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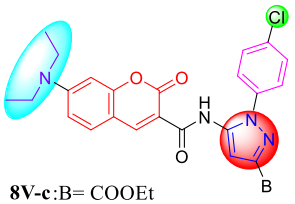
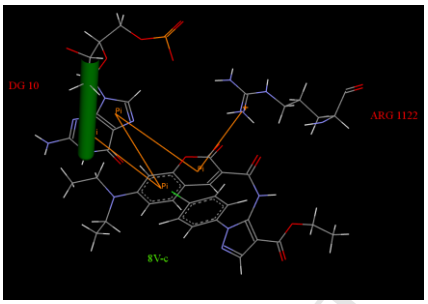


8V-c:B= COOEt
8V-k:B= COOH

Molecular docking(eg. 8V-c)

Topo II (PDB entry:2xcs)



	MIC(mg/L)			
	S. aureus	*L. monocytogenes*	*E. coli*	*Salmonella*
8V-c	1	0.5	2	0.05
8V-k	2	2	4	0.125

Novel Coumarin-Pyrazole Carboxamide derivatives as potential Topoisomerase II Inhibitors: Design, synthesis and antibacterial activity

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Abstract: The identification of novel Topoisomerase II (Topo II) inhibitors is one of the most attractive directions in the field of bactericide research and development. In our ongoing efforts to pursue the class of inhibitors, six series of 70 novel coumarin-pyrazole carboxamide derivatives were designed and synthesized. As a result of the evaluation against four destructive bacteria, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella*. Compound **8III-k** (MIC=0.25 mg/L) showed considerable inhibitory activity than ciprofloxacin (MIC=0.5 mg/L) against *Escherichia coli* and **8V-c** (MIC=0.05 mg/L) exhibited excellent antibacterial activity than ciprofloxacin (MIC=0.25 mg/L) against *Salmonella*. The selected compounds (**8III-k**, **8V-c** and **8V-k**) exhibit potent inhibition against Topo II and Topo IV with IC_{50} values (9.4**–**25mg/L). Molecular docking model showed that the compounds **8V-c** and **8V-k** can bind well to the target by interacting with amino acid residues. It will provide some valuable information for the commercial Topo II inhibiting bactericides.

Keywords: topoisomerase II inhibitors, coumarin, pyrazole, molecular docking, 3D-QSAR, antibacterial activity

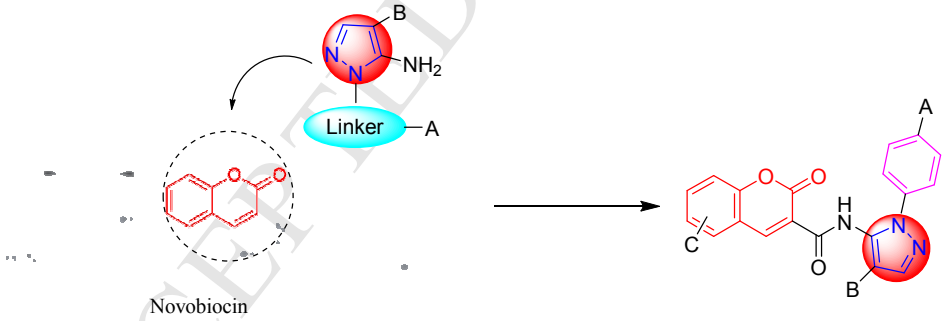
1 INTRODUCTION

Bacterial DNA topoisomerase II (Topo II, EC 5.99.1.3) has been extensively exploited as a target in antimicrobial chemotherapy.[1] As a subclass of the topoisomerase family, Topo II are found in all organisms from bacteria to human, and even in some viruses.[2-4] DNA Topo II enables cells to escape this fate by cutting the DNA duplex, passing the cleavage body through the second DNA duplex and connecting the cleaved DNA. There are two major structural types of Topo II inhibitors, the quinolones and the coumarins. However, mutations in the quinolone binding site of Topo II result in resistance to quinolones.[5, 6] Novobiocin as a representative of coumarin antibiotics, has a good inhibitory effect on DNA topoisomerase. Coumarins form a vast class of natural products widely occurring as secondary plant metabolites.[7, 8] More than 1800 different natural coumarins have been discovered and described to date, many of them exhibiting high levels of biological activity, such as insecticidal,[9] antioxidant,[10] anticancer,[11, 12] anti-HIV,[13] antifungal,[14] antibacterial,[15] antimicrobial,[16] and antibiotic activities.[17, 18] Because of its high bioactivities and naturally occurring scaffold, some efforts focus on the development of novel coumarin-based therapeutic agents.[19-21]

From the other hand, increasing bacterial resistance has led to the demand for new bactericides.[22] In addition, the toxicity of the novel bactericides and whether they are good for the environment account for a large proportion of current drug design. In recently years, pyrazole as an antimicrobial agent has a wide range of antimicrobial activity after the discovery of natural pyrazole C-glycosides such as pyrazolofuran, and is therefore of great interest.[23] Consequently, several pyrazole derivatives that exhibited antimicrobial activity are reported by Tanitame and coworkers.[24, 25] Therefore, such medicinal properties associated with these two heterocycles

render them as useful structural units in drug research. Our current research efforts are to find natural product-based antibacterial agents as Topo II inhibitors.

