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Synthesis of 4-(1-oxo-isoindoline) and 4-(5,6-dimethoxy-1-oxo-isoindoline)-substituted phenoxypropanolamines and their β_1-, β_2-adrenergic receptor binding studies

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Abstract

Phenoxypropanolamines with 1-oxo-isoindoline (**12**–**16**) and 5,6-dimethoxy-1-oxo-isoindoline groups (**17**–**20**) at the *para* position were synthesized. β_1, β_2-Adrenergic receptor binding affinities for the synthesized compounds were tested and compared with propranolol and atenolol. It was found that the incorporation of *para*-amidic functionality within the 1-oxo-isoindoline ring and 5,6-dimethoxy-1-oxo-isoindoline ring system led to a high degree of cardioselectivity in the phenoxypropanolamines. Two of the compounds **12** and **20** possessed β_1-adrenergic receptor affinity comparable with that of atenolol and both showed a better cardioselectivity than atenolol. Both **12** and **20** are undergoing further pharmacological evaluation.

Keywords: β-Adrenergic blocking agents; β-Adrenergic receptor binding; Phenoxypropanolamines; 1-Oxo-isoindoline; Atenolol; Propranolol

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1. Introduction

Cardiovascular diseases are the major cause of deaths worldwide, even surpassing deaths due to cancer. Among these, hypertension is central to the pathogenesis of coronary artery disease (angina, myocardial infarction), heart failure, cerebral (stroke), and peripheral vascular diseases [1]. Pharmacological treatment of hypertension includes mainly the use of six drug classes: diuretics, β-adrenergic blocking agents, calcium antagonist, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, and α-adrenergic blockers [2].

As a part of our efforts [3–6] to develop β-adrenergic blocking agents with better activity and cardioselectivity than the existing β-blockers, we have synthesized a new series of phenoxypropanolamines based on the structure of practolol (**1**), a cardioselective agent. Use of cardioselective β-adrenergic blockers has been shown better suited for patients suffering from asthma and other bronchial disease than a non-selective β-adrenergic blocker [7,8]. Following the discovery of practolol with cardioselective action, numerous attempts were made by various researchers to develop β-adrenergic blocking agents with cardioselectivity. All these efforts led to the conclusion that significant cardioselectivity could be achieved either by an appropriate substitution in the *para*-position of the phenyl ring or by appropriate substitution of the side chain amino group [9]. Presence of *para*-amidic functionality in phenoxypropanolamines has been found to confer cardioselectivity [3,9]. It was also found that cardioselectivity could be conferred to phenoxypropanolamine type compounds by replacing isopropyl/*tert*-butyl groups with 3,4-dimethoxyphenylethyl moiety as the amino substituent [10,11].

In our previous study, we have reported the synthesis and β-adrenergic receptor binding of a series of phenoxypropanolamines (Fig. 1, **2a** and **2b**) with a *para*-amidic functionality [3]. Herein, we report the synthesis of phenoxypropanolamines with the *para*-amidic functionality incorporated in 1-oxo-isoindoline and 4-(5,6-dimethoxy-1-oxo-isoindoline) ring system (Fig. 1). Incorporation of *para*-amidic functionality with in the ring system leads to rigidification of the functionality, whose effect on receptor affinity and selectivity could be evaluated in this study. Also, the amino side chain substituent was varied from isopropyl/*tert*-butyl moiety to 3,4-dimethoxyphenylethyl moiety, with the aim of obtaining phenoxypropanolamines with good activity and cardioselectivity.

2. Materials and methods

2.1. Chemical synthesis

2.1.1. Materials

Melting points reported are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC-300F, 300 MHz NMR instrument using tetramethylsilane (TMS) as the internal standard (chemical shifts in δ, ppm). IR spectra were recorded on Perkin-Elmer 882 spectrophotometer model. IR spectra were obtained with potassium

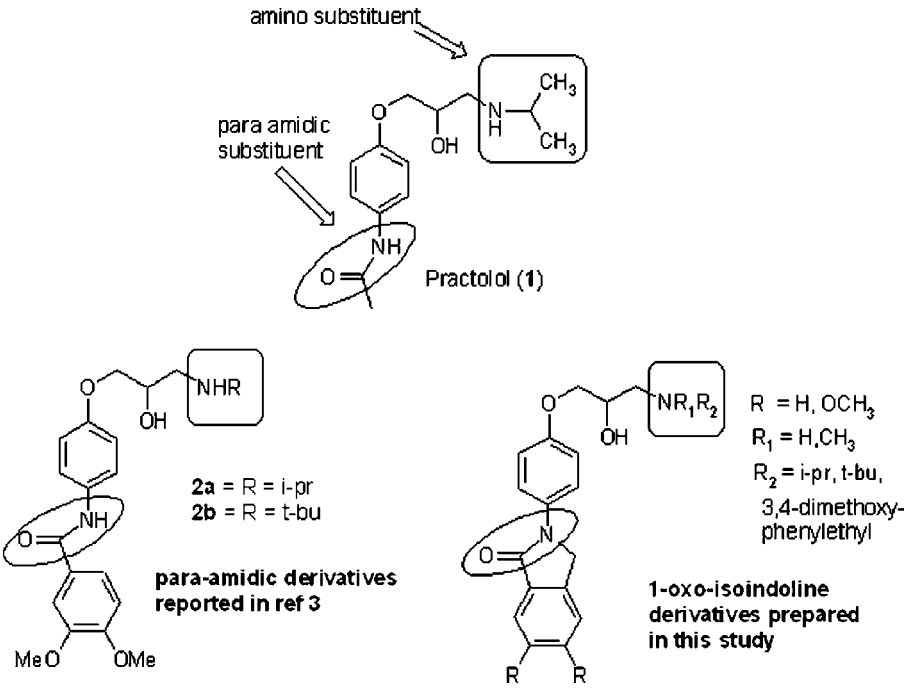


Fig. 1. *para*-Amidic group incorporation in 1-oxo-isoindoline ring system.

bromide pellets (γ max in cm^{-1}). The purity of the compounds were established by thin layer chromatography (TLC) and by elemental analysis (C, H, and N). Elemental analyses were carried out on a Perkin-Elmer-2400. Anhydrous sodium sulfate was used as drying agent. Plates for TLC were prepared with silica gel G using ethyl acetate. Iodine vapors were used to develop the plates.

