with ether, followed by solvent removal, a quantitative yield of the corresponding acid **4** as a white crystalline solid, mp 115–116 °C (benzene). Acid **4**, in THF, was amidated in the presence of Et_3N and EtOCOCl with 2-(2,6-dimethoxyphenoxy)ethylamine[12] (**5**) to give a 75% yield of amide **6** as a colorless oil, which was used without further purification. Reduction of amide **6** with borane–methyl sulfide complex in dry diglyme gave 70% of **1** as a white crystalline hydrochloride, mp 104–105 °C (*i*-PrOH–EtOAc–Et_2O).

Biology. Since benzodioxans have been extensively studied in isolated rat vas deferens preparations,[7] it was thought that keeping the bioassays constant would allow a better comparison with results obtained with use of related drugs. Each vas deferens was transversely bisected in two portions of ca. 15 mm in length. Owing to their different sensitivity to agonists and their different response to electrical stimulation,[13,14] prostatic and epididymal portions of the vas deferens were used to study pre- and postsynaptic α-adrenoreceptors, respectively.[15-18]

The biological profile of compound **1** at the rat vas deferens α-adrenoreceptors is shown in Table I together with pA_2 values of the most active and selective α_1-antagonists, that is, **2** and prazosin, in order to compare potency and selectivity for α_1- and α_2-adrenoreceptors. The biological activity of **1**, **2**, and prazosin was determined simultaneously to avoid possible variations from laboratory to laboratory.

Compound **1** displays a competitive mechanism of action toward both α_1- and α_2-adrenoreceptors as revealed by the slope of Schild plots and parallelism of curves. It is also clear that **1** is a very potent antagonist at the α_1-adrenoreceptor with an unusually high pA_2 value of 9.26. It suffices to say that on the same preparation **2** and prazosin were 3 and 3.6 times, respectively, less potent compared to **1** (Table I). However, the most striking finding of the present investigation was the unprecedented selectivity toward α_1-adrenoreceptors displayed by **1** as revealed by the α_1/α_2 selectivity ratio of 1000. Under the same conditions, **2** and prazosin had an α_1/α_2 selectivity ratio of 347 and 851, respectively. This is the first time that structural manipulation of the molecule **2** has resulted in increased affinity for the α_1-adrenoreceptor. It has been reported that both benzodioxan and (2,6-dimethoxyphenoxy)ethyl moieties are essential for activity.[19] Any structural modification resulting in a pronounced difference in pharmacological activity relative to the α_1-adrenoreceptor does not significantly affect the α_2-adrenoreceptor.[16,20] The substitution of oxygen at position 4 with a methylene group[21] (**3**) caused a 1000-fold decrease in potency compared to that of **2**. Furthermore, it is interesting to note that substitution of the oxygen at position 4 with a sulfur in other antagonists of the benzodioxan class, such as prosympal and piperoxan, gave rise to compounds with agonistic instead of antagonistic activity toward the α-adrenoreceptor.[11] Taken together, these results and ours emphasize the importance of the position 4 in both the benzodioxan and benzoxathian nucleus for the interaction at α_1-adrenoreceptors. Since sulfur cannot form a productive hydrogen bond, the position 4 of antagonists bearing a benzodioxan or a benzoxathian nucleus would interact with the receptor, either increasing the electron density of the phenyl ring by a way of an electron-releasing effect or giving rise to a dipole–dipole interaction.

In conclusion, compound **1** is a potent and selective α_1-adrenoreceptor antagonist that may represent a valuable tool in the characterization of α-adrenoreceptor subtypes.[23]

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(15) Compound **1** was incubated for 30 min before the initial challenge with the agonist. A 60-min incubation time gave identical results, suggesting that 30 min are sufficient to reach equilibrium conditions. Propranolol hydrochloride (1 μM), cocaine hydrochloride (10 μM), and deoxycorticosterone acetate (40 μM) were present in the Krebs solutions throughout the experiments to block β-adrenoreceptors, neuronal and extraneuronal uptake mechanisms, respectively. The experimental protocol was similar to that described in detail in ref 16 for other α-adrenoreceptor antagonists.
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(23) Detailed pharmacological characterization of **1** shall appear elsewhere together with the results of ongoing relevant research.

