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Original article

Synthesis and positive inotropic activity of novel pyrimido-[5,4-*b*][1,4]oxazin-7(8*H*)-ones

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Summary — Seventy-five compounds **I** were synthesized and tested for positive inotropic activity. Some derivatives (**22, 24, 54**) showed an activity comparable to that of amrinone. Compound **24** was selected for preclinical study. According to the biochemical and pharmacological data its activity may involve a novel mechanism(s).

Résumé — Synthèse et activité inotropie positive de nouveaux dérivés de la pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one. *75 composés **I** ont été synthétisés et testés pour leur activité inotrope. Certains dérivés ont montré une activité comparable à celle de l'amrinone. Le composé **24** a été sélectionné pour des études précliniques. Au vu des données biochimiques et pharmacologiques, son mécanisme d'action pourrait être nouveau.*

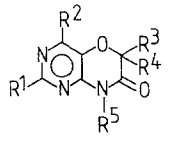
4-(substituted amino)-6,7-dihydropyrimido[5,4-*b*][1,4]-oxazin-7-(8*H*)-ones / positive inotropic activity

Introduction

Recently, a number of teams have been pursuing intensive work to prepare non-digitalis cardiotonic compounds which may be useful for the treatment of congestive heart failure (*eg* 1–5). These efforts express the aim to obtain active substances which are less toxic and more advantageous concerning the side effects in comparison to the long available cardiac glycosides.

These works have been evaluated in several excellent reviews [6–8] classifying the novel compounds according to their mechanism of action. It has been stated, however, that in some cases an inotropic component yet undefined in the cardiotonic effect may also play an important role.

In the course of our program focussing on the synthesis of novel, orally effective and safe positive inotropic substances [9, 10], we have found that certain 4-(substituted amino)-6,7-dihydropyrimido-[5,4-*b*][1,4]oxazin-7(8*H*)-one derivatives **I** (chart 1) could satisfy the therapeutic demands [11, 12].



I

Chart 1. R^1 = Me, Ph; R^2 = Cl, TosO, NH_2, subst amino; R^3, R^4 = H, Me, Ph; R^5 = subst alkyl.

Surprisingly, a few compounds of type **I** only have been published, with the exception of our patent application [13] relating to this class of compounds, and no cardiovascular effects of the previously known substances have been described [14, 17].

Now, we wish to report the synthesis and structure–activity relations of pyrimido[5,4-*b*][1,4]-oxazinones **I**.

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Abbreviations: BuOH: *n*-butanol; DMF: dimethylformamide; EtOH: ethanol; EtOAc: ethyl acetate; IS: isoproterenol; MeOH: methanol; MCF: myocardial contractile force; iPrOH: isopropanol; rt: room temperature; TBAB: tetrabutylammonium bromide; Ts: 4-toluenesulfonyl

Chemistry

The synthesis of compounds **I** is shown in the schemes 1–3.

The 4-(substituted amino) group was usually built in by reacting 4-chloro compounds **1, 2** [14], **3** [15] and **4** or 4-tosyloxy derivatives **5** and **6** respectively, with the corresponding primary or secondary amine. The substituent of the lactam nitrogen (R^5) was subsequently introduced through alkylation (Method *K–O*).

In consideration of the electrophilic centres of the starting lactams, a ring cleavage may also occur in the first step by the attack of the nucleophilic agent on the carbon of the lactam carbonyl (*C*–8) in addition to the S_NAr reaction desired. In this case, the corresponding acid amide of type **II** is formed. By using the tosyloxy derivatives, principally, another side reaction may also proceed to form 4-hydroxy-6,7-dihydropyrimido-[5,4-*b*][1,4]-oxazin-7-(8*H*)-one derivative and the corresponding toluenesulfonamide **(III)** by *O-S* bond fission through the attack of the nucleophile on the sulfur atom (scheme I). According to our observation, amide derivatives of the type **II** were obtained in the reaction of primary amines in amounts depending on the reaction conditions. Their formation, however, could be minimized in the presence of a tertiary amine as acid acceptor, in an aprotic solvent (Method *J*). By using the tosyloxy derivatives **5** or **6** in ethyl acetate, in the presence of potassium carbonate (Method **I**), the reaction almost did not proceed, although a toluenesulfonamide of type **III** and the corresponding 4-hydroxy-6,7-dihydropyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one were formed as side products in low yield.

