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Design and Facile Synthesis of 6-(Benzo Thiophen-3-YL)-3-para-Substituted-[1,2,4] Triazolo [3,4-a] Phthalazine Derivatives as Anti-Microbial Agents

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Abstract

The article is aimed to synthesize, characterize and screening the biological activity of novel a series of 6-(Benzo Thiophen-3-Yl)-3-Para-Substituted-[1,2,4] Triazolo[3,4-a] Phthalazine Derivatives (8 a-j)with good yields. The newly synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C NMR and Mass spectral data. The anti-microbial activity of the novel compounds were screened by disc diffusion method. Compounds 8h, 8g, and 8f demonstrated good antimicrobial activity against all the tested microbial strains. Fused Phialazine 1, 2,4 Triazole linked thiophene with 2,5 di fluoro nucleus has shown good antibacterial and antifungal activities.

Keywords: 1,4-di chloro Pthalazine, Tri azolo pthalazines, Hydrazine hydrate, Microwave irradiation, Suzuki Coupling, Antimicrobial activity.

Introduction

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural sub units exist in many natural products such as vitamins, hormones, and antibiotics[1,2]. Hence, they have attracted considerable attention in the design of biologically active molecules[3,4] and advanced organic chemistry[5,6]. Also in the family of heterocyclic compounds nitrogen containing Heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes[7].

Phthalazine derivatives, like the other members of the isomeric benzodiazine series, have been widely applied as therapeutic agents due to their anticonvulsant, cardiotonic, vasorelaxant and antiinflammatory properties[8,9]. Majorities of the drugs used in human medicine are hetero cyclic compounds. Common drugs such as Morphine, Lipitor, Penicillin and non steroidal anti-inflammatory agents contain at least one heteroatom in their structure[10]. Heterocyclic compounds containing nitrogen group have large are a in nature, and their utilizationis becoming progressively important as biologically active pharmaceuticals, agrochemicals, and functional materials[11]. In particular, hydrazine containing hetero cyclic compounds have been considered of great importance on account of pharmacological properties and clinical applications[12]. Moreover, these of combined phthalazines have biological properties such as inhibitionof p38MAPkinase[13] for selective binding of GABA receptor[14], antianxiety drug[15], antitumor agent[16], and high affinity ligand to the a2d-1 sub unit of calcium-channel[17].

Phthalazine derivatives have been greatly used as therapeutic agents owing to their anticonvulsant, cardiotonic, vasorelaxant, anti-inflammatory properties[18–23] and antimicrobial activity[24]. Like azelastine, the phthalazine derivatives have antihistaminic effects in the treatment of allergic rhinitis[25] and hydralazine is used as antihypertensive agent in the treatment of pulmonary hypertension[26–28]. Some commercially used phthalazine derivatives are shown in Figure-1.

Pthalazines are synthetically versatile substrates and hence can be used for the synthesis of a large variety of heterocyclic compounds. Pthalazines occupy a distinct and unique place in our life. This Hetero cyclic moiety has great biological and medicinal significance. Various synthetic aspects indicate that Pthalazine derivatives are easy to synthesize which can produce a wide variety of activity.

1, 2, 4-Triazole is one of a pair of Isomeric chemical compounds with molecular formula $C_2H_3N_3$, called Tri azoles (Figure-2), which have a five-membered ring of two carbon atoms and three nitrogen atoms. 1, 2, 4-Triazole is a basic aromatic hetero cycle.

The 1, 2, 4-triazole compounds are considered interesting heterocycles since they possess important pharmacological activities such as antifungal and antiviral activities. Examples of antifungal drugs[29,30] are i. fluconazole[31, 32], ii. itraconazole[33], iii. ravuconazole[34], iv. voriconazole[35,36], v. ICI 153066[37] and vi. posaconazole[38] (Table-1). 1,2,4 tri azole core structure was shown in blue colour in Table-1.

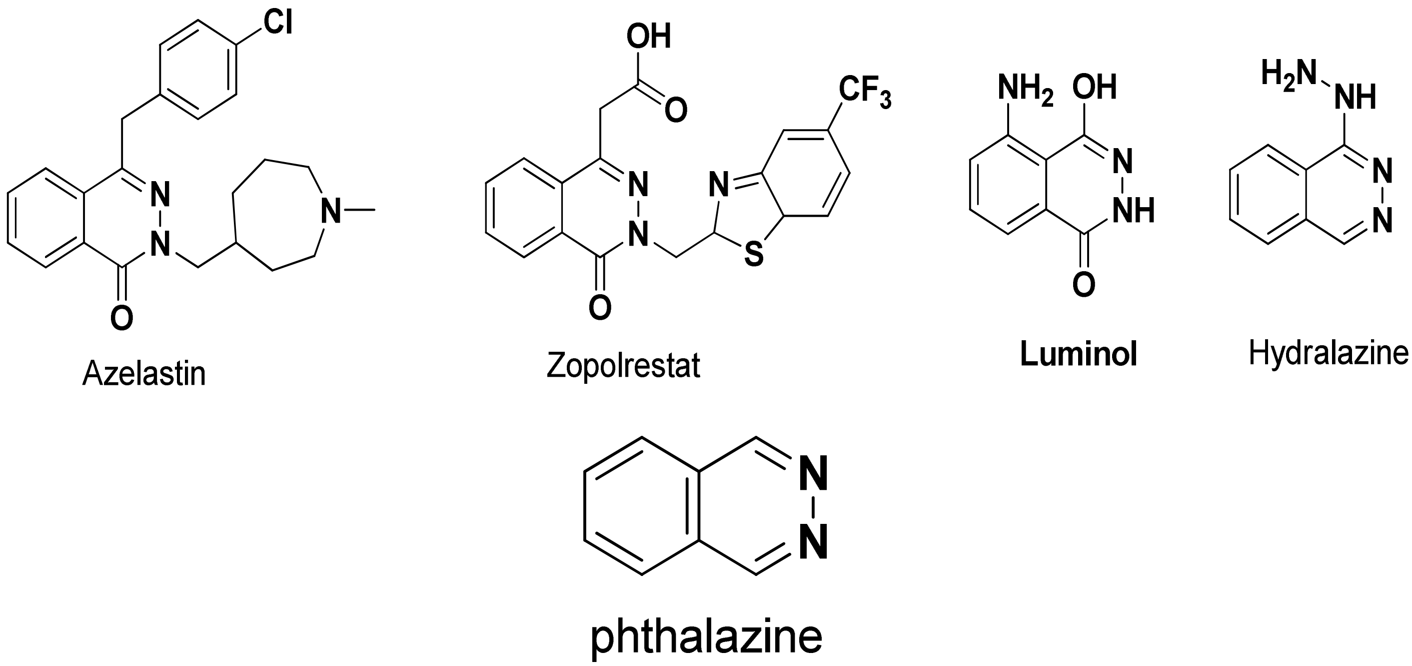


Figure-1
Some commercially used phthalazine derivatives and Structure of Pthalazine

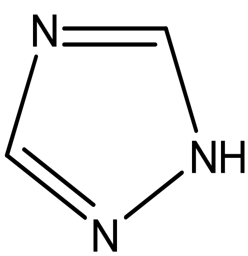


Figure-2
Structure of 1, 2, 4 tri azole

1,2,4-triazoles are very interesting targets for medicinal and pharmaceutical applications. 1,2,4-triazole derivatives investigated due to their wide range of biological activities such as antifungal[39], antitubercular[40], anticonvulsants[41,42] 5-lipoxygenase inhibitors[43] and as anticancer drugs[44], Platinum(II) complexes comprising 1,2,4-triazoles as ligands show antitumor activity similar to cis-platin[45-48].

Literature survey reveals that various 1,2, 4-triazole derivatives display signify cant biological activities such as Bactericidal[49], Diuretic[50], Fungicidal[51], Herbicidal[52], Insecticidal and acaricidal[53], Plant growth regulator[54], Anticancer and Anti-HIV[55], Anti leshmanial[56], Antitumor[57] activities.

Encouraged by the diverse biological activities of Pthalazine compounds, it was decided to prepare a new series of Pthalazines derivatives. The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H NMR spectral data. Further these compounds were subjected for antifungal and anti-bacterial activity.

Materials and Methods

Laboratory chemicals were provided by Rankem India Ltd. and Fisher Scientific Ltd. Melting points were determined by the open tube capillary method and are not correct. The purity of the compounds was determined by thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene:ethyl acetate (8:2). The spots were observed by exposure to iodine Vapours or by UV light or P-Anisaldehyde Stain Solution.

