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Synthesis and antitumor activity of 5-(5-halogenated-2-oxo-1*H*-pyrrolo[2,3-*b*]pyridin-(3*Z*)-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamides

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ABSTRACT

We report herein the design and synthesis of a series of novel 5-halogenated-7-azaindolin-2-one derivatives containing a 2,4-dimethylpyrrole moiety. Nine target compounds with ⩾70% inhibition against MCF-7 at 30 μM were further evaluated for their in vitro antitumor activity against seven human cancer cell lines by SRB assay. Results reveal that some compounds have potent antitumor activity, and the most active **13c7** (IC_{50}s: 4.49–15.39 μM) was found to be more active than Sunitinib (IC_{50}s: 4.70–>30 μM) against all of the tested cancer cell lines.

In the past decade, a numerous diverse scaffolds have been discovered as the multi-targeted tyrosine kinase inhibitors.[1–3] As an important class of compounds with potent antitumor activity, indolin-2-ones have been the subject of intense research recently. Sunitinib (Su11248, Fig. 1), a small-molecule multi-targeted receptor tyrosine kinase inhibitor with activity against many human cancer cell lines,[4] was approved for the treatment of advanced renal cell carcinoma (RCC) and irresponsible or intolerant to Imatinib mesilate therapy on gastrointestinal stromal tumor (GIST) by the US Food and Drug Administration (FDA) in 2006.[5]

Structural modifications mainly at the 3- and 5-positions of the indolin-2-one ring have made considerable progress in the ability to increase antitumor activity through inhibition on different receptors in recent years. It was reported that 5-carbamido-indolin-2-one derivative BX-517 (Fig. 1) has single-digit nanomolar activity against phosphoinositide-dependent kinase-1 (PDK1) and excellent selectivity against protein kinase A (PKA).[6,7] Sunitinib analogs **A** and **B** (Fig. 1) containing a pyrrolo-fused-heterocycle moiety at the 3-position of indolin-2-one core were reported to be potent inhibitors of vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit both enzymatically and cellularly (<50 nM).[8] In the above studies, however, any group was not placed at the N-1 position of the indolin-2-one scaffold. Therefore, we had focused our attention on introducing proper functional groups to this position in our previous work. As novel lead compounds discovered in our lab, Z24 (Fig. 1) and LK-B030 (Fig. 1) bearing a (piperidin-1-yl)methyl and a (3-dimethylamino)propyl group at the N-1 position, respectively, were found to have a broad spectrum of antitumor activity by inhibiting angiogenesis in new blood vessels.[9–11]

On the other hand, 7-azaindole (not indolin-2-one) scaffold displays many pharmacological profiles. Beside the antitumor activity, there are reports on antibacterial, antiviral, antihypertensive, antiasthmatic, antiosteoporotic and antidepressant activities of this class of compounds.[12–16] It is well-known that the replacement of the quinoline core with 1,8-naphthyridine has attracted great attention and led to the discovery of some new quinolone drugs, such as Gemifloxacin (one of the fourth generation of quinolone antibacterials) and Vosaroxin (the first and only quinolone antitumor drug).[17,18] However, 7-azaindolin-2-one antitumor agents, as we all know, have not been reported in the literature.

As part of our continuing modifications on Sunitinib as potential antitumor drug candidates, we planned to take the place of the CH at the 7-position of Sunitinib with N atom and meanwhile do structural modifications at the 5-position of the 7-azaindolin-2-one core and the 3′-position of the pyrrole ring. Thus, a series

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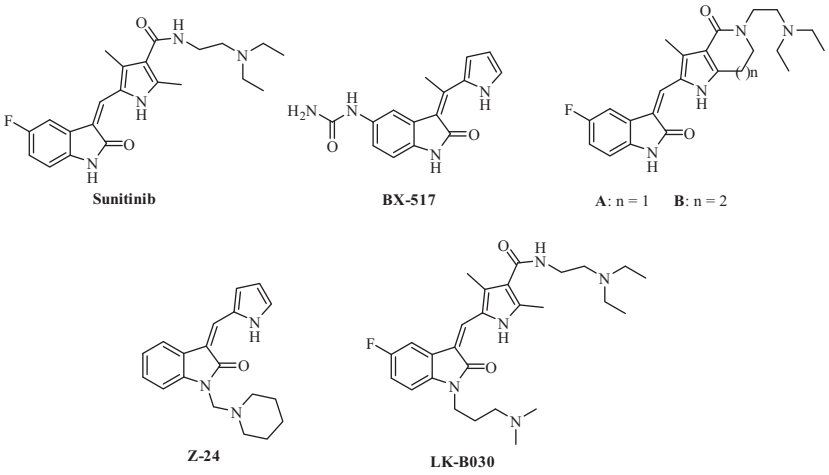


Figure 1. Structures of some indolin-2-ones.

of novel 5-halogenated-7-azaindolin-2-one derivatives containing a 2,4-dimethylpyrrole moiety were designed, synthesized and evaluated for their antitumor activity in this study. Our primary objective was to optimize the potency of these compounds against a set of solid tumors and contribute to the development of new antitumor agents. A preliminary structure–activity relationship (SAR) study is also explored to facilitate the further development of 7-azaindolin-2-ones.

