



Design, synthesis, characterization and anticancer activities of novel 3-(4-tert-butylbenzyl) [1,2,4] triazolo[3,4-a] phthalazine derivatives

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ABSTRACT

A novel series of 6-aryl substituted 3-(4-tert-butylbenzyl)[1,2,4]triazolo[3,4-a]phthalazines (**6a-g**) have been synthesized and characterized by spectral analysis and evaluated for their in-vitro cytotoxicity against a panel of five human cancer cell lines by MTT method. Results showed that these compounds selectively induced cell viability towards human breast cancer lines over the other cancer cell lines tested and compound **6g** exhibited good anticancer activities compared to 5-fluorouracil (standard used) against MCF-7 (Breast) cell lines with IC₅₀ value 16.5 ± 0.4 μM.

Keywords: Triazolophthalazine, Cytotoxic activity, Suzuki reaction, Boronic acids.



INTRODUCTION

Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells and is at present one of the leading causes of death in the United States and in the developed countries [1]. It is a fatal disease standing next to the cardiovascular disease in terms of morbidity and mortality. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future [2,3]. Currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs. Therefore, the design of new antitumor agents is one of the most emerging research areas in medicinal chemistry.

In the recent past, a large number of phthalazine derivatives have been prepared and studied for their antitumor activities [4-8]. Among them, Vatalanib (PTK-787) (Fig.1), a VEGFR (vascular endothelial growth factor receptor) inhibitor, is currently

in phase III clinical trials for advanced colorectal cancer [9] and additional phase II studies for leukemia [10]. In order to improve the antitumor efficacy of Vatalanib, many new phthalazine derivatives have been reported including 1,2,4-triazolo[3,4-a] phthalazine derivatives, which have been described as highly potent against human cancer cell lines [11].

In view of the above facts and based on the structures of phthalazine derivatives (**I-IV**), also in continuation of our research interest on the synthesis of biologically active heterocycles [12-15], we have designed and synthesized a novel series of 6-aryl substituted 3-(4-tert-butylbenzyl)[1,2,4]triazolo[3,4-a]phthalazine derivatives by an efficient synthetic route (Scheme-1). In addition, we screened them for their in-vitro cytotoxic activities against different cancer cell lines to find potent and selective agents. Some of these compounds showed promising cytotoxicity against human cancer cell lines and are found to be selective to breast cancer cell lines.

