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Synthesis and Antimicrobial Studies of Some New 3-Isoxazoline Substituted Phthalazine Methylsulfonyl Oxadiazoles

A.M. SRIDHARA[§], K. R. VENUGOPALA REDDY[*], J. KESHAVAYYA[¶], D.S. AMBIKA[#], SANATHKUMAR GOUD[§] and S.K. PEETHAMBAR

[§]Advinus Therapeutics Ltd, 21 & 22
Peenya II Phase, Bangalore-560058, India
[*]Department of Studies in Industrial Chemistry
Kuvempu University, Shankaragatta -577 451, Karnataka, India
[¶]Department of studies in Chemistry
Kuvempu University, Shankaragatta-577 451, Karnataka, India
[#]Dept. of Plant Pathology,
G.K.V.K, UAS, Bangalore-65, India
Department of Biochemistry, Kuvempu University
Shankaragatta-577 451, Karnataka, India
venurashmi30@rediffmail.com

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Abstract: A series of new 3-isoxazoline substituted phthalazine methylsulfonyl-oxadiazoles were prepared from methyl 2-(4-oxo-3,4-dihydrophthalazin-1-yl) acetate. The structure of synthesized compounds were characterized by spectral data and screened for their antimicrobial activities against various bacteria and fungi strains. Several of these compounds showed antimicrobial activity.

Keywords: Phthalazin-1(2*H*)-one, 1,3,4-Oxadiazole, Isoxazoline, Phthalazine acetic acid

Introduction

Phthalazin-1(2*H*)-ones, methylsulfanyl oxadiazoles and isoxazolines serves as some important building blocks for biological active molecules. Figure 1 has been revealed by these three important biological active pharmacophore component systems.

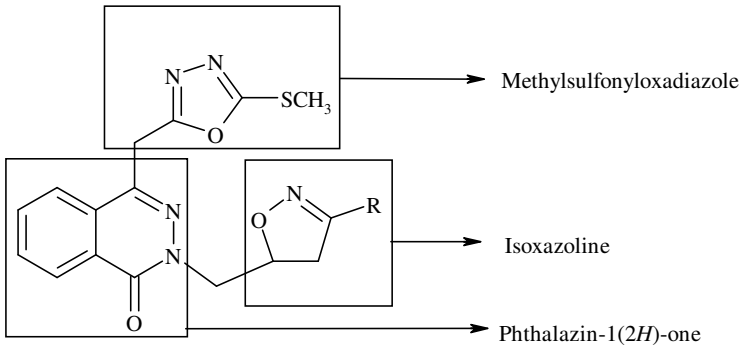


Figure 1. Phthalazine isoxazolines scaffold

Phthalazin-1(2*H*)-one derivatives are of considerable interest due to their antidiabatic[1] antiallergic[2], antiasthmatic[3], antihypertensive[4], vasorelaxant[5], aldose reductase inhibitor[6], and antimicrobial[7] activities. Moreover a number of established drug molecules like hydralazine[8], budralazine[9], azelastine[10], ponalrestat[11] and zopolrestat[12] are accessible starting from the corresponding phthalazinones. The second pharmacophore component is isoxazoline, which represents an important class of heterocyclic compounds with broad spectrum of biological activities. Substituted isoxazolines have revealed anti-influenza virus[13], antifungal[14], antitubercular[15], spermicidal and anti-HIV[16], β-adrenergic receptor antagonist properties[17]. Similarly the third pharmacophore methylsulfanyl oxadiazole unit is also one of important biologically active building block. The substituted methylsulfanyl-oxadiazoles have been found to exhibit diverse biological activities such as inhibitors of glycogen synthase kinase-3β[18], antifungal[19], antibacterial and anti-HIV[20], insecticidal[21], anti-inflammatory and analgesic[22], anticonvulsants and muscle relaxants[23].

