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SYNTHESIS, *IN VITRO* CYTOTOXICITY AND *IN VIVO* ANTI-INFLAMMATORY ACTIVITY OF LONG CHAIN 3-AMINO-1,2-DIOLS

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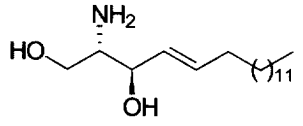
Abstract : The synthesis of long chain 3-amino-1,2-diols was carried out based on Sharpless asymmetric epoxidation of long chain allylic alcohols and regioselective nucleophilic ring opening by azido group. The *in vitro* cytotoxicity of the compounds prepared was evaluated against six solid tumor cell lines (A2780, H322, LL, WiDr, C26-10, UMSCC-22B). Free 3-amino-1,2-diols exhibited IC_{50} values between 1.45 μM and 32 μM. These compounds also presented interesting inhibition of carrageenin-induced paw edema in rats (85.3% - 79.6% at a concentration of 0.15 mmol/kg).

Sphingosine (**1**), a precursor and breakdown product of sphingolipids, as well as related compounds, have been found to inhibit protein kinase C, an enzyme that has been implicated in cell replication, tumor promotion, oncogenesis and signal transduction[1]. Structure-activity relationship studies showed that a long hydrophobic chain and a free amino group are the structural requirements for the inhibition of protein kinase C[2].



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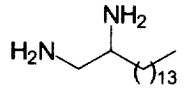

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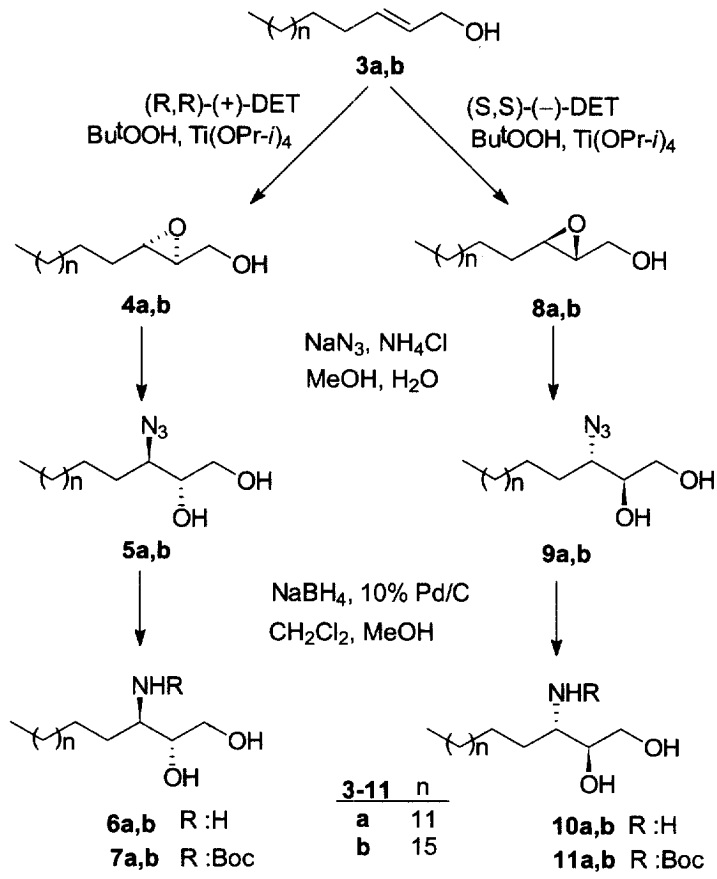
Long chain saturated amines (C12 to C18) and the unsaturated oleyl amine (C18:0) were found to inhibit the growth of *ras*-transformed NIH 3T3 cells (PAP2 cells)[3]. However, such a cell growth inhibition was attributed not only to protein kinase C inhibition but also to other factors[3]. On the other hand, sphingosine diversely affects a variety of enzymes such as phospholipases A_2 and D[4], phosphatidate phosphohydrolase[5] and calmodulin-depedent enzymes[6].

Two of us have been recently involved in the design and synthesis of new platinum(II) complexes[7,8] and novel lipid analogues[9] and the evaluation of their cytotoxic activity. According to this research, lipidic amines, such as oleyl amine and 1,2-hexadecanediamine[8,9] (**2**) showed interesting *in vitro* results against various cell lines. In this paper the enantiomeric synthesis of long chain 3-amino-1,2-diols, which can be considered sphingosine analogues, their *in vitro* cytotoxicity and their *in vivo* anti-inflammatory activity are described.



