Cationic Antiprotozoal Drugs. Trypanocidal Activity of 2-(4′-Formylphenyl)imidazo[1,2-*a*]pyridinium Guanylhydrazones and Related Derivatives of Quaternary Heteroaromatic Compounds

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A series of quaternary 2-phenylimidazo[1,2-*a*]pyridinum salts has been prepared and evaluated for antiparasitic activity. Primary attention was focused on derivatives with amido, substituted hydrazone, and heterocyclic functionality at the para position of the phenyl substituent. Guanylhydrazones and N-substituted guanylhydrazones of the 4′-formyl-substituted compounds are very active against the blood state *Trypanosoma rhodesiense* in mice by subcutaneous or oral administration. The most potent compounds attain 100% survival for 30 days at doses of <1.0 mg/kg (sc) and >5.0 mg/kg (po). Weaker activity is noted for certain other 4′-substituents such as carboxamidines and carboxamide oximes. Considerable variation in structure, including replacing of the imidazo[1,2-*a*]pyridinium ring by other cationic heterocyclic rings and insertion of linking groups between the heterocyclic ring and phenyl group, can be done, and a high level of activity is maintained. Relationships between these structural changes and biological activity are discussed.

Trypanosomiasis is a parasitic condition caused by blood protozoa of the order kinetoplastida.[1] The disease exerts a major impact on the practice of animal husbandry in Africa.[2] Human pathology due to various species of *Trypanosoma* is still present in Africa (sleeping sickness)[3] and in Central and South America (Chagas' disease).[4] We have synthesized and evaluated the trypanocidal activity of a series of cationic heteroaromatic compounds carrying at least one additional functional group. Many of these compounds are highly active in vivo in mice against the blood state of the *T. rhodesiense* strain of African trypanosome. No significant activity has been found against *Trypanosoma cruzi*, the causative organism of Chagas' disease. We report here the range of structural types which have been investigated and the level of activity associated with various structural types. The main emphasis has been on substituted imidazo[1,2-*a*]pyridines but imidazoles, thiazoles, pyridines, benzimidazoles, and imidazo[2,1-*b*]-thiazoles have also been examined to some extent.

Chemistry

We have already described the synthesis of the nitro, acylamido, cyano, and formyl derivatives which served as the precursors for the majority of the compounds tested.[5] The Experimental Section describes general procedures for preparation of the final target compounds. The target compounds were *N*-alkyl quaternary salts and were purified by crystallization. The compounds were characterized by NMR and IR spectra and by elemental analyses. The structures are specified by a numerical designation of the heterocyclic ring followed by a second number specifying the functional group. An alphabetical modifier of the first number indicates the substitution on the heteroaromatic ring. These descriptors are defined in Charts I and II.

Biological Evaluation

All of the compounds were tested in vivo by subcutaneous (sc) injection with groups of five mice at each dose.[6] Activity was recorded as toxicity, as a relative increase in life span, or as the fraction of animals cured (survival >30 days). In order to compare the relative activity of the compounds, it was desirable to have a numerical indicator of activity. To indicate *potency*, the lowest dose effecting 30-day cures of at least 50% of the test animals (CD_{50}) was adopted. However, this indicator is misleading since some of the very potent compounds are also toxic at relatively low doses. We therefore chose to use as the primary indicator of activity the fraction of all animals cured in the dose range 0.4–424 mg/kg. This choice weights compounds with low toxicity favorably, since these compounds have broader curative ranges. The 0.4 mg/kg cutoff is arbitrary but encompasses the curative dose for all but a few of the most potent compounds. Compounds were scored as 0 at all doses lower than the minimal curative dose. The data were normalized at each dose level so as to uniformly weight the results for each dose. For most compounds at least five mice were tested at 11 dose rates of $2^n \times 0.42$ mg/kg with $n = 1$–10. This numerical indicator is called the "indicator of curative effectiveness" (i.c.e.). Table I gives the complete data for two illustrative compounds. Table II gives a summary of biological activity for the most active of the compounds.

Results for all compounds tested are given in the supplemental material. The data in the supplemental material give the toxic dose range as the doses at which one or more animals died prematurely as an apparent toxic reaction to the compound. The 100% cure range is that in which all treated animals survived for 30 days after treatment. The partial cure range is the dose range in which some, but not all, of the test animals were 30-day survivors. Doses which were partially curative typically showed activity measured as an increase in length of survival after challenge, but these results are not reported.

Many compounds were also tested by oral administration (po). The results are reported as for the sc mode of administration. Representative compounds were also assayed in vitro by measurement of the inhibition of leucine and thymine uptake in cell cultures of *T. rhodesiense*.[7] These results are reported in Table II as concentrations

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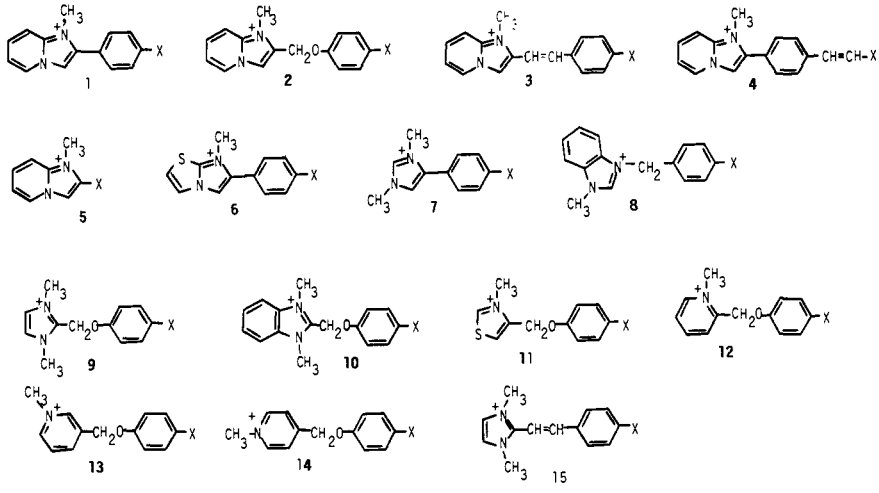
(1) Brown, J. R. *Crit. Rep. Appl. Chem.* **1987**, *21*, 72. Gutteridge, W. E. *Brit. Med. Bull.* **1985**, *41*, 162.
(2) Ormerod, W. E. *Pharmac. Ther.* **1979**, *6*, 1.
(3) Williamson, J. *Trop. Dis. Bull.* **1976**, *73*, 531.
(4) Brener, Z. *Pharmac. Ther.* **1979**, *7*, 71.
(5) Sundberg, R. J.; Dahlhausen, D. J.; Manikumar, G.; Mavunkel, B.; Biswas, A.; Srinivasan, V.; King, F., Jr.; Waid, P. *J. Heterocycl. Chem.* 1988, *25*, 129.
(6) Rane, L.; Rane, D. S.; Kinnamon, K. E. *Am. J. Trop. Med. Hyg.* **1976**, *25*, 395.
(7) Jennings, F. W.; Urquhart, G. M. *Z. Parasitenkd* **1983**, *69*, 577.

