Discovery of oxazolidinone-based heterocycles as subtype selective sigma-2 ligands

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Abstract
The sigma-2 (σ2) receptor, also known as the Transmembrane Protein 97 (TMEM97, and MAC30 (Meningioma-associated protein), has been linked to a number of conditions are disease states such as schizophrenia, cancer, Alzheimer’s disease, traumatic brain injury, and neuropathic pain. As part of our ongoing effort identify novel σ2 ligands, we have identified a series of novel, functionalized oxazolidin-2-one sigma-2 ligands (4). Our lead compound (4h) demonstrated high affinity (Ki = 36 nM) and excellent σ1/σ2 selectivity (79-fold). Evaluation of its affinity at key CNS targets via the Psychoactive Drug Screening Program (PDSP) also indicated a high degree of selectivity for σ2 over other receptors.

Graphical Abstract

Keywords Sigma-2 · Sigma-1 · Sigma receptor · Oxazolidin-2-one

Introduction
The identification and characterization of the sigma receptors began in 1976 when Martin et al. described their attempts to classify opioids based on pharmacological responses produced in chronic spinal dogs. Morphine (1), ketocyclazocine, (2), and SKF-100047 (3) (Fig. 1) each produced different results in this animal model. They proposed that each compound engaged a different receptor that they labeled the µ-opioid receptor (morphine type, MOR), the κ-opioid receptor (ketocyclazocine type, KOR), and the σ-opioid receptor (SKF-100047 like) [1]. These studies were conducted with racemic SKF-100047, and in the early 1980s, follow-up studies with (−)-SKF-100047 and (+)-SKF-100047 demonstrated that each enantiomer engaged different targets. While the (−)-SKF-100047 elicited opioid type responses through MOR and KOR, (+)-SKF-100047’s pharmacological activity was mediated by a previously unknown, non-opioid receptor that was designated the sigma receptor (σR) [2, 3]. It was later demonstrated by that there were two subtypes of this receptor, sigma-1 (σ1) and sigma-2 (σ2) [4]. The mammalian σ1 receptor was cloned and expressed in yeast cells in 1976 [5], and a crystal structure of the human σ1 was reported in 2016 [6], To date, however, there is no known ligand for this receptor.

Characterization of σ2 was significantly more challenging. The true nature of this protein was not determined until 2017, over 40 years after the original discovery of the sigma receptors, when it was demonstrated that σ2 is Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein) [7]. There are no known natural functional ligands for σ2, but this protein is present in the endoplasmic reticulum and lysosomes where it binds to
cholesterol [8]. Although the role of this protein in physiological processes has as yet to be determined, \( \sigma_2 \) has been linked to numerous disease states and conditions such as Niemann-Pick disease [9], schizophrenia [10], Alzheimer’s disease [11–13], neuropathic pain [14], traumatic brain injury [15], and cancer [16, 17]. Efforts to develop novel therapeutic agents based on these links has led to the identification of numerous, highly potent \( \sigma_2 \) ligands (Fig. 2). Many have in vivo efficacy in animal models, and some have advanced to human clinical trials. Siramesine (4, \( \sigma_2 K_i = 0.12 \text{nM} \)), ketocyclazocine (2), and (rac)-SKF-100047 (3) should further accelerate these efforts.

As part of an effort to identify novel, biologically active small molecules, we have been exploring the chemical space associated with a series of 5-(piperazin-1-ylmethyl) oxazolidin-2-ones with potent \( \sigma_2 \) binding was established. The synthesis, characterization and preliminary evaluation of these compounds as potential selective \( \sigma_2 \) ligands will be presented.

