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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of piperazine derivatives as novel isoform selective voltage-gated sodium (Na_v) 1.3 channel modulators

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Abstract Sponges of the genus *Agelas* produce compounds that modulate the activity of voltage-gated sodium ion channels and contribute novel scaffolds for the development of compounds with activity against a plethora of biological targets. In particular, clathrodin and dibromosceptrin were reported to decrease the average maximum amplitude of inward sodium currents in isolated chick embryo sympathetic ganglia cells; we envisaged these compounds as a starting point to design novel Na_v channel modulators. This endeavor was part of our long-term goal of designing a comprehensive library of *Agelas* alkaloid analogs that would cover a broader chemical space and allow us to examine the activity of such compounds on Na_v channels. Our series of compounds was designed by maintaining the terminal structural features found in clathrodin while rigidizing the central part of the molecule and replacing the 3-aminopropene linker with a 4-methylenepiperazine moiety. Synthesised compounds were screened for inhibitory action against the human voltage-gated sodium channel isoforms Na_v 1.3, Na_v 1.4, cardiac Na_v 1.5, and Na_v 1.7 using an automated patch clamp electrophysiology technique. The results demonstrate that we have obtained a series of compounds with a modest but selective inhibitory activity against the Na_v 1.3 channel isoform. The most potent compound showed selective activity against the Na_v 1.3 channel isoform with an IC_{50} of 19 μM and is a suitable starting point for further development of selective Na_v 1.3 channel modulators. Such compounds could prove to be beneficial as a pharmacological tool towards the development of novel therapeutically useful compounds in the treatment of pain.

Keywords Voltage-gated · Sodium channels · Na_v channels · Na_v channel modulators · Isoform selective modulators · Piperazine derivatives

Introduction

Voltage-gated sodium channels (Na_v channels) are large transmembrane proteins capable of selective sodium ion transmission, and they are responsible for the generation of action potentials. Na_v channels enable the spreading of electrical impulses through nerve, muscle, and endocrine cell systems (Catterall, 2000). Research on the mechanism of action of antiepileptic drugs and neurotoxins such as tetrodotoxin revealed that these molecules act as sodium channel modulators (Termin *et al.*, 2008). More recently, voltage-gated sodium channel α and β subunit mutations have been associated with pathological conditions such as cardiac arrhythmias, myotonia, periodic paralysis, familial hemiplegic migraine, epilepsy, congenital analgesia, and neuropathic pain (Andavan and Lemmens-Gruber, 2011). Moreover, research on voltage-gated sodium ion channels represents a significant portion of academic and industrial medicinal chemistry endeavors, and they are attractive therapeutic targets (Clare *et al.*, 2000). The Na_v 1.3 channel

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isoform is found in the embryonic CNS and is also expressed in damaged nerve tissues and could be associated with various pain states. Na_v channels have also been found in heart, uterus, lung, DRG, and glia tissues, where they contribute to pain and electrolyte imbalance conditions (England and de Groot, 2009; Jukič *et al.*, 2013). Non-selective voltage-gated sodium channel modulators are already present in *materia medica* and have been extensively reviewed (Bölcskei *et al.,* 2008; Anger *et al.*, 2001; Taylor, 1996). Such compounds are commonly developed without structural information on their respective targets, and they are commonly used as treatments for conditions such as epilepsy (lamotrigine, carbamazepine), cardiac arrhythmias (mexiletine), and numerous pain states (lidocaine).

Despite their widespread use, non-selective voltage-gated sodium channel modulators provide only modest therapeutic potential and often exhibit unwanted side effects. Non-selective Na_v channel modulators interact with α channel subunit S5–S6 pore lining segments; because the pore forming region is highly conserved among Na_v channel isoforms, selectivity of such compounds is low. Activity on distinct Na_v isoforms contributes to the adverse effects observed in clinically used therapies where the modulators presumably act on basal brain functions, skeletal muscle, and/or the heart (England and de Groot, 2009). With acquired and expanding knowledge on Na_v channel isoforms and their unique role(s), selective targeting and/or development of state-dependent modulators on individual Na_v channel isoforms could be beneficial (England and de Groot, 2009; Jukič *et al.*, 2013).

Interest in natural compounds as novel therapeutic leads was rekindled in recent years, as such compounds cover a significant portion of chemical space, and they often exert favorable pharmacokinetic profiles and have activity against numerous biological targets (Neuman, 2008; Vuorela *et al.*, 2004). Marine organisms represent an under-exploited source of natural compounds; sponges and their sponge-symbiotic microorganisms produce a variety of compounds with selectivity against biological targets, including cytotoxins, antibiotics, antivirals, anti-inflammatory compounds, and antifouling agents (Laport *et al.*, 2009). Sponges of the genus *Agelas* have been shown to produce compounds that modulate the activity of voltage-gated sodium ion channels and contribute novel scaffolds for the development of active compounds against a plethora of biological targets (Rivera Rentas *et al.*, 1995; Cafieri *et al.*, 1997; Richards *et al.*, 2008; Keifer *et al.*, 1991). In particular, alkaloids isolated from the marine sponge of the *Agelas* genus have shown modulatory activity on voltage-gated sodium and calcium channels. Clathrodin **1** (Fig. 1) and dibromosceptrin **2** (Fig. 1) decreased the average maximum amplitude of inward sodium currents in isolated chick embryo sympathetic ganglia cells by 27 and 40 %, respectively (Rivera Rentas *et al.*, 1995; Perdicaris *et al.*, 2013). *Agelas* sponge alkaloids have also been found to reduce voltage-dependent calcium elevation in PC12 cells (Bickmeyer *et al.*, 2002). Despite the very limited data on the activity of *Agelas* sponge alkaloids, we envisaged these compounds as a starting point to design novel potential Na_v channel modulators. This endeavor was a part of our long-term goal of designing a comprehensive library of *Agelas* alkaloid analogs that would cover a broader chemical space and allow us to examine the potential of such compounds as Na_v channel modulators (Tomašić *et al.*, 2013). The idea was also prosecuted by Hodnik *et al.*, who reported compound **3** (R_6, R_7 = Br, Fig. 1) as a Na_v 1.4 channel selective state-dependent modulator with an IC_{50} value of 15 μM for the open-inactivated state (Hodnik *et al.*, 2013). This series of compounds was designed by maintaining the terminal structural features found in clathrodin while rigidizing the central part of the molecule with a sterically restricted condensed cyclohexane ring and shortening the 3-aminopropene linker by one methylene group (compound **1**, Fig. 1).

In contrast to reported compound **3** (R_6, R_7 = Br, Fig. 1), our approach focused on the replacement of the native 3-aminopropene linker with the less sterically restricted 4-methylenepiperazine moiety (Teixeira *et al.*, 2013). Furthermore, we sought to investigate the importance of terminal structures found in the native clathrodin compound. 2-aminoimidazole is a common structural motif amongst *Agelas* alkaloids, so we postulated that the terminal basic 2-aminoimidazole is a key moiety responsible for activity against Na_v channels. Interestingly, 2-aminoimidazole, which is thought to be ionized in physiological conditions (Storey *et al.,* 1964), is commonly found in marine sponge alkaloids, and is a building block used in a multitude of small molecule drugs (Žula *et al.*, 2013). The pyrrole-2-carbonyl structure from clathrodin was replaced with various heteroaromatic structures and a series of compounds with this feature was synthesized (**4**). To determine the importance of the 2-aminoimidazole moiety, we have also replaced it with 2-aminothiazole or a 2-unsubstituted imidazole, while retaining the pyrrole-2-carbonyl substituent, to afford a small series of compounds (**5**). We also wanted to biologically evaluate the activity of the small focused library of synthesized piperazine analogs (**4**, **5**) against several Na_v channel isoforms using the voltage clamp technique (Fig. 1; Table 2).

Molecular modeling

Structural comparison of piperazine analogs to *Agelas* alkaloids clathrodin and oroidin was performed using TanimotoCombo scores calculated with the vROCS