0022-2623/90/1833-0298$02.50/0

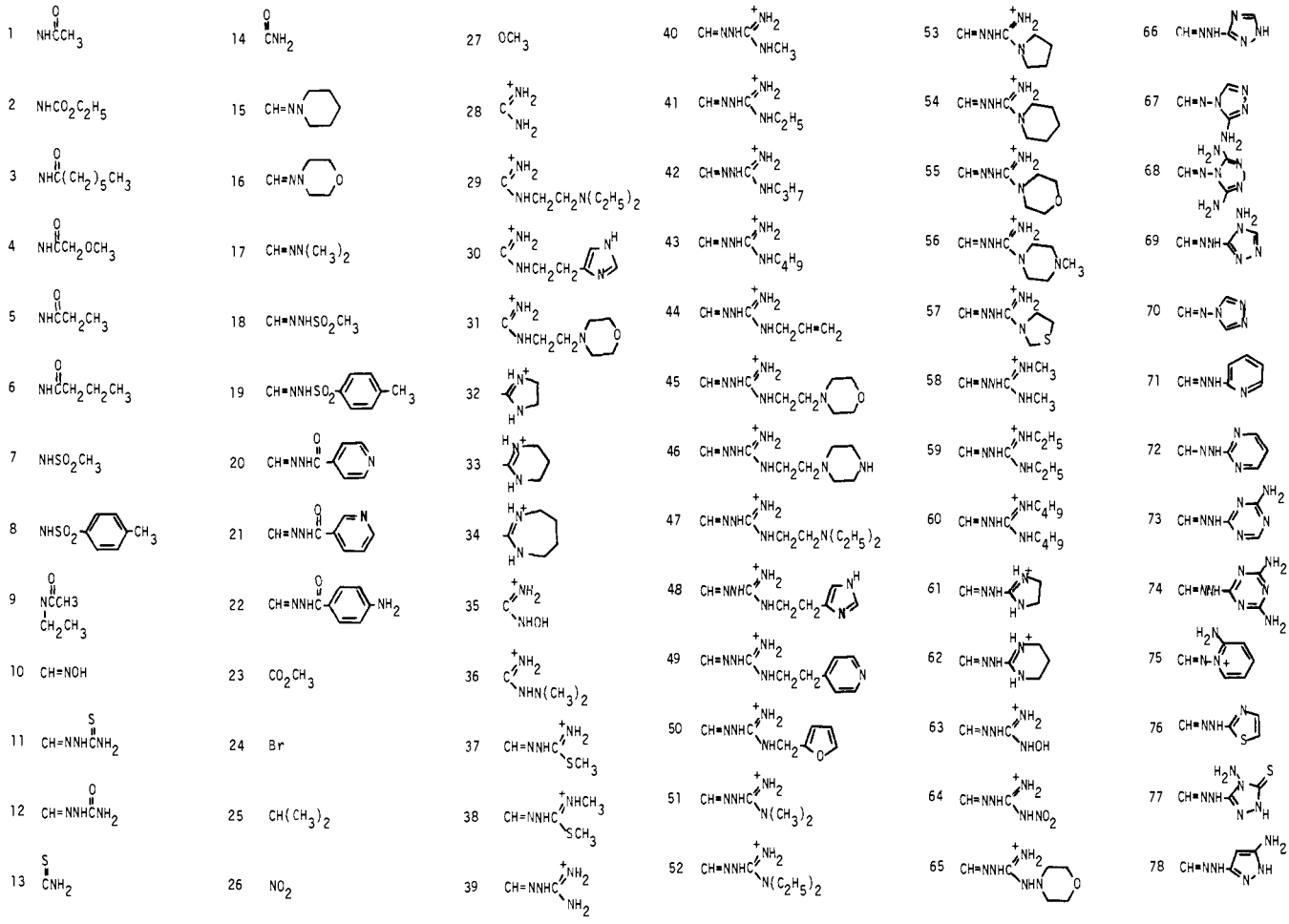
Chart I. Heteroaromatic Rings



Ring Modifiers for Structures 1-5

a	N-CH_3	e	6-Cl	f	7-CH_3	m	6-C_2H_5S	q	6-*n*-C_4H_9S	u	3-NO_2
b	N-$CH_2CH{=}CH_2$	f	6-I	j	6-CF_3	n	6-C_2H_5SO	r	6-C_6H_5S	v	3-Br
c	5,6-benzo	g	5-CH_3	k	6-CO_2CH_3	o	6-$C_2H_5SO_2$	s	6-CH_3O	w	3-CH_3
d	7,8-benzo	h	6-CH_3	l	7-CO_2CH_3	p	6-*n*-C_3H_7S	t	6-CH_3CONH	x	5,6,7,8-tetrahydro

Chart II. Functional Groups



which resulted in 50% inhibition of incorporation.

Structure–Activity Relationships for Subcutaneous Administration

A. Effect of Aryl Functional Group Substituent. The broad trends of structural effects on activity can best be organized in terms of the nature of the functional group on the phenyl substituent. The compounds are grouped into eight structural types according to the nature of this group (see Chart II). The following classifications were used: group 1, amido groups, 1–9; group 2, other neutral polar groups, **10–22**; group 3, lipophilic groups, **23–27**;

Table I. Curative Activity of Compounds 1a/39 and 1h/39

	dose, mg/kg											
	0.42	0.83	1.67	3.3	6.6	13	26	53	106	212	424	i.c.e.
						1a/39						
subcutaneous												
30-day survivors												
(toxic)	2/5	27/30	10/10	24/25	5/5	10/10	11/15 (4T)	(5T)	(10T)	(5T)	(10T)	0.54
fraction cured	0.4	0.9	1.0	0.96	1.0	1.0	0.73	0	0	0	0	
oral												
30-day survivors												
(toxic)	-	-	-	-	-	2/5	10/10	5/5	5/5	5/5	10/10	
fraction cured	0	0	0	0	0	0.4	1.0	1.0	1.0	1.0	1.0	0.49
						1h/39						
subcutaneous												
30-day survivors												
(toxic)	8/10	20/20	19/20	10/10	10/10	20/20	29/30	9/10	16/20 (1T)	5/10 (5T)	4/20 (16T)	
fraction cured	0.8	1.0	0.95	1.0	1.0	1.0	0.97	0.9	0.8	0.5	0.2	0.83
oral												
30-day survivors												
(toxic)	0	0	0	1/5	2/5	10/10	15/15	5/5	10/10	5/5	10/10	
fraction cured	0	0	0	0.2	0.4	1.0	1.0	1.0	1.0	1.0	1.0	0.60

group 4, amidines, amide oximes, and amidrazones, **28–36**; group 5, methylthio imidoyl hydrazones, **37–38**; group 6, guanylhydrazones and *N*-alkylguanylhydrazones, **39–62**; group 7, other substituted guanylhydrazones, **63–65**; and group 8, heterocyclic hydrazones, **66–78**.

The initial activity was detected for 2-(4′-acetamidophenyl)-1-methylimidazo[1,2-*a*]pyridinium iodide, **1a/1**. Subsequent exploration of the effect of variation of heterocyclic ring substituents or the acyl portion of the amide group resulted in no improvement in the level of activity. The maximum i.c.e. value for this group is 0.20 for compound **1b/1**.

More extensive exploration of the 4′-phenyl substituent showed that this group had a marked influence on activity. Significant levels of activity were seen for thiosemicarbazone (**1a/11**), (*p*-tolylsulfonyl)hydrazone (**1a/19**) and (*p*-aminobenzoyl)hydrazone (**1a/22**) derivatives of 1-methyl-2-(4′-formylphenyl)imidazo[1,2-*a*]pyridinium salts. The i.c.e. values for these compounds range from 0.17 to 0.57. Analogues with lipophilic substituents at C-4′ such as the carbomethoxy, bromo, isopropyl, methoxy, and nitro derivatives were devoid of activity (functional groups **23–27**). Several *N*,*N*-dialkylhydrazones were also inactive (functional groups **15–18**).

Since these results indicated activity was associated with the more polar nitrogen-containing functional groups, we examined amidines, amide oximes, and amidrazones, all of which were prepared from quaternary nitrile intermediates. A fairly consistent indication of activity was observed for amidines and amide oximes (functional groups **28** and **35**). Several of the compounds (**1a/28**, **1a/35**, and **6/28**) exhibited 100% curative activity, but only over rather narrow dose ranges, giving i.c.e. values of 0.21–0.29. Attachment of an additional basic group through an alkyl substituent at the amidine nitrogen had no beneficial effect (functional groups **29–31**). The cyclic amidine functional groups **32**, **33**, and **34** were completely inactive. No activity was seen for the amidrazones (Functional group **36**).

Methylthio imidoyl hydrazones (functional groups **37** and **38**) exhibited enhanced i.c.e. values over the amidines, primarily because of reduced toxicity. The imidazo[1,2-*a*]pyridinium derivative **1a/37** had an i.c.e. of 0.55 and was partially curative over the dose range 3.3–106 mg/kg. Several other methylthio imidoyl hydrazones, e.g. **1h/37**, **1i/38**, **1s/37**, and **2a/37** exhibited similar activity levels.

The most potent compounds found were guanylhydrazones and *N*-alkylguanylhydrazones. In general, the guanylhydrazones tended to be toxic above 26 mg/kg but 100% cures were observed down to about 1 mg/kg. This curative range gives rise to i.c.e. values of about 0.5. For 1-methyl-2-(4′-formylphenyl)-imidazo[1,2-*a*]pyridinium guanylhydrazone **1a/39** the i.c.e. was 0.54.

A number of N-alkylated guanylhydrazones were also prepared. These typically were very potent, but most were also more toxic than the unsubstituted guanylhydrazones. As a result, they exhibit lower i.c.e. values. Representative compounds are the *N*-ethyl, *N*-propyl, *N*,*N*-tetramethylene derivatives (**1a/41**, **1a/42**, **1a/53**) and the cyclic (2-imidazolinyl- and 3,4,5,6-tetrahydropyrimid-2-yl-) variants (**1a/61**, **1a/62**). The CD_{50} values were consistently <1.0 mg/kg but toxic deaths were frequently recorded at doses of 6.6–13 mg/kg.

Group 7 includes three other types of substituted guanylhydrazones, the *N*-hydroxy, *N*-nitro, and *N*-morpholino systems (functional groups **63–65**). The (*N*-hydroxyguanyl)hydrazones are essentially as active as the parent guanylhydrazones. The i.c.e. value for **1h/63** of 0.85 represents curative activity from 0.4 to 53 mg/kg. The (*N*-nitroguanyl)hydrazones (functional group **64**) were active but insufficient data were available for computing i.c.e. values. The *N*-morpholino compounds (functional group **65**) are quite toxic, leading to reduced i.c.e. values, but compound **1a/65** exhibited interesting po activity (vide infra).

A number of heterocyclic hydrazones were also investigated (group 8). For the most part they were considerably less active than the guanylhydrazones. The highest i.c.e. values were recorded for (4,6-diamino-1,3,5-triazin-2-yl)hydrazones, functional group **74**. These compounds were characterized by low toxicity and a broad curative range (e.g. **1a/74** (i.c.e. = 0.47) and **1m/74** (i.c.e. = 0.71)), but achievement of 100% cures required high doses.

