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Optimisation of Tetrahydroisoquinoline-Based Chimeric Microtubule Disruptors

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Tetrahydroisoquinoline (THIQ)-based "chimeric" microtubule disruptors were optimised through modification of the *N*-benzyl motif, in concert with changes at C3 and C7, resulting in the identification of compounds with improved in vitro antiproliferative activities (e.g. **15**: GI_{50} 20 nM in DU-145). The broad anticancer activity of these novel structures was confirmed in the NCI 60-cell line assay, with **12e,f** displaying MGM values in the 40 nM region. In addition, their profiles as inhibitors of tubulin polymerisation and colchicine binding to tubulin were confirmed. Compound **15**, for example, inhibited tubulin polymerisation with an IC_{50} of 1.8 μM, close to that of the clinical drug combretastatin A-4, and also proved effective at blocking colchicine binding. Additionally, compound **20b** was identified as the only phenol in the series to date showing both better in vitro antiproliferative properties than its corresponding sulfamate and excellent antitubulin data (IC_{50} = 1.6 μM). Compound **12f** was selected for in vivo evaluation at the NCI in the hollow fibre assay and showed very good activity and wide tissue distribution, illustrating the value of this template for further development.

Introduction

Chimeras in mythology are creatures composed from the elements of multiple animals or animals and man. Small or large molecules that combine the essential features of two or more separate entities are also termed chimeras. The chimeric fusion protein bcr-abl,[1] derived from the naturally occurring oncogenic gene fusion that drives the growth of chronic myelogenous leukemia,[2] serves as an example of the latter. A long-standing interest in the development of microtubule disruptors as anticancer agents led us to explore whether, through combining the key pharmacophore elements of two series of colchicine site binders, we might generate "chimeric" small molecules with similar activity. In a preliminary report[3] we outlined how, by introducing the A,B-ring elements of the pharmacophore from a series of steroidal microtubule disruptors **1**[4–9] (e.g., 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate, 2-MeOE2bisMATE, STX140, **1a**) into a tetrahydroisoquinoline (THIQ) motif and connecting this to a trimethoxyaryl motif commonly found in a range of colchicine site binding natural products (e.g., colchicine **2**), we could generate novel "chimeric" microtubule disruptors **3**,**4** (Figure 1).

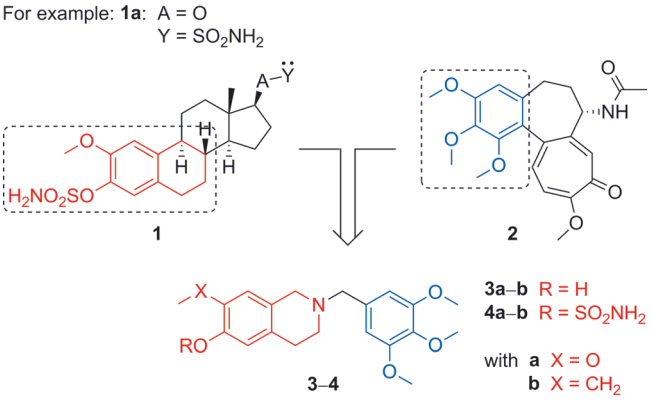

Figure 1. Generation of small-molecule chimeric microtubule disruptors (Y = H-bond acceptor).

These chimeras, and their sulfamates **4a**,**b** in particular, are notable for their excellent physicochemical properties, their in vitro and in vivo activity against various cancer cell lines, including drug-resistant types, and also their structural simplicity when compared to existing clinical agents. One notable advantage is the synthetic ease with which these compounds can be constructed. In parallel work, we also applied the same THIQ core to generate steroidomimetic microtubule disruptors that exhibit a distinct structure–activity relationship (SAR) to the chimeras, yet share their favourable physicochemical properties.[10] In the present study, we explore optimisation of our chimeric system through modification of the trimethoxyaryl component, knowing that in some colchicine site binding microtubule disruptor series replacement of this motif with alternate trisubstituted systems can have a dramatic effect on po-

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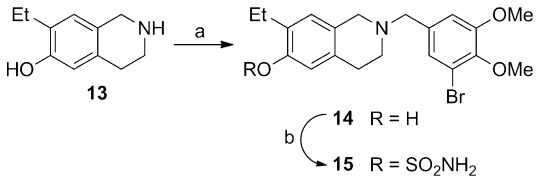
tency.[11] We therefore synthesised candidate chimeric microtubule disruptors in which one or more of the methoxy groups are exchanged for an alternate functionality.

Results and Discussion

Chemistry

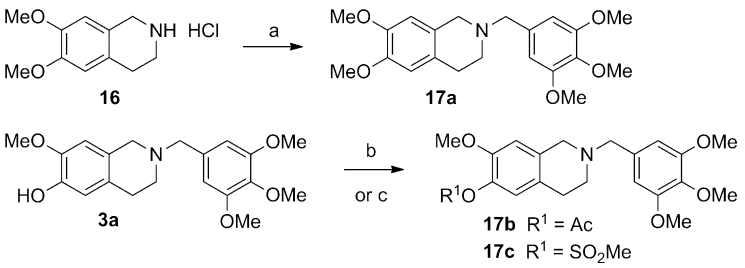
Having previously established synthetic approaches to protected 6-hydroxy-7-methoxy and 6-hydroxy-7-ethyl THIQs in preliminary studies, the logic was already in hand.[3] The major modification over foregoing work was introduction of an appropriately substituted benzyl motif at N2, followed by sequential deprotection and sulfamoylation of the 6-hydroxy group. This was achieved by transforming THIQs **5**[3] and **6a**,**b**[12,13] into the corresponding functionalised N-benzylated compounds **8a**–**d**[12] and **10a**–**f** using various direct N-benzylation methods or *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDCI) coupling with the corresponding benzoic acids and subsequent reduction of product with lithium aluminium hydride ($LiAlH_4$). The protected phenols were either treated with hydrogen and palladium on carbon (Pd/C; for **8a**–**d**) or tetra-*n*-butylammonium fluoride (TBAF; for **10a**–**f**), furnishing phenols **11a**–**j** in good yields. Subsequent treatment of **11a**–**j** with sulfamoyl chloride in *N*,*N*-dimethylacetamide (DMA)[14] gave the corresponding sulfamates **12a**–**j** (Scheme 1).

Compound **15** was elaborated in two synthetic steps by direct N-benzylation of the unprotected phenol **13**[3] with 3-bromo-4,5-dimethoxybenzyl bromide[15] and diisopropylethylamine (DIPEA) in *N*,*N*-dimethylformamide (DMF) and subsequent treatment of **14** with sulfamoyl chloride in DMA to give the corresponding sulfamate **15** in moderate overall yield (Scheme 2).

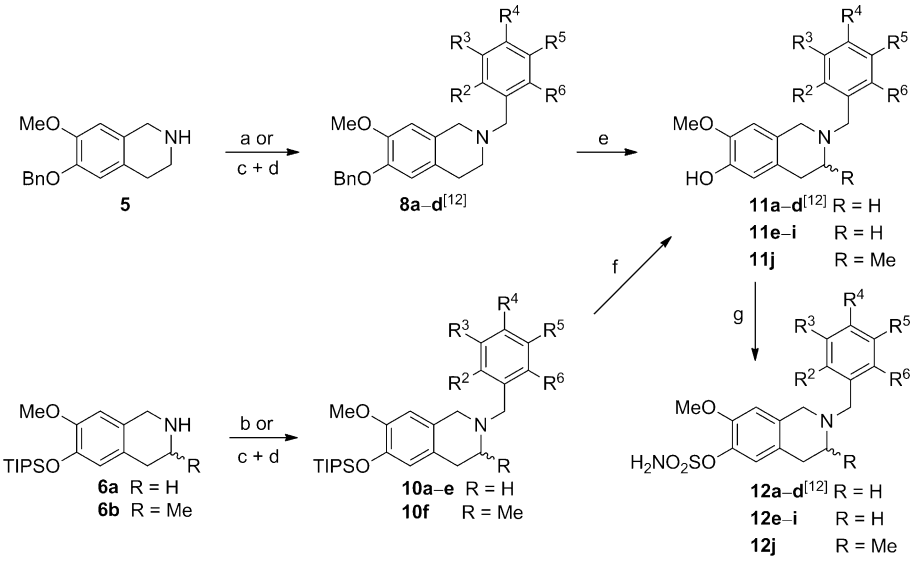


Scheme 2. Synthesis of 7-ethyl-THIQs. *Reagents and conditions*: a) 3-Bromo-4,5-dimethoxybenzyl bromide, DIPEA, DMF, 80 °C, 20 h, 40%; b) H_2NSO_2Cl, DMA, 25 °C, 20 h, 47%.

We also modified the potential hydrogen bonding effects around C6, while retaining the C7-methoxy and the *N*-2-(3′,4′,5′-trimethoxybenzyl) groups unchanged, to establish if in vitro activity could be further improved. Compound **17a** was synthesised from the commercially available 6,7-dimethoxy-THIQ salt **16**, 3,4,5-trimethoxybenzyl chloride and DIPEA in DMF. Compounds **17b**,**c** were accessed from phenol **3a** by treatment with acetic anhydride or methanesulfonyl chloride, respectively (Scheme 3).

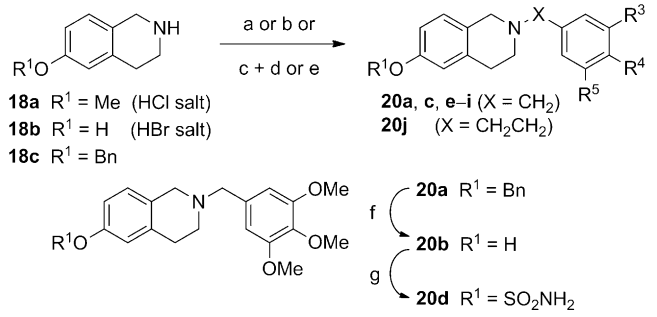


Scheme 3. Synthesis of C6-modified THIQs. *Reagents and conditions*: a) 3,4,5-Trimethoxybenzyl chloride, Et_3N, EtOH, 130 °C, MW, 1 h, 62%; b) Ac_2O, Et_3N, $CHCl_3$, 25 °C, 24 h, 79%; c) CH_3SO_2Cl, pyridine, 25 °C, 73%.



Scheme 1. Synthesis of 7-methoxy-THIQs. *Reagents and conditions*: a) $ArCH_2Cl$, Et_3N, EtOH, 130 °C, MW; b) $ArCH_2Br$, DIPEA, DMF, 80 °C; c) $ArCO_2H$, EDCI, CH_2Cl_2/THF, 25 °C; d) $LiAlH_4$, THF, reflux; e) H_2, Pd/C, THF/MeOH, 25 °C; f) TBAF, THF, 25 °C; g) H_2NSO_2Cl, DMA, 25 °C.

