Full Paper

Synthesis and Anti-Tumor Activities of a Novel Series of Tricyclic 1-Anilino-5*H*-pyridazino[4,5-*b*]indoles

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A novel series of 1-anilino-5*H*-pyridazino[4,5-*b*]indoles was designed and synthesized in order to find novel potent anti-tumor compounds. Their structures were confirmed by MS, ^{1}H-NMR, and elemental analysis. All compounds were screened for their cytotoxic activity against two human cancer cell lines (Bel-7420, HT-1080). The compounds **8**, **9**, and **17** showed 50% growth inhibitory activity in low micromolar concentration (IC_{50} = 7.7~12.8 μM). Among them, compound **17** displayed the most potent anti-tumor activity with IC_{50} values of 8.2 μM and 7.9 μM against Bel-7402 and HT-1080, respectively.

Keywords: Anilino-5*H*-pyridazino[4,5-*b*]indoles / Anti-tumor activities / Synthesis

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Introduction

Cancer is an important health problem that claims the lives of more than seven million people worldwide every year. Though a variety of approaches have been taken to cancer chemotherapy, and many anti-cancer drugs have been developed for clinical use, cancer still remains one of the leading causes of human mortality. It is urgently needed to develop more effective and reliable anti-cancer agents which can specifically block some targets in tumor cells. In recent years, a large number of heterocyclic compounds with targeted strategies were reported as potential anti-cancer drugs, which included 4-anilinoquinazolines, 4-anilino-3-quinolinecarbonitriles, pyrrolopyrimidines, 4-anilinopyrido[*d*]pyrimidines, imidazo[4,5-*g*]quinazolines, pyrazoloquinazolines, and pyrroloquinazolines, *etc* [1 – 5]. Among them, the 4-anilinoquinazolines targeting the epidermal growth factor receptor have shown great progress. Two of them, Iressa® (gefitinib) and Tarceva® (erlotinib) were approved by the FDA for use in patients with non-small cell lung cancer [6].

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Structure-activity relationship for 4-anilinoquinazoline derivatives demonstrated that cationic side chains in 7-position of quinazoline would produce an increase in potency of inhibition of EGFR (epidermal growth factor receptor) and a remarkable improvement in aqueous solubility [7]. Recently, it has been reported that tricyclic 4-anilinopyrimido[4,5-*b*]indoles (Fig. 1), which was developed from 4-anilinoquinazolines, have potential for cancer chemotherapy [8]. In view of these observations, we designed a novel series of 1-anilino-5*H*-pyridazino[4,5-*b*]indoles. Different substituted aminopropoxy moieties were introduced into the 8-position, and various anilines were introduced into the 1-position to investigate their influence on anti-tumor activity.

In this paper, we would like to report the synthesis and anti-tumor activities of a novel series of tricyclic 1-anilino-5*H*-pyridazino[4,5-*b*]indole derivatives represented by the general formula of **8 – 19** in Fig. 1.

Results and discussion

The synthetic route of the target compounds is shown in Scheme 1. The starting material ethyl 5-acetoxy-6-bromo-2-bromomethyl-1-cyclopropylindole-3-carboxylate **1** was prepared according to previously reported method [9]. Reaction of **1** with pyridine in trichloromethane afforded

