Bioorganic Chemistry 99 (2020) 103807

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Discovery of novel multi-substituted benzo-indole pyrazole schiff base derivatives with antibacterial activity targeting DNA gyrase

Hao Liu[a], Zhi-Wen Chu[a], Dong-Guo Xia[a], Hai-Qun Cao[b], Xian-Hai Lv[a,*]

[a] School of Science, Anhui Agricultural University, People's Republic of China
[b] School of Plant Protection, Anhui Agricultural University, People's Republic of China

ARTICLE INFO

Keywords:
DNA gyrase inhibitor
Indole
Pyrazole
Schiff base
Drug-resistant bacteria
Antibacterial activity

ABSTRACT

The design and synthesis of novel multi-substituted benzo-indole pyrazole Schiff base derivatives of potent DNA gyrase inhibitory activity were the main aims of this study. All the novel synthesized compounds were examined for their antibacterial activities against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella*. In addition, we selected 20 compounds for the *in vitro* antibacterial activities assay of 6 drug-resistant bacteria strains. The result revealed compound **8I-w** exhibited excellent antibacterial activity against 4 drug-resistant *E. coli* bacteria strains with IC_{50} values of 7.0, 17.0, 13.5, and 1.0 μM, respectively. *In vitro* enzyme inhibitory assay showed that compound **8I-w** displayed potent inhibition against DNA gyrase with IC_{50} values of 0.10 μM. The molecular docking model indicated that compounds **8I-w** can bind well to the DNA gyrase by interacting with various amino acid residues. This study demonstrated that the compound **8I-w** can act as the most potent DNA gyrase inhibitor in the reported series of compounds and provide valuable information for the commercial DNA gyrase inhibiting bactericides.

1. Introduction

For more than 60 years, antibacterial drugs have been regarded as the panacea to cure infections, which have been extensively misused in both humans and food-producing animals in ways that favor the selection and spread of resistant bacteria [1,2]. As a subclass of the topoisomerase family, Bacterial DNA gyrase is an attractive and well-established target for the development of antibacterial agents [3]. Its biological function is to control the topological state of DNA molecules, consists of two catalytic subunits: GyrA is responsible for DNA breakage and reunion, while the subunit GyrB contains the ATP-binding site [4–7]. Currently the most mainstream DNA gyrase inhibitors on the market are aminocoumarins and quinolones [8]. For example, novobiocin (aminocoumarins) inhibits the ATPase activity of DNA gyrase by blocking the binding of ATP to subunit GyrB, whereas ciprofloxacin (quinolones) exhibits the inhibitory effect by inhibiting GyrA [7]. With the widespread use of these drugs, the resistance of bacteria to such drugs is spreading. Therefore, it is very important to find a new type of DNA gyrase inhibitor with a new scaffold to alleviate the problem of bacterial resistance.

Indoles have been the subject of intense investigation in both academia and industry owing to its wide range of biological activities, such as anti-inflammatory [9,10], anti-viral [11], anti-hypoglycemic [12], anti-depressant [13], and anti-cancer activities [14,15]. A considerable number of natural products and currently available drugs carry an indole moiety. Thus, it is easy to understand why more and more researchers focus on the indole group to develop new inhibitors of DNA gyrase (Fig. 1)[16–21]. Among azole compounds, pyrazole plays an obvious and important role in combating bacterial infections owing to its unique structural features, which is more conducive to the interaction of DNA, enzymes, and receptors. These advantages can also be verified from our previous research [22].

In recent years, Schiff base structure has gained grow attention from the researchers due to their ease of synthesis, versatility, and large spectra of activities, such as antibacterial, [23] anti-inflammatory [24], anti-tumor [25], and antioxidant [26] activities. Besides, the imine bond in Schiff base provides the opportunities to bind with different nucleophiles and electrophiles, inhibiting enzymes or DNA replication [27]. Herein, we aim to find a novel class of bacteriostatic scaffold targeting DNA gyrase via binding the indole moiety to the pyrazole moiety through an imine bond. 48 novel multi-substituted benzo-indole pyrazole Schiff base derivatives targeting DNA gyrase designed and synthesized. All compounds were used to examined antibacterial activity against four bacteria strains (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella*). Moreover, computer-assisted molecular docking analysis and Lipinski's parameters were

* Corresponding author at: School of Science, Anhui Agricultural University, 230036 Hefei, People's Republic of China.
E-mail address: lvxianhai@ahau.edu.cn (X.-H. Lv).

https://doi.org/10.1016/j.bioorg.2020.103807
Received 4 February 2020; Received in revised form 25 March 2020; Accepted 28 March 2020
Available online 02 April 2020

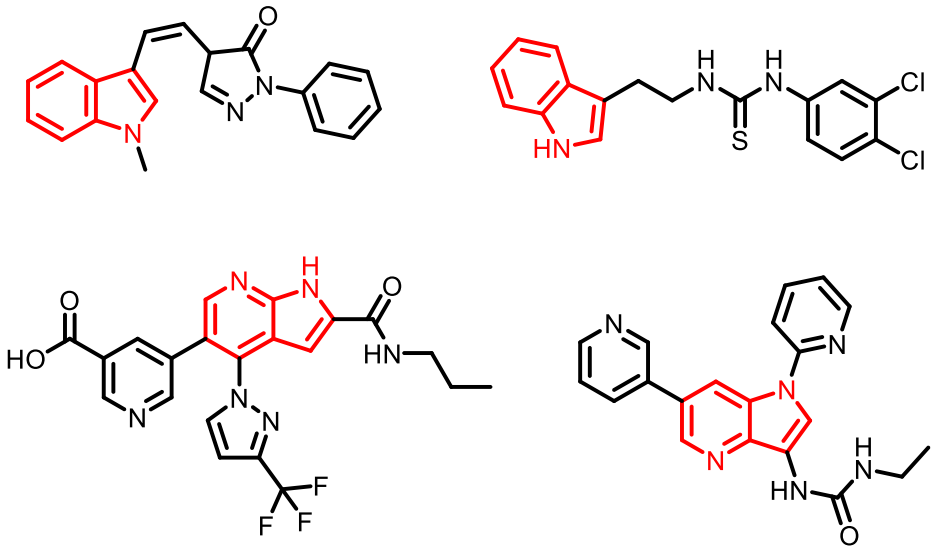

Fig. 1. Some previously reported indole-containing potential DNA gyrase inhibitors.

employed and measured on all derivatives to fully discussed structure–activity relationships.

2. Results and discussion

2.1. Molecular design

In the beginning, we synthesized 8 pyrazole intermediates **7** (**Table S2**). After measuring its MIC values against four bacteria, the results were not ideal. Therefore, we decided to combine the indole structure with the intermediate **7** through the Schiff base reaction to obtain the target scaffold. The data in Table 2 indicated that the introduction of the indole structure greatly enhances the antibacterial activity of the compounds, which demonstrated the rationality of our design strategy. The target compounds were designed based on Lipinski parameters (i.e., log*P*, molecular weight, number of hydrogen bond acceptors, and number of hydrogen bond donors) with computer aids. The aim of computational analysis is to filter the compounds considered unsuitable for screening purposes. "Rule of 5″ properties are set of simple molecular descriptors used by Lipinski in formulating his "Rule of 5″. These parameters (**Table S3**) showed that the compounds we designed and synthesized almost meet the requirements of the rule, which have no problem with bioavailability and making them play an important role in drug development.

2.2. Chemistry

The key reaction in the synthesis routes of target compounds was the Schiff base reaction. The synthetic routes of the target compounds were outlined in Fig. 2. The IDs and substituent details of all compounds were listed in the Table 1. Intermediate **3** was easily prepared by the reaction of *P*-substituted acetophenone **(1)** and phenyl-hydrazine **(2)** with CH_3COOH and CH_4O_3S. Intermediate **4** was obtained by using DMF and phosphoryl oxychloride with the intermediate **3**. As shown in Fig. 2**,** the intermediates **7** were synthesized by stirring the mixture of *P*-substituted phenyl-hydrazine and 2-(ethoxymethyl) malononitrile/2-cyano-3-ethoxypropanoic acid in the ethanol at 80 °C. The crude product was purified by recrystallization (ethanol/H_2O). The target compounds were prepared by mixing intermediate **4** and intermediate **7** with a catalytic amount of hydrochloric acid refluxed in ethanol. The chemical structures of all synthesized compounds were determined by ^{1}H NMR, ^{13}C NMR, and MS. ^{1}H NMR spectra and ^{13}C NMR spectra data for the target compounds are listed in Supporting Information. The check-cif files for compounds **8I-a** are listed in Supporting Information, crystal structures and data are shown in Fig. 3 and **Table S4**. By placing the single crystal figure horizontally, it can be seen that there is one dominant group on each side of the imine bond (**Fig. S1**), so this single crystal is in the *E* conformation. Crystallographic data (excluding structure factors) for the structure were deposited with the Cambridge Crystallographic Data Center (CCDC) as No. CCDC-1946882 (**8I-a**).

2.3. Antibacterial activity and SAR discussion

The IC_{50} values of the *in vitro* antibacterial activity screening of all compounds are presented in Table 2. When the IC_{50} value of the compound is greater than 100 μM, the specific value is not recorded. For two gram-positive bacteria, these compounds have not shown a strong and effective inhibitory effect, especially against *L. monocytogenes*. However, two of these compounds (i.e., **8I-g** and **8I-s**) showed superior inhibitory effects on *S. aureus* than four control drugs with IC_{50} values of 11.5 and 10.9 μM respectively, which indicated that the inhibitory effects of these compounds are remarkable. For two gram-negative bacteria, most compounds showed strong and effective inhibitory effects. Especially, among these derivates, seventeen compounds show better inhibitory effects on *E. coli* than control drugs with IC_{50} values less than 10 μM. For *Salmonella*, although most of the compounds showed bacteriostatic activity with IC_{50} values less than 100 μM, they are not good enough compared with the control drugs.

After integrating the antibacterial activity data in **Tables S1, S2** and 2, it can be observed that the target compounds have the best antibacterial inhibitory effect on *E. coli* in four typical bacteria strains. For *S. aureus*, when the R^2 position was substituted with chlorine, and the R^3 position was substituted with a cyano group, the antibacterial activities increased. For *L. monocytogenes*, almost all compounds exhibit poor effect, so it is beyond the discussion of their structure–activity relationships. For *E. coli*, when the R^1 position was substituted with a substituent, the antibacterial activities of compounds were enhanced. Whether the R^3 position is substituted by a cyano group or carboxyl, these compounds (expect **8II-q**, **8II-r**, **8II-s**, and **8II-t**) showed strong antibacterial activities when the R^2 position is substituted by chlorine or fluorine. For *Salmonella*, when the R^3 position was substituted with a cyano group, and the R^3 position was substituted with fluorine, the antibacterial activities of these compounds were significantly improved compared to other compounds. When the R^3 position was substituted with carboxyl, the compounds' antibacterial activities were not satisfactory. In summary, the *in vitro* antibacterial experiments' results

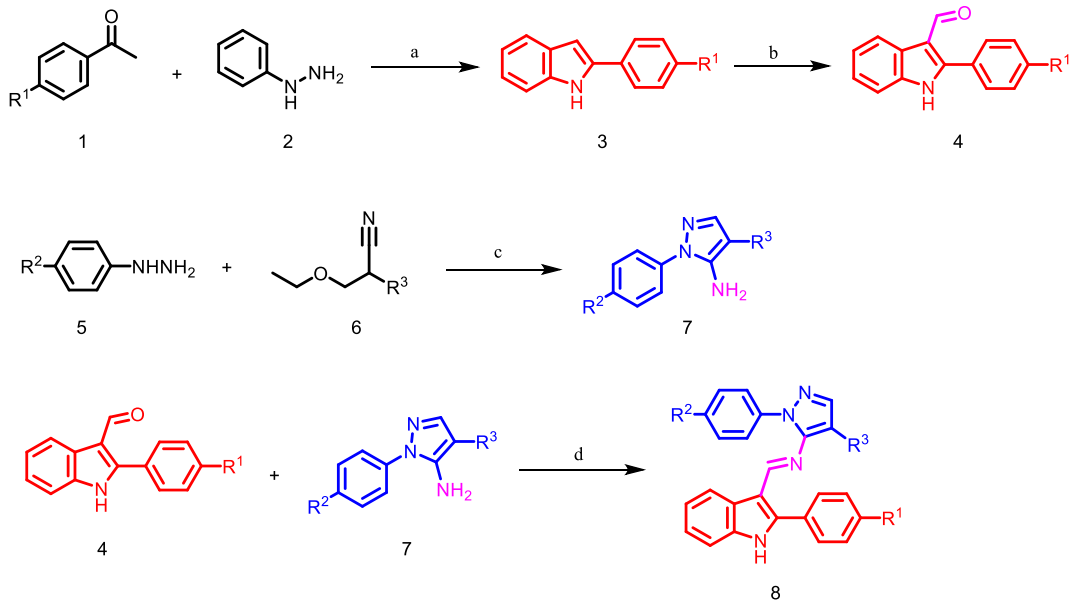

Fig. 2. Synthetic routes for intermediate **4**, **7**, and target derivatives. Reagents and conditions: (a) CH_3COOH, CH_4O_3S; 80 °C; (b) Phosphorus oxychloride, DMF, NaOH, DCM; (c) EtOH, reflux, 4 h; (d) EtOH, HCl, reflux.

