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Synthesis and biological evaluation of novel biaryl type α-noscapine congeners

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ABSTRACT

Natural α-noscapine, a known antitussive drug, is also now known to possess weak anticancer efficacy with relatively safe toxicity profile. In this study, we report synthesis and evaluation of novel biaryl type α-noscapine congeners designed by adding aryl unit to the tetrahydroisoquinoline part of natural α-noscapine core. Palladium catalyzed Suzuki cross coupling of 9-bromo α-noscapine with aryl boronic acids was employed using mild and inexpensive reagents to attain desired noscapinoids **5a–g** in excellent yields. Screening anti-proliferative activity for new noscapinoids **5b–g**, on human cancer cell lines resulted three compounds **5b**, **5d** and **5f** as potent analogues, active against human breast epithelial (MCF-7), human cervix cancer (HeLa) and human lung adenocarcinoma epithelial (A549) cell lines.

Noscapine, an opium alkaloid, non-narcotic, orally available, safe antitussive agent[1] was discovered as a tubulin-binding anticancer agent that arrests cancer cells in mitosis and induce apoptosis.[2] Unlike the other tubulin-targeting agents, α-noscapine and its derivatives do not alter the steady state monomer/polymer ratio of tubulin.[3] Rather they subtly attenuate microtubule dynamics just enough to activate the mitotic checkpoints to stop the cell cycle, particularly mitosis, leaving other processes dependent on less dynamic microtubules relatively unperturbed.[3] In addition, noscapine has some other advantage properties as lead molecule: (1) retains activity against paclitaxel-resistant cell lines (1A9/PTX10, 1A9/PTX22)[4] and epothilone-resistant cell line (1A9/A8); (2) a favorable pharmacokinetics (clearance in 6–10 h);[5] (3) a poor substrate for drug-pumps (polyglycoprotein and MDR-related proteins)[6] that comprise a major cause of drug resistance; (4) free from immunological and neurological toxicities.[7]

To enhance anticancer activity of noscapine, further efforts were made to add various functional substituents on noscapine core. For instance, 9-substituted analogues (9-bromo, 9-chloro, 9-iodo, 9-nitro, 9-iodo, 9-azido, 9-amino analogues, Fig. 1)[8] exhibited higher anticancer activity than natural α-noscapine without significant toxicity. Among them, 9-bromo α-noscapine **2c** (EM011) is recently introduced into clinical trials against non-Hodgkin's lymphoma and chronic lymphocytic leukemia.[9] Similarly, 9-nitro α-noscapine **2e** was active against T-cell lymphoma cells and drug-resistant ovarian cancer.[10] More recently our group synthesized 9-amino α-noscapine (**2g**, Fig. 1)[11] and was found to possess improved tubulin binding activity.[12] In continuation of our efforts[13] to develop new congeners of α-noscapine for improving the anticancer activity profile, in this manuscript we describe synthesis of a series of 9-aryl noscapinoids **5a–g** using palladium catalyzed Suzuki cross-coupling reaction conditions. All the new noscapinoids were screened for anti-proliferative activity against human breast epithelial (MCF-7), human cervix cancer (HeLa) and human lung adenocarcinoma epithelial (A549) cell lines.

It is known from the literature that natural α-noscapine share binding characteristics similar to Colchicine (**I**), having biaryl configuration.[14] However, due to toxic side effects, use of colchicine as anticancer agent is limited.[15] Later reports identified other natural products **II–VI** (Fig. 2) with biaryl (red color) architecture as effective antimitotic agents affecting the tubulin-microtubule equilibrium.[16] Jain et al.[17] explored this phenomenon and developed nitrovinyl biphenyl analogs as anti-mitotic agents and has shown to inhibit tubulin polymerization. Inspired by this we rationalize to design novel biaryl type α-noscapine congeners by hybridizing

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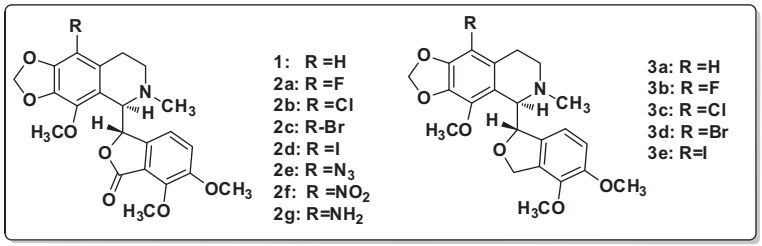


Figure 1. Natural α-noscapine and its congeners as potential chemotherapeutic cancer agents.

