MedChemComm

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Med. Chem. Commun.*, 2018, **9**, 1511

Synthesis and biological evaluation of novel 5-chloro-*N*-(4-sulfamoylbenzyl) salicylamide derivatives as tubulin polymerization inhibitors

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Received 22nd April 2018,
Accepted 15th July 2018

DOI: 10.1039/c8md00214b

rsc.li/medchemcomm

A novel series of sulfonamide derivatives, coupled with a salicylamide scaffold, was designed and synthesized. The structures of the synthesized compounds were established using ^{1}H NMR, ^{13}C NMR and high-resolution mass spectroscopy. The synthesized compounds were tested *in vitro* against five types of human cell lines. Two were breast adenocarcinoma, including the hormone-dependent MCF-7 and the hormone-independent MDA-MB-231. The others were the colorectal adenocarcinoma Caco-2, the carcinoma HCT-116 and the immortalized retinal-pigmented epithelium, hTERT-RPE1. Nine sulfonamides were able to inhibit the growth of the four tested cancer cells. Compound **33** was the most active against the selected colon cancer (Caco-2 and HCT-116) subtypes, while compound **24** showed the best efficacy against the examined breast cancer (MCF-7 and MDA-MB-231) cells. The selectivity index introduced compounds **24** and **33** as having the best selectivity among the breast and colon subtypes, respectively. *In vitro* tubulin polymerization experiments and flow cytometric assays showed that compounds **24** and **33** led to cell cycle arrest at the G2/M phase in a dose-dependent manner by effectively inhibiting tubulin polymerization. Furthermore, the results of the molecular docking studies indicate that this class of compounds can bind to the colchicine-binding site of tubulin.

1. Introduction

Cancer has been reported as the second leading cause of death worldwide. This catastrophic disease was responsible for 8.8 million death in 2015, as recently reported by WHO.[1] One of the well-defined features of cancer is the rapid division of abnormal cells, which leads to uncontrolled growth beyond the usual boundaries of the normal state. Accordingly, they can invade and metastasize (spread) to other organs.[1] As such, inhibiting cancer cell proliferation is an important approach to the disease therapy. Early on, cytotoxic compounds were used to treat cancer, but there was a high risk due to the potential for causing the destruction of healthy and normal cells.[2] The evaluation of the role of human enzymes in preventing diseases and the treatment of cancer has led the use of lower doses of chemotherapy, which reduces its side effects. However, developing new classes of anticancer agents with efficient and selective toxicity toward tumor cells is attracting significant attention due to the undesired side effects of several chemotherapeutic drugs.[3]

In 1952, George Gey established the first human cancer cell line in a Baltimore laboratory.[4] Over the last few decades, hundreds of cancerous cell lines have been sequentially developed. Consequently, thousands of synthetic and natural compounds can be explored for use as anticancer agents by applying the *in vitro* cell culture tools.[5] The National Cancer Institute was the first institute that established a program for this purpose. It introduced many agents that are now a part of standard cancer care after prolonged mechanistic studies to determine their actual pathways that affect the cancer cell growth.[6]

It is well known that sulfonamides have a wide spectrum of pharmacological activities, such as antibacterial,[7–9] antifungal,[10] anti-inflammatory,[11] anti-HIV[12–14] and anticancer agents.[15,16] Several classes of sulfonamide derivatives exert their effect by mediating different cellular mechanisms such as the inhibition of microtubule assembly,[17] inhibition of transcription factor NF-Y and matrix metalloproteinase (MMP),[18] inhibition of glucose-stimulated insulin release,[19] inhibition of carbonic anhydrase,[20–24] inhibition of male erectile dysfunction as phosphodiesterase-5[25] and inhibition of translation initiation.[26] In addition, sulfonamide derivatives inhibit multiple key oncogenic signalling pathways, *e.g.*, targeting protein kinases including vascular endothelial

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growth factors (VEGF), platelet-derived growth factors, and c-kit protein.[27,28]

The discovery of the potent anticancer 2-anilinopyridine sulfonamide (E7010) in 1992 has thrown the light on this class of compounds as anticancer agents.[15] At the cellular level, α and β tubulin heterodimer proteins are polymerized to build the microtubules that are responsible for many events including intracellular transport, motility and mitotic cell division.[29–35] E7010 exerts its antitumor effect by interacting with tubulin through colchicine-binding sites, resulting in the inhibition of tubulin polymerization and finally cell cycle arrest.[36] The same effect has been evidenced by many sulfonamides that continue their antitumor effect *via* arresting the G2/M phase.[17,37]

On the other hand, *N*-substituted salicylamides are well known derivatives that have a wide spectrum of pharmacological activities, such as antibacterial,[38–40] antimycobacterial[40–42] and antiviral.[43] Niclosamide, (5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide), is in the anthelmintic family of medications,[44] which is especially effective against cestodes.[45] Recently, several studies uncovered the antitumor activity of *N*-substituted salicylamide derivatives, which are being widely studied as potential anticancer agents that have been proved to effectively induce apoptosis in cancer cell lines.[46]

N-Substituted salicylamide derivatives inhibit multiple key oncogenic signalling pathways, *e.g.*, the erbB-2 tyrosine kinase receptor and arrests the cell cycle in the G1 phase,[47] the epidermal growth factor receptor protein tyrosine kinases,[48–50] the receptor activator of NF-kB ligand (RANKL)-induced osteoclastogenesis,[51,52] the TMPRSS4 serine protease,[53] the interleukin-12p40 production in dendritic cells,[54] and the histone deacetylases-epidermal growth factor receptor (HDAC-EGFR).[55]

Continuing the investigation of sulfonamides as potent anticancer agents,[37] a new series of sulfonamide derivatives with a salicylamide core were synthesized based on the strategy of drug design. The synthesized compounds were tested *in vitro* against four types of human cancer cell lines. Two are breast adenocarcinoma, including the hormone-dependent MCF-7 (ATCC® HTB-22™) and the hormone-independent MDA-MB-231 (ATCC® CRM-HTB-26™). The others are the colorectal adenocarcinoma Caco-2 (ATCC® HTB-37™), and the carcinoma HCT-116 (ATCC® CCL-247™). The selectivity of the active compounds towards the tested cancer type is discussed with respect to the excreted cytotoxicity on the immortalized retinal-pigmented epithelium, hTERT-RPE1 cell line as a model for normal cells. In addition, the interaction of the inhibitors with the colchicine-binding site of tubulin was performed by *Insilco* molecular docking calculations.

2. Results and discussion

2.1. Chemistry

In a previous study, the phenyl benzamide derivatives were achieved by the reaction of 5-chloro-2-methoxybenzoic acid with aniline.[37] Later, it was found that the produced compound was almost planar and the rotation around its single bonds was restricted due to the delocalization of both of π and n electrons all over the molecule.[56]

In the present study, aniline was replaced with benzylamine in order to increase the flexibility of the produced compounds by possible rotation around the single bonds. In addition, the methoxy group was replaced with a hydroxyl group to achieve the salicylamide moiety (Fig. 1).

Starting with the amidation of 5-chlorosalicylic acid with benzylamine followed by chlorosulfonation gave rise to a yield of less than 20%. However, starting with 5-chloro-2-methoxybenzoic acid increased the yield of the sulfonyl chloride derivative to 90%. Consequently, 5-chloro-2-methoxybenzoic acid **1** (Scheme 1) was reacted with benzylamine using ethyl chloroformate as the coupling reagent in the presence of triethylamine in dichloromethane (DCM), giving rise to 5-chloro-2- methoxy-*N*-benzyl benzamide **2** in a yield of 97%.[56] Subsequently, the chlorosulfonation of the amide **2** was achieved by the reaction with chlorosulfonic acid overnight to afford 4-(5-chloro-2-methoxybenzamido-*N*-methylene) benzene sulfonyl chloride **3** in a yield of 90%. The sulfonyl chloride **3** was coupled with aromatic, aliphatic and heterocyclic amines in the presence of sodium carbonate in a mixture of dichloromethane (DCM) and water (2 : 1 in ratio) to yield the 5-chloro-2-methoxy-*N*-(4-sulfamoylbenzyl) benzamide derivatives **4–22** in moderate to excellent yields (47–98%). Finally, the *o*-methoxy groups of the benzamide derivatives **4–22** were replaced with hydroxyl groups by refluxing with sodium cyanide in anhydrous dimethyl sulfoxide[57] to yield the desired 5-chloro-*N*-(4-sulfamoylbenzyl) salicylamide derivatives **23–41** in excellent yields (72–98%). The structures of the synthesized compounds were established using ^{1}H NMR, ^{13}C NMR and high-resolution mass spectroscopy.

2.2. Biological evaluation

2.2.1. *In vitro* antiproliferative activity and cytotoxicity effect. The novel *N*-(4-sulfamoylbenzyl) salicylamide derivatives **23–41** were evaluated *in vitro* for their antiproliferative activity against five types of human cell lines. Four were carcinoma cell lines and the immortalized retinal-pigmented epithelium, hTERT-RPE1 as a normal cell line; the well-known anticancer agent colchicine was used as a positive control (Table 1). The results were expressed in terms of GI_{50} values (the concentration that results in a 50% inhibition). In addition, the cytotoxicity on hTERT-RPE1 was expressed as CC_{50} (the concentration that produced 50% reduction in cell viability). It is well known that stopping proliferation is a characteristic feature of normal growing cells after they reach a confluent monolayer in a culture dish. Conversely, cancer cells continue their multiplication and they do not have the contact inhibition phenomenon (the process by which cells stops proliferating when they reach confluence, despite the availability of extracellular nutrients and growth factors).[58,59] Consequently, the four carcinoma cell lines were incubated overnight at 37 °C to get about 70–75% confluence (time

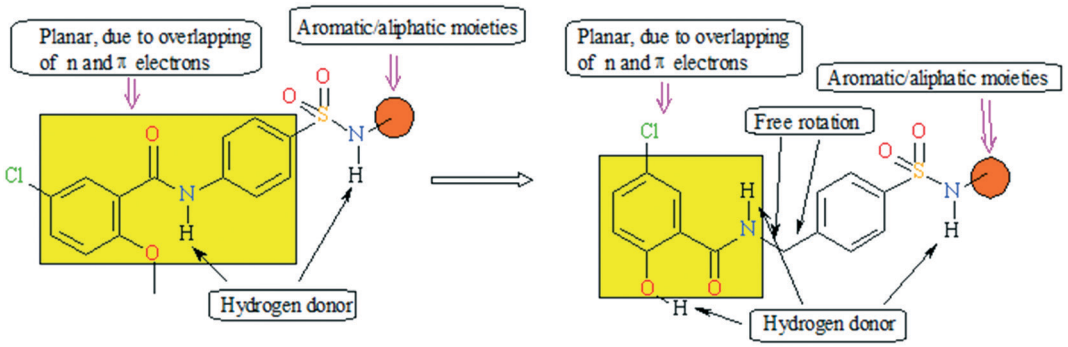
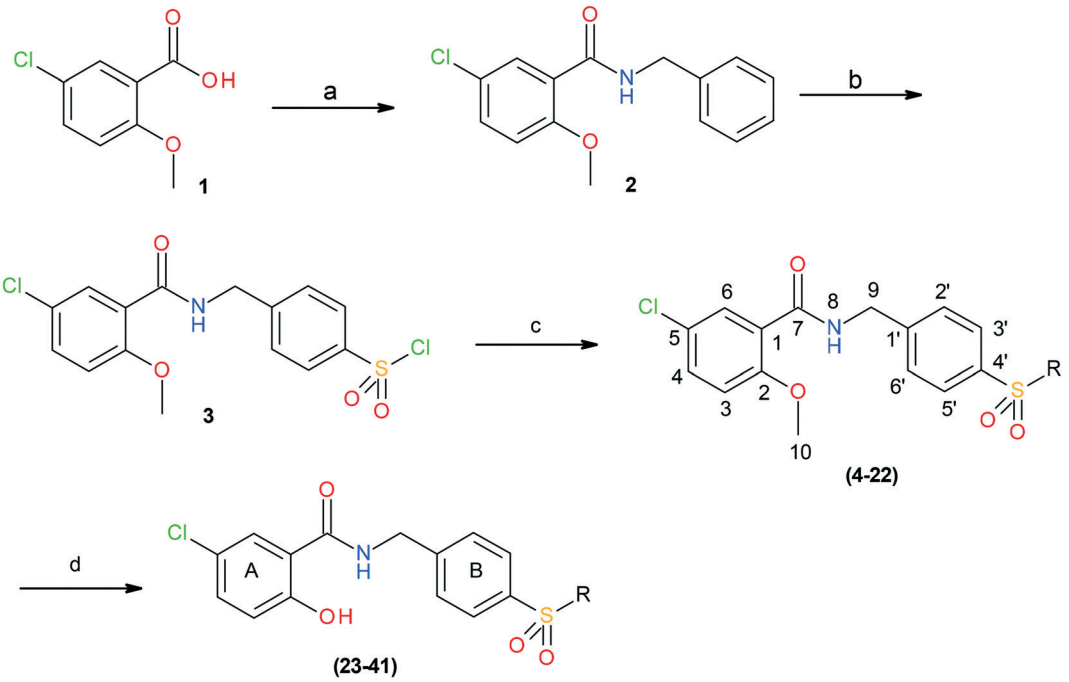


Fig. 1 Development of the previous 5-chloro-2-methoxy-*N*-(4-sulfamoylphenyl) benzamide derivatives to 5-chloro-*N*-(4-sulfamoylbenzyl) salicylamide derivatives.



