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Synthesis and biological evaluation of *N*-(7-indazolyl)benzenesulfonamide derivatives as potent cell cycle inhibitors

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Abstract—We herein describe a new synthesis of *N*-(7-indazolyl)benzenesulfonamide derivatives. These compounds were evaluated for their antiproliferative activities toward L1210 murine leukemia cells. One of them, 4-methoxy-*N*-(3-chloro-7-indazolyl)benzenesulfonamide, was identified as the most potent with an IC_{50} of 0.44 μM.

1. Introduction

The sulfonamide derivatives are known for their numerous pharmacological activities, with among others, antibacterial, insulin-release stimulation, carbonic anhydrase inhibition, and diuretic and antithyroid properties.[1] In addition, recent papers have reported novel sulfonamide drugs with other properties such as endothelin receptor antagonists,[2] 5-HT_6 receptor antagonists,[3] β_3 adrenergic receptor agonists,[4] thrombin inhibitors,[5] and matrix metalloproteinase inhibitors.[6] E7010 (Fig. 1), which is an original antitumor agent with a sulfonamide moiety, was found to induce cell cycle arrest in the M phase and apoptosis. This compound, which inhibits microtubule assembly owing to its reversible binding to the colchicine binding site on tubulin,[7] was found to be active in vivo against various rodent tumors and human tumor xenografts.[8] E7010 was the first drug candidate among the antitumor sulfonamides to be synthesized by Owa and co-workers.[9] Various pharmacomodulations performed by the same team led to the identification of *N*-(7-indolyl)benzenesulfonamide derivatives with different effects on cell cycle progression of P388 murine leukemia cells compared to E7010.[10]

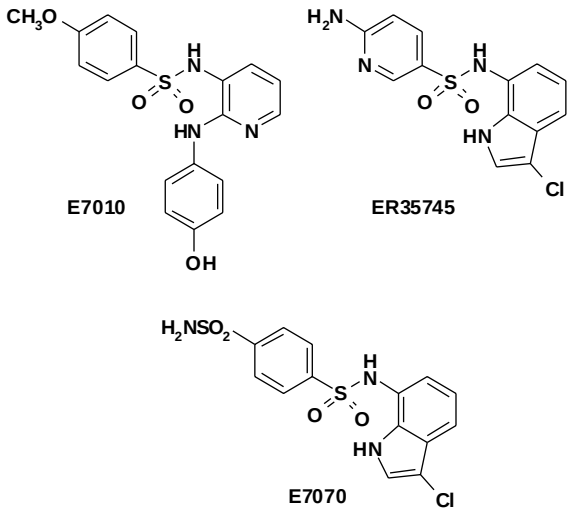

Figure 1.

Namely, accumulation of cells in the G1 phase was observed in a dose-dependent manner after a 24-h drug exposure. Growing interest for these G1-targeting sulfonamides has resulted in the discovery of *N*-(3-chloro-7-indolyl)-1,4-benzenesulfonamide E7070[11–13] (Fig. 1), which demonstrated excellent in vivo efficacy against

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various human tumor xenografts, for example, HCT116 colon carcinoma.

Studies performed with A549 cell line and its E7070-resistant subline A549/ER35745 further clarified that E7070 inhibits pRb phosphorylation, reduces protein expression of cyclin A, cyclin B1, CDK2, and CDC2, and represses CDK2 activity with the induction of p53 and p21 proteins, only in the parental A549 cells.[14] This compound also possesses a potent carbonic anhydrase inhibitory activity which may account for some of its antitumor properties.[15]

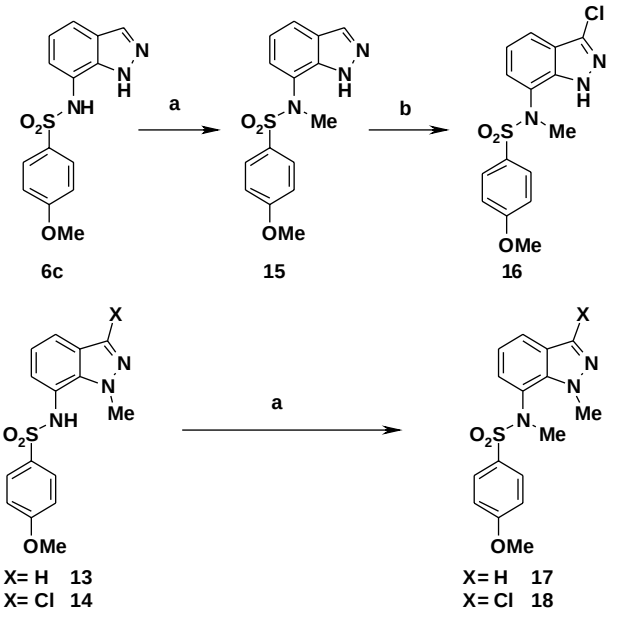
Recently, Owa and co-workers described the synthesis and antitumor activity of E7070 analogues containing a 3-pyridinesulfonamide moiety.[9,16] ER35745, which is a 6-amino-3-pyridinesulfonamide derivative, was found to display significant oral efficacy against the HCT116 human colon carcinoma xenograft in nude mice. Microarray-based gene expression analysis has shown that E7070 and ER-35745 could operate by the same mechanism of action.

In this paper, we describe the synthesis and in vitro biological evaluation on the murine L1210 and on the human DU145, HCT116, and HT29 (for DU145, HCT116, and HT29, see Section 4) cell lines of *N*-(7-indazolyl)benzenesulfonamides.

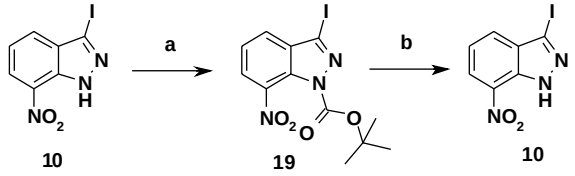
2. Chemistry

Eighteen new benzenesulfonamide derivatives have been prepared starting from 7-nitroindazole[17] (Schemes 1–4).

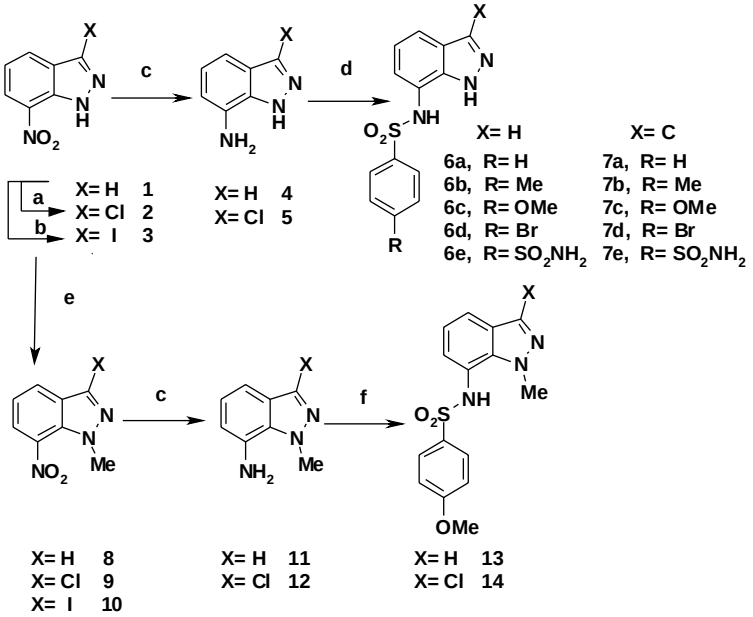
Compounds **6a–d** and **7a–d** were prepared according to the general route depicted in Scheme 1 from 7-nitroindazole.



