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Original article

Synthesis, antiproliferative and apoptotic activities of *N*-(6(4)-indazolyl)-benzenesulfonamide derivatives as potential anticancer agents

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ABSTRACT

Recently, it has been reported that compounds bearing a sulfonamide moiety possess many types of biological activities, including anticancer activity. The present work reports the synthesis and antiproliferative evaluation of some *N*-(6(4)-indazolyl)benzenesulfonamides and 7-ethoxy-*N*-(6(4)-indazolyl)benzenesulfonamides.

All compounds were evaluated for their *in vitro* antiproliferative activity against three tumor cell lines: A2780 (human ovarian carcinoma) A549 (human lung adenocarcinoma) and P388 (murine leukemia). The results indicated that sulfonamides **2c**, **3c**, **6d**, **8**, **13**, **3b** and **16** were endowed with a pharmacologically interesting antiproliferative activity with compounds **2c** and **3c** showing the lower IC_{50} (from 0.50 ± 0.09 to 1.83 ± 0.52 μM and from 0.58 ± 0.17 to 5.83 ± 1.83 μM, respectively). Moreover, these indazoles were able to trigger apoptosis through the upregulation of the typical apoptosis markers p53 and bax.

As regard to the hypothetic targets of these compounds, a preliminary docking analysis showed that all compounds seemed to interact with β-tubulin, in particular compound **3b** that showed the lower *Ki*. The cytofluorimetric analysis of the cell cycle phases indicates that all compounds, when administered at their IC_{75}, caused a block in the G2/M phase of the cell cycle with the generation of subpopulations of cells with a number of chromosome >4n. When the IC_{50}s were applied we observed a prevalent block in the G0/G1 phase except for compounds **16** and **8** where a partial G2/M block was present with a concomitant decrease of cells in the G0/G1 and S phases of the cell cycle. Altogether these results suggest a possible, but not exclusive, interaction with microtubules.

1. Introduction

Indazoles posses many types of biological activities [1,2] and representatives of this class of pharmacological agents are widely used as antibacterial [3], anti-inflammatory [4], anti-HIV [5], antiprotozoal [6] and antimalarial [7]. Recently, a host of structurally novel indazole derivatives have been reported to show substantial anticancer activities *in vitro* and/or *in vivo* [8–12], and our research group has reported the synthesis of some new *N*-(7-indazolyl)benzenesulfonamide derivatives of type **A** (Fig. 1). Some of these compounds exhibited significant cytotoxicity against human (colon and prostate) and murine (leukemia) cell lines [13]. The perturbations of the cell cycles induced by the most potent compounds were studied on the L1210 cell line by flow cytometry. All of these compounds induced a marked accumulation in G2/M + 8N phases of the cell cycle. This observation is typical of the modification of the cell cycle induced by numerous tubulin interacting drugs. Compound **B**, 4-methoxy-*N*-(3-chloro-7-indazolyl)benzenesulfonamide, was the most active of the series.

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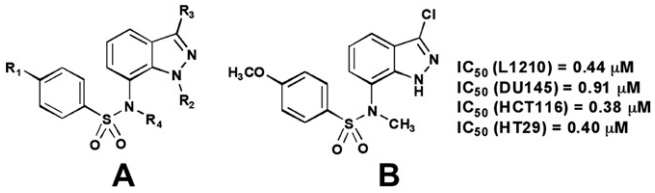


Fig. 1. Cytotoxic activity of *N*-(7-indazolyl)-benzenesulfonamides against human (colon and prostate) and murine (leukemia) cell lines.

In continuation of our efforts in searching for novel effective anticancer agents, we synthesized a series of *N*-(6(4)-indazolyl)benzenesulfonamide derivatives and evaluated their antiproliferative and apoptotic potential. Docking and cytometric analyses were also performed in order to identify the possible molecular target of these compounds.

2. Chemistry

Previously, we described the syntheses of various *N*-(alkoxy-1*H*-indazol-4(7)-yl)benzenesulfonamide derivatives [14,15] by reduction of nitroindazole derivatives with $SnCl_2$ in different alcohols followed by coupling the corresponding amine by benzenesulfonamide. This method was employed for the synthesis of *N*-(1*H*-indazol-4-yl)-benzenesulfonamides (**2a**–**c**) and *N*-(7-ethoxy-1*H*-indazol-4-yl)-benzenesulfonamides (**3a**–**c**) starting from 3-halogeno-4-nitroindazoles (**1a**–**c**) (Scheme 1). We observed a new kind of transformation of 4-nitroindazoles under the action of stannous chloride in ethanol leading to 7-ethoxy-4-aminoindazoles accompanied by the desired amine.

Similarly, we prepared the sulfonamides (**6d**,**e**) and (**7d**,**e**) by reduction of *N*-alkyl-4-nitroindazoles (**4d**,**e**) with $SnCl_2$ in ethanol followed by coupling of the corresponding amine with 4-methylbenzenesulfonamide (Scheme 2). Compounds **4** and **5** have been synthesized starting from alkylation of 4-nitroindazole **1a** according to a previously described procedure [16].

The structural assignments of the sulfonamides (**6d**,**e**) and (**7d**,**e**) are based on a full characterization by ^{1}H NMR and ^{13}C NMR spectra. The structure of the *N*-[7-ethoxy-1-(prop-2-en-1-yl)-1*H*-indazol-4-yl]-4-methylbenzenesulfonamide **7e**, was established by X-ray crystallography [17] (Fig. 2).

When we applied the same reduction conditions as previously described for 2-methyl-4-nitroindazole **5d**, we obtained only the *N*-(7-ethoxy-2-methyl-4-indazolyl)-4-methylbenzenesulfonamide **8** functionalized in position 7 in good yield (Scheme 3). This result indicates that alkylation of 4-nitroindazole at position 2 leads selectively to 7-ethoxyindazole derivatives with good yield.

Obviously, the position of nitro group of indazole plays an important role for the orientation of the nucleophilic substitution of ethoxy group in indazole. As an extension of these results, we continued our studies on reactions of the reduction of aromatic nitro compounds with anhydrous $SnCl_2$ in alcohol. Here, we report the reduction of 6-nitroindazole derivatives by anhydrous $SnCl_2$ in ethanol. Thus, compounds (**9a**,**b**) were reduced as previously described, and subsequent coupling was achieved with 4-methylbenzenesulfonyl chloride. We noticed that (**9a**,**b**) afforded only *N*-(3-halogeno-6-indazolyl)-benzenesulfonamides (**10a**,**b**) and (**11a**,**b**) (Scheme 4). No trace of nucleophilic substitution of ethoxy group was observed.

When we investigated the same conditions to 2-allyl-6-nitroindazole **12** we obtained a mixture of two products, 6-aminoindazole and 7-ethoxy-6-aminoindazole, which were immediately coupled by 4-methylbenzenesulfonamide chloride in pyridine (Scheme 5). In this case, it is clear that SN_H is improved by N-alkylation of the *N*-2 position.

The structure of compound **14** was unambiguously proven by single crystal X-ray analysis [18] (Fig. 3).

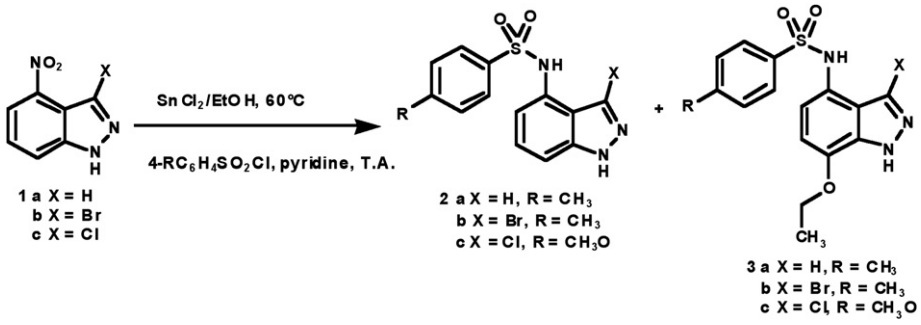
Interestingly, the reduction of 7-nitroindazole **10** as previously described in our work [14] delivered a mixture of sulfonamide **16** with ethoxy group at position 4 and compound **17** devoid of ethoxy group (Scheme 6).