4, 23: R = NH-C(2"/3"/4"/5"/6"/1")Ph
5, 24: R = NH-(4-Cl)Ph
6, 25: R = NH-(4-F)Ph
7, 26: R = NH-(4-CH_3)Ph
8, 27: R = NH-(4-OCH_3)Ph
9, 28: R = NH-(4-NH_2)Ph
10, 29: R = NH-(4-OH)Ph
11, 30: R = NH-(2-OH)Ph
12, 31: R = NH-(2-COOH)Ph
13, 32: R = NH-(2-NO_2-4-CH_3)Ph
14, 33: R = NH-CH_2-Ph
15, 34: R = NH-$(CH_2)_2$-Ph
16, 35: R = NH-$CH(CH_3)_2$
17, 36: R = NH-$(CH_2)_2$-OH
18, 37: R = NH-2-pyridinyl
19, 38: R = NH-2-(1,3-thiazolyl)
20, 39: R = morphinyl
21, 40: R = piperazinyl
22, 41: R = N-methylpiperazinyl

Scheme 1 Synthesis of 5-chloro-*N*-(4-sulfamoylbenzyl) salicylamide derivatives (**23**–**41**). Reagents and conditions: a) ethyl chloroformate, triethylamine, benzylamine, DCM, RT, 3 h, 97%; b) chlorosulfonic acid, RT, overnight, 90%; c) appropriate amine, sodium carbonate, DCM/H_2O (2 : 1), RT, 24 h, 47–98%; d) sodium cyanide, DMSO, reflux, 1 h, 72–98%.

zero). On the other hand, the normal cell lines did not increase as well as the cancer cell line during the screening time, since they were fully confluent at time zero. Finally, the number of cells corresponding to the zero time of carcinoma cell lines was subtracted from that obtained after treatment for 72 h to detect the actual growth inhibition.

In general, Caco-2 and MCF-7 cells seemed more sensitive to compounds with aromatic sulfonamide substitutions. This

Table 1 Antiproliferative activity (GI_{50}) of sulfonamides against colon and breast human cancer cell lines and their cytotoxic effects (CC_{50}) on the hTERT-RPE1 normal cell line

Compound	GI_{50}[a] (μM)				CC_{50}[b] (μM)
	Caco-2	HCT-116	MDA-MB-231	MCF-7	hTERT-RPE1
23	11.9 ± 0.3	30.0 ± 9.1	15.8 ± 0.3	9.8 ± 1.2	79.0
24	7.5 ± 1.0	10.7 ± 0.5	8.3 ± 1.1	5.6 ± 0.03	120.6
25	14.2 ± 0.9	23.0 ± 1.7	24.3 ± 1.2	13.7 ± 0.4	72.4
26	12.1 ± 1.0	19.3 ± 4.6	15.6 ± 0.9	8.0 ± 0.5	36.3
27	12.1 ± 0.4	19.7 ± 1.2	18.2 ± 1.3	13.9 ± 2.4	95.8
28	>50	>50	48.3 ± 3.4	>50	N
29	>50	>50	>50	>50	N
30	23.1 ± 3.5	28.5 ± 1.5	37.8 ± 2.8	33.1 ± 1.9	N
31	>50	>50	>50	>50	N
32	28.6 ± 1.3	42.1 ± 1.4	29.8 ± 2.8	23.7 ± 3.3	194.4
33	3.3 ± 0.7	5.9 ± 0.05	10.7 ± 1.3	5.8 ± 0.7	77.8
34	22.4 ± 1.6	19.4 ± 0.4	50	34.2 ± 0.8	N
35	28.0 ± 5.6	>50	>50	39.0 ± 1.0	N
36	34.0 ± 0.4	>50	>50	>50	N
37	>50	>50	50	>50	N
38	39.3 ± 0.7	>50	>50	31.1 ± 3.9	N
39	>50	>50	>50	>50	N
40	>50	>50	>50	>50	N
41	46.5 ± 3.5	>50	>50	38.2 ± 2.6	N
Colchicine	0.03 ± 0.01	1.2 ± 0.03	1.5 ± 0.07	0.4 ± 0.05	0.10

[a] GI_{50} is the concentration that causes 50% growth inhibition, displayed as mean ± SEM (standard error of the mean), and is derived from at least two independent experiments. [b] CC_{50} is the concentration that causes 50% cytotoxicity to the normal hTERT-RPE1 cell line. The CC_{50} of all compounds was firstly screened against the hTERT-RPE1 cell line in the range of 50 μM, while the most active compounds were only subjected to further examination to determine their defined CC_{50}. N: the indicated compound showed no or weak cytotoxicity at the screening concentration (50 μM) so no further test was done.

finding was supported by reports, wherein both types are frequently used as model systems for the sensitivity screening of antiproliferative examinations.[60] Like breast cancer, estrogen receptor (ER) status is implicated in the pathogenesis of colorectal cancer. The beta type (ER-β) is predominant in the colorectal epithelium where the ER-β expression is significantly reduced in colorectal cancer compared to normal colon tissue.[61] However, the level of ER-β is differentiated according to the disease stage and grade. An inverse correlation with the tumor progression has been previously evidenced in both cell lines and clinical samples.[62] It is noteworthy that Caco-2 cells constitutively express ER-β[63] while HCT116 cells have lower expression levels of the laterally mentioned receptor.[62,64,65]

Referring to the results in the present study, the growth inhibitory power of the potent sulfonamides was stronger in Caco-2 compared to HCT-116 cells as indicated by the recorded GI_{50} in both cells (Table 1). These results could be attributed to their effect through an ER-β-mediated mechanism and the fact that HCT-116 cells carry mutated KRAS and PIK3CA genes, while Caco-2 has a wild form of these genes;[66,67] this finding is supported by Tolba and Abdel-Rahman.[62] In their study, pterostilbene boosted the expression of ER-β in Caco-2 cells, while only inducing a slight increase in ER-β expression in HCT-116. On the other hand, the breast cell lines MCF-7 (inhibited with 12 compounds) were found to be more sensitive than MDA-MB-231 (inhibited with 9 compounds).

The examined sulfonamides are categorized as 5 heterocyclic, 2 aliphatic and 12 aromatic derivatives. The obtained results showed that all derivatives had weak cytotoxic effects on hTERT-RPE1 (normal cell line). On the other hand, the most active compounds against the other selected panel of cells were the aromatic derivatives. In addition, the substituent at the *para* position of ring C was more active than that at the *ortho* analogue. The only exception was compound **29** (*p*-hydroxyl), which showed $GI_{50} > 50$ μM against the four cell types, while compound **30** (*o*-hydroxyl) displayed moderate activities (GI_{50} ranged 23.1–37.8 μM).

Concerning the aliphatic and heterocyclic sulfonamide derivatives (**35–41**), they were considered weak, but the derivatives **35**, **36**, **38** and **41** were able to suppress the growth of Caco-2 and MCF-7 cell lines only at higher concentrations.

The structures of the derivatives **23**, **33** and **34** differ from each other by a group between the phenyl ring C and the sulfonamide group corresponding to O, CH_2 and $(CH_2)_2$ groups. The three derivatives strongly inhibited the four cancer cell lines, but compound **33** was more active than the other two derivatives. Moreover, it was found to be the most active derivative against Caco-2, HCT-116, MDA-MB-231 and MCF-7 cell lines with GI_{50} of 3.3, 5.9, 10.7 and 5.8 μM, respectively. Also, compound **24** with a chloride atom at the *para* position of ring C showed strong antiproliferative activity against the four cell lines with GI_{50} of 7.5, 10.7, 8.3 and 5.6 μM, respectively.

The substitution at the *para* position of ring C played an important role in the activity toward the four cancer cell lines and the activities differed according to the substituent and the cell line type. With respect to the colon human cancer

cell lines, the antiproliferative activity (GI_{50}) against Caco-2 was found to be in the order of Cl > H > CH_3 = OCH_3 > F > NH_2 = OH, while the order against HCT-116 was found to be Cl > CH_3 > OCH_3 > F > H > NH_2 = OH. With respect to the breast human cancer cell lines, the antiproliferative activity (GI_{50}) against MDA-MB-231 was found to be in the order of Cl > CH_3 > H > OCH_3 > F > NH_2 > OH, while against MCF-7, it was found to be in the order of Cl > CH_3 > H > F > OCH_3> NH_2 = OH (Table 1). The presence of a chromophore group at the *ortho* position of ring C showed weak to medium activity towards the four cell lines (compounds **30–32**).

The selectivity index (SI) of each active sulfonamide derivative was calculated in order to select the most potent and the safest one on a model of a normal cell line hTERT-RPE1 and the results are displayed in Table 2. The results obtained showed that the most potent compounds, **24** and **33**, had the higher selectivity indexes and were consequently selected for further mechanistic examinations. In addition, both compounds were found to be safer by comparing their SI with that obtained by colchicine.

2.2.2. *In vitro* test of tubulin polymerization inhibition. The most potent compounds **24** and **33** were evaluated in their capability to inhibit tubulin polymerization using the enzyme-linked immunosorbent assay (ELISA). In addition, colchicine as a standard tubulin inhibitor was matched on both tested cancer cell lines MCF-7 and Caco-2. As was displayed in Fig. 2 and 3, compound **33** against Caco-2 was found to be more efficient (IC_{50} = 6.44 μM) at suppressing tubulin and exerting a pattern similar to that obtained from colchicine (IC_{50} = 7.71 μM). On the other hand, compound **24** against MCF-7 exerted their effect (IC_{50} = 29.31 μM) at 6.4 fold of the colchicine concentration (IC_{50} = 4.59 μM). These results suggest that the growth inhibition associated with compounds **24** and **33** is correlated with the inhibition of tubulin polymerization. Consequently, the results support that compound **24** and **33** are potent inhibitors of tubulin assembly.

2.2.3. Flow cytometry analysis of cell cycle and apoptosis. To investigate whether compounds **24** and **33** could arrest cell cycle distribution, the effect of both compounds on the cell cycle was analysed by flow cytometry. MCF-7 cells were subjected to the effects of DMSO as the vehicle control or 5× of the obtained GI_{50} (28 μM) of compound **24** for 48 h. In addition, Caco2 cells were incubated with DMSO or 5× of the GI_{50} (16.5 μM) of compound **33** for 48 h. The induced apoptotic types of death for both compounds were compared with the corresponding vehicle control. As shown in Fig. 4 and 5, the treatment of Caco-2 cells with compound **24** at 28 μM resulted in substantial accumulation at the G2/M and Pre-G1 phases (31% and 12%), while the untreated cells showed 9% and 2%, respectively. Similarly, **33** caused a remarkable elevation in the cell population at G2/M and Pre-G1 from 9% and 2% in untreated control to 53% and 17%, respectively at 16.5 μM. These results suggest that compounds **24** and **33** could exert their anticancer effects through the arrest of G2/M and Pre-G1 phases as a consequence of suppressing the dynamic events of tubulin polymerization that lead to cell cycle arrest. Further, to assess the induced type of death, the treated MCF-7 and Caco-2 cells were stained with dual annexin V-FITC and propidium iodide (annexin V-FITC/PI). As shown in Fig. 5, the apoptotic cells indicated by annexin V+/PI− and annexin V+/PI+ increased by ~12% upon treatment with 28 μM of compound **24** when compared to the untreated cells (2%). On the other hand, 16.5 μM of compound **33** induced ~17% apoptotic cell death *versus* 2% for the untreated control.

Table 2 Determination of the selectivity index of the active sulfonamides

Compound	Caco-2	HCT-116	MDA-MB-231	MCF-7
23	6.6	2.6	5.0	8.1
24	16.1	11.3	14.5	21.5
25	5.1	3.1	3.0	5.3
26	3.0	1.9	2.3	4.5
27	7.9	4.9	5.3	6.9
32	6.8	4.6	6.5	8.2
33	23.6	13.2	7.3	13.4
Colchicine	3.33	0.08	0.07	0.25

Selectivity index was calculated by recording the ratio of CC_{50} against hTERT-RPE1 relative to the corresponding GI_{50} of every tested cell line. CC_{50}: the concentration that caused 50% cytotoxicity on hTERT-RPE1 normal cells. GI_{50}: the concentration that caused 50% growth inhibition of every selected cancer cell type.

2.3. Docking study

Tubulin inhibitors have gained much interest among antitumor drugs due to their success in clinical oncology. Colchicine was bound to the colchicine binding site of tubulin and formed a complex, which further prevented the polymerization of the microtubule. Consequently, it led to mitotic cell division arrest at the G2/M phase.[68,69]

Based on the results obtained from the *in vitro* testing of the tubulin polymerization inhibition and flow cytometry analysis of the cell cycle and apoptosis, and to understand the relation between the structure of the synthesized compounds and the antitumor activity, molecular docking studies were carried out using the Auto dock Vina software for compounds that have GI_{50} less than 50 μM. The aim of the docking calculations is to predict the correct binding geometry for each binder and compare it with its activity. The binding affinity (kcal mol^{-1}) and hydrogen bonds formed with the surrounding amino acids of the inhibitory pocket of the receptor tubulin were used to predict the test compounds binding modes (Table 3). Colchicine was used as a reference drug for the antitumor activity of the new derivatives.

The most potent inhibitors **23**, **24**, **25**, **26**, **27**, **33** and **34** were analysed in the inhibitory pocket of tubulin. Their binding affinities ranged from −10.2 to −10.9 kcal mol^{-1} (Table 3), while colchicine has an affinity of −9.5 kcal mol^{-1} and formed a strong hydrogen bond with VAL 181:A. The hydroxyl group at ring A of the compounds **23**, **24**, **25**, **27**, **28**, **32**, **33**, **35** and **41** formed a strong hydrogen bond with GLN 11:A,

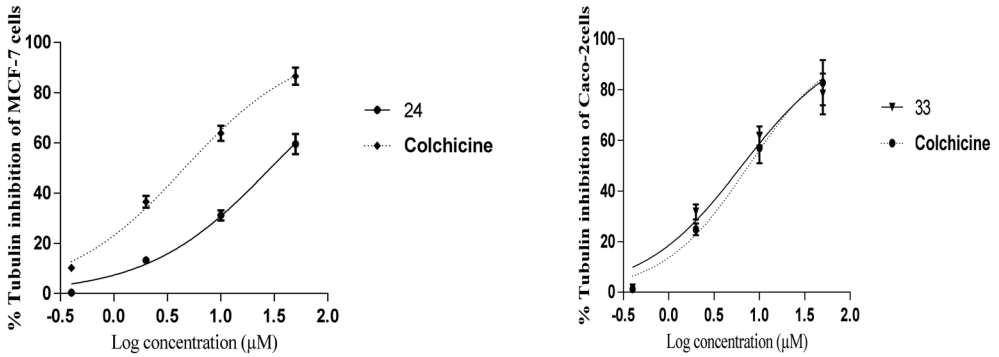


Fig. 2 Dose–response curves for tubulin polymerization inhibition. 48 h treatment of MCF-7 cancer cells with different concentrations of compounds **24** and colchicine (left), and Caco-2 with **33** and colchicine (right).

while those of compounds **26** and **36** formed a strong one with ASN 249:B and ASP 251:A, respectively. A moderate to strong hydrogen bond was formed between the oxygen at the sulfoxide group of the compounds **23**, **24**, **25**, **28**, **34** and **35** with ASN 258:B. In addition, the sulfonamide group of compounds **36** and **38** formed hydrogen bonds with ASN 101:A, and GLU 181:A. On the other hand, the oxygen of the methoxy group of compound **27** formed a strong hydrogen bond with TH 145:A. Fig. 6 and 7 represent the binding modes of compounds **24** and **33**. The 3D structures were created by PyMol 1 (www.pymol.org), while the 2D pose views were created using the Hamburg University, Centre of Bioinformatics server (http://proteinsplus.zbh.uni-hamburg.de/#poseview).

