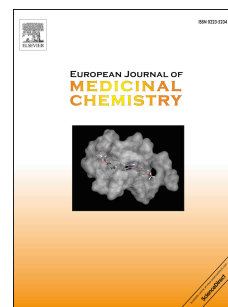


Journal Pre-proof

Promising anti-proliferative indolic benzenesulfonamides alter mechanisms with sulfonamide nitrogen substituents.

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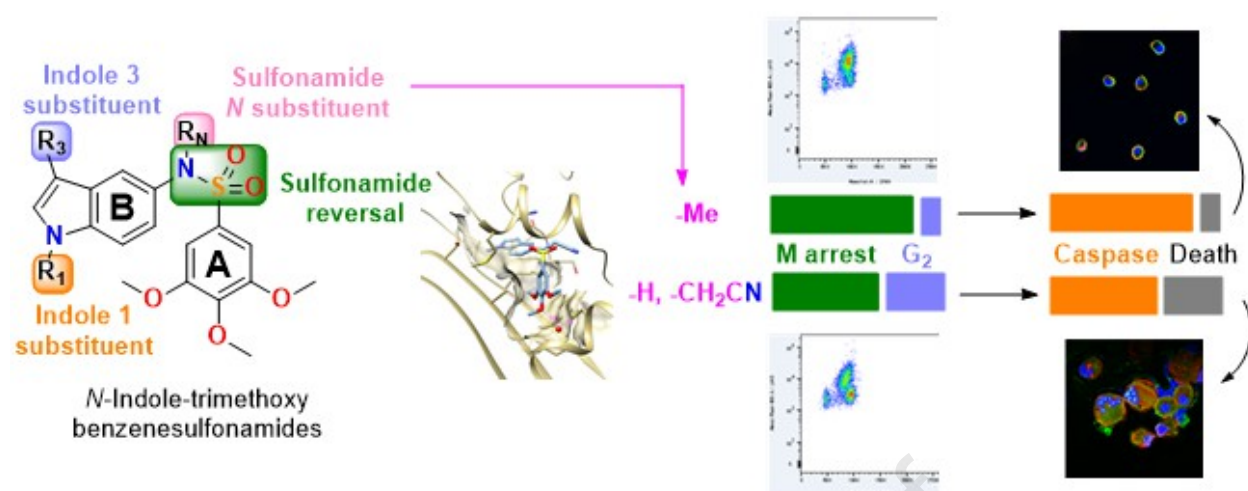
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Promising anti-proliferative indolic benzenesulfonamides alter mechanisms with sulfonamide nitrogen substituents.

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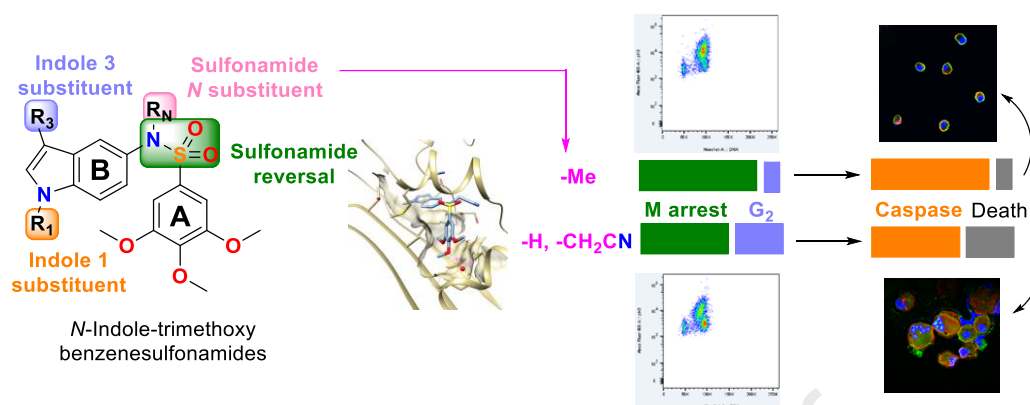
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Keywords:

Sulfonamides. Indole. Tubulin. SAR. Colchicine site. Antitumor. Mechanism. Resistance.

Journal Pre-proof

Graphical abstract



Highlights

- *N*-indolyl-3,4,5-trimethoxybenzenesulfonamides are potent anti-mitotics.
- Different tumor cell-lines show different sensitivities.
- Compounds cause G₂/M cell cycle arrest and induction of apoptosis.
- The substituent on the sulfonamide determines mechanism and cell fate.
- Binding at the colchicine site of tubulin.

Abstract:

Agents that cause apoptotic cell death by interfering with tubulin dynamics, such as vinblastine and paclitaxel, are an important class of chemotherapeutics. Unfortunately, these compounds are substrates for multidrug resistance (MDR) pumps, allowing cancer cells to gain resistance to these chemotherapeutics. The indolesulfonamide family of tubulin inhibitors are not excluded by MDR pumps and have a promising activity profile, although their high lipophilicity is a pharmacokinetic limitation for their clinical use. Here we present a new family of *N*-indolyl-3,4,5-trimethoxybenzenesulfonamide derivatives with modifications on the indole system at positions 1 and 3 and on the sulfonamide nitrogen. We synthesized and screened against HeLa cells 34 novel indolic benzenesulfonamides. The most potent derivatives (1.7 – 109 nM) were tested against a broad panel of cancer cell lines, which revealed that substituted benzenesulfonamides analogs had highest potency. Importantly, these compounds were only moderately toxic to non-tumorigenic cells, suggesting the presence of a therapeutic index. Consistent with known clinical anti-tubulin agents, these compounds arrested the cell cycle at G₂/M phase. Mechanistically, they induced apoptosis *via* caspase 3/7 activation, which occurred during M arrest. The substituents on the sulfonamide nitrogen appeared to determine different mechanistic results and cell fates. These results suggest that the compounds act differently depending on the bridge substituents, thus making them very interesting as mechanistic probes as well as potential drugs for further development.

Introduction

Tubulin, the microtubules, and the dynamic equilibria they are involved in are some of the most successful chemotherapeutic targets.[1-4] The microtubules are polymers of α - and β -tubulin dimers essential for the division of eukaryotic cells.[5] They are responsible for the chromosomes' segregation into two daughter cells during the anaphase. Microtubule-targeting agents (MTAs) bind to one of the many different drug-binding sites on tubulin, disrupting the polymerization and depolymerization dynamics and eventually triggering cellular death by apoptosis.[6] MTA are classified into two groups depending on the effects they have on microtubule stability: drugs such as colchicine, vinblastine, or maytansine are examples of microtubule destabilizing agents, which disrupt tubulin polymerization, whereas paclitaxel or laulimalide are microtubule-stabilizing agents promoting polymerization.[7] Vinblastine and paclitaxel are approved by the FDA for the treatment of different cancers. However, these drugs are substrates of the multidrug resistance (MDR) pumps leading to eventual resistance to the treatment.[8]

Drugs binding at the colchicine site are actively being explored as alternative anti-tubulin drugs because they are usually smaller and therefore have more favorable pharmacokinetic profiles than the larger drugs in clinical use and show lower sensitivity to MDR, and have vascular disrupting activity (VDA).[9] Combretastatin A-4 (CA4) has long been considered a prototype of the colchicine site ligands as its small size and high potency combine to a high ligand efficiency. Combretastatin A-4 is not a substrate of the MDR efflux proteins and has vascular disrupting activity. However, the combretastatins have not advanced in the clinic stages due to low aqueous solubility and double bond configurational and metabolic instability.[10] The diarylsulfonamides

(Figure 1) have emerged as a promising alternative, due to their preference for adopting the *cisoid* conformations required for binding at the colchicine site and their improved pharmacokinetic properties.[6] The B phenyl ring in colchicine site ligands has been linked to stability issues, and we have carried out many attempts to replace it by fused systems such as naphthalene or indole provided interesting results in different structural families, such as the combretastatins,[11-13] isocombretastatins,[14, 15] phenstatins,[16, 17] and sulfonamides.[18]

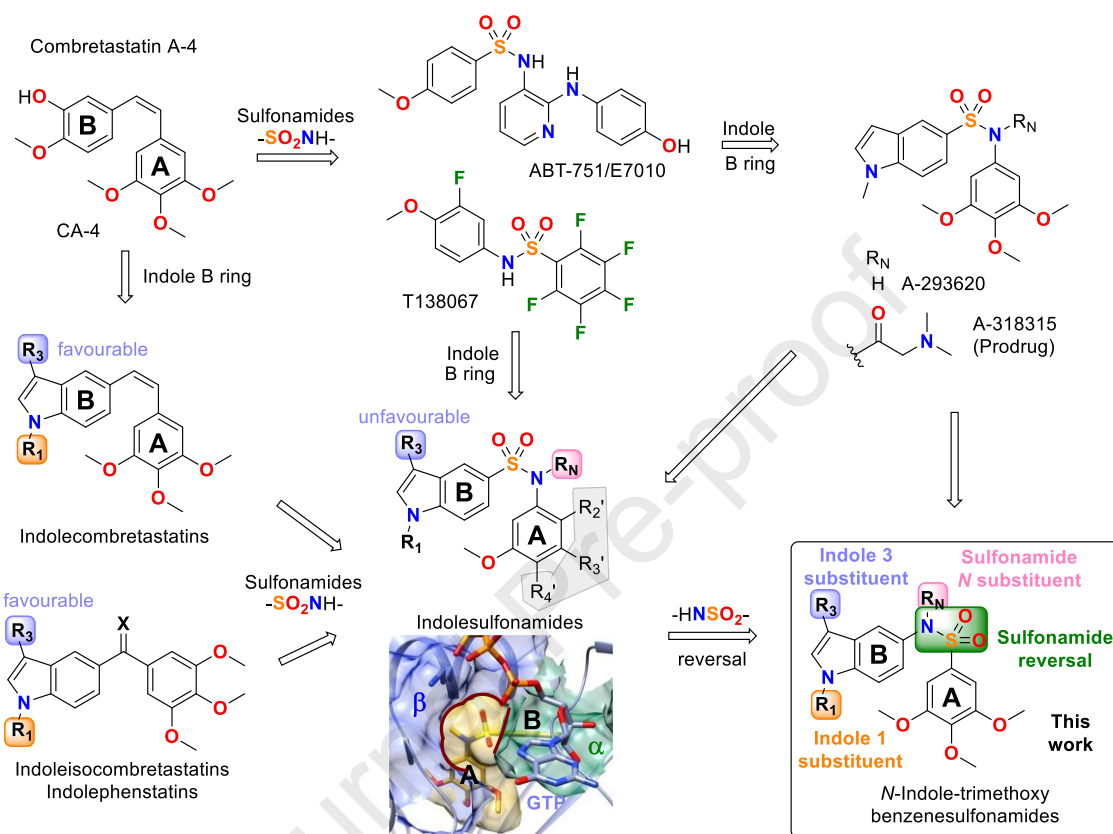


Figure 1. Chemical structures of colchicine site ligands: introduction of the sulfonamide and indole modifications and design rationale of the “reversed” sulfonamides of this work. A view down from the α subunit of the structure of the complex of indolesulfonamides with tubulin shows the sub-pocket of the sulfonamido group where the reversal could favor the introduction of substituents on the nitrogen atom.

The indolesulfonamides have a promising activity profile, as representatives A-293620 and its pro-drug A-318315 evidence.[19] However, they still suffer from limitations in the pharmacokinetic profile, and attempts to improve them by modifications on the indole, the sulfonamide substituents, and the aniline ring substituents have met with limited success.[18] Previous docking studies on the binding of indolesulfonamides to the colchicine site of tubulin showed that the N-methylsulfonamide moiety is located at the α,β interface, with the methyl contacting the sidechains of Leu242 β , Ala250 β , and Val255 β and the sulfonamide oxygens with Lys254 β and Asn258 β and the sidechains of Leu248 β and Thr179 α , packing itself close to the backbone of helix H8 of tubulin. This tight space explains the preference for small substituents at the sulfonamide nitrogen, as they bind to a pocket that cannot accommodate larger substituents that could help in improving the pharmacokinetic behavior. These models suggest that a reversal of the sulfonamido group giving N-indolyl-3,4,5-trimethoxybenzenesulfonamides,

might furnish additional space for sulfonamido-nitrogen substituents, thus allowing modulation of the pursued properties. It was previously shown that related trimethoxybenzenesulfonates benefit from this reversal.[20]

Following this rationale, we have synthesized a new family of *N*-indolyl-3,4,5-trimethoxybenzenesulfonamide derivatives with modifications on the indole system at positions 1 and 3 and on the sulfonamide nitrogen. We evaluated their anti-proliferative effect against the cancer human cell line HeLa to initially explore their structure-activity relationship (SAR) and compare it with that of related indolesulfonamides,[18] indolecombretastatins,[11, 13, 15] and indolephenstatins.[14, 17, 21] We have also studied the anti-proliferative effect of the most potent compounds against a panel of human cancer cell lines of different origin, and non-tumorigenic cell lines and found significant potency variations. We also explored their sensitivity to MDR pumps mediated resistance by co-treatments with the MDR inhibitor verapamil. The mechanism of action of the most potent compounds was assessed by their effect on intracellular microtubules and nuclei, and how their effects on the microtubule system induce anti-proliferative actions by their effects on the cell cycle populations and the cell-death induction mechanisms. We studied their structural interactions with tubulin through docking calculations and their pharmacokinetic profiles to provide explanations for the observed structure-activity relationships. These results show that the novel compounds are interesting new anti-mitotic agents for further development.

1. Chemistry results

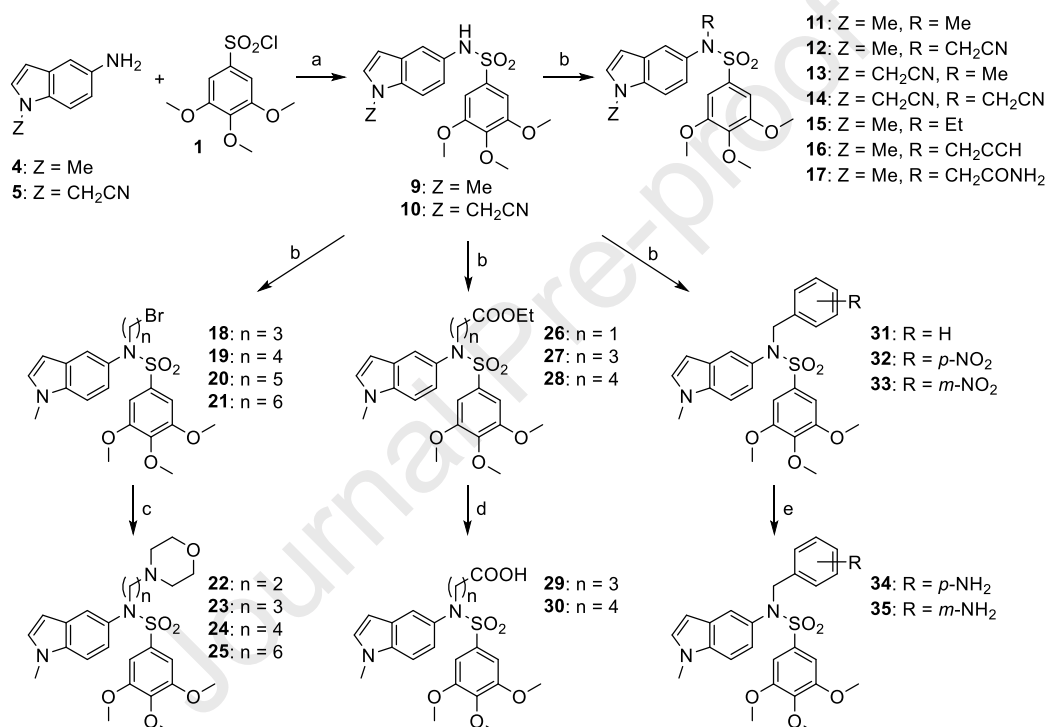
We prepared the sulfonamides by reaction of 3,4,5-trimethoxybenzenesulfonyl chloride (**1**) with the 5-aminoindoles, substitution of the sulfonamide nitrogen by alkylation reactions, and substitution at the indole 3 position by electrophilic aromatic substitution reactions followed by subsequent functional group transformations when necessary. 3,4,5-trimethoxybenzenesulfonyl chloride (**1**) was prepared in four steps from 3,4,5-trimethoxyaniline:[22, 23] i) diazotation, ii) substitution to a dithiocarbonate, iii) hydrolysis to the sulfide, and iv) oxidative chlorination. For production runs, the whole sequence of reactions was carried out without purifications due to the instability of the intermediates and product (Supplementary Material Scheme Suppl1a).

The indole amines were prepared from 5-nitro-1*H*-indole by alkylation with the corresponding halogen derivatives followed by reduction of the nitro groups to the amines. Alkylation of the 5-nitroindole under NaOH basic catalysis in phase exchange conditions with methyl iodide or 2-chloroacetonitrile led to compounds **2** and **3**, respectively (Supplementary Material Scheme Suppl1b). The 3-substituted indole-5-amines were prepared by electrophilic aromatic substitution and functional group interconversion on the alkylated nitro derivatives previously to the reduction step: i) Vilsmeier-Haack formylation, ii) oxime formation with hydroxylamine, and iii) thermal dehydration of the oxime with Ac₂O to obtain nitrile **7**. Reduction of the nitro groups provided the indole amines **4**, **5**, and **8** in quantitative yields.

Trimethoxybenzenesulfonamide scaffolds **9** and **10** were prepared by the reaction of 3,4,5-trimethoxybenzenesulfonyl chloride (**1**) and the indole amines **4** and **5** (Scheme 1). Substituents with different lengths, volumes, and polarities (alkanes, ω -haloalkanes, ω -aliphatic esters, and benzyls) were introduced on the sulfonamide nitrogen (presumed to extend towards the inter-subunit space of the tubulin dimer) to explore the structure-activity relationships. Previous

results with sulfonamides with the reverse bridge orientation show that the methylation of the sulfonamide nitrogen provides optimal cytotoxic potency.[18, 24, 25]

Alkylation of the sulfonamide nitrogens was carried out under basic (Cs_2CO_3) catalysis with halo derivatives for **11-17**, α,ω -dihaloalkanes for **18-21**, ω -halogenated esters for **26-28**, and benzyl halides for derivatives **31-33** in yields ranging from 22 to 96 %. The yields for dihalogenated alkyls **18-21** (22-45 %) fall well below those for monohalogenated alkyls (52-96 %), probably due to the formation of di-substitution products, even in the presence of a significant excess (larger than 3 fold mole excess) of the dihalogenated reagents. Further substitution of the bromine in bromoalkanes **18-21** with morpholine as a nucleophile yielded **22-25** in 66 to 93 % yields. Saponification of esters **26-28** gave carboxylic acids **29** and **30** in 61 and 51 % yields, respectively. Finally, the reduction of the nitrobenzyl derivatives with Cl_3Fe and Zn or H_2 under Pd-C catalysis led to the aminobenzyl derivatives **34** and **35** in 6 and 39 % yields, respectively.



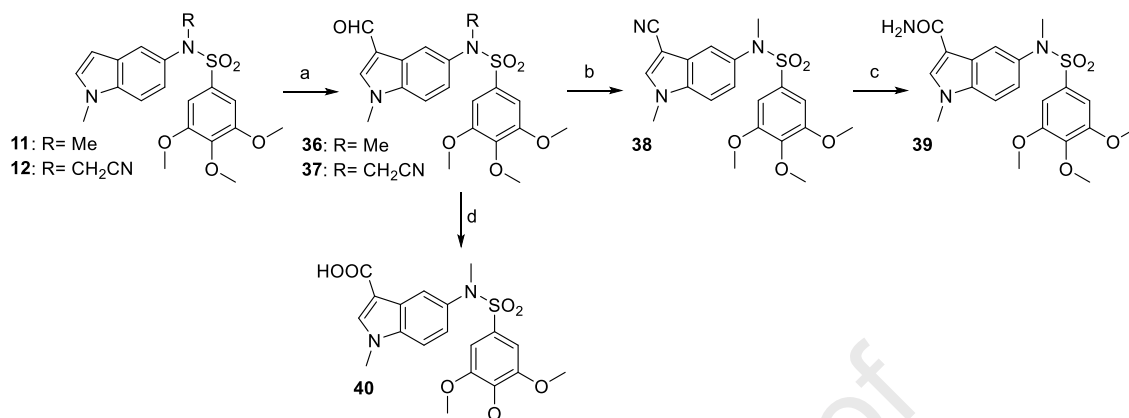
Scheme 1. Synthesis of trimethoxybenzenesulfonamides **9-35**. a) pyr, CH_2Cl_2 , rt, 48 h (30-52 %). b) R-X, Cs_2CO_3 , KI, CH_3CN , rt, 6-48 h (22-96 %). c) Morpholine, K_2CO_3 , KI, CH_3CN , rt, 72 h (66-93 %). d) NaOH, EtOH, rt, 3 h (51-61 %). e) H_2 , Pd/C, EtOAc/MeOH, rt, 12 h or Cl_3Fe , Zn, HCl, acetone/ H_2O , rt, 24 h (6-39 %).

Trimethoxybenzenesulfonamides **36-44** feature substituents at the indole 3 position. The synthesis of these derivatives was performed in two different ways depending on the substitution on the sulfonamide nitrogen (Scheme 2). For *N*-substituted sulfonamides (methyl or cyanomethyl) the indoles were formylated under Vilsmeier-Haack like conditions to give formyl sulfonamides **36** and **37**. Treatment of aldehyde **36** with hydroxylamine gave the mixture of oximes that was dehydrated to nitrile **38** by treatment with Ac_2O . Hydration of the nitrile of **38** gave amide **39**. Pinnick oxidation of aldehyde **36** led to carboxylic acid **40** (Scheme 2a).

