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Review Article

Stereochemical Comparison of Nebivolol with other β-Blockers

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ABSTRACT β-Blockers are widely used in the treatment of cardiovascular disease and act by antagonizing the effects of adrenaline (epinephrine) and noradrenaline (norepinephrine) on β-adrenergic receptors. All β-blockers currently used in the treatment of cardiovascular disease contain at least one chiral center and, while most are marketed as racemates, their cardiac antihypertensive activity generally resides in the *S*-enantiomer. Nebivolol is a third generation β-blocker that is highly selective for the β_1-adrenoceptor. The nebivolol molecule contains four chiral centers and is marketed as a racemate of (+)-nebivolol (*SRRR*-configuration) and (−)-nebivolol (*RSSS*-configuration). Nebivolol differs from all other β-blockers with a hydroxypropanolamine substructure in that its cardiac antihypertensive activity resides in the *R*-enantiomer at the hydroxy group, whereas all other β-blockers have antihypertensive activity in the *S*-enantiomer. Two of the four chiral centers in nebivolol are part of a ring structure and the increased rigidity of this structure may be related to nebivolol's divergence from the standard pharmacophore model of β-blockers. *Chirality 20:103–109, 2008.*

KEY WORDS: β-blockers; hydroxypropanolamine structure; enantiomer; eutomer; racemate

INTRODUCTION

Nebivolol (Nebilet®) is a racemic mixture of (+)-nebivolol, which has selective β_1-receptor blocking activity, and (−)-nebivolol, which causes vasodilation. The nebivolol molecule contains four chiral centers; (+)-nebivolol has the *SRRR*-configuration whereas its enantiomer (−)-nebivolol has the *RSSS*-configuration. The constitution of nebivolol is fundamentally different from the typical first generation β-blockers.[1] In this article, the absolute configuration of nebivolol and that of the hydroxypropanolamine substructure is discussed with regard to propranolol and the native enantiomerically pure neurotransmitters adrenaline (epinephrine) and noradrenaline (norepinephrine).

β-BLOCKERS: STEREOCHEMICAL CONSIDERATIONS

β-Blockers are used widely in the treatment of cardiovascular conditions including angina, hypertension, and arrhythmias. These agents act by antagonizing the actions of adrenaline (epinephrine) and noradrenaline (norepinephrine) on β-adrenoceptors. All currently available β-blockers contain at least one chiral center in their structure, and interaction of β-blockers with β-adrenoceptors is highly stereoselective[2]; the binding pocket of these receptors is a good example of chiral recognition.

Propranolol, the first successful β-blocker to be developed, is a nonselective β-adrenoceptor antagonist that blocks β_1- and β_2-receptors to similar extents. Like many β-blockers used in the treatment of cardiovascular disease, propranolol is marketed as a racemic mixture consisting of the *R*- and *S*-enantiomers in a fixed 1:1 ratio. Remarkably, the stereoisomer of propranolol with *R*-configuration at the hydroxy group (dexpropranolol; Fig. 1A) has no pharmaceutical importance and it is the *S*-enantiomer that accounts for the cardiac β-blocking effects of propranolol (Fig. 1B); a phenomenon that has been observed with other β-blockers.[3] In fact, certain β-blockers used for the treatment of hypertension are produced as enantiomerically pure compounds containing only the enantiomer which is more active (that is, the eutomer), including timolol (Dispatim®), levobunolol (Vistagan®), penbutolol (Betapressin®), and esatenolol (Atpure®).

