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Original article

Design, regioselective synthesis and cytotoxic evaluation of 2-aminoimidazole–quinoline hybrids against cancer and primary endothelial cells

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ABSTRACT

In search of new selective anti-cancer agents, a series of sixteen novel 2-aminoimidazole–quinoline hybrid compounds (**5a**–**5p**) have been designed and synthesized regioselectively. We have characterized the compounds extensively using IR, 1D and 2D NMR Spectroscopy and mass spectrometry. The cytotoxicity studies against different cancer cell lines showed that the compound **5a** (Imd–Ph) emerged as a potent cytotoxic scaffold. Imd–Ph (**5a**) exhibited a selective anticancer activity against human colon cancer cell line (HCT-116, DLD-1) and was found relatively non-toxic to breast cancer cells (MDA-MB-231) as well as to normal primary endothelial cells (HUVEC). Structure–activity relationship of imidazole–quinoline hybrid scaffolds revealed differential and selective toxicities exerted by the different derivatives against cancer and normal cells. Structural modification of the scaffold with library of a wide variety of substituents may lead to the development of novel selective anti-cancer agents in the future.

1. Introduction

Cancer, a life threatening disease is now affecting the people at all ages and is responsible for increase in the mortality rate globally [1–3]. In spite of availability of a large number of existing anti-cancer drugs, the development of new chemotherapeutics have always been one of the most noteworthy challenges due to non-selectivity and emergence of resistance by cancerous cells towards existing anti-cancer compounds. Therefore, a constant need to develop better alternatives to face such incoming problems in future is always in demand. There are a large number of heterocyclic compounds that have already been reported to exhibit anticancer properties [4].

Literature survey revealed that among heterocycles, imidazole derivatives have been proven as an excellent class of broad spectrum anti-cancer agents against a variety of cancer cell lines such as hepatocellular carcinoma, breast cancer, acute myelogenous leukemia, non-small cell lung carcinoma, etc. [5–9]. In past decades, 2-aminoimidazoles have gained much attention because of their high selectively as anti-cancer agents against some cancerous cells [10–12]. The dacarbazine, an antineoplastic drug bearing 2-aminoimidazole nucleus, has been utilized in the treatment of a variety of cancers such as malignant melanoma, Hodgkin lymphoma, sarcoma and carcinoma of the pancreas (Fig. 1).

Moreover, various 2-aminoimidazole alkaloids including Naamine A [13], Naamine G [14], Girolline [15], Preclathridine A [16] have been isolated from marine sources [17,18], which were found to possess excellent anti-cancer activities (Fig. 2). Further, some *bis*[2-chloroethyl]aminoimidazole derivatives have been found to act as DNA binding agents and topoisomerase II inhibitors [19].

A large number of 2-aminoimidazole derivatives have also been reported to possess diverse pharmacological properties such as antibacterial, anti-inflammatory and antiviral activity [20–22]. Similarly, quinoline and its derivatives have been described as another important class of pharmacologically active compounds that show broad spectrum of biological activities [23,24] such as antibacterial [25], antimalarial [26], anticancer [27], anti-inflammatory [28], antitumor [29], anti-HIV [30], antidepressant

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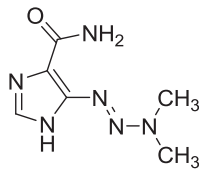


Dacarbazine

Fig. 1. Market available anticancer drug.

supported by the information obtained from DEPT-135, HSQC and HMBC of **5d**.

The reaction of more stable *trans*-conformer of hydrazones (**3**) and either α-bromoketones or α-tosyloxyketones (**4**) resulted in 2-amino-4-arylimidazole derivatives (**5**) as explained above. Further, the formation of **5** instead of isomeric 2-amino-5-arylimidazole derivative (**6**) was substantiated on the basis of analysis of 2D NMR spectra (COSY, ROESY, HSQC and HMBC). The correlation between the hydrogens through bond and in space was established by COSY and ROESY, respectively.

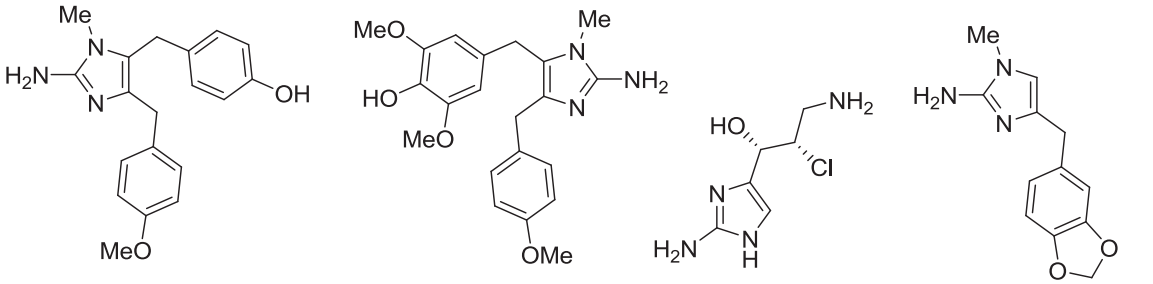


Fig. 2. Some naturally occurring 2-aminoimidazoles as anticancer agents.

[31] and antiallergic [32]. Vesnarinone and linomide have been reported to be effective against MH-134 tumor cells and prostate cancer [33] (Fig. 3).

Keeping in view of the above, it was thought of interest that a combination of imidazole and quinoline moiety may be more beneficial in inhibiting the cancer cells growth. Therefore, we report herein the regioselective synthesis of a new series of 2-aminoimidazole–quinoline hybrids (**5a**–**5p**) and cytotoxic evaluation against two cancer cell lines along with toxicity against normal endothelial cells.

2. Results and discussion

2.1. Chemistry

The regioselective synthesis of 2-aminoimidazole–quinoline hybrids has been accomplished according to the reaction sequence shown in Scheme 1. To achieve the synthesis of target compounds, firstly the reaction of amino guanidine carbonate (**1**) with various 2-chloroquinoline-3-carbaldehydes (**2**) was performed in a solution of hydrochloric acid and water under reflux to obtain corresponding hydrazones (**3**). Treatment of either α-bromoketones or α-tosyloxyketones (**4**) with **3** gave exclusively *E*-*N*-[(2-Chloro-6-substitutedquinolin-3-yl)methylene)-4-aryl]-1*H*-imidazole-1,2-diamines (**5**) in a regioselective manner. The use of α-tosyloxyketone was quite safe over lachrymatory α-bromoketones. The structure of **5** was established on the basis of FTIR, NMR (^{1}H and ^{13}C), 2D NMR spectroscopy and mass spectrometry.

