Bioorganic & Medicinal Chemistry 17 (2009) 1207–1213

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis, structure and antibacterial activity of new 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline derivatives

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ARTICLE INFO

Article history:
Received 18 October 2008
Revised 10 December 2008
Accepted 12 December 2008
Available online 24 December 2008

Keywords:
Synthesis
Methyloxazol pyrazole
Antibacterial activity
DNA gyrase inhibitor

ABSTRACT

A series of new 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline derivatives were synthesized. The results showed that compounds **9q** and **10q** can strongly inhibit *Staphylococcus aureus* DNA gyrase and *Bacillus subtilis* DNA gyrase (with IC_{50s} of 0.125 and 0.25 μg/mL against *S. aureus* DNA gyrase, 0.25 and 0.125 μg/mL against *B. subtilis* DNA gyrase). On the basis of the biological results, structure–activity relationships were also discussed.

1. Introduction

DNA gyrase, a typical of type II topoisomerase, has been known to cause DNA replication, transcription and recombination.[1] DNA gyrase catalyzes the ATP-dependent introduction of negative supercoils into bacterial DNA as well as the decatenation and unknotting of DNA.[2] DNA gyrase is mainly inhibited by quinolones and coumarins, some of which are widely used for the treatment of bacterial infectious diseases (e.g., ciprofloxacin).[3,4] Unfortunately, of late, multidrug-resistant Gram-positive bacteria have started posing serious issues in medical science to deal with. To overcome the limitations of the known DNA gyrase inhibitors, it has become imperative to identify new class of compounds.

Many pyrazole derivatives are well acknowledged to possess a wide range of antibacterial bioactivities.[5–8] Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole C-glycoside pyrazofurin which demonstrated a broad spectrum of antimicrobial activity.[9] Some pyrazole derivatives used as potent and selective inhibitors against DNA gyrase are capable to cause bacterial cell death, for example, Hoffmann–La Roche's group[10,11] has developed a new lead DNA gyrase inhibitor (compound **1**, Fig. 1). Recently, Tanitame et al.[12] have found compound **2** (Fig. 1) as potent and selective inhibitors of DNA gyrase. In our previous work, we[13] found compound **3** (Fig. 1) 2,5,5-trimethyl-1,5,6,10b-tetrahydropyrazolo(5,1-*a*)isoquinoline to possess potent antibacterial activity capable of inhibiting *Staphylococcus aureus* DNA gyrase and *Escherichia coli* DNA gyrase (with IC_{50s} of 0.25 μg/mL against *S. aureus* DNA gyrase, 0.125 μg/mL against *E. coli* DNA gyrase). We also reported that some 1-acetyl-5-(substituted-phenyl)-3-methy-4,5-dihydropyrazole derivatives and some 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl) ethanone oxime ester derivatives **4** (Fig. 1) showed antibacterial bioactivities.[13,14] All these selective inhibitors of DNA gyrase shown in Figure 1 contain diarylpyrazole template.

Oxazoles are key building elements of natural products. Among the numerous heterocyclic moieties of biological and pharmacological interest, the oxazole ring is endowed with various activi-

Abbreviations: *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *P. fluorescens*, *Pseudomonas fluorescens*; *S. aureus*, *Staphylococcus aureus*; MTT, 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide; MICs, minimum inhibitory concentrations; NMM, *N*-methylmorpholine; DMSO, dimethyl sulfoxide; DMF, dimethyl formamide, DMAP, *N*,*N*-dimethyl aminopyridine, MH, Mueller–Hinton; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; PMSF, phenylmethyl-sulfonyl fluoride.

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doi:10.1016/j.bmc.2008.12.034

