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Preliminary communication

Novel bradykinin-1 antagonists containing a (1,2,3,4-tetrahydro-isoquinolin-1-yl)acetic acid scaffold

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Abstract

A novel B_1 antagonist core was utilized and the effects of modification of its amide side chain on the biological activity were tested. The imino functional group of isoquinolin-1-ylacetic acid and its 6,7-dimethoxy variant was sulfonylated (4-toluenesulfonyl), while the acetyl side chain was converted to amides. Three of the synthesized compounds exhibited significant activity at the recombinant human B_1 receptors in binding tests and also in a functional assay.

Keywords: Bradykinin-1 receptor; B_1 antagonist; Bradykinin; Isoquinoline; Peptidomimetic

1. Introduction

A large number of people suffer from unnecessary persistent pain because, although really effective analgetics (typically non-steroidal antiinflammatory drugs and opioids) are available, they cause serious side-effects [1].

However, the recently discovered biological effects of oligopeptide hormones (e.g. kinins), modified peptides [2,3], pseudopeptides [4,5] and peptidomimetics with novel mechanisms of action might be utilized to counter chronic pain.

In humans, two endogenous peptides are classified as kinins: bradykinin (BK; Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9) and kallidin (Lys-BK; Lys^1-Arg^2-Pro^3-Pro^4-Gly^5-Phe^6-Ser^7-Pro^8-Phe^9-Arg^{10}), which are produced by the proteolytic cleavage of high- and low-molecular weight kininogen precursor proteins, respectively, by plasma or tissue serine proteases named kallikrein enzymes [6,7]. Kinins exert several modes of physiological action [8–10]. They are relatively selective endogenous agonists of B_2 receptors and are known to mediate acute inflammatory pain responses. The C-terminally truncated des-Arg carboxypeptidase metabolites of BK and Lys-BK, des-Arg^9-BK and des-Arg^{10}-Lys-BK are selective ligands at the B_1 receptors; they are generally absent in normal tissues, but are induced locally following inflammation, tissue injury, trauma or stimulation [11,12]. The role of the particularly important BK B_1 receptors (in addition to the B_2 receptors) in the chronic phase of pain responses is associated with inflammatory conditions [6,13–15].

Accordingly, there is great interest in investigations of the pathophysiological roles of BK B_1 receptors (and ligands) and in the development of potent, selective and orally bioavailable bradykinin B_1 antagonists, initially peptides [9,16], and later non-peptide analogues [17–22] as a novel class of antinociceptive and antiinflammatory drugs [2].

Novel BK_1 receptor antagonists containing rigid core molecules such as benzodiazepine were earlier revealed to demonstrate in vivo efficacy comparable to that of morphine for the suppression of hyperalgesia [22,23]. Optimization of a series

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of B_1 antagonists with 2,3-diaminopyridine [24] or 2-alkylamino-5-sulfamoylbenzamide [1] cores has been reported. Incorporation of piperidine-3-carboxylic acid and piperidine-4-carboxylic acid spacers and the use of morpholine and piperazine derivatives led to an increased receptor-binding affinity and oral absorption in animal models [1].

A highly potent and selective member of a new generation of arylsulfonamide dihydroquinoxalinone B_1 receptor antagonists has also been reported and characterized [17,23,25,26].

2. Results and discussion

Our goal was to design and synthesize a series of non-peptide bradykinin antagonists, using derivatives of the commercially available racemic (1,2,3,4-tetrahydroisoquinolin-1-yl)acetic acid (THIQAA) core. THIQAA is already mentioned in a patent application [27] as a potential B_1 antagonist, but it has not been utilized as a B_1 key moiety.

We performed a comparative analysis of a broad set of B_1 antagonists, that has indicated, that the phenylsulfonamide moiety plays a key role in the binding and in the in vivo antinociceptive efficacy. Thus, we decided to use the THIQAA core, sulfonylated on its nitrogen atom. The 4-toluenesulfonyl group was used to form the aromatic sulfonamide moiety. Changes were made in the THIQAA core at positions 6 and 7, by the insertion of methoxy groups, and in the amide side chain (Scheme 1).