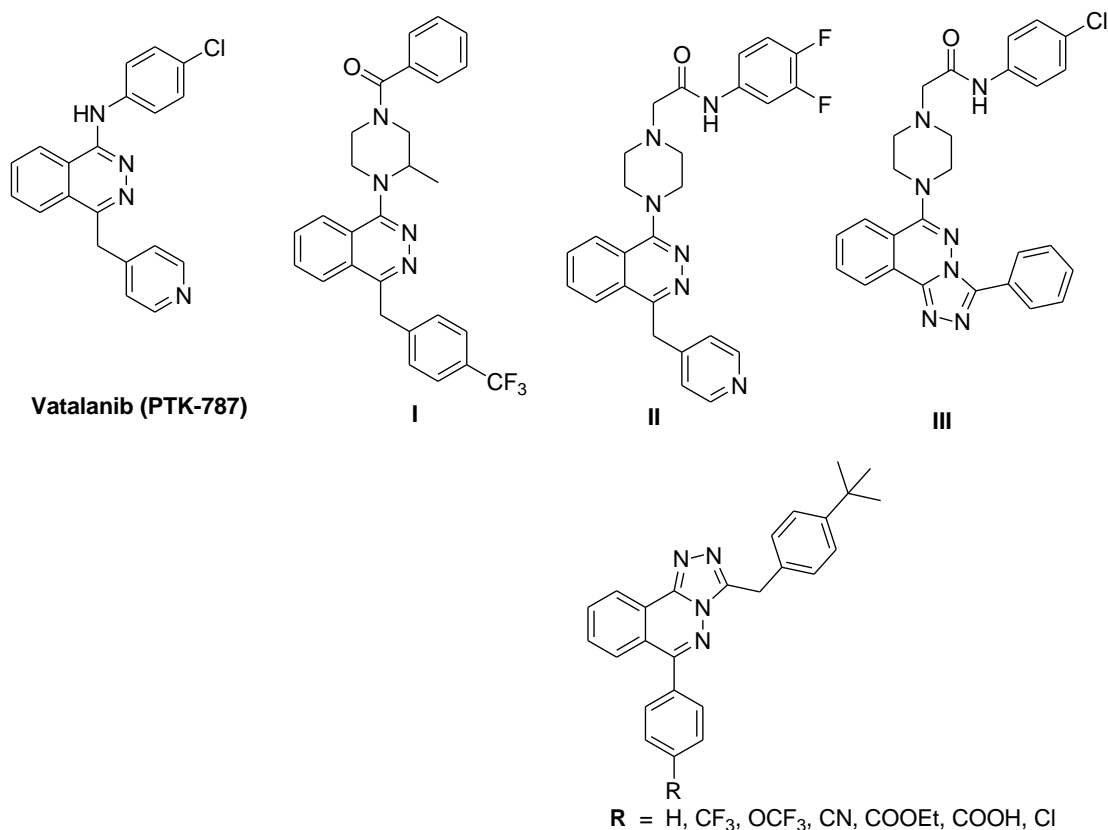


Fig. 1 Structures of PTK-787, I, II, III and the target compounds.

MATERIALS AND METHODS

Experimental: All reagents were of analytical grade, purchased from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was carried out on TLC Silica gel 60 F₂₅₄ plates (Merck). Column chromatography was performed using silica gel (60–120 mesh size; Merck). Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Nicolet Impact 410 FT IR spectrophotometer using KBr pellets. ¹H and ¹³C NMR were recorded on Bruker 300-MHz and 400-MHz FT NMR spectrometer in CDCl₃ and DMSO-d₆ by using TMS as internal standard. Chemical shifts are reported in ppm downfield (δ) from TMS. Mass spectra were recorded using Quadrupole LC-MS system with ESI resource.

Synthesis of 2,3-dihydrophthalazine-1,4-dione (1): Hydrazine hydrate (3.4 g, 67.7 mmol) was added to a stirred solution of phthalic anhydride (10.0 g, 61.6 mmol) in acetic acid (50 mL). The mixture was heated to 120 °C for 4 h. The reaction mixture was cooled to room temperature and filtered through a Buchner funnel. Then the solid product was washed with petroleum ether (50 mL)

and dried in vacuum to get 2,3-dihydrophthalazine-1,4-dione **1** as white solid in 92% yield. MP: 281–282 °C. ¹H-NMR (400 MHz, DMSO-d₆): δ 11.54 (s, 2H, D₂O exchangeable), 8.08 (dd, 2H), 7.88 (m, 2H).

Synthesis of 1,4-dichlorophthalazine (2): The compound **1** (8.0 g, 49.3 mmol) was added to a stirred solution of phosphorus oxychloride (100 mL) followed by 10 mL of triethylamine (TEA). The mixture was heated to 100 °C for 8 h. The progress of the reaction was monitored by TLC. After the reaction was complete, the reaction mixture was cooled to room temperature and distilled off the excess of phosphorus oxychloride. The residue was added dropwise to crushed ice with stirring for 10–20 min. Then the mixture was filtered through a Buchner funnel. The solid product was washed with H₂O until neutral and dried in vacuum to get 1,4-dichlorophthalazine **2** as white fluppy solid in 90% yield. MP: 163–165 °C; ¹H-NMR (400 MHz, CDCl₃): δ 8.34–8.31 (m, 2H), 8.28–8.24 (m, 2H).

Synthesis of 3-(4-tert-butylbenzyl)-6-chloro-[1,2,4]-triazolo[3,4-a]phthalazine (4): To a solution of 1,4-dichlorophthalazine **2** (5.0 g, 25.1

mmol) in o-xylene (50 mL) was added triethylamine hydrochloride (3.8 g, 27.6 mmol) and 2-(4-tert-butylphenyl)aceto hydrazide **3** (5.7 g, 27.6 mmol). The reaction mixture was heated to reflux for 12 h at 140 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was treated with water (50 mL), basified with saturated sodium carbonate solution and the separated solid was filtered, washed with excess of water and dried. The crude product was purified by recrystallization from ethanol.

