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Novel Carbazole Sulfonamide Derivatives of Antitumor Agent: Synthesis, Antiproliferative Activity and Aqueous Solubility

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Abstract:

The current optimization of IG-105 (**3**) on the carbazole–ring provided a series of new carbazole sulfonamides derivatives **13a-13m**. All of the compounds have been evaluated against HepG2 cells (hepatoma cancer) for antiproliferative activity. Compounds that showed activity better or comparable to that of **3** versus HepG2 were evaluated against MCF-7 (breast cancer), MIA PaCa-2 (pancreatic cancer), and Bel-7402 (hepatoma/liver cancer) for antiproliferative activity. Of the seven compounds selected for further study five **(13b**, **13g**, **13j**, **13k** and **13l)** were found to give IC_{50} values against the four cell lines comparable to those for **3**. Two compounds (**13f** and **13i)** were more active than **3** and their activity against HepG2 and MCF-7 (IC_{50}: 0.01-0.07 μM) approached that of the positive controls podophyllotoxin (**podo**) and **CA-4**. Most of compounds showed aqueous solubility (0.11-19.60 μg/mL at pH 7.4 and 2.0) better than **3.** These promising results warrant further development of new compounds **13f** and **13i** as potential potent antitumor drug candidates.

Keywords: carbazole sulfonamide derivatives; synthesis; antiproliferative activity; aqueous solubility

Microtubules, as key components of cytoskeleton, play important roles in a series of cellular processes such as regulation of motility, cell signaling, formation and maintenance of cell shape, secretion, and intracellular transport. Microtubule-binding agents (MBAs) are usually divided into 3 categories including taxol, vinblastine and colchicine binding site/domain inhibitors. [1, 2] Compounds like colchicine and podophyllotoxin (**podo**), discovered in the early days irreversibly bind at the colchicine domain thus inducing significant toxicities and limiting clinical use. The reversibly binding inhibitors discovered later, like Combretastatin A4 (CA-4, **1**), with effective dose much lower than maximum tolerated dose, have served as clinical candidates for further development.

<Figure1>

Combretastatin A4 (**1**), a novel small molecule tubulin binding agent which was isolated from the South African bush willow *Combretum caffrum* by Pettit et al., [3] strongly inhibits the polymerization of tubulin by binding to the colchicine binding site. [4] Potent cytotoxicity for **1** has been observed against a wide variety of cell lines, including multidrug resistant lines. However, the low water solubility of this compound has limited its efficacy in *vivo*. Its water-soluble prodrug，CA-4 phosphate disodium **2** is now in phase II clinical trials both as a single drug and in combination for anticancer therapy. [5] It appears to act by a unique irreversible and selective antivascular effect that disrupts immature endothelial cell's cytoskeleton in *vivo*. [6]

Over the years, a large number of structure-activity relationship (SAR) studies on **1** have been reported by several academic and industrial groups. In brief, it is thought that the presence of the 3, 4, 5-trimethoxy group on ring A, the *cis*-orientation of the two aromatic rings, and the *para*-methoxy group on ring B are essential for optimal cytotoxic activity. [7] Unfortunately, **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 alteration of the linker and the A, B-rings; we replaced the *cis*-olefin linker with a sulfonamide group, the 3,4,5-trimethoxyphenyl with 2,6-dimethoxylpyridine, the B ring with a carbazole-ring which led to the synthesis IG105 (**3**). [8]

<Figure 2 >

Our previous studies with **3** have shown a potent activity against human leukemia and solid tumors in breast, liver, prostate, lung, skin, colon and pancreas with IC_{50} values between 0.012 and 0.298 μM. In addition, **3** was shown to inhibit microtubule assembly by binding at colchicine pocket. Compound **3** shows a potent anticancer activity in *vitro* and in *vivo* and has good safety in mice, however its the poor water solubility limits oral absorbability. [9] Transforming the structure of **3** so as to significantly increase the water-solubility and improve the oral bioavailability while maintaining or increasing the anti-tumor activity is the goal of this work. The compounds selected for synthesis have various substitutions on the carbazole-ring

which will also expand the SAR of this series of carbazole sulfonamides. Thus, we focused on polar or ionizable groups on the 6- and 7-positions of the carbazole-ring which are expected to provide activity comparable to **3**. Furthermore, the presence of R substituent(s) which can serve as H-bond acceptors or donors is expected to be favorable for improving aqueous solubility. Therefore, a series of new carbazole sulfonamides derivatives with different R groups on the carbazole-ring are reported herein.