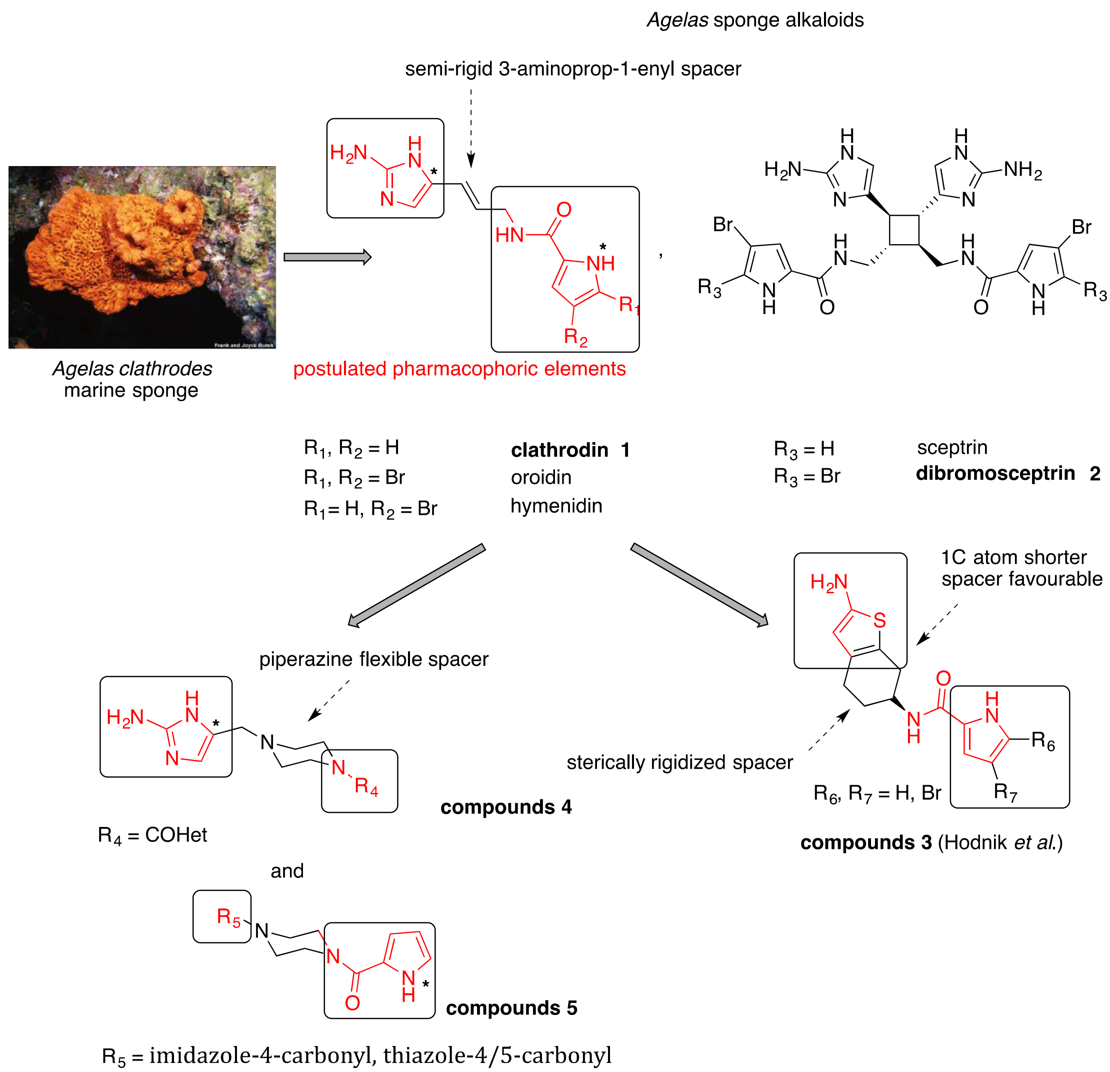
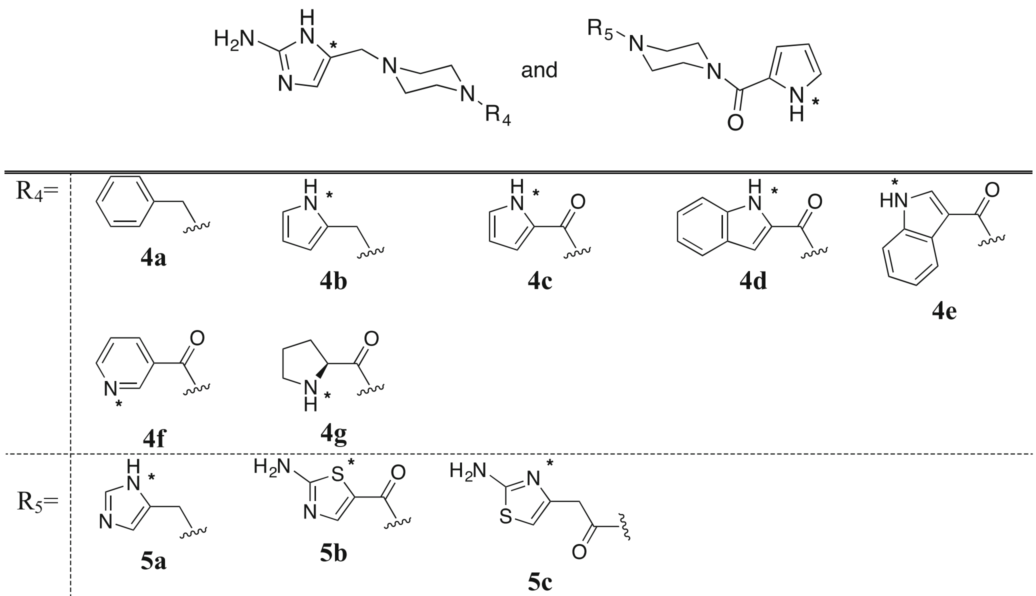


Fig. 1 Design of clathrodin-derived compounds. Photograph of *Agelas clathrodes* sponge from BioLib (Burek and Burek, 2013). *Asterisk* marked atoms were selected for distance measurements

software package (vROCS version 3.1.2. OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com, 2013; Grant *et al.*, 1996; Hawkins *et al.*, 2007; Tuccinardi *et al.*, 2009). The starting ligand geometries were optimized with ChemBio 3D Ultra 13.0 (CambridgeSoft) using the MM2 force field until a minimum 0.100 root mean square (RMS) gradient was reached. The optimized structure was further refined with the GAMESS interface using the semi-empirical PM3 method, the QA optimization algorithm and Gasteiger Hückel charges for all atoms for 100 steps. 2-aminoimidazoles were kept in their ionized state, corresponding to their ionization state in physiological pH (that is, 7.4). Conformer libraries were prepared for compounds **4a–g** and **5a–c** (Table 1; 151, 200, 74, 200, 200, 172, 136, 112, 106, and 200 conformers, respectively) using OMEGA software from OpenEye Scientific Software Inc. (OMEGA version 2.4.6. OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com, 2013; Hawkins *et al.*, 2010; Hawkins and Nicholis, 2012). vROCS overlays the conformer library on a query structure and performs ranking according to structure similarity based on molecular shape (shape score) and types of atoms (color score); molecules similar to the query structure obtain a favorable ROCS_TanimotoCombo score. The distances between two postulated key groups were also measured using Chem3D 13.0 Ultra. Atoms selected for distance measurements are designated in Fig. 1 and Table 1, respectively. Structure graphic overlays were performed using VIDA and vROCS software packages from OpenEye Scientific Software Inc. (VIDA version 4.2.1. OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com, 2013; Fig. 2).

Table 1 Synthesised clathrodin analogs



Atoms marked with an asterisk (*) were selected for distance measurements

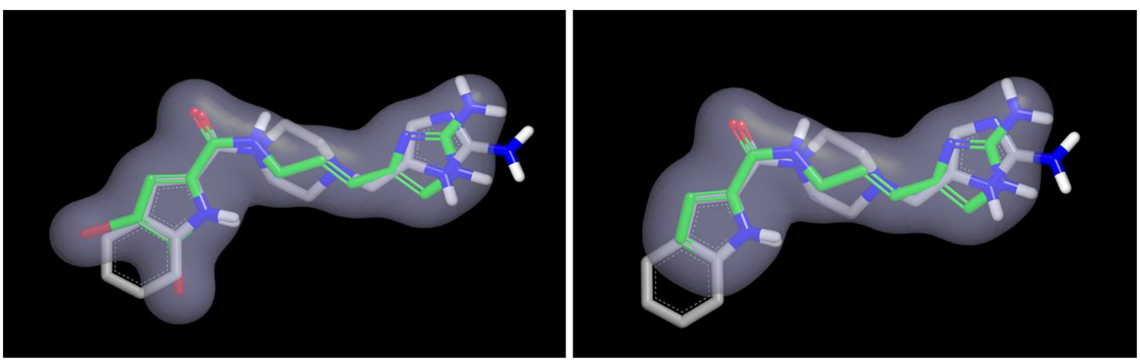


Fig. 2 *Left* Overlay of compound **4d** and oroidin structure (*green*); *right* overlay of compound 4d and clathrodin structure (*green*) (Color figure online)

Chemistry

The first two piperazine-linker analogs with an unsymmetrical substitution on the spacer moiety (compounds **4a** and **4b**) were synthesized using heteroaromatic aldehydes that were subjected to reductive amination with mono *N*-Cbz-protected piperazine and $Na(OAc)_3BH$ in dichloromethane or $NaCNBH_3$ in methanol as a solvent to give products **7a–b** in good yields (Abdel-Magid *et al.*, 1996). Further Cbz deprotection with H_2 and Pd/C (10 %) produced free-amino intermediates that were immediately subjected to similar reductive amination conditions with intermediate **6** to afford the unsymmetrical triple-Boc protected compound (Little and Webber, 1994). Due to the instability of the triple-Boc protected compound, deprotection with TFA in dichloromethane was promptly performed to afford the final product. Hydrochlorides of amino compounds **4a** and **4b** were obtained by subjecting the deprotected compounds to 1 M HCl in ethanol solution (Fig. 3; Table 1), or by direct deprotection with 1 M HCl solution.

The synthesis of other unsymmetrical piperazine-linker clathrodin analogs was envisioned through the coupling of heteroaromatic carboxylic acid **8** with intermediate **10** (Fig. 4, bottom) using an EDC/HOBt coupling procedure to afford Boc-protected target compounds (Anderluh *et al.*,

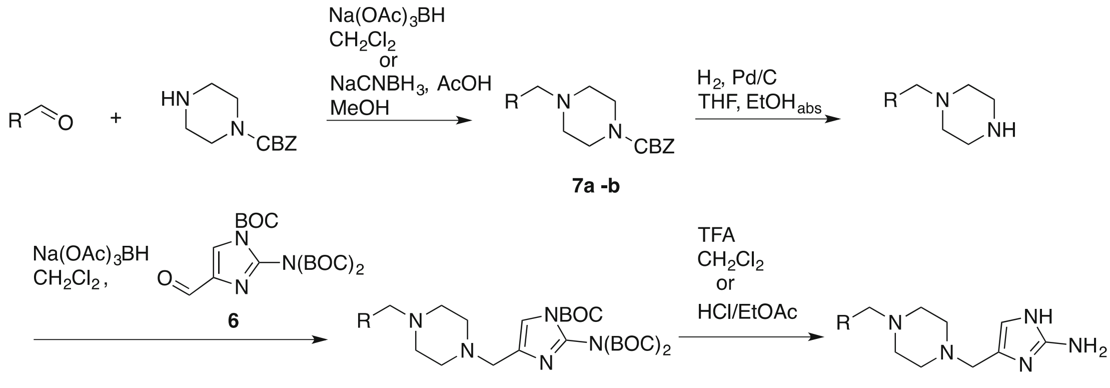


Fig. 3 Synthesis of compounds **4a** and **4b**

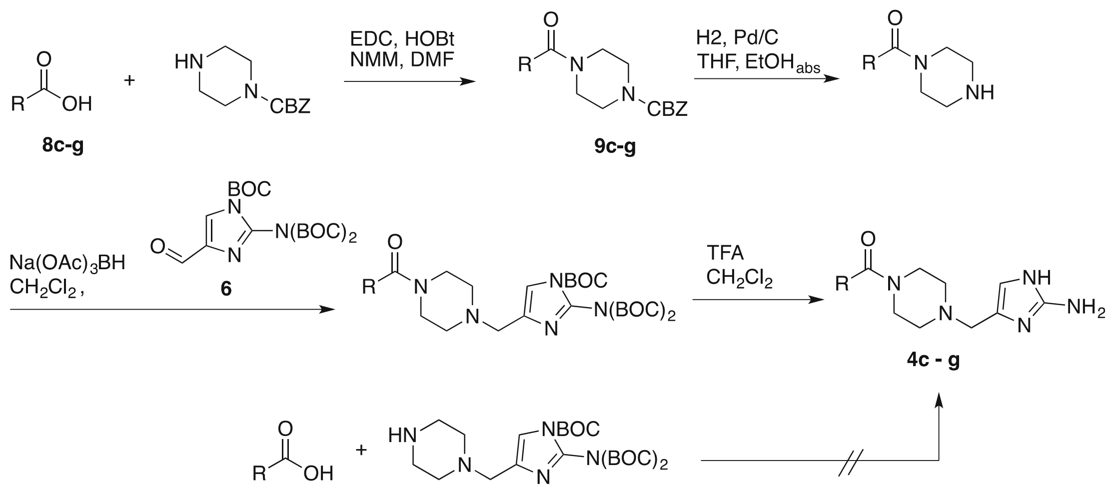


Fig. 4 Synthesis of compounds **4c–g**

2005). Compound **10** was obtained by reductive amination of mono *N*-Cbz piperazine with intermediate **6** (Fig. 4) using $Na(OAc)_3BH$ in dichloromethane and subsequent Cbz deprotection under H_2 and Pd/C (10 %), but **10** unfortunately proved to be unstable under storage and upon subjection to further reaction conditions (Little and Webber 1994). Because concise convergent synthesis could thus not be accomplished, an alternative synthesis from the heteroaromatic starting compound was pursued.

