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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of 1-cyano-2-amino-benzimidazole derivatives as a novel class of antitumor agents

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Abstract A series of 1-cyano-2-amino-benzimidazole derivatives were synthesized and evaluated for their cytotoxic activities in vitro against three human cancer cell lines (human lung carcinoma cell line: A549, human leukemia cell line: K562, and human prostate cancer cell line: PC-3). Most of these compounds showed potent activities against these tumor cell lines, especially against A549 and K562 cell lines. The preliminary structure–activity relationships of 1-cyano-2-amino-benzimidazole derivatives were also discussed. The cell cycle analysis was carried out in K562 cells and the results showed that compound **4d** caused a marked increase of cells in G_2/M phase.

Keywords 1-Cyano-2-amino-benzimidazole derivatives · Antitumor activity · Structure–activity relationships · Cell cycle analysis

Introduction

Cancer is one of the major leading causes of death around the world. Taking into account the existing cancer therapies, chemotherapy has turned out to be one of the most significant treatments in cancer management (Harrison *et al.*, 2009). Although the success of clinical practice in identifying a large number of potent chemotherapeutic anticancer agents has been significant, clinical treatments still suffer from many toxic side effects of the drugs such as bone marrow suppression, gastrointestinal tract lesions, nausea, hair loss, drug resistance, and so on (Nussbaumer *et al.*, 2011). Therefore, the development of novel, efficient, and less toxic anticancer agents is still of utmost importance.

The benzimidazole nucleus is an important pharmacophore and privileged structure in drug discovery. Benzimidazole derivatives have been found to possess a wide variety of biological activities including antiviral (Morningstar *et al.*, 2007), anti-inflammatory (Mader *et al.*, 2008), antihypertensive (Kaur *et al.*, 2008; Kohara *et al.*, 1996), antioxidant (Kuş *et al.*, 2008, 2009), antitubercular (Camacho *et al.*, 2011), antiulcer (Kühler *et al.*, 1998), and antimicrobial (Ansari and Lal, 2009; El-masry *et al.*, 2000). Particularly noteworthy is that benzimidazole derivatives have also been found to be of potent anticancer activities (Hranjec *et al.*, 2008a, b, 2010; Gellis *et al.*, 2008; Thimme Gowda *et al.*, 2008; Thimme Gowda *et al.*, 2009; Winfield *et al.*, 2008), and benzimidazole nucleus was considered to be an essential part of many antineoplastic derivatives (Badawey and Kappe, 1995). Bendamustine hydrochloride containing alkylating group and benzimidazole component has been approved by FDA to be used for the treatment of patients with chronic lymphocytic leukemia (Cheson and Rummel, 2009). Due to the significant medicinal importance, the synthesis and biological evaluation of benzimidazole derivatives have attracted considerable attention in the recent years.

The benzimidazole compounds with cyano substituent in general strongly enhance the cytotoxic activity. Some cyano-substituted benzimidazole compounds were found to possess cytotoxic activity on several human cancer cell lines (Hranjec *et al.*, 2010, 2008a, b). Earlier, we reported that the intramolecular C–N coupling reactions of various

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substituted aryl guanidines could be successfully carried out to form 1-cyanobenzimidazoles by employing a CuI/2,2′-biimidazole catalyst system (Hu *et al.*, 2011). In continuation with our efforts in search of potential anticancer agents, we have synthesized the novel series of cyano-substituted benzimidazole derivatives by introducing different substituted amino groups at the second position of benzimidazole nucleus. Full details about the synthesis, evaluation of antitumor activity in vitro of benzimidazole derivatives are reported herein.

Results and discussions

Chemistry

The synthesis of a series of 1-cyano-2-amino-benzimidazole derivatives were accomplished via intramolecular cyclization with a CuI/2,2′-biimidazole catalyst system. As shown in Scheme 1, condensation of the commercially available dimethyl *N*-cyanodithioiminocarbonate **1** with 2-iodoaniline in the presence of Cs_2CO_3 in DMF at 100 °C for 8 h gave adduct **2**. Compounds **2** reacted with the secondary amines in fusion to afford the corresponding aryl guanidines **3**. Finally, 1-cyano-2-amino-benzimidazole derivatives **4** were obtained by intramolecular cyclization of aryl guanidines **3** under the conditions of 5 mol% of CuI with 10 mol% of 2,2′-biimidazole in the presence of Cs_2CO_3. The final compounds **4** were purified by silica gel column chromatography using ethyl acetate/hexane as eluent and obtained in good yields. Structures of all the final compounds, **4,** were established through FTIR, NMR spectra, ESI–MS and elemental analysis.

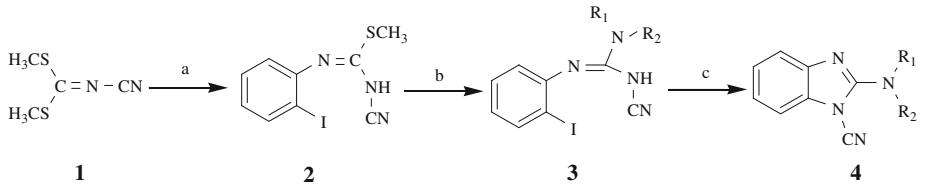
Biological activities

All the obtained 1-cyano-2-amino-benzimidazole derivatives were evaluated for their cytotoxic activities against three human cancer cell lines derived from the various human cancer types, including A549 (human lung carcinoma cells), K562 (human leukemia cells), and PC-3 (human prostate cancer cells). In vitro evaluation of the cytotoxic activities of these compounds was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Doxorubicin hydrochloride was employed as the reference drug. The cytotoxic potency of these compounds was indicated by IC_{50} values. The results are summarized in Table 1.