In IR spectrum, compound **5d** exhibited two characteristic bands at 3415 and 3275 cm^{-1} due to N–H stretching vibration of $-NH_2$ group attached to the position-2 of imidazole ring. In ^{1}H NMR spectrum of **5d**, appearance of a characteristic signal of 5-H as a singlet at 8.32 ppm established the formation of imidazole nucleus. Other two important singlets at δ 8.71 and 9.40 ppm were assigned to the hydrogen of imine (CH=N) and quinoline (4′-H), respectively, which supported the presence of quinoline moiety. Carbon-2, 4 and 5 of the imidazole nucleus resonated at 150.6, 133.6, and 102.7 ppm, respectively. The above results were further

The ROESY spectrum clearly indicated the formation of one of the rotational isomer of **5**, wherein 5-**H** and **H**–N=C hydrogens were found in close proximity, instead of isomeric compound **6**. In ROESY spectrum, it was observed that hydrogen at position-5 of imidazole nucleus is closely associated in space with imine hydrogen (CH=N) and *ortho* hydrogens of p-chlorophenyl ring. There was no proximity between $-NH_2$ and imine hydrogen, which confirmed the orientation of amino group away from imine group. All the spectral data confirmed the formation of compound **5** along with its *trans* geometry having imine-imidazole hydrogen in close proximity instead of isomeric compound **6**.

2.2. Cytotoxic activity against cancer cell lines

All the synthesized compounds (**5a**–**5p**) were screened for their anti-cancer potential by evaluating cytotoxicity by MTT assay. We have also checked toxicities of the compounds **5a**–**5p** on the proliferation of normal cell lines. The cytotoxicity studies were conducted against two human colon cancer cell lines (HCT-116, DLD-1), human breast cancer cell line (MDA-MB-231) and in normal human cell line (HUVEC) at different concentrations i.e. 10, 20, 30, 40 and 50 μM up to 48 h (Figs. 4–6).

The primary scaffold **5a** (Imd–Ph) showed good cytotoxicity profile in colon cancer cell lines with IC_{50} values around 6.92 μM and 16.37 μM against HCT-116 and DLD-1 cell lines respectively (Fig. 4, Table 1). Replacement of phenyl group at position-4 of imidazole ring in Imd–Ph (**5a**) with 1-naphthyl moiety (**5k**) preserved its anti-cancer activity in HCT-116 cell line (IC_{50} = 6.34 μM) and improved its activity in DLD-1 cell line (IC_{50} = 9.48 μM). However, replacement of phenyl group with 2-naphthyl group (**5l**) diminished anti-cancer activity against HCT-116 and DLD-1 as indicated by their IC_{50} values (>50 μM) in colon cancer cell lines. Similarly, attachment of 2-theinyl group (**5o**) in place of phenyl group in Imd–Ph (**5a**) causes no appreciable change in IC_{50} value in HCT-116 (7.22 μM) while lowered IC_{50} value in DLD-1 (10.27 μM) cell line. Any change in the electronic environment of the phenyl ring of Imd–Ph (**5a**) with respect to electron donating groups (–Me, **5b**; –OMe, **5c**) and electron withdrawing groups (–Cl, **5d**;

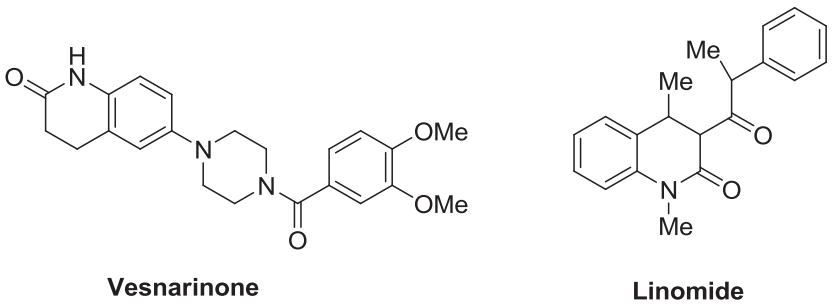
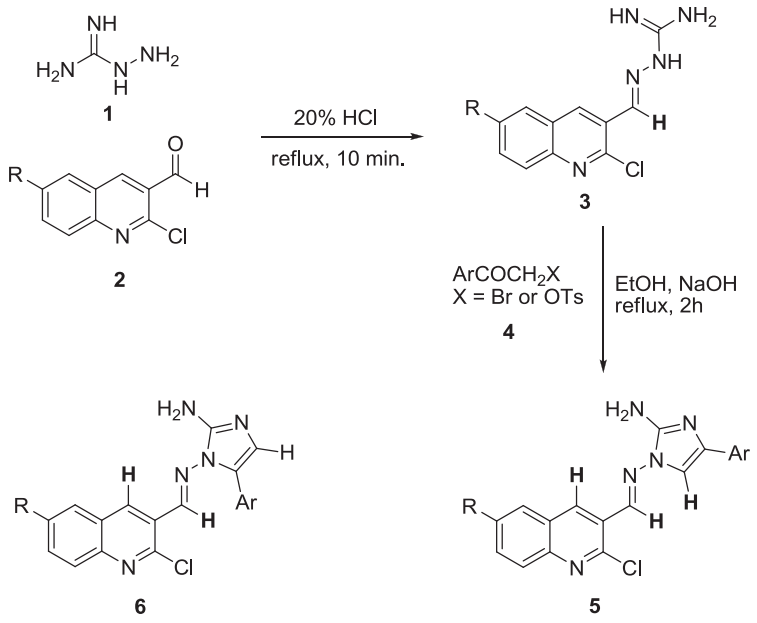


Fig. 3. Anti-tumor quinoline based compounds.



5a; R = H, Ar = C_6H_5; **5b**; R = H, Ar = $C_6H_4Me(4)$; **5c**; R = H, Ar = $C_6H_4OMe(4)$;
5d; R = H, Ar = $C_6H_4Cl(4)$; **5e**; R = H, Ar = $C_6H_4Br(4)$; **5f**; R = Me, Ar = C_6H_5;
5g; R = Me, Ar = $C_6H_4Me(4)$; **5h**; R = Me, Ar = C_6H_4OMe;**5i**; R = Me, Ar = C_6HCl;
5j; R = Me, Ar = $C_6HBr(4)$; **5k**; R = H, Ar = 1-naphthyl; **5l**; R = H, Ar = 2-naphthyl;
5m; R = Me, Ar = 1-naphthyl; **5n**; R = Me, Ar = 2-naphthyl; **5o**; R = H, Ar = 2-thienyl;
5p; R = Me, Ar = 2-thienyl

Scheme 1. Regioselective synthesis of imidazole-1,2-diamines (**5a**–**5p**).

–Br, **5e**) diminished the anticancer activity (IC_{50} > 50 μM) in colon cancer cell lines.

Introduction of methyl group at position-6 of quinoline ring in Imd–Ph (**5p**) did not affect anticancer activity in HCT-116 cell line (7.12 μM) and lowered IC_{50} value in DLD-1 (8.79 μM) as shown in Fig. 4 and Table 1. Similarly, anticancer activities of compounds **5m** and **5p** are unaltered with methyl group introduction on quinoline ring compared to compounds **5k** and **5o**, respectively. Replacement of hydrogen by methyl group in quinoline ring in compounds having phenyl group with electron withdrawing –Cl (**5i**), –Br (**5j**) and electron donating –Me (**5g**), and –OMe (**5h**) substituents causes increase in IC_{50} values thereby lowered their anticancer potential.

Surprisingly, neither the primary scaffold Imd–Ph (**5a**) nor its modified derivatives such as **5k**, **5l**, **5o** were found active (IC_{50} > 50 μM) against breast cancer cell line (MDA-MB-231). Introduction of methyl group in quinoline ring (**5f**, **5m**, **5n**, **5p**) as well as any change in the electronic environment of the phenyl group attached at position-4 of imidazole ring causes increase in IC_{50} values and thus lowered their anticancer activities (Fig. 5, Table 1). These results indicated that aminoimidazole–quinoline hybrid scaffold shows colon selective anticancer activities compared to standard drug doxorubicin, which showed no selectivity between different cancer types.