Another objective was to study the effect of deletion of the group at C7 since this had not previously been explored. A range of functionalised benzyl groups was then introduced at N2, as described above, starting from compounds **18a**–**c**.[16,17] Direct benzylation methods, or by coupling with the corresponding benzoic acids or benzoyl chlorides and successive reduction of product with $LiAlH_4$, afforded compounds **20a**–**i**, usually in moderate yield. An extension of the linker between the THIQ core and the aryl motif connected to it was also achieved by the same strategy and gave **20j** in good overall yield (Scheme 4).



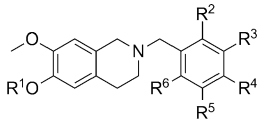
Scheme 4. Synthesis of C7-hydrogen-substituted THIQs. *Reagents and conditions*: a) $ArCH_2Cl$, Et_3N, EtOH, 130 °C, MW; b) $ArCH_2Br$, DIPEA, DMF, 80 °C; c) $ArCO_2H$, EDCI, Et_3N, CH_2Cl_2/THF, 25 °C; d) $LiAlH_4$, THF, 25 °C; e) ArCOCl, Et_3N, CH_2Cl_2, 25 °C; f) H_2, Pd/C, THF/MeOH, 25 °C, 1.5 h, 74%; g) H_2NSO_2Cl, DMA, 25 °C, 24 h, 88%.

Biology

Compounds were evaluated for their ability to inhibit DU-145 (prostate cancer) and MDA MB-231 (breast cancer) cell proliferation and compared to the first generation chimeras **3a** and **4a** and to 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (2-MeO-E2bisMATE; **1a**) and paclitaxel (Taxol) as benchmark drugs (Table 1). The results obtained across the two cell lines are in close agreement and therefore only the DU-145 data are used for SAR discussion herein. As reported previously, in both the trimethoxybenzyl phenol **3a** and **11a-c** and sulfamate series **4a** and **12a–c**, the 3′,4′,5′-trimethoxy system proves optimal (**4a** GI_{50} = 297 nM), although the 2′,4′,5′-trimethoxy sulfamate **12b** also displays good activity (GI_{50} = 660 nM).[12] Deletion of the 5′-methoxy group of **4a** to give the 3′,4′-dimethoxy compound **12d** results in a near 30-fold reduction in activity, thus illustrating the highly preferred status of trisubstitution in this series of chimeras.[12] Replacement of the 5′-methoxy with either chlorine in **12e**, or bromine in **12f**, however, delivers a dramatic 8- to 10-fold increase in antiproliferative activity, with the corresponding phenols **11e,f** also exhibiting significant activity. The 3′,4′-dimethoxy-5′-bromo derivatives **11f** and **12f** are exceptionally active. Substitution of the 4′-methoxy of **4a** with an ethoxy group also delivers improved activity for phenol **11g** and sulfamate **12g**, revealing a degree of steric flexibility at this position in the chimeric series in strong contrast to the steroidomimetic series.[12,13] Replacement of the 3′,4′,5′-trimethoxybenzyl group with a 3′,4′,5′-triethoxybenzyl group results in a >20-fold reduction in activity. Similarly, the 3′,4′,5′-triethyl compounds **11i** and **12i** are significantly less active than **3a** and **4a**, respectively.

Having established that the 3′,4′-dimethoxy-5′-bromobenzyl derivative is the most active of the various trisubstituted benzyl derivatives, we combined this motif with THIQ core modifications at C3 and C7 that had delivered enhanced activity in preceding studies. Introduction of a methyl group at C3 had delivered a modest enhancement in antiproliferative activity for the 3′,4′,5′-trimethoxybenzyl THIQs,[13] while replacement of the C7-methoxy group with an ethyl group had afforded a near 10-fold improvement in activity.[3] As can be seen in Table 2, when such modifications were made to the THIQ core bearing the 3′,4′-dimethoxy-5′-bromobenzyl group at N2, only relatively modest activity changes resulted with a slight improvement and a slight reduction in activity resulting from C7 and C3 modification, respectively. Note that the potential effect of individual enantiomers on the biological activity of **11j** and **12j** has not been pursued. Nonetheless, the C7 ethyl derivative **15** (GI_{50} = 20 nM) is in vitro the most active compound discovered in this series of chimeras to date. We then focused our attention on the C6 position. Changing the nature

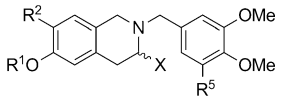
Table 1. Antiproliferative [μM] activity of THIQs against DU-145 human prostate and MDA MB-231 human breast cancer cells in vitro.[a]



Compd	R^1	R^2	R^3	R^4	R^5	R^6	DU-145	MDA MB-231
Taxol	N/A	N/A	N/A	N/A	N/A	N/A	0.004	0.002
1a	N/A	N/A	N/A	N/A	N/A	N/A	0.34	0.28
3a	H	H	OMe	OMe	OMe	H	0.65	0.62
4a	SO_2NH_2	H	OMe	OMe	OMe	H	0.297	0.329
11a	H	OMe	OMe	OMe	H	H	>100	>100
12a	SO_2NH_2	OMe	OMe	OMe	H	H	17	11.9
11b	H	OMe	H	OMe	OMe	H	7.8	4.17
12b	SO_2NH_2	OMe	H	OMe	OMe	H	0.66	0.491
11c	H	OMe	H	OMe	H	OMe	>100	>100
12c	SO_2NH_2	OMe	H	OMe	H	OMe	>100	>100
11d	H	H	OMe	OMe	H	H	>100	>100
12d	SO_2NH_2	H	OMe	OMe	H	H	8.54	3.15
11e	H	H	OMe	OMe	Cl	H	0.9	0.4
12e	SO_2NH_2	H	OMe	OMe	Cl	H	0.04	0.04
11f	H	H	OMe	OMe	Br	H	0.3	0.2
12f	SO_2NH_2	H	OMe	OMe	Br	H	0.03	0.03
11g	H	H	OMe	OEt	OMe	H	0.3	0.3
12g	SO_2NH_2	H	OMe	OEt	OMe	H	0.1	0.07
11h	H	H	OEt	OEt	OEt	H	>100	>100
12h	SO_2NH_2	H	OEt	OEt	OEt	H	8.5	4.4
11i	H	H	Et	Et	Et	H	63	26
12i	SO_2NH_2	H	Et	Et	Et	H	1.8	5.7

[a] Results are GI_{50} values in μM and are the mean of three determinations. Data for **1a**, **3a**, **4a**, **11a–d** and **12a–d** for comparison are taken from the literature [3,8,12]. N/A: not applicable.

Table 2. Antiproliferative activity [μM] of variously modified 3′,4′-dimethoxybenzyl-substituted THIQs against DU-145 human prostate and MDA MB-231 human breast cancer cells in vitro.[a]



Compd	R^1	X	R^2	R^5	DU-145	MDA MB-231
11j[b]	H	Me	OMe	Br	0.4	0.3
12j[b]	SO_2NH_2	Me	OMe	Br	0.05	0.04
14	H	H	Et	Br	1.2	0.4
15	SO_2NH_2	H	Et	Br	0.02	0.03
17a	Me	H	OMe	OMe	>100	77.6
17b	Ac	H	OMe	OMe	0.407	0.34
17c	SO_2Me	H	OMe	OMe	0.22	0.188

[a] Results are GI_{50} values in μM and are the mean of three determinations. [b] Compounds **11j** and **12j** are racemic mixtures.

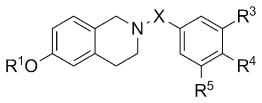
of the motif at C6 has a clear effect on in vitro antiproliferative activity (Table 2). Compounds bearing unhindered hydrogen-bond acceptors, such as in **3a** (see Table 1), prove much more active than hindered ones; for example, **17a** is 120-fold less active (GI_{50} = 77.6 μM in MDA MB-231) than **3a**. However, projection of a group bearing an unhindered hydrogen-bond acceptor at C6 proves positive with, for example, the sulfamoyl group of **4a** and the carbonyl and sulfonyl groups of **17b**,**c** delivering a ca. two- to three-fold better antiproliferative activity than that shown by phenol **3a**.

Next, we examined the effect of deletion of the C7 substituent that in the steroidomimetic series had proven essential for activity. In conjunction, we varied the C6 substituent and the linker at N2 and, in so doing, discovered an SAR strongly contrasting to that previously elucidated (Table 3). Here, the compound with a hydroxy group at C6, phenol **20b**, is by far the most active (GI_{50} = 151 nM in DU-145 and GI_{50} = 227 nM in MDA MB-231). Larger C6 substituents are universally less active, for example **20c** (about 20- to 200-fold) or inactive (e.g., **20a** and sulfamate **20d**) in the concentration range tested. Modifying the 3′,4′,5′-trimethoxybenzyl motif at N2 also proves to have a dramatic effect on antiproliferative activity. The only compounds to show modest activity are the 3′,4′-dimethoxy-5′-halobenzyl THIQs **20e**,**f**, albeit they are between 100- to 600-fold less active than **20b**. Increasing the size of one or more groups in this motif similarly delivers inactive compounds such as **20g**,**h**. Finally, exchanging the methylene linker between the 3′,4′,5′-trimethoxy aryl motif and N2 for a carbonyl group or extending it to ethylene proves fruitless (see compounds **20i**,**j**). Although not shown here, it should be mentioned that compounds wherein the 3′,4′,5′-trimethoxybenzyl motif at N2 in **20b** and **20d** is replaced by alternate mono-, di- and other trimethoxybenzyl motifs, and the sulfamoylated derivatives of **20e–j** show at best only modest activity (high micromolar GI_{50}s).

A selection of compounds was also tested at the US National Cancer Institute (NCI) in the full 60-cell-line assay (Table 4) that allows activity across a wide range of cancer types to be assessed. Data from six cell lines are presented along with the mean activity across the whole panel (MGM value). The data obtained in the assay are consistent with those obtained in the antiproliferative screens discussed above and confirm the potential of these compounds against a broad range of cancer phenotypes with, in particular, **12e**,**f** and **20b** proving highly active.

