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Synthesis and molecular docking studies of novel 2-chloro-pyridine derivatives containing flavone moieties as potential antitumor agents

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ABSTRACT

A series of novel 2-chloro-pyridine derivatives containing flavone, chrome or dihydropyrazole moieties as potential telomerase inhibitors were synthesized. The bioassay tests showed that compounds **6e** and **6f** exhibited some effect against gastric cancer cell SGC-7901 with IC_{50} values of 22.28 ± 6.26 and 18.45 ± 2.79 μg/mL, respectively. All title compounds were assayed for telomerase inhibition by a modified TRAP assay, the results showed that compound **6e** can strongly inhibit telomerase with IC_{50} value of 0.8 ± 0.07 μM. Docking simulation was performed to position compound **6e** into the active site of telomerase (3DU6) to determine the probable binding model.

Telomerase remains active in the early stages of life maintaining telomere length and the chromosomal integrity of frequently dividing cells. It turns dormant in most somatic cells during adulthood.[1] In cancer cells, however, telomerase gets reactivated and works tirelessly to maintain the short length of telomeres of rapidly dividing cells, leading to their immortality.[2] The essential role of telomerase in cancer and aging makes it an important target for the development of therapies to treat cancer and other age-associated disorders. Telomere and telomerase are closely related to the occurrence and development of gastric cancer.[3]

Pyridine derivatives find several applications in pharmaceutical and in agrochemical fields.[4] Kim et al. have reported bioisosteres of terpyridine with considerable protein kinase C (PKC) inhibitory activity and antitumor cytotoxicities against several human cancer cell lines.[5] Furthermore, 2-pyridine, a small bioactive molecule, is an important pharmacophore that can form hydrogen-bonded structures similar to those encountered with the base-pairing mechanism in DNA and RNA.[6,7] In view of their importance as drugs, biologically active natural products, and in other related applications, extensive studies have been carried out on the synthesis of pyridine compounds in recent years. On the other hand, flavonoids belong to an important class of compounds consisting of more than 5000 polyphenolic compounds that occur in nature in several foods of plant origin. These compounds are often characterized by the presence of a common phenylbenzopyrone linkage (C6–C3–C6) in their structures. They have a wide range of biological activities, for example, antimutagenic and antiproliferative activities, can act as antioxidants and are usually involved in cell signaling, cell cycle regulation, and angiogenesis.[8–11] A large numbers of in vitro studies have been conducted on the potential anticancer activity of flavonoids in various cells including human oral cancer system.[12] Significant contribution was made in this field by Elattar and Virji who reported appreciable inhibitory effects of tea polyphenols, curcumin, genistein, quercetin, and cisplatin on the growth of oral cancer cell lines SCC-25.[13]

Based on these reports, we considered the possibility of introducing heterocyclic flavone moiety into the parent 2-chloro-pyridine unit to design novel structures with enhanced anticancer activities. Since there are only a very few systematic reports on the synthetic methodology and evaluation of anticancer activities of these compounds, we prepared herein a series 2-chloro-pyridine derivatives containing flavone and other heteroaromatic derivatives

Abbreviations: MTT, 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2 h-tetrazolium bromide; DMSO, dimethyl sulfoxide; DMF, dimethyl formamide; MH, Mueller–Hinton; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; TRAP, telomere repeat amplification protocol.

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and tested their activities against gastric cancer cell SGC-7901. In order to elucidate the potential mechanism by which the title compounds induce anticancer activity, docking simulation was performed to position selected compounds into the active site of telomerase 3DU6.

The synthetic routes to intermediates **1**, **1′**, and **3** are shown in Scheme 1. Compound **1** was prepared according to the literature method as described.[14] Compounds **1′** and **3** were prepared by following the procedures published previously.[15,16]

The compounds **2a** and **2b** (Scheme 2) were synthesized by the reaction of 2-chloro-5-(chloromethyl) pyridine **1** with chromen **1′** in presence of catalyst Et_3N in chloroform at 40 °C. For the preparation of title compounds **4a**, **4b**, and **6a**–**6f**, 2-chloro-5-(chloromethyl) pyridine was slowly added to a well-stirred mixture of 5-aryl-dihydropyrazole, flavone, KCO_3, and KI in DMF at 40 °C. The temperature of the system was raised and the reaction mixture was refluxed for 5 h, the solvent was removed in vacuo and the crude residue was purified by chromatography on SiO_2 (acetone/petroleum, v/v = 3:1) to give the compounds as colorless solids. The spectral data can be found in the Supporting information.[17]

