Bioactive Ryanoids from Nucleophilic Additions to 4,12-Seco-4,12-dioxoryanodine

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Ryanoids are the most potent inhibitors known for the calcium-release channel (ryanodine receptor), and they are also botanical insecticides. Twenty-two new ryanoids are described in which the C-4, C-12 bond is ruptured or replaced with an oxygen bridge and in which substituents at C-4 and C-12 are modified to have a wide range of polarities. They are obtained by nucleophilic additions to the 4,12-seco-4,12-dioxo compounds or diketones prepared from ryanodine and dehydroryanodine by periodate oxidation. Structures of the new compounds are distinguished by changes in NMR chemical shifts of ^{13}C and ^{1}H nuclei in the regions of C-4 and C-12. The new ryanoids are compared with ryanodine as inhibitors of [^{3}H]ryanodine binding using a rabbit muscle sarcoplasmic reticulum preparation alone or with ATP and a mouse brain receptor with ATP. They are also examined as knockdown agents for houseflies pretreated with a cytochrome P_{450} oxidase inhibitor to suppress detoxification and then injection with the ryanoid. The diketones have very weak binding activity in the receptor assays and very low toxicity to flies. Activity approaching that of ryanodine in both the receptor and fly assays is obtained for ketals with small groups at C-12 and polar substituents such as OH or NHOH at C-4. The oximes range from low to moderate potency. Addition of thiols to the vinyl group of dehydroryanodine gives three thioethers all of low biological activity. With most ryanoids addition of ATP to the muscle system increases its sensitivity to near that found for the brain receptor with ATP; possible exceptions are compounds with phenyl substituents. Activity at the calcium-release channel generally follows housefly toxicity although the hydrazine and hydroxyamine adducts are much weaker than expected perhaps due to dissociation under the assay conditions.

Introduction

Ryanodine (1) and dehydroryanodine (2) are potent inhibitors of the calcium-release channel[1,2] and the principal active ingredients of the botanical insecticide ryania.[3-5] [^{3}H]Ryanodine is the standard radioligand for characterizing the calcium release channel (ryanodine receptor).[1,2,4,6] Although the physical structure of the muscle receptor has been revealed by electron microscopy[7] and it has been sequenced,[6,8,9] the localization of the ryanodine binding site within the channel is unknown. The nature of this site is deduced primarily from considerations of the relation between ryanoid structure and receptor potency or toxicity. The structural aspects of ryanodine action are defined on the basis of 10 natural ryanoids[5] and about 20 analogs from chemical modifications of 1 and 2.[2] Further progress requires a different approach to alter other parts of the molecule.

1 RYANODINE
2 DEHYDRORYANODINE (9,21-ene)
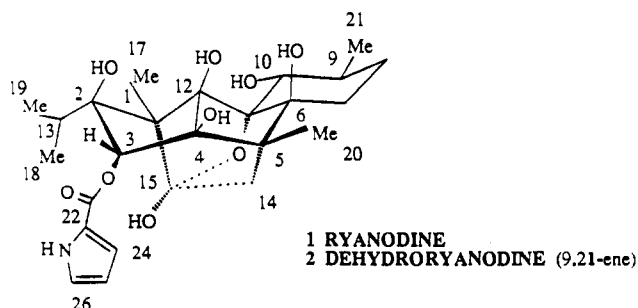
Ryanodine has a hydrophilic face (*cis*-hydroxyls at C-2, C-4, C-6, and C-12 and NH and C=O of the pyrrolecarboxylate) with a conformation fixed by the hemiketal ring

structure. Although much is understood about the effect of the structure of other molecular regions on activity,[2,5] little is known relative to the hydrophilic face. In the central portion of the molecule, one bridging ring is opened with acid at C-1/C-15 forming anhydroryanodine and another with periodate at C-4/C-12 forming 4,12-seco-4,-12-dioxoryanodine (3); both compounds were utilized by Wiesner's group in the classical structural proof for ryanodine,[3] and anhydroryanodol (the hydrolysis product of anhydroryanodine) was used by Deslongchamps and associates in the synthesis of ryanodol.[10] Anhydroryanodine is inactive,[2,5] but the potency of diketone 3 is not reported. Attention here was focused on 3 from ryanodine and the corresponding 4 from dehydroryanodine as starting materials for new compounds altered on the hydrophilic face (Figure 1).

Diketone 3 has several important features relative to availability, reactivity, and conformation. It is easily obtained in high yield[11] and is stable on storage. Both ketone groups are relatively hindered, and the β-hydroxycarbonyl functions are easily degraded by base.[3] Rupture of the C-4/C-12 bond reduces strain and allows conformational inversion. Conformer 3 of the cyclooctanedione ring retains much of the geometry of 1 whereas conformer 3A is more hindered than 3 with severe flag pole interactions between the C-2 isopropyl and the C-14 methylene.

This communication describes nucleophilic addition reactions at one or both carbonyl functions of 3 and 4 as a convenient approach to modify the central ring structure and the polar face of the molecule. It gives the preparations, structural assignments, and biological activities of 4- and 12-hydroxy ketal and 12-H ketal analogs with C-4 and/or C-12 hydroxy, hydroxyamino, alkoxyamino, oximino, hydrazino, and semicarbazide substituents. It

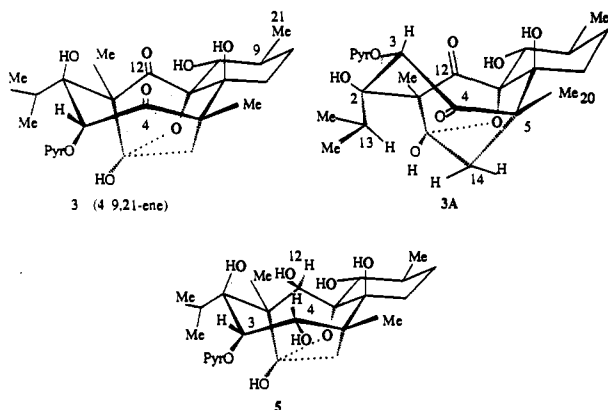† Present address: Sandoz Agro, Inc., Des Plaines, IL 60018.

Figure 1. Structures of 4,12-seco-4,12-dioxoryanodine (diketone 3) and its conformer (3A) and 9,21-ene (4) and diol (5) derivatives. Pyr = 1*H*-pyrrol-2-ylcarbonyl.
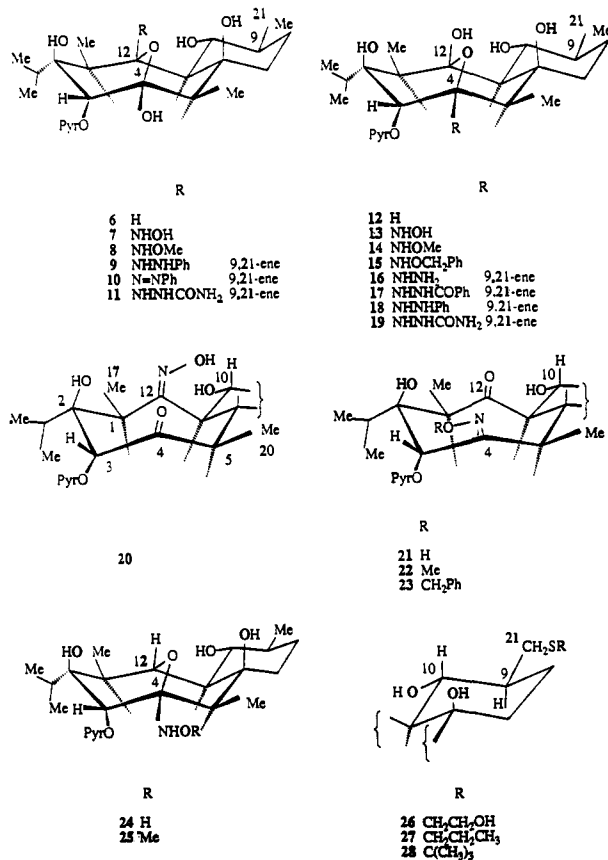

Figure 2. Partial structures showing modified portions of 4- and 12-hydroxy ketals 6–19, prepared from diketones 3 and 4, 12-H ketals 24 and 25 from oximes 21 and 22, oximes 20–23 from diketone 3, and thioethers 26–28 from 9,21-diene 2. Pyr = 1*H*-pyrrol-2-ylcarbonyl.

also reports a series of thioethers formed by addition of thiols to the vinyl group of dehydroryanodine. The derivatives vary over a wide range in structure (Figure 2) and biological activity and therefore help define the stereochemical and polarity features of the ryanodine binding site in the calcium-release channel.

