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Der Pharma Chemica, 2010, 2(5):201-211
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Synthesis and antimicrobial activity of phthalazine substituted 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles and 7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

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ABSTRACT

A series of 3,6-disubstituted 1,2,4–triazolo[3,4-b]-1,3,4-thiadiazoles and 7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine were synthesized from methyl {3-[(6-chloropyridin-3-yl)methyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetate in multiple steps. Most of the newly synthesized compounds were screened for their antimicrobial activity against variety of human pathogenic bacteria's. Many of the compounds were found to be potent as antimicrobials.

Keywords: Phthalazine acetic acid, 2-chloro-5-(chloromethyl)pyridine, triazolo-thiadiazole, triazolo-thiadiazine

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INTRODUCTION

Nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals. In recent years, the chemistry of 1,3,4-triazoles, 1,3,4-thiadiazoles, and 1,3,4-thiadiazines have received considerable attention owing to their synthetic and effective biological importance. The fused ring of thiadiazole and thiadiazine with triazole called [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (triazolo-thiadiazole) and 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (triazolo-thiadiazine) [1] reported to show a broad spectrum of pharmacological properties like antifungal [2], antibacterial [3], antiviral [4], anthelmentic [5], antitumor [6], anti-inflammatory

[7], antitubercular [8], diuretics [9], anticancer [10] and hypoglycaemic agents [11]. Phthalazin-1(2H)-ones are also one of the important biological active pharmacophore component in medicinal chemistry, which are of considerable interest due to their antidiabetic [12], antiallergic [13], Vasorelaxant [14], PDE4 inhibitors [15], VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer [16], antiasthmatic agents [17], herbicidal [18], like activities. A number of established drug molecules like Hydralazine [19], Budralazine [20], Azelastine [21], Ponalrestat [22] and Zopolrestat [23] are prepared from the corresponding phthalazinones. The diverse biological activities of phthalazin-1(2*H*)-one, triazolothiadiazole and triazolo-thiadiazine pharmacophores encouraged us for the construction of new molecular systems has biological active molecules. In finding new antimicrobials we designed new phthalazin-1(2H) one derivatives substituted with (6-chloropyridin-3-yl)methyl at 2nd position and triazolo-thiadiazole or triazolo-thiadiazine at 4th position. (6-chloropyridin-3-yl)methyl [24] is one of important biological active moiety explored industrially and commercially available due to its biological importance. The Figure 1 is revealed has the frame work of these important biological active pharmacophore component systems.

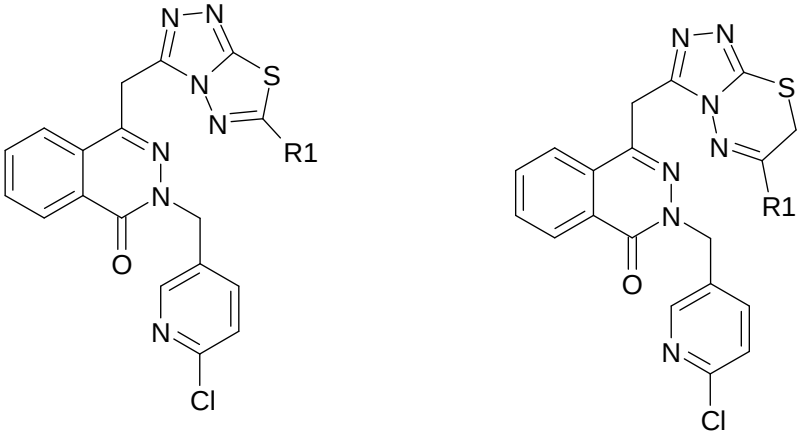


Figure 1. Phthalazin-1(2*H*)-one triazolo-thiadiazole and triazolo-thiadiazine scaffold

The synthesized new phthalazin-1(2*H*)-one derivatives were characterized by mass, IR, and NMR spectral data's and studies for their antimicrobial activity against different human pathogenic bacteria's.

MATERIALS AND METHODS

All chemicals used for the synthesis were of reagent grade and the intermediates prepared as per known literature procedures. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz Varian-AS NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using PerkinElmer 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 the reactions were monitored by thin layer chromatography (TLC) using precoated silica 60 F_{254}, 0.25 mm aluminum plates (Merck). The crude compounds were purified by using CombiFlash® Companion® flash chromatography system, Teledyne Isco, Inc USA using hexane and ethyl acetate as mobile phase and silica gel column.

