Compounds with Positive Inotropic Activity, III[1]:

Synthesis of 4-Aminoquinoline Derivatives as Potential Positive Inotropic Agents***)

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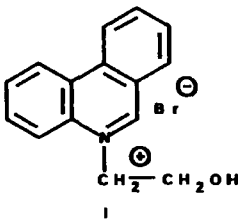
Anilinoacrylic acid derivatives **2-4** are the key intermediates from which 1-alkyl substituted 4-aminoquinoline **10-15** as well as 4-alkylaminoquinoline derivatives **16-23** are synthesized and examined for positive inotropic activity on isolated left atria and papillary muscles from guinea-pig. Structure activity relationships indicate that the effect depends on the alkyl side chain of the target compounds.

Positiv inotrop wirkende Verbindungen, 3. Mitt.[1]: Synthese und positiv inotrope Wirkung von 4-Aminochinolinen

Über die Anilinoacrylsäure-Derivate **2-4** werden die 1-alkylsubstituierten 4-Aminochinoline **10-15** sowie die 4-Alkylaminochinoline **16-23** dargestellt, um ihre Wirkung am isolierten linken Meerschweinchen-Vorhof sowie am Papillarmuskel zu untersuchen. Struktur-Wirkungs-Beziehungen zeigen, daß die Wirkung von der Alkylseitenkette abhängt.



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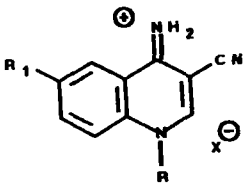
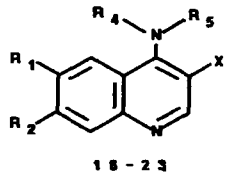


10 - 15

	R_1	R	X
10b	CH_3	CH_3	J
11b	CH_3	CH_2CH_3	Br
12b	CH_3	n-C_3H_7	J
13b	CH_3	CH_2-CH=CH_2	Br
14a	H	CH_2CH_2OH	Br
14b	CH_3	CH_2CH_2OH	Br
15b	CH_3	$CH_2CH_2CH_2OH$	Br



16 - 23

	R_1	R_2	R_4	R_5	X
16a	H	H	$CH_2CH_2CH_3$	H	CN
17a	H	H	CH_2-CH=CH_2	H	CN
18a	H	H	CH_2CH_2OH	H	CN
19a	H	H	$CH_2CH_2CH_2OH$	H	CN
20a	H	H	$CH_2CH_2OCH_2$	CH_2	CN
21a	H	H	$CH_2CH_2N(C_6H_5)CH_2$	CH_2	CN
22a	H	H	CH_2CH_2OH	H	$CO_2C_2H_5$
23a	H	H	$CH_2CH_2CH_2OH$	H	$CO_2C_2H_5$
22d	OCH_2	O	CH_2CH_2OH	H	$CO_2C_2H_5$
23d	OCH_2	O	$CH_2CH_2CH_2OH$	H	$CO_2C_2H_5$

Scheme 1: Structural modification of phenanthridinium salt **I** to 4-aminoquinoline derivatives **10-23**

***) Part of the dissertation *K. Eggert*, Kiel 1989; reported on the meeting of the German Pharmacological Society in Mainz, March 1988.

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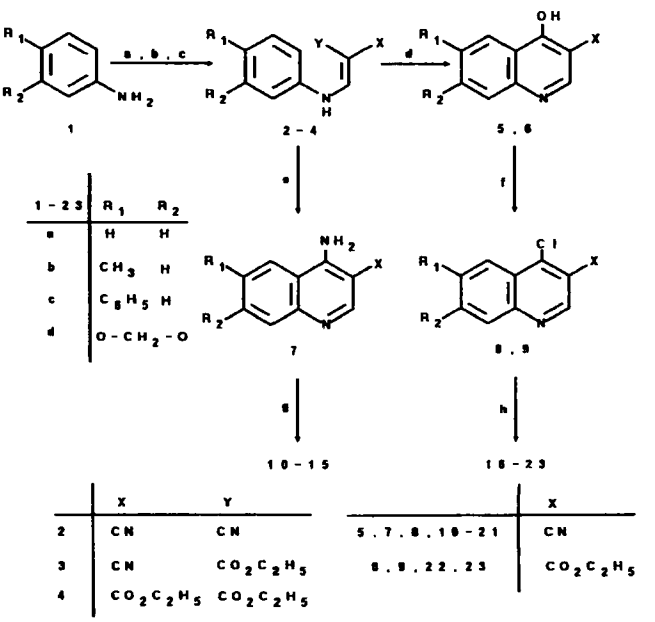
In a previous paper[1] we reported on the *in-vitro* positive inotropic activity of a series of phenanthridine derivatives. Structure-activity relationships revealed that compounds with propyl- or hydroxyethyl substitution at N-5 produce the best effect. Subsequently, our interest focussed on related bicyclic compounds that are substituted by an alkyl side chain in a position analogous to the phenanthridines. In particular, 4-amino-3-quinolinecarboxylic acid derivatives **10-23** in which either the N-1 position or the exocyclic amino group is alkylated were investigated. Methods of synthesis for such systems are known. Intensive work has been carried out on the development of antibacterial agents of the nalidixic acid type e.g. oxolinic acid, ciprofloxacin, and many others[2]. Only a few of the corresponding 4-substituted aminoquinoline-3-carboxylates exhibited low activity against *Staphylococcus aureus* [3]. 4-Anilino-3-quinolinecarboxylic acids and esters were tested but did not exhibit any significant diuretic activity [4]. Furthermore a series of 6,7-dimethoxy-4-aminoquinolines were synthesized and evaluated for hypotensive activity in dog [5]. But none of these compounds has ever been tested for cardiotonic activity. A potent new class of positive inotropic agents are heterocyclic-2(1H)-quinolinone derivatives which are inhibitors of phosphodiesterase, they have no structural similarity with our target compounds [6-9].