3. Biological results

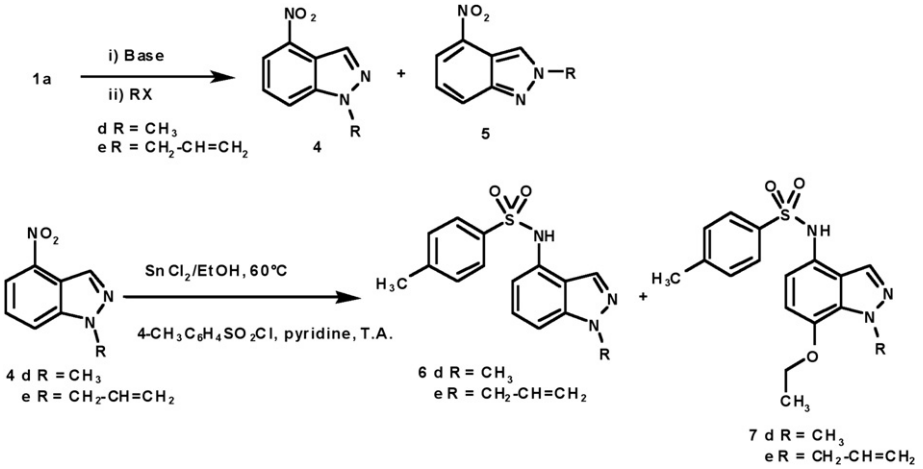
3.1. Inhibition of cell proliferation and triggering of apoptosis by indazole compounds

The analysis of the inhibition of cell proliferation obtained by the treatment for 72 h with our indazole compounds showed that at least 7 molecules had a significant inhibiting activity, here defined with an IC_{50} lower than the arbitrary value of 30 μM in all three cell lines used for the assays. In particular, **2c** and **3c** showed the lower IC_{50}s (from 0.50 ± 0.09 to 1.83 ± 0.52 μM and from 0.58 ± 0.17 to 5.83 ± 1.83 μM, respectively), demonstrating their greater activity compared to the other active compounds (from 3.40 ± 1.43 to 6.88 ± 2.10 μM, from 4.41 ± 0.52 to 11.9 ± 0.5 μM, from 5.21 ± 1.40 to 17.5 ± 4.0 μM, from 4.19 ± 1.70 to 14.4 ± 3.4, and from 5.70 ± 0.29 to 25.1 ± 8.6 μM for **6d**, **13**, **3b**, **8**, and **16**, respectively) (Table 1).

Considering the activation of the apoptotic death, in general, the most active compounds in terms of inhibition of cell proliferation (i.e. **2c** and **3c**) were also those displaying the higher activity in terms of apoptosis (Table 2). An interesting result is that, on average, while the sensitivity of the human lung A549 cell line to the antiproliferative activity of the active compounds was lower than that of human ovarian A2780 cancer cells (Table 1), in terms of induction of apoptosis and at equitoxic concentrations these cells seem to be in general (in particular for **2c**, **3c**, **13** and **6d**) more sensitive than A2780 cells ($p < 0.05$, Table 2).



Scheme 1.



Scheme 2.

3.2. Western blot analysis of p53 and bax apoptosis markers

Our compounds **3b**, **2c**, **3c**, **13**, **6d**, and **16**, all able to induce apoptosis, were also analyzed by western blot for the activation/upregulation of some apoptosis markers such as p53 and bax. To this end we studied the marker levels on A2780 cells after 24, 48 and 72 h incubation with their specific IC_{50}s.

Our results show (Fig. 4) that all tested compounds were able to upregulate both p53 and bax apoptosis markers, though in a different manner. In fact, p53 was greatly upregulated already after 24 h incubation by all active compounds, while bax upregulation generally appeared after 48 h incubation and was much less evident than that of p53 or, in case of compound **6d**, absent. Nevertheless, on the whole these data suggest a p53-dependent pathway of apoptosis induction.

3.3. Cell cycle analysis

To evaluate the modifications of the cell cycle phases due to the exposure to our indazole active compounds A2780 cells were analyzed after treatment by flow cytometry and propidium iodide staining. As shown in Table 3, our compounds **3b**, **3c**, **6d** and **13** were able to cause a block of cells in the G0/G1 phase of the cell cycle at their IC_{50}s, with a concomitant decrease of cells in the S phase. While we did not observe the presence of polyploid/aneuploid cells, a partial block of cells in the G2/M phase, when we used compounds **16** and **8**, suggested a possible mechanism of action starting from a direct interaction of these compounds with the microtubular structures. To verify this hypothesis we repeated the experiments using a higher compound concentration (IC_{75}) observing that all compounds in these culture conditions caused a typical and marked block of cells in the G2/M phase of the cell cycle and a simultaneous decrease of cells in the S and G0/G1 phases. Moreover, all compounds generated a cellular subpopulations with a number of chromosomes >4n.

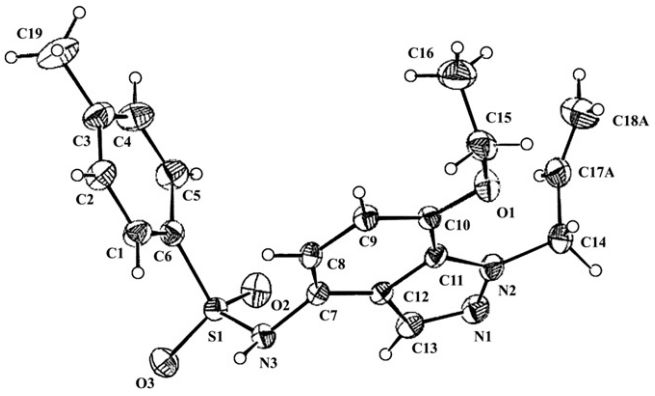


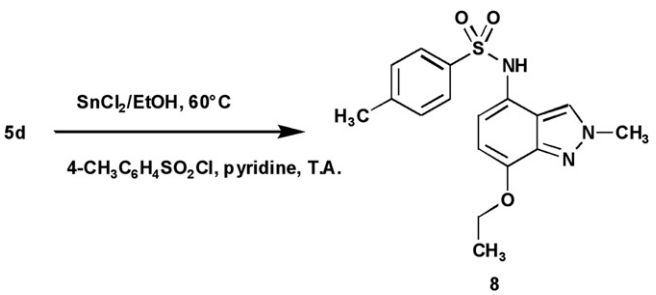
Fig. 2. ORTEP drawing of **7e**, showing the molecular numbering scheme. Ellipsoids are drawn at the 30% probability level.

3.4. Docking analysis

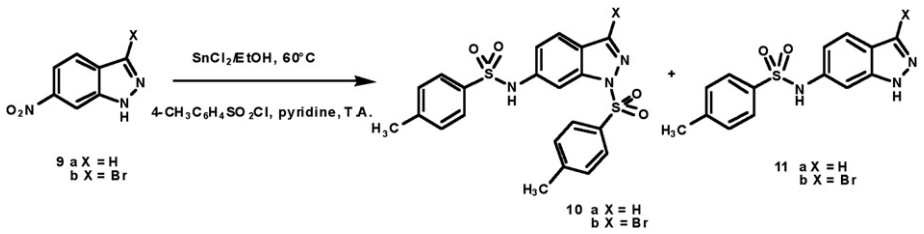
The docking procedure described below allowed us to identify the colchicine site as the binding pocket for the considered molecules. Particularly, all the ligand tested "in silico" adopted a bound conformation similar to that of colchicine where the ligand is mostly buried in the intermediate domain of the β subunit, boxed in by β-strands S8, S9, loop T7, and α-helices H7, H8. The ligands tested also interact with loop T5 of the neighboring α-subunit. Such a location explains how the binding of a colchicine molecule to tubulin prevents the curved complex from adopting a straight structure [19]. Among the molecules tested in silico, **3b** was identified as the best tubulin ligand displaying a calculated K_i of about 7.1×10^{-7} M and a favorable clustering. Fig. 5 shows the superposition of molecule **3b**, positioned according to the docking simulation results, to colchicine, as revealed by X-ray crystallography. Compound **2c** was shown to be an ideal candidate but with a slightly lower calculated K_i (3.2×10^{-6} M), the other candidates showed lower ranking scores and thus were considered as less good ligands.

4. Discussion and conclusion

Sulfonamides represent an important group of compounds endowed with different pharmacological activities such as diuretic,



Scheme 3.



