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RESEARCH ARTICLE

NEW N-THIOCARBAMIDE-BISPYRAZOLINE DERIVATIVES: SYNTHESIS AND ANTIMICROBIAL STUDIES

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ABSTRACT

A series of new N-thiocarbamide-bispyrazolines 2(a-g) have been synthesized from the cyclization reactions of bischalcones 1(a-g) with thiosemicarbazide by refluxing under KOH and dry alcoholic conditions. The structures of these compounds were analyzed on the basis of their detailed IR, ^{1}H-NMR, ^{13}C-NMR and ESI-MS spectral parameters. The prepared bispyrazolines were screened for their antimicrobial potencies against seven bacterial and five fungal strains. Most of the compounds displayed significant activity against tested microorganisms.

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INTRODUCTION

Infections caused by bacteria and fungi always remain a major health issue due to their resistance to existing antimicrobial products. The increasing incidences of gram-positive and gram-negative bacterial resistance to antibiotics have caused life-threatening infectious diseases (Chu *et al.*, 1996; Davies, 1996). The emergences of multiple drug resistant microorganisms have been responsible for the various major health issues overall world. Therefore, scientists are more interested in designing and synthesizing new antimicrobial agents with immense biological activities. The pyrazole moiety is one of the most ubiquitous heterocyclic scaffolds which are present in many natural and synthetic compounds. Considerable attentions have been focused upon the synthesis of these heterocyclics due to their broad range of bioactivities. These substances have been found to possess a variety of significant and diverse chemical and pharmacological behaviors such as antibacterial (Sharma *et al.*, 2007; Bakr *et al.*, 2009), antifungal (Ozdemir *et al.*, 2007; Andrea *et al.*, 2005), antiviral (Turan-Zitouni *et al.*, 2005; Mamolo *et al.*, 2003), anti-tubercular (Shaharyar *et al.*, 2007), antidepressant (Rajendra Prasad *et al.*, 2005; Shaharyar *et al.*, 2006), anti-amoebic (Palaska *et al.*, 2001; Palaska *et al.*, 1996), anti-inflammatory (Abid *et al.*, 2005; Abid *et al.*, 2006), anticonvulsant (Flora *et al.*, 2006), analgesic (Ozdemir *et al.*, 2007) and anticancer (Amir *et al.*, 2008) activities. Also, pyrazolines have been found to be useful material in drug research and are used extensively as a useful synthones in organic synthesis (Tomilovi *et al.*, 1995; Klimova *et al.*, 1999; Yusuf and Jain, 1999; Wanare *et al.*, 2010). In view of the above mentioned biological properties, it was thought worthwhile to develop the synthesis of new pyrazoline based symmetrical bisheterocyclics. In the recent years, our research group has been working upon the synthesis of symmetrical bisheterocycles (Yusuf and Jain, 2014; Yusuf and Jain, 2011; Yusuf *et al.*, 2012; Yusuf *et al.*, 2012; Yusuf and Kaur, 2015). In connection to these investigations, the present researches are oriented upon the synthesis of N-thiocarbamide-pyrazolines based new symmetrical bisheterocyclics 2(a-g) built around aliphatic linkers of varying lengths in order to study the influence of chain length upon the antimicrobial behavior of final compounds.

EXPERIMENTAL

Materials

All the chemicals used in this study were purchased from E. Merck, S. D. Fine Chem. Ltd., Mumbai and Sigma-Aldrich. The melting points were determined by using the open capillary method and are uncorrected.

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The Infrared (IR) spectra were scanned in KBr pellets on a Perkin Elmer RXIFT Infrared spectrophotometer. Both ^{1}H-NMR and ^{13}C-NMR spectra of the compounds were recorded on the Bruker Avance-II NMR Spectrometer at 400 MHz and 100 MHz respectively. The purity of the compounds was checked by TLC plates coated with silica gel (suspended in chloroform-methanol, 1:1) and iodine vapours were used as visualizing agent. The cultures required for the biological studies of prepared compounds were obtained from MTCC (Microbial Type Culture Collection & Gene Bank, Chandigarh-160036, India). The bischalcones 1(a-g) were prepared according to the literature methods (Yusuf and Kaur, 2015) and their physical and spectroscopic data was found to be similar as reported earlier. (Yusuf and Kaur, 2015)