Chemistry

For the preparation of 4-amino-3-quinolinecarboxylic acid derivatives we found it convenient to cyclize anilinoacrylic acid derivatives **2-4** which are easily synthesized as depicted in Scheme 2. First the combined action of orthoformate and appropriately substituted anilines upon malononitrile yields the corresponding arylaminomethylenemalononitriles **2**[10]. Their intramolecular *Friedel-Crafts* reaction



Scheme 2: Synthesis of 4-aminoquinoline derivatives **10-23**
[a] Reagents: (a) Triethyl orthoformate/malononitrile; (b) ethyl ethoxymethylenemalonate; (c) ethyl ethoxymethylenecyanoacetate; (d) diphenyl ether; (e) aluminium chloride; (f) phosphorus oxychloride; (g) alkyl halide; (h) alkyl amine

Tab. 1: Physical constants and analytical data for compounds **10-15**

Compound	Mp (°C) yield (%)	Mol.formula (Mol.wt.)	$M^{+\cdot}$ a)	IR (cm^{-1})	^{1}H-NMR b): δ (ppm)	HR-MS a) calcd./found
10b	296°	$C_{12}H_{12}N_3J$	197	1680	2.64 (s; 3H, CH_3), 4.78 (s; 3H, CH_3), 7.82 (d; J=9 Hz, 1H, H-8), 8.04	197, 0953
	86	(325.2)		2250 (CN)	(d; J= 9Hz, 1H, H-7), 8.24 (d; J=2 Hz, 1H, H-5), 8.79 (s; 1H, H-2)	197, 0949
11b	242°	$C_{13}H_{14}N_3Br$	211	1655	1.73 (t; 3H, CH_3), 2.68 (s; 3H, CH_3), 4.73 (q; 2H, N-CH_2), 7.85 (d;	211, 1109
	88	(292.2)		2220 (CN)	J=9 Hz, 1H, H-8), 8.07 (d; J=9 Hz, 1H, H-7), 8.22 (d; J=2H, 1H, H-5),	211, 1102
				3220 and 3320 (NH_2)	8.77 (s; 1H, H-2)	
12b	>350°	$C_{14}H_{16}N_3J$	225	1670	1.06 (t; 3H, CH_3), 2.04 (m; 2H, CH_2), 2.60 (s; 3H, CH_3), 4.55 (t; 2H,	225, 1266
	81	(353.2)		2220 (CN)	CH_2), 8.01 (d; J=9 Hz, 1H, H-8), 8.08 (d; J=9 Hz, 1H, H-7), 8.20 (d;	225, 1259
					J=2H, 1H, H-5), 8.76 (s; 1H, H-2)	
13b	224°	$C_{14}H_{14}N_3Br$	223	1660	5.18-5.33 (m; 2H, N-CH_2), 5.47-5.73 (m; 2H, =CH_2), 5.93-6.33 (m; 1H,	223, 1109
	78	(304.2)		2230 (CN)	CH=), 7.96 (d; J=9 Hz, 1H, H-8), 8.10 (d; J=9 Hz, 1H, H-7), 8.30 (d;	223, 1122
					J=2Hz, 1H, H-5), 8.77 (s; 1H, H-2)	
14a	226°	$C_{12}H_{12}N_3OBr$	213	1670	3.78 (t; 2H, CH_2O), 4.70 (t; 2H, N-CH_2), 7.70-8.20 (m; 3H, Ar),	213, 0902
	77	(294.2)		2230 (CN)	8.73 (d; J=8 Hz, 1H, H-5), 9.15 (s; 1H, H-2), 9.48 (s; broad, 1H, NH)	213, 0900
				3100 and 3270 (NH_2)	9.97 (s; broad, 1H, NH)	
14b	275°	$C_{13}H_{14}N_3OBr$	227	1660	2.52 (s; 3H, CH_3), 3.77 (t; 2H,CH_2O), 4.70 (t; 2H, N-CH_2), 7.90 (d;	227, 1058
	74	(308.2)		2230 (CN)	J=9 Hz, 1H, H-8), 8.20 (d; J=9 Hz, 1H, H-7), 8.55 (d; J=2 Hz, 1H, H-5),	227, 1062
				3120 and 3340 (NH_2)	9.11 (s; 1H, H-2), 9.40 (s; broad, 1H, NH), 9.90 (s; broad, 1H, NH)	
15b	199°	$C_{14}H_{16}N_3OBr$	241	1670	2.30-2.60 (m; 2H, CH_2), 2.70 (s; 3H, CH_3), 4.08 (t; 2H, CH_2O), 7.90	241, 1215
	67	(322.2)		2240 (CN)	(d; J=9 Hz, 1H, H-8), 8.12 (d; J=9 Hz, 1H, H-7), 8.18 (d; J=2 Hz, 1H,	241, 1205
				3200 and 3350 (NH_2)	H-5), 8.83 (s; 1H, H-2)	

a) Basing on the free bases.
b) DMSO-d_6 or CF_3COOD were used as solvents

Tab. 2: Physical constants and analytical data for 4-alkylaminoquinoline derivatives **16-23**

