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IMIDAZOTRIAZINONE INHIBITORS OF THE Ca^{2+}-CALMODULIN SENSITIVE PHOSPHODIESTERASE (PDE I)[1]

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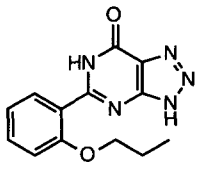
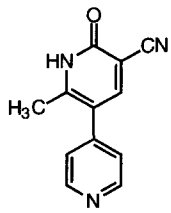
Abstract: Hybrid structural analogs **1** of the PDE V and PDE III inhibitors, zaprinast, milrinone, and CI-930 were prepared to identify dual PDE inhibitors. The SAR study led unexpectedly to the identification of WIN 61691 (**8d**), a potent inhibitor of PDE I (IC_{50} = 85 nM). A potent and selective inhibitor of PDE I would be a useful tool to elucidate the physiologic function of PDE I and other PDE isozymes in biological systems.

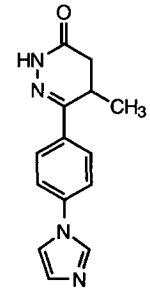
As a part of our program to identify novel PDE inhibitors and to identify mixed inhibitors of the cGMP-inhibitable cAMP phosphodiesterase (PDE III) and the cGMP phosphodiesterase (PDE V), we prepared the generic target compounds **1** as hybrid analogs of the selective PDE V inhibitor, zaprinast, and the specific PDE III inhibitors, milrinone and CI-930.[3] These mixed inhibitors were expected to have a unique cardiovascular profile by possessing cardiotonic/vasodilatory activity due to PDE III inhibition and diuretic/vasodilatory activity due to PDE V inhibition. Structural features of all three PDE inhibitors were incorporated in **1**.



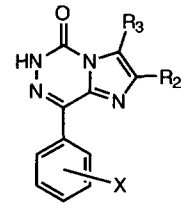
Zaprinast



Milrinone



CI-930



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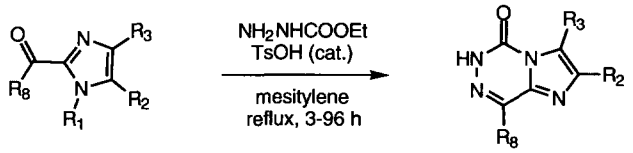
Micromolar inhibitors of the calcium-calmodulin sensitive phosphodiesterase (PDE I) have been described; however, most of these compounds are not selective and exhibit multiple pharmacologic effects.[3,4] This lack of selectivity limits their use as biochemical and pharmacological tools to study the role of PDE I in mammalian physiology. Only vinpocetine has been shown to selectively inhibit PDE I vs. PDE III and PDE V;[5] however, vinpocetine is also a known inhibitor of adenosine uptake, which complicates its use as a cardiovascular pharmacologic tool.[6] PDE I has been isolated from cardiac, vascular, and brain tissue, yet the physiological role of PDE I is not known.[3,4] More selective inhibitors of PDE I would be of great benefit in separating the physiological function(s) of PDE I from those of PDE III and PDE V.

The selective PDE I inhibitor **3c**, WIN 61626, from the series described in this paper, has already found use as a pharmacologic tool in separating out the vasorelaxant effects of PDEs. Compound **3c** was reported not to potentiate sodium nitroprusside-mediated vasorelaxation in rat aortic rings and was inactive or weakly active in other cardiovascular models.[7] This suggests that PDE I does not play a significant role in the physiology of vascular or cardiac tissue. These data also supported the postulate that PDE V inhibition is required for potentiation of nitroprusside-mediated vasorelaxation and that an inhibitor of PDE V may prove useful in disease states where increased cGMP levels are desirable (e.g., atrial natriuretic factor-mediated