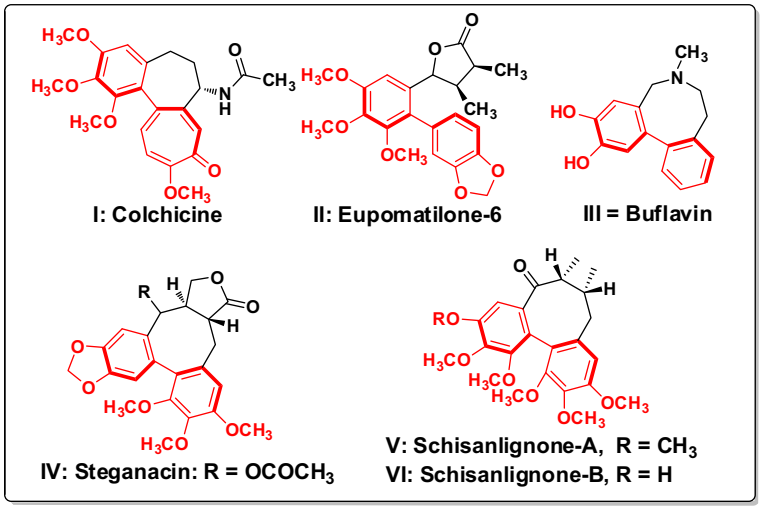


Figure 2. Cytotoxic natural lignans with biaryl pharmacophore.

biaryl ring architecture in to the natural α-noscapine skeleton to evaluate them as anticancer agents. The design strategy adopted is depicted in Figure 3.

α-Noscapine **1** is structurally consisting of two major constituents (isoquinoline and phthalide ring systems) connected with sensitive C–C bond which is labile to strong acids and bases. Therefore synthesis of noscapine analogues is always challenging.[18] In the present work we have optimized the reaction conditions for the synthesis of 9-aryl-α-noscapinods without affecting the sensitive C–C bond. The starting material 9-bromo noscapine (**2c**) required was synthesized from natural α-noscapine **1** in excellent yield (90%) using bromine water in 48% aqueous HBr by modifying the reaction conditions described in literature.[8] It was fully characterized by ^{1}H & ^{13}C NMR and mass (ESI & HRMS) spectral analysis and is in agreement with literature.[8b]

After synthesizing 9-bromo noscapine **2c** in good yield, we next examined palladium catalyzed Suzuki aryl coupling reaction with various aryl boronic acids **4a**–**g** (Fig. 4).

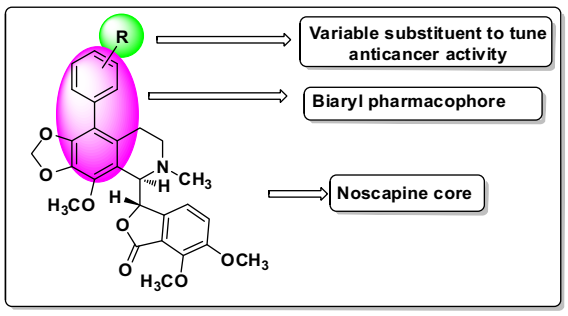


Figure 3. Design strategy for new biaryl type α-noscapine congeners.

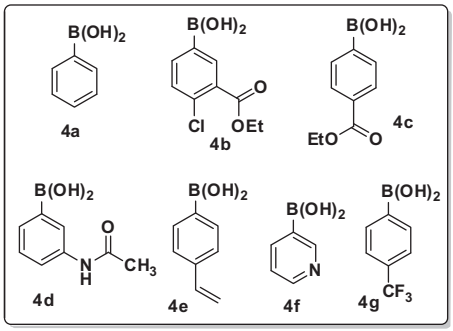
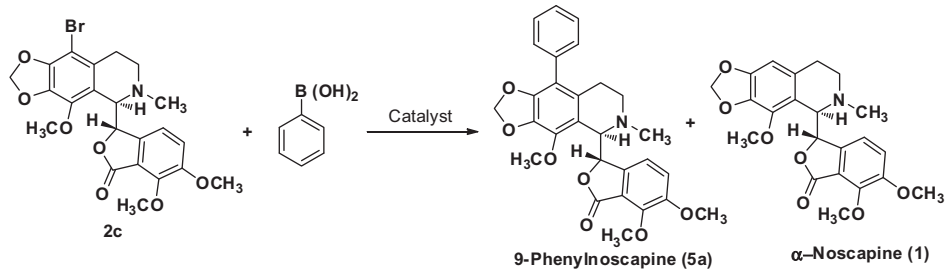


Figure 4. Aryl boronic acids **4a**–**g** used in the present study.

Initially, 9-bromo noscapine **2c** was treated with phenyl boronic acid **4a**, 3.0 mol % $Pd(PPh_3)_4$, and potassium carbonate (2 equiv) as base refluxing in toluene similar to the general Suzuki reaction conditions reported in the literature[19] (entry 1, Table 1). Disappointingly, no reaction took place and majority of bromo compound **2c** was recovered. Furthermore, changing the mole ratio of $Pd(PPh_3)_4$, potassium carbonate and solvent to dimethoxy ethane (DME) did not give any fruitful reaction (entries 2–4, Table 1). Even change of base to cesium carbonate and reaction medium to DMF did not help the progression of reaction (entries 5–6, Table 1). Further taking the clues from the general Suzuki aryl cross coupling reactions containing heterocyclic moieties, we next examined the reaction in mixed solvent system ethanol:toluene (1:1, v/v) as reaction medium at elevated temperatures. To our success, after series of experiments, 9-bromo noscapine **2c** was reacted with phenyl boronic acid **4a**, in the presence of 10 mol % of $Pd(PPh_3)_4$ and potassium carbonate (2 equiv) ethanol:toluene (1:1, v/v) at 120 °C for