Yield: 85%; Color: White crystalline solid; MP: 170-172 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.68 (d, *J* = 8.0 Hz, 1H), 8.24 (d, *J* = 8.4, 1H), 7.95 (t, *J* = 7.2, 1H), 7.85 (t, *J* = 7.2 Hz, 1H), 7.41 (d, *J* = 8.4, 2H), 7.33 (d, *J* = 8.4, 2H), 4.53 (s, 2H), 1.28 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 149.99, 149.92, 149.72, 142.33, 134.65, 132.33, 131.10, 128.79, 127.34, 125.83, 125.59, 124.06, 123.50, 122.11, 34.44, 31.32, 29.79. LC-MS (ESI): *m/z* calculated for C₂₀H₁₉ClN₄ ([M+H]) 351.84, found 351.4; Anal. Calcd (%) for C₂₀H₁₉ClN₄: C 68.47, H 5.46, N 15.97. Found: C 68.35, H 5.52, N 15.84.

General procedure for the preparation of title compounds 6a-g : To a well stirred solution of **4** (0.5 g, 2 mmol), p-substituted phenylboronic acids (2.2 mmol) and K₂CO₃ (3 equiv. 2M aqueous solution) in 1,4-dioxane (25 mL) was added Pd(dppf)Cl₂ (5 mol%). The resulting mixture was heated with stirring at 80 °C for 2-4 h. The progress of the reaction was monitored by TLC. After cooling, the solvent was evaporated under reduced pressure; the residue was dissolved in dichloromethane (50 mL) and water (25 mL). The separated organic layer was washed with excess of water followed by brine solution. Dried over magnesium sulfate, filtered and evaporated in reduced pressure. The residue was purified by column chromatography using ethyl acetate: hexane to obtain title compounds **6a-g** in 80-90% yield.

3-(4-tert-butylbenzyl)-6-phenyl[1,2,4]triazolo[3,4-a]phthalazine (6a): Yield: 84%; Color: White solid; MP: 180-181 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.66 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 8.4, 2H), 7.61-7.53 (m, 6H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4, 2H), 4.49 (s, 2H), 1.20 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 151.45, 149.64, 149.72, 142.33, 134.65, 132.33, 131.10, 128.79, 127.34, 126.62, 125.83, 125.59, 124.06, 123.50, 122.11, 34.44, 31.32, 29.79. LC-MS (ESI): *m/z* calculated for C₂₆H₂₄N₄ ([M+H]) 393.5, found 393.2; Anal. Calcd (%) for C₂₆H₂₄N₄: C 79.56, H 6.16, N 14.27. Found: C 79.38, H 6.08, N 14.35.

3-(4-tert-butylbenzyl)-6-(4-(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6b): Yield: 85%; Color: White solid; MP: 185-187 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.57 (d, *J* = 8.0 Hz, 1H), 7.74-7.50 (m, 7H) 7.20-7.06 (m, 4H), 4.36 (s, 2H), 1.08 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 155.37, 150.26, 149.05, 142.42, 137.96, 133.02, 132.84, 132.21, 130.69, 130.30, 128.45, 128.13, 125.82, 125.79, 125.62, 125.50, 125.19, 124.14, 123.87, 122.51, 34.46, 31.33, 30.01. ¹⁹F NMR (400 MHz, CDCl₃): δ -62.8 LC-MS (ESI): *m/z* calculated for C₂₇H₂₃F₃N₄ [M+H] 393.5, found 393.2; Anal. Calcd (%) for C₂₇H₂₃F₃N₄: C 70.42, H 5.03, N 12.17. Found: C 70.34, H 5.11, N 12.12.

