ORIGINAL ARTICLE

Synthesis and biological evaluation of indole core-based derivatives with potent antibacterial activity against resistant bacterial pathogens

Wei Hong[1], Jingyang Li[2], Zhe Chang[3], Xiaoli Tan[3], Hao Yang[3], Yifan Ouyang[3], Yanhui Yang[4], Sargit Kaur[5], Ian C Paterson[6], Yun Fong Ngeow[5] and Hao Wang[3,7]

The emergence of drug resistance in bacterial pathogens is a growing clinical problem that poses difficult challenges in patient management. To exacerbate this problem, there is currently a serious lack of antibacterial agents that are designed to target extremely drug-resistant bacterial strains. Here we describe the design, synthesis and antibacterial testing of a series of 40 novel indole core derivatives, which are predicated by molecular modeling to be potential glycosyltransferase inhibitors. Twenty of these derivatives were found to show *in vitro* inhibition of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*. Four of these strains showed additional activity against Gram-negative bacteria, including extended-spectrum beta-lactamase producing *Enterobacteriaceae*, imipenem-resistant *Klebsiella pneumoniae* and multidrug-resistant *Acinetobacter baumanii*, and against *Mycobacterium tuberculosis* H37Ra. These four compounds are candidates for developing into broad-spectrum anti-infective agents.

INTRODUCTION

Antibiotic resistance in bacterial pathogens has become a global public health threat associated with grave human and economic loss. This problem, previously characteristic of nosocomial infections, is now increasingly seen in community-acquired infections as well. Notable examples include the Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and drug-resistant *Streptococcus pneumoniae*, as well as multiply resistant Gram-negative bacilli, such as *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Escherichia coli*. These bacteria are common nosocomiants, but the emergence of multidrug resistance via multiple resistance mechanisms has made them deadly pathogens in a wide range of infections.[1–7] With the escalation of multidrug resistance, the need for new antibiotics is urgent. Unfortunately, very few antibiotics currently available can combat multidrug resistance and also very few are in the pipeline for future development.

Peptidoglycan is an essential component to keep the bacterial cell wall's strength and shape. The peptidoglycan glycosyltransferase (GT) and transpeptidase (TP) enzymes have a major role together in the synthesis of peptidoglycan. These enzymes are suitable targets for antibacterial drugs because they are not present in mammalian cells, and hence, drugs inhibiting them will not affect normal mammalian cells.[8,9]

TP enzymes, also called penicillin-binding proteins, are well-known targets of β-lactam antibiotics. GT inhibitors, on the other hand, have been less studied for their antibacterial activity. The best known peptidoglycan GT inhibitors are moenomycins, which are a family of phosphoglycolipids produced by the *Streptomyces* genus. These inhibitors have been used as growth promoters in animal feed but have not been developed as antibiotics for human infections.[10] Their activity against multidrug-resistant bacteria has not been reported.

Indoles are natural organic compounds with an aromatic heterocyclic structure that are widely used in the synthesis of pharmaceutical agents. Many synthesized derivatives have potent activity against fungi, viruses and Leishmania parasites as well as Gram-positive and Gram-negative bacteria and mycobacterial species.[11–14] This wide-spectrum activity makes them suitable candidates for the development of novel

[1]School of Chemistry and Chemical Engineering, Beifang University of Nationalities, Yinchuan, PR China; [2]Logistics University of the China Armed Police Force, Tianjin, PR China; [3]School of Pharmacy, Ningxia Medical University, Yinchuan, PR China; [4]School of Basic Medicine, Ningxia Medical University, Yinchuan, PR China; [5]Department of Pre-Clinical Sciences, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; [6]Department of Oral Biology and Biomedical Sciences and Oral Cancer Research and Coordinating Centre, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia and [7]Key Laboratory of Hui Ethnic Medicine Modernization, Ministry of Education, Ningxia Medical University, Yinchuan, PR China
Correspondence: Dr W Hong, School of Chemistry and Chemical Engineering, Beifang University of Nationalities, Yinchuan, Ningxia 750021, PR China.
E-mail: hongwei136@hotmail.com
or Professor YF Ngeow, Department of Pre-Clinical Sciences, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Sungai Long Campus, Selangor 43000, Malaysia.
E-mail: yunngeow@yahoo.com
or Professor H Wang, School of Pharmacy, Ningxia Medical University, Yinchuan 750004, PR China.
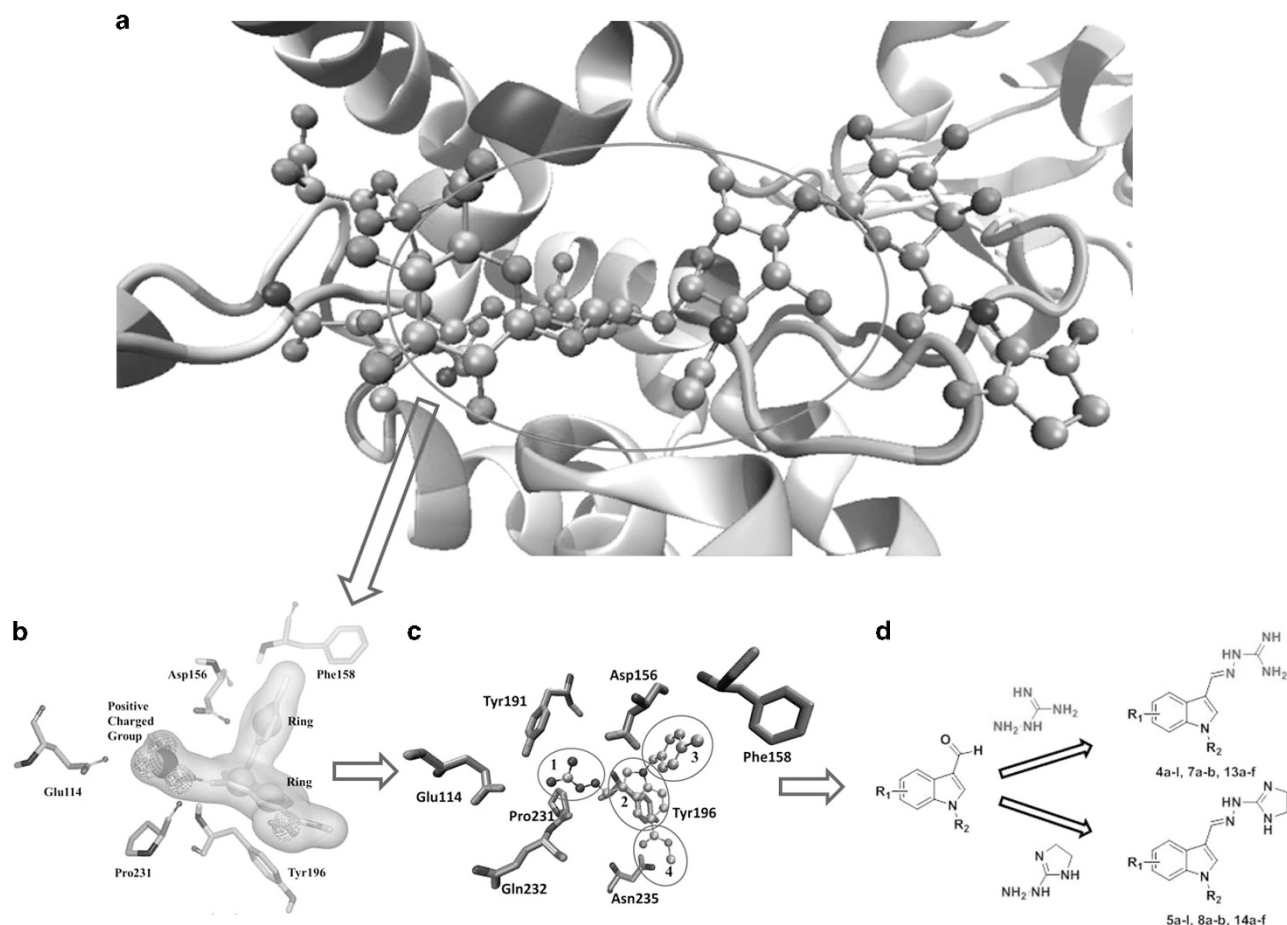E-mail: paxhw@yahoo.co.uk

Figure 1 The design of the glycosyltransferase inhibitors. (**a**) The binding site of moenomycin. The moenomycin is represented as a balls–sticks model, and the protein is representad as a ribbon; (**b**) the pharmacophore designed to fit the binding site; (**c**) the detailed interactions between the template compound and the GT-binding site; (**d**) combining bioactive molecules to generate target compounds. A full color version of this figure is available at *The Journal of Antibiotics* journal online.
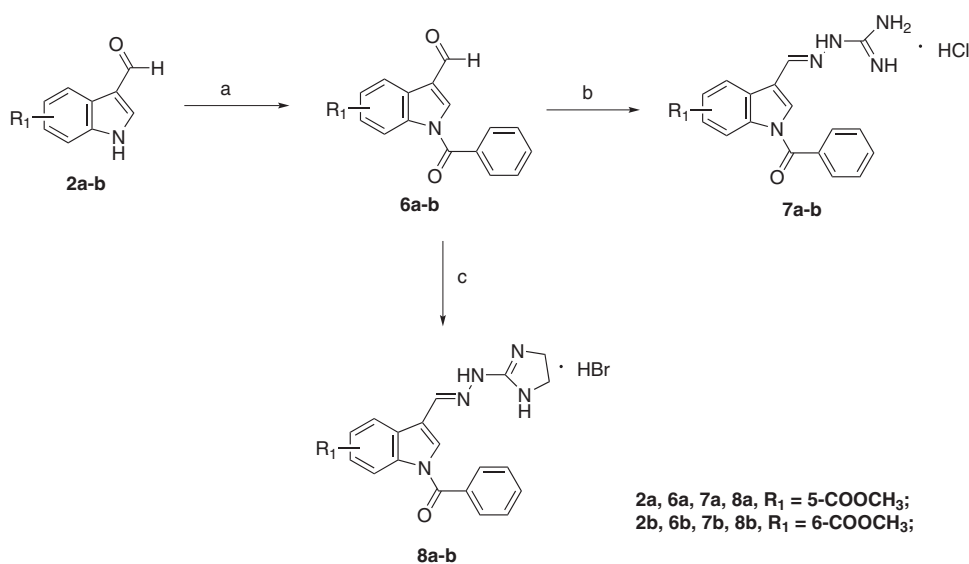
anti-infectives so urgently needed to overcome the current global problem of multidrug resistance.

Here we describe the discovery of new indole core derivatives showing activity against bacterial pathogens commonly isolated from clinical specimens. We synthesized indole compounds with substituted side-chains to act as potential GT inhibitors. In the bacterial cell wall, TP and GT work as bifunctional proteins, in which the TP domain is located in the C-terminal and the GT domain in the N-terminal, with a small linker region separating the two domains. The crystal structure of the GT-binding site of *S. aureus* complexed with moenomycin (PDB 2OLV) has been reported.[15] The moenomycin-binding site is a relatively big site. As Figure 1a shows, from studies on the crystal structure and drug-like properties of moenomycin, it appears unnecessary to design inhibitors to occupy the whole binding site. Hence, we chose the segment of the binding site composed by Asp156, Glu114, Pro231, Tyr196 and Phe158 (area in the red circle) as the target for our new indole derivatives. Based on the structure of the GT binding site (Figure 1b), it was noticed that Asp156, Glu114 and Pro231 form a hydrophilic pocket, which should be occupied by the positive-charged group to form strong static electricity interaction with the negative-charged residues (Asp156 and Glu114). Considering the Tyr196 in the binding site, it was believed that an aromatic ring around it should form strong π–π interactions, and another aromatic ring should be

added to form same interactions with Phe158. Recently, much attention has been paid to indole core derivatives due to their diverse biological activities, for instance, the inhibition of bacteria,[12] viruses,[13] leishmania parasites[14] and cancerous cells.[16] More recently, it has been discovered that aminoguanidine or 2-hydrazino-2-imidazoline derivatives display antitumor activities[17] and aminoguanidine derivatives show antibacterial activities.[18,19] Using this information, we designed a template compound (Figure 1c), which contains the indole core to form π–π interactions with Tyr196 (area 2), the aminoguanidine to form static electricity interactions with Asp156 and Glu114 and the *N*-1 substituted benzyl to form a potential π–π interaction with Phe158 (area 3). Based on the template compound, we noticed by the molecular docking results that aminoguanidine may form additional hydrogen bonds with Pro231 and Tyr191, and by adding methoxycarbonyl group on the indole core, hydrogen bonds may be formed with Asn235 (area 4). By further considering Pro231, which contains five-membered aromatic ring, it was thought an aromatic ring close by should increase the binding affinity. Therefore, in our second series, we used 2-(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone to form an extra π–π interaction with Pro231. Subsequently, we prepared indole core derivatives by combining 3-acylindoles with either aminoguanidine or 2-hydrazino-2-imidazoline (Figure 1d).
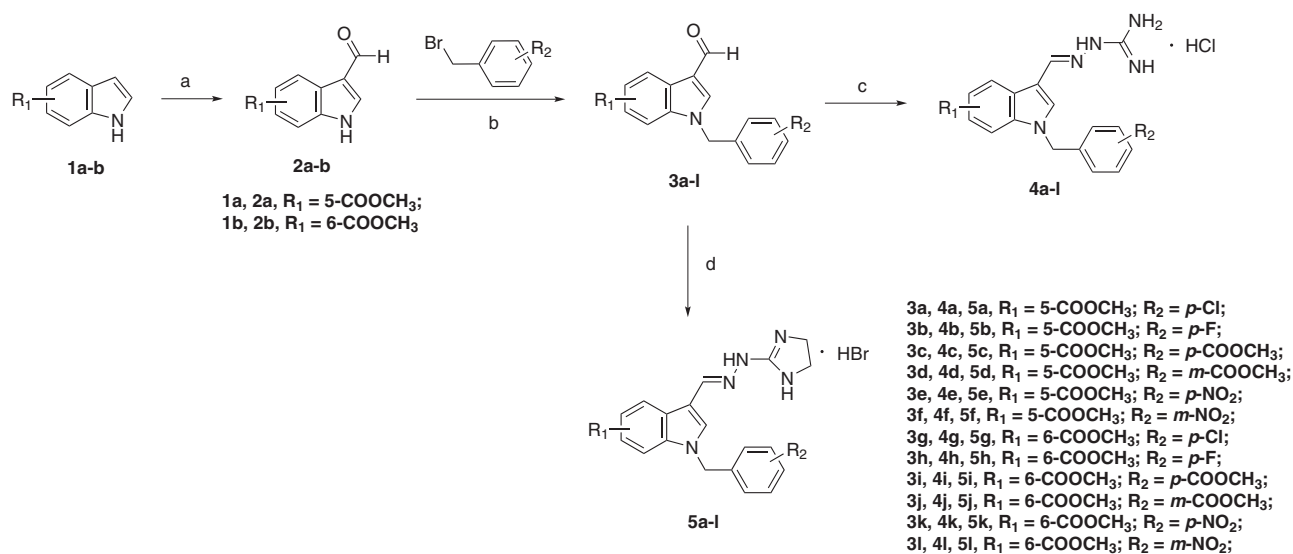
Scheme 1 (a) POCl₃ dry DMF; (b) substituted benzyl bromide, NaH, dry DMSO; (c) aminoguanidine hydrochloride, dry CH₃OH; (d) 2-hydrazino-2-imidazoline hydrobromide, dry CH₃OH.

