Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1165–1168

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel 5-Cyclopropyl-1,4-benzodiazepin-2-ones as Potent and Selective I_{Ks}-Blocking Class III Antiarrhythmic Agents

John W. Butcher,[a,*] Nigel J. Liverton,[a] David A. Claremon,[a] Roger M. Freidinger,[a] Nancy K. Jurkiewicz,[b] Joseph J. Lynch,[b] Joseph J. Salata,[b] Jixin Wang,[b] Christine M. Dieckhaus,[c] Donald E. Slaughter[c] and Kamlesh Vyas[c]

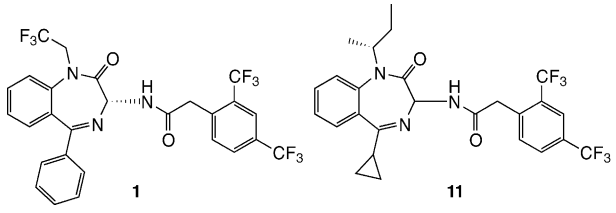
[a]*Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA*

Received 20 May 2002; accepted 6 December 2002

Abstract—Novel 5-cyclopropyl-1,4-benzodiazepin-2-ones having various N-l substituents were identified as potent and selective blockers of the slowly activating cardiac delayed rectifier potassium current (I_{Ks}). Compound **11** is the most potent I_{Ks} channel blocker reported to date.

The delayed rectifier potassium current, I_K, plays an important role in the repolarization of cardiac tissue.[1,2] Inhibition of I_K increases action potential duration, delays repolarization, and leads to an increase in the QT interval of the electrocardiogram (Class III antiarrhythmic effect).[3] Cardiac I_K consists of two kinetically and pharmacologically distinct currents, which have been identified as rapidly (I_{Kr}) and slowly (I_{Ks}) activating components.[4–6] Inhibition of I_{Kr} produces a Class III antiarrhythmic action that is typified by a significant reverse frequency dependence.[7] This profile limits effectiveness at fast 'arrhythmia' rates, and can lead to an exceptionally prolonged QT_c interval, especially at slow heart rates, and is therefore, potentially proarrhythmic.[8,9] Inhibition of I_{Ks}, on the other hand, partly due to its gating kinetics, may not result in reverse frequency dependence.[10,11] Thus, compounds with inhibitory activity on I_{Ks} channels may offer a potential therapeutic advantage over inhibitors of I_{Kr} channels as ventricular antiarrhythmic agents.

Previously, we have reported the discovery of compound **1**, a potent, selective and orally active I_{Ks}-blocking agent that demonstrates significant Class III antiarrhythmic activity in vivo.[12] In this communication, we describe the identification and optimization of a related series of 5-cyclopropyl-1,4-benzodiazepin-2-ones that show a significant potency improvement relative to the 5-phenyl-1,4-benzodiazepin-2-ones, making them the most potent I_{Ks} blocking Class III antiarrhythmic agents reported to date.[13–16]

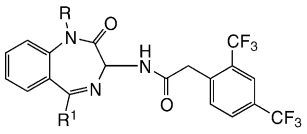


In an effort to increase structural diversity around the 1,4-benzodiazepin-2-one[12] framework, alternative 5-substituted-1,4-benzodiazepin-2-ones were targeted. Success was achieved with the 5-isopropyl analogue **2** (Table 1), which maintained both I_{Ks} activity and selectivity versus the I_{Kr} channel. Importantly, the profile of compound **2** clearly demonstrated that a 5-aryl substituent was not essential for activity. One potential liability that arose with the 5-isopropyl containing derivatives was their metabolic profile. Preliminary in vitro drug metabolism revealed rapid and extensive metabolic hydroxylation of the 5-isopropyl moiety