We also wished to establish the microtubule disruptor activity in particular of **12e**,**f**, **12j**, **15** and **20b** alongside the established potent disruptor combretastatin A-4 (**CA-4**) and the 3′,4′,5′-trimethoxybenzyl THIQ derivatives **3a** and **4a**,**b** (Table 5). The 3′,4′-dimethoxy-5′-halobenzyl THIQs are superior to the first generation chimeras **3a** and **4a** as inhibitors of tubulin assembly and approach the activity of **CA-4**, with **15** disrupting the polymerisation of tubulin with an IC_{50} value of 1.8 ± 0.04 μM. In tubulin-based assays, the concentration needed far exceeds the antiproliferative dose. It presumably suffices to disrupt microtubule dynamics to arrest the cell cycle. Additionally, of course, the nominal compound concentration recorded is that of agent added to the culture medium, and is not the concentration within cells. We also determined

Table 3. Antiproliferative activity [μM] of C7 hydrogen-substituted THIQs against DU-145 human prostate and MDA MB-231 human breast cancer cells in vitro.[a]



Compd	R^1	X	R^3	R^4	R^5	DU-145	MDA MB-231
20a	Bn	CH_2	OMe	OMe	OMe	>100	>100
20b	H	CH_2	OMe	OMe	OMe	0.151	0.227
20c	Me	CH_2	OMe	OMe	OMe	31	4.89
20d	SO_2NH_2	CH_2	OMe	OMe	OMe	>100	>100
20e	H	CH_2	OMe	OMe	Cl	86	74
20f	H	CH_2	OMe	OMe	Br	35	25
20g	H	CH_2	OMe	OEt	OMe	>100	>100
20h	H	CH_2	OEt	OEt	OEt	>100	>100
20i	H	CO	OMe	OMe	OMe	>100	>100
20j	H	CH_2CH_2	OMe	OMe	OMe	>100	>100

[a] Results are GI_{50} values in μM and are the mean of three determinations.

Table 4. Antiproliferative activity [μM] of selected compounds against various cancer cell lines from the NCI-60 cell line panel.[a]

Compd	Lung HOP-62	Colon HCT-116	CNS SF-539	Melanoma UACC-62	Ovarian OVCAR-3	Renal SN12-C	MGM
1a	0.051	0.045	0.036	<0.01	<0.01	0.126	0.087
11e	0.869	0.474	0.365	0.35	0.187	0.91	0.501
12e	0.056	0.039	0.019	0.025	0.02	0.066	0.039
11f	0.353	0.143	0.177	0.305	0.074	0.768	0.38
12f	0.08	0.038	0.022	0.028	0.018	0.055	0.044
12i	2.45	1.05	0.916	0.592	0.209	5.29	1.17
20b	0.062	0.035	0.036	0.049	0.019	0.049	0.045

[a] Results are GI_{50} values in μM and are the mean of three determinations. The MGM represents the mean concentration that caused 50% growth inhibition in all 60 cell lines. Data for **1a** are taken from the literature [9].

Table 5. Activity of selected THIQs as inhibitors of tubulin polymerisation and [^{3}H]colchicine binding to tubulin.

Compd	Tubulin assembly IC_{50} [μM][a]	Colchicine binding Inhibition at 5 μM inhibitor [%][a]
CA-4	1.2 ± 0.1	98 ± 0.7
3a	>20 (no activity)[b]	4.1 ± 2
4a	>20 (partial activity)[b]	10 ± 0.9
4b	2.5 ± 0.3	49 ± 0.5
12e	5.6 ± 0.7	32 ± 3
12f	2.4 ± 0.4	45 ± 0.6
12j[c]	2.4 ± 0.2	50 ± 4
15	1.8 ± 0.04	78 ± 2
20b	1.6 ± 0.1	73 ± 3

[a] Data are the mean ± SD of at least two determinations. [b] Compound **4a** inhibits tubulin assembly at 20 μM while **3a** is inactive at this concentration. [c] Compound **12j** is a racemic mixture. Data for **CA-4**, **3a** and **4a**,**b** are taken from the literature [12].

that sulfamates **12e**,**f**, **12j** and **15** inhibit colchicine binding to tubulin, with **15** being the best, showing 78% inhibition at 5 μM, approaching again the activity of **CA-4** (98%). Interestingly, deletion of the methoxy group at C7 in **3a** leads to phenol **20b** that shows slightly improved antiproliferative activity but also, somewhat surprisingly in comparison, excellent antitubulin data compared to both **3a** and its sulfamate **4a**. Moreover, **20b** is markedly better as an antiproliferative agent than its respective sulfamate derivative **20d**. The origin of these very considerable improvements remains to be determined, but might be due to steric effects leading to better accessibility of the unsubstituted phenolic hydroxy group and/or an alternate and stronger binding conformation at the tubulin binding site.

It thus appears reasonable to suggest that the interaction of the novel THIQ derivatives can at least partially be ascribed to their ability to disrupt the normal dynamic polymerisation of tubulin by interaction at, or around, the colchicine binding site. On the strength of their in vitro antiproliferative activity, **11f** and **12f** were selected for in vivo evaluation at the NCI in the hollow fibre assay that involves assessment of activity against the proliferation of various cancer lines in sealed polyvinylidine fluoride fibres implanted i.p. or s.c. in mice.[18] A 50% net cell growth inhibition is awarded a score of 2 and over 48 fibres (12 cell lines ×2 sites ×2 dose levels) a maximum score of 96 is possible. The results obtained for these compounds showed the strong difference in activity between the phenol **11f** and its corresponding sulfamate **12f**. Dosing of **11f** i.p. at 150 mg kg^{-1} resulted in 50% inhibition of cell growth in only one fibre and thus a score of only 2. In contrast, sulfamate **12f** when dosed by the same route at 75 mg kg^{-1} delivered a score of 34 (16 for i.p. fibres and 18 for s.c. fibres). This demonstrates both good activity and tissue distribution for the sulfamoylated THIQs and augers well for further development of this class of compounds as in vivo agents, with the anticipated better in vivo performance of the sulfamoyl ester versus the phenol. Although the activity surpasses normal criteria (a score >20) for further investigations at the NCI, compound **12f** was not selected for additional study. Despite the excellent in vitro and antitubulin data, and with in vivo data for the related phenol **11f** in hand, phenol **20b**, despite its clearly attractive antitubulin activities, is not very likely to show the same excellent in vivo and bioavailability properties as the sulfamoylated compound **12f**. Therefore, no further studies were carried out so far with this compound class.

The antimitotic natural product agents colchicine, combretastatin A-4, podophyllotoxin and steganacin interact with the colchicine site on tubulin and all possess a trimethoxyaryl unit. This unit in colchicine is thought to be derived via the shikimate biosynthetic pathway and that for combretastatin A-4, currently in clinical trials, is probably derived similarly. This A-ring unit in colchicine has long been thought to make an additive contribution to the strength of binding to tubulin, possibly through hydrogen bonding, and to serve also as an anchor to maintain the whole molecule in the proper orientation within the binding site. Alteration of the oxygenation pattern from a trimethoxy motif on the A-ring of the combretastatins was noted to adversely affect their biological properties and indeed, until recently, it was thought that the trimethoxyaryl group is critical for efficient binding. A range of combretastatins was synthesised with the trimethoxy motif substituted by other functionalities. This study demonstrated interestingly that modifying this motif could significantly reduce cytotoxicity and enhance antitubulin properties.[19] Our initial series of chimeric ligands[12] demonstrated that excellent antiproliferative in vitro and in vivo activities of this new class of compounds could be obtained with a chimera possessing a trimethoxyaryl motif, although the properties of such compounds with respect to inhibition of tubulin polymerisation and colchicine binding are somewhat less impressive. While it is as yet unclear if members of our prototype chimeric microtubule disruptors possessing the trimethoxyaryl motif (e.g., **3**–**4**) bind with this motif in a position comparable to that of colchicine, the present studies do generally support the previous observations that the trimethoxy motif is not critical[19] and demonstrate that molecules of a more optimised series possessing functionalities other than trimethoxy can interact potently with tubulin. Thus, in direct comparison, for example, the ca. 10-fold improvement in DU-145 antiproliferative activity of bromodimethoxyaryl-substituted **12f** versus the parent trimethoxyaryl **4a**, coupled with improvement in inhibition of tubulin assembly and colchicine binding, provide further evidence that excessive reverence for the trimethoxyaryl motif in optimisation of antimitotic properties should perhaps not remain. With antiproliferative activity maintained and enhanced in our more optimised series, together with the much enhanced antitubulin activity, members of the new series reported herein should yield even more impressive in vivo data in xenograft models of cancer.

Conclusions

A second generation class of tetrahydroisoquinoline (THIQ)-based chimeric microtubule disruptors with improved and excellent activity in vitro and in vivo, combined with a desirable drug-like profile, was identified. The best compounds possess antiproliferative activities in the 20–40 nM range, inhibit tubulin assembly, interfere with the colchicine binding site and possess a pendant *N*-aryl group without a trimethoxy motif. The sole sulfamoylated compound to be evaluated in vivo shows highly promising preliminary activity, validating the chimeric design and optimisation strategy for this new class of anticancer agents.

Experimental Section

Biology: In vitro studies

Cell Lines: DU-145 (brain metastasis carcinoma of the prostate) and MDA-MB-231 (metastatic pleural effusion of breast adenocarcinoma) established human cell lines were obtained from ATCC Global Bioresource Center. Cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, penicillin (100 U mL^{-1}), and streptomycin (0.1 mg mL^{-1}).

Antiproliferative assays: DU-145 and MDA-MB-231 cells were seeded into 96-well microtiter plates (5000 cells/well) and treated with 10^{-9}–10^{-4} M of compounds or with vehicle control. At 96 h post-treatment, live cell counts were determined by the WST-1 cell proliferation assay (Roche, Penzberg, Germany), as per the manufacturer's instructions. Viability results were expressed as a percentage of mean control values resulting in the calculation of the 50% growth inhibition (GI_{50}). All experiments were performed in triplicate.

Tubulin assays: Bovine brain tubulin, prepared as described previously,[20] was used in the studies presented here. Assembly IC_{50} values were determined as described in detail elsewhere.[21] Briefly, 1.0 mg mL^{-1} (10 μM) tubulin was preincubated without GTP with varying compound concentrations for 15 min at 30 °C. The reaction mixtures were placed on ice, and GTP (final concentration, 0.4 mM) was added. The reaction mixtures were transferred to cuvettes, held at 0 °C in a recording spectrophotometer. Baselines were established at 0 °C, and increase in turbidity was followed for 20 min following a rapid (< 30 s) jump to 30 °C. Compound concentrations required to reduce the turbidity increase by 50% were determined. The method for measuring inhibition of the binding of [^{3}H]colchicine to tubulin was described in detail previously.[22] Reaction mixtures contained 0.1 mg mL^{-1} (1.0 μM) tubulin, 5.0 μM [^{3}H]colchicine, and potential inhibitor at 5.0 μM. Compounds were compared to CA-4, a particularly potent inhibitor of the binding of colchicine to tubulin.[23] Reaction mixtures were incubated 10 min at 37 °C, a time point at which the binding of colchicine in control reaction mixtures is generally 40–60% complete. A minimum of two experiments was performed for each compound.

