Novel 3-(1-acetyl-5-(substituted-phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one Derivatives: Synthesis and Anticancer Activity

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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 showed that compound **3b** exhibited potentially high activity against human gastric cancer cell SGC-7901 with IC_{50} value was 2.98±0.16. Docking simulation was performed to position compound **3b** into the telomerase (3DU6) active site to determine the probable binding model. The result shows that some coumarin containing **4,5**-dihydropyrazole moiety can combine well with the telomerase active site and may have use as potential telomerase inhibitors.

Keywords: Coumarin, Dihydropyrazole, Molecular Docking, Antitumor Agent.

INTRODUCTION

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 ageing 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 and human liver cancer [3].

Coumarins are present in natural and synthetic compounds possessing biological activity. 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]. Based on above reports, we considered the possibility of introducing heterocyclic 4,5-dihydropyrazole moiety into the parent coumarin 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 novel coumarin derivatives containing 4,5-dihydropyrazole moiety and tested their activities against human gastric cancer cell SGC-7901 and human liver cancer cell Hep-G2. 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.

METHODS

1. Instruments

The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co.,China) and are not corrected. The ^1H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA300 (300 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Column chromatographic operations were performed on silica gel GF254. The reagents were all of analytical reagent-grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use.

2. Syntheses

The synthesis of compound **1** (Scheme **1**) started from 4-fluoro-2-hydroxybenzaldehyde and catalyzed by piperazine at 25 ^oC was added methyl-acetoacetate. Claisen-Schmidt condensation 3-acetyl-7-fluoro-2H-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**. To a

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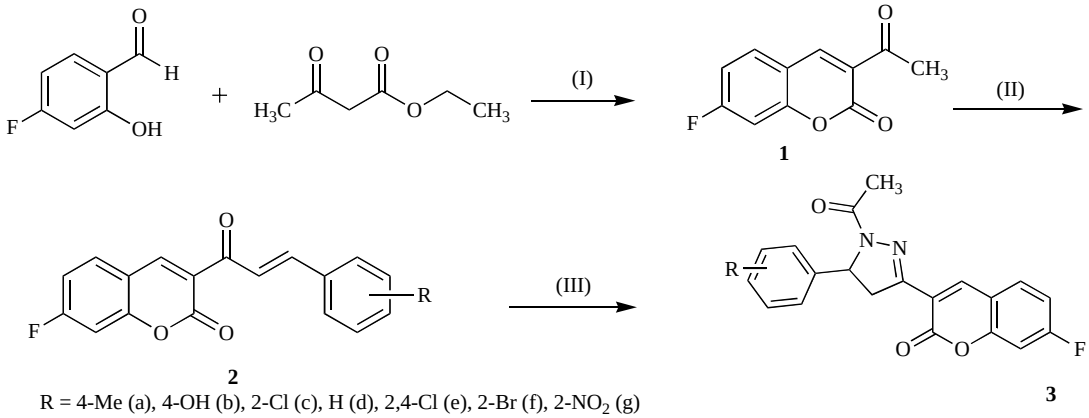

Scheme 1. Synthesis of title compounds. Reagent and conditions: (I) piperzine, 25 °C, 1 h. (II) substituted benzaldehyde, piperidine, ethanol, reflux, 8 h. (III) 60% NH_2-NH_2.H_2O, 98% CH_3COOH, reflux, 3 h.

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 3 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=3:1) to give title compounds **3** (Scheme **1**) as colorless solids.

3a: 3-(1-acetyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 70%, m.p., 186-187 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.27 (3H, s, -Me), 2.47 (3H, s, -Me), 3.39 (1H, dd, *J* 18.0 and 4.0 Hz, pyrazole, 4-H_a), 3.82 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.45 (1H, dd, *J* 12.0 and 4.1 Hz, pyrazole, 5-H), 6.77 (1H, s, C_8-H), 6.82 (1H, d, *J* 8.4 Hz, C_6-H), 7.10-7.15 (4H, m, ArH), 7.37 (1H, d, *J* 8.4 Hz C_5-H), 8.32 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.2, 25.1, 40.3, 56.2, 110.4, 111.7, 118.3, 124.0, 127.4, 128.5, 128.9, 134.2, 138.0, 142.1, 152.5, 157.2, 161.6, 164.3, 169.7; ESI-MS: 365.1 ($C_{21}H_{17}FN_2O_3$, $[M+H]^+$). Anal. calcd for $C_{21}H_{17}FN_2O_3$: C 69.22, H 4.70, N 7.69; found C 68.96, H 4.51, N 8.01.