Synthesis of (5R,5'S)-5,5'-(3,3'-(alkane-1,3-diylbis(oxy))bis(3,1-phenylene))bis(3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) 2(a-g)

A mixture of bischalcone (Yusuf and Kaur, 2015) **1(a-g)** (0.001mol), thiosemicarbazide (0.002 mol) and sodium hydroxide in dry EtOH (30 ml) was refluxed for 3-4 hr. TLC was used to check the progress of reactions (Hexane-Ethylacetate: 9:1). After the completion of reaction, whole reaction mixture were poured into ice to obtain solid products which were filtered, thoroughly washed with water, and dried. The crude products thus obtained were crystallized from MeOH to yield pure compounds **2(a-g)**.

(5R, 5'S)-5,5'-(3,3'-(propane-1,3-diylbis(oxy)) bis(3,1-phenylene)) bis(3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2a)

Off White, Yield 63%; m.p.: 110-112 °C; IR (KBr): υ_{max} cm^{-1}: 3434, 3273 (N-H), 2954, 2862 (methylene C-H), 1584 (C=N), 1159 (C=S); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 7.97 (2H, s, NH$_2$), 7.81 (4H, d, J_o=7.2 Hz, H-2', 6'), 7.42 (2H, t, J=7.1 Hz, H-4'), 7.39 (4H, d, J_o= 7.8 Hz, H-3', 5'), 7.19 (2H, brs, H-2''), 6.78 (2H, t, J_o=7.2 Hz, H-6''), 6.69 (4H, t, J=7.8 Hz, H-4'', 5''), 5.93 (2H, dd, J_{MX}= 11.5 HZ, J_{AX}= 8.5 HZ, H-X), 4.09 (4H, t, J_{vic}=6.1 Hz, OC$\boldsymbol{H_2}$), 3.86 (2H, dd, J_{MX}= 11.5 HZ, J_{MA}= 17.9 HZ, H-M), 3.14 (2H, dd, J_{AX}= 8.5 HZ, J_{AM}= 17.8 HZ, H-A), 2.18 (2H, quintet, J_{vic}=5.8 Hz, OCH$_2$C$\boldsymbol{H_2}$); ^{13}C-NMR (100 MHz, CDCl$_3$): 176.18 (C=S), 158.55 (C-3''), 154.78 (C-3), 144.34 (C-1''), 130.72 (C-1'), 130.33 (C-2', 6'), 129.54 (C-4'), 128.47 (C-3', 5'), 126.88 (C-6''), 117.15 (C-5''), 112.32 (C-4''), 111.69 (C-2''), 63.98 (O$\boldsymbol{C}$H$_2$), 62.67 (C-5), 42.38 (C-4), 28.67 (OCH$_2$$\boldsymbol{C}H_2$); ESI-MS: m/z 635 (M+1, 12%) 579 (7%), 541 (6%), 360 (9%), 342 (8%), 302 (15%), 301 (100%), 274 (5%), 246 (18%), 245 (10%), 170 (21%), 102 (3%); Anal. Calcd. for $C_{35}H_{34}O_2N_6S_2$: C-66.24, H-5.36, N-13.24, S-10.09; Found: C-66.28, H-5.32, N-13.21, S-10.09%.

(5R, 5'S)-5,5'-(3,3'-(butane-1,4-diylbis(oxy))bis(3,1-phenylene)) bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2b)