Scheme 4.

antibiotic, anti-hypertensive and hypoglycaemic. Obviously, the sulfonamide group represents a key structural motif for the interaction with many cell targets. In particular, in the last 15 years this motif has been considered as starting point for the synthesis of new anticancer compounds such as the chloroquinoxaline sulfonamide [20] or the diarylsulfonylureas [21] or again the sulfonamide E-7010, ER-34410 and E-7070 [22,23], some of which also entered the clinical phase of study [24].

On this base and taken into account our previously described synthesis of *N*-(7-indazolyl)benzenesulfonamide derivatives of type **A** [13], we have developed a method for the synthesis of a series of *N*-(6(4)-indazolyl)benzenesulfonamides and *N*-(7-ethoxy-6(4)-indazolyl)benzenesulfonamides and described their evaluation as anticancer agents. Our methodology for the synthesis of sulfonamides is based on the reduction of the nitro group of 6(4)-nitroindazoles using anhydrous stannous chloride in ethanol followed by coupling of the corresponding amine by benzenesulfonamides.

The sulfonamides **2c**, **3c**, **6d**, **13**, **3b**, **8**, and **16** exhibited high or very high activity against all the tumor cell lines investigated. Compounds **2c** and **3c** were the most potent of all derivatives tested with IC_{50}s near 1 μM in one or more cell lines. A similar result was found also considering their apoptotic activity, in fact active compounds were all able to trigger apoptosis, in particular compounds **2c** and **3c**, and to upregulate the p53 and bax markers, thus suggesting the mitochondrial way for the activation of this important mechanism of tumor cells killing.

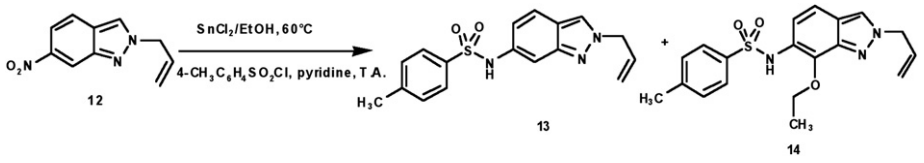
As regard to the structure, our data demonstrate that the biological activity depends mainly on the presence of a methoxy group in the benzene ring of the sulfonamide moiety associated with and a chlorine atom in position 3 of the indazole. This important role is suggested by the fact that the antiproliferative activity of the two molecules **2c** and **3c**, which are characterized by the presence of these residues, is significantly higher compared to that of the other active compounds. This finding is not surprising since other two classical antimitotic sulfonamides (E-7010 and ER-34410) were both characterized by a *para*-methoxy group linked to the benzenesulfonamide moiety [22–24]. On the other hand our docking analysis identifies as the better ligand for the β-tubulin binding site the molecule **3b** with a *Ki* of 7.1 $\times 10^{-7}$ M, and this molecule was, together with **16**, the less active compound among the active ones. This introduces the possibility that our compounds may bind to other possible molecular targets, probably more essential for cell survival. This results from the fact that **3b**, as already mentioned, does not carry a methoxy and/or a chlorine groups that seem to be linked to the higher activity of **2c** and **3c**. Our docking analysis also indicates that our indazoles may interact, although with significant differences, with the binding site of colchicine, a well-known tubulin interacting agent, disturbing the normal curvature of the tubulin complex and complicating the physiological growth of microtubules during polymerization with the subsequent stop or alteration of mitosis. This binding site was similar to that of the already mentioned antimitotic sulfonamides E7010 and ER-34410 and suggest that this kind of chemical structure has a particular binding "preference" for this molecular site.

Always as regard to the target, in a previous paper we showed that most of our previously synthesized *N*-(7-indazolyl)-benzenesulfonamide derivatives caused a deep perturbation of the cell cycles with a marked accumulation of cells in the G/M + 8N phases [13]. These results suggested the possibility that this different class of indazoles could have a mechanism of action starting from an initial binding to the cell microtubular system. On the basis of this hypothesis and to verify the hypothesis suggested by docking analysis we thus tried to evaluate by cytometry whether a similar molecular target could justify the activity of our new indazole derivatives.

Once analyzed for their ability to cause modifications of the cell cycle phases, all active compounds showed to cause a block in the G2/M phase of the cell cycle, and to generate subpopulations with a number of chromosome >4n. This effect clearly appeared at the higher concentration used for the treatment (IC_{75}) and, in analogy with the *N*-(7-indazolyl)-benzenesulfonamide derivatives, strongly suggests a possible interaction of indazoles with tubulin or with other macromolecules involved in the mitosis events such as the aurora kinases or the γ-tubulin, whose inhibition blocks the normal progression of the phases of mitosis with a subsequent polyploidization effect [25,26].

A G2/M block was also present, although to a smaller extent, at a lower applied concentration (IC_{50}) for compound **16** and **8**, and was linked to a decrease of cells in the G0/G1 or S phases. In the same culture conditions all the other compounds showed a typical block in the G0/G1 phase with a concomitant decrease of cells in the following S phase. As known cell cycle progression and apoptosis are the result of the interaction of a great number of biomolecular factors, whose study was outside our present aims; their definition in our culture conditions could help to better understand the partially different behavior of our compounds and to eventually enhance their efficacy *in vitro*.

In conclusion, in this paper we have described the synthesis, the antiproliferative and apoptotic activities of new *N*-(6(4)-indazolyl)-benzenesulfonamides and *N*-(7-ethoxy-6(4)-indazolyl)-benzenesulfonamides. Some selected compounds exerted a pharmacologically



Scheme 5.

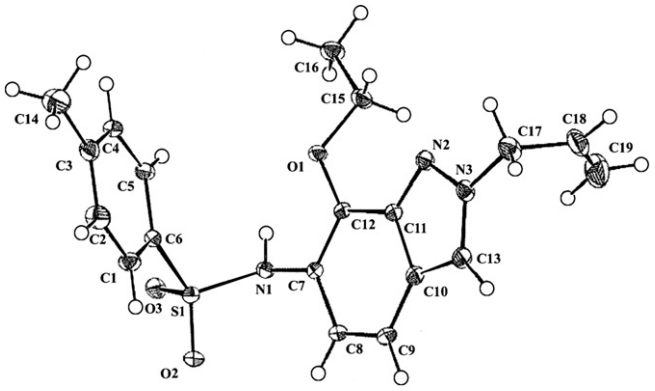


Fig. 3. ORTEP drawing of **14**, showing the molecular numbering scheme. Ellipsoids are drawn at the 30% probability level.

interesting antiproliferative/apoptotic activity against human and murine cell lines. Although with different characteristics all compounds induced a block of cells in the G2/M phase of the cell 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 suggest that our indazoles may represent good lead compounds for further studies aimed at the synthesis of new and more active anti-tumor agents.

5. Experimental

5.1. Chemistry

Melting points were determined using a Büchi-Tottoli apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 and solution (unless otherwise specified) with TMS as an internal reference using a Bruker AC 300 (^{1}H) or 75 MHz (^{13}C) instruments. Chemical shifts are given in δ parts per million (ppm). 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 (254 nm). Commercial reagents were used without further purification unless stated.

5.1.1. Synthesis of N-(1H-indazol-4-yl)-4-methylbenzenesulfonamides (**2a**–**c**) and N-(ethoxy-1H-indazol-4-yl)-4-methylbenzenesulfonamides (**3a**–**c**)

A mixture of 2-alkyl-4-nitroindazole (**3a**–**b**) (1.22 mmol) and anhydrous $SnCl_2$ (1.1 g, 6.1 mmol) in 25 mL of absolute EtOH was heated at 60 °C. After reduction, the starting material has disappeared and the solution is 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-methylbenzenesulfonyl 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).

Table 1
Concentrations of indazole compounds inhibiting 50% proliferation (IC_{50}) in A2780, A549 and P388 cells.

