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DESIGN, SYNTHESIS AND EVALUATION OF C/D-RING ANALOGS OF THE FUNGAL METABOLITE K-76 AS POTENTIAL COMPLEMENT INHIBITORS

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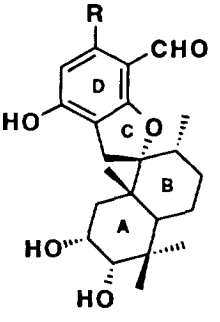
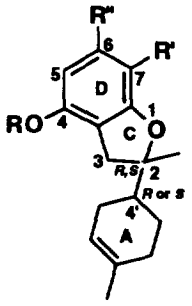
Abstract: A series of the C/D-ring analogs of the natural product complement inhibitor K-76 (**9-14**) and some of their bioisosteres (**19,20**) have been synthesized and evaluated for the inhibition of classical pathway activation of human complement and their intrinsic lytic activity. The *in vitro* assay results of the inhibition of complement-mediated hemolysis of these analogs indicate that the bioisosteric tetrazole ring significantly improves the human complement inhibitory potency. Additionally, most of this series of analogs do not exhibit lytic activity.

The greater understanding of the role of the complement system in the pathogenesis of several diseases has increased the need for more specific, more potent and less toxic complement inhibitors.[1-3] The terpenoid K-76 (**1a**; R = CHO), a natural product of fungal origin, and its partially oxidized derivative **1b** (K-76COOH; R = COOH) as well as its sodium salt **1c** (K-76COONa; R = COONa) have shown a unique complement inhibitory activity,[4] due to their ability to inhibit the generation of anaphylatoxin C5a, a peptide released upon complement activation.[5] In continuation of our ongoing research program for the development of new partial analogs of K-76,



1a: R = CHO; K-76
1b: R = COOH; K-76COOH
1c: R = COONa; K-76COONa

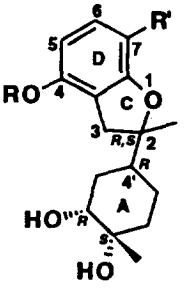
retaining the desired complement inhibiting potency, several A/C/D-ring analogs belonging to protoytpes **2** and **3** have been designed and synthesized in our laboratory.[6-8] The results of their *in vitro* human complement-mediated hemolysis and the structure-activity-relationship studies on these analogs have suggested that the lipophilic A-ring may be the source of the unwanted lytic activity.[8] This prompted us to synthesize partial analogs of K-76 devoid of the A-ring. Therefore, herein, we report the design, synthesis and evaluation of C/D-ring analogs (**4**) and their bioisosteric tetrazoles (**4**; R = tetrazole-5-yl) which have polar functional groups similar to those found on the natural product. This series of analogs may support not only our earlier hypothesis of significant hemolysis due to the A-ring on A/C/D-ring analogs but also may provide crucial pharmacophoric information about this important natural product.



2

R = H, Me, Ph

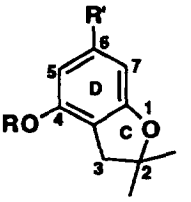
R' = R" = H, CHO, COOH



3

R = H, Me

R' = CHO, COOH



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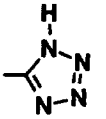
R = H, Me, Ph

