Journal of Pharmaceutical Research International

33(62A): 223-231, 2021; Article no.JPRI.82313
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Isoxazole Clubbed 1-carbothioamido-4,5-dihydro-1H-pyrazoles: Design, Synthesis, Characterization and Antitubercular Evaluation

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i62A35201

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/82313

Original Research Article

Received 24 October 2021
Accepted 27 December 2021
Published 28 December 2021

ABSTRACT

Tuberculosis (TB) is a global problem inspite the availability of drugs. This state of affairs is due to the limitations of existing drugs including muti-drug resistance and toxicites. As a result, there is a pressing need for new antitubercular medicines to be developed. In the present investigation we designed and synthesized a series of isoxazole clubbed 1-carbothioamido-4,5-dihydro-1*H*-pyrazoles (**16-30**) in considerable yeilds (43-78%). Further these compounds were purified by recrystallization and charcterized by spectral techniques-Mass, FT-IR and ^{1}H NMR and then evaluated for their antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain. Among the tested compounds, the analogues **24** bearing 3,5-dimethoxyphenyl scaffold at the 5th position of 4,5-dihydro-1*H*-pyrazole ring showed superior activity than isoniazid (MIC = 0.25 µg/mL) with MIC value 0.1 µg/mL whereas the compound **25** containing 2,3,4-trimethoxyphenyl had equal potency as that of isoniazid. Additionally, **24** and **25** were found to be less selective towards the human normal liver cell lines-LO2 in their cytotoxicity assays. Hence, these two compounds are safe and useful lead candidates for the development of novel antitubercular drugs.

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Keywords: Isoxazole; 1-carbothioamido-4,5-dihydro-1H-pyrazoles; antitubercular activity; mycobacterium tuberculosis H37Rv; MABA assay; cytotoxicity assay.

1. INTRODUCTION

Mycobacterium tuberculosis causes tuberculosis (TB), which is the greatest cause of single pathogen infection-related fatalities. TB is found worldwide. South-East Asia, with 44% of new TB cases, had the greatest number of new TB cases in 2019, followed by Africa, with 25%, and the Western Pacific, with 18%. In 2019, there are 87% of new TB cases in the 30 countries with the greatest TB burden in Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa account for two-thirds of new TB cases. Despite WHO's intention of reducing TB cases by 20% between 2015 and 2020, this target was not reached owing to MDR-TB and Rifampicin Resistant-TB (RR-TB). A total of 206,362 cases of MDR/RR-TB were recorded in 2019, a 10% rise from 186,883 in 2018. Multidrug-resistant tuberculosis affects mainly three countries: India, China, and Russia. Mycobacterium tuberculosis with drug-resistant isoniazid and rifampicin bacteria is a kind of MDR-TB. TB may be treated and cured. Second-line treatment choices are limited and need expensive and need intensive chemotherapy. Sometimes this may lead to severe drug resistance. TB which is not responsive to a second-line therapy, leaves patients with no further therapeutic choices [1]. To succeed, new antitubercular therapies are required to over come this global issue.

Five membered heterocyclic scaffolds constitute an important class of heterocyclics with potential bioactivities. Among them isoxazole and dihydropyrazole were of special interest to medicinal chemists, owing to their useful biological actions. Both these rings are not only the part of many drug molecules but their derivatives were reported with significant activities including antitubercular [2-9], antioxidant [10-11], anticancer [12,13], antibacterial [14,15], antifungal [16,17]. Hence, combining these two rings into a single molecule has a greater propensity of improving the activity of new analogues. In the previous study Kishor et al., synthesized isoxazole appended dihydropyrazole-1-carboxamides considering the presence of carbohydrazide portion of isoniazid and isoxazolidine ring of cycloserine and arrived with potential antitubercular lead molecules [18]. Ethionamide and thiacetazone are two drugs bearing carbothioamide ($S=C-NH_2$) functionality which is crucial for their potential antitubercular activity. In view of the above facts, here we synthesized and screened isoxazole clubbed 1-carbothioamido-4,5-dihydro-1*H*-pyrazoles as prospective antitubercular agents (Fig. 1).