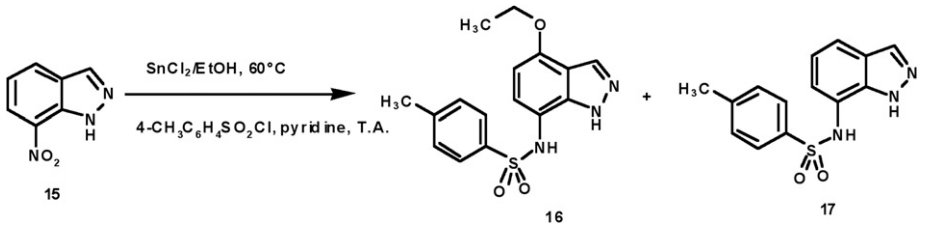
Compounds	Cell lines		
	A2780	A549	P388
2a	>100[a]	>100	85.5 ± 5.5
3a	55.1 ± 2.8	58.5 ± 3.4	43.5 ± 5.1
2b	70.9 ± 4.01	82.4 ± 19.5	53.9 ± 2.4
3b	10.5 ± 2.12	17.5 ± 4.0	5.21 ± 1.40
2c	0.86 ± 0.22	1.83 ± 0.52	0.50 ± 0.09
3c	1.23 ± 0.36	5.83 ± 1.83	0.58 ± 0.17
6d	6.62 ± 1.56	6.88 ± 2.1	3.40 ± 1.43
7e	61.6 ± 1.8	100.4 ± 2.7	51.8 ± 2.1
8	8.34 ± 1.53	14.4 ± 3.4	4.19 ± 1.70
10b	74.7 ± 12.8	103.6 ± 13.0	51.3 ± 1.2
11a	86.6 ± 6.7	>100	71.3 ± 5.7
11b	101.4 ± 3.9	>100	66.6 ± 4.9
13	4.89 ± 1.51	11.9 ± 0.5	4.41 ± 0.52
14	>100	>100	68.9 ± 1.4
16	8.00 ± 1.86	25.1 ± 8.6	5.70 ± 0.29

[a] Data represent the means ± SD of 3–9 experiments.

*5.1.1.1. N-(1H-4-Indazolyl)-4-methylbenzenesulfonamide (**2a**).* Yield: 35%; mp: 170–172 °C; IR (KBr, cm^{-1}): 3340, 3235 (NH), 1595 (CN), 1335, 1160 (SO_2); ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3), 6.92 (dd, 1H, J = 2.1 Hz and 6.1 Hz), 7.16 (d, 2H, J = 6.2 Hz), 7.28 (d, 2H, J = 8.3 Hz), 7.68 (d, 2H, J = 8.3 Hz), 8.22 (s, 1H), 10.51 (s, 1H, NH), 13.05 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.3 (CH_3), 106.7 (CH), 111.0 (CH), 117.3 (C), 126.9 (2CH), 127.2 (CH), 130.1 (2CH), 130.5 (C), 132.4 (CH), 137.3 (C), 141.4 (C), 143.7 (C); MS m/z = 288 [M + 1]$^+$. Anal. Calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 5.56; N, 14.62. Found: C, 58.76; H, 5.42; N, 14.77.

*5.1.1.2. N-(7-Ethoxy-1H-4-indazolyl)-4-methylbenzenesulfonamide (**3a**).* Yield: 33%, mp: 210–212 °C; IR (KBr, cm^{-1}): 3340, 3220 (NH), 1585 (CN), 1338, 1165 (SO_2); ^{1}H NMR (DMSO-d_6): δ 1.37 (t, 3H, CH_3, J = 6.9 Hz), 2.28 (s, 3H, CH_3), 4.12 (q, 2H, OCH_2, J = 6.9 Hz), 6.64 (d, 1H, J = 8.1 Hz), 6.72 (d, 1H, J = 8.1 Hz), 7.25 (d, 2H, J = 8.1 Hz), 7.58 (d, 2H, J = 8.1 Hz), 7.97 (s, 1H), 10.06 (s, 1H, NH), 13.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 15.1 (CH_3), 21.3 (CH_3), 64.2 (CH_2O), 106.1 (CH), 114.9 (CH), 120.6 (C), 122.3 (C), 127.2 (2CH), 129.9 (2CH), 132.7 (CH), 132.9 (C), 137.4 (C), 142.4 (C), 143.4 (C); MS m/z = 332 [M + 1]$^+$. Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68. Found: C, 57.86; H, 5.28; N, 12.57.

*5.1.1.3. N-(3-Bromo-1H-4-indazolyl)-4-methylbenzenesulfonamide (**2b**).* Yield: 25%; mp: 150–152 °C; IR (KBr, cm^{-1}): 3335, 3225 (NH),



Scheme 6.

Table 2
Triggering of apoptosis by indazole compounds, as evaluated by nuclear morphological analysis after DAPI staining.

Drug concentration		Anticancer compounds						
		3b	**13**	**2c**	**3c**	**6d**	**16**	**8**
A2780	CTR	2.8 ± 1.0[a]	3.2 ± 2	1.8 ± 1.3	2.2 ± 1.2	1.5 ± 1.4	1.8 ± 1.6	3.0 ± 1.0
	IC_{90}[b]	34.3 ± 3.4	66.5 ± 15	46.6 ± 10.1	36.8 ± 5.9	24.0 ± 6.6	27.0 ± 6.2	51.5 ± 10.8
	IC_{50}	12.8 ± 3.5	19.3 ± 6.5	23.6 ± 4.4	23.3 ± 5.3	7.3 ± 2.5	12.5 ± 2.1	33.5 ± 6.5
A549	CTR	1.8 ± 0.5	1.6 ± 0.5	2.0 ± 0.8	1.7 ± 0.5	1.0 ± 1.3	0.9 ± 1.2	3.0 ± 2.6
	IC_{90}	32.3 ± 9.4	29.2 ± 8.9	70.8 ± 12.3	64.5 ± 7.0	41.2 ± 5.8	24.9 ± 6.7	54.3 ± 1.5
	IC_{50}	2.8 ± 1.0	51.2 ± 11.2	34.3 ± 7.5	34.8 ± 9.7	28.0 ± 4.6	18.8 ± 7.7	22.3 ± 4.0
P388	CTR	2.5 ± 1.9	1.5 ± 0.5	2.5 ± 2.0	1.5 ± 1.0	1.5 ± 0.6	1.8 ± 1.0	1.5 ± 1.3
	IC_{90}	87.5 ± 6.4	68.7 ± 9.4	74.0 ± 12.5	98.0 ± 1.1	91.3 ± 3.9	56.0 ± 12.2	94.3 ± 4.9
	IC_{50}	9.8 ± 3.5	30.0 ± 12.3	11.5 ± 5.6	47.3 ± 10.5	37.3 ± 11.8	19.0 ± 6.7	10.3 ± 3.4

[a] The means ± SD (4–6 data) express the percentage of apoptotic cells.
[b] IC_{90} ranged from 0.97 μM to 957 μM. When IC_{90}s were higher than 200 μM (A2780, **13**, **16**, **8**, **6d**; A549, **13**, **8**, **6d**) this concentration was used to treat cells.

1595 (CN), 1340, 1156 (SO_2); ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 6.57 (d, 1H, J = 7.4 Hz), 7.25 (t, 1H, J = 7.4 Hz), 7.35 (d, 2H, J = 8.1 Hz), 7.41 (d, 1H, J = 7.3 Hz), 7.63 (d, 2H, J = 8.1 Hz), 9.69 (s, 1H, NH), 13.50 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.4 (CH_3), 109.8 (CH), 117.9 (C), 118.3 (CH), 118.7 (C), 127.4 (2CH), 127.9 (CH), 129.3 (C), 130.0 (2CH), 138.1 (C), 142.8 (C), 143.5 (C); MS m/z = 367 (^{79}Br) $[M + 1]^+$, 369 (^{81}Br) $[M + 3]^+$. Anal. Calcd. for $C_{14}H_{12}BrN_3O_2S$: C, 45.91; H, 3.30; N, 11.47. Found: C, 45.78; H, 3.44; N, 11.32.

*5.1.1.4. N-(3-Bromo-7-ethoxy-1H-4-indazolyl)-4-methyl-benzenesulfonamide (**3b**).* Yield: 30%; mp: 118–120 °C; IR (KBr, cm^{-1}): 3345, 3231 (NH), 1600 (CN), 1338, 1162 (SO_2); ^{1}H NMR (DMSO-d_6): δ 1.39 (t, 3H, CH_3, J = 7.0 Hz), 2.36 (s, 3H, CH_3), 4.15 (q, 2H, OCH_2, J = 7.0 Hz), 6.24 (d, 1H, J = 8.1 Hz), 6.38 (d, 1H, J = 8.1 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.57 (d, 2H, J = 8.0 Hz), 9.49 (s, 1H, NH), 13.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 15.0 (CH_3), 21.4 (CH_3), 64.5 (CH_2O), 106.6 (CH), 118.0 (C), 119.2 (C), 120.8 (C), 121.0 (CH), 127.4 (2CH), 130.0 (2CH), 134.4 (C), 138.4 (C), 142.1 (C), 143.5 (C); MS m/z = 411 (^{79}Br) $[M + 1]^+$, 413 (^{81}Br) $[M + 3]^+$. Anal. Calcd. for $C_{16}H_{16}BrN_3O_3S$: C, 46.84; H, 3.93; N, 10.24. Found: C, 46.96; H, 3.82; N, 10.37.