3a, 4a, 5a, R₁ = 5-COOCH₃; R₂ = *p*-Cl;
3b, 4b, 5b, R₁ = 5-COOCH₃; R₂ = *p*-F;
3c, 4c, 5c, R₁ = 5-COOCH₃; R₂ = *p*-COOCH₃;
3d, 4d, 5d, R₁ = 5-COOCH₃; R₂ = *m*-COOCH₃;
3e, 4e, 5e, R₁ = 5-COOCH₃; R₂ = *p*-NO₂;
3f, 4f, 5f, R₁ = 5-COOCH₃; R₂ = *m*-NO₂;
3g, 4g, 5g, R₁ = 6-COOCH₃; R₂ = *p*-Cl;
3h, 4h, 5h, R₁ = 6-COOCH₃; R₂ = *p*-F;
3i, 4i, 5i, R₁ = 6-COOCH₃; R₂ = *p*-COOCH₃;
3j, 4j, 5j, R₁ = 6-COOCH₃; R₂ = *m*-COOCH₃;
3k, 4k, 5k, R₁ = 6-COOCH₃; R₂ = *p*-NO₂;
3l, 4l, 5l, R₁ = 6-COOCH₃; R₂ = *m*-NO₂;

Scheme 2 (a) benzoyl chloride, NaH, dry DMF; (b) aminoguanidine hydrochloride, dry CH₃OH; (c) 2-hydrazino-2-imidazoline hydrobromide, dry CH₃OH.

2a, 6a, 7a, 8a, R₁ = 5-COOCH₃;
2b, 6b, 7b, 8b, R₁ = 6-COOCH₃;

RESULTS AND DISCUSSION

Chemistry

We synthesized a series of aminoguanidine derivatives of 3-acylindoles **4a-l**, **7a-b**, **13a-f** and 2-(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone derivatives of 3-acylindoles **5a-l**, **8a-b** and **14a-f** as illustrated in Schemes 1,2,3. In Scheme 1, the substituted indoles **2a-b** were generated in the presence of phosphorus oxychloride and dry dimethylformamide (DMF) by Vilsmeier–Haack reaction.[20] Treatment of **2a-b** with substituted benzyl bromide in the presence of sodium hydride in dry dimethyl sulfoxide (DMSO) generated **3a-l** in moderate-to-good yields (51–96%). Finally, target compounds **4a-l** were obtained by the reaction of **3a-l** with aminoguanidine hydrochloride under pH 3–4 in dry methanol in 76–97% yields, which were purified by tutrition with ethyl ether. Target compounds **5a-l** were generated by the reaction of **3a-l** with 2-hydrazino-2-imidazoline

hydrobromide in dry methanol under reflux, followed by purification by tutrition with ethyl ether in 82–96% yields.

In Scheme 2, treatment of compounds **2a-b** with benzoyl chloride in the presence of sodium hydride in dry DMF generated **6a-b** in 66–71% yields. Then target compounds **7a-b** and **8a-b** were obtained by the reaction of **6a-b** separately with aminoguanidine hydrochloride under pH 3–4 and 2-hydrazino-2-imidazoline hydrobromide under reflux, followed by the purification by tutrition with ethyl ether in 70–92% yields.

In Scheme 3, compound **10** was generated from **9** in the presence of phosphorus oxychloride and dry DMF by Vilsmeier–Haack reaction.[20] Treatment of **10** with benzenesulfonyl chloride in the presence of sodium hydride in dry DMF generated **11**, which was reacted with substituted phenylboronic acid in the presence of PdCl₂(dtbpf) and potassium carbonate in tetrahydrofuran/H₂O by Suzuki reaction[21] to
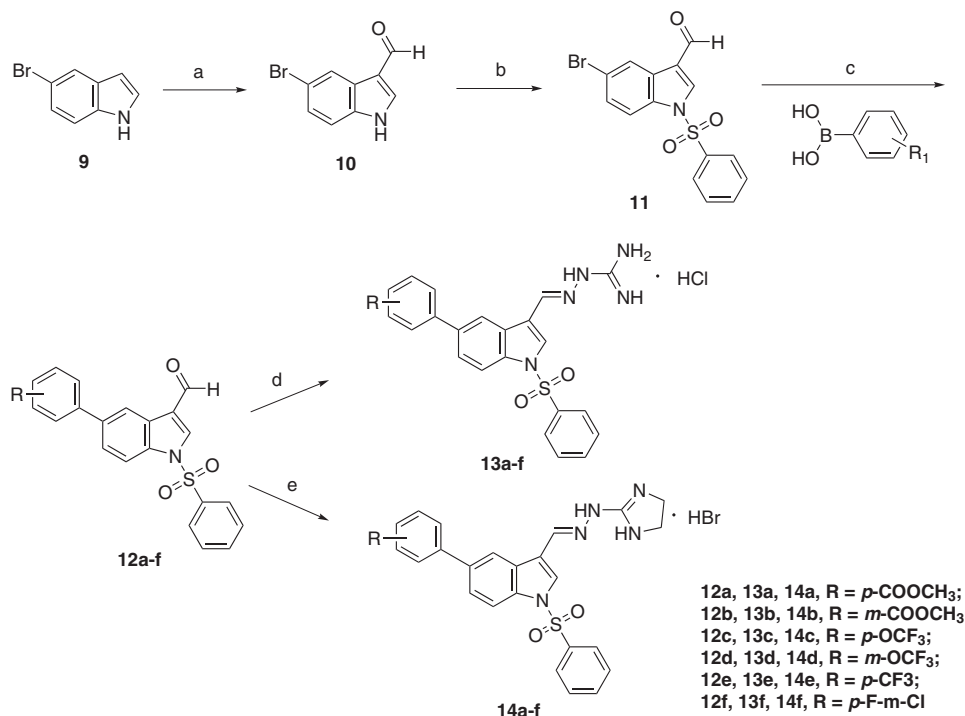
Scheme 3 (a) $POCl_3$, dry DMF; (b) benzenesulfonyl chloride, NaH, dry DMF; (c) substituted phenylboronic acid, K_2CO_3, Pd(dbpf)Cl_2, tetrahydrofuran/H_2O; (d) aminoguanidine hydrochloride, dry CH_3OH; (e) 2-hydrazino-2-imidazoline hydrobromide, dry CH_3OH.

give **12a-f** in moderate-to-good yields (53–89%). Finally, the target compounds **13a-f** and **14a-f** were obtained by the reaction of **12a-f** separately with aminoguanidine hydrochloride under pH 3–4 and 2-hydrazino-2-imidazoline hydrobromide under reflux, followed by the purification by tutrition with ethyl ether in 53–98% yields. All of the novel target compounds were characterized by ¹H-NMR, MS, HRMS and m.p.

Determination of in vitro antibacterial activity

In the preliminary screen where all 40 compounds were tested for antibacterial activity against *S. aureus*, 20 compounds showed *in vitro* growth inhibition with minimum inhibitory concentrations (MICs) ranging from 3.13 to 100 µg ml^{-1} and a median MIC of 12.5 µg ml^{-1} (data not shown).

Subsequent MIC and minimum bactericidal concentration (MBC) studies on these 20 compounds showed the inhibition of both methicillin-susceptible and methicillin-resistant *S. aureus* with median MICs of 12.5 and 6.25 µg ml^{-1}, respectively (Table 1). These six compounds (**4a**, **13a**, **13c**, **13d**, **13e**, **13f**) showed antimethicillin-resistant *Staphylococcus aureus* MICs that were almost the same as that of vancomycin, the standard antibiotic for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Eleven (58%) of the MICs and MBCs were within one doubling dilution difference, suggesting bactericidal activity from the majority of the compounds tested.

Four compounds, **4a**, **4g**, **5a** and **5h**, showed an MIC and MBC < 100 µg ml^{-1} against a strain of multidrug-resistant *A. baumanii*, and two of them, **4a** and **4g**, also inhibited a strain of multidrug-resistant *K. pneumoniae* that carried the *NDM-1* beta-lactamase gene.[22] With these encouraging preliminary results, we expanded our MIC determination to a larger set of both Gram-positive and -negative bacteria along with *Mycobacterium tuberculosis* H37Ra. The results (Table 2) showed all four 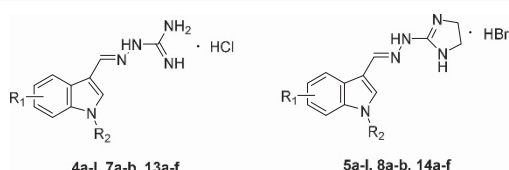compounds inhibiting all Gram-positive bacteria tested, including reference and clinical strains of methicillin-susceptible and -resistant *S. aureus*, *Streptococcus pyogenes* and *S. pneumoniae* from respiratory tract infections, a strain of vancomycin-resistant enterococcus from a urinary tract infection, a coagulase-negative *Staphylococcus*, a *Corynebacterium* sp. and *M. tuberculosis* H37Ra. Against Gram-negative bacilli, all four compounds inhibited *A. baumanii* strains, which were resistant to multiple antibiotics, including carbapenem and imipenem, and were susceptible to only colistin, the 'last-resort' antibiotic for many multidrug-resistant bacteria. **4a** and **4g** inhibited *E. coli* and *Klebsiella pneumoniae* strains that were producers of extended-spectrum beta-lactamases. None of the compounds, however, showed activity against *P. aeruginosa* at concentrations < 100 µg ml^{-1}.

Molecular docking

The molecular docking research showed that all the molecules were docked into a relative small area, although the binding site indicated by Moenomycin A is much larger than the synthesized molecules (Figure 2). By comparing with the literature, it was observed that the binding site of the current study is similar to that published before,[19] which is a site composed by Glu 114, TYR191, Gln 152, Asp 156, Ile 195, Phe 158, Gln 161, Tyr 196, Asn 235, Gln 232 and Pro234.

It was observed that, in the binding site, Asp156, Gln152, Tyr191, Glu114 and Gln232 form a hydrophilic and negative charged site, which is a perfect binding site for some positive charged groups to bind. For compound **4a** (Figure 3), it was observed that the aminoguanidine forms strong electric effects with Glu114 and Asp156 and hydrogen bonds with Gln152 and Pro231. Tyr196, Ile195, Gln161 and Phe158 contribute large Vdw interactions for the binding of **4a**, and Asn235 can form a potential link with the ester group. Chloride (Cl) on compound **4a** has polar effects with Gln161 and some other polar groups. The binding pose of compound **4g** is very similar to that of compound **4a**, but the difference is on the

Table 1 *In vitro* MIC and MBC values (μg ml^{-1}) of novel aminoguanidine and 2-hydrazone derivatives against multidrug-resistant nosocomiants

4a-l, 7a-b, 13a-f   5a-l, 8a-b, 14a-f

Compound	R1	R2	Staphylococcus aureus ATCC 29213 MIC	MRSA MIC	MRSA MBC	MDR Klebsiella pneumoniae MIC	MDR Klebsiella pneumoniae MBC	MDR Acinetobacter baumanii MIC	MDR Acinetobacter baumanii MBC
4a	5-COOCH$_3$	4-Chloro benzyl	3.13	3.13	3.13	12.5	12.5	50	100
4c	5-COOCH$_3$	4-(methoxycarbonyl) benzyl	12.5	50	100	>	>	>	>
4g	6-COOCH$_3$	4-Chloro benzyl	50	12.5	12.5	25	25	25	25
4k	6-COOCH$_3$	4-nitro benzyl	12.5	>	>	>	>	>	>
5a	5-COOCH$_3$	4-Chloro benzyl	12.5	6.25	6.25	>	>	25	100
5c	5-COOCH$_3$	4-(methoxycarbonyl) benzyl	6.25	>	>	>	>	>	>
5h	6-COOCH$_3$	4-Fluoro benzyl	6.25	12.5	25	–	>	50	100
5l	6-COOCH$_3$	3-Nitro benzyl	25	>	–	>	>	>	>
13a	4-(methoxycarbonyl) phenyl	Benzenesulfonyl	>	3.13	25	>	>	>	>
13b	3-(methoxycarbonyl) phenyl	Benzenesulfonyl	6.25	6.25	6.25	>	>	>	>
13c	4-(trifluoromethoxy) phenyl	Benzenesulfonyl	25	3.13	12.5	>	>	>	>
13d	3-(trifluoromethoxy) phenyl	Benzenesulfonyl	6.25	3.13	3.13	>	>	>	>
13e	4-(trifluoromethyl) phenyl	Benzenesulfonyl	25	3.13	100	>	>	>	>
13f	4-Fluoro-3-chloro phenyl	Benzenesulfonyl	>	3.13	50	>	>	>	>
14a	4-(methoxycarbonyl) phenyl	Benzenesulfonyl	12.5	12.5	12.5	>	>	>	>
14b	3-(methoxycarbonyl) phenyl	Benzenesulfonyl	>	50	100	>	>	>	>
14c	4-(trifluoromethoxy) phenyl	Benzenesulfonyl	12.5	6.25	12.5	>	>	>	>
14d	3-(trifluoromethoxy) phenyl	Benzenesulfonyl	25	6.25	25	>	>	>	>
14e	4-(trifluoromethyl) phenyl	Benzenesulfonyl	25	6.25	12.5	>	>	>	>
14f	4-Fluoro-3-chloro phenyl	Benzenesulfonyl	>	12.5	50	>	>	>	>
Levofloxacin			0.25	–	–	–	–	–	–
Vancomycin			–	2	–	–	–	–	–

Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MDR, multidrug resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; >, >100 μg ml^{-1}; –, not done.

Table 2 *In vitro* activity (MIC μg ml^{-1}) of four novel indole derivatives against common bacterial pathogens

Compound	*4a*	*4g*	*5a*	*5h*
MRSA ATCC-S-6007	3.13	12.5	6.25	25
MRSA (clinical strains) (n=8)	6.25–12.5 (6.25)	6.25–12.5 (12.5)	12.5–25 (12.5)	25 (25)
MSSA ATCC-25923	3.13	12.5	12.5	25
MSSA (clinical strain)	12.5	12.5	12.5	25
Staphylococcus saprophyticus	12.5	6.25	6.25	6.25
Streptococcus pyogenes	3.13	6.25	3.13	3.13
Streptococcus pneumoniae	1.56	6.25	6.25	1.56
VRE	6.25	6.25	12.5	6.25
Corynebacterium urealyticum	6.25	12.5	6.25	25
ESBL+ve *Escherichia coli* (n=10)	50–100 (100)	12.5–25 (25)	>	>
ESBL+ve *Klebsiella pneumoniae* (n=11)	12.5–100 (100)	12.5–100 (50)	>	>
MDR *Acinetobacter baumanii* (n=11)	6.25–100 (25)	12.5–25 (12.5)	12.5–100 (25)	25–100 (50)
Pseudomonas aeruginosa	>	100	>	>
M. tuberculosis H37Ra	12.5	12.5	3.13	6.25

Abbreviations: ESBL, extended-spectrum beta-lactamase; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; VRE, vancomycin-resistant *Enterococcus*; (value), (median MIC); +ve, positive; >, >100 μg ml^{-1}.

position of ester group. When the ester group is located at the indole 6-position, the distance between the ester group and Asn235 is increased so that a hydrogen bond cannot be formed. The docking

of compounds **5a** and **5h** shows binding poses similar to those of compounds **4a** and **4g** (Figure 3). They also form interactions similar to those of compounds **4a** and **4g**, but because of the 2-(4,5-dihydro-

1*H*-imidazol-2-yl)hydrazone group, they can form π–π interactions with Pro231. For compound **5h**, same as compound **4g**, as the ester group is located on the indole 6-position, it cannot form hydrogen bond with Asn235.

