Design and Facile Synthesis of 6-(Benzo Thiopen-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 Thiopen-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, 1H-NMR, 13C 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 Phthalazine 1, 2, 4 Triazole linked thiophene with 2, 5 di fluoro nucleus has shown good antibacterial and antifungal activities.

Keywords: 1,4-di chloro Phthalazine, Tri azolo phthalazines, 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 antibiotics1,2. Hence, they have attracted considerable attention in the design of biologically active molecules3,4 and advanced organic chemistry5,6. 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 processes7.

Phthalazine derivatives, like the other members of the isomeric benzodiazine series, have been widely applied as therapeutic agents due to their anticonvulsant, cardiotoxic, vasorelaxant and antiinflammatory properties8,9. Majorities of the drugs used in human medicine are hetero cyclic compounds. Common drugs such as Morphine, Lipitor, Penicillin and non steroid anti-inflammatory agents contain at least one heteroatom in their structure10. Heterocyclic compounds containing nitrogen group have large are a in nature, and their utilization is becoming progressively important as biologically active pharmaceuticals, agrochemicals, and functional materials11. In particular, hydrazine containing hetero cyclic compounds have been considered of great importance on account of pharmacological properties and clinical applications12. Moreover, these of combined phthalazines have biological properties such as inhibition of p38MAPKinase13 for selective binding of GABA receptor14, antianxiety drug15, antitumor agent16, and high affinity ligand to the a2d-1 sub unit of calcium-channel17.

Phthalazine derivatives have been greatly used as therapeutic agents owing to their anticonvulsant, cardiotoxic, vasorelaxant, anti-inflammatory properties18–23 and antimicrobial activity24. Like azelastine, the phthalazine derivatives have antihistaminic effects in the treatment of allergic rhinitis25 and hydralazine is used as antihypertensive agent in the treatment of pulmonary hypertension26–28. Some commercially used phthalazine derivatives are shown in Figure-1.

Phthalazines are synthetically versatile substrates and hence can be used for the synthesis of a large variety of heterocyclic compounds. Phthalazines occupy a distinct and unique place in our life. This Hetero cyclic moiety has great biological and medicinal significance. Various synthetic aspects indicate that Phthalazine 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 C₆H₆N₃, 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 drugs29,30 are i. fluconazole31, 32, ii. itraconazole33, iii. ravuconazole34, iv. voriconazole35,36, v. ICI 15306637 and vi. posaconazole38 (Table-1). 1,2,4 tri azole core structure was shown in blue colour in Table-1.
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 signifying 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 leishmanial\(^{56}\), 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 PerkinElmer 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 triazole nucleus

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Anti fungal Drug Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flucanazole</td>
<td><img src="image1" alt="Flucanazole Structure" /></td>
</tr>
<tr>
<td>2</td>
<td>Itraconazole</td>
<td><img src="image2" alt="Itraconazole Structure" /></td>
</tr>
<tr>
<td>3</td>
<td>Ravuconazole</td>
<td><img src="image3" alt="Ravuconazole Structure" /></td>
</tr>
<tr>
<td>4</td>
<td>Voriconazole</td>
<td><img src="image4" alt="Voriconazole Structure" /></td>
</tr>
<tr>
<td>5</td>
<td>Posaconazole</td>
<td><img src="image5" alt="Posaconazole Structure" /></td>
</tr>
<tr>
<td>6</td>
<td>ICI</td>
<td><img src="image6" alt="ICI Structure" /></td>
</tr>
</tbody>
</table>
Reagents and Reaction conditions: i. Acetic acid, Hydrazine hydrate, Reflux, 4 hrs ii. POCl₃, Reflux, 6 hrs iii. Ethanol, Hydrazine hydrate, Na₂CO₃, RT iv. POCl₃, Reflux v. K₂CO₃, PdCl₂(P₃H₃)₂, 1,4-dioxane, H₂O, micro wave, 120°C.