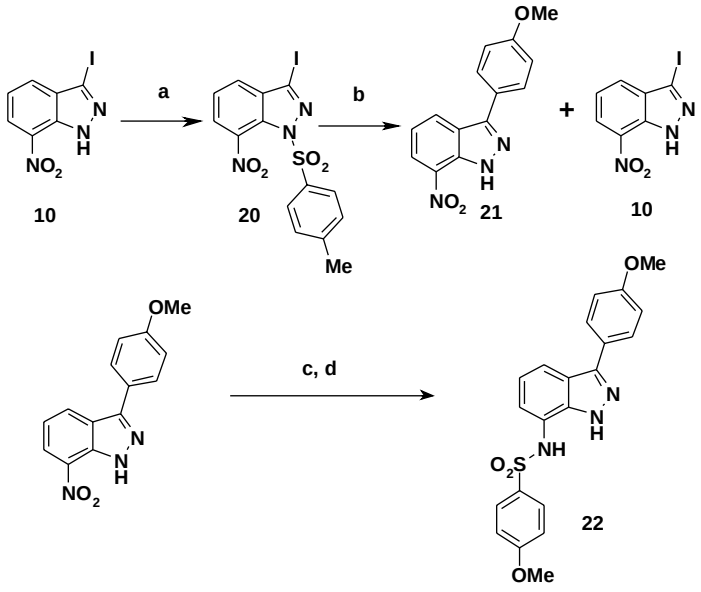
Scheme 2. Reagents and conditions: (a) MeI, KOH, acetone, 0 °C, 93–97%; (b) NaOCl, MeOH, NaOH (2 N), reflux, 98%.



Scheme 3. Reagents and conditions: (a) Boc_2O, DMAP, Et_3N, CH_2Cl_2, 93%; (b) 4-$MeOC_6H_4B(OH)_2$, Na_2CO_3, DME, $Pd(PPh_3)_4$, reflux, 95%.



Scheme 1. Reagents and conditions: (a) NaOCl, MeOH, NaOH (2 N), reflux, 98%; (b) I_2, KOH, DMF, rt, 95%; (c) H_2, Pd/C, MeOH; (d) arylsulfonyl chlorides, pyridine, rt, 63–95% (yields were calculated after two steps c and d); (e) MeI, KOH, acetone, 0 °C, 93–97%; (f) 4-methoxybenzenesulfonyl chloride, pyridine, rt, 92–93% (yields were calculated after two steps c and f).



Scheme 4. Reagents and conditions: (a) $PhSO_2Cl$, NaOH, $PHCH_2NEt_3Cl$, CH_2Cl_2, 76%; (b) 4-$MeOC_6H_4B(OH)_2$, Na_2CO_3, DME, $Pd(PPh_3)_4$, reflux, 45% (36% of **10**); (c) H_2, Pd/C, MeOH; (d) 4-methoxybenzenesulfonyl chloride, pyridine, rt, 33% (yield was calculated after two steps c and d).

Thus, after hydrogenation of **1** and **2** using 10% palladium on carbon in methanol, the corresponding amines **4** and **5** were immediately condensed with various arylsulfonyl chlorides in pyridine. Compounds **13** and **14** were synthesized in the same way. Compounds **6e** and **7e** were obtained by condensation of the corresponding amines with 4-sulfamoylbenzenesulfonyl chloride using 3 equivalents of pyridine in acetone. The treatment of 7-nitroindazole with sodium hypochlorite in the presence of sodium hydroxide (2 N) in MeOH at reflux afforded corresponding 3-chloro-7-nitroindazole **2**. Iodination of 7-nitroindazole using solid iodine and potassium hydroxide pellets in DMF gave the 3-iodo-7-nitroindazole **3**.

The 7-nitroindazole derivatives substituted at the 3-position were obtained in good yields. 7-Nitroindazole derivatives **1**–**3** have been alkylated with methyl iodide in the presence of potassium hydroxide in acetone. These reactions gave the corresponding *N*-alkylated derivatives **8**–**10**.

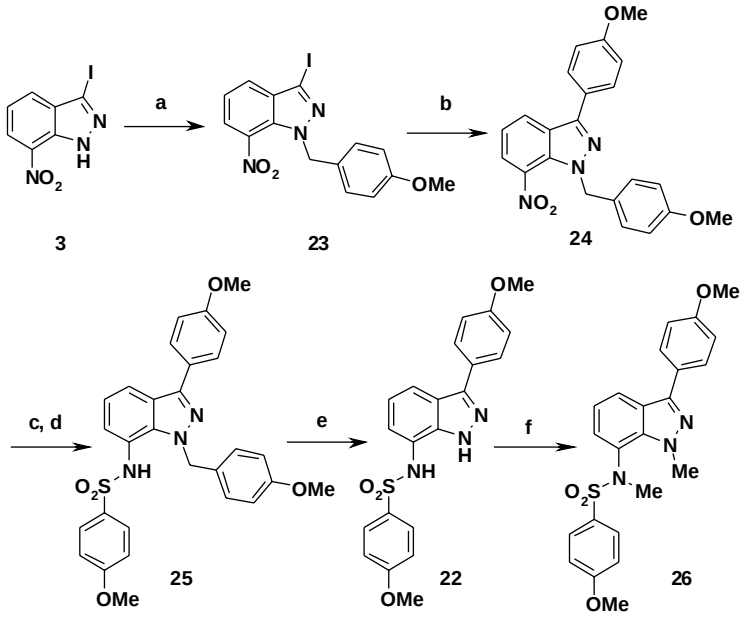
Reaction of **6c** with methyl iodide and potassium hydroxide in acetone gave the compound **15**, which was chlorinated to give **16**. In the same way, *N*-alkylation of **13** and **14** gave the compounds **17** and **18** (Scheme 2).

Suzuki cross-coupling reactions were used to introduce aryl groups at the 3-position. Our initial attempt to synthesize 4-methoxy-*N*-[3-(4-methoxyphenyl)-7-indazolyl]benzenesulfonamide **22** was performed under Suzuki cross-coupling conditions between 3-iodo-7-nitroindazole **3** and 4-methoxyphenyl boronic acid in the presence of catalytic amount of tetrakis(triphenylphosphine)palladium (0). Unfortunately, after 24 h in refluxing DME, no reaction was observed and we isolated only 3-iodo-7-nitroindazole **3** at the end of the reaction period in 95% yield. The same result was observed in the Suzuki cross-coupling reaction of 3-iodoindazoles.[18] Apparently, the Suzuki coupling of 3-iodo-7-nitroindazole **3** could need the protection of *N*-1, but treatment in the same conditions ($Pd(PPh_3)_4/Na_2CO_3$/DME at 92 °C) of 1-*N*-Boc-3-iodo-7-nitroindazole **19** gave only the deprotected parent compound **10** (Scheme 3).

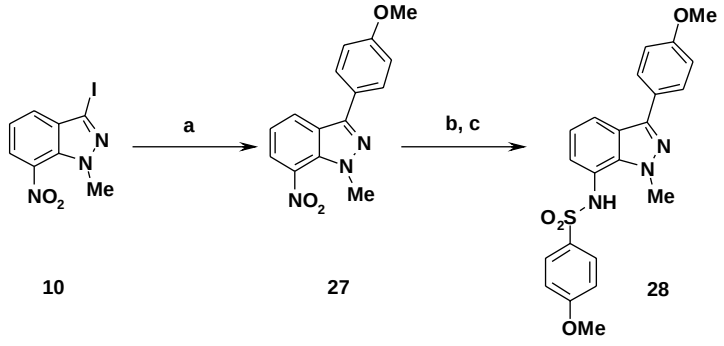
Replacement of the Boc protective group with a tosyl allowed the obtaining, in the same conditions, of the coupled product, but in a yield of only 45%. It is noteworthy that 36% of compound **10** was recovered (Scheme 4). The coupled product was hydrogenated in the presence of 10% palladium on carbon in methanol and then immediately treated with 4-methoxybenzenesulfonyl chloride in pyridine to gave desired product **22**, which was obtained with a relatively poor yield (33% after two steps).

