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INDOLOCARBAZOLES. 1. TOTAL SYNTHESIS AND PROTEIN KINASE INHIBITING CHARACTERISTICS OF COMPOUNDS RELATED TO K-252c.

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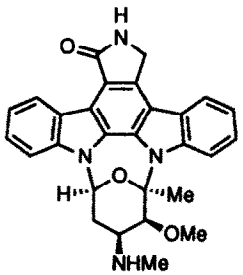
Abstract: The condensation of indolo[2,3-a]-carbazole (**12**) with 2,5-dimethoxytetrahydrofuran derivatives gave cyclofuranosylated compounds (e.g. **13**), which were converted *via* dibromocompounds to the dinitriles (e.g. **25**). Hydrolysis, hydrolysis-reduction and thiolysis afforded imides, lactams (e.g. **27**) and their thio analogs. These compounds were potent inhibitors of the protein kinase C family.

Introduction

Protein kinase C (PKC) consists of a family of highly homologous isozymes which phosphorylate serine/threonine residues on target proteins, processes which are vital for controlling cellular activation, differentation and proliferation.[3] PKC has attracted attention as a potential target for novel antitumor[4] and antiinflammatory[5] agents. The PKC isozymes are composed of a regulatory domain which interacts with the lipid cofactors responsible for activation, and a kinase domain which binds the substrates and catalyses phosphate transfer from ATP to the target protein.[3]

Most inhibitors which interact with the catalytic domain are ATP-competitive compounds, including isoquinoline sulfonamides[6] and erbstatin analogs.[7] Our own recent efforts have centered on the indolocarbazoles, which have been associated with the inhibition of inflammation[5] and tumor growth[8] and invasion.[9] Staurosporine[10] (**1**) and K-252a[11] (**2**) are the most well known members of this family. The aglycones K-252c (**3**) and arcyriaflavin A (**4**) also occur naturally, as does arcyriarubin A (**5**),[12] a diindolylmaleimide. The potential for selective kinase inhibition by the latter class has only been realised recently in an elegant series of chemical and biochemical investigations.[13] A variety of new fermentation products related to **1** and **2** have also been reported in the last few years.[14]



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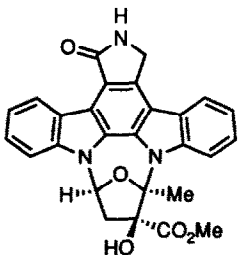

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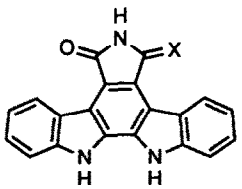
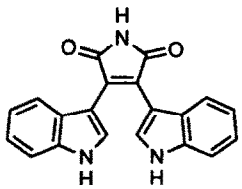


3 X = H_2
4 X = O

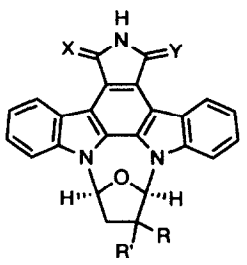


5

In this paper, we describe efficient synthetic routes to a series of compounds of general structure **6**, and report some of their kinase-inhibiting characteristics.

Synthetic studies

Several groups have synthezised the aglycones **3**[15] and **4**[16], but the only open literature example of a cyclocondensation with a simple carbohydrate was provided by Weinreb[17], who obtained **7** from the acid-catalysed reaction of **8** with 2,5-dimethoxytetrahydrofuran **9**. A similar reaction of **4** with **9** to give **10**, a process accompanied by some glycosidation of the imide nitrogen, was reported in a recent patent.[18]



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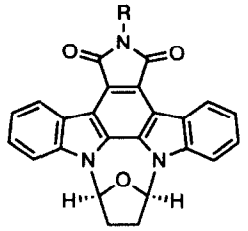
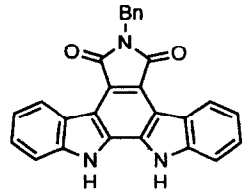


7 R = Bn
10 R = H



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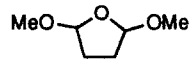
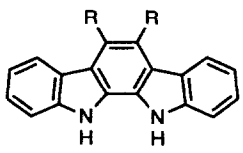
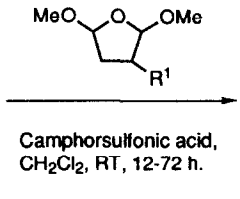


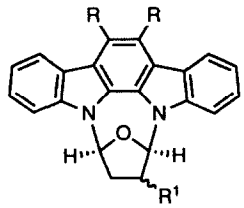
9

To avoid complications arising from the imide, and to delineate the effects of electron-withdrawing substituents on the key step, we compared "cycloglycosidations" of the diester (**11**)[19] and the parent heterocycle (**12**).[20] Under optimized conditions (sulfonic acid catalysts were superior to a variety of Lewis acids) the more nucleophilic **12** clearly was a better substrate than **11**, as shown in **Scheme 1**. The least substituted case which formed **13** was complete in 8-12 h. and afforded high yields, whereas the most substituted case which formed **15** took several days and gave poor yields with extensive decomposition.



