Synthesis of 4-(benzamide)-and 4-(phthalimide)-substituted phenoxypropanolamines and their β₁-, β₂-adrenergic receptor binding studies

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N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-1-oxo-isoindoline 3 possess a cardioselective β-adrenergic receptor binding affinity. Herein we attempted to synthesize the unreduced compound N-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]phthalimide 4. But, reaction of N-[4-(2,3-epoxypropoxy)phenyl]phthalimide 10 with isopropylamine opened the phthalimide ring to give N-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]-2-isopropylcarbamoylbenzamide 12 instead of 4 as expected. While treatment of 10 with tert-butylamine gives N-[4-(3-tert-butylamino-2-hydroxypropoxy)phenyl]phthalimide 15. Further, reaction of 15 with isopropylamine affords the phthalimide ring opened analogue N-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]-2-isopropylcarbamoyl-5,6-dimethoxybenzamide 13. Compounds 12, 13, 15 and 16 have been tested for their in vitro β₁- and β₂-adrenergic receptor binding affinity using turkey erythrocyte membrane (β₁) and lung homogenate of rats (β₂). The percentage inhibition of [³H]DHA binding to both β₁- and β₂-adrenergic receptors are compared with that of the standard non-selective β-adrenergic blocking agent propranolol 1 and selective agent atenolol. All the tested compounds exhibit binding affinity to β₁-adrenergic receptors at the tested concentration [10⁻⁵ M] and most of them (12, 15, 16) exhibit cardioselectivity (selectivity ratio > 1). The dimethoxy analogue 13 shows selectivity towards β₂-adrenergic receptor (selectivity ratio < 1).

Keywords: Phenoxypropanolamines, isoindoline, adrenergic receptor, 4-benzamide, 4-phthalimide

Use of β-adrenergic blocking agents for the treatment of hypertension has been well established and documented for the past 30 years or so. The most widely accepted β-blocker is propranolol 1, which is non-selective in its action, i.e., it blocks both β₁- and β₂-adrenergic receptors. Blockade of β₂-adrenergic receptor causes aggravation of asthma. Thus β-blockers which selectively block the β₁-adrenergic receptor were developed. Practolol 2 was one such compound developed very early in the quest for cardioselective β-blockers. This and other efforts have concluded that cardioselectivity could be imparted into the phenoxypropanolamine type of compounds either by the introduction of appropriate substitution in para-position of the phenyl ring or by appropriate substitution of the side chain amino group. Particularly, para-amidic substituents as that present in practolol 2 have been found to confer cardioselectivity to phenoxypropanolamines.

We have been involved in the development of cardioselective β-adrenoceptor blocking agents based on the structure of practolol. One of our recent paper describes N-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]-1-oxo-isoindoline 3, to possess a cardioselective β-adrenergic receptor binding affinity. This compound has been selected from a series of

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4-(1-oxo-isoindoline)-substituted phenoxypropanolamines for further pharmacological investigations. Herein, we report our attempt to synthesize the unreduced product of 3, N-[4-(2-hydroxy-3-isopropylaminoproxy)phenyl]phthalimide 4 and related compounds and test them for their β-receptor binding affinity.

Preparation of N-(4-hydroxyphenyl)phthalimide 8 by fusion of phthalic anhydride 5 with p-aminophenol 7 was carried out as reported earlier 5. Reaction of 8 with epichlorohydrin (Scheme I) in the presence of potassium carbonate gave N-[4-(2,3-epoxypropoxy)phenyl]phthalimide 10. Refluxing 10 in isopropylamine did not give the desired product 4 as expected but gave 12, which was found to have formed by the opening of the phthalimide ring. Compound 12 in its 1H NMR spectra showed proton peaks at δ 1.05 and 1.13 (2 × d, 12H) for two isopropyl groups (-CH(CH₃)₂). Moreover, four exchangeable protons were observed in 1H NMR spectrum. In IR spectrum the characteristic phthalimide carbonyl stretching vibrations were absent at 1655 cm⁻¹. This led us to the conclusion that the compound formed was 12 and not 4 as expected. Contrary to this, refluxing 10 with tert-butylamine (Scheme II) gave two compounds 14 (minor product) and 15 (major product). The structure of 14 was established as N-[4-(3-tert-butylamino-2-hydroxypropoxy)phenyl]phthalamic acid based on the absence of characteristic 5-membered imide absorption in IR spectrum. While compound 15 showed characteristic carbonyl stretching vibrations at 1662 cm⁻¹ for phthalimide ring system. But refluxing 10 with tert-butylamine in ethanol afforded only the ring opened analogue 14. As we have synthesized unexpectedly the ring opened analogue 12, we were interested to get other structurally related compounds to study the SAR. Thus, 15 was refluxed in isopropylamine to afford 16 (Scheme II), which was formed by the opening of the phthalimide ring. Also compound 13 (Scheme I) was prepared from 4,5-dimethoxyphthalic anhydride.

