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Full Paper

Synthesis and Antitumor Activity of Some Substituted Indazole Derivatives

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Some new *N*-[6-indazolyl]arylsulfonamides and *N*-[alkoxy-6-indazolyl]arylsulfonamides were prepared by the reduction of 2-alkyl-6-nitroindazoles with $SnCl_2$ in different alcohols, followed by coupling the corresponding amine with arylsulfonyl chlorides in pyridine. The newly synthesized compounds were evaluated for their antiproliferative and apoptotic activities against two human tumor cell lines: A2780 (ovarian carcinoma) and A549 (lung adenocarcinoma). Preliminary *in vitro* pharmacological studies revealed that *N*-(2-allyl-2*H*-indazol-6-yl)-4-methoxybenzenesulfonamide **4** and *N*-[7-ethoxy-2-(4-methylbenzyl)-2*H*-indazol-6-yl]-4-methyl-benzenesulfonamide **9** exhibited significant antiproliferative activity against the A2780 and A549 cell lines with IC_{50} values in the range from 4.21 to 18.6 μM, and also that they trigger apoptosis in a dose-dependent manner. Furthermore, both active compounds were able to cause an arrest of cells in the G2/M phase of the cell cycle, typical but not exclusive of tubulin interacting agents, although only infrequent interactions with the microtubule network were observed by immunofluorescence microscopy, while docking analysis showed a possible different behavior between the two active compounds.

Keywords: 2-Alkyl-6-nitroindazoles / Antiproliferative activity / Apoptosis / *N*-[6-Indazolyl]arylsulfonamides / $SnCl_2$/alcohols

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Introduction

The indazole ring is one of the intensively exploited templates in the medicinal chemistry. Different aspects of pharmaceutical and other useful applications of indazoles have been reviewed [1, 2]. Some substituted indazoles exhibit relevant biological properties for development as anticancer drugs [2–6]. Previously, we identified a series of indazoles bearing a sulfonamide moiety with good antiproliferative activities [7, 8]. In fact, some of the compounds exerted a pharmacologically interesting antiproliferative/apoptotic activity against human (DU145, HCT116, HT29, A2780, and A549) and murine (L1210 and P388) cell lines. 4-Methoxy-*N*-methyl-*N*-(3-chloro-7-indazolyl)benzene sulfonamide **A** and *N*-(3-chloro-1*H*-4-indazolyl)-4-methoxy-benzenesulfonamide **B** were the most active compounds of the series of indazoles (Fig. 1). Although with different characteristics some compounds induced a block of cells in the G2/M phase of the cell

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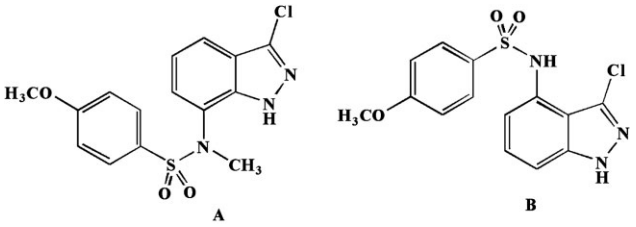


Figure 1. 4-Methoxy-*N*-methyl-*N*-(3-chloro-7-indazolyl)benzenesulfonamide **A** and *N*-(3-chloro-1*H*-4-indazolyl)-4-methoxy-benzenesulfonamide **B**.

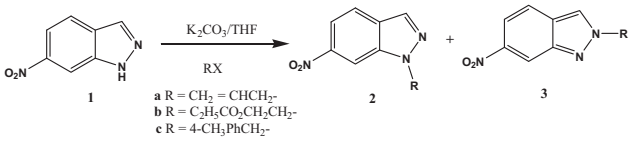
cycle and generate cells with an abnormal number of chromosomes, phenomena that are typical, although not exclusively, of tubulin interacting agents.

These findings together with docking results suggested that substitution of indazole at different positions may generate good lead compounds for further studies aimed at the synthesis of new and more active antitumor agents. Thus, as an extension of these results, in the present work, we investigated the synthesis of new series of *N*-(6-indazolyl)-benzenesulfonamide derivatives and evaluated their antiproliferative and apoptotic potential.

Results and discussion

Chemistry

We reported previously the reduction of some nitroindazoles, with anhydrous $SnCl_2$ in various alcohols, in the presence of arylsulfonyl chlorides and pyridine [8–11]. Thus, we observed a new kind of transformation: 4-, 6-, and 7-nitroindazoles, by the action of $SnCl_2$/EtOH, gave 4- or 7-ethoxy aminoindazoles, probably via an oxidative nucleophilic substitution of hydrogen (ONSH), along with the expected 4-, 6-, and 7-aminoindazoles. In view of all these facts, we continued our studies on the reactions of the reduction of aromatic nitro compounds with anhydrous $SnCl_2$ in alcohol. Here, we report the results of our more detailed study on the reduction of the nitro group in 2-alkyl-6-nitroindazoles **3a–c** by anhydrous $SnCl_2$ in different alcohols followed by coupling of the corresponding amine with arylsulfonyl chlorides. The *N*-alkyl-6-nitroindazoles **2a–c** and **3a–c** have been synthesized starting from alkylation of 6-nitroindazole **1** according to a previously described procedure [11] (Scheme 1).



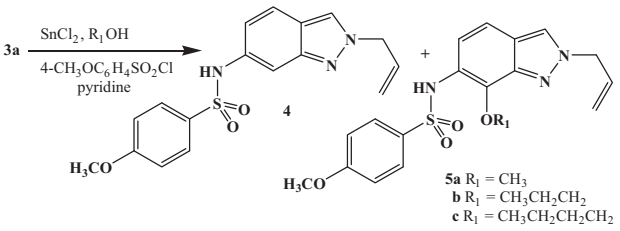
Scheme 1. Synthesis of 1-alkyl-6-nitroindazoles **2a–c** and 2-alkyl-6-nitroindazoles **3a–c**.