Fig. 8 illustrates all the docked compounds at the inhibitory pocket of tubulin (PDB: 4o2b). The structure of tubulin was deleted from the view to clarify the docked conformers. Compound **24** was defined as the reference compound because it was the most active compound against the carcinoma cell line tested. Compounds **23**, **25**, **26**, **28** and **32** were superimposed with compound **24** and have binding affinities greater than 10.4 kcal mol^{-1} (Fig. 8(A)). Moreover, they have good activities against the carcinoma cell line tested with the exception of compound **28** (weak) and compound **32** (medium) and all compounds formed hydrogen bonds with the amino acid residues GLN 11 (chain A) and/or ASN 258 (chain B). Introducing the methylene or ethylene group adjacent to the sulfonamide group led to the misaligning of the sulfonamide moiety of compounds **33** and **34** with that of compound **24**, while the salicylamide moiety was superimposed with that of compound **24** (Fig. 8(B)) but with little effect on the binding affinity and bioactivity. Introducing the aliphatic group directly to the sulfonamide residue led to a decrease in both the binding affinity and bioactivity of compound **35**; it was even aligned with compound **24** (Fig. 8(B)). Compounds **36**, **38** and **41** were totally misaligned with compound **24**, which could be the reason for their low binding affinity and their weak bioactivity. Compound **27** is the only exception, which was misaligned with compound **24** and had a high binding affinity and good results against the tested carcinoma cell lines.

As was reported before by Abdelaziz, Toner and Liu,[37,70,71] their synthesized sulfonamides mediated their anticancer action through the arresting cell cycle at the G2/M phase and induced the apoptosis of tumor cells. In addition, the present experimental data and docking studies at the inhibitory bucket of tubulin showed that compounds **23**, **24**, **25**, **26**, **27**, **28**, **30**, **32**, **33**, **34**, **35**, **36**, **38**, **41** can bind to the colchicine binding site of tubulin and exert part of their action through arresting cell division at the G2/M phase.

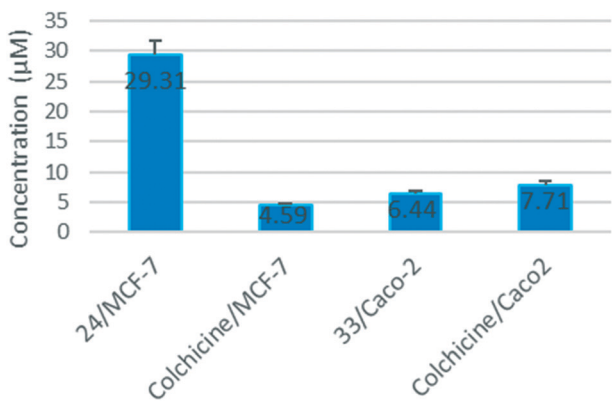


Fig. 3 Tubulin inhibition (IC_{50}) of compound **24** against MCF-7 and **33** against Caco-2 compared with colchicine.

3. Conclusion

In conclusion, a new series of sulfonamide derivatives having a salicylamide core were synthesized, based on the drug design strategy, and evaluated for their *in vitro* anticancer activity on four selected human cancer cell lines. The orientation and side chain substitutions effectively guided the differential activity of the selected sulfonamides towards cancer cell growth inhibition. Nine sulfonamides were able to inhibit the growth of the tested cancer cells. Compound **33** was the most active against selected colon cancer (Caco-2 and HCT-116) subtypes, while compound **24** showed the best efficacy against the examined breast cancer (MCF-7 and MDA-MB-231) cells. The selectivity index introduced compounds **24** and **33** with the best selectivity among breast and colon

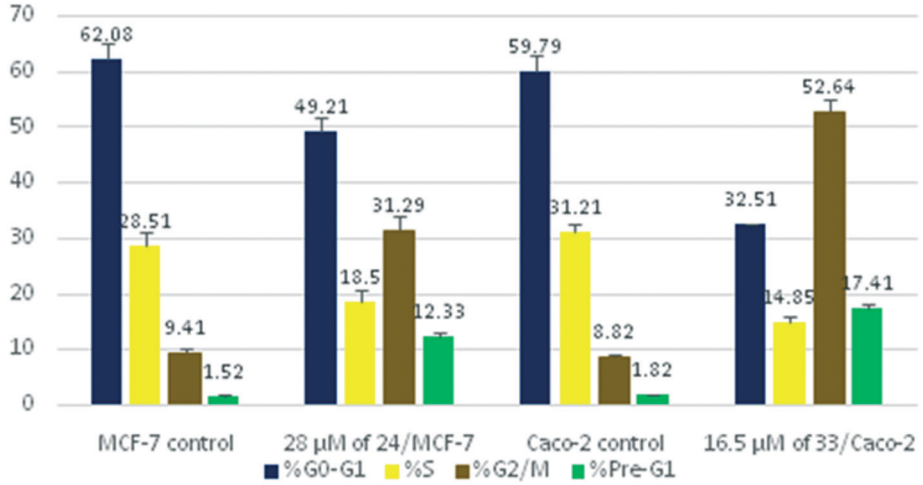


Fig. 4 Percentage of cell cycle phases after 48 h treatment of cancer cells as detected by flow cytometry.

subtypes, respectively. *In vitro* tubulin polymerization experiments and flow cytometric assay showed that compounds **24** and **33** led to cell cycle arrest at the G2/M phase in a dose-dependent manner by effectively inhibiting tubulin polymerization. Furthermore, the results of the molecular docking studies indicate that this class of compounds can bind to the colchicine-binding site of tubulin.

4. Experimental section

4.1. Chemistry

All materials, reagents and solvents were purchased from Sigma-Aldrich, Merck, Fisher chemicals, and Lab-Scan analytical sciences and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded at 298 K on a JEOL ECA-500 spectrometer (^{1}H at 500.16 MHz and ^{13}C NMR at 125.76 MHz), and were processed using the Bruker Topspin 3.2 software. ^{1}H and ^{13}C NMR spectra are referenced to ^{1}H signals of residual non-deuterated solvents and ^{13}C signals to the deuterated solvents. ^{1}H NMR signals are reported with chemical shift values δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), relative integral, coupling constants J (Hz) and assignments. Mass spectra were recorded on a JEOL DART+ HI RESOLUTION mass spectrometer, and ionization of all samples was carried out using ESI. Melting points were measured on an Electrothermal IA9100 digital melting point apparatus and were uncorrected. Analytical TLC was performed on Merck silica gel 60 F254 pre-coated aluminum plates (0.2 mm) and visualized under UV light (254 nm).

4.1.1. 5-Chloro-2-methoxy-*N*-benzyl benzamide (2)[56]. Triethylamine (1.5 ml, 10.7 mmol) was added slowly to a solution of 5-chloro-2-methoxybenzoic acid (**1**, 2.0 g, 10.7 mmol) in dichloromethane (DCM, 20 ml) at room temperature. Ethyl chloroformate (1.02 ml, 10.7 mmol) was added dropwise to the reaction mixture, which was stirred for 1 h at room temperature. Benzyl amine (1.2 ml, 10.7 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for a further 2 h. Distilled water (20 ml) was added. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was crystallized from methanol to give colorless transparent prism crystals (**2**; 2.85 g; yield 97%; mp 72–74 °C). ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 3H, CH_3), 4.45 (d, 2H, J = 5.75 Hz, CH_2), 7.13 (d, 1H, J = 8.6 Hz, Ar–H), 7.21 (d, 2H, J = 3.8 Hz, Ar–H), 7.29 (s, 3H, Ar–H), 7.48 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.65 (d, 1H, J = 2.5 Hz, Ar–H), 8.76 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.28 (C-9), 56.82 (C-10), 114.58 (C-3), 124.99 (C-1), 125.40 (C-5), 127.23 (C-4′), 127.61 (C-2′,C-6′), 128.81 (C-3′,C-5′), 130.22 (C-6), 132.10 (C-4), 139.97 (C-1′), 156.36 (C-2), 164.43 (C-7). HMS (ESI): m/z $[M + H]^+$ calcd. for $C_{15}H_{15}ClNO_2^+$ 276.07912 found 276.07527.

4.1.2. 4-(5-Chloro-2-methoxybenzamido-*N*-methylene) benzene sulfonyl chloride (3). 5-Chloro-2-methoxy-*N*-benzylbenzamide (**2**, 1.0 g, 3.6 mmol) was treated with chlorosulfonic acid (3 ml) on an ice bath with continuous stirring for 1 h. The ice bath was removed while stirring was continued overnight at room temperature. The reaction mixture was added slowly on ice to give a white precipitate. The precipitate was filtered, washed several times with distilled water until acid free and crystallized from dichloromethane to give white needles (**3**; 1.22 g; yield 90%; mp 113–115 °C). ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 3H, CH_3), 4.43 (d, 2H, J = 4.75 Hz, CH_2), 7.13 (d, 1H, J = 8.6 Hz, Ar–H), 7.26 (d, 2H, J = 7.2 Hz, Ar–H), 7.52 (d, 2H, J = 7.2 Hz, Ar–H), 7.54 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.62 (d, 1H, J = 2.5 Hz, Ar–H), 8.78 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.29 (C-9), 56.86 (C-10), 114.62 (C-3), 124.82 (C-1), 125.25 (C-5), 126.04 (C-3′,C-5′), 127.20 (C-2′,C-6′), 130.02 (C-6), 132.13 (C-4), 141.21 (C-1′), 145.70 (C-4′), 156.29 (C-2), 164.52 (C-7). HMS (ESI): m/z $[M + H]^+$ calcd. for $C_{15}H_{14}Cl_2NO_4S^+$ 374.00205 found 373.98679.

4.1.3. General synthetic procedures for 5-chloro-2-methoxy-*N*-(4-sulfamoylbenzyl) benzamide derivatives (4–22). A solution of sodium carbonate (0.25 g, 2.36 mmol) in distilled water (3 ml) was added to a solution of 4-(5-chloro-2-methoxybenzamido-*N*-methylene) benzene sulfonyl chloride

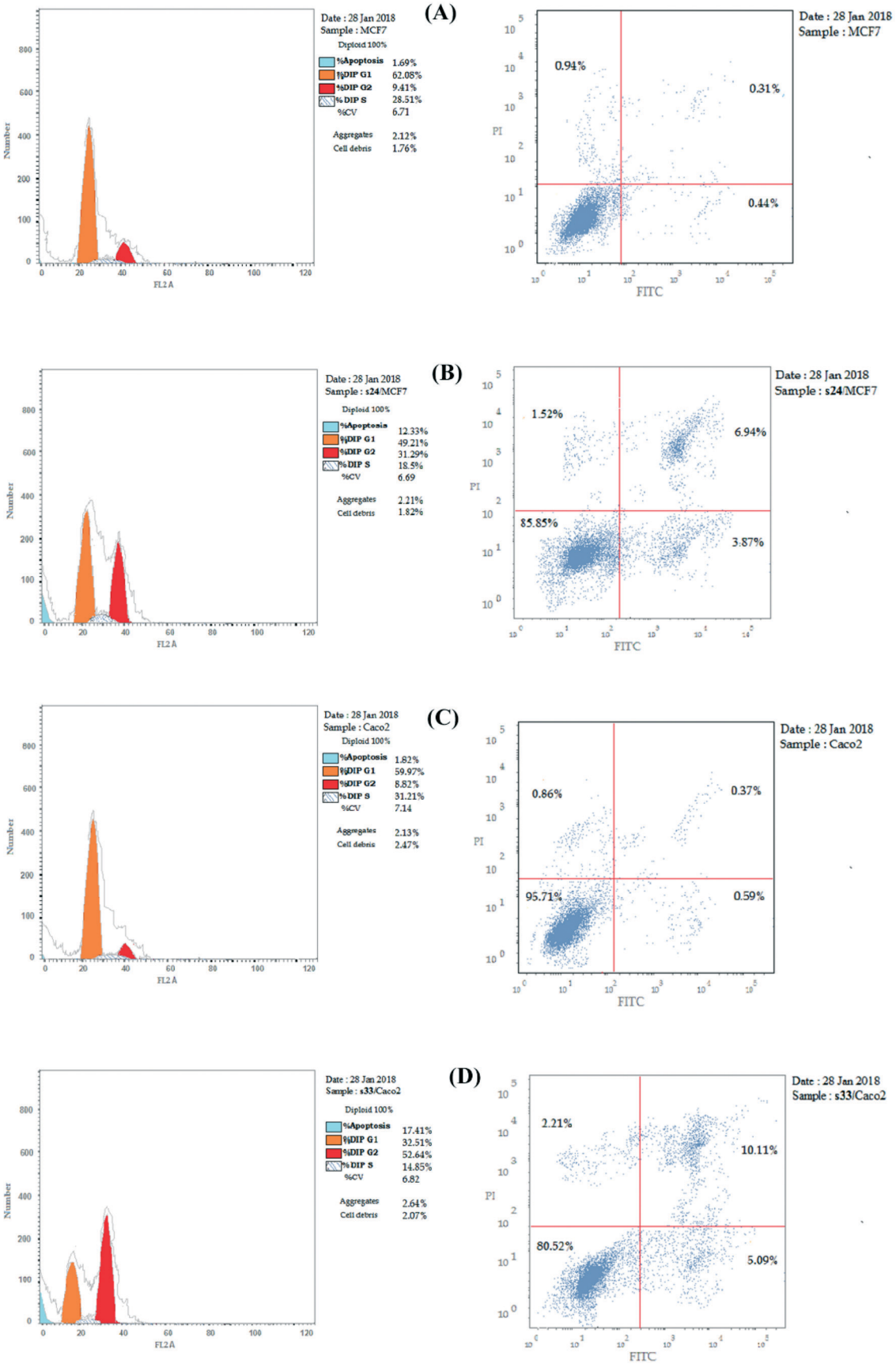


Fig. 5 Flow cytometry of cell cycle distribution of cancer cells and their apoptotic status. MCF-7 was treated for 48 h with either DMSO (A) or 28 μM of compound **24** (B). Caco-2 was treated for 48 h with either DMSO (C) or 16.5 μM of **33** (D).