A feature common to the chemical structure of β-blockers is the presence of at least one aromatic residue attached to a side alkyl chain that possesses hydroxy and amine functional groups; in all cases β-blockers contain one or more chiral centers, one of which is always a carbon atom in the alkyl chain directly attached to a hydroxy group.[2] The neurotransmitters adrenaline (Fig. 1C) and noradrenaline (Fig. 1D), which exert β-sympathomimetic activity in the body, contain just such a chiral center for

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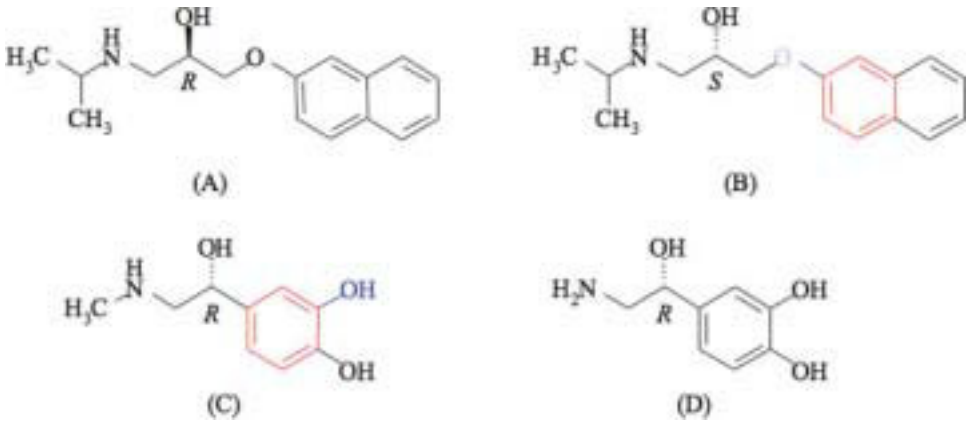

Fig. 1. Dexpropranolol (*R*-configuration) (**A**) is less potent than the *S*-enantiomer of propranolol (**B**). The neurotransmitters adrenaline (**C**) and noradrenaline (**D**) contain OH groups with *R*-configuration. Adrenaline (C) and *S*-propranolol (B) have different absolute configuration but the same spatial orientation (highlighted in color). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

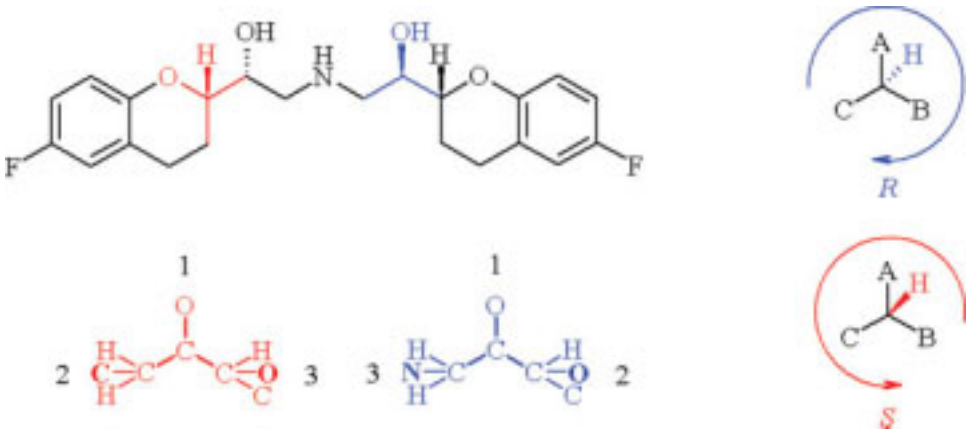

Fig. 2. (+)-Nebivolol is the *SRRR*-eutomer of racemic nebivolol. The absolute configuration of (+)-nebivolol is determined by digraphs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

which the absolute configuration has been determined to be *R*.

Binding at the β-adrenoceptor binding site requires hydrogen bonds, ionic, and π–π interactions. A comparison of adrenaline and propranolol reveals a distinct spatial orientation of the hydroxy group, the amino group, and the aromatic residue that is essential for binding at the adrenoceptors. Reversal of the hydroxybenzene moiety in adrenaline (Fig. 1C) as, for example, is seen in propranolol (Fig. 1B) leads to an antagonistic effect upon binding at the binding site of the receptor.[4]

As illustrated in Figure 1, the absolute configuration of adrenaline and *S*-propranolol is contrary although their spatial orientation is similar. This difference in absolute configuration (*R* vs. *S*) depends on the Cahn-Ingold-Prelog (CIP) rules for describing a chiral center. The spatial orientation of the ligand is critical to eliciting sympatholytic and sympathomimetic activity at the β-adrenoceptor.