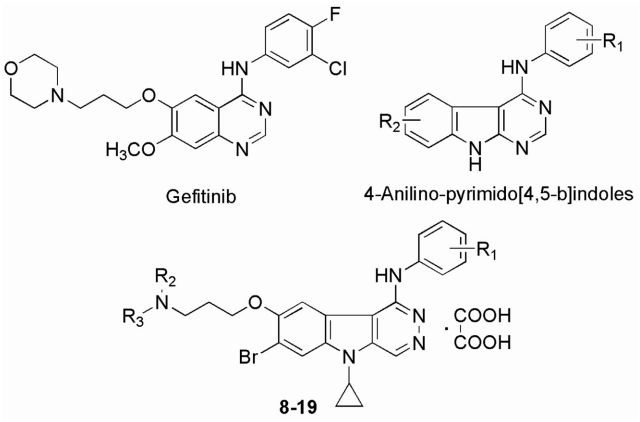
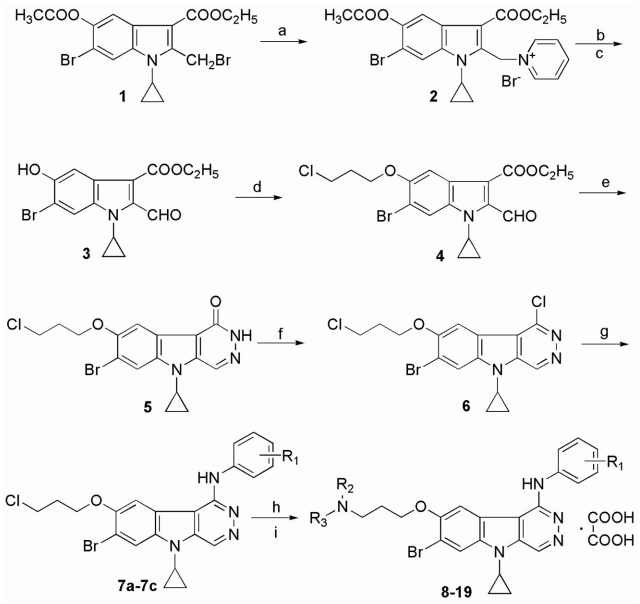


Figure 1. Structures of gefitinib, 4-anilinopyrimido[4.5-*b*]indoles, and compounds **8–19**.



Reagents and conditions: (a) Py, $CHCl_3$, reflux, 5 h; (b) EtOH, p-$Me_2NC_6H_4NO$, aq NaOH, r.t., 24 h; (c) H_2O, H_2SO_4; (d) K_2CO_3, DMF, $BrCH_2CH_2CH_2Cl$, 70°C, 4 h; (e) $NH_2NH_2.H_2O$, HAc, 70°C, 2 h; (f) $POCl_3$, DMF, reflux, 2 h; (g) R_1PhNH_2, *i*-PrOH, HCl (trace), reflux, 5 h; (h) K_2CO_3, DMF, R_2R_3NH, 60°C, 7 h; (i) acetone, oxalic acid, r.t.

Scheme 1. Synthetic route of compounds **8–19**.

pyridinium salt **2**. Compound **2** reacted with freshly prepared *p*-nitrosodimethylaniline hydrochloride in ethanol to yield the nitrone compound, which was hydrolyzed to give the desired key intermediate **3** [10]. The condensation of **3** with 1,3-bromochloropropane led to compound **4**, followed by cyclization with 80% hydrazine hydrate to give a good yield of 5*H*-pyridazino[4,5-*b*]indol-1-one **5**. Treatment of compound **5** with phosphorus oxychloride at reflux produced 1-chloro-5*H*-pyridazino[4,5-*b*]indole **6**. Compound **6** was converted to 1-anilino-5*H*-pyridazino[4,5-*b*]indole derivatives **7a–7c** by coupling with the appropriatly substituted anilines in isopropanol [2], and then reaction of **7a–7c** with the appropriate secondary amines in dimethylformamide afforded the corresponding target compounds **8–19** as oxalate.

Table 1. The substituents and anti-tumor effect of compounds **8–19**.