The reduction of 2-allyl-6-nitroindazole **3a** with $SnCl_2$ in different alcohols, followed by coupling of the obtained amines with 4-methoxybenzenesulfonyl chlorides in pyridine gave a mixture of two sulfonamides **4** and **5** in good combined yields (82–90%; Scheme 2 and Table 1). This result indicates that alkylation of 6-nitroindazole at position 2 plays an important role in the orientation of the nucleophilic substitution of alkoxy group in position 7 of indazole; in all cases we obtained a mixture of two compounds: the expected *N*-(2-allyl-2*H*-indazol-6-yl)-4-methoxybenzenesulfonamide **4** and the unexpected *N*-(2-allyl-7-alkoxy-2*H*-indazol-6-yl)-4-methoxy-benzenesulfonamides **5a–c**. In our previous work, the reduction of 1-allyl-6-nitroindazole with $SnCl_2$ in ethanol gave only aminoindazole [11]. No trace of nucleophilic substitution of the ethoxy group at position 7 of indazole was observed.

The structures of products **4** and **5a–c** were determined from their ^{1}H NMR and ^{13}C NMR spectra and by MS.

A plausible mechanism was proposed to explain the introduction of alkoxy group at position 7 of indazole **5**. First, the usual partial reduction of the nitro group generates the corresponding hydroxylamine. Hence, we assume that the nucleophilic attack of alcohol present in the reaction mixture occurs via the lone oxygen pair and leads to the intermediate **D**. Aromatization and coupling of the corresponding amine gives the product **5** (Scheme 3). It is noteworthy that reduction of aromatic nitro compounds with metals in protic solvents such as alcohols is usually presumed to proceed by way of hydroxylamine-like intermediates [12].

When we investigated the same conditions for 2-alkyl-6-nitroindazoles **3b** and **3c** in ethanol, we obtained a mixture of

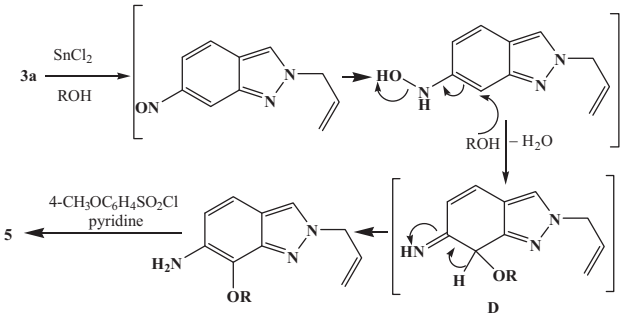


Scheme 2. Reduction of compound **3a** with $SnCl_2$ in ROH and protection with 4-methoxybenzenesulfonyl chloride.

Table 1. Reduction of **4** in different alcohols and protection with 4-methoxybenzenesulfonyl chloride in pyridine.

Entry	R_1	Yield of **4**[a)] (%)	Yield of **5**[a)] (%)	Yield of **4** + **5** (%)
1	CH_3	46	42 (**5a**)	88
2	$CH_3(CH_2)_2$	50	32 (**5b**)	82
3	$CH_3(CH_2)_3$	45	45 (**5c**)	90

[a)] Isolated yields over the two steps after separation by flash chromatography.



Scheme 3. Proposed mechanism for the formation of **5a–c**.

two compounds: 6-aminoindazole and 7-ethoxy-6-aminoindazole, which were immediately coupled by arylsulfonyl chloride in pyridine (Scheme 4). In this case, it is clear that SNH is improved by *N*-alkylation of the N-2 position of indazole.

The structural assignments of compounds **6–9** were based on the analytical and spectral data. The structure of compound **7** was unambiguously proven by single crystal X-ray analysis [13]. These data confirm the fixation of alkoxy group at position 7 of indazole (Fig. 2).

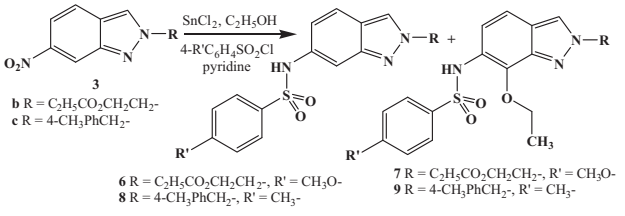
These results show that position of nitro group and alkylation of 6-nitroindazole at position 2 play an important role in the introduction of the alkoxy group in 7-position of indazole.

Biological activity

Inhibition of cell proliferation and apoptosis triggering

The study of inhibition of cell proliferation obtained by our *N*-[6-indazolyl]arylsulfonamides showed that the products **4** and **9** were significantly active. They, in fact, had, for both cell lines, IC_{50} values that fell in the micromolar range of concentrations (Table 2).

Furthermore, once we considered the activation of apoptotic cell death, the active compounds **4** and **9** displayed a significant activity (Table 3) on both A2780 and A549 cells treated with the specific IC_{50}, IC_{75}, or with 100 μM concentrations. In particular, compound **4** was highly active in human lung carcinoma A549 cells where it reached



Scheme 4. Reduction of compounds **3b**,**c** and protection with arylsulfonyl chloride.

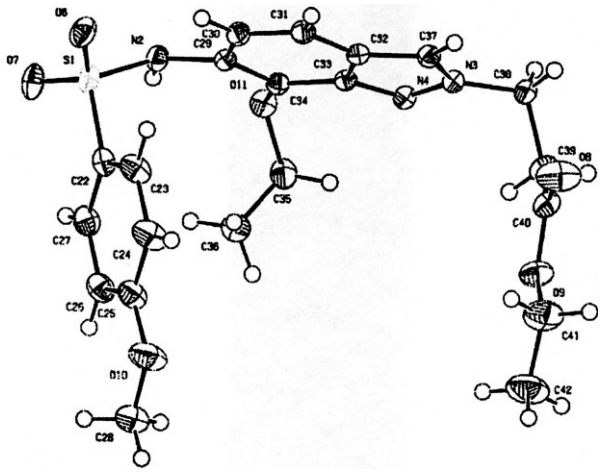


Figure 2. ORTEP drawing of **7**, showing the molecular numbering scheme. Ellipsoids are drawn at the 30% probability level.

about 36.0 ± 9.7% (23.5 ± 4.0% annexin$^+$/PI$^-$ and 12.5 ± 4.6% annexin$^+$/PI$^+$ cells) of apoptotic cells when administered at its IC_{50}.

In summary, these results showed that *N*-[6-indazolyl]-arylsulfonamides **4** and **9** had pharmacologically significant antiproliferative and apoptotic activities ranging in the micromolar field of concentrations.

