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Synthesis and anticancer studies of novel benzimidazole/benzoxazole-pyrazine derivatives

Y.Jayaprakash Rao,[a*]G. Umamaheswar Rao,[a,c] Y. Hemasri,[b] and Krishna Reddy Valluru[c]

[a]*Department of Chemistry, Telangana University, Nizamabad-503322 India*
[b]*Department of Chemistry, Nizam College, Osmania university, Hyderabad-500088 India*
[c]*Aragen Life Sciences Pvt. Ltd., Hyderabad-500076, Telangan, India*
Email: yjpr_19@yahoo.com

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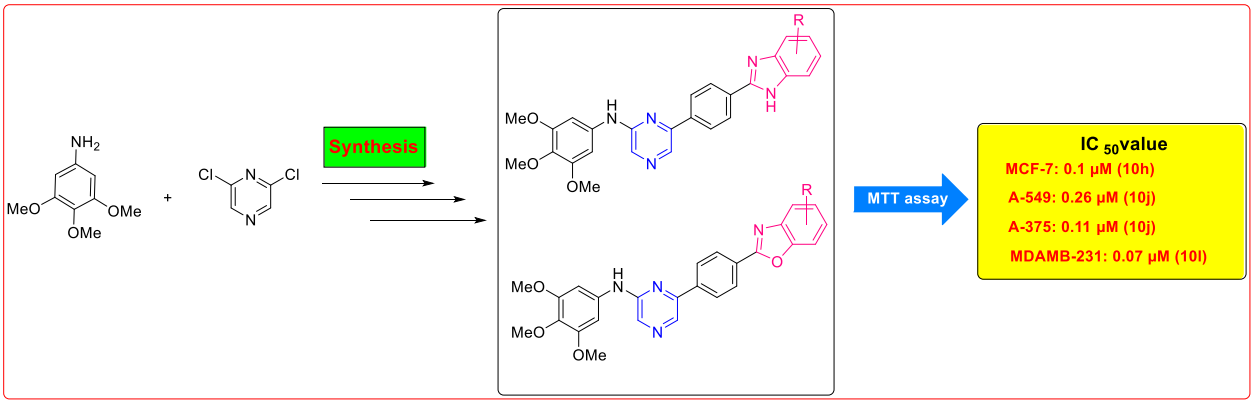
Abstract

A new series of aryl tethered benzimidazole-pyrazine and benzoxazole-pyrazine derivatives was synthesized *via* an intermediate chloro-aryl amine from 2,6-dichloropyrazine by adopting Pd(0)-catalysed Suzuki cross coupling and cyclo-condensation successively. The chloro-aryl amine was synthesised in high yield under transition metal free basic conditions. An efficient microwave mediated strategy was developed for the synthesis of benzoxazole–pyrazine derivatives. All the synthesized final targets were screened for *in vitro* cytotoxicity against human cancer cell lines MCF-7(breast), A-549(lung), A-375(melanoma) and DU-145(prostate) by employing *Doxorubicin* and *Etoposide* as controls.



Keywords:2,6-dichloropyrazine, benzimidazole, benzoxazole, microwave, cytotoxicity.

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Introduction

For many years, cancer has been a very serious health issue that kills people of all ages all over the world.[1,2] It caused 10 million fatalities worldwide in 2020, and by 2030, that number is predicted to rise to 13 million.[3–5] Lung, stomach, prostate, colorectal and liver cancer are the most common types of cancer in men, whereas breast, colorectal, lung, cervix and stomach cancer are more common in women.[6] Cancer typically develops because of genetic abnormalities and aberrant enzyme activity.[7] Avoiding exposure to carcinogenic chemicals, boosting the host defence system and using chemo preventive drugs that inhibit, reverse, or halt tumour formation can all help to prevent it.[8] However, it is still a condition that poses a serious risk to life and research into novel anticancer medications must continue.[9]

Pyrazines are a significant class of nitrogen heterocyclic motifs widely distributed in natural and synthetic molecules.[10] Pyrazine scaffolds and their derivatives exhibit a wide variety of biological activities such as anticancer,[11] diuretic,[12] anti-inflammatiory,[13] antidiabetic,[14] antimicrobial,[15] antiviral,[16] and DNA strand breakage agents.[17] In the anticancer area, *N*-(4-((6-((3-(oxazol-5-yl)phenyl)amino)pyrazin-2-yl)oxy)naphthalen-1-yl)acetamide (**1**) and *N*-(3-(6-((3,4,5-trimethoxyphenyl)amino)pyrazin-2-yl)phenyl)acetamide (**2**) act as BRAF inhibitors[18,19] (Figure 1).