Chemistry

All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK) or Alfa Aesar (Heysham, UK). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. The petroleum ether (PE) used for column chromatography was of fractions 40–60 °C. CH_2Cl_2, $CHCl_3$, *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMA) and tetrahydrofuran (THF) were purchased from Aldrich and stored under a positive pressure of N_2 after use. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[24] and was stored in the refrigerator under a positive pressure of N_2 as a solution in toluene as described by Woo et al.[25] An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. Compounds **5**, **6a**,**b**, **8a**–**d**, **11a**–**d**, **12a**–**d**, **13** and **18a**–**c** were prepared according to literature procedures.[3,12,13,16,17] Reactions were carried out at room temperature (RT) unless stated otherwise. Thin-layer chromatography (TLC) was performed on precoated aluminium plates (Merck, silica gel 60 F_{254}). Product spots were visualised either by UV irradiation at 254 nm or by staining with either alkaline $KMnO_4$ solution or 5% dodecamolybdophosphoric acid in EtOH, followed by heating. Flash column chromatography was performed using gradient elution (solvents indicated in text) on either prepacked columns (Isolute) on a Flashmaster II system (Biotage, Uppsala, Sweden) or on a CombiFlash R_f Automated Flash Chromatography System (Teledyne Isco, Lincoln, NE, USA) with RediSep R_f disposable flash columns. ^{1}H NMR and ^{13}C NMR spectra were recorded with either a Delta JMN-GX 270 (Jeol, Peabody, MA, USA) at 270 and 67.5 MHz, respectively, or a Mercury VX 400 NMR spectrometer (Varian, Paolo Alto, CA, USA) at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. Coupling constants *J* are recorded to the nearest 0.1 Hz. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath, UK. FAB-MS was carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Melting points were determined using a Stuart SMP3 or a Stanford research systems Optimelt MPA100 melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) and are uncorrected. All compounds were ≥98% pure by reverse phase HPLC run with CH_3CN/H_2O or MeOH/H_2O (Sunfire C18 reverse phase column, 4.6× 150 mm, 3.5 μm pore size).

2-(3-Chloro-4,5-dimethoxybenzoyl)-7-methoxy-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (9a): Compound **6a** (504 mg, 1.5 mmol) and 3-chloro-4,5-dimethoxybenzoic acid (487 mg, 2.25 mmol) were placed in an oven-dried tube and dissolved in CH_2Cl_2 (3.0 mL) and THF (3.0 mL). *N*′-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide (EDCI; 573 mg, 3.0 mmol) was added, and the reaction mixture was stirred at RT for 18 h. HCl (2 M, 30 mL) was then added, and the mixture was extracted with CH_2Cl_2 (2×50 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (hexane→hexane/EtOAc 4:1→EtOAc) afforded compound **9a** as a colourless oil (535 mg, 66%): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.04 (18H, d, *J* = 6.9 Hz), 1.11–1.30 (3H, m), 2.73 (2H, s, br), 3.59 and 3.69 (2H, m), 3.73 (3H, s, br), 3.84 (3H, s, br), 3.85 (3H, s), 4.49 and 4.73 (2H, s, br), 6.37 and 6.58 (1H, s), 6.61 (1H, s), 6.92 (1H, s), 7.03 ppm (1H, d, *J* = 1.9 Hz); HRMS (ES+): *m/z* $[M+H]^+$calcd for $C_{28}H_{41}ClNO_5Si^+$: 534.2437, found: 534.2431.

2-(3,5-Dimethoxy-4-ethoxybenzoyl)-7-methoxy-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (9b): Method as for **9a** using compound **6a** (503 mg, 1.5 mmol), 3,5-dimethoxy-4-ethoxybenzoic acid[26] (508 mg, 2.25 mmol) and EDCI (574 mg, 3.0 mmol) in CH_2Cl_2 (3.0 mL) and THF (3.0 mL) at RT for 18 h. Flash column chromatography (hexane→hexane/EtOAc 7:3→EtOAc) afforded compound **9b** as a colourless oil (449 mg, 55%): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.04 (18H, d, *J* = 6.9 Hz), 1.11–1.28 (3H, m), 1.32 (3H, t, *J* = 7.0 Hz), 2.73 and 2.80 (2H, s, br), 3.60 and 3.69 (2H, m), 3.73 (3H, s, br), 3.80 (6H, s), 4.03 (2H, q, *J* = 7.1 Hz), 4.48 and 4.74 (2H, s, br), 6.35 and 6.60 (1H, s), 6.60 (1H, s), 6.64 ppm (2H, s); HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{30}H_{46}NO_6Si^+$: 544.3089, found: 544.3093.

7-Methoxy-2-(3,4,5-triethoxybenzoyl)-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (9c): Method as for **9a** using compound **6a** (503 mg, 1.5 mmol), 3,4,5-triethoxybenzoic acid (571 mg, 2.25 mmol) and EDCI (573 mg, 3.0 mmol) in CH_2Cl_2 (3.0 mL) and THF (3.0 mL) at RT for 18 h. Flash column chromatography (hexane→hexane/EtOAc 7:3→EtOAc) afforded compound **9c** as a colourless oil (550 mg, 64%): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.06 (18H, d, *J* = 6.9 Hz), 1.14–1.29 (3H, m), 1.33 (3H, t, *J* = 7.2 Hz), 1.39 (6H, t, *J* = 6.9 Hz), 2.72 and 2.80 (2H, s, br), 3.60 and 3.69 (2H, m), 3.75 (3H, s, br), 4.03 (2H, q, *J* = 7.2 Hz), 4.06 (4H, q, *J* = 7.4 Hz), 4.48 and 4.74 (2H, s, br), 6.35 and 6.62 (1H, s), 6.62 ppm (3H, s); HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{32}H_{50}NO_6Si^+$: 572.3402, found: 572.3405.

7-Methoxy-2-(3,4,5-triethylbenzoyl)-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (9d): Method as for **9a** using compound **6a** (503 mg, 1.5 mmol) and 3,4,5-triethylbenzoic acid[27,28] (483 mg, 2.25 mmol) and EDCI (572 mg, 3.0 mmol) in CH_2Cl_2 (3.0 mL) and THF (3.0 mL) at RT for 18 h. Flash column chromatography using (hexane→hexane/EtOAc 9:1→EtOAc) afforded compound **9d** as a colourless oil (342 mg, 43%): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.07 (18H, d, *J* = 6.9 Hz), 1.10–1.31 (3H, m), 1.12 (3H, t, *J* = 7.7 Hz), 1.21 (6H, t, *J* = 7.7 Hz), 2.58–2.76 (2H, m), 2.66 (4H, q,

$J=7.7$ Hz), 2.68 (2H, q, $J=7.7$ Hz), 3.61 and 3.68 (2H, m), 3.76 and 3.93 (3H, s, br), 4.51 and 4.77 (2H, s, br), 6.35 and 6.62 (1H, s), 6.62 (1H, s), 7.10 ppm (2H, s); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{32}H_{50}NO_3Si^+$: 524.3555, found: 524.3562.

2-(3-Chloro-4,5-dimethoxybenzyl)-7-methoxy-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10a): $LiAlH_4$ (103 mg, 2.7 mmol) was placed in an oven-dried tube and covered with THF (1.0 mL). **9a** (481 mg, 0.9 mmol) was dissolved in THF (4.4 mL) and added dropwise via syringe. The reaction mixture was stirred at RT for 2 h. EtOAc (5 mL) was added carefully. The mixture was then diluted with EtOAc (100 mL) and left without stirring in a beaker for 0.5 h. The mixture was filtered through Celite that was then washed with EtOAc (4×10 mL), and the filtrate was concentrated in vacuo. Flash column chromatography (hexane/EtOAc 9:1→9:1 and 2% Et_3N) afforded compound **10a** as a pale yellow oil (328 mg, 70%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.07$ (18H, d, $J=6.9$ Hz), 1.13–1.32 (3H, m), 2.61–2.69 (2H, m), 2.70–2.78 (2H, m), 3.52 (2H, s), 3.54 (2H, s), 3.71 (3H, s), 3.85 (6H, s), 6.44 (1H, s), 6.58 (1H, s), 6.88 (1H, d, $J=1.7$ Hz), 6.96 ppm (1H, d, $J=1.6$ Hz); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{28}H_{43}ClNO_4Si^+$: 520.2644, found: 520.2632.

2-(3-Bromo-4,5-dimethoxybenzyl)-7-methoxy-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10b): Compound **6a** (369 mg, 1.1 mmol) was dissolved in DMF (3.3 mL), and diisopropylethylamine (DIPEA; 287 mg, 2.2 mmol) and 3-bromo-4,5-dimethoxybenzyl bromide[15] (335 mg, 1.2 mmol) were added. The mixture was stirred at 80 °C for 18 h, cooled to RT, diluted with EtOAc (100 mL) and washed with H_2O (100 mL) and NH_4Cl (sat., 5 mL). The aqueous layer was extracted with EtOAc (100 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (hexane/EtOAc 19:1→19:1 and 2% Et_3N) afforded compound **10b** as a yellow oil (272 mg, 43%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.06$ (18H, d, $J=6.9$ Hz), 1.13–1.32 (3H, m), 2.61–2.69 (2H, m), 2.70–2.78 (2H, m), 3.52 (2H, s), 3.55 (2H, s), 3.71 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 6.44 (1H, s), 6.58 (1H, s), 6.93 (1H, d, $J=1.7$ Hz), 7.12 ppm (1H, d, $J=1.4$ Hz); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{28}H_{43}BrNO_4Si^+$: 564.2139, found: 564.2132.

2-(3,5-Dimethoxy-4-ethoxybenzyl)-7-methoxy-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10c): Method as for **10a** using compound **9b** (435 mg, 0.8 mmol) and $LiAlH_4$ (92 mg, 2.4 mmol) in THF (4.8 mL) at RT for 2 h. Flash column chromatography (hexane/EtOAc 9:1→4:1→4:1 and 2% Et_3N) afforded compound **10c** as a viscous yellow oil (398 mg, 93%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.06$ (18H, d, $J=6.9$ Hz), 1.12–1.29 (3H, m), 1.33 (3H, t, $J=7.2$ Hz), 2.60–2.68 (2H, m) 2.69–2.77 (2H, m), 3.53 (2H, s), 3.56 (2H, s), 3.69 (3H, s), 3.80 (6H, s), 4.02 (2H, q, $J=7.2$ Hz), 6.44 (1H, s), 6.57 (1H, s), 6.59 ppm (2H, s); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{30}H_{48}NO_5Si^+$: 530.3296, found: 530.3303.