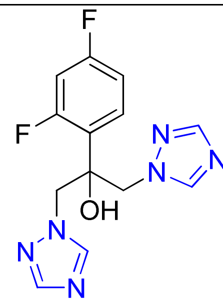
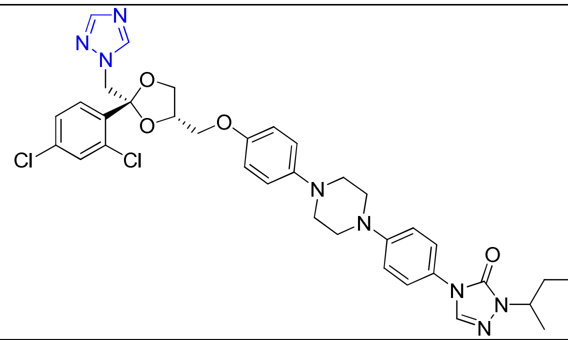
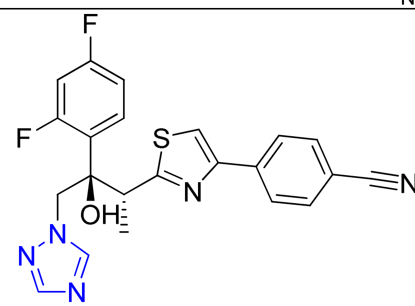
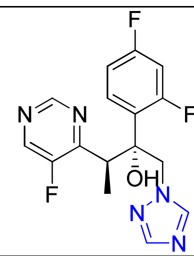
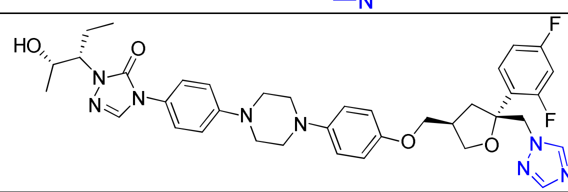
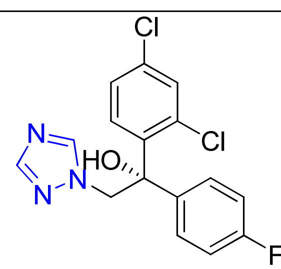
The IR spectra were received by PerkineElmer 1720 FT-IR spectrometer (KBr pellets). The ^{1}H NMR and ^{13}C NMR spectra were obtained by Bruker Advance II 400 spectrometer using TMS because the internal standard in $CDCl_3$.

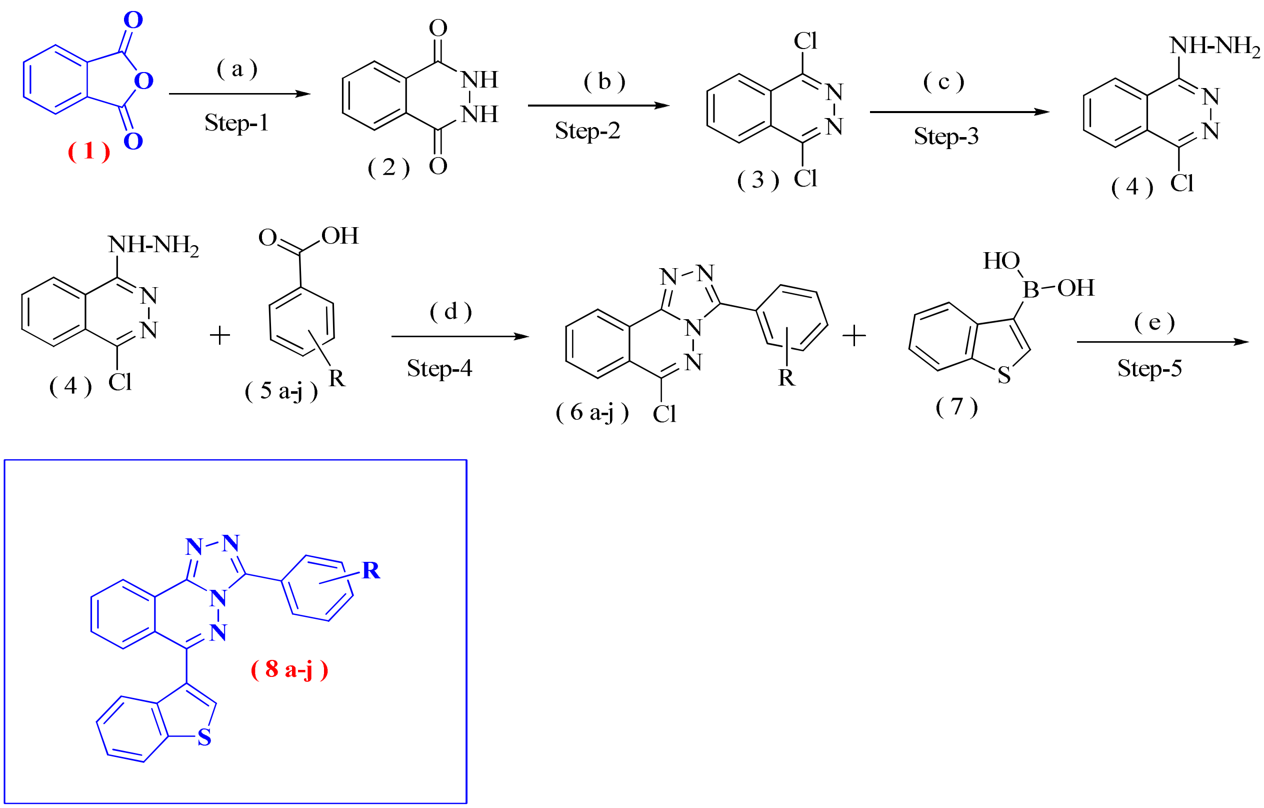
General Information: Commercial chemicals were treated as follows: 1,4 di oxane distilled from CaH_2 and degassed (freeze and thaw) three times prior to use; THF, ether, distilled from Na/benzophenone.

The synthetic route was depicted in scheme-I.

The title compounds 8(a-j) were synthesised in five sequential steps using different reagents and reaction conditions, the 8(a-j) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data.

Table-1
Examples of antifungal drugs containing 1, 2, 4 tri azole nucleus

S.NO	Anti fungal Drug Name	Structure
1	Flucanazole	
2	Itraconazole	
3	Ravuconazole	
4	Voriconazole	
5	Posaconazole	
6	ICI	



R = -H, -4 CH_3, -4 OCH_3, -4 NO_2, 3,4 di methoxy, -4 F, 2,5 DI Fluoro, -4 CF_3 , -4 OCF_3, -2,4 di nitro

Scheme-I
Synthetic

Reagents and Reaction conditions: i. Acetic acid, Hydrazine hydrate, Reflux, 4 hrs **ii.** $POCl_3$, Reflux, 6 hrs iii. Ethanol, Hydrazine hydrate, Na_2CO_3, RT **iv.** $POCl_3$, Reflux **v.** K_2CO_3, $PdCl_2(Ph_3P)_2$, 1,4-dioxane, H_2O, micro wave, 120^0C.

Experimental Section: General Methods: Column chromatography was performed using Silica gel 100-200 mesh size. THF and dioxane were distilled from sodium-benzo phenone and dried over MS $5A^0$ and MS $4A^0$, respectively. MeCN and 1,2-dichloroethane (DCE) were distilled from CaH_2. EtOH was distilled from Mg/I_2 and dried over MS $3A^0$. Prior to use, $POCl_3$ was distilled. All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. THF was distilled from sodium benzophenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20–30 Torr). Flash chromatography was performed with silica gel (200–100 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for ^{1}H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded using tetra methyl silane (TMS) in the solvent of $CDCl_3$-d_1 or DMSO-d_6 as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for the preparation of 2,3-dihydrophthalazine-1,4-dione[58] (Compound-2): The starting material Pthalic anhydride (1) (1 m.mol) was dissolved in Acetic acid (10 V.). To this mixture hydrazine hydrate (3m.mol) drop wise under ice bath. The reaction mixture was stirred at room temperature for 20 mints and then raises temperature at110^0C for 4 hrs. The off white solid was precipitated was collected through filtration and washed the chilled water and dried to afford compound 2 (Yield 80%).

m.p.: 300^0 C above also not melted.