Table 1
ID of the target compounds and their substituents.

No.	R^1	R^2	R^3	NO.	R^1	R^2	R^3	NO.	R^1	R^2	R^3
8I-a	H	H	CN	**8I-q**	OMe	H	CN	**8II-i**	Cl	H	COOH
8I-b	H	F	CN	**8I-r**	OMe	F	CN	**8II-j**	Cl	F	COOH
8I-c	H	Cl	CN	**8I-s**	OMe	Cl	CN	**8II-k**	Cl	Cl	COOH
8I-d	H	Me	CN	**8I-t**	OMe	ME	CN	**8II-l**	Cl	ME	COOH
8I-e	F	H	CN	**8I-u**	Br	H	CN	**8II-m**	Me	H	COOH
8I-f	F	F	CN	**8I-v**	Br	F	CN	**8II-n**	Me	F	COOH
8I-g	F	Cl	CN	**8I-w**	Br	Cl	CN	**8II-o**	Me	Cl	COOH
8I-h	F	Me	CN	**8I-x**	Br	ME	CN	**8II-p**	Me	ME	COOH
8I-i	Cl	H	CN	**8II-a**	H	H	COOH	**8II-q**	OMe	H	COOH
8I-j	Cl	F	CN	**8II-b**	H	F	COOH	**8II-r**	OMe	F	COOH
8I-k	Cl	Cl	CN	**8II-c**	H	Cl	COOH	**8II-s**	OMe	Cl	COOH
8I-l	Cl	Me	CN	**8II-d**	H	Me	COOH	**8II-t**	OMe	Me	COOH
8I-m	Me	H	CN	**8II-e**	F	H	COOH	**8II-u**	Br	H	COOH
8I-n	Me	F	CN	**8II-f**	F	F	COOH	**8II-v**	Br	F	COOH
8I-o	Me	Cl	CN	**8II-g**	F	Cl	COOH	**8II-w**	Br	Cl	COOH
8I-p	Me	ME	CN	**8II-h**	F	Me	COOH	**8II-x**	Br	Me	COOH

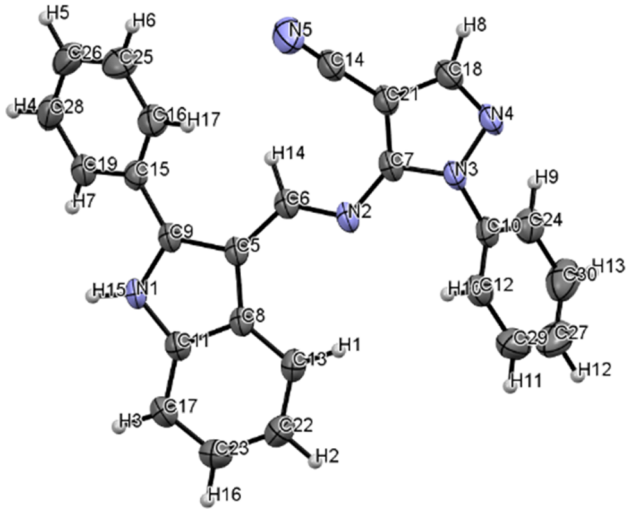

Fig. 3. The crystal structure of compound **8I-a.**

demonstrated that the improved antibacterial activities of these compounds may be attributed to the substitution (R^1) at the benzene ring attached to benzo-indole and various substituents on the pyrazole moiety (Fig. 4).

2.4. Antibacterial effect of selected compounds on the drug-resistant bacteria strains

After screening all the compounds for four bacteria strains, we found that two compounds (**8I-g** and **8I-s**) have a good inhibitory effect on *S. aureus* and most compounds have excellent inhibitory effects on *E. coli*. In order to verify whether these compounds have inhibitory effects on drug-resistant bacteria strains and further research on structure–-activity relationships, we selected 20 compounds (Table 3) to determine their IC_{50} values against *methicillin-resistant S. aureus* (*MRSA*), *vancomycin-resistant S. aureus* (*VRSA*) and 4 multidrug-resistant *E. coli* strains.

From Table 3, we can see that **8I-g** and **8I-s** have a poor inhibitory effect on *MRSA* and *VRSA*, which indicates that this kind of scaffold is not suitable for inhibiting drug-resistant *S. aureus*. On the contrary, these compounds have potent inhibitory effects on four drug-resistant *E. coli*. In particular, the two compounds **8I-w** and **8II-w** showed a promising inhibitory effect on 4 multidrug-resistant *E. coli* bacteria strains. The SARs of these compounds to drug-resistant bacteria strains are consistent with that described in Section 2.3. After combining and discussing the antibacterial activity data of Tables 2&**3**, we concluded that: **(1).** The target compounds synthesized in this work have a poor inhibitory effect on Gram-positive bacteria and a good effect on Gram-negative bacteria; **(2).** When the R^2 substituent is fluorine or chlorine, the bacteriostatic effect can be improved. When both R^1 and R^2 substituents are chlorine, the compounds in this work can show the strongest antibacterial effect. **(3).** Compound **8I-w** and **8II-w** can act as the most potent DNA gyrase inhibitors in the reported series of compounds.

2.5. Enzyme inhibitory activity

From the results of the *in vitro* biological assays, it can be concluded that the compound **8I-w** and **8II-w** have the value of further research, so they and two control drugs were selected for the *in vitro* enzyme inhibitory activity against DNA gyrase isolated from *E. coli*. As shown in

Table 2
IC_{50} Values of All Target Compounds against *S. aureus*, *L. monocytogenes*, *E. coli* and *Salmonella*.

Compd.	IC_{50} (μM) [a]			
	S. aureus	*L. monocytogenes*	*E. coli*	*Salmonella*
8I-a	> 100	> 100	64.2 ± 1.1	38.8 ± 0.3
8I-b	> 100	> 100	> 100	39.9 ± 0.3
8I-c	50.4 ± 0.3	> 100	> 100	14.6 ± 0.3
8I-d	27.1 ± 0.5	> 100	> 100	11.5 ± 0.3
8I-e	> 100	> 100	> 100	46.2 ± 0.3
8I-f	> 100	> 100	5.3 ± 0.7	9.3 ± 0.3
8I-g	11.5 ± 0.4	> 100	4.7 ± 1.1	29.5 ± 0.3
8I-h	> 100	> 100	12.7 ± 0.6	10.0 ± 0.3
8I-i	67.9 ± 0.4	> 100	8.2 ± 0.4	29.4 ± 0.3
8I-j	> 100	> 100	7.0 ± 1.5	8.0 ± 0.2
8I-k	30.2 ± 0.3	> 100	2.8 ± 1.5	67.1 ± 0.4
8I-l	> 100	> 100	4.9 ± 0.6	44.9 ± 0.3
8I-m	> 100	43.0 ± 0.3	21.3 ± 0.3	53.8 ± 0.2
8I-n	28.8 ± 0.3	> 100	1.2 ± 2.0	15.6 ± 0.4
8I-o	53.5 ± 0.3	> 100	0.7 ± 1.0	14.4 ± 0.3
8I-p	19.8 ± 0.3	> 100	10.3 ± 0.9	18.2 ± 0.3
8I-q	> 100	> 100	8.9 ± 0.3	98.8 ± 0.3
8I-r	> 100	> 100	> 100	9.1 ± 0.4
8I-s	10.9 ± 0.6	> 100	13.0 ± 0.6	17.7 ± 0.3
8I-t	> 100	> 100	7.8 ± 1.6	> 100
8I-u	> 100	> 100	16.1 ± 0.6	71.2 ± 0.2
8I-v	> 100	> 100	0.02 ± 1.7	19.4 ± 0.3
8I-w	47.8 ± 0.2	> 100	4.7 ± 0.7	25.3 ± 0.3
8I-x	> 100	> 100	6.2 ± 0.8	64.1 ± 0.2
8II-a	> 100	> 100	24.0 ± 2.8	43.3 ± 0.3
8II-b	43.5 ± 0.5	98.8 ± 0.5	68.5 ± 0.4	> 100
8II-c	> 100	> 100	99.0 ± 0.3	12.0 ± 0.4
8II-d	> 100	> 100	72.8 ± 0.2	> 100
8II-e	> 100	54.5 ± 0.3	82.2 ± 0.3	76.0 ± 0.2
8II-f	92.5 ± 0.3	95.2 ± 0.3	12.3 ± 0.4	30.0 ± 0.2
8II-g	> 100	> 100	2.9 ± 1.2	20.2 ± 0.3
8II-h	> 100	> 100	> 100	> 100
8II-i	83.1 ± 0.4	53.8 ± 0.3	28.0 ± 0.2	85.4 ± 0.4
8II-j	90.5 ± 0.2	22.9 ± 0.2	33.2 ± 0.2	> 100
8II-k	> 100	> 100	4.4 ± 0.6	22.1 ± 0.3
8II-l	> 100	> 100	> 100	> 100
8II-m	63.7 ± 0.4	21.0 ± 0.2	> 100	20.0 ± 0.3
8II-n	43.8 ± 0.2	32.7 ± 0.3	20.4 ± 0.4	53.8 ± 0.3
8II-o	57.7 ± 0.3	> 100	3.5 ± 0.9	24.2 ± 0.2
8II-p	> 100	40.3 ± 1.2	86.0 ± 0.2	> 100
8II-q	> 100	> 100	> 100	> 100
8II-r	> 100	> 100	> 100	> 100
8II-s	> 100	61.3 ± 0.3	> 100	> 100
8II-t	> 100	> 100	> 100	> 100
8II-u	> 100	> 100	79.8 ± 0.2	7.1 ± 0.3
8II-v	> 100	> 100	0.09 ± 1.2	65.9 ± 0.3
8II-w	> 100	> 100	7.8 ± 0.6	20.4 ± 0.2
8II-x	37.8 ± 0.4	23.8 ± 0.2	67.7 ± 0.3	> 100
CIP[a]	21.9 ± 0.4	35.9 ± 0.3	18.4 ± 0.3	1.45 ± 0.3
NB[b]	12.5 ± 0.2	29.5 ± 0.3	34.4 ± 0.4	28.7 ± 0.4
GAT[c]	14.7 ± 0.3	51.8 ± 0.2	31.3 ± 0.3	10.2 ± 0.3
LVX[d]	11.9 ± 0.3	24.7 ± 0.2	17.4 ± 0.3	2.5 ± 0.2

[a] *Ciprofloxacin*;
[b] *Novobiocin*;
[c] *Gatifloxacin*;
[d] *Levofloxacin*.

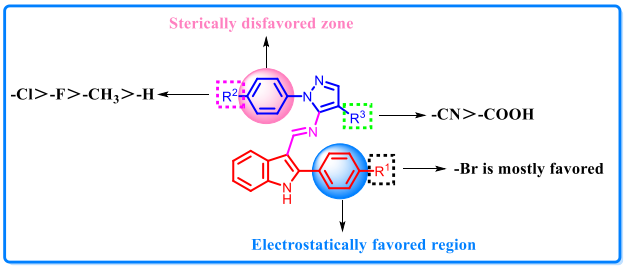

Fig. 4. Summarized SARs of the Target Compounds.