Table 1
Optimization of Suzuki reaction conditions



Entry	Catalyst	(mol %)	Solvent	Base (equiv)	Reaction time (h)	Temp (°C)	Products (%)[a]	
							5a	**1**
1	$Pd(TPP)_4$	3	Toulene	K_2CO_3 (2)	12	110	<1	3
2	$Pd(TPP)_4$	3	DME	K_2CO_3 (2)	12	100	20	5
3	$Pd(TPP)_4$	5	DME	K_2CO_3 (2)	24	100	27	5
4	$Pd(TPP)_4$	10	DME	K_2CO_3 (3)	24	100	35	8
5	$Pd(TPP)_4$	10	DME	$CsCO_3$ (2.0)	12	120	Sluggish reaction	
6	$Pd(TPP)_2Cl_2$	10	DMF	$CsCO_3$ (2.0)	12	130	Sluggish reaction	
7	$Pd(TPP)_2Cl_2$	10	DMF	K_2CO_3 (3.0)	12	130	30	19
8	$Pd(TPP)_2Cl_2$	15	DMF	K_2CO_3 (3.0)	24	130	45	22
9	$Pd(TPP)_4$	10	EtOH/Toluene (1:1)	K_2CO_3 (2.0)	24	120	62	15
10	$Pd(TPP)_4$	10	EtOH/Toluene (1:1)	K_2CO_3 (2.0)	48	120	65	15
11	$Pd(TPP)_4$	10	EtOH/Toluene (1:1)	$NaHCO_3$ (2.0)	24	120	60	—
12	$Pd(TPP)_4$	10	EtOH/Toluene (1:1)	$NaHCO_3$ (2.0)	48	120	75	2
13	$Pd(TPP)_4$	12	EtOH/Toluene (1:1)	$NaHCO_3$ (2.0)	48	120	80	2

[a] Isolated yields.

24 h to give 9-phenyl noscapine **5a** in 62% yield. At this stage we also observed formation of significant quantities (15%) of noscapine **1** through debromination of **2c** (entry 9, Table 1). The reaction was best progressed when bromo-α-noscapine **2c** was reacted with **4a** in ethanol:toluene (1:1, v/v) in the presence of 12 mol % of $Pd(PPh_3)_4$ and $NaHCO_3$ (2.0 equiv) as base, at 120 °C for 48 h (entry 13, Table 1) to give product **5a** in 80% yield.

Here we noticed significant reduction in the debromination and only 2% of noscapine **1** was isolated. The crude reaction mixture was purified over silica gel column chromatography to give pure 9-phenyl α-noscapine **5a** and was fully characterized by IR, ^1H & ^{13}C NMR and Mass (ESI and HRMS) spectra analysis.[20] Recent report from Porcu et al.[21] demonstrated synthesis of 9-aryl noscapines using palladium catalyzed Suzuki cross coupling reaction using 'Xphos'. In contrast, without using expensive ligand 'Xphos' in the Suzuki cross coupling reaction, we were able to attain the product **5a** in higher yield (80%). Here we also controlled the side reaction and significantly reduced the formation of noscapine **1**.

Having optimized Suzuki aryl coupling reaction conditions, we next employed the coupling of 9-bromo-α-noscapine (**2c**) with various aryl boronic acids. All the aryl boronic acids **4b–g** reacted well with bromonoscapine 2c to give biaryl type noscapine hybrids **5b–g** (Fig. 5) with excellent yields. All the compounds **5a–g** obtained were fully characterized by ^1H, ^{13}C NMR and mass (ESI

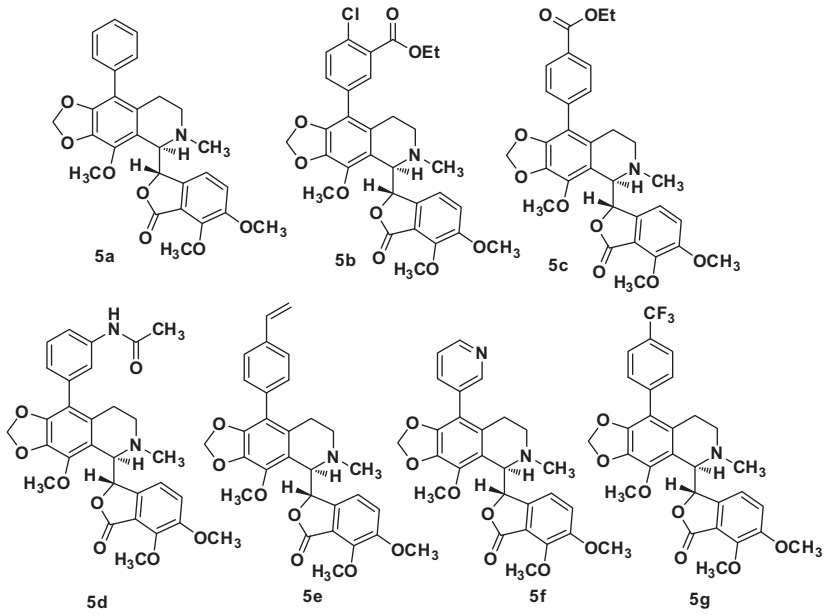


Figure 5. Novel biaryl type noscapine congeners **5a–g** synthesized.