2.1.2. Synthesis

The classical phenol–epoxide–aminoalcohol sequence of reactions was carried out to synthesize the new phenoxypropanolamines. The compounds prepared under this study have been divided into two structural groups for convenience of discussion, 1-oxo-isoindoline derivatives (**12**–**16**) and 5,6-dimethoxy-1-oxo-isoindoline derivatives (**17**–**20**). The synthetic scheme for both the series are shown in Fig. 2.

N-(Hydroxyphenyl)-1-oxo-isoindoline (**8**) was synthesized by condensing *p*-aminophenol (**5**) with phthalic anhydride (**3**), followed by zinc-acetic acid reduction. The intermediate oxirane (**10**) was prepared by condensing epichlorohydrin with **8**. Reaction of **10** with isopropylamine, *tert*-butylamine, *N*-methylpiperazine, homoveratrylamine, and *N*-methylhomoveratrylamine gave **12**, **13**, **14**, **15**, and **16**, respectively. While *N*-(hydroxyphenyl)-5,6-dimethoxy-1-oxo-isoindoline (**9**) was prepared from dimethoxyphthalic anhydride (**4**) and *p*-aminophenol, which was further elaborated to **17**, **18**, **19**, and **20** in similar fashion. All the compounds were converted to their

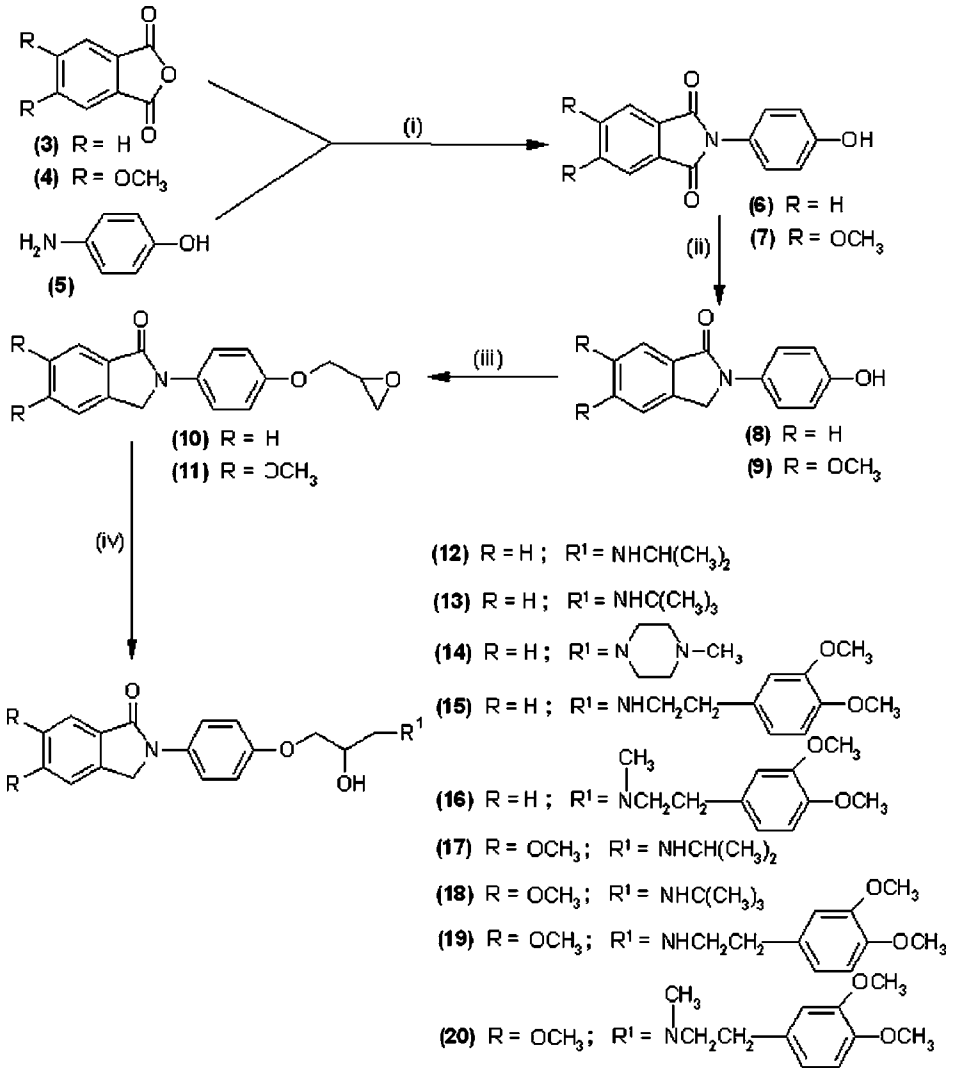


Fig. 2. Synthetic procedure to compounds **12**–**20**. Reagents and conditions: (i) toluene, reflux (**6**) or pyridine, reflux (**7**); (ii) zinc/glacial acetic acid; (iii) epichlorohydrin/K_2CO_3, reflux; (iv) isopropylamine (**12**, **17**) or *tert*-butylamine (**13**, **18**) or *N*-methylpiperazine (**14**) or homoveratrylamine/methanol, reflux (**15**) or *N*-methylhomoveratrylamine/methanol, reflux (**16**) or homoveratrylamine, 80 °C (**19**) or *N*-methylhomoveratrylamine, 80 °C (**20**).

hydrochloride salt by treatment with hydrogenchloride gas. The structures of all the compounds were established on the basis of ^{1}H NMR, IR, and elemental analyses.

2.1.3. General procedure for the preparation of HCl salt of amines (Method A)

Freshly prepared dry HCl gas was passed through a solution of amine in dry acetone (20 ml). The precipitated material was filtered and crystallized from appropriate solvent to afford HCl salt of the amine.