Interestingly, Imd–Ph (**5a**), Imd–Me–Ph (**5f**) and its naphthyl (**5k**, **5l**) modifications were found non-toxic to primary (normal) cell line (HUVEC) (Fig. 6, Table 1). Compounds with methyl group on quinoline ring (**5g**, **5h**, **5i**, **5j**, **5m**, **5n**) as well as *para* substitution on phenyl group attached at position-4 of imidazole nucleus resulted in decrease in IC_{50} values (10–30 μM) thereby increased the toxicity to normal cell lines. Collectively, these results indicated that the primary scaffold moiety is non-toxic to normal cell lines where as its structural cognomers are toxic to normal cell line (HUVEC) (Fig. 6, Table 1).

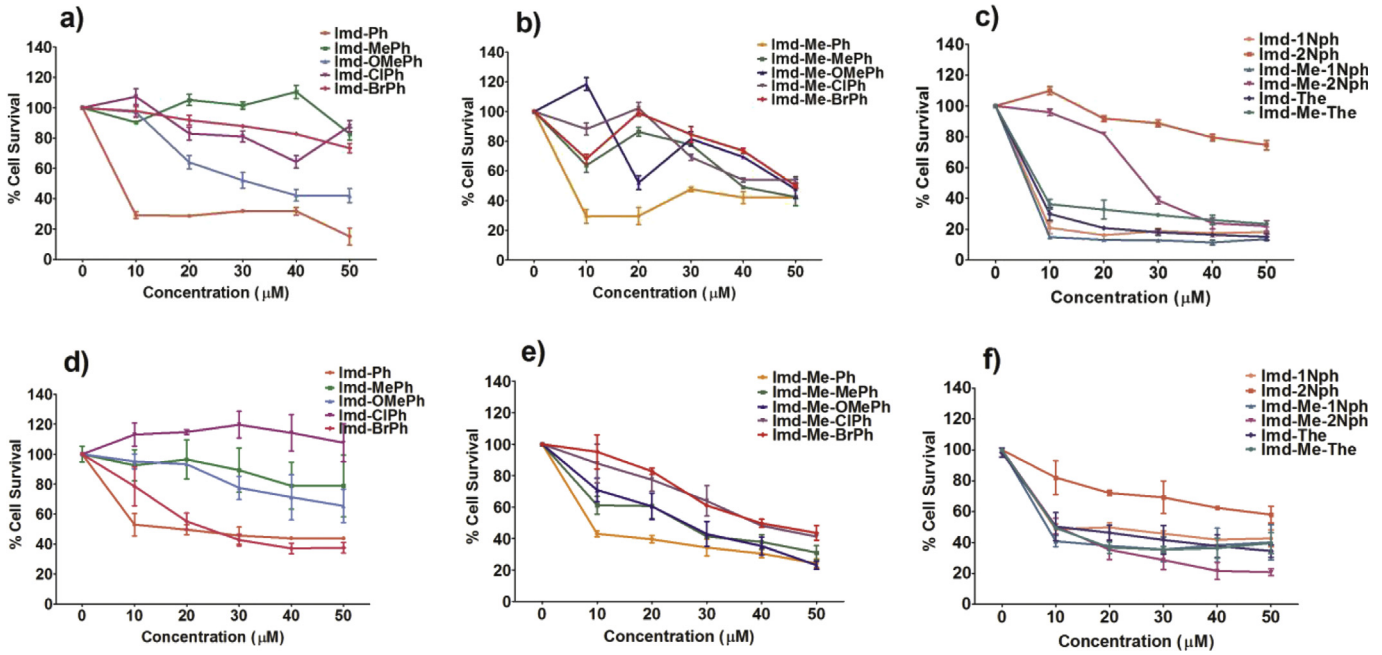


Fig. 4. Anticancer activities of compounds against colon cancer HCT-116 (a–c) and DLD-1 (d–f) cell lines at different concentrations.

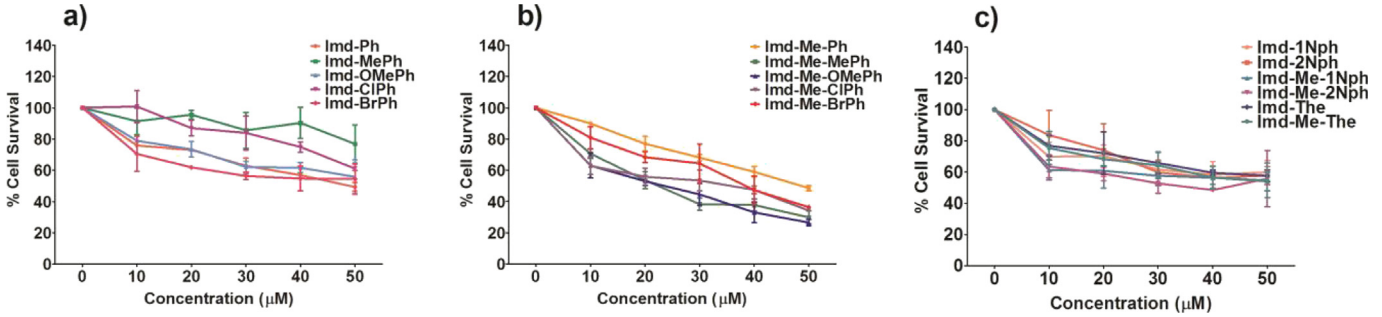


Fig. 5. Anticancer activities of compounds in breast cancer MDA-MB-231 (a–c) cell line at different concentrations.

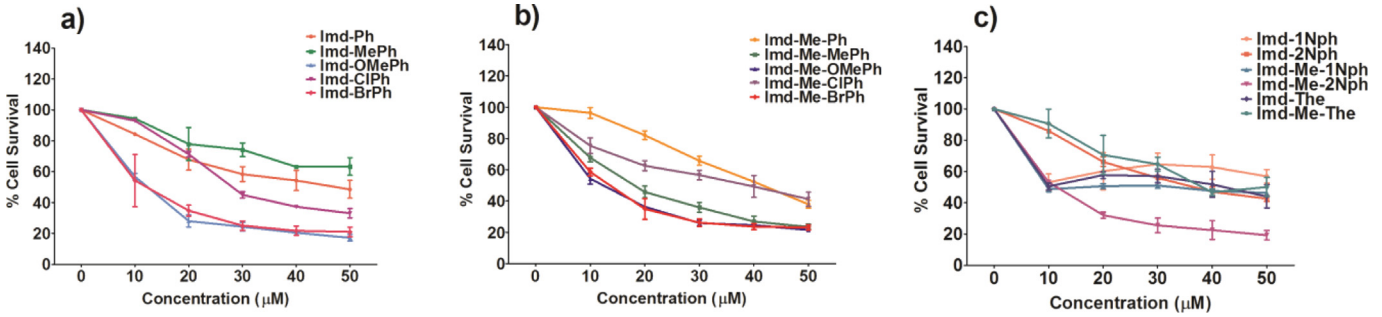


Fig. 6. Cytotoxicities of the compounds in primary Human Umbilical Vein Endothelial Cells (HUVEC) at different concentrations.

