β-Adrenoceptor Stimulant Properties of Amidoalkylamino-Substituted 1-Aryl-2-ethanols and 1-(Aryloxy)-2-propanols

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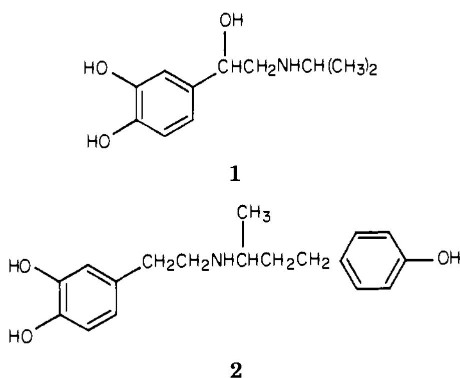
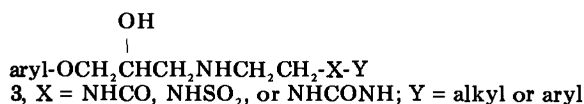
Received July 22, 1980

Parallel series of 2-[(2-amidoethyl)amino]-1-arylethanols and 1-[(2-amidoethyl)amino]-3-(aryloxy)-2-propanols have been prepared, and the compounds were tested as β-adrenoceptor stimulants on the heart and circulation of the dog. The corresponding 2-(alkylamino)-1-arylethanols and 3-(alkylamino)-1-(aryloxy)-2-propanols have been tested for comparison and the structure–activity relationships (SAR) examined. The arylethanols are potent full agonists, showing selectivity for the heart relative to blood vessels, while the (aryloxy)propanols are even more cardioselective and are partial agonists. Within a narrow series of 1-[(amidoethyl)amino]-3-(4-hydroxyphenoxy)-2-propanols, careful examination of the SAR of the amide group showed that great variation in cardioselectivity and degree of agonism may be produced. From this study ICI 118587, N-[2-[[2-hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl]-4-morpholinecarboxamide, was selected for its high cardioselectivity and 50% agonist properties. This compound is under clinical evaluation as a cardiac stimulant.

Heart failure is characterized by poor pumping performance of the cardiac muscle. Historically, treatment has been with cardiotonic digitalis glycosides, and more recently β-adrenergic stimulants and vasodilators, either independently[1–4] or in combination,[5] have been used in acute cases to increase cardiac contractile force and reduce vascular resistance. The disadvantages of catecholamine β-stimulants, such as isoproterenol (1), are their powerful



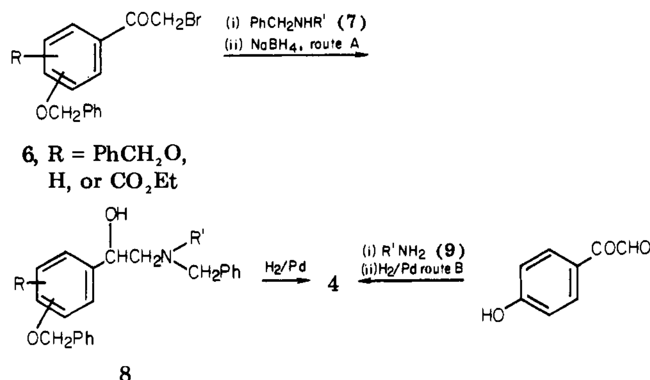


3, X = NHCO, $NHSO_2$, or NHCONH; Y = alkyl or aryl

vasodilating effect which causes a reflex rise in heart rate, their lack of oral absorption, and their short half-life. Although dobutamine (2) causes no vasodilation, it is still short acting. Infusions of 2 have been used successfully to treat chronic heart failure,[6] but only for short periods.

We wished to produce an orally active cardioselective β-stimulant as a long-term treatment of heart failure and were attracted by the properties of some acylaminoalkyl-substituted β-adrenergic blockers (3) which have been developed in our laboratories.[7] These compounds have

Scheme I[a]

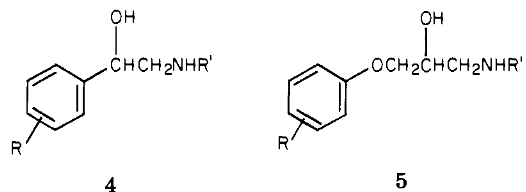


[a] R′ has the same meaning described for formulas 4 and 5.

a long duration of action and are selective in their actions on the heart relative to blood vessels (i.e., selective for β_1 vs. β_2 receptors in the classification of Lands[8]).

We have prepared a number of compounds containing amidoalkyl side chains and bearing phenolic substituents in various positions of the aromatic ring and have examined them for their cardiac stimulant (β_1) and vasodilating (β_2) effects. We were seeking a partial agonist, that is a compound with a lower maximum stimulant action on the cardiac β_1 adrenoceptors than isoproterenol (1), which we took as our standard. Such a compound would also be a β-adrenoceptor blocker and thus would attenuate the actions of natural catecholamines while exerting a stimulant action of its own. Stabilization of the level of cardiac stimulation should occur and this would, we believe, be safer than the use of a full agonist.

We have prepared compounds with two types of basic structure, the aminoarylethanols (4) and aminoaryloxypropanols (5). The compounds were tested for β-adre-



4 5

R = 3,4-$(OH)_2$, 3,5-$(OH)_2$, 4-OH, 3-OH, or 4-OH and 3-CH_2OH; R′ = $CH(CH_3)_2$, $CH_2CH_2NHCOCH(CH_3)_2$, $CH_2CH_2NHCONHC_6H_5$, or CH_2CH_2R''; R″ = some amidic grouping

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(2) D. T. Mason, N. A. Awan, A. N. DeMaria, G. Lee, and E. A. Amsterdam, *Drug. Ther.*, 8(6), 30 (1978).
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(4) E. Mikulic, J. N. Cohn, and J. A. Franciosa, *Circulation*, **56**(4), 528 (1977).
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(6) C. V. Leier, J. Webel, and C. A. Bush, *Circulation*, **56**(3), 468 (1977).
(7) L. H. Smith, *J. Appl. Chem. Biotechnol.*, **28**, 201 (1978); U.K. Patent 1 455 116.
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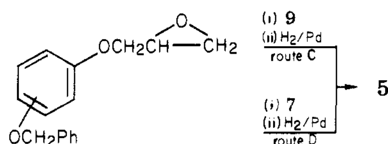
Scheme II



nergic stimulant action in anesthetized dogs as described under Pharmacology. We first compared compounds having representative amide (isobutyramidoethyl) and urea (phenylureidoethyl) side chains with their simple N-isopropyl counterparts. Structure **5** (R = 4-OH, R′ = CH_2CH_2R'') was then selected as being a compound with properties close to those we were seeking, and the effect of altering the amide function R″ on biological activity was examined in greater detail.

