One-pot synthesis and sigma receptor binding studies of novel spirocyclic-2,6-diketopiperazine derivatives

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A B S T R A C T
New spirocyclic-2,6-diketopiperazine derivatives containing benzylpiperidine and cycloalkane moieties were synthesized by a one-pot two-step sequential Ugi/intramolecular N-amidation process in moderate to good yields. The in vitro ligand-binding profile studies performed on the sigma-1 and sigma-2 receptors revealed that the sigma-1 affinities and subtype selectivities of three spirocyclic piperidine derivatives are generally comparable to those of spirocycloalkane analogues. Compared to the low sigma-1 affinities obtained for cycloalkyl-substituted spirocyclic-2,6-diketopiperazines with n = 2, those with n = 1 proved to have optimal fitting with sigma-2 subtype by exhibiting higher affinities. Moreover, the best binding affinity and subtype selectivity was identified for compound 3c with K_i,σ = 5.9 ± 0.5 nM and K_i,σ = 563 ± 21 nM as well as 95-fold σ1/σ2 selectivity ratio, respectively.

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Although more than three decades have passed of introducing of the sigma receptors (σ-Rs) as one of the opioid receptor binding sites,1 researches on the determination of their protein structure and also finding new ligands are still ongoing.2–4 However, it is approved that the σ-R is a unique binding site which includes at least two different subtypes called as sigma-1 (σ_1) and sigma-2 (σ_2) receptors.5,6 Respectively. Whereas the σ_1 receptor has been recently cloned from various sources and has a molecular weight of ~25.3 kDa,7–10 the elucidation of the σ_2 receptor with a molecular weight of ~21.5 kDa has proven more difficult.5,6 Both of these receptor subtypes have widespread distribution in the central nervous system (CNS) and in many of peripheral tissues.11,13-15 These receptor subtypes are also overexpressed in rapidly proliferating normal and cancer cells from animal and human origins.13,14 Although there are clear differences between the σ_1, and σ_2 receptors in terms of their tissue distribution patterns, sub-cellular localizations and apparent molecular weight, the function of both receptors are not clearly defined yet.4 While it is recently proposed that the σ_2 receptor may be a progesterone receptor complex, characterization of the σ_1 receptor as a chaperone protein has been proven.15,16 However, depending on the location of these receptors in the special tissues and organs, σ_1-R are involved several applications such as modulation of inositol phosphates,16 modulation of many biological mechanisms associated with neurodegeneration17 and modulation of voltage-gated K^+, Ca^{2+}, Na^+, and Cl^- channels.15,16 On the basis of their widespread modulatory role, high affinities of the selective σ_1 receptor ligands for typical neuroleptic drugs makes them perfect therapeutic agents for amnesic and cognitive deficits, depression and anxiety, schizophrenia, analgesia, and against some effects of abuse drugs such as cocaine and methamphetamine.18

Nevertheless, the main utilization of the selective σ_2 receptor ligands is in regulation of cell proliferation and apoptosis in human tumour cell lines of both neuronal and non-neuronal origin.19–22 The high σ_2 receptor density in a wide range of tumour types (about 10-fold more in proliferating tumour cells compared with quiescent tumour cells) suggests that σ_2 receptors could be a target for the development of new radiotracers for imaging tumours with positron emission tomography (PET) and/or single photon emission computed tomography (SPECT).23–25

Nowadays, it is well known that there are a wide variety of unrelated chemical scaffolds such as substituted-phenylmorphinan derivatives (CB64D and CB184), alkaloid ibogaine, (+)-pentazocine, N,N-di-(o-toly)guanidine (DTG) and haloperidol which selectively interact with σ_1 and σ_2 receptors (Fig. 1a).5,26,27 Overview of the literature revealed that a great series of the sigma ligands are those included the benzylpiperidine moiety as well as piperazine rings (Fig. 1b).28,29 Among the various classes of the σ_1 receptor ligands, the benzylpiperidine substituted spirocyclic compounds (spipethiane and their analogues) are potent σ_1 receptor ligands with high affinity (Fig. 1b).30–32 In particular, replacement of the...
pyran or thiopyran rings with a triazine ring (a piperazine analogue) in spipethiane analogues, compound III led to the increasing of $r_1$ affinity and $r_1/r_2$ selectivity (Fig. 1b, $K_{i_{r1}} = 0.6$ nM and $K_{i_{r1/r2}} = 4220$).33

The 2,5-diketopiperazine (2,5-DKP) as a ‘privileged’ scaffold present in many natural products.34 One particular significant approach has been identifying privileged structures as selective small molecule ligands of GPCRs.35 Additionally, among these variable privileged structures, the spirocyclic piperidine structure has been seen in various receptors including the sigma, serotonin, somatostatin, chemokine, melanocortin and ghrelin receptors.36

The main purpose of this research is the synthesis of compounds 3a–n (Fig. 1c) which have the spirocyclic backbone of both benzylpiperidine or cycloalkane and piperazine rings within a ligand.30–32,37,38 To the best of our knowledge, there has been no report in the literature on the sigma receptor study of such ligands.