As shown in Table 1, most of the 1-cyano-2-amino-benzimidazole derivatives exhibited inhibitions on the growth of selected tumor cell lines, especially on A549 and K562 cell lines. Several compounds showed moderate to potent cytotoxic activities against all the tested cell lines ($IC_{50} < 50$ μmol L^{-1}). Compounds **4c** and **4d** were the most promising compounds among the tested derivatives with IC_{50} values of 3.33–33.52 and 2.69–18.51 μmol L^{-1}, respectively. Preliminary structure–activity relationship of 1-cyano-2-amino-benzimidazole derivatives was investigated. In general, cyclic secondary amines with R_1R_2NH substitutions were beneficial for increasing the cytotoxicity of 1-cyano-2-amino-benzimidazoles compared with open chain secondary amines. It was found that the introduction of morpholine (**4c**) or methylpiperazine (**4d**) at the R_1R_2NH position led to compounds with good cytotoxic activity against A549 and K562 cell lines. Particularly noteworthy is that the methylpiperazine substitution yielded compound **4d** which was discovered as the most potent inhibitor against A549, K562 and PC-3 cell lines with IC_{50} values of 6.48, 2.69 and 18.51 μmol L^{-1}, respectively. Additionally, a heterocyclic derived piperazine analog, **4j** showed a similar potency against K562 with an IC_{50} of 3.39 μmol L^{-1} when compared with **4d**. Among R_1R_2NH substitutions of the open chain secondary amines, little steric hindrance substitutions were beneficial for 1-cyano-2-amino-benzimidazole's cytotoxicity on A549 and K562 cell lines, such as compounds **4k** and **4l**. And bulky groups were detrimental to the compounds' cytotoxicity, such as compounds **4m** and **4n**. When the acyclic aliphatic amines were replaced by diphenylamine, **4o** showed a decreased cytotoxic activity.

To shed light on the mechanisms responsible for the cytotoxic effect of compound **4d**, we examined its influence on the growth and division of K562 cells by measuring the DNA content of eukaryotic cells. Cells were cultured for 24 h in the presence of **4d** with 10, 30, and

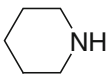
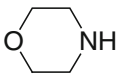
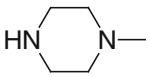
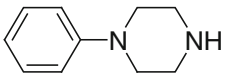
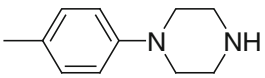
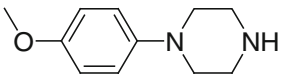
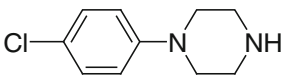
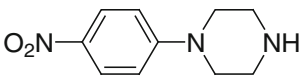
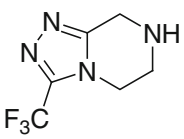
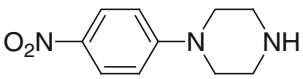
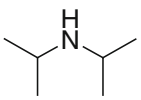
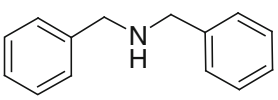
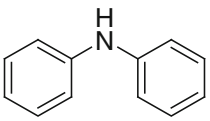
Scheme 1 Synthesis of 1-cyano-2-amino-benzimidazole derivatives



1 2 3 4

Reagents and conditions: (a) 2-iodoaniline, Cs_2CO_3, DMF, 100°C; (b) R_1R_2NH, ethanol, reflux; (c) CuI, 2,2'-biimidazole, Cs_2CO_3, DMF, 80°C.

Table 1 Cytotoxicity of 1-cyano-2-amino-benzimidazole derivatives against human cancer lines in vitro

Compounds	R_1R_2NH	Cytotoxicity (IC_{50}, μmol L^{-1})[a]		
		A549	K562	PC-3
Doxorubicin	–	3.36 ± 0.57	1.15 ± 0.18	2.37 ± 0.16
4a		19.11 ± 4.01	12.35 ± 3.16	38.76 ± 2.57
4b		12.38 ± 6.24	18.47 ± 5.17	41.39 ± 0.12
4c		8.55 ± 3.37	3.33 ± 2.15	33.52 ± 4.21
4d		6.48 ± 0.77	2.69 ± 0.65	18.51 ± 0.33
4e		13.21 ± 1.24	23.66 ± 3.03	>50
4f		17.16 ± 2.33	22.03 ± 5.12	39.65 ± 3.18
4g		15.69 ± 0.35	18.32 ± 4.11	>50
4h		16.22 ± 5.01	28.83 ± 2.35	42.15 ± 2.26
4i		11.16 ± 2.52	37.64 ± 1.20	>50
4j		28.26 ± 5.21	3.39 ± 0.26	31.25 ± 3.09
4k		17.03 ± 3.62	21.25 ± 3.15	>50
4l		18.31 ± 2.29	20.49 ± 2.15	>50
4m		40.35 ± 3.72	>50	>50
4n		37.13 ± 5.26	36.82 ± 3.47	>50
4o		>50	>50	>50

[a] Each experiment was independently performed three times and expressed as mean ± SD

Table 2 Cell cycle distribution by flow cytometry in K562 cells treated with compound **4d** for 24 h

Concentration (μmol L^{-1})	G_0/G_1 (%)	S (%)	G_2/M (%)
Control	44.7 ± 0.63	49.0 ± 0.31	6.3 ± 0.54
10	38.3 ± 3.26	46.5 ± 0.28	15.2 ± 2.59
30	27.0 ± 1.37	45.7 ± 0.86	27.3 ± 2.17
50	10.3 ± 1.72	44.5 ± 0.37	45.2 ± 0.53

50 μmol L^{-1}, and then evaluated. Flow cytometric analysis of cell cycle was carried out and reported in Table 2. The results showed that compound **4d** arrested the cells in the G_2/M phase of the cell cycle, which was accompanied by a dose-dependent manner.

Conclusion

A series of 1-cyano-2-amino-benzimidazole derivatives were synthesized via intramolecular cyclization. Their cytotoxicity against a variety of cancer cell lines was evaluated. Most of them displayed potent cytotoxic activities in the micromolar range, especially against A549 and K562 cell lines. The preliminary structure–activity relationship of these compounds was investigated. The cell cycle analysis in K562 cells showed that compound **4d** caused the significant arrest of the cell cycle at the G_2/M phase in a dose-dependent manner.

Experimental

Materials and methods

Unless otherwise indicated, all reactions were carried out under a dry nitrogen atmosphere. DMF was freshly distilled from calcium hydride. The other reagents were used directly without further purification. The melting points (mp) were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. FTIR spectra were recorded on a PerkinElmer Spectrum 100 infrared spectrophotometer (KBr pellet). ^{1}H NMR (400 MHz) or ^{13}C NMR (100 MHz) data were recorded on a DPX-400 instrument with $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in ppm and spin–spin coupling constants, J, are given in Hz. Abbreviations used were s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra (MS) were recorded on a HP5989A mass spectrometer. Elemental analyses were carried out on a PE EA2400 CHN analyzer.