B. Effect of Ring Substitution. The effect of ring substitution, particularly on guanylhydrazones and (*N*-alkylguanyl)hydrazones was also explored. Variations of the ring substituent led to improved curative ranges for the following substituents: 6-CH_3 (**1h/39**, i.c.e. = 0.83), 6-OCH_3 (**1s/39**, 0.87), 6-EtS (**1m/39**, 0.93), 6-CH_3CONH, (**1t/39**, 0.79), 6-PrS (**1p/39**, 0.92), 6-BuS (**1q/39**, 0.83). The improvement in the activity indicator reflects primarily reduced toxicity rather than enhanced potency. Compounds with this level of curative activity exhibit lethal toxicity only above 100 mg/kg and some exhibited curative activity as low as 0.1 mg/kg. The optimum i.c.e. values of the 6-EtS and 6-PrS derivatives represent curative activity in the dose range 0.1–53 mg/kg and 0.2–212 mg/kg,

respectively. The most extensively evaluated of the compounds to date is the 6-methyl derivative **1h/39** for which the range of curative activity spans 0.06–53 mg/kg. Complete data for this compound is included in Table I.

The reduced toxicity associated with ring substitution in the parent guanylhydrazone is also observed in the N-alkylated guanylhydrazones. The comparisons for the guanylhydrazone group (**39**), the (*N,N*-tetramethyleneguanyl)hydrazone (**53**), 2-imidazolinylhydrazone (**61**), 2-tetrahydropyrimidylhydrazone (**62**), and (*N*-hydroxyguanyl)hydrazone (**63**) functional groups are made in part A of Table III. The favorable effect of the 6-methyl 6-propylthio (**1p**) and 6-butylthio (**1q**) groups also have a favorable effect in the systems examined.

C. Effect of Changing the Separation of the Functional Group from the Heteroaromatic Ring. We investigated the influence of the distance between the functional group and the cationic heterocycle using ring structures **2–5**. In **2** a $-CH_2O-$ group links the imidazo[1,2-*a*]pyridinium and functionalized phenyl ring. In **3** a —CH=CH— unit is inserted at the same point. For **4**, the —CH=CH— group is inserted between the phenyl ring and the guanylhydrazone function. In **5**, the phenyl group is removed entirely, placing the guanylhydrazone group directly on the imidazo[1,2-*a*]pyridine ring. Section B of Table III shows the effect on the i.c.e. values for functional group **39**, **53**, and **61–63**, which represent the most consistently potent guanylhydrazone functions. The data shows that good activity is maintained for heterocyclic structures **2–4** but not for **5**. The vinyl-linked ring system **3** appears to be the best on this basis of comparison. The vinyl-linked series of compounds is just as potent, but less toxic, than structural series **1** and **2**. A limited number of ring-substituted derivatives of the **2** and **3** structural types were investigated and this data is given in section C of Table III. Methyl groups at C-5, C-6, or C-7 appear to reduce toxicity somewhat in the ether-linked series. Significant improvement is noted for **2h/39**, **2h/63**, and **2g/53**. In the vinyl-linked series, the 5-methyl derivatives **3g/39**, **3g/53**, **3g/61**, and **3g/62** all have i.c.e. > 0.8.

D. Other Heteroaromatic Cations. The replacement of the imidazo[1,2-*a*]pyridine ring by the isosteric imidazo[2,1-*b*]thiazole ring (**6**) maintains an approximately equivalent level of activity. The other heterocyclic ring substitutions are structurally comparable to the ether-linked system **2a**. The 2-imidazole (**9**) and 2-benzimidazole analogues (**10**) maintain a similar level of activity. The 4-thiazole analogues (**11**) are weakly active. For the pyridines, the 2-substituted systems (**12**) are quite toxic, but the 3-substituted system (**13**) is comparable in activity to the pyrido[1,2-*a*]imidazole series. Significant activity is also seen for the 4-substituted pyridine and for a vinyl-linked 1,3-dimethylimidazolium system **15**. In fact, the most potent of all the compounds tested was the 2-(styryl)imidazolium salt **15/53**, which cured 6/10 mice at 0.06 mg/kg. These results are summarized in section D of Table III.

Results of Other Methods of Evaluation of Activity

The activity by oral administration is included in Table II. The i.c.e. value is defined for the same dose range as for sc administration. Many of the compounds are able to effect partial or 100% cures. Acute toxicity by po administration is low. Only a few toxic deaths were recorded and only at the highest doses used. The potency by po administration is reduced relative to sc administration. Nevertheless, some of the compounds show 50% cures at doses as low as 6.6 mg/kg. The most effective compounds as measured by the i.c.e. (i.c.e. > 0.60) are **1a/51**, **1a/53**, **1a/62**, **1w/53**, **1x/61**, **2h/39**, **4a/39**, and **6/53**. These compounds typically effect 100% cures at doses of 12–26 mg/kg and above. The (*N*-morpholinoguanyl)hydrazone **1a/65** was very potent in one trial (complete cures at 3.3 mg/kg) but less so in a later trial.

Compounds **1a/61**, **1a/53**, **1h/39**, **1i/62**, **2h/63**, and **3g/53** were also screened in a *Trypanosoma brucei* model in mice with chronic central nervous system (CNS) involvement.[7] In this test the compounds were administered as either a single dose or four successive doses at 20 mg/kg. (10 mg/kg for **1a/53**). The animals showed a short period of aparasitema but relapsed after 10–40 days. It thus appears that the compounds are ineffective in clearing parasites in the central nervous system.

Data were obtained for representative compounds using an in vitro assay based on the uptake of radioactive thymidine and leucine by *T. rhodesiense* parasites in culture.[8] This data is included in Table II. A number of the compounds tested showed I_{50} values for thymidine inhibition at <10 μg/mL. The inhibition of thymidine uptake in the in vitro screen is very effective in recognizing the highly active guanylhydrazones (functional groups **39–63**). All such compounds with i.c.e. > 0.2 have an I_{50} < 6 μg/mL. Leucine uptake is only weakly inhibited by several active compounds. These include **1a/45** (i.c.e. = 0.45, I_{50} = 11.7), **1h/37** (i.c.e. = 0.38, I_{50} > 10), **1h/74** (i.c.e. = 0.71, I_{50} > 10), **1m/74** (i.c.e. = 0.71, I_{50} > 10), **1t/39** (i.c.e. = 0.79, I_{50} > 10), **3a/39** (i.c.e. = 0.89, I_{50} = 9.3), **10/62** (i.c.e. = 0.52, I_{50} > 10), **13/62** (i.c.e. = 0.45, I_{50} > 10), **14/53** (i.c.e. = 0.47, I_{50} = 13.6). Each of these compounds does show inhibition of thymidine uptake at <10 μg/mL. Since the in vitro results represent single-experiment screen procedures, the basis for mechanistic interpretation is limited. However, the correlation between observed in vivo activity and thymidine uptake appears to be more consistent than that with leucine uptake, suggesting that the compounds may act on the trypanosomes at the level of DNA synthesis.

Screening against Other Parasites

Representative compounds were screened for activity against *Plasmodium falciparum*, *Leishmania donovani*, *Trichomonas vaginalis*, and coccidia. Several of the compounds showed in vitro activity against malaria with I_{50} = 0.01–0.1 ng/mL (**1h/39**, **1a/53**, **1a/61**, **2i/53**, **12/61**, **1p/39**, **1p/53**, **2h/39**, **2h/53**, **2h/63**, **2i/53**, **2i/39**, **12/39**, **12/61**). However, no oral activity against malarial parasites was noted against *Plasmodium yoelii* in mice for **1h/39**, **1a/53**, or **1a/61**. Compounds **1a/53**, **1a/61**, **1a/65**, and **1h/39** showed no inhibition in cultured mouse peritoneal macrophages infected with *L. donovani*. Compound **2h/63** showed marginal activity (40% inhibition) at 1×10^{-5} M. Compounds **1a/53**, **1a/61**, **1a/65**, **1h/39**, and **2h/63** showed no significant activity against *T. vaginalis*. Compounds **1h/39** and **1a/61** showed no activity in chickens experimentally infected with *Eimeria acervulina* or *Eimeria tenella*.

Discussion

A crucial feature for the heteroaromatic ring is that it be cationic. The nonquaternized structural analogue of **1a/39** is inactive. The exact shape of the ring and its disposition with respect to the remainder of the molecule seems quite variable. The polar, or preferably, cationic functional group on the phenyl ring is also of major importance to activity. Again, its precise structural nature

(8) Desjardins, R. E.; Casero, R. A., Jr.; Willet, G. P.; Childs, G. E.; Canfield, C. J. *Exp. Parasitol.* **1980**, *50*, 260.