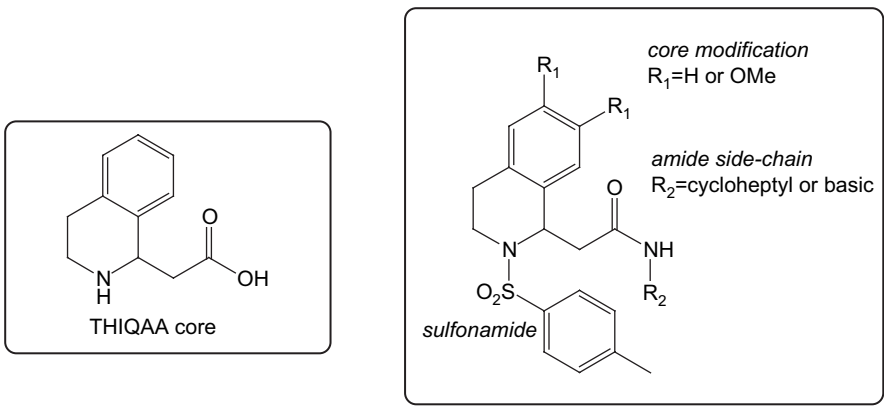
The first set of synthesized molecules, containing both the THIQAA and its 6,7-dimethoxy variant as holding scaffold for the sulfonamide and the basic side chain, included several inactive compounds. These contained the tosyl (4-toluenesulfonyl) sulfonamide moiety and different amides bound to the carboxyl functional group. There were apolar amides (cycloheptyl) and amides with slightly basic terminal primary amino groups, on alkyl spacers of different lengths, which had low activity in the 5 μM $[Ca^{2+}]_i$ assay (several examined examples, with no activity at all, are not presented in this report). However, when highly basic amides were applied (pK_a of the amine part >10), significant improvements were observed in the biological tests. Thus, a set of bifunctional basic amines were chosen and were used for conversion to the amide moiety of the THIQAA-containing B_1 antagonists.

There were primary amines with flexible alkyl linkers of different lengths. A guanidine-containing alkyl chain was also incorporated, as was an imidazoline-terminal apolar one containing a phenyl group [17].

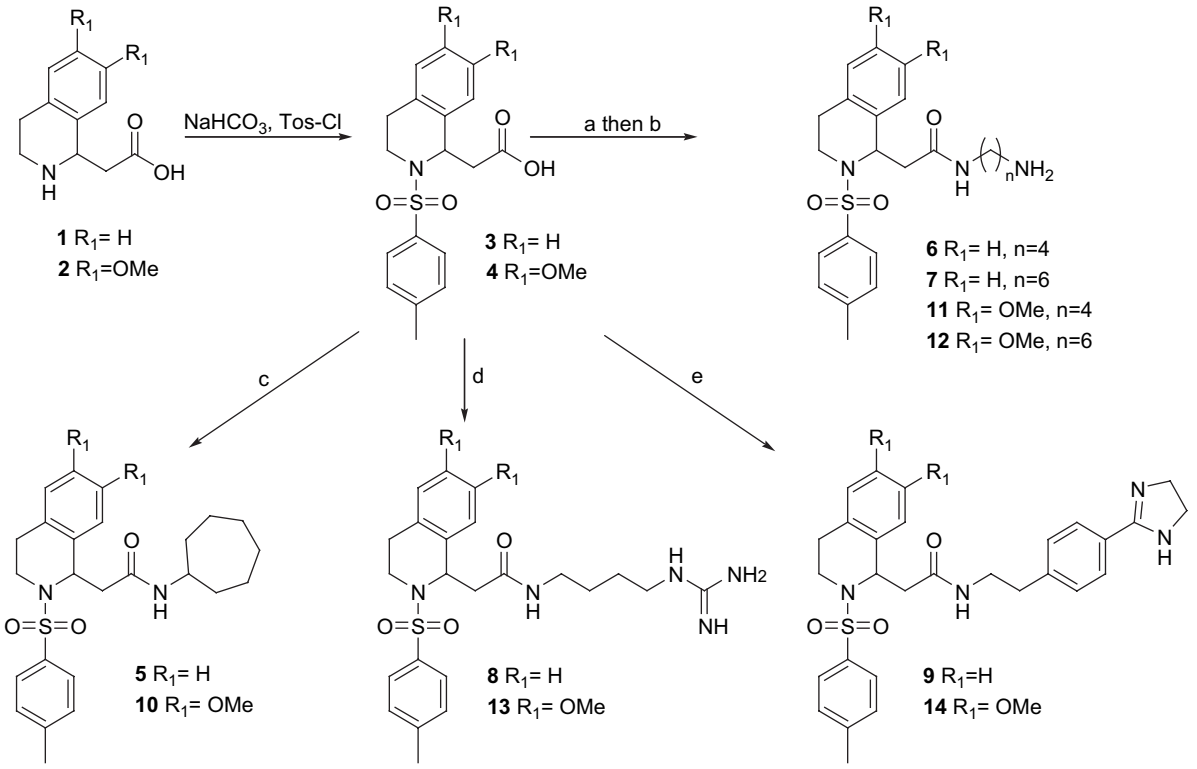
The racemic analogues were synthesized by simple chemical substitutions. Both THIQAA and its 6,7-dimethoxy variant are β-amino acids. The tosylate moiety was introduced into both the molecules first. The carboxylic acid function was then reacted with 5 different amines: cycloheptylamine, 1,4-diaminobutane, 1,4-diaminohexane, agmatine and 2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]ethylamine. In each series, one compound contained only the cycloheptylamine group, while three compounds included flexible alkyl chains of different lengths and a basic side-chain terminal (1,4-diaminobutane, 1,4-diaminohexane and agmatine). The dimethoxy derivative is rather rigid relative to the other amines used. All racemic products were purified by preparative HPLC, and appropriate fractions (>95%) were co-freeze-dried. Amines were gained as trifluoroacetate salts. Lyophilised products were tested in biological assays without desalting or further purification (Scheme 2, Table 1).