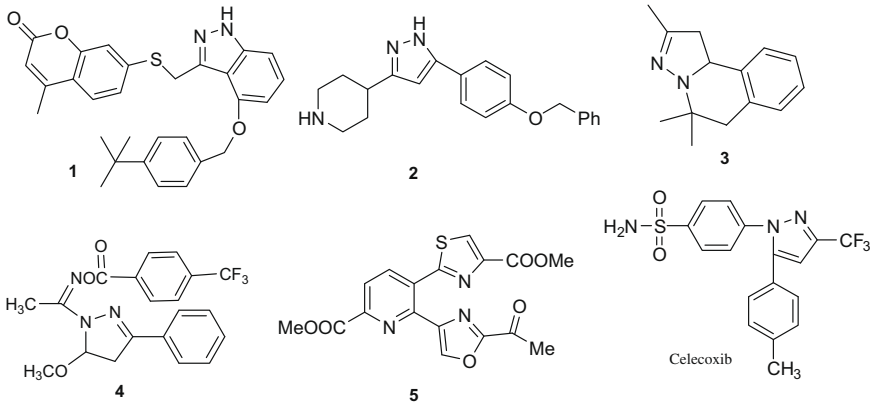


Figure 1. Recently disclosed pyrazole and oxazole as antibacterial inhibitors.

ties.[15,16] The substituted oxazole system occurs in various antivirals[17] and antibiotics.[18] For example, sulfomycin I, a novel thiopeptide antibiotic produced by a subspecies of *Streptomyces viridochromogenes*, which exhibits strong inhibitory activity against gram-positive bacteria (the oxazole structure **5** (Fig. 1)), was isolated from the sulfomycin I. Furthermore, it was found that the same heterocycle subunit is present in berninamycin A,[19] thioxamycin[20] and A10255.[21]

Motivated by the aforementioned findings, we anticipate that the presence of oxazole and aryl-4,5-dihydropyrazole moiety in quinoline part should play an important role in the antimicrobial activities. However, to date, only a few reports have been dedicated to the synthesis and antimicrobial activity evaluation of quinoline containing oxazole and aryl-4,5-dihydropyrazole moiety. Herein, in continuation to extend our research on antibacterial compounds containing aryl-4,5-dihydropyrazole group,[22,23] we designed a series of new 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline derivatives. Fluorinated compounds in general are of significant interest in modern medicinal chemistry. The trifluoromethyl-substituted compounds have been reported to possess biological activities and are often employed as fungicides,[24] analgesic agents,[25] antipyretic agents.[26] So, in our present design of new compounds with improved activity, the role of incorporation of fluorinated moiety into the parent structure was investigated. The results showed that some 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline compounds in this series exhibited potent antibacterial activity.

2. Chemistry

The synthetic route to target compounds is shown in Scheme 1. Dehydrogenation of ketone using mild oxidizing agent HIO_3·DMSO at 100 °C, by following a reported method,[27] proved to be an efficient alternative for the synthesis of α,β-unsaturated ketone **6**.

Compounds **7** were synthesized by cyclization of α,β-unsaturated ketone with hydrazine monohydrate in propionic acid.[28]

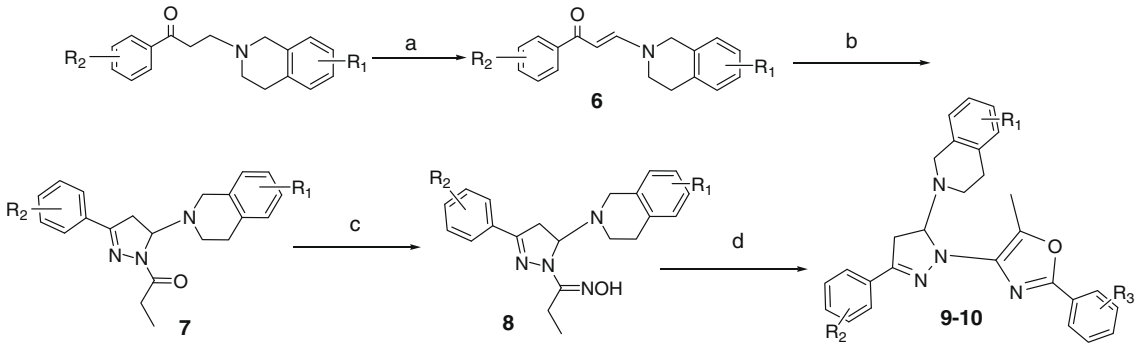
The Compounds **7** were converted into their corresponding oximes **8** by hydroxylamine. Spectroscopic studies of the oximes in solution confirmed the presence of *anti* configuration. The preparation of oximes was the key step for the synthesis of the title compounds. In order to optimize the reaction conditions for preparation of oximes, the syntheses were carried out in presence of various bases, for example: $NaHCO_3$, $KHCO_3$, $NaCH_3CO_2$, pyridine. It was found that a yield up to 65% could be attained when the reaction mixture was refluxed for 15 h in ethanol catalyzed by $NaCH_3CO_2$ and pyridine.