Table II. Trypanocidal Activity of Compounds Having I.C.E.[a] (sc) > 0.15

	sc administration		po administration		in vitro ID_{50}[c]			sc administration		po administration		in vitro ID_{50}[c]	
compound	CD_{50}[b]	i.c.e.	CD_{50}[b]	i.c.e.	thymidine	leucine	compound	CD_{50}[b]	i.c.e.	CD_{50}[b]	i.c.e.	thymidine	leucine
						Group 1							
1a/1 (I^-)	212	0.17	106	0.24	>10	>10	**1d**/1 (Br^-)	212	0.17			3.2	5.2
1b/1 (Br^-)	26	0.20											
						Group 2							
1a/11 (TsO^-)	106	0.24		0.01			**3g**/22 (TsO^-)	6.6	0.62				
1a/13 (TsO^-)	53	0.34					**10**/22 (TsO^-)	6.6	0.37				
1a/19 (Br^-)	53	0.17					**11**/22 (TsO^-)	53	0.18			10.1	>10
1a/22 (TsO^-)	0.8	0.57					**14**/22 (TsO^-)	26	0.27				
2g/22 (TsO^-)	13	0.44					**15**/22 (TsO^-)	26	0.32				
3a/22 (TsO^-)	6.6	0.58											
						Group 3							
						No Active Compounds							
						Group 4							
1a/28 ($2Cl^-$)	26	0.21					**2a**/28 ($2Cl^-$)	3.3	0.26			0.7	12
1a/35 (Cl^-)	53	0.29					**6**/28 ($2Cl^-$)	106	0.23			0.3	0.8
1h/35 (Cl^-)	106	0.16											
						Group 5							
1a/37 ($2Br^-$)	6.6	0.55			3.8	5.8	**1m**/37 ($2Br^-$)	106	0.16				
1a/38 ($2Br^-$)	26	0.24					**1s**/37 ($2Br^-$)	13	0.47				
1h/37 ($2Br^-$)	26	0.38			4.0	>10	**2a**/37 ($2Br^-$)	26	0.44			4.6	3.3
1i/38 ($2Br^-$)	26	0.38											
						Group 6							
1a/39 ($2TsO^-$)	0.8	0.54	26	0.48			**1u**/39 ($2Br^-$)	3.3	0.29			2.7	1.7
1a/40 ($2Br^-$)	0.8	0.41			0.5	0.7	**1u**/40 ($2Br^-$)	0.4	0.33			5.4	3.4
1a/41 ($2Br^-$)	0.1	0.59			0.4	0.5	**1u**/51 ($2Br^-$)	0.8	0.20	53	0.36		
1a/42 ($2Br^-$)	0.1	0.59					**1v**/39 ($2Br^-$)	1.6	0.32	26	0.43		
1a/43 ($2Br^-$)	0.8	0.50					**1v**/61 ($2Br^-$)	3.3				0.7	0.7
1a/44 ($2Br^-$)	0.2	0.65					**1w**/39 ($2Br^-$)	0.2	0.45				
1a/45 ($3Br^-$)	0.8	0.38			2.3	11.7	**1w**/53 ($2Br^-$)	0.2	0.47				
1a/48 ($3Br^-$)	1.6	0.38					**1x**/39 ($2Br^-$)	0.4	0.43				
1a/49 ($3Br^-$)	0.8	0.38					**1x**/61 ($2Br^-$)	0.4	0.44				
1a/50 ($2Br^-$)	1.6	0.46					**2a**/39 ($2Br^-$)	0.1	0.47			0.8	0.9
1a/51 ($2I^-$)	0.2	0.48	6.6	0.65			**2a**/51 ($2Br^-$)	3.3	0.26				
1a/52 ($2Br^-$)	0.2	0.35			1.1	1.0	**2a**/53 ($2Br^-$)	0.8	0.36	26	0.47		
1a/53 ($2Br^-$)	0.06	0.39	6.6	0.64	0.6	0.6	**2a**/58 ($2Br^-$)	0.8	0.45	53	0.35		
1a/54 ($2Br^-$)	0.8	0.44					**2a**/59 ($2Br^-$)	0.8	0.44	106	0.25		
1a/55 ($2Br^-$)	0.4	0.33					**2a**/59 ($2Br^-$)	0.8	0.44	106	0.25		
1a/56 ($2.75Br^-$)	3.3	0.21					**2a**/61 ($2Br^-$)	0.8	0.60	6.6	0.61		
1a/57 ($2TsO^-$)	13	0.30	212	0.20			**2g**/62 ($2Br^-$)	0.4	0.67				
1a/58 ($2I^-$)	0.2	0.58					**2g**/39 ($2Br^-$)	0.8	0.49	13	0.48		
1a/59 ($2Br^-$)	1.6	0.57	53	0.40			**2g**/41 ($2Br^-$)	0.4	0.52				
1a/61 ($2Br^-$)	0.8	0.57	13	0.56	0.8	0.9	**2g**/53 ($2Br^-$)	0.4	0.75	53	0.24		
1a/62 ($2Br^-$)	0.1	0.67	6.6	0.61			**2g**/58 ($2Br^-$)	3.3	0.32				
1b/39 ($2Br^-$)	0.4	0.36					**2g**/61 ($2Br^-$)	0.8	0.64				
1e/39 ($2TsO^-$)	1.6	0.39	13	0.50			**2g**/62 ($2Br^-$)	0.8	0.70				
1h/39 ($2TsO^-$)	0.2	0.82	6.6	0.56	0.4	0.5	**2h**/39 ($2Br^-$)	0.2	0.81	6.6	0.61		
1h/41 ($2Br^-$)	0.4	0.67	26	0.35			**2h**/41 ($2Br^-$)	0.4	0.67				
1h/51 ($2Br^-$)	0.8	0.43					**2h**/53 ($2Br^-$)	0.8	0.46	13	0.49		
1h/53 ($2Br^-$)	0.1	0.49					**2h**/59 ($2Br^-$)	1.6	0.36				
1h/55 ($2Br^-$)	0.8	0.47					**2h**/61 ($2Br^-$)	0.8	0.86				
1h/57 ($2TsO^-$)	6.6	0.39			>10	0.5	**2h**/62 ($2Br^-$)	0.8	0.69				
1h/59 ($2Br^-$)	1.6	0.66					**2i**/39 ($2Br^-$)	0.2	0.52	6.6	0.57		
1h/62 ($2Br^-$)	0.2	0.76					**2i**/53 ($2Br^-$)	0.4	0.32				
1i/39 ($2TsO^-$)	1.6	0.55			1.0		**2j**/39 ($2TsO^-$)	6.6	0.67				
1i/41 ($2Br^-$)	0.1	0.57	13	0.57			**2j**/53 ($2Br^-$)	26	0.27				
1i/42 ($2Br^-$)	0.2	0.63	26	0.45	1.9	4.6	**2j**/62 ($2Br^-$)	1.6	0.64	106	0.19	0.4	0.5
1i/43 ($2Br^-$)	0.4	0.71	26	0.32			**3a**/39 ($2TsO^-$)	0.1	0.89			2.7	9.3
1i/53 ($2Br^-$)	0.1	0.48	13	0.47			**3a**/41 ($2Br^-$)	0.1	0.66				
1i/55 ($2Br^-$)	0.4	0.53					**3a**/53 ($2TsO^-$)	0.8	0.74				
1i/56 ($2.75Br^-$)	3.3	0.24					**3a**/59 ($2Br^-$)	0.4	0.80				
1i/58 ($2Br^-$)	0.4	0.58					**3a**/61 ($2Br^-$)	0.2	0.79	13	>0.5		
1i/60 ($2Br^-$)	53	0.24					**3a**/62 (Br^-)	0.4	0.71	13	0.53		
1i/62 ($2Br^-$)	0.1	0.67	6.6	0.57	0.7	1.1	**3g**/39 ($2TsO^-$)	0.4	0.85	53	0.30		
1m/39 ($2TsO^-$)	0.1	0.93					**3g**/53 ($2Br^-$)	0.4	0.90	53	0.44		
1m/61 ($2Br^-$)	3.3	0.69					**3g**/59 ($2Br^-$)	0.4	0.65				
1n/39 ($2Br^-$)	1.6	0.64					**3g**/61 ($2Br^-$)	0.4	0.86	26	0.52		
1o/39 ($2Br^-$)	0.8	0.67					**3g**/62 ($2Br^-$)	0.4	0.84				
1p/39 ($2Br^-$)	0.2	0.92	26	0.27			**3h**/39 ($2Br^-$)	0.8	0.65				
1p/53 ($2Br^-$)	0.1	0.94	53	0.31			**3h**/53 ($2Br^-$)	0.4	0.77				
1q/39 ($2TsO^-$)	0.4	0.83	106	0.28			**3h**/62 ($2Br^-$)	1.6	0.41				
1q/53 ($2TsO^-$)	3.3	0.63	212	0.17			**4a**/39 ($2TsO^-$)	0.06	0.94	6.6	0.63		
1s/39 ($2TsO^-$)	0.4	0.87	6.6	0.55	5.9	4.3	**4a**/53 ($2Br^-$)	0.8	0.34	53	0.31		
1t/39 ($2TsO^-$)	0.4	0.79			1.5	>10	**4a**/59 ($2Br^-$)	13	0.34	53	>0.3		

Table II (Continued)

