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Synthesis and antimicrobial activity of novel fluorine containing 4-(substituted-2-hydroxybenzoyl)-1*H*-pyrazoles and pyrazolyl benzo[*d*]oxazoles

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ABSTRACT

A series of fluorine containing 4-(substituted-2-hydroxybenzoyl) pyrazoles and pyrazolyl benzo[*d*]oxazoles were synthesized and evaluated for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and antifungal activity against *Candida albicans*. The antibacterial activities were expressed as the minimum inhibitory concentration (MIC_{50}) in μg/ml. The compounds 1-(3,4-difluorophenyl)-4-(5-fluoro-2-hydroxybenzoyl)-1*H*-pyrazole (**4b**), oxime derivatives such as 1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)(2-hydroxy-4-methylphenyl)methanone oxime (**5b**) and (5-chloro-2-hydroxyphenyl)(1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)methanone oxime (**5e**) exhibited promising activities against tested bacterial strains. Except compound 1-(3,4-difluorophenyl)-4-(2-hydroxybenzoyl)-1*H*-pyrazole (**4d**), none of the other compounds showed promising antifungal activity.

The clinical applications of antibacterial chemotherapy began in 1935 with the discovery of prontosil and over the next three decades virtually all the major classes of antibiotics and synthetic antibacterial agents in current use were introduced. The widespread introduction of these antimicrobials into clinical practice have made possible the treatment of life threatening diseases and have enabled advances in surgical techniques by permitting the prophylaxis and treatment of surgery related infections.

However, the emergence of antibacterial drug resistance posed by bacteria is recognized as a serious problem worldwide.[1–4] It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets via genomics, improving the existing nature of antibiotics and identifying new antibacterial agents with novel mode of action and structure.[5–7]

Over the last decade, a variety of benzoxazole derivatives were synthesized and studied for their antibacterial activity.[8–13] A calcimycin (**I**) (Fig. 1), well known antibiotic contains a 2-substituted benzoxazole ring in its structure and is reported to be active against *Bacillus cereus*, *Bacillus megaterium* and *Micrococcus lutes*.[14] A careful literature survey has also revealed many hydrazone derivatives to be reported as anticancer and antibacterial agents (**II** and **III**) (Fig. 1).[15,16] Moreover, 4-(2-hydroxybenzoyl) pyrazoles are one of the precursors of benzoxazole derivatives and also important intermediates for the synthesis of biologically active benzofuran and coumarins systems.[17–19]

Although various synthetic methods are reported in literature for the synthesis of 4-(2-hydroxybenzoyl) pyrazoles,[20–22] but antimicrobial activities of these classes of compounds has received little attention.[23] Pyrazoles and substituted pyrazoles are also reported to be potent inhibitors of many bacteria.[24,25] Synthesis of compounds containing benzoxazole anchored with pyrazole ring system is known in the literature,[26] but there are no reports available describing antibacterial activity with both heterocycles in a single moiety. Therefore an attempt made here to synthesize series of 4-(2-hydroxybenzoyl) pyrazoles containing 3,4-difluorophenyl ring system in the present study. Further, it was extended to also cover corresponding fluorine containing pyrazolyl benzo[*d*]oxazoles and investigate their antimicrobial activities.

The synthetic route for the preparation of 4-(2-hydroxybenzoyl) pyrazoles and pyrazolyl benzo[*d*]oxazole derivatives are shown in Scheme 1. Substituted formyl chromones[27,28] **1a–h** were treated with 3,4-difluorophenyl hydrazine **2** in ethanol and catalytic amount of acetic acid at 40 °C to yield hydrazones **3a–h**. The hydrazones **3a–h** on further treatment with KOH at 50 °C yielded respective 1-(3,4-difluorophenyl)-4-(substituted-2-hydroxybenzoyl)-1*H*-pyrazoles **4a–h**.[29] The compounds **4a–h** on treatment with hydroxylamine hydrochloride in ethanol and sodium acetate yielded oxime **5a–e**, which underwent Beckmann rearrangement using $POCl_3$ to yield pyrazolyl benzo[*d*]oxazoles **6a–f**. All the