Preparation of methyl {3-[(6-chloropyridin-3-yl)methyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetate (2). A mixture of methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate [25] (**1**) (21.8 gm, 0.10 mol), dimethylformamide (250 mL), potassium carbonate (41.4 gm, 0.3 mol) and 2-chloro-5-(chloromethyl)pyridine (17.8 gm, 0.11 mol) was heated to 60-65 ^{o}C for 6 h. After completion of reaction, filtered the inorganics, and distilled the solvent completely under reduced pressure at 60-65 ^{o}C. The residue obtained was diluted with ice water (250 mL) and stirred for 30 minutes. The precipitated product was filtered, dried and recrystallised using isopropyl alcohol to yield (23.40 gm, 0.067 mol) compound **2** as white solid. Yield: 67.3 %; Mp= 178.2-183.5 ^{o}C; MS: m/z=344.1 (M+1); ^{1}H NMR (400 MHz , DMSO-d_6) δ: 3.64 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 7.48 (d, *J*=8.0 Hz, 1H, Ar-H), 7.79 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.80-7.99 (m, 3H, Ar-H), 8.30-8.32 (m, 1H, Ar-H), 8.42 (d, *J*=2.4 Hz, 1H, Ar-H).

Preparation of 2-{3-[(6-chloropyridin-3-yl) methyl]-4-oxo-3, 4-dihydrophthalazin-1-yl} aceto hydrazide (3). A mixture of ester **2** (20.0 gm, 0.058 mol) and hydrazine hydrate (10 gm, 0.20 mol) in ethanol (400 mL) were heated under reflux for 10 h. After completion of reaction, cooled to room temperature, the precipitated product was filtered and washed with cold ethanol to give the hydrazide **3** as white solid (18 gm). Yield: 89.6 %; Mp=206.2-210.0 ^{o}C; MS: m/z=344.1 (M+1); ^{1}H NMR: (400 MHz , DMSO-d_6) δ: 3.79 (s, 2H, CH_2), 4.27 (brs, 2H, NH_2), 5.34 (s, 2H, CH_2), 7.48-7.50 (d, *J*=8.4 Hz, 1H, Ar-H), 7.80-7.83 (dd, *J*=8.4, 2.4 Hz, 1H, Ar-H), 7.86-7.98 (m, 3H, Ar-H), 8.28-8.30 (m, 1H, Ar-H), 8.43-8.44 (d, *J*=2.4 Hz, 1H, Ar-H), 9.35 (brs, 1H, NH).

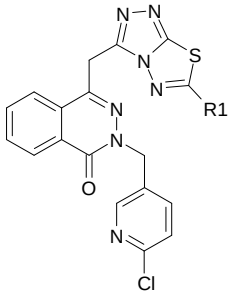
Preparation of 2-[(6-chloropyridin-3-yl)methyl]-4-[(5-sulfanyl-1,3,4-oxadiazol-2-yl) methyl] phthalazin-1(2H)-one (4). A solution of potassium hydroxide (8.2 gm, 0.113 mol) in alcohol (100 mL) was cooled in an ice bath and the hydrazide (**3**) (18.0 gm, 0.052 mol) was added with stirring. Then carbon disulphide (8.2 gm, 0.113 mol) was added in small portion with constant stirring. The reaction mixture 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 the compound (**4**) **(**15.4 gm) as white solid. Yield=77.0 %; m.p=195.2-199.3 ^{o}C; MS: m/z= 386.1 (M+1); IR (KBr) cm^{-1}: 2540, 1754, 1672; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.62 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 7.48-7.50 (d, J=8.4 Hz, 1H, Ar-H), 7.79-7.80 (d, J=2.0 Hz, 1H, Ar-H), 7.81-8.04 (m, 3H, Ar-H), 8.32-8.34 (d, J=8.4 Hz, 1H, Ar-H), 8.42 (d, J=2.0 Hz, 1H, Ar-H), 14.50 (brs, 1H, SH).

