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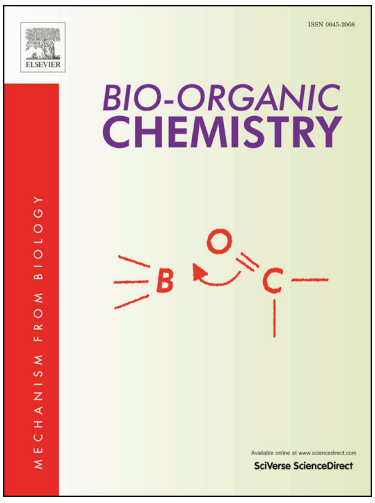

Design, Synthesis, and Evaluations of the Antiproliferative Activity and Aqueous Solubility of Novel Carbazole Sulfonamide Derivatives as Antitumor Agents

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Design, Synthesis, and Evaluations of the Antiproliferative Activity and Aqueous Solubility of Novel Carbazole Sulfonamide Derivatives as Antitumor Agents

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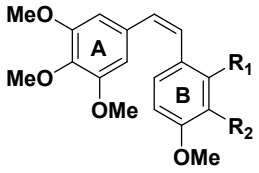
Abstract:

Optimization of **IG-105** (**1**) on the carbazole ring provided five series of new carbazole sulfonamides derivatives, **7a–e**, **8a–g**, **9a–g**, **10a–e**, and **11a–g**. All of the compounds were evaluated against HepG2, MCF-7, MIA PaCa-2, and Bel-7402 cells for antiproliferative activity. Each series of compounds was 2–5 times more active against HepG2 cells (IC_{50}: 1.00–10.0 μM) than the other three tumor cell lines. Several representative compounds, selected from each series, showed aqueous solubility (13.4–176.5 μg/mL at pH 7.4 and 2.0) better than **1,** with the aqueous solubility of corresponding salts > 30 mg/mL. From the results of evaluating the effects of the compounds **7b**, **8c**, **9c**, **10c** and **11c** on tubulin *in vitro*, we speculated that their targets were different from those of **1** and **CA-4P.** We tested the antitumor activity of the representative compound **7b·HCl (**10 mg/kg**)** in an *in vivo* study and found that its tumor growth inhibition rate was 41.1%. The tumor growth inhibition rate of **7b·HCl (**20 mg/kg**)** was 54.6%, whereas the tumor growth inhibition rate of **CA-4P (**50 mg/kg**)** was 48.3%. And in another batch of *in vivo* antitumor activity testing, **9c·HCl** and **11c·HCl** at doses of 10 mg/kg resulted in 61.1% and 50.0% inhibition, respectively. These promising results warrant further development of the derivatives, which may use a novel mechanism and show potential potency as antitumor drug candidates.

Keywords: Carbazole sulfonamide derivatives; Synthesis; Antiproliferative activity; Aqueous solubility.

1. Introduction

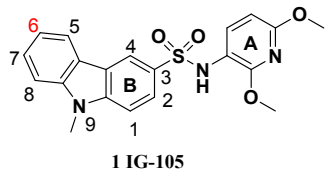


CA-4 : R_1 = H; R_2 = OH
CA-4P: R_1 = H; R_2 = OPO_3Na_2

Fig. 1 Structures of **CA-4** and **CA-4P**

Microtubules, as key components of cytoskeleton, have intrigued scientists since their discovery because of their critical importance to the cellular processes such as regulation of motility, cell signaling, formation and maintenance of cell shape, secretion, and intracellular transport [1]. Microtubule-active drugs generally bind to one of three main classes of sites on tubulin, the paclitaxel site, the *Vinca* domain and colchicine domain [2]. The compounds like colchicine and podophyllotoxin (**podo**), discovered in the early days irreversibly bind at the colchicine domain thus inducing great toxicities and limiting the use in clinical [3]. The reversibly binding inhibitors discovered in later, like Combretastatin A4 (**CA-4**), a novel small molecule tubulin binding agent which was isolated from the South African bush willow *Combretum caffrum*. (**Fig. 1**) [4]. **CA-4** strongly inhibits the polymerization of tubulin by binding to the colchicine binding site, and can be served as clinical candidates for further development [5]. However, the low water solubility of this compound has limited its efficacy in *vivo*. Its water-soluble prodrug, **CA-4** phosphate disodium (**CA-4P**) has good water solubility, stability and cytostatic activity and is in phase Ⅱ/Ⅲ clinical trials both as a single drug and in combination for anticancer therapy [6]. It appears to act by a unique irreversible and selective antivascular effect that disrupts immature endothelial cell's cytoskeleton in *vivo* [7]. In recent years, a large number of structure-activity relationship (SAR) studies on **CA-4** have been investigated. In brief, it is thought that the presence of the *cis*-orientation of the two aromatic rings, the 3,4,5-trimethoxy group on ring A, and the *para*-methoxy group on ring B were essential for optimal cytotoxic activity [8]. However, in later studies found that **CA-4**

and other olefinic analogues are prone to isomerize into their inactive *trans*-forms during storage and administration. To overcome this problem, varieties of conformationally restricted *cis*-locked analogues have been designed through the replacement of the olefinic bond with heterocyclic moieties. We also were interested in exploring the structure transformation of **CA-4**. We replaced the *cis*-olefin linker with sulfonamide group, the 3,4,5-trimethoxyphenyl with 2,6-dimethoxylpridine, the B ring with carbazole-ring to synthesize **IG-105** (**1**) [9].



1 IG-105

Fig. 2 Structure of **IG-105 (1)**

IG-105 (**1**, **Fig. 2**), exhibited potent activity against human leukemia and solid tumors in the breast, liver, prostate, lung, skin, colon, and pancreas, with IC_{50} values between 0.012 and 0.298 μM. However, only modest antitumor activity was observed *in vivo* when **1** was administered intraperitoneally to BDF1 mice bearing Lewis lung carcinoma cells at 40 mg/kg/day. The tumor mass inhibition rate of **1** was 59% compared to 71% produced by etoposide, a clinical anticancer drug that was used as a positive control [9]. The unexpectedly low antitumor activity was considered a result of its low bioavailability, which was caused, at least in part, by its poor aqueous solubility. Thus, improving aqueous solubility allows the administration dose to be increased, potentially increasing the therapeutic value of this antitumor agent.

To improve the aqueous solubility and bioavailability of **1**, the 6- and 7-positions of the carbazole rings were modified, yielding several antitumor-active compounds with water solubility (0.11–19.60 μg/mL at pH 7.4 and 2.0) better than that of **1** [10].。 The 6-position and 7-position are preferred for modification because the two positions on the carbazole ring are the most active, especially the 6-position, and the target product can be synthesized easily. At the same time, we learned to optimize the quinoline ring of receptor tyrosine kinase inhibitors (**Fig. 3**) [11]. Introduction of ionizable groups (nitrogen-containing heterocyclic groups) into compound structures

is a common approach to improving aqueous solubility. In this study, we introduced ionizable groups (benzylamines and phenyl ether alkylamines) into the 6-position of the carbazole ring of **1**, so as to significantly increase the water-solubility and may improve the oral bioavailability while maintaining or increasing the anti-tumor activity.

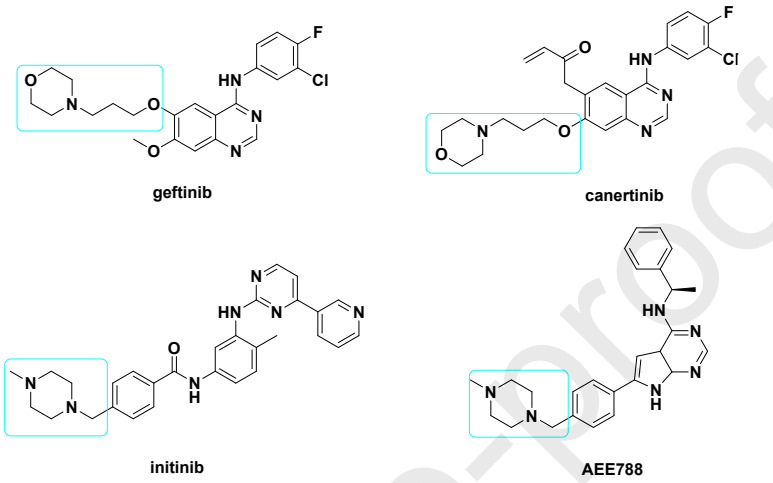
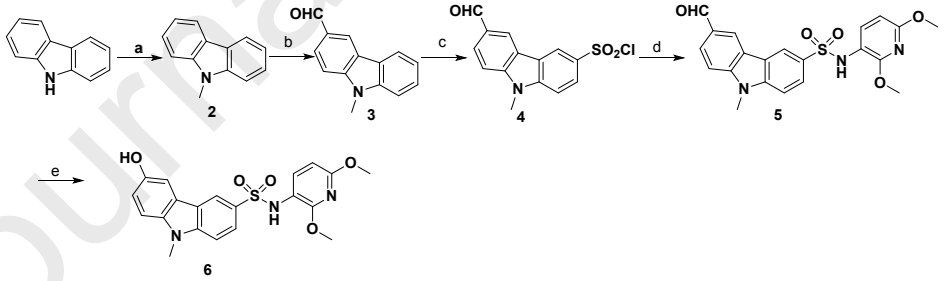


Fig. 3 Structures of receptor tyrosine kinase inhibitors

2. Results and Discussion

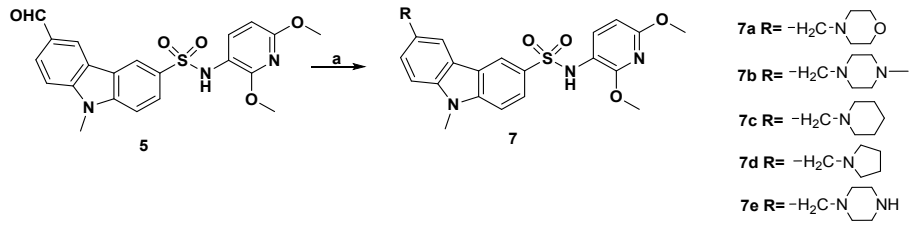
2.1 Chemistry

As shown Scheme **1**, compounds **5** and **6** were synthesized using our previously reported approach [10].



Scheme 1. Reagents and conditions: a: CH_3I, NaOH, DMF, 4 h; b: $POCl_3$, DMF, 0–80 °C, 3 h; c: $ClSO_3H$, 0 °C, 10 min; d: 3-amino-2, 6-dimethoxypyridine, K_2CO_3, DMF, rt.1 h; e: CH_3OH/DCM, H_2O_2, c H_2SO_4, 5 h.

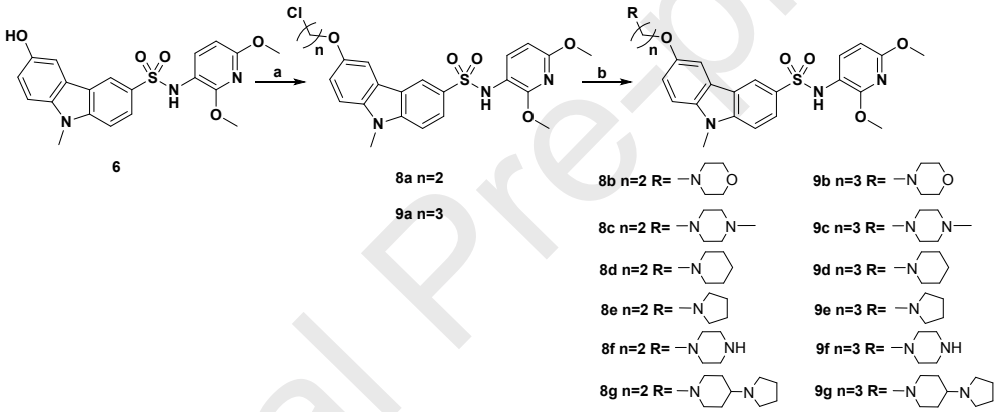
2.1.1 Benzylamine compounds：



Scheme 2. Reagents and conditions: a: amines, THF, $Ti(OiPr)_4$, $NaBH_3CN$, 3 h.

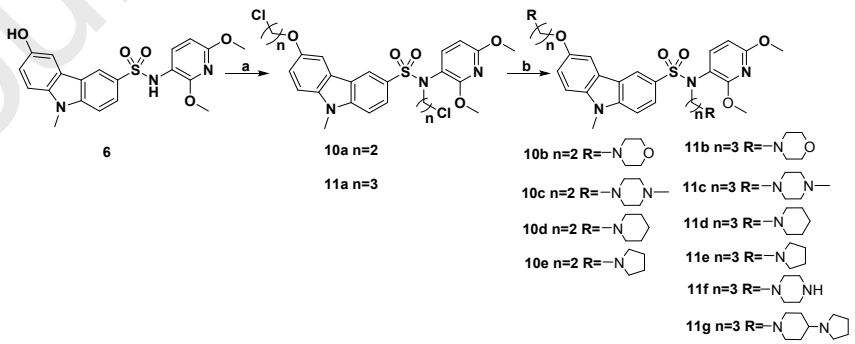
In Scheme **2**, a Borch reduction reaction of aldehyde **5** with different heterocyclic amines in $Ti(OiPr)_4$ was catalyzed by a sodium cyanoborohydride reduction to 40–70% yields of 6-modified benzylamine carbazole sulfonamide compounds **7a–g** [12]. A hydrochloric acid-methanol solution was added to **7a–g** in methanol and stirred for 1 h at 0 °C to produce the corresponding hydrochlorides.