2. MATERIALS AND METHODS

2.1 General

Isoxazolyl chalcones were used for the synthesis whereas thiosemicarbazide was purchased from Sigma Aldrich Chemical Co. (Milwaukee, Wisconsin, USA-53233). Merck grade silica gel-GF was used as the adsorbent for TLC to monitor the reactions. Boetius melting point apparatus was used to determine the melting points in open capillaries and the values are expressed in ^{o}C and are uncorrected. Mass spectra were recorded on Agilent LC-MS spectrometer whereas the FT-IR spectra were recorded on Bruker Vertex 80v spectrophotometer using potassium bromide disks and the wave numbers of the absorption bands are expressed in cm^{-1}. The ^{1}H NMR spectra were recorded by dissolving the compounds in deutereated chloroform on a Bruker AMX 400 MHz NMR spectrophotometer at an operating frequency of 400 MHz. TMS is used as an internal standard and the chemical shifts (δ) of the protons are expressed in ppm.

2.2 Chemistry

2.2.1 General method of synthesis of isoxazole clubbed 1-carbothioamido-4,5-dihydro-1H-pyrazoles

The intermediate (*E*)-1-(isoxazole-5-yl)-3-(aryl substituted)prop-2-en-1-one derivatives (**1-15**) were synthesized by using the protocol prescribed in the literature [19]. (E)-1-(isoxazole-5-yl)-3-(aryl substituted)prop-2-en-1-one derivatives (**1-15**) (1 mmol) and thiosemicarbazide (1 mmol) were refluxed for 8-11 h in 15-20 ml glacial acetic acid. After the completion of the reaction, excess acetic acid was removed under decreased pressure and then the reaction mixture was transferred into the crushed ice. The target isoxazole clubbed 1-carbothioamido-4,5-dihydro-1*H*-pyrazoles (**16-30**) were obtained by filtering, drying, and recrystallizing the solid mass using ethanol [20] (Scheme 1).