Some types of β-blocker have more than one chiral center. Labetalol, although not a propanolamino-type β-blocker, has two chiral centers and is marketed as a racemate of four stereoisomers. Its *RR*-stereoisomer accounts for most of its β-blocking activity, whereas the *SR*-stereoisomer is responsible for most of its α-blocking activity.[5]

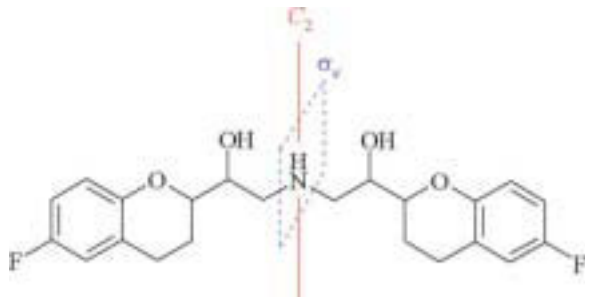

Fig. 3. The constitution (not the configuration) of nebivolol. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

When labetalol was tested in anaesthetized dogs, the isomer (−)-2-hydroxy-5-[(*R*)-1-hydroxy-2-((*R*)-1-methyl-3-phenylpropylamino)ethyl]benzamide (dilevalol) showed nonselective β-antagonism, 2-hydroxy-5-[(*S*)-1-hydroxy-2-((*R*)-1-methyl-3-phenylpropylamino)ethyl]benzamide was the most potent antagonist at α_1-adrenoceptors, whereas the enantiomer of dilevalol, (+)-2-hydroxy-5-[(*S*)-1-hydroxy-2-((*S*)-1-methyl-3-phenylpropylamino)ethyl]benzamide, showed weak activity at both adrenoceptors.[5] This example shows that the influence of stereoisomers is not limited to nebivolol, but the topology of the most active labetalol stereoisomer is the same as in adrenaline and noradrenaline. In both molecules the hydroxy group has *R*-configuration. Comparison of a 50:50 mixture of the *RR* and *SR* stereoisomers with racemic labetalol (*RR*, *RS*, *SR*, *SS*) in rats confirmed that these two labetalol isomers are responsible for the treatment effects in systemic hypertension.[5,6] Nevertheless, dilevalol was withdrawn from the market due to liver toxicity.

THE β-BLOCKER NEBIVOLOL

Unlike racemic propranolol, which shows no selectivity for β_1- over β_2-adrenergic receptors, nebivolol is highly selective for β_1-adrenergic receptors[7] and is not associated with β_2-mediated bronchoconstrictive effects. Nebivolol is at present the only β-blocker which differs fundamentally from the structure derived from propranolol. For all other β-blockers with a hydroxypropanolamine substructure, the *S*-configuration at the chirality center (i.e. at the chiral carbon attached to the hydroxy group) is responsible for the molecule's antihypertensive effect, with the *S*-enantiomers generally having about 100-fold greater cardiac β-blocking activity than the *R*-enantiomers.[2] To understand the unique properties of nebivolol, the precise orientation of the four chirality centers must be considered (Fig. 2).