The data in Table 1 allow the following conclusions. Modification of the core with methoxy groups decreased the biological activity. Incorporation of a cyclohexyl amide side chain resulted in inactive molecule. Amides of primary alkylamines did not display significant activity. The alkyl guanidino group increased the inhibitory potency of the molecule in the $[Ca^{2+}]_i$ assay, but the activity remained low. Significant activity was observed only for the phenylimidazoline side chain. The positive effect of this key moiety was also observed with the dimethoxy core **14**, although the activity was still almost 2 orders of magnitude lower than that of THIQAA **9**.

The imidazoline-containing side chain with the phenyl group may interact with apolar and aromatic amino acid side chains and is also a linear spacer that may keep the basic group in a position suitable for high affinity binding. Moreover, this side chain is the most rigid (conformationally most restricted) of our set. The positive effect of the non-basic cycloheptylamide on binding, as compared with the alkyl primary amine chains, suggests that the role of a bulky apolar part in the spacer may be important. The primary amine-containing ligands are flexible and might bend back on the core. On the



Scheme 1. The THIQAA core and its modifications.



Scheme 2. Synthesis of B_1 antagonists: (a) DCC/HOBt, (4-aminobutyl)carbamic acid *tert*-butyl ester or (6-aminohexyl)carbamic acid *tert*-butyl ester in DMF; (b) TFA/DCM; (c) DCC/HOBt, cycloheptylamine in DMF; (d) HBTU/HOBt/DIEA in DMF (agmatine was used dissolved in aq. $NaHCO_3$); (e) HBTU/HOBt/DIEA, 2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]ethylamine in DMF.

other hand, a more rigid side chain evidently favours long-distance binding without significant loss of entropy. Further investigations are under way to prove our hypotheses.

3. Conclusions

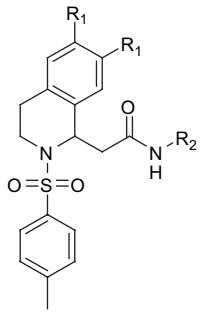
B_1 antagonists containing the THIQAA core and its 6,7-dimethoxy derivative were synthesized. The 6,7-methoxy substitution was found to lower the receptor-binding affinity markedly relative to the unsubstituted core. The best K_i obtained was 0.067 μM for the THIQAA core-containing compound and 1.996 μM for the 6,7-dimethoxy compound with the same substituents. Although the number of examined effective THIQAA-based BK_1 antagonists was not too high, one preliminary conclusion may be drawn from the results. The linear, rigid apolar or rather aromatic spacer with a phenyl group in the amide side chain bearing an imidazolidine basic terminal group may be important in the recognition of the B_1 antagonist by the BK B_1 receptor. Further investigations on the THIQAA core are under way.

