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Synthesis, structure and antibacterial activity of novel 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives

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Abstract—A series of novel 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives are synthesized. The results show that compounds **14** and **26c** can strongly inhibit *Staphylococcus aureus* DNA gyrase and *Escherichia coli* DNA gyrase (with IC_{50} of 0.25 and 0.125 μg/mL against *S. aureus* DNA gyrase, 0.125 and 0.25 μg/mL against *E. coli* DNA gyrase). On the basis of the biological results, structure–activity relationships are 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, such as methicillin-resistant *Staphylococcus aureus*, and penicillin-resistant *Streptococcus pneumoniae* 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] The pyrazole derivatives used as a potent and selective inhibitor against DNA gyrase capable of causing bacterial cell death have also been reported. 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 compounds **2a**, **2b**, and **3** (Fig. 1) as potent and selective inhibitors of DNA gyrase. From the structures shown in Figure 1, it may be noticed that all the selective inhibitors of DNA gyrase contain the characteristic arylpyrazole template. In our previous work, we reported that some 1-acetyl-5-(substituted-phenyl)-3-methy-4,5-dihydropyrazole derivatives showed antibacterial activity against *Bacillus subtilis* ATCC 6633 and *Pseudomonas fluorescens* ATCC 13525.[13] Earlier, the derivatives of 3,5-diaryl-4,5-dihydropyrazole were also well known to possess bioactivities, such as Christopher D. Cox[14,15] disclosed 3,5-diaryl-4,

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-2H-tetrazolium bromide; MICs, minimum inhibitory concentrations; NMM, *N*-methylmorpholine; DMAP, *N*,*N*-dimethyl-4-aminopyridine; DMSO, dimethyl sulfoxide; MH, Mueller–Hinton.

Keywords: Synthesis; 5-Arylpyrazole; Oxime ester; Antibacterial activity.

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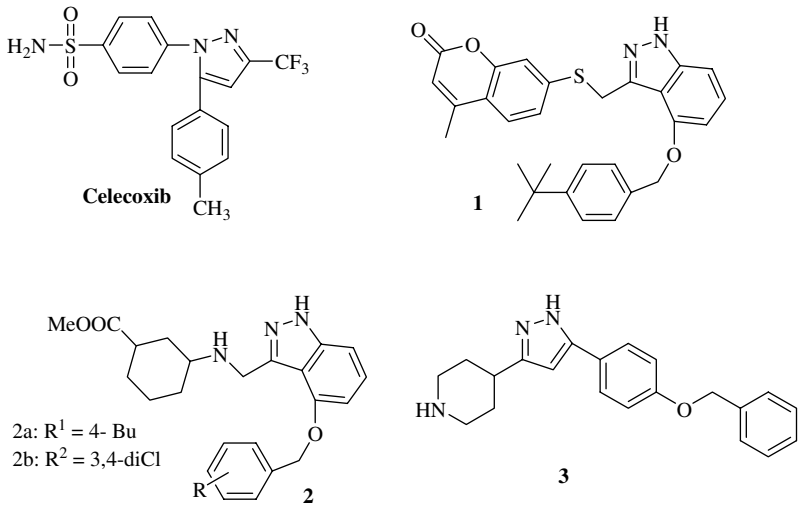


Figure 1. Recently disclosed pyrazole as antibacterial inhibitors.

5-dihydropyrazole as a potent, selective inhibitor of kinesin spindle protein.

Recently, oxime ester derivatives have attracted considerable attention in medicinal research due to their antiphytoviral, antitumor, and fungicidal bioactivities. A large number of investigations on their synthesis and biological activities have been reported during the last 20 years.[16,17]

Motivated by the afore-mentioned findings, we anticipate that the presence of aryl-4,5-dihydropyrazole group in the oxime ester part should play an important role in the antimicrobial activities. But few reports have been dedicated to the synthesis and antimicrobial activities evaluation of oxime ester containing aryl-4,5-dihydropyrazole group. Herein, in continuation to extend our research on antibacterial compounds containing aryl-4,5-dihydropyrazole group,[18,19] we designed a series of new aryl-4,5-dihydropyrazole oxime ester derivatives. In the molecule design against antimicrobial activities, considering to detecting the role of the presence position of aryl in the 4,5-dihydropyrazole, we designed and synthesized 3-methyl (or aryl)-5-aryl (or methoxyl)-pyrazole oxime ester and 3,5-diaryl-pyrazole oxime ester derivatives. The results showed that some 3-aryl-5-methoxyl-pyrazole oxime ester compounds in this series exhibited potent antibacterial activity.