Cell cycle analysis

As shown in Table 4, compound **9** caused an accumulation of A2780 cells in the S and G2/M cell cycle phases, when administered at both its IC_{50} and 100 μM. This effect was accompanied by a decrease of cells in the G0/G1 phase. When administered at its IC_{50} and compared to compound **9**, also the compound **4** caused a less evident increase of cells in the G2/M cell cycle phase with a corresponding loss of cells in the G0/G1. Differently, when administered at 100 μM, compound

Table 2. *In vitro* antiproliferative activity of new *N*-[6-indazolyl]-arylsulfonamides against A2780 and A549 tumor cell lines.

Compounds	Mean IC_{50} ± SD[a)]	
	A2780	A549
4	4.21 ± 1.19	18.6 ± 3.5
5a	>100	>100
5b	40.2 ± 8.6	45.2 ± 14.5
5c	75.6 ± 3.5	>100
6	111.6 ± 6.3	109.3 ± 3.7
7	>100	>100
8	69.7 ± 6.5	95.7 ± 8.9
9	5.47 ± 1.45	7.73 ± 1.66

[a)]The values represent the mean ± SD (μM) of three to four data. Analysis of curves was made by the Cricket Graph III software.

Table 3. *In vitro* apoptotic activity of active compounds against A2780 and A549 tumor cell lines.

	A2780		
Compounds	**CTR[a)]**	**IC_{50}[b)]**	**IC_{75}**
4	4.7 ± 2.6[c)]	22.0 ± 7.6	38.8 ± 11.9
9		19.3 ± 7.1	32.2 ± 13.7
	A549		
	CTR	**IC_{50}**	**100 μM**
4	4.2 ± 1.7	36.0 ± 9.7	44.7 ± 6.3
9		13.3 ± 2.4	21.9 ± 4.4

[a)] CTRs are the mean ± SD of control cells treated with both 0.6 and 1.2% DMSO. This was justified by the fact that no significant differences were found in terms of apoptotic death using the two different DMSO concentrations.
[b)] Applied concentrations, as determined by the MTT assay.
[c)] The mean ± SD ($N = 4–7$) represents the sum of early and late apoptotic cells (annexin$^+$/PI$^-$ and annexin$^+$/PI$^+$ cells, respectively), as determined by annexin V/PI staining.

4 caused a great cell accumulation in the G2/M phase with the additional generation of cell subpopulations with a number of chromosomes >4n and a corresponding loss of cells in the G0/G1 cell cycle phase.

In aneuploid A549 cells [14], compound **4** caused a great accumulation of cells in the G2/M cell cycle phase with a significant decrease of cells in the S and G0/G1 phases. In contrast, compound **9** caused a significant accumulation of A549 cells in the G0/G1 cell cycle phase when administered at its IC_{50} while, when used at 100 μM, it generated an increase of the G2/M peak with a concomitant decrease of cells above all in the G0/G1 cell cycle phase.

Immunofluorescence microscopy

In order to verify whether the arrest in the G2/M phase of the cell cycle and the generation of cells with a number of chromosomes >4n might depend on the binding of our *N*-[6-indazolyl]arylsulfonamides to microtubules, we verified the effect of these compounds on the microtubule network of A549 cells by immunofluorescence microscopy. Our images (Fig. 3) demonstrated that both **4** and **9** may alter the normal structure of the microtubule network similarly to the antimicrotubule agent vincristine, with the appearance of "dots" representing tubulin micro-aggregates. Nevertheless, this behavior was observed only in a relatively low number of non-apoptotic cells treated with 100 μM of compounds **4** and **9** ($10.7 \pm 09\%$, and 4.3 ± 0.9, respectively), while the remaining non-apoptotic cells showed a nearly normal architecture of microtubules. In similar culture conditions, vincristine (75 nM) caused $18.7 \pm 2.5\%$ cells presenting the same microtubule defects. It must be underlined that the compounds and drug concentrations used caused a comparable inhibition of cell proliferation ranging, on average, from 54 to 66%. Our observations seem to address toward a mechanism of action similar to that of the *N*-(7(4)-indazolyl)-4-methoxybenzenesulfonamides parent compounds, although the much lower concentrations applied to obtain a significant inhibition of cell proliferation (IC_{50}) do not exclude the possibility of other, still unknown, mechanisms of action.

Molecular docking

The docking procedure described in the Supplementary Data allowed us to identify the paclitaxel binding site as the binding pocket for compound **9**. Despite a favorable GOLD score for the adopted binding mode, molecule **9**, being much

Table 4. Percentage of A2780 and A549 cells in the different cell cycle phases after 72-h treatment with compounds **4** and **9** administered at their IC_{50} and 100 μM.

			Compounds			
			4		**9**	
	Cell cycle phases	**CTR[a)]**	**IC_{50}**	**100 μM[b)]**	**IC_{50}**	**100 μM[b)]**
A2780	G0/G1	55.9 ± 1.9[c)]	50.5 ± 7.3	4.3 ± 1.5	40.0 ± 7.5	30.5 ± 9.4
	S	25.0 ± 2.0	24.5 ± 2.6	21.7 ± 3.6	33.6 ± 6.7	37.6 ± 11.2
	G2/M	19.2 ± 1.5	25.0 ± 7.3	46.6 ± 7.2	29.7 ± 6.7	31.9 ± 1.9
	Cells with >4n	–	–	27.4 ± 2.1	–	–
A549	G0/G1	46.7 ± 3.2	35.1 ± 7.0	13.4 ± 5.5	57.8 ± 2.2	36.0 ± 14.1
	S	34.9 ± 4.8	25.0 ± 3.4	31.5 ± 9.4	18.8 ± 3.9	31.2 ± 7.4
	G2/M	18.5 ± 6.0	39.9 ± 9.4	55.2 ± 14.4	23.4 ± 3.8	32.8 ± 13.5
	Cells with >4n	–	–	–	–	–

[a)] The percentage of control cells in the various cell cycle phases, cultured in the presence of 0.6 or 1.2% DMSO, were statistically similar. This is the reason why they were pooled together.
[b)] At 100 μM, **4** expressed $86.9 \pm 4.3\%$ and $66.3 \pm 2.6\%$ cell proliferation inhibition against A2780 and aneuploid A549 cells, respectively. Compound **9** had $76.9 \pm 0.7\%$ and $53.5 \pm 5.4\%$ cell proliferation inhibition against the same cells.
[c)] The mean ± SD represents the percentage of cells ($N = 3–6$) in the various cell cycle phases.