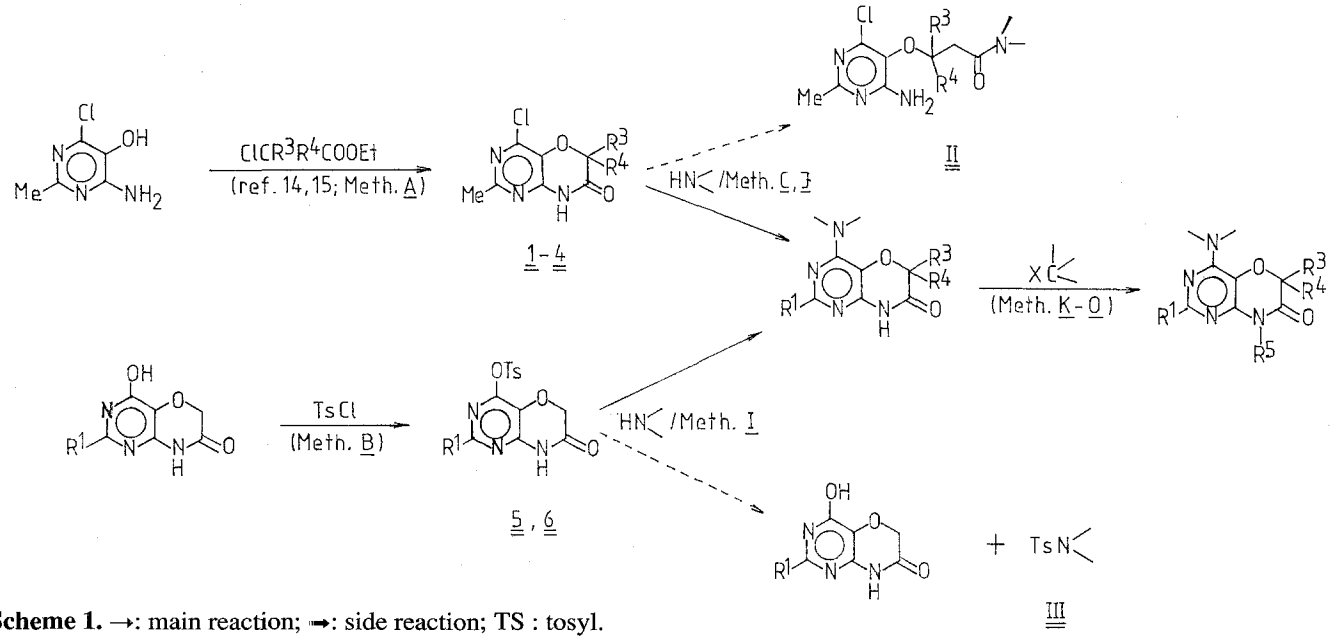
4-Amino derivatives **13** and **7** were prepared in two different ways. Compound **13** was obtained from the 4-hydrazino derivative **(7)** [18] in 3 steps (Methods *F–H*) in an excellent yield (scheme 2). The 8-morpholinoalkyl derivatives **(40, 41)** were prepared by isomerization of the appropriate 4-morpholinoalkyl derivatives (**24** and **27**, respectively) (scheme 3). This intramolecular reaction proceeds in EtOH or in BuOH by acid catalysis (Method *R*).

4-(Substituted amino)-8-alkyl derivatives containing an α-carbonyl or α-cyano group in the 8-alkyl substituent were obtained by alkylation with the appropriate α-halo compound (Method *K*), while the 4-(substituted amino)-8-(2,3-dihydroxypropyl) derivatives were prepared by using glycidol in the presence of triethylamine (Method *L*) or TBAB (Method *N*).

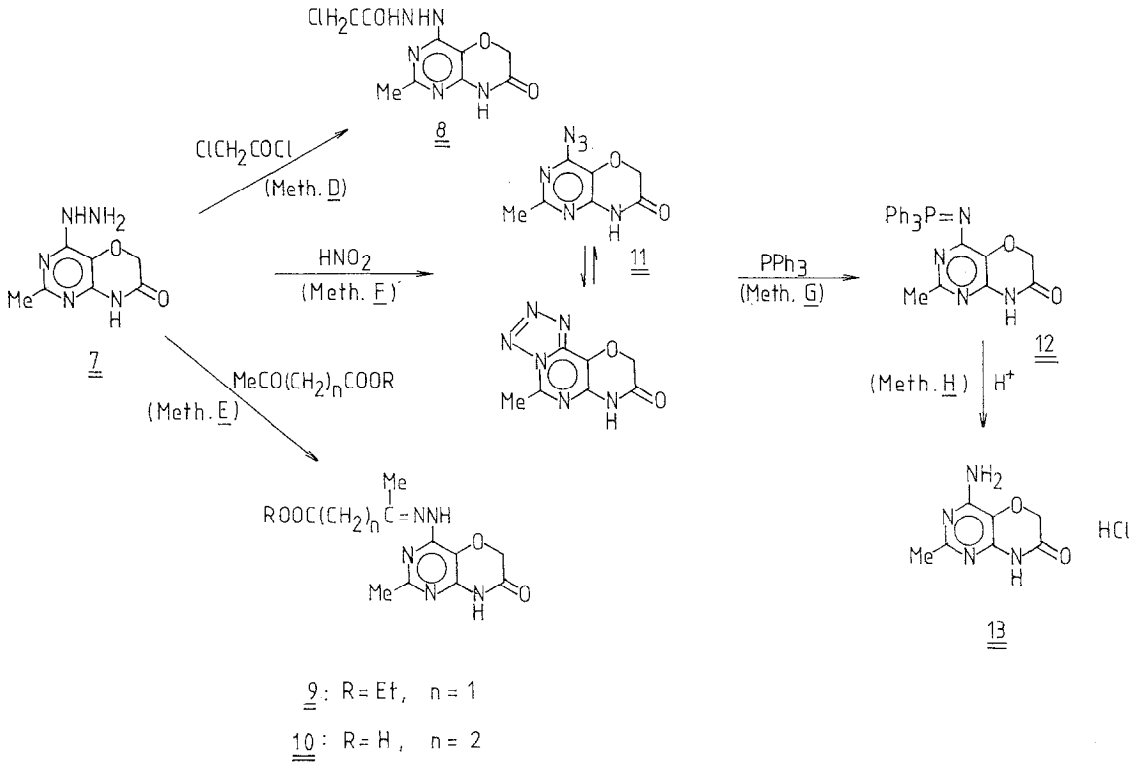
The compounds prepared, methods of their preparation, yields (not optimized) of the final step of their synthesis and melting points are summarized in table I.