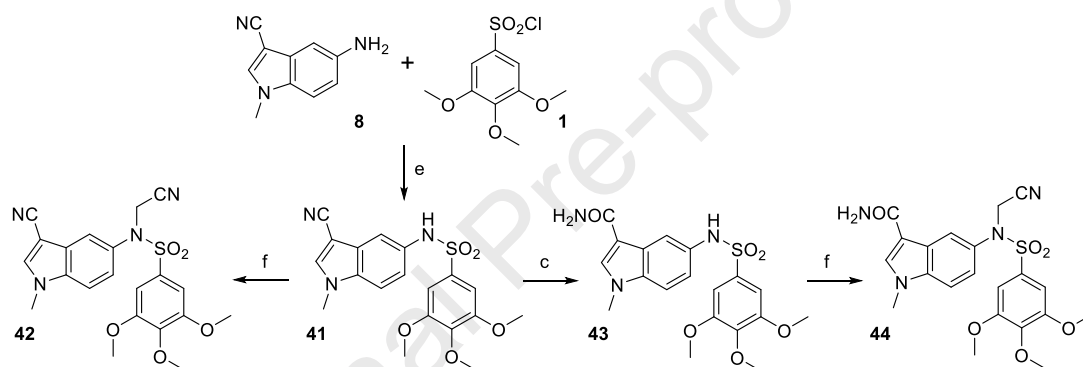
Selective hydration was not feasible in sulfonamides with two nitrile groups. In these cases, the functionalization of the indole 3 position was performed before the alkylation of the sulfonamide nitrogen. Cyanosulfonamide **41** was obtained by reacting 1-methyl-3-cyanoindole-5-amine (**8**)

with sulfonyl chloride **1**. Nitrile hydration yielded amide **43**. Both **41** and **42** were alkylated on the sulfonamide nitrogen by chloroacetonitrile to give **42** and **44**, respectively (Scheme 2b).

a) Synthesis of trimethoxybenzenesulfonamides **36-39**



b) Synthesis of trimethoxybenzenesulfonamides **41-44**



Scheme 2. Synthesis of 3' modified indoles **36-44**. a) POCl₃, DMF, 100 °C, 2 h. b) 1) NH₂OH, pyr, MeOH, 100 °C, 48 h 2) Ac₂O, pyr, 120 °C, 92 h. c) KO^tBu, ^tBuOH, 85 °C, 48-72 h. d) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, H₂O/^tBuOH, rt, 48 h. e) pyr, CH₂Cl₂, rt, 48 h. f) R-X, Cs₂CO₃, KI, CH₃CN, rt, 72 h.

2. Results and discussion

2.1 In vitro cell growth inhibitory activity

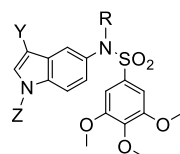
The biological activity of the synthesized compounds was initially assessed by the antiproliferative effect against HeLa cells (human cervix adenocarcinoma), a sensitive cell line that provides a first indication of the compounds' activity. The antiproliferative activity of 3,4,5-trimethoxybenzenesulfonamides **9-44** was measured by the cell viability reduction they induce upon treatment as measured by the MTT method. In this assay, alive cells metabolize the reagent giving a coloured furazan whose absorbance is directly proportional to the number of surviving cells.[26] We calculated the half-maximal inhibitory concentrations (IC₅₀) shown in Table 1 by fitting the inhibitory effects of the compounds at different concentrations. The *N*-(5-indolyl)-3,4,5-trimethoxybenzenesulfonamide scaffold revealed itself as a very favourable scaffold for the antiproliferative effect, with 17 out of the 36 assayed compounds (47%) showing sub-micromolar antiproliferative potencies (Table 1), 11 of them with IC₅₀ values below 100 nM, 8 under 25 nM,

and **4** with values lower than 10 nM. In most cases, compounds with IC₅₀ values above the 1 µM threshold carry substituents with more than 3 heavy atoms on the sulfonamide nitrogen (i.e. compounds **18-36**), thus suggesting that steric hindrance is a limiting factor in this region. Modifications to explore the structure – activity relationships in this series were performed in three zones of the basic scaffold: i) the indole nitrogen, ii) the sulfonamide nitrogen, and iii) the indole 3 position (Figure 1).

Two substitutions have been explored at the indole nitrogen: methylation and cyanomethylation. Pairwise comparison (i.e. compare **9** vs **10**, **11** vs **13**, and **14** vs **25**) of the results in these two series shows that methylation is much favoured over the longer cyanomethyl, with potency changes larger than 30-fold in all the cases. When the substituent on the sulfonamide nitrogen is a hydrogen atom (unsubstituted thereafter), **9** has an IC₅₀ value of 5.3 nM vs **10** that has an IC₅₀ value in the micromolar range of 2.6 µM (more than 500-fold difference). For the methylated sulfonamides **11** and **13** the IC₅₀ values are 3.2 nM and 114 nM, respectively with a larger than 30-fold increase, while the cyanomethylated sulfonamides **12** and **14** elicited potencies of 1.7 and 974 nM, respectively for a larger than 400-fold difference. This suggests that steric hindrance is a large restriction for the indole nitrogen substituent. Therefore, modifications at other parts of the skeleton focused only on *N*-methylated indoles.

We explored different substitutions at the sulfonamide nitrogen. The most potent derivatives are the unsubstituted or those with small substituents such as methyl or cyanomethyl substituents. Those with ethyl (**15**), propargyl (**16**), or carbamoylmethyl (**17**) substituents result in significant potency decreases to the 100 nM value range. A further potency reduction to above micromolar values was observed when longer alkyl substituents from two to six carbon atoms were employed, irrespective of the nature of the end-capping groups: halogens (**18-21**), morpholines (**22-25**), esters (**26-28**), carboxylic acids (**29-30**), or aromatic rings (**31-35**), with the only exception of the aminobenzyl group of **34**, that just caused it to drop to the high submicromolar level (IC₅₀ = 0.5 µM).

Several polar groups were introduced at the indole 3 position: formyl, carbamoyl, carboxylate, and cyano groups intended to increase the polarity and to improve the pharmacokinetics, and potentially make polar interactions with tubulin to enhance the potency. In all of them, the substituents stay within the ring plane with whom they conjugate. This extension of the B ring is compatible with high potency, as flat extended systems are known to provide good potencies.[27] The potency is reduced with respect to the corresponding unsubstituted analog for all the substituents: e.g. **11** (3.2 nM) is more potent than the analog with a formyl (**36**, 16.5 nM), a nitrile (**38**, 8.0 nM), a carboxylic acid (**40**, > 1 µM), or an amide group (**39**, 15.0 nM). This potency decrease could be due to the penalty associated with the desolvation of these polar groups in the binding site, but uptake can also be a determining factor, as the potency loss for the ionized carboxylates suggests. The observed potency reduction is similar to that observed for indolesulfonamides but with a somewhat smaller penalty,[6] although it does not provide the improvements observed for indolecombretastatins,[11] or indolephenstatins and indoleisocombretastatins.[14]

Table 1. Antiproliferative activity (IC₅₀) of sulfonamides **9-44** against HeLa cancer cell line.

Compound	Z	Y	R	IC ₅₀ HeLa (nM)
9	-CH ₃	-H	-H	5.3
10	-CH ₂ CN	-H	-H	2560
11	-CH ₃	-H	-CH ₃	3.2
12	-CH ₃	-H	-CH ₂ CN	1.7
13	-CH ₂ CN	-H	-CH ₃	114
14	-CH ₂ CN	-H	-CH ₂ CN	974
15	-CH ₃	-H	-CH ₂ CH ₃	109
16	-CH ₃	-H	-CH ₂ CCH	79.8
17	-CH ₃	-H	-CH ₂ CONH ₂	275
18	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ Br	>1000
19	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ Br	>1000
20	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ Br	>1000
21	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ Br	>1000
22	-CH ₃	-H	-CH ₂ CH ₂ Morf	>1000
23	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ Morf	>1000
24	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ Morf	>1000
25	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ Morf	>1000
26	-CH ₃	-H	-CH ₂ COOEt	>1000
27	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ COOEt	>1000
28	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ COOEt	>1000
29	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ COOH	>1000
30	-CH ₃	-H	-CH ₂ CH ₂ CH ₂ CH ₂ COOH	>1000
31	-CH ₃	-H	-Bn	>1000
32	-CH ₃	-H	-Bn- <i>p</i> -NO ₂	>1000
33	-CH ₃	-H	-Bn- <i>m</i> -NO ₂	>1000
34	-CH ₃	-H	-Bn- <i>p</i> -NH ₂	464
35	-CH ₃	-H	-Bn- <i>m</i> -NH ₂	>1000
36	-CH ₃	-CHO	-CH ₃	16.5
37	-CH ₃	-CHO	-CH ₂ CN	22.7
38	-CH ₃	-CN	-CH ₃	8.0
39	-CH ₃	-CONH ₂	-CH ₃	15.0
40	-CH ₃	-COOH	-CH ₃	>1000
41	-CH ₃	-CN	-H	43.0
42	-CH ₃	-CN	-CH ₂ CN	24.4
43	-CH ₃	-CONH ₂	-H	102
44	-CH ₃	-CONH ₂	-CH ₂ CN	50.9

IC₅₀: concentration of drug inhibiting cell growth by 50%. Morf = Morpholine.
(Supplementary Material Table Suppl4)

To further explore their anti-proliferative activity, the 12 more potent compounds against HeLa cells (i.e. **9**, **11**, **12**, **15**, **36-39**, and **41-44**) were evaluated against a panel of 10 human cancer cell lines (Table 2) following a semi-automatic standardized assay to assess drug sensitivity[28]: HeLa (cervix), MCF-7, BT-474, and MDA-MB468 (breast cancer); MES-SA (uterine sarcoma); HT-1080

(fibrosarcoma); MelMet5, A375 and WM-1366 (melanoma), and Caki1 (renal carcinoma). Three non-tumorigenic cell lines were included to study the tumour selectivity of the compounds: RPE-1 (retina cells), Hermes-3C (skin), and Hek293 (kidney) cells. Two microtubule stabilizer ligands (docetaxel (DT) and paclitaxel (PT)) and two destabilizer drugs (vincristine (VC) and vinorelbine (VN)) were included as positive controls. The anti-proliferative values against the HeLa cells determined in the two assays were quite consistent (for the sake of discussion, a threefold variation is taken as the threshold for considering differences significant throughout), although in some cases (e.g. compounds **11**, **12**, **41** and **42**) there are significant variations. These variations could arise from the methodological differences between the two assays: the semi-automatic vs the manual screening and the different means of measuring cell viability: for the first one, luminescence evolves from reaction with the cells' ATP while for the latter absorbance measures the MTT reduction to formazan. Nonetheless, despite these discrepancies, a consistent trend in activity was observed across both datasets.[26, 28]

There are variations in the IC_{50} values for each compound across the different cell-lines (Supplementary Material Figure Suppl1) that show that the selected cell-lines have varied sensitivities. These variations show distinct patterns for every compound, and they differ even for the reference compound pairs acting at the same tubulin sites (VC vs. VN and DT vs. PT). This variety supports the panel of cell-lines selected as a sampling of cancer cells' sensitivities. The reference compounds show potencies in the low nanomolar range, with VN showing the higher overall potency across all the cell-lines. PT and DT show significant variations across the panel, with several cell-lines having a significant reduction in sensitivity, especially the breast cancer cell-line BT-474. Taxane-based chemotherapy is the current basis for breast cancer treatments and this reduction uncovers a potential lower intrinsic responsiveness of some types of tumours.

The most sensitive cell-line is the melanoma cell-line MelMet5, closely followed by HeLa. The fibrosarcoma cell-line HT-1080, the uterine sarcoma MES-SA, and the breast cancer cell-lines MDA-MB468 and MCF7 have similar sensitivities to HeLa cells for the sulfonamides, but the last ones are less sensitive to some of the reference compounds (DT and VC for MCF7 and MES-SA and DT and PT for MDA-MB468). In the following, for the comparison of the relative potencies between cell lines, we will use the HeLa IC_{50} values as a reference, as it lies in between the sensitive ones, thus adjusting well to the 3-fold IC_{50} variation for considering a potency change significant. We have previously observed that similar 4-methoxybenzenesulfonamides are more potent (have lower IC_{50} values) against MCF7 than HeLa,[24, 29] but this is not a general trend, as their lower sensitivity to DT and VC illustrates. These results validate the use of HeLa for the initial screening. On the other hand, the breast cancer cell-line BT-474 is the least sensitive amongst all the cell lines, with melanoma WM-1366 and A375, and renal carcinoma Caki1 cell-lines showing also significantly higher IC_{50} values for the sulfonamides, except for **9** and the methylated sulfonamides **11**, **36**, **38**, and **39**. A375, WM-1366, and BT-474 are less sensitive to the taxanes, especially the latter, and A375, WM-1366, and Caki1 show lower sensitivity to VC.

Most of the assayed compounds present double-digit nanomolar IC_{50} values, thus validating the design proposal. Compound **9** emerges as the most potent ligand with single-digit nanomolar IC_{50} values across all tested tumour cell lines, except for the taxane-insensitive breast cancer BT-474 where its IC_{50} value drops to the 50 nM range. This potency reduction for the BT-474 cell-line extends to most of the sulfonamides, except to the indole-3-substituted *N*-methylsulfonamides **36** (3-formyl), **38** (3-cyano), and **39** (3-carbamoyl) that remain within the 15 nM range, better or equal than DT and PT. Both the *N*-methyl substituent and the polar substitution at the indole 3-position seem important for the sustained activity against the BT-474

cell-line as the unsubstituted (i.e. **9**, **41**, and **43**) or the cyanomethylated (i.e. **12**, **37**, **42**, and **44**) sulfonamides suffer the potency reduction to values about 50 nM, and the compound with the unsubstituted indole (i.e. **11**) is less potent as well (IC_{50} value of 34.5 nM). These modifications are also consistent with good potency (IC_{50} values below 25 nM) against the remainder of cell-lines, thus showing the most consistent anti-proliferative profile across all the cell-lines and therefore being the more promising representatives. Amongst the three compounds, **36** with a 3-formyl group on the indole is the most potent, with most IC_{50} values below 10 nM. *N*-Ethylsulfonamide **15** was the least potent ligand of the selection with most IC_{50} values above 100 nM, thus showing that the ethyl substituent is quite detrimental to the anti-proliferative activity.

Concerning the effects of the structural modifications on the potency, the effects of the indole 3 and the sulfonamide substituents seem to be somehow coupled to each other. Regarding the bridge substituent, the most potent sulfonamide overall is the 3 indole and sulfonamide unsubstituted **9**, but it has a potency drop of 10-fold (with respect to the HeLa IC_{50} value) against BT-474. The higher potency of the unsubstituted sulfonamide compared to the methylated analog **11** is different from the trend observed for sulfonamides oriented in the reverse direction (indolesulfonamides).[18] This is probably a result of the switch in the accommodation pockets of the sulfone and nitrogen in the two orientations, as predicted (Figure 1). The preference for a hydrogen over a methyl in this series (reversed sulfonamide) is opposite to that observed for the indolesulfonamides and further supports this assumption. For the unsubstituted sulfonamides **41** and **43**, the substituents at the indole 3 position decrease overall potency without improving the effect on the less sensitive BT-474 cells, and further result in potency drops for A375, WM-1366, and Caki1. The methylated sulfonamides **11**, **36**, **38**, and **39** as a group provide the best overall performance, with small potency reductions compared to **9** and not suffering the decrease against BT-474 when the indole 3 position bears a polar group. Differing from what is observed for the unsubstituted sulfonamides, in this group (methylsulfonamides) the substitution at the indole 3 position does not drastically modify the potency, with the formyl group providing the optimal overall potency, but is important for maintaining the relative potency against BT-474. This potency rank of the indole 3 substituents is different from that shown by the indolesulfonamides, where the nitrile is much more preferred to the formyl group.[18] The cyanomethylation of the sulfonamides results in changes in potency similar to the methylation, except for WM-1366, Caki1, and BT-474, which are less sensitive, thus worsening their overall performance. In this series, indole substitution is detrimental, except for the carbamoyl analog **44**, which shows an improvement in potency compared to HeLa for MDA-MB468, MES-SA, HT-1080, and MelMet5 cells.

When considering the effect of the substitution at the indole 3 position, the unsubstituted analogs (i.e. **9**, **11**, and **12**) are the most potent, but they all show lower potency against the BT-474 cell-line, and when combined with a cyanomethyl substituent on the bridge also WM-1366 and Caki1 become less sensitive. Formyl substitutions at the indole 3 position result in potencies similar to the unsubstituted indoles, with a methyl on the sulfonamide preferred to a cyanomethyl, but the former maintains the potency against BT-474, thus making it a very promising candidate. Nitriles at the indole 3 position behave differently depending on the sulfonamide substituent. For unsubstituted sulfonamides they behave like **12** (unsubstituted indole and cyanomethylated sulfonamide, a switch of the positions of the substituents). The methylated analogue behaves similarly to the formylated one (**36**) but with a slightly lower potency. This potency profile is quite different from that of combretastatins[11, 30] and isocombretastatins,[14] where the carbamoyl and the formyl groups are preferred over the

unsubstituted indoles. The cyanomethylation of the sulfonamide combined with the nitrile on the indole as in **44** greatly reduces the potency.

Table 2. Antiproliferative IC₅₀ values of active sulfonamides on HeLa, MCF7, BT-474, MDA-MB468, MES-SA, HT-1080, MelMet5, A375, WM-1366, and Caki1 cancer cell lines.

	HeLa	MCF7	BT-474	MDA-MB468	MES-SA	HT-1080	MelMet5	A375	WM-1366	Caki1
9	3.9	1.4	46.1	4.5	5.6	5.6	1.4	6.4	7.1	4.0
11	<u>14.5</u>	<u>15.8</u>	34.5	24.4	9.0	<u>11.8</u>	<u>12.9</u>	<u>17.5</u>	<u>13.5</u>	<u>11.0</u>
12	<u>11.0</u>	<u>13.7</u>	68.3	<u>13.0</u>	8.9	9.9	8.9	22.1	52.8	56.2
15	108.4	346.8	791.8	161.2	147.8	103.9	96.2	363.0	516.7	158.5
36	5.3	9.3	<u>10.6</u>	<u>17.2</u>	<u>17.9</u>	8.1	3.9	8.7	<u>11.6</u>	<u>12.9</u>
37	<u>18.4</u>	<u>15.3</u>	66.4	<u>15.2</u>	32.5	<u>14.0</u>	<u>12.5</u>	49.8	31.4	66.2
38	<u>16.0</u>	<u>12.3</u>	<u>16.3</u>	28.0	6.3	<u>15.3</u>	<u>10.6</u>	<u>11.7</u>	20.2	NA
39	<u>13.9</u>	44.0	<u>17.9</u>	26.0	<u>12.2</u>	<u>14.3</u>	<u>12.2</u>	22.8	21.3	29.6
41	<u>13.3</u>	<u>12.1</u>	57.5	<u>11.8</u>	<u>11.8</u>	<u>12.1</u>	1.6	33.2	65.7	32.4
42	77.3	60.8	71.1	31.6	80.3	65.8	32.3	86.8	81.3	29.5
43	93.0	108.0	125.5	100.7	99.9	69.2	29.5	245.3	277.5	310.0
44	68.8	48.6	40.8	20.3	<u>14.2</u>	<u>16.6</u>	<u>16.2</u>	48.3	36.0	98.3
DT	1.0	3.9	21.4	3.1	3.4	1.5	9.5	4.5	8.5	2.7
PT	0.9	2.2	<u>13.7</u>	3.5	1.1	1.8	1.4	5.1	4.8	0.9
VC	1.0	4.2	2.9	0.4	4.9	0.3	0.2	3.5	4.5	5.7
VN	0.3	0.8	0.3	0.1	0.4	0.1	0.1	0.6	0.9	0.3

DT: Docetaxel PT: Paclitaxel VC: Vincristine VN: Vinorelbine.

IC₅₀ in nM. Values below 10 nM are highlighted in bold. Values that represent significant potency variations discussed in the main text are underlined. (Supplementary Material Table Suppl5)

Indolesulfonamides show selectivity towards tumour cells.

We evaluated the anti-proliferative effect of the compounds against three non-tumorigenic human cell-lines: Hermes-3c (human extended-replication melanocytes-3C), Hek-293 (human embryonic kidney), and RPE-1 (human retinal pigment epithelial) cells (Table 3). The tumour-selectivity indexes (TS) are the ratios of the IC₅₀ values against these cell lines to those against the tumorigenic cell lines and are a surrogate of the window between anti-tumour activity and cell toxicity: $TS = (\text{non-tumorigenic IC}_{50} / \text{tumorigenic IC}_{50})$. TS values of 1 mean no selectivity, values higher than 1 imply selectivity towards the tumour cells, and values below 1 imply higher toxicity against the non-tumorigenic cells. TS could be thought of as a surrogate measure of the toxicity of the compounds. SuppTable3 in the Supplementary material shows the TS values for all the tumorigenic cell lines and their average and the three non-tumorigenic ones coloured as heat maps where red would mean toxicity and yellow to green increasing safety profiles.

As observed for the tumour cell lines, significant variations are observed in the toxicity of the controls and the sulfonamides against the three cell-lines. For the control compounds, the Hermes-3C line is the most sensitive, with IC₅₀ values close to those against the tumour cell lines, Hek-293 is the less sensitive, with IC₅₀ values in the 15 – 25 nM range, and RPE-1 is intermediate, with IC₅₀ values in the 5 – 10 nM range. The TSs accordingly vary for the tumorigenic cell lines. The situation is quite different for the sulfonamides, as the Hermes-3C line is the less sensitive

with IC₅₀ values in the 50 nM and above range, followed by RPE-1 with IC₅₀ values above 15 nM, and Hek-293 is the most sensitive of the three, thus providing the lower TS values. Overall, the sulfonamides provide acceptable TS values, superior as a class to the taxanes and the vinca alkaloids ligands assayed.

Table 3. Antiproliferative IC₅₀ values (nM) of active sulfonamides on non-tumoral cell lines Hermes-3C, Hek-293, and RPE-1.