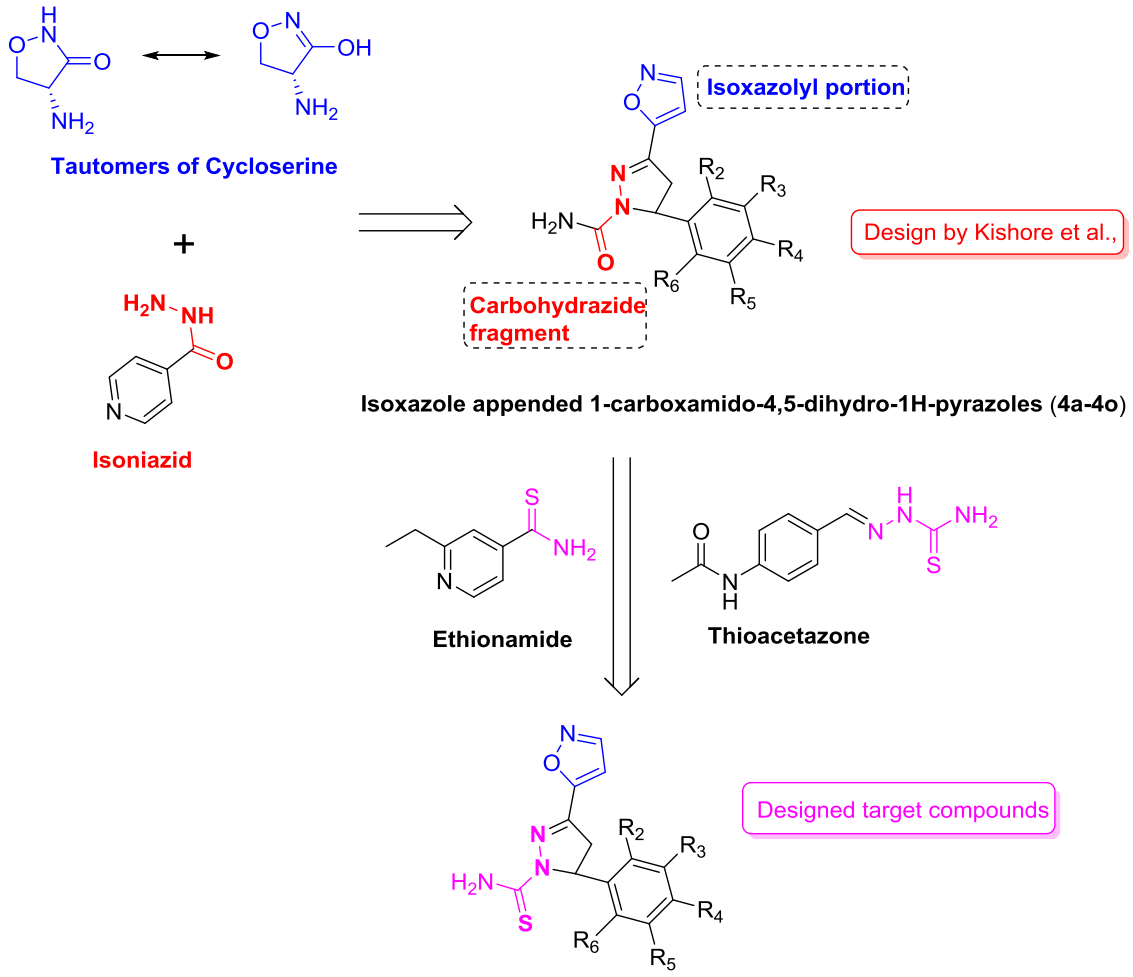
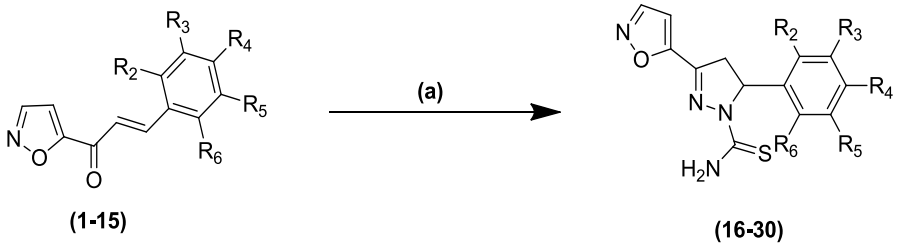


Fig. 1. Design of isoxazole clubbed 1-carbothioamido-4,5-dihydro-1*H*-pyrazoles



16: R_2=H; R_3=H; R_4=OCH_3; R_5=H; R_6=H
17: R_2=H; R_3=OCH_3; R_4=H; R_5=H; R_6=H
18: R_2=OCH_3; R_3=H; R_4=H; R_5=H; R_6=H
19: R_2=OCH_3; R_3=OCH_3; R_4=H; R_5=H; R_6=H
20: R_2=OCH_3; R_3=H; R_4=OCH_3; R_5=H; R_6=H
21: R_2=OCH_3; R_3=H; R_4=H; R_5= OCH_3; R_6=H
22: R_2=OCH_3; R_3=H; R_4=H; R_5=H; R_6=OCH_3
23: R_2=H; R_3= OCH_3; R_4= OCH_3; R_5=H; R_6=H
24: R_2=H; R_3= OCH_3; R_4=H; R_5=OCH_3; R_6=H
25: R_2=OCH_3; R_3=OCH_3; R_4=OCH_3; R_5=H; R_6=H
26: R_2=OCH_3; R_3=H; R_4=OCH_3; R_5= OCH_3; R_6=H
27: R_2=OCH_3; R_3=H; R_4=OCH_3; R_5=H; R_6=OCH_3
28: R_2=H; R_3= OCH_3; R_4=OCH_3; R_5=OCH_3; R_6=H
29: R_2=F; R_3=OCH_3; R_4=OCH_3; R_5=H; R_6=H
30: R_2=Cl; R_3=H; R_4=OCH_3; R_5=H; R_6=OCH_3

