Bioorganic & Medicinal Chemistry 18 (2010) 3910–3924

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological activity of *N*-aroyl-tetrahydro-γ-carbolines

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ARTICLE INFO

Article history:
Received 31 December 2009
Revised 13 April 2010
Accepted 14 April 2010
Available online 18 April 2010

Keywords:
5-LOX/COXs dual inhibitors
Cytotoxicity
Tetrahydro-γ-carbolines
2D NMR

ABSTRACT

Research on dual inhibitors of both 5-LOX and COXs gained interest due to the overexpressions of these enzymes during the malignant state of the evolution of prostate cancer. In order to take part in this research, new *N*-aroyl-tetrahydro-γ-carbolines issued from the modification of Indomethacin have been synthesised. As for the NSAIDs, the compounds have been tested for their activity against COX_1, COX_2 plus against 5-LOX and against the proliferation of malignant prostate cancer. Interesting cytotoxic activities and selectivities of some tetrahydro-γ-carboline derivatives have been obtained.

1. Introduction

Few chemotherapeutic agents have been tested to treat the hormono-independent state of prostate cancer and the reference treatment at this stage was changed in 2004 to the combination of docetaxel and prednisone (Fig. 1).[1] Moreover, the recent failures of molecules like atrasentan, a pyrrolidine-3-carboxylic acid derivative and an Endothelin A receptor antagonist (Fig. 1), during clinical evaluations led to consider new therapeutic approaches of the disease.[2] As the incidences of the analogous overexpressions of 5-lipoxygenase (5-LOX) and cyclooxygenases (COXs) enzymes were recently associated to the development of the malignancy of prostate cancer,[3–8] anti-inflammatory dual 5-LOX/COXs inhibitors such as the diaryl pyrazole derivatives, Tepoxaline (5-(4-chlorophenyl)-

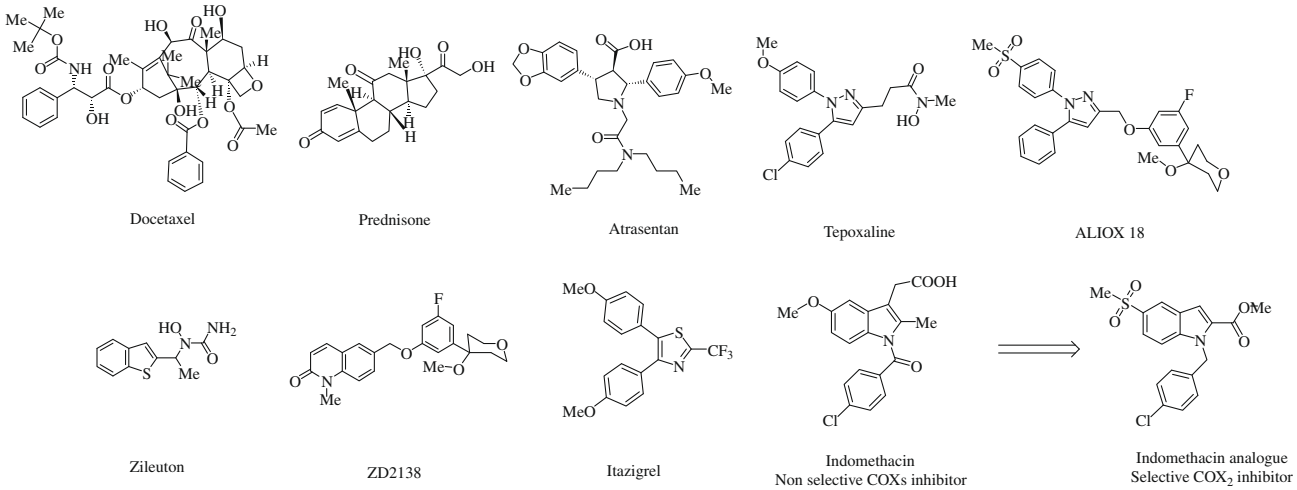

Figure 1. Selected anti-prostate cancer drugs and structures of dual 5-LOX/COXs inhibitors, 5-LOX inhibitors and of COX inhibitors.

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doi:10.1016/j.bmc.2010.04.034

Table 1
Relative potencies of selected molecules in ex vivo HWB assays on 5-LOX, COX_1 and COX_2

Inhibitor	5-LOX IC_{50} (μM)	COX_1 IC_{50} (μM)	COX_2 IC_{50} (μM)	COX_2/COX_1
ALIOX18[36]	0.2	>10	9.7	ND
ZD2138[26]	0.02	—	—	—
Zileuton[26]	0.7	—	—	—
Indomethacin[26]	—	0.2	0.5	2.9
Indomethacin analogue[30]	—	>10	4.3	<0.05

ND = not determinable.

N-hydroxy-(4-methoxyphenyl)-*N*-methyl-1*H*-pyrazole-3-propanamide)[9] and ALIOX18 (3-(3-fluoro-5-(4-methoxytetrahydro-2*H*-pyran-4-yl)phenoxymethyl)-1-[4-(methylsulfonyl)phenyl]-5-phenyl-1*H*-pyrazole),[10] have raised interest as one of these approaches (Fig. 1, Table 1). These were synthesised because inhibiting only a sole biosynthetic pathway has proven to switch the metabolism of arachidonic acid (AA) to the other pathway, thus leading to the overexpression of that one and to potential side effects.[11] In the literature, numerous NSAIDs, the reference in the inhibition of COXs, were also selected to be tested as anti-cancer drugs in clinical tests. These had a potential to inhibit the development of malignant cells.[12] On the other hand, the anti-cancer action of the best inhibitors have not yet been associated in full with COXs' enzymatic activities.[13] For instance, Indomethacin, a greater inhibitor of COX_1 than COX_2, proved to block the stimulation of the growth of very invasive and androgen-independent metastatic cells. The application of prostaglandins on the same cells did not correct the anti-cancer effect.[12,14] Some NSAIDs have also shown to suppress a crosstalk discovered between COX_2 and the sprouting of new vascular vessels mediated by the integrin $\alpha_v\beta_3$, a cell adhesion molecule (CAM), in cancer diseases.[15,16] In order to take share in the research on androgen-independent prostate cancer, new *N*-aroyl-tetrahydro-γ-carbolines intended to inhibit 5-LOX and COXs have been designed based on the template Indomethacin. They have then been synthesised and biologically tested for their activities against each enzyme and against the proliferation of malignant cells.

2. Rational design

At first, interesting structures have been selected from the literature and based on the structure–activity relationships (SARs) criteria found, a new general dual inhibitor template has been generated. In other words, ALIOX18 had previously revealed their potential to inhibit both COX_2 and 5-LOX at low concentrations (Table 1). It has thus seemed interesting to develop and then to test the unforeseen condensation of Indomethacin with a 5-LOX inhibitor. This new dual inhibitor template has been obtained from the condensation of an *N*-aroyl-8-substituted-tetrahydro-γ-carboline with a 5-LOX inhibitor—the methoxytetrahydro-pyran (MTHP), via a polymethylenic spacer (Fig. 2). In order to obtain a good fit of the potential ligands, the length of the linker has needed to be probed. Therefore, the docking of several compounds in comparison to ALIOX18 has been done in the COX_2 active site (Fig. 3, as an example). Using the docking method Gold, linkers bearing between one and three carbon atoms have been found suitable for the hit development phase of the study. In regards to the substituents, those on the R_1 position have been chosen to probe if their stereoelectronic effects (electron donating effect: F, OMe; electron withdrawing effect: SO_2Me, SO_2NH_2) may have generated different activity and selectivity towards COX_2. R_2 has been a suitable site to attach the linker and the 5-LOX inhibitor—the MTHP. On the R_3 position, substituents which were reported in the literature to optimise the selectivity of Indomethacin or the activity of any COX_2 inhibitors (C_2Is) when beard on the lower ring have been selected.[17–20] The 2,4,6-trichlorobenzoyl showed to reverse the selectivity of Indomethacin while halogen atoms and the sulfone derivatives at the *para* position enhanced the activities of the C_2Is. Several synthesis strategies have then been applied to synthesise these new compounds.

2.1. γ-Carbolines as new dual inhibitors

γ-Carbolines, or pyrido[4,3-*b*]indoles, were reported as planar tricyclic azacarbazoles composed of a homocycle, a pyrrole and a pyridine.[21] The reduced forms—dihydro-, tetrahydro- and hexahydro-γ-carbolines were also of potent interest in the past; here, about a better insertion in the COX active sites and due to some flexibility found in the piperidine ring, only the tetrahydro and hexahydro derivatives have been considered. Between the two core structures, only the *N*-aroyl-tetrahydro-γ-carboline model has been chosen because both aromatic rings (indole and benzoyl) formed a dihedral angle of 45° instead of 26° for the hexahydro derivative in the lowest-energy conformations. That arrangement paralleled the geometry of Indomethacin with a dihedral angle of 43° (calculated using the software Chem3D® Pro 5.0).

2.2. Leukotrienes biosynthesis inhibitors

5-LOX inhibitors were classified in three categories that differed in terms of enzyme selectivity and interaction with the iron atom, for example, in regards of their mechanism of action.[22] Tested redox inhibitors showed only weak selectivity for the enzyme, an activity which was rationalised by their ability to interact with other redox biochemical systems. For instance, the hydroxamic acid moiety found on several inhibitors was reported as being also a chelator of the zinc ion in Matrix Metallo Protease (MMP).[23] To the contrary, Zileuton (*N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea, Abbott) displayed the highest binding affinity and selectivity to chelate the iron ion of 5-LOX due to the *N*-hydroxyurea function (Fig. 1, Table 1).[24] The competitive non-re-

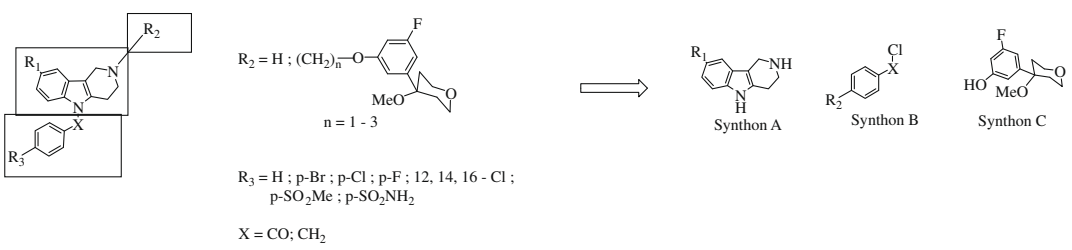


Figure 2. Novel dual 5-LOX/COXs inhibitors.

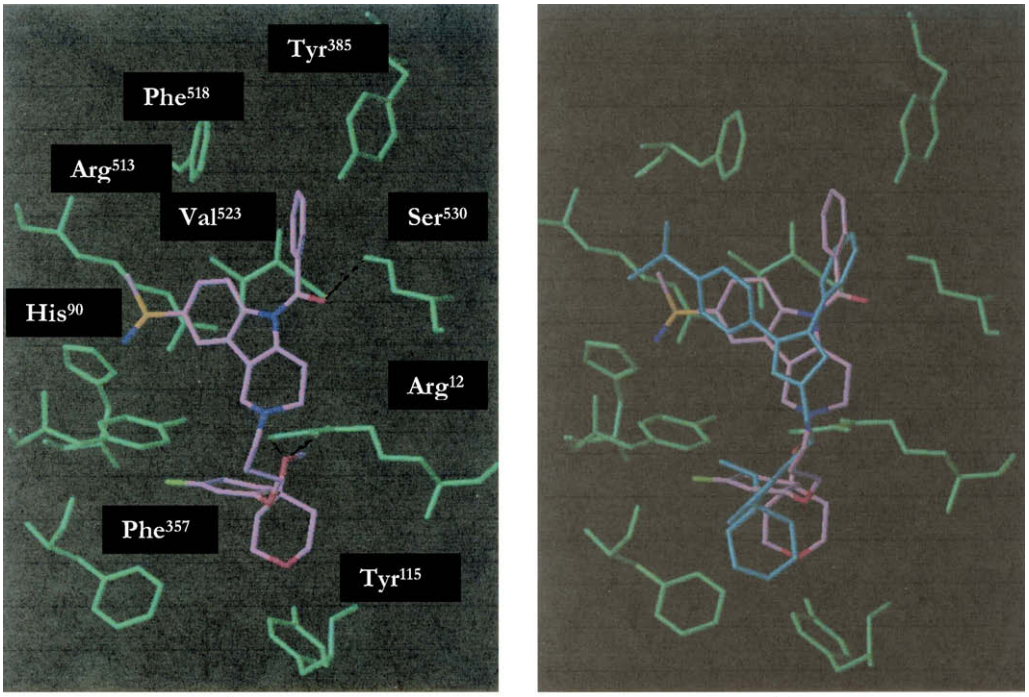


Figure 3. 3D docking. (A) Compound **41** in the COX_2 active site. (B) Superimposition of the compound **41** with ALIOX18.

dox class then arose from a research program which culminated with a lead compound ZD2138 (6-[3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-4*H*-pyran-4-yl)phenoxy]methyl]-1-methylquinol-2-one, Astra Zeneca) (Fig. 1, Table 1). This compound resulted from an optimisation study on an enantioselective methoxyethylthiazole hit compound.[25] In the structure of ZD2138, the methylquinolonyl was only used as a more soluble alternative to a naphthyl moiety and the SAR studies proved that only the achiral MTHP part held the activity. Further optimisation of that lead cleared up the fact that the oxygen on the saturated ring needed to be separated from the fluorophenyl ring of at least two saturated carbon atoms for a good activity and that the halogen on the phenyl ring blocked a potential site of metabolism. Molecular modelling studies also helped to visualise the active form of the MTHP, for example, when the phenyl ring was in the equatorial position.[25] As it was used to obtain one of the best known dual 5-LOX/COX_2 inhibitors,[10] the MTHP has also been the preferred 5-LOX inhibitor in this study.

