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Mediterranean Journal of Chemistry **2019**, 8(4), 261-269

Synthesis of new analogs of 3-methyl-[1,2,4] triazolo [3,4-*a*] phthalazines *via* Suzuki coupling and evaluation of their anticancer and antimicrobial activity

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Abstract: A series of new N-aryl substituted phenyl acetamide analogs of 3-methyl-[1,2,4] triazolo[3,4-*a*] phthalazines were synthesized starting from commercially available, in-expensive phthalic anhydride in good yields (65-75 %) *via* Suzuki Coupling. These compounds were tested for inhibition activity against HCT 116 cancer cell line by using MIT assay. Among the library of compounds, N-(3-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide followed by 2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(m-tolyl) acetamide and N-(3-chlorophenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide were found to be active compounds with IC_{50} of 70 to and 90 µg mL^{-1}. Further, the compounds were also screened for their antimicrobial activities.

Keywords: Phthalazine; Triazole; Anticancer; HCT 116; Antimicrobial activity.

Introduction

One among the clinical stage dependant deadliest disease is cancer. Cancer occupies its place next to cardiovascular disease and diabetes in decreasing the lifetime of a human being [1]. Recent statistics on cancer studies reveal the fact that several incidences of cancer and cancer deaths are crossing alarming levels. In 2012 globally 14.1 million new cancer cases were reported and out of which 8.2 million deaths were recorded [2].

Heterocycles are relevant pharmacophoric units in the natural products as well as in the synthetic drug molecules. They found in various biologically active agents and exhibits diverse bio-activities. Among these compounds, nitrogen-containing heterocyclic compounds have good applications in the biological system such as pharmaceuticals, pyrotechnics, explosives and chemotherapy [3]. Among the nitrogen-containing heterocyclic compounds, phthalazine derivatives form a structural profile for biologically active molecules [4-9,] and hence they play a vital role in the biological systems. Phthalazine derivatives show significant biological activity such as antifungal [10,11], antimicrobial and antitumor agents [12, 13]. Azelastine (**A**, Fig. 1) MY5445 (**B**, Fig. 1) which are the phthalazine derivatives having the remarkable active drugs. Azelastine is used for the treatment of allergic rhinitis [14]; it is an antihistamine drug. MY5445 is a suitable inhibitor of cGMP-inhibited phosphodiesterase (PDE) leads to inhibition of human platelet aggregation [15-18]. The phthalazine derivative Zopolrestat (C, Fig. 1) has been in the clinical trials; in the prevention of neuropathy, retinopathy and cataract formation in diabetes [19]. The compound luminol (D, Fig. 1) has found in analytical applications like chemiluminescent reactions [20-23].

Similarly, 1,2,4-triazole derivatives itself shows significant biological activities such as anti-inflammatory [24], anti-HIV [25], antibacterial [26,27] and antiplatelet [28] activities. Recently these derivatives show the thymidine phosphorylase inhibiting activity [29] Thymidine phosphorylase (TP) is over expressed in various solid tumors, and this inhibition activity can offer distinctive target suitable for the discovery of cancer drugs, and these derivatives have carbonic anhydrase II inhibition activity [30].

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DOI: http://dx.doi.org/10.13171/mjc841905257hbb