Results and interpretation of biological properties

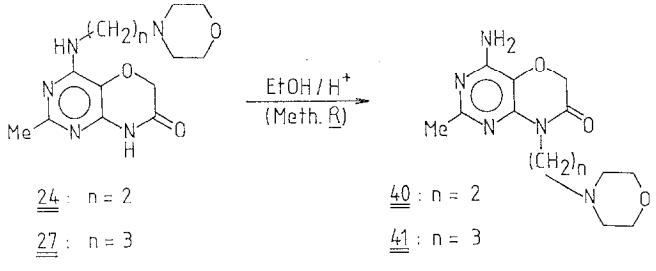
The inotropic activity, duration of the action as well as the effect on the heart rate in anaesthetized cats of compounds **I** are shown in table II. The myocardial reactivity was controlled before each experiment by intravenous administration of 0.2 μg/kg IS. The effi-



Scheme 1. →: main reaction; ⇢: side reaction; TS : tosyl.



Scheme 2.



Scheme 3.

ciency related to isoproterenol (T/IS) was calculated for the characterization of the activity.

Based on this test, the compounds having a quotient T/IS > 1, an effect lasting longer than 10 min and inducing a heart rate increase of at most 30 min^{-1} were selected for further examination.

It appears from the data of table II that the influence of the R^1–R^5 substituents on the positive inotropic effect can be scarcely evaluated as independent of one another. It seems to be more suitable to study their various combinations.

When R^3, R^4 and R^5 stand for hydrogen, R^1 is preferably 2-morpholinoethylamino or (4-benzyl-2-morpholinyl)methylamino group (**24, 22**). In the case of the former one, the lengthening of the alkyl group (**27**) or introducing a methyl group to the morpholino moiety (**25**) decrease the duration of action dramatically, while building-in of piperidino group instead of morpholino group (**26**) resulted in the loss of effectiveness. Within a series of compounds containing 2-hydroxyethylamino group as R^1 (**54–60**), CH_2COOEt group as R^5 (**54**) proved to be the most advantageous.

The positive inotropic action of the derivatives containing a hydrazino or amino group as R^1 (**7** and **13**, respectively) is not particularly strong but prolonged. The further investigation of **13**, however, was abandoned because of its strongly tachycardizing property. Surprisingly, compound **40**, which is the 8-(2-morpholinoethyl) derivative of **13** and a constitutional isomer of **24**, is quite inactive. For R^3 and R^4 hydrogen is most preferred within the scope of the compounds studied (*cf* **24, 73, 75** and **54, 68, 72**, respectively).

As a part of the study of the structure–activity relations, several other derivatives related to com-

Table I. List of pyrimido[5,4-*b*][1,4]oxazinones **I**.

Compound	*R1*	*R2*	*R3*	*R4*	*R5*	*Method*	*Mp (°C)* *base*	*salt*	*Yield (%) (for base)* [a]
1	Me	Cl	H	H	H	ref 14	175–177[d]		55
2	Me	Cl	Me	H	H	ref 14	167–168[e]		75
3	Me	Cl	Me	Me	H	ref 15	155–156[f]		65
4	Me	Cl	Ph	H	H	A	167–169		65
5	Me	tosyloxy	H	H	H	B	185–186		49
6	Ph	tosyloxy	H	H	H	B	230–232		91
7	Me	$NHNH_2$	H	H	H	ref 18	264–266		56
8	Me	$NHNHCOCH_2Cl$	H	H	H	D	265–266		55
9	Me	$NHN{=}C(Me)CH_2COOEt$	H	H	H	E	178–180		80
10	Me	$NHN{=}C(Me)CH_2CH_2COOH$	H	H	H	E	248–249		72
11	Me	N_3[b]	H	H	H	F	204–217[b]		93
12	Me	$N{=}PPh_3$	H	H	H	G	245–246		95
13	Me	NH_2	H	H	H	H	250–251	HCl 292–295	91
14	Me	morpholino	H	H	H	ref 16	244–245[g]		90
15	Me	4-methyl-1-piperazinyl	H	H	H			HCl 310–315	50
16	Me	4-(4-methoxyphenyl)-1-piperazinyl	H	H	H			HCl 243–244	35
17	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	H	C	197–198		87
18	Me	2-(4-methyl-1-piperazinyl)ethylamino	H	H	H	I		2 maleate 193–195	69
19	Me	cyclopropylamino	H	H	H	C		HCl 224–226	60
20	Me	3-pyridylmethylamino	H	H	H	J		2HCl 235–236	35
21	Me	(1-ethyl-2-pyrrolidinyl)methylamino	H	H	H	J		2HCl 196–198	42
22	Me	(4-benzyl-2-morpholinyl)methylamino	H	H	H	I		$2HCl{\cdot}2H_2O$ 196–199	15
23	Me	2-(*N*,*N*-diethylamino)ethylamino	H	H	H	J		2HCl 252–255	16
24	Me	2-morpholinoethylamino	H	H	H	I		fumarate[c] 215–216	50
25	Me	2-(2-methyl-4-morpholinyl)ethylamino	H	H	H	I		2HCl 186–189	18
26	Me	2-piperidinoethylamino	H	H	H	J		2HCl 134–138	25
27	Me	3-morpholinopropylamino	H	H	H	J		HCl 250–251	36
28	Me	bis(2-morpholinoethyl)amino	H	H	H	J	167–168	2HCl 162–163	30
29	Me	2-hydroxyethylamino	H	H	H	P	192–194	HCl 232–233	90
30	Me	*N*-methyl-*N*-(2-hydroxyethyl)amino	H	H	H	ref 18	150–151	HCl 165–167	92
31	Me	*N*-nicotinoyl-*N*-(2-hydroxyethyl)amino	H	H	H	C	202–204		73
32	Me	*N*-benzyl-*N*-(2-hydroxyethyl)amino	H	H	H	ref 18	135–137	HCl 165–166	75
33	Me	bis(2-hydroxyethyl)amino	H	H	H	C	170–172		35
34	Ph	2-morpholinoethylamino	H	H	H	I	225–226		35
35	Ph	2-hydroxyethylamino	H	H	H	I	182–184		46