Nucleophilic Additions to 4,12-Seco-4,12-dioxoryanodine

Reduction of Diketone 3 to Diol 5 and Hemiketals 6 and 12. Four steric approaches are available for nucleophilic addition to one or the other ketone group, but attack from within the ring would appear to be less

favored on steric grounds. The reaction is also likely to be influenced by complexation with hydroxyl groups. Further, attack from outside the ring could result in ketal formation by addition of the *endo*-hydroxyl to the unreacted carbonyl group. In the present study, reaction of 3 with excess borohydride in methanol slowly gave a mixture which could be separated by rotary chromatography. The main products 6 and 12 arose from monoreduction and were stable ketals which were not further reduced to diols. Although the 4-ketone appears slightly less hindered with its flanking 3-H, the major product 6 is formed by reduction of the 12-carbonyl probably due to complexation of the reagent with the 10-hydroxyl. Ryanodine and dehydroryanodine readily form borates with borohydride, but hemiketals 6 and 12 lack boron as judged by their mass spectra which gave the expected molecular ions (FAB).

A small quantity of diol 5 was also obtained, and this is thought to arise by prior reduction of the 4-carbonyl from within the cyclooctanedione ring which could be assisted by complexation with the 6- or 2-hydroxyl. Later reduction of the 12-carbonyl could then occur normally from the outside as observed for 6.

Other Addition Reactions. The high bioactivity of ketal 6 noted later encouraged us to look for other simple nucleophilic addition products from the diketone. Although reported to be readily degraded by mild bases,[3] we were pleased to note that 3 is stable in hot (70 °C) pyridine for several hours and when treated with hydroxyamine hydrochloride in this solvent at 50 °C formed two initial adducts. The major product 13, which corresponds to attack at the less hindered 4-position, dissociates in protic solvents ($t_{1/2}$ in MeOD ca. 8 h at 25 °C). The minor adduct 7 was more stable ($t_{1/2}$ in MeOD ca 4 h at 50 °C) and was characterized by its HRMS (FAB). Relatively stable solutions of both were obtained in dimethyl sulfoxide (DMSO) as observed by NMR, and these were used for bioassay.

On an analogous basis, treatment of diketone 3 with methoxyamine gave two adducts (8 and 14), which showed close agreement in their NMR spectra with the corresponding hydroxyamine products, were relatively stable in protic solvents and were fully characterized by HRMS and NMR spectra. A more lipophilic derivative was obtained through nucleophilic addition of (benzyloxy)-amine, with only one isomer (15) being isolated. Under more vigorous conditions the oximation mixture gave oximes 20 and 21. Under similar conditions only one methoxime (22) and one benzyloxime (23) were isolated.

Further adducts were obtained from the reaction of hydrazines with 4. Addition of hydrazine itself took place rapidly to give a hydrazinyl adduct (16) which dissociated easily ($t_{1/2}$ in MeOD ca. 6 h at 25 °C) but was sufficiently stable for spectral characterization and was converted to its stable benzoyl derivative (17). Phenylhydrazine formed isomeric adducts (9 and 18). In contrast with 16 the main product 9 corresponded to attack at C-12. Adduct 9 was oxidized by air and gave phenylazo compound 10. Semicarbazide added slowly to give a mixture of 11 and 19 with attack at C-4 predominating.

Nitrogen-bridged compounds are potentially accessible by reduction of the monooximes with cyanoborohydride resulting from preferential attack on the oxime group whereas borohydride should react more readily with the ketone function to give 12-H ketals.[12] Unfortunately 20

Table I. NMR Shifts for Environmentally-Sensitive Nuclei[a] of Cyclic Ketals 6–19, 24, and 25

			ppm (CD$_3$OD)								
substituent			C-4 region					C-12 region			
no.	C-4	C-12	H-3	C-3	C-4	C-20	H$_3$-20	C-1	C-12	C-17	H$_3$-17
				4-Hydroxy Ketals							
6	HO	H	5.43	84.1	101.8	17.9	0.90	55.1	83.8	15.9	1.28
7	HO	NHOH	5.45	85.5	101.0	18.0	0.89	60.3	100.0	12.1	1.36
8	HO	NHOMe	5.44	85.0	100.6	18.1	0.89	60.5	103.0	11.7	1.28
9	HO	NHNHPh	5.34[b]	85.3[b]	101.1[b]	18.1[b]	0.88[b]	60.5[b]	103.0[b]	13.1[b]	1.30[b]
10	HO	N=NPh	5.61	82.7	101.7	17.8	0.95	60.7	106.5	12.4	1.21
11	HO	NHNHCONH$_2$	5.40	83.7	101.0	18.1	0.91	59.4	101.4	12.2	1.28
				12-Hydroxy Ketals							
12	H	OH	5.64	76.7	74.3	19.3	0.76	59.2	109.8	12.2	1.24
13	NHOH	OH	6.08	75.2	93.0	17.4	0.79	58.9	109.0	11.9	1.22
14	NHOMe	OH	6.21	74.8	93.1	17.0	0.83	58.9	109.8	11.8	1.20
15	NHOCH$_2$Ph	OH	6.33	75.0	93.0	17.2	0.82	58.7	109.6	11.7	1.22
16	NHNH$_2$	OH	5.84	74.9	93.0	18.2	0.84	58.6	110.4	12.3	1.24
17	NHNHCOPh	OH	5.86	77.1	92.6	18.7	0.95	58.6	110.0	13.1	1.22
18	NHNHPh	OH	6.21	76.0	93.2	18.1	1.01	58.7	108.1	11.9	1.25
19	NHNHCONH$_2$	OH	5.85	78.7	92.5	17.9	0.92	58.4	109.5	11.7	1.22
				12-H Ketals							
24	NHOH	H	5.92	75.3	93.8	17.4	0.81	55.1	83.0	15.8	1.29
25	NHOMe	H	6.01	75.5	93.3	17.0	0.85	55.1	83.7	15.6	1.26

[a] C-5 and C-11 are relatively insensitive to substitution differences and are not included. [b] CD$_3$OD/CDCl$_3$.

and 21 were resistant to the less reactive cyanoborohydride, although sodium borohydride readily reduced the carbonyl groups. The less abundant 20 gave a mixture which was not further examined, but its isomer (21) easily gave a single 12-H ketal (24). The related methoxime (22) was reduced in the same way to give 25.

Thioethanol adds readily and stereospecifically to dehydroryanodine to give the more stable equatorial isomer 26. Propanethiol and 2-methylpropane-2-thiol require catalysis with AIBN and react in the same way to give thioethers 27 and 28.

Structural Assignments

Diol 5 and Hemiketals 6 and 12. Hydroxy ketals 6 and 12 lack ketone resonances and show the expected ketal carbons near 100 ppm. The isomers are distinguished by coupling (*J* = 7.4 Hz) for H-3 and H-4 in 12, whereas in 6 H-3 and the new hydroxymethine proton appear as singlets. The proton spectrum of 5 shows a singlet expected for H-12 and an AB pattern assigned to H-3 and H-4 with *J* = 2.1 Hz, much reduced from its value of 7.4 Hz in 12. The associated dihedrals for these protons are about 100° for the conformation shown as 5 and 60° if inversion of the large ring occurs, either of which could account for the coupling. In 12 the angle is near 10°, which predicts the larger value (*J* = 7.4 Hz). The chemical shift (5.47 ppm) of H-4 in 5 is well downfield from its position (4.32 ppm) in ketal 12, reflecting the proximity of one or more of the hydroxyls at C-2, C-6, or C-12.