compound	sc administration CD_{50}[b]	sc i.c.e.	po administration CD_{50}[b]	po i.c.e.	in vitro ID_{50}[c] thymidine	in vitro ID_{50}[c] leucine	compound	sc administration CD_{50}[b]	sc i.c.e.	po administration CD_{50}[b]	po i.c.e.	in vitro ID_{50}[c] thymidine	in vitro ID_{50}[c] leucine
4a/62 (2Br⁻)	0.06	0.58	13	0.51			**10/53** (2TsO⁻)	0.8	0.33	6.6	0.57		
5a/39 (2TsO⁻)	106	0.24	424	0.06	4.2	4.5	**10/58** (2Br⁻)	3.3	0.34				
6/39 (2TsO⁻)	0.4	0.65			1.4	1.3	**10/59** (2Br⁻)	6.6	0.29	212	0.10		
6/40 (2I⁻)	0.4	0.52	6.6	0.60	1.5	1.2	**10/61** (2Br⁻)	0.2	0.68	13	0.53		
6/51 (2I⁻)	0.2	0.56			1.0	1.0	**10/62** (2Br⁻)	1.6	0.52	6.6	0.57	4.4	>10
6/53 (2Br⁻)	0.1	0.54	3.3	0.70			**11/53** (2TsO⁻)	13	0.16		0.00	2.2	0.7
6/61 (2Br⁻)	0.8	0.44			0.6	0.8	**12/41** (2Br⁻)	6.6	0.18	212	0.10		
6/62 (2Br⁻)	0.2	0.47					**12/53** (2Br⁻)	0.1	0.19	106	0.17		
8/39 (2Br⁻)	6.6	0.19	424	0.06			**12/62** (2Br⁻)	6.6	0.31	212	0.16		
8/53 (2Br⁻)	6.6	0.23	424	0.12	0.6	0.7	**13/39** (2TsO⁻)	0.8	0.45	26	0.42	1.8	>10
8/59 (2Br⁻)	13	0.30	424	0.15			**13/53** (2Br⁻)	0.8	0.47	26	0.40	0.3	0.8
8/61 (2Br⁻)	3.3	0.19	424	0.10	11.1	>10	**13/61** (2Br⁻)	3.3	0.36	13	0.45		
8/62 (2Br⁻)	1.6	0.59	106	0.23			**13/62** (2Br⁻)	0.8	0.45	53	0.35	3.7	>10
9/39 (2Br⁻)	6.6	0.16					**14/53** (2TsO⁻)	0.8	0.47	424	0.04	9.3	13.6
9/41 (2TsO⁻)	6.6						**14/61** (2Br⁻)	13	0.21	424	0.12		
9/53 (2TsO⁻)	6.6	0.23					**15/41** (2TsO⁻)	0.11	0.61				
9/59 (2Br⁻)	13	0.30					**15/53** (2TsO⁻)	0.03	0.63				
9/61 (2Br⁻)	3.3	0.37					**15/59** (2Br⁻)	1.6	0.46				
9/62 (2Br⁻)		0.38					**15/61** (2Br⁻)	1.6	0.64		0.69		
10/39 (2Br⁻)	0.4	0.67	13	0.48	1.9	6.1	**15/62** (2Br⁻)	0.8	0.54	6.6	0.63		
10/41 (2Br)	1.6	0.60											
							Group 7						
1a/63 (2TsO⁻)	0.8	0.70					**2g/63** (2Br⁻)	0.8	0.74	13	0.49		
1a/64 (Br⁻)	<26						**2h/63** (2Br⁻)	0.2	0.85				
1a/65 (2Br⁻)	0.8	0.22		>0.5	6.7	9.7	**2j/63** (2Br⁻)	6.6	0.59				
1a/63 (2TsO⁻)	0.8	0.85	6.6	0.59	2.3	1.0	**3a/63** (2Br⁻)	0.2	0.72				
1h/64 (Br⁻)	<26						**3h/63** (2Br⁻)	3.3	0.69				
1h/65 (2Br⁻)	3.3	0.37					**5g/63** (2TsO⁻)	106	0.17		0.03		
1i/63 (2Br⁻)	0.2	0.71			0.9	0.7	**6/63** (2TsO⁻)	0.8	0.52			2.0	1.7
1i/65 (2Br⁻)	0.8	0.31					**13/63** (2TsO⁻)	1.6	0.22			3.8	10.3
1q/63 (2Br⁻)	0.8	0.84	26	0.40			**15/63** (2Br⁻)	0.8	0.47				
2a/63 (2Br⁻)	0.4	0.57											
							Group 8						
1a/66 (2Br⁻)	0.8	0.65			0.5	>10	**1h/76** (2Br⁻)	106	0.28				
1a/71 (TsO⁻)	212	0.18					**1h/78** (2Br⁻)	26	0.29			>10	>10
1a/72 (2Br⁻)	6.6	0.26					**1m/74** (TsO⁻)	1.6	0.71			0.6	>10
1a/74 (TsO⁻)	6.6	0.43					**2a/66** (2Br⁻)		0.23			0.5	>10
1a/77 (Br⁻)	53	0.18					**2a/71** (TsO⁻)	53	0.23			7.9	10.1
1a/78 (2Br⁻)	13	0.29					**2a/76** (2Br⁻)	53	0.27				
1h/66 (2Br⁻)	13	0.39			3.8		**6/66** (2Br⁻)	13	0.50				
1h/71 (2Br⁻)	0.8	0.48					**6/76** (2Br⁻)	26	0.31				
1h/74 (I⁻)		0.71			0.3	>10	**6/77** (Br⁻)	212	0.21			2.4	4.6

[a] See text and Table I for definition of i.c.e. [b] Minimum dose in mg/kg which effected cure of ≥50% of the test animals. [c] Concentration in μg/mL which effected 50% inhibition.

seems to be of subsidiary importance.

These structural features link these compounds to several other groups of bis-cationic drugs which have activity against *T. rhodesiense*. These include pentamidine,[9] berenil,[10] the bis-amidines developed by Dann,[11] and bis-amidines derived from 2,5-diphenylfuran.[12] Other examples of bis-cations with antitrypanosomal activity are bis-guanylhydrazones of 1,3-diacetylbenzene and related compounds,[13] the bis-amidines amicarbalide and imidocarb,[14] and the phthalanilides.[15] There is evidence of a general nature that these bis-cationic drugs interact with DNA, probably in a groove-binding mode.[16] Recent crystallographic work provides an example in the case of Hoechst 33258, which binds in the minor groove of oligonucleotides.[17] Another possible clue to the mechanism of action of the bis-cationic drugs is the interrelationship demonstrated in several cases with polyamine levels.[18] Enhanced levels of polyamines appear to reduce the effectiveness of the bis-cationic trypanocides.

A possible subset of active compounds are represented by the series of derivatives having functional groups **22**. All of the **22** compounds show significant activity, despite the fact that the *p*-amino group is too weakly basic for these compounds to be dications at physiological pH. The modest activity of the (*p*-tolylsulfonyl)hydrazone **1a/19**

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Table III. Effect of Structural Changes on Activity Index

A. Effect of Ring Substituents

	1a	1h	1i	1m	1p	1q
39	0.54	0.82	0.55	0.93	0.92	0.83
53	0.39	0.49	0.48		0.94	0.63
61	0.57			0.69		
62	0.67	0.76	0.67			
63	0.70	0.85	0.71			

B. Effect of Linking Groups

	1a	2a	3a	4a	5a
39	0.54	0.47	0.89	0.94	0.24
53	0.39	0.36	0.74	0.34	0.06
61	0.57	0.60	0.79		0.01
62	0.67	0.67	0.71	0.58	
63	0.70	0.57	0.72		0.01

C. Combined Effect of Methyl Groups and Linking Groups

	2a	2g	2h	2i	3a	3g	3h
39	0.47	0.49	0.81	0.52	0.89	0.85	0.65
53	0.36	0.75	0.46	0.32	0.74	0.90	0.77
61	0.60	0.64	0.86		0.79	0.86	
62	0.67	0.70			0.71	0.84	0.41
63	0.57	0.74	0.85		0.72		0.69

D. Effect of Heteroaromatic Rings

	1a	6	2a	9	10	11	12	13	14	15
39	0.54	0.65	0.47	0.16	0.67		0.07	0.45		
53	0.39	0.54	0.36	0.23	0.33	0.16	0.19	0.47	0.47	0.63
61	0.57	0.44	0.60	0.37	0.68	0.12	0.04	0.36	0.21	0.64
62	0.67	0.47	0.67	0.38	0.52	0.00	0.31	0.45		0.54
63	0.70	0.52	0.57	0.03				0.22		0.47

suggests that structures incorporating aromatic rings at the 4′-position may have some activity, despite being monocations.

The primary antitrypanosomal test system utilized evaluates efficacy against the blood state of the parasite. The most active compounds, from comparison of murine test data under essentially similar conditions, exceed both pentamidine ($CD_{50} \simeq 2$ mg/kg, i.c.e. $\simeq 0.75$) and berenil ($CD_{50} \approx 2$ mg/kg, i.c.e. $\simeq 0.75$) in potency in this screen. The failure to effect long-term cures in the chronic *T. brucei* model with CNS involvement indicates that the drugs do not cross the blood–brain barrier.

Experimental Section

General. All compounds which were submitted for biological evaluation were characterized by elemental analysis for at least three elements (C, H, N). Many of the samples were analyzed as hydrates. Melting points and elemental composition of all compounds are given in Table IV in the supplemental material. Infrared and, in most cases, ^{1}H NMR spectra were recorded. These spectra were consistent with the assigned structures. Full experimental details for preparation of individual compounds have been described in the final reports for contracts DAMD17-78-C-8016, DAMD17-83-C-3127, and DAMD17-85-C-5004. These reports are available through the Defense Technical Information Center (DTIC), Cameron Station, Alexandria, VA 22304-6145.

Method A. Acylamino and Sulfonylamido Heterocycles (Functional Groups 1–9). The acylamino or sulfonamido compounds were prepared by reducing and then acylating or sulfonylating the appropriate *p*-nitrophenyl heterocycle as described earlier.[5] The acylamino or sulfonamido derivative was dissolved or suspended in dry acetonitrile and refluxed overnight (16–20 h) with a 1.5–3-fold excess of the appropriate alkyl halide or tosylate. In many cases the product precipitated from the reaction mixture and was isolated by filtration. When this did not occur, the acetonitrile volume was reduced by about 70% by rotary evaporation and ether was added to induce crystallization.

Quaternization of Nitrile and Aldehyde Intermediates. Most of the compounds were prepared from quaternary derivatives of the 4′-cyanophenyl and 4′-formylphenyl heterocycles. The synthesis of these intermediates was described earlier.[5] The tosylate salts were prepared by heating the aldehyde or nitrile with 1.1–1.3 equiv of methyl tosylate in refluxing acetonitrile for 24 h. The mixture was then cooled to room temperature and a small amount of ether was added to induce crystallization. The precipitated salt could be recrystallized from ethanol–ether. *N*-Allyl salts were prepared similarly with allyl bromide as the alkylating agent. In cases where iodides were prepared, the quaternary iodide salt was prepared by alkylation with methyl iodide.

