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Articles

Synthesis and SAR of 6-Substituted Purine Derivatives as Novel Selective Positive Inotropes

Jeffery B. Press,* Robert Falotico, Zoltan G. Hajos, Rebecca Anne Sawyers, Ramesh M. Kanojia, Louella Williams, Barbara Haertlein, Jack A. Kauffman, Constance Lakas-Weiss, and Joseph J. Salata

The R.W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477 and Raritan, New Jersey 08869

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A series of purine derivatives was prepared and examined for selective inotropic activity in vitro and in vivo. Thioether-linked derivatives were superior to their oxygen and nitrogen isosteres. Substitution of electron-withdrawing groups on the benzhydryl moiety of these agents increased potency. The best compound of the study, **17** (carsatrin), was examined further and demonstrated selective oral activity as a positive inotrope. These compounds are presumed to act by affecting the kinetics of the cardiac sodium channel by analogy to the prototypic agent DPI 201106 (**1**). Their high selectivity for increasing contractile force and dP/dt without affecting blood pressure or heart rate is consistent with this mechanism. Carsatrin (**17**) was selected as a potential development candidate.

Congestive heart failure (CHF) is a serious health problem worldwide with exceedingly high rates (30–50%) of mortality in Class III and Class IV patients.[1,2] Historically, treatment of CHF has utilized sympathomimetics or cardiac glycosides to improve cardiac function due to their inotropic action in both acute and chronic heart failure.[3,4] In general, the use of sympathomimetic agents is limited by poor oral efficacy, short duration of action, and dangerous cardiac side effects. Cardiac glycosides such as digitalis have a narrow therapeutic window with arrhythmias, vasoconstriction, and other side effects which occur at relatively low doses.[5-8]

Intensive efforts to find effective therapeutic alternatives to digitalis have been underway in the past decade[9,10] and it remains an unmet need of cardiovascular therapy in the 1990s.[11] Various mechanisms have been explored to produce effective positive inotropes. One of the most studied approaches involves inhibition of cAMP phosphodiesterase III (PDE III) in cardiac cells. The resultant elevation in intracellular cAMP causes short-term increases in cardiac contractility and produces peripheral vasodilation which reduces cardiac afterload. The prototypic drug used parenterally is amrinone while milrinone,[12] enoximone,[13] imazodan,[14] and bemoradan[15] have been examined clinically. The development of side effects and tolerance has limited the use of these agents to short-

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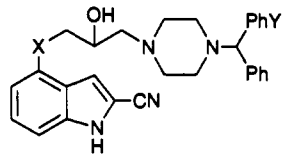
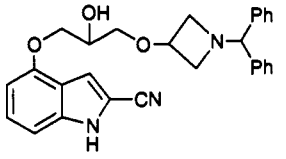
term treatment of end-stage CHF.[16-18] Another mechanistic approach to positive inotropy is selective histamine H_2 agonism which would activate adenylate cyclase and thus raise levels of intracellular cAMP. Impromidine and apromidine are protypic agents that may provide insight into the utility of this mechanism but, like PDE inhibitors, side effects may preclude extensive clinical use.[19-22]

A mechanism to increase contractile force by increasing intracellular calcium concentration $[Ca^{2+}]_i$ attracted us. Digitalis, by inhibiting Na^+/K^+ ATPase, increases intracellular Na^+ which leads to increased $[Ca^{2+}]_i$ by reversing Na^+/Ca^{2+} exchange. The prototypic agent DPI 201106 (1) facilitates Na^+ entry by altering the kinetics of the cardiac Na^+ channel.[23] Several recent reports of the effectiveness of sodium channel modulation in failing myocardium,[24] the possibility of Class III antiarrhythmic effects,[25] and distinction from PDE III inhibitors[26] suggest that this approach may well have the greatest potential to provide a viable alternative to cardiac glycosides in the treatment of CHF.

Recently several new agents have been reported to activate the Na^+ channel. In addition to 1, SDZ 210912 (2)[27] and BDF 9145 (3)[28] share the benzhydryl-substituted piperazine subunit while BDF 9148 (4)[29] utilizes a benzyhydrylazetidinoxy moiety as a structural alternative.

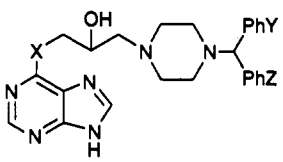


1, DPI 201106, X = O, Y = H
2, SDZ 210921, X = NH, Y = H
3, BDF 9145, X = O, Y = p-OCH_2CN



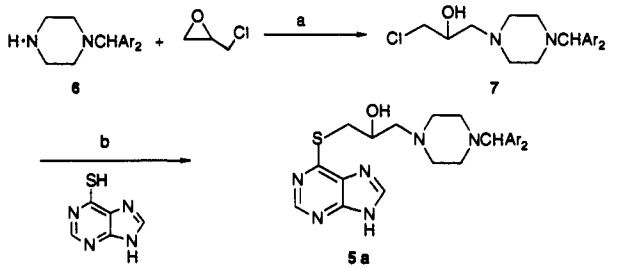
4, BDF 9148

We became interested in this class of compounds not only because of our long-standing interest in developing cardiotonic agents[15,30,31] but also because of an ongoing interest in purine chemistry in our laboratories. It seemed that the electron-withdrawing nature of the 2-cyanoindole moiety in 1-4 could be replaced by the electron-poor purine ring system while still maintaining the structural template seemingly necessary for inotropic activity. We were also struck by the relatively sparse information available on the SAR of the benzhydryl side chain. We thus undertook a study to examine the structure–activity requirements for general target structures **5a–c**.



5a, X = S
5b, X = NR
5c, X = O

Scheme I.[a] Synthesis of 6-S Analogues



[a] (a) EtOH, $NaHCO_3$; (b) NaH, DMF.