Other Addition Products. Structural assignments for cyclic ketals 6–19, 24, and 25 are made on the basis of NMR shifts for environmentally-sensitive nuclei in the regions of C-4 and C-12 (Table I). Some evidence comes from the proton shifts (5.3–5.6 ppm) for H-3 in the 4-hydroxy ketals (6–11) which is near that of ryanodine (5.62 ppm) whereas in the 12-hydroxy ketals this resonance is further downfield (5.64 ppm for 12 and 5.8–6.2 ppm for 13–19). A more secure assignment of structures was needed for those adducts which do not have protons at C-4 or C-12. We therefore carried out full assignments of the carbon spectra of ketal 6 and methoxyamine adduct 14 using short- and long-range correlation NMR methods

and applying the arguments we have used before to identify the quarternary carbons.[13–15] The structure assigned to 14 is further supported by the NOESY spectrum, which shows a strong signal for H-3 and the methyl of the methoxyamino group. 4-Hydroxy ketals 7–11 all show shifts for the C-4 region in fair agreement with those for resonances rigidly assigned for H-3, C-3, C-4, C-20, and H$_3$-20 in ketal 6. Similarly 12-hydroxy ketals 12, 13, and 15–19 have values for the shifts assigned to C-1, C-12, C-17, and H$_3$-17 which agree well with those for the C-12 region in the reference compound 14, with significant variation restricted to nuclei in the vicinity of the different groups at C-4. In comparison of one group with the other it is evident that the shifts for C-3 and the ketal carbons 4 and 12 are excellent indicators for the structure type. The NMR shifts in Table I also fully support the structural assignments for the hydroxy ketals derived from hydroxyamine (7 and 13), methoxyamine (8), (benzyloxy)-amine (15), phenylhydrazine (9, 10, and 18), hydrazine (16 and 17), and semicarbazide (11 and 19). 12-H ketal 24, formed from oxime 21 by hydride reduction, shows a partial structure centered on C-4 which corresponds with that in hydroxyamine adduct 13. In addition, NMR shifts for the C-12 region of 24 correspond to those in ketal 6. The shifts for the 12-H ketals 24 and 25 match well with the reference spectra (6, 13, and 14).

Table II gives the NMR shifts for environmentally-sensitive nuclei important in assigning the structures of diketone 3 and oximes 20–23. The oximes from hydroxyamine were provisionally distinguished on the basis of a profound downfield shift for H-3 to 7.01 ppm in 21 relative to 5.64 ppm in 20. The latter shows a large downfield shift for the H-10 proton, suggesting that the oxime group is at C-12 and is syn to C-10. The proton spectrum for methoxime 22 corresponds well with that for 21. Support for structure 23 was also evident from the low-field position of the H-3 signal. We have further substantiated the structures for the oximes by fully assigning the carbons of both the diketone 3 and the oxime 21 using correlation methods. It is evident that the shifts

Table II. NMR Shifts for Environmentally-Sensitive Nuclei[a] of Diketone 3 and Oximes 20–23

			ppm (CD$_3$OD)							
	substituent		C-4 region					C-12 region		
no.	C-4	C-12	H-3	C-3	C-4	C-5	C-20	H-10	C-12	C-17
3	=O	=O	5.44[b]	87.5[b]	215.0[b]	52.5[b]	21.7[b]	3.81[b]	222.0[b]	13.8[b]
20	=O	=NOH	5.64	89.1	212.1	52.5	21.8	4.38	168.3	16.9
21	=NOH	=O	7.01	72.3	160.2	46.5	24.5	3.76	221.7	13.4
22	=NOMe	=O	6.87	72.7	162.1	47.2	24.5	3.78	224.0	13.1
23	=NOCH$_2$Ph	=O	6.95	71.9	159.4	46.0	24.4	3.72	221.1	12.7

[a] H$_3$-17, H$_3$-20, C-1, and C-11 are relatively insensitive to substituent differences and are not included. [b] C$_5$D$_5$N.

Table III. Relation of Ryanoid Structure to Receptor Potency and Insecticidal Activity

		potency relative to ryanodine (100)				
		ryanodine receptor[a]			housefly[b]	
		rabbit muscle		mouse brain		
no.	compound and substituent	–ATP	+ATP	+ATP	KD$_{50}$	LD$_{50}$
	Ryanodines, Diketones, and Seco-diol					
1	ryanodine	100 ± 4	100 ± 11	100 ± 21	100	100
2	dehydroryanodine	91 ± 21	112 ± 7	77 ± 16	90	136
3	4,12-seco-4,12-dioxoryanodine	1.6 ± 0.3	1.8 ± 0.2		0.85	2.0
4	4,12-seco-4,12-dioxodehydroryanodine	0.79 ± 0.09	0.97 ± 0.07	1.1 ± 0.11	4.0	4.0
5	4,12-seco-4,12-dihydroryanodine	4.7 ± 0.7	5.3 ± 0.4	5.4 ± 0.6		
	Ketals[c]					
	C-4	C-12				
6	OH	H	62 ± 12	64 ± 16	54 ± 14	270	120
7	OH	NHOH	9.0 ± 1.5	11 ± 1.4	11 ± 0.3	20	12
8	OH	NHOMe				1.3	2
9	OH	NHNHPh	2.6 ± 0.2	3.0 ± 0.9	3.3 ± 0.4	<1.3	<3
10	OH	N=NPh	4.7 ± 0.5	6.3 ± 0.5	3.6 ± 0.4	6	6
11	OH	NHNHCONH$_2$	6.1[d]	4.4[d]		<4[d]	<4[d]
12	H	OH	28 ± 2	19 ± 2	21 ± 2	27	40
13	NHOH	OH	14 ± 2[e]	29 ± 3[e]	21 ± 2[e]	270	200
14	NHOMe	OH	23 ± 3	23 ± 2	27 ± 7	13	8
15	NHOCH$_2$Ph	OH	13 ± 2	21 ± 0.5	8.3 ± 0.8	2	2
16	NHNH$_2$	OH	11 ± 1[e]	14 ± 0.4[e]	14 ± 1.4[e]	50	60
17	NHNHCOPh	OH		6.5 ± 0.6		13	40
18	NHNHPh	OH	5.4 ± 0.5	6.1 ± 0.8	2.5 ± 0.5	<2	<2
19	NHNHCONH$_2$	OH	10 ± 1	6.6 ± 2.8		10	6
24	NHOH	H	50 ± 7	67 ± 15	43 ± 7	200	100
25	NHOMe	H	20 ± 2	34 ± 6	28 ± 3	7	20
	Oximes					
	C-4	C-12				
20	=O	=NOH	1.7 ± 0.2	3.1 ± 0.5	1.9 ± 0.3	8	30
21	=NOH	=O	0.76 ± 0.2	0.88 ± 0.1	0.78 ± 0.2	2.7	6
22	=NOMe	=O	0.75 ± 0.2	0.86 ± 0.1	0.70 ± 0.1	10	12
23	=NOCH$_2$Ph	=O	1.3 ± 0.1	2.1 ± 0.3	0.94 ± 0.2	0.8	1
	Thioethers					
	C-21					
26	SCH$_2$CH$_2$OH				0.86 ± 0.09	5	6
27	SCH$_2$CH$_2$CH$_3$				0.88 ± 0.18	4	6
28	SC(CH$_3$)$_3$				0.41 ± 0.23	2	2

[a] IC$_{50}$ values for 1 are 12.0 ± 0.5, 3.5 ± 0.4, and 3.3 ± 0.7 nM for rabbit muscle alone and with ATP and mouse brain with ATP, respectively. [b] KD$_{50}$ at 4 h and LD$_{50}$ at 24 h for 1 injected into PB-pretreated houseflies are 0.19 and 0.27 μg/g, respectively. [c] Ketals 9–11 and 16–19 are derived from 4 and the others from 3. [d] Average of duplicate experiments for receptors and estimate based on single determinations for flies. [e] Dissociate during receptor assay.

in the vicinity of both the 4- and 12-ketones are retaineed in the oximes which are readily distinguished on this basis. In comparison with the parent ketone, an oxime shields the syn carbon much greater than the anti.[16] The upfield shifts for C-3 and C-5 in 21 vs 3 are 15.2 and 6 ppm, respectively, validating the configuration for the 4-oxime as syn to C-3 in 21, and thus also for 22 and 23. Although not tabulated, only small differences are found for C-11 and C-1 in isomer 20, and assignment of the oxime syn to C-10 rests on the deshielding for H-10.