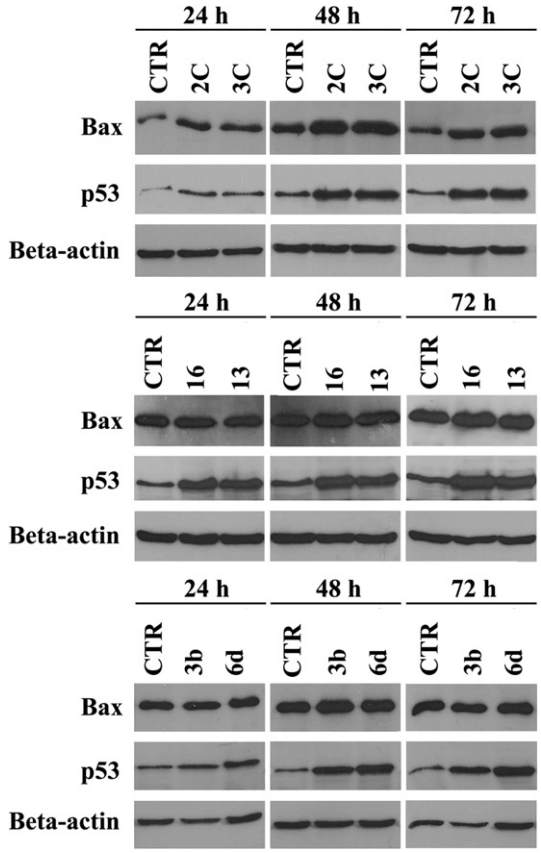


Fig. 4. Representative western blot analysis of p53 and bax after exposure of A2780 cells to compounds **3b**, **2c**, **3c**, **13**, **6d**, and **16** for 24, 48 and 72 h. Our molecules were administered at their specific IC_{50}s, as detected in MTT assays. Densitometric normalization was performed using the expression signal obtained by an anti-β-actin monoclonal antibody. Prestained molecular markers were always included as references. Fifty μg of protein extract from control and treated A2780 cells were seeded in each lane.

*5.1.1.5. N-(3-Chloro-1H-4-indazolyl)-4-methoxy-benzenesulfonamide (**2c**).* Yield: 27%; mp: 56–58 °C; IR (KBr, cm^{-1}): 3340, 3250 (NH), 1590 (CN), 1325, 1162 (SO_2); ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H, CH_3O), 6.78 (d, 1H, J = 7.8 Hz), 7.14 (t, 1H, J = 7.8 Hz), 6.72 (d, 2H, J = 8.4 Hz), 7.42 (d, 1H, J = 7.8 Hz), 7.60 (d, 2H, J = 8.4 Hz), 10.09 (s, 1H, NH), 13.25 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 55.5 (CH_3O), 105.8 (CH), 110.2 (CH), 112.1 (C), 114.0 (2CH), 126.7 (CH), 129.4 (2CH), 130.4 (C), 131.6 (C), 135.4 (C), 142.6 (C), 163.4 (C); MS m/z = 338 (^{79}Cl) $[M + 1]^+$, 339 (^{37}Cl) $[M + 3]^+$. Anal. Calcd. for $C_{14}H_{12}ClN_3O_3S$: C, 49.78; H, 3.58; N, 12.44. Found: C, 49.92; H, 3.49; N, 12.57.

*5.1.1.6. N-(3-Chloro -7-ethoxy-1H-4-indazolyl)-4-methoxy-benzenesulfonamide (**3c**).* Yield: 40%; mp: 68–70 °C; IR (KBr, cm^{-1}): 3338, 3260 (NH), 1580 (CN), 1345, 1150 (SO_2); ^{1}H NMR ($CDCl_3$): δ 1.47 (t, 3H, CH_3, J = 7.0 Hz), 3.77 (s, 3H, CH_3O), 4.16 (q, 2H, OCH_2, J = 7.0 Hz), 6.66 (d, 1H, J = 8.1 Hz), 6.79 (d, 2H, J = 8.6 Hz), 7.22 (d, 1H, J = 8.1 Hz), 7.64 (d, 2H, J = 8.6 Hz), 9.85 (s, 1H, NH), 13.1 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.7 (CH_3), 55.5 (CH_3O), 64.3 (CH_2O), 106.5 (C), 107.2 (CH), 112.1 (C), 114.0 (2CH), 116.5 (CH), 121.4 (C), 129.4 (2CH), 130.5 (C), 134.2 (C), 142.2 (C), 163.1 (C); MS m/z = 382 (^{35}Cl) $[M + 1]^+$, 384 (^{37}Cl) $[M + 3]^+$. Anal. Calcd. for $C_{16}H_{16}ClN_3O_4S$: C, 50.33; H, 4.22; N, 11.00. Found: C, 50.48; H, 4.11; N, 11.07.

*5.1.2. Preparation of N-alkylated indazoles **4** and **5***

To a solution of 4-nitroindazole **1a** (6.13 mmol) in THF (30 mL) cooled at 0 °C was added K_2CO_3 (9.2 mmol). After 15 min at 0 °C, allyl bromide/methyl iodide (6.13 mmol) was added dropwise. The solution was stirred for 16–20 h and 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).

*5.1.2.1. 1-Methyl-4-nitro-1H-indazole (**4d**).* Yield: 62%; mp 123–125 °C. ^{1}H NMR ($CDCl_3$): δ 4.17 (s, 3H, NCH_3), 7.50 (t, 1H, J = 8.0 Hz), 7.76 (d, 1H, J = 8.5 Hz), 8.13 (d, 1H, J = 8.0 Hz), 8.58 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 36.1 (NCH_3), 116.1 (CH-5), 117.0 (C-3a), 118.2 (CH-7), 125.4 (CH-6), 132.6 (CH-3), 140.5, 141.5 (C-4, C-7a).

Table 3
Percentage of A2780 cells in the different cell cycle phases after treatment for 72 h with the IC_{50} and IC_{75} of indazole compounds.

Cell cycle phases	IC_{50}							
	CTR	**3b**	**2c**	**3c**	**16**	**6d**	**13**	**8**
G0/G1	51.0 ± 6.2[a]	61.5 ± 3.3	56.1 ± 4.1	62.7 ± 0.7	53.7 ± 1.9	65.0 ± 4.2	66.0 ± 3.8	43.5 ± 2.9
S	25.9 ± 4.7	14.2 ± 2.9	18.0 ± 3.7	10.4 ± 4.6	12.6 ± 2.6	11.3 ± 5.3	9.8 ± 0.9	27.3 ± 5.0
G2/M	23.1 ± 2.5	24.3 ± 3.4	25.9 ± 1.6	26.9 ± 4.6	33.7 ± 3.2	23.7 ± 1.9	24.2 ± 4.1	29.3 ± 2.1
Cell cycle phases	IC_{75}[b]							
	CTR	**3b**	**2c**	**3c**	**16**	**6d**	**13**	**8**
G0/G1	51.0 ± 6.2	10.2 ± 1.1	9.4 ± 0.4	10.8 ± 1.8	8.9 ± 0.5	9.1 ± 1.0	10.8 ± 1.7	9.9 ± 2.3
S	25.9 ± 4.7	10.3 ± 1.0	7.8 ± 0.8	8.4 ± 6.9	8.2 ± 3.5	15.0 ± 2.1	5.5 ± 0.4	13.7 ± 3.1
G2/M	23.1 ± 2.5	71.5 ± 2.1	70.1 ± 0.2	69.6 ± 3.7	71.4 ± 1.5	69.2 ± 1.1	72.7 ± 1.4	64.5 ± 1.1
Cells with >4n	–	8.1 ± 1	12.8 ± 0.1	11.3 ± 1.4	11.6 ± 1.6	6.8 ± 0.9	11.1 ± 0.6	12.0 ± 0.4

[a] The means ± SD (3–4 data) express the percentage of cells in the various phases of the cell cycle.
[b] The IC_{75}s ranged from 6.8 μM to 102.3 μM.

