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THE ENANTIOSELECTIVE SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF NOVEL ARYL-SPHINGOLIPID PKC INHIBITORS

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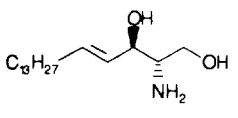
Abstract: Recently we identified three promising topically active antiinflammatory agents (**2**, **3**, and **4**) from a series of racemic aryl-sphingolipids that inhibit protein kinase C (PKC). We now wish to report the enantioselective synthesis of the aforementioned leads and their comparative biological profile. The individual enantiomers examined are equipotent to their racemate *in vitro* and *in vivo*.

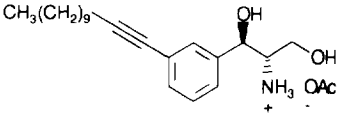
Protein kinase C (PKC) is a family of phospholipid-dependent proteins that mediate signal transduction and regulate cellular processes.[1] Specifically, PKC plays a major role in regulating epidermal cell proliferation and differentiation, and has recently been implicated in the pathophysiology of inflammatory-hyperproliferative skin diseases such as *psoriasis*.[2] For example, several reports now provide evidence of alterations in the PKC signal transduction pathway in psoriasis and indicate the potential therapeutic value of a PKC inhibitor for the treatment of this disease.[2-6]

The natural product sphingosine, D-(+)-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-octadecene (**1**), and related sphingolipids are known to inhibit PKC *in vitro*[7] and display antiinflammatory activity in human neutrophils.[8] Sphingosine (**1**) also reduces phorbol ester-induced inflammation and epidermal hyperplasia *in vivo*.[9]

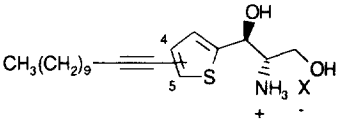
In our efforts to develop new topically active antiinflammatory and antiproliferative agents we have recently reported the synthesis and evaluation of a variety of racemic *erythro*-aryl-sphingosine analogs.[10] As a result of these studies we have identified three promising candidates for further study, including alkynyl-phenyl- and thiophene analogs **2**, **3** and **4**. We now wish to report the enantioselective synthesis and biological evaluation of these candidates.



Sphingosine (**1**)