Chemistry Long chain allylic alcohols **3a,b** may be prepared either by alkylation of a protected propargyl alcohol followed by deprotection and stereoselective reduction with $LiAlH_4$ or by Knoevenagel condensation of the suitable aldehyde with ethyl hemimalonate and reduction of the *E*-(α,β)-unsaturated ester with DIBAL®[10].

Alcohols **3a,b** were submitted to Sharpless asymmetric epoxidation with the expected yields and ee's (>80% yield and >95% ee) (Scheme 1)[11]. 2,3-Epoxy alcohols **4a,b** and **8a,b** were synthesized utilizing *(R,R)*-(+)- and *(S,S)*-(−)-diethyl tartrate respectively. The opening of epoxides using sodium azide and ammonium chloride yielded the azido diols **5a,b** and **9a,b** in good regioselectivity (>10:1) and high yield. The reduction of the azido group was carried out in high yield using $NaBH_4$ in the presence of 10% Pd/C in dichloromethane-methanol[9]. Boc-protected amino diols **7a,b** and **11a,b** were obtained when the reduction was performed in the presence of di-*tert*-butyl dicarbonate.

All intermediates and final products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures[12].

***In Vitro* Cytotoxicity Assay** Cell Culture: The *in vitro* experiments were performed with six different cell lines: A2780, a human ovarian cancer cell line; H322, a human NSCLC cell line (subtype BAC, NCI); LL, a murine NSCLC cell line; WiDr, a human colon carcinoma cell line; C26-10, a murine colon carcinoma cell line; and UMSCC-22B, a human head and neck squamous cell carcinoma cell line[13]. Cells were grown in monolayers in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM *L*-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinised and resuspended in antibiotic containing medium (50 μg gentamicin/mL); single cell suspensions displaying ≥97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto the microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities between 5,000 and 20,000 cells per well, based on their doubling times[14,15].

Chemosensitivity Testing: The assay was performed in 96-well plates using the National Cancer Institute protocol[16].Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Each agent was tested at five 10-fold dilutions, starting from a maximum concentration of 10^{-4} M. Immediately after preparation of these intermediate dilutions, 100 μL aliquots of each dilution were added to the appropriate 96-well plate wells. Drug incubation times were of 72 h after which cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min. Then the sulforhodamine B (SRB) assay was performed[17,18]. The optical density (OD) of each well was measured at 492 nm using a 96-well plate reader (Titertek Multiscan MCC/340; Flow Laboratories). The cytotoxicity of the compounds is expressed as IC_{50}, which is the drug concentration causing a 50% growth inhibition.

***In Vivo* Studies. Inhibition of Carrageenin-Induced Paw Edema in Rats** The experiment was conducted on Fisher 344 rats of either sex. Two groups, consisting of six animals each, were used for each compound. Pregnant females were excluded. Paw edema was induced as previously reported[19] by injecting intradermally a solution of carrageenin (type K100) in saline 2 % (0.1 mL), into the right hand paw and measuared 3.5 hrs later. A single dose of 0.15 mmol/kg body weight of compounds, was administered simultaneously (ip). Indomethacin (0.11 mmol/kg) was used as a standard drug.

Results and Discussion The hydrochloride salts of **6a,b** and **10a,b** together with the *N*-Boc protected derivative **11a** were tested for their cytotoxicity against six cell lines of various origin. Compound **2**, which has been previously reported to inhibit cell growth[8,9], was also tested against the same cell lines for comparison purposes. The IC_{50} values exhibited by the compounds are summarized in Table 1.

Table 1. *In Vitro* Cytotoxic Activity of Long Chain 3-Amino-1,2-diols

	IC_{50} (μM) ± SEM[a,b]					
Compound	A2780	H322	LL	WiDr	C26-10	22B
2	2.79 ± 0.11	2.94 ± 023	2.93 ± 0.08	24.18 ± 5.90	3.30 ± 0.53	1.89 ± 0.12
6a	2.63 ± 0.31	1.52 ± 0.04	1.87 ± 0.52	2.99 ± 0.03	3.58 ± 0.12	2.49 ± 0.09
6b	32.79 ± 10.59	24.35 ± 6.12	10.55 ± 5.16	13.28 ± 5.47	21.54 ± 5.36	3.50 ± 0.16
10a	2.66 ± 0.33	1.45 ± 0.23	2.86 ± 0.18	2.76 ± 0.06	3.68 ± 0.15	1.89 ± 0.23
10b	2.71 ± 0.38	1.71 ± 0.41	3.47 ± 1.09	3.47 ± 0.04	3.97 ± 0.25	2.34 ± 0.17
11a	30.40 ± 5.19	18.01 ± 3.12	24.18 ± 5.90	20.82 ± 6.77	29.34 ± 7.52	23.84 ± 5.74

[a]Mean values of 3 independent experiments; [b]Statistical significance of results was established using the Student's T test $p<0.05$.