Table I. *Continued.*

Compound	R^1	R^2	R^3	R^4	R^5	*Method*	*Mp (°C)* base		*salt*	*Yield (%) (for base)* a
36	Me	Cl	H	H	CH_2COOEt	K	68– 69			68
37	Me	Cl	H	H	$CH_2CH(OH)CH_2OH$	L	117–118			38
38	Me	Cl	H	H	Me	M	141–142[h]			60
39	Me	Cl	H	H	CH_2CN	K	147–149			79
40	Me	NH_2	H	H	2-morpholinoethyl	R		2HCl	236–238	40
41	Me	NH_2	H	H	3-morpholinopropyl	R	200–201			45
42	Me	2-morpholinoethylamino	H	H	$CH_2CH(OH)CH_2OH$	N		2HCl	158–159	30
43	Me	2-morpholinoethylamino	H	H	CH_2CN	K	126–128	HCl	228–229	91
44	Me	2-morpholinoethylamino	H	H	CH_2COOEt	K		HCl	176–179	77
45	Me	2-morpholinoethylamino	H	H	CH_2COCH_3	K	148–149			85
46	Me	morpholino	H	H	$CH_2CH(OH)CH_2OH$	N	153–155			80
47	Me	morpholino	H	H	CH_2CN	K	158–159			69
48	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	$CH_2CH(OH)CH_2OH$	N	146–148			83
49	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	CH_2CN	K	194–196			80
50	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	CH_2COCH_3	K	143–145			76
51	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	CH_2COOEt	K	105–106			79
52	Me	4-ethoxycarbonyl-1-piperazinyl	H	H	CH_2CONH_2	K	225–227			75
53	Me	2-hydroxyethylamino	H	H	$CH_2CH(OH)CH_2OH$	N	128–129			99
54	Me	2-hydroxyethylamino	H	H	CH_2COOEt	K		HCl	185–187	73
55	Me	2-hydroxyethylamino	H	H	CH_2CN	K	138–139	HCl	182–184	99
56	Me	2-hydroxyethylamino	H	H	CH_2COCH_3	K	150–152	HCl	185–187	83
57	Me	2-hydroxyethylamino	H	H	CH_2CONH_2	K	222–224			48
58	Me	2-hydroxyethylamino	H	H	3-pyridylmethyl	K	156–158	HCl	190–193	30
59	Me	2-hydroxyethylamino	H	H	CH_2COObu	K		HCl	142–145	62
60	Me	2-hydroxyethylamino	H	H	benzyl	C	124–126	HCl	153–157	84
61	Me	*N*-methyl-*N*-(2-hydroxyethyl)amino	H	H	CH_2COOEt	K		HCl	134–137	63
62	Me	*N*-methyl-*N*-(2-hydroxyethyl)amino	H	H	CH_2CN	K		HCl	145–148	96
63	Me	morpholino	H	H	CH_2COOEt	K	117–119			75
64	Me	H	H	H	$CH_2CH(OH)CH_2OH$	O	70– 72			15
65	Me	Cl	Ph	H	CH_2COOEt	K	68– 70			74
66	Me	morpholino	Ph	H	CH_2COOEt	K	85– 87			70
67	Me	2-hydroxyethylamino	Ph	H	H	C		HCl	118–119	
68	Me	2-hydroxyethylamino	Ph	H	CH_2COOEt	K		HCl	150–152	62
69	Me	2-morpholinoethylamino	Ph	H	H	C	168–169	maleate	194–196	26
70	Me	morpholino	Ph	H	H	C	201–202			92
71	Me	2-hydroxyethylamino	Me	H	H	C	195–197	maleate	165–167	49
72	Me	2-hydroxyethylamino	Me	H	CH_2COOEt	K		HCl	105–108	41
73	Me	2-morpholinoethylamino	Me	H	H	C	152–154			53
74	Me	Cl	Me	Me	CH_2COOEt	K	110–111			80
75	Me	2-morpholinoethylamino	Me	Me	H	J	148–149	HCl	225–228	71

[a]The preparation of the salt given is practically quantitative; [b]According to the spectral data exists as a mixture of azido and tetrazole form; [c]Base: fumaric acid = 2:1; [d]Reported value: 175.5–176°C; [e]Reported value: 169.5–170.5°C; [f]Reported value: 155–156°C; [g]Reported value: 243–245°C; [h]Reported value: 145.5–146°C (ref 16).