CONLUSIONS

Based on the crystal structure of GT, 40 novel indole core derivatives were designed and synthesized. Among these 40 compounds, 20 compounds showed anti-*staphylococcal* effects, of which 4 compounds can potentially be further developed into broad-spectrum anti-infective agents against common and multidrug-resistant bacterial and mycobacterial pathogens. Based on molecular modeling, we predicted that these compounds may target at GT, but further experiments are required for confirming this assumption, which is beyond our current research. Although β-lactam antibiotics inhibit the cross-linking of peptidoglycan chains by binding to the active site of the TP enzyme, GT inhibitors are expected to prevent the transfer of disaccharide peptides onto the growing glycan chains before the cross-linking step. Thus GT inhibitors
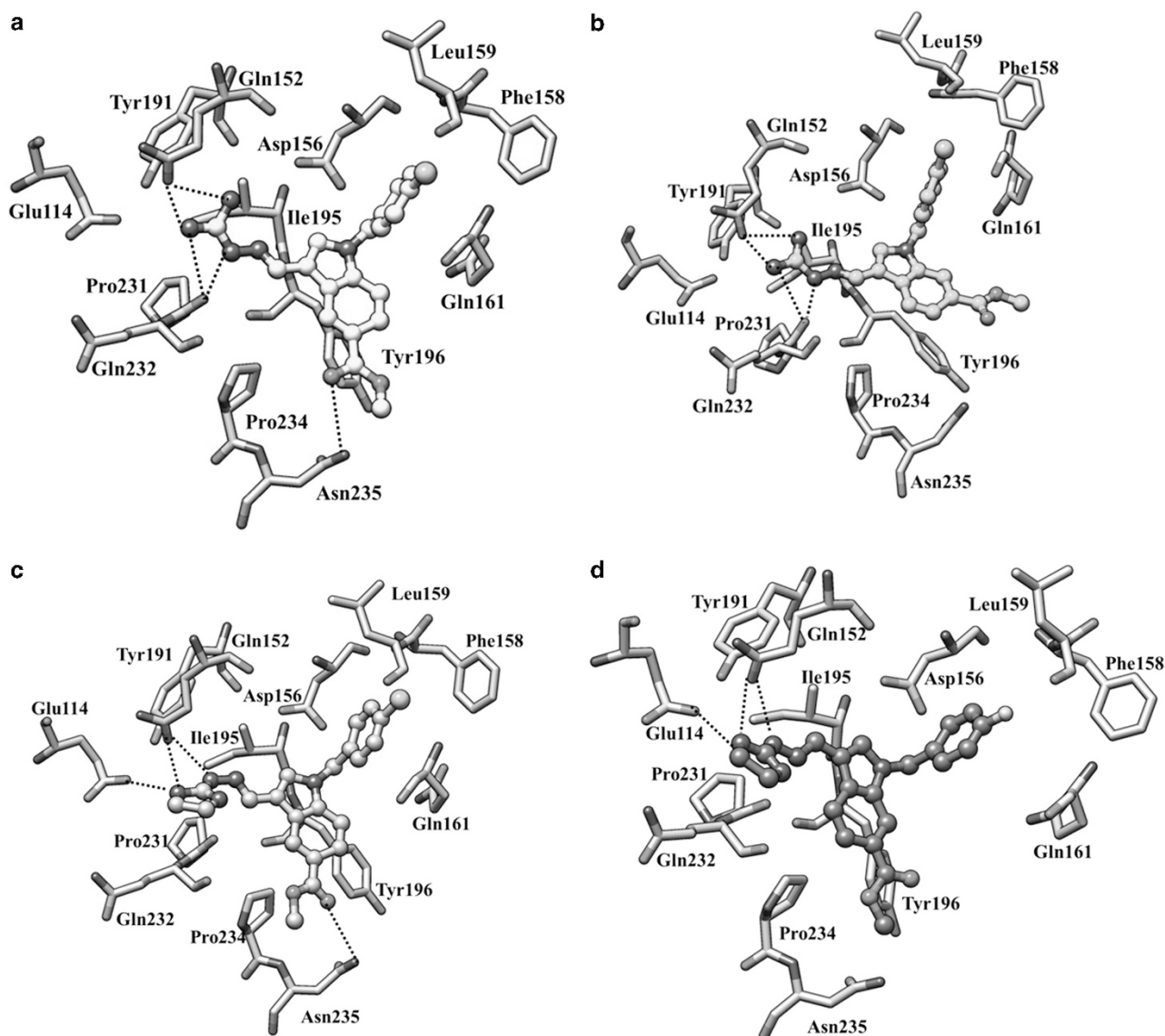

Figure 2 The compound **4a** (represented by balls and sticks model with N atoms colored in blue, O atoms in red, C atoms in yellow and C1 atom in green) occupies the Moenomycin A-binding site (represented by blue mesh surface). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Figure 3 The binding poses of compounds **4a** (a), **4g** (b), **5a** (c) and **5h** (d). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

are likely to act synergistically with β-lactam antibiotics to inhibit the polymerization and cross-linking of cell wall peptidoglycan.

METHODS
Chemistry
All chemicals were purchased from commercial sources and used as supplied unless stated. Dry CH_2Cl_2 was distilled over calcium hydride and dry tetrahydrofuran was distilled over sodium benzophenone. TLC was carried out using silica gel 60 precoated aluminium plates (0.20 mm thickness) from Macherey-Nagel (Damstadt, Germany) with visualization by UV light (254 nm). Flash chromatography was performed on silica gel (particle size 40–63 μm). ^{1}H-NMR spectra were obtained from Bruker AVANCE III 400 spectrometers (Fällanden, Switzerland). The chemical shifts, given as δ values, were quoted in p.p.m.; ^{1}H-NMR chemical shifts were measured relative to internal tetramethylsilane; apparent coupling constants (absolute values), J, were measured in Hertz and multiplicities quoted as singlet (s), doublet (d), triplet (t), quartet (q) or combinations thereof as appropriate. Mass spectra were obtained from Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies Inc., CA, USA). IR spectra were obtained from the solid phase using Bruker Tensor 27 spectrometer (Germany). Melting points were determined using WRS-1B melting point measurement (Shanghai, China) and were uncorrected.

Methyl 3-formyl-1H-indole-5-carboxylate (2a). To a solution of **1a** (5.00 g, 28.57 mmol) in dry DMF (20 ml) was added $POCl_3$ (3.62 ml, 38.86 mol) at 0 °C under N_2 and then stirred at room temperature for 2 h. The reaction was quenched with H_2O (60 ml) at 0 °C and adjusted the pH to 7–8 with 2 M NaOH. The resulting solution was heated to 70 °C and stirred for 30 min. After cooling to room temperature, the precipitate was filtered and washed with MeOH to give **2a** as a light pink solid (5.22 g, 90%), m.p. 231.4–233.0 °C; IR (KBr): υ max cm^{-1} 3232 (NH), 1712 (C=O), 1649 (C=O), 1622 (C=C), 1531 (C=C), 1448 (C=C), 1281 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.44 (brs, 1H, N*H*), 9.98 (s, 1H, C*H*O), 8.77 (d, $J=1.2$, 1H, Ar-*H*), 8.44 (s, 1H, Ar-*H*), 7.88 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-*H*), 7.61 (d, $J=8.8$, 1H, Ar-*H*), 3.87 (s, 3H, C*H*$_3$); ES-MS 204.1 (M+H)$^+$.

Methyl 3-formyl-1H-indole-6-carboxylate (2b). **2b** was obtained by reaction of **1b** as previously described for **1a** as a white solid (0.875 g, 76%), m.p. 221.4–221.7 °C; IR (KBr): υ max cm^{-1} 3218 (NH), 1707 (C=O), 1639 (C=O), 1572 (C=C), 1501 (C=C), 1430 (C=C), 1304 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.43 (brs, 1H, N*H*), 9.98 (s, 1H, C*H*O), 8.51 (s, 1H, Ar-*H*), 8.18 (d, $J=8.4$, 1H, Ar-*H*), 8.14 (s, 1H, Ar-*H*), 7.83 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-*H*), 3.87 (s, 3H, C*H*$_3$); ES-MS 204.1 (M+H)$^+$.

Methyl 1-(4-chlorobenzyl)-3-formyl-1H-indole-5-carboxylate (3a). To a solution of **2a** (500 mg, 2.46 mmol) in dry DMSO (8 ml) was added NaH (60%, 108 mg, 2.71 mmol) and stirred at room temperature for 1 h. 4-Chlorobenzyl bromide (557 mg, 2.71 mmol) was added and stirred at room temperature for 10 h. The reaction solution was quenched with saturated NH_4Cl and extracted with CH_2Cl_2 (150 ml×3), and the combined organic layers dried with Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as elunt to give **3a** as a white solid (417 mg, 52%), m.p. 160.2–160.7 °C; IR (KBr): υ max cm^{-1} 2360 (CH$_2$), 1700 (C=O), 1656 (C=O), 1616 (C=C), 1534 (C=C), 1433 (C=C), 1278 (C-O-C), 744 (C-Cl); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.06 (s, 1H, C*H*O), 9.03 (s, 1H, Ar-*H*), 8.02 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 7.35 (d, $J=8.4$, 2H, Ar-*H*), 7.33 (d, $J=8.4$, 1H, Ar-*H*), 7.10–7.12 (d, $J=8.4$, 2H, Ar-*H*), 5.37 (s, 2H, C*H*$_2$), 3.95 (s, 3H, C*H*$_3$); ES-MS 328.1 (M+H)$^+$.

Methyl 1-(4-fluorobenzyl)-3-formyl-1H-indole-5-carboxylate (3b). **3b** was obtained by reaction of **2a** as previously described for **3a** as a white solid (736 mg, 96%), m.p. 153.3–153.9 °C; IR (KBr): υ max cm^{-1} 2360 (CH$_2$), 1717 (C=O), 1658 (C=O), 1618 (C=C), 1532 (C=C), 1510 (C=C), 1273 (C-O-C), 1088 (C-F); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.05 (s, 1H, C*H*O), 9.02 (s, 1H, Ar-*H*), 8.03 (dd, $J_1=8.8$, $J_2=1.6$, 1H, Ar-*H*), 7.77 (s, 1H, Ar-*H*), 7.35 (d, $J=8.4$, 1H, Ar-*H*), 7.17 (dd, $J_1=8.8$, $J_2=5.2$, 2H, Ar-*H*),

7.06 (t, $J=8.8$, 2H, Ar-*H*), 5.36 (s, 2H, C*H*$_2$), 3.94 (s, 3H, C*H*$_3$); ES-MS 312.1 (M+H)$^+$.

Methyl 3-formyl-1-(4-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate (3c). **3c** was obtained by reaction of **2a** as previously described for **3a** as a white solid (767 mg, 92%), m.p. 203.6–204.0 °C; IR (KBr): υ max cm^{-1} 2360 (CH$_2$), 1716 (C=O), 1661 (C=O), 1615 (C=C), 1534 (C=C), 1436 (C=C), 1278 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.06 (s, 1H, C*H*O), 9.03 (d, $J=1.2$, 1H, Ar-*H*), 8.03 (d, $J=8.4$, 2H, Ar-*H*), 8.00 (dd, $J_1=8.8$, $J_2=1.6$, 1H, Ar-*H*), 7.81 (s, 1H, Ar-*H*), 7.30 (d, $J=8.8$, 1H, Ar-*H*), 7.21 (d, $J=8.4$, 2H, Ar-*H*), 5.46 (s, 2H, C*H*$_2$), 3.94 (s, 3H, C*H*$_3$), 3.91 (s, 3H, C*H*$_3$); ES-MS 352.1 (M+H)$^+$.

Methyl 3-formyl-1-(3-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate (3d). **3d** was obtained by reaction of **2a** as previously described for **3a** as a white solid (787 mg, 91%), m.p. 148.6–149.3 °C; IR (KBr): υ max cm^{-1} 2360 (CH$_2$), 1718 (C=O), 1667 (C=O), 1617 (C=C), 1532 (C=C), 1438 (C=C), 1292 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.06 (s, 1H, C*H*O), 9.03 (d, $J=1.2$, 1H, Ar-*H*), 8.02 (dd, $J_1=8.8$, $J_2=1.2$, 2H, Ar-*H*), 7.94 (s, 1H, Ar-*H*), 7.80 (s, 1H, Ar-*H*), 7.44 (t, $J=8.0$, 1H, Ar-*H*), 7.34 (d, $J=8.8$, 1H, Ar-*H*), 7.31 (d, $J=8.0$, 1H, Ar-*H*), 5.44 (s, 2H, C*H*$_2$), 3.94 (s, 3H, C*H*$_3$), 3.91 (s, 3H, C*H*$_3$); ES-MS 352.1 (M+H)$^+$.

Methyl 3-formyl-1-(4-nitrobenzyl)-1H-indole-5-carboxylate (3e). **3e** was obtained by reaction of **2a** as previously described for **3a** as an yellow solid (355 mg, 86%), m.p. 219.2–219.6 °C; IR (KBr): υ max cm^{-1} 2360 (CH$_2$), 1715 (C=O), 1671 (C=O), 1636 (C=C), 1540 (C=C), 1521 (NO$_2$), 1458 (C=C), 1339 (NO$_2$), 1282 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 10.01 (s, 1H, C*H*O), 8.80 (d, $J=1.2$, 1H, Ar-*H*), 8.66 (s, 1H, Ar-*H*), 8.21 (d, $J=8.8$, 2H, Ar-*H*), 7.88 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-H), 7.69 (d, $J=8.4$, 1H, Ar-*H*), 7.51 (d, $J=8.8$, 2H, Ar-*H*), 5.79 (s, 2H, C*H*$_2$), 3.87 (s, 3H, C*H*$_3$); ES-MS 339.1 (M+H)$^+$.

Methyl 3-formyl-1-(3-nitrobenzyl)-1H-indole-5-carboxylate (3f). **3f** was obtained by reaction of **2a** as previously described for **3a** as an yellow solid (588 mg, 71%), m.p. 190.9–193.9 °C; IR (KBr): υ max cm^{-1} 2361 (CH$_2$), 1712 (C=O), 1668 (C=O), 1617 (C=C), 1537 (C=C), 1521 (NO$_2$), 1459 (C=C), 1351 (NO$_2$), 1280 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.09 (s, 1H, C*H*O), 9.04 (s, 1H, Ar-*H*), 8.21 (d, $J=8.4$, 1H, Ar-*H*), 8.11 (s, 1H, Ar-*H*), 8.03 (dd, $J_1=8.8$, $J_2=1.6$, 1H, Ar-*H*), 7.85 (s, 1H, Ar-*H*), 7.55 (t, $J=8.0$, 1H, Ar-*H*), 7.41 (d, $J=8.0$, 1H, Ar-*H*), 7.30 (d, $J=8.8$ Hz, 1H, Ar-*H*), 5.52 (s, 2H, C*H*$_2$), 3.95 (s, 3H, C*H*$_3$); ES-MS 339.1 (M+H)$^+$.