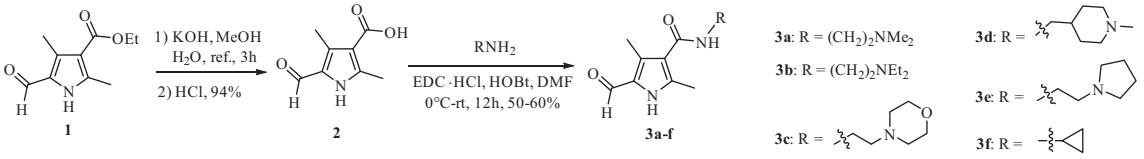
Detailed synthetic pathways to pyrrole-3-carboxamides (**3a**–**f**, **7a**,**b**, **11**) and 5-halogenated-7-azaindolin-2-one derivatives (**13a**–**c**) are depicted in Schemes 1–4, respectively. According to well-established literature procedures,[10,11,19,20] commercially available ethyl 5-formyl-2,4-dimethylpyrrole-3-carboxylate **1** was hydrolyzed to the corresponding carboxylic acid **2**, which upon amidation with different primary amines (RNH_2) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt) was converted to pyrrole-3-carboxamides **3a**–**f** (Scheme 1).

2-Phenoxyethanamine (**6a**) and 2-(benzyloxy)ethanamine (**6b**) were conveniently obtained from phenol (**4a**) and benzyl bromide (**4b**) respectively, by coupling with *N*-(2-hydroxyethyl)phthalimide and hydrazinolysis successively.[21] Condensation of the amines **6a**,**b** with the acid **2** gave pyrrole-3-carboxamides **7a**,**b** (Scheme 2).

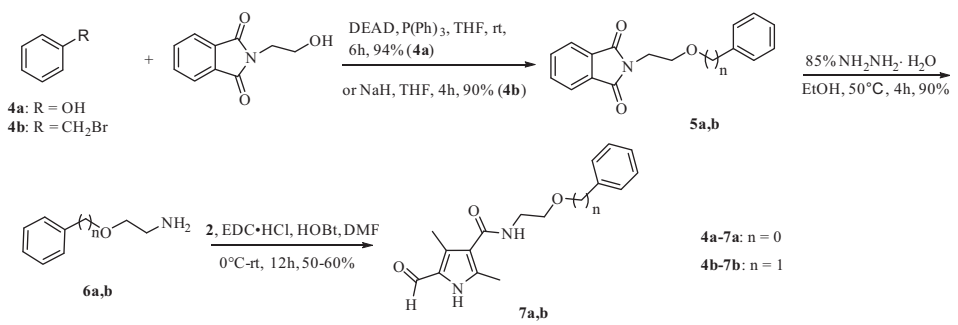
Treatment of 1-aminopropan-2-ol (**8**) with *tert*-butyldiphenylchlorsilane (TBDPSCl) in the presence of imidazole yielded selectively hydroxy-protected compound **9**, the latter condensed with the acid **2** and then the TBDPS protecting group was removed by tetra-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) to give 5-formyl-*N*-(2-hydroxyl-propyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**11**) (Scheme 3).

Finally, the target compounds **13a**–**c** were prepared via aldol condensation of 5-formyl-2,4-dimethylpyrrole derivatives (**1**–**3**, **7a**,**b**, **11**) with 5-halogenated(F, Cl, Br)-7-azaindolin-2-ones (**12a**–**c**) in the presence of piperidine (Scheme 4). All of the new synthetic compounds were well characterized by ^{1}H NMR, MS, HRMS and ^{13}C NMR in part.[22] As expected, the pyrrole-2-methylidene geometry at the 3-position of 7-azaindolin-2-one ring was confirmed to have the *Z*-configuration.[10,11]

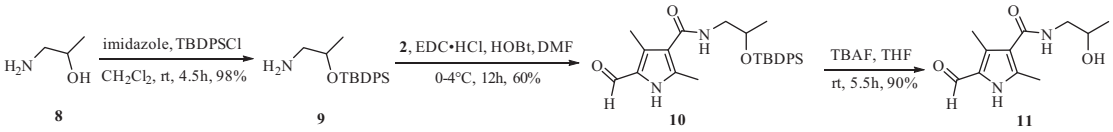
For preliminary screening of antitumor candidates, all the newly synthesized 7-azaindolin-2-one derivatives **13a**–**c** were first



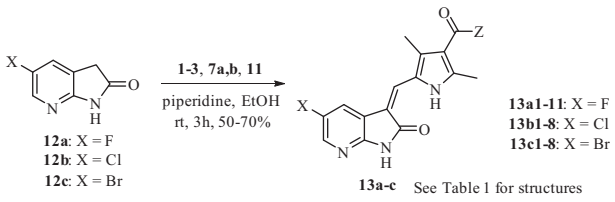
Scheme 1. Synthesis of pyrrole-3-carboxamides **3a**–**f**.