In thioethers 26–28, the C-9 chain is equatorial (as in ryanodine) based on the diaxial coupling for H-9 and H-10 of ~10 Hz.

Structure–Activity Relationships (SAR) (Table III)

Experimental Design and General Features. The study focused primarily on the C-4 and C-12 region in the central portion of the molecule involving nucleophilic additions to the 4,12-diketones which are in themselves of very low biological activity. In contrast, the modifications at C-21 as a peripheral substituent started with the high-potency dehydroryanodine. Ryanoid SAR was evaluated at both receptor and organismal levels.

The calcium-release channel/[³H]ryanodine receptor was examined under three sets of conditions.[1,2,17] Rabbit muscle sarcoplasmic reticulum (SR) preparations were

used in the presence and absence of ATP, and mouse brain membranes were evaluated with ATP. Both ATP and calcium ion are known to increase the open state of the calcium channel.[18] We find that the presence of ATP increases not only the receptor binding activity by (5.6 ± 0.5)-fold but also the apparent potency of the ryanoids by a similar amount $((4.5 \pm 0.2)$-fold), probably by facilitating channel opening. The muscle and brain receptors are very similar in sensitivity when ATP is present, and they are not differentiated by any of the ryanoid probes, with the possible exception of compounds with lipophilic phenyl groups (9, 10, 15, 18, and 23) which, except for 9, are 1.8–2.5 times less potent with brain than with muscle.

Organismal activity was evaluated primarily by injection of the ryanoids into houseflies to determine knockdown (KD_{50}) or kill (LD_{50}) after 4 and 24 h, respectively, using pretreatment with piperonyl butoxide (PB) to minimize cytochrome P_{450}-catalyzed detoxification and thereby more closely approximate intrinsic potency.[2] Mammalian toxicity of two ryanoids was evaluated intraperitoneally (ip) in mice.

C-21 Modifications. Our earlier studies[2] showed that addition of polar oxygenated substituents to the vinyl group of dehydroryanodine reduces receptor potency and fly toxicity. The present investigation therefore considered apolar thioether substituents from radical additions with thiols as a means of providing sulfur substitution, increasing lipophilicity in this area, and in the case of thioethanol, giving a spacer for potential attachment of various affinity labels through the primary alcohol. Unfortunately, thioethers 26–28 all have low potencies in both brain receptor and fly assays. Similar values for the propylthio and (2-hydroxyethyl)thio adducts coupled with the weaker activity for the (2-methylprop-2-yl)thio derivative indicate that steric factors rather than polarity limit their potency.

C-4 and C-12 Modifications. In the receptor assays, the diketo compounds (3 and 4) have about 1% of the potency of the parent ryanodines (1 and 2), consistent with their greatly reduced hydrophilicity. Seco-diol 5, with about 5% of the potency of ryanodine in preliminary assays, has steric crowding at the C-12 hydroxyl with H-3 and H-4 compared with 3 and may well adopt a conformation equivalent to 3A. Ketals 6–19, 24, and 25 provide a graded series of structural changes resulting in a wide range of potencies. One of the most active compounds, nearly as potent as ryanodine, is 4-hydroxy ketal 6. Other 4-hydroxy ketals (7 and 9–11) range from 3 to 11% of the potency of ryanodine, indicating that the H at C-12 in 6 is greatly preferred over NHOH, NHNHPh, N=NPh, or NHNHCONH₂. 12-Hydroxy ketal 12 is only about half as potent as the isomeric 4-hydroxy ketal 6, indicating the need for a polar group at C-4. Alternative functional groups at C-4 do not significantly increase the observed potency. However, the hydrazine (16) and hydroxyamine (13) adducts dissociate under the conditions of the test so their intrinsic potency is unknown; indeed, the hydrazine is fully dissociated into the low activity diketone when incubated in the rabbit muscle buffer for 2 h at 37 °C. On the other hand, all of the other ketals including the semicarbazide adduct 19 do not dissociate appreciably during the incubation time. The C-4 hydroxyamine and methoxyamine (24 and 25) have one-fifth to two-thirds the potency of ryanodine, reflecting the favorable hydrogen substitutent at C-12 and polar substituent at C-4 as in 6.

Oximes 20–23 approximate the potency of diketone 3, indicating that oxidation of either ketone substituent is insufficient to restore major activity.

The SAR for housefly KD generally follows the trends established by the receptor IC_{50}s, but there are also important differences. Diketone 3 is only 1–2% as potent as ryanodine. Compounds 6, 13, 16, and 24 are definitely better than the other hydroxy ketals. They share a polar substituent (OH, NHOH, or NHNH₂) at C-4 combined with H (6 and 24) or OH (13 and 16) at C-12. These findings are distinctly different than for the mammalian receptor binding site. The other hydroxy ketals are notable for their low potency. The most active oximes are 20 and 22 for which the enhanced organismal potency is not reflected in vitro in the receptor assay. Activity of the C-12 hydroxyamine 7 is higher than that of its more lipophilic analogs 8–10. For the 12-hydroxy ketals a polar substituent at C-4 is required for high toxicity, e.g. NHOH (13) and NHNH₂ (16) groups give ketals which have toxicity in the range of ryanodine. Ketals 13 and 16 must undergo rapid binding in vivo since they dissociate readily to low activity diketones based on in vitro studies. The polar semicarbazide substituent in 19 confers low activity. Substituents with lower polarity such as hydrogen (12), NHOMe (14 and 25), or phenyl (15 and 18 but not 17) give much lower toxicity. The low toxicity of benzyl derivative 15 relative to its receptor potency suggests that it may be readily detoxified. Oximes 20 and 22 but not 21 and 23 show significant toxicity by injection, and separate studies indicate that 20 and 22 are equally or more effective than ryanodine by topical application at 100 μg/g with PB.

4-Hydroxy ketal 6 is 3.5-fold less toxic than ryanodine by ip administration to mice, i.e. LD_{50}s of 0.49 and 0.14 mg/kg, respectively. On this basis, compound 6 has improved selectivity for toxicity to houseflies versus mice.

In summary, a hydrophilic group (OH, NHOH, or NHNH₂) is preferred at C-4 and a small group at C-12 for the most favorable interactions of the polar face of the new ryanoid probes with the calcium-release channel.

Experimental Section

Chemistry. General. Ryanodine (1) and dehydroryanodine (2) of above 95% purity were isolated as crystalline materials as previously described.[5] Reactions were carried out on a semimicroscale with product isolation by rotary chromatography as crystalline solids or resins. The purity of each product was >95% based on TLC, ¹H NMR, and ¹³C NMR. The structures were determined by ¹H and ¹³C NMR and HRMS.

Solvents used were HPLC grade. Separations were achieved by rotary chromatography with 1-mm silica gel PF₂₅₄ (E. Merck, Darmstadt, Germany) using a Model 8924 Chromatotron. Mass spectra were recorded with a Kratos MS-50 instrument.