In addition, benzimidazoles attract the attention of many organic and medicinal chemists in drug discovery.[20,21] Benzimidazole derivatives are potential pharmacological agents including anticancer,[22,23] antifungal,[24] Rho kinase inhibition,[25] antidiabetic,[26] antimicrobial,[27] anthelmintic,[28] antidepressant,[29] and antiprotozoal[30] compounds. Nocodazole (**3**), is a marketed antineoplastic drug which contains a benzimidazole nucleus and inhibits tubulin polymerization.[31] Benzoxazoles are among the most important heterocyclic compounds which exhibit remarkable pharmacological activities such as anticancer,[32] anthelmintic,[33] cyclooxygenase inhibitory,[34] antifungal,[35] antitubercular,[36] 5HT3 receptor antagonists,[37] anti-inflammatory,[38] analgesic,[39] and cyclin-dependent kinase inhibitory,[40] 5-lipoxygenase inhibitory,[41] melatonin receptor agonist,[42] antibacterial,[43] anti-HIV,[44] anticonvulsant,[45] antiviral,[46] antiallergic,[47] antihyperglycemic,[48] dopamineD4 agonists,[49] amyloidogenesis inhibitors,[50] and rho kinase inhibitors.[51]

Molecular hybridization is a tool which aims to combine two heterocyclic scaffolds in a single chemical entity to design multi target drug candidates for complex diseases.[52] We have applied this methodology in pursuit of benzimidazole-pyrazine and benzoxazole-pyrazine hybrid heterocyclic compounds with potential anticancer activity.