*Compound **2a**: 3-((6-Chloropyridin-3-yl)methoxy)-2-phenyl-4H-chromen-4-one:* Colorless crystals, yield, 71%; mp 235–236 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.05 (s, 2H, $-CH_2-$), 7.09–8.20 (m, 10H, flavone-H and pyridine-H), 8.21 (s, 1H, pyridine, 6-H), 8.24 (dd, 1H, J = 7.86 and 1.44 Hz, flavone, 5-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 71.2, 121.4, 123.0, 123.7, 124.0, 124.2, 131.8, 132.0, 135.5, 139.1, 140.6, 142.4, 146.7, 150.1, 151.5, 152.3, 158.7, 160.4, 182.3; ESI-MS: 364.2 ($C_{21}H_{14}ClNO_3$, $[M+H]^+$); Anal. Calcd for $C_{21}H_{14}ClNO_3$: C, 69.33; H, 3.88; N, 3.85. Found: C, 69.71; H, 4.11; N, 3.77.

*Compound **4a**: 1-(5-(2-((6-Chloropyridin-3-yl)methoxy)phenyl)-3-methyl-4,5-dihydropyrazol-1-yl): Ethanone*, Colorless crystals, yield, 75%; mp 156–157 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.94 (s, 3H, Me), 2.32 (s, 3H, Me), 2.58 (dd, J = 18.1 and 4.7 Hz, 1H, pyrazole, 4-H_a), 3.28 (dd, J = 11.8 and 18.2 Hz, pyrazole, 1H, 4-H_b), 5.08 (s, 2H, CH_2-), 5.68 (dd, J = 4.7 and 11.7 Hz, 1H, pyrazole, 5-H), 6.89–7.79 (m, 6H, ArH and pyridine, 3,4-H), 8.46 (s, 1H, pyridine, 6-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.9, 24.1, 70.8, 40.3, 55.3, 113.7, 120.4, 124.7, 127.5, 128.8, 130.1, 131.2, 138.0, 146.6, 149.9, 152.1, 158.7, 169.9; ESI-MS: 343.1 ($C_{18}H_{18}ClN_3O_2$, $[M+H]^+$); Anal. Calcd for $C_{18}H_{18}ClN_3O_2$: C, 62.88; H, 5.28; N, 12.22. Found: C, 63.05; H, 5.21; N, 12.44.

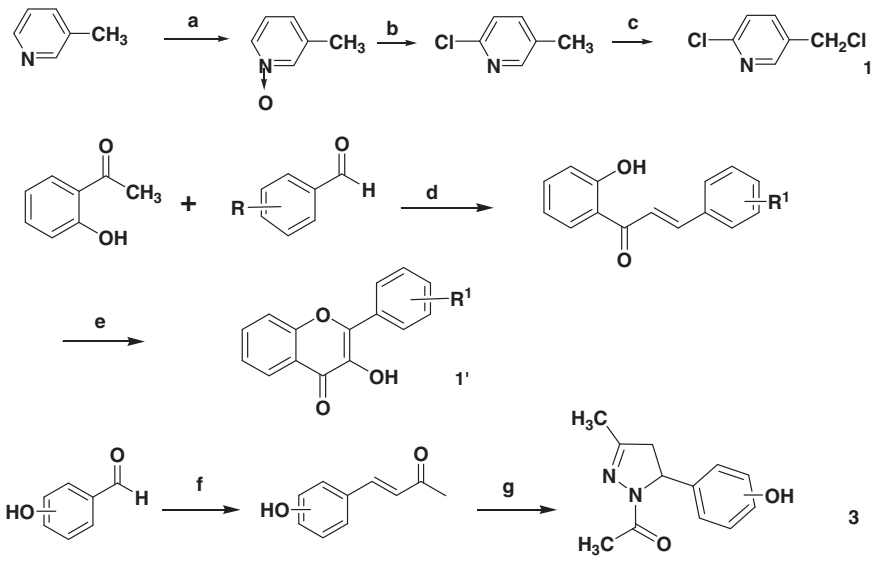
The single-crystal structure of compound **4b** was determined by X-ray crystallography. Colorless crystals, yield, 82%; mp 164–165 °C; crystal data of **4b**: $C_{18}H_{18}ClN_3O_2$, M = 343.8, monoclinic, space group $P2(1)/c$; a = 12.2753(12), b = 8.0425(8), c = 18.1766(19) (Å); α = 90, β = 109.74, γ = 90(°), V = 1689.1(3) nm^3, T = 173(2) K, Z = 4, D_c = 1.352 g/cm^3, F(0 0 0) = 720. Reflections collected/unique: 10,914/2941, fine, R_1 = 0.0431, $wR(F^2)$ = 0.1123.