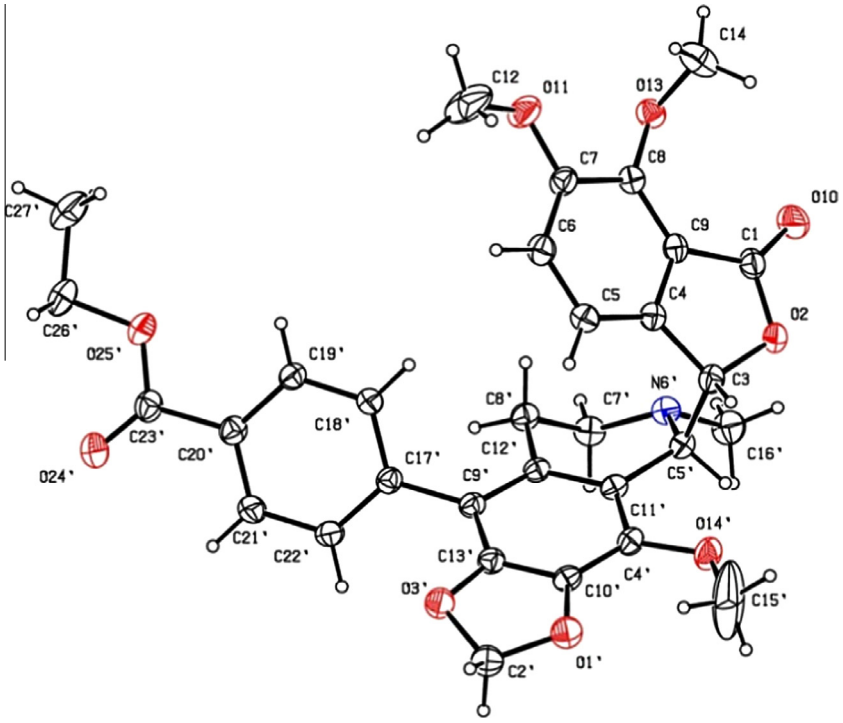


Figure 6. ORTEP diagram of **5c**, showing the atom-labeling scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are represented by circles of arbitrary radii.[22]

Table 2
IC_{50} values of noscapine derivatives **5a**–**g** for various cancer cell types[a]

Noscapine & analogues	HeLa (μM)	A549 (μM)	MCF-7 (μM)
1	24.0 ± 2.9	62.9 ± 4.6	42.3 ± 2.7
5a[24]	ND	ND	ND
5b	9.0 ± 1.5	33.8 ± 3.5	18.8 ± 2.7
5c	21.2 ± 3.7	53.1 ± 3.7	40.7 ± 3.3
5d	9.2 ± 1.4	32.6 ± 2.5	17.8 ± 2.5
5e	20.3 ± 2.5	42.7 ± 2.9	34.3 ± 2.5
5f	8.9 ± 1.7	31.6 ± 2.6	16.6 ± 2.9
5g	22.8 ± 2.8	57.3 ± 3.9	41.3 ± 2.4

[a] Cancer cells used in the assay namely, HeLa: human cervix cell line, A549: human lung adenocarcinoma epithelial cell line and MCF7: human breast epithelial cell line. Each value represents mean ± S.D. from three different experiments. ND, not determined.

and HR-MS) spectroscopic analysis.[20] Single crystal X-ray analysis unambiguously confirmed the structure of ethyl 4-((*R*)-5-((*S*)-4, 5-dimethoxy-3-oxo-1,3-dihydro isobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquin olin-9-yl) benzoate **5c** (Fig. 6).[22]

After successful synthesis of noscapinoids **5a**–**g**, we next examined whether these new analogues will affect cancer cell proliferation.[23] As a preliminary screen, all compounds including the lead molecule, noscapine were evaluated for their anti-proliferative activity in three human cancer cell lines; human breast epithelial cell (MCF-7), human cervix cancer cells (HeLa) and human lung adenocarcinoma epithelial cells (A549). The IC_{50} values for the test compounds 5**b**–**g** are included in Table 2.[24] Our results showed that all the cancer cell types were more susceptible to compounds

