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Design and synthesis of *N*-phenylacetyl (sulfonyl) 4,5-dihydropyrazole derivatives as potential antitumor agents

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ABSTRACT

A series of novel *N*-phenylacetyl (sulfonyl) 4,5-dihydropyrazole derivatives as potential telomerase inhibitors were synthesized. The bioassay tests show that compound **4a** exhibited high activity against human gastric cancer cell SGC-7901, liver cancer Hep-G2 and human prostate PC-3 cell lines with IC_{50} values of 21.23 ± 0.99, 29.43 ± 0.32 and 30.89 ± 1.07 μM, respectively. All title compounds were assayed for telomerase inhibition by a modified TRAP assay, the results show that compound **4a** can inhibit telomerase with IC_{50} value of 4.0 ± 0.32 μM. Docking simulation was performed to position compound **4a** 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 cancer.[3]

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 cannabinoid receptor 1 antagonist, monoamine oxidase inhibitors, and tumor necrosis inhibitors,[4,5] Many of them are currently being tested and/or clinically evaluated for new drug discovery. Of late, the dihydropyrazole derivatives used as potent and selective inhibitors capable of causing cancer cell death have also been reported.[6–10]

In an effort to synthesize novel 3,4-dihydropyrazole systems with potential anticancer activity, our group has recently reported on the anticancer activity of some acetyl 4,5-dihydropyrazole derivatives,[11] and a novel docking model based on the protein structure of telomerase inhibitors was developed using LigandFit module within Discovery Studio 2.1, we found 4,5-dihydropyrazole ring projects into a hydrophobic region, which is comprised of the side chains of 200–250, that is, important for the potent inhibitory activity. In current molecular design, when acetyl replaced by *N*-phenylacetyl, the 4,5-dihydropyrazole ring projects into a more stable hydrophobic region, which comprised of the side chains of 220–227, should be enhance the anticancer activity. 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 anticancer compounds, we prepared a series novel *N*-phenylacetyl 4,5-dihydropyrazole derivatives and tested their activities against human gastric cancer cell SGC-7901, liver cancer Hep-G2 and human prostate cancer PC-3 cell lines. In order to compare the activity of 4,5-dihydropyrazole moiety with carbonyl or sulfone, some sulfonyl-4,5-dihydropyrazole derivatives were synthesized.

The synthesis of compound **2** (Scheme 1) started from substituted-salicylaldehyde and catalyzed by NaOH at 20 °C was added

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, enzymelinked immunosorbentassay; TRAP, telomere repeat amplification protocol; DMAP, 4-dimethylaminopyridine.

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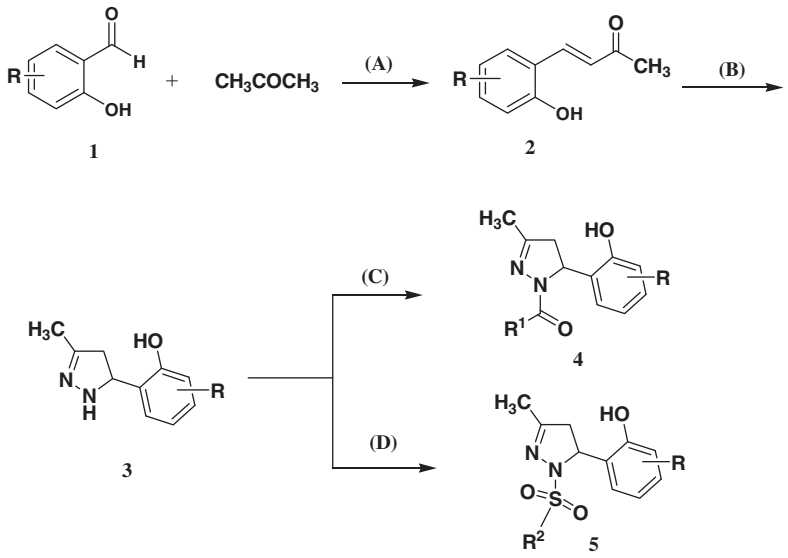

