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Synthesis, Characterization & Antimicrobial Evaluation of New *bis*(5-Biphenyl-3-alkoxyphenyl-4,5-dihydro-1*H*-pyrazole-*N*-carbothioamide) Derivatives

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ABSTRACT

In this study, a series of new symmetrical *N*-carbothioamide substituted bispyrazolines (**2a-f**) had been systematically synthesized by using ring closure reactions of bischalcones (**1a-f**) with thiosemicarbazide under the alkaline-alcoholic conditions. The structures of bisheterocyclic products have been fully characterized on the basis of their IR, ^{1}H & ^{13}C NMR and ESI-MS spectral strategies. All the prepared compounds were also evaluated for their *in vitro* antimicrobial assay with the help of serial tube dilution procedure against the selected numbers of microbes (seven bacterial and five fungal strains). The most of the synthesized bisheterocycles exhibited noticeable antimicrobial potencies against the tested strains.

KEYWORDS

Bischalcone, *N*-Carbothioamide-bispyrazolines, Aliphatic linkers, Antimicrobial activity.

INTRODUCTION

The chemistry of heterocyclic compounds has been responsible for the development of colossal innovations in the field of organic syntheses. These researches have encouraged the chemists throughout the world to prepare the variety of bioactive heterocycles [1]. More than half of known organic products are found to be belonging to the heterocyclic family [2]. These substrates by virtue of their particular mode of action could be effectively tested against a large number of infectious diseases that owe their usefulness in our lives. The capabilities of heterocyclic compounds to incorporate various substituents around their core moiety have increased the interest among the synthetic organic chemists to scrutinize their structural features. They can be arranged into a specialized group of organic molecules on the basis of their chemical properties which are certainly responsible to describe their affinity to behave as an electrophile or nucleophile [3-10].

Amongst the family of heterocyclic systems, the two nitrogen five-membered heterocycles have attracted tremendous attention due to their pervasiveness in plethora of natural and synthetic products along with their superlative bioactive behaviour. Pyrazolines are such compounds that are the most imposing functionalities having two adjoining nitrogen atoms and one double bond in a five-membered ring system [11]. The import-

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ant applications of these products in different fields provide their immense scope in the area of synthetic chemistry in order to design and develop the structurally novel and unique products, which may be associated to the specific biological activities [12,13].

The presence of pyrazoline moiety in large number of pharmacologically active products has prompted the necessities of intriguing and divergent approaches for the preparation of these heterocycles. They have also been enormously utilized as a very important synthons in organic synthesis [14-17]. Pyrazole scaffold forms the core structure of several well-known non steroidal anti-inflammatory drugs like cefoselis (antibacterial), crizotinib (anti-cancer), tolpiprazole (anxiolytic), tepoxalin (anti-arthritic), fezolamine (anti-depressant) and remogliflozin etabonate (antidiabetic) (Fig. 1) [18-20].

Most of the pyrazoline derivatives are exploited as insecticides, fungicides and herbicides for the safety of crops and these heterocycles also used in polymer and supramolecular chemistry, food industry, cosmetic colorings and UV stabilizers. The substituted pyrazolines have also been employed as bifunctional ligands for the metal complex reactions [21-23]. The literature review concedes that pyrazolines have been widely studied for their enormous chemotherapeutic properties such as antiviral [24], antimicrobial [25], antidepressant [26], anti-inflammatory [27], antitumor [28], anti-arthritic [29], anti-cancer [30], anticonvulsant [31], anti-HIV [32], antipyretic [33], hypoglycemic [34] and anxiolytic [35], *etc*.

In view of above described facts and in perpetuation of our research program upon the design and synthesis of biologically significant bisheterocycles [36-40], the present researches have been devoted upon the synthesis of *N*-carbothioamide substituted new bispyrazolines (**2a-f**) which have been assem-bled around the aliphatic linkers of varying lengths. The subst-antial interest behind these investigations was to explore the clout of interior alkyl chain lengths on the generation and anti-microbial profile of final symmetrical bisheterocyclic products (**2a-f**).

EXPERIMENTAL

All the chemicals and solvents used in this research work had been procured from the commercial traders (Loba Chemie, S. D. Fine, Sigma-Aldrich & TCI Chemical companies). The melting points of the synthesized products were determined with the help of open capillary equipment and are uncorrected. Infrared spectra (cm^{-1}) of the synthesized compounds were obtained on the Perkin-Elmer FT-IR instrument, whereas XEVO G2-XS QTOF spectrometer has been employed for obtaining the ESI-MS spectra. Bruker Advance-II Spectrometer working at 500 MHz & 125 MHz was used for scanning ^{1}H & ^{13}C NMR spectra of synthesized compounds, respectively by using TMS as the reference substance in the deuterated solvents (DMSO-d_6/$CDCl_3$). Silica-gel TLC (thin layer chromatography) plates run in hexane:ethyl acetate (9:1 v/v) eluent was used for analyzing the progress of reactions as well as the purity of synthesized products. The TLC plates were visualized by exposing them in the iodine vapours. The bischalcones (**1a-f**) were synthesized and identified according to the literature method [41].

Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-(4-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)butoxy)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2a): A mixture of bischalcone (**1a**) (1.5 g, 0.002 mol), thiosemicarbazide (0.4 g, 0.004 mol) and KOH (0.4 g, 0.008 mol) was dissolved in absolute ethanol (25.0 mL). This solution was allowed to reflux under conti-

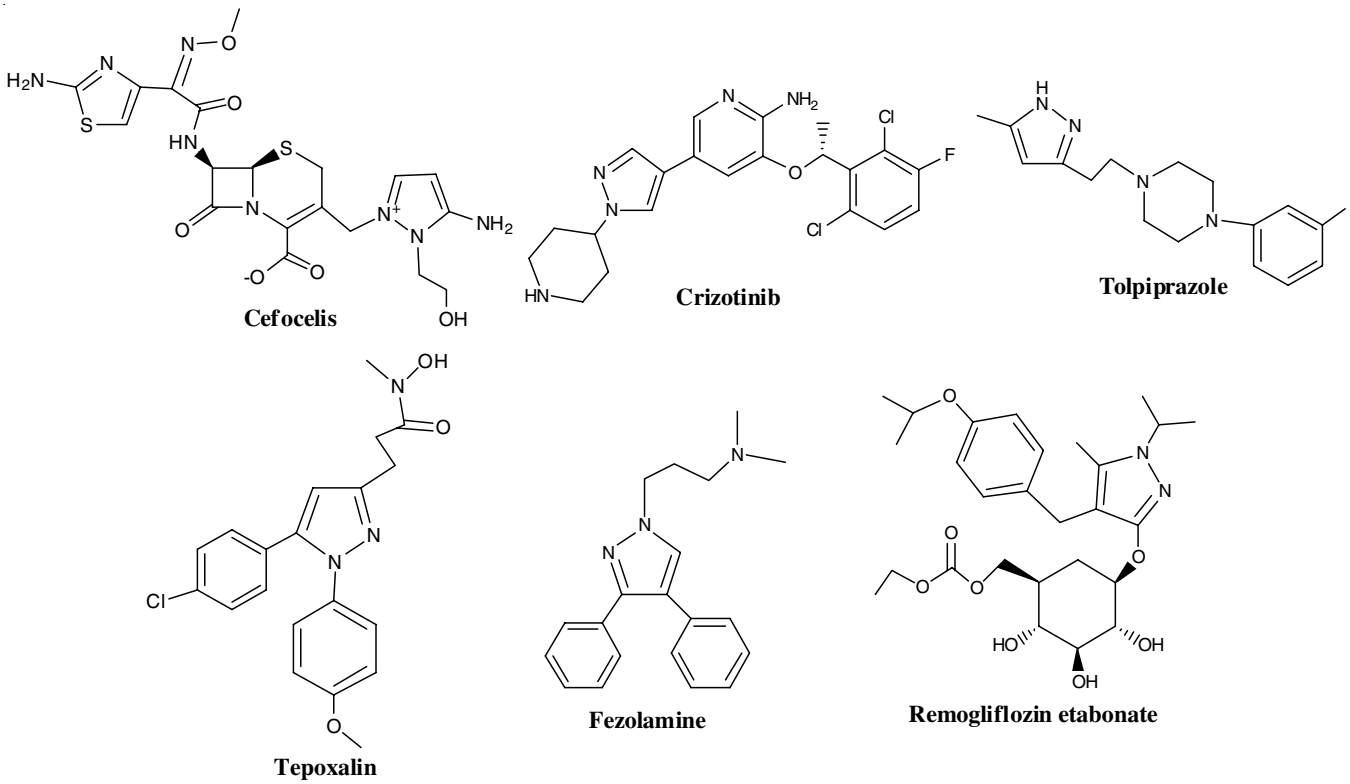


Fig. 1. The structures of six non steroidal anti-inflammatory drugs

nuous heating for 5 h. Upon the completion of reaction (as examined with the help of TLC), the solvent was distilled off under reduced pressure. The remaining residue was slowly poured into the iced-HCl to provide a solid substance. The crude product thus acquired was crystallized by using methanol as a solvent to afford a pure compound **2a**. Off white solid; m.p.: 146-148 °C; Yield: 72%; IR (KBr, ν_{max}, cm^{-1}): 3420, 3249 (N-H), 3028 (aromatic C-H), 2952 (methylene C-H), 1591 (C=N), 1215 (C=S) & 1289, 1091 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.09 (2H, brs, N***H***$_\alpha$), 7.95 (2H, brs, N***H***$_\beta$), 7.87 (2H, d, J_o = 8.2 Hz, H-4‴), 7.67 (4H, dd, $J_{m,o}$ = 2.0, 8.1 Hz, H-2″, 6″), 7.61 (4H, td, $J_{p,o}$ = 1.0, 8.2 Hz, H-3″, 5″), 7.52 (2H, s, H-2′), 7.41 (4H, m, H-2‴, 6‴), 7.31 (4H, m, H-3‴, 5‴), 7.01 (4H, d, J_o = 7.6 Hz, H-5′, 6′), 6.94 (2H, d, J_o = 7.8 Hz, H-4′), 5.99 (2H, dd, J_{XA} = 3.2 Hz, J_{XM} = 11.6 Hz, H_X), 4.11 (4H, brs, OC***H***$_2$), 3.89 (2H, dd, J_{MX} = 11.6 Hz, J_{MA} = 18.1 Hz, H_M), 3.20 (2H, dd, J_{AX} = 3.2 Hz, J_{AM} = 18.1 Hz, H_A), 1.93 (4H, brs, OCH_2C***H***$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 176.69 (C=S), 159.22 (C-3′), 156.04 (C-3), 140.66 (C-1″), 131.93 (C-4″), 129.98 (C-1‴), 128.93 (C-1′), 128.77 (C-5′), 127.95 (C-2″, 6″), 127.69 (C-3″, 5″), 127.45 (C-2‴, 6‴), 127.33 (C-3‴, 5‴), 127.09 (C-4‴), 125.89 (C-6′), 119.73 (C-4′), 117.42 (C-2′), 67.62 (O***C***H_2), 63.27 (C-5), 43.22 (C-4), 25.94 (OCH_2***C***H_2); ESI-MS: *m/z* 803 $[M+2]^+$ (23%), 802 $[M+1]^+$ (52%), 801 $[M]^+$ (100%); Anal. calcd. (%) for $C_{48}H_{44}N_6O_2S_2$: C, 71.97; H, 5.54; N, 10.49; S, 8.00; Found (%): C, 72.25; H, 5.56; N, 10.53; S, 7.97.

Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-((5-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)pentyl)oxy)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2b): The bisheterocycle (**2b**) was achieved by using the cyclocondensation reaction of compound **1b** (1.5 g, 0.002 mol) with thiosemicarbazide (0.4 g, 0.004 mol) under the identical conditions as used earlier for compound **2a**. Off white solid; m.p.: 110-112 °C; Yield: 70%; IR (KBr, ν_{max}, cm^{-1}): 3426, 3252 (N-H), 3024 (aromatic C-H), 2922 (methylene C-H), 1589 (C=N), 1211 (C=S) & 1288, 1044 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (2H, brs, N***H***$_\alpha$), 7.93 (2H, brs, N***H***$_\beta$), 7.88 (2H, d, J_o = 7.3 Hz, H-4‴), 7.65 (4H, dd, $J_{m,o}$ = 2.3, 7.8 Hz, H-2″, 6″), 7.62 (4H, td, $J_{p,o}$ = 1.0, 7.1 Hz, H-3″, 5″), 7.50 (2H, s, H-2′), 7.43 (4H, m, H-2‴, 6‴), 7.30 (4H, m, H-3‴, 5‴), 7.04 (4H, d, J_o = 7.7 Hz, H-5′, 6′), 6.93 (2H, d, J_o = 7.4 Hz, H-4′), 5.96 (2H, dd, J_{XA} = 3.5 Hz, J_{XM} = 11.8 Hz, H_X), 4.08 (4H, t, J_{vic} = 6.5 Hz, OC***H***$_2$), 3.90 (2H, dd, J_{MX} = 11.8 Hz, J_{MA} = 17.9 Hz, H_M), 3.16 (2H, dd, J_{AX} = 3.5 Hz, J_{AM} = 17.9 Hz, H_A), 1.91 (4H, quintet, J_{vic} = 6.5 Hz, OCH_2C***H***$_2$), 1.72 (2H, m, OCH_2CH_2C***H***$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 175.95 (C=S), 159.48 (C-3′), 156.23 (C-3), 140.78 (C-1″), 132.08 (C-4″), 129.35 (C-1‴), 128.89 (C-1′), 128.11 (C-5′), 127.99 (C-2″, 6″), 127.74 (C-3″, 5″), 127.60 (C-2‴, 6‴), 127.49 (C-3‴, 5‴), 127.12 (C-4‴), 126.65 (C-6′), 119.51 (C-4′), 118.22 (C-2′), 67.89 (O***C***H_2), 62.34 (C-5), 42.99 (C-4), 28.76 (OCH_2***C***H_2), 23.55 (OCH_2CH_2***C***H_2); ESI-MS: *m/z* 839 $[M+Na+1]^+$ (34%), 816 $[M+1]^+$ (78), 815 $[M]^+$ (100%); Anal. calcd. (%) for $C_{49}H_{46}N_6O_2S_2$: C, 72.21; H, 5.69; N, 10.31; S, 7.87; Found (%): C, 72.49; H, 5.66; N, 10.27; S, 7.90.

Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-((6-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)hexyl)oxy)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2c): The reaction of intermediate **1c** (1.5 g, 0.002 mol) with thiosemicarbazide (0.4 g, 0.004 mol) under the above mentioned procedure (for **2a**) provided the new bispyrazoline **2c**. Off white solid; m.p.: 130-132 °C; Yield: 78%; IR (KBr, ν_{max}, cm^{-1}): 3418, 3258 (N-H), 3020 (aromatic C-H), 2928 (methylene C-H), 1594 (C=N), 1216 (C=S) & 1286, 1046 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.09 (2H, brs, N***H***$_\alpha$), 7.96 (2H, brs, N***H***$_\beta$), 7.86 (2H, d, J_o = 7.5 Hz, H-4‴), 7.66 (4H, dd, $J_{m,o}$ = 2.1, 7.4 Hz, H-2″, 6″), 7.61 (4H, td, $J_{p,o}$ = 0.9, 7.3 Hz, H-3″, 5″), 7.52 (2H, s, H-2′), 7.44 (4H, m, H-2‴, 6‴), 7.29 (4H, m, H-3‴, 5‴), 7.02 (4H, d, J_o = 7.0 Hz, H-5′, 6′), 6.92 (2H, d, J_o = 8.0 Hz, H-4′), 5.98 (2H, dd, J_{XA} = 3.3 Hz, J_{XM} = 12.2 Hz, H_X), 4.06 (4H, t, J_{vic} = 6.2 Hz, OC***H***$_2$), 3.88 (2H, dd, J_{MX} = 12.2 Hz, J_{MA} = 18.0 Hz, H_M), 3.14 (2H, dd, J_{AX} = 3.3 Hz, J_{AM} = 18.0 Hz, H_A), 1.86 (4H, quintet, J_{vic} = 6.2 Hz, OCH_2C***H***$_2$), 1.53 (4H, m, OCH_2CH_2C***H***$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 176.08 (C=S), 159.67 (C-3′), 155.48 (C-3), 139.90 (C-1″), 133.08 (C-4″), 130.54 (C-1‴), 129.27 (C-1′), 129.44 (C-5′), 128.87 (C-2″, 6″), 128.19 (C-3″, 5″), 127.73 (C-2‴, 6‴), 127.56 (C-3‴, 5‴), 127.20 (C-4‴), 126.77 (C-6′), 118.23 (C-4′), 117.88 (C-2′), 67.26 (O***C***H_2), 62.95 (C-5), 43.74 (C-4), 29.37 (OCH_2***C***H_2), 25.42 (OCH_2CH_2***C***H_2); ESI-MS: *m/z* 852 $[M+Na]^+$ (28%), 831 $[M+2]^+$ (69%), 830 $[M+1]^+$ (100%); Anal. calcd. (%) for $C_{50}H_{48}N_6O_2S_2$: C, 72.43; H, 5.84; N, 10.14; S, 7.73; Found (%): C, 72.14; H, 5.86; N, 10.18; S, 7.76.

Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-((8-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)octyl)oxy)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2d): The product **2d** was synthesized from the treatment of bischalcone **1d** (1.5 g, 0.002 mol) with thiosemicarbazide (0.4 g, 0.004 mol) by following the same method as described formerly for **2a.** Off white solid; m.p.: 116-118 °C; Yield: 76%; IR (KBr, ν_{max}, cm^{-1}): 3420, 3256 (N-H), 3026 (aromatic C-H), 2925 (methylene C-H), 1598 (C=N), 1210 (C=S) & 1284, 1045 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.07 (2H, brs, N***H***$_\alpha$), 7.94 (2H, brs, N***H***$_\beta$), 7.88 (2H, d, J_o = 7.0 Hz, H-4‴), 7.67 (4H, td, $J_{m,o}$ = 2.4, 7.8 Hz, H-2″, 6″), 7.60 (4H, dd, $J_{p,o}$ = 0.6, 7.7 Hz, H-3″, 5″), 7.51 (2H, brs, H-2′), 7.45 (4H, m, H-2‴, 6‴), 7.30 (4H, m, H-3‴, 5‴), 7.01 (4H, d, J_o = 7.9 Hz, H-5′, 6′), 6.94 (2H, d, J_o = 8.2 Hz, H-4′), 5.92 (2H, dd, J_{XA} = 3.8 Hz, J_{XM} = 12.4 Hz, H_X), 4.04 (4H, t, J_{vic} = 6.0 Hz, OC***H***$_2$), 3.86 (2H, dd, J_{MX} = 12.4 Hz, J_{MA} = 17.5 Hz, H_M), 3.18 (2H, dd, J_{AX} = 3.8 Hz, J_{AM} = 17.5 Hz, H_A), 1.78 (4H, quintet, J_{vic} = 6.0 Hz, OCH_2C***H***$_2$), 1.48 (4H, m, OCH_2CH_2C***H***$_2$), 1.32 (4H, m, $OCH_2CH_2CH_2C$***H***$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 175.94 (C=S), 160.75 (C-3′), 156.44 (C-3), 140.75 (C-1″), 130.84 (C-4″), 130.18 (C-1‴), 129.66 (C-1′), 128.54 (C-5′), 128.13 (C-2″, 6″), 128.08 (C-3″, 5″), 127.79 (C-2‴, 6‴), 127.62 (C-3‴, 5‴), 127.24 (C-4‴), 126.50 (C-6′), 120.76 (C-4′), 118.31 (C-2′), 68.02 (O***C***H_2), 62.89 (C-5), 43.36 (C-4), 28.57 (OCH_2***C***H_2), 25.45 (OCH_2CH_2***C***H_2), 22.86 ($OCH_2CH_2CH_2$***C***H_2); ESI-MS: *m/z* 858 $[M+1]^+$ (92%), 857 $[M]^+$ (100%); Anal. calcd. (%) for $C_{52}H_{52}N_6O_2S_2$: C, 72.87; H, 6.12; N, 9.80; S, 7.48; Found (%): C, 73.16; H, 6.14; N, 9.83; S, 7.50.

**Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-((10-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)decyl)oxy)phenyl)-4,5-dihydro-

1*H*-pyrazole-1-carbothioamide (2e): The cyclocondensation reaction of compound **1e** (1.5 g, 0.002 mol) with thiosemicarbazide (0.4 g, 0.004 mol) could be able to afford new bisheterocycle **2e** by ensuing the similar protocol as stated above **2a**. Off white solid; m.p.: 132-134 °C; Yield: 75%; IR (KBr, ν_{max}, cm^{-1}): 3422, 3250 (N-H), 3028 (aromatic C-H), 2926 (methylene C-H), 1592 (C=N), 1218 (C=S) & 1283, 1049 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.09 (2H, brs, NH_α), 7.95 (2H, brs, NH_β), 7.87 (2H, d, J_o = 7.8 Hz, H-4‴), 7.66 (4H, td, $J_{m,o}$ = 2.3, 8.0 Hz, H-2″, 6″), 7.60 (4H, dd, $J_{p,o}$ = 0.8, 7.6 Hz, H-3″, 5″), 7.52 (2H, s, H-2′), 7.44 (4H, m, H-2‴, 6‴), 7.29 (4H, m, H-3‴, 5‴), 7.05 (4H, d, J_o = 7.3 Hz, H-5′, 6′), 6.92 (2H, d, J_o = 8.1 Hz, H-4′), 5.90 (2H, dd, J_{XA} = 4.0 Hz, J_{XM} = 12.6 Hz, H_X), 4.02 (4H, t, J_{vic} = 6.7 Hz, OCH_2), 3.89 (2H, dd, J_{MX} = 12.6 Hz, J_{MA} = 17.8 Hz, H_M), 3.21 (2H, dd, J_{AX} = 4.0 Hz, J_{AM} = 17.8 Hz, H_A), 1.84 (4H, quintet, J_{vic} = 6.7 Hz, OCH_2CH_2), 1.46 (4H, m, $OCH_2CH_2CH_2$), 1.33 (8H, brs, OCH_2CH_2 CH_2CH_2 CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 176.24 (C=S), 158.36 (C-3′), 156.23 (C-3), 140.55 (C-1″), 132.84 (C-4″), 130.75 (C-1‴), 129.28 (C-1′), 129.04 (C-5′), 128.28 (C-2″, 6″), 128.14 (C-3″, 5″), 127.56 (C-2‴, 6‴), 127.50 (C-3‴, 5‴), 127.28 (C-4‴), 126.30 (C-6′), 120.88 (C-4′), 116.99 (C-2′), 67.88 (OCH_2), 61.43 (C-5), 42.06 (C-4), 28.67 (OCH_2CH_2), 25.42 ($OCH_2CH_2CH_2$), 21.92 ($OCH_2CH_2CH_2CH_2$), 20.76 ($OCH_2CH_2CH_2CH_2CH_2$); ESI-MS: *m/z* 908 $[M+Na]^+$ (58%), 886 $[M+1]^+$ (100%); Anal. calcd. (%) for $C_{54}H_{56}N_6O_2S_2$: C, 73.27; H, 6.38; N, 9.49; S, 7.24; Found (%): C, 73.56; H, 6.40; N, 9.45; S, 7.27.