Methyl 1-(4-chlorobenzyl)-3-formyl-1H-indole-6-carboxylate (3g). **3g** was obtained by reaction of **2b** as previously described for **3a** as a white solid (515 mg, 64%), m.p. 153.1–154.2 °C; IR (KBr): υ max cm^{-1} 2924 (CH$_2$), 1714 (C=O), 1666 (C=O), 1617 (C=C), 1529 (C=C), 1456 (C=C), 1273 (C-O-C), 748 (C-Cl); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.03 (s, 1H, C*H*O), 8.36 (d, $J=8.0$, 1H, Ar-*H*), 8.09 (s, 1H, Ar-*H*), 8.02 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-*H*), 7.82 (s, 1H, Ar-*H*), 7.35 (d, $J=8.4$, 2H, Ar-*H*), 7.12–7.14 (d, $J=8.4$, 2H, Ar-*H*), 5.40 (s, 2H, C*H*$_2$), 3.94 (s, 3H, C*H*$_3$); ES-MS 328.1 (M+H)$^+$.

Methyl 1-(4-fluorobenzyl)-3-formyl-1H-indole-6-carboxylate (3h). **3h** was obtained by reaction of **2b** as previously described for **3a** as a white solid (170 mg, 56%), m.p. 155.6–155.7 °C; IR (KBr): υ max cm^{-1} 2926 (CH$_2$), 1713 (C=O), 1662 (C=O), 1618 (C=C), 1531 (C=C), 1439 (C=C), 1272 (C-O-C), 1095 (C-F); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.03 (s, 1H, C*H*O), 8.35 (d, $J=8.4$, 1H, Ar-*H*), 8.11 (s, 1H, Ar-*H*), 8.01 (dd, $J_1=8.4$, $J_2=1.6$, 1H, Ar-*H*), 7.81 (s, 1H, Ar-*H*), 7.20 (dd, $J_1=8.8$, $J_2=5.2$, 2H, Ar-*H*), 7.07 (t, $J=8.4$, 2H, Ar-*H*), 5.40 (s, 2H, C*H*$_2$), 3.94 (s, 3H, C*H*$_3$); ES-MS 312.1 (M+H)$^+$.

Methyl 3-formyl-1-(4-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate (3i). **3i** was obtained by reaction of **2b** as previously described for **3a** as a white solid (0.367 g, 58%), m.p. 169.9–171.1 °C; IR (KBr): υ max cm^{-1} 3102 (CH$_2$), 1714 (C=O), 1663 (C=O), 1616 (C=C), 1535 (C=C), 1437 (C=C), 1280 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.05 (s, 1H, C*H*O), 8.37 (d, $J=8.0$, 1H, Ar-*H*), 8.07 (s, 1H, Ar-*H*), 8.03 (d, $J=8.4$, 2H, Ar-*H*), 8.01–8.03 (dd, $J_1=8.4$, $J_2=1.2$, 1H, Ar-*H*), 7.85 (s, 1H, Ar-*H*), 7.23 (d, $J=8.4$ Hz, 2H,

Ar-*H*), 5.50 (s, 2H, C*H₂*), 3.93 (s, 3H, C*H₃*), 3.91 (s, 3H, C*H₃*); ES-MS 352.1 (M+H)⁺.

Methyl 3-formyl-1-(3-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate (3j). **3j** was obtained by reaction of **2b** as previously described for **3a** as a white solid (465 mg, 56%), m.p. 141.2–141.7 °C; IR (KBr): ν max cm⁻¹ 2953 (CH₂), 1719 (C=O), 1659 (C=O), 1619 (C=C), 1531 (C=C), 1432 (C=C), 1279 (C-O-C); ¹H-NMR (400 MHz, CDCl₃): δ 10.03 (s, 1H, C*H*O), 8.36 (d, *J*=8.0, 1H, Ar-*H*), 8.10 (s, 1H, Ar-*H*), 8.02 (d, *J*=8.0, 1H, Ar-*H*), 8.01 (dd, J_1=8.0, J_2=1.6, 1H, Ar-*H*), 7.94 (s, 1H, Ar-*H*), 7.84 (s, 1H, Ar-*H*), 7.45 (t, *J*=7.6, 1H, Ar-*H*), 7.34 (d, *J*=7.6, 1H, Ar-*H*), 5.47 (s, 2H, C*H₂*), 3.93 (s, 3H, C*H₃*), 3.91 (s, 3H, C*H₃*); ES-MS 352.1 (M+H)⁺.

Methyl 3-formyl-1-(4-nitrobenzyl)-1H-indole-6-carboxylate (3k). **3k** was obtained by reaction of **2b** as previously described for **3a** as an yellow solid (0.138 g, 80%), m.p. 184.5–184.9 °C; IR (KBr): ν max cm⁻¹ 2945 (CH₂), 1711 (C=O), 1663 (C=O), 1616 (C=C), 1516 (NO₂), 1436 (C=C), 1392 (C=C), 1344 (NO₂), 1278 (C-O-C); ¹H-NMR (400 MHz, CDCl₃): δ 10.08 (s, 1H, C*H*O), 8.39 (d, *J*=8.0, 1H, Ar-*H*), 8.22 (d, *J*=8.8, 2H, Ar-*H*), 8.02–8.04 (m, 2H, Ar-*H*), 7.89 (s, 1H, Ar-*H*), 7.30 (d, *J*=8.8, 2H, Ar-H), 5.56 (s, 2H, C*H₂*), 3.93 (s, 3H, C*H₃*); ES-MS 339.1 (M+H)⁺.

Methyl 3-formyl-1-(3-nitrobenzyl)-1H-indole-6-carboxylate (3l). **3l** was obtained by reaction of **2b** as previously described for **3a** as an yellow solid (580 mg, 70%), m.p. 153.8–155.3 °C; IR (KBr): ν max cm⁻¹ 3089 (CH₂), 1701 (C=O), 1669 (C=O), 1618 (C=C), 1571 (C=C), 1523 (NO₂), 1435 (C=C), 1348 (NO₂), 1280 (C-O-C); ¹H-NMR (400 MHz, CDCl₃): δ 10.07 (s, 1H, C*H*O), 8.38 (d, *J*=8.4, 1H, Ar-*H*), 8.21 (dd, J_1=8.4, J_2=1.2, 1H, Ar-*H*), 8.10 (s, 1H, Ar-*H*), 8.05 (s, 1H, Ar-*H*), 8.03 (dd, J_1=8.4, J_2=1.2, 1H, Ar-*H*), 7.89 (s, 1H, Ar-*H*), 7.55 (t, *J*=8.0, 1H, Ar-*H*), 7.42–7.44 (d, *J*=8.0, 1H, Ar-*H*), 5.55 (s, 2H, C*H₂*), 3.93 (s, 3H, C*H₃*); ES-MS 339.1 (M+H)⁺.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-chlorobenzyl)-1H-indole-5-carboxylate hydrochloride (4a). To a solution of **3a** (200 mg, 0.61 mmol) in dry MeOH was added aminoguanidine hydrochloride (260 mg, 0.61 mmol) and then the reaction solution was adjusted pH to 3–4 and stirred at room temperature for 1.5 h. After the completion of the reaction, the solvent was removed under reduced pressure, and then the residue was tutrited in Et₂O and filtered to give **4a** as a white solid (223 mg, 87%), m.p. 241.1–242.1 °C; IR (KBr): ν max cm⁻¹ 3337 (NH), 3272 (NH), 3099 (NH), 2361 (CH₂), 1709 (C=O), 1676 (C=O), 1641 (C=C), 1533 (C=C), 1433 (C=C), 1282 (C-O-C), 749 (C-Cl); ¹H-NMR (400 MHz,DMSO): δ11.75 (s, 1H, N*H*), 8.75 (d, *J*=1.2, 1H, Ar-*H*), 8.43 (s, 1H, Ar-*H*), 8.21 (s, 1H, C*H*), 7.84 (dd, J_1=8.8, J_2=1.6, 1H, Ar-*H*), 7.66 (d, *J*=8.4, 1H, Ar-H), 7.57 (s, 3H, N*H*), 7.40 (d, *J*=8.4, 2H, Ar-*H*), 7.27 (d, *J*=8.4, 2H, Ar-*H*), 5.54 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*); ES-MS 384.1 (M+H)⁺; HRMS calcd for C₁₉H₁₉ClN₅O₂⁺ 384.1227, found 384.1221.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-fluorobenzyl)-1H-indole-5-carboxylate hydrochloride (4b). **4b** was obtained by reaction of **3b** as previously described for **4a** as a white solid (195 mg, 76%), m.p. 237.6–238.4 °C; IR (KBr): ν max cm⁻¹ 3479 (NH), 3275 (NH), 3096 (NH), 2362 (CH₂), 1707 (C=O), 1674 (C=O), 1645 (C=C), 1533 (C=C), 1435 (C=C), 1280 (C-O-C), 1092 (C-F); ¹H-NMR (400 MHz, DMSO): δ 11.72 (s, 1H, N*H*), 8.75 (d, *J*=1.6, 1H, Ar-*H*), 8.42 (s, 1H, Ar-*H*), 8.21 (s, 1H, C*H*), 7.83 (dd, J_1=8.8, J_2=1.6, 1H, Ar-*H*), 7.69 (d, *J*=8.8, 1H, Ar-H), 7.56 (s, 3H, N*H*), 7.33 (dd, J_1=8.8, J_2=5.2, 2H, Ar-H), 7.15–7.20 (t, *J*=8.8, 2H, Ar-H), 5.52 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*); ES-MS 368.2 (M+H)⁺; HRMS calcd for C₁₉H₁₉FN₅O₂⁺ 368.1523, found 368.1514.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate hydrochloride (4c). **4c** was obtained by reaction of **3c** as previously described for **4a** as an yellow solid (207 mg, 82%), m.p. 225.7–228.6 °C; IR (KBr): ν max cm⁻¹ 3465 (NH), 3344 (NH), 2949 (NH), 2361 (CH₂), 1719 (C=O), 1672 (C=O), 1615 (C=C), 1537 (C=C), 1433 (C=C), 1281 (C-O-C); ¹H-NMR (400 MHz, DMSO): δ 11.72 (s, 1H, N*H*), 8.76 (d, *J*=1.2, 1H, Ar-*H*), 8.44 (s, 1H, Ar-*H*), 8.23 (s, 1H, C*H*), 7.92 (d, *J*=8.4, 2H, Ar-*H*), 7.82 (dd, J_1=8.4, J_2=1.6, 1H, Ar-*H*), 7.63 (d, *J*=8.8,

1H, Ar-*H*), 7.56 (s, 3H, N*H*), 7.34 (d, *J*=8.0, 2H, Ar-*H*), 5.65 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*), 3.82 (s, 3H, C*H₃*); ES-MS 408.2 (M+H)⁺; HRMS calcd for C₂₁H₂₂N₅O₄⁺ 408.1672, found 408.1663.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(3-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate hydrochloride (4d). **4d** was obtained by reaction of **3d** as previously described for **4a** as an yellow solid (198 mg, 79%), m.p. 137.3–138.7 °C; IR (KBr): ν max cm⁻¹ 3365 (NH), 3155 (NH), 2949 (NH), 2361 (CH₂), 1715 (C=O), 1672 (C=O), 1617 (C=C), 1536 (C=C), 1433 (C=C), 1281 (C-O-C); ¹H-NMR (400 MHz, DMSO): δ 11.70 (s, 1H, N*H*), 8.76 (d, *J*=1.2, 1H, Ar-*H*), 8.44 (s, 1H, Ar-*H*), 8.24 (s, 1H, C*H*), 7.86–7.88 (m, 2H, Ar-*H*), 7.83 (dd, J_1=8.8, J_2=1.6, 1H), 7.68 (d, *J*=8.8, 1H, Ar-*H*), 7.56 (s, 3H, N*H*), 7.50–7.52 (m, 2H, Ar-*H*), 5.64 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*), 3.81 (s, 3H, C*H₃*); ES-MS 408.2 (M+H)⁺; HRMS calcd for C₂₁H₂₂N₅O₄⁺ 408.1672, found 408.1663.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-nitrobenzyl)-1H-indole-5-carboxylate hydrochloride (4e). **4e** was obtained by reaction of **3e** as previously described for **4a** as an yellow solid (210 mg, 88%), m.p. 229.6–229.9 °C; IR (KBr): ν max cm⁻¹ 3363 (NH), 3113 (NH), 2950 (NH), 2361 (CH₂), 1701 (C=O), 1674 (C=O), 1620 (C=C), 1520 (NO₂), 1435 (C=C), 1392 (C=C), 1345 (NO₂), 1281 (C-O-C); ¹H-NMR (400 MHz, DMSO): δ 11.74 (s, 1H, N*H*), 8.77 (d, *J*=1.6, 1H, Ar-*H*), 8.44 (s, 1H, Ar-*H*), 8.25 (s, 1H, C*H*), 8.21 (d, *J*=8.8, 2H, Ar-H), 7.84 (dd, J_1=8.8, J_2=1.6, 1H, Ar-*H*), 7.64 (d, *J*=8.8, 1H, Ar-*H*), 7.56 (s, 3H, N*H*), 7.45 (d, *J*=8.4, 2H, Ar-*H*), 5.73 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*); ES-MS 395.1 (M+H)⁺; HRMS calcd for C₁₉H₁₉N₆O₄⁺ 395.1468, found 395.1460.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(3-nitrobenzyl)-1H-indole-5-carboxylate hydrochloride (4f). **4f** was obtained by reaction of **3f** as previously described for **4a** as an yellow solid (223 mg, 88%), m.p. 227.3–230.2 °C; IR (KBr): ν max cm⁻¹ 3368 (NH), 2361 (CH₂), 1699 (C=O), 1639 (C=O), 1517 (NO₂), 1435 (C=C), 1392 (C=C), 1355 (NO₂), 1278 (C-O-C); ¹H-NMR (400 MHz, DMSO): δ 11.72 (s, 1H, N*H*), 8.77 (d, *J*=1.2, 1H, Ar-*H*), 8.44 (s, 1H, Ar-*H*), 8.27 (s, 1H, C*H*), 8.15 (m, 2H, Ar-*H*), 7.84 (dd, J_1=8.8, J_2=1.6, 1H, Ar-*H*), 7.62–7.73 (m, 3H, Ar-*H*), 7.57 (s, 3H, N*H*), 5.71 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*); ES-MS 395.1 (M+H)⁺; HRMS calcd for C₁₉H₁₉N₆O₄⁺ 395.1468, found 395.1460.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-chlorobenzyl)-1H-indole-6-carboxylate hydrochloride (4g). **4g** was obtained by reaction of **3g** as previously described for **4a** as a white solid (223 mg, 87%), m.p. 249.6–250.9 °C; IR (KBr): ν max cm⁻¹ 3470 (NH), 3326 (NH), 3088 (NH), 1718 (C=O), 1645 (C=O), 1622 (C=C), 1513 (C=C), 1438 (C=C), 1287 (C-O-C), 741 (C-Cl); ¹H-NMR (400 MHz, DMSO): δ 11.71 (s, 1H, N*H*), 8.43–8.45 (d, *J*=8.4, 1H, Ar-*H*), 8.37 (s, 1H, Ar-*H*), 8.27 (s, 1H, C*H*), 8.17 (s, 1H, Ar-*H*), 7.76 (d, *J*=8.4, 1H, Ar-*H*), 7.57 (s, 3H, N*H*), 7.28 (dd, J_1=8.4, J_2=5.6, 2H, Ar-*H*), 7.18 (t, *J*=8.8, 2H, Ar-*H*), 5.59 (s, 2H, C*H₂*), 3.86 (s, 3H, C*H₃*); ES-MS 384.1 (M+H)⁺; HRMS calcd for C₁₉H₁₉ClN₅O₂⁺ 384.1227, found 384.1217.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-fluorobenzyl)-1H-indole-6-carboxylate hydrochloride (4h). **4h** was obtained by reaction of **3h** as previously described for **4a** as a white solid (251 mg, 97%), m.p. 261.4–261.9 °C; IR (KBr): ν max cm⁻¹ 3376 (NH), 3225 (NH), 3177 (NH), 1712 (C=O), 1668 (C=O), 1633 (C=C), 1459 (C=C), 1394 (C=C), 1269 (C-O-C), 1100 (C-F); ¹H-NMR (400 MHz, DMSO): δ 11.70 (s, 1H, N*H*), 8.45 (d, *J*=8.4 Hz, 1H, Ar-*H*), 8.38 (s, 1H, Ar-*H*), 8.28 (s, 1H, C*H*), 8.14 (s, 1H, Ar-*H*), 7.76 (dd, J_1=8.4, J_2=1.2, 1H, Ar-*H*), 7.57 (s, 3H, N*H*), 7.41 (d, *J*=8.4, 2H, Ar-*H*), 7.23 (d, *J*=8.4, 2H, Ar-*H*), 5.61 (s, 2H, C*H₂*), 3.85 (s, 3H, C*H₃*); ES-MS 368.2 (M+H)⁺; HRMS calcd for C₁₉H₁₉FN₅O₂⁺ 368.1523, found 368.1513.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate hydrochloride (4i). **4i** was obtained by reaction of **3i** as previously described for **4a** as a white solid (223 mg, 88%), m.p. 238.8–240.0 °C; IR (KBr): ν max cm⁻¹ 3471 (NH), 3334 (NH), 3159 (NH), 1715 (C=O), 1673 (C=O), 1616 (C=C), 1534 (C=C), 1438 (C=C), 1279