*N-(2-Iodophenyl)-N′-cyano-S-methylisothiourea (**2**)*

Dimethyl cyanodithioimido-carbonate (1.46 g, 10 mmol) was dissolved in DMF (20 mL). To the stirred solution were added 2-iodoaniline (10 mmol) and Cs_2CO_3 (4.89 g, 15 mmol). The solution was kept at 100 °C for 8 h and then poured into ice water. Precipitates formed immediately and were collected by filtration. The crude products were chromatographed on silica gel with petroleum ether/ethyl acetate to afford the desired compound. It was obtained as a yellow solid, mp 178–180 °C, yield 41 %. IR (KBr), ν (cm^{-1}): 3332 (NH), 3046 (Ar–H), 2241 (C≡N), 1587 (C=N). ^{1}H NMR (DMSO-d_6) δ: 2.63 (s, 3H, SCH_3), 7.12–7.14 (m, 1H, ArH), 7.36–7.48 (m, 2H, ArH), 7.92–7.94 (m, 1H, ArH), 10.35 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 13.3 (S–C), 83.6 (C–I), 116.1 (C≡N), 124.1, 128.5, 130.3, 139.2 (aromatic carbons), 158.1 (C–N, iodophenyl), 161.3 (C=N). MS: m/z $[M+1]^+$ 318.

*General procedure for the synthesis of aryl guanidines (**3a–o**)*

A mixture of **2** (10 mmol) and secondary amines (12 mmol) in ethanol (30 mL) was refluxed for 24 h, then the solvent was removed under the reduced pressure. The resulting residue was purified by column chromatography on silica gel with petroleum ether/ethyl acetate to provide the desired aryl guanidines **3a–o**.

*N′-(2-Iodophenyl)-N-cyanopyrrolidine-1-carboximidamide (**3a**)*

This compound was prepared by the reaction of **2** and pyrrolidine. It was obtained as a pale yellow solid, mp 82–84 °C, yield 63 %. IR (KBr), ν (cm^{-1}): 3386 (NH), 3075 (Ar–H), 2846 (CH_2, aliphatic), 2232 (C≡N), 1594 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.93 (t, 4H, CH_2 pyrrolidin), 3.44 (t, 4H, NCH_2, pyrrolidin), 6.91–6.94 (m, 1H, ArH), 7.34–7.36 (m, 2H, ArH), 7.82–7.84 (m, 1H, ArH), 9.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 25.2 (2C, pyrrolidin), 49.3 (2C, N–C, pyrrolidin), 83.5 (C–I), 116.4 (C≡N), 123.6, 128.7, 131.4, 139.5 (aromatic carbons), 159.3 (C–N, iodophenyl), 162.6 (C=N). MS: m/z $[M+1]^+$ 341.

*N′-(2-Iodophenyl)-N-cyanopiperidine-1-carboximidamide (**3b**)*

This compound was prepared by the reaction of **2** and piperidine. It was obtained as a pale yellow solid, mp 88–89 °C, yield 67 %. IR (KBr), ν (cm^{-1}): 3351 (NH), 3066 (Ar–H), 2911 (CH_2, aliphatic), 2238 (C≡N), 1610 (C=N). ^{1}H NMR (DMSO-d_6) δ: 1.57–1.59 (m, 6H, CH_2

piperidin), 3.51–3.53 (m, 4H, NCH_2, piperidin), 6.96–7.00 (m, 1H, ArH), 7.20–7.22 (m, 1H, ArH), 7.35–7.39 (m, 1H, ArH), 7.84–7.86 (m, 1H, ArH), 9.07 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 24.4 (piperidin), 25.6 (2C, piperidin), 49.5 (2C, N–C, pyrrolidin), 83.8 (C–I), 115.7 (C≡N), 123.9, 128.8, 130.2, 138.8 (aromatic carbons), 158.2 (C–N, iodophenyl), 159.7 (C=N). MS: *m/z* $[M+1]^+$ 355.

*N′-(2-Iodophenyl)-N-cyanomorpholine-4-carboximidamide (**3c**)*

This compound was prepared by the reaction of **2** and morpholine. It was obtained as a pale yellow solid, mp 163–165 °C, yield 61 %. IR (KBr), ν (cm^{-1}): 3375 (NH), 3053 (Ar–H), 2889 (CH_2, aliphatic), 2232 (C≡N), 1620 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.54 (t, 4H, NCH_2, morpholino), 3.66 (t, 4H, OCH_2, morpholino), 6.98–7.01 (m, 1H, ArH), 7.24–7.26 (m, 1H, ArH), 7.36–7.39 (m, 1H, ArH), 7.84–7.87 (m, 1H, ArH), 9.26 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.7 (2C, N–C, morpholino), 63.9 (2C, O–C, morpholino), 83.6 (C–I), 116.0 (C≡N), 123.3, 128.3, 130.7, 139.0 (aromatic carbons), 158.6 (C–N, iodophenyl), 160.1 (C=N). MS: *m/z* $[M+1]^+$ 357.

*N′-(2-Iodophenyl)-N-cyano-4-methylpiperazine-1-carboximidamide (**3d**)*

This compound was prepared by the reaction of **2** and 1-methylpiperazine. It was obtained as a pale yellow solid, mp 113–116 °C. yield 73 %; IR (KBr), ν (cm^{-1}): 3362 (NH), 3088 (Ar–H), 2867 (CH_2, aliphatic), 2236 (C≡N), 1608 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.20 (s, 3H, NCH_3), 2.35 (t, 4H, NCH_2, piperazin), 3.57 (t, 4H, NCH_2, piperazin), 6.96–6.99 (m, 1H, ArH), 7.21–7.23 (m, 1H, ArH), 7.35–7.37 (m, 1H, ArH), 7.83–7.85 (m, 1H, ArH), 9.10 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.6 (N–CH_3), 45.7 (2C, N–C, piperazin), 54.3 (2C, N–C, piperazin), 83.7 (C–I), 116.4 (C≡N), 123.1, 128.7, 131.2, 139.3 (aromatic carbons), 158.5 (C–N, iodophenyl), 161.6 (C=N). MS: *m/z* $[M+1]^+$ 370.