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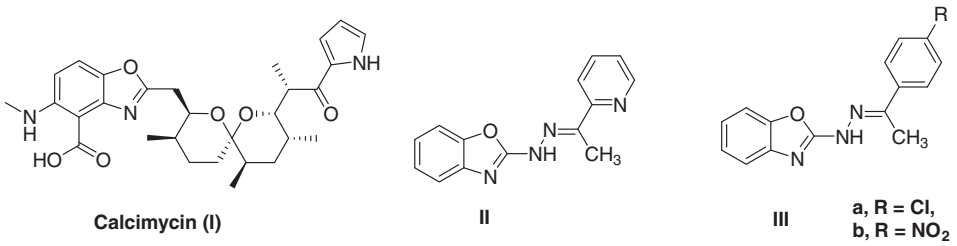
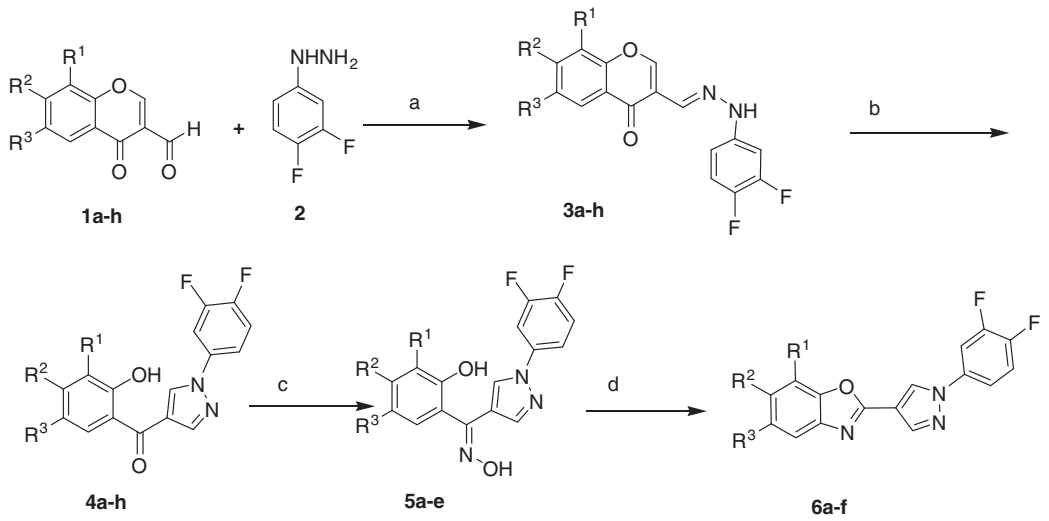
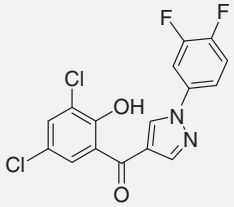
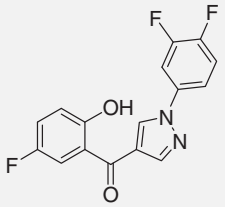
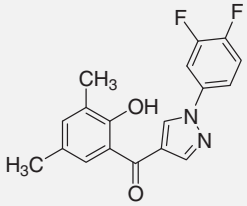


Figure 1.



Scheme 1. Reagents and conditions: (a) EtOH, 40 °C, Cat. AcOH, ~99%; (b) KOH, 50 °C, 3 h and HCl, 65–75%; (c) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, reflux, 80–90%; (d) $POCl_3$, 60–70%.

Table 1
Antibacterial and antifungal activities of newly synthesized compounds (**4a**–**h**), (**5a**–**e**) and (**6a**–**f**) indicated by MIC_{50} values (μg/ml)

Compound	Structures	MIC_{50} values (μg/mL)				
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
4a		500	500	1000	500	250
4b		62.5	50	100	62.5	500
4c		500	250	500	500	1000

(*continued on next page*)

Table 1 (*continued*)

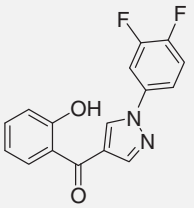
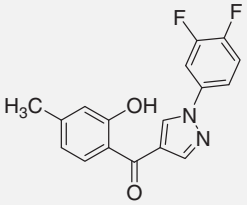
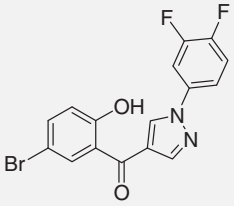
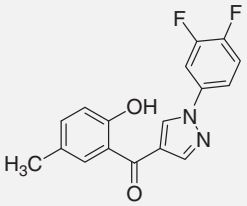
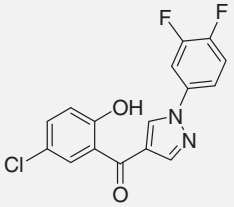
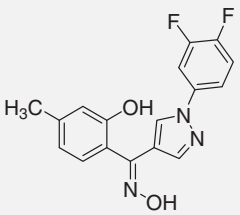
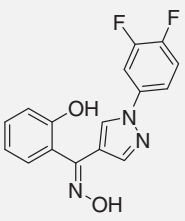
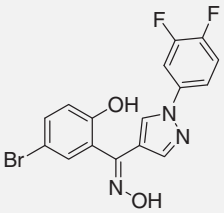
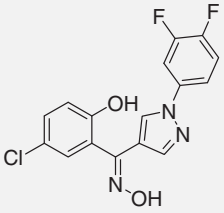
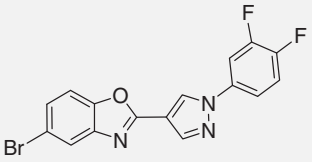
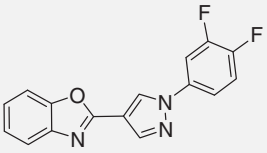
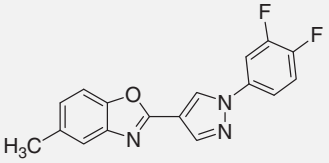
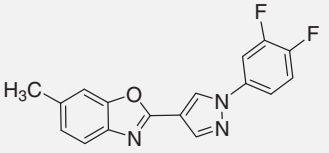
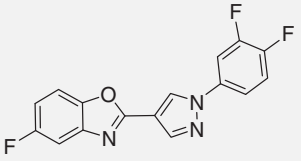
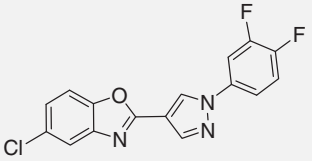
Compound	Structures	MIC_{50} values (μg/mL)				
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
4d		250	555	500	12.5	100
4e		125	62.5	200	100	500
4f		500	500	125	100	>1000
4g		500	250	500	500	1000
4h		250	500	1000	500	500
5a		500	500	500	250	1000
5b		25	50	250	500	500
5c		500	500	>1000	>1000	1000