Figs. 1 and 2 Structures of morphine (1), ketocyclazocine (2), and (rac)-SKF-100047 (3)

\[ \text{Fig. 1 Structures of morphine (1), ketocyclazocine (2), and (rac)-SKF-100047 (3)} \]

\[ \text{Fig. 2 Structures of Siramesine (4), UKH-1114 (5), CB-184 (6)} \]

Results and discussion

Synthesis of the 5-(piperazin-1-ylmethyl)oxazolidin-2-ones was conducted using the methods described in Schemes 1 and 2. The unsubstituted oxazolidin-2-one (9a) was prepared from the known primary chloride (10). Conversion of this starting material to the corresponding iodide using NaI in acetone was followed by displacement of the iodide with (11) to provide (9a). Synthesis of the remaining compounds (9b–9q) began with either epibromohydрин (12) or glycidol (13). Reaction of (12) with an amine (14) in methanolic \( \text{K}_2\text{CO}_3 \) provided oxazolidin-2-one (15). Alternatively, reaction of (13) with an amine (14), followed by ring closure with diethyl carbonate in the presence of sodium methoxide provided (15). Conversion of (15) to the corresponding tosylate (pTosCl, NEt3, THF), followed by displacement of the leaving group with an amine (11) provided the final target compounds (9b–9q).

Table 1 includes the in vitro binding (\( K_i \) at \( \sigma_2 \) and \( \sigma_1 \)) as well as the physicochemical properties (MW, TPSA, LogP) of target compounds. The compounds prepared and tested have TPSA and MW values that suggest they will cross the BBB, and all but three cLogP values are higher than the desired range of 2–4 for BBB penetration. The structure-activity relationship began with the unsubstituted oxazolidin-2-one (9a), which demonstrated minimal affinity for \( \sigma_1 \) and \( \sigma_2 \) (\( K_i = 10,000 \text{nM} \) at both \( \sigma_1 \) and \( \sigma_2 \)). Capping of the amide with a 3 to 6 membered cycloalkane (9b–9e) or a benzene ring (9f) produced compounds with moderate \( \sigma_2 \) binding affinity (\( K_i = 116–530 \text{nM} \)) that had limited capacity to bind to \( \sigma_1 \) (\( K_i = 10,000 \text{nM} \)). Interestingly, the impact of a benzyl substituent on \( \sigma_2 \) affinity was highly dependent on the electronic character of the benzene ring. In the absence of a substituent, the benzyl derivative (9g) is approximately twice as potent at \( \sigma_2 \) (\( K_i = 91 \text{nM} \)) as the corresponding phenyl derivative (9f), and the high degree of selectivity over \( \sigma_1 \) is maintained (\( K_i = 10,000 \text{nM} \)). \( \sigma_2 \) affinity increased three-fold when a 4-F substituent was appended to the benzene ring (9h, \( \sigma_2 K_i = 36 \text{nM} \)), and high degree of selectivity over \( \sigma_1 \) (\( K_i = 2847 \text{nM} \)) was maintained. In contrast, incorporation of the electron donating 4-OME (9i) or 4-Me (9j) produced compounds that had limited capacity to bind both sigma receptors (\( \sigma_2 \) and \( \sigma_1 K_i = 10,000 \text{nM} \)). Replacing the benzene ring of (9g) with either a cyclohexane ring (9k) or tetrahydropyran ring (9l) also lead to a substantial drop in \( \sigma_2 \).
affinity ($K_i = 1379$ and $2428$ nM, respectively). $\sigma_2$ binding affinity and selectivity over $\sigma_1$ were maintained when the benzyl group ($9g$) was extended by an additional methylene ($9m$, $\sigma_2 K_i = 49$ nM, $\sigma_1 K_i = 10,000$ nM), but incorporation of both electron withdrawing ($9n$) and electron donating substituents ($9o$, $9p$) led to a significant loss in $\sigma_2$ binding affinity, as did the addition of a third methylene unit ($9q$).