β-Adrenergic receptor binding affinity

Compounds 12, 13, 15 and 16 were subjected to in vitro β₁- and β₂-adrenergic receptor binding assay using turkey erythrocyte membrane (β₁) and lung homogenate of rats (β₂). The results of binding assay

Reagents and conditions: (i) toluene, reflux (8) or pyridine, reflux (9) (ii) epichlorohydrin / K₂CO₃, reflux (iii) isopropylamine, reflux.

Scheme I
and selectivity ratio ($\beta_1/\beta_2$) are shown in Table I. The percentage inhibition of $[^3H]$DHA binding to both $\beta_1$- and $\beta_2$-adrenergic receptors was compared with that of the standard non-selective $\beta$-adrenergic blocking agent propranolol 1 and atenolol, a cardioselective $\beta$-adrenergic blocking agent used clinically.

All the tested compounds exhibited binding affinity to $\beta_1$-adrenergic receptors at the tested concentration and most of them (12, 15, 16) exhibited cardioselectivity (selectivity ratio $>1$). In the 4-(benzamide) series of compounds (12, 13, 16) it was observed that 5,6-dimethoxy substitution (13 vs 12) leads to an increase in the affinity to $\beta_2$-adrenergic receptors. This finding is similar to our previous report in a series of 4-(1-oxo-isoindoline) 5 and 4-acylamino-substituted phenoxypropanolamines that the introduction of methoxy groups in the para-substituent increases the binding affinity to $\beta_2$-adrenergic receptor. As compound 13 exhibited very less affinity to $\beta_1$-adrenergic receptor compared to propranolol 1/atenolol and also showed tendency towards $\beta_2$-adrenergic receptor further plans to synthesize 5,6-dimethoxy substituted compounds were abandoned. Also in this series, the tert-butyl derivative 16 was found to have better affinity to $\beta_1$-adrenergic receptor than the isopropyl derivative 12 with cardioselectivity. In the 4-(phthalimide) series the desired compound 4 was not obtained but its tert-butyl analogue 15 was found to possess $\beta$-adrenergic receptor affinity without selectivity. Eventhough some of the compounds showed cardioselectivity as expected of a $para$-amidic substituted phenoxypropanolamines, but they were not significant for further pharmacological investigations due to lower affinity to $\beta_1$-adrenergic receptor compared to propranolol 1/atenolol.

**Experimental Section**

Melting points reported are uncorrected. $^1$H NMR spectra were recorded on a Bruker AC-300F, 300 MHz NMR instrument using TMS as the internal standard (chemical shifts in $\delta$, ppm); and IR spectra in KBr pellets on a Perkin-Elmer 882 spectrophotometer model ($\nu_{\text{max}}$ in cm$^{-1}$). The purity of the compounds was established by TLC and by elemental analysis (C, H, N). Elemental analyses were carried out on a Perkin-Elmer-2400. Anhydrous sodium sulfate was