The diverse biological activities of these pharmacophores; phthalazin-1(2*H*)-one, methylsulfanyl oxadiazole and isoxazoline pharmacophores, encouraged us to discover a new lead compounds, which contains all these pharmocophores in a single molecules, that may exhibit higher pharmacological activities. By combining these pharmacophore components in a single molecule, to give a compact system, we designed and synthesized a series of phthalazin-1(2*H*)-one derivatives containing methylsulfanyl oxadiazole and isoxazoline moieties. The synthesized new phthalazin-1(2*H*)-one derivatives were characterized by mass, IR and NMR spectral data's.

Experimental

NMR spectra were recorded on 400 MHz Varian-AS NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using Perkin Elmer Spectrum 100 Series FT-IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Buchi melting point B-545 instrument and are uncorrected. All reactions were monitored by thin layer chromatography (TLC) using Merck pre-coated TLC silica gel plates. The crude compounds were purified by using CombiFlash® Companion® flash chromatography system, Teledyne Isco, Inc USA. BPL Sanyo domestic microwave oven was used for reactions.

*Preparation of methyl 2-(3-allyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetate (**2**)*

Allyl bromide (60.5 g, 0.5 mol) was added to a stirred solution of methyl 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetate (**1**)[24] (21.8 g, 0.10 mol) in dimethylformamide (250 mL) and

potassium carbonate (69.0 g, 0.5 mol) at room temperature and heated to 60-65 ^{o}C for 6 h. After completion of reaction, filtered the inorganics, the filtrate obtained was distilled completely under reduced pressure at 60-65 ^{o}C. The residue obtained was diluted with ice water (500 mL) and stirred for 30 minutes. The precipitated product was filtered, dried and recrystallized using isopropyl alcohol to yield compound **2** as white solid. M.p.: 90.2-95.8 ^{o}C; MS: *m/z*=259.1 (M^{+}+1); IR (KBr) ν cm^{-1}: 3012, 1708, 1675, 1599; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.64 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 4.73-4.74 (d, *J*=5.6 Hz, CH_2), 5.08-5.19 (m, 2H, CH_2), 5.94-6.04 (m, 1H, CH_2), 7.87-7.92 (m, 3H, Ar-H), 8.30-8.32 (m, 1H, Ar-H).

*Preparation of 2-(3-allyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetohydrazide (**3**)*

To a solution of phthalazin-1(2*H*)-one acetic acid methyl ester (**2**), (20.0 g, 0.077 mol) in ethanol (300 mL) was added hydrazine hydrate (11.5 g, 0.22 mol). The reaction mixture was heated under reflux for 10 h. Cooled the reaction mixture to room temperature, the precipitate obtained was filtered, dried to yield compound **3** (11.5 g) as white solid. Yield: 74.6%; M.p.: 201.4-207.9 ^{o}C; MS: *m/z*=259.1(M+1); IR (KBr) ν cm^{-1}: 3202, 1758, 1673; ^{1}H NMR (400 MHz, DMSO-d_6): δ ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.75 (s, 2H, CH_2), 4.26-4.27 (d, *J*=4.0 Hz, 2H, CH_2), 4.72-4.73 (d, *J*=5.6 Hz, 2H, NH_2), 5.11-5.19 (m, 2H, CH_2), 5.94-6.04 (m, 1H, CH), 7.85-7.94 (m, 3H, Ar-H), 8.28-8.30 (d, *J*=8.0, 1H, Ar-H), 9.32 (s, 1H, NH).

*Preparation of 2-allyl-4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1 (2H)-one (**4**)*

Compound **3** (11.0 g, 0.042 mol) was added to a solution of KOH (3.8 g, 0.07 mol) in anhydrous ethanol (160 mL). A solution of CS_2 (8.2 g, 0.113 mol) in anhydrous ethanol (40 mL) was added drop wise to the vigorously stirred mixture, which was refluxed for 10 h. After completion of reaction, cooled and evaporated the solvent under reduced pressure. The residue obtained was dissolved in water (200 mL) and acidified to pH 5~6 with hydrochloric acid. The precipitated product was isolated by filtration and drying to yield compound **4** (8.5 g) as white solid. Yield: 66.6%; M.p.: 195.2-199.3 ^{o}C; MS: *m/z*= 301.1 (M^{+}+1); IR (KBr) νcm^{-1}: 2540, 1754, 1672; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.59 (s, 2H, CH_2), 4.70-4.72(d, *J*=5.6 Hz, 2H, CH_2), 5.09-5.19 (m, 2H, CH_2), 5.92-6.02 (m, 1H, CH), 7.88–8.27 (m, 3H, Ar-H), 8.27-8.33 (m, 1H, Ar-H), 14.37 (s, 1H, SH).