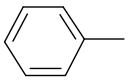
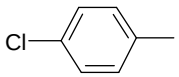
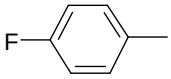
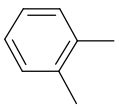
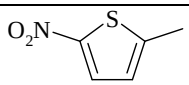
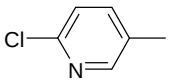
Preparation of 4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-2-[(6-chloropyridine-3-yl) methyl] phthalazin-1(2H)-one (5). Hydrazine hydrate (3.8 gm, 0,076 mol) was added to a suspension of amino thiol 4 (15.0 gm, 0.038 mol) in ethanol (100 ml) and refluxed for 8 hr. After completion of reaction, cooled and filtered the precipitated solid to get the titled compound 5 as white solid (10.0 gm). Yield=65.7 %; m.p=119.9-125.5 ^{o}C; MS: m/z= 400.0 (M+1); IR (KBr) cm^{-1}: 2935, 1711, 1652; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.23 (brs, S, -NH_2), 4.69 (s, 2H, CH_2), 5.38 (s, 2H, CH_2), 7.48-7.50 (d, J=8.4 Hz, 1H, Ar-H), 7.79-7.80 (d, J=2.0 Hz, 1H, Ar-H), 7.81-8.04 (m, 3H, Ar-H), 8.32-8.34 (d, J=8.4 Hz, 1H, Ar-H), 8.42 (d, J=2.0 Hz, 1H, Ar-H), 14.50 (brs, 1H, SH).

General method for the synthesis of 2-[(6-chloropyridin-3-yl)methyl]phthalazin-1(2H)-one-6-substituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (6a-f).

A mixture of amino thiol (**5**) (400 mg, 1.0 mmol) and aromatic acid (1.5 mmol) in $POCl_3$ (20 mL) was refluxed for 10 hr. The reaction mixture was gradually poured in to crushed ice with stirring. The solution was neutralized with solid K_2CO_3, till to pH~8. The solid which separated was filtered, washed with cold water, dried to get crude compounds, which were purified by using CombiFlash® Companion® flash chromatography system using hexane and ethyl acetate as mobile phase and silica gel as stationary phase to get pure compounds. The physical properties of the compounds were reported in table 1.

Table 1. Physical characteristics of the synthesized compounds 6(a-f)



Entry	R1	Yield %	MP ^{0}C	Physical state	Mass (M+1)
6a		53.2	189.5-191.8	Off-white solid	486.1
6b		49.0	205.0-207.2	Brown solid	520.6
6c		48.8	199.3-201.0	Off-white solid	504.0
6d		55.6	172.0-173.5	Off-white solid	516.1
6e		45.4	167.2-169.7	Brown solid	537.1
6f		50.8	177.6-179.8	Off-white solid	521.0

2-((6-chloropyridin-3-yl)methyl)-4-((6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl) phthalazin-1(2H)-one (6a). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.61 (s, 2H, -C-CH_2-), 5.35 (s, 2H, -N-CH_2-C-), 7.17-7.19 (d, *J*=8.0 Hz, 1H, Ar-H), 7.49-7.56 (m, 3H, Ar-H), 7.73-7.74 (d, J=2.4 Hz, 1H, Ar-H), 7.76-7.78 (d, J=8.0 Hz, 1H, Ar-H), 7.79-7.98 (m, 4H, Ar-H), 8.45-8.48 (m, 1H, Ar-H), 8.52-8.53 (d, J=2.4 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.8,

163.3, 158.4, 151.5, 150.2, 142.9, 141.2, 135.3, 132.9, 132.2, 128.5, 128.0, 126.8, 125.2, 124.9, 124.2, 50.8, 29.2; IR (KBr) cm^{-1}: 2830, 1719, 1652, 1599.

4-((6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)-2-((6-chloropyridin-3-yl)methyl)phthalazin-1(2H)-one (6b). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.61 (s, 2H, -C-CH2-C-), 5.35 (s, 2H, -N-CH2-C-), 7.49-7.50 (d, J = 8.0 Hz, 2H, Ar-H), 7.74-7.75 (d, J = 2.4 Hz, 1H, Ar-H), 7.74-7.77 (d, J = 8.0 Hz, 1H, Ar-H), 7.79-7.82 (m, 2H, Ar-H), 7.83-7.93 (m, 3H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51 (d, J=2.4 Hz 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.2, 161.8, 158.7, 149.8, 141.9, 139.9, 134.3, 132.9, 132.6, 129.5, 129.0, 128.9, 127.6, 126.0, 124.8, 50.8, 29.2, 17.0, 11.5; IR (KBr) cm^{-1}: 2923, 1718, 1656, 1587.