Received February 27, 2019
Accepted April 11, 2019
Published May 25, 2019

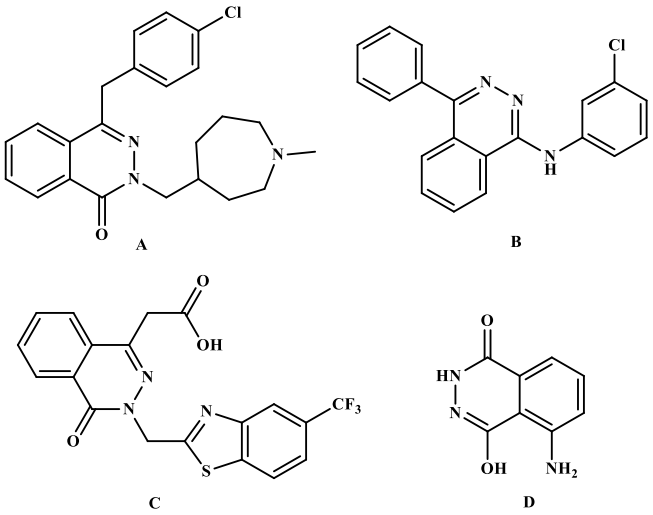
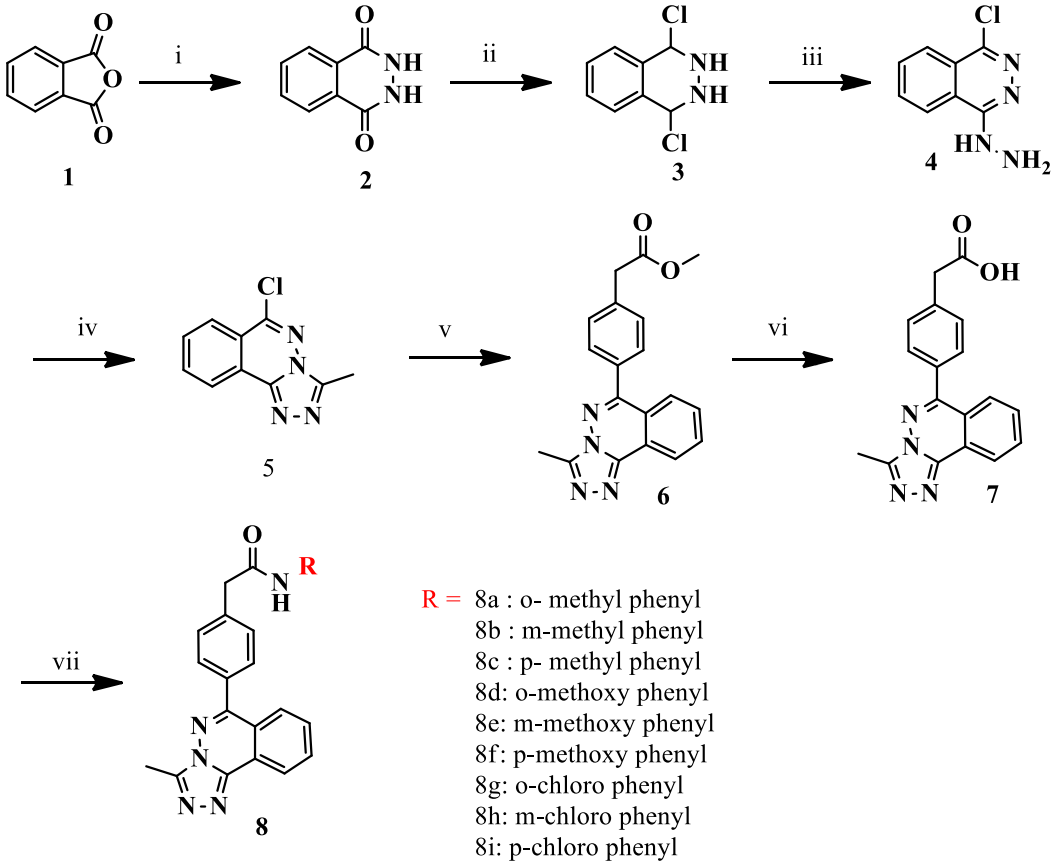


Figure 1. Important Phthalazine derivatives

So, in the present study, we considered it is worth to design a new derivative series, which are the combination of phthalazine and 1,2,4-Triazole units for potential anticancer compounds.

Day by day, the numbers of synthetic drugs are found to be increasing and are validated against clinical stage dependent carcinomas. One of the widely studied colorectal cancer cell line *in vitro* is HCT116. The tumor progression and drug resistance pathways are well reported in HCT116 CRC cell line [31, 32]. In the present study as a continuous effort for developing new anticancer compounds [33-36] we synthesized and screened some phthalazine analogs with the above discussed structural units and reported their anticancer activity on HCT116 colon carcinoma cell line and antimicrobial activities on five different organisms.



Scheme 1. Synthetic protocol of 3-Methyl-[1,2,4]triazolo [3,4-*a*] phthalazine analogs

Reagents and conditions: (i): Hydrazine hydrate, CH_3COOH, 120 °C, 4h, 83.77 %; (ii): $POCl_3$, 110 °C, 1h, 85.7 %; (iii): Hydrazine hydrate, C_2H_5OH, 78 °C, 1h, 81.84 %; (iv): CH_3COCl, Dioxane, $N(C_2H_5)_3$, 110 °C, 2h, 70.0 %; (v): (4-(2-methoxy-2-oxoethyl) phenyl) boronic acid, X-phos, $Pd_2(dba)_2$, K_2CO_3, H_2O, Dioxane, 110 °C, 3h, 45.4 %; (vi): KOH, CH_3OH, rt, 2h, 96.7 %; (vii) T3P, N,N-Diisopropylethylamine, DCM, rt, 3h.