We chose novobiocin as primer molecule, introduced the pyrazole ring and binding it to coumarin with an amide bond to design the target scaffold. In our previous work,[26] we found that the introduction of a benzene ring as a linker on the pyrazole ring can increase the number of the degree of combination of the compound and the target, finally got the target scaffold (**scheme 1**). In order to generate a new class of Topo II inhibitors, six series totaling 70 compounds were used for testing the antibacterial activity against two gram-positive bacteria, namely *Staphylococcus aureus* and *Listeria monocytogenes* and two gram-negative bacteria, namely *Escherichia coli* and *Salmonella*. In addition, molecular docking analysis and structure-activity relationship (SAR) studies were performed on all derivatives to determine critical structural factors responsible for their antibacterial efficacy.



Scheme 1. Design of the target scaffold

2 MATERIALS AND METHODS

2.1 General methods

All chemicals were purchased from Energy, Meryer, and Aladdin Chemicals and were used as received. ^{1}H NMR and ^{13}C NMR spectra were carried out using Agilent DD2 600 Hz spectrometer with $CDCl_3$ or DMSO as the solvent and tetramethylsilane as the internal standard. 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 Studio3.5. Crystal structure determination of compounds was carried out on a Bruker D8 VENTURE PHOTON.

2.2 General procedure for the synthesis of coumarin-3-carboxylic acids (4)

2-hydroxybenzaldehydes **(1)** (0.02 mol) and 2,2-dimethyl-1,3-dioxane-4,6-dione **(2)** (0.02 mol) were first reacted at room temperature in the presence of a catalytic amount of piperidinyl acetate and then refluxed in ethanol. The product then crystallizes in the solvent. After filtration and washing with ethanol, coumarin-3-carboxylic acid (**4**) was obtained in comparable yield and purity.

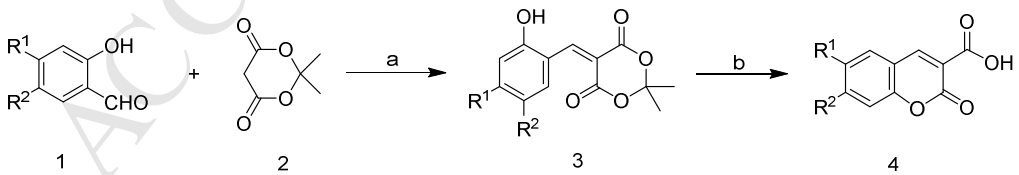


Figure 1. Synthetic route for intermediate **4**. Reagents and conditions: (a) piperidine, acetic acid, EtOH, 25°C; (b) reflux, 2 h.

2.3 General procedure for the synthesis of ethyl 5-amino-1-phenyl-1H-pyrazole-4-carboxylate derivatives (5)

Intermediates **5** was synthesized based on the literature of Stephane L.et al.[27] Dissolve phenylhydrazine hydrochloride with 1.5 equivalents of sodium acetate in ethanol, stir and reflux for 1 hour, filter to obtain filtrate, then add ethyl (E)-2-cyano-3-ethoxyacrylate, stir and reflux for 1 hour, then the solution was rotary evaporated until the volume of the solution was reduced by half. After cooling, solids intermediate **5** can be precipitated, then filtered under vacuum. The resulting crude products are subjected to recrystallization purification.

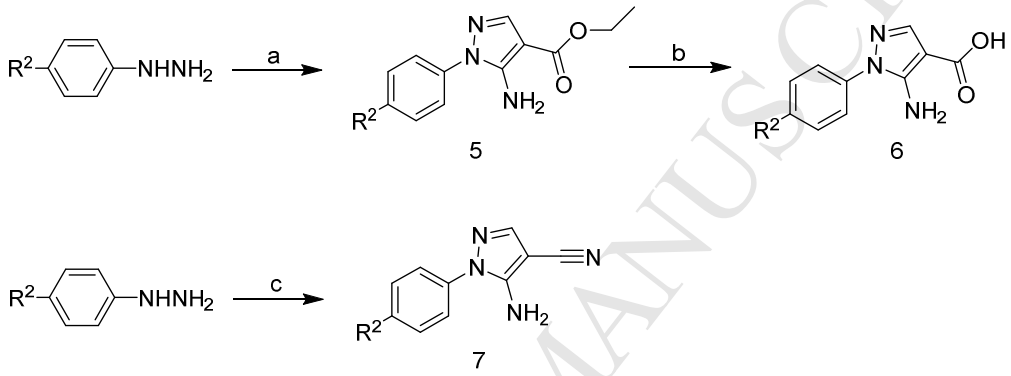


Figure 2. Synthetic route for intermediates **5,6** and **7**. Reagents and conditions: (a) ethyl (E)-2-cyano-3-ethoxyacrylate, H_2O, NaOH, EtOH, 3 h, reflux; (b) H_2O, NaOH, EtOH, 3 h, reflux; (c) 2-(ethoxymethyl)malononitrile, H_2O, NaOH, EtOH, 3 h, reflux.