11 R = CO_2Bn
12 R = H



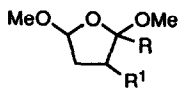


13 R = R^1 = H **(75-85%)**
14 R = CO_2Bn, R^1 = H (40-50%)
15 R = CO_2Bn, R^1 = CO_2Me **(20-25%)**

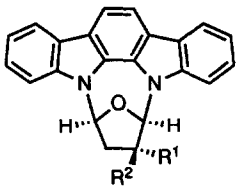
Scheme 1

In fact, cycloglycosidations on **12** were not unduly sensitive to substitution on the "carbohydrate" portion: reaction with 1.5 eq. of the dimethoxytetrahydrofuran (**16**)[21] (CH_2Cl_2, camphorsulfonic acid, RT, 24 h.) afforded 70-80% (based on **12**) of a readily separable, 1:1 mixture of the (racemic)[22] α-isomer (**17**, mp 152 °C) and the β-isomer (**18**, mp 161 °C). The ester (**19**) similarly afforded a mixture of **20** and **21**. These condensations also proceeded in high yields using excess $B(OH)_3$ in refluxing toluene. The epimers were easily distinguished by ^1H NMR, since the aromatic heterocycle strongly shielded protons on substituents located on the same side of the tetrahydrofuran ring, i.e. in the β-epimers (**18**) and (**21**).[23] The tetrahydrofuran (**22**) could

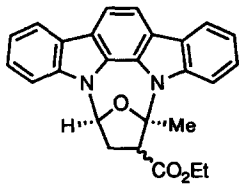
be efficiently converted to the anomeric *C*-methyl compound (**23**), a potential intermediate to synthetic K-252a. Under standard conditions only 15-20% of **23** was obtained, but using slow addition of **22** to refluxing 1,2-$C_2H_4Cl_2$ containing **12** and camphorsulfonic acid, the yield improved to 60%. These conditions minimised self-condensation of **22** (presumably *via* glycal intermediates) and promoted the initial, intermolecular reaction between **12** and the oxocarbonium ion derived from **22**.



16 R = H, R^1 = CH_2OSiPh_2Bu-t
19 R = H, R^1 = CO_2Et
22 R = Me, R^1 = CO_2Et

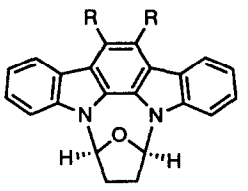


17 R^1 = CH_2OSiPh_2Bu-t, R^2 = H
18 R^1 = H, R^2 = CH_2OSiPh_2Bu-t
20 R^1 = CO_2Et, R^2 = H
21 R^1 = H, R^2 = CO_2Et

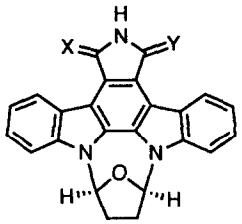


23

With the above results in hand, we sought a method to introduce the crucial[24] imide/lactam ring. Treatment of **13** with 2.05 eq. of *N*-bromosuccinimide or $PhNMe_3^+Br_3^-$ in DMF afforded the dibromocompound (**24**), contaminated with 10-15% of mono- and polybromo compounds. After considerable experimentation,[25] we were able to define conditions for conversion to the dicyanocompound (**25**), which was isolated in 72% yield after reacting **24** with CuCN[26] (4 eq.) and NaI (2 eq.) in $MeCONMe_2$ at 160-180°.[27] From **25**, controlled hydrolysis and thiolysis reactions led to imides and to novel thio analogs. Hydrolysis of **25** (KOH-H_2O-DMSO, 100 °C) produced the iminoimide (**26**), which was stable above pH ~5 but hydrolysed in acid (TFA-H_2O-DMSO, RT) to give the imide (**10**) in 90% yield from **25**. The lactam (**27**) was obtained efficiently by reducing either **26** ($NaBH_3CN$, HOAc, 60 °C) or **10** (BH_3, THF, reflux). Thiolysis of the dinitrile (NaSH, DMSO, 100 °C) gave 25% of the purple dithioimide (**28**), and similar treatment of **26** produced 15% of the red monothioimide (**29**). Reduction of **10** with $LiAlH_4$ (THF, RT) gave the hydroxylactam **30**.