Heteroaromatic carboxylic acids were coupled to monoprotected *N*-Cbz-piperazine using EDC/HOBt in excellent yields to afford key intermediates **9c–g**. The Cbz group was cleaved using H_2 and Pd/C (10 %) in THF/ $EtOH_{abs}$. Because of the instability of the key free-amino compounds, they were immediately subjected to reductive amination with compound **6** using $Na(OAc)_3BH$ (Abdel-Magid *et al.*, 1996) in dichloromethane to afford triple-Boc protected final compounds. Synthesis was concluded with direct and delicate Boc cleavage in trifluoroacetic acid (TFA)/dichloromethane to yield trifluoroacetate salts of compounds **4c–g**. Hydrochlorides were prepared by subjecting trifluoroacetates to 1 M HCl in ethanol solution (Fig. 4; Table 1).

Compounds **5a–c** were synthesized in a manner similar to compounds **4c–g** by initial coupling of pyrrole-2-carboxylic acid to mono *N*-protected Cbz-piperazine with EDC/HOBt and Cbz cleavage using H_2 and Pd/C (10 %) to give common intermediate **11**. **11** was subjected to reductive amination with 1*H*-imidazole-5-carbaldehyde in methanol using $NaCNBH_3$ and a catalytic amount of acetic acid to give compound **5a**. Boc protected **5b** was synthesized from **11** and 2 *N*-Boc-amino-thiazole-5-carboxylic acid using a coupling procedure with EDC in DMF solvent and Et_3N as a base. The deprotected trifluoroacetate salt of compound **5b** was obtained with trifluoroacetic acid in dichloromethane. Compound **5c** was obtained directly from

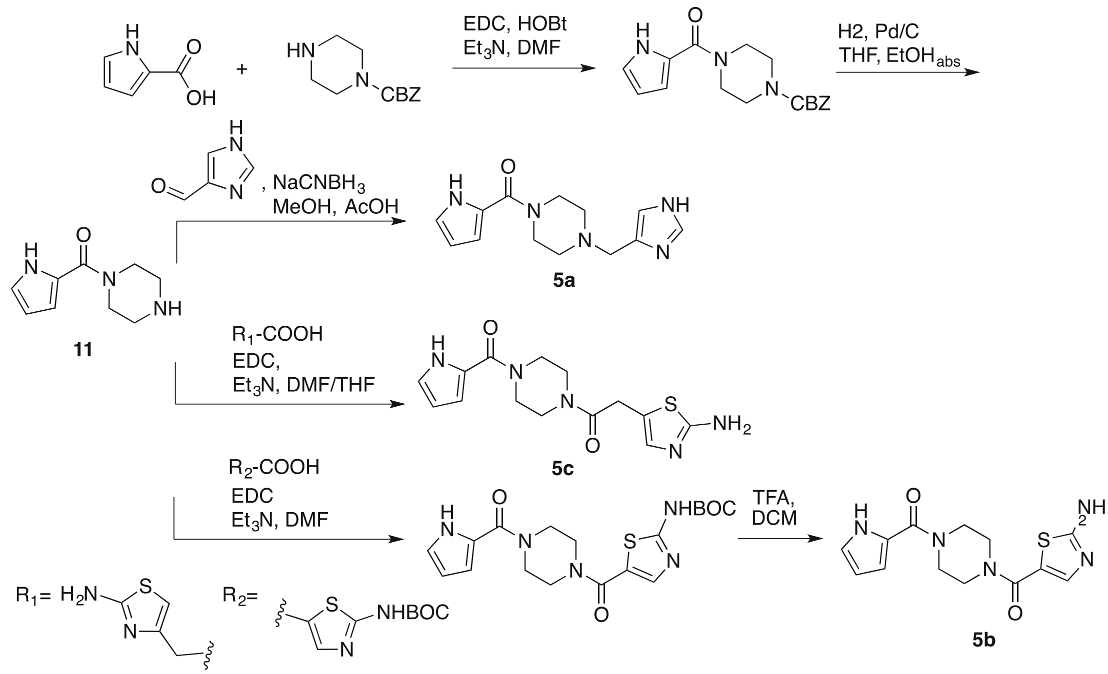


Fig. 5 Synthesis of compounds **5a–c**

11 with unprotected 2-amino-4-thiazoleacetic acid using a slightly modified coupling procedure with EDC in THF/DMF and Et_3N as a base (Fig. 5; Table 1).

Key intermediate **6** was synthesized starting with 1,1-dimethoxypropan-2-one that was subjected to α-keto bromination (Fig. 6). Due to compound instability, 3-bromo-1,1-dimethoxypropan-2-one was directly used in double nucleophilic substitution using BOC-protected guanidine to afford cyclized **12**. The free-amino group on compound **12** was additionally protected using a classic procedure with BOC-anhydride and DMAP catalyst in THF to afford triple BOC protected **13**. The last step was delicate dimethyl acetal deprotection towards the final aldehyde compound. This reaction presumably proceeds via a substrate exchange mechanism using elemental iodine. Deprotection was performed in dry acetone on an ice bath, followed by final quenching with sodium thiosulfate to afford **6** (Sun *et al.*, 2004).

Biological evaluation

Biological assays were conducted at Xention Limited, as previously described by Hodnik *et al.* (2013). Synthesised compounds were screened for inhibitory action on the human voltage-gated sodium channel isoforms Na_v 1.3, Na_v 1.4, cardiac Na_v 1.5, and Na_v 1.7 using the automated patch clamp electrophysiology technique on the Sophion QPatch HT system (Sophion Bioscience A/S). IC_{50} values were calculated from concentration–response graphs measured at 4 relevant concentrations ranging from 0.3 to 10 μM.

Cells were prepared by dissociation from T175 cell culture flasks using trypsin–EDTA (0.05 %) and were kept in serum free media on board the QPatch HT system. Cells were sampled, washed, and re-suspended in extracellular recording solution by the QPatch HT before application to well sites on the chip. 0.1 % v/v DMSO solution was applied to the control cells (4 min total) for comparison to cells treated with test sample concentrations (4 min incubation per test concentration). Samples were prepared in extracellular solution with serial dilutions from 10 to 0.3 μM concentration. Measurements were performed on Na_v 1.3, Na_v 1.4, and Na_v 1.7 cell lines using a standard two-pulse voltage protocol. From a holding potential of −100 mV, a 20 ms to −20 mV activating step was applied to measure the effect of the compounds on the resting state. The second activating pulse was applied following a 5-s pre-pulse to achieve half inactivation potential to assess the effect on the open-inactivated state of the channel. This protocol was applied at an interval of 0.067 Hz. For the Na_v 1.5 isoform, 10 pulses from −20 mV to a holding potential of −100 mV were applied at 1 Hz. This protocol was applied at an interval of 0.016 Hz for the duration of the experiment. Measurement of the effect of the compounds on the Na_v 1.3, Na_v 1.4, and Na_v 1.7 channel isoforms proceeded by determining the peak inward current for both the closed and open-inactivated test pulses for

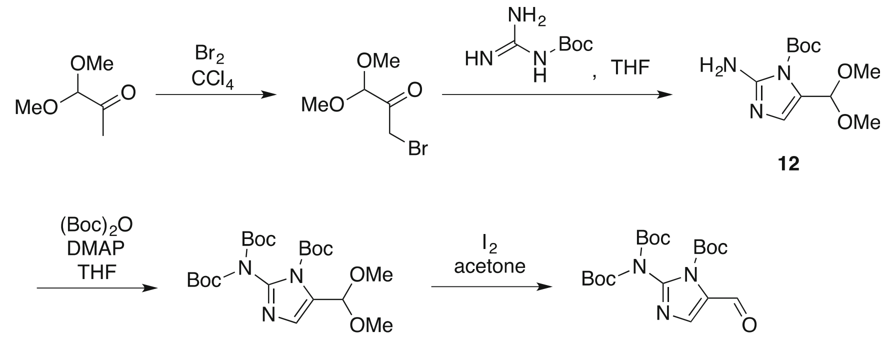


Fig. 6 Synthesis of key intermediate **6**

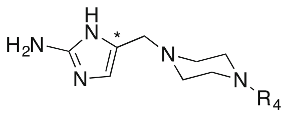
each sweep (the 10th pulse was used for the Na_v 1.5 channel isoform). Data were captured using QPatch assay software (v5.0). The percent inhibition of peak current was calculated as the mean peak current value for the last three sweeps measured in each concentration test period relative to the last three sweeps recorded during the control vehicle period. Sigmoidal concentration–response curves were fitted to the inhibition data using Xlfit (IDBS). Data are presented as the mean standard deviation for a minimum of three independent observations.

Results and discussion

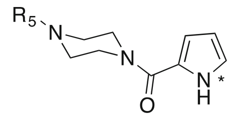
Previously published literature on the synthesis and activity of the *Agelas* sponge alkaloids was used to design the compounds aimed to selectively modulate voltage-gated sodium channels (Rasapalli *et al.*, 2013; Olofson *et al.*, 1998; Little and Webber 1994). As there are no reported crystal structures of voltage-gated sodium channels with bound clathrodin/oroidin or relevant analogs, we employed a simple ligand-based design by changing the central scaffold and performing small changes in the key structural elements of the compounds. We postulated that the terminal 2-aminoimidazole moiety of the *Agelas* alkaloid clathrodin separated by 3-aminopropene semi-rigid linker from the terminal pyrrole moiety was a key structural element. We also presumed that the two terminal moieties should be kept separate. A similar approach with rigidization and shortening of the linker was previously successful for the development of selective Na_v 1.4 channel isoform modulators (Hodnik *et al.*, 2013).