Compound	IC_{50} (μM)			
	R_1	NR_2R_3	Bel-7402	HT-1080
8	3-chloro-4-fluoro	dimethylamino	9.4	12.8
9	3-chloro-4-fluoro	pyrrolidinyl	7.7	12.3
10	3-chloro-4-fluoro	morpholinyl	157.2	84.2
11	3-chloro-4-fluoro	piperidinyl	13.6	12.1
12	4-trifluoromethyl	dimethylamino	37.7	19.6
13	4-trifluoromethyl	pyrrolidinyl	17.4	12.6
14	4-trifluoromethyl	morpholinyl	–	–
15	4-trifluoromethyl	piperidinyl	304.5	318.2
16	3,4-difluoro	dimethylamino	18.0	9.0
17	3,4-difluoro	pyrrolidinyl	8.2	7.9
18	3,4-difluoro	morpholinyl	35.8	18.8
19	3,4-difluoro	piperidinyl	14.7	11.9
Gefitinib			63.8	53.7

Table 1 compares the anti-tumor activities of compounds **8–19** against cancer cell lines Bel-7402 and HT-1080, as determined by MTT (MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. Of all the tested compounds, compound **17** possessed the most potent anti-tumor activity, with IC_{50} values of 8.2 μM and 7.9 μM against Bel-7402 and HT-1080 cancer cell lines, respectively. The data in Table 1 indicate that substituents on the aniline ring have substantial influence on anti-tumor activity. The 4-trifluoromethylanilino derivatives **12–15** were less effective than the corresponding 3,4-difluoro and 3-chloro-4-fluoro analogues. For example, compound **14** with 4-trifluoromethyl substituent showed no activity at all, while compound **18** with 3,4-difluoro substituent displayed moderate activity (IC_{50} = 35.8 μM, 18.8 μM, respectively). Introduction of the aminopropoxy moieties at C-8 position of the 1-anilino-5*H*-pyridazino[4,5-*b*]indole showed that the secondary amines at 3-position of propoxy produced notable effects. The morpholinylpropoxy led to loss of potency (**10** vs **9**, **14** vs **13**, and **18** vs **17**), while 1-pyrrolidinylpropoxy showed significant improvement in activity (**9**, **13**, and **17**).

Experimental

Chemistry

Melting points were determined with capillary tube method, and the thermometer was uncorrected. Mass spectra were obtained with a Finnigan LCQ HPLC-MS instrument (Thermo

Electron Corporation, Bremen, Germany). ^{1}H-NMR spectra were run on a Bruker ARX-300 instrument (Bruker Bioscience, Billerica, MA, USA), TMS as the internal stantard. Elemental analysis was performed by a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy).

5-Acetoxy-6-bromo1-cyclopropyl-3-ethoxycarbonyl-1H-indole-2-methylpyridinium bromide **2**

A mixture of ethyl 5-acetoxy-6-bromo-2-bromomethyl-1-cyclopropyl-1*H*-indole-3-carboxylate (91.8 g, 0.2 mol) and pyridine (23.8 g, 0.30 mol) in trichloromethane (500 mL) was heated under reflux for 5 h. The reaction mixture was cooled, the formed crystalline product was filtered and washed with trichloromethane to give **2** (101.0 g, 93.9%) as white powder. Mp. 234 – 236°C (dec.); MS [MH$^+$] (m/z): 457.3 (Br = 79), 459.3 (Br = 81).

Ethyl 6-bromo-1-cyclopropyl-2-formyl-5-hydroxy-1H-indole-3-carboxylate **3**

To a suspension of **2** (80.7 g, 0.15 mol) and *p*-nitrosodimethylaniline hydrochloride (278.8 g, 0.15 mol) in ethanol (400 mL), a solution of sodium hydroxide (12 g, 0.30 mol) in water (60 mL) was added dropwise with stirring at room temperature. Then, the resulting mixture was stirred at room temperature for 24 h. The precipitated product was collected by filtration and washed with water. Concentrated sulfuric acid (44.1 g, 0.45 mol) was added dropwise to a stirred suspension of the resulting wet solid and water (100 mL). Crushed ice was added after 20 min, and the crude solid was filtered, washed successively with dilute sodium bicarbonate solution and water, and then dried to yield **3** (42.6 g, 80.7%) as yellow solid. Mp. 240 – 242°C; ^{1}H-NMR (DMSO-d_6) δ: 10.75 (s, 1H), 7.45 (s, 1H), 7.06 (s, 1H), 4.43 (q, 2H), 3.25 (m, 1H), 1.36 (t, 3H), 1.25 (m, 2H), 1.08 (m, 2H).