Table 4, compound **8I-w** and **8II-w** both showed good suppression effect against DNA gyrase and are superior to the two control drugs. Among them, the IC_{50} value of **8I-w** is 0.10 μM, which is better than **8II-w**(IC_{50} = 0.39 μM). The results further proved that compound **8I-w** and **8II-w** can cause a lot of pressure on the survival of bacteria and can be further studied as potential DNA gyrase inhibitors.

2.6. Molecular docking analysis

To gain a deep understanding of the molecular docking relationships between compounds and DNA gyrase, all the compounds were docked into the active site of DNA gyrase. The representative docking details of compound **8I-w** and **8II-w** with DNA gyrase were shown in Fig. 5. The docking details of the 5 reference drugs (GSK299423, Novobiocin, Ciprofloxacin, Gatifloxacin, and Levofloxacin) were depicted in the Supporting Information (**Figs. S2-S6**). After analyzing their docking details, we concluded that the high-frequency key amino acid residues and bases of these drugs combined with DNA gyrase are Arg1122, Dg10, Dc11, and Ser1084. For compound **8I-w**, as shown in Fig. 5**(A&B)**, Arg1122(B) established cation-π interaction with the benzene ring which attached to indole; Dg10(E&F), Dc11(E&F) and the pyrazole ring formed 5 kinds of π-π interactions; Dg10(E&F), Dc11(E&F) and the indole ring erected 3 kinds of π-π interactions; Dg10(E&F), Dc11(E&F) and the benzene ring in benzo-indole constructed 4 kinds of π-π interactions. For compound **8II-w**, Dg10(E&F), Dc11(E&F) and the indole ring erected 5 kinds of π-π interactions; Dg10(E&F), Dc11(E&F) and the benzene ring in benzo-indole constructed 5 kinds of π-π interactions; Dc11(F) established π-π interaction with the benzene ring which attached to indole; Besides, Arg1122(B) formed a hydrogen bond (angle = 180°, distance = 2.40 Å) with the carboxyl group, and Asp1083(B) formed a hydrogen bond (angle = 180°, distance = 2.27 Å) with the Nitrogen atom in pyrazole ring. In summary, there are key amino acid residues and bases involved in the docking details of two compounds with DNA gyrase, and we speculate that the reason for the small gap in the biological activity of the two compounds may be the degree of binding to the DNA gyrase.

3. Conclusions

All the novel multi-substituted benzo-indole pyrazole Schiff base derivatives were successfully synthesized and evaluated for their antibacterial activity against four bacteria strains. In addition, we selected 20 of them and 6 drug-resistant bacteria strains for *in vitro* antibacterial activity determination. The experimental results indicated that most of the synthesized compounds showed moderate to potent antibacterial activities. Among these compounds, compound **8I-w** exhibited excellent antibacterial activity against four drug-resistant *E. coli* bacteria strains with IC_{50} values of 7.0, 17.0, 13.5, and 1.0 μM, respectively. SAR and molecular docking analysis indicated that the promising antibacterial efficacy can be attributed to the various substitutions on the two benzene rings and modification of the pyrazole ring. The results of the present work revealed that these Schiff base derivatives can be potential bactericides for the discovery of DNA gyrase inhibitors and worth further study.

4. Materials and methods

4.1. General methods

All chemicals and reagents used in this work were purchased from Energy, Meryer. ^{1}H NMR and ^{13}C NMR spectra were collected on Agilent DD2 600 Hz spectrometer with $CDCl_3$/DMSO as the solvent. ESI-MS spectra were carried out on a Mariner System 5304 mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. Quantitative structure–activity relationship (QSAR) analyses and molecular docking were performed with Discovery Studio

Table 3
IC_{50} Values of Selected Compounds against Drug-Resistant Bacteria Strains.

Compd.	IC_{50} (μM) *MRSA*[a]	*VRSA*[b]	*E. coli E564*[c]	*E. coli E68*[d]	*E. coli E48*[e]	*E. coli E109*[f]
8I-f	NT	NT	32.2 ± 0.3	19.0 ± 0.2	35.7 ± 0.2	4.9 ± 0.2
8I-g	> 100	> 100	57.4 ± 0.2	5.2 ± 0.2	> 100	2.9 ± 0.3
8I-j	NT	NT	36.4 ± 0.2	8.1 ± 0.2	67.1 ± 0.2	3.6 ± 0.2
8I-k	NT	NT	78.6 ± 0.3	11.0 ± 0.2	> 100	7.8 ± 0.1
8I-l	NT	NT	84.9 ± 0.2	3.1 ± 0.2	40.2 ± 0.2	11.6 ± 0.2
8I-n	NT	NT	40.0 ± 0.3	15.1 ± 0.3	15.5 ± 0.3	7.8 ± 0.2
8I-o	NT	NT	26.1 ± 0.3	7.8 ± 0.2	72.3 ± 0.2	13.8 ± 0.3
8I-s	> 100	45.0 ± 0.3	73.1 ± 0.3	99.0 ± 0.1	> 100	5.3 ± 0.2
8I-t	NT	NT	72.6 ± 0.3	9.8 ± 0.2	68.3 ± 0.2	8.4 ± 0.1
8I-v	NT	NT	50.0 ± 0.3	17.1 ± 0.2	13.3 ± 0.2	0.6 ± 0.5
8I-w	NT	NT	7.0 ± 0.3	17.0 ± 0.2	13.5 ± 0.3	1.0 ± 0.2
8I-x	NT	NT	61.8 ± 0.3	25.6 ± 0.3	28.9 ± 0.1	84.2 ± 0.3
8II-f	NT	NT	> 100	79.6 ± 0.2	44.0 ± 0.3	23.8 ± 0.3
8II-g	NT	NT	35.3 ± 0.4	59.6 ± 0.4	35.4 ± 0.3	13.8 ± 0.3
8II-j	NT	NT	43.5 ± 0.4	> 100	> 100	41.3 ± 0.2
8II-k	NT	NT	32.4 ± 0.3	32.1 ± 0.3	> 100	4.3 ± 0.1
8II-n	NT	NT	> 100	27.5 ± 0.3	85.3 ± 0.2	4.5 ± 0.2
8II-o	NT	NT	20. 4 ± 0.2	37.9 ± 0.3	28.4 ± 0.3	11.5 ± 0.3
8II-v	NT	NT	70.9 ± 0.3	52.7 ± 0.3	39.4 ± 0.2	4.0 ± 0.3
8II-w	NT	NT	6.3 ± 0.6	29.6 ± 0.4	31.0 ± 0.3	23.8 ± 0.3
CIP	91.6 ± 0.4	52.3 ± 0.3	18.3 ± 0.2	> 100	36.7 ± 0.3	22.7 ± 0.2
NB	> 100	> 100	> 100	58.0 ± 0.2	63.2 ± 0.3	41.8 ± 0.2
GAT	82.3 ± 0.3	28.9 ± 0.3	23.9 ± 0.2	42.7 ± 0.2	42.9 ± 0.2	10.8 ± 0.2
LVX	49.9 ± 0.4	31.2 ± 0.2	7.0 ± 0.2	50.1 ± 0.2	20.9 ± 0.2	12.5 ± 0.3

[a] *methicillin-resistant S. aureus* (*MRSA*, N315);
[b] *vancomycin-resistant S. aureus* (*VRSA*, Mu50);
[c] Multidrug-resistant *E. coli* (Cli122006P);
[d] Multidrug-resistant *E. coli* (Cli122202);
[e] Multidrug-resistant *E. coli* (Clu123302.3073);
[f] Multidrug-resistant *E. coli* (Clu120803); NT, Not tested.

Table 4
E. coli DNA gyrase supercoiling activity of selected compounds.

Compd.	IC_{50} (μM) DNA gyrase
8I-w	0.10
8II-w	0.39
CIP	0.75
NB	0.49

(version 3.5). Crystal data were collected with a Bruker D8 Venture diffractometer.

4.1.1. General procedures for the synthesis of 2-phenyl-1H-indole-3-carbaldehyde derivatives

P-substituted acetophenone (2 mmol) and phenyl-hydrazine (2 mmol) were poured into a round bottom flask, then CH_3COOH (0.5 mL) and CH_4O_3S (27 mmol) were added. After the thin layer chromatography (TLC) monitoring reaction was completed, the intermediate **3** was obtained by pouring the mixture into ice water. At −30 °C, $POCl_3$ (10 mmol) was slowly added dropwise to a round bottom flask containing DMF (10 mmol), and the reaction was stirred for 1 h. After that, intermediate **3** (1 mmol) was dissolved in DMF, slowly added dropwise to the flask, and the reaction was heated to 70 °C with stirring. Monitor the reaction through the TLC. Finally, the mixture was poured into ice water, washed with 40% NaOH solution, and then extracted with DCM to obtain intermediate **4**.

4.1.2. General procedure for the synthesis of 1-phenyl-1H-pyrazol-5-amine derivatives

A round bottom flask were charged with *P*-substituted phenyl-hydrazine (1 mmol) and 2-(ethoxymethyl) malononitrile/2-cyano-3-ethoxypropanoic acid (1.2 mmol) in the ethanol and the mixture was heated to 80 °C and refluxed for 4 h. After the reaction was completed, the mixture was vacuum filtered and concentrated to obtain intermediate **7**.

4.1.3. General procedure for the synthesis of target derivatives

Intermediate **4** (1 mmol) and the intermediate **7** (1 mmol) were dissolved in ethanol to the glass flask, then HCl solution was added as a catalyst refluxed at 80 °C for 15–18 h. After the TLC monitoring reaction was completed, the mixture was vacuum filtered and concentrated. Finally, the above crude product can be isolated and purified by column chromatography (Hexane/EtOAc = 8:1) to obtain target compounds.

4.1.4. (E)-1-phenyl-5-(((2-phenyl-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-a)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.27 (s, 1H, Indole-H), 8.71 (s, 1H, CH = N), 8.38 (d, J = 7.9 Hz, 1H, Ar-H), 7.87 (s, 1H, Pyrazole-H), 7.81 (d, J = 8.1 Hz, 2H, Ar-H), 7.70 (d, J = 7.3 Hz, 2H, Ar-H), 7.60 (t, J = 7.6 Hz, 2H, Ar-H), 7.54 (t, J = 7.2 Hz, 1H, Ar-H), 7.51 – 7.43 (m, 3H, Ar-H), 7.41 (t, J = 7.4 Hz, 1H, Ar-H), 7.34 (t, J = 7.6 Hz, 1H, Ar-H), 7.23 (t, J = 7.6 Hz, 1H, Ar-H). ^{13}C NMR (150 MHz, $CDCl_3$) δ161.0 (CH = N), 154.9 (Pyrazole-C), 148.3(Pyrazole-C), 142.3 (Ar-C), 138.5(Ar-C), 136.1(Ar-C), 130.1(Ar-C), 129.8(Ar-C), 129.5(Ar-C), 129.3(Ar-C), 128.7(Ar-C), 127.8(Ar-C), 126.2(Ar-C), 124.5(Ar-C), 124.4(Ar-C), 123.1(Indole-C), 123.0(Ar-C), 115.0(CN), 112.2(Ar-C), 111.1(Indole-C), 80.3(Pyrazole-C). MS (ESI): 388.15($C_{25}H_{17}N_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}N_5$: C, 77.50; H, 4.42; N, 18.08; Found: C, 77.51; H, 4.41; N, 18.06.