3-(4-tert-butylbenzyl)-6-(4-(trifluoromethoxy)phenyl)-[1,2,4]triazolo[3,4-a]phthalazine (6c): Yield: 88%; Color: White solid; MP: 179-180 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.62 (d, *J* = 8.0 Hz, 1H), 7.79 (m, 2H), 7.75 (t, 3H), 7.60 (m, 2H), 7.38 (d, 2H), 7.32 (d, 2H), 7.24 (d, 2H), 4.46 (s, 2H), 1.19 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 155.39, 150.26, 149.05, 142.49, 133.70, 132.97, 132.67, 131.55, 130.63, 128.91, 128.30, 125.50, 124.19, 123.87, 122.68, 121.77, 121.09, 119.20, 34.45, 31.34, 30.07. ¹⁹F NMR (400 MHz, CDCl₃): δ -57.6 LC-MS (ESI): *m/z* calculated for C₂₇H₂₃F₃N₄O [M+H] 477.49, found 477.31; Anal. Calcd (%) for C₂₇H₂₃F₃N₄O: C 68.06, H 4.87, N 11.76. Found: C 68.14, H 4.76, N 11.82.

4-(3-(4-tert-butylbenzyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzotrile (6d): Yield: 82%; Color: White solid; MP: 208-210 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.70 (d, *J* = 8.0 Hz, 1H), 7.84 (m, 3H), 7.68 (m, 4H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4, 2H), 4.48 (s, 2H), 1.21 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 157.85, 152.42, 150.63, 149.62, 142.43, 133.65, 132.38, 131.16, 128.79, 127.34, 126.62, 125.83, 125.59, 124.06, 123.50, 122.11, 34.44, 31.32, 29.79. LC-MS (ESI): *m/z* calculated for C₂₇H₂₃N₅ ([M+H]) 418.51, found 418.35; Anal. Calcd (%) for C₂₇H₂₃N₅: C 77.67, H 5.55, N 16.77. Found: C 77.76, H 5.46, N 16.82.

Ethyl 4-(3-(4-tert-butylbenzyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzoate (6e): Yield: 86%; Color: White solid; MP: 190-192 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.80 (d, *J* = 8.0 Hz, 1H), 8.31 (d, *J* = 8.4 Hz, 2H), 7.91 (t, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.59 (s, 2H), 4.51 (q, *J* = 7.2 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H), 1.31 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 164.13, 152.42, 150.63, 149.62, 142.43, 133.65, 132.38, 131.16, 128.79, 127.34, 126.62, 125.83, 125.59, 124.06, 123.50, 122.11, 61.26, 34.44, 31.32, 29.79, 14.20. LC-MS (ESI): *m/z* calculated for C₂₉H₂₈N₄O₂ ([M+H]) 465.56, found

465.41; Anal. Calcd (%) for C₂₉H₂₈N₄O₂: C 74.98, H 6.08, N 12.06. Found: C 74.82, H 6.11, N 12.02.

4-(3-(4-tert-butylbenzyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzoic acid (6f): Yield: 80%; Color: White solid; MP: 241-242 °C. ¹H-NMR (400 MHz, DMSO-d₆): δ 13.25 (bs, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 8.20 (d, *J* = 8.0 Hz, 2H), 8.08 (t, *J* = 7.2 Hz, 1H), 7.84 (m, 4H), 7.32 (m, 4H), 4.49 (s, 2H), 1.24 (s, 9H). ¹³C-NMR (100 MHz, DMSO-d₆): δ 168.32, 152.42, 150.63, 149.62, 142.43, 133.65, 132.38, 131.16, 128.79, 127.34, 126.62, 125.83, 125.59, 124.06, 123.50, 122.11, 61.26, 34.44, 31.32, 29.79, 14.20. LC-MS (ESI): *m/z* calculated for C₂₇H₂₄N₄O₂ ([M+H]) 437.51, found 437.48; Anal. Calcd (%) for C₂₉H₂₈N₄O₂: C 74.29, H 5.54, N 12.84. Found: C 74.32, H 5.48, N 12.91.

3-(4-tert-butylbenzyl)-6-(4-chlorophenyl)[1,2,4]triazolo[3,4-a]phthalazine (6g): Yield: 90%; Color: White solid; MP: 224-226 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.70 (d, *J* = 8.0 Hz, 1H), 7.85 (t, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.64 (t, 1H), 7.50 (d, 4H), 7.33 (d, 2H), 7.25 (d, 2H), 4.48 (s, 2H), 1.21 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 151.45, 149.64, 149.72, 142.33, 134.65, 132.33, 131.10, 128.79, 127.34, 126.62, 125.83, 125.59, 124.06, 123.50, 122.11, 34.44, 31.32, 29.79. LC-MS (ESI): *m/z* calculated for C₂₆H₂₃ClN₄ ([M+H]) 427.94, found 427.84; Anal. Calcd (%) for C₂₆H₂₃ClN₄: C 73.14, H 5.43, N 13.12. Found: C 73.14, H 5.43, N 13.12.