Chemistry. The arylethanols **17–30** were prepared by one of the routes shown in Scheme I. The saligenin derivatives **28–30** were made from **8** (4-OCH_2Ph, R = 3-CO_2Et) by reducing the ester group with sodium trimethoxyborohydride before hydrogenolysis.

(Aryloxy)propanols **31–45**, **47**, and **48** were prepared by routes previously described[9,10] and shown in Scheme II. Compounds **43–45** were made via the ester reduction described for **28–30** before hydrogenolysis (route D).

For convenience, the intermediate **11** was prepared in bulk from **10** (4-OCH_2Ph, R = H) and **7** (R′ = CH_2CH_2NHCO-i-C_3H_7) and hydrolyzed to the protected amine **12** (Scheme III). This was readily acylated by acid chlorides or isocyanates to **14**, which hydrogenolyzed to **15** (route E). For **55**, the acylating agent was potassium cyanate and hydrochloric acid. The same intermediate **12** reacted with phenyl chloroformate to give the crystalline carbamate **13** which, with amines, gave **14** (route F). For the preparation of **54**, the amine **12** was acylated with N-chlorosulfonylamide **16**, giving a mixture of sulfamide **14** (R″ = $NHSO_2NHPh$) and amide **14** (R″ = NHCOPh). After chromatography, hydrogenolysis of the former gave **54**.

The compounds are listed in Tables I–III. The synthesis of representative examples by routes A to F, and of intermediates **12** and **13**, is detailed under Experimental Section. All compounds are racemic.

Pharmacology. Experiments were performed in anesthetized dogs depleted of catecholamines, in which the vagal nerves to the heart and the main nerve trunks to a perfused hind limb were sectioned. The aim of the experiments was to determine the selectivity of the compounds as stimulants of cardiac β-adrenergic receptors (β_1) relative to vascular β-adrenergic receptors (β_2) and to measure the degree of intrinsic sympathomimetic activity (ISA) at each of these sites.

To this end, dose–response curves were constructed for the effect of isoproterenol (**1**) and the test compounds on peak changes in heart rate (HR) and hind limb perfusion pressure (HLPP). The detailed methods are described under Experimental Section.

A typical result is shown in Figure 1, exemplified by compound **36**. The ED_{50} values of **1** and **36** were calculated from the dose–response curves. The intrinsic sympathomimetic activity (ISA) of the compound was calculated as the maximum change in HR produced by **36** as a percentage of the maximum produced by **1**. The effects of

(9) A. F. Crowther, R. Howe, and L. H. Smith, *J. Med. Chem.*, 14, 511 (1971).

(10) C. Kaiser, T. Jen, E. Garvey, W. D. Bowen, D. F. Colella, and J. R. Wardell, Jr., *J. Med. Chem.*, 20, 687 (1977).

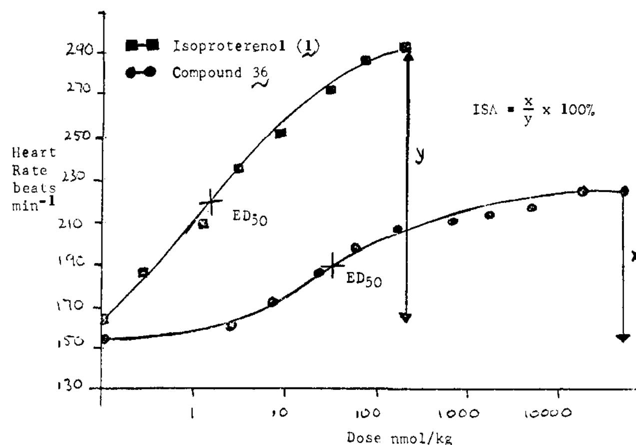


Figure 1. Effect of compound **36** on heart rate.

1 and test compounds on HLPP were defined in a similar manner.

To assess the reproducibility of the measurement of ED_{50} and ISA these values for **36** were determined several times. For a single estimate of an ED_{50}, the 95% confidence limits[11] were ±22% of the mean value and for ISA ±7% of the mean value for HR and ±9% for HLPP.

The values of ED_{50} and ISA for β_1 and β_2 effects are shown in Tables I–III, together with the values for cardioselectivity (ratio ED_{50} HLPP/ED_{50} HR).

Discussion

The acylaminoalkylamines described here have a good duration of action, at least 30 min following an iv dose of the ED_{50}, whereas that of **1** is only 2–3 min. Oral absorption has not been examined in all cases, but those tested (**21**, **39**, **50**, **60**) are orally effective when dosed to a conscious dog at 5 mg/kg. All the compounds are β-stimulants with cardiac potencies varying from 0.9 (**18**) to 755 nmol/kg (**27**).

3,4-Dihydroxy compounds (**17**, **18**, **31–33**) are comparable in potency to the standard, **1**.

Considering the compounds in Tables I and II, the only clear pattern regarding potency is that, with a few exceptions (**18** and **33**, **19** and **34**, **23** and **38**), amino(aryloxy)propanols are more potent than the corresponding aminoarylethanols (**17** and **32**, **20** and **35**, **21** and **36**, **22** and **37**, **24** and **39**, **26** and **41**, **27** and **42**, **28** and **43**, **29** and **44**).

