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Synthesis, characterization and antibacterial activity of isoniazide containing new arylazopyrazoles

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ABSTRACT

Various ethyl-2-(2- substituted phenyl hydrazono)-3-oxobutyrate (2a-h) condensation with isonicotinyl hydrazide (i.e. isoniazide) (3) to 1-isonicotinoyl-3-methyl-4-(2-substituted phenylhydrazono)-1H-pyrazol-5(4H)-one (4a-h). The structures of all these compounds (4a-h) were recognized on basis of analytical and spectral data. The newly synthesized compounds were evaluated for their antimicrobial activity against various bacteria and fungi.

Introduction

The arylazopyrazoles are generally prepared by combination of aryl-azo-ethyl actoacetate derivatives and hydrazine derivatives [1-6].Hydrazide and their heterocyclised products display diverse biological activities including antibacterial, antifungicidal, anticonvulsant, analgesic, anti-inflammatory properties [7-21].

These heterocyclic systems find wide use in medicine, agriculture and industry. One of the hydrazide, isonicotinyl hydrazide (i.e. isoniazide) of isonicotinic acid which is keystone of modern treatment of tuberculosis.[22] Isoniazide is bacteriostatic in resting bacilli but bactericidal for actively dividing Mycobacterium tuberculosis. It is suggested that it inhibits biosynthesis of mycotic acids, which are important constituents of the mycobacterial cell wall.[23]

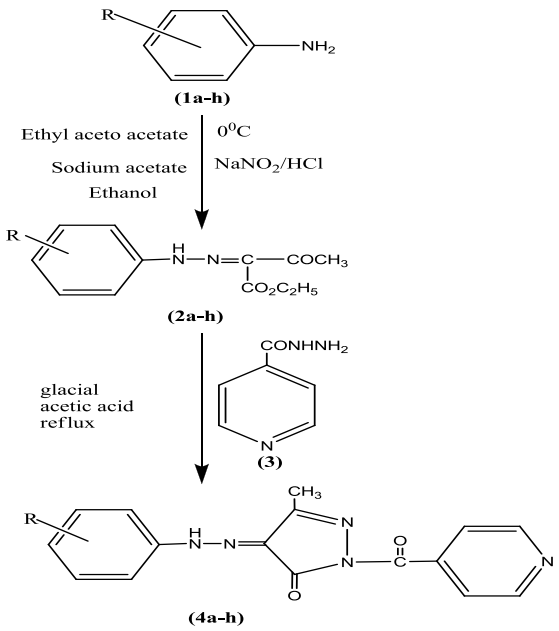
Hence, it was thought of interest to merge both of arylazopyrazole and isonicotinyl hydrazide moieties which may enhance the drug activity of compounds to some extent, or they might possess some of the above mentioned biological activities. From this point of view, the objective of the present work is to prepare new derivatives of isonicotinyl hydrazide containing arylazopyrazole moiety.

Hence the present communication comprises the synthesis of 1-isonicotinoyl-3-methyl-4-(2-substituted phenylhydrazono)-1H-pyrazol-5(4H)-one (4a-h). The synthetic approach is shown in scheme-1.

Experimental

General

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046.Purity of compound was checked by TLC on silica gel plates and the spots were visualized by UV lamp.



Where,R= (a) 4-H; (b) 2-CH_3; (c) 4-Cl; (d) 4-Br; (e) 4-Nitro; (f) 2,4-Dinitro; (g)2,4-Dichloro-6-Nitro; (h)2,4,6-tribromo.

Scheme-1

Synthesis of ethyl-2-(2-phenyl hydrazono)-3-oxobutyrate(2a): Aniline (1a) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 72%; m.p. 94-96°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH2), 2950, 1370 cm^{-1} (-CH_3,CH_2),1695-1750 cm^{-1}(C=O);

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1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH^3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH),6.89-7.37 (s,5H,ArH); ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N);114.6-143.7 (Ar-C); Anal. calcd. for $C_{12}H_{14}N_2O_3$: C, 63.15; H, 6.14; N,12.28. Found: C, 63.1; H, 6.1; N, 12.2.