*Preparation of 2-allyl-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl) phthalazin-1(2H) - one (**5**)*

The compound **4** (8.0 g, 0.026 mol) was dissolved in 10% potassium hydroxide in water (100 mL). To the clear solution, methyl iodide (4.4 g, 0.03 mol) was added and stirred for 4 h at room temperature. After completion of reaction (checked by TLC), precipitated product was filtered, washed with water, dried to get compound **5** (7.5 g) as white solid. Yield: 88.3%; M.p.: 119.9-125.5 ^{o}C; MS: *m/z*=315.1 (M^{+}+1); IR (KBr) cm^{-1}: 2935, 1711, 1652; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.60 (s, 3H, S-CH_3), 4.69 (s, 2H, CH_2), 4.71-4.72 (d, *J*=5.6 Hz, 2H, CH_2) 5.07-5.18 (m, 2H, CH_2), 5.91-6.00 (m, 1H, CH), 7.89–8.03 (m, 3H, Ar-H), 8.31-8.33 (d, *J*=7.6 Hz, 1H, Ar-H).

*General method for the preparation of 3-substituted phthalazin-1(2H)-one derivatives **6(a-i)***

An equimolar mixture of aldoxime (10.0 mmol) and *N*-chlorosuccinimide (10.0 mmol) were made paste with dimethylformamide (2 mL) and alumina (2 g) in a 10 mL beaker. The mixture was irradiated at 600 W for 2 min in domestic microwave oven. After adding

compound (**5**) (9 mmol), the reaction mixture was irradiated further for 6 min. After completion of reaction, diluted with water (25 mL) and extracted with ethyl acetate (3x50 mL). The organic layer was washed with water (10 mL) and then with saturated sodium chloride solution. After drying with anhydrous sodium sulphate and filtration, the solvent was evaporated to get crude product, which was purified by using flash chromatography eluting with ethyl acetate / hexane (1: 5) to get the corresponding phthalazin-1(2*H*)-ones **6(a-i).**

*2-((3-Methyl-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6a**)*

White solid; Yield: 52.1%; M.p.: 119.3-121.9 ^{o}C; MS: *m/z* =372.1 (M^{+}+1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.92 (s, 3H, C-CH_3), 2.65 (s, 3H, S-CH_3), 3.29-3.45 (m, 2H, CH_2), 4.34-4.39 (dd, *J*=6.8 Hz, 13.4 Hz, 1H, CH_2), 4.45-4.55 (m, 3H, two CH_2), 5.22-5.26 (m, 1H, oxazole-CH), 7.61-7.91 (m, 3H, Ar-H), 8.29-8.31 (m, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 163.7, 159.7, 156.1, 139.9, 141.9, 140.0, 134.35, 132.9, 132.7, 128.9, 127.6, 127.0, 126.0, 124.7, 50.9, 29.1, 10.68; IR (KBr) cm^{-1}: 2937, 1712, 1658, 1514.

*4-((5-(Methylthio)-1,3,4-oxadiazol-2-yl)methyl)-2-((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)phthalazin-1(2H)-one (**6b**)*

Yield: 65.6%; M.p.: 144.6-148.3 ^{o}C; MS: *m/z* =434.1 (M^{+}+1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.65 (s, 3H, S-CH_3), 3.29-3.45 (m, 2H, CH_2), 4.34-4.39 (dd, *J*=6.8, 13.4 Hz, 1H, CH_2), 4.45-4.55 (m, 3H, two CH_2), 5.22-5.26 (m, 1H, oxazole-CH), 7.38-7.39 (m, 3H, Ar-H), 7.67-7.87 (m, 5H, Ar-H), 8.45-8.47 (d, *J*=7.2 Hz, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.1, 163.9, 159.5, 156.5, 139.9, 133.6, 132.0, 130.1, 129.2, 128.6, 128.5, 127.5, 126.8, 124.3, 78.1, 77.4, 77.0, 76.7, 53.5, 38.4, 29.7, 14.4; IR (KBr) cm^{-1}: 2998, 1673, 1532.