Off White, Yield 66%; m.p.: 104-106 °C; IR (KBr): υ_{max} cm^{-1}: 3430, 3261 (N-H), 2943, 2868 (methylene C-H), 1586 (C=N), 1160 (C=S); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 7.92 (2H, s, NH$_2$), 7.80 (4H, d, J_o=7.2 Hz, H-2', 6'), 7.33 (2H, t, J_o=7.4 Hz, H-4'), 7.26 (4H, d, J_o= 7.8 Hz, H-3', 5'), 7.18 (2H, t, J_o=7.4 Hz, H-6''), 6.94 (2H, brs, H-2''), 6.75 (4H, t, J_o=7.9 Hz, H-4'', 5''), 5.92 (2H, dd, J_{MX}= 11.7 HZ, J_{AX}= 8.1 HZ, H-X), 4.05 (4H, t, J_{vic}=6.2 Hz, OC$\boldsymbol{H_2}$), 3.84 (4H, dd, J_{MX}= 11.7 HZ, J_{MA}= 17.8 HZ, H-M), 3.14 (2H, dd, J_{AX}= 8.1 HZ, J_{AM}= 17.8 HZ, H-A), 1.90 (4H, quintet, J_{vic}=4.9 Hz, OCH$_2$CH$_2$C$\boldsymbol{H_2}$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 176.16 (C=S), 158.69 (C-3''), 154.74 (C-3), 144.12 (C-1''), 130.63 (C-1'), 130.30 (C-2', 6'), 129.46 (C-4'), 128.41 (C-3', 5'), 126.77 (C-6''), 117.00 (C-5''), 112.27 (C-4''), 111.62 (C-2''), 66.87 (O$\boldsymbol{C}$H$_2$), 62.65 (C-5), 42.41 (C-4), 25.42 (OCH$_2$$\boldsymbol{C}H_2$); ESI-MS: m/z 649 (M+1, 22% 541 (6%), 360 (9%), 302 (19%), 301 (100%), 272 (13%), 245 (7%); Anal. Calcd. for $C_{36}H_{36}O_2N_6S_2$: C-66.66, H-5.55, N-12.96, S-9.87; Found: 66.70, H-5.51, N-12.90, S-9.91%.

(5R,5'S)-5,5'- (3,3'- (pentane-1,5-diylbis(oxy)) bis(3,1-phenylene))bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2c)

Light Yellow, Yield 64%; m.p.: 94-96 °C; IR (KBr): υ_{max} cm^{-1}: 3421, 3254 (N-H), 2942, 2870 (methylene C-H), 1585 (C=N), 1159 (C=S); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.01 (2H, s, NH$_2$), 7.77 (4H, d, J_o=7.1 Hz, H-2', 6'), 7.44 (2H, t, J_o=7.6 Hz, H-4'), 7.31 (4H, d, J_o= 7.4 Hz, H-3', 5'), 7.20 (2H, t, J_o=7.6 Hz, H-6''), 7.00 (2H, brs, H-2''), 6.77 (4H, t, J_o=7.7 Hz, H-4'', 5''), 5.99 (2H, dd, J_{MX}= 11.3 HZ, J_{AX}= 8.1 HZ, H-X), 4.11 (4H, t, J_{vic}=6.2 Hz, OC$\boldsymbol{H_2}$), 3.80 (2H, dd, J_{MX}= 11.4 HZ, J_{MA}= 17.6 HZ, H-M), 3.17 (2H, dd, J_{AX}= 8.1 HZ, J_{AM}= 17.7 HZ, H-A), 1.78 (4H, t, J_{vic}=5.9 Hz, OCH$_2$C$\boldsymbol{H_2}$), 1.02 (2H, t, J_{vic}=6.4 Hz, OCH$_2$CH$_2$C$\boldsymbol{H_2}$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 176.76 (C=S), 159.53 (C-3''), 156.03 (C-3), 143.37 (C-1''), 130.64 (C-1'), 130.33 (C-2', 6'), 129.48 (C-4'), 128.86 (C-3', 5'), 126.98 (C-6''), 117.41 (C-5''), 113.17 (C-4''), 111.84 (C-2''), 67.75 (O$\boldsymbol{C}$H$_2$), 63.41 (C-5), 43.13 (C-4), 29.19 (OCH$_2$$\boldsymbol{C}H_2$), 25.88 (OCH$_2CH_2$$\boldsymbol{C}H_2$); ESI-MS: m/z 685 (M+Na, 41%), 663 (M+1, 13%), 630 (5%), 587 (10%), 342 (8%), 302 (12%), 301 (100%), 274 (9%), 245 (20%), 218 (7%), 206 (7%); Anal. Calcd. for $C_{37}H_{38}O_2N_6S_2$: C-67.06, H-5.74, N-12.68, S-9.66; Found: C-67.10, H-5.77, N-12.64, S-9.61%.