As seen from TanimotoCombo score calculations, we accomplished the design of compounds similar to the parent clathrodin and oroidin structures (Fig. 2; Table 2). Furthermore, the piperazine linker confers a slightly increased flexibility and increases the distance between the key terminal heterocyclic moieties. Our direct clathrodin analog **4c** had a distance of 7.7 Å between designated atoms in comparison to 6.9 Å measured for the parent clathrodin structure. The IC_{50} of compound **4c** was determined to be 25 μM on the Na_v 1.3 channel isoform (Table 2). Interestingly, the carbonyl of the pyrrole-2-carboxylate was proven to be necessary as compound **4b** did not show any measurable activity on the tested channel isoforms. It should be noted, however, that during biological testing of the compounds on the panel of Na_v channel isoforms, we selected the IC_{50} value of 30 μM as a "cut-off" point, so all activities above this threshold were considered to be equivalent. The substitution of indole-2-carboxamide for the terminal pyrrole-2-carboxamide increased potency; the IC_{50} of compound **4d** was 19 μM on the Na_v 1.3 channel isoform. This compound could occupy the space filled by the two additional bromines in oroidin and possibly take advantage of the additional hydrophobic/dispersion interactions. The measured distances between the terminal structures of compounds **4c** and **4d** also nicely compared (7.7 and 7.4 Å, respectively) to parent clathrodin/oroidin structures. Compound **4e**, where indole-2-carboxylate was replaced with indole-3-carboxylate, also showed below-threshold activity with an IC_{50} of 29 μM on the Na_v 1.3 channel isoform. The observed decrease in potency was probably due to the greater distance measured between the two key structures (9.5 Å in **4e** compared with 7.4 Å for compound **4d**). The analogs **4f** and **4g** were potentially too long; their measured IC_{50} was above the "cut-off" point of 30 μM. Patch-clamp measurements confirmed the two terminal features were necessary, as the benzyl analog **4a** did not show any activity against the Na_v channel isoforms. In the final iteration, we selected our direct analog **4c** and replaced the 2-aminoimidazole structure with bioisosteric heteroaromatic moieties (Table 2). The measured distance between the key structures in compound **5c** was evidently longer than in the parent structure, and no activity was observed. However, compounds **5a** and **5b** compared nicely to the parent clathrodin structure distance-wise and according to TanimotoCombo score, but again, no activity was observed with IC_{50} measurements hovering above the "cut-off" point of

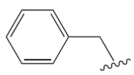
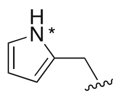
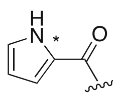
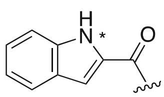
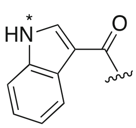
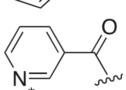
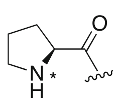
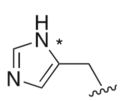
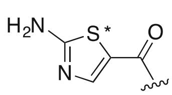
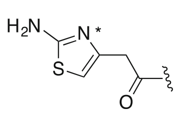
Table 2 Results of biological evaluation and molecular modeling



4a-f



5a-c

Compound	R_4	R_5	Distance (Å)[a]	*TanimotoCombo score*[b]	IC^{c}_{50} (μM)			
					Na_v 1.3	Na_v 1.4	Na_v 1.5	Na_v 1.7
1	/	/	6.9	/	>30	>30	>30	>30
4a			/	0.978 0.933	>30	n.t.	>30	>30
4b		/	7.6	1.040 0.972	30	>30	>30	>30
4c		/	7.7	1.197 1.116	25	>30	>30	>30
4d		/	7.4	1.067 1.082	19	>30	>30	>30
4e		/	9.5	1.007 0.991	29	>30	>30	>30
4f		/	9.3	1.057 1.009	>30	n.t.	>30	>30
4g		/	8.6	1.216 1.118	>30	n.t.	>30	>30
5a	/		7.5	1.238 1.144	>30	>30	>30	>30
5b	/		7.4	1.098 1.028	>30	n.t.	>30	>30
5c	/		8.0	0.919 0.858	>30	>30	>30	>30

[a] Measured distance between designated atoms (marked with asterisk *)

[b] TanimotoCombo score (*ROCS_TanimotoCombo*) calculated with OpenEye Scientific Software Inc. vROCS package. The first score is calculated against a clathrodin reference structure and the second value is calculated against an oroidin reference structure

[c] The concentration of compound that inhibited a sodium channel current by 50 %

30 μM. Based on these results, we conclude that the 2-aminoimidazole moiety is essential for activity against the Na_v channels.

Patch-clamp measurements revealed that all of our compounds displayed a trend towards selective modulation of the Na_v 1.3 channel isoform, while the IC_{50} of clathrodin was above the selected "cut-off" point (IC_{50} of clathrodin not shown). Nevertheless, our series of compounds demonstrate that the low-potency clathrodin structure was a solid starting point for ligand-based design, with compound **4d** reaching selective activity against the Na_v 1.3 channel isoform with an IC_{50} of 19 μM. When compared with the reported compounds by Hodnik *et al.,* where selectivity against the Na_v 1.4 channels was achieved, we observed a trend of activity towards the Na_v 1.3 channel isoform by clathrodin analogs with the less sterically restricted aminopropene linker substitution and a longer 4-methylene-piperazine moiety.

To conclude, our ligand-based design indicated a trend of increasing selectivity towards Na_v 1.3 inhibitory activity by slight prolongation of the linker between the clathrodin terminal heterocyclic moieties. The latter finding can be valuable for further development of selective Na_v 1.3 channel modulators. Compounds **4c–e** could also prove to be beneficial as pharmacological tools, especially with recent literature reports wherein the Na_v 1.3 channel isoform is associated with various pain conditions (Waxman *et al.*, 1994).

Experimental procedures

Chemistry: general

Chemicals were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA) and Acros Organics (Geel, Belgium) and were used without further purification. Analytical TLC was performed on silica gel Merck 60 G F_{254} plates (0.25 mm). Visualisation was performed with UV light and ninhydrin visualisation reagent. Column chromatography was conducted using silica gel 60 (particle size 240–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-d_6 or $CDCl_3$ solutions, with TMS used as the internal standard. IR spectra were recorded on a PerkinElmer Spectrum BX FT-IR spectrometer (PerkionElmer, Inc., Waltham, MA, USA) or Thermo Nicolet Nexus 470 ESP FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Mass spectra were obtained using a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, UK).

Synthesis of benzyl 4-benzylpiperazine-1-carboxylate (compound **7a**)

To a solution of *N*-Cbz piperazine (0.2 g, 0.88 mmol, 1.1 eq), benzaldehyde (81.5 μL, 0.80 mmol, 1 eq) in dry methanol (8 mL), activated molecular sieves (3 Å), and glacial acetic acid (50 μL) were added, and the reaction mixture was stirred for 20 min under argon atmosphere at room temperature. A solution of $NaCNBH_3$ (0.11 g, 1.68 mmol, 2.1 eq) in methanol (2 mL) was added dropwise and the reaction mixture was stirred for 14 h under argon atmosphere at room temperature. The reaction mixture was filtered, the solvent evaporated under reduced pressure, and the residue was redissolved in ethyl acetate, and washed with a saturated solution of $NaHCO_3$ in water (3 × 10 mL), brine (1 × 10 mL), dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:1) as an eluent to afford compound **7a**. Yield, 55 %, white solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 2.44$ (s, 4H, –$N(CH_2)_2$), 3.53–3.56 (m, 6H, –CH_2, –$N(CH_2)_2$), 5.17 (s, 2H, –O–CH_2), 7.35–7.36 (m, 5H, Ar), 7.37–7.39 (m, 5H, Cbz), (Compound reported by Shrikhande *et al.*, 2008, structure confirmed by ^{1}H NMR).

Synthesis of benzyl 4-((1*H*-pyrrol-2-yl)methyl)piperazine-1-carboxylate (compound **7b**)

To a solution of *N*-Cbz piperazine (1.01 g, 4.58 mmol, 1.1 eq) in dry dichloromethane (20 mL), 1*H*-pyrrole-2-carbaldehyde (0.40 g, 4.20 mmol, 1 eq) was added. The reaction mixture was stirred for 20 min under argon atmosphere at room temperature. It was then cooled to 0 °C, and $Na(OAc)_3BH$ (1.92 g, 9.62 mmol, 2.1 eq) was added. After stirring for 1 h with gradual warming to room temperature and additional stirring for 3 h at 40 °C, the dichloromethane phase was washed carefully with water (2 × 20 mL), brine (1 × 30 mL), dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate as an eluent to afford compound **7b**. Yield, 68 %, yellow-white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 2.41$–2.42 (m, 4H, $N(CH_2)_2$), 3.51–3.54 (m, 6H, –CH_2, $N(CH_2)_2$), 5.15 (s, 2H, O-CH_2), 6.05–6.1 (m, 1H, NCH_{Ar}), 6.13–6.16 (m, 1H, NCH_{Ar}), 6.77–6.79 (m, 1H, Ar), 7.32–7.40 (m, 5H, Cbz), 8.44–8.45 (m, 1H, NH) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 43.82$ (CH_2N), 52.67 (CH_2N), 55.42(CH_2N), 60.45 (CH_2O), 67.16 (CH_2N), 107.92 (CH_{Ar}), 108.04 (CH_{Ar}), 117.65 (CH_{Ar}), 127.92 (CH_{Ar}), 128.07 (CH_{Ar}), 128.18 (CH_{Ar}), 128.54 (CH_{Ar}), 136.72 (C_{Ar}), 155.26 (C_{Ar}), 171.23 (CO) ppm.; IR (cm^{-1}) $\nu = 3333$, 2899, 2864, 2809, 1685, 1428, 1285, 1238, 1118, 1093, 1024, 997, 785, 718, 696; MS (ESI) m/z (%) = 300 (MH^+); HRMS for $C_{17}H_{22}N_3O_2$: calculated 300.1712, found 300.1716.

Synthesis of 4-((4-benzylpiperazin-1-yl)methyl)-1*H*-imidazol-2-amine (compound **4a**)

Compound **7a** (0.20 g, 0.55 mmol) was dissolved in $EtOH_{abs}$ (10 mL), dry THF (10 mL), flushed with argon, and degassed under reduced pressure. Ten percent Pd/C (5 % m/m with respect to **7**) was added, and the reaction mixture was stirred under H_2 atmosphere for 3 h at room temperature. It was then filtered, and the solvent evaporated under reduced pressure. The residual oil (0.07 g, 0.39 mmol, 1.1 eq) was dissolved in dry dichloromethane (15 mL). Compound **6** (0.15 g, 0.36 mmol, 1 eq) was added, and the reaction mixture was stirred under argon for 20 min at room temperature. $Na(OAc)_3BH$ (0.15 g, 0.76 mmol, 2.1 eq) was slowly added, and the reaction mixture was stirred under argon atmosphere for 40 min at room temperature. The dichloromethane phase was washed carefully with water (2 × 10 mL), brine (1 × 50 mL), dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate/hexane (2:1) as an eluent to afford the Boc-protected compound. The Boc-protected compound (50 mg, 0.09 mmol) was dissolved in dichloromethane (2 mL), CF_3COOH (200 µL) was added, and the mixture was stirred under argon atmosphere for 2 h at 40 °C. Solvents were evaporated under reduced pressure and the resulting oil was redissolved in 1 M HCl in EtOH solution (2 mL). The white crystalline product was collected with filtration to afford compound **7a** in excellent yield (95 %). ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 2.09$ (s, 4H, –N(CH_2)$_2$), 4.06–4.10 (m, 2H, CH_2), 4.37 (s, 2H, CH_2), 7.03 (s, 1H, CH_{Ar}), 7.45–7.73 (m, 5H, CH_{Ar}), 12.14–12.24 (m, 2H, NH_2) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 30.68$ (CH_2), 47.13 (CH_2N), 48.16 (CH_2N), 58.36 (CH_2N), 128.74 (CH_{Ar}), 129.47 (CH_{Ar}), 129.56 (CH_{Ar}), 131.42 (CH_{Ar}), 131.56 (C_{Ar}), 147.31 (C_{Ar}), 206.54 (CNH_2) ppm.; IR (cm^{-1}) $\nu = 3360$, 3242, 3139, 2989, 2963, 2505, 2446, 1697, 1678, 1635, 1599, 1433, 1365, 953, 917, 747, 699; MS (ESI) *m*/*z* (%) = 342 (M−H$^-$); HRMS for $C_{15}H_{22}N_5Cl_2$: calculated 342.1252, found 342.1254.