Chemistry

The synthesis of compounds wherein the purine nucleus is coupled to side chains by sulfur, nitrogen, and oxygen heteroatoms requires different synthetic approaches. The synthetic pathway for the sulfur derivatives is the most straightforward and is shown in Scheme I. Piperazine derivative 6 is reacted with epichlorohydrin in the presence of sodium bicarbonate to give the chlorohydrin 7. Subsequent reaction of 7 with 6-mercaptopurine in the presence of sodium hydride in DMF gives the desired 6-S-substituted purine **5a**. Other bases such as sodium

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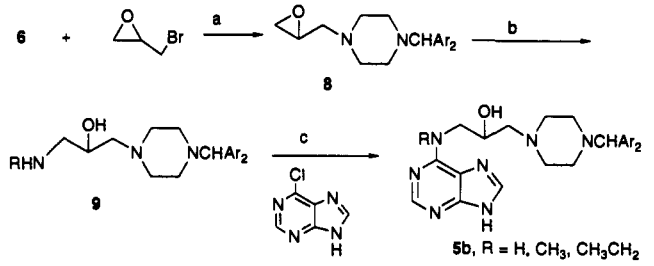
Scheme II.[a] Synthesis of 6-N Analogues



[a] (a) CH_3CN, K_2CO_3, 0 °C → room temperature; (b) liquid NH_3–EtOH, 110 °C, 28 h, or CH_3NH_2, or $CH_3CH_2NH_2$; (c) Et_3N, MeOH, reflux, 7 d.

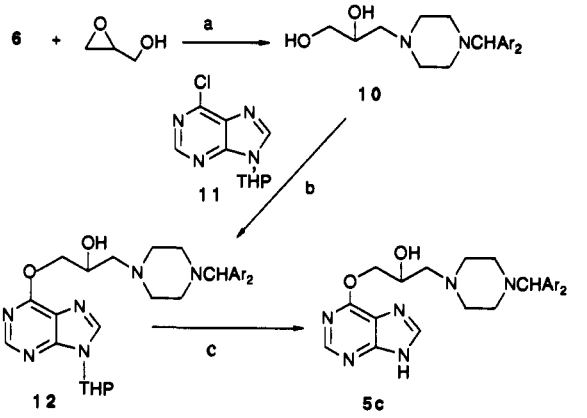
hydroxide, potassium hydroxide, or triethylamine may be used. The conversion of **7** to **5a** is particularily advantageous using dry sodium bicarbonate in 2-propanol. Analogues of the piperazine **6** may be readily prepared by reacting the appropriate halide derived from benzyhydrol or benzophenone derivatives and piperazine.[32] Several esters of the 2-hydroxyl moiety of **5a** were prepared with acid anhydrides using standard conditions. Compounds prepared are listed in Table I.

Ordinarily, β-blocking agents containing the 2-hydroxypropyl-substituted side chain are prepared using a pathway inverse from that depicted in Scheme I wherein epichlorohydrin is first condensed with the aromatic moiety. This derived intermediate is then condensed with a variety of amines to give target compounds. In the case of 6-mercaptopurine, this approach leads to an unusual rearrangement/thietane formation which interferes with the production and isolation of the target compounds.[33]

Scheme II depicts the synthesis of **5b**, the 6-nitrogen analogues. Piperazine derivative **6** is reacted with epibromohydrin to give epoxide **8**. Reaction with liquid ammonia produces amine **9** (R = H) which is then reacted with unprotected 6-chloropurine in the presence of triethylamine to give the target compound **5b**. Alternatively, using methylamine or ethylamine in the preparation of **9**, *N*-alkyl derivatives may be formed. Compounds prepared in this series are listed in Table II.

The O-linked derivatives **5c** proved the most difficult to prepare (Scheme III). Piperazine **6** is reacted with glycidol in methanol to give the diol **10**. The reduced nucleophilicity of the oxygen of **10** vis-á-vis sulfur or nitrogen required N-9 protection of the purine to prevent undesired N-9 nucleophilic displacement and dimerization. Reaction of **10** with **11**, the *N*-9-tetrahydropyranyl derivative of 6-chloropurine,[34] using potassium hydroxide and 18-crown-6 gives the 6-O, 9-*N*-THP-protected derivative **12**. Removal of the N-9 protecting THP group in aqueous acetic acid gives the target compounds **5c**. Compounds prepared in this series are also listed in Table II.

Scheme III.[a] Synthesis of 6-O Analogues



[a] (a) $MeOH,N_2$, room temperature, 18 h; (b) KOH, toluene, 18-c-6, room temperature, 3 h; (c) $AcOH–H_2O$, room temperature, 18 h.

Results and Discussion

Cardiotonic activity of these new 6-substituted purine derivatives was evaluated both in vitro using isolated ferret papillary muscle and in vivo using anesthetized instrumented dogs according to procedures described previously.[31] In vitro, changes in contractile force (CF) were quantitated by determining the maximal increase in isometric tension as a percent of control tension (max %). Relative changes in CF were determined by calculating the effective concentration that produced a 50% of maximum increase in tension (EC_{50}). These measurements provide indices of activity and potency, respectively. In vivo, drug effects on CF, dP/dt_{max}, heart rate (HR), and mean arterial blood pressure (MAP) were measured in dogs. Results are reported as percent change from pretreatment control parameters. ED_{50} values (dose to produce a 50% increase of CF) were determined to compare potencies for several of the more interesting compounds. Data are summarized in Tables I and II.