Results and Discussion

The synthetic protocol for the synthesis of new analogs of 3-methyl-[1,2,4] triazolo[3,4-*a*] phthalazines has shown in Scheme 1. In the first step of the synthesis, commercially available, in-expensive phthalic anhydride (**1**) was reacted with hydrazine hydrate in acetic acid under reflux conditions afforded phthalazine (**2**) in excellent yield. In the next step, phthalazine was treated with phosphorus oxychloride at 110 °C to get 1,4-dichlorophthalazine [37-39] (**3**) in excellent yield. In step three of the synthetic sequence, compound **3** was treated with hydrazine hydrate in ethanol solvent at refluxing temperature produced 1-choro-4-hydrazinophthalazine (**4**) in very excellent yield. The obtained compound **4** was dissolved in dioxane, acetyl chloride and triethylamine were added. The reaction mixture was subjected to reflux for 12 h, to get the compound **5** in good yield.

Upon achieving the compound **5**, it was treated with Boronic acid Tris(dibenzylidene acetone) dipalladium (0) ($Pd_2(dba)_2$), compound **6** was obtained in poor yield through Suzuki reaction. The formed ester derivative (**6**) undergoes ester hydrolysis by using potassium hydroxide in methanol as solvent yields acid derivative (**7**) in quantitative yield. Finally, the novel acid derivative on reaction with various aromatic amines and propylphosphonic anhydride (T_3P) reagent in DCM solvent yields compounds **8a-8i,** and the results were mentioned in Table 1.

Table 1. Synthetic and *In vitro* anticancer activity results of the compounds **7, 8a-8i.**

Compound	R-	Yield %	IC_{50} in µg mL^{-1}
8a	*o*-methyl phenyl	65.2	162.4
8b	*m*-methyl phenyl	69.2	74.6
8c	*p*-methyl phenyl	72.2	98.6
8d	*o*-methoxy phenyl	74.1	125.1
8e	*m*-methoxy phenyl	70.1	57.4
8f	*p*-methoxy phenyl	66.2	101.1
8g	*o*-chloro phenyl	78.2	132.9
8h	*m*-chloro phenyl	68.12	75.6
8i	*p*-chloro phenyl	75.22	98.6
7	-	96.7	>200
Doxorubicin	-	-	0.260

The compound **8g** with the *ortho*-chloro substitution was obtained in a higher yield of 78.2 % and followed by compounds **8i** and **8d** of about 75 % and 74 % yield with *para*- chloro and *ortho*- methoxy substitutions respectively. The compound **8a** with *ortho*- methyl substitution was found to be obtained with the lowest yield.

All the molecules were tested for their anticancer activity on HCT116 cancer cell line, and results were presented in Table 1.

The compounds with a *meta*-methoxy substitution (**8e**) were found to possess the highest activity (IC_{50} = 57.4 µg mL^{-1}) moreover it was also observed that the compounds **8b** and **8h** with *meta*-methyl and *meta*- chloro substitutions were found to possess almost equal activities (IC_{50} = 74.6; 75.6 µg mL^{-1}**)**. Similarly, the compound **8c** and **8i** with *para* methyl and *para*- chloro substitutions were also shown similar activity, i.e. IC_{50} = 98.6 µg mL^{-1}. Further, close observation on the substituted compounds showed the highest activity for meta-substitutions followed by *para*- substituted compounds. The *ortho*- substituted compounds possess the lowest activity. Further, compound **7** with acid functionality before conversion into its amide possesses activity >200 µg mL^{-1}, which established the importance of the present structural unit.

The compounds were screened for their antimicrobial activities on five different pathogens, and the results were presented in Table 2. The results showed that the acid compound **7** displayed a significant activity on *Candida albicans* (with a zone of inhibition of 0.8 Cm) followed by compounds **8h** and **8a** with a zone of inhibition zones 0.7 and 0.6 Cm respectively, whereas the positive tetracycline control was showed a zone of inhibition of 1.3 Cm. The compounds **8c** and **8h** were found to be moderately active against *Staphylococcus aureus* when compared to Tetracycline. Similarly, the compounds **7** and **8f** were also moderately active against *Escherichia coli.*

Table 2. The results of antimicrobial activities of the phthalazine derivatives.