4.1.5. (E)-1-(4-fluorophenyl)-5-(((2-phenyl-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-b)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.09 (s, 1H), 8.84 (s, 1H), 8.45 – 8.42 (m, 1H), 7.67 – 7.62 (m, 3H), 7.54 (d, J = 4.5 Hz, 4H), 7.49 (dd, J = 8.7, 4.7 Hz, 2H), 7.46 – 7.42 (m, 2H), 7.33 (dd, J = 5.6, 3.4 Hz, 2H), 7.22 (t, J = 8.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.7,

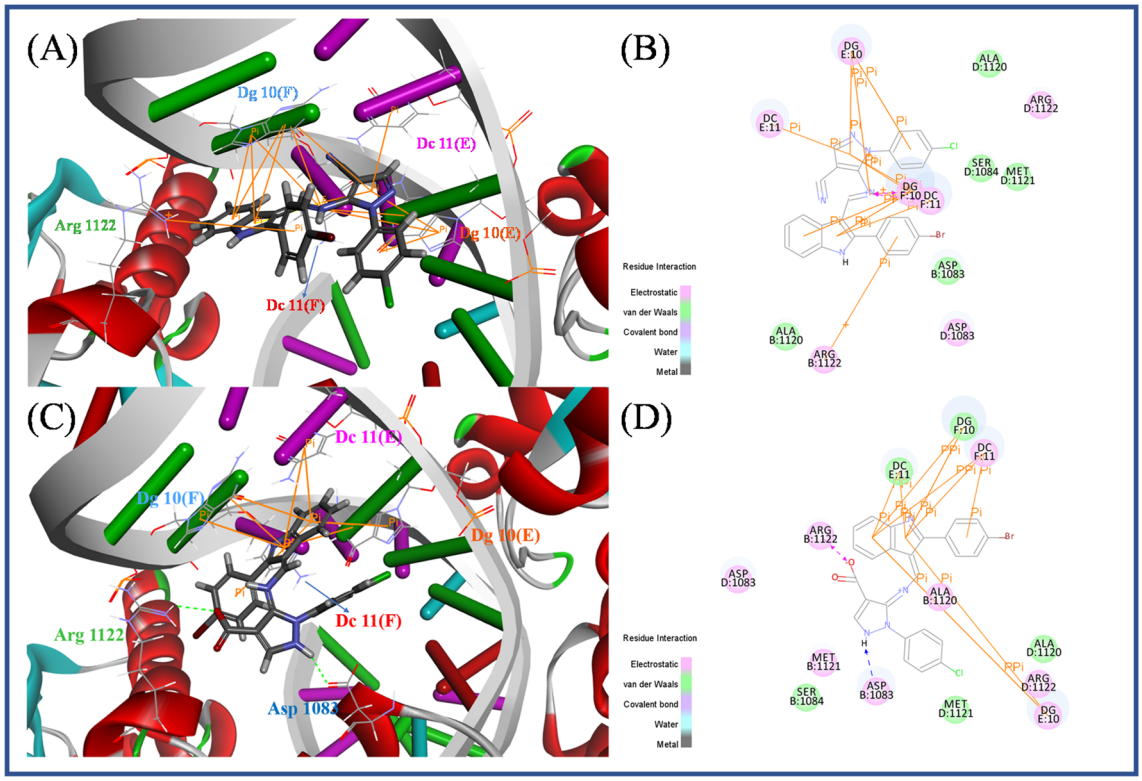

Fig. 5. Binding models of compound **8I-w(A&B)** and **8II-w (C&D)** with DNA gyrase (3D&2D diagram).

186.6, 148.8, 141.3, 135.4, 130.0, 129.5, 129.1, 126.4, 126.4, 126.2, 124.4, 123.2, 122.4, 117.0, 116.9, 115.0, 110.9. MS (ESI): 406.14($C_{25}H_{16}FN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}FN_5$: C, 74.06; H, 3.98; F, 4.69; N, 17.27; Found: C, 74.04; H, 3.97; F, 4.68; N, 17.25.

4.1.6. (E)-1-(4-chlorophenyl)-5-(((2-phenyl-1H-indol-3-yl)methylene) amino)-1H-pyrazole-4-carbonitrile (8I-c)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.76 (s, 1H), 8.45 – 8.41 (m, 1H), 7.55 (d, J = 3.4 Hz, 3H), 7.50 (d, J = 8.6 Hz, 4H), 7.46 (d, J = 8.6 Hz, 4H), 7.35 – 7.31 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.7, 149.7, 148.8, 141.5, 135.5, 135.4, 134.8, 130.1, 130.1, 130.0, 129.5, 129.2, 125.4, 124.4, 123.2, 122.4, 113.6, 110.9. MS (ESI): 422.11($C_{25}H_{16}ClN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}ClN_5$: C, 71.17; H, 3.82; Cl, 8.40; N, 16.60; Found: C, 71.18; H, 3.81; Cl, 8.41; N, 16.62.

4.1.7. (E)-5-(((2-phenyl-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carbonitrile (8I-d)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.68 (s, 1H), 8.39 (d, J = 7.6 Hz, 1H), 7.85 (s, 1H), 7.73 – 7.64 (m, 4H), 7.61 (dd, J = 16.0, 8.9 Hz, 2H), 7.57 – 7.53 (m, 2H), 7.45 (t, J = 8.7 Hz, 1H), 7.35 (dd, J = 19.0, 7.3 Hz, 3H), 2.43 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 149.2, 142.9, 138.0, 137.2, 136.2, 130.2, 130.1, 129.7, 129.5, 129.4, 126.1, 124.6, 124.4, 122.8, 122.5, 118.2, 115.4, 112.6, 111.0, 79.8, 21.1. MS (ESI): 402.16($C_{26}H_{19}N_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44; Found: C, 77.77; H, 4.75; N, 17.43.

4.1.8. (E)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carbonitrile (8I-e)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.57 (s, 1H), 8.44 – 8.41 (m, 1H), 8.32 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.78 (dd, J = 8.7, 4.9 Hz, 1H), 7.70 (dd, J = 8.4, 5.2 Hz, 1H), 7.65 (dd, J = 7.6, 4.2 Hz, 3H), 7.36 – 7.33 (m, 2H), 7.30 (dd, J = 18.3, 9.7 Hz, 2H), 7.22 (d, J = 8.4 Hz, 1H), 7.18 (t, J = 9.1 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.8,161.0, 153.4, 151.9, 148.3, 146.8, 139.0, 135.3, 131.4, 131.3, 126.7, 124.5, 123.3, 122.3, 120.8, 117.2, 116.5, 116.4, 110.9, 105.6, 80.7. MS (ESI): 406.14($C_{25}H_{16}FN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}FN_5$: C, 74.06; H, 3.98; F, 4.69; N, 17.27; Found: C, 74.05; H, 3.99; F, 4.67; N, 17.26.

4.1.9. (E)-1-(4-fluorophenyl)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carbonitrile (8I-f)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.23 (s, 1H), 8.65 (s, 1H), 8.32 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.81 – 7.75 (m, 2H), 7.73 – 7.67 (m, 2H), 7.46 (d, J = 8.0 Hz, 1H), 7.33 (dt, J = 16.3, 7.8 Hz, 3H), 7.18 (t, J = 8.2 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.1, 160.8, 160.8, 159.5, 154.3, 142.3, 131.3, 131.3, 126.3, 126.2, 126.1, 124.7, 123.2, 122.9, 120.7, 116.9, 116.8, 115.7, 115.5, 111.3, 80.9. MS (ESI): 424.13($C_{25}H_{17}F_2N_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}F_2N_5$: C, 70.92; H, 3.57; F, 8.97; N, 16.54; Found: C, 70.90; H, 3.55; F, 8.98; N, 16.55.

4.1.10. (E)-1-(4-chlorophenyl)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carbonitrile (8I-g)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.71 (s, 1H), 8.36 (d, J = 7.8 Hz, 1H), 7.86 (s, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.72 – 7.67 (m, 2H), 7.46 (t, J = 9.6 Hz, 3H), 7.38 – 7.35 (m, 1H), 7.33 – 7.28 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.7, 160.9, 154.3, 153.1, 142.4, 141.1, 139.2, 131.3, 131.3, 128.8, 125.4, 124.8, 123.3, 122.8, 118.0, 116.9, 116.8, 115.3, 113.1, 111.3, 80.5. MS (ESI): 440.10($C_{25}H_{15}ClFN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{15}ClFN_5$: C, 68.26; H, 3.44; Cl, 8.06; F, 4.32; N, 15.92; Found: C, 68.28; H, 3.42; Cl, 8.04; F, 4.33; N, 15.94.

4.1.11. (E)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carbonitrile (8I-h)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.70 (s, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.85 (s, 1H), 7.68 (dd, J = 11.2, 5.9 Hz, 4H), 7.36 – 7.32 (m, 1H), 7.28 (dd, J = 10.9, 8.5 Hz, 4H), 7.24 (d, J = 7.4 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.7, 163.0, 160.6, 154.7, 147.0, 142.1, 137.8, 136.1, 131.3, 131.2, 129.2, 126.1, 126.0, 125.9, 124.6, 124.2, 123.1, 123.0, 116.8, 116.7, 115.1, 112.3, 111.1, 80.1,

21.1. MS (ESI): 420.15($C_{26}H_{18}FN_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}FN_5$: C, 74.45; H, 4.33; F, 4.53; N, 16.70; Found: C, 74.43; H, 4.31; F, 4.52; N, 16.71.

4.1.12. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carbonitrile (8I-i)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.21 (s, 1H, Indole-H), 8.40 (d, J = 7.4 Hz, 1H, CH = N), 8.35 (d, J = 7.9 Hz, 1H, Ar-H), 7.87 (s, 1H, Pyrazole-H), 7.79 (d, J = 8.1 Hz, 1H, Ar-H), 7.65 – 7.60 (m, 2H, Ar-H), 7.59 – 7.38 (m, 7H, Ar-H), 7.33 (t, J = 6.9 Hz, 1H, Ar-H), 7.22 (t, J = 7.5 Hz, 1H, Ar-H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8 (CH = N), 147.1, 142.4 (Pyrazole-C), 135.4 (Ar-C), 130.6 (Ar-C), 130.5 (Ar-C), 129.8 (Ar-C), 129.5 (Ar-C), 128.8 (Ar-C), 128.4 (Ar-C), 125.4 (Ar-C), 124.9 (Ar-C), 124.6 (Ar-C), 123.4 (Indole-C), 123.3 (Ar-C), 122.9 (Ar-C), 122.3 (Ar-C), 121.0 (Ar-C), 111.3 (Indole-C), 111.0 (Pyrazole-C), 81.7(Pyrazole-C). MS (ESI): 422.11($C_{25}H_{16}ClN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}ClN_5$: C, 71.17; H, 3.82; Cl, 8.40; N, 16.60; Found: C, 71.15; H, 3.81; Cl, 8.42; N, 16.61.

4.1.13. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-fluorophenyl)-1H-pyrazole-4-carbonitrile (8I-j)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.23 (s, 1H), 8.78 (s, 1H), 8.32 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.78 (dd, J = 6.9, 3.8 Hz, 2H), 7.63 (d, J = 7.2 Hz, 2H), 7.57 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.0 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 7.17 (t, J = 8.4 Hz, 2H), 6.99 (d, J = 21.1 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.7, 146.9, 142.3, 136.6, 130.5, 129.8, 129.3, 128.2, 126.3, 126.3, 126.2, 126.1, 124.8, 123.2, 122.9, 115.7, 115.5, 112.3, 111.3, 80.2. MS (ESI): 440.10($C_{25}H_{15}ClFN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{15}ClFN_5$: C, 68.26; H, 3.44; Cl, 8.06; F, 4.32; N, 15.92; Found: C, 68.24; H, 3.45; Cl, 8.08; F, 4.31; N, 15.94.

4.1.14. (E)-1-(4-chlorophenyl)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-k)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.07 (s, 1H), 8.42 (d, J = 6.0 Hz, 1H), 8.36 (d, J = 8.1 Hz, 1H), 7.87 (s, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 7.58 (t, J = 9.4 Hz, 3H), 7.53 (d, J = 8.3 Hz, 2H), 7.46 – 7.42 (m, 2H), 7.37 – 7.32 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8, 147.1, 135.4, 130.6, 130.5, 129.8, 129.5, 128.8, 128.4, 125.4, 124.9, 124.6, 123.4, 123.3, 122.9, 122.3, 121.0, 115.3 111.3, 111.0, 81.7. MS (ESI): 456.07($C_{25}H_{15}Cl_2N_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{15}Cl_2N_5$: C, 65.80; H, 3.31; Cl, 15.54; N, 15.35; Found: C, 65.82; H, 3.30; Cl, 15.52; N, 15.34.

