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Synthesis and molecular docking study of novel coumarin derivatives containing 4,5-dihydropyrazole moiety as potential antitumor agents

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ABSTRACT

A series of novel coumarin derivatives containing 4,5-dihydropyrazole moiety as potential telomerase inhibitors were synthesized. The bioassay tests show that compound **3d** exhibited potentially high activity against human gastric cancer cell SGC-7901 with IC_{50} value of 2.69 ± 0.60 μg/mL. All title compounds were assayed for telomerase inhibition by a modified TRAP assay, the results show that compounds **3d and 3f** can strongly inhibit telomerase with IC_{50} values of 2.0 ± 0.07 and 1.8 ± 0.35 μM, respectively. Docking simulation was performed to position compound **3d** into the telomerase (3DU6) active site 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 human gastric cancer.[3]

Coumarins are present in natural and synthetic compounds possessing biological activity. They are acting at different stages of cancer formation. Some of them have cytostatic properties and the others have cytotoxic activity.[4] Two naturally occurring coumarins have been found to exhibit cytotoxicity against a panel of mammalian cancer cell lines.[5] 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 coumarin compounds in recent years. On the other hand, many literatures discussed the antitumor activity of pyrazole derivatives.[6,7] Furthermore, 3,4-dihydropyrazole, a small bioactive molecule, is a prominent structural motif found in numerous pharmaceutically active compounds. Many 3,4-dihydropyrazole-based derivatives have shown several biological activities as seen in CB1 antagonist,[8] and tumor necrosis inhibitor.[9] Many of them are currently being tested and/or clinically evaluated for new drug discovery.

In an effort to synthesize novel 3,4-dihydropyrazole heterocyclic systems with potential biological activity, our group has recently reported on the activity of some 3,4-dihydropyrazole derivatives.[10,11] At present, we focus on screening of novel precursor structure with anti-gastric cancer activity and found some 2-chloro-pyridine derivatives containing phenylpropanoid (flavone, chrome) or dihydropyrazole unit used as potential telomerase inhibitors.[12] Based on above reports, we considered the possibility of introducing heterocyclic 4,5-dihydropyrazole moiety into the parent coumarin unit (phenylpropanoids) 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, Herein, in continuation to extend our research on antiproliferative activity against cancer cells, we prepared a series novel coumarin

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

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(phenylpropanoids) derivatives containing 4,5-dihydropyrazole moiety and tested their activities against SGC-7901 (human gastric cancer), PC-3 (human prostate cancer) cell, and A431 (human epidermoid carcinoma) cell. 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 synthesis of compound **1** (Scheme 1) started from salicylaldehyde and catalyzed by piperazine at 30 °C was added acetoacetate. Claisen–Schmidt condensation 3-acetyl-2*H*-chromen-2-one and substituted-benzaldehyde using mild catalyst piperidine, by following a reported method,[10] proved to be an efficient alternative for the synthesis of α,β unsaturated ketone **2**. The general synthetic procedure process and spectral data of compounds **3** can be found in Ref. 14. Compound **4** was prepared according to a previously published Letter.[11]

Compound **2a**: *(E)-3-(3-(4-methoxyphenyl)acryloyl)-2H-chromen-2-one*, colorless crystals, yield, 60%; mp 153–154 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.85 (3H, s, –OMe), 6.93 (2H, d, *J* = 8.8 Hz, ArH), 7.32 (2H, d, *J* = 7.5 Hz, ArH), 7.37–8.37 (6H, m, C_{5-8}-H and –CH=CH–), 8.57 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 56.3, 113.7, 122.9, 124.7, 124.9, 126.6, 126.9, 128.0, 128.2, 128.7, 134.3, 148.1, 152.0, 154.1, 159.4, 160.3, 184.2; ESI-MS: 306.3 ($C_{19}H_{14}O_4$, $[M+H]^+$); Anal. Calcd for $C_{19}H_{14}O_4$: C, 74.5; H, 4.6. Found: C, 74.9; H, 4.2.