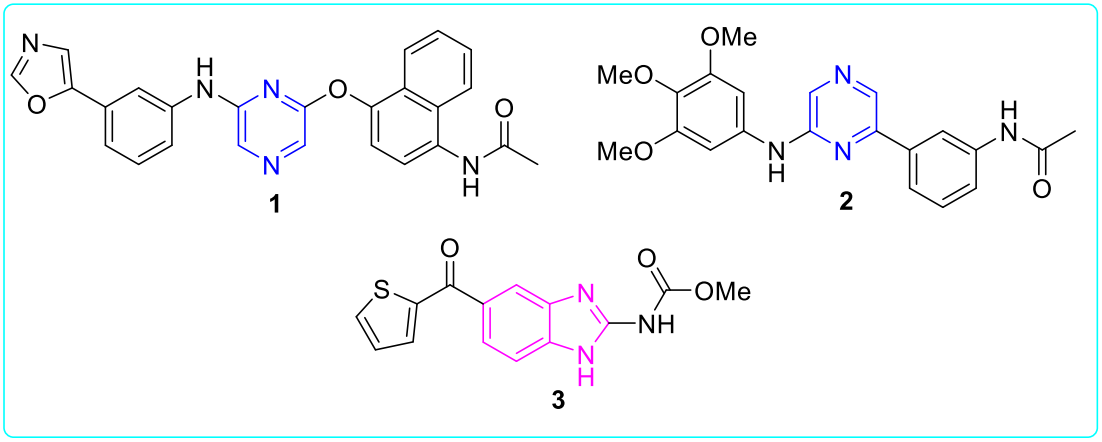
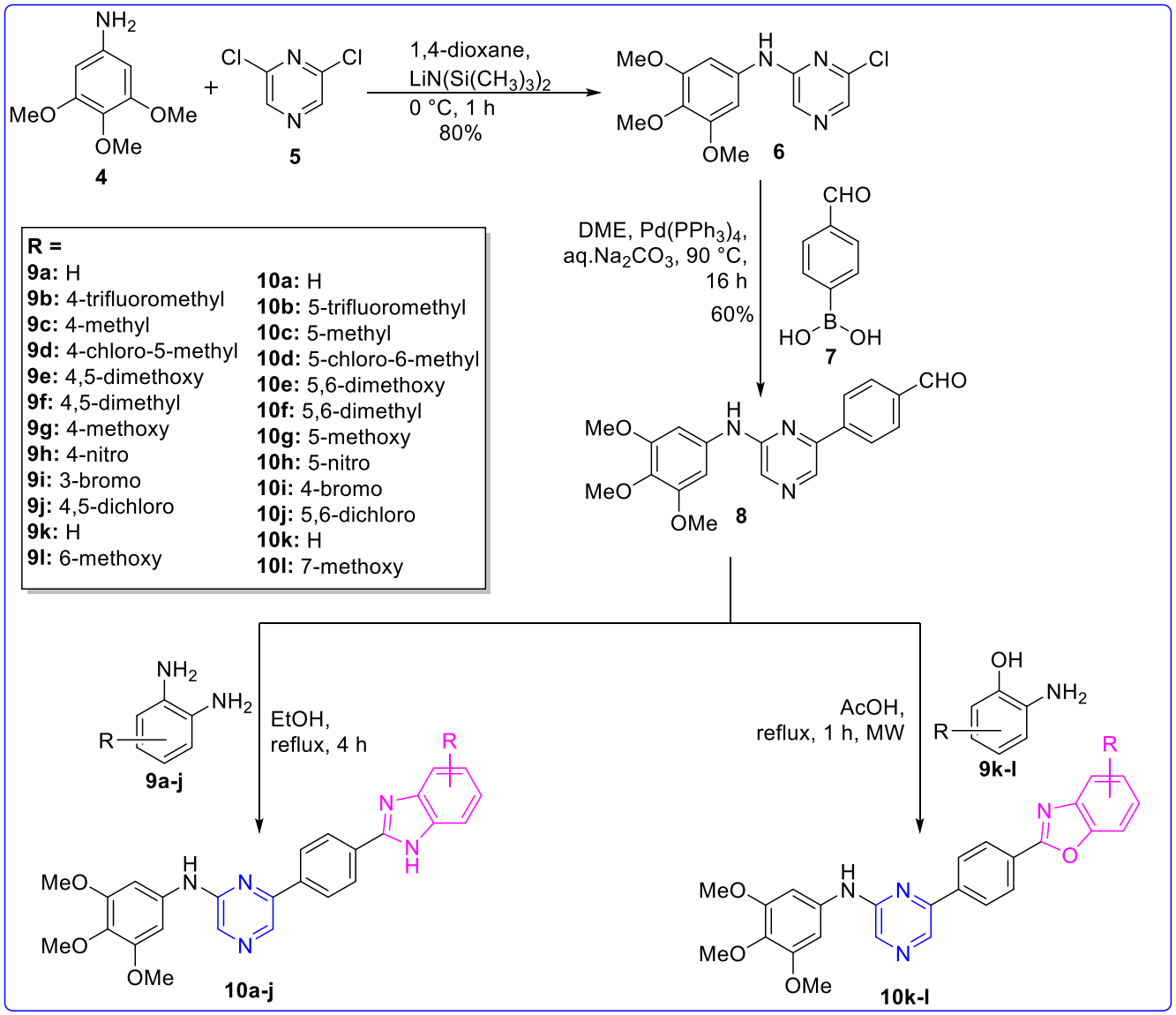


Figure 1. Biologically active pyrazine & benzimidazole molecules.

In the present study a new series of compounds with benzimidazole-pyrazine and benzoxazole-pyrazine ring systems have been synthesised and tested for *in vitro* anticancer activity.

Results and Discussion

Syntheses of benzimidazole-pyrazine **10a-j** and benzoxazole-pyrazine **10k-l** derivatives are outlined in Scheme 1. Initially, trimethoxy aniline **4** was coupled with 2,6-dichloropyrazine **5** in presence of $LiN(Si(CH_3)_3)_2$ in 1,4-dioxane to afford intermediate 6-chloro-N-(3,4,5-trimethoxyphenyl)pyrazine-2-amine **6** with high yield (80%). In earlier reported methods, low yields of intermediate **6** were obtained.[18] To introduce the desired aldehyde function, Compound **6** was subjected to Pd-catalysed Suzuki cross coupling reaction with 4-formylphenyl boronic acid **7** in Na_2CO_3 and DME medium to obtain 4-(6-((3,4,5-trimethoxyphenyl)amino)pyrazin-2-yl)benzaldehyde **8**. The aldehyde function of **8** was confirmed by appearance of a singlet at δ 10.07 in the ^{1}H NMR spectrum.



Scheme 1. Synthesis of novel pyrazine based benzimidazoles **10a-j** and benzoxazole derivatives **10k** and **10l**.