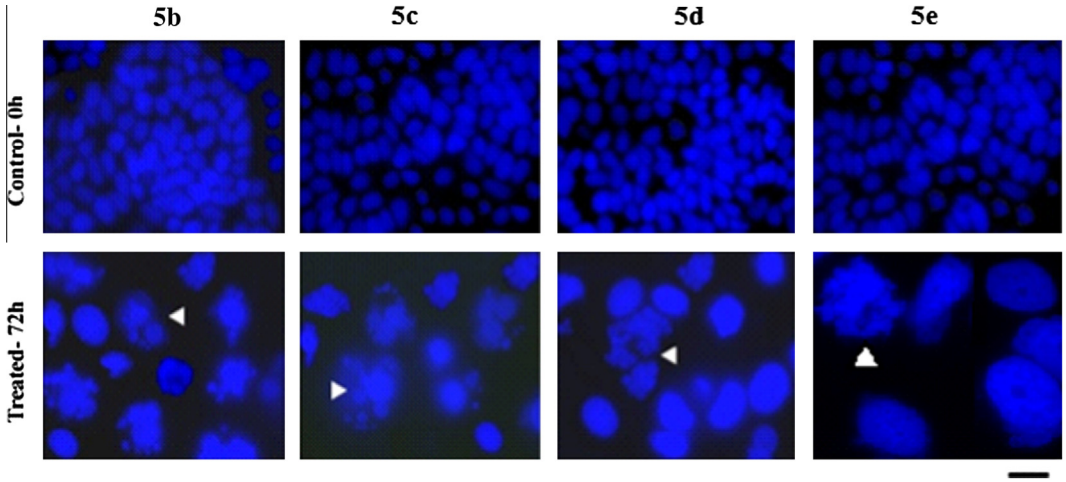


Figure 7. Representative figures show morphological evaluation of nuclei stained with DAPI in the absence and presence of the analogues (25 μM each). Several typical features of apoptotic cells such as condensed chromosomes, numerous fragmented micronuclei, and apoptotic bodies are evident (indicated by white head arrows) upon 72 h of drug treatment. (Scale bar = 15 μm).

5**b**–**g** in comparison to the lead compound, noscapine, with lower IC_{50} value. Especially three compounds **5b**, **5d** and **5f** possess potent cytotoxic activity. The IC_{50} value amounted to 9.0 μM, 9.2 μM and 8.9 μM with 5**b**, **5d** and **5f**, respectively, for HeLa cells, which reflects a pronounced anti-proliferative activity. Parenthetically, it is worth mentioning that a similar low IC_{50} value of 18.8 μM, 17.8 μM and 16.6 μM was measured using **5b**, **5d** and **5f**, respectively, for the MCF-7 cells. In contrast, modest anti-proliferative activity for these compounds was noted against A549 cell line. Thus this preliminary screen with the three chosen cell lines revealed 5**b**, **5d** and **5f** as potent cytotoxic compounds compared to noscapine. Besides the anti-proliferative effect, DAPI staining[25] of the cells treated with noscapinoids showed condensed chromatin along with numerous fragmented nuclei (shown by white arrow heads), indicative of apoptotic cell death (Fig. 7).

In conclusion we have designed a series of novel biaryl type noscapine analogues and synthesized using optimized Suzuki reaction conditions in good yields. Evaluation of all the new derivatives (**5a**–**g**) impacts their therapeutic potential for a variety of cancer cell types, indicating a great potential for further preclinical and clinical evaluation.

Acknowledgments

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Supplementary data

Supplementary data (complete experimental details, copies of ^1H, ^{13}C NMR and mass spectra (ESI and HR-MS) of synthesized products **5a**–**g**; single crystal X-ray diffraction data for compound for **5c** (CCDC 944746) can be found free of charge from the Cambridge crystallographic data center via www.ccdc.cam.ac.uk/data_ request/cif) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.046.