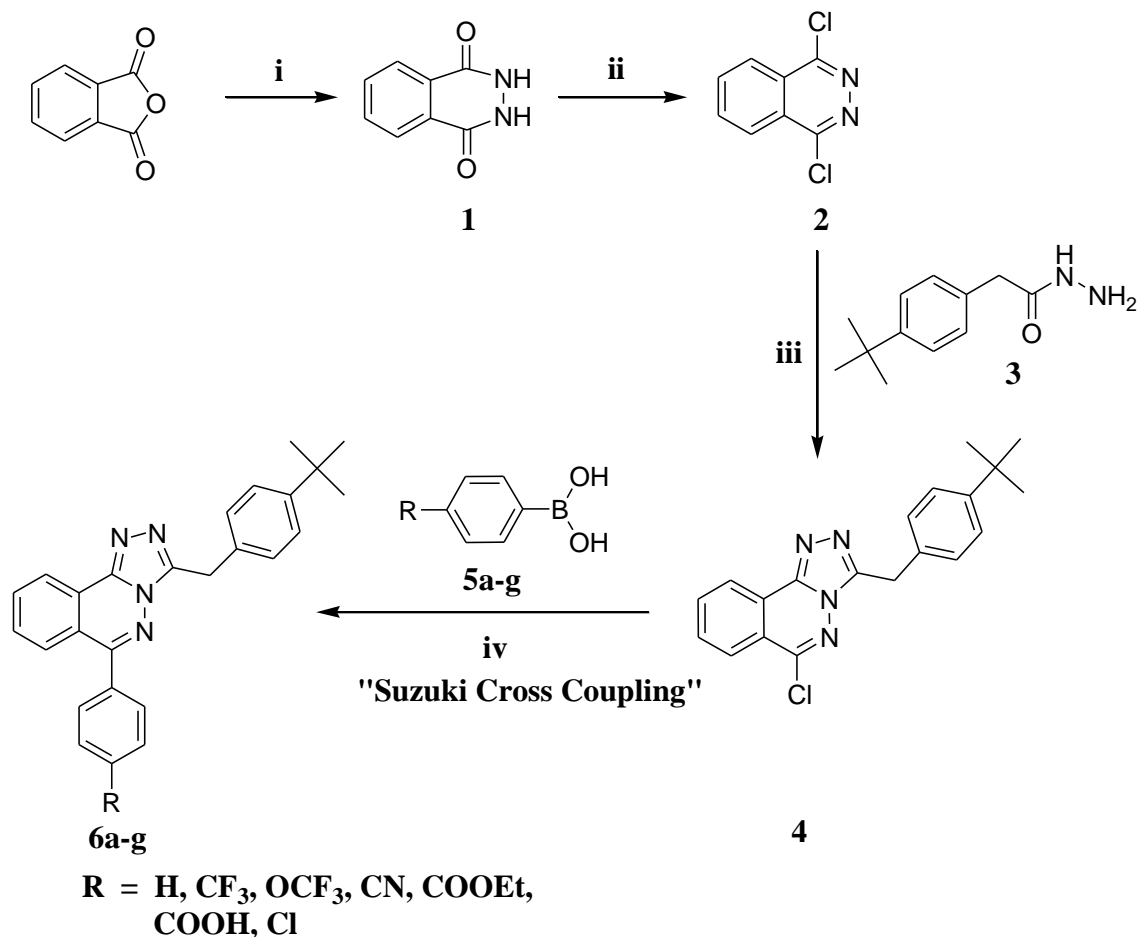
Anticancer activity-MTT assay: The cytotoxic activity of the compounds was determined by MTT assay as described earlier. This assay measures the percentage viability of the cells in response to different concentrations of the compounds. Active mitochondrial dehydrogenases of living cells convert the water soluble yellow tetrazolium salt to an insoluble purple formazan. The intensity of color developed is an indicator of percentage of viable cells present. In brief, cells (3000/well) were plated in 96-well plates and kept overnight at 37 °C after which, the cells were incubated with and without various concentrations of the compounds (50 and 100 μM). 5-Fluorouracil (5FU) was used as the positive control. At the end of the incubation, medium was removed and fresh medium containing 20% MTT solution (2 mg/mL in PBS) was added to each well. After 2 h, 0.1 ml of the extraction buffer (20% SDS and 50% DMF) was added, and the optical density was measured at 570 nm using a plate reader (Bio-Rad) after 1 h and compared with that of the untreated control. The percentage of inhibition of cell viability was determined with reference to the untreated control. The data were subjected to linear regression analysis and the regression lines were plotted for