*N′-(2-Iodophenyl)-N-cyano-4-phenylpiperazine-1-carboximidamide (**3e**)*

This compound was prepared by the reaction of **2** and 1-phenylpiperazine. It was obtained as a pale yellow solid, mp 121–123 °C, yield 56 %. IR (KBr), ν (cm^{-1}): 3379 (NH), 3051 (Ar–H), 2883 (CH_2, aliphatic), 2238 (C≡N), 1607 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.68 (t, 4H, NCH_2, piperazin), 3.20 (t, 4H, NCH_2, piperazin), 6.92–6.94 (m, 2H, ArH), 6.98–7.02 (m, 2H, ArH), 7.23–7.26 (m, 3H, ArH), 7.34–7.36 (m, 1H, ArH), 7.79–7.82 (m, 1H, ArH), 9.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.9 (2C, N–C, piperazin), 54.6 (2C, N–C, piperazin), 83.6 (C–I), 116.2 (C≡N), 116.6 (2C), 120.8, 123.5, 128.4, 130.3, 130.7 (2C), 138.9 (aromatic carbons), 155.0 (C–N, phenyl), 158.2 (C–N, iodophenyl), 160.5 (C=N). MS: *m/z* $[M+1]^+$ 432.

*N′-(2-Iodophenyl)-N-cyano-4-p-tolylpiperazine-1-carboximidamide (**3f**)*

This compound was prepared by the reaction of **2** and 1-*p*-tolylpiperazine. It was obtained as a pale yellow solid, mp 106–108 °C, yield 58 %. IR (KBr), ν (cm^{-1}): 3358 (NH), 3079 (Ar–H), 2942 (CH_3), 2879 (CH_2, aliphatic), 2235 (C≡N), 1600 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.18 (s, 3H, CH_3), 2.72 (t, 4H, NCH_2, piperazin), 3.20 (t, 4H, NCH_2, piperazin), 6.76–6.78 (d, $J = 7.2$ Hz, 2H, ArH), 6.93–6.95 (d, $J = 7.2$ Hz, 2H, ArH), 7.01–7.03 (m, 1H, ArH), 7.23–7.26 (m, 1H, ArH), 7.33–7.35 (m, 1H, ArH), 7.77–7.81 (m, 1H, ArH), 9.10 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 24.1 (CH_3), 47.3 (2C, N–C, piperazin), 53.6 (2C, N–C, piperazin), 83.5 (C–I), 116.3 (C≡N), 115.4 (2C), 123.1, 128.0, 128.4, 130.0 (2C), 130.3, 139.1 (aromatic carbons), 154.1 (C–N, 1-*p*-tolyl), 158.3 (C–N, iodophenyl), 160.3 (C=N). MS: *m/z* $[M+1]^+$ 446.

*N′-(2-Iodophenyl)-N-cyano-4-(4-methoxyphenyl)piperazine-1-carboximidamide (**3g**)*

This compound was prepared by the reaction of **2** and 1-(4-methoxyphenyl)piperazine. It was obtained as a pale yellow solid, mp 138–140 °C, yield 62 %. IR (KBr), ν (cm^{-1}): 3350 (NH), 3086 (Ar–H), 2956 (CH_3), 2892 (CH_2, aliphatic), 2243 (C≡N), 1603 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.70 (t, 4H, NCH_2, piperazin), 3.16 (t, 4H, NCH_2, piperazin), 3.76 (s, 3H, OCH_3), 6.82–6.84 (d, $J = 7.2$ Hz, 2H, ArH), 6.88–6.90 (d, $J = 7.2$ Hz, 2H, ArH), 6.99–7.02 (m, 1H, ArH), 7.24–7.26 (m, 1H, ArH), 7.34–7.36 (m, 1H, ArH), 7.79–7.82 (m, 1H, ArH), 9.09 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.5 (2C, N–C, piperazin), 54.7 (2C, N–C, piperazin), 57.2 (OCH_3), 83.7 (C–I), 116.1 (C≡N), 116.5 (2C), 118.6(2C), 123.3, 128.5, 130.6, 139.0 (aromatic carbons), 154.9 (C–N, methoxyphenyl), 156.5 (C–O, methoxyphenyl), 158.4 (C–N, iodophenyl), 160.8 (C=N). MS: *m/z* $[M+1]^+$ 462.

*N′-(2-Iodophenyl)-N-cyano-4-(4-chlorophenyl)piperazine-1-carboximidamide (**3h**)*

This compound was prepared by the reaction of **2** and 1-(4-chlorophenyl)piperazine. It was obtained as a pale yellow solid, mp 131–133 °C, yield 42 %. IR (KBr), ν (cm^{-1}): 3346 (NH), 3072 (Ar–H), 2881 (CH_2, aliphatic), 2237 (C≡N), 1602 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.71

(t, 4H, NCH_2, piperazin), 3.42 (t, 4H, NCH_2, piperazin), 6.83–6.85 (d, $J = 7.2$ Hz, 2H, ArH), 7.00–7.03 (m, 1H, ArH), 7.21–7.24 (m, 1H, ArH), 7.32–7.34 (m, 1H, ArH), 7.36–7.39 (d, $J = 7.2$ Hz, 2H, ArH), 7.82–7.84 (m, 1H, ArH), 9.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.3 (2C, N–C, piperazin), 54.1 (2C, N–C, piperazin), 83.5 (C–I), 116.3 (C≡N), 116.5 (2C), 123.5, 124.2, 127.6 (2C), 128.3, 130.1, 138.9 (aromatic carbons), 157.8 (C–N, 4-chlorophenyl), 158.1 (C–N, iodophenyl), 160.5 (C=N). MS: *m/z* $[M+1]^+$ 466.

*N′-(2-Iodophenyl)-N-cyano-4-(4-nitrophenyl)piperazine-1-carboximidamide (**3i**)*

This compound was prepared by the reaction of **2** and 1-(4-nitrophenyl)piperazine. It was obtained as a pale yellow solid, mp 126–129 °C, yield 48 %. IR (KBr), ν (cm^{-1}): 3411 (NH), 3079 (Ar–H), 2886 (CH_2, aliphatic), 2228 (C≡N), 1610 (C=N), 1521, 1372 (NO_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.68 (t, 4H, NCH_2, piperazin), 3.44 (t, 4H, NCH_2, piperazin), 6.81–6.83 (d, $J = 7.2$ Hz, 2H, ArH), 7.01–7.03 (m, 1H, ArH), 7.25–7.27 (m, 1H, ArH), 7.33–7.35 (m, 1H, ArH), 7.82–7.85 (m, 1H, ArH), 8.09–8.11 (d, $J = 7.2$ Hz, 2H, ArH), 9.13 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.8 (2C, N–C, piperazin), 54.6 (2C, N–C, piperazin), 83.7 (C–I), 116.1 (C≡N), 116.9 (2C), 123.4, 127.2 (2C), 128.4, 130.4, 138.8 (aromatic carbons), 140.5 (C–NO_2, nitrophenyl), 158.2 (C–N, nitrophenyl), 158.5 (C–N, iodophenyl), 160.9 (C=N). MS: *m/z* $[M+1]^+$ 477.

