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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Coumarinyl pyrazole derivatives of INH: promising antimycobacterial agents

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Abstract The purpose of this study was to evaluate the antimycobacterial activity of various pyrazole derivatives derived from the isoniazid pharmacophore along with coumarin scaffold. The synthesized title compounds (**4a**–**4k**) were investigated for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using Resazurin MIC assay. The synthesized compounds exhibited MIC ranging from 0.625 to 2.50 μg/ml. Among the series tested, compound 3-[3-(4-fluorophenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4i** was found to be the most active with MIC of 0.625 μg/ml.

Keywords Antimycobacterial activity · Coumarin · Isonicotinic acid hydrazide · Pyrazole · *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB) is still a challenging worldwide health problem and *Mycobacterium tuberculosis* remains one of the single most deadly human pathogens. The resurgence of TB over the last two decades, even in industrialized countries where it was almost eradicated, has been favored by the pathogenic synergy with human immunodeficiency virus (HIV) infection. In fact, TB and other atypical mycobacterioses are now diseases frequently associated with AIDS; HIV infection significantly increases the risk that new or latent TB infections will progress to active diseases (Collins, 1989; Graham *et al*., 1996; Halsey *et al*., 1998; Inderlied *et al*., 1993). The emergence of TB has also been accompanied by the appearance of single-drug-resistant (SDR) and multidrug-resistant (MDR) strains of *M. tuberculosis* which are insensitive to one or more of the first-line anti-TB drugs (isoniazid [INH], rifampin, ethambutol, streptomycin, and pyrazinamide) (Telzak *et al*., 1995). Indeed, a great amount of work has been done in order to acquire useful knowledge about the mechanisms of action and resistance to available anti-TB drugs (Dessen *et al*., 1995). *M. tuberculosis* often becomes drug resistant as a consequence of spontaneous genetic mutations involving the molecular targets of drugs. The primary mechanism of multidrug resistance in TB is the accumulation of mutations in individual drug target genes (Morris *et al*., 1995). However, such knowledge is not sufficient to rationally overcome drug resistance in mycobacteria. In fact, currently, combinations of two or more anti-TB drugs are used to prevent the development of resistant mycobacteria; sometimes it is also necessary to resort to second-line drugs (ciprofloxacin, ethionamide, kanamycin, amino salicylic acid, etc.) (Mandell and Petri, 1996; Sensi and Grassi, 1996). Consequently, the present anti-TB regimen is rather complex and lengthy. In immunosuppressed patients, it is also unsatisfactory. All of these serious concerns require particular attention and stimulate the continuing search for new anti-TB agents and therapeutic regimens.

The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The synthesis of

Authors sincerely dedicate the article in the name of Late. Dr. V. S. Maddi.

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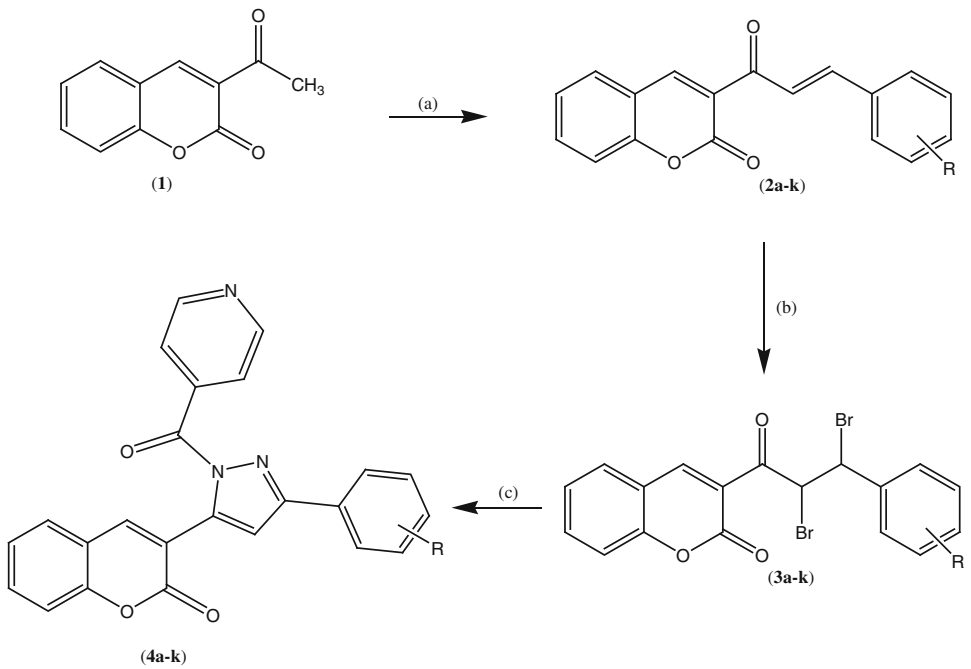
novel pyrazole derivatives and investigation of their chemical and biological behavior has gained more importance in recent decades for biological, medicinal, and agricultural reasons. Living organisms find difficulty in construction of N–N bonds which limits the natural abundance of compounds having such bonds. Pyrazole and their derivatives, a class of compounds containing the N–N bond exhibits a wide range of biological activities (Kucukguzel and Rollas, 2000; Nauduri and Reddy, 1998; Ali *et al*., 2007; Shaharyar *et al*., 2006a, b). Much attention is given to pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside like pyrazofurin which demonstrated a broad spectrum antimicrobial activity (Genin *et al*., 2000). A literature survey has revealed that pyrazole derivatives are active against many mycobacterias (Kucukguzel and Rollas, 2002; Shenoy *et al*., 2001).