^{1}H NMR (DMSO-d_6, 400 MHz): 8.1(d, 2H), 7.9 (d, 2H), 11.2 (bs, 2 H). **^{13}C NMR (DMSO-d_6, 100 MHz):** 120,134,117,169 **IR (KBr, cm^{-1}):** O-H (3510, sharp), Ar stretch C-H (3130.34), C-O (1060), C=N (1608.69), C=C (1344.43). m/z (LC-MS Shows 95% purity.): 163 $[M+H]^+$

General procedure for the preparation of 1,4-dichlorophthalazine[59] (Compound-3): The compound (2) (**10 m.mol**) was added to a stirred solution of phosphoruso xychloride (15 ml). The mixture was heated to 110°C for 1 h. After the reaction wascomplete (monitored by TLC). The reaction mixture was cooled to room temperature. The mixture was added dropwise to crushed ice with stirring for 10 minutes. Then themixture was filtered through a Buchner funnel. The filter cae was washed with H_2O until neutral and dried in a vacuum. Compound (3) (Yield 90%) was obtained as a white solid.

m.p.: 160–162^0 C. **^{1}H NMR (DMSO-d$_6$, 400 MHz)**: 8.1(d, 2H), 7.9 (d, 2H), 11.2 (bs, 2 H). **^{13}C NMR (DMSO-d$_6$, 100 MHz):** 120,134,117,169. **IR (KBr, cm^{-1}):** O-H (3510, sharp), Ar stretch C-H (3130.34), C-O (1060), C=N (1608.69), C=C (1344.43). **EI-MS (m/z):** 199 [M$^+$], 201[M+2], 203[M+4] (9:6:1, it indicates molecule contains two chlorine atoms).

General procedure for the preparation of 1-chloro-4-hydrazinylphthalazine[60] (Compound-4): 1, 4-Dichlorophthalazine (20.0 g, 0.100 mol) was added to a boilingsolution of hydrazine monohydrate (37.3 ml, 0.765 mol) in ethanol (500 ml) and the mixture heated at reflux for 0.5 h. The mixture was cooled to room temperature and the solid collected by filtration. The material was washed with ether, azeotroped with ethanol and dried in vacuo to afford the compound.
m.p.: 256–257^0 C.

^{1}H NMR (**400 MHz, d$_6$-DMSO**) 7.72-8.35 (4H, m, 4 of Ar-H). 4.64 (2H, bs), 7.2 (1H, bs). **^{13}C NMR (DMSO-d$_6$, 100 MHz):** 120,134,132, 124, 165, 146, 118,125. **IR (KBr, cm^{-1}):** 3368 and 3272 (-NH_2), 3066 (Ar-H), 1574 (C=C), 1468 (C=N), 660 (C-Cl).

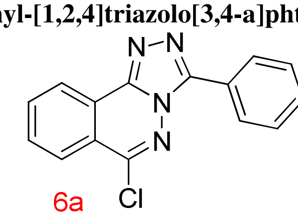
General procedure for the preparation of 6-chloro-3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (6a), 6-chloro-3-p-tolyl-[1,2,4] triazolo [3,4-a] phthalazine (6b), 6-chloro-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6c), 6-chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6d), 6-chloro-3-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6e), 6-chloro-3-(4-fluorophenyl)-[1,2,4] triazolo[3,4-a]phthalazine (6f), 6-chloro-3-(2,5-difluorophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6g), 6-chloro-3-(4-(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6h), 6-chloro-3-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6i), 6-chloro-3-(2,4-dinitrophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6j)[61] :

Compound (4) (0.1 m.mol), and substituted benzoic acids (5 a-j) (0.15 **m.mol**) were taken in $POCl_3$ (5 ml) and heated to reflux for 7 hrs. The reaction mass was concentrated under reduced pressure and then quenched in ice. The Solid obtained was filtered off, washed with aqueous $NaHCO_3$ Solution and dried.

Table-2
Yields and Melting Points of Corresponding Compounds (6 a-6j)

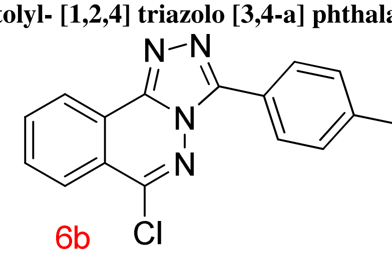
S.NO	Yield (%)	Melting Point (^{o}C)	Physical Appearence
6a	80	102-104	White Solid
6b	82	183-184	Off white Solid
6c	76	126-127	White Solid
6d	76	143-144	Pale Yellow Solid
6e	73	115-116	Pale brown solid
6f	71	124-126	Brown solid
6g	78	190-191	Off white Solid
6h	75	168-169.2^{0}C	White Solid
6i	73	115-116	Pluppy White Solid
6j	72	127-128	Off white Solid.

6-chloro-3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (6a) :



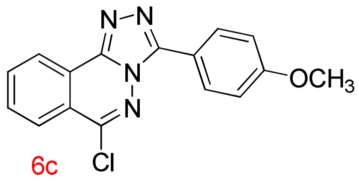
6a

^{1}H NMR (**400 MHz, d$_1$-CDCl$_3$**) 8(2H,d), 7.9(2H,d), 7.4-8.3 (5H,m). **^{13}C NMR (d$_1$-CDCl$_3$, 100 MHz):** 120-156 (13 Aromatic carbons). **IR (KBr, cm^{-1}):** 3056 (Ar-H), 1544 (C=C), 1428 (C=N), 680 (C-Cl). **EI-MS (m/z):** 280 [M$^+$], 282[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-p-tolyl- [1,2,4] triazolo [3,4-a] phthalazine (6b):**



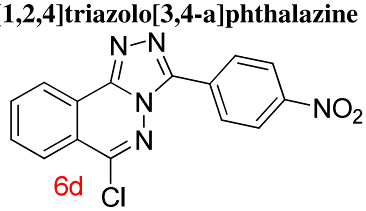
6b

^{1}H NMR (**400 MHz, d$_1$-CDCl$_3$**) 8(2H,d), 7.9(2H,d), 2.4 (3H,S), 8.6(2H,d), 7.3(2H,d).. **^{13}C NMR (d$_1$-CDCl$_3$, 100 MHz):** 123-156 (13 Aromatic carbons), 23(Aromatic methyl carbon). **IR (KBr, cm^{-1}):** 2957 (SP^3 C-H), 3066 (Ar-H), 1564 (C=C), 1468 (C=N), 670 (C-Cl). **EI-MS (m/z):** 294 [M$^+$], 296[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-a] phthalazine (6c) :**



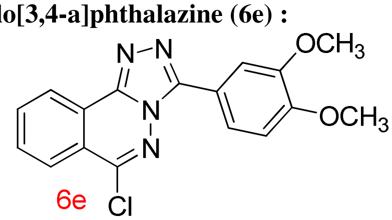
6c

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 8(2H,d), 7.85(2H,d), 3.85 (3H,S), 8.1(2H,d), 7.03(2H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 113-2-163 (13 Aromatic carbons), 56.5(Aromatic methoxy carbon). **IR (KBr, cm^{-1}):** 2968 (SP^3 C-H), 3066 (Ar-H), 1564 (C=C), 1468 (C=N), 656 (C-Cl), C-O-C (1060 and 1230). **EI-MS (m/z):** 310 [M$^+$], 312[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6d) :**



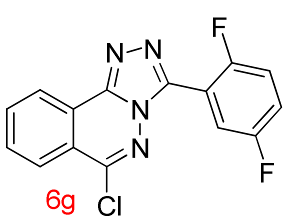
6d

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 8(2H,d), 7.83(2H,d), 8.1(2H,d), 8.4 (2H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 124-156 (13 Aromatic carbons). **IR (KBr, cm^{-1}):** 1360 & 1520(N-O Symmetric and asymmetric Stretching in nitro group), 3046 (Ar-H), 1574 (C=C), 1468 (C=N), 636 (C-Cl), **EI-MS (m/z):** 325 [M$^+$], 327[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6e) :**



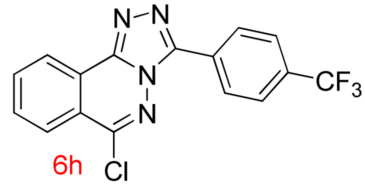
6e

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 8(2H,d), 7.83(2H,d), 3.85 (3H,S), 3.88(3H,S), 7.3(1H,d, J=2.4 Hz), 7.53(1H,d), 6.9(1H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 110-157 (15 Aromatic carbons), 56.5(Aromatic methoxy carbons). **IR (KBr, cm^{-1}):** 2988 (SP^3 C-H), 3046 (Ar-H), 1554 (C=C), 1438 (C=N), 676 (C-Cl), C-O-C (1060 & 1230). **EI-MS (m/z):** 340 [M$^+$], 342[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(2,5-difluorophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6g) :**