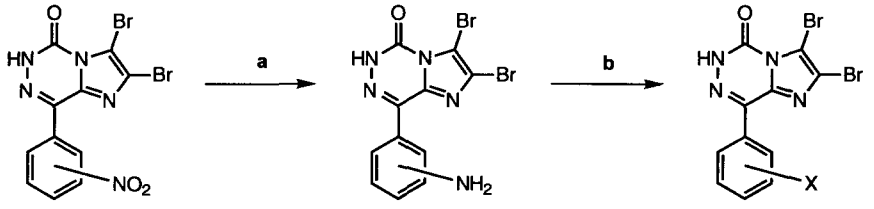
diuresis/natriuresis or nitric oxide-mediated enhancement of pulmonary ventilation/perfusion). This paper describes the structure-activity relationship study that was undertaken to improve the PDE inhibitory potency of the initial lead compound **2a**. Unexpectedly, the SAR did not lead to potent PDE III and/or PDE V inhibitors; instead, potent PDE I inhibitors such as **3c** and **8d** were identified.

Chemistry: The imidazotriazinones **2a-2d** and **3** shown in Table 1 were prepared by either Method A or B as shown in Scheme 1. Thermolysis of the starting 2-aroylimidazoles (where R_1 = H or CH_2OCH_3) in the presence of ethyl carbazate and catalytic *p*-toluenesulfonic acid afforded the desired imidazotriazinones. The syntheses of the remaining derivatives **2e**,[8] **2f**,[9] **5**,[10] and **6**[11] are described in the References and Notes section. The phenyl substituted analogs **7-9** (Table 2) were prepared by the method shown below in Scheme 2. The starting 2,3-dibromo-8-nitrophenylimidazotriazinones **4** were prepared by bromination of the corresponding 2-(nitrobenzoyl)imidazoles[12] followed by condensation with ethyl carbazate and cyclization to afford these starting materials. Reduction of the imidazotriazinones **4** with stannous chloride gave the aniline intermediates **7a**, **8a**, and **9a** in good yields that were then acylated or sulfonylated to yield the desired compounds **7-9** for PDE inhibition studies.



2a-d, 3

Scheme 1. *Method A* - R_1 = H. *Method B* - R_1 = CH_2OCH_3.



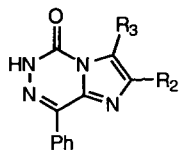
4 **7a, 8a, 9a** **7-9**

X = $NHCOCH_3$, NHCONH-*n*-Bu, or $NHSO_2$-*n*-Bu

Scheme 2. *Reagents and Conditions:* **(a)**, $SnCl_2$, HCl, H_2O, EtOH, reflux, 4 h (60-85% yield). **(b)**, Ac_2O, pyridine, rt, 1 h (80-82% yield); *n*-Bu-NCO, DMF, CH_3CN, reflux, 3-24 h (64-80% yield); or *n*-$BuSO_2Cl$, Et_3N, pyridine, rt, 16 h (48-57% yield).

PDE I SAR Study: The selective PDE V inhibitor, zaprinast, and the specific PDE III inhibitors, milrinone and CI-930, are included in Table 2 as reference compounds. The effects of substitution modifications at positions 2, 3, 5, 6, and 8 of the imidazotriazinone ring system are shown in Table 1. Hydrophobic electron withdrawing groups at R_2 and R_3 appeared to increase PDE I potency. The 2,3-dimethyl (**2b**) and dichloro (**2c**) analogs were more potent than the unsubstituted derivative **2a**, but were significantly less potent than the 2,3-dibromo analog **2d**. The 3-position is sensitive to substitution, since the 3-bromo derivative **2e** was at least ten times more potent than the 2-bromo analog **2f**. In the 2,3-dibromo-

Table 1. ***Inhibition of Phosphodiesterases by Substituted Imidazotriazinones.***



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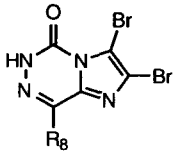

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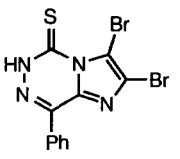
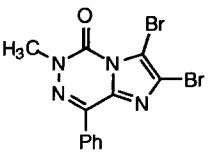