*Corresponding author. Fax: +1-215-652-3971; e-mail: john_butcher@merck.com

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00049-0

Table 1. Effect of substitution on I_{Ks} potency



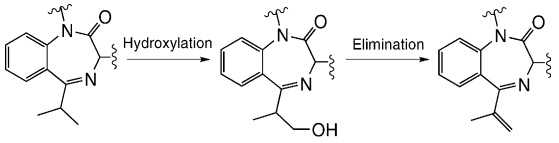
Compd[a]	Stereochemistry[b]	*R*	R^1	I_{Ks} IC_{50} (nM)[c]	I_{Kr} IC_{50} (nM)[d]	Mp °C
1	*R*(−)	F_3CCH_2	Phenyl	6	6000	132–134
2	(+)	F_3CCH_2	Isopropyl	6	>1000	78–80
3	(+)	F_3CCH_2	Cyclopropyl	1.5	>1000	71–74
4	(+)	Me	Cyclopropyl	38	>1000	158–159
5	(−)	Me	Cyclopropyl	280	>1000	158–160
6	(+)	Et	Cyclopropyl	200	>1000	120–121
7	(−)	Et	Cyclopropyl	4	>1000	124–125
8	(+)	Propyl	Cyclopropyl	165	>1000	94–95
9	(−)	Propyl	Cyclopropyl	1.2	>1000	78–80
10	(+)	(*R*)-2-Butyl	Cyclopropyl	50	>1000	108–109
11	(−)	(*R*)-2-Butyl	Cyclopropyl	0.08	>1000	119–120
12	(+)	(*S*)-2-Butyl	Cyclopropyl	180	>1000	109–110
13	(−)	(*S*)-2-Butyl	Cyclopropyl	0.40	>1000	118–120
14	(+)	(*R*)-2-Butyl	Phenyl	3.7	>1000	125–126
15	(−)	(*R*)-2-Butyl	Phenyl	44	>1000	124–126

[a]All compounds gave satisfactory spectral and analytical data.
[b]Stereochemistry at the 3-position of compounds **2**–**15** is undetermined.
[c]Nanomolar concentrations of compounds required to inhibit 50% of I_{Ks} current measured in isolated guinea pig myocytes during a 1 s voltage clamp step from −50 to +50 mV (see ref 21 for details of protocol). IC_{50} values determined from the fits of a Hill equation to the concentration–response relationships.
[d]Nanomolar IC_{50} values for inhibition of I_{Kr} current during a 0.5 s voltage clamp step from −50 to −10 mV (see ref 21 for details of protocol).

followed by elimination to the corresponding 5-isopropenyl derivative (Scheme 1). Our attention then turned to installation of alternative 5-substituents as a strategy to avoid formation of this potentially reactive metabolite. Incorporation of a cyclopropyl substituent at the same position would be expected to eliminate this undesired metabolic pathway.

We were gratified to see that this change to the 5-cyclopropyl substituent (**3**, Table 1) resulted in a 4-fold increase in potency. Moreover, comparison of **2** and **3** in an in vitro human microsomal incubation assay[17] (Fig. 1) confirmed the enhanced metabolic stability of the cyclopropyl functionality relative to the isopropyl group. At the end of a 60-min experiment, only ~15% of the cyclopropyl compound **3** had been metabolized, versus ~70% for the corresponding isopropyl derivative **2**.

Preparation of the N-1 methyl (**4**), ethyl (**7**), and *n*-propyl (**9**) analogues, demonstrated that activity increased as the size of the substituent increased. In each case, I_{Ks} activity resided almost entirely in a single enantiomer. Based on data from the 5-phenylbenzodiazepine series showing that N-1 branched substituents were tolerated, a series of *sec*-butyl diastereomers (**10**–**13**) were prepared. The (*R*)-2-butyl analogue (**11**) proved to be an extremely potent blocker of I_{Ks} with an IC_{50} of 0.08 nM, representing the most potent I_{Ks} channel blocker we have identified to date, with a >10,000 fold selectivity versus the I_{Kr} channel.



Scheme 1.

In an effort to determine whether the (*R*)-2-butyl substituent would have the same potency-enhancing effect in the 5-phenylbenzodiazepines, compounds **14** and **15** were prepared. Interestingly, these compounds did not show any significant enhancement of activity relative to the trifluoroethyl analogue (**1**).