*2-((3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6c**)*

White solid; Yield: 53.2%; M.p.: 122.3-127.9 ^{o}C; MS: *m/z* = 468.1 (M^{+}+1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.67 (s, 3H, S-CH_3), 3.28-3.43 (m, 2H, CH_2), 4.32-4.37 (dd, *J*=6.8, 13.4 Hz, 1H, CH_2), 4.48-4.58 (m, 3H, two CH_2), 5.23-5.27 (m, 1H, oxazole-CH), 7.34-7.36 (d, *J*=8.0 Hz, 2H, Ar-H), 7.76-7.88 (m, 3H, Ar-H), 8.43-8.45 (d, *J*=7.6 Hz, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.0, 163.9, 159.4, 155.6, 140.0, 136.0, 133.7, 132.0, 129.1, 128.6, 128.0, 127.7, 127.4, 124.3, 78.4, 77.4, 77.1, 76.8, 53.3, 38.2, 29.6, 14.4; IR (KBr) cm^{-1}: 2989, 1681, 1542.

*2-((3-(4-Fluorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6d**)*

Yield: 53.8%; White solid; M.p.: 130.2-133-8 ^{o}C; MS: *m/z* = 452.1 (M^{+}+1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.67 (s, 3H, S-CH_3), 3.28-3.43 (m, 2H, CH_2), 4.32-4.37 (dd, *J*=6.8, 13.4 Hz, 1H, CH_2), 4.48-4.58 (m, 3H, two CH_2), 5.23-5.27 (m, 1H, oxazole-CH), 7.34-7.36 (d, *J*=8.0 Hz, 2H, Ar-H), 7.56-7.62 (d, *J*=8.0 Hz, 2H, Ar-H), 7.76-7.88 (m, 3H, Ar-H), 8.43-8.45 (d, *J*=7.6 Hz, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.0, 163.9, 159.4, 155.6, 140.0, 136.0, 1133.7, 132.0, 129.1, 128.5, 128.0, 127.7, 127.4, 124.3, 78.4, 77.4, 77.1, 76.8, 53.3, 38.2, 29.6, 14.4; IR (KBr) cm^{-1}: 2989, 1752, 1681, 1542.

*2-((3-(4-Methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6e**)*

Yield: 59.3%; White solid; M.p.: 151.6-155.8 ^{o}C; MS: *m/z*= 464.1 (M^{+}+1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.65 (s, 3H, S-CH_3), 3.27-3.42 (m, 2H, CH_2), 3.82 (s, 3H, -OCH_3),

4.30-4.35 (dd, *J*= 6.4 Hz, 1H, CH_2), 4.46-4.52 (m, 3H, two CH_2), 5.18-5.21 (m, 1H, oxazole-CH), 6.88 (dd, *J*=8.8 Hz, 2H, Ar-H), 7.58-7.60 (d, *J*=8.8 Hz, 2H, Ar-H), 7.75-7.87 (m, 3H, Ar-H), 8.42 (d, *J*=7.6 Hz, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.0, 163.9, 161.0, 159.4, 156.1, 139.9, 133.6, 131.9, 129.3, 128.2, 127.3, 124.3, 122.3, 114.0, 77.5, 76.9, 55.3, 53.4, 38.6, 36.3, 29.6, 14.4; IR (KBr) cm^{-1}: 2988, 1753, 1657, 1545.