An alternative synthetic approach led us to investigate the reactivity of 3-iodo-1-(4-methoxybenzyl)-7-nitroindazole **23**. This compound was obtained by protecting the NH group with 4-methoxybenzyl chloride in acetone at 0 °C in the presence of potassium hydroxide. The compound **24** was subsequently obtained by reaction of **23** with 4-methoxyphenyl boronic acid under Suzuki cross-coupling conditions in 80% yield (Scheme 5). After hydrogenation using 10% palladium on carbon in methanol, the corresponding crude amine was coupled with 4-methoxybenzenesulfonyl chloride in pyridine at rt to afford in good yield (76% after two steps) the 4-methoxy-*N*-[1-(4-methoxybenzyl)-3-(4-methoxyphenyl)-7-indazolyl]benzenesulfonamide **25** which, after deprotection in refluxing TFA,[19] gave the desired compound **22**. Alkylation of **22** with methyl iodide in acetone in the presence of potassium hydroxide at 0 °C provided the desired compound **26** in good yield.

Reaction of 3-iodo-1-methyl-7-nitroindazole **10** with 4-methoxyphenyl boronic acid under Suzuki-type reaction conditions gave compound **27** in 80% yield (Scheme 6). After reduction of the nitro by hydrogenation in the



Scheme 5. Reagents and conditions: (a) 4-Methoxybenzyl chloride, KOH, acetone, 0 °C, 90%; (b) 4-$MeOC_6H_4B(OH)_2$, Na_2CO_3, DME, $Pd(PPh_3)_4$, reflux, 80%; (c) H_2, Pd/C, MeOH; (d) 4-methoxybenzenesulfonyl chloride, pyridine, rt, 76% (after two steps c and d); (e) TFA, reflux, 97%; (f) MeI, KOH, acetone, 0 °C, 95%.



Scheme 6. Reagents and conditions: (a) 4-$MeOC_6H_6B(OH)_2$, Na_2CO_3, DME, $Pd(PPh_3)_4$, reflux, 80%; (b) H_2, Pd/C, MeOH; (c) 4-methoxybenzenesulfonyl chloride, pyridine, rt, 90%.

presence of 10% palladium on carbon in methanol, the corresponding crude amine was coupled with 4-methoxybenzenesulfonyl chloride in pyridine leading to 4-methoxy-*N*-[3-(4-methoxyphenyl)-1-methyl-7-indazolyl]benzenesulfonamide **28**.

3. Pharmacological results

3.1. Antiproliferative activity on the murine leukemia L1210 cell line

The antiproliferative properties of various *N*-(7-indazolyl)benzenesulfonamide derivatives on the murine leukemia L1210 cell line are reported in Table 1.

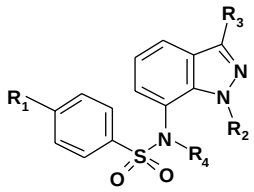
The *N*-(7-indazolyl)benzene sulfonamides **6b**–**c**, **7c** and **13**–**16** were the most potent with IC_{50} values ranging from 0.44 to 1.43 μM. These compounds are substituted on the phenylsulfonyl moiety (R_1 = Me or OMe) and bear relatively small substituents on position 3 of the indazole (R_3 = H or Cl).

Compounds that are not substituted on the aryl sulfonyl (R_1 = H, **6a**) or bear an aminosulfonyl group (**6e** and **7e**) are inactive with $IC_{50} > 50$ μM.

Compounds **22**, **26**, and **28**, with a 4-methoxyphenyl group on the indazole, were significantly less active than their analogues substituted with less bulky groups (H or Cl) with IC_{50} ranging from 14.2 to 29.1 μM. We can conclude that both positions 3 of indazole and 4 of the phenylsulfonamide are sensitive to the sizes of their substituents.

The perturbations of the cell cycles induced by the most potent compounds were studied on the L1210 cell line by flow cytometry. Except for **7c**, all of these compounds induced a marked accumulation in G2M + 8N phases of the cell cycle. This observation is typical of

Table 1. In vitro antiproliferative activity of *N*-(7-indazolyl)benzenesulfonamide derivatives on the murine L1210 cell line



Compound	R_1	R_2	R_3	R_4	IC_{50} (μM)	Cell cycle analysis
6a	H	H	H	H	>49.9	Not tested
6b	Me	H	H	H	1.43	93% G2M + 8N at 5 μM
6c	OMe	H	H	H	0.98	93% G2M + 8N at 2.5 μM
6d	Br	H	H	H	18.3	Not tested
6e	SO_2NH_2	H	H	H	68.5	Not tested
7a	H	H	Cl	H	51.5	Not tested
7b	Me	H	Cl	H	2.52	92% G2M + 8N at 5 μM
7c	OMe	H	Cl	H	0.923	27% G2M at 1 to 10 μM
7d	Br	H	Cl	H	25.3	Not tested
7e	SO_2NH_2	H	Cl	H	85.5	Not tested
13	OMe	Me	H	H	1.16	86% G2M + 8N at 5 μM
14	OMe	Me	Cl	H	1.16	88% G2M + 8N at 5 μM
15	OMe	H	H	Me	1.18	93% G2M + 8N at 5 μM
16	OMe	H	Cl	Me	0.44	92% G2M + 8N at 1 μM
18	OMe	Me	Cl	Me	1.9	90% G2M + 8N at 10 μM
22	OMe	H	4-MeO-C_6H_4	H	14.2	Not tested
26	OMe	Me	4-MeO-C_6H_4	Me	24.3	Not tested
28	OMe	Me	4-MeO-C_6H_4	H	29.1	Not tested

the modification of the cell cycle induced by numerous tubulin interacting drugs.

3.2. Antiproliferative activity on human DU145, HCT116, and HT29 cell lines

The antiproliferative properties of most of the compounds were also evaluated on human DU145, HCT116, and HT29 cell lines (results reported in Table 2).

Table 2. In vitro antiproliferative activity (IC_{50} in μM) of *N*-(7-indazolyl)benzenesulfonamide derivatives on the DU145, HCT116, and HT29 cell lines

Compound	DU145	HCT116	HT29
6a	NT	NT	NT
6b	2.39	1.2	1.2
6c	1.18	0.6	0.5
6d	NT	NT	NT
6e	88.25	37.2	78.8
7a	NT	NT	NT
7b	1.70	0.7	2.1
7c	0.96	0.6	0.9
7d	NT	NT	NT
7e	7.07	0.9	23.5
13	1.98	0.7	0.8
14	1.91	0.8	1.0
15	3.53	1.2	1.0
16	0.91	0.38	0.40
18	3.2	2.1	2.1
22	30.5	11.2	10.8
26	38.4	31.2	32.5
28	47.9	27.0	34.7

NT, Not tested.

There again, compounds **6c**, **7c**, and **16** were the most potent with IC_{50} values ranging from 0.38 to 1.18 μM on human DU145, HCT116, and HT29 cell lines.

In conclusion, we have described here the synthesis and the antiproliferative activities of new *N*-(7-indazolyl)benzenesulfonamide derivatives. Some of these compounds exhibit significant cytotoxicity against human (colon and prostate) and murine (leukemia) cell lines. The most potent compounds induced cell cycle modifications typical of tubulin interacting agents (tetraploid cells). Compound **16**, 4-methoxy-*N*-(3-chloro-7-indazolyl)benzenesulfonamide, was the most active of the series.

4. Experimental

4.1. General

Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance DPX250 spectrometer (250.19 MHz ^1H, 62.89 MHz ^{13}C) using tetramethylsilane as the internal standard, multiplicities determined by the DEPT 135 sequence. Chemical shifts were reported in parts per million (ppm, δ units). Coupling constants were reported in units of hertz (Hz). Splitting patterns were designated as s, singlet; d, doublet, and t, triplet. Low-resolution mass spectra (MS) were recorded on a Perkin-Elmer SCIEX API 3000 spectrometer. All commercial solvents were used without further purification. The following solvents and reagents have been abbreviated: dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethyl

acetate (EtOAc), methanol (MeOH), trifluoroacetic acid (TFA), and ethylene glycol dimethyl ether (DME). Thin layer chromatography (TLC) was carried out on Merck silica gel $60F_{254}$ precoated plates. Visualization was made with ultraviolet light.