As shown Scheme 1, carbazole, a commercially available and inexpensive compound, was allowed to react with CH_3I in DMF in the presence of excess NaOH at room temperature to produce 9-methyl-9H-carbazole (**4**) with a yield 95%. Compound **4** was separately brominated, nitrated, and formylated to afford **11a**, **11b**, and **11c**. However, **11d** and **11e** were synthesized by a 4 steps process of nitration, Suzuki coupling, cyclization to the carbzole and finally methylation starting with 4-bromobenzaldehyde or 1-Bromo-4-nitrobenzene, respectively.

In Scheme 2, compounds **12a**, **12b** and **12d** were obtained from compounds **11a**, **11b** and **11d** using Gupta's method. [10] However, compounds **12c** and **12e** can't be afforded using the same method from **11c** and **11e**. Finally, we found that **12c** and **12e** could be afforded successfully through **11c** and **11e** reacting with excess $ClSO_3H$ at 0 °C in 70%-80% yields. [11] Intermediates **12a-12e** were reacted with 3-amino-2, 6-dimethoxypyridine in DMF in the presence of potassium carbonate with stirring at room temperature for about 2-3 h to afford **13a-13e** in yields of 70%-85%. In addition, the aldehyde group in **13c** and **13e** were reduced with $NaBH_4$ to provide compounds

13g and **13j** with a hydroxymethyl substituent in yields of 65% and 70%, respectively. The aldehydes group in **13c** and **13e** were oxidized with H_2O_2 (35% in water) and conc. sulfuric acid in methanol at room temperature to provide the carboxylic acid **13k** in yield 71% and the ester **13h** in yield 45%. **13e** was allowed to react with *m*-chloroperbenzoic acid (*m*-CPBA) in DCM at room temperature for about 24 h to give the corresponding formate ester. Subsequently, the formate ester was hydrolyzed to afford **13f** in 60% yield. Catalytic hydrogenation of **13b or 13d** with hydrogen (40 PSI) in the presence of Pd/C (5-10%) in anhydrous methanol afforded **13l or 13i** in 75% yield. **13k** was allowed to react with CH_3I in DMF in the presence of excess NaOH at room temperature to produce **13m** in 50% yield.

<Scheme 1>

<Scheme 2>

All of the target compounds were evaluated against HepG2 cells for antiproliferative activity by the standard sulforhodamine B (SRB) assay.[12] Seven compounds, which showed activity versus HepG2 better than or comparable to that of **3**, were evaluated against MCF-7, MIA PaCa-2, and Bel-7402 for antiproliferative activity. The data are summarized in Table 1 and Table 2. Compounds **13f**, **13i** were found to have lower IC_{50} values against the four cell lines than **3**. Against HepG2 and MCF-7 their IC_{50} values (IC_{50}: 0.01-0.07 μM) approach that of the positive control drugs **podo** and **CA-4**. Compounds **13b**, **13g**, **13j**, **13k** and **13l** were found to have IC_{50} values equivalent to that of **3** against four cell lines. On the other hand, compounds **13a**, **13c**, **13d**, **13e** and **13m** were significantly less potent than **3** against HepG2 cells (IC_{50}: 0.26μM, 0.26μM, >25μM, 0.22μM and 7.22 μM, respectively). Thus, the current

results demonstrated that the R substituent on the carbazole-ring was modifiable and could greatly affect potency. When the R substituent is either a hydroxyl group or an amino group the highest antitumor activity is observed.

It is very interesting that compounds **13e**, **13f**, **13g** and **13i** which have the R groups at the 7-position of the carbazole ring generally showed cytotoxic activity greater than that of compounds **13c**, **13k**, **13j** and **13l** with the R group at the 6-position of the carbazole ring. Interestingly, the most active compounds **13f** and **13i** (substituted in the 7-position) exhibited greater potency against four tumor cell lines in *vitro*, than the corresponding 6-substituted compounds **13k** and **13l**.

