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Synthesis and Biological Evaluation of *N*-Substituted Noscapine Analogues

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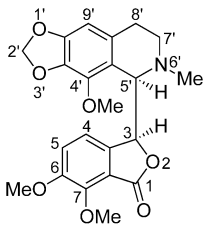
Noscapine is a phthalideisoquinoline alkaloid isolated from the opium poppy *Papaver somniferum*. It has long been used as an antitussive agent, but has more recently been found to possess microtubule-modulating properties and anticancer activity. Herein we report the synthesis and pharmacological evaluation of a series of 6′-substituted noscapine derivatives. To underpin this structure–activity study, an efficient synthesis of *N*-nornoscapine and its subsequent reduction to the cyclic ether derivative of *N*-nornoscapine was developed. Reaction of the latter with a range of alkyl halides, acid chlorides, isocyanates, thioisocyanates, and chloroformate reagents resulted in the formation of the corresponding *N*-alkyl, *N*-acyl, *N*-carbamoyl, *N*-thiocarbamoyl, and *N*-carbamate derivatives, respectively. The ability of these compounds to inhibit cell proliferation was assessed in cell-cycle cytotoxicity assays using prostate cancer (PC3), breast cancer (MCF-7), and colon cancer (Caco-2) cell lines. Compounds that showed activity in the cell-cycle assay were further evaluated in cell viability assays using PC3 and MCF-7 cells.

Introduction

Microtubule-interfering agents for cancer therapy have been validated through the use of taxanes and vinca alkaloids in the treatment of various cancers.[1] There are a variety of antimitotic agents that perturb microtubule dynamics: vinca alkaloids (vinblastine, vincristine, vindesine and others) and taxanes (paclitaxel and docetaxel). These antitumour agents are of significant importance and are commonly used in current chemotherapy regimens. However, the emergence of multidrug-resistance phenotypes and the inability of current antitumour agents to selectively target malignant cells have led to a global search for new anticancer drugs.



Noscapine (**1**)

Noscapine (**1**) is a phthalideisoquinoline alkaloid used medicinally as an antitussive drug in humans. Its nontoxicity and oral availability allow noscapine to bypass the problems associated with the administration methodologies of current cancer chemotherapy agents such as parenteral injections and intravenous infusion. These routes of administration generally result in such complications as anaphylactic reactions at the injection site causing severe pain and thrombosis of blood vessels or embolisms.[2,3]

The discovery of noscapine originated from its isolation from *Papaver somniferum*, more commonly known as the opium poppy. Noscapine's potential as an anticancer microtubule binding agent was subsequently discovered by Ye et al. in the 1990s.[2] The rational structure-based screening of naturally derived compounds sharing structural similarities with toxic microtubule-depolymerising agents such as colchicine and podophyllotoxin demonstrated noscapine to possess both the structural and binding similarities of these previously known microtubule (MT) depolymerising compounds.[4] Subsequent biological testing of noscapine showed enhanced qualities over current tubulin binding agents. Noscapine has the ability to evade noncancerous cells and thus does not halt the segregation of chromosomes in cells undergoing normal mitotic separation. In comparison, currently available anticancer agents either over-polymerise (taxanes) or depolymerise (vinca alkaloids) MTs with no selectivity for malignant over normal cells.[2] Noscapine does not appear to significantly change the MT polymer mass even at higher concentrations.[5]

Noscapine does not alter the organisation of MT arrays at the interphase stage of the cell cycle. Noscapine binds to tubulin stoichiometrically during the pro-metaphase of mitosis and induces subtle effects on the kinetic parameters of MTs resulting in the instability of MTs. These subtle effects in MT dynamics increase the elapsed time the MTs spend in an attenuated state, when neither microtubule shortening nor growth is detected.[2,5] The subtle induced suppression of the MT dynamics is sufficient to interfere with proper attachment of chromosomes to the kinetochore microtubules. This suppresses the tension across the paired kinetochores, resulting in the spindle checkpoints to block cells at a metaphase-like state. This is similar to the actions of taxanes and vinca alkaloids at low con-

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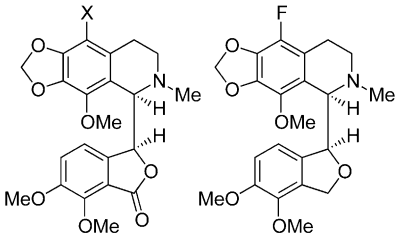
centrations, at which the chromosomes do not complete congression to the equatorial plane.[5] Noscapine then induces apoptosis of malignant cells.

Binding studies are yet to ascertain specifically where noscapinoids bind tubulin. A recent binding study by Joshi and coworkers used the known binding interaction of colchicine in an attempt to identify the binding site of noscapine on tubulin.[6] These authors suggested that noscapine binds at or near the binding site of colchicine.[6] To understand the binding of noscapinoids, a competitive binding study between colchicine and noscapine was conducted. Colchicine is nonfluorescent, but shows clear fluorescence properties when bound to tubulin.[7] The binding of colchicine is a somewhat complex biphasic reaction. The initial phase results in the binding of colchicine to a low-affinity site in which binding depends directly on the intracellular diffusion of drug into the cell. Colchicine then inhabits a site where it binds practically irreversibly and cannot be dislodged by competing ligands of the same site.[6] This observable fact allows determination of whether other drugs compete for the same site. To test this hypothesis, tubulin was pre-incubated with the test ligands noscapine (**1**) and 9-bromonoscapine (**4**). Three different concentrations were used for the colchicine competition studies. The highest concentration of noscapine used (100 μM) had little inhibitory effect (<5%) on the binding of colchicine. However, at the same concentration, the 9-bromonoscapine analogue showed a significant 66±5% decrease in colchicine binding.[6,8] A competitive binding study was also carried out at concentrations of 25 and 50 μM, and the respective inhibition values for colchicine were found to be 23±7 and 40±4%. Lower concentrations of the 9-bromonoscapine analogue were not tested owing to the inability to statistically measure inhibition of <5%.[6] The precise site where noscapinoid binding occurs is not known, and thus the probable binding site can only be inferred. The evidence suggests that the site of noscapinoid binding is at or near the colchicine site.

Derivatisation of noscapine by Aneja et al. resulted in the synthesis of the 9-halogenated analogues: 9-fluoronoscapine (**2**), 9-chloronoscapine (**3**), 9-bromonoscapine (**4**), and 9-iodonoscapine (**5**), which possess higher binding affinities for tubulin than noscapine.[9] The 9-halogenated analogues showed a pronounced increase in the inhibition of cancer cell proliferation over noscapine. The 9-iodo analogue showed varying abilities to inhibit cancer cell proliferation in the cell lines chosen. The activity of 9-iodonoscapine in MDA-MB-231 cells showed higher activity than noscapine, but lower activity in other cell lines.[9] Two breast cancer cell lines (MCF-7 and MDA-MB-231) and a T-cell lymphoma line, CEM, showed the cancer-active halogenated analogues to have markedly lower IC_{50} values than noscapine. The inhibition of cell proliferation was observed after 4,6-diamidino-2-phenylindole (DAPI) staining to reveal the appearance of numerous fragmented nuclei after drug exposure and pronounced multi-polar spindles, which are characteristic of apoptotic cell death.[9] From these results it is evident that tubulin is a potential target for these compounds; however, each of these analogues are cell-type dependent in their anticancer effect.



2: X = F
3: X = Cl
4: X = Br
5: X = I

6: CEFNA

The effects of reducing the lactone moiety of noscapine to the corresponding cyclic ether was also investigated; the cyclic ether noscapinoid was fluorinated at the C9 position (**6**, CEFNA), and its anticancer activity was analysed.[10] The emergence of multidrug resistance is a major limitation to current chemotherapeutics. The anticancer activity of CEFNA was monitored with the MCF-7/Adr cancer cell line. This cancerous cell line exhibits the multidrug-resistance phenotype through an overexpression of the drug efflux pump P-glycoprotein, where substrates such as paclitaxel are actively transported out of the cell.[11,12] To determine whether the cyclic fluorinated analogue is also a substrate for cellular efflux, the IC_{50} values of CEFNA in both normal and MDR phenotype breast cancer cell lines (MCF-7 and MCF-7/Adr) were found to be 6.2 and 6.9 μM, respectively, suggesting that CEFNA is a weak substrate for drug efflux.[10] The inherent base sensitivity of the lactone functionality makes it an undesirable moiety if the intended route of drug administration is oral. The retention of activity by cyclic ether noscapinoids invites further research.

Anderson et al. investigated the manipulation of the benzofuranone moiety through regioselective O-demethylation at the 7-position. This yielded a battery of O-alkylated derivatives, including the hydroxy analogue, which was shown to be ~100-fold more potent than the parent noscapine.[13] This suggests that chemical manipulations of the benzofuranone ring do impact the biological activity of noscapine. Anderson et al. further explored the 7-position, as the respective hydroxy moiety allowed functionalisation via conversion into its trifluoromethanesulfonate. The 7-amino analogue showed inhibition of tubulin polymerisation in adenocarcinoma human alveolar basal epithelial (A549) cells, with an EC_{50} value of 0.3 μM, which is similar to those of the 7-hydroxy analogue and vinblastine: 0.7 and 0.2 μM, respectively.[14]

A series of second-generation 7-position analogues were synthesised and showed diminished steric hindrance through selective O-demethylation at the 7-position, resulting in the 7-hydroxy analogue, with a 100-fold improvement in efficacy over noscapine.[14,15] Mishra et al. explored the hypothesis of whether introducing a large group at this position would have the opposite effect and decrease activity.[15] Acylation of the hydroxy group led to synthesis of the acetyl and benzoyl analogues; the phenolic group was also treated with available isocyanates to yield a small number of 7-carbamate compounds. The two acyl analogues, 7-acetyl and 7-benzoyl, with the 7-ethyl, phenyl, and benzyl carbamates, showed varying degrees of in vitro cytotoxicity. The A549, CEM, MCF-7, PC3, and MIA

PaCa-2 cell lines were used to analyse the cytotoxicity of these second-generation benzofuranone-substituted analogues. These compounds showed varying results, with some analogues exhibiting better activity than noscapine in a particular cell line, and weaker activity than noscapine in others.[15] It was expected that bulkier substituents at the 7-position would lead to lower activity; however, these analogues were shown to be significantly better than the parent compound noscapine. The cancer cell sensitivity to these analogues is, however, cell-type dependent.[15]

Noscapine was recently demonstrated to inhibit tumour growth in temozolomide (TMZ)-resistant gliomas.[16] In order to target glioblastoma multiforme (GMB), the drug must penetrate the blood–brain barrier (BBB). Landen et al. demonstrated the ability of noscapine to cross the BBB using an in vitro model.[3] Noscapine was evaluated against TMZ-resistant gliomas and was found to inhibit the proliferation of TMZ-resistant human glioma cells. The highly vascularised GBM is characterised by extensive endothelial cell proliferation which migrates readily. Noscapine administration resulted in significantly decreased migration and invasion of these cells in vitro.[16] The co-administration of noscapine and TMZ exhibited a significant additive effect for TMZ-sensitive cells relative to each drug alone.[16]

Herein we describe an improved synthesis of *N*-nornoscapine (**7**) and subsequent reduction to the cyclic ether *N*-nornoscapine analogue **9** in good yield allowing synthesis of a small series of N-substituted analogues. Polonovski-type reaction conditions for N-demethylation of apomorphine using $FeSO_4 \cdot 7H_2O$ at low temperature[17] was shown to be the most efficient method for the preparation of **7**. Several years ago, Joshi and colleagues[18] described the N-demethylation of noscapine via the *N*-oxide hydrochloride, to afford 1,3-dihydro-6,7-dimethoxy-3-[1,2,3,4-tetrahydro-8-methoxy-6,7-(methylenedioxy)isoquinolin-1-yl]isobenzofuran-1-one (**7**, *N*-nornoscapine), using ferric citrate in a buffer solution of citric acid at pH 1–2. The reported isolated yield was 35%. A modified procedure for the reduction of **7** using $BF_3 \cdot Et_2O$ and $NaBH_4$ in THF[18] delivered the N-demethylated cyclic ether analogue in good yield (78%).