Table 3 Docking calculation results for compounds that have $GI_{50} < 50$ μM

Compound	Binding affinity kcal mol^{-1}	No. of H.B.[a]	Amino acid residues forming H.B.[a]	
			Chain A	Chain B
23	−10.4	2	GLN 11	ASN 258
24	−10.7	2	GLN 11	ASN 258
25	−10.6	2	GLN 11	ASN 258
26	−10.9	1	—	ASN 249
27	−10.2	2	GLN 11; TH 145	—
28	−10.5	2	GLN 11	ASN 258
30	−10.3	2	ALA 12; SER 140	—
32	−11.2	2	GLN 11	—
33	−10.3	2	GLN 11	—
34	−10.4	1	—	ASN 258
35	−9.0	2	GLN 11	ASN 258
36	−8.9	3	ASN 101; GLU 183	ASP 251
38	−9.7	2	ASN 101; GLU 183	—
41	−9.5	1	GLN 11	—
Colchicine	−9.5	1	VAL 181	—

[a] H.B. = hydrogen bond.

(3, 0.88 g, 2.35 mmol) in dichloromethane (DCM, 6 ml). The appropriate amine (3.59 mmol) was added portionwise to the reaction mixture with continuous stirring. The stirring was continued for 24 h at room temperature. The organic solvent was evaporated under vacuum. The residue was acidified by 1N HCl. The precipitate was filtered, washed several times with distilled water and crystallized from methanol to give benzamide derivatives (**4**–**22**).

4.1.3.1. 5-Chloro-2-methoxy-N-[(N-phenyl) 4-sulfamoylbenzyl] benzamide (4). Greenish white granules; yield 78%; mp 163–165 °C. ^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H, CH_3), 4.49 (d, 2H, J = 5.75 Hz, CH_2), 6.98 (t, 1H, J = 5.7 and 7.2 Hz, Ar–H), 7.06–7.18 (m, 5H, Ar–H), 7.42 (d, 2H, J = 8.6 Hz, Ar–H), 7.44 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.67 (d, 1H, J = 5.7 Hz, Ar–H), 7.7 (d, 2H, J = 7.2 Hz, Ar–H), 8.84 (s, 1H, NH), 10.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.89 (C-9), 56.78 (C-10), 114.55 (C-3), 120.41 (C-2″,C-6″), 124.59 (C-4″), 124.87 (C-1), 124.95 (C-5), 127.05 (C-3′,C-5′), 127.36 (C-2′,C-6′), 129.70 (C-3″,C-5″), 130.04 (C-6), 132.33 (C-4), 138.17 (C-1″), 138.38 (C-4′), 145.30 (C-1′), 156.35 (C-2), 164.78 (C-7). HMS (ESI): m/z $[M + H]^+$ calcd. for $C_{21}H_{20}ClN_2O_4S^+$ 431.08322 found 431.07731.

4.1.3.2. 5-Chloro-2-methoxy-N-[(N-4-chlorophenyl) 4-sulfamoylbenzyl] benzamide (5). White crystals; yield 83%; mp 191–193 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.49 (d, 2H, J = 5.75 Hz, CH_2), 7.07 (d, 2H, J = 8.6 Hz, Ar–H), 7.09 (d, 1H, J = 8.6 Hz, Ar–H), 7.25 (d, 2H, J = 8.6 Hz, Ar–H), 7.43 (d, 2H, J = 8.6 Hz, Ar–H), 7.45 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.67 (d, 1H, J = 5.75 Hz, Ar–H), 7.7 (d, 2H, J = 7.2 Hz, Ar–H), 8.84 (s, 1H, NH), 10.43 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.91 (C-9), 56.77 (C-10), 114.52 (C-3), 121.95 (C-2″,C-6″), 124.97 (C-1,C-5), 127.36 (C-3′,C-5′), 128.16 (C-2′,C-6′), 128.70 (C-4″), 129.66 (C-3″,C-5″), 130.22 (C-6), 132.27 (C-4), 137.24 (C-1″), 138.09 (C-4′), 145.55 (C-1′), 156.36 (C-2), 164.70 (C-7). HMS (ESI): m/z $[M + H]^+$ calcd. for $C_{21}H_{19}Cl_2N_2O_4S^+$ 465.04425 found 465.03892.

4.1.3.3. 5-Chloro-2-methoxy-N-[(N-4-fluorophenyl) 4-sulfamoylbenzyl] benzamide (6). White crystals; yield 71%; mp 155–157 °C. ^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H, CH_3), 4.49 (d, 2H, J = 4.75 Hz, CH_2), 7.05 (d, 4H, J = 8.6 Hz, Ar–H), 7.15 (d, 1H, J = 8.6 Hz, Ar–H), 7.42 (d, 2H, J = 7.65 Hz, Ar–H), 7.48 (dd, 1H, J = 8.6 and 2.85 Hz, Ar–H), 7.64 (d, 2H, J = 7.65 Hz,

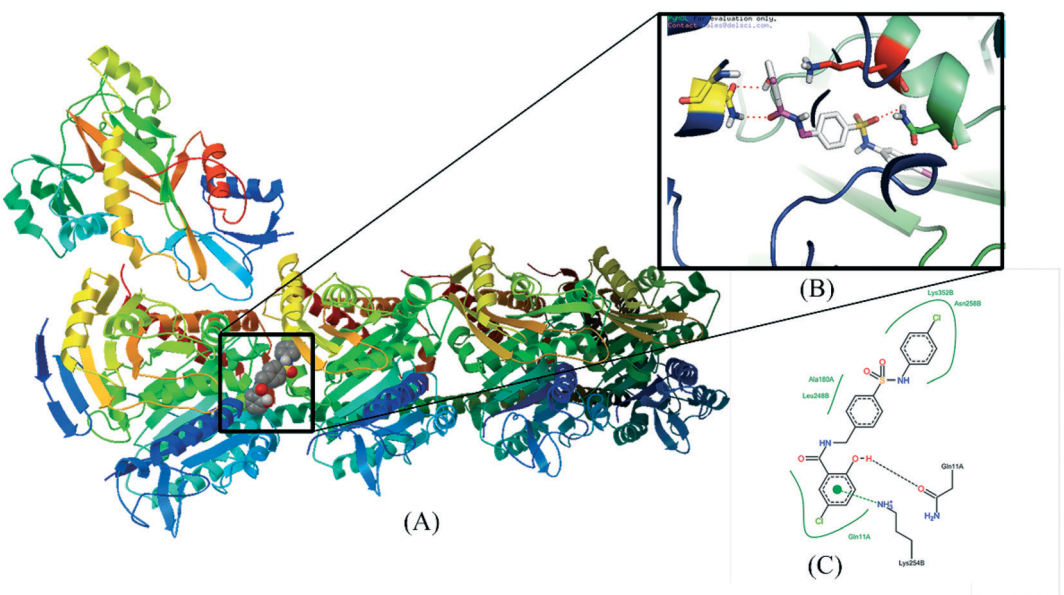


Fig. 6 (A) The binding mode of compound **24** (represented as CPK) in the LOC binding pocket of tubulin (PDB: 4o2b), where the protein is shown as a cartoon representation. (B) The 3D binding mode showing intermolecular hydrogen bonds (red dashed lines) with GLN 11: A (yellow colour) and ASN 258: B (green colour). (C) Residues in the active site of tubulin interacting with compound **24**. The black dashed lines indicate hydrogen bonds, the green solid lines show hydrophobic interactions and the green dashed line shows π–π cation interactions.

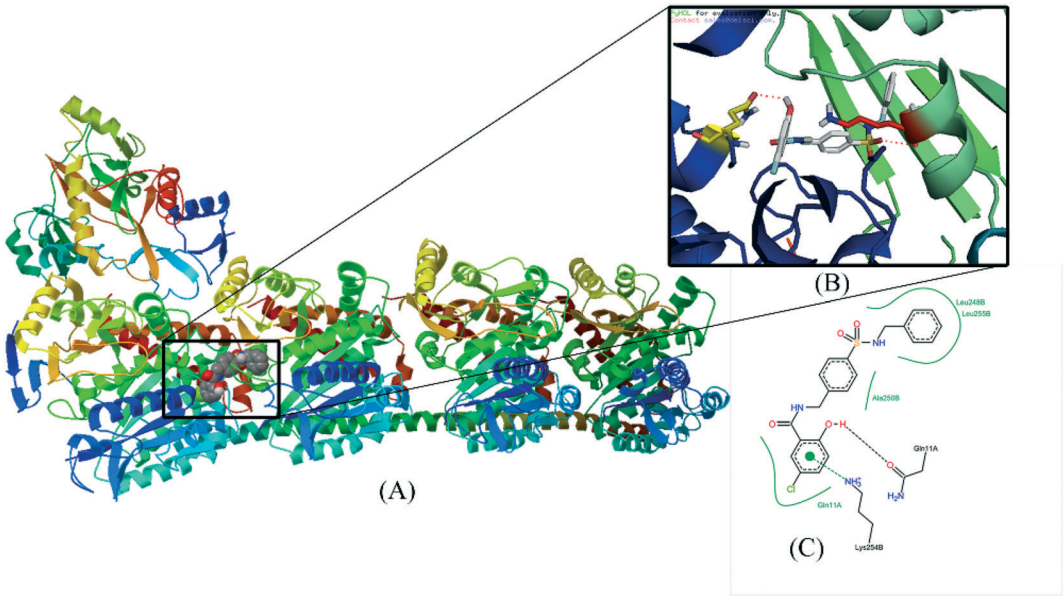


Fig. 7 (A) The binding mode of compound **33** (represented as CPK) in the LOC binding pocket of tubulin (PDB: 4o2b), where the protein is shown as a cartoon representation. (B) The 3D binding mode showing intermolecular hydrogen bonds (red dashed lines) with GLN 11: A (yellow colour). (C) Residues in the active site of tubulin interacting with compound **33**. The black dashed lines indicate a hydrogen bond, the green solid line shows hydrophobic interactions and the green dashed line shows π–π cation interactions.

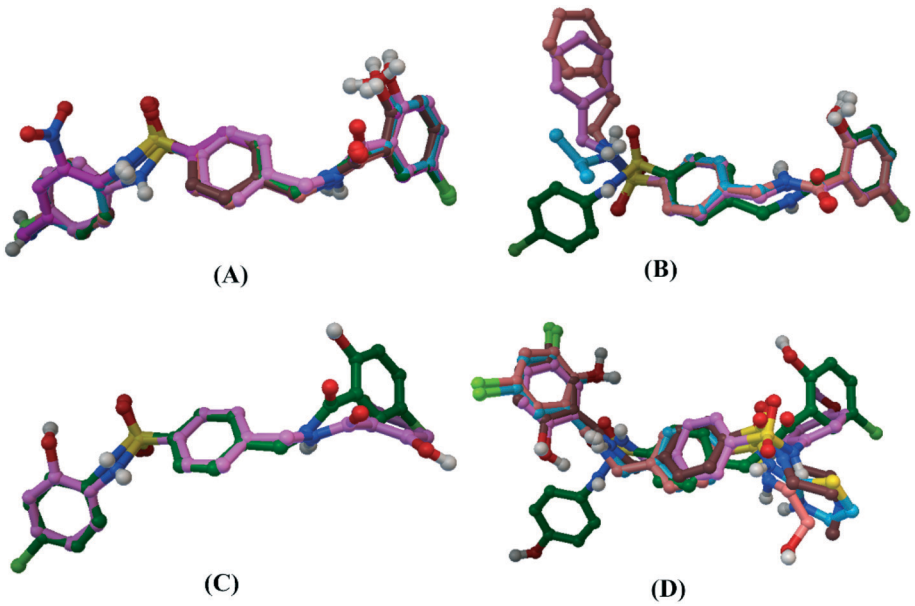


Fig. 8 (A) A superimposition of the docked conformers corresponding to the compounds **33** (pink), **24** (green), **25** (pale brown), **26** (blue), **28** (brown) and **32** (magenta). (B) The 5-chloro salicylamide moiety of the docked conformers corresponding to the compounds 33 (magenta), **34** (pale brown) and **35** (blue) was aligned with that of compound **24** (green), while the sulfonamide moiety was misaligned. (C) The sulfonamide moiety of the docked conformer of compound **30** was aligned with that of compound **24** (green), while the 5-chloro salicylamide moiety was misaligned. (D) The docked conformers corresponding to the compounds **27** (magenta), **36** (pale yellow), **38** (blue) and **41** (brown) were misaligned with compound **24** (green). The structure of tubulin (PDB: 4o2b) was deleted from the view to clarify the docked conformers.

Ar–H), 7.66 (s, 1H, Ar–H), 8.81 (s, 1H, NH), 10.2 (s, 1H, NH). ^{13}C NMR (DMSO-d$_6$): δ 42.91 (C-9), 56.84 (C-10), 114.64 (C-3), 116.49 (C-3″,C-5″), 123.08 (C-2″,C-6″), 124.90 (C-1), 125.23 (C-5), 127.32 (C-3′,C-5′), 128.07 (C-2′,C-6′), 130.10 (C-6), 132.20 (C-4), 134.45 (C-1″), 138.09 (C-4′), 145.39 (C-1′), 156.34 (C-2), 158.59 (C-4″), 164.67 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{19}ClFN_2O_4S^+$ 449.07380 found 449.07089.