*5.1.2.2. 2-Methyl-4-nitro-2H-indazole (**5d**).* Yield: 34%; mp 75–77 °C. ^{1}H NMR ($CDCl_3$): δ 4.31 (s, 3H, NCH_3), 7.40 (t, 1H, *J* = 8.0 Hz), 8.06 (d, 1H, *J* = 8.2 Hz), 8.16 (d, 1H, *J* = 8.0 Hz), 8.54 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 40.9 (NCH_3), 120.6 (CH-5), 115.1 (C-3a), 124.4 (CH-7), 125.3, 125.8 (CH-6, CH-3), 140.5 (C-4), 149.9 (C-7a).

*5.1.2.3. 1-Allyl-4-nitro-1H-indazole (**4e**).* Yield: 54; mp 63–65 °C. ^{1}H NMR ($CDCl_3$): δ 5.07–5.10 (m, 2H, NCH_2), 5.15–5.26 (m, 2H, =CH_2), 5.95–6.08 (m, 1H, =CH), 7.44 (t, 1H, *J* = 7.8 Hz), 7.75 (d, 1H, *J* = 8.4 Hz), 8.04 (d, 1H, *J* = 7.8 Hz), 8.51 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 52.2 (NCH_2), 116.5 (CH-5), 117.1 (C-3a), 118.2 (CH-7), 118.5 (=CH_2), 125.4 (CH-6), 132.0 (=CH), 132.8 (CH-3), 140.6, 141.0 (C-4, C-7a).

*5.1.2.4. 2-Allyl-4-nitro-2H-indazole (**5e**).* Yield: 30; mp 68–70 °C. ^{1}H NMR ($CDCl_3$): δ 5.10–5.13 (m, 2H, NCH_2), 5.35–5.42 (m, 2H, =CH_2), 6.09–6.22 (m, 1H, =CH), 7.37 (t, 1H, *J* = 7.8 Hz), 8.07 (d, 1H, *J* = 8.4 Hz), 8.15 (d, 1H, *J* = 7.8 Hz), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.7 (NCH_2), 115.0 (C-3a), 120.4 (=CH_2), 120.6 (CH-5), 124.2 (CH-3), 124.4 (CH-7), 126.0 (CH-6), 131.4 (=CH), 140.6 (C-4), 149.9 (C-7a).

*5.1.3. Synthesis of sulfonamides (**6d**,**e**) and (**7d**,**e**)*

These compounds were prepared from 1-alkyl-4-nitroindazole (**4d**,**e**) by using the same procedure applied to 3-halogeno-4-nitroindazoles (**1a**–**c**).

*5.1.3.1. N-(1-Methyl-2H-indazol-4-yl)-4-methylbenzenesulfonamide (**6d**).* Yield: 75%, mp 204–206 °C. ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 3.92 (s, 3H, NCH_3), 6.90 (d, 1H, *J* = 7.2 Hz), 7.20–7.28 (m, 4H), 7.65 (d, 2H, *J* = 7.4 Hz), 8.15 (s, 1H, H-3), 10.52 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.4 (CH_3), 35.9 (NCH_3), 106.2 (CH), 110.9 (CH), 117.7 (C), 127.0 (CH), 127.2 (2CH), 130.1 (2CH), 130.6 (C), 131.2 (CH), 137.2 (C), 141.0 (C), 143.8 (C). MS: *m*/*z* 302 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{15}N_3O_2S$: C, 59.78; H, 5.02; N, 13.94. Found: C, 59.92; H, 5.14; N, 13.81.

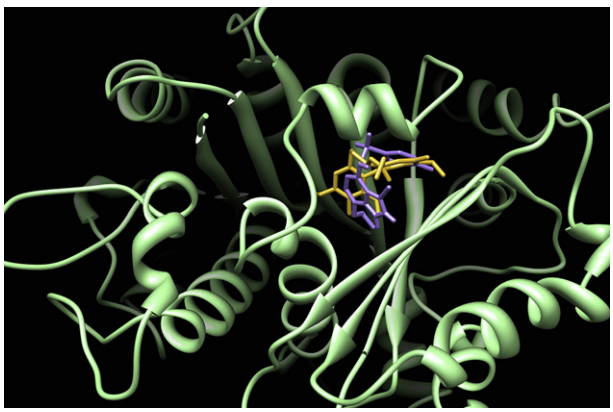


Fig. 5. Representation of the interaction between compound **3b** (violet) and colchicine (yellow) and β-tubulin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*5.1.3.2. N-(1-Methyl-7-ethoxy-2H-indazol-4-yl)-4-methylbenzenesulfonamide (**7d**).* Yield: 14%, mp 190–192 °C. ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, CH_3, *J* = 7.2 Hz), 2.21 (s, 3H, CH_3), 3.90 (s, 3H, NCH_3), 4.10 (q, 2H, CH_2O, *J* = 7.2 Hz), 6.45 (d, 1H, H-6, *J* = 8.0 Hz), 6.60 (d, 1H, H-5, *J* = 8.0 Hz), 7.15 (d, 2H, ArH, *J* = 7.8 Hz), 7.45 (d, 2H, ArH, *J* = 7.8 Hz), 8.14 (s, 1H, H-3), 10.34 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 15.1 (CH_3), 21.5 (CH_3), 36.1 (NCH_3), 63.0 (CH_2O), 104.5 (CH), 116.8 (CH), 117.4 (C), 127.2 (2CH), 129.8 (2CH), 130.6 (C), 132.0 (CH), 136.9 (C), 142.5 (C), 143.1 (C), 148.2 (C). MS: *m*/*z* 346 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.16. Found: C, 59.24; H, 5.40; N, 12.25.

*5.1.3.3. N-(1-Allyl-2H-indazol-4-yl)-4-methylbenzenesulfonamide (**6e**).* Yield: 68%, mp 124–126 °C. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.86–4.92 (m, 2H, NCH_2), 5.30–5.33 (m, 2H, =CH_2), 5.97–6.07 (m, 1H, =CH), 6.97 (d, 1H, *J* = 8.1 Hz), 7.18–7.21 (m, 3H), 7.43 (d, 1H, *J* = 8.0 Hz), 7.55 (d, 2H, *J* = 8.4 Hz), 7.99 (s, 1H, H-3), 8.38 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.5 (CH_3), 53.2 (NCH_2), 113.5 (CH), 117.1 (=CH_2), 121.0 (C), 127.1 (CH), 127.4 (2CH), 128.0 (CH), 128.3 (C), 129.7 (2CH), 130.9 (CH), 133.8 (CH), 135.8 (C), 136.4 (C), 144.3 (C). MS: *m*/*z* 328 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{17}N_3O_2S$: C, 62.37; H, 5.23; N, 12.83. Found: C, 62.48; H, 5.30; N, 12.72.

*5.1.3.4. N-(1-Allyl-7-ethoxy-2H-indazol-4-yl)-4-methylbenzenesulfonamide (**7e**).* Yield: 18%, mp 133–135 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (t, 3H, CH_3, *J* = 7.0 Hz), 2.33 (s, 3H, CH_3), 4.11 (q, 2H, CH_2O, *J* = 7.0 Hz), 4.94–5.10 (m, 2H, NCH_2), 5.18–5.23 (m, 2H, =CH_2), 5.94–6.05 (m, 1H, =CH), 6.54 (d, 1H, H-6, *J* = 8.1 Hz), 6.84 (d, 1H, H-5, *J* = 8.1 Hz), 7.14 (d, 2H, *J* = 7.8 Hz), 7.61 (d, 2H, *J* = 7.8 Hz), 7.78 (s, 1H, H-3), 8.84 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.7 (CH_3), 21.5 (CH_3), 53.8 (NCH_2), 64.2 (CH_2O), 106.3 (CH), 117.0 (=CH_2), 117.5 (CH), 121.1 (C), 122.3 (C), 127.3 (2CH), 129.5 (2CH), 130.6 (CH), 131.4 (C), 134.1 (CH), 136.2 (C), 143.7 (C), 144.2 (C). MS: *m*/*z* 372 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{21}N_3O_3S$: C, 61.44; H, 5.70; N, 11.31. Found: C, 61.56; H, 5.52; N, 11.46.