(C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.70 (s, 1H, N*H*), 8.45 (d, *J* = 8.4, 1H, Ar-*H*), 8.39 (s, 1H, Ar-*H*), 8.30 (s, 1H, C*H*), 8.10 (s, 1H, Ar-*H*), 7.92 (d, *J* = 8.0, 2H, Ar-*H*), 7.77 (dd, *J*₁ = 8.4, *J*₂ = 1.2, 1H, Ar-*H*), 7.52 (s, 3H, N*H*), 7.30 (d, *J* = 8.0, 2H, Ar-*H*), 5.72 (s, 2H, C*H*₂), 3.84 (s, 3H, C*H*₃), 3.82 (s, 3H, C*H*₃); ES-MS 408.2 (M+H)⁺; HRMS calcd for $C_{21}H_{22}N_5O_4^+$ 408.1672, found 408.1665.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(3-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate hydrochloride (4j). **4j** was obtained by reaction of **3j** as previously described for **4a** as a white solid (225 mg, 89%), m.p. 228.8–230.9 °C; IR (KBr): υ max cm⁻¹ 3470 (NH), 2956 (NH), 2359 (CH₂), 1717 (C=O), 1667 (C=O), 1640 (C=C), 1535 (C=C), 1435 (C=C), 1284 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.70 (s, 1H, N*H*), 8.45 (d, *J* = 8.4, 1H, Ar-*H*), 8.39 (s, 1H, Ar-*H*), 8.31 (s, 1H, Ar-*H*), 8.16 (s, 1H, Ar-*H*), 7.86–7.88 (d, *J* = 6.8, 1H, Ar-*H*), 7.83 (s, 1H, Ar-*H*), 7.77 (d, *J* = 8.0, 1H, Ar-*H*), 7.65 (s, 3H, N*H*), 7.49–7.51 (m, 2H, Ar-*H*), 5.71 (s, 2H, C*H*₂), 3.85 (s, 3H, C*H*₃), 3.82 (s, 3H, C*H*₃); ES-MS 408.2 (M+H)⁺; HRMS calcd for $C_{21}H_{22}N_5O_4^+$ 408.1672, found 408.1666.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(4-nitrobenzyl)-1H-indole-6-carboxylate hydrochloride (4k). **4k** was obtained by reaction of **3k** as previously described for **4a** as an yellow solid (230 mg, 96%), m.p. 259.8–260.8 °C; IR (KBr): υ max cm⁻¹ 3469 (NH), 2363 (CH₂), 1711 (C=O), 1673 (C=O), 1644 (C=C), 1519 (NO₂), 1436 (C=C), 1395 (C=C), 1349 (NO₂), 1277 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.71 (s, 1H, N*H*), 8.47 (d, *J* = 8.4, 1H, Ar-*H*), 8.39 (s, 1H, Ar-*H*), 8.31 (s, 1H, C*H*), 8.21 (d, *J* = 8.8, 2H, Ar-*H*), 8.13 (s, 1H, Ar-*H*), 7.79 (dd, *J*₁ = 8.4, *J*₂ = 1.2, 1H, Ar-*H*), 7. 57 (s, 3H, N*H*), 7.41 (d, *J* = 8.8, 2H, Ar-*H*), 5.80 (s, 2H, C*H*₂), 3.84 (s, 3H, C*H*₃); ES-MS 395.1 (M+H)⁺; HRMS calcd for $C_{19}H_{19}N_6O_4^+$ 395.1468, found 395.1462.

Methyl (E)-3-((2-carbamimidoylhydrazono)methyl)-1-(3-nitrobenzyl)-1H-indole-6-carboxylate hydrochloride (4l). **4l** was obtained by reaction of **3l** as previously described for **4a** as an yellow solid (199 mg, 79%), m.p. 248.9–249.3 °C; IR (KBr): υ max cm⁻¹ 3479 (NH), 3402 (NH), 3274 (NH), 2361 (CH₂), 1707 (C=O), 1676 (C=O), 1643 (C=C), 1531 (NO₂), 1439 (C=C), 1393 (C=C), 1348 (NO₂), 1271 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.68 (s, 1H, N*H*), 8.47 (d, *J* = 8.4, 1H, Ar-*H*), 8.39 (s, 1H, Ar-*H*), 8.33 (s, 1H, C*H*), 8.21 (s, 1H, Ar-*H*), 8.15 (dd, *J*₁ = 5.6, *J*₂ = 2.0, 1H, Ar-*H*), 8.12 (s, 1H, Ar-H), 7.78 (d, *J* = 8.4, 1H, Ar-*H*), 7.64–7.67 (m, 2H, Ar-*H*), 7. 50 (s, 3H, N*H*), 5.79 (s, 2H, C*H*₂), 3.85 (s, 3H, C*H*₃); ES-MS 395.1 (M+H)⁺; HRMS calcd for $C_{19}H_{19}N_6O_4^+$ 395.1468, found 395.1458.

Methyl (E)-1-(4-chlorobenzyl)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1H-indole-5-carboxylate hydrobromide (5a). To a solution of **3a** (50 mg, 0.15 mmol) in dry MeOH (2 ml) was added 2-hydrazino-2-imidazoline hydrobromide (28 mg, 0.15 mmol), and then the reaction solution was stirred at 75 °C. After the completion of the reaction, the solvent was removed under reduced pressure, and then the residue was washed by Et₂O and filtered to give **4a** as a white solid (65 mg, 87%), m.p. 243.6–244.3 °C; IR (KBr): υ max cm⁻¹ 3426 (NH), 3185 (NH), 2950 (NH), 2361 (CH₂), 1708 (C=O), 1669 (C=O), 1603 (C=C), 1539 (C=C), 1453 (C=C), 1246 (C-O-C), 745 (C-Cl); ^{1}H-NMR (400 MHz, DMSO): δ 11.95 (s, 1H, N*H*), 8.79 (d, *J* = 1.2, 1H, Ar-*H*), 8.41 (s, 2H, C*H*, N*H*), 8.19 (s, 1H, Ar-*H*), 7.84 (dd, *J*₁ = 8.8, *J*₂ = 1.6, 1H, Ar-*H*), 7.68 (d, *J* = 8.8, 1H, Ar-*H*), 7.41 (d, *J* = 8.4, 2H, Ar-*H*), 7.29 (d, *J* = 8.4, 2H, Ar-*H*), 5.54 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.73 (s, 4H, C*H*₂); ES-MS 410.1 (M+H)⁺; HRMS calcd for $C_{21}H_{21}ClN_5O_2^+$ 410.1384, found 410.1374.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-fluorobenzyl)-1H-indole-5-carboxylate hydrobromide (5b). **5b** was obtained by reaction of **3b** as previously described for **5a** as a white solid (279 mg, 92%), m.p. 241.5–243.7 °C; IR (KBr): υ max cm⁻¹ 3175 (NH), 3051 (NH), 2952 (NH), 2361 (CH₂), 1706 (C=O), 1668 (C=O), 1602 (C=C), 1510 (C=C), 1451 (C=C), 1246 (C-O-C), 1096 (C-F); ^{1}H-NMR (400 MHz, DMSO): δ 12.00 (s, 1H, N*H*), 8.79 (s, 1H, Ar-*H*), 8.41 (s, 2H, C*H*, N*H*), 8.19 (s, 1H, Ar-*H*), 7.84 (dd, *J*₁ = 8.8, *J*₂ = 1.2, 1H, Ar-*H*), 7.71 (d, *J* = 8.4, 1H, Ar-*H*), 7.33–7.36 (dd, *J*₁ = 8.4, *J*₂ = 1.6, 2H, Ar-*H*), 7.16–7.20 (t, *J* = 8.8, 2H, Ar-*H*), 5.52 (s, 2H,

CH₂), 3.86 (s, 3H, CH₃), 3.73 (s, 4H, CH₂); ES-MS 394.2 (M+H)⁺; HRMS calcd for $C_{21}H_{21}FN_5O_2^+$ 394.1679, found 394.1670.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate hydrobromide (5c). **5c** was obtained by reaction of **3c** as previously described for **5a** as a white solid (264 mg, 90%), m.p. 238.7 °C (dec); IR (KBr): υ max cm⁻¹ 3184 (NH), 2950 (NH), 1721 (C=O), 1666 (C=O), 1603 (C=C), 1538 (C=C), 1453 (C=C), 1281 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.99 (s, 1H, N*H*), 8.80 (d, *J* = 0.8, 1H, Ar-*H*), 8.42 (s, 2H, C*H*, N*H*), 8.21 (s, 1H, Ar-*H*), 7.93 (d, *J* = 8.0, 2H, Ar-*H*), 7.83 (dd, *J*₁ = 8.4, *J*₂ = 1.2, 1H, Ar-*H*), 7.64 (d, *J* = 8.4, 1H, Ar-*H*), 7.35 (d, *J* = 8.4, 2H, Ar-*H*), 5.65 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.82 (s, 3H, C*H*₃), 3.74 (s, 4H, C*H*₂); ES-MS 434.2 (M+H)⁺; HRMS calcd for $C_{23}H_{24}N_5O_4^+$ 434.1828, found 434.1817.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(3-(methoxycarbonyl)benzyl)-1H-indole-5-carboxylate hydrobromide (5d). **5d** was obtained by reaction of **3d** as previously described for **5a** as a white solid (271 mg, 93%), m.p. 243.4–246.0 °C; IR (KBr): υ max cm⁻¹ 3192 (NH), 3086 (NH), 2951 (NH), 1704 (C=O), 1666 (C=O), 1601 (C=C), 1539 (C=C), 1449 (C=C), 1291 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.98 (s, 1H, N*H*), 8.80 (d, *J* = 1.2, 1H, Ar-*H*), 8.42 (s, 2H, C*H*, N*H*), 8.23 (s, 1H, Ar-*H*), 7.83–7.89 (m, 3H, Ar-*H*), 7.69 (d, *J* = 8.4, 1H, Ar-*H*), 7.51–7.53 (m, 2H, Ar-*H*), 5.64 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.81 (s, 3H, C*H*₃), 3.74 (s, 4H, C*H*₂); ES-MS 434.2 (M+H)⁺; HRMS calcd for $C_{23}H_{24}N_5O_4^+$ 434.1828, found 434.1820.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-nitrobenzyl)-1H-indole-5-carboxylate hydrobromide (5e). **5e** was obtained by reaction of **3e** as previously described for **5a** as an yellow solid (271 mg, 93%), m.p. 247.3–247.7 °C; IR (KBr): υ max cm⁻¹ 3463 (NH), 3268 (NH), 3107 (NH), 2362 (CH₂), 1692 (C=O), 1661 (C=O), 1613 (C=C), 1539 (C=C), 1514 (NO₂), 1435 (C=C), 1347 (NO₂), 1248 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.99 (s, 1H, N*H*), 8.81 (s, 1H, Ar-*H*), 8.44 (s, 2H, C*H*, N*H*), 8.23 (s, 1H, Ar-*H*), 8.21 (d, *J* = 8.8, 2H, Ar-*H*), 7.84 (dd, *J*₁ = 8.8, *J*₂ = 1.2, 1H, Ar-*H*), 7.65 (d, *J* = 8.8, 1H, Ar-*H*), 7.46 (d, *J* = 8.4, 2H, Ar-*H*), 5.73 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.74 (s, 4H, C*H*₂); ES-MS 421.2 (M+H)⁺; HRMS calcd for $C_{21}H_{21}N_6O_4^+$ 421.1624, found 421.1617.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(3-nitrobenzyl)-1H-indole-5-carboxylate hydrobromide (5f). **5f** was obtained by reaction of **3f** as previously described for **5a** as an yellow solid (265 mg, 90%), m.p. 246.9–250.9 °C; IR (KBr): υ max cm⁻¹ 3424 (NH), 3198 (NH), 2361 (CH₂), 1703 (C=O), 1665 (C=O), 1604 (C=C), 1532 (NO₂), 1452 (C=C), 1395 (C=C), 1346 (NO₂), 1248 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.99 (s, 1H, N*H*), 8.81 (s, 1H, Ar-*H*), 8.43 (s, 2H, C*H*, N*H*), 8.27 (s, 1H, Ar-*H*), 8.15 (m, 2H, Ar-*H*), 7.85 (d, *J* = 8.4, 1H, Ar-*H*), 7.74–7.63 (m, 3H, Ar-*H*), 5.72 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.74 (s, 4H, C*H*₂); ES-MS 421.2 (M+H)⁺; HRMS calcd for $C_{21}H_{21}N_6O_4^+$ 421.1624, found 421.1615.