2.4 General procedure for the synthesis of 5-amino-1-phenyl-1H-pyrazole-4-carboxylic acid derivatives (6)

Intermediates **6** was synthesized according to the literature by ML Bender.[28] Intermediates **5** was dissolved in a solution of ethanol and potassium hydroxide and refluxed for 2 h. After cooling, the pH of the mixture was adjusted to 5-6 by the dropwise addition of 10% HCl solution to give an intermediate **6,** which was filtered under vacuum and recrystallized from ethanol to give the pure products.

2.5 General procedure for the synthesis of 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile

derivatives (7)

Intermediates **7** were synthesized according to the literature by Harden et al.[29] A stirred mixture of p-substituted phenylhydrazine hydrochloride was dissolved in EtOH, then the pH was adjusted to alkaline by the addition of 10% NaOH solution to remove the hydrochloride, which were then refluxed for 3 h with 2-(ethoxymethyl)malononitrile in ethanol. Finally, the mixture is cooled at room temperature, and the intermediates **7** were filtered under vacuum and recrystallized from ethanol to give the pure products.

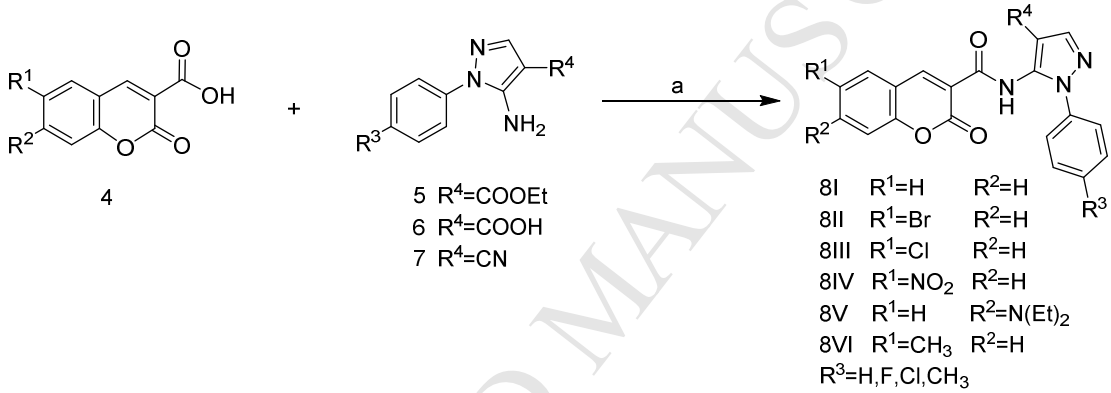


Figure 3. Synthetic routes for target compounds **8I−VI**. Reagent and condition: (a) $POCl_3$, pyridine, 40°C.

2.6 General procedure for the synthesis of

2-oxo-N-(1-phenyl-1H-pyrazol-5-yl)-2H-chromene-3-carboxamide derivatives(8I-8VI)

Intermediate **4** (0.001 mol) and **5** (0.001 mol) were dissolved in pyridine, phosphorus oxychloride is slowly added dropwise under ice bath conditions, then at 40-60° C reacted for 5-8 hours. Finally, the mixture was poured into 30 mL saturated Na_2CO_3 solution and stirred well. After the mixture was acidified, the saturated $CuSO_4$ solution was used to remove pyridine, which greatly improved the purity and yield of the product. The data for compounds **8I-8VI** are provided in the supporting information.

2.7 Biological assay

2.7.1 Minimum inhibitory concentration (MIC)[30]

The *in-vitro* antibacterial activity for synthesized compounds **8I-a−8VI-l** were evaluated using the agar-dilution method.[31, 32] Twofold serial dilutions of the compounds and reference drugs (ciprofloxacin and novobiocin) were prepared in LB-Broth-Agar-Medium. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted LB-Broth-Agar-Medium was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 mg/L, and the MIC values were calculated separately.

2.7.2 Enzyme Inhibitory effects against Topo II and Topo IV

The *in vivo* antibacterial activity of the target compounds was carried out on by the methods of Sato et al.[33] and Peng and Marians[34].