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 5A⁰ and MS 4A⁰, respectively. MeCN and 1,2-dichloroethane (DCE) were distilled from CaH₂. EtOH was distilled from Mg/I₂ and dried over MS 3A⁰. Prior to use, POCl₃ 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 Na₂SO₄, 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 ¹H for ¹³C, respectively, in CDCl₃ 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 (¹H NMR and ¹³C NMR) were recorded using tetra methyl silane (TMS) in the solvent of CDCl₃-d₁ or DMSO-d₆ as the internal standard (¹H NMR: TMS at 0.00 ppm, CDCl₃ at 7.26 ppm, DMSO at 2.50 ppm; ¹³C NMR: CDCl₃ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for the preparation of 2,3-dihydrophthalazine-1,4-dione⁸ (Compound-2): The starting material Phthalic 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°C 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.: 300⁰C above also not melted.

¹H NMR (DMSO-d₆, 400 MHz): 8.1(d, 2H), 7.9 (d, 2H), 11.2 (bs, 2s). ¹³C NMR (DMSO-d₆, 100 MHz): 120,134,117,169 IR (KBr, cm⁻¹): O-H (3510, sharp), Ar (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\(^9\) (Compound-3): The compound (2) (10 m.mol) was added to a stirred solution of phosphorus oxychloride (15 ml). The mixture was heated to 110°C for 1 h. After the reaction was complete (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 the mixture was filtered through a Buchner funnel. The filter cake was washed with H\(_2\)O 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\(^6\) (Compound-4): 1,4-Dichlorophthalazine (20.0 g, 0.100 mol) was added to a boiling solution 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, 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)\(^6\)

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

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Yield (%)</th>
<th>Melting Point (°C)</th>
<th>Physical Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>80</td>
<td>102-104</td>
<td>White Solid</td>
</tr>
<tr>
<td>6b</td>
<td>82</td>
<td>183-184</td>
<td>Off white Solid</td>
</tr>
<tr>
<td>6c</td>
<td>76</td>
<td>126-127</td>
<td>White Solid</td>
</tr>
<tr>
<td>6d</td>
<td>76</td>
<td>143-144</td>
<td>Pale Yellow Solid</td>
</tr>
<tr>
<td>6e</td>
<td>73</td>
<td>115-116</td>
<td>Pale brown solid</td>
</tr>
<tr>
<td>6f</td>
<td>71</td>
<td>124-126</td>
<td>Brown solid</td>
</tr>
<tr>
<td>6g</td>
<td>78</td>
<td>190-191</td>
<td>Off white Solid</td>
</tr>
<tr>
<td>6h</td>
<td>75</td>
<td>168-169.2(^0)C</td>
<td>White Solid</td>
</tr>
<tr>
<td>6i</td>
<td>73</td>
<td>115-116</td>
<td>Plumpy White Solid</td>
</tr>
<tr>
<td>6j</td>
<td>72</td>
<td>127-128</td>
<td>Off white Solid</td>
</tr>
</tbody>
</table>

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

\(^1\)H NMR (400 MHz, d\(_6\)-CDCl\(_3\)) 8(2H,d), 7.9(2H,d), 7.4-8.3 (5H,m). \(^13\)C NMR (d\(_6\)-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], 31 (1, it indicates molecule contains one chlorine atom).

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

\(^1\)H NMR (400 MHz, d\(_6\)-CDCl\(_3\)) 8(2H,d), 7.9(2H,d), 7.4-8.3 (5H,m). \(^13\)C NMR (d\(_6\)-CDCl\(_3\), 100 MHz): 120-156 (13 Aromatic carbons). IR (KBr, cm\(^{-1}\)): 2957 (SP-H), 3066 (Ar-H), 1574 (C=C), 1468 (C=N), 660 (C-Cl).