2.3. Structure–activity relationship of imidazole–quinoline scaffold

2.3.1. Effect of substituents on position-4 of imidazole ring

On the basis of the results, it has been concluded that Imd–Ph (**5a**) shows selective toxicity against colon cancer cells and has relatively non-toxic to breast cancer cells as well as normal cells (Fig. 7a). Moreover, presence of 1-naphthyl substituent instead of 2-naphthyl moiety is responsible for increased selective anticancer activity. Replacement of phenyl group by classical ring equivalent bioisostere such as theinyl moiety at position-4 of imidazole ring also affect the anticancer activity and is found to be more effective as compared to substituted aryl moiety but less selective to cancer cells. On the other hand, any substitution on phenyl ring at position-4 leads to decrease in anticancer potential and increase in toxicity to normal cells (Fig. 7c and d).

Table 1
IC_{50} (μM) values after treatment with compounds against four cell lines.

Compound		HCT-116	DLD-1	MDA-MB-231	HUVEC
Imd–Ph	**5a**	6.92 ± 2.22	16.37 ± 3.37	49.04 ± 2.87	46.67 ± 5.80
Imd–MePh	**5b**	>50	>50	>50	>50
Imd–OMePh	**5c**	31.82 ± 5.82	>50	>50	12.43 ± 2.01
Imd–ClPh	**5d**	>50	>50	>50	27.97 ± 1.99
Imd–BrPh	**5e**	>50	23.94 ± 5.55	>50	11.94 ± 2.40
Imd–Me–Ph	**5f**	7.12 ± 4.74	8.79 ± 1.86	48.14 ± 1.64	41.46 ± 1.28
Imd–Me–MePh	**5g**	39.59 ± 0.42	25.22 ± 0.77	22.27 ± 5.41	18.04 ± 3.76
Imd–Me–OMePh	**5h**	>50	25.71 ± 7.84	23.55 ± 2.81	12.24 ± 3.49
Imd–Me–ClPh	**5i**	>50	38.90 ± 1.08	35.16 ± 2.47	38.50 ± 7.01
Imd–Me–BrPh	**5j**	49.92 ± 1.34	39.29 ± 2.67	38.21 ± 8.98	13.42 ± 2.40
Imd–1Nph	**5k**	6.34 ± 3.77	9.48 ± 3.45	>50	>50
Imd–2Nph	**5l**	>50	>50	>50	36.24 ± 2.97
Imd–Me–1Nph	**5m**	5.84 ± 1.11	8.30 ± 3.61	>50	9.58 ± 1.66
Imd–Me–2Nph	**5n**	27.39 ± 2.30	10.17 ± 5.17	35.55 ± 0.61	11.35 ± 1.21
Imd–The	**5o**	7.22 ± 4.20	10.27 ± 8.97	>50	10.17 ± 2.23
Imd–Me–The	**5p**	7.71 ± 3.20	8.99 ± 0.50	>50	38.02 ± 2.11
Doxorubicin	Dox	0.34 ± 0.03	0.84 ± 0.05	1.47 ± 0.22	–

2.3.2. Effect of methyl group introduction at position-6 of quinoline ring

Replacement of hydrogen atom by the methyl group at position-6 of quinoline ring leads to significant increase in cytotoxicity (Fig. 7b, e).

3. Conclusions

In conclusion, in this investigation sixteen novel 2-aminoimidazole–quinoline hybrid compounds (**5a**–**5p**) have been designed and synthesized in a regioselective manner. Out of possible geometrical and conformational isomers, structure of product (**5**) along with its *trans* geometry having imine-imidazole hydrogen in close proximity was established on the basis of a rigorous analysis of their IR, ^{1}H, ^{13}C NMR, HRMS and 2D NMR (HSQC, COSY, ROESY, HMBC) spectral data. The cytotoxicity studies against different cancer cell lines showed that the

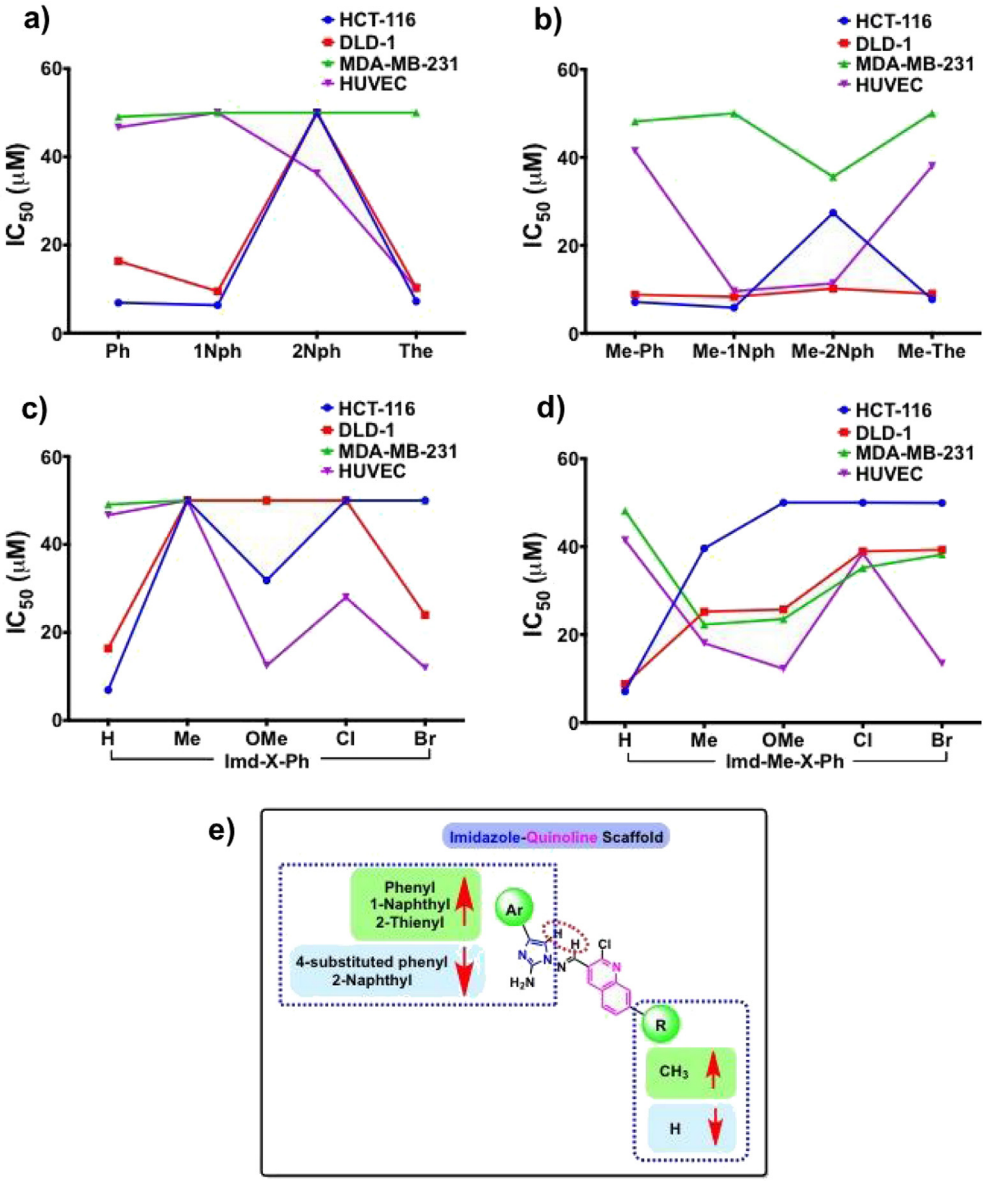


Fig. 7. Structure–activity relationship of imidazole–quinoline compound screened for anticancer activity (a–e). a) Effect of substituents on position-4 of imidazole ring on selective anticancer activity. b) Effect of methyl group introduction at position-6 of quinoline ring on selective anticancer activity. Influence of electronic environment on anticancer activities of Imd–Ph. (c) on Imd–Me–Ph (d) molecules. e) Graphical representation of structure–activity relationship.