2.3. Prostaglandin biosynthesis inhibitors

Non-selective COXs inhibitors (NSAIDs)—Aspirin (acetyl salicylic acid, Bayer), Indomethacin, the 'Profen' family, Ketorolac ((±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, Roche) and Diclofenac (2-[2-(2,6-dichlorophenyl)aminophenyl] ethanoic acid, Novartis)[26] were mainly approved for the relief of inflammation and pain. To avoid gastric side effects, research led to the C_2Is from a lead optimisation study on the structure of the diaryl heterocycle Itazigrel (4,5-bis(4-methoxyphenyl)-2-trifluoromethylthiazole, Pfizer) (Fig. 1).[27] These new drugs were introduced on the market to treat acute arthritis symptoms; some even entered some clinical trials against cancer before cardiovascular side effects precluded them from any new trials.[28] These baleful effects have then been rationalised by the fact that C_2Is, as they respect COX_1, broke the equilibrium between prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) in the benefit of the proaggregating and vasoconstrictor TXA_2.[29] This trial unfortunately shaded the development of the selective dual 5-LOX/COX_2 inhibitors based on the structure of the diaryl heterocycles. Meanwhile, a novel C_2I evolved from Indomethacin (Fig. 1, Table 1) was reported.[30] The selectivity for COX_2 emerged because the substituent on the aromatic ring was bulky enough to prevent the entry of the compound in the COX_1 active site. The relative safe profile of the drug thus allowed considering with ease the modification of the *N*-benzyl or *N*-benzoyl-5-indole framework of these compounds by ring closure into a semi-rigid analogue—a *N*-aroyl-tetrahydro-γ-carboline.

3. Results and discussion

A synthesis strategy of the potential dual inhibitors has been developed starting from 4-substituted-phenylhydrazine hydrochlorides and 4-piperidine derivatives which were cyclised in tetrahydro-γ-carbolines by acid catalysis.[31] Then a NMR study has helped to check the stereochemistry and the conformation of the synthesised compounds in organic solvents.

3.1. Synthesis strategy

The strategy has relied on the condensation of three synthons: an 8-substituted-tetrahydro-γ-carboline (synthon A); a *para* substituted aroyl chloride (synthon B) and the MTHP framework (synthon C) (Fig. 2). The MTHP has been synthesised by a reaction sequence adapted from a published procedure.[32] The synthesis of 8-substituted-tetrahydro-γ-carbolines has called out an exothermic aza-Cope [3,3] sigmatropic reaction as an intermediate step in the cyclisation process.[31] The compounds that have been obtained with the highest yield have then served as building blocks in the first pursued strategy. In this linear strategy, the target compounds have easily appeared accessible when looking at the condensation of a N-alkylated tetrahydro-γ-carboline intermediate by the spacer chain with the phenol (synthon C). Despite some intermediate molecules have successfully resulted from the con-

densation of the protected synthon A with the synthon B, this strategy has revealed a weakness: an unforeseen debenzylation. This self dissociation was reported on the structure of Indomethacin only in aqueous condition; here, an inorganic material has been enough to remove the benzoyl group in harsh organic conditions.[33] The consideration of a new approach has led to a convergent, more efficient and economic strategy which has used the difference in basicity of the indole amine (pK_a = 21.0) and the piperidine amine (pK_a = 11.0) on the tetrahydro-γ-carboline framework to selectively alkylate the piperidine amine. This one has revealed to be successful.

3.2. Synthesis

Fischer indole synthesis has been applied to prepare 8-substituted-tetrahydro-γ-carbolines **1–5** by reacting equimolar quantities of substituted arylhydrazines with *N*-protected-4-piperidinones or 4-piperidinone hydrochloride, as reported.[31] Then, starting from compounds **1** and **4**, compounds **13** and **14** have been obtained by the method listed in Figure 4. This method has allowed synthesising N-benzoylated analogues (compounds **6–10**) in yields ranging in between 30% and 67% depending on the stereoelectronic nature of the substituent on the tetrahydro-γ-carboline; compounds, obtained as oils, have been converted to the corresponding hydrochlorides. The benzoylated compounds have then been reacted under catalytic hydrogenation conditions. Compounds **7**, **8** and **10** have not reacted under such conditions whereas compounds **11** and **12** have been obtained quantitatively. Nevertheless, compound **10** has alternatively been debenzylated following the method using vinyl chloroformate (VOC-Cl); via a peculiar SN_1 substitution of the benzyl group.[34] Further decarbamoylation of the vinylcarbamate intermediate, which existence has been monitored by the full analysis of analytical samples, has occurred using HCl gas in MeOH at reflux in 70% yield (Fig. 4). Key to the following convergent strategy has been the optimisation of the phenol alkylation step which has clearly justified 1-bromo-3-chloropropane as the best alkylating agent for an efficient synthesis (Fig. 5). The selective alkylation of the amine has resulted in intermediate molecules (compounds **26**–**29**). Those have been aroylated and alkylated to get the desired compounds **30–41**. Interestingly, alkylation of compound **25** using an excess of compound **19** and base has resulted in a surprising double alkylation. Compound **42** has thus been obtained in addition to compound **29** and in the same yield (Fig. 5) after purification by silica gel chromatography. Compound **38** has been understood as the result of the in situ reaction of the aminosulfone group on the benzoyl with a molecule of dimethylformamide (DMF). Deprotection of the dimethylaminomethylene group has been tried by refluxing the compound in an aqueous HCl (0.5 N) solution in order to keep the unstable MeO (methoxy) moiety on the MTHP but has not been successful.[35]

3.3. Structural characterisation

From the beginning, the carbonyl of the *N*-benzoyltetrahydro-γ-carboline structure has been hypothesised to lie between the two protons of the β-carbon of the piperidine and the aromatic ring to position itself perpendicularly to the indole ring. A fine 2D NMR study combined to molecular modelling has helped to determine their geometry and their true orientation in deuterated solvent conditions. On the obtained spectrum after the NMR analysis, an Overhauser correlation peak has been assigned between the protons of the benzoyl ($H_8 + H_{11}$) and the homocycle (H_4) (Fig. 6). Furthermore, a clear shielding effect of the homocycle protons has been assigned on the ^1H NMR spectra of all compounds after benzoylation. Together with the detected high field shift of the protons on the α- and β-carbons of the piperidine ring, the conclusion has been made that the *N*-benzoyltetrahydro-γ-carboline structure had a twisted structure with a benzoyl that drifted to be perpendicular to the homocycle plane to optimise the π–H contacts (Fig. 6). The compounds had thus the same conformation as Indomethacin when tested in such conditions.

3.4. Biological evaluation

Ex vivo and in vitro cytotoxic potencies of the novel compounds **30–42** have been reported in Tables 2 and 3. These tests have been done to evaluate the hybrid *N*-aroyl-tetrahydro-γ-carboline molecules as dual 5-LOX/COXs inhibitors and as cytotoxic compounds. The tetrahydro-γ-carbolines **11**, **12** and **16** have been assayed as negative indicators.

The screening of dual inhibitors was relatively easy in vitro by using classical enzyme models and the purified or recombinant proteins or using other techniques to quantify the resulting eicosanoid products. However, these methods did not reflect the situation in the whole organism where the COX/LOX systems mutually interact and the end result as a balance between both metabolic pathways. Thus a technique using a human whole blood assay has been considered a more appropriate screening system.[36]

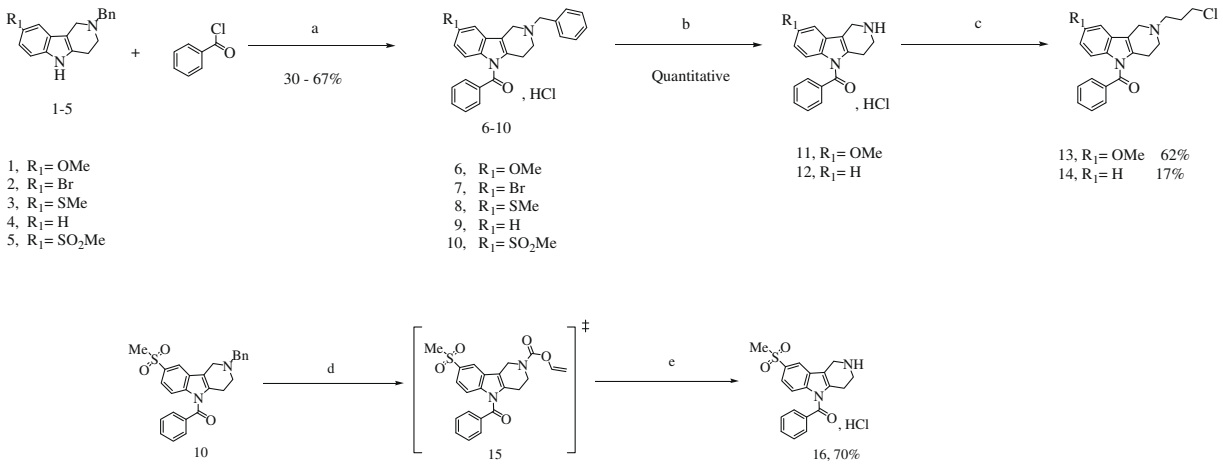


Figure 4. Linear synthesis and debenzylation using the VOC method. Reagents and conditions: (a) (1) NaH 60% (1.0 equiv), DMF, 2 h, 0 °C, N_2; (2) benzoyl chloride (1.0 equiv), 2 h, rt, 30–67%; (b) Pd/C 10%, H_2, solvent, atm P., 48 h, rt, quantitative; (c) (1) DIEA (2.0 equiv), DCM, 30 min, rt; (2) 1-bromo-3-chloropropane (1.0 equiv), DCM, 24 h, rt; (d) VOC-Cl (1.0 equiv), DCM, 1 h, reflux, N_2; (e) (1) HCl, DCM, rt; (2) MeOH, 2 h, reflux, N_2, 70%.

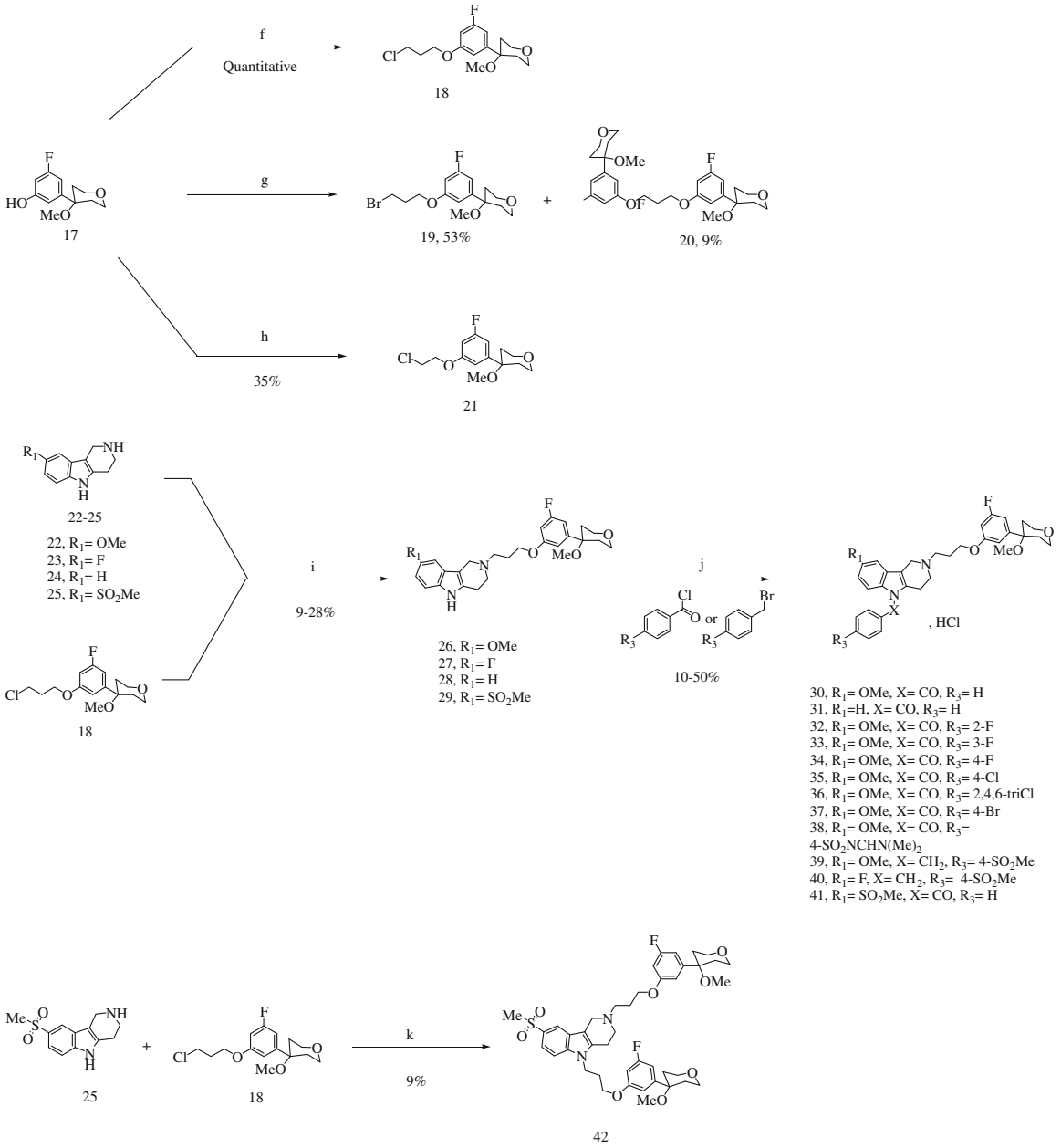


Figure 5. Convergent synthesis and synthesis of compound **42**. Reagents and conditions: (f) 1-bromo-3-chloropropane (1.0 equiv), Cs_2CO_3 (1.5 equiv), DMF, 2 h, rt, N_2, quantitative; (g) 1,3-dibromopropane (1.0 equiv), Cs_2CO_3 (3.0 equiv), DMF, 17 h, rt, N_2; (h) 1-bromo-2-chloropropane (2.0 equiv), Cs_2CO_3 (2.0 equiv), acetone, 24 h, reflux, N_2, 35%; (i) compound **18** (1.0 equiv), Cs_2CO_3 (2.0 equiv), acetone, 24 h, reflux, N_2, 9–28%; (j) (1) NaH 60% (1.0 equiv), DMF, 2 h, 0 °C, N_2; (2) benzoyl chloride or benzyl bromide derivative (1.0 equiv), 2 h, rt, N_2; (3) *i*PrOH–HCl (5–6 M) (1.0 equiv), 10–50%; (k) compound **18** (2.6 equiv), Cs_2CO_3 (2.6 equiv), acetone, 24 h, reflux, N_2, 9%.