3b: 3-(1-acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 64%, m.p., 201-202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (3H, s, -Me), 3.30 (1H, dd, *J* 18.0 and 4.1 Hz, pyrazole, 4-H_a), 3.75 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.60 (1H, dd, *J* 12.0 and 4.1 Hz, pyrazole, 5-H), 6.61-7.03 (6H, m, C_6-H, C_8-H, ArH), 7.39 (1H, d, *J* 8.4 Hz C_5-H), 8.20 (1H, s, C_4-H), 8.85 (1H, s, -OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.0, 39.7, 55.5, 109.7, 112.9, 118.0, 119.3, 124.1, 129.0,129.1, 134.5, 137.2, 152.7, 157.3, 158.0, 160.2, 163.3, 169.9; ESI-MS: 367.0 ($C_{20}H_{15}FN_2O_4$, $[M+H]^+$). Anal. calcd for $C_{20}H_{15}FN_2O_4$: C 65.57, H 4.13, N 7.65; found C 65.67, H 3.94, N 7.82.

3c: 3-(1-acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-One

Colorless crystals; yield: 58%, m.p., 190-191 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.30 (3H, s, -Me), 3.37 (1H, dd, *J* 18.0 and 4.1 Hz, pyrazole, 4-H_a), 3.68 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.65 (1H, dd, *J* 12.0 and 4.0 Hz, pyrazole, 5-H), 6.76-7.16 (6H, m, C_6-H, C_8-H, ArH), 7.42 (1H, d, *J* 8.4 Hz C_5-H), 8.28 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 39.2, 56.4, 110.2, 113.3, 118.7, 124.0, 127.5, 128.2, 128.7, 128.9, 129.1, 132.1, 134.3, 144.9, 152.2, 156.8, 160.8, 163.7, 169.9; ESI-MS: 384.1 ($C_{20}H_{14}ClFN_2O_3$, $[M+H]^+$). Anal. calcd for $C_{20}H_{14}ClFN_2O_3$: C 62.43, H 3.67, N 7.28; found C 62.07, H 4.01, N 7.44.

3d: 3-(1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 76%, m.p., 178-179 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.31 (3H, (3H, s, -Me), 3.42 (1H, dd, *J* 18.0 and 4.0 Hz, pyrazole, 4-H_a), 3.76 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.65 (1H, dd, *J* 12.0 and 4.0 Hz, pyrazole, 5-H), 6.70 (1H, s, C_8-H), 6.84 (1H, d, *J* 8.3 Hz, C_6-H), 7.00-7.17 (5H, m, ArH), 7.41 (1H, d, *J* 8.4 Hz C_5-H), 8.18 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 39.8, 57.2, 110.7, 113.4, 119.0, 124.2, 126.6, 127.7, 128.0, 129.1, 134.5, 145.2, 152.3, 156.7, 160.9, 163.8, 170.0; ESI-MS: 351.1 ($C_{20}H_{15}FN_2O_3$, $[M+H]^+$). Anal. calcd for $C_{20}H_{15}FN_2O_3$: C 68.57, H 4.32, N 8.00; found C 68.83, H 4.70, N 8.38.