¹H and ¹³C NMR spectra were acquired at 300 and 75 MHz, respectively, using a Bruker AM-300 spectrometer and CD₃OD, CD₃OD/CDCl₃, or C₅D₅N as the solvent. The chemical shift reference was tetramethylsilane. All ¹³C spectra, acquired using a 60° pulse (4.1 μs) and 1.5-s interpulse delay, had a digital resolution of 2.3 Hz. Two-dimensional spectra included in these studies were ¹H–¹H correlation spectroscopy (COSY); homonuclear nuclear Overhauser effect (NOESY) spectra; long- and short-range heteronuclear C–H correlation; inverse C–H correlation (INVXCOR). Both COSY and NOESY spectra were acquired with spectral widths varied from 2100 to 2400 Hz by using a 1024 × 256 data set, zero filling by a factor of 2, and multiplication by an unshifted sine-bell function prior to Fourier transformation. In the long-range C–H correlation, short-range C–H correlation, and INVXCOR experiments, the spectral widths varied from 12 800 to 16 800 Hz (¹³C) and 2160 to 2700 Hz (¹H). These experiments were acquired using 2048 × 256 data set, zero filling

to 4096 × 256, and transforming after multiplication by an exponential function (line-broadening factor of 6 Hz) in the ^{13}C dimension and an unshifted sine-bell function in the ^{1}H dimension. The number of scans in each block of the 2D acquisitions varied with sample concentration. Spectral data of ketals and oximes which differ little from the fully assigned reference spectral data are given only in abbreviated form in Tables I and II.

Diketones 3 and 4 from 1 and 2 with Periodic Acid. Oxidation of 1 with periodic acid gave the diketone "oxoryanodine"[11] (3) in 80–85% yield. ^{1}H NMR.[15] ^{13}C NMR (C$_5$D$_5$N): 60.3 (C-1), 80.5 (C-2), 87.5 (C-3), 215.0 (C-4), 52.5 (C-5), 77.0 (C-6), 28.0 and 28.5 (C-7 and -8), 35.0 (C-9), 71.0 (C-10), 90.7 (C-11), 222.0 (C-12), 37.0 (C-13), 41.5 (C-14), 103.7 (C-15), 13.8 (C-17), 18.5 and 18.3 (C-18 and C-19), 21.7 (C-20), 19.1 (C-21), 160.0 (C-22), 122.3 (C-23), 116.9 (C-24), 111.0 (C-25), 126.3 (C-26). Treatment of 2 with periodic acid in the same way gave the corresponding diketone (4) which crystallized from methanol, mp 220–222 °C (yield 80–85%). ^{1}H NMR (CD$_3$OD): 7.03 (m, H-26), 6.93 (m, H-24), 6.23 (m, H-25), 5.51 (s, H-3), 4.99, 4.90 (H$_2$-21), 4.71 (H-10), 3.58 (d, 14 Hz) and 2.15 (d, 14 Hz), AB H$_2$-14, 2.51 (m, H-8$_{ax}$), 2.23 (m, H-8$_{eq}$), 2.15 (m, H-13), 2.00 (m, H-7$_{ax}$), 1.60 (m, H-7$_{eq}$), 1.31 (s, H$_3$-17), 1.37 (d, 7.0 Hz) and 1.00 (d, 7.1 Hz), (H$_3$-18 and -19), 0.93 (s, H$_3$-20). ^{13}C NMR (C$_5$D$_5$N): 60.2 (C-1), 80.5 (C-2), 87.5 (C-3), 213.5 (C-4), 52.3 (C-5), 77.3 (C-6), 29.7 and 28.8 (C-7 and -8), 148.3 (C-9), 67.8 (C-10), 91.8 (C-11), 221.2 (C-12), 37.1 (C-13), 41.4 (C-14), 103.7 (C-15), 13.8 (C-17), 18.4 and 18.2 (C-18 and -19), 21.7 (C-20), 108.1 (C-21), 160.0 (C-22), 122.2 (C-23), 117.0 (C-24), 111.1 (C-25), 126.4 (C-26), HRMS (FAB): m/z calcd for C$_{25}$H$_{31}$NO$_9$Na$^+$ 512.1900, found 512.1903.

Diol 5 and Hemiketals 6 and 12 from 3. Diketone 3 (50 mg, 0.10 mmol) in THF (2 mL) and MeOH (2 mL) was treated with NaBH$_4$ (76 mg, 2.0 mmol) at –20 °C during 2 h. After addition of aqueous NH$_4$OAc the products were isolated by ethyl acetate extraction and separated by rotary chromatography using CHCl$_3$–MeOH–40% aqueous MeNH$_2$ mixtures (90:8:2 to 73:15:2) which gave first the less polar ketal 12 (14 mg, 28%). ^{1}H NMR (CD$_3$-OD): 7.05 (m, H-26), 6.83 (m, H-24), 6.23 (m, H-25), 5.64 (d, 7.4 Hz, H-3), 4.22 (d, 7.4 Hz, H-4) AB, 3.78 (d, 10.3 Hz, H-10), 2.78 (d, 14.2 Hz) and 1.82 (d, 14.2 Hz) AB H$_2$-14, 1.53 (m, 8-H$_{ax}$), 1.50 (m, H-7$_{eq}$), 1.33 (m, H-8$_{eq}$), 1.24 (s, H$_3$-17), 1.22 (d, 6.9 Hz) and 1.01 (d, 6.8 Hz) (H$_3$-18 and -19), 1.00 (d, 6.4 Hz, H$_3$-21), 0.76 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 59.2 (C-1), 78.7, 80.5, and 82.8 (C-2, -6, and -11), 76.7 (C-3), 74.3 (C-4), 41.6 and 40.4 (C-5 and -14), 27.1 and 28.5 (C-7 and -8), 34.9 and 35.5 (C-9 and -13), 73.4 (C-10), 109.8 (C-12), 40.4 (C-14), 103.3 (C-15), 12.2 (C-17), 18.9 and 19.1 (C-18 and -19), 19.3 (C-20), 20.6 (C-21), 161.2 (C-22), 123.0 (C-23), 117.0 (C-24), 111.0 (C-25), 125.9 (C-26). HRMS (FAB): m/z calcd for C$_{25}$H$_{35}$NO$_9$Na$^+$ 516.2210, found 516.2208. This was followed by a mixed fraction (5 mg) and then the isomeric ketal 6 (25 mg, 50%). ^{1}H NMR (CD$_3$OD): 7.04 (m, H-26), 6.83 (m, H-24), 6.23 (m, H-24), 5.43 (s, H-3), 4.32 (s, H-12), 3.57 (d, 10.2 Hz, H-10), 2.94 (d, 14.5 Hz) and 1.90 (d, 14.5 Hz) AB H$_2$-14, 2.01 (m, H-7$_{ex}$), 1.50 (m, H-8$_{ax}$), 1.33 (m, H-7$_{eq}$), 1.28 (s, H$_3$-17), 1.07 (d, 6.4) and 1.01 (d, 7 Hz) (H$_3$-18 and -19), 1.02 (d, 7 Hz, H$_3$-21), 0.90 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 55.1 (C-1), 78.1 (C-2), 84.1 (C-3), 101.8 (C-4), 45.2 (C-5), 81.2 (C-6), 27.1 (C-7), 28.3 (C-8), 35.4 (C-9), 73.2 (C-10), 81.4 (C-11), 83.8 (C-12), 34.0 (C-13), 42.3 (C-14), 103.8 (C-15), 15.9 (C-17), 19.3 and 19.4 (C-18 and -19), 17.9 (C-20), 20.0 (C-21), 161.5 (C-22), 123.3 (C-23), 116.8 (C-24), 110.8 (C-25), 125.6 (C-26). HRMS (FAB): m/z calcd for C$_{25}$H$_{35}$NO$_9$Na$^+$ 516.2210, found 516.2211. Further elution of the plate gave diol 5 (3 mg, 6%). ^{1}H NMR (CD$_3$OD/CDCl$_3$): 6.98 (m, H-26), 6.85 (m, H-24), 6.20 (m, H-25), 5.69 (d, 2.1 Hz) and 5.57 (d, 2.1 Hz) AB H-3 and H-4, 3.33 (d, 10.5 Hz, H-10), 3.22 (d, 14.6 Hz) and 1.80 (d, 14.6 Hz) AB H$_2$-14, 2.05 (m, H-13), 1.78 (m, H-7$_{ax}$), 1.60 (m, H-9), 1.29 (s, H$_3$-17), 1.10 (d, 6.3 Hz, H$_3$-21), 0.92 (d, 6.9 Hz) and 1.21 (d, 7.0 Hz) (H$_3$-18 and -19), 0.81 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD/CDCl$_3$): 53.2 (C-1), 79.4, 81.2 and 83.4 (C-2, -6 and -11), 84.3 and 83.4 (C-3 and -12), 72.5 and 68.8 (C-4 and -10), 44.5 and 45.5 (C-5 and -14), 28.3 and 30.0 (C-7 and C-8), 35.2 and 37.9 (C-9 and -13), 104.2 (C-15), 17.2, 17.5, 19.7, 21.6, and 23.7 (C-17, -18, -19, -20, and -21), 162.2 (C-22), 122.9 (C-23), 116.4 (C-24), 110.4 (C-25), 124.7 (C-26). HRMS (FAB): m/z calcd for C$_{25}$H$_{37}$NO$_9$Na$^+$ 518.2366, found 518.2360.