Compound **4**: *5-(2-hydroxyphenyl)-3-methyl-4,5-dihydropyrazole-1-carbaldehyde*, colorless crystals, yield, 90%; mp 155–156 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (3H, s, –Me), 3.10 (1H, dd, *J* = 18.7 and 3.7 Hz, pyrazole, 4-H_a), 3.39 (1H, dd, *J* = 11.2 and 18.6 Hz, pyrazole, 4-H_b), 5.67 (1H, dd, *J* = 11.6 and 3.5 Hz, pyrazole, 5-H), 6.89–7.26 (5H, m, ArH and –OH), 8.63 (1H, s, –COH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 21.2, 43.1, 48.5, 115.4, 121.9, 129.6, 129.9, 131.3, 149.7, 155.0, 160.6; ESI-MS: 205.1 ($C_{11}H_{12}N_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.7; H, 5.9; N, 13.7. Found: C, 64.5; H, 6.1; N, 14.0.

In the screening assay studies, compounds **2a** and **4** and all the compounds **3** were evaluated for their cytotoxic activity against gastric SGC-7901, PC-3 (human prostate cancer), and A431 (human

Table 1
Cytotoxic activity of test compounds against SGC-7901 cell line[a] negative control DMSO, no activity

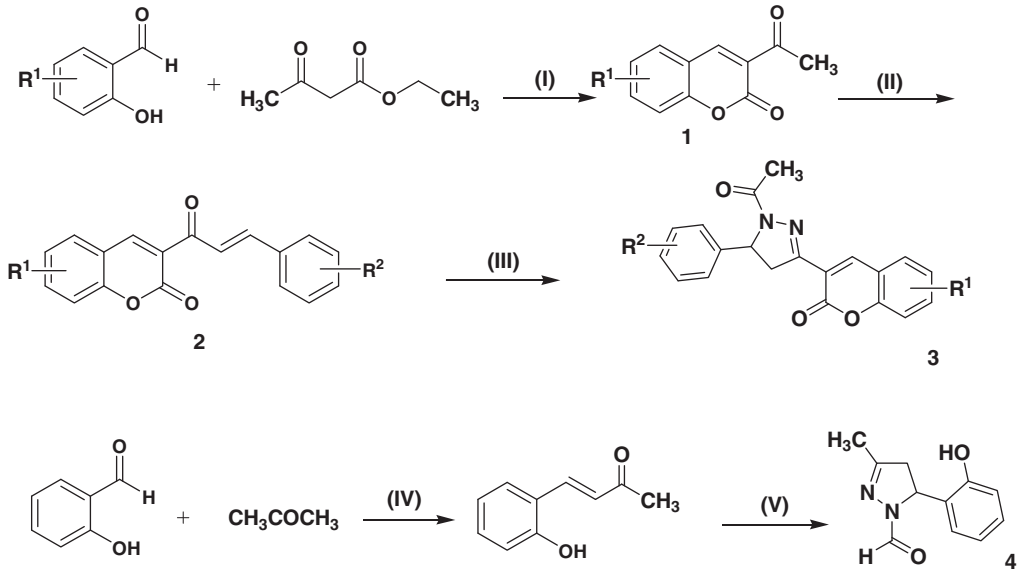
Compound	IC_{50}[b] (μg/mL) SGC-7901	IC_{50}[b] (μg/mL) PC-3	IC_{50}[b] (μg/mL) A431
2a	—[d]	—[d]	—[d]
4	—[d]	65.10 ± 1.22	—[d]
3a	48.74 ± 2.40	—[d]	68.74 ± 0.15
3b	35.93 ± 0.77	58.74 ± 0.40	72.97 ± 2.11
3c	29.93 ± 0.09	55.08 ± 1.95	50.65 ± 2.00
3d	2.69 ± 0.60	40.16 ± 2.31	51.74 ± 0.09
3e	39.93 ± 0.49	46.74 ± 2.00	68.74 ± 1.12
3f	28.93 ± 0.77	58.09 ± 1.08	48.12 ± 1.40
3g	22.35 ± 1.66	38.77 ± 0.98	68.35 ± 0.56
5-Fluorouracil[c]	7.38 ± 0.98	2.48 ± 0.11	2.17 ± 0.20

[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	No	—
4	No	No	—
3a	No	36.7 ± 1.1	—
3b	58.5 ± 1.8	29.1 ± 1.03	2.0
3c	16.8 ± 2.0	6.5 ± 0.16	2.6
3d	6.0 ± 2.2	2.0 ± 0.16	3.0
3e	20.7 ± 1.9	9.6 ± 0.09	2.1
3f	4.0 ± 2.0	1.8 ± 0.35	2.2
3g	10.0 ± 2.0	4.0 ± 0.25	2.5
Ethidium bromide[b]		2.5 ± 0.8	

No, 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.



