Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 209–212

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

New Series of Aryloxypropanolamines with Both Human β_3-Adrenoceptor Agonistic Activity and Free Radical Scavenging Properties

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Received 16 July 2001; revised 22 October 2001; accepted 23 October 2001

Abstract—A series of 13 novel hybrid molecules designed to possess both free radical scavenging activity and to stimulate the β_3-adrenoceptors in order to improve antidiabetic effect and to restore insulin sensitivity was synthesized and evaluated. Compounds were of quinolyl-, isoquinolyl-, pyridoindolyl- or carbazolyloxypropanolamine structure with a terminal amino group of benzopyranolyl-, di-*tert*-butylphenolyl- or methoxyindolyl-type. Some of the products possessed both the expected activities.

Non insulin-dependent diabetes mellitus (NIDDM) is an expanding disease throughout industrialized countries. It is characterized by a resistance to the action of insulin on peripheral or hepatic tissues, leading to high levels of circulating glucose. In nearly 50% of the time, overweight and obesity are present in the same times. In diabetic patients, free radical overproduction is observed participating to vascular complications. Moreover, it has been shown that food supplementation with antioxidants improves insulin sensitivity in diabetic patients.[1]

The discovery in 1989 of the β_3-adrenoceptor present in brown adipose (BAT) and white adipose tissues (WAT) where it stimulates thermogenesis and lipolysis, respectively, has open a new way for antidiabetes drug design.[2–4] There is now evidence showing that β_3-adrenergic stimulation can increase insulin sensitivity and glucose tolerance, leading to selective loss of adipose tissue mass in animal models.[5,6] In fact, BAT is present in hibernating animals and in new-borns but disappears by adulthood; this explains in part why there are important differences in the in vivo results between humans and smaller mammals. It has been also demonstrated that β_3-adrenergic stimulation leads to reappearence of brown adipocytes suggesting a possible antidiabetic effect in humans.[7] Clinical investigations were led on many β_3-adrenergic agonists, but it is generally admitted these products lacked either of selectivity, potency or biodisponibility.

In order to improve activity of potential β_3-adrenoceptor agonists, we hypothesized that an original hybrid molecule possessing both β_3-adrenergic and antioxidant structures might lead to compounds useful for the treatment of insulin resistance syndrome and its vascular complications. On the basis of this hypothesis, we designed and synthesized structures corresponding to the general formula given in Figure 1, bearing various aromatic rings and different terminal amines derived

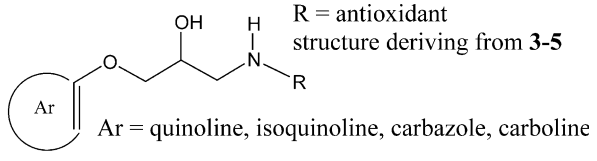

Figure 1. General structures of aryloxypropanolamines.

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00716-8

from known radical scavenging structures such as Trolox (benzopyranol derivative) or di-*tert*-butyl-4-hydroxytoluene (BHT) or 5-methoxytryptamine.

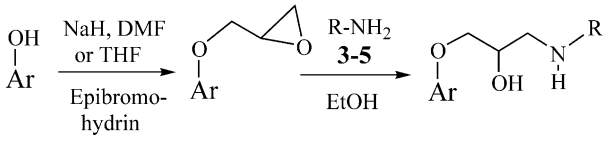
The anti-oxidant activity was evaluated compared to Trolox and also to carvedilol (4-hydroxycarbazole derivative), a β_2-adrenergic blocker with potent anti-oxidant properties.[8] β_3-Adrenergic activity was compared to L-755,507, the most potent and selective β_3-adrenoceptor agonist for human β_3-receptors actually known ($EC_{50} = 0.43$ nM).[9] In terms of structural features, the importance of an oxypropanolamines moiety for β_3-adrenergic agonist activity is well documented. In addition, it should also be noted that in most β_3-adrenergic agonists, an intra- or extracyclic heteroatom in the *meta* position of the oxypropanolamine chain is present; this is true for BRL 37344, CL 316243, carazolol and cyanopindolol. Some results obtained in our laboratory have underscored the importance of one of these two *meta* positions for β_3-adrenoceptor affinity (unpublished results). So, we tried to use these observations to investigate the quinoline, isoquinoline, carbazole and carboline rings (Fig. 1).

Another important feature appearing now, especially in L-755,507 is the importance of the length and the bulkiness of the substituent linked to the nitrogen of the oxypropanolamine for selectivity toward the β_3-adrenoreceptor subtype. For the present study, we have chosen three different well known antioxidants: a BHT derivative, a benzopyranol derivative whose structure is encountred in tocopherols and, finally, a melatonin derivative (5-methoxytryptamine) which is a highly studied derivatives for its free radicals scavenging properties.