Microwave chemistry, a non conventional popular technique, has been successfully employed in the preparation of oxazole. Lee et al. developed a solvent free method in which (hydroxy-(2,4-dinitrobenzenesulfonyloxy)iodo)benzene reacted with various ketones in a household microwave to form α-((2,4-dinitrobenzene)sulfonyl)oxy ketones, which were then converted into oxazoles through treatment with amides.[29] Microwave irradiation is also known to promote the rapid O,N-acylation–cyclodehydration cascade reaction of oximes and acid chlorides to give oxazoles.[30] Malamas et al.'s method[31] for converting oxime to oxazole under microwave irradiation at various temperatures in pyridine/toluene (5.6:1, moL) actually turned out to be a failure in our hands. Catalytic amount of NMM or DMAP in DMF was added in an attempt to facilitate acyl transfer process which led to a much faster conversion. The major advantage from using microwave irradiation was noted in terms of considerable increase in the product yield. Control reactions with oximes **8** and acid chloride under a range of thermal and optimized microwave conditions are shown in Table 1. Physical appearance, melting points and yields of title compounds **9–10** are shown in Table 2.

3. In vitro antibacterial assay

The activities of synthesized compounds were tested against *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525 and *S. aureus* ATCC 6538 which may be causal agents of some serious infections in humans using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the compounds against four bacteria are presented in Table 3. Also included are the activities of reference compounds kanamycin, penicillin and novobiocin. The results revealed that some of the synthesized compounds exhibited significant antibacterial activity, especially against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538.

The compounds **9e, 9s, 9r** and **10s**, **10r**, **10f** showed antibacterial activities against *B. subtilis* with the MIC of 1.562 μg/mL, comparable to that of positive control penicillin. Compounds **10e** and **10q** with MIC value of 0.78 μg/mL exhibited promising antibacterial activities against *B. subtilis* which were even better than that of the commercial penicillin. Compound **9q** with the MIC of 0.39 μg/mL exhibited potent antibacterial activity against *B. subtilis* which was even better than those of the commercial penicillin, kanamycin and novobiocin. The compounds **9g, 9h, 9r, 9s, 10h** and **10t** showed antibacterial activities against *S. aureus* with the MIC of 3.125 μg/mL, comparable to that of positive control novobi-



9a-9t
R_1: H
R_2: 2-CF_3, 2,4-2Cl, 2-F, -H, 4-furan
R_3: 2,4-2F, 2-F, 2-CF_3, 2-CH=CH_2

10a-10t
R_1: 7-OMe
R_2: 2-CF_3, 2,4-2Cl, 2-F, -H, 4-furan
R_3: 2,4-2F, 2-F, 2-CF_3, 2-CH=CH_2

Scheme 1. Synthesis of new 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline derivatives. Reagent and conditions: (a) HIO_3, DMSO, 100 °C, 15 h; (b) $N_2H_4{\cdot}H_2O$, 98% CH_3CH_2COOH, reflux, 4 h; (c) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, pyridine, reflux, 15 h; (d) substituted-benzoyl chloride, catalytic NMM, DMSO (dry 4 h), 100 °C, 10 min.

Table 1
Yields of **9a** under various reaction conditions

Compd	Solvent	Conditions	Additive	Yield (%)
9a	Pyridine	μW, 180 °C, 10 min	—	—
9a	1,2-Dichlorobenzene	μW, 160 °C, 10 min	—	—
9a	DMF	μW, 100 °C, 10 min	NMM[a]	36
9a	DMF	μW, 120 °C, 20 min	DMAP[b]	32
9a	DMF	μW, 100 °C, 10 min	NMM[a]	40

—: No product.
[a] 5 mol % of NMM was used.
[b] 7 mol % of DMAP was used.

ocin, compounds **9e, 9q, 9t** and **10e, 10f, 10g, 10r, 10s** with the MIC of 1.562 μg/mL, exhibited antibacterial activities against *S. aureus* surpassing that of the commercial novobiocin. The compound **10d** showed antibacterial activity against *P. fluorescens* with MIC of 3.125 μg/mL, comparable to the positive control kanamycin and even better than that of the commercial penicillin. The compounds **9a, 9n, 9p, 10c** and **10j** showed moderate antibacterial activities against *P. fluorescens* with MIC of 12.5 μg/mL. The compound **9n** showed antibacterial activity against *E. coli* with MIC of 6.25 μg/mL, comparable to the positive control penicillin. The compounds **9m** and **9p** showed moderate antibacterial activities against *E. coli* with MIC of 12.5 μg/mL.