3.4.1. Dual inhibitor activity

The dual inhibitory effects have been measured ex vivo on 5-LOX and COXs on heparinised human whole blood (HWB). Heparin has been used to prevent coagulation. Also, a simplified extraction procedure and a modified activation procedure have been used in an attempt to differentiate between COX_1 and COX_2 activities. Then a simple HPLC method has been operated in order to evaluate the selectivity of the synthesised compounds in a more representative manner than the human whole cell assay (Table 2). Leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE) have been used as indicators of 5-LOX activity. Similarly, measuring the production of 12-hydroxyheptadecatrienoic acid (12-HHTr) and of 12-HHTr* has provided information on the activities of COX_1 and COX_2, respectively. The longer incubation time and additional stimulation by lipopolysaccharide (LPS) gave information on the extent of induction of COX_2 and permitted the determination of an apparent COX_2/COX_1 selectivity for the compounds studied.

The activities of Indomethacin ($IC_{50}(COX_1) = 0.3\ \mu M$; $IC_{50}(COX_2) = 0.5\ \mu M$) and Zileuton ($IC_{50}(5\text{-LOX}) = 0.8\ \mu M$) have ranged in the same order as the ones described in the literature whereas Celecoxib ($IC_{50}(COX_1) = 13.0\ \mu M$; $IC_{50}(COX_2) = 0.8\ \mu M$) had a weaker activity.[24,26] At first glimpse, the first results justified the importance of the MTHP for a balanced activity. The compound **34** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = 4-F; IC_{50}(5-LOX) = 60.0 μM; % of inhibition of COX_1 at 10 μM = 71.6%; $IC_{50}(COX_2) = 54.0\ \mu M$) has revealed the best balanced activity. On the whole, these compounds had an activity with IC_{50} values a hundred times higher than the ones of the reference compounds and were only COX_1 selective. The activities on 5-LOX were higher than expected and varied greatly from one compound to another. The compound **35** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = 4-Cl;

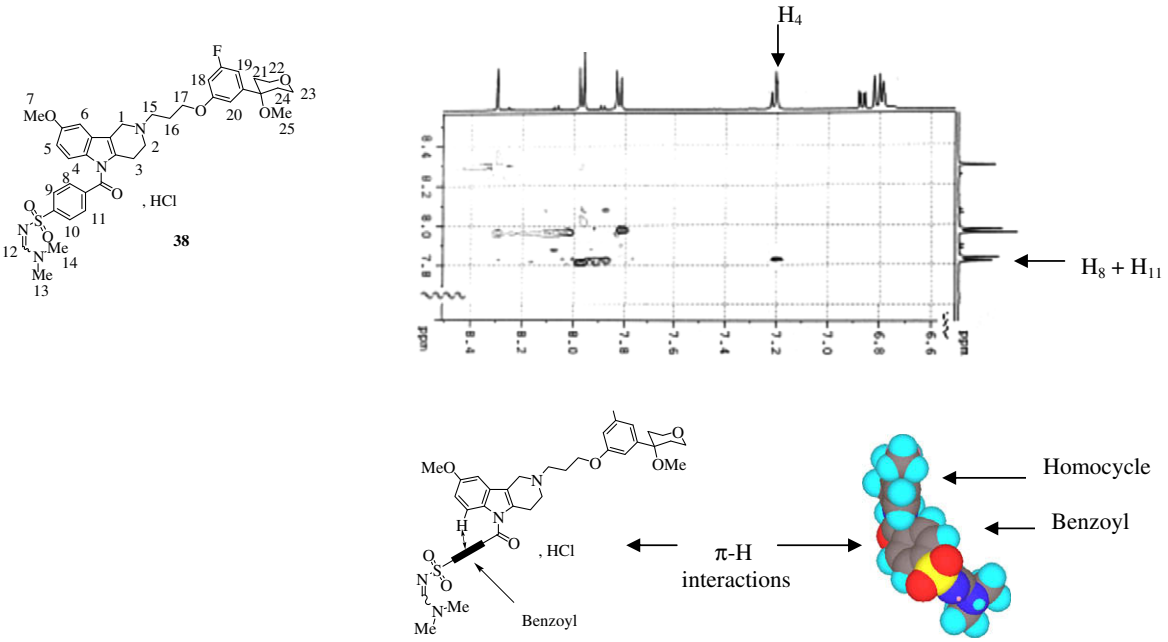


Figure 6. 2D NMR (ROESY) study of compound **38**; 2D and space conformation (rear view) of compound **38**.

IC_{50}(5-LOX) = 119.0 μM; $IC_{50}(COX_1)$ = 7.0 μM; $IC_{50}(COX_2)$ = 198.0 μM), closely related to Indomethacin, had the best activity against COX_1 of the series. Nevertheless, this activity remained 10 times higher than the one of the reference compound. Overall, the selectivity indexes (S) have shown the deterioration of the selectivity. On the contrary of what has been stated in the literature, a bulky trichlorobenzoyl, compound **36** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = 2,4,6-triCl; IC_{50}(5-LOX) = 55.0 μM; $IC_{50}(COX_1)$ = 37.0 μM; $IC_{50}(COX_2)$ = 100.0 μM; S = 2.7), has not reversed the selectivity between the COXs. The $MeSO_2$ group either on the γ-carboline ring, compound **41** ($R_1 = SO_2Me$, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = H; $IC_{50}(COX_1)$ = 24.0 μM; % of inhibition of COX_2 at 10 μM = 8.4%), have not induced a large selectivity towards COX_2.

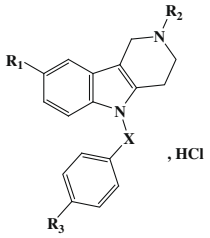
3.4.2. Cytotoxic activity

In parallel, the inhibition of cellular proliferation has been evaluated against prostate adenocarcinoma cell line (PC-3), an androgen-independent human cell line which originated from a bone metastasis and which was considered as very invasive. Other tests have been performed against murine leukaemia cell line (L-1210) and against human androgen-dependent breast carcinoma cell line (MCF-7) for comparison purposes. The MTT assay has monitored the change of MTT (3-(4,5-dimethyl-1,3-thiazolidin-2-yl)-2,5-diphenyl-2*H*-tetrazol-3-ium bromide) to formazan (4,5-dimethyl-2-{(*E*)-[(*Z*)-phenyl(phenylhydrazono)methyl]diazenyl}-1,3-thiazole) in viable cells; thus, the less formazan measured by a spectrophotometer the greatest the effectiveness of the compound in causing death. Compared to Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide, Pfizer), the *N*-benzoyl-tetrahydro-γ-carbolines (compounds **11** and **16**) have appeared to be deprived of activity; nevertheless, some of these compounds bearing the MTHP and substituted on the R_1 and R_3 positions have shown interesting activities. Compound **35** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = 4-Cl; IC_{50} (L-1210) = 8.0 μM and IC_{50} (MCF-7) = 8.0 μM), compound **38** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, $R_3 = 4\text{-}SO_2NCHN(Me)_2$; IC_{50} (L-1210) = 6.0 μM), compound **39** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CH_2, $R_3 = 4\text{-}SO_2Me$; IC_{50} (L-1210) = 5.0 μM and IC_{50} (PC-3) = 8.0 μM), compound **40** (R_1 = F, $R_2 = (CH_2)_3$MTHP, X = CH_2, $R_3 = 4\text{-}SO_2Me$; IC_{50} (L-1210) = 5.0 μM and IC_{50} (PC-3) = 8.0 μM) and compound **42** ($R_1 = SO_2Me$, $R_2 = (CH_2)_3$MTHP, X = $(CH_2)_3$MTHP, no benzoyl); IC_{50} (L-1210) = 3.0 μM and IC_{50} (PC-3) = 6.0 μM have shown better potencies in comparison to Celecoxib (IC_{50} (L-1210) = 44.0 μM; IC_{50} (MCF-7) = 50.0 μM and IC_{50} (PC-3) = 48.0 μM) (Table 3). Among the most active compounds, none of those have exhibited cytotoxicity toward the hormono-dependent breast cancer (MCF-7) cell lines. Compound **35** (R_1 = OMe, $R_2 = (CH_2)_3$MTHP, X = CO, R_3 = 4-Cl; IC_{50} (L-1210) = 8.0 μM and IC_{50} (MCF-7) = 8.0 μM), the closest in structure to Indomethacin, has proven to be active against L-1210 and MCF-7 cells but not against hormono-independent cells. Nonetheless, the $MeSO_2$ has been identified as a privileged group on the R_3 position from the activities displayed in Table 3 against prostate cancer cell lines. On the whole, the most noteworthy events were that the most Indomethacin-like compound (compound **35**) induced cytotoxicity on the androgen-dependent cell line and modulating its structure with $R_3 = 4\text{-}SO_2Me$ (compound **39**) shifted the selectivity toward the androgen-independent cell line.

4. Conclusion

New therapeutic goals were set towards the inhibition of the proliferation of prostate cancer. To reach these goals, a new concept has been applied in enzymatic and cancer cell assays. It consisted in the modification of Indomethacin (a COX inhibitor and a potential anti-cancer agent) in tetrahydro-γ-carbolines and their condensation with a known 5-LOX inhibitor. These novel compounds have been synthesised by a simple four-step synthesis. Unfortunately, maybe because of the size of the linker, the compounds had a low dual 5-LOX/COX inhibitory profile in the HWB assay. This has precluded most of them from carrying interesting antiproliferative activities against androgen-independent prostate adenocarcinoma, androgen-dependent cancer and leukaemia cell lines. More, as what was observed for Indomethacin, some tetrahydro-γ-carbolines remained active against very invasive androgen-independent cell lines without any found relationship with the results on the HWB tests. The activity on cancer cell lines depends upon multifactorial mechanisms and it is obvious that 5-LOX/COX enzymes inhibition is only one (but important) effect. Nevertheless, it seems that the strong/weak activity on enzyme has been correlated with the activity ratio on cancer cell lines. For those tet-

Table 2
Relative potencies of compounds **11**, **16**, **30–42** in the ex vivo HWB assay



Molecules	Structures	IC50 (μM) and *(mean % of inhibition at 10.0 μM)*				Selectivity
		LTB4	5-HETE	12-HHTr	12-HHTr*	
Zileuton	—	0.7 ± 4.5	0.8 ± 11.2	ND	ND	ND
Indomethacin (NS)	—	ND	ND	0.3 ± 0.0	0.5 ± 0.0	0.55
Celecoxib (S)	—	ND	ND	13.1 ± 8.2	0.8 ± 2.7	0.06
	R_2 = H					
11	R_1 = OMe; X = CO; R_3 = H	ND	ND	6.0 ± 2.1	87.6 ± 13.7	>10
16	R_1 = SO_2Me; X = CO; R_3 = H R_2 = $(CH_2)_3$MTHP	ND	ND	24.4 ± 17.9	ND	ND
30	R_1 = OMe; X = CO; R_3 = H	66.4 ± 2.0	107.5 ± 20.7	(*84.3%*)	78.3 ± 6.7	>7.8
31	R_1 = H; X = CO; R_3 = H	92.9 ± 9.4	205.9 ± 155.7	18.2 ± 5.6	101.9 ± 9.8	5.6
32	R_1 = OMe; X = CO; R_3 = 2-F	39.3 ± 4.7	>10	(*83.5%*)	126.0 ± 34.6	>10.0
33	R_1 = OMe; X = CO; R_3 = 3-F	38.0 ± 7.1	40.5 ± 15.3	7.8 ± 2.3	357.2 ± 24.8	>10.0
34	R_1 = OMe; X = CO; R_3 = 4-F	58.0 ± 4.3	59.9 ± 1.3	(*71.6%*)	53.6 ± 7.4	>5.4
35	R_1 = OMe; X = CO; R_3 = 4-Cl	59.3 ± 0.7	118.6 ± 23.1	7.4 ± 8.0	197.9 ± 29.0	>10.0
36	R_1 = OMe; X = CO; R_3 = 2,4,6-triCl	45.4 ± 1.6	55.0 ± 0.0	36.7 ± 2.2	100.0 ± 0.0	2.7
37	R_1 = OMe; X = CO; R_3 = 4-Br	47.4 ± 1.2	62.0 ± 2.9	16.5 ± 6.1	85.4 ± 9.1	5.2
38	R_1 = OMe; X = CO; R_3 = 4-$SO_2NCHN(Me)_2$	ND	(*81.4%*)	ND	ND	ND
39	R_1 = OMe; X = CH_2; R_3 = 4-SO_2Me	76.6 ± 45.3	109.7 ± 82.5	ND	109.2 ± 0.0	ND
40	R_1 = F; X = CH_2; R_3 = 4-SO_2Me	29.5 ± 0.0	24.6 ± 0.2	180.3 ± 53.7	ND	ND
41	R_1 = SO_2Me; X = CO; R_3 = H	ND	ND	24.0 ± 14.0	(*8.4%*)	ND
42	R_1 = SO_2Me; no benzoyl; X = $(CH_2)_3$MTHP	ND	ND	ND	ND	ND

S = selective.
NS = non-selective.
ND = not determinable.