2.8 3D(Three-dimensional)-QSAR analysis

The three-dimensional(3D) Quantitative structure–activity relationship was performed with Discovery Studio3.5, using genetic function algorithm (GFA).[35]

2.9 Molecular docking

The crystal structure of bacterial DNA Topo II (PDB code:2xcs) was downloaded from the RCSB Protein Data Bank. The molecular docking procedures were performed by using CDOCKER protocol for receptor–ligand interactions section of Discovery Studio3.5.[36]

3 RESULTS AND DISCUSSION

3.1 Chemistry

The chemical synthesis routes of **8I-a−8VI-l** are shown in **Figure 1−3**. For the synthesis of intermediate **4**(**Figure 1**), 2-hydroxybenzaldehydes (**1**) and 2,2-dimethyl-1,3-dioxane-4,6-dione (**2**) were first reacted at room temperature in the presence of a catalytic amount of piperidinyl acetate and then refluxed in ethanol. The synthesis process is very easy because of the good solubility in the solvent and the very fast crystal formation. After filtration and washing with ethanol, coumarin-3-carboxylic acids derivatives (**4**) were obtained with considerable yields and purity. The intermediates **5** and **7** were synthesized by stirring and refluxing the mixture of p-substituted phenyl hydrazine hydrochlorides and ethyl (E)-2-cyano-3-ethoxyacrylate/ethoxymethylenemalononitrile in the ethanol medium respectively. Intermediate **5** can be hydrolyzed to get **6**. The target compound under mild conditions by using $POCl_3$ in pyridine amine and the acid converted to the amide by convenient and efficient one-pot process is obtained. We optimized the post-treatment conditions of the products, using saturated Na_2CO_3 to liberate pyridine. After the system was acidified, the saturated $CuSO_4$ was used to remove pyridine, which greatly improved the purity and yield of the product. The chemical structures of all synthesized compounds were determined by ^{1}H NMR, ^{13}C NMR, and MS. The crystal data and checkcif files for compounds **8IV-a, 8V-f** and **8V-g** are listed in supporting information, the crystal structures are shown in **Figure 4**. Crystallographic data (excluding structure factors) for the structure were deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-1842674 (**8IV-a**), No. CCDC-1842675(**8V-f**), No. CCDC-1842676(**8V-g**).

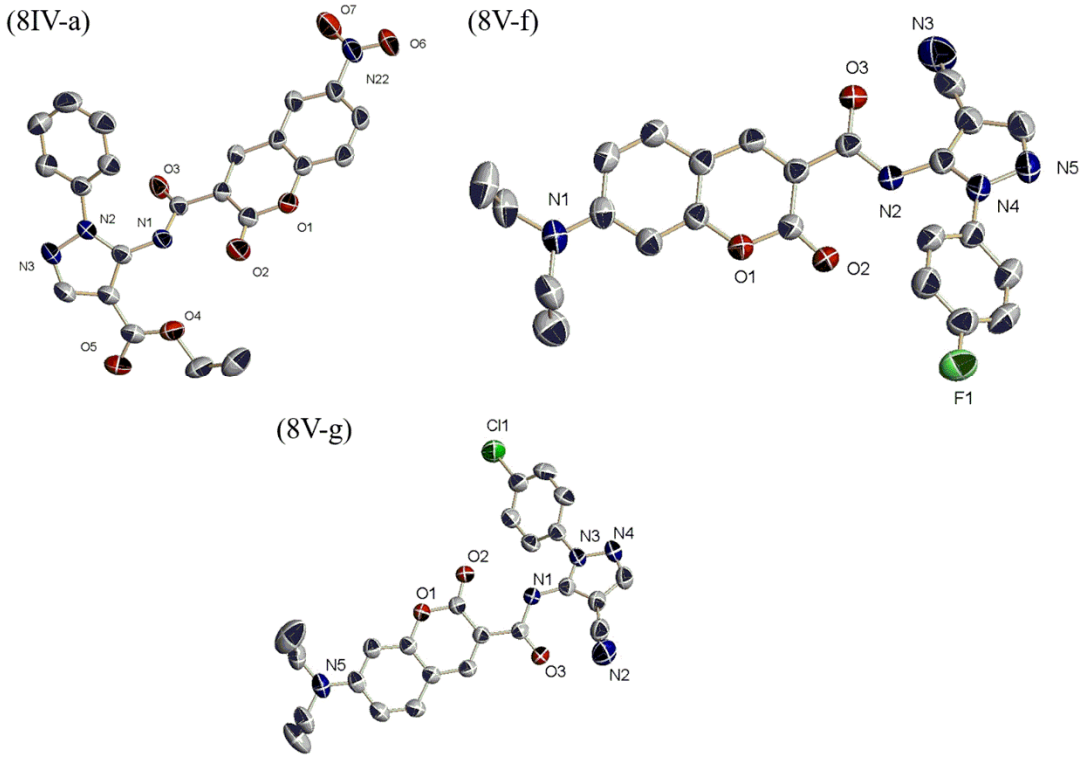


Figure 4. The crystal structures of **8IV-a**, **8V-f** and **8V-g** displacement ellipsoids.