4-((6-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)-2-((6-chloropyridin-3-yl)methyl)phthalazin-1(2H)-one (6c). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.82 (s, 2H, -C-CH2-C-), 5.27 (s, 2H, -N-CH2-C-), 7.14-7.24 (m, 2H, Ar-H), 7.25-7.26 (d, J=8.0 Hz, 1H, Ar-H), 7.74-7.76 (dd, J=8.0, 2.4 Hz, 1H, Ar-H), 7.77-7.83 (m, 2H, Ar-H), 7.85-7.99 (m, 3H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51-8.52 (d, J=2.4 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 164.7, 163.7, 150.7, 150.1, 149.9, 141.9, 140.0, 134.2, 134.3, 132.9, 132.7, 128.9, 127.6, 126.7, 124.7, 123.2, 122.7, 49.8, 29.2; IR (KBr) cm^{-1}: 2930, 1719, 1657, 1591.

2-((6-chloropyridin-3-yl)methyl)-4-((6-(2-methylphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thidiazol-3-yl)methyl)phthalazin-1(2H)-one (6d). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 4.62 (s, 2H, -C-CH_2-C-), 5.35 (s, 2H, -N-CH_2-C-), 7.17-7.19 (d, *J*=8.0 Hz, 1H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 7.40-7.43 (m, 1H, Ar-H), 7.73-7.75 (dd, *J*=8.0, 1.6 Hz, 1H, Ar-H), 7.79-7.95 (m, 4H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51-8.52 (d, *J*=1.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.8, 164.1, 163.6, 150.6, 150.0, 149.2, 141.1, 139.1, 133.2, 134.2, 132.8, 131.7, 128.9, 127.2, 126.8, 126.6, 125.8, 125.0, 124.8, 123.1, 122.9, 50.1, 29.1, 22.2.

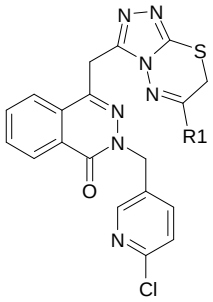
2-[(6-chloropyridin-3-yl)methyl]-4-{[6-(5-nitrothiophen-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}phthalazin-1(2H)-one (6e). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.62 (s, 2H, -C-CH2-C-), 5.34 (s, 2H, -N-CH2-C-), 7.23-7.24 (m, 1H, Ar-H), 7.60-7.61 (m, 1H, Ar-H), 7.75-7.77 (m, 1H, Ar-H), 7.78-7.81 (m, 1H, Ar-H), 7.82-7.99 (m, 3H, Ar-H), 8.46-8.49 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.1, 161.8, 158.7, 149.9, 141.8, 139.9, 134.4, 132.9, 132.5, 129.5, 129.0, 128.8, 127.6, 127.1, 126.0, 125.8, 124.7, 124.6, 123.7, 50.8, 29.1; IR (KBr) cm^{-1}: 2924, 1790, 1653, 1585;

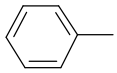
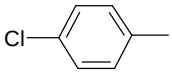
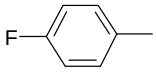
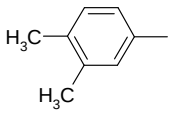
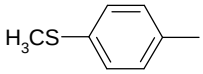
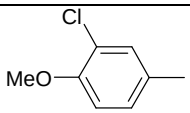
4-((6-(6-chloropyridin-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)-2-((6-chloropyridin-3-yl)methyl)phthalazin-1(2H)-one (6f). 4.63 (s, 2H, -C-CH2-C-), 5.34 (s, 2H, -N-CH2-C-), 7.22-7.24 (d, *J*=8.0 Hz, 1H, Ar-H), 7.51-7.53 (d, *J*= 8.4 Hz, 1H, Ar-H), 7.74-7.76 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.80-7.92 (m, 3H, Ar-H), 8.20-8.23 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 8.47-8.49 (d, *J*=2.4 Hz, 2H, Ar-H), 8.99-9.00 (d, *J*=2.4 Hz, 1H, Ar-H); 13C NMR (100 MHz, CDCl3) δ: 164.9, 164.2, 163.5, 152.1, 150.6, 149.4, 141.2, 139.8, 133.1, 134.1, 132.2, 131.6, 128.0, 127.1, 126.3, 124.7, 123.1, 122.6, 50.1, 29.1.