*2.1.4. N-(4-Hydroxyphenyl)phthalimide (**6**)*

4-Aminophenol **5** (4.0 g, 36.7 mmol) and triethylamine (2 ml) were added to a solution of phthalic anhydride **3** (6.0 g, 40.5 mmol) in ethanol (150 ml) and the reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the solid residue was dried in a vacuum desiccator for 24 h. The residue so obtained was refluxed in dry toluene (250 ml) for 4 h using Dean and Stark apparatus to effect cyclization. The solvent was removed under reduced pressure and the solid was washed with ether. The residue was refluxed in distilled water for 0.5 h to remove unreacted materials. The solid obtained was crystallized from methanol to afford **6** (4.75 g, 58.78%), mp. 291–293 °C. IR (KBr): 3415, 1711, 1690, 1593, 1515, 1462, 1439, 1275, 1200, and 721 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 6.92 (d, 2H, $J = 9$ Hz, Ar_o to hydroxy), 7.26 (d, 2H, $J = 9$ Hz, Ar_o to imide), 7.92 (s, 4H, Ar) and 9.70 ppm (br, 1H, disappeared on D_2O exchange, –O*H*).

*2.1.5. N-(4-Hydroxyphenyl)-5,6-dimethoxypthalimide (**7**)*

4-Aminophenol **5** (0.52 g, 4.8 mmol) was added to a solution of 4,5-dimethoxyphthalic anhydride **4** (1.0 g, 4.8 mmol) in dry pyridine (15 ml) and the reaction mixture was refluxed for 2 h. The excess of pyridine was removed under reduced pressure, the solid obtained was taken in water, filtered, and washed with distilled water till its free from pyridine. The residue given washings with methanol and crystallized from ethanol to yield **7** (0.82 g, 57.04%), mp 278–280 °C. IR (KBr): 3534, 3115, 2961, 1709, 1690, 1598, 1466, 1391, 1266, 1082, and 840 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 3.98 (s, 6H, 2 × –OCH_3), 6.73 (d, 2H, $J = 9$ Hz, Ar_o to hydroxy), 7.08 (d, 2H, $J = 9$ Hz, Ar_o to imide), 7.41 (s, 2H, Ar) and 9.43 ppm (br, 1H, disappeared on D_2O exchange, –O*H*). Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 63.90; H, 4.31; N, 4.74.

*2.1.6. N-(4-Hydroxyphenyl)-1-oxo-isoindoline (**8**)*

To a solution of *N*-(4-hydroxyphenyl)phthalimide **6** (3.0 g, 12.5 mmol) in glacial acetic acid (700 ml), zinc dust (2.0 g) was added and the reaction mixture was shaken manually for 2 h, then refluxed for 2 h, cooled and filtered. The glacial acetic acid was removed under reduced pressure. The solid residue obtained was washed with distilled water and crystallized from methanol to give **8** (1.98 g, 70.10%), mp 222–225 °C. IR (KBr): 3173, 1656, 1615, 1591, 1515, 1443, 1399, 1236, and 834 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.85 (s, 2H, methylene protons of isoindoline), 6.86–7.88 (m, 8H, Ar) and 9.29 ppm (s, 1H, disappeared on D_2O exchange, –O*H*). Calcd. for $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.32; H, 4.81; N, 6.15.

*2.1.7. N-(4-Hydroxyphenyl)-5,6-dimethoxy-1-oxo-isoindoline (**9**)*

N-(4-Hydroxyphenyl)-5,6-dimethoxypthalimide **7** (2.0 g, 6.7 mmol) was reduced with zinc dust (5.0 g) in glacial acetic acid (700 ml) as for **8** and the resulting product

was crystallized from methanol to afford **9** (1.68 g, 88.12%), mp 140–142 °C. IR (KBr): 3159, 1658, 1615, 1558, 1462, 1259, 1057, 1031, and 833 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 3.89 (s, 6H, 2 × –OCH_3), 4.70 (s, 2H, methylene protons of isoindoline), 6.86 (d, 2H, $J = 9$ Hz, Ar_o to hydroxy), 7.10 (s, 1H, Ar), 7.32 (s, 1H, Ar), 7.62 (d, 2H, $J = 9$ Hz, Ar_o to imide) and 9.20 ppm (br, 1H, disappeared on D_2O exchange, –O*H*). Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.22; H, 5.20; N, 4.90.

*2.1.8. N-[4-(2,3-Epoxypropoxy)phenyl]-1-oxo-isoindoline (**10**)*

A mixture of *N*-(4-hydroxyphenyl)-1-oxo-isoindoline **8** (1.0 g, 4.4 mmol), epichlorohydrin (25 ml) and potassium carbonate (1.0 g) was refluxed for 6 h. The reaction mixture was filtered and the excess of epichlorohydrin was removed under reduced pressure. The white solid residue was crystallized from methanol to give **10** (0.88 g, 70.46%), mp 162–164 °C. IR (KBr): 3114, 2924, 1674, 1514, 1389, 1299, 1237, 1029, and 817 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.76 and 2.91 (dd, 1H; t, 1H, –CH_2 of oxirane ring), 3.36 (m, 1H, –C*H* of oxirane ring), 3.95 and 4.24 (2 × dd, 2H, –OCH_2–), 4.79 (s, 2H, methylene protons of isoindoline) and 6.79–7.89 ppm (m, 8H, Ar). Calcd. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.38; N, 4.98. Found: C, 72.25; H, 5.31; N, 5.03.

*2.1.9. N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline (**11**)*

N-(4-Hydroxyphenyl)-5,6-dimethoxy-1-oxo-isoindoline **9** (0.5 g, 1.8 mmol) was condensed with epichlorohydrin (10 ml) in the presence of potassium carbonate (1.0 g) as for **10**, and the resulting product crystallized from ethanol to give **11** (0.42 g, 70.21%), mp 158–160 °C. IR (KBr): 3054, 2937, 2832, 1681, 1518, 1450, 1388, 1244, 1056, and 820 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.76 and 2.85 (dd, 1H; t, 1H, –CH_2 of oxirane ring), 3.35 (m, 1H, –C*H* of oxirane ring), 4.00 (s, 6H, 2 × –OCH_3), 4.18 and 4.24 (2 × d, 2H, –OCH_2–), 4.86 (s, 2H, methylene protons of isoindoline) and 6.92–7.79 ppm (m, 6H, Ar). Calcd. for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10. Found: C, 66.58; H, 5.57; N, 4.12.