R^1=H, R^2 = 4-OMe (a); R^1=H, R^2 =4-OH (b); R^1=H, R^2= 2-Cl (c); R^1=H, R^2 =H (d); R^1=H, R^2 =2,4-2Cl (e); R^1=5-Br (f), R^2=H; 5-Br, R^2=4-OH (g)

Scheme 1. Synthesis of title compounds. Reagent and conditions: (I) piperzine, 25–30 °C, 1 h; (II) substituted-benzaldehyde, piperzine, ethanol, reflux, 6 h; (III) 80% NH_2–NH_2·H_2O, 98% CH_3COOH, reflux, 2 h; (IV) NaOH, CH_3COCH_3, HCl, 20 °C, 15 h; (V) 80% NH_2–NH_2·H_2O, HCOOH, ethanol, reflux, 1 h.

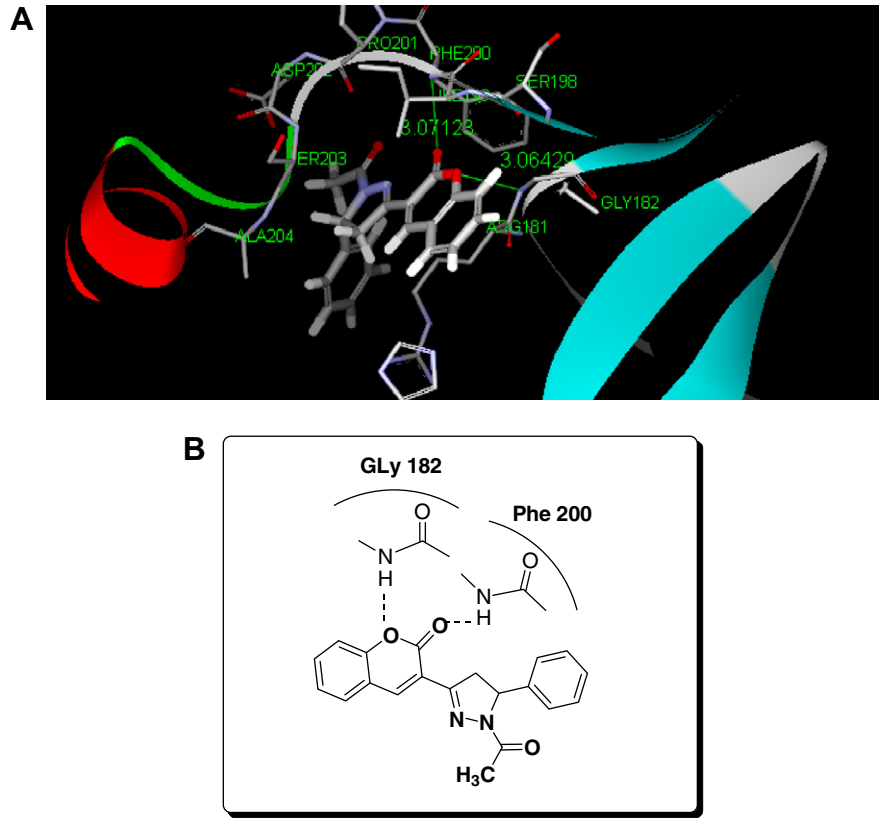


Figure 1. Molecular docking modeling of compound **3d** (A) with telomerase; the small molecule and the critical interaction of 3DU6 are represented by sticks. Panel is a view into the active site cavity. (B) Schematic representation of the binding mode of **3d** in the ATP binding site of 3DU6.

epidermoid carcinoma) cell lines.[15] The cells were 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 compound **3d** exhibited high activity against the human gastric cell SGC-7901 with the IC_{50} value of 2.69 ± 0.60 μg/mL, which was even better than that of the 5-fluorouracil, while all the synthesized compounds exhibited poor activity against PC-3 cell (human prostate cancer) and A431 (human epidermoid carcinoma) cell.