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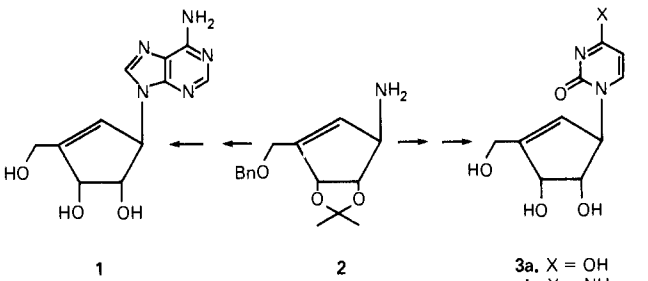
Cyclopentenyluridine and Cyclopentenylcytidine Analogues as Inhibitors of Uridine-Cytidine Kinase

Sir:

The unusual cyclopentene moiety and the antitumor properties of the antibiotic nucleoside neplanocin A[1] (**1**)



1 **2** **3a.** X = OH **b.** X = NH_2

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Table I. Inhibition of Uridine Kinase and L1210 Cell Growth

	uridine kinase[a]						
	% inhibn			inhibition of cell growth:[b] % inhibn			
compd	100 μM	1000 μM	K_i, μM	0.03 μM	100 μM	500 μM	1000 μM
3a	35	78	230		2	12	36
3b	57	89	60	58	99	96	99

[a] Uridine kinase from L1210 cells was prepared and assayed as described by Ahmed and Baker.[12] In brief, the phosphorylation of [^{14}C]-uridine (40 μM) at 37 °C was measured by the retention of phosphorylated product on DE81 filter paper disks. K_i values were determined from the Dixon plots obtained with data from determinations with uridine present at 10, 40, and 200 μM and with inhibitor at 1, 0.5, 0.2, and 0.1 mM. The time of incubations was 5 min. [b] Cell growth inhibiton: L1210 cells were grown as stationary suspension cultures of 1-mL volume in 24 well tissue culture plates in Fishers medium containing 10% heat-inactivated horse serum. Cells were exposed to drug continuously for 72 h, and the cell number was determined by use of a Coulter counter. Percent inhibition is given relative to control cultures which grew from 25 000 to 600 000 cells/mL in 72 h.

has prompted several laboratories, including our own, to develop practical synthetic methodologies for its preparation.[2-4] Our synthetic approach resulted in the enantioselective 15-step synthesis of (−)-neplanocin A starting with D-(+)-ribonic acid γ-lactone.[3] A key intermediate in this synthesis is the 2-cyclopentenylamine **2**, which can be used as a common precursor for the preparation of other cyclopentene-containing purine and pyrimidine nucleosides. Such versatility in the neplanocin synthesis was desirable since we were interested in studying the effects of the novel carbocyclic "sugar" on the biological activity of a wide variety of purine and pyrimidine bases. A recent brief communication reporting on the synthesis and cytotoxicity to KB cells of several cyclopentene nucleosides[5] has prompted us to disclose our preliminary results on the synthesis and unique biological properties of the cyclopentene-containing uridine (**3a**) and cytidine (**3b**) analogues.

Chemistry. These cyclopentenyl nucleosides were prepared as shown in Scheme I by starting with optically pure cyclopentenylamine **2**, obtained as reported earlier.[3] Following Holy's procedure,[6] condensation of **2** with 1.2 equiv of **4** and excess of triethylamine in ethanol (80 °C, 16 h), afforded the intermediate **5**, which was then subsequently cyclized to the uracil derivative **6a** (mp 160–162 °C) in 80% yield. At this stage, two synthetic routes for the decarboethoxylation of **6a** were investigated. The first involved the reduction of **6a** to **7** (74%) with 1.1 equiv of sodium borohydride in ethanol (−10 °C, 10 min), followed by decarboethoxylation[7] with sodium chloride in wet Me_2SO (reflux, 1 h) to produce the dihydrouracil **8** in 83% yield. Silylation-mediated oxidation[8] of **8** with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile (80 °C, 24 h) afforded the uracil derivative **9a** in 20–50% yield. The yields obtained with this procedure, however, were erratic, and an alternative approach was sought. In the second approach, hydrolysis of **6a** with 4 equiv of LiOH in water–methanol (24 °C, 1.5 h), followed by neutralization with Dowex 50W-X8(H^+) resin, produced the acid **6b**, which was decarboxylated without purification in the presence of copper powder in refluxing quinoline (1 h). This method gave 66% of the desired uracil derivative **9a**. Debenzylation of **9a** with 5 equiv of BCl_3 in dichloromethane (−78 → 24 °C, 2 h) was accompanied by the simultaneous removal of the isopropylidene group during the acidic workup and afforded the target cyclopentenyluracil **3a**[9] [55%; $[\alpha]_D^{24}$ −62° (*c* 0.47, H_2O); UV (H_2O) λ_{max} 266 nm (log ε 4.07); ^{1}H NMR (D_2O, 200 MHz) δ 4.18 (m, 1 H), 4.32 (m, 2 H), 4.62 (d, *J* = 5.5 Hz, 1 H), 5.52 (m, 1 H), 5.82 (m, 1 H), 5.90 (d, *J* = 8.0 Hz, 1 H), 7.52 (d, *J* = 8.0 Hz, 1 H); MS,[10] *m/z* 241 (MH^+)]. Individual proton assignments were made on the basis of proton decoupling experiments. For example, irradiation of the C-1′ proton at δ 5.52, readily allowed identification of the C-2′ proton multiplet at δ 4.18 after its collapse to a doublet (*J* = 5.5 Hz). Similarly, decoupling of the C-2′ proton caused the C-3′ doublet at δ 4.62 to become a singlet. These ^{1}H NMR absorptions were characteristic of the other nucleosides in this series.