From the structure–activity relationships presented in Table 3, it can be concluded that some 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline derivatives showed good activity against Gram positive strains (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 65385), but most of the derivatives displayed poor activity against Gram negative strain (*P. fluorescens* ATCC 13525 and *E. coli* ATCC 35218).

Among all the synthetic compounds, two classifications can be made, one containing furan-phenyl moieties which include **9q–9t** and **10q–10t**, and the other containing substituted phenyl moieties (see Scheme 1). From Table 3 we can see that compounds **9q–9t** (with MICs of 0.39, 1.562, 1.562 and 3.125 μg/mL against *B. subtilis* ATCC 6633, with MICs of 1.562, 3.125, 3.125 and 1.562 μg/mL against *S. aureus* ATCC 65385) and **10q–10t** (with MICs of 0.78, 1.562, 1.562 and 3.125 μg/mL against *B. subtilis* ATCC 6633, with MICs of 0.78, 1.562, 1.562 and 3.125 μg/mL against *S. aureus* ATCC 65385) showed obvious antibacterial activity against the *B. subtilis* ATCC 6633 and *S. aureus* ATCC 65385 strains, while most of the other compounds containing substituted phenyl moieties only showed moderate antibacterial activity (**9a–9d**, **9i–9p**, **10i–10p**). So the group of compounds having furan-phenyl moieties has a much higher inhibitory potency than the rest.

Among the other compounds containing substituted phenyl moieties (difference **R_2**, Scheme 1), they could be divided into two subunits: A-substituted phenyl ring and A, B-substituted phenyl ring. A comparison of the substitution pattern against the *B. subtilis* ATCC 6633 and *S. aureus* ATCC 65385 strains demonstrated that 2,4-disubstituted-phenyl analogs afforded compounds with much higher potency (**9e, 9f, 9g, 9h, 10e, 10f, 10g** and **10h**) than 2 or 4-substituted-phenyl analogs (**9i–9p** and **10i–10p**).

The rationale behind selecting a large number of compounds bearing fluorinated functionalities is to ascertain the role of fluorine in imparting bioactivity. The influence of fluorinated substituent on bioactivity is clearly demonstrated by the observed higher activities against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538. Similarly, it can be derived from the data presented in Table 3 that the nature of **R_3** group in the title compounds significantly influences the antibacterial activity. With a fluorinated substituent (2,4-2F) in the phenyl ring, the compounds exhibited enhanced bioactivity against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538 (**9e**, **9q**, **10e**, **10q**).

To elucidate the mechanism by which the pyrazole derivatives induce antibacterial activity, the inhibitory activities of selected compounds (**9e, 9l, 9f, 9q, 10e, 10j, 10q** and **10s**) were examined against DNA gyrase isolated from *B. subtilis* and *S. aureus*. As shown in Table 4, compounds **9q** and **10q** with potent antibacterial activities strongly inhibited *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase (with IC_{50s} of 0.125 and 0.25 μg/ml against *S. aureus* DNA gyrase, 0.25 and 0.125 μg/ml against *B. subtilis* DNA gyrase). Compound **9f** showed moderate inhibition against the *S. aureus* DNA gyrase (IC_{50} = 1.0 μg/mL). Compound **9l** showed poor inhibition against the *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase (IC_{50} >125 μg/mL).There was a good correlation between the MICs and the IC_{50s} of **9q** and **10q** (Tables 3 and 4), indicating that inhibition of the DNA gyrase by the pyrazole-oxazole derivatives caused inhibition of bacterial cell growth. Bacterial topoisomerase inhibitors sometimes have poor selectivity against human topoisomerase, for example, the compound **9f** showed the same activities against *S. aureus* and *B. subtilis* with the MIC of 3.125 μg/mL, but it showed different inhibition against the *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase (IC_{50} = 5.5 μg/mL, 1.0 μg/mL respectively).