From the data presented in Table 1, it can be concluded that coumarin α,β unsaturated ketone (**2a**) and 4,5-dihydropyrazole (**4**), respectively, displayed poor activity against human gastric cell SGC-7901, whereas coumarin derivatives containing 4,5-dihydropyrazole moiety, in general, showed relatively higher activity against the gastric human SGC-7901 cell, so, some compounds in this series deserve further investigation.

All purified title compounds were assayed for telomerase inhibition by a modified TRAP[17] 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.[18] 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 coumarin derivatives containing 4,5-dihydropyrazole moiety were strong. Especially compounds **3d and 3f** with IC_{50} values of 2.0 ± 0.16 and 1.8 ± 0.35 μM, respectively, which were even better than that of the ethidium bromide.

In an effort to elucidate the mechanism by which the title compound can induce anticancer activity in the human gastric cell SGC-7901, molecular docking of the potent inhibitors **3d** into ATP binding site of telomerase was performed to simulate a binding model derived from telomerase structure (3DU6.pdb) (see Fig. 1).[19] Visual inspection of the pose of **3d** into the ATP-site revealed that two more optimal intramolecular hydrogen bonds are observed (N–H···O: 3.06 Å, with amino hydrogen group of Gly 182; N–H···O: 3.07 Å, with amino hydrogen group of Phe 200). Also the 4,5-dihydropyrazole ring projects into a hydrophobic region, which is comprised of the side chains of Pro 201, Asp 202, Ser 203, Ala 204, that is, important for the potent inhibitory activity of **3d**. 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 O of dihydropyrazole acetyl interacted with the residue Ile 199, which made the 3D structure more stable.

In summary, we prepared a series of novel coumarin derivatives containing 4,5-dihydropyrazole moiety as potential telomerase inhibitors. The result showed that compound **3d** had strongly high activity against human gastric cancer cell SGC-7901. Docking simulation was performed to position compounds **3d** into the telomerase 3DU6 active site, the result shows compound **3d** can bind well with the telomerase active site and act as telomerase inhibitor.