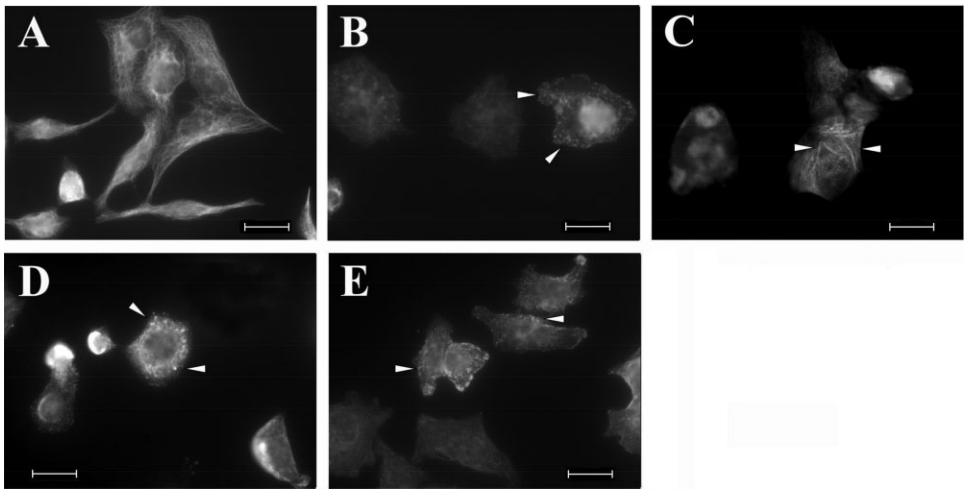


Figure 3. (A): Microtubular effect of compounds **4** and **9** in A549 cells. Untreated control aneuploid A549 cells. (B) and (C): Cells treated for 24 h with 75 nM and 100 nM vincristine and paclitaxel, respectively. The treatment caused the formation of many round microstructures and bundles, as indicated by the arrowheads in (B) and (C), respectively. (D) and (E): A549 cells treated for 24 h with 100 nM **4** and **9**, respectively. Note the round microstructure in some cells interpreted as tubulin aggregates (arrowheads) similar to those found after vincristine treatment. Bar = 20 mm.

flatter than paclitaxel, was predicted to bind deeply in the protein cleft as shown in Fig. 4A. Therefore, **9** does not seem to be able to perturb the tubulin superstructure by stabilizing the formation of the microtubule like paclitaxel does. Surprisingly, the mitotic figure of bundles typical of microtubules stabilizing agents was not present after the immunofluorescence microscopy analysis of compound **9**, at least in the cell culture conditions used for our experiments, and this evidence seems to indirectly confirm that the different steric hindrance of our compound, compared to the more bulky paclitaxel or docetaxel, may be responsible of their different behavior.

On the other hand, the molecule **4** was predicted to bind in a previously not identified binding site on the α/β subunits interface (Fig. 4B) distinct from the colchicine binding site already identified for the parent *N*-(4-indazolyl)-benzenesulfonamide derivatives by docking analysis [8]. However, the inhibition constants for our active indazoles calculated by the program AutoDock are relatively low (K_i ca. 1.4×10^{-6} and 3.4×10^{-6} for **4** and **9**, respectively); therefore, neither **4** nor **9** can be considered as highly effective ligands, and this result could in part justify the lower microtubular effect observed at the microscope.

These biological results showed that *N*-[6-indazolyl]arylsulfonamides **4** and **9** exhibited a significant antiproliferative activity against the tumor cell lines used as target for our tests. Moreover, both these active compounds were also able to significantly trigger apoptosis, with compound **4** that caused on average 36% apoptotic cells when used at its IC_{50} against lung carcinoma A549 cells. In our previous papers, we showed that *N*-(4-indazolyl)benzenesulfonamide derivatives caused a perturbation of the cell cycle with an accumulation of cells

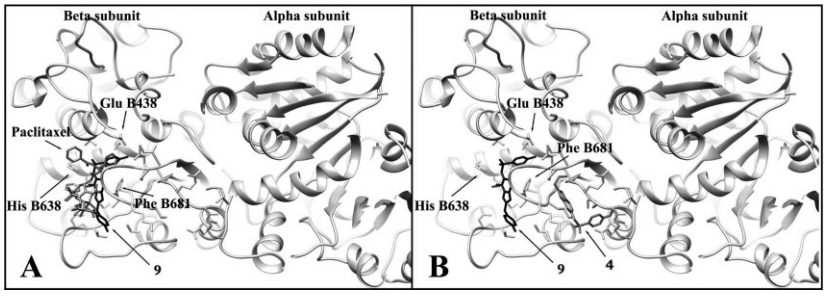


Figure 4. The figure reports the ligand binding modes to tubulin, represented as light gray ribbons. Panel (A) reports the crystallographic structure of the dimeric tubulin with a bound paclitaxel moiety (dim gray sticks). Molecule **9** is reported in black. As shown in the figure, the two ligands share the same binding site with compound **9** being positioned more deeply in the binding cleft. Panel (B) reports how the binding site of compound **4** (dim gray sticks) is located at the interface between the alpha and beta tubulin subunits. Compound **9** bound to the paclitaxel binding cleft is also reported for reference.

in the G_2/M phase [8]. This is typical, but not exclusive, of compounds having a mechanism of action directly or indirectly involving the microtubular system as target.

On the basis of these results, we thus studied the cell cycle modification of the active compounds **4** and **9**. As expected, also these products caused a typical arrest in the G2/M phase of the cell cycle, in particular when used at the highest concentration tested (100 μM), but only compound **4** was able to generate cells with a number of chromosomes >4n. Although this result reinforced the idea of a possible interaction of indazoles with microtubules, this hypothesis was only partially confirmed by immunofluorescence microscopy. In fact, both compounds were able to alter the microtubule network causing the condensation of microtubules into dots likely representing tubulin aggregates, similarly to the anticancer agent vincristine. However, the microtubules aggregates were detected in a smaller number of treated cells (in particular for compound **9**), as compared to vincristine, in spite of the nearly equitoxic concentrations used. Furthermore, good results of inhibition of cell proliferation were obtained at much lower concentrations. All these results seem to suggest that, although compounds **4** and **9** may share with their parent compounds a mechanism of action passing through the inhibition of tubulin functions, they may also posseses other relevant but still unknown mechanisms of action, which cooperated to inhibit cell proliferation and induce apoptosis.