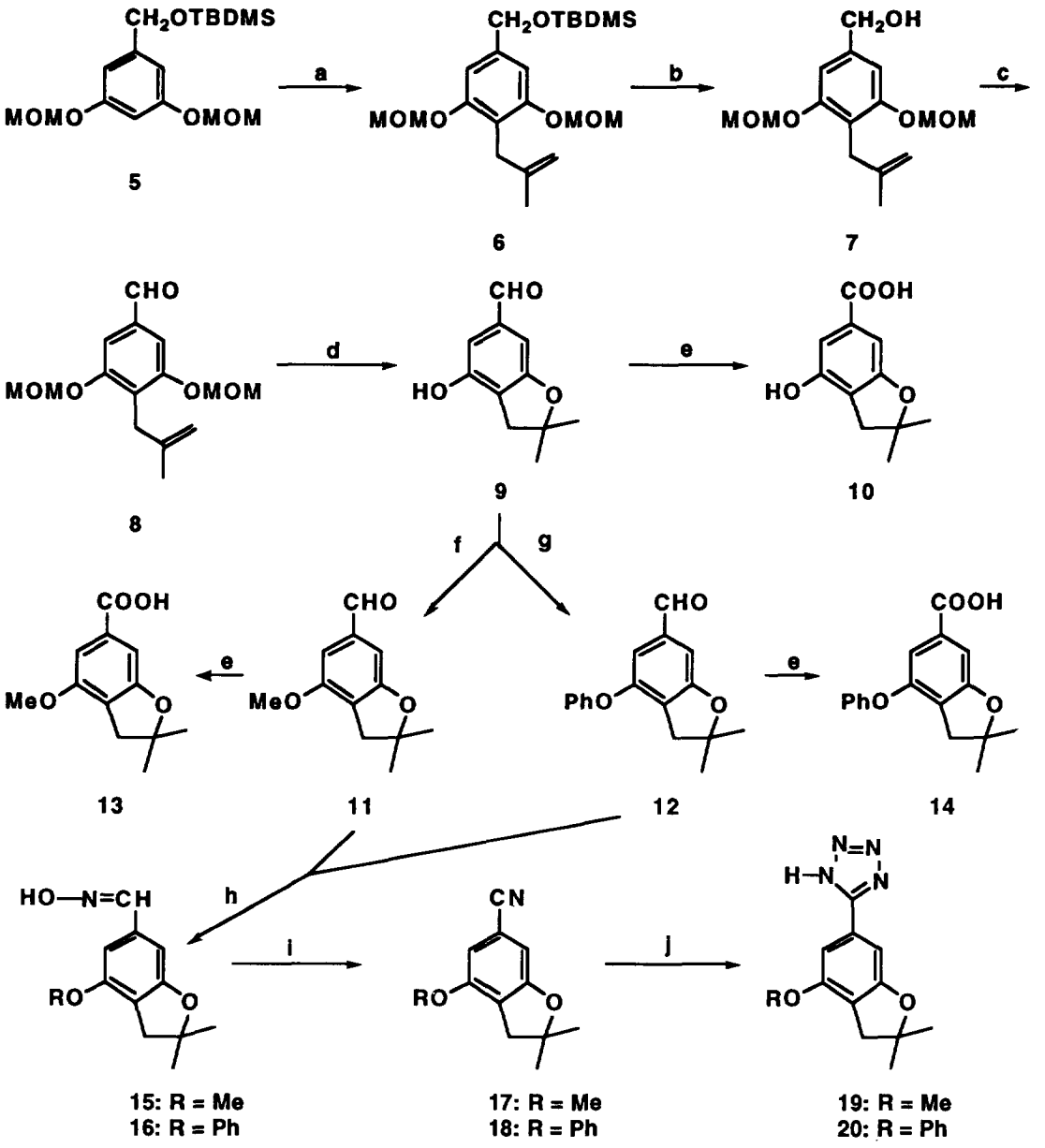
R' = CHO, COOH,



The synthetic strategy is outlined in Scheme 1.[9] The appropriate silyl ether **5** which will introduce ring-D of the proposed analogs, was prepared according to the method reported earlier.[10,11] The coupling of the arylcuprate reagent, formed by the reaction of **5** with TMEDA–*n*–BuLi complex and cuprous iodide in anhydrous tetrahydrofuran, with 3-chloro-2-methylpropene as electrophile furnished the key intermediate **6** in 83% yield. The deprotection of the *tert*-butyldimethylsilyl protected hydroxyl in **6** by tetrabutylammonium fluoride in THF gave the benzyl alcohol **7** which after PCC oxidation yielded the benzaldehyde **8** as a colorless oil (89%). Compound **8** was then subjected to mild acidic hydrolysis by stirring with 3 N HCl/2-propanol at room temperature for 8h which afforded exclusively the desired prototype compound **9** in 92% yield. In the next step, benzofuran **9** was oxidized by silver nitrate and potassium hydroxide in ethanol to yield the corresponding acid **10** as a white crystalline solid (64%). The methoxy- and phenoxy- derivatives of **9** were successfully prepared by the reaction of **9** with iodomethane/silver oxide or triphenylbismuth diacetate/Cu,[12] furnishing **11** or **12** in 68% and 61% yields, respectively. Compounds **11** and **12** were then easily converted to their corresponding acids **13** and **14** following the same oxidation procedure as described earlier. The observation[13] that the tetrazole acts as an excellent carboxylic acid bioisostere, prompted us to introduce this ring onto the proposed C/D-ring analogs. This was accomplished by three sequential reactions. Compound **11** or **12** was treated with hydroxylamine hydrochloride and sodium acetate in methanol to afford oxime **15** or **16** in 85% and 91% yields, respectively. Then, the reaction of **15** or **16** with thionyl chloride in benzene under reflux gave nitrile **17** or **18** in good yield. Finally, compound **17** or **18** was heated at reflux with ammonium azide (prepared *in situ* by the decomposition reaction of sodium azide and ammonium chloride) and lithium chloride in DMF for 52h. Following chromatographic separation of the reaction mixture, two fractions were obtained which were characterized as recovered **17** or **18** (53% and 60% respectively) and as the desired tetrazole **19** or **20** in 44% and 30% yields, respectively.