2

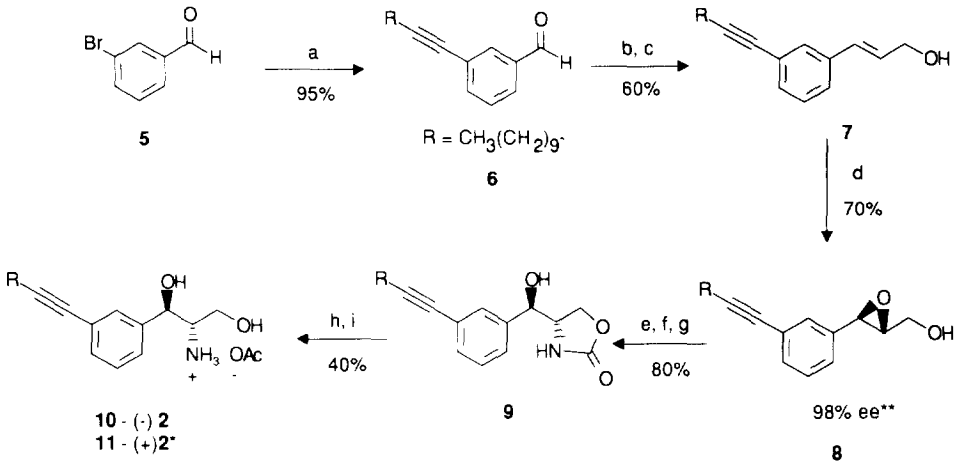


3 4-yl X = Maleate
3a 4-yl X = HCl
4 5-yl X = Acetate

Chemistry

Three unique enantioselective routes for the preparation of the aforementioned PKC inhibitors were investigated. These routes were adopted from literature methods for the preparation of D-(+)-*erythro*-sphingosine (**1**) and related sphingolipids. We began with preparation of the phenyl-sphingosine enantiomers of **2** using Sharpless epoxidation and carbamate cyclization methodology (Scheme I).[11] Thus, coupling of bromobenzaldehyde **5** with 1-dodecyne afforded the alkynyl-benzaldehyde **6** in 95% yield. Treatment of **6** with (carbethoxymethylene)triphenylphosphorane followed by reduction of the resulting α,β-unsaturated ester using diisobutylaluminum hydride afforded allylic alcohol **7**. Sharpless epoxidation of **7** using (-)-diisopropyltartrate afforded epoxy alcohol **8** in 98% ee (70%).[12] Treatment of **8** with benzoylisocyanate followed by carbamate cyclization using sodium hydride and hydrolysis of the benzoyl ester gave **9** in 80% yield for three steps. Carbamate **9** was hydrolyzed under more vigorous conditions and the resulting aminodiol converted to the corresponding acetate salt **10** (40%), ($[\alpha]_D^{25}$ = -17.3°, c. 0.53, EtOH). Enantiomer **11** ($[\alpha]_D^{25}$ = +16.8°, c. 0.50, EtOH) was prepared under similar conditions using (+)-diisopropyltartrate for the epoxidation step **7**→**8**.

Scheme I



(a) 1-Dodecyne, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, THF; (b) $Ph_3P{=}CHCO_2Et$, Toluene, Δ; (c) Dibal-H, THF, -78°C; (d) (-)-DIPT, *t*BuOOH, $Ti(iOPr)_4$, 4A Molecular Sieves; (e) PhC(O)NCO, CH_2Cl_2; (f) NaH, THF; (g) NaOH, MeOH-H_2O; (h) NaOH, EtOH, Δ; (i) AcOH

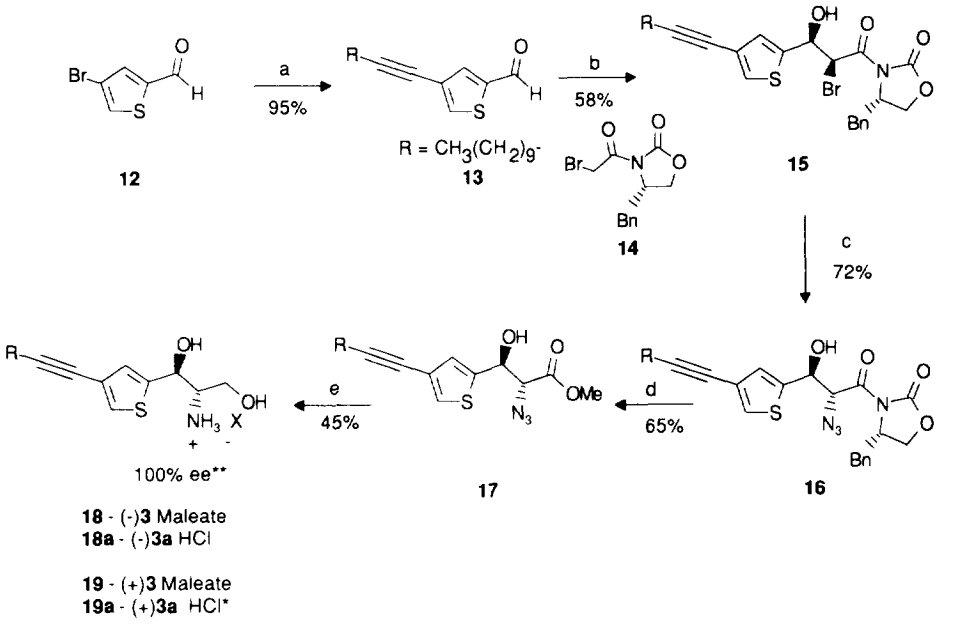
* (+)-**2** (**11**) was prepared using (+)-DIPT in the Sharpless epoxidation **7** → **8**