*2.1.10. N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-1-oxo-isoindoline (**12**)*

Isopropylamine (2 ml, 23.3 mmol) was added to a solution of *N*-[4-(2,3-epoxypropoxy)phenyl]-1-oxo-isoindoline **10** (0.25 g, 0.9 mmol) in aldehyde free ethanol (10 ml) and the reaction mixture was refluxed for 5 h. The solvent and excess of isopropylamine were removed under reduced pressure and the solid obtained was crystallized from methanol to yield **12** (0.25 g, 82.64%), mp 138–141 °C. IR (KBr): 3095, 2961, 1683, 1582, 1450, 1392, 1336, 1241, 1064, and 826 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.09 (d, 6H, –CH(CH_3)$_2$), 2.21 (br, 2H, disappeared on D_2O exchange, –O*H* and –N*H*–), 2.69–2.92 (m, 3H, –CH_2NHC*H*<), 4.01 (m, 3H, –OCH_2C*H*<), 4.81 (s, 2H, methylene protons of isoindoline) and 6.98–7.91 ppm (m, 8H, Ar). Calcd. for $C_{20}H_{24}N_2O_3$: C, 70.56; H, 7.11; N, 8.23. Found: C, 70.30; H, 7.13; N, 8.21.

*2.1.11. N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-1-oxo-isoindoline (**12**) HCl*

Method A. Crystallization solvent: methanol–acetone mixture; yield: 75.87%; mp 212–214 °C. IR (KBr): 3319, 2971, 1688, 1512, 1445, 1387, 1298, 1241, 1030, 843,

and 731 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.35 (d, 6H, –CH(CH_3)$_2$), 3.03 and 3.16 (2 × m, 2H, –CH_2NH–), 3.36 (m, 1H, –CH(CH_3)$_2$), 4.04 (m, 2H, –OCH_2–), 4.32 (m, 1H, –CH(OH)–), 4.93 (s, 2H, methylene protons of isoindoline), 5.89 (d, 1H, disappeared on D_2O exchange, –CH(O*H*)–), 7.00–7.80 (m, 8H, Ar) and 8.69 ppm (br, 1H, disappeared on D_2O exchange, –N*H*–). Calcd. for $C_{20}H_{25}N_2O_3Cl$: C, 63.73; H, 6.69; N, 7.44. Found: C, 63.55; H, 6.61; N, 7.48.

*2.1.12. N-[4-(3-tert-Butylamino-2-hydroxypropoxy)phenyl]-1-oxo-isoindoline (**13**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-1-oxo-isoindoline **10** (0.25 g, 0.9 mmol) was reacted with *tert*-butylamine (2 ml, 19.0 mmol) as for **12** and the resultant product crystallized from methanol to afford **13** (0.25 g, 79.36%), mp 150–153 C. IR (KBr): 3062, 2971, 1670, 1580, 1466, 1388, 1334, 1245, 1066, and 827 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.13 (s, 9H, –C(CH_3)$_3$), 2.17 (br, 2H, ‘ on D_2O exchange, –O*H* and –N*H*–), 2.69 and 2.86 (2 × dd, 2H, –CH_2NH–), 3.98 (m, 3H, –CH_2CH(OH)–), 4.81 (s, 2H, methylene protons of isoindoline) and 6.99–7.92 ppm (m, 8H, Ar). Calcd. for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.40; N, 7.91. Found: C, 70.90; H, 7.39; N, 7.96.

*2.1.13. N-[4-(3-tert-Butylamino-2-hydroxypropoxy)phenyl]-1-oxo-isoindoline (**13**) HCl*

Method A. Crystallization solvent: acetone; yield: 77.07%; mp 212–215 °C. IR (KBr): 2976, 1686, 1615, 1581, 1442, 1393, 1246, 1066, and 832 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.24 (s, 9H, –C(CH_3)$_3$), 3.10 and 3.34 (2 × m, 2H, –CH_2NH–), 3.36 (m, 1H, –CH(CH_3)$_2$), 4.00 and 4.10 (2 × m, 2H, –OCH_2–), 4.64 (m, 1H, –CH(OH)–), 4.71 (s, 2H, methylene protons of isoindoline), 5.71 (d, 1H, disappeared on D_2O exchange, –CH(O*H*)–), 6.95–7.86 (m, 8H, Ar) and 8.23 (br, 1H, disappeared on D_2O exchange, –N*H*–). Calcd. for $C_{21}H_{27}N_2O_3Cl$: C, 64.52; H, 6.96; N, 7.17. Found: C, 64.26; H, 6.98; N, 7.17.

*2.1.14. N-{4-[2-Hydroxy-3-(4-methylpiperazino)propoxy]phenyl}-1-oxo-isoindoline (**14**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-1-oxo-isoindoline **10** (0.25 g, 0.9 mmol) was reacted with *N*-methylpiperazine (1 ml, 9.0 mmol) as for **12** and the resultant product crystallized from acetone to afford **14** (0.23 g, 67.84%), mp 92–95 °C. IR (KBr): 3412, 2941, 1685, 1460, 1296, 1248, 1043, and 831 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.24 (s, 3H, –NCH_3), 2.30–3.03 (m, 10H, –CH_2N(CH_2CH_2)$_2$N–), 4.00 (m, 3H, –CH_2CH(OH)–), 4.78 (s, 2H, methylene protons of isoindoline) and 7.02–7.84 ppm (m, 8H, Ar). Calcd. for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.14; N, 11.02. Found: C, 69.00; H, 7.18; N, 10.98.