4.1.15. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carbonitrile (8I-l)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.43 – 8.39 (m, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.85 (s, 1H), 7.58 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.34 (dt, J = 14.3, 8.6 Hz, 4H), 7.28 (s, 1H), 7.23 (d, J = 7.3 Hz, 1H), 2.42 (d, J = 4.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 142.1, 141.0, 139.2, 136.4, 130.7, 130.5, 130.5, 129.7, 129.4, 129.2, 124.6, 124.6, 124.2, 123.3, 123.1, 123.0, 122.3, 111.2, 111.0, 80.7, 21.1. MS (ESI): 436.13($C_{26}H_{18}ClN_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}ClN_5$: C, 71.64; H, 4.16; Cl, 8.13; N, 16.07; Found: C, 71.65; H, 4.18; Cl, 8.12; N, 16.06.

4.1.16. (E)-1-phenyl-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-m)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.64 (s, 1H), 8.37 (d, J = 7.8 Hz, 1H), 7.87 (s, 1H), 7.81 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 7.8 Hz, 2H), 7.48 (t, J = 7.8 Hz, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.5 Hz, 3H), 7.32 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.1, 155.9, 152.1, 142.3, 140.5, 139.1, 138.6, 135.7, 131.7, 130.2, 129.2, 128.6, 127.8, 126.3, 126.2, 124.4, 124.4, 123.0, 122.9, 111.0, 87.6, 18.4. MS (ESI): 402.16($C_{26}H_{19}N_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44; Found: C, 77.77; H, 4.76; N, 17.42.

4.1.17. (E)-1-(4-fluorophenyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-n)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.68 (s, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.85 (s, 1H), 7.79 (dd, J = 8.7, 4.8 Hz, 2H), 7.60 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 7.8 Hz, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.17 (t, J = 8.5 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.2, 154.9, 148.8, 142.4, 140.6, 136.1, 130.2, 129.2, 126.9, 126.9, 126.3, 126.2, 125.0, 124.5, 123.0, 122.8, 115.6, 115.5, 114.9, 111.2, 80.1, 21.4. MS (ESI): 420.15($C_{26}H_{18}FN_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}FN_5$: C, 74.45; H, 4.33; F, 4.53; N, 16.70; Found: C, 74.46; H, 4.32; F, 4.52; N, 16.71.

4.1.18. (E)-1-(4-chlorophenyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-o)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.08 (s, 1H), 9.25 (s, 1H), 8.80 (s, 1H), 8.42 (d, J = 8.8 Hz, 1H), 8.36 (d, J = 7.7 Hz, 1H), 7.85 (s, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.53 (dd, J = 10.2, 8.6 Hz, 1H), 7.45 (t, J = 7.7 Hz, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.0 Hz, 1H), 7.32 (dd, J = 6.0, 3.1 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 2.46–2.45 (d, J = 6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 154.4,137.4, 136.4, 132.2, 130.2, 129.9, 129.3, 129.2, 128.8, 125.4, 124.5, 124.4, 124.3, 123.1, 122.8, 122.3, 114.8, 111.2, 110.8, 80.4, 22.7. MS (ESI): 436.13($C_{26}H_{18}ClN_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}ClN_5$: C, 71.64; H, 4.16; Cl, 8.13; N, 16.07; Found: C, 71.62; H, 4.18; Cl, 8.12; N, 16.06.

4.1.19. (E)-1-(p-tolyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-p)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.79 (s, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.84 (s, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.62 (s, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 8.7 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 8.0, 5.7 Hz, 2H), 7.28 (s, 1H), 7.22 (t, J = 7.5 Hz, 1H), 2.44 (dd, J = 18.5, 3.8 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.0, 154.5, 151.9, 141.0, 140.5, 137.7, 136.1, 130.5, 132.6, 130.5, 130.2, 129.2, 129.2, 124.3, 124.2, 124.2, 123.1, 122.8, 114.0, 111.1, 104.8, 81.7, 21.1. MS (ESI): 416.18($C_{27}H_{21}N_5$, [M + H] $^+$). Anal. Calcd for $C_{27}H_{21}N_5$: C, 78.05; H, 5.09; N, 16.86; Found: C, 78.04; H, 5.08; N, 16.85.

4.1.20. (E)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carbonitrile (8I-q)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.75 (s, 1H), 8.43 – 8.38 (m, 1H), 7.64 (s, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.55 – 7.51 (m, 3H), 7.50 (t, J = 4.3 Hz, 3H), 7.47 – 7.43 (m, 2H), 7.06 (d, J = 8.7 Hz, 2H), 3.89 (d, J = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.2, 161.2, 151.9, 149.7, 149.0, 141.2, 137.0, 135.4, 130.8, 129.9, 128.9, 126.3, 124.2, 124.1, 123.1, 122.3, 122.2, 114.7, 113.9, 110.8, 80.4, 55.4. MS (ESI): 418.16($C_{26}H_{19}N_5O$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}N_5O$: C, 74.80; H, 4.59; N, 16.78; O, 3.83; Found: C, 74.82; H, 4.58; N, 16.76; O, 3.82.

4.1.21. (E)-1-(4-fluorophenyl)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carbonitrile (8I-r)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.69 (s, 1H), 8.31 (d, J = 7.9 Hz, 1H), 7.85 (s, 1H), 7.79 (dd, J = 8.7, 4.8 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.23 (d, J = 7.3 Hz, 1H), 7.17 (t, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 3.89 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 161.3, 161.0, 155.0, 148.8, 142.3, 136.1, 130.7, 126.3, 126.2, 126.2, 124.4, 122.9, 122.7, 122.0, 115.6, 115.5, 115.1, 111.6, 111.1, 80.1, 55.5. MS (ESI): 436.15($C_{26}H_{18}FN_5O$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}FN_5O$: C, 71.71; H, 4.17; F, 4.36; N, 16.08; O, 3.67; Found: C, 71.70; H, 4.16; F, 4.35; N, 16.09; O, 3.65.

4.1.22. (E)-1-(4-chlorophenyl)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carbonitrile (8I-s)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.60 (dd, J = 8.3, 3.0 Hz, 3H), 7.53 (d, J = 8.6 Hz, 1H), 7.43 – 7.33 (m, 3H), 7.17 (t, J = 7.5 Hz, 1H), 7.11 (dd, J = 14.8, 7.0 Hz, 2H), 7.04 (d, J = 9.0 Hz, 1H), 6.98 (d, J = 8.6 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.4, 159.3, 138.4, 136.7, 131.0, 129.4, 126.5, 125.4, 125.2, 124.4, 124.3, 123.9, 121.9, 120.3, 120.1, 119.5, 119.1, 114.5, 110.7, 98.9, 84.0, 55.4. MS (ESI): 452.12($C_{26}H_{18}ClN_5O$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}ClN_5O$: C, 69.10; H, 4.01; Cl, 7.84; N, 15.50; O, 3.54; Found: C, 69.12; H, 4.00; Cl, 7.85; N, 15.52; O, 3.55.

4.1.23. (E)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carbonitrile (8I-t)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.60 (s, 1H), 8.36 (s, 1H), 7.84 (s, 1H), 7.72 – 7.59 (m, 4H), 7.42 (s, 1H), 7.24 (s, 3H), 7.10 (s, 2H), 3.88 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.2, 161.0, 154.9, 148.4, 142.1, 137.7, 136.0, 130.8, 130.7, 129.2, 126.3, 124.3, 124.2, 123.0, 122.8, 122.1, 115.1, 114.7, 111.7, 111.0, 80.0, 55.5, 21.1. MS (ESI): 388.15($C_{27}H_{21}N_5O$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}N_5$: C, 75.16; H, 4.91; N, 16.23; O, 3.71; Found: C, 75.18; H, 4.90; N, 16.21; O, 3.70.

4.1.24. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carbonitrile (8I-u)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.76 (s, 1H), 8.44 – 8.40 (m, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.65 (s, 1H), 7.58 – 7.49 (m, 5H), 7.45 (dd, J = 7.8, 4.5 Hz, 2H), 7.34 (dd, J = 9.0, 5.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.2, 158.2, 153.4, 149.7, 143.1, 141.2, 137.0, 135.4, 132.4, 130.9, 129.9, 128.9, 126.1, 124.2, 123.3, 122.3, 120.6, 113.9, 111.6, 111.0, 76.2. MS (ESI): 466.06($C_{25}H_{16}BrN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}BrN_5$: C, 64.39; H, 3.46; Br, 17.13; N, 15.02; Found: C, 64.38; H, 3.48; Br, 17.12; N, 15.00.

4.1.25. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-fluorophenyl)-1H-pyrazole-4-carbonitrile (8I-v)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.07 (s, 1H), 8.67 (s, 1H), 8.44 – 8.40 (m, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.63 (s, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.46 – 7.42 (m, 1H), 7.38 – 7.31 (m, 7H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 160.1, 147.1, 143.8, 135.5, 132.4, 131.6, 130.8, 128.9, 126.5, 126.4, 126.1, 124.7, 124.6, 123.4, 122.3, 117.0, 116.9, 115.2, 111.0. MS (ESI): 484.05($C_{25}H_{15}BrFN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{15}BrFN_5$: C, 62.00; H, 3.12; Br, 16.50; F, 3.92; N, 14.46; Found: C, 62.02; H, 3.10; Br, 16.51; F, 3.91; N, 14.47.

4.1.26. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile (8I-w)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.78 (s, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.79 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.45 (t, J = 9.6 Hz, 3H), 7.37 (t, J = 7.8 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8, 154.9, 147.1, 142.4, 137.0, 136.2, 133.6, 132.8, 130.7, 128.8, 128.6, 126.1, 125.4, 124.9, 124.2, 123.3, 122.9, 114.9, 112.3, 111.4, 80.6. MS (ESI): 500.02($C_{25}H_{15}BrClN_5$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{15}BrClN_5$: C, 59.96; H, 3.02; Br, 15.96; Cl, 7.08; N, 13.99; Found: C, 59.98; H, 3.00; Br, 15.95; Cl, 7.06; N, 13.98.

4.1.27. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carbonitrile (8I-x)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.61 (s, 1H), 8.42 (d, J = 5.0 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.63 (s, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 7.35 (dd, J = 25.9, 7.9 Hz, 6H), 2.42 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.2, 149.6, 147.0, 141.0, 139.2, 135.4, 134.3, 132.5, 130.8, 130.5, 128.9, 126.1, 124.7, 124.2, 123.4, 122.4, 119.0, 113.9, 111.0, 101.8, 76.0, 21.1. MS (ESI): 478.07($C_{26}H_{18}BrN_5$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{18}BrN_5$: C, 65.01; H, 3.78; Br, 16.63; N, 14.58; Found: C, 65.00; H, 3.77; Br, 16.62; N, 14.59.

4.1.28. (E)-1-phenyl-5-(((2-phenyl-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-a)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.49 (d, J = 7.8 Hz, 2H), 7.91 (d, J = 7.6 Hz, 1H), 7.62 (d, J = 6.6 Hz, 2H), 7.59 – 7.53 (m, 3H), 7.49 (t, J = 7.9 Hz, 2H), 7.43 (d, J = 8.1 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.32 (t, J = 7.6 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H).^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8, 160.9, 156.0, 145.2, 140.0, 139.9, 136.0, 130.9, 129.6, 129.2, 129.2, 128.4, 126.6, 126.4, 124.4, 124.2, 123.2, 122.4, 112.6, 110.8, 92.2. MS (ESI): 407.14($C_{25}H_{18}N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78; O, 7.87; Found: C, 73.86; H, 4.45; N, 13.76; O, 7.86.