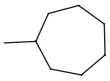
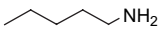
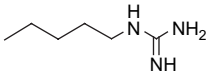
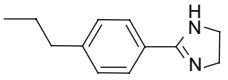
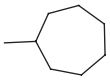
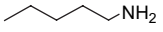
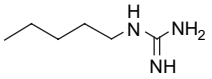
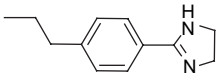
4. Experimental protocols

4.1. Fluorometric measurement of cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$)

All materials and cell culture reagents were from Invitrogen (Carlsbad, CA, USA) or Sigma–Aldrich (St. Louis, MO, USA). The human B_1 receptor-expressing cell line was purchased from Euroscreen (Belgium). For the $[Ca^{2+}]_i$ measurements, we used Chinese hamster ovary (CHO) cells stably expressing recombinant human B_1 receptors. Cells were cultured in Dulbecco's modified Eagle medium containing 10% foetal bovine serum, 1% MEM non-essential amino acid solution, 400 μg/cm^3 G418 and antibiotics (0.25 μg/cm^3 amphotericin B, 100 U/cm^3 penicillin G, and 100 μg/cm^3 streptomycin). Cells were kept at 37 °C in a humidified atmosphere of 5% CO_2/95% air. They were plated into 96-well microplates at a density of 2.5×104 cells/well and measurements of $[Ca^{2+}]_i$ were carried out 1 day after cell plating. After removal of the medium, the cells were loaded with a Ca^{2+}-sensitive fluorescent dye, fluo-4 (2 μM), for 40–120 min in the following buffer: 2 mM $MgCl_2$, 140 mM NaCl, 5 mM KCl, 5 mM HEPES–Na, 5 mM HEPES, 2 mM $CaCl_2$, 20 mM D-glucose, 2 mM probenecid. To remove excess dye, the cells were washed twice with buffer. After washing, the test compounds were added and the cells were incubated for 20–25 min at 37 °C. The test compounds were diluted in buffer from a DMSO stock solution; the final DMSO concentration was <0.1%. After the incubation period, the baseline and the agonist-evoked fluorescence changes were monitored with a plate reader fluorometer (Fluoroskan Ascent, Thermo-Labsystems, Finland). The agonist was [Lys-des-Arg^9]bradykinin applied at EC80 concentration. Excitation was at 485 nm, and the fluorescence emission was detected at 538 nm. The whole measurement process was performed at 37 °C and was controlled

Table 1
Biological assay results on Chinese hamster ovary cells expressing human B_1 receptors (CHO-hB_1)



Compd	R_1	R_2	$[Ca^{2+}]_i$ assay CHO-hB_1, Inhibition (%) (at 5 μM)	Binding assay, CHO-hB_1 K_i (μM)
5	H		37.0	–
6	H		15.9	–
7	H		16.3	–
8	H		44.9	–
9	H		99.0	0.067
10	OMe		9.3	–
11	OMe		15.3	–
12	OMe		10.9	–
13	OMe		19.0	–
14	OMe		85.7	1.996

by custom software. Raw fluorescence data were expressed as $\Delta F/F$ values (fluorescence changes normalized to the baseline). The inhibitory effects of the test compounds were expressed as percentage inhibition of the control agonist response. Data analysis was carried out with Microsoft Excel.

4.2. Human recombinant bradykinin-1 receptor-binding assay

4.2.1. Preparation of membrane

Membrane was prepared from hB_1-A5 cells (expressed in CHO cells) according to the Euroscreen Technical Data Sheet (Cat. No.: ES-091). The cells were dissociated in Dulbecco's phosphate-buffered saline and centrifuged (1500 rpm, 10 min, 4 °C). The pellet was resuspended in 15 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 0.3 mM EDTA, 1 mM EGTA, and homogenized in a glass homogenizer. The crude membrane fraction was collected through two consecutive centrifugation steps at 40,000*g* for 25 min at 4 °C separated by a washing step in the above buffer. The final pellet was resuspended in the buffer 75 mM Tris–HCl (pH 7.5), 12.5 mM $MgCl_2$, 0.3 mM EDTA, 1 mM EGTA, 250 mM sucrose. The membrane was divided into aliquots, flash-frozen and stored at −80 °C until use. Protein concentrations were determined with the BCA method.

