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**COMBINACIONES QUE COMPRENDEN AGENTES
ANTIMUSCARÍNICOS Y AGONISTAS BETA-
ADRENÉRGICOS**
Solicitante : LABORATORIOS ALMIRALL, S.A.
Clase : A61K 045/000
Fecha de Solicitud : 30 de Noviembre de 2006

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al Oficio No. 00960 notificado por fijación en lista del 21 de Enero de 2011.

2. Mediante memorial radicado el 13 de Abril de 2011 solicité plazo de treinta (30) días para atender el oficio No. 00960.

3. Mediante el Oficio No. 00960 se puso en conocimiento del solicitante el informe del examen de fondo que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente.

4. Nivel Inventivo

Para la evaluación de este requisito, el Examinador ha citado las siguientes anterioridades:

- D1: WO 01/04118, "Novel quinuclidine derivates and medicinal compositions containing the same"
- D2: WO 02/053564, "Novel quinuclidine derivates and medicinal compositions containing the same"
- D3: WO 03/087094, "New pyrrolidinium derivates"

La anterioridad D1 enseña los derivados de quinuclidina y su efecto como antagonistas de los receptores muscarínicos M3 para el tratamiento de enfermedades respiratorias. De la misma manera, dicha anterioridad describe de la combinación de los compuestos mencionados en dicha anterioridad, como lo es (3R)-1-fenetil-3-(9H-xanten-9-carboniloxi)-1-azoniabicyclo[2.2.2] octano, junto con agonistas $\beta 2$, esteroides, antialérgicos o inhibidores de fosfodiesterasa IV.

Por su parte, el documento D2 describe los derivados de quinuclidina y composiciones que los contienen, como agentes antimuscarínicos de efecto potente y de larga duración, los cuales pueden administrarse en forma simultánea, por separado o en forma secuencial junto con agonistas $\beta 2$, esteroides, antialérgicos, inhibidores de fosfodiesterasa IV y/o antagonistas de leucotrieno D4.

Así mismo, el documento D3 describe la combinación de los derivados de pirrolidina como agentes antimuscarínicos M3, junto con agonistas $\beta 2$, esteroides, antialérgicos, inhibidores de fosfodiesterasa IV y/o antagonistas de leucotrieno D4 para el tratamiento de enfermedades respiratorias.

En este sentido, el Examinador afirma que la presente solicitud no menciona características que concedan un efecto inesperado o mejorado de las combinaciones descritas con relación a aquellas mencionadas en las anterioridades citadas. Siendo así las cosas, una persona conocedora de la materia lograría el objeto de la presente solicitud partiendo de los compuestos reclamados en las combinaciones de las anterioridades D1 y D3, así como D2 y D3.

5. Respuesta a las objeciones

De este modo y con el objeto de atender las objeciones arriba mencionadas, se pone de presente al Despacho las siguientes modificaciones, aclaraciones y argumentos:

5.1 Nivel Inventivo

El Examinador cita los documentos D1 a D3 y manifiesta que estos afectan el nivel inventivo de la presente invención, puesto que la invención no presenta característica alguna que conceda un efecto inesperado o mejorado de las combinaciones descritas. A este respecto solicitamos la reconsideración de la objeción, con base en los siguientes argumentos:

La presente invención esta dirigida a las combinaciones de un antagonista de M3, específicamente de (3R)-1-fenetil-3-(9H-xanten-9-carboniloxi)-1-azoniabicyclo[2.2.2] octano (referenciado como el compuesto **LAS 35201**), junto con un agonista $\beta 2$. Contrario a la

afirmación de ese Despacho, dicha combinación alcanza una atenuación sorprendente de los efectos secundarios cardíacos observados normalmente durante el uso de broncodilatadores.

Así pues, es de conocimiento común de una persona conocedora de enfermedades respiratorias causadas por broncoespasmo y sus respectivos tratamientos, que la taquicardia es un efecto secundario asociado con el uso de todos los agonista β_2 . Dichos compuestos, al lograr de manera efectiva la relajación de los músculos lisos de los bronquios, se usan comúnmente para el tratamiento agudo de enfermedades respiratorias. Como es mencionado en el libro "Goodman and Gilman's The Pharmacological Basis of Therapeutics", específicamente en la página 231, columna derecha, párrafo 2:

*"La taquicardia es un efecto secundario adverso de la administración sistemática de los agonistas β adrenérgicos."*¹ [subrayado fuera del texto]

De la misma manera, en la página 231, columna izquierda, cuarto párrafo se menciona:

*"Los pacientes que padecen enfermedades cardiovasculares se encuentran particularmente en riesgo de sufrir reacciones significativas."*²

Siguiendo los lineamientos del libro anteriormente citado, resulta claro que los agonista β_2 causan comúnmente taquicardia en pacientes, y dicho efecto secundario puede representar una complicación específica significativa y determinante en pacientes con condiciones cardíacas preexistentes.

Resulta igual de importante traer a colación el libro "Textbook of Respiratory Medicine", el cual proporciona un análisis general o resumen de las propiedades de los agonista β_2 . Los efectos secundarios de esta clase de medicamentos son discutidos en la página 271, columna izquierda:

"La taquicardia y las palpitaciones son causadas por una estimulación secundaria de reflejo cardíaco frente a la vasodilatación periférica, por estimulación directa de los receptores atriales beta 2 (el corazón)"

¹HARDMAN, Joel Griffith; LIMBIRD, Lee E.; GILMAN, Alfred G.: "Goodman & Gilman's The Pharmacological Basis of Therapeutics". Décima Edición, McGraw-Hill Professional. 2001. Página 231.

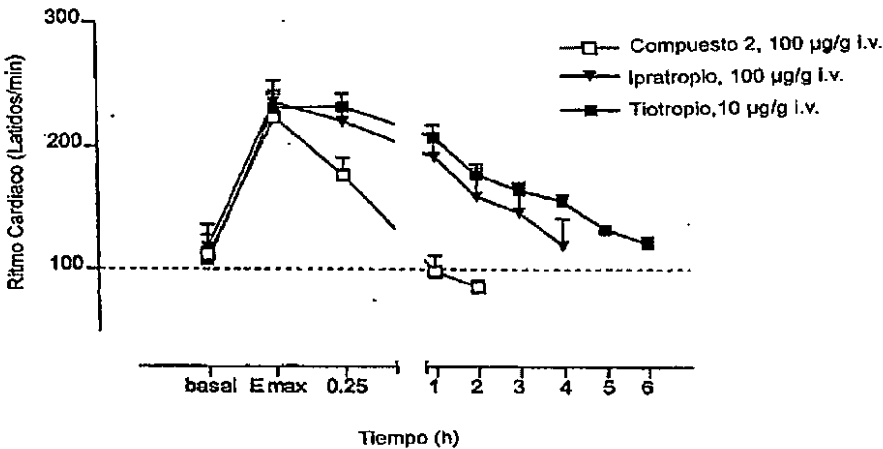
² Ibidem.

humano es inusual al presentar una proporción alta de receptores beta
2)³ [subrayado fuera del texto]

Por otro lado, solicitamos al Señor Examinador tener de presente el anexo 2 adjunto a este memorial, el cual compara las estructuras de los agonistas $\beta 2$ comúnmente usados, incluyendo salmeterol, compuesto usado en los ejemplos incluidos en la memoria descriptiva de la presente solicitud. Resulta evidente a partir de dicho documento que todos los agonistas $\beta 2$ se basan en un núcleo de p-hidroxifenil etanol amino. Este aspecto común permite que todos los agonistas $\beta 2$ logren la relajación de los músculos lisos de los bronquios, pero a su vez generan una actividad taquicárdica indeseable. En este sentido se puede concluir que la taquicardia es un efecto que surge por el mecanismo de acción de todos los agonistas $\beta 2$.

La presente invención presenta como una actividad favorable o perfil de efecto secundario particular del compuesto **LAS 35201** sobre otras composiciones farmacéuticas que contienen este tipo de medicamentos. Con relación a la anterior afirmación, se desea llamar la atención del Señor Examinador a la gráfica comparativa anexa a la respuesta de la primera acción oficial, y la cual se presente a continuación. Dicha gráfica compara el efecto cardiaco cronotrópico del compuesto **LAS 35201** en perros, frente al efecto observado para otras sustancias como ipatropio y tiotropio, correspondientes a antagonistas muscarínicos M3 convencionalmente comercializados para el tratamiento de enfermedades respiratorias.

Efectos en el curso del tiempo del compuesto 2, tiotropio e ipratropio en el ritmo cardiaco de perros Beagle consentidos



N=3 por tratamiento. Los datos del Ipratropio corresponden a 2 animales a 100 µg/kg y el otro a 10 µg/kg

³ MASON, Robert J. MD; V. BROADDUS, Courtney MD; MARTIN, Thomas; KING, Talmadge Jr. MD; SCHRAUFNAGEL, Dean; MURRAY, John F.; NADEL, Jay A.: "Murray and Nadel's Textbook of Respiratory Medicine". Tercera Edición, W. B. Saunders Company. 2000. Página 271.

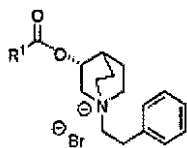
A partir de la grafica resulta evidente que el efecto cronotrópico del compuesto **LAS 35201** es significativamente menor que al observado para los fármacos comúnmente utilizados en el tratamiento de enfermedades respiratorias. Por lo tanto, el compuesto **LAS 35201**, contrario a otros compuestos farmacológicos de su clase, presenta un perfil de efecto cardiaco ventajoso.

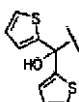
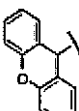
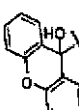
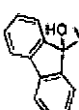
A pesar que el mecanismo exacto responsable de dicho efecto ventajoso todavía no es comprendido en su totalidad, se cree que este se encuentra relacionado con el hecho que el compuesto **LAS 35201** resulta sorprendentemente lábil en el plasma humano. En línea con lo anterior, dicho compuesto se degrada rápidamente una vez se absorbe en el pulmón y se encuentra en la circulación sanguínea. Contrario a otros antagonistas M3 o anticolinérgicos, el compuesto de acuerdo a la invención no presente actividad sistémica significativa. En particular, el compuesto **LAS 35201** no genera taquicardia en la misma medida que otros broncodilatadores agonistas β_2 .

La naturaleza lábil del compuesto **LAS 35201** en el plasma humano resulta evidente, por ejemplo, a partir de los resultados expuestos en la tabla 6 de la página 5085 del artículo "*Discovery of Novel Quaternary Ammonium Derivatives of (3R)-Quinuclidinol Esters as Potent and Long-Acting Muscarinic Antagonists with Potential for Minimal Systemic Exposure after Inhaled Administration: Identification of (3R)-3-[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (Aclidinium Bromide)*"⁴. En dicho cuadro comparativo, **LAS 35201**, correspondiente al compuesto 43, mostró una degradación del 53% después de 60 minutos de encontrarse en el plasma humano. En contraste, los antagonistas de M3 *ipratropio* y *tiotropio*, cuyos resultados se presentan al final de la tabla 6, se consideran estables en el plasma humano.

⁴ PRAT, María; FERNANDEZ, Dolors; BUIL, M. Antonia; CRESPO, María I.; CASALS, Gaspar; FERRER, Manuel; TORT, Laia; CASTRO, Jordi; MONLEÓN, Juan M.; GAVALDÀ, Amadeu; MIRALPEIX, Montserrat; RAMOS, Israel; DOMÉNECH, Teresa; VILELLA, Dolors; ANTÓN, Francisca; HUERTA, Josep M.; ESPINOSA, Sonia; LÓPEZ, Manuel; SENTELLAS, Sonia; GONZÁLEZ, Marisa; ALBERTÍ, Joan; SEGARRA, Victor; CÁRDENAS, Alvaro; BELETA, Jorge; RYDER, Hamish.: "*Discovery of Novel Quaternary Ammonium Derivatives of (3R)-Quinuclidinol Esters as Potent and Long-Acting Muscarinic Antagonists with Potential for Minimal Systemic Exposure after Inhaled Administration: Identification of (3R)-3-[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (Aclidinium Bromide)*". JOURNAL OF MEDICINAL CHEMISTRY. 2009, 52, 5076–5092.

Table 6. Effect of R¹ Substitution on the in Vitro Human Plasma Stability of Compound 54 Analogues



R ¹	compd	binding affinity ^a (IC ₅₀ , nM)			human plasma stability			
		M ₁	M ₂	M ₃	(% disappearance) ^b			t _{1/2} (min) ^c
					5 min	15 min	60 min	
	54	0.20 ^b (0.003)	0.18 ^b (0.06)	0.14 ^b (0.04)	NE ^d	68 (3)	99 (0)	7.9
	43	0.31 (0.02)	1.82 (0.19)	0.44 (0.03)	NE ^d	22 (6)	53 (4)	56.1
	44	0.28 (0.12)	0.54 (0.15)	0.29 (0.10)	NE ^d	7 (11)	15 (3)	> 60
	47	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	NE ^d	35 (4)	90 (1)	17.6
flutropium bromide		0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)	NE ^d	5 (6)	35 (3)	> 60
ipratropium bromide		2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)	NE ^d	< 5	< 5	> 60

^aValues shown are the mean, *n* = 2 (SD). ^bValues shown are the mean, *n* = 3 (SD). ^cHalf-life calculated with at least three experimental time points. ^dNE = not evaluated.

Una vez combinado el compuesto **LAS 35201** con cualquier tipo de agonista β_2 , la reducción benéfica de los efectos secundarios cardiacos se debe únicamente a las propiedades de LAS 35201. En este sentido, los ejemplos muestran que la combinación del compuesto (2) LAS 35201 con un agonista β_2 representativo (salmeterol) se traduce en un ritmo cardiaco máximo más bajo en comparación con aquel observado para la combinación de *tiotropio* y el mismo agonista β_2 . Adicionalmente, se observó que la duración del efecto cronotrópico se redujo significativamente para la combinación farmacológica de acuerdo a la invención, como se muestra en la tabla 1 y figura 4.

En este sentido se desea aclarar que la reducción ventajosa en los efectos secundarios cardiacos se observa cuando el compuesto **LAS 35201** se combina con cualquier agonista

β 2, puesto que la taquicardia es un efecto secundario asociado a la clase entera de fármacos de agonistas de β 2. De manera resumida, el ahorrador cardíaco antagonista de M3 **LAS 35201**, se considera particularmente apropiado para la combinación con cualquier medicamento de la clase de los broncodilatadores que estén asociados con efectos secundarios taquicárdicos.

Por las razones arriba expuestas, corresponde entonces a un resultado sorprendente de la presente invención que la combinación del antagonista específico **LAS 35201** junto con un agonista β 2 puede lograr una broncodilatación efectiva a través de dos tipos de receptores diferentes y sorprendentemente mejorar el perfil de efectos secundarios taquicárdicos.

Es así como las anterioridades citadas no presentan indicio alguno que lleve a una persona versada en la materia a combinar específicamente el compuesto **LAS 35201** junto con cualquier tipo de agonista β 2 para lograr ventajosamente un balance entre las propiedades de ambos compuestos farmacológicos para el tratamiento de enfermedades respiratorias.

En este sentido, el documento D1 a pesar de reclamar varios antagonistas de M3, no hace mención alguna o sugiere dentro de sus especificaciones las propiedades de ahorro cardíaco ventajosas alcanzadas cuando el compuesto **LAS 35201** se combina con un antagonista de β 2. Por lo anterior, el documento D1 no resulta una anterioridad válida capaz de afectar el nivel inventivo de la solicitud al no revelar o sugerir la combinación específica de los compuestos tal como se reivindican y los cuales poseen propiedades para alcanzar una broncodilatación efectiva, disminuyendo la taquicardia producida como efecto secundario de su administración.

Así las cosas, consideramos que la presente invención goza de nivel inventivo a la luz del arte previo.

6. Conclusiones

Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

Así mismo, manifiesto expresamente a ese Despacho que renuncio al término de 30 días solicitado, teniendo en cuenta que con este escrito presento la información y documentación necesaria para continuar con el trámite de la solicitud de patente de la referencia.

7. Anexos

- Copia de las páginas 215 a 232 del libro "Goodman & Gilman's The Pharmacological Basis of Therapeutics". Décima Edición, McGraw-Hill Professional. 2001. Página 231.
- Copia de las páginas 267 a 272 del libro "Murray and Nadel's Textbook of Respiratory Medicine". Tercera Edición, W. B. Saunders Company. 2000.
- Copia del artículo "Discovery of Novel Quaternary Ammonium Derivatives of (3R)-Quinuclidinol Esters as Potent and Long-Acting Muscarinic Antagonists with Potential for Minimal Systemic Exposure after Inhaled Administration: Identification of (3R)-3-[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (Aclidinium Bromide)". JOURNAL OF MEDICINAL CHEMISTRY. 2009, 52, 5076–5092.
- Anexo 2: tabla comparativa de las estructuras de una variedad de agonistas β_2 comunes.

Señor Director,


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CHAPTER 10

CATECHOLAMINES, SYMPATHOMIMETIC DRUGS, AND ADRENERGIC RECEPTOR ANTAGONISTS

Brian B. Hoffman

Catecholamines released by the sympathetic nervous system and adrenal medulla are involved in regulating a host of physiological functions, particularly in integrating responses to a range of stresses that would otherwise threaten homeostatic mechanisms. Norepinephrine is the major neurotransmitter in the peripheral sympathetic nervous system, whereas epinephrine is the primary hormone secreted by the adrenal medulla in mammals. Activation of the sympathetic nervous system occurs in response to diverse stimuli, including physical activity, psychological stress, blood loss, and many other normal or disease-related provocations. Because the functions mediated or modified by the sympathetic nervous system are diverse, drugs that mimic, alter, or antagonize its activity are useful in the treatment of many clinical disorders, including hypertension, cardiovascular shock, arrhythmias, asthma, and anaphylactic reactions. Some of these indications are discussed elsewhere (see Chapters 28, 32, 33, 34, and 35).

The physiological and metabolic responses that follow stimulation of sympathetic nerves in mammals usually are mediated by the neurotransmitter norepinephrine, although cotransmitters such as peptides potentially may contribute to sympathetic effects. As part of the response to stress, the adrenal medulla also is stimulated, resulting in elevation of the concentration of epinephrine in the circulation; epinephrine functions as a hormone, acting at distant sites in the circulation. The actions of these two catecholamines are very similar at some sites but differ significantly at others. For example, both compounds stimulate the myocardium; however, epinephrine dilates blood vessels to skeletal muscle, whereas norepinephrine causes constriction of blood vessels in skin, mucosa, and kidney.

Dopamine is a third naturally occurring catecholamine. Although it is found predominantly in the basal ganglia of the central nervous system (CNS), dopaminergic nerve endings and specific receptors for this catecholamine have been identified elsewhere in the CNS and in the periphery. The role of catecholamines in the CNS is detailed in Chapter 12 and elsewhere. As might be expected, sympathomimetic amines—naturally occurring catecholamines and drugs that mimic their actions—and adrenergic receptor antagonists—drugs that block the effects of sympathetic stimulation—constitute two of the more extensively studied groups of pharmacological agents.

Many of the actions of agonists or antagonists that activate or inhibit adrenergic receptors are understandable in terms of the known physiological effects of catecholamines. Whereas endogenous catecholamines such as epinephrine are sometimes used as drugs, most of the available agonists are structural analogs of epinephrine or norepinephrine. These synthetic compounds have a variety of advantages as therapeutic agents—such as oral bioavailability, prolonged duration of action, and specificity for particular subtypes of adrenergic receptors—which serve to enhance their therapeutic actions and to diminish potential adverse effects. The structure, cellular function, and physiological effects of adrenergic agonists and antagonists are outlined in this chapter.

I. CATECHOLAMINES AND SYMPATHOMIMETIC DRUGS

Most of the actions of catecholamines and sympathomimetic agents can be classified into seven broad types: (1) a peripheral excitatory action on certain types of smooth muscle, such as those in blood vessels supplying skin, kidney, and mucous membranes, and on gland cells, such as those in salivary and sweat glands; (2) a peripheral inhibitory action on certain other types of smooth muscle, such as those in the wall of the gut, in the bronchial tree, and in blood vessels supplying skeletal muscle; (3) a cardiac excitatory action, responsible for an increase in heart rate and force of contraction; (4) metabolic actions, such as an increase in rate of glycogenolysis in liver and muscle and liberation of free fatty acids from adipose tissue; (5) endocrine actions, such as modulation (increasing or decreasing) of the secretion of insulin, renin, and pituitary hormones; (6) central nervous system (CNS) actions, such as respiratory stimulation and, with some of the drugs, an increase in wakefulness and psychomotor activity and a reduction in appetite; and (7) presynaptic actions that result in either inhibition or facilitation of the release of neurotransmitters such as norepinephrine and acetylcholine; physiologically, the inhibitory action is more important than the excitatory action. Many of these actions and the receptors that mediate them are summarized in Tables 6-1 and 6-3. Not all sympathomimetic drugs show each of the above types of action to the same degree. However, many of the differences in their effects are only quantitative, and descriptions of the effects of each compound would be unnecessarily repetitive. Therefore, the pharmacological properties of these drugs as a class are described in detail for the prototypical agent, epinephrine.

Appreciation of the pharmacological properties of the drugs that are described in this chapter is critically dependent on understanding the classification, distribution, and mechanism of action of the various subtypes of adrenergic receptors (α , β) (Figure 10-1). This information is presented in Chapter 6.

History. The pressor effect of adrenal extracts was first shown by Oliver and Schäfer in 1895. The active principle was named *epinephrine* by Abel in 1899 and synthesized independently by Stolz and Dakin (see Hartung, 1931). The development of our knowledge of epinephrine and *norepinephrine* as neurohumoral transmitters is outlined in Chapter 6. Barger and Dale (1910) studied the pharmacological activity of a large series of synthetic amines related to epinephrine and termed their action *sympathomimetic*. This important study determined the basic structural requirements for activity. When it was later found that cocaine or chronic denervation of effector organs reduced the responses to ephedrine and tyramine but enhanced the ef-

fects of epinephrine, it became clear that the differences between sympathomimetic amines were not simply quantitative. It was suggested that epinephrine acted directly on the effector cell, whereas ephedrine and tyramine had an indirect effect by acting on the nerve endings. The discovery that reserpine depletes tissues of norepinephrine (Bertler *et al.*, 1956) was followed by evidence that tyramine and certain other sympathomimetic amines do not act on tissues from animals that have been treated with reserpine; this, too, indicated that they act by releasing endogenous norepinephrine (Burn and Rand, 1958).

Chemistry and Structure-Activity Relationship of Sympathomimetic Amines. β -Phenylethylamine (Table 10-1) can be viewed as the parent compound of the sympathomimetic amines, consisting of a benzene ring and an ethylamine side chain. The structure permits substitutions to be made on the aromatic ring, the α - and β -carbon atoms, and the terminal amino group to yield a variety of compounds with sympathomimetic activity. Norepinephrine, epinephrine, *dopamine*, *isoproterenol*, and a few other agents have hydroxyl groups substituted at positions 3 and 4 of the benzene ring. Since *o*-dihydroxybenzene also is known as catechol, sympathomimetic amines with these hydroxyl substitutions in the aromatic ring are termed *catecholamines*.

Many directly acting sympathomimetic drugs influence both α and β receptors, but the ratio of activities varies among drugs in a continuous spectrum from predominantly α activity (*phenylephrine*) to predominantly β activity (*isoproterenol*). Despite the multiplicity of the sites of action of sympathomimetic amines, several generalizations can be made (see Table 10-1). **Separation of Aromatic Ring and Amino Group.** By far the greatest sympathomimetic activity occurs when two carbon atoms separate the ring from the amino group. This rule applies with few exceptions to all types of action.

Substitution on the Amino Group. The effects of amino substitution are most readily seen in the actions of catecholamines on α and β receptors. Increase in the size of the alkyl substituent increases β -receptor activity (e.g., *isoproterenol*). Norepinephrine has, in general, rather feeble β_2 activity; this activity is greatly increased in epinephrine with the addition of a methyl group. A notable exception is phenylephrine, which has an *N*-methyl substituent but is an α -selective agonist. β_2 -Selective compounds require a large amino substituent, but depend on other substitutions to define selectivity for β_2 rather than for β_1 receptors. In general, the smaller the substitution on the amino group the greater the selectivity for α activity, although *N*-methylation increases the potency of primary amines. Thus, α activity is maximal in epinephrine, less in norepinephrine, and almost absent in isoproterenol.

Substitution on the Aromatic Nucleus. Maximal α and β activity depends on the presence of hydroxyl groups on positions 3 and 4. When one or both of these groups are absent, with no other aromatic substitution, the overall potency is reduced. Phenylephrine is thus less potent than epinephrine at both α and β receptors, with β_2 activity almost completely absent. Studies of the β -adrenergic receptor suggest that the hydroxyl groups on serine residues 204 and 207 probably form hydrogen bonds with the catechol hydroxyl groups at positions 3 and 4, respectively (Strader *et al.*, 1989). It also appears that aspartate 113 is a point of electrostatic interaction with the amine group on

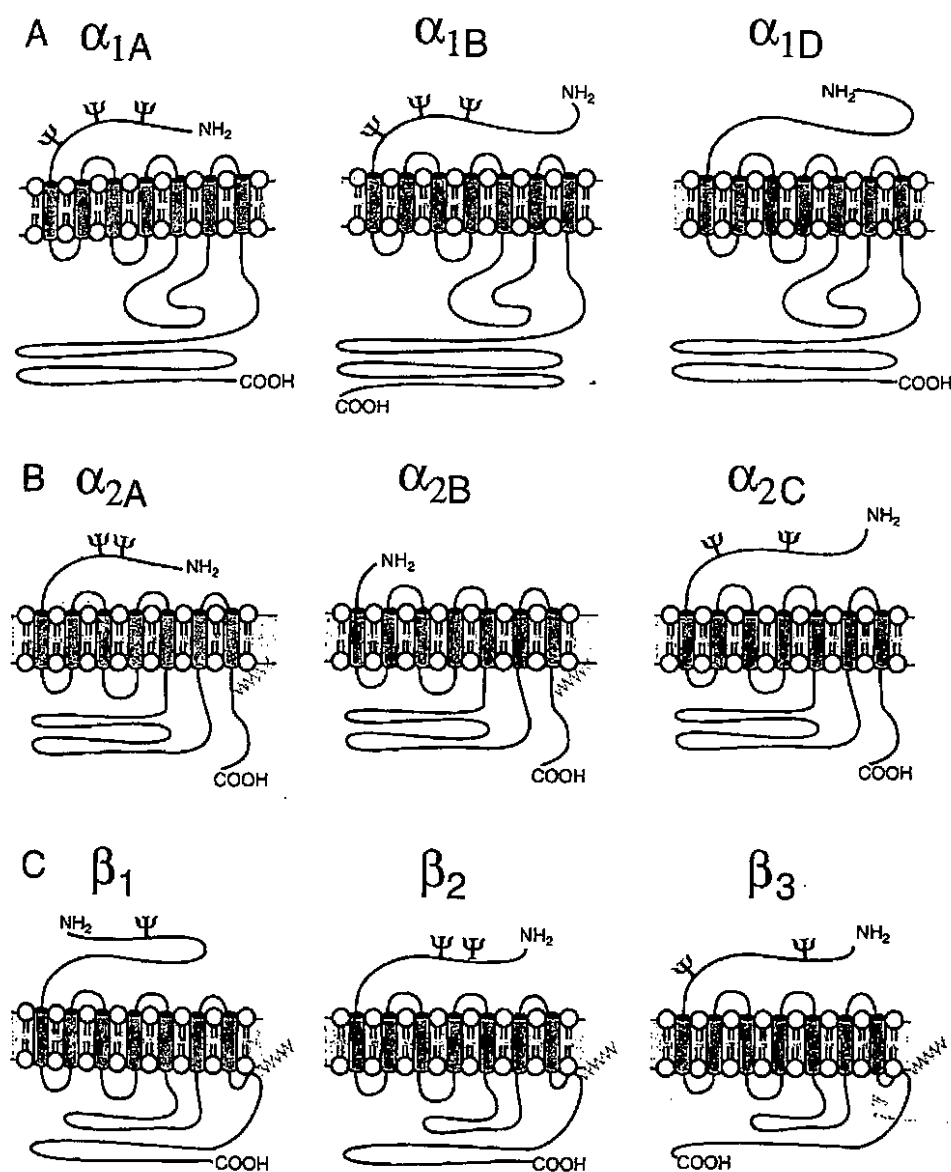


Figure 10-1. Subtypes of adrenergic receptors.

There are three known subtypes of each of the α_1 -, α_2 -, and β -adrenergic receptor populations. All β -adrenergic receptor subtypes are coupled to stimulation of adenylyl cyclase activity; similarly, all α_2 -adrenergic receptor subtypes affect the same effector systems, *i.e.*, inhibition of adenylyl cyclase, activation of receptor-operated K^+ channels, and inhibition of voltage-sensitive Ca^{2+} channels. In contrast, there is evidence that different α_1 -adrenergic receptor subpopulations couple to different effector systems. Ψ indicates a site for *N*-glycosylation; w/w/w indicates a site for thio-acylation.

the ligand. Since the serines are in the fifth membrane-spanning region and the aspartate is in the third (see Chapter 6), it is likely that catecholamines bind parallel to the plane of the membrane, forming a bridge between the two membrane spans. However, models involving dopamine receptors suggest alternative possibilities (Hutchins, 1994).