The thioether series examines effects on variation of the benzhydryl substituent as well as substitution on the purine ring and side-chain modification. As may be seen from the in vitro data (Table I), a number of thioethers are more active and at least as potent as **1** (DPI 201106) (cf. **13**, **15**, **17**, **26**, **28** to **1**). Small electron-withdrawing substituents on the benzhydryl moiety, such as fluoro- (**17**) or chloro- (**15**) or no substitution (**13**), give molecules with the best activity while larger electron-withdrawing groups such as bromo- (**16**), dichloro- (**18**), trifluoromethyl- (**19**, **20**), or electron-donating groups (**21**) are less active. Altering the benzhydryl to a benzyl (**22**) or to a trityl (**23**) gives compounds with no activity in vitro. Substitution of methyl at N-9 of the purine ring (**26**) does not greatly affect activity (cf. **17**) but reduces potency; other substitutions are less active (**24**, **27**). Acetylation of the hydroxyl on the propyl side chain (**28**) increases activity but other esters (**29**–**32**) as well as the removal of the hydroxyl group (**33**) are less active. The EC_{50} data for the thioethers shows that almost all the derivatives active in vitro are at least as potent as **1** with the exceptions of **13**, **18**, **19**, **24**, **26**, and **30**. However, when potency and activity are considered together, compounds **15**, **17**, and **28** are the most outstanding derivatives.

The amine and oxygen analogues (Table II) are considerably less interesting than the thioethers. While amines **34** and **37** enhance contractile force, they are not as active as the thioethers **17** and **28**, respectively. The other amine and oxygen derivatives have no in vitro activity. It is noteworthy that **38**, the direct purine analogue of **1**, is inactive.

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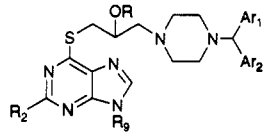
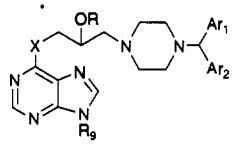
Table I. Cardiotonic Activity of Thioether Derivatives



						papillary muscle[a]		canine[b]					
no.	Ar_1	Ar_2	R_2	R_9	R	max %	EC_{50}	CF	dP/dt_{max}	HR	MAP	n	ED_{50}
						Benzhydryl Variation							
13	Ph	Ph	H	H	H	249 ± 81	1.1 (0.5–2.6)	86 ± 18	60 ± 13	−1 ± 2.5	−12 ± 4	3	0.63 (0.39–1.12)
14	4-ClPh	4-ClPh	H	H	H	–	–	151 ± 43	99 ± 18	−4 ± 3	9 ± 2	3	0.33 (0.12–0.58)
15	4-ClPh	Ph	H	H	H	242 ± 71	0.44 (0.3–0.5)	100 ± 3	75 ± 1	−4 ± 4	+2 ± 1	2	0.49[c]
16	4-BrPh	4-BrPh	H	H	H	64 ± 20	0.47 (0.1–0.5)	148 ± 9	108 ± 8	−2 ± 4	11 ± 2	2	0.31[c]
17	4-FPh	4-FPh	H	H	H	189 ± 52	0.3 (0.2–0.5)	224 ± 37	150 ± 25	0 ± 2	−5 ± 1	12	0.15 (0.07–0.22)
18	2,4-ClPh	2,4-ClPh	H	H	H	49 ± 10	1.6 (0.7–3.6)	69 ± 17	47 ± 3	6 ± 7	−10 ± 11	2	0.90[c]
19	3-CF_3Ph	3-CF_3Ph	H	H	H	106 ± 28	3.4 (1.2–9.5)	68 ± 24	46 ± 16	−6 ± 1	8 ± 2	2	1.1[c]
20	4-CF_3Ph	4-CF_3Ph	H	H	H	47 ± 9	1.0 (0.4–2.2)	71	47	0	5	1	–
21	4-CH_3OPh	4-CH_3OPh	H	H	H	98 ± 26	0.5 (0.4–0.7)	45 ± 15	18 ± 12	−2 ± 1	−7 ± 0	2	–
22	Ph	H	H	H	H	IA	IA	30 ± 12	34 ± 24	0 ± 4	1 ± 0	2	–
23	$(Ph)_2$	Ph	H	H	H	IA	IA	22 ± 9	23 ± 21	4 ± 7	−14 ± 13	2	–
						Substitution on Purine							
24	4-FPh	4-FPh	NH_2	H	H	74 ± 4	1.9 (1.0–3.3)	74 ± 9	38 ± 16	12 ± 3	−9 ± 6	2	0.85[c]
25	4-FPh	4-FPh	Me	H	H	–	–	23 ± 9	17 ± 1	1 ± 1	1 ± 2	2	–
26	4-FPh	4-FPh	H	Me	H	142 ± 54	0.6 (0.1–2.7)	50 ± 17	11 ± 4	−2 ± 1	−6 ± 0	2	1.9[c]
27	4-FPh	4-FPh	H	Ph	H	IA	IA	12 ± 3	5 ± 4	4 ± 1	−3 ± 1	2	–
						Esterification							
28	4-FPh	4-FPh	H	H	COMe	248 ± 65	0.7 (0.5–0.9)	145 ± 12	62 ± 19	−4 ± 7	−12 ± 8	2	0.30[c]
29	4-FPh	4-FPh	H	H	CO-2,4,6-$(MeO)_3$Ph	IA	IA	17	15	3	0	1	–
30	4-FPh	4-FPh	H	H	CO-2-(AcO)Ph	34 ± 19	2.5 (0.4–14.6)	56 ± 26	34 ± 22	1 ± 10	0 ± 6	2	1.6[c]
31	4-FPh	4-FPh	H	H	$COCMe_3$	43 ± 16	0.17	12 ± 2	24 ± 10	4 ± 3	−5 ± 3	2	–
32	4-FPh	4-FPh	H	H	SO_2OH	57 ± 3	0.39	159 ± 21	110 ± 9	6 ± 2	6 ± 3	2	0.26[c]
						Removal of OH							
33	4-FPh	4-FPh	H	H	–	IA	IA	38 ± 13	17 ± 7	2 ± 2	−6 ± 4	4	1.3[c]
1	(DPI 201106)					131 ± 46	0.5 (0.4–0.6)	98 ± 16	88 ± 17	−12 ± 4	−21 ± 3	5	0.43 (0.25–0.72)