** Epoxide **8** was analyzed by chiral HPLC using a Chiralcel OD column.

Unfortunately, the Sharpless epoxidation methodology failed when applied to the synthesis of the corresponding thiophene analogs. As a result we employed chiral oxazolidinone technology to prepare the

enantiomers of thiophene analog **3** (Scheme II).[13] Thus, treatment of thiophene carboxaldehyde **13** with the boron enolate derived from (*S*)-bromoacetyl-oxazolidinone **14** gave diastereomerically pure bromohydrin **15** (58%). Treatment of **15** with sodium azide afforded hydroxy-azide **16** in 72% yield. Subsequent removal of the chiral auxiliary using methoxy magnesium bromide and reduction of the resulting azide-ester **17** with lithium aluminum hydride gave the desired target **18** ($[\alpha]_D^{25}$ = -18.0°, c. 0.54, EtOH). Enantiomer **19** ($[\alpha]_D^{25}$ = +18.9°, c. 0.57, EtOH) was prepared in comparable yield using the (*R*)-oxazolidinone of **14** for the conversion **13** → **15**.

Scheme II



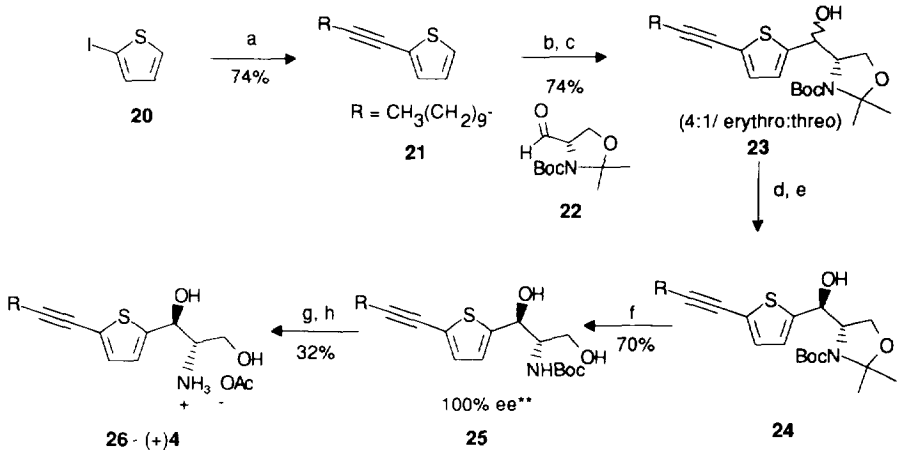
(a) 1-Dodecyne, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, THF; (b) 4(*S*)-oxazolidinone **14**, nBu_2BOTf, Et_3N, Et_2O; (c) NaN_3, DMSO; (d) MeO^-MgBr^+, MeOH; (e) LAH, Et_2O

* (+)-**3** (**19**) was prepared using the 4(*R*)-oxazolidinone enantiomer of **14** for the conversion **13**→**15**.
** Compound **18** was derivatized to its *N*-Boc carbamate and analyzed by chiral HPLC using a Chralcel OD column.

Finally, a third independent route was explored for the preparation of a thiophene **4** enantiomer (Scheme III). The key step involved the *ortho*-metallation of thiophene **21** followed by condensation with the optically pure serine derived aldehyde **22** to afford aldol product **23** as a mixture of diastereomers (erythro:threo = 4:1) (74%).[14,15] The mixture was acetylated and separated by chromatography. The major *erythro* component was hydrolyzed using potassium carbonate in methanol to give diasteromerically pure **24**. Selective hydrolysis of the

dimethylacetonide using *p*-toluenesulfonic acid in methanol gave *N*-Boc diol **25** in 70% yield. A more vigorous hydrolysis using methanolic HCl gave optically pure target **26** (32%), ($[\alpha]_D^{25}$ = +7.0°, c. 0.63 EtOH).

Scheme III



(a) 1-Dodecyne, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, THF; (b) nBuLi, THF, -78°C (c) aldehyde **22**; (d) Ac_2O, Et_3N, DMAP; Chromatograph (e) K_2CO_3, MeOH; (f) *p*-TSA, MeOH; (g) HCl, MeOH; (h) AcOH

** Compound **25** was analyzed by chiral HPLC using a Chiracel OD column.

Pharmacology

In vitro PKC Inhibition (PKC). The radiometric PKC assay was modified from the method of Bell et al.[16] PKC was isolated from rat brain by the method of Kikkawa.[17] Inhibition was determined by the blockade of ^{32}P incorporation into histone type III upon phosphatidyl serine (PS) : diacylglycerol (DAG) (2 : 1; 8 μg total) stimulation in the presence of Ca^{2+}. Compounds were tested at 10^{-6} -10^{-4} M. The known PKC inhibitor, D-(+)-sphingosine (Sigma), derived from bovine brain cerebroside, was utilized as a comparative standard.