Compound	Mp. (°C) yield (%)	Mol.formula (Mol. wt.)	Analysis calcd./found	IR (cm^{-1})	^{1}H-NMR[a]: δ (ppm)
16a	149° 77	$C_{13}H_{13}N_3$ (211.3)	C 73.9 H 6.20 N 19.9 C 73.8 H 6.16 N 19.9	2220 (CN) 3240 (NH)	0.96 (t; 3H, CH_3), 1.68 (m; 2H, CH_2), 3.73 (q; 2H, N-CH_2), 7.33-7.83 (m; 3H, Ar), 8.10 (s; broad, 1H, NH), 8.32 (dd; J=8/2 Hz, 1H, H-5), 8.40 (1H, H-2)
17a	175° 86	$C_{13}H_{11}N_3$ (209.3)	C 74.6 H 5.30 N 20.1 C 74.6 H 5.33 N 20.0	2210 (CN) 3240 (NH)	4.27-4.57 (m; 2H, N-CH_2), 4.93-5.37 (m; 2H, =CH_2), 5.75-6.37 (m; 1H, CH=), 7.35-7.87 (m; 3H, Ar), 8.28 (s; broad, 1H, NH), 8.38 (dd; J=8/2Hz, 1H, H-5), 8.42 (s; 1H, H-2)
18a	188° 71	$C_{12}H_{11}N_3O$ (213.2)	C 67.6 H 5.20 N 19.7 C 67.6 H 5.20 N 19.7	2220 (CN) 3000-3600 (broad,OH,NH)	4.10-4.66 (m; 4H, 2xCH_2), 7.77-8.10 (m; 3H, Ar), 8.40 (dd; J=8/2 Hz, 1H, H-5), 8.73 (s; 1H, H-2)
19a	156° 83	$C_{13}H_{13}N_3O$ (227.3)	C 68.7 H 5.77 N 18.5 C 68.9 H 5.77 N 18.5	2210 (CN) 3300 (NH)	2.35 (m; 2H, CH_2), 4.18 (t; 2H, CH_2-O), 4.43 (t; 2H, N-CH_2), 7.67-8.10 (m; 3H, Ar), 8.32 (dd; J=8/2 Hz, 1H, H-5), 8.70 (s; 1H, H-2),
20a	112-113° 78	$C_{14}H_{13}N_3O$ (239.3)	C 70.3 H 5.48 N 17.6 C 70.3 H 5.42 N 17.6	2210 (CN)	3.62-3.82 (m; 4H, CH_2-N-CH_2), 3.88-4.08 (m; 4H, CH_2-O-CH_2), 7.32-8.10 (m; 4H, Ar), 8.65 (s; 1H, H-2)
21a	139-140° 73	$C_{20}H_{18}N_4$ (314.4)	C 76.4 H 5.77 N 17.8 C 76.5 H 5.78 N 17.8	2220 (CN) 2840 and 2890 (CH)	3.27-3.57 (m; 4H, CH_2-CH_2), 3.67-3.95 (m; 4H, CH_2-CH_2), 6.68-7.32 (m; 5H, Ar), 7.32-7.85 (m; 2H, Ar), 8.00 (dd; J=8/2 Hz, 2H, Ar), 8.65 (s; 1H, H-2)
22a	167° 92	$C_{14}H_{16}N_2O_3$ (260.3)	C 64.6 H 6.19 N 10.8 C 64.5 H 6.13 N 10.8	1665 (CO) 3000-3300 (broad,OH,NH)	1.33 (t; 3H, CH_3), 3.40-3.90 (m; 4H, N-CH_2-CH_2), 4.33 (q; 2H, OCH_2), 5.00 (s; broad, 1H, OH), 7.23-7.83 (m; 3H, Ar), 8.33 (dd; J=7/2 Hz, 1H, H-5), 8.86 (s; 1H, H-2), 9.10 (s; broad, 1H, NH)
23a	138° 89	$C_{15}H_{18}N_2O_3$ (274.4)	C 65.7 H 6.61 N 10.2 C 65.7 H 6.67 N 10.2	1660 (CO) 3160 (NH)	1.35 (t; 3H, CH_3), 1.85 (m; 2H, CH_2), 3.46-4.00 (m; 4H, 2xCH_2), 4.33 (q; 2H, OCH_2), 4.63 (s; broad, 1H, OH), 7.29-7.85 (m; 3H, Ar), 8.37 (dd; J=8/2 Hz, 1H, H-5), 8.90 (s; broad, 2H, NH, H-2)
22d	209° 88	$C_{15}H_{16}N_2O_5$ (304.3)	C 59.2 H 5.30 N 9.2 C 59.2 H 5.30 N 9.2	1670 (CO) 3230 (NH)	1.50 (t; 3H, CH_3), 4.20-4.73 (m; 6H, 3xCH_2), 6.23 (s; 2H, O-CH_2-O), 7.20 (s; 1H, H-8), 7.77 (s; 1H, H-5), 8.80 (s; 1H, H-2)
23d	175° 77	$C_{16}H_{18}N_2O_5$ (318.3)	C 60.4 H 5.70 N 8.8 C 60.3 H 5.67 N 8.9	1660 (CO) 3100-3500 (broad,OH,NH)	1.47 (t; 3H, CH_3), 2.13-2.53 (m; 2H, CH_2), 4.00-4.75 (m; 6H, 3xCH_2), 6.22 (s; 2H, O-CH_2-O), 7.17 (s; 1H, H-8), 7.83 (s; 1H, H-5), 8.77 (s; 1H, H-2)

a) DMSO-d_6 or CF_3COOD were used as solvents.

with $AlCl_3$ in chlorobenzene to give **7** has been described by *Schäfer* and *Gewald* [11]. We improved this procedure by carrying out the ring closure without any solvent at elevated temp. resulting in easier work up and better yields. The 4-aminoquinoline-3-carbonitriles **7** so obtained are alkylated with alkyl halides in boiling nitromethane to give the quaternary quinoline derivatives **10-15** with exocyclic positive charge listed in Tab. 1, the alkylation of **7c** failed to occur. Second the *Gould-Jacobs* reaction[12] is well known as the most useful method for the synthesis of 4-hydroxy-3-quinoline carboxylic acid derivatives. It involves condensation of aromatic amines with ethoxymethylenemalonates (EMME) and thermal cyclization of the resulting anilinomethylenemalonates. Using this procedure we obtained the 4-hydroxyquinolines **5** and **6** by refluxing anilinoacrylic acids **3** and **4** in boiling diphenyl ether. Treatment of **5** and **6** with $POCl_3$ produced the 4-chloroquinoline derivatives **8** and **9**. Nucleophilic displacement of the 4-chloro substituent by the appropriate amino group occured in high yield after 30 min in refluxing ethanol. The relative ease of displacement is presumably due to the activating effect of the carbonitrile resp. carboxylic ester adjacent to the reaction center. Tab. 2 lists 4-alkylaminoquinolines **16-23**.

