Original Article

Synthesis, Characterization, And Antiproliferative Studies Of Isoxazole Clubbed 1-Carbothioamido-4,5-Dihydro-1H-Pyrazoles

Pasumarthy N V Gopal[1], Usha Kiranmayi M[2], Sudhakar Poda[3]*

[1]Department of Biotechnology, Acharya Nagarjuna University, Guntur, India–522510; email: gopalpnv@gmail.com
[2]Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur-522 510, India. Email: drmangamuri@gmail.com
[3]*Department of Biotechnology, Acharya Nagarjuna University, Guntur-522 510, India.

***Corresponding Author:** Sudhakar Poda

*Department of Biotechnology, Acharya Nagarjuna University, Guntur-522 510, India.Email: podasudhakar@gmail.com
DOI:10.47750/pnr.2023.14.S01.105

Abstract

Cancer remains a global problem despite the availability of drugs due to this issues like multi-drug resistance and toxicities. As a result, there is a pressing need for new anticancer medicines to be developed. In the present investigation, we designed and synthesized a series of Isoxazole clubbed 1-carbothioamido-4,5-dihydro-1H-pyrazoles (16-30) in considerable yields. Further, these compounds were purified by recrystallization and characterized by spectral techniques-Mass, FT-IR, and 1H NMR and then evaluated for their antiproliferative activity against prostate cancer DU-145 cell line. Among the tested compounds, The dihydro pyrazole-1-carbothioamides 28 and 29 showed the highest activity with IC50 value 1 µg/mL and the activity of these two compounds was four-fold more than that of the standard, Docetaxel. In addition, the less selectivity of these compounds against normal human liver cells suggests the significance of these compounds in the further discovery and development of novel anticancer drugs.

Keywords: Cancer, Isoxazole, 1-carbothioamido-4,5-dihydro-1H-pyrazoles, antiproliferative activity, prostate cancer, DU-145.

1. INTRODUCTION

Heterocyclic compounds are a class of cyclic organic compounds containing heteroatoms like nitrogen, sulfur, oxygen etc., along with the carbon framework. The heterocyclic compounds possess diverse pharmacological activities and are employed in the treatment of a variety of diseases. Most of the therapeutic agents employed in the present-day therapy contain heterocyclic ring as the major structural component. Among these compounds, nitrogen containing heterocyclic rings is distinctive not only because of their ease of synthesis but also due their widespread distribution and biological profiles. Chalcones are open chain flavonoids containing the reactive propenone linker connected to two aryl rings. Literature survey revealed that chalcones and nitrogen containing heterocycles i.e., isoxazoles and dihydropyrazoles possess a broad spectrum of biological activities like antimicrobial, anticancer, antimalarial, antidepressant, antihistaminic, antitubercular and anti-inflammatory [1-31].

Isoxazole, a five membered heterocyclic ring containing oxygen and nitrogen present in the drugs used in the therapy including Isoxazole, a five membered heterocyclic ring is present in the drugs used in the therapy including the β-lactam antibiotics-cloxacillin and dicloxacillin, sulfisoxazole and sulfamethoxazole (antibacterials), valdecoxib (selective COX-II inhibitor) and leflunomide {immunosuppressive disease-modifying antirheumatic drug (DMARD)}. The completely reduced form of isoxazole i.e., isoxazoline and isoxazolidine scaffolds are seen in the antifungal agent drazoxolon and antitubercular antibiotic cycloserine respectively. Dihydropyrazole is another interesting heterocyclic compound with two nitrogen atoms present in the adjacent positions of a five membered ring. This ring can be conveniently synthesized from α, β-unsaturated carbonyl compounds by reacting with different kinds of hydrazines. The dihydropyrazole scaffold constitutes the part of drugs including diuretic-Muzolimine; analgesic-Antipyrine, Aminopyrine, Ramifenazone and Dipyrone; anti-obesity cannabinoid receptor type 1 (CB_1) antagonist-Ibipinabant (**Figure 1**).