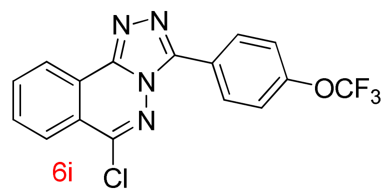
6g

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 7.9(2H,d), 7.83(2H,d), 7.3(1H,d,), 7.2(1H,dd), 7.5(1H,dd, J_{H-F}= , J_{H-H} = 2.4HZ). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 115-158 (15 Aromatic carbons). **IR (KBr, cm^{-1}):** 3036 (Ar-H), 1582 (C=C), 1438 (C=N), 654 (C-Cl), C-F (1260). **EI-MS (m/z):** 316 [M$^+$], 318[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(4-(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6h) :**



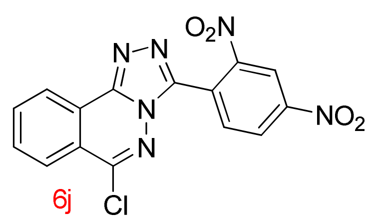
6h

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 7.9(2H,d), 7.85(2H,d), 8.6(2H,d,), 7.7(2H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 125-156 (13 Aromatic carbons), 124.3(Tr fluoro methyl carbon). **IR (KBr, cm^{-1}):** 3066 (Ar-H), 1584 (C=C), 1448 (C=N), 664 (C-Cl), C-F (1278). **EI-MS (m/z):** 348 [M$^+$], 350[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6i) :**



6i

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 7.9(2H,d), 7.85(2H,d), 8.02(2H,d,), 7.06(2H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 123-156 (13 Aromatic carbons), 129.8(Tr fluoro methyl carbon). **IR (KBr, cm^{-1}):** 3066 (Ar-H), 1584 (C=C), 1448 (C=N), 664 (C-Cl), C-F (1278), 1084(C-O-C). **EI-MS (m/z):** 364 [M$^+$], 366[M+2], (3:1, it indicates molecule contains one chlorine atom). **6-chloro-3-(2,4-dinitrophenyl)-[1,2,4]triazolo [3,4-a]phthalazine (6j) :**



6j

^{1}H NMR (**400 MHz, d_1-$CDCl_3$**) 8(2H,d), 7.83(2H,d), 8.95(1H,s), 8.74(1H,d),8.34 (1H,d). **^{13}C NMR (d_1-$CDCl_3$, 100 MHz):** 123-156 (15 Aromatic carbons). **IR (KBr, cm^{-1}):** 1350 & 1540(N-O Symmetric and asymmetric Stretching in nitro group), 3046 (Ar-H), 1574 (C=C), 1468 (C=N), 667 (C-Cl), **EI-MS (m/z):** 370 [M$^+$], 372[M^{+2}], (3:1, it indicates molecule contains one chlorine atom).

General procedure for the preparation of 6-(benzo[b]thiophen-3-yl) -3-phenyl- [1,2,4] triazolo [3,4-*a*] phthalazine (8a), 6-(benzo[b]thiophen-3-yl)-3-p-tolyl-[1,2,4]triazolo[3,4-*a*]phthalazine (8b), 6-(benzo[b]thiophen-

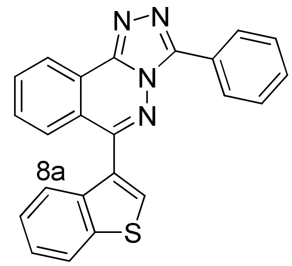
3-yl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (8c), 6-(benzo[b]thiophen-3-yl)-3-(4-nitrophenyl)-[1,2,4] triazolo[3,4-*a*]phthalazine (8d), 6-(benzo[b]thiophen-3-yl)-3-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (8e), 6-(benzo[b]thiophen-3-yl)-3-(4-fluorophenyl)-[1,2,4] triazolo [3,4-*a*]phthalazine (8f), 6-(benzo[b]thiophen-3-yl)-3-(2,5-difluorophenyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (8g), 6-(benzo[b]thiophen-3-yl)-3-(4-(trifluoromethyl)phenyl)-1,2,4] triazolo[3,4-*a*]phthalazine (8h), 6-(benzo[b]thiophen-3-yl)-3-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-*a*] phthalazine (8i), 6-(benzo[b]thiophen-3-yl)-3-(2,4-dinitrophenyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (8j)[62] :

A mixture of 6a-6j (**0.6 m.mole**), benzo[b]thiophen-3-ylboronic acid (7) (**0.9 m.mol**), K_2CO_3 (**3.3 m.mol**) and $PdCl_2(Ph_3P)_2$ (**0.03 m.mol**), in 5 ml Solvent (DME/Water/Ethanol 7:3:2) was placed in a sealed tube and heated to 120°C for 30 min using microwave irradiation. The reaction mixture was diluted with water and extracted with EtoAc, Dried with Na_2SO_4 filtered and evaporated to dryness. The Crude product was purified by preparative TLC, affording products (8a-8j). Yields are 60-65%.

Table-3
Yields & Melting Points of Corresponding Compounds (8a-8j)

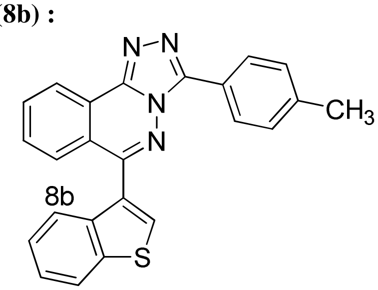
S.NO	Yield (%)	Melting Point (°C)	Physical Appearence
8a	60	135-136	White Solid
8b	62	139-141	Off white Solid
8c	60	205-206	White Solid
8d	61	202-204	white Solid
8e	63	219-221	Off white solid
8f	64.2	225-227	White solid
8g	63	199-201	White solid
8h	62	213-214	White solid
8i	61	115-117	White solid
8j	62.3	232-234	White solid

6-(benzo[b]thiophen-3-yl)-3-phenyl-[1,2,4]triazolo[3,4-*a*] phthalazine (8a) :



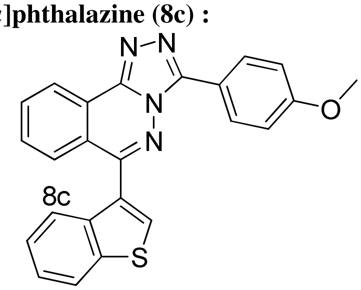
^{1}H NMR (400 MHz, d_6-DMSO) 7.88(2H,d), 7.9(2H,d), 7.4-8.3 (5H,m), 7.95(1H,d, J=3HZ), 7.7(1H,dd, J=7.3HZ, J=3HZ), 7.3(1H,d, J=7.3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **^{13}C NMR (d_6-DMSO, 100 MHz):** 120,125,128,130,, 132, 139, 148, 151,153. **IR (KBr, cm^{-1}):** 3066 (Ar-H), 1544 (C=C), 1428 (C=N), 687(C-S-C). **EI-MS (m/z):** 379 [M+H].

6-(benzo[b]thiophen-3-yl)-3-p-tolyl-[1,2,4]triazolo[3,4-*a*] phthalazine (8b) :



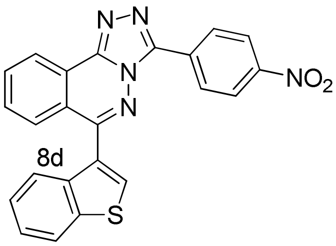
^{1}H NMR (400 MHz, d_6-DMSO) 7.8(2H,d), 7.9(2H,d), 2.4 (3H,S), 8.6(2H,d), 7.3(2H,d), 7.95(1H,d, J=3HZ), 7.7(1H,dd, J=7.3HZ, J=3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d),7.3(1H,d, J=7.3HZ). **^{13}C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 153, 23 (Aromatic methyl carbon). **IR (KBr, cm^{-1}):** 2959 (SP^3 C-H), 3068 (Ar-H), 1584 (C=C), 1458 (C=N), 677 (C-S-C). **EI-MS (m/z):** 393 [M+H].