(5R,5'S)-5,5'-(3,3'- (hexane-1,6-diylbis(oxy))bis(3,1-phenylene))bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2d)

Off White, Yield 69%; m.p.: 85-87 °C; IR (KBr): υ_{max} cm^{-1}: 3421, 3250 (N-H), 2940, 2863 (methylene C-H), 1591 (C=N), 1162 (C=S); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.00 (2H, s, NH$_2$), 7.73 (4H, d, J_o=7.0 Hz, H-2', 6'), 7.46 (2H, t, J_o=7.6 Hz, H-4'), 7.33 (4H, d, J_o= 7.2 Hz, H-3', 5'), 7.18 (2H, t, J_o=7.4 Hz, H-6''), 7.19 (2H, brs, H-2''), 6.76 (4H, t, J_o=7.6 Hz, H-4'', 5''), 5.94 (2H, dd, J_{MX}= 11.3 HZ, J_{AX}= 7.9 HZ, H-X), 4.11 (4H, t, J_{vic}=6.2 Hz, OCH_2), 3.81 (2H, dd, J_{MX}= 11.4 HZ, J_{MA}= 17.5 HZ, H-M), 3.14 (2H, dd, J_{AX}= 7.9 HZ, J_{AM}= 17.5 HZ, H-A), 1.91 (4H, quintet, J_{vic}=6.0 Hz, OCH$_2$CH_2), 1.22 (4H, q, J_{vic}=6.1 Hz, OCH$_2$CH$_2$CH_2); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 176.72 (C=S), 159.53 (C-3''), 156.05 (C-3), 143.39 (C-1''), 130.64 (C-1'), 130.01 (C-2', 6'), 129.32 (C-4'), 128.72 (C-3', 5'), 126.98 (C-6''), 117.41 (C-5''), 113.19 (C-4''), 111.83 (C-2''), 67.76 (OCH$_2$), 63.40 (C-5), 43.13 (C-4), 29.19 (OCH$_2$$CH_2$), 25.86 (OCH$_2CH_2$$CH_2$); ESI-MS: m/z 677 (M+1, 11%), 579 (12%), 342 (8%), 302 (14%), 301 (100%), 245 (16%), 243 (8%), 218 (11%); Anal. Calcd. for $C_{39}H_{40}O_2N_6S_2$: C-69.23, H-5.91, N-12.42, S-9.46; Found: C-69.19, H-5.95, N-12.39, S-9.42%.

(5R, 5'S)-5,5'-(3,3'-(octane-1, 8-diylbis(oxy))bis(3,1-phenylene)) bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2e)

Off White, Yield 71%; m.p.: 78-80 $^{\circ}$C; IR (KBr): υ_{max} cm^{-1}: 3429, 3264 (N-H), 2951, 2869 (methylene C-H), 1588 (C=N), 1161 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.79 (2H, s, NH_2), 7.71 (4H, d, J_o=7.1 Hz, H-2', 6'), 7.43 (2H, t, J_o=7.4 Hz, H-4'), 7.26 (4H, d, J_o= 7.2 Hz, H-3', 5'), 7.20 (2H, t, J_o=7.6 Hz, H-6''), 7.00 (2H, brs, H-2''), 6.77 (4H, t, J_o=7.7 Hz, H-4'', 5''), 5.98 (2H, dd, J_{MX}= 11.7 HZ, J_{AX}= 7.8 HZ, H-X), 4.21 (4H, t, J_{vic}=6.3 Hz, OCH_2), 3.84 (4H, dd, J_{MX}= 11.7 HZ, J_{MA}= 17.6 HZ, H-M), 3.21 (2H, dd, J_{AX}= 7.8 HZ, J_{AM}= 17.6 HZ, H-A), 1.97 (4H,quintet, J_{vic}=5.9 Hz, OCH_2CH_2), 1.44 (4H, quintet, J_{vic}=6.0 Hz, $OCH_2CH_2CH_2$), 1.02 (4H, m, $OCH_2CH_2CH_2CH_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.16 (C=S), 158.69 (C-3''), 156.03 (C-3), 143.37 (C-1''), 130.64 (C-1'), 130.03 (C-2', 6'), 129.99 (C-4'), 128.86 (C-3', 5'), 126.97 (C-6''), 117.36 (C-5''), 113.12 (C-4''), 111.89 (C-2''), 67.88 (OCH_2), 63.41 (C-5), 43.13 (C-4), 32.18 (OCH_2CH_2), 29.99 ($OCH_2CH_2CH_2$), 25.99 ($OCH_2CH_2CH_2CH_2$); ESI-MS: m/z 727 (M+Na, 7%), 630 (15%), 509 (5%), 420 (4%), 274 (3%), 245 (4%), 243 (14%), 242 (100%), 218 (8%); Anal. Calcd. for $C_{41}H_{44}O_2N_6S_2$: C-69.88, H-6.25, N-11.93, S-9.09; Found: C-69.92, H-6.21, N-11.89, S-9.13%.