Moreover, as known, the mechanism of action of paclitaxel and docetaxel passes through the stabilization of microtubules leading to mitotic arrest [15]. In the past years, many compounds have been synthesized showing a similar mechanism of action and all were able to stabilize the microtubule network with the formation, similarly to paclitaxel and docetaxel, of typical bundles [16].

Surprisingly, this mitotic figure was not present after the immunofluorescence microscopy analysis of compound **9**, at least in the cell culture conditions used for our experiments, and although our docking analysis showed that this compound was able to bind to the classical binding site of β-tubulin for paclitaxel. This evidence makes our result quite unexpected and makes necessary other analyses to verify this apparent disagreement. On the other hand, it is not possible to exclude that the different steric hindrance of our compound, compared to the more bulky paclitaxel or docetaxel, may be responsible for their different behavior.

On the contrary, compound **4** binds in a novel binding site on the α/β subunits interface that is separated from the colchicine binding site already identified for the parent *N*-(6(4)-indazolyl)-benzenesulfonamide derivatives by docking analysis [8]. Nevertheless, this binding seems pretty weak and this could, in part, justify the lower depolymerizing microtubular effect observed at the microscope.

Conclusion

We have described the synthesis and some antitumor pharmacological properties of the newly designed *N*-[6-indazolyl]arylsulfonamides and *N*-[7-alkoxy-6-indazolyl]arylsulfonamides, two of which showed relevant antiproliferative and apoptotic activities against tumor cell lines. As compared to the previously described *N*-(4-indazolyl)-benzenesulfonamide derivatives [8], the molecules described in the present work seem to have a different interaction with the microtubules that, also based on the results of the cell cycle and of immunofluorescence microscopy, does not seem to represent their unique molecular target. Our results suggest that these new compounds could represent alternative lead compounds for the synthesis of new alternative antitumor indazoles.

Experimental

Chemistry

Melting points were determined using a Büchi-Tottoli apparatus. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6, and solution (unless otherwise specified) with tetramethylsilane (TMS) as an internal reference using a Bruker AC 300 (^{1}H) or 75 MHz (^{13}C) instrument. Chemical shifts are given in δ parts per million (ppm) downfield from TMS. Multiplicities of ^{13}C NMR resources were assigned by distortionless enhancement by polarization transfer (DEPT) experiments. Low-resolution mass spectra (MS) were recorded on a Perkin-Elmer Sciex API 3000 spectrometer. Column chromatography was carried out on SiO_2 (silica gel 60 Merck 0.063–0.200 mm). Thin-layer chromatography (TLC) was carried out on SiO_2 (silica gel 60, F 254 Merck 0.063–0.200 mm), and the spots were located with UV light. Commercial reagents were used without further purification unless stated.

*General synthetic procedure for 1-alkyl-6-nitro-1H-indazoles **2a–c** and 2-alkyl-6-nitro-2H-indazoles **3a–c***

To a solution of 6-nitroindazole **1** (6.13 mmol) in tetrahydrofuran (THF) (30 mL), K_2CO_3 (9.2 mmol) was added. After 15 min at room temperature, alkyl halide (6.13 mmol) was added dropwise. The solution was heated and stirred for 16 h. The resulting mixture was evaporated. The crude material was dissolved with EtOAc (50 mL), washed with water and brine, dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The resulting residue was purified by column chromatography (EtOAc/hexane 3:7).

*1-Allyl-6-nitro-1H-indazole (**2a**)*

Yield: 43%, yellow solid, m.p. 56–58°C. ^{1}H NMR ($CDCl_3$): δ 5.10–5.13 (m, 2H, NCH_2), 5.16–5.30 (m, 2H, $=CH_2$), 6.01–6.10 (m, 1H, =CH), 7.84 (d, 1H, $J = 9.1$ Hz), 8.01 (dd, 1H, $J = 9.1$ Hz, 1.8 Hz), 8.13 (s, 1H), 8.37 (d, 1H, $J = 1.6$ Hz). ^{13}C NMR ($CDCl_3$): δ 52.3 (NCH_2), 106.1 (CH), 115.5 (CH), 118.7 ($=CH_2$), 121.9 (CH), 127.3 (C-3a), 131.9, 133.6 (CH-3, =CH), 138.2 (C), 146.5 (C). Anal. calcd. for $C_{10}H_9N_3O_2$: C, 59.11; H, 4.46; N, 20.68. Found: C, 59.23; H, 4.58; N, 20.61.

*2-Allyl-6-nitro-2H-indazole (**3a**)*

Yield: 49%, yellow solid, m.p. 48–50°C. ^{1}H NMR ($CDCl_3$): δ 5.10–5.12 (m, 2H, NCH_2), 5.34–5.43 (m, 2H, $=CH_2$), 6.13–6.18

(m, 1H, =CH), 7.75 (d, 1H, $J = 9.3$ Hz), 7.88 (dd, 1H, $J = 9.3$ Hz, 2.1 Hz), 8.08 (s, 1H), 8.68 (d, 1H, $J = 1.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 56.8 (NCH_2), 115.5 (CH), 115.9 (CH), 120.7 (=CH_2), 121.5 (CH), 123.7 (CH-3), 124.4 (C), 131.2 (=CH), 146.6 (C), 146.8 (C). Anal. calcd. for $C_{10}H_9N_3O_2$: C, 59.11; H, 4.46; N, 20.68. Found: C, 59.18; H, 4.50; N, 20.57.

*Ethyl 3-(6-nitro-1H-indazol-1-yl)propanoate (**2b**)*

Yield: 35%, yellow solid, m.p. 42–44°C. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_3, $J = 7.2$ Hz), 3.05 (t, 2H, CH_2, $J = 6.4$ Hz), 4.11 (q, 2H, CH_2O, $J = 7.2$ Hz), 4.77 (t, 2H, NCH_2, $J = 6.4$ Hz), 7.83 (d, 1H, $J = 8.7$ Hz), 8.04 (dd, 1H, $J = 8.7$ Hz, 1.8 Hz), 8.15 (s, 1H), 8.51 (d, 1H, $J = 1.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.3 (CH_3), 34.4 (CH_2), 44.8 (NCH_2), 60.6 (CH_2O), 107.6 (CH), 115.4 (CH), 122.6 (CH), 126.9 (C), 134.4 (CH-3), 138.6 (C), 146.3 (C), 171.2 (CO). Anal. calcd. for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.64; H, 5.08; N, 15.84.