Hydroxyl groups in positions 3 and 5 confer β_2 -receptor selectivity on compounds with large amino substituents. Thus, metaproterenol, terbutaline, and other similar compounds relax the bronchial musculature in patients with asthma but cause less direct cardiac stimulation than do the nonselective drugs. The response to noncatecholamines is in part determined by their capacity to release norepinephrine from storage sites. These agents thus cause effects that are mostly mediated by α and β_1 receptors, since norepinephrine is a weak β_2 agonist. Phenylethyl-

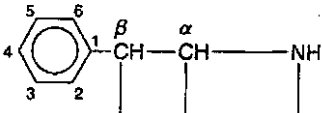
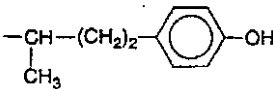
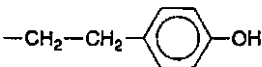
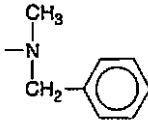
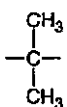
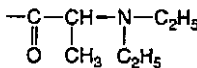
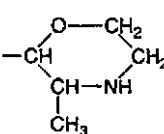
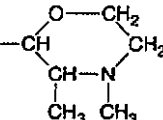
amines that lack hydroxyl groups on the ring and the β -hydroxyl group on the side chain act almost exclusively by causing the release of norepinephrine from adrenergic nerve terminals.

Since substitution of polar groups on the phenylethylamine structure makes the resultant compounds less lipophilic, unsubstituted or alkyl-substituted compounds cross the blood-brain barrier more readily and have more central activity. Thus, *ephedrine*, *amphetamine*, and *methamphetamine* exhibit considerable CNS activity. In addition, as noted above, the absence of polar hydroxyl groups results in a loss of direct sympathomimetic activity.

Catecholamines have only a brief duration of action and are ineffective when administered orally, because they are rapidly inactivated in the intestinal mucosa and in the liver before

Table 10-1

Chemical Structures and Main Clinical Uses of Important Sympathomimetic Drugs†

						MAIN CLINICAL USES		
						α Receptor A N P V	β Receptor B C U	CNS, O
Phenylethylamine			H	H	H			
Epinephrine	3-OH,4-OH		OH	H	CH ₃	A, P, V	B, C	
Norepinephrine	3-OH,4-OH		OH	H	H	P		
Dopamine	3-OH,4-OH		H	H	H	P		
Dobutamine	3-OH,4-OH		H	H	1*		C	
Colterol	3-OH,4-OH		OH	H	C(CH ₃) ₃		B	
Ethylnorepinephrine	3-OH,4-OH		OH	CH ₂ CH ₃	H		B	
Isoproterenol	3-OH,4-OH		OH	H	CH(CH ₃) ₂		B, C	
Isoetharine	3-OH,4-OH		OH	CH ₂ CH ₃	CH(CH ₃) ₂		B	
Metaproterenol	3-OH,5-OH		OH	H	CH(CH ₃) ₂		B	
Terbutaline	3-OH,5-OH		OH	H	C(CH ₃) ₃		B, U	
Metaraminol	3-OH		OH	CH ₃	H	P		
Phenylephrine	3-OH		OH	H	CH ₃	N, P		
Tyramine	4-OH		H	H	H			
Hydroxyamphetamine	4-OH		H	CH ₃	H			
Ritodrine	4-OH		OH	CH ₃	2*		U	
Prenalator	4-OH		OH†	H	-CH(CH ₃) ₂		C	
Methoxamine	2-OCH ₃ ,5-OCH ₃		OH	CH ₃	H	P		
Albuterol	3-CH ₂ OH,4-OH		OH	H	C(CH ₃) ₃		B, U	
Amphetamine			H	CH ₃	H			CNS, O
Methamphetamine			H	CH ₃	CH ₃			CNS, O
Benzphetamine			H	CH ₃	3*			O
Ephedrine			OH	CH ₃	CH ₃	N, P	B, C	
Phenylpropanolamine			OH	CH ₃	H	N		O
Mephentermine			H	4*	CH ₃	N, P		O
Phentermine			H	4*	H			O
Propylhexedrine	5*		H	CH ₃	CH ₃	N		O
Diethylpropion				6*				O
Phenmetrazine				7*				O
Phendimetrazine				8*				O
1			2			3		
								
4			5			6		
								
7			8			9		
								

α Activity

A = Allergic reactions (includes β action)

N = Nasal decongestion

P = Pressor (may include β action)

V = Other local vasoconstriction

(e.g., in local anesthesia)

β Activity

B = Bronchodilator

C = Cardiac

U = Uterus

CNS = Central nervous system

O = Anorectic

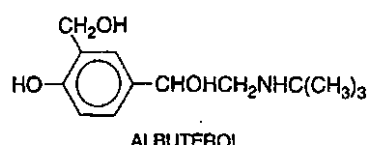
*Numbers bearing an asterisk refer to the substituents numbered in the bottom rows of the table; substituent 3 replaces the N atom, substituent 5 replaces the phenyl ring, and 6, 7, and 8 are attached directly to the phenyl ring, replacing the ethylamine side chain.

†The α and β in the prototypical formula refer to positions of the C atoms in the ethylamine side chain.

‡Prenalator has -OCH₂- between the aromatic ring and the carbon atom designated as β in the prototypical formula.

reaching the systemic circulation (*see* Chapter 6). Compounds without one or both hydroxyl substituents are not acted upon by catechol-*O*-methyltransferase (COMT), and their oral effectiveness and duration of action are enhanced.

Groups other than hydroxyls have been substituted on the aromatic ring. In general, potency at α receptors is reduced and β -receptor activity is minimal; the compounds may even block β receptors. For example, *methoxamine*, with methoxy substituents at positions 2 and 5, has highly selective α -stimulating activity and, in large doses, blocks β receptors. *Albuterol*, a β_2 -selective agonist, has a substituent at position 3 and is an important exception to the general rule of low β -receptor activity. The structure of albuterol is as follows:



Substitution on the α -Carbon Atom. This substitution blocks oxidation by monoamine oxidase (MAO), greatly prolonging the duration of action of noncatecholamines because their degradation depends largely on the action of MAO. The duration of action of drugs such as ephedrine or amphetamine is thus measured in hours rather than in minutes. Similarly, compounds with an α -methyl substituent persist in the nerve terminal and are more likely to release norepinephrine from storage sites. Agents such as *metaraminol* exhibit a greater degree of indirect sympathomimetic activity.

Substitution on the β Carbon. Substitution of a hydroxyl group on the β carbon generally decreases actions within the CNS, largely because of the lower lipid solubility of such compounds. However, such substitution greatly enhances agonist activity at both α and β receptors. Although ephedrine is less potent than methamphetamine as a central stimulant, it is more powerful in dilating bronchioles and increasing blood pressure and heart rate.

Optical Isomerism. Substitution on either α or β carbon yields optical isomers. Levorotatory substitution on the β carbon confers the greater peripheral activity, so that the naturally occurring *l*-epinephrine and *l*-norepinephrine are at least ten times as potent as their unnatural *d*-isomers. Dextrorotatory substitution on the α carbon generally results in a more potent compound. *d*-Amphetamine is more potent than *l*-amphetamine in central but not peripheral activity.

Physiological Basis of Adrenergic Receptor Function.

An important factor in the response of any cell or organ to sympathomimetic amines is the density and proportion of α - and β -adrenergic receptors. For example, norepinephrine has relatively little capacity to increase bronchial airflow, since the receptors in bronchial smooth muscle are largely of the β_2 subtype. In contrast, isoproterenol and epinephrine are potent bronchodilators. Cutaneous blood vessels physiologically express almost exclusively α receptors; thus, norepinephrine and epinephrine cause constriction of such vessels, whereas isoproterenol

has little effect. The smooth muscle of blood vessels that supply skeletal muscles has both β_2 and α receptors; activation of β_2 receptors causes vasodilation, and stimulation of α receptors constricts these vessels. In such vessels, the threshold concentration for activation of β_2 receptors by epinephrine is lower than that for α receptors, but when both types of receptors are activated at high concentrations of epinephrine, the response to α receptors predominates. Physiological concentrations of epinephrine cause primarily vasodilation.

The ultimate response of a target organ to sympathomimetic amines is dictated not only by the direct effects of the agents but also by the reflex homeostatic adjustments of the organism. One of the most striking effects of many sympathomimetic amines is a rise in arterial blood pressure caused by stimulation of vascular α receptors. This stimulation elicits compensatory reflexes which are mediated by the carotid aortic baroreceptor system. As a result, sympathetic tone is diminished and vagal tone is enhanced; each of these responses leads to slowing of the heart rate. This reflex effect is of special importance for drugs that have little capacity to activate β -adrenergic receptors directly. With diseases such as atherosclerosis, which may impair baroreceptor mechanisms, the effects of sympathomimetic drugs may be magnified (Chapleau *et al.*, 1995).

Indirectly Acting Sympathomimetic Drugs. For many years, it was presumed that sympathomimetic amines produced their effects by acting directly on adrenergic receptors. However, this notion was dispelled by the finding that the effects of tyramine and many other noncatecholamines were reduced or abolished after chronic postganglionic denervation or treatment with cocaine or reserpine. Under these circumstances, the effects of exogenously administered epinephrine, and especially norepinephrine, often were enhanced. These observations led to the proposal that tyramine and related amines act indirectly, following uptake into the adrenergic nerve terminal, by displacing norepinephrine from storage sites in the synaptic vesicles or from extravesicular binding sites. Norepinephrine could then exit from the adrenergic nerve terminal and interact with receptors to produce sympathomimetic effects. The depletion of tissue stores of catecholamines that follows treatment with reserpine or degeneration of adrenergic nerve terminals would explain the lack of effect of tyramine under these conditions. In the presence of cocaine, the high-affinity neuronal transport system for catecholamines and certain congeners is inhibited, and tyramine and related amines are unable to enter the adrenergic nerve terminal. In this manner, cocaine inhibits the actions

of indirectly acting sympathomimetic amines while potentiating the effects of directly acting agents that are normally removed from the synaptic cleft by this transport system (see Chapter 6).

In assessing the proportion of direct and indirect actions of a sympathomimetic amine, the most common experimental procedure is to compare the dose-response curves for the agent on a particular target tissue before and after treatment with reserpine (Trendelenburg, 1972). Those drugs whose actions are essentially unaltered after treatment with reserpine are classified as *directly acting* sympathomimetic amines (e.g., norepinephrine, phenylephrine), whereas those whose actions are abolished are termed *indirectly acting* (e.g., tyramine). Many agents exhibit some degree of residual sympathomimetic activity after the administration of reserpine, but higher doses of these amines are required to produce comparable effects. These are classified as mixed-acting sympathomimetic amines; that is, they have both direct and indirect actions. The proportion of direct and indirect actions can vary considerably among different tissues and species. In some cases, relatively little is known about these properties in human beings.

Since the actions of norepinephrine are more marked on α and β_1 receptors than on β_2 receptors, many noncatecholamines that release norepinephrine have predominantly α -receptor-mediated and cardiac effects. However, certain noncatecholamines with both direct and indirect effects on adrenergic receptors show significant β_2 activity and are used clinically for these effects. Thus, ephedrine, although dependent on release of norepinephrine for some of its effects, relieves bronchospasm by its action on β_2 receptors in bronchial muscle, an effect not seen with norepinephrine. It also must be recalled that some noncatecholamines—phenylephrine, for example—act primarily and directly on effector cells. It is therefore impossible to predict precisely the characteristic effects of noncatecholamines simply on the basis that they may provoke the release of at least some norepinephrine.

False-Transmitter Concept. As indicated above, indirectly acting amines are taken up into adrenergic nerve terminals and storage vesicles, where they replace norepinephrine in the storage complex. Phenylethylamines that lack a β -hydroxyl group are retained there poorly, but β -hydroxylated phenylethylamines and compounds that subsequently become hydroxylated in the synaptic vesicle by dopamine β -hydroxylase are retained in the synaptic vesicle for relatively long periods of time. Such substances can produce a persistent diminution in the content of norepinephrine at functionally critical sites. When the nerve is stimulated, the contents of a relatively constant number of synaptic vesicles are apparently released by exocytosis. If these vesicles contain phenylethylamines that are much less potent

than norepinephrine, activation of postsynaptic adrenergic receptors will be diminished.

This hypothesis, known as the *false-transmitter concept*, is a possible explanation for some of the effects of inhibitors of MAO. Phenylethylamines normally are synthesized in the gastrointestinal tract as a result of the action of bacterial tyrosine decarboxylase. The tyramine formed in this fashion usually is oxidatively deaminated in the gastrointestinal tract and the liver, and the amine does not reach the systemic circulation in significant concentrations. However, when a MAO inhibitor is administered, tyramine may be absorbed systemically and is transported into adrenergic nerve terminals, where its catabolism is again prevented because of the inhibition of MAO at this site; it is then β -hydroxylated to octopamine and stored in the vesicles in this form. As a consequence, norepinephrine gradually is displaced, and stimulation of the nerve terminal results in the release of a relatively small amount of norepinephrine along with a fraction of octopamine. The latter amine has relatively little ability to activate either α - or β -adrenergic receptors. Thus, a functional impairment of sympathetic transmission parallels long-term administration of MAO inhibitors.

Despite such functional impairment, patients who have received MAO inhibitors may experience severe hypertensive crises if they ingest cheese, beer, or red wine. These and related foods, which are produced by fermentation, contain a large quantity of tyramine and, to a lesser degree, other phenylethylamines. When gastrointestinal and hepatic MAO are inhibited, the large quantity of tyramine that is ingested is absorbed rapidly and reaches the systemic circulation in high concentration. A massive and precipitous release of norepinephrine can result, with consequent hypertension that can be severe enough to cause myocardial infarction or a stroke. The properties of various MAO inhibitors (reversible or irreversible; selective or nonselective at MAO-A and MAO-B) are discussed in Chapter 19.

ENDOGENOUS CATECHOLAMINES

Epinephrine

Epinephrine (adrenaline) is a potent stimulant of both α - and β -adrenergic receptors, and its effects on target organs are thus complex. Most of the responses listed in Table 6-1 are seen after injection of epinephrine, although the occurrence of sweating, piloerection, and mydriasis depends on the physiological state of the subject. Particularly prominent are the actions on the heart and on vascular and other smooth muscle.

Blood Pressure. Epinephrine is one of the most potent vasopressor drugs known. If a pharmacological dose is given rapidly by an intravenous route, it evokes a characteristic effect on blood pressure, which rises rapidly to a peak that is proportional to the dose. The increase in systolic pressure is greater than the increase in diastolic

pressure, so that the pulse pressure increases. As the response wanes, the mean pressure may fall below normal before returning to control levels.

The mechanism of the rise in blood pressure due to epinephrine is threefold: (1) a direct myocardial stimulation that increases the strength of ventricular contraction (positive inotropic action); (2) an increased heart rate (positive chronotropic action); and (3) vasoconstriction in many vascular beds—especially in the precapillary resistance vessels of skin, mucosa, and kidney—along with marked constriction of the veins. The pulse rate, at first accelerated, may be slowed markedly at the height of the rise of blood pressure by compensatory vagal discharge. Small doses of epinephrine ($0.1 \mu\text{g/kg}$) may cause the blood pressure to fall. The depressor effect of small doses and the biphasic response to larger doses are due to greater sensitivity to epinephrine of vasodilator β_2 receptors than of constrictor α receptors.

The effects are somewhat different when the drug is given by slow intravenous infusion or by subcutaneous injection. Absorption of epinephrine after subcutaneous injection is slow due to local vasoconstrictor action; the effects of doses as large as 0.5 to 1.5 mg can be duplicated by intravenous infusion at a rate of 10 to 30 μg per minute. There is a moderate increase in systolic pressure due to increased cardiac contractile force and a rise in cardiac output (Figure 10-2). Peripheral resistance decreases, owing to a dominant action on β_2 receptors of vessels in skeletal muscle, where blood flow is enhanced; as a consequence, diastolic pressure usually falls. Since the mean blood pressure is not, as a rule, greatly elevated, compensatory baroreceptor reflexes do not appreciably antagonize the direct cardiac actions. Heart rate, cardiac output, stroke

volume, and left ventricular work per beat are increased as a result of direct cardiac stimulation and increased venous return to the heart, which is reflected by an increase in right atrial pressure. At slightly higher rates of infusion, there may be no change or a slight rise in peripheral resistance and diastolic pressure, depending on the dose and the resultant ratio of α to β responses in the various vascular beds; compensatory reflexes also may come into play. The details of the effects of intravenous infusion of epinephrine, norepinephrine, and isoproterenol in human beings are compared in Table 10-2 and Figure 10-2.

Vascular Effects. The chief vascular action of epinephrine is exerted on the smaller arterioles and precapillary sphincters, although veins and large arteries also respond to the drug. Various vascular beds react differently, which results in a substantial redistribution of blood flow.

Injected epinephrine markedly decreases cutaneous blood flow, constricting precapillary vessels and small venules. Cutaneous vasoconstriction accounts for a marked decrease in blood flow in the hands and feet. The "after-congestion" of mucosae following the vasoconstriction from locally applied epinephrine probably is due to changes in vascular reactivity as a result of tissue hypoxia rather than to β -receptor activity of the drug on mucosal vessels.

Blood flow to skeletal muscles is increased by therapeutic doses in human beings. This is due in part to a powerful β_2 -receptor vasodilator action that is only partially counterbalanced by a vasoconstrictor action on the α receptors that also are present in the vascular bed. If an α -adrenergic receptor antagonist is given, the vasodilation in muscle is more pronounced, the total peripheral resistance is decreased, and the mean blood pressure falls

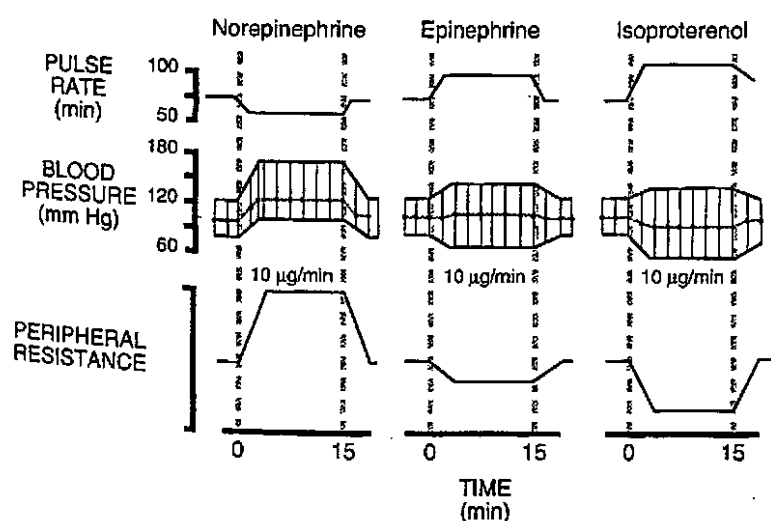


Figure 10-2. Effects of intravenous infusion of norepinephrine, epinephrine, or isoproterenol in human beings. (Modified from Allwood et al., 1963, with permission.)

Table 10-2
Comparison of the Effects of Intravenous Infusion of
Epinephrine and Norepinephrine in Human Beings*

EFFECT	EPINEPH- RINE	NOREPINEPH- RINE
Cardiac		
Heart rate	+	-†
Stroke volume	++	++
Cardiac output	+++	0,-
Arrhythmias	++++	++++
Coronary blood flow	+++	++
Blood pressure		
Systolic arterial	+++	+++
Mean arterial	+	++
Diastolic arterial	+,0,-	++
Mean pulmonary	++	++
Peripheral circulation		
Total peripheral resistance	-	++
Cerebral blood flow	+	0,-
Muscle blood flow	+++	0,-
Cutaneous blood flow	--	--
Renal blood flow	-	-
Splanchnic blood flow	+++	0,+
Metabolic effects		
Oxygen consumption	++	0,+
Blood glucose	+++	0,+
Blood lactic acid	+++	0,+
Eosinopenic response	+	0
Central nervous system		
Respiration	+	+
Subjective sensations	+	+

*0.1 to 0.4 $\mu\text{g/kg}$ per minute.

Abbreviations: + = increase; 0 = no change; - = decrease; † = after atropine, +

SOURCE: After Goldenberg *et al.*, 1950. Courtesy of *Archives of Internal Medicine*.

(epinephrine reversal). After the administration of a non-selective β -adrenergic receptor antagonist, only vasoconstriction occurs, and the administration of epinephrine is associated with a considerable pressor effect.

The effect of epinephrine on cerebral circulation is related to systemic blood pressure. In usual therapeutic doses, the drug has relatively little constrictor action on cerebral arterioles. It is physiologically advantageous that the cerebral circulation does not constrict in response to activation of the sympathetic nervous system by stressful stimuli. Indeed, autoregulatory mechanisms tend to limit the increase in cerebral blood flow caused by increased blood pressure.

Doses of epinephrine that have little effect on mean arterial pressure consistently increase renal vascular resistance and reduce renal blood flow by as much as 40%. All segments of the renal vascular bed contribute to the increased resistance. Since the glomerular filtration rate is only slightly and variably altered, the filtration fraction is consistently increased. Excretion of Na^+ , K^+ , and Cl^- is decreased; urine volume may be increased, decreased, or unchanged. Maximal tubular reabsorptive and excretory capacities are unchanged. The secretion of renin is increased as a consequence of a direct action of epinephrine on β_1 receptors in the juxtaglomerular apparatus.

Arterial and venous pulmonary pressures are raised. Although direct pulmonary vasoconstriction occurs, redistribution of blood from the systemic to the pulmonary circulation, due to constriction of the more powerful musculature in the systemic great veins, doubtless plays an important part in the increase in pulmonary pressure. Very high concentrations of epinephrine may cause pulmonary edema precipitated by elevated pulmonary capillary filtration pressure and possibly by "leaky" capillaries.

Coronary blood flow is enhanced by epinephrine or by cardiac sympathetic stimulation under physiological conditions. The increased flow occurs even with doses that do not increase the aortic blood pressure and is the result of two factors. The first is the increased relative duration of diastole at higher heart rates (*see below*); this is partially offset by decreased blood flow during systole because of more forceful contraction of the surrounding myocardium and an increase in mechanical compression of the coronary vessels. The increased flow during diastole is further enhanced if aortic blood pressure is elevated by epinephrine, and, as a consequence, total coronary flow may be increased. The second factor is a metabolic dilator effect that results from the increased strength of contraction and myocardial oxygen consumption due to the direct effects of epinephrine on cardiac myocytes. This vasodilation is mediated in part by adenosine released from cardiac myocytes, which tends to override a direct vasoconstrictor effect of epinephrine that results from activation of α receptors in coronary vessels.

Cardiac Effects. Epinephrine is a powerful cardiac stimulant. It acts directly on the predominant β_1 receptors of the myocardium and of the cells of the pacemaker and conducting tissues; β_2 and α receptors also are present in the heart, although there are considerable species differences. Considerable recent interest has focused on the role of β_1 and β_2 receptors in the human heart, especially in heart failure. The heart rate increases, and the rhythm often is altered. Cardiac systole is shorter and more powerful, cardiac output is enhanced, and the work of the heart and its oxygen consumption are markedly increased. Cardiac efficiency (work done relative to oxygen consumption) is lessened. Direct responses to epinephrine include increases in contractile force, accelerated rate of rise of isometric tension, enhanced rate of relaxation, decreased time to peak tension, increased excitability, acceleration of the rate of spontaneous beating, and induction of automaticity in specialized regions of the heart.

In accelerating the heart, epinephrine preferentially shortens systole so that the duration of diastole usually is not reduced. Indeed, activation of β receptors increases the rate of relaxation of ventricular muscle. Epinephrine speeds the heart by accelerating the slow depolarization of sinoatrial (SA) nodal cells that takes place during diastole, i.e., during phase 4 of the action potential (see Chapter 35). Consequently, the transmembrane potential of the pacemaker cells rises more rapidly to the threshold level at which the action potential is initiated. The amplitude of the action potential and the maximal rate of depolarization (phase 0) also are increased. A shift in the location of the pacemaker within the SA node often occurs, owing to activation of latent pacemaker cells. In Purkinje fibers, epinephrine also accelerates diastolic depolarization and may cause activation of latent pacemaker cells. These changes do not occur in atrial and ventricular muscle fibers, where epinephrine has little effect on the stable, phase 4 membrane potential after repolarization. If large doses of epinephrine are given, premature ventricular systoles occur and may herald more serious ventricular arrhythmias. This rarely is seen with conventional doses in human beings, but ventricular extrasystoles, tachycardia, or even fibrillation may be precipitated by release of endogenous epinephrine when the heart has been sensitized to this action of epinephrine by certain anesthetics or in cases of myocardial infarction. The mechanism of induction of these cardiac arrhythmias is not clear.

Some effects of epinephrine on cardiac tissues are largely secondary to the increase in heart rate, and are small or inconsistent when the heart rate is kept constant. For example, the effect of epinephrine on repolarization of atrial muscle, Purkinje fibers, or ventricular muscle is small if the heart rate is unchanged. When the heart rate is increased, the duration of the action potential is consistently shortened, and the refractory period is correspondingly decreased.

Conduction through the Purkinje system depends on the level of membrane potential at the time of excitation. Excessive reduction of this potential results in conduction disturbances, ranging from slowed conduction to complete block. Epinephrine often increases the membrane potential and improves conduction in Purkinje fibers that have been excessively depolarized.

Epinephrine normally shortens the refractory period of the human atrioventricular (AV) node by direct effects on the heart, although doses of epinephrine that slow the heart through reflex vagal discharge may indirectly tend to prolong it. Epinephrine also decreases the grade of AV block that occurs as a result of disease, drugs, or vagal stimulation. Supraventricular arrhythmias are apt to occur from the combination of epinephrine and cholinergic stimulation. Depression of sinus rate and AV conduction by vagal discharge probably plays a part in epinephrine-induced ventricular arrhythmias, since various drugs that block the vagal effect confer some protection. The action of epinephrine in enhancing cardiac automaticity and its action in causing arrhythmias are effectively antagonized by β -adrenergic receptor antagonists such as propranolol. However, α_1 receptors exist in most regions of the heart, and their activation prolongs the refractory period and strengthens myocardial contractions.

Cardiac arrhythmias have been seen in patients after inadvertent intravenous administration of conventional subcutaneous doses of epinephrine. Ventricular premature systoles can appear, which may be followed by multifocal ventricular tachycardia or ventricular fibrillation. Pulmonary edema also may occur.

Epinephrine decreases the amplitude of the T wave of the electrocardiogram (ECG) in normal persons. In animals given relatively larger doses, additional effects are seen on the T wave and the ST segment. After decreasing in amplitude, the T wave may become biphasic, and the ST segment can deviate either above or below the isoelectric line. Such ST-segment changes are similar to those seen in patients with angina pectoris during spontaneous or epinephrine-induced attacks of pain. These electrical changes therefore have been attributed to myocardial ischemia. Also, epinephrine as well as other catecholamines may cause myocardial cell death, particularly after intravenous infusions. Acute toxicity is associated with contraction band necrosis and other pathological changes. Recent interest has focused on the possibility that prolonged sympathetic stimulation of the heart, such as in congestive cardiomyopathy, may promote apoptosis of cardiomyocytes.

Effects on Smooth Muscles. The effects of epinephrine on the smooth muscles of different organs and systems depend on the type of adrenergic receptor in the muscle (see Table 6-1). The effects on vascular smooth muscle are of major physiological importance, whereas those on gastrointestinal smooth muscle are relatively minor. Gastrointestinal smooth muscle is, in general, relaxed by epinephrine. This effect is due to activation of both α - and β -adrenergic receptors. Intestinal tone and the frequency and amplitude of spontaneous contractions are reduced. The stomach usually is relaxed and the pyloric and ileocecal sphincters are contracted, but these effects depend on the preexisting tone of the muscle. If tone already is high, epinephrine causes relaxation; if low, contraction.

The responses of uterine muscle to epinephrine vary with species, phase of the sexual cycle, state of gestation, and dose given. Epinephrine contracts strips of pregnant or nonpregnant human uterus *in vitro* by interaction with α receptors. The effects of epinephrine on the human uterus *in situ*, however, differ. During the last month of pregnancy and at parturition, epinephrine inhibits uterine tone and contractions. β_2 -Selective agonists, such as ritodrine or terbutaline, have been used to delay premature labor, although their efficacy is limited. Effects of these and other drugs on the uterus are discussed later in this chapter.

Epinephrine relaxes the detrusor muscle of the bladder as a result of activation of β receptors and contracts the trigone and sphincter muscles owing to its α -agonist activity. This can result in hesitancy in urination and may contribute to retention of urine in the bladder. Activation of smooth muscle contraction in the prostate promotes urinary retention.