*2-((3-(3,4-Dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6f**)*

Yield: 45.0%; White solid; M.p.: 146.0-149.8 °C; MS *m/z* = 494.1 (M^++1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.68 (s, 3H, S-CH_3), 3.25-3.45 (m, 1H, CH_2), 3.74 (m, 1H, CH_2), 3.91 (s, 6H, two -OCH_3), 4.37-4.48 (dd, *J*= 6.4 Hz, 1H, CH_2), 4.52-4.57 (m, 3H, two CH_2), 5.18-5.24 (m, 1H, oxazole-CH), 6.93-6.95 (dd, *J*=8.0 Hz, 2H, Ar-H), 7.05-7.14 (d, *J*=8.8 Hz, 2H, Ar-H), 7.38-7.40 (m, 1H, Ar-H), 7.57-7.7.99 (m, 2H, Ar-H), 8.47-8.49 (d, *J*=7.2 Hz, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.0, 163.9, 161.0, 159.4, 156.1, 139.9, 133.6, 131.9, 129.3, 128.4, 128.2, 127.3, 124.3, 122.3, 121.6, 114.0, 77.7, 77.5, 77.2, 76.9, 55.3, 53.4, 38.6, 36.3, 29.6, 14.4; IR (KBr) cm^{-1}: 2979, 17650, 1673, 1545.

*2-((3-(3-(4-Fluorophenoxy)phenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(5-methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6g**)*

Yield: 52.8%; White solid; M.p.: 171.1-174.9 °C; MS: *m/z* = 544.1 (M^++1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.66 (s, 3H, S-CH_3), 3.24-3.40 (m, 2H, CH_2), 4.34-4.39 (dd, *J*=6.8, 13.4 Hz, 1H, CH_2), 4.45-4.56 (m, 3H, CH_2), 5.20-5.34 (m, 1H, oxazole-CH), 6.98-7.00 (d, *J*=7.6 Hz, 2H, Ar-H), 7.09-7.11 (t, *J*=7.6, 7.2 Hz, 1H, Ar-H), 7.21-7.23 (m, 1H, Ar-H), 7.33-7.45 (m, 4H, Ar-H), 7.78-7.88 (m, 3H, Ar-H), 8.45-8.47 (m, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.1, 163.9, 159.5, 156.7, 155.2, 153.9, 144.2, 139.9, 133.7, 132.0, 129.8, 128.5, 127.8, 127.5, 126.4, 123.6, 123.3, 119.7, 117.6, 117.2, 78.4, 77.4, 77.1, 76.8, 53.4, 38.3, 29.6, 14.1; IR (KBr) cm^{-1}: 2979, 1775, 1673, 1544.

*4-((5-(Methylthio)-1,3,4-oxadiazol-2-yl)methyl)-2-((3-(thiophen-2-yl)-4,5-dihydro isoxazol-5-yl)methyl)phthalazin-1(2H)-one (**6h**)*