7-Methoxy-2-(3,4,5-triethoxybenzyl)-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10d): Method as for **10a** using compound **9c** (515 mg, 0.9 mmol) and $LiAlH_4$ (104 mg, 2.7 mmol) in THF (5.4 mL) at RT for 2 h. Flash column chromatography (hexane/EtOAc 9:1→4:1→4:1 and 2% Et_3N) afforded compound **10d** as a viscous yellow oil (379 mg, 75%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.07$ (18H, d, $J=6.9$ Hz), 1.14–1.30 (3H, m), 1.34 (3H, t, $J=7.2$ Hz), 1.39 (6H, t, $J=7.2$ Hz), 2.59–2.68 (2H, m) 2.69–2.78 (2H, m), 3.53 (2H, s), 3.55 (2H, s), 3.71 (3H, s), 4.05 (6H, q, $J=7.2$ Hz), 6.44 (1H, s), 6.59 ppm (3H, s); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{32}H_{52}NO_5Si^+$: 558.3609, found: 558.3616.

7-Methoxy-2-(3,4,5-triethylbenzyl)-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10e): Method as for **10a** using compound **9d** (340 mg, 0.65 mmol) and $LiAlH_4$ (74 mg, 1.95 mmol) in THF (4.0 mL) at RT for 2 h. Flash column chromatography (hexane/EtOAc 19:1→19:1 and 2% Et_3N) afforded compound **10e** as a viscous yellow oil (289 mg, 87%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.07$ (18H, d, $J=6.9$ Hz), 1.13–1.32 (3H, m), 1.15 (3H, t, $J=7.6$ Hz), 1.23 (6H, t, $J=7.6$ Hz), 2.61–2.69 (2H, m) 2.66 (4H, q, $J=7.5$ Hz), 2.67 (2H, q, $J=7.7$ Hz), 2.70–2.78 (2H, m), 3.56 (2H, s), 3.60 (2H, s), 3.72 (3H, s), 6.45 (1H, s), 6.58 (1H, s), 7.04 ppm (2H, s); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{32}H_{52}NO_2Si^+$: 510.3762, found: 510.3769.

(±)-2-(3-Bromo-4,5-dimethoxybenzyl)-7-methoxy-3-methyl-6-(triisopropylsilyloxy)-1,2,3,4-tetrahydroisoquinoline (10f): Method as for **10b** using compound **6b** (419 mg, 1.2 mmol), 3-bromo-4,5-dimethoxybenzyl bromide[15] (449 mg, 1.45 mmol) and DIPEA (310 mg, 2.4 mmol) in DMF (3.6 mL) at 80 °C for 18 h. Flash column chromatography (hexane/EtOAc 9:1→9:1 and 2% Et_3N) afforded compound **10f** as a yellow oil (598 mg, 86%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.07$ (18H, d, $J=6.6$ Hz), 1.09 (3H, d, $J=5.0$ Hz), 1.14–1.32 (3H, m), 2.45 (1H, dd, $J=16.1$, 5.9 Hz), 2.85 (1H, dd, $J=16.0$, 4.7 Hz), 3.04 (1H, sext, $J=6.1$ Hz), 3.40–3.73 (4H, m), 3.71 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 6.41 (1H, s), 6.57 (1H, s), 6.89 (1H, d, $J=1.9$ Hz), 7.11 ppm (1H, d, $J=1.6$ Hz); HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{29}H_{45}BrNO_4Si^+$: 578.2296, found: 578.2251.

2-(3-Chloro-4,5-dimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (11e): Compound **10a** (286 mg, 0.55 mmol) was dissolved in THF (2.5 mL). Tetra-*n*-butylammonium fluoride (TBAF; 0.66 mL, 1 M in THF, 0.66 mmol) was added dropwise via syringe, and the reaction mixture was stirred at 0 °C for 0.5 h. MeOH (5 mL) and CH_2Cl_2 (30 mL) were added, and the mixture was concentrated in vacuo. Flash column chromatography ($CHCl_3$/acetone 9:1→9:1 and 2% MeOH) afforded compound **11e** as a white solid (168 mg, 84%): mp: 193–195 °C; ^1H NMR (270 MHz, $CDCl_3$): $\delta=2.61$–2.68 (2H, m), 2.69–2.76 (2H, m), 3.46 (2H, s), 3.51 (2H, s), 3.75 (3H, s), 3.80 (6H, s), 6.42 (1H, s), 6.57 (1H, s), 6.88 (1H, d, $J=1.6$ Hz), 6.90 ppm (1H, d, $J=1.6$ Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta=28.0$, 50.6, 55.5, 55.8, 56.0, 60.6, 62.0, 108.9, 111.5, 114.3, 122.2, 125.3, 126.4, 127.6, 134.6, 144.1, 145.1, 153.7 ppm; LC-MS (ES+): m/z 364.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{19}H_{23}ClNO_4^+$: 364.1310, found: 364.1305.

2-(3-Bromo-4,5-dimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (11f): Method as for **11e** using compound **10b** (254 mg, 0.45 mmol) and TBAF (0.54 mL, 1 M in THF, 0.54 mmol) in THF (2.7 mL) at 0 °C for 0.5 h. Flash column chromatography ($CHCl_3$/acetone 9:1→9:1 and 2% MeOH) gave compound **11f** as a pale yellow solid (148 mg, 80%): mp: 195–198 °C; ^1H NMR (270 MHz, $CDCl_3$): $\delta=2.59$–2.67 (2H, m), 2.68–2.76 (2H, m), 3.45 (2H, s), 3.50 (2H, s), 3.74 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 6.41 (1H, s), 6.55 (1H, s), 6.91 (1H, d, $J=1.6$ Hz), 7.05 ppm (1H, d, $J=1.6$ Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta=27.9$, 50.6, 55.5, 55.8, 55.9, 60.4, 61.9, 108.9, 112.3, 114.3, 117.0, 125.0, 125.2, 126.4, 135.2, 144.1, 145.2, 153.5 ppm; LC-MS (ES+): m/z 408.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{19}H_{23}BrNO_4^+$: 408.0805, found: 408.0796.

2-(3,5-Dimethoxy-4-ethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (11g): Method as for **11e** using compound **10c** (371 mg, 0.7 mmol) and TBAF (0.84 mL, 1 M in THF, 0.84 mmol) in THF (4.2 mL) at 0 °C for 0.5 h. Flash column chromatography ($CHCl_3$/acetone 9:1→4:1→4:1 and 2% MeOH) afforded compound **11g** as a yellow glass (194 mg, 74%): ^1H NMR (270 MHz, $CDCl_3$): $\delta=1.34$ (3H, t, $J=7.0$ Hz), 2.65–2.72 (2H, m),

2.72–2.79 (2H, m), 3.53 (2H, s), 3.58 (2H, s), 3.78 (3H, s), 3.80 (6H, s), 4.03 (2H, q, J=7.1 Hz), 5.52 (1H, s, br), 6.45 (1H, s), 6.60 (1H, s), 6.61 ppm (2H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.5, 28.2, 50.6, 55.7, 55.9, 56.0, 62.8, 68.8, 105.7, 108.8, 114.3, 125.7, 126.8, 133.7, 135.8, 144.1, 145.0, 153.3 ppm; LC-MS (ES+): m/z 374.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{21}H_{28}NO_5^+$: 374.1962, found: 374.1953.

6-Hydroxy-7-methoxy-2-(3,4,5-triethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (11h): Method as for **11e** using compound **10d** (336 mg, 0.6 mmol) and TBAF (0.72 mL, 1 M in THF, 0.72 mmol) in THF (3.6 mL) at 0 °C for 0.5 h. Flash column chromatography ($CHCl_3$/acetone 9:1→9:1 and 2% MeOH) afforded compound **11h** as a pale yellow solid (189 mg, 78%): mp: 119–121 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.34 (3H, t, J=6.3 Hz), 1.38 (6H, t, J=6.9 Hz), 2.62–2.70 (2H, m), 2.71–2.79 (2H, m), 3.51 (2H, s), 3.55 (2H, s), 3.79 (3H, s), 4.03 (6H, q, J=7.0 Hz), 5.63 (1H, s, br), 6.45 (1H, s), 6.58 (1H, s), 6.62 ppm (2H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.0, 15.6, 28.4, 50.6, 55.8, 56.0, 62.8, 64.6, 68.7, 107.4, 108.8, 114.2, 126.1, 127.0, 133.6, 144.0, 144.9, 152.8 ppm; LC-MS (ES+): m/z 402.3 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{23}H_{32}NO_5^+$: 402.2275, found: 402.2268.

6-Hydroxy-7-methoxy-2-(3,4,5-triethylbenzyl)-1,2,3,4-tetrahydroisoquinoline (11i): Method as for **11e** using compound **10e** (255 mg, 0.5 mmol) and TBAF (0.6 mL, 1 M in THF, 0.6 mmol) in THF (3.0 mL) at 0 °C for 0.5 h. Flash column chromatography ($CHCl_3$/acetone 9:1→9:1 and 2% MeOH) afforded compound **11i** as a yellow glass (134 mg, 75%): ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.16 (3H, t, J=7.6 Hz), 1.24 (6H, t, J=7.4 Hz), 2.67 (4H, q, J=7.4 Hz), 2.67–2.82 (6H, m), 3.57 (2H, s), 3.63 (2H, s), 3.80 (3H, s), 5.49 (1H, s, br), 6.48 (1H, s), 6.61 (1H, s), 7.06 ppm (2H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.3, 15.8, 21.2, 25.7, 28.2, 50.5, 55.7, 55.9, 62.5, 108.9, 114.3, 126.0, 126.9, 127.1, 135.2, 138.2, 141.9, 144.0, 144.9 ppm; LC-MS (ES+): m/z 354.3 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{23}H_{32}NO_2^+$: 354.2428, found: 354.2414.

(±)-2-(3-Bromo-4,5-dimethoxybenzyl)-6-hydroxy-7-methoxy-3-methyl-1,2,3,4-tetrahydroisoquinoline (11j): Method as for **11e** using compound **10f** (520 mg, 0.9 mmol) and TBAF (1 M in THF, 1.08 mL, 1.08 mmol) in THF (4.5 mL) at 0 °C for 0.5 h. Flash column chromatography ($CHCl_3$/acetone 9:1→9:1 and 2% MeOH) afforded compound **11j** as a yellow solid (342 mg, 90%): mp: 111–114 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.11 (3H, d, J=6.3 Hz), 2.46 (1H, dd, J=16.2, 6.1 Hz), 2.87 (1H, dd, J=16.1, 4.8 Hz), 3.06 (1H, sext, J=6.1 Hz), 3.41–3.73 (4H, m), 3.78 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 5.32 (1H, s, br), 6.43 (1H, s), 6.59 (1H, s), 6.92 (1H, d, J=1.9 Hz), 7.10 ppm (1H, d, J=1.7 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.2, 34.4, 51.2, 52.2, 55.8, 56.0, 56.3, 60.5, 108.6, 112.0, 114.5, 117.1, 124.5, 125.0, 126.2, 136.7, 144.0, 144.9, 145.1, 153.6 ppm; LC-MS (ES+): m/z 422.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{20}H_{25}BrNO_4S^+$: 422.0962, found: 422.0942.