Follow-up assessment of our most potent $\sigma_2$ ligand ($9h$) at a series of key CNS targets available through the PDSP demonstrated that this compound is selective for $\sigma_2$ over a variety of important CNS targets (Table 2). Specifically, ($9h$) is >100-fold selective for $\sigma_2$ over, $\alpha_{1B}$, $\alpha_{1D}$, $\alpha_{2A}$, the benzodiazepine receptor (brain and peripheral), $\beta_1$, $\beta_2$, $\beta_3$ D1, D5, GABA-A, H2, H3, 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT2C, M1, M2, M3, M4, M5, the $\delta$-opioid receptor, the $\mu$-Opioid receptor, and the serotonin transporter. In addition, ($9h$) was >25 fold $\alpha_{1A}$, $\alpha_{2B}$, $\alpha_{2C}$, D2, D3, 5-HT2A, the $\kappa$-opioid receptor, and the norepinephrine transporter. Less selectivity was observed over D4 (521 nM), 5HT2b (201 nM), DAT (186 nM), and H1 (99 nM).

### Conclusion

In summary, we have identified a series of 5-(piperazin-1-ylmethyl)oxazolidin-2-ones (9) $\sigma_2$ ligands that are highly selective for this receptor over $\sigma_1$. In addition, we have established preliminary SAR that could be used to extend our exploration of this series and identified a novel compound ($9h$) that is selective for $\sigma_2$ over a range of key CNS targets. Future efforts are focused on determining the in vitro ADME
Table 2 Off-target assessment of (9h) at key CNS targets

| α2 | α1 | α1A | α1B | α1D | α2A | α2B | α2C | β1 | β2 |
|----|----|-----|-----|-----|-----|-----|-----|----|----|
| 36 ± 6.5 | 2847 ± 456 | 1705 ± 196 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 |
| β3 | BZP-B | BZP-P | D1 | D2 | D3 | D5 | DAT | DOP |
| >10,000 | >10,000 | >10,000 | 1508 ± 368 | 1293 ± 148 | 521 ± 61 | >10,000 | 186 ± 46 | >10,000 |
| GABA_A | H1 | H2 | H3 | 5HT1A | 5HT1B | 5HT1D | 5HT1E | 5HT2A | 5HT2B |
| >10,000 | 99 ± 13.5 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 |
| 5HT2C | KOP | M1 | M2 | M3 | M4 | M5 | MOP | NET | SERT |
| >10,000 | 1007 ± 69 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 |

Tosylation of compounds (normal phase and reverse phase) was carried out on a Teledyne Isco Combiflash RF system. Chemical shift values (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 × 30 mm, 3.5 μm, 100% water – 0.1% formic acid to 100% acetonitrile – 0.1% formic acid over 40 min, 1.0 ml/min.) with a diode array detector from 210–400 nm and Agilent 6130 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and 1H-NMR. Melting points were recorded on a capillary melting point apparatus.

Properties (e.g., solubility, CYP450 inhibition, microsomal stability) of our compounds and exploring the impact of additional substituents on the embedded benzene rings.

**Experimental methods and materials**

Reagents were purchased from Fisher Scientific, VWR International, Sigma Aldrich, and Combi-Blocks, Inc. Chromatographic purification of compounds (normal phase and reverse phase) was carried out on a Teledyne Isco CombiFlash RF system. 1H-NMR spectra were obtained on a Bruker 400-MHz NMR. Chemical shift values (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 × 30 mm, 3.5 μm, 100% water – 0.1% formic acid to 100% acetonitrile – 0.1% formic acid over 40 min, 1.0 ml/min.) with a diode array detector from 210–400 nm and Agilent 6130 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and 1H-NMR. Melting points were recorded on a capillary melting point apparatus.

**Preparation of 5-(4-Benzhydryl-piperazin-1-ylmethyl)oxazolidin-2-one (9a)**

Sodium iodide (13.19 g, 87.9 mmol) in acetonitrile (35 ml) was added to a round bottom flask containing 5-chloromethyl-oxazolidin-2-one (0.8 g, 5.90 mmol) and the mixture was refluxed for 48 h. The reaction was later quenched with ice water and extracted with ether. The organic layer was washed with water, brine and dried over MgSO4 to obtain the product as a light-yellow solid. This material was dissolved in THF (50 ml) that contained diphenylmethylpiperazine (2.43 g, 9.629 mmol) and triethylamine (1.299 g, 12.83 mmol). The reaction was stirred at reflux under a nitrogen atmosphere for 48 h. The mixture was filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane and purified by flash silica gel chromatography using methanol (0–10%) in dichloromethane and reverse phase chromatography using acetonitrile:water (0–100%) to obtain the desired product: Yield: 18.0%, 2 steps (0.203 g).