The molecular structure of the compound **4b** is shown in Figure 1. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-762238.

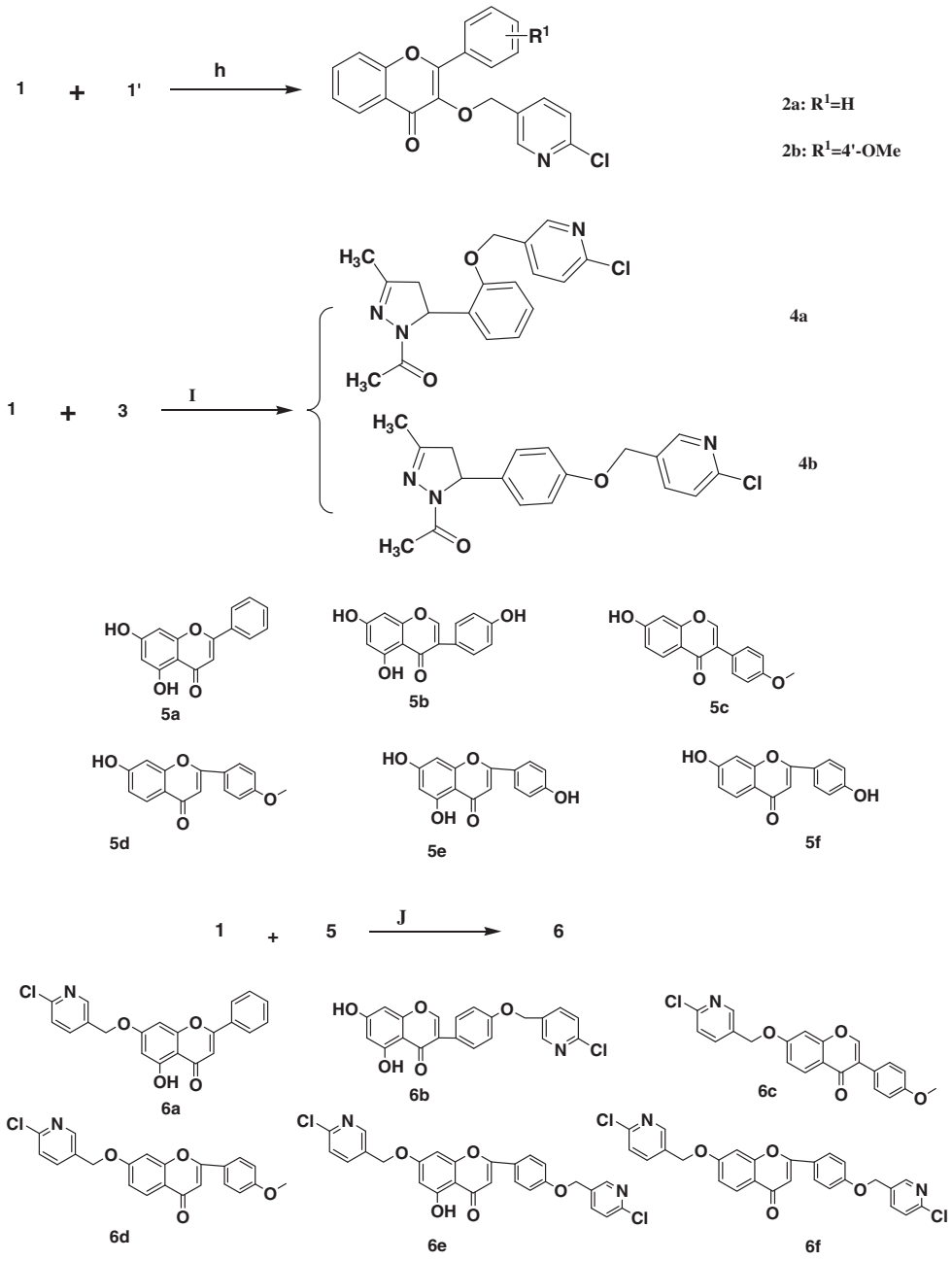
*Compound **6a**: 7-((6-Chloropyridin-3-yl)methoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one:* Colorless crystals, yield, 64%; mp 221–222 °C; ^{1}H NMR (DMSO, 300 MHz): δ 5.32 (s, 2H, $-CH_2-$), 6.53 (s, 1H, flavone, 6-H), 6.95 (s, 1H, flavone, 8-H), 7.07 (s, 1H, flavone, 3-H), 7.58–8.09 (m, 5H, flavone, 2′,3′,4′,5′,6′-H), 8.10–8.56 (m, 2H, pyridine, 3,4-H), 8.57 (s, 1H, pyridine, 6-H), 12.83 (s, 1H, flavone, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 72.0, 95.0, 96.9, 104.8, 105.1, 123.2, 126.1, 128.4, 129.0, 131.1, 132.4, 139.2, 148.1, 150.3, 150.9, 163.7, 163.9, 167.8, 183.1; ESI-MS: 380.0 ($C_{21}H_{14}ClNO_4$, $[M+H]^+$); Anal. Calcd for $C_{21}H_{14}ClNO_4$: C, 66.41; H, 3.72; N, 3.69. Found: C, 66.31; H, 4.04; N, 3.77.