Cyclo-condensation of intermediate aldehyde **8** with various substituted aromatic 1,2-diamines **9a-j** individually in ethanol solvent under reflux condition afforded compounds **10a-j**, respectively, in good yields. In addition, we synthesised benzoxazole–pyrazine derivatives **10k** and **10l** by cyclo-condensation of intermediate **8** with substituted 2-aminophenols **9k** and **9l** in AcOH at 120 °C under microwave irradiation conditions. The formation of benzoxazole was explored under different conditions like i) EtOH, reflux, ii) EtOH, microwave, iii) toluene, *p*-TSA, heating, iv) ethanol, AcOH, heating, and v) AcOH, reflux. However, under these conditions no significant conversion was observed. A microwave assisted protocol in acetic acid medium gave benzoxazoles in low yields (<10%). The structures of all the synthesised compounds were confirmed by ^{1}HNMR, ^{13}CNMR and mass spectral analysis.

Anti-cancer activity

All the final target compounds were screened for *in vitro* cytotoxicity against human cancer cell lines MCF-7(breast), A-549(lung), A-375(melanoma) and DU-145(prostate) by using *Doxorubicin* and *Etoposide* as controls. Benzimidazole compounds **10b, 10d, 10g, 10h** and **10j** showed superior activity against human breast cancer cell line MCF-7, human lung cancer cell line A-549, and human melanoma cell line A-375 (Table 1).

Table 1. IC_{50} values for benzimidazole compounds **10a-j** in µM.

Compound	MCF-7	A-549	A-375
10a	2.9±0.07	3.78±0.24	-
10b	0.19±0.02	0.76±0.03	0.12±0.02
10c	4.78±0.55	2.45±0.12	9.78±0.78
10d	1.24±0.10	1.33±0.05	0.56±0.02
10e	7.45±0.55	3.55±0.51	6.49±0.62
10f	5.12±0.57	10.3±0.03	2.65±0.07
10g	1.9±0.02	0.89±0.02	0.34±0.07
10h	0.1±0.03	1.78±0.07	0.22±0.03
10i	7.34±0.84	12.9±1.10	3.56±0.55
10j	0.98±0.01	0.26±0.05	0.11±0.02
Doxorubicin	2.02± 0.05	2.18±0.02	5.51±0.04

The benzoxazole analogues **10k** and **10l**, displayed outstanding activity against all the three MCF-7, MDA-MB-231 and A-549 cell lines (Table-2). Replacing one of the N atoms in the benzimidazole ring with an O atom enhanced activity.

Table 2. IC_{50} values for benzoxazole compounds **10k** and **10l** in µM.

Compound	MCF-7	A-549	MDA-MB-231	DU-145
10k	0.23±0.04	0.34±0.05	0.92±0.02	1.45±0.04
10l	0.04±0.01	0.87±0.01	0.07±0.01	-

Etoposide	2.11±0.02	1.91±0.01	1.97±0.02	0.01±0.04

Conclusions

We have developed a simple and efficient strategy for the synthesis of new benzimidazole-pyrazine derivatives **10a-j** and established a practical microwave mediated synthesis of benzoxazole-pyrazines **10k** and **10l**. The intermediate chloro aryl amine was prepared under transition metal free conditions. The results of anticancer evaluation of **10a-l** against MCF-7, MDA-MB-231 and A-549 cell lines revealed that the pyrazine-benzimidazole and pyrazine-benzoxazole derivatives could be staring points for potential anticancer agents and can be further investigated for the development new chemotherapeutics.

Experimental Section

General. All the chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Microwave reactions were performed in an Anton Paar 450 apparatus at 250W power. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualisation on TLC was achieved by UV light or iodine indicator. All the final compounds were purified on a Waters HPLC system. ^{1}H and ^{13}C NMR spectra were recorded on Gemini Varian-VXR-unity (400 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. Solvents were purified according to standard procedures.

Synthesis of 6-chloro-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (6). To a mixture of 2,6-dichloropyrazine (**5**) (10 g, 67.11 mmol) and 3,4,5-trimethoxyaniline (**4)** (12.3 g, 67.11 mmol) in 1,4-dioxane (50 mL) was added $LiN(Si(CH_3)_3)_2$ (67.11 mL, 201.33 mmol, 3M in THF) at 0°C and stirred at same temperature for 1 h. The reaction mixture was quenched with ice water and the resulting solid was filtered, washed with ice cold water and dried under reduced pressure to afford pure compound **6**, as off white solid (16 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.80 (s, 1H), 8.13 (s, 1H), 7.96 (s, 1H), 7.04 (s, 2H), 3.77 (s, 6H), 3.64 (s, 3H);MS (ESI): 296 $[M+H]^+$.