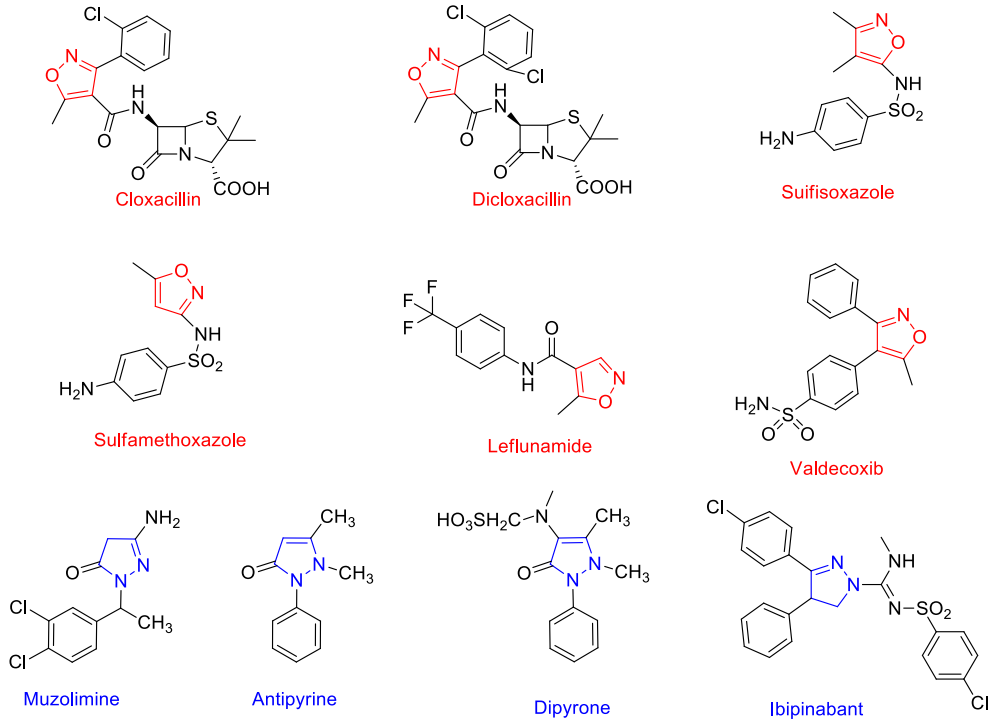
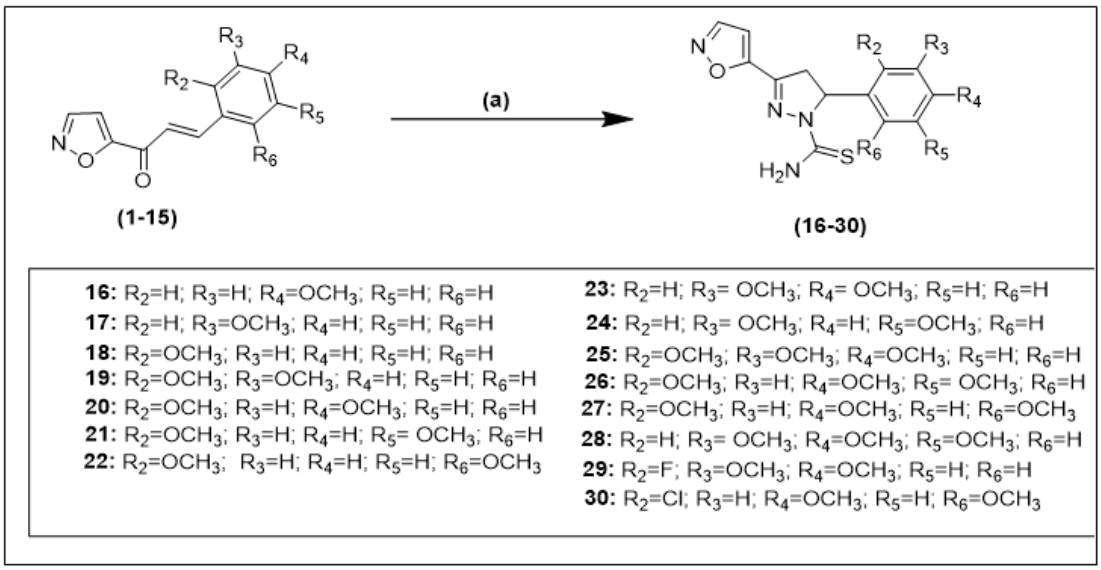