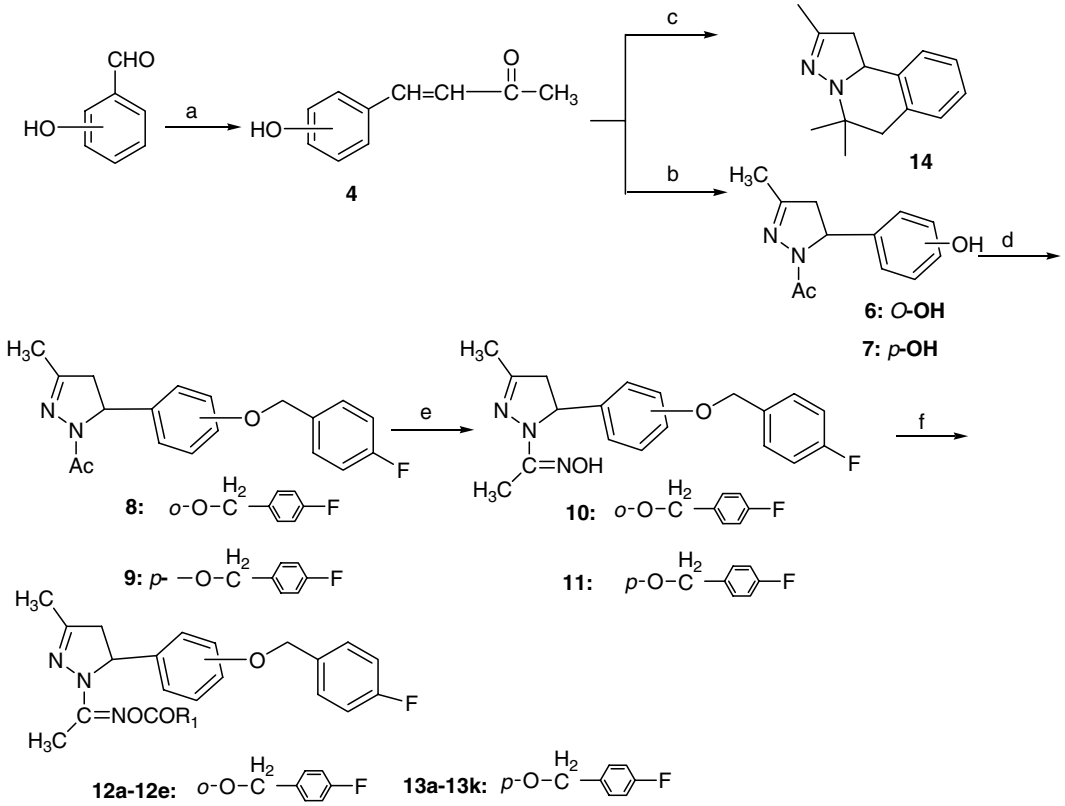
2. Chemistry

The synthetic routes to target compounds are shown in Schemes 1–3. Claisen–Schmidt condensation[20] of a ketone with an aldehyde in the presence of NaOH or H_2SO_4 gave the enones (**4**, **15**, and **16**) in generally good yields (highest for acetone). Dehydrogenation of ketone using mild oxidizing agent HIO_3·DMSO, by following a reported method,[21] proved to be an efficient alternative for the synthesis of α, β unsaturated ketone **23**.

