Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 18, pp. 2151-2154, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00365-7

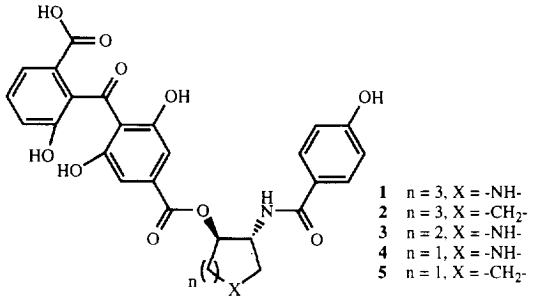
RING SIZE EFFECT IN THE PKC INHIBITORY ACTIVITIES OF PERHYDROAZEPINE ANALOGS OF BALANOL[1]

Yen-Shi Lai,* David S. Menaldino, Jeffrey B. Nichols,[2] G. Erik Jagdmann, Jr.
Frankie Mylott, Jan Gillespie, and Steven E. Hall

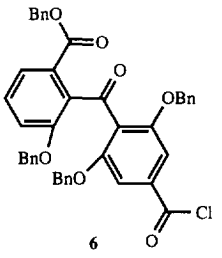
Sphinx Pharmaceuticals
A Division of Eli Lilly and Company
4615 University Drive
Durham, NC 27707

Abstract: Analogs **3-5** of balanol (-)-**1**, a potent protein kinase C (PKC) inhibitor, were synthesized with the perhydroazepine moiety replaced by a smaller ring. Their *in vitro* inhibitory activities against PKC revealed a significant relationship between ring size and potency.

The protein kinase C (PKC) family of enzymes is known to play a crucial role in a range of cellular processes.[3] Compounds that specifically inhibit PKC may develop into useful human therapeutic agents since activated PKC has been suggested to underlie several disease states.[4] Balanol (-)-**1**, recently isolated in our laboratories, is a metabolite produced by the fungus *Verticillium Balanoides* that inhibits PKC in low nanomolar concentrations.[5] As we pursued an understanding of the structure-activity relationships of balanol analogs, we sought to define the correlation between the ring size of the perhydroazepine moiety and the potency of these compounds against PKC. Also desirable was a potent and chemically more accessible[6] ring size that would allow large scale preparation of research material. We report herein the syntheses, shown in **Scheme 1-3**, of three such analogs **3-5**[7] and a comparison of their PKC inhibitory activities with those of balanol and the cycloheptane analog **2**.[8]



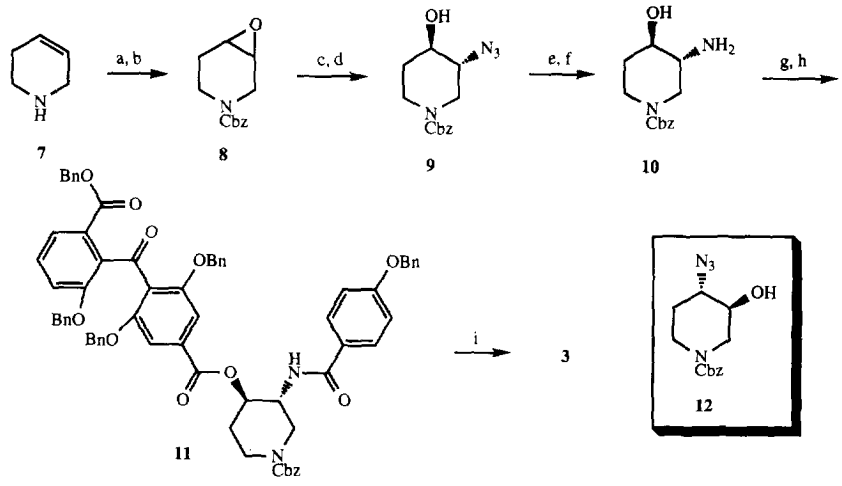
1 n = 3, X = -NH-
2 n = 3, X = -CH_2-
3 n = 2, X = -NH-
4 n = 1, X = -NH-
5 n = 1, X = -CH_2-