Scheme 1. Synthesis of title compounds. Reagent and conditions: (**A**) CH_3COCH_3, NaOH, C_2H_5OH, 20 °C, 15 h. (**B**) $N_2H_4{\cdot}H_2O$, 98% CH_3CH_2OH, reflux, 2 h. (**C**) carboxylic acid, tosyl chloride, DMAP, reflux, 3 h. (**D**) substituted-benzenesulfonyl chloride, $CHCl_3$, reflux. **R** = H, $\mathbf{R^1}$ = phenyl (**4a**); **R** = H, $\mathbf{R^1}$ = 4-methylphenyl (**4b**); **R** = H, $\mathbf{R^1}$ = 2-methylphenyl (**4c**); **R** = H, $\mathbf{R^1}$ = 4-nitrophenylethyl (**4d**); **R** = H, $\mathbf{R^1}$ = chloromethyl (**4e**); **R** = H, $\mathbf{R^1}$ = 2-chlorophenyl) (**4f**); **R** = 3-methyl, $\mathbf{R^1}$ = phenyl (**4g**); **R** = H, $\mathbf{R^2}$ = phenyl (**5h**); **R** = H, $\mathbf{R^2}$ = *p*-methylphenyl (**5i**); **R** = H, $\mathbf{R^2}$ = 4-nitrophenylethyl (**5j**).

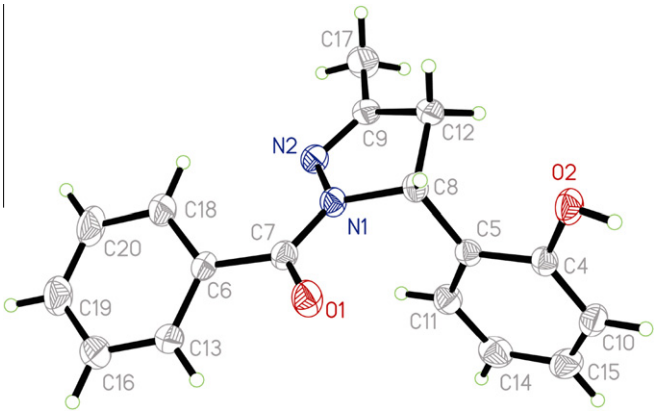

Figure 1. ORTEP drawing of **4a**.

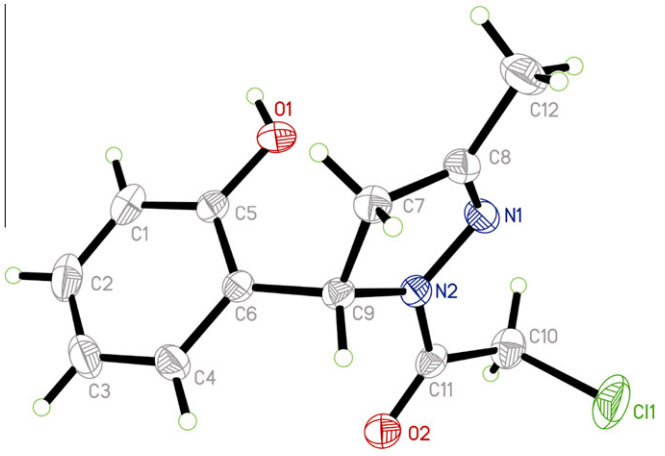

Figure 2. ORTEP drawing of **4e**.

acetone. Compound **3** was prepared according to a previously published report.[9] Using catalysts tosyl chloride and DMAP, proved to be an efficient alternative for the synthesis of title compounds **4**. The general synthetic procedure process and spectral data of compounds **4 and 5** can be found in the Supplementary data.[12]

In order to facilitate molecular docking, we trained a single-crystal of compounds **4a**, **4e** and the single-crystal structure of compounds **4a** and **4e** were determined by X-ray crystallography. Compound **4a**: Colorless crystals, yield, 70%; mp 202–203 °C; crystal data: $C_{17}H_{16}N_2O_2$, M = 280.3, monoclinic, space group $P2(1)/c$; a = 8.666(3), b = 16.043(5), c = 10.585(4) (Å); α = 90, β = 99.551, γ = 90(°), V = 1451.2(8) nm^3, T = 296(2) K, Z = 4, Dc = 1.283 g/cm^3,