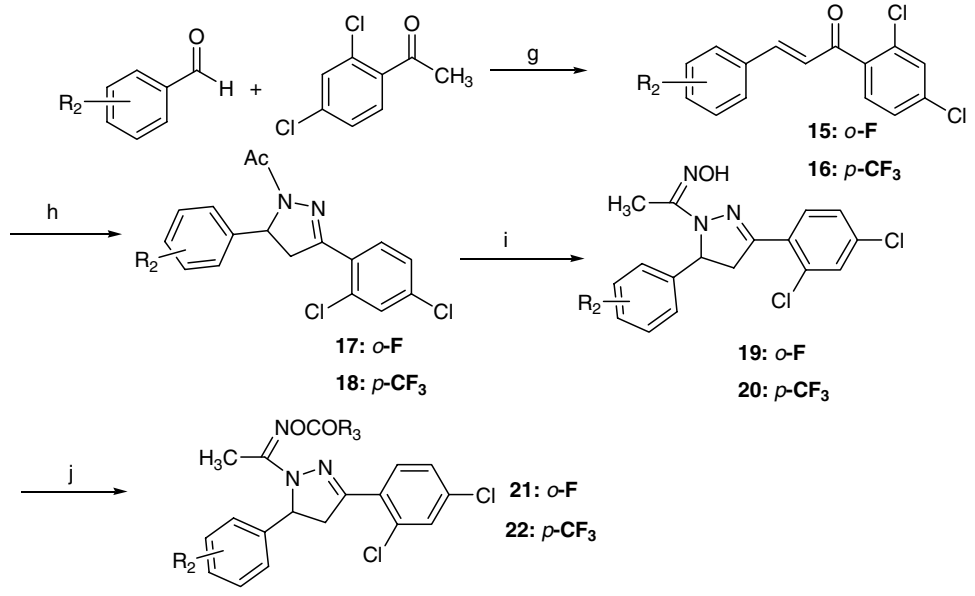
Compounds **6**, **7**, **17**, **18**, and **24** were synthesized by cyclization of α, β-unsaturated ketone with hydrazine monohydrate in acetic acid.[22] But, on the other hand, when the cyclization with hydrazine monohydrate was carried out under neutral condition, instead of the reported 3-methyl-4,5-dihydro-*1H*-pyrazole,an unusual product 2,5,5-trimethyl-1,5,6,10b-tetrahydropyrazolo[5,1-*a*]isoquinoline (**14**) was formed which was characterized by single X-ray crystallographic data.

The ketones **6**, **7**, **17**, **18**, and **24** were converted into their corresponding oximes by hydroxylamine. Spectroscopic studies of the oximes and the oxime esters 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 synthesis was carried out in presence of various bases (Table 1), for example: $NaHCO_3$, $KHCO_3$, $NaCH_3CO_2$, NaOH, pyridine. It was found that a yield up to 60% (62) could be attained when the reaction mixture was refluxed for 10 h (15) in ethanol catalyzed by $NaCH_3CO_2$.

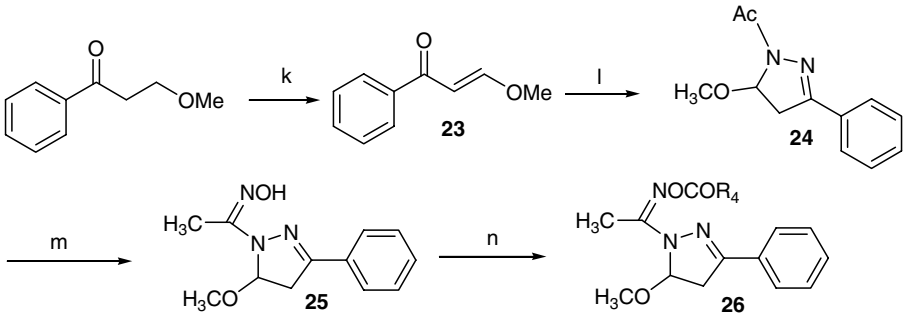
The esterification of compounds **10**, **11**, **19**, **20**, and **25** with acyl chloride produced **12**, **13**, **21**, **22**, and **26**, respectively, in moderate yield. In order to optimize the reaction conditions, the esterification reaction was carried out under various conditions. First, the effect of temperature was studied and the best result could be obtained between 0 and 20 °C. While negligible amount of product was obtained at temperature below 0 °C, on increasing the reaction temperature above 20 °C, a number of side products were obtained causing significant reduction in the yield of title compounds. In addition, role of various bases, such as pyridine, triethylamine, sodium carbonate, potassium carbonate, NMM, and DMAP was also studied. The result demonstrated that the presence of NMM could accelerate the esterification reaction. Further, the effect of solvent, reaction time, and molar ratio of the components on the esterification reaction was examined. The best result was obtained when oxime was reacted with 1.25 equiv of acyl chloride and 1.5 equiv of NMM in chloroform at 0–20 °C for 10 h.