Scheme 2. Synthesis of pyrrole-3-carboxamides **7a**,**b**.



Scheme 3. Synthesis of pyrrole-3-carboxamide **11**.



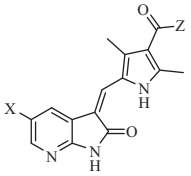
Scheme 4. Synthesis of 7-azaindolin-2-one derivatives **13a–c**.

investigated for cytotoxic activity in vitro against MCF-7 (human breast cancer cell line), and the compounds having ⩾70% inhibition at the concentration of 30 μM were subjected to IC_{50}s (50 % inhibition concentrations) determination. Nine target compounds (**13a4**, **13a6**, **13b4**, **13b6**, **13b7**, **13c3**, **13c4**, **13c6**, **13c7**) meeting this criterion (Table 1) were further evaluated for their in vitro antitumor activity in seven human cancer cell lines, including MCF-7 (breast cancer), HepG2 (liver carcinoma), HT-29 (colon adenocarcinoma), A549 (lung adenocarcinoma), PANC-1 (pancreatic carcinoma), Hela (cervical cancer) and Skov-3 (ovarian carcinoma) by SRB assay.[23] The IC_{50} values were compared with those of Sunitinib (Table 2).

The selected target 7-azaindolin-2-ones have potent in vitro antitumor activity against the tested human cancer cell lines. Among them, compounds **13c3**, **13c4** and **13c7** (IC_{50}s: 3.99–27.8 μM) are more active than or comparable to Sunitinib (IC_{50}s: 4.70–>30 μM) against all of the cell lines. In particular, the most active compound **13c7** (IC_{50}s: 4.49–15.39 μM) was found to be more potent than the reference drug against these cancer cell lines.

According to the biological evaluation results showed in Tables 1 and 2, antitumor activity of the 7-azaindolin-2-one derivatives

Table 1
Structures and in vitro activity of compounds **13a–c** against MCF-7 (at 30 μM)



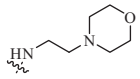
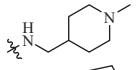
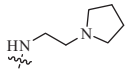
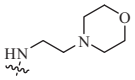
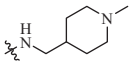
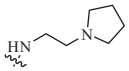
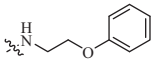
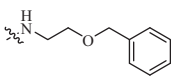
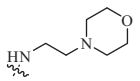
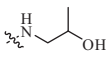
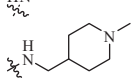
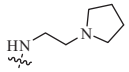
Compounds	X	Z	% inhibition	Compounds	X	Z	% inhibition
13a1	F	OEt	60.4	**13b4**	Cl	$NH(CH_2)_2NEt_2$	88.9
13a2	F	OH	20.5	**13b5**	Cl		6.0
13a3	F	$NH(CH_2)_2NMe_2$	61.1	**13b6**	Cl		88.4
13a4	F	$NH(CH_2)_2NEt_2$	70.7	**13b7**	Cl		91.3
13a5	F		15.6	**13b8**	Cl		6.2
13a6	F		78.0	**13c1**	Br	OEt	6.3
13a7	F		37.5	**13c2**	Br	OH	19.6
13a8	F		6.3	**13c3**	Br	$NH(CH_2)_2NMe_2$	79.4
13a9	F		2.8	**13c4**	Br	$NH(CH_2)_2NEt_2$	89.7
13a10	F		4.1	**13c5**	Br		6.0
13a11	F		0	**13c6**	Br		82.8
13b1	Cl	OEt	54.8	**13c7**	Br		86.9
13b2	Cl	OH	15.8	**13c8**	Br		5.5
13b3	Cl	$NH(CH_2)_2NMe_2$	45.7	Sunitinib			94.0

Table 2
In vitro activity of selected compounds against seven cells

Cell lines	IC_{50} (μM)						
Compounds	MCF-7	HepG2	HT-29	A549	PANC-1	Hela	Skov-3
13a4	>30	14.99	6.27	—	>30	—	—
13a6	>30	21.16	5.72	—	>30	—	—
13b4	26.28	12.41	6.93	10.32	13.98	29.35	8.28
13b6	>30	12.69	5.57	16.88	>30	>30	16.40
13b7	15.58	7.87	12.51	11.43	10.35	24.46	16.12
13c3	14.09	6.57	5.04	4.57	11.55	17.86	4.98
13c4	27.8	12.03	3.99	6.46	15.58	26.66	11.79
13c6	>30	21.81	5.57	15.17	26.94	>30	19.20
13c7	15.09	9.36	4.49	4.82	6.20	15.39	5.15
Sunitinib	25.41	16.06	4.70	7.93	14.94	>30	9.21

