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ANTI-ALLERGY AGENTS. 3.[1] ANGULAR TRICYCLIC ETHER DERIVATIVES OF THE 1,8-NAPHTHYRIDIN-2(1H)-ONE RING SYSTEM[2]

David J. Blythin,* Ho-Jane Shue, Emilie Carlon, James Spitler, and William Kreutner§

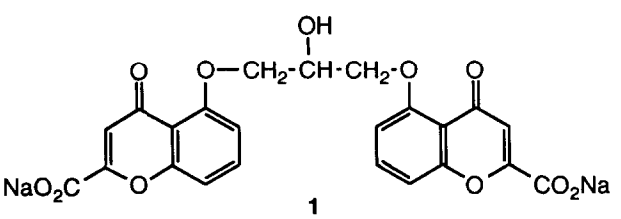
Chemical Research Department and Department of Allergy and Immunology,§
Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033

Abstract

The compounds described are potent, orally active inhibitors of the release of the leukotriene mediators of anaphylaxis *in vitro* and *in vivo*. Unlike most "Mediator Release Inhibitors" (MRI's) described in the literature, they do not contain an acidic function.

Since the introduction of sodium cromoglycate (**1**; Intal®) as a prophylactic antiasthmatic/antiallergy agent more than 20 years ago an enormous amount of effort has been expended in both industrial and academic laboratories to design and synthesize more potent, more effective, and/or orally active analogues.[3] The vast majority of the SAR data generated between 1970 and 1980 was derived from the Rat PCA (Passive Cutaneous Anaphylaxis) assay.[4] This methodology was, in one sense, developed as an animal model for a compound, **1**, already known to show anti-asthmatic effects - the fact that the first "animal model" was actually a human is well known.[5] However, it became evident that the Rat PCA Assay was not predictive of anti-asthmatic activity in humans. Not one of the multitude of structures showing activity in the PCA model has been developed into a viable, orally-active anti-asthma drug.

The use of an assay more closely paralleling the biochemical processes occurring in asthma was a priority in our laboratories, and our screening methodology emphasizes the important role of the leukotrienes in allergic processes and asthma. Thus, the tissues utilized for *in vitro* screening are from lung,[6] and the *in vivo* model utilizes an allergically provoked bronchospasm in guinea pigs, a species which many consider to be closer in lung physiology and pathology to that of the human than are other species available for large scale screening. In addition, bronchial effects caused by histamine or prostaglandin release are eliminated by the addition of an antihistamine and a cyclooxygenase inhibitor.[7]



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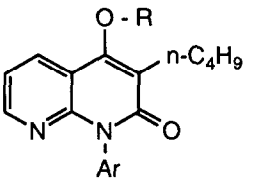


2a; R = H; **2b**; R = C_2H_5

During the course of searching for new structural leads fitting these criteria, we evaluated 3-n-butyl-4-hydroxy-l-phenyl[1,8]naphthyridinone (**2a**) in these assays.[8] Of interest and relevance to the current work was the observation that most of the 4-O-alkylated derivatives, such as **2b**, showed essentially no activity, thus strengthening the thesis that an acid-equivalent function was needed for anti-allergic activity. That this was not the case was shown when the cyclic ether derivative, **7** (Scheme 1,) was synthesized and tested.