Examples (9b), (9c), (9d), and (9e) were prepared according to previously published methods [26]. (2-oxo-3-phenyloxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to previously published methods [27].

**General procedure for the synthesis of toslyates from epibromohydrine and an amine**

To a suspension of K2CO3 (2.5 g, 18.08 mmol) in anhydrous methanol (30 ml) containing epibromohydrin (4.965 g, 36.24 mmol) was added an amine (36.12 mmol) and the reaction was stirred overnight. The reaction mixture was then filtered and the organic solvent was removed under reduced pressure to obtain a liquid residue that was utilized for the toslylation step without further purification.

p-Toluene sulfonyl chloride (13.5 g, 7.096 mmol) in methylene chloride (50 ml) was added dropwise to a 0 °C solution of the crude alcohol (35.52 mmol) and triethylamine (7.1874 g, 71.028 mmol) in methylene chloride (60 ml). The reaction was stirred at 0 °C for 1 h followed by overnight stirring at room temperature. It was then quenched with ice water and the organic layer was washed successfully with 10% HCl, saturated aqueous sodium bicarbonate and brine. The organic layer was dried over MgSO4 and concentrated under reduced pressure to obtain a thick oil which was
purified on a silica gel column using hexane: ethyl acetate (0–100%) to obtain the desired product.

(3-(cyclohexylmethyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using cyclohexylmethanamine: Yield 22.81% (2.98 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 0.89–0.99 (m, 2H), 1.11–1.28 (m, 3H), 1.47–1.61 (m, 1H), 1.63–1.74 (m, 6H), 2.47 (s, 3H), 3.00–3.11 (m, 2H), 3.42–3.46 (m, 1H), 3.63 (t, 1H), 4.11–4.18 (m, 2H), 4.66–4.71 (m, 1H), 7.35–7.39 (m, 2H), 7.79–7.81 (m, 2H). LC-MS (ESI) (m/z) 368.1 (M$^+$+1)$^\dagger$.

(3-benzyl-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using benzyl amine. Yield: 68.7% (8.95 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 2.45 (s, 3H), 3.23–3.27 (m, 1H), 3.48 (t, 1H), 4.07–4.14 (m, 1H), 4.39 (s, 2H), 4.62–4.68 (m, 1H), 7.24–7.26 (m, 2H), 7.30–7.38 (m, 5H), 7.73–7.76 (m, 2H). LC-MS (ESI) (m/z) 363.1 (M$^+$+1)$^\dagger$.

(3-(4-fluorobenzyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using 4-fluorobenzyl amine. Yield: 48.9% (6.7 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 2.48 (s, 3H), 3.28–3.30 (m, 1H), 3.50 (t, 1H), 4.11–4.12 (m, 2H), 4.34–4.43 (m, 2H), 4.64–4.70 (m, 1H), 7.03–7.07 (m, 2H), 7.22–7.27 (m, 2H), 7.38–7.39 (m, 2H), 7.76–7.78 (m, 2H). LC-MS (ESI) (m/z) 380.1 (M$^+$+1)$^\dagger$.

(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using 4-methoxybenzyl amine. Yield: 79.7% (11.26 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 2.46 (s, 3H), 3.44 (t, 1H), 3.81 (s, 3H), 4.04–4.13 (m, 2H), 4.33 (s, 1H), 4.60–4.66 (m, 1H), 6.86–6.90 (m, 2H), 7.15–7.19 (m, 2H), 7.35–7.37 (m, 2H), 7.74–7.76 (m, 2H). LC-MS (ESI) (m/z) 392.1 (M$^+$+1)$^\dagger$.