Synthesis of 4-(6-(3,4,5-trimethoxyphenylamino)pyrazin-2-yl)benzaldehyde (8). To a stirred and degassed solution of 6-chloro-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amine **(6)** (13 g, 44 mmol) in DME (40 mL) and water (10 mL) were added tetrakis(triphenylphosphine)palladium(0) (2.5 g, 2.2 mmol), 4-formylphenylboronic acid (**7**) (6.6 g, 44 mmol) followed by sodium carbonate (9.3 g, 88 mmol). The mixture was heated at 90 °C for 16 h. After cooling, the reaction mixture was diluted with ethyl acetate and washed with water, brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified on silica gel column with 20% EtOAc/hexane to give compound **8** as off white solid (9.6 g, 60 % yield). ^{1}H NMR (400 MHz, DMSO-d_6, δppm):10.07 (s, 1H), 9.69 (s, 1H), 8.64 (s, 1H), 8.35 (d, 2H, *J* = 8.2 Hz), 8.23 (s, 1H), 8.05 (d, 2H, *J*= 8.2 Hz), 7.25 (s, 2H), 3.84 (s, 6H), 3.65 (s, 3H); MS (ESI): 366 $[M+H]^+$.

Synthesis of 6-(4-(1*H*-benzo[d]imidazol-2-yl)phenyl)-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amines 10a-j. Mixtures of aldehyde **8** (200 mg, 0.54 mmol) and each of o-phenylenediamines **9a-j** (0.82 mmol) individually in absolute ethanol (10 mL) were heated at reflux for 4 h. After cooling to room temperature, diethyl ether (20 mL)

was added to the reaction mixture, and the resulting precipitate was filtered off. The crude product was purified by preparative HPLC to afford pure compounds **10a-j**.

Prep-HPLC conditions for **10a**, **10c**, **10d**, **10e**, **10f**, **10g**, **10i**, **10j**:
Mobile phase: A: 0.1% Formic acid in water; B: Acetonitrile
Column: X-Bridge 19 X 150 mm, 5µm
Gradient: Time/%B: 0/30; 10/80, 10.1/98; 13/98, 13.1/30, 16/30
Flow rate: 19 mL/ min

Prep-HPLC conditions for **10b**, **10h**:
Mobile phase: A: 10mM Ammonium bicarbonate in water; B: Acetonitrile
Column: X-SELECT CSH C18 19 X 150 mm, 5µm
Gradient: Time/%B: 0/50; 11/80, 11.1/98; 13/98, 13.1/50, 15/50
Flow rate: 15 mL/ min

6-(4-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10a). Off white solid; Yield: 48%; mp 120-122 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.96 (brs, 1H, NH), 9.83 (brs, 1H, NH), 8.92 (d, 2H, *J* = 8.12 Hz, Ar-H), 8.63 (s, 1H, Ar-H), 8.53 (s, 1H, Ar-H), 8.12 (d, 2H, *J* = 8.12 Hz, Ar-H), 7.38-7.42 (m, 2H, Ar-H), 7.33-7.35 (m, 2H, Ar-H), 7.23 (s, 2H, Ar-H), 3.87 (s, 3H, OCH_3), 3.84 (s, 6H, 2xOCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.3, 153.8, 152.8, 144.8, 142.7, 140.4, 136.7, 134.8, 133.4, 131.2, 129.8, 129.3, 124.8, 117.4, 93.5, 61.9, 57.6; MS (ESI): 454 $[M+H]^+$. Anal calcd. for $C_{26}H_{23}N5O_3$: C, 68.86; H, 5.14; N, 15.39%. Found: C, 68.80; H, 5.09; N, 15.40%.

6-(4-(5-(Trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10b). Pale yellow solid; Yield: 42%;mp 127–129 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.80 (brs, 1H, NH), 9.63 (brs, 1H, NH),9.05 (d, 2H, *J* = 8.14 Hz, Ar-H),8.94 (s, 1H, Ar-H),8.83 (s, 1H, Ar-H),8.79 (s, 1H, Ar-H),8.61 (s, 1H, Ar-H),7.98-8.09 (m, 2H, Ar-H),7.62-7.63 (m, 1H, Ar-H),7.44 (s, 2H, Ar-H),3.70 (s, 6H, 2xOCH_3),3.65 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.8, 153.8, 152.3, 145.6, 142.8, 141.5, 140.5, 136.8, 135.7, 134.5, 131.7, 129.9, 129.7, 126.7, 123.8, 121.6, 119.7, 116.5, 93.8, 61.9, 57.7; MS (ESI): 522 $[M+H]^+$. Anal calcd. for $C_{27}H_{22}F_3N_5O_3$: C, 62.19; H, 4.25; N, 13.43; Found: C, 62.65; H, 4.39; N, 13.52%