6-(benzo[b]thiophen-3-yl)-3-(4-methoxyphenyl)-[1,2,4] triazolo [3,4-*a*]phthalazine (8c) :



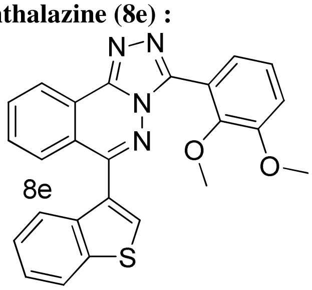
^{1}H NMR (400 MHz, d_6-DMSO) 7.8(2H,d), 7.9(2H,d), 3.8 (3H,S), 7.9(2H,d), 7.03(2H,d), 7.95(1H,d, J=3HZ), 7.75(1H,dd, J=7.3HZ, J=3HZ), 7.23(1H,d,J=7.3HZ), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d). **^{13}C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 153, 56 (Aromatic methyl carbon). **IR (KBr, cm^{-1}):** 2969 (SP^3 C-H), 3066 (Ar-H), 1564 (C=C), 1458 (C=N), C-O-C (1060 and 1230), 667 (C-S-C). **EI-MS (m/z):** 409 [M+H].

6-(benzo[b]thiophen-3-yl)-3-(4-nitrophenyl)-[1,2,4] triazolo [3,4-*a*] phthalazine (8d) :



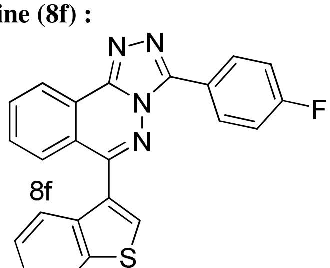
[1]H NMR (**400 MHz, d_6-DMSO**) 7.85(2H,d), 7.9(2H,d), 8.09(2H,d), 8.33(2H,d), 7.95(1H,d,J=3HZ), 7.75(1H,dd, J=7.3HZ,J=3HZ), 7.23(1H,d,J=7.3HZ), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 155. **IR (KBr, cm^{-1}):** 1350 and 1540(N-O Symmetric and asymmetric Stretching in nitro group), 3046 (Ar-H), 1574 (C=C), 1468 (C=N), 665 (C-S-C). **EI-MS (m/z):** 424[M+H].

6-(benzo[b]thiophen-3-yl)-3-(3,4-dimethoxyphenyl)-[1,2,4] triazolo[3,4-*a*]phthalazine (8e) :



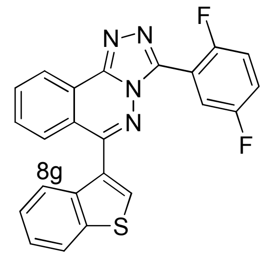
[1]H NMR (**400 MHz, d_6-DMSO**) 7.8(2H,d), 7.9(2H,d), 3.8 (3H,S), 3.85(3H,S), 7.6(1H,d), 7.53(1H,dd,J=7HZ,J=3HZ), 7.25(1H,d,J=3HZ), 7.23(1H,d, J=7.3HZ), 7.8(1H,dd, J=7.3 HZ, J=3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d), 8(1H,d,J=3HZ). **[13]C NMR (d_6-DMSO, 100 MHz)** 120,125,128,130,, 132, 139, 148, 151,153,58(Aromatic methoxy carbon). **IR (KBr, cm^{-1}):** 2969 (SP^3 C-H), 3066 (Ar-H), 1564 (C=C), 1458 (C=N), C-O-C (1060 & 1230), 667 (C-S-C). **EI-MS (m/z):** 439 [M+H].

6-(benzo[b]thiophen-3-yl)-3-(4-fluorophenyl)-[1,2,4] triazolo [3,4-*a*]phthalazine (8f) :



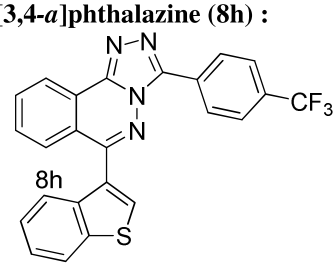
[1]H NMR (**400 MHz, d_6-DMSO**) 7.85(2H,d), 7.9(2H,d), 7.8(2H,d), 7.33(2H,d), 7.95(1H,d, J=3HZ), 7.75(1H,dd, J=7.3HZ, J=3HZ), 7.23(1H,d, J=7.3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120,125,128,130,, 132, 139, 148, 151,153,165. **IR (KBr, cm^{-1}):** 3054 (Ar-H), 1582 (C=C), 1438 (C=N), C-F (1260),662 (C-S-C). **EI-MS (m/z):** 397[M+H].

6-(benzo[b]thiophen-3-yl)-3-(2,5-difluorophenyl)-[1,2,4] triazolo [3,4-*a*]phthalazine (8g) :



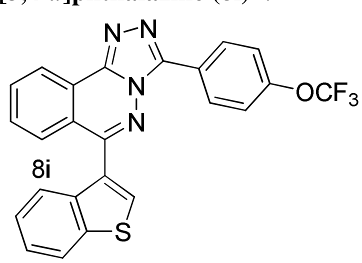
[1]H NMR (**400 MHz, d_6-DMSO**) 7.85(2H,d), 7.9(2H,d), 7.5(1H,d, J=3HZ), 7.33(1H,d, J=7.3HZ,), 7.25(1H,dd, J=7.3HZ, J=3HZ), 7.75(1H,dd, J=7.3HZ, J=3HZ), 8(1H,d, J=3HZ), 7.24(1H,d, J=7.3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 153, 163. **IR (KBr, cm^{-1}):** 3036 (Ar-H), 1582 (C=C), 1438 (C=N), C-F (1255), 662 (C-S-C). **EI-MS (m/z):** 413[M-H].

6-(benzo[b]thiophen-3-yl)-3-(4-(trifluoro methyl)phenyl)-[1,2,4] triazolo[3,4-*a*]phthalazine (8h) :



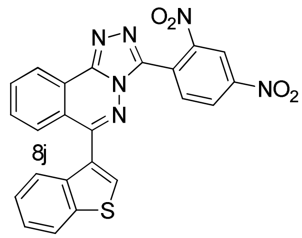
[1]H NMR (**400 MHz, d_6-DMSO**) 7.85(2H,d), 7.9(2H,d), 8.6(2H,d), 7.7(2H,d), 7.95(1H,d, J=3HZ), 7.75(1H,dd, J=7.3HZ, J=3HZ), 7.23(1H,d, J=7.3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120,125,128,130, 132, 139, 148, 151, 155. **IR (KBr, cm^{-1}):** 3064 (Ar-H), 1562 (C=C), 1428 (C=N), C-F (1250), 662 (C-S-C). **EI-MS (m/z):** 447[M+H].

6-(benzo[b]thiophen-3-yl)-3-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (8i) :



[1]H NMR (**400 MHz, d_6-DMSO**) 7.85(2H,d), 7.9(2H,d), 7.9(2H,d), 7.03(2H,d), 7.95(1H,d, J=3HZ), 7.75(1H,dd, J=7.3HZ, J=3HZ), 7.23(1H,d, J=7.3HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 153, 132 (Tri fluoro methyl carbon). **IR (KBr, cm^{-1}):** 3066 (Ar-H), 1582 (C=C), 1438 (C=N), C-F (1250), 662 (C-S-C). **EI-MS (m/z):** 463[M+H].

6-(benzo[b]thiophen-3-yl)-3-(2,4-dinitrophenyl)-[1,2,4] triazolo [3,4-*a*]phthalazine (8j) :



[1]H NMR (400 MHz, d_6-DMSO) 7.85(2H,d), 7.9(2H,d), 8.99 (1H,d, J=3HZ), 8.33(1H,d, J=7.3 HZ), 8.75(1H,dd, J=7.3 HZ, J=3HZ), 7.75(1H,dd, J=6.3HZ, J=2.8HZ), 7.23(1H,d, J=6.3HZ), 8(1H,d, J=2.8HZ), 7.5(2H,q), 8.5(1H,d),7.8(1H,d). **[13]C NMR (d_6-DMSO, 100 MHz)** 120, 125, 128, 130, 132, 139, 148, 151, 155. **IR (KBr, cm^{-1}):** 1350 and 1540 (N-O Symmetric and asymmetric Stretching in nitro group), 3066 (Ar-H), 1584 (C=C), 1478 (C=N), 660 (C-S-C). **EI-MS (m/z):** 469[M+H].

Biological Activity: Antibacterial activity: Antimicrobial screening The samples of synthesized Compounds (8a-8j) for antimicrobial activity were prepared at concentration 40µg/ml in DMSO solvent. In case of antibacterial activity, the plates were incubated at 37°C for 24 hours and for antifungal activity the plates were incubated at 30°C for 48 hours. The antibacterial activity was checked against Gram positive bacteria Staphylococcus aureus (S. aureus) and Bacillus subtilis (B. subtilis), Gram negative bacteria Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli). The antifungal activity was checked against fungi Aspergillus niger (A. niger) and Candida albicans (C. albicans). The results were compared with stand drugs Sparfloxacin, Benzyl penicillin and Fluconazole. The Pthalazine-1,2,4 triazole derivates containing Thiophene core structure with 2,5 di flouro (8g) and $-CF_3$ (8h) showed more activity than other substituent's 8g>8h>8i> 8j>8f >8d>8b>8a>8c>8e.