The extensive use of multicomponent reactions (MCRs) relies on their powerful synthetic strategies for the construction of biologically interesting compounds. In this regard, the isocyanide-based multicomponent reactions (IMCRs) have emerged particularly as efficient tools in this area based on their high efficiency, rich diversity, and easy operation.39,40 Recently, there have been efforts on the synthesis of more complex structures with the tandem Ugi/post-Ugi reactions.41–44

As a part of our own interest in the synthesis of biologically active heterocyclic compounds by multi-component reactions45,46 and the discovery of new sigma receptors ligands,47 herein we describe a one-pot synthesis of a number of spirocyclic-2,6-diketopiperazines via Ugi-4CR/intramolecular annulation reactions in order to explore their $\sigma$-Rs bindings.

Four component reaction employing acids 1a–d, cyclic ketones 2a–d with ethylglycinate hydrochloride and cyclohexyl isocyanide in the presence of triethylamine in MeOH were stirred at room temperature for 6 h. After removing the solvent under reduced pressure, the residue was heated in DMF at $140^\circ$C for 36 h in the presence of an equimolar amount of K$_2$CO$_3$ to afford the spirocyclic-2,6-diketopiperazine 3a–n in satisfactory yields (Scheme 1, Table 1).

Our described method proved to be successful, providing the target spirocyclic-2,6-diketopiperazines 3a–n with yields ranging from 70% to 88% (Table 1). Since these values refer to the overall yield of the apparently two synthetic Ugi/post-Ugi N-amidation cyclization processes, it can be concluded that reactions have proceeded with excellent yields for each simple step. All data provided in Table 1 also supports the rather excellent reactivity for all components.

Particularly significant is that whereas the first Ugi step proceeded successfully using other isocyanides such as tert-butyl,
values of Haloperidol, DTG and (+)-pentazocine (in this case, the σ₂ affinity of (+)-pentazocine was determined in the presence of AC915 as a σ₁ antagonist blocking agent) as reference compounds are also included in Table 2 in order to make the comparison more convenient.

Indeed in current study, we designed the rather rigid scaffolds which contain flexible hydrophobic parts which at least two hydrophobic parts are required to be matched into the sigma receptor pharmacophore models. Among the spirocyclic-2,6-diketopiperazine derivatives 3a–n prepared in this presentation, the benzyl piperidine substituted 3a–c exhibited moderate to good affinities ranging from 5.9 to 11.1 nM for σ₁ and 134.0 to 563.0 nM for σ₂ subtypes, respectively (entries 1–3, Table 2). In particular, the N-(4-MeO-benzoyl)-substituted spirocyclic-diketopiperazine 3c has a Kᵦ₁ value of 5.9 nM and good σ₁/σ₂ selectivity ratios (95-fold). Particularly significant is that changing the substituent on the benzoyl ring attached to the piperazine nitrogen in N-benzyl piperidine derivatives 3a–c has a minor effect on σ₁ binding affinities (entries 1–3, Table 2).

Compared to cyclohexyl-substituted analogues 3g–j (entries 7–10, Table 2), cyclopentyl-substituted spirocyclic-2,6-diketopiperazine derivatives 3d–f showed higher σ₁-Rs affinities (entries 4–6, Table 2). As such, it can be concluded that reducing the size of the cycloalkyl ring from six to five-member ring also leads to an increase in σ₁-Rs binding affinities. Moreover, within cyclopentyl-substituted spirocyclic-2,6-diketopiperazines 3d–f, compound 3f exhibited the best σ₂ binding affinity (Kᵦ₂ = 98 ± 18 nM) (entry 6, Table 2). Dramatic reduction in σ₁-Rs binding affinities were observed for the spirocyclic-2,6-diketopiperazine derivatives 3k–n (entries 11–14, Table 2) in which bulky tert-butyl group is present in the position 4 of the six-member ring (compare Kᵦ₁ = 227 ± 99 nM and Kᵦ₂ = 1388 ± 143 nM for compound 3g with Kᵦ₁ = 7896 ± 976 nM and Kᵦ₂ = >10,000 for compound 3k) (entry 11, Table 2).

Based on Glennon’s pharmacophore model for σ₁ receptor, it is possible that the benzyl piperidine moiety in compounds 3a–c plays an important role as a hydrophobic part (Fig. 2a).