Table 1 (*continued*)

Compound	Structures	MIC_{50} values (μg/mL)				
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
5d		200	100	500	1000	500
5e		12.5	125	100	62.5	1000
6a		250	250	250	250	500
6b		125	100	125	200	1000
6c		500	62.5	50	200	500
6d		200	500	200	25	500
6e		250	50	500	200	250
6f		100	125	125	200	500
	Gentamycin	0.05	1	0.25	1	
	Ampicillin	100	100	250	250	
	Chloramphenicol	50	50	50	50	
	Ciprofloxacin	25	25	50	50	
	Norfloxacin	10	10	10	100	
	Nystatin					100
	Greseofulvin					500

compounds were isolated in pure form with a yield of 60–70%. The compounds were analyzed by ^{1}H NMR, LC–MS spectral methods.

^{1}H NMR spectrum of (3,5-dichloro-2-hydroxyphenyl)(1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)methanone (**4a**) showed broad singlet at δ 9.9 corresponds to phenolic –OH group and singlets

at δ 8.3 and δ 9.2 showed characteristic peak for pyrazole ring. Molecular mass was confirmed by LC–MS at *m*/*z* 368.8 (M+1, 100%). The ^{1}H NMR spectrum of (5-fluoro-2-hydroxyphenyl)(1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)methanone oxime (**5a**) showed one more deshielded singlet at δ 11.8 which corresponds to hydroxyl group of oxime in addition to phenolic –OH proton at δ 9.8. The compound exhibited a mass peak at *m*/*z* 334 (M+1, 100%) which further confirms the structure. For the compound 5-bromo-2-(1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)benzo[*d*]oxazole (**6a**), we observed characteristic singlets at δ 8.4 and δ 9.4 due to pyrazole ring. Molecular mass has been confirmed by LC–MS with a peak at *m*/*z* 375.9 (M+1, 100%).

All newly synthesized compounds were evaluated for their antimicrobial activity against four antibacterial strains, *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688) and *Bacillus subtilis* (MTCC 441) and one antifungal strain, *Candida albicans* (MTCC 227), using broth dilution method and serial dilution techniques. The results were determined using minimum inhibitory concentration (MIC) values in μg/ml.[30] Ciprofloxacin, Norfloxacin, Gentamycin, Ampicillin, Chloramphenicol were used as standard drugs. A detailed observation of the set of 4-(2-hydroxybenzoyl) pyrazoles and pyrazolyl benzoxazoles derivatives showed MIC values to be generally within a range of 12–500 μg/ml. Antimicrobial activities of newly synthesized compound are mentioned in Table 1.

Among the newly synthesized compounds, 1-(3,4-difluorophenyl)-4-(5-fluoro-2-hydroxy benzoyl)-1*H*-pyrazole derivative (**4b**), oxime derivatives 1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)(2-hydroxy-4-methylphenyl)methanone oxime (**5b**) and (5-chloro-2-hydroxyphenyl)(1-(3,4-difluorophenyl)-1*H*-pyrazol-4-yl)methanone oxime (**5e**) exhibited broad-spectrum antibiotic activity against the mentioned strains of gram-positive and gram-negative bacteria. Similarly benzoxazole derivatives substituted with methyl, chloro and fluoro groups (**6b, 6c, 6e**, and **6f**) exhibited promising results against *S. aureus* and *P. aeruginosa*.

For antifungal studies, the compounds were tested with a pathogenic fungi *C. albicans*. Greseofulvin and Nystatin were used as reference for inhibitory activity against fungi. The compound 1-(3,4-difluorophenyl)-4-(2-hydroxybenzoyl)-1*H*-pyrazole (**4d**) exhibited excellent activity against *C. albicans* in comparison to Nystatin. None of the other compounds were promising in terms of antifungal activity. A detailed structure activity relationship could not be evolved from the present studies.

In conclusion, we have designed and synthesized a series of fluorine containing 4-(substituted-2-hydroxybenzoyl) pyrazoles and pyrazolyl benzoxazoles, which exhibited promising antibacterial activity. We believe the insights gained in this study would be useful for the development of potential drug candidates derived from 4-(2-hydroxybenzoyl) pyrazoles to pyrazolyl benzo[*d*]oxazoles in the development of novel anti-infective agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.019.

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