13a–c depends on both of the halogen atom at the 5-position of the 7-azaindolin-2-one ring and the side chain at the 3′-position of the pyrrole ring. In general, 5-bromo-7-azaindolin-2-ones are generally more active than the corresponding 5-chloro analogs, and 5-fluoro analogs have the worst rating of the threes with a few exceptions (Table 2). As expected, converting the ester (**13a1**, Z = OEt) and acid (**13a2**, Z = OH) into carboxamides [Z = $NH(CH_2)_2NMe_2$ (**13a3**) or $NH(CH_2)_2NEt_2$ (**13a4**)], just like in the case of Sunitinib, enhances the antitumor activity (Table 1). For heterocyclic groups, pyrrolidine (equivalent to the closed-ring one of diethylamine) makes more contribution to antitumor activity than piperidine (**13b7** vs **13b6**, **13c7** vs **13c6**, Table 2), while morpholine is the lowest (Table 1). Surprisingly, replacing the diethylamine in **13a4** by either 2-phenoxy (**13a9**) or benzyloxy (**13a10**) markedly reduces activity (although O atom is a isostere of N), and introduction of a free hydroxyl group (**13a11**) leads to the loss of antitumor activity. In addition, *N*-cyclopropylcarboxamide at the 3′-position of the pyrrole ring (**13a8**, **13b8**, **13c8**) is not preferred (Table 1). The above SARs suggest that candidates with better activity than Sunitinib, may be obtained from a good combination of the two substituents at the 5-position of the 7-azaindolin-2-one ring and the side chain at the 3′-position of the pyrrole ring. Based on this, further studies on structural modifications and related mechanism of action are currently in progress.

In summary, a series of novel 5-halogenated-7-azaindolin-2-one derivatives containing a 2,4-dimethylpyrrole moiety were designed, synthesized and characterized by ^1H NMR, MS, HRMS and ^{13}C NMR in part. Nine target compounds having ⩾70% inhibition against MCF-7 at 30 μM were further evaluated for their in vitro antitumor activity against seven human cancer cell lines by SRB assay. Results reveal that some compounds show potent antitumor activity, and the most active compound **13c7** is found to have better activity than Sunitinib against all of the tested cancer cell lines. The results reported here provide a foundation for further improvement of the potency of these compounds to discover more potent novel 7-azaindolinone antitumor agents in future studies.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.017. These data include MOL files and InChiKeys of the most important compounds described in this article.

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22. To a stirring solution of **1**(19.4 g, 100 mmol) in methanol (100 mL) and water (400 mL) was added 5 N KOH solution (200 mL). The reaction mixture was heated to refluxing and stirred for 3 h. Cooled to room temperature, the mixture was adjusted to pH 3 with 5 N HCl, and then filtered. The precipitate was washed with water and dried in vacuo to give the title compound **2** (15.5 g, 94 %) as an yellowish brown solid. A solution of compound **2** (167 mg, 1 mmol), EDC·HCl (230 mg, 1.2 mmol) and HOBt (162 mg, 1.2 mmol) in DMF (15 mL) was stirred at 0–4 °C under the atmosphere of nitrogen for 20 min. The substituted amine **a–f** (2 mmol) was added to the mixture and stirred at the same temperature for 30 min and then overnight at room temperature. The mixture was diluted with water and adjusted to pH 9 with saturated Na_2CO_3 solution and then extracted with dichloromethane and methanol (9:1, v/v). The combined extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to provide crude product **3a–f** (50–60%) as red oils.
To a solution of **4a** (1.88 g, 20 mmol), *N*-(2-hydroxyethyl)phthalimide (3.82 g, 20 mmol) and triphenylphosphine(7.87 g, 30 mmol) in tetrahydrofuran (30 mL) was added dropwise a solution of diethyl azodicarboxylate (4.7 mL, 30 mmol) in tetrahydrofuran (10 mL) at 0 °C over 0.5 h. The mixture was stirred at the same temperature for 6 h and concentrated under reduced pressure. The residue was diluted in diethyl ether and stirred at −5 to 0 °C for 30 min and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel), eluting with petroleum ether and ethyl acetate (6:1, v/v) to give **5a** (5.02 g, 94 %) as a off-white solid. To a solution of *N*-(2-hydroxyethyl)phthalimide (2 g, 10.5 mmol) in anhydrous tetrahydrofuran (30 mL) was added 60% NaH (0.84 g, 21 mmol) at −5 °C. The mixture was stirred at the same temperature for 20 min. Commercially available **4b** (1.5 mL, 12.6 mmol)was added to the mixture and was stirred at the same temperature for 30 min and then stirred at room temperature for 4 h and filtered. The filtrate was concentrated under reduced pressure to give **5b** (2.65 g, 90%) as a off-white solid. To a solution of **5a**,**b** (3 mmol) in ethanol (30 mL) was added 85% hydrazine hydrate (0.19 mL, 3.3 mmol) at 50 °C. The reaction mixture was stirred at the same temperature for 4 h, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give crude **6a**,**b** (90%). **7a**,**b** were obtained as red oils