Scheme 1. Synthetic strategy employed for the preparation of Isoxazole clubbed 1-carbothioamido-4,5-dihydro-1H-pyrazoles (16-30); (a) glacial acetic acid, reflux

3-(isoxazol-5-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (16): Yield 62%; Molecular Weight: 302.35; **m.p.** 95-97 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1589 (C=N), 1239 (C=S), 3359 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.05 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB}=16 Hz), 3.65 (1H, H_B, dd, J_{AB} = 16Hz, dd, J_{BX}= 12 Hz), 5.05 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 9.65 (2H, s, NH_2, D_2O exchangeable), 3.54 (3H, s, Ar-OCH_3), 6.06-8.11 (6H, Ar-H); **MS** (*m/z*, %): 303.25 (M+1, 98.59).

3-(isoxazol-5-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (17): Yield 43%; Molecular Weight: 302.35; **m.p.** 88-89 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1591 (C=N), 1233 (C=S), 3366 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.08 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.66 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.11 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX}=12 Hz), 9.82 (2H, s, NH_2, D_2O exchangeable), 3.77 (3H, s, Ar-OCH_3), 6.06-7.95 (6H, Ar-H); **MS** (*m/z*, %): 303.25 (M+1, 98.56).

3-(isoxazol-5-yl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (18): Yield 49%; Molecular Weight: 302.35; **m.p.** 92-94 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1588 (C=N), 1245 (C=S), 3312 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.09 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} =16 Hz), 3.74 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.13 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX}=12 Hz), 9.66 (2H, s, NH_2, D_2O exchangeable), 3.62 (3H, s, Ar-OCH_3), 6.11-8.11 (6H, Ar-H); **MS** (*m/z*, %): 303.25 (M+1, 99.85).

3-(isoxazol-5-yl)-5-(2,3-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (19): **Yield** 44%; Molecular Weight: 332.38; **m.p.** 115-117 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1573 (C=N), 1241 (C=S), 3321 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.11 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB}=16Hz), 3.76 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.14 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX}=12 Hz), 9.79 (2H, s, NH_2, D_2O exchangeable), 3.51 (3H, s, Ar-OCH_3), 3.64 (3H, s, Ar-OCH_3), 6.15-8.15 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.51).

3-(isoxazol-5-yl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (20): **Yield** 65%; Molecular Weight: 332.38; **m.p.** 132-134 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1566 (C=N), 1244 (C=S), 3322 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.12 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB}=16Hz), 3.78 (1H, H_B, dd, J_{AB} = 16Hz, dd, J_{BX}= 12Hz), 5.09 (1H, H_x, dd, J_{AX} = 3.6Hz, dd, J_{BX}=12Hz), 9.41 (2H, s, NH_2, D_2O exchangeable), 3.46 (3H, s, Ar-OCH_3), 3.52 (3H, s, Ar-OCH_3), 6.12-8.05 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.12).

3-(isoxazol-5-yl)-5-(2,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (21): **Yield** 66%; Molecular Weight: 332.38; **m.p.** 141-143 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1568 (C=N), 1230 (C=S), 3312 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.06 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB}=16Hz), 3.79 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.16 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX}=12 Hz), 9.22 (2H, s, NH_2, D_2O exchangeable), 3.59 (3H, s, Ar-OCH_3), 3.68 (3H, s, Ar-OCH_3), 6.01-7.98 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.31).

3-(isoxazol-5-yl)-5-(2,6-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (22): **Yield** 51%; Molecular Weight: 332.38; **m.p.** 119-121 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1571 (C=N), 1232 (C=S), 3319 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.09 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.22 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 9.54 (2H, s, NH_2, D_2O exchangeable), 3.55 (3H, s, Ar-OCH_3), 3.73 (3H, s, Ar-OCH_3), 6.02-8.15 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.31).