Scheme 1. Synthesis of novel isoquinoline and 1-(5-aryl-3-methyl-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives. Reagents and conditions: (a) CH_3COCH_3, NaOH, C_2H_5OH, 25 °C, 15 h; (b) $N_2H_4{\cdot}H_2O$, 98% CH_3COOH, reflux, 2 h; (c) $N_2H_4{\cdot}H_2O$, *n*-butanol, reflux, 10 h; (d) *p*-F-Ph-CH_2Cl, NaOH, $CHCl_3$, reflux, 3 h; (e) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, pyridine, reflux, 8 h; (f) RCOCl, NMM, $CHCl_3$.



Scheme 2. Synthesis of novel 1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives. Reagents and conditions: (g) H_2SO_4, MeOH, reflux, 10 h; (h) $N_2H_4{\cdot}H_2O$, 98% CH_3COOH, reflux, 4 h; (i) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, pyridine, reflux, 8 h; (j) RCOCl, NMM, $CHCl_3$.



R4: CH_2CH_3, C_6H_4-*p*-CF_3, C_6H_4-*p*-F, C_6H_4-*p*-Cl, Ph-CH=CH, C_6H_4-*o*-NO_2, C_6H_4-*o*-Me, 2-furan

Scheme 3. Synthesis of novel 1-(5-methoxy-3-aryl-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives and isoquinoline. Reagents and conditions: (k) HIO_3·DMSO, 60 °C, 15 h; (l) N_2H_4·H_2O, 98% CH_3COOH, reflux, 2 h; (m) NH_2OH·HCl, $NaCH_3CO_2$, reflux, 10 h; (n) RCOCl, NMM, $CHCl_3$.

Table 1. Yields of **10** under various reaction conditions

Entry	Solvent	Base	Time (h)	Temperature	Yield (%)
1	*n*-Butanol	$NaHCO_3$	10	Reflux	—
2	*n*-Butanol	$KHCO_3$	10	Reflux	
3	C_2H_5OH	NaOH	10	Reflux	10
4	CH_3OH	NaOH	15	Reflux	—
5	*n*-Butanol	NaOH	20	Reflux	6
6	C_2H_5OH	$NaCH_3CO_2$	15	Reflux	62
7	C_2H_5OH	Pyridine	10	Reflux	32

2.1. Crystal structure analysis

The skeleton of the new unusual compound **14** contains a benzene ring, a six-membered non-aromatic heterocyclic ring, a five-membered ring with two nitrogens, and three methyl groups directly attached to the ring carbons (Fig. 2). The bond length of C(9)–N(1) (1.320 (12) Å) is slightly shorter than that of typical C=N (1.34 Å), which is indicative of significant double bond character; C(11)–N(2) (1.426 (11) Å) is in good agreement with normal single C–N (1.47 Å) bond, N(1)–N(2) (1.379 (10) Å) is comparable with the general N–N single bond length. The bond angles of C(11)–N(2)–C(7) and C(9)–N(1)–N(2) are 112.9 (6)° and 108.9 (7)°, respectively. The angle between the six-membered ring [C(3)–C(4)–C(12)–C(11)–C(7)–N(2)] and the benzene ring is 7.44 (48)°. The angle between the five-membered ring [N(1)–N(2)–C(7)–C(8)–C(9)] and the aromatic ring is 77.40 (28)°.

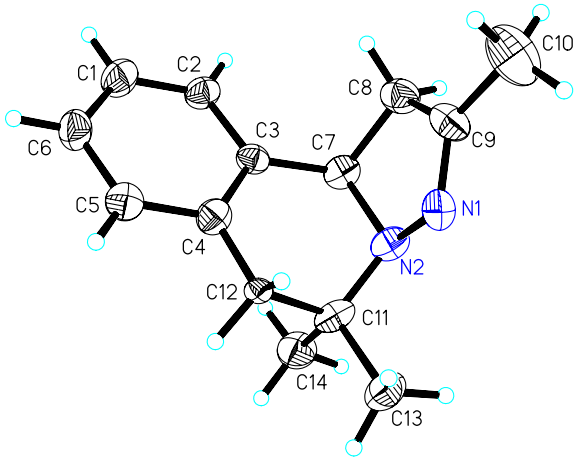


Figure 2. The molecular structure of compound **14**.