Synthesis of ethyl 3-oxo-2-(2-o-tolylhydrazono)butanoate (2b): o-toluidine (1b) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 80%; m.p. 98-99°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2),1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 2.36 (s,3H,CH_3) , 6.74-7.19 (s,4H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N); 17.9 (CH_3), 113.4-142.1 (Ar-C); Anal. calcd. for $C_{13}H_{16}N_2O_3$: C, 64.46; H, 6.61; N, 11.57. Found: C, 64.4; H, 6.6; N, 11.4.

Synthesis of ethyl 2-(2-(4-chlorophenyl)hydrazono)-3-oxobutanoate (2c): p-chloro aniline (1c) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 76%; m.p. 112-115°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2),1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 7.11-7.26 (s,4H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N);118.2-130.4(Ar-C); Anal. calcd. for $C_{12}H_{13}N_2O_3Cl$: C, 53.63; H, 4.84; N, 10.42. Found: C, 53.6; H, 4.8; N, 10.3

Synthesis of ethyl 2-(2-(4-bromophenyl)hydrazono)-3-oxobutanoate (2d): p-bromo aniline (1d) (0.01 mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 82%; m.p. 114-117°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2),1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 6.56-7.38 (s,4H, ArH) ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N); 116.9-142.5(Ar-C);Anal. calcd. for $C_{12}H_{13}N_2O_3Br$: C, 46.00; H, 4.15; N, 8.94. Found: C, 45.9; H, 4.1; N, 8.8.

Synthesis of ethyl-2-(2- nitro phenyl hydrazono)-3-oxobutyrate (2e): p-nitro aniline (1e) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 65%; m.p. 82-84°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2),1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 7.24-8.08 (s,4H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N); 113.7-149.5(Ar-C);Anal. calcd. for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.65; N, 15.05. Found: C, 51.5; H, 4.6; N, 14.9.

Synthesis of ethyl-2-(2,4-dinitro phenyl hydrazono)-3-oxobutyrate (2f): 2,4-dinitro aniline (1f) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 66%; m.p. 118-120°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2), 1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 8.01-8.92(s,3H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1 (CH_3), 163.5-196.4 (-CO), 126.9 (C=N),116.9-139.1(Ar-C); Anal. calcd. for $C_{12}H_{12}N_4O_7$: C, 44.44; H, 3.70; N, 17.28. Found: C, 44.4; H, 3.6; N, 17.2.

Synthesis of ethyl-2-(2,4-dichloro-6-nitro phenyl hydrazono)-3-oxobutyrate (2g): 2,4-dichloro-6-nitro aniline (1g) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 77%; m.p. 126-128°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2), 1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH),8.04-8.20 (s,2H,ArH); ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N) , 125.1-140.8(Ar-C);Anal. calcd. for $C_{12}H_{11}N_3O_5C_{l2}$: C, 41.37; H, 3.16; N, 12.06. Found: C, 41.3; H, 3.1; N, 11.9.

Synthesis of ethyl-2-(2,4,6-tribromo phenyl hydrazono)-3-oxobutyrate (2h): 2,4,6-tribromo aniline (1h) (0.01mole) was dissolved in a mixture of HCl (8ml) and water (6ml) and cooled to 0˚c in ice bath. To it a cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl actoacetate (0.01mole) and sodium acetate (0.12mole) in ethanol (50ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yield 84%; m.p. 122-125°C; IR (KBr) νcm^{-1} 3090, 1620-1640 cm^{-1}(C=N), 3030-3080 cm^{-1}(C-H of Ar.), 2815-2850 cm^{-1} (-OCH_2), 2950, 1370 cm^{-1} (-CH_3,CH_2), 1695-1750 cm^{-1}(C=O); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) 1.25(t,3H,CH_3), 2.35 (s,3H,$COCH_3$), 4.29 (q,2H,$COCH_2$), 11.62 (s,1H,NH); 7.01-7.08 (s,2H,ArH); ^{13}C ¬NMR (100MHz,DMSO,δ/ppm):

14.2(CH_3),62.6 (OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N); 109.8-152.7(Ar-C);Anal. calcd. for $C_{12}H_{11}N_2O_3Br_3$: C, 30.57; H, 2.33; N, 5.94. Found: C, 30.5; H, 2.3; N, 5.8.