2-(3-Chloro-4,5-dimethoxybenzyl)-7-methoxy-6-sulfamoyloxy-1,2,3,4-tetrahydroisoquinoline (12e): Compound **11e** (146 mg, 0.4 mmol) was placed in an oven-dried 50 mL round-bottom flask and dissolved in DMA (2.0 mL). Sulfamoyl chloride (0.57 M in toluene, 2.1 mL, 1.2 mmol) was concentrated in vacuo and re-dissolved in DMA (1.0 mL). This solution was added dropwise via syringe at 0 °C. The reaction mixture was stirred at RT for 2 h. EtOAc (100 mL) was added, and the mixture was washed with $NaHCO_3$ (saturated, 50 mL) and H_2O (4×50 mL). The organic layer was dried (NaCl), filtered and concentrated in vacuo. The residue was stirred in CH_2Cl_2/Et_2O/hexane (~1:2:2), filtered and dried to afford compound **12e** as a white solid (85 mg, 48%): mp: 130–134 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=2.62–2.70 (2H, m), 2.73–2.81 (2H, m), 3.50 (2H, s), 3.52 (2H, s), 3.76 (3H, s), 3.81 (6H, s), 5.98 (2H, s, br), 6.56 (1H, s), 6.83 (1H, d, J=1.6 Hz), 6.92 (1H, d, J=1.7 Hz), 7.04 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=28.0, 50.4, 55.5, 56.1, 56.1, 60.6, 61.8, 110.9, 111.2, 121.8, 123.8, 126.9, 127.8, 134.0, 134.8, 137.4, 144.3, 149.6, 153.7 ppm; LC-MS (ES+): m/z 443.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{19}H_{24}ClN_2O_6S^+$: 443.1038, found: 443.1034.

2-(3-Bromo-4,5-dimethoxybenzyl)-7-methoxy-6-sulfamoyloxy-1,2,3,4-tetrahydroisoquinoline (12f): Method as for **12e** using compound **11f** (122 mg, 0.3 mmol) and sulfamoyl chloride (0.9 mmol) in DMA (2.5 mL) at RT for 2 h. The residue was stirred in CH_2Cl_2/Et_2O/hexane (~1:2:2), filtered and dried to afford compound **12f** as a pale yellow solid (54 mg, 37%): mp: 132–136 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=2.62–2.70 (2H, m), 2.73–2.81 (2H, m), 3.50 (2H, s), 3.53 (2H, s), 3.76 (3H, s), 3.80 (3H, s), 3.80 (3H, s), 5.99 (2H, s, br), 6.56 (1H, s), 6.87 (1H, d, J=1.6 Hz), 7.04 (1H, s), 7.07 ppm (1H, d, J=1.6 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=28.0, 50.4, 55.5, 56.0, 56.1, 60.4, 61.7, 110.9, 112.0, 117.2, 123.8, 124.6, 126.8, 134.0, 135.4, 137.4, 145.4, 149.6, 153.6 ppm; LC-MS (ES+): m/z 487.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{19}H_{24}BrN_2O_6S^+$: 487.0533, found: 487.0511.

2-(3,5-Dimethoxy-4-ethoxybenzyl)-7-methoxy-6-sulfamoyloxy-1,2,3,4-tetrahydroisoquinoline (12g): Method as for **12e** using compound **11g** (169 mg, 0.45 mmol) and sulfamoyl chloride (1.35 mmol) in DMA (4.0 mL) at RT for 2 h. The residue was stirred in CH_2Cl_2/Et_2O (~1:4), filtered and dried to afford compound **12g** as a pale yellow solid (125 mg, 61%): mp: 127–130 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.34 (3H, t, J=7.0 Hz), 2.66–2.75 (2H, m), 2.76–2.85 (2H, m), 3.57 (2H, s), 3.59 (2H, s), 3.79 (3H, s), 3.82 (6H, s), 4.03 (2H, q, J=7.1 Hz), 5.05 (2H, s, br), 6.59 (3H, s), 7.06 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.5, 27.9, 50.2, 55.6, 56.1, 56.2, 62.6, 68.9, 105.8, 111.1, 124.0, 127.3, 133.3, 134.4, 136.0, 137.3, 149.4, 153.4 ppm; LC-MS (ES+): m/z 453.2 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{21}H_{29}N_2O_7S^+$: 453.1690, found: 453.1688.

7-Methoxy-6-sulfamoyloxy-2-(3,4,5-triethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (12h): Method as for **12e** using compound **11h** (160 mg, 0.4 mmol) and sulfamoyl chloride (1.2 mmol) in DMA (3.0 mL) at RT for 2 h. The residue was stirred in CH_2Cl_2/Et_2O/hexane (~1:2:2), filtered and dried to afford compound **12h** as a pale yellow solid (84 mg, 46%): mp: 133–134 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.33 (3H, t, J=6.9 Hz), 1.39 (6H, t, J=6.9 Hz), 2.64–2.72 (2H, m), 2.75–2.83 (2H, m), 3.54 (2H, s), 3.55 (2H, s), 3.79 (3H, s), 4.03 (2H, q, J=7.0 Hz), 4.04 (4H, q, J=7.0 Hz), 5.07 (2H, s, br), 6.57 (2H, s), 6.59 (1H, s), 7.05 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=14.9, 15.6, 28.0, 50.2, 55.6, 56.2, 62.6, 64.6, 68.8, 107.4, 111.1, 124.0, 127.5, 133.1, 134.6, 137.0, 137.3, 149.3, 152.8 ppm; LC-MS (ES+): m/z 481.3 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{23}H_{33}N_2O_7S^+$: 481.2003, found: 481.1989.

7-Methoxy-6-sulfamoyloxy-2-(3,4,5-triethylbenzyl)-1,2,3,4-tetrahydroisoquinoline (12i): Method as for **12e** using compound **11i** (106 mg, 0.3 mmol) and sulfamoyl chloride (0.9 mmol) in DMA (2.5 mL) at RT for 2 h. The residue was stirred in CH_2Cl_2/hexane (~1:4), filtered and dried to afford compound **12i** as a pale yellow solid (65 mg, 50%): mp: 138–140 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=1.14 (3H, t, J=7.4 Hz), 1.22 (6H, t, J=7.6 Hz), 2.61–2.75 (4H, m), 2.65 (4H, q, J=7.6 Hz), 2.77–2.84 (2H, m), 3.59 (2H, s), 3.61 (2H, s), 3.79 (3H, s), 5.07 (2H, s, br), 6.61 (1H, s), 7.02 (2H, s), 7.06 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ=15.5, 27.9, 50.2, 55.6, 56.1, 56.2, 62.6, 68.9, 105.8, 111.1, 123.9, 127.3, 133.3, 134.4, 136.0, 137.3,

149.3, 153.4 ppm; LC-MS (ES+): *m/z* 433.3 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{23}H_{33}N_2O_4S^+$: 433.2156, found: 433.2147.

(±)-2-(3-Bromo-4,5-dimethoxybenzyl)-7-methoxy-3-methyl-6-sulfamoyloxy-1,2,3,4-tetrahydroisoquinoline (12j): Method as for **12e** using compound **11j** (212 mg, 0.5 mmol) and sulfamoyl chloride (1.5 mmol) in DMA (3.0 mL) at RT for 2 h. The residue was stirred in CH_2Cl_2, filtered and dried to afford compound **12j** as a pale yellow solid (241 mg, 96%): mp: 155–158 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 0.98$ (3H, d, $J = 6.6$ Hz), 2.39 (1H, dd, $J = 16.6$, 5.6 Hz), 2.81 (1H, dd, $J = 16.2$, 4.4 Hz), 2.95 (1H, sext, $J = 5.6$ Hz), 3.30–3.62 (4H, m), 3.66 (3H, d, $J = 2.2$ Hz), 3.69 (3H, d, $J = 2.2$ Hz), 3.71 (3H, d, $J = 2.2$ Hz), 6.45 (2H, s), 6.50 (1H, s), 6.76 (1H, s), 6.94 (1H, s), 6.97 ppm (1H, d, $J = 1.7$ Hz); LC-MS (ES+): *m/z* 501.2 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{26}BrN_2O_6S^+$: 501.0617, found: 501.0644;.

2-(3-Bromo-4,5-dimethoxybenzyl)-7-ethyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (14): Method as for **10b** using compound **13**[3] (266 mg, 1.5 mmol), DIPEA (581 mg, 4.5 mmol) and 3-bromo-4,5-dimethoxybenzyl bromide[15] (511 mg, 1.65 mmol) in DMF (5 mL) at 80 °C for 20 h. Flash column chromatography ($CHCl_3$/acetone 4:1) afforded compound **14** as a tan solid (246 mg, 40%): mp: 156–160 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.12$ (3H, t, $J = 7.4$ Hz), 2.50 (2H, q, $J = 7.4$ Hz), 2.60 (2H, d, $J = 5.2$ Hz), 2.67 (2H, d, $J = 5.2$ Hz), 3.51 (2H, s), 3.57 (2H, s), 3.75 (3H, s), 3.84 (3H, s), 6.12 (1H, s), 6.70 (1H, s), 6.98 (1H, s), 7.12 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 14.1$, 22.7, 28.1, 50.7, 55.4, 55.9, 60.5, 62.1, 112.6, 114.8, 117.1, 125.3, 127.0, 128.5, 132.1, 134.8, 145.5, 145.5, 152.5, 153.6 ppm; LC-MS (ES+): *m/z* 406.2 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{25}BrNO_3^+$: 406.1012, found: 406.1005.

2-(3-Bromo-4,5-dimethoxybenzyl)-7-ethyl-6-sulfamoyloxy-1,2,3,4-tetrahydroisoquinoline (15): Method as for **12e** using compound **14** (150 mg, 0.37 mmol) and sulfamoyl chloride (1.48 mmol) in DMA (1.0 mL) at RT for 20 h. Flash column chromatography ($CHCl_3$/acetone 4:1) afforded compound **15** as a pale yellow foam (85 mg, 47%): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.16$ (3H, t, $J = 7.6$), 2.64 (2H, q, $J = 7.6$ Hz), 2.70 (2H, t, $J = 5.5$ Hz), 2.84 (2H, t, $J = 5.5$ Hz), 3.56–3.58 (4H, m), 3.84 (6H, s), 6.88–6.93 (2H, m), 7.08–7.13 ppm (2H, m); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 14.3$, 22.7, 28.4, 50.1, 55.2, 56.1, 60.6, 61.7, 112.3, 117.3, 121.4, 125.0, 127.9, 133.1, 133.2, 134.4, 135.0, 145.6, 146.7, 153.7 ppm; LC-MS (ES+): *m/z* 485.2 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{26}BrN_2O_5S^+$: 485.0740, found: 485.0736.