Figure 1: Structures of some clinically useful drugs containing isoxazole and dihydropyrazole rings.

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). 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 ºC and are uncorrected. Mass spectra were recorded on Agilent LC-MS spectrometer whereas the FT-IR spectra were recorded on Bruker Vertex 80v spectrometer using potassium bromide disks and the wave numbers of the absorption bands are expressed in cm^{-1}. ^1H spectra were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the chemical shifts (δ) are expressed in ppm.

2.2 Chemistry: 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-1H-pyrazoles (**16-30**) were obtained by filtering, drying, and recrystallizing the solid mass using ethanol (Scheme 1) [32].



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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 ºC; **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 °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 °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 °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 °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); **MS** (*m/z*, %): 369.82 (M+1, 33.13).

2.3 In vitro antiproliferative activity

The target compounds were evaluated for their antiproliferative activity against prostate cancer cell lines DU-145 using Mosmanns MTT assay (Mosmann T *et al*., 1983) 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 IC50 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

3.1 Chemistry: The synthesis of new isoxazole linked 1-carbothioamido-4,5-dihydro-1H-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 Antiproliferative activity and SAR

All the fifteen compounds were evaluated for their antiproliferative potency against prostate cancer cell line, **DU-145**, employing MTT assay. Dihydropyrazole-1-carbothioamides (**16-30**) exhibited superior activity than the dihydropyrazole-1-carboxamides (**4a-4o**) (**Table 1**). The dihydropyrazole-1-carbothioamides **28** and **29** showed the highest activity with IC_{50} value 1 µg/mL and the activity of these two compounds was four-folds more than that of the standard, Docetaxel (MIC = 5 µg/mL). The dihydropyrazole-1-carbothioamide derivatives **23** and **24** were 2.25 and 1.25-times more active than docetaxel with MIC 2 and 4 µg/mL respectively. All the other compounds exhibited modest anticancer activity with MIC's ranging from 8-28 µg/mL. The SAR features indicated that heterocyclic

pyrazoline ring bearing a carbothioamide linkage is more essential for the antiproliferative activity than the carboxamide linkage seen in dihydropyrazole-1-carboxamides (**4a-4o**). The compounds were also tested against the human normal cells (**L02**) and were found to be nontoxic to these cells as they have high IC_{50} values.

Table 1. Comparison of the MTT assay antiproliferative results of isoxazole linked dihydropyrazole-1-carboxamides (**4a-4o**) Vs isoxazole clubbed dihydropyrazole-1-carbothioamides (**16-30**) (IC_{50} ±SD, µg/mL)[a,b].

Compound	DU-145	Human normal cells (L02)	Compound	DU-145	Human normal cells (L02)
4a	28 ± 1	>40	**16**	16 ± 1	>60
4b	18 ± 2	>40	**17**	12 ± 2	>50
4c	32 ± 2	>40	**18**	28 ± 2	>50
4d	16 ± 2	>40	**19**	10 ± 2	>50
4e	26 ± 2	>40	**20**	14 ± 2	>50
4f	31 ± 2	>40	**21**	21 ± 2	>75
4g	20 ± 2	>40	**22**	8 ± 2	>50
4h	4 ± 2	>40	**23**	2 ± 2	>60
4i	8 ± 2	>40	**24**	4 ± 2	>75
4j	9 ± 1	>40	**25**	6 ± 1	>75
4k	18 ±2	>40	**26**	11 ±2	>75
4l	21 ± 2	>40	**27**	19 ± 2	>40
4m	5 ± 2	>40	**28**	1 ± 2	>60
4n	2 ± 1	>40	**29**	1 ± 1	>60
4o	12 ± 2	>40	**30**	12 ± 2	>75
Docetaxel 5 ± 1					