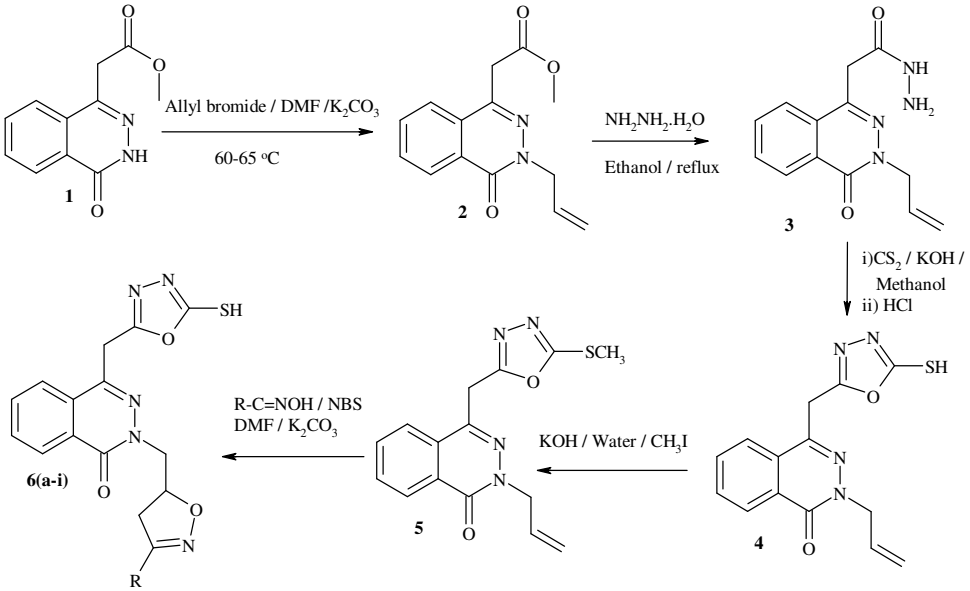
Yield: 70.2%; Off-white solid; M.p.: 139.5-143.1 °C; MS: *m/z* = 440.1 (M^++1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.091.23 (m, 1H, CH_2), 2.66 (s, 3H, S-CH_3), 3.51-3.58 (dd, *J*=10.4, 16.8 Hz, 1H, CH_2), 4.05-4.13 (m, 1H, CH_2), 4.41-4.54 (m, 1H, CH_2), 4.71-4.87 (m, 2H, CH_2), 5.08-5.12 (m, 1H, Oxazole CH), 7.17-7.22 (m, 1H, Ar-H), 7.38-7.39 (d, *J*=3.6 Hz, 1H, Ar-H), 7.70-7.71 (d, *J*=4.8 Hz, 1H, Ar-H), 7.85-8.15 (m, 3H, Ar-H), 8.32-8.34 (d, *J*=7.6 Hz, 1H, Ar-H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 159.4, 155.6, 140.0, 136.0, 133.7, 132.0, 129.1, 128.9, 128.6, 128.4, 127.7, 127.7, 124.3, 77.4, 77.1, 76.8, 53.4, 38.3, 29.6, 14.1; IR (KBr) cm^{-1}: 2989, 1760, 1681, 1542.

*2-((3-(2-Hydroxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one (**6i**)*

Yield: 45.6%; White solid; M.p.: 169.2-172.9 °C; MS: *m/z* = 450.1 (M^++1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.66 (s, 3H, S-CH_3), 3.41-3.48 (m, 1H, CH_2), 3.60-3.67 (dd, *J*=10.4, 17.2 Hz, 1H, CH_2),4.16-4.20 (dd, *J*=5.6, 13.6 Hz, 1H, CH_2), 4.39-4.44 (q, *J*=7.6, 13.4 Hz, 1H, CH_2), 4.66-4.75 (m, 2H, CH_2), 5.07-5.11 (m, 1H, oxazole-CH), 6.90-6.97 (q, *J*=7.2, 8.4, 17.8 Hz, 2H, Ar-H). 7.31-7.35 (t, *J*=7.2, 7.6 Hz, 1H, Ar-H), 7.42-7.44 (d, *J*=7.6 Hz, 1H, Ar-H), 7.90-8.05 (m, 3H, Ar-H), 8.32-8.34 (d, *J*=8.0 Hz, 1H, Ar-H), 9.84 (s, 1H, OH); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.1, 163.9, 159.5, 156.5, 139.9, 133.6, 132.0, 130.1, 129.2, 128.6, 128.5, 127.5, 126.8, 124.3, 78.1, 77.4, 77.0, 76.7, 53.5, 38.4, 29.7, 14.4; IR (KBr) cm^{-1}: 3326, 2975, 1675.

Results and Discussion

The key intermediate in the synthesis of new phthalazine-1(2*H*)-one isoxazolines **6(a-i)** is methyl 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetate **(1)**, which was prepared from phthalic anhydride in good yield[24].