General method for the synthesis of 2-((6-chloropyridin-3-yl)methyl)-4-((6-substituted-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)phthalaz in-1(2H)-one (7a-f).

A mixture of compound **5** (400 mg, 1.0 mmol), different acyl bromides (1.1 mmol) and anhydrous sodium acetate (400 mg) in anhydrous ethanol (10 ml) was refluxed for 8 hr. After completion of reaction, the solvent was evaporated under reduced pressure. The residue was diluted with water and the precipitated solid was filtered and dried to get the crude compounds. The compounds were purified by using CombiFlash® Companion® flash chromatography system using hexane and ethyl acetate as mobile phase and silica gel as stationary phase to get pure compounds **7(a-f). The** physical constants of the synthesised compounds were reported in table 2.

Table 2. Physical characteristics of the synthesized compounds 7(a-f)



Entry	R1	Yield %	Melting point	Physical state	Mass (M+1)
7a		36.9	201-203 ^{o}C	White solid	500.1
7b		39.2	192-193 ^{o}C	Off-white solid	534.1
7c		30.0	180-183 ^{o}C	Off-white solid	518.1
7d		40.5	_	Semi solid	528.1
7e		42.3	174-175 ^{o}C	Brown solid	546.1
7f		39.0	210-211 ^{o}C	White sold	564.1

2-((6-chloropyridin-3-yl)methyl)-4-((6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)methyl)phthalazin-1(2H)-one (7a). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.32-4.45 (m, 4H, CH_2 and two protons of triazolothiadiazine), 5.36 (s, 2H, -N-CH_2-C), 7.17-7.19 (d, *J*=8.0 Hz, 1H, Ar-H), 7.49-7.56 (m, 3H, Ar-H), 7.73-7.74 (d, J=2.4 Hz, 1H, Ar-H), 7.76-7.78 (d, J=8.0 Hz, 1H, Ar-H), 7.79-7.98 (m, 4H, Ar-H), 8.45-8.48 (m, 1H, Ar-H), 8.52-8.53 (d, J=2.4 Hz, 1H, Ar-H).

4-((6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)-2-((6-chloro pyridin-3-yl)methyl)phthalazin-1(2H)-one (7b). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4-4.41-4.62 (m, 4H, CH_2 and two protons of triazolothiadiazine), 5.35 (s, 2H, -N-CH_2-C-), 7.19-7.25 (m, 2H, Ar-H), 7.24-7.26 (d, *J*=8.0 Hz, 1H, Ar-H), 7.74-7.76 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.77-7.83 (m, 2H, Ar-H), 7.85-7.99 (m, 3H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51-8.52 (d, *J*=2.4 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 164.7, 163.7, 150.7, 150.1, 149.9, 141.9, 140.0, 134.2, 134.3, 132.9, 132.7, 128.9, 127.6, 126.7, 124.7, 123.2, 122.7, 49.8, 29.2; IR (KBr) cm^{-1}: 2930, 1719, 1657, 1591.

2-((6-chloropyridin-3-yl)methyl)-4-((6-(4-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin -3-yl)methyl)phthalazin-1(2H)-one (7c). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.32-4.43 (m, 4H, and two protons of triazolothiadiazine), 5.37 (s, 2H, -N-CH_2-C-), 7.19-7.21 (d, *J*=8.0 Hz, 1H, Ar-H), 7.49-7.51 (d, *J*=8.1 Hz, 2H, Ar-H), 7.74-7.77 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.79-7.83 (d, *J*=8.1 Hz, 2H, Ar-H), 7.85-7.93 (m, 3H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51-8.52 (d, *J*=2.4 Hz 1H, Ar-H); IR (KBr) cm^{-1}: 2928, 1718, 1658, 1592.

