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MEDICINAL CHEMISTRY RESEARCH

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Microwave-assisted synthesis and in vitro antiproliferative activity of some novel 1,2,3-triazole-based pyrazole aldehydes and their benzimidazole derivatives

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Abstract

A series of new 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **4(a–g)** and their benzimidazole derivatives **6(a–g)** were synthesized under both conventional and microwave irradiation methods. However, we successfully achieved good yields in shorter reaction times under microwave irradiation. All the synthesized scaffolds were characterized by ^{1}H NMR, ^{13}C NMR, IR and mass spectral analyses. The synthesized compounds have been evaluated as potential antiproliferative agents. The antiproliferative activity of the synthesized compounds was studied against two cancer cell lines C6 (nerve cells) and MCF-7 (human breast adenocarcinoma cells) and among them, compounds **4g** and **6b** exhibited highly potent activity against C6 cell line and **6b–c** exhibited highly potent activity against MCF-7 cell line.

Keywords Pyrazole aldehyde · Benzimidazole · 1,2,3-triazole · Microwave irradiation · Antiproliferative

Introduction

Cancer is one of the fastest-growing disease caused due to abnormal cell division is a major concern from decades (Kidwai et al. 2002). About 10 million new cancer cases are diagnosed every year which represents a real crisis in public health in the world (Husain et al. 2012). The searching of new motif for the treatment of cancer is an important tool of medicinal chemistry. Developments of new scaffolds with a novel mechanism of action to fight cancer are urgently needed, as most anticancer drugs are ineffective due to drug resistance.

Drugs like vinblastine, vincristine, taxol, and camptothecin have improved the chemotherapy of some cancers. In spite of the recent domination of the usage of natural products to discover and produce drugs, the potential usage of synthetic chemistry as a method to provide new and novel products for antitumor treatment and prevention is still enormous. Now a day's researchers are focusing on the development of new indole, triazole, pyrazole, benzimidazole, their fused compounds and are widely studied for their significant biological activities, especially as antiproliferative agents.

Benzimidazole derivatives are very useful scaffolds in medicinal and pharmaceutical chemistry due to wide range of biological activities. Specifically, this nucleus is a constituent of vitamin B_{12} (Höllriegl et al. 1982) and exhibits antimicrobial (Madabhushi et al. 2014), anticancer (Paul et al. 2013), chemotherapeutic (Boiani and González 2005), antihistaminic (Wang et al. 2012), antiproliferative (Abdelgawad et al. 2017), anticoagulant (Kuo et al. 2010), antihypertensive (Zhu et al. 2014), anti-hepatitis (Li et al. 2006), analgesic and anti-inflammatory activities (Achar et al. 2010). Compounds containing pyrazole ring have been investigated to possess various biological activities

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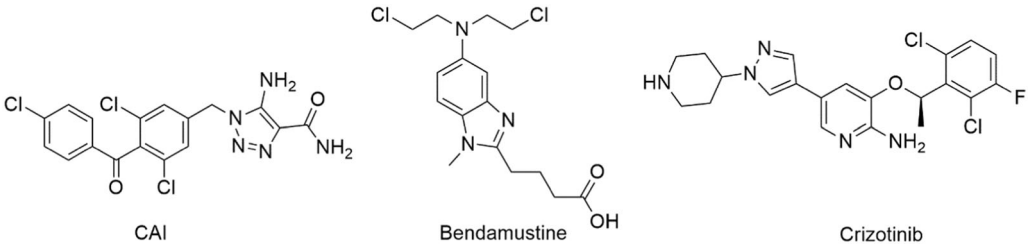

Fig. 1 Anticancer drugs available in the market

such as anticancer (Li et al. 2012), antihypertensive (Arya et al. 1969), antiproliferative (Ravula et al. 2016), antiviral (Sujatha et al. 2009), antimycobacterial (Taban et al. 2017), and hydrogen receptor modulator activities (Zhang et al. 2007). Similarly, 1,2,3-triazole derivatives have gained enormous attention in recent years owing to their broad spectrum of pharmaceutical activities and their highly diverse biological activities, such as anticancer (Yan et al. 2010), antimicrobial (Wang et al. 2010), antiviral (Jordao et al. 2009), antiproliferative (Duan et al. 2013) and antimycobacterial agents (Gallardo et al. 2007). Benzimidazole-, pyrazole- and triazole-based drugs such as Bendamustine (Leoni et al. 2008), Crizotinib (Forde and Rudin 2012), and CAI (Soltis et al. 1996) are available in the market to treat the cancer (Fig. 1).