*2.1.15. N-{4-[2-Hydroxy-3-(4-methylpiperazino)propoxy]phenyl}-1-oxo-isoindoline (**14**) HCl*

Method A. Crystallization solvent: methanol; yield: 67.12%; mp 250–253 °C. IR (KBr): 3399, 2925, 1691, 1515, 1442, 1390, 1244, 1159, 1066, and 828 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.89 (s, 3H, –NCH_3), 3.37 (m, 2H, –CH_2N<), 3.56 (br, 9H, 1H disappeared on D_2O exchange, –N(CH_2CH_2)$_2$N–, –O*H*), 3.99 (m, 2H, –OCH_2–), 4.35

(m, 1H, –C*H*(OH)–), 4.84 (s, 2H, methylene protons of isoindoline) and 7.01–8.14 ppm (m, 8H, Ar). Calcd. for $C_{22}H_{28}N_3O_3Cl$: H, 6.75; N, 10.06. Found: H, 6.69; N, 9.94.

*2.1.16. N-{4-[2-Hydroxy-3-(3,4-dimethoxyphenylethylamino)propoxy]phenyl}-1-oxo-isoindoline (**15**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-1-oxo-isoindoline **10** (0.25 g, 0.9 mmol) was reacted with homoveratrylamine (1 ml, 6.0 mmol) as for **12** and the resultant product was given washings with hot hexane and then crystallized from acetone to afford **15** (0.19 g, 46.22%), mp 118–120 °C. IR (KBr): 3061, 2940, 1696, 1588, 1447, 1385, 1296, 1242, 1024, and 818 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.18 (br, 2H, disappeared on D_2O exchange, –O*H* and –N*H*–), 2.74 (m, 6H, –CH_2NHCH_2CH_2–), 3.75 (m, 9H, –OCH_2C*H*< and 2 × –OCH_3), 4.66 (s, 2H, methylene protons of isoindoline) and 6.50–7.75 ppm (m, 11H, Ar). Calcd. for $C_{27}H_{30}N_2O_5$: C, 70.11; H, 6.54; N, 6.06. Found: C, 69.98; H, 6.50; N, 6.08.

*2.1.17. N-{4-[2-Hydroxy-3-(3,4-dimethoxyphenylethylamino)propoxy]phenyl}-1-oxo-isoindoline (**15**) HCl*

Method A. Crystallization solvent: acetone; yield: 88.99%; mp 178–180 °C. IR (KBr): 3345, 2946, 1678, 1591, 1466, 1391, 1243, 1155, 1066, and 825 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.23–3.31 (br, 6H, –CH_2NHCH_2CH_2–), 3.78 and 3.82 (2 × s, 6H, 2 × OCH_3), 3.93–4.07 (m, 2H, –OCH_2–), 4.61 (m, 3H, methylene protons of isoindoline, –C*H*(OH)–), 5.51 (br, 1H, disappeared on D_2O exchange, –O*H*), 6.71–7.81 (m, 11H, Ar) and 8.94 ppm (br, 1H, disappeared on D_2O exchange, –N*H*–). Calcd. for $C_{27}H_{31}N_2O_5Cl$: C, 64.99; H, 6.26; N, 5.62. Found: C, 64.74; H, 6.19; N, 5.57.

*2.1.18. N-{4-[2-Hydroxy-3-(3,4-dimethoxy-N-methylphenylethylamino) propoxy]phenyl}-1-oxo-isoindoline HCl (**16**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-1-oxo-isoindoline **10** (0.25 g, 0.9 mmol) was reacted with *N*-methylhomoveratrylamine (1 ml, 5.4 mmol) as for **12** and the resultant product was given washings with hot hexane. The residue was not crystallizable and converted to HCl salt by the general procedure (Method A) and then crystallized from methanol to afford HCl salt of **16** (0.23 g, 50.45%), mp 186–188 °C. IR (KBr): 3282, 3077, 2938, 1681, 1613, 1449, 1334, 1243, 1040, and 819 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.0 (s, 3H, –NCH_3), 3.14–3.49 (m, 7H, 1H disappeared on D_2O exchange, –OCH_2(O*H*)CH_2N(CH_3)CH_2CH_2–), 3.85 (d, 6H, 2 × –OCH_3), 3.95 and 4.15 (2 × m, 2H, –OCH_2–), 4.75 (m, 3H, methylene protons of isoindoline and –C*H*(OH)–) and 6.80–7.88 ppm (m, 11H, Ar). Calcd. for $C_{28}H_{33}N_2O_5Cl$: C, 65.55; H, 6.48; N, 5.46. Found: C, 65.52; H, 6.43; N, 5.41.

*2.1.19. N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline (**17**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline **10** (0.5 g, 1.5 mmol) was reacted with isopropylamine (2 ml, 23.3 mmol) as for **12** and the resultant product crystallized from acetone to afford **17** (0.43 g, 73.31%), mp 138–141 °C. IR (KBr): 3084, 2966, 1678, 1450, 1388, 1244, 1058, and 827 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 1.12 (d, 6H, –CH(CH_3)$_2$), 2.56 (br, 2H disappeared on D_2O exchange, –O*H* and –N*H*–), 2.75 (m, 1H, –C*H*(CH_3)$_2$), 2.88 (m, 2H, –CH_2NH–), 3.96 (m, 8H, –OCH_2– and 2 × –OCH_3), 4.06 (m, 1H, –C*H*(OH)–), 4.69 (s, 2H, methylene protons of isoindoline) and 6.95–7.68 ppm (m, 6H, Ar). Calcd. for $C_{22}H_{28}N_2O_5$: C, 65.98; H, 7.05; N, 7.00. Found: C, 65.76; H, 6.99; N, 6.91.

*2.1.20. N-[4-(2-Hydroxy-3-isopropylaminopropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline (**17**) HCl*

Method A. Crystallization solvent: methanol–acetone mixture; yield: 84.32%; mp 245–248 °C. IR (KBr): 3268, 2945, 1665, 1606, 1449, 1390, 1258, 1061, and 820 cm^{-1}. Calcd. for $C_{22}H_{29}N_2O_5Cl$: C, 60.47; H, 6.69; N, 6.41. Found: C, 60.39; H, 6.65; N, 6.39

*2.1.21. N-[4-(3-tert-Butylamino-2-hydroxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline (**18**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline **11** (0.5 g, 1.5 mmol) was reacted with *tert*-butylamine (2 ml, 19.0 mmol) as for **12** and the resultant product crystallized from acetone to afford **18** (0.42 g, 69.18%), mp 142–145 °C. IR (KBr): 3344, 2964, 2865, 1670, 1609, 1462, 1387, 1226, 1059, and 825 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 9H, –C(CH_3)$_3$), 2.86 (m, 4H, 2H disappeared on D_2O exchange, –CH(O*H*)-CH_2N*H*–), 4.06 (m, 9H, 2 –OCH_3, –OCH_2C*H*<), 4.72 (s, 2H, methylene protons of isoindoline) and 7.01–7.75 ppm (m, 6H, Ar). Calcd. for $C_{23}H_{30}N_2O_5$: C, 66.64; H, 7.30; N, 6.76. Found: C, 66.43; H, 7.34; N, 6.72.