Synthesis of 4-((4-((1*H*-pyrrol-2-yl)methyl)piperazin-1-yl)methyl)-1*H*-imidazol-2-amine (compound **4b**)

Compound **7b** (0.40 g, 1.34 mmol) was dissolved in the mixture of $EtOH_{abs}$ (10 mL) and dry THF (10 mL), flushed with argon, and degassed under reduced pressure. Ten percent Pd/C (5 % m/m with respect to **7**) was added, and the reaction mixture was stirred under H_2 atmosphere for 2 h at room temperature. The reaction mixture was filtered, and the solvent evaporated under reduced pressure. The residual oil (0.22 g, 1.34 mmol, 1.1 eq) was dissolved in dry dichloromethane (20 mL). Compound **6** (0.49 g, 1.20 mmol, 1 eq) was added, and the mixture was stirred under argon for 20 min at room temperature. $Na(OAc)_3BH$ (0.55 g, 2.59 mmol, 2.1 eq) was added and again stirred under argon atmosphere for 40 min at room temperature. The dichloromethane phase was washed carefully with water (2 × 20 mL), brine (1 × 20 mL), dried over Na_2SO_4, and filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate/methanol (9:1) as an eluent to afford the Boc-protected compound. The Boc-protected compound (50 mg, 0.09 mmol) was dissolved in ethyl acetate (3 mL), 1 M HCl solution was added (1.5 mL), and the reaction mixture was stirred for 50 min at room temperature. The water phase was alkalinized to pH 13 by adding 10 M NaOH, and extracted with ethyl acetate (2 × 5 mL). The organic phases were joined and the solvent evaporated under reduced pressure. The crude residue was purified by flash column chromatography using dichloromethane/methanol (5:1) as an eluent to afford compound **4b**. Yield, 20 %, yellow oil; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 2.59$ (s, 4H, –N(CH_2)$_2$), 3.08 (s, 4H, N(CH_2)$_2$), 3.44 (s, 2H, CH_2), 6.81 (s, 1H, CH_{Ar}), 7.54 (s, 2H, –NH_2), 8.79–8.81 (m, 2H, CH_{Ar}), 12.12 (2, 1H, NH), 12.43–12.46 (m, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 45.31$ (CH_2N), 47.45 (CH_2N), 48.56 (CH_2N), 114.44 (CH_{Ar}), 114.98 (CH_{Ar}), 143.22 (CH_{Ar}), 147.30 (CH_{Ar}), 158.14 (C_{Ar}), 161.59 (C_{Ar}), 188.23 (CNH_2) ppm.; IR (cm^{-1}) $\nu = 3148$, 3006, 2781, 1676, 1619, 1433, 1410, 1259, 1082, 1068, 1019, 931, 799; MS (ESI) *m*/*z* (%) = 334 (MH$^+$).

General procedure for synthesis of compounds **9c–g**

N-Cbz piperazine (0.55 g, 2.48 mmol, 1 eq) and carboxylic acid **8c–g** (2.48 mmol, 1 eq) were dissolved in dry DMF (10 mL), the reaction mixture flushed with argon and cooled to 0 °C. *N*-methyl morpholine (NMM; 7.44 mmol, 3 eq), hydroxybenzotriazole hydrate (HOBt; 2.98 mmol, 1.2 eq) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride HCl salt (EDC; 3.22 mmol, 1.3 eq) were slowly added. The reaction mixture was stirred under argon atmosphere for 5 h at 0 °C and an additional 15 h at room temperature. DMF was evaporated under reduced pressure and the residue redissolved in dichloromethane (10 mL). The dichloromethane phase was washed with H_2O (1 × 10 mL), a 1 M HCl solution (3 × 10 mL), saturated aqueous $NaHCO_3$ solution (3 × 10 mL), brine (1 × 20 mL), dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate/hexane solvents as eluents to afford compounds **9c–g**.

*Benzyl 4-(1H-pyrrole-2-carbonyl)piperazine-1-carboxylate (compound **9c**)*

Yield, 86 %, white crystalline solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 3.60$–3.64 (m, 4H, N(CH_2)$_2$), 3.87 (s, 4H,

$N(CH_2)_2$), 5.19 (s, 2H, O-CH_2), 6.25–6.27 (m, 1H, N-CH_{Ar}), 6.52–6.54 (m, 1H, N-CH_{Ar}), 6.93–6.94 (m, 1H, Ar), 7.37–7.41 (m, 5H, Cbz), 10.11–10.14 (m, 1H, NH) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 43.73$ (CH_2N), 43.78 (CH_2N), 67.50 (OCH_2), 109.55 (CH_{Ar}), 112.39 (CH_{Ar}), 121.56 (CH_{Ar}), 124.14 (CH_{Ar}), 128.09 (CH_{Ar}), 128.25 (C_{Ar}), 128.61 (CH_{Ar}), 136.41 (C_{Ar}), 155.22 (CO), 162.18 (CO) ppm.; IR (cm^{-1}) $v = 3255$, 3104, 2905, 2851, 1695, 1593, 1545, 1473, 1446, 1421, 1358, 1281, 1245, 1232, 1191, 1131, 1100, 1014, 961, 849, 783, 742, 697, 680; MS (ESI) *m/z* (%) = 312 (M−H$^-$); HRMS for $C_{17}H_{18}N_3O_3$: calculated 312.1348, found 312.1342.

*Benzyl 4-(1H-indole-2-carbonyl)piperazine-1-carboxylate (compound **9d**)*

Yield, 92 %, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 3.66$–3.68 (m, 4H, –$N(CH_2)_2$), 3.97 (s, 4H, –$N(CH_2)_2$), 5.21 (s, 2H, O-CH_2), 6.79–6.80 (m, 1H, Ar), 7.17 (ddd, $J = 8.01$, 7.01, 0.98 Hz, 1H, Ar), 7.32 (ddd, $J = 8.24$, 7.01, 1.14, 1H, Ar), 7.36–7.42 (m, 5H, Ar), 7.46 (dd, J = 8.29, 0.90 Hz, 1H, Ar), 7.67 (dd, $J = 8.03$, 0.89 Hz, 1H, Ar), 9.68 (s, 1H, NH) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 43.73$ (CH_2N), 43.76 (CH_2N), 43.79 (CH_2N), 43.82 (CH_2N), 67.59 (OCH_2), 105.49 (CH_{Ar}), 111.87 (CH_{Ar}), 120.71 (CH_{Ar}), 121.94 (CH_{Ar}), 124.65 (CH_{Ar}), 127.37 (CH_{Ar}), 128.11 (CH_{Ar}), 128.29 (CH_{Ar}), 128.62 (C_{Ar}), 128.78 (C_{Ar}), 135.84 (C_{Ar}), 136.34 (C_{Ar}), 155.20 (CO), 162.81 (CO) ppm.; IR (cm^{-1}) $v = 3252$, 3058, 2878, 1700, 1595, 1530, 1460, 1443, 1408, 1346, 1221, 1120, 804, 765, 745, 727, 693, 678; MS (ESI) *m/z* (%) = 362 (M−H$^-$); HRMS for $C_{21}H_{20}N_3O_3$: calculated 362.1505, found 362.1498.

*Benzyl 4-(1H-indole-3-carbonyl)piperazine-1-carboxylate (compound **9e**)*

Yield, 82 %, yellow-white solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.38$ (s, 1H, NCH_2), 3.52–3.59 (m, 5H, $N(CH_2)_2$), 3.73 (s, 2H, NCH_2), 5.19 (s, 2H, –OCH_2), 7.21–7.28 (m, 2H, Ar), 7.34–7.40 (m, 8H, Ar) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 43.41$ (CH_2N), 43.63 (CH_2N), 44.50 (CH_2N), 44.95 (CH_2N), 66.93 (CH_2O), 112.20 (CH_{Ar}), 120.48 (CH_{Ar}), 121.19 (CH_{Ar}), 122.24 (CH_{Ar}), 128.13 (CH_{Ar}), 128.30 (CH_{Ar}), 128.39 (CH_{Ar}), 128.92 (CH_{Ar}), 134.14 (C_{Ar}), 136.02 (C_{Ar}), 137.18 (C_{Ar}), 154.85 (C_{Ar}), 161.56 (CO), 166.69 (CO) ppm.; IR (cm^{-1}) $v = 3513$, 3032, 2916, 2863, 1696, 1425, 1357, 1281, 1230, 1199, 1119, 1069, 1002, 732, 697; MS (ESI) *m/z* (%) = 362 (M-H$^-$); HRMS for $C_{21}H_{20}N_3O_3$: calculated 362.1505, found 362.1507.

*Benzyl 4-nicotinoylpiperazine-1-carboxylate (compound **9f**)*

Yield, 98 %, yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 3.44$–3.76 (m, 8H, –$N(CH_2)_2$), 5.51 (s, 2H, O-CH_2), 7.32–7.39 (m, 6H, Cbz, CH_{Ar}), 7.75–7.78 (m, 1H, CH_{Ar}), 8.66–8.68 (m, 2H, CH_{Ar}) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 42.14$ (CH_2N), 43.80 (CH_2N), 47.53 (CH_2N), 67.59 (CH_2O), 123.65 (CH_{Ar}), 128.07 (CH_{Ar}), 128.28 (CH_{Ar}), 128.60 (CH_{Ar}), 131.18 (CH_{Ar}), 135.22 (CH_{Ar}), 136.22 (C_{Ar}), 147.84 (CH_{Ar}), 151.00 (CO), 155.07 (C_{Ar}), 167.96 (CO) ppm.; IR (cm^{-1}) $v = 3386$, 3027, 2919, 2835, 2857, 1697, 1627, 1472, 1426, 1410, 1278, 1225, 1159, 1115, 1071, 1003, 980, 861, 830, 754, 746, 743, 698; MS (ESI) *m/z* (%) = 326 (MH$^+$); HRMS for $C_{18}H_{20}N_3O_3$: calculated 326.1505, found 326.1499.