4.1.29. (E)-1-(4-fluorophenyl)-5-(((2-phenyl-1H-indol-3-yl)methylene) amino)-1H-pyrazole-4-carboxylic acid (8II-b)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.85 (s, 1H), 8.41 (d, J = 7.7 Hz, 1H), 7.87 – 7.82 (m, 1H), 7.61 – 7.48 (m, 5H), 7.42 (d, J = 8.0 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.24 – 7.20 (m, 1H), 7.19 – 7.14 (m, 2H).^{13}C NMR (150 MHz, $CDCl_3$) δ 168.1, 159.7, 156.2, 140.0, 136.1, 130. 9, 129.6, 129.3, 129.2, 126.1, 126.1, 124.2, 123.0, 122.4, 117.5, 115.3, 115.1, 111.0, 110.2, 100.1, 92.1. MS (ESI): 425.13($C_{25}H_{17}FN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}FN_4O_2$: C, 70.75; H, 4.04; F, 4.48; N, 13.20; O, 7.54; Found: C, 70.74; H, 4.02; F, 4.49; N, 13.21; O, 7.56.

4.1.30. (E)-1-(4-chlorophenyl)-5-(((2-phenyl-1H-indol-3-yl)methylene) amino)-1H-pyrazole-4-carboxylic acid (8II-c)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.56 (s, 1H), 8.46 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 7.1 Hz, 3H), 7.56 (dd, J = 16.4, 9.0 Hz, 3H), 7.44 (d, J = 8.0 Hz, 3H), 7.34 (t, J = 7.3 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 162.1, 156.3, 145.5, 140.2, 138.4, 132.8, 132.0, 130.8, 129.6, 129.2, 128.5, 125.3, 124.3, 123.0, 123.0, 119.5, 119.2, 110.9. MS (ESI): 441.10($C_{25}H_{17}ClN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}ClN_4O_2$: C, 68.11; H, 3.89; Cl, 8.04; N, 12.71; O, 7.26; Found: C, 68.10; H, 3.88; Cl, 8.02; N, 12.70; O, 7.25.

4.1.31. (E)-5-(((2-phenyl-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carboxylic acid (8II-d)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.62 (d, J = 11.9 Hz, 1H), 7.79 (d, J = 13.0 Hz, 1H), 7.71 – 7.65 (m, 2H), 7.52 (dd, J = 18.8, 8.2 Hz, 3H), 7.44 – 7.38 (m, 3H), 7.35 (s, 1H), 7.33 – 7.29 (m, 2H), 7.20 (d, J = 8.1 Hz, 1H), 7.12 (dd, J = 8.6, 2.4 Hz, 1H), 2.41 (d, J = 6.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.4, 160.6, 141.7, 136.7, 134.9, 134.3, 130.4, 129.8, 129.1, 128.5, 128.1, 127.0, 124.7, 124.5, 123.9, 123.8, 123.7, 119.1, 114.0, 107.4, 21.1. MS (ESI): 421.16($C_{20}H_{20}N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{20}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33; O, 7.61; Found: C, 74.26; H, 4.80; N, 13.32; O, 7.60.

4.1.32. (E)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carboxylic acid (8II-e)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.70 (s, 1H), 7.68 – 7.65 (m, 2H), 7.62 (d, J = 1.9 Hz, 1H), 7.55 – 7.46 (m, 5H), 7.44 – 7.38 (m, 3H), 7.30 (t, J = 7.4 Hz, 1H), 7.12 (dd, J = 8.6, 2.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.7, 169.7, 154.1, 150.9, 146.9, 141.6, 140.2, 139.6, 137.4, 129.8, 129.4, 128.5, 128.1, 127.0, 124.7, 124.4, 123.7, 119.1, 114.0, 102.9. MS (ESI): 425.13($C_{25}H_{17}FN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}FN_4O_2$: C, 70.75; H, 4.04; F, 4.48; N, 13.20; O, 7.54; Found: C, 70.72; H, 4.02; F, 4.47; N, 13.21; O, 7.55.

4.1.33. (E)-1-(4-fluorophenyl)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-f)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.64 (s, 1H), 8.41 (d, J = 7.8 Hz, 1H), 7.87 – 7.83 (m, 2H), 7.59 (d, J = 7.7 Hz, 2H), 7.42 (d,

J = 8.1 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.28 (s, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.17 (t, J = 8.4 Hz, 2H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7, 161.3, 160.8, 155.7, 152.0, 140.0, 136.0, 131.1, 131.1, 126.1, 126.1, 124.3, 123.0, 122.6, 116.5, 116.4, 115.3, 115.1, 112.7, 110.9. MS (ESI): 443.12($C_{25}H_{16}F_2N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}F_2N_4O_2$: C, 67.87; H, 3.65; F, 8.59; N, 12.66; O, 7.23; Found: C, 67.86; H, 3.66; F, 8.58; N, 12.65; O, 7.22.

4.1.34. (E)-1-(4-chlorophenyl)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-g)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.70 (s, 2H), 7.78 (s, 2H), 7.53 – 7.46 (m, 9H), 7.36 (t, J = 6.5 Hz, 1H), 7.12 (dd, J = 8.6, 2.3 Hz, 1H).^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 163.2, 161.6, 145.3, 137.0, 136.9, 129.2, 128.8, 128.7, 126.9, 126.8, 124.5, 124.0, 122.4, 120.6, 120.3, 116.0, 115.9, 110.9, 99.9. MS (ESI): 459.09($C_{25}H_{16}ClFN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}ClFN_4O_2$: C, 65.44; H, 3.51; Cl, 7.73; F, 4.14; N, 12.21; O, 6.97; Found: C, 65.42; H, 3.50; Cl, 7.72; F, 4.12; N, 12.20; O, 6.98.

4.1.35. (E)-5-(((2-(4-fluorophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carboxylic acid (8II-h)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (d, J = 7.7 Hz, 1H), 7.55 – 7.50 (m, 5H), 7.48 (t, J = 7.8 Hz, 2H), 7.41 – 7.37 (m, 2H), 7.36 (d, J = 8.4 Hz, 2H), 7.03 (t, J = 8.6 Hz, 2H), 2.46 (s, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 167.9, 163.5, 160.4, 143.7, 139.0, 138.3, 138.0, 135.1, 129.5, 127.8, 125.6, 124.4, 124.2, 123.9, 123.4, 119.9, 115.8, 115.6, 112.6, 101.5, 22.7. MS (ESI): 439.15($C_{26}H_{19}FN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}FN_4O_2$: C, 71.22; H, 4.37; F, 4.33; N, 12.78; O, 7.30; Found: C, 71.20; H, 4.36; F, 4.32; N, 12.79; O, 7.32.

4.1.36. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carboxylic acid (8II-i)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.54 – 7.50 (m, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.43 – 7.39 (m, 1H), 7.34 (dd, J = 15.7, 7.9 Hz, 2H), 7.29 (dd, J = 14.8, 7.4 Hz, 2H), 7.17 – 7.03 (m, 6H), 6.94 – 6.87 (m, 1H).^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 161.1, 135.9, 133.7, 129.9, 129.6, 129.4, 129.1, 128.4, 126.3, 123.9, 122.6, 122.2, 121.2, 121.0, 120.7, 120.4, 120.0, 119.1, 106.3. MS (ESI): 441.10($C_{25}H_{17}ClN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{17}ClN_4O_2$: C, 68.11; H, 3.89; Cl, 8.04; N, 12.71; O, 7.26; Found: C, 68.10; H, 3.90; Cl, 8.03; N, 12.70; O, 7.28.

4.1.37. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid (8II-j)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.57 – 7.50 (m, 1H), 7.44 (dd, J = 7.7, 4.9 Hz, 1H), 7.36 (s, 1H), 7.32 (t, J = 8.0 Hz, 2H), 7.26 (d, J = 7.7 Hz, 1H), 7.19 – 7.08 (m, 6H), 6.92 (t, J = 7.5 Hz, 1H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7, 162.3, 147.1, 141.6, 141.0, 135.9, 133.8, 131.2, 129.6, 128.5, 128.2, 125.9, 123.9, 122.3, 120.9, 120.0, 116.3, 114.4, 110.8, 106.4. MS (ESI): 459.09($C_{25}H_{16}ClFN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}ClFN_4O_2$: C, 65.44; H, 3.51; Cl, 7.73; F, 4.14; N, 12.21; O, 6.97; Found: C, 65.42; H, 3.50; Cl, 7.71; F, 4.12; N, 12.22; O, 6.98.

4.1.38. (E)-1-(4-chlorophenyl)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-k)

^{1}H NMR (600 MHz, DMSO) δ 9.59 (s, 1H), 8.65 (s, 1H), 8.46 (s, 2H), 7.64 (d, J = 8.7 Hz, 3H), 7.59 (d, J = 8.4 Hz, 3H), 7.55 – 7.47 (m, 3H), 7.43 (d, J = 8.4 Hz, 1H).^{13}C NMR (150 MHz, DMSO) δ 176.2, 163.5, 162.4, 154.5, 142.9, 140.4, 138.2, 135.6, 133.6, 130.2, 129.8, 129.8, 129.4, 129.1, 127.0, 125.7, 125.0, 124.3, 110.7, 109.0. MS (ESI): 475.07($C_{25}H_{16}Cl_2N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{25}H_{16}Cl_2N_4O_2$: C, 63.17; H, 3.39; Cl, 14.92; N, 11.79; O, 6.73; Found: C, 63.16; H, 3.40; Cl, 14.91; N, 11.80; O, 6.75.

4.1.39. (E)-5-(((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carboxylic acid (8II-l)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 7.4 Hz, 1H), 7.55 – 7.45 (m, 5H), 7.45 – 7.39 (m, 2H), 7.36 (d, J = 8.5 Hz, 2H), 7.33 – 7.28 (m, 1H), 7.12 (dd, J = 8.5, 2.2 Hz, 1H), 2.34 – 2.32(t, J = 6 Hz, 3H) .^{13}C NMR (150 MHz, $CDCl_3$) δ 166.4, 158.8, 152.9, 147.7, 147.1, 138.5, 134.3, 129.8, 124.7, 124.4, 124.3, 124.2, 123.9, 123.7, 121.6, 119.1, 117.8, 116.0, 109.0, 103.8, 22.6. MS (ESI): 455.12($C_{26}H_{19}ClN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}ClN_4O_2$: C, 68.65; H, 4.21; Cl, 7.79; N, 12.32; O, 7.03; Found: C, 68.64; H, 4.20; Cl, 7.78; N, 12.31; O, 7.02.

4.1.40. (E)-1-phenyl-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-m)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.72 (s, 2H), 7.80 (s, 2H), 7.53 (d, J = 3.5 Hz, 8H), 7.43 (d, J = 4.6 Hz, 2H), 7.35 (s, 1H), 7.14 – 7.10 (m, 1H), 2.35 – 2.25 (m, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 184.3, 148.4, 142.0, 138.1, 137.6, 136.7, 129.9, 129.7, 129.6, 129.4, 128.5, 125.1, 123.8, 122.1, 120.5, 120.2, 110.8, 107.4, 99.4, 21.2. MS (ESI): 421.16($C_{26}H_{20}N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33; O, 7.61; Found: C, 74.26; H, 4.78; N, 13.31; O, 7.60.

4.1.41. (E)-1-(4-fluorophenyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene) amino)-1H-pyrazole-4-carboxylic acid (8II-n)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.38 – 8.34 (m, 1H), 8.09 (s, 1H), 7.91 (s, 1H), 7.72 (s, 1H), 7.53 (d, J = 8.6 Hz, 3H), 7.35 (s, 3H), 7.12 (d, J = 8.5 Hz, 3H), 2.34 (d, J = 9.2 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 167.0, 164.4, 159.2, 155.2, 147.6, 147.1, 146.1, 139.8, 138.5, 137.8, 132.0, 125.5, 124.4, 123.9, 122.8, 120.4, 119.1, 116.7, 109.9, 94.4, 22.6. MS (ESI): 439.15($C_{26}H_{19}FN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}FN_4O_2$: C, 71.22; H, 4.37; F, 4.33; N, 12.78; O, 7.30; Found: C, 71.20; H, 4.35; F, 4.31; N, 12.79; O, 7.32.