To investigate the stereochemical properties of nebivolol, all possible stereoisomers were synthesized by enantioselective reactions.[8,9] The starting materials were (*R*)-6-fluorochroman-2-carboxylic acid and (*S*)-6-fluorochroman-2-carboxylic acid, respectively.[9] In the next step, the carboxylic acid was reduced to yield the aldehyde,[10] which was converted into 6-fluoro-2-oxiranylchroman. The four 6-fluoro-2-oxiranylchroman key intermediates were prepared in the desired stereochemical configuration: (*R*,*R*), (*R*,*S*), (*S*,*R*), and (*S*,*S*). These compounds were separated by reverse-phase liquid chromatography. Subsequent ring-opening of two building blocks with benzylamine delivered 2-{benzyl-[2-(6-fluorochroman-2-yl)-2-hydroxyethyl]amino}-1-(6-fluorochroman-2-yl)ethanol, the *N*-protected (±)-nebivolol, and its stereoisomers. After debenzylation, all isomers were purified by chiral chromatography (preparative chiral chromatography on a Chiralpak AD column using MeOH [0.1% *N*,*N*-diisopropylethylamine] as the eluent)[11] and isolated as hydrochloride salts. The stereochemical purity of all isomers is shown in Table 1; impurities of more than 1% were analyzed to exclude the existence of constitutional byproducts. Single crystals were prepared and the unit cells were investigated by X-ray crystallography,[12] additionally ^{1}H and ^{13}C NMR data confirmed the structures. The spatial orientation of all isomers was found to correlate with the four chirality centers and the crystallized compounds demonstrated definite differences in extension. Notably, (±)-nebivolol is less flexible than other stereoisomers and the two aromatic moieties are approximately coplanar. To understand the geometric properties a stereochemical consideration is helpful.

TABLE 1. Stereochemical purity of nebivolol isomers

Stereoisomer	Stereochemical purity (area %)	Impurities (area %)
SRRS	99.7	–
RSSR	99.9	–
RSRS	99.0	–
RRRS	99.9	–
RSSS	99.9	–
RRRR	99.9	–
RRSR	97.0	1.6 *RSSS*, 1.3 *SRSS*
SRSS	99.0	–
RRSS	99.2	–
SSSS	96.7	3.3 *RRSS*

STEREOCHEMICAL RESEARCH

The constitution of nebivolol is symmetric in that the two substituents on nitrogen have the same constitution (although not the same configuration). The absolute configuration of both enantiomers leads to C_1-symmetry in the molecule. A C_2-axis and a mirror plane σ_v do not exist. In Figure 3 the two elements of symmetry are drawn in the constitution formula.

The number of possible stereoisomers for a chiral organic compound with *unsymmetric* constitution is determined by $N_r = 2^n$ stereoisomers (N_r = real number of stereoisomers, n = number of chirality centers). Figure 4 shows all 16 of the theoretically possible stereoisomers of nebivolol. Reflecting the images on the mirror planes σ_x and σ_y reduces the number of stereoisomers from eight enantiomeric pairs (16 compounds) to 10 compounds (Table 2). It is important to realize that reflection on σ_x and σ_y gives the same result (Fig. 5). This theoretical treatment is sufficient and drawing of all stereoisomers is not necessary—this is very useful if a number of compounds with many stereogenic units are of interest.

Thus, nebivolol (1-(6-fluorochroman-2-yl)-2-[2-(6-fluorochroman-2-yl)-2-hydroxyethylamino]ethanol), with its four chiral centers, exists as only 10 possible stereoisomers. Two of them are meso-compounds (i.e. they have an intramolecular mirror plane) and the others are enantiomeric pairs. All other relationships between the stereoisomers are diastereomeric. All actual stereoisomers are listed in Table 2. Only two of them have intrinsic activity: (+)-nebi-