4-Sulfamoylbenzenesulfonyl chloride was prepared according to methods described in the literature.[20,21]

4.1.1. 7-Nitroindazole (1). This compound was prepared according to the method described in the literature[17] (65% yield). Mp 185–186 °C (lit.[17] mp 186 °C). ^{1}H NMR (DMSO-d_6) δ 7.32 (t, 1H, J = 7.8 Hz), 8.32 (d, 1H, J = 7.8 Hz), 8.35 (d, 1H, J = 7.8 Hz), 8.41 (s, 1H), 13.94 (s, NH). ^{13}C NMR (DMSO-d_6) δ 120.2, 123.5, 127.1, 129.9, 131.9, 132.1, 135.6. MS m/z = 164.1 $[M+1]^+$.

4.1.2. 3-Chloro-7-nitroindazole (2). To 7-nitroindazole (1 g, 6.13 mmol) dissolved in 25 ml MeOH was added 2 N aq sodium hydroxide (20 ml) and then sodium hypochlorite (6 ml, 98.2 mmol) was added to the solution. The mixture was refluxed for 1 h. After cooling, the solution was acidified with acetic acid. The precipitated solid was filtered, washed with water, and dried in vacuo to give a yellow solid (98% yield). Mp 167–168 °C (lit.[22] mp 166–168 °C). ^{1}H NMR (DMSO-d_6) δ 7.45 (t, 1H, J = 7.8 Hz), 8.20 (d, 1H, J = 7.8 Hz), 8.44 (d, 1H, J = 7.8 Hz), 14.13 (s, NH). ^{13}C NMR (DMSO-d_6) δ 121.3, 123.4, 125.1, 127.8, 132.5, 133.2, 134.7. MS m/z = 198.1 (^{35}Cl) $[M+1]^+$, 201.1 (^{37}Cl) $[M+3]^+$.

4.1.3. 3-Iodo-7-nitroindazole (3). To a solution of 7-nitroindazole (1 g, 6.13 mmol) in DMF (60 ml) were added iodine (3.1 g, 12.26 mmol) and potassium hydroxide pellets (1.28 g, 23 mmol) at rt under stirring. After 1 h, the reaction mixture was poured into 10% aq $NaHSO_3$ (200 ml) and extracted with Et_2O (2 × 150 ml). The combined organic layers were washed with water and brine, dried over $MgSO_4$, and the solvent was evaporated to give a light yellow solid (95% yield). Mp 188–189 °C. ^{1}H NMR (DMSO-d_6) δ 7.44 (t, 1H, J = 7.8 Hz), 8.00 (d, 1H, J = 7.8 Hz), 8.46 (d, 1H, J = 7.8 Hz), 14.32 (s, NH). ^{13}C NMR (DMSO-d_6) δ 96.8, 120.9, 124.6, 129.6, 130.8, 132.1, 132.6. MS m/z = 290.2 $[M+1]^+$. Anal. Calcd for $C_7H_4IN_3O_2$: C, 29.09; H, 1.39; I, 43.91; N, 14.54. Found: C, 29.20; H, 1.51; I, 43.78; N, 14.50.

4.1.4. *N*-(7-Indazolyl)benzenesulfonamide (6a). A solution of 7-nitroindazole (200 mg, 1.23 mmol) in MeOH (20 ml) was hydrogenated over 10% palladium on carbon (20 mg) under H_2 at 1 atm overnight. After the catalyst was filtered off, the filtrate was evaporated to give 7-aminoindazole **4**. The crude amine was immediately dissolved in pyridine (5 ml) and then reacted with benzenesulfonyl chloride (180 µl, 1.35 mmol) at rt for 8 h. After the reaction mixture was concentrated in vacuo, the resulting residue was purified by flash chromatography using EtOAc/EP to afford a colorless solid in 95% yield. Mp 215–216 °C. ^{1}H NMR (DMSO-d_6) δ 6.90 (d, 1H, J = 8.5 Hz), 7.04 (dd, 1H, J = 7.5, 7.9 Hz), 7.42 (d, 1H, J = 7.9 Hz), 7.54 (d, 2H, J = 8.5 Hz), 7.62 (d, 1H, J = 7.5 Hz), 7.74 (d, 2H, J = 8.5 Hz), 10.12 (s, 1H), 12.71 (s, NH). ^{13}C NMR (DMSO-d_6) δ 117.7, 118.4, 120.6, 124.5, 126.8, 129.2, 133.1, 133.9, 134.8, 135.1, 139.2. MS m/z = 274 $[M+1]^+$. Anal. Calcd for $C_{13}H_{11}N_3O_2S$: C, 57.13; H, 4.06; N, 15.37; S, 11.73. Found: C, 57.06; H, 4.21; N, 15.25; S, 11.66.

4.2. General procedure for the synthesis of 6b–d

These products were synthesized as described for **6a** by using the appropriate sulfonyl chlorides.

4.2.1. 4-Methyl-*N*-(7-indazolyl)benzenesulfonamide (6b). Obtained as a colorless solid in 93% yield. Mp 207–208 °C. ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 6.94 (d, 2H, J = 8.1 Hz), 7.30 (d, 2H, J = 7.8 Hz), 7.48 (t, 1H, J = 7.8 Hz), 7.65 (d, 2H, J = 8.1 Hz), 8.04 (s, 1H), 10.04 (s, NH), 12.67 (s, NH). ^{13}C NMR (DMSO-d_6) δ 20.9, 113.9, 117.4, 117.9, 120.6, 121.0, 124.6, 126.9, 129.6, 131.2, 136.4, 143.4. 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; S, 11.16. Found: C, 58.34; H, 4.45; N, 14.88; S, 11.29.

4.2.2. 4-Methoxy-*N*-(7-indazolyl)benzenesulfonamide (6c). Obtained as a colorless solid in 92% yield. Mp 230–231 °C. ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H, OCH_3), 6.9 (d, 2H, J = 7.9 Hz), 7.25 (d, 2H, J = 8.7 Hz), 7.49 (t, 1H, J = 7.9 Hz), 7.59 (d, 2H, J = 8.7 Hz), 8.04 (s, 1H), 9.97 (s, NH), 12.66 (s, NH). ^{13}C NMR (DMSO-d_6) δ 55.6, 114.3, 117.4, 117.9, 120.6, 120.9, 124.4, 129.1, 130.7, 133.9, 134.6, 162.3. MS m/z = 304 $[M+1]^+$. Anal. Calcd for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85; S, 10.57. Found: C, 55.56; H, 4.20; N, 13.62; S, 10.71.

4.2.3. 4-Bromo-*N*-(7-indazolyl)benzenesulfonamide (6d). Obtained as a colorless solid in 71% yield. Mp 224–225 °C. ^{1}H NMR (DMSO-d_6) δ 6.88 (d, 1H, J = 7.5 Hz), 6.95 (dd, 1H, J = 7.5, 7.7 Hz), 7.54 (d, 1H, J = 7.7 Hz), 7.65 (d, 2H, J = 8.7 Hz), 7.74 (d, 2H, J = 8.7 Hz), 8.06 (s, 1H), 10.19 (s, NH), 14.32 (s, NH). ^{13}C NMR (DMSO-d_6) δ 118.2, 119.2, 120.2, 120.6, 125.4, 126.8, 128.8, 132.3, 133.9, 135.1, 138.5. MS m/z = 353 $[M+1]^+$ (^{79}Br), 354 $[M+3]^+$ (^{81}Br). Anal. Calcd for $C_{13}H_{10}BrN_3O_2S$: C, 44.33; H, 2.86; Br, 22.69; N, 11.93; S, 9.10. Found: C, 44.20; H, 2.75; Br, 22.85; N, 11.98; S, 9.21.