4.2.2. Binding conditions

Binding assays were performed in triplicate in DeepWell plates containing the binding buffer [50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$], hB_1 membrane (20 μg protein/tube), and [^{3}H]Lys-BK (Perkin Elmer Life Sciences) as radioligand. Non-specific binding was determined in the presence of

10 μM Lys-des-Arg^9-BK. The samples were incubated in a final volume of 0.25 cm^3 for 15 min at 25 °C. Binding reactions were terminated by rapid filtration through a 96-well GF/B Unifilter presoaked for at least 1 h in 0.5% PEI. The filters were washed four times with 1 cm^3 of ice-cold washing buffer (same composition as the binding buffer), using a Brandel harvester. The filters were allowed to dry, Perkin Elmer Microscint 20 scintillant was added (20 μl/well) and the bound radioactivity was determined with a Packard TopCount scintillation counter for 3 min. Data were analysed with Prism, Graph Pad Software.

4.3. Chemistry

Commercial chemicals and solvents were of reagent grade and used without further purification. The following abbreviations are used: DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCM, dichloromethane; DIEA, ethyldiisopropylamine; DCU, *N*,*N*′-dicyclohexylurea; DMF, dimethyl formamide; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; and TFA, trifluoroacetic acid. Preparative reverse-phase HPLC was performed on a Shimadzu SPD-6AV 3000 liquid chromatograph, using a C18 reverse-phase silica gel column (Phenomenex, Jupiter 250 × 21.2 mm, 15 μ, 300 Å). Eluent: acetonitrile (20–100%, v/v) in water containing 0.1% (v/v) TFA. Peak detection was at 254 nm. NMR: with a Bruker Avance DRX 500 Spectrometer (^{1}H: 500.13 MHz), $CDCl_3$ or DMSO-d_6 solutions, δ (ppm), J (Hz). Spectral patterns: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad. Mass spectra (MS) were measured on Bruker Esquire 3000+ and Waters Q-Tof Premier instruments. Low-resolution MS were acquired on a Bruker Esquire 3000+ ion trap mass spectrometer, equipped with an electrospray ionization source. High-resolution exact mass measurements were performed on a hybrid, quadrupole-orthogonal acceleration time-of-flight mass spectrometer (Waters Q-Tof Premier), equipped with an electrospray ionization source. Samples were dissolved in acetonitrile:water = 1:1, 0.1% acetic acid (v/v) solvent mixture. High-resolution experiments were performed in the presence of 0.2 ng/μl leucine enkephalin peptide as lock mass calibration standard. Chemical yields were not optimized.

4.3.1. [1-(Toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetic acid **3**

$NaHCO_3$ (672 mg, 8 mmol) was added to a solution of (1,2,3,4-tetrahydroisoquinolin-2-yl)acetic acid (**1**, 765 mg, 4 mmol) in distilled water (3 cm^3), followed by the dropwise addition of 4-methylbenzenesulfonyl chloride (915 mg, 4.8 mmol) in acetone (5 cm^3) and the reaction mixture was then stirred for 4 h at room temperature at pH 8. Acetone was removed under vacuum and the residue was extracted with $NaHCO_3$/diethyl ether (5 cm^3). The basic solution was acidified with solid $NaHSO_4$ to pH 4–5 and extracted with ethyl acetate (3 × 10 cm^3). The combined organic phases were dried, filtered and concentrated. The crude product was crystallized from ethyl acetate/diethyl ether and gave **3** (1040 mg, 75%). MS (APCI) Calcd for $C_{18}H_{19}NO_4S$: *m*/*z* 345.41 (M^+). Found: *m*/*z* 345.97 (M^+). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.6 (2H, d, J = 8 Hz), 7.25 (2H, d, J = 8 Hz), 7.15 (1H, m), 7.09 (2H, m), 6.97 (1H, m), 5.33 (1H, t, J = 7 Hz), 3.63 (2H, m), 3.49 (2H, m), 2.6 (2H, m), 2.3 (3H, s).

4.3.2. [6,7-Dimethoxy-2-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-1-yl]acetic acid **4**

Compound **4** (1.035 g, 63%) was prepared from **2** according to the method for the preparation of **3**. MS (ESI) Calcd for $C_{20}H_{23}NO_6S$: *m*/*z* 406.46 ($M + H^+$). Found: *m*/*z* 409.02 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (2H, d, J = 8 Hz), 7.26 (2H, d, J = 8 Hz), 6.75 (1H, s), 6.52 (1H, s), 5.28 (1H, t, J = 7 Hz), 3.68 (3H, s), 3.66 (2H, m), 3.64 (3H, s), 3.42 (2H, m), 2.64 (2H, t, J = 6 Hz), 2.31 (3H, s).