4.1.42. (E)-1-(4-chlorophenyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene) amino)-1H-pyrazole-4-carboxylic acid (8II-o)

^{1}H NMR (600 MHz, DMSO) δ 9.47 (s, 1H), 8.65 (s, 1H), 8.39 (d, J = 26.3 Hz, 2H), 7.80 (s, 1H), 7.68 – 7.39 (m, 8H), 6.83 (s, 1H), 2.58 (s, 3H).^{13}C NMR (150 MHz, DMSO) δ 171.6, 161.1, 158.3, 154.5, 143.0, 139.2, 138.7, 135.6, 133.7, 132.0, 129.8, 129.4, 129.1, 127.0, 125.7, 125.4, 125.0, 124.8, 121.1, 109.0, 24.2. MS (ESI): 455.12($C_{26}H_{19}ClN_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{26}H_{19}ClN_4O_2$: C, 68.65; H, 4.21; Cl, 7.79; N, 12.32; O, 7.03; Found: C, 68.66; H, 4.20; Cl, 7.77; N, 12.31; O, 7.01.

4.1.43. (E)-1-(p-tolyl)-5-(((2-(p-tolyl)-1H-indol-3-yl)methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-p)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.70 (s, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.62 (s, 1H), 7.52 (dd, J = 19.3, 8.0 Hz, 4H), 7.45 – 7.38 (m, 3H), 7.30 (dd, J = 13.8, 6.9 Hz, 1H), 7.14 (dd, J = 16.9, 8.1 Hz, 1H), 2.34 (d, J = 8.7 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 166.5, 160.0, 154.0, 150.9, 141.6, 140.2, 139.6, 137.4, 129.8, 129.5, 128.5, 128.1, 127.0, 124.7, 124.4, 123.9, 123.7, 119.1, 102.9, 91.8, 22.7. MS (ESI): 434.17($C_{27}H_{22}N_4O_2$, [M + H] $^+$). Anal. Calcd for $C_{27}H_{22}N_4O_2$: C, 74.64; H, 5.10; N, 12.89; O, 6.97; Found: C, 74.65; H, 5.12; N, 12.88.

4.1.44. (E)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carboxylic acid (8II-q)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.57 (d, J = 8.7 Hz, 1H), 8.31 (s, 1H), 7.93 (d, J = 9.2 Hz, 1H), 7.87 (s, 1H), 7.76 (s, 1H), 7.69 (d, J = 9.4 Hz, 1H), 7.54 (d, J = 8.6 Hz, 3H), 7.36 (s, 2H), 7.12 (dd, J = 8.6, 2.1 Hz, 3H), 7.04 (d, J = 6.6 Hz, 1H), 3.71 (dd, J = 14.2, 7.2 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.4, 160.9, 153.4, 148.3, 147.1, 142.3, 139.3, 134.5, 130.4, 130.1, 129.5, 124.4, 123.9, 121.0, 119.1, 113.9, 111.8, 104.2, 60.0. MS (ESI): 437.15($C_{26}H_{20}N_4O_3$,

[M + H] $^{+}$). Anal. Calcd for $C_{26}H_{20}N_4O_3$: C, 71.55; H, 4.62; N, 12.84; O, 11.00; Found: C, 71.54; H, 4.64; N, 12.82; O, 11.01.

4.1.45. (E)-1-(4-fluorophenyl)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-r)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.51 (d, J = 9.7 Hz, 1H), 8.32 (s, 1H), 7.87 (d, J = 14.7 Hz, 1H), 7.74 (s, 1H), 7.69 (d, J = 9.4 Hz, 1H), 7.54 (d, J = 8.8 Hz, 3H), 7.36 (s, 2H), 7.12 (d, J = 8.7 Hz, 3H), 3.71 (d, J = 18.5 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.4, 160.5, 159.4, 147.6, 147.1, 137.9, 136.7, 129.4, 126.5, 125.2, 124.4, 123.9, 121.9, 120.3, 120.1, 119.1, 114.5, 110.7, 98.8, 55.3. MS (ESI): 455.14($C_{26}H_{19}FN_4O_3$, [M + H] $^{+}$). Anal. Calcd for $C_{26}H_{19}FN_4O_3$: C, 68.72; H, 4.21; F, 4.18; N, 12.33; O, 10.56; Found: C, 68.70; H, 4.20; F, 4.17; N, 12.32; O, 10.58.

4.1.46. (E)-1-(4-chlorophenyl)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl) methylene)amino)-1H-pyrazole-4-carboxylic acid (8II-s)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.39 (s, 1H), 7.99 (s, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.54 (d, J = 8.2 Hz, 3H), 7.35 (d, J = 11.3 Hz, 4H), 7.12 (d, J = 8.6 Hz, 3H), 3.75 – 3.69 (m, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 160.2, 152.7, 147.6, 147.1, 138.5, 136.4, 133.0, 129.3, 128.6, 124.4, 123.9, 122.1, 119.3, 119.1, 116.1, 113.4, 111.9, 108.1, 102.9, 56.0. MS (ESI): 471.11($C_{26}H_{19}ClN_4O_3$, [M + H] $^{+}$). Anal. Calcd for $C_{26}H_{19}ClN_4O_3$: C, 66.32; H, 4.07; Cl, 7.53; N, 11.90; O, 10.19; Found: C, 66.30; H, 4.05; Cl, 7.54; N, 11.92; O, 10.18.

4.1.47. (E)-5-(((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carboxylic acid (8II-t)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.39 (s, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.77 (s, 1H), 7.70 – 7.67 (m, 1H), 7.53 (d, J = 8.6 Hz, 3H), 7.35 (s, 3H), 7.14 – 7.10 (m, 3H), 3.86 – 3.75 (m, 3H), 2.34 (t, J = 7.6 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 161.9, 159.4, 138.4, 137.7, 136.8, 132.3, 130.8, 129.3, 129.2, 129.0, 128.6, 128.5, 128.3, 126.6, 126.3, 125.7, 119.5, 119.0, 54.3, 21.2. MS (ESI): 451.17($C_{27}H_{22}N_4O_3$, [M + H] $^{+}$). Anal. Calcd for $C_{27}H_{22}N_4O_3$: C, 71.99; H, 4.92; N, 12.44; O, 10.65; Found: C, 72.00; H, 4.94; N, 12.45; O, 10.66

4.1.48. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-phenyl-1H-pyrazole-4-carboxylic acid (8II-u)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.53 (s, 1H), 8.47 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 1.1 Hz, 2H), 7.69 (dd, J = 8.4, 1.8 Hz, 2H), 7.48 (d, J = 8.2 Hz, 3H), 7.42 (d, J = 8.1 Hz, 1H), 7.34 (dt, J = 21.9, 7.7 Hz, 3H), 7.23 (t, J = 7.6 Hz, 1H).^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 163.6, 155.3, 152.0, 140.8, 136.1, 135.1, 132.5, 130.8, 130.6, 129.8, 126.7, 124.4, 124.4, 123.4, 123.2, 122.6, 122.4, 110.9, 94.0. MS (ESI): 485.05($C_{25}H_{17}BrN_4O_2$, [M + H] $^{+}$). Anal. Calcd for $C_{25}H_{17}BrN_4O_2$: C, 61.87; H, 3.53; Br, 16.46; N, 11.54; O, 6.59; Found: C, 61.88; H, 3.54; Br, 16.47; N, 11.55; O, 6.60.

4.1.49. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid (8II-v)

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.07 (s, 1H), 8.67 (s, 1H), 8.56 (s, 1H), 8.44 – 8.41 (m, 1H), 7.84 (dd, J = 8.9, 5.0 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 5.4 Hz, 2H), 7.36 – 7.33 (m, 2H).^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6, 160.3, 144.2, 135.5, 132.5, 132.4, 130.8, 130.6, 128.9, 126.1, 124.7, 124.6, 123.4, 123.0, 122.3, 116.3, 115.2, 112.9, 111.0, 110.0. MS (ESI): 503.04($C_{25}H_{16}BrFN_4O_2$, [M + H] $^{+}$). Anal. Calcd for $C_{25}H_{16}BrFN_4O_2$: C, 59.66; H, 3.20; Br, 15.88; F, 3.77; N, 11.13; O, 6.36; Found: C, 59.68; H, 3.21; Br, 15.89; F, 3.76; N, 11.12; O, 6.38.

4.1.50. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(4-chlorophenyl)-1H-pyrazole-4-carboxylic acid (8II-w)

^{1}H NMR (600 MHz, DMSO) δ 12.29 (s, 1H), 8.81 (s, 1H), 8.27 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 7.9 Hz, 2H), 7.79 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.5 Hz, 2H), 7.62 (s, 1H), 7.56 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 7.6 Hz, 1H), 7.27 (t, J = 7.1 Hz, 1H), 7.17 (t, J = 6.8 Hz, 1H).^{13}C NMR (150 MHz, DMSO) δ 157.2, 152.8, 145.3, 140.9, 138.8, 137.0, 132.5, 132.0, 131.2, 130.1, 129.0, 126.3, 125.64, 124.2, 123.5, 122.4, 112.4, 111.5, 93.7. MS (ESI): 519.01 ($C_{25}H_{16}BrClN_4O_2$, [M + H] $^{+}$). Anal. Calcd for $C_{25}H_{16}BrClN_4O_2$: C, 57.77; H, 3.10; Br, 15.37; Cl, 6.82; N, 10.78; O, 6.16; Found: C, 57.78; H, 3.12; Br, 15.38; Cl, 6.81; N, 10.79; O, 6.14.

4.1.51. (E)-5-(((2-(4-bromophenyl)-1H-indol-3-yl)methylene)amino)-1-(p-tolyl)-1H-pyrazole-4-carboxylic acid (8II-x)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.42 (d, J = 7.8 Hz, 2H), 7.88 (d, J = 7.5 Hz, 2H), 7.58 (s, 1H), 7.54 (d, J = 7.5 Hz, 2H), 7.45 (t, J = 7.8 Hz, 2H), 7.40 (s, 1H), 7.37 – 7.30 (m, 1H), 7.17 (dd, J = 18.1, 10.4 Hz, 1H), 7.11 (d, J = 8.6 Hz, 1H), 2.46 (s, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 165.2, 155.4, 147.1, 144.7, 140.3, 138.6, 132.4, 130.7, 129.4, 128.4, 127.4, 126.6, 124.4, 124.0, 123.2, 122.5, 119.1, 111.0, 103.7, 22.7. MS (ESI): 499.07($C_{26}H_{19}BrN_4O_2$, [M + H] $^{+}$). Anal. Calcd for $C_{26}H_{19}BrN_4O_2$: C, 62.54; H, 3.84; Br, 16.00; N, 11.22; O, 6.41; Found: C, 62.55; H, 3.85; Br, 16.02; N, 11.24; O, 6.40.

4.2. Biological assay

4.2.1. Minimum inhibitory concentration (MIC)

The *in vitro* antibacterial activity for intermediate **4**, **7** were evaluated using the agar-dilution method [22]. The MIC values were calculated separately.

4.2.2. Half maximal inhibitory concentration (IC_{50})

IC_{50} values of all target compounds were determined by MTT assay in the 96-well plates [28]. Compounds and control drugs (1.0 mg) were dissolved in DMSO (5 mL) and the solution was diluted with water (5 mL). The prepared drug solution was diluted with a solvent in Eppendorf tubes to a concentration gradient of 50 μg/mL, 12.5 μg/mL, 6.25 μg/mL and 3.125 μg/mL. A small amount of the lawn was taken from the slope of the fresh strain, inoculated in a medium suitable for the growth of the experimental bacteria, and the bacteria were cultured in an incubator at 37 °C for 24 h. After the culture, 2–3 mL of sterile physiological saline was added, and the cells were thoroughly washed with a sterile inoculation needle, and then the suspension was aspirated from the inclined surface by a sterile pipette with a cotton mouth and counted in sterile test tubes. The bacteria were further diluted 50-fold with LB-Broth-Agar-Medium to a final concentration of 10^3–10^5 CFU/mL. The first strip of the 96-well plate was used as a blank control, 100 μL of the medium was added, and the second was a positive control, and 100 μL of the bacterial suspension was added. 90 μL of the bacterial suspension and 10 μL of the drug solution were added to the remaining wells. The concentration of each drug solution was treated 3 times. The 96-well plates were then placed in a 38 °C incubator for 24 h. Then, 10 μL MTT solution (4 mg/mL) was added per well and incubated for 4 h. Finally, 100 μL SDS lysate was added to each well for further 12 h, and the OD (optical density) values at a wavelength of 600 nm were measured with Synergy™ HTX Multi-Mode Microplate Reader. The statistical analyses were performed by SPSS software (SPSS Statistic 23.0).