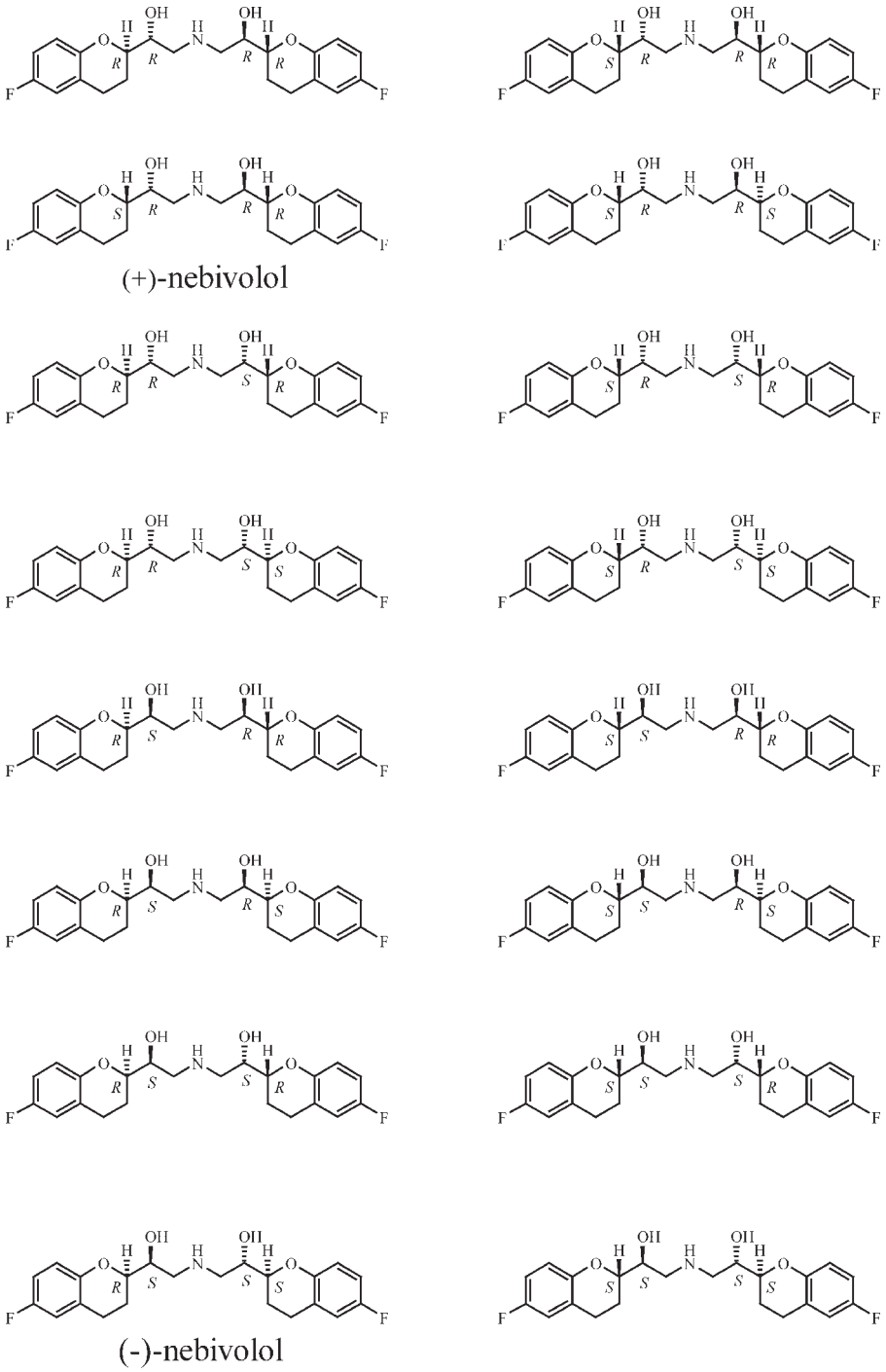

Fig. 4. Sixteen theoretically possible stereoisomers of nebivolol.

volol (*SRRR*) and (−)-nebivolol (*SSSR*). The term intrinsic activity is used to denote that a ligand–receptor complex shows a desired effect in the organism, in this instance antihypertensive activity; the assumption is that this results from the binding of a drug molecule at its receptor.