Among the standard antimycobacterial agents, in spite of toxicity on repeated dosing INH is still considered to be a first-line drug for chemotherapy of TB. INH has very high in vivo inhibitory activity against *M. tuberculosis* H_{37}Rv. Enzymatic acetylation of INH by *N*-acetyltransferase represents a major metabolic pathway for INH in human beings. Acetylation greatly reduces the therapeutic activity of the drug, resulting in under dosing, decreased bioavailability, and acquired INH resistance (Kopec and Zwolska, 2002). Chemical modification of INH with a functional group that blocks acetylation, while maintaining a strong antimycobacterial action, may improve clinical outcomes and facilitate to reduce the rise of INH resistance. The aim of our research work was to investigate such chemical modification of INH. On the other hand, pyrazoline derivatives were active against many mycobacterias. Therefore, such medicinal properties associated with these two heterocycles render them as useful structural units in drug research. Recently, we have reported the synthesis and antibacterial activity of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives (Aragade *et al*., 2009). In view of these observations and in continuation of our research program on the synthesis of biheterocyclic compounds (Khode *et al*., 2009), we report herein the antimycobacterial activity of title compounds (**4a**–**4k**), which mainly describes the impact of incorporation of INH in a pyrazole along with coumarin moiety for their antimycobacterial activity against *M. tuberculosis* H_{37}Rv.

Results and discussion

Chemistry

The synthesis of series of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a**–**4k**) was achieved through the versatile and efficient synthetic route outlined in Scheme 1, as reported earlier by our group. It is apparent from the scheme that the new target molecules possess both coumarin and pyrazole units. Reaction of 3-[2, 3-dibromo-3-(substituted phenyl)propanoyl]-2*H*-chromen-2-one with INH seemed to be a convenient route for the synthesis of desired molecules. Starting material 3-acetyl-2*H*-chromen-2-one (**1**) was synthesized by the reaction of salicylaldehyde with ethylacetoacetate in presence of catalytic amount of piperidine at room temperature following the



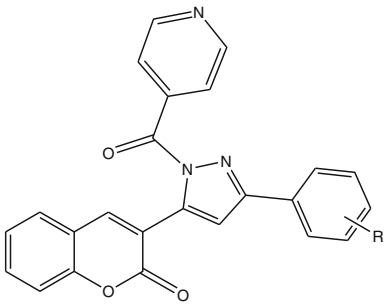
Scheme 1 Synthesis of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a**–**4k**) (Aragade *et al*., 2009). *Reagents and conditions*. (*a*) Ar–CHO, piperidine/*n*-butanol, reflux, 4 h; (*b*) Br_2/$CHCl_3$, stirr r.t. 12 h; (*c*) isonicotinic acid hydrazide, TEA/ethanol, reflux, 12 h