Ethyl 6-bromo-5-(3-chloropropoxy)-1-cyclopropyl-2-formyl-1H-indole-3-carboxylate **4**

A mixture of **3** (42.3 g, 0.12 mol), potassium carbonate (24.8 g, 0.18 mol) and 1,3-bromochloropropane (28.4 g, 0.18 mol) in *N,N*-dimethylformamide (150 mL) was stirred and heated at 70°C for 4 h. The mixture was cooled and then poured into water, the resulting precipitate was collected and dried to give **4** (46.0 g, 89.5%) as light brown solid. Mp. 137-139°C; ^{1}H-NMR (DMSO-d_6) δ: 10.77 (s, 1H), 7.47 (s, 1H), 7.42 (s, 1H), 4.44 (q, 2H), 4.26 (t, 2H), 3.83 (t, 2H), 3.25 (m, 1H), 2.25 (m, 2H), 1.37 (t, 3H), 1.28 (m, 2H), 1.08 (m, 2H).

7-Bromo-8-(3-chloropropoxy)-5-cyclopropyl-1,2-dihydro-5H-pyridazino[4,5-b]indol-1-one **5**

A suspension of **4** (42.9 g, 0.10 mol) and 80% hydrazine hydrate (7.5 g, 0.12 mol) in glacial acetic acid (100 mL) was stirred and heated at 70°C for 2 h, after cooling to room temperature, the formed precipitate was filtered, washed with ethanol and dried to give **5** (33.8 g, 85.2%) as white powder. mp 221 – 223°C; MS [M$^+$] (m/z): 418.2 (Br = 79), 420.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 10.53 (s, 1H), 9.41 (s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 4.25 (t, 2H), 3.82 (t, 2H), 3.48 (m, 1H), 2.26 (m, 2H), 1.32 (m, 2H), 1.10 (m, 2H).

7-Bromo-1-chloro-8-(3-chloropropoxy)-5-cyclopropyl-5H-pyridazino[4,5-b]indole **6**

A solution of **5** (31.7 g, 0.08 mol) in phosphorus oxychloride (100 mL) containing *N,N*-dimethylformamide (10 drops) was stirred and heated to reflux for 2 h. The solution was evaporated and the residue was triturated with water, and saturated aqueous sodium hydrogen carbonate was added. The precipitate was filtered, dried, and then recrystallized from methanol to give **6** (26.9 g, 81.0%) as light yellow crystals. Mp. 197 – 199°C; MS [MH$^+$] (m/z): 414.1 (Br = 79), 416.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.42 (s, 1H), 8.09 (s, 1H), 8.07 (s, 1H), 4.26 (t, 2H), 3.84 (t, 2H), 3.50 (m, 1H), 2.27 (m, 2H), 1.33 (m, 2H), 1.10 (m, 2H).

General procedure for the synthesis of 7-bromo-8-(3-chloropropoxy)-5-cyclopropyl-1-substituted-5H-pyridazino[4,5-b]indoles **7a – c**

To a mixture of **6** (24.9 g, 0.06 mol) and R_1PhNH_2 (0.072 mol) in isopropanol (250 mL) was added saturated ethanol hydrochloride (0.5 mL). After heating to reflux for 5 h, the mixture was cooled, sufficient triethylamine was added to basify the mixture, and then filtered to give **7** as light yellow solid (85 – 90%).

7a:

R_1 = 3-chloro-4-fluoro: MS [MH$^+$] (m/z): 523.3 (Br = 79), 525.3 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H), 8.06 (s, 1H), 8.04 (s, 1H), 7.99 (dd, 1H), 7.65 (brs, 1H), 7.43 (t, 1H), 4.25 (t, 2H), 3.83 (t, 2H), 3.50 (m, 1H), 2.25 (m, 2H), 1.34 (m, 2H), 1.10 (m, 2H).