The number of actual stereoisomers ($n = 10$) is less than the number of possible stereoisomers ($2^4 = 16$) because the constitution of nebivolol is symmetric. The identical sets of stereodescriptors are named in Table 3. Now it becomes much clearer that (+)-nebivolol can be described stereochemically by two sets of stereodescriptors: *SRRR* and *RRRS*. This knowledge is helpful when searching and reading scientific literature: for chiral molecules with symmetric constitutions, pairs of molecules are

TABLE 2. Actual stereoisomers of nebivolol

Image	Reflected image	Isomeric relationship	Point group	Σ = 10
RRRR	*SSSS*	Enantiomeric	C_2	2
RRSR	*SRSS*	Enantiomeric	C_1	2
SRRR [(+)-nebivolol]	*SSSR* [(−)-nebivolol]	Enantiomeric	C_1	2
RRSS	*RRSS*	Meso-compounds	C_s	1
RSRS	*RSRS*	Meso-compounds	C_s	1
RSSR	*SRRS*	Enantiomeric	C_2	2

identical when the sequence of stereodescriptors for one is exactly the reverse of another, e.g. an *RRRS*-configuration is identical with an *SRRR*-configuration. Reading forward or backward is allowed.[4]

To help elucidate the stereochemistry at the hydroxy group, the configuration of the enantiomerically pure β-blockers timolol, penbutolol, levobunolol, and esatenolol is shown in Figure 6. Levobunolol is an ingredient of racemic bunolol and esatenolol is the *S*-enantiomer of atenolol (Atehexal®).

It can be seen from Figure 6 that the hydroxy group is always in the *S*-configuration to get an antihypertensive systemic effect (excepting ophthalmic use[13]). Conversely, the pure *R*-configuration enantiomer dexpropranolol (Fig. 1A) has no pharmacological relevance. Nevertheless, most of the more than 100 agents with β-blocking activity are administered as racemates.

The determination of the absolute configuration of nebivolol is described in detail in Figure 2. The CIP rules for the determination of priority are applied to specify the configuration of a chiral center. The substituents are ranked and the position of the hydrogen atom which is above or below the plane of the paper must be considered. A clockwise numbering with hydrogen (the substituent of lowest priority) below the plane of the paper results is deemed the *R*-configuration. An anticlockwise counting is deemed the *S*-configuration.

Fig. 5. Nebivolol contains 10 possible stereoisomers instead of the $n = 2^4 = 16$ stereoisomers. For nebivolol pairs of isomers are identical when the sequence of stereodescriptors for one is exactly the reverse of another, e.g. the *RRRS*-configuration is identical with an *SRRR*-configuration. This simple rule allows elimination of identical stereoisomers without the necessity of drawing all 16 compounds by reflection on σ_x and σ_y. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Comparison of the first generation β-blockers and nebivolol indicates that the orientation of the hydroxy group relative to the neighboring aryl residue is important (*R*-configuration in nebivolol and *S*-configuration in enantiomerically pure aryloxyaminopropanol derivatives). The topology of these structural elements is not comparable although their intrinsic activity (i.e. cardiac antihypertensive activity) is comparable.

The binding of (+)-nebivolol, (−)-nebivolol and the eight stereoisomers to β_1-adrenoceptors from rabbit lung membrane preparations was tested in an in vitro assay.[14] The IC_{50} values of the stereoisomers are listed in order of decreasing potency in Table 4. The binding of the *SSSS*-stereoisomer was about 1200-fold weaker than that of (+)-nebivolol, and binding of the *RSSS*-stereoisomer, (−)-nebivolol, was 175-fold weaker than that of (+)-nebivolol. Most of the stereoisomers with a hydroxy group in the *S*-configuration had weak binding affinities. Both symmetric meso-compounds showed only high nanomolar activity. All enantiomeric couples had very different binding affinities. It is obvious from these findings that a relationship between configuration at the chirality centers and binding affinity is not simple.

