Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 21, pp. 2477-2482, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00427-0

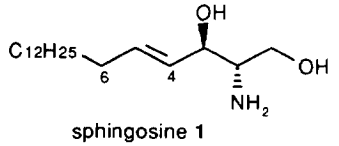
ARYL-FUSED SPHINGOLIPIDS AS NOVEL PKC INHIBITORS WITH TOPICAL ANTIINFLAMMATORY ACTIVITY

John J. Tegeler,* Barbara S. Rauckman, R. Richard L. Hamer, Brian S. Freed, Gregory H. Merriman, Luther Hellyer, Marie Ortega-Nanos, Steven C. Bailey, Ellen S. Kurtz

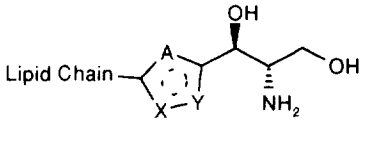
Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey 08876

Abstract: Novel aryl-fused sphingolipids, in which aryl-/heteroaryl-moieties were incorporated into the allylic 4,5,6-positions of sphingosine, were prepared and found to possess good *in vitro* PKC inhibitory activity. (3-(1-Dodecynyl)phenyl)- and (4- and (5-(1-dodecynyl)-2-thienyl)-2-amino-1,3-propanediols were found to have topical antiinflammatory activity comparable to sphingosine.

Protein kinase C (PKC) is a major player in signal transduction and the regulation of numerous cellular processes.[1] Evidence demonstrating alterations in the PKC signal transduction system in psoriasis has been reviewed.[2] The natural product sphingosine, D(+)-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-octadecene (**1**), and related long-chain bases have PKC inhibitory properties.[3] Sphingoid bases also displayed *in vitro* antiinflammatory properties in human neutrophils.[4] *In vivo* studies confirmed the ability of topically applied sphingosine to reduce phorbol ester-induced inflammation and epidermal hyperplasia and suggested its potential for the treatment of psoriasis and other inflammatory skin disorders.[5] With this in mind, we undertook a program to identify novel topically effective PKC inhibitors. With the knowledge that novel 1,3-pyridinyl-fused LTB_4 analogs[6] had displayed interesting biological properties, we initiated our studies with the synthesis and evaluation of a series of 1,3-pyridinyl-fused sphingosine derivatives (**2**). This report reveals the promising *in vitro* PKC inhibitory activity found in these and numerous other aryl- and heteroaryl- derivatives (**3-7**) and the topical antiinflammatory activity displayed by several lead compounds.