4.1.3.4. 5-Chloro-2-methoxy-N-[(N-4-methylphenyl) 4-sulfamoylbenzyl] benzamide (7). Fine yellow crystals; yield 72%; mp 152–154 °C. ^{1}H NMR (DMSO-d$_6$): δ 2.13 (s, 3H,

CH_3), 3.84 (s, 3H, CH_3), 4.48 (d, 2H, J = 5.7 Hz, CH_2), 6.93 (d, 2H, J = 4.8 Hz, Ar–H), 6.97 (d, 2H, J = 4.8 Hz, Ar–H), 7.15 (d, 1H, J = 4.85 Hz, Ar–H), 7.41 (d, 2H, J = 4.8 Hz, Ar–H), 7.5 (dd, 1H, J = 4.85 and 0.75 Hz, Ar–H), 7.67 (d, 2H, J = 4.8 Hz, Ar–H), 7.7 (d, 1H, J = 4.85 Hz, Ar–H), 8.84 (s, 1H, NH), 10.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.81 (C-7″), 42.91 (C-9), 56.82 (C-10), 114.62 (C-3), 120.94 (C-2″,C-6″), 124.87 (C-1), 125.25 (C-5), 127.33 (C-3′,C-5′), 128.00 (C-2′,C-6′), 130.10 (C-6,C-3″,C-5″), 132.19 (C-4), 133.79 (C-4″), 135.61 (C-1″), 138.41 (C-4′), 145.21 (C-1′), 156.34 (C-2), 164.63 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{22}H_{22}ClN_2O_4S^+$ 445.09887 found 445.09058.

4.1.3.5. 5-Chloro-2-methoxy-N-[(N-4-methoxyphenyl) 4-sulfamoylbenzyl] benzamide (8). Dark brown granules; yield 82%; mp 172–174 °C. ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 4.64 (d, 2H, J = 5.7 Hz, CH_2), 6.64 (d, 2H, J = 8.6 Hz, Ar–H), 6.87 (d, 2H, J = 8.65 Hz, Ar–H), 7.1 (d, 1H, J = 8.6 Hz, Ar–H), 7.25 (d, 2H, J = 8.6 Hz, Ar–H), 7.38 (d, 2H, J = 8.65, Ar–H), 7.5 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.7 (d, 1H, J = 8.6 Hz, Ar–H), 8.12 (s, 1H, NH), 8.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.91 (C-9), 55.61 (C-7″), 56.80 (C-10), 114.54 (C-3), 114.80 (C-3″,C-5″), 123.78 (C-2″,C-6″), 124.97 (C-1), 125.06 (C-5), 127.38 (C-3′,C-5′), 127.99 (C-2′,C-6′), 130.22 (C-6), 130.75 (C-1″), 132.25 (C-4), 138.45 (C-4′), 145.14 (C-1′), 156.38 (C-2), 157.01 (C-4″), 164.69 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{22}H_{22}ClN_2O_5S^+$ 461.09378 found 461.08841.

4.1.3.6. 5-Chloro-2-methoxy-N-[(N-4-aminophenyl) 4-sulfamoylbenzyl] benzamide (9). Fine brownish white crystals; yield 98%; mp 118–120 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.49 (d, 2H, J = 4.8 Hz, CH_2), 5.72 (s, 2H, NH_2), 6.5 (d, 2H, J = 7.65 Hz, Ar–H), 6.74 (d, 2H, J = 7.65 Hz, Ar–H), 7.15 (d, 1H, J = 7.65 Hz, Ar–H), 7.41 (d, 2H, J = 7.65 Hz, Ar–H), 7.5 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.61 (d, 2H, J = 7.65, Ar–H), 7.66 (d, 1H, J = 7.65 Hz, Ar–H), 8.84 (s, 1H, NH), 9.63 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.91 (C-9), 56.85 (C-10), 114.65 (C-3), 116.24 (C-3″,C-5″), 124.35 (C-2″,C-6″), 124.86 (C-1), 125.32 (C-5), 127.39 (C-3′,C-5′), 127.82 (C-2′,C-6′), 130.08 (C-6,C-1″), 132.17 (C-4), 138.55 (C-4′), 143.90 (C-4″), 144.91 (C-1′), 156.34 (C-2), 164.65 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{21}ClN_3O_4S^+$ 446.09412 found 446.09313.

4.1.3.7. 5-Chloro-2-methoxy-N-[(N-4-hydroxyphenyl) 4-sulfamoylbenzyl] benzamide (10). Dark brown plates; yield 98%; mp 130–132 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.48 (d, 2H, J = 5.7 Hz, CH_2), 6.56 (d, 2H, J = 8.6 Hz, Ar–H), 6.81 (d, 2H, J = 8.65 Hz, Ar–H), 7.14 (d, 1H, J = 8.6 Hz, Ar–H), 7.4 (d, 2H, J = 8.6 Hz, Ar–H), 7.5 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.59 (d, 2H, J = 7.65, Ar–H), 7.65 (d, 1H, J = 8.6 Hz, Ar–H), 8.83 (s, 1H, NH), 9.27 (s, 1H, NH), 9.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.92 (C-9), 56.78 (C-10), 114.53 (C-3), 116.13 (C-3″,C-5″), 124.48 (C-2″,C-6″), 124.98 (C-1), 125.12 (C-5), 127.40 (C-3′,C-5′), 127.88 (C-2′,C-6′), 129.11 (C-6), 130.21 (C-1″), 132.21 (C-4), 138.53 (C-4′), 144.96 (C-1′), 155.37 (C-4″), 156.36 (C-2), 164.70 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{20}ClN_2O_5S^+$ 447.07814 found 447.07571.

4.1.3.8. 5-Chloro-2-methoxy-N-[(N-2-hydroxyphenyl) 4-sulfamoylbenzyl] benzamide (11). Fine yellowish white needles; yield 87%; mp 95–97 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, CH_3), 4.49 (d, 2H, J = 5.75 Hz, CH_2), 6.63–6.7 (m, 3H, Ar–H), 6.88 (t, 1H, J = 6.7 and 7.65 Hz, Ar–H), 7.1 (d, 1H, J = 7.65 Hz, Ar–H), 7.16 (d, 1H, J = 9.55 Hz, Ar–H), 7.4 (d, 2H, J = 7.65 Hz, Ar–H), 7.49 (dd, 1H, J = 8.6 and 2.9 Hz, Ar–H), 7.65 (d, 1H, J = 2.85 Hz, Ar–H), 7.7 (d, 1H, J = 8.6 Hz, Ar–H), 8.82 (s, 1H, NH), 9.18 (s, 1H, NH), 9.55 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.82 (C-9), 56.84 (C-10), 114.64 (C-3), 116.06 (C-3″), 119.45 (C-6″), 124.77 (C-1,C-4″), 125.37 (C-5), 126.56 (C-5″), 127.36 (C-3′,C-5′), 127.69 (C-2′,C-6′), 130.03 (C-6,C-1″), 132.15 (C-4), 139.53 (C-4′), 144.89 (C-1′), 150.57 (C-2″), 156.30 (C-2), 164.66 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{20}ClN_2O_5S^+$ 447.07814 found 447.07881.

4.1.3.9. 5-Chloro-2-methoxy-N-[(N-2-carboxyphenyl) 4-sulfamoylbenzyl] benzamide (12). Yellowish brown plates; yield 79%; mp 224–226 °C. ^{1}H NMR (DMSO-d_6): δ 3.14 (s, 1H, NH), 3.83 (s, 3H, CH_3), 4.48 (d, 2H, J = 4.75 Hz, CH_2), 7.13 (d, 1H, J = 2.5 Hz, Ar–H), 7.24 (d, 1H, J = 7.2 Hz, Ar–H), 7.44–7.49 (m, 6H, Ar–H), 7.63 (s, 1H, Ar–H), 7.78 (d, 1H, J = 6.2 Hz, Ar–H), 7.8 (d, 1H, J = 5.75 Hz, Ar–H), 8.84 (s, 1H, NH), 11.15 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ 42.82 (C-9), 56.77 (C-10), 114.54 (C-3, C-2″), 116.89 (C-6″), 118.58 (C-4″), 123.77 (C-1), 124.92 (C-5), 127.56 (C-3′,C-5′), 128.33 (C-2′,C-6′), 130.14 (C-6), 132.13 (C-4), 132.28 (C-3″), 135.08 (C-5″), 137.41 (C-1″), 140.36 (C-4′), 146.13 (C-1′), 156.33 (C-2), 164.77 (C-7), 170.39 (C-7″). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{22}H_{20}ClN_2O_6S^+$ 475.07305 found 475.06793.

4.1.3.10. 5-Chloro-2-methoxy-N-[(N-2-nitro-4-methylphenyl) 4-sulfamoylbenzyl] benzamide (13). Orange red crystals; yield 98%; mp 145–147 °C. ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.45 (d, 2H, J = 5.7 Hz, CH_2), 6.91 (d, 1H, J = 8.6 Hz, Ar–H), 7.11 (d, 1H, J = 8.6 Hz, Ar–H), 7.14 (d, 2H, J = 8.6 Hz, Ar–H), 7.19 (d, 2H, J = 8.6 Hz, Ar–H), 7.36 (dd, 1H, J = 6.85 and 1.5 Hz, Ar–H), 7.48 (dd, 1H, J = 8.6 and 2.9 Hz, Ar–H), 7.66 (d, 1H, J = 8.6 Hz, Ar–H), 7.7 (d, 1H, J = 7.65 Hz, Ar–H), 8.85 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.47 (C-7″), 42.82 (C-9), 56.82 (C-10), 114.61 (C-3), 119.77 (C-3″,C-6″), 124.64 (C-1,C-5″), 125.04 (C-5), 127.47 (C-3′,C-5′), 128.15 (C-2′,C-6′), 130.11 (C-6), 130.42 (C-4″), 132.18 (C-4), 135.19 (C-2″), 137.82 (C-4′,C-1″), 144.95 (C-1′), 156.34 (C-2), 164.65 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{22}H_{21}ClN_3O_6S^+$ 490.08395 found 490.07029.

4.1.3.11. 5-Chloro-2-methoxy-N-[(N-benzyl) 4-sulfamoylbenzyl] benzamide (14). Brownish yellow granules; yield 76%; mp 87–89 °C. ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, CH_3), 3.94 (s, 2H, CH_2), 4.54 (d, 2H, J = 5.7 Hz, CH_2), 7.17–7.25 (m, 6H, Ar–H), 7.5 (d, 4H, J = 7.65 Hz, Ar–H), 7.65 (dd, 1H, J = 6.65 and 1.5 Hz, Ar–H), 7.75 (d, 1H, J = 7.65 Hz, Ar–H), 8.15 (br s, 1H, NH), 8.91 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.90 (C-9), 47.21 (C-7″), 56.88 (C-10), 114.65 (C-3), 124.91 (C-1), 125.31 (C-5), 127.17 (C-3′,C-5′), 127.66 (C-3″,C-5″), 128.11 (C-2″, C-4″,C-6″), 128.76 (C-2′,C-6′), 130.14 (C-6), 132.21 (C-4), 138.25 (C-4′), 139.60 (C-1″), 144.80 (C-1′), 156.37

(C-2), 164.64 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{22}H_{22}ClN_2O_4S^+$ 445.09887 found 445.08754.

4.1.3.12. 5-Chloro-2-methoxy-N-[(N-2-phenylethyl) 4-sulfamoylbenzyl] benzamide (15). White crystals; yield 97%; mp 152–154 °C. ^{1}H NMR (DMSO-d_6): δ 2.62 (t, 2H, J = 7.65 and 7.65 Hz, CH_2), 2.9 (q, 2H, J = 6.7, 6.7 and 6.7 Hz, CH_2), 3.39 (s, 1H, NH), 3.85 (s, 3H, CH_3), 4.52 (d, 2H, J = 6.65 Hz, CH_2), 7.09 (d, 1H, J = 7.65 Hz, Ar–H), 7.11–7.25 (m, 5H, Ar–H), 7.46 (d, 2H, J = 7.65 Hz, Ar–H), 7.52 (dd, 1H, J = 5.85 and 1.5 Hz, Ar–H), 7.65 (d, 1H, J = 2.9, Ar–H), 7.7 (d, 2H, J = 7.65 Hz, Ar–H), 8.86 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 35.91 (C-7″), 42.90 (C-9), 44.55 (C-8″), 56.85 (C-10), 114.65 (C-3), 124.89 (C-1), 125.22 (C-5), 126.77 (C-4″), 127.16 (C-3′,C-5′), 128.14 (C-2″,C-6″), 128.85 (C-3″,C-5″), 129.17 (C-2′,C-6′), 130.09 (C-6), 132.24 (C-4), 139.19 (C-4′,C-1″), 144.77 (C-1′), 156.35 (C-2), 164.69 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{23}H_{24}ClN_2O_4S^+$ 459.11452 found 459.10252.

4.1.3.13. 5-Chloro-2-methoxy-N-[(N-iso-propyl) 4-sulfamoylbenzyl] benzamide (16). White crystals; yield 57%; mp 138–140 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (d, 6H, J = 6.7 Hz, $2CH_3$), 3.42 (q, 1H, J = 2.5, 5.75 and 2.5 Hz, CH), 3.93 (s, 3H, CH_3), 4.61 (d, 1H, J = 5.75, NH), 4.71 (d, 2H, J = 4.75 Hz, CH_2), 6.94 (d, 1H, J = 8.6 Hz, Ar–H), 7.4 (d, 2H, J = 7.7 Hz, Ar–H), 7.44 (d, 2H, J = 7.7 Hz, Ar–H), 7.82 (d, 2H, J = 8.6 Hz, Ar–H), 8.2 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.76 (C-2″, C3″), 43.29 (C-9), 46.00 (C-1″), 56.86 (C-10), 114.66 (C-3), 124.86 (C-1), 125.28 (C-5), 127.01 (C-3′,C-5′), 128.00 (C-2′,C-6′), 130.08 (C-6), 132.16 (C-4), 140.78 (C-4′), 144.52 (C-1′), 156.34 (C-2), 164.64 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{18}H_{22}ClN_2O_4S^+$ 397.09887 found 397.08438.