Respiratory Effects. Epinephrine affects respiration primarily by relaxing bronchial muscle. It has a powerful bronchodilator action, most evident when bronchial muscle

is contracted because of disease, as in bronchial asthma, or in response to drugs or various autacoids. In such situations, epinephrine has a striking therapeutic effect as a physiological antagonist to substances that cause bronchoconstriction.

The beneficial effects of epinephrine in asthma also may arise from inhibition of antigen-induced release of inflammatory mediators from mast cells, and to a lesser extent from diminution of bronchial secretions and congestion within the mucosa. Inhibition of mast cell secretion is mediated by β_2 -adrenergic receptors, while the effects on the mucosa are mediated by α receptors; however, other drugs, such as glucocorticoids and leukotriene antagonists, have much more profound antiinflammatory effects in asthma (*see Chapter 28*).

Effects on the Central Nervous System. Because of the inability of this rather polar compound to enter the CNS, epinephrine in conventional therapeutic doses is not a powerful CNS stimulant. While the drug may cause restlessness, apprehension, headache, and tremor in many persons, these effects in part may be secondary to the effects of epinephrine on the cardiovascular system, skeletal muscles, and intermediary metabolism; that is, they may be the result of somatic manifestations of anxiety. Some other sympathomimetic drugs more readily cross the blood-brain barrier.

Metabolic Effects. Epinephrine has a number of important influences on metabolic processes. It elevates the concentrations of glucose and lactate in blood by mechanisms described in Chapter 6. Insulin secretion is inhibited through an interaction with α_2 receptors and is enhanced by activation of β_2 receptors; the predominant effect seen with epinephrine is inhibition. Glucagon secretion is enhanced by an action on the β receptors of the α cells of pancreatic islets. Epinephrine also decreases the uptake of glucose by peripheral tissues, at least in part because of its effects on the secretion of insulin, but also possibly due to direct effects on skeletal muscle. Glycosuria rarely occurs. The effect of epinephrine to stimulate glycogenolysis in most tissues and in most species involves β receptors (*see Chapter 6*).

Epinephrine raises the concentration of free fatty acids in blood by stimulating β receptors in adipocytes. The result is activation of triglyceride lipase, which accelerates the breakdown of triglycerides to form free fatty acids and glycerol. The calorogenic action of epinephrine (increase in metabolism) is reflected in human beings by an increase of 20% to 30% in oxygen consumption after conventional doses. This effect is mainly due to enhanced breakdown of triglycerides in brown adipose tissue, providing an increase in oxidizable substrate (*see Chapter 6*).

Miscellaneous Effects. Epinephrine reduces circulating plasma volume by loss of protein-free fluid to the extracellular space,

thereby increasing erythrocyte and plasma protein concentrations. However, conventional doses of epinephrine do not significantly alter plasma volume or packed red-cell volume under normal conditions, although such doses are reported to have variable effects in the presence of shock, hemorrhage, hypotension, and anesthesia. Epinephrine rapidly increases the number of circulating polymorphonuclear leukocytes, likely due to β -receptor-mediated demargination of these cells. Epinephrine accelerates blood coagulation in laboratory animals and human beings and promotes fibrinolysis.

The effects of epinephrine on secretory glands are not marked; in most glands secretion usually is inhibited, partly owing to the reduced blood flow caused by vasoconstriction. Epinephrine stimulates lacrimation and a scanty mucous secretion from salivary glands. Sweating and pilomotor activity are minimal after systemic administration of epinephrine, but occur after intradermal injection of very dilute solutions of either epinephrine or norepinephrine. Such effects are inhibited by α -receptor antagonists.

Mydriasis is readily seen during physiological sympathetic stimulation but not when epinephrine is instilled into the conjunctival sac of normal eyes. However, epinephrine usually lowers intraocular pressure from normal levels and in wide-angle glaucoma; the mechanism of this effect is not clear, but both reduced production of aqueous humor due to vasoconstriction and enhanced outflow probably occur (*see Chapter 66*).

Although epinephrine does not directly excite skeletal muscle, it facilitates neuromuscular transmission, particularly that following prolonged rapid stimulation of motor nerves. In apparent contrast to the effects of α -receptor activation at presynaptic nerve terminals in the autonomic nervous system (α_2 receptors), stimulation of α receptors causes a more rapid increase in transmitter release from the somatic motor neuron, perhaps as a result of enhanced influx of Ca^{2+} . These responses likely are mediated by α_1 receptors. These actions may explain in part the ability of epinephrine (given intraarterially) to cause a brief increase in motor power of the injected limb of patients with myasthenia gravis. Epinephrine also acts directly on white, fast-contracting muscle fibers to prolong the active state, thereby increasing peak tension. Of greater physiological and clinical importance is the capacity of epinephrine and selective β_2 -agonists to increase physiological tremor, at least in part due to β -receptor-mediated enhancement of discharge of muscle spindles.

Epinephrine promotes a fall in plasma K^+ largely due to stimulation of K^+ uptake into cells, particularly skeletal muscle, due to activation of β_2 receptors. This is associated with decreased renal K^+ excretion. These receptors have been exploited in the management of hyperkalemic familial periodic paralysis, which is characterized by episodic flaccid paralysis, hyperkalemia, and depolarization of skeletal muscle. The β_2 -selective agonist albuterol apparently is able to ameliorate the impairment in the ability of the muscle to accumulate and retain K^+ .

Large or repeated doses of epinephrine or other sympathomimetic amines given to experimental animals lead to damage to arterial walls and myocardium, which is so severe as to cause the appearance of necrotic areas in the heart indistinguishable from myocardial infarcts. The mechanism of this injury is not yet clear, but α - and β -receptor antagonists and Ca^{2+} channel blockers may afford substantial protection against the damage.

Similar lesions occur in many patients with pheochromocytoma or after prolonged infusions of norepinephrine.

Absorption, Fate, and Excretion. As indicated above, epinephrine is not effective after oral administration, because it is rapidly conjugated and oxidized in the gastrointestinal mucosa and liver. Absorption from subcutaneous tissues occurs relatively slowly because of local vasoconstriction and the rate may be further decreased by systemic hypotension, for example in a patient with shock. Absorption is more rapid after intramuscular injection. In emergencies, it may be necessary in some cases to administer epinephrine intravenously. When relatively concentrated solutions (1%) are nebulized and inhaled, the actions of the drug largely are restricted to the respiratory tract; however, systemic reactions such as arrhythmias may occur, particularly if larger amounts are used.

Epinephrine is rapidly inactivated in the body. The liver, which is rich in both of the enzymes responsible for destroying circulating epinephrine (COMT and MAO), is particularly important in this regard (see Figure 6-5). Although only small amounts appear in the urine of normal persons, the urine of patients with pheochromocytoma may contain relatively large amounts of epinephrine, norepinephrine, and their metabolites.

Epinephrine is available in a variety of formulations geared for different clinical indications and routes of administration, such as by injection (usually subcutaneously but sometimes intravenously), by inhalation, or topically. Several practical points are worth noting. First, epinephrine is unstable in alkaline solution; when exposed to air or light, it turns pink from oxidation to adrenochrome and then brown from formation of polymers. *Epinephrine injection* is available in 1:1,000, 1:10,000, and 1:100,000 solutions. The usual adult dose given subcutaneously ranges from 0.3 mg to 0.5 mg. The intravenous route is used cautiously if an immediate and reliable effect is mandatory. If the solution is given by vein, it must be adequately diluted and injected very slowly. The dose is seldom as much as 0.25 mg, except for cardiac arrest, when larger doses may be required. Epinephrine suspensions (e.g., SUS-PHRINE) are used to slow subcutaneous absorption and must never be injected intravenously. *Also, a 1% (1:100) formulation is available for administration via inhalation; every precaution must be taken not to confuse this 1:100 solution with the 1:1000 solution designed for parenteral administration, because inadvertent injection of the 1:100 solution can be fatal.*

Toxicity, Adverse Effects, and Contraindications. Epinephrine may cause disturbing reactions, such as rest-

lessness, throbbing headache, tremor, and palpitations. The effects rapidly subside with rest, quiet, recumbency, and reassurance.

More serious reactions include cerebral hemorrhage and cardiac arrhythmias. The use of large doses or the accidental, rapid intravenous injection of epinephrine may result in cerebral hemorrhage from the sharp rise in blood pressure. Ventricular arrhythmias may follow the administration of epinephrine. Angina may be induced by epinephrine in patients with coronary artery disease.

The use of epinephrine generally is contraindicated in patients who are receiving nonselective β -adrenergic receptor blocking drugs, since its unopposed actions on vascular α_1 -adrenergic receptors may lead to severe hypertension and cerebral hemorrhage.

Therapeutic Uses. Epinephrine has limited clinical uses. In general, these are based on the actions of the drug on blood vessels, heart, and bronchial muscle. In the past, the most common use of epinephrine was to relieve respiratory distress due to bronchospasm; however, β_2 -selective agonists now are preferred. A major use is to provide rapid relief of hypersensitivity reactions, including anaphylaxis, to drugs and other allergens. Also, epinephrine may be used to prolong the action of local anesthetics, presumably by decreasing local blood flow. Its cardiac effects may be of use in restoring cardiac rhythm in patients with cardiac arrest due to various causes. It also is used as a topical hemostatic agent on bleeding surfaces such as in the mouth or in bleeding peptic ulcers during endoscopy of the stomach/duodenum. Systemic absorption of the drug can occur with dental application. In addition, inhalation of epinephrine may be useful in the treatment of postintubation and infectious croup. The therapeutic uses of epinephrine are discussed later in this chapter, in relation to other sympathomimetic drugs.

Norepinephrine

Norepinephrine (levarterenol, *l*-noradrenaline, *l*- β -[3,4-dihydroxyphenyl]- α -aminoethanol) is the major chemical mediator liberated by mammalian postganglionic adrenergic nerves. It differs from epinephrine only by lacking the methyl substitution in the amino group (see Table 10-1). Norepinephrine constitutes 10% to 20% of the catecholamine content of human adrenal medulla and as much as 97% in some pheochromocytomas, which may not express the enzyme phenylethanolamine-*N*-methyltransferase. The history of its discovery and its role as a neurohumoral mediator are discussed in Chapter 6.

Pharmacological Properties. The pharmacological actions of norepinephrine and epinephrine have been extensively compared *in vivo* and *in vitro* (see Table 10-2). Both drugs are direct agonists on effector cells, and their actions differ mainly in the ratio of their effectiveness in stimulating α and β_2 receptors. They are approximately equipotent in stimulating β_1 receptors. Norepinephrine is a potent agonist at α receptors and has relatively little action on β_2 receptors; however, it is somewhat less potent than epinephrine on the α receptors of most organs.

Cardiovascular Effects. The cardiovascular effects of an intravenous infusion of 10 μ g of norepinephrine per minute in human beings are shown in Figure 10-2. Systolic and diastolic pressures and, usually, pulse pressure are increased. Cardiac output is unchanged or decreased, and total peripheral resistance is raised. Compensatory vagal reflex activity slows the heart, overcoming a direct cardioaccelerator action, and stroke volume is increased. The peripheral vascular resistance increases in most vascular beds, and blood flow is reduced to the kidneys. Norepinephrine constricts mesenteric vessels and reduces splanchnic and hepatic blood flow. Coronary flow usually is increased, probably owing both to indirectly induced coronary dilation, as with epinephrine, and to elevated blood pressure. However, patients with Prinzmetal's variant angina may be supersensitive to the α -adrenergic vasoconstrictor effects of norepinephrine (see Chapter 32).

Unlike epinephrine, small doses of norepinephrine do not cause vasodilation or lower blood pressure, since the blood vessels of skeletal muscle constrict rather than dilate; α -adrenergic receptor blocking agents therefore abolish the pressor effects but do not cause significant reversal, *i.e.*, hypotension.

Other Effects. Other responses to norepinephrine are not prominent in human beings. The drug causes hyperglycemia and other metabolic effects similar to those produced by epinephrine, but these are observed only when large doses are given; that is, norepinephrine is not as effective a "hormone" as epinephrine. Intradermal injection of suitable doses causes sweating that is not blocked by atropine.

Absorption, Fate, and Excretion. Norepinephrine, like epinephrine, is ineffective when given orally and is absorbed poorly from sites of subcutaneous injection. It is rapidly inactivated in the body by the same enzymes that methylate and oxidatively deaminate epinephrine (see above). Small amounts normally are found in the urine. The excretion rate may be greatly increased in patients with pheochromocytoma.

Toxicity, Adverse Effects, and Precautions. The untoward effects of norepinephrine are similar to those of epinephrine,

although there is typically greater elevation of blood pressure with norepinephrine. Excessive doses can cause severe hypertension, so careful blood pressure monitoring generally is indicated during systemic administration of this agent.

Care must be taken that necrosis and sloughing do not occur at the site of intravenous injection owing to extravasation of the drug. The infusion should be made high in the limb, preferably through a long plastic cannula extending centrally. Impaired circulation at injection sites, with or without extravasation of norepinephrine, may be relieved by infiltrating the area with phentolamine, an α -receptor antagonist. Blood pressure must be determined frequently during the infusion and particularly during adjustment of the rate of the infusion. Reduced blood flow to organs such as kidney and intestines is a constant danger with the use of norepinephrine.

Therapeutic Uses and Status. Norepinephrine (*norepinephrine bitartrate*, LEVOPHED) has only limited therapeutic value. The use of it and other sympathomimetic amines in shock is discussed later in this chapter. In the treatment of low blood pressure, the dose is titrated to the desired pressor response.

Dopamine

Dopamine (3,4-dihydroxyphenylethylamine) (see Table 10-1) is the immediate metabolic precursor of norepinephrine and epinephrine; it is a central neurotransmitter particularly important in the regulation of movement (Chapters 12, 20, and 22) and possesses important intrinsic pharmacological properties. Dopamine is a substrate for both MAO and COMT and thus is ineffective when administered orally. Classification of dopamine receptors is described in Chapter 22.

Pharmacological Properties. Cardiovascular Effects. The cardiovascular effects of dopamine are mediated by several distinct types of receptors that vary in their affinity for dopamine (Goldberg and Rajfer, 1985). At low concentrations, the primary interaction of dopamine is with vascular D_1 -dopaminergic receptors, especially in the renal, mesenteric, and coronary beds. By activating adenylyl cyclase and raising intracellular concentrations of cyclic AMP, D_1 -receptor stimulation leads to vasodilation (Missale *et al.*, 1998). Infusion of low doses of dopamine causes an increase in glomerular filtration rate, renal blood flow, and Na^+ excretion. As a consequence, dopamine has pharmacologically appropriate effects in the management of states of low cardiac output associated with compromised renal function, such as severe congestive heart failure.

At somewhat higher concentrations, dopamine exerts a positive inotropic effect on the myocardium, acting on β_1 -adrenergic receptors. Dopamine also causes the release

of norepinephrine from nerve terminals, which contributes to its effects on the heart. Tachycardia is less prominent during infusion of dopamine than of isoproterenol (*see below*). Dopamine usually increases systolic blood pressure and pulse pressure and either has no effect on diastolic blood pressure or increases it slightly. Total peripheral resistance usually is unchanged when low or intermediate doses of dopamine are given, probably because of the ability of dopamine to reduce regional arterial resistance in some vascular beds, such as mesenteric and renal, while causing only minor increases in other vascular beds. At high concentrations, dopamine activates vascular α_1 -adrenergic receptors, leading to more general vasoconstriction.

Other Effects. Although there are specific dopamine receptors in the CNS, injected dopamine usually has no central effects because it does not readily cross the blood-brain barrier.

Precautions, Adverse Reactions, and Contraindications.

Before dopamine is administered to patients in shock, hypovolemia should be corrected by transfusion of whole blood, plasma, or other appropriate fluid. Untoward effects due to overdosage generally are attributable to excessive sympathomimetic activity (although this also may be the response to worsening shock). Nausea, vomiting, tachycardia, anginal pain, arrhythmias, headache, hypertension, and peripheral vasoconstriction may be encountered during an infusion of dopamine. Extravasation of large amounts of dopamine during infusion may cause ischemic necrosis and sloughing. Rarely, gangrene of the fingers or toes has followed the prolonged infusion of the drug.

Dopamine should be avoided or used at a much reduced dosage (one-tenth or less) if the patient has received a MAO inhibitor. Careful adjustment of dosage also is necessary for the patient who is taking tricyclic antidepressants, as responses may be particularly variable.

Therapeutic Uses. Dopamine (*dopamine hydrochloride*; INTROPIN) is used in the treatment of severe congestive failure, particularly in patients with oliguria and with low or normal peripheral vascular resistance. The drug also may improve physiological parameters in the treatment of cardiogenic and septic shock. While dopamine may acutely improve cardiac and renal function in severely ill patients with chronic heart disease or renal failure, there is relatively little evidence supporting long-term changes in clinical outcome (Marik and Iglesias, 1999). The management of shock is discussed later in this chapter.

Dopamine hydrochloride is used only intravenously. The drug is initially administered at a rate of 2 to 5 $\mu\text{g/kg}$ per minute; this rate may be increased gradually up to 20 to 50 $\mu\text{g/kg}$ per minute or more as the clinical situation dictates. During the infusion, patients require clinical assessment of myocardial function, perfusion of vital organs such as the brain, and the production of urine. Most patients should receive intensive care, with monitoring of arterial and venous pressures and the ECG. Reduction in urine flow, tachycardia, and the development of arrhythmias may be indications to slow or terminate the infusion. The duration of action of dopamine is brief, and hence the rate of administration can be used to control the intensity of effect.

Related drugs include *fenoldopam* and *dopexamine*. Fenoldopam (CORLOPAM) is a D_1 -receptor-selective agonist, which lowers blood pressure in severe hypertension (Elliott *et al.*, 1990; Nichols *et al.*, 1990). Fenoldopam does not appear to activate α - or β -adrenergic receptors. Intravenous infusions of fenoldopam dilate a variety of blood vessels including coronary, renal (both afferent and efferent arterioles), and mesenteric arteries (Brogden and Markham, 1997). The drug is indicated for short-term management of severe hypertension where rapid reduction of blood pressure is clinically indicated. Dopexamine (DOPACARD) is a synthetic analog related to dopamine with intrinsic activity at dopamine receptors as well as at β_2 -adrenergic receptors; it may have other effects such as inhibition of catecholamine uptake (Fitton and Benfield, 1990). It appears to have favorable hemodynamic actions in patients with severe congestive heart failure, sepsis, and shock. Dopexamine is not available in the United States.

β -ADRENERGIC AGONISTS

β -Adrenergic receptor agonists have been utilized in many clinical settings but now play a major role only in the treatment of bronchoconstriction in patients with asthma (reversible airway obstruction) or chronic obstructive pulmonary disease.

Epinephrine was first used as a bronchodilator at the beginning of this century, and ephedrine was introduced into western medicine in 1924, although it had been used in China for thousands of years (Chen and Schmidt, 1930). The next major advance was the development in the 1940s of isoproterenol, a β -receptor-selective agonist; this provided a drug for asthma that lacked α -adrenergic activity. The recent development of β_2 -selective agonists has resulted in drugs with even more valuable characteristics, including adequate oral bioavailability, lack of α -adrenergic activity, and diminished likelihood of adverse cardiovascular effects.

β -Adrenergic agonists may be used to stimulate the rate and force of cardiac contraction. The chronotropic effect is useful in the emergency treatment of arrhythmias such as *torsades de pointes*, bradycardia, or heart block (Chapter 35), whereas the inotropic effect is useful when it is desirable to augment myocardial contractility. The various therapeutic uses of β -adrenergic agonists are discussed later in the chapter.

Isoproterenol

Isoproterenol (isopropylarterenol, isopropylnorepinephrine, isoprenaline, isopropylnoradrenaline, *d,l*- β -[3,4-dihydroxyphenyl]- α -isopropylaminoethanol) (see Table 10-1) is a potent, nonselective β -adrenergic agonist with very low affinity for α -adrenergic receptors. Consequently, isoproterenol has powerful effects on all β receptors and almost no action at α receptors.

Pharmacological Actions. The major cardiovascular effects of isoproterenol (compared with epinephrine and norepinephrine) are illustrated in Figure 10-2. Intravenous infusion of isoproterenol lowers peripheral vascular resistance, primarily in skeletal muscle but also in renal and mesenteric vascular beds. Diastolic pressure falls. Systolic blood pressure may remain unchanged or rise, although mean arterial pressure typically falls. Cardiac output is increased because of the positive inotropic and chronotropic effects of the drug in the face of diminished peripheral vascular resistance. The cardiac effects of isoproterenol may lead to palpitations, sinus tachycardia, and more serious arrhythmias; large doses of isoproterenol may cause myocardial necrosis in animals.

Isoproterenol relaxes almost all varieties of smooth muscle when the tone is high, but this action is most pronounced on bronchial and gastrointestinal smooth muscle. It prevents or relieves bronchoconstriction. Its effect in asthma may be due in part to an additional action to inhibit antigen-induced release of histamine and other mediators of inflammation; this action is shared by β_2 -receptor-selective stimulants.

Absorption, Fate, and Excretion. Isoproterenol is readily absorbed when given parenterally or as an aerosol. It is metabolized primarily in the liver and other tissues by COMT. Isoproterenol is a relatively poor substrate for MAO and is not taken up by sympathetic neurons to the same extent as are epinephrine and norepinephrine. The duration of action of isoproterenol therefore may be longer than that of epinephrine, but it still is brief.

Toxicity and Adverse Effects. Palpitations, tachycardia, headache, and flushed skin are common. Cardiac ischemia and arrhythmias may occur, particularly in patients with underlying coronary artery disease.

Therapeutic Uses. Isoproterenol (*isoproterenol hydrochloride*; ISUPREL) may be used in emergencies to stimulate heart rate in patients with bradycardia or heart block, particularly in anticipation of inserting an artificial cardiac pacemaker or in patients with the ventricular arrhythmia *torsades de pointes*. In disorders such as asthma and shock, isoproterenol largely has been replaced by other sympathomimetic drugs (see below and Chapter 28).

Dobutamine

Dobutamine resembles dopamine structurally but possesses a bulky aromatic substituent on the amino group (see Table 10-1). The pharmacological effects of dobutamine are due to direct interactions with α - and β -adrenergic receptors; its actions do not appear to be a result of release of norepinephrine from sympathetic nerve endings, nor are they exerted via dopaminergic receptors. Although dobutamine originally was thought to be a relatively selective β_1 -adrenergic agonist, it is now clear that its pharmacological effects are complex. Dobutamine possesses a center of asymmetry; the two enantiomeric forms are present in the racemic mixture that is used clinically (Ruffolo *et al.*, 1981). The (–) isomer of dobutamine is a potent agonist at α_1 receptors and is capable of causing marked pressor responses (Ruffolo and Yaden, 1983). In contrast, (+)-dobutamine is a potent α_1 -adrenergic receptor antagonist, which can block the effects of (–)-dobutamine. The effects of these two isomers are mediated via β -adrenergic receptors. The (+) isomer is about ten times more potent as a β -adrenergic receptor agonist than is the (–) isomer. Both isomers appear to be full agonists.

Cardiovascular Effects. The cardiovascular effects of racemic dobutamine represent a composite of the distinct pharmacological properties of the (–) and (+) stereoisomers. Dobutamine has relatively more prominent inotropic than chronotropic effects on the heart compared to isoproterenol. The explanation for this useful selectivity is not clear. It may be due in part to the fact that peripheral resistance is relatively unchanged. Alternatively, cardiac α_1 receptors may contribute to the inotropic effect. At equivalent inotropic doses, dobutamine enhances automaticity of the sinus node to a lesser extent than does isoproterenol; however, enhancement of atrioventricular and intraventricular conduction is similar for the two drugs.

In animals, administration of dobutamine at a rate of 2.5 to 15 $\mu\text{g/kg}$ per minute increases cardiac contractility and cardiac output. Total peripheral resistance is not greatly affected. The relative constancy of peripheral resistance presumably reflects counterbalancing of α_1 -adrenergic receptor-mediated vasoconstriction and β_2 -receptor-mediated vasodilation (Ruffolo, 1987). The heart rate increases only modestly when the rate of administration of dobutamine is maintained at less than 20 $\mu\text{g/kg}$ per minute. After administration of β -adrenergic blocking agents, infusion of dobutamine fails to increase cardiac output, but total peripheral resistance increases, confirming that dobutamine does have modest direct effects on α receptors in the vasculature.

Adverse Effects. In some patients, blood pressure and heart rate may increase significantly during administration of dobutamine; this may require reduction of the rate of infusion. Patients with a history of hypertension may be at greater risk of developing an exaggerated pressor response. Since dobutamine facilitates atrioventricular conduction, patients with atrial fibrillation are at risk of marked increases in ventricular response rates; digoxin or other measures may be required to prevent this from occurring. Some patients may develop ventricular ectopic activity. As with any inotropic agent, dobutamine potentially may increase the size of a myocardial infarct by increasing myocardial oxygen demand. This risk must be balanced against the patient's overall clinical status. The efficacy of dobutamine over a period of more than a few days is uncertain; there is evidence for the development of tolerance (Unverferth *et al.*, 1980).

Therapeutic Uses. Dobutamine (*dobutamine hydrochloride*; DOBUTREX) is indicated for the short-term treatment of cardiac decompensation that may occur after cardiac surgery or in patients with congestive heart failure or acute myocardial infarction. Dobutamine increases cardiac output and stroke volume in such patients, usually without a marked increase in heart rate. Alterations in blood pressure or peripheral resistance usually are minor, although some patients may have marked increases in blood pressure or heart rate. Clinical evidence of longer-term efficacy remains uncertain. Interestingly, an infusion of dobutamine in combination with echocardiography is useful in the noninvasive assessment of patients with coronary artery disease (Madu *et al.*, 1994). Stressing of the heart with dobutamine may reveal cardiac abnormalities in carefully selected patients.

Dobutamine has a half-life of about 2 minutes; the major metabolites are conjugates of dobutamine and 3-*O*-methyl-dobutamine. The onset of effect is rapid. Consequently, a loading dose is not required, and steady-state concentrations generally are achieved within 10 minutes of initiation of an infusion. The rate of infusion required to increase cardiac output is typically

between 2.5 and 10 $\mu\text{g/kg}$ per minute, although higher infusion rates occasionally are required. The rate and duration of the infusion are determined by the clinical and hemodynamic responses of the patient.

β_2 -Selective Adrenergic Agonists

Some of the major adverse effects of β -adrenergic agonists in the treatment of asthma are caused by stimulation of β_1 -adrenergic receptors in the heart. Accordingly, drugs with preferential affinity for β_2 receptors compared with β_1 receptors have been developed. However, this selectivity is not absolute, and it is lost at sufficiently high concentrations of these drugs.

A second strategy that has increased the usefulness of several β_2 -selective agonists in the treatment of asthma has been structural modification that results in lower rates of metabolism and enhanced oral bioavailability (compared with catecholamines). Modifications have included placing the hydroxyl groups at positions 3 and 5 of the phenyl ring or substituting another moiety for the hydroxyl group at position 3. This has yielded drugs such as *metaproterenol*, *terbutaline*, and *albuterol*, which are not substrates for COMT. Bulky substituents on the amino group of catecholamines contribute to potency at β -adrenergic receptors with decreased activity at α -adrenergic receptors and decreased metabolism by MAO (Nelson, 1982).

A final strategy to enhance preferential activation of pulmonary β_2 receptors is the administration by inhalation of small doses of the drug in aerosol form. This approach typically leads to effective activation of β_2 receptors in the bronchi but very low systemic drug concentrations (Newhouse and Dolovich, 1986). Consequently, there is less potential to activate cardiac β_1 receptors or to stimulate β_2 receptors in skeletal muscle, which can cause tremor and thereby limit oral therapy.

Administration of β -adrenergic agonists by aerosol (see Chapter 28) typically leads to a very rapid therapeutic response, generally within minutes, although some agonists such as salmeterol have a delayed onset of action. While subcutaneous injection also causes prompt bronchodilation, the peak effect of a drug given orally may be delayed for several hours. Aerosol therapy depends on the delivery of drug to the distal airways. This, in turn, depends on the size of the particles in the aerosol and respiratory parameters such as inspiratory flow rate, tidal volume, breath-holding time, and airway diameter (Newhouse and Dolovich, 1986). Only about 10% of an inhaled dose actually enters the lungs; much of the remainder is swallowed and ultimately may be absorbed. Successful aerosol

therapy requires that each patient master the technique of drug administration. Many patients, particularly children and the elderly, do not use optimal techniques, often because of inadequate instructions (Kelly, 1985; Newhouse and Dolovich, 1986). In these patients, spacer devices may enhance the efficacy of inhalation therapy (see Chapter 28).

In the treatment of asthma, β -adrenergic agonists are used to activate pulmonary receptors that relax bronchial smooth muscle and decrease airway resistance. Although this action appears to be a major therapeutic effect of these drugs in patients with asthma, evidence suggests that β -adrenergic agonists also may suppress the release of leukotrienes and histamine from mast cells in lung tissue (Hughes *et al.*, 1983), enhance mucociliary function, decrease microvascular permeability, and possibly inhibit phospholipase A₂ (Seale, 1988). The relative importance of these actions in the treatment of human asthma remains to be determined. However, it is becoming increasingly clear that airway inflammation is directly involved in airway hyperresponsiveness (see Chapter 28); consequently, the use of antiinflammatory drugs such as inhaled steroids may have primary importance. The use of β -adrenergic agonists for the treatment of asthma is discussed in Chapter 28.