It is surprising that (+)-nebivolol (*SRRR*) is about 175 times more active than (−)-nebivolol (*RSSS*). It is difficult to reconcile this finding with the stereochemistry of other β_1-selective blocking agents; that is, the orientation of the hydroxy group and the neighboring bicyclic system is not in accordance with that observed in all other β-blockers. Obviously, the established pharmacophore

TABLE 3. *SRRR* and *RRRS* are identical because the sequence for one stereodescriptor is exactly the reverse of another

Stereo-descriptor 1	Stereo-descriptor 2	Isomeric relationship	Point group	Σ = 6
RSSS	*SSSR* [(−)-nebivolol]	Identical	C_1	1
SRSS	*SSRS*	Identical	C_1	1
RRSS	*SSRR*	Identical	C_s	1
RSRS	*SRSR*	Identical	C_s	1
SRRR [(+)-nebivolol]	*RRRS*	Identical	C_1	1
RSRR	*RRSR*	Identical	C_1	1

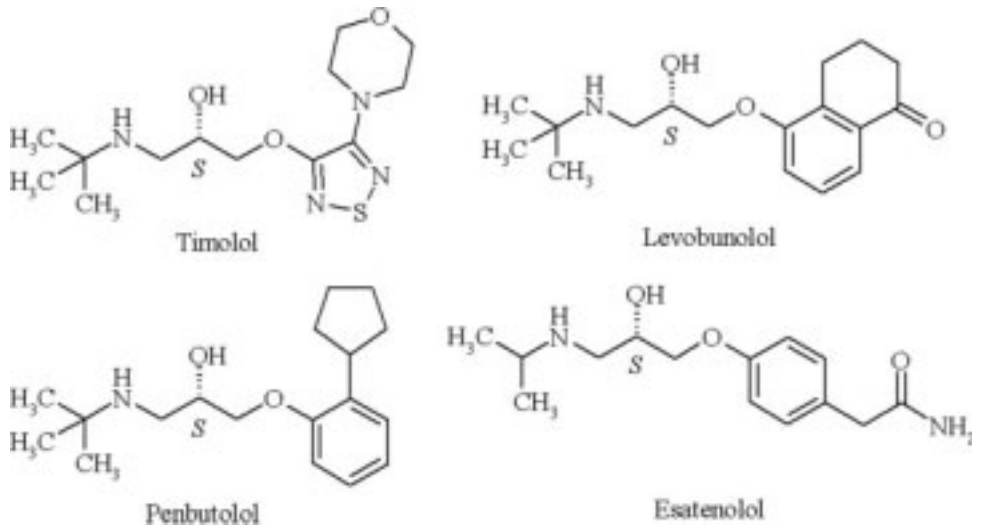

Fig. 6. β-Blockers marketed as pure enantiomers.

model for the interaction of ligands at the receptor binding site must be extended. All other sympatholytics contain only 1 or 2 chiral centers and have more degrees of freedom because of their linear carbon chains. In the case of nebivolol, in which two chirality centers are part of a ring structure, increased rigidity seems to be important for this deviation from the standard pharmacophore model of β-blockers.

Nebivolol is a β-blocker with an unusual constitution and configuration and has an interesting pharmaceutical profile. Racemic nebivolol differs from all other β-blockers and its antihypertensive properties and beneficial effects on left ventricular function result from the combined and synergistic actions of both enantiomers. The (+)-enantiomer shows β_1-blocking activity and the (−)-enantiomer acts as a vasodilator.[13–17] Vasodilation may be achieved by NO release, as indicated by studies in animal models and in humans, with possible mechanisms including an increase in NO synthase and stimulation of P2Y-purinoceptor-mediated NO release.[18] Consideration of stereochemical properties and the application of modern analytic methods delivers deeper insights into the antihypertensive profile of nebivolol.

TABLE 4. IC_{50} values in a rabbit lung β_1-adrenoceptor binding assay[14]

Stereoisomer	IC_{50} (nM)
SRRR [(+)-nebivolol]	0.8
SRRS	2.7
RRSR	3.8
RRRR	11
SRSS	30
SRSR	38
RSSR	84
RRSS	133
RSSS [(−)-nebivolol]	140
SSSS	945

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