4.1.3.14. 5-Chloro-2-methoxy-N-[(N-2-hydroxyethyl) 4-sulfamoylbenzyl] benzamide (17). White granules; yield 47%; mp 146–148 °C. ^{1}H NMR (DMSO-d_6): δ 2.74 (t, 2H, J = 5.75 and 6.7 Hz, CH_2), 3.36 (d, 2H, J = 11.45, CH_2), 3.87 (s, 3H, CH_3), 4.53 (d, 2H, J = 5.7 Hz, CH_2), 4.68 (s, 1H, NH), 7.15 (d, 1H, J = 8.6 Hz, Ar–H), 7.48 (d, 2H, J = 6.7 Hz, Ar–H), 7.54 (s, 1H, Ar–H), 7.66 (s, 1H, Ar–H), 7.75 (d, 2H, J = 7.65 Hz, Ar–H), 8.88 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.29 (C-9), 45.00 (C-1″), 56.86 (C-10), 60.45 (C-2″), 114.66 (C-3), 124.88 (C-1), 125.34 (C-5), 127.14 (C-3′,C-5′), 128.06 (C-2′,C-6′), 130.09 (C-6), 132.18 (C-4), 139.45 (C-4′), 144.72 (C-1′), 156.35 (C-2), 164.67 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{17}H_{20}ClN_2O_5S^+$ 399.07814 found 399.06069.

4.1.3.15. 5-Chloro-2-methoxy-N-[(N-2-pyridinyl) 4-sulfamoylbenzyl] benzamide (18). Fine white crystals; yield 69%; mp 197–199 °C. ^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H, CH_3), 4.5 (d, 2H, J = 6.7 Hz, CH_2), 6.04 (s, 1H, NH), 6.88 (s, 1H, Ar–H), 7.12 (s, 2H, Ar–H), 7.3 (s, 1H, Ar–H), 7.45 (s, 3H, Ar–H), 7.66 (s, 1H, Ar–H), 7.82 (d, 2H, J = 6.7 Hz, Ar–H), 8 (s, 1H, Ar–H), 8.86 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.82 (C-9), 56.82 (C-10), 114.56 (C-3,C-6″), 116.17 (C-4″), 124.90 (C-1), 125.29 (C-5), 127.20 (C-3′,C-5′), 127.92 (C-2′,C-6′), 130.15 (C-6), 132.13 (C-4), 140.67 (C-4′), 141.36 (C-5″), 143.40 (C-3″), 144.70 (C-1′), 153.42 (C-1″), 156.32 (C-2), 164.65 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{20}H_{19}ClN_3O_4S^+$ 432.07847 found 432.07109.

4.1.3.16. 5-Chloro-2-methoxy-N-[(N-2-1, 3-thiazolyl) 4-sulfamoylbenzyl] benzamide (19). Fine brownish yellow crystals; yield 93%; mp 170–172 °C. ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H, CH_3), 4.64 (d, 2H, J = 8.6 Hz, CH_2), 6.4 (s, 1H, NH), 6.91 (d, 1H, J = 8.6 Hz, Ar–H), 7.33–7.37 (m, 4H, Ar–H), 7.7 (d, 1H, J = 7.65 Hz, Ar–H), 7.86 (dd, 1H, J = 7.65 and 1.5 Hz, Ar–H), 8.08 (t, 1H, J = 7.65 and 7.65 Hz, Ar–H), 8.15 (t, 1H, J = 6.75 and 7.65 Hz, Ar–H), 8.43 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.82 (C-9), 56.82 (C-10), 108.69 (C-3″), 114.46 (C-3), 124.94 (C-1), 125.03 (C-5), 126.51 (C-3′,C-5′), 127.94 (C-2′,C-6′), 130.30 (C-6,C-4″), 132.25 (C-4), 141.21 (C-4′), 144.42 (C-1′), 156.39 (C-2), 164.63 (C-7), 169.45 (C-1″). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{18}H_{17}ClN_3O_4S_2^+$ 438.03489 found 438.02437.

4.1.3.17. 5-Chloro-2-methoxy-N-[4-(morpholinosulfonyl)benzyl] benzamide (20). Yellowish white plates; yield 62%; mp 75–77 °C. ^{1}H NMR (DMSO-d_6): δ 2.81 (s, 4H, $2CH_2$), 3.57 (s, 4H, $2CH_2$), 3.88 (s, 3H, CH_3), 4.59 (d, 2H, J = 5.7 Hz, CH_2), 7.15 (d, 1H, J = 7.65 Hz, Ar–H), 7.48 (dd, 1H, J = 6.65 and 1.5 Hz, Ar–H), 7.57 (d, 2H, J = 7.65, Ar–H), 7.62 (d, 1H, J = 6.7 Hz, Ar–H), 7.69 (d, 2H, J = 7.65 Hz, Ar–H), 8.92 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.97 (C-9), 46.85 (C-2″,C-6″), 56.83 (C-10), 65.81 (C-3″,C-5″), 114.56 (C-3), 124.96 (C-1), 125.05 (C-5), 128.29 (C-3′,C-5′), 128.34 (C-2′,C-6′), 130.23 (C-6), 132.26 (C-4), 141.84 (C-4′), 145.97 (C-1′), 156.40 (C-2), 164.70 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{19}H_{22}ClN_2O_5S^+$ 425.09378 found 425.06734.

4.1.3.18. 5-Chloro-2-methoxy-N-[4-(piperazin-1-ylsulfonyl) benzyl] benzamide (21). Fine white crystals; yield 66%; mp 215–217 °C. ^{1}H NMR (DMSO-d_6): δ 2.92 (t, 4H, J = 8.8 and 8.8 Hz, $2CH_2$), 3.16 (d, 4H, J = 9.5 Hz, $2CH_2$), 3.87 (s, 3H, CH_3), 4.58 (d, 2H, J = 5.7 Hz, CH_2), 7.22 (d, 1H, J = 6.7 Hz, Ar–H), 7.49 (dd, 1H, J = 6.65 and 1.5 Hz, Ar–H), 7.53 (d, 2H, J = 6.7 Ar–H), 7.58 (d, 1H, J = 6.65 Hz, Ar–H), 7.85 (d, 2H, J = 6.7 Hz, Ar–H), 8.73 (s, 1H, NH), 8.96 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.84 (C-9), 43.85 (C-2″,C-6″), 45.55 (C-3″,C-5″), 56.86 (C-10), 114.63 (C-3), 124.88 (C-1), 125.40 (C-5), 128.10 (C-3′,C-5′), 128.39 (C-2′,C-6′), 130.09 (C-6), 132.25 (C-4), 141.77 (C-4′), 146.11 (C-1′), 156.32 (C-2), 164.73 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{19}H_{23}ClN_3O_4S^+$ 424.10977 found 424.08815.

4.1.3.19. 5-Chloro-2-methoxy-N-[4-(4-methylpiperazin-1-ylsulfonyl)benzyl] benzamide (22). White granules; yield 60%; mp 189–191 °C. ^{1}H NMR (DMSO-d_6): δ 2.69 (s, 11H, $4CH_2$ and CH_3), 3.87 (s, 3H, CH_3), 4.58 (d, 2H, J = 5.7 Hz, CH_2), 7.15 (d, 1H, J = 8.6 Hz, Ar–H), 7.5 (dd, 1H, J = 6.65 and 1.5 Hz, Ar–H), 7.59 (d, 2H, J = 8.6, Ar–H), 7.67 (d, 1H, J = 6.7 Hz, Ar–H), 7.72 (d, 2H, J = 8.6, Ar–H), 8.98 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 42.84 (C-9), 43.18 (C-7″), 43.85 (C-2″,C-6″), 52.01 (C-3″,C-5″), 56.91 (C-10), 114.67 (C-3), 124.86 (C-1), 125.18 (C-5), 128.25 (C-3′,C-5′), 128.48 (C-2′,C-6′), 130.09 (C-6), 132.27 (C-4), 133.47 (C-4′), 146.36 (C-1′), 156.38 (C-2), 164.76 (C-7). HMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_{20}H_{25}ClN_3O_4S^+$ 438.12542 found 438.11765.

4.1.4. General synthetic procedures of 5-chloro-*N*-(4-sulfamoylbenzyl) salicylamide derivatives (23–41)[57]. Sodium cyanide (0.29 g, 6 mmol) and the appropriate 5-chloro-2-

methoxy-*N*-(4-sulfamoylbenzyl) benzamide derivatives [(**4–22**), 4 mmol] were added to anhydrous dimethyl sulfoxide (5 ml). The reaction mixture was refluxed for 1 h under stirring and then cold water (50 ml) was added to the reaction mixture. The solution was acidified with 1N HCl, the precipitate was filtered, washed several times with distilled water and crystallized from benzene to give salicylamide derivatives (**23–41**).

4.1.4.1. 5-Chloro-N-[(N-phenyl) 4-sulfamoylbenzyl] salicylamide (23). Brown crystals; yield 87%; mp 97–99 °C. ^{1}H NMR (DMSO-d_6): δ 4.5 (d, 2H, J = 4.8 Hz, CH_2), 6.95 (d, 1H, J = 6.65 Hz, Ar–H), 7.06 (d, 2H, J = 7.65 Hz, Ar–H), 7.25 (d, 2H, J = 7.65 Hz, Ar–H), 7.37 (dd, 1H, J = 7.65 and 1.5 Hz, Ar–H), 7.43 (d, 2H, J = 7.65 Hz, Ar–H), 7.47 (d, 2H, J = 4.8 Hz, Ar–H), 7.7 (d, 1H, J = 6.65 Hz, Ar–H), 7.92 (d, 1H, J = 6.65 Hz, Ar–H), 9.38 (br s, 1H, NH), 10.29 (br s, 1H, NH), 12.28 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.89 (C-9), 117.46 (C-3), 119.84 (C-1), 120.35 (C-2″,C-6″), 122.98 (C-5), 124.50 (C-4″), 126.61 (C-6), 127.41 (C-3′,C-5′), 128.32 (C-2′,C-6′), 129.69 (C-3″,C-5″), 133.89 (C-4), 138.24 (C-1″), 138.88 (C-4′), 144.55 (C-1′), 158.80 (C-2), 168.00 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{20}H_{18}ClN_2O_4S^+$ 417.06757 found 417.06214.

4.1.4.2. 5-Chloro-N-[(N-4-chlorophenyl) 4-sulfamoylbenzyl] salicylamide (24). Yellowish white crystals; yield 97%; mp 137–139 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, J = 4.8 Hz, CH_2), 6.94 (d, 1H, J = 6.65 Hz, Ar–H), 7.06 (d, 1H, J = 8.6 Hz, Ar–H), 7.11 (d, 1H, J = 8.6 Hz, Ar–H), 7.26 (d, 1H, J = 8.6 Hz, Ar–H), 7.37 (d, 1H, J = 8.6 Hz, Ar–H), 7.43 (d, 2H, J = 8.6 Hz, Ar–H), 7.47 (d, 2H, J = 8.6 Hz, Ar–H), 7.7 (d, 1H, J = 7.65 Hz, Ar–H), 7.92 (d, 1H, J = 7.65 Hz, Ar–H), 9.37 (br s, 1H, NH), 10.44 (br s, 1H, NH), 12.27 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.88 (C-9), 117.46 (C-3), 119.85 (C-1), 121.91 (C-2″,C-6″), 123.01 (C-5), 127.40 (C-6), 128.18 (C-3′,C-5′), 128.43 (C-3″,C-5″), 129.42 (C-2′,C-6′), 129.68 (C-4″), 133.89 (C-4), 137.25 (C-1″), 138.33 (C-4′), 145.19 (C-1′), 158.81 (C-2), 167.98 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{20}H_{17}Cl_2N_2O_4S^+$ 451.02860 found 451.02613.

4.1.4.3. 5-Chloro-N-[(N-4-fluorophenyl) 4-sulfamoylbenzyl] salicylamide (25). Black crystals; yield 95%; mp 117–119 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, J = 5.7 Hz, CH_2), 6.93 (t, 1H, J = 9.6 and 9.6 Hz, Ar–H), 7.05 (d, 4H, J = 8.6 Hz, Ar–H), 7.43 (d, 2H, J = 7.6 Hz, Ar–H), 7.53 (dd, 1H, J = 7.6 and 2.5 Hz, Ar–H), 7.66 (d, 2H, J = 7.65 Hz, Ar–H), 7.91 (s, 1H, Ar–H), 9.36 (s, 1H, NH), 10.24 (s, 1H, NH), 12.28 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.89 (C-9), 116.32 (C-3″,C-5″), 116.50 (C-2″,C-6″), 117.45 (C-3), 119.83 (C-1), 123.06 (C-5), 127.40 (C-3′,C-5′), 128.09 (C-6), 128.36 (C-2′,C-6′), 133.88 (C-4), 134.59 (C-1″), 138.35 (C-4′), 144.61 (C-1′), 158.87 (C-2), 160.55 (C-4″), 168.04 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{20}H_{17}ClFN_2O_4S^+$ 435.05815 found 435.05613.

4.1.4.4. 5-Chloro-N-[(N-4-methylphenyl) 4-sulfamoylbenzyl] salicylamide (26). Yellowish white crystals; yield 87%; mp 167–169 °C. ^{1}H NMR (DMSO-d_6): δ 2.13 (s, 3H, CH_3), 4.5 (d, 2H, J = 5.7 Hz, CH_2), 6.93 (d, 2H, J = 8.6 Hz, Ar–H), 6.97 (d, 2H, J = 8.6 Hz, Ar–H), 7.1 (d, 1H, J = 7.65 Hz, Ar–H), 7.42 (d, 2H, J = 8.6 Hz, Ar–H), 7.46 (d, 1H, J = 7.65 Hz, Ar–H), 7.67 (d, 2H, J = 8.6 Hz, Ar–H), 7.91 (s, 1H, Ar–H), 9.36 (br s, 1H, NH), 10.12 (s, 1H, NH), 12.29 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 20.79 (C-7″), 42.90 (C-9), 117.45 (C-3), 119.84 (C-1), 120.92 (C-2″,C-6″), 123.00 (C-5), 126.75 (C-6), 127.41 (C-3′,C-5′), 128.30 (C-2′,C-6′), 130.10 (C-3″,C-5″), 133.81 (C-4″), 133.90 (C-4), 135.79 (C-1″), 138.55 (C-4′), 144.51 (C-1′), 158.91 (C-2), 168.06 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{20}ClN_2O_4S^+$ 431.08322 found 431.07984.