imidazole–quinoline hybrid compound **5a** (Imd–Ph) is a potent scaffold for anticancer drug discovery. This scaffold showed a selective anticancer activity against human colon cancer cell lines (HCT-116, DLD-1) over breast cancer cell line (MDA-MB-231) where standard drug doxorubicin showed a broad-spectrum anticancer activity. Imd–Ph (**5a**) scaffold is relatively non-toxic to normal primary endothelial cells (HUVEC). Replacement of phenyl in Imd–Ph scaffold by 1-naphthyl and 2-thienyl groups leads to increase in anticancer activity. Further, presence of 6-methyl group on qunioline ring also causes increase in anticancer potential. However, the changes in electronic environment of the phenyl ring with electron donating or electron withdrawing groups lead to substantial decrease in anticancer activities and increase in normal cell toxicities. The overall studies presented in this manuscript provide imidazole–quinoline hybrids as an excellent class of novel, selective anticancer agents that may lead to the development of more potent anticancer drugs in the future.

4. Experimental

Melting points were determined in open capillaries and are uncorrected. The FTIR spectra were obtained in KBr on either Shimadzu FTIR 8210 PC or Perkin Elmer Spectrum RX1 instruments and are reported in cm^{-1}. The ^1H and ^{13}C NMR spectra were scanned on a Bruker Avance III NMR spectrometer at operating frequency 400 MHz and 100 MHz, respectively in $CDCl_3$ or $CDCl_3$ + DMSO-d_6 using TMS as an internal standard. The chemical shifts are expressed in ppm with respect to TMS. The COSY (Correlation spectroscopy), HSQC (Heteronuclear single-quantum coherence), HMBC (Heteronuclear multiple bond correlation) and ROESY (Rotating-frame Overhauser effect spectroscopy) spectra were scanned on a Bruker Avance III NMR spectrometer. The HRMS were recorded on VG 70 EB and an accurate mass measurement was made on PE-Biosystems Mariner ESI-TOF mass spectrometer. All the solvents were dried according to standard procedures.

4.1. Synthesis of E-N-[(2-chloro-6-substitutedquinolin-3-yl) methylene]-4-aryl-1H-imidazole-1,2-diamines (5)

4.1.1. General procedure

2-Chloro-6-substitutedquinoline-3-carbaldehyde (**2**, 15.8 mmol) and hydrochloric acid (20%, 4 mL) were mixed together and slowly added to a solution of aminoguanidine bicarbonate (**1**, 2.2 g, 15.8 mmol) in water (12 mL). After liberation of carbon dioxide, the reaction mixture was heated to reflux for 1 h and then cooled to room temperature. A solution of 40% aqueous potassium hydroxide (7 mL) was then added and the mixture was heated at reflux for an additional 10 min. The solid so obtained was filtered, washed with water until the washed water was having pH 7. The crude solid was crystallized from ethanol to give guanidine derivative **3**.

The guanidine derivative (**3**, 4.06 mmol) was added to an ethanolic solution of α-bromoketones or α-tosyloxyketone (**4**, 2.03 mmol in 10 mL) and the mixture was heated to reflux for 2 h. Aqueous sodium hydroxide solution was added drop-wise to precipitate the solid. The reaction mixture was then cooled to room temperature for 10 h. The precipitates were then filtered, washed with hot water and recrystallized from ethanol to afford **5**.

*4.1.2. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-phenyl-1H-imidazole-1,2-diamine (**5a**)*

Yield: 74 mg (26%); M.p. 243 °C; IR (KBr): 3400 and 3269 (NH str.), 3128, 3196 and 3066 (CH str.), 2357, 1467, 1325, 1627, 1466, 750, 702 cm^{-1}; ^1H NMR: (400 MHz, DMSO-d_6) δ: 6.51 (s, 2H, NH_2), 7.23 (t, 1H, J = 7.2 Hz, 4′-H), 7.38 (t, 2H, J = 7.6 Hz, 3″-H and 5″-H), 7.74–7.78 (m, 1H, 6′-H), 7.80 (d, 2H, J = 7.2 Hz, 2″-H and 6″-H), 7.90 (m, 1H, J = 8.0 and 7.2 Hz, 7′-H), 8.02 (d, 1H, J = 8.4 Hz, 8′-H), 8.10 (d, 1H, J = 8.0 Hz, 5′-H), 8.27 (s, 1H, 5-H), 8.72 (s, 1H, CH=N), 9.40 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 102.1 (C-5), 126.4 (C-2″ and C-6″), 124.8 (C-3′), 127.1 (C-4′a), 127.4 (C-8′), 128.2 (C-6′), 128.5 (C-5′), 128.8 (C-3″ and C-5″), 129.2 (C-1″), 132.4 (C-7′), 134.7 (C-4″), 137.4 (C-4), 138.0 (C-4′), 140.7 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.5 (C-2); HRMS: (m/z) M^+ calcd. for $C_{19}H_{14}ClN_5$: 347.0938, Found: 348.1047 (M^++H).

*4.1.3. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-(4″-methylphenyl)-1H-imidazole-1,2-diamine (**5b**)*

Yield: 84 mg. (30%); M.p. 241 °C; IR (KBr): 3404 and 3265 (NH str.), 3197, 3115, 3076 and 3032 (CH str.), 2355, 1627, 1465, 1327, 742 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, 4″-CH_3), 6.46 (s, 2H, NH_2), 7.19 (d, 2H, J = 8.0 Hz, 3″-H and 5″-H), 7.69 (d, 2H, J = 8.0 Hz, 2″-H and 6″-H), 7.75 (t, 1H, J = 7.6 Hz, 6′-H),7.90 (t, 1H, J = 8.8 Hz, 7′-H), 8.02 (d, 1H, J = 8.4 Hz, 8′-H), 8.09 (d, 1H, J = 8.0 Hz, 5′-H), 8.19 (s, 1H, 5-H), 8.68 (s, 1H, CH=N), 9.39 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO d_6) δ: 21.3 (C-4″-CH_3), 101.5 (C-5), 124.8 (C-2″ and C-6″), 126.4 (C-3′), 127.4 (C-4′a), 128.2 (C-8′), 128.5 (C-6′), 129.1 (C-5′), 129.4 (C-3″ and C-5″), 131.9 (C-1″), 132.4 (C-7′), 136.2 (C-4″), 137.3 (C-4), 138.2 (C-4′), 140.4 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.5 (C-2); HRMS: (m/z) M^+ calcd. for $C_{20}H_{16}ClN_5$: 361.1094, Found: 362.1191 (M^++H).