3-(isoxazol-5-yl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (23): **Yield** 55%; Molecular Weight: 332.38; **m.p.** 164-168 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1574 (C=N), 1241 (C=S), 3331 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.16 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.72 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.24 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 9.41 (2H, s, NH_2, D_2O exchangeable), 3.58 (3H, s, Ar-OCH_3), 3.81 (3H, s, Ar-OCH_3), 6.19-8.38 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.31).

3-(isoxazol-5-yl)-5-(3,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (24): **Yield** 69%; Molecular Weight: 332.38; **m.p.** 155-157 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1577 (C=N), 1242 (C=S), 3329 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.17 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} =16 Hz), 3.78 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.28 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} =12 Hz), 9.58 (2H, s, NH_2, D_2O exchangeable), 3.48 (3H, s, Ar-OCH_3), 3.94 (3H, s, Ar-OCH_3), 6.29-8.58 (5H, Ar-H); **MS** (*m/z*, %): 333.38 (M+1, 99.31).

3-(isoxazol-5-yl)-5-(2,3,4-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (25): Yield 75%; Molecular Weight: 362.10; **m.p.** 191-193 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1578 (C=N), 1238 (C=S), 3338 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.06 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.77 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.34 (1H, H_x, dd, J_{AX} = 3.6Hz, dd, J_{BX} =12 Hz), 9.48 (2H, s, NH_2, D_2O exchangeable), 3.61 (3H, s, Ar-OCH_3), 3.94 (6H, s, 2x Ar-OCH_3), 6.32-8.65 (4H, Ar-H); **MS** (*m/z*, %): 363.10 (M+1, 99.56).

3-(isoxazol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (26): Yield 71%; Molecular Weight: 362.10; **m.p.** 186--188 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1574 (C=N), 1239 (C=S), 3328 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.08 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.68 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.38 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} =12 Hz), 9.51 (2H, s, NH_2, D_2O exchangeable), 3.76 (3H, s, Ar-OCH_3), 3.92 (6H, s, 2x Ar-OCH_3), 6.46-8.75 (4H, Ar-H); **MS** (*m/z*, %): 363.10 (M+1, 99.88).

3-(isoxazol-5-yl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (27): Yield 77%; Molecular Weight: 362.10; **m.p.** 165-167 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1579 (C=N), 1238 (C=S), 3341 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.09 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB}=16Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.25 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} =12 Hz), 9.29 (2H, s, NH_2, D_2O exchangeable), 3.72 (3H, s, Ar-OCH_3), 3.95 (6H, s, 2x Ar-OCH_3), 6.44-8.58 (4H, Ar-H); **MS** (*m/z*, %): 363.10 (M+1, 99.45).

3-(isoxazol-5-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (28): Yield 71%; Molecular Weight: 362.10; **m.p.** 181-183 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1578 (C=N), 1243 (C=S), 3344 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.10 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.76 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.18 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} =12 Hz), 9.38 (2H, s, NH_2, D_2O exchangeable), 3.66 (3H, s, Ar-OCH_3), 3.97 (6H, s, 2x Ar-OCH_3), 6.22-8.44 (4H, Ar-H); **MS** (*m/z*, %): 363.10 (M+1, 99.71).

3-(isoxazol-5-yl)-5-(2-fluoro-3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (29): Yield 76%; Molecular Weight: 350.37; **m.p.** 136-138 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1584 (C=N), 1248 (C=S), 3349 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.11 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} =16 Hz), 3.82 (1H, H_B, dd, J_{AB} = 16Hz, dd, J_{BX}= 12Hz), 5.35 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 9.56 (2H, s, NH_2, D_2O exchangeable), 3.65 (3H, s, Ar-OCH_3), 3.91 (3H, s, 2x Ar-OCH_3), 6.48-8.65 (4H, Ar-H); **MS** (*m/z*, %): 351.37 (M+1, 99.16).