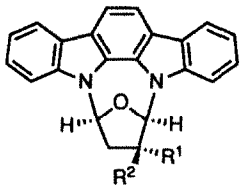
24 R = Br
25 R = CN



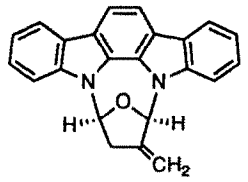
26 X = O, Y = NH
10 X = Y = O
27 X = O, Y = H_2
28 X = Y = S
29 X = O, Y = S
30 X = O, Y = H, OH

In addition to facilitating the coupling step, delaying the introduction of the imide and related systems permitted manipulation of the stereochemistry and functionality on the carbohydrate portion. Thus, >95% stereocontrol for either the α or β series was achieved as follows: hydrolysis of the ester mixture (**20**)/(**21**) in DMSO-H_2O-KOH was accompanied by equilibration, and provided the α-acid (**31**). Reduction ($LiAlH_4$) gave the alcohol (**32**), which was converted ($MsCl$-NEt_3; NaI) to the iodocompound (**33**). Reaction with DBU (DMSO, 50 °C) produced[28] the exocyclic olefin (**34**), which underwent highly stereoselective hydroboration-

oxidation (BH_3, THF; $NaOH-H_2O-H_2O_2$) to afford the β-alcohol (**35**). Disubstituted compounds could also be readily prepared: *C*-hydroxylation of the ester enolate from (**20**)/(**21**) (LDA, THF, -70 °C) with camphorsulfonyloxaziridine[29] gave the hydroxyester (**36**) as a single isomer in 77% yield.



31 $R^1 = CO_2H$, $R^2 = H$
32 $R^1 = CH_2OH$, $R^2 = H$
33 $R^1 = CH_2I$, $R^2 = H$
35 $R^1 = H$, $R^2 = CH_2OH$
36 $R^1 = OH$, $R^2 = CO_2Et$



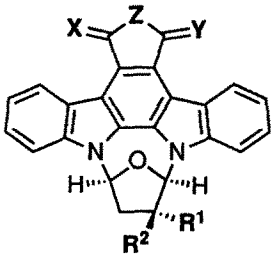
34

Application of the dibromination - cyanation - hydrolysis sequence described above for the conversion of **13** to **10** to these and related intermediates introduced the imide ring in ~60% overall yield, affording a variety of analogs of K-252. Results of preliminary *in vitro* evaluation of selected compounds *vs* protein kinases are presented in the next section; cellular and *in vivo* studies will be reported elsewhere.

In Vitro Biological Evaluation

These compounds were tested for their ability to inhibit partially purified rat brain PKC, a mixture of isozymes, under the conditions described for the evaluation of erbstatin analogs.[7] The results are shown in **Table 1**, grouped into modifications to the "northern" and "southern" rings of the polycyclic system.[30]

Table 1: *In vitro* PKC Inhibition



#	X	Y	Z	R1	R2	PKC (nM)
10	O	O	NH	H	H	19
26	O	NH	NH	H	H	13
27	O	H_2	NH	H	H	18
28	S	S	NH	H	H	42
29	O	S	NH	H	H	58
30	O	H,OH	NH	H	H	50
37	O	O	NMe	H	H	>5000
38	O	O	NHNH	H	H	>5000
39	O	O	NH	H	CH_2OH	1.7
40	O	O	NH	CH_2OH	H	2.4
41	O	O	NH	CO_2H	H	20
42	O	O	NH	$=CH_2$		5.0
43	O	O	NH	CONHMe	H	0.5
44	O	O	NH	CH_2NMe_2	H	4.0
45	O	O	NH	CO_2H	OH	6.0

One general observation is that the high potency characteristic of the natural products (staurosporine (**1**): IC_{50} = 8 nM) is achieved by structurally simpler systems. In fact, all of the active compounds in the table are more potent than K-252a (**2**: IC_{50} = 60 nM). With regard to SAR in the "northern" section, the imides, lactams

and iminoimides were comparably potent. The thio analogs and the hydroxylactam were somewhat less active. In marked contrast, *N*-alkylation (entry **37**) or ring expansion to the cyclic hydrazide (**38**) resulted in complete loss of activity.

The "southern" ring proved to be quite tolerant of substitution. Entries **39** - **45**, selected from an extensive series, show that potent compounds may be neutral or may bear acidic (entries **41** and **45**) or basic (entry **44**) substituents. This tolerance has potential for tailoring characteristics such as cellular penetration, absorption and tissue distribution which are important in secondary assays and for *in vivo* activity.