	Hermes-3c (IC ₅₀)	Hek-293 (IC ₅₀)	RPE-1 (IC ₅₀)
9	61.0	4.9	5.7
11	67.4	12.3	20.0
12	44.7	23.4	80.4
15	964.9	323.0	334.7
36	52.8	50.2	12.0
37	81.3	70.9	41.3
38	45.6	29.0	13.2
39	31.8	92.2	22.7
41	87.1	18.2	52.8
42	70.5	17.7	76.3
43	465.8	491.5	173.0
44	88.5	281.8	33.0
DT	1.7	7.5	8.2
PT	1.0	26.8	5.1
VC	2.8	25.8	7.6
VN	0.5	15.9	9.3

DT: Docetaxel PT: Paclitaxel VC: Vincristine VN: Vinorelbine.

Indolesulfonamides are not substrates of the P-GP-1 pump, but PT-resistant MDA-MB468-PR cells are less sensitive to the indolesulfonamides, except for those with the cyanomethylated sulfonamides

The resistance to the sulfonamides achieved by the paclitaxel-resistant MDA-MB468-PR cell line (Table 4) was assessed by the change in IC₅₀ value with respect to that against the wild-type cell line. Taxol derivatives PT and DT presented a 2.7-fold increase in their IC₅₀ values. Sulfonamides **11**, **38**, **39**, **43**, and **44** suffered a loss of potency in the same range, between 2.5 to 3.6 fold. The biggest difference was for compound **9** with an IC₅₀ value 6.6 times that of the wild type. The potency reduction did not affect the sulfonamides with a cyanomethyl substituent, with the only exception of **44**, the indole-3-carboxamide analogue. The formyl groups at the indole 3 position seem also to have a protective effect against this resistant phenotype.

One common resistance mechanism against anticancer treatments involves efflux pumps, specifically multidrug resistance (MDR) pumps like P-glycoprotein. We compared the IC₅₀ values for HeLa cells obtained with co-treatment with 10 μ M verapamil, a P-GP/MDR1 inhibitor that does not affect the cell viability at this concentration with those obtained without.[11, 31, 32] The well-known P-GP substrates colchicine and PT have their IC₅₀ values reduced by 1.80 and 2.07 times respectively in the presence of verapamil (Table 4), and 1.5 was taken as the threshold for considering a compound a substrate. Most of the sulfonamides showed ratios lower than this

threshold, thus indicating that they are not substrates of the efflux pumps. Among the tested compounds only compounds **43** and **44**, with carbamoyl groups at the indole 3 position, have ratios of 1.83 and 1.93 respectively, thus suggesting that these compounds are probably substrates of the pumps. For the rest of the sulfonamides suffering a potency reduction against the PT-resistant MDA-MB468 cell-line alternative mechanism(s) must be in place.

Table 4. Antiproliferative activity (IC_{50}) of active sulfonamides on HeLa cancer cell line, with and without verapamil (MDR inhibitor), and MDA-MB468 wild type and paclitaxel-resistant.

	IC_{50} (HeLa)	IC_{50} (HeLa + verpamil)	Times	MDA- MB468	MDA- MB468-PR	Times
9	22.9	17.9	1.28	4.5	29.6	6.6
11	50.1	40.9	1.22	24.4	67.6	2.8
12	22.3	16.3	1.37	13.0	14.8	1.1
15	122.7	104.7	1.17	161.2	145.1	1.1
36	18.4	14.6	1.27	17.2	32.9	1.9
37	57.4	52.4	1.10	15.2	14.7	1.0
38	25.7	24.6	1.04	28.0	69.5	2.5
39	24.3	18.2	1.34	26.0	70.8	2.7
41	43.0	29.1	1.48	11.8	12.4	1.1
42	34.5	27.2	1.27	31.6	34.4	1.1
43	101.7	55.4	1.83	100.7	283.8	2.8
44	50.9	26.4	1.93	20.3	72.9	3.6
Colchicine	16.7	9.3	1.80	nd	nd	-
PT	6.2	3.0	2.07	3.5	9.4	2.7
DT	nd	nd	-	3.1	8.3	2.7

IC_{50} expressed in nanomolar. nd: non-determined. PT = Paclitaxel. DT = Docetaxel. Values indicative of resistance are highlighted in cream. (Supplementary Material Table Suppl6)

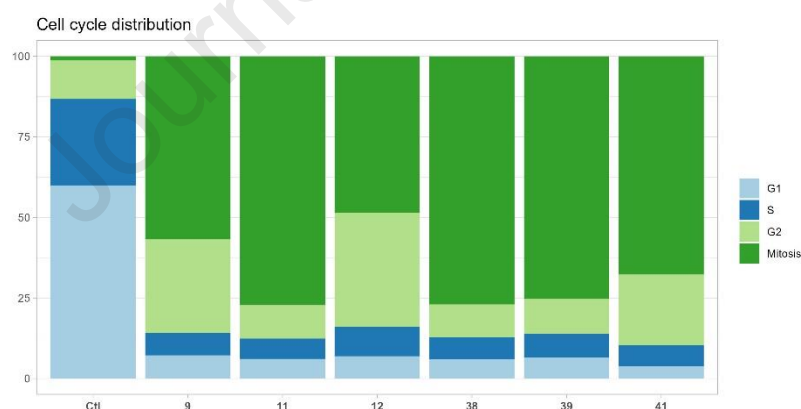
2.2 Cell cycle analysis

MTAs typically arrest cell cycle progression during the mitotic phase (M phase), due to the inability of the mitotic spindle to segregate the two copies of the chromosomes.[5] We performed an analysis of the changes in the cell cycle distribution profiles as measured by multichannel flow cytometric measurements after incubating the cells for 24 h with representative sulfonamides **9**, **11**, **12**, **38**, **39**, and **41** to assist in the establishment of their mechanism of action. The MES-SA cells (human uterine sarcoma) were chosen for the assay as the selected compounds show IC_{50} values similar to each other around 5 - 10 nM at the 72 h time point. We used a concentration of 100 nM for 24 hours for all the compounds.

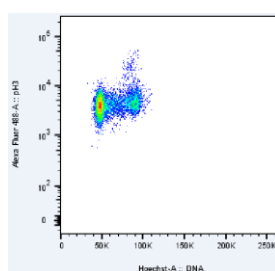
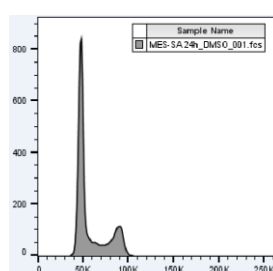
Alive cells were selected by Live/Dead Orange staining and DNA was stained with Hoechst 33258, enabling us to classify the cells into three groups based on their DNA content:[33] Cells in the G₁ state contain only one copy of DNA, those in G₂/M possess two copies, and cells in the S phase have intermediate amounts.[34, 35] To differentiate between the cells in the G₂ and M phases, we employed phospho-histone H3 staining. Histone H3 phosphorylation occurs during late G₂ and its dephosphorylation takes place between the late anaphase and early telophase therefore phospho-histone H3 labels and identifies cells in mitosis.[36]

The normal cell cycle distribution of cells along the different phases for the MES-SA cells is represented by the untreated control. 60 % of the alive cells are in the G1 phase, with 1.3 % of mitotic cells and 12 % in the G₂ phase. The results depicted in Figure 2 illustrate that treatment with compounds **9**, **11**, **12**, **38**, **39**, and **41** leads to a very similar change in the cell cycle populations' distributions for every compound. An increase in the percentage of cells in the G₂/M ensemble from 13.3 % to 83.6 - 89.7 % occurs for all of them at the expense of the S (from 27 % to 6 - 9 %) and G1 phases (from 60 % to 4 - 7 %). Interestingly, the only significant differences observed correspond to the G₂/M ratios. A higher percentage of M cells is obtained for the *N*-methylated benzenesulfonamides **11**, **38**, and **39**, with population values from 75.3-77.2 %, and correspondingly lower G₂ percentages between 10 - 11 %, representing almost no variation with respect to the untreated control. Progressively lower percentages of cells in the M phase are observed for the unsubstituted sulfonamides **41** (nitrile at the indole 3 position, 67.7 %) and **9** (unsubstituted indole 3 position, 56.8 %) and finally for the *N*-cyanomethyl sulfonamide **12** with just a 48.6 % of cells in mitosis. This descending trend in cells in the M phase is accompanied by an increase in the G₂ phase, with values of 22 %, 29 %, and 35 % respectively to conform to the almost constant G₂/M combined values. These patterns suggest that the substituent on the bridge might have an impact on the G₂/M ratios after 24 h in MES-SA cells. Interestingly, the first group of highly M accumulating compounds corresponds to compounds that became less potent against the PT-resistant MDA-MB468 in a non-MDR dependent way, whereas the second group shows no differences between the resistant and sensitive MDA-MB468 cells, except for **9**, whose potency change against the resistant cells is also of different magnitude, which might suggest a different mechanism of resistance applying. These results suggest an intriguing connection between the chemical structure, the mechanism of action, and the susceptibility to resistance mechanisms, thus making them interesting probes for further analysis.

A



B



C

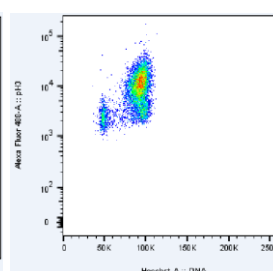
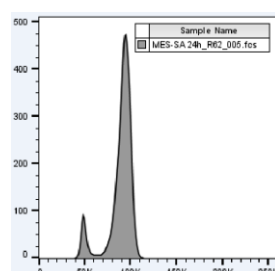


Figure 2. Effects of the most promising compounds on the cell cycle profiles of MES-SA cells. A) Flow cytometry analysis of MES-SA cells stained by Phospho-Histone H3 content (M marker) and Hoechst 33258 (DNA content) after treatment for 24 h at 100 nM (Supplementary Material Fig

Suppl69 and Table Suppl7). B) Cell cycle histograms and dot plots of control MES-SA cells. (Supplementary Material Fig Suppl2 and 3). C) Cell cycle histograms and dot plots of MES-SA cells after treatment with **11** for 24 hours. The rest of the compounds are shown in the Supplementary Material (Figure Suppl2 and Figure Suppl3).

2.3 Cell apoptosis and caspase-3 activation

Chemotherapy-injured cells subsequently die by programmed cell death programs, one of which being apoptosis.[5] Its measurement would thus be useful for the understanding of the mechanisms of action of the sulfonamides. Apoptotic cells eventually activate nucleases that cleave their DNA (karyorrhexis) leaving free 3'OH ends which can be labelled by the TUNEL assay.[37, 38] Cells positive for the TUNEL reagent identified as undergoing apoptosis were categorized as live or dead by Live/Dead Orange staining. Dead cells negative to TUNEL are classified as necrotic cells, although unspecific DNA cleavage can occur in necrotic cells which might fail to discriminate between these two processes.[39] Two-dimensional flow cytometry analysis of the MES-SA cells treated with the sulfonamides **9**, **11**, **12**, **38**, **39**, and **41** at 100 nM for 24 hours were classified as non-apoptotic (TUNEL negative, live cells), in early apoptosis (TUNEL positive, live cells), late apoptosis (TUNEL positive, dead cells), or necrotic (TUNEL negative, dead cells).

The percentage of death cells is similar for all the treatments (Table 5 and Supplementary Material Figure Suppl4), ranging from 12 % for **41** to 8 % for **12**. Interestingly, the percentage of necrotic cells is somewhat different for the two groups uncovered in the cell cycle analysis: those that induce a higher mitotic arrest result in lower necrotic death (between 1.8 and 3.4 %) whereas those compounds that retained a larger percentage of cells in G₂ result in higher percentages of necrotic cells (ca 4.5 %). The apoptotic cell death does not follow the same pattern, with changes accounting for the total cell death count. There is also a slight indication that indole substituted sulfonamides (i.e. **38**, **39**, and **41**) reach faster the late apoptosis than the unsubstituted ones (i.e. **9**, **11**, and **12**).

Table 5. Cell death analyses by flow cytometry with Live/Dead Orange and TUNEL staining of MES-SA cells treated with the selected compounds for 24 hours.

	Necrotic	Late Apoptotic	Apoptotic	Live
9	4.7 %	0.8 %	4.2 %	90.3 %
11	3.4 %	0.9 %	5.7 %	90.0 %
12	4.5 %	0.5 %	3.1 %	92.0 %
38	2.3 %	1.3 %	5.0 %	91.4 %
39	1.8 %	1.6 %	4.8 %	91.8 %
41	4.5 %	1.8 %	5.8 %	88.0 %

% of the single cells

To further characterize the apoptosis induction by the sulfonamides, we conducted a specific assay monitoring effector caspases 3 and 7 activation as they play a key role in the apoptotic process, using a fluorescent assay with the live-cell imaging platform Incucyte®.[40] Caspase activation was assessed every 24 hours for 3 days with the treatments at 100 nM. 1 μ M Staurosporin was used as a positive control. The results, presented in Figure 3, demonstrate that all the compounds induced caspase activation and therefore cell apoptosis in a time-dependent manner. For all the sulfonamides caspase activation increases from the 24 to the 48 hours

timepoints, while for the last 24 hours the increase is small or even absent. This is somewhat different for staurosporin, which causes a higher caspase activation at the 24 h timepoint that is followed by a neat overtime decrease at the subsequent timepoints, probably due to a decrease in the viable cell population that translates into a reduction in active caspase concentration (data not shown). For the sulfonamides, the active caspase concentrations at the 24-hour timepoint cluster in the two groups observed for G₂ and M accumulation profiles in the cell cycle: sulfonamides **11**, **38**, and **39** show more caspase activation than **9**, **12**, and **41** (this one to a slightly higher extent). In the subsequent time-point, the active caspase concentrations increase in all cases, but the pattern is still patent. At the final timepoint of 72 hours, the compounds with a higher activation profile do not further increase the activated caspase concentration, an increase that does occur for the other set, but does not reach the levels of the former group in any case. This pattern is not likely due to a reduction of cell viability, as the reduction in cell confluency shows an opposing trend and is similar for all of the sulfonamides (Supplementary Material Figure Suppl5). Quite interestingly, this compound clustering closely reproduces the one observed for the cell cycle populations effects, and the apoptosis induction shown in this experiment parallel the mitotic arrest, as would be the expected behaviour if mitotic arrest and spindle assembly checkpoint were the apoptotic trigger. G₂ accumulation in turn seems to be related to the necrotic cell death.

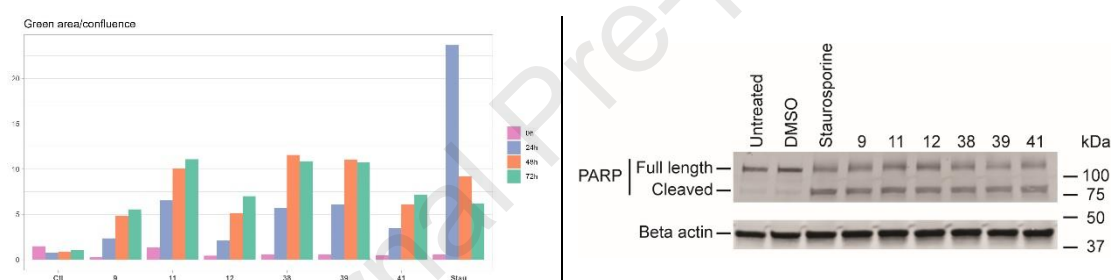


Figure 3. Left) Quantification of the caspase 3/7 signal in MES-SA cells after treatment with the selected compounds. Detection of caspases 3/7 after staining and analyses with Incucyte®. Right) Western Blot image showing PARP cleavage as a result of caspase activation after treatment with compounds 9, 11, 12, 38, 39 and 41 for 24 h. Staurosporine was used as a positive control.

2.4 Effect of the compounds on tubulin polymerization

Microtubules play crucial roles not only in the process of mitosis but also in the intracellular trafficking and the maintenance of cell shape and volume.[41] Antimitotic drugs commonly arrest cells in mitosis and cause cells to lose their characteristic shape and assume a rounded appearance. We conducted an immunofluorescence assay after incubating MES-SA cells with the compounds at a concentration of 10 nM for 24 hours. Vincristine was included at the same concentration as a positive control. Nuclei stained in blue with DAPI, α -Tubulin in green to observe the microtubules, and F-actin with fluorescent phalloidin in red to monitor changes in the cell shape (Supplementary Material, Figure Suppl6).

Figure 4 illustrates the effects on the cells' shape, microtubule network, and nuclei of the treatment with vincristine and the sulfonamides. Vincristine, a microtubule-destabilizing compound causes cells to adopt a spherical shape. This happens because the microtubule

becomes non-functional, undergoes depolymerization, and consequently, the cell loses its characteristic shape. Compounds **11**, **38**, and **39** showed a similar effect on tubulin polymerization as vincristine. Indolesulfonamides **9**, **12**, **41** behave similarly to each other and induce different shapes from the previous group, larger volume increases, and changes in the nuclei, with patent tubulin bundles. These compound clusters are also the same previously observed for the caspase activation levels and the G₂/M relative populations in the cell cycle, therefore suggesting that different substituents on the sulfonamide nitrogen result in distinct phenotypes arising from different mechanistic outcomes.

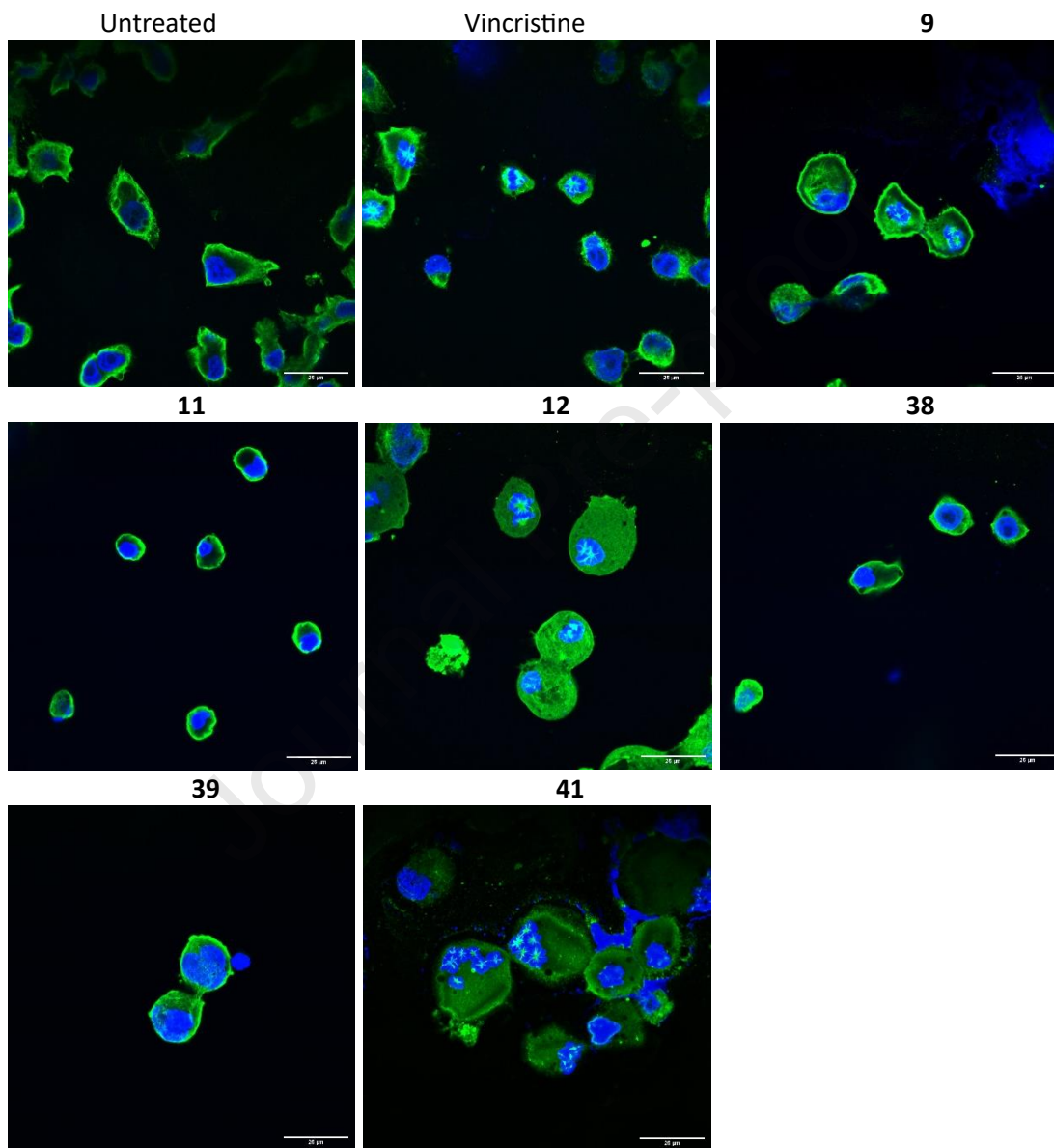


Figure 4. Effects of the selected compounds on MES-SA cells. MES-SA cells incubated with 10 nM of the antimitotic compounds or 0.1 % DMSO for 24 hours are stained in green for α -Tubulin and nuclei in blue with DAPI. Scale bar: 25 μ M

2.5 Physicochemical properties, ADMET, and toxicity prediction

We have evaluated the most promising sulfonamides **9**, **11**, **12**, **15**, **36**, **37**, **38**, **39**, **41**, **42**, **43** and **44** in SwissADME online web tool to assess their physicochemical and pharmacokinetic properties, and drug-likeness (Table 6). The topological polar surface area, considered a predictor of membrane penetration, of the compounds varies from 78.38 to 121.47 Å², higher values than those of CA-4 (57.15 Å²) and above the desired safety threshold of 75 Å² Pfizer's optimal drug-like properties for reduced toxicity.[42] The incorporation of polar groups at the indole 3 position pushes the compounds above this threshold. The calculated partition coefficients varied from 1.70 to 2.58, close to the optimal value of 3. All tested compounds exhibited less lipophilicity than CA-4 (cLogP of 3.27). Regarding the solubility, the predicted logS values ranged from -4.20 to -3.66, indicating moderate to good water solubility. The prediction of the susceptibility to P-GP only picked the amides **39**, **43**, and **44** as potential substrates. This is in good agreement with our experiments that suggest that only **43** and **44** enhance their antiproliferative effect in the presence of verapamil, a P-GP inhibitor. Finally, all compounds revealed good drug-likeness without any violation of the Lipinski, Ghose, Veber, Egan, and Muegge models.