4.2.4. *N*-(7-Indazolyl)-1,4-benzenedisulfonamide (6e). A solution of 7-nitroindazole (200 mg, 1.23 mmol) in MeOH (20 ml) was hydrogenated over 10% palladium on carbon (20 mg) under H_2 at 1 atm during 2 h. After the catalyst was filtered off, the filtrate was evaporated to give almost pure 7-aminoindazole **4**. The crude amine was dissolved in acetone (5 ml) followed by the immediate addition of 4-sulfamoylbenzenesulfonyl chloride (408 mg, 1.67 mmol) and pyridine (300 µl, 3.6 mmol). The reaction mixture was stirred at rt overnight. After the reaction mixture was concentrated in vacuo, the resulting residue was purified by flash chromatography (eluted with EtOAc/hexane) afforded as a colorless solid in 76% yield. Mp 240–241 °C. ^{1}H NMR (DMSO-d_6) δ

6.87 (d, 1H, $J = 7.3$ Hz), 6.96 (dd, 1H, $J = 7.3$, 7.5 Hz), 7.53 (d, 1H, $J = 7.5$ Hz), 7.56 (s, 2H, NH_2), 7.90–7.95 (m, 4H), 8.06 (s, 1H), 10.34 (s, NH). ^{13}C NMR (DMSO-d_6) δ 118.2, 119.0, 120.2, 120.2, 121.5, 124.5, 126.5, 127.8, 131.7, 142.0, 147.7. MS $m/z = 353$ $[M+1]^+$. Anal. Calcd for $C_{13}H_{12}N_4O_4S_2$: C, 44.31; H, 3.43; N, 15.90; S, 18.20. Found: C, 44.50; H, 3.48; N, 15.72; S, 18.04.

4.3. General procedure for the synthesis of 7a–d

These products were synthesized as described for **6a** by using the appropriate sulfonyl chlorides.

4.3.1. *N*-(3-Chloro-7-indazolyl)benzenesulfonamide (7a). Obtained as a colorless solid in 93% yield. Mp 198–199 °C. ^{1}H NMR (DMSO-d_6) δ 6.90 (d, 1H, $J = 7.2$ Hz), 7.04 (dd, 1H, $J = 7.5$, 7.8 Hz), 7.42 (d, 1H, $J = 7.8$ Hz), 7.54 (d, 2H, $J = 7.2$ Hz), 7.60 (d, 1H, $J = 7.5$ Hz), 7.74 (d, 2H, $J = 7.2$ Hz), 10.2 (s, NH), 13.00 (s, NH). ^{13}C NMR (DMSO-d_6) δ 116.1, 120.5, 121.1, 121.2, 121.7, 126.9, 129.2, 132.5, 133.2, 136.5, 138.9. MS $m/z = 308.5$ (^{35}Cl) $[M+1]^+$, 310.5 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$: C, 50.74; H, 3.28; Cl, 11.52; N, 13.65; S, 10.42. Found: C, 50.96; H, 3.44; Cl, 11.44; N, 13.49; S, 10.41.

4.3.2. 4-Methyl-*N*-(3-chloro-7-indazolyl)benzenesulfonamide (7b). Obtained as a colorless solid in 88% yield. Mp 176–177 °C. ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 6.94 (d, 1H, $J = 7.5$ Hz), 7.05 (dd, 1H, $J = 7.5$, 7.8 Hz), 7.30 (d, 2H, $J = 8.1$ Hz), 7.40 (d, 1H, $J = 7.8$ Hz), 7.64 (d, 2H, $J = 8.1$ Hz), 10.11 (s, NH), 13.01 (s, NH). ^{13}C NMR (DMSO-d_6) δ 20.9, 115.8, 120.2, 121.1, 121.4, 121.8, 126.9, 129.7, 132.6, 136.1, 136.3, 143.5. MS $m/z = 322$ (^{35}Cl) $[M+1]^+$, 324 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{14}H_{12}ClN_3O_2S$: C, 52.26; H, 3.76; Cl, 11.02; N, 13.06; S, 9.96. Found: C, 52.40; H, 3.52; Cl, 10.91; N, 13.21; S, 9.83.

4.3.3. 4-Methoxy-*N*-(3-chloro-7-indazolyl)benzenesulfonamide (7c). Obtained as a colorless solid in 90% yield. Mp 201–202 °C. ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H, OCH_3), 6.95 (d, 1H, $J = 7.5$ Hz), 7.01 (dd, 1H, $J = 7.5$, 7.8 Hz), 7.07 (d, 2H, $J = 8.7$ Hz), 7.24 (d, 1H, $J = 7.8$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 10.02 (s, NH), 13.00 (s, NH). ^{13}C NMR (DMSO-d_6) δ 55.6, 114.3, 115.7, 120.0, 120.1, 121.5, 121.8, 129.2, 130.5, 132.5, 136.2, 162.6. MS $m/z = 338$ (^{35}Cl) $[M+1]^+$, 340 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{14}H_{12}ClN_3O_2S$: C, 49.78; H, 3.58; Cl, 10.50; N, 12.44; S, 9.46. Found: C, 49.60; H, 3.61; Cl, 10.77; N, 12.32; S, 9.30.

4.3.4. 4-Bromo-*N*-(3-chloro-7-indazolyl)benzenesulfonamide (7d). Obtained as a colorless solid in 63% yield. Mp 220–221 °C. ^{1}H NMR (DMSO-d_6) δ 6.89 (d, 1H, $J = 7.5$ Hz), 7.08 (dd, 1H, $J = 7.5$, 8.1 Hz), 7.47 (d, 1H, $J = 8.1$ Hz), 7.65 (d, 2H, $J = 8.5$ Hz), 7.78 (d, 2H, $J = 8.5$ Hz), 10.27 (s, NH), 13.10 (s, NH). ^{13}C NMR (DMSO-d_6) δ 116.5, 120.8, 121.3, 121.8, 122.5, 127.0, 128.9, 132.3, 132.5, 136.8, 138.2. MS $m/z = 387$ (^{35}Cl) $[M+1]^+$, 389 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{13}H_9BrClN_3O_2S$: C, 40.38; H, 2.35; Br, 20.67; Cl, 9.17; N, 10.87; S, 8.29. Found: C, 40.20; H, 2.31; Br, 20.88; Cl, 9.29; N, 10.66; S, 8.37.

4.3.5. *N*-(3-Chloro-7-indazolyl)-1,4-benzenedisulfonamide (7e). This compound was synthesized from 3-chloro-7-nitroindazole as described for **6e**. Chromatography eluted with EtOAc/hexane afforded a colorless solid in 72% yield. Mp > 250 °C. ^{1}H NMR (DMSO-d_6) δ 6.88 (d, 1H, $J = 7.5$ Hz), 7.06 (dd, 1H, $J = 7.5$, 7.9 Hz), 7.47 (d, 1H, $J = 7.9$ Hz), 7.58 (s, 2H, NH_2), 7.92–7.97 (m, 4H), 10.42 (s, NH). ^{13}C NMR (DMSO-d_6) δ 116.6, 122.1, 122.6, 125.4, 126.6, 127.4, 128.6, 132.5, 136.7, 141.8, 147.8. MS $m/z = 387$ (^{35}Cl) $[M+1]^+$, 389 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{13}H_{11}ClN_4O_4S_2$: C, 40.36; H, 2.87; Cl, 9.16; N, 14.48; S, 16.58. Found: C, 40.51; H, 2.62; Cl, 9.02; N, 14.73; S, 16.40.

4.4. General method for alkylation of 7-nitroindazole derivatives

To a solution of 7-nitroindazole derivatives (6.13 mmol) in acetone (15 ml) cooled at 0 °C was added potassium hydroxide (9.2 mmol). After 15 min at 0 °C, iodomethane or 4-methoxybenzyl chloride (6.13 mmol) was added dropwise. Upon disappearance of the starting material as indicated by TLC, 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 removed in vacuo to give the corresponding compounds.

4.4.1. 1-Methyl-7-nitroindazole (8). Chromatography using EtOAc/hexane gave a yellow solid in 93% yield. Mp 99–100 °C (lit.[22] mp 98 °C). ^{1}H NMR (DMSO-d_6) δ 4.16 (s, 3H, CH_3), 7.40 (t, 1H, $J = 7.7$ Hz), 7.90 (d, 1H, $J = 7.7$ Hz), 8.29 (d, 1H, $J = 7.7$ Hz). ^{13}C NMR (DMSO-d_6) δ 40.3, 120.1, 124.5, 125.4, 128.6, 130.4, 134.3, 206.4. MS $m/z = 178.1$ $[M+1]^+$. Anal. Calcd for $C_8H_7N_3O$: C, 54.24; H, 3.98; N, 23.72. Found: C, 54.02; H, 4.17; N, 23.83.