in a similar manner as for the preparation of **3a–f**, yield 50–60%.

To a solution of **8** (5 g, 66.6 mmol) and imidazole (18 g, 266 mmol) in methylene chloride (40 mL) was added dropwise a solution of *tert*-butylchlorodiphenylsilane (22 g, 80 mmol) for 10 min. The mixture was stirred at the same temperature for 4.5 h. The mixture was washed with water , saturated brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give crude **9** (20.46 g, 98%) as a colorless oil. **10** was obtained as a red oil from **9** (6.27 g, 20 mmol) in a similar manner as for the preparation of **3a–f**, yield 60%. To a solution of **10** (13.78 g, 30 mmol) in tetrahydrofuran (40 mL) was added a solution of tetrabutylammonium fluoride (1 M in tetrahydrofuran) (36 mL, 36 mmol) and stirred at room temperature for 5.5 h and concentrated under reduced pressure. The residue was diluted with ethyl acetate (20 mL), washed with water, saturated brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give crude **11** (6.06 g, 90%) as a red solid.

A solution of **1–3**, **7a,b**, **11** (1 mmol) and **12a–c** (0.7 mmol) in anhydrous ethanol (10 mL) and piperidine (6 drops) was stirred at room temperature under atmosphere of nitrogen for 2–3 h. The yellow precipitate was collected by suction and washed with ethanol and then dried under vacuum to afford the targeted compounds **13a1–11**, **13b1–8**, **13c1–8** (50–70%) as yellow solids.