Table 1
Cytotoxic activity of the synthesized compounds against SGC-7901, Hep-G2 and PC-3 cell lines[a]

	IC_{50} (μM)[b]				IC_{50} (μM)[b]		
Compound	SGC-7901	Hep-G2	PC-3	Compound	SGC-7901	Hep-G2	PC-3
4a	21.23 ± 0.99	29.43 ± 0.32	30.89 ± 1.07	**4f**	103.02 ± 2.16	95.55 ± 2.86	84.21 ± 2.35
4b	51.48 ± 0.68	78.87 ± 0.92	115.53 ± 0.85	**4g**	149.81 ± 2.96	177.40 ± 1.87	154.29 ± 2.07
4c	103.77 ± 1.49	95.17 ± 1.29	106.32 ± 3.06	**5h**	66.40 ± 2.84	57.24 ± 2.05	50.60 ± 2.43
4d	97.26 ± 0.85	107.28 ± 2.51	132.15 ± 1.74	**5i**	73.64 ± 0.67	66.77 ± 1.76	85.47 ± 2.03
4e	26.83 ± 0.79	47.92 ± 3.60	20.10 ± 1.27	**5j**	52.82 ± 1.30	83.04 ± 1.66	38.77 ± 0.83
5-Fluorouracil[c]	31.46 ± 4.38	18.85 ± 0.85	15.69 ± 1.92	5-Fluorouracil[c]	31.46 ± 4.38	18.85 ± 0.85	15.69 ± 1.92

[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.

Table 2
Inhibitory effects of the title compounds against telomerase

Compound	IC_{50} (μM) *Taq* polymerase	IC_{50} (μM) telomerase	Selectivity index[a] (SI)
4a	8.0 ± 2.0	4.0 ± 0.32	2
4b	no	31.5 ± 1.1	—
4c	50.5 ± 1.80	24.5 ± 0.87	2.1
4d	no	no	—
4e	20.4 ± 2.0	9.5 ± 0.46	2.1
4f	36.0 ± 2.2	no	—
4g	no	no	—
5h	31.0 ± 2.0	17.3 ± 0.21	1.8
5i	30.0 ± 1.0	16.8 ± 0.38	1.9
5j	23.5 ± 0.29	12.5 ± 0.25	1.9
Ethidium bromide[b]		2.6 ± 0.7	

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.

$F(0\,0\,0) = 592$. Reflections collected/unique: 6878/2818, Fine, $R_1 = 0.0450$, $wR(F^2) = 0.1070$. Compound **4e**: Colorless crystals, yield, 91%; mp 174–175 °C; crystal data: $C_{12}H_{13}ClN_2O_2$, $M = 252.7$, monoclinic, space group $P2(1)/c$; $a = 9.646(3)$, $b = 9.558(3)$, $c = 13.364(4)$ (Å); $\alpha = 90$, $\beta = 90.536(4)$, $\gamma = 90(^\circ)$, $V = 1232.1(7)$ nm^3, $T = 296(2)$ K, $Z = 4$, $Dc = 1.362$ g/cm^3, $F(0\,0\,0) = 528$. Reflections collected/unique: 5246/2374, Fine, $R_1 = 0.0427$, $wR(F^2) = 0.1045$.

The molecular structures of compounds **4a** and **4e** are shown in Figures 1 and 2. Crystallographic data (excluding structure factors) for the structures have been deposited with the Cambridge Crystallographic Data Center as Supplementary publication No. CCDC-808895 and CCDC-808896.[14]

In the screening assay studies, compounds **4** and **5** were evaluated for their cytotoxic activity against SGC-7901, Hep-G2 and PC-3 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 **4a** exhibited high activity against the human gastric cell SGC-7901 with the IC_{50} value of 21.23 ± 0.99 μM, comparable to the positive control 5-fluorouracil. Also compound **4a** can inhibit telomerase with IC_{50} value of 4.0 ± 0.32 μM.