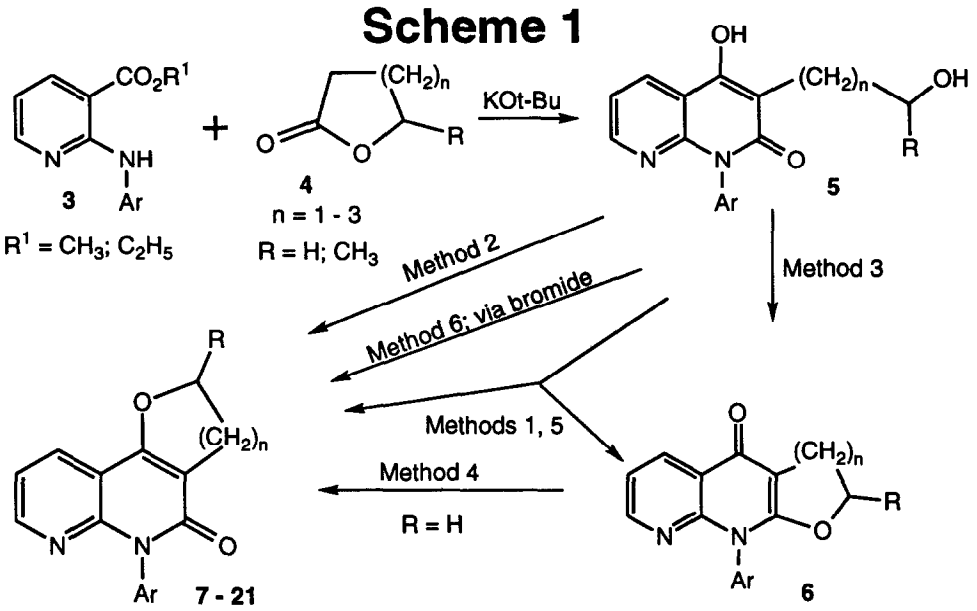
Chemistry Scheme 1 shows the general synthetic pathways to this class of compound, and the individual products are listed in Table 1. Synthesis of the prototype of the series, **7**, was accomplished in several ways. Initial condensation of the readily available 2-anilinonicotinic ester (**3**; $R^1 = CH_3$; Ar = C_6H_5) (methyl and ethyl esters were used interchangeably in this work) with an excess of γ-butyrolactone (**4**; R = H; n = 1) and KOt-Bu, at elevated temperature, resulted in a thick syrupy mass which, when treated with dilute base followed by acidification, resulted in a reasonable yield of the 3-(2-hydroxyethyl)-[1,8]naphthyridinone derivative (**5**; R = H; n = 1; Ar = C_6H_5). Acid catalyzed ring closure utilizing 30% or 40% H_2SO_4 at reflux (**General Method 2**) resulted in an equilibrium mixture containing about 80-85% of **7**, the remainder being **5**. By pouring the crude mixture carefully into an excess of dilute base it was possible to retain **5** in solution while **7** precipitated out.

An alternate procedure, which proved to be more flexible and to provide material of high purity, involved two steps. Initial closure of **5** to the linear tricyclic system **6** (**General Method 3**) took place in excellent yield when cyclization was carried out using Eaton's Reagent[9] at about 50°C. Subsequent heating of a solution of **6** in dimethylacetamide or DMF with a catalytic quantity of KI or NaI (**General Method 4**) resulted in clean conversion to the angular ether **7** with no trace of the linear isomer **6** or non-cyclized material **5** remaining. However, this method could not be applied in cases where there was an alkyl substituent on the α-carbon (eg **10**). Alternate routes to the angular analogues were used in these cases.

The first method used to synthesize **7** from **5** utilized 48% HBr at 90-100°C (**General Method 1**). Although this method caused complete loss of starting material, it produced both linear- (**6**) and angular-fused (**7**) products, which were separated by column chromatography. A similar result was obtained when the intermediate **5** was refluxed with a catalytic amount of p-TSA in toluene, using a Dean and Stark separator to remove water (**General Method 5**). This method was used to prepare products having alkyl groups α- to the ether oxygen (eg **10**, **20**). The products were purified by column chromatography to afford modest yields of the angular isomers. Finally, products with a 7-membered ring (**9**, **21**) were prepared via the intermediate ω-bromobutyl derivatives, which were cyclized under basic conditions (**General Method 6**.)

Structure confirmation of the isomeric ring fusion products was accomplished by comparison of the ^{1}H-NMR line positions of the γ-proton of the pyridine ring. In the angular fusion product, **7**, this proton, having coupling constants of 2 and 5Hz, appeared at δ = 8.02 ppm (in $CDCl_3$) whereas in the linear isomer, **6**, the corresponding proton appeared at δ = 8.77 ppm because of the strong deshielding effect of the coplanar carbonyl group. The UV spectra of the isomers differ also. Whereas the peak at

around 320nm showed two definite shoulders in the angular isomer, the same peak in the linear isomer was smooth and almost symmetrical.