The molecular hybridization approach of biologically active molecules in drug design and development is one of the most powerful techniques in research field. It involves the structural modification and mixing of two or more pharmacophores of different biologically active molecules to deliver a new hybrid with improved affinity and efficiency than the parent drug. Moreover, the hybrid drug can result a modified selectivity, more specific, different mode of action and reduced unwanted side effects than the parent drug (Viegas-Junior et al. 2007).

Several methods are known for the synthesis of benzimidazoles and some of them use strong acid catalysts (Bougrin et al. 2001), Lewis acids (Tandon and Kumar 2004), metal catalysts, inorganic clays (Rastogi and Sharma 1983) and solid phase (Mazurov 2000; Jing et al. 2006). But, the traditional way for the synthesis of these derivatives is from the key intermediate aldehydes and OPDA. Furthermore, there are several methods for the formylation of pyrazoles at 4-position of the heteroaromatic ring (Arbačiauskienė et al. 2011; Vera-DiVaio et al. 2009; Luo et al. 2008; Arbačiauskienė et al 2010). Among these the most popular one is Vilsmeier–Haack reaction (Fig. 2).

Microwave-assisted synthesis has developed as a new tool for the decades in the research field as this technique offers shorter reaction time, higher yields, selective heating, easy to carry out and eco-friendly as compared with the traditional heating method. The reason for the observed rate enhancements is purely thermal/kinetic effects, which are consequence of high temperature that can be rapidly attained when exposing the polar reacting materials in a microwave field (Ashok et al. 2017).

It was thought worthwhile to prepare a new hybrid with triazole, pyrazole and benzimidazole ring systems with a view to produce promising antiproliferative agents. We designed and synthesized new pyrazole aldehydes **4(a–g)** and respective benzimidazoles **6(a–g)** using conventional and microwave irradiation methods.

Results and discussion

Chemistry

The protocol adopted for synthesis of compounds 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **4(a–g)** and their benzimidazole derivatives **6(a–g)** is shown in Scheme 1. Compounds **1(a–g)** were prepared according to reported procedure (Kamalraj et al. 2008). The carbaldehyde intermediates **4(a–g)** were synthesized in two steps. The hydrazones **3(a–g)** were initially prepared from ketones **1(a–g)** and phenylhydrazine, which on further reaction with Vilsmeier–Haack reagent (DMF/$POCl_3$) gave carbaldehyde intermediates **4(a–g)**. Benzimidazole derivatives **6(a–g)** were obtained by the reaction of carbaldehydes **4(a–g)** with ortho phenylene diamine in DMF. In order to improve the yield, a preliminary study of effect of solvents on synthesis of compounds **6(a–g)** has been carried out by synthesizing compound **6a** using different solvents as shown in Table 1. With the use of high boiling solvents, the rate of formation of **6a** showed an increase. Among all the solvents, DMF was found to be a better solvent in microwave irradiation method with relatively better yield and easy work-up when compared with conventional heating.

To investigate the general scope and versatility of this procedure in the synthesis of novel scaffolds **4(a–g)** and **6(a–g)**, the reaction has been carried out under conventional heating and microwave irradiation methods. The reaction carried out under microwave irradiation method is proved to