[a] In vitro assay of inotropic activity using ferret papillary muscle suspended in Tyrode's sodium max % ± SEM was determined by serially increasing concentration of test compound to produce maximal increase in tension. EC_{50} is the concentration of test compound that produces 50% of maximal increase in tension. IA = inactive (<30% increase in tension at 10 μM concentration). See Experimental Section for detail. [b] In vivo assay of inotropic activity (±SEM). Test compounds were initially administered ca. 1.875 mg/kg, iv in mongrel dogs. CF = cardiac force, HR = heart rate, MAP = mean arterial pressure. ED_{50} is the dose of compound that produces 50% increase of CF from base line and was determined from dose–response studies. See Experimental Section for detail. [c] Estimated from graphical analysis.

Table II. Cardiotonic Activity of Amine and Ether Derivatives



							papillary muscle[a]		canine[b]					
no.	X	Ar_1	Ar_2	R_9	R	max %	EC_{50}	CF	dP/dt_{max}	HR	MAP	n	ED_{50}
							Amine Derivatives						
34	NH	4-FPh	4-FPh	H	H	86 ± 43	0.5 (0.2–1.0)	154 ± 34	83 ± 27	2 ± 7	−2 ± 2	3	0.28 (0.09–0.51)
35	NMe	4-FPh	4-FPh	H	H	IA	IA	82 ± 14	66 ± 17	7 ± 9	4 ± 6	2	0.74[c]
36	NEt	4-FPh	4-FPh	H	H	IA	IA	13 ± 3	16 ± 3	2 ± 4	5 ± 2	2	–
							Esterification						
37	NH	4-FPh	4-FPh	H	$COCH_3$	45 ± 17	2.1 (1.0–4.2)	144 ± 16	70 ± 17	−12 ± 3	−26 ± 0	2	0.27[c]
							Oxygen Derivatives						
38	O	Ph	Ph	H	H	IA	IA	69 ± 13	37 ± 1	2 ± 1	4 ± 1	2	0.90[c]
39	O	4-FPh	4-FPh	H	H	IA	IA	80 ± 1	58 ± 11	12 ± 8	4 ± 2	2	0.72[c]
40	O	Ph	Ph	THP	THP	IA	IA	24 ± 19	13 ± 6	0 ± 2	−2 ± 3	2	–
41	O	4-FPh	4-FPh	THP	THP	IA	IA	34 ± 3	38 ± 22	9 ± 9	−5 ± 8	2	–
1	(DPI 201106)					131 ± 46	0.5 (0.4–0.6)	98 ± 16	88 ± 17	−12 ± 4	−21 ± 3	5	0.43 (0.25–0.75)

[a–c] See footnotes in Table I.

Examination of these compounds in vivo shows qualitatively similar trends (Table I). Thioether derivatives **14–17, 28,** and **32** all enhance CF and dP/dt_{max} more than **1** at the screening dose. It is unclear why electron-withdrawing substituents such as bromo- (**16**) cause better in vivo activity than is predicted by in vitro tests. Similarly, the origins of the unpredicted reduced activity for acetate **28** and enhanced activity for sulfonic acid **32** are unclear. Preliminary calculations of log *P* or molecular modeling studies give no insight into these differences. All of these compounds have equal or even less cardiovascular side effects on HR or MAP as compared to **1**. Most interesting

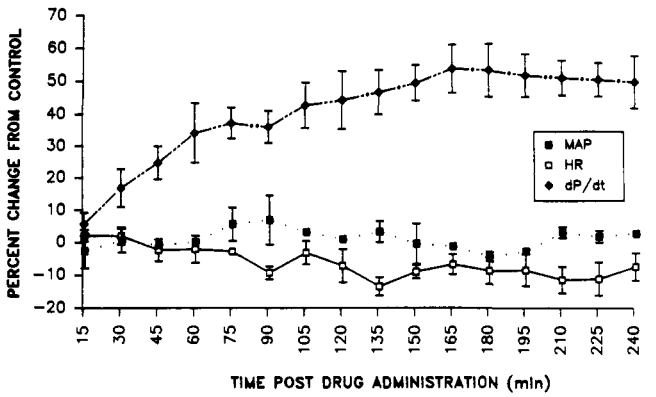


Figure 1. Hemodynamic effects of orally administered compound 17 in conscious instrumented dogs. MAP = mean arterial blood pressure; HR = heart rate; dP/dt = rate of pressure development over time. Determination in conscious instrumented dogs (n = 3) after oral administration at 10 mg/kg.

is the greatly enhanced in vivo activity of 17 as compared to other members of this group or compared to 1. In addition to its remarkable inotropic efficacy (CF and dP/dt_{max}), 17 has the greatest potency of the series with an ED_{50} of 0.15 mg/kg, essentially 4-fold better than 1, whose ED_{50} is 0.43 mg/kg.

Amine derivatives (Table II) have substantial activity in vivo despite their rather poor activity in vitro. Amine **34** is among the more active compounds in vivo with good increases in CF and dP/dt_{max} and is at least as potent as 1. Compound **37**, the ester derivative of **34**, has very similar activity and potency. Alkylation of the amine nitrogen (**35, 36**) reduces in vivo activity. Similar to the in vitro data, the oxygen derivatives are also the least interesting compounds in vivo. As noted earlier, **38**, the direct purine analogue of 1, is not nearly as active as other compounds in this series or as active as 1.