5



6

Cmpd	Substitution		IC50 (μM)[a] PDE I	PDE III	PDE V	PDE Selectivity III/I	PDE Selectivity V/I	Synthetic Route[b]
R_2, R_3 Modifications:								
2a	R_2=H	R_3=H	>10	6.4 (5.6-7.3)	>10	<0.6	–	A-1
2b	CH_3	CH_3	3.5 (3.3-3.7)	2.1 (1.8-2.4)	22.7 (18.7-27.5)	0.6	19	A-1
2c	Cl	Cl	4.0 (3.4-4.7)	8.5 (7.4-9.6)	56.1 (50.0-62.9)	2.1	14	B-2
2d	Br	Br	0.77 (0.69-0.85)	2.1 (1.6-2.6)	2.9 (2.3-3.8)	2.7	3.8	A-3
2e	H	Br	3.1 (2.8-3.4)	14.4 (9.5-21.6)	≥ 100	4.6	≥32	ref 8
2f	Br	H	≥30	>10	66.7 (59.8-74.4)	–	–	ref 9
R_8 Modifications:								
3a	R_8 = cyclohexyl		1.6 (1.5-1.8)	3.5 (3.2-3.8)	3.9 (3.4-4.3)	2.2	2.4	A-4
3b	4-pyridyl		4.6 (2.9-7.2)	3.6 (3.2-4.0)	≥ 30	0.8	≥7	A-3
3c	Ph-4-OCH_3		0.45 (0.37-0.55)	1.1 (0.9-1.3)	> 30	2.4	>67	A-3
Amide Modifications:								
5	–		2.5 (1.8-3.5)	3.9 (3.1-4.9)	19.7 (17.8-21.8)	1.6	7.9	ref 10
6	–		>10	>10	>10	–	–	ref 11

[a]The PDE isozymes were prepared and purified and the IC_{50} determined by the methods described in ref 7. [b]The synthetic route is listed as X-Y where X = the method of imidazotriazinone ring formation in Scheme 1, while Y = the method for the preparation of the imidazole starting materials. Method 1 - imidazole, R_8COCl (2 equiv), pyridine, Et_3N, rt; then 35% NaOH, reflux, 1 h (ref 12). Method 2 - 4,5-dichloro-1-methoxymethyl-imidazole, *n*-BuLi/TMEDA, THF, $PhCON(OCH_3)(CH_3)$, -78 °C. Method 3 - (1) imidazole, R_8COCl (2 equiv), pyridine, Et_3N, rt; then 35% NaOH, reflux, 1 h (ref 12); then (2) Br_2, HOAc, rt. Method 4 - (1) 1-methoxymethyl-imidazole, Et_3N, CH_3CN, cyclohexyl-COCl, rt, 5 h (ref 13); (2) 3 N HCl, reflux, 45 min; then (3) Br_2, HOAc, rt, 2 h.

imidazotriazinone subseries **3a-3c**, potency was affected by replacement or substitution of the R_8 phenyl. The 4-methoxyphenyl derivative **3c** was the most potent compound with the following order of potency Ph-4-OCH_3 > Ph > cyclohexyl > 4-pyridyl. The thione (**5**) and *N*-methyl (**6**) analogs of **2d** did not lead to new SAR directions. The thione **5** offered no advantage and was less potent than **2d**, while the *N*-H of **2d** was shown to be important for PDE I inhibition, since the *N*-methyl compound **6** was inactive.

Because we had demonstrated the potential for improving PDE I inhibitory potency via phenyl substitution (see **3c** vs. **2d**), we chose to explore this SAR direction. Computer models of cGMP (in the anti conformation) and inhibitor **2d** were built in the molecular modeling program SYBYL. The structures were minimized and the atom charges and dipole moment vectors were displayed. The imidazotriazinone of **2d** and the guanine of cGMP were overlaid by aligning the dipole moments and matching the atom charges. The model showed that a hydrogen bond donor/acceptor at the 3'- or 4'-phenyl position of **2d** could substitute for the phosphate of cGMP.[14] As an inhibitor of PDE I, imidazotriazinone **2d** is competitive with the substrate cGMP (data not shown); however, there is no direct evidence that **2d** binds to PDE I in the conformation suggested by these models. To test this hypothesis, the compounds listed in Table 2 were prepared. *This approach led to the most potent PDE I inhibitor in this series*, the butanesulfonamido derivative **8d** with an

