Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 11, pp. 1369-1374, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/94 $7.00+0.00

0960-894X(94)00158-8

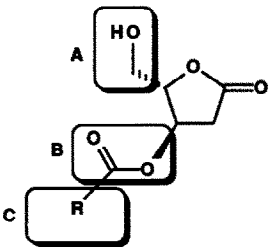
CONFORMATIONALLY CONSTRAINED ANALOGUES OF DAG .8.[1] CHANGES IN PK-C BINDING AFFINITY PRODUCED BY ISOSTERIC GROUPS OF THE 3-*O*-ACYL FUNCTION IN 2-DEOXY-L-RIBONOLACTONES

Jeewoo Lee[†], Rajiv Sharma[†], Kelly Teng[†], Nancy E. Lewin[‡], Peter M. Blumberg[‡] and Victor E. Marquez[†]*

[†]Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment and [‡]Molecular Mechanisms of Tumor Promotion Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, NIH, Bethesda, Maryland 20892 (USA)

Abstract: Syntheses of a series of 2-deoxy-L-ribonolactones modified at C-3 with groups isosteric to the 3-*O*-acyl moiety of the parent 3-*O*-tetradecanoyl-2-deoxy-L-ribonolactone (**1**) are reported. Lipophilicity of the molecules was kept constant by maintaining invariant the length of the aliphatic chain. Compound **5** was identified as having an affinity equal to that of **1** in a competitive binding assay that measured the ability of the ligands to displace [^{3}H]-phorbol-12,13-dibutyrate from PK-C. The reverse ester function in **5** makes this compound more stable than **1** by preventing β-elimination. Other changes in **1** led to a reduction in affinity.

Protein kinase C (PK-C) is a family of closely related phospholipid-dependent enzymes that differ in their mode of activation.[2-4] The involvement of PK-C isozymes in cell signal transduction phenomena, which results in either uncontrolled cellular proliferation or differentiation,[5] makes them attractive targets for chemotherapeutic intervention. In most isozymes, activation follows rapidly after PK-C binds to diacylglycerol (DAG), which is released as part of a receptor-mediated hydrolysis process of membrane phosphatidylinositol-4,5-biphosphate.[6] In our search for a conformationally restricted analogue of DAG that might function as a potent PK-C activator, we have shown that 3-*O*-tetradecanoyl-2-deoxy-L-ribonolactone (**1**) behaved as a good DAG surrogate based on its ability to inhibit the binding of [^{3}H]-phorbol-12,13-dibutyrate (^{3}H-PDBU) to PK-C.[7] In order to develop a systematic structure-activity approach based on this lactone system, we have divided the molecule, **1**, into three regions labeled A, B, and C. Based on molecular modeling studies,[8] the A region could not be extensively altered and changes were limited to extending the length of the alcohol chain by one methylene unit. The C region, which controls lipophilicity and hence the ability of the molecule to partition adequately between lipid and aqueous phases has been studied and optimized.[9] In this paper, we wish to report the effects of changes in the B region, while holding the other regions constant.[10] The modified elements of the B region appear highlighted in bold letters in the structures of the compounds (**1–8**) selected for this study (Table 1).

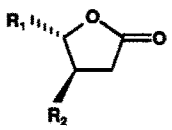


1, R = $CH_3(CH_2)_{12}$-

Chemistry[11]

Synthesis of the ether analogue **2** started with the common intermediate (2S,3R)-3,4-epoxy-1,2-*O*-