Pharmacological Activity The compounds **7-10** were prepared and tested first as typical examples of ring-size variation and alkyl-substitution on the tricyclic ring system. Screening results are shown in Table 1. A trend toward greater activity in the 5- and 7-membered ring systems (n = 1 and 3 *vs.* n = 2) was observed, and methyl substitution on the position adjacent to the ether oxygen (**10, 20**)appeared to give comparable activity. The series of compounds (**7, 11-14** and **8, 15-18**) comprise the base set for what has become known as the "Topliss Tree" approach to structure-activity relationships.[10] The substituents represented possess significant variations in electronic and steric factors, and the overall trend in biological activity across each series has been used to predict the next members of the series that ought to be synthesized in attempts to optimize potency. The particular order seen here for the 5-membered ring compounds (3,4-Cl_2 and H as most potent, followed by p-Cl and p-CH_3, with p-CH_3O as least potent) suggested that a σ effect might be operable. However, the inversion of order of H vs. p-Cl suggested to us the possibility of an adverse steric effect at the para-position. We reasoned that the 3-Cl derivative (**19**) might be a desirable follow-up compound. In fact, several 3-Cl derivatives were synthesized (**19 - 21**) and were found to be the most active compounds in the series.

TABLE 1

Cpd. No.	n	R	Ar	Method of Prepn.	Yield (%)	mp (°C)	*in vitro*[a] Inhibn (%) at: 10μM	3μM
7	1	H	C_6H_5-	1,2,3,4	97	277-278	72	54
8	2	H	C_6H_5-	1	27	253-255	62	26
9	3	H	C_6H_5-	6	21	180-181		75
10	1	CH_3	C_6H_5-	5	62	223-224	60	
11	1	H	4-$CH_3C_6H_5$	5	41	258.5-260	59	28
12	1	H	4-$CH_3OC_6H_4$	3,4	90	282-284		17
13	1	H	4-ClC_6H_4	5	<10	>260		33
14	1	H	3,4-$Cl_2C_6H_3$	5	12	256.5-258	63	62
15	2	H	4-$CH_3C_6H_5$	3,4	76	>280		27
16	2	H	4-$CH_3OC_6H_4$	3,4	72	>270		28
17	2	H	4-ClC_6H_4	3,4	88	236-237		33
18	2	H	3,4-$Cl_2C_6H_3$	5	23	>265		48
19	1	H	3-ClC_6H_5	3,4	59	205-207		71
20	1	CH_3	3-ClC_6H_5	5	57	254-256		64 (1μM)
21	3	H	3-ClC_6H_5	6	<10	151-153		100 62(1μM)

[a] Inhibition of LT-D_4 release from sensitized guinea pig lung fragments.[6] Values are the average of assays using three separate lung tissues. Compounds were added 12 min before challenge with ovalbumin.

Most of the derivatives active *in vitro* were next studied in an *in vivo* model of anaphylactic bronchospasm.[7] Pharmacological manipulation in this model results in a bronchospasm that is mainly leukotriene mediated. Test compounds are administered orally, usually 2h before challenge, although the assay can be used to measure duration of action by varying the time before challenge. In addition, a sub-acute dosing regimen (five doses given over a 2.5 day period before challenge) is used routinely to detect any potential problems with tachyphylaxis, and to assess if a cumulative effect might be seen. The results obtained for this series of compounds are shown in Table 2. One of the 3,4-dichloro-substituted compounds (**14**) which showed good *in vitro* activity was inactive *in vivo* so this substitution pattern was not studied further. Although not all doses were run for the unsubstituted compounds (**7**, **8**, and **9**) it looked as if the 7-membered ring ether gave the best overall activity. This was confirmed when the 3-chloro derivatives (**19**, **20**, and **21**) were studied. Although the ED_{50} was not determined, it is clear that compound **21** is effective at doses below 1mg/Kg both acutely and in the sub-acute regimen.

Plasma Levels Before one of the compounds from this group could be recommended for further development, such as pre-clinical toxicology, we wished to know if a significant plasma level was obtained after oral dosing. Compound **7** was chosen for this study because its activity profile was

TABLE 2

In vivo Antianaphylactic Activity of some [1,8] Naphthyridinone Derivatives

Cpd. No.	Inhibition of antigen-induced bronchospasm[7,a] (PO; 2h) in sensitized guinea pigs			
	Single Dose[b]		Multiple Dose[c]	
	Dose (mg/Kg)	Inhibition (%)	Dose (mg/Kg)	Inhibition (%)
7	25	79	12.5	90
	12	86	6.25	65
	3	42		
8	25	62	12.5	43
			6.25	51
			3.13	20
9	2	71		
14	5	0		
19	10	93	2	62
	2	87	1	21
20	2	38		
21	2	85	0.5	50
	1	74		
	0.5	62		
	0.25	27		

a Male Hartley guinea pigs were sensitized to ovalbumin. Preparation for the assay has been described elsewhere.[11] The animals were pre-treated with propranolol, indomethacin, and mepyramine to enhance the leukotriene component of bronchospasm.
b Groups of 4 to 6 animals were used for each of the determinations, and results are reported as averages.
c The protocol for the assay was the same as the single dose except that the test substance was given BID for 2 days prior to the experiment and again 2h before challenge on the third day.