Similarly, the benzoyl group attached to the piperazine nitrogen may acts as another hydrophobic part (Fig. 2a). In addition, according to this pharmacophoric model, a crucial feature for high σ₁ binding affinity is the existence of ionizable basic nitrogen in the structure which N-atom of piperidine ring in compound 3a–c can act as suitable hydrogen binding sites. On the other hand, low σ₁ binding affinities obtained for compounds 3d–n may be

Table 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>n</th>
<th>X</th>
<th>Kᵦ₁ (nM)ᵃ</th>
<th>Kᵦ₂ (nM)ᵇ</th>
<th>Selectivity (σ₁/σ₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>N-CH₂Ph</td>
<td>7.2 ± 0.7</td>
<td>248 ± 18</td>
<td>34.4</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>H</td>
<td>Me</td>
<td>1</td>
<td>N-CH₂Ph</td>
<td>11.1 ± 1.0</td>
<td>134 ± 9</td>
<td>12.7</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>H</td>
<td>OMe</td>
<td>1</td>
<td>N-CH₂Ph</td>
<td>5.9 ± 0.5</td>
<td>563 ± 21</td>
<td>95.4</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>H</td>
<td>H</td>
<td>0</td>
<td>CH₂</td>
<td>79.8 ± 9.1</td>
<td>118 ± 21</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>H</td>
<td>Me</td>
<td>0</td>
<td>CH₂</td>
<td>185 ± 34</td>
<td>134 ± 15</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>3f</td>
<td>H</td>
<td>OMe</td>
<td>0</td>
<td>CH₂</td>
<td>429 ± 89</td>
<td>98 ± 18</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>3g</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>CH₂</td>
<td>227 ± 99</td>
<td>138 ± 143</td>
<td>6.1</td>
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<tr>
<td>8</td>
<td>3h</td>
<td>H</td>
<td>Me</td>
<td>1</td>
<td>CH₂</td>
<td>399 ± 116</td>
<td>2189 ± 314</td>
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<tr>
<td>9</td>
<td>3i</td>
<td>H</td>
<td>OMe</td>
<td>1</td>
<td>CH₂</td>
<td>1370 ± 201</td>
<td>5381 ± 657</td>
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</tr>
<tr>
<td>10</td>
<td>3j</td>
<td>OMe</td>
<td>OMe</td>
<td>1</td>
<td>CH₂</td>
<td>968 ± 173</td>
<td>3174 ± 319</td>
<td>3.3</td>
</tr>
<tr>
<td>11</td>
<td>3k</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>CH-Bu-t</td>
<td>7896 ± 976</td>
<td>&gt;10,000</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>3l</td>
<td>H</td>
<td>Me</td>
<td>1</td>
<td>CH-Bu-t</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>3m</td>
<td>H</td>
<td>OMe</td>
<td>1</td>
<td>CH-Bu-t</td>
<td>9365 ± 1241</td>
<td>&gt;10,000</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>3n</td>
<td>OMe</td>
<td>OMe</td>
<td>1</td>
<td>CH-Bu-t</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>Haloperidol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.6 ± 0.4</td>
<td>54.5 ± 5.1</td>
<td>12.6</td>
</tr>
<tr>
<td>16</td>
<td>(+)-Pentazocine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.0 ± 1.0</td>
<td>1824 ± 36</td>
<td>365</td>
</tr>
<tr>
<td>17</td>
<td>DTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.7 ± 6.4</td>
<td>59.1 ± 12</td>
<td>1.6</td>
</tr>
</tbody>
</table>

ᵃ σ₁ Affinities were determined in rat liver homogenates using [³H]-(+)-pentazocine.
ᵇ σ₂ Affinities were determined in guinea pig brain using [³H]-DTG in the presence of (+)-pentazocine to block σ₁ receptors. Nonspecific binding was determined in the presence of 10 μM haloperidol. The values are means ± SEM of three experiments performed in duplicate.

The Kᵦ₁ for (+)-pentazocine was determined using [³H]-DTG in the presence of AC915 (sigma-1 antagonist) as σ₁ blocking agent.
due to changes in one of the hydrophobic parts from cyclopentyl-substituted spirocyclic moiety to the cyclohexyl analogue attached to the piperazine nitrogen (Fig. 2b). As such, ring expansion from n = 1 to n = 2 together with introduction of bulky tert-Bu group in position 4 of the six-member ring could lead to lower σ-Rs binding affinities due to increase in steric hindrance. Another reason for lower σ binding affinity of compounds 3d–n in comparison to those of 3a–c may be the absence of a basic nitrogen atom in these structures (Fig. 2b).51

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbmc.2016.04.010. These data include MOL files and InChlKeys of the most important compounds described in this article.

References and notes