3,4-Dihydroxy compounds (**17**, **18**, **31–33**) are virtually full agonists on both cardiac (β_1) and hind-limb (β_2) receptors. The other arylethanols in Table I have high ISA on both receptors (average ~81% of isoproterenol maximum on β_1 and 85% on β_2 effects) with the exception of compound **24**. In the (aryloxy)propanol series (Table II), however, partial agonists are the rule, noncatechols having 19–57% ISA (mean 36%) on β_1 receptors and slightly less (mean 28%) on β_2.

When selectivity for cardiac vs. vascular effects is examined, several trends emerge, though each has exceptions. In general, amides are more β_1 selective than isopropyl compounds (**17** vs. **1**, **23** vs. **22**, **26** vs. **25**, **29** vs. **28**, **35** vs. **34**, **44** vs. **43**) with **20** vs. **19** and **38** vs. **37** as exceptions. Ureas are often more selective than amides (**18** vs. **17**, **21** vs. **20**, **30** vs. **29**, **33** vs. **32**, **39** vs. **38**, **42** vs. **41**) with again some exceptions (**24** vs. **23**, **27** vs. **26**, **36** vs. **35**, **45** vs. **44**). (Aryloxy)propanols are more β_1 selective than arylethanols (**31** vs. **1**, **33** vs. **18**, **35** vs. **20**, **36** vs. **21**, **37** vs. **22**, **39** vs. **24**, **42** vs. **27**, **43** vs. **28**, **44** vs. **29**), excepting **32** and **17**, **34** and

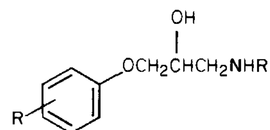
(11) G. W. Snedecor and W. G. Cochran "Statistical Methods", 6th ed., Iowa State University Press, Ames, Iowa, 1967.

Table I. 2-(Substituted-amino)-1-arylethanols

			heart rate		hind limb perf. pressure								
no.	R	R'	ED_{50},[a] nmol/kg	ISA,[b] %	ED_{50},[a] nmol/kg	ISA,[b] %	selectivity[c]	salt	synth. method[d]	crystn. solvent	yield,[e] %	mp, °C	formula[f]
1	3-OH, 4-OH	i-C_3H_7	1.4	100	0.8	100	0.5						
17	3-OH, 4-OH	CH_2CH_2NHCO-i-C_3H_7	2.5	84	6.0	100	2.4	0.5-oxalate, $0.5H_2O$	A	EtOH-Et_2O	22	150-151	$C_{15}H_{23}N_2O_6 \cdot 0.5H_2O$
18	3-OH, 4-OH	$CH_2CH_2NHCONHPh$	0.9	100	5.4	100	6.0	0.5-oxalate, H_2O	A	MeCN	38	98-100	$C_{18}H_{22}N_3O_6 \cdot H_2O$
19	4-OH	i-C_3H_7	10	88	77	55	7.7	0.5-oxalate	A	EtOH	41	216-218	$C_{12}H_{18}NO_4$
20	4-OH	CH_2CH_2NHCO-i-C_3H_7	56	90	71	82	1.3	0.5-oxalate	B	H_2O	20	215-216	$C_{15}H_{23}N_2O_5$
21	4-OH	$CH_2CH_2NHCONHPh$	98	71	228	70	2.3	0.5-oxalate, $0.5H_2O$	B	i-PrOH-EtOH	19	160-162	$C_{18}H_{22}N_3O_5 \cdot 0.5H_2O$
22	3-OH	i-C_3H_7	71	100	138	100	1.9	0.5-oxalate, $0.75H_2O$	A	EtOH-Et_2O	70	144	$C_{12}H_{18}NO_4 \cdot 0.75H_2O$
23	3-OH	CH_2CH_2NHCO-i-C_3H_7	38	75	132	75	3.5	0.5-oxalate, $0.5H_2O$	A	EtOH-Et_2O	20	165-166	$C_{15}H_{23}N_2O_5 \cdot 0.5H_2O$
24	3-OH	$CH_2CH_2NHCONHPh$	143	42	286	55	2.0	oxalate, $0.5H_2O$	A	EtOH-Et_2O	39	111-112	$C_{19}H_{23}N_3O_7 \cdot 0.5H_2O$
25	3-OH, 5-OH	i-C_3H_7	14	83	14	100	1.0						
26	3-OH, 5-OH	CH_2CH_2NHCO-i-C_3H_7	35	89	105	100	3.0	oxalate, H_2O	A	EtOH-Et_2O	45	118-120	$C_{16}H_{24}N_2O_8 \cdot H_2O$
27	3-OH, 5-OH	$CH_2CH_2NHCONHPh$	755	80	755	100	1.0	0.5-oxalate, $0.5H_2O$	A	MeOH	8	203-205	$C_{18}H_{22}N_3O_6 \cdot 0.5H_2O$
28	3-CH_2OH, 4-OH	i-C_3H_7	222	100	9	100	0.04	base, $0.25H_2O$	A	freeze-dried from H_2O	10	oil	$C_{12}H_{19}NO_3 \cdot 0.25H_2O$
29	3-CH_2OH, 4-OH	CH_2CH_2NHCO-i-C_3H_7	77	85	152	100	2.0	0.5-oxalate, $0.5H_2O$	A	EtOH-H_2O-Et_2O	27	176-178	$C_{16}H_{25}N_2O_6 \cdot 0.5H_2O$
30	3-CH_2OH, 4-OH	$CH_2CH_2NHCONHPh$	38	76	304	88	8.0	0.75-oxalate	A	EtOH-Et_2O	42	132-134	$C_{19.5}H_{24.5}N_3O_7$

[a] The dose to produce 50% of the maximum effect achieved by the compound. [b] The maximum effect of the compound as a percentage of that achieved by 1. [c] Selectivity is expressed as the ratio of the compound's ED_{50} on hind-limb perfusion pressure to the ED_{50} on heart rate. [d] Refers to route shown in Schemes I-III. [e] The yield quoted is for isolated purified product. [f] All microanalyses were within ±0.4% of theory for C, H, and N.