Synthesis of 1-isonicotinoyl-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (4a): To ethyl-2-(2-phenyl hydrazono)-3-oxobutyrate(2a) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 57%; m.p. 176-178°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH),6.90-8.92(s,9H,ArH);^{13}C ¬NMR (100MHz, DMSO, δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N),114.2-156.7 (Ar-C); Anal. calcd. for $C_{16}H_{13}N_5O_2$: C, 62.54; H, 4.23; N, 22.80. Found: C, 62.5; H, 4.2; N, 22.7.

Synthesis of 1-isonicotinoyl-3-methyl-4-(2-o-tolylhydrazono)-1H-pyrazol-5(4H)-one (4b): To ethyl-2-(2- o-tolylhydrazono)-3-oxobutyrate(b) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 64%; m.p. 189-191°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH) , 2.24 (s,3H,CH_3) , 6.84-8.92 (s,8H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N),17.8 (CH_3),113.5-156.6 (Ar-C);Anal. calcd. for $C_{17}H_{15}N_5O_2$: C, 63.55; H, 4.67; N, 21.80. Found: C, 63.5; H, 4.6; N, 21.7.

Synthesis of 1-isonicotinoyl-4-(2-(4-chlorophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (4c): To ethyl-2-(2-(4-chlorophenyl)hydrazono)-3-oxobutyrate (2c) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 55%; m.p. 198-201°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6,δ/ppm):2.42 (s,3H,CH_3), 11.62 (s,1H, NH),7.20-8.94(s,8H,ArH);^{13}C-¬NMR(100MHz,DMSO, δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N) ,118.4-156.6 (Ar-C);Anal. calcd. for $C_{16}H_{12}N_5O_2Cl$: C, 56.22; H, 3.51; N, 20.49. Found: C, 56.2; H, 3.5; N, 20.4.

Synthesis of 1-isonicotinoyl-4-(2-(4-bromophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (4d): To ethyl-2-(2-(4-bromophenyl)hydrazono)-3-oxobutyrate (2d) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 57%; m.p. 206-208°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH, 6.85-8.93 (s,8H,ArH); ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N) ,117.9-156.5(Ar-C) ;Anal. calcd. for $C_{16}H_{12}N_5O_2Br$: C, 49.74; H, 3.10; N, 18.13. Found: C, 49.7; H, 3.1; N, 18.0.

Synthesis of 1-isonicotinoyl-4-(2-(4-nitrophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (4e): To ethyl-2-(2-(4-nitrophenyl) hydrazono)-3-oxobutyrate(2e) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 46%; m.p. 183-185°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH),6.85-8.93 (s,8H,ArH) ;^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N),117.9-156.5(Ar-C);Anal. calcd. for $C_{16}H_{12}N_6O_4$: C, 54.54; H, 3.40; N, 23.86. Found: C, 54.5; H, 3.4; N, 23.8.

Synthesis of 1-isonicotinoyl-4-(2-(2,4-dinitrophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (4f): To ethyl-2-(2-(2,4-dinitrophenyl) hydrazono)-3-oxobutyrate (2f) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 58%; m.p. 194-196°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH) , 7.90-8.92(s,7H,ArH) ; ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N),118.1-156.6 (Ar-C) ; Anal. calcd. for $C_{16}H_{11}N_7O_6$: C, 48.36; H, 2.77; N, 24.68. Found: C, 48.3; H, 2.7; N, 24.6.