Comp.	Zone of inhibition (cm)				
	SA	**BM**	**PA**	**CA**	**EC**
8a	0.5	0.5	0.5	0.6	-
8b	0.5	0.6	0.6	0.4	-
8c	0.9	0.5	0.5	0.2	-
8d	0.6	0.6	0.6	0.2	0.5
8e	-	-	-	-	-
8f	0.5	0.5	-	0.2	0.7
8g	0.5	0.5	-	-	0.5
8h	0.8	0.8	0.5	0.7	0.6
8i	0.5	0.5	-	0.5	-
7	0.5	0.5	0.8	0.8	0.8
Tetracycline	1.6	1.7	1.7	1.3	1.6

SA - Staphylococcus aureus; BA - Bacillus Megaterium; PA-Pseudomonas aeuroginosa; CA-Candida albicaus; EC - Escherichia coli.

Conclusion

The synthesized novel target molecules were tested against the Human carcinoma cell line. Among the synthesized compounds N-(3-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide showed prominent activity, 2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(o-tolyl) acetamide and N-(3-chloro-phenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide compounds showed good activity at nearly same concentration. Among the novel derivatives, *meta*-substituted derivatives showed better results than the other compounds. After to *meta*-substituted compounds *para* substituted derivatives showed average results against HCT 116 cell line. However, *ortho* substituted compounds showed poor results. The compounds 2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)acetic acid**,** N-(3-chlorophenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide **,** 2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(p-tolyl) acetamide, and N-(4-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]-triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide were significant in respect of antimicrobial activities.

Experimental

The progress of the reactions was monitored by thin layer silica gel plates (Merck Manufactures). All the compounds synthesized (**8a-8i**) were characterized by ^{1}H NMR (at 400 MHz), ^{13}C NMR (at 100 MHz) with Tetramethylsilane as an internal standard and HRMS spectral data.

Preparation of 2,3-di hydro phthalazine-1,4-dione (2)

The phthalic anhydride (6.01 g 40 mmol) was taken in 25 mL of the acetic acid solution, and the mixture was heated to 60 **°C**. At this stage (2.8 mL, 44 mmol) of hydrazine hydrate was added slowly and was heated to 120 **°C** for 4 hours. TLC observed the completion of the reaction, and the reaction mixture was cooled to room temperature after completion. The formed precipitate was filtered through Buckner funnel. Later the precipitate was washed with petroleum ether (2 x 320 mL) and dried under vacuum, the compound **2** (5.50 g, 83.77 %) was obtained as white solid. M.p. 181-183 °C.
^{1}H NMR (300 MHz, DMSO): δ 7.59-7.63 (m, 2H), 8.14 (d, *J* = 7.9 Hz, 2H), 9.04 (s, 2H).

Preparation of 1,4-dichlorophthalazine (3)

The compound (**2**) (2.0 g 12 mmol) was added to a stirred solution of Phosphoryl chloride (5 mL). Now the reaction mixture was heated to 110 **°C** until compound **2** was completely soluble. TLC monitored the reaction completion. The reaction mixture cooled to room temperature. The reaction mixture was poured in crushed ice which was taken in ethyl acetate (500 mL) with continuous stirring. Then the ethyl acetate layer was separated and neutralised with triethylamine and then washed with water (2 x 500 mL). The ethyl acetate layer was separated, dried with Na_2SO_4 and concentrated under vacuum, the compound **3** (2.1 g, 85.7 %) was obtained as white solid. Mp: 192 – 193 °C;
^{1}H NMR (400 MHz, DMSO) δ 8.29 (d, *J* = 7.6 Hz, 1H), 8.11- 7.94 (m, 3H).

Preparation of 1-chloro-4-hydrazinyl Phthalazine (4)

The compound (**3**) (2.0 g, 10 mmol) was dissolved in ethanol (10 mL). Hydrazine hydrate (3.73 mL, 76.5 mmol) was added to the reaction mixture, heated to reflux for 0.54 h. TLC monitored reaction completion. The reaction mixture was cooled to room temperature and filtered through Buckner funnel. The filter cake was washed with diethyl ether (2 x10 mL)

and dried under vacuum, the compound **4** (1.6 g, 81.84 %;) was obtained. Mp: 259-260 °C;
^{1}H NMR (400 MHz, DMSO) δ 8.32 - 8.27 (m, 1H), 8.21 (s, 1H, NH), 8.07 - 8.03 (m, 1H), 8.03 - 7.99 (m, 1H), 7.98 - 7.95 (m, 1H), 7.89 (s, 2H, NH_2).