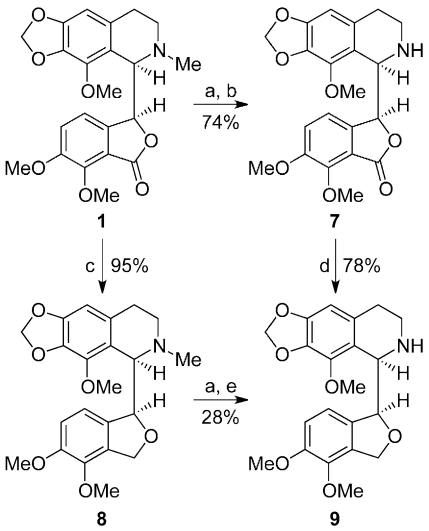
The cell-cycle arrest and decrease in proliferation of rapidly dividing cells by cyclic ether noscapine analogues was investigated by flow cytometry (using a DNA binding dye) and use of the CellTiter-Blue® assay as a surrogate marker of cell growth. We observed that some of the novel cyclic ether noscapine analogues are able to cause G_2/M arrest in prostate (PC3), breast (MCF-7), and colon (Caco-2) cancer cell lines.

Results and Discussion

Synthesis of N-substituted noscapine analogues was hampered by the relatively low yields in the N-demethylation of **1** for the synthesis of the secondary amine *N*-nornoscapine (**7**). This compound is a key synthetic intermediate that provides the scaffold for N-derivatisation and is not commercially available. Our research group used a nonclassical Polonovski reaction for the synthesis of **8**. The Polonovski reaction conditions described by Scammells and co-workers for the N-demethylation of various opiate alkaloids using $FeSO_4 \cdot 7H_2O$ at low temperatures[17] was shown to be the most efficient method for the generation of **7** in good yield (78%). In this reaction, the ferrous ion coordinates to the protonated *N*-oxide species and promotes a single electron transfer resulting in the formation of an aminium radical cation. The species then loses an α-proton, undergoing an electron reorganisation to form the more stable carbon-centered radical, which undergoes another single electron transfer to afford the iminium ion intermediate. Hydrolysis then affords the desired secondary amine.

The novel N-substituted cyclic ether analogues were synthesised by using noscapinoid **9** as the scaffold. Previous research by Aneja et al. showed the 9-fluorinated cyclic ether noscapine analogue to exhibit greater potency against the MCF-7 cell line.[10] The benefits of a reduced lactone are twofold: the cyclic ether noscapine analogue improves the in vivo potential of novel analogues, and decreases the in vivo base sensitivity of compounds that may result in ring opening in situ, thereby decreasing alkaloid binding through loss of the active structure.

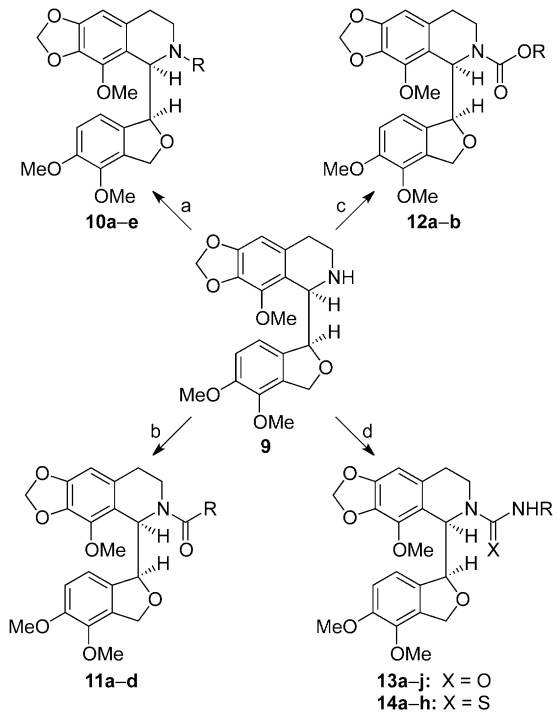
Scheme 1 shows that there are two pathways to access the secondary amine **9**, thereby allowing structural diversification



Scheme 1. *Reagents and conditions:* a) 1. *m*-CPBA, $CHCl_3$, 0 °C, 2. HCl, 95%; b) $FeSO_4 \cdot 7H_2O$, MeOH, −5 °C, 78%; c) $NaBH_4/BF_3 \cdot Et_2O$, THF, reflux, 95%; d) $NaBH_4/BF_3 \cdot Et_2O$, THF, RT, 78%; e) $FeSO_4 \cdot 7H_2O$, MeOH, −5 °C, 29%.

of noscapine through N-derivatisation. One pathway begins with the synthesis of noscapine-*N*-oxide isolated as the hydrochloride salt. Recent work in our lab has shown that the N-demethylation of opiate *N*-oxide hydrochlorides improves the yield of the desired noropiates relative to other opiate *N*-oxide salts. A number of different conditions were trialed, including temperature and solvent effects with various iron catalysts. It was discovered that the most suitable conditions for N-demethylation of noscapine involve $FeSO_4 \cdot 7H_2O$ in methanol at −5 °C. A respectable yield of 78% was attained for the desired secondary amine *N*-nornoscapine scaffold, which was sequen-

tially reduced to the cyclic ether analogue by the procedure described by Ye et al.[2] Accordingly, the resultant yield for the reduction was a modest 50%. A slight modification to the reaction procedure, allowing the reduction reaction to proceed at room temperature overnight rather than at reflux for 2 h resulted in a modest increase in the yield of the desired cyclic ether *N*-nornoscapine to 78%. The pathway that involved first the reduction of the lactone moiety (95% yield) followed by N-demethylation of cyclic *N*-oxide, which previously proved unsuccessful,[18] gave a low yield of 29% of **9** using the iron sulfate method described herein. Given the greater mass recovery of the pathway that involves N-demethylation followed by lactone reduction and ease of purification, this pathway was used for the synthesis of the cyclic ether nornoscapine scaffold **9**. The synthesis of **9** allowed for derivatisation of the secondary amine, to furnish a number of novel N-substituted cyclic ether noscapine analogues. Compound **9** was subsequently treated with a number of alkyl halides, acid chlorides, chloroformate reagents, isocyanates, and isothiocyanates (Scheme 2), to yield a focused library of novel cyclic ether N-substituted analogues.



Scheme 2. *Reagents and conditions:* a) RX, Cs_2CO_3, MeCN, reflux, 18 h, 56–81%; b) RCOCl, Et_3N, CH_2Cl_2, RT, 3 h, 53–68%; c) ROCOCl, Et_3N, CH_2Cl_2, RT, 24 h, 83–85%; d) RNCO/RNCS, MeCN, RT, 3 h, 59–98%.

Given the activity of noscapine was shown to inhibit the progression of a number of tumour cell lines, investigation into the tumour cell anti-proliferation effect of the presence of larger alkyl chains was investigated. The synthesis of the *N*-methyl, ethyl, propyl, hydroxyethyl, benzyl, and phenethyl analogues followed. The initial reaction procedure involved dissolution of **9** in acetone in the presence of triethylamine (2 equiv), followed by the addition of the appropriate alkyl iodide (e.g. ethyl iodide for the preparation of **10a**); this was held at reflux overnight. The presence of two products was observed by TLC analysis following reaction completion after 18 h. Column purification of the crude material using petroleum ether (PE)/ethyl acetate could not separate the desired compound from other impurities present in the reaction mixture. The reaction was then attempted using Cs_2CO_3 in acetonitrile instead of acetone to decrease the likelihood of reagent–solvent reaction. The reaction was held at reflux overnight, and TLC analysis showed completion within 18 h. The reaction mixtures were subsequently quenched with water and extracted with chloroform. The crude material was then purified by flash chromatography using PE/EtOAc to give the desired *N*-alkyl derivatives. The straight-chain alkyl analogues *N*-ethyl, *N*-propyl, and *N*-hydroxyethyl appeared to be relatively unstable, and once purified, were subsequently converted into their hydrochloride salts.

A small series of *N*-acyl analogues were synthesised, whereby **9** was dissolved in dichloromethane in the presence of triethylamine (2 equiv); the reaction mixture was then cooled to 0 °C followed by the addition of the appropriate acid chloride. If the acid chloride was not available, it was synthesised in situ using the corresponding acid with DMF and oxalyl chloride. After the addition of reagents, the reaction was allowed to warm to ambient temperature until completion (2 h). The reaction was quenched with water and extracted with dichloromethane. The solvent was removed in vacuo, and flash chromatography with PE/EtOAc gave moderate yields of the desired amide analogues. NMR analysis of the *N*-amides showed the presence of two rotameric forms of each of the *N*-acyl analogues. The corresponding urea and thiourea analogues were prepared by a procedure similar to that described by Aggarwal et al.[18] This involved cooling of **9** in acetonitrile to 0 °C in the presence of Cs_2CO_3 (2 equiv). The isocyanate/isothiocyanate reagent was added to the solution, and the mixture was allowed to warm to ambient temperature. Reactions were generally complete within 2 h of isocyanate addition. Reactions were quenched with cold H_2O and extracted in $CHCl_3$. Purification of crude reaction products was carried out by means of flash chromatography using PE/EtOAc. Generally the compounds were quite nonpolar and as a result elution could be facilitated using 15% EtOAc in PE. The synthesis of the urea/thiourea analogues proved efficient, with yields ranging from 59 to 98%, with most analogues being prepared in yields of >85%.

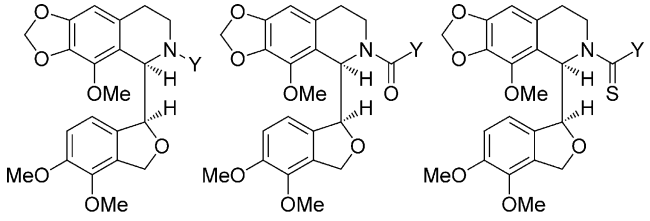
The final class of N-substituted analogues is the carbamate derivatives, which were prepared as follows: compound **9** was cooled in dry dichloromethane in the presence of triethylamine. The solution was cooled to -5 °C to which the chloroformate reagent was added, and the reaction was allowed to proceed at ambient temperature for 2 h. After this time, the reaction mixture was quenched with water and extracted into an organic solvent. The resulting crude material was purified by flash chromatography using PE/EtOAc to give the desired carbamates in good yield. NMR analysis of the *N*-carbamates showed the presence of two rotameric forms of each of these analogues.

Biological data

We investigated the possibility that the novel *N*-nornoscapine derivatives (Table 1) cause G_2/M-phase arrest through a disruption of microtubule structures. Our results suggest that specific *N*-nornoscapine analogues show anticancer activity by arresting cells in mitosis in all three of the cell lines tested, whereas at similar concentrations, noscapine (**1**) is ineffective. The commercially available microtubule inhibitor vincristine, which has been in common use for decades as an anticancer drug, was used as a positive control.

