Bioorganic & Medicinal Chemistry Letters 20 (2010) 4163–4167

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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ARTICLE INFO

Article history:
Received 28 February 2010
Revised 16 April 2010
Accepted 14 May 2010
Available online 24 May 2010

Keywords:
Synthesis
2-Chloro-pyridine
Flavone
Molecular docking
Antitumor agent

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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doi:10.1016/j.bmcl.2010.05.080

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.

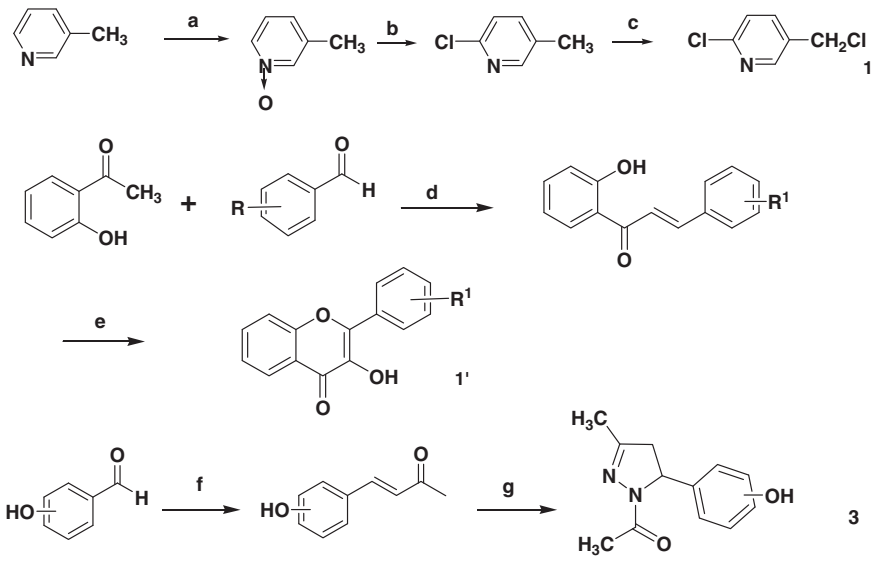
The single-crystal structure of compound **4b** was determined by X-ray crystallography. Colorless crystals, yield, 82%; mp 164–165 °C; crystal data of **4b**: $C_{18}H_{18}ClN_3O_2$, M = 343.8, monoclinic, space group $P2(1)/c$; a = 12.2753(12), b = 8.0425(8), c = 18.1766(19) (Å); α = 90, β = 109.74, 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.