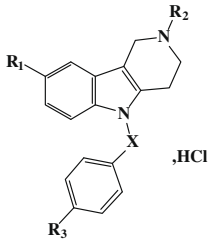
rahydro-γ-carbolines which could have been linked to the NSAIDs' anti-cancer activities: perhaps they expressed their activities because they were very close in structure to Indomethacin. These active tetrahydro-γ-carbolines, which have been 10 times more potent than the reference drug, have deserved to get improved. The synthesis of the 8-methylsulfonyl tetrahydro-γ-carboline bearing two MTHP groups (compound **42**, IC_{50} (PC-3) = 6.0 μM, IC_{50} (L-1210) = 3.0 μM) have unexpectedly led to the most promising cytotoxic tetrahydro-γ-carboline. Therefore, the $MeSO_2$ group has been selected as a privileged functional group to keep in the hit-to-lead development phase of this research study.

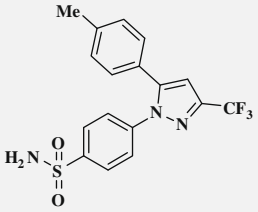
5. Experimental

5.1. Chemistry

Unless otherwise stated, moisture-sensitive reactions were stirred under a dry nitrogen atmosphere. Thin-layer chromatography (TLC) was performed on precoated Kieselgel $60F_{254}$ plates (Merck); compounds were visualised by UV and/or with iodine or ninhydrin. Flash chromatography (FC) and column chromatography were performed with silica gel Kieselgel Si 60, 0.040–0.063 mm (230–400 mesh ASTM) (Merck) and Geduran Si 60, 0.063–0.200 mm (70–230 mesh ASTM) (Merck). Melting points were determined on a Büchi 535 capillary melting point apparatus and remain uncorrected. The structures of all compounds were supported by IR spectra on Brucker Vector 22 instrument and ^{1}H NMR at 300 MHz on a Brucker AC300P spectrometer or a Brucker DPX 300 AVANCE spectrometer. Chemical shifts (δ) were reported as δ (ppm) relative to trimethylsilane (TMS) as internal standard, *J* in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; ds, doublet of singlet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet; br, broad. Atmospheric pressure chemical ionisation (APCI) mass spectra were obtained on a liquid chromatography coupled to mass spectrometry (LC–MS) system Thermo Electron Surveyor MSQ. The purity of some compounds was characterised

Table 3
Relative potencies of compounds **11**, **16**, **30**–**42** in the in vitro cytotoxic assay



Molecules	Structures	IC_{50} (μM) and *(mean % of inhibition at 10.0 μM)*		
		L-1210	MCF-7	PC-3
Celecoxib		44.0	50.2	48.5
11	R_2 = H R_1 = OMe; X = CO; R_3 = H	(*32%*)	(*0%*)	(*0%*)
16	R_1 = SO_2Me; X = CO; R_3 = H R_2 = $(CH_2)_3$MTHP	(*0%*)	(*0%*)	(*7%*)
30	R_1 = OMe; X = CO; R_3 = H	(*20%*)	(*18%*)	(*20%*)
31	R_1 = H; X = CO; R_3 = H	(*24%*)	(*30%*)	(*6%*)
32	R_1 = OMe; X = CO; R_3 = 2-F	(*45%*)	(*21%*)	(*15%*)
33	R_1 = OMe; X = CO; R_3 = 3-F	(*40%*)	(*8%*)	(*44%*)
34	R_1 = OMe; X = CO; R_3 = 4-F	(*44%*)	(*37%*)	(*0%*)
35	R_1 = OMe; X = CO; R_3 = 4-Cl	8.0	8.0	(*9%*)
36	R_1 = OMe; X = CO; R_3 = 2,4,6-triCl	(*11%*)	(*36%*)	(*0%*)
37	R_1 = OMe; X = CO; R_3 = 4-Br	(*42%*)	(*39%*)	(*0%*)
38	R_1 = OMe; X = CO; R_3 = 4-$SO_2NCHN(Me)_2$	6.0	(*21%*)	(*42%*)
39	R_1 = OMe; X = CH_2; R_3 = 4-SO_2Me	5.0	(*27%*)	8.0
40	R_1 = F; X = CH_2; R_3 = 4-SO_2Me	(*38%*)	(*34%*)	8.0
41	R_1 = SO_2Me; X = CO; R_3 = H	(*38%*)	(*15%*)	(*37%*)
42	R_1 = SO_2Me; no benzoyl; X = $(CH_2)_3$MTHP	3.0	(*36%*)	6.0

by their retention time (rt.) by high-performance liquid chromatography (HPLC) on a Kontron 325 System apparatus equipped with a UV detector DAD 440L, in isocratic mode. Elemental analyses were determined by the Service Central d'Analyses (CNRS, Vernaison, France) and all analyses were within ±0.4% of theory. 5-HETE, LTB_4, 12-HHTr, LPS B4 from *Escherichia* coli and calcium ionophore (A23187) were purchased from Sigma. The tetrahydrofuran (THF) was dried and distilled over Na and benzophenone. EtOH and MeOH were dried over molecular sieves 3.0 Å. Acetone, acetonitrile (MeCN), DCM and dioxane were previously dried over potassium carbonate (K_2CO_3) then over calcium chloride ($CaCl_2$). All the organic solutions were dried over $MgSO_4$.

5.2. Syntheses of compounds 17–21

5.2.1. 3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenol (17)

The title compound was prepared as described by Pommery et al.[27]

5.2.2. 4-[3-(3-Chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (18)

A solution of 1-bromo-3-chloropropane (2.5 mL, 25.2 mmol, 1.0 equiv) diluted in DMF (5 mL) was added dropwise to a mixture of 3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenol (**17**)

(3.8 g, 16.8 mmol, 1.0 equiv) dissolved in DMF (30 mL) and Cs_2CO_3 (8.3 g, 25.2 mmol, 1.5 equiv) at rt. After stirring for 17 h, the reaction was quenched by adding H_2O (100 mL) and the aqueous solution was stirred during 1 h and then extracted with DCM (3 × 100 mL). The organic layer was filtered and evaporated under vacuum. Yield 92%; mp 73 °C (from Et_2O); colourless crystals; TLC R_f 0.68 (DCM/AcOEt, 90/10); IR cm^{-1}: 1139, 1105, 1073; ^1H NMR ($CDCl_3$) δ: 1.96 (m, 4H, $2CH_2$), 2.24 (m, 2H, CH_2), 3.00 (s, 3H, CH_3), 3.78 (m, 6H, $3CH_2$), 4.11 (t, J = 6.0 Hz, 2H, CH_2), 6.55 (dd, J = 10.0 Hz, J = 2.0 Hz, 1H, ArH), 6.71 (m, 1H, ArH), 6.76 (m, 1H, ArH); MS (+APCI) m/z 271 (5.0) $[M+H-MeOH]^+$ (calcd for $C_{14}H_{17}ClFO_2$ 271.7 $[M+H-MeOH]^+$).

5.2.3. 4-[3-(3-Bromopropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (19) and 1,3-di-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propane (20)

A solution of 3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenol (**17**) (3.3 g, 14.4 mmol, 1.0 equiv) dissolved in DMF (25 mL) was added dropwise to a mixture of 1,3-dibromopropane (1.6 mL, 14.4 mmol, 1.0 equiv) diluted in DMF (25 mL) and Cs_2CO_3 (14.1 g, 43.2 mmol, 3.0 equiv) at rt. After stirring for 24 h, the reaction was quenched by adding H_2O (100 mL), and extracted with AcOEt (3 × 100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under vacuum. The residue was purified by FC over silica gel using chromatography solvent (cyclohexane/AcOEt, 90/10) to give a solution which was filtered and concentrated in vacuo.

Compound **19**. Yield 53%; colourless oil; TLC R_f 0.80 (DCM/AcOEt, 50/50); IR cm^{-1}: 1138, 1105, 1073; ^1H NMR ($CDCl_3$) δ: 1.86 (m, 4H, $2CH_2$), 2.26 (m, 2H, CH_2), 2.94 (s, 3H, CH_3), 3.54 (t, J = 6.0 Hz, 2H, CH_2), 3.75 (m, 4H, $2CH_2$), 4.04 (t, J = 6.0 Hz, 1H, ArH), 6.50 (d, J = 10.0 Hz, 1H, ArH), 6.68 (d, J = 10.0 Hz, 1H, ArH), 6.70 (br, 1H, ArH); MS (+APCI) m/z 315 (5.0) [M−MeOH], 317 (5.0) [M+2MeOH] (calcd for $C_{14}H_{16}BrFO_2$ 315.1 [M−MeOH]).

Compound **20**. Yield 7%; yellow oil; TLC R_f 0.63 (DCM/AcOEt, 50/50); IR cm^{-1}: 1138, 1104, 1073; ^1H NMR ($CDCl_3$) δ: 1.93 (m, 8H, $4CH_2$), 2.27 (m, 2H, CH_2), 2.98 (s, 6H, $2CH_3$), 3.79 (m, 8H, $4CH_2$), 4.14 (t, J = 6.0 Hz, 4H, $2CH_2$), 6.54 (dd, J = 10.0 Hz, J = 2.0 Hz, 2H, ArH), 6.68 (dd, J = 10.0 Hz, J = 2.0 Hz, 2H, ArH), 6.74 (m, 2H, ArH); MS (+APCI) m/z 429 (4.4) $[M+H-2MeOH]^+$ (calcd for $C_{25}H_{27}F_2O_4$ 429.4 $[M+H-2MeOH]^+$).

5.2.4. 4-[3-(2-Chloroethyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (21)

A solution of 3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenol (**17**) (1.0 g, 4.4 mmol, 1.0 equiv) dissolved in acetone (10 mL) was added dropwise to a mixture of 1-bromo-2-chloroethane (736 μL, 8.8 mmol, 2.0 equiv) diluted in DMF (25 mL) and Cs_2CO_3 (4.3 g, 13.2 mmol, 3.0 equiv) at rt. After stirring for 24 h, the reaction medium was filtered and the filtrate was evaporated under vacuum. The solid that formed was then dissolved in DCM (10 mL). The organic layer was washed with aqueous KOH solution (1 N), dried over $MgSO_4$, and evaporated under vacuum. The residue was purified by FC over silica gel using chromatography solvent (heptane/AcOEt, 60/40) to give a solution which was filtered and concentrated in vacuo. Yield 35%; colourless oil; TLC R_f 0.51 (DCM/AcOEt, 90/10); IR cm^{-1}: 1140, 1104, 1073; ^1H NMR ($CDCl_3$) δ: 1.95 (m, 4H, $2CH_2$), 3.00 (s, 3H, CH_3), 3.83 (m, 6H, $3CH_2$), 4.23 (t, J = 5.0 Hz, 2H, CH_2), 6.55 (dd, J = 10.0 Hz, J = 2.0 Hz, 1H, ArH), 6.72 (m, 1H, ArH), 6.76 (m, 1H, ArH); MS (+APCI) m/z 257 (5.0) $[M+H-MeOH]^+$ (calcd for $C_{13}H_{15}ClFO_2$ 257.7 $[M+H-MeOH]^+$).

5.3. General procedures for the synthesis of compounds 6–16 and compounds 26–42

General procedure for benzoylation of 2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indoles: To a stirred suspension of NaH (60% dispersion in mineral oil, 1.0 equiv) at 0 °C in DMF (10 mL) was added slowly an anhydride solution of 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole derivative (1.0 equiv) dissolved in DMF (20 mL). The medium was stirred for 2 h at 0 °C. Then benzoyl chloride (1.0 equiv) was added dropwise at 0 °C. The reaction medium was then stirred during 2 h at rt. After filtration, the filtrate was evaporated.