The site of alkylation in neutral medium, whether on the acyclic or heterocyclic nitrogen, depends upon the volume of the alkylating reagent and the steric hindrance and electron density of the resp. N-atoms[13]. All ring alkylations of **7a** and **7b** are performed without any problems yielding generally 1:1 adducts. Their structural assignment as vinylogous amidinium salts with exocyclic positive charge, as depicted in Scheme 1, is established according to ^{1}H-NMR- and IR-spectroscopy. The imino form is demonstrated in the IR-spectra by an intensive absorption band at ν = 1660-1670 cm^{-1}; in contrast the spectra of **16-23** produced by aminolysis of the 4-chloroquinolines **8** and **9** as well as their HBr-salts do not show any comparable absorption band. Furthermore the ^{1}H-NMR spectra are characteristic for the alkylation at the heterocyclic nitrogen and the formation of the vinylogous amidinium salt form. The spectra show a triplet between δ = 4.6-4.8 ppm for the (NCH_2) protons and

Tab. 3: The effects of quinoline derivatives in left and right atria, and in right ventricular papillary muscle of guinea-pig hearts.
Increase in force of contraction in electrically stimulated left atria induced by the test compound (10^{-4} mol/l) and expressed in percent of the increase observed with isoprenaline (3×10^{-7} mol/l, column[a]) given at the beginning of the experiment. Column[b]: Positive inotropic effect of the test compound in the presence of propranolol (10^{-5} mol/l), values expressed again in percent of the isoprenaline effect prior to addition of propranolol. Mean values ± S.E.M. from 4-6 experiments. Chronotropic effect of test compounds in spontaneously beating right atria. Column[c]: change in beat frequency expressed as percent of the pre-drug control value (=100%). Column[d]: positive inotropic effect of test compound (10^{-4} mol/l) in spontaneously beating right atria expressed in percent of the isoprenaline effect (3×10^{-7} mol/l, 100%). Mean values from 4-5 experiments.
Maximum change in force of contraction (expressed in percent of the pre-drug control value (=100%), column[e]) and in action potential duration at 90% of repolarization (in ms, column[f]) of right ventricular papillary muscles under the influence of several quinoline derivatives (10^{-4}mol/l). Values from individual experiments or means ± S.E.M. from 4-5 experiments are given.

Test	left atria		right atria		papillary muscles	
compound	PIE in % of IPN eff.[a]	Propranolol[b]	change in frequency[c]	PIE in % of IPN eff.[d]	change in force[e]	change in APD[f]
2b	9.7 ± 0.9	–	-10 ± 5	4.4 ± 2.2	–	–
2c	20.6 ± 5.8	–	- 8 ± 4	8.4 ± 1.3	–	–
3a	33.0 ± 4.7	–	- 2 ± 2	7.4 ± 2.2	–	–
7b	14.7 ± 2.2	–	–	–	–	–
10b	44.3 ± 4.1	16.2 ± 2.3	-28 ± 2	25.8 ± 2.9	+21, +30	+85, +85
11b	67.8 ± 3.5	–	+14 ± 7	42.2 ± 9.1	–	–
	(46.3 ± 4.0)	–	(- 2 ± 2)*	(20.6 ± 0.8)*	(+123, +156)	(-93, –)
	43.4 ± 4.8**	–	–	–	–	–
12b	64.9 ± 3.7	39.6 ± 2.6	-22 ± 4	48.8 ± 5.4	+ 59 ± 39	+81 ± 19
	(69.5 ± 1.6)*	–	–	–	–	–
13b	71.5 ± 4.0	–	- 9 ± 4	47.7 ± 5.5	+364	–
14b	60.6 ± 5.8	47.7 ± 2.9	+30 ± 8	40.2 ± 3.3	+374 ± 29	+14 ± 4
	(37.8 ± 8.4)*	–	(+28 ±11)*	(27.4 ± 2.8*	(+125)	(+15)
	30.0 ± 3.1**	29.8 ± 4.7**	–	–	+101	+20
14a	52.3 ± 6.0	–	+23 ±12	16.3 ± 6.3	–	–
15b	51.1 ± 8.6	–	-18 ± 2	30.7 ± 5.2	–	–
16a	68.0 ± 3.3	–	+17 ±10	37.9 ± 3.0	–	–
	(62.5 ± 1.8)*	–	(+ 7 ± 4)*	(23.4 ± 3.0)*	–	–
17a	42.8 ± 5.8	–	+ 5 ± 3	17.8 ± 2.7	–	–
18a	11.2 ± 1.6	–	+ 2 ± 2	14.6 ± 4.5	–	–
19a	28.7 ± 1.3	–	+ 4 ± 2	24.8 ± 1.2	–	–
20a	18.1 ± 2.4	–	- 3 ± 4	3.8 ± 1.5	–	–
21a	37.1 ± 3.6	–	+16 ± 4	34.1 ± 4.9	–	–
22a	67.6 ± 2.1	–	-16 ± 2	46.6 ± 5.2	- 48, -30	+74, +67
23a	59.5 ± 9.8	–	-17 ± 4	46.0 ± 7.0	- 48, -74	+58, +38
22d	72.6 ± 3.5	106.7 ± 15.6	-35 ± 4	55.1 ± 3.8	+ 43 ± 5	+62 + 8
	(85.9 ± 5.2)*	–	–	–	–	–
23d	60.7 ± 6.2	94.1 ± 9.0	-14 ± 4	50.4 ± 3.5	+ 13, +31	+55, +65
	(87.7 ± 2.1)*	–	(-14 ± 2)*	(47.9 ± 3.4)*	–	–