<table>
<thead>
<tr>
<th>Compound</th>
<th>Code</th>
<th>%I $\beta_1$ (M ± s.d.)</th>
<th>%I $\beta_2$ (M ± s.d.)</th>
<th>Selectivity ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>DPJ-276</td>
<td>34.01±0.42</td>
<td>None</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>DPJ-561</td>
<td>43.92±0.64</td>
<td>56.50±2.40</td>
<td>0.78</td>
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<tr>
<td>15</td>
<td>DPJ-290</td>
<td>47.35±5.30</td>
<td>38.43±4.17</td>
<td>1.23</td>
</tr>
<tr>
<td>16</td>
<td>DPJ-454</td>
<td>48.15±3.75</td>
<td>6.89±0.99</td>
<td>6.99</td>
</tr>
<tr>
<td>Propranolol</td>
<td></td>
<td>97.12±3.00</td>
<td>99.80±0.28 (K_i:1.60±0.134 M)</td>
<td>0.97</td>
</tr>
<tr>
<td>Atenolol</td>
<td></td>
<td>73.06±1.25</td>
<td>34.34±0.84 (K_i:2.70±0.401 M)</td>
<td>2.13</td>
</tr>
</tbody>
</table>

$^a$ expressed as (%I $\beta_1$/%I $\beta_2$)

$K_i$ - apparent inhibition constants
used as a drying agent. Plates for TLC were prepared with silica gel G using ethyl acetate. Iodine vapours were used to develop the plates.

N-[4-(2,3-Epoxypropoxy)phenyl]phthalimide 10. A mixture of N-(4-hydroxyphenyl)phthalimide 8 (2.0 g, 8.4 mmole), epichlorohydrin (30 mL) and anhyd. potassium carbonate (1.0 g) was refluxed for 6 hr. The reaction mixture was cooled and filtered. The excess of epichlorohydrin was removed under reduced pressure and the residue obtained was crystallized from ethanol to afford 10 (1.6 g, 64.78%), mp 168-70°C; IR (KBr): 2919, 1715, 1655, 1609, 1514, 1389, 1299, 1237, 1029 and 817 cm⁻¹; ¹H NMR (CDCl₃): δ 2.78 and 2.93 (dd, 2H, -OCH₂ of oxirane ring), 3.39 (m, 1H, -CH of oxirane ring); 4.01 and 4.26 (2 × dd, 2H, -OCH₂N); 7.05 (dd, 2H, J = 9, 3 Hz, ArH), 7.34 (dd, 2H, J = 9, 3 Hz, ArH), 7.79-7.95 (m, 6H, ArH) (Found: C, 68.46; H, 6.57; N, 7.61%).

N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxyphthalimide 11. A mixture of N-(4-hydroxyphenyl)-5,6-dimethoxyphthalimide 9 (0.5 g, 1.7 mmole), epichlorohydrin (20 mL) and anhyd. potassium carbonate (1.0 g) was refluxed for 12 hr. Processing the reaction mixture as for 10 and crystallization from ethanol afforded 11 (0.43 g, 72.43%), mp 220-223°C; IR (KBr): 2919, 1715, 1655, 1609, 1514, 1389, 1299, 1237, 1029 and 817 cm⁻¹; ¹H NMR (CDCl₃): δ 2.75 (m, 2H, -CH₂ of oxirane ring), 3.16-3.32 (m, 1H, -CH of oxirane ring), 4.06 (s, 6H, 2 × OCH₃), 4.07-4.25 (m, 2H, -OCH₂N), 6.98-7.34 (m, 6H, ArH) (Found: C, 63.97; H, 4.72; N, 4.20. C₁₁H₁₃N₃O₆ requires C, 64.22; H, 4.82; N, 3.94%).

N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-2-isopropylcarbamoyl-4,5-dimethoxbenzamide 12. Iso- propylamine (2 mL, 23.3 mmole) was added to a solution of 10 (0.5 g, 1.7 mmole) and the reaction mixture was refluxed for 4 hr maintaining anhydrous conditions. The excess of isopropylamine was removed under reduced pressure and the solid obtained was crystallized from acetone to afford 12 (0.29 g, 87.04%), mp 144-149°C; IR (KBr): 3095, 2961, 1683, 1582, 1450, 1392, 1336, 1241, 1064 and 826 cm⁻¹; ¹H NMR (CDCl₃): δ 1.05 and 1.13 (2 × d, 12H, 2 × -CH(CH₃)₂), 2.40-2.49 (br, 2H, disappeared on D₂O exchange, -OH and -NH₂), 2.69-2.90 (m, 3H, -CH₂NHCH₂), 3.92-4.00 (m, 3H, -OCH₂CH₂), 4.16 (m, 1H, -CONHCH₂), 6.52 (br, 1H, disappeared on D₂O exchange, -CONH₂), 6.84-7.66 (m, 8H, ArH), 7.66 (br, 1H, disappeared on D₂O exchange, ArCONHAr) (Found: C, 66.64; H, 7.41; N, 9.92. C₂₃H₃₁N₃O₄ requires C, 66.80; H, 7.56; N, 10.16%).