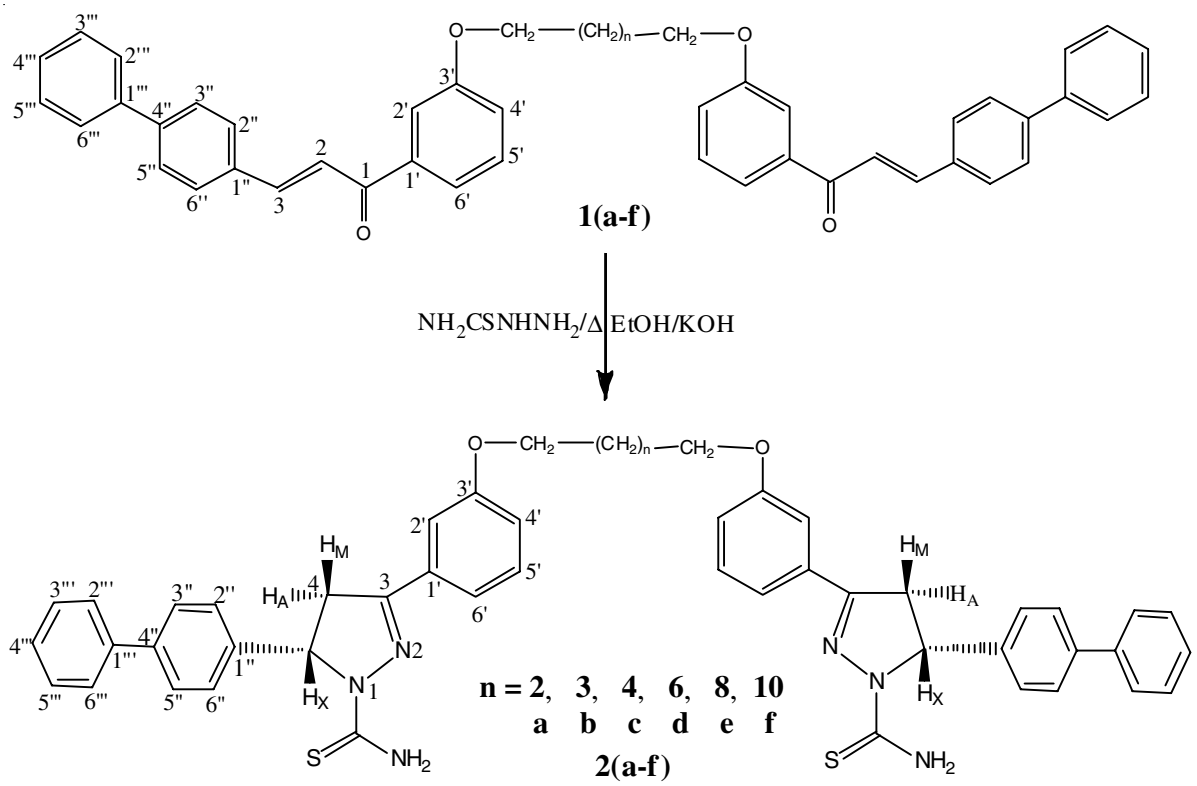
Synthesis of (*S*)-5-([1,1′-biphenyl]-4-yl)-3-(3-((12-(3-((*R*)-5-([1,1′-biphenyl]-4-yl)-1-carbamothioyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)dodecyl)oxy)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2f): The reaction of compound **1f** (1.5 g, 0.002 mol) with thiosemicarbazide (0.4 g, 0.004 mol) by applying the similar reaction condition as affirmed previously for **2a** furnished the new product **2f**. Off white solid; m.p.: 144-146 °C; Yield: 69%; IR (KBr, ν_{max}, cm^{-1}): 3428, 3256 (N-H), 3025 (aromatic C-H), 2930 (methylene C-H), 1596 (C=N), 1215 (C=S) & 1280, 1041 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (2H, brs, NH_α), 7.97 (2H, brs, NH_β), 7.86 (2H, d, J_o = 7.5 Hz, H-4‴), 7.67 (4H, td, $J_{m,o}$ = 2.0, 7.9 Hz, H-2″, 6″), 7.62 (4H, dd, $J_{p,o}$ = 0.5, 8.0 Hz, H-3″, 5″), 7.53 (2H, s, H-2′), 7.45 (4H, m, H-2‴, 6‴), 7.30 (4H, m, H-3‴, 5‴), 7.03 (4H, d, J_o = 7.4 Hz, H-5′, 6′), 6.91 (2H, d, J_o = 7.0 Hz, H-4′), 5.97 (2H, dd, J_{XA} = 3.5 Hz, J_{XM} = 11.9 Hz, H_X), 4.00 (4H, t, J_{vic} = 6.3 Hz, OCH_2), 3.87 (2H, dd, J_{MX} = 11.9 Hz, J_{MA} = 17.4 Hz, H_M), 3.15 (2H, dd, J_{AX} = 3.5 Hz, J_{AM} = 17.4 Hz, H_A), 1.80 (4H, quintet, J_{vic} = 6.3 Hz, OCH_2CH_2), 1.47 (4H, m, $OCH_2CH_2CH_2$), 1.29 (12H, m, $OCH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 175.88 (C=S), 159.44 (C-3′), 157.26 (C-3), 140.34 (C-1″), 133.18 (C-4″), 130.64 (C-1‴), 129.14 (C-1′), 128.81 (C-5′), 128.34 (C-2″, 6″), 128.18 (C-3″, 5″), 127.93 (C-2‴, 6‴), 127.40 (C-3‴, 5‴), 127.28 (C-4‴), 126.30 (C-6′), 119.58 (C-4′), 117.33 (C-2′), 68.04 (OCH_2), 63.55 (C-5), 43.18 (C-4), 29.96 (OCH_2CH_2), 28.74 ($OCH_2CH_2CH_2$), 28.16 ($OCH_2CH_2CH_2CH_2$), 25.67 ($OCH_2CH_2CH_2CH_2CH_2$), 20.88 ($OCH_2CH_2CH_2CH_2CH_2CH_2$); ESI-MS: *m/z* 915 $[M+2]^+$ (45%), 914 $[M+1]^+$ (28), 913 $[M]^+$ (100%); Anal. calcd. (%) for $C_{56}H_{60}N_6O_2S_2$: C, 73.65; H, 6.62; N, 9.20; S, 7.02; Found (%): C, 73.94; H, 6.65; N, 9.24; S, 7.05.