In general, we did not find high specificity in this series for individual PKC isozymes or for PKC *vs* other serine/threonine protein kinases. The β-(hydroxymethyl) compound (**39**) inhibited cAMP-dependent protein kinase (IC_{50} = 18.5 nM). Kinetic analyses indicated that **39** inhibits myosin light chain kinase in an ATP-competitive manner. (K_i = 0.7 nM). In contrast, **39** was a much less potent inhibitor of the *neu (erb-B2)* oncogene tyrosine kinase (IC_{50} = 250 nM), and did not inhibit the epidermal growth factor receptor kinase.

Conclusions

We have developed the first efficient syntheses of cyclofuranosylated indolocarbazole imides, lactams and thio analogs. These compounds are highly potent inhibitors of the PKC family. We will report cellular and *in vivo* studies of selected compounds in later papers.

Acknowledgements: we thank the Physical-Analytical department for spectra and microanalyses, D. H. R. Barton, R. Breslow A. K. Ganguly and S. F. Vice for helpful discussions, and M. Cohen and his associates for large-scale preparation of **12**.

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19. Dibenzyl diindolylsuccinate was prepared by oxidative coupling: Bergman, J.; Pelcman, J. *Tetrahedron Lett.*, **1987**, *28*, 4441-4444. Treatment of this diester with CF_3CO_2H resulted in 2,2' coupling, and subsequent oxidation with dichlorodicyano-1,4-benzoquinone afforded **11** in ~60% overall yield.
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21. These dimethoxytetrahydrofuran derivatives were prepared from the corresponding furans by treatment with Br_2 or NBS in MeOH-$NaHCO_3$, followed by catalytic hydrogenation (Pd-C).
22. All new compounds gave microanalytical and mass spectrometric data consistent with the assigned structures. Excepting **1** and **2**, all chiral compounds are racemates.
23. For example, in ester (**20**) in $CDCl_3$ solution, the $OC\underline{H}_2C\underline{H}_3$ signals appeared at 4.30 and 1.35 ppm, whereas in ester (**21**) they were considerably upfield, at 3.84 and 0.72 ppm. All assignments were confirmed by NOE studies.
24. In addition to the compounds described in this paper, we have prepared a large number of "acyclic" analogs with other functional groups replacing the imide system. None of these compounds approached the imides and lactams in terms of *in vitro* potency. We will detail this chemistry elsewhere.
25. Attempted Pd(0)-catalysed bis-carbonylations of the dibromocompound (**13**) were not satisfactory.
26. The conversion of monohaloarenes to nitriles with CuCN is well known: a) Friedman, L.; Shechter, H. *J. Org. Chem.*, **1961**, *26*, 2522-2524. b) Newman, M. S.; Boden, H. *ibid*, 2526. These reactions may be catalysed by the product nitrile, and by Cu(II) salts: c) Koelsch, C. F.; Whitney, A. G. *J. Org. Chem.*, **1941**, *6*, 795-803. NaI catalysis appears to be novel, and was particularly useful with these relatively unreactive dibromoindolocarbazoles. It should be generally applicable to ArBr ---> ArCN conversions.
27. Earlier attempts to prepare 1,2-dinitriles or 2-cyanoacetophenones resulted in further reactions to produce Cu complexes of phthalocyanines and tetra-aza tetrabenzporphyrins: a) Diesbach, H.; Van der Weid, E. *Helv. Chim. Acta*, **1927**, *10*, 886-889. b) Helberger, J. H. *Annalen*, **1937**, *529*, 205-218.
28. The diastereoisomeric alcohol mixture prepared by reduction of **20/21** ($LiAlH_4$, THF) could also be utilised in this sequence. With alkoxide bases instead of DBU, elimination was followed by epimerisation to the endocyclic olefin, indicating a substantial acidifying effect from the adjacent anomeric centers.
29. Davis, F. A.; Haque, M. S.; Ulatowski, T. G.; Towson, J. C. *J. Org. Chem.*, **1986**, *51*, 2402-2404. Either enantiomer could be used, since these reactions were run to completion, with no attempt to achieve kinetic resolution. The less hindered 2-benzenesulfonyl-3-phenyloxaziridine gave poor yields.
30. Some aromatic ring substitution is tolerated. We prepared the 5,15-dimethyl substituted analogs of imide (**10**) and lactam (**27**) and found them to be potent PKC inhibitors. A propoxy-substituted analog of K-252a is a selective MLCK inhibitor: Nakanishi, S.; Yamada, K.; Iwahashi, K.; Kuroda, K.; Kase, H. *Molecular. Pharmacol.*, **1990**, **37**, 482-488.