Synthesis of 1-isonicotinoyl-4-(2-(2,4-dichloro-6-nitrophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one 4g):

To ethyl-2-(2-(2,4-dichloro-6-nitrophenyl) hydrazono)-3-oxobutyrate (2g) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight.

The resulting solid was filtered off dried and crystallized from methanol. Yield 57%; m.p. 180-183°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH) , 7.94-8.95 (s,6H,ArH) ; 13C ¬NMR (100MHz,DMSO,δ/ppm): 12.1 (CH3), 163.7-172.4 (-CO), 129.4 (C=N),125.3-156.8(Ar-C);Anal. calcd. for $C_{16}H_{10}N_6O_4C_{l2}$: C, 45.60; H, 2.37; N, 19.95. Found: C, 45.6; H, 2.3; N, 19.9.

Synthesis of 1-isonicotinoyl-4-(2-(2,4,6-tribromophenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (4h): To ethyl-2-(2-(2,4,6tribromophenyl) hydrazono)-3-oxobutyrate (2h) (0.002mole) dissolved in glacial acetic acid (20ml), a solution of isonicotinyl hydrazide (i.e. isoniazide) (3) (0.002mole) in 25ml of glacial acetic acid was added and the mixture was refluxed 10-12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. Yield 59%; m.p. 211-213°C; IR (KBr)νcm^{-1} 1624-1640 cm^{-1}(C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C); 1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42 (s,3H, CH3),11.62 (s,1H,NH) ,7.71-8.95 (s,6H,ArH); ^{13}C ¬NMR (100MHz,DMSO,δ/ppm): 12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N),110.7-156.4(Ar-C);Anal. calcd. for $C_{16}H_{10}N_5O_2Br_3$: C, 35.29; H, 1.83; N, 12.86. Found: C, 35.2; H, 1.8; N, 12.8.

Results and Discussion

In the present research we have synthesized novel pyrazolone derivatives from precursors ethyl -2-(2- substituted phenyl hydrazono)-3-oxobutyrate and isoniazide.The reaction sequences employed for the synthesis of pyrazolone derivatives is shown in scheme-1. Formation of pyrazolone was characterized by by their elemental analysis, IR, 1H-NMR, ^{13}C-¬NMR and LC-MS spectral studies. From the structural investigation, IR spectra showed the stretching frequency range between 1624-1640 cm^{-1}, which evidenced the presence of C=N linkage and also the absence of -NH_2 peak for the synthesized compound derivatives., Further it was also supported by 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH3), 1705-1765(C=O), 3330 and 3155 cm^{-1}(NH), and 1585, 1548, and 1530 cm^{-1} (C=C).

1H NMR (400MHz , DMSO - d6 ,δ / ppm) : 2.42(s,3H,CH_3), 11.62 (s,1H,NH); (4a): 6.90-8.92(s,9H, ArH); (4b): 2.24 (s,3H,CH_3) , 6.84-8.92 (s,8H,ArH) ; (4c): 7.20-8.94 (s,8H,ArH) ; (4d): 6.85-8.93 (s,8H,ArH) ; (4e): 7.24-8.94 (s,8H,ArH) ; (4f): 7.90-8.92(s,7H,ArH) ; (4g): 7.94-8.95 (s,6H,ArH) ; (4h): 7.71-8.95 (s,6H, ArH).

^{13}C ¬NMR(100MHz,DMSO,δ/ppm):12.1(CH_3),163.7-172.4 (-CO), 129.4 (C=N); (4a): 114.2-156.7 (Ar-C); (4b): 17.8 (CH_3), 113.5-156.6 (Ar-C); (4c): 118.4-156.6 (Ar-C); (4d): 117.9-156.5(Ar-C); (4e) 116.8-159.9 (Ar-C); (4f)118.1-156.6 (Ar-C) ; (4g) 125.3-156.8(Ar-C);(4h)110.7-156.4(Ar-C). The C, H, N analysis data of all compounds are presented in Table -2.