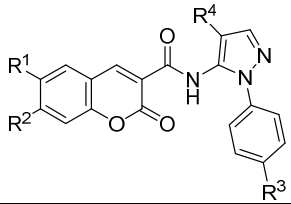
3.3 Antibacterial activity and SAR Discussion

All newly synthesized compounds were evaluated for *in-vitro* antibacterial activity against two gram-positive bacteria, namely *Staphylococcus aureus* and *Listeria monocytogenes* and two gram-negative bacteria, namely *Escherichia coli* and *Salmonella*. Novobiocin and ciprofloxacin were used as a reference standard. The results of the *in-vitro* antibacterial activity screening of the test compounds are summarized in **Table 1**.

Most of the derivatives exhibited moderate to potent antibacterial activities against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella*, demonstrating the rationality of our design strategy. First, we discuss the activities of compounds against gram-positive bacteria. Among them, nine compounds (i.e., **8II-f**, **8III-k**, **8III-a**, **8V-a**, **8V-l**, **8V-I**, **8V-g**, **8VI-g** and **8VI-l**) were found to display improved antibacterial activities against

Staphylococcus aureus compared with novobiocin (MIC = 2 mg/L), with MIC values ranging from 0.5 to 2 mg/L. Particularly, compound **8III-k**, determined as the most potent inhibitor against *Staphylococcus aureus* with a MIC value of 0.5 mg/L, showed four-fold higher inhibitory activity than novobiocin. However, the MIC value of ciprofloxacin is better than it. Three derivatives (i.e., **8II-c**, **8III-k** and **8V-c**) exhibited improved antibacterial activity against *Listeria monocytogenes* in comparison to ciprofloxacin (MIC = 1 mg/L), with MIC values ranging from 0.5 to 1 mg/L. In these derivatives, compound **8V-c**, showed antibacterial activity against *Listeria monocytogenes* with a MIC value at 0.5 mg/L. Obviously, it was better than ciprofloxacin, but much worse than novobiocin. Then, the data on the activity of compounds inhibiting gram-negative bacteria are discussed below. Among them, sixteen derivatives were found to show improved antibacterial activities against *Escherichia coli* compared with novobiocin (MIC = 4 mg/L), with MIC values ranging from 0.25 to 4 mg/L. Especially, compound **8III-k**, identified as the most potent inhibitor against *Escherichia coli* with a MIC value at 0.25 mg/L, showed 16-fold higher inhibitory activity than novobiocin and two-fold than ciprofloxacin. Five derivatives (i.e., **8II-g**, **8IV-g**, **8V-c**, **8V-i** and **8V-k**) were found to display improved antibacterial activities against *Salmonella* compared with ciprofloxacin (MIC = 0.25 mg/L), with MIC values ranging from 0.05 to 0.25 mg/L. Noticeably, compound **8V-c**, identified as the most potent inhibitor against *Salmonella* with a MIC value at 0.05 mg/L, showed five-fold higher inhibitory activity than novobiocin and ten-fold than ciprofloxacin.

Table 1. Chemical Structures of Compounds 8I-VI and MIC Values against *S. aureus, L. monocytogenes, E. coli* and *Salmonella*.



Compd.	R^1	R^2	R^3	R^4	MIC(mg/L)			
					S.a[a]	*L.m*[a]	*E.c*[a]	*S.e*[a]
8I-a	H	H	H	COOEt	16	64	64	2
8I-b	H	H	F	COOEt	> 128	> 128	> 128	> 128
8I-c	H	H	Cl	COOEt	16	64	64	16
8I-d	H	H	CH_3	COOEt	> 128	32	> 128	> 128
8I-e	H	H	H	CN	16	64	64	32
8I-f	H	H	F	CN	> 128	64	> 128	> 128
8I-g	H	H	Cl	CN	4	32	32	16
8I-h	H	H	CH_3	CN	8	> 128	32	> 128
8I-i	H	H	H	COOH	> 128	32	> 128	> 128
8I-j	H	H	F	COOH	> 128	> 128	> 128	> 128
8I-k	H	H	Cl	COOH	32	32	64	32
8I-l	H	H	CH_3	COOH	64	> 128	64	> 128
8II-a	Br	H	H	COOEt	16	16	16	64
8II-b	Br	H	F	COOEt	64	> 128	4	> 128
8II-c	Br	H	Cl	COOEt	4	1	8	32
8II-d	Br	H	CH_3	COOEt	32	2	32	2
8II-e	Br	H	H	CN	> 128	> 128	> 128	> 128
8II-f	Br	H	F	CN	2	64	64	> 128
8II-g	Br	H	Cl	CN	4	16	8	0.25
8II-h	Br	H	CH_3	CN	> 128	8	> 128	64
8II-i	Br	H	H	COOH	> 128	> 128	> 128	> 128