To contribute to the structural understanding of the activity of these compounds, we performed docking studies with four compounds at the colchicine binding pocket using the CDOCKER program in the Discovery Studio 3.0 software and the tubulin crystal structure (PDB code: 4O2B)[13] in comparison with the **3**. As shown in Figure **3**, a key amino acid Asn258 potentially can form hydrogen bonds with the same group of compounds **13f**, **13i**, **13k**, **13l** and **3**. In addition, **13f**, **13i** could form an additional hydrogen bond with amino acid Val238, and similarly **13k**, **13l** could form an additional hydrogen bond with amino acid Asp251. These additional H-bonds may enhance the binding affinity of these four compounds (**13f**, **13i**, **13k**, **13l**) with tubulin and subsequently lead to the increase antitumor activity observed. All the calculated distances between ligands and amino acids is less than 3 Å, and the distance between hydroxyl group and amino acid is less than the distance between amino group and amino acid, which may contribute to the explanation for the higher antitumor activity

of **13f**, **13k (**hydroxyl group-substituted**)** than that of **13i**, **13l (**amino group-substituted**)** .

<Figure 3 >

An important goal of this research was to increase the aqueous solubility of the new compounds compared to that of **3**. To assess this property we measured their aqueous solubility by using an HPLC/UV method under two conditions: pH = 2.0 and pH = 7.4 as shown in Table 1. [14] All of the tested compounds, except **13b** and **13d**, showed improved solubility at both pH 2.0 and pH 7.4 compared with that of **3**. Notably, the most active compound **13f** had aqueous solubility of 0.84μg/mL at pH 2.0 and 1.53 μg/mL at pH 7.4 which represents a 100-fold increase over that of **3**. Generally, most of compounds showed equivalent aqueous solubility under the two conditions, but the compounds **13i** and **13l** were more soluble in the acidic solution than neutral one, presumably because of the presence of the amine group. A comparison of the 6-substituted and 7-substituted series showed that the 6-substituted compounds were generally more soluble than the corresponding 7-substituted compounds.

<Table 1>

<Table 2>

Following our optimization strategy, 13 novel carbazole sulfonamide derivatives were synthesized by modifying the R group on the carbazole-ring of **3**. Compounds **13b**, **13g**, **13j**, **13k** and **13l** showed significant cytotoxic activity against HepG2、MIA PaCa-2、MCF-7 and Bel-7402 cell lines with IC_{50} values at a sub-micromole level (IC_{50}: 0.05-0.1 μM) comparable to that of **3**. Compounds **13f**, **13i** were found to have

lower IC_{50} values against the four cell lines than **3**. Against HepG2 and MCF-7 the IC_{50} values for **13f** and **13i** (IC_{50}: 0.01-0.07μM) approached those of the positive controls **podo** and **CA-4**. The modifications of the R substituent on the carbazole-ring provided the following new SAR information: (1) when the R substituent is the hydroxyl group or the amino group, compounds show the highest antitumor activity and solubility; (2) the antitumor activity of the compounds with substituted at the 7-position of carbazole ring ware generally more potent than the activity of the corresponding compounds with substituted at the 6-position of the carbazole ring. Furthermore, most of compounds showed aqueous solubility (0.11-19.60 μg/mL at pH 7.4 and 2.0) better than **3**. A comparison of the 6-substituted and 7-substituted series showed that the 6-substituted compounds were generally more soluble than the corresponding 7-substituted ones. These promising results for new compounds **13f** and **13i** as potential potent antitumor drug candidates warrant further development.

Acknowledgements:

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12. **The standard sulforhodamine B (SRB) assay**. The SRB assay was used for in vitro anticancer study of the synthesized compounds. The human cancer cells were inoculated into 96-well microtiter plates at plating densities of 4000-6000 cells/well and were incubated for 24 h. Treatment of the cells with the solutions of compounds in DMSO was done after 24 h, and watered down in medium to yield 4 different concentrations of 2.5, 5, 10 and 20 µg/ml, while cells that contained no drugs/sample were used as control and blanks comprised complete medium with no cells. Incubation of plates for 48 h was followed with addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, and washed, dried and dyed by SRB. The boundless dye was detached, while 10 mM Tris base was used for the extraction of protein-bound dye, and its optical density determination achieved using a multi-well

spectrophotometer at the wavelength 570 nm. 50% of cell growth inhibition was calculated.

