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Original article

Design, synthesis and characterization of fluoro substituted novel pyrazolylpyrazolines scaffold and their pharmacological screening

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ABSTRACT

A novel series of fluoro substituted pyrazolylpyrazolines **7a**–**l** was synthesized in good to excellent yield (77–88%) from pyrazole chalcones **5a**–**d** and substituted phenyl hydrazine hydrochlorides **6a**–**c** under microwave irradiation. The newly synthesized compounds were screened for their preliminary *in vitro* antibacterial activity against a panel of pathogenic stains of bacteria and fungi, antituberculosis activity against *Mycobacterium tuberculosis* H37Rv and antimalarial activity against *Plasmodium falciparum*. Compounds **7a**, **7b**, **7g**, **7h**, **7j** and **7k** displayed excellent activity against *P. falciparum* stain as compared to quinine IC_{50} 0.268. Good antitubercular activity was exhibited by compounds **7a**, **7e**, **7h** and **7k**. Some of them also exhibited superior antibacterial activity as compared to the first line drugs.

1. Introduction

The most imperative vector-borne infectious diseases malaria is caused by the protozoan parasite *Plasmodium falciparum* [1]. The most recent report from the WHO states that malaria is responsible for the death of over 1 million persons every year including children under the age of five. It is most common in subtropical and tropical areas and 90% of the cases are originated in sub-Saharan Africa [2]. Tuberculosis is the second most common lethal infectious disease subsequent to AIDS and HIV [3]. About one-third of the world's population is infected by *Mycobacterium tuberculosis* every year and more than 2 million deaths are reported [4]. In this context, it was thought worth to synthesize novel compounds which may exhibit synergistic potency to be employed as antimicrobial, antituberculosis and antimalarial agents.

The substitution of fluorine in to a potential drug molecule can improve efficacy of drugs by extending pharmacokinetic and pharmacodynamics properties [5]. Trifluoromethyl group is a well-known substituent of unique qualities. Its high lipophilicity enables to improve pharmacological activities of the molecule [6,7]. Pyrazoles and their derivatives possess numerous medicinal applications because of their versatile biological activities [8–14]. They have occupied a distinct place due to a range of bioactivities such as antiproliferative [15], antimicrobial [16–18], antidepressant [19], antipyretic [20], anti-inflammatory [21] and anticonvulsant [22]. Pyrazoline is also an important nitrogenous heterocyclic moiety in many drugs. Literature survey revealed that various pyrazoline derivatives have displayed significant biological roles [23–29].

Microwave irradiation as a source of energy leads to environmentally benign protocols in terms of reduction in reaction time, energy saving with high efficiency, improved yields and selectivity [30]. In context of the above consequences and in continuation to our previous studies directed toward the synthesis of biologically active novel heterocyclic scaffolds [30–37], herein we report microwave assisted synthesis of some fluorinated novel pyrazolylpyrazoline derivatives. The synthesized compounds exhibited an interesting profile as antimalarial, antitubercular and antimicrobial agents.

2. Chemistry

The synthesis of novel series of pyrazolylpyrazolines **7a**–**l** was performed as outlined in Scheme 1. The starting material 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **1** was prepared according to Vilsmeier–Haack reaction of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one [38]. 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a**–**d** were prepared by refluxing compound **1** and substituted phenols **2a**–**d** in presence of anhydrous K_2CO_3 as basic catalyst in DMF as solvent. 3-methyl-5-

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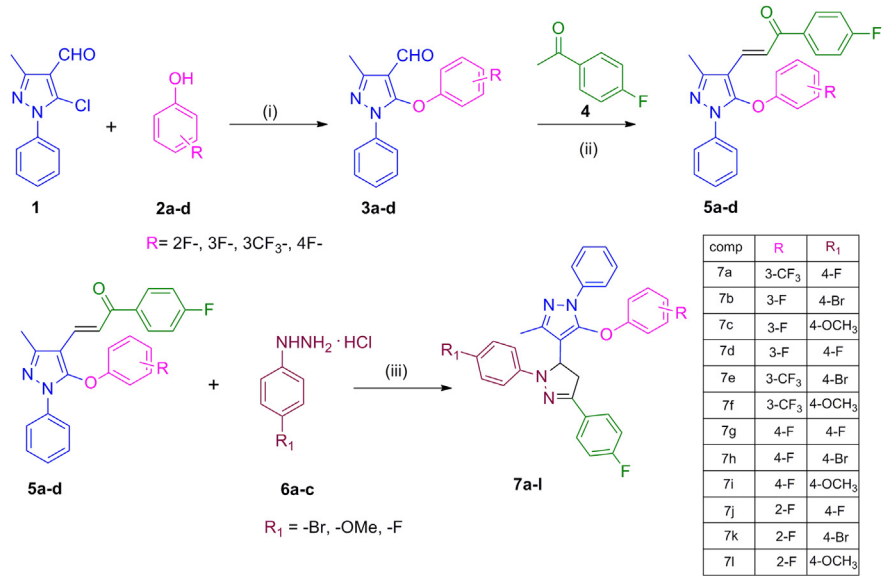

comp	R	R_1
7a	3-CF_3	4-F
7b	3-F	4-Br
7c	3-F	4-OCH_3
7d	3-F	4-F
7e	3-CF_3	4-Br
7f	3-CF_3	4-OCH_3
7g	4-F	4-F
7h	4-F	4-Br
7i	4-F	4-OCH_3
7j	2-F	4-F
7k	2-F	4-Br
7l	2-F	4-OCH_3