a singlet for H-2 at δ = 9.1 ppm. In the case of exocyclic alkylation the (NCH_2) protons are shifted to a higher field and are split by the (NH) proton of the (4-alkyl-NH) substituent to form a quartet ($J_{NH\text{-}CH} = J_{CH\text{-}CH}$) (e.g. **16a**). Finally comparison with the spectra of the HBr-salts of 4-alkylaminoquinoline derivatives shows different resonance pictures.

Pharmacology

In analogy to our study with phenanthridine derivatives[1] we have tested the quinoline compounds and their precursors for positive inotropic activity in electrically stimulated left atria of guinea-pig hearts. Typical contractile responses are depicted in Fig. 1. Compounds **2b, 2c** and **3a** slightly increased force of contraction, the maximum effect was observed with **3a** and amounted to approximately one third of the inotropic stimulation obtained with isoprenaline (3×10^{-7} mol/l) as a standard (Tab. 3). N-1 alkylation of the unsubstituted quinoline molecule as well as of 4-aminoquinaldine increased force only marginally (5-9 % of isoprenaline maximum)[14]. However, alkyl substituents in the same position of the 4-aminoquinoline-3-carbonitriles **7** yielded active compounds **10-15** with inotropic effects of 44-72 % of the isoprenaline maximum. These results showed the importance of the electron withdrawing group at C-3 for the positive inotropic activity. 4-Amino-6-methylquinoline-3-carbonitrile (**7b**) had only a small positive inotropic action. N-1 methylation (**10b**) yielded a compound with a larger effect and even longer alkyl chains resulted in strongly inotropic agents with up to two thirds of the isoprenaline effect (Tab. 3). Thus the length of the alkyl chain seemed to be important for the size of the effect. With compounds **11b, 13b, 14a** and **14b**, the positive inotropic effect was multiphasic, i.e. it declined after passing through a maximum but recovered later. Furthermore, baseline tension increased. - Compounds **16a-23d** are characterized by alkylation of the exocyclic amino group. Their positive

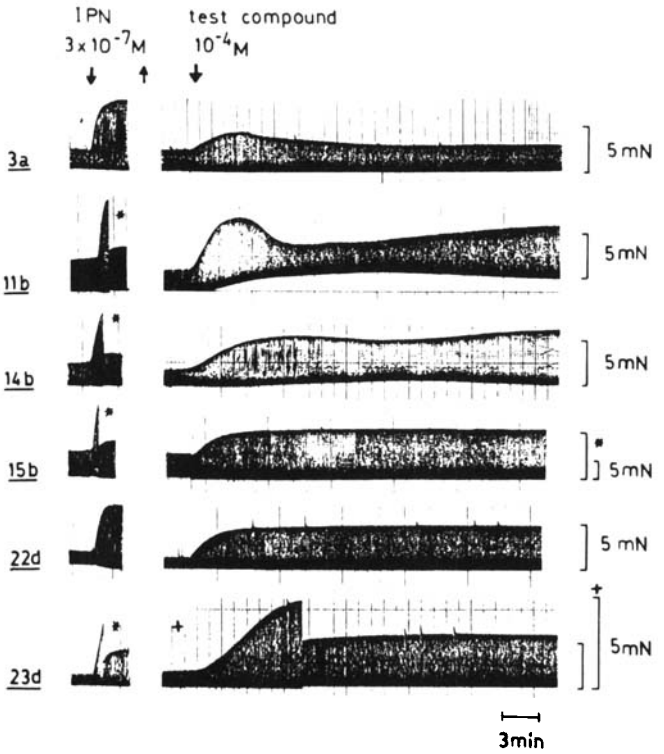


Fig. 1: Original mechanograms of isolated, electrically stimulated left atria from guinea-pig hearts showing the effects of several quinoline derivatives. Isoprenaline (3 x 10^{-7} mol/l) was added as a standard. After wash, the test compound was added in a concentration of 10^{-4} mol/l. The maximum of the positive inotropic response to the test compound was expressed in % of the isoprenaline effect (Tab. 3). Calibrations as indicated by the bars. Please note the changes in sensitivity of the recorder (marked by asterisks).

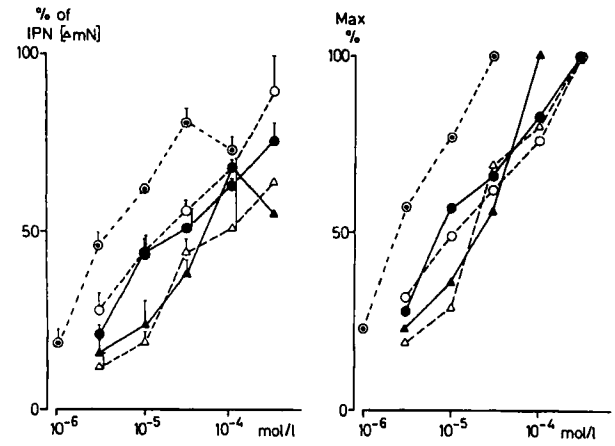


Fig. 2: Concentration-response curves for some test compounds in isolated left atria from guinea-pig hearts (one concentration only in each atrium). A: Increase in force of contraction expressed in % of the maximum effect produced by isoprenaline (IPN, 3 x 10^{-7} mol/l). B: Increase in force of contraction normalized to the maximum response obtained with each compound (calculated from the data in A). Abscissae, concentration in mol/l. Mean value ± S.E.M. from 4-5 experiments for each concentration. ⊙--⊙ **22d** EC_{50} 2.4 x 10^{-6} mol/l, ●—● **11b** EC_{50} 7.7 x 10^{-6} mol/l, Δ—Δ **15b** EC_{50} 1.8 x 10^{-5} mol/l, ▲—▲ **16a** EC_{50} 2.2 x 10^{-5} mol/l, ○—○ **22a** EC_{50} 1.1 x 10^{-5} mol/l.