General procedure for catalytic hydrogenation of 5-benzoyl-2-benzyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indoles: A solution of 5-benzoyl-2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole derivative and Pd/C 10% in a mixture of solvents including an alcohol was stirred under atmospheric H_2 pressure for 48 h at rt. The solution was then filtered with Celite and the filtrate was concentrated in vacuo.

General procedure for alkylation of 5-benzoyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indoles. Method A: A solution of 5-benzoyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole chloride derivative (1.0 equiv) dissolved in DCM (50 mL) and *N*,*N*-diisopropylethylamine (DIEA) (2.0 equiv) was stirred at rt during 30 min. The solution was then added to a stirred solution of 1-bromo-3-chloropropane (1.0 equiv) in DCM (7 mL). The reaction medium was stirred at rt for 24 h, concentrated under vacuo and the residual oil was then diluted in AcOEt (100 mL). The organic solution was washed with a 10% K_2CO_3 solution (3 × 100 mL), dried over $MgSO_4$, filtered and evaporated. The residual oil was then purified by FC over silica gel to give a solution which was filtered and evaporated.

Method B: To a mixture of 5-benzoyl-2-(3-chloropropyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole derivative (1.0 equiv) and Cs_2CO_3 (1.5 equiv) in acetone (30 mL) was added a solution of 3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenol (**17**) (1.0 equiv) dissolved in acetone (10 mL). The stirred medium was refluxed during 24 h, filtered after cooling to rt and the filtrate was evaporated under vacuum. The residual oil was then purified by FC over silica gel to give a solution which was filtered and evaporated.

Method C: To a mixture of 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole derivative (1.0 equiv) and Cs_2CO_3 (1.0–2.6 equiv) in acetone (30 mL) was added a solution of 4-[3-(3-chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (**18**) (1.5–2.6 equiv) dissolved in acetone (10 mL). The stirred medium was refluxed during 24 h, filtered after cooling to rt and the filtrate was evaporated under vacuum. The residual oil was then purified by FC over silica gel to give a solution which was filtered and evaporated.

General procedure for benzoylation and benzylation of 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4H-4-pyranyl)phenoxy]propyl}-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole: To a stirred suspension of NaH (60% dispersion in mineral oil, 1.0 equiv) at 0 °C in DMF (10 mL) was added slowly an anhydride solution of 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole derivative (1.0 equiv) dissolved in DMF (20 mL). The medium was stirred for 2 h at 0 °C. Then a benzoyl chloride derivative (1.0 equiv) or 4-methanesulfonylbenzyl bromide (1.0 equiv) was added dropwise at 0 °C. The reaction medium was stirred during 2 h at rt, filtrated and the filtrate was evaporated. The residual oil was then diluted in a minimum volume of acetone, added dropwise to a 10% K_2CO_3 aqueous solution (150 mL) and was allowed to precipitate during the night. After filtration, the crude product was purified by column chromatography (230–400 mesh) over silica gel to give a solution which was filtered and evaporated. The residue was then diluted in chloroform (CH_3Cl) (10 mL) and transformed to a hydrochloride using a solution of HCl in isopropanol (*i*PrOH) (5.0–6.0 M) (0.2–1.0 mmol). The solution was evaporated and the residue was sonicated in Et_2O (20 mL) to give a solid which was filtrated and dried over phosphorous pentoxide (P_2O_5).

5.3.1. 5-Benzoyl-2-benzyl-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (6)

Following general procedure for benzoylation, NaH (958 mg, 24.0 mmol, 1.0 equiv), 2-benzyl-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**1**) (7.0 g, 24.0 mmol, 1.0 equiv) and benzoyl chloride (2.8 mL, 24.0 mmol, 1.0 equiv) gave the product which was filtered and washed with EtOH 95% (25 mL). Yield 48%; mp 118 °C (from EtOH 95%); white crystals; TLC R_f 0.51 (DCM/EtOAc, 80/20); IR cm^{-1}: 2923, 1672, 1466; ^1H NMR ($CDCl_3$) δ: 2.72 (m, 4H, $2CH_2$), 3.67 (m, 2H, CH_2), 3.79 (m, 2H, CH_2), 3.83 (s, 3H, CH_3), 6.73 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H, ArH), 6.78 (ds, J = 2.0 Hz, 1H, ArH), 7.26–7.58 (m, 11H, ArH); MS (+APCI) m/z 397 (5.5) $[M+H]^+$ (calcd for $C_{26}H_{25}N_2O_2$ 397.4 $[M+H]^+$).

5.3.2. 5-Benzoyl-2-benzyl-8-bromo-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (7)

Following general procedure for benzoylation, NaH (293 mg, 7.3 mmol, 1.0 equiv), 2-benzyl-8-bromo-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**2**) (2.5 g, 7.3 mmol, 1.0 equiv) and benzoyl chloride (850 μL, 7.3 mmol, 1.0 equiv) gave the product as an orange oil which crystallised in MeCN (60 mL). Yield 55%; mp >230 °C (from EtOH); white crystals; TLC R_f 0.94 (DCM/MeOH, 90/10); IR cm^{-1}: 2920, 1686; ^1H NMR ($CDCl_3$) δ: 2.77 (m, 1H, C*H*H′), 3.00 (m, 1H, CH*H*′), 3.19 (m, 1H, C*H*H′), 3.52 (m, 1H, CH*H*′), 4.27 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 7.04–7.77 (m, 13H, ArH), 11.59 (br, 1H, NH^+); MS (+APCI) m/z 445 (3.5) [M], 447 (3.5) [M+2] (calcd for $C_{25}H_{21}BrN_2O$ 445.3 [M]).

5.3.3. 5-Benzoyl-2-benzyl-8-methylthio-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (8)

Following general procedure for benzoylation, NaH (221 mg, 5.5 mmol, 1.0 equiv), 2-benzyl-8-methylthio-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**3**) (1.7 g, 5.5 mmol, 1.0 equiv) and benzoyl chloride (641 μL, 5.5 mmol, 1.0 equiv) gave the product which was filtered and washed with EtOH 95% (25 mL). Yield 48%; mp >230 °C (from EtOH); white crystals; TLC R_f 0.69 (heptane/EtOAc, 60/40); IR cm^{-1}: 2929, 1674, 1325; ^1H NMR ($CDCl_3$) δ: 2.50 (s, 3H, CH_3), 2.76 (m, 2H, CH_2), 2.88 (m, 2H, CH_2), 3.79 (s, 2H, CH_2), 3.91 (m, 2H, CH_2), 7.07 (d, J = 8.5 Hz, J = 2.0 Hz, 1H, ArH), 7.23 (d, J = 8.5 Hz, 1H, ArH), 7.32 (m, 6H, ArH), 7.60 (t, J = 7.0 Hz, 3H, ArH), 7.67 (d, J = 7.0 Hz, 2H, ArH); MS (+APCI) m/z 413 (3.5) $[M+H]^+$ (calcd for $C_{26}H_{25}N_2OS$ 413.5 $[M+H]^+$).

5.3.4. 5-Benzoyl-2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (9)

Following general procedure for benzoylation, NaH (1.2 g, 28.9 mmol, 1.1 equiv), 2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**4**) (6.9 g, 26.3 mmol, 1.0 equiv) and benzoyl chloride (3.0 mL, 26.3 mmol, 1.0 equiv) gave the product which was filtered and washed with EtOH 95% (25 mL). Yield 67%; mp 143 °C (from EtOH 95%); white crystals; TLC R_f 0.92 (DCM/MeOH, 90/10); IR cm^{-1}: 2922, 1676; ^1H NMR ($CDCl_3$) δ: 2.73 (m, 4H, $2CH_2$), 3.71 (s, 2H, $2CH_2$), 3.79 (s, 2H, $2CH_2$), 7.15 (m, 2H, CH_2), 7.30–7.80 (m, 12H, ArH); MS (+APCI) m/z 367 (4.4) $[M+H]^+$ (calcd for $C_{25}H_{23}N_2O$ 367.4 $[M+H]^+$).

5.3.5. 5-Benzoyl-2-benzyl-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (10)

Following general procedure for benzoylation, NaH (350 mg, 10.4 mmol, 1.0 equiv), 2-benzyl-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**5**) (5.0 g, 10.4 mmol, 1.0 equiv) and benzoyl chloride (1.2 mL, 10.4 mmol, 1.0 equiv) gave an oil which was diluted in AcOEt (50 mL), washed with a 10% K_2CO_3 aqueous solution. The aqueous layer was extracted with Et_2O (3 × 150 mL). The combined organic layer was dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by FC over silica gel using the mixture of solvents for chromatography (DCM/AcOEt, 90/10) to give a solution which was filtered and concentrated in vacuo. Yield 30%; mp 175 °C (from MeOH); white crystals; TLC R_f 0.24 (heptane/EtOAc, 50/50); IR cm^{-1}: 1674, 1318, 1148; ^1H NMR ($CDCl_3$) δ: 2.75 (m, 2H, CH_2), 2.79 (m, 2H, CH_2), 3.07 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 7.32–7.56 (m, 8H, ArH), 7.59 (m, 4H, ArH), 7.97 (d, J = 2.0 Hz, 1H, ArH); MS (+APCI) m/z 445 (3.0) $[M+H]^+$ (calcd for $C_{26}H_{25}N_2O_3S$ 445.5 $[M+H]^+$).

5.3.6. 5-Benzoyl-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (11)

Following general procedure for catalytic hydrogenation, 5-benzoyl-2-benzyl-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (**6**) (5.0 g, 10.5 mmol, 1.0 equiv) in the mixture of solvents (DCM/EtOH, 50/50) (200 mL) gave the product. Yield 98%; mp >230 °C (from EtOH); grey crystals; TLC R_f 0.54 (DCM/MeOH, 80/20); IR cm^{-1}: 3245, 1683; ^1H NMR ($CDCl_3$) δ: 1.26 (br, 2H, NH, NH^+), 3.15 (m, 2H, CH_2), 3.43 (m, 2H, CH_2), 3.79 (s, 3H, CH_3), 4.41 (m, 2H, CH_2), 6.67 (d, J = 9.0 Hz, 1H, ArH), 6.85 (m, 1H, ArH), 6.88 (t, J = 9.0 Hz, 1H, ArH), 7.48 (d, J = 7.0 Hz, 1H, ArH), 7.51 (d, J = 7.0 Hz, 1H, ArH), 7.63 (t, J = 7.0 Hz, 3H, ArH); HPLC (Kromasil C18, H_2O/MeOH/TFA (46:54:0.1), λ = 220 nm) rt.: 5.1 min (100%); Anal. Calcd for $C_{19}H_{18}N_2O_2{\cdot}HCl{\cdot}1/2H_2O$ (351.8): C, 64.96; H, 5.70; N, 7.98. Found: C, 65.06; H, 5.79; N, 8.18; MS (+APCI) m/z 307 (2.7) $[M+H]^+$ (calcd for $C_{19}H_{19}N_2O_2$ 307.3 $[M+H]^+$).

5.3.7. 5-Benzoyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (12)

Following general procedure for catalytic hydrogenation, 5-benzoyl-2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (7.1 g, 17.6 mmol, 1.0 equiv) in the mixture of solvents (DCM/MeOH, 25/75) (400 mL) gave the product. Yield 90%; mp >230 °C (from EtOH 95%); orange crystals; TLC R_f 0.12 (DCM/MeOH, 90/10); IR cm^{-1}: 3395, 1632; ^1H NMR (DMSO-d_6) δ: 2.90 (m, 2H, CH_2), 3.36 (m, 2H, CH_2), 4.35 (m, 2H, CH_2), 7.18 (m, 3H, ArH), 7.57–7.76 (m, 6H, ArH), 9.78 (br, 2H, NH, NH^+); HPLC (Kromasil C18, H_2O/MeOH/TFA (50:50:0.1), λ = 220 nm) rt.: 9.7 min (99.6%); MS (+APCI) m/z 277 (2.6) $[M+H]^+$ (calcd for $C_{18}H_{17}N_2O$ 277.3 $[M+H]^+$).

5.3.8. 5-Benzoyl-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (16)

Vinyl chloroformate (150 μL, 1.75 mmol, 1.0 equiv) was added dropwise to a solution of 5-benzoyl-2-benzyl-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (780 mg, 1.75 mmol, 1.0 equiv) dissolved in DCM (25 mL). The reaction medium was refluxed during 1 h then allowed to cool to rt and concentrated in vacuo. The residue was triturated in a mixture of solvents (heptane/AcOEt, 70/30) to give the intermediate (compound **15**) as a solid which was sonicated, filtered and gave an analytical sample after recrystallisation. The reaction intermediate was dissolved in DCM (10 mL) and the solution was saturated with HCl and then evaporated in vacuum. The red oily residue was refluxed in MeOH (25 mL) during 2 h then allowed to cool to rt and concentrated in vacuo. The product was precipitated in MeOH (10 mL) and filtered.

Compound **15**. Yield 76%; mp 180 °C (from EtOH); yellow crystals; TLC R_f 0.43 (heptane/AcOEt, 50/50); IR cm^{-1}: 1709, 1681, 1326, 1146; ^1H NMR (DMSO-d_6) δ: 2.59 (m, 2H, CH_2), 3.23 (s, 3H, CH_3), 3.68 (m, 2H, CH_2), 4.57 (m, 1H, C*H*H′), 4.74 (m, 1H, CH*H*′), 4.88 (m, 2H, CH_2 vinyl), 7.18 (dd, J = 6.0 Hz, J = 6.0 Hz, CH vinyl), 7.59 (m, 3H, ArH), 7.73 (m, 4H, ArH), 8.24 (s, 1H, ArH); MS (+APCI) m/z 355 (3.0) $[M+H]^+$ (calcd for $C_{19}H_{19}N_2O_3$ 355.4 $[M+H]^+$).