Results and Discussion

The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial activity of the newly synthesized Pthalazine triazole derivatives. The yield of the products ranged from 55-90%. The purity was checked by TLC. The structures of the newly synthesized compounds [8a-8j] are characterized and confirmed by spectral data viz. IR, [1]H and [13]C NMR and Mass spectra and all the synthesized compounds [8a-8j] were screened for antimicrobial activity.

Chemistry: The Title Compounds Novel 6-(benzo Thiophen-3-yl)-3-Para-Substituted-[1,2,4] Triazolo[3,4-a] Phthalazine Derivatives were synthesized in good yields (**scheme-I**). All these compounds were tested for Anti-microbial activity showed considerable activity when compared to the standard drugs.

Table-4
In vitro antibacterial and antifungal activities of the synthesized compounds (8a-8j)

Compound	Anti-Bacterial activity (Zone if Inhibition in mm)				Anti-Fungal Activity (Zone if Inhibition in mm)	
	Staphylococcus aureus	Bacillus subtilis	P. aeruginosa	E. coli	A. niger	C. albicans
8a	12	11	07	11	23	19
8b	12	16	10	14	09	12
8c	10	28	17	21	14	12
8d	13	17	30	13	11	18
8e	10	19	09	09	12	16
8f	16	12	13	11	10	18
8g	23	13	18	15	18	10
8h	19	10	12	11	17	15
8i	18	16	08	08	17	09
8j	16	12	13	11	10	18
Sparfloxacin	**24**	**25**	**25**	**22**	**---**	**---**
Benzyl penicillin	**18**	**17**	**16**	**16**	**---**	**---**
Fluconazole	**---**	**---**	**---**	**---**	**22**	**20**

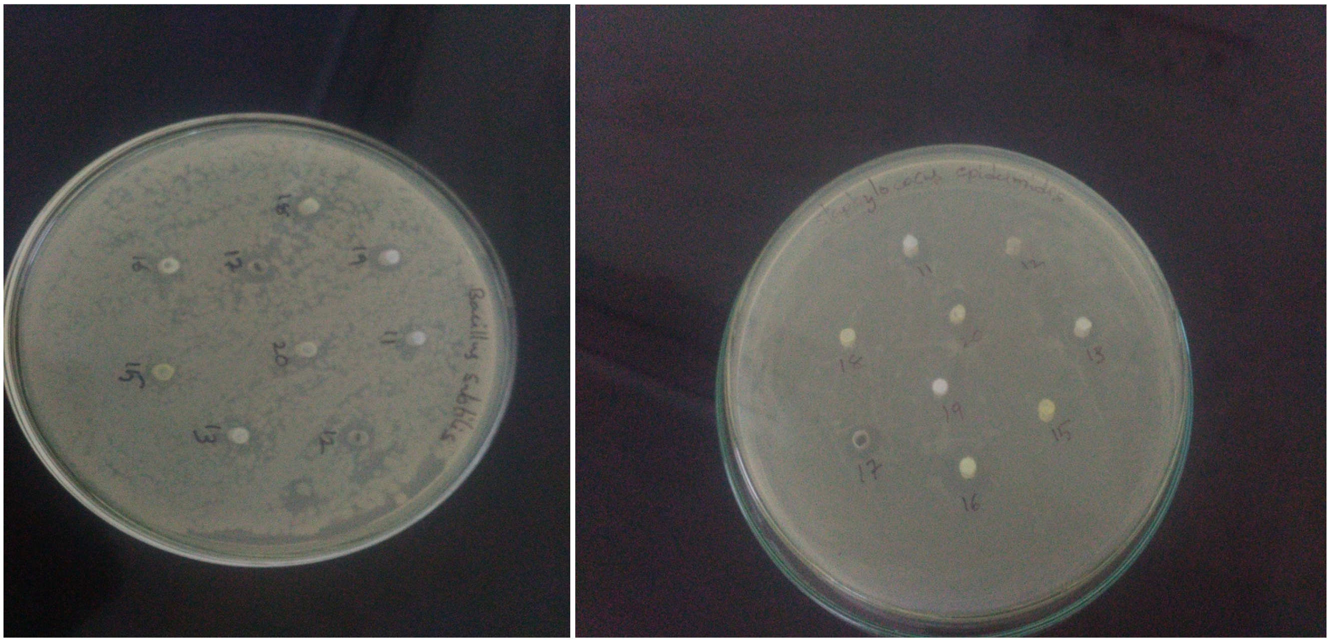


Figure-3
Antibacterial activity of Novel Pthalazine derivatives (8a-8j)

2,3-dihydrophthalazine-1,4-dione (2) was synthesised from Pthalic anhydride (1), hydrazine hydrate in Acetic acid at reflux for 4 hrs, Compound (2) was converted in to 1,4-dichlorophthalazine compound (3) by using $POCl_3$ at reflux for 6 hrs, Compound (3) was converted into 1-chloro-4-hydrazinylphthalazine compound (4) by using hydrazine hydrate in Ethanol at reflux for 4 hrs, Compound (4) reacts with different substituted benzoic acids (5 a-5j) in $POCl_3$ at reflux to form fused 1,2,4 tri azole Pthalazine derivatives (6a-6j), Compounds (6a-6j) were reacted with benzo(b)thiophen-3-yl-boronic acid (7) under Suzuki reaction conditions in microwave to get target Pthalazine derivatives (8a-8j). Structures of Compounds 8a-8j were confirmed by IR, ^{1}H and ^{13}C NMR, mass Spectroscopic Techniques. All of the Pthalazine tri azoles possess similar basic skeletal structure.

Characterization: The FT-IR spectra of 8a–8j were recorded using KBr pellets in the range of 4,000–400 cm-^{1}.The IR spectrum of the title Compounds 8(a-j) has given stretching vibration 3420 cm^{-1} due to the stretching vibration corresponding to N-H Stretching vibrations. 3100cm^{-1}, due to the stretching vibration corresponding to Ar-H Stretching vibrations.

The absorption peak at 2935 cm^{-1} is due to The stretching vibration corresponding to the SP^3 C-H (methyl gp). The strong Intensity absorption at 1300 and 1500 cm^{-1} is due to the stretching vibration of -N-O Stretching in Nitro group, 1350 cm^{-1} is due to the stretching vibration of C-F bond. 760 cm^{-1} is due to the stretching vibration of C-Cl bond. 660 cm^{-1} is due to the stretching vibration of C-S-C bond. The weak Intensity absorption at 1620 cm^{-1} corresponds to a C=N Stretching vibration.1160cm^{-1} corresponding to C-O-C Stretching.

It has been observed from chemical structure of compound 8(a-j) that different pair of protons. The protons of Methyl group which is attached to benzene ring appeared as a singlet at δ =2.3 ppm, The protons of Methoxy group appeared as a Singlet at δ =3.85 ppm, The protons attached benzene ring appeared between δ =7.2-8.3 ppm respectively.

The chemical shifts of the final compound carbon vary from δ = 160 to 23 ppm. The carbon nucleus under the influence of a strong electronegative environment appeared down field, The carbon chemical shift of the methyl group at δ= 23 ppm. The carbon chemical shift of the Methoxy group at δ= 55 ppm.

From antimicrobial screening data (Table-4) of synthesized directives show that the compounds 8h, 8g, and 8i have good antibacterial activity against S. aureus, B. subtilis (Gram positive bacteria) respectively compare to Bacteriomycin. The compounds 2c, 2e, 3b, 3f and 3h have good antibacterial activity against P. aeruginosa (Gram negative bacteria) respectively compare to Benzyl penicillin and Sparfloxacin. The compounds 8h, 8g, and 8i have very good antifungal activity against C. albicans and compounds 8h, 8g, and 8i have good antifungal activity against A. niger compare to Flucanazole.

Readily available starting materials and Simple Synthesizing procedures make this method very attractive and convenient for the synthesis of Fused Pthalazine triazole derivatives. Formation of products was confirmed by recording their ^{1}H NMR, ^{13}C, FT-IR, mass spectra.