*Benzyl 4-((tert-butoxycarbonyl)-L-prolyl)piperazine-1-carboxylate (compound **9g**)*

Yield, 99 %, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 1.41$ (s, 4H, CH), 1.47 (s, 5H, CH), 1.82–1.91 (m, 2H, CH_2), 1.97–2.21 (m, 2H, CH_2), 3.45–3.65 (m, 8H, –$N(CH_2)_2$), 4.53–4.68 (m, 1H, CH), 5.17 (d, $J = 3.64$ Hz, 1H, NH), 5.32 (s, 2H, O-CH_2), 7.35-7.39 (m, 5H, Cbz) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 22.96$ (CH_2), 28.01 (CH_3), 28.14 (CH_3), 29.85 (CH_2), 41.11 (CH_2N), 43.34 (CH_2N), 43.41 (CH_2N), 44.12 (CH_2N), 46.29 (CH_2), 56.38 (CH), 56.22 (CH_2N), 66.39 (CH_2O), 78.15 (CO), 127.59 (CH_{Ar}), 127.88 (CH_{Ar}), 128.41 (CH_{Ar}), 136.71 (CH_{Ar}), 153.07 (CH_{Ar}), 153.25 (CH_{Ar}), 154.40 (CO), 170.22 (CO), 170.55 (CO) ppm.; IR (cm^{-1}) $v = 2979$, 2954, 2866, 1704, 1680, 1658, 1450, 1404, 1364, 1246, 1210, 1156, 1114, 1074, 1044, 947, 928, 754, 698; MS (ESI) *m/z* (%) = 418 (MH$^+$); HRMS for $C_{22}H_{32}N_3O_5$: calculated 418.2342, found 418.2353.

General procedure for synthesis of compounds **4c–g**

Compound **9c–g** (0.55 mmol) was dissolved in the mixture of $EtOH_{abs}$ (10 mL) and dry THF (10 mL), flushed with argon, and degassed under reduced pressure. Ten percent Pd/C (5 % m/m with respect to compound **9**) was added, and the reaction mixture was stirred under H_2 atmosphere for 3 h at room temperature. The catalyst was then filtered off, and the solvent evaporated under reduced pressure. The residue (0.55 mmol, 1.1 eq) was dissolved in dry dichloromethane (15 mL). Compound **6** (0.50 mmol, 1 eq) was added, and the mixture was stirred under argon for 20 min at room temperature. $Na(OAc)_3BH$ (0.23 g, 1.05 mmol, 2.1 eq) was added and 40 min of stirring at room temperature followed. Dichloromethane was washed carefully with water (2 × 15 mL), brine (1 × 15 mL), dried over Na_2SO_4, and filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate as an eluent to afford the Boc-protected compound. The Boc-protected compound (0.14 mmol) was dissolved in dichloromethane (5 mL), CF_3COOH (1 mL) was added, and the mixture was stirred under argon atmosphere for 1 h at 40 °C. The solvents were evaporated under reduced pressure, and the residue was dissolved in a 1 M ethanolic solution of HCl (2 mL). The product was obtained by adding dry ether (500 µL) and collection by filtration.

*(4-((2-Amino-1H-imidazol-4-yl)methyl)piperazin-1-yl)(1H-pyrrol-2-yl)methanone (compound **4c**)*

Yield, 77 %, white solid; ^{1}H NMR (DMSO-d6, 400 MHz) $\delta = 3.05$–3.10 (m, 2H, –NCH_2), 4.27 (s, 2H, –NCH_2), 4.48–4.52 (m, 2H, –NCH_2), 6.15 (s, 1H, CH_{Ar}), 6.58 (s, 1H, CH_{Ar}), 6.94 (s, 1H, CH_{Ar}), 7.10 (s, 1H, CH_{Ar}), 7.81 (s, 2H, –NH_2), 11.58 (s, 1H, NH), 12.20–12.21 (m, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 41.36$ (CH_2N), 48.38 (CH_2N), 48.45 (CH_2N), 50.08 (CH_2N), 108.63 (CH_{Ar}), 112.57 (CH_{Ar}), 115.08 (CH_{Ar}), 116.59 (CH_{Ar}), 121.90 (C_{Ar}), 123.23 (C_{Ar}), 147.43 (CNH_2), 161,47 (CO) ppm.; IR (cm^{-1}) $v = 3246$, 3106, 2945, 2680, 1680, 1583, 1548, 1428, 1290, 1115, 1048, 944, 747; MS (ESI) *m/z* (%) = 309 (M-HCl$^-$); HRMS for $C_{13}H_{18}N_6OCl$: calculated 309.1231, found 309.1223.

*(4-((2-Amino-1H-imidazol-4-yl)methyl)piperazin-1-yl)(1H-indol-2-yl)methanone (compound **4d**)*

Yield, 93 %, yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.06$–3.17 (m, 2H, –NCH_2), 4.29 (s, 2H, –NCH_2), 4.57–4.59 (m, 2H, –NCH_2), 6.90 (s, 1H, CH_{Ar}), 7.07 (t, $J = 7.53$ Hz, 1H, CH_{Ar}), 7.11 (s, 1H, CH_{Ar}), 7.21 (t, $J = 7.54$ Hz, 1H, CH_{Ar}), 7.45 (d, $J = 7.85$ Hz, 1H, CH_{Ar}), 7.62 (d, $J = 7.74$ Hz, 1H, CH_{Ar}) 7.82 (s, 2H, NH_2), 11.69 (s, 1H, NH), 12.21 (s, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 30.68$ (CH_2N), 45.27 (CH_2N), 48.46 (CH_2N), 50.03 (CH_2N), 104.80 (CH_2N), 112.16 (CH_{Ar}), 115.01 (CH_{Ar}), 116.60 (CH_{Ar}), 119.88 (CH_{Ar}), 121.43 (CH_{Ar}), 123.55 (C_{Ar}), 126.68 (C_{Ar}), 128.84 (C_{Ar}), 136.10 (C_{Ar}), 147.45 (CNH_2), 162.14 (CO) ppm.; IR (cm^{-1}) $v = 3272$, 3093, 2932, 2662, 2571, 1677, 1604, 1423, 1249, 1199, 948, 733; MS (ESI) *m/z* (%) = 359 (M-HCl$^-$); HRMS for $C_{17}H_{20}N_6OCl$: calculated 359.1387, found 359.1385.

*(4-((2-Amino-1H-imidazol-4-yl)methyl)piperazin-1-yl)(1H-indol-3-yl)methanone (compound **4e**)*

Yield, 66 %, yellow–brown solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.06$–3.12 (m, 2H, NCH_2), 4.26–4.28 (m, 2H, –NCH_2), 4.39–4.43 (m, 2H, NCH_2), 7.11–7.18 (m, 3H, CH_{Ar}), 7.47 (d, $J = 7.42$ Hz, 1H, CH_{Ar}), 7.72–7.79 (m, 2H, CH_{Ar}), 11.47–11.55 (s, 1H, NH), 11.77 (s, 1H, NH), 12.04–12.05 (m, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 50.23$ (CH_2N), 50.32 (CH_2N), 67.96 (CH_2N), 97.96 (CH_{Ar}), 112.05 (CH_{Ar}), 120.09 (CH_{Ar}), 120.41 (CH_{Ar}), 122.05 (CH_{Ar}), 125.89 (CH_{Ar}), 128.79 (C_{Ar}), 135.75 (C_{Ar}), 147.43 (C_{Ar}), 155.04 (C_{Ar}), 165.73 (CNH_2), 178.58 (CO) ppm.; IR (cm^{-1}) $v = 3218$, 3131, 2997, 2956, 2992, 2561, 1679, 1606, 1522, 1421, 1105, 947, 768, 755, MS (ESI) *m/z* (%) = 359 (M-HCl$^-$).

*(4-((2-Amino-1H-imidazol-4-yl)methyl)piperazin-1-yl)(pyridin-3-yl)methanone (compound **4f**)*

Yield, 65 %, yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.12$–3.15 (m, 8H, –$N(CH_2)_2$), 4.27 (s, 2H, CH_2), 7.12 (s, 1H, CH_{Ar}), 7.72 (dd, $J = 7.34$, 5.11 Hz, 1H, CH_{Ar}), 7.81 (s, 1H, CH_{Ar}), 8.14 (d, $J = 7.65$ Hz, 1H, CH_{Ar}), 8.79–8.82 (m, 2H, CH_{Ar}, NH), 12.18–12.20 (m, 2H, NH_2) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 43.79$ (CH_2N), 48.31 (CH_2N), 49.49 (CH_2N), 49.60 (CH_2N), 114.79 (CH_{Ar}), 116.78 (CH_{Ar}), 125.19 (CH_{Ar}), 132.07 (CH_{Ar}), 138.85 (CH_{Ar}), 144.79 (C_{Ar}), 147.43 (C_{Ar}), 147.48 (CNH_2), 165.62 (CO) ppm.; IR (cm^{-1}) $v = 3374$, 3326, 3166, 3076, 3050, 2984, 2928, 2836, 2756, 2365, 1682, 1642, 1632, 1604, 1493, 1476, 1443, 1300, 1285, 953, 807, 685; MS (ESI) *m/z* (%) = 321 (M-HCl$^-$); HRMS for $C_{14}H_{18}N_6OCl$: calculated 321.1231, found 321.1235.

*(S)-1-((2-Amino-1H-imidazol-4-yl)methyl)-4-prolylpiperazine (compound **4g**)*

Yield, 55 %, white solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 1.87$–1.92 (m, 3H, CH_2), 2.34–2.35 (m, 1H, CH_2), 3.19–3.21 (m, 6H, –$N(CH_2)_2$), 4.22–4.29 (m, 3H, CH_2), 4.62–4.63 (s, 1H, CH), 7.09–7.11 (s, 1H, CH_{Ar}), 7.75–7.81 (m, 2H, NH_2), 8.51–8.52 (s, 1H, NH), 12.16 (s, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 23.63$ (CH_2), 28.46 (CH_2), 30.70 (CH_2), 41.64 (CH_2N), 45.63 (CH_2N), 48.29 (CH_2N), 49.35 (CH_2N), 49.66 (CH_2N), 57.19 (CH), 115.01 (CH_{Ar}), 116.58 (C_{Ar}), 147.41 (CNH_2), 166.87 (CO) ppm.; IR (cm^{-1}) $v = 3345$, 2928, 2753, 2574, 1679, 1653, 1479, 1445, 1255, 1201, 1123, 945; MS (ESI) *m/z* (%) = 349 (M-H$^-$); HRMS for $C_{13}H_{23}N_6OCl_2$: calculated 349.1310, found 349.1320.