*2.1.22. N-[4-(3-tert-Butylamino-2-hydroxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline (**18**) HCl*

Method A. Crystallization solvent: methanol–acetone mixture; yield: 73.53%; mp 224–226 °C. IR (KBr): 3365, 3055, 2978, 1670, 1605, 1518, 1449, 1386, 1317, 1250, 1129, and 822 cm^{-1}. ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ 1.40 (s, 9H, –C(CH_3)$_3$), 2.99 and 3.16 (t, 1H; d, 1H, –CH_2NH–), 3.92 and 3.95 (2 × s, 6H, 2 × –OCH_3), 4.05 (m, 2H, –OCH_2–), 4.33 (m, 1H, –C*H*(OH)–), 4.81 (s, 2H, methylene protons of isoindoline), 5.95 (br, 1H, disappeared on D_2O exchange, –O*H*), 7.01–7.75 (m, 6H, Ar) and 8.85 ppm (br, 1H, disappeared on D_2O exchange, –N*H*–). Calcd. for $C_{23}H_{31}N_2O_5Cl$: C, 61.26; H, 6.93; N, 6.21. Found: C, 60.99; H, 6.90; N, 6.26.

*2.1.23. N-{4-[2-Hydroxy-3-(3,4-dimethoxyphenylethylamino)propoxy]phenyl}-5,6-dimethoxy-1-oxo-isoindoline (**19**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline **11** (0.25 g, 0.7 mmol) and homoveratrylamine (1 ml, 6.0 mmol) were heated at 80 °C for 8 h, added distilled water and left overnight. The solid obtained was filtered and crystallized from acetone to afford **19** (0.35 g, 91.45%), mp 60–62 °C. IR (KBr): 2936, 1675, 1609, 1455, 1385, 1263, 1057, and 822 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.15 (br, 2H, disappeared on D_2O exchange, –O*H* and –N*H*–), 2.80 (m, 6H, –CH_2NHCH_2CH_2–), 3.68–4.10 (m, 15H, –OCH_2C*H*< and 4 –OCH_3), 4.67 (s, 2H, methylene protons of isoindoline) and 6.70–7.65 ppm (m, 9H, Ar). Calcd. for $C_{29}H_{34}N_2O_7$: C, 66.65; H, 6.56; N, 5.36. Found: C, 66.50; H, 6.46; N, 5.32.

*2.1.24. N-{4-[2-Hydroxy-3-(3,4-dimethoxyphenylethylamino)propoxy]phenyl}-5,6-dimethoxy-1-oxo-isoindoline (**19**) HCl*

Method A. As no precipitation occurs the solvent was removed under reduced pressure to give an oily residue, which on treatment with ethyl acetate afforded the HCl salt of **19** (40.73%; mp 188–190 °C). IR (KBr): 3394, 2836, 1679, 1606, 1507, 1456, 1385, 1024, and 816 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.27 (br, 6H, –CH_2NHCH_2CH_2–), 3.78 and 3.82 (2 × s, 6H, 2 × –OCH_3), 3.91 (s, 6H, 2 × –OCH_3), 3.95–4.06 (m, 3H, –OCH_2CH<), 4.47 (s, 2H, methylene protons of isoindoline), 5.58 (br, 1H, disappeared on D_2O exchange, –O*H*), 6.70–7.53 (m, 9H, Ar) and 8.95 ppm (br, 1H, disappeared on D_2O exchange, –N*H*–). Calcd. for $C_{29}H_{35}N_2O_7Cl$: C, 62.30; H, 6.31; N, 5.01. Found: C, 62.13; H, 6.21; N, 4.93.

*2.1.25. N-{4-[2-Hydroxy-3-(3,4-dimethoxy-N-methylphenylethylamino)propoxy]phenyl}-5,6-dimethoxy-1-oxo-isoindoline (**20**)*

N-[4-(2,3-Epoxypropoxy)phenyl]-5,6-dimethoxy-1-oxo-isoindoline **11** (0.50 g, 1.5 mmol) and *N*-methylhomoveratrylamine (1 ml, 5.4 mmol) were heated at 80 °C for 7 h, added distilled water and left overnight. The solid obtained was filtered and crystallized from acetone to afford **20** (0.75 g, 95.42%), mp 74–76 °C. IR (KBr): 3437, 2842, 1671, 1604, 1559, 1466, 1388, 1262, 1057, and 827 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, –NCH_3), 2.53–2.82 (m, 7H, –CH(O*H*)CH_2N(CH_3)CH_2CH_2–, 1H D_2O exchanged), 3.85 and 3.88 (2 × s, 6H, 2 × –OCH_3), 4.05 (m, 2H, –OCH_2–), 4.71 (m, 3H, –C*H*(OH)–, methylene protons of isoindoline), and 6.72–7.69 ppm (m, 9H, Ar). Calcd. for $C_{30}H_{36}N_2O_7$: C, 67.14; H, 6.76; N, 5.22. Found: C, 66.92; H, 6.73; N, 5.21.