From the data presented in Table 1, it can be concluded that phenylsulfonyl-4,5-dihydropyrazole derivatives showed moderate inhibition against SGC-7901, Hep-G2 and PC-3 cell lines, 2-hydroxy-substitutedphenyl-4,5-dihydropyrazole (**4g**) displayed poor activity against above cell lines, also poor activity against telomerase, whereas 2-hydroxy-phenyl-4,5-dihydropyrazole derivatives,

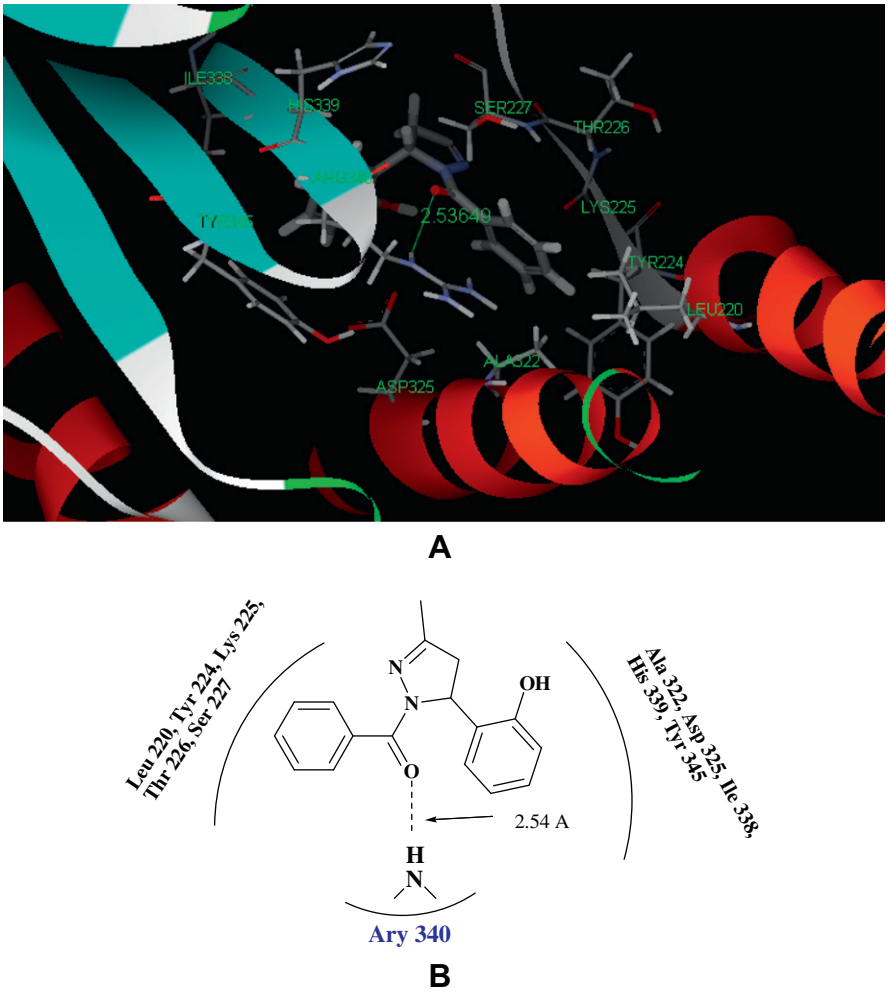

Figure 3. Molecular docking modeling of compound **4a** (A) with telomerase; the small molecule and the critical interaction of 3DU6 are represented by sticks. Panel is a view into the active site cavity. (B) Schematic representation of the binding mode of **4a** in the ATP binding site of 3DU6.

in general, showed relatively higher activity against the above cells, so, some compounds in this series deserve further investigation.

All purified title compounds were assayed for telomerase inhibition by a modified telomere repeat amplification protocol (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] Telomerase activity was detected by a modified version of the TRAP protocol. Telomerase products were resolved by 10% polyacrylamide gel electrophoresis and visualized by staining with SYBER Green. As a source of telomerase, the total cell lysates derived from SGC-7901 cell line was used. Protein concentration of the lysates was assayed using Bio-Rad protein assay kit using BSA standards.