literature procedure (Bolakatti *et al*., 2008; Knoevenagel, 1898). 3-[(2*E*)-3-Substituted-prop-2-enoyl]-2*H*-chromen-2-one (chalcones, **2a–k**) were obtained by Claisen–Schmidt condensation of 3-acetyl-2*H*-chromen-2-one (**1**) with various substituted benzaldehydes in presence of mixture of piperidine and *n*-butanol. Efforts to convert compounds **2a–2k** into target molecules (**4a–4k**) under a variety of conditions were not successful. Hence an alternative method was adopted. This involved the bromination of chalcones (**2a–2k**) and subsequent ring closure using isonicotinic acid hydrazide. Bromination of chalcones (**2a–2k**) was carried out in chloroform using bromine in chloroform (Karthikeyan *et al*., 2007). The resulting dibromo compounds (**3a–3k**) on further treatment with isonicotinic acid hydrazide in the presence of triethylamine in absolute ethanol yielded the desired compounds 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a–4k**). Structures of the synthesized compounds were established on the basis of physicochemical, elemental analysis, and spectral data (IR and ^{1}H NMR), which were reported earlier by our group (Aragade *et al*., 2009).

Antimycobacterial activity

The antimycobacterial evaluation of the title compounds was carried out at the Tuberculosis Antimicrobial Acquistition Co-ordinating Facility (TAACF), USA, antituberculosis drug discovery program coordinated by the Southern Research Institute (Birmingham, USA) under the direction of the National Institute of Allergy and Infectious Diseases (NIAID, USA). All the compounds were screened against *M. tuberculosis* strain H_{37}Rv in Middlebrrok 7H9 medium using the Resazurin minimum inhibitory concentration (MIC) assay. This methodology is nontoxic, uses a thermally stable reagent and shows a good correlation as that of BACTEC radiometric methods (Reis *et al*., 2004). The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*. MIC was recorded as the lowest concentration of a compound that inhibits the growth of tested microorganism. The antimycobacterial activity data of test compounds were compared with the standard drug INH and rifampin which exhibited a MIC value of 0.06 and 0.003 μg/ml, respectively. The results of the in vitro antimycobacterial activity screening of the test compounds are summarized in Table 1. Among the newly synthesized compounds, 3-[3-(4-fluorophenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4i**) especially showed excellent activity at MIC 0.625 μg/ml and exhibited 80 % growth inhibition, while compounds **4c**, **4e**, **4f**, and **4h** showed good activity (MIC = 1.25 μg/ml) with 75.39, 69.09, 65.39, and 65.14 % growth inhibition, respectively. Further, compounds **4a**, **4b**, **4d**, **4j**, and **4k** showed respectable antimycobacterial activity (MIC = 2.5 μg/ml) with 75.39, 66.67, 73.82, 67.83, and 62.63 % growth inhibition, respectively. Whereas compound **4g** displayed the least activity (MIC = 5.0 μg/ml) with 62.63 % growth inhibition as compared to the standard drugs, INH, and rifampicin, respectively. In general, the brief structure–activity relationship (SAR) studies revealed that the presence of electron withdrawing groups such as F, Cl, and NO_2 on the phenyl ring of pyrazole moiety at C-3 position may be attributed for enhanced antimycobacterial activity in the series. The presence of 4-fluoro group in (**4a–4k**) derivatives, i.e., compound **4i** displayed relatively higher inhibitory activity. Similarly, the chloro and nitro substituted analogs, such as 4-chlorophenyl (**4c**), 2,4-dichlorophenyl (**4e**), and 3-nitrophenyl (**4f**) showed moderate inhibitory activity against *M. tuberculosis*. These reports clearly showed that the compound **4i** containing a 4-fluoro group at C-3 phenyl ring of pyrazole nucleus gave better result and has emerged as promising antimycobacterial agents.

Table 1 In vitro antimycobacterial activity of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a–4k**)



(**4a-k**)

Compounds	R	MIC[a]	IC_{50}	IC_{90}	% growth inhibition
4a	H–	2.5	1.92	2.26	73.53
4b	4-OMe–	2.5	1.21	1.35	66.67
4c	4-Cl–	1.25	0.75	1.22	75.39
4d	4-NMe_2–	2.5	1.21	2.3	73.82
4e	2,4-$(Cl)_2$–	1.25	0.74	1.46	69.09
4f	3-NO_2–	1.25	0.89	1.67	65.39
4g	4-Me–	5	2.46	2.69	62.63
4h	3-OMe–	1.25	1.17	1.29	65.14
4i	4-F–	0.625	0.41	0.73	80.09
4j	2-NO_2–	2.5	1.9	2.74	67.83
4k	4-OH–	2.5	1.51	2.85	69.91
INH	–	0.06			
Rifampicin	–	0.003			