Based on its intriguing in vitro potency, the electrophysiologic and hemodynamic profile of compound **11** was evaluated, particularly for its Class III activity in vivo.[18] in a rising dose study conducted in chloralose anesthetized dogs, compound **11** significantly increased

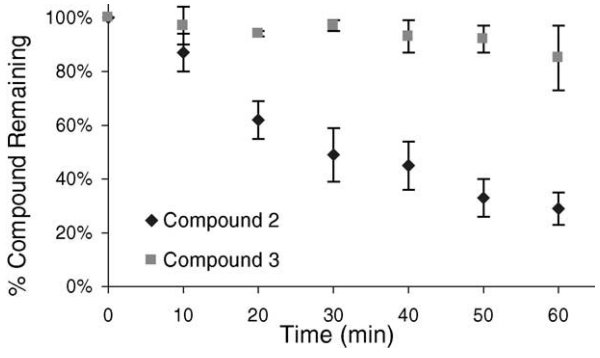
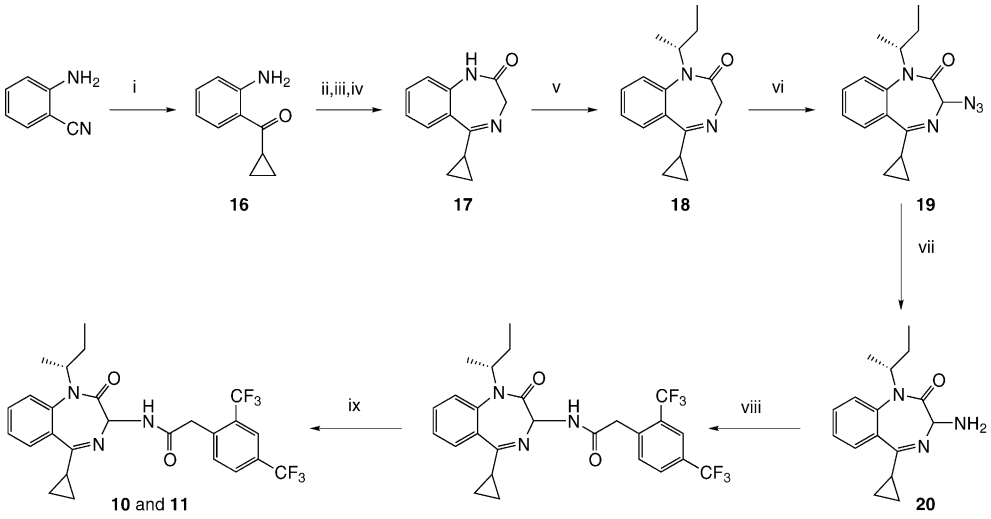


Figure 1. Relative metabolic stability of **2** and **3** in human liver microsome incubations.[17]

Table 2. In vivo Class III activity of compound **11**

Dose (mg/kg iv) Vehicle–10% ethanol/PEG-200	Vehicle	0.001	0.01	0.1
QT_c Interval (msec/$\sqrt{}$ sec)	360±19	384±21	392±19	454±19
Ventricular relative Refractory period (msec)	167±7	176±7	180±6	198±5



Scheme 2. Synthesis of compounds **10** and **11**: (i) cyclopropylmagnesium bromide, THF, −10 °C; (ii) bromoacetyl bromide, dichloromethane, 3 N NaOH, 0 °C; (iii) 1:1 ethanol/aq NH_4OH; (iv) NaOH, pH 12.0 (71%, four steps); (v) *S*-(+)-methanesulfonic acid *sec*-butyl ester, cesium carbonate, DMF, 50 °C (66%); (vi) (a) K-O*t*Bu, trisyl azide, THF, −78 °C; (b) AcOH (79%); (vii) H_2, 10% Pd/C, EtOH (100%); (viii) EDC, HOBT, triethylamine, DMF, 2,4-bis(trifluoromethyl)phenylacetic acid (90%); (ix) HPLC separation of diastereomers.

the QT_c interval and ventricular relative refractory period (Table 2), even at extremely low doses of 0.001 mg/kg, paralleling the in vitro potency. Other key cardiovascular parameters (including mean arterial pressure, QRS interval, PR interval, sino-atrial conduction time, and atrial excitation threshold) were unchanged.