To understand the cytotoxic activity of these compounds, a cell-cycle cytotoxicity assay was performed by treating prostate cancer (PC3), breast cancer (MCF-7), and colon cancer (Caco-2) cell lines with compounds at 10 μM, as listed in Table 1. Cells were then stained with Cell Cycle Blue (a cell-cycle distribution dye) and cell-cycle phase distribution was analysed by flow cytometry (Figure 1). Compounds that were able to cause ≥50% change in the number of cells in G_2/M arrest were considered to be highly active; compounds effecting changes between 10 and 50% were considered to be active.

Table 1. Changes in the percentage of cells arrested at the G_2/M phase following treatment with noscapine analogues versus vehicle control, along with EC_{50} values for active compounds.



1, **10a–e** **11a–d**, **12a–b**, **13a–j** **14a–h**

Compd	Y	Change in cell population at G_2/M [%][a]			EC_{50} [μM][b]	
		PC3	MCF-7	Caco-2	PC3	MCF-7
1	Me	2	2	3	NA	NA
10a	Et	1	−2	2	NA	NA
10b	Pr	1	3	−2	NA	NA
10c	Bn	−2	2	2	NA	NA
10d	$(CH_2)_2Ph$	2	2	2	NA	NA
10e	$(CH_2)_2OH$	96	86	43	6.70	≥10
11a	Me	2	−2	2	NA	NA
11b	Et	3	3	−2	NA	NA
11c	Ph	86	58	47	≥10	5.27
11d	Bn	81	168	48	≥10	3.91
12a	OPh	67	105	28	≥10	≥10
12b	OBn	79	170	27	7.70	4.18
13a	NHEt	133	172	60	6.7	3.58
13b	NH*n*Pr	123	156	47	14.40	12.1
13c	NH*n*Bu	−2	2	−4	NA	NA
13d	NH*n*Pent	3	−2	3	NA	NA
13e	NHPh	134	155	61	8.40	7.53
13f	NHBn	3	3	2	NA	NA
13g	NH(2-ClPh)	68	14	49	≥10	≥10
13h	NH(3-ClPh)	78	99	42	≥10	≥10
13i	NH(4-ClPh)	102	117	33	≥10	11.1
13j	NH(3,5-$(CF_3)_2$Ph)	−2	3	1	NA	NA
14a	NHEt	147	153	20	≥10	4.87
14b	NH*n*Pr	3	−2	3	NA	NA
14c	NHPh	1	3	3	NA	NA
14d	NHBn	−1	2	1	NA	NA
14e	NH(2-ClPh)	3	1	2	NA	NA
14f	NH(3-ClPh)	3	1	2	NA	NA
14g	NH(4-ClPh)	−1	2	2	NA	NA
14h	NH(3,5-$(CF_3)_2$Ph)	1	2	1	NA	NA

[a] The percentage arrested was determined by reference to the number of cells in G_2/M phase in vehicle control samples through the following formula: $[(Cells_{compd} - Cells_{vc})/Cells_{vc}] \times 100\%$, in which $Cells_{compd}$ is the number of compound-treated cells arrested in G_2/M phase, and $Cells_{vc}$ is the number of vehicle control cells arrested in G_2/M phase. [b] Values estimated using the CellTiter-Blue® assay; NA: not assayed.

Only compounds which showed activity in the cell-cycle assay were tested for potency. This was determined by determination of an EC_{50} within PC3 and MCF-7 cells. Caco-2 cells were not used to calculate potency because active compounds displayed consistently lower cell-cycle assay activity in this cell line. PC3 and MCF-7 cell lines were treated with a range of concentrations that were able to cause G_2/M arrest. Cell viability was measured by CellTiter-Blue® assay (Table 1). Most of the analogues showed low potency, with an EC_{50} value ≥10 μM, whereas **10e**, **12b**, **13a**, and **13e** had EC_{50} values of ~7 μM in PC3 and similar potency in MCF-7 cells. However, **11c**, **11d**, and **14a** had much lower EC_{50} values in MCF-7 cells, indicating that the efficacy of *N*-nornoscapine derivatives may be cell-line dependent.

Of the *N*-alkyl analogues synthesised (compounds **10a–e**), only the hydroxyethyl analogue **10e** was found to exhibit prominent activity (95% more cells were arrested in G_2/M relative to control in PC3 cells), with an EC_{50} value of 6.7 μM. Activity was also maintained for two of the amide analogues, namely, the *N*-benzoyl and *N*-phenacyl cyclic ether noscapine analogues (**11c** and **11d**, respectively). The *N*-carbamate analogues **12a**,**b** showed moderate activity with 67 and 79% of PC3 cells trapped in G_2/M relative to the vehicle control. For the structurally related amide analogues **11c** and **11d**, it is clear that the activities are quite similar for both PC3 and MCF-7 cell lines; however, the activities of carbamates **12a** and **12b** in the Caco-2 cell line are half those of their respective amides. This illustrates the cell-dependent nature of such compounds in various cancer cell lines. Examination of *N*-urea and *N*-thiourea analogues shows similar trends, whereby the ureas and

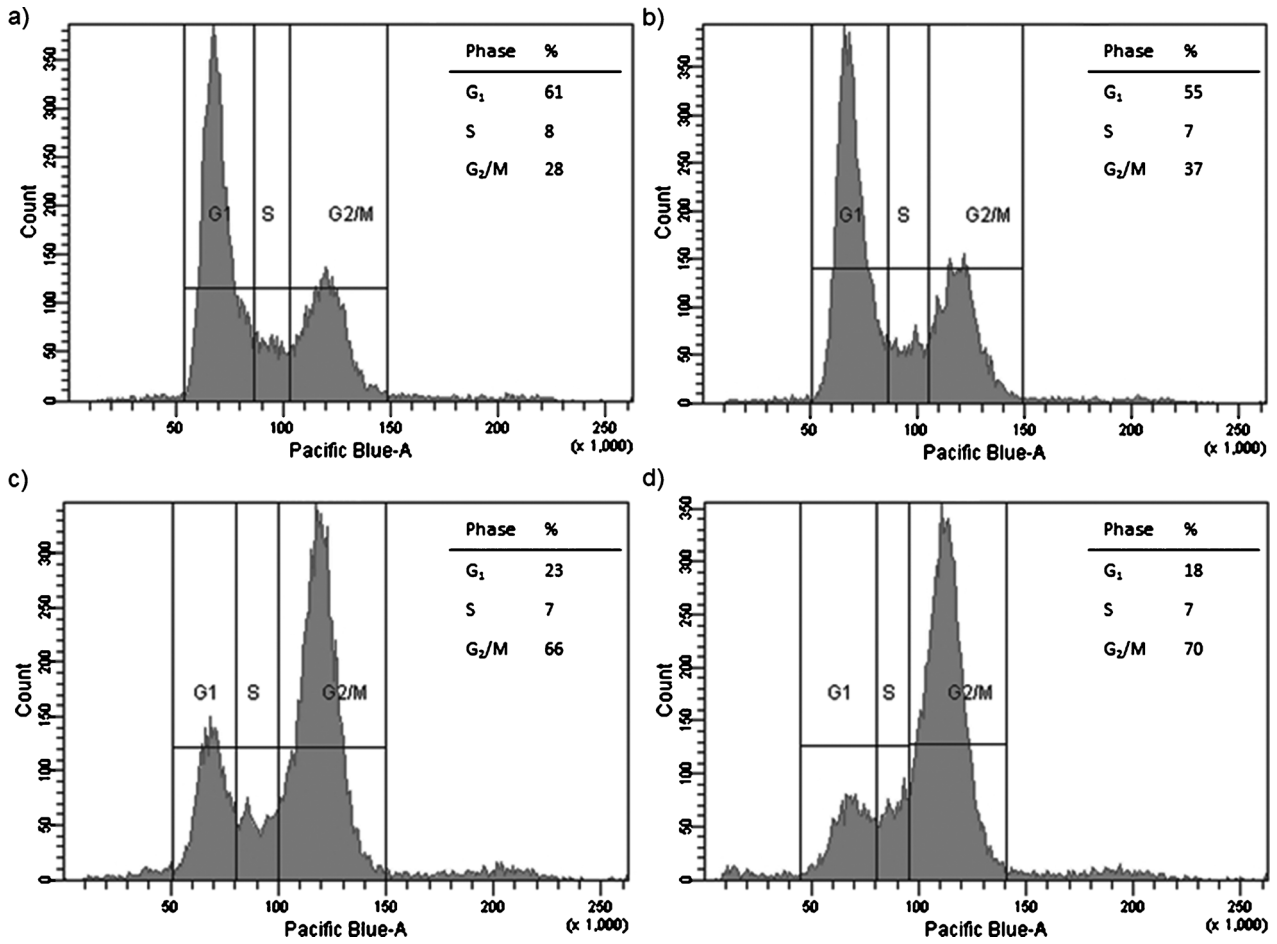


Figure 1. FACS analysis of PC3 cells treated for 16 h with a) vehicle control, b) noscapine (10 μM), c) vincristine (5.36 nM [EC_{80}]), and d) *N*-ethylurea cyclic ether noscapine **13a** (10 μM). Cells were stained with Cell Cycle 405-blue (Invitrogen) after treatment. The cell population in G_2/M, having replicated its DNA, was considered to be in G_2 or mitosis. The percentage of cells arrested in G_2/M (Table 1) revealed that the cyclic ether noscapine analogues have promising activity, given noscapine itself is inactive at 10 μM. Most of the active compounds show selectivity for prostate and breast cancer cells over colon cancer cells.

thioureas with short alkyl chains (**13a**/**13b**, **14a**) show promising activity in PC3 and MCF-7 cells. On average, 140% of cells were trapped in G_2/M relative to vehicle control in both the PC3 and MCF-7 cell lines. The alkyl ureas exhibited threefold better activity than their respective thiourea counterparts in the Caco-2 cell line, with the propyl thiourea analogue **14b** having very little activity in either of the cell lines used. The phenyl urea **13e** and chloro-substituted phenyl ureas **13g**–**i** showed moderate results in terms of percentage of cells trapped; however, this was not reciprocated for their thiourea complements with little to no activity observed. The noscapine analogue with an ethyl urea (compound **13a**) was shown to be the most potent compound tested, having activity equal to that of vincristine with 133, 171, and 60% increases in cells trapped in the G_2/M phase of PC3, MCF-7, and Caco-2 cells, respectively.

In summary, by studying the inhibition of cellular proliferation by cyclic ether N-substituted noscapine analogues, analysed with Cell Cycle 405-blue in PC3, MCF-7, and Caco-2 cell lines, we identified that *N*-alkyl analogues did not improve in activity relative to noscapine, with the exception of **10e**. It was observed that a phenyl group is essential to arrest cells in the G_2/M phase for both *N*-acyl and *N*-carbamate analogues. The *N*-urea and *N*-thiourea class produced the most potent analogues within the series. The *N*-urea compounds showed more activity than their *N*-thiourea counterparts, with **13a** exhibiting the most promising activity. Analogue **13e** showed moderate activity within the chosen cell lines; mono-chloro substitution around the phenyl ring (compounds **13g**–**i**) also showed activity but not as profound as the phenyl urea **13e**.

Conclusions

An improved method for the N-demethylation of noscapine (**1**) was developed which proceeded in 74% yield. The yield for the reduction of the lactone moiety of *N*-nornoscapines was also optimised (78%) to provide ready access to the cyclic ether *N*-nornoscapine (**9**). A series of N-substituted noscapine analogues were synthesised using **9** as the scaffold and were evaluated for their ability to cause G_2/M-phase arrest and cytotoxic activity. These compounds were initially tested in a cell inhibition study using the PC3 cell line. Compounds that exhibited notable anticancer activity were then tested against the MCF-7 and Caco-2 cell lines, and the respective EC_{50} values

were calculated for these compounds using the PC3 cell line. Some of these compounds exhibited a vast improvement in anticancer activity relative to parent compound **1**, with EC_{50} values ranging between 6.7 and 14.4 μM. Compound **13a**, containing the ethyl urea function, was found to be the most active within the series and showed potent activity across all three cancer cell lines used.