In the screening assay studies, all the compounds were evaluated for their cytotoxic activity against gastric SGC-7901 cell line.[18] The cell was allowed to proliferate in presence of tested material for 48 h, and the results are reported in terms of IC_{50} values (Table 1). It is obvious from the data that compounds **6e** and **6f** exhibited inhibitory activities to a certain degree against the gastric cell SGC-7901 with the IC_{50} values of 22.28 ± 6.26 and 18.45 ± 2.79 μg/mL, respectively.

Among the different groups of synthesized compounds, 2-chloro-pyridine derivatives **2a**,**b** and **4a**,**b** containing chrome and



Scheme 1. Synthesis of intermediate compounds. Reagent and conditions: (a) H_2O_2, CH_3COOH, 15–20 atm, 115–190 °C, 6 h; (b) $POCl_3$, CH_2Cl_2, −10 °C, 3 h; (c) Cl_2, KCO_3, CCl_4, 60 °C, 6 h; (d) KOH, CH_3OH, HCl, 25 °C, 10 h; (e) KOH, CH_3OH, H_2O_2, HCl, 25 °C, 4 h; (f) NaOH, CH_3COCH_3, HCl, 20 °C, 15 h; (g) 80% NH_2–NH_2·H_2O, 98% CH_3COOH, reflux, 2 h.



Scheme 2. Synthesis of title compounds. Reagent and conditions: (h) $CHCl_3$, Et_3N, 40 °C, 4 h; (I) KCO_3, KI, DMF, reflux 5 h; (J) KCO_3, KI, DMF, reflux 5–8 h.

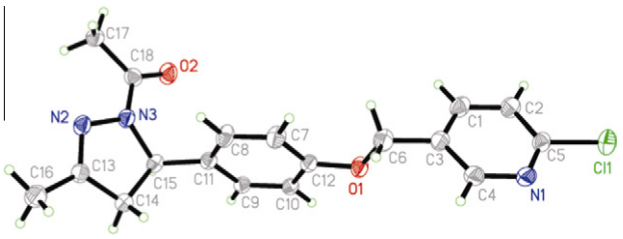


Figure 1. ORTEP drawing of **4b**.

5-aryl-dihydropyrazole moieties, respectively, displayed poor activity against gastric cell SGC-7901, whereas 2-chloro-pyridine derivatives **6a**–**6f** containing flavones, in general, showed rela-

Table 1
Cytotoxic activity of the synthesized compounds against SGC-7901 cell line[a]

Compound	IC_{50} (μg/mL)[b] SGC-7901	Compound	IC_{50} (μg/mL)[b] SGC-7901
2a	—[d]	**6b**	25.94 ± 2.41
2b	—[d]	**6c**	31.93 ± 4.49
4a	—[d]	**6d**	35.17 ± 4.28
4b	—[d]	**6e**	22.28 ± 6.26
6a	—[d]	**6f**	18.45 ± 2.79
5-Fluorouracil[c]	7.38 ± 0.98	5-Fluorouracil[c]	7.38 ± 0.98

Negative control DMSO, no activity.
[a] The data represented the mean of three experiments in triplicate and were expressed as means ± SD; only descriptive statistics were done in the text.
[b] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed. The results are listed in the table.
[c] Used as a positive control.
[d] No significant activity.