The superior intravenous profile of the best compound in these series is also demonstrated after oral administration. The results in Figure 1 show that 17 (10 mg/kg, po) selectively increases myocardial contractility (dP/dt_{max}). The onset of activity occurs within 30 min, peaks within the 3rd hour, and gradually returns to control within 12 h (not shown). Mean arterial pressure does not change while heart rate declines modestly over this interval. The lack of hypotension and tachycardia in conscious dogs distinguishes 17 from other inotropes (including those of the PDE inhibitor class) and highlights the unique activity of this new inotropic agent.

Conclusions

A series of 6-substituted (benzhydrylpiperazino)propanol purine derivatives has been synthesized. Many of these compounds were active, and some of the more potent compounds were highly selective positive inotropic agents. Compound 17 (carsatrin, 4-[bis(4-fluorophenyl)methyl]-α-[(9*H*-purin-6-ylthio)methyl]-1-piperazineethanol) has been selected for further study because of its superior, selective cardiovascular profile and excellent oral activity. The best compounds in this study are highly selective, positive inotropes that most likely act through a mechanism involving the cardiac sodium fast channel as suggested by its structural similarity to 1 as well as data reported earlier.[35,36] Compound 17 fulfills the criteria of a potential drug candidate for digitalis replacement therapy and may therefore be useful in the treatment of mild to severe congestive heart failure.

Table III. Physical Properties of 6-Substituted-1*H*-imidazo[4,5-*d*]pyrimidines

no.	mp	anal. (C, H, N)	method	yield (%)
13	105–110 dec	$C_{25}H_{28}N_6OS \cdot {}^1/_2H_2O$	A	15
14	120–124 dec	$C_{25}H_{26}Cl_2N_6OS \cdot {}^5/_4H_2O$	A	22.3
15	117–120 dec	$C_{25}H_{27}ClN_6OS \cdot H_2O$	A	40.5
16	134–136 dec	$C_{25}H_{26}Br_2N_6OS \cdot {}^1/_2H_2O$	A	46
17	115–116	$C_{25}H_{26}F_2N_6OS \cdot {}^1/_2H_2O$	A	25.4
18	120–123 dec	$C_{25}H_{24}Cl_4N_6OS \cdot {}^1/_4H_2O$	A	34.7
19	100–105 dec	$C_{27}H_{26}F_6N_6OS \cdot {}^1/_2H_2O$	A	49.3
20	108–110 dec	$C_{27}H_{26}F_6N_6OS$	A	12.8
21	110–113 dec	$C_{27}H_{32}N_6O_3S \cdot {}^1/_5H_2O$	A	10
22	175 dec	$C_{19}H_{24}N_6OS \cdot C_3H_4O_4$	A	48.8
23	158–161 dec	$C_{31}H_{32}N_6OS \cdot H_2O$	A	38.2
24	118–122 dec	$C_{25}H_{27}F_2N_7OS \cdot {}^1/_2H_2O$	A	55.1
25	83–86 dec	$C_{26}H_{26}F_2N_6OS \cdot {}^3/_4H_2O$	A	23.2
26	68–70 dec	$C_{26}H_{26}F_2N_6OS \cdot {}^1/_2H_2O$	A	19.2
27	119–122 dec	$C_{31}H_{30}F_2N_6O \cdot {}^1/_3H_2O$	A	61.9
28	105–109 dec	$C_{27}H_{26}F_2N_6O_2S \cdot {}^3/_4H_2O$	D	64.8
29	114–118 dec	$C_{35}H_{26}F_2N_6O_5S$	D	30
30	110–111 dec	$C_{34}H_{32}F_2N_6O_4S \cdot {}^1/_3H_2O$	D	18.5
31	102–104 dec	$C_{30}H_{34}F_2N_6O_2S$	D	37.5
32	197–202 dec	$C_{25}H_{26}F_2N_6O_4S_2 \cdot {}^3/_4H_2O$	D	75
33	90–93	$C_{25}H_{26}F_2N_6S$	A	41
34	140–170	$C_{25}H_{27}F_2N_7O$	B	70
35	100–115	$C_{26}H_{29}F_2N_7O \cdot {}^1/_2H_2O$	B	86.5
36	105–110	$C_{27}H_{31}F_2N_7O$	B	87
37	110–155	$C_{27}H_{29}F_2N_7O_2 \cdot {}^7/_4H_2O$	D	66
38	120–130	$C_{25}H_{28}N_6O_2$	C	79
39	147–155	$C_{25}H_{26}F_2N_6O_2$	C	53
40	100–110	$C_{30}H_{35}N_6O_3 \cdot {}^1/_4H_2O$	C	10.2
41	120–125	$C_{30}H_{34}F_2N_6O_3 \cdot {}^1/_4H_2O$	C	20.4

Experimental Section

Compounds listed in Tables I, II and III were prepared using procedures exemplified below. All compounds were homogeneous by TLC analysis and gave satisfactory elemental, IR, and ^1H NMR (Bruker AC300) analysis. The majority of the compounds were isolated as amorphous solids with poorly defined handling characteristics and variable amounts of hydration.