[a] MIC is expressed in μg/ml

Conclusion

We have described earlier an efficient synthesis and antibacterial activity of a novel series of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a**–**4k**). In these novel heterocyclic compounds INH has been converted into a pyrazole nucleus which also contains of coumarin ring system. Herein, we report the antimycobacterial activity of title compounds (**4a**–**4k**). In general, the results of the in vitro antimycobacterial activity tests were encouraging as out of eleven compounds tested, one compound **4i** exhibited significant antimycobacterial activity, which is comparable to the reference drugs. The MIC values of these novel compounds evidenced that the presence of fluorine, chlorine, and nitro groups in the phenyl ring at C-3 position of the pyrazole nucleus gave rise to increased antimycobacterial potency. In conclusion, the compound **4i** may serve as a promising lead molecule for new anti-tubercular drug development and our findings will have an impact on medicinal chemists and pharmacists for further investigations in this field in search of potent anti-tubercular agents.

Experimental

Chemistry

All research chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent grade were dried and purified, when necessary, according to the literature. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from Merck (Darmstadt, Germany). Melting points of synthesized compounds were determined in Thermonik melting point apparatus (Thermonik, Mumbai, India) and are uncorrected, UV spectra were recorded on Thermospectronic spectrometer (Rochester, NY, USA) and IR spectra were recorded on Thermo Nicolet IR200 FT-IR spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}H NMR was recorded on Bruker AVANCE 300 (Bruker, Rheinstetten/Karlsruhe, Germany) using DMSO-d_6 as solvent. Chemical shifts are given in δ ppm units with respect to TMS as internal standard. The elemental analyzes (C, H, and N) of the compounds were performed on Heraus CHN–O rapid elemental analyzer (Heraeus, Hanau, Germany). Results of elemental analysis were within $\pm$0.4 % of the theoretical values. The purity of compounds was examined by TLC on silica gel plate using chloroform and methanol (10:1) as mobile phase and iodine vapors as visualizing agent. The starting material 3-acetyl coumarin (**1**) was synthesized by our earlier reported method (Bolakatti *et al*., 2008). The synthesis and spectral characterization of a series of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives (**4a**–**4k**) were reported earlier by our group (Aragade *et al*., 2009).

Antimycobacterial activity

Medium

Middlebrook 7H9 broth medium (7H9 medium) was used for testing antimycobacterial activity. Middle brook 7H9 broth medium supplemented with 0.2 % (v/v) glycerol, 10 % (v/v) albumin, dextrose, catalase (ADC), and 0.05 % (v/v) Tween 80.

Test microorganism

Mycobacterium tuberculosis $H_{37}Rv$ obtained from Colorado State University, Fort Collins, CO was used for testing antimycobacterial activity.

Inoculum preparation

The mycobacterium was inoculated in 50 ml of 7H9 medium in 1 l bottles that were placed on a roller bottle apparatus in an ambient 37 °C incubator. When the cells were reached OD_{600} of 0.150 (equivalent to $\sim 1.5 \times 10^7$ CFU/ml), they were diluted 200-fold in 7H9 medium.

Minimum inhibitory concentration

The in vitro antimycobacterial activity for newly synthesized compounds **4a**–**4k** was evaluated using Resazurin MIC assay (Collins and Franzblau, 1997). 20 µl of the 3.2 mg/ml test compound is added to 96-well microtiter plate. Twofold dilutions were made by the addition of 20 µl of diluents. Further progressive double dilution with dilutent was performed to obtain the required concentrations 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 µg/ml. Then each dilution was further diluted 1:10 in sterile water. 6.25 µl of each dilution was transferred to duplicate 96-well test plates. The cell suspension (93.75 µl $\sim 10^4$ bacteria) in 7H9 medium was added to the test plate. The 96-well test plates were incubated in an ambient 37 °C incubator for 6 days. At the end of incubation period, sterile resazurin solution (5 µl of 0.05 %) was added to each cell of the 96-well plate and placed in an ambient 37 °C incubator for 2 days. After completion of 2 day incubation, the MIC (a visual evaluation) and IC_{50} and IC_{90} (fluorometric readout) were determined.

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Conflict of interest The authors have declared no conflict of interest.

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