the best straight-line fit. The IC₅₀ concentrations were calculated using the respective regression analysis.

RESULTS AND DISCUSSION

Chemistry: The synthetic pathway leading to the target compounds **6a-g** is outlined in Scheme 1. The compound **1** was prepared by the reaction of phthalic anhydride with hydrazine hydrate in acetic acid, which reacted further with the refluxing phosphorus oxychloride to yield the 1,4-dichlorophthalazine **2** [16]. Furthermore, compound **2** was refluxed with 2-(4-tert-butylphenyl)acetohydrazide **3** and triethyl amine hydrochloride in xylene as solvent to afford the 3-(4-tert-butylbenzyl)-6-chloro-[1,2,4]triazolo[3,4-a]phthalazine **4** as per the reported procedure [17]. After the cyclization, the 6-chloro intermediate compound **5** was subjected to Suzuki-Miyaura cross coupling reaction with various p-substituted phenylboronic acids (**5a-g**) using K₂CO₃ as base, in a mixture of 1,4-dioxane and water as solvent under heating at 80 °C afforded the target 6-arylated triazolo phthalazines (**6a-g**) in good yields. All the newly synthesized compounds were characterized, unambiguously, by ¹H NMR, ¹³C NMR, ¹⁹F NMR, LC-MS analysis and Elemental analysis.

Anticancer activity: All the synthesized novel compounds **6a-g** was tested for their in-vitro screening for their cytotoxicity toward human cancer cell lines using MTT assay [18]. The cytotoxicity studies were determined against five human cancer cell lines, MCF-7 (Breast), SK-BR-3 (Breast), A-549 (Lung), HL-60 (Leukemia) and HeLa (Cervical) and the percentage cytotoxic activity (dose dependent) of the synthesized compounds at concentrations 50 and 100 μM are presented in Table 1. 5-Fluorouracil (5FU) was used as reference compound. It is interesting to note that all these compounds selectively cytotoxic towards human breast cancer cell lines over other human cancer cell lines. The percentage of cell viability for human breast cancer lines (MCF-7 and SK-BR-3) is in the range of 55.2 to 91.5% and did not exhibit significant activity for other human cancer cell lines such as A-549, HL-60 and HeLa (percentage of cell viability is in the range of 18.4 to 51.4%).



Scheme 1 Reagents and conditions: (i): H₂N-NH₂.H₂O, CH₃COOH, 120 °C, 80%; (ii): POCl₃, TEA, 100 °C, 75%; (iii): 2-(4-tert-butylphenyl)acetohydrazide (3), Triethylamine hydrochloride, 1,4-Dioxane, 140 °C, 8 h, 85%; (iv): p-substituted phenylboronic acids (5a-g), Pd(dppf)Cl₂, K₂CO₃, H₂O, 1,4-Dioxane, 80 °C, 2-4 h, 80-90%.

In Table 2, we presented the IC₅₀ values of compounds **6a-g** against two human breast cancer lines. IC₅₀ values are in the range of 16.5 to 64.2 μM. All these compounds possess common 3-(4-tert-butylbenzyl)-6-chloro-[1,2,4]triazolo[3,4-a]phthalazine nucleus. The substitution at C-6 position of the phthalazine moiety plays an important role in determining the potency of the compounds. Among

the compounds, **6c** and **6g** bearing OCF₃ and Cl groups exhibited maximum cytotoxic activity over the remaining compounds against MCF-7 and SK-BR-3 cell lines. These results revealed that compound **6c** exhibited good cytotoxic activities compared to 5-fluorouracil against the MCF-7 cell lines with IC₅₀ value 16.5 ± 0.4 μM.