Ketals 7 and 13 from 3 with Hydroxyamine. Diketone 3

(50 mg, 0.1 mmol) and hydroxyamine hydrochloride (200 mg, 2.9 mmol) were heated in pyridine (1 mL) at 50 °C for 2 h and left overnight at 20 °C. Most of the pyridine was removed under reduced pressure and the product acidified at 0 °C to pH 2 and isolated with ethyl acetate. Rotary chromatography using ether–ethyl acetate–methanol (73:25:2) gave unchanged diketone mixed with a little oxime (15 mg) (see below) followed by the adduct 7 (9 mg, 17%). HRMS (FAB): m/z calcd for C$_{25}$H$_{36}$N$_2$O$_{10}$H$^+$ 525.2446, found 525.2452. Further elution and evaporation at 20 °C gave the isomeric adduct 13 (17 mg, 32%). ^{1}H NMR (CD$_3$-OD): 7.05 (m, H-26), 6.81 (m, H-24), 6.23 (m, H-25), 6.08 (s, H-3), 3.80 (d, 10.3 Hz, H-10), 3.07 (d, 14.5 Hz) and 1.87 (d, 14.5 Hz) AB H$_2$-14, 2.05 (m, H-7$_{ax}$), 2.09 (m, H-13), 1.85 (m, H-9), 1.22 (s, H$_3$-17), 1.03 (d, 6.0 Hz, H$_3$-21), 1.17 (d, 7.0 Hz) and 1.01 (d, 6.1) (H$_3$-18 and -19), 0.79 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 58.9 (C-1), 81.4 (C-2), 75.2 (C-3), 93.0 (C-4), 44.0 and 43.3 (C-5 and -14), 78.6 (C-6), 28.8 (C-7), 28.0 (C-8), 35.3 (C-9), 73.5 (C-10), 82.7 (C-11), 109.0 (C-12), 34.8 (C-13), 102.6 (C-15), 11.9 (C-17), 18.6 and 19.0 (C-18 and -19), 17.4 (C-20), 19.3 (C-21), 161.6 (C-22), 123.2 (C-23), 116.9 (C-24), 111.0 (C-25), 126.7 (C-26).

Ketals 8 and 14 from 3 with Methoxyamine. Diketone 3 (54 mg, 0.11 mmol) was heated with methoxyamine hydrochloride (1 mL, 30% aqueous, 3.6 mmol) in pyridine (2 mL) for 9 h at 65 °C. Workup as above for the hydroxyamine adducts and rotary chromatography using ethyl acetate–hexane mixtures (2:3 and 1:1) gave methoxime 22 (7 mg, 12%, see below) and then the less polar adduct 8 (12 mg, 20%). HRMS (FAB): m/z calcd for C$_{26}$H$_{38}$N$_2$O$_{10}$H$^+$ 539.2603, found 539.2620. Later fractions were the more polar adduct 14 (27 mg, 46%). ^{1}H NMR (CD$_3$OD): 7.04 (m, H-26), 6.77 (m, H-24), 6.22 (m, H-25), 6.21 (s, H-3), 3.81 (d, 10.3 Hz, H-10), 3.58 (OCH$_3$), 3.18 (d, 14.5 Hz) and 1.88 (d, 14.5 Hz) AB H$_2$-14, 2.05 (m, H-13), 2.10 (m, H-7$_{ax}$), 1.86 (m, H-9), 1.20 (s, H$_3$-17), 1.01 (d, 6.9 Hz, H$_3$-21), 1.19 (d, 6.8 Hz) and 1.02 (d, 7.0 Hz) (H$_3$-18 and -19), 0.83 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 58.9 (C-1), 81.7 (C-2), 74.8 (C-3), 93.1 (C-4), 44.2 (C-5), 78.7 (C-6), 28.3 and 28.8 (C-7 and -8), 34.8 (C-9), 73.5 (C-10), 82.7 (C-11), 109.8 (C-12), 35.3 (C-13), 43.2 (C-14), 102.7 (C-15), 11.8 (C-17), 18.5 and 19.1 (C-18 and -19), 17.0 (C-20), 19.0 (C-21), 160.8 (C-22), 123.4 (C-23), 116.6 (C-24), 110.8 (C-25), 125.5 (C-26), 62.6 (OCH$_3$). HRMS (FAB): m/z C$_{26}$H$_{38}$N$_2$O$_{10}$H$^+$ 539.2603, found 539.2605.

Ketals 9, 10, and 18 from 4 with Phenylhydrazine. Diketone 4 (30 mg, 0.061 mmol) in EtOH (1 mL) was heated with phenylhydrazine (150 mg, 1.39 mmol) under nitrogen at 60 °C for 4 h and set aside for 48 h. The solvent was removed and water added, and acidification (pH 2), extraction with ethyl acetate, and rotary chromatography with chloroform–methanol (20:1) gave an early fraction of phenylazo compound 10 (3 mg, 8%). HRMS (FAB): m/z calcd for C$_{31}$H$_{37}$N$_3$O$_9$H$^+$ 596.2608, found 596.2627. After a mixed fraction (9 mg), phenylhydrazine adduct 9 (12 mg, 33%) was obtained. HRMS (FAB): m/z calcd for C$_{31}$H$_{39}$N$_3$O$_9$H$^+$ 598.2765, found 598.2773. The mixed fraction was separated by rotary chromatography with ether–hexane (2:1) containing MeOH (1%) to give the less polar adduct 18 (1.7 mg, 5%). HRMS (FAB): m/z calcd for C$_{31}$H$_{39}$N$_3$O$_9$H$^+$ 598.2765, found 598.2779.

Ketals 11 and 19 from 4 with Semicarbazide. Diketone 4 (20 mg, 0.04 mmol) in a solution of semicarbazide hydrochloride (0.3 g, 2.7 mmol), NaOAc·3H$_2$O (0.5 g, 3.7 mmol), ethanol (0.5 mL), and THF (0.75 mL) was set aside for 3 weeks. Isolation with ethyl acetate and rotary chromatography using chloroform–methanol (1:15) gave a mixed fraction of semicarbazide adducts which was separated using rotary chromatography in ethyl acetate to give the less polar 19 (7 mg, 31%). HRMS (FAB): m/z calcd for C$_{26}$H$_{36}$N$_4$O$_{10}$H$^+$ 565.2510, found 565.2505. Further elution gave the more polar 11 (3 mg, 13%). HRMS (FAB): m/z calcd for C$_{26}$H$_{36}$N$_4$O$_{10}$H$^+$ 565.2510, found 565.2500.