Scheme 1. Synthesis of 5-(4-fluorophenyl)-3′-methyl-5′-substituted aryloxy-1′,2-diphenyl-3,4-dihydro-1′H,2H-3,4′-bipyrazole (**7a**–**l**) (i) DMF, K_2CO_3, Reflux 2 h. (ii) 20% ethanolic NaOH, room temperature. (iii) Ethanol, catalytic glacial acetic acid, MW, 8–10 min, 350 W.

substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a**–**d** were subjected to base catalysed Claisen–Schmidt condensation reaction with 4-Fluoro acetophenone **4** generating the required (*E*)-1-(4-fluorophenyl)-3-(3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-ones **5a**–**d**. Finally pyrazolyl-pyrazolines **7a**–**l** were obtained by the condensation of **5a**–**d** and substituted phenyl hydrazine hydrochlorides **6a**–**c** in ethanol containing catalytic amount of glacial acetic acid under microwave irradiation at 350 W power level for 8–10 min.

Table 1
In vitro antimicrobial activity (MIC, μg/mL) of compounds **7a**–**l**.

Comp.	*Gram positive bacteria*			*Gram negative bacteria*			*Fungi*	
	S.P.	B.S.	C.T.	E.C.	S.T.	V.C.	C.A.	A.F.
	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC
	1936	441	449	443	98	3906	227	3008
7a	500	500	500	200	250	125	1000	250
7b	500	**250**	**250**	500	200	125	**250**	>1000
7c	**100**	**200**	**200**	500	500	200	1000	>1000
7d	200	**250**	500	200	200	250	**250**	1000
7e	500	**250**	**250**	250	200	250	1000	500
7f	200	**100**	**200**	**100**	**100**	250	**250**	500
7g	**100**	500	**250**	500	250	200	**200**	>1000
7h	500	500	**125**	200	200	500	**500**	>1000
7i	125	**62.5**	**200**	500	500	**100**	**500**	250
7j	250	500	**250**	200	250	250	1000	**100**
7k	250	**200**	500	**100**	200	500	**250**	1000
7l	500	**100**	500	500	500	500	**500**	250
A	100	250	250	100	100	100	n. t.[a]	n. t.
B	10	100	50	10	10	10	n. t.	n. t.
C	50	50	50	50	50	50	n. t.	n. t.
D	25	50	100	25	25	25	n. t.	n. t.
E	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	100	100
F	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	500	100

S.P.: *Streptococcus pneumoniae*, B.S.: *Bacillus subtilis*, C.T.: *Clostridium tetani*, E.C.: *Escherichia coli* S.T.: *Salmonella typhi*, V.C.: *Vibrio cholerae*, C.A.: *Candida albicans*, A.F.: *Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Norfloxacin, C: Chloramphenicol, D: Ciprofloxacin, E: Nystatin, F: Griseofulvin. The bold values indicate comparable/superior potency as compared to the reference drugs.

[a] n.t.: not tested.

3. Pharmacology

3.1. In vitro antimicrobial activity

The synthesized pyrazolylpyrazoline derivatives **7a**–**l** were evaluated for their antimicrobial activity by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [39]. The compounds were screened for anti-bacterial activity employing three Gram positive (*Clostridium tetani* MTCC 449, *Bacillus subtilis* MTCC 441, and *Streptococcus pneumoniae* MTCC 1936) and three Gram negative (*Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98 and *Vibrio cholerae* MTCC 3906) bacteria against ampicillin, norfloxacin, ciprofloxacin and chloramphenicol as the reference drugs. Antifungal activity was screened against two fungal species (*Candida albicans* MTCC 227 and *Aspergillus fumigats* MTCC 3008) where nystatin and griseofulvin were used as the standard drugs. The result of the antimicrobial screening data is shown in Table 1.

3.2. In vitro antituberculosis activity

A primary *in vitro* antituberculosis activity of novel pyrazolylpyrazolines **7a**–**l** was conducted at 250 μg/mL against *M. tuberculosis* H37Rv stain by using Lowensteine–Jensen medium as described by Rattan [40]. The obtained result is presented in Table 2 in the form of % inhibition. Rifampicin and Isoniazid were employed as the standard drugs.

Table 2
In vitro antituberculosis activity (% inhibition) of compounds **7a**–**l** against *M. tuberculosis* H37Rv (at concentration 250 μg/mL).

Comp.	% Inhibition	Comp.	% Inhibition
7a	**90**	**7h**	**96**
7b	56	7i	74
7c	56	7j	10
7d	65	**7k**	**94**
7e	**91**	7l	22
7f	52	**Rifampicin**	98
7g	40	**Isoniazid**	99

The bold values indicate comparable/superior potency as compared to the reference drugs.