The general synthetic route used to prepare the target compounds **6**–**18** is outlined in Scheme 1.

All the compounds were prepared by alkylation of hydroxylated rings with epibromohydrin to obtain the target epoxides which were opened by the different amines **3**–**5** in ethanol, either at room temperature or at reflux.[10] The resulting aryloxypropanolamines are described in Table 1.

The hydroxylated rings were either commercially available [5-hydroxyisoquinoline, 7-hydroxyquinoline] or obtained either by known methods or by new synthesis. 5-Hydroxyquinoline was prepared according to Bücherer synthesis on 5-aminoquinoline.[11] 7-Hydroxyisoquinoline (**1**) was prepared according to Pomeranz–Fritsch synthesis.[12] 5-Hydroxy-α-carboline synthesis was assessed by Suzuki coupling between 2-fluoro-3-iodopyridine and *N*-Boc-*m*-anisidine boronic acid, according to the method described by Rocca et al. (Scheme 2A).[13] 4-Hydroxycarbazole was obtained in a three steps synthesis via phenylhydrazone, Fisher cyclization and oxidation with carbon on activated palladium.[14]



Scheme 1. General scheme of synthesis.

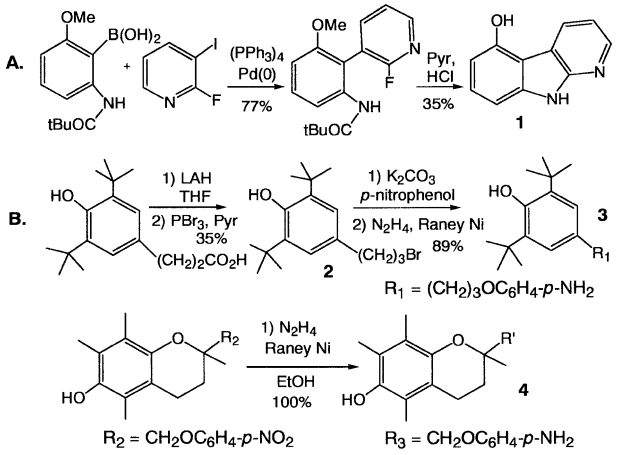
Amine **3** derived from BHT, was prepared in four steps by reduction of 3,5-di-*tert*-butyl-4-hydroxyphenylpropionic acid with LAH, followed by bromination with PBr_3 (Scheme 2B). Alkylation of *p*-nitrophenol by the brominated derivative **2** followed by reduction by hydrazine and Raney nickel, afforded to amine **3**. The precursor of compound **4**, an intermediate of the Troglitazone synthesis was obtained according to Yoshioka et al.[15] 5-Methoxytryptamine (**5**) is commercially available and was used such as. In all the cases, racemic forms were then biologically evaluated.

Free radical scavenging effects

In diabetes, protein oxidation and lipid peroxidation lead to vascular degenerative lesions. For these reasons, the representative derivatives were evaluated for antioxidant activity by determining their ability in vitro to protect against protein oxidation and plasma lipid peroxidation induced by the highly reactive hydroxyl radicals generated by a Fenton reaction (Fe^{2+}/H_2O_2). The extent of oxidation was assessed indirectly by measuring

Table 1. Aryloxypropanolamines **6**–**18**: chemical characteristics

Compd	Ar	R
6	5-Quinoline	BHT
7	5-Quinoline	Trolox
8	5-Quinoline	Tryptamine
9	7-Quinoline	BHT
10	7-Quinoline	Tryptamine
11	7-Quinoline	Trolox
12	5-Isoquinoline	BHT
13	5-Isoquinoline	Trolox
14	7-Isoquinoline	BHT
15	7-Isoquinoline	Trolox
16	4-Carbazole	Trolox
17	4-Carbazole	Tryptamine
18	5-Carboline	Trolox



Scheme 2. Synthesis of 5-hydroxycarboline (A) and terminal amines (B).