Table 6. Physicochemical, pharmacokinetic, and drug-likeness properties predictions by SwissADME

	TPSA	CLogP	LogS	SClass	GIA	BBB	P-GP	Drug-likeness
9	87.17	2.29	-3.91	S	High	No	No	Yes (0)
11	78.38	2.58	-3.92	S	High	No	No	Yes (0)
12	102.17	2.28	-4.20	MS	High	No	No	Yes (0)
15	78.38	2.86	-4.30	MS	High	No	No	Yes (0)
36	95.45	2.28	-3.71	S	High	No	No	Yes (0)
37	119.24	2.01	-4.00	S	High	No	No	Yes (0)
38	102.17	2.34	-4.12	MS	High	No	No	Yes (0)
39	121.47	1.7	-3.66	S	High	No	Yes	Yes (0)
41	110.96	2.11	-4.12	MS	High	No	No	Yes (0)
42	125.96	2.08	-4.41	MS	High	No	No	Yes (0)
43	130.26	1.49	-3.65	S	High	No	Yes	Yes (0)
44	145.26	1.52	-3.93	S	Low	No	Yes	Yes (1)
CA-4	57.15	3.27	-4.61	S	High	Yes	No	Yes (0)
ABT751	108.93	2.27	-4.73	S	High	No	No	Yes (0)
Colchicine	83.09	2.36	-2.36	S	High	No	Yes	Yes (0)
PT	221.29	3.54	-8.00	PS	Low	No	Yes	No (1-3)
VC	171.17	3.38	-6.39	PS	Low	No	Yes	No (1-4)

TPSA: topological polar surface area (Å²).

CLogP: consensus Log P (average of iLOGP, XLOGP3, WLOGP, MLOGP, and Silicos-IT Log P).

LogS: Ali topological method Log S.

SClass: Ali solubility class (insoluble (IS); <-10<poorly soluble (PS); <-6<moderately soluble (MS); <-4<soluble (S); <-2<very soluble (VS); <0<highly soluble (HS)).

GI Absorption: gastrointestinal absorption.

BBB permeant: blood-brain barrier permeation

P-GP: p-glycoprotein substrates.

Drug-likeness: (Number of violations of the Lipinski, Ghose, Veber, Egan, and Muegge filters).

We also studied the mutagenic potential of the compounds with two different software, Derek (mathematical model) and Sarah (statistical data). None of them predicted either relevant

mutagenic or skin sensitization profiles, which are good settings for future *in vivo* studies (Supplementary Material Table Suppl2).

2.6. Docking studies

We studied the binding modes of the most potent compounds at the colchicine site of tubulin (Figure 5) employing ensemble docking experiments.[43] The X-ray crystal structures of complexes of colchicine site ligands with tubulin show binding to three sub-pockets (called A, B, and C) for structurally diverse ligands.[6, 44, 45] We have taken into account the protein flexibility in the docking experiments by using an ensemble of all the available X-ray structures that represent different protein arrangements in the complexes of tubulin with different ligands, therefore efficiently sampling the interactional space and the ligands' pharmacophore configurations.[43] We further increased the protein configurational space by adding to the proteins' ensemble structures with waters in direct contact (less than 5Å) with the ligands as they improve the binding mode predictive capability[43] and also with 6 additional structures from a previous molecular dynamics simulation obtained as described.[14] As a result, 153 structures were used to represent the different possible configurations of the colchicine site of tubulin.

We used two docking software with very different scoring functions (PLANTS[46] and AutoDock 4.2[47]) for the docking experiments, and we considered as the binding mode for every ligand the common pose with the best scores. The pose similarity and optimal combined scoring were assessed by means of automated geometry and scoring comparison procedures. The geometrical comparisons of the poses were based on pocket occupancy: sub-pocket binding was assigned to the poses if the shortest atomic distances to geometrical centers of the sub-pockets (determined by the pharmacophores derived from the X-ray structures of colchicine site ligands in complex with tubulin)[43] were under a 3Å threshold. Program scores were made comparable by scaling for each ligand the absolute scores to relative ones from 0 (worse) and 1 (best) and to Z-scores. A consensus binding-mode was proposed for each ligand as the pair of common poses for the two programs with the best possible Z-values for each (Supplementary Material Table Suppl3). Applying this procedure to control examples with representative colchicine-site ligands of known X-ray structures in complex with tubulin correctly retrieved the experimental poses in most cases, as previously shown.[18]

Sulfonamides **9** to **44** bind to the colchicine site of tubulin (Figure 5) with the trimethoxyphenyl rings allocated within the A sub-pocket, placing it similarly to that of combretastatin A4 (Supplementary Material Figure Suppl7). The trimethoxyphenyl moieties are placed between the sidechains of the residues of sheets S9 and S8 (contacting the methoxy groups) and helix H7 (contacting the thiol group of Cys241β) that conform the A sub-pocket and slightly protruding towards the C sub-pocket between sheets S5, S6 and S7 and helix H7.[6, 44] The indole systems bind at the B zone, stacked behind helix H8 with the sidechain of Asn258β (H8) piled onto it making carbonyl-π interactions and making hydrophobic contacts with Met259β (H8), Thr314β (S8), and the methylenes of Lys352β (S9).[27, 48] The *N*-substituents closely contact Met259β (H8), thus explaining the preference for small aliphatic chains (methyl preferred to acetonitrile). The substituents at the indole 3 position project towards the inter-subunit space, making hydrogen bonds with Thr179α. Binding to the A-B zones requires a folded arrangement of the

two aryl systems. The sulfonamide bridge protrudes towards the interfacial space between subunits with the substituents on the nitrogen extending in different directions.

The application of ensemble docking approaches renders the most favorable poses for each ligand and pinpoints the preferred protein structures for binding, thus providing information on the interaction modes. The most frequently retrieved pdb ID are 6XES and 6O5M, whose ligands have a trimethoxybenzoyl moiety (Figure Suppl8 in the supplemental material) that closely resembles the trimethoxyphenylsulfonamido moiety. The trimethoxybenzoyls are connected to biaryl systems (phenyl-pyridazine and 4-indolyl-imidazole for 6EXS's and 6O5M's ligands, respectively). As previously observed, the proteins in every pair of consensus poses are different for AutoDock and PLANTS, probably reflecting their different scoring schemes.[24, 25, 29, 49, 50] These results validate the use of as many protein structures as possible in the docking experiments (ensemble docking strategy) and reinforce the application of consensus scoring approaches. Remarkably, one of the most selected protein models includes structural waters in the proximity of the methoxy groups of the trimethoxyphenyl (Figure 5), thus reassessing the inclusion of these models in the docking protocol. The scoring results of neither program correlate well with the biological ranking obtained for the ligands. This probably results from limitations of the scoring functions and the cytotoxic potency being dependent on more events than just binding (as the potency variations observed against different cell lines suggest).

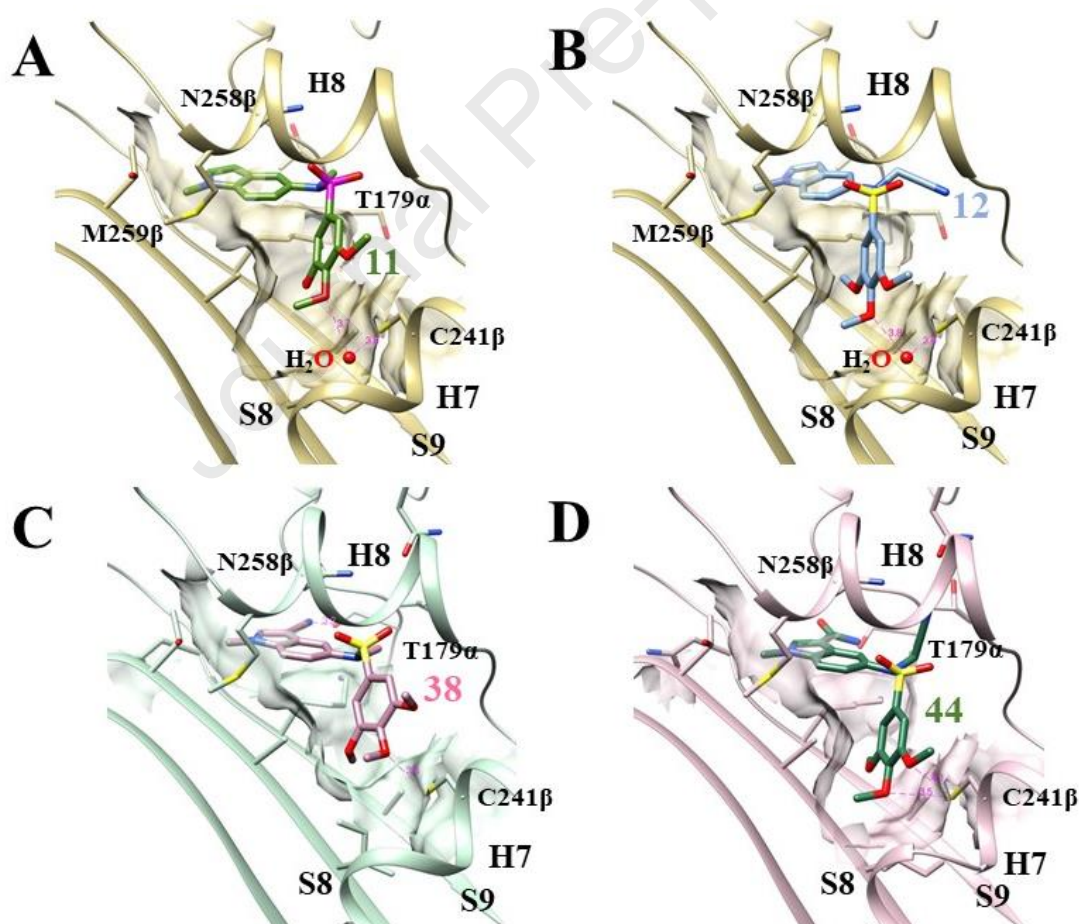


Figure 5. Selected docking poses for representative ligands **11** (A), **12** (B), **38** (C), and **44** (D) in complex with the protein configurations they prefer: 6XES in ochre for **11** and **12**, 5JVD in light greenish blue for **38**, and 6O5M in pale pink for **44**. The poses of **11** and **12** select protein with

one structural water, indicated as a red sphere. Labels indicate the structural elements mentioned in the main text and the protein surface contacting the ligands from behind is shown.

3. Conclusion

The indolesulfonamides are highly potent antimitotic compounds binding at the colchicine site of tubulin, but structural models suggest that their engagement with the pocket seems to be in non-optimal orientation for the sulfonamide bridge.[18] We have therefore synthesized and evaluated a new family of benzenesulfonamides with the reverse bridge orientation with the aim of better fitting the pocket and achieving compounds with more favourable profiles for their development. 36 *N*-(5-1*H*-indolyl)-3,4,5-trimethoxybenzenesulfonamides with variations on the indole nitrogen, the indole 3 position, and the sulfonamide nitrogen were synthesized and assayed as antiproliferative agents against the cervix human cancer cell line HeLa. The antiproliferative potencies show that the selected scaffold is a very favourable one, with many compounds in the low nanomolar range. The results on HeLa cells allowed for the establishment of structure-activity relationships: 1) the more favourable substituents on the indole nitrogen is the methyl; 2) substituents at the indole 3 position do not improve the potency, and 3) substitutions on the sulfonamide nitrogen are worse than the unsubstituted one, although some methyl and cyanomethyl compounds are still highly potent, even when combined with modifications on the indole 3 position. The antiproliferative activity was further evaluated against a panel of human cancer cell lines of different origin, showing varying sensitivities towards each compound and different sensitivity profiles depending on the chemical structures. The compounds showed reasonable tumor selectivity vs non-tumorigenic cells. The benzene sulfonamides are not substrates of the P-GP efflux protein, one of the main causes of cancer cell resistance against taxane and vinca alkaloids.

We have studied the mechanism of action of the most promising compounds and shown that they arrest the cell cycle in G₂/M, as expected for anti-tubulin drugs. Depending on the sulfonamide bridge substitution, a different profile of G₂ vs M accumulation was observed in the cell cycle analysis, suggesting different cellular outcomes arising from structural variations. Mechanistic studies showed that the compounds induce apoptosis by activation of the caspases 3/7, an activation that correlates with the extent of mitotic arrest, an observation coherent with mitotic arrest and spindle assembly checkpoint activation. The sulfonamides with a methyl group on the nitrogen cause mitotic arrest and induce apoptotic cell death. On the other hand, those with hydrogen or cyanomethyl groups on the sulfonamide nitrogen result in a higher extent of G₂ cells, less apoptotic induction and more necrotic cell death. This suggests that these compounds induce mitotic arrest and partially activate the spindle assembly checkpoint (SAC) but slippage takes place causing mitotic stopwatch accumulation and the activation of the mechanisms of cell demise. These results suggest alternative mechanisms of cell death being at work depending on compound structure. This structural split is sustained by cell morphological studies that confirm the effect of the compounds on tubulin and further show the different clustering depending on the sulfonamide substituent. Furthermore, the sensitivity of the cell lines to the two classes of compounds is also different, in particular the cell lines with lower intrinsic sensitivities to the sulfonamides (methylated sulfonamides are more potent) and that of a PT induced resistant cell line (cyanomethylated sulfonamides are more potent). These results suggest that the sulfonamide substituents determine different effects of the compounds

and cell fates, even if they act on the same target, binding pocket, and binding mode. These compounds seem to exert their antiproliferative action by a distinct mechanism, thus making them very interesting tools for further characterization it and making them interesting new anti-tumor drugs for further development.

4. Experimental section

4.1. Chemistry

4.1.1 General chemical techniques

Commercial reagents (Aldrich, Alpha, Fischer, SDS) and solvents (Fischer, SDS, Scharlau) were used as purchased without further purification. Thin Layer Chromatography (TLC) for monitoring reactions was performed on pre-coated silica gel polyester plates (Kieselgel 60 F254, E. Merck, Germany). Spots were detected by exposure to UV light at 254 nm (Polychrome SI F254) and, if it is necessary, colorization with 10% ninhydrin or spray. Flash column chromatography was taken with silica Kieselgel 40 (0.040-0.063; Merck). Melting points (mp) were determined on a Mel-Temp apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were performed using a Varian Mercury 400 MHz (400/100 MHz). The spectra were recorded in CDCl_3 , methanol- d_4 or $\text{DMSO}-d_6$. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. High resolution mass spectra (HRMS) were obtained by electron spray ionization-mass spectrometry (ESI-MS) technique (5 kV) on a QSTAR XL mass spectrometer. Yields were of purified product and were not optimized, with the exception of the starting materials. The non-crystalline compounds were purified by column chromatography using mixtures of hexane/EtOAc as the mobile phase.

4.1.2 Chemical synthesis

Procedure for synthesis of O-ethyl S-(3,4,5-trimethoxyphenyl)carbonodithioate

A solution of 3,4,5-trimethoxyaniline (16.40 mmol, 1.0 eq) in 1N HCl (45 mL) was cooled at 0 °C, added dropwise to a solution of NaNO_2 (21.30 mmol, 1.3 eq) in milliQ H_2O (15 mL) at 0 °C and stirred for 1 hour. Then, the mixture was poured onto a solution of ethyl xanthogenate (49.19 mmol, 3.0 eq) in milliQ H_2O (10.5 mL) at 50 °C. After 1 hour stirring, the reaction was extracted with ethyl acetate (EtOAc) washing the organic layers with 2N HCl, 5 % NaHCO_3 and brine. The organic layers were dried over anhydrous Na_2SO_4 , filtered and evaporated.

Procedure for synthesis of 3,4,5-trimethoxybenzenethiol

3M NaOH (6.5 mL) was added to a solution of O-ethyl S-(3,4,5-trimethoxyphenyl)carbondithioate (1.77 mmol) in ethanol (6.5 mL). After 2 hours of stirring at 65 °C, the reaction was acidified by adding 2N HCl until pH 5 and extracted with EtOAc. The organic layers were dried over anhydrous Na_2SO_4 , filtered and vacuum concentrated to obtain 3,4,5-trimethoxybenzenethiol.

Procedure for synthesis of 3,4,5-trimethoxybenzenesulfonyl chloride 1

KNO_3 (6.15 mmol, 3.7 eq) and trimethylchlorosilane (6.60 mmol, 4.0 eq) were added to a solution of 3,4,5-trimethoxybenzenethiol (1.65 mmol, 1.0 eq) in dichloromethane. After 72 hours of stirring at 50 °C, the reaction was filtered and extracted with dichloromethane. Organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered, and vacuum concentrated. The compound was purified by column chromatography (hexane/EtOAc 7:3) obtaining 3,4,5-trimethoxybenzenesulfonyl chloride (**1**).

Orange oil. $^1\text{H-NMR}$ (CDCl_3): 7.24 (2H, s, H-2); 3.95 (3H, s, OCH_3); 3.94 (6H, s, OCH_3).

General synthetic procedure for the alkylation of the indole nitrogen

To a solution of 5-nitro-1*H*-indole (1.0 eq) in dichloromethane, crushed NaOH (2.0 eq), and a catalytic amount of tetrabutylammonium hydrogen sulfate were added. The reaction was stirred at room temperature for 30 minutes. After this time, the halogenated derivative (2.0-3.0 eq) was added. The progress of the reaction was monitored by thin-layer chromatography, and once complete, the mixture was filtered and extracted with dichloromethane. The organic phases obtained were washed with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

1-methyl-5-nitro-1*H*-indole (2)

Yellow powder; Yield, 91 %. ¹H-NMR (400 MHz, CDCl₃) δ: 8.59 (1H, *d*, *J* = 2.0, H-4); 8.13 (1H, *dd*, *J* = 9.2; 2.0, H-6); 7.34 (1H, *d*, *J* = 9.2, H-7); 7.21 (1H, *d*, *J* = 3.2, H-2); 6.67 (1H, *dd*, *J* = 3.2, H-3); 3.86 (3H, *s*, NCH₃). ¹³C-RMN (100 MHz, CDCl₃) δ: 141.5 (C, C-7a); 139.4 (C, C-5); 132.1 (CH, C-4); 127.6 (C, C-3a); 118.1 (CH, C-2); 117.2 (CH, C-6); 109.1 (CH, C-7); 103.8 (CH, C-3); 33.3 (CH₃, NCH₃).

2-(5-nitro-1*H*-indol-1-yl)acetonitrile (3)

Yellow powder; Yield, 77 %. ¹H-NMR (400 MHz, C₂D₆OS) δ: 8.59 (1H, *d*, *J* = 2.4 Hz, H-4); 8.12 (1H, *dd*, *J* = 8.8; 2.4 Hz, H-6); 7.79 (1H, *d*, *J* = 8.8 Hz, H-7); 7.69 (1H, *d*, *J* = 3.6 Hz, H-2); 6.83 (1H, *d*, *J* = 3.6 Hz, H-3); 5.64 (2H, *s*, CH₂). ¹³C-RMN (100 MHz, C₂D₆OS) δ: 142.1 (C, C-7a); 139.0 (C, C-5); 132.8 (CH, C-4); 128.3 (C, C-3a); 118.2 (CH, C-2); 117.8 (CH, C-6); 116.6 (C, CN); 110.9 (CH, C-7); 105.7 (CH, C-3); 34.8 (CH₂).

General synthetic procedure for reduction of a nitro group to an amino group in aromatic rings (procedure 1).

The nitrated aromatic compound (1.0 eq) was dissolved in a mixture of EtOAc /methanol, and a catalytic amount of palladium on carbon (Pd/C) was added. The reaction was vigorously stirred under a hydrogen atmosphere at room temperature, monitoring its progress by thin-layer chromatography. Upon completion, the solution was filtered through celite and evaporated under vacuum.

1-methyl-1*H*-indol-5-amine (4)

Red powder; Yield, 96 %. ¹H-NMR (400 MHz, CDCl₃) δ: 7.12 (1H, *d*, *J* = 8.8, H-7); 6.96 (1H, *d*, *J* = 3.2, H-2); 6.93 (1H, *d*, *J* = 2.4, H-4); 6.70 (1H, *dd*, *J* = 8.8; 2.4, H-6); 6.29 (1H, *d*, *J* = 3.2, H-3); 3.73 (3H, *s*, NCH₃). ¹³C-RMN (100 MHz, CDCl₃) δ: 139.4 (C, C-5); 131.9 (C, C-3a); 129.4 (C, C-7a); 129.2 (CH, C-2); 112.6 (CH, C-7); 109.7 (CH, C-6); 105.8 (CH, C-4); 99.5 (CH, C-3); 33.9 (CH₃, NCH₃).

5-amino-1-methyl-1*H*-indole-3-carbonitrile (8)

Brown powder; Yield, 99 %. ¹H-NMR (400 MHz, CDCl₃) δ: 7.44 (1H, *s*, H-2); 7.18 (1H, *d*, *J* = 8.4 Hz, H-7); 7.00 (1H, *d*, *J* = 2.4 Hz, H-4); 6.77 (1H, *dd*, *J* = 8.4; 2.4 Hz, H-6); 3.78 (3H, *s*, NCH₃); 3.68 (2H, *s*, NH₂). ¹³C-RMN (100 MHz, C₂D₆OS) δ: 142.6 (C, C-5); 135.8 (CH, C-2); 130.7 (C, C-3a); 128.8 (C, C-7a); 116.3 (C, CN); 114.4 (CH, C-7); 110.9 (CH, C-6); 103.2 (CH, C-4); 82.1 (C, C-3); 32.3 (CH₃, NCH₃).