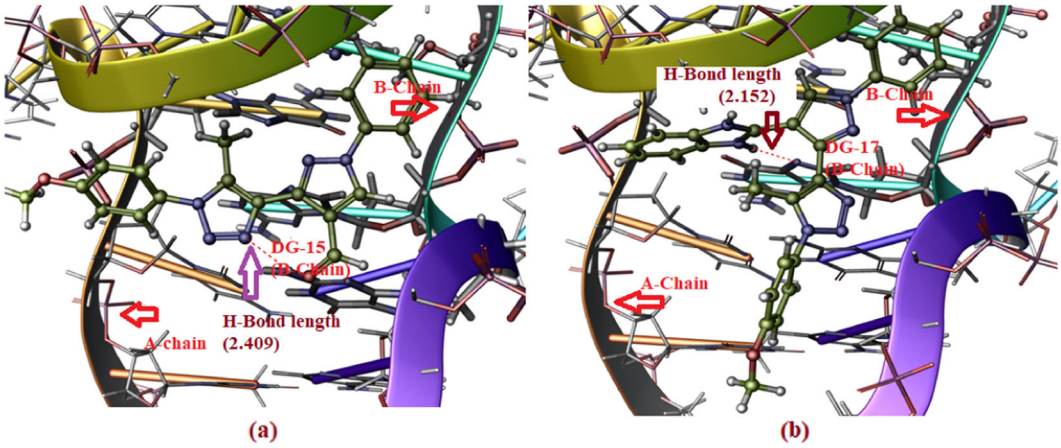

Fig. 2 Molecular interaction of molecule **4(b)** and **6(b)** showing hydrogen bond interaction with deoxy guanosine (DG-15 and DG-17)

Scheme 1 Synthetic pathway for compounds **4(a–g)** and **6 (a–g)**

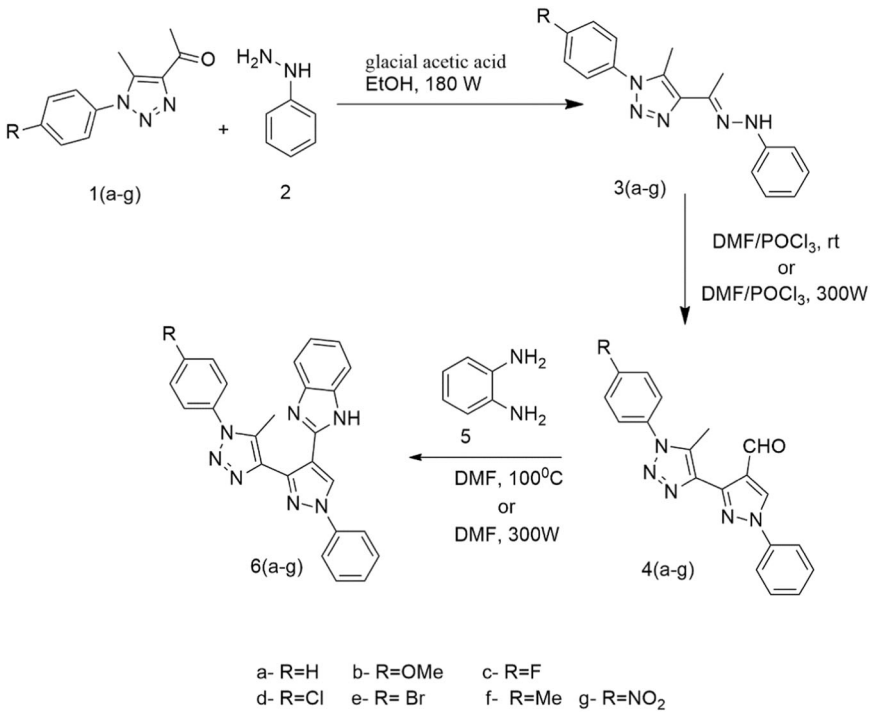

Table 1 Effect of solvent on the synthesis of compound **6a**

Entry	Solvent	Yield (%)	
		Conventional method	MWI method
1	CH_2Cl_2	15	29
2	Et_2O	12	25
3	THF	22	30
4	MeCN	28	40
5	EtOH	40	51
6	MeOH	42	55
7	1,4-dioxane	53	73
8	DMF	59	83

give better yield as compared with conventional method with shorter reaction time as shown in Table 2.

Biological evaluation

All the synthesized compounds **4(a–g)** and **6(a–g)** were screened for in vitro antiproliferative activity against C6 (nerve cells) and MCF-7 (human breast adenocarcinoma cells) cell lines by using the MTT assay method. Cisplatin, one of the most effective anticancer drugs is used for this study as a reference drug. The cell culture used for this study were grown in a controlled environment for 24 h at 37 °C in humidified 5% CO_2 incubator. The titled compounds were

Table 2 Comparison of reaction time and yields of the synthesized compounds **4(a–g)** and **6(a–g)**

Compound	Conventional method		MWI method	
	Time (h)	Yield (%)	Time (min)	Yield (%)
4a	6	60	8	81
4b	6	64	7	83
4c	5.5	63	9	82
4d	6	65	8	87
4e	5.6	60	7	85
4f	5.5	58	9	83
4g	6	59	7	78
6a	7	59	9	83
6b	7	62	7	84
6c	7	60	8	81
6d	7.5	66	7	89
6e	7	64	8	87
6f	7.5	59	9	85
6g	7	57	7	77

dissolved in DMSO and added to the cell culture. The IC_{50} values were determined and are summarized in the Table 3. and these results were compared with the standard drug.