Experimental Section

General procedures: All solvents used were reagent grade. Dry solvents were dispensed from the MBraun SPS-800 solvent purification system under nitrogen. NMR spectra were obtained on a Bruker 400 WB (400 MHz) spectrometer with Bruker Avance console. NMR spectra were run in $CDCl_3$ unless otherwise stated; ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ at 400.13 and 100.62 MHz, respectively. Chemical shifts were measured relative to the residual solvent peaks of the deuterated solvent: $\delta = 7.26$ ppm (^1H NMR) and $\delta = 77.16$ ppm (^{13}C NMR). Signal multiplicities are reported as: s = singlet; d = doublet; t = triplet; dd = doublet of doublets; m = multiplet. Chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (*J*) in Hertz (Hz). Mass spectra were collected on a Waters Micromass LCT Premier XE time-of-flight mass spectrometer fitted with an electrospray (ESI) ion source and controlled with MassLynx software version 4.5. Low-resolution mass spectrometry analysis was performed using a Micromass Platform II single-quadrupole mass spectrometer equipped with an atmospheric pressure (ESI/APCI) ion source. Chromatography was carried out on silica gel 60, particle size 40–63 μm from Merck (Darmstadt, Germany) by gradient elution. Solution-phase reactions were monitored by thin-layer chromatography (TLC). The samples were spotted on analytical aluminum plates pre-coated with silica gel 60 F_{254} from Merck (Darmstadt). Noscapine used in the project was supplied by GlaxoSmithKline Australia (Port Fairy, Victoria, Australia).

***N*-Nornoscapine; (*R*)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline (7)**: To a cooled solution (−5 °C) of noscapine (2.00 g, 4.80 mmol, 97.4%) in $CHCl_3$ (50 mL) was added *m*-CPBA (1.60 g, 9.70 mmol, 77%) portionwise. When the reaction was complete (1 h), cold $CHCl_3$ (100 mL) and *i*PrOH (15 mL) were added to the reaction mixture. The resultant solution was washed with cold 10% NaOH (3×10 mL), cold water (2×20 mL) and finally with 10% HCl (2×15 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield the *N*-oxide as a hydrochloride salt in the form of a light-yellow foam (2.30 g, 4.90 mmol, 100%); R_f ($CHCl_3$/MeOH/NH_3; 85:14:1) 0.56, 0.26; ^1H NMR: $\delta = 8.01$ (d, J = 8.1 Hz, 1H), 7.35 (d, $J = 8.7$ Hz, 1H), 6.60 (s, 1H), 6.35 (s, 1H), 6.24 (s, 1H), 5.86 (m, 2H), 4.25 (m, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.83 (m, 1H), 3.67 (s, 3H), 3.44 (m, 1H), 3.28 (s, 3H), 3.12 ppm (m, 1H); HRMS: $m/z = 430.1490$, calcd for $[M+H]^+$ $C_{22}H_{23}NO_8$: 430.1496. A solution of noscapine-*N*-oxide·HCl (0.100 g, 0.220 mmol) in MeOH (10 mL) was prepared and the mixture was stirred at −5 °C. $FeSO_4 \cdot 7H_2O$ (0.120 g, 0.430 mmol) was added and stirring was continued until complete consumption of noscapine-*N*-oxide·HCl was observed by TLC. The solvent was removed in vacuo to yield crude product which was then taken up in $CHCl_3$ (15 mL) and washed with aqueous acidic 0.1 M EDTA (3×5 mL). The organic layer was subsequently washed with 1 M NaOH (2×5 mL) to yield the *N*-nornoscapine in the free-base form. The organic layer was dried over anhydrous Na_2SO_4 and the crude product was purified by flash chromatography (EtOAc/PE; 2:1) and reduced to give title compound **3** (0.070 g, 0.130 mmol, 78%) as a yellow foam; R_f (EtOAc) 0.4; ^1H NMR: $\delta = 6.93$ (d, $J = 8.1$ Hz,1H), 6.33 (s, 1H), 5.95 (m, 3H), 5.91 (d, $J = 3.3$ Hz, 1H), 4.84 (d, $J = 3.3$ Hz, 1H) 4.09 (s, 3H), 4.06 (s, 3H), 3.84 (s, 3H), 2.63 (m, 1H), 2.49 (m, 1H), 2.31 (m, 1H), 2.16 (m, 1H), 1.74 ppm (br s, 1H); ^{13}C NMR: $\delta = 168.5$, 152.2, 148.4, 147.9, 141.0, 140.4, 134.2, 131.9, 119.6, 118.5, 117.6, 116.9, 103.2, 100.8, 80.6, 62.3, 59.5, 56.7, 52.8, 39.5, 29.6 ppm; HRMS: $m/z = 400.1409$, calcd for $[M+H]^+$ $C_{21}H_{21}NO_7$: 400.1391.

Synthesis of (*R*)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline (8): To a solution of $NaBH_4$ (0.20 g, 5.00 mmol) in dry THF (20 mL) was added a solution of noscapine (1.00 g, 2.42 mmol) in $BF_3 \cdot Et_2O$ (12 mL) dropwise at −5 °C. The mixture was stirred at −5 °C for 1 h and then held at reflux for another 2 h. The reaction was placed on ice and quenched with cold HCl (10%, 10 mL) and allowed to stir for a further 1 h. The reaction mixture was then brought to pH 8 with 2.5 M NaOH, extracted with $CHCl_3$ (3×30 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent afforded a crude product, which was purified by flash chromatography (EtOAc/hexane; 4:1) to afford the title compound as white amorphous solid (0.91 g, 2.28 mmol, 94%); R_f (EtOAc) 0.26; ^1H NMR: $\delta = 6.65$ (d, $J = 8.1$ Hz, 1H), 6.30 (s, 1H), 6.09 (d, $J = 8.1$, 1H), 5.89 (s, 2H), 5.38 (d, $J = 2.7$ Hz, 1H), 5.15 (dd, $J = 1.2$ Hz, 2H), 4,22 (d, $J = 2.7$ Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 2.82 (m, 1H), 2.56 (s, 3H), 2.43 (m, 2H), 2.16 ppm (m, 1H); ^{13}C NMR: $\delta =$ 150.9, 147.8, 142.6, 140.7, 134.3, 133.9, 133.1, 131.6, 118.4, 117.3, 111.6, 102.2, 100.5, 86.8, 71.5, 63.3, 59.9, 59.1, 56.2, 49.5, 45.7, 27.4 ppm; HRMS: $m/z = 400.1766$, calcd for $[M+H]^+$ $C_{22}H_{25}NO_6$: 400.1755.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline (9): A solution of $NaBH_4$ (0.22 g, 5.8 mmol) in THF (18 mL) was stirred at −5 °C. *N*-Nornoscapine (0.75 g, 1.88 mmol) dissolved in $BF_3 \cdot Et_2O$ (9 mL) was added dropwise at −5 °C. The reaction was allowed to stir at −5 °C for 1 h, then allowed to warm to room temperature and stirred overnight. The reaction was quenched with HCl (10%, 10 mL) and allowed to stir for 1 h before being extracted with $CHCl_3$ (3×30 mL). The organic layer was then washed with 2.2 M NaOH (20 mL) to yield the free-base form. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/NH_4OH; 85:14:1) as an eluent to afford the title compound as a white foam (0.54 g, 1.39 mmol, 78%); R_f ($CHCl_3$/MeOH/NH_4OH; 85:14:1) 0.25; ^1H NMR: $\delta = 6.59$ (d, $J = 8.2$ Hz, 1H), 6.33 (s, 1H), 5.92 (q, $J = 1.5$ Hz, 2H), 5.83 (dd, $J = 8.2$, 0.9 Hz, 1H), 5.77 (td, $J = 2.6$, 1.3 Hz, 1H), 5.36 (dd, $J = 12.4$, 2.8 Hz, 1H), 5.19–5.16 (m, 1H), 4.62 (d, $J = 4.0$ Hz, 1H), 3.99 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 2.69–2.54 (m, 3H), 2.35–2.26 ppm (m, 2H); ^{13}C NMR: $\delta = 151.3$, 148.1, 143.0, 140.7, 134.4, 133.5, 132.7, 131.4, 118.7, 117.4, 112.2, 103.2, 100.7, 85.0, 71.8, 60.1, 59.5, 56.3, 55.3, 39.7, 29.9 ppm; HRMS: $m/z = 386.1612$, calcd for $[M+H]^+$ $C_{21}H_{23}NO_6$: 386.1598.

General procedure for tertiary amine synthesis: Compound **9** (0.100 g, 0.238 mmol) was dissolved in MeCN (10 mL) and Cs_2CO_3 (0.240 g, 0.737 mmol) was added. The alkyl halide (0.48 mmol) was subsequently added and the reaction was heated at reflux overnight. Reaction progress was monitored by TLC until complete (18 h). The reaction volume was then decreased in vacuo and taken up in $CHCl_3$ (20 mL). The organic layer was washed with H_2O (5 mL) and then dried over anhydrous Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by flash chromatography. The desired compound was then washed with 2 M

HCl (5 mL) to give the *N*-alkylated derivative as the hydrochloride salt.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-6-ethyl-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline hydrochloride (10a): Yield: 75%; chromatography eluent: PE/EtOAc (1:1); R_f (PE/EtOAc; 2:1) 0.12; ^{1}H NMR: δ = 7.00 (d, J = 8.2 Hz, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.39 (s, 1H), 6.27 (d, J = 1.0 Hz, 1H), 5.87 (dd, J = 12.3, 1.4 Hz, 2H), 4.94 (s, 1H), 4.91 (d, J = 11.6 Hz, 1H), 4.59 (d, J = 11.6 Hz, 1H), 4.00–3.96 (m, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.37 (s, 4H), 3.08–3.00 (m, 3H), 2.94–2.87 (m, 1H), 1.52 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR: δ = 151.8, 150.0, 143.0, 140.6, 133.8, 131.8, 131.5, 127.3, 118.0, 112.8, 109.7, 102.4, 101.2, 83.8, 72.2, 60.6, 60.2, 58.8, 56.5, 47.9, 42.9, 22.2, 10.2 ppm; HRMS: m/z = 414.1927, calcd for $[M+H]^+$ $C_{23}H_{27}NO_6$: 414.1911.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-propyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline hydrochloride (10b): Yield: 81%; chromatography eluent: PE/EtOAc (1:1); R_f (PE/EtOAc; 1:1) 0.29; ^{1}H NMR: δ = 6.92 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.31 (s, 1H), 6.21 (d, J = 1.0 Hz, 1H), 5.79 (dd, J = 13.9, 1.4 Hz, 2H), 4.86 (d, J = 1.0 Hz, 1H), 4.83 (d, J = 12.1 Hz, 1H), 4.49 (d, J = 12.1 Hz, 1H), 3.92–3.88 (m, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.32–3.26 (m, 4H), 2.98–2.92 (m, 1H), 2.87–2.77 (m, 3H), 1.97–1.90 (m, 2H), 0.88 ppm (t, J = 7.4 Hz, 3H); ^{13}C NMR: δ = 151.8, 150.0, 143.0, 140.6, 133.8, 131.8, 131.5, 127.3, 118.0, 112.8, 109.7, 102.4, 101.2, 83.8, 72.2, 61.2, 60.1, 58.8, 56.5, 54.4, 43.3, 22.2, 18.1, 11.3 ppm; HRMS: m/z = 428.2081, calcd for $[M+H]^+$ $C_{24}H_{29}NO_6$: 428.2068.