R = a) Methyl, b) Phenyl, C) 4-Chlorophenyl, d) 4-Fluorophenyl, e) 4-methoxyphenyl, f) 3-4-Dimethoxyphenyl, g) 4-Fluoro(3-phenoxy)phenyl, h) Thiophene-2yl, i) 2-hydroxyphenyl

Scheme 1. Synthesis of phthalazine isoxazoline derivatives **6(a-i)**

N-allylation of compound (**1**) with allyl bromide in presence of potassium carbonate gave methyl 2-(3-allyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetate (**2**). The compound **2** on reaction with hydrazine hydrate afford hydrazide **3.** Oxadiazolinethione (**4**) was prepared from reaction of the hydrazide **3** with carbon disulphide and potassium hydroxide, in ethanol, followed by acidification. On treatment with methyl iodide, the compound (**4**) gave 2-allyl-4-(5-mercapto-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2*H*)-one (**5**). Allyl function of compound (**5**) undergoes 1,3 dipolar cyclization[25] with aldoximes[26] via nitrile oxide in presence of *N*-chlorosuccinimide, potassium carbonate and dimethylformamide gave desired compounds **6(a-i)**. The synthesised new compounds were characterised by mass, IR, NMR and elemental analysis studies.

Biological screening

The *in vitro* antimicrobial activity was carried out against 24 h old cultures of two bacteria and two fungi by cup-plate method in duplicate. Compounds **(6a-i)** has been tested for their antimicrobial activity against *E. coli* and *S. aureus* and antifungal activity against *M. gypsum* and *A. flavus* at a concentration of 100 µg/mL in DMSO using cup plate diffusion method. Nutrient agar and potato dextrose agars were used to culture the bacteria and fungus respectively. The solution of Amoxicillin 100 µg/mL and metronidazole at 100 µg/mL were prepared in DMSO and used as standard for comparison of antibacterial and antifungal activities respectively the results were discussed in Table 1**.**

Table 1. Antimicrobial activity of compounds **6(a-i)**

Synthesized compounds	Zone of Inhibition measured in mm			
	Antibacterial activity		Antifungal activity	
	Escherichia Coli ± S.D	*Staphylococcus Aureus* ± S.D	*Microsporum Gypsum* ± S.D	*Aspergillus flavus*[d] ± S.D
	100 µg/mL	100 µg/mL	100 µg/mL	100 µg/mL
6a	2.50 ± 0.70	4.50 ± 0.70	4.50 ± 0.70	4.50 ± 0.70
6b	6.50 ± 0.70	8.5 ± 0.70	3.50 ± 0.70	11.50 ± 0.70
6c	5.00 ± 0.00	7.5 ± 0.70	4.50 ± 0.70	7.50 ± 0.70
6d	3.00 ± 0.00	4.50 ± 0.70	5.50 ± 0.70	5.50 ± 0.70
6e	3.50 ± 0.70	3.00 ± 0.00	3.00 ± 0.00	5.00 ± 0.00
6f	7.50 ± 0.70	8.00 ± 0.00	2.50 ± 0.70	7.50 ± 0.70
6g	8.50 ± 0.70	11.50 ± 0.70	7.00 ± 0.00	11.00 ± 0.00
6h	6.00 ± 0.70	7.50 ± 0.70	4.50 ±0.70	4.50 ± 0.70
6i	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	5.00 ± 0.00
Metronidazole	-	-	17.50 ± 0.70	17.50 ± 0.70
Amoxicillin	9.00 ± 0.00	15.50 ± 0.70	-	-

*The compounds **6b** and **6f-g** exhibiting good activity against E. coli and compounds **6b, 6c, 6f** and **6g** show good activity of against S. Aureus. Similarly the compounds **7g** showed good activity against M.gypsum and the compounds **6b** and **6g** showed activity against A. and all remaining compounds exhibiting moderate activity against all the four organisms used for screening*

Conclusion

In this article we report the synthesis of (**6a-i**), new phthalazine isoxazolines substituted at C_4 position with methylsulfonyl oxadiazole starting from commercially available phthalic anhydride. Investigation of their antimicrobial activity revealed that isoxazolines with substitution with thiophene (**6h**) shows the most active compound although it was significantly less than that of positive control. The fact that the compounds prepared in this study are chemically unrelated to the current medication suggests that the further work is clearly warranted.

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