*Ethyl 3-(6-nitro-2H-indazol-2-yl)propanoate (**3b**)*

Yield: 59%, yellow solid, m.p. 54–56°C. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_3, $J = 7.2$ Hz), 3.09 (t, 2H, CH_2, $J = 6.4$ Hz), 4.14 (q, 2H, CH_2O, $J = 7.2$ Hz), 4.78 (t, 2H, NCH_2, $J = 6.4$ Hz), 7.73 (d, 1H, $J = 9.0$ Hz), 7.88 (dd, 1H, $J = 9.0$ Hz, 1.2 Hz), 8.15 (s, 1H), 8.65 (d, 1H, $J = 1.2$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3), 34.6 (CH_2), 49.6 (NCH_2), 61.2 (CH_2O), 115.4 (CH), 115.8 (CH), 121.6 (CH), 124.1 (C), 124.9 (CH-3), 146.7 (C), 146.9 (C), 170.6 (CO). Anal. calcd. for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.61; H, 5.12; N, 15.88.

*1-(4-Methyl-benzyl)-6-nitro-1H-indazole (**2c**)*

Yield: 42%, yellow solid, m.p. 96–98°C. ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 5.63 (s, 2H, NCH_2), 7.14 (m, 4H, H–Ar), 7.82 (d, 1H, $J = 8.7$ Hz), 7.99 (dd, 1H, $J = 8.7$ Hz, 1.5 Hz), 8.15 (s, 1H), 8.31 (d, 1H, $J = 1.5$ Hz). ^{13}C NMR ($CDCl_3$): δ 21.1 (CH_3), 53.5 (NCH_2), 106.1 (CH), 115.5 (CH), 121.9 (CH), 127.1 (C), 127.3 (2CH), 129.7 (2CH), 132.7 (C), 133.6 (CH-3), 138.1 (C), 146.5 (C). Anal. calcd. for $C_{15}H_{13}N_3O_2$: C, 67.41; H, 4.90; N, 15.72. Found: C, 67.58; H, 4.78; N, 15.85.

*2-(4-Methyl-benzyl)-6-nitro-2H-indazole (**3c**)*

Yield: 54%, yellow solid, m.p. 84–86°C. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 5.61 (s, 2H, NCH_2), 7.21 (m, 4H, H–Ar), 7.70 (d, 1H, $J = 9.0$ Hz), 7.87 (dd, 1H, $J = 8.7$ Hz, 1.8 Hz), 7.97 (s, 1H), 8.71 (d, 1H, $J = 1.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 21.2 (CH_3), 58.1 (NCH_2), 115.6 (CH), 115.8 (CH), 121.5 (CH), 123.6 (CH-3), 124.5 (C), 128.4 (2CH), 129.8 (2CH), 131.6 (C), 138.9 (C), 146.6 (C), 146.8 (C). Anal. calcd. for $C_{15}H_{13}N_3O_2$: C, 67.41; H, 4.90; N, 15.72. Found: C, 67.49; H, 4.84; N, 15.81.

*General synthetic procedure for N-(2-allyl-2H-indazol-6-yl)-4-methoxybenzenesulfonamide **4** and N-(2-allyl-7-alkoxy-2H-indazol-6-yl)-4-methoxybenzenesulfonamides **5a–c***

A mixture of 2-allyl-6-nitroindazole **3a** (1.22 mmol) and anhydrous $SnCl_2$ (1.1 g, 6.1 mmol) in 25 mL of absolute ROH was heated at 60°C. After reduction, the starting material disappeared and the solution was allowed to cool down. The pH was made slightly basic (pH 7–8) by addition of 5% aqueous potassium bicarbonate before being extracted with EtOAc. The organic phase was washed with brine and dried over magnesium sulfate. The solvent was removed to afford the amine, which was immediately dissolved in pyridine (5 mL) and then reacted with 4-methoxybenzenesulfonyl chloride (0.26 g, 1.25 mmol) at room temperature for 24 h. After the reaction mixture was concentrated *in vacuo*, the resulting residue was purified by flash chromatography (eluted with EtOAc/hexane 1:9).

*N-(2-Allyl-2H-indazol-6-yl)-4-methoxybenzenesulfonamide (**4**)*

M.p. 168–170°C. ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, CH_3O), 4.94–4.97 (m, 2H, NCH_2), 5.13–5.21 (m, 2H, =CH_2), 5.99–6.08 (m, 1H, =CH), 6.82 (dd, 1H, $J = 8.7$ Hz), 7.00 (d, 2H, $J = 9.0$ Hz), 7.19 (s, 1H, H-Ar), 7.53 (d, 1H, $J = 8.7$ Hz), 7.68 (d, 2H, $J = 9.0$ Hz), 8.21 (s, 1H, H-3), 10.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.5 (NCH_2), 56.0 (CH_3O), 105.8 (CH), 114.8 (2CH), 117.4 (CH), 118.9 (=CH_2), 119.1 (C), 121.9 (CH), 124.2 (CH), 129.4 (2CH), 131.6 (C), 133.9 (CH), 135.8 (C), 148.5 (C), 162.8 (C). MS: *m/z* 344 $[M+1]^+$. Anal. calcd. for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24. Found: C, 59.54; H, 4.91; N, 12.16.

*N-(2-Allyl-7-methoxy-2H-indazol-6-yl)-4-methoxybenzenesulfonamide (**5a**)*

Yield: 42%, m.p. 110–112°C. ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H, CH_3O), 3.92 (s, 3H, CH_3O), 5.04–5.07 (m, 2H, NCH_2), 5.32–5.38 (m, 2H, =CH_2), 6.05–6.14 (m, 1H, =CH), 6.82 (d, 2H, $J = 8.7$ Hz), 7.10 (s, 1H, NH), 7.29 (d, 1H, H-5, $J = 9.0$ Hz), 7.45 (d, 1H, H-4, $J = 9.0$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 7.92 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 55.5 (CH_3O), 56.0 (NCH_2), 60.8 (CH_3O), 114.0 (2CH), 115.1 (CH), 118.8 (CH), 120.5 (=CH_2), 122.1 (C), 123.9 (CH), 124.6 (C), 129.3 (2CH), 130.8 (C), 131.4 (CH), 138.2 (C), 140.6 (C), 163.0 (C). MS: *m/z* 374 $[M+1]^+$. Anal. calcd. for $C_{18}H_{19}N_3O_4S$: C, 57.90; H, 5.13; N, 11.25. Found: C, 57.98; H, 5.02; N, 11.36.