2.1.2 Phenylethylamine compounds：



Scheme 3. Reagents and conditions: a: 1-bromo-3-chloropropane or 1-bromo-2- chloroethane, ACE (acetone), K_2CO_3, reflux, 5-10h; b: amine, CH_3CN, reflux, 24-48h.

2.1.3 Double substituted alkanines：



Scheme 4. Reagents and conditions: a: 1-bromo-3-chloropropane or 1-bromo-2-chloroethane, ACE (acetone), K_2CO_3, reflux, 5-10 h; b: amine, CH_3CN, reflux, 24-48h.

In Scheme **3**, Compound **6** reacted with 1-bromo-3-chloropropane or

1-bromo-2-chloroethane and potassium carbonate under reflux in acetone for 5–10 h to produce ether compounds **8a** and **9a** [13]. The ether compounds were refluxed with different heterocyclic amines in acetonitrile to give carbazole sulfonamide compounds **8b-g** and **9b-g** [14]. The nitrogen on the sulfonamide also underwent a coupling reaction with 1-bromo-3-chloropropane or 1-bromo-2-chloroethane during the formation of **8a** and **9a** to obtain compounds **10a** and **11a**, in which the hydroxyl group and sulfonamide were disubstituted as shown in Scheme **4**. And then react with different heterocyclic amines to obtain disubstituted alkylamine compounds **10b-e** and **11b-g**.

A hydrochloric acid-methanol solution was added to the final compounds having nitrogen-containing heterocycles in methanol, and the new solution was stirred for 1 h at 0 °C to produce the corresponding hydrochloride.

2.2 *In vitro* antitumor activity

All of the target compounds were evaluated against HepG2 (hepatoma cancer), MCF-7 (breast cancer), MIA PaCa-2 (pancreatic cancer), and Bel-7402 (hepatoma/liver cancer) cells for antiproliferative activity using the standard sulforhodamine B (SRB) assay [15-17]. The data are summarized in **Table 1**, **Table 2**, and **Table 3**.

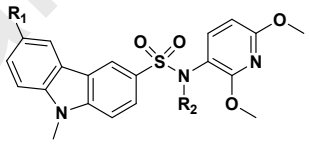


Table 1. Antiproliferative activities of compounds **7a-e** against four tumor cell lines

Comp.	R_1	R_2	$IC_{50}(\mu M)^a$			
			HepG2	MIA PaCa-2	MCF-7	Bel-7402
7a	6-$CH_2N(CH_2CH_2)_2O$	H	>25	NT[b]	NT[b]	NT[b]
7b	6-$CH_2N(CH_2CH_2)_2NCH_3$	H	3.98	10.48	15.70	5.38
7c	6-$CH_2N(CH_2)_5$	H	2.24	5.28	8.57	10.06
7d	6-$CH_2N(CH_2)_4$	H	7.17	12.10	15.32	18.90
7e	6-$CH_2N(CH_2CH_2)_2NH$	H	2.62	6.34	9.85	7.27
1	H	H	0.095	0.18	0.68	1.07
Podo			0.003	0.012	0.020	0.016
CA-4			0.002	0.003	0.005	0.020

[a]: IC_{50} values, concentrations required to inhibit 50% proliferation of human tumor cells after 48 h of treatment.

b: NT: Not tested.

Table 2. Antiproliferative activities of compounds **8a-g** and **9a-g** against four tumor cell lines

Comp.	R_1	R_2	$IC_{50}(\mu M)^a$			
			HepG2	MIA PaCa-2	MCF-7	Bel-7402
8a	6-O$(CH_2)_2$Cl	H	1.10	2.15	2.87	2.64
8b	6-O$(CH_2)_2$N$(CH_2CH_2)_2$O	H	4.28	5.27	6.86	6.19
8c	6-O$(CH_2)_2$N$(CH_2CH_2)_2$NCH_3	H	4.55	4.25	8.88	4.59
8d	6-O$(CH_2)_2$N$(CH_2)_5$	H	5.50	5.34	6.15	6.37
8e	6-O$(CH_2)_2$N$(CH_2)_4$	H	4.95	5.13	6.00	6.26
8f	6-O$(CH_2)_2$N$(CH_2CH_2)_2$NH	H	5.93	6.25	7.85	9.11
8g	6-O$(CH_2)_2$N$(CH_2CH_2)_2$CHN$(CH_2)_4$	H	2.76	4.59	5.10	4.76
9a	6-O$(CH_2)_3$Cl	H	1.69	2.56	3.21	3.14
9b	6-O$(CH_2)_3$N$(CH_2CH_2)_2$O	H	8.12	9.31	9.55	10.26
9c	6-O$(CH_2)_3$N$(CH_2CH_2)_2$NCH_3	H	5.68	6.00	6.73	5.67
9d	6-O$(CH_2)_3$N$(CH_2)_5$	H	5.79	6.01	5.98	6.32
9e	6-O$(CH_2)_3$N$(CH_2)_4$	H	4.94	5.40	6.34	5.76
9f	6-O$(CH_2)_3$N$(CH_2CH_2)_2$NH	H	10.91	11.13	15.44	20.08
9g	6-O$(CH_2)_3$N$(CH_2CH_2)_2$CHN$(CH_2)_4$	H	5.15	5.78	6.05	5.93
1	H	H	0.095	0.18	0.68	1.07
Podo			0.003	0.012	0.020	0.016
CA-4			0.002	0.003	0.005	0.020

[a]: IC_{50} values, concentrations required to inhibit 50% proliferation of human tumor cells after 48 h of treatment.

Table 3. Antiproliferative activities of compounds **10a-e** and **11a-g** against four tumor cell lines

Comp.	R_1	R_2	$IC_{50}(\mu M)^a$			
			HepG2	MIA PaCa-2	MCF-7	Bel-7402
10a	6-O$(CH_2)_2$Cl	$(CH_2)_2$Cl	1.78	2.54	3.17	2.96
10b	6-O$(CH_2)_2$N$(CH_2CH_2)_2$O	$(CH_2)_2$N$(CH_2CH_2)_2$O	5.41	5.79	6.33	5.85
10c	6-O$(CH_2)_2$N$(CH_2CH_2)_2$NCH_3	$(CH_2)_2$N$(CH_2CH_2)_2$NCH_3	5.60	5.94	6.21	5.90
10d	6-O$(CH_2)_2$N$(CH_2)_5$	$(CH_2)_2$N$(CH_2)_5$	5.23	5.38	6.24	5.90
10e	6-O$(CH_2)_2$N$(CH_2)_4$	$(CH_2)_2$N$(CH_2)_4$	4.89	5.05	6.52	6.19
11a	6-O$(CH_2)_3$Cl	$(CH_2)_3$Cl	2.50	3.03	3.45	3.18
11b	6-O$(CH_2)_3$N$(CH_2CH_2)_2$O	$(CH_2)_3$N$(CH_2CH_2)_2$O	7.41	6.86	7.54	7.47
11c	6-O$(CH_2)_3$N$(CH_2CH_2)_2$NCH_3	$(CH_2)_3$N$(CH_2CH_2)_2$NCH_3	4.22	8.15	20.54	4.75
11d	6-O$(CH_2)_3$N$(CH_2)_5$	$(CH_2)_3$N$(CH_2)_5$	1.53	2.00	2.57	2.32
11e	6-O$(CH_2)_3$N$(CH_2)_4$	$(CH_2)_3$N$(CH_2)_4$	3.32	5.10	5.63	4.95
11f	6-O$(CH_2)_3$N$(CH_2CH_2)_2$NH	$(CH_2)_3$N$(CH_2CH_2)_2$NH	10.35	11.11	14.36	13.75
11g	6-O$(CH_2)_3$N$(CH_2CH_2)_2$CHN$(CH_2)_4$	$(CH_2)_3$N$(CH_2CH_2)_2$CHN$(CH_2)_4$	6.12	6.50	6.94	6.20
1	H	H	0.095	0.18	0.68	1.07
Podo			0.003	0.012	0.020	0.016
CA-4			0.002	0.003	0.005	0.020

[a]: IC_{50} values, concentrations required to inhibit 50% proliferation of human tumor cells after 48 h

of treatment.

Compounds **7a–e** are derivatives of aldehydes with antitumor activity at the same levels and IC_{50} values against HepG2 at 1–10 μM, which are at least 10 times lower than that of compound **1**. Compounds **8a-g**, **9a-g**, **10a-e**, and **11a-g** are hydroxyl group derivatives, with **10a-e** and **11a-g** having the same groups introduced at hydroxyls and sulfonamides. The antitumor activity of each compound was generally reduced. In addition to **8a**, **9a**, **10a**, and **11a**, the four compounds used originally as key intermediates against HepG2 cells had IC_{50} values of approximately 1–2 μM, other compounds containing water-soluble groups IC_{50} at 5–10 μM were tested. The antitumor activities of compounds containing two C atoms in the ether chain were equivalent to those of compounds containing three C atoms, and the IC_{50} values against HepG2 were 1–10 μM. The antitumor activity of compounds **10a–e** and **11a–g** which introduce group on the hydroxy and sulfonamide were equivalent to that of the compounds **8a–g** and **9a–g** which only introduce the same group on the hydroxyl; the IC_{50} against HepG2 was generally 5–10 μM. The antitumor activity of all derivatives against the other three tumor cells (IC_{50}: 5–20 μM) was generally weaker or equivalent to their activity against HepG2.

Table 4. Antitumor activities of compounds and their corresponding hydrochlorides against four tumor cell lines

Comp.	R_1	R_2	IC_{50}(μM)[a]			
			HepG2	MIA PaCa-2	MCF-7	Bel-7402
7b	6-$CH_2N(CH_2CH_2)_2NCH_3$	H	3.98	10.48	15.70	5.38
7b•HCl	6-$CH_2N(CH_2CH_2)_2NCH_3$	H	5.76	NT[b]	NT[b]	NT[b]
8f	6-$O(CH_2)_2N(CH_2CH_2)_2NH$	H	5.93	6.25	7.85	9.11
8f•HCl	6-$O(CH_2)_2N(CH_2CH_2)_2NH$	H	10.17	NT[b]	NT[b]	NT[b]
8g	6-$O(CH_2)_2N(CH_2CH_2)_2CHN(CH_2)_4$	H	2.76	4.59	5.10	4.76
8g•HCl	6-$O(CH_2)_2N(CH_2CH_2)_2CHN(CH_2)_4$	H	8.28	NT[b]	NT[b]	NT[b]
9c	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	H	5.68	6.00	6.73	5.67
9c•HCl	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	H	5.41	NT[b]	NT[b]	NT[b]
9d	6-$O(CH_2)_3N(CH_2)_5$	H	5.79	6.01	5.98	6.32
9d•HCl	6-$O(CH_2)_3N(CH_2)_5$	H	8.65	NT[b]	NT[b]	NT[b]
10b	6-$O(CH_2)_2N(CH_2CH_2)_2O$	$(CH_2)_2N(CH_2CH_2)_2O$	5.41	5.79	6.33	5.85
10b•HCl	6-$O(CH_2)_2N(CH_2CH_2)_2O$	$(CH_2)_2N(CH_2CH_2)_2O$	6.37	NT[b]	NT[b]	NT[b]
10c	6-$O(CH_2)_2N(CH_2CH_2)_2NCH_3$	$(CH_2)_2N(CH_2CH_2)_2NCH_3$	5.60	5.94	6.21	5.90

10c•HCl	6-O$(CH_2)_2$N$(CH_2CH_2)_2NCH_3$	$(CH_2)_2N(CH_2CH_2)_2NCH_3$	6.16	5.08	8.17	4.96
10d	6-O$(CH_2)_2$N$(CH_2)_5$	$(CH_2)_2N(CH_2)_5$	5.23	5.38	6.24	5.90
10d•HCl	6-O$(CH_2)_2$N$(CH_2)_5$	$(CH_2)_2N(CH_2)_5$	5.87	NT[b]	NT[b]	NT[b]
10e	6-O$(CH_2)_2$N$(CH_2)_4$	$(CH_2)_2N(CH_2)_4$	4.89	5.05	6.52	6.19
10e•HCl	6-O$(CH_2)_2$N$(CH_2)_4$	$(CH_2)_2N(CH_2)_4$	5.17	NT[b]	NT[b]	NT[b]
11c	6-O$(CH_2)_3$N$(CH_2CH_2)_2NCH_3$	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	4.22	8.15	20.54	4.75
11c•HCl	6-O$(CH_2)_3$N$(CH_2CH_2)_2NCH_3$	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	14.64	NT[b]	NT[b]	NT[b]
11d	6-O$(CH_2)_3$N$(CH_2)_5$	$(CH_2)_3N(CH_2)_5$	1.53	2.00	2.57	2.32
11d•HCl	6-O$(CH_2)_3$N$(CH_2)_5$	$(CH_2)_3N(CH_2)_5$	2.37	NT[b]	NT[b]	NT[b]
11e	6-O$(CH_2)_3$N$(CH_2)_4$	$(CH_2)_3N(CH_2)_4$	3.32	5.10	5.63	4.95
11e•HCl	6-O$(CH_2)_3$N$(CH_2)_4$	$(CH_2)_3N(CH_2)_4$	2.15	NT[b]	NT[b]	NT[b]

[a]: IC_{50} values, concentrations required to inhibit 50% proliferation of human tumor cells after 48 h of treatment.