Compound **16**. Yield 70%; mp >230 °C (from MeOH); yellow crystals; TLC R_f 0.63 (DCM/MeOH, 90/10 + NH_4OH); IR cm^{-1}: 1697, 1298, 1143; ^1H NMR ($CDCl_3$) δ: 2.90 (m, 2H, CH_2), 3.34 (s, 3H, CH_3), 3.39 (m, 2H, CH_2), 4.46 (s, 2H, CH_2), 7.39 (d, J = 9.0 Hz,

1H, ArH), 7.62 (m, 2H, ArH), 7.70–8.32 (m, 4H, ArH), 8.27 (s, 1H, ArH), 9.55 (br, 2H, NH, NH^+); Anal. Calcd for $C_{19}H_{18}N_2O_2{\cdot}HCl{\cdot}3/4H_2O$ (403.5): C, 56.50; H, 5.08; N, 6.94. Found: C, 56.63; H, 4.95; N, 6.99; MS (+APCI) *m*/*z* 355 (2.7) $[M+H]^+$, 396 (2.7) $[M+H+MeCN]^+$ (calcd for $C_{19}H_{19}N_2O_3S$ 355.4 $[M+H]^+$).

5.3.9. 5-Benzoyl-2-(3-chloropropyl)-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (13)

Following general method A for alkylation, 5-benzoyl-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (**11**) (3.5 g, 10.3 mmol, 1.0 equiv), DIEA (4.3 mL, 20.7 mmol, 2.0 equiv) and 1-bromo-3-chloropropane (1.1 mL, 11.4 mmol, 1.0 equiv) gave the product. Column eluent (DCM/AcOEt, 80/20). Yield 62%; white oil; TLC R_f 0.48 (DCM/AcOEt, 50/50); IR cm^{-1}: 1720, 1452; ^1H NMR ($CDCl_3$) δ: 2.13 (m, 2H, CH_2), 2.75 (m, 6H, $3CH_2$), 3.68 (t, *J* = 8.0 Hz, 2H, CH_2), 3.70 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 6.73 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.81 (d, *J* = 2.5 Hz, 1H, ArH), 7.27 (m, 1H, ArH), 7.47–7.61 (m, 5H, ArH); MS (+APCI) *m*/*z* 383 (5.0) $[M+H]^+$ (calcd for $C_{22}H_{24}ClN_2O_2$ 383.8 $[M+H]^+$).

5.3.10. 5-Benzoyl-2-(3-chloropropyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (14)

Following general method A for alkylation, 5-benzoyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (**12**) (4.9 g, 15.6 mmol, 1.0 equiv), DIEA (2.7 mL, 31.2 mmol, 2.0 equiv) and 1-bromo-3-chloropropane (1.5 mL, 15.6 mmol, 1.0 equiv) gave the product. Column eluent (DCM/AcOEt, 90/10). Yield 17%; white oil; TLC R_f 0.54 (DCM/AcOEt, 90/10); IR cm^{-1}: 1680; ^1H NMR ($CDCl_3$) δ: 2.08 (m, 2H, CH_2), 2.75 (m, 6H, $3CH_2$), 3.69 (t, *J* = 7.0 Hz, 2H, CH_2), 3.71 (s, 2H, CH_2), 7.10–7.85 (m, 9H, ArH); MS (+APCI) *m*/*z* 353 (5.5) $[M+H]^+$ (calcd for $C_{21}H_{22}ClN_2O$ 353.8 $[M+H]^+$).

5.3.11. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl) phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (26)

Following general method C for alkylation, 8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**22**) (3.0 g, 14.8 mmol, 1.0 equiv), Cs_2CO_3 (9.7 g, 29.7 mmol, 2.0 equiv) and 4-[3-(3-chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (**18**) (4.5 g, 14.8 mmol, 1.0 equiv) gave the product. Column eluent (DCM/MeOH, 90/10). Yield 22%; white oil; TLC R_f 0.50 (DCM/MeOH, 90/10); IR cm^{-1}: 3270, 1460, 1455, 1076; ^1H NMR (DMSO-d_6) δ: 1.87 (m, 4H, $2CH_2$), 2.31 (m, 2H, $2CH_2$), 2.89 (s, 3H, CH_3), 3.36 (m, 2H, $2CH_2$), 3.65 (m, 8H, $4CH_2$), 3.74 (m, 1H, C*H*H′), 3.79 (s, 3H, CH_3), 3.88 (m, 1H, CH*H*′), 4.14 (m, 2H, CH_2), 6.78 (m, 3H, ArH), 6.92 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.54 (d, *J* = 9.0 Hz, 1H, ArH), 7.61 (d, *J* = 2.0 Hz, 1H, ArH), 10.05 (br, 1H, NH); MS (+APCI) *m*/*z* 469 (3.3) $[M+H]^+$ (calcd for $C_{27}H_{34}FN_2O_4$ 469.5 $[M+H]^+$).

5.3.12. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl) phenoxy]propyl}-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (27)

Following general method C for alkylation, 8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**23**) (2.8 g, 14.8 mmol, 1.0 equiv), Cs_2CO_3 (6.5 g, 20.0 mmol, 2.0 equiv) and 4-[3-(3-chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (**18**) (4.5 g, 14.8 mmol, 1.0 equiv) gave the product. Column eluent (AcOEt/EtOH, 90/10). Yield 13%; brown oil; TLC R_f 0.40 (AcOEt/EtOH, 90/10); IR cm^{-1}: 3303, 1079; ^1H NMR ($CDCl_3$) δ: 1.89 (m, 1H, C*H*H′), 1.94 (m, 4H, $2CH_2$), 2.07 (m, 1H, CH*H*′), 2.15 (m, 2H, CH_2), 2.85 (m, 2H, CH_2), 2.91 (m, 2H, CH_2), 3.00 (s, 3H, CH_3), 3.72 (m, 2H, CH_2), 3.83 (m, 4H, $2CH_2$), 4.08 (m, 2H, CH_2), 6.56 (ddd, *J* = 10.0 Hz, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.70 (dd, *J* = 10.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.74 (m, 1H, ArH), 6.84 (ddd, *J* = 10.0 Hz, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.04 (dd, *J* = 10.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.15 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H, ArH), 8.15 (br, 1H, NH); MS (+APCI) *m*/*z* 457 (3.2) $[M+H]^+$ (calcd for $C_{26}H_{31}F_2N_2O_3$ 457.5 $[M+H]^+$).

5.3.13. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl) phenoxy]propyl}-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (28)

Following general method C for alkylation, 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**24**) (1.2 g, 6.6 mmol, 1.0 equiv), Cs_2CO_3 (3.3 g, 9.9 mmol, 1.5 equiv) and 4-[3-(3-chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (**18**) (2.0 g, 6.6 mmol, 1.0 equiv) gave the product. Column eluent (AcOEt/EtOH, 90/10). Yield 28%; white oil; TLC R_f 0.20 (DCM/MeOH, 90/10); IR cm^{-1}: 3300, 1460, 1455, 1072; ^1H NMR (DMSO-d_6) δ: 1.87 (m, 4H, $2CH_2$), 2.31 (m, 2H, $2CH_2$), 2.89 (s, 3H, CH_3), 3.36 (m, 2H, $2CH_2$), 3.65 (m, 8H, $4CH_2$), 3.74 (m, 1H, C*H*H′), 3.79 (s, 3H, CH_3), 3.88 (m, 1H, CH*H*′), 4.14 (m, 2H, CH_2), 6.78 (m, 3H, ArH), 6.92 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.54 (d, *J* = 9.0 Hz, 1H, ArH), 7.61 (d, *J* = 2.0 Hz, 1H, ArH), 10.05 (br, 1H, NH); MS (+APCI) *m*/*z* 439 (4.4) $[M+H]^+$ (calcd for $C_{26}H_{32}FN_2O_3$ 439.5 $[M+H]^+$).

5.3.14. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl) phenoxy]propyl}-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (29) and 2,5-di{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (42)

Following general method C for alkylation, 8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**25**) (2.4 g, 9.6 mmol, 1.0 equiv), Cs_2CO_3 (8.3 g, 25.4 mmol, 2.6 equiv) and 4-[3-(3-chloropropyloxy)-5-fluorophenyl]-4-methoxytetrahydro-4*H*-pyran (**18**) (7.7 g, 25.4 mmol, 2.6 equiv) gave the two products. Column eluent (AcOEt/EtOH, 80/20).

Compound **29**. Yield 9%; mp 223 °C (from EtOH); yellow crystals; TLC R_f 0.39 (DCM/MeOH, 90/10); IR cm^{-1}: 3333, 1460, 1300, 1140, 1073; ^1H NMR ($CDCl_3$) δ: 1.94 (m, 4H, $2CH_2$), 2.14 (m, 2H, $2CH_2$), 2.86 (t, *J* = 7.0 Hz, 2H, CH_2), 2.96 (m, 4H, $2CH_2$), 3.01 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 3.84 (m, 4H, $2CH_2$), 4.11 (t, *J* = 7.0 Hz, 2H, CH_2), 6.57 (ddd, *J* = 10.0 Hz, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.71 (ddd, *J* = 10.0 Hz, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.76 (dd, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.43 (dd, *J* = 8.5 Hz, *J* = 1.0 Hz, 1H, ArH), 7.68 (dd, *J* = 8.5 Hz, *J* = 2.0 Hz, 1H, ArH), 8.07 (d, *J* = 2.0 Hz, 1H, ArH), 8.29 (br, 1H, NH); MS (+APCI) *m*/*z* 517 (3.1) $[M+H]^+$, 485 (3.1) $[M+H-MeOH]^+$ (calcd for $C_{27}H_{34}FN_2O_5S$ 517.6 $[M+H]^+$).

Compound **42**. Yield 10%; mp 223 °C (from EtOH); yellow crystals; TLC R_f 0.52 (DCM/MeOH, 80/20); IR cm^{-1}: 3333, 1460, 1300, 1136, 1071; ^1H NMR ($CDCl_3$) δ: 1.94 (m, 8H, $4CH_2$), 2.12 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 2.83 (t, *J* = 7.3 Hz, 2H, CH_2), 2.91 (m, 4H, $2CH_2$), 3.00 (s, 3H, CH_3), 3.01 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 3.77 (s, 2H, CH_2), 3.86 (m, 10H, $5CH_2$), 3.88 (t, *J* = 6.0 Hz, 2H, CH_2), 4.08 (t, *J* = 6.0 Hz, 2H, CH_2), 4.34 (t, *J* = 6.0 Hz, 2H, CH_2), 6.57 (ddd, *J* = 10.5 Hz, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H, CH_2), 6.72 (m, 4H, ArH), 7.40 (d, *J* = 9.0 Hz, 1H, ArH), 7.68 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, ArH), 8.07 (d, *J* = 2.0 Hz, 1H, ArH), 8.29 (s, 1H, ArH); Anal. Calcd for $C_{42}H_{52}F_2N_2O_8S{\cdot}1/2H_2O$ (790.9): C, 63.71; H, 6.57; N, 3.54. Found: C, 63.86; H, 6.69; N, 3.74; MS (+APCI) *m*/*z* 783 (3.6) $[M+H]^+$, 751 (3.6) $[M+H-MeOH]^+$, 719 (3.6) $[M+H-2MeOH]^+$ (calcd for $C_{41}H_{49}F_2N_2O_7S$ 751.8 $[M+H-MeOH]^+$).

5.3.15. 5-Benzoyl-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (30)

Following general procedure for benzoylation and benzylation, NaH (23 mg, 0.6 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (270 mg, 0.6 mmol, 1.0 equiv),

benzoyl chloride (67 μL, 0.6 mmol, 1.0 equiv) and HCl–*i*PrOH (50 μL, 0.3 mmol, 1.0 equiv) gave the product. Column eluent (DCM/EtOAc, 90/10). Yield 57%; mp 183 °C (from *i*PrOH); light brown crystals; TLC R_f 0.78 (DCM/MeOH, 90/10); IR cm^{-1}: 2956, 1678, 1464; ^1H NMR ($CDCl_3$) δ: 1.92 (m, 4H, $2CH_2$), 2.62 (m, 2H, CH_2), 2.99 (s, 3H, CH_3), 3.00 (m, 1H, C*H*H′), 3.25 (m, 3H, CH*H*′, CH_2), 3.47 (m, 4H, $2CH_2$), 3.70 (m, 1H, C*H*H′), 3.84 (s, 3H, CH_3), 3.90 (m, 1H, CH*H*′), 4.14 (m, 2H, CH_2), 4.15 (m, 1H, C*H*H′), 4.85 (m, 1H, CH*H*′), 6.53 (m, 1H, ArH), 6.74 (m, 4H, ArH), 6.91 (d, J = 9.0 Hz, 1H, ArH), 7.56 (t, J = 8.0 Hz, 1H, ArH), 7.63–7.73 (m, 4H, ArH), 13.40 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{37}FN_2O_5{\cdot}HCl{\cdot}H_2O$ (627.1): C, 65.12; H, 6.43; N, 4.47. Found: C, 64.99; H, 6.21; N, 4.45; MS (+APCI) m/z 573 (3.6) $[M+H]^+$ (calcd for $C_{34}H_{38}FN_2O_5$ 573.6 $[M+H]^+$).