3. In vitro antibacterial assay

The activities of synthesized compounds were tested against *B. subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *P. fluorescens* ATCC 13525, and *S. aureus* ATCC 6538 using MH medium. The MICs of the compounds against four bacteria are presented in Table 2. Also included was the activities of reference compounds kanamycin, penicillin, and novobiocin. The compounds **22k**, **22n**, **26c**, and **26k** showed antimicrobial activities against *B. subtilis* with the MIC of 1.562 μg/mL, comparable to that of positive control penicillin. Compounds **14** and **26n** with the MIC of 0.78 and 1.25 μg/mL, respectively, exhibited promising antimicrobial activities against *B. subtilis* which were even better than that of the commercial fungicide penicillin. The compounds **14**, **21j**, and **22n** showed antimicrobial activities against *S. aureus* with the MIC of 3.125 μg/mL, comparable to that of positive control novobiocin, compounds **21n** and **26c** with the MIC of 1.562 μg/mL, exhibited antimicrobial activities against *S. aureus* surpassing that of the commercial fungicide novobiocin. The compounds **14**, **21k**, **22k**, **22n**, **26c**, **26k**, and **26n** showed antimicrobial activities against *P. fluorescens* with MIC values in the range 1.562–3.125 μg/mL, comparable to the positive control kanamycin and penicillin. The compounds **14**, **21n**, **26c**, and **26n** showed promising antimicrobial activities comparable to the positive control kanamycin and novobiocin and even better than that of the commercial fungicide penicillin against *E. coli* with MIC values ranging from 1.562–3.125 μg/mL.

From the structure–activity relationships presented in Table 2, it can be concluded that all 5-phenyl-3-methyl-4,5-dihydropyrazole derivatives displayed poor activity against four strains, but only some 3-phenyl-5-phenyl-4,5-dihydropyrazole derivatives showed good activity against bacterial strains, specially against *S. aureus* ATCC 6538 and *Pseudomonas eruginosa* ATCC 13525. The most active agent against the bacterial

Table 2. Minimum inhibitory concentrations (MIC-μg/mL) of the title compounds negative control DMSO, no activity

Compound		Gram-positive		Gram-negative	
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Escherichia coli*
12a	CH_2CH_3	50	50	50	50
12b	$(CH_2)_2CH_3$	50	50	50	50
12c	C_6H_4-*p*-CF_3	50	50	50	50
12d	C_6H_4-*o*-F	50	50	50	50
12e	C_6H_4-*p*-F	>50	>50	>50	>50
13a	CH_2CH_3	50	50	50	50
13b	$(CH_2)_2CH_3$	50	>50	50	>50
13c	C_6H_4-*p*-CF_3	25	25	25	50
13d	C_6H_4-*o*-F	25	50	25	50
13e	C_6H_4-*p*-F	50	50	50	50
13f	C_6H_4-*p*-Cl	>50	>50	>50	>50
13g	C_6H_4-*o*-Cl	50	50	50	12.5
13h	2,4-2Cl–C_6H_3	12.5	12.5	50	12.5
13i	3-Pyridine	6.25	6.25	12.5	6.25
13j	CH_2=CH	12.5	25	50	50
13K	Ph-CH=CH	12.5	12.5	12.5	12.5
14	Isoquinoline	1.25	3.125	3.125	1.562
21a	CH_2CH_3	50	50	50	50
21c	C_6H_4-*p*-CF_3	12.5	12.5	50	12.5
21d	C_6H_4-*o*-F	12.5	25.0	6.25	12.5
21e	C_6H_4-*p*-F	25.0	25.0	25.0	12.5
21f	C_6H_4-*p*-Cl	12.5	50	50	50
21j	CH_2=CH	3.125	3.125	12.5	25.0
21k	Ph-CH=CH	6.25	6.25	3.125	50
21l	C_6H_4-*o*-NO_2	25	50	50	50
21m	C_6H_4-*o*-Me	50	50	50	50
21n	2-Furan	3.125	1.562	6.25	3.125
22a	CH_2CH_3	50	50	50	50
22c	C_6H_4-*p*-CF_3	25.0	50	50	50
22d	C_6H_4-*o*-F	12.5	50	12.5	50
22e	C_6H_4-*p*-F	25.0	25.0	25.0	50
22f	C_6H_4-*p*-Cl	12.5	50	50	50
22j	CH_2=CH	3.125	6.25	12.5	12.5
22k	Ph-CH=CH	1.562	12.5	3.125	6.25
22l	C_6H_4-*o*-NO_2	50	50	50	50
22m	C_6H_4-*o*-Me	50	50	50	50
22n	2-Furan	1.562	3.125	1.562	12.5
26a	CH_2CH_3	12.5	12.5	12.5	25.0
26c	C_6H_4-*p*-CF_3	1.562	1.562	1.562	3.125
26e	C_6H_4-*p*-F	3.125	6.25	12.5	6.25
26f	C_6H_4-*p*-Cl	3.125	6.25	6.25	25.0
26k	Ph-CH=CH	1.562	6.25	3.125	12.5
26l	C_6H_4-*o*-NO_2	6.25	6.25	12.5	12.5
26m	C_6H_4-*o*-Me	12.5	12.5	25.0	12.5
26n	2-Furan	0.78	6.25	1.562	3.125
Penicillin		1.562	1.562	6.25	6.25
Kanamycin		0.39	1.562	3.125	3.125
Novobiocin		0.78	3.125	1.562	3.125