4-[Bis(4-fluorophenyl)methyl]-α-[(9*H*-purin-6-ylthio)methyl]-1-piperazineethanol (17). Method A. To epichlorohydrin (3.5 mL, 0.05 mol) in EtOH (12 mL) at 0 °C with anhydrous $NaHCO_3$ (4.2 g, 0.05 mol) was added [bis(4-fluorophenyl)methyl]-piperazine (14.4 g, 0.05 mol) in EtOH (200 mL) dropwise over 45 min under N_2. The ice bath was removed, and the mixture was allowed to warm to room temperature. After 18 h, the $NaHCO_3$ was removed by filtration and the filtrate was evaporated to give the crude product (21.3 g). Silica gel flash chromatography using 2.0% $MeOH/CH_2Cl_2$ gave pure 1-(1-chloro-2-hydroxy-3-propanyl)-4-[bis(4-fluorophenyl)methyl]piperazine monohydrate (7, 10.05 g, 52.9%) as an amber oil: DCI/MS (M + 1) 381; 300-MHz ^1H NMR ($CDCl_3$) δ 7.3 (m, 4 H), 6.95 (m, 4 H), 4.2 (s, 1 H), 3.95 (m, 1 H), 3.55 (m, 2 H), 2.7 (m, 2 H), 2.5 (m, 4 H), 2.4 (m, 4 H). Anal. ($C_{20}H_{23}ClF_2N_2O \cdot H_2O$) C, H, N.

To DMF (7 mL) was added 6-mercaptopurine (5 mmol, 0.85 g) in portions, and the solution was stirred at room temperature under N_2 for 5 min. Et_3N (5 mmol, 0.69 mL) was added dropwise and, after 5 min, 7 (5 mmol, 1.9 g) in DMF (5 mL) was then added dropwise over 5 min at room temperature under N_2. After 22 h, the solution was filtered and the filtrate was evaporated (1.0 mmHg, 50 °C). Silica gel flash chromatography of the crude product (2.34 g) using 10% $MeOH/CH_2Cl_2$ gave pure 17: yield

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0.630 g (25.4%); mp 115–116 °C dec; DCI/MS (M + 1) 497; 300-MHz ^{1}H NMR ($CDCl_3$) δ 8.6 (s, 1 H), 8.25 (s, 1 H), 7.35 (m, 4 H), 6.95 (m, 4 H), 4.2 (s, 1 H), 4.15 (m, 1 H), 3.45 and 3.6 (m, 2 H), 2.65 (m, 2 H), 2.6 (m, 4 H), 2.4 (m, 4 H). Anal. ($C_{25}H_{26}F_2N_6OS \cdot {}^1/_2H_2O$) C, H, N.

4-[Bis(4-fluorophenyl)methyl]-α-[(9*H*-purin-6-ylthio)methyl]-1-piperazineethanol Acetate (28). Method D. To 17 (1.0 g, 0.002 mol) in CH_2Cl_2 (7 mL) was added acetic anhydride (0.2 mL, 0.002 mol) in Et_3N (0.2 mL, 0.002 mol) dropwise over 5 min at room temperature under N_2. After 70 h, CH_2Cl_2 (50 mL) was added and the solution was extracted with saturated $NaHCO_3$ (2×), H_2O (1×), and saturated brine (1×). The organic layer was dried over Na_2SO_4 and concentrated to give pure **28** which was dried in vacuo at 40 °C (0.7 g, 64.8%): mp 105–109 °C dec; DCI/MS (M + 1) 539; 300-MHz ^{1}H NMR ($CDCl_3$) δ 8.7 (s, 1 H), 8.2 (s, 1 H), 7.3 (m, 4 H), 6.95 (m, 4 H), 5.3 (m, 1 H), 4.2 (s, 1 H), 3.4 and 4.0 (m, 2 H), 2.65 (m, 2 H), 2.6 (m, 4 H), 2.4 (m, 4 H), 2.0 (s, 3 H). Anal. ($C_{27}H_{28}F_2N_6O_2S \cdot {}^3/_4H_2O$) C, H, N.

1-[1-(2,3-Epoxypropyl)]-4-[bis(4-fluorophenyl)methyl]-piperazine (8). To an ice-cold mixture of epibromohydrin (9.1 mL, 110 mmol) and anhydrous potassium carbonate (15.2 g, 110 mmol) in acetonitrile (150 mL) was added a solution of 4,4′-difluorobenzhydrylpiperazine (28.83 g, 100 mmol) in acetonitrile (250 mL) over a period of 40 min. The mixture was stirred at room temperature for 4 days and filtered, and the solids were washed with CH_2Cl_2. The combined filtrates were concentrated to dryness to give an oil which was purified by flash chromatography on silica gel column using 2–3% MeOH/CH_2Cl_2 to give **8** as a glass: yield 23.98 g (69.6%); 300-MHz ^{1}H NMR ($CDCl_3$) δ 7.4–6.9 (m, 8 H), 4.22 (s, 1 H), 3.09 (br m, 1 H), 2.8–2.25 (m, 12 H); MS 345 (MH^+). Anal. ($C_{20}H_{22}F_2N_2O$) C, H, N.

4-[Bis(4-fluorophenyl)methyl]-α-[(9*H*-purin-6-ylamino)methyl]-1-piperazineethanol (34). Method B. A solution of 1-[1-(2,3-epoxypropyl)]-4-[bis(4-fluorophenyl)methyl]piperazine (**8**, 8.9 g, 25.8 mmol) and liquid ammonia (20 mL) in EtOH (40 mL) was heated in a teflon reaction vessel in a bomb at 110 °C for 28 h. The solution was evaporated to dryness to give ≃10 g of a glass which was purified using flash chromatography on silica gel and increasing proportions of MeOH in CH_2Cl_2 to give 1-amino-3-[4-[bis(4-fluorophenyl)methyl]-1-piperazinyl]-2-propanol (**9**) as an oil which solidified upon vacuum drying: yield 5.7 g (61%); mp 45–47 °C; IR (neat) 3350 cm^{-1}; 300-MHz ^{1}H NMR ($CDCl_3$) δ 7.4–6.9 (m, 8 H), 4.21 (s, 1 H), 3.68 (br m, 1 H), 2.8–2.2 (m, 12 H); MS 362 (MH^+). Anal. ($C_{20}H_{25}F_2N_3O$) C, H, N.