2-((6-chloropyridin-3-yl)methyl)-4-((6-(3,4-dimethylphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)methyl)phthalazin-1(2H)-one (7d). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.62 (s, 6H, two CH_3), 4.37-4.42 (m, 4H, CH_2 and two protons of triazolothiadiazine), 5.35 (s, 2H, -N-CH_2-C-), 7.20-7.21 (d, *J*=8.0 Hz, 1H, Ar-H), 7.33-7.36 (m, 1H, Ar-H), 7.41-7.44 (m, 1H, Ar-H), 7.63-7.65 (dd, *J*=8.0, 1.6 Hz, 1H, Ar-H), 7.79-7.95 (m, 4H, Ar-H), 8.46-8.48 (m, 1H, Ar-H), 8.51-8.52 (d, *J*=1.6 Hz, 1H, Ar-H).

2-((6-chloropyridin-3-yl)methyl)-4-((6-(4-(methylthio)phenyl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thia diazin-3-yl)methyl)phthalazin-1(2H)-one (7e). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.63 (s, 3H, SCH_3), 4.42-4.52 (m, 4H, CH_2 and two protons of triazolothiadiazine), 5.34 (s, 2H, -N-CH_2-C-), 7.22-7.24 (d, *J*=8.0 Hz, 1H, Ar-H), 7.51-7.53 (d, *J*= 8.4 Hz, 1H, Ar-H), 7.74-7.76 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.80-7.92 (m, 3H, Ar-H), 8.20-8.23 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 8.47-8.48 (d, *J*=2.4 Hz, 2H, Ar-H), 8.49 (m, 1H, Ar-H) 8.99-9.00 (d, *J*=2.4 Hz, 1H, Ar-H).

2-((6-chloropyridin-3-yl)methyl)-4-((6-(3-fluoro-4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazin-3-yl)methyl)phthalazin-1(2H)-one (7f). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.86 (s, 3H, OCH_3), 4.51-4.63 (m, 4H, CH_2 and two protons of triazolo thiadiazine), 5.34 (s, 2H, -N-CH_2-C-), 7.22-7.24 (d, *J*=8.0 Hz, 1H, Ar-H), 7.51-7.53 (d, *J*= 8.4 Hz, 1H, Ar-H), 7.74-7.76 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 7.80-7.92 (m, 3H, Ar-H), 8.20-8.23 (dd, *J*=8.0, 2.4 Hz, 1H, Ar-H), 8.47-8.48 (d, *J*=2.4 Hz, 1H, Ar-H), 8.49 (m, 1H, Ar-H) 8.99-9.00 (d, *J*=2.4 Hz, 1H, Ar-H).

Pharmacological Activity

The antimicrobial activity of newly synthesized compounds **6a-f** and **7a-f** were was determined by well plate method in nutrient agar (antibacterial activity) and Sabouraud dextrose agar (antifungal activity). The *in vitro* antimicrobial activity was carried out against 24 h old cultures of bacterial and 72 h old cultures of fungal strain. In this work, Escherichia coli (ATCC-10536), Salmonella typhi (ATCC-14028), Bacillus subtilis (ATCC-6633), Staphylococcus aureus (ATCC-29737) were used to investigate the antibacterial activities and Aspergillums niger

(ATCC-16404), Candida albicans (ATCC-10231) were used to investigate the antifungal activities.

The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentration of 200, 100, 50, 25, 12.5 and 6.25 mg/mL. MIC readings were performed twice for each chemical agent. In order to ensure that the solvent perse had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium. Approximately 1 cm3 of a 24 h broth culture was placed in sterile Petri dishes. Molten nutrient agar kept at 45 °C was then poured into the Petri dishes and allowed to solidify. Six millimeter diameter holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. The inhibition zone if appeared after 24 h, around the holes in each plate was measured as zone of inhibition in mm. MIC was defined as the lowest concentration of inhibitor at which bacterial growth was not visually apparent. The antimicrobial results were compared with amoxicillin and ciprofloxacin as positive controls and summarized in Table 1.