inotropic effects in electrically stimulated left atria varied with the length of the side chain, the propyl-moiety seemed to yield an optimum structure, whereas the hydroxyethyl derivative **18a** was the least effective compound of this group. - Compounds **22a, 22d** and **23a, 23d** increased force of contraction of left atria by 60-73% of the isoprenaline maximum. For some compounds concentration-response curves were obtained for a wider concentration range in a non-cumulative manner (one concentration only in each preparation), the results are shown in Fig. 2. The EC_{50} for **22d** was 2.4 x 10^{-6} mol/l, thus it was the most potent compound of this arbitrarily assembled group (see legend to Fig. 2 for the other EC_{50} values).

In order to estimate whether stimulation of β-adrenoceptors either directly or indirectly contributes to this positive inotropic effect, some representative compounds, i.e. **10b, 12b, 14b,** and **23d** were chosen for investigation after blockade of β-adrenoceptors with propranolol (10^{-5} mol/l). In all cases, propranolol partially abolished but did not completely suppress the effects with the exception of compound **23d**, which was actually more effective in the presence of propranolol ($p<0.05$) (Tab. 3). Compounds **11b, 12b, 14b,** and **16a** were also tested in atria from guinea pigs pretreated with reserpine (see methods). The positive intropic effect of **12b** was uneffected by reserpine pretreatment, whereas that of **11b** and **14b** was significantly lower than in control preparations (see Tab. 3, $p<0.01$) and **22d** and **23d** were somewhat more effective after reserpine pretreatment (statistically not significant). - The positive inotropic response of compound **12b** was also not changed in the presence of the ganglion blocker hexamethonium (3 x 10^{-5} mol/l), i.e. **12b** enhanced force by 59.1 ± 4.3 % in the presence and 64.9 ± 3.8 % in the absence of hexamethonium.

We further tested for direct β-adrenoceptor stimulating activity by measuring the influence of the compounds on the spontaneous beat frequency of right atria (Tab. 3). Isoprenaline (3 x 10^{-7} mol/l) increased the frequency of contractions from 176 ± 1.4 beats/min to 297 ± 1.5 beats/min which corresponds to an increase of 68.2 % of the control value (n = 139). Compounds **11b, 14a,** and **14b** increased the right atrial beat frequency by 14-30 %, whereas **10b, 12b, 13b,** and **15b** did in fact decrease frequency by 9-28 %. It should be noted that these compounds enhanced force in spite of the decrease in spontaneous frequency. The negative chronotropic effect was not modified by blocking muscarinic receptors: in atria pre-exposed to atropine (3 x 10^{-5} mol/l), the N_1-propyl derivative **12b** chosen as a representative compound was just as effective as in control atria (-20 ± 3 % vs -22 ± 4 %, respectively, $p<0.10$). In right atria from reserpine-pretreated animals, the positive chronotropic effect of **11b** was abolished. The increase in rate of spontaneously beating right atria observed with **14a** and **16a** suggested that β-adrenergic stimulation is involved, although this was not verified in experiments with tissue from reserpinized animals. Compounds **22a, 22d** and **23a, 23d** decreased beating frequency of right atria by 14-35 %. Irrespective of either negative or positive chronotropic effects,

Tab. 4: pD_2 values for isoprenaline in the absence and presence of some test compounds (guinea pig left atria)

test compound (10^{-4})	control	test compound	wash	n[1]
no drug	7.31 ± 0.12	7.21 ± 0.10	6.94 ± 0.12	10
7b	7.16 ± 0.09	7.27 ± 0.11	6.68 ± 0.11	5
10b	7.08 ± 0.07	7.39 ± 0.09[2]	6.64 ± 0.06	6
12b	7.03 ± 0.07	8.07 ± 0.12[2]	7.21 ± 0.09	5
13b	7.80 ± 0.15	-[3]	7.80 ± 0.06	4
($8x10^{-6}$)	7.12 ± 0.11	7.67 ± 0.04[2]	7.31 ± 0.04	4
14b	7.44 ± 0.09	7.72 ± 0.12	7.14 ± 0.12	6
15b	7.58 ± 0.12	8.43 ± 0.09[2]	7.80 ± 0.06	4
16a	7.63 ± 0.4	-[3]	7.29	4
22a	7.44 ± 0.08	-[3]	7.41 ± 0.09	4
22d	7.43 ± 0.10	8.47 (n=1)[3]	7.56 ± 0.15	4
quinidine[4]	7.90 ± 0.06	7.91 ± 0.05	-	4

[1] number of experiments
[2] difference statistically significant ($p < 0.05$)
[3] no concentration-response curve for isoprenaline could be determined in the presence of the test compound (positive inotropic effect was too large to obtain a further increase)
[4] measured in rat atria

all those quinoline derivatives that increased force in left atria also enhanced contraction amplitudes of spontaneously beating right atria (Tab. 3).