*4.1.4. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-(4″-methoxyphenyl)-1H-imidazole-1,2-diamine (**5c**)*

Yield: 95 mg (30%); M.p. 255 °C; IR (KBr): 3419 and 3269 (NH str.), 3128, 3196, 3066 (CH str.), 2355, 1463, 1325, 1627, 1466, 740 cm^{-1}; ^1H NMR: (400 MHz, DMSO-d_6) δ: 3.79 (s, 3H, 4″-OCH_3), 6.47 (s, 2H, NH_2), 6.96 (d, 2H, J = 8.8 Hz, 3″-H and 5″-H), 7.71–7.77 (m, 3H, 6′-H, 2″-H and 6″-H), 7.90 (t, 1H, J = 8.0 and 7.2 Hz 7′-H), 8.02 (d, 1H, J = 8.4 Hz, 8′-H), 8.09 (d, 1H, J = 7.2 Hz, 5′-H), 8.12 (s, 1H, 5-H), 8.66 (s, 1H, CH=N), 9.39 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.5 (C-4″-OCH_3), 100.7 (C-5), 114.3 (C-3″ and C-5″), 126.1 (C-2″ and C-6″), 126.5 (C-3′), 127.4 (C-1″), 127.5 (C-4′a), 128.2 (C-8′), 128.5 (C-6′), 129.1 (C-5′), 132.4 (C-7′), 137.3 (C-4), 138.1 (C-4′), 140.0 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.5 (C-2), 158.7 (C-4″); HRMS: (m/z) M^+ calcd. for $C_{20}H_{16}ClN_5O$: 377.1043, Found: 378.1159 (M^++H).

*4.1.5. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-(4″-chlorophenyl)-1H-imidazole-1,2-diamine (**5d**)*

Yield: 190 mg (58%); M.p: 250 °C; IR (KBr): 3415 and 3275 (NH str.), 3068 (CH str.), 2355, 1471, 1325, 699 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.50 (s, 2H, NH_2), 7.42 (d, 2H, J = 8.4 Hz, 3″-H and 5″-H), 7.72–7.80 (m, 3H, 6′-H, 2″-H and 6″-H), 7.89 (t, 1H, J = 6.9 and 7.4 Hz, 7′-H), 8.01 (d, 1H, J = 8.0 Hz, 8′-H), 8.09 (d, 1H, J = 7.9 Hz, 5′-H), 8.30 (s, 1H, 5-H), 8.70 (s, 1H, CH=N), 9.38 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 102.7 (C-5), 126.3 (C-3′), 126.4 (C-2″ and C-6″), 127.4 (C-4′a), 128.2 (C-8′), 128.5 (C-6′), 128.9 (C-3″ and C-5″), 129.2 (C-5′), 131.2 (C-1″), 132.5 (C-7′), 133.6 (C-4″), 136.9 (C-4), 137.5 (C-4′), 141.0 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.6 (C-2); HRMS: (m/z) M^+ calcd. for $C_{19}H_{13}Cl_2N_5$: 381.0548, Found: 382.0611 (M^++H).

*4.1.6. (E)-4-(4″-bromophenyl)-N1-[(2′-chloroquinolin-3′-yl) methylene]-1H-imidazole-1,2-diamine (**5e**)*

Yield: 112 mg (32%); M.p. 261 °C; IR (KBr): 3404 and 3263 (NH str.), 3116, 3194 and 3074 (CH str.), 2360, 1625, 1473, 1327, 750 cm^{-1}; ^1H NMR: (400 MHz, DMSO-d_6) δ: 6.56 (s, 2H, NH_2), 7.57 (d, 2H, J = 8.4 Hz, 3″-H and 5″-H), 7.73 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H), 7.75–7.77 (m, 1H, 6′-H), 7.91 (t, 1H, J = 8.4 and 7.2 Hz, 7′-H), 8.02 (d, 1H, J = 8.8 Hz, 8′-H), 8.04 (d, 1H, J = 8.4 Hz, 5′-H), 8.35 (s, 1H,

5-H), 8.71 (s, 1H, CH=N), 9.41 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 102.8 (C-5), 119.7 (C-4″), 126.3 (C-3′), 126.7 (C-2″ and C-6″), 127.4 (C-4′a), 128.2 (C-8′), 128.5 (C-6′), 129.2 (C-5′), 131.8 (C-3″ and C-5″), 132.5 (C-7′), 134.0 (C-1″), 136.91 (C-4), 137.5 (C-4′), 141.0 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.6 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{19}H_{13}BrClN_5$: 425.0043, Found: 426.0154 (M^++H).

*4.1.7. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-phenyl-1H-imidazole-1,2-diamine (**5f**)*

Yield: 67 mg (25%); M.p. 269 °C; IR (KBr): 3408 and 3282 (NH str.), 3078, 3209 and 2920 (CH str.), 2360, 1327, 696 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, 6′-CH_3), 6.44 (s, 2H, NH_2), 7.23 (br s, 1H, 4″-H), 7.37 (br s, 2H, 3″-H and 5″-H), 7.74 (d, 2H, *J* = 7.6 Hz, 2″-H and 6″-H), 7.80 (d, 1H, *J* = 6.4 Hz, 8′-H), 7.84 (s, 1H, 5′-H), 7.91 (d, 1H, *J* = 8.0 Hz, 7′-H), 8.23 (s, 1H, 5-H), 8.69 (s, 1H, CH=N), 9.27 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′ −CH_3), 102.1 (C-5), 124.8 (C-2″ and C-6″), 126.3 (C-3′), 127.4 (C-4′a), 127.7 (C-5′), 128.0 (C-8′), 128.8 (C-3″ and C-5″), 129.3 (C-1″) 132.4 (C-7′), 134.6 (C-6′), 134.7 (C-4″), 138.0 (C-4), 138.1 (C-4′), 140.9 (CH=N), 146.4 (C-8′), 148.1 (C-2″), 150.5 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{20}H_{16}ClN_5$: 361.1094, Found: 362.1190 (M^+ + H).

*4.1.8. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-(4″-methylphenyl)-1H-imidazole-1,2-diamine (**5g**)*

Yield: 69 mg (25%); M.p. 271 °C; IR (KBr): 3406 and 3263 (NH str.), 3076, 3115, 3030 and 2989, 2360 (CH str.), 1463, 1600, 1327, 823 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, 4″-CH_3), 2.55 (s, 3H, 6′-CH_3), 6.44 (s, 2H, NH_2), 7.19 (d, 2H, *J* = 8.0 Hz, 3″-H and 5″-H), 7.68 (d, 2H, *J* = 7.6 Hz, 2″-H and 6″-H), 7.74 (d, 1H, *J* = 8.4 Hz, 7′-H), 7.84 (s, 1H, 5′-H), 7.91 (d, 1H, *J* = 8.4 Hz, 8′-H), 8.18 (s, 1H, 5-H), 8.67 (s, 1H, CH=N), 9.28 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-4″−CH_3), 21.3 (C-6′ −CH_3), 101.5 (C-5), 124.8 (C-2″ and C-6″), 126.3 (C-3′), 127.4 (C-4′a), 127.7 (C-5′), 128.0 (C-8′), 129.4 (C-3″ and C-5″), 130.09 (C-7′) 131.9 (C-1″), 134.6 (C-6′), 136.2 (C-4″), 136.6 (C-4), 138.1 (C-4′), 140.5 (CH=N), 146.3 (C-8′a), 148.1 (C-2′), 150.4 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{21}H_{18}ClN_5$: 375.1251, Found: 376.3037 (M^++H).