Table 2
Structure, appearance, melting points and yields of title compounds **9**–**10**

Compound	R_1	R_2	R_3	Appearance	Mp (°C)	Yield[a] (%)
9a	H	2-CF_3	2,4-2F	Colorless crystals	62–63	40
9b	H	2-CF_3	2-F	Colorless crystals	59–60	38
9c	H	2-CF_3	2-CF_3	Colorless crystals	59–60	39
9d	H	2-CF_3	2-CH=CH_2	Colorless crystals	61–63	34
9e	H	2,4-2Cl	2,4-2F	Colorless crystals	66–67	35
9f	H	2,4-2Cl	2-F	Colorless crystals	65–66	38
9g	H	2,4-2Cl	2-CF_3	Colorless crystals	66–67	33
9h	H	2,4-2Cl	2-CH=CH_2	Colorless crystals	65–66	36
9i	H	2-F	2,4-2F	Colorless crystals	60–61	41
9j	H	2-F	2-F	Colorless crystals	57–58	42
9k	H	2-F	2-CF_3	Colorless crystals	61–62	36
9l	H	2-F	2-CH=CH_2	Colorless crystals	62–63	33
9m	H	H	2,4-2F	Colorless crystals	60–61	45
9n	H	H	2-F	Colorless crystals	59–60	35
9o	H	H	2-CF_3	Colorless crystals	61–61	33
9p	H	H	2-CH=CH_2	Colorless crystals	62–63	31
9q	H	4-Furan	2,4-2F	Colorless crystals	68–69	42
9r	H	4-Furan	2-F	Colorless crystals	66–67	38
9s	H	4-Furan	2-CF_3	Colorless crystals	68–69	36
9t	H	4-Furan	2-CH=CH_2	Colorless crystals	67–68	38
10a	7-OMe	2-CF_3	2,4-2F	Colorless crystals	65–66	32
10b	7-OMe	2-CF_3	2-F	Colorless crystals	64–65	31
10c	7-OMe	2-CF_3	2-CF_3	Colorless crystals	65–66	32
10d	7-OMe	2-CF_3	2-CH=CH_2	Colorless crystals	66–67	35
10e	7-OMe	2,4-2Cl	2,4-2F	Colorless crystals	70–71	34
10f	7-OMe	2,4-2Cl	2-F	Colorless crystals	68–69	31
10g	7-OMe	2,4-2Cl	2-CF_3	Colorless crystals	69–70	40
10h	7-OMe	2,4-2Cl	2-CH=CH_2	Colorless crystals	70–71	30
10i	7-OMe	2-F	2,4-2F	Colorless crystals	64–65	41
10j	7-OMe	2-F	2-F	Colorless crystals	63–64	43
10k	7-OMe	2-F	2-CF_3	Colorless crystals	64–65	44
10l	7-OMe	2-F	2-CH=CH_2	Colorless crystals	65–66	39
10m	7-OMe	H	2,4-2F	Colorless crystals	63–64	38
10n	7-OMe	H	2-F	Colorless crystals	61–62	42
10o	7-OMe	H	2-CF_3	Colorless crystals	63–64	42
10p	7-OMe	H	2-CH=CH_2	Colorless crystals	64–65	39
10q	7-OMe	4-Furan	2,4-2F	Colorless crystals	71–72	34
10r	7-OMe	4-Furan	2-F	Colorless crystals	69–70	36
10s	7-OMe	4-Furan	2-CF_3	Colorless crystals	68–69	37
10t	7-OMe	4-Furan	2-CH=CH_2	Colorless crystals	99–70	33

[a] Yields are based on oxime.

4. Conclusion

A series of new 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substitued-1,2,3,4-tetrahydroisoquinoline derivatives **9** and **10** were synthesized. The compounds were evaluated and assayed for their antibacterial (*B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525 and *S. aureus* ATCC 6538) activities by MTT method. The results show that compounds **9p** and **10p** possess potent antibacterial activity and can strongly inhibit *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase, with IC_{50s} of 0.125 and 0.25 μg/ml against *S. aureus* DNA gyrase, 0.25 and 0.125 μg/ml against *B. subtilis* DNA gyrase.