The examination of data reveals that the elemental contents are consistence with the predicted structure shown in scheme-1.The IR data also direct for assignment of the predicted structure. The LC-MS of one sample i.e.4a shows the peak of M+ ion at 328.5 which is consistent of molecular weight of 4a i.e. 307. All these facts confirm the structures 4a-h.

All the compounds were evaluated for their antimicrobial properties. MIC's were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms. As shown in table- 3 and 4. The study of antibacterial screening expose that the compounds 4c and 4h have exhibited good antibacterial activity comparable to the standard ampicilin, while compounds 4f and 4h displayed better antifungal activity.

Biological Activity

Antibacterial Activities

Antibacterial activities of all the compounds were studied against gram-positive Bacteria (Bacillus subtilis and Staphylococcus aurous) and gram-negative Bacteria (E.coil, Salmonella typhi and Klebsiella promioe) at a concentration of 50μg/ml by agar cup plate method.[24] Methanol system was used as control in this method. Under similar conditions, using ampicilin as a standard for comparison, we carried out a control experiment. The area of inhibition of measured in millimeters. Compounds 4c and 4h were found more toxic for microbes. Other compounds found to be less or moderate active than ampicilin(Table-3).

Antifungal Activity

The fungicidal activity of all the compounds (4a-h) was studied at 1000 ppm concentration in vitro plant pathogenic organisms listed in Table-4. The antifungal activities of all the samples were measured on each of these plant pathogenic strains on potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200 gms, dextrose 20gms, agar 20 gms and water 1 litre five days old cultures were employed. The compounds to be tested were suspended (1000ppm) in a PDA medium and autoclaved at 120° C for 15 min. at 15 atm pressure. These medium were poured into sterile Petri plate and the organisms were inoculated after cooling the petri plate. The percentage inhabitation for fungi was calculated after 5 days using the formula given below.

Percentage of inhibition = 100(X-Y) / X

Where, X: Area of colony in control plate

Y: Area of colony in test plate

The fungicidal activity all compounds (4a-h) are shown in Table-4.

Conclusion

The present study reports the synthesis of novel heterocyclic pyrazolinone from the corresponding precursors ethyl-2-(2-substituted phenyl hydrazono)-3-oxobutyrateand isoniazide. The investigation of antibacterial screening reveals that the compounds 4c and 4h have exhibited good antibacterial activity comparable to the standard ampicilin, while compounds 4f and 4h displayed better antifungal activity.

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Table 1 Physical and Analytical Data of the Compounds Synthesized (2a-h).

Com.	R	Molecular Formula (Mol.wt.)	LC-MS Data	M.P.* °C	Yield %	Elemental Analysis					
						C%		H%		N%	
						Found	Calcd.	Found	Calcd.	Found	Calcd.
2a	Hydrogen	$C_{12}H_{14}N_2O_3$ (228)	245	94-96	72	63.1	63.15	6.1	6.14	12.2	12.28
2b	2-Methyl	$C_{13}H_{16}N_2O_3$ (242)	260	98-99	80	64.4	64.46	6.6	6.61	11.4	11.57
2c	4-Chloro	$C_{12}H_{13}N_2O_3Cl$ (268.5)	281	112-115	76	53.6	53.63	4.8	4.84	10.3	10.42
2d	4-Bromo	$C_{12}H_{13}N_2O_3Br$ (313)	324	114-117	82	45.9	46.00	4.1	4.15	8.8	8.94
2e	4-Nitro	$C_{12}H_{13}N_3O_5$ (279)	297	82-84	65	51.5	51.61	4.6	4.65	14.9	15.05
2f	2,4-Dinitro	$C_{12}H_{12}N_4O_7$ (324)	339	118-120	66	44.4	44.44	3.6	3.70	17.2	17.28
2g	2,4-Dichloro-6-Nitro	$C_{12}H_{11}N_3O_5Cl_2$ (348)	364	126-128	77	41.3	41.37	3.1	3.16	11.9	12.06
2h	2,4,6-Tribromo	$C_{12}H_{11}N_2O_3Br_3$ (471)	492	122-125	84	30.5	30.57	2.3	2.33	5.8	5.94