4.1.4.5. 5-Chloro-N-[(N-4-methoxyphenyl) 4-sulfamoylbenzyl] salicylamide (27). Fine white needles; yield 98%; mp 107–109 °C. ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 3H, CH_3), 4.51 (d, 2H, J = 8.6 Hz, CH_2), 6.76 (d, 1H, J = 8.6 Hz, Ar–H), 6.84 (d, 2H, J = 8.6 Hz, Ar–H), 6.94 (d, 2H, J = 6.65 Hz, Ar–H), 7.42 (d, 2H, J = 7.65 Hz, Ar–H), 7.47 (d, 2H, J = 6.7 Hz, Ar–H), 7.61 (dd, 1H, J = 8.6 and 1.5 Hz, Ar–H), 7.93 (t, 1H, J = 10.55 and 10.55 Hz, Ar–H), 9.37 (br s, 1H, NH), 10.44 (br s, 1H, NH), 12.27 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.88 (C-9), 55.63 (C-7″), 114.82 (C-3″,C-5″), 117.29 (C-3), 119.83 (C-1), 123.00 (C-5), 123.70 (C-2″,C-6″), 127.41 (C-6), 128.09 (C-3′,C-5′), 128.23 (C-2′,C-6′), 133.90 (C-4), 135.39 (C-1″), 138.65 (C-4′), 144.76 (C-1′), 156.82 (C-4″), 158.82 (C-2), 168.01 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{21}H_{20}ClN_2O_5S^+$ 447.07814 found 447.06813.

4.1.4.6. 5-Chloro-N-[(N-4-aminophenyl) 4-sulfamoylbenzyl] salicylamide (28). Brown crystals; yield 91%; mp 207–209 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, J = 5.7 Hz, CH_2), 6.6 (d, 2H, J = 7.65 Hz, Ar–H), 6.8 (d, 2H, J = 7.65 Hz, Ar–H), 7.15 (d, 1H, J = 7.65 Hz, Ar–H), 7.41 (d, 2H, J = 7.65 Hz, Ar–H), 7.47 (d, 1H, J = 7.65 Hz, Ar–H), 7.7 (d, 2H, J = 7.65, Ar–H), 7.93 (d, 1H, J = 7.65 Hz, Ar–H), 9.43 (br s, 1H, NH), 10.31 (s, 1H, NH), 12.28 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.91 (C-9), 115.84 (C-3″,C-5″), 117.49 (C-3), 119.81 (C-1), 122.33 (C-5), 123.37 (C-2″,C-6″), 126.17 (C-6), 127.48 (C-3′,C-5′), 128.32 (C-2′,C-6′), 133.82 (C-4), 135.79 (C-1″), 138.75 (C-4′), 143.75 (C-4″), 144.85 (C-1′), 158.78 (C-2), 168.01 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{20}H_{19}ClN_3O_4S^+$ 432.07847 found 432.07558.

4.1.4.7. 5-Chloro-N-[(N-4-hydroxyphenyl) 4-sulfamoylbenzyl] salicylamide (29). Fine black crystals; yield 92%; mp 176–178 °C. ^{1}H-NMR (DMSO-d_6): δ 4.56 (d, 2H, J = 7.65 Hz, CH_2), 6.91 (d, 1H, J = 9.55 Hz, Ar–H), 7.15–7.3 (m, 4H, Ar–H), 7.42–7.64 (m, 4H, Ar–H), 7.87 (d, 1H, J = 7.65 Hz, Ar–H), 7.93 (s, 1H, Ar–H), 8.72 (br s, 1H, NH), 9.33 (br s, 1H, NH), 12.29 (br s, 1H, OH), 13.34 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.89 (C-9), 115.24 (C-3″,C-5″), 117.65 (C-3), 119.83 (C-1), 122.98 (C-5), 124.57 (C-2″,C-6″), 126.65 (C-6), 127.59 (C-3′,C-5′), 128.61 (C-2′,C-6′), 133.84 (C-4), 135.89 (C-1″), 140.23 (C-4′), 145.54 (C-1′), 156.16 (C-4″), 159.02 (C-2), 168.04 (C-7). HMS (ESI): m/z [M + H]$^+$ calcd. for $C_{20}H_{18}ClN_2O_5S^+$ 433.06249 found 433.05792.

4.1.4.8. 5-Chloro-N-[(N-2-hydroxyphenyl) 4-sulfamoylbenzyl] salicylamide (30). Fine black crystals; yield 89%; mp 144–146 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, J = 3.8 Hz, CH_2), 6.67 (dd, 1H, J = 6.7 and 1.5 Hz, Ar–H), 6.92 (d, 2H, J = 8.6 Hz, Ar–H), 7.1 (d, 1H, J = 6.7 Hz, Ar–H), 7.41 (d, 4H, J = 6.7 Hz, Ar–H), 7.55 (dd, 1H, J = 6.7 and 1.5 Hz, Ar–H), 7.7 (d, 1H, J = 7.65 Hz, Ar–H), 7.92 (s, 1H, Ar–H), 9.23 (s, 1H, NH), 9.38 (s, 1H, NH), 9.57 (br s, 1H, OH), 12.31 (br s, 1H, OH). ^{13}C NMR

(DMSO-d_6): δ 42.92 (C-9), 116.10 (C-3″), 117.63 (C-3), 119.48 (C-6″), 119.84 (C-1), 123.00 (C-5), 124.68 (C-4″), 126.67 (C-6), 127.45 (C-3′,C-5′), 128.02 (C-5″), 128.10 (C-2′,C-6′), 133.88 (C-4,C-1″), 139.50 (C-4′), 144.12 (C-1′), 150.56 (C-2″), 158.83 (C-2), 167.99 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{20}H_{18}ClN_2O_5S^+$ 433.06249 found 433.05631.

4.1.4.9. 5-Chloro-N-[(N-2-carboxyphenyl) 4-sulfamoylbenzyl] salicylamide (31). Grey granules; yield 91%; mp 129–131 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, *J* = 3.8 Hz, CH_2), 6.91 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.06 (t, 1H, *J* = 4.8 and 5.7 Hz, Ar–H), 7.13 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.32 (s, 1H, Ar–H), 7.41 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.46 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.78 (d, 2H, *J* = 6.65 Hz, Ar–H), 7.87 (t, 1H, *J* = 4.75 and 8.6 Hz, Ar–H), 8.83 (s, 1H, NH), 9.37 (s, 1H, NH), 11.26 (br s, 1H, COOH), 12.28 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.93 (C-9), 114.50 (C-2″), 117.00 (C-6″), 117.64 (C-3), 118.49 (C-4″), 119.83 (C-1), 122.98 (C-5), 127.66 (C-3′,C-5′), 128.11 (C-6), 128.61 (C-2′,C-6′), 132.13 (C-3″), 133.90 (C-4), 135.06 (C-5″), 137.59 (C-1″), 140.05 (C-4′), 145.02 (C-1′), 158.80 (C-2), 167.98 (C-7), 170.38 (C-7″). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{21}H_{18}ClN_2O_6S^+$ 461.05740 found 461.05062.

4.1.4.10. 5-Chloro-N-[(N-2-nitro-4-methylphenyl) 4-sulfamoylbenzyl] salicylamide (32). Brown crystals; yield 77%; mp 96–98 °C. ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H, CH_3), 4.53 (d, 2H, *J* = 5.75 Hz, CH_2), 6.9 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.07 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.2 (d, 2H, *J* = 8.6 Hz, Ar–H), 7.39 (d, 2H, *J* = 8.6 Hz, Ar–H), 7.46 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.65 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.7 (s, 1H, Ar–H), 7.83 (d, 1H, *J* = 8.6 Hz, Ar–H), 9.4 (br s, 1H, NH), 10.16 (s, 1H, NH), 12.3 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 20.52 (C-7″), 42.90 (C-9), 116.34 (C-3″), 117.45 (C-3), 119.74 (C-1,C-6″), 123.00 (C-5), 124.59 (C-5″), 127.53 (C-3′,C-5′), 128.08 (C-6), 128.41 (C-2′,C-6′), 130.39 (C-4″), 133.85 (C-4), 135.17 (C-2″), 137.78 (C-4′,C-1″), 144.91 (C-1′), 158.83 (C-2), 167.97 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{21}H_{19}ClN_3O_6S^+$ 476.06830 found 476.05972.

4.1.4.11. 5-Chloro-N-[(N-benzyl) 4-sulfamoylbenzyl] salicylamide (33). Fine yellow needles; yield 72%; mp 152–154 °C. ^{1}H NMR (DMSO-d_6): δ 3.93 (d, 2H, *J* = 5.7 Hz, CH_2), 4.54 (d, 2H, *J* = 5.75 Hz, CH_2), 6.94 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.16–7.24 (m, 5H, Ar–H), 7.43 (dd, 1H, *J* = 8.6 and 1.5 Hz, Ar–H), 7.49 (d, 2H, *J* = 8.6 Hz, Ar–H), 7.74 (d, 2H, *J* = 8.6 Hz, Ar–H), 7.94 (d, 1H, *J* = 1.9 Hz, Ar–H), 8.11 (br s, 1H, NH), 9.44 (s, 1H, NH), 12.27 (br s, 1H, OH). ^{13}C NMR (DMSO-d6): δ 42.88 (C-9), 46.65 (C-7″), 117.52 (C-3), 119.89 (C-1), 122.97 (C-5), 127.21 (C-3′,C-5′), 127.64 (C-6), 128.08 (C-2″,C-4″,C-6″), 128.42 (C-3″,C-5″), 128.75 (C-2′,C-6′), 133.93 (C-4), 138.17 (C-4′), 139.75 (C-1″), 144.01 (C-1′), 158.89 (C-2), 167.96 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{21}H_{20}ClN_2O_4S^+$ 431.08322 found 431.07824.

4.1.4.12. 5-Chloro-N-[(N-2-phenylethyl) 4-sulfamoylbenzyl] salicylamide (34). Pale yellow needles; yield 76%; mp 155–157 °C. ^{1}H NMR (DMSO-d_6): δ 2.62 (t, 2H, *J* = 7.65 and 7.65 Hz, CH_2), 2.9 (d, 2H, *J* = 6.7 Hz, CH_2), 4.55 (d, 2H, *J* = 5.75 Hz, CH_2), 6.98 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.09 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.13 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.19 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.41 (dd, 1H, *J* = 8.65 and 2.5 Hz, Ar–H), 7.49 (d, 2H, *J* = 8.65 Hz, Ar–H), 7.68 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.71 (d, 2H, *J* = 8.65 Hz, Ar–H), 7.98 (s, 1H, NH), 9.43 (s, 1H, NH), 12.34 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 35.89 (C-7″), 42.92 (C-9), 44.59 (C-8″), 117.47 (C-3), 119.86 (C-1), 122.99 (C-5), 126.75 (C-6), 127.24 (C-3′,C-5′), 128.09 (C-4″), 128.42 (C-2″,C-6″), 128.83 (C-3″,C-5″), 129.16 (C-2′,C-6′), 133.92 (C-4), 139.19 (C-4′), 139.50 (C-1″), 143.99 (C-1′), 158.90 (C-2), 168.01 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{22}H_{22}ClN_2O_4S^+$ 445.09887 found 445.09126.

4.1.4.13. 5-Chloro-N-[(N-iso-propyl) 4-sulfamoylbenzyl] salicylamide (35). Yellowish white crystals; yield 87%; mp 174–176 °C. ^{1}H NMR (DMSO-d_6): δ 0.9 (d, 6H, *J* = 5.75 Hz, $2CH_3$), 3.18 (s, 1H, CH), 4.55 (d, 2H, *J* = 5.75 Hz, CH_2), 6.92 (d, 1H, *J* = 9.55 Hz, Ar–H), 7.41 (d, 2H, *J* = 6.7 Hz, Ar–H), 7.48 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.53 (t, 1H, *J* = 5.75 and 6.7 Hz, Ar–H), 7.72 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.93 (s, 1H, NH), 9.41 (s, 1H, NH), 12.35 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 23.75 (C-2″,C3″), 42.90 (C-9), 45.90 (C-1″), 117.47 (C-3), 119.86 (C-1), 123.00 (C-5), 127.09 (C-3′,C-5′), 128.09 (C-6), 128.29 (C-2′,C-6′), 133.91 (C-4), 141.01 (C-4′), 143.73 (C-1′), 158.88 (C-2), 168.02 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{17}H_{20}ClN_2O_4S^+$ 383.08322 found 383.07519.

4.1.4.14. 5-Chloro-N-[(N-2-hydroxyethyl) 4-sulfamoylbenzyl] salicylamide (36). Yellowish brown crystals; yield 77%; mp 163–165 °C. ^{1}H NMR (DMSO-d_6): δ 2.74 (d, 2H, *J* = 5.75 Hz, CH_2), 3.33 (d, 2H, *J* = 5.75 Hz, CH_2), 4.55 (d, 2H, *J* = 5.75 Hz, CH_2), 6.94 (d, 1H, *J* = 9.55 Hz, Ar–H), 7.41 (d, 2H, *J* = 6.7 Hz, Ar–H), 7.5 (d, 2H, *J* = 7.65 Hz, Ar–H), 7.56 (t, 1H, *J* = 5.75 and 6.7 Hz, Ar–H), 7.74 (d, 1H, *J* = 7.65 Hz, Ar–H), 7.94 (s, 1H, NH), 9.43 (s, 1H, NH), 12.35 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.94 (C-9), 45.80 (C-1″), 60.44 (C-2″), 117.46 (C-3), 119.86 (C-1), 123.01 (C-5), 127.23 (C-3′,C-5′), 128.08 (C-6), 128.36 (C-2′,C-6′), 133.92 (C-4), 139.70 (C-4′), 143.94 (C-1′), 158.88 (C-2), 168.02 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{16}H_{18}ClN_2O_5S^+$ 385.06249 found 385.05627.

4.1.4.15. 5-Chloro-N-[(N-2-pyridinyl) 4-sulfamoylbenzyl] salicylamide (37). Fine yellowish white crystals; yield 89%; mp 167–169 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, *J* = 6.7 Hz, CH_2), 6.95 (s, 1H, Ar–H), 7.4 (s, 2H, Ar–H), 7.53 (s, 2H, Ar–H), 7.67 (s, 1H, Ar–H), 7.78 (s, 2H, Ar–H), 7.91 (s, 2H, Ar–H), 8.06 (s, 1H, Ar–H), 8.84 (br s, 1H, NH), 9.39 (s, 1H, NH), 12.33 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.90 (C-9), 111.81 (C-6″), 116.25 (C-4″), 117.50 (C-3), 119.85 (C-1), 122.99 (C-5), 127.22 (C-3′,C-5′), 128.04 (C-6), 128.39 (C-2′,C-6′), 133.91 (C-4), 138.70 (C-4′), 141.68 (C-5″), 142.26 (C-3″), 144.85 (C-1′), 153.72 (C-1″), 158.93 (C-2), 168.01 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{19}H_{17}ClN_3O_4S^+$ 418.06282 found 418.05565.