In the presence of an inhibitor of phosphodiesterases (PDE) the concentration-response curve for isoprenaline is shifted to the left[14]. This assay was used as a first estimate for a possible influence on PDE in guinea-pig left atria. Tab. 4 summarizes the pD_2 values for IPN in the presence and absence of the agent under investigation (10^{-4} mol/l). A significant shift to the right was observed with compounds **10b** and **12b** whereas **7b** and **14b** were ineffective.

Since positive inotropic agents are to be used in the management of cardiac failure which involves the ventricles rather than the atria, we also investigated some of our compounds in isolated papillary muscles of the guinea pig. **22a** and **23a** slightly reduced force of contraction in ventricular tissue, **10b, 11b** (in hearts from reserpinized animals), **12b, 22d,** and **23d** had a marginal positive inotropic effect, but all five agents prolonged the action potential duration. Compound **14b** was investigated in some detail, it had a remarkable positive inotropic action and oscillatory afterpotentials were recorded upon prolonged exposure.

The time courses of the effects of **14b** are depicted in Fig. 3 which also includes the effects of **22d** for comparison. The increase in force by **14b** was accompanied by a shortening of the plateau phase of the action potential, whereas **22d** prolonged the action potential duration at all stages of repolarization. The intropic effect of **14b** was partially abolished in papillary muscles from reserpine-pretreated animals.

Discussion

Similar to the phenanthridine derivatives investigated previously[1] the quinoline derivatives also had a positive inotropic effect in isolated left atria. Our results with the β-adrenoceptor blocking agent propranolol and in atria from reserpinized animals suggest, that at least some indirect sympathomimetic effect may contribute to the total positive inotropic action. However, when this is eliminated, the compounds decrease the spontaneous beating frequency of right atria, and therefore, direct stimulation of β-adrenoceptors is unlikely to occur. This conclusion is further supported by the high concentration of agents required for the positive inotropic effect and by their lack of positive inotropic activity (with a few exceptions) in papillary muscles. For some of our compounds we could demonstrate a significant shift to the left in the concentration-response curve for isoprenaline in guinea-pig left atria. This finding suggests that these agents may possess some phosphodiesterase activity[15]. Incidentally, quinidine was ineffective in this assay although it also produces a positive inotropic response in atria [16]. Because of some similarity in pharmacological profiles observed with quinidines and phenanthridines we have suggested previously that the latter compounds could increase atrial force of contraction indirectly by prolonging the action potential duration[1,16,17]. In the present study we

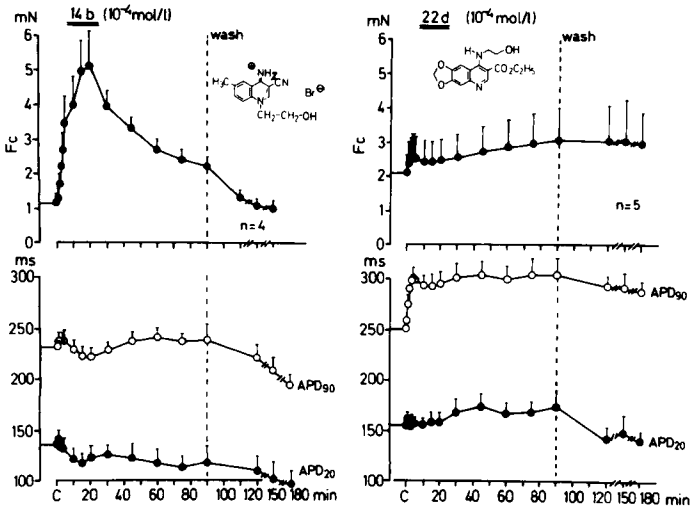


Fig. 3: Time courses of the duration of action potential (open circles) and force of contraction (closed circles) of guinea-pig papillary muscles in the presence of the quinoline derivatives **14b** (10^{-4} mol/l, left) and **22d** (10^{-4} mol/l, right). Ordinates: action potential duration at 90 % and 20 % of repolarization (ADP_{90}, APD_{20}) in ms and force of contraction (F_c) in mN, respectively; abscissa: time in min after addition of test compound. The vertical dashed lines indicate the removal of compound from the organ bath. Mean values ± S.E.M. from four experiments.

have measured action potentials in ventricular rather than atrial tissue and as with the phenanthridines [1], a small prolongation in action potential duration was seen with those compounds which had only a small positive inotropic effect in papillary muscles. One of the compounds that developed contracture in left atria, e.g. **11b, 13b, 14a** and **14b**, strongly enhanced force of contraction also in ventricular muscles but shortened the plateau phase of the action potential (i.e. **14b**). Some indirect sympathomimetic action seems to contribute to its positive inotropic effect.

In conclusion, different mechanisms of action seem to be contributing to the positive inotropic effect of the quinoline derivatives described in this paper. However, details of the various sites involved must await further experimental clarification.

Experimental Part

Chemistry

Elementary analysis: Microlaboratory of Ilse Beetz, 8640 Kronach. - Mp's: Reichert microhotstage (uncorr.). - IR spectra: Beckman Acculab 10, KBr disk. - ^{1}H-NMR spectra: Varian EM-360 and A-60, TMS int. stand. - MS: Finnigan MAT 8230, at 70 eV and Hewlett-Packard GC-MS 5985, at 70 eV. - Compounds **2a** and **2b**[10], **3a** and **5a**[18], **4a**[19], **6a**[20], **6d**[21], **7a** and **7b**[11], **8a**[22], **9a**[23] and **9d**[24] are described in the literature. - **6d** was a gift from Fa. Dynamit Nobel.