*2.1.26. N-{4-[2-Hydroxy-3-(3,4-dimethoxy-N-methylphenylethylamino)propoxy]phenyl}-5,6-dimethoxy-1-oxo-isoindoline (**20**) HCl*

Method A. As no precipitation occurs the solvent was removed under reduced pressure to give an oily residue, which on treatment with ether and then with ethyl acetate afforded a solid residue which was crystallized from acetone to afford HCl salt of **20** (44.95%; mp 202–205 °C). IR (KBr): 3295, 2942, 2541, 1673, 1615, 1512, 1466, 1389, 1238, and 828 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H, –NCH_3), 3.41 (br, 6H, –CH_2N(CH_3)CH_2CH_2–), 3.85 and 3.88 (2 × s, 6H, 2 × –OCH_3), 3.96 (s, 6H, 2 × –OCH_3), 4.16 (m, 2H, –OCH_2–), 4.68 (m, 3H, –C*H*(OH)–, methylene protons of isoindoline), 5.60 (br, 1H, disappeared on D_2O exchange, –O*H*), and 6.81–7.68 ppm (m, 9H, Ar). Calcd. for $C_{30}H_{37}N_2O_7Cl$: C, 62.87; H, 6.51; N, 4.89. Found: C, 62.69; H, 6.56; N, 4.87.

2.2. Pharmacological methods

2.2.1. Materials

All the used solvents and powders were for analysis (J.T. Baker, Deventer, Holland). Propranolol HCl and ICI 118551 were purchased from Sigma Chemical, St. Louis, MO, USA. [^{3}H]dihydroalprenolol ([^{3}H]DHA) (New England Nuclear, Boston, MA), having a specific activity of 99.9 Ci/Mol and radiochemical purity >98.5%, was used as ligand.

2.2.2. β_1-Adrenergic receptor binding assay

Pellets containing β_1-type adrenergic receptors were obtained from turkey erythrocyte membranes as described in the literature [12].

β_1-Adrenergic receptor binding assay was determined as follows: 300 μl of membrane (~1.2 mg/ml protein, dilution 1:8 v/v) were incubated for 15 min at 37 °C with 100 μl of 4 nM [^{3}H]DHA and 100 μl of various concentrations of the test compounds (dissolved in water and diluted with saline buffer) and 12 M Tris–HCl, pH 7.5 (total vol. 1 ml). The incubations were stopped adding 4 ml of cold buffer (12 M Tris–HCl) followed by rapid filtration through glass fiber filter Whatman GF/B disks (Brandel Biomedical Research and Laboratories, Gaithersburg, MD). The samples were subsequently washed 3 times with 4.5 ml of the same buffer and placed into scintillation vials 10 ml Filter-Count liquid scintillation cocktail (Packard BioScience s.r.l., Pero, Milan, Italy) was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB 2000CA—Packard BioScience s.r.l., Pero, Milan, Italy).

Non-specific binding was defined as non-displaceable binding in the presence of 100 μl of 10 μM propranolol.

Competition experiments were analyzed by the "Easy Fit" program (EasyFit 1.4, 1989–1991, Matteo Vaccari and Mario Negri Institute, Milan, Italy) to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}), with six concentrations or displacers, each performed in triplicate.

The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prussoff [13], from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis [14], for [^{3}H]DHA is 3.6×10^{-9} M).

2.2.3. β_2-Adrenergic receptor binding assay

Preparation of lung homogenate: male Sprague–Dawley rats (Harlan Italy s.r.l., Correzzana, Milan) were sacrificed by decapitation. The right lung was removed free of the major bronchi. Lungs were homogenized with Polytron Apparatus—Kinematica GmbH, Littau, Switzerland (setting 5 for 15 s) in 50 volumes buffer, 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$ and then centrifuged at 30,000*g* for 10 min twice. The resulting pellets were resuspended in 100 volumes of buffer 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$, then were frozen at −80 °C before being assayed [15,16]. [^{3}H]Dihydroalprenolol was used as ligand.

Three hundred microliters of membrane of lung (~13.05 mg of fresh tissue, dilution 1:10) were incubated for 30 min at 37 °C with 100 μl of 6 nM [^{3}H]DHA, 100 μl ketanserine 10^{-7} M 5HT antagonist and 100 μl of various concentrations of the test compounds (dissolved in water and diluted with buffer) and 75 M Tris–HCl (pH 7.65), 25 M $MgCl_2$ (total vol. 1 ml). The samples were subsequently washed with 4.5 ml of the same buffer and placed into scintillation vials. 10 ml of Filter-Count liquid scintillation cocktail (Packard BioScience s.r.l., Pero, Milan, Italy) was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB 2000CA—Packard BioScience s.r.l., Pero, Milan, Italy).

Non-specific binding was measured in the presence of 100 μl of 10 μM unlabeled ICI 118551, and specific binding as the difference between total and non-specific binding.

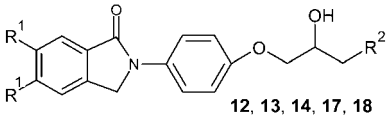
The concentration of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined as above reported.

3. Results and discussion

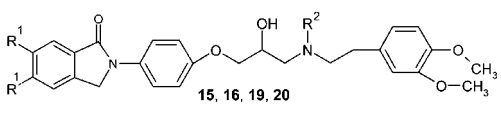
HCl salts of compounds **12**–**20** were subjected to in vitro β_1- and β_2-adrenergic receptor binding assay using turkey erythrocyte membrane (β_1) and lung homogenate of rats (β_2). The percentage inhibition of [^{3}H]DHA binding to both β_1- and β_2-adrenergic receptors and selectivity ratio (β_1/β_2) are shown in Table 1 and their K_i values shown in Table 2. The results of binding assay were compared with that of the standard non-selective β-adrenergic blocking agent propranolol and cardioselective β-adrenergic blocking agent atenolol used clinically.