3-(isoxazol-5-yl)-5-(2-chloro-4,6-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (30): Yield 78%; Molecular Weight 366.82; **m.p.** 172-174 ^{o}C; **FT-IR** (KBr, cm^{-1}): 1588 (C=N), 1246 (C=S), 3351 (-NH_2); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.08 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} =16 Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.36 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} =12 Hz), 9.52 (2H, s, NH_2, D_2O exchangeable), 3.48 (3H, s, Ar-OCH_3), 3.94 (3H, s, 2x Ar-OCH_3), 6.38-8.25 (4H, Ar-H); **MS** (*m/z*, %): 367.82 (M+1, 99.38); 369.82 (M+2, 33.13).

2.3 *In vitro* Antitubercular Activity

The target isoxazole clubbed 1-carbothioamido-4,5-dihydro-1*H*-pyrazoles were screened for their antimycobacterial activity against M. tuberculosis H37Rv strain. The minimum inhibitory concentration (MIC) of all the compounds was obtained using a broth dilution assay and is defined as the lowest concentration of the drug that inhibits ≤ 99% of the bacteria present at the start of the assay. The MIC of the test compounds were compared with that of the standard Isoniazid. The culture was thawed and diluted in broth to 10^5 cfu mL-1 (colony forming unit/mL) dilutions using Middlebrook 7H9 broth enriched with 10% albumin-dextrose-catalase and 0.2 percent glycerol. All the target compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted twice in broth to achieve the appropriate concentration. The assay medium contained 1.3 percent DMSO at its final concentration. Following that, each U-tube was injected with 0.05 mL of standardised culture and cultured for 21 days at 37°C. The appearance of the growth in the U-tubes was observed and compared in contrast to isoniazid (positive control) and ioculum without drug (negative control) [21-23].

2.4 Cytotoxicity Studies

The target compounds with the highest activity 24 and 25 were evaluated for their cytotoxic properties using Mosmanns MTT assay against normal human liver cell lines-LO2. In MTT assay,

mitochondrial reducatse of living cells converts solube MTT (0.5 mg mL^{-1}, 100 µL), into a formazan (bluish-purple) product.Cells used in cytotoxicity assays were grown in RPMI 1640 media supplemented with 10% foetal calf serum, penicillin, and streptomycin at 37 °C and 5% CO2. Twenty-four hours after seeding, the cells were transferred to 96-well plates at 100 µL per well and allowed to adhere overnight before treatment with the compounds in DMSO solution (10^{-5}, 10^{-6} and 10^{-7} mol/Lfinal concentration). Three times, the same treatment was provided. After continuous compound exposure with MTT, cell viability was measured after 96 hours. 150 µL of DMSO solution was applied to each well. The plates were mechanically mixed until the colour response was homogeneous and the OD570 was measured using a micro plate reader. The IC_{50} was determined as the concentration that decreased the absorbance of the untreated wells by 50% relative to vehicle in the MTT test. Triplicate assays were conducted and the reproducibility of the findings was excellent with standard errors below 10% [24,25].

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthesis of new isoxazole linked 1-carbothioamido-4,5-dihydro-1*H*-pyrazole derivatives was achieved by the condensation of isoxazolyl chalcones (1-15) with thiosemicarbazide using glacial acetic acid. All the compounds were purified by recrystallization. Mass spectrometry, FT-IR and 1H NMR techniques enabled to elucidate the structures of the purified compounds. The compounds showed M+1 peak corresponding to their molecular weights in their positive ion mass spectrum. Additionally, the compound 30 also dsiplayed a sattelite peak due to the ^{37}Cl isotope at m/z value 369.82 (M+1, 33.13). In their FT-IR spectra, the compounds exhibited three diagnostic absorption bands corresponding to C=S, C=N and NH_2 around wave numbers 1230-1248 cm^{-1}, 1566-1588 cm^{-1} and 3312-3359 cm^{-1} respectively. The three diagnostic peaks of 2-pyrazoline scaffold in the ^1H NMR spectra of target compounds corresponding to the ABX system was observed at chemical shift values 3.05-3.17 ppm (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB}=16 Hz), 3.65-3.82 ppm (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), and 5.05-5.38 ppm (1H, Hx, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz) respectively. Furthermore, the other characteristic peaks corresponding to the amino group (9-10 ppm), aromatic protons (Ar-H, 6-8.5 ppm) and methoxyl groups (2-4 ppm) in their ^1H NMR spectrum had confirmed the structures of the target compounds.