[b]: NT: not tested.

Because the introduced ionizable groups contained free amines, the corresponding hydrochloride compounds were synthesized. We selected the hydrochloride of the representative compound from each series, and tumor cell inhibitory activity of the hydrochloride compounds was evaluated. The data in **Table 4** shows that the *in vitro* antitumor activities of most compounds, except **8g** and **11c**, were equivalent to those of their corresponding hydrochloride salts.

2.3 The aqueous solubility

An important goal of this research was to increase the aqueous solubility of the new compounds compared to that of **1**. To assess this property, we measured the aqueous solubility of several representative compounds in each series using an high-performance liquid chromatography/ultraviolet (HPLC/UV) method (method A) under two conditions [18], pH = 2.0 and pH = 7.4, as shown in **Table 5**. Each tested compound showed improved solubilities at both pH 2.0 and pH 7.4 compared with those of **1**. The water solubility of the polar group-containing compounds were 10–100 times higher than those of **1** under at pH = 2.0 and pH = 7.4, especially with the introduction of an ionizable group (i.e., a nitrogen-containing heterocycle). Most of the compounds showed comparable water solubilities under both conditions, whereas amino acids and most ionizable groups have water solubilities at pH = 2.0 that are significantly better than those at pH = 7.4. However, there were exceptions. Compounds **9e**, **9g**, **11e**, and **11g** had water solubilities at pH = 7.4 that were 2–3

times higher than those at pH = 2.0.

The aqueous solubilities of the corresponding hydrochloride salts of compounds **7a–e**, **8b–g**, **9b–g**, **10b–e**, and **11b-g**, were measured by adding excess quantities the hydrochlorides to a predetermined volume of distilled water (**method B**) [19]. Due to limited quantities of the hydrochlorides, no further effort was made to measure the maximum solubility of these compounds. Their aqueous solubilities (> 30 mg/mL at pH 7.0) were improved 300,000 times relative to that of **1**, so they may exhibit more antitumor activity *in vivo* than *in vitro*.

Table 5. Aqueous solubilities of representative compounds

Comp.	R_1	R_2	Aqueous solubility (μg/mL)	
			pH = 2.0	pH = 7.4
7a	6-$CH_2N(CH_2CH_2)_2O$	H	13.4	43.4
7b	6-$CH_2N(CH_2CH_2)_2NCH_3$	H	118.8	30.2
7c	6-$CH_2N(CH_2)_5$	H	75.7	21
8d	6-$O(CH_2)_2N(CH_2)_5$	H	112.8	36.6
8f	6-$O(CH_2)_2N(CH_2CH_2)_2NH$	H	122.0	59.4
9c	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	H	52.9	76.4
9e	6-$O(CH_2)_3N(CH_2)_4$	H	22.4	66.3
9g	6-$O(CH_2)_3N(CH_2CH_2)_2CHN(CH_2)_4$	H	36.9	91.3
10d	6-$O(CH_2)_2N(CH_2)_5$	$(CH_2)_2N(CH_2)_5$	70.8	55.3
11c	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	71.2	85.5
11e	6-$O(CH_2)_3N(CH_2)_4$	$(CH_2)_3N(CH_2)_4$	35.7	62.7
11g	6-$O(CH_2)_3N(CH_2CH_2)_2CHN(CH_2)_4$	$(CH_2)_3N(CH_2CH_2)_2CHN(CH_2)_4$	76.2	176.5
1	H	H	0.10	< 0.10

2.4 Inhibition of tubulin polymerization *in vitro*

1, **CA-4P** inhibited tubulin polymerization in a colchicine-like manner [20]. We further evaluated the effects of the compounds **7b**, **8c**, **9c**, **10c** and **11c**, which selected from each series and their 6-position have the same derived group, on tubulin *in vitro*. The effects on the assembly of purified porcine tubulin were evaluated by measuring an increase in absorbance at 340 nm at 37 ℃, by using **1**, **CA-4P** and **Taxol** as comparative agents.

As depicted in **Fig.4** and **Table 6**, Compounds **7b**, **8c**, **9c**, **10c** and **11c** showed little inhibition of tubulin polymerization at concentrations of 10 μM. At a

concentration of 100 μM, the inhibition of tubulin polymerization was improved, but was significantly weaker than that of **CA-4P** and **1**, which was basically consistent with the results of anti-tumor activity *in vitro*. At 100 μM, the inhibition rate of all compounds was lower than 50% except compound **9c**, which reached 84%, indicating that these compounds had little effect on tubulin. According to the above results, although these compounds were derived from **1**, their targets were different from those of **1** and **CA-4P**.

Table 6. Inhibition of tubulin polymerization

Comp.	R_1	R_2	Inhibition of tubulin polymerization (%)[a]	
			10 μM	100 μM
7b	6-$CH_2N(CH_2CH_2)_2NCH_3$	H	0	27
8c	6-$O(CH_2)_2N(CH_2CH_2)_2NCH_3$	H	3	49
9c	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	H	4	84
10c	6-$O(CH_2)_2N(CH_2CH_2)_2NCH_3$	$(CH_2)_2N(CH_2CH_2)_2NCH_3$	3	8
11c	6-$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	6	12
1	-	-	91	94
CA-4P	-	-	95	94
Taxol	-	-	-	-

[a]: inhibition% = 100 - activity%, activity% = $A_{test}/A_{control}$

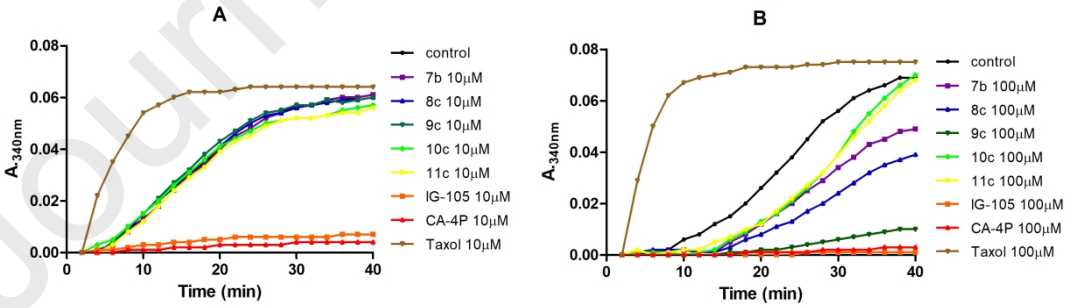


Fig. 4. Effects of test compounds on tubulin polymerization *in vitro*. Test compounds, **IG-105**, **CA-4P**, **Taxol** and DMSO were added in the plates and then polymerizations were tested as an increase in absorbance at 340 nm over 40 min at 37 ℃. (**Fig. 4A** 10 μM and **Fig. 4B** 100 μM)

2.5 *In vivo* antitumor activity

The antitumor activities of novel derivatives (IC_{50}: 1–20 μM) were significantly

reduced compared with that of compound **1**, but the aqueous solubilities of the compounds were significantly improved. To investigate the effect of the aqueous solubilities of the compounds on its antitumor activity *in vivo*, and consider the antitumor activity and water solubility of the compound comprehensively, we selected compound **7b·HCl** for *in vivo* antitumor activity testing, and established a HepG2 model in BALB/c nude mice [21]. Remarkably, **7b·HCl** inhibited tumor growth in a dose-dependent manner. **CA-4P** at a dose of 50 mg/kg inhibited tumor growth by 48.3%, whereas **7b·HCl** at doses of 10 mg/kg and 20 mg/kg resulted in 41.1% and 54.6% inhibition, respectively. **Taxol** at a dose of 10 mg/kg inhibited tumor growth by 78.3% (**Fig. 5A**).

To investigate *in vivo* the antitumor activity of other series of hydrochlorides, we selected compounds **9c·HCl** and **11c·HCl** for *in vivo* antitumor activity testing, and established another HepG2 model in BALB/c nude mice. They were randomized into 4 groups named blank control (saline solution), **7b·HCl** (positive control), **9c·HCl** and **11c·HCl**. **7b·HCl** at a dose of 20 mg/kg inhibited tumor growth by 63.6%， **9c·HCl and 11c·HCl** at doses of 10 mg/kg resulted in 61.1% and 50.0% inhibition, respectively (**Fig. 6A**).

The inhibition rate of **Taxol** (dose 10 mg/kg) reached 78.3%, but the weight of mice in this group was significantly decreased, which indicated that cytotoxicity of **Taxol** was greater than that of **CA-4P** and **7b•HCl**. During the experiments, there were no deaths and no significant adverse reactions such as lethargy or anorexia, and the changes in body weight were in an acceptable range (**Fig. 5B**, **Fig.6B**).

The tumor inhibition rate of **7b·HCl** (20 mg/kg) was 54.6% and 63.6%, respectively, which was within the acceptable range considering that the two batches of animal experiments were conducted by different companies. Although the antitumor activity against HepG2 of **CA-4** (IC_{50}: 0.002 μM) was 2000 times higher than that of compound **7b** (IC_{50}: 3.98 μM) *in vitro*, the inhibition rate of **7b·HCl** (dose, 20 mg/kg) was higher than that of **CA-4P** (dose, 50 mg/kg) *in vivo*. Compounds **9c** (IC_{50}: 5.68 μM) and **11c** (IC_{50}: 4.22 μM), which have poor activity *in vitro*, and the tumor inhibition rate of their hydrochloride salts *in vivo* can reach 61.1% and 50.0%,

showing efficient *in vivo*. This is a very interesting result, suggesting that the derivatives may have novel anti-tumor mechanisms, and we will have further studies in the future.

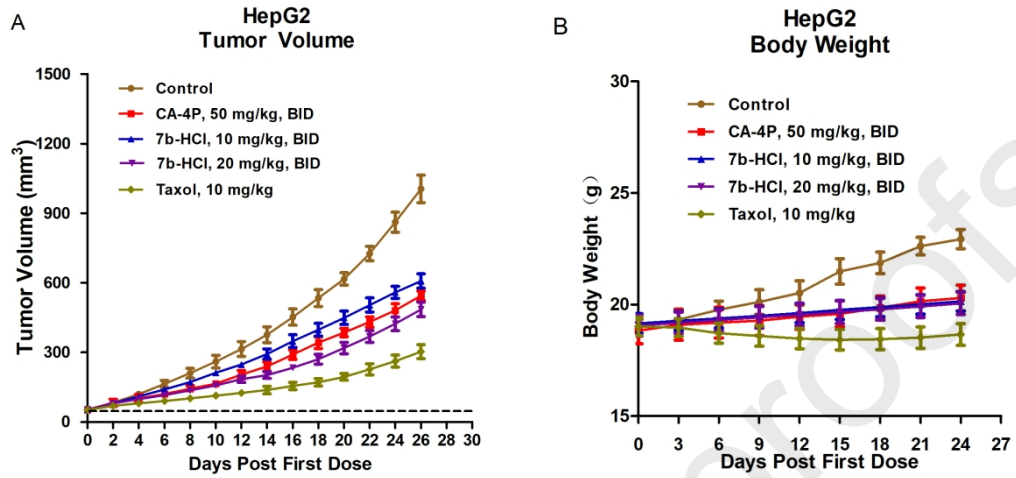


Fig 5. *In vivo* efficacy of **7b·HCl** against HepG2 cells. (A) Antitumor effects of **7b·HCl** in BALB/c nude mice bearing HepG2. (B) Body weight changes in HepG2-bearing mice.

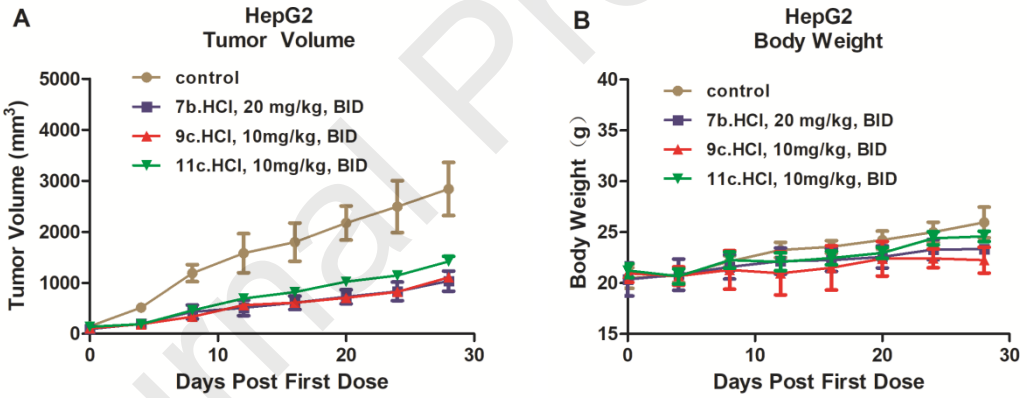


Fig 6. *In vivo* efficacy of **7b·HCl, 9c·HCl and 11c·HCl** against HepG2 cells. (A) Antitumor effects of **7b·HCl, 9c·HCl and 11c·HCl** in BALB/c nude mice bearing HepG2. (B) Body weight changes in HepG2-bearing mice.