5.3.16. 5-Benzoyl-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (31)

Following general procedure for benzoylation and benzylation, NaH (15 mg, 0.4 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-2,3,4,5-tetrahy-dro-1*H*-pyrido[4,3-*b*]indole (**28**) (160 mg, 0.4 mmol, 1.0 equiv), benzoyl chloride (43 μL, 0.4 mmol, 1.0 equiv) and HCl–*i*PrOH (34 μL, 0.2 mmol, 1.0 equiv) gave the product. Column eluent (DCM/EtOAc, 50/50). Yield 48%; mp 174 °C (from DCM/Et_2O, 50/50); light brown crystals; TLC R_f 0.78 (DCM/MeOH, 90/10); IR cm^{-1}: 2976, 1685; ^1H NMR ($CDCl_3$) δ: 1.92 (m, 4H, $2CH_2$), 2.64 (m, 2H, CH_2), 2.99 (s, 3H, CH_3), 3.25 (m, 1H, C*H*H′), 3.45 (m, 2H, CH_2), 3.58 (m, 1H, CH*H*′), 3.71 (m, 1H, C*H*H′), 3.82 (m, 4H, $2CH_2$), 3.86 (m, 1H, CH*H*′), 4.14 (m, 2H, CH_2), 4.23 (m, 1H, C*H*H′), 4.82 (m, 1H, CH*H*′), 6.51 (ddd, J = 10.0 Hz, J = 2.0 Hz, J = 2.0 Hz, 1H, ArH), 6.71 (m, 1H, ArH), 6.73 (d, J = 2.0 Hz, 1H, ArH), 7.12–7.72 (m, 9H, ArH), 13.35 (br, 1H, NH^+); HPLC (Kromasil C18, H_2O/MeOH/TFA (26:74:0.1), λ = 220 nm) rt.: 7.6 min (90%); Anal. Calcd for $C_{33}H_{35}FN_2O_3{\cdot}HCl{\cdot}1¾H_2O$ (609.5): C, 64.97; H, 6.19; N, 4.59. Found: C, 64.93; H, 6.15; N, 4.85 (the degradation of the product was observed during the analysis); MS (+APCI) m/z 543 (3.6) $[M+H]^+$ (calcd for $C_{33}H_{36}FN_2O_4$ 543.6 $[M+H]^+$).

5.3.17. 5-(2-Fluorobenzoyl)-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (32)

Following general procedure for benzoylation and benzylation, NaH (77 mg, 1.7 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (800 mg, 1.7 mmol, 1.0 equiv), 2-fluorobenzoyl chloride (205 μL, 1.7 mmol, 1.0 equiv) and HCl–*i*PrOH (136 μL, 0.3 mmol) gave the product. Column eluent (DCM/EtOAc, 60/40). Yield 16%; mp >230 °C (from *i*PrOH); white crystals; TLC R_f 0.81 (EtOAc/EtOH, 80/20); IR cm^{-1}: 2930, 1676; ^1H NMR (DMSO-d_6) δ: 1.87 (m, 4H, $2CH_2$), 2.31 (m, 2H, CH_2), (m, 1H, C*H*H′), 2.90 (s, 3H, CH_3), 2.97 (m, 1H, CH*H*′), 3.42 (m, 2H, CH_2), 3.65 (m, 1H, C*H*H′), 3.66 (m, 4H, $2CH_2$), 3.75 (m, 1H, CH*H*′), 3.79 (s, 3H, CH_3), 4.15 (m, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.71 (m, 1H, CH*H*′), 6.82 (m, 3H, ArH), 6.85 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H, ArH), 7.19 (m, 2H, ArH), 7.46 (m, 2H, ArH), 7.73 (m, 2H, ArH), 10.72 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{36}F_2N_2O_5{\cdot}HCl{\cdot}H_2O$ (645.1): C, 63.03; H, 6.09; N, 4.34. Found: C, 63.21; H, 6.11; N, 4.32; MS (+APCI) m/z 591 (3.7) $[M+H]^+$ (calcd for $C_{34}H_{37}F_2N_2O_5$ 591.6 $[M+H]^+$).

5.3.18. 5-(3-Fluorobenzoyl)-2-{3-[3-fluoro-5-(4-methoxy tetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (33)

Following general procedure for benzoylation and benzylation, NaH (70 mg, 1.7 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (800 mg, 1.7 mmol, 1.0 equiv), 3-fluorobenzoyl chloride (416.5 μL, 3.4 mmol, 2.0 equiv) and HCl–*i*PrOH (48 μL, 0.2 mmol) gave the product. Column eluent (DCM/EtOAc, 60/40). Yield 10%; mp 213 °C (from *i*PrOH); light brown crystals; TLC R_f 0.62 (EtOAc/EtOH, 90/10); IR cm^{-1}: 2954, 1687; ^1H NMR (DMSO-d_6) δ: 1.87 (m, 4H, $2CH_2$), 2.34 (m, 2H, CH_2), 2.85 (m, 1H, C*H*H′), 2.90 (s, 3H, CH_3), 3.08 (m, 1H, CH*H*′), 3.36 (m, 2H, CH_2), 3.67 (m, 1H, C*H*H′), 3.68 (m, 4H, $2CH_2$), 3.79 (s, 3H, CH_3), 3.87 (m, 1H, CH*H*′), 4.15 (m, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.70 (m, 1H, CH*H*′), 6.81 (m, 4H, ArH), 7.13 (d, J = 9.0 Hz, 1H, ArH), 7.19 (m, 1H, ArH), 7.56 (m, 4H, ArH), 11.33 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{36}ClF_2N_2O_5{\cdot}HCl{\cdot}1½H_2O$ (654.1) requires C, 62.37; H, 6.11; N, 4.28; found C, 62.36; H, 6.18; N, 4.09; MS (+APCI) m/z 591 (3.7) $[M+H]^+$ (calcd for $C_{34}H_{37}F_2N_2O_5$ 591.6 $[M+H]^+$).

5.3.19. 5-(4-Fluorobenzoyl)-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (34)

Following general procedure for benzoylation and benzylation, NaH (50 mg, 1.1 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (500 mg, 1.1 mmol, 1.0 equiv), 4-fluorobenzoyl chloride (128 μL, 1.1 mmol, 1.0 equiv) and HCl–*i*PrOH (20 μL, 0.1 mmol) gave the product. Column eluent (EtOAc/EtOH, 90/10). Yield 11%; mp 177 °C (from *i*PrOH); light brown crystals; TLC R_f 0.69 (AcOEt/EtOH, 90/10); IR cm^{-1}: 2946, 1679; ^1H NMR ($CDCl_3$) δ: 1.88 (m, 4H, $2CH_2$), 2.35 (m, 2H, CH_2), 2.82 (m, 1H, C*H*H′), 2.91 (s, 3H, CH_3), 3.09 (m, 1H, CH*H*′), 3.37 (m, 1H, C*H*H′), 3.44 (m, 2H, CH_2), 3.68 (m, 4H, $2CH_2$), 3.76 (m, 1H, CH*H*′), 3.80 (s, 3H, CH_3), 4.16 (m, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.75 (m, 1H, CH*H*′), 5.72 (br, 1H, NH^+), 6.82 (m, 4H, ArH), 7.18 (d, J = 9.0 Hz, 1H, ArH), 7.19 (d, J = 2.0 Hz, 1H, ArH), 7.44 (dd, J = 8.5 Hz, J = 8.5 Hz, 2H, ArH), 7.72 (dd, J = 8.5 Hz, J = 5.0 Hz, 2H, ArH); Anal. Calcd for $C_{34}H_{36}F_2N_2O_5{\cdot}HCl{\cdot}1/2H_2O$ (636.1): C, 64.23; H, 5.82; N, 4.41. Found: C, 64.05; H, 6.13; N, 4.51; MS (+APCI) m/z 591 (3.7) $[M+H]^+$ (calcd for $C_{34}H_{37}F_2N_2O_5$ 591.6 $[M+H]^+$).

5.3.20. 5-(4-Chlorobenzoyl)-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (35)

Following general procedure for benzoylation and benzylation, NaH (85 mg, 2.1 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (1.0 g, 2.1 mmol, 1.0 equiv), 4-chlorobenzoyl chloride (271 μL, 2.1 mmol, 1.0 equiv) and HCl–*i*PrOH (135 μL, 0.7 mmol) gave the product. Column eluent (DCM/MeOH, 99/1). Yield 33%; mp 172 °C (from DCM/cyclohexane 50/50); light brown crystals; TLC R_f 0.67 (DCM/MeOH, 90/10); IR cm^{-1}: 2948, 1681; ^1H NMR ($CDCl_3$) δ: 1.93 (m, 4H, $2CH_2$), 2.60 (m, 2H, $2CH_2$), 2.90 (m, 1H, C*H*H′), 2.98 (s, 3H, CH_3), 3.16 (m, 1H, CH*H*′), 3.42 (m, 2H, CH_2), 3.60 (m, 1H, C*H*H′), 3.65 (m, 4H, $2CH_2$), 3.83 (s, 3H, CH_3), 3.90 (m, 1H, CH*H*′), 4.07 (m, 2H, CH_2), 4.20 (m, 1H, C*H*H′), 4.78 (m, 1H, CH*H*′), 6.50 (d, J = 9.0 Hz, 1H, ArH), 6.73 (m, 3H, ArH), 6.83 (m, 1H, ArH), 6.92 (d, J = 9.0 Hz, ArH), 7.51 (d, J = 8.0 Hz, ArH), 7.67 (d, J = 8.0 Hz, ArH), 13.24 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{36}ClFN_2O_5{\cdot}HCl{\cdot}1½H_2O$ (670.5): C, 60.84; H, 5.96; N, 4.17. Found: C, 60.95; H, 5.99; N, 4.37; MS (+APCI) m/z 607 (3.8) [M], 609 (3.8) [M+2] (calcd for $C_{34}H_{36}ClFN_2O_5$ 607.1 [M]).

5.3.21. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl) phenoxy]propyl}-8-methoxy-5-(2,4,6-trichlorobenzoyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (36)

Following general procedure for benzoylation and benzylation, NaH (85 mg, 2.1 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (1.0 g, 2.1 mmol, 1.0 equiv), 2,4,6-trichlorobenzoyl chloride (667 μL, 4.3 mmol, 2.0 equiv) and

HCl–*i*PrOH (210 μL, 1.0 mmol) gave the product. Column eluent (DCM/EtOAc, 80/20). Yield 50%; mp >230 °C (from *i*PrOH); light brown crystals; TLC R_f 0.80 (EtOAc/EtOH, 90/10); IR cm^{-1}: 2954, 1700; ^1H NMR (DMSO-d_6) δ: 1.86 (m, 4H, $2CH_2$), 2.30 (m, 2H, $2CH_2$), 2.70 (m, 1H, C*H*H′), 2.89 (s, 3H, CH_3), 3.17 (m, 1H, CH*H*′), 3.59 (m, 2H, CH_2), 3.67 (m, 4H, $2CH_2$), 3.76 (m, 2H, CH_2), 3.83 (s, 3H, CH_3), 4.15 (m, 2H, CH_2), 4.36 (m, 1H, C*H*H′), 4.67 (m, 1H, CH*H*′), 6.77 (m, 3H, ArH), 7.05 (d, J = 9.0 Hz, 1H, ArH), 7.22 (m, 1H, ArH), 7.99 (d, J = 2.0 Hz, 1H, ArH), 8.05 (d, J = 1.5 Hz, 1H, ArH), 8.39 (d, J = 9.0 Hz, 1H, ArH), 11.19 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{34}Cl_3FN_2O_5{\cdot}HCl{\cdot}1/2H_2O$ (721.4): C, 56.55; H, 4.71; N, 3.88. Found: C, 56.50; H, 4.97; N, 3.93; MS (+APCI) m/z 675 (3.9) [M], 677 (3.9) [M+2] (calcd for $C_{34}H_{34}Cl_3FN_2O_5$ 676.0 [M]).

5.3.22. 5-(4-Bromobenzoyl)-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (37)

Following general procedure for benzoylation and benzylation, NaH (60 mg, 1.3 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (600 mg, 1.3 mmol, 1.0 equiv), 4-bromobenzoyl chloride (281 mg, 1.3 mmol, 1.0 equiv) and HCl–*i*PrOH (60 μL, 0.3 mmol) gave the product. Column eluent (DCM/EtOAc, 10/90). Yield 20%; mp 165 °C (from *i*PrOH); white crystals; TLC R_f 0.53 (EtOAc/EtOH, 90/10); IR cm^{-1}: 2954, 1700; ^1H NMR (DMSO-d_6) δ: 1.88 (m, 4H, $2CH_2$), 2.35 (m, 2H, CH_2), 2.81 (m, 1H, C*H*H′), 2.81 (s, 3H, CH_3), 3.10 (m, 1H, CH*H*′), 3.30 (m, 1H, C*H*H′), 3.37 (m, 2H, CH_2), 3.64 (m, 4H, $2CH_2$), 3.90 (m, 1H, CH*H*′), 3.80 (s, 3H, CH_3), 4.16 (m, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.71 (m, 1H, CH*H*′), 6.81 (m, 4H, ArH), 7.16 (m, 2H, ArH), 7.62 (d, J = 8.0 Hz, 2H, ArH), 7.81 (d, J = 8.0 Hz, 2H, ArH), 11.32 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{36}BrFN_2O_5{\cdot}HCl{\cdot}1/2H_2O$ (697.0): C, 58.53; H, 5.16; N, 4.01. Found: C, 58.60; H, 5.42; N, 4.22; MS (+APCI) m/z 651 (3.8) [M], 653 (3.8) [M+2] (calcd for $C_{34}H_{36}BrFN_2O_5$ 651.5 [M]).