Compounds **6a**, **10a**, **10b** exhibited similar activity against all cell lines and comparable to that presented by compound **2**. Comparing the activities of (2*S*,3*S*) derivatives **10a** and **10b**, it seems that the IC_{50} values were not depended on the chain length. However, in the case of (2*R*,3*R*) derivatives **6a**, **6b** the increase of the chain length from C17 to C21 caused a decrease of the activity, apart from the cell line 22B. A similar decrease of the activity was observed when the amino group of **10a** was protected (compound **11a**), indicating that the presence of a free amino group is critical.

Taking into consideration that sphingosine can act as an anti-inflammatory agent[20], the compounds prepared were tested for such an activity. The rat carrageenin-induced paw edema assay was employed as a model for acute inflammation and indomethacin was included as a reference drug. The *in vivo* data are summarized in Table 2. All the long chain 3-amino-1,2-diols **6a,b**, **10a,b** exhibited interesting inhibition of paw edema (85.3%-79.6% at a concentration of 0.15 mmol/kg). It seems that neither the stereochemistry, nor the chain length significantly influenced the inhibition of edema. Comparing the activities of compounds **10** and **11**, it is concluded that the presence of a free amino group is essential also for the expression of *in vivo* anti-inflammatory activity. 1,2-Hexadecanediamine (**2**) was also tested under the same conditions and found to exhibit 93.5% inhibition of paw edema.

Although during last years accumulating evidence from epidemiological and laboratory animal studies has indicated that chronic ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a

reduced risk of death from colon cancer[21,22], the molecular mechanism by which NSAIDs inhibit carcinogenesis remains unknown. The results obtained in this study show that there is a correlation between cytotoxicity and CPE inhibition. The compounds that present *in vitro* cytotoxicity, exhibit also *in vivo* anti-inflammatory activity. On the contrary compound **11a** proved inactive in both systems.

Table 2. *In Vivo* Anti-inflammatory Activity of Long Chain 3-Amino-1,2-diols

Compound[a]	% Inhibition of CPE ± SEM[b]
2	93.50 ± 0.36
6a	85.30 ± 0.98
6b	80.04 ± 0.48
10a	81.10 ± 1.07
10b	79.60 ± 0.94
11a	45.20 ± 1.75
Indomethacin[c]	53.30 ± 5.60

[a]Dose: 0.15 mmol/kg; [b]Statistical significance of results was established using the Student's T test $p<0.001$; [c]Dose: 0.11 mmol/kg.

Acknowledgement

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12. For example: Compound **6b**: $[\alpha]_D$ +5.4 (*c* 0.5, MeOH). ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 0.85 (t, 3H, *J*=7 Hz), 1.25 (m, 32H), 1.44 (m, 1H), 1.64 (m, 1H), 3.26 (m, 1H), 3.59 (dd, 1H, *J*=11 and *J*=5 Hz), 3.67 (dd, 1H, *J*=11 and *J*=4.5 Hz), 3.75 (dd, 1H, *J*=4.5 and *J*=4.5 Hz). FAB MS: m/e 367 (M+23-HCl, 40 %), 3.45 (M+H-HCl, 100), 283 (17), 176 (56). Analysis for $C_{21}H_{45}NO_2$·HCl (380.05): Calc C 66.37, H 12.20, N 3.69 %; Found C 66.47, H 12.33, N 3.81 %. Compound **7b**: $[\alpha]_D$ +9.6 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.88 (t, 3H, *J*=7 Hz), 1.26 (m, 32H), 1.45 (s, 9H), 1.75 (m, 1H), 1.85 (m, 1H), 2.83 (d, 1H, *J*=8 Hz), 3.30 (b s, 2H), 3.50 (m, 1H), 3.61 (m, 1H), 3.68 (m, 1H), 4.53 (d, 1H, *J*=7.5 Hz). Analysis for $C_{26}H_{53}NO_4$ (443.71): Calc C 70.38, H 12.04, N 3.16 %; Found C 70.26, H 11.99, N 3.20 %. Compound **10b**: $[\alpha]_D$ -5.4 (*c* 0.5, MeOH). Analysis for $C_{21}H_{45}NO_2$·HCl (380.05): Calc C 66.37, H 12.20, N 3.69 %; Found C 66.36, H 12.37, N 3.42 %.
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