Table II. The inotropic activity of 5 mg/kg iv of compounds **I** in anesthetized cats.

Compound	*Increase in MCF (%)*	*T/IS*	*Influence on heart rate (min^{-1})*	*Duration of action (min)*
7	36	0.8	10	140
8	91.6	1.95	0	9
9	25	0.26	40	21
10	23.1	1.13	0	17
11	18.5	0.64	–15	10
12	12.5	0.4	0	43
13	55	1.1	60	96
15	–[a]			
16	–[b]			
17	50	0.71	20	2
18	33	0.76	15	7
19	0	–	30	
20	15	0.35	10	
21	15	0.35	10	
22	130	2.95	15	13
23	18.2	0.29	30	5
24	110[c]	1.0	15	120
25	50	1.16	15	4
26	15	0.35	0	5
27	51	1.19	15	5
28	13.3	0.43	37	3
29	20	0.46	10	5
30	67	0.96	60	5
31	0	–	0	–
32	83	1.1	50	5
34	10	0.2	0	12
35	15	0.2	0	3
36	41.6	1.08	10	5
37	100	1.49	0	8
39	5.9	0.16	–5	3
40	0	–	50	–
41	14	0.33	–10	5
42	6	0.16	–5	3
43	9	0.39	–10	10
44	29.4	1.32	–10	15
45	16.7	0.17	10	8
46	45	1.28	0	10
47	5.5	0.15	15	6
49	33.3	1.04	10	13
50	10	0.26	0	3
51	29.4	1.32	–10	15
52	25	0.7	10	5
54	55.5	1.2	20	190
55	35	0.98	35	6
56	40	0.9		
57	114	1.82	25	6
58	17	0.71	30	5
59	37	1.8	40	6
60	66	0.87		
61	41.6	0.94	25	11
62	0	–	–	–
63	30.4	0.58	10	30
64	0	–	20	–
65	0	–	0	–
66	52	1.73	35	8
68	7.5	1.00	0	6
69	–	1.58	0	7
70	40	1.98	25	1
71	15.8	1.58	0	7
72	10.5	0.21	15	4
73	33.3	0.53	20	7
74	22.2	0.74	20	5
75	200	2.05	115	3
amrinone		1.5	40	60

Table II. *Continued.* [a]It has negative inotropic effect; [b]It has toxic effects; [c]In a dose of 2 mg/kg iv.

pounds of type **I** have been prepared, also. Thus, tricyclic substances obtained from the 4-hydrazino or 4-(2-hydroxyethylamino) derivatives possess no positive inotropic effect, indicating the availability of the *N*-3 to be important for this effect [18].

Based on results obtained in anaesthetized cats, the compounds **22, 24** and **54**, promising to be more advantageous than amrinone, were also examined in dogs. The data are summarized in table III. Compounds **22** and **54** showed about the same activity as amrinone on this model, while **24** increased the MCF stronger than amrinone did.

The heart effects of this latter substance and amrinone were also compared by intraduodenal administration (table IV). It appears that the positive inotropic action of **24** is preferably completed by its mild antihypertensive and coronary flow-increasing

Table III. Cardiac effects of 1 mg/kg iv of compounds **22, 24, 54** and amrinone in anesthetized open chest dogs after 10 min. [a]$P < 0.05$; [b]$P < 0.01$

Compound	*Heart rate (min^{-1}) ($x \pm SE$)*	*MCF (%) ($x \pm SE$)*
22	base: 167.0 ± 7.7	100
	+9.0 ± 3.3	+17.8[a] ± 6.3
24	base: 174.0 ± 18.3	100
	22.0[a] ± 6.6	+98.0[b] ± 17.1
54	base: 173.3 ± 4.4	100
	+8.3[a] ± 2.5	+26.9[b] ± 5.7
amrinone		+24.6[b] ± 8.5

Table IV. Cardiac effects of compound **24** and amrinone in anesthetized dogs after id administration.

Compound (Dose, mg/kg)		*Blood pressure (mmHg)*		*Heart rate (min–1)*	*MCF (%)*		*Coronary flow (%)*	
		Systolic	*Diastolic*		*10 min*	*30 min*	*10 min*	*30 min*
24 (1)	Base:	153.2±12.9	98.3±12.8	169.4±3.8	100		100	
	Peak:	147.2±10.2	82.2±12.8	188.0±4.9				
	Change:	–6.0± 3.8	–16.1± 5.6	18.5±3.8	38.0±6.3 ($P < 0.01$)	37.5±6.2 ($P < 0.01$)	19.4±9.4	32.3±11.2
amrinone (5)	Base:	170.0±20.8	110.0±20.8	156.7±8.8	100		100	
	Peak:	145.0±38.2	88.3±22.0	186.7±8.3				
	Change:	–28.3±14.2	–21.6± 1.4 ($P < 0.01$)	30.0±2.9 ($P < 0.05$)	27.9±6.3 ($P < 0.05$)	16.7±5.6	19.8±13.3	10.0± 5.8

effects. When given in a dose of 1 mg/kg id, its tachycardizing effect proved to be insignificant.