4.3.3. N-Cycloheptyl-2-[1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetamide **5**

To a solution of [1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetic acid (**3**, 100 mg, 0.29 mmol) in DMF (1 cm^3), DCC (72 mg, 0.35 mmol) and HOBt (50 mg, 0.35 mmol) were added, the reaction mixture was stirred for 30 min, and DCU was then filtered off. Cycloheptylamine (44 μl, 0.35 mmol) was next added to the solution, which was subsequently stirred for 4 h at room temperature. DMF was removed under vacuum and the residue was dissolved in ethyl acetate (20 cm^3), washed three times with saturated $NaHSO_4$, and with saturated $NaHCO_3$ and once with brine, dried with Na_2SO_4, filtered and concentrated. The crude residue was purified by HPLC to give **5** (79 mg, 62%). HRMS (EI) Calcd for $C_{25}H_{32}N_2O_3S$: *m*/*z* 441.2212 ($M + H^+$). Found: *m*/*z* 441.2224 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (2H, d, J = 8 Hz), 7.09 (5H, m), 6.88 (1H, d, J = 8 Hz), 6.13 (1H, d, J = 7 Hz), 5.38 (1H, d, J = 7 Hz), 3.96 (1H, m), 3.8 (1H, m), 3.52 (1H, m), 2.81 (1H, dd, J = 8 Hz), 2.61 (1H, d, J = 6 Hz), 2.57 (2H, m), 2.32 (3H, s), 1.94 (2H, m), 1.61 (4H, m), 1.44 (6H, m).

4.3.4. N-(4-Aminobutyl)-2-[1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydro-isoquinolin-2-yl]acetamide **6**

To a solution of [1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetic acid (**3**, 75 mg, 0.22 mmol) in DMF (1 cm^3), DCC (54 mg, 0.26 mmol) and HOBT (35 mg, 0.26 mmol) were added, the reaction mixture was stirred for 30 min, and DCU was then filtered off. (4-Aminobutyl)carbamic acid *tert*-butyl ester (62 mg, 0.33 mmol) was added to the solution, which was next stirred for 4 h at room temperature. DMF was removed under vacuum and the residue was dissolved in ethyl acetate (20 cm^3), washed three times with saturated $NaHSO_4$, $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was dissolved in 50% TFA/DCM (5 cm^3), the reaction mixture was stirred for 20 min at room temperature and the solvent was then removed. The crude product was purified by HPLC to give **6** (39 mg, 43%). HRMS (EI) Calcd for $C_{22}H_{29}N_3O_3S$: *m*/*z* 416.2008 ($M + H^+$). Found: *m*/*z* 416.2021 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (1H, m), 7.73 (3H, m), 7.59

(2H, d, $J = 8$ Hz), 7.25 (2H, d, $J = 8$ Hz), 7.08 (3H, m), 6.97 (1H, m), 5.35 (1H, t, $J = 7$ Hz), 3.66 (2H, m), 2.98 (2H, m), 2.76 (2H, m), 2.63 (2H, m), 2.5 (2H, m), 2.29 (3H, s), 1.48 (2H, m), 1.37 (2H, m).

4.3.5. N-(6-Aminohexyl)-2-[1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetamide ***7***

Compound **7** (28 mg, 29%) was prepared from **3** according to the method for the preparation of **6** using (4-aminohexyl)-carbamic acid *tert*-butyl ester instead of (4-aminobutyl)carbamic acid *tert*-butyl ester. HRMS (EI) Calcd for $C_{24}H_{33}N_3O_3S$: *m/z* 444.2321 ($M + H^+$). Found: *m/z* 444.2306 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 (1H, m), 7.59 (2H, d, $J = 8$ Hz), 7.25 (2H, d, $J = 8$ Hz), 7.09 (3H, m), 6.98 (1H, m), 5.35 (1H, t, $J = 7$ Hz), 3.66 (2H, m), 3.00 (1H, m), 2.92 (1H, m), 2.76 (2H, m), 2.65 (2H, m), 2.3 (3H, s), 1.5 (2H, m), 1.26 (5H, m), 1.16 (3H, m) (NH_3^+ signal is likely under the water peak).