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20. *General procedure for the preparation of 9-arylnoscapines* **5a**–**g**: To a solution of 9-bromonoscapine (200 mg, 0.41 mmol) in ethanol/toluene (1:1, v/v, 10 mL) was added $Pd(PPh_3)_4$ (0.049 mmol), sodium bicarbonate (0.82 mmol) and **4a**–**g** (0.82 mmol), under nitrogen. Reaction mixture was heated at 120 °C for 48 h, cooled to room temperature, solvents were removed under reduced pressure, water (10 mL) was added, extracted with dichloromethane (3 × 25 mL), and combined organic portions were washed with water, dried over anhydrous sodium sulphate and concentrated. Crude product was purified over silica gel column chromatography eluted with 25% ethyl acetate in hexanes to give pure compounds **5a**–**g** as colorless solids.
(S)-6,7-Dimethoxy-3-((R)-4-methoxy-6-methyl-9-phenyl-5,6,7,8-tetra hydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one (**5a**): Yield: 80%; m.p: 110 °C; $[\alpha]_D^{25}$ −76 (*c* = 1, dichloromethane); ^1H NMR (300 MHz, $CDCl_3$): δ 7.48–7.20 (m, 5H), 7.02 (d, *J* = 8.3 Hz, 1H), 6.13 (d, *J* = 8.1 Hz, 1H), 6.00 (s, 1H), 5.92 (s, 1H), 5.56 (d, *J* = 3.7 Hz, 1H), 4.51 (d, *J* = 3.7 Hz, 1H), 4.13 (s, 6H), 3.90 (s, 3H), 2.56 (s, 3H), 2.30–2.09 (m, 2H), 1.78–1.55 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 152.2, 147.6, 145.9, 140.8, 139.5, 134.1, 133.7, 130.7, 129.8, 128.1,127.3, 120.4, 117.7, 116.4, 100.8, 81.9, 62.2, 61.0, 59.4, 56.8, 50.8, 46.7, 27.0; IR (KBr): 2942, 2900, 2791, 1751, 1605, 1497, 1437, 1380, 1310, 1270, 1034, 1006, 974, 791 cm^{-1}; HRMS (ESI): *m*/*z* Calcd for $C_{28}H_{28}NO_7$ $(M+H)^+$, 490.18603; found: 490.18509.
Ethyl 2-chloro-5-((R)-5-((S)-4,5-dimethoxy-3-oxo-1,3-dihydroiso benzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo [4,5-g]isoquinolin-9-yl)benzoate (**5b**): Yield: 70%; m.p: 152 °C; $[\alpha]_D^{25}$ −127.28 (*c* = 1, dichloromethane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.64 (d, *J* = 2.0 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.34–7.27 (dd, *J* = 2.0, 8.1 Hz, 1H), 7.26 (s, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.16–6.05 (d, *J* = 7.9 Hz, 1H), 6.00 (s, 1H), 5.92 (s, 1H), 5.45 (d, *J* = 3.7 Hz, 1H), 4.49–4.34 (m, 3H), 4.10 (s, 6H), 3.91 (s, 3H), 2.72–2.53 (m, 4H), 2.26–2.09 (m, 2H), 1.66 (s, 1H), 1.42 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 167.9, 165.6, 152.3, 147.6, 146.0, 140.8, 140.1, 133.8 133.7, 132.9, 132.5, 130.8, 130.5, 130.3, 120.4, 118.1, 117.7, 117.6, 114.1, 100.9, 81.8, 62.9, 61.6, 61.0, 59.5, 56.8, 50.5, 46.6, 26.9, 14.1; IR (KBr): 3418, 2922, 2853, 2795, 1756, 1633, 1597, 1498, 1363, 1274, 1159, 1036, 940, 813, 728, 506 cm^{-1}; MS (ESI): *m*/*z* 618 $(M+Na)^+$; HRMS (ESI): *m*/*z* Calcd for $C_{31}H_{30}NO_9ClNa$ $(M+Na)^+$, 618.1506; found: 618.1476.
Ethyl 4-((R)-5-((S)-4,5-dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-9-yl)benzoate (**5c**): Yield: 67%; m.p: 143 °C; $[\alpha]_D^{25}$ −140.52 (*c* = 1, dichloromethane); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.09 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.16 (d, *J* = 8.1 Hz, 1H), 6.00 (d, *J* = 1.3 Hz, 1H), 5.93 (d, *J* = 1.3 Hz, 1H), 5.54 (d, *J* = 4.1 Hz, 1H), 4.49 (d, *J* = 4.3 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 4.12 (s, 3H), 4.11 (s, 3H), 3.91 (s, 3H), 2.67–2.52 (m, 4H), 2.27–2.11 (m, 2H), 1.76–1.65 (m, 1H), 1.41 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.9, 166.2, 152.3, 147.7, 146.0, 140.9, 140.0, 138.9, 133.7, 130.6, 130.0, 129.3, 120.5, 118.1, 117.7, 117.7, 115.4, 100.9, 81.8, 62.2, 61.1, 60.9, 59.5, 56.9, 56.7, 46.7, 27.0, 14.3; IR (KBr): 3413, 2915, 1767, 1697, 1616, 1498, 1464, 1443, 1381, 1306, 1262, 1165, 1088, 1034, 1012, 821, 621 cm^{-1}; MS (ESI): *m*/*z* 584 $(M+Na)^+$; HRMS (ESI): *m*/*z* Calcd for $C_{31}H_{31}NO_9Na$ $(M+Na)^+$, 584.1896; found: 584.1881.
N-(3-((R)-5-((S)-4,5-Dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquin olin-9-yl)phenyl)acetamide (**5d**): Yield: 55%; m.p: 240 °C; $[\alpha]_D^{25}$ −138.68 (*c* = 1, dichloromethane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.68 (s, 1H), 7.46–7.28 (m, 2H), 7.25–7.12 (d, *J* = 8.3 Hz, 1H), 7.07–6.93 (d, *J* = 7 .2 Hz, 1H), 6.23–6.10 (d, *J* = 8.3 Hz, 1H), 5.98 (s, 1H), 5.91 (s, 1H), 5.54 (d, *J* = 3.9 Hz, 1H), 4.50 (d, *J* = 3.9 Hz, 1H), 4.10 (s, 6H), 3.94 (s, 3H), 2.54 (s, 4H), 2.27–2.10 (m, 5H), 1.77–1.60 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 168.4, 152.2, 147.3, 145.9, 140.6, 139.5, 138.2, 134.9, 133.6, 130.8, 128.5, 125.5, 121.3, 120.2, 118.3, 117.9, 117.6,