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

4. CONCLUSİON

A novel series of isoxazole clubbed dihydropyrazole-1-carbothioamides (**16-30**) were synthesized, chaarcterized and screened for their antiproliferative activity against prostate cancer cell line (DU-145). Dihydropyrazole-1-carbothioamides (**16-30**) exhibited superior activity than the dihydropyrazole-1-carboxamides (**4a-4o**). The dihydropyrazole-1-carbothioamides **28** and **29** showed the highest activity with IC_{50} value 1 µg/mL and the activity of these two compounds was four-folds more than that of the standard, Docetaxel. 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 anticancer drugs. Further studies are under process inorder to elucidate the plausiable mode of action for the proposed actvity.

Conflict of interests

The authors declared “no conflict of interest” in the manuscript.

REFERENCES

1. Gibson, M.Z,; Nguyen, M.A.; Zingales, S.K. Design, synthesis and evaluation of (2-(Pyridinyl)methylene)-1-tetralone chalcones for Anticancer and Antimicrobial Activity. Med. Chem. **2018**, 14, 333-343.
2. Afzal, B.S.; Lohitha S.V.K.; Puttagunta S.B.; Shaik A.; Supraja, K.; Sai, H.K. Synthesis and screening of novel lipophilic diarylpropeones as prospective antitubercular, antibacterial and antifungal agents. Biointerface Res. Appl. Chem. **2019**, 9, 3912-3918.
3. RamirezPrada, J.; Robledo, S.M.; Velez, I.D.; Crespo, M.D.P.; Quiroga, J.; Abonia, R.; Montoya, A.; Svetaz, L.; Zacchino, S.; Insuasty, B. Synthesis of novel quinoline-based 4,5-dihydro-1-H-pyrazoles as potential anticancer, antifungal, antibacterial, antiprotozoal agents. Med. Chem. **2017**, 131, 237-254.
4. Sowmya, D.V.; Lakshmi Teja, A.; Padmaja, A.; Kamala Prasad, V.; Padmavathi, V. Green approach for the synthesis of thiophenyl pyrazoles and isoxazoles by adopting 1,3-dipolar cycloaddition methodology and their antimicrobial activity. Eur. J. Med. Chem. **2018**, 143, 891-898.
5. Lavanya, G.; Mallikarjunareddy, L.; Padmavathi, V.; Padmaja, A^2.Synthesis and antimicrobial activity of (1,4-phenylene)bis(arylsulfonylpyrazoles and isoxazoles). Eur. J. Med. Chem. **2014**, 73, 187-194.
6. Abdelhamid, A.O.; EI Sayed, I.E,.; Zaki, Y.H.; Hussein, A.M.; Mangoud, M.M.; Hosny, M.A. Utility of 5-(furan-2-yl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide in the synthesis of heterocyclic compounds with antimicrobial activity. Biorg. Med. Chem. **2019**, 13, 48.
7. Hassan, S.Y. Synthesis, antibacterial and antifungal activity of some new pyrazoline and pyrazole derivatives. Molecules. **2013**, 18, 2683-2711.
8. Afzal, B.S.; Yejella, R.P.; Shaik S. Design, Facile Synthesis, Characterization and Computational Evaluation of Novel Isobutylchalcones as Cytotoxic Agents: Part-A. FABAD J. Pharm. Sci. **2015**, 40, 7-22.
9. Pallepati, K.; Venkata, R.K.; Afzal, B.S.; Antitubercular evaluation of isoxazole appended 1-carboxamido-4,5-dihydro-1H-pyrazoles. J. Res. Pharm. **2019**, 23, 156-163.
10. Caliskan, B.; Sinoplu, E.; Ibis, K.; Akhan Guzelcan, E.; Cetin Ataly, R.; Banoglu, E. Synthesis and cellular bioactivities of novel isoxazole derivatives incorporating an arylpiperazine moiety as anticancer agents. J. Enzyme Inhib. Med. Chem. **2018**, 33, 1352-1361.
11. Havrylyuk, D.; Kovach, N.; Zimenkovsky, B.; Vasylenko, O.; Lesyk, R. Synthesis and anticancer activity of isatin-based pyrazolines and thiazolidines conjugates. Arch. Pharm. **2011**, 344, 514-522.
12. Insuasty, B.; Montoya, A.; Becerra, D.; Quiroga, J.; Abonia, R.; Robledo, S.; Velez, I.D.; Upegui, Y.; Nogueras, M.; Cobo, J. Synthesis of novel analogs of 2-pyrazoline obtained from [(7-chloroquinolin-4-yl)amino]chalcones and hydrazine as potential antitumor and antimalarial agents. Eur. J. Med. Chem. **2013**, 67, 252-262.
13. Fernandez, J.; Chicharro, J.; Bueno, J.M.; Lorenzo, M. Isoxazole mediated synthesis of 4-(1H)pyridones: improved preparation of antimalarial candidate GSK932121. Chem. Commun(camb). **2016**, 52, 10190-10192.
14. Bueno, J.M.; Herreros, E.; Angulo-Barturen, I.; Ferre, S.; Fiandor, J.M.; Gamo, F.J.; Gargallo-Viola, D.; Derimanov, G. Exploration of 4(1H)-pyridones as a novel family of potent antimalarial inhibitors of the plasmodial cytochrome bc1. Fut. Med. Chem. **2012**, 4, 2311-2323.