Antimicrobial activity: The newly synthesized bisheterocycles **2a-f** were also appraised for their *in vitro* antibacterial and antifungal assay and serial tube dilution process [42] was applied for obtaining the minimum inhibitory concentrations (MICs) values of these products. These assessments was performed by taking five fungal strains (*Aspergillus sclerotiorum*, *Fusarium oxysporum*, *Aspergillus janus*, *Penicillium glabrum* and *Aspergillus niger*), three Gram-positive pathogens (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and four Gram-negative species (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*). Dimethyl sulphoxide and standard drugs were used in the form of negative and positive control, respectively. The least concentration of tested products in µg/mL which obstructs the conspicuous growth of the used bacterial and fungal species was ascribed as the minimum inhibitory concentration. The stockpile solutions of all the tested compounds and references drugs were made in DMSO after that these solutions was sequentially diluted in order to obtain different concentrations of 128, 64, 32, 16, 8, 4 and 2 µg/mL into a series of sterilized test tubes. Fluconazole and amoxicillin were employed as the reference antifungal and antibacterial drugs respectively while nutrient broth and malt extract medium were served as the growth media for listed bacterial and fungal pathogens, respectively. The test tubes immunized with bacterial culture were incubated at 37 ºC for 24 h, whereas test tubes inoculated with fungal pathogens were placed in incubator at 28 ºC for 72 h. The subsistence of microbes in the studied products has been checked through the appearance of turbidity inside the inoculated test tubes after the above illustrated time period.

RESULTS AND DISCUSSION

The present research work has been undertaken to synthesize a series of novel *N*-carbothioamide substituted bispyrazolines **2a-f** through the cyclocondensation reactions of bischalcones **1a-f** with thiosemicarbazide in the presence of alkaline-alcohlic conditions. The preparations of compounds **1a-f** have been carried out according to the known protocol in the literature [41]. The modern spectroscopic techniques like IR, ^{1}H & ^{13}C NMR & ESI-MS had been scrupulously utilized to characterize the structures of six newly synthesized bisheterocyclic products. The purity of these bisheterocycles was also corroborated from their melting points, satisfactory elemental analysis as well as TLC outcomes. The synthetic pathway for the preparations of target compounds **2a-f**) is shown in **Scheme-I**.

In the IR spectra of bisheterocycles **2a-f**, the materialization of intense absorption in the region 1598-1589 cm^{-1} could be ascertained to the stretching frequency of C=N moiety of pyrazoline ring which ostensibly authenticate that carbonyl functionality (1662-1659 cm^{-1}) of **1a-f** have participated in the ring closure reactions. The N-H stretching vibrations of the amino groups ($CSNH_2$) were noticed in the form two distinctive bands at 3428-3418 cm^{-1} (asymmetric) & 3258-3250 (symmetric) cm^{-1}. The noticeable band emerged at 1218-1210 cm^{-1} might be credited to C=S stretching frequency. Here, other important absorptions were observed at 3028-3020 cm^{-1} (aromatic C-H) and 2930-2920 (aliphatic C-H) cm^{-1}.



Scheme-I: Synthesis of *N*-carbothioamide-bispyrazolines (**2a-f**)

The formation of bisheterocycles **2a-f** was further substantiated from their ^{1}H NMR (500 MHz, $CDCl_3$/DMSO-d_6) spectra. Two protons N-H_α & N-H_β protons of thiocarbamide moiety ($CSNH_2$) were found to be centered in the form of a two broad singlets (exchangeable with D_2O) integrating for two protons each at δ 8.09-8.07 and 7.97-7.93 ppm. The salient characteristic of these spectra was the prevalence of AMX splitting system furnished by one stereogenic proton present at C-5 position and two methylenic protons situated at C-4 carbon of the pyrazoline ring. These three hydrogens were resonating each in the form of well splitted doublet of doublets which were centered at δ 5.99-5.90 (2H, C_5-H_X), 3.90-3.86 (2H, C_4-H_M) and 3.21-3.14 (2H, C_4-H_A) ppm, respectively. The interrelationship among these protons could be validated from their mutual coupling values. The vicinal coupling constant of J_{XA} = 4.0-3.3 Hz demonstrated that H_X and H_A were *trans*- to each other while *cis*-orientation of H_X and H_M around C_4-C_5 bond of pyrazoline moiety was endorsed from their coupling value of J_{XM} = 12.6-11.6 Hz. The existence of two diastereotopic protons H_M and H_A at C_4-postion of pyrazoline ring was corroborated from their geminal coupling constant of J_{MA} = 18.1-17.4 Hz. The OCH_2 groups associated to the intervening chains were responsible to generate a broad singlet or triplet (J_{vic} = 6.7-6.0 Hz) in the upfield region at δ 4.11-4.00 ppm, while other methylene groups [$(CH_2)_n$] protons of same chains were found to resonating in the form of quintet and multiplet situated at δ 1.93-1.29 ppm. The rest of the aromatic protons (C_3-phenyl & C_5-biphenyl) could be able to afford their appropriate signals in the aromatic region.