Compound **13a1**: mp 275–276 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.72 (s, 1H), 11.60 (s, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 8.00 (dd, J = 2.4, 2.0 Hz, 1H), 7.85 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 2.55 (s, 3H), 2.52 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H). MS-ESI (m/z): 330.23 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{17}H_{17}O_3N_3F$ (M+H)$^+$: 330.12485; Found: 330.12481. Compound **13a2**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.68 (s, 1H), 12.25 (s, 1H), 11.59 (s, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 8.01 (dd, J = 2.5, 1.8 Hz, 1H), 7.86 (s, 1H), 2.56 (s, 3H), 2.53 (s, 3H). MS-ESI (m/z): 302.29 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{15}H_{13}O_3N_3F$ (M+H)$^+$: 302.09355; Found: 302.09350. Compound **13a3**, mp 272–274 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.7, 1.7 Hz, 1H), 7.80 (s, 1H), 7.53–7.56 (t, J = 5.6 Hz, 1H), 3.30-3.34 (m, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 2.39 (t, J = 6.8 Hz, 2H), 2.20 (s, 6H). MS-ESI (m/z): 372.23 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{19}H_{23}O_2N_5F$ (M+H)$^+$: 372.18303; Found: 372.18296. Compound **13a4**, mp 220–221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.7, 1.7 Hz, 1H), 7.81 (s, 1H), 7.48 (t, J = 5.6 Hz, 1H), 3.28 (q, J = 6.4 Hz, 2H), 2.57–2.51 (m, 6H), 2.46 (s, 3H), 2.43 (s, 3H), 0.97 (t, J = 7.1 Hz, 6H). MS-ESI (m/z): 400.69 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{27}O_2N_5F$ (M+H)$^+$: 400.21433; Found: 400.21404. Compound **13a5**, mp 249–251 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.8, 1.7 Hz, 1H), 7.80 (s, 1H), 7.53 (t, J = 5.3 Hz, 1H), 3.59–3.56 (t, J = 4.4 Hz, 4H), 3.35 (q, J = 6.4z Hz, 2H), 2.45–2.46 (m, 5H), 2.44–2.39 (m, 7H). MS-ESI (m/z): 414.85 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_3N_5F$ (M+H)$^+$: 414.19359; Found: 414.19351. Compound **13a6**, mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.7, 1.7 Hz, 1H), 7.80 (s, 1H), 7.70 (t, J = 5.8 Hz, 1H), 3.11 (t, J = 6.3 Hz, 2H), 2.77–2.72 (m, 2H), 2.44 (s, 3H), 2.41 (s, 3H), 2.14 (s, 3H), 1.84–1.77 (m, 2H), 1.67–1.62 (m, 2H), 1.49–1.43 (m, 1H), 1.24–1.14 (m, 2H). MS-ESI (m/z): 412.89 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{22}H_{27}O_2N_5F$ (M+H)$^+$: 412.21433; Found: 412.21410. Compound **13a7**, mp 249–250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.49 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.7, 1.7 Hz, 1H), 7.80 (s, 1H), 7.58 (t, J = 5.6 Hz, 1H), 3.34 (q, J = 6.4 Hz, 2H), 2.57 (t, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 1.71–1.67 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 169.61, 164.59, 155.52, 148.89, 137.47, 131.59, 130.88(d, J = 27.3 Hz), 126.62, 125.89, 121.66, 121.37(d, J = 6.4 Hz), 113.72(d, J = 23.7 Hz), 111.87(d, J = 2.4 Hz), 56.06, 55.16 (2C), 35.41, 18.60 (2C), 13.41, 10.57. MS-ESI (m/z): 398.63 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_2N_5F$ (M+H)$^+$: 398.19868; Found: 398.19890. Compound **13a8**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.49 (s, 1H), 11.53 (s, 1H), 8.20 (dd, J = 9.2, 2.7 Hz, 1H), 7.99 (dd, J = 2.6, 1.8Z Hz, 1H), 7.80 (s, 1H), 7.78 (d, J = 4.0 Hz, 1H), 2.80–2.77 (m, 1H), 2.42 (s, 3H), 2.39 (s, 3H), 0.72–0.66 (m, 2H), 0.55–0.50 (m, 2H). MS-ESI (m/z): 341.14 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{18}H_{18}O_2N_4F$ (M+H)$^+$: 341.14083; Found: 341.14074. Compound **13a9**, mp 243–244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 8.00–7.96 (m, 1H), 7.86 (t, J = 5.6 Hz, 1H), 7.80 (s, 1H), 7.32–7.27 (m, 2H), 6.99–6.92 (m, 3H), 4.10 (t, J = 5.8 Hz, 2H), 3.60 (q, J = 5.7 Hz, 2H), 2.44 (s, 3H), 2.42 (s, 3H). MS-ESI (m/z): 421.26 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{23}H_{22}O_3N_4F$ (M+H)$^+$: 421.16705; Found: 421.16697. Compound **13a10**, mp 216–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 11.52 (s, 1H), 8.19 (dd, J = 9.2, 2.7 Hz, 1H), 8.00–7.96 (m, 1H), 7.80 (s, 1H), 7.73 (t, J = 5.7 Hz, 1H), 7.35 (d, J = 4.4 Hz, 4H),7.32–7.26 (m, 1H), 4.52 (s, 2H), 3.57 (t, J = 5.8 Hz, 2H), 3.44 (q, J = 5.7 Hz, 2H), 2.44 (s, 3H), 2.41 (s, 3H). MS-ESI (m/z): 435.25 (M+H)$^+$, 457.10 (M+Na)$^+$. HRMS-ESI (m/z): Calcd for $C_{24}H_{24}O_3N_4F$ (M+H)+: 435.18270; Found: 435.18290. Compound **13a11**, mp 281–282 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.51 (s, 1H), 11.52 (s, 