(3-(4-methylbenzyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using 4-methylbenzyl amine. Yield: 64.8% (8.77 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 2.27 (s, 3H), 2.38 (s, 3H), 3.15–3.19 (m, 1H), 3.38 (t, 1H), 3.96–4.04 (m, 2H), 4.23–4.32 (m, 2H), 4.52–4.58 (m, 1H), 7.02–7.09 (m, 4H), 7.28 (d, 2H), 7.67–7.69 (m, 2H). LC-MS (ESI) (m/z) 376.1 (M$^+$+1)$^\dagger$.

(2-oxo-3-(tetrahydropyran-4-ylmethyl)-oxazolidin-5-ylmethyl ester was prepared according to the general procedure using (tetrahydro-2H-pyran-4-yl)methanamine. Yield: 63.6% (8.48 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 1.23–1.36 (m, 2H), 1.54–1.58 (m, 2H), 1.78–1.89 (m, 1H), 2.45 (s, 3H), 3.01–3.06 (m, 1H), 3.14–3.19 (m, 1H), 3.32–3.38 (m, 2H), 3.46–3.50 (m, 1H), 3.66 (t, 1H), 3.93–3.97 (m, 2H), 4.15 (d, 2H), 4.66–4.74 (m, 1H), 7.37 (d, 2H), 7.77 (d, 2H). LC-MS (ESI) (m/z) 370.1 (M$^+$+1)$^\dagger$.

(2-oxo-3-phenethyloxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using phenylethylamine. Yield: 81.7% (11.1 g). $^1$H-NMR (CDCl$_3$, 400 MHz, δ (ppm)) 2.46 (s, 3H), 2.83 (t, 1H), 3.25–3.29 (m, 1H), 3.45–3.52 (m, 3H), 4.01–4.09 (m, 2H), 4.56–4.61 (m, 1H), 7.20–7.26 (m, 3H), 7.30–7.33 (m, 2H), 7.38 (d, 2H), 7.77–7.80 (m, 2H). LC-MS (ESI) (m/z) 376.1 (M$^+$+1)$^\dagger$. 
(3-(4-fluorophenethyl)-2-oxo-oxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using 4-fluorophenylethylamine. Yield: 61.1% (8.67 g). 1H-NMR (CDCl3, 400 MHz, δ (ppm)) 2.47 (s, 3H), 2.84 (t, 2H), 3.32–3.36 (m, 1H), 3.39–3.54 (m, 3H), 4.02–4.16 (m, 3H), 6.98–7.03 (m, 2H), 7.15–7.20 (m, 2H), 7.38 (d, 2H), 7.78–7.80 (m, 2H). LC-MS (ESI) (m/z) 394.1 (M+1)+.

Toluene-4-sulfonic acid 3-[2-(4-methoxy-phenyl)-ethyl]-2-oxo-oxazolidin-5-ylmethyl ester was prepared according to the general procedure using 4-methoxyphenylethylamine. Yield: 84.5% (12.36 g). 1H-NMR (CDCl3, 400 MHz, δ (ppm)) 2.47 (s, 3H), 2.78 (t, 2H), 3.26–3.30 (m, 1H), 3.42–3.52 (m, 3H), 3.81 (s, 3H), 4.01–4.16 (m, 3H), 4.56–4.64 (m, 1H), 6.85 (d, 2H), 7.11 (d, 2H), 7.38 (d, 2H), 7.78–7.80 (d, 2H). LC-MS (ESI) (m/z) 406.1 (M+1)+.

Toluene-4-sulfonic acid 2-oxo-3-(2-p-tolyl-ethyl)-oxazolidin-5-ylmethyl ester was prepared according to the general procedure using 4-methylphenylethylamine. Yield: 75.9% (10.66 g). 1H-NMR (CDCl3, 400 MHz, δ (ppm)) 2.34 (s, 3H), 2.47 (s, 3H), 2.81 (t, 2H), 3.26–3.30 (m, 1H), 3.44–3.53 (m, 3H), 4.01–4.16 (m, 2H), 4.56–4.62 (m, 1H), 7.08–7.13 (m, 4H), 7.37–7.39 (d, 2H), 7.78–7.80 (d, d, 2H). LC-MS (ESI) (m/z) 390.1 (M+1)+.