*N-(2-Allyl-7-propoxy-2H-indazol-6-yl)-4-methoxybenzenesulfonamide (**5b**)*

Yield: 32%, m.p. 88–90°C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, CH_3, $J = 7.25$ Hz), 1.66–1.69 (m, 2H, CH_2), 3.76 (s, 3H, CH_3O), 4.18 (t, 2H, CH_2O, $J = 6.9$ Hz), 4.98–5.14 (m, 2H, NCH_2), 5.36–5.41 (m, 2H, =CH_2), 6.05–6.14 (m, 1H, =CH), 6.82 (d, 2H, $J = 8.7$ Hz), 7.13 (s, 1H, NH), 7.30 (d, 1H, H-5, $J = 9.0$ Hz), 7.52 (d, 1H, H-4, $J = 9.0$ Hz), 7.66 (d, 2H, $J = 8.7$ Hz), 7.96 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 10.2 (CH_3), 23.3 (CH_2), 55.5 (CH_3O), 55.8 (NCH_2), 74.9 (CH_2O), 114.1 (2CH), 115.0 (CH), 118.8 (CH), 121.2 (=CH_2), 122.7 (C), 124.7 (CH), 125.9 (C), 129.2 (2CH), 130.9 (C), 131.0 (CH), 136.5 (C), 139.6 (C), 163.1 (C). MS: *m/z* 402 $[M+1]^+$. Anal. calcd. for $C_{20}H_{23}N_3O_4S$: C, 59.83; H, 5.77; N, 10.47. Found: C, 59.95; H, 5.62; N, 10.58.

*N-(2-Allyl-7-butoxy-2H-indazol-6-yl)-4-methoxybenzenesulfonamide (**5c**)*

Yield: 45%, m.p. 75–77°C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, CH_3, $J = 7.5$ Hz), 1.34–1.42 (m, 2H, CH_2), 1.57–1.64 (m, 2H, CH_2), 3.77 (s, 3H, CH_3O), 4.26 (t, 2H, CH_2O, $J = 6.9$ Hz), 4.95–5.09 (m, 2H, NCH_2), 5.33–5.39 (m, 2H, =CH_2), 6.05–6.11 (m, 1H, =CH), 6.81 (d, 2H, $J = 9.0$ Hz), 7.10 (s, 1H, NH), 7.28 (d, 1H, H-5, $J = 9.0$ Hz), 7.50 (d, 1H, H-4, $J = 9.0$ Hz), 7.66 (d, 2H, $J = 9.0$ Hz), 7.92 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 13.9 (CH_3), 19.0 (CH_2), 32.1 (CH_2), 55.5 (CH_3O), 55.9 (NCH_2), 73.1 (CH_2O), 114.0 (CH), 114.7 (2CH), 118.7 (CH), 120.7 (=CH_2), 122.6 (C), 124.1 (CH), 125.0 (C), 129.2 (2CH), 130.8 (C), 131.3 (CH), 136.9 (C), 140.3 (C), 163.0 (C). MS: *m/z* 416 $[M+1]^+$. Anal. calcd. for $C_{21}H_{25}N_3O_4S$: C, 60.70; H, 6.06; N, 10.11. Found: C, 60.82; H, 6.14; N, 10.01.

*Synthesis of sulfonamides **6** and **7***

These compounds were prepared from ethyl 3-(6-nitro-1*H*-indazol-2-yl)propanoate **3b** by using the same procedure applied to **3a**.

3-[6-(4-Methoxybenzenesulfonylamino)-indazol-2-yl]-propionic acid ethyl ester **6**

Yield: 38%, m.p. 60–62°C. ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, CH_3, $J = 7.2$ Hz), 3.10 (t, 2H, CH_2, $J = 6.0$ Hz), 3.76 (s, 3H, CH_3O), 4.09 (q, 2H, CH_2O, $J = 7.2$ Hz), 4.82 (t, 2H, NCH_2, $J = 6.0$ Hz), 6.83 (d, 2H, $J = 9.0$ Hz), 7.00 (dd, 1H, $J = 8.7$ and 1.2 Hz), 7.37 (s, 1H, H–Ar), 7.52 (d, 1H, $J = 8.7$ Hz), 7.77 (d, 2H, $J = 9.0$ Hz), 8.10 (s, 1H, H-3), 8.20 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3), 34.4 (CH_2), 48.8 (NCH_2), 55.5 (CH_3O), 61.2 (CH_2O), 104.1 (CH), 114.3 (2CH), 118.9 (CH), 119.7 (C), 122.0 (CH), 126.9 (CH), 129.5 (2CH), 130.6 (C), 138.1 (C), 145.6 (C), 163.1 (C), 170.8 (C). MS: m/z 404 $[M+1]^+$. Anal. calcd. for $C_{19}H_{21}N_3O_5S$: C, 56.56; H, 5.25; N, 10.41. Found: C, 56.45; H, 5.12; N, 10.52.

3-[7-Ethoxy-6-(4-methoxybenzenesulfonylamino)-indazol-2-yl]propionic acid ethyl ester **7**

Yield: 45%, m.p. 108–110°C. ^{1}H NMR ($CDCl_3$): δ 1.19 (t, 3H, CH_3, $J = 7.2$ Hz), 1.23 (t, 3H, CH_3, $J = 7.2$ Hz), 3.10 (t, 2H, CH_2, $J = 6.0$ Hz), 3.77 (s, 3H, CH_3O), 4.10 (q, 2H, CH_2O, $J = 7.2$ Hz), 4.29 (q, 2H, CH_2O, $J = 7.2$ Hz), 4.82 (t, 2H, NCH_2, $J = 6.0$ Hz), 6.81 (d, 2H, $J = 8.7$ Hz), 7.12 (s, 1H, NH), 7.27 (d, 1H, H-5, $J = 9.0$ Hz), 7.45 (d, 1H, H-4, $J = 9.0$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 7.98 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3), 15.6 (CH_3), 34.7 (CH_2), 48.8 (NCH_2), 55.5 (CH_3O), 61.1 (CH_2O), 68.7 (CH_2O), 114.0 (2CH), 114.8 (CH), 118.5 (CH), 121.6 (C), 124.8 (C), 125.2 (CH), 129.3 (2CH), 131.0 (C), 136.8 (C), 140.9 (C), 163.0 (C), 170.8 (C). MS: m/z 448 $[M+1]^+$. Anal. calcd. for $C_{21}H_{25}N_3O_6S$: C, 56.36; H, 5.63; N, 9.39. Found: C, 56.45; H, 5.52; N, 9.48.