5. Experimental

5.1. Analysis and instruments

Melting points were measured and not corrected. The ^{1}H NMR spectra were recorded on a Varian INOVA500 (500 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$. Elemental analysis was performed by a Vario-III CHN analyzer and were within ±0.4% of the theoretical values. ESI mass spectra were obtained on a Mariner System 5303 mass spectrometer. Analytical TLC was performed on Silica Gel GF254. Column chromatographic purification was carried out using silica gel. All reagents were of analytical grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use. Compound **6** was prepared according to a previously published report.[27] Compounds **7** and **8** were prepared according to literature method as described.[5]

5.2. Syntheses

5.2.1. General synthetic procedure for 1-(5-(substituted-3,4-dihydroisoquinolin-2(1*H*)-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydropyrazol-1-yl)propan-1-one oxime (8)

The appropriate 1-(5-(substituted-3,4-dihydroisoquinolin-2(1*H*)-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydropyrazol-1-yl) propan-1-one (1 mol equiv), hydroxylamine hydrochloride (3.0 mol equiv), pyridine (2.0 mol equiv) and sodium acetate (3.0 mol equiv) in absolute ethanol (20 L per mol of ethanone) were heated under reflux for 15 h. The mixture was allowed to cool to room temperature and water (30 L per mol ethanone oxime) was added. The aqueous layer was extracted with dichloromethane, washed with water and dried. The solvent was removed in vacuo and the crude oxime mixture was recrystallized from ethanol to give the compounds **8**. The structures were confirmed by ^{1}H NMR and ^{13}C NMR spectral data.

5.2.1.1. 8a:1-(5-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydropyrazol-1-yl)propan-1-oneoxime. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.18 (t, 3H, Me, J = 7.2 Hz), 2.07 (q,

Table 3
Minimum inhibitory concentrations (MIC-μg/mL) of the title compounds Negative control DMSO, no activity

Compd	Gram positive		Gram negative	
	Bacillus subtilis	*Staphylococs aureus*	*Pseudomonas fluorescens*	*Escherichia coli*
9a	12.5	6.25	12.5	25
9b	12.5	6.25	50	50
9c	6.25	6.25	50	50
9d	12.5	12.5	50	>50
9e	1.562	1.562	>50	>50
9f	3.125	3.125	>50	50
9g	3.125	3.125	>50	>50
9h	6.25	3.125	50	>50
9i	25.0	6.25	25	25
9j	12.5	12.5	50	>50
9k	6.25	6.25	>50	>50
9l	50	50	50	50
9m	12.5	12.5	50	12.5
9n	25.0	6.25	12.5	6.25
9o	12.5	25	50	50
9p	12.5	12.5	12.5	12.5
9q	0.39	1.562	25.0	>50
9r	1.562	3.125	25.0	>50
9s	1.562	3.125	50	50
9t	3.125	1.562	50	>50
10a	3.125	12.5	25.0	25.0
10b	12.5	50	50	50
10c	3.125	3.125	12.5	25.0
10d	6.25	6.25	3.125	50
10e	0.78	1.562	50	50
10f	1.562	1.562	>50	>50
10g	3.125	1.562	>50	>50
10h	3.125	3.125	>50	50
10i	3.125	6.25	50	50
10j	12.5	50	12.5	50
10k	25.0	25.0	25.0	50
10l	12.5	50	50	50
10m	6.25	6.25	25.0	50
10n	25.0	25.0	>50	>50
10o	12.5	25.0	>50	50
10p	50	50	>50	>50
10q	0.78	0.78	50	25.0
10r	1.562	1.562	>50	25.0
10s	1.562	1.562	25.0	50
10t	3.125	3.125	25.0	>50
Penicillin	1.562	1.562	6.25	6.25
Kanamyci	0.39	1.562	3.125	3.125
Novobiocin	0.78	3.125	1.562	3.125

Table 4
Inhibitory effects of the selected title compounds against DNA gyrase

Compounds	IC_{50}[a] (μg/mL)	
	S. aureus DNA gyrase	*B. subtilis DNA gyrase*
9e	0.5	0.25
9f	5.5	1.0
9l	>125	>125
9q	0.125	0.25
10e	0.5	0.25
10j	4.0	>125
10q	0.25	0.125
10s	0.25	0.25
Novobiocin	0.25	0.5

[a] DNA gyrase supercoiling activity.