sphingosine **1**

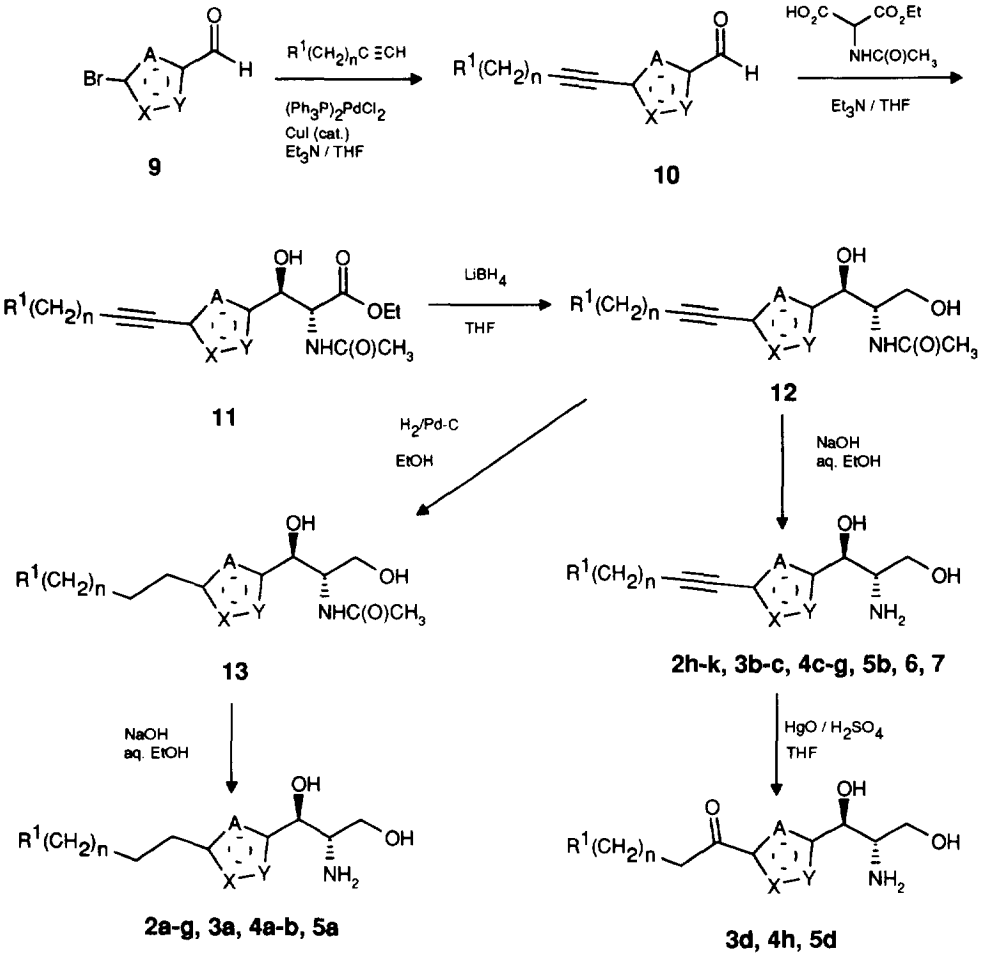


	A	X	Y
2	N	CH	CH-CH
3	CH	CH	CH-CH
4	S	CH	CH
5	CH	CH	S
6	CH	S	CH
7	N	CH	S
8	CH	N	O

A generic synthesis of alkyl-, alkynyl-, and α-acylaryl- and -heteroaryl-*erythro*-2-amino-1,3-propanediols is outlined in Scheme 1. Refer to Table 1 for specific examples of these compounds (**2-7**). Acetylenic coupling of bromo(aryl, -heteroaryl)aldehydes **9** in the presence of copper (I) iodide/bis[triphenylphospine]palladium chloride[7] in triethylamine/THF at 40°C provided alkyn-1-yl(pyridinyl, phenyl, thienyl, thiazolyl)aldehydes **10**. Aldol condensation[8] with acetamidomalonic acid-monoethyl ester[9] in triethylamine/THF at rt followed by recrystallization of the resulting 13:1 *erythro:threo* mixture gave pure ethyl *erythro*-3-hydroxy-2-acetamidopropionates **11**. Lithium borohydride reduction of esters **11** in THF yielded alkynyl-chain *erythro*-2-acetamido-1,3-propanediols **12**. Hydrogenation of alkynes **12** using 5% Pd-C in ethanol gave alkyl-chain

erythro-2-acetamido-1,3-propanediols **13**. Alkaline hydrolysis of amides **13** in 95% ethanol at 80°C provided alkyl-chain *erythro*-2-amino-1,3-propanediols including 2,6-pyridines **2a-g**, 1,3-benzene **3a**, 2,5-thiophenes **4a-b**, and 4,2-thiophene **5a**. Alkaline hydrolysis of amides **12** similarly gave alkynyl-chain *erythro*-2-amino-1,3-propanediols including 2,6-pyridines **2h-k**, 1,3-benzenes **3b-c**, 2,5-thiophenes **4c-g**, 4,2-thiophene **5b**, 2,4-thiophene **6**, and 5,2-thiazole **7**. Several alkynyl-chain *erythro*-2-amino-1,3-propanediols (**3c, 4e, 5b**) were treated with mercuric oxide and sulfuric acid in THF to provide the corresponding α-acyl-chain *erythro*-2-amino-1,3-propanediols including 1,3-benzene **3d**, 2,5-thiophene **4h**, and 4,2-thiophene **5d**.