1H), 8.20 (dd, J = 9.2, 2.7 Hz, 1H), 7.98 (dd, J = 2.7, 1.7 Hz, 1H), 7.81 (s, 1H), 7.58 (t, J = 5.8 Hz, 1H), 4.72 (q, J = 4.8 Hz, 2H), 3.81–3.73 (m, 1H), 3.22–3.17 (m, 2H), 2.47 (s, 3H), 2.43 (s, 3H), 1.10 (d, J = 6.4 Hz, 3H). MS-ESI (m/z): 359.23 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{18}H_{20}O_3N_4F$ (M+H)$^+$: 359.31539; Found: 359.31540. Compound **13b1**, mp 294–295 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.66 (s, 1H), 11.69z (s, 1H), 8.40 (d, J = 2.2 Hz, 1H), 8.05 (d, J = 2.2 Hz, 1H), 7.90 (s, 1H), 4.23 (q, J = 7.1 Hz, 2H), 2.56 (s, 3H), 2.52 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H). MS-ESI (m/z): 346.23 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{17}H_{17}O_3N_3Cl$ (M+H)$^+$: 346.09530; Found: 346.09519. Compound **13b2**, mp 274–276 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.61 (s, 1H), 12.24 (s, 1H), 11.67 (s, 1H), 8.39 (d, J = 2.2 Hz, 1H), 8.04 (d, J = 2.2 Hz, 1H), 7.89 (s, 1H), 2.55 (s, 3H), 2.52 (s, 3H). MS-ESI (m/z): 318.42 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{15}H_{13}O_3N_3Cl$ (M+H)$^+$: 318.06400; Found: 318.06403. Compound **13b3**, mp 275–277 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.61 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.84 (s, 1H), 7.55 (t, J = 5.6 Hz, 1H), 3.34–3.28 (m, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 2.39 (t, J = 6.8 Hz, 2H), 2.19 (s, 6H). MS-ESI (m/z): 388.79 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{19}H_{23}O_2N_5Cl$ (M+H)$^+$: 388.15348; Found: 388.15355. Compound **13b4**, mp 225–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45 (s, 1H), 11.61 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.84 (s, 1H), 7.48 (t, J = 5.2 Hz, 1H), 3.28 (q, J = 6.2 Hz, 2H), 2.55 – 2.50 (m, 6H), 2.46 (s, 3H), 2.43 (s, 3H), 0.98 (t, J = 7.1 Hz, 6H). MS-ESI (m/z): 416.31 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{27}O_2N_5Cl$ (M+H)$^+$: 416.18478; Found: 416.18492. Compound **13b5**, mp 274–275 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.46 (s, 1H), 11.57 (s, 1H), 8.37 (d, J = 2.2 Hz, 1H), 8.03 (d, J = 2.2 Hz, 1H), 7.86 (s, 1H), 7.55 (t, J = 5.5 Hz, 1H), 3.59 (t, J = 4.6 Hz, 4H), 3.36 (q, J = 6.4 Hz, 2H), 2.47 (s, 3H), 2.45 (s, 3H), 2.46–2.41 (m, 6H). MS-ESI (m/z): 430.87 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_3N_5Cl$ (M+H)$^+$: 430.16404; Found: 430.16419. Compound **13b6**, mp 276–277 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45 (s, 1H), 11.60 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.84 (s, 1H), 7.71 (t, J = 5.8 Hz, 1H), 3.11 (t, J = 6.2 Hz, 2H), 2.72–2.78 (m, 2H), 2.44 (s, 3H), 2.41 (s, 3H), 2.15 (s, 3H), 1.83 (t, J = 10.8 Hz, 2H), 1.67–1.62 (m, 2H), 1.53–1.40 (m, 1H), 1.26–1.13 (m, 2H). MS-ESI (m/z): 428.88 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{22}H_{27}O_2N_5Cl$ (M+H)$^+$: 428.18478; Found: 428.18502. Compound **13b7**, mp 265–267 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.55 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.85 (s, 1H), 7.58 (t, J = 5.6 Hz, 1H), 3.30–3.34 (m, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 2.39 (t, J = 6.8 Hz, 2H), 3.33 (q, J = 6.8 Hz, 2H), 2.56 (t, J = 6.9 Hz, 3H), 2.49–2.46 (m, 4H), 2.45 (s, 3H), 2.42 (s, 3H), 1.72–1.65 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 169.30, 164.34, 150.95, 142.10, 137.89, 131.87, 126.79, 126.04, 125.28, 124.36, 121.72, 121.47, 111.17, 54.78 (2C), 53.54, 38.06, 23.22 (2C), 13.31, 10.50. MS-ESI (m/z): 414.84 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_2N_5Cl$ (M+H)$^+$: 414.16913; Found: 414.16917. Compound **13b8**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.61 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.84 (s, 1H), 7.77 (d, J = 4.1 Hz, 1H), 2.82–2.77 (m, 1H), 2.41 (s, 3H), 2.39 (s, 3H), 0.71–0.66 (m, 2H), 0.54–0.50 (m, 2H). MS-ESI (m/z): 357.07 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{18}H_{18}O_2N_4Cl$ (M+H)+: 357.11128; Found: 357.11123. Compound **13c1**, mp 296–298 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.66 (s, 1H), 11.69 (s, 1H), 8.51 (d, J = 2.1 Hz, 1H), 8.12 (d, J = 2.1 Hz, 1H), 7.90 (s, 1H), 4.23 (q, J = 7.1 Hz, 2H), 2.56 (s, 3H), 2.52 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H). MS-ESI (m/z): 390.20 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{17}H_{17}O_3N_3Br$ (M+H)$^+$: 390.04478; Found: 390.04491. Compound **13c2**, mp 