Metaproterenol. *Metaproterenol* (called *orciprenaline* in Europe), along with terbutaline and fenoterol, belongs to the structural class of resorcinol bronchodilators that have hydroxyl groups at positions 3 and 5 of the phenyl ring (rather than at positions 3 and 4 as in catechols) (see Table 10-1). Consequently, metaproterenol is resistant to methylation by COMT, and a substantial fraction (40%) is absorbed in active form after oral administration. It is excreted primarily as glucuronic acid conjugates. Metaproterenol is considered to be β_2 -selective, although it probably is less selective than albuterol or terbutaline. Effects occur within minutes of inhalation and persist for several hours. After oral administration, onset of action is slower, but effects last 3 to 4 hours. Metaproterenol (*metaproterenol sulfate*; ALUPENT) is used for the long-term treatment of obstructive airway diseases and for treatments of acute bronchospasm (see Chapter 28).

Terbutaline. *Terbutaline* is a β_2 -selective bronchodilator. It contains a resorcinol ring and thus is not a substrate for methylation by COMT. It is effective when taken orally, subcutaneously, or by inhalation. Effects are observed rapidly after inhalation or parenteral administration; after inhalation its action may persist for 3 to 6 hours. With oral administration, the onset of effect may be delayed for 1 to 2 hours. Terbutaline (*terbutaline sulfate*;

BRETHINE, others) is used for the long-term treatment of obstructive airway diseases and for treatment of acute bronchospasm; furthermore, it is available for parenteral use for the emergency treatment of status asthmaticus (see Chapter 28).

Albuterol. *Albuterol* (salbutamol; VENTOLIN, PROVENTIL, others) is a selective β_2 -adrenergic agonist with pharmacological properties and therapeutic indications similar to those of terbutaline. It is administered either by inhalation or orally for the symptomatic relief of bronchospasm. When administered by inhalation, it produces significant bronchodilation within 15 minutes, and effects are demonstrable for 3 to 4 hours. The cardiovascular effects of albuterol are considerably weaker than those of isoproterenol when doses that produce comparable bronchodilation are administered by inhalation.

Isoetharine. *Isoetharine* was the first drug with β_2 selectivity widely used for the treatment of airway obstruction. However, its degree of selectivity for β_2 -adrenergic receptors may not approach that of some of the other agents. Although resistant to metabolism by MAO, it is a catecholamine and thus is a good substrate for COMT (see Table 10-1). Consequently, it is used only by inhalation for the treatment of acute episodes of bronchoconstriction.

Pirbuterol. *Pirbuterol* is a relatively selective β_2 agonist. It is structurally identical to albuterol except for the substitution of a pyridine ring for the benzene ring (Richards and Brogden, 1985). Pirbuterol acetate (MAXAIR) is available for inhalation therapy; dosing is typically every 4 to 6 hours.

Bitolterol. *Bitolterol* (*bitolterol mesylate*; TORNALATE) is a novel β_2 agonist in which the hydroxyl groups in the catechol moiety are protected by esterification with 4-methylbenzoate. Esterases in the lung and other tissues hydrolyze this pro-drug to the active form, colterol, or terbutyl-norepinephrine (see Table 10-1). Results of animal studies have suggested that these esterases are present in higher concentration in lung than in tissues such as the heart (Nelson, 1986; Fridel and Brogden, 1988). The duration of effect of bitolterol after inhalation ranges from 3 to 6 hours.

Fenoterol. *Fenoterol* (BEROTEC) is a β_2 -selective adrenergic receptor agonist. After inhalation, it has a prompt onset of action, and its effect typically is sustained for 4 to 6 hours. Fenoterol is not available in the United States. The possible association of fenoterol use with increased deaths from asthma in New Zealand is controversial (Pearce *et al.*, 1995; Suissa and Ernst, 1997).

Formoterol. *Formoterol* (FORADIL) is a long-acting β_2 -selective adrenergic receptor agonist. Significant bronchodilation occurs within minutes of inhalation of a therapeutic dose, and this action may persist for up to 12 hours (Faulds *et al.*, 1991). Its major advantage over many other β_2 -selective agonists is this prolonged duration of action, which may be particularly

advantageous in settings such as nocturnal asthma. Formoterol is not available in the United States.

Procaterol. *Procaterol* (MASCACIN, others) is a β_2 -selective adrenergic receptor agonist. After inhalation, it has a prompt onset of action, and action is sustained for about 5 hours. Procaterol is not available in the United States.

Salmeterol. *Salmeterol* (SEREVENT) is a β_2 -selective adrenergic receptor agonist with a prolonged duration of action, about 12 hours. However, it has a relatively slow onset of action after inhalation, so is not suitable alone for prompt relief of breakthrough attacks of bronchospasm (Lötvall and Svedmyr, 1993; Brogren and Faulds, 1991).

Ritodrine. *Ritodrine* is a selective β_2 -adrenergic agonist that was developed specifically for use as a uterine relaxant. Nevertheless, its pharmacological properties closely resemble those of the other agents in this group. Ritodrine is rapidly but incompletely (30%) absorbed following oral administration, and 90% of the drug is excreted in the urine as inactive conjugates; about 50% of ritodrine is excreted unchanged after intravenous administration. The pharmacokinetic properties of ritodrine are complex and incompletely defined, especially in pregnant women.

Therapeutic Uses. Ritodrine may be administered intravenously to selected patients to arrest premature labor. Ritodrine and related drugs can prolong pregnancy (King *et al.*, 1988). However, β_2 -selective agonists may not have clinically significant benefits on perinatal mortality and may actually increase maternal morbidity (The Canadian Preterm Labor Investigators Group, 1992; Higby *et al.*, 1993; Johnson, 1993). Given modern improvements in the care of premature babies, it is possible that existing clinical trials may not have had sufficient statistical power to demonstrate subtle, but potentially important, clinical effects. Many other drugs are used to delay labor (Bossmar, 1998; Norwitz *et al.*, 1999). Magnesium therapy may prolong labor in preterm women. There is some evidence suggesting that indomethacin may prolong preterm labor, but a favorable risk-to-benefit ratio has not been established (Panter *et al.*, 1999). Also, while calcium channel blockers prolong preterm labor, their long-term benefits are unclear (see Chapter 32; Carr *et al.*, 1999).

Adverse Effects of β_2 -Selective Agonists. The major adverse effects of β -adrenergic agonists occur as a result of excessive activation of β -adrenergic receptors. Patients with underlying cardiovascular disease are particularly at risk for significant reactions. However, the likelihood of adverse effects can be greatly decreased in patients with lung disease by administering the drug by inhalation rather than orally or parenterally.

Skeletal muscle tremor is a relatively common adverse effect of the β_2 -selective adrenergic agonists. Tolerance generally develops to this effect; it is not clear whether tolerance reflects desensitization of the β_2 receptors of skeletal muscle or adaptation within the CNS. This adverse effect can be minimized by starting oral therapy with a low dose of drug and progressively increasing the dose as tolerance to the tremor develops. Feelings of restlessness, apprehension, and anxiety may limit therapy with these drugs, particularly after oral or parenteral treatment.

Tachycardia is a common adverse effect of systemically administered β -adrenergic agonists. Stimulation of heart rate occurs primarily via β_1 receptors. It is uncertain to what extent the increase in heart rate also is due to activation of cardiac β_2 receptors or to reflex effects that stem from β_2 -receptor-mediated peripheral vasodilation. However, during a severe asthmatic attack, heart rate may actually decrease during therapy with a β -adrenergic agonist, presumably because of improvement in pulmonary function with consequent reduction in endogenous cardiac sympathetic stimulation. In patients without cardiac disease, β agonists rarely cause significant arrhythmias or myocardial ischemia; however, patients with underlying coronary artery disease or preexisting arrhythmias are at greater risk. The risk of adverse cardiovascular effects also is increased in patients who are receiving MAO inhibitors.

Arterial oxygen tension may fall when treatment of patients with an acute exacerbation of asthma is begun; this may be due to drug-induced pulmonary vascular dilation, which leads to increased mismatching of ventilation and perfusion. This effect usually is small and transient. Supplemental oxygen should be given if necessary. Severe pulmonary edema has been reported in women receiving ritodrine or terbutaline for premature labor.

The results of a number of epidemiologic studies have suggested a possible adverse connection between prolonged use of β -adrenergic agonists and death or near-death from asthma (Suisa *et al.*, 1994). While exact interpretation of these results is difficult, these studies have raised questions about the role of β -adrenergic agonists in the treatment of chronic asthma. Tolerance to effects of β -adrenergic agonists has been studied extensively, both *in vitro* and *in vivo* (see Chapter 6). Long-term systemic administration of β -adrenergic agonists leads to down-regulation of β receptors in some tissues and decreased pharmacological responses. However, it appears likely that tolerance to the pulmonary effects of these drugs is not a major clinical problem for the majority of asthmatics who do not exceed recommended dosages of β -adrenergic agonists over prolonged periods (Jenne, 1982; Tattersfield, 1985).

There is some evidence suggesting that regular use of β_2 -selective agonists may cause increased bronchial hyperreactivity and deterioration in disease control (Lipworth and McDevitt, 1992; Hancox *et al.*, 1999). To what extent this potential adverse association may be even more unfavorable for very long-acting β agonists or excess doses of medication is not yet known (Beasley *et al.*, 1999). However, for patients requiring regular use of these drugs over prolonged periods, strong consideration should be given to additional or alternative therapy, such as the use of inhaled corticosteroids.

Large doses of β -adrenergic agonists cause myocardial necrosis in laboratory animals. When given parenterally, these drugs also may increase the concentrations of glucose, lactate, and free fatty acids in plasma and decrease the concentration of K^+ . The decrease in K^+ concentration may be especially important in patients with cardiac disease, particularly those taking cardiac glycosides and diuretics. In some diabetic patients, hyperglycemia may be worsened by these drugs, and higher doses of insulin may be required. All these adverse effects are far less likely with inhalation therapy than with parenteral or oral therapy.

α_1 -SELECTIVE ADRENERGIC AGONISTS

The major clinical effects of a number of sympathomimetic drugs are due to activation of α -adrenergic receptors in vascular smooth muscle. As a result, peripheral vascular resistance is increased and blood pressure is maintained or elevated. Although the clinical utility of these drugs is limited, they may be useful in the treatment of some patients with hypotension or shock. *Phenylephrine* and *methoxamine* are direct-acting vasoconstrictors and are selective activators of α_1 receptors. *Mephentermine* and *metaraminol* act both directly and indirectly; *i.e.*, a portion of their effects is mediated through the release of endogenous norepinephrine.

Methoxamine

Methoxamine (*methoxamine hydrochloride*; VASOXYL; *see* Table 10-1) is a relatively specific α_1 -receptor-selective adrenergic agonist; as such, it causes a dose-related increase in peripheral vascular resistance. The drug may have different intrinsic activities at α_1 receptors in different tissues (Garcia-Sainz *et al.*, 1985). Methoxamine does not activate β -adrenergic receptors, nor does it cause stimulation of the CNS. However, at high concentrations, methoxamine has some capacity to block β receptors. The

major cardiovascular response to the drug is a rise in blood pressure, which is associated with sinus bradycardia because of activation of vagal reflexes; the slowing of the heart rate is largely blocked by atropine. Methoxamine may be used intravenously in the treatment of hypotensive states.

Phenylephrine

Phenylephrine is an α_1 -selective agonist; it activates β -adrenergic receptors only at much higher concentrations. Chemically, phenylephrine differs from epinephrine only in lacking a hydroxyl group at position 4 on the benzene ring (*see* Table 10-1). The pharmacological effects of phenylephrine are similar to those of methoxamine. The drug causes marked arterial vasoconstriction during intravenous infusion. Phenylephrine (*phenylephrine hydrochloride*; NEOSYNEPHRINE, others) also is used as a nasal decongestant and as a mydriatic in various nasal and ophthalmic formulations (*see* Chapter 66 for ophthalmic uses).

Mephentermine

Mephentermine (*see* Table 10-1) is a sympathomimetic drug that acts both directly and indirectly; it has many similarities to ephedrine (*see* below). After an intramuscular injection, the onset of action is prompt (within 5 to 15 minutes), and effects may last for several hours. Since the drug releases norepinephrine, cardiac contraction is enhanced, and cardiac output and systolic and diastolic pressures usually are increased. The change in heart rate is variable, depending on the degree of vagal tone. Adverse effects are related to CNS stimulation, excessive rises in blood pressure, and arrhythmias. *Mephentermine* (*mephentermine sulfate*; WYAMINE SULFATE) is used to prevent hypotension, which frequently accompanies spinal anesthesia.

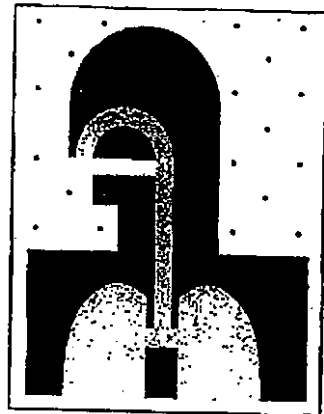
Metaraminol

Metaraminol (*metaraminol bitartrate*; ARAMINE) (*see* Table 10-1) is a sympathomimetic drug with prominent direct effects on vascular α -adrenergic receptors. Metaraminol also is an indirectly acting agent that stimulates the release of norepinephrine. The drug has been used in the treatment of hypotensive states or to relieve attacks of paroxysmal atrial tachycardia, particularly those associated with hypotension (*see* Chapter 35 for preferable treatments of this arrhythmia).

Midodrine

Midodrine (PROAMATINE) is an orally effective, α_1 -adrenergic agonist (Fouad-Tarazi *et al.*, 1995). It is a prodrug; its activity is due to its conversion to an active metabolite, desglymidodrine, which achieves peak concentrations about 1 hour after a dose of midodrine. The half-life of desglymidodrine is about 3 hours. Consequently, the duration of action is about 4 to 6 hours. Midodrine-induced rises in blood pressure are associated with both arterial and venous smooth muscle contraction. This is

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Textbook of Respiratory Medicine

Third Edition

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Airway Pharmacology

PETER J. BARNES, M.A., D.M., D.Sc., F.R.C.P.

Introduction

Bronchodilators

- Beta₂-Adrenergic Agonists
- Theophylline
- Anticholinergics
- New Bronchodilators

Controller Drugs

- Corticosteroids
- Cromones

Mediator Antagonists

- Antihistamines
- Anti-Leukotrienes

Steroid-Sparing Therapies

- Methotrexate
- Gold
- Cyclosporine
- Intravenous Immunoglobulin
- Immunotherapy

New Drugs for Asthma and Chronic Obstructive Pulmonary Disease

- Mediator Antagonists
- Anti-Cytokines
- Phosphodiesterase Inhibitors
- Adhesion Molecule Blockers
- Immunomodulators
- Furosemide
- Neuromodulators
- Enzyme Inhibitors
- Transcription Factor Blockers
- Impact of Molecular Genetics

Other Drugs

- Mucoregulators
- Antitussives
- Drugs for Breathlessness
- Ventilatory Stimulants

Drugs Contraindicated in Airway Disease

- Sedatives
- Beta-Blockers
- Aspirin and Other Cyclooxygenase Inhibitors
- Angiotensin-Converting Enzyme Inhibitors
- Local Anesthetics

INTRODUCTION

The pharmacology of the airways is largely concerned with the treatment of airway obstruction, particularly asthma. Important advances in the understanding of asthma as an inflammatory disease of the airways have resulted in a shift of emphasis from bronchodilator to anti-inflammatory therapy. Chronic obstructive pulmonary disease (COPD) has received less attention but appears also to be an inflammatory disease, although the inflammatory response differs from that in asthma and is largely unresponsive to currently available anti-inflammatory treatments.¹ This chapter discusses the pharmacology of the drugs used in the treatment of obstructive airway disease, including bronchodilators, which act mainly by reversing airway smooth muscle contraction, and anti-inflammatory drugs, which suppress inflammatory responses in the airways. Drugs that play a less important role include mediator antagonists, mucolytics, antitussives, and respiratory stimulants. Antibiotics, antituberculosis therapy, and oxygen are considered elsewhere.

BRONCHODILATORS

Bronchodilator drugs have an "anti-bronchoconstrictor" effect, which may be demonstrated directly *in vitro* by a relaxant effect on precontracted airways. Bronchodilators cause rapid reversal of airway obstruction in asthma *in vivo*, and this is believed to result from an effect on airway smooth muscle, although additional pharmacologic effects on other airway cells (such as reduced microvascular leakage and reduced release of bronchoconstrictor mediators from inflammatory cells) may contribute to the reduction in airway narrowing.

Three classes of bronchodilators are in current clinical use: beta-adrenergic agonists (sympathomimetics), theophylline (methylxanthines), and anticholinergic drugs.

Drugs such as cromolyn sodium, which prevent bronchoconstriction, have no direct bronchodilator action and are ineffective once bronchoconstriction has occurred. Anti-leukotrienes (leukotriene receptor antagonists and 5-lipoxygenase inhibitors) have a small bronchodilator effect in some patients. Corticosteroids, although they gradually improve airway obstruction, have no direct effect on contraction of airway smooth muscle and therefore are not considered to be bronchodilators.

Beta₂-Adrenergic Agonists

Inhaled beta₂-agonists are the bronchodilator treatment of choice in asthma, because they are the most effective bronchodilators and have minimal side effects when used correctly.² There is no place for short-acting and nonselective beta-agonists such as isoproterenol or metaproterenol.

Chemistry

Beta₂-agonists were developed logically by substitutions in the catecholamine structure of norepinephrine. The catechol ring consists of hydroxyl groups in the 3 and 4 positions of the benzene ring (Fig. 11.1). Norepinephrine differs from epinephrine only in the terminal amine group, which therefore indicates that modification at this site confers beta-receptor selectivity. Further substitution of the terminal amine resulted in beta₂-receptor selectivity, as in albuterol and terbutaline. Catecholamines are rapidly metabolized by the enzyme catechol-*o*-methyl transferase (COMT), which methylates in the 3-hydroxyl position and accounts for the short duration of action of catecholamines. Modification of the catechol ring, as in albuterol and terbutaline, prevents this degradation and therefore prolongs the drugs' effect. Catecholamines are also broken down by monoamine oxidase (MAO) in sympathetic nerve terminals and in the gastrointestinal tract through cleavage of the side chain of catecholamines. Isoproterenol, which is a substrate for MAO, is therefore metabolized in the gut, making absorption variable. Substitution in the amine group confers resistance to MAO and ensures reliable absorption. Many other beta₂-selective agonists have now been introduced and, although there may be differences in potency, there are no clinically significant differences in selectivity.

Inhaled beta₂-selective drugs in current clinical use (apart from rimeterol, which is broken down by COMT) have a similar duration of action of 3 to 6 hours. Inhaled beta₂-selective drugs that have a much longer duration of effect (more than 12 hours), such as salmeterol and formoterol, have been developed. Formoterol has a bulky

substitution in the aliphatic chain and has a high lipophilicity, which keeps the drug within the membrane, close to the receptor.³ Salmeterol has a long aliphatic chain, and its long duration of action may be a result of binding within the receptor binding cleft ("exosite") that anchors the drug in the binding cleft.⁴

Mode of Action

Beta-agonists produce bronchodilatation by directly stimulating beta₂-receptors in airway smooth muscle, which leads to relaxation.⁵ This can be demonstrated *in vitro* by the relaxant effect of isoproterenol on human bronchi and lung strips (indicating an effect on peripheral airways) and *in vivo* by a rapid decrease in airway resistance. Beta-receptors have been demonstrated in airway smooth muscle by direct receptor binding techniques, and autoradiographic studies indicate that beta-receptors are located in smooth muscle of all airways from the trachea to terminal bronchioles.⁶ The molecular mechanisms by which beta-agonists induce relaxation of airway smooth muscle have been extensively investigated.

Occupation of beta₂-receptors by agonists results in the activation of adenylyl cyclase via the stimulatory G protein (G_s). This increases intracellular cyclic 3',5'-adenosine monophosphate (cAMP), leading to activation of a specific kinase (protein kinase A); this kinase then phosphorylates several target proteins within the cell, leading to relaxation⁶ (Fig. 11.2).

These processes include lowering of intracellular calcium ion (Ca²⁺) concentration by active removal of Ca²⁺ from the cell and into intracellular stores; an inhibitory effect on phosphoinositide hydrolysis; direct inhibition of myosin light chain kinase; and opening of a large-conductance calcium-activated potassium channel (K_{Ca}), which repolarizes the smooth muscle cell and may stimulate the sequestration of Ca²⁺ into intracellular stores.^{7,8} Beta₂-receptors are directly coupled to K_{Ca} via G_s, so that relaxation of airway smooth muscle may occur independently of an increase in cAMP.⁹

Beta₂-agonists act as *functional antagonists* and reverse

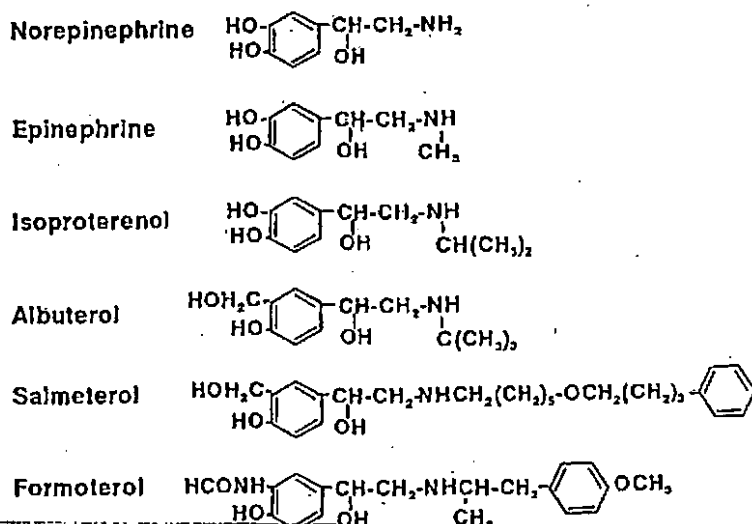
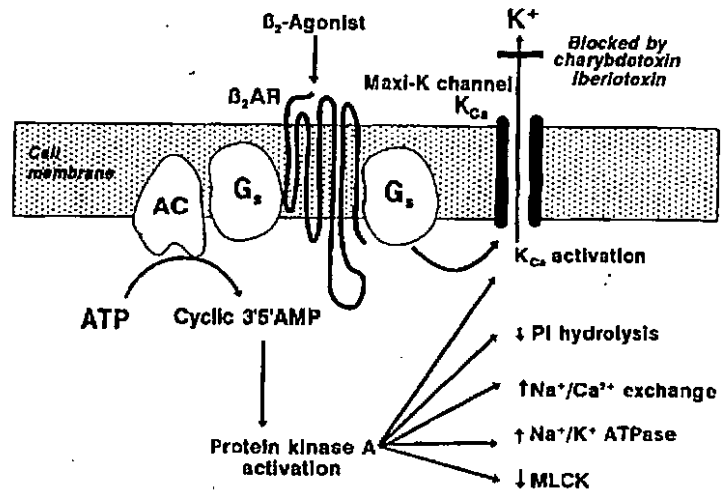


Figure 11.1 Chemical structures of some adrenergic agonists, showing development from catecholamines, are presented.

Figure 11.2 Molecular mechanism of action of beta₂-agonists on airway smooth muscle cells is presented. Activation of beta₂-receptors (β₂AR) results in activation of adenylyl cyclase (AC) via a stimulatory G protein (G_s) and increase in cyclic 3'5'-adenosine monophosphate (AMP). This activates protein kinase A, which then phosphorylates several target proteins, resulting in opening of calcium-activated potassium channels (K_{Ca}) or maxi-K channels, decreased phosphoinositide (PI) hydrolysis, increased sodium/calcium ion (Na⁺/Ca²⁺) exchange, increased sodium-potassium adenosine triphosphatase (Na⁺/K⁺ATPase) production, and decreased myosin light chain kinase (MLCK) activity. In addition, beta₂-receptors may be coupled directly via G_i to K_{Ca}.



bronchoconstriction regardless of the contractile agent. This is an important property, because many bronchoconstrictor mechanisms (neural and mediator) are likely to be contributory in asthma.

Beta₂-agonists have additional effects on airways, and beta-receptors are located in several different types of airway cells (Table 11.1). Beta₂-agonists can cause bronchodilatation by a direct action on airway smooth muscle, but also indirectly by inhibiting the release of bronchoconstrictor mediators from inflammatory cells and of bronchoconstrictor neurotransmitters from airway nerves (Fig. 11.3). Other effects of beta₂-agonists include

- Prevention of *mediator release* from isolated human lung mast cells (via beta₂-receptors)
- Prevention of *microvascular leakage* and thus the development of bronchial mucosal edema after exposure to mediators such as histamine and leukotriene D₄
- Increase in *mucus secretion* from submucosal glands and *ion transport* across airway epithelium; these effects may enhance mucociliary clearance and therefore reverse the defect in clearance found in asthma. Beta₂-agonists appear to selectively stimulate mucous rather than serous cells, which may result in a more viscous mucus secretion, although the clinical significance of this is uncertain.
- Reduction in *neurotransmission* in human airway cholinergic nerves by an action at prejunctional beta₂-receptors to inhibit acetylcholine release. This

may contribute to their bronchodilator effect by reducing cholinergic reflex bronchoconstriction. In animal studies, beta₂-receptors act on sensory nerves to inhibit the release of bronchoconstrictor and inflammatory peptides such as substance P.

Although these additional effects of beta₂-agonists may be relevant to the prophylactic use of these drugs against various challenges, their rapid bronchodilator action can probably be attributed to a direct effect on airway smooth muscle.

Anti-Inflammatory Effects

Whether beta₂-agonists have anti-inflammatory effects in asthma has become an important issue, particularly with their increasing use and the introduction of long-acting inhaled beta₂-agonists. The inhibitory effects of beta₂-agonists on mast cell mediator release and microvascular leakage are clearly anti-inflammatory, suggesting that beta₂-agonists may modify *acute* inflammation. However beta₂-agonists do not appear to have a significant inhibitory effect on the *chronic* inflammation of asthmatic airways, which is controlled by corticosteroids. This has been confirmed by biopsy and bronchoalveolar lavage studies in patients with asthma who were taking beta₂-agonists regularly; no significant reduction in the number or activation in inflammatory cells in the airways was demonstrated, in contrast to resolution of the inflammation that occurs with inhaled corticosteroids.⁹⁻¹¹ This finding is likely to be related to the fact that beta₂-agonists do not have an important inhibitory effect on macrophages, eosinophils, or lymphocytes.^{12, 13}

Clinical Use

Inhaled beta₂-agonists are the most widely used and effective bronchodilators in the treatment of asthma.² When inhaled as an aerosol from a metered-dose inhaler (MDI), they are convenient, easy to use, rapid in onset, and without significant side effects. In addition to having an acute bronchodilator effect, they are effective in protecting against various challenges, such as exercise, cold air, and allergens. They are the bronchodilators of choice

Table 11.1 EFFECTS OF BETA₂-AGONISTS ON AIRWAYS

Effect	Site of Action
Bronchodilation	Smooth muscle
Inhibition of mediator release	Mast cells
Inhibition of microvascular leakage	Endothelial cells
Increase in mucus secretion	Submucosal glands
Increase in ion transport	Airway epithelium
Inhibition of neurotransmission	Prejunctional receptors on cholinergic nerves

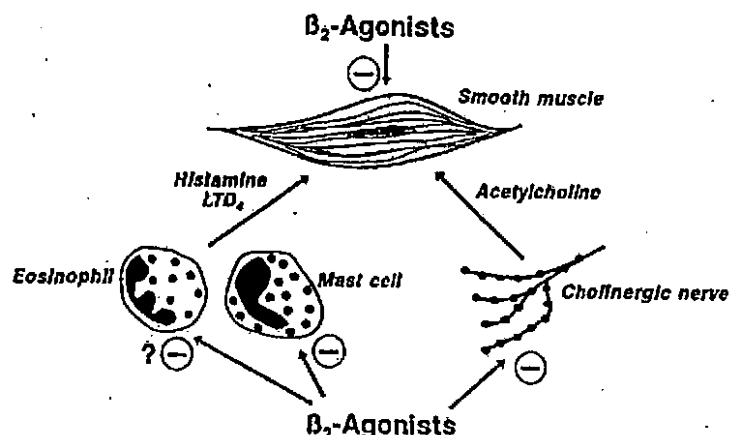


Figure 11.3 Direct and indirect effects of beta₂-agonists are shown. Beta₂-agonists relax airway smooth muscle cells directly but may also cause bronchodilatation indirectly by inhibiting the release of mediators from inflammatory cells or neurotransmitters from cholinergic nerves. LTD₄ = leukotriene D₄.

for the treatment of acute severe asthma when the nebulized route of administration is as effective as and easier than intravenous use. The inhaled route is preferable to the oral route because side effects are less and also because it may be more effective. Short-acting inhaled beta₂-agonists, such as albuterol and terbutaline, should not be used on a regular basis in the treatment of mild asthma but should be used "as required" by symptoms, because increased usage serves as an indicator for the need for more anti-inflammatory therapy.