116.3, 100.8, 82.1, 62.1, 61.0, 59.4, 56.6, 56.4, 50.8, 46.7, 27.0, 24.4; IR (KBr): 3329, 2920, 2791, 1739, 1682, 1586, 1548, 1503, 1384, 1274, 1086, 1037,797, 620 cm^{-1}; MS (ESI): *m/z* 569 $(M+Na)^+$; HRMS (ESI): Calcd for $C_{30}H_{30}N_2O_8Na$ $(M+Na)^+$, 569.1899; found: 569.1920.

(S)-6,7-Dimethoxy-3-((R)-4-methoxy-6-methyl-9-(4-vinylphenyl)-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one (**5e**): Yield: 60%; m.p: 120 °C; $[\alpha]_D^{25}$ −120.22 (*c* = 1, dichloromethane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.74–6.66 (dd, *J* = 10.8 Hz, 17.5 Hz, 1H) 6.10 (s, 1H), 5.98 (s, 1H), 5.91 (s, 1H), 5.74 (d, *J* = 17.5 Hz, 1H), 5.48 (s, 1H), 5.25 (d, *J* = 10.8 Hz, 1H), 4.47 (s, 1H), 4.10 (s, 6H), 3.90 (s, 3H), 2.66–2.54 (m, 4H), 2.27–2.13 (m, 2H), 1.77–1.64 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 152.2, 147.7, 146.0, 143.6, 140.9, 139.6, 136.7, 133.7, 133.5, 130.7, 130.1, 126.0, 120.4, 117.8, 116.1, 114.2, 100.8, 81.9, 62.3, 61.1, 59.5, 56.9, 50.8, 46.6, 27.0, 23.2, 29.6 cm^{-1}; MS (ESI): *m/z* 538 $(M+Na)^+$; HRMS (ESI): Calcd for $C_{30}H_{29}NO_7Na$ $(M+Na)^+$, 538.1841; found: 538.1848.

(S)-6,7-Dimethoxy-3-((R)-4-methoxy-6-methyl-9-(pyridin-3-yl)-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one (**5f**): Yield: 62%; mp: 193 °C; $[\alpha]_D^{25}$ −124.25 (*c* = 1, dichloro methane); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.52 (s, 1H), 8.43 (s, 1H) 7.56 (d, *J* = 7.6 Hz, 1H), 7.30 (t, *J* = 6.6 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.12 (d, *J* = 7.6 Hz, 1H), 5.99 (s, 1H), 5.92 (s, 1H), 5.43 (d, *J* = 4.7 Hz, 1H), 4.43 (d, *J* = 4.7 Hz, 1H), 4.11 (s, 3H), 4.08 (s, 3H), 3.88 (s, 3H), 2.67–2.60 (m, 1H), 2.55 (s, 3H), 2.22–2.14 (m, 2H), 1.79–1.69 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 167.9, 152.3, 150.7, 148.36, 147.6, 146.4, 140.7, 140.2, 137.3, 133.7, 130.7, 130.3, 130.2, 123.1, 120.4, 118.2, 117.5, 100.9, 81.8, 62.2, 61.0, 59.4, 56.8, 50.6, 46.6, 26.8; IR (KBr): 3412, 2938, 1756, 1637, 1497, 1445, 1273, 1082, 1032, 943, 815, 714 cm^{-1}; MS (ESI): *m/z* 513 $(M+Na)^+$; HRMS (ESI): Calcd for $C_{27}H_{26}N_2O_7Na$ $(M+Na)^+$, 513.1637; found: 513.1615.

(S)-6,7-Dimethoxy-3-((R)-4-methoxy-6-methyl-9-(4-(trifluoromethyl) phenyl)-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)iso benzofuran-1(3H)-one (**5g**): Yield: 65%; mp: 135 °C; $[\alpha]_D^{25}$ −62.22 (*c* = 1, dichloromethane); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60–7.40 (m, 4H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.05 (d, *J* = 8.3 Hz, 1H), 6.02 (d, *J* = 1.3 Hz, 1H), 5.93 (d, *J* = 1.3 Hz, 1H), 5.44 (d, *J* = 4.1 Hz, 1H), 4.46 (d, *J* = 4.3 Hz, 1H), 4.14 (s, 3H), 4.10 (s, 3H), 3.89 (s, 3H), 2.65–2.51 (m, 4H), 2.20–2.00 (m, 2H), 1.70–1.56 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.9, 152.4, 147.5, 146.0, 140.5, 140.0, 134.9, 133.2, 128.7, 126.7, 126.6, 124.1, 120.5, 118.1, 117.2, 114.7, 100.9, 81.8, 62.2, 61.0, 59.5, 56.5, 50.7, 46.7, 29.6, 27.2; IR (KBr): 3413, 2948, 1765, 1614, 1497, 1422, 1237, 1158, 1120, 1039, 946, 806, 732, 701 cm^{-1}; MS(ESI): *m/z* 580 $(M+Na)^+$; HRMS (ESI): Calcd for $C_{29}H_{26}NO_7F_3Na$ $(M+Na)^+$, 580.1559; found: 580.1568