6,7-Dimethoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (17a): Compound **16** (0.23 g, 1.0 mmol), 3,4,5-trimethoxybenzyl chloride (0.26 g, 1.2 mmol) and Et_3N (0.5 mL, 3.6 mmol) were placed in a 10 mL microwave vessel and dissolved in EtOH (2.5 mL). The mixture was then irradiated at 130 °C for 1 h in the microwave oven. After cooling to RT, the mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (hexane/EtOAc 6:1→1:1) gave a white solid that was stirred in Et_2O, filtered and dried to afford compound **17a** as a white powder (230 mg, 62%): mp: 118–119 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.70$ (2H, t, $J = 5.6$ Hz), 2.81 (2H, t, $J = 5.6$ Hz), 3.55 (2H, s), 3.59 (2H, s), 3.81 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 3.85 (6H, s), 6.50 (1H, s), 6.60 (1H, s), 6.62 ppm (2H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.8$, 50.7, 55.9, 56.0, 56.2, 61.0, 63.1, 105.6, 109.5, 111.4, 126.3, 126.8, 134.5, 136.9, 147.3, 147.6, 153.2 ppm; LC-MS (ES+): *m/z* 374.27 $[M+H]^+$; Anal. calcd for $C_{21}H_{27}NO_5$: C 67.54, H 3.75, N 7.29, found: C 67.5, H 3.76, N 7.10.

6-Acetoxy-7-methoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (17b): Compound **3a** (108 mg, 0.3 mmol), Et_3N (0.21 mL, 1.6 mmol) and Ac_2O (0.16 mL, 1.6 mmol) were stirred in $CHCl_3$ (10 mL) at RT for 24 h. The reaction mixture was diluted with $CHCl_3$ (30 mL) and washed with H_2O (4×30 mL) and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was stirred in Et_2O, filtered and dried in vacuo to afford compound **17b** as a white powder (95 mg, 79%): mp: 143–144 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.28$ (3H, s), 2.69–2.83 (4H, m), 3.60 (4H, s), 3.75 (3H, s), 3.84 (3H, s), 3.85 (6H, s), 6.58 (1H, s), 6.62 (2H, s), 6.77 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 20.7$, 28.3, 50.5, 55.9, 56.0, 56.2, 60.9, 62.8, 105.7, 110.5, 122.7, 124.1, 126.5, 133.3, 136.9, 138.1, 149.1, 153.3, 169.4 ppm; LC-MS (ES+): *m/z* 402.24 $[M+H]^+$; Anal. calcd for $C_{22}H_{27}NO_6$: C 65.82, H 6.78, N 3.49, found: C 65.8, H 6.81, N 3.49.

7-Methoxy-6-methanesulfonyloxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (17c): Compound **3a** (80 mg, 0.22 mmol) was dissolved in pyridine (1.0 mL) and cooled to 0 °C. Methanesulfonyl chloride (20 μL, 0.26 mmol) was added, and the reaction mixture was stirred at 0 °C for 2 h and then at RT for 4 h. The mixture was diluted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (hexane/EtOAc 1:1) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **17c** as a white solid (70 mg, 73%): mp: 134–135 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.68$–2.84 (4H, m), 3.15 (3H, s), 3.57 (2H, s), 3.59 (2H, s), 3.81 (3H, s), 3.83 (3H, s), 3.85 (6H, s), 6.61 (3H, s), 7.04 ppm (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.3$, 38.2, 50.4, 55.9, 56.1, 56.2, 61.0, 62.9, 105.6, 110.9, 124.5, 127.4, 134.2, 135.0, 136.7, 137, 149.3, 153.3 ppm; LC-MS (ES+): *m/z* 438.14 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{21}H_{28}NO_7S^+$: 438.1581, found: 438.1569.

2-(3-Chloro-4,5-dimethoxybenzoyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (19a): Method as for **9a** using compound **18b** (345 mg, 1.5 mmol), 3-chloro-4,5-dimethoxybenzoic acid (357 mg, 1.65 mmol), EDCI (575 mg, 3.0 mmol) and Et_3N (0.25 mL, 1.8 mmol) in CH_2Cl_2 (10 mL) at RT for 18 h. The mixture was diluted with CH_2Cl_2, washed with H_2O (10 mL), citric acid (10%, 10 mL), Na_2CO_3 (sat.) and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (hexane→hexane/EtOAc 1:1) afforded compound **19a** as an off-white solid (313 mg, 60%): mp: 151–164 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.75$–2.82 (2H, m), 3.61 (1H, s, br), 3.81 (3H, s, br), 3.86 (3H, s), 3.85–3.92 (1H, m), 4.49 (1H, s, br), 4.74 (1H, s, br), 6.56–6.90 (4H, m), 7.02 (1H, s), 7.80 ppm (1H, s, br); LC-MS (ES+): *m/z* 348.4 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{18}H_{19}ClNO_4^+$: 348.0997, found: 348.0987.

2-(3,5-Dimethoxy-4-ethoxybenzoyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (19b): Method as for **19a** using compound **18b** (345 mg, 1.5 mmol), 4-ethoxy-3,5-dimethoxybenzoic acid[26] (373 mg, 1.65 mmol), EDCI (575 mg, 3.0 mmol) and Et_3N (0.25 mL, 1.8 mmol) in CH_2Cl_2 (10 mL) at RT for 18 h. Flash column chromatography (hexane→hexane/EtOAc 1:4) afforded compound **19b** as a white foam (296 mg, 55%): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 1.34$ (3H, t, $J = 6.9$ Hz), 2.75–2.86 (2H, m), 3.60–3.67 (1H, m), 3.81 (6H, s), 3.81–3.90 (1H, m), 4.06 (2H, q, $J = 6.9$ Hz), 4.51 (1H, s, br), 4.76 (1H, s, br), 6.60–6.97 ppm (5H, m); LC-MS (ES+): *m/z* 358.1 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}NO_5^+$: 358.1649, found: 358.1637.

6-Hydroxy-2-(3,4,5-triethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline (19c): Method as for **19a** using compound **18b** (690 mg, 3.0 mmol), 3,4,5-triethoxybenzoic acid (1.14 g, 4.5 mmol), EDCI (1.15 g, 6.0 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (10 mL)

and THF (5 mL) at RT for 18 h. Flash column chromatography (hexane→hexane/EtOAc 3:2) afforded compound **19c** as a white solid (731 mg, 63%): mp: 157–158 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.32–1.42 (9H, m), 2.80 (2H, s, br), 3.62 and 3.92 (2H, s, br), 3.98–4.11 (6H, m), 4.51 and 4.75 (2H, s, br), 5.97 (1H, s, br), 6.62–6.66 (4H, m), 6.67 and 7.00 ppm (1H, s, br); LC-MS (APCI+): *m/z* 386.5 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}NO_5^+$: 386.1962, found: 386.1960.

6-Hydroxy-2-(3,4,5-trimethoxyphenacetyl)-1,2,3,4-tetrahydroisoquinoline (19d): Method as for **19a** using compound **18b** (345 mg, 1.5 mmol) and 3,4,5-trimethoxyphenylacetic acid (373 mg, 1.65 mmol), EDCI (575 mg, 3.0 mmol) and Et_3N (0.25 mL, 1.8 mmol) in CH_2Cl_2 (10 mL) at RT for 18 h. Flash column chromatography (hexane→EtOAc) afforded compound **19d** as a gummy foam (470 mg, 88%): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.66 and 2.78 (2H, t, *J* = 6.2 Hz), 3.64 and 3.81 (2H, t, *J* = 6.2 Hz), 3.70–3.82 (11H, m), 4.55 and 4.67 (2H, s), 5.50 (1H, s, br), 6.39 and 6.46 (2H, s), 6.56–6.68 (2H, m), 6.83 and 6.97 ppm (1H, d, *J* = 8.1 Hz); LC-MS (APCI): *m/z* 358.3 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}NO_5^+$: 358.1649, found: 358.1643.

6-Benzyloxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (20a): Method as for **17a** using compound **18c** (431 mg, 1.8 mmol), 3,4,5-trimethoxybenzyl chloride (433 mg, 2 mmol) and Et_3N (0.5 mL, 3.6 mmol) in EtOH (2.5 mL) at 130 °C for 1.5 h in the microwave oven. Flash column chromatography (hexane/EtOAc 3:1→1:1) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **20a** as a yellow powder (450 mg, 60%): mp: 103–104 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.69 (2H, t, *J* = 5.8 Hz), 2.86 (2H, t, *J* = 5.8 Hz), 3.58 (2H, s), 3.60 (2H, s), 3.84 (3H, s), 3.85 (6H, s), 5.03 (2H, s), 6.62 (2H, s), 6.74–6.78 (2H, m), 6.92 (1H, d, *J* = 8.0 Hz), 7.26–7.43 ppm (5H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 29.4, 50.3, 55.7, 56.1, 60.8, 63.0, 69.9, 105.5, 112.8, 114.3, 127.4, 127.5, 127.9, 128.5, 134.4, 135.6, 136.7, 137.1, 153.1, 157.1 ppm; LC-MS (ES+): *m/z* 420.25 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{26}H_{30}NO_4^+$: 420.2169, found: 420.2158; Anal. calcd for $C_{26}H_{29}NO_4$: C 74.44, H 6.97, N 3.34, found: C 74.3, H 6.91, N 3.30.

6-Hydroxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (20b): Compound **20a** (300 mg, 0.72 mmol) was treated with Pd/C (10%, 40 mg) in THF (20 mL) and MeOH (20 mL) under H_2 at RT for 1.5 h. The reaction mixture was then filtered through Celite and washed with MeOH (4×10 mL). The combined filtrates were concentrated in vacuo, and the residue was stirred in EtOAc, filtered and dried to afford compound **20b** as a white powder (175 mg, 74%): mp: 197–199 °C; ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 2.60 (2H, t, *J* = 5.4 Hz), 2.72 (2H, t, *J* = 5.4 Hz), 3.45 (2H, s), 3.54 (2H, s), 3.64 (3H, s), 3.75 (6H, s), 6.48–6.53 (2H, m), 6.65 (2H, s), 6.81 (1H, d, *J* = 8.0 Hz), 9.11 ppm (1H, s); ^{13}C NMR (67.5 MHz, [D$_6$]DMSO): δ = 28.8, 50.2, 55.2, 55.8, 60.0, 62.1, 105.5, 113.0, 114.6, 125.2, 127.3, 134.3, 135.1, 136.2, 152.8, 155.4 ppm; LC-MS (ES−): *m/z* 328.16 $[M-H]^-$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}NO_4^+$: 330.1700, found: 330.1689; Anal. calcd for $C_{19}H_{23}NO_4$: C 69.28, H 7.04, N 4.25, found: C 69.1, H 7.33, N 4.56.