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 compound **4a** showed strong telomerase inhibitory ability with IC_{50} value of 4.0 ± 0.32 μM, which comparable to 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 **4a** into ATP binding site of telomerase was performed to simulate a binding model derived from telomerase structure (PDB: 3DU6).[19] See Figure 3. Visual inspection of the pose of **4a** into the ATP-site revealed that optimal intramolecular hydrogen bond is observed (N–H···O: 2.54 Å, with amino hydrogen group of Ary 340). Also the **4,5**-dihydropyrazole ring projects into a hydrophobic region, which is comprised of the side chains of Ala 220, Tyr 224, Lys 225, Thr 226, Ser 227, that is, important for the potent inhibitory activity of **4a**. 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 2-hydroxyphenyl interacted with the residue His 339, which made the 3D structure more stable.

In summary, we prepared a series of novel *N*-phenylacetyl 4,5-dihydropyrazole derivatives as potential telomerase inhibitors. The result showed that compound **4a** had high activity against SGC-7901, Hep-G2 and PC-3 cell lines. Compounds **5h–5j** showed moderate inhibition against above cell lines. Docking simulation result shows compound **4a** can bind well with the telomerase active site and act as potential telomerase inhibitor.

Supplementary data: CCDC-808895 and CCDC-808896 contain 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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12. *General synthetic procedure process for compounds* **4**: To a chloroform (20 ml) solution of carboxylic acid (12 mmol), tosyl chloride (12 mmol) and DMAP (15 mmol) was added 2-(3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol **3** (10 mmol), then the reaction mixture was refluxed for 3 h. The mixture was cooled, washed with water, and allowed to stand at 0 °C over night. The product was collected by filtration and the crude residue was purified by chromatography on SiO_2 (acetone/petroleum, v:v = 3:1) to give title compounds **4** (Scheme 1) as colorless solids.[13]
Compound ***4a***. *(5-(2-Hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl) (phenyl)methanone*: colorless crystals, yield, 60%; mp 202–204 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.20 (3H, s, –Me), 3.09 (1H, dd, *J* = 18.6 and 3.9 Hz, pyrazole, 4-H_a), 3.40 (1H, dd, *J* = 11.4 and 18.6 Hz, pyrazole, 4-H_b), 5.91 (1H, dd, *J* = 11.4 and 3.9 Hz, pyrazole, 5-H), 6.89–7.90 (9H, m, ArH), 9.31 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.7, 44.1, 55.0, 118.1, 122.4, 127.2, 129.1, 129.6, 130.7, 132.2, 132.9, 135.1, 144.2, 150.9, 156.8; ESI-MS: 280.9 ($C_{17}H_{16}N_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 73.08; H, 6.00; N, 9.62.
Compound ***4b***. *(5-(2-Hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl) (p-tolyl)methanone*: colorless crystals, yield, 54%; mp 200–201 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.18 (3H, s, –Me), 2.39 (3H, s, –Me), 3.12 (1H, dd, *J* = 18.6 and 3.9 Hz, pyrazole, 4-H_a), 3.45 (1H, dd, *J* = 11.4 and 18.6 Hz, pyrazole, 4-H_b), 5.86 (1H, dd, *J* = 11.4 and 3.9 Hz, pyrazole, 5-H), 6.82–7.96 (8H, m, ArH), 9.27 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.9, 25.5, 43.8, 54.7, 117.7, 122.8, 128.4, 129.7, 130.0, 131.8, 132.4, 133.9, 141.5, 143.9, 152.7, 156.0; ESI-MS: 293.8 ($C_{18}H_{18}N_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.17; H, 6.32; N, 9.88.