Methyl (E)-1-(4-chlorobenzyl)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1H-indole-6-carboxylate hydrobromide (5g). **5g** was obtained by reaction of **3g** as previously described for **5a** as a white solid (281 mg, 94%), m.p. 176.7–179.6 °C; IR (KBr): υ max cm⁻¹ 3408 (NH), 2951 (NH), 2361 (CH₂), 1713 (C=O), 1667 (C=O), 1617 (C=C), 1511 (C=C), 1437 (C=C), 1277 (C-O-C), 748 (C-Cl); ^{1}H-NMR (400 MHz, DMSO): δ 12.00 (s, 1H, N*H*), 8.58 (brs, 1H, N*H*), 8.48 (d, *J* = 8.4, 1H, Ar-*H*), 8.38 (s, 1H, Ar-*H*), 8.28 (s, 1H, C*H*), 8.18 (s, 1H, Ar-*H*), 7.77 (dd, *J*₁ = 8.4, *J*₂ = 1.2, 1H, Ar-*H*), 7.30 (dd, *J*₁ = 8.4, *J*₂ = 1.6, 2H, Ar-*H*), 7.19 (t, *J* = 8.8, 2H, Ar-*H*), 5.59 (s, 2H, C*H*₂), 3.86 (s, 3H, C*H*₃), 3.74 (s, 4H, C*H*₂); ES-MS 410.1 (M+H)⁺; HRMS calcd for $C_{21}H_{21}ClN_5O_2^+$ 410.1384, found 410.1374.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-fluorobenzyl)-1H-indole-6-carboxylate hydrobromide (5h). **5h** was obtained by reaction of **3h** as previously described for **5a** as a white solid (257 mg, 85%), m.p. 213.4–214.6 °C; IR (KBr): υ max cm⁻¹ 3403 (NH), 3168 (NH), 2948 (NH), 2361 (CH₂), 1709 (C=O), 1665 (C=O), 1617 (C=C), 1511 (C=C), 1437 (C=C), 1277 (C-O-C), 1097 (C-F); ^{1}H-NMR (400 MHz, DMSO):

δ 11.98 (s, 1H, N*H*), 8.58 (brs, 1H, N*H*), 8.48 (d, *J*=8.4, 1H, Ar-*H*), 8.38 (s, 1H, Ar-*H*), 8.28 (s, 1H, C*H*), 8.18 (s, 1H, Ar-*H*), 7.78 (dd, *J*$_1$=8.4, *J*$_2$=1.2, 1H, Ar-*H*), 7.30 (dd, *J*$_1$=8.4, *J*$_2$=5.6, 2H, Ar-*H*), 7.19 (t, *J*=8.8, 2H, Ar-*H*), 5.59 (s, 2H, C*H*$_2$), 3.86 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 394.2 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{21}$FN$_5$O$_2^+$ 394.1679, found 394.1670.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate hydrobromide (5i). **5i** was obtained by reaction of **3i** as previously described for **5a** as a white solid (280 mg, 96%), m.p. 239.1 °C (dec); IR (KBr): υ max cm^{-1} 3416 (NH), 2951 (NH), 2361 (CH$_2$), 1718 (C=O), 1670 (C=O), 1617 (C=C), 1459 (C=C), 1436 (C=C), 1281 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.99 (s, 1H, N*H*), 8.49 (d, *J*=8.4, 2H, Ar-*H*, N*H*), 8.39 (s, 1H, Ar-*H*), 8.30 (s, 1H, C*H*), 8.12 (s, 1H, Ar-*H*), 7.93 (d, *J*=8.4, 2H, Ar-*H*), 7.78 (dd, *J*$_1$=8.4, *J*$_2$=1.2, 1H, Ar-*H*), 7.31 (d, *J*=8.0, 2H, Ar-*H*), 5.72 (s, 2H, C*H*$_2$), 3.84 (s, 3H, C*H*$_3$), 3.82 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 434.2 (M+H)$^+$; HRMS calcd for C$_{23}$H$_{24}$N$_5$O$_4^+$ 434.1828, found 434.1816.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(3-(methoxycarbonyl)benzyl)-1H-indole-6-carboxylate hydrobromide (5j). **5j** was obtained by reaction of **3j** as previously described for **5a** as a white solid (240 mg, 82%), m.p. 203.7–206.2 °C; IR (KBr): υ max cm^{-1} 3405 (NH), 2950 (NH), 2361 (CH$_2$), 1716 (C=O), 1665 (C=O), 1618 (C=C), 1536 (C=C), 1457 (C=C), 1287 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.98 (s, 1H, N*H*), 8.49 (d, *J*=8.4, 2H, Ar-*H*, N*H*), 8.40 (s, 1H, Ar-*H*), 8.31 (s, 1H, C*H*), 8.17 (s, 1H, Ar-*H*), 7.89–7.87 (m, 1H, Ar-*H*), 7.84 (s, 1H, Ar-*H*), 7.78 (dd, *J*$_1$=8.4, *J*$_2$=1.6, 1H, Ar-*H*), 7.52–7.50 (m, 2H, Ar-*H*), 5.71 (s, 2H, C*H*$_2$), 3.85 (s, 3H, C*H*$_3$), 3.82 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 434.2 (M+H)$^+$; HRMS calcd for C$_{23}$H$_{24}$N$_5$O$_4^+$ 434.1828, found 434.1821.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(4-nitrobenzyl)-1H-indole-6-carboxylate hydrobromide (5k). **5k** was obtained by reaction of **3k** as previously described for **5a** as an yellow solid (260 mg, 88%), m.p. 253.6–255.6 °C; IR (KBr): υ max cm^{-1} 3404 (NH), 3108 (NH), 2910 (NH), 2361 (CH$_2$), 1712 (C=O), 1662 (C=O), 1615 (C=C), 1522 (NO$_2$), 1490 (C=C), 1391 (C=C), 1350 (NO$_2$), 1276 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.01 (d, *J*=3.6, 1H, N*H*), 8.51 (d, *J*=8.4, 2H, Ar-*H*, NH), 8.40 (s, 1H, C*H*), 8.32 (s, 1H, Ar-*H*), 8.22 (d, *J*=8.8, 2H, Ar-*H*), 8.14 (s, 1H, Ar-*H*), 7.79 (dd, *J*$_1$=8.4, *J*$_2$=1.6, 1H, Ar-*H*), 7.42 (d, *J*=8.8, 2H, Ar-*H*), 5.81 (s, 2H, C*H*$_2$), 3.84 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 421.2 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{21}$N$_6$O$_4^+$ 421.1624, found 421.1618.

Methyl (E)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(3-nitrobenzyl)-1H-indole-6-carboxylate hydrobromide (5l). **5l** was obtained by reaction of **3l** as previously described for **5a** as an yellow solid (278 mg, 94%), m.p. 252.0–253.8 °C; IR (KBr): υ max cm^{-1} 3422 (NH), 3234 (NH), 2361 (CH$_2$), 1708 (C=O), 1661 (C=O), 1619 (C=C), 1531 (NO$_2$), 1462 (C=C), 1438 (C=C), 1351 (NO$_2$), 1286 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.01 (s, 1H, N*H*), 8.51 (d, *J*=8.4, 2H, Ar-*H*, NH), 8.40 (s, 1H, C*H*), 8.34 (s, 1H, Ar-*H*), 8.22 (s, 1H, Ar-*H*), 8.15–8.17 (m, 1H, Ar-*H*), 8.12 (s, 1H, Ar-*H*), 7.79 (dd, *J*$_1$=8.4, *J*$_2$=1.2, 1H, Ar-*H*), 7.67–7.65 (m, 2H, Ar-*H*), 5.79 (s, 2H, C*H*$_2$), 3.85 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 421.2 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{21}$N$_6$O$_4^+$ 421.1624, found 421.1618.

Methyl 1-benzoyl-3-formyl-1H-indole-5-carboxylate (6a). To a solution of **2a** (100 mg, 0.49 mmol) in dry DMF (2 ml) was added NaH (60%, 22 mg, 0.54 mmol) at 0 °C under argon and then stirred at room temperature for 1 h. The reaction solution was cooled to 0 °C, benzoyl chloride (0.07 ml, 0.54 mmol) was added and then stirred at room temperature overnight. The reaction solution was poured into H$_2$O (20 ml), and the precipitate was filtered. The crude product was purified by column chromatography on silica gel using EtOAc/PE (1/8, V/V) as elute to give **6a** as a white solid (107 mg, 71%), m.p. 172.7–173.3 °C; IR (KBr): υ max cm^{-1} 1721 (C=O), 1675 (C=O), 1611 (C=C), 1552 (C=C), 1452 (C=C), 1267 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.10 (s, 1H, C*H*O), 9.00 (d, *J*=1.2, 1H, Ar-*H*), 8.35 (d, *J*=8.8, 1H, Ar-*H*), 8.18 (dd, *J*$_1$=8.8, *J*$_2$=1.6, 1H, Ar-*H*), 8.03 (s, 1H, Ar-*H*), 7.81–7.79 (m, 2H, Ar-*H*), 7.71 (t, *J*=7.6, 1H, Ar-*H*), 7.61 (t, *J*=7.6, 2H, Ar-*H*), 3.98 (s, 3H, C*H*$_3$); ES-MS 308.1 (M+H)$^+$.

Methyl 1-benzoyl-3-formyl-1H-indole-6-carboxylate (6b). **6b** was obtained by reaction of **2b** as previously described for **6a** as a white solid (99 mg, 66%), m.p. 176.1–176.4 °C; IR (KBr): υ max cm^{-1} 1718 (C=O), 1672 (C=O), 1612 (C=C), 1547 (C=C), 1431 (C=C), 1283 (C-O-C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.09 (s, 1H, C*H*O), 9.00 (s, 1H, Ar-*H*), 8.38 (d, *J*=8.0, 1H, Ar-*H*), 8.15 (dd, *J*$_1$=8.4, *J*$_2$=1.2, 1H, Ar-*H*), 8.08 (s, 1H, Ar-*H*), 7.81–7.79 (m, 2H, Ar-*H*), 7.73 (t, *J*=7.6, 1H, Ar-*H*), 7.62 (t, *J*=7.6, 2H, Ar-*H*), 3.97 (s, 3H, C*H*$_3$); ES-MS 308.1 (M+H)$^+$.

Methyl (E)-1-benzoyl-3-((2-carbamimidoylhydrazono)methyl)-1H-indole-5-carboxylate hydrochloride (7a). **7a** was obtained by reaction of **6a** as previously described for **4a** as a white solid (245 mg, 94%), m.p. 204.6–206.0 °C; IR (KBr): υ max cm^{-1} 3446 (NH), 1685 (C=O), 1627 (C=O), 1551 (C=C), 1371 (C=C), 1298 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.92 (s, 1H, N*H*), 8.82 (s, 1H, Ar-*H*), 8.43 (s, 1H, Ar-*H*), 8.0 (d, *J*=8.8, 1H, Ar-*H*), 8.20 (s, 1H, C*H*), 8.07 (d, *J*=8.4, 1H, Ar-*H*), 7.85 (d, *J*=7.6, 2H, Ar-*H*), 7.63–7.77 (m, 6H, Ar-*H*, N*H*), 3.90 (s, 3H, C*H*$_3$); ES-MS 364.1 (M+H)$^+$; HRMS calcd for C$_{19}$H$_{18}$N$_5$O$_3^+$ 364.1410, found 364.1403.

Methyl (E)-1-benzoyl-3-((2-carbamimidoylhydrazono)methyl)-1H-indole-6-carboxylate hydrochloride (7b). **7b** was obtained by reaction of **6b** as previously described for **4a** as a white solid (239 mg, 92%), m.p. 229.0 °C (dec); IR (KBr): υ max cm^{-1} 3423 (NH), 2361 (CH$_2$), 1680 (C=O), 1632 (C=O), 1545 (C=C), 1432 (C=C), 1372 (C=C), 1242 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 11.93 (s, 1H, N*H*), 8.94 (d, *J*=1.2, 1H, Ar-*H*), 8.59 (d, *J*=8.4, 1H, Ar-*H*), 8.38 (s, 1H, Ar-*H*), 8.28 (s, 1H, C*H*), 7.99 (dd, *J*$_1$=8.4, *J*$_2$=1.6, 1H, Ar-*H*), 7.83–7.85 (m, 2H, Ar-*H*), 7.63–7.77 (m, 6H, Ar-*H*, N*H*), 3.92 (s, 3H, C*H*$_3$); ES-MS 364.1 (M+H)$^+$; HRMS calcd for C$_{19}$H$_{18}$N$_5$O$_3^+$ 364.1410, found 364.1404.

Methyl (E)-1-benzoyl-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1H-indole-5-carboxylate hydrobromide (8a). **8a** was obtained by reaction of **6a** as previously described for **5a** as a white solid (212 mg, 70%), m.p. 215.7–216.7 °C; IR (KBr): υ max cm^{-1} 3407 (NH), 3291(NH), 2950 (NH), 1699 (C=O), 1664 (C=O), 1615 (C=C), 1447 (C=C), 1369 (C=C), 1272 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.24 (s, 1H, N*H*), 8.86 (d, *J*=0.8, 1H, Ar-*H*), 8.42–8.39 (m, 2H, Ar-*H*, C*H*), 8.18 (s, 1H, Ar-*H*), 8.07 (dd, *J*$_1$=8.8, *J*$_2$=1.6, 1H, Ar-*H*), 7.85 (d, *J*=7.2, 2H, Ar-*H*), 7.76 (t, *J*=7.2, 1H, Ar-*H*), 7.65 (t, *J*=7.6, 2H, Ar-*H*), 3.91 (s, 3H, C*H*$_3$), 3.75 (s, 4H, C*H*$_2$); ES-MS 390.2 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{20}$N$_5$O$_3^+$ 390.1566, found 390.1562.

Methyl (E)-1-benzoyl-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1H-indole-6-carboxylate hydrobromide (8b). **8b** was obtained by reaction of **6b** as previously described for **5a** as a white solid (217 mg, 80%), m.p. 218.6–219.6 °C; IR (KBr): υ max cm^{-1} 3428 (NH), 3366(NH), 2360 (CH$_2$), 1712 (C=O), 1661 (C=O), 1613 (C=C), 1432 (C=C), 1380 (C=C), 1230 (C-O-C); ^{1}H-NMR (400 MHz, DMSO): δ 12.28 (s, 1H, N*H*), 8.95 (d, *J*=0.8, 1H, Ar-*H*), 8.61 (d, *J*=8.4, 1H, Ar-*H*), 8.37 (s, 1H, C*H*), 8.27 (s, 1H, Ar-*H*), 7.99 (dd, *J*$_1$=8.4, *J*$_2$=1.6, 1H, Ar-*H*), 7.86–7.84 (m, 2H, Ar-*H*), 7.76 (t, *J*=7.6, 1H, Ar-*H*), 7.65 (t, *J*=7.4, 2H, Ar-*H*), 3.92 (s, 3H, C*H*$_3$), 3.77 (s, 4H, C*H*$_2$); ES-MS 390.2 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{20}$N$_5$O$_3^+$ 390.1566, found 390.1599.

5-Bromo-1H-indole-3-carbaldehyde (10). To a solution of **9** (5.00 g, 25.5 mmol) in dry DMF (20 ml) was added POCl$_3$ (3.22 ml, 34.68 mol) at 0 °C under N$_2$ and then stirred at room temperature for 2 h. The reaction was quenched with H$_2$O (60 ml) at 0 °C and adjusted the pH to 7–8 with 2 M NaOH. The resulting solution was heated to 70 °C and stirred 30 min. After cooling to room temperature, the precipitate was filtered and washed with MeOH to give **10** as a white solid (5.19 g, 99%), m.p. 203.5–204.3 °C; IR (KBr): υ max cm^{-1} 3217 (NH), 2361 (C-N), 1645 (C=O), 1523 (C=C), 1437 (C=C), 1395 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 12.28 (s, 1H, N*H*), 9.93 (s, 1H, C*H*O), 8.35 (s, 1H, Ar-*H*), 8.21 (d, *J*=2.0, 1H, Ar-*H*), 7.49 (d, *J*=8.4, 1H, Ar-*H*), 7.43 (dd, *J*$_1$=8.8, *J*$_2$=2.0, 1H, Ar-*H*); ES-MS 225.1 (M+H)$^+$.