4.1.4.16. 5-Chloro-N-[(N-2-1,3-thiazolyl) 4-sulfamoylbenzyl] salicylamide (38). Fine brownish white crystals; yield 96%; mp 196–198 °C. ^{1}H NMR (DMSO-d_6): δ 4.51 (d, 2H, *J* = 4.8 Hz, CH_2), 6.93 (d, 1H, *J* = 4.8 Hz, Ar–H), 7.33 (d, 2H, *J* = 4.8 Hz, Ar–H), 7.42 (d, 2H, *J* = 4.8 Hz, Ar–H), 7.64 (d, 1H, *J* = 4.8 Hz, Ar–H), 7.75 (d, 2H, *J* = 4.8 Hz, Ar–H), 7.92 (d, 1H, *J* = 4.8 Hz, Ar–H), 8.85 (br s, 1H, NH), 9.4 (s, 1H, NH), 12.31 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.89 (C-9), 107.20 (C-3″), 117.46 (C-3),

119.84 (C-1), 123.00 (C-5), 126.68 (C-6), 127.87 (C-3′,C-5′), 128.20 (C-2′,C-6′), 129.86 (C-4″), 133.90 (C-4), 141.25 (C-4′), 143.68 (C-1′), 158.88 (C-2), 166.64 (C-1″), 167.99 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{17}H_{15}ClN_3O_4S_2^+$ 424.01924 found 424.00748.

*4.1.4.17. 5-Chloro-N-[4-(morpholinosulfonyl)benzyl] salicylamide (**39**).* Fine yellowish white needles; yield 84%; mp 164–166 °C. ^{1}H NMR (DMSO-d_6): δ 2.81 (s, 4H, $2CH_2$), 3.58 (s, 4H, $2CH_2$), 4.59 (s, 2H, CH_2), 6.94 (d, 1H, J = 8.6 Hz, Ar–H), 7.43 (dd, 1H, J = 6.65 and 1.5 Hz, Ar–H), 7.57 (d, 2H, J = 8.6, Ar–H), 7.68 (d, 2H, J = 7.65 Hz, Ar–H), 7.93 (d, 1H, J = 2.85 Hz, Ar–H), 8.9 (br s, 1H, NH), 9.42 (br s, 1H, NH), 12.29 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.93 (C-9), 46.45 (C-2″,C-6″), 65.80 (C-3″,C-5″), 117.56 (C-3), 119.83 (C-1), 123.02 (C-5), 128.13 (C-6), 128.41 (C-3′,C-5′), 128.60 (C-2′,C-6′), 133.91 (C-4), 141.44 (C-4′), 145.18 (C-1′), 158.84 (C-2), 168.02 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{18}H_{20}ClN_2O_5S^+$ 411.07814 found 411.07086.

*4.1.4.18. 5-Chloro-N-[4-(piperazin-1-ylsulfonyl)benzyl] salicylamide (**40**).* Fine white crystals; yield 92%; mp 253–255 °C. ^{1}H NMR (DMSO-d_6): δ 2.92 (t, 4H, J = 6.5 and 6.5 Hz, $2CH_2$), 3.16 (d, 4H, J = 7.5 Hz, $2CH_2$), 4.58 (d, 2H, J = 5.7 Hz, CH_2), 6.93 (d, 1H, J = 5.7 Hz, Ar–H), 7.42 (d, 1H, J = 5.7 Hz, Ar–H), 7.54 (d, 2H, J = 6.7 Ar–H), 7.64 (d, 2H, J = 6.7 Hz, Ar–H), 7.93 (d, 1H, J = 5.7 Hz, Ar–H), 8.52 (br s, 1H, NH), 9.42 (s, 1H, NH), 12.3 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.88 (C-9), 43.95 (C-2″,C-6″), 45.50 (C-3″,C-5″), 116.88 (C-3), 119.84 (C-1), 123.04 (C-5), 128.14 (C-6), 128.50 (C-3′,C-5′), 128.66 (C-2′,C-6′), 133.90 (C-4), 139.69 (C-4′), 145.32 (C-1′), 158.83 (C-2), 168.02 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{18}H_{21}ClN_3O_4S^+$ 410.09412 found 410.08127.

*4.1.4.19. 5-Chloro-N-[4-(4-methylpiperazin-1-ylsulfonyl) benzyl] salicylamide (**41**).* Fine yellowish white crystals; yield 81%; mp 233–235 °C. ^{1}H NMR (DMSO-d_6): δ 2.69 (s, 11H, $4CH_2$ and CH_3), 4.58 (d, 2H, J = 5.75 Hz, CH_2), 6.94 (d, 1H, J = 7.65 Hz, Ar–H), 7.44 (d, 1H, J = 7.65 Hz, Ar–H), 7.55 (d, 2H, J = 8.6 Hz, Ar–H), 7.66 (d, 2H, J = 8.6 Hz, Ar–H), 7.93 (d, 1H, J = 7.65 HZ, Ar–H), 8.52 (br s, 1H, NH), 9.42 (s, 1H, NH), 12.3 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 42.94 (C-9), 43.42 (C-7″), 44.03 (C-2″,C-6″), 52.32 (C-3″,C-5″), 117.23 (C-3), 119.83 (C-1), 123.01 (C-5), 128.09 (C-6), 128.42 (C-3′,C-5′), 128.56 (C-2′,C-6′), 133.89 (C-4), 135.31 (C-4′), 146.18 (C-1′), 158.79 (C-2), 167.98 (C-7). HMS (ESI): *m*/*z* $[M + H]^+$ calcd. for $C_{19}H_{23}ClN_3O_4S^+$ 424.10977 found 424.09783.

4.2. Biological evaluation

All ingredients used in preparing the required buffers were analytical grade; Trypsin-Versene, DMSO for preparing all tested compounds and non-essential amino acids (NEAA) were tissue culture grade (Lonza). Sulforhodamine-B (SRB), RPMI-1640 (RPMI) and Dulbecco's modified Eagle's medium (DMEM) were from Sigma. Fetal bovine serum (FBS) was purchased from LSP, Life science production. All plastic wares, *e.g.* serological pipettes and 96-well plates that directly came in contact with cell cultures were purchased sterile from LSP or Greiner. Colchicine was the selected standard, purchased from BDH Laboratory supplies.

4.2.1. Cultures for cancer and normal cell lines. Four human cancer cell lines were selected to examine the ability of the synthesized sulfonamides as cancer growth inhibitors and were obtained from the American Type and Culture Collection (ATCC). Two were breast adenocarcinoma, including the hormone-dependent MCF-7 (ATCC® HTB-22™) and the hormone-independent MDA-MB-231 (ATCC® CRM-HTB-26™). The rest were the colorectal adenocarcinoma Caco-2 (ATCC® HTB-37™) and the carcinoma HCT-116 (ATCC® CCL-247™). The immortalized retinal pigmented epithelium, hTERT-RPE1 was kindly provided by Prof. Stig Linder, Karolinska Institute, Stockholm, Sweden and was used as a model to investigate the cytotoxicity effects of the synthesized sulfonamides on normal cells.

Except for MCF-7, all cell types were cultured in DMEM supplemented with 2 mM L-glutamine and 10% FBS. In addition, 1% NEAA, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin sulfate were also provided in the prepared growth media. The basic medium of MCF-7 culture was RPMI1640 and all supplements provided lacked 1% NEAA. Cells were routinely incubated at 37 °C and 5% CO_2 in a humidified CO_2 incubator (Certomat-20S, Sartorius group, NY, USA) as monolayer adherent cells in either T75 cm^2 or T25 cm^2 culture flasks.

4.2.2. Preparation of sulfonamide stock solutions. All of the compounds were initially solubilized as 40 mM stock solutions in DMSO and stored in dry conditions. Screening of the prepared compounds at a top final 50 μM concentration was accomplished by diluting the stock solutions into culture growth medium free of FBS to get 2× the final concentration and they were freshly prepared prior to the performed test.

4.2.3. *In vitro* antiproliferative activity and cytotoxicity effects. The exponentially growing cancer cells were collected and seeded into 96-well plates according to the doubling time and were incubated overnight at 37 °C to get about 70–75% confluency, while normal cells were plated out to get full confluence overnight. The 10-fold dilution series was prepared from the compound stock solutions in 100 μL of serum-free medium, added to cells, and incubated at 37 °C for further 72 h. At the end of the indicated time, the cells were fixed with 3.3% trichloroacetic acid for 1/2 h at 4 °C, where the sulforhodamine-B assay[72,73] was used to evaluate the growth inhibition effect of every cell culture and the final intensity of the color in every well was measured spectrophotometrically at 540 nm with an ELISA microplate reader (Fluostar Optima, BMG technologies, Germany). The compounds that showed GI_{50} less than 50 μM were further tested by preparing a two-fold series: 5 dilutions in 100 μl serum-free medium (50–3.125 μM). The GI_{50} values were calculated using the nonlinear regression-fitting model of Graph Pad, Prism, and version 6.

4.2.4. Selectivity index (SI) calculations. To select the most potent sample as the anti-cancer treatment and the safest on hTERT-RPE1 cells as the model of normal cell type active

compounds for further mechanistic investigations, the selectivity index (SI) was calculated. The concentration that caused 50% growth inhibition (GI_{50}) of every tested cancer type was determined for all synthesized sulfonamides while the concentration that caused 50% cytotoxicity on hTERT-RPE1 normal cells (CC_{50}) was also determined. Finally, the SI was calculated from the following equation:

$$SI = CC_{50}/GI_{50}$$

Equation: calculation of the selectivity index (SI)

4.2.5. *In vitro* testing of tubulin polymerization inhibition. The most potent samples as indicated in our screening results are **24** and **33** and were further investigated by the enzyme-linked immunosorbent Assay to check their efficiency to suppress the tubulin polymerization process of MCF-7 and Caco-2 cancer cells, respectively. SEB870Hu Kit, Cloud-Clone Corp., U.S.A for Tubulin Beta (TUBb) was followed to detect and measure the β-tubulin residue in the presence and absence of the selected compounds. Briefly, concentrations ranging from 50–0.4 μM were separately prepared in DMSO prior to 48 h incubation with a culture of Caco-2 or MCF-7. Colchicine with the same concentration range was used as the positive control against both cancer types. At the end of the indicated time, cells were detached by trypsin and washed with cold phosphate buffered saline (PBS). The washed cells were suspended in PBS and subjected 3 times to freeze/thaw cycles for lysing the cells. The obtained cell lysates were centrifuged at 1500 × *g* for 10 minutes at 2–8 °C to remove cellular debris while detecting β-tubulin residues in the supernatant according to the recommended steps of the kit; finally, the detected color was measured at 450 nm using an ELISA reader (ROBONIK P2000).

4.2.6. Cell cycle analysis. MCF-7 and Caco-2 cells were separately plated out into six-well plates at a density of 1×10^6/well in RPMI 1640 medium supplemented with 10% FBS. Cells were incubated for 24 h at 37 °C and 5% CO_2 prior to the exposure of 28 μM of compound **24** (5× of its GI_{50}) and 16.5 μM of compound **33** (5× of its GI_{50}), respectively. Both cell types were exposed to the effects of the DMSO as a vehicle for the untreated control, synchronized with the treated cells; all were left for another 48 h. At the indicated time, the cells were washed with cold PBS after trypsinization and fixation with 70% ethanol. The fixed cells were further washed prior to being lysed in a prepared solution of propidium iodide, Triton X-100 and RNase, and kept at 37 °C for 15 min according to the manufacturer's instructions for the propidium iodide (ab139418) Flow Cytometry Kit, Abcam, UK. Every analysed sample consisted of 10 000 cells, using the FACS Caliber flow cytometer (BD Biosciences & Co., Franklin Lakes, NJ).

4.2.7. Measurements of apoptosis using Annexin-V FITC/PI staining. Apoptosis was detected in both treated and untreated MCF-7 and Caco-2 cells after staining with the Annexin-V fluorescein isothiocyanate (Annexin V- FITC) then counterstaining with propidium iodide (PI). The instruction manual of the Annexin V-FITC/PI apoptosis detection kit (556547, BD Biosciences & Co., Franklin Lakes, NJ) was followed. In brief, 28 μM of compound **24** and 16.5 μM of **33** were incubated with 1×10^6 cells of MCF-7 and Caco-2, respectively, while untreated samples with equivalent DMSO concentrations were used as the vehicle control under the same conditions for both cell types. At the end of 48 h, the cells were detached by trypsinization, washed twice with cold PBS and stained with 5 μL of Annexin V-FITC and 5 μL of PI in the binding buffer for 15 min at 37 °C. Finally, 10 000 cells were analysed from each sample using the FACS Calibre flow cytometer (BD Biosciences & Co., Franklin Lakes, NJ).

4.3. Molecular docking

http://www.rcsb.org/pdb/welcome.do) with resolution 2.3 A°. Its energy was minimized using the YASARA Energy Minimization Server (http://www.rcsb.org/pdb/welcome.do) and all bound water, ligands and cofactors were removed. The pdbqt file format of the protein and the synthesized compounds were created using MGL Tools 1.5.6 (www.mgltools.scripps.edu). Docking calculation was performed using Auto Dock Vena[74] and PyRx 0.8 (www.mgltools.scripps.edu). The results were analysed based on the binding of the ligand at the inhibitory pocket using PyMol 1 (www.pymol.org).

Future perspective

The present study explores the potential of the newly synthesized compounds as tubulin inhibitors. Some of the active compounds that were found will be subjected to *in vivo* studies to evaluate their activities in addition to the side effects. This study sheds light on the importance of the design and synthesis of new derivatives based on the salicylamide moiety incorporated with sulfonamide groups.

Conflicts of interest

The authors declare no competing interest.

Acknowledgements

The authors would like to thank the National Research Centre, Egypt for the laboratory facilities and financial support provided by the project number 11010324.

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