*N′-(2-Iodophenyl)-N-cyano-3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-carboximidamide (**3j**)*

This compound was prepared by the reaction of **2** and 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazine. It was obtained as a pale yellow solid, mp 173–175 °C, yield 43 %. IR (KBr), ν (cm^{-1}): 3369 (NH), 3060 (Ar–H), 2869 (CH_2, aliphatic), 2235 (C≡N), 1601 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.32 (s, 2H, NCH_2, piperazin), 4.44 (t, 2H, NCH_2, piperazin), 5.36 (t, 2H, NCH_2, piperazin), 7.00–7.03 (m, 1H, ArH), 7.24–7.26 (m, 1H, ArH), 7.33–7.35 (m, 1H, ArH), 7.80–7.83 (m, 1H, ArH), 9.08 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 29.0 (N–C, piperazin), 42.7 (N–C, piperazin), 43.0 (N–C, piperazin), 83.6 (C–I), 116.0 (C≡N), 119.9 (C–F), 123.5, 128.6, 130.5, 139.0 (aromatic carbons), 143.3 (C=N, triazolo), 153.0 (C=N, triazolo), 158.6 (C–N, iodophenyl), 160.8 (C=N). MS: *m/z* $[M+1]^+$ 462.

*1,1-Dimethyl-2-(2-iodophenyl)-3-cyanoguanidine (**3k**)*

This compound was prepared by the reaction of **2** and dimethylamine. It was obtained as a pale yellow solid, mp 89–92 °C, yield 55 %. IR (KBr), ν (cm^{-1}): 3386 (NH), 3083 (Ar–H), 2972 (CH_3), 2864 (CH_2), 2240 (C≡N), 1613 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.52 (s, 6H, CH_3), 6.98–7.01 (m, 1H, ArH), 7.20–7.23 (m, 1H, ArH), 7.33–7.35 (m, 1H, ArH), 7.81–7.84 (m, 1H, ArH), 9.14 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 35.2 (2C, N–CH_3), 83.3 (C–I), 116.5 (C≡N), 123.1, 128.6, 130.7, 139.0 (aromatic carbons), 159.2 (C–N, iodophenyl), 162.5 (C=N). MS: *m/z* $[M+1]^+$ 315.

*1,1-Diethyl-2-(2-iodophenyl)-3-cyanoguanidine (**3l**)*

This compound was prepared by the reaction of **2** and diethylamine. It was obtained as a pale yellow solid, mp 76–79 °C, yield 64 %. IR (KBr), ν (cm^{-1}): 3393 (NH), 3071 (Ar–H), 2987 (CH_3), 2848 (CH_2), 2242 (C≡N), 1622 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (t, 6H, CH_3), 3.32 (q, 4H, NCH_2), 6.97–7.00 (m, 1H, ArH), 7.22–7.25 (m, 1H, ArH), 7.33–7.36 (m, 1H, ArH), 7.84–7.86 (m, 1H, ArH), 9.16 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.4 (2C, CH_3), 43.0 (2C, N–C), 83.2 (C–I), 116.3 (C≡N), 123.4, 128.8, 131.0, 139.2 (aromatic carbons), 159.1 (C–N, iodophenyl), 162.3 (C=N). MS: *m/z* $[M+1]^+$ 343.

*1,1-Diisopropyl-2-(2-iodophenyl)-3-cyanoguanidine (**3m**)*

This compound was prepared by the reaction of **2** and diisopropylamine. It was obtained as a pale yellow solid, mp 71–73 °C, yield 58 %. IR (KBr), ν (cm^{-1}): 3426 (NH), 3063 (Ar–H), 2953 (CH_3), 2924 (CH), 2239 (C≡N), 1617 (C=N), 1386 (CH_3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.40–1.41 (12H, d, $J = 6.8$ Hz, CH_3), 3.11 (m, 2H, CH), 6.98–7.00 (m, 1H, ArH), 7.23–7.25 (m, 1H, ArH), 7.30–7.34 (m, 1H, ArH), 7.82–7.85 (m, 1H, ArH), 9.07 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 22.1 (4C, CH_3), 43.3 (2C, N–C), 83.3 (C–I), 116.2 (C≡N), 123.5, 128.6, 131.0, 139.0 (aromatic carbons), 158.9 (C–N, iodophenyl), 160.5 (C=N). MS: *m/z* $[M+1]^+$ 371.

*1,1-Dibenzyl-2-(2-iodophenyl)-3-cyanoguanidine (**3n**)*

This compound was prepared by the reaction of **2** and dibenzylamine. It was obtained as a pale yellow solid, mp 103–105 °C, yield 46 %. IR (KBr), ν (cm^{-1}): 3395 (NH), 3078 (Ar–H), 2936 (CH_2), 2223 (C≡N), 1583 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.18 (s, 4H, CH_2), 6.97–7.00 (m, 1H, ArH), 7.22–7.27 (m, 5H, ArH), 7.31–7.38 (m, 7H, ArH), 7.84–7.86 (m, 1H, ArH), 9.19 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 50.5 (2C, CH_2), 83.4 (C–I), 116.2 (C≡N), 123.3, 126.9 (2C), 128.3 (4C), 128.6, 128.8 (4C), 131.1, 138.4 (2C), 139.3 (aromatic carbons), 158.9 (C–N, iodophenyl), 161.7 (C=N). MS: *m/z* $[M+1]^+$ 467.

*1,1-Diphenyl-2-(2-iodophenyl)-3-cyanoguanidine (**3o**)*

This compound was prepared by the reaction of **2** and diphenylamine. It was obtained as a pale yellow solid, mp 121–123 °C, yield 48 %. IR (KBr), ν (cm^{-1}): 3376 (NH), 3073 (Ar–H), 2246 (C≡N), 1620 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.97–7.00 (m, 1H, ArH), 7.03–7.07 (m, 2H, ArH), 7.15–7.26 (m, 5H, ArH), 7.31–7.39 (m, 5H, ArH), 7.82–7.85 (m, 1H, ArH), 9.22 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 83.2 (C–I), 116.5 (C≡N), 119.2 (2C), 120.2 (4C), 123.7, 128.3, 130.0 (4C), 131.0, 139.6 (aromatic carbons), 144.6 (2C, C–N, phenyl), 159.2 (C–N, iodophenyl), 161.4 (C=N). MS: *m/z* $[M+1]^+$ 439.