(5R, 5'S)-5, 5'-(3,3'-(decane-1,10-diylbis(oxy))bis(3,1-phenylene)) bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2f)

The bispyrazoline 4f was prepared from the reaction of bischalcone 3f (0.001 mol) with thiosemicarbazide (0.002 mol) under the similar conditions as described earlier for 4a. Light Yellow, Yield 73%; m.p.: 70-72 $^{\circ}$C; IR (KBr): υ_{max} cm^{-1}: 3429, 3271 (N-H), 2943, 2870 (methylene C-H), 1586 (C=N), 1160 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.91 (2H, s, NH_2), 7.85 (4H, d, J_o=7.4 Hz, H-2', 6'), 7.39 (2H, t, J_o=7.1 Hz, H-4'), 7.28 (4H, d, J_o= 7.8 Hz, H-3', 5'), 7.20 (2H, t, J_o=7.7 Hz, H-6''), 6.94 (2H, brs, H-2''), 6.75 (4H, t, J_o=7.9 Hz, H-4'', 5''), 5.92 (2H, dd, J_{MX}= 11.7 HZ, J_{AX}= 8.1 HZ, H-X), 3.99 (4H, t, J_{vic}=6.2 Hz, OCH_2), 3.84 (4H, dd, J_{MX}= 11.7 HZ, J_{MA}= 17.6 HZ, H-M), 3.13 (2H, dd, J_{AX}= 8.1 HZ, J_{AM}= 17.6 HZ, H-A),2.65 (4H, quintet, J_{vic}=5.9 Hz, OCH_2CH_2), 2.11 (4H, quintet, J_{vic}=6.4 Hz, $OCH_2CH_2CH_2$), 1.54 (4H, m, $OCH_2CH_2CH_2CH_2$), 1.19 (4H, m, $OCH_2CH_2CH_2CH_2CH_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.13 (C=S), 158.75 (C-3''), 154.92 (C-3), 144.49 (C-1''), 130.83 (C-1'), 130.51 (C-2', 6'), 129.65 (C-4'), 128.64 (C-3', 5'), 127.04 (C-6''), 117.08 (C-5''), 112.25 (C-4''), 111.71 (C-2''), 67.24 (OCH_2), 62.73 (C-5), 42.34 (C-4), 31.10 (OCH_2CH_2), 28.90 ($OCH_2CH_2CH_2$), 25.52 ($OCH_2CH_2CH_2CH_2$), 18.53 ($OCH_2CH_2CH_2CH_2CH_2$); ESI-MS: m/z 727 (M+Na, 7%), 587 (13%), 420 (4%), 301 (5%), 274 (3%), 243 (16%), 242 (100%); Anal. Calcd. for $C_{43}H_{48}O_2N_6S_2$: C-70.49, H-6.55, N-11.47, S-8.74; Found: C-69.92, H-6.21, N-11.89, S-8.69%.