5-Bromo-1-(phenylsulfonyl)-1H-indole-3-carbaldehyde (11). To a solution of **10** (2 g, 8.88 mmol) in dry DMSO (15 ml) was added NaH (60%, 0.392 g, 9.77 mmol) and stirred at room temperature for 1 h. After the reaction solution

was cooled to 0 °C, benzenesulfonyl chloride (1.36 ml, 10.66 mmol) was added and then stirred at room temperature overnight. The reaction solution was quenched with sat. NH$_4$Cl and extracted with CH$_2$Cl$_2$ (200 ml x 3), and the combined organic layers were dried with Na$_2$SO$_4$, filtered and concentrated. The residue was recrystallized by acetone/hexane to give **11** as a white solid (2.67 g, 83%), m.p. 235.2–239.3 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1677 (C=O), 1539 (C=C), 1441 (C=C), 1376 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 10.06 (s, 1H, CHO), 8.96 (s, 1H, Ar-H), 8.22 (d, J=2.0, 1H, Ar-H), 8.14–8.12 (m, 2H, Ar-H), 7.95 (d, J=8.8, 1H, Ar-H), 7.81–7.77 (m, 1H, Ar-H), 7.69–7.65 (m, 2H, Ar-H), 7.62 (dd, J_1=8.8, J_2=2.0, 1H, Ar-H); ES-MS 365.2 (M+H)$^+$.

4-(3-Formyl-1-(phenylsulfonyl)-1H-indol-5-yl)phenyl acetate (12a). To a solution of **11** (0.5 g, 1.37 mmol) in a solution of tetrahydrofuran/H$_2$O (20 ml, 1/1, v/v) was added K$_2$CO$_3$ (0.28 g, 2.05 mmol), 4-(methoxycarbonyl)benzeneboronic acid (0.24 g, 1.64 mmol) and Pd(dbpf)Cl$_2$ (8.9 mg, 0.0137 mmol) and then stirred at 60 °C for 8 h. The reaction solution was extracted with EtOAc (100 ml × 3), and the combined organic layers were dried with Na$_2$SO$_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/PE (1/7, v/v) as elunt to give **12a** as a white solid (620 mg, 86%), m.p. 166.5–166.8 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1720 (C=O), 1678 (C=O), 1541 (C=C), 1455 (C=C), 1378 (C=C); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 10.13 (s, 1H, CHO), 8.52 (d, J=2.0, 1H, Ar-H), 8.27 (s, 1H, Ar-H), 8.11 (d, J=8.4, 2H, Ar-H), 8.04 (d, J=8.8, 1H, Ar-H), 8.02–7.99 (m, 2H, Ar-H), 7.70–7.63 (m, 4H, Ar-H), 7.57–7.53 (m, 2H, Ar-H), 3.94 (s, 3H, OCH_3); ES-MS 420.5 (M+H)$^+$.

Methyl 3-(3-formyl-1-(phenylsulfonyl)-1H-indol-5-yl)benzoate (12b). **12b** was obtained by reaction of **11** as previously described for **12a** as a white solid (328 mg, 53%), m.p. 186.8–187.1 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1714 (C=O), 1681 (C=O), 1542 (C=C), 1453 (C=C), 1379 (C=C); 1H NMR (400 MHz, CDCl$_3$): δ 10.13 (s, 1H, CHO), 8.50 (d, J=1.6, 1H, Ar-H), 8.28–8.27 (m, 2H, Ar-H), 8.05–7.99 (m, 4H, Ar-H), 7.83–7.80 (m, 1H, Ar-H), 7.69 (dd, J_1=8.8, J_2=2.0, 1H, Ar-H), 7.66–7.63 (m, 1H, Ar-H), 7.57–7.50 (m, 3H, Ar-H), 3.95 (s, 3H, OCH_3); ES-MS 420.5 (M+H)$^+$.

1-(Phenylsulfonyl)-5-(4-(trifluoromethoxy)phenyl)-1H-indole-3-carbaldehyde (12c). **12c** was obtained by reaction of **11** as previously described for **12a** as a white solid (360 mg, 89%), m.p. 180.4–181.3 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1678 (C=O), 1542 (C=C), 1457 (C=C), 1380 (C=C); 1H NMR (400 MHz, DMSO): δ 10.12 (s, 1H, CHO), 8.98 (s, 1H, Ar-H), 8.34 (d, J=1.6, 1H, Ar-H), 8.18–8.16 (m, 2H, Ar-H), 8.07 (d, J=8.8, 1H, Ar-H), 7.80–7.75 (m, 2H, Ar-H), 7.69–7.65 (m, 3H, Ar-H), 7.61–7.57(m, 2H, Ar-H), 7. 37 (d, J=8.4, 1H, Ar-H); ES-MS 446.4 (M+H)$^+$.

1-(Phenylsulfonyl)-5-(3-(trifluoromethoxy)phenyl)-1H-indole-3-carbaldehyde (12d). **12d** was obtained by reaction of **11** as previously described for **12a** as a white solid (300 mg, 74%), m.p. 150.4–151.0 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1677 (C=O), 1541 (C=C), 1450 (C=C), 1379 (C=C); 1H NMR (400 MHz, DMSO): δ 10.12 (s, 1H, CHO), 8.98 (s, 1H, Ar-H), 8.33 (d, J=1.6, 1H, Ar-H), 8.18–8.16 (m, 2H, Ar-H), 8.07 (d, J=8.8, 1H, Ar-H), 7.78–7.74 (m, 4H, Ar-H), 7.70–7.66 (m, 2H, Ar-H), 7.45 (d, J=8.0, 2H, Ar-H); ES-MS 446.4 (M+H)$^+$.

1-(Phenylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-1H-indole-3-carbaldehyde (12e). **12e** was obtained by reaction of **11** as previously described for **12a** as a white solid (85 mg, 67%), m.p. 216.5–216.8 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1677 (C=O), 1542 (C=C), 1451 (C=C), 1380 (C=C); 1H NMR (400 MHz, CDCl$_3$): δ 10.13 (s, 1H, CHO), 8.50 (d, J=1.6, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.05 (d, J=8.8, 1H, Ar-H), 8.03–8.00 (m, 2H, Ar-H), 7.74–7.68 (m, 4H, Ar-H), 7.68–7.63(m, 2H, Ar-H), 7.58–7.53 (m, 2H, Ar-H); ES-MS 430.4 (M+H)$^+$.

5-(3-Chloro-4-fluorophenyl)-1-(phenylsulfonyl)-1H-indole-3-carbaldehyde (12f). **12f** was obtained by reaction of **11** as previously described for **12a** as a white solid (510 mg, 83%), m.p. 171.1–171.6 °C; IR (KBr): υ max cm^{-1} 2361 (C-N), 1677 (C=O), 1542 (C=C), 1453 (C=C), 1379 (C=C); 1H NMR (400 MHz, CDCl$_3$): δ 10.12 (s, 1H, CHO), 8.41 (d, J=1.6, 1H, Ar-H), 8.27

(s, 1H, Ar-H), 8.03–7.99 (m, 3H, Ar-H), 7.67–7.62 (m, 2H, Ar-H), 7.58–7.53 (m, 3H, Ar-H), 7.46 (ddd, J_1=8.6, J_2=4.4, J_3=2.4, 1H, Ar-H), 7.21 (t, J=8.6, 1H, Ar-H); ES-MS 414.9 (M+H)$^+$.

Methyl (E)-4-(3-((2-carbamimidoylhydrazono)methyl)-1-(phenylsulfonyl)-1H-indol-5-yl)benzoate hydrochloride (13a). To a solution of **12a** (100 mg, 0.22 mmol) in dry MeOH was added aminoguanidine hydrochloride (24 mg, 0.22 mmol), and then the reaction solution was adjusted pH to 3–4 and stirred at room temperature for 1.5 h. After completion of the reaction, the solvent was removed under reduced pressure and then the residue was tutrited in EtOAc and filtered to give **13a** as a white solid (100 mg, 89%), m.p. 229.4–232.4 °C; IR (KBr): υ max cm^{-1} 3736 (NH), 3420 (NH), 3276 (NH), 2361 (C-N), 1677 (C=O), 1540 (C=C), 1452 (C=C), 1379 (C=C); ^{1}H NMR (400 MHz, DMSO): δ 11.90 (s, 1H, NH), 8.56 (s, 1H, Ar-H), 8.49 (d, J=1.5, 1H, Ar-H), 8.42 (s, 1H, CH), 8.02–8.09 (m, 5H, Ar-H), 7.89 (d, J=8.5, 2H, Ar-H), 7.61–7.82 (m, 7H, Ar-H and NH), 3.88 (s, 3H, CH_3); ES-MS 476.1 (M+H)$^+$; HRMS calcd for C$_{24}$H$_{22}$N$_5$O$_4$S$^+$ 476.1393, found 476.1383.

Methyl (E)-3-(3-((2-carbamimidoylhydrazono)methyl)-1-(phenylsulfonyl)-1H-indol-5-yl)benzoate hydrochloride (13b). **13b** was obtained by reaction of **12b** as previously described for **13a** as a white solid (56 mg, 73%), m.p. 206.6–208.6 °C; IR (KBr): υ max cm^{-1} 3735 (NH), 3396 (NH), 3275 (NH), 2361 (C-N), 1673 (C=O), 1539 (C=C), 1453 (C=C), 1377 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 12.08 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.47 (s, 1H, Ar-H), 8.44 (s, 1H, CH), 8.23 (s, 1H, Ar-H),7.99–8.10 (m, 4H, Ar-H), 7.95 (d, J=7.7, 1H, Ar-H), 7.67–7.87 (m, 5H, Ar-H and NH), 7.60–7.66 (m, 3H, Ar-H), 3.89 (s, 3H, CH_3); ES-MS 476.1 (M+H)$^+$; HRMS calcd for C$_{24}$H$_{22}$N$_5$O$_4$S$^+$ 476.1393, found 476.1388.

(E)-2-((1-(Phenylsulfonyl)-5-(4-(trifluoromethoxy)phenyl)-1H-indol-3-yl)methylene)hydrazine-1-carboximidamide hydrochloride (13c). **13c** was obtained by reaction of **12c** as previously described for **13a** as a white solid (134 mg, 83%), m.p. 268.9–269.4 °C; IR (KBr): υ max cm^{-1} 3735 (NH), 3488 (NH), 3255 (NH), 2361 (C-N), 1674 (C=O), 1540 (C=C), 1455 (C=C), 1380 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 11.93 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.43 (d, J=1.5, 1H, Ar-H), 8.41 (s, 1H, CH), 8.02–8.10 (m, 3H, Ar-H), 7.81–7.87 (m, 2H, Ar-H), 7.60–7.80 (m, 7H, Ar-H and NH), 7.44 (d, J=8.0, 2H, Ar-H); ES-MS 502.1 (M+H)$^+$; HRMS calcd for C$_{23}$H$_{19}$F$_3$N$_5$O$_3$S$^+$ 502.1161, found 502.1152.

(E)-2-((1-(Phenylsulfonyl)-5-(3-(trifluoromethoxy)phenyl)-1H-indol-3-yl)methylene)hydrazine-1-carboximidamide hydrochloride (13d). **13d** was obtained by reaction of **12d** as previously described for **13a** as a white solid (170 mg, 65%), m.p. 218.6–220.8 °C; IR (KBr): υ max cm^{-1} 3468 (NH), 3255 (NH), 3128 (NH), 2361 (C-N), 1674 (C=O), 1538 (C=C), 1452 (C=C), 1380 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 12.08 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.45 (d, J=1.5, 1H, Ar-H), 8.43 (s, 1H, CH), 8.05–8.07 (m, 3H, Ar-H), 7.70–7.81 (m, 6H, Ar-H and NH), 7.56–7.70 (m, 4H, Ar-H), 7.36 (d, J=8.2, 1H, Ar-H); ES-MS 502.1 (M+H)$^+$; HRMS calcd for C$_{23}$H$_{19}$F$_3$N$_5$O$_3$S$^+$ 502.1161, found 502.1156.

(E)-2-((1-(Phenylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-1H-indol-3-yl)methylene)hydrazine-1-carboximidamide hydrochloride (13e). **13e** was obtained by reaction of **12e** as previously described for **13a** as a white solid (55 mg, 89%), m.p. 249.0–249.3 °C; IR (KBr): υ max cm^{-1} 3735 (NH), 3396 (NH), 3216 (NH), 2361 (C-N), 1673 (C=O), 1539 (C=C), 1454 (C=C), 1379 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 12.05 (s, 1H, NH), 8.57 (s, 1H, Ar-H), 8.49 (d, J=1.5, 1H, Ar-H), 8.43 (s, 1H, CH), 8.02–8.13 (m, 3H, Ar-H), 7.95 (d, J=8.1, 2H, Ar-H), 7.70–7.85 (m, 7H, Ar-H and NH), 7.64 (t, J=7.8, 2H, Ar-H); ES-MS 486.1 (M+H)$^+$; HRMS calcd for C$_{21}$H$_{19}$F3N$_5$O$_2$S$^+$ 486.1212, found 486.1201.

(E)-2-((5-(3-Chloro-4-fluorophenyl)-1-(phenylsulfonyl)-1H-indol-3-yl)methylene)hydrazine-1-carboximidamide hydrochloride (13f). **13f** was obtained by reaction of **12f** as previously described for **13a** as a white solid (110 mg, 98%), m.p. 234.2–237.3 °C; IR (KBr): υ max cm^{-1} 3736 (NH), 3462 (NH), 3128 (NH), 2361 (C-N), 1672 (C=O), 1539 (C=C), 1455 (C=C), 1379 (C=C); ^{1}H-NMR (400 MHz, DMSO): δ 11.91 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.41

(s, 1H, Ar-*H*), 8.40 (s, 1H, C*H*), 8.02–8.07 (m, 3H, Ar-*H*), 7.95 (dd, *J* = 7.1, 1H, Ar-*H*), 7.70–7.77 (m, 5H, Ar-*H* and N*H*), 7.63–7.66 (m, 3H, Ar-*H*), 7.50 (t, *J* = 9.1, 1H, Ar-*H*); ES-MS 470.1 (M+H)$^+$; HRMS calcd for $C_{22}H_{18}ClFN_5O_2S^+$ 470.0854, found 470.0849.