4.4.2. 3-Chloro-1-methyl-7-nitroindazole (9). This compound was similarly prepared from 3-chloro-7-nitroindazole. Chromatography using EtOAc/EP gave a yellow solid in 96% yield. Mp 150–151 °C (lit.[23] mp 148–150 °C). ^{1}H NMR (DMSO-d_6) δ 4.11 (s, 3H, CH_3), 7.42 (t, 1H, $J = 7.8$ Hz), 8.11 (d, 1H, $J = 7.8$ Hz), 8.28 (d, 1H, $J = 7.8$ Hz). ^{13}C NMR (DMSO-d_6) δ 40.9, 121.2, 124.6, 126.1, 126.5, 132.0, 132.8, 206.5. MS $m/z = 212$ (^{35}Cl) $[M+1]^+$, 214 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_8H_6ClN_3O$: C, 45.41; H, 2.86; Cl, 16.75; N, 19.86. Found: C, 45.55; H, 2.90; Cl, 16.49; N, 19.77.

4.4.3. 3-Iodo-1-methyl-7-nitroindazole (10). This compound was similarly prepared from 3-iodo-7-nitroindazole. Chromatography using EtOAc/EP gave a yellow solid in 97% yield. Mp 171–172 °C. ^{1}H NMR (DMSO-d_6) δ 4.16 (s, 3H, CH_3), 7.40 (t, 1H, $J = 7.7$ Hz), 7.9 (d, 1H, $J = 7.7$ Hz), 8.29 (d, 1H, $J = 7.7$ Hz). ^{13}C NMR (DMSO-d_6) δ 40.8, 95.1, 120.8, 125.7, 128.6, 131.4, 132.1, 134.7. MS $m/z = 304$ $[M+1]^+$. Anal. Calcd

for $C_7H_4IN_3O$: C, 29.09; H, 1.39; I, 43.91; N, 14.54. Found: C, 29.20; H, 1.45; I, 43.70; N, 14.37.

4.4.4. 4-Methoxy-*N*-(1-methyl-7-indazolyl)benzenesulfonamide (13). This compound was synthesized as described for **6a** as a colorless solid in 92% yield. Mp 163–164 °C. ^{1}H NMR (DMSO-d_6) δ 3.48 (s, 3H, CH_3), 4.23 (s, 3H, OCH_3), 6.43 (d, 1H, J = 7.5 Hz), 6.90 (dd, 1H, J = 7.5, 7.9 Hz), 7.10 (d, 2H, J = 8.5 Hz), 7.59 (d, 2H, J = 8.5 Hz), 7.65 (d, 1H, J = 7.9 Hz), 8.06 (s, 1H), 9.83 (s, NH). ^{13}C NMR (DMSO-d_6) δ 38.7, 55.7, 114.0, 114.3, 120.2, 120.6, 123.1, 125.4, 127.6, 129.6, 133.7, 137.8, 162.8. MS m/z = 318 $[M+1]^+$. Anal. Calcd for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 13.24; S, 10.10. Found: C, 56.58; H, 4.49; N, 13.50; S, 10.25.

4.4.5. 4-Methoxy-*N*-(3-chloro-1-methyl-7-indazolyl)benzenesulfonamide (14). This compound, synthesized from **12** as described for **6a**, was obtained as a colorless solid in 93% yield. Mp 160–161 °C. ^{1}H NMR (DMSO-d_6) δ 3.24 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.63 (d, 1H, J = 7.5 Hz), 6.91 (d, 2H, J = 8.5 Hz), 7.05 (dd, 1H, J = 7.5, 7.9 Hz), 7.47 (d, 2H, J = 8.5 Hz), 7.60 (d, 1H, J = 7.9 Hz), 10.49 (s, NH). ^{13}C NMR (DMSO-d_6) δ 38.6, 55.7, 114.4, 118.5, 121.4, 121.3, 122.5, 124.5, 126.1, 127.0, 130.0, 130.1, 162.9. MS m/z = 352 (^{35}Cl) $[M+1]^+$, 354 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{15}H_{14}ClN_3O_3S$: C, 51.21; H, 4.01; Cl, 10.08; N, 11.94; S, 9.11. Found: C, 51.00; H, 4.23; Cl, 9.87; N, 11.99; S, 8.93.

4.4.6. 4-Methoxy-*N*-methyl-*N*-(7-indazolyl)benzenesulfonamide (15). This compound was prepared from 4-methoxy-*N*-(7-indazolyl)benzenesulfonamide as described in general method for alkylation of 7-nitroindazole derivatives. Chromatography using EtOAc/hexane as eluent afforded a colorless solid in 95% yield. Mp 165–166 °C. ^{1}H NMR (DMSO-d_6) δ 3.21 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.67 (d, 1H, J = 7.5 Hz), 6.98 (dd, 1H, J = 7.5, 7.8 Hz), 7.11 (d, 2H, J = 8.7 Hz), 7.53 (d, 2H, J = 8.7 Hz), 7.71 (d, 1H, J = 7.8 Hz), 8.10 (s, 1H), 13.27 (s, NH). ^{13}C NMR (DMSO-d_6) δ 38.7, 55.7, 114.3, 120.2, 120.4, 123.1, 124.9, 125.4, 127.6, 130.1, 133.7, 137.8, 162.8. MS m/z = 318 $[M+1]^+$. Anal. Calcd for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 13.24; S, 10.10. Found: C, 56.86; H, 4.92; N, 13.04; S, 9.91.

4.4.7. 4-Methoxy-*N*-methyl-*N*-(3-chloro-7-indazolyl)benzenesulfonamide (16). Obtained as a colorless solid in 98% yield. Mp 198–199 °C. ^{1}H NMR (DMSO-d_6) δ 3.20 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.77 (d, 1H, J = 7.2 Hz), 7.07–7.13 (m, 3H), 7.52 (d, 2H, J = 8.5 Hz), 7.61 (d, 1H, J = 8.3 Hz), 13.56 (s, NH). ^{13}C NMR (DMSO-d_6) δ 38.6, 55.7, 114.4, 118.5, 121.3, 121.3, 121.4, 124.5, 126.1, 127.1, 130.1, 132.3, 139.1, 162.9. MS m/z = 352 (^{35}Cl) $[M+1]^+$, 354 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{15}H_{14}ClN_3O_3S$: C, 51.21; H, 4.01; Cl, 10.08; N, 11.94; S, 9.11. Found: C, 51.12; H, 4.15; Cl, 10.17; N, 12.20; S, 8.95.

4.4.8. 4-Methoxy-*N*-methyl-*N*-(1-methyl-7-indazolyl)benzenesulfonamide (17). This compound was prepared from 4-methoxy-*N*-methyl-*N*-(7-indazolyl)benzenesulfonamide as described in general method for alkylation of 7-nitroindazole derivatives. Chromatography using EtOAc/hexane as eluent afforded a colorless solid in 97% yield. Mp 164–165 °C. ^{1}H NMR (DMSO-d_6) δ 3.22 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 4.29 (s, 3H, OCH_3), 6.54 (d, 1H, J = 7.5 Hz), 6.97 (dd, 1H, J = 7.5, 7.8 Hz), 7.17 (d, 2H, J = 8.7 Hz), 7.58 (d, 2H, J = 8.7 Hz), 7.74 (d, 1H, J = 7.8 Hz), 8.11 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 38.1, 39.6, 55.7, 114.5, 120.4, 121.4, 124.5, 125.4, 125.8, 127.5, 130.2, 132.5, 136.6, 163.0. 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; S, 9.68. Found: C, 57.60; H, 5.36; N, 12.45; S, 9.61.