Anti-microbial Screening: The results of Anti -microbial studies of newly synthesized compounds reveal that the compounds possess significant Anti-microbial activities. The results of these studies are given in Table-4. From Anti -Microbial screening results, it has been observed that compounds 8g, 8h and 8i possess good activity.

In the present work, a series of Pthalazine triazole derivatives have been synthesized using new substituted benzoic acids and hydrazine hydrate with moderate to good yield. The antimicrobial activities of synthesized derivatives show that some derivatives have good results compared to standard drugs data. Further investigation with appropriate structural modification of the above compounds may result in therapeutically useful products. The analytical data and spectral data support the structure and geometry of the Pthalazine triazole derivatives (8a-8j).

Conclusion

In conclusion, a simple and effective procedure for the preparation of novel 1,2,4-triazoles from a common 1,4-dichlorophthalazine intermediate was developed. The method is very simple, clean and applicable to a variety of reactants. Finally In conclusion, a series of novel Pthalazine 1,2,4 tri azole derivatives 8 (a-j) were synthesised in good yield, characterised by different spectral studies and their anti-microbial activity have been evaluated. Among the synthesised compounds 8g, 8h, and 8i showed more anti-microbial activity when compared to other compounds in the series.

Acknowledgment

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References

1. Ju Y. and Varma R.S. (2006).Aqueous N-Hetero cyclization of primary amines and hydrazines with dihalides: micro wave assisted Syntheses of N-azacycloalkanes, isoindole, pyrazole, pyrazolidine, and phthalazine derivatives. *Journal of Organic Chemistry*, 71(1), 135–141.

2. Ju Y., Kumar D. and Varma R.S. (2006). Revisiting nucleophilic substitution reactions: microwave-assisted synthesis of azides, thiocyanates, and sulfones in an aqueous medium. *Journal of Organic Chemistry,* 71(17), 6697–6700.

3. Lokhande P.D., Waghamare B.Y. and Sakate S.S. (2005). Regioselective one-pot synthesis of 3,5-diarylpyrazoles. *Indian Journal of Chemistry B,* 44(11), 2338–2342.

4. Reddy G.J., Manjula D., Rao K.S., Khalilullah M. and Latha D. (2005). A Direct single step synthesis of 1,3-diaryl-4-cyanopyrazoles and their conversion to 1,3-diaryl-4-(4,6-diamino 1,3,5-triazin-2-yl) pyrazoles. *Indian Journal of Chemistry B,* 44, 2412–2415.

5. Zificsak C.A. and Hlasta D.J. (2004). Current methods for the synthesis of 2-substituted azoles. *Tetrahedron,* 60(41), 8991– 9016.

6. Haino T., Tanaka M., Ideta K., Kubo K., Mori A. and Fukazawa Y. (2004). Solid-phase synthesis of liquid crystalline isoxazole library. *Tetrahedron Letters,* 45(11), 2277–2279.

7. García-Valverde M. and Torroba T. (2005). Special issue: sulfur-nitrogen heterocycles. *Molecules,* 10(2), 318–320.

8. Tsoungas P.G. and Searcey M. (2001). A convenient access to benzo-substituted phthalazines as potential precursors to DNA intercalators. *Tetrahedron Lett.,* 42, 6589-6592.

9. Sivakumar R., Gnanasam S.K., Ramachandran S. and Leonard J.T. (2002). Pharmacological evaluation of some new 1-substituted-4-hydroxyphthalazines. *Eur. J. Med. Chem*., 37, 793-801.

10. Joule J.A. and Mills K. (2012). Heterocyclic Chemistry at a Glance. John Wiley & Sons, NewYork, NY, USA, 2012.

11. Franklin E.C. and Bergstrom F.W. (1944). Heterocyclic nitrogen compounds: part-IIA. Hexa cyclic compounds: pyridine, quinoline, and isoquinoline. *Chemical Reviews,* 35(2), 77– 277.

12. Turk C., Svete J. and Stanovniketal B. (2001). Regioselective 1, 3-dipolar cycloadditions of (1Z)-1-(arylmethylidene)-5, 5-dimethyl-3 oxopyrazolidin-1-ium-2-ide azomethine imines to acetylenic dipolarophiles. *Helvetica Chimica Acta,* 84(1), 146–156.

13. Mavel S., Thery L. and Gueiffier A. (2002). Synthesis of imidazo[2, 1-a]phthalazines, potential inhibitors of p38 MAP kinase. Prediction of binding affinities of protein ligands. *Archiv der Pharmazie Medicinal Chemistry,* 335(1), 7–14.

14. Carling R.W., Moore K.W., Street L.J. et al. (2004). 3-Phenyl-6-(2pyridyl)methyloxy-1,2,4-triazolo[3,4-a]phthalazines and Analogues: high-affinity γ-aminobutyric acid-a benzodiazepine receptorligandswith α2, α3,and α5-subtypebindingselectivity over α1. *Journal of Medicinal Chemistry,* 47(7), 1807–1822.

15. Imamura Y., Noda A., Imamura T., Ono Y., Okawara T. and Noda H. (2003). A novel methylthio metabolite of s-triazolo[3,4a]phthalazine, a lead compound for the development of antianxiety drugs, in rats. *Life Sciences,* 74(1), 29–36.

16. Kim J.S., Lee H.J. and Suhetal M.E. (2004). Synthesisandcytotoxicity of 1-substituted 2-methyl-1H-imidazo[4,5-g] phthalazine-4, 9 dionederivatives. *Bioorganic and Medicinal Chemistry,* 12(13), 3683–3686.

17. Lebsack A.D., Gunzner J., Wang B. et al. (2004). Identification and synthesis of [1,2,4]triazolo[3,4-a]phthalazine derivatives as high-affinity ligands to the $\alpha 2\delta$ -1 subunit of voltage gated calcium channel. *Bioorganic and Medicinal Chemistry Letters,* 14(10), 2463–2467.

18. Tsoungas P.G. and Searcey M. (2001). A convenient access to benzo substituted phthalazines as potential precursors to DNA intercalators. *Tetrahedron Letters,* 42(37), 6589–6592.

19. Sivakumar R., Gnanasam S.K., Ramachandran S. and Leonard J.T. (2002). Pharmacological evaluation of some new 1-substituted-4-hydroxy-phthalazines. *European Journal of Medicinal Chemistry,* 37(10), 793–801.

20. Coelho A., Sotelo E. and Fraizetal N. (2004). Pyridazines Part 36: synthesis and anti platelet activity of 5-substituted-6-phenyl-3(2H) pyridazinones. *Bioorganic and Medicinal Chemistry Letters,* 14(2), 321–324.

21. Demirayak S., Karaburun A.C. and Beis R. (2004). Some pyrrole substituted aryl pyridazinone and phthalazinone derivatives and their anti hyper tensive activities. *European Journal of Medicinal Chemistry,* 39(12), 1089–1095.

22. Dogruer D.S., ahin M.F.S¸, upeli E.K¨ and Ye silada E. (2003). Synthesis and analgesic and anti-Inflammatory activity of new pyridazinones. *Turkish Journal of Chemistry,* 27(6), 727–738.

23. Dogruer D.S., Kupeli E., Yesilada E. and Sahin M.F. (2004). Synthesis of new 2-[1(2H)-phthalazinon-2-yl]-acetamide and 3-[1(2H)phthalazinon-2-yl]-propanamide derivatives as antinociceptive and anti-inflammatory agents. *Archiv der Pharmazie*, 337(6), 303–310.

24. Sonmez M., Berber I. and Akba¸s E. (2006). Synthesis, antibacterial and antifungal activity of some new pyridazinone metal complexes. *European Journal of Medicinal Chemistry,* 41(1), 101–105.

25. Tanizaki Y., Ohtani J. and Kimura I. (1992). Actions and cross reactivity of antiallergic agents and a calcium channel antagonist on rat peritoneal mast cells. Difference in the action mechanisms and cross-reactivity among the agents. *Agents and Actions,* 37(1-2), 8–15.

26. Groves B.M., Rubin L.J. and M.F. Frosolono (1985). A comparison of the acute hemodynamic effects of prostacyclin and hydralazine in primary pulmonary hypertension. *American Heart Journal,* 110(6), 1200–1204.

27. Packer M., Greenberg B., Massie B. and Dash H. (1982).Deleterious effects of hydralazine in patients with pulmonary hypretension. *The New England Journal of Medicine,* 306(22), 1326–1331.

28. Keller C.A., Shepard Jr. J.W. and Chun D.S. (1984). Effects of hydralazine on hemodynamics, ventilation, and gas exchange in patients with chronic obstructive pulmonary disease and pulmonary hypertension 1-4. *American Review of Respiratory Disease,* 130(4), 606–611.