Table 1. Target Compounds

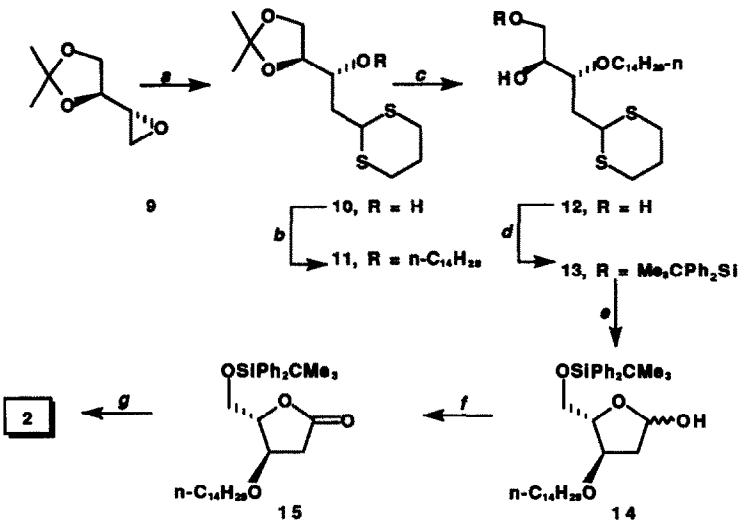


Cpd. #	R_1	R_2
1	$HOCH_2$—	$CH_3(CH_2)_{12}C(O)O$—
2	$HOCH_2$—	$CH_3(CH_2)_{13}O$—
3	$HOCH_2$—	$CH_3(CH_2)_{13}C(O)$—
4	$HOCH_2$—	$CH_3(CH_2)_{13}OC(O)$—
5	$HOCH_2$—	$CH_3(CH_2)_{13}OC(O)CH_2$—
6	$HOCH_2$—	$CH_3(CH_2)_{12}C(O)OCH_2$—
7	$HOCH_2CH_2$—	$CH_3(CH_2)_{12}C(O)O$—
8	$HOCH_2CH_2$—	$CH_3(CH_2)_{13}OC(O)CH_2$—

isopropylidenebutane-1,2-diol (**9**, Scheme 1) which was also used in the synthesis of **1**.[7] This compound, which was obtained from L-ascorbic acid, was converted to the dithiane **10**[7] and then reacted with bromotetradecane/NaH to form the corresponding *O*-alkyl derivative **11**. The rest of the synthesis involved removal of the isopropylidene group and selective protection of the primary alcohol as the *tert*-butyldiphenylsilyl (TBDPS) ether **13**. Unmasking of the dithiane function in **13** gave the aldehyde which cyclized *in situ* to give 2-deoxy-L-ribopentofuranoside **14** (mixture of C-1 anomers). Oxidation of **14** using a mixture of tetrapropylammonium perruthenate (TPAP) and 4-methylmorpholine N-oxide (4-NMO) afforded the corresponding lactone **15** and removal of the hydroxyl-protecting group produced the desired target, the ether lactone **2**.

The rest of the target compounds were prepared as outlined in Scheme 2. The key butenolides **18a** and **18b** were prepared, respectively, from protected D-gulono-1,4-lactone[12] (**16**) and (R)-(-)-5-oxo-2-tetrahydrofuran carboxylic acid (**17**). Conversion of **17** to **18a** has already been reported[13] and formation of **18b** proceeded through the thermal elimination of the 2,3-*O*-(trimethylammonio)methylidene intermediate generated from **16** in the same manner as reported for the isomeric L-gulono lactone.[14,15] Reactions of **18a** and **18b** with a vinylcuprate derived from vinylmagnesium bromide and Cu(I)Br•Me_2S gave the 1,4-addition products **19a** and **19b**, respectively. In each case, a single diastereoisomer corresponding to the *trans* product was isolated. This outcome is in agreement with results obtained with the optical antipode of **18a** under identical conditions.[16] Both olefin intermediates (**19a** and **19b**) were converted to the corresponding alcohols **27a** and **27b**, while **19a** was oxidized under different conditions to give either aldehyde **20a** or acid **25a**. Hydride reduction of aldehyde **20a** to alcohol **22a** followed by acylation with myristoyl chloride and removal of the TBDPS group afforded target compound **6**. Grignard addition of $C_{14}H_{29}MgCl$ on aldehyde