Table II. 3-(Substituted-amino)-1-(aryloxy)propan-2-ols



no.	R	R'	heart rate: ED_{50},[a] nmol/kg	heart rate: ISA,[b] %	hind limb perf. pressure: ED_{50},[a] nmol/kg	hind limb perf. pressure: ISA,[b] %	selectivity[c]	salt	synth. method[d]	crystn solvent	yield,[e] %	mp, °C	formula[f]
31	3-OH, 4-OH	i-C_3H_7	1.2	100	1.2	100	1.0	0.5-oxalate	D	MeOH	80	205–207	$C_{13}H_{20}NO_6$
32	3-OH, 4-OH	CH_2CH_2NHCO-i-C_3H_7	1.6	100	1.6	100	1.0	0.5-oxalate	D	i-PrOH–MeOH	38	168	$C_{16}H_{25}N_2O_7$
33	3-OH, 4-OH	$CH_2CH_2NHCONHPh$	4.1	80	100	100	24.4	0.5-oxalate, $0.25H_2O$	D	i-PrOH	40	122	$C_{19}H_{24}N_3O_7 \cdot 0.25H_2O$
34[g]	4-OH	i-C_3H_7	31	56	6	31	0.8						
35	4-OH	CH_2CH_2NHCO-i-C_3H_7	20	30	78	40	3.9	0.5-oxalate, $0.25H_2O$	C	EtOH	86	213	$C_{16}H_{25}N_2O_6 \cdot 0.25H_2O$
36	4-OH	$CH_2CH_2NHCONHPh$	58	52	165	45	2.8	0.5-oxalate	C	MeOH–Et_2O	32	164–165	$C_{19}H_{24}N_3O_6$
37	3-OH	i-C_3H_7	18	57	67	33	3.7	0.5-oxalate	C	EtOH trituration	25	193–195	$C_{13}H_{20}NO_5$
38	3-OH	CH_2CH_2NHCO-i-C_3H_7	209	25	260	33	1.2	0.5-oxalate, $0.75H_2O$	D	i-PrOH–EtOAc	37	73	$C_{16}H_{25}N_2O_6 \cdot 0.75H_2O$
39	3-OH	$CH_2CH_2NHCONHPh$	9	21	>1500	0	>170	0.5-oxalate, $0.5H_2O$	C	EtOH–H_2O	10	153–154	$C_{19}H_{24}N_3O_6 \cdot 0.5H_2O$
40	3-OH, 5-OH	i-C_3H_7	12	32	12	25	1.0	oxalate	D	EtOH–Et_2O	48	157–159	$C_{14}H_{21}NO_8$
41	3-OH, 5-OH	CH_2CH_2NHCO-i-C_3H_7	19	19	16	18	0.8	oxalate, H_2O	D	EtOH–Et_2O	20	86–88	$C_{17}H_{26}N_2O_9 \cdot H_2O$
42	3-OH, 5-OH	$CH_2CH_2NHCONHPh$	41	28	>5500	0	>135	oxalate, $0.25H_2O$	D	MeCN	20	176	$C_{20}H_{25}N_3O_9 \cdot 0.25H_2O$
43	3-CH_2OH, 4-OH	i-C_3H_7	67	44	98	55	1.5	0.5-oxalate, $0.25H_2O$	D	EtOH	35	213–214	$C_{14}H_{22}NO_6 \cdot 0.25H_2O$
44	3-CH_2OH, 4-OH	CH_2CH_2NHCO-i-C_3H_7	43	26	270	30	6.3	0.5-oxalate, $0.5H_2O$	D	MeOH–Et_2O	40	148–149	$C_{17}H_{27}N_2O_7 \cdot 0.5H_2O$
45	3-CH_2OH, 4-OH	$CH_2CH_2NHCONHPh$	45	42	112	32	2.5	0.5-oxalate, $0.5H_2O$	D	EtOH trituration	75	135–137	$C_{20}H_{26}N_3O_7 \cdot 0.5H_2O$

[a–f] See corresponding footnotes in Table I. [g] Also known as Hassle H80/62.

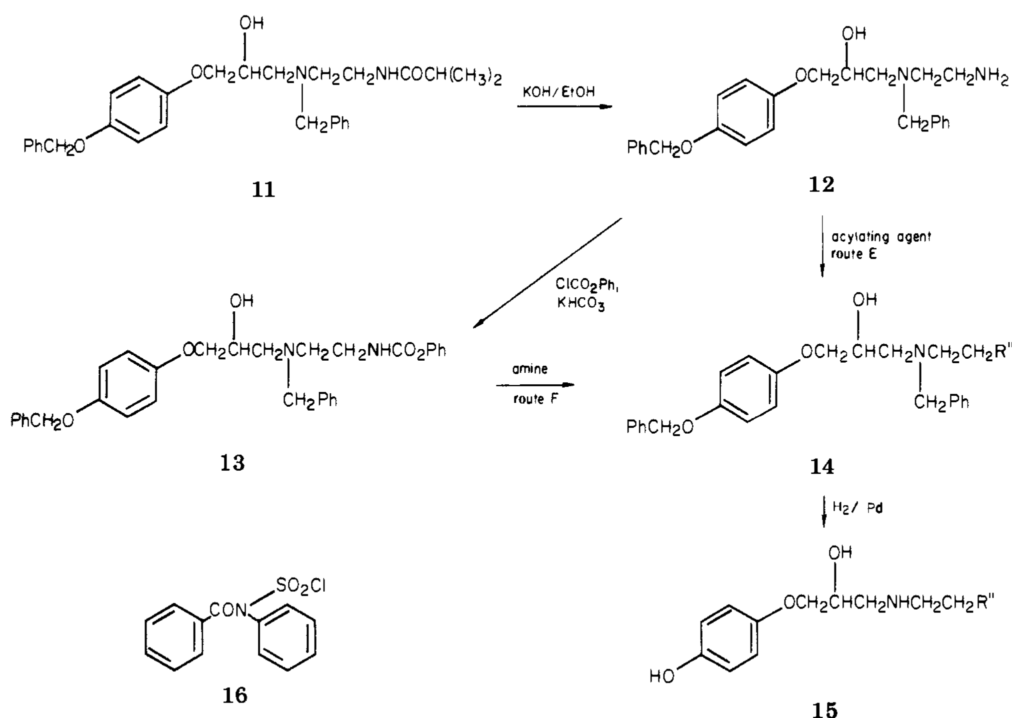
Table III. 1-[(Amidoethyl)amino]-3-(4-hydroxyphenoxy)-2-propanols