From Table 3 it is clear that, all the synthesized compounds exhibited better activity against MCF-7 and moderate activity against C6 Cell line. The IC_{50} values reported in Table 3 indicates that benzimidazole derivatives **6(a–g)** exhibited better activity when compared with their corresponding aldehydes **4(a–g)** against the MCF-7 cell line, this higher activity is attributed to the presence of benzimidazole moiety. Most of the derivatives displayed promising activity than the standard cisplatin. Moreover, the derivatives **4g**, **6b** and **6f** exhibit higher activity and the derivatives **4e**, **6c** and **6e** exhibit equipotent activity when compared with the standard drug against the C6 cell line. Furthermore, most of the compounds showed better activity against the MCF-7 cell line and among them, the compounds **4f, 6a, 6b, 6c**, and **6d** displayed very high activity and the compounds **6e, 6f, 6g** and **4b** exhibited good activity and the remaining showed equipotent activity as the standard. From these results, we concluded that the potent activity of compounds might be due to the presence of electron-donating substituents on the core nucleus.

The increased activity of the benimidazole derivatives, when compared with pyrazole aldehydes, is supported by docking data. Although both triazole and benzimidazole have shown ideal H-Bond interaction with deoxy guanosine (DG-15 and DG-17) along with good docking scores as well, which is responsible for disrupting the DNA cross-linking, hydrogen bond length exhibited by benzimidazole (2.152) is shorter than triazole (2.409). Thus, stating that shorter H-Bond, ligand has more affinity toward DNA.

Table 3 IC_{50} values after 24 h drug incubation with C6 and MCF-7 cell lines by MTT assay

Compound	IC_{50} in μM	
	C6	MCF-7
4a	1.515	0.790
4b	3.440	0.354
4c	1.658	0.524
4d	0.496	0.505
4e	0.283	0.556
4f	0.457	0.158
4g	0.097	0.538
6a	0.564	0.253
6b	0.102	0.110
6c	0.243	0.144
6d	3.220	0.185
6e	0.219	0.450
6f	0.120	0.303
6g	0.560	0.452
Cisplatin	0.122	0.596

Conclusion

In conclusion, we have reported a series of novel 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **4(a–g)** and their benzimidazole derivatives **6(a–g)** in excellent yields under microwave irradiation method and evaluation of their in vitro antiproliferative activity. Among all the compound **4g** emerged as potent antiproliferative agent against C6 cell line and compound **6b** emerged as better antiproliferative agent against MCF-7 cell line.

Experimental

Materials and methods

All the chemicals and solvents were purchased from sigma Aldrich and other commercial suppliers. Progress of the reaction was monitored by thin-layer chromatography (TLC) on silica gel plates (60 F_{254}), visualizing with ultraviolet light. Column chromatography was performed on silica gel (60–120 mesh) using distilled hexane, ethyl acetate. ^{1}H NMR and ^{13}C NMR spectra were determined on Bruker AVANCE-400 spectrometer using $CDCl_3$ and DMSO solvents at 400 and 100 MHz, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiple). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a Shimadzu FT-IR-8400s

spectrometer. Melting points were determined using Stuart SMP3 melting point apparatus and are uncorrected. All the microwave irradiation experiments were performed in a CEM Discover microwave system and reaction temperatures were monitored by an equipped IR sensor.

General procedure for the synthesis of 5-methyl-1-aryl-4-(2-phenylhydrazono)ethyl)-1*H*-1,2,3-triazoles (3a–g)

A mixture of phenyl hydrazine (1.08 g, 0.01 mol), ketone **(1a–g)** (0.01 mol) and ethanol (5 mL) in glacial acetic acid (2 drops) was subjected to microwave irradiation at 180 W for 5–6 min with 5–10 s intervals and the progress of the reaction was monitored by TLC. After completion of the reaction, mixture was poured into crushed ice. The solid obtained was filtered, washed with water, dried and purified by recrystallization from ethanol to give **(3a–g)**.