*5.1.4. N-(2-Methyl-7-ethoxy-2H-indazol-4-yl)-4-methylbenzenesulfonamide (**8**)*

This compound was prepared from 2-Methyl-4-nitro-2*H*-indazole (**5d**) by using the same procedure applied to 3-halogeno-4-nitroindazoles (**1a**–**c**).

Yield: 72%, mp 66–68 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (t, 3H, CH_3, *J* = 7.2 Hz), 2.32 (s, 3H, CH_3), 4.00 (s, 3H, NCH_3), 4.15 (q, 2H, CH_2O, *J* = 7.2 Hz), 6.34 (d, 1H, H-6, *J* = 8.0 Hz), 6.56 (d, 1H, H-5, *J* = 8.0 Hz),

7.12 (d, 2H, $J = 7.8$ Hz), 7.58 (d, 2H, $J = 7.8$ Hz), 8.38 (s, 1H, H-3), 10.14 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.6 (CH_3), 21.3 (CH_3), 40.4 (NCH_3), 63.9 (CH_2O), 103.1 (CH), 117.8 (CH), 120.3 (C), 124.1 (CH), 127.3 (2CH), 129.5 (2CH), 130.6 (C), 135.6 (C), 142.2 (C), 143.7 (C), 147.9 (C). MS: m/z 346 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.16. Found: C, 59.01; H, 5.65; N, 12.20.

*5.1.5. Synthesis of compounds (**10a**,**b**) and (**11a**,**b**)*

These compounds were prepared from 3-halogeno-6-nitroindazoles (**9a**,**b**) by using the same procedure applied to 3-halogeno-4-nitroindazoles (**1a**–**c**).

*5.1.5.1. 4-Methyl-N-[1-(toluene-4-sulfonyl)-1H-indazol-6-yl]-benzenesulfonamide (**10a**).* Yield: 27%. Mp 140–142 °C. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.20–7.28 (m, 4H), 7.49–7.68 (m, 2H), 7.79–7.86 (m, 3H), 8.05 (d, 2H), 8.41 (s, 1H, H-3), 9.03 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.6 (CH_3), 21.7 (CH_3), 103.3 (CH), 117.8 (CH), 122.2 (CH), 122.5 (C), 127.2 (2CH), 127.6 (2CH), 129.8 (2CH), 129.9 (2CH), 134.3 (C), 135.9 (C), 138.5 (C), 140.7 (C), 141.0 (CH), 144.2 (C), 145.5 (C). MS: m/z 442 $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{19}N_3O_4S_2$: C, 57.13; H, 4.34; N, 9.52. Found: C, 57.21; H, 3.20; N, 9.68.

*5.1.5.2. N-(1H-Indazol-6-yl)-4-methylbenzenesulfonamide (**11a**).* Yield: 60%. Mp 166–168 °C. ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H, CH_3), 6.90 (dd, 1H, $J = 8.6$ Hz, 1.4 Hz), 7.24 (t, 1H, $J = 8.6$ Hz), 7.29 (d, 2H, $J = 8.0$ Hz), 7.58 (dd, 1H, $J = 8.6$ Hz, 1.4 Hz), 7.65 (d, 2H, $J = 8.0$ Hz), 7.93 (s, 1H, H-3), 10.37 (s, 1H, NH), 12.87 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.4 (CH_3), 100.2 (CH), 115.2 (CH), 120.2 (C), 121.7 (CH), 127.1 (2CH), 130.1 (2CH), 133.8 (CH), 136.5 (C), 137.0 (C), 140.6 (C), 143.7 (C). MS: m/z 288 $[M + 1]^+$. Anal. Calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.71; H, 4.42; N, 14.68.

*5.1.5.3. 4-Methyl-N-[1-(toluene-4-sulfonyl)-3-bromo-1H-indazol-6-yl]-benzenesulfonamide (**10b**).* Yield: 40%; mp 180–182 °C. ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.21 (d, 1H, $J = 8.4$ Hz), 7.35 (t, 1H, $J = 8.4$ Hz), 7.50 (d, 1H, $J = 8.4$ Hz), 7.61 (d, 2H, $J = 8.1$ Hz), 7.73 (d, 2H, $J = 8.1$ Hz), 10.85 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.4 (CH_3), 21.6 (CH_3), 101.9 (CH), 118.6 (CH), 121.8 (C), 122.4 (CH), 127.3 (2CH), 127.4 (2CH), 130.3 (2CH), 130.8 (2CH), 131.6 (C), 133.4 (C), 136.8 (C), 141.5 (C), 141.8 (C), 144.3 (C), 146.8 (C). MS: m/z 521 (^{79}Br) $[M + 1]^+$, 523 (^{81}Br) $[M + 3]^+$. Anal. Calcd. for $C_{21}H_{18}BrN_3O_4S_2$: C, 48.47; H, 3.49; N, 8.07. Found: C, 48.64; H, 3.34; N, 8.18.

*5.1.5.4. N-(3-Bromo-1H-indazol-6-yl)-4-methylbenzenesulfonamide (**11b**).* Yield: 32%. Mp 218–220 °C. ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3), 6.97 (d, 1H, $J = 8.7$ Hz), 7.25 (t, 1H, $J = 8.7$ Hz), 7.36 (d, 2H, $J = 8.1$ Hz), 7.40 (d, 1H, $J = 8.7$ Hz), 7.63 (d, 2H, $J = 8.1$ Hz), 10.46 (s, 1H, NH), 13.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.4 (CH_3), 100.2 (CH), 116.1 (CH), 119.4 (C), 120.6 (CH), 120.8 (C), 127.1 (2CH), 130.2 (2CH), 136.9 (C), 138.1 (C), 141.8 (C), 143.9 (C). MS m/z = 367 (^{79}Br) $[M + 1]^+$, 369 (^{81}Br) $[M + 3]^+$. Anal. Calcd. for $C_{14}H_{12}BrN_3O_2S$: C, 45.91; H, 3.30; N, 11.47. Found: C, 45.82; H, 3.38; N, 11.38.

*5.1.6. Synthesis of sulfonamides (**13**) and (**14**)*

These compounds were prepared from 2-allyl-6-nitroindazole (**12**).

*5.1.6.1. N-(2-Allyl-2H-indazol-6-yl)-4-methylbenzenesulfonamide (**13**).* Yield: 54%, mp 138–140 °C. ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 4.94–4.98 (m, 2H, NCH_2), 5.13–5.21 (m, 2H, $=CH_2$), 5.99–6.08 (m, 1H, =CH), 6.82 (t, 1H, $J = 8.0$ Hz), 7.18 (d, 1H, $J = 8.2$ Hz), 7.29 (d, 2H, $J = 8.4$ Hz), 5.54 (d, 1H, $J = 8.0$ Hz), 7.63 (d, 2H, $J = 8.4$ Hz), 8.21 (s, 1H, H-3), 10.19 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.4 (CH_3), 55.5 (NCH_2), 105.8 (CH), 117.3 (CH), 118.9 ($=CH_2$), 119.1 (C), 122.0 (CH), 124.3 (CH), 127.2 (2CH), 130.1 (2CH), 135.9 (C), 137.1 (C), 143.6 (C), 148.4 (C). MS: m/z 328 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{17}N_3O_2S$: C, 62.37; H, 5.23; N, 12.83. Found: C, 62.50; H, 5.20; N, 12.68.

*5.1.6.2. N-(2-Allyl-7-ethoxy-2H-indazol-6-yl)-4-methylbenzenesulfonamide (**14**).* Yield: 36%, mp 108–110 °C. ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, CH_3, $J = 7.0$ Hz), 2.31 (s, 3H, CH_3), 4.26 (q, 2H, CH_2O, $J = 7.0$ Hz), 4.98–5.06 (m, 2H, NCH_2), 5.30–5.35 (m, 2H, $=CH_2$), 6.04–6.13 (m, 1H, =CH), 7.11 (d, 2H, $J = 8.4$ Hz), 7.27 (d, 1H, H-6, $J = 8.1$ Hz), 7.45 (d, 1H, H-5, $J = 8.1$ Hz), 7.61 (d, 2H, $J = 8.4$ Hz), 7.88 (s, 1H, H-3), 9.05 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 15.5 (CH_3), 21.5 (CH_3), 56.1 (NCH_2), 68.6 (CH_2O), 114.6 (CH), 118.5 (CH), 120.2 ($=CH_2$), 122.2 (C), 123.5 (CH), 124.0 (C), 127.1 (2CH), 129.4 (2CH), 131.7 (CH), 136.1 (C), 137.4 (C), 141.3 (C), 143.7 (C). MS: m/z 372 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{21}N_3O_3S$: C, 61.44; H, 5.70; N, 11.31. Found: C, 61.62; H, 5.60; N, 11.38.