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13. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were collected on PX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ).
14. General synthetic procedure process for compounds **3**: To a solution of α,β unsaturated ketone **2** (10 mmol) in acetic acid (20 mL) was added hydrazine monohydrate (40 mmol) and the reaction mixture was refluxed for 2 h. The mixture was cooled, adjusted pH to 7 with 10% Na_2CO_3 solution, poured into crush ice, and allowed to stand at room temperature over night. The product was collected by filtration and the crude residue was purified by chromatography on SiO_2 (acetone/petroleum, *V*:*V* = 2:1) to give title compounds **3** (Scheme 1) as colorless solids.[13]
Compound **3a**: *3-(1-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one*, colorless crystals, yield, 64%; mp 180–182 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.34 (3H, s, –Me), 3.34 (1H, dd, *J* = 19.0 and 4.7 Hz, pyrazole, 4-H_a), 3.70 (3H, s, –OMe), 3.86 (1H, dd, *J* = 12.0 and 19.0 Hz, pyrazole, 4-H_b), 5.48 (1H, dd, *J* = 11.7 and 4.6 Hz, pyrazole, 5-H), 6.77 (2H, d, *J* = 8.6 Hz, ArH), 7.08 (2H, d, *J* = 8.6 Hz, ArH), 7.20–7.95 (4H, m, C_{5-8}-H), 8.35 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.2, 40.0, 54.9, 56.3, 115.0, 121.9, 123.4, 123.7, 125.0, 127.3, 128.5, 128.8, 134.2, 136.8, 152.1, 158.2, 159.0, 160.5, 169.2; ESI-MS: 362.0 ($C_{21}H_{18}N_2O_4$, $[M+H]^+$); Anal. Calcd for $C_{21}H_{18}N_2O_4$: C, 69.6; H, 5.0; N, 7.7. Found: C, 70.0; H, 4.6; N, 8.0.
Compound **3b**: *3-(1-acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one*, colorless crystals, yield, 61%; mp 167–168 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.44 (3H, s, –Me), 3.45 (1H, dd, *J* = 19.0 and 4.6 Hz, pyrazole, 4-H_a), 3.95 (1H, dd, *J* = 11.9 and 19.0 Hz, pyrazole, 4-H_b), 5.41 (1H, s, –OH), 5.55 (1H, dd, *J* = 11.9 and 4.6 Hz, pyrazole, 5-H), 6.71 (2H, d, *J* = 8.4 Hz, ArH), 6.99 (2H, d, *J* = 8.4 Hz, ArH), 7.27–8.09 (4H, m, C_{5-8}-H), 8.44 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.7, 38.9, 55.4, 114.7, 121.0, 122.9, 124.2, 126.1, 127.7, 128.9, 129.0, 134.1, 137.3, 150.9, 156.1, 158.8, 160.0, 169.4; ESI-MS: 349.1 ($C_{20}H_{16}N_2O_4$, $[M+H]^+$); Anal. Calcd for $C_{20}H_{16}N_2O_4$: C, 69.0; H, 4.6; N, 8.0. Found: C, 68.6; H, 4.9; N, 7.8.
Compound **3c**: *3-(1-acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one*, colorless crystals, yield, 62%; mp 184–186 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.41 (3H, s, –Me), 3.23 (1H, dd, *J* = 19.0 and 4.2 Hz, pyrazole, 4-H_a), 3.98 (1H, dd, *J* = 12.2 and 19.0 Hz, pyrazole, 4-H_b), 5.83 (1H, dd, *J* = 12.0 and 4.9 Hz, pyrazole, 5-H), 6.95–7.55 (8H, m, C_{5-8}-H and ArH), 8.34 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.2, 39.3, 54.7, 122.1, 123.3, 124.5, 126.1, 127.0, 127.2, 128.8, 128.9, 129.1, 129.5, 133.5, 133.9, 144.7, 151.7, 157.8, 160.3, 169.0; ESI-MS: 367.9 ($C_{20}H_{15}ClN_2O_3$, $[M+H]^+$); Anal. Calcd for $C_{20}H_{15}ClN_2O_3$: C, 65.5; H, 4.1; N, 7.6. Found: C, 65.2; H, 3.8; N, 7.5.
Compound **3d**: *3-(1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one*, colorless crystals, yield, 71%; mp 199–200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.36 (3H, s, –Me), 3.34 (1H, dd, *J* = 19.0 and 4.8 Hz, pyrazole, 4-H_a), 3.89 (1H, dd, *J* = 12.0 and 19.0 Hz, pyrazole, 4-H_b), 5.52 (1H, dd, *J* = 12.0 and 4.8 Hz, pyrazole, 5-H), 7.12–7.95 (9H, m, C_{5-8}-H and ArH), 8.37 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.0, 39.8, 58.6, 122.0, 123.1, 124.0, 126.7, 126.9, 127.5, 127.8, 128.9, 129.2, 134.3, 144.4, 151.7, 157.2, 161.7, 169.8; ESI-MS: 333.0 ($C_{20}H_{16}N_2O_3$, $[M+H]^+$); Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.3; H, 4.9; N, 8.4. Found: C, 72.8; H, 5.3; N, 8.0.