The synthesis of 5-cyclopropylbenzodiazepines **10** and **11** began with treatment of anthranilonitrile with cyclopropylmagnesium bromide (Scheme 2). Acetylation of the resulting *ortho*-aminophenylcyclopropylketone (**16**) with bromoacetyl bromide, followed by treatment with aqueous ammonia/ethanol resulted in cyclization to the 5-cyclopropylbenzodiazapine (**17**). Alkylation of the amide nitrogen with *S*-(+)-methanesulfonic acid *sec*-butyl ester occurred smoothly with no detectable loss of stereochemistry using cesium carbonate in DMF at 50 °C. Introduction of the C-3 substituent began with direct azidation of **18** with trisyl azide,[19] followed by reduction of the azide (**19**) with 10% Pd/C in ethanol to give the amine (**20**). Coupling of the amine with 2,4-bis(trifluoromethyl)phenylacetic acid followed by separation of the diastereomers using a Chiralpak® AD preparative HPLC column[20] gave the (+)-diastereomer (**10**) and the (−)-diastereomer (**11**). 5-Isopropylbenzodiazepines were prepared in a similar fashion, utilizing isopropylmagnesium chloride in the first step.

In summary, the cyclopropyl moiety has been identified as a useful replacement for the 5-phenyl group in a series of benzodiazepine based blockers of the I_{Ks} channel. These compounds also retain selectivity over the I_{Kr} channel. Subsequent optimization of the N-1 substituent provided compound **11**, with sub-nanomolar potency of inhibition of the I_{Ks} channel. This compound also demonstrated significant Class III antiarrhythmic activity in vivo.

References and Notes

1. Bigger, J. T.; Hoffman, B. F. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; McGraw-Hill: New York, 1996; p 840.
2. Gerlach, U. *Drugs Future* **2001**, *26*, 473.
3. Vaughan Williams, E. M. *J. Clin. Pharmacol.* **1984**, *24*, 129.
4. Sanguinetti, M. C.; Jurkiewicz, N. K. *Gen. Physiol.* **1990**, *96*, 195.
5. Barhanin, J.; Lesage, F.; Guillemare, E.; Fink, M.; Lazdunski, M.; Romey, G. *Nature* **1996**, *384*, 78.
6. Sanguinetti, M. C.; Curran, M. E.; Zou, A.; Shen, J.; Spector, P. S.; Atkinson, D. L.; Keating, M. T. *Nature* **1996**, *384*, 80.
7. Baskin, E. P.; Serik, C. M.; Wallace, A. A.; Brookes, L. M.; Selnick, H. G.; Claremon, D. A.; Lynch, J. J. *J. Cardiovasc. Pharm.* **1991**, *18*, 406.
8. Hondeghem, L. M.; Snyders, D. J. *Circulation* **1990**, *81*, 686.
9. Smith, P. L.; Baukrowitz, T.; Yellen, G. *Nature* **1996**, *379*, 833.
10. Hondeghem, L. M. *J. Cardiovasc. Pharmacol.* **1995**, *20* (suppl. 4A), 28.
11. Li, G.-R.; Feng, J.; Yue, L.; Carrier, M.; Nattel, S. *Circulation Res.* **1996**, *78*, 689.
12. Selnick, H. J.; Liverton, N. J.; Baldwin, J. J.; Butcher, J. W.; Claremon, D. A.; Elliott, J. M.; Freidinger, R. M.; King, S. A.; Libby, B. E.; McIntyre, C. J.; Pribush, D. A.; Remy, D. C.; Smith, G. R.; Tebben, A. J.; Jurkiewicz, N. K.;