29. Yagisawa M. (2006). 1,2,4-Triazoles: Synthetic approaches and pharmacological importance. *Jpn. J. Med. Mycol*., 45, 77.

30. Johnson E.M., Szekely A., Warnock D.W. (1999). Antimicrob. Agents Chemother, 43, 1260.

31. Tsukuda Y., Shiratori M., Watanabe H., Ontsuka H., Hattori K., Shirai M. and Shimma N. (1998). Modeling, synthesis and biological activity of novel antifungal agents. *Bioorg. Med. Chem. Lett*., 8, 1819.

32. Narayanan A., Chapman D.R., Upadhyaya S.P. and Bauer L. (1993). Conversion of 4-amino-4*H*-1,2,4-triazole to 1,3-bis(1*H*-azol-l-yl)-2-aryl-2-propanols and 1-phenacyl-4-[(benzoyl or 4-toluenesulfonyl)-imino]-(1*H*-1,2,4-triazolium) Ylides. *J. Heterocycl. Chem.*, 30, 1405.

33. Bailey E.M. and Krakovsky D.J. (1990). The triazole antifungal agents: a review of itraconazole and fluconazole. *J. Pharmacotherapy* , 10, 146-153.

34. Roberts J., Schock K., Marino S. and Andriole V.T. (2000). Efficacies of Two New Antifungal Agents, the Triazole Ravuconazole and the Echinocandin LY-303366, in an Experimental Model of Invasive Aspergillosis. *Antimicrob. Agents Chemother.*, 44, 3381-3388.

35. Sanati H., Belanger P., Fratti R. and Ghannoum M. (1997). A new triazole, voriconazole (UK-109,496), blocks sterol biosynthesis in Candida albicans and Candida krusei. *Antimicrob. Agents Chemother.* , 41, 2492-2496.

36. Espinel-Ingroff A. (1998). Comparison of In Vitro Activities of the New Triazole SCH56592 and the Echinocandins MK-0991 (L-743,872) and LY303366 against Opportunistic Filamentous and Dimorphic Fungi and Yeasts. *J. Clin. Microbiol*., 36, 198-202.

37. Urbina J.A., Lazardi K. and Aguirre T. (1991). Antiproliferative effects and mechanism of action of ICI 195,739, a novel bis-triazole derivative, on epimastigotes and amastigotes of Trypanosoma (Schizotrypanum) cruzi. *J. M. Ann. N.Y. Acad. Sci*., 544, 86.

38. Oakley K.L., Moore C.B. and Denning D.W. (1997). In vitro activity of SCH-56592 and comparison with activities of amphotericin B and itraconazole against Aspergillus spp. *Antimicrob. Agents Chemother.* , 41, 1124-1126.

39. Zitouni G.T., Kaplancikh Z.A., Yildiz M.T., Chevallet P. and Kaya D. (2005). Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. *Eur. J. Med. Chem*., 40, 607-613.

40. Walczak K., Gondela A. and Suwin´ski (2004). Synthesis and anti-tuberculosis activity of *N*-aryl-*C*-nitroazoles. *J. Eur. J. Med. Chem*., 39, 849-853.

41. Ainsworth C., Easton N.R., Livezey M., Morrison D.E. and Gibson W.R. (1962). The Anticonvulsant Activity of 1,2,4-Triazoles. *J. Med. Pharm. Chem*., 5, 383-389.

42. Kane J.M., Baron B.M., Dudley M.W., Sorensen S.M., Staeger M.A. and Miller F.P. (1990). 2,4-Dihydro-3H-1,2,4-triazol-3-ones as anticonvulsant agents. *J. Med. Chem*., 33, 2772-2777.

43. Mano T., Stevens R.W., Okumura Y., Kawai M., Okumura T. and Sakakibara M. (2005). 5-Lipoxygenase inhibitors: convenient synthesis of 4-[3-(4-heterocyclylphenylthio)phenyl]-3,4,5,6-tetrahydro-2*H*-pyran-4-carboxamide analogues. *Bioorg. Med. Chem. Lett*., 15, 2611-2615.

44. Holla B.S., Veerendra B., Shivananda M.K. and Poojary B. (2003). Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. *Eur. J. Med. Chem*., 38, 759-767.

45. Matesanz A.I., Joie C. and Souza P. (2010). Chemistry, anti proliferative activity and low nephrotoxicity of 3,5-diacetyl-1,2,4-triazol bis(^{4}N-thiosemicarbazone) ligands and their platinum(II) complexes. *J. Chem. Soc. Dalton Trans*., 7059-7065.

46. Souza P., Matesanz A.I. and Pastor C. (2002). Preparation and structural characterisation of a novel palladium (II) binuclear complex containing triazole bis thio semi carbazone bridges. *Inorg. Chem. Commun*., *5, 344-346*.

47. Holm S.C. and Straub B.F. (2011). Synthesis of *N*-Substituted 1,2,4-Triazoles. A Review.

48. Janam S.R. and Kumar S.D. (2010). Novel Syntheses of Some 1, 2, 4-Triazoles as Potent Bacteriocidal Agents. *Journal of Chemistry,* 7(1), 37-40.

49. Kaplaushenko A.G., Panasenko A.I., Knysh E.G. and Svintozelsky A.A. (2008). Farmatsevtichnii Zhurnal Kiev, Ukraine. 4, 57.

50. Zhong X.L., Shu-sheng Z., Zhi-qiang H. and Kui J. (2003). Studies on Synthesis and Biological Activities of Novel Triazole compounds Containing Thiophene Groups. *Chemical Research in Chinese Universities*. 19(3), 310-313.

51. Kim S.H., Kang S.W., Ahn H.J., Kim H.R. and YH Kim - KR (2009). Repub. Korean Kongkae Taeho Kongbo. 34.

52. Hull Jr J.W., Romer D.R. and Adaway T.J. (2009). Development of Manufacturing Processes for a New Family of 2, 6-dihaloaryl 1, 2, 4-triazole Insecticides. 13, 1125.

53. Churilov I.S., Popkov S.V., Grishina A.A., Chembarova E.V. and Mironova O.Y. (2008). Khimicheskaya Promyshlennost Segodnya. 8, 31.

54. Rida S.M., E.L. Hawash A.M., Hesham T.Y., Hazzaa A.A., Meligy E. and Mostafa M.M. (2006). Synthesis of novel benzofuran and related benzimidazole derivatives for evaluation of in vitro anti-HIV-1, anticancer and antimicrobial activities. *Archives of Pharmacal Research.* 29(10), 826-833.

55. Meide W.F., Leslie O.A., Jensema A.J., Peekel I., William R.F., Schallig F.H., Fat R.F. and Lai M.A. (2009). Evaluation of treatment with pentamidine for cutaneous leishmaniasis in Suriname. *International Journal of Dermatology.* 48, 52.

56. Qian G., Chun-ling Z., Wei-wei F. and Ju-zheng F. (2009). Huaxi Yaoxue Zazhi. 24, 475.

57. Bayer H.O., Cook R.S. and von Meyer W.C. (1972). 1 2 4-triazole metal salt complexes. *Chem. Abstr*., 76, 113224.

58. Jianxin GUO, Na XUE, Ting KANG, Bingqiang YE, Xinfa CHEN and Xin ZHANG (2014). Tao Patent: EP2799435A1, Location in patent: Paragraph 0049; 0068 ; 0069. Chengdu Di'ao Pharmaceutical Group Co. Ltd.

59. Zhang Qiu-Rong, Xue Deng-Qi He, Peng Shao, Kun-Peng, Chen Peng-Ju, Gu Yi-Fei, Ren Jing-Li, Shan Li-Hong, Liu Hong-Min (2014). Bio organic and Medicinal Chemistry Letters. 24(4), 1236 - 1238.

60. Merck Sharp and Dohme (2001). Therapeutically active 1,2,4-triazolo[4.,3-B] pyridazine derivatives as ligands for GABA receptors. Patent: US6313125 B1 2001.

61. Kikkeri N. Mohana, Basava Patna N., Kumar Prasanna and Lingappa Mallesha (2013). Synthesis and antiproliferative activity of some new fluorinated Schiff bases derived from 1,2,4-triazoles. *Journal of Fluorine Chemistry*, 156, 15-20.

62. Pelle Lidström, Jason Tierney, Bernard Wathey and Jacob Westman (2001). Microwave assisted organic synthesis-a review microwave assisted microwave assisted synthesis. *Tetrahedron*, 57, 9225-9283.