*General procedure for the synthesis of 1-cyano-2-amino-benzimidazole derivatives (**4a–o**)*

A mixture of aryl guanidine **3** (0.5 mmol), CuI (0.025 mmol), 2,2′-biimidazole (0.05 mmol), Cs_2CO_3 (1.0 mmol) and DMF (5 mL) was heated at 80 °C for 10 h under nitrogen atmosphere. The resulting suspension was cooled to room temperature, diluted with ethyl acetate, filtered through a pad of silica gel, and washed with ethyl acetate. The combined filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel with cyclohexane/ethyl acetate to provide the desired product.

*2-(Pyrrolidin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4a**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3a**. It was obtained as a white solid; mp 59–60 °C. IR (KBr), ν (cm^{-1}): 3081 (Ar–H), 2886 (CH_2, aliphatic), 2235 (C≡N), 1612 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.00 (t, 4H, CH_2, pyrrolidin), 3.75 (t, 4H, NCH_2, pyrrolidin), 7.03–7.07 (m, 1H, ArH), 7.16–7.22 (m, 1H, ArH), 7.26–7.32 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 25.6 (2C, pyrrolidin), 49.0 (2C, N–C, pyrrolidin), 105.7 (C≡N), 109.4, 117.0, 121.2, 125.4 (aromatic carbons), 132.4 (C-5, imidazol), 142.2 (C-4, imidazol), 151.5 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 213. Anal. Calcd. for $C_{12}H_{12}N_4$ (%): C, 67.90; H, 5.70; N, 26.40. Found: C, 67.79; H, 5.81; N, 26.26.

*2-(Piperidin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4b**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3b**. It was obtained as a white solid; mp 68–69 °C. IR (KBr), ν (cm^{-1}): 3093 (Ar–H), 2891 (CH_2, aliphatic), 2241 (C≡N), 1618 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.61–1.67 (m, 6H, CH_2 piperidin), 3.53–3.59 (m, 4H, NCH_2, piperidin), 7.16–7.20 (m, 1H, ArH), 7.25–7.28 (m, 1H, ArH), 7.40–7.44 (m, 2H, ArH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23.9 (piperidin), 25.1 (2C, piperidin), 49.8 (2C, N–C, pyrrolidin), 105.6 (C≡N), 109.8, 117.9, 122.4, 125.9 (aromatic carbons), 132.6 (C-5, imidazol), 141.4 (C-4, imidazol), 154.8 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 227. Anal. Calcd. for $C_{13}H_{14}N_4$ (%): C, 69.00; H, 6.24; N, 24.76. Found: C, 68.79; H, 6.21; N, 24.70.

*2-Morpholino-1H-benzo[d]imidazole-1-carbonitrile (**4c**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3c**. It was obtained as a white solid; mp 83–85 °C. IR (KBr), ν (cm^{-1}): 3088 (Ar–H), 2883 (CH_2, aliphatic), 2239 (C≡N), 1621 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.75 (t, 4H, NCH_2, morpholino), 3.91 (t, 4H, OCH_2, morpholino), 7.01–7.05 (m, 1H, ArH), 7.13–7.18, (m, 1H, ArH), 7.27–7.31 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 44.3 (2C, N–C, morpholino), 65.6 (2C, O–C, morpholino), 105.2 (C≡N), 108.6, 117.1, 121.4, 125.8 (aromatic carbons), 132.9 (C-5, imidazol), 143.1 (C-4, imidazol), 152.3 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 229. Anal. Calcd. for $C_{12}H_{12}N_4O$ (%): C, 63.15; H, 5.30; N, 24.55. Found: C, 63.32; H, 5.41; N, 24.47.

*2-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4d**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3d**. It was obtained as a white solid; mp 78–81 °C. IR (KBr), ν (cm^{-1}): 3091 (Ar–H), 2932 (CH_3), 2856 (CH_2, aliphatic), 2234 (C≡N), 1611 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.23 (s, 3H, NCH_3), 2.47 (t, 4H, NCH_2, piperazin), 3.92 (t, 4H, NCH_2, piperazin), 7.00–7.06 (m, 1H, ArH), 7.15–7.20 (m, 1H, ArH), 7.28–7.34 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 42.3 (N–CH_3), 45.9 (2C, N–C, piperazin), 54.1 (2C, N–C, piperazin), 105.4 (C≡N), 108.9, 117.2, 121.5, 126.0 (aromatic carbons), 133.2 (C-5, imidazol), 143.3 (C-4, imidazol), 152.6 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 242. Anal. Calcd. for $C_{13}H_{15}N_5$ (%): C, 64.71; H, 6.27; N, 29.02. Found: C, 64.57; H, 6.20; N, 28.91.

*2-(4-Phenylpiperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4e**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3e**. It was obtained as a white solid; mp 128–130 °C. IR (KBr), ν (cm^{-1}): 3057 (Ar–H), 2879 (CH_2, aliphatic), 2236 (C≡N), 1603 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.31 (t, 4H, NCH_2, piperazin), 3.97

(t, 4H, NCH_2, piperazin), 6.90–6.93 (m, 2H, ArH), 6.99–7.05 (m, 2H, ArH), 7.14–7.18 (m, 1H, ArH), 7.23–7.25 (m, 2H, ArH), 7.37–7.39 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 49.1 (2C, N–C, piperazin), 54.9 (2C, N–C, piperazin), 105.6 (C≡N), 109.2, 116.6 (2C), 117.3, 120.8, 122.1, 126.5 (aromatic carbons), 130.6 (2C), 134.4 (C-5, imidazol), 142.8 (C-4, imidazol), 153.3 (C-2, imidazol), 155.1 (N–C, phenyl). MS: *m/z* $[M+1]^+$ 304. Anal. Calcd. for $C_{18}H_{17}N_5$ (%): C, 71.27; H, 5.65; N, 23.09. Found: C, 71.31; H, 5.57; N, 23.01.