***N*-(3-aminobenzyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (35)**

Brown oil; Yield, 38 %. ¹H-NMR (400 MHz, CDCl₃) δ: 7.16 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.06 (1H, *d*, *J* = 8.8 Hz, H-7'); 6.93 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.88 (1H, *t*, *J* = 7.6 Hz, H-5''); 6.80 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.78 (2H, *s*, H-2); 6.59 (1H, *t*, *J* = 2.0 Hz, H-2''); 6.50 (1H, *d*, *J* = 7.6 Hz, H-6''); 6.40 (1H,

dd, $J = 7.6$; 2.0 Hz, H-4''); 6.28 (1H, *d*, $J = 3.2$ Hz, H-3'); 4.59 (2H, *s*, CH₂); 3.83 (3H, *s*, OCH₃); 3.68 (6H, *s*, OCH₃); 3.65 (3H, *s*, NCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ : 153.0 (2C, C-3); 146.4 (C, C-3''); 141.3 (C, C-4); 137.6 (C, C-1''); 136.0 (C, C-7'a); 133.9 (C, C-1); 131.1 (C, C-5'); 129.8 (CH, C-2'); 129.1 (CH, C-5''); 128.3 (C, C-3'a); 123.2 (CH, C-6'); 121.5 (CH, C-4'); 118.9 (CH, C-6''); 115.2 (CH, C-2''); 114.3 (CH, C-4''); 109.2 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 55.7 (CH₂); 32.9 (CH₃, NCH₃). **HRMS** calculated for C₂₅H₂₈N₃O₅S⁺ 482.1744, found: 482.1742. **HPLC** (C18): $t_R = 4.33$ min, 96.1 %.

General synthetic procedure for reduction of a nitro group to an amino group in aromatic rings (procedure 2).

The nitrated aromatic compound (1.0 eq) was dissolved in a mixture of acetone/Milli-Q water (4:1) and maintained at 0 °C. Chlorine trifluoride (Cl₃Fe) (4.0 eq) was added carefully, followed by powdered metallic zinc (10.0 eq), and the mixture was stirred for 30 minutes. After this period, 6 drops of 37 % HCl were added, and the reaction was continued with stirring. After 24 hours, the mixture was filtered through celite, evaporated and extracted with EtOAc, washing the organic phases with brine. The organic phases were dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

2-(5-amino-1*H*-indol-1-yl)acetonitrile (**5**)

Brown powder; Yield, 95 %. **¹H-NMR (400 MHz, CDCl₃)** δ : 7.16 (1H, *d*, $J = 8.8$ Hz, H-7); 7.00 (1H, *d*, $J = 3.2$ Hz, H-2); 6.93 (1H, *d*, $J = 2.0$ Hz, H-4); 6.75 (1H, *dd*, $J = 8.8$; 2.0 Hz, H-6); 6.41 (1H, *d*, $J = 3.2$ Hz, H-3); 4.93 (2H, *s*, CH₂). **¹³C-RMN (100 MHz, CDCl₃)**: 140.9 (C, C-5); 130.4 (C, C-7a); 129.9 (C, C-3a); 128.0 (CH, C-2); 115.4 (C, CN); 113.3 (CH, C-7); 109.6 (CH, C-6); 105.9 (CH, C-4); 102.6 (CH, C-3); 33.9 (CH₂).

N-(4-aminobenzyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**34**)

Brown crystals (MeOH); Yield, 6 %. **M. p.** 136-137 °C. **¹H-NMR (400 MHz, CDCl₃)** δ : 7.20 (1H, *d*, $J = 8.8$ Hz, H-7'); 7.14 (1H, *d*, $J = 3.2$ Hz, H-2'); 7.09 (1H, *d*, $J = 2.0$ Hz, H-4'); 6.92 (2H, *d*, $J = 8.0$ Hz, H-2''); 6.84 (2H, *s*, H-2); 6.74 (1H, *dd*, $J = 8.8$; 2.0 Hz, H-6'); 6.53 (2H, *d*, $J = 8.0$ Hz, H-3''); 6.32 (1H, *d*, $J = 3.2$ Hz, H-3'); 4.65 (2H, *s*, CH₂); 3.84 (3H, *s*, OCH₃); 3.74 (3H, *s*, NCH₃); 3.71 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ : 153.0 (2C, C-3'); 145.6 (C, C-4''); 141.2 (C, C-4'); 135.9 (C, C-5'); 134.0 (C, C-1); 130.9 (C, C-5'); 129.9 (2CH, C-2''); 129.8 (CH, C-2'); 128.2 (C, C-3'a); 126.1 (C, C-1''); 123.3 (CH, C-6'); 121.6 (CH, C-4'); 114.9 (2CH, C-3''); 109.2 (CH, C-7'); 105.2 (2CH, C-2); 101.2 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 55.3 (CH₂); 32.9 (CH₃, OCH₃). **HRMS** calculated for C₂₅H₂₈N₃O₅S⁺ 482.1744, found: 482.1731 (M+H⁺); calculated for C₂₅H₂₇N₃NaO₅S⁺ 504.1564, found: 504.1548 (M+Na⁺). **HPLC** (C18): $t_R = 3.63$ min, 96.0 %.

General synthetic procedure for formylation of indoles

POCl₃ (2.0 eq) was slowly added to dimethylformamide at 0 °C and stirred for 30 minutes. Then, this solution was slowly poured over the indole compound (1.0 eq) dissolved in dimethylformamide at 0 °C, and the reaction was subsequently heated for 2 hours at 100 °C. After this period, it was poured into a saturated aqueous solution of sodium acetate and kept at 4 °C, resulting in a precipitate that was vacuum-filtered.

1-methyl-5-nitro-1*H*-indole-3-carbaldehyde (**6**)

Yellow powder; Yield, 93 %. **M. p.** 202-203 °C. **¹H-NMR (400 MHz, CDCl₃)** δ : 10.06 (1H, *s*, CHO); 9.21 (1H, *d*, $J = 2.0$ Hz, H-4); 8.26 (1H, *dd*, $J = 9.2$; 2.0 Hz, H-6); 7.84 (1H, *s*, H-2); 7.43 (1H, *d*, $J = 9.2$ Hz, H-7); 3.96 (3H, *s*, NCH₃). **¹³C-RMN (C₂D₆OS)**: 185.4 (C, CHO); 144.9 (CH, C-2); 143.5 (C, C-

7a); 140.9 (C, C-5); 124.2 (C, C-3a); 119.1 (CH, C-4); 118.3 (C, C-3); 117.4 (CH, C-6); 112.3 (CH, C-7); 34.3 (CH₃, NCH₃).

N-(3-formyl-1-methyl-1*H*-indol-5-yl)-3,4,5-trimethoxy-*N*-methylbenzenesulfonamide (**36**)

Brown crystals (Hex/DCM); Yield, 36 %. **M. p.** 146-147 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 9.93 (1H, s, CHO); 7.90 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.70 (1H, s, H-2'); 7.41 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 7.36 (1H, *d*, *J* = 8.8 Hz, H-7'); 6.80 (2H, s, H-2); 3.92 (3H, s, OCH₃); 3.89 (3H, s, NCH₃); 3.72 (6H, s, OCH₃); 3.25 (3H, s, H-1''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 184.1 (C, CHO); 153.1 (2C, C-3); 141.8 (C, C-4); 140.3 (CH, C-2'); 137.5 (C, C-7'a*); 136.9 (C, C-5'*); 131.5 (C, C-1); 125.2 (CH, C-6'); 125.2 (C, C-3'a); 119.6 (CH, C-4'); 118.0 (C, C-3'); 110.3 (CH, C-7'); 105.6 (2CH, C-2); 61.1 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 39.0 (CH₃, C-1''); 34.0 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₃N₂O₆S⁺ 419.1271, found: 419.1263 (M+H⁺). **HPLC** (C18): *t*_R = 4.96 min, 97.0 %.

N-(cyanomethyl)-*N*-(3-formyl-1-methyl-1*H*-indol-5-yl)-3,4,5-trimethoxybenzenesulfonamide (**37**)

Brown crystals (Hex/DCM); Yield, 5 %. **M. p.** 208-209 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 9.96 (1H, s, CHO); 8.08 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.75 (1H, s, H-2'); 7.38 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.29 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.93 (2H, s, H-2); 4.61 (2H, s, H-1''); 3.94 (3H, s, OCH₃); 3.90 (3H, s, NCH₃); 3.78 (6H, s, OCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 183.9 (C, CHO); 153.3 (2C, C-3); 142.5 (C, C-4); 140.3 (CH, C-2'); 137.6 (C, C-7'a); 133.6 (C, C-1); 132.1 (C, C-5'); 125.6 (C, C-3'a); 125.3 (CH, C-6'); 122.1 (CH, C-4'); 118.1 (C, C-3'); 115.0 (C, CN); 111.0 (CH, C-7'); 105.4 (2CH, C-2); 61.1 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 40.4 (CH₂, C-1''); 34.0 (CH₃, NCH₃). **HRMS** calculated for C₂₁H₂₂N₃O₆S⁺ 444.1224, found: 444.1220 (M+H⁺). **HPLC** (C18): *t*_R = 3.91 min, 97.1 %.

General synthetic procedure for the transformation of the aldehyde group into a nitrile

The formylated indole (1.0 eq) was dissolved in methanol with a few drops of pyridine and hydroxylamine hydrochloride (10.0 eq) was added. The reaction was stirred at 100 °C for 48 hours, after which it was evaporated under vacuum. The residue was redissolved in pyridine, acetic anhydride was added, and the mixture was refluxed for 24-96 hours. After completion of the reaction, it was poured over ice, and extracted with dichloromethane, washing with 2N HCl, 5 % NaHCO₃, and brine. The organic phases were dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

1-methyl-5-nitro-1*H*-indole-3-carbonitrile (**7**)

Yellow powder; Yield, 24 %. **¹H-NMR (400 MHz, CDCl₃)** δ: 8.74 (1H, *d*, *J* = 1.6 Hz, H-4); 8.27 (1H, *dd*, *J* = 9.2; 1.6 Hz, H-5); 7.75 (1H, s, H-2); 7.49 (1H, *d*, *J* = 9.2 Hz, H-6); 3.95 (3H, s, NCH₃). **¹³C-RMN (100 MHz, C₂D₆OS)**: 143.1 (C, C-7a); 142.0 (CH, C-2); 139.2 (C, C-5); 126.6 (C, C-3a); 118.9 (CH, C-4); 115.6 (CH, C-6); 115.0 (C, CN); 112.9 (CH, C-7); 86.3 (C, C-3); 34.4 (CH₃, NCH₃).

N-(3-cyano-1-methyl-1*H*-indol-5-yl)-3,4,5-trimethoxy-*N*-methylbenzenesulfonamide (**38**)

Soft yellow crystals (Hex/DCM); Yield, 21 %. **M. p.** 188-189 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.60 (1H, s, H-2'); 7.38 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.37 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.32 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.78 (2H, s, H-2); 3.92 (3H, s, OCH₃); 3.87 (3H, s, NCH₃); 3.74 (6H, s, OCH₃); 3.25 (3H, s, H-1''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.1 (2C, C-3); 141.9 (C, C-4); 136.7 (C, C-7'a); 136.6 (CH, C-2'); 135.0 (C, C-5'); 131.2 (C, C-1); 127.7 (C, C-3'a); 124.6 (CH, C-6'); 117.6 (CH, C-4'); 115.1 (C, CN); 110.7 (CH, C-7'); 105.5 (2CH, C-2); 85.8 (C, C-3'); 61.1 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 39.0 (CH₃, C-1''); 33.9 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₂N₃O₅S⁺ 416.1275, found: 416.1267 (M+H⁺). **HPLC** (C18): *t*_R = 6.11 min, 97.8 %.

General synthetic procedure for the preparation of sulfonamides

A solution of the sulfonyl chloride derivate (1.0 eq) was dissolved with the corresponding amine (1.0-2.0 equiv) in dichloromethane in the presence of pyridine (1.0-0.2 mL). The reaction was stirred at room temperature under an argon atmosphere, and its progress was monitored by thin-layer chromatography. Upon completion, the reaction mixture was poured over ice and extracted with dichloromethane, washed with 2N HCl, 5 % NaHCO₃ and brine. The organic phases were dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (9)

Purple crystals (Hex/DCM); Yield, 52 %. **M. p.** 146-147 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.32 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.18 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.05 (1H, *d*, *J* = 2.8 Hz, H-2'); 6.90 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.85 (2H, *s*, H-2); 6.39 (1H, *d*, *J* = 2.8 Hz, H-3'); 6.26 (1H, *s*, NH); 3.84 (3H, *s*, OCH₃); 3.76 (3H, *s*, NCH₃); 3.67 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.0 (2C, C-3); 141.4 (C, C-4); 135.3 (C, C-7'a); 133.7 (C, C-1); 130.0 (CH, C-2'); 128.6 (C, C-5'*); 128.1 (C, C-3'a*); 119.0 (CH, C-6'); 116.8 (CH, C-4'); 109.6 (CH, C-7'); 104.7 (2CH, C-2); 101.1 (CH, C-3'); 60.9 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 33.0 (CH₃, NCH₃). **HRMS** calculated for C₁₈H₂₀N₂NaO₅S⁺ 399.0985, found: 399.0976 (M+Na⁺). **HPLC** (C18): t_R = 5.37 min, 98.0 %.

***N*-(1-(cyanomethyl)-1*H*-inden-5-yl)-3,4,5-trimethoxybenzenesulfonamide (10)**

White crystals (Hex/EtOAc); Yield, 2 %. **M. p.** 199-198 °C. **¹H-NMR (400 MHz, CD₃OD)** δ: 7.39 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.30 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.26 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.98 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.84 (2H, *s*, H-2); 6.49 (1H, *d*, *J* = 3.2 Hz, H-3'); 5.30 (2H, *s*, CH₂); 3.74 (3H, *s*, OCH₃); 3.64 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, C₂D₆SO)** δ: 153.1 (2C, C-3); 141.0 (C, C-4); 134.8 (C, C-7'a); 133.8 (C, C-1); 130.9 (C, C-5'); 130.1 (CH, C-2'); 129.0 (C, C-3'a); 118.5 (CH, C-6'); 116.9 (C, CN); 115.1 (CH, C-4'); 110.5 (CH, C-7'); 104.7 (2CH, C-2); 103.3 (CH, C-3'); 60.6 (CH₃, OCH₃); 56.6 (2CH₃, OCH₃); 34.3 (CH₂, NCH₂). **HRMS** calculated for C₁₉H₁₉N₃NaO₅S⁺ 424.0938, found: 424.0934 (M+Na⁺). **HPLC** (C18): t_R = 3.32 min, 97.0 %.

***N*-(3-cyano-1-methyl-1*H*-indol-5-yl)-3,4,5-trimethoxybenzenesulfonamide (41)**

Orange crystals (Hex/DCM/MeOH); Yield, 9 %. **M. p.** 216-217 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.56 (1H, *s*, H-2''); 7.41 (1H, *s*, H-4''); 7.30 (1H, *d*, *J* = 8.8 Hz, H-7''); 7.16 (1H, *d*, *J* = 8.8 Hz, H-6''); 6.94 (2H, *s*, H-2); 6.42 (1H, *s*, NH); 3.86 (3H, *s*, OCH₃); 3.83 (3H, *s*, NCH₃); 3.76 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, C₂H₆OS)** δ: 153.2 (2C, C-3); 141.1 (C, C-4); 138.7 (CH, C-2'); 134.6 (C, C-7'a); 133.7 (C, C-1); 132.8 (C, C-5'); 127.8 (C, C-3'a); 118.9 (CH, C-6'); 116.2 (C, CN); 112.6 (CH, C-4'/7'); 111.2 (CH, C-4'/7'); 104.8 (2CH, C-2); 83.3 (C, C-3'); 60.6 (CH₃, OCH₃); 56.6 (2CH₃, OCH₃); 33.9 (CH₃, NCH₃). **HRMS** calculated for C₁₉H₂₀N₃O₅S⁺ 402.1118, found: 402.1115 (M+H⁺); calculated for C₁₉H₁₉N₃NaO₅S⁺ 424.0938, found: 424.0932 (M+Na⁺). **HPLC** (C18): t_R = 3.43 min, 97.6 %.

General synthetic procedure for the alkylation of the NH on the sulfonamide

The corresponding sulfonamide (1.0 eq) was dissolved in acetonitrile and cesium carbonate (1.3-2.0 eq) was added. After ten minutes of stirring, this solution was added to a solution of the halogenated derivative (2.0-4.0 eq) in acetonitrile in the presence of potassium iodide. The reaction was stirred at room temperature under an argon atmosphere, with the progress monitored by thin-layer chromatography. Upon completion, the reaction mixture was evaporated under vacuum, water was added, and a liquid-liquid extraction with EtOAc was performed. The organic phases were washed with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

3,4,5-trimethoxy-*N*-methyl-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**11**)

Brown crystals (Hex/DCM); Yield, 52 %. **M. p.** 137-138 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.28 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.06 (1H, *d*, *J* = 3.2 Hz, H-2'); 7.02 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.76 (2H, *s*, H-2); 6.40 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.78 (3H, *s*, NCH₃); 3.70 (6H, *s*, OCH₃); 3.23 (3H, *s*, H-1''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.4 (C, C-4); 135.8 (C, C-7'a); 133.7 (C, C-1*); 131.5 (C, C-5'*); 130.0 (CH, C-2'); 128.3 (C, C-3'a); 121.5 (CH, C-6'); 119.4 (CH, C-4'); 109.2 (CH, C-7'); 105.5 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 39.2 (CH₃, C-1''); 33.0 (CH₃, NCH₃). **HRMS** calculated for C₁₉H₂₂N₂NaO₅S⁺ 413.1142, found: 413.1134 (M+Na⁺). **HPLC** (C18): *t_R* = 8.16 min, 98.5 %.

N-(cyanomethyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**12**)

Yellow oil; Yield, 68 %. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.42 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.26 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.10 (1H, *d*, *J* = 3.2 Hz, H-2'); 7.02 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.89 (2H, *s*, H-2); 6.44 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.62 (2H, *s*, H-1''); 3.91 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.74 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.1 (2C, C-3); 142.1 (C, C-4); 136.5 (C, C-7'a); 132.2 (C, C-1); 130.6 (CH, C-2'); 130.2 (C, C-5'); 128.6 (C, C-3'a); 121.9 (CH, C-6'); 121.4 (CH, C-4'); 115.4 (C, CN); 110.0 (CH, C-7'); 105.5 (2CH, C-2); 101.7 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 40.6 (CH₂, C-1''); 33.1 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₂N₃O₅S⁺ 416.1275, found: 416.1269 (M+H⁺). **HPLC** (C18): *t_R* = 6.95 min, 97.4 %.

N-(1-(cyanomethyl)-1*H*-inden-5-yl)-3,4,5-trimethoxy-*N*-methylbenzenesulfonamide (**13**)

Brown crystals (Hex/DCM); Yield, 59 %. **M. p.** 137-138 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.38 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.31 (1H, *d*, *J* = 8.4 Hz, H-7'); 7.13 (1H, *d*, *J* = 2.8 Hz, H-2'); 7.10 (1H, *dd*, *J* = 8.4; 2.0 Hz, H-6'); 6.73 (2H, *s*, H-2); 6.55 (1H, *d*, *J* = 2.8 Hz, H-3'); 5.01 (2H, *s*, CH₂); 3.90 (3H, *s*, OCH₃); 3.70 (6H, *s*, OCH₃); 3.23 (3H, *s*, H-1''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.0 (2C, C-3); 141.5 (C, C-4); 135.1 (C, C-7'a); 134.7 (C, C-1*); 131.1 (C, C-5'*); 129.0 (C, C-3'a); 128.6 (CH, C-2'); 122.5 (CH, C-6'); 120.2 (CH, C-4'); 114.2 (C, CN); 109.0 (CH, C-7'); 105.4 (2CH, C-2); 104.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 39.0 (CH₃, NCH₃); 34.4 (CH₂, NCH₂). **HRMS** calculated for C₂₀H₂₁N₃NaO₅S⁺ 538.1094, found: 538.1090 (M+Na⁺); calculated for C₂₄H₃₃N₄O₅S⁺ 489.2166, found: 489.2162 (M+(CH₃)₄N⁺). **HPLC** (C18): *t_R* = 6.03 min, 97.0 %.

N-(cyanomethyl)-*N*-(1-(cyanomethyl)-1*H*-inden-5-yl)-3,4,5-trimethoxybenzenesulfonamide (**14**)

Yellow crystals (MeOH); Yield, 25 %. **M. p.** 186-187 °C. **¹H-NMR (400 MHz, C₂D₆SO)** δ: 7.61 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.52 (1H, *d*, *J* = 3.2 Hz, H-2'); 7.41 (1H, *d*, *J* = 2.0 Hz, H-4'); 6.98 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.83 (2H, *s*, H-2); 6.59 (1H, *d*, *J* = 3.2 Hz, H-3'); 5.54 (2H, *s*, H-1''); 4.92 (2H, *s*, CH₂); 3.75 (3H, *s*, OCH₃); 3.70 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, C₂D₆SO)** δ: 153.3 (2C, C-3); 142.0 (C, C-4); 135.6 (C, C-7'a); 132.2 (C, C-1); 131.7 (C, C-5'); 130.8 (CH, C-2'); 128.9 (C, C-3'a); 122.7 (CH, C-6'); 121.6 (CH, C-4'); 117.0 (C, C-2''); 116.8 (C, CN); 110.9 (CH, C-7'); 105.6 (2CH, C-2); 103.7 (CH, C-3'); 60.8 (CH₃, OCH₃); 56.7 (2CH₃, OCH₃); 44.7 (CH₂, C-1''); 34.5 (CH₂). **HRMS** calculated for C₂₁H₂₀N₄NaO₅S⁺ 463.1047, found: 463.1034 (M+Na⁺). **HPLC** (C18): *t_R* = 4.98 min, 97.6 %.