15. Patel, P.; Koregaokar, S.; Shah, M.; Parekh, H. Synthesis of some novel pyrazoline and cyanopyridine derivatives as antimicrobial agents. Farmaco. **1996**, 51, 59-63.
16. Guan, L.P.; Zhao, D.H.; Chang, Y.; Wen, Z.S.; Tang, L.M.; Huang, F.F. Synthesis of 2,4-dihydroxychalcone derivatives as potential antidepressant effect. Drug. res(stuttg). **2013**, 63, 46-51.
17. Yu, L.F.; Tuckmantel, W.; Eaton, J.B.; Caldarone, B.; Fedolak, A.; hanania, T.; Brunner, D.; Lukas, R.J.; Kozikowski, A.P. Identification of novel α4β2-nicotinic acetylcholine receptor (nAChR) agonists based on an isoxazole ether scaffold that demonstrate antidepressant-like activity. J. Med. Chem. **2012**, 55812-823.
18. Liu, J.; Yu, L.F.; Eaton, J.B.; Caldarone, B.; Cavino, K.; Ruiz, C.; Terry, M.; Fedolak, A.; Wang, D.; Ghavami, A.; Lowe, D.A.; Brunner, D.; Lukas, R.J.; Kozikowsi, A.P. Discovery of isoxazole analogues of sazetidine-A as selective α4β2-nicotinic acetylcholine receptor partial agonists for the treatment of depression. J. Med. Chem. **2011**, 54, 7280-7288.
19. Rajendra Prasad, Y.; Lakshmana Rao, A.; Prasoona, L.; Murali, K.; Ravi Kumar, P. Synthesis and antidepressant activity of some 1,3,5-triphenyl-2-pyrazolines and 3-(2"-hydroxy naphthalen-1"-yl)-1,5-diphenyl-2-pyrazolines. Bioorg. Med. Chem. Lett. **2005**, 15, 5030-5034.
20. Palaska, E.; Aytemir, M.; Uzbay, I.T.; Erol, D. Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. Eur. J. Med. Chem. **2001**, 36, 539-543.
21. Lin, Y.M.; Zhou, Y.; Flavin, M.T.; Zhou, L.M.; Nie, W.; Chen, F.C. Chalcones and flavonoids as anti-tuberculosis agents. Bioorg. Med. Chem. **2002**, 10, 2795-2802.
22. Azzali, E.; Machado, D.; Kaushik, A.; Vacondio, F.; Flisi, S.; Cabassi, C.S.; Lamichhane, G.; Viveiros, M.; Costantino, G.; Pieroni, M. Substituted N-Phenyl-5-(2-(phenylamino)thiazol-4-yl)isoxazole-3-carboxamides are valuable antitubercular candidates that evade innate efflux machinery. J. Med. Chem. **2017**, 60, 7108-7122.
23. Balaji, N.V.; HariBabu, B.; Rao, V.U.; Subbaraju, G.V.; Nagasree, K.P.; Kumar, M.M.K. Synthesis, screening and docking analysis of hispolon pyrazoles and isoxazoles as potential antitubercular agents. Curr. Top. Med. Chem. **2019**, 19, 662-682.