Oral beta₂-agonists are indicated as an additional bronchodilator. Slow-release preparations (such as slow-release albuterol or bambuterol) may be indicated for nocturnal asthma but are less useful than inhaled beta₂-agonists because of an increased risk of side effects.

Therapeutic Choices

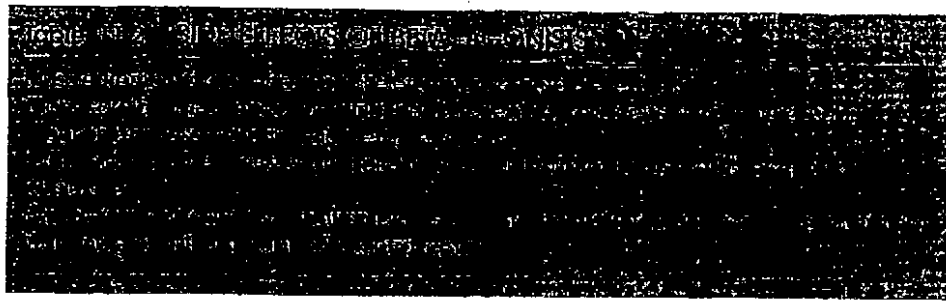
Several beta₂-selective agonists are now available. These drugs are as effective as nonselective agonists in their bronchodilator action, because beneficial airway effects are mediated only by beta₂-receptors. However, they are less likely to produce cardiac stimulation than isoproterenol because beta₁-receptors are stimulated relatively less. With the exception of rimeterol (which retains the catechol ring structure and is therefore susceptible to rapid enzymatic degradation), they have a longer duration of action because they are resistant to uptake and enzymatic degradation by COMT and MAO. There is little to choose among the various short-acting beta₂-agonists currently available; all are usable by inhalation and orally, have a similar duration of action (usually 3 to 4 hours but less in severe asthma), and have similar side effects. Differences in beta₂-selectivity have been claimed but are not clinically important. Drugs in clinical use include albuterol, terbutaline, fenoterol, tulobuterol, rimeterol, and pirbuterol. It has been claimed that fenoterol is less beta₂-selective than albuterol and terbutaline, resulting in increased cardiovascular side effects,¹⁴ but this evidence is controversial, because all of these effects are mediated via beta₂-receptors.¹⁵ The increased incidence of cardiovascular effects is more likely to be related to the greater effective dose of fenoterol that is used and perhaps to more rapid absorption into the circulation.

Long-Acting Inhaled Beta₂-Agonists

The long-acting inhaled beta₂-agonists salmeterol and formoterol have been a major advance in asthma therapy. Both drugs have a bronchodilator action of longer than 12 hours and also protect against bronchoconstriction for a similar period.^{16,17} They are particularly useful in treating nocturnal asthma. Both improve asthma control (when given twice daily), compared with regular treatment with short-acting beta₂-agonists four times daily. Both drugs are well tolerated. Tolerance to the bronchodilator effect of formoterol and to the bronchoprotective effects of formoterol and salmeterol has been demonstrated,¹⁸⁻²¹ but this is not a loss of protection, does not appear to be progressive, and is of doubtful clinical significance. Although both drugs have a similar duration of effect in clinical studies, there are some differences. Formoterol has a more rapid onset of action and is a fuller agonist than salmeterol. This might confer a theoretical advantage in more severe asthma, but it might also make it more likely to induce tolerance. Studies comparing the clinical efficacy of both drugs in mild and severe asthmatic patients are now needed.

Studies have suggested that an inhaled long-acting beta₂-agonist might be introduced earlier in therapy. In patients with asthma not controlled by either 400 or 800 µg of inhaled corticosteroids, the addition of salmeterol gave better control than did increasing the dose of inhaled steroid.^{22,23} A similar result was found with formoterol, which also reduces the frequency of asthma attacks.²⁴ This suggests that a long-acting inhaled beta₂-agonist may be added to low-dose inhaled steroids if asthma is not controlled, as an alternative (and perhaps in preference) to increasing the dose of inhaled corticosteroids. Long-acting inhaled beta₂-agonists have also been shown to help patients with COPD.²⁵

At present it is recommended that long-acting inhaled beta₂-agonists should be used only in patients who are also prescribed inhaled steroids. In the future, long-acting inhaled beta₂-agonists may be used in fixed-combination inhalers (salmeterol + fluticasone, formoterol + budesonide), to improve compliance and to reduce the risk of patients' using these drugs as sole long-term treatment.



Side Effects

Unwanted effects are dose-related and are caused by stimulation of extrapulmonary beta-receptors (Table 11.2). Side effects are not common with inhaled therapy but are more common with oral or intravenous administration.

- *Muscle tremor* is caused by stimulation of beta₂-receptors in skeletal muscle and is the most common side effect. It may be more troublesome with elderly patients.
- *Tachycardia and palpitations* are caused by reflex cardiac stimulation secondary to peripheral vasodilatation, by direct stimulation of atrial beta₂-receptors (the human heart is unusual in having a relatively high proportion of beta₂-receptors), and possibly also by stimulation of myocardial beta₁-receptors as the doses of beta₂-agonist are increased. These side effects tend to disappear with continued use of the drug, reflecting the development of tolerance.
- *Metabolic effects* (increase in free fatty acids, insulin, glucose, pyruvate, and lactate) are usually seen only after large systemic doses.
- *Hypokalemia* is a potentially more serious side effect. It is caused by beta₂-receptor stimulation of potassium entry into skeletal muscle, which may be secondary to a rise in insulin secretion. Hypokalemia might be serious in the presence of hypoxia, as in acute asthma, when there may be a predisposition to cardiac dysrhythmias. However, in practice, significant arrhythmias after use of nebulized beta₂-agonist have not been reported in acute asthma.
- *Ventilation-perfusion mismatching* is caused by pulmonary vasodilatation in blood vessels previously constricted by hypoxia, resulting in the shunting of blood to poorly ventilated areas and a fall in arterial PO₂. Although in practice the effect of beta₂-agonists on arterial PO₂ is usually very small (a fall of less than 5 mm Hg), occasionally in severe chronic airway obstruction it is large, although it may be prevented by giving additional inspired oxygen.

Desensitization

Continuous treatment with an agonist often leads to desensitization (tolerance, subsensitivity), perhaps because of down-regulation of the receptor. For this reason, there have been many studies of bronchial beta-receptor function after prolonged therapy with beta-agonists.²⁶ Tolerance of nonairway beta₂-receptor mediated responses,

such as tremor and cardiovascular and metabolic responses, is readily induced in normal and asthmatic subjects. Tolerance of human airway smooth muscle to beta₂-agonists has been demonstrated in vitro,⁵ although the concentration of agonist necessary is high and the degree of desensitization is variable. Animal studies suggest that airway smooth muscle beta₂-receptors may be more resistant to desensitization than beta₂-receptors elsewhere owing to a high receptor reserve.⁵ In normal subjects, bronchodilator tolerance was demonstrated in some studies after high-dose inhaled albuterol, but not in others. In asthmatic patients, tolerance to the bronchodilator effects of beta₂-agonists has not usually been found.²⁶ However, tolerance develops to the bronchoprotective effects of beta₂-agonists, and this is more marked with indirect constrictors such as adenosine, allergen, and exercise (which activate mast cells) than with direct constrictors such as histamine and methacholine.^{27, 28} The reason for the relative resistance of airway smooth muscle beta₂-receptors to desensitization remains uncertain, but it may reflect the fact that there is a large receptor reserve, so that more than 90% of beta₂-receptors may be lost without any reduction in the relaxation response. The high level of beta₂-receptor gene expression in airway smooth muscle compared with peripheral lung²⁹ may also contribute to the resistance to tolerance, because there is likely to be a high rate of beta₂-receptor synthesis. Another possibility is that the expression of the enzyme beta-adrenergic receptor kinase (βARK), which phosphorylates and inactivates the occupied beta₂-receptor, is very low in airway smooth muscle.³⁰ By contrast, there is no receptor reserve in inflammatory cells and βARK expression is high, so that indirect effects of beta₂-agonists are more readily lost. However, tolerance to the bronchodilator effects of the long-acting beta₂-agonist formoterol has been reported.^{19, 21}

Experimental studies have shown that corticosteroids prevent the development of tolerance in airway smooth muscle and prevent and reverse the fall in pulmonary beta-receptor density.³¹ However, inhaled corticosteroids do not appear to prevent the tolerance to the bronchoprotective effect of inhaled beta₂-agonists, possibly because they do not reach airway smooth muscle in a sufficient concentration.^{32, 33}

Long-Term Safety

Because of a possible relationship between adrenergic drug therapy and the rise in asthma deaths in several countries during the early 1960s, doubts were cast on the

safety of beta-agonists. A causal relationship between beta-agonist use and mortality has never been firmly established, although in retrospective studies this would not be possible. A particular beta₂-agonist, fenoterol, was linked to the more recent rise in asthma deaths in New Zealand, because significantly more of the patients who died than case-matched control patients were prescribed fenoterol. This association was strengthened by two subsequent studies, and since fenoterol has not been available the asthma mortality has fallen dramatically.³⁴ An epidemiologic study based in Saskatchewan, Canada, examined the links between drugs prescribed for asthma and death or near-death from asthma attacks, based on computerized records of prescriptions. There was a marked increase in the risk of death with high doses of all inhaled beta₂-agonists.³⁵ The risk was greater with fenoterol, but when the dose was adjusted to the equivalent dose for albuterol there was no significant difference in risk for these two drugs. The link between high beta₂-agonist usage and increased asthma mortality does not prove a causal association, because patients with more severe and poorly controlled asthma, who are more likely to have an increased risk of fatal attacks, are also more likely to use higher doses of beta₂-agonist inhalers and less likely to use effective anti-inflammatory treatment. Among the patients who used regular inhaled steroids, there was a significant reduction in risk of death.

Regular use of inhaled beta₂-agonists has also been linked to increased asthma morbidity. In a study carried out in New Zealand, the regular use of fenoterol was associated with poorer control and with a small increase in airway responsiveness compared with patients using fenoterol "on demand" for symptom control over a 6-month period.³⁶ However, this association was not found in studies with albuterol.^{37,38} There is some evidence that regular use of inhaled albuterol may increase exercise-induced asthma³⁹ and increase inflammation in asthmatic airways. One possible mechanism is that beta-agonists may inhibit the anti-inflammatory action of glucocorticoids.⁴⁰

Although it is unlikely that normally recommended doses of beta₂-agonists worsen asthma, it is possible that this could occur with larger doses. Furthermore, some patients may be more susceptible if they have polymorphic forms of the beta₂-receptor that more rapidly down-regulate.⁴¹ Short-acting inhaled beta₂-agonists should be used only "on demand" for symptom control; if they are required frequently (more than three times weekly), then an inhaled anti-inflammatory drug is needed. There is an association between increased risk of death from asthma and use of high doses of inhaled beta₂-agonists; although this may reflect severity, it is also possible that high doses of beta₂-agonists have a deleterious effect on asthma. High concentrations of beta₂-agonists interfere with the anti-inflammatory action of steroids.⁴² Patients receiving high doses of beta₂-agonists (more than 1 canister per month) should be treated with effective anti-inflammatory treatments, and attempts should be made to reduce the daily dose of inhaled beta₂-agonist.

Future Trends

Beta₂-agonists will continue to be the bronchodilators of choice for the foreseeable future, because they are effective

in all patients and have few or no side effects when used in low doses. It will be very difficult to find a bronchodilator that improves on the efficacy and safety of inhaled beta₂-agonists. Although some concerns have been expressed about the long-term effects of inhaled beta₂-agonists, the evidence suggests that they are safe when used as required for symptom control. If patients are using large doses, their asthma should be assessed; an appropriate anti-inflammatory treatment should be given, and attempts should be made to reduce the dose. Long-acting inhaled beta₂-agonists are a very useful addition for long-term control in asthma and COPD. In the future, these drugs may also be useful for treatment of acute exacerbations. Improvement in inhaled beta₂-agonists might include an increased selectivity for beta₂-receptors and a longer duration of action. There is little advantage to be gained by improving beta₂-receptor selectivity, because most of the side effects of beta-agonists are caused by beta₂-receptor stimulation (muscle tremor, tachycardia, hypokalemia). It may be possible to develop once-daily inhaled beta₂-agonists in the future. *R*-albuterol is the active enantiomer of albuterol; it was developed because of animal studies showing that the *S*-albuterol in the normal racemic (*RS*) mixture that is used clinically could induce airway hyperresponsiveness in guinea pigs.⁴³ In single doses, inhaled *R*-albuterol has an equivalent effect to *RS*-albuterol, whereas *S*-albuterol has a weak effect, providing little indication that the *R*-enantiomer will have any clinical advantage.⁴⁴

Theophylline

Methylxanthines such as theophylline, which are related to caffeine, have been used in the treatment of asthma since 1930. Theophylline is still the most widely used antiasthma therapy worldwide because it is inexpensive. Theophylline became more useful with the availability of rapid plasma assays and the introduction of reliable slow-release preparations.⁴⁵ However, the frequency of side effects and the relatively low efficacy of theophylline have led to reduced usage; inhaled beta₂-agonists are far more effective as bronchodilators, and inhaled steroids have a greater anti-inflammatory effect. However, in patients with severe asthma, theophylline still remains a very useful drug. There is increasing evidence that theophylline has an anti-inflammatory or immunomodulatory effect.⁴⁶

Chemistry

Theophylline is a methylxanthine similar in structure to the common dietary xanthines caffeine and theobromine. Several substituted derivatives have been synthesized, but none has any advantage over theophylline,⁴⁷ apart from the 3-propyl derivative, enprofylline, which is more potent as a bronchodilator and may have fewer toxic effects. Many salts of theophylline have also been marketed, the most common being aminophylline, which is the ethylenediamine salt used to increase solubility at neutral pH. Other salts, such as choline theophyllinate, do not have any advantage, and some, such as acepifylline, are virtually inactive.⁴⁸

Discovery of Novel Quaternary Ammonium Derivatives of (3*R*)-Quinuclidinol Esters as Potent and Long-Acting Muscarinic Antagonists with Potential for Minimal Systemic Exposure after Inhaled Administration: Identification of (3*R*)-3-[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (Aclidinium Bromide)

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The objective of this work was to discover a novel, long-acting muscarinic M₃ antagonist for the inhaled treatment of chronic obstructive pulmonary disease (COPD), with a potentially improved risk–benefit profile compared with current antimuscarinic agents. A series of novel quaternary ammonium derivatives of (3*R*)-quinuclidinol esters were synthesized and evaluated. On the basis of its overall profile, (3*R*)-3-[[hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (aclidinium bromide) emerged as a candidate for once-daily maintenance treatment of COPD. This compound is a potent muscarinic antagonist, with long duration of action in vivo, and was found to have a rapid hydrolysis in human plasma, minimizing the potential to induce class-related systemic side effects. Aclidinium bromide is currently in phase III development for maintenance treatment of patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD^a) is a major global health concern, which is characterized by airflow obstruction that deteriorates progressively and has a limited reversibility after bronchodilator therapy.¹ Projections from the World Health Organization predict that COPD will become the fourth most common cause of death by 2030 and the third most common cause of chronic disability by 2020.^{2,3} Acetylcholine is involved in airway smooth muscle contraction and narrowing, and cholinergic tone appears to be the major reversible component of airway obstruction in COPD.^{4–6}

Three subtypes of muscarinic receptor are expressed in the human airway: M₁, M₂, and M₃ receptors.⁷ The M₃ receptor is the primary subtype responsible for bronchial and tracheal smooth muscle contraction.⁸ M₂ receptors localized in cholinergic nerve endings act as negative feedback of acetylcholine release from the nerve.^{9,10} Finally, M₁ receptors are localized to parasympathetic ganglia in the airways, facilitating ganglionic neurotransmission.¹¹

Inhaled anticholinergics are an effective class of bronchodilators in the management of symptomatic patients with

COPD: ipratropium is a short-acting agent requiring up to four doses per day, whereas tiotropium is a long-acting, once-daily treatment (Figure 1).^{11–13} Certain systemic anticholinergic effects are associated with these compounds, including dry mouth (reported in 11.6% of patients treated with tiotropium bromide versus 3.5% of patients treated with placebo), glaucoma, constipation, increased heart rate, and urinary retention.^{14,15} Therefore, a compound that has similar or improved bronchodilatory efficacy to these agents but causes fewer unwanted anticholinergic effects would be desirable as a monotherapy or as part of a combination therapy because of its lower potential risk of additive systemic pharmacology and drug–drug interactions.

The objective of our work was to discover a novel and potent inhaled muscarinic M₃ antagonist suitable for once-daily administration with an improved safety profile for the maintenance treatment of COPD.

Chemistry

3-Quinuclidinol esters are known to be potent muscarinic antagonists, and various examples of these compounds have been described in the literature.^{16–22} Notably, the (3*R*)-quinuclidinol ester revatropate (Figure 1), an M₃, M₁ selective muscarinic antagonist, was developed as a potential treatment for COPD.^{23–25} This compound entered phase II clinical trials, although the development of revatropate has since been discontinued, probably because of its short duration of action.^{23,24}

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^aAbbreviations: CHO-K1, Chinese hamster ovary K1; COPD, chronic obstructive pulmonary disease; QNB, quinuclidinyl benzylate; MQNB, quinuclidinyl benzylate methiodide; PET, positron emission tomography.

Quaternization of the tertiary amino function is a common chemical manipulation in this class of compounds, widely used to minimize absorption across membranes, resulting in low oral bioavailability and blood–brain barrier permeability.^{26,27} Antimuscarinic activity has been reported for certain quaternary ammonium derivatives of quinuclidinyl benzylate (QNB), with aliphatic chains of different length on the N⁺ of the azoniabicyclo ring,^{28–30} although the potency and duration of action of these agents in the lung are unknown. Radiolabeled QNB and its methiodide salt MQNB are also used as muscarinic ligands in positron emission tomography (PET) to investigate receptor distribution and expression changes in several pathologies.^{31,32}

Here we describe the synthesis of a variety of new (3*R*)-quinuclidinol esters and their corresponding quaternary ammonium derivatives, which were prepared with the objective of studying the potential of this scaffold to deliver the desired activity profile. These compounds were synthesized as outlined in Schemes 1–4.

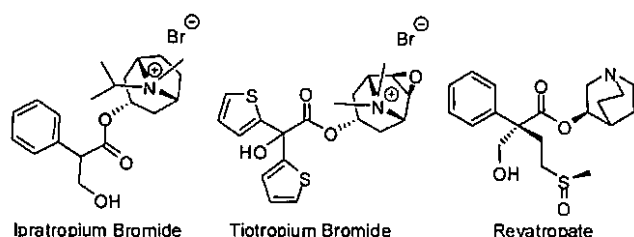
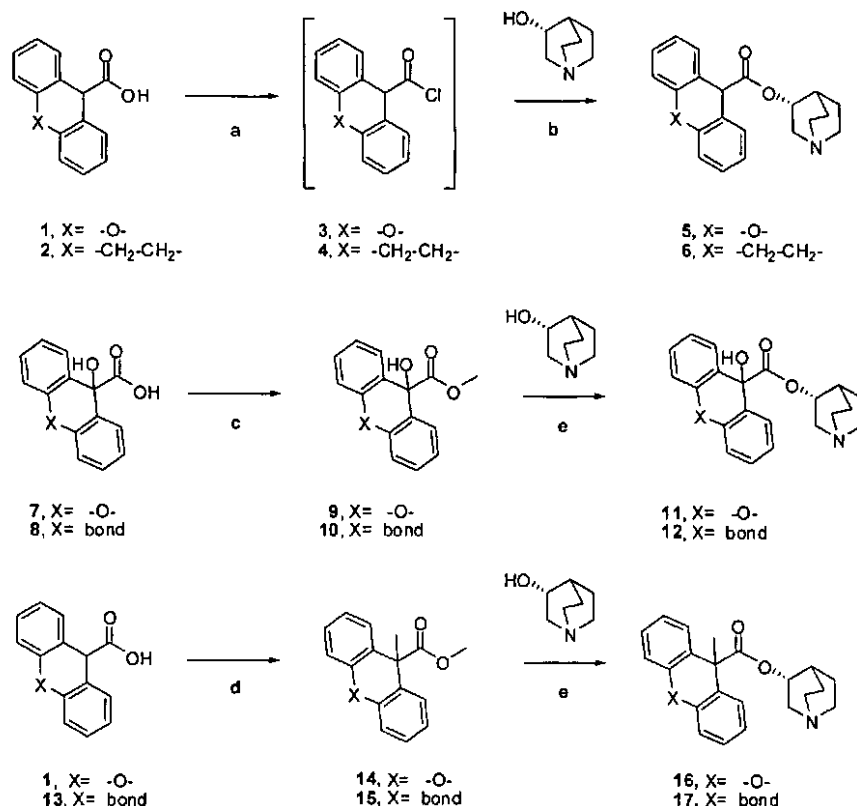


Figure 1

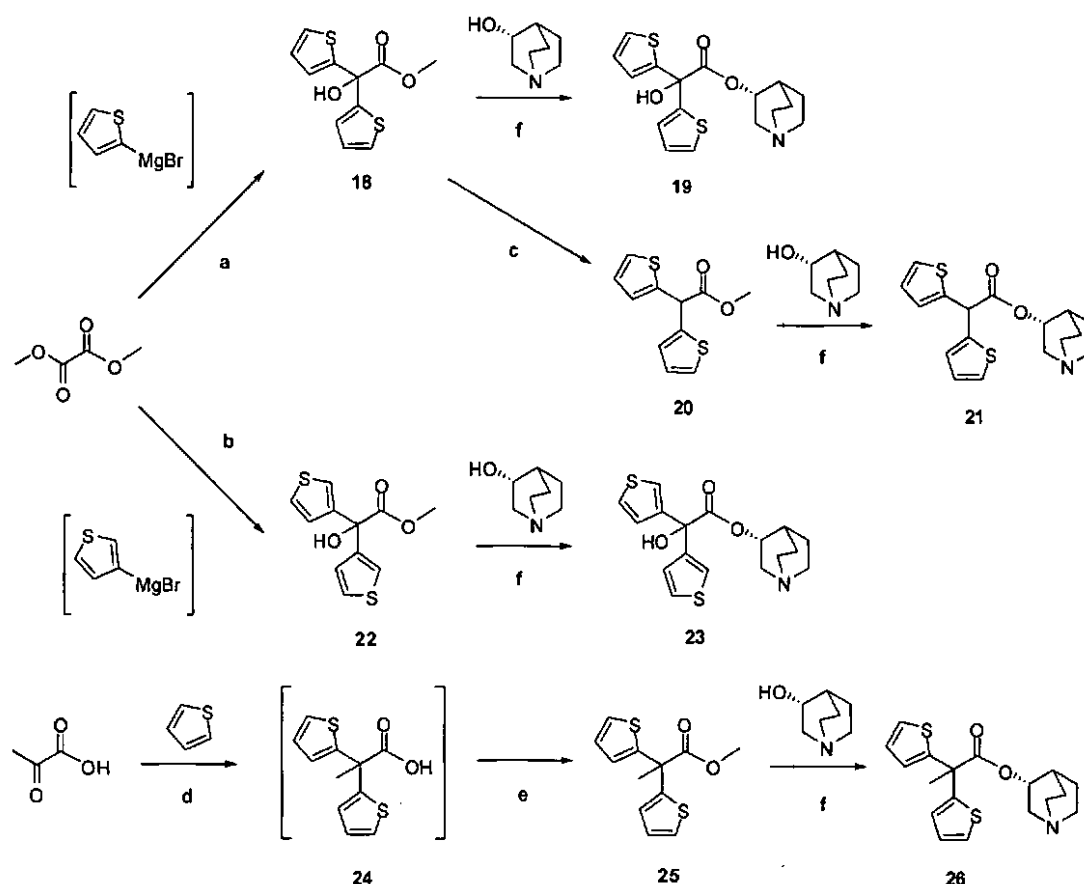
(3*R*)-Quinuclidinol esters 5,^{19,20} 6,^{11,33} 12,¹⁶ and 17 were prepared from carboxylic acids 1, 2,³⁴ 7,³⁵ 8, and 13 (Scheme 1). Transformation of acids 1 and 2 into their acyl chloride derivatives (compounds 3 and 4), followed by reaction with commercially available (3*R*)-quinuclidinol, led to the esters 5 and 6. (3*R*)-Quinuclidinol esters 11 and 12 were obtained from their corresponding methyl ester derivatives, 9 and 10, which were synthesized from α -hydroxy acids 7 and 8, respectively. Acids 1 and 13 were methylated in the α position and esterified in a one-pot reaction to give methyl ester derivatives 14 and 15. Transesterification of compounds 14 and 15 with (3*R*)-quinuclidinol yielded compounds 16 and 17.

Scheme 2 shows the synthesis of (3*R*)-quinuclidinol esters with a dithienyl moiety (compounds 19, 21, 23, and 26). The reaction of dimethyl oxalate with 2-thienylmagnesium bromide and 3-thienylmagnesium bromide afforded methyl esters 18 and 22, respectively.³⁶ Reduction of compound 18 by reaction with stannous chloride in acidic conditions³⁷ yielded the methyl ester 20. The reaction of 2-oxopropanoic acid with thiophene in acidic media gave acid 24,³⁸ which was transformed to its methyl ester 25 by standard methods. Reaction of synthesized methyl esters 18, 20, 22, and 25 with (3*R*)-quinuclidinol by means of sodium hydride in refluxing toluene afforded the desired compounds 19, 21, 23, and 26, respectively.

The synthesis of (3*R*)-quinuclidinol esters 33 ((*R*)-QNB¹⁹), 35, 36, 38, and 39 are described in Scheme 3. Transformation of commercially available acids 27 and 28 to their corresponding acyl chloride derivatives 29 and 30, followed by reaction with (3*R*)-quinuclidinol, afforded the glyoxalate derivatives

Scheme 1. Synthesis of (3*R*)-Quinuclidinol Esters 5, 6, 11, 12, 16, and 17^a

^a Reagents and conditions: (a) CHCl₃, oxalyl chloride, DMF (catalytic), room temp; (b) toluene, reflux, or CHCl₃, room temp; (c) MeOH, H₂SO₄ (catalytic), reflux, or acetone, K₂CO₃, (CH₃O)₂SO₂, reflux; (d) (i) THF, LDA (addition at 0 °C), reflux, (ii) CH₃I, room temp, (iii) MeOH, H₂SO₄, reflux; (e) toluene, HNa, reflux.

Scheme 2. Synthesis of (3*R*)-Quinuclidinol Esters 19, 21, 23, and 26^a

^a Reagents and conditions: (a) diethyl ether, reflux; (b) (i) diethyl ether, $-30\text{ }^{\circ}\text{C}$, (ii) diethyl ether, $0\text{--}5\text{ }^{\circ}\text{C}$; (c) AcOH glacial, HCl gas, $\text{Cl}_2\text{Sn}\cdot 2\text{H}_2\text{O}$, $15\text{ }^{\circ}\text{C}$; (d) H_2O , H_2SO_4 , $40\text{--}50\text{ }^{\circ}\text{C}$; (e) acetone, K_2CO_3 , $(\text{CH}_3\text{O})_2\text{SO}_2$, reflux; (f) toluene, HNa, reflux.

31³⁹ and 32, respectively. Reaction of compounds 31 and 32 with commercially available solutions of different organometallic reagents yielded esters 33, 34, and 37. Compounds 34 and 37 were both obtained as a mixture of diastereomers that were further separated by crystallization, yielding compounds 35, 36, 38, and 39, respectively.

The stereochemistry of the ester moiety in compounds 35, 36, 38, and 39 was deduced from the configuration of acids 40, 41, and 42, obtained by hydrolysis (Scheme 3). The configuration of these acids was elucidated by comparison of their optical properties (specific optical rotation $[\alpha]$, circular dichroism (CD) curve $[\Delta\epsilon]$) with published values of structurally related acids of known configuration.^{40–42}

Quaternary ammonium derivatives 43–64 were obtained by reaction of selected (3*R*)-quinuclidinol esters with a variety of bromoalkyl or bromoalkylaryl derivatives (Scheme 4).