Compound **3e**: *3-(1-acetyl-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one*, colorless crystals, yield, 68%; mp 197–199 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.40 (3H, s, –Me), 3.22 (1H, dd, *J* = 19.0 and 5.5 Hz, pyrazole, 4-H_a), 3.97 (1H, dd, *J* = 12.2 and 19.2 Hz, pyrazole, 4-H_b), 5.76 (1H, dd, *J* = 12.1 and 5.5 Hz, pyrazole, 5-H), 6.89–7.94 (7H, m, C_{5-8}-H and ArH), 8.35 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.6, 38.2, 53.1, 122.0, 123.1, 124.5, 126.2, 127.7, 127.8, 128.9, 131.2, 131.5, 133.3, 133.8, 140.0, 142.8, 150.3, 156.7, 160.5, 169.4; ESI-MS: 401.9 ($C_{20}H_{14}Cl_2N_2O_3$, $[M+H]^+$); Anal. calcd for $C_{20}H_{14}Cl_2N_2O_3$: C, 59.9; H, 3.5; N, 7.0. Found: C, 60.3; H, 3.8; N, 6.6.
Compound **3f**: *3-(1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-bromo-2H-chromen-2-one*, colorless crystals, yield, 57%; mp 158–159 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.30 (3H, s, –Me), 3.38 (1H, dd, *J* = 19.0 and 4.5 Hz, pyrazole, 4-H_a), 3.98 (1H, dd, *J* = 11.8 and 19.0 Hz, pyrazole, 4-H_b), 5.29 (1H, dd, *J* = 11.8 and 4.5 Hz, pyrazole, 5-H), 6.99 (H, d, *J* = 8.2 Hz, C_8-H), 7.08–7.36 (6H, m, ArH and C_7-H), 7.67 (1H, s, C_5-H), 8.28 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 25.1, 39.3, 56.2, 121.5, 123.3, 124.0, 125.2, 126.7, 128.0, 129.4, 130.9, 131.4, 134.5, 144.9, 150.3, 156.7, 160.8, 169.9; ESI-MS: 410.8 ($C_{20}H_{15}BrN_2O_3$, $[M+H]^+$); Anal. Calcd for $C_{20}H_{15}BrN_2O_3$: C, 58.4; H, 3.7; N, 6.8. Found: C, 58.6; H, 4.0; N, 6.5.
Compound **3g**: *3-(1-acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-bromo-2H-chromen-2-one*, colorless crystals, yield, 63%; mp 183–185 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (3H, s, –Me), 3.32 (1H, dd, *J* = 19.0 and 4.5 Hz, pyrazole, 4-H_a), 3.85 (1H, dd, *J* = 11.8 and 19.0 Hz, pyrazole, 4-H_b), 5.35 (1H, dd, *J* = 11.8 and 4.5 Hz, pyrazole, 5-H), 5.75 (1H, s, –OH), 6.74 (2H, d, *J* = 8.4 Hz, ArH), 6.98–7.32 (4H, m, ArH and C_7, C_8-H), 7.60 (1H, s, C_5-H), 8.21 (1H, s, C_4-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.5, 40.0, 57.4, 116.2, 120.4, 123.0, 124.2, 124.7, 129.1, 130.8, 131.6, 134.2, 138.5, 150.6, 156.9, 157.1, 160.7, 169.2; ESI-MS: 426.4 ($C_{20}H_{15}BrN_2O_4$, $[M+H]^+$); Anal. Calcd for $C_{20}H_{15}BrN_2O_4$: C, 56.2; H, 3.5; N, 6.6. Found: C, 55.9; H, 3.2; N, 6.5.
15. The cytotoxicity evaluation was conducted by using a modified procedure as described in the literature.[16] Briefly, target tumor cells were grown to log phase in *RPMI 1640* medium supplemented with 10% fetal bovine serum. After diluting to 3×10^4 cells mL^{-1} with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was performed at 37 °C, 5% CO_2 atmosphere for 24 h before subjecting to cytotoxicity assessment. Tested samples at pre-set concentrations were added to 6 wells with 5-fluorouracil co-assayed 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 t* test. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.
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19. Discovery Studio 2.1 (DS 2.1, Accelrys Software Inc., San Diego, California, USA). Crystal structure of telomerase (PDB entry 3DU6) was used as template. Hydrogen atoms were added to protein model. The added hydrogen atoms were minimized to have stable energy conformation and to also relax the conformation from close contacts. The active site was defined and sphere of 5 Å was generated around the active site pocket, with the active site pocket of BSAI model using C-DOCKER, a molecular dynamics (MD) simulated-annealing-based algorithm module from DS 2.1. Random substrate conformations are generated using high-temperature MD. Candidate poses are then created using random rigid-body rotations followed by simulated annealing. The structure of protein, substrate were subjected to energy minimization using CHARMm forcefield as implemented in DS 2.1. A full potential final minimization was then used to refine the substrate poses. Based on C-DOCKER, energy docked conformation of the substrate was retrieved for postdocking analysis.