4. Conclusion

Based on our optimization strategy, 31 novel carbazole sulfonamide derivatives were synthesized by modifying the R group on the carbazole ring of **1**. All of the compounds showed moderate antitumor activity against HepG2, MIA PaCa-2, MCF-7, and Bel-7402 cell lines, with micromolar IC_{50} values (1.00–10.0 μM) higher than **1**. The water solubility of compounds at both pH levels was higher than that of **1**, with

the water solubility of corresponding hydrochlorides > 30 mg/mL. From the results of evaluating the effects of the compounds **7b**, **8c**, **9c**, **10c** and **11c** on tubulin *in vitro*, we speculated that their targets were different from those of **1** and **CA-4P**. The *in vitro* antitumor activity against HepG2 of compound **7b (**IC_{50}: 3.98 μM**)** was significantly lower than that of **CA-4** (IC_{50}: 0.002 μM), but the inhibition rate of **7b·HCl** (20 mg, 54.6%) was higher than that of **CA-4P** (50 mg, 48.3%) *in vivo*. And in another batch of *in vivo* antitumor activity testing, **9c·HCl** and **11c·HCl** at doses of 10 mg/kg resulted in 61.1% and 50.0% inhibition, respectively. Studies have shown that derivatives with poor *in vitro* efficacy like **7b·HCl, 9c·HCl** and **11c·HCl** are efficient *in vivo* owing to their excellent biodistribution or novel antitumor mechanism, which will be further explored in future research.

Modifications of the R substituent on the carbazole ring provided the following new structure-activity relationships (SAR) information: (1) the 6 position on carbazole rings were active variable sites, and the long chain nitrogen-containing heterocyclic compounds derived from aldehydes and hydroxyl groups at the 6-position R_1, both alkanes and ethers, were associated with a significant decrease in antitumor activity *in vitro*; (2) the antitumor activities of compounds (**8a–g**) containing two C atoms in the ether chain were equivalent to those of compounds (**9a–g**) containing three C atoms, the length of the ether chain has little effect on *in vitro* antitumor activity; (3) R_3 is also a reactive variable group, and *in vitro* antitumor activity decreased after introduction of the alkyl chain; (4) the *in vitro* antitumor activity of compounds **8a**, **9a**, **10a** and **11a** is higher than that of other compounds in respective series. The introduction of a larger group at the end of the alkyl chain may decrease the activity *in vitro*, and the activity of compounds having different nitrogen-containing heterocycles is equal; (5) at both pH 2.0 and 7.4, the solubilities of all derivatives were much higher than those of **1**, and the structures of nitrogen-containing heterocycles improved the water solubility of the compounds.

4. Experimental section

4.1. Chemistry

Melting points were measured using a Mettler Toledo MP90 melting apparatus

without correction. The nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were measured on a MERCURY-400 spectrometer using tetramethylsilane (TMS) as the internal standard. Unless otherwise indicated, d-DMSO was the solvent used. Mass spectra were obtained on an AutoSpec Ultima-Tof mass spectrometer with electrospray ionization, and the relative intensity of each ion peak is presented as a percentage (%). The microwave reactions were performed on a microwave reactor from CEM Inc. Thin-layer chromatography (TLC) and preparative TLC plates were used with silica gel GF254 (200-300 mesh) purchased from Qingdao Haiyang Chemical Company. Medium-pressure column chromatography was performed with a CombiFlash companion purification system. All chemical reagents and solvents were obtained from Beijing Chemical Works or J&K.

4.2. Synthesis

4.2.1. Final compounds 7a-e

General procedures used to prepare 7a–e. To a solution of **5** (425 mg, 1.0 mmol) in THF (10 mL), different heterocyclic amines (2 mmol) and $Ti(OiPr)_4$ (0.36 mL, 1.2 mmol) were added at room temperature. After stirring for 1.0 h, sodium cyanoborohydride ($NaBH_3CN$, 252 mg, 4.0 mmol) was added slowly, and the mixture was stirred for 1.0–2.0 h. The reaction was quenched with cold water and subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography to obtain the corresponding compounds.

4.2.1.1.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(morpholinomethyl)-9*H*-carbazole-3-sulfonamide (7a).** Starting with **5** (425 mg, 1.0 mmol), morpholine (0.18 mL, 2.0 mmol) was added to produce 200 mg of **7a** in 40.3% yield, white solid, mp 193–195°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.39 (4H, m), 3.43 (3H, s), 3.58 (4H, m), 3.63 (2H, s), 3.72 (3H, s), 3.91 (3H, s), 6.29 (1H, d, J = 8.4 Hz), 7.43 (1H, d, J = 8.4 Hz), 7.50 (1H, dd, J = 8.4, 1.2 Hz), 7.62 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 8.4 Hz), 7.74 (1H, dd, J = 8.4, 1.2 Hz), 8.12 (1H, d, J = 1.2 Hz), 8.46 (1H, d, J = 1.2 Hz), 9.31 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.83, 53.40, 53.59, 53.85,

63.10, 66.69, 101.13, 109.59, 110.06, 112.73, 120.23, 121.43, 121.47, 121.91, 124.68, 128.60, 129.83, 130.78, 139.69, 141.18, 142.91, 157.17, 160.64; **HRMS (ESI+)** 497.18525 calculated for $C_{25}H_{29}O_5N_4S$ 497.18532 [M+H] +.

7a·HCl

7a (100 mg, 0.20 mmol) was directly dissolved in methanol (5 mL), 3 M HCl-methanol (0.04 mL) was added, and the solution was stirred in an ice bath for 1 h. After removing the solvent, the residue was purified by added anhydrous ether, and the solution was stirred at room temperature for 0.5 h and filtered to obtain the corresponding approximately 100 mg of the hydrochloride salt **7a ·HCl**. **MS *m/z* (%)** 497 (M+1, 100).

4.2.1.2.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-((4-methylpiperazin-1-yl)methyl)-9*H*-carbazole-3-sulfonamide (7b).** Starting with **5** (425 mg, 1.0 mmol), N-methyl piperazine (0.22 mL, 2.0 mmol) was added to produce 250 mg of **7b** in 49.1% yield, white solid, mp 177.5–179.5°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.17 (3H, s), 2.22-2.46 (8H, m), 3.43 (3H, s), 3.63 (2H, s), 3.72 (3H, s), 3.92 (3H, s), 6.30 (1H, d, *J* = 8.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.49 (1H, dd, *J* = 8.4, 1.2 Hz), 7.62 (1H, d, *J* = 8.4 Hz), 7.71 (1H, d, *J* = 8.4 Hz), 7.74 (1H, dd, *J* = 8.4, 1.2 Hz), 8.11 (1H, d, *J* = 1.2 Hz), 8.46 (1H, d, *J* = 1.2 Hz), 9.32 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.83, 46.13, 52.87, 53.40, 53.85, 55.18, 62.68, 101.13, 109.57, 110.03, 112.74, 120.20, 121.27, 121.49, 121.89, 124.65, 128.52, 130.34, 130.76, 139.67, 141.15, 142.90, 157.16, 160.63; **HRMS (ESI+)** 510.21684 calculated for $C_{26}H_{32}O_4N_5S$ 510.21695 [M+H] +.

7b·HCl

This compound was synthesized using the same procedure described for **7a** with **7b** (100 mg, 0.20 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **7b·HCl**. **MS *m/z* (%)** 510 (M+1, 100).

4.2.1.3.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(piperidin-1-ylmethyl)-9*H*-carbazole-3-sulfonamide (7c).** Starting with **5** (425 mg, 1.0 mmol), piperidine (0.20 mL, 2.0

mmol) was added to produce 300 mg of **7c** in 60.7% yield, white solid, mp 213–215°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.39 (2H, m), 1.47-1.50 (4H, m), 2.36 (4H, m), 3.43 (3H, s), 3.59 (2H, s), 3.72 (3H, s), 3.91 (3H, s), 6.29 (1H, d, *J* = 8.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.4Hz), 7.61 (1H, d, *J* = 8.4 Hz), 7.71 (1H, d, *J* = 8.4 Hz), 7.74 (1H, d, *J* = 8.4Hz), 8.09 (1H, s), 8.46 (1H, s), 9.32 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 24.55, 26.04, 29.83, 53.40, 53.84, 54.25, 63.45, 101.13, 109.55, 109.96, 112.75, 120.19, 121.21, 121.51, 121.86, 124.63, 128.52, 130.62, 130.74, 139.65, 141.11, 142.89, 157.15; **HRMS (ESI+)** 495.20596 calculated for $C_{26}H_{31}O_4N_4S$ 495.20605 [M+H] +.

7c·HCl

This compound was synthesized using the same procedure described for **7a** with **7c** (100 mg, 0.20 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **7c·HCl**. **MS *m/z* (%)** 495 (M+1, 100).

4.2.1.4.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(pyrrolidin-1-ylmethyl)-9*H*-carbazole-3-sulfonamide (7d).** Starting with **5** (425 mg, 1.0 mmol), pyrrolidine (0.17 mL, 2.0 mmol) was added to produce 200 mg of **7d** in 41.7% yield, white solid, mp 182–184°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.71 (4H, m), 2.48 (4H, m), 3.43 (3H, s), 3.72 (3H, s), 3.75 (2H, s), 3.91 (3H, s), 6.29 (1H, d, *J* = 8.4 Hz),7.43 (1H, d, *J* = 8.4 Hz), 7.50 (1H, dd, *J* = 8.4, 1.2 Hz), 7.60 (1H, d, *J* = 8.4 Hz), 7.70 (1H, d, *J* = 8.4 Hz), 7.74 (1H, dd, *J* = 8.4, 1.2 Hz), 8.11 (1H, s), 8.45 (1H, d, *J* = 1.2Hz), 9.31 (1H, s); **^{13}CNMR (DMSO-d_6, 100 MHz) δppm** 23.55, 29.84, 53.39, 53.83, 53.84, 60.04, 101.12, 109.59, 110.01, 112.76, 120.20, 120.91, 120.95, 121.53, 121.92, 124.65, 128.30, 130.78, 139.64, 141.11, 142.92, 157.16, 160.64; **HRMS (ESI+)** 481.19019 calculated for $C_{25}H_{29}O_4N_4S$ 481.19040 [M+H] +.

7d·HCl

This compound was synthesized using the same procedure described for **7a** with **7d** (100 mg, 0.21 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **7d·HCl**. **MS *m/z* (%)** 481 (M+1, 100).

4.2.1.5.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(piperazin-1-ylmethyl)-9*H*-carbazole-3-sulfonamide (7e).** Starting with **5** (425 mg, 1.0 mmol), piperazine (172 mg, 2.0 mmol) was added to produce 250 mg of **7e** in 50.5% yield, white solid, mp 168–170°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.41, 2.95 (4H, m), 3.34 (4H, m), 3.44 (3H, s), 3.68 (2H, s), 3.73 (3H, s), 3.92 (3H, s), 6.30 (1H, d, *J* = 8.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.50 (1H, dd, *J* = 8.4Hz, 1.2 Hz), 7.63 (1H, d, *J* = 8.4 Hz), 7.72 (1H, d, *J* = 8.4 Hz), 7.76 (1H, dd, *J* = 8.8 Hz, 1.4 Hz), 8.14 (1H, d, *J* = 1.2 Hz), 8.46 (1H, d, *J* = 1.2 Hz); **^{13}CNMR (DMSO-d_6, 100 MHz) δppm** 29.86, 44.53, 51.49, 53.42, 53.87, 62.70, 101.13, 109.66, 110.08, 112.76, 120.24, 121.44, 121.49, 121.94, 124.73, 128.61, 129.61, 130.84, 139.72, 141.22, 142.91, 157.22, 160.65; **HRMS (ESI+)** 496.20117 calculated for $C_{25}H_{30}O_4N_5S$ 496.20130 [M+H] +.

7e·HCl

This compound was synthesized using the same procedure described for **7a** with **7e** (100 mg, 0.20 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **7e·HCl**. **MS *m/z* (%)** 496 (M+1, 100).

4.2.2. Final compounds 8a-g

4.2.2.1.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-chloroethoxy)-9H-carbazole-3-sulfonamide (8a).** To a solution of **6** (413 mg, 1.0 mmol) and 1-bromo-2-chloroethane (0.17 mL, 2.0 mmol) in ACE, K_2O_3 (276 mg, 2.0 mmol) was added, and the mixture was refluxed for 5 h. The reaction mixture was concentrated under reduced pressure, quenched with cold water, and then subjected to EtOAc extraction three times. Then, the combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography (PE/EtOAc = 3/1) to give 354 mg of **8a** in 74.5% yield, white solid, mp 118–120°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 3.29 (3H, s), 3.61 (2H, t, 6.0 Hz), 3.79 (2H, t, 6.0 Hz), 3.81 (3H, s), 3.88 (3H, s), 6.40 (1H, d, *J* = 8.4 Hz), 7.05 (1H, dd, *J* = 8.8, 2.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.50 (1H, d, *J* = 8.4 Hz), 7.56 (1H, d, *J* = 2.4 Hz), 7.60 (1H, dd, *J* = 8.8, 1.8 Hz), 7.65 (1H, d, *J* = 8.8 Hz), 8.33 (1H, d, *J* = 1.8 Hz), 9.20 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.81, 43.29, 51.19, 53.43,

54.07, 101.62, 105.97, 109.42, 110.84, 113.70, 116.70, 120.80, 121.63, 122.91, 124.81, 128.45, 136.00, 143.30, 144.84, 152.10, 159.21, 162.32; **HRMS (ESI+)** 476.10407 calculated for $C_{22}H_{23}O_5N_3ClS$ 476.10415 [M+H] +.