IC_{50} of 85 nM. The 2'-substituted derivatives **7a** and **7c** were inactive. The 2'-substituents would affect the conformation of the R_8 phenyl ring relative to the imidazotriazinone ring, presumably leading to an unfavorable conformation for PDE binding. In the 3'-substitution series the 3'-carbonylamino (**8b**, **8c**) and the 3'-sulfonamido (**8d**) analogs were all more potent than the corresponding 4'-substituted analogs **9b-9d**. Also, the analogs **9b-9d** were not more potent than the 4'-amino analog **9a** or even the unsubstituted inhibitor **2d**, suggesting that a 4'-hydrogen bond donor/acceptor does not interact with the PDE enzyme surface. Within the 3'- and 4'-series only the 3'-sulfonamido analog **8d** showed a substantial increase in PDE I potency. This result suggests that the 3'-hydrogen bond donor/acceptors may mimic the cyclic phosphate of cGMP (as proposed in the model) or that the 3'-substituents may bind to another site on the PDE surface, thereby resulting in more potent inhibitors (**8**).

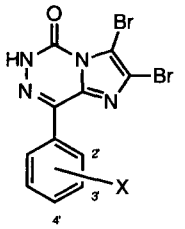
PDE Selectivity SAR: All of the active imidazotriazinone PDE I inhibitors also inhibited PDE III; however, they were significantly less active or inactive as inhibitors of PDE V. Substituent effects that increased PDE I inhibitory activity also generally increased selectivity over PDE V, while only small changes in PDE III inhibition were noted. When comparing a series of phenyl substituted analogs, specifically in the progression of **2d** -> **3c** -> **8d**, the PDE I IC_{50} decreased (i.e., potency increased) from 0.77 -> 0.45 -> 0.085 μM and the V/I selectivity increased from 3.8 -> >67 -> 36, respectively, while the PDE III/I selectivity was unchanged (2.7 -> 2.4 -> 3.4, respectively). In addition, when comparing the PDE I and PDE V inhibition of the monobromides **2e** and **2f**, **2e** was at least ten times more potent as a PDE I inhibitor than **2f** and was less active against PDE V. These results show that differences exist in the SAR for PDE I and PDE V inhibition in this series. However, the SAR for PDE I and PDE III inhibition appear to be related, since structural modifications that led to an increase or decrease in PDE I inhibitory potency led to corresponding changes in PDE III inhibitory potency. Also, while the 4-pyridyl substituent was important for the PDE III inhibitory potency of milrinone,[15] this modification had no effect in this series and actually reduced PDE III inhibitory potency (see **2d** vs. **3b** in Table 1). This suggests that **2d** and **3b** bind to PDE III in a different manner than does milrinone.

We have identified the imidazotriazinone **8d** as a potent (IC_{50} = 85 nM) and selective inhibitor of PDE I. The PDE V/I selectivity ratio of 36 will permit the use of **8d** as a pharmacologic tool for elucidating the functional roles of PDE I and PDE V. In fact, the analog **3c** has recently been used in this fashion.[7] The PDE III/I selectivity ratio of 3.4 for **8d** will limit the potential for its use in separating out functional effects of PDE III and PDE I. Nonetheless, these new inhibitors provide an additional tool for the elucidation of the function of the various PDE isozymes in mammalian physiology.

Synthetic Methods:

Preparation of Imidazotriazinone 8d: 4,5-Dibromo-2-(3-nitrobenzoyl)imidazole. To a mechanically stirred suspension of the 2-(3-nitrobenzoyl)imidazole[12] (72.0 g, 0.33 mol) in 1 L of HOAc was added dropwise bromine (34 mL, 0.66 mol), and stirring continued for 3 h. More bromine (34 mL, 0.66 mol) was added and the mixture warmed on a steam bath for 1 h. On cooling, the mixture was poured onto ice and diluted to 4 L with water. A yellow precipitate formed, was collected, and recrystallized from ethanol to give 71.7 g (58%) of the dibromo-imidazole as tan crystals: mp 244-246 °C; IR (KBr) 3223, 2626 cm^{-1}; NMR (DMSO-d_6) δ 7.89 (t, J = 7.6 Hz, 1H), 8.52 (dd, J = 6.9 Hz, J = 1.9 Hz) and 8.72 (d, 7.3 Hz)(2H), 9.12 (d, J = 1.9 Hz, 1H), 14.93 (bs, 1H). Anal. calcd for $C_{10}H_5Br_2N_3O_3$: C, 32.03; H, 1.34; N, 11.21. Found: C, 32.05; H, 1.40; N, 11.28.