4.4.9. 4-Methoxy-*N*-methyl-*N*-(3-chloro-1-methyl-7-indazolyl)benzenesulfonamide (18). This compound was prepared from 4-methoxy-*N*-(3-chloro-1-methyl-7-indazolyl)benzenesulfonamide as described in general method for alkylation of 7-nitroindazole derivatives. Chromatography using EtOAc/hexane gave a colorless solid in 93% yield. Mp 181–182 °C. ^{1}H NMR (DMSO-d_6) δ 3.21 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 4.26 (s, 3H, OCH_3), 6.67 (d, 1H, J = 7.8 Hz), 7.11 (t, 1H, J = 7.8 Hz), 7.17 (d, 2H, J = 8.7 Hz), 7.56 (d, 2H, J = 8.7 Hz), 7.65 (d, 1H, J = 7.8 Hz). ^{13}C NMR (DMSO-d_6) δ 38.5, 39.4, 55.7, 114.5, 119.5, 121.7, 122.6, 125.4, 126.0, 127.0, 130.2, 130.7, 138.1, 163.1. MS m/z = 366 (^{35}Cl) $[M+1]^+$, 368 (^{37}Cl) $[M+3]^+$. Anal. Calcd for $C_{16}H_{16}N_3O_3S$: C, 52.53; H, 4.44; Cl, 9.69; N, 11.49; S, 8.76. Found: C, 52.19; H, 4.69; Cl, 9.55; N, 11.66; S, 8.51.

4.4.10. 1-*N*-Boc-3-iodo-7-nitroindazole (19). To a solution of 3-iodo-7-nitroindazole (1.65 mmol) in CH_2Cl_2, were added Et_3N (1.82 mmol), DMAP (1.65 mmol), and di-*tert*-butyl dicarbonate (1.82 mmol). The reaction mixture was refluxed with vigorous stirring for 20 h. The organic solvent was removed under reduced pressure and the crude product was purified by chromatography (silica gel, EtOAc/EP) to give compound **19** as a yellow solid in 93% yield. Mp 108–109 °C. ^{1}H NMR ($CDCl_3$) δ 1.64 (s, 9H, $3CH_3$), 7.48 (dd, 1H, J = 8.1, 7.8 Hz), 7.79 (d, 1H, J = 8.1 Hz), 8.08 (d, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 28.4, 88.2, 101.9, 124.3, 126.1, 128.0, 130.9, 133.9, 137.9, 174.8. MS m/z=390 $[M+1]^+$. Anal. Calcd for $C_{12}H_{12}IN_3O_4$: C, 37.04; H, 3.11; I, 32.61; N, 10.80. Found: C, 37.25; H, 3.02; I, 32.77; N, 10.69.

4.4.11. 1-Benzenesulfonyl-3-iodo-7-nitroindazole (20). To a solution of sodium hydroxide (5.1 mmol) in 10 ml of CH_2Cl_2 at 0 °C, 3-iodo-7-nitroindazole (1.65 mmol) and benzyltriethylammonium chloride (0.04 mmol) were added. To the mixture, benzenesulfonyl chloride (2 mmol) was added dropwise. The reaction mixture was refluxed with vigorous stirring overnight. The organic solvent was removed under reduced pressure and the crude product was purified by chromatography (silica gel, EtOAc/EP) to give compound **20** as a yellow solid in 76% yield. Mp 185–186 °C. ^{1}H NMR (DMSO-d_6) δ 7.47 (d, 2H, J = 7.5 Hz), 7.57 (dd, 1H, J = 8.1, 7.9 Hz), 7.63–7.68 (m, 3H), 8.04 (d, 1H, J = 7.9 Hz), 8.10 (d, 1H, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6) δ 115.5, 117.1, 127.9, 128.1, 129.5, 130.2, 133.8, 134.7,

139.6, 138.2, 171.7. MS $m/z = 444$ $[M+1]^+$. Anal. Calcd for $C_{14}H_{10}IN_3O_4S$: C, 37.94; H, 2.27; I, 28.63; N, 9.48. Found: C, 37.99; H, 2.49; I, 28.41; N, 9.30.

4.5. General procedure for Suzuki-type cross-coupling reaction of 3-iodo-7-nitroindazole derivatives

To a solution of 3-iodo-7-nitroindazole derivatives (1.65 mmol) and 4-methoxyphenyl boronic acid (2.47 mmol) in DME (10 ml), sodium carbonate (4.95 mmol) dissolved in H_2O (5 ml) was added followed by the addition of $Pd(PPh_3)_4$ (0.165 mmol). The reaction mixture was refluxed with vigorous stirring under argon atmosphere for 2 h. The organic solvent was removed under reduced pressure and the crude product was purified by chromatography (silica gel, EtOAc/EP) to give the corresponding compounds. Spectral data for representative compounds were as follows.

4.5.1. 3-(4-Methoxyphenyl)-7-nitroindazole (21). Obtained as a colorless solid in 92% yield. Mp 209–210 °C. ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H, OCH_3), 7.12 (d, 2H, $J = 8.5$ Hz), 7.43 (dd, 1H, $J = 7.7$, 8.1 Hz), 7.94 (d, 2H, $J = 8.5$ Hz), 8.41 (d, 1H, $J = 7.7$ Hz), 8.55 (d, 1H, $J = 8.1$ Hz). ^{13}C NMR (DMSO-d_6) δ 55.3, 114.5, 120.7, 123.7, 124.4, 124.6, 128.7, 130.0, 132.1, 133.5, 145.3, 159.7. MS $m/z = 270$ $[M+1]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_4$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.31; H, 4.00; N, 15.88.

4.5.2. 4-Methoxy-*N*-[3-(4-methoxyphenyl)-7-indazolyl]benzenesulfonamide (22). A mixture of 4-methoxy-*N*-[1-(4-methoxybenzyl)-3-(4-methoxyphenyl)-7-indazolyl] benzenesulfonamide (200 mg, 0.37 mmol) and TFA (5 ml) was refluxed for 6 h. After the reaction mixture had been concentrated in vacuo to dryness, water was added to the residue and then extracted with EtOAc. The organic layer was washed successively with satd aq $NaHCO_3$, water, and brine, and dried over $MgSO_4$. The solvent was concentrated in vacuo. The resulting residue was purified by flash chromatography (eluted with EtOAc/hexane) to give a colorless solid in 97% yield. Mp 170–171 °C. ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.82 (d, 2H, $J = 9.1$ Hz), 7.03 (d, 2H, $J = 8.8$ Hz), 7.11 (d, 1H, $J = 7.5$ Hz), 7.20 (d, 1H, $J = 8.1$ Hz), 7.70 (dd, 1H, $J = 7.5$, 8.1 Hz), 7.74 (d, 2H, $J = 9.1$ Hz), 7.77 (d, 2H, $J = 8.8$ Hz). ^{13}C NMR ($CDCl_3$) δ 56.0, 56.2, 114.9, 115.4, 120.0, 122.5, 123.6, 123.9, 124.2, 129.9, 130.1, 130.3, 136.7, 144.8, 161.5, 164.0, 172.4. MS $m/z = 410$ $[M+1]^+$. Anal. Calcd for $C_{21}H_{19}N_3O_4S$: C, 61.60; H, 4.68; N, 10.26; S, 7.83. Found: C, 61.41; H, 4.48; N, 10.56; S, 7.92.

4.5.3. 3-Iodo-1-(4-methoxybenzyl)-7-nitroindazole (23). This compound was prepared from 3-chloro-7-nitroindazole as described in general method for alkylation of 7-nitroindazole derivatives. Chromatography using EtOAc/hexane gave a yellow solid in 90% yield. Mp 125–126 °C. ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H, OCH_3), 5.69 (s, 2H, CH_2), 6.80 (d, 2H, $J = 8.7$ Hz), 6.87 (d, 2H, $J = 8.7$ Hz), 7.40 (dd, 1H, $J = 7.8$, 8.3 Hz), 7.94 (d, 1H, $J = 8.3$ Hz), 8.21 (d, 1H, $J = 7.8$ Hz). ^{13}C NMR (DMSO-d_6) δ 55.0, 55.7, 114.1, 121.3, 126.0, 128.1, 128.4, 128.9, 130.4, 132.6, 134.7, 158.6, 206.4. MS $m/z = 410$ $[M+1]^+$. Anal. Calcd for $C_{15}H_{12}IN_3O_3$: C, 44.03; H, 2.96; I, 31.01; N, 10.27. Found: C, 43.92; H, 3.15; I, 31.20; N, 10.11.