Table 1. Percentage of cell viability induced by the compounds **6a–g**

| Compound Cell lines | <u>MCF-7 (Breast)</u> | | <u>SK-BR-3 (Breast)</u> | | <u>A-549 (Lung)</u> | | <u>HL-60 (Leukemia)</u> | | <u>HeLa (Cervical)</u> | |
|------------------------|-----------------------|------|-------------------------|------|---------------------|------|-----------------------------|------|----------------------------|------|
| | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 |
| 6a | 65.4 | 54.0 | 71.2 | 63.4 | 35.0 | 32.5 | 46.9 | 45.1 | 38.7 | 33.2 |
| 6b | 75.2 | 73.7 | 78.4 | 72.1 | 51.4 | 48.5 | 35.8 | 32.2 | 42.0 | 44.4 |
| 6c | 88.4 | 75.3 | 91.5 | 82.2 | 45.5 | 52.4 | 40.0 | 42.5 | 52.1 | 60.6 |
| 6d | 70.0 | 66.2 | 63.1 | 55.3 | 36.8 | 40.4 | 42.8 | 39.2 | 45.1 | 50.2 |
| 6e | 66.4 | 56.2 | 71.6 | 65.4 | 33.6 | 42.5 | 25.4 | 35.4 | 22.3 | 28.5 |
| 6f | 55.2 | 43.8 | 60.6 | 58.8 | 18.4 | 21.8 | 32.6 | 36.9 | 26.7 | 25.8 |
| 6g | 90.5 | 82.4 | 78.6 | 75.5 | 33.3 | 31.2 | 24.5 | 26.8 | 35.6 | 40.1 |
| 5-FU | 86.2 | 72.5 | 78.6 | 65.4 | 74.4 | 70.3 | 66.8 | 64.2 | 80.3 | 70.8 |

Table 2 Cytotoxic activity (IC_{50} , μM) of compounds **6a–g** against two Human breast cancer cell lines

| Compounds | R | IC_{50} (μM) ^a | |
|-----------|------------------|------------------------------------|----------------|
| | | <u>MCF-7</u> | <u>SK-BR-3</u> |
| 6a | H | 56.8 ± 0.8 | 51.4 ± 0.4 |
| 6b | CF ₃ | 34.5 ± 0.3 | 32.5 ± 0.6 |
| 6c | OCF ₃ | 23.5 ± 0.9 | 22.7 ± 1.2 |
| 6d | CN | 48.4 ± 0.7 | 50.8 ± 0.6 |
| 6e | COOEt | 52.6 ± 0.4 | 44.2 ± 0.6 |
| 6f | COOH | 64.2 ± 1.4 | 60.8 ± 0.6 |
| 6g | Cl | 16.5 ± 0.4 | 22.8 ± 0.6 |
| 5-FU | - | 20.7 ± 0.2 | 24.4 ± 0.4 |

^aAnticancer activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). IC_{50} values are presented as the means ± SDs of three independent experiments.

CONCLUSION

As part of our continuous search for the potential anticancer heterocyclic compounds, a series of novel 6-aryl substituted 3-(4-tert-butylbenzyl) [1,2,4]triazolo[3,4-a]phthalazine derivatives **6a–g** were synthesized and evaluated for their anticancer activity. The synthesis involves the cyclization leading to the formation 6-chloro-3-(4-tert-butylbenzyl)[1,2,4]triazolo[3,4-a]phthalazine, which is derivatized by Suzuki cross coupling reaction with various p-substituted phenylboronic acids. The products were obtained in high purity with excellent yields, which have been, unambiguously, characterized by ¹H NMR, ¹³C NMR, ¹⁹F NMR and LC-MS analysis. All the newly synthesized compounds were screened for anticancer activity against five human cancer lines. Hence, our

preliminary results indicate that these compounds **6a–g** selectively induced cell viability towards human breast cancer (MCF-7 and SF-BR-3) cell lines over other human cancer (A-549, HL-60 and HeLa) cell lines tested. Among the compounds, **6g** exhibited good anticancer activities compared to 5-fluorouracil against MCF-7 (Breast) cell lines with IC_{50} value 16.5 ± 0.4 μM .

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