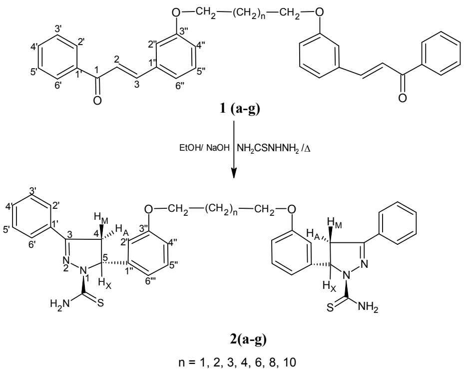
(5R,5'S)-5, 5'-(3,3'-(dodecane-1,12-diylbis(oxy))bis(3,1-phenylene)) bis (3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide) (2g)

Off white, Yield 68%; m.p.: 76-78 $^{\circ}$C; IR (KBr): υ_{max} cm^{-1}: 3426, 3259 (N-H), 2952, 2870 (methylene C-H), 1591 (C=N), 1161 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (2H, s, NH_2), 7.80 (4H, d, J_o=7.2 Hz, H-2', 6'), 7.34 (2H, t, J_o=7.4 Hz, H-4'), 7.26 (4H, d, J_o= 7.8 Hz, H-3', 5'), 7.18 (2H, t, J_o=7.4 Hz, H-6''), 6.96 (2H, brs, H-2''), 6.76 (4H, t, J_o=7.9 Hz, H-4'', 5''), 5.94 (2H, dd, J_{MX}= 11.4 HZ, J_{AX}= 7.8 HZ, H-X), 4.05 (4H, t, J_{vic}=6.3 Hz, OCH_2), 3.84 (4H, dd, J_{MX}= 11.4 HZ, J_{MA}= 17.6 HZ, H-M), 3.19 (2H, dd, J_{AX}= 7.8 HZ, J_{AM}= 17.6 HZ, H-A), 1.99 (4H, quintet, J_{vic}=5.9 Hz, OCH_2CH_2), 1.54 (4H, quintet, J_{vic}=6.4 Hz, $OCH_2CH_2CH_2$), 1.25 (4H, m, $OCH_2CH_2CH_2CH_2$), 1.26 (8H, m, $OCH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.11 (C=S), 158.74 (C-3''), 154.94 (C-3), 144.48 (C-1''), 130.80 (C-1'), 130.50 (C-2', 6'), 129.66 (C-4'), 128.60 (C-3', 5'), 127.06 (C-6''), 117.09 (C-5''), 112.24 (C-4''), 111.70 (C-2''), 67.24 (OCH_2), 62.73 (C-5), 43.34 (C-4), 31.08 (OCH_2CH_2), 28.95 ($OCH_2CH_2CH_2$), 28.11 ($OCH_2CH_2CH_2CH_2$), 25.52 ($OCH_2CH_2CH_2CH_2CH_2$), 19.11 ($OCH_2CH_2CH_2CH_2CH_2CH_2$); ESI-MS: m/z 761 (M+1, 14%), 630 (19%), 509 (6%), 302 (13%), 301 (100%), 245 (3%), 243 (6%), 242 (50%); Anal. Calcd. for $C_{45}H_{52}O_2N_6S_2$: C-71.05, H-6.84, N-11.05, S-8.42; Found: C-71.10, H-6.79, N-11.01, S-8.39%.

RESULTS AND DISSCUSSION

Chemistry

The title compounds 2(a-g) has been obtained in good yields from the cyclization reactions of bischalcones[28] 1(a-g) with thiosemicarbazide in the presence of KOH/EtOH (Scheme-I). The bischalcones were prepared according to the method as described in the litrature.[28] The structures of seven newly prepared symmetrical bisheterocycles 2(a-g) have been fully characterized by using their various spectroscopic data such as IR, ^{1}H-NMR, ^{13}C-NMR & ESI-MS. The elemental analysis also confirmed the purity of these compounds.