3,4,5-trimethoxy-*N*-ethyl-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**15**)

White crystals (Hex/DCM); Yield, 11 %. **M. p.** 118-119 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.95 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.83 (2H, *s*, H-2); 6.40 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.74 (6H, *s*, OCH₃); 3.63 (1H, *q*, *J* = 7.2 Hz, Et); 1.09 (1H, *t*, *J* = 7.2 Hz, Et). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.2 (C, C-4); 136.0 (C, C-7'a); 133.5 (C, C-1); 130.6 (C, C-5'); 130.0 (CH,

C-2'); 128.4 (C, C-3'a); 123.1 (CH, C-6'); 121.4 (CH, C-4'); 109.3 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 46.2 (CH₂, C-1''); 33.0 (CH₃, NCH₃); 14.1 (CH₃, C-2''). **HRMS** calculated for C₂₀H₂₅N₂O₅S⁺ 405.1479, found: 405.1475 (M+H⁺). **HPLC** (C18): t_R = 9.56 min, 97.8 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(prop-2-yn-1-yl)benzenesulfonamide (**16**)

Brown crystals (MeOH); Yield, 8 %. **M. p.** 88-89 °C. **¹H-NMR (400 MHz, C₂D₆SO)** δ: 7.43 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.08 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 7.05 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.90 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.49 (2H, *s*, H-1''); 3.90 (3H, *s*, OCH₃); 3.77 (3H, *s*, NCH₃); 3.75 (6H, *s*, OCH₃); 2.19 (1H, *s*, H-3''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.8 (2C, C-3); 142.4 (C, C-4); 137.1 (C, C-7'a); 134.6 (C, C-1); 131.9 (C, C-5'); 131.1 (CH, C-2'); 129.3 (C, C-3'a); 123.6 (CH, C-6'); 122.4 (CH, C-4'); 110.4 (CH, C-7'); 106.5 (2CH, C-2); 102.4 (CH, C-3'); 79.7 (C, C-2''); 74.5 (CH, C-3''); 61.9 (CH₃, OCH₃); 57.3 (2CH₃, OCH₃); 42.9 (CH₂, C-1''); 33.9 (CH₃, NCH₃). **HRMS** calculated for C₂₁H₂₃N₂O₅S⁺ 415.1322, found: 415.1309 (M+H⁺); calculated for C₂₁H₂₂N₂NaO₅S⁺ 437.1142, found: 437.1128 (M+Na⁺). **HPLC** (C18): t_R = 8.19 min, 97.6 %.

2-((3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)phenyl)sulfonamide)acetamide (**17**)

Yellow oil; Yield, 24 %. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.34 (1H, *d*, *J* = 2.0, H-4'); 7.25 (1H, *d*, *J* = 8.8, H-7'); 7.10 (1H, *d*, *J* = 2.8 Hz, H-2'); 6.95 (1H, *dd*, *J* = 8.8; 2.0, H-6'); 6.76 (2H, *s*, H-2); 6.70 (2H, *s*, NH₂); 6.43 (1H, *d*, *J* = 2.8, H-3'); 5.52 (1H, *s*, NH); 4.23 (2H, *s*, CH₂); 3.91 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.70 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 170.8 (C, CO); 153.0 (2C, C-3); 141.9 (C, C-4); 136.0 (C, C-7'a); 131.5 (C, C-1); 131.2 (C, C-5'); 130.4 (CH, C-2'); 128.4 (C, C-3'a); 121.5 (CH, C-6'); 120.6 (CH, C-4'); 109.6 (CH, C-7'); 105.4 (2CH, C-2); 101.4 (CH, C-3'); 60.9 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 55.2 (CH₂, C-1''); 33.0 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₄N₃O₆S⁺ 434.1380, found: 434.1377 (M+H⁺); calculated for C₂₀H₂₃N₃NaO₆S⁺ 456.1200, found: 456.1194 (M+Na⁺). **HPLC** (C18): t_R = 4.02 min, 99.2 %.

N-(3-bromopropyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**18**)

Brown crystals (MeOH); Yield, 6 %. **M. p.** 127-128 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.25 (2H, *m*, H-2' y H-7'); 7.08 (1H, *d*, *J* = 3.2 Hz, H-3'); 6.94 (1H, *d*, *J* = 8.8; 2.0 Hz, H-6'); 6.80 (2H, *s*, H-2); 6.42 (1H, *d*, *J* = 2.0 Hz, H-4'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.74-3.71 (8H, *m*, H-3'' y OCH₃); 3.45 (2H, *t*, *J* = 6.4 Hz, H-1''); 2.03-2.00 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.0 (2C, C-3); 141.4 (C, C-4); 136.1 (C, C-7'a); 132.9 (C, C-1); 130.7 (C, C-5'); 130.2 (CH, C-2'); 128.5 (C, C-3'a); 122.6 (CH, C-6'); 121.2 (CH, C-4'); 109.5 (CH, C-7'); 105.3 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 49.8 (CH₂, C-1''); 33.0 (CH₃, NCH₃); 31.5 (CH₂, C-3''); 30.2 (CH₂, C-2''). **HRMS** calculated for C₂₁H₂₉BrN₂O₅S⁺ 497.0740, found: 497.0731 (M+H⁺). **HPLC** (C18): t_R = 11.07 min, 95.2 %.

N-(4-bromobutyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**19**)

Brown crystals (MeOH); Yield, 10 %. **M. p.** 132-133 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.24 (2H, *m*, H-2' y H-7'); 7.08 (1H, *d*, *J* = 3.2 Hz, H-3'); 6.94 (1H, *d*, *J* = 8.8; 2.4 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.42 (1H, *d*, *J* = 2.4 Hz, H-4'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.73 (6H, *s*, OCH₃); 3.61 (2H, *t*, *J* = 6.4 Hz, H-4''); 3.41 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.00-1.96 (2H, *m*, H-3''); 1.61-1.55 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.0 (2C, C-3); 141.3 (C, C-4); 136.0 (C, C-7'a); 133.1 (C, C-1); 130.5 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 122.7 (CH, C-6'); 121.2 (CH, C-4'); 109.4 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 50.0 (CH₂, C-1'');

33.3 (CH₂, C-4''); 33.0 (CH₃, NCH₃); 29.4 (CH₂, C-3''); 26.5 (CH₂, C-2''). **HRMS** calculated for C₂₂H₂₈BrN₂O₅S⁺ 511.0897, found: 511.0893 (M+H⁺). **HPLC** (C18): t_R = 12.02 min, 95.4 %.

N-(5-bromopentyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**20**)

Brown crystals (MeOH); Yield, 20 %. **M. p.** 117-118 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.24 (2H, *m*, H-2' y H-7'); 7.08 (1H, *d*, *J* = 3.2 Hz, H-3'); 6.95 (1H, *d*, *J* = 8.8; 2.4 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.42 (1H, *d*, *J* = 2.4 Hz, H-4'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.73 (6H, *s*, OCH₃); 3.57 (2H, *t*, *J* = 6.8 Hz, H-5''); 3.35 (2H, *t*, *J* = 6.4 Hz, H-1''); 1.83-1.79 (2H, *m*, H-4''); 1.55-1.45 (4H, *m*, H-2'' y H-3''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.3 (C, C-4); 136.0 (C, C-7'a); 133.2 (C, C-1); 130.7 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 122.9 (CH, C-6'); 121.3 (CH, C-4'); 109.4 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 50.9 (CH₂, C-1''); 33.6 (CH₂, C-5''); 33.0 (CH₃, NCH₃); 32.2 (CH₂, C-4''); 27.4 (CH₂, C-2''); 25.0 (CH₂, C-3''). **HRMS** calculated for C₂₃H₃₀BrN₂O₅S⁺ 525.1053, found: 525.1051 (M+H⁺). **HPLC** (C18): t_R = 12.88 min, 96.0 %.

N-(6-bromohexyl)-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**21**)

White crystals (MeOH); Yield, 3 %. **M. p.** 134-135 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.26-7.24 (2H, *m*, H-4' y H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *d*, *J* = 8.8; 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.72 (6H, *s*, OCH₃); 3.56 (2H, *t*, *J* = 6.8 Hz, H-6''); 3.36 (2H, *t*, *J* = 6.4 Hz, H-1''); 1.84-1.76 (2H, *m*, H-5''); 1.43-1.38 (6H, *m*, H-2'', H-3'' y H-4''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.2 (C, C-4); 136.0 (C, C-7'a); 133.3 (C, C-1); 130.8 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 122.8 (CH, C-6'); 121.2 (CH, C-4'); 109.3 (CH, C-7'); 105.2 (2CH, C-2); 101.2 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 51.0 (CH₂, C-1''); 33.8 (CH₂, C-6''); 33.0 (CH₃, NCH₃); 32.6 (CH₂, C-5''); 28.0 (CH₂, C-4''); 27.6 (CH₂, C-2''); 25.5 (CH₂, C-3''). **HRMS** calculated for C₂₄H₃₂BrN₂O₅S⁺ 539.1210, found: 539.1204 (M+H⁺); calculated for C₂₄H₃₁BrN₂NaO₅S⁺ 561.1024, found: 561.1029 (M+Na⁺). **HPLC** (C18): t_R = 13.87 min, 95.8 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(2-morpholinoethyl)benzenesulfonamide (**22**)

Brown crystals (Hex/EtOAc); Yield, 31 %. **M. p.** 158-159 °C. **¹H-NMR (400 MHz, CD₃OD)** δ: 7.34 (1H, *d*, *J* = 8.8 Hz, H-4'); 7.26 (1H, *d*, *J* = 2.0 Hz, H-7'); 7.21 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.90 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.40 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.83 (3H, *s*, OCH₃); 3.80 (3H, *s*, NCH₃); 3.76 (2H, *t*, *J* = 6.8 Hz, H-1''); 3.70 (6H, *s*, OCH₃); 3.62 (4H, *t*, *J* = 4.4 Hz, H-5''); 2.46 (2H, *t*, *J* = 6.8 Hz, H-2''); 2.42 (4H, *t*, *J* = 4.4 Hz, H-4''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.3 (C, C-4); 136.0 (C, C-7'a); 133.3 (C, C-1); 130.9 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 123.0 (CH, C-6'); 121.5 (CH, C-4'); 109.3 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 66.8 (2CH₂, C-5''); 61.0 (CH₃, OCH₃); 56.8 (CH₂, C-2''); 56.3 (2CH₃, OCH₃); 53.6 (2CH₂, C-4''); 48.6 (CH₂, C-1''); 33.0 (CH₃, NCH₃). **HRMS** calculated for C₂₄H₃₂N₃O₆S⁺ 490.2006, found: 490.1996 (M+H⁺). **HPLC** (C18): t_R = 2.07 min, 99.3 %.

Ethyl *N*-(1-methyl-1*H*-indol-5-yl)-*N*-((3,4,5-trimethoxyphenyl)sulfonyl)glycinate (**26**)

Brown crystals (Hex/DCM); Yield, 55 %. **M. p.** 120-121 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.44 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.23 (1H, *d*, *J* = 9.2 Hz, H-7'); 7.11 (1H, *dd*, *J* = 9.2; 2.0 Hz, H-6'); 7.06 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.44 (2H, *s*, H-1''); 4.13 (2H, *q*, *J* = 7.2 Hz, Et); 3.89 (3H, *s*, OCH₃); 3.77 (3H, *s*, NCH₃); 3.75 (6H, *s*, OCH₃); 1.21 (3H, *t*, *J* = 7.2 Hz, Et). **¹³C-NMR (100 MHz, CDCl₃)** δ: 169.1 (C, CO); 152.9 (2C, C-3); 141.4 (C, C-4); 136.0 (C, C-7'a); 133.9 (C, C-1); 131.6 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 123.0 (CH, C-7'); 121.8 (CH, C-6'); 109.5 (CH, C-4'); 105.4 (2CH, C-2); 101.5 (CH, C-3'); 61.3 (CH₂, Et); 60.9 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃);

53.6 (CH₂, C-1''); 33.0 (CH₃, NCH₃); 14.1 (CH₃, Et). **HRMS** calculated for C₂₂H₂₇N₂O₇S⁺ 463.1533, found: 463.1526 (M+H⁺). **HPLC** (C18): t_R = 9.01 min, 96.0 %.

Ethyl 4-((3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)phenyl)sulfonamido)butanoate (**27**)

Brown-red crystals (Hex/DCM); Yield, 52 % yield. **M. p.** 111-112 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 9.2 Hz, H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *dd*, *J* = 9.2, 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.09 (2H, *q*, *J* = 7.6 Hz, Et); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.72 (6H, *s*, OCH₃); 3.63 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.42 (2H, *t*, *J* = 7.6 Hz, H-3''); 1.77-1.73 (2H, *m*, H-2''); 1.22 (3H, *t*, *J* = 6.8 Hz, Et). **¹³C-NMR (100 MHz, CDCl₃)** δ: 173.0 (C, CO); 152.9 (2C, C-3); 141.3 (C, C-4); 136.0 (C, C-7'a); 133.1 (C, C-1); 130.6 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 122.7 (CH, C-6'); 121.2 (CH, C-4'); 109.4 (CH, C-7'); 105.2 (2CH, C-2); 101.2 (CH, C-3'); 60.9 (CH₃, OCH₃); 60.3 (CH₂, Et); 56.3 (2CH₃, OCH₃); 50.4 (CH₂, C-1''); 33.0 (CH₃, NCH₃); 30.9 (CH₂, C-3''); 23.4 (CH₂, C-2''); 14.2 (CH₃, Et). **HRMS** calculated for C₂₄H₃₁N₂O₇S⁺ 491.1846, found: 491.1838 (M+H⁺). **HPLC** (C18): t_R = 10.26 min, 95.4 %.

Ethyl 5-((3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)phenyl)sulfonamido)pentanoate (**28**)

White crystals (Hex/DCM); Yield, 46 %. **M. p.** 143-144 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.26 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.07 (2H, *q*, *J* = 7.6 Hz, Et); 3.90 (3H, *s*, OCH₃); 3.77 (3H, *s*, NCH₃); 3.73 (6H, *s*, OCH₃); 3.57 (2H, *t*, *J* = 7.2 Hz, H-1''); 2.26 (2H, *t*, *J* = 7.6 Hz, H-4''); 1.72-1.68 (2H, *m*, H-3''); 1.48-1.45 (2H, *m*, H-2''); 1.20 (3H, *t*, *J* = 7.2 Hz, Et). **¹³C-NMR (100 MHz, CDCl₃)** δ: 173.3 (C, CO); 152.9 (2C, C-3); 141.2 (C, C-4); 136.0 (C, C-7'a); 133.2 (C, C-1); 130.7 (C, C-5'); 130.0 (CH, C-2'); 128.4 (C, C-3'a); 122.8 (CH, C-6'); 121.2 (CH, C-4'); 109.3 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 60.2 (CH₂, Et); 56.3 (2CH₃, OCH₃); 50.7 (CH₂, C-1''); 33.7 (CH₂, C-4''); 33.0 (CH₃, NCH₃); 27.6 (CH₂, C-2''); 21.8 (CH₂, C-3''); 14.2 (CH₃, Et). **HRMS** calculated for C₂₅H₃₃N₂O₇S⁺ 505.2003, found: 505.1998 (M+H⁺). **HPLC** (C18): t_R = 10.96 min, 95.2 %.

N-benzyl-3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)benzenesulfonamide (**31**)

Brown crystals (MeOH); Yield, 24 %. **M. p.** 149-150 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.25 (3H, *m*); 7.23-7.21 (2H, *m*); 7.19-7.17 (1H, *m*); 7.14 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.02 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.85 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.86 (2H, *s*, H-2); 6.35 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.76 (2H, *s*, CH₂); 3.92 (3H, *s*, OCH₃); 3.74 (6H, *s*, OCH₃); 3.73 (3H, *s*, NCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.0 (2C, C-3); 141.3 (C, C-4); 136.5 (C, C-1''); 135.9 (C, C-7'a); 133.8 (C, C-1); 131.0 (C, C-5'); 129.9 (C, C-3'a); 128.6 (CH, C-2'); 128.3 (2CH, C-3''*); 128.3 (2CH, C-2''*); 127.5 (CH, C-4''); 123.2 (CH, C-6'); 121.6 (CH, C-4'); 109.2 (CH, C-7'); 105.3 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 55.7 (CH₂); 32.9 (CH₃, NCH₃). **HRMS** calculated for C₂₅H₂₇N₂O₅S⁺ 467.1635, found: 467.1635 (M+H⁺). **HPLC** (C18): t_R = 11.48 min, 95.6 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(4-nitrobenzyl)benzenesulfonamide (**32**)

Pink crystals (DCM/MeOH); yield, 39 %. **M. p.** 190-191 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 8.09 (2H, *d*, *J* = 8.8 Hz, H-3''); 7.47 (2H, *d*, *J* = 8.8 Hz, H-2''); 7.23 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.16 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.04 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.85 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.84 (2H, *s*, H-2); 6.37 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.84 (2H, *s*, CH₂); 3.93 (3H, *s*, OCH₃); 3.74 (9H, *s*, OCH₃ y NCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.1 (2C, C-3); 147.3 (C, C-1''); 144.1 (C, C-4''); 141.7 (C, C-4); 136.0 (C, C-7'a); 132.9 (C, C-1); 130.5 (C, C-5'); 130.2 (CH, C-2'); 129.2 (2CH, C-2''); 128.4 (C, C-3'a); 123.6 (2CH, C-3''); 122.6 (CH, C-6'); 121.3 (CH, C-4'); 109.5 (CH, C-7'); 105.3 (2CH, C-2); 101.3 (CH, C-

3'); 61.0 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 55.0 (CH₂); 33.0 (CH₃, NCH₃). **HRMS** calculated for C₂₅H₂₆N₃O₇S⁺ 512.1486, found: 512.1473 (M+H⁺); calculated for C₂₅H₂₅N₃NaO₇S⁺ 534.1305, found: 534.1290 (M+Na⁺). **HPLC** (C18): t_R = 11.16 min, 98.1 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(3-nitrobenzyl)benzenesulfonamide (**33**)

Orange crystals (DCM/MeOH); Yield, 15 %. **M. p.** 184-185 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 8.09 (1H, *d*, *J* = 2.0 Hz, H-2''); 8.05 (1H, *d*, *J* = 8.0 Hz, H-4''); 7.73 (1H, *d*, *J* = 8.0 Hz, H-6''); 7.43 (1H, *t*, *J* = 8.0 Hz, H-5''); 7.24 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.17 (1H, *d*, *J* = 8.4 Hz, H-7'); 7.04 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.88 (1H, *dd*, *J* = 2.0 Hz, H-6'); 6.86 (2H, *s*, H-2); 6.37 (1H, *d*, *J* = 3.2 Hz, H-3'); 4.86 (2H, *s*, CH₂); 3.93 (3H, *s*, OCH₃); 3.74 (9H, *s*, OCH₃ y NCH₃). **¹³C-NMR (100 MHz, CDCl₃)** δ: 153.1 (2C, C-3); 148.2 (C, C-3''); 141.6 (C, C-4'); 138.8 (C, C-1''); 136.0 (C, C-7'a); 134.6 (CH, C-6''); 133.0 (C, C-1); 130.5 (C, C-5'); 130.2 (CH, C-2'); 129.4 (CH, C-5''); 128.4 (C, C-3'a); 123.2 (CH, C-2''); 122.7 (CH, C-4''); 122.6 (CH, C-6'); 121.4 (CH, C-4'); 109.5 (CH, C-7'); 105.3 (2CH, C-2); 101.4 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.4 (2CH₃, OCH₃); 54.9 (CH₂); 33.0 (CH₃, NCH₃). **HRMS**: Calculated for C₂₅H₂₆N₃O₇S⁺ 512.1486, found: 512.1476 (M+H⁺); calculated for C₂₅H₂₅N₃NaO₇S⁺ 534.1295, found: 534.1395 (M+Na⁺). **HPLC** (C18): t_R = 10.89 min, 96.8 %.

N-(3-cyano-1-methyl-1*H*-indol-5-yl)-*N*-(cyanomethyl)-3,4,5-trimethoxybenzenesulfonamide (**42**)

Brown crystals (DCM/MeOH); Yield, 20 %. **M. p.** 226-227 °C. **¹H-NMR (400 MHz, C₂H₆OS)** δ: 8.30 (1H, *s*, H-2'); 7.67 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.29 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.14 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.83 (2H, *s*, H-2); 4.94 (2H, *s*, H-1''); 3.84 (3H, *s*, NCH₃); 3.73 (3H, *s*, OCH₃); 3.70 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, C₂H₆OS)** δ: 153.4 (2C, C-3); 142.2 (C, C-4); 139.8 (CH, C-2'); 135.9 (C, C-7'a); 133.3 (C, C-5'); 132.0 (C, C-1); 127.5 (C, C-3'a); 124.6 (CH, C-6'); 118.9 (CH, C-4'); 116.8 (C, C-2''); 115.7 (C, 3'-CN); 112.9 (CH, C-7'); 105.7 (2CH, C-2); 84.0 (C, C-3'); 60.8 (CH₃, OCH₃); 56.7 (2CH₃, OCH₃); 40.3 (CH₂); 34.1 (CH₃, NCH₃). **HRMS** calculated for C₂₁H₂₄N₅O₅S⁺ 458.1493, found: 458.1488 (M+NH₄⁺); calculated for C₁H₂₀N₄NaO₅S⁺ 463.1047, found: 463.1041 (M+Na⁺). **HPLC** (C18): t_R = 4.61 min, 97.8 %.

5-((*N*-(cyanomethyl)-3,4,5-trimethoxyphenyl)sulfonamido)-1-methyl-1*H*-indole-3-carboxamide (**44**)

Orange oil; Yield, 17 %. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.86 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.70 (1H, *s*, H-2'); 7.35 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.16 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.93 (2H, *s*, H-2); 5.50 (2H, *s*, NH₂); 4.64 (2H, *s*, H-1''); 3.93 (3H, *s*, OCH₃); 3.85 (3H, *s*, NCH₃); 3.78 (6H, *s*, OCH₃). **¹³C-NMR (100 MHz, C₂H₆OS)** δ: 166.1 (C, C=O); 153.4 (2C, C-3); 142.0 (C, C-4); 136.8 (C, C-7'a); 134.2 (CH, C-2'); 132.7 (C, C-1); 132.2 (C, C-5'); 127.2 (C, C-3'a); 122.6 (CH, C-6'); 122.1 (CH, C-4'); 117.0 (C, CN); 111.4 (CH, C-7'); 110.4 (CH, C-3'); 105.6 (2CH, C-2); 60.7 (CH₃, OCH₃); 56.6 (2CH₃, OCH₃); 40.8 (CH₂, C-1''); 33.6 (CH₃, NCH₃). **HRMS** calculated for C₂₁H₂₃N₄O₆S⁺ 459.1333, found: 459.1326 (M+H⁺); calculated for C₂₁H₂₂N₄NaO₆S⁺ 416.1152, found: 481.1146 (M+Na⁺). **HPLC** (C18): t_R = 3.39 min, 99.9 %.