8II-j	Br	H	F	COOH	> 128	32	4	32
8II-k	Br	H	Cl	COOH	32	32	4	32
8II-l	Br	H	CH_3	COOH	64	> 128	64	> 128
8III-a	Cl	H	H	COOEt	4	64	16	64
8III-b	Cl	H	F	COOEt	> 128	> 128	64	64
8III-c	Cl	H	Cl	COOEt	2	32	4	32
8III-d	Cl	H	CH_3	COOEt	4	2	64	> 128
8III-e	Cl	H	CH_3	CN	16	32	32	64
8III-f	Cl	H	F	CN	32	32	32	64
8III-g	Cl	H	Cl	CN	4	8	4	64
8III-h	Cl	H	CH_3	CN	> 128	> 128	> 128	> 128
8III-i	Cl	H	H	COOH	32	32	32	> 128
8III-j	Cl	H	F	COOH	64	32	32	> 128
8III-k	Cl	H	Cl	COOH	0.5	0.5	0.25	4
8III-l	Cl	H	CH_3	COOH	> 128	16	> 128	> 128
8IV-a	NO_2	H	H	COOEt	> 128	> 128	> 128	> 128
8IV-b	NO_2	H	F	COOEt	32	8	64	> 128
8IV-c	NO_2	H	Cl	COOEt	32	32	64	64
8IV-d	NO_2	H	CH_3	COOEt	> 128	> 128	> 128	> 128
8IV-e	NO_2	H	H	CN	> 128	> 128	> 128	> 128
8IV-f	NO_2	H	F	CN	> 128	> 128	> 128	> 128
8IV-g	NO_2	H	Cl	CN	16	8	4	0.125
8IV-h	NO_2	H	CH_3	CN	> 128	> 128	> 128	> 128
8IV-i	NO_2	H	H	COOH	64	> 128	64	64
8IV-j	NO_2	H	F	COOH	> 128	> 128	> 128	> 128
8IV-k	NO_2	H	Cl	COOH	32	32	32	32
8IV-l	NO_2	H	CH_3	COOH	> 128	> 128	4	64

8V-a	H	$N(Et)_2$	H	COOEt	64	64	64	64
8V-b	H	$N(Et)_2$	F	COOEt	> 128	> 128	> 128	> 128
8V-c	H	$N(Et)_2$	Cl	COOEt	1	0.5	2	0.05
8V-d	H	$N(Et)_2$	CH_3	COOEt	> 128	> 128	> 128	> 128
8V-e	H	$N(Et)_2$	H	CN	4	16	32	32
8V-f	H	$N(Et)_2$	F	CN	64	> 128	64	64
8V-g	H	$N(Et)_2$	Cl	CN	2	8	2	16
8V-h	H	$N(Et)_2$	CH_3	CN	> 128	> 128	> 128	> 128
8V-i	H	$N(Et)_2$	H	COOH	2	4	8	0.25
8V-j	H	$N(Et)_2$	F	COOH	32	16	32	16
8V-k	H	$N(Et)_2$	Cl	COOH	2	2	4	0.125
8V-l	H	$N(Et)_2$	CH_3	COOH	64	64	64	64
8VI-a	CH_3	H	H	COOEt	32	32	16	16
8VI-c	CH_3	H	Cl	COOEt	8	16	4	8
8VI-d	CH_3	H	CH_3	COOEt	32	> 128	64	> 128
8VI-e	CH_3	H	H	CN	64	64	8	8
8VI-f	CH_3	H	F	CN	> 128	> 128	> 128	> 128
8VI-g	CH_3	H	Cl	CN	2	16	4	2
8VI-i	CH_3	H	H	COOH	64	32	32	32
8VI-j	CH_3	H	F	COOH	64	32	4	32
8VI-k	CH_3	H	Cl	COOH	16	16	8	16
8VI-l	CH_3	H	CH_3	COOH	32	> 128	64	> 128
CIP[b]					0.125	1	0.5	0.25
NB[c]					2	0.25	4	0.5

[a]Abbreviations: *Staphylococcus aureus* (ATCC-12600); *Listeria monocytogenes*(ATCC-15313); *Escherichia coli*(ATCC-25922); *Salmonella*(ATCC-9184). [b]Ciprofloxacin. [c] Novobiocin.

It can be observed from **Table 1** that chlorine substituted on the benzene ring at the R^3 site was beneficial to antibacterial activity of the compounds when substitution groups on the benzopyran ring were determined. Intriguingly, the introduction of diethylamino (**8V series**) at R^2 showed noticeable enhanced effects compared with other series. For *Staphylococcus aureus*, when R^1 position was substituted with other moieties except nitro, the antibacterial activities

