Bioorganic & Medicinal Chemistry Letters 21 (2011) 5259–5261

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel antitubercular 3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide/carbothioamide analogues

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ARTICLE INFO

Article history:
Received 9 June 2011
Revised 6 July 2011
Accepted 8 July 2011
Available online 19 July 2011

Keywords:
Pyrazolines
Antitubercular agents

ABSTRACT

In the present investigation, a series of 3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide/carbothioamide analogues were synthesized and were evaluated for antitubercular activity by two fold serial dilution technique. All the newly synthesized compounds showed low to high inhibitory activities against *Mycobacterium tuberculosis* $H_{37}Rv$ and INH resistant *M. tuberculosis*. The compound 3-(4-fluorophenyl)-6,7-dimethoxy-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carbothioamide (**4o**) was found to be the most promising compound active against *M. tuberculosis* $H_{37}Rv$ and isoniazid resistant *M. tuberculosis* with minimum inhibitory concentration 3.12 μM and 6.25 μM, respectively.

Mycobacterium tuberculosis infections are responsible for one in four preventable adult deaths in developing countries. The current strategies for the treatment of tuberculosis (TB) are very complicated as it takes several months of chemotherapy to eliminate persistent bacteria. Also widespread non-compliance has contributed to the emergence of multidrug-resistant (MDR) and extensively drug-resistent (XRD) TB.[1,2] There are estimated 1.3 million multi/extensively drug resistant TB (M/XDR-TB) cases will need to be treated between 2010 and 2015.[3] Hence there is a need for fast acting drugs that are capable of eliminating an infection just in a few weeks.

In an effort to discover new and effective therapeutic agents, we recently reported the in vitro antimycobacterial activity of novel diketones and pyrazoline derivatives.[4,5] Pyrazoline is a class of heterocyclic compounds possess significant pharmacological activities including anticancer, antitubercular, anticonvulsant, antiamoebic, antimicrobial, anti-inflammatory, antiviral, antiarrhythmic, antidepressant, antidiabetic, etc.[6–15] In the current work we have synthesized eighteen 3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide/carbothioamide analogues based on the structure of the known antitubercular agent, thiacetazone (Fig 1). All the synthesized compounds were evaluated for their anti-tubercular activities.

The 3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide/carbothioamide analogues (**4a**–**r**) described in this study are shown in Table 1 and the reaction sequence for the synthesis is summarised in Scheme 1. In the initial step 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one (0.1 mol) and appropriate aromatic aldehydes (0.1 mol) in diluted methanolic sodium hydroxide solution stirred at room temperature giving the (2*E*)-2-substituted-5,6-dimethoxy-2,3-dihydro-1*H*-indene-1-one derivatives (**3a–f**). In the subsequent step 2-substituted-5,6-dimethoxy-2,3-dihydro-1*H*-indene-1-one derivatives treated with appropriate semicarbazides furnished the titled compounds (**4a–r**). The substituted phenyl semicarbazides were synthesized as per the reported method.[16,17] The yields of the titled compounds were ranging from 66% to 87% after recrystallization with absolute ethanol. The purity of the compounds was checked by TLC using eluant benzene–acetone (9:1) and elemental analyses. Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on TLC plates (silica gel G) using eluants benzene–acetone (9:1), the spots were located under iodine vapours or UV light. The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). IR spectra were obtained on a Schimadzu 8201 PC, FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO. Mass spectra were recorded on a Bruker Esquire LCMS using ESI and elemental analyses were performed on Perkin–Elmer 2400 Elemental Analyzer. Both the analytical and spectral data (IR, ^{1}H NMR) of all the synthesized compounds were in full accordance with the proposed structures. In general, the IR spectra of the compounds afforded pyrazoline C=N stretching at 1501–1576 cm^{-1}, C–H deformation at 1362–1464 cm^{-1}, C_2–N_1 stretching at 1069–1189 cm^{-1}, and carbamoyl

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doi:10.1016/j.bmcl.2011.07.035