7b:

R_1 = 4-trifluoromethyl: MS [MH$^+$] (m/z): 539.4 (Br = 79), 541.4 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.38 (s, 1H), 8.05 (s, 1H), 7.84 – 7.81 (d, 3H), 7.67 (d, 2H), 4.20 (t, 2H), 3.81 (t, 2H), 3.50 (m, 1H), 2.22 (m, 2H), 1.30 (m, 2H), 1.08 (m, 2H).

7c:

R_1 = 3,4-difluoro: MS [MH$^+$] (m/z): 507.2 (Br = 79), 509.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.36 (s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 7.86-7.76 (m, 1H), 7.48 – 7.41 (m, 2H), 4.25 (t, 2H), 3.82 (t, 2H), 3.50 (m, 1H), 2.24 (m, 2H), 1.33 (m, 2H), 1.10 (m, 2H).

General procedure for the synthesis of compounds **8 – 19**

A mixture of **7** (0.05 mol), potassium carbonate (13.8 g, 0.10 mol) and R_2R_3NH (0.06 mol) in *N,N*-dimethylformamide (100 mL) was stirred and heated at 60°C for 7 h. It was cooled to room temperature, and then poured into water. The resulting precipitate was collected, dried, and then dissolved in acetone. The solution of oxalic acid (0.06 mol) in acetone (20 mL) was added, the formed solid was filtered and dried to give **8 – 19** as light yellow solid (80 – 87%).

7-Bromo-1-(3-chloro-4-fluorophenylamino)-5-cyclopropyl-8-[3-(dimethylamino)propoxy]-5H-pyridazino[4,5-b]indole oxalate **8**

Yield: 81%; mp. 153 – 155°C; MS [MH$^+$] (m/z): 532.2 (Br = 79), 534.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.32 (s, 1H, C4-H), 8.05 (s, 1H, C6-H), 8.03 (s, 1H, C9-H), 7.97 (dd, 1H, *J* = 6.6, 2.5 Hz, ph-H), 7.64 (brs, 1H, ph-H), 7.40 (t, 1H, *J* = 9.0 Hz ph-H), 4.25 (t, 2H, *J* = 5.7 Hz, $CH_2CH_2CH_2O$), 3.50 (m, 1H, NCH), 3.31 (t, 2H, *J* = 7.0 Hz, $NCH_2CH_2CH_2$), 2.85 (s, 6H, $N(CH_3)_2$), 2.23 (brs, 2H, $CH_2CH_2CH_2$),

1.35 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{24}H_{24}BrClFN_5O \cdot C_2H_2O_4$: C 50.14, H 4.21, N 11.24; Found: C 50.02, H 4.31, N 11.41.

7-Bromo-1-(3-chloro-4-fluorophenylamino)-5-cyclopropyl-8-[3-(pyrrolidin-1-yl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***9***

Yield: 83%; mp. 167 – 169°C; MS [MH^+] (m/z): 558.1 (Br = 79), 560.1 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.29 (s, 1H, C_4-H), 8.02 (s, 2H, $C_{6,9}$-H), 7.97 (dd, 1H, *J* = 6.5, 2.4 Hz, ph-H), 7.65 (brs, 1H, ph-H), 7.39 (t, 1H, *J* = 9.0 Hz ph-H), 4.25 (t, 2H, *J* = 5.8 Hz, $CH_2CH_2CH_2O$), 3.47 (m, 1H, NCH), 3.38 (brs, 4H, pyrrolidinyl-2,5-2H), 3.31 (brs, 2H, $NCH_2CH_2CH_2$), 2.24 (brs, 2H, $CH_2CH_2CH_2$), 1.97 (brs, 4H, pyrrolidinyl-2,5-2H), 1.34 (m, 2H, CH_2CH_2), 1.08 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{26}H_{26}BrClFN_5O \cdot C_2H_2O_4$: C 51.83, H 4.35, N 10.79; Found: C 51.72, H 4.39, N 10.62.