Scheme 1



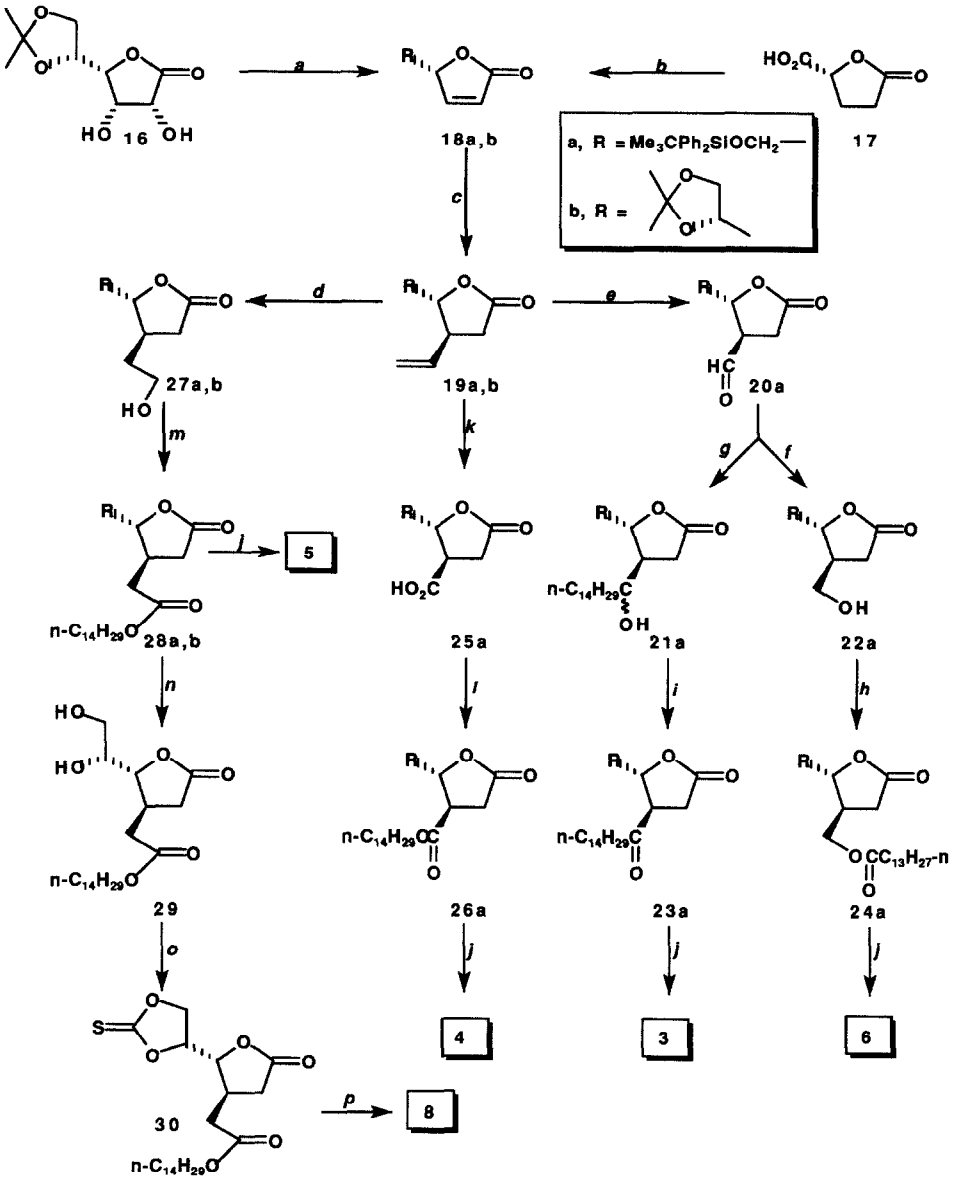
Reagents. (*a*) ref. 7 (*b*) $C_{14}H_{29}Br$, NaH, DMF, 0 °C (87%) (*c*) 1M HCl, THF (95%) (*d*) Me_3CSiPh_2Cl, DMAP, pyr. 0° to 5 °C (83%) (*e*) $CaCO_3$, MeI, CH_3CN–H_2O, 50 °C (70%) (*f*) TPAP, 4-NMO, 4 Å molecular sieves, CH_2Cl_2 (95%) (*g*) HF-pyr., THF (84%).

20a gave alcohol **21a**, which was oxidized with pyridinium dichromate (PDC) to the corresponding ketone **23a**. Removal of the TBDPS group afforded target compound **3**. Acid **25a** was esterified with 1-tetradecanol in the presence of 1,3-dicyclohexylcarbodiimide (DCC) to give ester **26a**, and removal of the TBDPS group afforded target compound **4**. Alcohols **27a** and **27b** were oxidized to the acid stage under different conditions and the corresponding acid intermediates were coupled with 1-tetradecanol in the presence of DCC to give esters **28a** and **28b**. Cleavage of the TBDPS group from **28a** produced target compound **5**. Removal of the isopropylidene protection in compound **28b** was followed by reaction with thiophosgene to produce the cyclic thionocarbonate **30**. As established earlier during the similar synthesis of **7**,[8] the cyclic thionocarbonate underwent radical deoxygenation with tributyltin hydride/azobis(isobutyronitrile) (AIBN) with the expected regioselectivity to give the desired target **8**.