Ketal 15 from 3 with (Benzyloxy)amine. Diketone 3 (29 mg, 0.059 mmol) was added to a solution of (benzyloxy)amine hydrochloride (200 mg, 1.25 mmol) in ethanol–water (1.5 mL) which had been adjusted to pH 7 with 5% NaOH solution. A few drops of THF were added, and the solution was set aside for 5 days. Acidification, isolation with ethyl acetate, and rotary chromatography with ether–hexane mixtures gave adduct 15 (20 mg, 55%). ^{1}H NMR (CD$_3$OD): 7.45–7.25 (m, C$_6$H$_5$), 7.06 (m, H-26), 6.79 (m, H-24), 6.23 (m, H-25), 6.33 (s, H-3), 5.00 and 4.88

(CH$_2$-21), 4.90 (d, 10.9 Hz) and 4.70 (d, 10.9 Hz) AB ArCH$_2$, 4.75 (b, s, H-10), 3.19 (d, 14.5 Hz) and 1.85 (d, 14.5 Hz) AB H$_2$-14, 2.50 (m, H-8$_{ax}$), 2.20 (m, H-13 and H-7$_{ax}$), 1.8 (m, H-9), 1.22 (s, H$_3$-17), 1.20 (d, 7 Hz) and 1.05 (d, 7 Hz) (H$_3$-18 and -19), 0.82 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 58.7 (C-1), 81.8 C-2), 75.0 (C-3), 93.0 (C-4), 44.4 and 43.3 (C-5 and -14), 78.7 (C-6), 29.3 and 29.6 (C-7 and -8), 147.2 (C-9), 69.9 (C-10), 84.0 (C-11), 109.6 (C-12), 35.3 (C-13), 43.3 (C-14), 102.7 (C-15), 11.7 (C-17), 18.5 and 19.3 (C-18 and -19), 17.2 (C-20), 108.0 (C-21), 161.0 (C-22), 123.5 (C-23), 116.6 (C-24), 110.9 (C-25), 125.6 (C-26), 78.8 (CH$_2$Ar), 139.1, 130.2, 129.2, and 128.1 (C-1, -3, -2, -4 of C$_6$H$_5$). HRMS (FAB): *m/z* calcd for C$_{32}$H$_{40}$N$_2$O$_{10}$H$^+$ 613.2761, found 613.2775.

Ketals 16 and 17 from 4 with Hydrazine. Diketone 4 (15 mg, 0.031 mmol) was dissolved in MeOH (0.5 mL), and NH$_2$-NH$_2$·H$_2$O (50 mg, 1.0 mmol) was added. After 15 min the product 16 was isolated with water and ethyl acetate. ^{1}H NMR (CD$_3$-OD): 7.08 (m, H-26), 6.81 (m, H-24), 6.24 (m, H-25), 5.84 (s, H-3), 4.80 (d, 10 Hz, H-10), 3.02 (d, 14.6 Hz) and 1.86 (d, 14.5 Hz) AB H$_2$-14, 2.15 (m, H-13), 2.57 (m, H-8$_{ax}$), 2.26 (m, H-8$_{eq}$), 2.05 (m, H-7$_{ex}$), 1.43 (m, H-7$_{eq}$), 1.24 (s, H$_3$-17), 1.13 (d, 6.8 Hz) and 1.04 (d, 6.7 Hz) (H$_3$-18 and H$_3$-19), 0.84 (s, H$_3$-20). ^{13}C NMR (CD$_3$-OD): 58.6 (C-1), 78.8 and 81.3 (C-2 and -6), 74.9 (C-3), 93.0 (C-4), 43.4 and 44.3 (C-5 and -14), 29.6 and 29.6 (C-7 and -8), 148.0 (C-9), 70.0 (C-10), 84.5 (C-11), 110.0 (C-12), 35.3 (C-13), 102.5 (C-15), 12.3 (C-17), 18.9 and 19.6 (C-18 and -19), 18.2 (C-20), 107.8 (C-21), 161.7 (C-22), 123.2 (C-23), 117.1 (C-24), 111.0 (C-25), 126.1 (C-26). Adduct 16 (15 mg, 0.028 mmol) in THF (1 mL) was treated with benzoyl chloride (50 mg, 0.35 mmol) and NEt$_3$ (50 mg, 0.50 mmol). After 15 min the product 17 was isolated with ethyl acetate and purified by rotary chromatography using THF–CHCl$_3$ mixtures (1:50,1:20). HRMS (FAB): *m/z* calcd for C$_{32}$H$_{39}$N$_3$O$_{10}$H$^+$ 626.2714, found 626.2734.

Oximes 20 and 21 from 3 with Hydroxyamine. Diketone 3 (24 mg, 0.049 mmol) and hydroxyamine hydrochloride (100 mg, 1.44 mmol) were heated in pyridine (1 mL) at 65 °C for 12 h. Isolation as for 7 and 13 with ethyl acetate and rotary chromatography with ether–hexane–methanol (74:25:1) gave the less polar 21 (14 mg, 56%). ^{1}H NMR (CD$_3$OD): 7.05 (m, H-26), 7.01 (s, H-3), 6.87 (m, H-24), 6.23 (m, H-25), 3.76 (d, 10.0 Hz, H-10), 3.50 (d, 15 Hz) and 2.21 (d, 15 Hz) AB H$_2$-14, 1.28 (s, H$_3$-17), 1.00 (d, 6.9 Hz, H$_3$-21), 1.37 (d, 6.9 Hz) and 1.03 (d, 6.9 Hz) (H$_3$-19 and -18), 0.96 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 60.7 (C-1), 83.7 and 81.1 (C-2 and -6), 72.3 (C-3), 160.2 (C-4), 46.5 (C-5), 29.4 and 29.1 (C-7 and -8), 38.1 (C-9), 72.3 (C-10), 90.8 (C-11), 221.7 (C-12), 35.3 (C-13), 42.3 (C-14), 104.0 (C-15), 13.4 (C-17), 18.0 and 18.2 (C-18 and -19), 24.5 (C-20), 19.2 (C-21), 160.2 (C-22), 123.0 (C-23), 117.2 (C-24), 111.2 (C-25), 126.4 (C-26). HRMS (FAB): *m/z* calcd for C$_{25}$H$_{34}$N$_2$O$_9$H$^+$ 507.2341, found 507.2339. A later fraction consisted of 20 (8 mg, 32%). ^{1}H NMR (CD$_3$OD): 7.12 (m, H-26), 6.93 (m, H-25), 6.28 (m, H-24), 5.64 (s, H-3), 4.38 (d, 10.7 Hz, H-10), 3.48 (d, 15.4 Hz) and 2.18 (d, 15.4 Hz) AB H$_2$-14, 2.26 (m, H-13), 2.10 (m, H-7$_{ax}$), 1.88 (m, H-9), 1.44 (s, H$_3$-17), 0.99 (d, 6.7 Hz, H$_3$-21), 1.29 (d, 7.0 Hz) and 1.05 (d, 6.7 Hz) (H$_3$-18 and -19), 0.91 (s, H$_3$-20). ^{13}C NMR (CD$_3$OD): 59.6 (C-1), 82.0 and 83.0 (C-2 and -6), 89.1 (C-3), 212.1 (C-4), 52.5 (C-5), 28.8 and 29.0 (C-7 and -8), 36.6 (C-9), 72.6 (C-10), 88.7 (C-11), 168.3 (C-12), 35.1 (C-13), 40.7 (C-14), 103.6 (C-15), 16.9 C-17), 18.8, 19.1, 19.2 (C-18, -19 and -21), 21.8 (C-20), 160.2 (C-22), 122.3 (C-23), 117.2 (C-24), 111.2 (C-25), 126.4 (C-26). HRMS (FAB): *m/z* calcd for C$_{25}$H$_{34}$N$_2$O$_9$H$^+$ 507.2341, found 507.2357.