Table 2
Biological properties of test compounds

Compound	IC_{50} (μM) *Taq* polymerase	IC_{50} (μM) telomerase	Selectivity index[a] (SI)
2a	No[c]	No[c]	–
2b	No[c]	No[c]	–
4a	53.2 ± 2.2	No[c]	–
4b	No[c]	46.7 ± 1.1	–
6a	27.5 ± 2.2	20.9 ± 0.78	1.3
6b	6.8 ± 2.2	3.5 ± 0.16	1.9
6c	7.5 ± 2.2	2.5 ± 0.06	3.0
6d	6.4 ± 2.2	3.1 ± 0.09	2.1
6e	2.0 ± 2.2	0.8 ± 0.07	2.5
6f	5.0 ± 2.2	3.0 ± 0.13	1.6
Ethidium bromide[b]		2.4 ± 0.8	

No, means were not observed in the tested concentration range 0–60 μM.
[a] Ratio between the drug concentrations at which 50% *Taq* polymerase/telomerase inhibition was observed.
[b] Ethidium bromide is reported as a control. The inhibition constant of ethidium toward telomerase has been reported previously.
[c] Indicates the highest concentration the compounds were tested.

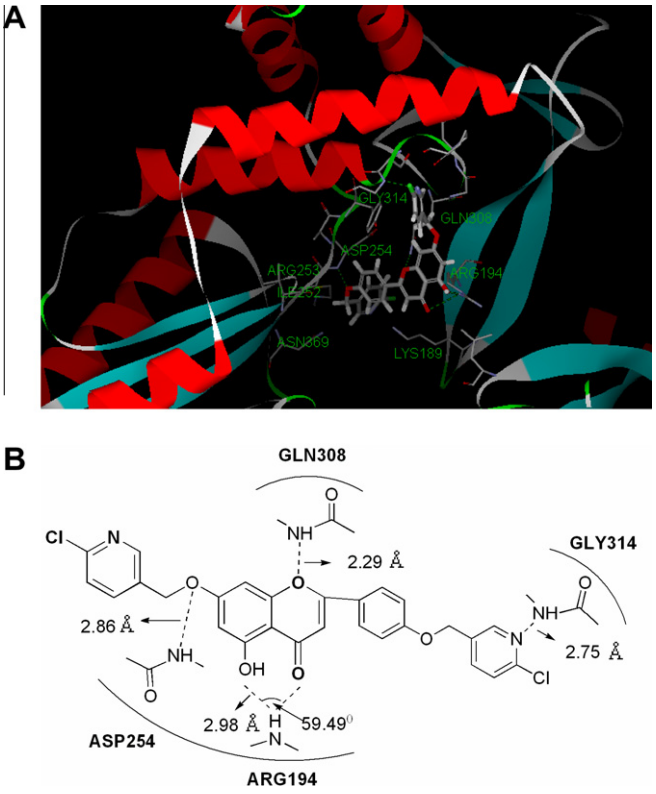


Figure 2. Molecular docking modeling of compound **6e** (A) with telomerase; the small molecule and the critical interaction of 3DU6 are represented by sticks. Panel is a view into the active site cavity. (B) Schematic representation of the binding mode of **6e** in the ATP binding site of 3DU6.

tively higher inhibitory potency against the gastric SGC-7901 cell (Table 1).

All purified title compounds were assayed for telomerase inhibition by a modified TRAP[20] assay, using a SGC-7901 cell extract. Modified TRAP is a powerful technique and could give us some information about small molecules inhibiting telomere elongation qualitatively and quantitatively.[21] To avoid false positive results due to drug interference with the amplification step, *Taq* polymerase inhibition was additionally monitored. The results are summarized in Table 2, where a selectivity index (SI, ratio between IC_{50} for *Taq* polymerase vs telomerase inhibition) is also included. The results suggested that the telomerase inhibitory abilities of certain 2-chloro-pyridine derivatives containing flavone were potent. Especially the IC_{50} value of compound **6e** was as low as 0.8 ± 0.07 μM, which was even better than that of the ethidium bromide.