4.2.3. Enzyme inhibitory activity assay

The *in vitro* enzyme inhibitory activity of the selected compound was carried out by the literature [29].

4.3. 3D(Three-dimensional) Quantitative structure activity relationships

The 3D QSAR simulation processes were performed with Discovery Studio (version 3.5, Supporting Information), using genetic function algorithm (GFA) [30].

4.4. Molecular docking

The crystal structure of bacterial Topo II (PDB ID:2xcs) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). The molecular docking procedures were performed by using CDOCKER protocol for Receptor–Ligand interactions section of Discovery Studio (version 3.5) [31].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was financially supported by National Key R&D Program of China (2017YFD0600805) and Natural Science Foundation of Education Committee of Anhui Province (KJ2018A0162). We are grateful to Prof. Hai-Liang Zhu (State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing) for the computational molecular docking assistance.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103807.

References

[1] Antimicrobial Resistance: Global Report on Surveillance; World Health Organization: Geneva, 2014. http://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/ (accessed Nov 11, 2018).
[2] Chenghui; Shi; Yinyong; Zhang; Ting; Wang; Wenchao; Lu; Shuhua; Bin, Design, Synthesis, and Biological Evaluation of Novel DNA Gyrase-Inhibiting Spiropyrimidinetriones as Potent Antibiotics for Treatment of Infections Caused by Multidrug-Resistant Gram-Positive Bacteria. J. Med. Chem. 62(2019) 2950 − 2973, https://doi.org/10.1021/acs.jmedchem.8b01750.
[3] P. Yorgey, J. Lee, J. Kordel, E. Vivas, P. Warner, D. Jebaratnam, Posttranslational modifications in Microcin B17 define an additional class of DNA gyrase inhibitor, P. Natl. Acad. Sci. USA 91 (1994) 4519–4523, https://doi.org/10.2307/2364688.
[4] Nitiss, John L . DNA topoisomerase II and its growing repertoire of biological functions. Nat. Rev. Cancer. 9 (2009) 327-337, https://doi.org/10.1038/nrc2608.
[5] J.C. Wang, Cellular roles of DNA topoisomerases: a molecular perspective, Nat. Rev. Mol. Cell. Bio. 3 (2002) 430–440, https://doi.org/10.1038/nrm831.
[6] J.J. Champoux, DNA topoisomerases: structure, function, and mechanism, Annu. Rev. Biochem. 70 (2001) 369–413, https://doi.org/10.1146/annurev.biochem.
[7] M. Oblak, M. Kotnik, Solmajer, discovery and development of ATPase Inhibitors of DNA gyrase as antibacterial agents, Curr. Med. Chem. 14 (2007) 2033–2047, https://doi.org/10.2174/092986707781368414.
[8] S.L. Badshah, A.J. Ullah, New developments in non-quinolone-based antibiotics for the inhibiton of bacterial gyrase and topoisomerase IV, Eur. J. Med. Chem. 152 (2018) 393–400, https://doi.org/10.1016/j.ejmech.2018.04.059.
[9] A. Ozdemir, M.D. Altintop, G. Turan-Zitouni, G.A. Ciftci, I. Ertorun, O. Alatas, Z.A. Kaplancikli, Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents, Eur. J. Med. Chem. 89 (2015) 304–309, https://doi.org/10.1016/j.ejmech.2014.10.056.
[10] A.V. Bogdanov, I.F. Zaripova, A.D. Voloshina, A.S. Sapunova, N.V. Kulik, J.K. Voronina, Mironov, VF synthesis and antimicrobial study of novel 1-benzylated water-soluble isatin-3-hydrazones, Chem. Biodivers. 15 (2018) 1800088, https://doi.org/10.1002/cbdv.201800088.
[11] Ming-Zhi Zhang, Qiong Chen, Guang-Fu Yang, A review on recent developments of indole-containing antiviral agents, Eur. J. Med. Chem. 89 (2015) 421–441, https://doi.org/10.1016/j.ejmech.2014.10.065.
[12] Tzvetkov, NT; Kuppers, P; Gastreich, M; Muller, CE. Indazole- and Indole-5-carboxamides: Selective and Reversible Monoamine Oxidase B Inhibitors with Subnanomolar Potency. J. Med. Chem. 57 (2014) 6679-6703, https://doi.org/10.1021/jm500729a.
[13] S.B. Somappa, J.S. Biradar, P. Rajesab, S. Rahber, M.A. Sundar, one-pot synthesis of indole-appended heterocycles as potent anti-inflammatory, analgesic, and CNS depressant agents, Monatsh. Chem. 146 (2015) 2067–2078, https://doi.org/10.1007/s00706-015-1476-x.
[14] G. La Regina, R. Bai, A. Coluccia, V. Famiglini, S. Pelliccia, S. Passacantilli, C. Mazzoccoli, V. Ruggieri, A. Verrico, A. Miele, New indole tubulin assembly inhibitors cause stable arrest of mitotic progression, enhanced stimulation of natural killer cell cytotoxic activity, and repression of hedgehog-dependent cancer, J. Med. Chem. 58 (2015) 5789–5807, https://doi.org/10.1021/acs.jmedchem.5b00310.
[15] P.R. Kamath, D. Sunil, A.A. Ajees, K.S.R. Pai, S.J. Das, Some new indole-coumarin hybrids; synthesis, anticancer and Bcl-2 docking studies, Bioorg. Chem. 63 (2015) 101–109, https://doi.org/10.1016/j.bioorg.2015.10.001.
[16] Indrasena, A.; Riyaz, S.; Mallipeddi, P.L.; Padmaja, P.; Sridhar, B.; Dubey, P.K. Design, synthesis, and biological evaluation of indolylidinepyrazolones as potential anti-bacterial agents. 55 (2014) 5014-5018, https://doi.org/10.1016/j.tetlet.2014.05.131.
[17] J.I. Manchester, D.D. Dussault, J.A. Rose, P.A. Boriack-Sjodin, M. Uria-Nickelsen, G. Ioannidis, S. Bist, P. Fleming, K.G.H.J. Bioorganic, Discovery of a novel azaindole class of antibacterial agents targeting the ATPase domains of DNA gyrase and Topoisomerase IV, Bioorg. Med. Chem. Lett. 22 (2012) 5150–5156, https://doi.org/10.1016/j.bmcl.2012.05.128.
[18] N. Zidar, H. Macut, T. Tomasic, M. Brvar, S. Montalvao, P. Tammela, T. Solmajer, L.P. Masic, J. Ilas, D. Kikelj, N-Phenyl-4,5-dibromopyrrolamides and N-Phenylindolamides as ATP Competitive DNA gyrase B inhibitors: design, synthesis, and evaluation, J. Med. Chem. 58 (2015) 6179–6194, https://doi.org/10.1021/acs.jmedchem.5b00775.
[19] Zhang, J.; Yang, Q.; Cross, J.B.; Romero, J.A.C.; Poutsiaka, K.M.; Epie, F.; Bevan, D.; Wang, B.; Zhang, Y.; Chavan, A.; Zhang, X.; Moy, T.; Daniel, A.; Kien, N.; Chamberlain, B.; Carter, N.; Shotwell, J.; Silverman, J.; Metcalf, C.A., III; Ryan, D.; Lippa, B.; Dolle, R.E., Discovery of Azaindole Ureas as a Novel Class of Bacterial Gyrase B Inhibitors. J. Med. Chem. 58 (2015) 8503-8512, https://doi.org/10.1021/acs.jmedchem.5b00961.
[20] G. Sanna, S. Madeddu, G. Giliberti, S. Piras, M. Struga, M. Wrzosek, G. Kubiak-Tomaszewska, A.E. Koziol, O. Savchenko, T. Lis, J. Stefanska, P. Tomaszewski, M. Skrzycki, D. Szulczyk, Synthesis and biological evaluation of novel indole-derived thioureas, Molecules 23 (2018) 2067–2078, https://doi.org/10.3390/molecules23102554.
[21] A. Singh, N. Kumar, D. Sood, S. Singh, A. Awasthi, V. Tomar, R. Chandra, Designing of a novel indoline scaffold based antibacterial compound and pharmacological evaluation using chemoinformatics approach, Curr. Top. Med. Chem. 18 (2018) 2056–2065, https://doi.org/10.2174/1568026619666181129125524.
[22] L. Hao, R. Zi-Li, W. Wei, G. Jie-Xiu, C. Ming-Jie, M. Quan-Wei, W. Jie-Chun, Novel coumarin-pyrazole carboxamide derivatives as potential topoisomerase II inhibitors: Design, synthesis and antibacterial activity, Eur. J. Med. Chem. 157 (2018) 81–87, https://doi.org/10.1016/j.ejmech.2018.07.059.
[23] S. Rostamizadeh, Z. Daneshfar, H. Moghimi, Synthesis of sulfamethoxazole and sulfabenzamide metal complexes; evaluation of their antibacterial activity, Eur. J. Med. Chem. 171 (2019) 364–371, https://doi.org/10.1016/j.ejmech.2019.03.002.
[24] Z. Ju, M. Su, J. Hong, E.L. Kim, H.R. Moon, H.Y. Chung, S. Kim, J.H. Jung, Design of balanced COX inhibitors based on anti-inflammatory and/or COX-2 inhibitory ascidian metabolites, Eur. J. Med. Chem. 180 (2019) 86–98, https://doi.org/10.1016/j.ejmech.2019.07.016.
[25] L.P. Lu, F.Z. Suo, Y.L. Feng, L.L. Song, Y. Li, Y.J. Li, K.T. Wang, Synthesis and biological evaluation of vanadium complexes as novel anti-tumor agents, Eur. J. Med. Chem. 176 (2019) 1–10, https://doi.org/10.1016/j.ejmech.2019.04.073.
[26] D.K. Mahapatra, S.K. Bharti, V. Asati, S.K. Singh, Perspectives of medicinally privileged chalcone based metal coordination compounds for biomedical applications, Eur. J. Med. Chem. 174 (2019) 142–158, https://doi.org/10.1016/j.ejmech.2019.04.032.
[27] C. Nastasa, D.C. Vodnar, I. Ionut, A. Stana, D. Benedec, R. Tamaian, O. Oniga, B. Tiperciuc, Antibacterial evaluation and virtual screening of new thiazolyl-triazole schiff bases as potential DNA-gyrase inhibitors, Int. J. Mol. Sci. 19 (2018) 222, https://doi.org/10.3390/ijms19010222.
[28] L. Rojas, L. Monzote, I. Sariego, Setting a colorimetric assay with MTT for assessment of trichomonicidal activity, Curr. Clin. Pharmacol. 9 (2014) 283–287, https://doi.org/10.2174/1574884709031408061202l8.
[29] Y. Oyamada, J.-I. Yamagishi, T. Kihara, H. Yoshida, M. Wachi, H. Ito, Mechanism of inhibition of DNA gyrase by ES-1273, a novel DNA gyrase inhibitor, Microbiol. Immunol. 51 (2007) 977–984, https://doi.org/10.1111/j.1348-0421.2007.tb03994.x.
[30] Y. Dai, Q. Wang, X. Zhang, S. Jia, H. Zheng, D. Feng, P. Yu, Molecular docking and QSAR study on steroidal compounds as aromatase inhibitors, Eur. J. Med. Chem. 45 (2010) 5612–5620, https://doi.org/10.1016/j.ejmech.2010.09.011.
[31] G.S. Wu, D.H. Robertson, C.L. Brooks, M. Vieth, Detailed analysis of grid-based molecular docking: a case study of CDOCKER—A CHARMm-based MD docking algorithm, J. Comput. Chem. 24 (2003) 1549–1562, https://doi.org/10.1002/jcc.10306.