7-Bromo-1-(3-chloro-4-fluorophenylamino)-5-cyclopropyl-8-[3-(4-morpholinyl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***10***

Yield: 84%; mp. 125 – 127°C; MS [MH^+] (m/z): 574.2 (Br = 79), 576.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.33 (s, 1H, C_4-H), 8.05 (s, 1H, C_6-H), 8.03 (s, 1H, C_9-H), 7.97 (d, 1H, *J* = 6.8 Hz, ph-H), 7.68 (brs, 1H, ph-H), 7.41 (t, 1H, *J* = 9.1 Hz ph-H), 4.25 (t, 2H, *J* = 5.6 Hz, $CH_2CH_2CH_2O$), 3.83 (s, 4H, morpholinyl-3,5-2H), 3.50 (m, 1H, NCH), 3.21 (brs, 6H, morpholinyl-2,6-2H, $NCH_2CH_2CH_2$), 2.22 (brs, 2H, $CH_2CH_2CH_2$), 1.35 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{26}H_{26}BrClFN_5O_2 \cdot C_2H_2O_4$: C 50.58, H 4.24, N 10.53; Found: C 50.32, H 4.36, N 10.35.

7-Bromo-1-(3-chloro-4-fluorophenylamino)-5-cyclopropyl-8-[3-(piperidin-1-yl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***11***

Yield: 86%; mp. 122 – 124°C; MS [MH^+] (m/z): 572.1 (Br = 79), 574.1 (Br = 81); ^{1}H-NMR (DMSO-d_6) d: 9.32 (s, 1H, C_4-H), 8.05 (s, 1H, C_6-H), 8.03 (s, 1H, C_9-H), 7.96 (d, 1H, *J* = 6.8 Hz, ph-H), 7.68 (brs, 1H, ph-H), 7.41 (t, 1H, J = 8.9 Hz ph-H), 4.25 (t, 2H, *J* = 5.7 Hz, $CH_2CH_2CH_2O$), 3.50 (m, 5H, NCH, piperidinyl-2,6-2H), 2.96 (brs, 2H, $NCH_2CH_2CH_2$), 2.25 (brs, 2H, $CH_2CH_2CH_2$), 1.78 (brs, 6H, piperidinyl-3,4,5-2H), 1.35 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{27}H_{28}BrClFN_5O \cdot C_2H_2O_4$: C 52.54, H 4.56, N 10.56; Found: C 52.17, H 4.12, N 10.72.

7-Bromo-1-(4-trifluoromethylphenylamino)-5-cyclopropyl-8-[3-(dimethylamino)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***12***

Yield: 84%; mp. 164 – 166°C; MS [MH^+] (m/z): 548.2 (Br = 79), 550.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.40 (s, 1H, C_4-H), 8.05 (s, 1H, C_6-H), 7.83 – 7.79 (d, 3H, C_9-H, ph-2H), 7.67 (d, 2H, *J* = 8.5 Hz, ph-2H), 4.16 (t, 2H, *J* = 5.5 Hz, $CH_2CH_2CH_2O$), 3.50 (m, 1H, NCH), 3.31 (t, 2H, *J* = 7.0 Hz, $NCH_2CH_2CH_2$), 2.85 (s, 6H, $N(CH_3)_2$), 2.23 (brs, 2H, $CH_2CH_2CH_2$), 1.34 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{25}H_{25}BrF_3N_5O.C_2H_2O_4$: C 50.80, H 4.26, N 10.97; Found: C 50.52, H 4.35, N 11.05.

7-Bromo-1-(4-trifluoromethylphenylamino)-5-cyclopropyl-8-[3-(pyrrolidin-1-yl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***13***

Yield: 85%; mp. 120 – 122°C; MS [MH^+] (m/z): 574.1 (Br = 79), 576.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.41 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 7.81 – 7.78 (d, 3H, C_9-H, ph-2H), 7.67 (d, 2H, *J* = 8.4 Hz, ph-2H), 4.15 (t, 2H, *J* = 5.6 Hz, $CH_2CH_2CH_2O$), 3.51 (m, 1H, NCH), 3.35 (brs, 6H, pyrrolidinyl-2,5-2H, $NCH_2CH_2CH_2$), 2.21 (brs, 2H, $CH_2CH_2CH_2$), 1.96 (brs, 4H, pyrrolidinyl-2,5-2H), 1.36 (m, 2H, CH_2CH_2), 1.11 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{27}H_{27}BrF_3N_5O \cdot C_2H_2O_4$: C 52.42, H 4.40, N 10.54; Found: C 52.52, H 4.55, N 10.36.