Table 2. Aryloxypropanolamines **6**–**18**: β-adrenergic and anti-oxidant activities

Compd	cAMP (pmol/min/well)			Ellman's test % protection[c]		TBARs test % protection[c]		
	Transfected cells[a]	Transfected cells[b]	Untransfected cells [b]	500 μM	1 mM	10 μM	100 μM	500 μM
Basal		20.6 (±0.1)	28.8 (±0.9)					
6		42.2 (±3.5)	21.6 (±1.6)	47.3 (±17.9)	120.3 (±36.6)*,**	30.3 (±0.6)**	33.3 (±0.9)*,**	50.4 (±2.8)*
7	102.6 (±13.8)	28.6 (±1.9)	26.8 (±0.7)	49.2 (±16.5)	82.9 (±25.7)*	37.8 (±1.5)*,**	64.6 (±1.8)*,**	78.2 (±0.2)*,**
8		24.1 (±0.2)	29.9 (±1.1)			43.6 (±4.7)*,**	52.0 (±4.2)*,**	50.7 (±2.8)*
9	97.4 (±11.4)	21.4 (±0.6)	23.3 (±0.5)					
10		1.8 (±0.1)	32.5 (±1.5)					
11				46.0 (±7.7)*	66.3 (±3.4)*	26.5 (±1.5)**	30.6 (±0.7)**	50.8 (±2.6)*
12	129.6 (±2.8)	76.3 (±6.1)	24.9 (±0.6)			43.6 (±3.3)*,**	32.8 (±4.6)**	49.3 (±1.9)*
13	142.1 (±28.1)	41.6 (±3.7)	28.7 (±0.6)	54.8 (±4.9)*,**	115.2 (±36.6)*,**	39.4 (±3.9)*,**	49.3 (±2.9)*,**	94.4 (±9.2)*,**
14		23.9 (±1.4)	26.9 (±1.6)					
15		33.2 (±0.7)	33.0 (±2.2)					
16		22.3 (±0.6)	31.5 (±0.7)					
17		34.4 (±3.7)	27.9 (±1.6)					
18						44.3 (±3.5)*,**	53.6 (±3.3)*,**	75.5 (±1.8)*,**
Trolox				31.4 (±5.1)	75.2 (±3.3)*	−21.4 (±3.7)	7.6 (±2.8)	64.8 (±2.8)*
Carvedilol				20.0 (±9.5)	−88.6 (±17.5)	1.0 (±0.8)	−0.6 (±0.6)	6.2 (±2.5)
L-755,507	166.6 (±1.8)	173.6 (±17.5)	30.4 (±1.1)					

$*p < 0.05$ versus BSA or plasma oxidation without tested molecule. $**p < 0.05$ versus Trolox.
[a]Without propranolol.
[b]With propranolol.
[c]Values are means ±(SEM) of three different experiments.

the protection of bovine serum albumin (BSA) thiol protection by using Ellman's test[16] and formation of thiobarbituric acide reactive substances (TBARs).[17] Results are expressed as percentage of protection in comparison to the benzopyranol structure Trolox and are presented in Table 2. Statistical method of calculation was ANOVA test ($p < 0.05$).

As far as the TBARs test is concerned, the closely related molecules derived from benzopyranol, compounds **7, 13, 18** had similar antioxidant activity, between themselves, and are better ($p < 0.05$) from Trolox at 10 and 100 μM but not 500 μM except for compounds **7**, **13** and **18**. Moreover, no significant difference was observed in the antioxidant activity of compounds bearing different aromatic moities i.e. quinoline, isoquinoline or carboline but the same terminal amine derived from benzopyranol structure ($p < 0.05$). Thus, the introduction of different aromatic moities seems to bring no significant changes in the free radical scavenging. So, only one aromatic structure of each lateral amine was subsequently tested. On the other side, the di-*tert*-butylphenol derivative **12**, did not develop the same scavenging properties compared to benzopyranol derivatives, which developed dose–response curve activity. Concerning the tryptamine derivative **8**, no change in anti-oxidant protection was observed between the three concentration levels. Surprisingly, and in contradiction with literature, carvedilol showed in these models, weak scavenging properties. Yet, data of literature referred to a protection against $^{\bullet}OH$ exclusively, generated by a Fenton reaction. The oxidative system used in the previous works produced less reactive free radicals (such as $O_2^{\bullet -}$) and it is probable that carvedilol is a better scavenger for $O_2^{\bullet -}$ than $^{\bullet}OH$.

Besides the Ellman's test, it is interesting to observe that the thiol group protection increased with the concentration of the tested molecules.