In an effort to elucidate the mechanism by which the title compounds can induce anticancer activity in the gastric cell SGC-7901 and to establish an SAR based on our experimental studies, molecular docking of the potent inhibitors **6e** into ATP binding site of telomerase was performed to simulate a binding model derived from telomerase structure (3DU6 PDB). See Figure 2. Visual inspection of the pose of **6e** into the ATP-site revealed that four more optimal intramolecular hydrogen bonds are observed (N–H···O: 2.98 Å, with amino hydrogen group of Arg 194; N–H···O: 2.67 Å, with amino hydrogen group of Arg 194, O···N···O, 59.49°; N–H···O: 2.86 Å, with amino hydrogen group of Asp 254; N–H···O: 2.29 Å, with amino hydrogen group of Gln 308). Also the 2-chloro-pyridine ring projects into a hydrophobic region, which is comprised of the side chains of Lys 189, Asn 369, Ile 252, Arg 253, that is important for the potent inhibitory activity of **6e**. These residues influenced the accessibility of the hydrophobic pocket that flanks the ATP binding site, and their size can be a key factor in controlling telomerase selectivity. In the other end of the ATP-binding pocket, the N of another 2-chloro-pyridine interacted with the residue Gly 314, which made the 3D structure more stable.

In summary, we prepared a series of novel 2-chloro-pyridine derivatives containing flavone, chrome or dihydropyrazole unit as potential telomerase inhibitors. A systematic SAR study illustrated that the optimal activity could be obtained with a selected class of compounds. Thus, the best potency was achieved when the 2-chloro-pyridine core was attached with flavone. The results showed that compounds **6e** and **6f** had certain activity against gastric cell SGC-7901, compound **6e** can strongly inhibit telomerase with IC_{50} value of 0.8 ± 0.07 μM. Docking simulation was performed to position compound **6e** into the telomerase 3DU6 active site. The results show that compound **6e** can bind well with the telomerase active site and act as potential telomerase inhibitor.