(*R*)-6-Benzyl-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline (10c): Yield: 76%; chromatography eluent: PE/EtOAc (3:1); R_f (PE/EtOAc; 1:1) 0.83; ^{1}H NMR: δ = 7.28 (d, J = 4.6 Hz, 4H), 7.24–7.22 (m, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.35 (s, 1H), 6.21 (d, J = 8.1 Hz, 1H), 5.91 (q, J = 2.1 Hz, 2H), 5.48 (dd, J = 2.6, 0.2 Hz, 1H), 5.23–5.19 (m, 1H), 5.09 (d, J = 12.1 Hz, 1H), 4.43 (d, J = 3.9 Hz, 1H), 4.18 (d, J = 13.9 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.72 (d, J = 13.9 Hz, 1H), 2.84–2.78 (m, 1H), 2.55–2.48 (m, 1H), 2.40–2.34 (m, 1H), 2.30–2.23 ppm (m, 1H); ^{13}C NMR: δ = 151.1, 148.0, 142.9, 141.1, 140.4, 134.8, 134.2, 132.9, 131.8, 128.40, 128.29, 126.8, 119.0, 117.5, 111.9, 102.5, 100.7, 87.2, 71.8, 62.2, 61.4, 60.0, 59.3, 56.4, 45.5, 27.1 ppm; HRMS: m/z = 476.2067, calcd for $[M+H]^+$ $C_{28}H_{29}NO_6$: 476.2068.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-phenethyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline (10d): Yield: 63%; chromatography eluent: PE/EtOAc (2:1); R_f (PE/EtOAc; 2:1) 0.73; ^{1}H NMR: δ = 7.28–7.24 (m, 2H), 7.20–7.16 (m, 3H), 6.68 (d, J = 8.2 Hz, 1H), 6.32 (m, 2H), 5.88 (q, J = 1.9 Hz, 2H), 5.37 (s, 1H), 5.10 (d, J = 12.4 Hz, 1H), 5.04 (d, J = 11.9 Hz, 1H), 4.34 (d, J = 3.8 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 2.96–2.80 (m, 5H), 2.64–2.57 (m, 2H), 2.27–2.22 ppm (m, 1H); ^{13}C NMR: δ = 151.2, 148.0, 142.9, 141.1, 140.8, 134.8, 134.1, 132.9, 131.4, 128.9, 128.4, 126.0, 119.1, 117.6, 111.9, 102.6, 100.6, 86.5, 71.7, 61.6, 60.1, 59.2, 58.6, 56.4, 45.6, 34.6, 26.3 ppm; HRMS: m/z = 490.2203, calcd for $[M+H]^+$ $C_{29}H_{31}NO_6$: 490.2224.

2-((*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinolin-6(5*H*)-yl)ethanol (10e): Yield: 56%; chromatography eluent: PE/EtOAc (1:4); R_f (PE/EtOAc; 1:4) 0.25; ^{1}H NMR: δ = 6.87 (s, 2H), 6.39 (s, 1H), 6.20 (s, 1H), 5.88 (d, J = 7.5 Hz, 2H), 5.23 (s, 1H), 5.06 (s, 1H), 4.93 (d, J = 12.2 Hz, 1H), 4.70 (d, J = 13.0 Hz, 1H), 4.05 (s, 2H), 3.97–3.91 (m, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.58–3.52 (m, 1H), 3.44 (s, 3H), 3.20–3.16 (m, 2H), 3.01–2.97 (m, 2H), 2.11–1.94 ppm (m, 1H); ^{13}C NMR: δ = 151.9, 150.2, 143.0, 140.6, 133.8, 131.8, 131.0, 127.2, 118.0, 112.9, 109.5, 102.4, 101.2, 83.5, 72.2, 62.6, 60.2, 58.9, 56.68, 56.51, 44.7, 22.2 ppm; HRMS: m/z = 430.1854, calcd for $[M+H]^+$ $C_{23}H_{27}NO_7$: 430.1860.

General procedure for amide synthesis: Compound **9** (0.100 g, 0.238 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and Et_3N (0.050 g, 0.480 mmol) was added. The reaction was cooled to 0 °C. The acid chloride (0.36 mmol) was added to the stirred solution, and the reaction was then allowed to warm to room temperature. Reaction progress was monitored by TLC until complete (2 h). The reaction was quenched with H_2O (5 mL) and extracted with CH_2Cl_2 (3× 10 mL). The organic layer was dried (anhydrous Na_2SO_4), reduced, and then purified by flash chromatography (PE/EtOAc; 1:4) to give the title compound as a colourless oil.

1-((*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinolin-6(5*H*)-yl)ethanone (11a): Yield: 68%; chromatography eluent: PE/EtOAc (1:4); R_f (PE/EtOAc; 1:4) 0.32; ^{1}H NMR: (rotamers: 5$^{\#}$:2*) δ = 6.89* (dd, J = 8.2, 0.8 Hz, 1H), 6.82* (d, J = 8.2 Hz, 1H), 6.64$^{\#}$ (d, J = 8.2 Hz, 1H), 6.35$^{\#}$ (s, 1H), 6.34* (s, 1H), 6.09* (d, J = 2.8 Hz, 1H), 5.98* (d, J = 0.8 Hz, 1H), 5.95$^{\#}$* (dd, J = 6.4, 1.4 Hz, 2H), 5.83* (dd, J = 7.9, 1.5 Hz, 2H), 5.71–5.69$^{\#}$ (m, 1H), 5.61–5.59* (m, 1H), 5.36$^{\#}$ (d, J = 5.1 Hz, 1H), 5.21–5.13 (m, 2H), 4.96* (d, J = 12.2 Hz, 1H), 4.60* (dd, J = 12.3, 2.8 Hz, 1H), 4.31–4.25$^{\#}$ (m, 1H), 4.09$^{\#}$ (s, 3H), 3.85* (s, 3H), 3.83$^{\#}$ (s, 3H), 3.81$^{\#}$ (s, 3H), 3.76* (s, 3H), 3.56* (s, 3H), 3.52–3.48* (m, 1H), 3.41–3.35* (m, 1H), 3.21–3.15* (m, 1H), 2.68–2.60$^{\#}$* (m, 2H), 2.43–2.37$^{\#}$ (m, 1H), 2.23$^{\#}$ (s, 3H), 2.16* (s, 3H), 2.15–2.09$^{\#}$ ppm (m, 1H); ^{13}C NMR: δ = 171.1, 170.3, 151.7, 151.3, 148.8, 148.4, 143.5, 142.7, 140.6, 139.9, 134.3, 133.9, 133.7, 132.5, 132.2, 131.9, 131.2, 130.0, 118.2, 117.52, 117.36, 116.9, 112.31, 112.28, 102.9, 102.0, 101.0, 100.6, 87.4, 84.3, 71.9, 71.7, 60.2, 60.0, 59.6, 58.9, 56.5, 56.4, 56.3, 52.9, 43.1, 35.8, 29.3, 27.9, 22.6, 22.4 ppm; HRMS: m/z = 428.1703, calcd for $[M+H]^+$ $C_{23}H_{25}NO_7$: 428.1704.

1-((*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinolin-6(5*H*)-yl)propanone (11b): Yield: 53%; chromatography eluent: PE/EtOAc (1:1); R_f (PE/EtOAc; 1:1) 0.33; ^{1}H NMR: (rotamers; 1.8$^{\#}$:1*) δ = 6.96* (d, J = 8.2 Hz, 1H), 6.82* (d, J = 8.2 Hz, 1H), 6.63$^{\#}$ (d, J = 8.2 Hz, 1H), 6.33$^{\#}$ (s, 1H), 6.33* (s, 1H), 6.07* (d, J = 2.4 Hz, 1H), 5.98$^{\#}$ (d, J = 8.2 Hz, 1H), 5.93$^{\#}$ (dd, J = 6.0, 1.4 Hz, 2H), 5.80* (dd, J = 9.6, 1.5 Hz, 2H), 5.66$^{\#}$ (m, 1H), 5.58–5.57* (m, 1H), 5.40$^{\#}$ (d, J = 5.2 Hz, 1H), 5.17–5.10$^{\#}$ (m, 2H), 4.92* (d, J = 12.2 Hz, 1H), 4.51* (dd, J = 12.2, 2.8 Hz, 1H), 4.31–4.26$^{\#}$ (m, 1H), 4.06$^{\#}$ (s, 3H), 3.83* (s, 3H), 3.82$^{\#}$ (s, 3H), 3.80$^{\#}$ (s, 3H), 3.73* (s, 3H), 3.55* (m, 1H), 3.49* (s, 3H), 3.37–3.31* (m, 1H), 3.25–3.18* (m, 1H), 2.65–2.58$^{\#}$* (m, 3H), 2.40$^{\#}$* (m, 4H), 2.18$^{\#}$ (m, 1H), 1.18* (t, J = 7.4 Hz, 3H), 1.10$^{\#}$ ppm (t, J = 7.4 Hz, 3H); ^{13}C NMR: δ = 174.3, 173.5, 151.7, 151.2, 148.8, 148.3, 143.4, 142.7, 140.6, 139.9, 134.3, 133.79, 133.77, 132.6, 132.2, 131.8, 131.6, 130.1, 118.3, 117.49, 117.37, 117.1, 112.3, 103.0, 101.9, 101.0, 100.6, 87.8, 84.2, 71.86, 71.74, 60.17, 59.98, 59.6, 58.8, 56.46, 56.35, 55.2, 53.3, 42.2, 36.0, 29.4, 27.9, 27.4, 26.9, 9.7, 9.3 ppm; HRMS: m/z = 442.1861, calcd for $[M+H]^+$ $C_{24}H_{27}NO_7$: 442.1862.

1-((*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinolin-6(5*H*)-yl)-(phenyl)methanone (11c): Yield: 54%; chromatography eluent: PE/EtOAc (1:4); R_f (PE/EtOAc; 1:1) 0.18; ^{1}H NMR: (rotamers; 1.5$^{\#}$:1*) δ = 7.39–7.33$^{\#}$* (m, 10H), 6.77* (d, J = 8.2 Hz, 1H), 6.68$^{\#}$ (d, J = 8.2 Hz, 1H), 6.60* (d, J = 8.3 Hz, 1H), 6.42$^{\#}$ (s, 1H), 6.35* (d, J = 3.3 Hz, 1H), 6.32* (s, 1H), 5.97$^{\#}$ (d, J = 8.3 Hz, 1H), 5.95$^{\#}$ (s, 2H), 5.90* (s, 2H), 5.83–5.81$^{\#}$ (m, 1H), 5.48–5.42* (m, 2H), 5.19–5.10$^{\#}$ (m, 3H), 4.98–4.95* (m, 1H), 4.36–4.31$^{\#}$ (m, 1H), 3.85* (s, 3H), 3.84$^{\#}$ (s,

9H), 3.82 (s, 3H), 3.78* (m, 3H), 3.45–3.40* (m, 1H), 3.07–2.99* (m, 1H), 2.92–2.82* (m, 1H), 2.71–2.54#* ppm (m, 4H); ^{13}C NMR: δ = 172.3, 171.2, 151.7, 151.5, 148.9, 148.5, 143.5, 143.0, 140.6, 139.9, 137.04, 137.02, 134.6, 134.2, 133.8, 132.8, 132.1, 130.0, 129.8, 129.5, 129.4, 128.6, 128.3, 127.4, 126.7, 118.0, 117.4, 117.2, 112.4, 112.3, 103.2, 102.3, 101.1, 100.8, 86.4, 84.3, 72.1, 71.5, 60.2, 60.1, 59.5, 59.2, 57.0, 56.4, 56.4, 52.9, 43.1, 36.8, 29.3, 27.9 ppm; HRMS: m/z = 490.1877, calcd for $[M+H]^+$ $C_{28}H_{27}NO_7$: 490.1860.