Compound ***4c***. *(5-(2-Hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl) (o-tolyl)methanone:* colorless crystals, yield, 56%; mp 198–199 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.10 (3H, s, –Me), 2.42 (3H, s, –Me), 3.06 (1H, dd, *J* = 18.5 and 3.9 Hz, pyrazole, 4-H_a), 3.40 (1H, dd, *J* = 11.4 and 18.5 Hz, pyrazole, 4-H_b), 5.95 (1H, dd, *J* = 11.4 and 3.9 Hz, pyrazole, 5-H), 6.89–8.04 (8H, m, ArH), 9.15 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2, 18.9, 44.2, 55.1, 117.5, 121.9, 126.3, 127.2, 129.0, 129.5, 130.5, 131.6, 132.1, 136.7, 137.0, 144.1, 152.4, 157.8; ESI-MS: 294.5 ($C_{18}H_{18}N_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.61; H, 6.04; N, 9.27.
Compound ***4d***. *1-(5-(2-Hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl)-2-(4-nitrophenyl)ethanone*: colorless crystals, yield, 72%; mp 210–212 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.17 (3H, s, –Me), 3.05 (1H, dd, *J* = 18.6 and 3.9 Hz, pyrazole, 4-H_a), 3.27 (1H, dd, *J* = 11.4 and 18.6 Hz, pyrazole, 4-H_b), 3.51 (2H, s, –CH_2–), 5.84 (1H, dd, *J* = 11.4 and 3.9 Hz, pyrazole, 5-H), 6.85–8.18 (8H, m, ArH), 9.26 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2, 40.8, 43.7, 54.5, 117.1, 122.4, 123.0, 129.1, 129.6, 130.2, 130.8, 143.1, 148.6, 149.1, 154.0, 158.9; ESI-MS: 340.2 ($C_{18}H_{17}N_3O_4$, $[M+H]^+$); Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.45; H, 4.91; N, 12.57.
Compound ***4e***. *2-Chloro-1-(5-(2-hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl)ethanone*: colorless crystals, yield, 91%; mp 174–175 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (3H, s, –Me), 3.05 (1H, dd, *J* = 18.6 and 3.6 Hz, pyrazole, 4-H_a), 3.39 (1H, dd, *J* = 11.1 and 18.6 Hz, pyrazole, 4-H_b), 4.40 (2H, s, –CH_2–), 5.71 (1H, dd, *J* = 11.1 and 3.6 Hz, pyrazole, 5-H), 6.86–7.26 (4H, m, ArH), 8.53 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.8, 43.2, 44.5, 54.3, 116.8, 122.3, 128.6, 129.0, 130.5, 144.0, 152.0, 158.9; ESI-MS: 253.3 ($C_{12}H_{13}ClN_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{12}H_{13}ClN_2O_2$: C, 57.04; H, 5.19; N, 11.09. Found: C, 57.33; H, 5.42; N, 11.34.
Compound ***4f***. *(2-Chlorophenyl) (5-(2-hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl)methanone*: colorless crystals, yield, 62%; mp 207–209 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.10 (3H, s, –Me), 3.08 (1H, dd, *J* = 18.6 and 3.3 Hz, pyrazole, 4-H_a), 3.45 (1H, dd, *J* = 11.1 and 18.6 Hz, pyrazole, 4-H_b), 5.90 (1H, dd, *J* = 11.1 and 3.3 Hz, pyrazole, 5-H), 6.74–7.40 (8H, m, ArH), 8.97 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2, 44.2, 54.0, 117.0, 121.2, 126.0, 126.6, 128.7, 129.6, 129.9, 130.7, 132.3, 132.4, 133.4, 155.0, 160.3, 165.9; ESI-MS: 316.1 ($C_{17}H_{15}ClN_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{17}H_{15}ClN_2O_2$: C, 64.87; H, 4.80; N, 8.90. Found: C, 65.06; H, 4.92; N, 9.11.
Compound ***4g***. *(5-(2-Hydroxy-3-methylphenyl)-3-methyl-4,5-dihydropyrazol-1-yl) (phenyl)methanone*: colorless crystals, yield, 54%; mp 195–196 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.09 (3H, s, –Me), 2.32 (3H, s, –Me), 3.17 (1H, dd, *J* = 18.6 and 3.6 Hz, pyrazole, 4-H_a), 3.37 (1H, dd, *J* = 11.4 and 18.6 Hz, pyrazole, 4-H_b), 5.95 (1H, dd, *J* = 11.4 and 3.6 Hz, pyrazole, 5-H), 6.77–7.99 (8H, m, ArH), 9.04 (1H, br s, –OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.9, 16.2, 43.3, 55.1, 121.7, 125.8, 126.3, 127.8, 128.9, 129.3, 130.7, 132.9, 134.5, 143.6, 152.2, 156.8; ESI-MS: 293.2 ($C_{18}H_{18}N_2O_2$, $[M+H]^+$); Anal. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.66; H, 6.08; N, 9.35.
General synthetic procedure process for compounds **5**: To a chloroform (20 ml)

solution of 2-(3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol **3** (10 mmol) was added tosyl chloride (10 mmol) and DMAP (12 mmol), the reaction mixture was refluxed for 1 h. The mixture was cooled, washed with water, and allowed to stand at 0–5 °C 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 **5** (Scheme 1) as colorless solids.[13]