4.3.6. N-(4-Guanidinobutyl)-2-[1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetamide ***8***

To a solution of [1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetic acid (**3**, 150 mg, 0.43 mmol) in DMF (2 cm^3), HBTU (330 mg, 0.86 mmol), HOBt (117 mg, 0.86 mmol) and DIEA (139 μl, 0.86 mmol) were added. The solution was next added dropwise to a solution of agmatine sulfate (200 mg, 0.86 mmol) in aqueous $NaHCO_3$ (147 mg, 1.72 mmol in 2 cm^3 water), and the reaction mixture was then stirred at room temperature overnight. The solvents were removed under vacuum and the residue was dissolved in ethyl acetate (20 cm^3), washed three times with saturated $NaHSO_4$, $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by HPLC to give **8** (98 mg, 45%). HRMS (EI) Calcd for $C_{23}H_{31}N_5O_3S$: *m/z* 458.2226 ($M + H^+$). Found: *m/z* 458.2229 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.83 (1H, t, $J = 6$ Hz), 7.59 (2H, d, $J = 8$ Hz), 7.49 (1H, t, $J = 5$ Hz), 7.25 (2H, d, $J = 8$ Hz), 7.08 (3H, m), 6.97 (1H, t, $J = 4$ Hz), 6.9 (4H, m), 5.35 (1H, t, $J = 7$ Hz), 3.65 (2H, m), 3.05 (2H, m), 2.95 (2H, m), 2.65 (2H, m), 2.5 (2H, m), 2.29 (3H, s), 1.36 (4H, m).

4.3.7. N-{2-[4-(4,5-Dihydro-1H-imidazol-2-yl)phenyl]ethyl}-2-[1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetamide ***9***

To a solution of [1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetic acid (**3**, 138 mg, 0.40 mmol) in DMF (1 cm^3), HBTU (303 mg, 0.8 mmol), HOBt (108 mg, 0.80 mmol) and DIEA (128 μl, 0.80 mmol) were added and the reaction mixture was stirred for 15 min. 2-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]ethylamine dihydrochloride (126 mg, 0.48 mmol) was then added, and the solution was stirred for 4 h at room temperature. DMF was removed under vacuum and the residue was dissolved in ethyl acetate (20 cm^3), washed three times with saturated $NaHSO_4$, saturated $NaHCO_3$ and once with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by HPLC to give **9** (60 mg, 29%). HRMS (EI) Calcd for $C_{29}H_{32}N_4O_3S$: *m/z* 517.2273 ($M + H^+$). Found: *m/z* 517.2265 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.46 (2H, s), 7.96 (1H, t, $J = 7$ Hz), 7.84 (2H, d, $J = 8$ Hz), 7.61 (2H, d, $J = 8$ Hz), 7.45 (2H, d, $J = 8$ Hz), 7.26 (2H, d, $J = 8$ Hz), 7.1 (3H, m), 7.0 (1H, t, $J = 5$ Hz), 5.37 (1H, t, $J = 7$ Hz), 4.01 (4H, s), 3.69 (2H, m), 3.5 (2H, m), 3.27 (2H, t, $J = 4$ Hz), 2.77 (2H, m), 2.66 (2H, m), 2.22 (3H, s).

4.3.8. N-Cycloheptyl-2-[6,7-dimethoxy-1-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl]acetamide ***10***

Compound **10** (81 mg, 44%) was prepared from **4** according to the method for the preparation of **5**. HRMS (EI) Calcd for $C_{27}H_{36}N_2O_5S$: *m/z* 501.2423 ($M + H^+$). Found: *m/z* 501.2426 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (2H, d, $J = 8$ Hz), 7.13 (2H, d, $J = 8$ Hz), 6.62 (1H, s), 6.38 (1H, d, $J = 8$ Hz), 6.32 (1H, s), 5.32 (1H, t, $J = 5$ Hz), 3.97 (2H, m), 3.87 (3H, s), 3.83 (3H, s), 3.46 (1H, m), 2.82 (1H, dd, $J = 8$ Hz), 2.65 (1H, dd, $J = 5$ Hz), 2.44 (2H, m), 2.34 (3H, s), 1.94 (2H, m), 1.61 (4H, m), 1.51 (6H, m).