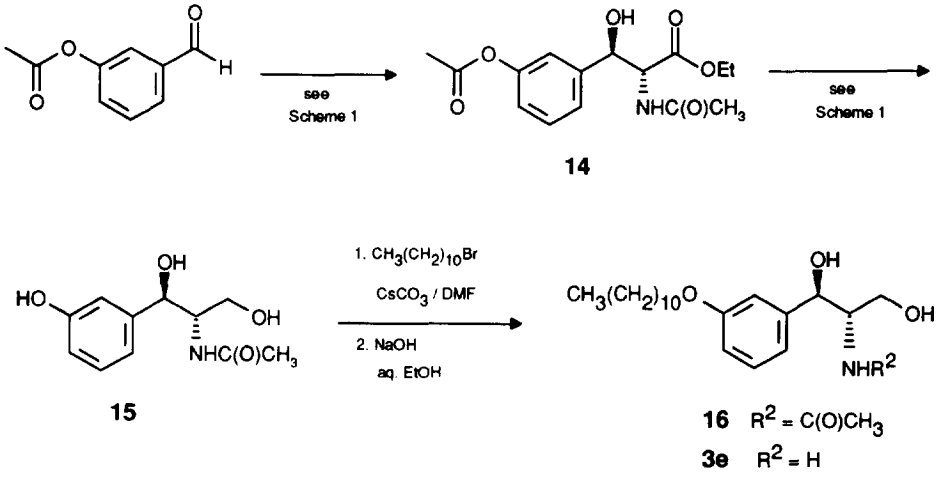
Scheme 1



The undecyloxy-chain benzene derivative **3e** was prepared as outlined in Scheme 2. Aldol condensation with 3-acetoxybenzaldehyde gave acetoxyphenyl acetamidopropionate **14**. Borohydride reduction yielded

hydroxyphenyl acetamidodiol **15**. Alkylation with undecyl bromide and cesium carbonate in DMF followed by hydrolysis provided undecyloxyphenyl acetamidodiol **16** and the corresponding aminodiol **3e**.

Scheme 2



14

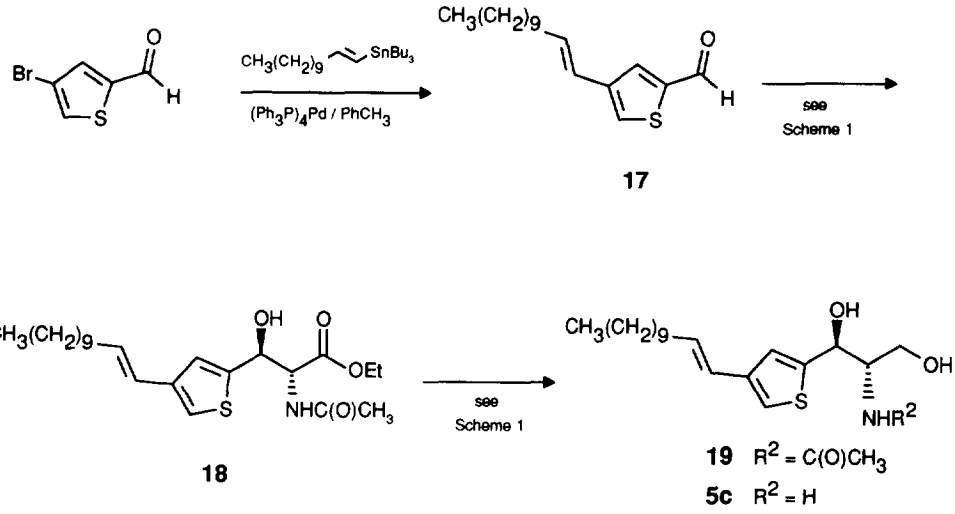
15

16 $R^2 = C(O)CH_3$

3e $R^2 = H$

The 4-(E)-1-dodecenyl-chain 2-thiophene chemistry is outlined in Scheme 3. Stille coupling of (E)-1-(tributylstannyl)-1-undecene and 4-bromo-2-thiophenecarboxaldehyde with tetrakis(triphenylphosphine)-palladium in toluene provided (E)-dodecenyl-chain carboxaldehyde **17**. Aldol condensation, borohydride reduction and hydrolysis gave acetamidopropionate **18**, acetamidopropanediol **19** and the desired aminodiol **5c**.