6-(4-(5-Methyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10c). Pale brown solid; Yield: 37%; mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO- d_6, δppm): 9.64-9.67 (m, 2H, NH), 9.32 (d, 2H, *J* = 8.13 Hz, Ar-H), 8.09 (s, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.74 (d, 2H, *J* = 8.13 Hz, Ar-H), 7.22-7.24 (m, 2H, Ar-H), 7.08 (d, 1H, *J* = 8.04 Hz, Ar-H), 6.99 (s, 2H, Ar-H), 3.83 (s, 6H, 2xOCH_3), 3.80 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.8, 153.8, 152.4, 145.8, 142.3, 138.6, 136.7, 133.7, 133.4, 131.3, 129.8, 129.5, 127.8, 123.5, 119.6, 93.6, 61.7, 57.6, 23.4; MS (ESI): 468 $[M+H]^+$. Anal calcd. for $C_{27}H_{25}N_5O_3$: C, 69.36; H, 5.39; N, 14.98. Found: C, 69.56; H, 5.41; N, 14.99%.

6-(4-(5-Chloro-6-methyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10d). Pale yellow solid; Yield: 23%; mp 139–141 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.95 (brs, 1H, NH), 9.84 (brs, 1H, NH), 9.34 (d, 2H, *J* = 8.15 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.73 (d, 2H, *J* = 8.15 Hz, Ar-H), 7.65 (s, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.18 (s, 2H, Ar-H), 3.88 (s, 3H, OCH_3), 3.86 (s, 6H, 2xOCH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.7, 153.7, 152.3, 145.8, 142.5, 136.9, 136.4, 135.7, 134.6, 133.7, 133.5, 131.5, 129.8, 129.6, 128.6, 122.5, 116.7, 93.8, 61.8, 57.6, 22.7; MS (ESI): 502.1 $[M+H]^+$. Anal calcd. for $C_{27}H_{24}ClN_5O_3$: C, 64.60; H, 4.82; N, 13.95. Found: C, 64.79; H, 4.99; N, 14.11%.

***N*-6-[4-(5,6-Dimethoxy-1*H*-benzo[*d*]imidazol-2-yl)phenyl]-2-pyrazinyl-*N*-(3,4,5-trimethoxyphenyl)amine (10e).** Off white solid; yield: 32%; mp 143–145 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.91 (s, 1H, NH), 9.42 (s, 1H, NH), 9.22 (d, 2H, *J* = 8.15 Hz, Ar-H), 8.12 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 7.73 (d, 2H, *J* = 8.15 Hz, Ar-H), 6.98 (s, 2H, Ar-H), 6.79 (s, 2H, Ar-H), 3.95 (s, 6H, 2xOCH_3), 3.88 (s, 3H, OCH_3), 3.86 (s, 6H, 2xOCH_3); ^{13}C NMR (100

MHz, DMSO-d_6, δppm): 156.8, 154.7, 153.6, 152.7, 148.6, 145.8, 142.3, 137.6, 136.9, 136.4, 134.7, 133.4, 131.5, 129.9, 129.5, 99.5, 93.5, 61.8, 57.8; MS (ESI): 514.47 $[M+H]^+$. Anal calcd. for $C_{28}H_{27}N_5O_5$: C, 65.49; H, 5.30; N, 13.64; Found: C, 65.73; H, 5.66; N, 13.88%.

***N*-6-[4-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl]-2-pyrazinyl-*N*-(3,4,5-trimethoxyphenyl)amine(10f).** Pale brown solid; Yield: 30%; mp 148–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 10.04 (s, 1H, NH), 9.47 (s, 1H, NH), 9.15 (d, 2H, *J* = 8.14 Hz, Ar-H), 8.09 (s, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.77 (d, 2H, *J* = 8.14 Hz, Ar-H), 7.29 (s, 2H, Ar-H), 6.89 (s, 2H, Ar-H), 3.87 (s, 3H, OCH_3), 3.86 (s, 6H, 2xOCH_3), 2.29 (s, 6H, 2xCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.8, 153.8, 152.3, 145.8, 142.4, 135.9, 135.8, 135.6, 134.5, 133.4, 131.2, 129.8, 129.3, 114.5, 93.8, 61.8, 57.4, 21.6; MS (ESI): 482.46 $[M+H]^+$. Anal calcd. for $C_{28}H_{27}N_5O_3$: C, 69.84; H, 5.65; N, 14.54; Found: C, 69.97; H, 5.88; N, 14.98%.

