.24 (d, $J$ = 9.1 Hz, 2H); 8.45 (t, $J$ = 5.6 Hz, 1H); 9.33 (s, 1H); 10.66 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 42.48; 43.45; 113.83; 116.53; 119.18; 120.22; 125.55; 129.57; 137.84; 142.63; 145.52; 157.66; 169.31; 171.22; EIMS $m/z$ 329 ($M^+$). Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76; found: C, 58.41; H, 4.58; N, 12.74.

*4.4.2.31. N2-[(2-Hydroxyphenyl)acetyl]-N-(4-methoxyphenyl)glycinamide (**3be**).* White powder, 57%, mp 184–186 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 3.48 (s, 2H); 3.72 (s, 3H); 3.88 (d, $J$ = 5.6 Hz, 2H); 6.75 (t, $J$ = 7.4 Hz, 1H); 6.81 (d, $J$ = 7.9 Hz, 1H); 6.89 (d, $J$ = 9.0 Hz, 2H); 7.07 (td, $J$ = 7.8 Hz, $J$ = 1.5 Hz, 1H); 7.13 (dd, $J$ = 7.4 Hz, $J$ = 1.2 Hz, 1H); 7.48 (d, $J$ = 9.0 Hz, 2H); 8.23 (t, $J$ = 5.6 Hz, 1H); 9.67 (s, 1H); 9.81 (s, 1H); EIMS $m/z$ 329 ($M^+$). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91; found: C, 64.88; H, 5.78; N, 8.93.

*4.4.2.32. N2-[(3-Chlorophenyl)acetyl]-N-(4-methylphenyl)glycinamide (**3bf**).* White powder, 38%, mp 173–174 °C, $^1$H NMR (500 MHz, DMSO-$d_6$): 2.25 (s, 3H); 3.55 (s, 2H); 3.90 (d, $J$ = 5.7 Hz, 2H); 7.11 (d, $J$ = 8.3 Hz, 2H); 7.26 (d, $J$ = 7.4 Hz, 1H); 7.30 (d, $J$ = 8.1 Hz, 1H); 7.34 (t, $J$ = 7.6 Hz, 1H); 7.39 (s, 1H); 7.46 (d, $J$ = 8.3 Hz, 2H); 8.44 (t, $J$ = 5.6 Hz, 1H); 9.90 (s, 1H); EIMS $m/z$ 316 ($M^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_2$: C, 64.46; H, 5.41; Cl, 11.19; N, 8.84; found: C, 64.54; H, 5.41; Cl, 11.17; N, 8.83.

*4.4.2.33. N2-[(4-Methylphenyl)acetyl]-N-(4-nitrophenyl)glycinamide (**3bg**).* Light yellow powder, 47%, mp over 300 °C, $^1$H NMR (500 MHz, DMSO-$d_6$): 2.27 (s, 3H); 3.48 (s, 2H); 3.96 (d, $J$ = 5.8 Hz, 2H); 7.11 (d, $J$ = 7.9 Hz, 2H); 7.19 (d, $J$ = 7.9 Hz, 2H); 7.83 (d, $J$ = 9.2 Hz, 2H); 7.19 (d, $J$ = 9.2 Hz, 2H); 8.42 (t, $J$ = 5.7 Hz, 1H); 10.63 (s, 1H); EIMS $m/z$ 327 ($M^+$). Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84; found: C, 62.16; H, 5.24; N, 12.87.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.08.025.

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