4.3.9. N-(4-Aminobutyl)-2-[6,7-dimethoxy-2-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-1-yl]acetamide ***11***

Compound **11** (44 mg, 25%) was prepared from **4** according to the method for the preparation of **6**. HRMS (EI) Calcd for $C_{24}H_{33}N_3O_5S$: *m/z* 476.2219 ($M + H^+$). Found: *m/z* 476.2217 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (1H, t, $J = 5$ Hz), 7.73 (3H, m), 7.58 (2H, d, $J = 8$ Hz), 7.26 (2H, d, $J = 8$ Hz), 6.66 (1H, s), 6.52 (1H, s), 5.28 (1H, t, $J = 7$ Hz), 3.66 (3H, s), 3.64 (3H, s), 3.5 (2H, m), 3.43 (2H, m), 2.98 (2H, m), 2.76 (2H, m), 2.52 (2H, m), 2.3 (3H, s), 1.47 (2H, m), 1.38 (2H, m).

4.3.10. N-(6-Aminohexyl)-2-[6,7-dimethoxy-2-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-1-yl] acetamide ***12***

Compound **12** (50 mg, 27%) was prepared from **4** according to the method for the preparation of **7**. HRMS (EI) Calcd for $C_{26}H_{37}N_3O_5S$: *m/z* 504.2532 ($M + H^+$). Found: *m/z* 504.2533 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.78 (3H, m), 7.59 (2H, d, $J = 8$ Hz), 7.26 (2H, d, $J = 8$ Hz), 6.65 (1H, s), 6.53 (1H, s), 5.28 (1H, t, $J = 7$ Hz), 3.67 (5H, m), 3.64 (3H, s), 3.38 (2H, m), 2.97 (2H, m), 2.75 (2H, m), 2.53 (2H, m), 2.31 (3H, s), 1.49 (2H, m), 1.17 (6H, m).

4.3.11. 2-[6,7-Dimethoxy-2-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-1-yl]-N-(4-guanidinobutyl) acetamide ***13***

Compound **13** (71 mg, 32%) was prepared from **4** according to the method for the preparation of **8**. HRMS (EI) Calcd for $C_{25}H_{35}N_5O_5S$: *m/z* 518.2437 ($M + H^+$). Found: *m/z* 518.2437 ($M + H^+$). ^{1}H NMR (500 MHz, DMSO-d_6) 7.85 (1H, t, $J = 6$ Hz), 7.59 (2H, d, $J = 8$ Hz), 7.48 (1H, t, $J = 5$ Hz), 7.26 (2H, d, $J = 8$ Hz), 6.9 (4H, m), 6.65 (1H, s), 6.52 (1H, s), 5.28 (1H, t, $J = 7$ Hz), 3.69 (2H, m), 3.66 (3H, s), 3.64 (3H, s), 3.42 (2H, m), 3.06 (2H, m), 3.00 (2H, m), 2.5 (2H, m), 2.3 (3H, s), 1.41 (2H, m), 1.36 (2H, m).

*4.3.12. N-{2-[4-(4,5-Dihydro-1H-pyrrol-2-yl)phenyl]ethyl}-2-[6,7-dimethoxy-2-(toluene-4-sulfonyl)-1,2,3,4-tetrahydroisoquinolin-1-yl] acetamide **14***

Compound **14** (36 mg, 29%) was prepared from **4** according to the method for the preparation of **9**. HRMS (EI) Calcd for $C_{31}H_{36}N_4O_5S$: *m/z* 577.2485 ($M + H^+$). Found: *m/z* 577.2488 ($M + H^+$). ^1H NMR (500 MHz, DMSO-d_6) δ 10.43 (2H, s), 7.96 (1H, t, $J = 6$ Hz), 7.83 (2H, d, $J = 8$ Hz), 7.59 (2H, d, $J = 8$ Hz), 7.44 (2H, d, $J = 8$ Hz), 7.25 (2H, d, $J = 8$ Hz), 6.66 (1H, s), 6.53 (1H, s), 5.29 (1H, t, $J = 7$ Hz), 4.00 (4H, s), 3.68 (2H, m), 3.67 (3H, s), 3.64 (3H, s), 3.52 (2H, s), 3.41 (2H, m), 3.26 (2H, m), 2.76 (2H, m), 2.21 (3H, s).

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