Based on preliminary biochemical studies, it has been found that **24** has no influence on the sarcolemmal (K^+–Na^+)-ATPase, the adenyl cyclase, the phosphodiesterase (I, II, III) enzymes or the adrenergic receptors. However, similar to ouabain and BayK 8644, it increased the unstable Ca^{2+} pool of the sarcolemmal membrane which may be an important factor of the positive inotropic effect. Moreover, it protected the mitochondria of cardiac cells against Ca^{2+} overload by suppressing an excessive Ca^{2+} uptake.

Considering both the above results and toxicity data (table V), **24** (GYKI-12 735) promises to be a valuable positive inotropic drug.

Table V. Acute toxicity of compound **24** and amrinone.

Compound	*Animal*	*Route of administration*	LD_{50} *(mg/kg)*
24	mouse	po	1388
		iv	329
	rat	po	820
		iv	243
amrinone[a]	mouse	po	288
		iv	150
	rat	po	363
		iv	130

[a]According to reference 19.

Conclusion

Several compounds of the presented chemical class of pyrimido[5,4-*b*][1,4]oxazines exhibit pronounced positive inotropic activity. Compound **24** (GYKI-12 735) was selected for a detailed preclinical study. In contrast to typical cardiotonics, however, its activity probably involves a novel mechanism. Further experiments to clear this point are in progress and the results will be published elsewhere.

Experimental protocols

Chemistry

Melting points were determined on a Boetius apparatus and are uncorrected. The elementary analyses (C, H, N) of the new compounds were within ± 0.4% of the theoretical values. The IR and ^1H NMR spectrum data were in accordance with the structure of compounds prepared.

Hydrochlorides, fumarates and maleates were prepared in the usual way.

6-Amino-4-chloro-2-methyl-pyrimidin-5-ol used as starting material for method A and 4-hydroxy-2-methyl-6,7-dihydropyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one used for method B were synthesized according to the published procedures [14].

*4-Chloro-2-methyl-6-phenyl-6,7-dihydropyrimido[5,4-*b*][1,4]-oxazin-7(8*H*)-one* **4**

Method A. A solution of 1.60 g (0.01 mol) of 6-amino-4-chloro-2-methyl-pyrimidin-5-ol, 1.11 g (0.011 mol) of triethylamine and 2.98 g (0.015 mol) of ethyl α-chloro-phenylacetate in 12 ml of EtOH was heated under reflux for 7 h. From the stirred solution, crystallization started at 0°C. To this mixture 12 ml of water were added and the product was filtered. The substance is sufficiently pure of subsequent use. An analytical sample was recrystallized from EtOH.

*Preparation of 4-tosyloxy-6,7-dihydropyrimido[5,4-*b*][1,4]-oxazin-7(8*H*)-ones (***5***: R^1 = Me, **6**: R^1 = Ph)*

Method B. To a solution of 0.01 mol of the appropriate 4-hydroxy-6,7-dihydropyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one in 18 ml of 1 n NaOH, a solution of 0.012 mol of tosyl chloride in 9 ml of acetone was added dropwise at rt (for **5**) or at 35°C (for **6**) and the mixture was stirred at the same temperature for 5 h. Then the product was isolated in the following manner: **5**: the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The precipitate was filtered and washed with cold acetone; **6**: The crystalline product was filtered off, washed once with water and twice with an aqueous solution of NaOH (2%).

The starting material for **6**, *ie* 4-hydroxy-2-phenyl-6,7-dihydropyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one, was obtained from 6-amino-2-phenyl-pyrimidin-4(3*H*)-one in the way described for the 2-methyl derivative.

*Reaction of 4-chloro-6,7-dihydropyrimido[5,4-*b*][1,4]oxazin-7(8H)-ones with amines in BuOH*
Method C. A mixture of 0.01 mol of the appropriate 4-chloro compound (**1, 2** or **4**, resp) and 0.02 mol of amine in 20 ml of BuOH was heated under reflux for several h (according to TLC). The solvent was removed *in vacuo* and the residue was treated with water. The crude product obtained by filtration or extraction was either recrystallized from EtOH or converted to the given salt.

*4-(2-Chloroacetylhydrazino)-2-methyl-6,7-dihydropyrimido-[5,4-b][1,4]oxazin-7(8H)-one **8***
Method D. A mixture of 0.78 g (0.004 mol) of **7** in 10 ml of DMF was treated dropwise with 0.56 g (0.005 mol) of chloroacetyl chloride at 0°C. After stirring for 5 h at rt, the reaction mixture was allowed to stand overnight. It was then poured into 15 ml of ice-cold water and the precipitate was filtered, washed with water and dried.
Method E. A mixture of 1.95 g (0.01 mol) of **7** and 0.011 mol of the appropriate β-ketoester in 20 ml of EtOH was stirred at rt for 6 h and allowed to stand overnight. The crude product was filtered and recrystallized from EtOH.

*4-Azido-2-methyl-6,7-dihydropyrimido[5,4-b][1,4]oxazin-7(8H)-one **11***
Method F. To a stirred suspension of 4.88 g (0.025 mol) of **7** in aqueous acetic acid (containing 56 ml of water and 8.4 ml of acetic acid), 1.73 g (0.025 mol) sodium nitrite in 14 ml of water was added dropwise at 0–5°C. The reaction mixture was stirred at rt for 0.5 h and filtered to give **11**. An analytical sample was prepared by recristallization from DMF.