Biological Results and Discussion

Results from the inhibition of ^{3}H-PDBU binding to PK-C by the non-radioactive ligands **1**-**8** appear on Table 2.[17] During the course of the synthetic work, the PK-C preparation used for the bioassay was changed from a PK-C mixture to a purified preparation containing the single isozyme PK-Cα. We have observed little variance between the results obtained with these two assays using either enzyme preparation (e.g. compound **1**, Table 2).

Scheme 2

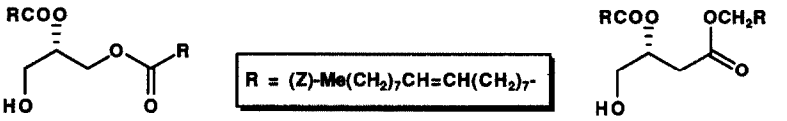


Reagents. (*a*) ref. 13 (*b*) ref. 12 (*c*) $CH_2CHMgBr$, $CuBr{\cdot}SMe_2$, THF-ether (62%) (*d*) $BH_3{\cdot}SMe_2$, $NaBO_3$, THF, –78 °C to rt (56%) (*e*) OsO_4, 4-NMO, $NaIO_4$, acetone-H_2O (96%) (*f*) $NaBH_4$, MeOH (98%) (*g*) $C_{14}H_{29}MgCl$, ether (76%) (*h*) $C_{13}H_{27}COCl$, DMAP, pyr.–CH_2Cl_2 (98%) (*i*) PDC, CH_2Cl_2 (82%) (*j*) HF-pyr., THF (96%) (*k*) $KMnO_4$, *n*-Bu_4Br, benzene–H_2O (95%) (*l*) $C_{14}H_{29}OH$, DCC, CH_2Cl_2 (98%) (*m*) for **27a** (*i*) PDC, DMF; for **27b** (*i*) $RuCl_3{\cdot}H_2O$, $NaIO_4$, $CHCl_3$-CCl_4; for **27a,b** (*ii*) $C_{14}H_{29}OH$, DCC, DMAP, CH_2Cl_2 (55%-78%) (*n*) 1M HCl, THF (78%) (*o*) $Cl_2C{=}S$, DMAP, pyr. (77%) (*p*) *n*-Bu_3SnH/AIBN, benzene, Δ, (47%).

Table 2. Apparent K_i (μM) values for ligands 1-8 as inhibitors of PDBU binding to PK-C

	1	2	3	4	5	6	7	8
PK-C mix	2.50[7] ±0.30	3.31 ±0.83	50.75 ±1.25	8.07 ±0.50	—	—	—	—
PK-C α	1.50 ±0.11	—	—	—	1.47 ±0.14	3.10 ±0.22	0.88[8] ±0.13	18.63 ±2.30

Since the ester group is considered to be a key element of the pharmacophore for good PK-C binding in **1**, this functionality was dissected into its component ether (compound **2**) and ketone (compound **3**) functions to investigate the importance of each function separately. The results showed that while the ether oxygen alone in **2** was capable of maintaining good binding affinity, the isolated ketone carbonyl in **3** caused a significant reduction in binding. Next, based on the reported bio-equivalence between the 3,4-dihydroxybutyrate moiety and the backbone of diacylglycerol[18] — which was proposed to demonstrate the structural link between DAG and the natural PK-C agonist aplysiatoxin— compound **4** was synthesized. The relationship between the DAG analogue, *sn*-1,2-diolein,[18] and the 3,4-dihydroxybutyrate dioleate ester[18] is equivalent to that between compound **1** and **4** in our series. Structurally, it corresponds to a simple transposition of the ester function. Based on the results obtained here, there appears to be no



sn-1,2-diolein 3,4-dihydroxybutyrate dioleate

absolute bio-equivalence between the ester and the transposed ester functions since there is a three-fold decrease in binding affinity towards PK-C for the transposed ester function in compound **4**. The increased affinity of the rigid DAG structure **1** over the rigid 3,4-dihydroxybutyrate structure **4** in our assay is commensurate to the superior binding reported for the *sn*-1,2-diolein versus the 3,4-dihydroxybutyrate dioleate in a partially purified rat brain PK-C preparation.[18] Swapping the oxygen and the methylene groups of the ester function in **1**, which moves the oxygen from one side of the carbonyl to the other, resulted in the reverse ester **5**. Such a molecular alteration produced no change in binding affinity with respect to **1** and demonstrates that the two carbonyl esters in **1** and **5** are perfectly bio-equivalent The transposition of the ester in **5**, represented by structure **6**, resulted in a two-fold reduction in binding which suggests that the carbonyl ester function in **6** was perhaps moved to a less than ideal position. We have