1-((*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinolin-6(5*H*)-yl)-2-phenylethanone (11 d): Yield: 62%; chromatography eluent: PE/EtOAc (1:4); R_f (PE/EtOAc;1:4) 0.42; ^{1}H NMR: δ (rotamers; 1.8#:1*) δ = 7.33–7.14#* (m, 10H), 6.78* (q, J = 8.2 Hz, 2H), 6.63#(d, J=8.2 Hz, 1H), 6.32# (s, 1H), 6.29* (s, 1H), 6.13* (d, J = 2.7 Hz, 1H), 5.95# (d, J = 8.2 Hz, 1H), 5.92# (dd, J = 3.9, 1.4 Hz, 2H), 5.81* (dd, J = 6.9, 1.4 Hz, 2H), 5.70–5.68# (m, 1H), 5.63–5.62* (m, 1H), 5.48# (d, J = 5.1 Hz, 1H), 5.25–5.16# (m, 2H), 4.92* (d, J = 12.3 Hz, 1H), 4.50* (dd, J = 12.3, 2.8 Hz, 1H), 4.33–4.27# (m, 1H), 3.99# (d, J = 15.45 Hz, 1H), 3.91–3.86# (s, 4H), 3.84# (s, 3H), 3.82* (s, 3H), 3.81# (s, 3H), 3.79* (d, J = 4.1 Hz, 2H), 3.71* (s, 3H), 3.55* (s, 3H), 3.45–3.41* (m, 2H), 3.07–3.00* (m, 1H), 2.67–2.59# (m, 1H), 2.54–2.47* (m, 1H), 2.43–2.37# (m, 1H), 2.24–2.16# ppm (m, 1H); ^{13}C NMR: δ = 171.6, 170.7, 151.8, 151.2, 148.7, 148.4, 143.5, 142.7, 140.5, 139.9, 135.8, 135.1, 134.5, 133.85, 133.65, 132.4, 132.2, 131.7, 131.1, 129.8, 129.0, 128.8, 128.5, 126.8, 126.5, 118.1, 117.44, 117.27, 116.9, 112.32, 112.22, 103.0, 102.0, 101.0, 100.6, 87.2, 84.3, 71.9, 71.7, 60.2, 60.0, 59.6, 58.9, 56.4, 56.3, 55.6, 53.3, 42.5, 41.8, 41.1, 36.3, 29.2, 27.8 ppm; HRMS: m/z = 504.2041, calcd for $[M+H]^+$ $C_{29}H_{29}NO_7$: 504.2017.

General procedure for carbamate synthesis: Compound **9** (0.143 g, 0.340 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and Et_3N (0.075 g, 0.74 mmol) was added. The reaction was cooled to −5 °C and the appropriate chloroformate was added (0.55 mmol). The reaction was then allowed to warm to room temperature and stir overnight. The reaction was quenched with H_2O (5 mL) and extracted with CH_2Cl_2 (3×10 mL). The organic layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by flash chromatography.

(*R*)-Phenyl 5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxylate (12 a): Yield: 83%; chromatography eluent: PE/EtOAc (2:1); R_f (PE/EtOAc; 2:1) 0.40; ^{1}H NMR: δ (rotamers; 1.6:1) δ = 7.38–7.33 (m, 5H), 7.21–7.08 (m, 5H), 6.79 (s, 2H), 6.70 (d, J = 8.2 Hz, 1H), 6.39 (s, 1H), 6.38 (s, 1H), 6.24 (d, J = 8.2 Hz, 1H), 5.93 (q, J = 1.3 Hz, 2H), 5.87 (d, J = 4.9 Hz, 1H), 5.85 (dd, J = 9.0, 1.3 Hz, 2H), 5.76 (d, J = 2.9 Hz, 1H), 5.70–5.69 (m, 2H), 5.21–5.13 (m, 2H), 5.02 (d, J = 12.2 Hz, 1H), 4.70 (dd, J = 12.2, 2.9 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.83 (s, 7H), 3.78 (s, 3H), 3.65–3.60 (m, 4H), 3.17–3.09 (m, 1H), 2.83–2.63 ppm (m, 5H); ^{13}C NMR: δ = 154.86, 154.76, 151.76, 151.67, 151.4, 148.72, 148.55, 143.4, 142.9, 140.5, 140.2, 134.4, 133.9, 133.4, 133.2, 132.3, 132.0, 131.4, 130.1, 129.36, 129.30, 125.3, 122.1, 122.0, 118.1, 117.5, 117.4, 117.3, 112.4, 112.3, 102.8, 102.3, 101.0, 100.7, 87.2, 85.2, 71.9, 71.8, 60.2, 60.1, 59.4, 59.0, 56.5, 55.3, 54.6, 41.4, 39.4, 29.0, 28.2 ppm; HRMS: m/z = 506.1827, calcd for $[M+H]^+$ $C_{28}H_{27}NO_8$: 506.1809.

(*R*)-Benzyl 5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxylate (12 b): Yield: 85%; chromatography eluent: PE/EtOAc (2:1); R_f (PE/EtOAc; 2:1) 0.35; ^{1}H NMR: δ (rotamers; 1:1) δ = 7.39–7.27 (m, 10H), 6.72 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 6.59 (d, J = 8.1 Hz, 1H), 6.35 (s, 1H), 6.32 (s, 1H), 6.23 (d, J = 8.2 Hz, 1H), 5.89 (s, 2H), 5.85 (d, J = 4.2 Hz, 2H), 5.76 (d, J = 3.5 Hz, 1H), 5.69 (d, J = 4.5 Hz, 1H), 5.64–5.63 (m, 1H), 5.58–5.56 (m, 1H), 5.24–5.11 (m, 4H), 5.06–4.98 (m, 2H), 4.91 (dd, J = 12.4, 2.4 Hz, 1H), 4.73 (dd, J = 12.4, 2.5 Hz, 1H), 3.83–3.81 (m, 9H), 3.78 (s, 3H), 3.76 (s, 3H), 3.70 (s, 4H), 3.58–3.52 (m, 1H), 3.24–3.17 (m, 1H), 2.92–2.84 (m, 2H), 2.71–2.68 (m, 2H), 2.65–2.57 ppm (m, 1H); ^{13}C NMR: δ = 156.2, 156.1, 151.5, 151.3, 148.5, 148.4, 143.2, 142.9, 140.4, 140.2, 137.2, 134.3, 134.0, 133.5, 133.4, 132.2, 132.1, 130.9, 130.5, 128.6, 128.5, 128.0, 127.9, 117.9, 117.7, 117.4, 112.2, 112.2, 102.8, 102.3, 100.8, 100.7, 86.6, 85.6, 77.2, 71.8, 71.71, 67.3, 67.1, 60.1, 60.0, 59.3, 59.1, 56.4, 54.7, 54.2, 40.3, 39.6, 28.8, 28.3 ppm; HRMS: m/z = 520.1979, calcd for $[M+H]^+$ $C_{29}H_{29}NO_8$: 520.1966.

General procedure for urea synthesis: Compound **9** (0.110 g, 0.261 mmol) was dissolved in MeCN (2.5 mL) and cooled to −5 °C. The appropriate isocyanate (0.360 mmol) dissolved in MeCN (2.5 mL) was then added dropwise. The reaction was then stirred at room temperature until complete (2 h). The reaction was quenched with cold water (1 mL) and extracted with $CHCl_3$ (3× 10 mL). Organic layers were combined and dried with anhydrous Na_2SO_4 and reduced in vacuo. The crude product was purified by flash chromatography.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-*N*-ethyl-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13 a): Yield: 84%; chromatography eluent: PE/EtOAc (3:1); R_f (PE/EtOAc; 4:1) 0.1; ^{1}H NMR: δ = 6.62 (d, J = 8.2 Hz, 1H), 6.32 (s, 1H), 6.01 (d, J = 8.2 Hz, 1H), 5.98 (bs, 1H), 5.93 (m, 2H), 5.80 (m, 1H), 5.32 (d, J = 3.22 Hz, 1H), 5.12 (m, 2H), 4.04 (s, 3H), 3.82 (s, 3H), 3.81 (s, 4H), 3.33 (m, 1H), 3.21 (m, 1H), 2.57 (m, 1H), 2.21 (m, 2H), 1.19 ppm (t, J = 7.19 Hz, 3H); ^{13}C NMR: δ = 160.0, 151.7, 148.5, 143.3, 139.9, 134.3, 132.2, 131.9, 131.1, 117.7, 116.6, 112.2, 103.0, 100.9, 86.2, 71.9, 60.3, 59.6, 56.6, 56.4, 38.1, 35.8, 28.3, 15.9 ppm; HRMS: m/z = 457.1991, calcd for $[M+H]^+$ $C_{24}H_{28}N_2O_7$: 457.2029.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-*N*-propyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13 b): Yield: 91%; chromatography eluent: PE/EtOAc (2:1); R_f (PE/EtOAc; 5:1) 0.1.^{1}H NMR: δ = 6.62 (d, J = 8.2 Hz, 1H), 6.33 (s, 1H), 6.06 (bs, 1H), 6.02 (d, J = 8.2 Hz, 1H), 5.94 (dd, J = 6.0, 1.4 Hz, 2H), 5.82–5.80 (m, 1H), 5.32 (d, J = 3.6 Hz, 1H), 5.15 (d, J = 2.4 Hz, 1H), 5.12 (d, J = 12.0 Hz, 1H), 4.04 (s, 3H), 3.83 (s, 4H), 3.81 (s, 3H), 3.29–3.25 (m, 1H), 3.18–3.11 (m, 1H), 2.56 (m, 1H), 2.21 (s, 2H), 1.60–1.56 (m, 2H), 0.98 ppm (t, J = 7.4 Hz, 3H); ^{13}C NMR: δ = 160.1, 151.7, 148.5, 143.3, 139.8, 134.3, 132.2, 131.9, 131.1, 117.7, 116.6, 112.2, 102.9, 100.9, 86.1, 71.9, 60.2, 59.5, 56.6, 56.3, 42.8, 38.1, 28.3, 23.6, 11.7 ppm; HRMS: m/z = 471.2140, calcd for $[M+H]^+$ $C_{25}H_{30}N_2O_7$: 471.2175.