3.2 Antitubercular Activity and SAR

The antimycobacterial activity of all the target compounds (**16-30**) was evaluated against H37Rv strain of *Mycobacterium tuberculosis*. Some of the compounds were more active than isoniazid and previously investigated carboxamide derivatives (**4a-4o**) by Kishor et al (Fig. 1) [19]. In the **4a-4o** series, the compounds with methoxy group at the meta-positions were poorly active. However, in our case compounds substituted with meta-methoxyl groups had contributed positively for the activity. For instance, compound **24** containing 3,5-dimethoxy groups at meta positions was the most active analogue with an MIC value of 0.1 µg/mL. Its activity was similar to compounds **4n** and **4o** containing 2-fluoro-3,4-dimethoxy and 2-chloro-3,4-dimethoxy rings respectively in the carboxamide series. The compound **25** containing 2,3,4-trimethoxy substituents was equipotent to isoniazid (MIC, 0.25 µg/mL). İn a similar way the compounds-**26** and **28** containing 2,4,5-trimethoxy and 3,4,5-trimethoxy groups showed more activity than the carboxamide counters but less activity than isoniazid with MIC 1 and 2 µg/mL respectively. The other compounds elicited activity ranging between 4-128 µg/mL.

3.3 Cytotoxicity Studies

The MTT assay result of compounds 24 and 25 for their cytotoxicity against L02 (human normal cell line) showed that the compunds were less selective against the human cells as their IC50 values were more than 70 µg/mL (Table 2) indicating the usefulness of these analogues for further studies.

Table 1. Antitubercular activity of isoxazole linked dihydropyrazole-1-carboxamides (4a-4o) Vs isoxazole clubbed dihydropyrazole-1-carbothioamides (16-30)

Compound code	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$	Compound code	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$
4a	32	16	128
4b	62.5	17	2
4c	126	18	64
4d	62.5	19	32
4e	0.5	20	128
4f	32	21	32
4g	16	22	128
4h	32	23	32
4i	126	24	0.1
4j	8	25	0.25
4k	8	26	1
4l	0.25	27	128
4m	8	28	2
4n	0.1	29	4
4o	0.1	30	8
Isoniazid	0.25		0.25

Table 2. Cytotoxicity of compounds 24 and 25 against human normal cells (IC_{50} ±SD, µg/mL)[a,b].

S.No	Compounds	Human liver normal cells (L02)
1	24	>70
2	25	>70

[a] *Mean value ±SD (standard deviation from three experiments).*
[b] *Boldface: IC_{50} ≤ the control, (IC_{50} , µg mL^{-1})*

4. CONCLUSION

A novel series of isoxazole clubbed dihydropyrazole-1-carbothioamides (16-30) were synthesized, chaarcterized and screened for their antimiycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain. The compounds 24 and 25 bearing methoxyl groups at the meta positions were found to be the most promising lead molecules considering their potential antitubercular activity with MIC values 0.1 and 0.25 µg/mL respectively. In addition the less selectivity of these compounds against the normanl human liver cells suggests the significance of these compounds in the further discovery and development of novel antitubercular drugs. Further studies are under process inorder to elucidate the plausiable mode of action for the proposed actvity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applıcable.

ETHICAL APPROVAL

It is not applıcable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/82313