5.3.23. 5-(4-Dimethylaminomethylenaminosulfonylbenzoyl)-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (38)

Following general procedure for benzoylation and benzylation, NaH (68 mg, 1.7 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (800 mg, 1.7 mmol, 1.0 equiv), 4-sulfaminobenzoyl chloride–DMF complex (750 mg, 2.6 mmol, 1.5 equiv) and HCl–*i*PrOH (85 μL, 0.4 mmol) gave the product. Column eluent (EtOAc/EtOH, 90/10). Yield 24%; mp >230 °C; light brown powder; TLC R_f 0.36 (EtOAc/EtOH, 90/10); IR cm^{-1}: 1625, 1340, 1149; ^1H NMR (DMSO-d_6) δ: 1.88 (m, 4H, $2CH_2$), 2.34 (m, 2H, CH_2), 2.83 (m, 1H, C*H*H′), 2.92 (s, 3H, CH_3), 2.96 (s, 3H, CH_3), 3.05 (m, 1H, CH*H*′), 3.19 (s, 3H, CH_3), 3.32 (m, 1H, C*H*H′), 3.38 (m, 4H, $2CH_2$), 3.44 (m, 2H, CH_2), 3.76 (m, 1H, CH*H*′), 3.81 (s, 3H, CH_3), 4.16 (m, 2H, CH_2), 4.32 (m, 1H, C*H*H′), 4.73 (m, 1H, CH*H*′), 6.80 (m, 3H, ArH), 6.54 (d, J = 9.5 Hz, 1H, ArH), 7.19 (m, 2H, ArH), 7.82 (d, J = 8.0 Hz, 2H, ArH), 7.97 (d, J = 8.0 Hz, 2H, ArH), 8.30 (s, 1H, CH_2), 11.09 (br, 1H, NH^+); MS (+APCI) m/z 707 (3.4) $[M+H]^+$ (calcd for $C_{37}H_{44}FN_4O_7S$ 707.8 $[M+H]^+$).

5.3.24. 2-{3-[3-Fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-5-(4-methanesulfonylbenzyl)-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (39)

Following general procedure for benzoylation and benzylation, NaH (43 mg, 1.1 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methoxy-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**26**) (500 mg, 1.1 mmol, 1.0 equiv), 4-methanesulfonylbenzyl bromide (266 mg, 1.1 mmol, 1.0 equiv) and HCl–*i*PrOH (78 μL, 0.4 mmol) gave the product. Column eluent (DCM/EtOAc, 20/80). Yield 36%; mp >230 °C (from EtOH); white crystals; TLC R_f 0.53 (EtOAc/EtOH, 80/20); IR cm^{-1}: 2954, 1310, 1146; ^1H NMR (DMSO-d_6) δ: 1.86 (m, 4H, $2CH_2$), 2.27 (m, 2H, CH_2), 2.72 (m, 1H, C*H*H′), 2.89 (s, 3H, CH_3), 3.09 (m, 1H, CH*H*′), 3.17 (s, 3H, CH_3), 3.45 (m, 2H, CH_2), 3.46 (m, 1H, C*H*H′), 3.67 (m, 4H, $2CH_2$), 3.76 (s, 3H, CH_3), 3.87 (m, 1H, CH*H*′), 4.14 (t, J = 5.0 Hz, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.75 (m, 1H, CH*H*′), 5.52 (q, J_{AB} = 17.5 Hz, 2H, CH_2), 6.78 (m, 4H, ArH), 7.09 (d, J = 2.0 Hz, 1H, ArH), 7.27 (d, J = 8.0 Hz, 2H, ArH), 7.37 (d, J = 9.0 Hz, 1H, ArH), 7.85 (d, J = 8.0 Hz, 2H, ArH), 10.10 (br, 1H, NH^+); Anal. Calcd for $C_{35}H_{41}FN_2O_6S{\cdot}HCl{\cdot}H_2O$ (691.2): C, 60.81; H, 6.42; N, 4.05. Found: C, 60.45; H, 6.32; N, 3.94; MS (+APCI) m/z 637 (3.5) $[M+H]^+$ (calcd for $C_{35}H_{42}FN_2O_6S$ 637.7 $[M+H]^+$).

5.3.25. 8-Fluoro-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-5-(4-methanesulfonylbenzyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (40)

Following general procedure for benzoylation and benzylation, NaH (42 mg, 1.1 mmol, 1.0 equiv), 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**27**) (500 mg, 1.1 mmol, 1.0 equiv), 4-methanesulfonylbenzyl bromide (259 mg, 1.1 mmol, 1.0 equiv) and HCl–*i*PrOH (62 μL, 0.3 mmol) gave the product. Column eluent (EtOAc/EtOH, 95/5). Yield 30%; mp >230 °C (from EtOH); white crystals; TLC R_f 0.37 (EtOAc/EtOH, 80/20); IR cm^{-1}: 1306, 1149, 1070; ^1H NMR (DMSO-d_6) δ: 1.86 (m, 4H, $2CH_2$), 2.29 (m, 2H, CH_2), 2.72 (m, 1H, C*H*H′), 2.89 (s, 3H, CH_3), 3.12 (m, 1H, CH*H*′), 3.17 (s, 3H, CH_3), 3.44 (m, 2H, CH_2), 3.63 (m, 1H, C*H*H′), 3.66 (m, 4H, $2CH_2$), 3.88 (m, 1H, CH*H*′), 4.15 (m, 2H, CH_2), 4.33 (m, 1H, C*H*H′), 4.75 (m, 1H, CH*H*′), 5.57 (q, J_{AB} = 17.0 Hz, 2H, CH_2), 6.77 (m, 3H, ArH), 7.01 (dd, J = 9.0 Hz, J = 8.5 Hz, 1H, ArH), 7.28 (d, J = 8.0 Hz, 2H, ArH), 7.38 (dd, J = 10.0 Hz, J = 2.5 Hz, 1H, ArH), 7.50 (dd, J = 8.5 Hz, J = 4.0 Hz, 1H, ArH), 7.86 (d, J = 8.0 Hz, 2H, ArH), 10.31 (br, 1H, NH^+); Anal. Calcd for $C_{34}H_{38}F_2N_2O_5S{\cdot}HCl{\cdot}H_2O$ (679.2): C, 61.76; H, 5.95; N, 4.24. Found: C, 61.54; H, 6.11; N, 4.12; MS (+APCI) m/z 625 (3.4) $[M+H]^+$ (calcd for $C_{34}H_{39}F_2N_2O_5S$ 625.7 $[M+H]^+$).

5.3.26. 5-Benzoyl-2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (41)

To a stirred suspension of 4-dimethylaminopyridine (90 mg, 0.7 mmol, 1.0 equiv) at 0 °C in DMF (5 mL) was added slowly an anhydride solution of 2-{3-[3-fluoro-5-(4-methoxytetrahydro-4*H*-4-pyranyl)phenoxy]propyl}-8-methanesulfonyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**29**) (380 mg, 0.7 mmol, 1.0 equiv) dissolved in DMF (5 mL). NaH (60% dispersion in mineral oil, 30 mg, 0.7 mmol, 1.0 equiv) was added portionwise and the medium was stirred for 2 h at 0 °C. Then benzoyl chloride derivative (171 μL, 1.5 mmol, 2.0 equiv) was added dropwise at 0 °C. The reaction medium was stirred during 2 h at rt, filtered and the filtrate was evaporated. The residual oil was then diluted in EtOAc (10 mL). The solution was added dropwise to a 10% K_2CO_3 aqueous solution (15 mL) and was allowed to precipitate during the night. The organic product was extracted with EtOAc (3 × 15 mL). The organic phase was dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography (230–400 mesh) over silica gel using the solvent for chromatography (EtOAc) to give a solution which was filtered and evaporated. The residue was then diluted in $CHCl_3$ (5 mL) and transformed to a hydrochloride using a solution of HCl in *i*PrOH (5–6 M) (10 μL, 0.3 mmol). The solution was evaporated in vacuo and the residue was sonicated in Et_2O (25 mL) to give the title compound as a solid which was filtered and dried over P_2O_5. Yield 31%; mp 183 °C (from MeCN); white crystals; TLC R_f 0.49 (EtOAc/EtOH, 90/10); IR cm^{-1}: 3441, 1695,

1303, 1140; ^{1}H NMR (DMSO-d_6) δ: 1.95 (m, 4H, $2CH_2$), 2.13 (m, 2H, CH_2), 2.81 (m, 6H, $3CH_2$), 3.01 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 3.77 (m, 1H, C*H*H′), 3.82 (m, 4H, $2CH_2$), 4.09 (t, J = 6.0 Hz, 2H, CH_2), 6.58 (ddd, J = 10.5 Hz, J = 2.0 Hz, J = 2.0 Hz, 1H, ArH), 6.71 (ddd, J = 10.0 Hz, J = 2.0 Hz, J = 2.0 Hz, 1H, ArH), 6.75 (dd, J = 2.0 Hz, J = 2.0 Hz, 1H, ArH), 7.50 (m, 3H, ArH), 7.67 (m, 4H, ArH), 8.03 (d, J = 1.5 Hz, 1H, ArH); Anal. Calcd for $C_{34}H_{37}FN_2O_6S{\cdot}HCl{\cdot}H_2O$ (675.2): C, 60.48; H, 5.97; N, 4.15. Found: C, 60.50; H, 5.95; N, 4.13; MS (+APCI) m/z 621 (3.2) $[M+H]^+$, 589 (3.2) $[M+H-MeOH]^+$ (calcd for $C_{34}H_{38}FN_2O_6S$ 621.7 $[M+H]^+$).

5.4. Biological tests

5.4.1. Analysis of arachidonic metabolites by high-performance liquid chromatography

Human peripheral venous blood samples were taken from healthy volunteers and collected into heparinised tubes. Aliquots were then transferred to tubes containing either the vehicle (DMSO) or the test compounds and incubated for 15 min at 37 °C under continuous agitation. A23187 (40.0 mM) alone or in combination with LPS (500 mg) was then added and incubation continued for either 30 min in the case of A23187 alone, or 24 h for samples stimulated with A23187 + LPS. Eicosanoids were extracted from the samples into EtOAc. Samples were then evaporated to dryness under nitrogen, re-suspended in the mobile phase to precipitate the proteins and then centrifuged. The supernatant was then directly analysed by HPLC using Hypersil ODS 3 mM columns (12.5 × 0.46 mm), a MeOH/H_2O/AcOH (80:20:0.1) mobile phase at a flow rate of 1 mL/min and UV detection firstly at 270 nm and 12 min later at 245 nm. Eicosanoids were separated on the following gradients: 70–80% MeOH for 10 min, 100% MeOH on 19 min for 5 min and 70% MeOH for 7 min.[36]

5.4.2. MTT test

PC-3 cells (3200 cells/well in 96-well culture plates) were incubated for 72 h with different concentrations of compounds **11**, **16**, **30**–**42** dissolved in DMSO. After treatment, MTT solution (4.0 mg/mL in 10% PBS) was added to each well. After 18 h incubation lysis buffer (100 µL SDS/HCl (7% of SDS in HCl 0.01 N)) was added to each well to dissolve the formazan. The absorbency was measured at 570 nm and then corrected at 630 nm with a microplate reader. All experiments were performed at least three times and average of the growth percentage was plotted against time. The results were expressed as percentage relative to untreated control (set to 100%) which allowed determining the percentage of inhibition of the cellular growth.

5.4.3. Statistical analysis

All results were shown as means ± standard error of the mean (SEM) of three experiments performed in triplicate. P values <0.05 were considered significant.

5.4.4. Molecular modelling. General methodology

All the calculations were realised on Silicon Graphics Octane 2 workstations running under Irix. A homology model of human COX_2 was built from the murine enzyme co-crystallised with SC-558 taken from the PDB entry 6COX.[37] As the identity of the sequences was very high, the achieved model was directly minimised with the AMBER95 forcefield and associated partial charges down to a 0.001 kcal/mol Å gradient using Sybyl 6.91 with no further investigations. The structures of the inhibitors were also built in Sybyl and minimised to a 0.001 gradient before their docking in the human COX_2 model with Gold. The best docking conformation was chosen by taking into account a consensus scoring function including Goldscore and Xscore as well as a visual inspection of the coherency of the docking results.[38] Therefore, the chosen conformation was the best scored one.

SYBYL 6.9.1—Molecular modelling software **2001**, Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144-2913, USA.

Acknowledgements

This study has been part of the Ph.D. thesis defended by Dr. Alexandre BRIDOUX on 11/04/2005. The authors would like to thank Ms. Amélie LEMOINE (Institut de Chimie Pharmaceutique Albert Lespagnol) for her technical assistance in this study, Mrs. Fabienne BRIDOUX for editing this manuscript and her availability. The molecular modelling studies have been achieved at the Laboratoire de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques de Lille. This study has been funded by the Institut de Chimie Pharmaceutique Albert Lespagnol.

Supplementary data

Supplementary data (the structure of the internal standard used during the HWB analyses was added) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.034.

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