Table 3. Antibacterial activity of compounds 6(a-f)

	Minimum inhibitory concentration (MIC) in µg/mL			
Compounds	*S.aureus*	*B.subtilis*	*S.typhi*	*E.coli*
6a	25	200	50	100
6b	25	50	50	50
6c	25	50	50	50
6d	100	100	100	100
6e	12.5	12.5	12.5	25
6f	50	50	100	50
Ciprofloxacin	6.25	6.25	6.25	6.25
Amoxicillin	6.25	6.25	6.25	6.25

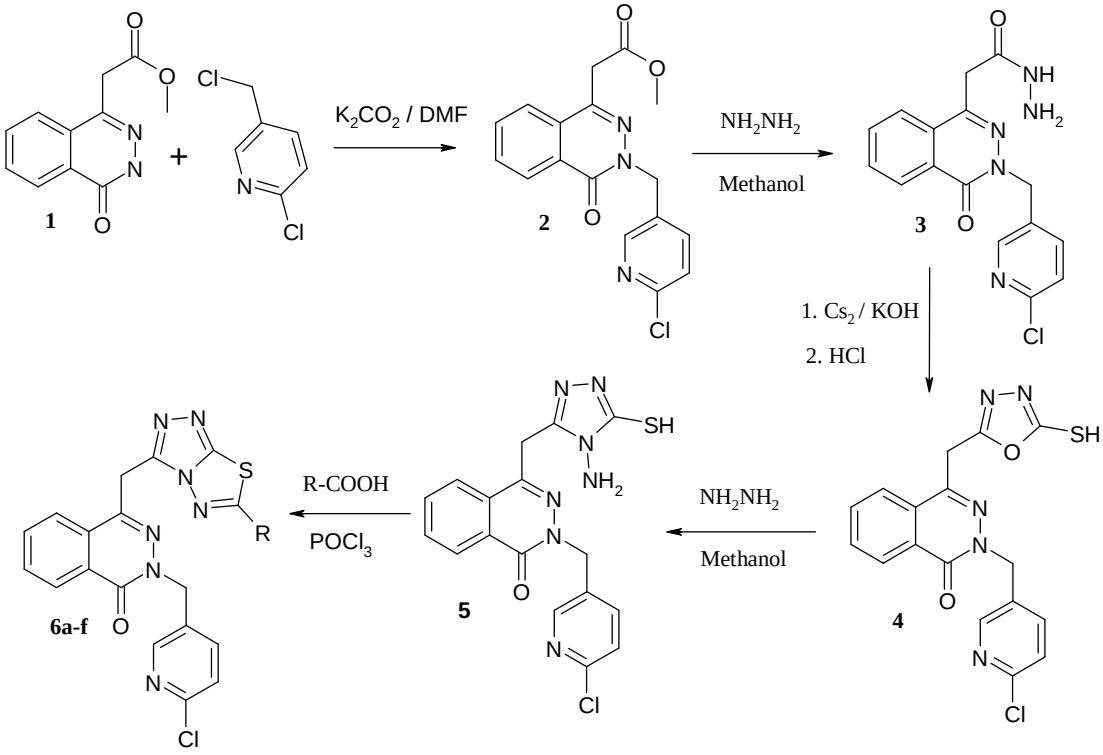
Similarly the antifungal activity of compounds **7a-f** were determined by using 72 h old broth culture. The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentration of 500, 250, 125, and 62.5 mg/mL. The results were compared with ketaconazole and azoxystrobin as positive control and summarized in Table 2.

Table 2. Antifungal activity of compounds 7(a-f)

Compounds	Minimum inhibitory concentration (MIC) in µg/mL	
	Aspergillums niger	*Candida albicans*
8a	250	125
8b	125	125
8c	125	125
8d	250	250
8e	62.5	62.5
8f	250	250
Ketaconazole	31.25	31.25
Azoxystrobin	62.5	62.5