General procedure to prepare 8b-g

Sodium iodide and different heterocyclic amines were added to **8a** in DMF (15 mL), and the mixture was refluxed overnight. The reaction was quenched with cold water, and subjected to EtOAc extraction three times. Then, the combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography to obtain the corresponding compounds.

4.2.2.2.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-morpholinoethoxy)-9H-carbazole-3-sulfonamide (8b).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and morpholine (0.87 mL, 10 mmol) were added to produce 300 mg of **8b** in 57.0% yield, white solid, mp 88–90°C. **^{1}HNMR (DMSO-d_6, 600 MHz) δppm** 2.25 (4H, m), 2.31 (2H, t, *J* = 6.4 Hz), 3.41 (3H, s), 3.43 (4H, m), 3.59 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 3.88 (3H, s), 6.36 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8 Hz, 2.4 Hz), 7.45 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.57 (1H, d, *J* = 2.4 Hz), 7.66 (2H, m), 8.36 (1H, s), 9.20 (1H, s); **^{13}CNMR (DMSO-d_6, 150 MHz) δppm** 29.80, 46.34, 53.44, 53.56, 54.01, 57.20, 66.54, 101.30, 105.95, 109.37, 110.80, 114.35, 116.64, 120.72, 121.59, 122.92, 124.86, 128.96, 135.98, 143.17, 144.49, 152.05, 159.54, 161.99; **HRMS (ESI+)** 527.19568 calculated for $C_{26}H_{31}O_6N_4S$ 527.19588 [M+H] +.

8b·HCl

This compound was synthesized using the same procedure described for **7a** with **8b** (100 mg, 0.19 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **8b·HCl**. **MS *m/z* (%)** 527 (M+1, 100).

4.2.2.3.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(4-methylpiperazin-1-yl)ethoxy)-9H-carbazole-3-sulfonamide (8c).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and *N*-methyl piperazine (1.1 mL, 10 mmol) were added to produce

305 mg of **8c** in 56.5% yield, white solid, mp 84–86°C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 2.07 (3H, s), 2.21-2.25 (4H, m), 2.30 (2H, m), 3.33-3.37 (4H, m), 3.41 (3H, s), 3.57 (2H, m), 3.82 (3H, s), 3.88 (3H, s), 6.35 (1H, d, *J* = 8.4 Hz),7.05 (1H, dd, *J* = 8.8, 2.4 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.57 (1H, d, *J* = 2.4 Hz), 7.66 (2H, m), 8.35 (1H, s), 9.20 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.79, 46.16, 46.65, 52.96, 53.43, 53.99, 55.06, 56.84, 101.29, 105.96, 109.36, 110.78, 114.40, 116.63, 120.71, 121.59, 122.92, 124.86, 128.98, 135.98, 143.17, 144.42, 152.04, 159.55, 161.95; **HRMS (ESI+)** 540.22742 calculated for $C_{21}H_{228}O_5N_3S$ 540.22752 [M+H] +.

8c·HCl

This compound was synthesized using the same procedure described for **7a** with **8c** (100 mg, 0.18 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **8c·HCl**. **MS *m/z* (%)** 540 (M+1, 100).

4.2.2.4.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(piperidin-1-yl)ethoxy)-9H-carbazole-3-sulfonamide (8d).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and piperidine (0.99 mL, 10 mmol) were added to produce 300 mg of **8d** in 57.3% yield, white solid, mp 93–95°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.30 (2H, m), 1.36 (4H, m), 2.22 (4H, m), 2.29 (2H, m), 3.41 (3H, s), 2.31 (2H, m), 3.82 (3H, s), 3.89 (3H, s), 6.36 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8 Hz, 2.0 Hz), 7.41 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.57 (1H, d, *J* = 2.0 Hz), 7.66 (2H, s), 8.35 (1H, s), 9.21 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 24.32, 25.89, 29.80, 46.75, 53.43, 54.01, 54.38, 57.57, 101.28, 105.95, 109.37, 110.80, 114.41, 116.63, 120.72, 121.58, 122.93, 124.87, 128.98, 135.98, 143.18, 144.47, 152.06, 159.53, 161.97; **HRMS (ESI+)** 525.21637 calculated for $C_{27}H_{33}O_5N_4S$ 525.21662 [M+H] +.

8d·HCl

This compound was synthesized using the same procedure described for **7a** with **8d** (100 mg, 0.19 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **8d·HCl**. **MS *m/z* (%)** 525 (M+1, 100).

4.2.2.5.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(pyrrolidin-1-yl)ethoxy)-9H-carbazole-3-sulfonamide (8e).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and pyrrolidine (0.85 mL, 10 mmol) were added to produce 300 mg of **8e** in 58.8% yield, white solid, mp 98–100°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.60 (4H, m), 2.36 (4H, m), 2.43 (2H, t, 6.0 Hz), 3.40 (3H, s), 3.58 (2H, t, 6.0 Hz), 3.82 (3H, s), 3.86 (3H, s), 6.37 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8 Hz, 2.0 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.57 (1H, d, *J* = 2.0 Hz), 7.66 (2H, s), 8.35 (1H, s), 9.21 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 23.56, 29.79, 48.30, 53.45, 53.89, 54.03, 54.55, 101.39, 105.94, 109.38, 110.81, 114.20, 116.64, 120.73, 121.58, 122.93, 124.85, 128.91, 135.98, 143.19, 144.49, 152.07, 159.50, 162.05; **HRMS (ESI+)** 511.20088 calculated for $C_{26}H_{31}O_5N_4S$ 511.20097 [M+H] +.

8e·HCl

This compound was synthesized using the same procedure described for **7a** with **8e** (100 mg, 0.20 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **8e·HCl**. **MS *m/z* (%)** 511 (M+1, 100).

4.2.2.6.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(piperazin-1-yl)ethoxy)-9H-carbazole-3-sulfonamide (8f).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and piperazine (860 mg, 10 mmol) were added to produce 318 mg of **8f** in 60.6% yield, white solid, mp 130–132°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.27 (4H, m), 2.31 (2H, t, *J* = 6.4 Hz), 2.66 (4H, m), 3.40 (3H, s), 3.57 (2H, t, *J* = 6.4 Hz), 3.82 (3H, s), 3.88 (3H, s), 6.36 (1H, d, *J* = 8.4 Hz), 7.05 (1H, dd, *J* = 8.8, 2.4 Hz), 7.44 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.56 (1H, d, *J* = 2.4 Hz), 7.64-7.66 (2H, m), 8.34 (1H, s), 9.22 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.33, 44.59, 46.00, 52.35, 52.97, 53.55, 56.69, 100.85, 105.48, 108.91, 110.35, 113.87, 116.18, 120.24, 121.12, 122.44, 124.38, 128.46, 135.51, 142.71, 144.07, 151.60, 159.05, 161.53; **HRMS (ESI+)** 526.21179 calculated for $C_{26}H_{32}O_5N_5S$ 526.21187 [M+H] +.

8f·HCl

This compound was synthesized using the same procedure described for **7a** with **8f** (100 mg, 0.19 mmol) instead of **7a** to obtain approximately 100 mg of the

corresponding hydrochloride salt **8f·HCl**. **MS *m/z* (%)** 526 (M+1, 100).

4.2.2.7.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(4-(pyrrolidin-1-yl)piperidin-1-yl)ethoxy)-9H-carbazole-3-sulfonamide (8g).** Starting with **8a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and 4-(1-pyrrolidinyl) piperidine (1.54 g, 10 mmol) were added to produce 325 mg of **8g** in 54.8% yield, white solid, mp 123–125°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.17-1.23 (2H, m), 1.61-1.62 (4H, m), 1.65 (2H, m), 1.79-1.84 (4H, m), 2.06 (1H,s), 2.27 (2H, m), 2.37 (4H, m), 3.41 (3H, s), 3.57 (2H, m), 3.82 (3H, s), 3.88 (3H, s), 6.36 (1H, d, J = 8.4 Hz), 7.05 (1H, dd, J = 8.8, 2.4 Hz), 7.42 (1H, d, J = 8.4 Hz), 7.48 (1H, d, J = 8.8 Hz), 7.56 (1H, d, J = 2.4 Hz), 7.64-7.66 (2H, m), 8.35 (1H, s), 9.25 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 23.33, 29.79, 31.27, 45.63, 46.88, 51.17, 52.27, 53.45, 54.01, 61.40,101.29, 105.92, 109.38, 110.79, 114.35, 116.63, 120.71, 121.61, 122.93, 124.86, 129.02, 135.98, 143.18, 144.46, 152.07, 159.56, 161.98; **HRMS (ESI+)** 594.27466 calculated for $C_{31}H_{40}O_5N_5S$ 594.27447 [M+H] +.

8g·HCl

This compound was synthesized using the same procedure described for **7a** with **8g** (100 mg, 0.017 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **8g·HCl**. **MS *m/z* (%)** 594 (M+1, 100).

4.2.3. Final compounds 9a-g

4.2.3.1.

6-(3-chloropropoxy)-*N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-9H-carbazole-3-sulfonamide (9a). To a solution of **6** (413 mg, 1.0 mmol) and 1-bromo-3-chloropropane (0.20 mL, 2.0 mmol) in ACE (10 mL), K_2O_3 (276 mg, 2.0 mmol) was added, and the mixture was refluxed for 5 h. The reaction mixture was concentrated under reduced pressure, quenched with cold water, and then subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography (PE/EtOAc = 4/1) to give 355 mg of **9a** in 72.6% yield, white solid, mp 148–150°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.78 (2H, m),

3.44 (3H, s), 3.60 (2H, t, *J* = 6.4 Hz), 3.66 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 3.89 (3H, s), 6.37 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8, 2.4 Hz), 7.41 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.56 (1H, d, *J* = 2.4 Hz), 7.63 (1H, dd, *J* = 8.8, 2.0 Hz), 7.68 (1H, d, *J* = 8.8 Hz), 8.32 (1H, d, *J* = 2.0 Hz), 9.21 (1H, s); **^{13}CNMR (DMSO-d_6, 151 MHz) δppm** 29.81, 31.86, 42.82, 47.14, 53.56, 54.06, 101.64, 105.91, 109.50, 110.85, 114.12, 116.71, 120.77, 121.63, 122.90, 124.82, 128.40, 136.00, 143.25, 143.95, 152.10, 159.71, 162.14;**HRMS (ESI+)** 490.11980 calculated for $C_{23}H_{25}O_5N_3ClS$ 490.11980 [M+H] +.

General procedure to prepare 9b–g:

To a solution of **9a** (1.0 mmol) in CH_3CN or DMF (15 mL), added different heterocyclic amines were added, and the mixture was refluxed for 3–24 h. The reaction was quenched with cold water and subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography to obtain the corresponding compounds.

4.2.3.2.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-morpholinopropoxy)-9H-carbazole -3-sulfonamide (9b).** Starting with **9a** (489 mg, 1.0 mmol), morpholine (0.87 mL, 10 mmol) was added to produce 290 mg of **9b** in 53.7% yield, white solid, mp 85–87°C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 1.45 (2H, m), 2.19 (4H, m), 2.24 (2H, t, *J* = 6.0 Hz), 3.44 (4H, m), 3.46 (3H, s), 3.49 (2H, t, *J* = 6.0 Hz), 3.83 (3H, s), 3.88 (3H, s), 6.38 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.4, 2.0 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.55 (1H, d, *J* = 2.0 Hz), 7.64 (1H, dd, *J* = 8.4, 1.2 Hz), 7.67 (1H, d, *J* = 8.8 Hz), 7.95 (1H, s), 8.33 (1H, d, *J* = 1.2 Hz), 9.21 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 25.57, 29.80, 47.54, 53.50, 53.59, 54.04, 55.44, 66.55, 101.50, 105.86, 109.46, 110.85, 114.24, 116.68, 120.76, 121.57, 122.89, 124.86, 128.68, 135.99, 143.19, 144.06, 152.09, 159.70, 162.02; **HRMS (ESI+)** 541.21143 calculated for $C_{27}H_{33}O_6N_4S$ 541.21253 [M+H] +.

9b·HCl

This compound was synthesized using the same procedure described for **7a** with **9b**

(100 mg, 0.19 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **9b·HCl**. **MS *m/z* (%)** 541 (M+1, 100).