were increased. When R^4 is cyano group, there are not much different among them. When R^4 is carboxyl or ester group, 8III series exhibit a very good activity than other series. For *Listeria monocytogenes*, when R^4 position was substituted with ester group or carboxyl, **8II-c** and **8III-k** displayed improved antibacterial activities than others respectively. When R^4 is cyano group, there are not much different among them. For *Escherichia coli*, when R^1 position was substituted with other moieties, the antibacterial activities were increased. And when R^1 is chlorine, **8III-k** exhibit best antibacterial activity than others. However, when R^1 is other moieties, their performance is general. For *Salmonella*, when R^4 is carboxyl or ester group, their antibacterial activities are less than ideal. When R^1 is bromine and R^4 is cyano group, **8II-g** showed excellent antibacterial effect. Next, we discuss the structure-activity relationship between the **8IV** and **8V** series. The overall antibacterial activities of 8IV series are not ideal except **8IV-g** against *Salmonella*. Compared with other series, we speculate that the antibacterial activity is decreased because of the introduction of nitro groups. In addition, 8V series displayed significantly enhanced effects against *Salmonella* than other series. Their difference lies in the introduction of diethylamino so that we believe that diethylamino can act as a special pharmacophore for *Salmonella* inhibition. Every nutshell has a concave and convex side. From the data we observed that the introduction of fluorine and methyl groups resulted in a decrease in antibacterial activity. Taking into account the above SAR results, these findings indicate that the antibacterial efficacy of the designed compounds may be attributed to a combination of factors, such as chlorine substitution at the benzene ring which is attached to pyrazole ring, changes in substituents on the benzopyran ring and diversity of substituents on pyrazole ring.

3.4 Inhibitory effects against Topo II and Topo IV

In order to determine the relationships by which the chlorine, diethylamino, ester group and carboxyl group substituents inducing their antibacterial activity, the inhibitory activity of selected compounds (**8III-k , 8V-c** and **8V-k**) against topoisomerase II and topoisomerase IV isolated from *E. coli* was examined[33, 34]. As shown in **Table 2**, compounds **8V-c** and **8V-k** having the same three substituents but **8V-c** showed more potent inhibition than **8V-k** against the two enzymes. Compounds **8III-k** showed the best suppression effect among them. We suspect that the reason for this difference is that **8III-k** contains two chlorine groups. On the other hand, there was a good correlation between the MICs and the IC_{50}s (**Tables 1 and 2**), suggesting that inhibition of the Topo II and Topo IV by the chlorine, diethylamino, ester group and carboxyl group substituents suppresses bacterial cell growth. These results indicate that the chlorine, diethylamino, ester and carboxyl substituents put a great deal of pressure on the survival of the bacteria.

Table 2. Inhibitory effects of selected compounds against topoisomerase II and topoisomerase IV

Compd.	IC_{50}(mg/L)	
	Topo II [a]	Topo IV [b]
8III-k	0.125	8
8V-c	0.25	8
8V-k	0.25	16
CIP	0.5	8
NB	0.5	4

[a] Topoisomerase II supercoiling activity. [b] Topoisomerase IV decatenation activity.

3.5 3D Quantitative Structure−Activity Relationships

In this study, a total of 3D-QSAR models for four different bacteria were established (see the Support Information for the remaining models). Here, salmonella is used as a target and

compound **8V-k** is taken as the example to show the structure−activity relationships. The pMIC values were calculated on the basis of the *in vitro* activity experiment results and we get the linear regression equation through GFA, r^2 value is 0.964 which explains the accuracy and reliability of the experimental results. The 3D-CoMFA contour maps are shown in **Figure 5**. In the electrostatic map **Figure 5(A)**, the red region indicates that the stronger the negative charge of the substituent in this region is, the more favorable the activity of the compound, and the blue region indicates that the stronger the positive charge of the substituent in this region is, the more favorable the activity of the compound. In the steric map **Figure 5(B)**, the yellow region indicates that the increase of substituents in this region is not conducive to the activity of the compound, while the blue region is the opposite. It can be observed that the electrostatically favorable zone is located in the pyrazole ring, while the electrostatically unfavorable zone covers the space around the carboxyl group. In the steric contour map, the sterically favored region covers a small space near the phenyl ring. The 3D-CoMFA contour maps provide valuable clues for further structural optimization of this class of new Topo II inhibitors.

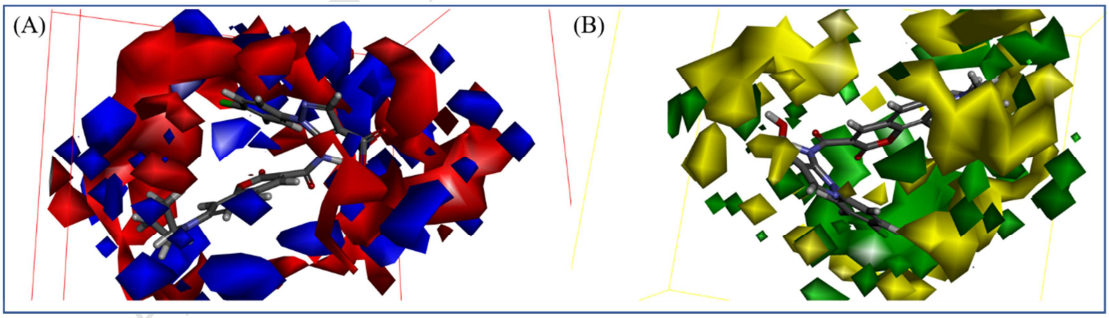


Figure 5. Electrostatic map **(A)** and steric map **(B)** for the CoMFA model.