6

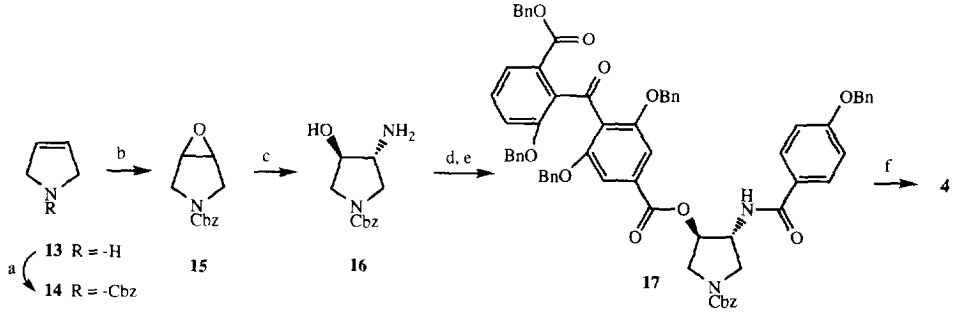
1,2,3,6-Tetrahydropyridine was protected as the benzylcarbamate and epoxidized to give **8**. Epoxide ring opening of **8** with NaN_3 led to formation of **9** and **12**, in a 4.8:1 ratio in favor of the desired regioisomer. Compound **9** was separated from **12** by chromatography and converted to **10** by a modified Staudinger reaction,[9] employing PPh_3 followed by methanolic NaOH. Reaction of **10** with 4-benzyloxybenzoyl chloride under the Schotten-Baumann conditions allowed for exclusive N-acylation and the resultant amidoalcohol was

coupled with **6**[10] to give **11**, which was debenzylated by catalytic hydrogenolysis to provide the piperidine analog **3**.



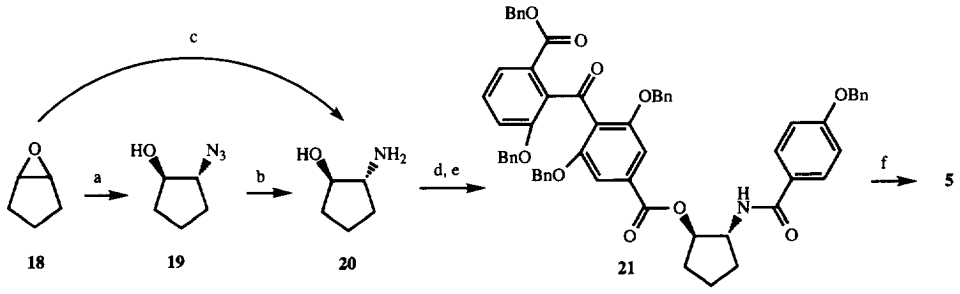
Scheme 1: (a) BnOCOCl, Et_3N, CH_2Cl_2, rt, quant; (b) mCPBA, CH_2Cl_2, rt, quant; (c) NaN_3, NH_4Cl, MeOH-H_2O, reflux, 83%; (d) separation on silica gel; (e) PPh_3, THF, rt; (f) 0.5N aq. NaOH, MeOH-THF, rt, 86%, 2 steps; (g) 4-benzyloxybenzoic acid, oxalyl chloride, aq. NaOH, CH_2Cl_2, rt, 80%; (h) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 83%; (i) H_2, $Pd(OH)_2$-C, EtOH, rt, 70%.

Commercially available 75% pure pyrroline **13** was similarly N-protected with the Cbz group and purified by chromatography to provide **14**. Epoxidation of this compound with mCPBA followed by a sequence of (i) epoxide ring opening with NaN3; (ii) O-silylation with the TBDMS group; and (iii) azide-to-amine conversion using the PPh3/basic hydrolysis method was used to prepare a synthetic equivalent of **16** that was manipulated to give **17** and **4** for our initial biological screening. Preferably, as we discovered later, the epoxide of **14** was treated with aqueous ammonia to give **16** directly. Subsequent N- and O-acylation of **16** followed by hydrogenolysis of the resultant **17**, using the same protocols used for **11**, gave the pyrrolidine analog **4**.



Scheme 2: (a) BnOCOCl, Et_3N, CH_2Cl_2, rt; then chromatography on silica gel, 92%; (b) mCPBA, CH_2Cl_2, rt, 78%; (c) aq. NH_3, 55 °C, 85%; (d) 4-benzyloxybenzoyl chloride, aq. NaOH, CH_2Cl_2, rt, 85%; (e) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 75%; (f) H_2, $Pd(OH)_2$-C, THF- EtOH, rt, 72%.

The cyclopentane analog **5** was prepared similarly with the added convenience that the required cyclopentene oxide is commercially available.



Scheme 3: (a) NaN_3, NH_4Cl, MeOH-H_2O, reflux, 70%; (b) PPh_3, THF, rt; then aq. NaOH, MeOH, rt, 88%; (c) aq. NH_3, 65 °C, 75%; (d) 4-benzyloxybenzoyl chloride, aq. NaOH, CH_2Cl_2, rt, 81%, (e) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 78%; (f) H_2, $Pd(OH)_2$-C, EtOH, rt, 85%.

Compounds **3-5** were screened against human PKC isoenzymes α, β-1, β-2, γ, δ, ε, η, and ζ[11] and the results are summarized in **Table 1**, together with racemic balanol and the cycloheptane analog **2**.