Results and Discussion

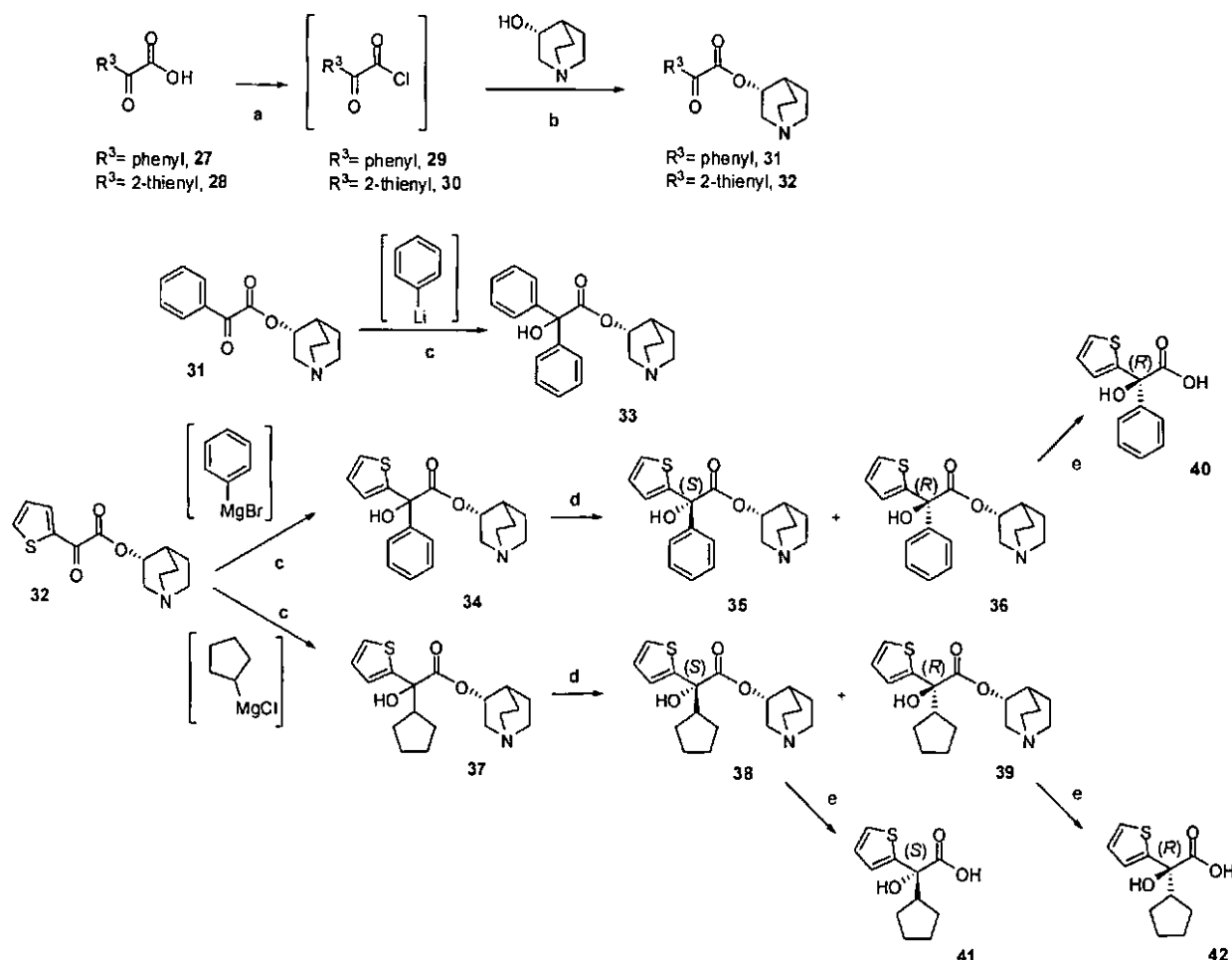
In Vitro Receptor-Binding Studies. We evaluated the muscarinic receptor-binding affinities of the synthesized compounds using membranes expressing the human M_1 , M_2 , and M_3 receptors transfected into Chinese hamster ovary-K1 (CHO-K1) cells. [^3H]-*N*-Methylscopolamine was used as a radioligand in displacement assays. Nonspecific binding was measured in the presence of atropine ($1\text{ }\mu\text{M}$).

The influence of R^1 in the binding affinity (IC_{50} , defined as the concentration required to induce 50% inhibition of muscarinic receptor activity) for some of the nonquaternized compounds previously described is shown in Table 1. The

affinities of tiotropium bromide and ipratropium bromide were also determined for reference.⁴³ When R^1 was a non-cyclized moiety, all tested derivatives were potent M_3 receptor ligands, with affinities at the low nanomolar range; however, like the reference compounds, they were not selective for M_3 receptors over M_1 and M_2 receptors. Among them, compound 19, with two 2-thienyl substituents, had the highest affinity for the M_3 receptor, with an IC_{50} 1.5- and 9-fold lower than tiotropium bromide and ipratropium bromide, respectively. The tested R^1 -cyclized subset of compounds maintained the low nanomolar muscarinic activity, with the exception of compound 6.

To explore the SAR around R^2 , we picked up two of the nonquaternized compounds previously described. We chose the most active derivative, compound 19, and also compound 12 as a representative of the R^1 -cyclized subset of compounds. Muscarinic receptor-binding affinities (IC_{50}) for their quaternized derivatives are shown in Table 2. Among the derivatives of compound 12 evaluated (compounds 46–48), the highest binding affinity was obtained for compound 48, with a phenoxypropyl chain. The binding affinity profile of compound 19 was retained for almost all of the quaternized derivatives tested (compounds 51–56). The highest potencies were obtained for compound 54, with a phenethyl chain, and compound 56, with a phenoxypropyl chain also.

On the basis of the in vitro profile, compounds 12 and 19 and their active derivatives were selected for further evaluation.

Scheme 3. Synthesis of (3*R*)-Quinuclidinol Esters 33, 35, 36, 38, and 39^a

^a Reagents and conditions: (a) CHCl_3 , oxalyl chloride, DMF (catalytic), room temp; (b) CHCl_3 , room temp; (c) THF, from $t \leq -40^\circ\text{C}$ to room temp; (d) separation of diastereomers by crystallization; (e) MeOH, NaOH, room temp or EtOH, NaOH 2 N, room temp.

In Vivo Duration of Action Studies. The duration of action of selected compounds was evaluated by measuring the inhibition of bronchospasm induced by acetylcholine in anesthetized guinea pigs at different time points.⁴⁴ Duration of action results for compounds 12 and 19 and their corresponding quaternized derivatives are shown in Table 2, along with data for reference compounds, tiotropium bromide and ipratropium bromide.

Compound 12 had a short duration of action (3.9 h), comparable to that of ipratropium bromide (3.4 h). However, quaternization with a heptyl group (compound 46), a phenethyl group (compound 47), or a phenoxypropyl group (compound 48) resulted in a longer duration of action (> 6 h), similar to that of tiotropium bromide. Most of the quaternized derivatives of compound 19 that were evaluated (compounds 51, 52, 54, and 56) retained the long duration of action observed for compound 19 (> 6 h). These results were comparable to those obtained for tiotropium bromide.

In conclusion, quaternization appeared to retain or improve the duration of action of these compounds relative to the corresponding unquaternized compounds.

On the basis of the results of M_3 receptor-binding affinity and duration of action studies, a series of compounds with phenethyl (compounds 43, 44, and 47) or phenoxypropyl (compounds 45, 48, 60, 63, and 64) as R^2 substituents and with diverse R^1 groups on the ester moiety were synthesized

and evaluated. M_3 receptor-binding affinities and duration of action data for these compounds are presented in Table 3. All tested compounds showed high M_3 muscarinic affinity, ranging from 0.18 to 1.62 nM, and exhibited long durations of action (> 6 h). Compounds with the highest M_3 receptor-binding affinities (compounds 54, 56, and 63) were selected for further evaluation.

In Vivo Characterization of Selected Compounds. Table 4 describes the duration of action and potency (IC_{50}) for compounds 54, 56, and 63, reverting the bronchospasm induced by acetylcholine in guinea pigs. These compounds showed relevant potency in this in vivo model, comparable to tiotropium bromide and ipratropium bromide.

We further evaluated the preliminary in vivo safety profiles of compounds 54, 56, and 63 in mice using an observational test focusing on antimuscarinic side effects. Tiotropium bromide and ipratropium bromide were also evaluated for reference. Each compound was administered intraperitoneally at a dose of 30 mg/kg. Compound 56 did not produce mydriasis, a class-related adverse effect of muscarinic antagonists, and had the most favorable safety profile of the three compounds tested. For all of the other compounds tested in mice, including tiotropium bromide and ipratropium bromide, mydriasis was observed. At this dose, all the compounds tested showed no other adverse effects.

Scheme 4. Preparation of Quaternary Ammonium Derivatives 43 to 64^a

starting compd	quaternary ammonium derivative				
	R ²				
	CH ₃ -	CH ₃ (CH ₂) ₆ -	PhCH ₂ -	Ph(CH ₂) ₂ -	PhO(CH ₂) ₃ -
5, 11, 12, 16, 17, 19, 21, 23, 26, 33, 35, 36, 38, 39					
5				43	
11				44	45
12		46		47	48
16					49
17					50
19	51	52	53	54	55
21					57
23					58
26					59
33					60
35 (3 <i>R</i> , 2 <i>S</i>)					61
36 (3 <i>R</i> , 2 <i>R</i>)					62
38 (3 <i>R</i> , 2 <i>S</i>)					63
39 (3 <i>R</i> , 2 <i>R</i>)					64

^a Reagents and conditions: (a) THF reflux or CHCl₃ room temp or CHCl₃/acetonitrile room temp.

On the basis of its *in vitro* binding affinity, *in vivo* potency, duration of action, and preliminary safety profile, compound **56** was selected for further biological characterization.⁴⁵

Human Plasma Stability Studies. To better understand the reasons for the favorable *in vivo* safety profile of compound **56** and to evaluate its potential for low systemic class-related side effects in humans, stability studies in human plasma for compounds **54**, **56**, and **63** were performed (Table 4). The plasma stability of tiotropium bromide and ipratropium bromide was also evaluated for reference. Each compound was incubated at 37 °C in human plasma, and the amount of remaining compound after 15 min were monitored. The degradation of this type of compound in human plasma would be expected to be mediated by ester cleavage. Surprisingly, compound **56** was almost fully degraded at the 15 min time point, providing a rationale for a wider safety margin of this compound. In order to elucidate the mechanism responsible of such a quick plasma degradation, we evaluated human plasma and chemical stability half-lives of these compounds at the same experimental conditions (37 °C and pH 7.4). In comparison with reference compounds, compounds **54** and **56** showed human plasma stability half-lives much shorter than the corresponding chemical stability half-lives, suggesting that plasma degradation of compounds **54** and **56** is mainly driven by enzymatic cleavage.

This interesting result led us to investigate the human plasma stability of several (3*R*)-quinuclidinol esters up to 60 min.

Table 5 shows the influence of R² substitution on plasma stability for analogues of compound **56**. All the quinuclidinol

esters described in Table 5 were less stable in human plasma than tiotropium bromide and ipratropium bromide (scopine and tropine derivatives). Quaternized compounds **51**, **54**, and **56** were less stable than the unquaternized compound **19**. Thus, to achieve rapid hydrolysis in human plasma, quaternization of the (3*R*)-quinuclidinol ester **19** is required.

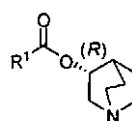
Tables 6 and 7 show the influence of the R¹ group on the rate of degradation in human plasma. The presence of two 2-thienyl substituents on the R¹ group (compound **56**) reduced stability in human plasma compared with the presence of two 3-thienyl substituents (compound **58**) or two phenyl substituents (compound **60**). Replacing one 2-thienyl group with a phenyl group (compounds **61** and **62**) or a cyclopentyl group (compounds **63** and **64**) increased plasma stability compared with compound **56**. For these compounds with a chiral α carbon, the 2*R*-enantiomers (compounds **62** and **64**) were less stable than the corresponding 2*S*-enantiomers (compounds **61** and **63**).

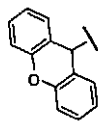
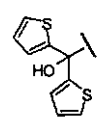
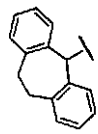
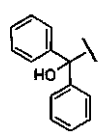
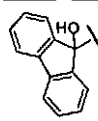
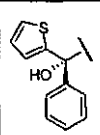
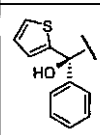
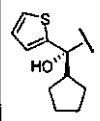
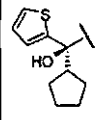
Bridging the two phenyl groups of compound **60** (compound **48**) resulted in lower stability. Substitution of the fluorenyl group (compounds **47** and **48**) by a xanthenyl group (compounds **44** and **45**) resulted in greater stability.

The human plasma stability was greater for compounds with a methyl substituent (compounds **49**, **50**, and **59**) than for compounds with a hydroxyl substituent (compounds **45**, **48**, and **56**), and compounds with a hydrogen substituent were the least stable (compound **43** vs compound **44**; and compound **57** vs compound **56**).

In summary, among the (3*R*)-quinuclidinol esters evaluated for human plasma stability, compounds **51**, **54**, **56**, and **57** were found to degrade most rapidly in human plasma.

Table 1. Effects of R¹ Substitution on M₁, M₂, and M₃ Muscarinic Receptor-Binding Affinities for (3*R*)-Quinuclidinol Ester Derivatives



R ¹	compd	binding affinity ^a (IC ₅₀ , nM)			R ¹	compd	binding affinity ^a (IC ₅₀ , nM)		
		M ₃	M ₂	M ₁			M ₃	M ₂	M ₁
	5	0.76 (0.01)	2.83 (0.37)	0.31 (0.04)		19	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)
	6	94 (1)	79 (1)	15 (1)		33	0.90 (0.05)	0.40 (0.06)	0.36 (0.004)
	12	0.92 (0.13)	1.14 (0.18)	0.26 (0.02)		35 (2 <i>S</i> -isomer)	0.31 (0.03)	0.21 (0.02)	0.20 (0.04)
						36 (2 <i>R</i> -isomer)	0.42 (0.001)	0.26 (0.01)	0.23 (0.01)
						38 (2 <i>S</i> -isomer)	0.39 (0.05)	0.23 (0.04)	0.19 (0.02)
						39 (2 <i>R</i> -isomer)	0.70 (0.14)	0.54 (0.005)	0.24 (0.08)
tiotropium bromide		0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)					
ipratropium bromide		2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)					

^a Values shown are the mean, *n* = 2 (SD). ^b Values shown are the mean, *n* = 3 (SD).

These results help us to understand the more favorable in vivo safety profile of compound **56** compared with the other compounds tested and reinforce the decision to take compound **56** forward as a candidate for further studies.

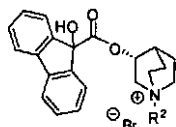
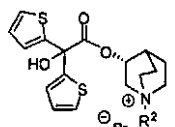
Conclusion

With the objective of identifying a novel, once-daily anti-muscarinic agent for the maintenance treatment of COPD by inhalation, we synthesized a series of quaternized (3*R*)-quinuclidinol esters. In vitro and in vivo tests were then conducted to evaluate the potential beneficial characteristics of these compounds in terms of clinical efficacy (muscarinic receptor-binding affinity, duration of action) and low class-related side effects (preliminary in vivo safety, human plasma stability). On the basis of these preclinical results, compound **56** (acridinium bromide) was selected as a candidate for a complete biological characterization. Acridinium bromide

showed high M₃ receptor-binding affinity and had duration of action longer than that of ipratropium bromide and comparable to that of tiotropium bromide. Acridinium bromide also demonstrated a favorable in vivo safety profile likely to be due to its rapid human plasma hydrolysis, indicating low potential for systemic class-related side effects. The combination of high potency, long duration of action, low oral absorption, and rapid plasma degradation in the same molecule led us to a novel, effective, and long-acting anticholinergic compound with the potential for a wider safety margin than currently available inhaled anticholinergic therapies. Overall, this profile supports the potential for this compound as a safe and effective monotherapy or as part of a combination therapy for COPD.

Acridinium bromide is currently in phase III development for maintenance treatment of patients with COPD and in phase II studies in combination with a long-acting β₂-agonist.

Table 2. Effects of R² Substitution on the M₁, M₂, and M₃ Muscarinic Receptor-Binding Affinities and in Vivo Duration of Action for Novel Quaternized (3*R*)-Quinuclidinol Ester Derivatives

R ²												
	binding affinity ^a (IC ₅₀ , nM)					duration of action	binding affinity ^a (IC ₅₀ , nM)					duration of action
	compd	M ₃	M ₂	M ₁	T (h)		compd	M ₃	M ₂	M ₁	T (h)	
–	12	0.92 (0.13)	1.14 (0.18)	0.26 (0.02)	3.9 ^c		19	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)	> 6 ^c	
CH ₃ –							51	0.60 (0.01)	0.46 (0.003)	0.43 (0.12)	> 6 ^c	
CH ₃ (CH ₂) ₆ –	46	1.68 (0.08)	4.50 (0.03)	3.10 (0.17)	> 6 ^d		52	0.39 (0.003)	0.36 (0.09)	0.41 (0.04)	> 6 ^c	
PhCH ₂ –							53	200 (1)	12 (1)	100 (52)	NE ^f	
Ph(CH ₂) ₂ –	47	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	> 6 ^d		54	0.20 ^b (0.003)	0.18 ^b (0.06)	0.14 ^b (0.04)	> 6 ^c	
Ph(CH ₂) ₄ –							55	0.45 (0.07)	0.38 (0.12)	0.29 (0.02)	6 ^d	
PhO(CH ₂) ₃ –	48	0.71 (0.07)	1.08 (0.14)	0.63 (0.02)	> 6 ^c		56	0.17 ^b (0.05)	0.17 ^b (0.05)	0.14 ^b (0.03)	> 6 ^d	
							tiotropium bromide	0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)	> 6 ^d	
							ipratropium bromide	2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)	3.4 ^e	

^a Values shown are the mean, *n* = 2 (SD). ^b Values shown are the mean, *n* = 3 (SD). ^c Administered concentration of 3 mg/mL, *n* = 4–6 animals.^d Administered concentration of 1 mg/mL, *n* = 4–6 animals. ^e Administered concentration of 0.3 mg/mL, *n* = 4–6 animals. ^f NE = not evaluated. The duration of action for compound 53 was not evaluated because its muscarinic receptor binding affinity was too low.

Experimental Section

Biology: General. (3*R*)-Quinuclidinol ester compounds and tiotropium bromide were synthesized by the Department of Medicinal Chemistry (Laboratorios Almirall, Barcelona, Spain). Ipratropium bromide was purchased from Sigma Chemicals (Tres Cantos, Spain). All antagonists were dissolved in dimethyl sulfoxide.

In Vitro Receptor-Binding Studies. All binding assays were performed in 96-well plates (Nunc, Thermo Fischer Scientific, Roskilde, Denmark) with membrane preparations expressing human M₁, M₂, and M₃ receptors (transfected CHO-K1 cells; Membrane Target Systems, Perkin-Elmer Life and Analytical Sciences, Boston, MA). 1-[*N*-Methyl-³H]scopolamine methyl chloride ([³H]NMS specific activity of 82 Ci mmol) from Perkin-Elmer Life and Analytical Sciences (Boston, MA) was used in all binding affinity studies.

The membrane protein concentrations were 8.1, 5, and 4.9 μg/well for M₁, M₂, and M₃ receptors, respectively. The [³H]NMS concentration was 0.3 nM for M₁ and 1 nM for both M₂ and M₃ assays. A range of antagonist concentrations (10^{−14}–10^{−5} M) was tested to generate competition curves. Nonspecific binding was determined in the presence of 1 μM atropine. Reagents were dissolved in assay binding buffer (phosphate buffered saline with calcium and magnesium) to a total volume of 200 μL. After a 4 h incubation period at room temperature in order to reach equilibrium, 150 μL samples of the reaction mixtures were transferred to GF/C filter plates pretreated for 1 h with wash buffer containing 0.05% polyethylenimine. Bound and free [³H]NMS samples were then separated by rapid vacuum filtration and four washes with ice-cold wash buffer. Samples were dried for 30 min before addition of 30 μL of OptiPhase Supermix

(Perkin-Elmer Life and Analytical Sciences, Boston, MA), and radioactivity was quantified using a MicroBeta microplate scintillation counter (Trilux, Perkin-Elmer Boston, MA). The binding affinities (IC₅₀ values) for each antagonist were determined for the three muscarinic receptors using GraphPad Prism software (San Diego, CA).

In Vitro Human Plasma Stability Studies. The stability of the test compounds was assessed using pooled human plasma samples from 10 healthy volunteers. Blood samples were collected in lithium heparin tubes, and the plasma was separated by centrifugation, pooled, and stored at −20 °C until use.

Stock solutions of test compounds (2 mM) were prepared by dissolving corresponding amounts of compounds in 0.4 mL of 0.1 N hydrochloric acid before adding acetonitrile to a final volume of 2 mL. Working solutions of test compounds were prepared by diluting from 2 mM stock solutions in water. All working solutions were prepared immediately before use. The final concentration of the test compounds was 1 μM.

Human plasma samples were incubated in triplicate for 5 min at 37 °C (Unitronic 320 OR, JPselecta, Spain) before the addition of the test compound to initiate the reaction (final concentration, 1 μM). At predetermined times during incubation (0, 15, and 60 min), aliquots of the incubation mixtures (100 μL) were transferred to another tube containing 300 μL of cold acetonitrile/1 N HCl (90/10, v/v) to stop the reaction. Samples were then centrifuged at 2800g for 10 min at 4 °C. Control incubations in the absence of the test compound were also performed. Additional incubations for the most unstable compounds (51, 56, and 57) were also performed at predetermined times (0, 5, 10, and 15 min) and assayed as indicated above.

Table 3. Effects of R¹ Substitution on the M₃ Muscarinic Receptor-Binding Affinities and the in Vivo Duration of Action for Novel Quaternized (3*R*)-Quinuclidinol Ester Derivatives (R² = Phenethyl; R² = Phenoxypropyl)

R ¹	R ² =					R ² =				
	binding affinity ^a (IC ₅₀ , nM)				duration of action	binding affinity ^a (IC ₅₀ , nM)				duration of action
	cmpd	M ₃	M ₂	M ₁	T (h)	cmpd	M ₃	M ₂	M ₁	T (h)
	54	0.20 ^b (0.003)	0.18 ^b (0.06)	0.14 ^b (0.04)	> 6 ^c	56	0.17 ^b (0.05)	0.17 ^b (0.05)	0.14 ^b (0.03)	> 6 ^d
	43	0.31 (0.02)	1.82 (0.19)	0.44 (0.03)	> 6 ^d					
	44	0.28 (0.12)	0.54 (0.15)	0.29 (0.10)	> 6 ^d	45	0.42 (0.03)	0.36 (0.06)	0.23 (0.01)	> 6 ^d
	47	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	> 6 ^d	48	0.71 (0.07)	1.08 (0.14)	0.63 (0.02)	> 6 ^c
						60	0.61 (0.03)	0.44 (0.01)	0.31 (0.05)	> 6 ^c
						63 (2 <i>S</i> - isomer)	0.18 ^b (0.02)	0.14 ^b (0.04)	0.15 ^b (0.01)	> 6 ^d
						64 (2 <i>R</i> - isomer)	1.62 (0.21)	1.01 (0.29)	0.81 (0.17)	> 6 ^c
	tiotropium bromide	0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)	> 6 ^d					
	ipratropium bromide	2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)	3.4 ^e					

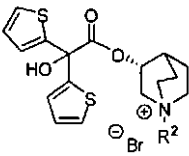
^a Values shown are the mean, *n* = 2 (SD). ^b Values shown are the mean, *n* = 3 (SD). ^c Administered concentration of 3 mg/mL, *n* = 4–6 animals. ^d Administered concentration of 1 mg/mL, *n* = 4–6 animals. ^e Administered concentration of 0.3 mg/mL, *n* = 4–6 animals.

Table 4. Overall in Vitro and in Vivo Characterization of Selected Compounds

compd	binding affinity, ^a <i>K</i> _i (nM)			in vivo model		safety observational test muscarinic-related side effects (30 mg/kg, ip) ^d	human plasma stability, ^{a,b}		chemical stability, ^{a,c} <i>t</i> _{1/2} (min) ^e
	<i>M</i> ₃	<i>M</i> ₂	<i>M</i> ₁	duration of action, <i>T</i> (h)	IC ₅₀ (μg/mL)		% disappearance (15 min)	<i>t</i> _{1/2} (min) ^e	
54	0.08 (0.001)	0.08 (0.02)	0.08 (0.02)	> 6 ^f	54	mydriasis	68 (3)	7.9	56
56	0.07 (0.02)	0.08 (0.02)	0.08 (0.02)	> 6 ^g	140	none	99 (0)	2.2	60
63	0.07 (0.01)	0.06 (0.01)	0.09 (0.004)	> 6 ^g	120	mydriasis	< 5	> 60	> 60
tiotropium bromide	0.14 (0.002)	0.10 (0.02)	0.12 (0.02)	> 6 ^g	45	mydriasis	5 (6)	> 60	> 60
ipratropium bromide	0.83 (0.01)	1.02 (0.07)	1.51 (0.08)	3.4 ^h	68	mydriasis	< 5	> 60	> 60

^a Values shown are the mean, *n* = 3 (SD). ^b Experimental conditions: pH 7.4, *T* = 37 °C. ^c Values shown are the mean, *n* = 2 (SD). ^d ip = intraperitoneal. ^e Half-life calculated with at least three experimental time points. ^f Administered concentration of 3 mg/mL, *n* = 4–6 animals. ^g Administered concentration of 1 mg/mL, *n* = 4–6 animals. ^h Administered concentration of 0.3 mg/mL, *n* = 4–6 animals.

Table 5. Effect of R² Substitution on the in Vitro Human Plasma Stability of Compound 19 Derivatives



R ²	compd	binding affinity, ^a IC ₅₀ (nM)			human plasma stability			
		<i>M</i> ₃	<i>M</i> ₂	<i>M</i> ₁	% disappearance ^b			<i>t</i> _{1/2} (min) ^c
					5 min	15 min	60 min	
	19	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)	NE ^d	14 (5)	53 (6)	54.5
CH ₃ –	51	0.60 (0.01)	0.46 (0.003)	0.43 (0.12)	51 (1)	94 (0)	NE ^d	3.6
Ph(CH ₂) ₂ –	54	0.20 ^b (0.003)	0.18 ^b (0.06)	0.14 ^b (0.04)	NE ^d	68 (3)	99 (0)	7.9
PhO(CH ₂) ₃ –	56	0.17 ^b (0.05)	0.17 ^b (0.05)	0.14 ^b (0.03)	78 (2)	99 (0)	NE ^d	2.2
	tiotropium bromide	0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)	NE ^d	5 (6)	35 (3)	> 60
	ipratropium bromide	2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)	NE ^d	< 5	< 5	> 60

^a Values shown are the mean, *n* = 2 (SD). ^b Values shown are the mean, *n* = 3 (SD). ^c Half-life calculated with at least three experimental time points. ^d NE = not evaluated.

The analyses of test compounds were performed by ultra-performance liquid chromatography (Acquity Ultra Performance LC, Waters, Milford, MA) with a Quattro Premier mass spectrometry detector operating in positive mode (Micromass Technologies, Waters, Milford, MA).

In all experiments, the stability of test compounds was determined as the percentage of remaining compound at the time point of interest (assuming 100% of the compound is present at time 0).

The test compound elimination half-life (*t*_{1/2}) in human plasma was estimated using the WinNonlin software, version 5.0.1. (Pharsight Corporation). The slope of the linear regression from log [S] versus time plot (–*k*) was determined, and the in vitro plasma half-life was determined as *t*_{1/2} = –0.693/*k*.

Chemical Stability Studies. The stability of the test compounds was assessed by monitoring for 24 h the 210 nm UV–UPLC chromatograms of 0.05 mg/mL compound solutions at 37 °C and pH 7.4.

An amount of 1 mg/mL test solution of each compound in DMSO (dimethyl sulfoxide) was prepared. At time zero, 50 μL of the test solution was added to 1 mL of pH 7.4 buffer solution (final concentration, 0.05 mg/mL). This solution was placed in the UPLC sample manager thermostabilized at 37 °C, and an amount of 5 μL was injected into the UPLC system (time zero). Final test solutions were prepared in duplicate. At predetermined times (0, 1, 4, 8, 12, 16, 20, and 24 h), an amount of 5 μL of each solution was injected in the UPLC system.

Control solutions for each compound were prepared using acetonitrile instead of pH 7.4 buffer solution. All compounds were stable in acetonitrile solutions.

The analyses of test compounds were performed by ultra-performance liquid chromatography (Acquity Ultra Performance LC, Waters, Milford, MA) with a PDA Acquity detector (Waters, Milford, MA).

In all experiments, the stability of test compounds was determined by integration of 210 nm extracted and blank subtracted chromatogram.

All obtained chromatograms were processed using Empower software (Waters, Milford, MA). The 210 nm chromatograms were extracted from 3D diode array signal, and a blank chromatogram was subtracted. Purity of compounds tested at each time point was determined by area normalization.

The test compound stability half-life (*t*_{1/2}) at 37 °C and pH 7.4 was estimated using the Kinetica software, version 4.2 (InnaPhase). The slope of the linear regression from log [S] versus time plot (–*k*) was determined, and the chemical stability half-life was determined as *t*_{1/2} = –0.693/*k*.

In Vivo Studies. Animals. Male Dunkin–Hartley guinea pigs (400–600 g) or male Swiss mice (20–25 g) were obtained from Harlan (Interfauna Ibérica, Spain). Animals were housed in groups of four or five at 20–24 °C under a 12 h light/dark cycle. Food and water were available ad libitum. All experiments were carried out with the approval of the Animal Ethical Committee of Almirall.