*4.1.9. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-(4″-methoxyphenyl)-1H-imidazole-1,2-diamine (**5h**)*

Yield: 88 mg (38%); M.p. 261 °C; IR (KBr): 3424 and 3269 (NH str.), 3064 and 2345 (CH str.), 2355, 1465, 1327, 1047, 671 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.33 (s, 3H, 6′-CH_3), 3.79 (s, 3H, 4″-OCH_3), 6.41 (s, 2H, NH_2), 6.96 (br s, 2H, 3″-H and 5″-H), 7.67 (br s, 3H, 2″-H, 6″-H and 7′-H), 7.85 (m, 2H, 8′-H and 5′-H), 8.10 (s, 1H, 5-H), 8.65 (s, 1H, CH=N), 9.27 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′ −CH_3), 55.5 (C-4″−OCH_3), 100.7 (C-5), 114.3 (C-3″ and C-5″), 126.1 (C-2″ and C-6″), 126.4 (C-3′), 126.44 (C-1″), 126.5 (C-4′a) 127.7 (C-5′), 128.0 (C-8′), 131.3 (C-7′), 134.5 (C-6′), 136.6 (C-4), 138.1 (C-4′), 140.2 (CH=N), 146.3 (C-8′a), 148.1 (C-2′), 150.4 (C-2), 158.7 (C-4″); HRMS: (*m*/*z*) M^+ calcd. for $C_{21}H_{18}ClN_5O$: 391.1200, Found: 392.1421 (M^++H).

*4.1.10. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-(4″-chlorophenyl)-1H-imidazole-1,2-diamine (**5i**)*

Yield: 87 mg (29%); M.p. 271 °C; IR (KBr): 3417 and 3275 (NH str.), 3109, 3074 and 2922 (CH str.), 2360, 1635, 1463, 1327, 823 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, 6′-CH_3), 6.52 (s, 2H, NH_2), 7.44 (d, 2H, *J* = 8.4 Hz, 3″-H and 5″-H), 7.75 (d, 1H, *J* = 7.2 Hz, 7′-H), 7.79 (d, 2H, *J* = 8.4 Hz, 2″-H and 6″-H), 7.84 (s, 1H, 5′-H), 7.92 (d, 1H, *J* = 8.4 Hz, 8′-H), 8.31 (s, 1H, 5-H), 8.70 (s, 1H, CH=N), 9.29 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′ −CH_3), 102.7 (C-5), 126.2 (C-3′) 126.4 (C-2″ and C-6″), 127.3 (C-4′a), 127.7 (C-5′), 127.9 (C-8′), 128.9 (C-3″ and C-5″), 131.3 (C-1″), 130.8 (C-4′a), 134.02 (C-4″), 134.7 (C-6′), 136.8 (C-4), 138.2 (C-4′), 141.4 (CH=N), 146.4 (C-8′a), 148.1 (C-2′), 150.5 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{20}H_{15}Cl_2N_5$: 395.0705, Found: 396.0831 (M^++H).

*4.1.11. (E)-4-(4″-bromophenyl)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-1H-imidazole-1,2-diamine (**5j**)*

Yield: 98 mg (29%); Mp: 271 °C; IR (KBr): 3414 and 3271 (NH str.), 3074, 3194, 2924 and 2866 (CH str.), 2351, 1463, 1327, 1060, 699 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, 6′-CH_3), 6.51 (s, 2H, NH_2), 7.57 (d, 2H, *J* = 8.4 Hz, 3″-H and 5″-H), 7.73 (d, 2H, *J* = 8.4 Hz, 2″-H and 6″-H), 7.74 (d, 1H, *J* = 8.0 Hz, 7′-H), 7.84 (s, 1H, 5′-H), 7.92 (d, 1H, *J* = 8.4 Hz, 8′-H), 8.33 (s, 1H, 5-H), 8.70 (s, 1H, CH=N), 9.29 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′ −CH_3), 102.8 (C-5), 119.7 (C-4″), 126.2 (C-3′), 126.7 (C-2″ and C-6″), 127.4 (C-4′a), 127.7 (C-5′), 128.0 (C-8′), 131.4 (C-7′), 131.7 (C-3″ and C-5″), 133.9 (C-1″), 134.6 (C-6′), 136.8 (C-4), 138.2 (C-4′), 141.3 (CH=N), 146.4 (C-8′a), 148.1 (C-2′), 150.5 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{20}H_{15}BrClN_5$: 439.0199, Found: 440.0311 (M^++H).

*4.1.12. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-(naphthalen-1″-yl)-1H-imidazole-1,2-diamine (**5k**)*

Yield: 60 mg (20%); M.p. 263 °C. IR (KBr): 3464 and 3286 (NH str.) 3059, 3205, 3286 and 2922 (CH str.), 2357, 1656, 1462, 1321, 763 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.53 (s, 2H, NH_2), 7.55–8.02 (m, 10H, 2″-H, 3″-H, 4″-H 5″-H, 6″-H, 7″-H, 8″-H, 6′-H, 7′-H and 8′-H), 8.14 (d, 1H, *J* = 8.3 Hz, 5′-H), 8.84 (s, 1H, 5-H), 8.99 (s, 1H, CH=N), 9.42 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 104.9 (C-5), 125.9 (C-7″), 126.1 (C-5″), 126.3 (C-2″), 126.5 (C-3″ and C-6″), 126.9 (C-3′) 127.4 (C-4′a), 127.8 (C-8″), 128.2 (C-8′) 128.5 (C-6′), 128.7 (C-4″), 129.2 (C-5′), 130.9 (C-1″), 132.2 (C-4′a), 132.5 (C-7′), 134.1 (C-8′a), 137.6 (C-4), 138.0 (C-4′), 141.4 (CH=N), 147.8 (C-8′a), 149.0 (C-2′), 150.1 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{23}H_{16}ClN_5$: 397.1094, Found: 398.1176 (M^++H).

*4.1.13. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene-4-(naphthalen-2″-yl)]-1H-imidazole-1,2-diamine (**5l**)*

Yield: 85 mg (25%); M.p. 274 °C; IR (KBr): 3419 and 3278 (NH str.), 3055.24 (CH str.), 1643, 1463, 1327, 748 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.53 (s, 2H, NH_2), 7.48–8.28 (m, 11H, 1″-H, 3″-H, 4″-H 5″-H, 6″-H, 7″-H, 8″-H, 6′-H, 7′-H, 8′-H and 5′-H), 8.40 (s, 1H, 5-H), 8.76 (s, 1H, CH=N), 9.40 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 103.0 (C-5), 122.6 (C-8″a*), 123.9 (C-3″*), 126.0 (C-4″*), 126.1 (C-8″*), 126.3 (C-3′), 126.4 (C-5″*), 126.7 (C-1″*), 127.4 (C-4′a), 128.0 (C-6″ and C-7″*), 128.2 (C-8′), 128.5 (C-6′), 129.2 (C-5′), 132.1 (C-2″*), 132.4 (C-4), 132.6 (C-7′), 137.49 (C-4″a*), 138.2 (C-4′), 141.0 (CH=N), 147.7 (C-8′a), 149.0 (C-2′), 150.7 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{23}H_{16}ClN_5$: 397.1094, Found: 398.2543 (M^++H). *Exchangeable carbon values.