3.6 Molecular Docking Analysis

After the previous bioactivity test, in order to further obtain the relationship between the structure of the compounds and the inhibition of bacterial activity, all synthesized compounds were molecularly docked using Discover Studio 3.5. The detailed interactions of representative derivative compounds **8V-c** and **8V-k** with Topo II are shown in **Figure 6**. In the binding models of compound **8V-c** and **8V-k**, their scaffolds and amino acid residues linked together tightly, suggesting the pose of **8V-c** and **8V-k** into the Topo II-binding site which revealed that it has suitable shape complementarity with the binding pocket, which means that the rationality of our design scheme. As show in the **Figure 6**, Dg10 established π-π interactions between benzene and furan ring, Arg1122 established cation-π interactions with furan ring. The simulated docking results of **8V-c** and **8V-k** are the same, which may account for their antibacterial effect are almost the same.

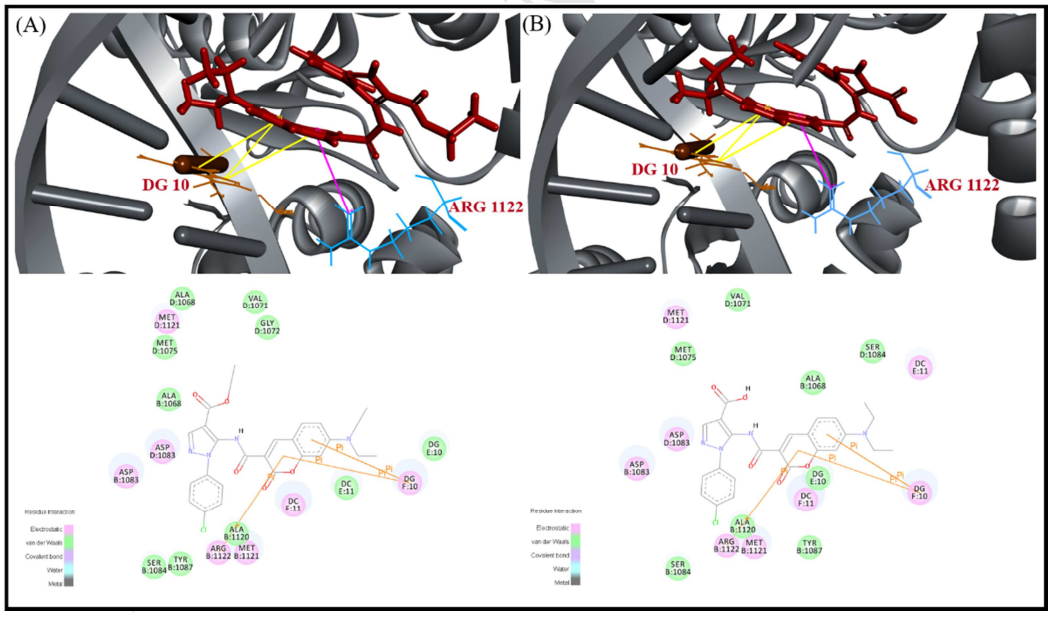


Figure 6. (A) Binding model of **8V-c**. **(B)** Binding model of **8V-k**. The π-π interactions are displayed as yellow solid line, cation-π interactions are displayed as rose-carmine solid line

4. CONCLUSIONS

All of the novel coumarin-pyrazole carboxamide derivatives were synthesized and evaluated. Furthermore, their antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella*, indicating that most of compounds exhibited moderate to potent antibacterial activities. Most surprisingly, **8II-g**,**8IV-g**, **8V-c** and **8V-k** exhibited excellent antibacterial activity against *Salmonella* and compound **8III-k** showed considerable inhibitory activity against three bacteria except *Salmonella*. SAR analysis and molecular docking simulations show that the promising antibacterial efficacy of our designed compounds can be attributed to structural changes including the substitution of chlorine on the benzene and benzofuran rings and modification of the pyrazole ring. Therefore, the rationality of the molecules we designed was fully confirmed in experiments. These results demonstrate that the chlorine groups at different substitution positions and the modification on the pyrazole ring have a very good effect on the antibacterial effect. We are further modifying the coumarin-pyrazole scaffold to obtain more effective Topo II and Topo IV inhibitors and looking for valuable information for further structural optimization and development of novel Topo II inhibitors.

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Highlights

1. Structure-based design and synthesis of novel coumarin-pyrazole carboxamide derivatives are reported
2. Molecular docking and 3D-QSAR were carried out to verify the rationality of the design and the reliability of the activity data of the compounds.
3. *In vitro* and *in vivo* studies reveal that some of the reported compounds possessed good antibacterial activities.