Synthesis of sulfonamides **8** *and* **9**

These compounds were prepared from 2-(4-methyl-benzyl)-6-nitro-1*H*-indazole **3c** by using the same procedure applied to **3a**.

4-Methyl-N-[2-(4-methyl-benzyl)-2H-indazol-6-yl]benzene-sulfonamide **8**

Yield: 50%, m.p. 124–126°C. ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 5.45 (s, 2H, NCH_2), 6.80 (dd, 1H, $J = 8.7$ Hz, 1.8 Hz), 7.10–7.19 (m, 5H, H–Ar), 7.28 (d, 2H, $J = 7.8$ Hz), 7.52 (d, 1H, $J = 8.7$ Hz), 7.63 (d, 2H, $J = 7.8$ Hz), 8.30 (s, 1H), 10.16 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.1 (CH_3), 21.4 (CH_3), 56.4 (NCH_2), 105.8 (CH), 117.3 (CH), 119.2 (C), 122.0 (CH), 124.5 (CH), 127.2 (2CH), 128.5 (2CH), 129.5 (2CH), 130.1 (2CH), 134.4 (C), 135.8 (C), 137.2 (C), 137.6 (C), 143.6 (C), 148.6 (C). MS: m/z 392 $[M+1]^+$. Anal. calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.64; H, 5.30; N, 10.82.

N-[7-Ethoxy-2-(4-methyl-benzyl)-2H-indazol-6-yl]-4-methyl-benzenesulfonamide **9**

Yield: 46%, m.p. 135–137°C. ^{1}H NMR ($CDCl_3$): δ 1.23 (t, 3H, CH_3, $J = 7.2$ Hz), 2.32 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.31 (q, 2H, CH_2O, $J = 7.2$ Hz), 5.55 (s, 2H, NCH_2), 7.14–7.24 (m, 8H, H–Ar), 7.45 (d, 1H, $J = 9.0$ Hz), 7.63 (d, 2H, $J = 7.8$ Hz), 7.76 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.5 (CH_3), 21.2 (CH_3), 21.5 (CH_3), 57.2 (NCH_2), 68.8 (CH_2O), 114.7 (CH), 118.5 (CH), 122.0 (C), 123.9 (CH), 124.6 (C),127.1 (2CH), 128.5 (2CH), 129.4 (2CH), 129.7 (2CH), 131.7 (C), 136.2 (C), 136.9 (C), 138.7 (C), 140.7 (C), 143.7 (C). MS: m/z 436 $[M+1]^+$. Anal. calcd. for $C_{24}H_{25}N_3O_3S$: C, 66.18; H, 5.79; N, 9.65. Found: C, 66.31; H, 5.68; N, 9.75.

Biological assays

Evaluation of antiproliferative activity (MTT assay)

Human cell lines A549 (lung and carcinoma) and A2780 (ovary and adenocarcinoma) were seeded at 0.89×10^5/mL and 10^5/mL, respectively, in 180 μL of RPMI 1640 complete medium into flat-bottomed microtiter plates. After 6–8 h, the cells were treated with five 1:10-fold concentrations of compounds (20 μL) diluted in FCS containing 6% (**4**, final concentration 0.6%) or 12% (**9**, final concentration 1.2%) DMSO. Three days later cells were processed with the addition of MTT (Sigma, St. Louis, MO) solution (2 mg/mL in PBS, phosphate-buffered saline), as described elsewhere [17]. The IC_{50}s (and IC_{75}s) were calculated on the basis of concentration–response curves, analyzing each single curve by the Cricket Graph III software. Each experiment was repeated four to eight times.

Evaluation of apoptosis by annexin-V/PI staining

Apoptosis was evaluated in A2780 and A549 cells treated for 72 h with *N*-[6-indazolyl]arylsulfonamides **4** (IC_{50} and IC_{75}) and **9** (IC_{50} and 100 μM). After treatment, cells were harvested, washed with cold PBS containing 2% fetal calf serum, and apoptosis triggering evaluated by double staining with FITC-annexin-V/PI (rh Annexin-V/FITC Kit, Bender MedSystem GmbH, Vienna, Austria) and cytofluorimetric analysis [18].

Cell cycle analysis

A549 and A2780 cells were also studied for the effect of compounds **4** and **9** on the cell cycle phases. Cells (1×10^4/mL and 0.88×10^4/mL in 10 mL for A2780 and A549, respectively) were treated for 72 h with the IC_{50}s and 100 μM concentrations of both **4** and **9**. Floating and adherent cells were then harvested, washed with PBS, and fixed overnight at −20°C in 70% ethyl alcohol. Once fixed, cells were treated as described elsewhere [19] and analyzed using a FACSort flow cytometer (BD Biosciences, Mountain View, CA). The ModFit LT software was used to evaluate the distribution of cells in various cell cycle phases.

Microtubule immunofluorescence

Ten to five A549 cells were cultured in chamber slides and 24 h later treated for another 24 h with 100 μM of selected active substances and with 75 nM vincristine as positive control. After 24-h incubation, cells were washed with PBS and fixed for 5 min with 3.7% paraformaldehyde in PBS plus 2% sucrose (PBS-S) at room temperature. After further washing with cold PBS-S on ice, cells were fixed again with methanol for another 5 min at 20°C. Additional washes with cold PBS-S ended the fixation procedure. The labeling procedure with monoclonal anti-α-tubulin (T5168, Sigma) antibodies is described elsewhere [20]. Images were acquired with an Axiovert 200M microscope (Carl Zeiss MicroImaging

GmbH, Goettingen, Germany) and microphotographs rendered by Photoshop 5.0 software.

Molecular docking

Molecular docking simulations were performed using the program GOLD (CCDC, Cambridge, UK) and, as molecular target, the three-dimensional coordinates of α/β-tubulin stabilized by paclitaxel (REF1, PDB code 1JFF). The searching grid was extended over a sphere of 20 Å radius centered at the O atom of Asp357 of the β chain. The "Goldscore" scoring function as furnished by the program was adopted.

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