A mixture of 6-chloropurine (0.728 g, 4.7 mmol), **9** (1.73 g, 4.77 mmol), and Et_3N (1.36 mL, 9.5 mmol) in MeOH (20 mL) was heated to reflux for 7 days, and the solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ and extracted with aqueous sodium bicarbonate (2 × 100 mL); the organic phase was dried over sodium sulfate and evaporated to give a solid which was purified by flash chromatography on silica gel with 5% MeOH in $CHCl_3$. The product **34**, a colorless solid, was triturated with ether: yield 1.5 g (70%); mp 140–170 °C; IR (KBr) 3000 cm^{-1}; 300-MHz ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1 H), 8.11 (s, 1 H), 7.5–7.0 (m, 8 H), 4.90 (br s, 1 H), 4.34 (s, 1 H), 3.87 (br s, 1 H), 3.7–2.0 (m, 12 H); MS 480 (MH^+). Anal. ($C_{25}H_{27}F_2N_7O$) C, H, N.

6-[3-[4-[Bis(4-fluorophenyl)methyl]piperazin-1-yl]-2-hydroxypropoxy]-9-(tetrahydropyran-2-yl)purine (41). Method C. To a stirred and warmed solution of 4,4′-difluorobenzhydrylpiperazine (6.343 g, 22 mmol) in MeOH (75 mL) was slowly added a solution of glycidol (1.63 g, 22 mmol) in MeOH (25 mL) under nitrogen. The mixture was stirred at room temperature for 18 h, refluxed for 2 h, and evaporated to dryness. The syrupy residue was dissolved in CH_2Cl_2 (4 × 100 mL), evaporated to dryness, and purified by chromatography on a silica gel column (medium pressure) eluting with 2–5% MeOH/CH_2Cl_2 to give 3-[4-[bis(4-fluorophenyl)methyl]-1-piperazinyl]-1,2-propanediol (**10**) as a colorless syrup which upon prolonged evacuation formed a hygroscopic foam (5.84 g, 73%): mp 40–50 °C; IR (KBr) cm^{-1} 3625, 3575; 300-MHz ^{1}H NMR ($CDCl_3$) δ 6.9–7.4 (m, 8 H, Ar-H), 4.21 [s, 1 H, C*H*($C_6H_4F)_2$], 3.80 (m, 1 H, HCOH), 3.73 and 3.49 (each m, each 1 H, $HOCH_2$), 3.8–2.3 (m, 10 H, NCH_2); MS (DCI) 363 (MH^+). Anal. ($C_{20}H_{24}F_2N_2O_2 \cdot {}^1/_4H_2O$) C, H, N.

To a warmed (60 °C) slurry of 6-chloropurine (20 g, 0.1294 mol) and *p*-toluenesulfonic acid monohydrate (0.35 g) was added dihydropyran (13.4 mL, 0.172 mol) over a period of 30 min with stirring. After an additional 30 min of heating, the mixture was allowed to cool to room temperature for 1 h. Concentrated ammonium hydroxide (12 mL) was added and stirring was continued for 5 min. The solution was washed with water (4 × 70 mL) and the organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a syrup (~29 g) which slowly crystallized upon standing. Extraction with boiling hexane gave 6-chloro-9-(tetrahydropyran-2-yl)purine (**11**) as a solid: yield 24.36 g in two crops, 78%; mp 70–71 °C (lit.[34] 69–71°).

To a stirred mixture of **11** (2.387 g, 10 mmol) in toluene (40 mL), powdered KOH (1.22 g, 21.4 mmol), and 18-crown-6 (0.132 g, 0.5 mmol) was added dropwise a solution of **10** (3.8 g, 10.25 mmol) in toluene (80 mL) over a period of 5 min. After 3 h of stirring at room temperature the reaction mixture was treated with ice-cold water (70 mL). The organic layer was separated and washed with ice-water (4 × 70 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo to yield a foam (~6 g) which was eluted through a silica gel column at medium pressure using increasing proportions of MeOH in CH_2Cl_2 as eluant. The middle fraction was **41** (1.26 g, colorless foam): mp 120–125 °C; IR (KBr) cm^{-1} 3400, 1602, 1578, 1506, 1341, 1224; 300-MHz ^{1}H NMR ($CDCl_3$) δ 8.52 (s, 1 H, 2 or 8-H), 8.14 (s, 1 H, 2 or 8-H), 6.9–7.4 (m, 8 H, Ar-H), 5.76 (d, 1 H, NCHOC), 4.63 (m, 2 H, OCH_2), 4.21 [s, 1 H, C*H*($C_6H_4F)_2$], 4.20 (m), 3.79 (m), 1.5–2.9 (m); MS 565 (MH^+). Anal. ($C_{30}H_{34}F_2N_6O_3 \cdot {}^1/_4H_2O$) C, H, N.

4-[Bis(4-fluorophenyl)methyl]-α-[(9*H*-purin-6-yloxy)methyl]-1-piperazineethanol (39). A sample of **41** (0.84 g, 1.488 mmol) was dissolved in glacial acetic acid (50 mL) and diluted with water (30 mL). The solution was stirred at room temperature for 18 h. The mixture was evaporated to dryness, and the residue was treated with saturated aqueous sodium bicarbonate. The precipitated solid was collected by filtration and washed with water and ether. The ether insoluble portion was reextracted with boiling ether/CH_2Cl_2 mixture and the insoluble solid isolated to give **39** (380 mg, 53%): mp 147–155 °C; IR (KBr) cm^{-1} 3327, 3365, 1605, 1506; 300-MHz ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H, 2 or 8-H), 8.37 (brs, 1 H, 2 or 8-H), 7.05–7.50 (m, 8 H, Ar-H), 4.54 (m, 1 H, OC*H*H), 4.38 (m, 1 H, OCH*H*); MS (DCI) 481 (MH^+). Anal. ($C_{25}H_{26}F_2N_6O_2$) C, H, N.