In Vivo Duration of Action Studies. Guinea pigs were anesthetized with an intraperitoneal injection of 1 g/kg urethane and 20 mg/kg sodium pentobarbital. Additional anesthetic was administered after 60 min, as required. The trachea was cannulated, and the lungs were artificially ventilated with a small rodent ventilator (Ugo Basile, Biological Research Apparatus, Comerio-Varese, Italy) at a rate of 60 strokes/min and a tidal volume of 10 mL/kg. Animals were maintained at 37 °C with a homeothermic blanket throughout the experiment. Blood pressure was measured from the carotid artery, and acetylcholine was administered via the jugular vein. Intrapulmonary pressure and blood pressure were measured by blood pressure transducers (MLT0699, ADInstruments-Panlab, Barcelona, Spain) connected to a bridge amplifier (PowerLab/8sp, ADInstruments-Panlab, Madrid, Spain).

Table 6. Effect of R¹ Substitution on the in Vitro Human Plasma Stability of Compound 54 Analogues

R ¹	compd	binding affinity ^a (IC ₅₀ , nM)			human plasma stability			
		M ₃	M ₂	M ₁	(% disappearance) ^b			t _{1/2} (min) ^c
					5 min	15 min	60 min	
	54	0.20 ^b (0.003)	0.18 ^b (0.06)	0.14 ^b (0.04)	NE ^d	68 (3)	99 (0)	7.9
	43	0.31 (0.02)	1.82 (0.19)	0.44 (0.03)	NE ^d	22 (6)	53 (4)	56.1
	44	0.28 (0.12)	0.54 (0.15)	0.29 (0.10)	NE ^d	7 (11)	15 (3)	> 60
	47	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	NE ^d	35 (4)	90 (1)	17.6
tiotropium bromide		0.35 ^b (0.01)	0.21 ^b (0.04)	0.22 ^b (0.03)	NE ^d	5 (6)	35 (3)	> 60
ipratropium bromide		2.07 ^b (0.03)	2.24 ^b (0.16)	2.65 ^b (0.13)	NE ^d	< 5	< 5	> 60

^a Values shown are the mean, *n* = 2 (SD). ^b Values shown are the mean, *n* = 3 (SD). ^c Half-life calculated with at least three experimental time points. ^d NE = not evaluated.

The data were recorded using Chart 5 software (ADInstruments-Panlab, Barcelona, Spain).

After induction of anesthesia and preparation, animals were allowed to stabilize for 10 min before bronchoconstriction was induced by an intravenous administration of acetylcholine. Acetylcholine (Sigma, Tres Cantos, Spain) was administered at a dose (10–60 μg/kg) that approximately doubled the basal intrapulmonary pressure. Repeated bolus injections of acetylcholine at the selected dose were administered until two reproducible responses were obtained; the mean of the two final responses before the addition of the test compound corresponded to the maximal response to acetylcholine and was used to evaluate the antibronchoconstrictor effect of the test compounds.

Five minutes after the last administration of acetylcholine that was used to calculate maximal bronchoconstriction, the test compound was administered via a nebulizer (5 s duration; Mumed ultrasonic nebulizer, Mumed Systems Ltd., London, U.K.) to investigate the reversal of the bronchoconstriction. Test compounds were delivered in the concentration range 0.3–3 mg/mL. Acetylcholine doses were then administered 5, 10, 20, 30, 60, 120, 180, 240, 300, and 360 min after the administration of the test compound to evaluate the antibronchoconstrictor effects of each compound. This was expressed as a percentage of the maximal response to acetylcholine. Duration

of action (*T*, h) was defined as the time taken to recover 50% of the maximum inhibitory effect achieved by the test compound and was derived from a time-course bronchoconstriction curve and calculated with a one-phase exponential decay formula using GraphPad Prism software (San Diego, CA).

In Vivo Bronchospasm Inhibitory Activity. The in vivo potency (IC₅₀) of selected compounds was determined using the guinea pig model described above, at the *t*_{max} (data not shown), from a sigmoidal dose–response curve. *T*_{max} was defined as the time taken from the administration of the test compound to the time at which maximum inhibition of the acetylcholine-induced bronchoconstriction was achieved. The IC₅₀ was calculated using GraphPad Prism software (San Diego CA).

In Vivo Safety Observational Test. Assessment of safety was performed for selected compounds administered intraperitoneally at a dose of 30 mg/kg, using an in vivo safety observational test focusing on antimuscarinic side effects (e.g., mydriasis, xerostomia) in Swiss mice. This assay comprises standardized observational tests that assess the neurobiological state of rodents and include measures of autonomic and sensorimotor functions, convulsive behavior, and excitability up to 24 h after drug administration.

Chemistry: General. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as

received. Concentration refers to evaporation under vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40–63 μm) with the solvent system indicated. Melting points were recorded on a Büchi B-540 instrument. ^1H NMR spectra were performed using a Varian Gemini 2000 spectrometer operating at a frequency of 200 or 300 MHz. Samples were dissolved in deuterated chloroform (CDCl_3) or deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$). Tetramethylsilane (TMS) was used as reference. The following abbreviations were used to assign spectra: (s) singlet, (d) doublet, (dd) double doublet, (ddd) double double doublet, (t) triplet, (dt) double triplet, (td) triple doublet, (m) multiplet, (br s) broad signal. HPLC–UV–MS chromatograms were acquired in a Waters Alliance 2695 chromatographer equipped with a Waters 2996 diode-array detector and a Waters ZQ mass spectrometer detector. HPLC analysis was conducted according to method A, B, or C, with the retention time (t_R) expressed in min. UV chromatograms were processed at 210 nm with blank subtraction. For HPLC method A, chromatography was performed on a Kromasil C18 column (100 mm $\times$ 4.6 mm, 5 μm). The mobile phase, at a flow of 1 mL/min, was a 30 min binary gradient (5–85%) of water (containing 0.01 M phosphoric acid at pH 3.0 with sodium hydroxide, 0.1 N) and acetonitrile. The total run time was 35 min. For HPLC method B, chromatography was performed on a Symmetry C18 column (100 mm $\times$ 2.16 mm, 3.5 μm). The mobile phase, at a flow of 0.4 mL/min, was a 20 min binary gradient of water (containing 0.01 M ammonium formate at pH 3.0) and a mixture of acetonitrile–methanol 50:50 (containing 0.01 M ammonium formate) (0–95%). The total run time was 26 min. For HPLC method C, chromatography was performed on a Symmetry C18 column (100 mm $\times$ 2.16 mm, 3.5 μm). The mobile phase, at a flow of 0.4 mL/min, was a 20 min binary gradient of water (containing 0.01 M ammonium formate at pH 7.4) and a mixture of acetonitrile/methanol, 50:50 (containing 0.01 M ammonium formate) (0–95%). The total run time was 26 min. Specific optical rotations were measured in a Perkin-Elmer 241 MC polarimeter at the wavelength of the D-line of sodium ($\lambda = 589.3$ nm) at 25 or 22 $^\circ\text{C}$ using a layer of 1 dm. Circular dichroism spectra were recorded on a Jasco J-720 spectropolarimeter controlled by J-700 software using the following acquisition conditions: optical path 0.1 cm, temperature 25 $^\circ\text{C}$, purge gas nitrogen at 5 L/min, spectral width 1.0 nm, response time 4 s, wavelength 200–300 nm, scan speed 10 nm/min, number of scans 2. Samples were dissolved in methanol (concentration, 0.43 mM).

Tiotropium bromide was synthesized by the Department of Medicinal Chemistry (Laboratorios Almirall, Barcelona, Spain). Ipratropium bromide was purchased from Sigma Chemicals (Tres Cantos, Spain). The purity of these compounds was determined by HPLC–MS.

The methods used for the preparation of compounds **2**,³⁴ **7**,³⁵ **9**, **10**, **14**, and **15** (Scheme 1); **18**,³⁶ **20**, **22**,³⁶ and **25** (Scheme 2); and **31**³⁹ and **32** (Scheme 3) are available as Supporting Information.

Preparation of (3R)-Quinuclidinol Esters 5^{19,20} and 6 (Scheme 1). (3R)-1-Azabicyclo[2.2.2]oct-3-yl 9H-xanthene-9-carboxylate (**5**)^{19,20}. To a solution of the acid **1** (5 g, 22.1 mmol) in 65 mL of CHCl_3 (amylene stabilized), cooled to 0 $^\circ\text{C}$, was added DMF (2 drops) followed by oxalyl chloride (3.1 mL, 36.6 mmol) dissolved in 5 mL of CHCl_3 . After 1.5 h of stirring at room temperature, the reaction mixture was concentrated and the residue was coevaporated with CHCl_3 three times to give the acyl chloride **3** (100%). Compound **3**, dissolved in 30 mL of CHCl_3 , was slowly added to a solution of (3R)-quinuclidinol (3.05 g, 23.9 mmol) in 50 mL of CHCl_3 . The mixture was stirred at room temperature overnight. After this reaction time, solvent was evaporated and the residue was dissolved in toluene and extracted with 2 N HCl. The acid layers were combined and basified with solid K_2CO_3 . The basic solution was extracted with

CHCl_3 . The organic layers were combined, washed with water, dried over MgSO_4 , and concentrated. The obtained residue was purified by formation of the hydrochloride salt. The residue was dissolved in diethyl ether (150 mL), and a solution of EtOH/HCl (g) was slowly added. The solid obtained was filtered and washed with diethyl ether to give 5.6 g (68%) of the hydrochloride salt of compound **5**, mp 227 $^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.55–1.93 (m, 4 H), 2.06–2.17 (m, 1 H), 2.82–2.99 (m, 2 H), 2.98–3.15 (m, 2 H), 3.15–3.29 (m, 1 H), 3.48–3.63 (dd, $J = 12.8, 9.2$ Hz, 1 H), 4.88–4.99 (m, 1 H), 5.27 (s, 1 H), 7.11–7.28 (m, 4 H), 7.38 (dd, $J = 8.2, 7.0$ Hz, 2 H), 7.49 (dd, $J = 7.0, 5.5$ Hz, 2 H). HPLC (method B): 96.6%, $t_R = 11.0$ min. MS (ESI) 336 m/z ($M + \text{H}$)⁺.

(3R)-1-Azabicyclo[2.2.2]oct-3-yl 10,11-dihydro-5H-dibenzo[*a,d*][7]annulene-5-carboxylate (**6**) was prepared as described for compound **5** starting from the acid **2**³⁴ (2.15 g, 9.0 mmol) and (3R)-quinuclidinol (1.26 g, 9.9 mmol). The reaction conditions used in the second step were as follows: toluene, reflux, 2 h. Purification by column chromatography (silica gel, chloroform/methanol/ NH_4OH , 95:5:0.5, as eluent) afforded 1.5 g (48%) of compound **6**, mp 112–113 $^\circ\text{C}$. ^1H NMR (300 MHz, CDCl_3) δ ppm 1.07–1.36 (m, 2 H), 1.36–1.53 (m, 1 H), 1.54–1.69 (m, 1 H), 1.84–1.93 (m, 1 H), 2.47–2.58 (m, 2 H), 2.59–2.76 (m, 3 H), 2.80–3.00 (m, 2 H), 3.06–3.18 (ddd, $J = 14.7, 7.9, 2.1$ Hz, 1 H), 3.25–3.43 (m, 2 H), 4.75–4.83 (m, 1 H), 4.82 (s, 1 H), 7.09–7.33 (m, 8 H). HPLC (method B): 99.8%, $t_R = 11.4$ min. MS (ESI) 348 m/z ($M + \text{H}$)⁺.

Preparation of (3R)-Quinuclidinol Esters 11,³³ 12, 16, and 17 (Scheme 1) and **19**, **21**, **23**, and **26** (Scheme 2). (3R)-1-Azabicyclo[2.2.2]oct-3-yl 9-hydroxy-9H-xanthene-9-carboxylate (**11**)³³. To a stirred solution of the methyl ester **9** (2 g, 7.8 mmol) and (3R)-quinuclidinol (1.16 g, 9.1 mmol) in 50 mL of toluene was added NaH 60% in mineral oil (0.125 g, 3.12 mmol) in several portions. The mixture was refluxed with continuous removal of distillate and replacement with fresh toluene over 1.5 h. After this time, the reaction mixture was cooled to room temperature and extracted with 2 N HCl. The acid extracts were combined, washed with a small volume of AcOEt , basified by addition of solid K_2CO_3 , and extracted with CHCl_3 . The organic extracts were combined, washed with water, dried over Na_2SO_4 , and concentrated. The obtained product was purified by dissolving in diethyl ether and repeating the acid/base extraction process to afford 1.86 g (68%) of compound **11**, mp 175 $^\circ\text{C}$. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 0.88–1.09 (m, 2 H), 1.22–1.49 (m, 2 H), 1.53–1.62 (m, 1 H), 1.88–1.98 (d, $J = 14.3$ Hz, 1 H), 1.99–2.14 (m, 1 H), 2.37–2.57 (m, 3 H), 2.81–2.93 (ddd, $J = 14.6, 8.0, 2.4$ Hz, 1 H), 4.49–4.58 (m, 1 H), 7.01–7.09 (br s, 1 H), 7.13–7.28 (m, 4 H), 7.33–7.45 (m, 2 H), 7.58–7.65 (dd, $J = 7.6, 1.8$ Hz, 2 H). HPLC (method A): 99.9%, $t_R = 10.4$ min. MS (ESI) 352 m/z ($M + \text{H}$)⁺.

(3R)-1-Azabicyclo[2.2.2]oct-3-yl 9-hydroxy-9H-fluorene-9-carboxylate (**12**) was prepared as described for compound **11** starting from the methyl ester **10** (5 g, 20.8 mmol). The obtained product was purified by crystallization from CHCl_3 /diisopropyl ether to afford 4.63 g (66%) of compound **12**, mp 217 $^\circ\text{C}$. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 0.90–1.08 (m, 2 H), 1.23–1.50 (m, 2 H), 1.54–1.67 (m, 1 H), 1.95–2.17 (m, 2 H), 2.35–2.58 (m, 3 H), 2.82–2.98 (dd, $J = 14.3, 7.9$ Hz, 1 H), 4.50–4.63 (m, 1 H), 6.74 (br s, 1 H), 7.27–7.38 (m, 2 H), 7.38–7.48 (m, 2 H), 7.51 (d, $J = 6.41$ Hz, 2 H), 7.81 (d, $J = 7.32$ Hz, 2 H). HPLC (method B): 99.7%, $t_R = 8.0$ min. MS (ESI) 336 m/z ($M + \text{H}$)⁺.

(3R)-1-Azabicyclo[2.2.2]oct-3-yl 9-methyl-9H-xanthene-9-carboxylate (**16**) was prepared as described for compound **11** starting from the methyl ester **14** (2.65 g, 10.4 mmol). The yield was 1.91 g (53%) of **16** as an oil. Compound **16** (0.3 g, 0.86 mmol) was treated with oxalic acid (0.077 g, 0.86 mmol) in acetone/diethyl ether to give 0.25 g (66%) of the oxalate salt, mp 152 $^\circ\text{C}$. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.18–1.33 (m, 1 H), 1.39–1.56 (m, 1 H), 1.59–1.82 (m, 2 H), 1.90 (s, 3 H),

1.94–2.04 (m, 1 H), 2.53–2.67 (m, 1 H), 2.69–2.79 (d, $J = 14.0$ Hz, 1 H), 2.90–3.14 (m, 3 H), 3.42–3.55 (ddd, $J = 14.2, 8.4, 2.7$ Hz, 1 H), 4.84–4.96 (m, 1 H), 7.11–7.21 (m, 4 H), 7.30–7.40 (m, 2 H), 7.41–7.50 (m, 2 H), 7.80–10.80 (br s, 2 H). HPLC (method A): 100.0%, $t_R = 15.1$ min. MS (ESI) 350 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9-methyl-9*H*-fluorene-9-carboxylate (**17**) was prepared as described for compound **11** starting from the methyl ester **15** (3.15 g, 13.2 mmol). The obtained product was purified by column chromatography (silica gel, chloroform/isopropanol, 80:20, as eluent) to give 3.44 g (80%) of compound **17** as an oil. Compound **17** (0.3 g, 0.89 mmol) was treated with oxalic acid (0.080 g, 0.89 mmol) in acetone/diethyl ether to give 0.26 g (68%) of the oxalate salt, mp 186.3 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.34–1.61 (m, 2 H), 1.61–1.86 (m, 2 H), 1.75 (s, 3 H), 1.98–2.07 (m, 1 H), 2.72–2.87 (m, 1 H), 2.90–2.99 (d, $J = 14.6$ Hz, 1 H), 2.99–3.21 (m, 3 H), 3.47–3.59 (ddd, $J = 14.0, 8.2, 2.4$ Hz, 1 H), 4.82–4.95 (m, 1 H), 7.33–7.41 (m, 2 H), 7.42–7.50 (m, 2 H), 7.60–7.66 (d, $J = 7.0$ Hz, 1 H), 7.66–7.72 (d, $J = 7.3$ Hz, 1 H), 7.87–7.93 (d, $J = 7.3$ Hz, 2 H), 6.50–11.50 (br s, 2 H). HPLC (method A): 100.0%, $t_R = 14.7$ min. MS (ESI) 334 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(di-2-thienyl)acetate (**19**) was prepared as described for compound **11** starting from the methyl ester **18**³⁶ (6.0 g, 23.6 mmol). The obtained product was purified by treatment with diisopropyl ether to afford 4.11 g (50%) of compound **19**, mp 180 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.12–1.34 (m, 1 H), 1.37–1.68 (m, 3 H), 1.87–1.95 (m, 1 H), 2.34–2.75 (m, 5 H), 3.04–3.15 (ddd, $J = 14.7, 8.1, 2.0$ Hz, 1 H), 4.76–4.87 (m, 1 H), 6.98–7.04 (m, 2 H), 7.09–7.12 (m, 2 H), 7.37 (br s, 1 H), 7.46–7.54 (m, 2 H). HPLC (method B): 98.8%, $t_R = 7.7$ min. MS (ESI) 350 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl di-2-thienylacetate (**21**) was prepared as described for compound **11** starting from the methyl ester **20** (1.67 g, 7.0 mmol). The obtained product was purified by column chromatography (silica gel, chloroform/methanol/NH₄OH, 95:5:0.5, as eluent) to give 0.82 g (35%) of compound **21** as an oil. Compound **21** (0.82 g, 2.5 mmol) was treated with fumaric acid (0.144 g, 1.23 mmol) in acetone/diethyl ether to give 0.58 g (60%) of the fumarate salt, mp 122 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.34–1.52 (m, 1 H), 1.52–1.78 (m, 3 H), 1.98–2.07 (m, 1 H), 2.62–2.72 (d, $J = 14.6$ Hz, 1 H), 2.72–2.97 (m, 4 H), 3.27–3.37 (ddd, $J = 14.5, 8.4, 2.1$ Hz, 1 H), 4.84–4.96 (m, 1 H), 5.85 (s, 1 H), 6.55 (s, 2 H), 6.97–7.03 (dd, $J = 5.2, 3.7$ Hz, 2 H), 7.07–7.12 (m, 2 H), 7.47–7.52 (dd, $J = 5.2, 1.2$ Hz, 2 H). HPLC (method B): 98.7%, $t_R = 10.7$ min; MS (ESI) 334 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(di-3-thienyl)acetate (**23**) was prepared as described for compound **11** starting from the methyl ester **22**³⁶ (3.55 g, 14.0 mmol). The obtained product was purified by crystallization in acetonitrile. The resulting solid was filtered and washed with diethyl ether to afford 1.54 g (31.5%) of compound **23**, mp 151 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.10–1.31 (m, 1 H), 1.31–1.68 (m, 3 H), 1.82–1.94 (m, 1 H), 2.36–2.45 (d, $J = 14.6$ Hz, 1 H), 2.45–2.72 (m, 4 H), 3.02–3.15 (ddd, $J = 14.6, 8.2, 2.3$ Hz, 1 H), 4.73–4.83 (m, 1 H), 6.54–6.68 (br s, 1 H), 7.06–7.12 (dt, $J = 5.2, 1.5$ Hz, 2 H), 7.34–7.40 (td, $J = 3.3, 1.4$ Hz, 2 H), 7.46–7.54 (dt, $J = 5.1, 3.4$ Hz, 2 H). HPLC (method A): 99.0%, $t_R = 10.0$ min. MS (ESI) 350 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 2,2-di-2-thienylpropanoate (**26**) was prepared as described for compound **11** starting from the methyl ester **25** (0.86 g, 3.4 mmol). The yield was 1.11 g (94.1%) of compound **26** as an oil. Compound **26** (0.25 g, 0.72 mmol) was treated with oxalic acid (65 mg, 0.72 mmol) in acetone/diethyl ether to give 0.25 g (79%) of the oxalate salt, mp 126.7–128.6 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.50–1.70 (m, 2 H), 1.69–1.93 (m, 2 H), 2.07 (s, 3 H), 2.14–2.24 (m, 1 H), 2.78–2.95 (m, 1 H), 2.95–3.24 (m, 4 H), 3.54–3.67 (ddd, $J = 13.9, 8.4, 1.8$ Hz, 1 H), 5.01–5.15 (m, 1 H), 6.97–7.04 (ddd, $J = 5.2, 3.5, 1.7$ Hz, 2 H), 7.04–7.10 (m, 2 H), 7.47–7.52 (m, 2 H), 7.75–10.75 (br s,

2 H). HPLC (method B): 99.3%, $t_R = 11.1$ min. MS (ESI) 348 m/z ($M + H$)⁺.

Preparation of (3*R*)-Quinuclidinol Esters **33,¹⁹ **34**, **35**, **36**, **37**, **38**, and **39** (Scheme 3).**

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(diphenyl)acetate (**33**)¹⁹. To a solution of compound **31**³⁹ (1 g, 3.86 mmol) in 2 mL of THF, cooled to –40 °C, was slowly added phenyllithium (1.8 M solution in cyclohexane/diethyl ether, 2.4 mL, 4.32 mmol). The mixture was stirred for 10 min at –40 °C. Then it was warmed to room temperature and the stirring was continued for 1 h. The reaction mixture was poured over a saturated aqueous NH₄Cl solution and extracted with diethyl ether. The ether extracts were combined, washed with brine, dried over Na₂SO₄, and concentrated. The obtained residue was purified by treatment with diisopropyl ether to afford 0.46 g (35%) of compound **33**, mp 188 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.07–1.24 (m, 1 H), 1.24–1.40 (m, 1 H), 1.39–1.65 (m, 2 H), 1.82–1.90 (m, 1 H), 2.30–2.46 (m, 2 H), 2.52–2.67 (m, 3 H), 3.00–3.15 (ddd, $J = 14.7, 8.1, 2.3$ Hz, 1 H), 4.75–4.86 (m, 1 H), 6.59 (br s, 1 H), 7.16–7.47 (m, 10 H). HPLC (method B): 95.6%, $t_R = 9.4$ min. MS (ESI) 338 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(phenyl)2-thienylacetate (**34**). To a solution of compound **32** (16.6 g, 62.5 mmol) in 300 mL of THF, cooled to –70 °C, was added phenylmagnesium bromide (1.0 M solution in THF, 75 mL, 75 mmol). The mixture was stirred for 15 min at –60 °C, and then it was warmed to room temperature and the stirring continued for 2 h. The reaction mixture was poured over a saturated aqueous NH₄Cl solution and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over Na₂SO₄, and concentrated. The obtained solid was treated with isopropanol at room temperature, filtered, and washed with diethyl ether to yield 9.85 g (38%) of compound **34** as a mixture of diastereomers **35** and **36**, which were separated by crystallization. The diastereomeric resolution was monitored by ¹H NMR, and crystallizations were repeated until the signals of the minor diastereomer were negligible.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*R*)-hydroxy(phenyl)2-thienylacetate (**36**). After five successive crystallizations of compound **34** from boiling isopropanol, an amount of 2.08 g (16%) of diastereomer **36** was obtained. Diastereomeric purity by ¹H NMR was $\geq 95\%$, mp 174–175 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.07–1.27 (m, 1 H), 1.27–1.41 (m, 1 H), 1.41–1.64 (m, 2 H), 1.80–1.87 (m, 1 H), 2.36–2.71 (m, 5 H), 3.03–3.15 (ddd, $J = 14.6, 8.2, 1.9$ Hz, 1 H), 4.74–4.86 (m, 1 H), 7.00 (s, 1 H), 7.01–7.10 (m, 2 H), 7.24–7.53 (m, 6 H). HPLC (method B): 99.9%, $t_R = 8.6$ min. MS (ESI) 344 m/z ($M + H$)⁺.

The absolute configuration of the ester moiety of compound **36** was determined by correlation with the absolute configuration of (+)-hydroxy(phenyl)2-thienylacetic acid (compound **40**). Diastereomer **36** was hydrolyzed (MeOH, NaOH, 30 min, at room temperature) to give compound **40**. $[\alpha]_D^{25} = +25.4^\circ$ ($c = 2$, EtOH). The *R* configuration for the acid **40** was assigned by comparison of its $[\alpha]$ value with the data described in the literature for (2*S*)-hydroxy(phenyl)2-thienylacetic acid: $[\alpha]_D^{25} = -20^\circ$ ($c = 2$, EtOH).⁴⁰

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*S*)-hydroxy(phenyl)2-thienylacetate (**35**). The mother liquors from the first crystallization of compound **34** were enriched with the diastereomer **35**. The solution was concentrated and cooled. The precipitated solid was filtered and discarded. The filtrate was concentrated and treated with diethyl ether, and the resulting solid was filtered to give 0.89 g (7%) of compound **35**. Diastereomeric purity by ¹H NMR was $\geq 95\%$, mp 151.8–152.6 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.11–1.32 (m, 1 H), 1.35–1.64 (m, 3 H), 1.87–1.94 (m, 1 H), 2.28–2.46 (m, 2 H), 2.46–2.69 (m, 3 H), 2.97–3.15 (ddd, $J = 14.7, 8.1, 2.0$ Hz, 1 H), 4.71–4.86 (m, 1 H), 6.98–7.10 (m, 3 H), 7.26–7.52 (m, 6 H). HPLC (method B): 94.5%, $t_R = 8.3$ min. MS (ESI) 344 m/z ($M + H$)⁺.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl cyclopentyl(hydroxy)2-thienylacetate (**37**) was prepared as described for compound **34**

starting from a solution of compound **32** (39.0 g, 147 mmol) in THF (325 mL) and cyclopentyl magnesium chloride (2.0 M solution in diethyl ether, 86 mL, 172 mmol). Purification by column chromatography (silica gel, chloroform/methanol/ NH_4OH , 97:3:0.5 to 97:4:0.5, as eluent) afforded 22.8 g (46%) of compound **37** as a mixture of diastereomers **38** and **39**, which were separated by crystallization. The diastereomeric resolution was monitored by ^1H NMR, and crystallizations were repeated until the signals of the minor diastereomer were negligible.

(3R)-1-Azabicyclo[2.2.2]oct-3-yl (2S)-cyclopentyl(hydroxy)2-thienylacetate (38). After successive macerations of the mixture **37** with diisopropyl ether (twice) and with diethyl ether (twice), an amount of 3.8 g (15%) of diastereomer **38** was obtained. Diastereomeric purity by ^1H NMR was $\geq 95\%$, mp 157.3–158.7 °C. ^1H NMR (300 MHz, CDCl_3) δ ppm 1.36–1.83 (m, 11 H), 1.83–1.97 (m, 1 H), 2.03–2.15 (m, 1 H), 2.50–2.64 (m, 1 H), 2.64–2.98 (m, 5 H), 3.09–3.23 (dd, $J = 14.8, 8.1$ Hz, 1 H), 4.08 (br s, 1 H), 4.87–4.98 (m, 1 H), 6.96–7.04 (m, 1 H), 7.12–7.19 (m, 1 H), 7.25–7.31 (m, 1 H). HPLC (method B): 99.2%, $t_R = 9.8$ min. MS (ESI) 336 m/z ($M + \text{H}$) $^+$.

The absolute configuration of the ester moiety of compound **38** was determined by correlation with the absolute configuration of (–)-cyclopentyl(hydroxy)2-thienylacetic acid⁴² (compound **41**). Diastereomer **38** was hydrolyzed (EtOH/NaOH , 2 N, 2 h at room temperature and 2 h at 60 °C) to give compound **41**, $[\alpha]_D^{25} = -6.44^\circ$ ($c = 1$, EtOH). CD curve: λ (nm) = 233, $\Delta\epsilon$ ($M^{-1}\text{cm}^{-1}$) = -4.19 . Configuration *S* was assigned by comparison of the CD curve with that of the acid **68**⁴¹ (see the Supporting Information for more details).