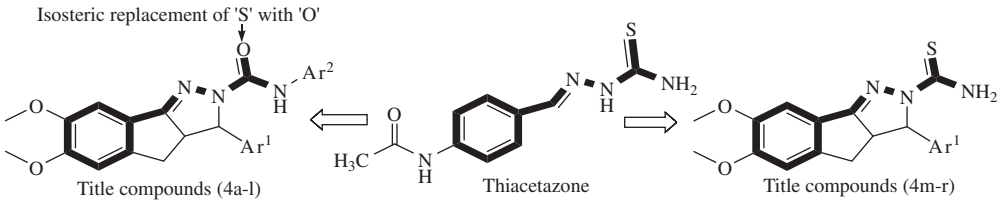
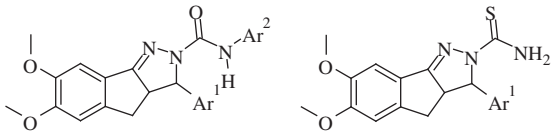


Figure 1. Design of the title compounds based on known antitubercular drug.

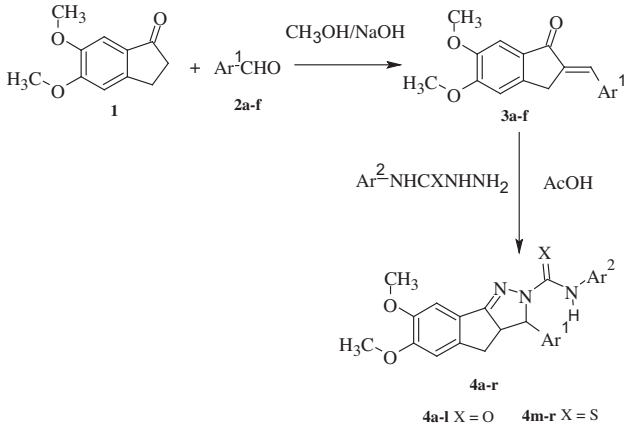
Table 1
Physical constant and antimycobacterial activity of the synthesized compounds



4a-4l **4m-4r**

Compound	Ar^1	Ar^2	Yield (%)	Mp (°C)	MIC (μM)	
					MTB[a]	MTB[b]
4a	4-Pyridinyl-	2,4-Dimethylphenyl-	78	176	10	>12.5
4b	2-Chlorophenyl-	2,4-Dimethylphenyl-	72	186	6.25	10
4c	4-Fluorophenyl-	2,4-Dimethylphenyl-	68	180	6.25	12.5
4d	3,4-Dimethoxyphenyl-	2,4-Dimethylphenyl-	84	188	12.5	>12.5
4e	Phenyl-	2,4-Dimethylphenyl-	67	150	>12.5	>12.5
4f	4-Methoxyphenyl-	2,4-Dimethylphenyl-	79	124	>12.5	>12.5
4g	4-Pyridinyl-	2,6-Dimethylphenyl-	82	190	>10	>12.5
4h	2-Chlorophenyl-	2,6-Dimethylphenyl-	87	160	6.25	12.5
4i	4-Fluorophenyl-	2,6-Dimethylphenyl-	85	178	6.25	10
4j	3,4-Dimethoxyphenyl-	2,6-Dimethylphenyl-	73	174	12.5	>12.5
4k	Phenyl-	2,6-Dimethylphenyl-	66	148	12.5	>12.5
4l	4-Methoxyphenyl-	2,6-Dimethylphenyl-	69	164	>12.5	>12.5
4m	4-Pyridinyl-	–	82	194	6.25	10
4n	2-Chlorophenyl-	–	84	202	6.25	10
4o	4-Fluorophenyl-	–	68	212	3.12	6.25
4p	3,4-Dimethoxyphenyl-	–	66	262	12.5	12.5
4q	Phenyl-	–	76	232	12.5	12.5
4r	4-Methoxyphenyl-	–	75	104	12.5	>12.5
INH	–	–	–	–	0.78	12.5
Rifampin	–	–	–	–	0.006	3.12

[a] *M. tuberculosis* $H_{37}Rv$.
[b] INHR-TB.



Scheme 1. Protocol for the synthesis.

group N–H stretching at 3112–3481 cm^{-1} and C=O stretching at 1675–1685 cm^{-1} bands. In the Nuclear Magnetic Resonance spectra (^1H NMR) the signals of the respective protons of the synthesized titled compounds were verified on the basis of their chemical shift, multiplicities and coupling constants in DMSO-d_6. The spectra showed singlet at δ 2.21–2.37 ppm corresponding to CH_3; triplet at δ 3.20–3.57 ppm corresponding to CH; a doublet at δ 3.43–3.48 ppm corresponding to CH_2 group; a singlet at δ 3.79–3.83 ppm corresponding to OCH_3 group; a doublet at 4.9–5.4 ppm corresponding to CH; a broad singlet at 5.2–5.6 ppm corresponding to NH_2; multiplet at δ 6.51–8.38 ppm corresponding to aromatic protons; broad singlet at δ8.88–11.52 ppm corresponding to CONH. The elemental analysis results were within ±0.4% of the theoretical values.