Conversion of **9a** to the thio analogue **9b** was accomplished in 71% yield after reacting with 1.3 equiv of 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide (Lawesson reagent)[11] in benzene (reflux, 45 min). Reaction of **9b** with methyl iodide and potassium carbonate in methanol (25 °C, 45 min) readily afforded the methylthio compound **10** in 97% yield. Deprotection of **10** under the conditions described above led to the free nucleoside analogue **11** [(80%); mp 161–63 °C; $[\alpha]_D^{24}$ −34° (*c* 0.14, H_2O); UV (CH_3OH) λ_{max} 277 nm (log ε 4.51), 303 (4.54); ^{1}H NMR (D_2O, 200 MHz) δ 2.57 (s, 3 H), 4.24 (m, 1 H), 4.34 (s, 2 H), 4.68 (d, *J* = 5.5 Hz, 1 H), 5.56 (m, 1 H), 5.86 (m, 1 H), 6,68 (d, *J* = 8.0 Hz, 1 H), 7.72 (d, *J* = 8.0 Hz, 1 H)]. Finally, treatment of **11** with saturated methanolic ammonia (80 °C, 48 h) afforded the corresponding target cyclopentenyl cytosine **3b**[9] [73%; $[\alpha]_D^{24}$ −54° (*c* 0.14, H_2O); UV (H_2O) λ_{max} 274 nm (log ε 3.91); ^{1}H NMR (Me_2SO-d_6 + D_2O, 200 MHz) δ 3.79 (m, 1 H), 4.03 (s, 2 H), 4.30 (d, *J* = 5.5 Hz, 1 H), 5.34 (m, 1 H), 5.45 (m, 1 H), 5.71 (d, *J* = 7.3 Hz, 1 H), 7.28 (d, *J* = 7.3 Hz, 1 H); MS,[10] *m/z* 240 (MH^+)].

Biology. Several laboratories have attempted to develop inhibitors of uridine kinase (UK) as a means to blocking the salvage pathway of uracil nucleotide synthesis. Such inhibitors may be of use as chemotherapeutic agents particularly in combination with previously synthesized inhibitors of pyrimidine synthesis de novo. Effective inhibitors of uridine kinase would also be valuable biochemical tools for the evaluation of the physiological significance of circulating pyrimidines. To date no potent

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Table II. Inhibition of Nucleoside Salvage[a]

precursor	% inhibn (0.1 mM) 3a	3b	precursor	% inhibn (0.1 mM) 3a	3b
[^{14}C]uridine	58	93	[^{14}C]deoxycytidine	17	2
[^{14}C]cytidine	53	85	[^{14}C]thymidine	2	39