Preparation of 6-chloro-3-methyl-[1,2,4]-triazolo-[3,4-a]phthalazine (5)

The compound (**4**) (1.6 g, 8.22 mmol) was dissolved in Dioxane. TEA (1.14 mL, 8.22 mmol) and acetyl chloride (9.868 mmol) were added. The reaction mixture was heated to 110 °C. TLC monitored reaction completion. The solvent was evaporated, left solid mixtures portioned between DCM (200 mL) and water (3x100 mL). The DCM layer was dried with Na_2SO_4, filtered and concentrates on yielding a solid. The obtained compound purified by silica gel column chromatography to give compound (**5**) (1.25 g, 70 %). Mp: 169–170 °C;
^{1}H NMR (400 MHz, CDCl3) δ 8.64 (d, *J* = 7.9 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 7.99 (t, *J* = 7.6 Hz, 1H), 7.85 (t, *J* = 7.8 Hz, 1H), 2.81 (s, 3H, CH_3).

Preparation of methyl 2-(4-(3-methyl-[1,2,4-]triazolo[3,4-a]phthalazin-6- yl)phenyl)acetate (6)

The compound (**5**) (1.25 g, 5.71 mmol) was added to a stirred solution of dry Dioxane (10 mL). Later the Boronic acid (1.781 g 8.565 mmol) was added to the stirred solution. Further, X-phos (0.6 mmol) was added to the above mixture, potassium carbonate (11.42 mmol) and 1 mL water were added. Degassing the reaction mixture using Nitrogen gas. Tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_2$) (0.2 mmol) was added to the reaction mixture. Then the mixture was heated to reflux 110 ˚C for 3 h. The TLC monitored the reaction completion. The reaction mixture filtered through celite bed. Later the solvent present in the filtrate was removed under reduced pressure, purification carried by silica gel column chromatography to yield compound **6** (0.9 g, 45.4 %).

Methyl-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]-phthalazin-6-yl)phenyl)acetate (6)

Brown solid, yield 45.4 %; Mp: 151-153 °C; R_f 0.6 (methanol: dichloro methane, 0.05: 1.0);
^{1}H NMR (400 MHz, DMSO) δ 8.59 (d, *J* = 7.02 Hz, 1H), 8.08 - 8.04 (m, 1H), 7.87 (d, *J* = 4Hz, 2H), 7.68 (d, *J* = 4 Hz, 2H), 7.53 (d, *J* = 4 Hz, 2H), 4.10 (s, 3H), 3.75 (s, 2H), 2.72 (s, 3H).
^{13}C NMR (100 MHz, DMSO) δ 171.9, 154.3, 152.8, 146.1, 144.1, 135.7, 135.9, 132.8, 132.5, 127.2, 125.2, 125.0, 122.9, 121.9, 120.7, 120.3, 118.2, 53.9, 44.2, 9.9.
HRMS (ESI): Calcd. $[M+H]^+$m/z: 333.1280, Found: 333.1273.

Preparation of 2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)acetic acid (7)

The compound (**6**) (0.9 g, 12.60 mmol) was dissolved in methyl alcohol, and KOH (2.60 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 2 h. The reaction completion was monitored by the TLC. The reaction mixture is neutralised with 2M HCl formed solid was filtered. The obtained compound **7** (0.8 g, 96.7 %;) is in brown.

2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)acetic acid (7)

Brown solid, yield 96.7 %; Mp: 211-213 **°C**; R_f 0.30 (methanol: dichloro methane, 0.1: 1.0);
^{1}H NMR (400 MHz, DMSO) δ 12.43 (s, 1H), 8.56 (d, J = 4 Hz, 1H), 8.06 - 8.00 (m, 1H), 7.85 - 7.82 (m, 2H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.50 (d, *J* = 7.6 Hz, 2H), 3.72 (s, 2H), 2.69 (s, 3H).
^{13}C NMR (100 MHz, DMSO) δ 177.8, 155.5, 153.8, 146.9, 144.4, 135.9, 132.9, 132.5, 127.4, 125.2, 125.1, 123.0, 121.9, 120.7, 120.3, 118.3, 49.1, 9.6.
HR-MS(ESI): Calcd. $[M+H]^+$m/z: 319.1156, Found: 319.1184.