reported earlier that the homologue of **1** in the A region ($R_1 = CH_2CH_2OH$, compound **7** in Table 1) was slightly more potent than **1**.[8] Unfortunately, this change was incompatible with the reverse ester function of **5**, as the binding constant deteriorated significantly for compound **8** which bears both modifications.

From this investigation we can surmise that the hydrogen-bonding capacity of the ester function in **1** can be reasonably maintained by the ether function in **2** and to a lesser extent by the transposed ester function in **4**. An important finding for future studies is that the reverse ester function in **5** is completely bio-equivalent to the original ester function in **1**. The advantage of this discovery is that compound **5** is chemically more stable than **1** by not being susceptible to β-elimination which often times leads to the formation of inactive unsaturated lactones. Other changes that either weakened the hydrogen-bonding capacity of the ester function (compound **3**), or displaced it from its ideal position (compound **8**), were accompanied by a reduction in binding.

References and Notes

1. Paper # 7 in the series: Lee, J.; Marquez, V. E.; Lewin, N. E.; Blumberg, P. M. *BioMed. Chem. Lett.* **1994**, *4*, 543.
2. Nishizuka, Y. *Nature* **1988**, *334*, 661.
3. Nishizuka, Y. *J. Amer. Med. Assoc.* **1989**, *262*, 1826.
4. Parker, P. J.; Kour, G.; Marais, R. M.; Mitchell, F.; Pears, C.; Schaap, D.; Stabel, S.; Webster, C. *Mol. Cell Endocrinol.* **1989**, *65*, 1.
5. Clemens, M. J.; Trayner, I.; Menaya, J. *J. Cell Science* **1992**, *103*, 881.
6. For a review, see *Protein Kinase C. Current Concepts and Future Perspectives*; Lester, D. S.; Epand, R. M. (Eds.), Ellis Horwood: New York, 1992.
7. Teng, K.; Marquez, V. E.; Milne, G.W. A.; Barchi, Jr. J. J.; Kazanietz, M. G.; Lewin, N. E.; Blumberg, P. M.; Abushanab, E. *J. Am. Chem. Soc.* **1992**, *114*, 1059.
8. Sharma, R.; Marquez, V. E.; Milne, G. W. A.; Lewin, N. E.; Blumberg, P. M. *BioMed. Chem. Lett.* **1993**, *3*, 1993.
9. Marquez, V. E.; Lee, J.; Sharma, R.; Teng, K.; Wang, S.; Lewin, N. E.; Bahador, A.; Kazanietz, M. G.; Blumberg, P. M. *BioMed. Chem. Lett.* **1994**, *4*, 355.
10. Based on the availability of the appropriate reagents the C region shows a variance of ± CH_2. The small contribution of a single methylene unit does not translate into a major change in K_i (see ref. 9).
11. All final products and intermediates were fully characterized by ^1H NMR, ^{13}C NMR and IR spectroscopy, FAB mass spectral analysis, optical rotation, and elemental analysis.
12. Copeland, C.; Stick, R. V. *Austr. J Chem.* **1978**, *31*, 1371.
13. Lee, J.; Marquez, V. E.; Lewin, N. E.; Kazanietz, M. G.; Bahador, A.; Blumberg, P. *BioMed. Chem. Lett.* **1993**, *3*, 1101.
14. Vekemans, J. A. J. M.; Boerekamp, J.; Godefroi, E. F.; Chittenden, G. J. F. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 266.
15. The $[\alpha]_D^{20}$ + 131.5° for **18b** compares well with the value of –132.0° reported for the enantiomer in ref. 14.
16. Sahlberg, C. *Tetrahedron Lett.* **1992**, *33*, 679.
17. PK-C binding assays were performed as reported in ref. 7.
18. Kong, F.; Kishi, Y.; Perez-Sala, D.; Rando, R. R. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1973.

(*Received in USA* 16 *March* 1994; *accepted* 26 *April* 1994)