strains was 5-methoxy-3-phenyl-4,5-dihydropyrazol, such as **26c** (with MICs of 1.562, 1.562, 1.562, and 3.125 μg/mL against *B. subtilis* ATCC 6633, *S. aureus* ATCC 65385, *P. fluorescens* ATCC 13525, and *E. coli* ATCC 35218) and **26n** (with MICs of 0.78, 1.562, and 3.125 μg/mL against *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 13525, and *E. coli* ATCC 35218). Further, the presence of furan group in the oxime ester part played an important role in the antimicrobial activities (such as **21n**, **22n**, and **26n**), however, introduction of alkyl group in the oxime ester depressed the antimicrobial activities. Compound **14** (a saturated analogue of isoquinoline) has potentially high antimicrobial activity.

To elucidate the mechanism by which the pyrazole derivatives induce antibacterial activity, the inhibitory activity of selected compounds (**12a**, **13i**, **14**, **21n**, **21j**, **22n**, **26c**, and **26n**) was examined against DNA gyrase isolated from *S. aureus* and *E. coli*. As shown in Table 3, compounds **14** and **26c** with potent antibacterial activities strongly inhibited *S. aureus* DNA gyrase and *E. coli* DNA gyrase (with IC_{50}s of 0.25 and 0.125 μg/mL against *S. aureus* DNA gyrase, 0.125 and 0.25 μg/mL against *E. coli* DNA gyrase). Compounds **21j** and **22n** showed moderate inhibition against the *S. aureus* DNA gyrase (IC_{50} = 0.5 μg/mL). There was a good correlation between the MICs and the IC_{50}s of **14** and **26c**

Table 3. Inhibitory effects of the selected title compounds against DNA gyrase

Compound	IC_{50}[a] (μg/mL)	
	S. aureus DNA gyrase	*E. coli* DNA gyrase
12a	>125	>125
13i	3.5	4.0
14	0.25	0.125
21n	0.125	1.0
21j	0.5	100
22n	0.5	8.0
26c	0.125	0.25
26n	4.0	0.25
Novobiocin	0.28	0.31

[a] DNA gyrase supercoiling activity.

(Tables 2 and 3), indicating that inhibition of the DNA gyrase by the pyrazole derivatives caused inhibition of bacterial cell growth.

4. Conclusion

A series of novel 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives and an unexpected compound 2,5,5-trimethyl-1,5,6,10b-tetrahydro-pyrazolo[5,1-*a*]isoquinoline (**14**) were synthesized. The compounds are 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 **14** and **26c** possess potent antibacterial activity and can strongly inhibit *S. aureus* DNA gyrase and *E. coli* DNA gyrase (with IC_{50}s of 0.25 and 0.125 μg/mL against *S. aureus* DNA gyrase, 0.125 and 0.25 μg/mL against *E. coli* 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 INOVA300 (300 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$. Elemental analysis was performed by a Vario-III CHN analyzer and was 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. Compounds **4**, **6**, **7**, **8**, **9**, **15**, **16**, **17**, **18** and **24** were prepared according to literature method as described.[13,18] Compound **23** was prepared according to a previously published report.[21]