General procedure for the synthesis of 8a to 8i

The compound **7** (50 mg 0.157 mmol) was added to a stirred solution of DCM (3 mL). After dissolving propyl phosphonic anhydride solution (T_3P) (0.157 mmol) was added to the reaction mixture. N, N- Diisopropyl ethyl amine (0.188 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 h. After the reaction completion, the reaction mixture was portioned between DCM (1x20 mL) and water (2x10 mL). The DCM layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography to give 8a-8i with good yield, ^{1}H NMR, HR-MS**(ESI) data of each compound were given below.**

2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(o-tolyl) acetamide (8a)

Brown solid, yield 65.2 %; Mp: 163-165 °C; R_f 0.55 (methanol: dichloro methane, 0.1: 1.0);
^{1}H NMR (400 MHz, DMSO) δ 9.55 (s, 1H), 8.57 (d, *J* = 8 Hz, 1H), 8.069 - 8.028 (m, 1H), 7.87 - 7.84 (m, 2H), 7.68 (d, *J* = 8 Hz, 2H), 7.60 (d, *J* = 8 Hz, 2H), 7.40 (d, *J* = 8 Hz, 1H), 7.40 (d, *J* = 8 Hz, 1H), 7.20 (d, *J* = 8 Hz, 1H), 7.15 (t, *J* = 8 Hz, 1H), 7.073 (t, *J* = 8 Hz, 1H), 3.82 (s, 2H), 2.69 (s, 3H), 3.82 (s, 3H).
^{13}C NMR (100 MHz, DMSO) δ 169.1, 156.5, 147.8, 142.2, 138.7, 136.6, 134.3, 132.2, 131.2, 130.7, 129.8, 129.1, 126.3, 125.7, 123.8, 122.9, 122.8, 42.9, 18.3, 9.9.
HR-MS(ESI): Calcd. $[M+H]^+$m/z: 408.1355, found: 408.1368.

2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(m-tolyl) acetamide (8b)

Brown solid, yield 69.2 %; Mp: 160-162 ˚C; $R_{f\ 0.55}$ (methanol: dichloro methane, 0.1: 1.0);
^{1}H NMR (400 MHz, DMSO) δ 10.14 (s, 1H, NH), 8.56 (d, *J* = 8 Hz, 1H, ArH), 8.06 -8.02 (m, 1H, ArH), 7.83 (d, *J* = 4 Hz, 2H, ArH), 7.67 (d, *J* = 8 Hz, 2H, ArH), 7.57 (d, *J* = 8 Hz, 2H, ArH), 7.45 (s, 1H, ArH), 7.39 (d, *J* = 8 Hz, 1H, ArH), 7.17 (t, *J* = 8 Hz, 1H, ArH), 6.85 (d, *J* = 8 Hz, 1H, ArH), 3.77 (s, 2H, CH_2), 2.68 (s, 3H, CH_3), 2.26 (s, 3H, CH_3),
^{13}C NMR (100 MHz, DMSO) δ 169.1, 156.5, 147.8, 142.2, 139.5, 138.5, 138.3, 134.3, 132.9, 131.2, 130.2, 129.9, 129.1, 129.0, 124.4, 123.7, 122.9, 122.8, 120.1, 116.8, 43.6, 21.6, 9.9.
HR-MS(ESI): Calcd. $[M+H]^+$m/z: 408.1355, found: 408.1323

2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)-N-(p-tolyl) acetamide (8c)

Light cream solid, yield 72.2 %; Mp: 166-168 ˚C; R_f 0.55 (methanol: dichloro methane, 0.1: 1.0);
^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H, NH), 8.56 (d, *J* = 8 Hz, 1H, ArH), 8.06 -8.02 (m, 1H, ArH), 7.84 (d, *J* = 4 Hz, 2H, ArH), 7.67 (d, *J* = 8 Hz, 2H, ArH), 7.57 (d, *J* = 8 Hz, 2H, ArH), 7.49 (d, *J* = 8 Hz, 2H, ArH), 7.09 (d, *J* = 8 Hz, 2H, ArH), 3.76 (s, 2H, CH_2), 2.65 (s, 3H, CH_3), 2.23 (s, 3H, CH_3).
^{13}C NMR (100 MHz, DMSO) δ 169.2, 156.5, 147.9, 142.3, 139.6, 138.6, 134.4, 133.0, 131.4, 131.0, 129.5, 129.2, 129.0, 124.6, 123.9, 123.0, 122.9, 120.0, 116.9, 43.9, 21.8, 9.9.
HR-MS(ESI): Calcd. $[M+H]^+$m/z: 408.1355, found: 408.1357.