*2-Methyl-4-triphenylphosphoranylidenamino-6,7-dihydropyrimido- [5,4-b][1,4]oxazin-7(8H)-one **12***
Method G. To a stirred solution of 4.48 g (0.017 mol) of triphenylphosphine in 140 ml of dichloromethane, 3.30 g (0.017 ml) of **7** was added. After stirring for 3.5 h at rt, the mixture was allowed to stand overnight. The solvent was removed *in vacuo* and the residue was triturated with ether. After filtration the analytical pure product was obtained.

*4-Amino-2-methyl-6,7-dihydropyrimido[5,4-b][1,4]oxazin-7(8H)-one **13***
Method H. To a stirred suspension of 4.00 g (0.009 mol) of **13** in 25 ml of EtOH, 25 ml of aqueous HCl (20%) was added dropwise at rt. After stirring for 5 h, the precipitate was filtered and washed several times with EtOH and ether to give pure **13** as its hydrochloride salt.

*Reaction of **5** with amines*
Method I. A mixture of 0.15 mol of **5**, 0.15 mol of anhydrous K_2CO_3 in 1250 ml of EtOAc was treated with 0.15 mol of the appropriate amine. After stirring at rt for 72 h the suspension was filtered and the filtrate was extracted with aqueous HCl (4%). The aqueous layer was adjusted to pH 4-5 with an aqueous solution of NaOH (4%) and extracted with dichloromethane. The aqueous phase was separated, adjusted to pH 9-10 with an aqueous solution of NaOH (4%) and extracted with dichloromethane. The organic layer was separated and after evaporation of the solvent, the product was obtained by treatment of the crude base with the appropriate acid.

*Reaction of **1** with amines in the presence of triethyl amine*
Method J. A mixture of 0.01 mol of **1**, 0.015 mol of triethyl amine and 0.01 mol of the appropriate amine in 50 ml of benzene (for **23, 26** and **27**) or dioxane (for **20, 21, 28** and **75**) was heated under reflux for 20 h. The solvent was evaporated *in vacuo* and the residue was treated with water. The product was isolated according to method I.

Alkylation of 2-methyl-6,7-dihydropyrimido[5,4-b][1,4]-oxazin-7(8H)-ones
Method K. A solution of 0.011 mol of the appropriate alkylating agent in 20 ml of 2-butanone ($ClCH_2CN$, $ClCH_2COOEt$, $ClCH_2CONH_2$ or $ClCH_2COCH_3$) was added dropwise to a stirred mixture of 0.01 mol of the appropriate pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one, 0.012 mol of anhydrous K_2CO_3 in 50 ml of 2-butanone. After heating under reflux for 8 h, the hot mixture was filtered and the filtrate was evaporated *in vacuo*. The product was obtained either as base after recrystallization from iPrOH or as its hydrochloride salt by treatment of the crude base with a solution of HCl in EtOH.

*4-Chloro-8-(2,3-dihydroxypropyl)-2-methyl-6,7-dihydropyrimido[5,4-b][1,4]oxazin-7(8H)-one **37***
Method L. A solution of 10.0 g (0.05 mol) of **1**, 0.70 g (0.005 mol) of triethyl amine and 7.40 g (0.1 mol) of 2,3-epoxy-1-propanol in 300 ml of anhydrous benzene was heated under reflux for 4 h. The solution was washed twice with water and the combined aqueous layers were extracted with chloroform. The organic phase was evaporated *in vacuo*, the residue was treated with a 1:1 mixture of ether-petrolether. The precipitate was filtered and recrystallized from ether to give pure **37**.

*4-Chloro-2,8-dimethyl-6,7-dihydropyrimido[5,4-b]-[1,4]oxazin-7(8H)-one **38***
Method M. A solution of 2.0 g (0.001 mol) of **1** in 10 ml of dioxane was added dropwise at rt to a stirred solution of 0.4 g (0.01 mol) of diazomethane in 20 ml of ether prepared from Diazald® (Aldrich) by the standard procedure. After standing at rt for 5 h, the reaction mixture was quenched by hydrochloric acid and then evaporated to dryness. The crude product was recrystallized from EtOH.
Method N. To a reaction mixture described in method L containing the appropriate pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one instead of **1**, 0.05 mol of TBAB was also added. The crude base was recrystallized from diisopropyl ether and/or converted to its hydrochloride salt.
*8-(2,3-Dihydroxypropyl)-2-methyl-6,7-dihydropyrimido[5,4-b]-[1,4]oxazin-7(8H)-one **64***
Method O. In a Parr apparatus a mixture of 2.0 g (0.0074 mol) of **37**, 0.74 g (0.0074 mol) of triethyl amine and 0.20 g of palladium on charcoal (5%) catalysator in 50 ml of EtOH was hydrogenated. After the calculated value had been reached, the catalysator was filtered off. The solvent was removed *in vacuo* and the residue was treated with water, then extracted with chloroform. The crude product was recrystallized from petrolether to give pure **64**.