4.2.3.3.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(4-methylpiperazin-1-yl)propoxy)-9H-carbazole-3-sulfonamide (9c).** Starting with **9a** (489 mg, 1.0 mmol), N-methyl piperazine (1.1 mL, 10 mmol) was added to produce 280 mg of **9c** in 50.6% yield, white solid, mp 96–98 °C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 1.43 (2H, m), 2.07 (3H, s), 2.17 (4H, m), 2.19 (4H, m), 2.22 (2H, t, *J* = 6.8 Hz), 3.46 (3H, s), 3.47 (2H, t, *J* = 6.8 Hz), 3.83 (3H, s), 3.88 (3H, s), 6.37 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.4, 2.2 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.55 (1H, d, *J* = 2.2 Hz), 7.64 (1H, dd, *J* = 8.8, 1.2 Hz), 7.67 (1H, d, *J* = 8.8 Hz), 8.32 (1H, d, *J* = 1.2 Hz), 9.24 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 25.95, 29.80, 46.16, 47.55, 52.93, 53.49, 54.04, 54.98, 55.14, 101.48, 105.88, 109.46, 110.82, 114.26, 116.67, 120.78, 121.57, 122.90, 124.85, 128.67, 135.98, 143.19, 144.04, 152.10, 159.69, 161.99; **HRMS (ESI+)** 554.24298 calculated for $C_{28}H_{36}O_5N_5S$ 554.24317 [M+H] +.

9c·HCl

This compound was synthesized using the same procedure described for **7a** with **9c** (100 mg, 0.18 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **9c·HCl**. **MS *m/z* (%)** 554 (M+1, 100).

4.2.3.4.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(piperidin-1-yl)propoxy)-9H-carbazole-3-sulfonamide (9d).** Starting with **9a** (489 mg, 1.0 mmol), piperidine (0.99 mL, 10 mmol) was added to produce 280 mg of **9d** in 52.0% yield, white solid, mp 125–127°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.39 (8H, m), 2.18 (6H, m), 3.45 (3H, s), 3.48 (2H, m), 3.83 (3H, s), 3.88 (3H, s), 6.38 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8, 2.4 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.54 (1H, d, *J* = 2.4 Hz), 7.63 (1H, dd, *J* = 8.8, 2.4 Hz), 7.67 (1H, d, *J* = 8.8 Hz), 8.32 (1H, d, *J* = 2.4 Hz), 9.20 (1H, s); **^{13}CNMR ($CDCl_3$, 101 MHz) δppm** 23.87, 25.11, 25.33, 29.49, 47.77, 53.09, 53.82, 54.41, 56.29, 101.24, 106.22, 107.97, 109.68, 113.46, 116.23, 120.95, 121.89, 123.16, 125.08, 128.93, 136.43, 143.20, 143.72, 150.56, 159.47, 162.30;

HRMS (ESI+) 539.23197 calculated for $C_{28}H_{35}O_5N_4S$ 539.23227 [M+H] +.

9d·HCl

This compound was synthesized using the same procedure described for **7a** with **9d** (100 mg, 0.190 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **9d·HCl**. **MS *m/z* (%)** 539 (M+1, 100).

4.2.3.5.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(pyrrolidin-1-yl)propoxy)-9H-carbazole-3-sulfonamide (9e).** Starting with **9a** (489 mg, 1.0 mmol), pyrrolidine (0.85 mL, 10 mmol) was added to produce 290 mg of **9e** in 55.3% yield, white solid, mp 88–90°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.45 (2H, m), 1.58 (4H, m), 2.26 (4H, m), 2.34 (2H, t, *J* = 6.4 Hz), 3.45 (3H, s), 3.51 (2H, t, *J* = 6.4 Hz), 3.84 (3H, s), 3.88 (3H, s), 6.38 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.4, 2.4 Hz), 7.41 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.4 Hz), 7.55 (1H, d, *J* = 2.4 Hz), 7.63 (1H, dd, *J* = 8.4, 1.6 Hz), 7.67 (1H, d, *J* = 8.4 Hz), 8.32 (1H, d, *J* = 1.6 Hz), 9.21 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 23.47, 27.91, 29.79, 47.79, 53.04, 53.47, 53.87, 54.04, 101.49, 105.88, 109.44, 110.83, 114.16, 116.66, 120.72, 121.57, 122.90, 124.84, 128.71, 135.98, 143.19, 144.08, 152.08, 159.70, 162.02; **HRMS (ESI+)** 525.21619 calculated for $C_{27}H_{33}O_5N_4S$ 525.21662 [M+H] +.

9e·HCl

This compound was synthesized using the same procedure described for **7a** with **9e** (100 mg, 0.19 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **9e·HCl**. **MS *m/z* (%)** 525 (M+1, 100).

4.2.3.6.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(piperazin-1-yl)propoxy)-9H-carbazole-3-sulfonamide (9f).** Starting with **9a** (489 mg, 1.0 mmol), piperazine (860 mg, 10 mmol) was added to produce 280 mg of **9f** in 51.9% yield, white solid, mp 118–120°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.40-1.47 (2H, m), 2.12 (4H, m), 2.20 (2H, m), 2.57 (4H, m), 3.46 (3H, s), 3.50 (2H, m), 3.84 (3H, s), 3.89 (3H, s), 6.31 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8 Hz, 2.2 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.55 (1H, d, *J* = 2.2 Hz), 7.64 (1H, dd, *J* = 8.8 Hz, 1.6 Hz), 7.67 (1H, d, *J*

= 8.8 Hz), 8.33 (1H, d, *J* = 1.6 Hz), 9.24 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 25.75, 29.80, 45.82, 47.63, 53.48, 54.04, 54.19, 55.75, 101.48, 105.86, 109.44, 110.83, 114.27, 116.67, 120.73, 121.58, 122.89, 124.85, 128.70, 135.98, 143.19, 144.05, 152.09, 159.68, 162.00 ; **HRMS (ESI+)** 540.22751 calculated for $C_{27}H_{34}O_5N_5S$ 540.22752 [M+H] +.

9f·HCl

This compound was synthesized using the same procedure described for **7a** with **9f** (100 mg, 0.18 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **9f·HCl**. **MS *m/z* (%)** 540 (M+1, 100).

4.2.3.7.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(4-(pyrrolidin-1-yl)piperidin-1-yl)propoxy)-9H-carbazole-3-sulfonamide (9g).** Starting with **9a** (489 mg, 1.0 mmol), 4-(1-pyrrolidinyl) piperidine (1.54 g, 10 mmol) was added to produce 280 mg of **9g** in 46.1% yield, white solid, mp 110–112°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.20 (2H, m), 1.42 (2H, m),1.62-1.66 (6H, m), 1.69-1.76 (2H, m), 1.80 (1H, m), 2.19 (2H, m),2.38 (4H, m), 2.58 (2H, m), 3.47 (2H, m), 3.48 (3H, s),3.84 (3H, s), 3.89 (3H, s), 6.38 (1H, d, *J* = 8.4 Hz), 7.06 (1H, dd, *J* = 8.8 Hz, 2.0 Hz), 7.44 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 7.55 (1H, d, *J* = 2.0 Hz),7.67 (2H, s), 8.34 (1H, s), 9.25 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 23.35, 26.15, 29.79, 31.54, 47.62, 51.21, 52.27, 53.50, 54.03, 55.10, 61.64, 101.49, 105.87, 109.46, 110.81, 114.25, 116.65, 120.78, 121.58, 122.91, 124.86, 128.69, 135.98, 143.19, 144.04, 152.11, 159.71, 161.99; **HRMS (ESI+)** 608.28839 calculated for $C_{32}H_{42}O_5N_5S$ 608.28839 [M+H] +.

9g·HCl

This compound was synthesized using the same procedure described for **7a** with **9g** (100 mg, 0.16 mmol) instead of **7a** to obtain approximately 100 mg of the corresponding hydrochloride salt **9g·HCl**. **MS *m/z* (%)** 608 (M+1, 100).

4.2.4. Final compounds 10a-e

4.2.4.1.

6-(2-chloroethoxy)-*N*-(2-chloroethyl)-*N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-9H-carbazole-3-sulfonamide (10a). To a solution of **6** (413 mg, 1.0 mmol) and

1-bromo-2-chloroethane (0.33 mL, 4.0 mmol) in ACE (10 mL), K_2O_3 (276 mg, 2.0 mmol) was added, and the mixture was refluxed for 8 h. The reaction mixture was concentrated under reduced pressure, quenched with cold water, and then subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography（PE/EtOAc = 5/1）to obtain 340 mg of **10a** in 63.2% yield, white solid, mp 130–132°C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 3.29 (3H, s), 3.63 (2H, t, 5.8 Hz), 3.81 (3H, s), 3.81 (2H, t, 5.8 Hz), 3.93 (3H, s), 4.01 (2H, t, *J* = 5.2 Hz), 4.38 (2H, t, *J* = 5.2 Hz), 6.41 (1H, d, *J* = 8.4 Hz), 7.22 (1H, dd, *J* = 8.8, 2.0 Hz), 7.52 (1H, d, *J* = 8.4 Hz), 7.61 (1H, s), 7.63 (1H, s), 7.70 (1H, d, *J* = 8.8 Hz), 8.04 (1H, d, *J* = 2.0 Hz), 8.57 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.89, 43.25, 43.68, 51.22, 53.43, 54.07, 69.13, 101.67, 105.36, 109.64, 111.19, 113.66, 117.18, 121.32, 121.87, 122.73, 124.98, 128.91, 136.99, 143.32, 144.90, 153.06, 159.19, 162.34;**HRMS (ESI+)** 539.0998 calculated for $C_{25}H_{25}Cl_2N_3O_5S$ 539.09967 [M+H] +.

General procedure to prepare 10b–e

Sodium iodide and different heterocyclic amines were added to **10a** in DMF (15 mL), and the mixture was refluxed overnight. The reaction was quenched with cold water and subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography (DCM/MeOH/TEA = 50/1/1) to obtain the corresponding compounds.

4.2.4.2.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-morpholinoethoxy)-*N*-(2-morpholinoethyl)-9H-carbazole-3-sulfonamide (10b).** Starting with **10a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and morpholine (0.87 mL, 10 mmol) were added to produce 300 mg of **10b** in 46.9% yield, white solid, mp 62–64°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.25 (4H, m), 2.32 (2H, t, *J* = 6.4 Hz), 2.53 (4H, m), 2.76 (2H, t, *J* = 6.4 Hz), 3.36, 3.58 (2H, t, *J* = 4.8 Hz), 3.39 (3H, s), 3.44 (4H, m), 3.60 (4H, m), 3.82 (3H, s), 3.92 (3H, s), 4.21 (2H, t, *J* = 6.0 Hz), 6.36 (1H, d, *J* = 8.4

Hz), 7.18 (1H, dd, 8.8 Hz, 2.2 Hz), 7.45 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.66 (1H, d, *J* = 8.8 Hz), 7.69 (1H, d, *J* = 8.8 Hz), 8.00 (1H, d, *J* = 2.2 Hz), 8.56 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.86, 46.36, 53.43, 53.57, 54.01, 54.14, 57.22, 57.66, 66.44, 66.54, 66.66 , 101.34, 104.98, 109.56, 111.04, 114.30, 117.09, 121.18, 121.86, 122.75, 124.94, 129.34, 136.73, 143.15, 144.59, 153.57, 159.55, 162.01; **HRMS (ESI+)** 640.27977 calculated for $C_{32}H_{42}O_7N_5S$ 640.27995 [M+H] +.

10b·HCl

This compound was synthesized using the same procedure described for **7a** with **10b** (100 mg, 0.16 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **10b·HCl**. **MS *m/z* (%)** 640 (M+1, 100).

4.2.4.3.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(4-methylpiperazin-1-yl)ethoxy)-*N*-(2-(4-methylpiperazin-1-yl)ethyl)-9H-carbazole-3-sulfonamide (10c).** Starting with **10a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and N-methyl piperazine (1.1 mL, 10 mmol) were added to produce 325 mg of **10b** in 48.9% yield, white solid, mp 58–60°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 2.07 (3H, s), 2.16 (3H, s), 2.18-2.23 (4H, m), 2.26-2.31 (4H, m), 2.32-2.41 (4H, m), 2.51-2.58 (4H, m), 2.74 (2H, t, *J* = 6.0 Hz), 3.36 (2H, t, *J* = 6.0 Hz), 3.39 (3H, s), 3.55 (2H, t, *J* = 6.0 Hz), 3.82 (3H, s), 3.91 (3H, s), 4.18 (2H, t, *J* = 6.0 Hz), 6.35 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 8.8 Hz, 2.2 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.65 (1H, dd, *J* = 8.8 Hz, 2.2 Hz), 7.69 (1H, d, *J* = 8.8 Hz), 8.00 (1H, d, *J* = 2.2 Hz), 8.56 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.86, 46.12, 46.19, 46.67, 52.93, 53.42, 53.51, 53.99, 55.04, 55.20, 56.85, 57.27, 66.73, 101.33, 104.99, 109.53, 111.01, 114.36, 117.09, 121.18, 121.88, 122.76, 124.92, 129.35, 136.71, 143.14, 144.52, 153.60, 159.55, 161.97; **HRMS (ESI+)** 666.34308 calculated for $C_{34}H_{48}O_5N_7S$ 666.34321 [M+H] +.

10c·HCl

This compound was synthesized using the same procedure described for **7a** with **10c** (100 mg, 0.15 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **10c·HCl**. **MS *m/z* (%)** 666 (M+1, 100).