General procedure for the synthesis of 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes (4a–g)

Conventional method Vilsmeier–Haack reagent was prepared by the drop wise addition of 3 mL of $POCl_3$ to a 15 mL of ice-cooled DMF and substituted 5-methyl-1-aryl-4-(2-phenylhydrazono)ethyl)-1*H*-1,2,3-triazoles **(3a–g)** (0.01 mol) were added in portions. After complete addition, the reaction mixture was stirred for 5–6 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, mixture was poured into crushed ice. The solid obtained was filtered, washed with water, dried and purified by column chromatography to give **(4a–g)**.

Microwave irradiation method To a freshly prepared Vilsmeier–Haack reagent, obtained by the drop wise addition of 3 mL of $POCl_3$ to a 15 mL of ice-cooled DMF, substituted 5-methyl-1-aryl-4-(2-phenylhydrazono)ethyl)-1*H*-1,2,3-triazoles **(3a–g)** (0.01 mol) were added in portions. After completion of the addition, the reaction mixture was irradiated in a microwave oven at 300 W for 5 s with 6–10 min intervals. The progress of the reaction was monitored by TLC. After completion of the reaction, mixture was poured into crushed ice. The solid obtained was filtered, washed with water, dried and purified by column chromatography to give **(4a–g)**.

General procedure for the synthesis of 2-(3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazoles (6a–g)

Conventional method A mixture of aldehydes (**4a–g**) (0.01 mol) and ortho phenylene diamine (**5**) (1.30 g, 0.012 mol) in DMF (15 mL) were stirred at 100 °C for 7–8 h and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into crushed ice, the solid obtained was filtered, washed with water, dried and purified by column chromatography to give **(6a–g)**.

Microwave irradiation method A mixture of aldehydes **(4a–g)** (0.01 mol) and ortho phenylene diamine **(5)** (0.012 mol) in DMF (5 mL) were treated under microwave irradiation for 6–9 min at 300 W and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into crushed ice. The solid obtained was filtered, washed with water, dried and purified by column chromatography to give **(6a–g)**.

3-(5-methyl-1-phenyl-1H-1.2.3-triazol-4yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (4a) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4a** as a white solid. mp 142–144 °C; IR (KBr) υ_{max} 3124, 2879, 1666, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 10.79$ (1H, s, CHO), 8.59 (1H, s, Ar-H), 7.78 (2H, d, $J = 8.0$ Hz, 2H, Ar-H), 7.61-7.50 (7H, m, Ar-H), 7.39 (3H, t, $J = 7.0$ Hz, Ar-H), 2.71 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta =$ 188.0, 146.7, 139.0, 138.2, 136.0, 132.9, 129.8, 129.7, 129.6, 129.0, 127.9, 125.2, 123.9, 119.4, 10.4; ESIMS *m/z* 330 $[M + H]^+$.

3-(1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4b) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4b** as a white solid. mp 134–136 °C; IR (KBr) υ_{max} 3120, 2879, 1662, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta =$ 10.78 (1H, s, CHO), 8.59 (1H, s, Ar-H), 7.79 (2H, d, $J =$ 8.5 Hz, Ar-H), 7.53 (2H, t, $J = 8.0$ Hz, Ar-H), 7.45 (2H, d, $J = 8.5$ Hz, Ar-H), 7.40 (1 H, t, $J = 7.3$ Hz, Ar-H), 7.09 (2H, d, $J = 9.0$ Hz, Ar-H), 3.91(3H, s, OCH_3), 2.68 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 188.1$, 160.5, 146.8, 139.1, 138.0, 133.0, 129.7, 129.0, 128.9, 127.9, 126.7, 124.0, 119.4, 114.8, 55.7, 10.3; ESIMS *m/z* 360 $[M + H]^+$.

3-(1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4c) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4c** as a white solid. mp 125–127 °C; IR (KBr) υ_{max} 3128, 2862, 1670, 1595 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 10.77$ (1H, s, CHO), 8.60 (1H, s, Ar-H), 7.79 (2H, d, $J = 8.0$ Hz, Ar-H), 7.56-7.51 (4H, m, Ar-H), 7.41 (1H, t, $J = 7.2$ Hz, Ar-H), 7.31 (2H, t, $J = 8.5$ Hz, Ar-H), 2.70 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 187.9$, 164.3, 161.8, 146.5, 139.0, 138.3, 132.9, 132.1, 132.1, 129.7, 129.1, 127.9, 127.3, 127.2, 124.0, 119.4, 116.9, 116.6, 10.3; ESIMS *m/z* 348 $[M + H]^+$.