Table 1: PKC Isozyme Inhibition by Balanol Analogs **1-5** (IC_{50} values in μM)

Compd	α	β–1	β–2	γ	δ	ε	η	ζ
(±)-**1***	0.074	0.032	0.044	0.034	0.032	0.049	0.022	3.5
2	0.27	0.22	0.43	0.06	0.09	0.28	0.06	>150
3	5.1	2.2	4.7	2.2	0.05	1.8	0.08	>150
4	0.022	0.017	0.033	0.013	0.005	0.01	0.004	>0.15
5	0.04	0.04	0.05	0.01	0.0009	0.05	0.0006	22

*synthetic material, see ref. 6a and 6d.

Piperidine analog **3** was found to be 30-100 fold less potent than racemic balanol. However, the high potency against the δ and η isozymes was selectively retained. This drastic loss of potency is contrasted by a marginal but uniform increase in potency against all 8 isozymes moving from balanol to pyrrolidine analog **4**. These results suggest that PKC inhibitory activity of this series of compounds is dependent on the conformation of substituents about the central cyclic structure.[12] A similar but more appreciable change in potency is observed in the all-carbon series where the five-membered analog **5** is found to be 10 times (100 times for δ and η) more potent than the seven-membered analog **2**. Analog **5** is also a selective inhibitor, with subnanomolar IC_{50} values, for the δ and η isozymes, which it inhibits 10-80 times more effectively than other isozymes. Thus it was

demonstrated not only with the heterocyclic analogs, but also in the carbocyclic series, that five is a preferred ring size over seven in terms of potency against PKC.

In summary we have shown that a five-membered ring is favored over a seven-membered ring for PKC inhibitory activity in the balanol series of compounds, and that six proved to be an unfavorable ring size. Importantly, the two five-membered ring analogs, **4** and **5**, are also more readily available synthetically than balanol as exemplified by the fact that the 4-benzyloxybenzamide of **16** has been prepared on hundred-gram scale.

Acknowledgement: We thank Joseph W. Wilson for providing us the precursor to compound **6** and Thomas Mitchell for performing elemental analyses and FTIR on compounds presented in this article.

References and Notes:

(1) Presented as part of medicinal chemistry poster #75 at the 208th ACS National Meeting, Washington, D.C., August 21-25, 1994.

(2) Deceased December 19, 1993.

(3) (a) Nishizuka, Y. *Nature* **1988**, *334*, 661. (b) Parker, P. J.; Kour, G.; Marais, R. M.; Mitchell, F.; Pears, C.; Schaap, D.; Stabel, S.; Webster, C. *Mol. Cell. Endocrinol.* **1989**, *65*, 1. (c) Stabel, S.; Parker, P. J. *Pharmacol. Ther.* **1991**, *51*, 71.

(4) Bradshaw, D.; Hill, C. H.; Nixon, J. S.; Wilkinson, S. E. *Agents Actions* **1993**, *38*, 135.

(5) Kulanthaivel, P.; Hallock, Y. F.; Boros, C.; Hamilton, S. M.; Janzen, W. P.; Ballas, L. M.; Loomis, C. R.; Jiang, J. B. *J. Am. Chem. Soc.* **1993**, *115*, 6452.

(6) For synthesis of the perhydroazepine moiety, see: (a) Lampe, J. W.; Hughes, P. F.; Biggers, C. K.; Smith, S. H.; Hu, H. *J. Org. Chem.* **1994**, *59*, 5147. (b) Hughes, P. F.; Smith. S. H.; Olson, J. T. *J. Org. Chem.* **1994**, *59*, 5799. (c) Nicolaou, K. C.; Bunnage, M. E.; Koide, K. *J. Am. Chem. Soc.* **1994**, *116*, 8402. (d) Hu, H.; Jagdmann G. E., Jr.; Hughes, P. F.; Nichols, J. B. *Tetrahedron Lett.* **1995**, *36*, 3659.

(7) All these balanol analogs were characterized by ^{1}H NMR, FTIR, and elemental analysis, and were homogeneous by TLC and/or HPLC.

(8) Lai, Y. S.; Stamper, M. *Bioorg. & Med. Chem. Lett.*, preceding article.

(9) Staudinger, H.; Hauser, E. *Helv. Chim. Acta* **1921**, *4*, 21.

(10) For the preparation of **6**, see: (a) Ref. 6a. (b) Hollinshead, S. P.; Nichols, J. B.; Wilson, J. W. *J. Org. Chem.* **1994**, *59*, 6703.

(11) (a) Kikkawa, U.; Go, M.; Komoto, J.; Nishizuka, Y. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 636. (b) Basta, P.; Strickland, M. B.; Holmes, W.; Loomis, C. R.; Ballas, L. M.; Burns, D. J. *Biochem. Biophys. Acta* **1992**, *1132*, 154. (c) Kashiwada, Y.; Huang, L.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 195.

(12) A computational evaluation of this possible relationship between potency and certain conformational indices is being undertaken in our laboratories.

(*Received in USA* 7 *July* 1995; *accepted* 16 *August* 1995)