Method B. Oximes, Semicarbazones, and Thiosemicarbazones (Functional Groups 10–12). A solution of the quaternary heterocyclic aldehyde in ethanol was treated with hydroxylamine hydrochloride–pyridine, semicarbazide, or thiosemicarbazide and heated for 1 h. The solution was then refrigerated and the precipitated product was recrystallized from absolute ethanol.

Method C. 1-Methyl-2-[4′-(aminothiocarbonyl)phenyl]imidazo[1,2-*a*]pyridinium Tosylate (Functional Group 13). 1-Methyl-2-(4′-cyanophenyl)imidazo[1,2-*a*]pyridinium tosylate (15 mmol) was dissolved in 25 mL of dimethylformamide. Diethylamine (2 mL) was added and the mixture was warmed to 55–58 °C. A moderate stream of H_2S was passed through the mixture for 3.5 h after which excess H_2S was expelled by a stream of N_2. The solution was diluted by addition of 25 mL of ether and then filtered. The yellow solid was washed with dry ether, and then recrystallized from 95% ethanol. Concentration of the mother liquid gave an additional crop.

Method D. Quaternization of 4′-Substituted Phenylimidazo[1,2-*a*]pyridine Derivatives (Functional Groups 14, 23, 24, 25, 26, 27). The appropriate 2-phenylimidazo[1,2-*a*]-pyridine derivative[5] was added to dry acetonitrile. Methyl tosylate (1.1–1.5 equiv) was added and the mixture was heated. When necessary for complete conversion, the reaction was run at 120–140° C in a sealed tube. The product was filtered and crystallized from ethanol–ether.

Method E. Substituted Hydrazones (Functional Groups 15–22). A mixture of the quaternary tosylate salt of the appropriate aldehyde and 1.1 equiv of the hydrazine or hydrazide was refluxed in absolute ethanol for 4 h. The solution was then acidified with hydrochloric acid or hydrobromic acid, resulting

in precipitation of the corresponding salt. The precipitated salts were recrystallized from ethanol, adding ether if necessary.

Method F. Carboxamidines (Functional Group 28). The appropriate *N*-methyl-4′-cyanophenyl heterocyclic quaternary tosylate salt (20 mmol) was suspended in 100 mL of dry nitrobenzene. Absolute ethanol (10 mL) was added and, with ice cooling, HCl gas was passed through the mixture, with stirring, for 6 h. The mixture was then allowed to stand at room temperature for 2 days. Dry ether was then added to precipitate the imino ether hydrochloride. It was filtered (or decanted when the compound formed as an oil), washed with dry ether, and then stirred with 60 mL of saturated alcoholic ammonia for 24 h. The amidine was then precipitated as the hydrochloride by addition of dry ether. The precipitate was dissolved in 3 N HCl. On addition of acetone, the compound reprecipitated. This process was repeated and finally the precipitate was recrystallized from 95% ethanol.

Method G. N-Substituted Amidines (Functional Groups 29–31). 1-Methyl-2-(4′-cyanophenyl)imidazo[1,2-*a*]pyridinium tosylate was treated with gaseous HCl and absolute ethanol in dry nitrobenzene as for the preparation of the unsubstituted amidines. The precipitated imino ether hydrochloride was filtered, washed with dry ether, and suspended in absolute ethanol. The appropriate amine was added until the solution was basic and the mixture was then stirred at room temperature for 4 h. On addition of ether, an oily layer separated. The solvent was decanted and the residue was dissolved in 3 N HCl. Acetone was added and the product precipitated. The product was recrystallized from ethanol–acetone until satisfactory purity was achieved.

Method H. Cyclic *N*,*N*′-Dialkylamidines (Functional Groups 32–34). 1-Methyl-2-(4′-cyanophenyl)imidazo[1,2-*a*]-pyridinium tosylate was converted to the imino ether hydrochloride as in method F. The imino ether salt was then dissolved in ethanol and treated with 2.4 equiv of the appropriate diamine. The solution was heated for several hours, filtered, and cooled, and the product precipitated with ether. The solid was then recrystallized from 95% ethanol containing a small amount of HCl.

Method I. Carboxamide Oximes (Functional Group 35). The appropriate *N*-methyl-4′-cyanophenyl heterocyclic tosylate salt (4 mmol) was stirred with a mixture of ethanol (10 mL), pyridine (20 mL), and hydroxylamine hydrochloride (2.5 g). The product was recrystallized from ethanol containing a small amount of water.

Method J. Carboximide *N*,*N*-Dimethylhydrazides (Functional Group 36). The appropriate *N*-methyl-2-(4′-cyanophenyl)imidazo[1,2-*a*]pyridinium salt was converted to the imino ether as described for the amidines. The imino ether hydrochloride was dissolved in absolute ethanol and *N*,*N*-dimethylhydrazine was added until the solution became slightly basic. The mixture was stirred at room temperature for 5 h, after which dry ether was added. An oil separated and was isolated by decanting. The oil was dissolved in 3 N HCl, acetone was added, and the mixture was refrigerated. Slow crystallization ensued. The crystallization process was repeated until satisfactory purity was achieved.

Method K. *S*-Methylthio Imidoyl Hydrazones (Functional Groups 37 and 38). A mixture of the quaternary *N*-methyl tosylate salt of the appropriate aldehyde and 1.1 equiv of *S*-methyl thiosemicarbazide hydroiodide in ethanol (100 mL) was refluxed for 4 h. The solution was then cooled and the resulting solid was collected and converted to a bromide salt by recrystallization from ethanol containing hydrobromic acid.

Method L. Guanylhydrazones (Functional Group 39). The appropriate quaternary heterocyclic aldehyde salt was dissolved in ethanol and treated with 1.2 equiv of *N*-aminoguanidinium bicarbonate and 1.5 equiv of *p*-toluenesulfonic acid. The mixture was refluxed for 1 h and chilled overnight, and the precipitate was collected and recrystallized. Compounds isolated as bromides were obtained by acidifying the reaction mixture with hydrobromic acid and recrystallizing the precipitate from ethanol containing a small amount of hydrobromic acid.

Method M. (*N*-Alkylguanyl)hydrazones (Functional Groups 40–60). a. *N*-Amino-*N*′-alkylguanidinium Salts. The appropriate amine (2 equiv) and *S*-methylthiosemicarbazide hydriodide were refluxed together in absolute ethanol until the evolution of methanethiol was judged to be complete (several hours). The solution was then cooled and the product was precipitated with ether. In most cases, the product was purified by an additional cycle of dissolution in alcohol and precipitation with ether. When the product was not readily crystallized, the oily precipitate was thoroughly dried in vacuo and used without purification.

b. (*N*-Alkylguanyl)hydrazone Derivatives. The substituted aminoguanidinium iodides prepared as above were heated with the appropriate quaternary aldehyde (usually a tosylate) for several hours in ethanol. The solutions were then cooled and acidified by addition of hydrobromic acid. The product was usually precipitated by addition of ether and chilling and could be purified by recrystallization several times from ethanol containing hydrobromic acid. In a few cases the salts crystallized as mixed iodide–bromide salts in which the iodide content was not reduced by further recrystallization.

c. *N*-2-Imidazolinylhydrazones and *N*-(1,4,5,6-Tetrahydropyrimid-2-yl)hydrazones (Functional Groups 61 and 62). The commercially available 1*H*-imidazoline-2(3*H*)-thione and 3,4,5,6-tetrahydro-2(1*H*)-pyrimidinethione were converted to the *S*-methyl salts with methyl iodide and then heated with hydrazine in ethanol until evolution of methanethiol ceased. The residue obtained by evaporation and drying was used for reaction with the appropriate quaternary aldehyde as described in b.

Method N. (*N*-Hydroxyguanyl)hydrazones (Functional Group 63). An equimolar mixture of the appropriate quaternary aldehyde and *N*-amino-*N*′-hydroxyguanidinium tosylate[19] was refluxed in absolute ethanol for 2 h. After cooling to room temperature, the product was precipitated by addition of ether. The compound was recrystallized from acetonitrile.

Method O. (*N*-Nitroguanyl)hydrazones (Functional Group 64). An equimolar mixture of the appropriate quaternary aldehyde and *N*-amino-*N*′-nitroguanidine[20] was refluxed in ethanol for 60 h. Concentrated HBr was then added and the solution was cooled. The precipitated product was recrystallized from hot ethanol.

Method Q. (*N*-Morpholinoguanyl)hydrazones (Functional Group 65). A mixture of *N*-aminomorpholine (1.2 g) and *S*-methylthiosemicarbazide hydriodide (2.3 g) was refluxed for 24 h in absolute ethanol (40 mL). The volume of the solution was then reduced by about one-half and ether was added, resulting in an oily precipitate. This was redissolved in ethanol and reprecipitated with ether. The precipitate was dried in vacuo to give a solid (1.8 g). This solid and the appropriate quaternary aldehyde (6 mmol) were refluxed for 15 h in ethanol. The solution was then cooled and treated cautiously with 8 mL of concentrated hydrobromic acid. On further cooling, the product precipitated and was recrystallized from ethanol.