Scheme 3



17

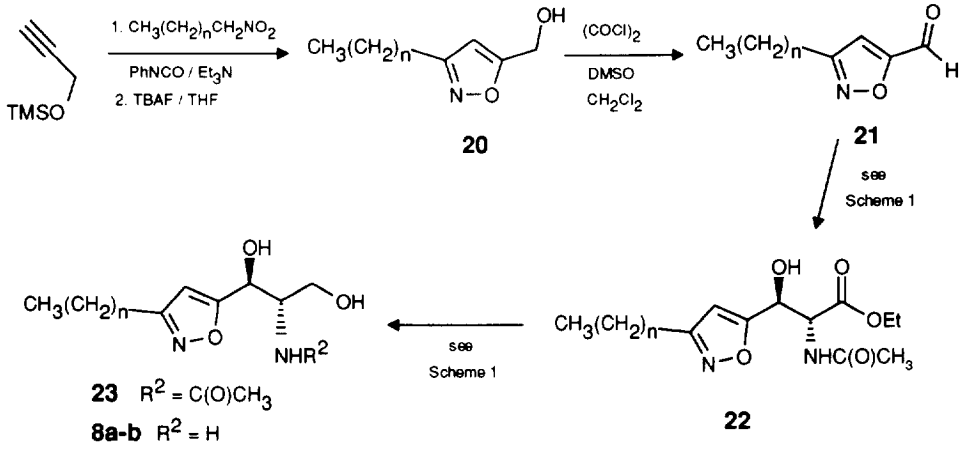
18

19 $R^2 = C(O)CH_3$

5c $R^2 = H$

The 3-alkyl-chain 5-isoxazole derivatives **8a-b** were prepared as depicted in Scheme 4. Nitrile oxide cycloaddition[10] to 3-(trimethylsilyloxy)-1-propyne followed by desilylation with tetrabutylammonium fluoride in THF resulted in isoxazole-5-methanols **20**. Barton oxidation gave the desired isoxazole-5-carboxaldehydes **21**. Aldol condensation, borohydride reduction and alkaline hydrolysis, as before, provided amide-ester **22**, amide-diol **23**, and 3-alkyl-chain 5-isoxazole aminodiols **8a-b**.

Scheme 4

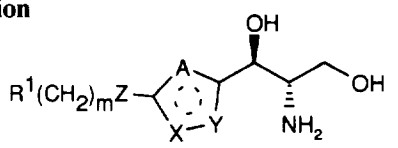


The PKC inhibitory activity of these aryl-fused sphingolipids is illustrated in Table 1. The *in vitro* assay utilized a modification of the radiometric mixed micelle procedure of Bell[11] with PKC isolated from rat brain.[12]

The SAR of pyridinyl derivatives **2a-k** reveals a correlation between the size of the lipid side chain and *in vitro* PKC inhibitory potency. A straight alkyl chain of ten carbons (**2c**) appears to be required for significant activity while potency gradually increases up to sixteen carbons (**2f**). Terminal phenyl substitution (**2g**) is similar to three straight chain carbons. In general, alkynyl-, alkenyl-, alkoxy-, and acyl-chain derivatives are equipotent or slightly more potent than their alkyl homologs.

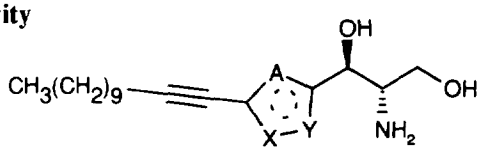
These novel PKC inhibitors were also evaluated for topical antiinflammatory activity using a modification of the phorbol ester-induced mouse ear edema assay of Young.[13] Table 2 reveals three dodecynyl analogs, including the 1,3-benzene **3c**, the 2,5-thiophene **4e**, and the 2,4-thiophene **5b**, that displayed consistent potent antiinflammatory activity comparable to sphingosine (**1**). The enantiospecific synthesis and biological evaluation of specific enantiomers of these compounds is the subject of an accompanying report.