Table 2. ***Inhibition of Phosphodiesterases by 2,3-Dibromo-8-Aryl-Imidazotriazinones.***



Cmpd	X=	IC_{50} (μM)			PDE Selectivity	
		PDE I	PDE III	PDE V	III/I	V/I
7a	2'-NH_2	>10	>>1[a]	>10	-	-
7c	2'-NHCONH-*n*-Bu	>>10	>>1[a]	>10	-	-
8a	3'-NH_2	1.15 (1.09-1.22)	2.5 (2.1-3.0)	17.7 (16.4-19.1)	2.2	15
8b	3'-$NHCOCH_3$	1.22 (1.09-1.36)	4.4 (3.9-5.0)	9.7 (6.3-15.0)	3.6	8.0
8c	3'-NHCONH-*n*-Bu	0.65 (0.53-0.79)	1.2 (0.86-1.6)	14.9 (13.6-16.3)	1.8	23
8d	3'-$NHSO_2$-*n*-Bu	0.085 (0.076-0.093)	0.29 (0.26-0.31)	3.1 (2.2-4.3)	3.4	36
9a	4'-NH_2	0.91 (0.85-0.97)	1.5 (1.3-1.7)	12.1 (10.8-13.5)	1.6	13
9b	4'-$NHCOCH_3$	1.82 (1.4-2.3)	2.3 (2.2-2.5)	21.2 (19.2-23.3)	1.3	12
9c	4'-NHCONH-*n*-Bu	1.8 (1.5-2.1)	4.5 (3.5-5.6)	18.2 (16.4-20.3)	2.5	10
9d	4'-$NHSO_2$-*n*-Bu	1.02 (0.76-1.36)	0.84 (0.70-1.0)	1.7 (1.4-1.9)	0.8	1.7
Zaprinast		6.0 (5.0-7.2)	133 (109-162)	0.30 (0.25-0.36)	22	0.05
Milrinone		>100	0.93 (0.80-1.13)	>10	<0.01	-
CI-930		>100	0.26 (0.22-0.31)	>10	<0.003	-

[a]Insoluble at higher concentrations and an IC_{50} could not be determined.

2,3-Dibromo-8-(3-nitrophenyl)imidazo[1,2-*d*][1,2,4]triazin-5(6*H*)-one. A mixture of the 4,5-dibromo-2-(3-nitrobenzoyl)imidazole (65.6 g, 0.175 mol), ethyl carbazate (32.8 g, 0.315 mol), and toluenesulfonic acid (0.3 g) in 875 mL of mesitylene was refluxed for 3.5 h. While still hot, the clear brown solution was decanted from a dark brown gum. On cooling, crystals formed from the decanted solution and were collected to give 67.6 g (93%) of the nitro compound as a tan solid: mp 267-269 °C; IR (KBr) 1720 cm^{-1}; NMR (DMSO-d_6) δ 7.85 (t, *J* = 8.0 Hz, 1H), 8.36 (dd, *J* = 8.1 Hz, *J* = 1.9 Hz, 1H), 8.63 (d, *J* = 8.3 Hz, 1H), 9.03 (t, *J* = 1.7 Hz, 1H), 13.31 (bs, 1H). Anal. calcd for $C_{11}H_5Br_2N_5O_3$: C, 31.84; H, 1.21; N, 16.88. Found: C, 31.87; H, 1.31; N, 16.66.