PKC-dependent neutrophil superoxide burst assay (NSOB). Superoxide anion production by neutrophils (NSOB) was performed by modification of the method of Babior.[18] Canine neutrophils were isolated by a modification of Boyum[19] from whole blood and combined with salt solution, cytochrome C (170μM) and vehicle or PKC inhibitor. After pretreatment with inhibitors for 3 min, the reaction was initiated with (12-*O*-tetra-decanoyl)phorbol-13-acetate (TPA) and production of reduced cytochrome C was monitored at 550 nm over 4 min.

In vivo antiinflammatory assay (TPAEE). Phorbol ester-induced ear edema determinations were performed using a modification of Young et al.[20] Male CFW (Charles River) control mice (n=8) received a solution containing 2 μg of TPA on the right ear and vehicle on the left ear. Drug treated animals received drug and TPA (2μg) on the right ear and vehicle on the left ear. After 5 h the mice were sacrificed, ear punches were

taken from each ear (4mm) and weighed on an analytical balance. The difference in punch weights (right ear - left ear) was determined and the percent change from the control group quantified.[21]

Results

The enantiomers of three lead aryl-sphingosine racemates **2**, **3** and **4** were prepared and tested as topical antiinflammatory agents (Table). Thus, all of the enantiomers inhibited PKC in an isolated enzyme assay at comparable levels to both the racemate and sphingosine (**1**). The enantiomers were also equipotent in a PKC-regulated neutrophil superoxide burst assay (NSOB). The activity of the compounds in this whole cell assay is indicative of their high membrane permeability and potent inhibition of neutrophil function. Finally, the enantiomers also exhibited similar topical antiiflammatory activity *in vivo* using a phorbol ester-induced ear edema model (TPAEE). In conclusion, the enantiomers of leads **2-4** appear to be equal in activity to their respective racemates and similar in activity to the known PKC inhibitor sphingosine (**1**). Thus, as seen with sphingosine enantiomers,[22] there appears to be no stereochemical specificity for the *erythro*-2-amino-1,3-propanediol moiety on these analogs at their PKC binding site. With this in mind, racemic compound **3a** was selected for toxicological evaluation based on its superior solubility and stability characteristics.

Table

Compound	**PKC Inhibition** (IC_{50} μM)	**NSOB** (IC_{50} μM)	**TPAEE** % change from control @ 1 mg/ear screening dose (ED_{50} mg/ear)
2 (±)	22.2	2.4	-82% (0.34)
10 (–)	21.8	2.2	-74%
11 (+)	19.5	2.4	-74%
3 (±)	23.7	1.5	NT
3a (±)	28.8	NT	-81% (0.23)
18 (–)	20.1 (**18a**)	1.3	-73%
19 (+)	23.0 (**19a**)	1.0	-63%
4 (±)	26.2	1.5	-81% (0.24)
26 (+)	21.3	1.8	-81%
1 (Sphingosine)	23.4	4.2	-74% (0.31)