5.2. Syntheses

5.2.1. Synthesis of 2,5,5-trimethyl-1,5,6,10b-tetrahydro-pyrazolo[5,1-*a*]isoquinoline (14). To a solution of the 4-(2-hydroxyphenyl) but-3-en-2-one (2 mmol) was added hydrazine hydrate (2.2 mmol) in *n*-butanol (30 mL). The reaction mixture was refluxed for 8 h and washed with 5% HCl solution (10 mL). The solvent was removed in vacuo, added dropwise acetone/petroleum (10 mL, volume ratio 2:1). The resultant solution was kept to evaporate slowly in air. When the solvent volume was reduced to almost half of the original, large brown slab crystals of the title compound were deposited and collected by filtration (yield 56%). Mp 155–156 °C. Elemental analysis: Anal. Calcd for $C_{14}H_{18}N_2$: C, 78.46; H, 8.47; N, 13.07. Found: C, 78.40; H, 8.33; N, 12.97.

5.2.2. General synthetic procedure process for 1-(5-substituted-3-substituted-4,5-dihydro-pyrazol-1-yl)ethanone oxime (10, 11, 19, 20, and 25). The appropriate 1-(5-substituted-3-methyl-4,5-dihydropyrazol-1-yl)ethanone (1 mol equiv), hydroxylamine hydrochloride (4.0 mol equiv), and sodium acetate (2.5 mol equiv) in absolute ethanol (30 L per mol of ethanone) were heated under reflux for 10 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 compound (**10**, **11**, **19**, **20**, and **25**). The structure was confirmed by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis (see the Supporting information).

5.2.3. General synthetic procedure for 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl)ethanone oxime ester (12, 13, 21, 22, and 26). To a solution of 1-(5-substituted-3-methyl-4,5-dihydropyrazol-1-yl)ethanone oxime (2 mmol) and NMM (0.010 mmol) in chloroform (30 mL) at 10 °C was added dropwise acid chloride (3.0 mmol) for 30 min. The reaction mixture was stirred at room temperature for 8 h and washed with 20 mL H_2O, 10 mL 5% $NaHCO_3$, respectively, then dried on anhydrous $MgSO_4$. The solvent was removed in vacuo and the crude residue was purified by chromatography on SiO_2 (acetone/petroleum, 5:1, v/v) to give title compound as a colorless solid. The spectral data can be found in the Supporting information.

Compound **12a**: Colorless crystals, yield, 51%; mp 170–171 °C,^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.16 (t, 3H, Me, J = 7.2 Hz), 2.22 (s, 3H, Me), 2.35 (q like, 2H, $COCH_2$), 2.40 (s, 3H, Me), 3.02 (dd, 1H, J_1 = 18.3, J_2 = 3.1 Hz, 4-H_a), 3.44 (dd, 1H, J_1 = 18.3, J_2 = 11.1 Hz, 4-H_b), 5.33 (s, 2H, CH_2–O), 5.59 (dd, 1H, J_1 = 11.1, J_2 = 3.1 Hz, 5-H), 6.81–7.32 (m, 8H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.7, 16.4, 21.5, 26.1, 43.4 (CH_2-4), 53.8 (CH-5), 73.5, 114.9, 115.8, 117.8, 122.3, 128.8, 129.5, 130.9, 138.0, 157.2, 158.4 (C-3), 163.3, 169.6, 178.5 (C=N–OCO); ESI-MS: 397.0 ($C_{22}H_{24}FN_3O_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{24}FN_3O_3$: C, 66.48; H, 6.09; N, 10.57. Found: C, 66.23; H, 6.21; N, 10.39.

5.3. Crystal structure determination

A sample of size $0.25 \times 0.21 \times 0.16$ mm^3 was selected for the crystallographic study. The diffraction measurement

was performed at room temperature (293 K) using graphite monochromated MoKα radiation (λ = 0.71073 Å) and an Enraf-Nonius CAD-4 four-circle diffractometer. Accurate cell parameters and orientation matrix were obtained by least-squares refinement of the setting angles of 1542 reflections at the θ range of $3.00 < \theta < 24.99$. The systematic absences and intensity symmetries indicated the Monoclinic Cc space group. The corrections for *LP* factors were applied. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 with anisotropic thermal parameters for all non-hydrogen atoms. The calculations were performed with SHELXL-97 program.[23] The molecular structure of the compound **14** is shown in Figure 2. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-668105.

5.4. Bioassay conditions

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.[24] 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.

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Supplementary data

CCDC-668105 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the URL http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.01.035.

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