***N*-6-[4-(5-Methoxy-1*H*-benzo[*d*]imidazol-2-yl)phenyl]-2-pyrazinyl-*N*-(3,4,5-trimethoxyphenyl)amine (10g).** Off white solid; Yield: 45%; mp 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.89 (s, 1H, NH), 9.53 (s, 1H, NH), 9.29 (d, 2H, *J* = 8.13 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.75 (d, 2H, *J* = 8.13 Hz, Ar-H), 7.08-7.11 (m, 2H, Ar-H), 7.04 (s, 1H, Ar-H), 6.96 (s, 2H, Ar-H), 3.88 (s, 3H, OCH_3), 3.86 (s, 6H, 2xOCH_3), 3.85 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 158.5, 156.7, 154.7, 153.6, 152.4, 145.6, 142.7, 142.3, 136.7, 135.7, 134.6, 133.4, 131.5, 129.8, 129.4, 116.7, 109.5, 101.5, 93.6, 61.9, 57.2; MS (ESI): 484 $[M+H]^+$. Anal calcd. for $C_{27}H_{25}N_5O_4$: C, 67.07; H, 5.21; N, 14.48; Found: C, 67.51; H, 5.63; N, 14.77%.

***N*-6-[4-(5-Nitro-1*H*-benzo[*d*]imidazol-2-yl)phenyl]-2-pyrazinyl-*N*-(3,4,5-trimethoxyphenyl)amine (10h).** Yellow solid; Yield: 29%; mp 157–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.81 (brs, 1H, NH), 9.61 (brs, 1H, NH), 8.98 (d, 2H, *J* = 8.19 Hz, Ar-H), 8.81 (s, 1H, Ar-H), 8.76-8.83 (m, 3H, Ar-H), 8.09 (d, 2H, *J* = 8.19 Hz, Ar-H), 7.57 (d, 1H, *J* = 8.14 Hz, Ar-H), 7.29 (s, 2H, Ar-H), 3.94 (s, 6H, 2xOCH_3), 3.87 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.8, 153.7, 152.6, 145.9, 145.7, 142.9, 142.6, 140.7, 136.8, 134.6, 131.5, 129.8, 129.4, 128.4, 120.8, 116.9, 115.6, 93.8, 61.9, 57.9; MS (ESI): 499 $[M+H]^+$. Anal calcd. for $C_{26}H_{22}N_6O_5$:C, 62.64; H, 4.45; N, 16.86; Found: C, 62.92; H, 4.65; N, 17.25%.

***N*-6-[4-(4-Bromo-1*H*-benzo[*d*]imidazol-2-yl)phenyl]-2-pyrazinyl-*N*-(3,4,5-trimethoxyphenyl)amine (10i).** Pale yellow solid; Yield: 42%; mp 160–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.64 (brs, 1H, NH); 9.28 (d, 2H, *J* = 8.16 Hz, Ar-H), 8.09 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.72 (d, 2H, *J* = 8.16 Hz, Ar-H), 7.32-7.39 (m, 3H, Ar-H), 6.91(s, 2H, Ar-H), 3.88 (s, 3H, s, 3H, OCH_3), 3.81 (s, 6H, 2xOCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.7, 153.7, 151.5, 145.6, 142.5, 137.8, 136.6, 135.7, 134.6, 133.5, 132.0, 131.6, 129.9, 129.7, 125.7, 122.3, 117.8, 93.8, 61.9, 57.6; MS (ESI): 532 $[M+H]^+$. Anal calcd. for $C_{26}H_{22}BrN_5O_3$: C, 58.66; H, 4.17; N, 13.15; Found: C, 58.75; H, 4.31; N, 13.45%.