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.
\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.85(2H,d), 3.85 (3H, s), 8.1(2H,d), 7.03(2H,d). \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.83(2H,d), 8.1(2H,d), 8.4 (2H,d), 1.3(2H,d), 3.75(2H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 113-2-163 (13 Aromatic carbons), 56.5 (Aromatic methoxy carbon). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 3036 (Ar-H), 1582 (C=C), 1438 (C=N), 654 (C-Cl), C-F (1260). \]

\[ \text{EI-MS (m/z):} \quad 316 [M^+], 318[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6d):} \]

\[ \text{C NMR (d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.83(2H,d). \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.85(2H,d), 3.85 (3H, s), 8.1(2H,d), 7.03(2H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 124-156 (13 Aromatic carbons). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 3160 & 1520(N-O Symmetric and asymmetric Stretching in nitro group), 3046 (Ar-H), 1574 (C=C), 1468 (C=N), 636 (C-Cl), \]

\[ \text{EI-MS (m/z):} \quad 325 [M^+], 327[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6e):} \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.83(2H,d), 3.85 (3H, s), 3.88(3H, s), 7.3(1H,d, J=2.4 Hz), 7.53(1H,d), 6.9(1H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 110-157 (15 Aromatic carbons), 56.5 (Aromatic methoxy carbons). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 2988 (SP^3 C-H), 3046 (Ar-H), 1554 (C=C), 1438 (C=N), 676 (C-Cl), C-O-C (1060 & 1230). \]

\[ \text{EI-MS (m/z):} \quad 340 [M^+], 342[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(2,5-difluorophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6g):} \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 7.9(2H,d), 7.83(2H,d), 7.3(1H,d), 7.2(2H,dd), 7.5(1H,dd, J_{H-H} = 2.4Hz). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 115-158 (15 Aromatic carbons). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 3036 (Ar-H), 1582 (C=C), 1438 (C=N), 654 (C-Cl), C-F (1260). \]

\[ \text{EI-MS (m/z):} \quad 316 [M^+], 318[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(4-(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6h):} \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 7.9(2H,d), 7.85(2H,d), 8.6(2H,d), 7.7(2H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 125-156 (13 Aromatic carbons), 124.3 (Tr fluoro methyl carbon). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 3066 (Ar-H), 1584 (C=C), 1448 (C=N), 664 (C-Cl), C-F (1278). \]

\[ \text{EI-MS (m/z):} \quad 348 [M^+], 350[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6i):} \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 7.9(2H,d), 7.85(2H,d), 8.02(2H,d), 7.06(2H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 123-156 (13 Aromatic carbons), 129.8 (Tr fluoro methyl carbon). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 3066 (Ar-H), 1584 (C=C), 1448 (C=N), 664 (C-Cl), C-F (1278), 1084(C-O-C). \]

\[ \text{EI-MS (m/z):} \quad 364 [M^+], 366[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

\[ \text{6-chloro-3-(2,4-dinitrophenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6j):} \]

\[ \text{H NMR (400 MHz, d}_1\text{-CDCl}_3) \quad 8(2H,d), 7.83(2H,d), 8.95(1H,s), 8.74(1H,d), 8.34 (1H,d). \]

\[ ^{13}\text{C NMR (d}_1\text{-CDCl}_3, 100 MHz): \quad 123-156 (15 Aromatic carbons). \]

\[ \text{IR (KBr, cm}^{-1}) : \quad 1350 & 1540(N-O Symmetric and asymmetric Stretching in nitro group), 3046 (Ar-H), 1574 (C=C), 1468 (C=N), 667 (C-Cl), \]

\[ \text{EI-MS (m/z):} \quad 370 [M^+], 372[M+2], (3:1, it indicates molecule contains one chlorine atom). \]

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-y1)-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)32:

A mixture of 6a-6j (0.6 mmole), benzo[b]thiophen-3-ylboronic acid (7) (0.9 mmol), K2CO3 (3.3 mmol) and PdCl2(PPh3)2 (0.03 mmol), 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 Na2SO4 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)

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

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

1H NMR (400 MHz, d6-DMSO) 7.88(2H,d), 7.92(2H,d), 7.4-8.3 (5H, m), 7.95(1H,d, J=3Hz), 7.7(1H,dd, J=7Hz, J=3Hz), 7.3(1H,d, J=7Hz), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d).