2H, CH_2), 2.50 (dd, J = 18.0 and 3.0 Hz, 1H, pyrazole, 4-H_a), 2.68 (t, 2H, isoquinoline-H), 2.73 (t, 2H, isoquinoline-H), 2.85 (dd, J = 18.0 and 11.0 Hz, pyrazole, 1H, 4-H_b), 3.77 (t, 2H, isoquinoline-H), 4.52 (dd, J = 11.0 and 3.0 Hz, 1H, pyrazole, 5-H), 6.80-7.94 (m, 8H, ArH), 10.01 (S, 1H, –NOH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 5.7, 19.1, 27.9, 42.5, 48.2, 57.0, 65.2, 119.3, 126.1, 126.6, 126.7, 128.1, 128.4, 128.8, 129.6, 130.4, 131.3, 133.1, 135.2, 138.2, 154.3, 169.3.

5.2.1.2. 8b: 1-(5-(7-Methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydropyrazol-1-yl)propan-1-one oxime. ^1H NMR ($CDCl_3$, 500 MHz): δ 1.21 (t, 3H, Me, J = 7.2 Hz), 2.01 (q, 2H, CH_2), 2.52 (dd, J = 18.0 and 3.0 Hz, 1H, pyrazole, 4-H_a), 2.64 (t, 2H, isoquinoline-H), 2.79 (t, 2H, isoquinoline-H), 2.81 (dd, J = 18.0 and 11.0 Hz, pyrazole, 1H, 4-H_b), 3.68 (t, 2H, isoquinoline-H), 3.79 (S, 3H, OMe), 4.55 (dd, J = 11.0 and 3.0 Hz, 1H, pyrazole, 5-H), 6.46–7.88 (m, 7H, ArH), 10.08 (S, 1H, –NOH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 5.9, 19.0, 27.7, 42.9, 48.0, 55.5, 57.7, 65.8, 119.0, 126.3, 126.7, 126.9, 128.1, 128.4, 128.6, 129.9, 130.6, 131.2, 133.4, 135.6, 138.2, 154.1, 168.7.

5.2.2. General synthetic procedure for 2-(1-(2-(substituted-phenyl)-5-methyloxazol-4-yl)-3-(2-(substituted-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-substituted-1,2,3,4-tetrahydroisoquinoline (9, 10)

To a solution of oxime **8** (2 mmol) and *N*-methylmorpholine (0.010 mmol) in DMF (30 mL) at 20 °C was added dropwise acid chloride (3.0 mmol) for 30 min. The reaction mixture was heated in the microwave for 10 min at 100 °C and poured into water (50 mL), then the solution was maintained at 0–5 °C for 10 h. The product was collected by filtration, and the crude residue was purified by chromatography on SiO_2 (acetone/petroleum, v/v = 4:1) to

give **9** and **10** as colorless solids. Their spectra are provided in the Supplementary data.

5.2.2.1. 2-(1-(2-(2,4-Difluorophenyl)-5-methyloxazol-4-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (9a). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.38 (s, 3H, Me), 2.55 (dd, J = 18.0 and 3.0 Hz, 1H, pyrazole, 4-H_a), 2.62 (t, 2H, isoquinoline-H), 2.73 (t, 2H, isoquinoline-H), 2.87 (dd, J = 18.0 and 11.0 Hz, pyrazole, 1H, 4-H_b), 3.67 (t, 2H, isoquinoline-H), 4.48 (dd, J = 11.0 and 3.0 Hz, 1H, pyrazole, 5-H), 6.80–7.91 (m, 11H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 5.8, 28.2, 37.6, 47.9, 55.9, 69.2, 106.3, 111.0, 119.2, 119.8, 125.4, 125.5, 125.9, 126.0, 127.9, 128.1, 128.4, 128.8, 129.7, 130.6, 131.0, 132.9, 133.6, 134.1, 138.0, 139.2, 152.3, 161.2, 164.8; ESI-MS: 538.4 ($C_{29}H_{23}F_5N_4O$, $[M+H]^+$); Anal. Calcd for $C_{29}H_{23}F_5N_4O$: C, 64.68; H, 4.30; N, 10.40. Found: C, 65.03; H, 4.21; N, 10.08.