HO–C6H4–OCH2CH(OH)CH2NHCH2CH2R''

no.	R''	heart rate ED50,[a] nmol/kg	heart rate ISA,[b] %	hind limb perf. pressure ED50,[a] nmol/kg	hind limb perf. pressure ISA,[b] %	selectivity[c]	salt	synth. method[d]	crystn solvent	yield,[e] %	mp, °C	formula[f]
46	$NHCON(CH_3)_2$	24	29	>1000	0	>40	oxalate, $0.5H_2O$	E	MeOH	37	181	$C_{16}H_{25}N_3O_4\cdot 0.5H_2O$
47	$NHCOCH_2Ph$	27	40	90	40	3.3	0.5-oxalate, H_2O	C	EtOH	13	110	$C_{20}H_{25}N_2O_6\cdot H_2O$
48	$NHSO_2Ph$	74	55	55	45	0.7	0.5-oxalate, $0.5H_2O$	C	MeOH	30	158–159	$C_{20}H_{23}N_2O_7S\cdot 0.5H_2O$
49	$NHCONHCH(CH_3)_2$	235	60	193	26	0.8	0.5-oxalate	E	EtOH–H_2O	25	204	$C_{16}H_{26}N_3O_6$
50	$NHCONHCH_2CH_2OH$	77	69	236	49	3.1	0.5-oxalate, $0.25H_2O$	F	EtOH	17	151–155	$C_{15}H_{24}N_3O_7\cdot 0.25H_2O$
51	$NHCONHCH_2CH_2OCH_3$	21	74	52	58	2.5	0.5-oxalate, $0.25H_2O$	F	EtOH	45	126–128	$C_{16}H_{26}N_3O_7\cdot 0.25H_2O$
52	$NHSO_2N(CH_3)_2$	69	80	175	80	2.5	0.5-oxalate, $0.5H_2O$	E	EtOH trituration	30	171–172	$C_{14}H_{24}N_3O_7S\cdot 0.5H_2O$
53	$NHCONHCH_2Ph$	19	80	81	70	4.3	HCl, H_2O	F	EtOH–Et_2O	10	113	$C_{18}H_{26}ClN_3O_4\cdot H_2O$
54	$NHSO_2NHPh$	18	92	52	79	2.9	0.5-oxalate	E	EtOH	35	139–140	$C_{18}H_{24}N_3O_7S$
55	$NHCONH_2$	78	92	134	50	1.7	acetate	E	*i*-PrOH	21	135–137	$C_{14}H_{23}N_3O_6$
56	$NHCON(C_2H_5)_2$	37	28	36	18	10.0	0.5-oxalate, $0.5H_2O$	F	EtOH	18	169–171	$C_{17}H_{28}N_3O_6\cdot 0.5H_2O$
57	$NHCON(CH_3)CH_2CH_2OH$	37	43	1170	48	33.0	HCl, $2H_2O$	F	freeze-dried	30	oil	$C_{15}H_{26}ClN_3O_5\cdot 2H_2O$
58	$NHCON(CH_2CH_2OH)_2$	145	41	2330	42	16.0	HCl, $3H_2O$	F	freeze-dried	50	oil	$C_{16}H_{28}ClN_3O_6\cdot 3H_2O$
59	$NHCON(CH_3)CH_2CH_2OCH_3$	12	27	>3000	0	>250	0.5-oxalate	F	EtOH trituration	70	158–160	$C_{17}H_{28}N_3O_7$
60	NHCON(morpholino ring)O	12	50	>3000	0	>250	0.5-oxalate	F	EtOH	52	168–169	$C_{17}H_{26}N_3O_7$

[a–f] See corresponding footnotes in Table I.

Scheme III[a]



[a] R'' is a variety of amide or urea groups.

19, **38** and **23**, **41** and **26**, and **45** and **30**. These differences may be large, for example, **28**, which is a close analogue of the bronchodilator salbutamol, is 200 times less cardioselective than the phenylurea **30**, and the arylethanol **24** is 85 times less selective than the (aryloxy)propanol **39**.

In summary, arylethanols tend to be full agonists, while (aryloxy)propanols are partial agonists, and cardioselectivity tends to increase from isopropyl to amide to urea and also from arylethanol to (aryloxy)propanol.

Thus, (aryloxy)propanols, probably with a urea side chain, offer the best chance of achieving our objective of a cardioselective partial agonist.

We have investigated the effects of the nature of the amidic group in more detail using the compounds listed in Table III. Cardiac potency varies very little within this group (excepting **49** and **58**), but the degree of agonism varies widely, between 29 (**46**) and 92% (**54** and **55**) of that achieved by isoproterenol (**1**). Changing from the carbonyl group of amides (e.g., **47**) to the sulfonyl group of sulfonamides (e.g., **48**) increases the ISA from 40 to 55%. This is shown even more dramatically when changing from urea **46** (40% ISA) to sulfamide **52** (80% ISA) and from urea **36** (52% ISA) to sulfamide **54** (92% ISA), the first reported example of a noncatechol that is virtually a full agonist. This is matched by the simple urea **55**. The biochemical basis for this is discussed by Franklin et al.[12] Monoalkylation of the primary urea **55** (e.g, **49**, 60% ISA) and dialkylation (**46**, 29% ISA; **56**, 28% ISA) progressively lower the degree of agonism, while introduction of an electronegative group, as in alcohol **50** (69% ISA) or ether **51** (74% ISA), increases the amount of agonist activity compared to an alkylurea, e.g., **49** (60% ISA).

Dialkylation of ureas increases cardioselectivity. Thus, compounds **50**, **51**, **53**, etc. are cardioselective, but compound **46** is at least 10 times more so, having a selectivity ratio of more than 40:1. This exciting discovery prompted the synthesis of compounds **56**–**60** in an attempt to produce a very cardioselective compound with an ISA nearer to 50% than compound **46** (29%). All these compounds are very selective, and compounds **57**, **58**, and **60** have much higher ISA (43, 41, and 50%, respectively).