N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-2-isopropylcarbamoyl-4,5-dimethoxbenzamide 13. Isopropylamine (2 mL, 23.3 mmole) was added to a solution of 11 (0.25 g, 0.7 mmole) in ethyl free ethanol (10 mL) and the reaction mixture was refluxed for 8 hr. The solvent and excess of isopropylamine were removed under reduced pressure and the solid obtained was crystallized from acetone to give 13 (0.29 g, 87.04%), mp 152-54°C; IR (KBr): 3095, 2961, 1683, 1582, 1450, 1392, 1336, 1241, 1064 and 826 cm⁻¹; ¹H NMR (CDCl₃): δ 1.09 and 1.15 (2 × d, 6H, 2 × -CH(CH₃)₂), 2.15 (br, 2H, disappeared on D₂O exchange, -OH and -NH₂), 2.69-2.91 (m, 3H, -CH₂NHCH₂), 3.89 (s, 6H, 2 × OCH₃), 4.01 (m, 3H, -OCH₂CH₂), 4.12 (m, 1H, -CONHCH₂), 6.59 (br, 1H, disappeared on D₂O exchange, -CONH), 6.83-7.59 (m, 6H, ArH), 9.61 (br, 1H, disappeared on D₂O exchange, ArCONHAr) (Found: C, 63.00; H, 7.05; N, 8.48. C₂₅H₃₅N₃O₆ requires C, 63.40; H, 7.45; N, 8.87%).

N-[4-(3-tert-Butylamino-2-hydroxypropoxy)phenyl]phthalimide 15. tert-Butylamine (25 mL) was added to a solution of 10 (0.5 g, 1.7 mmole) and the reaction mixture was refluxed for 24 hr maintaining anhydrous conditions. The excess of tert-butylamine was removed under reduced pressure and the solid obtained was dissolved in acetone. The insoluble material was filtered to give 14 (0.05 g, 7.64%), mp 200-202°C; IR (KBr): 3095, 2961, 1683, 1582, 1450, 1392, 1336, 1241, 1064 and 826 cm⁻¹; ¹H NMR (TFA-DMSO-d₆): δ 1.39 (s, 9H, -C(CH₃)₃), 3.02 and 3.17 (2 × m, 2H, -CH₂NH-), 4.00 (m, 2H, -OCH₂-), 4.27 (m, 1H, -OCH₂CH₂), 6.98 (d, 1H, disappeared on D₂O exchange, -CONH), 7.32-8.53 (m, 8H, ArH), 9.80 (s, 1H, disappeared on D₂O exchange, ArCOOH) (Found: C, 65.20; H, 6.60; N, 6.88. C₂₁H₁₈N₂O₂ requires C, 65.27; H, 6.78; N, 7.25%).

The filtrate was concentrated and the crystals obtained were filtered to afford 15 (0.24 g, 38.47%), mp 160-63°C; IR (KBr): 1597, 1520, 1371, 1249, 1145, 995, 874 cm⁻¹; ¹H NMR (CDCl₃-DMSO-d₆): δ 1.10 (s, 9H, -C(CH₃)₃), 2.31 (br, 2H, disappeared on D₂O exchange, -OH and -NH₂), 2.68-2.87 (2 × dd, 2H, -CH₂NH-), 4.02 (m, 3H, -OCH₂CH₂), 7.05 (dd, 2H, J = 9, 3 Hz, ArH₃ to alkox), 7.34 (dd, 2H, J = 9, 3 Hz, ArH₃ to imide), 7.79-7.88 (m, 4H, ArH) (Found: C, 68.28; H, 6.83; N, 7.30. C₂₇H₂₄N₂O₄ requires C, 68.46; H, 6.57; N, 7.61%).
**Pharmacological methods**

Both $\beta_1$- & $\beta_2$-adrenergic receptor binding assays were carried out as previously described by us.$^4$

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**References**