References and Notes

1. Nishizuka, Y. *Science* **1986**, *233*, 305.
2. Hegemann, L.; Mahrle, G.; *Pharmacology of the Skin;* Muktar, H., Ed.; CRC: Boca Raton, 1991, 357.
3. Horn, F.; Fisher, G. J.; Marcelo, C. L.; Voorhees, J. J. *J. Invest. Dermatol.* **1990**, *95*, 428.
4. Fisher, G. J.; Talwar, H. S.; Baldassare, J. J.; Voorhees, J. J. *J. Invest. Dermatol.* **1993**, *101 (4)*, 560.
5. Fisher, G. J.; Talwar, H. S.; Tavakkol, A.; Esmann, J.; Baldassare, J. J.; Elder, J. T.; Griffiths, C. E. M.; Baadsgaard, O.; Cooper, K. D.; Voorhees, J. J. *J. Invest. Dermatol.* **1990**, *95*, 15s.
6. Rasmussen, H. H.; Celis, J. E. *J. Invest. Dermatol.* **1993**, *101 (4)*, 560.
7. Hannun, Y. A.; Loomis, C. R.; Merrill, A. H.; Bell, R. M. *J. Biol.Chem.* **1986**, *261*, 12604.
8. Wilson, E.; Olcott, M. C.; Bell, R. M.; Merrill, A. H. Jr.; Lambert, J. D. *J. Biol. Chem.***1986**, *261*, 12616.
9. Gupta, A. K.; Fisher, G. J.; Elder, J. T.; Nickoloff, B. J.; Voorhees, J. J. *J. Invest. Dermatol.***1988**, *91 (5)*, 486.
10. Kurtz, E. S.; Bailey, S. C.; Hellyer, L. D.; Dominguez, A. A.; Prezkop, P. A.; Ortega-Nanos, M. C.; Freed, B. S.; Hamer, R. R. *J. Invest. Dermatol.* **1993**, *100 (4)*, 518. Tegeler, J. J.; Rauckman, B. S.; Hamer, R. R. L.; Freed, B. S.; Merriman, G. H.; Hellyer, L.; Ortega-Nanos, M.; Bailey, S. C.; Kurtz, E. S. *Abstracts of Papers* 209th National Meeting of the American Chemical Society, Los Angeles, Ca.; **1995**, Abstracts MEDI-179, 180 and 181.
11. Knapp, S.; Kukkola, P. J.; Sharma, S.; Murali Dhar, T. G.; Naughton, A. B. J. *J. Org. Chem.* **1990**, *55*, 5700. Roush, W. R.; Adam, M. A. *J. Org. Chem.* **1985**, *50*, 3752. Vasella, A.; Bernet, B. *Tetrahedron Lett.* **1983**, *24*, 5491. Mori, K.; Umemura, T. *Agric. Biol. Chem.* **1987**, *51 (7)*, 1973. Mori, K.; Umemura, T. *Tetrahedron Lett.* **1981**, *22*, 4433. Vasella, A.; Bernet, B.; Herzig, T.; Julina, R. *Helv. Chim. Acta.* **1986**, *69*, 368.
12. For a reveiw of the Sharpless epoxidation see: Pfenninger, A. *Synthesis*, **1986**, 89.
13. Nicolaou, K. C.; Caufield, T.; Kataoka, H.; Kumazawa, T *J. Am. Chem. Soc.* **1988**, *110*, 7910. Nakagawa, M.; Tsuruoka, A.; Yoshida, J.; Hino, T. *J. Chem. Soc., Chem. Commun.* **1990**, 603.
14. Liotta, D.; Nimkar, S.; Menaldino, D.; Merrill, A. H. *Tetrahedron Lett.* **1988**, 3073. Dondoni, A.; Fantin, G.; Fogagnolo, M.; Medici, A. *J. Chem. Soc., Chem. Commun.* **1988**, 10. Eiermann, V.; Devant, R. M.; Radunz, H.-E. *Liebigs Ann. Chem.* **1988**, 1103.
15. For preparation of **22** see: Garner, P.; Park, J.-M. *J. Org. Chem.* **1987**, *52*, 2361.
16. Bell, R. M.; Hannun, Y.; Loomis, C. ; *Methods in Enzymology*, Academic: New York 1986, Vol. 124, 353.
17. Kikkawa, U.; Go, M.; Kuomoto, J.; Nishizuka, Y. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 626.
18. Babior, B. M. *J. Clin. Invest.* **1984**, *73*, 599.
19. Boyum, A. Scand. *J. Immunol.* **1976**, *5*, 9.
20. Young, J. M.; Wagner, B. M.; Spires, D. A. *J. Invest. Dermatol.* **1983**, *80*, 48.
21. % change from control = (x control ear weights - x treatment ear weights/ x control ear weights) x 100
22. Merrill, A. H.; Nimkar, S.; Menaldino, D.; Hannun, Y. A.; Loomis, C.; Bell, R. M.; Tyagi, S. R.; Lambeth, J. D.; Stevens, V.; Hunter, R.; Liotta, D. *Biochemistry*, **1989**, *28*, 3138.

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