All the synthesized compounds (**4a–r**) were tested for their in vitro antimycobacterial activity against MTB and INHR-MTB by agar dilution method using double dilution technique similar to that recommended by the National Committee for Clinical Laboratory Standards.[18] The MTB and INHR-MTB clinical isolate was obtained from Tuberculosis Research Center, Alwar, India. The MIC was defined as the minimum concentration of compound required to inhibit 90% of bacterial growth and MIC of the compounds were reported in Table 1 with standard drug INH and rifampin for comparison.

Among the 18 synthesized compounds, seven compounds were found to be active with minimum inhibitory concentration of

3.12–6.25 μM. The compound **4o** was found to be active against MTB and INH-MTB at a MIC of 3.12 μM and 6.25 μM, respectively. When compared with INH the compound, **4o** was four folds less active than INH against MTB, while compound, **4o** was two folds more active than INH against INHR-MTB. In the title compounds (**4a–r**), both the C_3 aryl group and the *N*-aryl group influenced the antitubercular activity. The 3-substituted compounds with electron withdrawing groups such as 4-flourophenyl produced more inhibitory activity than 2-chlorophenyl and 2-pyridyl substitution, while the electron releasing groups such as 4-methoxyphenyl, 3,4-dimethoxyphenyl showed less inhibitory activity. *N*-Aryl substitution with electron releasing group showed decreased activity when compared with reported earlier screening.[5] Also substitution of 'O' with 'S' produced more pronounced activity in the series of title compounds (**4a–4r**), might be due to increased lipophilicity. The compounds **4b**, **4c**, **4h**, **4i**, **4m, 4n** and **4a** showed good to moderate inhibitory activity against MTB at MIC 6.25 μM and 10 μM.

All the active compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/mL or 10 times the MIC. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method. Most of the active compounds were found to be non-toxic up to 62.5 μg/mL.[19] All these derivatives can be further modified to exhibit more potency. Further studies to acquire more information about Quantitative Structure Activity Relationships (QSAR) and MDR are in progress in our laboratory. The pyrazoline derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of tubercular disease.

Acknowledgments

Authors are thankful to the management people of Alwar Pharmacy College, Alwar, Rajasthan, India for providing research facilities. The authors are also thankful to Dr. K.P. Singh and Dr. (Mrs.) Shobha Tomar, National Institute of Medical Sciences University for their guidance.

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19. Gundersen, L. L.; Nissen-Meyer, J.; Spilsberg, B. *J. Med. Chem.* **2002**, *45*, 1383. Compound (**4o**): 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one (1.92 g, 0.001 mol) with 4-fluorobenzaldehyde (1.24 g, 0.001 mol) in diluted methanolic sodium hydroxide solution was stirred under room temperature for 4 h. The reaction mixture was then poured in crushed ice neutralized with diluted HCl, giving the (2*E*)-2-(4-fluorobenzylidene)-5,6-dimethoxy-2,3-dihydro-1*H*-indene-1-one which was then refluxed with thiosemicarbazide in glacial acetic acid for 12 h. Excess of solvent was removed and the reaction mixture was poured in crushed ice then filtered and washed furnished 3-(4-fluorophenyl)-6,7-dimethoxy-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carbothioamide (**4o**). IR (KBr) cm^{-1}: 3364 (NH), 1680 (C=O), 1579 (C=N), 1389 (CH), 1180 (C–N), 789 (C–F); ^1H NMR (DMSO-d_6) ppm: 3.23–3.35 (1H, t, CH), 3.41–3.45 (2H, d, J = 6.3 Hz, CH_2), 3.81 (6H, s, OCH_3), 5.2 (1H, d, J = 6.1 Hz, CH), 5.4 (2H, br s, NH_2), 6.88–8.16 (6H, m, Ar); Mass (m/z) 371 (M^+); Cacld/Anal. [C (61.44) 61.43, H (4.88) 4.87, N (11.31) 11.32].