(*R*)-*N*-Butyl-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13 c): Yield: 76%; chromatography eluent: CH_2Cl_2/EtOAc (4:1), R_f (CH_2Cl_2/EtOAc; 4:1) 0.27; ^{1}H NMR: δ = 6.62 (d, J = 8.1 Hz, 1H), 6.32 (s, 1H), 6.01 (d, J = 8.1 Hz, 2H), 5.93 (dd, J = 6.0, 1.4 Hz, 2H), 5.81–5.80 (m, 1H), 5.31 (d, J = 3.6 Hz, 1H), 5.17–5.09 (m, 2H), 4.04 (s, 3H), 3.83 (s, 4H), 3.81 (s, 3H), 3.35–3.28 (m, 1H), 3.22–3.15 (m, 1H), 2.64–2.56 (m, 1H), 2.27–2.21 (m, 2H), 1.60–1.52 (m, 2H), 1.45–1.36 (m, 2H), 0.96 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR: δ = 160.2, 151.8, 148.6, 143.3, 139.8, 134.3, 132.2, 131.7, 131.0, 117.7, 116.5, 112.3, 103.0, 101.0, 86.2, 71.9, 60.3, 59.6, 56.7, 56.4, 41.0, 38.4, 32.6, 28.3, 20.4, 14.0 ppm; HRMS: m/z = 485.2274, calcd for $[M+H]^+$ $C_{26}H_{32}N_2O_7$: 485.2282.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-*N*-pentyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13 d): Yield: 78%; chromatography eluent: CH_2Cl_2/

EtOAc (4:1); R_f (CH_2Cl_2/EtOAc; 4:1) 0.13; ^1H NMR: δ = 6.62 (d, J = 8.1 Hz, 1H), 6.32 (s, 1H), 6.02 (d, J = 8.1 Hz, 2H), 5.93 (dd, J = 6.0, 1.4 Hz, 2H), 5.81–5.79 (m, 1H), 5.31 (d, J = 3.6 Hz, 1H), 5.17–5.09 (m, 2H), 4.04 (s, 3H), 3.82 (s, 4H), 3.81 (s, 3H), 3.33–3.25 (m, 1H), 3.20–3.14 (m, 1H), 2.61–2.53 (m, 1H), 2.27–2.17 (m, 2H), 1.59–1.53 (m, 2H), 1.38–1.34 (m, 4H), 0.92 ppm (t, J = 7.0 Hz, 3H); ^{13}C NMR: δ = 160.1, 151.7, 148.5, 143.3, 139.9, 134.3, 132.2, 131.9, 131.1, 117.7, 116.7, 112.2, 103.0, 100.9, 86.2, 71.9, 60.3, 59.6, 56.6, 56.4, 41.1, 38.2, 30.2, 29.5, 28.3, 22.6, 14.3 ppm; HRMS: m/z = 499.2463, calcd for $[M+H]^+$ $C_{27}H_{34}N_2O_7$: 499.2439.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-*N*-phenyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13e): Yield: 98%; chromatography eluent: PE/EtOAc (4:1) R_f (PE/EtOAc; 4:1) 0.05; ^1H NMR: δ = 8.66 (bs, 1H), 7.39 (d, J = 7.6 Hz, 2H), 7.29 (t, J = 7.0 Hz, 2H), 6.99 (t, J = 7.6 Hz, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.35 (s, 1H), 5.99 (d, J = 8.2 Hz, 1H), 5.97–5.93 (m, 3H), 5.50 (d, J = 3.6 Hz, 1H), 5.33 (dd, J = 12.2, 2.7 Hz, 1H), 5.22 (d, J = 12.2 Hz, 1H), 4.09 (s, 3H), 3.96–3.89 (m, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 2.69–2.60 (m, 1H), 2.28–2.15 ppm (m, 2H); ^{13}C NMR: δ = 157.4, 151.9, 148.8, 143.4, 140.2, 139.9, 134.4, 132.0, 131.3, 130.8, 129.0, 122.4, 119.4, 117.7, 116.1, 112.3, 103.0, 101.0, 86.2, 72.1, 60.3, 59.7, 57.0, 56.4, 38.2, 28.2 ppm; HRMS: m/z = 505.1993, calcd for $[M+H]^+$ $C_{28}H_{28}N_2O_7$: 505.1969.

(*R*)-*N*-Benzyl-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13f): Yield: 91%; chromatography eluent: PE/EtOAc (7:1); R_f (PE/EtOAc; 4:1) 0.08; ^1H NMR: δ = 7.40–7.33 (m, 4H), 7.29–7.27 (m, 1H), 6.61 (d, J = 8.1 Hz, 1H), 6.40–6.36 (bs, 1H), 6.33 (d, J = 3.3 Hz, 1H), 6.01 (d, J = 8.1 Hz, 1H), 5.93 (dd, J = 5.3, 1.4 Hz, 2H), 5.79 (td, J = 3.0, 1.1 Hz, 1H), 5.38 (d, J = 3.0 Hz, 1H), 5.07–4.99 (m, 2H), 4.51 (dd, J = 14.6, 6.1 Hz, 1H), 4.38 (td, J = 8.0, 4.3 Hz, 1H), 4.01 (s, 3H), 3.90–3.83 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.59 (m, 1H), 2.29–2.22 ppm (m, 2H); ^{13}C NMR: δ = 159.9, 151.7, 148.6, 143.3, 140.3, 139.9, 134.3, 132.1, 131.8, 131.0, 128.6, 128.0, 127.2, 117.7, 116.5, 112.2, 102.9, 100.9, 86.0, 71.8, 60.2, 59.6, 56.50, 56.37, 45.2, 38.3, 28.3 ppm; HRMS: m/z = 519.2109, calcd for $[M+H]^+$ $C_{29}H_{30}N_2O_7$: 519.2126.

(*R*)-*N*-(2-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13g): Yield: 80%; chromatography eluent: PE/EtOAc (5:1); R_f (PE/EtOAc; 5:1) 0.03; ^1H NMR: δ = 8.85 (bs, 1H), 7.86 (dd, J = 8.3, 1.3 Hz, 1H), 7.36 (dd, J = 8.0, 1.4 Hz, 1H), 7.20 (ddd, J = 8.4, 7.3, 1.3 Hz, 1H), 6.93 (ddd, J = 8.4, 7.3, 1.3 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.36 (s, 1H), 6.03 (d, J = 8.2 Hz, 1H), 5.97–5.94 (m, 3H), 5.55 (d,, J = 3.5 Hz, 1H), 5.38 (d, J = 11.9 Hz, 1H), 5.22 (d, J = 11.9 Hz, 1H), 4.07 (s, 3H), 3.98 (s, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.69 (m, 1H), 2.34–2.29 (m, 1H), 2.15 ppm (m, 1H); ^{13}C NMR: δ = 157.1, 151.8, 148.7, 143.4, 139.9, 137.1, 134.4, 132.0, 131.5, 130.5, 129.2, 127.3, 123.2, 122.9, 122.1, 117.6, 116.1, 112.2, 103.0, 101.0, 85.5, 72.2, 60.2, 59.7, 57.1, 56.3, 38.1, 28.1 ppm; HRMS: m/z = 539.1606, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_7$: 539.1580.

(*R*)-*N*-(3-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13h): Yield: 85%; chromatography eluent: PE/EtOAc (5:1); R_f (PE/EtOAc; 4:1) 0.14; ^1H NMR: δ = 8.78 (bs, 1H), 7.47 (t, J = 2.0 Hz, 1H), 7.29–7.26 (m, 1H), 7.20 (t, J = 8.0 Hz, 1H), 6.98–6.95 (m, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.36 (s, 1H), 5.99–5.95 (m, 3H), 5.93 (t, J = 3.6 Hz, 1H), 5.46 (d, J = 3.2 Hz, 1H), 5.32 (dd, J = 12.2, 2.6 Hz, 1H), 5.23 (d, J = 12.2 Hz, 1H), 4.10 (s, 3H), 3.93–3.88 (m, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.65 (s, 1H), 2.22 ppm (m, 2H); ^{13}C NMR: δ = 157.1, 151.8, 148.7, 143.4, 139.9, 137.1, 134.4, 132.0, 131.5, 130.5, 129.2, 127.3, 123.2, 122.9, 122.1, 117.6, 116.1, 112.2, 103.0, 101.0, 85.5, 72.2, 60.2, 59.7, 57.1, 56.3, 38.1, 28.1 ppm; HRMS: m/z = 539.1601, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_7$: 539.1580.

(*R*)-*N*-(4-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13i): Yield: 59%; chromatography eluent: PE/EtOAc (5:1); R_f (PE/EtOAc; 4:1) 0.12; ^1H NMR: δ = 8.71 (s, 1H), 7.33 (d, J = 8.9 Hz, 2H), 7.24 (d, J = 8.9 Hz, 2H), 6.63 (d, J = 8.2 Hz, 1H), 6.35 (s, 1H), 5.99–5.93 (m, 4H), 5.46 (d, J = 3.2 Hz, 1H), 5.30 (dd, J = 12.2, 2.4 Hz, 1H), 5.21 (d, J = 12.2 Hz, 1H), 4.10 (s, 3H), 3.92–3.87 (m, 1H), 3.83 (s, 3H), 3.18 (s, 3H) 2.68–2.59 (m, 1H), 2.26–2.18 ppm (m, 2H); ^{13}C NMR: δ = 157.2, 151.9, 148.8, 143.4, 139.8, 138.8, 134.4, 131.9, 131.0, 130.7, 128.9, 127.2, 120.61, 120.52, 117.7, 115.8, 112.3, 103.0, 101.0, 86.2, 72.1, 60.3, 59.7, 56.9, 56.4, 38.2, 28.1 ppm; HRMS: m/z = 539.1595, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_7$: 539.1580.

(*R*)-*N*-(3,5-Bis(trifluoromethyl)phenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo-[4,5-*g*]isoquinoline-6(5*H*)-carboxamide (13j): Yield: 85%; chromatography eluent: PE/EtOAc (8:1); R_f (PE/EtOAc; 8:1) 0.05; ^1H NMR: δ = 9.17 (s, 1H), 7.89 (s, 2H), 7.49 (s, 1H), 6.66 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 6.00–5.97 (m, 4H), 5.49 (d, J = 3.6 Hz, 1H), 5.32–5.25 (m, 2H), 4.13 (s, 3H), 3.95–3.90 (m, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 2.67 (m, 1H), 2.30–2.21 ppm (m, 2H); ^{13}C NMR: δ = 156.8, 152.1, 149.0, 143.4, 141.8, 139.8, 134.4, 132.2 (q, J = 32.9), 131.8, 130.60, 130.51, 123.5 (q, J = 271), 118.9 (q, J = 3), 117.7, 115.4 (m), 115.3, 112.5, 103.0, 101.1, 86.2, 72.2, 60.3, 59.7, 56.9, 56.3, 38.5, 28.0 ppm; HRMS: m/z = 641.1729, calcd for $[M+H]^+$ $C_{30}H_{26}F_6N_2O_7$: 641.1717.

General procedure for thiourea synthesis: Compound **9** as the free-base form (0.088 g, 0.228 mmol) was dissolved in MeCN (3.0 mL) and cooled to 5 °C. The appropriate isothiocyanate (0.270 mmol) in MeCN (3.0 mL) was then added dropwise and the reaction was then stirred at room temperature for 2 h. The reaction was quenched with cold water (1 mL) and extracted with $CHCl_3$ (3×10 mL). The combined organic layer was dried (anhydrous Na_2SO_4) and reduced to give crude product. The product was purified by column chromatography.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-*N*-ethyl-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14a): Yield: 93%; chromatography eluent: toluene/EtOAc (10:1); R_f (toluene/EtOAc; 10:1) 0.10; ^1H NMR: δ = 7.48 (bs, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.33 (s, 1H), 6.11 (bs, 1H), 5.95 (m, 1H), 5.93 (dd, J = 5.8, 1.4 Hz, 2H), 5.57 (bs, 1H), 5.08 (m, 2H), 4.62 (s, 1H), 4.02 (s, 3H), 3.82 (s, 3H), 3.81 (s, 4H), 3.63–3.56 (m, 1H), 2.77 (m, 1H), 2.37 (s, 2H), 1.29 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR: δ = 185.6, 151.8, 148.8, 143.4, 139.6, 134.3, 131.8, 131.4, 131.0, 117.6, 115.8, 112.4, 103.0, 101.0, 85.9, 71.7, 60.3, 59.70, 59.58, 56.4, 44.0, 40.8, 27.8, 14.8 ppm; HRMS: m/z = 473.1743, calcd for $[M+H]^+$ $C_{24}H_{28}N_2O_6S$: 473.1741.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-*N*-propyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14b): Yield: 91%; chromatography eluent: toluene/EtOAc (8:1); R_f (toluene/EtOAc; 7:1) 0.38; ^1H NMR: δ = 7.59 (s, 1H), 6.65 (d, J = 8.2 Hz, 1H), 6.34 (s, 1H), 6.09 (bs, 1H), 5.96 (m, 1H), 5.94 (dd, J = 6.1, 1.4 Hz, 2H), 5.56 (bs, 1H), 5.10 (s, 2H), 4.64 (bs, 1H), 4.03 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.76–3.71 (m, 1H), 3.53 (m, 1H), 2.83–2.75 (m, 1H), 2.35 (bs, 2H), 1.75–1.69 (m, 2H), 1.02 ppm (t, J = 7.4 Hz, 3H); ^{13}C NMR: δ = 185.7, 151.8, 148.8, 143.4, 139.6, 134.3, 131.7, 131.3, 130.9, 117.6, 115.8, 112.4, 103.0, 101.0, 85.8, 71.7, 60.3, 59.8, 59.6, 56.3, 47.9, 44.0, 27.8, 22.6, 11.7 ppm; HRMS: m/z = 487.1917, calcd for $[M+H]^+$ $C_{25}H_{30}N_2O_6S$: 487.1897.