*Synthesis of (4-((1H-imidazol-4-yl)methyl)piperazin-1-yl)(1H-pyrrol-2-yl)methanone (compound **5a**)*

Compound **9c** (4.30 g, 13.7 mmol, 1 eq) was dissolved in absolute ethanol (100 mL), and glacial acetic acid (20 mL) was added. The reaction mixture was flushed with argon and degassed under reduced pressure, and 10 % Pd/C (5 % m/m with respect to **9c**) was added. The reaction mixture was stirred under H_2 atmosphere for 1 h at room temperature, and the catalyst was filtered off. The solvent was evaporated under reduced pressure, and the resulting compound **11** was used in the next reaction step without further purification. Compound **11** (0.70 g, 3.91 mmol, 3.5 eq) was dissolved in methanol (8 mL) and glacial acetic acid (64 μL, 0.11 mmol, 0.1 eq) was added. 1*H*-imidazole-4-carbaldehyde (0.108 g, 1.16 mmol, 1 eq) in methanol (4 mL) was added dropwise, and the mixture was left stirring under argon atmosphere for 30 min at room temperature. $NaCNBH_3$ (0.20 g, 3.35 mmol, 3 eq) dissolved in methanol (5 mL) was added dropwise via syringe with further stirring under argon atmosphere at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (20 mL). 1 M aqueous NaOH was added until alkaline pH was reached, and the water phase was extracted with ethyl acetate (6 × 30 mL). The combined organic phases were dried above Na_2SO_4 and evaporated under reduced pressure, and the resulting crude product was purified by flash column chromatography using dichloromethane/methanol (9:1) as an eluent to afford compound **5a**. Yield, 51 %, yellow–white solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 2.37$–2.40 (m, 4H, –N(CH_2)$_2$–], 3.43 (s, 2H, –CH_2–), 3.62–3.69 (m, 4H, –N(CH_2)$_2$–), 6.07–6.10 (m, 1H, Ar), 6.43–6.47 (m, 1H, Ar), 6.84–6.91 (m, 2H, Ar, Ar-imi.), 7.52–7.56 (m, 1H, Ar-imi.), 11.40 (s, 1H, Ar–NH), 11.93 (s, 1H, Ar-imi.-NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 44.41$ (CNH_2), 44.52 (CNH_2), 52.47 (CNH_2), 108.27 (CH_{Ar}), 111.65 (CH_{Ar}), 120.99 (CH_{Ar}), 124.19 (CH_{Ar}), 134.81 (C_{Ar}), 134.85 (C_{Ar}), 134.96 (CH_{Ar}), 161.32 (CO) ppm.; IR (cm^{-1}) $\nu = 3122$, 3072, 2955, 2868, 2820, 2778, 1597, 1459, 1436, 1293, 1266, 1141, 1090, 995, 849, 730, 633; MS (ESI) *m/z* (%) = 260 (MH^+); HRMS for $C_{13}H_{18}N_5O$: calculated 260.1511, found 260.1514.

*Synthesis of 1-(4-(1H-pyrrole-2-carbonyl)piperazin-1-yl)-2-(2-aminothiazol-5-yl)ethan-1-one (compound **5c**)*

Compound **11** (0.30 g, 1.64 mmol, 1.1 eq) and 2-amino-4-thiazole acetic acid (0.24 g, 1.52 mmol, 1 eq) were dissolved in anhydrous THF (20 mL). The reaction mixture was flushed with argon and cooled to 0 °C. Triethylamine (0.84 mL, 6.09 mmol, 4 eq) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride HCl salt (EDC; 0.38 g, 2.74 mmol, 1.8 eq) were added, and the reaction mixture was removed from the ice bath. DMF (20 mL) was added, and the reaction mixture was left stirring under argon for 20 h at 60 °C. The solvents were evaporated under reduced pressure and the crude product was purified by flash column chromatography using dichloromethane/methanol (20:1 + 1 % AcOH) as the eluent to afford compound **5c**. Yield, 14 %, yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.50$–3.56 (m, 4H, –N(CH_2)$_2$–), 3.59–3.63 (m, 2H, –CO–CH_2–), 3.64–3.72 (m, 4H, –N(CH_2)$_2$–), 6.12 (td, $J = 3.49$, 2.45, 2.45 Hz, 1H, Ar), 6.26 (s, 1H, –S–CH=C–), 6.51 (ddd, $J = 3.67$, 2.50, 1.35 Hz, 1H, Ar), 6.85 (s, 2H, –NH_2), 6.89 (dt, $J = 2.80$, 2.70, 1.37 Hz, 1H, Ar), 11.44 (s, 1H, Ar–NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 21.04$ (CH_2), 36.69 (CNH_2), 41.25 (CNH_2), 45.57 (CNH_2), 102.28 (CNH_2), 108.42 (CH_{Ar}), 112.02 (CH_{Ar}), 121.30 (CH_{Ar}), 123.99 (C_{Ar}), 145.56 (C_{Ar}), 161.58 (CH_{Ar}), 168.03 (CNH_2), 168.14 (CO), 172.01 (CO) ppm.; IR (cm^{-1}) $\nu = 3122$, 2954, 2915, 2868, 2820, 1597, 1460, 1434, 1292, 1141, 849, 730; MS (ESI) *m/z* (%) = 320 (MH^+); HRMS for $C_{14}H_{18}N_5O_2S$: calculated 320.1181, found 320.1185.

*Synthesis of (4-(1H-pyrrole-2-carbonyl)piperazin-1-yl)(2-aminothiazol-5-yl)methanone (compound **5b**)*

Compound **11** (0.394 g, 1.83 mmol, 1.1 eq) was dissolved in anhydrous DMF (20 mL), and the reaction mixture was cooled to 0 °C. Triethylamine (0.81 mL, 5.81 mmol, 3.5 eq) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride HCl salt (EDC; 0.48 g, 2.49 mmol, 1.5 eq) were added. The reaction mixture was removed from the ice bath and stirred under argon for 20 h at room temperature. The solvents were evaporated under reduced pressure, and the crude product was purified by flash column chromatography using hexane/ethyl acetate/methanol (5:4:1) as the eluent to afford the Boc-protected compound. The latter (0.71 g, 1.75 mmol, 1 eq) was dissolved in dichloromethane (15 mL), and CF3COOH was added (5 mL). The reaction mixture was stirred under argon for 1 h at room temperature. Solvents were evaporated under reduced pressure to afford compound **5b**. Yield, 21 %, brown solid; ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 3.73$ (s, 4H, –N(CH_2)$_2$), 3.79 (s, 4H, –N(CH_2)$_2$), 6.14 (s, 1H, CH_{Ar}), 6.51–6.55 (m, 1H, CH_{Ar}), 6.92 (s, 1H, CH_{Ar}), 7.54 (s, 1H, CH_{Ar}), 8.09–8.12 (m, 2H, –NH_2). 11.52 (s, 1H, NH) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 44.43$ (CNH_2), 44.45 (CNH_2), 108.49 (CH_{Ar}), 112.14 (CH_{Ar}), 119.59 (CH_{Ar}), 121.42 (CH_{Ar}), 123.99 (C_{Ar}), 139.05 (C_{Ar}), 160.72 (CO), 161.51 (CO), 170.83 (CNH_2) ppm.; IR (cm^{-1}) $\nu = 3254$, 3076, 2734, 1663, 1585, 1425, 1180, 1126, 998, 834, 723; MS (ESI) *m/z* (%) = 306 (MH^+); HRMS for $C_{13}H_{16}N_5O_2S$: calculated 306.1025, found 306.1020.

*Synthesis of tert-butyl 2-amino-5-(dimethoxymethyl)-1H-imidazole-1-carboxylate (compound **12**)*

1,1-Dimethoxypropan-2-one (200 g, 1.7 mol) was dissolved in tetrachloromethane (800 mL) and cooled in an ice bath. Bromine (87.2 mL, 1.7 mol) was added dropwise over 10 min, and the reaction mixture was left stirring in argon atmosphere for 15 h at room temperature. Sodium bicarbonate (178 g, 2.1 mol) was then added, and the resulting mixture was left stirring for 30 min and transferred to a separatory funnel. The organic phase was washed carefully with water (2 × 100 mL), brine (1 × 100 mL), dried over Na_2SO_4, and evaporated under reduced pressure to afford 3-bromo-1,1-dimethoxypropan-2-one as a clear pale-yellow oil in 89 % yield.

As this compound was proven to be intrinsically instable, it was used directly in a subsequent reaction step. BOC-guanidine (5.14 g, 32 mmol) was dissolved in dry THF (50 mL), activated molecular sieves (3 Å) were added, and the reaction mixture was left stirring for 10 min under argon atmosphere at 0 °C. 3-Bromo-1,1-dimethoxypropan-2-one (6.4 g, 32 mmol, 1 eq) dissolved in dry THF (50 mL) was then added dropwise. The mixture was left stirring under argon for 20 h at room temperature. Na_2SO_4 was added and the reaction mixture filtered to remove solids, the solvents were evaporated under reduced pressure and the crude product was purified using column chromatography with ethyl acetate +1 % Et_3N as an eluent to afford an orange solid (**12**). Yield, 12 %, white solid; 1H NMR ($CDCl_3$, 400 MHz) $\delta = 1.53$ (s, 9H, Boc), 3.30 (s, 6H, OCH_3), 5,22 (s, 1H, CH), 6.02 (s, 2H, NH_2), 6.78 (s, 1H, CH_{Ar}) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 28.08$ (CH_3), 52.76 (OCH_3), 85.18 (CH), 99.41 (CH), 109.43 (CH_{Ar}), 135.59 (C_{Ar}), 149.47 (CO), 150.74 (CNH_2) ppm.; IR (cm^{-1}) $\nu = 3240$, 3180, 3110, 2980, 2950, 2886, 2828, 1735, 1631, 1452, 1385, 1336, 1274, 1249, 1153, 1112, 1054, 978, 907, 843, 765, 711.; MS (ESI) *m/z* (%) = 258.1 (MH^+); HRMS for $C_{11}H_{19}N_3O_4$: calculated 258.1454, found 258.1460.