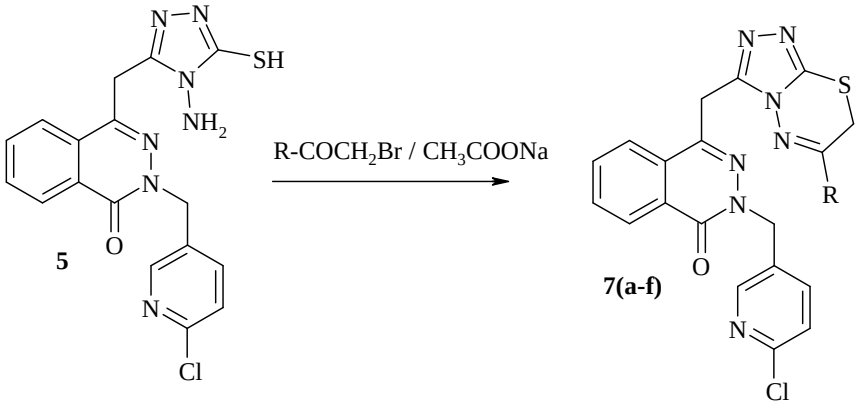
RESULTS AND DISCUSSION

The reaction sequences employed for the synthesis of title compounds are depicted in the Scheme-1 and 2.



R: a) Phenyl b) 4-chlorophenyl c) 4-Fluorophenyl
d) 3,4-dimethylphenyl e) 4-methylthiophenyl f) 3-chloro-4-methoxy phenyl

Scheme 1: Synthetic scheme for the preparation of triazolothiadiazole (9a-j)



R: a) Phenyl b) 4-chlorophenyl c) 4-Fluorophenyl
d) 3,4-dimethylphenyl e) 4-methylthiophenyl f) 3-chloro-4-methoxy phenyl

Scheme 2. Synthetic scheme for the preparation of phthalazine triazolothiadiazines(7a-f)

The key raw material for the synthesis of titled compounds is methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate which was prepared as per the literature [25] starting from phthalic anhydride. Condensation of methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate with 2-chloro-5-(chloromethyl) pyridine in presence of base gave {3-[(6-chloropyridin-3-yl)methyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetate (**2**), followed by reaction with hydrazine hydrate resulted in compound **3**. The acid hydrazide **3** on reaction with carbon disulphide with ethanolic potassium hydroxide and neutralisation with dilute hydrochloric acid affords 2-[(6-chloropyridin-3-yl)methyl]-4-[(5-sulfanyl-1,3,4-oxadiazol-2-yl) methyl] phthalazin-1(2H)-one (**4**). The triazole 4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-2-[(6-chloro pyridine-3-yl)methyl]phthalazin-1(2H)-one (**5**) was synthesized by refluxing compound **4** with excess of hydrazine hydrate and condensation of **5** with various aromatic acids in the presences of phosphorous oxy chloride yielded 1, 2, 4-triazolo-[3, 4-b]-1,3,4-thiadiazoles (**6a-f)**. Further the triazole 5 reacts with different acylbromides gave 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**7a-f**). The structural assignments of new compounds were based on their analytical and spectral data. The IR spectra of the compound **3** showed a characteristic weak absorption band at 2608 cm^{-1} attributed to SH group, and which is disappeared in the compounds **4** indicates the formation of oxadiazole thiol ring system. Further the ^{1}H NMR spectra of the synthesized oxadiazole **4** showed a broad singlet at δ 13.6 due to SH and disappearance of signals due to –NH-NH_2 further confirms the cyclization. The structure of **5** was confirmed by the two characteristic broad singlets at δ 13.6 and 5.9, due to SH proton and NH_2 groups respectively. The absence of these absorption due to SH and NH2 in compound **6a-f** and **7a-f** established that the triazole **5** had converted in to triazole-thiadiazole and triazole-thiadiazines by reacting with the -COOH group of various acid and acyl bromide. The mass spectra of all the synthesised compounds were in agreement with molecular formulas. In summary all the synthesized compounds exhibited satisfactory spectral data consistent with their structures. The ^{13}C NMR spectrum further confirms the structures of the cyclised compounds.

CONCLUSION

In this article we report the synthesis of a new 6-chloropyridin-3-yl)methyl substituted phthalazine 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**6a-f)** and 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**7a-f**) starting from commercially available phthalic anhydride. Investigation on antimicrobial data of synthesised compounds revealed that, compounds substituted with 5-nitro-thiazole to triazolothiadiazole (**6f**) and methylthiophenyl to triazolothiadiazines (**7e**) showed better activity compared to other analogues. Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication suggests that, further work with analogs is clearly warranted

Acknowledgments
The author A.M. Sridhara thankful to Advinus Therapeutics Pvt. Ltd, Bangalore, Chairman, Department of studies in Industrial Chemistry, Kuvempu University, Shimoga, India for their help and support to the research.

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