(*R*)-5-((*S*)-4,5-Dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-*N*-phenyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14c): Yield: 89%; chromatography eluent: toluene/EtOAc (8:1); R_f (toluene/EtOAc; 8:1) 0.15; ^{1}H NMR: δ = 9.64 (bs, 1H), 7.45 (dd, 2H, J = 8.4, 1.2 Hz), 7.36 (dd, 2H, J = 8.4, 7.6 Hz), 7.26 (t, 1H, J = 7.3 Hz), 6.67 (d, 1H, J = 8.2 Hz), 6.38 (s, 1H), 6.09–6.07 (m, 1H), 6.05 (bs, 1H), 5.96 (dd, 2H, J = 9.7, 1.4 Hz), 5.84 (bs, 1H), 5.35 (dd, 1H, J = 11.9, 0.2 Hz), 5.25 (d, 1H, J = 11.9 Hz), 4.87 (bs, 1H), 4.08 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 2.91–2.83 (m, 1H), 2.36 ppm (m, 2H); ^{13}C NMR: δ = 185.3, 152.0, 149.0, 143.6, 140.6, 139.6, 134.3, 131.6, 131.0, 130.7, 128.7, 124.7, 124.1, 117.6, 115.4, 112.6, 103.0, 101.1, 85.9, 71.9, 60.4, 59.9, 59.7, 56.4, 43.9, 27.7 ppm; HRMS: m/z = 521.1766, calcd for $[M+H]^+$ $C_{28}H_{28}N_2O_6S$: 521.1741.

(*R*)-*N*-benzyl-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14d): Yield: 65%; chromatography eluent: CH_2Cl_2/EtOAc; (8:1); R_f (CH_2Cl_2/EtOAc; 8:1) 0.25; ^{1}H NMR: δ = 7.78 (s, 1H), 7.42–7.35 (m, 4H), 7.33–7.28 (m, 1H), 6.65 (d, J = 8.2 Hz, 1H), 6.34 (s, 1H), 6.14 (bs, 1H), 5.94–5.92 (m, 3H), 5.66 (bs, 1H), 5.06 (dd, J = 14.7, 5.3 Hz, 1H), 5.01–4.98 (m, 2H), 4.78 (dd, J = 14.7, 4.9 Hz, 1H), 4.64 (s, 1H), 3.99 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 2.84–2.76 ppm (m, 1H), 2.57–2.47 (m, 2H); ^{13}C NMR: δ = 185.9, 151.8, 148.8, 143.4, 139.7, 138.7, 134.3, 131.7, 131.4, 130.9, 128.7, 128.0, 127.5, 117.7, 115.8, 112.5, 102.9, 101.0, 85.8, 71.7, 60.2, 59.7, 59.5, 56.4, 50.2, 44.4, 27.8 ppm; HRMS: m/z = 535.1916, calcd for $[M+H]^+$ $C_{29}H_{30}N_2O_6S$: 535.1897.

(*R*)-*N*-(2-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14e): Yield: 88%; chromatography eluent: PE/EtOAc (6:1); R_f (PE/EtOAc; 6:1) 0.13; ^{1}H NMR: δ = 9.63 (bs, 1H), 7.78 (dd, J = 8.0, 0.9 Hz, 1H), 7.42 (dd, J = 8.0, 1.3 Hz, 1H), 7.23 (td, J = 7.8, 1.5 Hz, 1H), 7.09 (ddd, J = 8.0, 7.4, 1.5 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 6.38 (s, 1H), 6.09–6.07 (m, 1H), 6.01 (bs, 1H), 5.96 (dd, J = 9.7, 1.3 Hz, 2H), 5.89 (bs, 1H), 5.43 (d, J = 11.8 Hz, 1H), 5.24 (d, J = 11.8 Hz, 1H), 4.88 (bs, 1H), 4.08 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 2.93–2.85 (m, 1H), 2.36–2.32 ppm (m, 2H); ^{13}C NMR: δ = 185.9, 151.9, 149.0, 143.5, 139.6, 137.9, 134.3, 131.7, 131.1, 130.5, 129.5, 127.8, 127.3, 126.6, 125.7, 117.5, 115.4, 112.6, 103.0, 101.1, 85.0, 72.3, 60.3, 60.3, 59.7, 56.3, 44.1, 27.6 ppm; HRMS: m/z = 555.1375, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_6S$: 555.1351.

(*R*)-*N*-(3-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14f): Yield: 93%; chromatography eluent: PE/EtOAc (6:1); R_f (PE/EtOAc; 6:1) 0.08; ^{1}H NMR: δ = 9.66 (s, 1H), 7.46 (t, J = 2.0 Hz, 1H), 7.37 (ddd, J = 8.1, 2.0, 1.0 Hz, 1H), 7.27 (s, 1H), 7.09 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 6.37 (s, 1H), 6.07 (dd, J = 3.8, 2.9 Hz, 1H), 6.01 (d, J = 6.9 Hz, 1H), 5.96 (dd, J = 9.8, 1.4 Hz, 2H), 5.79 (bs, 1H), 5.32 (d, J = 12.0 Hz, 1H), 5.25 (d, J = 12.1 Hz, 1H), 4.86–4.83 (m, 1H), 4.08 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 2.89–2.80 (m, 1H), 2.35 ppm (m, 2H); ^{13}C NMR: δ = 184.9, 152.0, 149.0, 143.5, 141.8, 139.6, 134.3, 134.1, 131.4, 130.69, 130.53, 129.5, 124.4, 123.7, 122.0, 117.6, 115.1, 112.6, 102.9, 101.1, 85.8, 71.9, 60.3, 59.9, 59.7, 56.3, 43.9, 27.6 ppm; HRMS: m/z = 555.1375, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_6S$: 555.1351.

(*R*)-*N*-(4-Chlorophenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14g): Yield: 76%; chromatography eluent: toluene/EtOAc (8:1); R_f (toluene/EtOAc; 6:1) 0.27. Yield: 81%; ^{1}H NMR: δ = 9.60 (s, 1H), 7.34 (d, J = 8.9 Hz, 2H), 7.25 (d, J = 8.9 Hz, 2H), 6.63 (d, J = 8.2 Hz, 1H), 6.33 (s, 1H), 6.05–6.04 (m, 1H), 5.99 (s, 1H), 5.91 (dd, J = 8.9, 1.4 Hz, 2H), 5.77 (s, 1H), 5.28 (d, J = 11.5 Hz, 1H), 5.20 (d, J = 12.1 Hz, 1H), 4.81 (d, J = 6.8 Hz, 1H), 4.04 (s, 3H), 3.82 (s, 3H), 3.78 (s, 3H), 2.80 (ddd, J = 16.7, 11.4, 5.6 Hz, 1H), 2.33–2.27 ppm (m, 2H); ^{13}C NMR: δ = 185.2, 152.0, 149.1, 143.5, 139.6, 139.2, 134.3, 131.5, 130.76, 130.6, 129.8, 128.7, 125.3, 117.6, 115.2, 112.7, 103.0, 101.1, 85.9, 72.0, 60.4, 59.9, 59.7, 56.4, 44.0, 27.7 ppm; HRMS: m/z = 555.1341, calcd for $[M+H]^+$ $C_{28}H_{27}ClN_2O_6S$: 555.1351.

(*R*)-*N*-(3,5-bis(trifluoromethyl)phenyl)-5-((*S*)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro[1,3]dioxolo-[4,5-*g*]isoquinoline-6(5*H*)-carbothioamide (14h): Yield: 62%; chromatography eluent: toluene/EtOAc (20:1); R_f (toluene/EtOAc; 20:1) 0.50; ^{1}H NMR: δ = 9.94 (s, 1H), 7.92 (s, 2H), 7.58 (s, 1H), 6.68 (d, J = 8.0 Hz 1H), 6.39 (s, 1H), 6.12–6.11 (m, 1H), 6.01 (d, J = 8.0 Hz, 1H), 5.97 (dd, J = 10.8, 1.4 Hz, 2H), 5.83–5.83 (m, 1H), 5.36–5.28 (m, 2H), 4.89–4.82 (m, 1H), 4.11 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 2.88–2.81 (m, 1H), 2.39–2.37 ppm (m, 2H); ^{13}C NMR: δ = 184.8, 152.2, 149.2, 143.6, 142.2, 139.6, 134.4, 131.6 (q, J = 33.3), 131.4, 130.4, 130.4, 123.4 (q, J = 271), 123.2 (q, J = 1.4), 117.6, 117.3 (m), 114.8, 112.8, 103.0, 101.2, 85.8, 72.1, 60.4, 60.0, 59.7, 56.4, 44.2, 27.6 ppm; HRMS: m/z = 657.1481, calcd for $[M+H]^+$ $C_{30}H_{26}F_6N_2O_6S$: 657.1489.

Pharmacology

Cell culture and reagents: Three human cancer cell lines (PC3, MCF-7, and Caco-2) were purchased from ATCC and cultured in 5% CO_2 at 37 °C in F-12K, α-MEM and DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and penicillin–streptomycin solution (100 U; Invitrogen). Cells were seeded at 10^4 cells per well in 24-well plates for 48 h. Cells were then treated with analogues, vincristine, or vehicle control at 10 μM for 16 h. Culture medium with 0.1% DMSO was used as a vehicle control.

Cell-cycle assay: After the incubation, cells were detached using 0.05% trypsin (Invitrogen) for 5 min and fixed with 4% paraformaldehyde (PFA) for 15 min. Cells were then washed with phosphate-buffered saline (PBS) and permeablised with 0.5% Triton X-100 for 30 min. Cells were stained with Cell Cycle 405-blue (Invitrogen) for 30 min and analysed by flow cytometry (FACSCanto II analyser, BD Biosciences, Australia) using the pacific blue channel (λ 450 nm) to detect the various stages of the cell proliferation cycle.

EC_{50} determination: EC_{50} values were determined for all compounds that were observed to cause arrest of cells in G_2/mitosis. PC3 and MCF-7 cells were seeded at 2000 cells per well in 96-well plates, 48 h before treatment. After five days treatment, the CellTiter-Blue® assay (Promega G8080) was performed according to the manufacturer's protocol. Briefly, a fluorescent product was produced by viable cells and detected using an Envision Microplate reader (PerkinElmer) at λ 560 nm excitation. Higher fluorescence indicated the presence of greater numbers of viable cells. Fluorescence values were plotted against log[inhibitor], and EC_{50} values were calculated using GraphPad Prism 5 statistical software.

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Keywords: antitumor agents · cytotoxic activity · natural products · noscapine analogues · structure–activity relationships

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