3e:3-(1-acetyl-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 62%, m.p., 200-202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (3H, (3H, s, -Me), 3.48 (1H, dd, *J* 18.0 and 4.0 Hz, pyrazole, 4-H_a), 3.70 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.58 (1H, dd, *J* 12.0 and 4.0 Hz, pyrazole, 5-H), 6.79 (1H, s, C_8-H), 6.89 (1H, d, *J* 8.4 Hz, C_6-H), 6.96-7.25 (3H, m, ArH), 7.49 (1H, d, *J* 8.4 Hz C_5-H), 8.29 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 39.1, 55.2, 110.3, 112.7, 118.8, 124.4, 127.1, 129.0, 130.3, 131.5, 134.4, 134.6, 134.8, 142.5, 152.7, 157.0, 161.2, 163.0, 169.9; ESI-MS: 420.2 ($C_{20}H_{13}Cl_2FN_2O_3$, $[M+H]^+$). Anal. calcd for $C_{20}H_{13}Cl_2FN_2O_3$: C 57.30, H 3.13, N 6.68; found C 56.91, H 2.84, N 6.92.

3f: 3-(1-acetyl-5-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 55%, m.p., 196-197 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (3H, s, -Me), 3.31 (1H, dd, *J* 18.0 and 4.1 Hz, pyrazole, 4-H_a), 3.71 (1H, dd, *J* 12.0 and

Table 1. Cytotoxic Activity of the Synthesized Compounds Against SGC-7901 and Hep-G2 Cell Lines[a]

Compound	IC_{50} (μg/mL)[b]		Compound	IC_{50} (μg/mL)[b]	
	SGC-7901	Hep-G2		SGC-7901	Hep-G2
3a	35.74±2.14	47.12±1.18	**3e**	22.61±0.68	40.03±1.40
3b	2.98±0.16	48.20±0.99	**3f**	23.95±0.29	49.70±0.88
3c	28.93±0.11	40.02±2.00	**3g**	38.74±1.27	39.94±1.00
3d	8.51±0.70	39.73±1.01	5-Fluorouracil[c]	7.30±0.67	2.58±0.29
5-Fluorouracil[c]	7.30±0.67	2.58±0.29			

[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.
Negative control DMSO, no activity.

18.0 Hz, pyrazole, 4-H_b), 5.70 (1H, dd, *J* 12.0 and 4.0 Hz, pyrazole, 5-H), 6.70 (1H, s, C_8-H), 6.81 (1H, d, *J* 8.4 Hz, C_6-H), 6.99-7.44 (5H, m, C_5-H, ArH), 8.11 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.7, 38.7, 53.2, 110.7, 112.9, 118.4, 122.6, 124.0, 127.6, 128.9, 129.9, 130.2, 132.3, 134.5, 147.0, 152.7, 156.8, 160.8, 162.9, 169.4; ESI-MS: 428.2 ($C_{20}H_{14}BrFN_2O_3$, $[M+H]^+$). Anal. calcd for $C_{20}H_{14}BrFN_2O_3$: C 55.96, H 3.29, N 6.53; found C 56.21, H 3.08, N 6.81.

3g: 3-(1-acetyl-5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-fluoro-2H-chromen-2-one

Colorless crystals; yield: 60%, m.p., 214-216 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.32 (3H, s, -Me), 3.38 (1H, dd, *J* 18.0 and 4.1 Hz, pyrazole, 4-H_a), 3.65 (1H, dd, *J* 12.0 and 18.0 Hz, pyrazole, 4-H_b), 5.58 (1H, dd, *J* 12.0 and 4.0 Hz, pyrazole, 5-H), 6.79 (1H, s, C_8-H), 6.88 (1H, d, *J* 8.4 Hz, C_6-H), 7.33-8.08 (5H, m, C_5-H, ArH), 8.23 (1H, s, C_4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.6, 39.1, 52.2, 110.8, 113.0, 118.6, 121.5, 124.2, 127.8, 128.0, 129.3, 134.5, 135.6, 138.9, 148.7, 152.5, 156.9, 159.4, 163.4, 169.0; ESI-MS: 396.7 ($C_{20}H_{14}FN_3O_5$, $[M+H]^+$). Anal. calcd for $C_{20}H_{14}FN_3O_5$: C 60.76, H 3.57, N 10.63; found C 60.82, H 3.89, N 11.00.

3. Cytotoxic Assay

The cytotoxicity evaluation was conducted by using a modified procedure. 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 (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added to each well. After 4h, 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. The results are listed in the Table **1**.