Cardioselective compounds do not show an unusually high affinity for β_1 receptors, as might be expected at first sight, but rather a lack of effect on β_2 receptors. Thus, for example, the morpholine **60** with a cardiac ED_{50} of 12 nmol/kg has no vasodilator (β_2) effects at doses up to at least 3000 nmol/kg.

As a result of the above study, compound **60** (ICI 118587) was chosen as a development candidate for the treatment of heart failure and other cardiac disorders. This compound is a potent orally active β_1 partial agonist (50% ISA) with no measurable stimulant action on blood vessels up to at least 250 times its cardiac ED_{50} dose. A preliminary publication of the pharmacology of this compound has appeared recently.[13]

Experimental Section

All melting points were obtained using a Buchi capillary apparatus and are uncorrected. Where analyses are indicated only by the symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values. NMR data were consistent with the structures, and the spectra were recorded on a Varian HA 100, Varian A60, or Perkin-Elmer R12 spectrometer, using Me_4Si as internal standard and usually Me_2SO-d_6 as solvent.

***N*-[2-[[2-Hydroxy-2-(3-hydroxyphenyl)ethyl]amino]-ethyl]-2-methylpropanamide (23; Route A).** 3-(Benzyloxy)-phenacyl bromide (7.0 g at 90% estimated purity, 0.02 mol), *N*-[2-(benzylamino)ethyl]isobutyramide (8.8 g, 0.04 mol), and dioxane (150 mL) were stirred for 16 h at ambient temperature. The solvent was evaporated, and the residue was shaken with water and extracted with EtOAc. After evaporation, the product in 50 mL of EtOH was reduced with excess $NaBH_4$ (0.5 h). Dilution with water and extraction with ethyl acetate gave an oil, which was dissolved in 50 mL of AcOH and hydrogenated over

(12) T. J. Franklin and P. A. Twose, *J. Cyclic Nucleotide Res.*, 5(1), 19 (1979).

(13) J. J. Barlow, B. G. Main, A. Nuttall, J. Moors, and H. M. Snow, *Br. J. Pharmacol.*, 67(3), 412p (1979).

(14) J. Meybeck, *Ann. Chim.*, 17, 129–206 (1932).

100 mg of 30% Pd/C, 790 mL of hydrogen being taken up (1.5 h). The catalyst and solvent were removed, and the resulting oil was dissolved in EtOH. Addition of excess ethereal oxalic acid gave the product as a solid, which was recrystallized from EtOH–Et_2O to give 1.28 g (20%) of **23** hemioxalate hemihydrate, mp 164–166 °C dec. Anal. ($C_{15}H_{23}N_2O_5$·$0.5H_2O$) C, H, N.

***N*-[2-[[2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl]amino]ethyl]-2-methylpropanamide (29; Route A).** *N*-[2-[*N*-Benzyl-2-hydroxy-2-[4-(benzyloxy)-3-carboethoxy-phenyl]amino]ethyl]-2-methylpropanamide (7.9 g, 0.0152 mol), prepared as above, was dissolved in 30 mL of THF and 15 mL of MeOH and stirred for 72 h with $NaBH_4$ (3.8 g, 0.1 mol), for 48 h with a further 3.8 g, and finally for 10 days with an additional 7.6 g (a total of 0.4 mol). After dilution with water, the product was extracted with EtOAc and hydrogenolyzed as before to give **29** hemioxalate hemihydrate, which was recrystallized from a mixture of 10% aqueous EtOH and Et_2O to yield 1.3 g (27%), mp 176–178 °C dec. Anal. ($C_{16}H_{25}N_2O_6$·$0.5H_2O$) C, H, N.

1-[2-[[2-Hydroxy-2-(4-hydroxyphenyl)ethyl]amino]-ethyl]-3-phenylurea (21; Route B). 2-(Phenylureido)ethylamine (1.79 g, 0.01 mol) and 100 mg of 30% Pd/C were stirred in 25 mL of EtOH under 1 atm pressure of H_2. 4-Hydroxyphenylglyoxal (1.68 g, 0.01 mol) in 15 mL of EtOH was added over 0.5 h. Hydrogen (215 mL) was absorbed. AcOH was added to dissolve the acetate salt formed, and stirring was continued until H_2 absorption ceased (325 mL). After the solution was evaporated, the salt was prepared as before and crystallized from *i*-PrOH-EtOH to give 700 mg of **21** hemioxalate hemihydrate (20%), mp 160–162 °C. Anal. ($C_{18}H_{22}N_3O_5$·$0.5H_2O$) C, H, N.

1-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)propyl]amino]-ethyl]-3-phenylurea (36; Route C). 4-(Benzyloxy)phenylglycidyl ether (2.56 g, 0.01 mol) and 2-(phenylureido)ethylamine (1.79 g, 0.01 mol) were refluxed for 3 h in 20 mL of *i*-PrOH. After the solution was evaporated, the residue was hydrogenolyzed in AcOH as before to give **36** hemioxalate, which was recrystallized from MeOH–Et_2O to yield 1.27 g (32%), mp 164–165 °C. Anal. ($C_{19}H_{24}N_3O_6$) C, H, N.

***N*-[2-[[2-Hydroxy-3-(3,4-dihydroxyphenoxy)propyl]-amino]ethyl]-2-methylpropanamide (32; Route D).** Similarly to route C, reaction of 3,4-bis(benzyloxy)phenylglycidyl ether with an equimolar quantity of *N*-benzyl-2-(isobutyramido)ethylamine in *i*-PrOH, followed by hydrogenolysis, gave 38% **32** hemioxalate, mp 168 °C dec. Anal. ($C_{16}H_{26}N_2O_7$) C, H, N.

This compound, and all other 3,4-dihydroxyphenyl compounds prepared, is extremely sensitive to light and air in solution. Prolonged exposure to air should be avoided and a nitrogen blanket used as much as possible. The crystalline salts of these compounds, however, are air and light stable.