Diphenylyl-4-aminomethylene-malononitrile (**2c**)

A solution of 5.08 g (30 mmol) 4-aminobiphenyl, 4.45 g (30 mmol) triethyl orthoformate, and 1.98 g (30 mmol) malononitrile in 50 ml of EtOH was refluxed for 2 h. The crude product obtained was collected by filtration, washed with EtOH and purified by recrystallization from EtOH. Colourless crystals, m.p. 301°C, yield 7.0 g (95%). - $C_{16}H_{11}N_3$ (245.3) Calcd. C 78.3 H 4.52 H 17.1 Found C 78.3 H 4.52 N 17.2. - IR: 3220 (NH); 2220 (CN); 1650 cm^{-1} (C=C). - ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.26-7.77 (m; 9H aromatic), 8.50 (s; 1H, =CH), 11.13 (s; 1H, NH).

General procedure for intramolecular Friedel-Crafts-Reactions of **2a-c**

The mixture of 3.00 g of the appropriate **2a-c** and a fourfold molar amount of $AlCl_3$ was heated at 140°C for 1 h, then cooled and poured into ice-water. The crude product was collected by filtration, washed with water and dried at 110°C.

4-Amino-quinoline-3-carbonitrile (**7a**)

Colourless crystals, m.p. 306°C (Lit.[11]: 305-307°), yield 2.88 g (96%). - $C_{10}H_7N_3$ (169.2). - IR: 3380; 3180 (NH_2); 2230 (CN); 1680. - ^{1}H-NMR (CF_3COOD): δ (ppm) = 7.77-8.20 (m; 3H aromatic), 8.43 (d; J = 8 Hz, H-5), 8.70 (s; 1H, H-2).

4-Amino-6-methyl-quinoline-3-carbonitrile (**7b**)

Colourless crystals, m.p. 304-306°C (Lit.[11]: 303-306°), yield 2.83 g (95%). - $C_{11}H_9N_3$ (183.2). - IR: 3380; 3180 (NH_2): 2230 (CN); 1680. - ^{1}H-NMR (CF_3COOD): δ (ppm) = 2.68 (s; 3H, CH_3), 7.91 (s; 2H aromatic), 8.20 (s; 1H, H-5), 8.72 (s; 1H, H-2).

4-Amino-6-phenyl-quinoline-3-carbonitrile (**7c**)

Pale brown crystals, m.p. 260°C, yield 2.76 g (92 %). - $C_{16}H_{11}N_3$ (245.3). - MS: m/z = 245 (100; M^+). - IR: 3390; 3230 (NH_2); 2240 (CN); 1665. - ^{1}H-NMR (CF_3COOD): δ (ppm) = 7.30-8.10 (m; 7H aromatic), 8.25 (s; 2H, NH_2), 8.55 (s; 1H, H-2), 8.75 (d; J = 2 Hz, 1H, H-5).

General procedure for the alkylation of **7** *to form quinoline derivatives* **10-15**

A suspension of 6 mmol **7**, 18 mmol of the appropriate alkyl halide and 40 ml of nitromethane was refluxed for 16 h. The crude product was collected by filtration, washed with nitromethane and dried *in vacuo*. **14b** and **15b** were purified by refluxing in boiling water for 10 min, hot filtration, and evaporation of the solvent *in vacuo*. Some physical and spectral properties are given in Tab. 1.

General procedure for the amination of the 4-chloro-quinolines **8a, 9a,** *and* **9d** *to form quinoline derivatives* **16-23**

A solution of 5 mmol of **8a, 9a,** and **9d** resp. and 50 mmol of the appropriate amine in 50 ml of EtOH was refluxed for 30 min. After cooling at 0°C the crude product was collected by filtration, washed with ice-cold EtOH, and recrystallized from EtOH. Some physical and spectral properties are given in Tab. 2.

Pharmacology

a) Force of contraction measured in left atria from guinea-pig hearts

Left atria were isolated from the hearts of guinea-pigs and suspended vertically in a small organ bath (15 ml). The muscles were pinned on two platinum wires that served as electrodes for electrical stimulation (parameters: frequency 1 Hz, duration of impulse 2 ms, amplitude 5 V). The free end of the atrium was tied with a silk thread to make connection with the strain gauge for measurement of contractile tension. The preparations were preloaded with 3 to 5 mN to yield an optimum contraction amplitude. Force of contraction was recorded on a pen writer (Hellige).

The bathing solution was continuously bubbled with a mixture of 95 % O_2 and 5 % CO_2. Its composition was (in mmol/l): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.2, NaH_2PO_4 0.21, $NaHCO_3$ 12.0, glucose 5.5. The pH was 7.4, the temp. was maintained at 35°C. The test compounds were dissolved in dimethylsulfoxide (DMSO) as concentrated stock solutions, and a small volume was added to the organ bath to yield the final concentration desired.

Right atria were mounted in the same way, but were allowed to beat spontaneously without electrical stimulation. Recordings of contractions at high paper speed were used for measurement of beating frequency. Some guinea pigs received reserpine, 5 mg/kg body weight, by i.p. injection in a volume of less than 0.2 ml. They were treated 24 h prior to the experiment.

b) Blockade of β-adrenoceptors

After an equilibration period of 30 min the atria were exposed to isoprenaline (3 x 10^{-7} mol/l) until a steady state positive inotropic effect had developed. The increase in force of contraction was set to 100 % and served as an internal standard. After wash in normal bathing solution, the atria were exposed to propranolol (10^{-5} mol/l) for 30 min before the test compound was added. Propranolol had no negative inotropic effect of its own.

c) Electrophysiological experiments

Right ventricular papillary muscles were isolated from guinea-pig hearts and mounted horizontally in a small muscle chamber (3 ml) which was continuously perfused with oxygenated Tyrode solution. Action potentials were recorded with conventional glass microelectrodes, force of contraction was measured isometrically via a force transducer (Statham UC2 cell). The preparations were stimulated at a frequency of 1 Hz, the voltage was adjusted to 20 % above threshold.

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[Ph716]