*2-(4-p-tolylpiperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4f**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3f**. It was obtained as a white solid; mp 115–117 °C. IR (KBr), *v* (cm^{-1}): 3083 (Ar–H), 2962 (CH_3), 2885 (CH_2, aliphatic), 2240 (C≡N), 1608 (C=N). ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.18 (s, 3H, CH_3), 3.30 (t, 4H, NCH_2, piperazin), 3.89 (t, 4H, NCH_2, piperazin), 6.83–6.85 (d, $J = 7.2$ Hz, 2H, ArH), 6.85–6.89 (d, $J = 7.2$ Hz, 2H, ArH), 7.01–7.05 (m, 1H, ArH), 7.18–7.21 (m, 1H, ArH), 7.28–7.36 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 24.2 (CH_3), 47.5 (2C, N–C, piperazin), 53.8 (2C, N–C, piperazin), 105.5 (C≡N), 109.2, 115.4 (2C), 117.3, 120.8, 122.1, 128.1, 130.2 (2C, aromatic carbons), 134.2 (C-5, imidazol), 142.4 (C-4, imidazol), 153.6 (C-2, imidazol), 154.1 (N–C, 1-*p*-tolyl). MS: *m/z* $[M+1]^+$ 318. Anal. Calcd. for $C_{19}H_{19}N_5$ (%): C, 71.90; H, 6.03; N, 22.07. Found: C, 71.78; H, 6.09; N, 21.98.

*2-(4-(4-Methoxyphenyl)piperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4g**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3g**. It was obtained as a white solid; mp 152–154 °C. IR (KBr), *v* (cm^{-1}): 3103 (Ar–H), 2954 (CH_3), 2885 (CH_2, aliphatic), 2241 (C≡N), 1594 (C=N). ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.32 (t, 4H, NCH_2, piperazin), 3.76 (s, 3H, OCH_3), 3.95 (t, 4H, NCH_2, piperazin), 6.81–6.84 (d, $J = 7.2$ Hz, 2H, ArH), 6.87–6.91 (d, $J = 7.2$ Hz, 2H, ArH), 6.99–7.06 (m, 1H, ArH), 7.15–7.19 (m, 1H, ArH), 7.26–7.33 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.3 (2C, N–C, piperazin), 54.6 (2C, N–C, piperazin), 57.2 (OCH_3), 105.6 (C≡N), 108.8, 116.4 (2C), 117.2, 118.5 (2C), 121.3, 126.2 (aromatic carbons), 134.2 (C-5, imidazol), 142.5 (C-4, imidazol), 153.1 (C-2, imidazol), 154.8 (C–N, methoxyphenyl), 156.3 (C–O, methoxyphenyl). MS: *m/z* $[M+1]^+$ 334. Anal. Calcd. for $C_{19}H_{19}N_5O$ (%): C, 68.45; H, 5.74; N, 21.01. Found: C, 68.27; H, 5.83; N, 21.11.

*2-(4-(4-Chlorophenyl)piperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4h**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3h**. It was obtained as a white solid; mp 141–143 °C. IR (KBr), *v* (cm^{-1}): 3069 (Ar–H), 2870 (CH_2, aliphatic), 2238 (C≡N), 1605 (C=N). ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.73 (t, 4H, NCH_2, piperazin), 3.44 (t, 4H, NCH_2, piperazin), 6.84–6.86 (d, $J = 7.2$ Hz, 2H, ArH), 6.97–7.03 (m, 1H, ArH), 7.15–7.20 (m, 1H, ArH), 7.23–7.30 (m, 2H, ArH), 7.35–7.39 (d, $J = 7.2$ Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 48.5 (2C, N–C, piperazin), 54.2 (2C, N–C, piperazin), 105.3 (C≡N), 109.0, 116.7 (2C), 117.5, 122.0, 123.6, 126.4, 127.5 (2C, aromatic carbons), 134.4 (C-5, imidazol), 142.1 (C-4, imidazol), 153.3 (C-2, imidazol), 157.6 (C–N, 4-chlorophenyl). MS: *m/z* $[M+1]^+$ 338. Anal. Calcd. for $C_{18}H_{16}ClN_5$ (%): C, 64.00; H, 4.77; N, 20.73. Found: C, 64.23; H, 4.81; N, 20.65.

*2-(4-(4-Nitrophenyl)piperazin-1-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4i**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3i**. It was obtained as a white solid; mp 147–149 °C. IR (KBr), *v* (cm^{-1}): 3084 (Ar–H), 2877 (CH_2, aliphatic), 2239 (C≡N), 1616 (C=N), 1530, 1366 (NO_2). ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.35 (t, 4H, NCH_2, piperazin), 3.98 (t, 4H, NCH_2, piperazin), 6.83–6.87 (d, $J = 7.2$ Hz, 2H, ArH), 6.98–7.05 (m, 1H, ArH), 7.16–7.20 (m, 1H, ArH), 7.25–7.32 (m, 2H, ArH), 8.08–8.11 (d, $J = 7.2$ Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 49.0 (2C, N–C, piperazin), 54.7 (2C, N–C, piperazin), 105.7 (C≡N), 109.1, 117.0 (2C), 117.3, 122.3, 126.2, 127.4 (2C, aromatic carbons), 134.2 (C-5, imidazol), 140.8 (C-NO_2, nitrophenyl), 142.6 (C-4, imidazol), 153.5 (C-2, imidazol), 158.4 (C–N, nitrophenyl). MS: *m/z* $[M+1]^+$ 349. Anal. Calcd. for $C_{18}H_{16}N_6O_2$ (%): C, 62.06; H, 4.63; N, 24.12. Found: C, 62.31; H, 4.58; N, 24.01.

*2-(3-(Trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)-1H-benzo[d]imidazole-1-carbonitrile (**4j**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3j**. It was obtained as a white solid; mp 216–219 °C. IR (KBr), *v* (cm^{-1}): 3062 (Ar–H), 2865 (CH_2, aliphatic), 2225 (C≡N), 1610 (C=N). ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.34 (s, 2H, NCH_2, piperazin), 4.45 (t, 2H, NCH_2, piperazin), 5.31 (t, 2H, NCH_2, piperazin), 7.00–7.08 (m, 1H, ArH), 7.17–7.21 (m, 1H, ArH), 7.30–7.36 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 29.1 (N–C, piperazin), 42.9 (N–C, piperazin), 43.2 (N–C,

piperazin), 105.7 (C≡N), 109.3, 117.1, 119.9 (C–F), 121.3, 125.8 (aromatic carbons), 133.0 (C-5, imidazol), 142.7 (C-4, imidazol), 143.4 (C=N, triazolo), 151.6 (C-2, imidazol), 153.1 (C=N, triazolo). MS: *m/z* $[M+1]^+$ 334. Anal. Calcd. for $C_{14}H_{10}F_3N_7$ (%): C, 50.45; H, 3.02; N, 29.42. Found: C, 50.62; H, 3.06; N, 29.33.