N-(2-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide (8d)

Dark brown solid, yield 74.1 %; Mp: 175-177 **°C;** R_f 0.40 (methanol: dichloro methane, 0.1: 1.0); ^{1}H NMR (400 MHz, DMSO) δ 9.39 (s, 1H), 8.54 (d, *J* = 8 Hz, 1H), 8.05-8.01 (m, 1H), 7.94 (d, *J* = 4 Hz, 2H), 7.82 (d, *J* = 4Hz, 2H, ArH), 7.67 (d, *J* = 8 Hz, 2H, ArH), 7.07 to 7.02 (m, 2H, ArH), 6.86 to 6.90 (m, 1H), 3.89 (s, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 2.68 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 169.0, 156.6, 150.1, 147.9, 142.3, 138.7, 134.3, 131.3, 130.2, 130.0, 129.2, 127.7, 125.0, 123.8, 123.0, 122.9, 122.3, 120.7, 111.6, 56.2, 43.2, 9.9. HR-MS(ESI): Calcd. $[M+H]^+$m/z: 424.1280, found: 424.1225.

N-(3-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide (8e)

White solid, yield 70.1 %; Mp: 172-174 **°C;** R_f 0.40 (methanol: dichloro methane, 0.1: 1.0); ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.56 (d, *J* = 8 Hz, 1H), 8.06-8.02 (m, 1H), 7.84 (d, *J* = 3.6Hz, 2H), 7.67 (d, *J* = 8 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.32 (s, 1H), 7.22 - 7.13 (m, 2H), 6.62 (d, *J* = 4 Hz, 1H), 3.78 (s, 2H), 3.71 (s, 3H), 2.65 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 169.3, 160.0, 156.5, 147.9, 142.3, 140.8, 138.4, 134.4, 133.0, 131.3, 130.2, 130.0, 129.9, 129.2, 123.8, 122.9, 122.8, 122.7, 111.9, 109.3, 105.2, 55.4, 43.6, 9.9. HR-MS(ESI): Calcd. $[M+H]^+$m/z: 424.1280, found: 424.1281.

N-(4-methoxyphenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide (8f)

White solid, yield 66.2 %; Mp: 178-180 **°C;** Rf 0.40 (methanol: dichloro methane, 0.1: 1.0); ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 8.55 (d, *J* = 8 Hz, 1H), 8.04 (d, *J* = 4 Hz, 1H), 7.84 (s, 1H), 7.68 (d, J = 8 Hz, 2H), 7.59 (d, *J* = 4 Hz, 2H), 7.54 (d, *J* = 8 Hz, 2H), 6.88 (d, *J* = 12 Hz, 2H), 3.77 (s, 2H), 3.71 (s, 3H), 2.69 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 168.7, 156.0, 155.2, 138.1, 133.8, 132.4, 132.3, 130.8, 129.4, 128.7, 123.3, 122.5, 122.3, 120.6, 113.8, 55.1, 43.0, 9.5. HR-MS(ESI): Calcd. $[M+H]^+$ m/z: 424.1280, found: 424.1285.

N-(2-chlorophenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl) acetamide (8g)

Brown solid, yield 78.2 %; Mp: 167-169 **°C;** R_f 0.50 (methanol: dichloro methane, 0.1: 1.0); ^{1}H NMR (400 MHz, DMSO) δ 9.62 (s, 1H), 8.37 (d, J = 8 Hz, 1H), 7.87 - 7.83 (m, 1H), 7.67 - 7.63 (m, 2H), 7.54 - 7.48 (m, 3H), 7.41 (d, *J* = 4Hz, 2H), 7.32 - 7.29 (m, 1H), 7.15 - 7.11 (m, 1H), 7.02 - 6.98 (m, 1H), 3.70 (s, 2H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 170.1, 155.2, 151.3, 146.3, 143.4, 139.4, 135.9, 135.1, 132.9, 132.8, 130.3, 130.1, 127.2, 126.9, 124.9, 123.2, 121.6, 121.4, 120.2, 115.3, 42.7, 9.1. HR-MS(ESI):Calcd. $[M+H]^+$m/z: 428.0764, found: 428.0769.