Supporting information: CCDC-762238 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the URL http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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17. *Compound **2b**:* Colorless crystals, yield, 76%; mp 226–227 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.89 (s, 3H, –OMe), 5.31 (s, 2H, $-CH_2-$), 6.89–8.48 (m, 11H, flavone-H and pyridine-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 56.2, 69.4, 118.5, 123.8, 124.0, 125.3, 125.5, 128.0, 131.3, 131.8, 135.0, 140.1, 140.7, 144.3, 146.2, 150.9, 157.5, 160.2, 161.3, 181.7; ESI-MS: 393.1 ($C_{22}H_{16}ClNO_4$, $[M+H]^+$); Anal. Calcd for $C_{22}H_{16}ClNO_4$: C, 67.10; H, 4.10; N, 3.56. Found: C, 66.89; H, 4.39; N, 3.21. *Compound **6b**:* Colorless crystals, yield, 60%; mp 190–192 °C; ^{1}H NMR (DMSO, 300 MHz): δ 5.31 (s, 2H, $-CH_2-$), 6.52–8.56 (m, 10H, flavone-H and pyridine-H), 9.60 (s, 1H, flavone, 7-OH), 12.97 (s, 1H, flavone, 5-OH); ^{13}C NMR (DMSO, 125 MHz): δ 70.7, 98.1, 98.5, 104.3, 113.7, 123.0, 123.3, 124.7, 128.2, 133.0, 138.3, 146.2, 151.1, 154.4, 155.9, 160.9, 163.2, 167.1, 179.9; ESI-MS: 396.9 ($C_{21}H_{14}ClNO_5$, $[M+H]^+$); Anal. Calcd for $C_{21}H_{14}ClNO_5$: C, 63.73; H, 3.57; N, 3.54. Found: C, 64.01; H, 3.35; N, 3.72. *Compound **6c**:* Colorless crystals, yield, 78%; mp 188–189 °C; ^{1}H NMR (DMSO, 300 MHz): δ 3.84 (s, 3H, OMe), 5.16 (s, 2H, $-CH_2-$), 6.91–8.49 (m, 11H, flavone-H and pyridine-H); ^{13}C NMR (DMSO, 125 MHz): δ 56.3, 70.8, 103.2, 110.1, 113.8, 117.7, 123.9, 124.1, 125.2, 128.0, 132.3, 132.6, 137.8, 146.4, 150.9, 153.7, 158.6, 160.3, 166.5, 177.6; ESI-MS: 393.4 ($C_{22}H_{16}ClNO_4$, $[M+H]^+$); Anal. Calcd for $C_{22}H_{16}ClNO_4$: C, 67.10; H, 4.10; N, 3.56. Found: C, 66.84; H, 4.41; N, 3.25. *Compound **6d**:* Colorless crystals, yield, 81%; mp 205–206 °C; ^{1}H NMR (DMSO, 300 MHz): δ 3.84 (s, 3H, OMe), 5.16 (s, 2H, $-CH_2-$), 6.91–8.50 (m, 11H, flavone-H and pyridine-H); ^{13}C NMR (DMSO, 125 MHz): δ 56.1, 71.2, 103.3, 103.7, 109.6, 113.4, 117.6, 123.0, 124.1, 128.0, 131.3, 131.9, 138.1, 144.7, 151.8, 155.2, 160.1, 163.3, 166.9, 182.9; ESI-MS: 394.2 ($C_{22}H_{16}ClNO_4$, $[M+H]^+$); Anal. Calcd for $C_{22}H_{16}ClNO_4$: C, 67.10; H, 4.10; N, 3.56. Found: C, 66.99; H, 4.05; N, 3.83. *Compound **6e**:* Colorless crystals, yield, 42%; mp 202–203 °C; ^{1}H NMR (DMSO, 300 MHz): δ 5.29 (s, 4H, $2CH_2-$), 6.50–8.55 (m, 13H, flavone-H and pyridine-H), 12.91 (s, 1H, flavone, 5-OH); ^{13}C NMR (DMSO, 125 MHz): δ 71.1, 97.3, 98.2, 103.7, 104.8, 113.7, 123.1, 123.9, 128.0, 132.1, 138.9, 146.6, 151.4, 161.5, 162.3, 163.8, 164.7, 169.7, 182.9; ESI-MS: 522.0 ($C_{27}H_{18}Cl_2N_2O_5$, $[M+H]^+$); Anal. Calcd for $C_{27}H_{18}Cl_2N_2O_5$: C, 62.20; H, 3.48; N, 5.37. Found: C, 62.04; H, 3.17; N, 5.65. *Compound **6f**:* Colorless crystals, yield, 39%; mp 214–215 °C; ^{1}H NMR (DMSO, 300 MHz): δ 5.11 (s, 2H, $-CH_2-$), 5.16 (s, 2H, $-CH_2-$), 6.90–8.54 (m, 14H, flavone-H and pyridine-H); ^{13}C NMR (DMSO, 125 MHz): δ 71.2, 103.7, 104.2, 108.8, 113.2, 117.1, 121.9, 124.1, 128.0, 132.3, 132.5, 138.7, 146.4, 151.5, 155.7, 163.2, 163.7, 168.2, 182.9; ESI-MS: 506.4 ($C_{27}H_{18}Cl_2N_2O_4$, $[M+H]^+$); Anal. Calcd for $C_{27}H_{18}Cl_2N_2O_4$: C, 64.17; H, 3.59; N, 5.54. Found: C, 64.00; H, 3.81; N, 5.79.
18. The cytotoxicity evaluation was conducted by using a modified procedure as described in the literature.[19] Briefly, target tumor cells were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After reaching a dilution of 3×10^4 cells mL^{-1} with the medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. Subsequently, incubation was performed at 37 °C in 5% CO_2 atmosphere for 24 h before subjecting to cytotoxicity assessment. Tested samples at pre-set concentrations were added to six-wells with 5-fluorouracil being employed as a positive reference. After 48 h exposure period, 25 μL of PBS containing 2.5 mg mL^{-1} of MTT was added to each well. After 4 h, the medium was replaced by 150 μL DMSO to dissolve the purple formazan crystals produced. The absorbance at 570 nm of each well was measured on an ELISA plate reader. The data represented the mean of three experiments in triplicate and were expressed as means ± SD using Student's *t* test. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.
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