The target compounds described above were bioassayed[14,15] for their ability to inhibit the classical pathway activation of human complement and also incubated with sensitized sheep erythrocytes in the absence of complement to determine their lytic activity. Compounds were tested at a maximum of 300 μg/mL. The results are summarized as IC_{50} or EC_{50} values in Table 1 which revealed that none of this series of C/D-ring analogs

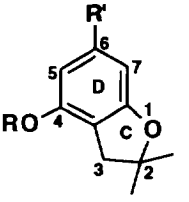
Scheme 1[a]



[a] Reagents: (a) *n*–BuLi, TMEDA, CuI, $H_2C{=}C(CH_3)CH_2Cl$; (b) $(Bu)_4NF$, THF; (c) PCC, CH_2Cl_2; (d) 3 N HCl, 2-PrOH; (e) $AgNO_3$, KOH, EtOH, H_2O; (f) CH_3I, Ag_2O, $CHCl_3$; (g) $Ph_3Bi(OAc)_2$, Cu, CH_2Cl_2; (h) $NH_2OH.HCl$, CH_3COONa, MeOH; (i) $SOCl_2$, C_6H_6; (j) NaN_3, NH_4Cl, LiCl, DMF

exhibit the unwanted lysis except compound **20**. Also, target compounds **9-14** exhibited less potent complement inhibition with respect to the natural product. It was interesting to note that the complement inhibiting potency of C/D-ring analogs was significantly improved by the replacement of the carboxylic acid functionality by a tetrazole ring as compounds **19** and **20** appeared to have approximately a three fold increase in potency in comparison to K-76COONa. These results suggest that the ionizable functional group at position 6 plays an important role in the inhibition of complement activation. Additionally, the absence of intrinsic lytic activity in C/D-ring analogs **9-14** and **19** confirms indirectly our assumption that the significant unwanted lysis exhibited by the A/C/D-ring analogs is caused by the A-ring.

Table 1. The Inhibition of Human Complement Activation and Intrinsic Lytic Activity of Target Compounds.



Compound	R	R'	IC_{50} (μM)[a] Complement Inhibition	EC_{50} (μM)[b] Lysis
1c			680	>680
9	H	CHO	>1560	>1560
10	H	COOH	>1442	>1442
11	Me	CHO	1100	>1456
12	Ph	CHO	>1120	>1120
13	Me	COOH	>1350	>1350
14	Ph	COOH	>1056	>1056
19	Me	tetrazole-5-yl	245	>1220
20	Ph	tetrazole-5-yl	195	650

[a] The concentration of compound required to inhibit complement induced hemolysis by 50% comparable to vehicle (DMSO). Values reported are interpolated from concentration/inhibition plots of mean values (n = 3 at each concentration).
b The concentration of compound required to cause 50% hemolysis in the absence of complement. Values are interpolated from concentration/lysis plots of mean values (n = 3 at each concentration).

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