Table 1. In Vitro Protein Kinase C Inhibition



Compound	R	m	Z	A	X	Y	PKC (IC_{50} μM)[14]
2a	H	5	CH_2	N	CH	CH-CH	NA@100
2b	H	7	CH_2	N	CH	CH-CH	>100
2c	H	9	CH_2	N	CH	CH-CH	30.5
2d	H	10	CH_2	N	CH	CH-CH	10.0
2e	H	11	CH_2	N	CH	CH-CH	10.9
2f	H	15	CH_2	N	CH	CH-CH	4.5
2g	Ph	4	CH_2	N	CH	CH-CH	NA@100
2h	H	8	C≡C	N	CH	CH-CH	13.3
2i	H	9	C≡C	N	CH	CH-CH	10.0
2j	Ph	3	C≡C	N	CH	CH-CH	NA@100
2k	Ph	5	C≡C	N	CH	CH-CH	15.9
3a	H	11	CH_2	CH	CH	CH-CH	3.7(17.3[15])
3b	H	9	C≡C	CH	CH	CH-CH	9.7
3c	H	10	C≡C	CH	CH	CH-CH	2.7(22.2[15])
3d	H	11	C(O)	CH	CH	CH-CH	20.7[15]
3e	H	11	O	CH	CH	CH-CH	24.1[15]
4a	H	8	CH_2	S	CH	CH	10.0
4b	H	11	CH_2	S	CH	CH	14.0
4c	H	7	C≡C	S	CH	CH	19.9
4d	H	9	C≡C	S	CH	CH	4.7
4e	H	10	C≡C	S	CH	CH	2.9(26.2[15])
4f	H	11	C≡C	S	CH	CH	3.5
4g	Ph	4	C≡C	S	CH	CH	36.0
4h	H	11	C(O)	S	CH	CH	22.4[15]
5a	H	11	CH_2	CH	CH	S	13.3[15]
5b	H	10	C≡C	CH	CH	S	10.8(23.7[15])
5c	H	10	(E)-C=C	CH	CH	S	5.0
5d	H	11	C(O)	CH	CH	S	22.4[15]
6	H	10	C≡C	CH	S	CH	34.5[15]
7	H	10	C≡C	N	CH	S	15.2[15]
8a	H	9	CH_2	CH	N	O	43.9
8b	H	11	CH_2	CH	N	O	9.1
1	sphingosine						6.7(23.4[15])

Table 2. Topical Antiinflammatory Activity



Compound	A	X	Y	PKC[14] (IC_{50} μM)	TPAEE[16] (ED_{50} mg/ear)
3c	CH	CH	CH-CH	2.7(22.2[15])	0.34
4e	S	CH	CH	2.9(26.2[15])	0.24
5b	CH	S	CH	10.8(23.7[15])	0.23
1	sphingosine			6.7(23.4[15])	0.31

References and Notes

1. Nishizuka, Y. *Science* **1986**, *233*, 305.
2. Hegemann, L.; Mahrle, G. In *Pharmacology of the Skin*; Mukhtar, H., Ed.; CRC: Boca Raton, 1991; pp 357-368.
3. Hannun, Y. A.; Loomis, C. R.; Merrill, A. H. Jr.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604.
4. Wilson, E.; Olcott, M. C.; Bell, R. M.; Merrill, A. H. Jr.; Lambert, J. D. *J. Biol. Chem.* **1986**, *261*, 12616.
5. Gupta, A. K.; Fisher, G. J.; Elder, J. T.; Nickoloff, B. J.; Voorhees, J. J. *J. Invest. Dermatol.* **1988**, *91 (5)*, 486.
6. Morris, J.; Wishka, D. G. *Tetrahedron Lett.* **1988**, *29*, 143.
7. Takahashi, S.; Kuroyama, Y.; Sonogashira, K.; Hagihara, N. *Synthesis*, **1980**, 627.
8. Hellmann, H.; Piechota, H. *Justus Liebigs Ann. Chem.* **1960**, *631*, 175.
9. Hellman, H.; Teichmann, K.; Lingens, F. *Chem. Ber.* **1958**, *91*, 2427.
10. Mukiayama, T.; Hoshino, T. *J. Am. Chem. Soc.* **1960**, *82*, 5339.
11. Bell, R. M.; Hannun, Y.; Loomis, C. In *Methods in Enzymology*, Vol. 124, Academic: New York, 1986; pp 353-359.
12. Kikkawa, U.; Go, M.; Koumoto, J.; Nishizuka, Y. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 626: This method uses whole rat brain and results in a mixture of the PKC isozymes. See reference 2 for a review of some of the known isozyme differences between rat brain and human skin.
13. Young, J. M.; Wagner, B. M.; Spires, D. A. *J. Invest. Dermatol.* **1983**, *80*, 48.
14. The PKC assay[11] used for this data was performed with 4μg phosphatidyl serine:diacylglycerol (2:1).
15. The PKC assay[11] used for this data was performed with 8μg phosphatidyl serine:diacylglycerol (2:1).
16. The method of Young[13] was modified measuring differences in ear punch (4mm) weights from vehicle control. TPA [(12-O-tetradecanoyl)phorbol-13-acetate, Sigma] (2 μg/ear) was applied. The vehicle used was propylene glycol:ethanol (3:7).

(*Received in USA* 4 *August* 1995; *accepted* 20 *September* 1995)