All the newly synthesized compounds exhibited affinity to β_1/β_2-adrenergic receptor in the concentration range tested. In general the 5,6-dimethoxy-1-oxo-isoindoline derivatives (**17**–**19**) were less active than the 1-oxo-isoindoline derivatives (**12**–**15**) at the β_1-adrenergic receptor site, while the reverse is true at β_2-adrenergic receptor site, i.e., 5,6-dimethoxy-1-oxo-isoindoline derivatives (**17**, **18**) were more potent than the unsubstituted derivative (**12**, **13**). Thus, it seems that incorporation of dimethoxy

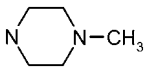
Table 1
Percentage inhibition of [^{3}H]DHA binding on β_1- and β_2-adrenergic receptor and selectivity ratio of compounds **12**–**20** (hydrochloride salt) at the highest used concentration [10^{-5} M]



12, **13**, **14**, **17**, **18**



15, **16**, **19**, **20**

Compound[a]	R^1	R^2	%I β_1	%I β_2	Selectivity ratio[b]
12	H	$NHCH(CH_3)_2$	77.24 ± 13.79	None	—
13	H	$NHC(CH_3)_3$	45.75 ± 6.79	None	—
14	H		26.44 ± 5.59	None	—
15	H	H	36.02 ± 0.71	16.22 ± 1.58	2.22
16	H	CH_3	52.31 ± 6.01	None	—
17	OCH_3	$NHCH(CH_3)_2$	17.18 ± 0.96	82.51 ± 2.05	0.21
18	OCH_3	$NHC(CH_3)_3$	2.91 ± 0.50	39.00 ± 5.94	0.08
19	OCH_3	H	23.90 ± 1.19	11.59 ± 0.47	2.06
20	OCH_3	CH_3	68.81 ± 5.30	None	—
Propranolol			97.12 ± 3.00	99.80 ± 0.28	0.97
Atenolol			73.06 ± 1.25	34.34 ± 0.84	2.13

[a] HCl salt of the compounds were used for binding studies.
[b] Expressed as (%I β_1/%I β_2).

Table 2
Inhibition of [^{3}H]DHA binding on β_1- and β_2-adrenergic receptor of compounds **2a**, **2b**, **12**, **13**, **16**, **17**, and **20**

Compound[a]	$K_i\beta_1 \pm$ SD (M)	$K_i\beta_2 \pm$ SD (M)
2a (Ref. [3])	$4.85 \times 10^{-7} \pm 0.23$	$1.72 \times 10^{-5} \pm 0.98$
2b (Ref. [3])	$1.62 \times 10^{-9} \pm 0.37$	—
12	$1.36 \times 10^{-7} \pm 0.40$	—
13	$1.44 \times 10^{-5} \pm 0.15$	—
16	$2.20 \times 10^{-6} \pm 0.85$	—
17	—	$1.47 \times 10^{-9} \pm 0.33$
20	$1.66 \times 10^{-6} \pm 0.24$	—
Propranolol	$1.60 \times 10^{-9} \pm 0.13$	$2.50 \times 10^{-9} \pm 0.18$
Atenolol	$2.70 \times 10^{-8} \pm 0.40$	—

[a] HCl salt of the compounds were used for binding studies.

substituent at 5,6-position of 1-oxo-isoindoline derivatives leads to a decrease in β_1-adrenergic receptor affinity and increase in β_2-adrenergic receptor affinity. This finding is in accordance to our previous report, i.e., introduction of additional methoxy group in *para*-substituent in a series of 4-acylamino substituted phenoxypropanolamines decreases affinity to β_1-adrenergic receptor and increases the binding affinity to β_2-adrenergic receptor [3].

In both the series the isopropyl derivative (**12**, **17**) is more active than the *tert*-butyl derivative (**13**, **18**), while the *N*-methylhomoveratrylamine derivative (**16**, **20**) is more active than the homoveratrylamine derivative (**15**, **19**) at β_1-adrenergic receptor. In 1-oxo-isoindoline series most of the compounds (**12**–**14**, **16**) do not exhibit affinity to β_2-adrenergic receptor. While in 5,6-dimethoxy-1-oxo-isoindoline series isopropyl derivative is the most active and *N*-methylhomoveratrylamine derivative is the least active with respect to β_2-adrenergic receptor.

Regarding the selectivity of compounds, 1-oxo-isoindoline series of compounds were more selective to β_1-adrenergic receptor than the 5,6-dimethoxy-1-oxo-isoindoline series of compounds (**12/13/15** vs **17/18/19**). In the 5,6-dimethoxy-1-oxo-isoindoline series *N*-methylhomoveratrylamine derivative (**20**) is the most cardioselective compound, while the isopropyl derivative (**17**) being selective towards the β_2-adrenergic receptor.

Comparison of the K_i values of the previously reported compounds **2a** and **2b** [3], with the compounds **12** and **13** (Table 2), show that the incorporation of *para*-amidic functionality within the 1-oxo-isoindoline ring system retain the affinity to β-adrenergic receptor with cardioselectivity. While incorporation of *para*-amidic functionality within the 5,6-dimethoxy-1-oxo-isoindoline ring system still retains affinity for β-adrenergic receptor, but the selectivity is reversed to β_2-adrenergic receptor as exemplified by compounds **17** and **18.** So it can be said rigidification of the *para*-amidic substituent in phenoxypropanolamines retain affinity to β-adrenergic receptor, but the selectivity could be determined by the nature of amino substituent and the substituents in the 1-oxo-isoindoline ring system.

Among the two series of compounds, **12** and **20** possessed good affinity comparable with that of atenolol, a commonly used cardioselective beta blocker. Both **12** and

20 showed a high degree of cardioselectivity (they did not show affinity to β_2-adrenergic receptor) and were far higher than the selectivity ratio of atenolol, which was 2.13. Propranolol a non-selective β-adrenergic blocking agent had showed better affinity than **12** and **20**, but did not posses cardioselectivity. Both **12** and **20** have been selected from this series of compounds for further pharmacological investigations and the results will be published elsewhere.

Use of *para*-amidic substituents in phenoxypropanolamines has been well documented to lead to cardioselective beta blocking action [3,9]. In this study, the incorporation of amidic functionality within the 1-oxo-isoindoline ring and 5,6-dimethoxy-1-oxo-isoindoline ring system retained the high degree of cardioselectivity normally seen in open chain analogues. Thus, it could be concluded that the affinity and selectivity to β_1- and β_2-adrenergic receptor of phenoxypropanolamines could be influenced by the nature of the substituent present at both the *para*-position of the phenyl ring and also by the amino substituent. Skillful manipulation of these two substituents could lead to phenoxypropanolamines with a good affinity and selectivity to β_1-adrenergic receptor.

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