*5.1.7. Synthesis of sulfonamides (**16**) and (**17**)*

These compounds were prepared from 7-nitroindazole (**15**).

*5.1.7.1. N-(4-ethoxy-1H-7-indazolyl)-4-methylbenzenesulfonamide (**16**).* Yield: 36%, mp: 170–172 °C; IR (KBr, cm^{-1}): 3335, 3240 (NH), 1580 (CN), 1335, 1160 (SO_2); ^{1}H NMR ($CDCl_3$): δ 1.45 (t, 3H, CH_3, $J = 7.2$ Hz), 2.30 (s, 3H, CH_3), 4.08 (q, 2H, OCH_2, $J = 7.2$ Hz), 6.24 (d, 1H, $J = 8.1$ Hz), 6.81 (d, 1H, $J = 8.1$ Hz), 7.11 (d, 2H, $J = 8.4$ Hz), 7.60 (d, 2H, $J = 8.4$ Hz), 8.15 (s, 1H), 10.15 (s, 1H, NH), 13.35 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.7 (CH_3), 21.5 (CH_3), 64.0 (CH_2O), 100.9 (CH), 112.6 (C), 116.1 (C), 120.8 (CH), 124.3 (C), 127.4 (2CH), 129.6 (2CH), 133.6 (CH), 135.4 (C), 138.9 (C), 144.0 (C); MS m/z = 332 $[M + 1]^+$. Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68. Found: C, 58.05; H, 5.12; N, 12.76.

*5.1.7.2. N-(1H-7-Indazolyl)-4-methylbenzenesulfonamide (**17**).* Yield: 35%; mp: 202–204 °C; IR (KBr, cm^{-1}): 3340, 3235 (NH), 1595 (CN), 1335, 1160 (SO_2); ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 6.99 (t, 1H, $J = 7.8$ Hz), 7.04 (d, 1H, $J = 7.8$ Hz), 7.14 (d, 2H, $J = 8.4$ Hz), 7.48 (d, 1H, $J = 7.8$ Hz), 7.66 (d, 2H, $J = 8.3$ Hz), 8.18 (s, 1H), 10.58 (s, 1H, NH), 13.93 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 21.3 (CH_3), 118.7 (CH), 122.0 (CH), 116.1 (C), 124.2 (C), 125.3 (CH), 127.5 (2CH), 129.8 (2CH), 131.7 (CH), 135.4 (C), 141.4 (C), 143.8 (C); MS m/z = 288 $[M + 1]^+$. Anal. Calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 5.56; N, 14.62. Found: C, 58.65; H, 5.51; N, 14.68.

5.2. Biology

5.2.1. Chemicals

Because of the low water solubility of our indazole compounds they were firstly dissolved in 100% dimethylsulfoxide (DMSO) to 100 mM concentration and subsequently diluted in fetal calf serum, the final DMSO concentrations on cells being 0.6% for **6d** and **7e** and 0.2% for all other compounds.

5.2.2. 3-(2,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

A2780 (human ovarian carcinoma) A549 (human lung adenocarcinoma) and P388 (murine leukemia) cells were plated at opportune densities/well (range: 900–1800/well) in order to maintain an exponential growth into 96-well microtiter plates. They were then centrifuged at 275 g for 2 min and maintained for about 6–8 h before treatment. Our indazole compounds were added in duplicate at the appropriate 10× concentrations for a minimum of 5 concentrations (1:10 fold serial dilutions) reaching a final volume of 200 μL. Opportune controls were always made. The MTT assay was performed as described elsewhere [27,28]. IC_{50}s were calculated by the analysis of single concentration–response curves. Each final mean ± SD was calculated from 3 to 9 experiments.

5.2.3. Visualization of apoptotic cells by 4′-6-diamidino-2-phenylindole (DAPI) staining

Cells were plated in 1 mL at different densities/well (range: 5000–10000/well) into 24-well microtiter plates for about 6–8 h, then all active indazole compounds were added in order to obtain their specific IC_{50} and IC_{90}, as determined with the MTT assay. After 3 days, floating and adherent cells were harvested and washed twice with cold PBS, fixed with 100 μL of 70% ethanol in PBS and maintained at 4 °C until analysis. Five μL of a solution of 10 μg/mL DAPI in water were added to the samples just before examination at the microscope, and the percentage of apoptotic segmented nuclei/cells stained with the fluorescent dye evaluated.

5.2.4. Western blot analysis

Analysis by western blots of p53, bax, and β-actin was carried on A2780 after treatment with the IC_{50}s of the active compounds **3b**, **2c**, **3c**, **13**, **16**, and **6d**, as determined by the MTT assay. At 24, 48 and 72 h culture aliquots of treated and untreated cells were harvested, washed twice with normal saline and treated at 4 °C for 30 min with a lysis buffer containing 1% Triton X-100, 0.15 M NaCl and 10 mM Tris (pH 7.4), 2 mg/mL aprotinin and 50 mg/mL phenylmethylsulphonyl fluoride. The protein concentration was determined by the Bradford method (Sigma Chemical Co., St. Louis, MO, USA). Fifty μg of protein were separated on a 12% polyacrylamide gel (SDS-PAGE), and then transferred onto a nitrocellulose membrane (Hybond C-Extra, Amersham Italia Srl, Milan, Italy). Protein loading was verified by Ponceau S staining. Nonspecific binding was inhibited by overnight incubation at 4 °C with 2% bovine albumin (Sigma) in TBST solution containing 0.15 M NaCl, 10 mM Tris (pH 8.0), and 0.05% Tween 20. Blots were probed with anti-p53 DO-1 1:2000, and anti-bax sc-20067 1:800 (Santa Cruz, CA, USA). After incubation with horseradish peroxidase-conjugated antimouse IgG, bands were visualized by chemiluminescent detection (ECL™ Western blotting analysis system, Amersham Italia Srl) as recommended by the suppliers. Prestained molecular weight markers (New England Biolabs, Beverly, MA, USA) were used as reference. Densitometric normalization was performed by using the expression signal obtained by an anti-β-actin monoclonal antibody (AC-74, 1:10,000, Sigma).

5.2.5. Cell cycle analysis

A2780 cells were also analyzed for the effect of active compounds **3b**, **2c**, **3c**, **13**, **6d**, **8**, and **16** on the cell cycle phases. Cells (1×10^4/mL in 10 mL) were treated in flask for 72 h with their IC_{50}s, then floating and adherent cells were harvested, washed twice with normal saline and fixed in 70% ethyl alcohol at −20 °C overnight. After fixation, cells were treated as described elsewhere [29] and analyzed using a FACSort flow cytometer (BD Bioscences, Mountain. View, CA). The ModFit LT software was then used to evaluate the distribution of cells in the various cell cycle phases.

5.2.6. Molecular docking

Molecular docking simulations were performed by the programs Autodock 4.2 and GOLD using, as a target, the atomic coordinates of β-tubulin [[30], PDB code 1SA0]. In a first phase, for each ligand, we performed a "blind docking": the docking of small molecules to their targets was done without *a priori* knowledge of the location of the binding site by the system. Each moiety was docked to the protein using the program Autodock with the protein considered as a rigid body and the ligands being flexible. The searching grid was extended over the whole receptor protein. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 Å. The search was carried out with the Lamarckian Genetic Algorithm: populations of 256 individuals with a mutation rate of 0.02 were evolved for 100 generations. Evaluation of the results was performed by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values, with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most promising bioactive candidate. When clusters of molecules were almost equipopulated, and their energy distribution is spread, the corresponding moiety was not considered a good ligand. In a second phase, we validated the correct positioning of the ligands within the active site cleft by using a second docking program, GOLD (CCDC, Cambridge, UK). The results obtained by GOLD confirmed what already achieved with Autodock.

5.2.7. Statistical analysis

The Student's *t* test was used for the statistical analysis of data.

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