7-Bromo-1-(4-trifluoromethylphenylamino)-5-cyclopropyl-8-[3-(4-morpholinyl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***14***

Yield: 82%; mp. 143 – 145°C; MS [MH^+] (m/z): 590.2 (Br = 79), 592.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.42 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 7.79 – 7.77 (d, 3H, C_9-H, ph-2H), 7.67 (d, 2H, *J* = 8.6 Hz, ph-2H), 4.13 (t, 2H, *J* = 5.8 Hz, $CH_2CH_2CH_2O$), 3.81 (s, 4H, morpholinyl-3,5-2H), 3.52 (m, 1H, NCH), 3.17 (brs, 6H, morpholinyl-2,6-2H, $NCH_2CH_2CH_2$), 2.18 (brs, 2H, $CH_2CH_2CH_2$), 1.36 (m, 2H, CH_2CH_2), 1.11 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{27}H_{27}BrF_3N_5O_2 \cdot C_2H_2O_4$: C 51.19, H 4.30, N 10.29; Found: C 51.37, H 4.38, N 10.42.

7-Bromo-1-(4-trifluoromethylphenylamino)-5-cyclopropyl-8-[3-(piperidin-1-yl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***15***

Yield: 85%; mp. 134 – 135°C; MS [MH^+] (m/z): 588.2 (Br = 79), 590.2 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.41 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 7.81 – 7.78 (d, 3H, C_9-H, ph-2H), 7.67 (d, 2H, *J* = 8.6 Hz, ph-2H), 4.15 (t, 2H, *J* = 5.7 Hz, $CH_2CH_2CH_2O$), 3.51 (m, 5H, NCH, piperidinyl-2,6-2H), 2.99 (brs, 2H, $NCH_2CH_2CH_2$), 2.23 (brs, 2H, $CH_2CH_2CH_2$), 1.77 (brs, 6H, piperidinyl-3,4,5-2H), 1.36 (m, 2H, CH_2CH_2), 1.11 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{28}H_{29}BrF_3N_5O \cdot C_2H_2O_4$: C 53.11, H 4.61, N 10.32; Found: C 53.38, H 4.45, N 10.47.

7-Bromo-1-(3,4-difluorophenylamino)-5-cyclopropyl-8-[3-(dimethylamino)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***16***

Yield: 81%; mp. 160 – 162°C; MS [MH^+] (m/z): 516.4 (Br = 79), 518.4 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 8.04 (s, 1H, C_9-H), 7.85 – 7.77 (m, 1H, ph-H), 7.46 – 7.40 (m, 2H, ph-H), 4.26 (t, 2H, *J* = 5.5 Hz, $CH_2CH_2CH_2O$), 3.51 (m, 1H, NCH), 3.30 (t, 2H, *J* = 7.1 Hz, $NCH_2CH_2CH_2$), 2.85 (s, 6H, $N(CH_3)_2$), 2.24 (brs, 2H, $CH_2CH_2CH_2$), 1.35 (m, 2H, CH_2CH_2), 1.08 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{24}H_{24}BrF_2N_5O \cdot C_2H_2O_4$: C 51.50, H 4.32, N 11.55; Found: C 51.31, H 4.43, N 11.32.