Scheme 1. Synthesis of N-thiocarbamide-bispyrazolines 2(a-g)

IR spectra of 2(a-g) exhibited major absorption bands at 3434-3421, 3273-3250 (N-H), 2954-2940, 2870-2862 (methylene C-H), 1591-1584 (C=N) and 1162-1159 (C=S) cm^{-1}. The absence of any absorption in the carbonyl region (1661-1660 cm^{-1}) in their IR spectra suggested the involvement of carbonyl group during the cyclization reaction of 1(a-g). In the ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of 2(a-g), 3-aryl ring protons (H-2', 6' & 3', 5') produced well-defined two doublets at δ 7.85-7.71 (J_o =7.4-7.0 Hz) and 7.39-7.26 (J_o = 7.8-7.2 Hz) integrating for four hydrogens each. The D_2O exchangeable N-*H* protons of the thiocarbamide moiety generated four hydrogens singlets at δ 8.01-7.79. Also, triplets was found to be well resonated at δ 7.44-7.32 (2H, J_o =7.6-6.9 Hz) could be assignable to H-4'

Table 1. MIC (μg/ml) data of bispyrazolines 2(a-g)

	Gram (-ve) bacteria				Gram (+ve) bacteria			Fungi				
Compound no.	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*P. fluorescens*	*S. aureus*	*B. subtilis*	*S. pyogenes*	*A. janus*	*P. glabrum*	*A. niger*	*F.oxysporum*	*A.sclerotiorum*
2a	32	32	8	64	64	32	32	8	16	8	32	8
2b	64	4	32	16	32	8	16	8	32	16	8	32
2c	64	2	4	64	32	32	64	32	8	8	8	16
2d	8	64	4	4	64	32	64	16	16	8	8	32
2e	64	4	16	16	16	32	32	16	8	8	16	8
2f	64	32	8	32	8	32	64	4	64	8	8	8
2g	4	16	16	8	8	8	16	4	8	4	8	8
Amoxicillin	4	4	4	4	2	2	4	-	-	-	-	-
Fluconazole	-	-	-	-	-	-	-	2	2	2	2	2

proton. Regarding the remaining signals in the aromatic region, triplets at δ 7.20-6.78 (J_o =7.6-7.1Hz) and broad singlets at δ 7.19-6.94 integrating for two protons each could be ascribed to H-6’’ & H-2’’ respectively while four hydrogens triplets resonating at δ 6.77-6.69 (4H, t, J_o=7.9-7.5 Hz) were easily attributed by H-5’’, 4’’. The protons of benzene ring present at 5 position of pyrazoline moiety furnished a two protons doublet, two protons broad singlet and four protons triplet at δ 7.21-6.78 (J_o=7.7-7.2 Hz, H-6’’), 7.19-6.94 (H-2’’) and δ 6.77-6.69 (J_o=7.7-7.2 Hz, H-4’’, 5’’) respectively. Protons H-X, H-M & H-A belonging to pyrazoline moiety produced three well resolved doublet of doublets at δ 5.99-5.92 (2H), 3.86-3.80 (2H) and 3.21-3.14 (2H) respectively.

The coupling value of 11.8-11.4 Hz between H-X and H-M suggests their *cis* disposition and J_{XA}= 8.0-7.7 Hz and J_{MA}= 17.9-17.5 Hz informs that H-X and H-A are *trans* to each other while H-M and H-A are located together at C-4. In the upfield region, triplets placed at δ 5.08-4.53 (4H, J_{vic}=6.2-6.1 Hz may be given by $OC\mathit{H}_2$ group of internal aliphatic chain and the inner methylene group $OCH_2C\mathit{H}_2$ were easily situated at δ 2.18-1.78 (*see experimental*). The carbon framework of the compounds 2(a-g) were fully explained on the basis of their ^{13}C NMR spectra (100 MHz, DMSO-d_6). Carbon atoms C=S, C-3’’ & C=N were found to be resonating at δ 176.76-176.11, 159.53-158.55, 154.94-154.03 respectively. The remaining aromatic carbons (?) provided appropriate signals in the region δ 144.49-111.62 (*see experimental*). The pyrazoline ring carbons C-5 and C-4 resulted in the generation of two signals at δ 63.41-62.65 and 43.34-42.38 respectively while signal placed at δ 67.88-63.98 could be ascribed to aliphatic chain $O\boldsymbol{C}H_2$ group due to its bonding to oxygen atom.

The remaining methylene carbons also produced suitable resonances in the aliphatic region (*see experimental*). ESI-MS fragmentation pattern of the bispyrazolines **2(a-g)** also proved helpful to corroborate their proposed structures (*see experimental*).