21. Porcù, E.; Sipos, A.; Basso, G.; Hamel, E.; Bai, R.; Stempfer, V.; Udvardy, A.; Bényei, A. C.; Schmidhammer, H.; Antus, S.; Viola, G. *Eur. J. Med. Chem.* **2014**, *84*, 476.

22. X-ray data for the compound **5c** was collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoKα radiation (λ = 0.71073 Å) with ω-scan method. Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Integration and scaling of intensity data were accomplished using SAINT program. The structure was solved by direct methods using SHELXS97 and refinement was carried out by full-matrix least-squares technique using SHELXL97. Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were located in difference Fourier maps and subsequently geometrically optimized and allowed for as riding atoms, with C–H = 0.93–0.97 Å, with $U_{iso}(H) = 1.5U_{eq}(C)$ for methyl H or $1.2U_{eq}(C, N)$. The methyl groups were allowed to rotate but not to tip. The absolute configuration of the procured material was known in advance and was confirmed by unambiguous refinement of the absolute structure parameter. In the absence of significant anomalous scattering efforts, Friedel pairs were merged for **5c**. Crystal data for **5c**: $C_{31}H_{31}NO_9$, *M* = 561.57, colorless needle, 0.16 × 0.08 × 0.06 mm^3, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 11.0875(8), *b* = 15.3517(11), *c* = 17.0221(12) Å, *V* = 2897.4(4) $Å^3$, *Z* = 4, D_c = 1.287 g/cm^3, F_{000} = 1184, CCD Area Detector, MoKα radiation, λ = 0.71073 Å, *T* = 294(2) K, $2\theta_{max}$ = 50.0°, 27964 reflections collected, 2891 unique (R_{int} = 0.0196). Final *GooF* = 1.111, *R1* = 0.0402, *wR2* = 0.1212, *R* indices based on 2737 reflections with $I>2\sigma(I)$ (refinement on F^2), 375 parameters, 0 restraints, μ = 0.095 mm^{-1}. CCDC 944746 contains supplementary Crystallographic data for the structure. (a) Bruker (2001). SAINT (Version 6.28a) & SMART (Version 5.625) (b) Sheldrick G. M. *Acta Crystallogr.* **2008**, *A64*, 112. (c) Flack, K.; Bernardinelli, G. J. *Appl. Cryst.* **2000**, *i*, 1143.

23. *In vitro cell proliferation assays (MTS assay)*: Cell culture reagents were obtained from Sigma and Invitrogen. MCF-7, a human breast epithelial cancer cell line; HeLa, a human cervix cell line and A549, a human lung cancer cell line were obtained from the National Repository of Animal Cell Culture, National Centre for Cell Sciences, Pune (NCCS), India. The cell lines were maintained in Dulbecco's Modification of Eagle's Medium 1× (DMEM) with 4.5 g/L glucose and L-glutamine (Sigma) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). Suspension cells (MCF-7, HeLa and A549) were seeded into 96-well plates at a density of 5×10^3 cells per well and were treated with increasing gradient concentrations of noscapinoids, **5b**–**g** for 72 h. Measurement of cell proliferation was performed calorimetrically by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2-(4-sulpho phenyl)-2H-tetrazolium, inner salt (MTS) assay, using the CellTiter 96 AQueous One Solution Reagent (Sigma). Cells were exposed to MTS for 3 h and absorbance was measured using a microplate reader (Molecular Devices, Sunnyvale, CA) at an optical density (OD) of 490 nm. The percentage of cell survival as a function of drug concentration was then plotted to determine the IC_{50} value, which stands for the drug concentration needed to prevent cell proliferation by 50%.

24. Due to solubility issues, we could not report a conclusive anticancer activity for **5a**.

25. *DAPI staining:* Nuclear morphology of cells treated with the noscapinoids was evaluated by staining the cells with DAPI and imaging with fluorescence microscopy. Briefly, MCF-7 cells (2×10^3 cells) were grown on poly-L-lysine coated cover slips in 6-well plates and were treated with the compounds at 25 μM for 72 h. After incubation, cover slips were fixed in cold methanol and washed with PBS, stained with DAPI, and mounted on slides. Images were captured using a BX60 fluorescence microscope (Olympus, Tokyo, Japan) with an 8-bit camera (Dage-MTI, Michigan City, IN) and IP Lab software (Scanalytics, Fairfax, VA). Apoptotic cells were identified by features characteristic of apoptosis (e.g., nuclear condensation, formation of membrane blebs and apoptotic bodies).