(2-oxo-3-(3-phenylpropyl) oxazolidin-5-yl)methyl-4-methylbenzenesulfonate was prepared according to the general procedure using phenylpropylamine. Yield: 76.9% (10.8 g). 1H-NMR (CDCl3, 400 MHz, δ (ppm)) 1.83–1.90 (m, 2H), 2.45 (s, 3H), 2.61–2.66 (m, 2H), 3.25–3.32 (m, 2H), 3.38–3.42 (m, 1H), 3.58 (t, 1H), 4.11–4.13 (m, 2H), 4.59–4.65 (m, 1H), 7.19–7.23 (m, 3H), 7.28–7.32 (m, 2H), 7.34–7.36 (m, 2H), 7.77–7.83 (m, 2H). LC-MS (ESI) (m/z) 390.1 (M+1)+.

General procedure for the preparation of functionalized 5-((4-benzhydrylpiperazin-1-yl)methyl)oxazolidin-2-one (9f–9q)

Functionalized (2-oxo-oxazolidin-5-yl)methyl-4-methylbenzenesulfonate (2.955 mmol) diphenylmethylpiperazine (1.49 g, 5.90 mmol), and triethylamine (1.1648 g, 11.51 mmol) in anhydrous tetrahydrofuran (5 ml) were stirred under microwave at 120 °C for 1 h. After 1 h, the solvent was stripped off, residue was dissolved in dichloromethane, and purified by silica gel column using hexane-ethyl acetate (0–100%) to obtain the desired product.
5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-fluorobenzyl) oxazolidin-2-one (9h) was prepared according to the general procedure using (3-(4-fluorobenzyl)-2-oxooxazolidin-5-yl) methyl-4-methylbenzenesulfonate. Yield: 65.8% (0.8941 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 2.37-2.67 (m, 10H), 3.17-3.19 (m, 1H), 3.43 (s, 1H), 4.21 (s, 1H), 4.39 (s, 2H), 4.56-4.69 (m, 1H), 7.01-7.07 (m, 2H), 7.17-7.21 (m, 2H), 7.25-7.30 (m, 6H), 7.40-7.42 (m, 4H). \]

LC-MS (ESI) (m/z) 460.2 (M + 1)^+.

5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-methoxybenzyl) oxazolidin-2-one (9i) was prepared according to the general procedure using (3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 70.8% (0.951 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 2.87-3.03 (m, 1H), 3.07 (m, 1H), 3.30-3.32 (m, 1H), 3.57 (t, 1H), 4.22 (s, 1H), 4.58-4.70 (m, 1H), 7.17-7.21 (m, 2H), 7.26-7.30 (m, 4H). \]

LC-MS (ESI) (m/z) 546.2 (M + 1)^+.

5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-methylbenzyl) oxazolidin-2-one (9j) was prepared according to the general procedure using (3-(4-Methylbenzyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 59.4% (0.829 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 2.67 (m, 10H), 3.03 (m, 1H), 3.30-3.32 (m, 1H), 3.57 (t, 1H), 4.22 (s, 1H), 4.58-4.70 (m, 1H), 7.17-7.21 (m, 2H), 7.26-7.30 (m, 4H), 7.40-7.42 (m, 4H). \]

LC-MS (ESI) (m/z) 448.3 (M + 1)^+.

5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-methoxybenzyl) oxazolidin-2-one (9k) was prepared according to the general procedure using (2-oxo-3-(tetrahydro-2H-pyran-4-yl)methyl)oxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 75.8% (1.006 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 1.31-1.41 (m, 2H), 1.58-1.61 (m, 2H), 1.74 (s, 1H), 1.81-1.92 (m, 1H), 2.41-2.70 (m, 10H), 3.08-3.18 (m, 2H), 3.31-3.41 (m, 3H), 3.60 (t, 1H), 3.97-4.01 (m, 2H), 4.22 (s, 1H), 4.60-4.66 (m, 1H), 7.17-7.21 (m, 2H), 7.26-7.30 (m, 4H), 7.40-7.42 (m, 4H). \]

LC-MS (ESI) (m/z) 450.3 (M + 1)^+.