*4-(2-Hydroxyethylamino)-2-methyl-6,7-dihydropyrimido[5,4-b]-[1,4]oxazin-7(8H)-one **29***
Method P. In a Parr apparatus a mixture of 2.00 g of **32** and 1.2 g of palladium on charcoal (10%) in 60 ml of methanol was hydrogenated at rt. After the calculated hydrogen consumption, the mixture was refluxed for 15 min and filtered. After evaporation of the solvent and recrystallization from methanol, the pure **29** was obtained.

Preparation of 8-(N-morpholinoalkylamino)-6,7-dihydropyrimido-[5,4-b][1,4]oxazin-7(8H)-ones ***40, 41***

Method R. A solution of 0.005 mol of the appropriate 4-(*N*-morpholinoalkylamino) derivative (**24** or **27**, resp) in the form of dihydrochloride salt in 15 ml of BuOH or EtOH was heated under reflux for 14 h. The solvent was evaporated *in vacuo* and the residue was recrystallized from EtOH.

Biological test

The cardiotonic activity of compounds was tested by a modified strain gauge method of Walton and Brodie [20].

Screening in anesthetized open chest cat

Cats of both sexes were anesthetized with a 1:5 mixture of chlorolose and urethane. After arranging the artificial respiration through a tracheal cannule with a Harvard 665 A model respirator equipped with phase control, the chest and epicardium were opened. A strain gauge sheet was sutured onto the epicardial surface of the left ventricle and the myocardial contractile force was measured. The systemic blood pressure was continuously recorded by a cannule inserted into the femoral artery and joined to a Statham (P23Db) pressure transducer and electromanometer. The heart rate was continuously recorded by a pulsotachometer. The positive inotropic effect was also determined by measuring dP/dt_{max}. Compounds **I** were dissolved in distilled water and administered intravenously through the femoral vein. In some cases, the effect was also studied by intraduodenal administration.

Positive inotropic activity in anesthetized dog model

Cross-bred dogs of both sex were anesthetized with pentobarbital sodium (30 mg/kg, iv). Contractile force, heart rate and blood pressure were recorded on Beckman 612 Dynograph as described above. For measuring the blood flow in the coronary artery, one of the branches of ramus descendens a, coronariae circum flexae sin was prepared and fitted with electromagnetic flow probe.

Experiments on rabbit papillary muscle

Male rabbit heart was placed into Locke solution. The right papillary muscle was isolated and suspended in an organ bath (32°C, pH = 7.5), then it was driven electrically by square impulses having a frequency of 60 min and duration of 4 ms. The compound tested was dissolved in water. The myocardial contractile force was continuously recorded by an auxotonic strain gauge.

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References

1 Miller JP, Boswell KH, Meyer RB, Christensen LF, Robins RK (1980) *J Med Chem* 23, 242–251
2 Seamon KB, Daly JW, Metzger H, de Souza NJ, Reden J (1983) *J Med Chem* 26, 436–439
3 Sircar I, Bobowski G, Bristol JA, Weishaar RE, Evans DB (1986) *J Med Chem* 29, 261–267
4 Mertens A, Müller-Beckmann B, Kampe W, Hölck JP, won der Saal W (1987) *J Med Chem* 30, 1279–1287
5 Spitzer WA, Victor F, Pollock DG, Hayes SJ (1988) *J Med Chem* 31, 1590–1595
6 Rakhit S, Marciniak G, Leclerc G, Schwartz J (1986) *Eur J Med Chem Chim Ther* 21, 511–515
7 Erhardt PW (1987) *J Med Chem* 30, 231–237
8 Wetzel B, Hauel N (1988) *Trends Pharmacol Sci* 9, 166–170
9 Mátyus P, Szilágyi G, Kasztreiner E, Rabloczky Gy, Sohár P (1988) *J Heterocycl Chem* 25, 1535–1542
10 Kosáry J, Kasztreiner E, Rabloczky Gy, Kürthy M (1989) *Eur J Med Chem Chim Ther* 24 (in press)
11 Kürthy M, Rabloczky Gy, Heltai K, Kasztreiner E, Mader M (1987) *J Mol Cell Cardiol* 19, S50
12 Mátyus P, Kasztreiner E, Makk N, Kosáry J, Diesler E, Czakó K, Tegdes A, Rabloczky Gy, Kürthy M (1988) Poster presentation at the Xth Int Symp Med Chem 15–19 August, Budapest EFMC Abstr, 252 p
13 Chinoin-Gyógyszer (1987) Eur Patent Appl 228, 094 (July 8, 1987); (1987) *Chem Abstr* 107, 176062p
14 Sazonov NV, Safonova TS (1972) *Khim Geterotsikl Soedin* 1285–1288
15 Sazonov NV, Safonova TS (1976) *Khim Geterotsikl Soedin* 681–685
16 Sazonov NV, Safonova TS (1973) *Khim Geterotsikl Soedin* 171–174
17 Ito I, Oda N, Kato T (1976) *Chem Pharm Bull* 24, 1189–1196
18 Mátyus P, Szalay L, Kasztreiner E, Jerkovich Gy, Rabloczky Gy (1989) *J Heterocycl Chem* 26, 739
19 Alousi AA, Farah AE (1980) *Trends Pharmacol Sci* 1, 143–146
20 Walton RP, Brodie OJ (1947) *J Pharm Exp Ther* 90, 26–41