285–286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.61 (s, 1H), 12.25 (s, 1H), 11.67 (s, 1H), 8.50 (d, J = 2.0 Hz, 1H), 8.11 (d, J = 2.0 Hz, 1H), 7.90 (s, 1H), 2.55 (s, 3H), 2.52 (s, 3H). MS-ESI (m/z): 362.12 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{15}H_{13}O_3N_3Br$ (M+H)+: 362.01348; Found: 362.01347. Compound **13c3**, mp 272–273 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.60 (s, 1H), 8.46 (d, J = 2.1 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H), 7.85 (s, 1H), 7.55 (t, J = 5.6 Hz, 1H), 3.31 (q, J = 5.6 Hz, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 2.39 (t, J = 6.8 Hz, 2H), 2.19 (s, 6H). MS-ESI (m/z): 432.15 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{19}H_{23}O_2N_5Br$ (M+H)$^+$: 432.10296; Found: 432.10328. Compound **13c4**, mp 242–244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.61 (s, 1H), 8.47 (d, J = 2.1 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H), 7.85 (s, 1H), 7.49 (t, J = 5.5 Hz, 1H), 3.28 (q, J = 6.4 Hz, 2H), 2.53 (q, J = 7.6 Hz, 6H), 2.46(s, 3H), 2.43 (s, 3H), 0.98 (s, 6H). MS-ESI (m/z): 460.20 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{27}O_2N_5Br$ (M+H)$^+$: 460.13426; Found: 460.13460. Compound **13c5**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.62 (s, 1H), 8.47 (d, J = 1.7 Hz, 1H), 8.10 (d, J = 1.7 Hz, 1H), 7.85 (s, 1H), 7.57 (s, 1H), 3.63–3.58 (m, 4H), 3.40–3.32 (m, 2H), 2.47 (s, 3H), 2.44 (s, 3H), 2.48–2.36 (m, 6H). MS-ESI (m/z): 474.17 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_3N_5Br$ (M+H)$^+$: 474.11353; Found: 3474.11374. Compound **13c6**, mp 283–285 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 11.58 (s, 1H), 8.46 (d, J = 2.1 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H), 7.84 (s, 1H), 7.70 (t, J = 5.8 Hz, 1H), 3.11 (t, J = 6.3 Hz, 2H), 2.78–2.72 (m, 2H), 2.44 (s, 3H), 2.41 (s, 3H), 2.14 (s, 3H), 1.84–1.77 (m, 2H), 1.68–1.62 (m, 2H), 1.50–1.40 (m, 1H), 1.24–1.14 (m, 2H). MS-ESI (m/z): 472.22 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{22}H_{27}O_2N_5Br$ (M+H)$^+$: 472.13426; Found: 472.13457. Compound **13c7**, mp 282–283 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.43 (s, 1H), 11.59 (s, 1H), 8.46 (d, J = 2.1 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H), 7.85 (s, 1H), 7.58 (t, J = 5.6 Hz, 1H), 3.33 (q, J = 5.2 Hz, 2H), 2.56 (t, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 1.68 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 169.15, 164.34, 151.17, 144.22, 137.91, 131.92, 127.83, 126.83, 126.06, 122.29, 121.51, 112.59, 111.06, 54.78(2C), 53.55, 38.04, 23.21(2C), 13.32, 10.50. MS-ESI (m/z): 458.26 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{21}H_{25}O_2N_5Br$ (M+H)$^+$: 458.11861; Found: 458.11868. Compound **13c8**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.43 (s, 1H), 11.60 (s, 1H), 8.46 (d, J = 2.2 Hz, 1H), 8.10 (d, J = 2.2 Hz, 1H), 7.84 (s, 1H), 7.77 (d, J = 4.2 Hz, 1H), 2.82–2.76 (m, 1H), 2.41 (s, 3H), 2.39 (s, 3H), 0.71–0.65 (m, 2H), 0.54–0.49 (m, 2H). MS-ESI (m/z): 401.08 (M+H)$^+$. HRMS-ESI (m/z): Calcd for $C_{18}H_{18}O_2N_4Br$ (M+H)$^+$: 401.06076; Found: 401.06073.

23. *SRB assay*: Various human cancer cell lines were cultured in minimum essential medium (MEM), supplemented with 10% fetal calf serum, 100 units/mL penicillin and 100 mg/mL streptomycin in a humidified atmosphere in 5% CO_2 at 37 °C. Cell culture media were renewed every three days, up to the confluence of the monolayer. Cell culture was washed upon formation of confluent cultures, using trypsine-EDTA to detach the cells from their culture flasks or dishes. Test compounds were stored at −18 °C and solubilized in 100% dimethyl sulfoxide. Cells were seeded in 96-well plates at a density of 10^4 for 24 h. After 24 h, cells were cultured with test compounds. Drug concentrations were based on preliminary experiments and were

adjusted experimentally. After additional incubation of test compounds for 48 h, cells were fixed with 50% trichloroacetic acid(TCA) and SRB (sulforhodamine B) at 0.4% (w/v) in 1% acetic acid was added to stain cells. Unbound SRB was washed out by 1% acetic acid and SRB-bound cells were solubilized with 10 mM trizma base. The absorbance was read at a wavelength of 570 nm. The percentage of cell survival was calculated, and IC_{50} for the compounds could be derived using curve-fitting methods with statistical analysis software SigmaPlot 10.0.