5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-fluorophenethyl) oxazolidin-2-one (9l) was prepared according to the general procedure using with (2-oxo-3-((tetrahydro-2H-pyran-4-yl)methyl)oxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 47.9% (0.778 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 1.61 (m, 2H), 1.74 (s, 1H), 1.81-1.92 (m, 1H), 2.41-2.70 (m, 10H), 3.08 (m, 1H), 3.30 (m, 1H), 3.37 (s, 3H), 4.22 (s, 1H), 4.50-4.57 (m, 1H), 7.17-7.33 (m, 11H), 7.40-7.42 (m, 4H). \]

LC-MS (ESI) (m/z) 548.3 (M + 1)^+.

5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-fluorophenethyl) oxazolidin-2-one (9m) was prepared according to the general procedure using (3-(4-phenethyloxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 66.5% (0.878 g).

\[ \text{H-NMR: } \{\text{CDCl}_3, 400 MHz, } \delta (\text{ppm})\} \]
\[ 0.92-1.01 (m, 2H), 1.12-1.29 (m, 3H), 1.52-1.76 (m, 7H), 2.42-2.70 (m, 10H), 3.02-3.11 (m, 2H), 3.28-3.32 (m, 1H), 3.57 (t, 1H), 4.22 (s, 1H), 4.58-4.70 (m, 1H), 7.17-7.21 (m, 2H), 7.26-7.30 (m, 4H), 7.40-7.42 (m, 4H). \]

LC-MS (ESI) (m/z) 548.3 (M + 1)^+.
5-(4-Benzhydryl-piperazin-1-ylmethyl)-3-[(2-(4-methoxy-phenyl)-ethy1)]-oxazolidin-2-one (90) was prepared according to the general procedure using (3-(4-methoxyphenethyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 48.2% (0.690 g). 1H-NMR: {CDCl3, 400 MHz, δ (ppm)} 2.31–2.58 (m, 2H), 2.31–2.58 (m, 2H), 3.13–3.27 (m, 3H), 3.79 (s, 3H), 4.23 (m, 1H), 4.50–4.57 (m, 1H), 6.84–6.87 (m, 2H), 7.13–7.21 (m, 4H), 7.27–7.30 (m, 4H). LC-MS (ESI) (m/z) 470.3 (M + 1)+.

5-(4-Benzhydryl-piperazin-1-ylmethyl)-3-(2-p-tolyl-ethyl)-oxazolidin-2-one (9p) was prepared according to the general procedure using (3-(4-methoxyphenethyl)-2-oxooxazolidin-5-yl)methyl-4-methylbenzenesulfonate. Yield: 63.2% (0.875 g). 1H-NMR: {CDCl3, 400 MHz, δ (ppm)} 2.23–2.53 (m, 13H), 2.72–2.77 (m, 2H), 3.02–3.06 (m, 1H), 3.32 (t, 1H), 3.40 (t, 2H), 4.12 (s, 1H), 4.40–4.47 (m, 1H), 7.07–7.11 (m, 2H), 7.16–7.20 (m, 4H), 7.30–7.33 (m, 4H). LC-MS (ESI) (m/z) 470.3 (M + 1)+.

5-((benzhydrylpiperazin-1-ylmethyl)-3-(3-phenylpropyl) oxazolidin-2-one (9q) was prepared according to the general procedure using (2-oxo-3-(3-phenylpropyl)oxazolidin-5-yl) methyl-4-methylbenzenesulfonate. Yield: 24.8% (0.343 g). 1H-NMR: {CDCl3, 400 MHz, δ (ppm)} 1.75–1.82 (pentet, 2H), 2.31–2.58 (m, 12H), 3.13–3.27 (m, 3H), 3.43 (t, 1H), 4.10 (s, 1H), 4.44–4.50 (m, 1H), 7.07–7.14 (m, 5H), 7.16–7.23 (m, 6H), 7.30–7.32 (m, 4H). LC-MS (ESI) (m/z) 470.3

Computational values

TPSA and cLogP values were calculated using the Dotmatics software suite (Dotmatics LLC The Old Monastery, Windhill Bishops, Stortford Herts, CW23 2ND UK).