7-Bromo-1-(3,4-difluorophenylamino)-5-cyclopropyl-8-[3-(pyrrolidin-1-yl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***17***

Yield: 83%; mp. 151 – 152°C; MS [MH^+] (m/z): 542.4 (Br = 79), 544.3 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 8.02 (s, 1H, C_9-H), 7.87 – 7.80 (m, 1H, ph-H), 7.46 – 7.37 (m, 2H, ph-H), 4.26 (t, 2H, *J* = 5.5 Hz, $CH_2CH_2CH_2O$), 3.51 (m, 1H, NCH), 3.42 – 3.35 (m, 6H, pyrrolidinyl-2,5-2H, $NCH_2CH_2CH_2$), 2.23 (brs, 2H, $CH_2CH_2CH_2$), 1.97 (brs, 4H, pyrrolidinyl-2,5-2H), 1.35 (m, 2H,

CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{26}H_{26}BrF_2N_5O \cdot C_2H_2O_4$: C 53.17, H 4.46, N 11.07; Found: C 53.36, H 4.55, N 10.95.

7-Bromo-1-(3,4-difluorophenylamino)-5-cyclopropyl-8-[3-(4-morpholinyl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***18***

Yield: 82%; mp. 143 – 145°C; MS [MH^+] (m/z): 558.5 (Br = 79), 560.5 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 8.02 (s, 1H, C_9-H), 7.86 – 7.77 (m, 1H, ph-H), 7.46 – 7.37 (m, 2H, ph-H), 4.25 (t, 2H, *J* = 5.7 Hz, $CH_2CH_2CH_2O$), 3.83 (s, 4H, morpholinyl-3,5-2H), 3.51 (m, 1H, NCH), 3.24 (brs, 6H, morpholinyl-2,6-2H, $NCH_2CH_2CH_2$), 2.24 (brs, 2H, $CH_2CH_2CH_2$), 1.35 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{26}H_{26}BrF_2N_5O_2 \cdot C_2H_2O_4$: C 51.86, H 4.35, N 10.80; Found: C 51.37, H 4.48, N 10.49.

7-Bromo-1-(3,4-difluorophenylamino)-5-cyclopropyl-8-[3-(4-morpholinyl)propoxy]-5H-pyridazino[4,5-b]indole oxalate ***19***

Yield: 82%; mp. 138 – 140°C; MS [MH^+] (m/z): 556.4 (Br = 79), 558.4 (Br = 81); ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H, C_4-H), 8.06 (s, 1H, C_6-H), 8.02 (s, 1H, C_9-H), 7.85 – 7.76 (m, 1H, ph-H), 7.46 – 7.37 (m, 2H, ph-H), 4.25 (t, 2H, *J* = 5.8 Hz, $CH_2CH_2CH_2O$), 3.50 (m, 5H, NCH, piperidinyl-2,6-2H), 3,00 (brs, 2H, $NCH_2CH_2CH_2$), 2.25 (brs, 2H, $CH_2CH_2CH_2$), 1.77 (brs, 6H, piperidinyl-3,4,5-2H), 1.35 (m, 2H, CH_2CH_2), 1.09 (m, 2H, CH_2CH_2); Anal. Calcd. for $C_{27}H_{28}BrF_2N_5O \cdot C_2H_2O_4$: C 53.88, H 4.68, N 10.83; Found: C 53.68, H 4.47, N 10.57.

Pharmacology

The anti-tumor activities of compounds **8 – 19** were evaluated with Bel-7402 (Human Liver Cancer Cell Lines) and HT-1080 (Human Fibro Sarcoma Cell Lines) *in vitro* through measuring cell viability by the MTT method, with gefitinib as the positive control. The cells were seeded in 96-well plate at the concentration of 4000 cells per well in 100 μL RPMI 1640 medium. After cultured for 12 h at 37°C and 5% CO_2, cells were incubated with various concentrations of samples for 24 h. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was added at a terminal concentration of 5 μg/mL and incubated with cells for 4 h. The formazane crystals were dissolved in 100 μL DMSO each well, and the optical density was measured at 492 nm (for absorbance of MTT formazane) and 630 nm (for the reference wavelength). The IC_{50} was calculated by Bliss [11].

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