*Synthesis of tert-butyl 2-(di-(tert-butoxycarbonyl)amino)-5-(dimethoxymethyl)-1H-imidazole-1-carboxylate (compound **13**)*

Compound **12** (1.7 g, 6.62 mmol, 1 eq) was dissolved in dry THF (10 mL), DMAP (242 mg, 2 mmol, 0.3 eq) was added, and the reaction mixture was left stirring under argon atmosphere for 20 min at 0 °C. Di-*tert*-butyl dicarbonate (4.5 g, 19.9 mmol, 3 eq) dissolved in dry THF (5 mL) was added and stirring was continued for 20 h at room temperature. The solvents were evaporated under reduced pressure and the resulting crude product was purified using column chromatography with ethyl acetate +1 % Et_3N as an eluent to afford compound **13** as bright-yellow crystals. Yield, 78 %, yellow crystalline solid; 1H NMR ($CDCl_3$, 400 MHz) $\delta = 1.34$ (s, 18H, Boc), 1.53 (s, 9H, Boc), 3.20 (s, 6H, OCH_3), 5.36 (s, 1H, CH), 7.42 (s, 1H, CH_{Ar}) ppm.; ^{13}C NMR ($CDCl_3$, 100 MHz) $\delta = 27.24$ (CH_3), 27.38 (CH_3), 51.69 (OCH_3), 83.24 (CH), 86.29 (CH), 97.85 (CH), 116.77 (CH_{Ar}), 136.43 (C_{Ar}), 137.54 (CO), 145.88 (CO), 148.74 (CN) ppm.; IR (cm^{-1}) $\nu = 3166$, 3138, 2977, 2942, 2834, 1745, 1724, 1538, 1456, 1361, 1318, 1249, 1211, 1146, 1109, 1058, 1022, 985, 908, 872, 842, 816, 768.; MS (ESI) *m/z* (%) = 458.2 (MH^+); HRMS for $C_{21}H_{35}N_3O_8$: calculated 458.2502, found 458.2502.

*Synthesis of tert-butyl 2-(di-(tert-butoxycarbonyl)amino)-5-formyl-1H-imidazole-1-carboxylate (compound **6**)*

Acetal intermediate **13** (0.2 g, 0.44 mmol, 1 eq) was dissolved in dry acetone (5 mL) and cooled to 0 °C. A 0.05 M solution of iodine in dry acetone (0.9 mL) was added via syringe and the reaction was left to stir under argon atmosphere at 0 °C for 4 h. The reaction was quenched with the addition of aqueous 5 % m/m solution of $Na_2S_2O_3$ in water (1 mL). The aqueous mixture was then extracted with ethyl acetate (3 × 3 mL), the organic fractions were combined and washed with brine (1 × 5 mL), and the solvent was evaporated under reduced pressure. The crude product was purified using column chromatography with ethyl acetate/hexane (1:2) as an eluent. Yield, 56 %, white–yellow crystalline solid; 1H NMR (DMSO-d_6, 400 MHz) $\delta = 1.37$ (s, 18H, $N(Boc)_2$), 1.56 (s, 9H, NBoc), 8.52 (s, 1H, CH_{Ar}), 9.77 (s, 1H, CHO) ppm.; ^{13}C NMR (DMSO-d_6, 100 MHz) $\delta = 27.20$ (CH_3), 27.40 (CH_3), 83.93 (CH), 87.76 (CH), 127.75 (CH_{Ar}), 137.66 (C_{Ar}), 139.12 (CO), 145.39 (CO), 148.62 (CN), 184.85 (CO) ppm.; IR (cm^{-1}) $\nu = 3142$, 2980, 2935, 1751, 1726, 1688, 1545, 1457, 1399, 1349, 1313, 1277, 1248, 1209, 1146, 1112, 1017, 991, 873, 845, 768, 734.; MS (ESI) *m/z* (%) = 412.2 (MH^+); HRMS for $C_{19}H_{29}N_3O_7$: calculated 412.2084, found 412.2076 (Peat *et al.*, 2008, Yoke Chong *et al.*, 2010).

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References

Abdel-Magid AF, Carson KG, Harris BD, Maryanoff CA, Shah RD (1996) Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedures. J Org Chem 61:3849–3862

Andavan GSB, Lemmens-Gruber R (2011) Voltage-gated sodium channels: mutations, channelopathies and targets. Curr Med Chem 18:377–397

Anderluh M, Cesar J, Štefanič P, Kikelj D, Janeš D, Murn J, Nadrah K, Tominc M, Addicks E, Giannis A, Stegnar M, Sollner Dolenc M (2005) Design and synthesis of novel platelet fibrinogen receptor antagonists with 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold. A systematic study. Eur J Med Chem 40:25–49

Anger T, Madge DA, Mulla M, Riddall D (2001) Medicinal chemistry of neuronal voltage-gated sodium channel blockers. J Med Chem 44:115–137

Bickmeyer U, Drechsler C, Kock M, Assmann M (2002) Brominated pyrrole alkaloids from marine *Agelas* sponges reduce depolarization-induced cellular calcium elevation. Toxicon 44:45–51

Bölcskei H, Tarnawa I, Kocsis P (2008) Voltage-gated sodium channel blockers. 2001–2006: an overview. Med Chem Res 17:356–368

Burek F, Burek J (2013) BioLib: taxonomic tree of plants and animals with photos. http://www.biolib.cz/en/image/id26477/. Accessed 12 Dec 2013

Cafieri F, Carnuccio R, Fattorusso E, Taglialatela Scafati O, Vallefuoco T (1997) Antihistaminic activity of bromopyrrole alkaloids isolated from Caribbean *Agelas* sponges. Bioorg Med Chem Lett 7:2283–2288

Catterall WA (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26:13–25

Clare JJ, Tate SN, Nobbs M, Romanos MA (2000) Voltage-gated sodium channels as therapeutic targets. Drug Discov Today 5:506–520

England S, de Groot MJ (2009) Subtype-selective targeting of voltage-gated sodium channels. Brit J Pharmacol 158:1413–1425

Grant JA, Gallardo MA, Pickup BT (1996) A fast method of molecular shape comparison: a simple application of a Gaussian description of molecular shape. J Comput Chem 17:1653–1666

Hawkins PC, Nicholis A (2012) Conformer generation with OMEGA: learning from the data set and the analysis of failures. J Chem Inf Model 52:2919–2936

Hawkins PCD, Skillman AG, Nicholis A (2007) Comparison of shape-matching and docking as virtual screening tools. J Med Chem 50:74–82

Hawkins PCD, Skillman AG, Warren GL, Ellingson BA, Stahl MT (2010) Conformer generation with OMEGA: algorithm and validation using high quality structures from the protein databank and the Cambridge structural database. J Chem Inf Model 50:572–584

Hodnik Ž, Tomašič T, Mašič LP, Chan F, Kirby RW, Madge DJ, Kikelj D (2013) Novel state-dependent voltage-gated sodium channel modulators, based on marine alkaloids from *Agelas* sponges. Eur J Med Chem 70:154–164

Jukič M, Kikelj D, Anderluh M (2013) Isoform selective voltage-gated sodium channel modulators and the therapy of pain. Curr Med Chem 20:1–24

Keifer PA, Schwartz RE, Moustapha ESK, Hughes RG Jr, Rittschof D, Rinehart KL (1991) Bioactive bromopyrrole metabolites from the Caribbean sponge *Agelas conifer*. J Org Chem 56:2965–2975

Laport MS, Santos OCS, Muricy G (2009) Marine sponges: potential sources of new antimicrobial drugs. Curr Pharm Biotechnol 10:86–105

Little TL, Webber SE (1994) A simple and practical synthesis of 2-aminoimidazoles. J Org Chem 59:7299–7305

Neuman DJ (2008) Natural products as leads to potential drugs: an old process or the new hope for drug discovery. J Med Chem 51:2589–2599

OEDocking, version 3.0.1, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com, 2013

Olofson A, Yakushijin K, Horne DA (1998) Synthesis of marine sponge alkaloids oroidin, clathrodin, and dispacamides. Preparation and transformation of 2-amino-4,5-dialkoxy-4,5-dihydroimidazolines from 2-aminoimidazoles. J Org Chem 63:1248–1253

OMEGA, version 2.4.6, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com, 2013

Shrikhande JJ, Gawande MB, Jayaram RV (2008) A catalyst-free *N*-benzyloxycarbonylation of amines in aqueous micellar media at room temperature. Tetrahedron Lett. 49:4799–4803

Peat AJ, Sebahar PR, Youngman M, Chong PY, Zhang H (2008) Chemical Compounds WO2008154271-A1

Perdicaris S, Vlachogianni T, Valavanidis A (2013) Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals. Nat Prod Chem Res 1:1–8

Rasapalli S, Kumbam V, Dhawane AN, Golden JA, Lovely CJ, Rheingold AL (2013) Total synthesis of oroidin, hymenidin and clathrodin. Org Biomol Chem 11:4133–4137

Richards JJ, Ballard TE, Huigens RW, Melander C (2008) Synthesis and screening of an oroidin library against *Pseudomonas aeruginosa* biofilms. ChemBioChem 9:1267–1279

Rivera Rentas AL, Rosa R, Rodriguez AD, De Motta GE (1995) Effect of alkaloid toxins from tropical marine sponges on membrane sodium currents. Toxicon 33:491–497

Storey BT, Sullivan WW, Moyer CL (1964) The pKa values of some 2-amino imidazolium ions. J Org Chem 29:3118–3120

Sun J, Dong Y, Cao L, Wang X, Wang S, Hu Y (2004) Highly efficient chemoselective deprotection of *O*,*O*-acetals and *O*,*O*-ketals catalyzed by molecular iodine in acetone. J Org Chem 69:8932–8934

Taylor CP (1996) Voltage-gated Na^+ channels as targets for anticonvulsant, analgesic and neuroprotective drugs. Curr Pharm Des 2:375–388

Teixeira C, Serradji N, Amroune S, Storck K, Rogez-Kreus C, Clayette P, Barbault F, Maurel F (2013) Is the conformational flexibility of piperazine derivatives important to inhibit HIV-1 replication? J Mol Graph Model 44:91–103

Termin A, Martinborough E, Wilson D (2008) Chapter 3. Recent advances in voltage-gated sodium channel blockers: therapeutic potential as drug targets in the CNS. Annu Rep Med Chem 43:43–60

Tomašić T, Hartzoulakis B, Zidar N, Chan F, Kirby RW, Madge DJ, Peigneur S, Tytgat J, Kikelj D (2013) Ligand- and structure-based virtual screening for clathrodin-derived human voltage-gated sodium channel modulators. J Chem Inf Model 53:3223–3232

Tuccinardi T, Ortore G, Amelia Santos M, Marques SM, Nuti E, Rosello A, Martinelli AJ (2009) Multitemplate Alignment method for the development of a reliable 3D-QSAR model for the analysis of MMP3 inhibitors. J Chem Inf Model 49:1715–1724

vROCS, version 3.2.0.4, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com, 2013

Vuorela P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, Vuorela H (2004) Natural products in the process of finding new drug candidates. Curr Med Chem 11:1375–1389

Waxman SG, Kocsis JD, Black JA (1994) Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. J Neurophysiol 72:466–471

Yoke Chong P, Peat AJ, Sebahar PR, Youngman M, Zhang H (2010) Chemical Compounds US Patent 2010/0216746 A1 26th Aug 2010

Žula A, Kikelj D, Ilaš J (2013) 2-Aminoimidazoles in medicinal chemistry. Mini-Rev Med Chem 13:1921–1943