8-(3-Aminophenyl)-2,3-dibromoimidazo[1,2-*d*][1,2,4]triazin-5(6*H*)-one (8a). To a mechanically stirred mixture of the nitro compound (10.4 g, 25 mmol) in 90 mL conc. HCl, 45 mL water and 135 mL of ethanol was added $SnCl_2$ (16.9 g, 75 mmol). The reaction mixture was heated on a steam bath for 4 h, cooled, and poured onto ice-35% NaOH-saturated $NaHCO_3$ (1:1). The mixture was extracted while cold with ethyl acetate (3X) and the combined extracts washed with water and then brine, dried over $MgSO_4$, filtered, and concentrated to give 9.40 g of a yellow solid. Recrystallization from acetonitrile gave 8.20 g (85%) of the aniline **8a** as a light-yellow powder: mp 258 °C (dec.); IR (KBr) 3265, 1731, 1620 cm^{-1}; NMR (DMSO-d_6) δ 5.27 (bs, 2H), 6.70 (d, *J* = 8.4 Hz, 1H), 7.14 (t, *J* = 7.9 Hz) and 7.32 (m)(3H), 13.01 (bs, 1H). Anal. calcd for $C_{11}H_7Br_2N_5O$: C, 34.32; H, 1.83; N, 18.19. Found: C, 34.46; H, 1.91; N, 18.26.

8-(3-Butylsulfonamidophenyl)-2,3-dibromoimidazo[1,2-*d*][1,2,4]triazin-5(6*H*)-one (8d). To a mixture of the aniline **8a** (3.0 g, 7.79 mmol) in 15 mL of pyridine and 15 mL of triethylamine was added dropwise *n*-butylsulfonyl chloride (1.1 mL, 8.6 mmol). The reaction mixture was stirred at rt for 16 h, and then poured onto water. When the pH was adjusted to 5.5 with dilute HCl a gum separated. The aqueous phase was decanted off and the gum taken up in hot chloroform. On cooling a reddish powder formed, was collected, and recrystallized from ethanol to give 2.23 g (57%) of **8d** as a light tan solid: mp 199-201 °C; IR (KBr) 3254, 1739 cm^{-1}; NMR (DMSO-d_6) δ 0.87 (t, J = 7 Hz, 3H), 1.35 (m, 2H), 1.67 (m, 2H), 3.15 (~t, J = 7 Hz, 2H), 7.34 (d, J = 8 Hz, 1H), 7.49 (t, J = 8 Hz, 1H), 7.93 (d, J = 8 Hz, 1H), 8.02 (s, 1H), 10.04 (s, 1H), 13.16 (s, 1H). Anal. calcd for $C_{15}H_{15}Br_2N_5O_3S$: C, 35.66; H, 2.99; N, 3.86. Found: C, 35.62; H, 3.05; N, 13.91.

2,3-Dibromo-8-(4-methoxyphenyl)imidazo[1,2-*d*][1,2,4]triazin-5(6*H*)-one (3c). This compound was prepared in two steps from 2-(4-methoxybenzoyl)imidazole[12] following the procedures described above to give **3c** (60% overall) as an off-white powder: mp 260-261 °C; IR (KBr) 1730, 1710, 1613 cm^{-1}; NMR (DMSO-d_6) δ 3.84 (s, 3H), 7.10 (d, J = 8 Hz, 2H), 8.18 (d, J = 8 Hz, 2H), 13.02 (bs, 1H). Anal. calcd for $C_{12}H_8Br_2N_4O_2$: C, 36.03; H, 2.02; N, 14.01. Found: C, 36.23; H, 2.21; N, 14.01.

Acknowledgment: We wish to acknowledge the assistance of Dr. Paul Kowalczyk in performing the computer modeling studies described in this letter.

References and Notes:

1. This research was conducted at Sterling Winthrop Pharmaceuticals Research Division prior to its acquisition by Sanofi, Inc.
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8. Reaction of **2a** with three portions of bromine in acetic acid at rt for 5 days gave **2e** (20% yield): mp 250-252 °C.
9. Reaction of **2d** with KH in THF at rt and then *n*-BuLi at -78 °C followed by H_3O^+ quench gave **2f** (40% yield): mp 224-226 °C. The structure was determined by X-ray crystallography.
10. The hydrazone of 4,5-dibromo-2-benzoylimidazole was treated with thiocarbonyl diimidazole in CH_2Cl_2 and refluxed to give **5** (20% yield): mp 216-217 °C.
11. Reaction of **2d** with K_2CO_3 and MeI in DMF at 40 °C gave **6** (90% yield): mp 169-171 °C.
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