Methyl (E)-4-(3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(phenyl-sulfonyl)-1H-indol-5-yl)benzoate dihydrobromide (14a). To a solution of **12a** (50 mg, 0.11 mmol) in dry MeOH (2 ml) was added 2-hydrazino-2-imidazoline hydrobromide (20 mg, 0.11 mmol), and then the reaction solution was stirred at 75 °C. After the completion of the reaction, the solvent was removed under reduced pressure and then the residue was tutrited in EtOAc and filtered to give **14a** as a white solid (48 mg, 88%), m.p. 228.9–230.3 °C; IR (KBr): *υ* max cm^{-1} 3735 (NH), 3439 (NH), 3174 (NH), 2361 (C-N), 1661 (C=O), 1541 (C=C), 1454 (C=C), 1376 (C=C); ^{1}H-NMR (400 MHz, DMSO): *δ* 12.39 (s, 1H, N*H*), 8.55 (s, 1H, Ar-*H*), 8.51 (d, *J* = 1.6, 1H, Ar-*H*), 8.42 (s, 1H, C*H*), 8.11–8.01 (m, 5H, Ar-*H*), 7.88 (d, *J* = 8.5, 2H, Ar-*H*), 7.79 (dd, *J*₁ = 8.6, *J*₂ = 1.8, 1H, Ar-*H*), 7.75 (d, *J* = 7.6, 1H, Ar-*H*), 7.65 (t, *J* = 7.8, 2H, Ar-*H*), 3.88 (s, 3H, C*H₃*), 3.72 (s, 4H, C*H₂*); ES-MS 502.2 (M+H)$^+$; HRMS calcd for $C_{26}H_{24}N_5O_4S^+$ 502.1549, found 502.1538.

Methyl (E)-3-(3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(phenyl-sulfonyl)-1H-indol-5-yl)benzoate hydrobromide (14b). **14b** was obtained by reaction of **12b** as previously described for **14a** as a white solid (187 mg, 97%), m.p. 237.4–240.4 °C; IR (KBr): *υ* max cm^{-1} 3736 (NH), 3459 (NH), 3123 (NH), 2361 (C-N), 1665 (C=O), 1541 (C=C), 1452 (C=C), 1378 (C=C); ^{1}H-NMR (400 MHz, DMSO): *δ* 12.31 (s, 1H, N*H*), 8.56 (s, 1H, Ar-*H*), 8.49 (s, 1H, Ar-*H*), 8.43 (s, 1H, C*H*), 8.25 (s, 1H, Ar-*H*), 8.07–8.09 (m, 3H, Ar-*H*), 8.01 (d, *J* = 8, 1H, Ar-*H*), 7.96 (d, *J* = 7.6, 1H, Ar-*H*), 7.76 (t, *J* = 7.6, 2H, Ar-*H*), 7.61–7.67 (m, 3H, Ar-*H*), 3.89 (s, 3H, OC*H₃*), 3.74 (s, 4H, C*H₂*); ES-MS 502.2 (M+H)$^+$; HRMS calcd for $C_{26}H_{24}N_5O_4S^+$ 502.1549, found 502.1539.

(E)-3-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(phenylsulfonyl)-5-(4-(trifluoromethoxy)phenyl)-1H-indole hydrobromide (14c). **14c** was obtained by reaction of **12c** as previously described for **14a** as a white solid (100 mg, 60%), m.p. 254.4–254.5 °C; IR (KBr): *υ* max cm^{-1} 3732 (NH), 3445 (NH), 3179 (NH), 2361 (C-N), 1667 (C=O), 1541 (C=C), 1455 (C=C), 1378 (C=C); ^{1}H NMR (400 MHz, DMSO): *δ* 12.28 (s, 1H, N*H*), 8.55 (s, 1H, Ar-*H*), 8.44(s, 1H, Ar-*H*), 8.42 (s, 1H, C*H*), 8.06–8.09 (m, 3H, Ar-*H*), 7.83 (d, *J* = 8.6, 2H, Ar-*H*), 7.62–7.77 (m, 4H, Ar-*H*), 7.46 (d, *J* = 8.1, 2H, Ar-*H*), 3.74 (s, 4H, C*H₂* C*H₂*); ES-MS 528.1 (M+H)$^+$; HRMS calcd for $C_{25}H_{21}F_3N_5O_3S^+$ 528.1317, found 528.1306.

(E)-3-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(phenylsulfonyl)-5-(3-(trifluoromethoxy)phenyl)-1H-indole hydrobromide (14d). **14d** was obtained by reaction of **12d** as previously described for **14a** as a white solid (183 mg, 53%), m.p. 243.2 °C; IR (KBr): *υ* max cm^{-1} 3736 (NH), 3435 (NH), 3174 (NH), 2361 (C-N), 1666 (C=O), 1541 (C=C), 1452 (C=C), 1376 (C=C); ^{1}H-NMR (400 MHz, DMSO): *δ* 12.33 (s, 1H, N*H*), 8.57 (s, 1H, Ar-*H*), 8.47 (d, *J* = 1.2, 1H, Ar-*H*), 8.42 (s, 1H, C*H*), 8.06–8.09 (m, 3H, Ar-*H*), 7.74–7.78 (m, 4H, Ar-*H*), 7.58–7.68 (m, 3H, Ar-*H*), 7.37 (d, *J* = 8.4, 1H, Ar-*H*), 3.73 (s, 4H, C*H₂*); ES-MS 528.1 (M+H)$^+$; HRMS calcd for $C_{25}H_{21}F_3N_5O_3S^+$ 528.1317, found 528.1305.

(E)-3-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)methyl)-1-(phenylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-1H-indole hydrobromide (14e). **14e** was obtained by reaction of **12e** as previously described for **14a** as a white solid (204 mg, 90%), m.p. 249.2–249.7 °C; IR (KBr): *υ* max cm^{-1} 3735 (NH), 3449 (NH), 3178 (NH), 2361 (C-N), 1666 (C=O), 1542 (C=C), 1451 (C=C), 1379 (C=C); ^{1}H-NMR (400 MHz, DMSO): *δ* 12.31 (s, 1H, N*H*), 8.58 (s, 1H, Ar-*H*), 8.49 (d, *J* = 1.2, 1H, Ar-*H*), 8.43 (s, 1H, C*H*), 8.08–8.10 (m, 3H, Ar-*H*), 7.93 (d, *J* = 8.0, 2H, Ar-*H*), 7.82 (d, *J* = 8.4, 2H, Ar-*H*), 7.77 (m, 2H, Ar-*H*), 7.66 (t, *J* = 7.8, 2H, Ar-*H*), 3.74 (s, 4H, C*H₂*); ES-MS 512.1 (M+H)$^+$; HRMS calcd for $C_{23}H_{18}F_3N_5O_2S^+$ 512.1368, found 512.1359.

(E)-5-(3-Chloro-4-fluorophenyl)-3-((2-(4,5-dihydro-1H-imidazol-2-yl)hydra-zono)methyl)-1-(phenylsulfonyl)-1H-indole hydrobromide (14f). **14f** was obtained by reaction of **12f** as previously described for **14a** as a white solid

(205 mg, 91%), m.p. 256.3–256.5 °C; IR (KBr): *υ* max cm^{-1} 3735 (NH), 3281 (NH), 2925 (NH), 2361 (C-N), 1664 (C=O), 1546 (C=C), 1457 (C=C), 1373 (C=C); ^{1}H-NMR (400 MHz, DMSO): *δ* 12.31 (s, 1H, N*H*), 8.56 (s, 1H, Ar-*H*), 8.42 (d, *J* = 1.6, 1H, Ar-*H*), 8.41 (s, 1H, C*H*), 8.03–8.08 (m, 3H, Ar-*H*), 7.94 (dd, *J*₁ = 7.0, *J*₂ = 2.2, 1H, Ar-*H*), 7.71–7.77 (m, 3H, Ar-*H*), 7.65 (t, J = 7.8, 2H), 7.52 (t, *J* = 8.8, 1H, Ar-*H*), 3.74 (s, 4H, C*H₂*); ES-MS 496.1 (M+H)$^+$; HRMS calcd for $C_{24}H_{20}ClFN_5O_2S^+$ 496.1010, found 496.1001.

Biology

Bacterial strains. S. aureus strains ATCC 29213, ATCC 25923 and ATCC-S-6007 were purchased from the American Type Culture Collection (Manassas, VA, USA). When required for testing, these strains were retrieved from storage at − 80 °C and subcultured on Luria-Bertani agar for viability and purity.

The clinical strains used for MIC determinations were isolated from hospital in-patients as part of their routine investigations for the diagnosis of various infections. They were identified by standard microbiological methods and tested for antibiotic susceptibilities by the Kirby Bauer method[23,24] as well as the semiautomated BD Phoenix system (Becton Dickinson, Franklin Lakes, NJ, USA).

As these strains were archived routine isolates made anonymous by the removal of all labels that could be traced back to the patient source, ethical approval for this study was exempted.

Determination of MIC and MBC. All bacterial strains were initially screened with 100 μg ml^{-1} of each compound in Trypticase Soy Broth with 1% DMSO. The compounds showing bactericidal activity at this concentration were then further tested in two-fold dilutions from 50 to 0.39 μg ml^{-1}. The inoculum used was an overnight culture diluted 1:1000 for Gram-negative bacteria and 1:100 for Gram-positive bacteria, to make approximately 10^5 cfu ml^{-1}.

Each well in a sterile 96-well microtiter plate was filled with 150 μl of compound in Trypticase Soy Broth or Middlebrook broth with 1% DMSO. Each compound concentration was pipetted into duplicate wells. The wells were then inoculated with 10 μl of culture, covered with a plate cover and incubated at 36 °C for 48 h. OD readings (at 600 nm) were taken at 0, 24 and 48 h. After 48 h of incubation, each well was subcultured onto a blood agar plate to demonstrate bacterial growth. Viability and 1% DMSO controls were used in every plate.

For the determination of MIC, OD readings for test wells were compared with those of the control wells; an arrested or at least 50% retarded increase in OD reading was taken to indicate bacterial growth inhibition. MBC was defined as the lowest compound concentration to cause complete absence of bacterial growth on subculture.

For Mycobacterium H37Ra, inoculations were made in Middlebrook 7H9 broth and incubated up to 4 weeks. Subcultures were made onto Middlebrook 7H10 agar at 2 weeks and again at 4 weeks of incubation.

Molecular docking

In total, 40 compounds were designed and synthesized, and molecular docking was performed by using GOLD (v 5.2.2 Genetic Optimization for Ligand Docking, Cambridge, UK). Each ligand was docked 10 times, starting each time from a different random population of ligand orientations and using the default automatic genetic algorithm parameter settings. The Moenomycin A in the crystal structure was used as the reference to indicate the binding site for the molecular docking. All torsion angles in each compound were allowed to rotate freely and the results of the different docking runs were ranked using Gold Score.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Yang, Z. et al. Proportions of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in patients with surgical site infections in mainland China: a systematic review and meta-analysis. PLoS ONE 10, e0116079 (2015).

2 Kali, A. Antibiotics and bioactive natural products in treatment of methicilln resistant Staphylococcus aureus: a brief review. Pharmacogn. Rev. 9, 29–34 (2015).

3 Kumar, A., Verma, N., Agrawal, D. & Sharma, B. S. Endoscopic lavage for antibiotic unresponsive severe Acinetobacter baumanii ventriculitis: an unexplored treatment option. Acta Neurochir. (Wien) 157, 1225–1227 (2015).

4 Ahmed, M. U. et al. Sensitive, resistant and multi-drug resistant Acinetobacter baumanii at Saudi Arabia hospital eastern region. Pak. J. Pharm. Sci. 28, 825–832 (2015).

5 Lee, C. Y., Tsai, H. C., Lee, S. S. & Chen, Y. S. Concomitant emphysematous prostatic and periurethral abscesses due to Klebsiella pneumoniae: a case report and review of the literature. Southeast Asian J. Trop. Med. Public Health 45, 1099–1106 (2014).

6 Huang, C. H. et al. Risk factors for in-hospital mortality in patients with type 2 diabetes complicated by community-acquired Klebsiella pneumoniae bacteremia. J. Formos Med. Assoc. 114, 916–922 (2015).

7 Yaita, K., Komatsu, M., Oshiro, Y. & Yamaguchi, Y. Postoperative meningitis and epidural abscess due to extended-spectrum β-lactamase-producing Klebsiella pneumoniae: a case report and a review of the literature. Intern. Med. 51, 2645–2648 (2012).

8 Wright, G. D. A new target for antibiotic development. Science 315, 1373–1374 (2007).

9 Halliday, J., McKeveney, D., Muldoon, C., Rajaratnam, P. & Meutermans, W. Targeting the forgotten transglycosylases. Biochem. Pharmacol. 71, 957–967 (2006).

10 Bohdan, O. B. & Walker, S. Moenomycin family antibiotics: chemical synthesis, biosynthesis, biological activity. Nat. Prod. Rep. 27, 1594–1617 (2010).

11 Xu, H., Wang, Q. & Yang, W. B. Antifungal activities of some indole derivatives. Z. Naturforsch C. 65, 437–439 (2010).

12 Zhang, M. Z. et al. Synthesis and antifungal activity of 3-(1,3,4-oxadiazol-5-yl)-indoles and 3-(1,3,4-oxadiazol-5-yl)methyl-indoles. Eur. J. Med. Chem. 63, 22–32 (2013).

13 Zhao, F., Liu, N., Zhan, P., Jiang, X. & Liu, X. Discovery of HCV NS5B thumb site I inhibitors: core-refining from benzimidazole to indole scaffold. Eur. J. Med. Chem. 94, 218–228 (2015).

14 Giraud, F. et al. Design, synthesis and evaluation of 3-(imidazol-1-ylmethyl)indoles as antileishmanial agents. Part II. J. Enzyme Inhib. Med. Chem. 24, 1067–1075 (2009).

15 Andrew, L. L. et al. Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. Science 315, 1402–1405 (2007).

16 Penthala, N. R., Yerramreddy, T. R. & Crooks, P. A. Synthesis and in vitro screening of novel N-benzyl aplysinopsin analogs as potential anticancer agents. Bioorg. Med. Chem. Lett. 21, 1411–1413 (2011).

17 Andreani, A. et al. Imidazo[2,1-b]thiazole guanidinylhydrazones as RSK2 inhibitors. Eur. J. Med. Chem. 46, 4311–4323 (2011).

18 Mohammad, H. et al. Synthesis and antibacterial evaluation of a novel series of synthetic phenylthiazole compounds against methicillin-resistant Staphylococcus aureus (MRSA). Eur. J. Med. Chem. 94, 306–316 (2015).

19 Wang, Y. et al. Structure-based design, synthesis, and biological evaluation of isatin derivatives as potential glycosyltransferase inhibitors. Chem. Biol. Drug Des. 84, 685–696 (2014).

20 Xu, H. & Fan, L. Antifungal agents. Synthesis and antifungal activities of novel indole [1,2-c]-1,2,4-benzotriazine derivatives against phytopathogenic fungi in vitro. Eur. J. Med. Chem. 46, 364–369 (2011).

21 Moseley, J. D., Murray, P. M., Turp, E. R., Tyler, S. N. G. & Burn, R. T. A mild robust generic protocol for the Suzuki reaction using an air stable catalyst. Tetrahedron 68, 6010–6017 (2012).

22 Al-Marzooq, F., Ngeow, Y. F. & Tay, S. T. Emergence of Klebsiella pneumoniae producing dual carbapenemases (NDM-1 and OXA-232) and 16S rRNA methylase (armA) isolated from a Malaysian patient returning from India. Int. J. Antimicrob. Agents 45, 445–446 (2015).

23 Clinical and Laboratory Standards Institute. Approved Standard M2-A10. Performance Standards for Antimicrobial Disk Susceptibility Tests 10th edn. (CLSI, Wayne, PA, USA, 2009).

24 Clinical and Laboratory Standards Institute. M100-S21 (M2). Disk Diffusion Supplemental Tables (CLSI, Wayne, PA, USA, 2011).