(3R)-1-Azabicyclo[2.2.2]oct-3-yl (2R)-cyclopentyl(hydroxy)2-thienylacetate (39). The mother liquors from the first treatment of compound **37** with diisopropyl ether were enriched with the diastereomer **39**. The solution was concentrated, and the residue was purified by column chromatography (silica gel, chloroform/methanol/ NH_4OH , 97:3:0.5 to 97:4:0.5, as eluent). The obtained product was treated with diisopropyl ether (twice) and then with chloroform/diethyl ether to give 1.2 g (5%) of compound **39**. Diastereomeric purity by HPLC (method B) was 92.1%; mp was not determined. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.18–1.69 (m, 12 H), 1.80–1.89 (m, 1 H), 2.40–2.89 (m, 6 H), 3.05–3.22 (dd, $J = 14.5, 8.1$ Hz, 1 H), 4.64–4.74 (m, 1 H), 5.99 (br s, 1 H), 6.95–7.00 (dd, $J = 4.9, 3.7$ Hz, 1 H), 7.10 (d, $J = 3.7$ Hz, 1 H), 7.40 (d, $J = 4.9$ Hz, 1 H). HPLC (method B): 95.7% (compound **39**, 88.1%; compound **38**, 7.5%), $t_R = 9.5$ min. MS (ESI) 336 m/z ($M + \text{H}$) $^+$.

The absolute configuration of the ester moiety of compound **39** was determined by correlation with the absolute configuration of (+)-cyclopentyl(hydroxy)2-thienylacetic acid⁴² (compound **42**). Diastereomer **39** was hydrolyzed (EtOH/NaOH , 2 N, 2 h at room temperature and 2 h at 60 °C) to give compound **42**, $[\alpha]_D^{25} = +6.63^\circ$ ($c = 1$, EtOH). CD curve: λ (nm) = 233, $\Delta\epsilon$ ($M^{-1}\text{cm}^{-1}$) = $+4.18$. Configuration *R* was assigned by comparison of the CD curve with that of the acid **69**.⁴¹ (see the Supporting Information section for more details).

Preparation of Quaternary Ammonium Derivatives 43–64 (Scheme 4). **(3R)-1-(2-Phenylethyl)-3-[(9H-xanthen-9-ylcarbonyl)oxy]-1-azoniabicyclo[2.2.2]octane bromide (43)**. To a solution of compound **5** (5.05 g, 15.05 mmol) in 100 mL of THF was added (2-bromoethyl)benzene (6.88 g, 37.20 mmol). The mixture was refluxed for 9 h and then stirred at room temperature for 80 h. The precipitated solid was filtered and washed with diethyl ether to obtain 6.5 g (83%) of compound **43**, mp 258 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.65–2.03 (m, 4 H), 2.14–2.24 (m, 1 H), 2.89–3.10 (m, 2 H), 3.18–3.34 (m, 1 H), 3.35–3.55 (m, 5 H), 3.55–3.70 (m, 1 H), 3.85–3.98 (ddd, $J = 13.7, 8.2, 2.1$ Hz, 1 H), 5.01–5.12 (m, 1 H), 5.33 (s, 1 H), 7.14–7.22 (m, 3 H), 7.22 (s, 1 H), 7.24–7.42 (m, 7 H), 7.46–7.60 (ddd, $J = 17.0, 7.6, 1.5$ Hz, 2 H). HPLC (method B): 100.0%, $t_R = 12.3$ min. MS (ESI) 440 m/z ($M - \text{Br}$) $^+$.

(3R)-3-[(9-Hydroxy-9H-xanthen-9-yl)carbonyl]oxy-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (44). To a solution of compound **11** (0.3 g, 0.85 mmol) in 4 mL of acetonitrile and 6 mL of chloroform was added (2-bromoethyl)benzene (0.79 g, 4.25 mmol). The mixture was stirred at room temperature for 6 days. The solvents were evaporated, and the obtained residue was treated with diethyl ether. The resulting solid was filtered and washed with diethyl ether to obtain 0.29 g (64%) of compound **44**, mp 221 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.20–1.42 (m, 1 H), 1.51–1.73 (m, 1 H), 1.73–1.99 (m, 2 H), 2.01–2.14 (m, 1 H), 2.66–2.94 (m, 3 H), 2.98 (d, $J = 13.7$ Hz, 1 H), 3.23–3.54 (m, 5 H), 3.81 (dd, $J = 12.1, 9.0$ Hz, 1 H), 4.98–5.11 (m, 1 H), 7.13–7.39 (m, 10 H), 7.44 (t, $J = 7.5$ Hz, 2 H), 7.67 (t, $J = 8.5$ Hz, 2 H). HPLC (method B): 95.1%, $t_R = 11.4$ min. MS (ESI) 456 m/z ($M - \text{Br}$) $^+$.

(3R)-3-[(9-Hydroxy-9H-xanthen-9-yl)carbonyl]oxy-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (45) was prepared as described for compound **43** starting from compound **11** (0.95 g, 2.70 mmol) and (3-bromopropoxy)benzene (0.87 g, 4.05 mmol). The reaction time was 20 h at reflux temperature. Trituration with diethyl ether gave 1.33 g (87%) of compound **45**, mp 112.7–114.9 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.24–1.40 (m, 1 H), 1.56–1.71 (m, 1 H), 1.71–1.93 (m, 2 H), 1.91–2.13 (m, 3 H), 2.62–2.78 (m, 1 H), 2.86–2.98 (d, $J = 13.7$ Hz, 1 H), 3.19–3.48 (m, 5 H), 3.74–3.88 (ddd, $J = 13.7, 7.9, 1.5$ Hz, 1 H), 3.93–4.05 (t, $J = 5.7$ Hz, 2 H), 4.97–5.07 (m, 1 H), 6.91–7.02 (m, 3 H), 7.19 (s, 1 H), 7.19–7.37 (m, 6 H), 7.38–7.49 (m, 2 H), 7.64–7.71 (ddd, $J = 6.4, 6.4, 1.2$ Hz, 2 H). HPLC (method B): 98.7%, $t_R = 12.1$ min. MS (ESI) 486 m/z ($M - \text{Br}$) $^+$.

(3R)-1-Heptyl-3-[(9-hydroxy-9H-fluoren-9-yl)carbonyl]oxy-1-azoniabicyclo[2.2.2]octane bromide (46) was prepared as described for compound **44** starting from compound **12** (0.25 g, 0.75 mmol) and 1-bromoheptane (0.67 g, 3.75 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.33 g (85.5%) of compound **46**, mp 217 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 0.89 (t, $J = 7.0$ Hz, 3 H), 1.13–1.70 (m, 12 H), 1.70–1.93 (m, 2 H), 2.03–2.11 (m, 1 H), 2.68–2.84 (m, 1 H), 2.98–3.19 (m, 3 H), 3.18–3.43 (m, 4 H), 3.78 (ddd, $J = 13.6, 7.8, 2.4$ Hz, 1 H), 4.97–5.05 (m, 1 H), 7.36 (dd, $J = 7.5, 1.1$ Hz, 2 H), 7.45 (dd, $J = 7.3, 7.3$ Hz, 2 H), 7.59 (d, $J = 7.0$ Hz, 1 H), 7.64 (d, $J = 7.6$ Hz, 1 H), 7.84 (dd, $J = 7.3, 1.1$ Hz, 2 H). HPLC (method B): 99.6%, $t_R = 12.6$ min. MS (ESI) 434 m/z ($M - \text{Br}$) $^+$.

(3R)-3-[(9-Hydroxy-9H-fluoren-9-yl)carbonyl]oxy-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (47) was prepared as described for compound **43** starting from compound **12** (0.7 g, 2.1 mmol) and (2-bromoethyl)benzene (0.58 g, 3.15 mmol). The reaction time was 31 h at reflux and 6 days at room temperature. Trituration with diethyl ether/acetonitrile gave 0.89 g (81.5%) of compound **47**, mp 118 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.34–1.54 (m, 1 H), 1.60–1.76 (m, 1 H), 1.76–2.03 (m, 2 H), 2.07–2.15 (m, 1 H), 2.78–3.06 (m, 3 H), 3.21 (d, $J = 13.4$ Hz, 1 H), 3.28–3.58 (m, 5 H), 3.88 (dd, $J = 5.3, 2.6$ Hz, 1 H), 5.01–5.13 (m, 1 H), 6.87 (s, 1 H), 7.27–7.41 (m, 7 H), 7.47 (dd, $J = 7.2$ Hz, 2 H), 7.60 (d, $J = 7.6$ Hz, 1 H), 7.66 (d, $J = 7.2$ Hz, 1 H), 7.85 (d, $J = 7.6$ Hz, 2 H). HPLC (method B): 95.5%, $t_R = 10.2$ min. MS (ESI) 440 m/z ($M - \text{Br}$) $^+$.

(3R)-3-[(9-Hydroxy-9H-fluoren-9-yl)carbonyl]oxy-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (48) was prepared as described for compound **43** starting from compound **12** (2.2 g, 6.6 mmol) and (3-bromopropoxy)benzene (2.86 g, 13.3 mmol). The reaction time was 18 h at reflux temperature. Trituration with diethyl ether/acetonitrile gave 3.17 g (88%) of compound **48**, mp 108 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 1.35–1.54 (m, 1 H), 1.57–1.75 (m, 1 H), 1.75–1.96 (m, 2 H), 1.96–2.18 (m, 3 H), 2.77–2.96 (m, 1 H), 3.16 (d, $J = 13.7$ Hz, 1 H), 3.24–3.54 (m, 5 H), 3.86 (ddd, $J = 8.1, 5.2, 2.0$ Hz, 1 H), 4.01 (t, $J = 5.8$ Hz, 2 H), 4.96–5.08 (m, 1 H), 6.86 (s, 1 H), 6.90–7.03 (m, 3 H), 7.26–7.40 (m, 4 H), 7.45 (dd, $J = 7.3$ Hz, 2 H), 7.61 (d, $J = 7.6$ Hz, 1 H), 7.66 (d, $J = 7.3$ Hz, 1 H), 7.84 (d, $J = 7.3$ Hz, 2 H). HPLC (method A): 99.9%, $t_R = 15.8$ min. MS (ESI) 470 m/z ($M - \text{Br}$) $^+$.

(3*R*)-3-[[[9-Methyl-9*H*-xanthen-9-yl)carbonyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**49**) was prepared as described for compound **44** starting from compound **16** (0.10 g, 0.28 mmol) and (3-bromopropoxy)benzene (0.30 g, 1.40 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.11 g (69%) of compound **49**, mp 195 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.33–1.48 (m, 1 H), 1.59–1.74 (m, 1 H), 1.74–1.96 (m, 2 H), 1.92 (s, 3 H), 1.97–2.18 (m, 3 H), 2.74–2.91 (m, 1 H), 3.05–3.18 (d, *J* = 13.7 Hz, 2 H), 3.23–3.50 (m, 5 H), 3.79–3.92 (m, 1 H), 3.97–4.07 (t, *J* = 5.8 Hz, 2 H), 5.00–5.10 (m, 1 H), 6.91–7.02 (m, 3 H), 7.12–7.23 (m, 4 H), 7.27–7.42 (m, 4 H), 7.44–7.50 (dd, *J* = 8.1, 1.7 Hz, 1 H), 7.50–7.55 (dd, *J* = 8.1, 1.4 Hz, 1 H). HPLC (method A): 98.4%, *t*_R = 21.3 min. MS (ESI) 484 *m/z* (M – Br)⁺.

(3*R*)-3-[[[9-Methyl-9*H*-fluoren-9-yl)carbonyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**50**) was prepared as described for compound **44** starting from compound **17** (0.20 g, 0.6 mmol) and (3-bromopropoxy)benzene (0.64 g, 3.0 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.20 g (61%) of compound **50**, mp 204 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.42–1.63 (m, 1 H), 1.63–1.97 (m, 3 H), 1.78 (s, 3 H), 1.97–2.25 (m, 3 H), 2.94–3.15 (m, 1 H), 3.20–3.60 (m, 6 H), 3.80–3.97 (m, 1 H), 3.96–4.18 (t, *J* = 5.5 Hz, 2 H), 4.91–5.05 (m, 1 H), 6.87–7.01 (m, 3 H), 7.24–7.50 (m, 6 H), 7.62–7.70 (d, *J* = 6.7 Hz, 1 H), 7.72–7.80 (d, *J* = 7.0 Hz, 1 H), 7.86–7.95 (d, *J* = 6.7 Hz, 2 H). HPLC (method A): 99.4%, *t*_R = 20.8 min. MS (ESI) 468 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-methyl-1-azoniabicyclo[2.2.2]octane bromide (**51**) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and bromomethane (2.85 mL of a 1 M solution in acetonitrile, 2.85 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.20 g (79%) of compound **51**, mp 251 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.60–1.84 (m, 2 H), 1.82–2.07 (m, 2 H), 2.29 (m, 1 H), 3.02 (s, 3 H), 3.19–3.58 (m, 5 H), 3.90–4.03 (ddd, *J* = 13.3, 8.4, 2.1 Hz, 1 H), 5.15–5.27 (m, 1 H), 6.97–7.07 (dt, *J* = 5.0, 3.8 Hz, 2 H), 7.16–7.24 (ddd, *J* = 8.1, 3.5, 1.2 Hz, 2 H), 7.49 (s, 1 H), 7.50–7.55 (ddd, *J* = 5.1, 3.7, 1.2 Hz, 2 H). HPLC (method B): 99.7%, *t*_R = 7.7 min. MS (ESI) 364 *m/z* (M – Br)⁺.

(3*R*)-1-Heptyl-3-[[[hydroxy(di-2-thienyl)acetyl]oxy]-1-azoniabicyclo[2.2.2]octane bromide (**52**). To a solution of compound **19** (0.50 g, 1.43 mmol) in 10 mL of chloroform was added 1-bromoheptane (1.02 g, 5.52 mmol). The mixture was stirred at room temperature for 7 days. The solvent was evaporated, and the residue was treated with diethyl ether. The resulting solid was filtered and washed with diethyl ether to obtain 0.49 g (66%) of compound **52**, mp 134 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 0.88 (t, *J* = 6.7 Hz, 3 H), 1.16–1.39 (m, 8 H), 1.5–1.8 (m, 4 H), 1.83–2.06 (m, 2 H), 2.25–2.35 (m, 1 H), 3.03–3.54 (m, 7 H), 3.89 (ddd, *J* = 13.9, 8.2, 1.7 Hz, 1 H), 5.16–5.29 (m, 1 H), 7.02 (ddd, *J* = 5.1, 3.6, 2.3 Hz, 2 H), 7.17 (td, *J* = 3.6, 1.2 Hz, 2 H), 7.49 (s, 1 H), 7.52 (ddd, *J* = 5.1, 2.3, 1.2 Hz, 2 H). HPLC (method A): 99.5%, *t*_R = 17.9 min. MS (ESI) 448 *m/z* (M – Br)⁺.

(3*R*)-1-Benzyl-3-[[[hydroxy(di-2-thienyl)acetyl]oxy]-1-azoniabicyclo[2.2.2]octane bromide (**53**) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and (bromomethyl)benzene (0.34 mL, 2.85 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.23 g (77%) of compound **53**, mp 89.0–90.6 °C (dec). ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.57–2.03 (m, 4 H), 2.26 (m, 1 H), 2.98–3.15 (m, 1 H), 3.22–3.54 (m, 4 H), 3.81–3.94 (m, 1 H), 4.47 (d, *J* = 12.8 Hz, 1 H), 4.54 (d, *J* = 12.8 Hz, 1 H), 5.21 (m, 1 H), 6.96 (dd, *J* = 8.6, 3.6 Hz, 2 H), 7.01–7.11 (d, *J* = 3.1 Hz, 2 H), 7.44 (s, 1 H), 7.45–7.61 (m, 7 H). HPLC (method B): 95.6%, *t*_R = 10.3 min. MS (ESI) 440 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (**54**) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and (2-bromoethyl)benzene (0.53 g, 2.86 mmol).

The reaction time was 72 h. Crystallization from acetonitrile gave 0.18 g (59%) of compound **54**, mp 216 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.60–1.89 (m, 2 H), 1.89–2.13 (m, 2 H), 2.27–2.40 (m, 1 H), 2.89–3.12 (m, 2 H), 3.17–3.32 (m, 1 H), 3.37–3.67 (m, 6 H), 3.92–4.08 (m, 1 H), 5.22–5.34 (m, 1 H), 7.03 (dd, *J* = 3.4 Hz, 2 H), 7.19 (dd, *J* = 3.7 Hz, 2 H), 7.24–7.42 (m, 5 H), 7.49 (s, 1 H), 7.54 (dd, *J* = 3.4 Hz, 2 H). HPLC (method A): 99.6%, *t*_R = 15.5 min. MS (ESI) 454 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(4-phenylbutyl)-1-azoniabicyclo[2.2.2]octane bromide (**55**) was prepared as described for compound **52** starting from compound **19** (0.50 g, 1.43 mmol) and (4-bromobutyl)benzene (1.22 g, 5.73 mmol). The reaction time was 7 days. Trituration with diethyl ether gave 0.32 g (41%) of compound **55**, mp 64 °C. ¹H NMR (300 MHz, CDCl₃) δ ppm 1.50–1.72 (m, 4 H), 1.72–2.12 (m, 5 H), 2.34–2.44 (m, 1 H), 2.61 (t, *J* = 6.6 Hz, 2 H), 3.34–3.59 (m, 5 H), 3.60–3.69 (m, 1 H), 3.74 (d, *J* = 13.7 Hz, 1 H), 4.20 (dd, *J* = 13.0, 8.4 Hz, 1 H), 5.18–5.32 (m, 1 H), 6.93 (dd, *J* = 5.0, 3.8 Hz, 2 H), 7.09–7.33 (m, 9 H). HPLC (method B): 98.6%, *t*_R = 12.5 min. MS (ESI) 482 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Hydroxy(di-2-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**56**) was prepared as described for compound **44** starting from compound **19** (5.0 g, 14.33 mmol) and (3-bromopropoxy)benzene (15.97 g, 74.23 mmol). The reaction time was 18 h. Trituration with acetonitrile gave 7.20 g (89%) of compound **56**, mp 230 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.63–1.87 (m, 2 H), 1.85–2.06 (m, 2 H), 2.06–2.24 (m, 2 H), 2.26–2.38 (m, 1 H), 3.13–3.29 (m, 1 H), 3.32–3.64 (m, 6 H), 3.91–4.07 (m, 1 H), 4.03 (t, *J* = 5.8 Hz, 2 H), 5.19–5.31 (m, 1 H), 6.91–7.00 (m, 3 H), 7.00–7.06 (m, 2 H), 7.17–7.22 (m, 2 H), 7.28–7.36 (m, 2 H), 7.50 (s, 1 H), 7.50–7.56 (m, 2 H). HPLC (method B): 99.8%, *t*_R = 11.9 min. MS (ESI) 484 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Di-2-thienylacetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**57**) was prepared as described for compound **44** starting from compound **21** (0.25 g, 0.75 mmol) and (3-bromopropoxy)benzene (0.81 g, 3.75 mmol). The reaction time was 24 h. Trituration with diethyl ether gave 0.34 g (83%) of compound **57**, mp 148 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.72–2.05 (m, 4 H), 2.05–2.23 (m, 2 H), 2.25–2.36 (m, 1 H), 3.20–3.34 (m, 1 H), 3.36–3.64 (m, 4 H), 3.40–3.48 (t, *J* = 7.9 Hz, 2 H), 3.90–4.03 (m, 1 H), 4.00–4.06 (t, *J* = 5.8 Hz, 2 H), 5.14–5.24 (m, 1 H), 5.91 (s, 1 H), 6.92–6.99 (m, 3 H), 6.99–7.04 (ddd, *J* = 5.0, 3.6, 1.2 Hz, 2 H), 7.12–7.18 (dd, *J* = 7.3, 3.4 Hz, 2 H), 7.26–7.36 (m, 2 H), 7.48–7.54 (d, *J* = 5.2 Hz, 2 H). HPLC (method B): 94.5%, *t*_R = 13.2 min. MS (ESI) 468 *m/z* (M – Br)⁺.

(3*R*)-3-[[[Hydroxy(di-3-thienyl)acetyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**58**) was prepared as described for compound **43** starting from compound **23** (0.5 g, 1.43 mmol) and (3-bromopropoxy)benzene (0.46 g, 2.15 mmol). The reaction time was 6 h at reflux and 72 h at room temperature. Trituration with diethyl ether gave 0.68 g (86%) of compound **58**, mp 219 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.50–1.66 (m, 1 H), 1.66–1.82 (m, 1 H), 1.82–2.03 (m, 2 H), 2.03–2.24 (m, 2 H), 2.25–2.37 (m, 1 H), 3.08–3.25 (m, 1 H), 3.28–3.60 (m, 6 H), 3.86–4.02 (m, 1 H), 4.01–4.08 (t, *J* = 5.7 Hz, 2 H), 5.15–5.26 (m, 1 H), 6.78 (s, 1 H), 6.91–7.01 (m, 3 H), 7.11–7.17 (d, *J* = 5.2 Hz, 2 H), 7.32 (m, 2 H), 7.42–7.48 (m, 2 H), 7.48–7.55 (m, 2 H). HPLC (method B): 99.7%, *t*_R = 11.8 min. MS (ESI) 484 *m/z* (M – Br)⁺.

(3*R*)-3-[[[2,2-Di-2-thienylpropanoyl]oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**59**) was prepared as described for compound **44** starting from compound **26** (0.25 g, 0.72 mmol) and (3-bromopropoxy)benzene (0.77 g, 3.60 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.35 g (92%) of compound **59**, mp 170 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.60–2.25 (m, 6 H), 2.10 (s, 3 H), 2.25–2.34 (m, 1 H), 3.02–3.21 (m, 1 H), 3.30–3.63 (m, 6 H), 3.90–4.08 (m, 1 H), 4.01–4.09 (t, *J* = 5.5 Hz,

2 H), 5.14–5.26 (m, 1 H), 6.92–7.00 (m, 3 H), 7.00–7.06 (dd, $J = 5.0, 3.6$ Hz, 2 H), 7.08–7.14 (m, 2 H), 7.26–7.36 (m, 2 H), 7.48–7.54 (d, $J = 5.19$ Hz, 2 H). HPLC (method B): 97.9%, $t_R = 14.0$ min. MS (ESI) 482 m/z ($M - Br$)⁺.

(3*R*)-3-([Hydroxy(diphenyl)acetyl]oxy)-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**60**) was prepared as described for compound **44** starting from compound **33** (0.40 g, 1.18 mmol) and (3-bromopropoxy)benzene (1.23 g, 5.69 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.28 g (42%) of compound **60**, mp 199 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.44–1.62 (m, 1 H), 1.61–1.79 (m, 1 H), 1.80–2.01 (m, 2 H), 2.01–2.22 (m, 2 H), 2.22–2.34 (m, 1 H), 2.99–3.18 (m, 1 H), 3.19–3.57 (m, 6 H), 3.85–3.99 (m, 1 H), 3.99–4.09 (t, $J = 5.5$ Hz, 2 H), 5.14–5.33 (m, 1 H), 6.89–7.02 (m, 3 H), 7.20–7.51 (m, 13 H). HPLC (method B): 94.9%, $t_R = 12.6$ min. MS (ESI) 472 m/z ($M - Br$)⁺.

(3*R*)-3-([(2*S*)-2-Hydroxy-2-phenyl-2-(2-thienyl)acetyl]oxy)-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**61**) was prepared as described for compound **43** starting from compound **35** (0.5 g, 1.50 mmol) and (3-bromopropoxy)benzene (0.62 g, 2.92 mmol). The reaction time was 3 h at reflux and overnight at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 0.66 g (79%) of compound **61**, mp 206.4–207.9 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.57–1.82 (m, 2 H), 1.82–2.03 (m, 2 H), 2.03–2.20 (m, 2 H), 2.25–2.35 (m, 1 H), 3.03–3.19 (m, 1 H), 3.24–3.55 (m, 6 H), 3.87–3.99 (ddd, $J = 13.6, 8.2, 1.7$ Hz, 1 H), 3.99–4.07 (t, $J = 6.0$ Hz, 2 H), 5.18–5.28 (m, 1 H), 6.91–7.00 (m, 3 H), 7.00–7.04 (dd, $J = 5.0, 3.5$ Hz, 1 H), 7.1–7.14 (dd, $J = 3.7, 1.2$ Hz, 1 H), 7.19 (s, 1 H), 7.27–7.43 (m, 5 H), 7.43–7.55 (m, 3 H). HPLC (method B): 99.7%, $t_R = 12.3$ min. MS (ESI) 478 m/z ($M - Br$)⁺.

(3*R*)-3-([(2*R*)-2-Hydroxy-2-phenyl-2-(2-thienyl)acetyl]oxy)-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**62**) was prepared as described for compound **43** starting from compound **36** (0.5 g, 1.50 mmol) and (3-bromopropoxy)benzene (0.62 g, 2.92 mmol). The reaction time was 3 h at reflux and overnight at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 0.69 g (82%) of compound **62**, mp 240.8–241.4 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.45–1.61 (m, 1 H), 1.63–1.79 (m, 1 H), 1.81–2.03 (m, 2 H), 2.03–2.23 (m, 2 H), 2.22–2.33 (m, 1 H), 3.05–3.21 (m, 1 H), 3.30–3.57 (m, 6 H), 3.88–4.01 (m, 1 H), 3.99–4.07 (t, $J = 5.8$ Hz, 2 H), 5.19–5.29 (m, 1 H), 6.92–7.00 (m, 3 H), 7.01–7.06 (dd, $J = 4.9, 3.7$ Hz, 1 H), 7.14–7.17 (dd, $J = 3.5, 1.1$ Hz, 1 H), 7.20 (s, 1 H), 7.27–7.41 (m, 5 H), 7.45–7.57 (m, 3 H). HPLC (method B): 99.9%, $t_R = 12.2$ min. MS (ESI) 478 m/z ($M - Br$)⁺.

(3*R*)-3-([(2*S*)-2-Cyclopentyl-2-hydroxy-2-(2-thienyl)acetyl]oxy)-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**63**) was prepared as described for compound **43** starting from compound **38** (1.0 g, 2.98 mmol) and (3-bromopropoxy)benzene (0.96 g, 4.44 mmol). The reaction time was 8.5 h at reflux and 16 h at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 1.53 g (93%) of compound **63**, mp 168–170 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.27–1.67 (m, 8 H), 1.79–2.05 (m, 4 H), 2.05–2.19 (m, 2 H), 2.26–2.36 (m, 1 H), 2.75–2.91 (m, 1 H), 3.17–3.27 (d, $J = 13.4$ Hz, 1 H), 3.27–3.62 (m, 6 H), 3.85–3.97 (ddd, $J = 13.4, 8.7, 2.3$ Hz, 1 H), 4.04 (t, $J = 5.8$ Hz, 2 H), 5.11–5.19 (m, 1 H), 6.19 (s, 1 H), 6.91–6.98 (m, 3 H), 6.98–7.03 (dd, $J = 4.9, 3.7$ Hz, 1 H), 7.15–7.18 (dd, $J = 3.7, 1.2$ Hz, 1 H), 7.28–7.35 (dd, $J = 7.8$ Hz, 2 H), 7.42–7.45 (dd, $J = 4.7, 1.1$ Hz, 1 H). HPLC (method B): 99.7%, $t_R = 13.1$ min. MS (ESI) 470 m/z ($M - Br$)⁺.

(3*R*)-3-([(2*R*)-2-Cyclopentyl-2-hydroxy-2-(2-thienyl)acetyl]oxy)-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (**64**) was prepared as described for compound **43** starting from compound **39** (1.0 g, 2.98 mmol) and (3-bromopropoxy)benzene (0.96 g, 4.44 mmol). The reaction time was 6 h at reflux and 12 h at room temperature. The precipitated solid was filtered and washed

with diethyl ether to obtain 1.20 g (73%) of compound **64**, mp 181 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.27–1.67 (m, 8 H), 1.67–2.05 (m, 4 H), 2.05–2.20 (m, 2 H), 2.20–2.28 (m, 1 H), 2.76–2.95 (m, 1 H), 3.18–3.27 (d, $J = 13.7$ Hz, 1 H), 3.28–3.62 (m, 6 H), 3.95–3.97 (ddd, $J = 13.7, 8.9, 2.1$ Hz, 1 H), 4.05 (t, $J = 5.5$ Hz, 2 H), 5.10–5.19 (m, 1 H), 6.18 (s, 1 H), 6.93–6.99 (m, 3 H), 6.98–7.03 (dd, $J = 4.9, 3.7$ Hz, 1 H), 7.17–7.20 (dd, $J = 3.7, 1.2$ Hz, 1 H), 7.28–7.73 (dd, $J = 7.8$ Hz, 2 H), 7.42–7.46 (dd, $J = 4.9, 1.2$ Hz, 1 H). HPLC (method B): 99.8%, $t_R = 13.1$ min. MS (ESI) 470 m/z ($M - Br$)⁺.

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Supporting Information Available: (i) Purity of compounds (HPLC); (ii) preparation of the acids **68** and **69**; (iii) assignment of the absolute configuration of acids **41** and **42** and circular dichroism (CD) curves; (iv) preparation of compounds **2**, **7**, **9**, **10**, **14**, and **15** (Scheme 1); (v) preparation of compounds **18**, **20**, **22**, and **25** (Scheme 2); (vi) preparation of compounds **31** and **32** (Scheme 3); (vii) references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Annex 2