Method Q. Heterocyclic Hydrazones (Functional Groups 66–78). The heterocyclic hydrazone functional groups 66–78 were prepared with the following heterocyclic hydrazine derivatives prepared according to literature methods: **66**, 1*H*-1,2,4-triazol-3-ylhydrazine;[21] **67**, 3,4-diamino-1,2,4-triazole;[22] **68**, 3,4,5-triamino-4*H*-1,2,4-triazole;[23] **69**, (4-amino-4*H*-1,2,4-triazol-3-yl)-hydrazine;[24] **70**, 4-amino-1,2,4-triazole;[25] **71**, 2-pyridylhydrazine;[26] **72**, pyrimid-2-ylhydrazine;[27] **73**, 4-aminopyrimid-2-ylhydrazine;[28]

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74, (4,6-diaminopyrimid-2-yl)hydrazine;[29] 75, 1,2-diaminopyridinium iodide;[30] 76, thiazol-2-ylhydrazine;[31] 77, 3,4-diamino-1,2,4-triazole-5-thione;[32] and 78, 5-amino-4*H*-pyrazol-2-ylhydrazine.[33]

The appropriate quaternary aldehyde, usually as the tosylate salt, and 1.1 equiv of the appropriate heterocyclic hydrazine were refluxed together in ethanol for 4–6 h. In most cases, the hydrazones were isolated as bromide salts by adding concentrated hydrobromic acid to the resulting solution. In cases where the tosylate salt precipitated during the reaction, it was redissolved in ethanol and then treated with concentrated hydrobromic acid. The bromide salts were then recrystallized from ethanol or ethanol–ether.

In Vivo Antitrypanosomal Activity. The method of Rane, Rane, and Kinnamon was employed.[6] Groups of five mice were administered with a standard parasite inoculum which produces trypanosomiasis which is lethal to 100% of untreated controls (mean survival time of 4.45 ± 0.25 days). The mice were treated with the test drugs by sc or po administration. The life span of treated animals was recorded. Those with decreased survival time relative to untreated controls were scored as toxic deaths. Increased survival spans were recorded as T-C, the number of days the treated animals survived beyond the controls. Animals that survived >30 days were scored as cured.

Activity in the Chronic *Trypanosoma brucei*[7] Model. Groups of six mice were infected on day one with *T. brucei*. The test compounds were injected on day 21 for a single dose and on days 21, 24, 26, and 28 for multiple doses. Effectiveness was determined by the number of days to relapse.

In Vitro Trypanosomal Activity. The assay used was that described by Desjardins, Casero, Willet, Childs, and Canfield.[8] Parasites harvested from infected rats were cultured for 3 hours in the presence of several concentrations of the test compound, usually 0.1–100 μg/mL. The extent of uptake of radiolabeled thymidine and L-leucine was measured. Active compounds showed a sigmoidal curve from which the ED_{50}, the 50% inhibitory concentration, was determined.

Acknowledgment. This research was carried out under the auspices of the Walter Reed Army Institute of Research, U.S. Army Medical Research and Development Command, Contracts DAMD17-78-C-8016, 17-83-C-3127, and 17-85-C-5004. This paper is designated Contribution No. 1845 to the U.S. Army Drug Development Program. The evaluation in the chronic *T. brucei* assay was done by Dr. F. W. Jennings, Faculty of Veterinary Medicine, Glasgow. We also thank the Wellcome Research Laboratories, Kent, UK, for the oral malarial, *L. donovani*, and *T. vaginalis* evaluations. We thank Merck Sharp and Dohme, Rahway, NJ, for evaluation of **1h/39** and **1a/61** against coccidiosis.