The preparation of the following intermediates is described in ref 15: 2-(isobutyramido)ethylamine and its *N*-benzyl derivative; 2-(phenylureido)ethylamine and its *N*-benzyl derivative; 3,5-bis(benzyloxy)phenol and its glycidyl ether; 4-(benzyloxy)-3-(hydroxymethyl)phenol and its glycidyl ether.

***N*-[2-[*N*-Benzyl[3-[4-(benzyloxy)phenoxy]-2-hydroxy-propyl]amino]ethyl]ethylamine (12).** *N*-[[*N*-Benzyl[3-[4-(benzyloxy)phenoxy]-2-hydroxypropyl]amino]ethyl]-2-methyl-propanamide (95.2 g, 0.2 mol), KOH (200 g), and EtOH (400 mL) were stirred and refluxed for 4 days. After the solution was cooled and diluted with water, the product was extracted with toluene. Evaporation of the extract gave **12** as an oil, which was then used directly. A portion, however, was converted to the oxalate salt, which was recrystallized from H_2O, mp 187–188 °C. Anal. ($C_{29}H_{34}N_2O_{11}$) C, H, N.

1,1-Dimethyl-3-[2-[[2-hydroxy-3-(4-hydroxyphenoxy)-propyl]amino]ethyl]urea (49; Route E). Amine **12** (8.12 g, 0.02 mol) in toluene (50 mL) was stirred with dimethylcarbamoyl chloride (2.15 g, 0.02 mol) and anhydrous K_2CO_3 (2.7 g, 0.02 mol) for 2 h. After the solution was diluted with water, the product was extracted with EtOAc and the evaporated extract hydrogenolyzed as before to give **49** monoxalate hemihydrate, which was recrystallized from MeOH to give 3.0 g (37%), mp 181 °C. Anal. ($C_{16}H_{25}N_3O_8$·$0.5H_2O$) C, H, N.

A similar procedure was followed when acid chlorides, sulfonyl chlorides, and dialkylsulfamoyl chlorides were used. Where isocyanates were used, K_2CO_3 was omitted.

1-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)propyl]amino]-ethyl]urea (55; Route E). A solution of **12** (4.06 g, 0.01 mol) in toluene (15 mL) was stirred for 18 h with 1 N HCl (20 mL) and KCNO (0.85 g, 0.01 mol). KCNO (0.85 g) was then added and the mixture stirred for another 1 h. Ether extraction and hydrogenolysis of the evaporated extract gave **55** monoxalate, which was recrystallized from *i*-PrOH to give 700 mg (21%), mp 135–137 °C. Anal. ($C_{14}H_{21}N_3O_8$) C, H, N.

1-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)propyl]amino]-ethyl]-3-phenylsulfamide (54; Route E). A solution of amine **12** (7.3 g, 0.018 mol) in toluene (20 mL) was treated with *N*-(chlorosulfonyl)benzanilide[14] (1.5 g, 0.05 mol) and the mixture was stirred for 48 h. The solution was chromatographed on silica gel (160 g; British Drug Houses) using 10% EtOAc in $CHCl_3$ as eluant and gave three products: 450 mg, R_f 0.6; 50 mg, R_f 0.4; 8.3 g, R_f <0.1. This third fraction was rechromatographed on silica gel (160 g; Merck) using 50% EtOAc in $CHCl_3$ and gave two products, one (R_f 0.7) was compound **14** (R″ = NHCOPh) and the other (R_f 0.6–3 g) was **14** (R = $NHSO_2NHPh$). The latter was hydrogenated in the usual fashion to give **54** hemioxalate: yield 0.75 g [35% based on *N*-(chlorosulfonyl)benzanilide] after recrystallization from EtOH; mp 139–140 °C. Anal. ($C_{19}H_{24}N_3O_7S$) C, H, N.

Phenyl *N*-[2-[*N*-Benzyl[3-[4-(benzyloxy)phenoxy]-2-hydroxypropyl]amino]ethyl]carbamate. A mixture of amine **12** (4.06 g, 0.01 mol), $NaHCO_3$ (2.5 g, 0.03 mol), and toluene (15 mL) was stirred and treated with phenyl chloroformate (1.6 g, 0.01 mol). An exotherm raised the temperature to 50 °C. When cool, water was added, and the solid product was collected, washed, and dried to give 2.6 g of **13** (50%), mp 90–91 °C. Anal. ($C_{32}H_{34}N_5O_5$) C, H, N.

1-Benzyl-3-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)-propyl]amino]ethyl]urea. A mixture of carbamate **13** (2.63 g, 0.005 mol) and benzylamine (0.6 g, 0.0055 mol) in toluene (25 mL) was heated for 18 h at 100 °C. When the mixture cooled, 2.05 g of crystals separated. This product was debenzylated as usual to give **53** as an oil, which was converted to 0.2 g (10%) of the hydrochloride with HCl in EtOH–ether, mp 113 °C. Anal. ($C_{19}H_{26}ClN_3O_4$·H_2O) C, H, N.

Pharmacological Methods. Beagle dogs of either sex weighing between 10 and 16.5 kg were treated with syrosingopine for 2 days (dose 5 mg/kg sc per day) in order to deplete catecholamines. The dogs were anesthetized with pentobarbital (20 mg/kg iv) and maintained on a dose of 2 mg/kg iv every 30 min. A tracheotomy was performed and artificial ventilation was started using a mixture of 40% oxygen in room air supplied by a Starling ideal pump.

To perfuse the left hind limb the abdominal aorta was exposed through a flank incision, and all branches below the renal arteries were tied off. Blood was drawn through a cannula in the abdominal aorta and returned to the animal via a roller pump (Watson Marlow) through a cannula inserted into the left femoral artery. Coagulation of blood was prevented by an iv injection of heparin (dose 500 IU/kg). The pump was primed with 150-mL dextran 150 injection (British Pharmacopoaea) in 5%, w/v, dextrose.

The following nerves were divided: the left femoral, the left spinal nerves and sympathetic chain at the level of L7 to denervate the vascular supply to the hind limb, and the vagal nerves in the neck to complete the denervation of the heart.