Compound ***5h****. 2-(3-Methyl-1-(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol*: colorless crystals, yield, 78%; mp 218–219 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.02 (3H, s, –Me), 2.89 (1H, dd, J = 4 and 12 Hz, pyrazole, 4-H_a), 3.08 (1H, dd, J = 12 and 18 Hz, pyrazole, 4-H_b), 4.92 (1H, dd, J = 4 and 12 Hz, pyrazole, 5-H), 5.94 (1H, s, –OH), 6.82–7.81 (9H, m, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.4, 45.4, 60.5, 117.1, 122.0, 126.8, 128.0, 129.8, 130.5, 131.7, 138.9, 144.0, 152.9, 161.4; ESI-MS: 317.1 ($C_{16}H_{16}N_2O_3S$, $[M+H]^+$); Anal. Calcd for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10; N, 8.85. Found: C, 61.03; H, 5.29; N, 9.04.

Compound ***5i****. 2-(3-Methyl-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl)phenol*: colorless crystals, yield, 82%; mp 221–222 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.00 (3H, s, –Me), 2.82 (1H, dd, J = 18 and 3.6 Hz, pyrazole, 4-H_a), 2.44 (3H, s, –Me), 3.03 (1H, dd, J = 12 and 18 Hz, pyrazole, 4-H_b), 4.89 (1H, dd, J = 12 and 3.6 Hz, pyrazole, 5-H), 5.99 (1H, s, –OH), 6.85–7.78 (8H, m, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2, 21.8, 45.9, 61.2, 117.7, 121.1, 126.1, 128.3, 129.4, 129.6, 131.6, 138.1, 144.7, 153.6, 161.0; ESI-MS: 331.0 ($C_{17}H_{18}N_2O_3S$, $[M+H]^+$); Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48. Found: C, 61.51; H, 5.68; N, 8.60.

Compound ***5j****. 2-(3-Methyl-1-(4-nitrophenylsulfonyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol*: colorless crystals, yield, 87%; mp 232–233 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.02 (3H, s, –Me), 2.81 (1H, dd, J = 3.6 and 12 Hz, pyrazole, 4-H_a), 3.16 (1H, dd, J = 10.5 and 17.7 Hz, pyrazole, 4-H_b), 4.89 (1H, dd, J = 3.6 and 10.5 Hz, pyrazole, 5-H), 6.74–8.36 (9H, m, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.7, 44.9, 60.7, 116.5, 121.0, 121.6, 128.7, 128.9, 129.2, 131.3, 145.2, 148.7, 152.5, 156.7; ESI-MS: 362.0 ($C_{16}H_{15}N_3O_5S$, $[M+H]^+$); Anal. Calcd for $C_{16}H_{15}N_3O_5S$: C, 53.18; H, 4.18; N, 11.63. Found: C, 53.42; H, 4.01; N, 12.00.

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). Elemental analysis was performed by a Vario-III CHN analyzer and was within ±0.4% of the theoretical values.
14. *Crystal structure determination*
A sample of size 0.26 × 0.23 × 0.15 mm^3 was selected for the crystallographic study. The diffraction measurement was performed at room temperature (296 K) using graphite monochromated MoKα radiation (λ = 0.71073 Å) and an Enraf-Nonius CAD-4 four-circle diffractometer. The systematic absences and intensity symmetries indicated the Monoclinic Cc space group. The corrections for *LP* factors were applied. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 with anisotropic thermal parameters for all non-hydrogen atoms. The calculations were performed with SHELXL-97 program.
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 six 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 4 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.