In the ^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6) spectra of bisheterocycles **2a-f**, the three well defined resonances emerging at δ 157.26-155.48, 63.55-61.43 and 43.74-42.06 ppm could be assuredly ascribed to C-3, C-5 and C-4 carbon atom of pyrazoline moiety. The downfield signal located at δ 176.69-175.88 ppm may be easily accredited to C=S carbon atom whereas C-3′ carbon atom produced downfield resonances at δ 160.75-158.36 ppm because of its correlation with oxygen atom. Towards the upfield region, the internal spacer methylene groups may be able to generate their respective resonances at δ 68.04-67.26 (OCH_2) and 29.96-20.76 [$(CH_2)_n$] ppm. The carbon atoms of C_3-phenyl and C_5-biphenyl rings were very well resonated at their appropriate δ values.

The ESI-MS spectral fragmentation data of bisheterocyclic products **2a-f** was further very advantageous to prove their prospective structures and found to be definitive with their molecular formulas.

Antimicrobial activity: It is apparent from Table-1 that bispyrazoline **2a** demonstrated a potent behaviour against bacterial pathogen *Streptococcus pyogene* and fungal strain *Aspergillus niger* at the MIC value of 8 µg/mL and also furnished the modest potency (MIC-16 µg/mL) against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Compound **2b** was found to be incredibly potent (MIC-8 µg/mL) against *P. aeruginosa*, *P. fluorescens*, *P. glabrum* and *A. sclerotiorum*, while it displayed the MIC value of 16 µg/mL against *E. coli*, *S. aureus*, *A. janus* and *F. oxysporum*. Compound **2c** displayed the perceptible activity (MIC-8 µg/mL) against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Aspergillus janus*, and *Fusarium oxysporum*. The bisheterocyclic **2d** was found to exhibit MIC value of 8 µg/mL against *P. aeruginosa*, *S. aureus* and *A. niger*.

Bisheterocyles **2c** & **2d** also displayed a moderate action (MIC-16 µg/mL) against the strains *Pseudomonas fluorescens*, *Streptococcus pyogene*s, *Aspergillus niger*, *Aspergillus sclerotiorum* and *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus*

TABLE-1
MIC (µg/mL *in vitro*) VALUES OF BISPYRAZOLINES (**2a-f**)

Compound	Gram-negative bacteria				Gram-positive bacteria			Fungi				
	EC	KP	PA	PF	SA	BS	SP	AJ	PG	AN	FO	AS
2a	32	16	16	32	16	32	8	32	32	8	32	32
2b	16	32	8	8	16	32	32	16	8	32	16	8
2c	8	8	32	16	32	8	16	8	32	16	8	16
2d	16	16	8	32	8	16	32	32	16	8	16	32
2e	32	32	32	16	32	8	16	32	32	32	32	16
2f	16	32	16	32	32	32	32	16	32	16	32	16
Amoxicillin	4	4	4	4	2	2	4	–	–	–	–	–
Fuluconazole	–	–	–	–	–	–	–	2	2	2	2	2

EC = *E. coli*, KP = *K. pneumoniae*, PA = *P. aeruginosa*, PF = *P. fluorescens*, SA = *S. aureus*, BS = *B. subtilis*, SP = *S. pyogenes*, AJ = *A. janus*, PG = *P. glabrum*, AN = *A. niger*, FO = *F. oxysporum*, AS = *A. sclerotiorum*

subtilis, *Penicillium glabrum*, *Fusarium oxysporum*, respectively. The bispyrazoline **2e** was found to obstruct the growth of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptococcus pyogenes* and *Aspergillus sclerotiorum* at the MIC value of 8 and 16 µg/mL, respectively. Compound **2f** showed a moderate activity (MIC-16 µg/mL) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus janus*, *Aspergillus niger* and *Aspergillus sclerotiorum*.

The antimicrobial examination results showed that bis-heterocyclic products linked through five, six and eight methylene units (**2b**, **2c** & **2d**) could be able to exhibit auspicious antibacterial and antifungal activities which certainly describe our intention of using varied length of the internal chain.

Conclusion

The present investigation describes the efficient method for the syntheses of new *N*-carbothioamide substituted bispyrazolines built around the methylene chains of different lengths. These bisheterocycles have been successfully achieved under the usual conditions without using any noxious and costly reagents. Among the newly prepared bisheterocyclic products, the pentyl, hexyl and octyl chain linked bispyrazolines (**2b**, **2c** & **2d**) were found to evince potential antibacterial and antifungal properties as compared to other products in the series.

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