N-(3-chlorophenyl)-2-(4-(3-methyl-[1,2,4]-triazolo[3,4-a]phthalazin-6-yl)phenyl)acetamide (8h)

Brown solid, yield 68.12 %; Mp: 165-167 **°C;** R_f 0.50 (methanol: dichloro methane, 0.1: 1.0); ^{1}H NMR (400 MHz, DMSO) δ 10.42 (s, 1H), 8.56 (d, *J* = 8 Hz, 1H), 8.06 - 8.02 (m, 1H), 7.87 - 7.79 (m, 2H, ArH), 7.68 - 7.63 (m, 3H), 7.57 (d, *J* = 4 Hz, 2H), 7.46 (t, *J* = 8Hz, 2H), 7.33 (t, *J* = 8 Hz, 1H), 7.10 (d, *J* = 8 Hz, 1H), 3.80 (s, 2H), 2.69 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 169.9, 155.2, 152.2, 146.0, 143.6, 139.7, 135.9, 135.5, 132.7, 132.6, 130.2, 130.1, 128.2, 126.9, 124.9, 122.9, 121.6, 121.4, 121.2, 115.9, 42.9, 9.3. HR-MS(ESI): Calcd. $[M+H]^+$m/z: 428.0764, found: 428.0765.

N-(4-chlorophenyl)-2-(4-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)acetamide (8i)

Brown solid, yield 75.22 %; Mp: 169-171 **°C;** R_f 0.50 (methanol: dichloro methane, 0.1: 1.0);
1H NMR (400 MHz, DMSO) δ 10.36 (s, 1H), 8.56 (d, *J* = 8 Hz, 1H), 8.04 (p, *J* = 4 Hz 1H), 7.83 (d, *J* =

4 Hz, 2H), 7.68 - 7.63 (m, 4H), 7.57 (d, *J* = 8 Hz, 2H), 7.35 (d, *J* = 8 Hz, 2H), 3.79 (s, 2H), 2.68 (s, 3H).
^{13}C NMR (100 MHz, DMSO) δ 169.9, 155.1, 152.1, 145.9, 143.1, 139.1, 135.6, 135.1, 132.7, 132.5, 130.1, 129.9, 127.1, 126.8, 124.8, 122.9, 121.1, 120.9, 120.1, 115.2, 42.9, 9.2.
HR-MS(ESI): Calcd. $[M+H]^+$m/z: 428.0764, found: 428.0766.

Procedures for evaluation of anticancer and antimicrobial activities

MTT cell *proliferation assay method*

The present compounds were tested on HCT116 cell lines using MTT cell proliferation assay method [33] (Table 1). The HCT-116 cell line was obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10 % fetal bovine serum (FBS). The cells (2000 cells per well) were seeded in a 96-well microplate containing 100 μL of DMEM +10 % FBS medium per well and incubated at 37 ºC with 5 % CO_2. The cells were treated with different concentrations of compounds up to 72 h for every 24 h interval. Controls were maintained with 0.5 % DMSO. After 72 h treatment, 5μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (R & D Systems, USA) along with 45 μL of phenol red-free DMEM (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37 ºC with 5 % CO_2 for 4 h. After that, 50 μL of solubilisation buffer (R & D Systems, USA) was added to each well to dissolve the coloured formazan crystals produced by the reduction of MTT. After 24 h the optical density was measured at 550 nm using a micro plate reader (Bio-Rad, USA).

Antimicrobial activity

The antimicrobial activity of the compounds was evaluated by agar well diffusion protocol [40] against a panel of human pathogens (Table 2). Nutrient agar media was used for cultivating the test bacteria. Nutrient agar media was sterilized at 15 lbs pressure at 12l °C for 15 minutes, cooled and inoculated with test bacteria. After thoroughly mixing, the inoculated media was poured into Petri plates under aseptic condition and allow them to solidify, wells of about 6 mm diameter were made in each Petri plate with the help of sterilized cork borer. The compounds were dissolved in DMSO at a concentration of 1 mg/ 1 µL about 200 µL of dissolved compounds were added to each well. Adding DMSO alone to wells served a control. The commercially available tetracycline in DMSO serves as a positive control. The inoculated plates were incubated at 37 °C temperature for 24 h, the zone of inhibition produced by each compound was measured in centimetres (Cm).

Acknowledgements

We are grateful to DST for providing FIST Level-I funding for the Department of Chemistry and thankful to Acharya Nagarjuna University, NNagar-522510, Guntur, AP-India for constant support.

Conflict of interest

The authors declare no conflict of interest

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