6-Methoxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (20c): Method as for **17a** using compound **18a** (300 mg, 1.5 mmol), 3,4,5-trimethoxybenzyl chloride (325 mg, 1.5 mmol) and Et_3N (0.5 mL, 3.6 mmol) in EtOH (5 mL) at 120 °C for 1 h in the microwave oven. Flash column chromatography (hexane→hexane/EtOAc 1:1) afforded compound **20c** as a white solid (81 mg, 16%): mp: 102–103 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.69 (2H, t, *J* = 7.8 Hz), 2.87 (2H, t, *J* = 7.8 Hz), 3.58 (2H, s), 3.59 (2H, s), 3.77 (3H, s), 3.84 (3H, s), 3.85 (6H, s), 6.62–6.71 (4H, m), 6.92 ppm (1H, d, *J* = 8.2 Hz); ^{13}C NMR (67.5 MHz, [D$_6$]DMSO): δ = 15.5, 28.5, 50.5, 55.4, 56.0, 63.0, 68.9, 106.3, 113.7, 115.1, 125.4, 127.5, 132.8, 135.0, 135.9, 153.3, 154.9 ppm; LRMS (ES+): *m/z* 344.4 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{20}H_{26}NO_4^+$: 344.1856, found: 344.1842.

6-Sulfamoyloxy-2-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (20d): Method as for **12e** using compound **20b** (100 mg, 0.3 mmol) and sulfamoyl chloride (0.6 mmol) in DMA (1.0 mL) at RT for 24 h. Flash column chromatography (hexane/EtOAc 3:1→EtOAc) afforded compound **20d** as a white powder (110 mg, 88%): mp: 173–174 °C; ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 2.65 (2H, t, *J* = 5.5 Hz), 2.84 (2H, t, *J* = 5.5 Hz), 3.56 (2H, s), 3.58 (2H, s), 3.64 (3H, s), 3.75 (6H, s), 6.65 (2H, s), 6.98–7.02 (2H, m), 7.12 (1H, d, *J* = 8.0 Hz), 7.91 ppm (2H, br); ^{13}C NMR (67.5 MHz, [D$_6$]DMSO): δ = 28.8, 49.7, 55.1, 55.8, 60.0, 61.8, 105.4, 119.5, 121.8, 127.7, 133.3, 134.2, 135.8, 136.3, 148.3, 152.8 ppm; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_6S^+$: 409.1428, found: 409.1428; Anal. calcd for $C_{19}H_{24}N_2O_6S$: C 55.87, H 5.92, N 6.86, found: C 55.6, H 6.02, N 6.51.

2-(3-Chloro-4,5-dimethoxybenzyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (20e): Method as for **10a** using compound **19a** (203 mg, 0.58 mmol) and $LiAlH_4$ (111 mg, 2.9 mmol) in THF (8 mL) at RT for 18 h. The reaction mixture was cooled to 0 °C, and H_2O (0.15 mL) was added slowly followed by aq NaOH (15%, 0.15 mL) and then H_2O (0.45 mL). The mixture was stirred at RT for 0.25 h, then diluted with EtOAc (100 mL) and stirred another 0.25 h, filtered, dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (hexane→hexane/EtOAc 2:3) afforded compound **20e** as a colourless solid (134 mg, 69%): mp: 155–157 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.69 (4H, s), 3.51 (2H, s), 3.56 (2H, s), 3.73 (3H, s), 3.84 (3H, s), 6.25 (1H, d, *J* = 2.3 Hz), 6.45 (1H, dd, *J* = 8.3, 2.3 Hz), 6.76 (1H, d, *J* = 8.3 Hz), 6.93 ppm (2H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 28.6, 50.5, 55.4, 56.0, 60.6, 62.2, 111.8, 113.6, 115.1, 122.5, 125.5, 127.6, 127.7, 134.1, 135.1, 144.4, 153.8, 154.6 ppm; LC-MS (ES+): *m/z* 333.9 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}ClNO_3^+$: 334.1204, found: 334.1197; Anal. calcd for $C_{18}H_{20}ClNO_3$: C 64.77, H 6.04, N 4.20, found: C 64.7, H 6.03, N 4.19.

2-(3-Bromo-4,5-dimethoxybenzyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (20f): Method as for **10b** using compound **18b** (200 mg, 0.87 mmol), 3-bromo-4,5-dimethoxybenzyl bromide[15] (296 mg, 0.96 mmol), DIPEA (790 mg, 6.1 mmol) in DMF (5.0 mL) at 80 °C for 60 h. Flash column chromatography (hexane→hexane/EtOAc 2:3) afforded compound **20f** as a white solid (246 mg, 85%): mp: 161–165 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.68–2.72 (4H, m), 3.52 (2H, s), 3.56 (2H, s), 3.76 (3H, s), 3.83 (3H, s), 6.33 (1H, d, *J* = 2.2 Hz), 6.49 (1H, dd, *J* = 8.1, 2.2 Hz), 6.78 (1H, d, *J* = 8.1 Hz), 6.96 (1H, d, *J* = 1.5 Hz), 7.09 ppm (1H, d, *J* = 1.5 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 28.7, 50.5, 55.4, 56.0, 60.6, 62.1, 112.4, 113.5, 115.0, 117.2, 125.2, 125.8, 127.6, 135.0, 135.2, 145.5, 153.7, 154.5 ppm; LC-MS (ES+): *m/z* 377.7 $[M+H]^+$; HRMS (ES+): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}BrNO_3^+$: 378.0699, found: 378.0685; Anal. calcd for $C_{18}H_{20}BrNO_3$: C 57.15, H 5.33, N 3.70, found: C 57.0, H 5.39, N 3.61.

2-(3,5-Dimethoxy-4-ethoxybenzyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (20g): Method as for **20e** using compound **19b** (280 mg, 0.78 mmol) and $LiAlH_4$ (149 mg, 3.9 mmol) in THF (8 mL) at RT for 18 h. Flash column chromatography (hexane→EtOAc) afforded compound **20g** as a colourless solid (179 mg, 67%): mp: 154–157 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.33 (3H, t, *J* = 7.2 Hz), 2.68 (4H, s), 3.52 (2H, s), 3.59 (2H, s), 3.75 (6H, s), 4.02 (2H, q, *J* = 7.2 Hz), 6.23 (1H, d, *J* = 1.9 Hz), 6.44 (1H, dd, *J* = 8.3, 1.9 Hz), 6.61

(2H, s), 6.74 ppm (1H, d, $J=8.3$ Hz); LC-MS (ES+): m/z 344.0 $[M+H]^+$; HRMS (ES+): m/z calcd for $C_{20}H_{26}NO_4^+$: 344.1856, found: 344.1842; Anal. calcd for $C_{20}H_{25}NO_4$: C 69.95, H 7.34, N 4.08, found: C 69.60, H 7.35, N 4.00.

6-Hydroxy-2-(3,4,5-triethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (20h): Method as for **20e** using compound **19c** (258 mg, 0.67 mmol) and $LiAlH_4$ (127 mg, 3.4 mmol) in THF (8 mL) at RT for 18 h. Flash column chromatography (hexane→hexane/EtOAc 1:4) afforded compound **20h** as a white solid (75 mg, 38%): mp: 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.34$ (3H, t, $J=7.2$ Hz), 1.35 (6H, t, $J=6.8$ Hz), 2.68 (4H, s), 3.52 (2H, s), 3.57 (2H, s), 3.97 (4H, q, $J=6.8$ Hz), 4.04 (2H, q, $J=7.2$ Hz), 6.27 (1H, d, $J=2.4$ Hz), 6.45 (1H, dd, $J=8.4$, 2.4 Hz), 6.59 (2H, s), 6.75 ppm (1H, d, $J=8.4$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=14.9$, 15.6, 28.6, 50.5, 55.4, 63.0, 64.5, 68.8, 107.7, 113.6, 115.1, 125.5, 127.5, 132.6, 135.1, 136.8, 152.7, 154.7 ppm; LC-MS (ES+): m/z 372.4 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{22}H_{30}NO_4^+$: 372.2169, found: 372.2156; Anal. calcd. for $C_{22}H_{29}NO_4$: C 71.13, H 7.87, N 3.77, found: C 70.9, H 7.87, N 3.77.

6-Hydroxy-2-(3,4,5-trimethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline (20i): Compound **18b** (200 mg, 0.87 mmol) and 3,4,5-trimethoxybenzoyl chloride (200 mg, 0.87 mmol) were dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. Et_3N (0.36 mL, 2.6 mmol) was added. The reaction mixture was stirred at RT for 18 h, then diluted with CH_2Cl_2, washed with H_2O (10 mL) and brine, dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (hexane→hexane/EtOAc 1:4) afforded compound **20i** as an off-white solid (200 mg, 67%): mp: 175–176 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=2.78$–2.86 (2H, m), 3.62 (1H, s, br), 3.82–3.88 (10H, m), 4.51 (1H, s, br), 4.76 (1H, s, br), 6.60–6.70 ppm (5H, m); LC-MS (ES+): m/z 344.0 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{19}H_{22}NO_5^+$: 344.1492, found: 344.1480.

6-Hydroxy-2-(3,4,5-trimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinoline (20j): Method as for **20e** using compound **19d** (200 mg, 0.56 mmol) and $LiAlH_4$ (106 mg, 2.79 mmol) in THF (8 mL) at RT for 18 h. Flash column chromatography (hexane→EtOAc) afforded compound **20j** as a colourless solid (120 mg, 63%): mp: 180–186 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=2.72$–2.87 (8H, m), 3.64 (2H, s), 3.82 (3H, s), 3.84 (6H, s), 5.02 (1H, s, br), 6.44 (2H, s), 6.54 (1H, d, $J=2.5$ Hz), 6.59 (1H, dd, $J=8.3$, 2.5 Hz), 6.89 ppm (1H, d, $J=8.3$ Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta=28.7$, 34.0, 51.0, 55.4, 56.1, 60.2, 60.8, 105.6, 113.8, 115.2, 125.4, 127.6, 135.1, 135.8, 136.3, 153.1, 154.9 ppm; LC-MS (ES+): m/z 344.3 $[M+H]^+$; HRMS (ES+): m/z $[M+H]^+$ calcd for $C_{20}H_{26}NO_4^+$: 344.1856, found: 344.1840; Anal. calcd for $C_{20}H_{25}NO_4\cdot0.25H_2O$: C 69.04, H 7.39, N 4.03, found: C 69.1, H 7.31, N 3.91.

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