24. Lokesh, B.V.S.; Prasad, Y.R.; Shaik, A.B. Synthesis, Biological evaluation and molecular docking studies of new pyrazolines as an antitubercular and cytotoxic agents. Infect. Disord. Drug. Targets. **2019**, 19, 310-321.
25. Dixit, S.R.; Joshi, S.D.; Kulkarni, V.H.; Jalalpure, S.S.; Kumbar, V.M.; Mudaraddi, T.Y.; Nadagouda, M.N.; Aminabhavi, T.M. Pyrrolyl pyrazoline carbaldehydes as Enoyl-ACP reductase inhibitors. Design, synthesis and antitubercular activity. Open. Med. Chem J. **2017**, 11, 92-108.
26. Mahapatra DK, Bharti SK, Asati V. Chalcone Derivatives: Anti-inflammatory Potential and Molecular Targets Perspectives. Curr. Top. Med. Chem. **2017**, 17, 3146-3169.
27. Ozdemir, A.; Altintop, M.D.; Turan-Zitouni, G.; Çiftçi, G.A.; Ertorun, I.; Alataş, O.; Kaplancikli, Z.A. Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents. Eur. J. Med. Chem. **2015**, 89, 304-309.
28. Filali, I.; Romdhane, A.; Znati, M.; Jannet, H.B.; Bouajila, J. Synthesis of new harmine isoxazoles and evaluation of their potential anti-alzheimer, anti-inflammatory, and anticancer activities. Med. Chem. **2016**, 12, 184-190.
29. Gawad, N.M.; Georgey, H.H.; Ibrahim, N.A.; Amin, N.H.; Abdelsalam, R.M. Synthesis of novel pyrazole and dihydropyrazoles derivatives as potential anti-inflammatory and analgesic agents. Chem. Commun (Camb). **2016**, 52, 14490-14493.
30. Kharbanda, C.; Alam, M.S.; Hamid, H.; Javed, K.; Bano, S.; Dhulap, A.; Ali, Y.; Nazreen, S.; Haider, S. Synthesis and evaluation of pyrazolines bearing benzothiazole as anti-inflammatory agents. Bioorg Med Chem. **2014**, 22, 5804-12.
31. Gao, Z.; Hurst, WJ.; Czechtizky, W.; Hall, D.; Moindrot, N.; Nagorny, R.; Pichat, P.; Stefany, D.; Hendrix, J.A.; George, P.G. Identification and profiling of 3,5-dimethyl-isoxazole-4-carboxylic acid [2-methyl-4-((2S,3'S)-2-methyl-[1,3']bipyrrolidinyl-1'-yl)phenyl] amide as histamine H(3) receptor antagonist for the treatment of depression. Bioorg. Med. Chem. Lett. **2013**, 23, 6269-6273.