4.5.4. 1-(4-Methoxybenzyl)-3-(4-methoxyphenyl)-7-nitroindazole (24). The title compound was synthesized from 3-iodo-1-(4-methoxybenzyl)-7-nitroindazole. Chromatography using EtOAc/hexane afforded a yellow solid in 80% yield. Mp 144–145 °C. ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.72 (s, 2H, CH_2), 6.80 (d, 2H, $J = 8.7$ Hz), 6.90 (d, 2H, $J = 8.7$ Hz), 7.14 (d, 2H, $J = 8.8$ Hz), 7.39 (dd, 1H, $J = 7.7$, 7.9 Hz), 7.92 (d, 2H, $J = 8.8$ Hz), 8.14 (d, 1H, $J = 7.7$ Hz), 8.45 (d, 1H, $J = 7.9$ Hz). ^{13}C NMR (DMSO-d_6) δ 55.0, 55.3, 55.7, 114.1, 114.6, 120.9, 123.7, 125.4, 128.1, 128.5, 128.7, 129.1, 130.9, 135.1, 144.7, 144.9, 158.6, 159.8. MS $m/z = 390$ $[M+1]^+$. Anal. Calcd for $C_{22}H_{19}N_3O_4$: C, 67.86; H, 4.92; N, 10.79. Found: C, 67.63; H, 4.82; N, 10.94.

4.5.5. 4-Methoxy-*N*-[1-(4-methoxybenzyl)-3-(4-methoxyphenyl)-7-indazolyl]benzenesulfonamide (25). The title compound was synthesized from 3-iodo-1-methyl-7-nitroindazole as described for **6a** as a colorless solid in 76% yield. Mp 132–133 °C. ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.78 (s, 2H, CH_2), 6.72 (d, 1H, $J = 7.2$ Hz), 6.82 (d, 2H, $J = 8.8$ Hz), 6.91 (d, 2H, $J = 8.8$), 6.97 (d, 1H, $J = 7.5$ Hz), 7.04 (d, 2H, $J = 8.8$ Hz), 7.13 (d, 2H, $J = 8.8$ Hz), 7.62 (d, 2H, $J = 8.8$ Hz), 7.84–7.89 (m, 3H). ^{13}C NMR (DMSO-d_6) δ 54.1, 55.4, 55.5, 55.7, 114.3, 114.5, 114.5, 119.2, 121.0, 121.4, 125.1, 125.8, 126.1, 128.1, 129.0, 130.3, 130.5, 130.9, 131.0, 136.9, 159.3, 160.0, 163.4. MS $m/z = 530$ $[M+1]^+$. Anal. Calcd for $C_{29}H_{27}N_3O_4S$: C, 65.77; H, 5.14; N, 7.93. S, 6.05. Found: C, 65.44; H, 5.02; N, 8.25. S, 6.19.

4.5.6. 4-Methoxy-*N*-methyl-*N*-[3-(4-methoxyphenyl)-1-methyl-7-indazolyl]benzenesulfonamide (26). This compound was prepared from 4-methoxy-*N*-[3-(4-methoxyphenyl)-7-indazolyl]benzenesulfonamide as described in general method for alkylation of 7-nitroindazole derivatives using 2 equivalents of iodomethane. Chromatography using EtOAc/hexane gave a colorless solid in 95% yield. Mp 204–205 °C. ^{1}H NMR ($CDCl_3$) δ 3.27 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.47 (s, 3H, OCH_3), 6.50 (d, 1H, $J = 7.8$ Hz), 6.95 (dd, 1H, $J = 7.5$, 7.8 Hz), 7.00 (d, 2H, $J = 8.7$ Hz), 7.04 (d, 2H, $J = 8.7$ Hz), 7.65 (d, 2H, $J = 8.8$ Hz), 7.82 (d, 2H, $J = 8.8$ Hz), 7.89 (d, 1H, $J = 7.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 38.8, 40.2, 55.4, 55.8, 113.2, 114.2, 114.4, 120.3, 121.6, 122.0, 124.5, 126.0, 126.2, 128.2, 129.0, 130.7, 138.6, 159.6, 163.4. MS $m/z = 438$ $[M+1]^+$. Anal. Calcd for $C_{23}H_{23}N_3O_4S$: C, 63.14; H, 5.30; N, 9.60; S, 7.33. Found: C, 63.51; H, 5.12; N, 9.87; S, 7.11.

4.5.7. 3-(4-Methoxyphenyl)-1-methyl-7-nitroindazole (27). The title compound was synthesized from 3-iodo-1-methyl-7-nitroindazole as described in general procedure for Suzuki-type cross-coupling reaction of 3-iodo-7-nitroindazole derivatives. Chromatography

using EtOAc/hexane gave a yellow solid in 80% yield. Mp 175–176 °C. ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 3H, CH_3), 4.16 (s, 3H, OCH_3), 7.11 (d, 2H, J = 8.7 Hz), 7.38 (dd, 1H, J = 7.7, 7.9 Hz), 7.86 (d, 2H, J = 8.7 Hz), 8.22 (d, 1H, J = 7.7 Hz), 8.41 (d, 1H, J = 7.9 Hz). ^{13}C NMR (DMSO-d_6) δ 40.4, 55.3, 114.6, 120.5, 123.9, 124.7, 125.4, 128.4, 128.8, 132.5, 135.0, 143.9, 159.7. MS m/z = 284 $[M+1]^+$. Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.86; H, 4.41; N, 15.13.

4.5.8. 4-Methoxy-*N*-[3-(4-methoxyphenyl)-1-methyl-7-indazolyl]benzenesulfonamide (28). This compound was synthesized as described for **6a** as a colorless solid in 90% yield. Mp 159–160 °C. ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.24 (s, 3H, CH_3), 6.47 (d, 1H, J = 7.3 Hz), 6.97 (dd, 1H, J = 7.3, 8.1 Hz), 7.06 (d, 2H, J = 8.9 Hz), 7.14 (d, 2H, J = 8.9 Hz), 7.61 (d, 2H, J = 8.7 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.89 (d, 1H, J = 8.1 Hz), 9.88 (s, NH). ^{13}C NMR (DMSO-d_6) δ 38.6, 55.2, 55.7, 114.3, 114.4, 115.1, 120.0, 120.6, 120.8, 121.2, 123.8, 124.7, 126.1, 128.3, 129.3, 130.5, 137.4, 141.3. MS m/z = 424 $[M+1]^+$. Anal. Calcd for $C_{22}H_{21}N_3O_4S$: C, 62.40; H, 5.00; N, 9.92; S, 7.57. Found: C, 62.19; H, 4.82; N, 10.20; S, 7.42.

4.6. Cell culture and cytotoxicity

4.6.1. Cell lines. The human cell lines, HCT116 colorectal carcinoma, HT29 colorectal adenocarcinoma, DU145 prostate carcinoma, and the murine L1210 lymphocytic leukemia, were obtained from the American type culture collection (Rockville, MD).

Cells were maintained in RPMI 1640 medium supplemented with 10% decomplemented fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM Hepes, pH 7.4. Cells were grown at 37 °C in 5% CO_2/95% air. All media and supplements were from Life Technologies (Cergy_Pontoise, France), except for FCS, which was purchased from Sigma Chemical, Co. (St. Louis, MO).

4.6.2. Standard proliferation assay. Cytotoxicity was measured by the microculture tetrazolium assay as described.[24] Briefly, adherent cells were seeded in 96-well microplates and incubated for 2 days. Tested compounds were then added and plates were incubated for 4 doubling times. The nonadherent L1210 cells were directly incubated for 48 h with the compounds. At the end of this period, 15 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was added to each well and the plates were incubated for 4 h at 37 °C. The medium was aspirated and the formazan was solubilized by 100 μl DMSO. The IC_{50}, concentration reducing by 50% the optical density at 540 nm, was calculated by a linear regression performed on the linear zone of the dose–response curve. All the measurements were performed in triplicate. For the cell cycle analysis, (2.5×10^5 cells/ml) were incubated for 21 h with various concentrations of the compounds. Cells were then fixed with 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/ml RNase and 25 μg/ml propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analyzed on an Epics XL/MCL flow cytometer (Beckman Coulter, France). Results are expressed as the percentage of cells found in the different phases of the cell cycle.

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