Together, these results showed that bulky polyphenol derivatives are far more potent to scavenge OH than indole or carbazole derivatives and that quinonic structures (BHT and Trolox) are the most potent amongst them. In addition, it should be noted that, at the tested concentrations, the novel molecules were generally better free radical scavengers against lipid peroxidation than Trolox which is though the parent molecule for compounds **7**, **13** and **18.**

β-Adrenergic activity

β-Adrenergic activities were calculated by measuring of cAMP production on human β_3-adrenergic transfected and untransfected COS-7 cells.[18] These activities were compared to L-755,507, one of the most potent and selective β_3 agonists.[9] The results are shown in Table 2, given in pmol of cAMP produced per min and per well for each compound (10 μM) in the absence or presence of 0.1 μM propranolol, a β_1/β_2 antagonist. The levels of cAMP produced in transfected cells without propranolol is a reflection of the total β-adrenergic agonistic activity (β_1, β_2 and β_3) while cAMP levels in transfected cells in the presence of propranolol reflects the selective β_3-adrenergic activity. When untransfected cells were treated with all the compounds in the presence of propranolol, all activity levels were equivalent. Together, these data show that compounds **7**, **9**, **12** and **13** possess both β_2 and β_3 adrenergic agonistic activity. Compounds **7**, **8**, **9**, **14**, **15** and **16** were inactive to stimulate cAMP production in β_3-transfected COS-7 cells.[19] The

results indicated that compounds **6**, **12**, **13** and **17** exhibited selective stimulation of cAMP production in human β_3-transfected cells. The compound with the best β_3-adrenergic activity was **12**, which was derived from 5-hydroxyisoquinoline and BHT, giving cAMP production in transfected cells of 40% of L-755,507 activity. It should be noted that one compound (**10**), derived from 7-hydroxyquinoline and tryptamine, appeared to possess reverse agonistic activity as demonstrated by a decrease in cAMP production in cells compared to the cAMP basal production of untransfected preincubated (1.8 vs 32.5 pmol/min/well). Evidently, this finding will require further investigations.

Concerning the structure–activity relationship, and in opposition to that suggested in literature, the presence of an heteroatom in the *meta* position of the aryloxypropanolamine lateral chain does not seem to have significance for the activity since the 5-hydroxyisoquinoline derivatives (i.e., **12** and **13**) developed a better activity than their quinoline analogues. On the other hand, in the same manner we previously noted for carbazole and indole derivatives (unpublished data), the 5-position for the aryloxypropanolamine chain of isoquinoline and quinoline seemed to have some significance for the activity since no 7-substituted compound developed any agonistic activity (**9**, **14**, **15**). Moreover, the presence of a bulky amine substituent appears to be favorable for the β_3-adrenergic activity, in this way, it came to light that BHT derivatives were the best structures compared to the benzopyranol derivatives which were more potent than their 5-methoxytryptamine analogues. In addition, flexibility of this chain seems to play an important role since BHT derivatives were better than their Trolox analogues (**6** > **7** and **12** > **13**).

In conclusion, this study brought to light a new series of aryloxypropanolamines appearing to be unique in that they possess both antioxidant and β_3-adrenergic agonistic activity. The most potent β_3 agonist (**12**) was a good radical scavenger so, taken on the whole, these molecules could open the field of a new therapeutic strategy in diabetic patients, as the improvement of insulin activity has an indirect beneficial effect on antioxidant defence system. Further synthesis will be directed towards improving the activation of cAMP production in transfected cells, and selectivity toward β_3-adrenoceptors by lengthening the lateral chain in order to improve its bulkiness.

Acknowledgements

We would like to thank Roche Laboratories and Merck Research Laboratories for providing us with Carvedilol and L-755,507, respectively. We thank the US NIDDK National Hormone and Pituitary Program for the antisera to cAMP. We are grateful to Dr. A. Boumendjel and Pr. C. Ribuot for their profitable advices.

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18. **cAMP measurement:** sCOS-7 cells were cultured in DMEM with 10% foetal bovine serum (Hyclone, UT, USA), 100 units/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Cells were seeded into 6-well cluster plates (Falcon) and transfected at 70–80% confluence with 2 mg pBC12-h$\beta_3$adrenergic receptor plasmid DNA per well with 5 mL lipofectamine (Life Technologies, Gaithersburg, USA) in 1 mL of serum-free media. Four hours after addition of DNA, the cells were gently washed with serum-free media and incubated in complete growth media for an additional 24 h. Adenylyl cyclase assay cells were treated in 6-well cluster plates according method of Cotecchia et al.[19] Attached cells were incubated for 30 min at 37 °C in serum-free DMEM/25 mM HEPES, pH 7.5. The medium was replaced with the same medium containing 0.24 mM IBMX and the drugs for 20 min. Treatment was terminated by rapidly aspirating the medium and adding cold 5% TCA (500 mL/well). Adenylyl cyclase was assessed in 20 mL of the TCA extract by measuring the cAMP formed by RIA[20] using a polyclonal antiserum iodinated to cAMP.[21]
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