Radiolabel binding studies for the sigma-2 receptor

A solution of the test compound is prepared as a 1-mg/ml stock in Assay Buffer or DMSO according to its solubility. A similar stock of the reference compound Haloperidol is also prepared as a positive control. Eleven dilutions (5 × assay concentration) of the test compound and Haloperidol are prepared in the Assay Buffer by serial dilution to yield final corresponding assay concentrations ranging from 10 pM to 10 μM.

A stock concentration of 5 nM 3H-1,3-di-(2-tolyl) guanidine (3H-DTG) is prepared in 50 mM Tris-HCl, 10 mM MgCl2, 1 mM EDTA, pH 7.4 (Assay Buffer). Aliquots (50 μl) of radioligand are dispensed into the wells of a 96-well plate containing 100 μl of Assay Buffer. Duplicate 50-μl aliquots of the test compound and Haloperidol positive control reference compound serial dilutions are added.

Membrane fractions of cells expressing recombinant σ2 (50 μl) are dispensed into each well. The membranes are prepared from stably transfected cell lines expressing sigma-2 receptors cultured on 10-cm plates by harvesting PBS-rinsed monolayers, resuspending and lysing in chilled, hypotonic 50 mM Tris-HCl, pH 7.4, centrifuging at 20,000 × g, decanting the supernatant and storing at \(-80\) °C; the membrane preparations are resuspended in 3 ml of chilled Assay Buffer and homogenized by several passages through a 26 guage needle before using in the assay.

The 250-μl reactions are incubated at room temperature for 1.5 h, then harvested by rapid filtration onto 0.3% polyethyleneimine-treated, 96-well filter mats using a 96-well Filtermate harvester. Four rapid 500-μl washes are performed with chilled Assay Buffer to reduce non-specific binding. The filter mats are dried, then scintillant is added to the filters and the radioactivity retained on the filters is counted in a Microbeta scintillation counter.

Raw data (dpm) representing total radioligand binding (i.e., specific + non-specific binding) are plotted as a function of the logarithm of the molar concentration of the competitor (i.e., test or reference compound). Non-linear regression of the normalized (i.e., percent radioligand binding) compared to that observed in the absence of test or reference compound) raw data is performed in Prism 4.0 (GraphPad Software) using the built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites:

\[
y = \text{bottom} + \left\{\text{top} - \text{bottom}\right\}/\left(1 + 10^{x - \text{logIC}_{50}}\right)
\]

where bottom equals the residual radioligand binding measured in the presence of 10 μM reference compound (i.e., non-specific binding) and top equals the total
radioligand binding observed in the absence of competitor. The log IC_{50} (i.e., the log of the ligand concentration that reduces radioligand binding by 50%) is thus estimated from the data and used to obtain the K_i by applying the Cheng–Prusoff approximation:

\[ K_i = \frac{IC_{50}}{1 + [\text{ligand}] / K_D} \]

where [ligand] equals the assay radioligand concentration and K_D equals the affinity constant of the radioligand for the target receptor [28].

Radiolabel binding studies for the sigma-1 receptor

Binding assays to determine test compound affinity at \( \sigma_1 \) was determined using the methods described for \( \sigma_2 \) using membrane fractions of cells expressing recombinant \( \sigma_1 \). The radioligand employed was \(^{3}H\)-DTG and the control compound was Haloperidol [28].

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Compliance with ethical standards

Conflict of interest BEB and DJC both have equity interests in Praeventix LLC, which have been reviewed and approved by Temple University in accordance with its conflict of interest policies. Questions regarding this interest may be directed to the Temple University Conflict of Interest Program. No other author has reported conflicts of interest to disclose at the time of publication.

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