13C NMR (d6-DMSO, 100 MHz): 120, 125, 128, 130, 132, 139, 148, 151, 153. IR (KBr, cm-1): 691 (C=C), 1544, 1428 (C=N), 678 (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):

1H NMR (400 MHz, d6-DMSO) 7.8(2H,d), 7.92(2H,d), 7.4-8.3 (5H, m), 7.95(1H,d, J=3Hz), 7.7(1H,dd, J=7Hz, J=3Hz), 7.3(1H,d, J=7Hz), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d), 7.3(1H,d).

13C NMR (d6-DMSO, 100 MHz): 120, 125, 128, 130, 132, 139, 148, 151, 153, 23 (Aromatic methyl carbon).

IR (KBr, cm-1): 2959 (SP C-H), 3068 (Ar-H), 1544 (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):

1H NMR (400 MHz, d6-DMSO) 7.8(2H,d), 7.9(2H,d), 7.4-8.3 (5H, m), 7.95(1H,d, J=3Hz), 7.7(1H,dd, J=7Hz, J=3Hz), 7.3(1H,d, J=7Hz), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d).

13C NMR (d6-DMSO, 100 MHz): 120, 125, 128, 130, 132, 139, 148, 151, 153, 56 (Aromatic methyl carbon).

IR (KBr, cm-1): 2959 (SP C-H), 3068 (Ar-H), 1544 (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):

1H NMR (400 MHz, d6-DMSO) 7.8(2H,d), 7.9(2H,d), 7.4-8.3 (5H, m), 7.95(1H,d, J=3Hz), 7.7(1H,dd, J=7Hz, J=3Hz), 7.3(1H,d, J=7Hz), 7.5(2H,q), 8.5(1H,d), 7.8(1H,d).

13C NMR (d6-DMSO, 100 MHz): 120, 125, 128, 130, 132, 139, 148, 151, 153, 56 (Aromatic methyl carbon).
6-(benzo[b]thiophen-3-yl)-3-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (8c):

6-(benzo[b]thiophen-3-yl)-3-(4-trifluoromethylphenyl)-[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):
Biological Activity: Antibacterial activity: The antibacterial activity was checked against fungi Aspergillus niger (A. niger) and Candida albicans (C. albicans). The results were compared with stand drugs Sparfloxacin, Benzylic penicillin and Fluconazole. The Pthalazine-1,2,4 triazole derivates containing Thiophene core structure with 2,5 di flouro (8g) and –CF₃ (8h) showed more activity than other substituents’ 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 derivates. 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, ¹H and ¹³C NMR and Mass spectra and all the synthesized compounds [8a-8j] were screened for antimicrobial activity.

Chemistry: The Title Compounds Novel 6-(benzo Thiophene-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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anti-Bacterial activity (Zone if Inhibition in mm)</th>
<th>Anti-Fungal Activity (Zone if Inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>8a</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>8b</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>8c</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>8d</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>8e</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>8f</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>8g</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>8h</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>8i</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>8j</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Benzylic penicillin</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

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2,3-dihydrophthalazine-1,4-dione (2) was synthesised from Phthalic 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 POCl₃ 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 (5a-5j) in POCl₃ at reflux to form fused 1,2,4 triazole 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, ¹H and ¹³C NMR, mass Spectroscopic Techniques. All of the Pthalazine triazoles 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. 3100 cm⁻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 SP³ 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. 1160 cm⁻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 Bacterio mycin. 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 ¹H NMR, ¹³C, 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 triazole derivatives 8 (a-j) were synthesised in good yield, characterised by different spectral studies and their antimicrobial 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