3-(1-(4-chlorophenyl)-5-methyl-1*H*-1,2,3-triazol-4yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4d) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4d** as a white solid. mp 147–149 °C; IR (KBr) υ_{max} 3120, 2879, 1670, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 10.76$ (1H, s, CHO), 8.60 (1H, s, Ar-H), 7.78 (2H, d, $J = 7.8$ Hz, Ar-H), 7.59 (2H, d, $J = 8.5$ Hz, Ar-H), 7.55-7.50 (4H, m, Ar-H), 7.41 (1H, t, $J = 7.3$ Hz, Ar-H), 2.72 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 187.9$, 146.4, 139.0, 138.4, 135.9, 134.5, 132.8, 129.9, 129.7, 129.1, 127.9, 126.4, 124.0, 119.4, 10.4; ESIMS *m/z* 364 $[M + H]^+$.

3-(1-(4-bromophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4e) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4e** as a white solid. mp 137–139°C; IR (KBr) υ_{max} 3122, 2879, 1674, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 10.76$ (1H, s, CHO), 8.59 (1H, s, Ar-H), 7.78 (2H, d, $J = 8.0$ Hz, Ar-H), 7.75 (2H, d, $J = 8.5$ Hz, Ar-H) 7.53 (2H, t, $J =$ 7.8 Hz, Ar-H), 7.45 (2H, d, $J = 8.5$ Hz, Ar-H), 7.40 (1H, t, $J = 7.3$ Hz, Ar-H), 2.72 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 187.9$, 146.4, 139.0, 138.5, 135.0, 132.9, 132.7, 129.7, 129.1, 128.0, 126.7, 124.0, 123.9, 119.4, 10.4; HRMS *m/z* 408.0452 $[M + H]^+$.

3-(5-methyl-1-(p-tolyl)-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4f) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4f** as a white solid. mp 150–152 °C; IR (KBr) υ_{max} 3120, 2873, 1668, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 10.78$ (1H, s, CHO), 8.59 (1H, s, Ar-H), 7.79 (2H, d, $J = 8.5$ Hz, Ar-H), 7.52 (2H, t, $J = 8.5$ Hz, Ar-H), 7.44-7.38 (5H, m, Ar-H), 2.69 (3H, s, OCH_3), 2.48 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 188.1$, 146.8, 140.0, 139.1, 138.1, 133.6, 132.8, 130.2, 129.7, 129.0, 127.8, 125.1, 124.0, 119.4, 21.3, 10.3; ESIMS *m/z* 344 $[M + H]^+$.

3-(5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4 carbaldehyde (4g) Purified by column chromatography (ethyl acetate/hexane, 1:4) which gave **4g** as a pale yellow solid. mp 157–159 °C; IR (KBr) υ_{max} 3126, 2883, 1658, 1595 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) $\delta =$ 10.75 (1 H, s, CHO), 8.61 (1 H, s, Ar-H), 8.50 (2 H, d, $J =$ *9*.0 Hz, Ar-H), 7.83 (2 H, d, $J = 9.0$ Hz, Ar-H), 7.79 (2 H, d, $J = 7.8$ Hz, Ar-H), 7.54 (2 H, t, $J = 8.3$ Hz, Ar-H), 7.42 (1 H, t, $J = 7.5$ Hz, Ar-H), 2.81 (3 H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 187.6$, 148.0, 145.9, 140.9, 139.1, 138.9, 132.7, 129.8, 129.2, 128.1, 125.5, 125.2, 124.0, 119.5, 10.6; ESIMS *m/z* 375 $[M + H]^+$.

2-(3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6a) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6a** as a white solid. mp 183–185 °C; IR (KBr) υ_{max} 3153, 3059, 1591, 1273 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.35$ (1H, s, NH), 9.42 (1H, s, Ar-H), 8.09 (2H, d, $J = 8.0$ Hz, Ar-H), 7.78-7.66 (7H, m, Ar-H), 7.60 (2H, t, $J = 7.3$ Hz, Ar-H), 7.42 (1H, t, $J = 7.0$ Hz, Ar-H), 7.23-7.21 (2H, m, Ar-H), 2.68 (3H, s, CH_3); ^{13}C NMR (DMSO, 100 MHz) $\delta = 145.5$, 141.3, 138.8, 137.6, 135.6, 133.5, 130.0, 129.9, 129.8, 129.7, 127.2, 125.4, 122.1, 121.7, 118.6, 118.1, 114.0, 111.6, 10.3; ESIMS *m/z* 418 $[M + H]^+$.