4.2.4.4.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(piperidin-1-yl)ethoxy)-*N*-(2-(piperidin-1-yl)ethyl)-9H-carbazole-3-sulfonamide (10d).** Starting with **10a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and piperidine (0.99 mL, 10 mmol) were added to produce 300 mg of **10d** in 47.2% yield, white solid, mp 64–66°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.39-1.42 (4H, m), 1.46-1.50 (8H, m), 1.57-1.62 (8H, m), 2.27 (2H, m), 3.27 (2H, m), 3.38 (3H, s), 3.58 (2H, t, *J* = 6.0 Hz), 3.81 (3H, s), 3.91 (3H, s), 4.19 (2H, t, *J* = 6.0 Hz), 6.35 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 8.8 Hz, 2.2 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.65 (1H, dd, *J* = 8.8 Hz, 2.2 Hz), 7.69 (1H, d, *J* = 8.8 Hz), 8.00 (1H, d, *J* = 2.2 Hz), 8.56 (1H, *J* = 2.2 Hz); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 24.25, 25.85, 29.87, 46.73, 53.42, 54.00, 54.36, 54.70, 54.73, 57.56, 101.33, 105.03, 109.56, 111.03, 114.36, 117.11, 121.19, 121.86, 122.76, 124.95, 129.35, 136.75, 143.15, 144.56, 153.50, 159.51, 162.00; **HRMS (ESI+)** 636.32111 calculated for $C_{34}H_{46}O_5N_5S$ 636.32142 [M+H] +).

10d·HCl

This compound was synthesized using the same procedure described for **7a** with **10d** (100 mg, 0.16 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **10d·HCl**. **MS *m/z* (%)** 636 (M+1, 100).

4.2.4.5.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(2-(pyrrolidin-1-yl)ethoxy)-*N*-(2-(pyrrolidin-1-yl)ethyl)-9H-pyrido[2,3-b]indole-3-sulfonamide (10e).** Starting with **10a** (475 mg, 1.0 mmol), sodium iodide (1.5 g, 10 mmol) and pyrrolidine (0.85 mL, 10 mmol) were added to produce 310 mg of **10e** in 51.1% yield, white solid, mp 68–70°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.59 (4H, m), 1.69-1.72 (4H, m), 2.34 (4H, m), 2.42 (2H, t, *J* = 6.4 Hz), 2.57 (4H, m), 2.85 (2H, t, *J* = 6.0 Hz), 3.22,3.44 (2H, t, *J* = 6.4 Hz), 3.39 (3H, s), 3.82 (3H, s), 3.92 (3H, s), 4.19 (2H, t, *J* = 6.0 Hz), 6.36 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, 8.8 Hz, 2.2 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.65 (1H, d, *J* = 8.8 Hz), 7.67 (1H, d, *J* = 8.8 Hz), 7.80 (1H, d, *J* = 2.2 Hz), 8.57 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 23.57, 23.60, 29.86, 43.13, 45.81, 48.34, 53.44, 53.90, 54.51, 54.92, 67.74, 101.41, 104.90, 109.53, 111.04,

114.18, 117.07, 121.19, 121.86, 122.76, 124.92, 129.30, 136.70, 143.15, 144.58, 153.59, 159.49, 162.05; **HRMS (ESI+)** 608.29114 calculated for $C_{32}H_{42}O_5N_5S$ 608.29012 [M+H] +.

10e·HCl

This compound was synthesized using the same procedure described for **7a** with **10e** (100 mg, 0.16 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **10e·HCl**. **MS *m/z* (%)** 608 (M+1, 100).

4.2.5. Final compounds 11a-g

4.2.5.1.

6-(3-chloropropoxy)-*N*-(3-chloropropyl)-*N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-9H-carbazole-3-sulfonamide (11a). To a solution of **6** (413 mg, 1.0 mmol) and 1-bromo-3-chloropropane (0.40 mL, 4.0 mmol) in ACE (10 mL), K_2O_3 (276 mg, 2.0 mmol) was added, and the mixture was refluxed for 8 h. The reaction mixture was concentrated under reduced pressure, quenched with cold water, and then subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, the crude product was purified by column chromatography (PE/EtOAc = 7/1) to obtain 347 mg of **11a** in 61.3% yield, white solid, mp 138–139°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.79 (2H, m), 2.23 (2H, m), 3.43 (3H, s), 3.63 (2H, t, *J* = 6.4 Hz), 3.68 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 3.86 (2H, t, *J* = 6.4 Hz), 3.92 (3H, s), 4.22 (2H, t, *J* = 6.4 Hz), 6.38 (1H, d, *J* = 8.4 Hz), 7.20 (1H, dd, *J* = 8.8, 2.4 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.61 (1H, d, *J* = 8.8 Hz), 7.63 (1H, dd, *J* = 8.8, 1.8 Hz), 7.71 (1H, d, *J* = 8.8 Hz), 8.01 (1H, d, *J* =2.4 Hz), 8.56 (1H, d, *J* = 1.8 Hz); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 29.88, 31.88, 32.37, 42.61, 42.80, 47.15, 53.54, 54.05, 65.49, 101.67, 105.12, 109.67, 111.10, 114.08, 117.13, 121.29, 121.90, 122.74, 124.93, 128.80, 136.83, 143.24, 144.03, 153.50, 159.69, 162.15; **HRMS (ESI+)** 566.12788 calculated for $C_{26}H_{30}O_5N_3Cl_2S$ 566.12777 [M+H] +.

General procedure to prepare 11b–g

To a solution of **11a** (1.0 mmol) in acetonitrile or DMF (15 mL), different heterocyclic amines were added, and the mixtures were refluxed for 3–24 h. The

reactions were quenched with cold water and subjected to EtOAc extraction three times. The combined organics were washed with brine (10 mL) and dried with Na_2SO_4. After removing the solvent *in vacuo*, crude products were purified by column chromatography to obtain the corresponding compounds.

4.2.5.2.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-morpholinopropoxy)-*N*-(3-morpholinopropyl)-9H-carbazole-3-sulfonamide (11b).** Starting with **11a** (565 mg, 1.0 mmol), morpholine (0.87 mL, 10 mmol) was added to produce 330 mg of **11b** in 49.5% yield, white solid, mp 68–70°C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 1.64 (2H, m), 2.06 (2H, m), 2.41 (4H, m), 2.43 (2H, t, *J* = 5.6 Hz), 2.54 (4H, m), 2.62 (2H, t, *J* = 6.8 Hz), 3.40 (3H, s), 3.63 (2H, t, *J* = 5.6 Hz), 3.68 (4H, m), 3.76 (4H, m), 3.86 (3H, s), 3.87 (3H, s), 4.14 (2H, t, *J* = 6.8Hz), 6.29 (1H, d, *J* = 8.4 Hz), 7.18 (1H, d, *J* = 8.8 Hz), 7.32-7.38 (2H, m), 7.46 (1H, d, *J* = 8.4 Hz), 7.56 (1H, s), 7.74 (1H, d, *J* = 8.8 Hz), 8.38 (1H, s); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 25.59, 26.49, 29.86, 47.58, 53.48, 53.60, 53.86, 54.03, 55.46, 55.47, 66.54, 66.64, 66.95, 101.52, 104.89, 109.61, 111.04, 114.23, 117.11, 121.25, 121.84, 122.72, 124.92, 129.04, 136.69, 143.15, 144.14, 153.75, 159.68, 162.02; **HRMS (ESI+)** 668.31080 calculated for $C_{34}H_{46}O_7N_5S$ 668.31125 [M+H] +.

11b·HCl

This compound was synthesized using the same procedure described for **7a** with **11b** (100 mg, 0.15 mmol) instead of 7**a** to obtain approximately 100 mg of the corresponding hydrochloride salt **11b·HCl**. **MS *m/z* [%]** 668 [M+1, 100].

4.2.5.3.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(4-methylpiperazin-1-yl)propoxy)-*N*-(3-(4-methylpiperazin-1-yl)propyl)-9H-carbazole-3-sulfonamide (11c).** Starting with **11a** (565 mg, 1.0 mmol), N-methyl piperazine (1.1 mL, 10 mmol) was added to produce 300 mg of **11c** in 43.3% yield, white solid, mp 88-90 °C. **^{1}HNMR ($CDCl_3$, 400 MHz) δppm** 1.40-1.47 (2H, m), 1.88-1.95 (2H, m), 2.07 (3H, s), 2.15 (3H, s), 2.23 (2H, t, *J* = 6.4 Hz), 2.08-2.44 (16H, m), 2.46 (2H, t, *J* = 6.4 Hz), 3.46 (3H, s), 3.49 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 3.92 (3H, s), 4.12 (2H, t, *J* = 6.4 Hz), 6.38 (1H,

d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 8.8, 2.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.65 (1H, dd, *J* = 8.8, 1.6 Hz), 7.70 (1H, d, *J* = 8.8 Hz), 7.94 (1H, d, *J* = 2.4 Hz), 8.55 (1H, d, *J* = 1.6 Hz); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 25.98, 26.88, 29.86, 46.14, 46.20, 47.57, 52.92, 53.24 , 53.48, 54.03, 55.00, 55.12, 55.24, 67.03, 101.51, 104.88, 109.61, 111.03, 114.26, 117.11, 121.26, 121.84, 122.74, 124.91, 129.05, 136.69, 143.15, 144.12, 153.78, 159.68, 162.00; **HRMS (ESI+)** 694.37428, Calculated for $C_{36}H_{52}O_5N_7S$ 694.37451 [M+H] +.

11c·HCl

This compound was synthesized using the same procedure described for **7a** with **11c** (100 mg, 0.14 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **11c·HCl**. **MS *m/z* (%)** 694 (M+1, 100).

4.2.5.4.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(piperidin-1-yl)propoxy)-*N*-(3-(piperidin-1-yl)propyl)-9H-carbazole-3-sulfonamide (11d).** Starting with **11a** (565 mg, 1.0 mmol), piperidine (0.99 mL, 10 mmol) was added to produce 330 mg of **11d** in 49.8% yield, white solid, mp 116–118°C. **^{1}HNMR (DMSO-d_6, 600 MHz) δppm** 1.30 (2H, m), 1.36 (2H, m), 1.37-1.39 (4H, m), 1.43 (2H, m), 1.48-1.52 (4H, m), 1.91 (2H, m), 2.16 (4H, m), 2.20 (2H, t, *J* = 7.2 Hz), 2.35 (4H, m), 2.43 (2H, t, *J* = 7.2 Hz), 3.44 (3H, s), 3.49 (2H, t, *J* = 6.6 Hz), 3.83 (3H, s), 3.91 (3H, s), 4.11 (2H, t, *J* = 6.6 Hz), 6.37 (1H, d, *J* = 8.4 Hz), 7.16 (1H, dd, *J* = 8.8, 2.4 Hz), 7.41 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.63 (1H, dd, *J* = 8.8, 1.8 Hz), 7.69 (1H, d, *J* = 8.8 Hz), 7.93 (1H, d, *J* = 2.4 Hz), 8.54 (1H, d, *J* = 1.8 Hz); **^{13}CNMR (DMSO-d_6, 151 MHz) δppm** 24.54, 24.62, 25.98, 26.08, 26.93, 29.86, 40.53, 47.70, 53.46, 54.01, 54.34, 54.63, 55.75, 55.82, 67.13, 101.49, 104.87, 109.57, 111.03, 114.27, 117.13, 121.20, 121.86, 122.73, 124.90, 129.09, 136.69, 143.15, 144.14, 153.78, 159.66, 162.00;**HRMS (ESI+)** 664.35220 calculated for $C_{36}H_{50}O_5N_5S$ 664.35272 [M+H] +.

11d·HCl

This compound was synthesized using the same procedure described for **7a** with **11d** (100 mg, 0.15 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **11d·HCl**. **MS *m/z* (%)** 664 (M+1, 100).

4.2.5.5.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(pyrrolidin-1-yl)propoxy)-*N*-(3-(pyrrolidin-1-yl)propyl)-9H-carbazole-3-sulfonamide (11e).** Starting with **11a** (565 mg, 1.0 mmol), pyrrolidine (0.85 mL, 10 mmol) was added to produce 350 mg of **11e** in 55.1% yield, mp 76–78°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.44-1.51 (2H, m), 1.61 (4H, m), 1.71 (4H, m), 1.92-1.99 (2H, m), 2.33 (4H, m), 2.41 (2H, t, *J* = 7.0 Hz), 2.63 (4H, m), 3.36 (2H, t, *J* = 7.0 Hz), 3.42 (3H, s), 3.53 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 3.91 (3H, s),4.14 (2H, t, *J* = 6.4 Hz), 6.38 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 8.8 Hz, 2.4 Hz), 7.41 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.63 (1H, dd, *J* = 8.8 Hz, 1.8 Hz), 7.70 (1H, d, *J* = 8.8 Hz), 7.96 (1H, d, *J* = 2.4 Hz), 8.55 (1H, d, *J* = 1.8 Hz); **^{13}CNMR (DMSO-d_6, 101MHz) δppm** 22.98, 23.08, 27.35, 28.24, 29.38, 47.32, 52.31, 52.56, 52.98, 53.41, 53.55, 53.63, 66.49, 101.04, 104.37, 109.10, 110.55, 113.65, 116.63, 120.73, 121.39, 122.26, 124.41, 128.61, 136.20, 142.67, 143.69, 153.30, 159.21, 161.56; **HRMS (ESI+)** 636.32137 calculated for $C_{34}H_{46}O_5N_5S$ 636.32142 [M+H] +.

11e·HCl

This compound was synthesized using the same procedure described for **7a** with **11e** (100 mg, 0.16 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **11e·HCl**. **MS *m/z* (%)** 636 (M+1, 100).