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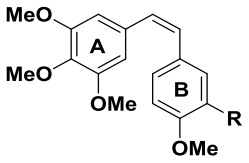
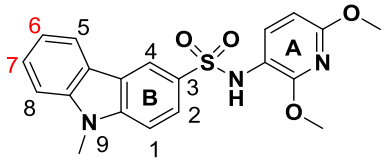
14. **Aqueous Solubility Determination.** Solubility was measured separately at pH 7.4 and pH 2.0 by using an HPLC-UV method. Test compounds were initially dissolved in DMSO at 10 mg/mL. Ten microliters of this stock solution was spiked into either pH 7.4 phosphate buffer (1.0 mL) or 0.01 M HCl (approximately pH 2.0, 1 mL) with the final DMSO concentration being 1%. The mixture was stirred for 4 h at r.t., 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.



1 CA-4 : R = OH
2 CA-4P: R = OPO_3Na_2

Figure1 Structures of **CA-4** and **CA-4P**



3 IG-105

Figure 2 Structure of **IG-105**

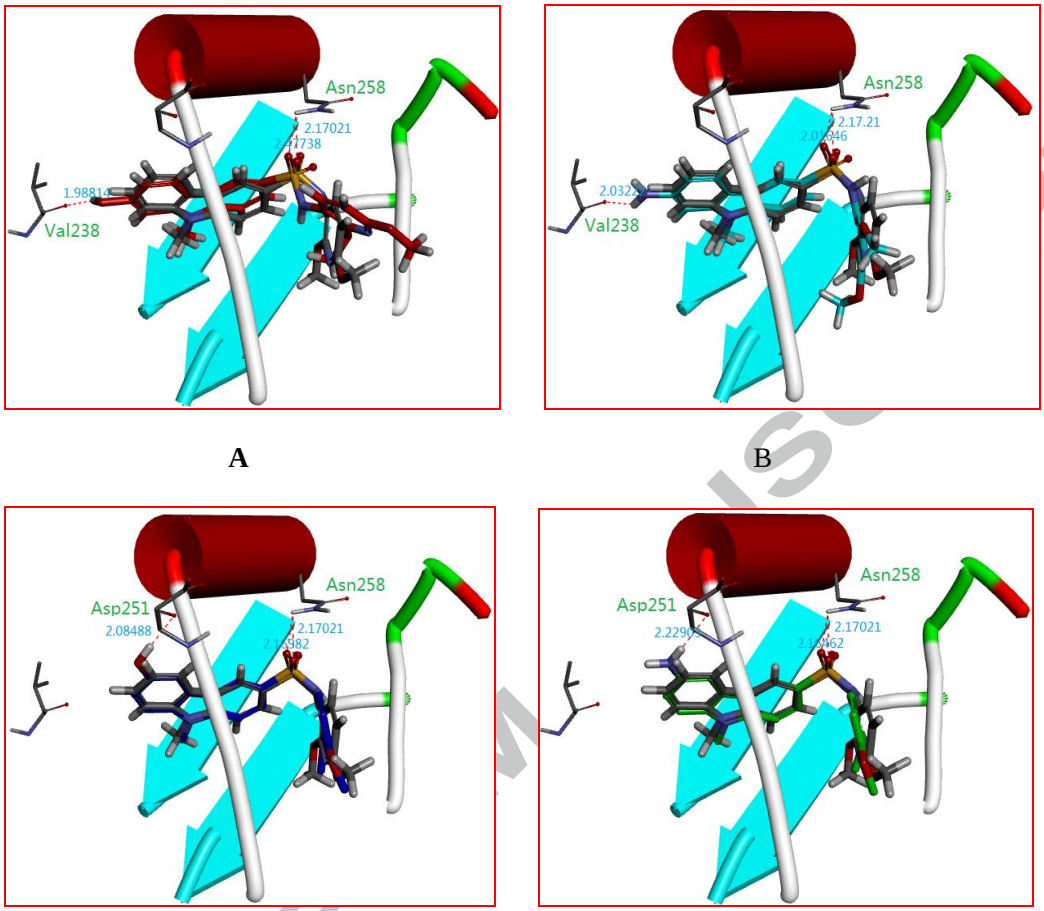
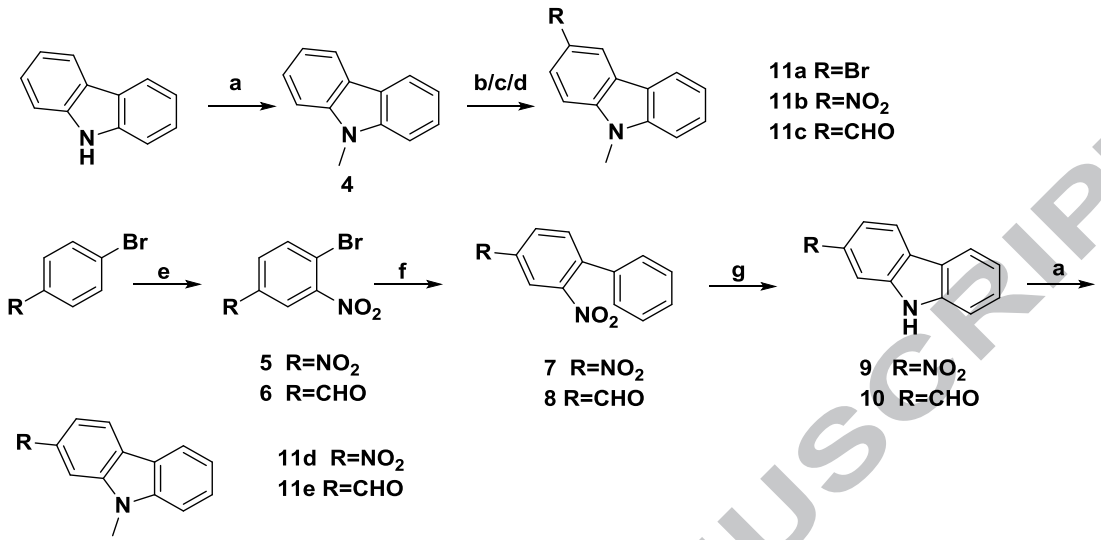
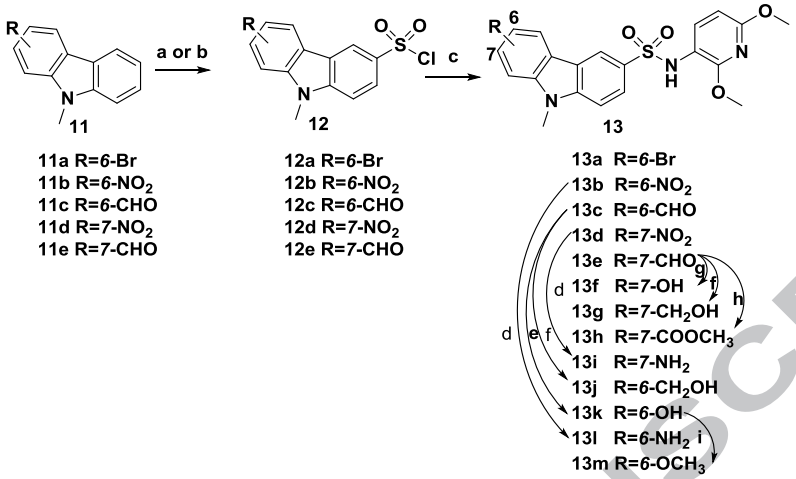


Figure3 Predicted binding modes of four compounds (different colors) and **3** (grey) with tubulin, respectively (PDB code: 4O2B). A: **13f** (red); B: **13i** (cyan); C: **13k** (blue); D: **13l** (green).



Reagent and conditions: a: CH_3I, NaOH, DMF, 4 h; b: NBS, DCM, rt. 2 h; c: HNO_3, HOAC, rt. 0.5h; d: $POCl_3$, DMF, 0-80 °C, 3 h; e: $NaNO_3$, c H_2SO_4, 1 h; f: phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, 12 h; g: PPh_3, 1,2-dichlorobenzene, reflux, 6 h.