Lynch, J. J.; Salata, J. J.; Sanguinetti, M. C.; Siegl, P. K. S.; Slaughter, D. E.; Vyas, K. *J. Med. Chem.* **1997**, *40*, 3865.
13. Lloyd, J.; Schmidt, J. B.; Rovnyak, G.; Ahmad, S.; Atwal, K. S.; Bisaha, S. N.; Doweyko, L. M.; Stein, P. D.; Traeger, S. C.; Mathur, A.; Conder, M. L.; DiMarco, J.; Harper, T. W.; Jenkins-West, T.; Levesque, P. C.; Normandin, D. E.; Russell, A. D.; Serafino, R. P.; Smith, M. A.; Lodge, N. J. *J. Med. Chem.* **2001**, *44*, 3764.
14. Gerlach, U.; Brendel, J.; Lang, H.; Paulus, E. F.; Weidman, K.; Bruggemann, A.; Busch, A. E.; Suessbrich, H.; Bleich, M.; Greger, R. *J. Med. Chem.* **2001**, *44*, 3831.
15. Busch, A. E.; Suessbrich, H.; Waldegger, S.; Sailer, E.; Greger, R.; Lang, H.-J.; Lang, F.; Gibson, K. J.; Maylie, J. G. *Pfluger's Arch* **1996**, *432*, 1094.
16. Suessbrich, H.; Bleich, M.; Ecke, D.; Rizzo, M.; Waldegger, S.; Lang, F.; Szabo, I.; Lang, H.-J.; Kunzelmann, K.; Greger, R.; Busch, A. E. *FEBS Lett.* **1996**, *396*, 271.
17. Microsomal incubations were conducted using pooled human liver microsomes performed in triplicate at 37 °C. Each incubation contained a final concentration of 2 mg/mL microsomal protein, 1 μM of the respective compound and NADPH-regenerating system (3 units of glucose-6-phosphate dehydrogenase, 10 mM D-glucose-6-phosphate, 1 mM β-nicotinamide adenine dinucleotide phosphate) in 100 mM phosphate buffer, pH 7.4. At each time point, a 100 μL aliquot was removed and mixed with 100 μL acetonitrile containing compound **11** at a final concentration of 5 μM. The samples were spun at 3800 rpm for 5 min and then 100 μL of the supernatant was added to wells containing 100 μL water. An aliquot (50 μL) was injected for analysis via LC-MS/MS in using a Sciex API3000 with SRM monitoring. A linear calibration curve was achieved between 50 to 1000 nM. Data are reported as the percentage of compound remaining as compared to time zero.
18. The hemodynamic profile was obtained using the protocol described in: Lynch, J. J.; Wallace, A. A.; Stupenski, R. F.; Baskin, P. B.; Beare, C. M.; Appleby, S. D.; Salata, J. J.; Jurkiewicz, N. K.; Sanguinetti, M. C.; Stein, R. B.; Gehret, J. R.; Kothstein, T.; Claremon, D. A.; Elliott, J. M.; Butcher, J. W.; Remy, D. C.; Baldwin, J. J. *J. Pharm. Exp. Ther.* **1994**, *269*, 541.
19. Butcher, J. W.; Liverton, N. J.; Selnick, H. G.; Elliott, J. M.; Smith, G. R.; Tebben, A. J.; Pribush, D. A.; Wai, J. S.; Claremon, D. A. *Tetrahedron Lett.* **1996**, *37*, 6685.
20. Separation using a Chiralpak® AD preparative HPLC column (25×2 cm), eluent 1/1 hexane/ethanol with 0.1% diethylamine buffer, 7.0 mL/min.
21. Fermini, B.; Jurkiewicz, N. K.; Jow, B.; Guinoso, P. J., Jr.; Baskin, E. P.; Lynch, J. J., Jr.; Salata, J. J. *J. Cardiovasc. Pharmacol.* **1995**, *26*, 259.