4.2.5.6.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(piperazin-1-yl)propoxy)-*N*-(3-(piperazin-1-yl)propyl)-9H-carbazole-3-sulfonamide (11f).** Starting with **11a** (565 mg, 1.0 mmol), piperazine (860 mg, 10 mmol) was added to produce 330 mg of **11f** in 49.6% yield, mp 88–90°C. **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 1.42-1.48 (2H, m), 1.89-1.95 (2H, m), 2.14 (4H, m), 2.20 (2H, t, *J* = 7.2 Hz), 2.33 (4H, m), 2.44 (2H, t, *J* = 7.2 Hz), 2.59 (4H, m), 2.72 (4H, m), 3.44 (3H, s), 3.49 (2H, t, *J* = 6.8 Hz), 3.83 (3H, s), 3.92 (3H, s), 4.12 (2H, t, *J* = 6.8 Hz), 6.38 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 8.8 Hz, 2.4 Hz), 7.42 (1H, d, *J* = 8.4 Hz), 7.58 (1H, d, *J* = 8.8 Hz), 7.64 (1H, dd, *J* = 8.8 Hz, 1.8 Hz), 7.70 (1H, d, *J* = 8.8 Hz), 7.94 (1H, d, *J* = 2.4 Hz), 8.54 (1H, d, *J* = 1.8 Hz); **^{13}CNMR (DMSO-d_6, 100 MHz) δppm** 25.80, 26.68, 29.86, 45.86, 45.97, 47.67,

53.47, 54.03, 54.29, 54.56, 55.76, 67.08, 101.50, 104.90, 109.58, 111.03, 114.27, 117.11, 121.22, 121.85, 122.73, 124.91, 129.08, 136.69, 143.16, 144.14, 153.78, 159.66, 162.01; **HRMS (ESI+)** 666.34308 calculated for $C_{34}H_{48}O_5N_7S$ 666.34321 [M+H] +.

11f·HCl

This compound was synthesized using the same procedure described for **7a** with **11f** (100 mg, 0.15 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **11f·HCl**. **MS *m/z* (%)** 666 (M+1, 100).

4.2.5.7.

***N*-(2,6-dimethoxypyridin-3-yl)-9-methyl-6-(3-(4-(pyrrolidin-1-yl)piperidin-1-yl)propoxy)-*N*-(3-(4-(pyrrolidin-1-yl)piperidin-1-yl)propyl)-9H-carbazole-3-sulfonamide (11g).** Starting with **11a** (565 mg, 1.0 mmol), 4-(1-pyrrolidinyl) piperidine (1.54 g, 10 mmol) was added to produce 410 mg of **11g** in 51.2% yield, mp 86–88°C, **^{1}HNMR (DMSO-d_6, 400 MHz) δppm** 0.93-1.01 (4H, m), 1.16-1.29 (8H, m), 1.38-1.44 (4H, m), 1.63 (4H, m), 1.65 (2H, m), 1.66 (4H, m), 2.18-2.22 (2H, t, *J* = 6.8 Hz) 2.42-2.46 (8H, m), 2.57-2.60 (2H, m), 2.84-2.87 (2H, m), 3.00-3.07 (2H, t, *J* = 6.8 Hz), 3.33-3.39 (2H, t, *J* = 6.4 Hz), 3.46 (3H, s), 3.83 (3H, s), 3.91 (3H, s), 4.11 (2H, t, *J* = 6.4 Hz) 6.37 (1H, d, *J* = 8.4 Hz), 7.16 (1H, dd, *J* = 8.8 Hz, 2.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.57 (1H, d, *J* = 8.8 Hz), 7.65 (1H, dd, *J* = 8.8 Hz, 1.8 Hz), 7.69 (1H, d, *J* = 8.8 Hz), 7.93 (1H, d, *J* = 2.4 Hz), 8.55 (1H, d, *J* = 1.8 Hz); **^{13}CNMR (DMSO-d_6, 101 MHz) δppm** 21.30, 22.62, 22.67, 22.91, 25.66, 26.64, 29.38, 30.80, 30.96, 33.27, 50.67, 50.80, 51.75, 51.99, 52.04, 53.02, 53.55, 54.61, 54.68, 61.10, 61.26, 66.65, 101.57, 104.90, 109.60, 111.00, 114.23, 117.07, 121.26, 121.86, 122.75, 124.92, 129.07, 136.69, 143.16, 144.11, 153.78, 159.70, 162.00; **HRMS (ESI+)** 802.46594 calculated for $C_{44}H_{64}O_5N_7S$ 802.46842 [M+H] +.

11g·HCl

This compound was synthesized using the same procedure described for **7a** with **11g** (100 mg, 0.12 mmol) instead of **7a**, to obtain approximately 100 mg of the corresponding hydrochloride salt **11g·HCl**. **MS *m/z* (%)** 802 (M+1, 100).

4.3. Evaluation of final compounds for tumor cell killing *in vitro*. The SRB assay

was used for an *in vitro* anticancer study of the synthesized compounds. Human cancer cells were inoculated into 96-well microtiter plates at plating densities of 4000–6000 cells/well and incubated for 24 h. The cells were treated with compounds in DMSO after 24 h and then diluted in medium for concentrations of 2.5, 5, 10, and 20 μg/mL, with cells that contained no drugs/sample used as controls and blanks comprising complete medium with no cells. Incubation of plates for 48 h was followed by addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried, and dyed with SRB. The unbound dye was detached, 10 mM Tris base was used to extract protein-bound dye, and the optical densities of wells were determined using a multi-well spectrophotometer at the wavelength 490 nm. Inhibition of 50% cell growth inhibition was determined.

4.4. Aqueous Solubility Determination. Method A: Solubility was measured separately at pH 7.4 and pH 2.0 by using an HPLC-UV method. Test compounds were initially dissolved in DMSO at 10 mg/mL. Ten microliters of this stock solution was spiked into either pH 7.4 phosphate buffer (1.0 mL) or 0.01 M HCl (approximately pH 2.0, 1 mL) with the final DMSO concentration being 1%. The mixture was stirred for 4h at room temperature, and then concentrated at 3000 rpm for 10 min. The saturated supernatants were transferred to other vials for analysis by HPLC-UV. Each sample was performed in triplicate. For quantification, a model 1200 HPLC-UV (Agilent) system was used with an Agilent TC-C18 column (250 × 4.6 mm, 5 μm) and gradient elution of acetonitrile (ACN) in water, starting with 0% of ACN, which was linearly increased up to 70% over 10 min, then slowly increased up to 98% over 15 min. The flow rate was 1.0 mL/min and injection volume was 15 μL. Aqueous concentration was determined by comparison of the peak area of the saturated solution with a standard curve plotted peak area versus known concentrations, which were prepared by solutions of test compound in ACN at 50 μg/mL, 12.5 μg/mL, 3.125 μg/mL, 0.781μg/mL, and 0.195 μg/mL.

Method B: The solubility of the hydrochlorides was measured by adding excess quantities the hydrochlorides to a predetermined volume of distilled water. The

resulting mixtures were stirred at 25 °C for 24 h and the solutions were filtered, so concentration of the saturated solution is the corresponding water solubility.

4.5. *In vitro* tubulin polymerization inhibitory assay. Pig brain microtubule protein was isolated by three cycles of temperature-dependent assembly/disassembly according to Shelanski et al in 100 mM PIPES (pH 6.5), 1 mM $MgSO_4$, 2 mM EGTA, 1 mM GTP and 1 mM 2-Tubulin polymerization captoethanol [22]. In the first cycle of tubulin polymerization, glycerol and phenylmethylsulfonyl fluoride were added to 4 M and 0.2 mM, respectively. Homogeneous tubulin was prepared from microtubule protein by phosphocellulose (P11) chromatography. The purified proteins were stored in aliquots at -70℃.

Tubulin protein was mixed with different concentrations (10μM, 100μM) of compound **7b**, **8c**, **9c**, **10c**, **11c**, **1**, **CA-4P** and **Taxol** in PEM buffer (100 mM PIPES, 1 mM $MgCl_2$, and 1 mM EGTA) containing 1 mM GTP and 5 % glycerol. Microtubule Tubulin polymerization was monitored at 37°C by light scattering at 340 nm using a SPECTRA MAX 190 (Molecular Device) spectrophotometer. The plateau absorbance values were used for calculations.

4.6. Evaluation of 7b·HCl for antitumor efficacy *in vivo*. The animal testing procedures were carried out by Saier Biotechnology Inc. Briefly, 2×10^6/100 μL HepG2 tumor cells were implanted subcutaneously into the right armpits of BALB/c nude mice. Therapy was started on the 10^{th} day when tumor volumes reached approximately 50 mm^3. Then, the mice were randomly divided into five groups (n = 7 per group) named blank control (saline solution), positive control (**CA-4P** and **Taxol**), low dosage experiment group and high dosage experiment group, and intravenously injected with **Taxol** (10 mg/kg, one rejection) and **CA-4P** (50 mg/kg), **7b·HCl** (10 mg/kg), or **7b·HCl** (20 mg/kg) every two days for a total of 10 injections. No drug was given to the control group. The tumors were measured 2–3 times per week using calipers. Tumor volumes were calculated according to the formula $V = L \times W^2/2$, where L is the longest diameter of the tumor and W is the shortest diameter perpendicular to L. The tumor growth inhibition rate was calculated as [1 – (tumor volume $_{\text{treated final}}$ – tumor volume $_{\text{treated initial}}$ / (tumor volume $_{\text{control final}}$ – tumor volume

$_{control\ initial}$)] × 100%. At the end of the experiment, the mice were euthanized. Results of all experiment were presented as mean values ± standard deviation (SD) calculated using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA).

Notes

The authors declare no competing financial interest.

Abbreviations

SAR, structure-activity relationships; DMF, N,N-Dimethylformamide; DCM, dichloromethane; THF, tetrahydrofuran; ACE, acetone; SRB, sulforhodamine B; IC_{50}, concentration required to inhibit 50%; HPLC, high performance liquid chromatography; UV, ultraviolet rays; DMSO, dimethyl sulfoxide; ACN, acetonitrile; EtOAc, ethyl acetate; SD, standard deviation.

Ethical statement

All animal experiments were carried out in accordance with the guidelines of the Chinese Association for Laboratory Animal Sciences, and approved by the institutional ethical committee (IEC) of Peking Union Medical College.

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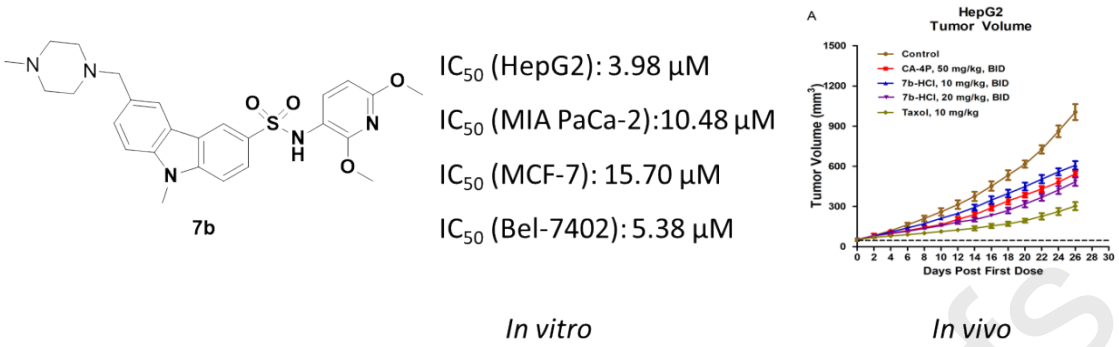
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphic abstracts:



Optimization of **IG-105** (**1**) on the carbazole ring provided five series of new carbazole sulfonamides derivatives. All of the compounds showed moderate cytotoxic activity against HepG2, MIA PaCa-2, MCF-7, and Bel-7402 cell lines, with micromolar IC_{50} values (IC_{50}: 1.00–10.0 μM). The water solubility of compounds at both pH levels was higher than that of **1**, with the water solubility of corresponding hydrochlorides > 30 mg/mL. From the results of evaluating the effects of the compounds **7b**, **8c**, **9c**, **10c** and **11c** on tubulin *in vitro*, we speculated that their targets were different from those of **1** and **CA-4P.** The *in vitro* antitumor activity against HepG2 of compound **7b (**IC_{50}: 3.98 μM**)** was significantly lower than that of **CA-4** (IC_{50}: 0.002 μM), but the inhibition rate of **7b·HCl** (20 mg, 54.6%) was higher than that of **CA-4P** (50 mg, 48.3%) *in vivo*. And in another batch of *in vivo* antitumor activity testing, **9c·HCl** and **11c·HCl** at doses of 10 mg/kg resulted in 61.1% and 50.0% inhibition, respectively. Studies have shown that derivatives with poor *in vitro* efficacy like **7b·HCl, 9c·HCl** and **11c·HCl** are efficient *in vivo* owing to their excellent biodistribution or novel antitumor mechanism, which will be further explored in future research.

Highlights

IG-105 optimization on the carbazole ring provided carbazole sulfonamides derivatives

In vitro and *in vivo* methods showed promising antitumor activity

These compounds may use a novel mechanism and serve as antitumor drugs