*4.1.14. (E)-N1-[(2′-chloro-6′-methylquinolin-3″-yl)methylene]-4-(naphthalen-1″-yl)-1H-imidazole-1,2-diamine (**5m**)*

Yield: 60 mg (22%); M.p. 265 °C; IR (KBr): 3448 and 3228 (NH str.) 2918, 2956, 2850 (CH str.), 1645, 1462, 1325, 769 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.52 (s, 3H, 6′-CH_3), 6.51 (s, 2H, NH_2), 7.55–8.13 (m, 9H, 2″-H, 3″-H, 4″-H 5″-H, 6″-H, 7″-H, 8″-H, 7′-H and 8′-H), 8.12 (s, 1H, 5′-H), 8.82 (s, 1H, 5-H), 8.90 (s, 1H, CH=N), 9.30 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′-CH_3), 104.9 (C-5), 125.9 (C-7″), 126.1 (C-5″), 126.3 (C-2″), 126.4 (C-3″ and C-6″), 126.9 (C-3′), 127.4 (C-4′a), 127.7 (C-5′), 128.0 (C-8′), 128.7 (C-4″), 129.3 (C-5′) 130.9 (C-1″), 132.2 (C-4′a), 134.1 (C-8′a), 134.6 (C-6′), 136.9 (C-4), 138.0 (C-8′), 138.1 (C-4′), 141.6 (CH=N), 146.4 (C-8′a), 148.1 (C-2′), 150.1 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{24}H_{18}ClN_5$: 411.1251, Found: 412.1330 (M^++H).

*4.1.15. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-(naphthalen-2″-yl)-1H-imidazole-1,2-diamine (**5n**)*

Yield: 50 mg (27%); M.p. 274 °C; IR (KBr): 3425 (NH str.) 3057 (CH str.), 2360, 1645, 1463, 1325, 748 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, 6′-CH_3), 6.51 (s, 2H, NH_2), 7.48–7.91 (m, 9H, 1″-H, 3″-H, 4″-H 5″-H, 6″-H, 7″-H, 8″-H, 8.0 Hz and 8′-H), 8.27 (s, 1H, 5′-H), 8.39 (s, 1H, 5-H), 8.74 (s, 1H, CH═N), 9.29 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′-CH_3), 103.0 (C-5), 122.6 (C-8″a*), 123.9 (C-3″*), 125.9 (C_8″*), 126.0 (C-4″*), 126.2 (C-3′), 126.4 (C-5″*), 126.7 (C-1″*), 127.4 (C-4′a), 127.7 (C-5′), 128.1 (C-8′), 128.2 (C-6″ and C-7″), 132.6 (C-7′), 133.7 (C-2″*), 134.6 (C-6′), 136.7 (C-4), 136.8 (C-4″a*), 138.2 (C-4′), 141.1 (CH═N), 146.4 (C-8′a), 148.1 (C-2′), 150.7 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{24}H_{18}ClN_5$: 411.1251, Found: 412.3954 (M^++H). *Exchangeable carbon atoms.

*4.1.16. (E)-N1-[(2′-chloroquinolin-3′-yl)methylene]-4-(thien-2″-yl)-1H-imidazole-1,2-diamine (**5o**)*

Yield: 60 mg (23%); M.p. 275 °C; IR (KBr): 3421 and 3267 (NH str.), 3120, 3190, 2920 (CH str.), 1629, 1463, 1317, 688 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.37 (s, 2H, NH_2), 7.08–7.39 (m, 3H, 3″-H, 4″-H and 5″-H), 7.75 (br s, 1H, 6′-H), 7.90 (br s, 1H, 7′-H), 8.01–8.03 (m, 2H, 8′-H and 5′-H), 8.10 (s, 1H, 5-H), 8.72 (s, 1H, CH═N), 9.37 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 101.0 (C-5), 122.1 (C-3″), 124.3 (C-2″), 126.4 (C-3′), 127.4 (C-4′a), 128.2 (C-8′), 128.3 (C-4″), 128.5 (C-6″), 129.2 (C-5′), 132.4 (C-7′), 133.8 (C-1″), 137.4 (C-4), 139.1 (C-4′), 140.8 (CH═N), 147.7 (C-8′a), 149.0 (C-2′), 150.4 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{17}H_{12}ClN_5S$: 353.0502, Found: 354.0692 (M^++H).

*4.1.17. (E)-N1-[(2′-chloro-6′-methylquinolin-3′-yl)methylene]-4-(thien-2″-yl)-1H-imidazole-1,2-diamine (**5p**)*

Yield: 60 mg (24%); M.p. 258 °C; IR (KBr): 3415 and 3271 (NH str.), 3116, 3192 and 2922 (CH str.), 1639, 1463, 1313, 700 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.54 (s, 3H, 6′-CH_3), 6.52 (s, 2H, NH_2), 7.08 (s, 1H, 4″-H) 7.33–7.39 (m, 2H, 3″-H and 5″-H), 7.73 (d, 1H, J = 7.2 Hz, 7′-H), 7.83 (s, 1H, 5′-H), 7.91 (d, 1H, J = 7.6 Hz, 8′-H), 8.08 (s, 1H, 5-H), 8.64 (s, 1H, CH═N), 9.26 (s, 1H, 4′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6 (C-6′-CH_3), 101.2 (C-5), 122.1 (C-3″), 124.3 (C-2″), 126.2 (C-3′), 127.4 (C-4′a), 127.7 (C-5′), 128.0 (C-8′), 128.2 (C-4″), 133.7 (C-1″) 134.6 (C-6′), 136.7 (C-4), 138.1 (C-4′), 139.0 (C-3′), 140.9 (CH═N), 146.4 (C-8′a), 148.1 (C-2′), 150.4 (C-2); HRMS: (*m*/*z*) M^+ calcd. for $C_{18}H_{14}ClN_5S$: 367.0658, Found: 368.0743 (M^++H).

4.2. Cell cultures

HCT116 and DLD1 (colon cancer cells), MDA MB 231 (Breast cancer cell) were maintained as monolayers for experiments. HCT116 cells were cultured in McCoy's medium (Hyclone, USA), DLD1 cells were cultured in RPMI-1640 medium and MDA MB 231 cells were maintained in DMEM containing sodium pyruvate. 10% (w/v) Fetal bovine serum, penicillin 100 μg/mL, streptomycin 100 μg/mL, gentamicin 45 μg/mL were added to each cell culture medium and cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. Subcultures were made by trypsinization and reseeded for experiments. Cells were treated with different compounds at 10, 20, 30, 40 and 50 μM concentration and treatment was continued up to 48 h. HUVEC cells were maintained in EGM2 media. Cytotoxicity and ROS studies were performed as described earlier [34].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.055.

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