Table 2. Physical and Analytical Data of the Compounds Synthesized (4a-h)

Com.	R	Molecular Formula	LC-MS Data	M.P.* °C	Yield %	Elemental Analysis					
						C%		H%		N%	
						Found	Calcd.	Found	Calcd.	Found	Calcd.
4a	Hydrogen	$C_{16}H_{13}N_5O_2$ (307)	328.5	176-178	57	62.5	62.54	4.2	4.23	22.7	22.80
4b	2-Methyl	$C_{17}H_{15}N_5O_2$ (321)	341	189-191	64	63.5	63.55	4.6	4.67	21.7	21.80
4c	4-Chloro	$C_{16}H_{12}N_5O_2Cl$ (341.5)	366	198-201	55	56.2	56.22	3.5	3.51	20.4	20.49
4d	4-Bromo	$C_{16}H_{12}N_5O_2Br$ (386)	408	206-208	57	49.7	49.74	3.1	3.10	18.0	18.13
4e	4-Nitro	$C_{16}H_{12}N_6O_4$ (352)	374	183-185	46	54.5	54.54	3.4	3.40	23.8	23.86
4f	2,4-Dinitro	$C_{16}H_{11}N_7O_6$ (397)	422	194-196	58	48.3	48.36	2.7	2.77	24.6	24.68
4g	2,4-Dichloro-6-Nitro	$C_{16}H_{10}N_6O_4Cl_2$ (421)	447	180-183	57	45.6	45.60	2.3	2.37	19.9	19.95
4h	2,4,6-Tribromo	$C_{16}H_{10}N_5O_2Br_3$ (544)	560	211-213	59	35.2	35.29	1.8	1.83	12.8	12.86

Table 3 Antibacterial Activity of Compounds (4a-h)

Com.	Zone of Inhibition(mm) (Activity Index)^{std}				
	Gram +ve		Gram -ve		
	Bacillus Subtilis	Staphylococcus aureus	Kllebsiella promioe	Salmonella Typhl	E.coil
4a	57 (0.72)	42 (0.76)	49 (0.56)	46 (0.60)	57 (0.79)
4b	52 (0.65)	47 (0.85)	60 (0.68)	57 (0.75)	60 (0.83)
4c	72 (0.91)	48 (0.87)	78 (0.89)	69 (0.90)	65 (0.90)
4d	69 (0.87)	43 (0.78)	74 (0.85)	65 (0.85)	61 (0.84)
4e	56 (0.70)	46 (0.83)	70 (0.80)	45 (0.59)	59 (0.81)
4f	67 (0.84)	44 (0.81)	57 (0.65)	66 (0.86)	58 (0.80)
4g	68 (0.86)	47 (0.85)	74 (0.85)	64 (0.84)	54 (0.75)
4h	71 (0.89)	50 (0.90)	79 (0.90)	68 (0.89)	63 (0.87)
Ampicilin	79	55	87	76	72

(Activity Index) std = Zone of Inhibition of the sample/ Zone of Inhibition of the standard.

Table 4 Antifungal Activity of Compounds (4a-h)

Com.	Zone of Inhibition at 1000 ppm(%)				
	Botrydepladia Thiobromine	Nigrosspora Sp.	Penicillium Expansum	Trichothesium Sp.	Rhizopus Nigricuns
4a	63	72	75	55	50
4b	74	66	63	56	67
4c	66	59	52	75	57
4d	68	67	68	68	64
4e	57	64	55	64	69
4f	75	79	73	78	72
4g	74	63	63	59	54
4h	79	76	74	76	68