Registry No. **1a**/1 (I^-), 123508-26-9; **1a**/11 (TsO^-), 123508-30-5; **1a/13** (TsO^-), 123508-32-7; **1a/19** (Br^-), 123508-33-8; **1a/22** (TsO^-), 123508-35-0; **1a/28** ($2Cl^-$), 123508-48-5; **1a/35** (Cl^-), 123508-49-6; **1a/37** ($2Br^-$), 123508-53-2; **1a/38** ($2Br^-$), 123508-54-3; **1a/39** ($2TsO^-$), 123508-62-3; **1a/40** ($2Br^-$), 123508-63-4; **1a/41** ($2Br^-$), 123508-64-5; **1a/42** ($2Br^-$), 123508-65-6; **1a/43** ($2Br^-$), 123508-66-7; **1a/44** ($2Br^-$), 123508-67-8; **1a/45** ($2Br^-$), 123508-68-9; **1a/48** ($2Br^-$), 123508-69-0; **1a/49** ($2Br^-$), 123508-70-3; **1a/50** ($2Br^-$), 123508-71-4; **1a/51** ($2I^-$), 123508-72-5; **1a/52** ($2Br^-$), 123508-73-6; **1a/53** ($2Br^-$), 123508-74-7; **1a/54** ($2Br^-$), 123508-75-8; **1a/55** ($2Br^-$), 123508-76-9; **1a/56** ($3Br^-$), 123508-77-0; **1a/57** ($2TsO^-$), 123508-80-5; **1a/58** ($2I^-$), 123508-81-6; **1a/59** ($2Br^-$), 123508-82-7; **1a/61** ($2Br^-$), 123508-83-8; **1a/62** ($2Br^-$), 123508-84-9; **1a/63** ($2TsO^-$), 123510-38-3; **1a/64** (Br^-), 123510-39-4; **1a/65** ($2Br^-$), 123510-40-7; **1a/66** ($2Br^-$), 123510-65-6; **1a/71** (TsO^-), 123510-67-8; **1a/72** ($2Br^-$), 123510-68-9; **1a/74** (TsO^-), 123510-70-3; **1a/77** (Br^-), 123510-71-4; **1a/78** ($2Br^-$), 123510-72-5; **1b**/1 (Br^-), 123508-27-0; **1b/39** ($2Br^-$), 123508-85-0; **1d**/1 (Br^-), 123508-28-1; **1e/39** ($2TsO^-$), 123508-88-3; **1h/35** (Cl^-), 123508-50-9; **1h/37** ($2Br^-$), 123508-55-4; **1h/39** ($2TsO^-$), 123508-91-8; **1h**/41 ($2Br^-$), 123508-92-9; **1h/51** ($2Br^-$), 123508-93-0; **1h/53** ($2Br^-$), 123508-94-1; **1h/55** ($2Br^-$), 123508-95-2; **1h/57** ($2TsO^-$), 123508-98-5; **1h/59** ($2Br^-$), 123508-99-6; **1h/62** ($2Br^-$), 123509-00-2; **1h/63** ($2TsO^-$), 123510-43-0; **1h/64** (Br^-), 123510-44-1; **1h/65** ($2Br^-$), 123510-45-2; **1h/66** ($2Br^-$), 123510-73-6; **1h/71** ($2Br^-$), 123510-74-7; **1h/74** (I^-), 123510-75-8; **1h/74** ($2Br^-$), 123510-76-9; **1h/78** ($2Br^-$), 123510-77-0; **1i/38** ($2Br^-$), 123508-56-5; **1i/39** ($2TsO^-$), 123509-03-5; **1i/41** ($2Br^-$), 123509-04-6; **1i/42** ($2Br^-$), 123509-05-7; **1i/43** ($2Br^-$), 123509-06-8; **1i/53** ($2Br^-$), 123509-07-9; **1i/55** ($2Br^-$), 123509-08-0; **1i/56** ($2Br^-$), 123509-09-1; **1i/58** ($2Br^-$), 123509-10-4; **1i/60** ($2Br^-$), 123509-11-5; **1i/62** ($2Br^-$), 123509-12-6; **1i/63** ($2Br^-$), 123510-46-3; **1i/65** ($2Br^-$), 123510-47-4; **1m/37** ($2Br^-$), 123508-57-6; **1m/39** ($2TsO^-$), 123509-15-9; **1m/61** ($2Br^-$), 123509-16-0; **1m/74** (TsO^-), 123510-79-2; **1n/39** ($2Br^-$), 123509-17-1; **1o/39** ($2Br^-$), 123509-18-2; **1p/39** ($2Br^-$), 123509-19-3; **1p/53** ($2Br^-$), 123509-20-6; **1q/39** ($2TsO^-$), 123509-23-9; **1q/53** ($2TsO^-$), 123509-26-2; **1q/63** ($2Br^-$), 123510-48-5; **1s/37** ($2Br^-$), 123508-58-7; **1s/39** ($2TsO^-$), 123509-29-5; **1t/39** ($2TsO^-$), 123509-32-0; **1u/39** ($2Br^-$), 123509-33-1; **1u/40** ($2Br^-$), 123509-34-2; **1u/51** ($2Br^-$), 123509-35-3; **1v/39** ($2Br^-$), 123509-36-4; **1v/61** ($2Br^-$), 123509-37-5; **1w/39** ($2Br^-$), 123509-38-6; **1w/53** ($2Br^-$), 123509-39-7; **1x/39** ($2Br^-$), 123509-40-0; **1x/61** ($2Br^-$), 123540-34-1; **2a/28** ($2Cl^-$), 123508-51-0; **2a/37** ($2Br^-$), 123508-59-8; **2a/39** ($2Br^-$), 123509-41-1; **2a/51** ($2Br^-$), 123509-42-2; **2a/53** ($2Br^-$), 123509-43-3; **2a/58** ($2Br^-$), 123509-44-4; **2a/59** ($2Br^-$), 123540-35-2; **2a/61** ($2Br^-$), 123540-36-3; **2a/63** ($2Br^-$), 123510-49-6; **2a/66** ($2Br^-$), 123510-80-5; **2a/71** (TsO^-), 123510-82-7; **2a/76** ($2Br^-$), 123510-83-8; **2g/22** (TsO^-), 123508-37-2; **2g/39** ($2Br^-$), 123509-46-6; **2g**/41 ($2Br^-$), 123509-47-7; **2g/53** ($2Br^-$), 123509-48-8; **2g/58** ($2Br^-$), 123509-49-9; **2g/61** ($2Br^-$), 123509-50-2; **2g/62** ($2Br^-$), 123509-45-5; **2g/63** ($2Br^-$), 123510-50-9; **2h/39** ($2Br^-$), 123509-51-3; **2h**/41 ($2Br^-$), 123509-52-4; **2h/53** ($2Br^-$), 123509-53-5; **2h/59** ($2Br^-$), 123509-54-6; **2h/61** ($2Br^-$), 123540-37-4; **2h/62** ($2Br^-$), 123509-55-7; **2h/63** ($2Br^-$), 123510-51-0; **2i/39** ($2Br^-$), 123509-56-8; **2i/53** ($2Br^-$), 123509-57-9; **2j/39** ($2TsO^-$), 123540-40-9; **2j/53** ($2Br^-$), 123509-58-0; **2j/62** ($2Br^-$), 123509-59-1; **2j/63** ($2Br^-$), 123510-52-1; **3a/22** (TsO^-), 123508-39-4; **3a/39** ($2TsO^-$), 123509-62-6; **3a**/41 ($2Br^-$), 123509-63-7; **3a/53** ($2TsO^-$), 123540-43-2; **3a/59** ($2Br^-$), 123509-64-8; **3a/61** ($2Br^-$), 123509-65-9; **3a/62** ($2Br^-$), 123540-44-3; **3a/63** ($2Br^-$), 123510-53-2; **3g/22** (TsO^-), 123508-41-8; **3g/39** ($2TsO^-$), 123509-68-2; **3g/53** ($2Br^-$), 123540-45-4; **3g/59** ($2Br^-$), 123509-69-3; **3g/61** ($2Br^-$), 123509-70-6; **3g/62** ($2Br^-$), 123509-71-7; **3h/39** ($2Br^-$), 123509-72-8; **3h/53** ($2Br^-$), 123509-73-9; **3h/62** ($2Br^-$), 123540-46-5; **3h/63** ($2Br^-$), 123510-54-3; **4a/39** ($2TsO^-$), 123509-76-2; **4a/53** ($2Br^-$), 123509-77-3; **4a/59** ($2Br^-$), 123509-78-4; **4a/62** ($2Br^-$), 123509-79-5; **5a/39** ($2TsO^-$), 123509-82-0; **5g/63** ($2TsO^-$), 123510-57-6; **6/28** ($2Cl^-$), 123508-52-1; **6/39** ($2TsO^-$), 123509-85-3; **6/40** ($2I^-$), 123509-86-4; **6/51** ($2I^-$), 123509-87-5; **6/53** ($2Br^-$), 123509-88-6; **6/61** ($2Br^-$), 123509-89-7; **6/62** ($2Br^-$), 123509-90-0; **6/63** ($2TsO^-$), 123510-60-1; **6/66** ($2Br^-$), 123510-84-9; **6/76** ($2Br^-$), 123510-85-0; **6/77** (Br^-), 123540-52-3; **8/39** ($2Br^-$), 123509-91-1; **8/53** ($2Br^-$), 123509-92-2; **8/59** ($2Br^-$), 123509-93-3; **8/61** ($2Br^-$), 123509-94-4; **8/62** ($2Br^-$), 123540-47-6; **9/39** ($2Br^-$), 123509-95-5; **9**/41 ($2TsO^-$), 123509-98-8; **9/53** ($2TsO^-$), 123510-01-0; **9/59** ($2Br^-$), 123510-03-2; **9/61** ($2Br^-$), 123510-04-3; **9/62** ($2Br^-$), 123510-05-4; **10/22** (TsO^-), 123540-33-0; **10/39** ($2Br^-$), 123510-06-5; **10**/41 ($2Br^-$), 123510-07-6; **10/53** ($2Br^-$), 123510-10-1; **10/58** ($2Br^-$), 123510-11-2; **10/59** ($2Br^-$), 123510-12-3; 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79-19-6; 1-methyl-2-(4′-cyanophenyl)imidazo[1,2-*a*]pyridinium tosylate, 123510-87-2; 2-phenylimidazo[1,2-*a*]pyridine, 4105-21-9; hydrazine, 302-01-2; hydrazide, 25415-88-7; *S*-methyl thiosemicarbazide hydriodide, 35600-34-1; *N*-aminoguanidinium bicarbonate, 2582-30-1; *p*-toluenesulfonic acid, 104-15-4; 1*H*-imidazoline-2(3*H*)-thione, 872-35-5; 3,4,5,6-tetrahydro-2(1*H*)-pyrimidinethione, 2055-46-1; *N*-amino-*N*′-hydroxyguanidium tosylate, 36826-58-1; *N*-amino-*N*′-nitroguanidine, 18264-75-0; *N*-aminomorpholine, 4319-49-7; 1,2,4-triazol-3-ylhydrazine, 38767-33-8; 3,4-diamino-1,2,4-triazole, 38104-45-9; 3,4,5-triamino-(4*H*)-1,2,4-triazole, 473-96-1; (4-amino-4*H*-1,2,4-triazol-3-yl)hydrazine, 6421-06-3; 4-aminopyrimid-2-ylhydrazine, 584-13-4; 2-pyridylhydrazine, 4930-98-7; pyrimid-2-ylhydrazine, 7504-94-1; 4-aminopyrimid-2-ylhydrazine, 123510-88-3; (4,6-diaminopyrimid-2-yl)hydrazine, 123510-89-4; diaminopyridinium iodide, 4931-36-6; thiazol-2-ylhydrazine, 30216-51-4; 3,4-diamino-1,2,4-triazole-5-thione, 3529-50-8; 5-aminopyrazol-2-ylhydrazine, 123510-91-8.

Supplementary Material Available: Tables showing melting point and elemental composition and a summary of biological data for all compounds tested (27 pages). Ordering information is given on any current masthead page.

Synthesis of Some 3-(1-Azabicyclo[2.2.2]octyl) 3-Amino-2-hydroxy-2-phenylpropionates: Profile of Antimuscarinic Efficacy and Selectivity

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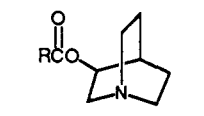
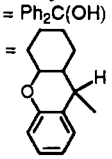
Nova Pharmaceutical Corporation, Baltimore, Maryland 21224-2788. *Received March 21, 1989*

A series of 3-quinuclidinyl atrolactate [3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-2-phenylpropionate, QNA] derivatives in which the methyl group of the parent is substituted with a tertiary amino substituent was prepared and tested for antimuscarinic activity. In general, potency was markedly decreased, although the morpholinyl and thiomorpholinyl derivatives retained significant activity. These compounds were also examined for muscarinic receptor subtype selectivity. Their subtype selectivities were comparable to that of (*R*,*R*)-QNA. The results of this investigation suggest possible differences in the accessory binding sites of the proteinaceous receptor subtypes.

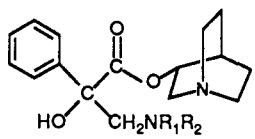
A principle utilized for derivation of structurally novel anticholinergics[1] entails appropriate substitution[2,3] of the acetyl group of an agonist and incorporation of the ethanolamine chain into a rigid cyclic system. Exemplary of such compounds are ones in which the acetyl group of 3-(1-azabicyclo[2.2.2]octyl) acetate (3-quinuclidinyl acetate, aceclidine, **1a**), a potent and selective muscarinic receptor agonist,[4-6] is properly substituted. This has resulted in the potent antimuscarinic agents 3-quinuclidinyl benzilate (QNB, **1b**),[7,8] 3-quinuclidinyl xanthene-9-carboxylate (QNX, **1c**),[9] and 3-quinuclidinyl atrolactate (QNA, **1d**).[10,11]



1a: R = CH_3
b: R = $Ph_2C(OH)$
c: R =

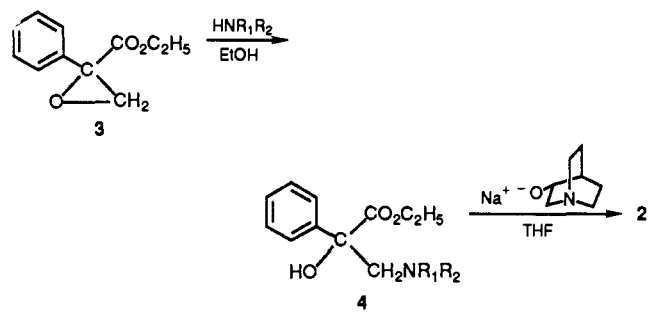


d: R = $PhC(CH_3)(OH)$



2

Scheme I



Examination of a large number of analogues and derivatives of QNB (**1b**), QNX (**1c**), and QNA (**1d**)[9-13] has resulted in the observation of remarkable muscarinic M_1 receptor subtype selectivity for some of the optical isomers of QNA in particular.[14] To further study this subtype selectivity, a series of QNA derivatives **2** bearing a tertiary amine functionality on the atrolactic acid methyl group, i.e., 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-

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