Antimicrobial activity

The prepared compounds **2(a-g)** were screened for their *in vitro* antibacterial and antifungal activity against seven bacterial and five fungal species namely *Klebsellia pneumonia* (MTCC 3384)*, Pseudomonas aeruginosa* (MTCC 424)*, Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Pseudomonas fluorescens (MTCC 103)*, *Streptococcus pyogenes (MTCC 442)* and *Aspergillus janus* (MTCC 2751), *Pencillium glabrum* (MTCC 4951), *Fusarium oxysporum (MTCC 2480)*, *Aspergillus sclerotiorum (MTCC 1008)*, *Aspergillus niger (MTCC 281)* respectively.

Minimum inhibitory concentrations (MICs) were determined by using Serial dilution technique (Pandey and and Khan, 2008). Amoxicillin and fluconazole were used as the standard drug as positive control while the DMSO was used as negative control. This control did not show any activity against the strains of micro-organisms used. Normal saline was used to make a suspension of spore of bacterial and fungal strain for lawning. . A loopful of particular microbial strain was transferred to 10 ml saline to get a suspension of corresponding species After this, 1 ml volume of nutrient broth was added to each tube successively and all the tubes were seeded with the bacterial and fungal strains.

The minimum inhibitory concentration (MIC-µg/ml) was determined by using different dilutions of the concerned compound. The lowest concentration required to arrest the growth of bacterial and fungal strains was regarded as minimum inhibitory concentration (MIC). The results were compared with positive controls, the standard drug amoxicillin and fluconazole. Serial dilution of the test compounds previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml. All the bacteria strains were grown at 37˚C for 24 hr in nutrient broth and fungi were grown in malt extract at 28˚C for 72 hr.

Each test compound was dissolved in DMSO and MIC thus obtained were compared with control. The susceptibility of the bacteria and fungi to the test compounds was determined by the appearance of turbidity after the above said time period. The MIC determination results of 2(a-g) have been presented in Table 1. It is evident from Table-1 data describes that compounds 2(a-g) mainly prohibited the growth of the fungal strains. The 2a compound was potent against *Pseudomonas aeruginosa, Aspergillus janus* and *Aspergillus niger* at MIC-8 µg/ml. 2b was found to possess significant activity against *Klebsellia pneumonia* (MIC-4 µg/ml), *Bacillus subtilis Aspergillus janus* and *Fusarium oxysporum* (MIC-8 µg/ml). The compound 2c also seems to be most active against bacterial strains *Klebsellia pneumonia* (MIC-2 µg/ml), *Pseudomonas aeruginosa* (MIC-4 µg/ml) and fungal strains *Pencillium glabrum*, *Aspergillus niger, Fusarium oxysporum* at MIC of 8 µg/ml. 2d exhibited noticeable activity (MIC-8 & 4 µg/ml) against *Escherichia coli*, *Pseudomonas aeruginosa, P. fluorescens* and *Aspergillus niger, Fusarium* oxysporum.

The compounds 2e and 2f were found to be significant activity against *Klebsellia pneumonia, Pencillium glabrum, Aspergillus niger, Pseudomonas aeruginosa, Staphylococcus aureus and Aspergillus niger, Fusarium oxysporum Aspergillus sclerotiorum* (MIC-8 & 4 µg/ml). 2g was found to be most active (MIC-8 & 4 µg/ml) against all bacterial and fungal strains *Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, Bacillus subtilis* and *Aspergillus janus, Pencillium glabrum, Aspergillus niger, Fusarium oxysporum and Aspergillus sclerotiorum* respectively. Antimicrobial analysis reveals that bispyrazolines linked through the longer methylene chains showed promising antibacterial and antifungal properties.

Conclusion

This study provides simple and general method for the preparation of N-thiocarbamide-bispyrazolines 2(a-g) under the ordinary conditions. The *in vitro* antibacterial and antifungal evaluations of the prepared compounds reveals that longer chain linked bisheterocycles seems to be significant antimicrobial products and this observation clearly signifies the purpose of studied synthetic reactions.

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