5.2.2.2. 2-(1-(2-(2,4-Difluorophenyl)-5-methyloxazol-4-yl)-3-(2-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-methoxy-1,2,3,4-tetrahydroisoquinoline (10a). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.34 (s, 3H, Me), 2.57 (dd, J = 18.0 and 3.0 Hz, 1H, pyrazole, 4-H_a), 2.68–2.73 (m, 4H, isoquinoline-H), 2.89 (dd, J = 18.0 and 11.0 Hz, pyrazole, 1H, 4-H_b), 3.63 (t, 2H, isoquinoline-H), 3.78 (s, 3H, OMe), 4.51 (dd, J = 11.0 and 3.0 Hz, 1H, pyrazole, 5-H), 6.58–7.87 (m, 10H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 5.2, 28.0, 38.1, 47.9, 56.2, 56.4, 68.8, 105.4, 112.3, 112.7, 113.0, 119.0, 120.2, 125.2, 125.8, 126.0, 126.2, 128.9, 129.1, 130.0, 131.5, 131.7, 132.2, 135.4, 138.8, 152.5, 158.7, 161.0, 162.0, 164.1; ESI-MS: 567.7 ($C_{30}H_{25}F_5N_4O_2$, $[M+H]^+$); Anal. Calcd for $C_{30}H_{25}F_5N_4O_2$: C, 63.38; H, 4.43; N, 9.85. Found: C, 63.01; H, 4.67; N, 10.00.

5.3. Bioassay conditions

5.3.1. In vitro antibacterial activity

The antibacterial activities of the synthesized compounds were tested against *B. subtilis*, *E. coli*, *P. fluorescens* and *S. aureus* using MH medium (casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the test compounds were determined by a colorimetric method using the dye MTT.[32] A stock solution of the synthesized compound (100 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO for testing and incubation at 37 °C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (Phosphate Buffered Saline 0.01 mol/L, pH 7.4: $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density was measured with a microplate reader at 550 nm. The MICs were observed.

5.3.2. Enzyme inhibition

5.3.2.1. *S. aureus* DNA gyrase supercoiling. The *S. aureus* DNA gyrase were purified by the Blanche, F.[33] from a crude extract of *S. aureus* and cultivated with medium B[34], which was composed of 10 g of polypeptone, 2 g of yeast extract, 8 g of Na_2HPO_4, 2 g of KH_2PO_4, 1.2 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4$, 4 g of glucose per liter of distilled water. Supercoiling and decatenation were performed according to Blanche, F.[33]

5.3.2.2. *B. subtilis* DNA gyrase supercoiling. The *B. subtilis* DNA gyrase were purified by the methods of Elisha, O.[35]: Cells were suspended in an equal volume of 25 mM HEPES-KOH (pH 8.0)–100 mM KCl and stored frozen at −70 °C. The frozen cell suspension was thawed and diluted with an equal volume of 25 mM HEPES-KOH (pH 8.0)–0.4 M sucrose–20 mM magnesium acetate–1 mM dithiothreitol–5 mM PMSF. All operations were performed at 0–4 °C. Lysozyme was added to a final concentration, and the mixture was incubated for 2.5 h. One-third volume of 2 M KCl–1.5% Brij was added, and the incubation was continued for 15 min. The lysate was then centrifuged for 90 min in tirotor. The supernatant was adjusted to a KCl concentration of 0.2 M by dilution with 25 mM HEPES–KOH (pH 8.0)–1 mM dithiothreitol–1 mM EDTA–0.5 mM pmsf–10% ethylene glycol and applied to a column. The column was washed with starting buffer and eluted successively with buffer containing 20 mM ATP–25 mM magnesium acetate–0.2 M KCI, buffer (2 M KCI), and 5 M urea in buffer (0.2 M KCl). Protein-containing fractions were dialyzed against buffer (0.05 M KCI). Supercoiling and decatenation were carried out by the Blanche, F.[33] method.

Acknowledgment

The authors wish to thank Universities natural science key research projects of Anhui Province (2009KJ0101AZC) and the opening foundation of the Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, grant No.2008GDGP0105. The work was supported by the Young College Teachers Research Projects of Anhui Province (2008JQ1030) and we also thank the Young College Teachers Research Projects of Anhui Technology University (QZ200809).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.12.034.

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