In Vitro Cardiotonic Activity: Ferret Papillary Muscle. Male ferrets (700–1200 g) were deeply anesthetized with xylazine (2 mg/kg) and ketamine hydrochloride (50 mg/kg). Following a midsternal thoracotomy, the heart was quickly excised and placed in oxygenated Tyrode's solution of the following composition in millimoles: NaCl, 130.0; KCl, 4.0; $MgCl_2$, 1.0; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; $CaCl_2$, 2.0; dextrose, 11.0; pH 7.3. The tendons of papillary muscles from the right ventricle were tied securely with a fine silk suture and then cut proximal to the tie. The distal connection of the muscle in the ventricular wall was cut and secured to a holder/stimulator. The tied tendon was attached to a force transducer, and the muscle was stretched to approximately 0.4 g of tension. The papillary muscles were placed in a tissue bath containing 55 mL of Tyrode's solution which was gassed continuously with 5% CO_2–95% O_2. Temperature was maintained at 37 °C. The papillary muscles were stimulated at a frequency of 1 Hz using platinum bipolar electrodes at a stimulus duration of 3 ms and a current intensity of 2 times threshold. The preparations were allowed to equilibrate for 2 h during which time the bath of Tyrode's solution was exchanged three times before control measurements were obtained. Timolol (1 × 10^{-7} M, Sigma) was present to prevent variations in tension that result from spontaneous or electrical stimulation-induced release of catecholamines. Stable measurements were obtained during two successive 15-min control periods prior to the addition of any test agents and then 15 min after addition of each concentration. Tension measurements were made directly from strip chart recordings (Gould, TA4000) and from an automated data acquisition system (MI^2).

Test compounds were dissolved in 100% dimethyl sulfoxide (DMSO) at a stock concentration of 1 × 10^{-2} M. Dilutions were made directly in Tyrode's solution to achieve the final concentration. Test compounds were evaluated by serially increasing concentrations.

Data in the table are values estimated from graphing the mean

data of $n \geq 3$ observations for each compound. The effective concentration producing a 50% increase in tension (EC_{50}) is calculated relative to the maximum increase produced by a given compound at any concentration. In other words, the EC_{50} is the concentration at which 50% of the maximum increase in tension occurred and is thus a measure of potency. The maximum percent change indicates the absolute maximum increase in tension that was produced and thus serves as an index of activity. The concentration at which that maximum increase occurred is also reported. A compound is listed as inactive (IA) if less than a 30% increase in tension occurred up to the highest concentration tested (10 μM); beyond this concentration most compounds were insoluble.

In Vivo Cardiotonic Activity: Anesthetized Mongrel Dogs. Adult mongrel dogs were anesthetized with sodium pentobarbital (45 mg/kg, ip) and artifically respired.[12] Mean arterial pressure (MAP) was recorded from a cannulated femoral artery, and drugs were infused into a cannulated femoral vein. The arterial pressure pulse was used to trigger a cardiotachometer for determination of heart rate (HR). Left ventricular pressure was measured with a Millar catheter and $+dP/dt_{max}$ was derived. A right thoracotomy was performed, and myocardial contractile force (CF) was measured with a Walton Brodie strain gauge sutured to the right ventricle. The ventricular muscle was stretched to produce a base line tension of 100 g. A standard dose of dopamine (10–15 μg/kg per min for 3 min) was administered to ensure myocardial responsiveness to inotropic stimulation.

Test compounds were solublized in a small volume of DMF diluted to a final concentration of 10% in physiological saline. Alternatively, where possible, a soluble hydrochloride salt was prepared by addition of 0.1 N HCl diluted in physiological saline. Vehicles were tested in appropriate volumes and found to exert less than a 5% effect on contractile force. Compounds were administered by infusion at rates of 0.58–2.2 mL/min in three to four stepwise increasing doses. Each dose was infused over 5 min immediately after the effect of the previous dose peaked. MAP, HR, dP/dt_{max}, and CF responses were continuously monitored on a Beckman or Gould recorder and expressed as a percent change from predrug control values vs the cumulative dose of drug administered. For these studies, an n of one to five test animals were used.

Quantitation of the inotropic potency was obtained by calculation of the contractile force (CF) ED_{50}. This was defined as the dose of compound that produced a 50% increase above base line in myocardial contractile force. The value was obtained from three to four point dose–response curves using either graphical estimation ($n < 3$) or linear regression analysis ($n \geq 3$). Data from this evaluation are shown in Tables I and II.

In Vivo Cardiotonic Activity: Instrumented Conscious Mongrel Dogs. Mongrel dogs were anesthetized through the cephalic vein with 5% surital. Using aseptic technique, heparin-filled Tygon catheters were inserted into a femoral artery and vein and exteriorized at the neck above the shoulder blades. A left thoracotomy was performed at the fifth intercostal space, and a calibrated Konigsberg pressure transducer was inserted into the left ventricle through an incision in the apex of the heart. Left ventricular pressure was monitored, and its first derivative (LV $+dP/dt_{max}$) was used as an index of contractility. Animals were placed on a regimen of antibiotics and given a week to recover.

The dogs were trained to lie quietly in an isolated cage. Base line values for MAP, HR, and $+dP/dt_{max}$ were obtained immediately prior to drug administration. For oral evaluation, drugs were administered through a gavage tube passed into the esophagus. Compound was prepared in 0.5% methyl cellulose immediately prior to use in a 10-mL volume. Hemodynamic parameters were monitored for 8 h after dosing. Data are expressed as a percent change from the predrug control. Data are reported as the percent change from base line in Figure 1.

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