2-(3-(1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6b) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6b** as a white solid. mp 205–207 °C; IR (KBr) υ_{max} 3122, 3057, 1595, 1274 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.43$ (1H, s, NH), 9.43 (1H, s, Ar-H), 8.09 (2H, d, $J = 8.5$ Hz, Ar-H), 7.69-7.67 (4H, m, Ar-H), 7.59 (2H, t, $J = 8.0$ Hz, Ar-H), 7.42 (1H, t, $J = 7.3$ Hz, Ar-H), 7.24-7.21 (4H, m, Ar-H), 3.89 (3H, s, OCH_3), 2.65 (3H, s, CH_3); ^{13}C NMR (DMSO, 100 MHz) $\delta = 160.2$, 145.6, 141.3, 138.8, 137.3, 133.6, 129.9, 129.7, 128.3, 127.1, 127.0, 121.9, 118.6, 114.8, 113.9, 55.6, 10.2; HRMS *m/z* 448.1891 $[M + H]^+$.

2-(3-(1-(4-fluorophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6c) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6c** as a white solid. mp 246–248 °C; IR (KBr) υ_{max} 3149, 3084, 1591, 1273 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.31$ (1H, s, NH), 9.42 (1H, s, Ar-H), 8.09 (2H, d, $J = 7.8$ Hz, Ar-H), 7.86-7.83 (2H, m, Ar-H), 7.69-7.65 (2H, m, Ar-H), 7.62-7.54 (4H, m, Ar-H), 7.42 (1H, t, $J = 7.3$ Hz, Ar-H), 7.23-7.21 (2H, m, Ar-H), 2.66 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 146.3$, 141.4, 139.3, 138.8, 133.3, 131.9, 129.7, 129.1, 127.5, 127.4, 127.3, 122.4, 118.8, 117.0, 116.8, 114.4, 10.9 ESIMS *m/z* 436 $[M + H]^+$.

2-(3-(1-(4-chlorophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6d) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6d** as a white solid. mp 200–202 °C; IR (KBr) υ_{max} 3140, 3061, 1591, 1271 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.27$ (1H, s, NH), 9.41 (1H, s, Ar-H), 8.08 (2H, d, $J = 7.8$ Hz, Ar-H), 7.83-7.77 (4H, m, Ar-H), 7.67-7.65 (2H, m, Ar-H), 7.59 (2H, t, $J =$ 8.0 Hz, Ar-H), 7.42 (1H, t, $J = 7.3$ Hz, Ar-H), 7.23-7.20 (2H, dd, $J = 3.0$, 5.5 Hz, Ar-H), 2.67 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 146.2$, 141.3, 139.3, 138.9, 136.3, 134.3, 133.2, 130.1, 129.7, 129.1, 127.3, 126.6, 122.5, 122.3, 118.8, 114.5, 111.6, 11.0; ESIMS *m/z* 452 $[M + H]^+$.

2-(3-(1-(4-bromophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6e) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6e** as a white solid. mp 168–170 °C; IR (KBr) υ_{max} 3120,3059, 1595, 1273 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.26$ (1H, s, NH), 9.41 (1H, s, Ar-H), 8.08 (2H, d, $J = 8.0$ Hz, Ar-H), 7.92 (2H, d, $J = 8.5$ Hz, Ar-H), 7.75 (2H, d, $J = 8.5$ Hz, Ar-H), 7.69-7.65 (2H, m, Ar-H), 7.59 (2H, t, $J = 7.8$ Hz, Ar-H), 7.42 (1H, t, $J = 7.3$ Hz, Ar-H), 7.23-7.21 (2H, m, Ar-H), 2.67 (3H, s, CH_3); ^{13}C NMR (DMSO, 100 MHz) $\delta = 145.5$, 143.6, 141.2, 138.8, 137.7, 134.8, 133.9, 133.6, 132.8, 129.9, 129.7, 127.5, 127.2, 123.2, 122.1, 121.7, 118.6, 118.2, 114.0, 111.6, 10.2; HRMS *m/z* 496.0899 $[M + H]^+$.