6-(4-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10j). White solid; Yield 21%; mp 164–166 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.73 (s, 1H, NH),9.59 (s, 1H, NH), 9.33 (d, 2H, *J* = 8.19 Hz, Ar-H), 8.11 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.79 (d, 2H, *J* = 8.19 Hz, Ar-H), 7.72 (s, 2H, Ar-H), 7.12 (s, 2H, Ar-H), 3.95 (s, 3H, OCH_3), 3.88 (s, 6H, 2xOCH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δppm): 156.9, 154.8, 153.7, 152.6, 145.8, 142.7, 137.5, 136.5, 135.8, 134.7, 133.6, 131.8, 129.8, 129.6, 128.5, 119.9, 93.8, 61.9, 57.7; MS (ESI): 522 $[M+H]^+$. Anal calcd. for $C_{26}H_{21}Cl_2N_5O_3$: C, 59.78; H, 4.05; N, 13.41; Found: C, 60.15; H, 4.37; N, 13.67%.

General procedure for synthesis of 6-(4-(benzo[*d*]oxazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine derivatives 10k and 10l.

Mixtures of aldehyde **8** (200 mg, 0.82 mmol) and each of 2-aminophenols **9k** and **9l** (0.82 mmol) individually in glacial acetic acid (15 mL) were irradiated at 120 °C in a microwave reactor at 250W power for 1 h. The reaction mixture was cooled to room temperature, diethyl ether (20 mL) was added, and the resulting precipitate was filtered off. The crude product was purified by preparative HPLC to afford pure compound.

Prep-HPLC conditions:
Mobile phase: A: 10mM Ammonium bicarbonate in water; B: Acetonitrile
Column: X-SELECT CSH C18 19 X 150 mm, 5µm
Gradient: Time/%B: 0/50; 11/90, 11.1/100; 13/100, 13.1/50, 15/50
Flow rate: 19 mL/ min

6-(4-(Benzo[*d*]oxazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10k). Pale yellow solid; Yield: 8%; mp 144–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.67 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.33-8.39 (m, 5H, Ar-H), 8.23 (s, 1H, Ar-H), 7.82-7.86 (m, 2H, Ar-H), 7.44-7.47 (m, 2H, Ar-H), 7.27 (s, 1H, Ar-H), 3.87 (s, 6H, 2xOCH_3), 3.65 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 161.8, 152.8, 151.4, 150.2, 146.3, 141.5, 139.6, 136.7, 134.5, 132.1, 130.0, 127.6, 127.1, 127.0, 125.7, 124.9, 119.8, 110.9, 95.8, 60.1, 55.6; MS (ESI): 455 $[M+H]^+$. Anal calcd. for $C_{26}H_{22}N_4O_4$: C, 68.71; H, 4.88; N, 12.33; Found: C, 68.97; H, 4.93; N, 12.81%**6-(4-(7-Methoxybenzo[*d*]oxazol-2-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (10l).** Off white solid; Yield: 6%; mp 164–186 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δppm): 9.68 (s, 1H, NH), 8.64 (s, 1H, Ar-H), 8.37 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.30 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.22 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.21 (s, 2H, Ar-H), 7.00 (m, 1H, Ar-H), 4.01 (s, 3H, OCH_3), 3.86 (s, 6H, 2xOCH_3), 3.65 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δppm): 161.2, 153.4, 152.0, 151.5, 148.1, 139.2, 135.6, 133.8, 132.1, 131.7, 131.4, 127.9, 127.8, 126.9, 126.0, 105.8, 103.3, 97.4, 61.0, 56.1, 56.0; MS (ESI): 485 $[M+H]^+$. Anal calcd. for $C_{27}H_{24}N_4O_5$: C, 66.93; H, 4.99; N, 11.56; Found: C, 67.11; H, 5.13; N, 12.01%.

MTT assay. The cytotoxic activity of the compounds was determined using MTT assay. MTT [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were procured from Gibco. 25 cm^2 and 75 cm^2 flasks and 96 well plates were purchased from Eppendorf India. The cell lines were purchased from NCCS, Pune, India. 1×10^4 cells/well were seeded in 200 ml DMEM [Dulbecco's Modified Eagle Medium], supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 hours at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 hours of incubation, 10 ml MTT (5 mg/ml) was added to each well and the plates were further incubated for 4 hours. The supernatant from each well was carefully removed, formazan crystals were dissolved in 100 ml of DMSO and absorbance at 540 nm wavelength was recorded. The experimental procedure was performed in triplicate with 6 variable µM concentrations.

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Supplementary Material

Copies of all ^{1}H and ^{13}C NMR and Mass spectra along with anticancer activity data of all products are available in the Supplementary material.

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