General synthetic procedure for nucleophilic substitution of bromine by morpholines

The brominated derivative (1.0 eq) was dissolved in acetonitrile in the presence of potassium carbonate (2.0-3.0 eq) and potassium iodide. To this solution, morpholine (2.0-4.0 eq), previously dissolved in acetonitrile, was added. The reaction was stirred at room temperature under an argon atmosphere, and its progress was monitored by thin-layer chromatography. Upon completion, the reaction mixture was evaporated under vacuum, water was added, and

extracted with EtOAc. The organic phases were washed with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(3-morpholinopropyl)benzenesulfonamide (**23**)

Soft brown powder (MeOH); Yield, 58 %. **M. p.** 102-103 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.23 (2H, *m*, H-4' y H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.95 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.73 (6H, *s*, OCH₃); 2.64-2.60 (6H, *m*, H-1'' y H-6''); 2.40 (2H, *t*, *J* = 7.6 Hz, H-3''); 2.35-2.32 (4H, *m*, H-5''); 1.65-1.61 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.3 (C, C-4); 136.0 (C, C-7'a); 133.2 (C, C-1); 130.8 (C, C-5'); 130.0 (CH, C-2'); 128.4 (C, C-3'a); 122.8 (CH, C-6'); 121.3 (CH, C-4'); 109.3 (CH, C-7'); 105.3 (2CH, C-2); 101.3 (CH, C-3'); 66.9 (2CH₂, C-6''); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 55.9 (CH₂, C-1''); 53.7 (2CH₂, C-5''); 49.5 (CH₂, C-3''); 33.0 (CH₃, NCH₃); 25.4 (CH₂, C-2''). **HRMS** calculated for C₂₅H₃₄N₃O₆S⁺ 504.2163, found: 504.2158 (M+H⁺). **HPLC** (C18): t_R = 1.99 min, 98.6 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(4-morpholinobutyl)benzenesulfonamide (**24**)

Brown powder (MeOH); Yield, 59 %. **M. p.** 120-121 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.27-7.23 (2H, *m*, H-4' y H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.78 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.72 (6H, *s*, OCH₃); 3.65 (4H, *t*, *J* = 4.8 Hz, H-7''); 3.57 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.38-2.34 (4H, *m*, H-6''); 2.28-2.24 (2H, *m*, H-4'); 1.56-1.53 (2H, *m*, H-3'); 1.46-1.45 (2H, *m*, H-2'). **¹³C-NMR (100 MHz, CDCl₃)**: 152.9 (2C, C-3); 141.2 (C, C-4); 136.0 (C, C-7'a); 133.2 (C, C-1); 130.7 (C, C-5'); 130.0 (CH, C-2'); 128.4 (C, C-3'a); 122.9 (CH, C-6'); 121.3 (CH, C-4'); 109.3 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 66.9 (2CH₂, C-7''); 61.0 (CH₃, OCH₃); 58.3 (CH₂, C-1''); 56.3 (2CH₃, OCH₃); 53.6 (2CH₂, C-6''); 50.9 (CH₂, C-4''); 30.0 (CH₃, NCH₃); 26.0 (CH₂, C-3''); 23.3 (CH₂, C-2''). **HRMS** calculated for C₂₆H₃₆N₃O₆S⁺ 518.2319, found: 518.2316 (M+H⁺). **HPLC** (C18): t_R = 2.08 min, 97.0 %.

3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)-*N*-(6-morpholinohexyl)benzenesulfonamide (**25**)

White powder; Yield, 66 %. **M. p.** 98-99 °C. **¹H-NMR (400 MHz, CDCl₃)** δ: 7.23-7.20 (2H, *m*, H-4' y H-7'); 7.03 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.90 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.77 (2H, *s*, H-2); 6.37 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.86 (3H, *s*, OCH₃); 3.74 (3H, *s*, NCH₃); 3.68 (6H, *s*, OCH₃); 3.67-3.64 (4H, *m*, C-9''); 3.52 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.35-2.34 (4H, *m*, C-8''); 2.23-2.20 (2H, *m*, H-6''); 1.39-1.31 (6H, *m*, H-3'', H-4'' y H-5''); 1.22-1.21 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ: 152.9 (2C, C-3); 141.2 (C, C-4); 135.9 (C, C-7'a); 133.3 (C, C-1); 130.8 (C, C-5'); 130.0 (CH, C-2'); 128.3 (C, C-3'a); 122.9 (CH, C-6'); 121.2 (CH, C-4'); 109.2 (CH, C-7'); 105.2 (2CH, C-2); 101.2 (CH, C-3'); 66.9 (2CH₂, C-9''); 60.9 (CH₃, OCH₃); 58.9 (CH₂, C-1''); 56.3 (2CH₃, OCH₃); 53.7 (2CH₂, C-8''); 51.1 (CH₂, C-6''); 30.0 (CH₃, NCH₃); 28.1 (CH₂, C-5''); 26.9 (CH₂, C-4''); 26.3 (CH₂, C-2''); 26.2 (CH₂, C-3''). **HRMS** calculated for C₂₈H₄₀N₃O₆S⁺ 546.2632, found: 546.2626 (M+H⁺). **HPLC** (C18): t_R = 2.42 min, 99.5 %.

General synthetic procedure for hydrolysis of ester groups to carboxylic acids

The ester (1.0 eq) was dissolved in methanol or ethanol and crushed NaOH (2.0-4.0 eq) was added. The mixture was stirred at room temperature for 3 hours. After completion of the reaction, the solvent was evaporated, water was added and the compound was extracted with EtOAc at acidic pH. The organic phases were dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum.

4-((3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)phenyl)sulfonamido)butanoic acid (**29**)

Brown crystals (Hex/DCM); Yield, 61 %. **M. p.** 162-163 °C. **¹H-NMR (400 MHz, CDCl₃)** δ : 7.27 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.24 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.72 (6H, *s*, OCH₃); 3.64 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.50 (2H, *t*, *J* = 7.6 Hz, H-3''); 1.77-1.73 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ : 178.8 (C, CO); 152.9 (2C, C-3); 141.3 (C, C-4); 136.1 (C, C-7'a); 133.1 (C, C-1); 130.5 (C, C-5'); 130.1 (CH, C-2'); 128.5 (C, C-3'a); 122.7 (CH, C-6'); 121.2 (CH, C-4'); 109.5 (CH, C-7'); 105.2 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 50.3 (CH₂, C-1''); 33.0 (CH₃, NCH₃); 30.6 (CH₂, C-3''); 23.1 (CH₂, C-2''). **HRMS** calculated for C₂₂H₂₇N₂O₇S⁺ 463.1533, found: 463.1526 (M+H⁺). **HPLC** (C18): *t_R* = 6.97 min, 99.2 %.

5-((3,4,5-trimethoxy-*N*-(1-methyl-1*H*-indol-5-yl)phenyl)sulfonamido)pentanoic acid (**30**)

Soft brown crystals (Hex/DCM); Yield, 51 %. **M. p.** 123-124 °C. **¹H-NMR (400 MHz, CDCl₃)** δ : 7.27 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.25 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.07 (1H, *d*, *J* = 3.2 Hz, H-2'); 6.94 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 6.41 (1H, *d*, *J* = 3.2 Hz, H-3'); 3.90 (3H, *s*, OCH₃); 3.79 (3H, *s*, NCH₃); 3.72 (6H, *s*, OCH₃); 3.58 (2H, *t*, *J* = 6.8 Hz, H-1''); 2.32 (2H, *t*, *J* = 7.6 Hz, H-4''); 1.74-1.70 (2H, *m*, H-3''); 1.50-1.46 (2H, *m*, H-2''). **¹³C-NMR (100 MHz, CDCl₃)** δ : 178.8 (C, CO); 152.9 (2C, C-3); 141.2 (C, C-4); 136.0 (C, C-7'a); 133.1 (C, C-1); 130.6 (C, C-5'); 130.1 (CH, C-2'); 128.4 (C, C-3'a); 122.8 (CH, C-6'); 121.3 (CH, C-4'); 109.4 (CH, C-7'); 105.3 (2CH, C-2); 101.3 (CH, C-3'); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 50.6 (CH₂, C-1''); 33.3 (CH₂, C-4''); 33.0 (CH₃, NCH₃); 27.4 (CH₂, C-2''); 21.5 (CH₂, C-3''). **HRMS** calculated for C₂₃H₂₉N₂O₇S⁺ 477.1690, found: 477.1684 (M+H⁺). **HPLC** (C18): *t_R* = 7.90 min, 95.2 %.

General synthetic procedure for hydrolysis of the nitrile functional group to form an amide.

Upon a mixture of the corresponding nitrile (1.0 equiv) in tert-butanol, KO^tBu (3.0 equiv) is added, and the reaction is heated to reflux for 48-72 hours. After this period, the compound is isolated by precipitation at acidic pH followed by vacuum filtration of the solid.

1-methyl-5-((3,4,5-trimethoxy-*N*-methylphenyl)sulfonamido)-1*H*-indole-3-carboxamide (**39**)

Soft brown crystals (Hex/DCM); Yield, 20 %. **M. p.** 184-185 °C. **¹H-NMR (400 MHz, CDCl₃)** δ : 7.75 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.67 (1H, *s*, H-2'); 7.31 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.15 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.79 (2H, *s*, H-2); 5.58 (2H, *s*, CONH₂); 3.91 (3H, *s*, OCH₃); 3.83 (3H, *s*, NCH₃); 3.73 (6H, *s*, OCH₃); 3.25 (3H, *s*, H-1''). **¹³C-NMR (100 MHz, CDCl₃)** δ : 166.6 (C, CONH₂); 153.0 (2C, C-3); 141.6 (C, C-4); 136.3 (C, C-7'a); 135.9 (C, C-5'); 133.3 (CH, C-2'); 131.6 (C, C-1); 126.1 (C, C-3'a); 122.8 (CH, C-6'); 119.7 (CH, C-4'); 110.1 (CH, C-3'); 110.0 (C, C-3'); 105.5 (2CH, C-2); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 39.1 (CH₃, C-1''); 33.5 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₄N₃O₆S⁺ 434.1380, found: 434.1378 (M+H⁺). **HPLC** (C18): *t_R* = 3.84 min, 96.0 %.

1-methyl-5-((3,4,5-trimethoxyphenyl)sulfonamido)-1*H*-indole-3-carboxamide (**43**)

White powder; Yield, 61 %. **M.p.** 269-268 °C. **¹H-NMR (400 MHz, C₂H₆OS)** δ : 9.82 (1H, *s*, NH); 7.96 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.89 (1H, *s*, H-2'); 7.33 (1H, *d*, *J* = 9.2 Hz, H-7'); 6.99 (2H, *s*, H-2); 6.97 (1H, *dd*, *J* = 9.2; 2.0 Hz, H-6'); 3.70 (3H, *s*, OCH₃); 3.69 (6H, *s*, OCH₃); 3.62 (3H, *s*, NCH₃). **¹³C-NMR (100 MHz, C₂H₆OS)** δ : 167.2 (C, CO); 153.0 (2C, C-3); 140.8 (C, C-4); 134.9 (C, C-7'a); 134.4 (C, C-5); 133.7 (CH, C-2'); 131.1 (C, C-1); 126.9 (C, C-3'a); 118.3 (CH, C-6'); 115.1 (CH, C-4'); 111.1 (CH, C-7'); 108.8 (C, C-3'); 104.7 (2CH, C-2); 60.7 (CH₃, OCH₃); 56.5 (2CH₃, OCH₃); 33.4 (CH₃, NCH₃). **HRMS** calculated for C₁₉H₂₂N₃O₆S⁺ 420.1224, found: 420.1219 (M+H⁺); calculated for C₁₉H₂₁N₃NaO₆S⁺ 442.1043, found: 442.1038 (M+Na⁺). **HPLC** (C18): *t_R* = 2.55 min, 96.8 %.

Procedure for synthesis of 1-methyl-5-((3,4,5-trimethoxy-N-methylphenyl)sulfonamido)-1H-indole-3-carboxylic acid (40)

A solution of *N*-(3-formyl-1-methyl-1H-indol-5-yl)-3,4,5-trimethoxy-N-methylbenzenesulfonamide (**36**) (0.75 mmol, 1 eq) in ^tBuOH (20 mL) and 2-methyl-2-butene (10 mL) was poured onto a solution of NaClO₂ (15.16 mmol, 20.0 eq) and NaH₂PO₄·H₂O (9.85 mmol, 13.1 eq) in water (10 mL) at 4 °C. The mixture was stirred at room temperature under argon atmosphere for 48 hours. Then, the reaction was vacuum concentrated and extracted with dichloromethane washing the organic phases with brine. The organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated. The compound was purified by preparative chromatography obtaining 1-methyl-5-((3,4,5-trimethoxy-N-methylphenyl)sulfonamido)-1H-indole-3-carboxylic acid (**40**). Yield, 10 %.

Soft brown powder; Yield 10 %. **M. p.** 205-204 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 7.86 (1H, s, H-2'); 7.78 (1H, *d*, *J* = 2.0 Hz, H-4'); 7.35 (1H, *d*, *J* = 8.8 Hz, H-7'); 7.29 (1H, *dd*, *J* = 8.8; 2.0 Hz, H-6'); 6.82 (2H, s, H-2); 3.90 (3H, s, OCH₃); 3.87 (3H, s, NCH₃); 3.73 (6H, s, OCH₃); 3.26 (3H, s, H-1''). ¹³C-NMR (100 MHz, CDCl₃) δ: 169.7 (C, CO); 153.0 (2C, C-3); 141.6 (C, C-4); 137.4 (CH, C-2'); 136.8 (C, C-7'a); 136.4 (C, C-5'); 131.7 (C, C-1); 126.7 (C, C-3'a); 123.9 (CH, C-6'); 119.6 (CH, C-4'); 110.3 (CH, C-7'); 106.4 (C, C-3'); 105.5 (2CH, C-2); 61.0 (CH₃, OCH₃); 56.3 (2CH₃, OCH₃); 39.1 (CH₃, C-1''); 33.8 (CH₃, NCH₃). **HRMS** calculated for C₂₀H₂₃N₂O₇S⁺ 435.1220, found: 435.1217 (M+H⁺). **HPLC** (C18): t_R = 5.42 min, 96.1 %.

4.2. Biological evaluation

4.2.1 Cell culture

HeLa, RPE-1, HT-1080 and Hek-293 cell lines were cultured in DMEM culture medium containing 10 % heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin. MCF7, MDA-MB 468, MDA-MB 468-PR, BT474, A375, MelMet5, and WM1366 were grown in RPMI-1640 medium supplemented with 10 % FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. MES-SA and Caki-1 were cultured in McCoy's 5A culture medium supplemented with 10 % FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. Hermes 3C cells were cultured in 256 medium supplemented with 10 % FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin 200 nM phorbol-12-myristate-13-acetate (TPA), 10 ng/mL human stem cell factor (hSCF), 10 nM endothelin (EDN1) and 200 pM cholera toxin. All cell lines were grown in humidified environment at 37 °C and 5 % CO₂.

4.2.2. Antiproliferative activity

Antiproliferative studies against HeLa (human cervical carcinoma) cell line were performed using the MTT assay. Cells were seeded with the density of 1.5·10⁴ cells/mL in 96-well plates and were incubated for attachment for 24 hours at 37 °C in a humidified atmosphere with 5 % CO₂. Then, cells were treated with different concentrations of tested compounds or DMSO (0.05 %). After incubation in the same conditions for an additional 72 hours, 10 µL of MTT reagent dissolved in PBS (5 mg/mL) were added to each well. The plates were incubated for another 4 hours. The culture medium was removed and the MTT crystals were dissolved in 100 µL of DMSO per well. The intensity of the absorbance signal was measured at 570 nm. Each experiment was repeated, at least, three times and the IC₅₀ (50% inhibitory concentration) value was determined for each compound using GraphPad Prism 8 software using non-linear regression curves fitting the data. To study the sensitivity to MDR efflux pumps, the IC₅₀ values were determined using the identical methodology, but performing the assay in parallel with and without verapamil (final concentration of 10 µM).

4.2.3 Semi-automatic standardized drug-sensitivity assays

Semi-automatic standardized drug-sensitivity assays followed Ayuda-Duran *et al* settings.[28] 384-well plates with compounds were printed with five final concentrations ranging from 10^{-5} to 10^{-10} M in triplicates. Each plate included positive controls (benzethonium chloride) and negative controls (DMSO). Single-cell suspensions were seeded at a density of $2 \cdot 10^4$ - $1.5 \cdot 10^5$ cells/mL depending on the cell line using the CERTUS Flex liquid dispenser (Fritz Gyger, Thun, Switzerland). The plates were incubated at 37 °C for 72 h. Then, cell viability was measured using the CellTiterGlo 2.0 (Promega, Madison, WI, USA) luminescent assay according to the manufacturer's instructions. Luminescence was recorded with an EnVision Xcite 2105 Multimode Plate Reader. The response readout was normalized to the negative (DMSO) and positive (100 µM benzethonium chloride) controls.

4.2.4. Flow cytometry assay: cell cycle distribution and cell death analyses

MES-SA cells were seeded at a density of $3.3 \cdot 10^5$ cells/mL in a 6-well plate which was incubated for 24 h at 37 °C, 5% CO₂. Subsequently, the culture medium was removed, and fresh medium was added with the final concentration of treatments or DMSO (0.1 %). All the samples were performed in duplicates. After incubating for 24 hours, cells were detached and stained as follows, involving sequential steps of staining and washing with PBS. Samples were stained with Live/Dead Orange reagent (1 µL/mL in PBS) for 30 minutes at 4 °C, fixed with ice-cold methanol for 2 hours at -20 °C, stained with TUNEL kit prepared according to the manufacturer's instructions for 30 minutes at 37 °C, anti-Phospho-Histone H3 (1 µL/mL in 5 % non-fat dry milk PBS) at room temperature for 30 min in the dark, anti-Mouse Alexa488 (2 µL/mL in 5 % non-fat dry milk PBS) and Cy5 Conjugate Streptavidin (2.5 µL/mL in 5 % non-fat dry milk PBS) for 30 min in the dark at room temperature and finally, stained with Hoechst 33258 (1.5 µL/mL in PBS) at 4 °C in the dark overnight. Samples were analysed by flow cytometry with LSR II Yellow Laser Flow Cytometer and analysed using the software FloJo.

4.2.5. Apoptosis assay

MES-SA cells were seeded at a density of $5 \cdot 10^4$ cells/mL in 384-well plates with the reagent Incucyte® Caspase-3/7 Dye for Apoptosis Green solved in the culture medium (1:1000). After 24 hours of incubation at 37 °C, treatments were added in triplicates using staurosporine as positive control and DMSO as a negative control. The cells were incubated at 37 °C inside the Incucyte® S3 taking pictures every 24 hours with the x10 confocal objective in the green channel. The results were analysed with the proprietary Incucyte® software.

4.2.6 Confocal microscopy

MES-SA cells were grown on coverslips pretreated with 10 % poly-L-lysine, seeded at $2.5 \cdot 10^5$ cells/mL. After incubating for 24 hours, the culture medium was removed and fresh medium was added with the final concentration of treatments or DMSO (0.1 %). Vincristine was used as a positive control, and all the samples were performed in duplicates. The cells were incubated at 37 °C for 24 hours and fixed with 4 % formaldehyde in PEM buffer (80 mM PIPES, 5 mM EGTA, 1 mM MgCl₂, pH 6.8-7.0) for 10 min. The coverslips were washed three times with PEM buffer and permeabilized with 0.15 % Triton X-100 in PEM for 5 minutes. The samples were washed three times with PEM and stained independently with anti-α-tubulin (2 µL/mL in PEM) and anti-Mouse Alexa488 (2 µL/mL in PEM) + Phalloidin-CF568 (5 µL/mL in PEM). After 1 hour of incubation in the dark with the antibodies, the samples were washed three times with PEM. The coverslips were mounted on slides using ProLong Diamond Antifade Mountant with DAPI, which labels the cellular nuclei. The samples were stored at 4 °C in darkness for a minimum of 12 hours before reading the results with the ZEISS LSM 880 AiryScan confocal microscope.

4.2.7 Western Blot assays

MES-SA cells were seeded at a density of 10^6 cells/well in 6-well plates. After 24 hours, the culture medium was removed and fresh medium was added with the final concentration of treatments (100 nM). Staurosporine was used as a positive control, and DMSO and untreated samples were used as negative controls. DMSO % was the same for all the samples, kept at 0,05%, except on the untreated sample. The cells were incubated at 37 °C for 24 hours. Subsequently, cells were scraped and collected, pelleted by centrifugation and washed twice with PBS. Cells were lysed with RIPA buffer with PhosStop and Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) at 4 °C for 30 min. The supernatants were collected and measured for its protein concentrations with Bradford assay (Bio-Rad), and all the extracts were diluted to match the lowest concentration (0.6 µg/µl). Proteins (6.75 µg) were separated by 4–20% Mini-PROTEAN TGX Precast Protein Gel and transferred to nitrocellulose membranes. The membranes were washed with H₂O, stained with Ponceau, blocked in TBS-T 5% milk and incubated overnight at 4 °C with the corresponding antibodies (rabbit anti-PARP (Cell Signalling)). After incubation, the membranes were washed with TBS-T and incubated in the secondary antibody (Donkey anti-rabbit IRDye 680RD, Li-Cor Biosciences, 926-32212) and imaged in a Sapphire biomolecular imager (azure biosystems). After reading the membranes were stripped with Restore™ PLUS Western Blot Stripping Buffer (Thermo Fisher, 46430) according to vendor instructions, blocked in TBS-T milk 5%, and re-probed for actin (primary antibody, Anti-beta Actin, abcam, ab170325; secondary antibody IRDye 800CW Donkey Anti-Mouse, Li-Cor Biosciences, 926-32212).