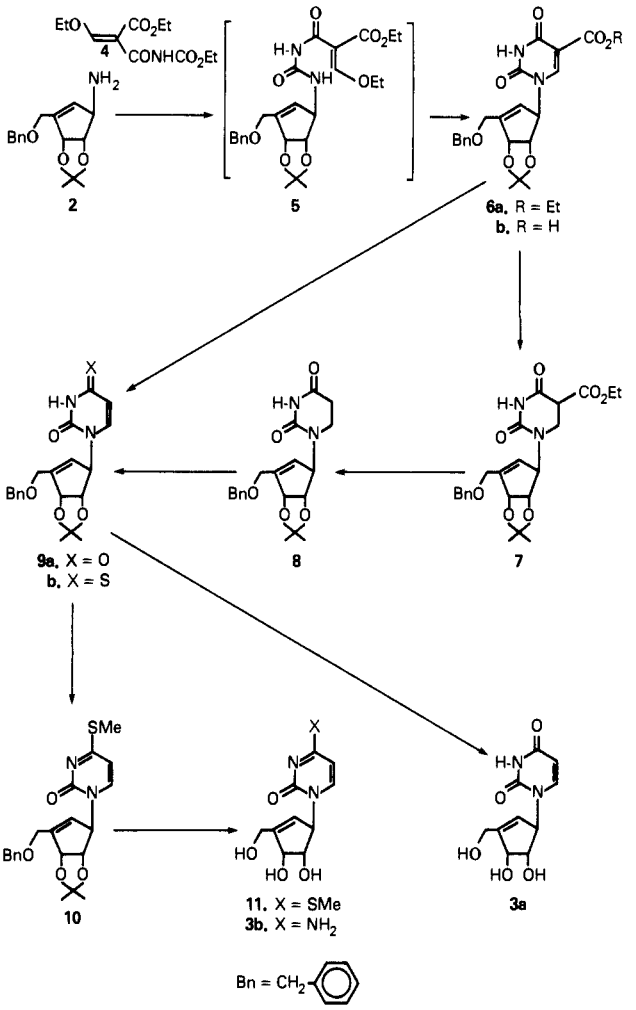
[a] L1210 cells at 250 000/mL were incubated at 37 °C in Fishers medium with 10% horse serum, in the presence or absence of 0.1 mM of **3a** or **3b**. Precursors were added at final concentrations of 2.5 μCi/mL. After 30 min of incubation the label incorporated into nucleotides and nucleic acids was determined by measuring the radiolabel adsorbed on DE81 filters (Whatman). Incorporation for control cells (in the absence of drugs) was as follows: [^{14}C]-uridine, 2100 cpm/10^5 cells; [^{14}C]cytidine, 1040 cpm/10^5 cells; [^{14}C]deoxycytidine, 380 cpm/10^5 cells; [^{14}C]thymidine, 740 cpm/10^5 cells. The values given are the average of two experiments done in duplicate. The difference between the two experiments was <10%.

inhibitors of uridine kinase have been identified despite considerable effort.[12-14] The cyclopentenyluridine and -cytidine analogues (**3a** and **3b**) appeared worth examining as inhibitors of UK in view of their chemical resemblance to the natural substrates. In the present communication we report the results of our studies with these compounds in relation to their (a) cytotoxicity to L1210 cells, (b) inhibition of UK, (c) stability toward the degradative enzyme, uridine phosphorylase, and (d) inhibition of pyrimidine nucleoside salvage by intact cells. These results are summarized in Tables I and II.

Both **3a** and **3b** were found to be competitive inhibitors of UK with the cytosine derivative approximately 4-fold more potent (Table I). This compound appears to be 6 times more potent that 5′-azido-5′-deoxycytidine[12] and nearly a 100-fold more potent than 3-deaza-6-azauridine.[14] Preliminary results indicate that both **3a** and **3b** are phosphorylated by enzymes present in L1210 cells, but more conclusive studies of the metabolism of these compounds await synthesis of the corresponding radiolabeled compounds. Marked differences were found between **3a** and **3b** in their ability to inhibit cell growth: the uracil derivative **3a** showed only very modest inhibition of cell growth even at 1 mM, whereas the cytosine derivative **3b** completely blocked cell replication at a concentration of 0.1 mM and significantly inhibited cell replication even at concentrations as low as 30 nM (Table I). Both **3a** and **3b** inhibited the salvage of [^{14}C]uridine and [^{14}C]cytidine (both phosphorylated by uridine kinase) in intact L1210 cells in culture (Table II) consistent with their inhibition of UK. This inhibition appeared to be highly selective. Only a slight inhibition of the salvage of [^{14}C]deoxycytidine or [^{14}C]thymidine, which are phosphorylated by kinases distinct from uridine kinase, was observed.

An additional consideration in the use of uridine analogues in vivo is the high activity of the degradative enzyme uridine phosphorylase. If adequate concentrations of any analogue of uridine are to be maintained in vivo, it is desirable for the drug to be resistant to cleavage by this enzyme. Neither **3a** nor **3b** was cleaved by uridine phosphorylase after 30 min of incubation at 37 °C in 20 mM potassium phosphate (pH 7.2) with 1 U/mL of *Escherichia coli* uridine phosphorylase.[15] By comparison, uridine was

Scheme I



92% cleaved to uracil in only 10 min by incubation with 0.01 U/mL of enzyme under these conditions.

This pair of compounds appears to be quite diverse pharmacologically. The cyclopentenylcytosine **3d** has potent cytotoxic activity against L1210 cells, in agreement with the earlier report of marked toxicity to KB cells.[5] The cyclopentenyluracil **3a** is promising as an inhibitor of uridine salvage since it selectively blocks the salvage of uridine by cells, is resistant to phosphorolysis, and is not cytotoxic at biochemically active concentrations. Although not a particularly potent inhibitor of uridine kinase, **3a** may nonetheless be effective in vivo, because physiological levels of uridine phosphorylase should prevent accumulation of uridine behind the enzymic block. Further development and assessment of the in vivo effectiveness of these analogues is anticipated.

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