2-(3-(5-methyl-1-(p-tolyl)-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6f) Purified by column chromatography (ethyl acetate/hexane, 7:130 which gave **6f** as a white solid. mp 187–189 °C; IR (KBr) υ_{max} 3120,3053, 1593, 1271 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.39$ (1H, s, NH), 9.42 (1H, s, Ar-H), 8.09 (2H, d, $J = 8.5$ Hz, Ar-H), 7.70-7.66 (2H, m Ar-H), 7.63 (2H, d, $J = 8.5$ Hz, Ar-H), 7.59 (2H, t, $J = 8.3$ Hz, Ar-H), 7.50 (2H, d, $J = 8.0$ Hz, Ar-H), 7.42 (1H, t, $J = 7.3$ Hz, Ar-H), 7.23-7.21 (2H, m, Ar-H), 2.67 (3H, s, CH_3), 2.46 (3H, s, CH_3); ^{13}C NMR (DMSO, 100 MHz) $\delta = 145.6$, 141.3, 139.8, 138.8, 137.4, 133.4, 133.1, 130.2, 129.9, 129.7, 127.1, 125.2, 121.8, 118.6, 114.0, 20.7, 10.3; HRMS *m/z* 432.1939 $[M + H]^+$.

2-(3-(5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[d]imidazole (6g) Purified by column chromatography (ethyl acetate/hexane, 7:13) which gave **6g** as a pale yellow solid. mp 228–230 °C; IR (KBr) 3153, 3086, 1595, 1294 cm^{-1}; ^1H NMR (DMSO, 400 MHz) $\delta = 13.07$ (1H, s, NH), 9.40 (1H, s, Ar-H), 8.54 (2h, d, $J =$ 9.0 Hz, Ar-H), 8.12-8.07 (4H, m, Ar-H), 7.67-7.65 (2H, m, Ar-H), 7.60 (2H, t, $J = 8.5$ Hz, Ar-H), 7.43 (1H, t, $J =$ 7.3 Hz, Ar-H), 7.23-7.20 (2H, dd, $J = 2.8$, 6.0 Hz, Ar-H), 2.73 (3H, s, CH_3); ^{13}C NMR (DMSO, 100 MHz) $\delta = 147.8$, 145.3, 141.0, 140.4, 138.8, 138.1, 133.8, 130.0, 129.7, 127.2, 126.3, 125.2, 122.1, 121.7, 118.6, 118.2, 114.2, 111.6, 10.3; ESIMS *m/z* 463 $[M + H]^+$.

Biology

MTT assay for antiproliferative screening

Under sterile conditions, in vitro cytotoxicity assay for synthesized compounds (**4a–g** and **6a–g**) was carried out by employing a standard MTT assay. The MCF 7 (breast adenocarcinoma cell line) tumor cell line in Dulbecco's modified eagles medium (DMEM) (Gibco NY, USA) supplemented with 10% Fetal bovine serum (FBS) (Bio cell, CA, USA) and 1x antibiotic-antimycotic solution (contains Penicillin, Streptomycin, Amphotericin-D). 5×10^5 cells/mL was used to test the growth inhibition activity of synthesized compounds. MCF 7 cells growing exponentially were added to 96-well micro assay culture plate at a density of 3×10^3 cells per well in 200 µL culture media and were grown for 24 h at 37 °C in humidified 5% CO_2 incubator. The synthesized compounds were initially solubilised in phosphate-buffered saline (PBS) and serial dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 were made in PBS having concentrations of 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.0312 mg/mL, and 0.0156 mg/mL, respectively. After 24 h of seeding cells in 96-well plate, under sterile conditions the MCF7 cells and C6 cells were subjected to given synthesized compounds of respective dilutions to make final concentrations in triplicates. Cis-platin is taken as standard also prepared in same manner in triplicates. After treatment the plates were incubated for 24 h at 37 °C in humidified 5% CO_2 incubator. After 24 h, 20 µL of MTT dye solution (5 mg/mL) was added to each well and incubated for 1 h at 37 °C in humidified 5% CO_2 incubator. MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-di phenyl tetrazolium bromide) is cleaved by mitochondrial succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan is directly proportional to the number of viable cells and inversely proportional to the degree of cytotoxicity. After 1 h the MTT dye solution was aspirated and 100 µL of DMSO was added to solubilize the crystals formed. The absorbance was measured at 590 nm in ELISA reader. The graph was plotted between the concentration of synthesized compounds on *x*-axis and percentage of cell viability on *y*-axis. The data reported percent growth of treated cells which is presented as percentage growth of inhibition (GI%). IC_{50} values were calculated from the percentage of cell death.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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