*2-(Dimethylamino)-1H-benzo[d]imidazole-1-carbonitrile (**4k**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3k**. It was obtained as a white solid; mp 86–88 °C. IR (KBr), *v* (cm^{-1}): 3089 (Ar–H), 2978 (CH_3), 2837 (CH_2), 2236 (C≡N), 1605 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 2.53 (s, 6H, NCH_3), 7.05–7.08 (m, 1H, ArH), 7.21–7.25 (m, 1H, ArH), 7.29–7.35 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 35.1 (2C, N–CH_3), 106.0 (C≡N), 109.1, 117.4, 121.5, 125.7 (aromatic carbons), 132.6 (C-5, imidazol), 143.1 (C-4, imidazol), 152.1 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 187. Anal. Calcd. for $C_{10}H_{10}N_4$ (%): C, 64.50; H, 5.41; N, 30.09. Found: C, 64.43; H, 5.45; N, 29.95.

*2-(Diethylamino)-1H-benzo[d]imidazole-1-carbonitrile (**4l**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3l**. It was obtained as a white solid; mp 72–74 °C. IR (KBr), *v* (cm^{-1}): 3075 (Ar–H), 2981 (CH_3), 2845 (CH_2), 2232 (C≡N), 1602 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.32 (t, 6H, CH_3), 3.76 (q, 4H, NCH_2), 7.03–7.06 (m, 1H, ArH), 7.19–7.25 (m, 1H, ArH), 7.27–7.34 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 13.7 (2C, CH_3), 43.3 (2C, N–C), 105.6 (C≡N), 109.2, 117.2, 121.3, 125.8 (aromatic carbons), 132.8 (C-5, imidazol), 143.0 (C-4, imidazol), 151.9 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 215. Anal. Calcd. for $C_{12}H_{14}N_4$ (%): C, 67.27; H, 6.59; N, 26.15. Found: C, 67.34; H, 6.51; N, 26.02.

*2-(Diisopropylamino)-1H-benzo[d]imidazole-1-carbonitrile (**4m**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3m**. It was obtained as a white solid; mp 69–71 °C. IR (KBr), *v* (cm^{-1}): 3059 (Ar–H), 2968 (CH_3), 2921 (CH), 2235 (C≡N), 1606 (C=N), 1382 (CH_3). ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.42–1.44 (12H, d, $J = 6.8$ Hz, CH_3), 3.12 (m, 2H, CH), 7.00–7.05 (m, 1H, ArH), 7.20–7.26 (m, 1H, ArH), 7.28–7.36 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 22.4 (4C, CH_3), 48.7 (2C, N–C), 105.3 (C≡N), 109.7, 117.6, 122.3, 125.6 (aromatic carbons), 132.7 (C-5, imidazol), 142.2 (C-4, imidazol), 152.4 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 243. Anal. Calcd. for $C_{14}H_{18}N_4$ (%): C, 69.39; H, 7.49; N, 23.12. Found: C, 69.23; H, 7.53; N, 23.06.

*2-(Dibenzylamino)-1H-benzo[d]imidazole-1-carbonitrile (**4n**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3n**. It was obtained as a white solid; mp 113–115 °C. IR (KBr), *v* (cm^{-1}): 3062 (Ar–H), 2924 (CH_2), 2241 (C≡N), 1596 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 4.64 (s, 4H, CH_2), 7.05–7.08 (m, 1H), 7.14–7.19 (m, 1H, ArH), 7.23–7.32 (m, 4H, ArH), 7.34–7.37 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 50.5 (2C, CH_2), 105.2 (C≡N), 108.7, 117.0, 121.1, 125.6, 126.6 (2C), 128.1 (4C), 128.5 (4C, aromatic carbons), 132.7 (C-5, imidazol), 138.1 (2C), 142.5 (C-4, imidazol), 151.7 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 339. Anal. Calcd. for $C_{22}H_{18}N_4$ (%): C, 78.08; H, 5.36; N, 16.56. Found: C, 78.31; H, 5.29; N, 16.35.

*2-(Diphenylamino)-1H-benzo[d]imidazole-1-carbonitrile (**4o**)*

This compound was prepared by the intramolecular C–N coupling reaction of **3o**. It was obtained as a white solid; mp 156–159 °C. IR (KBr), *v* (cm^{-1}): 3061 (Ar–H), 2240 (C≡N), 1611 (C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.00–7.06 (m, 3H, ArH), 7.13–7.23 (m, 5H, ArH), 7.29–7.38 (m, 6H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 105.7 (C≡N), 109.4, 117.0, 117.6 (2C), 119.3 (4C), 121.2, 125.4, 130.5 (4C, aromatic carbons), 132.4 (C-5, imidazol), 142.2 (C-4, imidazol), 146.1 (2C, C–N, phenyl), 151.5 (C-2, imidazol). MS: *m/z* $[M+1]^+$ 311. Anal. Calcd. for $C_{20}H_{14}N_4$ (%): C, 77.40; H, 4.55; N, 18.05. Found: C, 77.52; H, 4.48; N, 17.89.

Cytotoxic assay

The tumor cell lines panel consisted of A549, K562, and PC-3. The tumor cells were cultured in proper medium in a 5 % CO_2 atmosphere at 37 °C. The cells were seeded to 96-well plates at a density of 5×10^3 cells/well. After 24 h, the cells were treated with 0.01, 0.1, 1, 10, and 100 μmol L^{-1} chemicals dissolved in DMSO (final concentration 0.1 %) and the positive control cells were treated with doxorubicin hydrochloride. 72 h later, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 5 mg mL^{-1}) solution was added per well and the cells were cultured for another 4 h. Following this, the medium was discarded, and DMSO was added. The optical density (OD) of the resultant solution was measured at a wavelength of 570 nm with a microplate reader. Doxorubicin hydrochloride was used as a control antitumor agent.

The IC_{50} values were calculated with LOGIT method. Assays were performed in triplicate on three independent experiments (Hu *et al.*, 2009).

Cell cycle analysis

K562 cells were seeded into 6-well plates and treated with different concentrations of **4d** or without any addition for 24 h. The cells were harvested, washed with PBS, and fixed overnight in 70 % ethanol at −20 °C. The fixed cells were treated with 100 μg mL^{-1} Rnase A in PBS for 1 h at room temperature and followed by staining with 50 μg mL^{-1} propidium iodide for 30 min in the dark. DNA content was analyzed by flow cytometry.

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