Arterial pressure in the perfused hind limb was recorded through a polyethylene cannula inserted through the lumen of the perfusion cannula. Systemic arterial pressure was recorded through a cannula inserted into the right carotid artery. To each cannula was attached a strain gauge manometer (Hewlett Packard 4-442-001) and, after amplification by means of a DC bridge amplifier (Devices N3552), the pressures were recorded using a UV light recorder (S. E. Laboratories Model 6008). The ECG lead II was recorded and the signal used to drive a cardiotachometer (Devices Model 4521).

Samples of arterial blood were withdrawn at intervals throughout each experiment, and pH, pO_2, and pCO_2 were measured using a Corning EEL Model blood gas analyzer. End tidal pCO_2 was continuously measured by aspirating expired air

(15) U.K. Patent Application 57970 (1972); 43478 (1970).

from the trachea into an infrared carbon dioxide analyzer (P. K. Morgan Ltd.). The arterial pCO_2 was kept within the limits of 34–42 mmHg and the arterial pH between 7.35 and 7.43 by either the adjustment of respiration or the iv injection of a 1.0 M $NaHCO_3$ solution. Esophageal temperature was recorded from a thermister probe (Yellow Springs Instrument Co., Inc.) and maintained at 37 ± 1 °C by heating lamps above and below the animal.

In each dog, response curves relating iv injections of racemic isoproterenol (1) in the range 0.05 to 10 μg/kg to peak changes in heart rate (HR) and hind limb perfusion pressure (HLPP) were obtained. About 45 min after the final injection of 1 when HR and HLPP had returned to control values, cumulative dose–response curves relating changes in HR and HLPP to the dose of a test compound were obtained. The compounds were administered as solutions in physiological saline.

Acknowledgment. The authors thank B. J. Ball and I. J. Edwards for their expert technical assistance in synthesizing the compounds, A. Nuttall and Mrs. J. Moors in testing them, and C. J. Howarth for providing analytical data.

Fibrin Polymerization. 1. Alkylating Peptide Inhibitors of Fibrin Polymerization[1]

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A series of analogues relating to the NH_2-terminal region of the fibrin α chain, i.e., Gly-Pro-Arg-Pro, were prepared by stepwise solid-phase synthesis, and their abilities to inhibit fibrin polymerization and to prolong thrombin-initiated clotting time were evaluated. Among the analogues systematically modified at different positions, replacement of the NH_2-terminal three residues of Gly-Pro-Arg-Pro by either chlorambucil, p-nitrophenyl-L-alanine, or p-aminophenyl-L-alanine gave inactive compounds in the thrombin time assay, whereas similar substitution or extension of the COOH terminus produced the highly active analogues Gly-Pro-Arg-Phe(4-NH_2), 22%; Gly-Pro-Arg-Pro-Phe(4-NO_2), 88%; and Gly-Pro-Arg-Pro-Phe(4-NH_2), 105%; relative to Gly-Pro-Arg-Pro = 100% in the fibrin polymerization inhibitory assay. As potential photoaffinity labeling probes, analogues containing a nitrophenylalanine residue in position 4 or 5 underwent photolysis under the experimental photoactivation conditions. As a potential alkylating probe, Chl-Pro-Arg-Pro was selectively effective in inhibiting thrombin amidolysis and fibrin polymerization. In the latter assay, Chl-Pro-Arg-Pro was approximately 20 times more potent than Gly-Pro-Arg-Pro in inhibiting fibrin aggregation.

The enzymatic conversion of the soluble plasma glycoprotein fibrinogen to its spontaneously aggregating polymer fibrin by thrombin, leading to clot formation, is central to normal hemostasis.[2] This event[3,4] has been proposed to initially involve binding of the newly released fibrin NH_2 termini (E domain) with the COOH termini (D domain) of an adjacent unit, resulting in an end to end aggregate of the type I fibrin, which is subsequently reinforced by lateral association forming the more compact type II fibrin.

In an attempt to localize these two sets of polymerization sites, Laudano and Doolittle[5] synthesized the tetrapeptides Gly-Pro-Arg-Pro and Gly-His-Arg-Pro corresponding to the NH_2 termini of human fibrin α and β chains and observed that both peptides selectively bound to fragment D (80 000 daltons) of fibrinogen in a reversible manner but not to fragment E.[6] Furthermore, the more tightly bound Gly-Pro-Arg-Pro ($K_a = 5 \times 10^4\ M^{-1}$) could prevent fibrin polymerization in vitro. Although these studies localized part of the "E" binding sites, precise localization of the complementary "D" domain polymerization sites, as well as further characterization of the "E" sites, is not feasible due to the reversible nature of their binding. One approach to circumvent this limitation is to develop probes which may label these binding sites selectively and irreversibly. Such affinity-labeling probes can lead to better understanding of the detailed mechanism of fibrin polymerization, as well as its causal relationship in thrombotic and other disorders, and are also useful as potential therapeutic anticoagulants.

Among the active-site-directed affinity labels,[7] the chemical alkylating agents, such as the nitrogen mustards,[8] have the advantage of being effective in vivo[9] but require appropriate steric alignment of the label with a suitable recipient on the receptor in order for covalent linking to occur. On the other hand, the photoaffinity labeling agents have the advantage of being unusually reactive upon irradiation,[7,10] such that alkylation of the receptor can occur

(1) (a) The abbreviations used to denote amino acids and peptides are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1972); *Biochemistry*, **14**, 449 (1975)]. Other abbreviations include: Phe(4-NO_2), 4-nitrophenylalanine; Phe(4-NH_2), 4-aminophenylalanine; Chl, chlorambucil [4-[p-[bis(2-chloroethyl)amino]phenyl]butyric acid]; X, photolysis products of 4-nitrophenylalanine. (b) Peptides prepared for this series are also referred by their compound number shown in Table II. (c) This report was presented in part; see "Abstracts of Papers", Second Chemical Congress of the North American Continent, San Francisco, CA, Aug 1980, American Chemical Society, Washington, D.C., 1980, Abstr MEDI 111.
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