RESULTS AND DISCUSSION

1. Biological Activity

In the screening assay studies, all the compounds **3a-g** were evaluated for their cytotoxic activity against gastric SGC-7901 and human liver cancer Hep-G2 cell lines. Also included was the activities of reference compound 5-Fluorouracil. 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**). From the data presented in Table **1**, it can be concluded that title compounds displayed certain activity against human gastric cell SGC-7901, whereas showed relatively poor activity against the human liver cancer Hep-G2 cell. It is obvious from the data that compound **3b** exhibited potentially high activity against the human gastric cell SGC-7901 with IC_{50} value of 2.98±0.16 μg/mL, exceed to that of positive control 5-Fluorouracil, compound **3d** showed anticancer activity against the human gastric cell SGC-7901 with IC_{50} value of 8.51±0.70 μg/mL, comparable to that of positive control 5-Fluorouracil.

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 inhibitor **3b** into binding site of telomerase was performed to simulate a binding model derived from telomerase structure (3DU6. pdb). The binding model of compounds **3b** with telomerase is depicted in Fig. (**1**).

CONCLUSIONS

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

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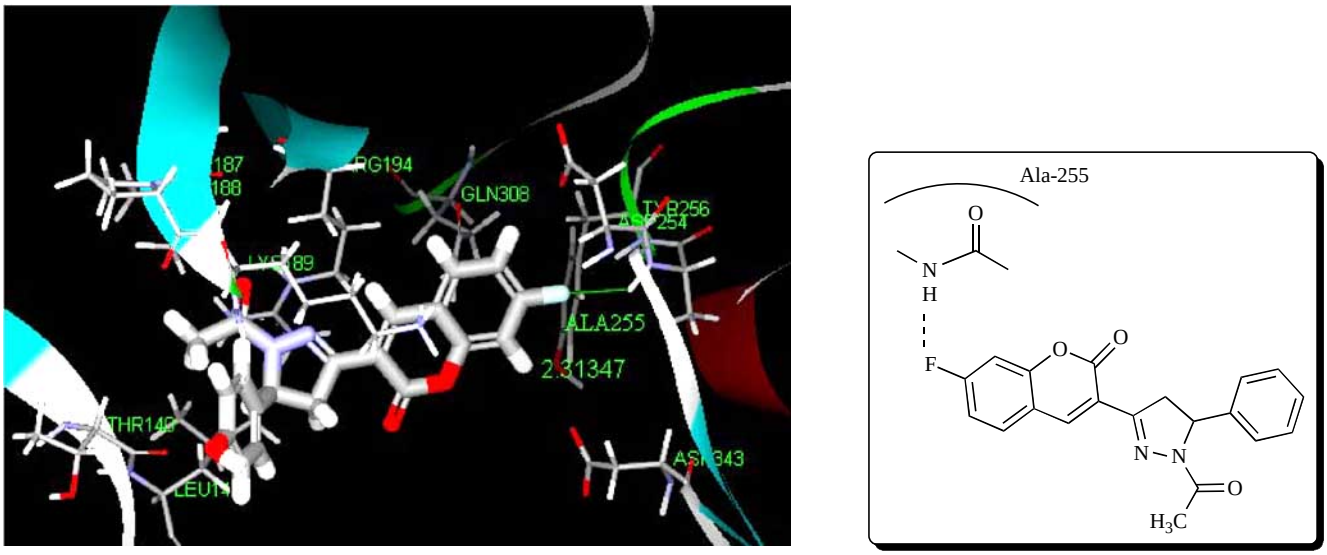

(**A**) (**B**)

Fig. (1). Molecular docking modeling of compound **3b** (**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. Compound **3b** resides in a novel location, binds in a distinct manner among the residues (140–343). They can bind well with the active site. An intramolecular hydrogen bond is observed between the N–H⋯F: 2.31347 Å, with amino hydrogen group of Ala-255. (**B**) Schematic representation of the binding mode of **3b** in the ATP binding site of 3DU6.

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