Methoxime 22 from 3 with Methoxyamine. Diketone 3 (27 mg, 0.055 mmol) was treated as for 8 and 14 at 65 °C for 10 h and set aside for 2 days. Workup and rotary chromatography as for 8 and 14 gave methoxime 22 (10 mg, 35%). HRMS (FAB): *m/z* calcd for C$_{26}$H$_{36}$N$_2$O$_9$H$^+$ 521.2499, found 521.2515.

Benzyloxime 23 from 3 with (Benzyloxy)amine. Diketone 3 (20 mg, 0.041 mmol) in pyridine (3 mL) was heated for 12 h at 60 °C with (benzyloxy)amine hydrochloride (200 mg, 1.25 mmol). Isolation as for 15 and rotary chromatography using hexane–ethyl acetate (2:1) gave benzyloxime 23 (11 mg, 45%) and then a small amount of the more polar unreacted 3. HRMS (FAB): *m/z* calcd for C$_{32}$H$_{40}$N$_2$O$_9$H$^+$ 597.2812, found 597.2825.

12-H Ketals 24 and 25 from Oximes 21 and 22 with Sodium Borohydride. Oxime 21 (28 mg, 0.055 mmol) in MeOH (1.5 mL) was treated with NaBH$_4$ (100 mg, 2.65 mmol) during 1 h at −15 °C when more hydride (50 mg, 1.32 mmol) was added with

MeOH (1 mL). After 2 h the mixture was acidified (pH 2) and the product (24, 25 mg, 89%) isolated with ethyl acetate. HRMS (FAB): *m/z* calcd C$_{25}$H$_{36}$N$_2$O$_9$H$^+$ 509.2499, found 509.2485. Similar treatment of methoxime 22 gave methoxyamine 25 which was purified by rotary chromatography using CHCl$_3$–MeOH (20:1). HRMS (FAB): *m/z* calcd for C$_{26}$H$_{38}$N$_2$O$_9$Na$^+$ 545.2475, found 545.2468.

Thioethers 26–28 from 2 with Thiols. (a) Thioethanol. Dehydroryanodine (2, 12 mg, 0.024 mmol) in THF (0.5 mL) was left overnight with thioethanol (50 mg, 0.64 mmol). Extraction with ethyl acetate from 5% NaOH gave a crude product which was purified by rotary chromatography using MeOH–CHCl$_3$ mixtures (1:10, 1:6) to give thioether 26 (8 mg, 58%). ^{1}H NMR (CDCl$_3$/CD$_3$OD): 7.00 (m, H-26), 6.87 (m, H-24), 6.21 (m, H-25), 5.53 (s, H-3), 4.01 (d, 10.3 Hz, H-10), 3.66 (t, 6.3 Hz, CH$_2$OH), 2.63 (dt, 6.3, 2.7 Hz, SCH$_2$CH$_2$OH), 2.95 (dd, 13.0, 3.1 Hz) and 2.45 (obsc) H$_2$-21, 2.45 (d, 13.3 Hz) and 1.90 (d, 13.3 Hz) AB H$_2$-14, 2.25 (m, H-13), 2.15 (m, H-7$_{ax}$), 1.85 (m, H-9), 1.34 (s, H$_3$-17), 1.08 (d, 7 Hz) and 0.75 (d, 7 Hz) (H$_3$-18 and -19), 0.9 (s, H$_3$-20). ^{13}C NMR (CDCl$_3$/CD$_3$OD): 65.1 (C-1), 86.6, 85.4 and 83.7 (C-2, -6 and -11), 90.7 (C-3), 91.4 (C-4), 48.9 (C-5), 25.9 and 25.1 (C-7 and -8), 40.8 and 40.3 (C-9 and -14), 69.5 (C-10), 95.9 (C-12), 30.0 (C-13), 102.2 (C-15), 9.6 (C-17), 18.5 and 19.1 (C-18 and -19), 12.7 (C-20), 35.6 (C-21), 161.6 (C-22), 122.2 (C-23), 116.8 (C-24), 110.6 (C-25), 125.2 (C-26), 35.9 and 61.7 (-SCH$_2$CH$_2$OH). HRMS (FAB): *m/z* calcd for C$_{27}$H$_{39}$NO$_{10}$SNa$^+$ 592.2192, found 592.2204. **(b) Propanethiol.** Dehydroryanodine (2, 15 mg, 0.03 mmol) in MeOH (0.5 mL), propanethiol (0.5 mL, 0.66 mmol), and AIBN (5 mg) were sealed in an ampoule and heated at 75 °C for 2 h when more AIBN (5 mg) was added, and the mixture was heated for another 2 h. The product was isolated and purified by rotary chromatography as above to give thioether 27 (12 mg, 69%). HRMS (FAB): *m/z* calcd for C$_{28}$H$_{41}$NO$_9$SNa$^+$ 590.2400; found 590.2389. **(c) 2-Methylpropane-2-thiol.** Adduct 28 was prepared as in (b) above. HRMS (FAB): *m/z* calcd for C$_{29}$H$_{43}$-NO$_9$SNa$^+$ 604.2556, found 604.2554.

Biology. [^{3}H]Ryanodine Binding Site in Calcium-Release Channel. The potency of each compound was compared with that of ryanodine in rabbit muscle SR preparations[5,19] alone and with ATP and in mouse brain preparations with ATP. The procedure of McPherson and Campbell[17] was modified to prepare mouse brain membranes (35000g pellet) without the addition of protease inhibitors. Protein was determined,[20] and the preparations were stored as aliquots at −70 °C until required for assays. Three assay buffers were used: a, 50 μM CaCl$_2$, 15 mM NaCl, and 250 mM KCl in 20 mM Hepes, pH 7.1; b, 10 mM ATP, 800 μM CaCl$_2$, and 1.5 M KCl in 10 mM Hepes, pH 7.4; c, 0.303 M sucrose in 20 mM Tris maleate, pH 7.0.

Assay mixtures of rabbit muscle SR without ATP involved 20 μg of protein and 1 nM [^{3}H]ryanodine in 500 μL of assay buffer a. Assays with ATP utilized rabbit muscle SR (20 μg protein) or brain membranes (500 μg protein) and 1 nM [^{3}H]rhanodine in 400 μL of 1:1 assay buffers b and c. Samples were incubated at 37 °C for 2 h for rabbit SR alone or for 1 h with both membrane preparations in the presence of ATP. They were then filtered through Whatman GF/C glass fiber filters and washed three times each with 5 mL of ice-cold a (except replacing Hepes with tris HCl) for rabbit SR alone or 150 mM KCl in 10 mM Hepes, pH 7.4, for the other two systems. Nonspecific binding was determined with 10 μM dehydroryanodine. Specific binding was >90% with both rabbit muscle SR systems and >80% for the mouse brain membranes.

The potency of various ryanoids was determined by introducing the unlabeled ryanoid in DMSO (1% final concentration) prior to incubation. Inhibitor concentrations for 50% inhibition (IC$_{50}$s) were calculated using the LIGAND computer program.[21]

Toxicity to Houseflies and Mice. The procedures used for earlier SAR studies[2,5] were followed in the present investigation to facilitate direct comparisons of the data. The houseflies (*Musca domestica*, adult females, SCR susceptible strain) were pretreated with PB (5 μg) applied in acetone (0.5 μL) to the ventrum of the abdomen. The ryanoid was administered 1–2 h later by injection into the thorax of a 0.22-μL solution in DMSO. KD$_{50}$ and LD$_{50}$ values were determined at 4 and 24 h, respectively, and are based on two or more experiments with 10 flies at each dose and a dose differential of 1.50–2-fold. Mice (male albino

Swiss-Webster, 18–22 g) were administered the ryanoid in methoxytriglycol (50 µL) by the ip route with mortality determination at 24 h.

Supplementary Material Available: ^{13}C and ^{1}H NMR data are listed for compounds 3–28 (6 pages). Ordering information is given on any current masthead page.

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