Scheme 1

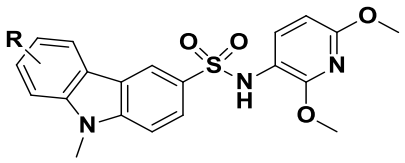


Reagent and conditions: a: 1) $ClSO_3H$, DCM, 0°C to rt. 2 h; 2) $SOCl_2$, DMF, 80°C, 1 h; b: $ClSO_3H$, 0°C, 10 min; c: 3-amino-2,6-dimethoxypyridine, K_2CO_3, DMF, rt. 1h; d: H_2, Pd/C, 3 h; e: CH_3OH/DCM, H_2O_2, c H_2SO_4, 5 h; f: $NaBH_4$, THF, 0.5 h ; g: m-CPBA, DCM, 24 h; h: CH_3OH, H_2O_2, c H_2SO_4, 24 h; i: CH_3I, NaOH, DMF, 4 h.

Scheme 2

Table 1

Antiproliferative activity of **13a-13m** in HepG2 and Aqueous solubility.



Comp.	R	IC_{50} (μM)[a]	Aqueous solubility (μg/mL)[b]	
		HepG2	pH 2.0	pH 7.4
13a	6-Br	0.26	-[c]	-[c]
13b	6-NO_2	0.082	0.17	0.11
13c	6-CHO	0.26	1.7	1.4
13d	7-NO_2	>25	0.11	< 0.10
13e	7-CHO	0.22	0.41	0.23
13f	7-OH	0.012	0.84	1.53
13g	7-CH_2OH	0.051	0.14	0.13
13h	7-$COOCH_3$	7.22	0.21	0.17
13i	7-NH_2	0.070	15.8	1.0
13j	6-CH_2OH	0.11	0.5	1.3
13k	6-OH	0.096	3.4	3.1
13l	6-NH_2	0.036	19.6	2.0
13m	6-OCH_3	0.23	1.32	0.31
3	H	0.095	0.10	< 0.10
Podo		0.003	N[d]	N[d]
CA-4		0.002	N[d]	N[d]

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

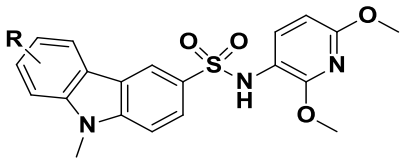
[b] Compounds were measured at pH 7.4 and pH 2.0.

c "-": No solution.

[d] "N": Not Tested.

Table 2

Data for **13b**, **13f**, **13g** and **13i-13l** against four tumor cells.



Comp.	R	IC_{50} (μM)[a]			
		HepG2	MIA PaCa-2	MCF-7	Bel-7402
13b	6-NO_2	0.082	0.18	0.36	1.99
13f	7-OH	0.012	0.051	0.014	0.056
13g	7-CH_2OH	0.051	2.38	0.69	0.99
13i	7-NH_2	0.070	0.092	0.036	0.18
13j	6-CH_2OH	0.11	0.91	0.87	1.28
13k	6-OH	0.096	0.43	0.45	1.83
13l	6-NH_2	0.036	0.29	0.42	0.58
3	**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, concentration required to inhibit 50% of human tumor cells proliferation after 48 h treatment.

Graphical abstract

Novel Carbazole Sulfonamide Derivatives of Antitumor Agent: Synthesis, Antiproliferative Activity and Aqueous Solubility

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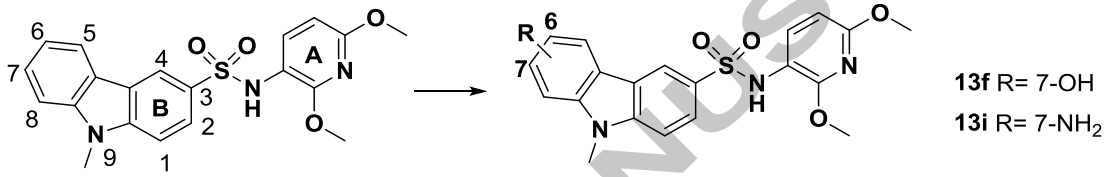

Data of **13f**, **13i** and **3** against four tumor cells and aqueous solubility.

Compound	IC_{50} (μM)				Aqueous solubility (μg/mL)	
	HepG2	MIA PaCa-2	MCF-7	Bel-7402	pH 2.0	pH 7.4
13f	0.012	0.051	0.014	0.056	0.84	1.53
13i	0.070	0.092	0.036	0.18	15.8	1.0
3	0.095	0.18	0.68	1.07	0.10	< 0.10