4.2.8 ADME, pharmacokinetic and toxicity properties prediction

The study of pharmacokinetic properties was performed with the Swiss ADME web tool of the Swiss Institute of Bioinformatics (SIB).[51] This web page predicts the pharmacokinetic characteristics and Absorption, Distribution, Metabolism, and Excretion (ADME) parameters of the synthesized compounds. The molecular structures of the final compounds were converted into Simplified Molecular Input Line Entry System (SMILES) codes, and subsequent calculations were executed through submission to the online server.

Evaluation of toxicity parameters for the synthesized compounds was undertaken utilizing the program Nexus by two different software; Derek Nexus,[52] based on mathematical models to determine general toxicity and Sarah Nexus,[53] founded on statistical information to evaluate the mutagenicity.

4.2.9. Molecular docking studies

Structural preferences for the compounds were calculated by means of RB3LYP DFT calculations at the 6-31G(D) level with Spartan.[54] The compounds were subjected to conformational searches at the molecular mechanics (MMFF force field) level followed by energy minimizations with RB3LYP DFT calculations at the 6-31G(D) level and the lowest energy configurations amongst all the possible outcomes selected as the most stable forms for every compound.

We performed ensemble docking on different tubulin-ligand complexes' models to sample the protein conformational space.[29, 43] Briefly, all the different complexes of tubulin with colchicine site ligands were retrieved from the pdb,[55] the ligands were removed and the waters in the binding site were either completely removed or conserved in different models. 5 representative structures from a previous molecular dynamics simulation run on a tubulin – podophyllotoxin complex were also used, giving a total of 153 tubulin models. The compounds were docked in parallel into their colchicine sites using AutoDock 4.2 with the Lamarckian genetic algorithm (LGA) 100–300 times for a maximum of 2.5 million energy evaluations, 150 individuals,

and a maximum of 27000 generations and with PLANTS with default settings and 10 runs per ligand. For ligands with different possible configurations (protonation states or amide rotamers) we run every one and selected as the docking result for every ligand the best scored one amongst all the configurations. We converted the scores of the different programs into relative scales and calculated the Z-scores so that the different scoring scales could be easily compared. We selected as the docking results the common poses for the two programs with the best consensus Z-scores. For every pose, we automatically assigned them to the colchicine subzones using in-house KNIME pipelines.[56] The RMSD between every pose and model scaffolds with no substituents and with colchicine binding site ligands representative of binders occupying different subzones were calculated with LigRMSD.[57] Docked poses were analyzed with Chimera,[58] Marvin,[59] OpenEye,[60] and JADOPPT.[61]

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Appendix A. Supplementary data

References

- [1] C. Dumontet, M.A. Jordan, Microtubule-binding agents: a dynamic field of cancer therapeutics, *Nat Rev Drug Discov*, 9 (2010) 790-803. doi: 10.1038/nrd3253
- [2] L. Chen, Y. Hu, Z. Lu, Z. Lin, L. Li, J.Q. Wu, Z.L. Yu, C. Wang, W.H. Chen, J. Hu, Design, Synthesis, and Antitumor Efficacy of Substituted 2-Amino[1,2,4]triazolopyrimidines and Related Heterocycles as Dual Inhibitors for Microtubule Polymerization and Janus Kinase 2, *J Med Chem*, 66 (2023) 15006-15024. doi: 10.1021/acs.jmedchem.3c01690
- [3] M. Barreca, V. Spano, R. Rocca, R. Bivacqua, G. Gualtieri, M.V. Raimondi, E. Gaudio, R. Bortolozzi, L. Manfreda, R. Bai, A. Montalbano, S. Alcaro, E. Hamel, F. Bertoni, G. Viola, P. Barraja, Identification of pyrrolo[3',4':3,4]cyclohepta[1,2-d][1,2]oxazoles as promising new candidates for the treatment of lymphomas, *Eur J Med Chem*, 254 (2023) 115372. doi: 10.1016/j.ejmech.2023.115372
- [4] P. Barraja, L. Caracausi, P. Diana, V. Spano, A. Montalbano, A. Carbone, B. Parrino, G. Cirrincione, Synthesis and antiproliferative activity of the ring system [1,2]oxazolo[4,5-g]indole, *ChemMedChem*, 7 (2012) 1901-1904. doi: 10.1002/cmdc.201200296
- [5] A.C. Henriques, D. Ribeiro, J. Pedrosa, B. Sarmiento, P.M.A. Silva, H. Bousbaa, Mitosis inhibitors in anticancer therapy: When blocking the exit becomes a solution, *Cancer Lett*, 440-441 (2019) 64-81. doi: 10.1016/j.canlet.2018.10.005

- [6] A. Vicente-Blazquez, M. Gonzalez, R. Alvarez, S. Del Mazo, M. Medarde, R. Pelaez, Antitubulin sulfonamides: The successful combination of an established drug class and a multifaceted target, *Med Res Rev*, 39 (2019) 775-830. doi: 10.1002/med.21541
- [7] S. Eli, R. Castagna, M. Mapelli, E. Parisini, Recent Approaches to the Identification of Novel Microtubule-Targeting Agents, *Front Mol Biosci*, 9 (2022) 841777. doi: 10.3389/fmolb.2022.841777
- [8] R.J. van Vuuren, M.H. Visagie, A.E. Theron, A.M. Joubert, Antimitotic drugs in the treatment of cancer, *Cancer Chemother Pharmacol*, 76 (2015) 1101-1112. doi: 10.1007/s00280-015-2903-8
- [9] E.C. McLoughlin, N.M. O'Boyle, Colchicine-Binding Site Inhibitors from Chemistry to Clinic: A Review, *Pharmaceuticals (Basel)*, 13 (2020). doi: 10.3390/ph13010008
- [10] L.M. Nainwal, M.M. Alam, M. Shaquiquzzaman, A. Marella, A. Kamal, Combretastatin-based compounds with therapeutic characteristics: a patent review, *Expert Opin Ther Pat*, 29 (2019) 703-731. doi: 10.1080/13543776.2019.1651841
- [11] R. Alvarez, C. Gajate, P. Puebla, F. Mollinedo, M. Medarde, R. Pelaez, Substitution at the indole 3 position yields highly potent indolecombretastatins against human tumor cells, *Eur J Med Chem*, 158 (2018) 167-183. doi: 10.1016/j.ejmech.2018.08.078
- [12] A.B. Sanchez Maya, C. Perez-Melero, N. Salvador, R. Pelaez, E. Caballero, M. Medarde, New naphthylcombretastatins. Modifications on the ethylene bridge, *Bioorg Med Chem*, 13 (2005) 2097-2107. doi: 10.1016/j.bmc.2005.01.012
- [13] A.B. Maya, C. Perez-Melero, C. Mateo, D. Alonso, J.L. Fernandez, C. Gajate, F. Mollinedo, R. Pelaez, E. Caballero, M. Medarde, Further naphthylcombretastatins. An investigation on the role of the naphthalene moiety, *J Med Chem*, 48 (2005) 556-568. doi: 10.1021/jm0310737
- [14] R. Alvarez, P. Puebla, J.F. Diaz, A.C. Bento, R. Garcia-Navas, J. de la Iglesia-Vicente, F. Mollinedo, J.M. Andreu, M. Medarde, R. Pelaez, Endowing indole-based tubulin inhibitors with an anchor for derivatization: highly potent 3-substituted indolephenstatins and indoleisocombretastatins, *J Med Chem*, 56 (2013) 2813-2827. doi: 10.1021/jm3015603
- [15] S. Pecnard, A. Hamze, J. Bignon, B. Prost, A. Deroussent, L. Gallego-Yerga, R. Pelaez, J.Y. Paik, M. Diederich, M. Alami, O. Provot, Anticancer properties of indole derivatives as IsoCombretastatin A-4 analogues, *Eur J Med Chem*, 223 (2021) 113656. doi: 10.1016/j.ejmech.2021.113656
- [16] C. Alvarez, R. Alvarez, P. Corchete, C. Perez-Melero, R. Pelaez, M. Medarde, Naphthylphenstatins as tubulin ligands: synthesis and biological evaluation, *Bioorg Med Chem*, 16 (2008) 8999-9008. doi: 10.1016/j.bmc.2008.08.040
- [17] C. Alvarez, R. Alvarez, P. Corchete, J.L. Lopez, C. Perez-Melero, R. Pelaez, M. Medarde, Diarylmethyloxime and hydrazone derivatives with 5-indolyl moieties as potent inhibitors of tubulin polymerization, *Bioorg Med Chem*, 16 (2008) 5952-5961. doi: 10.1016/j.bmc.2008.04.054
- [18] A. Vicente-Blazquez, M. Gonzalez, M. Medarde, F. Mollinedo, R. Pelaez, New indolesulfonamide derivatives targeting the colchicine site of tubulin: synthesis, anti-tumour activity, structure-activity relationships, and molecular modelling, *J Enzyme Inhib Med Chem*, 36 (2021) 2025-2044. doi: 10.1080/14756366.2021.1975277
- [19] J.A. Segreti, K.A. Brooks, K.C. Marsh, J.L. Bauch, Y. Lan, C. Thomas Lin, S.H. Rosenberg, H.L. Sham, G.A. Reinhart, B.F. Cox, J.S. Polakowski, Tumour-selective antivascular effects of the novel anti-mitotic compound A-318315: An in vivo rat regional haemodynamic study, *Clin Exp Pharmacol Physiol*, 37 (2010) 636-640. doi: 10.1111/j.1440-1681.2010.05365.x
- [20] S.L. Gwaltney, 2nd, H.M. Imade, K.J. Barr, Q. Li, L. Gehrke, R.B. Credo, R.B. Warner, J.Y. Lee, P. Kovar, J. Wang, M.A. Nukkala, N.A. Zielinski, D. Frost, S.C. Ng, H.L. Sham, Novel sulfonate analogues of combretastatin A-4: potent antimitotic agents, *Bioorg Med Chem Lett*, 11 (2001) 871-874. doi: 10.1016/s0960-894x(01)00098-1

- [21] C. Alvarez, R. Alvarez, P. Corchete, C. Perez-Melero, R. Pelaez, M. Medarde, Exploring the effect of 2,3,4-trimethoxy-phenyl moiety as a component of indolephenstatins, *Eur J Med Chem*, 45 (2010) 588-597. doi: 10.1016/j.ejmech.2009.10.047
- [22] G.K. Prakash, T. Mathew, C. Panja, G.A. Olah, Chlorotrimethylsilane-nitrate salts as oxidants: direct oxidative conversion of thiols and disulfides to sulfonyl chlorides, *J Org Chem*, 72 (2007) 5847-5850. doi: 10.1021/jo070907g
- [23] L. Llauger, H. He, J. Kim, J. Aguirre, N. Rosen, U. Peters, P. Davies, G. Chiosis, Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90, *J Med Chem*, 48 (2005) 2892-2905. doi: 10.1021/jm049012b
- [24] M. Gonzalez, M. Ovejero-Sanchez, A. Vicente-Blazquez, M. Medarde, R. Gonzalez-Sarmiento, R. Pelaez, Methoxy and bromo scans on N-(5-methoxyphenyl) methoxybenzenesulphonamides reveal potent cytotoxic compounds, especially against the human breast adenocarcinoma MCF7 cell line, *J Enzyme Inhib Med Chem*, 36 (2021) 1029-1047. doi: 10.1080/14756366.2021.1925265
- [25] M. Gonzalez, M. Ovejero-Sanchez, A. Vicente-Blazquez, R. Alvarez, A.B. Herrero, M. Medarde, R. Gonzalez-Sarmiento, R. Pelaez, Microtubule Destabilizing Sulfonamides as an Alternative to Taxane-Based Chemotherapy, *Int J Mol Sci*, 22 (2021). doi: 10.3390/ijms22041907
- [26] R.A.M. T.L. Riss, A.L. Niles, S. Duellman, H.A. Benink, T.J. Worzella, L. Minor, Cell Viability Assays, in: G.A. Markossian S, Arkin M, et al. (Ed.) *The Assay Guidance Manual*, Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD), 2013.
- [27] T. Naret, I. Khelifi, O. Provot, J. Bignon, H. Levaique, J. Dubois, M. Souce, A. Kasselouri, A. Deroussent, A. Paci, P.F. Varela, B. Gigant, M. Alami, A. Hamze, 1,1-Diheterocyclic Ethylenes Derived from Quinaldine and Carbazole as New Tubulin-Polymerization Inhibitors: Synthesis, Metabolism, and Biological Evaluation, *J Med Chem*, 62 (2019) 1902-1916. doi: 10.1021/acs.jmedchem.8b01386
- [28] P. Ayuda-Duran, J.U. Hermansen, M. Giliberto, Y. Yin, R. Hanes, S. Gordon, H. Kuusanmaki, A.M. Brodersen, A.N. Andersen, K. Tasken, K. Wennerberg, J.M. Enserink, S.S. Skanland, Standardized assays to monitor drug sensitivity in hematologic cancers, *Cell Death Discov*, 9 (2023) 435. doi: 10.1038/s41420-023-01722-5
- [29] M. Gonzalez, Y. Ellahioui, L. Gallego, A. Vicente-Blazquez, R. Alvarez, M. Medarde, R. Pelaez, Novel amino analogs of the trimethoxyphenyl ring in potent colchicine site ligands improve solubility by the masked polar group incorporation (MPGI) strategy, *Bioorg Chem*, 131 (2023) 106282. doi: 10.1016/j.bioorg.2022.106282
- [30] M. Ovejero-Sanchez, G. Asensio-Juarez, M. Gonzalez, P. Puebla, M. Vicente-Manzanares, R. Pelaez, R. Gonzalez-Sarmiento, A.B. Herrero, Panobinostat Synergistically Enhances the Cytotoxicity of Microtubule Destabilizing Drugs in Ovarian Cancer Cells, *Int J Mol Sci*, 23 (2022). doi: 10.3390/ijms232113019
- [31] A. Rogalska, M. Szwed, B. Rychlik, The connection between the toxicity of anthracyclines and their ability to modulate the P-glycoprotein-mediated transport in A549, HepG2, and MCF-7 cells, *ScientificWorldJournal*, 2014 (2014) 819548. doi: 10.1155/2014/819548
- [32] S. Merry, E.R. Courtney, C.A. Fetherston, S.B. Kaye, R.I. Freshney, Circumvention of drug resistance in human non-small cell lung cancer in vitro by verapamil, *Br J Cancer*, 56 (1987) 401-405. doi: 10.1038/bjc.1987.214
- [33] R. Bernander, T. Stokke, E. Boye, Flow cytometry of bacterial cells: comparison between different flow cytometers and different DNA stains, *Cytometry*, 31 (1998) 29-36. doi: 10.1002/(sici)1097-0320(19980101)31:1<29::aid-cyto4>3.0.co;2-e
- [34] Z. Darzynkiewicz, X. Huang, H. Zhao, Analysis of Cellular DNA Content by Flow Cytometry, *Curr Protoc Immunol*, 119 (2017) 5 7 1-5 7 20. doi: 10.1002/cpim.36
- [35] K.H. Kim, J.M. Sederstrom, Assaying Cell Cycle Status Using Flow Cytometry, *Curr Protoc Mol Biol*, 111 (2015) 28 26 21-28 26 11. doi: 10.1002/0471142727.mb2806s111
- [36] J.Y. Kim, H.S. Jeong, T. Chung, M. Kim, J.H. Lee, W.H. Jung, J.S. Koo, The value of phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers: A

- comparative study with Ki67, Oncotarget, 8 (2017) 65064-65076. doi: 10.18632/oncotarget.17775
- [37] K. Kyrylkova, S. Kyryachenko, M. Leid, C. Kioussi, Detection of apoptosis by TUNEL assay, *Methods Mol Biol*, 887 (2012) 41-47. doi: 10.1007/978-1-61779-860-3_5
- [38] R. Wieder, TUNEL assay as a measure of chemotherapy-induced apoptosis, *Methods Mol Med*, 111 (2005) 43-54. doi: 10.1385/1-59259-889-7:043
- [39] K.J. Kelly, R.M. Sandoval, K.W. Dunn, B.A. Molitoris, P.C. Dagher, A novel method to determine specificity and sensitivity of the TUNEL reaction in the quantitation of apoptosis, *Am J Physiol Cell Physiol*, 284 (2003) C1309-1318. doi: 10.1152/ajpcell.00353.2002
- [40] K.M. Hanson, J.N. Finkelstein, An accessible and high-throughput strategy of continuously monitoring apoptosis by fluorescent detection of caspase activation, *Anal Biochem*, 564-565 (2019) 96-101. doi: 10.1016/j.ab.2018.10.022
- [41] T. Hohmann, F. Dehghani, The Cytoskeleton-A Complex Interacting Meshwork, *Cells*, 8 (2019). doi: 10.3390/cells8040362
- [42] J.D. Hughes, J. Blagg, D.A. Price, S. Bailey, G.A. Decrescenzo, R.V. Devraj, E. Ellsworth, Y.M. Fobian, M.E. Gibbs, R.W. Gilles, N. Greene, E. Huang, T. Krieger-Burke, J. Loesel, T. Wager, L. Whiteley, Y. Zhang, Physiochemical drug properties associated with in vivo toxicological outcomes, *Bioorg Med Chem Lett*, 18 (2008) 4872-4875. doi: 10.1016/j.bmcl.2008.07.071
- [43] L. Gallego-Yerga, R. Ochoa, I. Lans, C. Pena-Varas, M. Alegria-Arcos, P. Cossio, D. Ramirez, R. Pelaez, Application of ensemble pharmacophore-based virtual screening to the discovery of novel antimitotic tubulin inhibitors, *Comput Struct Biotechnol J*, 19 (2021) 4360-4372. doi: 10.1016/j.csbj.2021.07.039
- [44] R. Alvarez, M. Medarde, R. Pelaez, New ligands of the tubulin colchicine site based on X-ray structures, *Curr Top Med Chem*, 14 (2014) 2231-2252. doi: 10.2174/1568026614666141130092637
- [45] A. Massarotti, A. Coluccia, R. Silvestri, G. Sorba, A. Brancale, The tubulin colchicine domain: a molecular modeling perspective, *ChemMedChem*, 7 (2012) 33-42. doi: 10.1002/cmdc.201100361
- [46] O. Korb, T. Stutzle, T.E. Exner, Empirical scoring functions for advanced protein-ligand docking with PLANTS, *J Chem Inf Model*, 49 (2009) 84-96. doi: 10.1021/ci800298z
- [47] S. Forli, R. Huey, M.E. Pique, M.F. Sanner, D.S. Goodsell, A.J. Olson, Computational protein-ligand docking and virtual drug screening with the AutoDock suite, *Nat Protoc*, 11 (2016) 905-919. doi: 10.1038/nprot.2016.051
- [48] C. Jimenez, Y. Ellahoui, R. Alvarez, L. Aramburu, A. Riesco, M. Gonzalez, A. Vicente, A. Dahdouh, A. Ibn Mansour, C. Jimenez, D. Martin, R.G. Sarmiento, M. Medarde, E. Caballero, R. Pelaez, Exploring the size adaptability of the B ring binding zone of the colchicine site of tubulin with para-nitrogen substituted isocombretastatins, *Eur J Med Chem*, 100 (2015) 210-222. doi: 10.1016/j.ejmech.2015.05.047
- [49] L. Gallego-Yerga, A.J. Chilibingua, R. Pelaez, Novel Tetrazole Derivatives Targeting Tubulin Endowed with Antiproliferative Activity against Glioblastoma Cells, *Int J Mol Sci*, 24 (2023). doi: 10.3390/ijms241311093
- [50] L. Gallego-Yerga, V. Cena, R. Pelaez, Potent and Selective Benzothiazole-Based Antimitotics with Improved Water Solubility: Design, Synthesis, and Evaluation as Novel Anticancer Agents, *Pharmaceutics*, 15 (2023). doi: 10.3390/pharmaceutics15061698
- [51] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Sci Rep*, 7 (2017) 42717. doi: 10.1038/srep42717
- [52] Derek Nexus KB 1.0, in, 2023.
- [53] Sarah Nexus, in, 2023.
- [54] Spartan, in, 2008.

- [55] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, <http://www.rcsb.org/> The Protein Data Bank, *Nucleic Acids Res*, 28 (2000) 235-242. doi: 10.1093/nar/28.1.235
- [56] Berthold MR, Cebron N, Dill F, e. al., KNIME: The Konstanz Information Miner. , in: *Studies in Classification, Data Analysis, and Knowledge Organization.*, Springer Berlin, Berlin, 2007, pp. 319-326
- [57] J.L. Velazquez-Libera, F. Duran-Verdugo, A. Valdes-Jimenez, G. Nunez-Vivanco, J. Caballero, LigRMSD: a web server for automatic structure matching and RMSD calculations among identical and similar compounds in protein-ligand docking, *Bioinformatics*, 36 (2020) 2912-2914. doi: 10.1093/bioinformatics/btaa018
- [58] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis, *J Comput Chem*, 25 (2004) 1605-1612. doi: 10.1002/jcc.20084
- [59] Marvin 17.8 in, ChemAxon (<http://www.chemaxon.com>), 2017.
- [60] <https://www.eyesopen.com/>, in.
- [61] C. Garcia-Perez, R. Pelaez, R. Theron, J. Luis Lopez-Perez, JADOPPT: java based AutoDock preparing and processing tool, *Bioinformatics*, 33 (2017) 583-585. doi: 10.1093/bioinformatics/btw677

Highlights

- *N*-indolyl-3,4,5-trimethoxybenzenesulfonamides are potent anti-mitotics.
- Different tumor cell-lines show different sensitivities.
- compounds cause G₂/M cell cycle arrest and induction of apoptosis.
- The substituent on the sulfonamide determines mechanism and cell fate.
- Binding at the colchicine site of tubulin.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.