superior to other members of the series. A simple HPLC method for estimating blood or plasma levels in rats was used. This involved PO dosing at 25mg/Kg followed 2h later by removal of 1mL of blood. Cells were removed, and 4mL of EtOH was added. After shaking and filtering, the residue was evaporated to dryness in a stream of N_2. The residue was redissolved in 1.0mL of HPLC solvent, generally a mixture of CH_3CN, H_2O and AcOH, and was analyzed by HPLC on a Whatman Partisil 5, ODS-3 column using UV detection at 320nm, the λ_{max} for this class of compound. Even after this high dose of test compound, plasma levels were very low, being 1.18μg/mL in fasted animals and only 0.4μg/mL in fed animals.

Conclusions The class of compound described here includes several highly potent, orally active inhibitors of leukotriene-mediated anaphylactic bronchospasm in guinea pigs. This is an assay thought to bear some resemblances to asthma in humans. These compounds do not inhibit 5-LO significantly, nor are they antagonists of LT-B_4, C_4 or D_4. They appear to act by inhibiting the release of the leukotrienes, although their precise mechanism of action remains unknown. However, the low plasma levels shown by the prototype of the group precluded further development of this series. A related compound, Sch 37224, having an improved pharmacokinetic profile over the compounds described here, has been shown to possess useful activity in at least one clinical trial, against the bronchoconstriction induced by cold air isocapnic hyperventilation.[12]

Experimental

General procedures for the preparation of compounds **7 - 21:**

Preparation of intermediates 5

A mixture of methyl or ethyl 2-arylamino-3-pyridine carboxylate (**3**; 1g) and the lactone (**4**; *ca.* 10mL) was treated with KOt-Bu (*ca.* 2g). The reaction mixture was stirred under nitrogen at 95 - 110°C for 1 - 6h then it was poured on to ice and 5% NaOH. The mixture was stirred overnight. Organic-soluble material was extracted into ether then the aqueous layer was acidified with HCl or AcOH to pH *ca.* 4.5 - 5.0. The solid product that precipitated was collected by filtration and was purified by recrystallization, usually from mixtures of CH_2Cl_2 and isopropanol and characterized by microanalysis, MS, ^{1}H-NMR, and mp.

General Method 1 - mixtures of linear- and angular-fused analogues 6 - 21

A suspension of **5** (5g) in 47% HBr (50mL), under N_2, was stirred and heated to 90°C and kept there for about 4 - 6 h. The product was cooled, poured into water, and the pH was adjusted to 4.5. The resulting solid was purified first by chromatography on silica gel, eluting with a CH_2Cl_2/Et_2O mixture, then by recrystallization from a suitable solvent, such as $CHCl_3$/IPA.

General Method 2 - acid-catalyzed cyclizations of 5

A solution of **5** (10g) in 30% (v/v) H_2SO_4 (200mL) was heated at reflux, under N_2, until TLC showed that no further change was occurring (*ca.* 5 - 8h). The product was cooled then added very slowly and carefully -CAUTION- to an ice-cold 50% NaOH solution (200mL.) After standing overnight the product was filtered off, air dried, and recrystallized from a suitable solvent, such as DMF/H_2O.

General Method 3 - preparation of linear-fused analogues 6

A solution of the product from step 1 (**5**; 5g) in Eaton's Reagent[9] (100mL; made by dissolving 10% P_2O_5 in methanesulfonic acid) was heated at about 50 - 70°C for 2h. The product was isolated by pouring the reaction mixture into water (with cooling) and adjusting the pH to about 7.0 with $NaHCO_3$. The products were purified by recrystallization.

General Method 4 - rearrangement of linear to angular analogues; R = H

A solution of **6** (1g), and NaI (1g) in dry DMF or DMA (10mL), under N_2, was heated at 70°C for 4h. The product was cooled, poured into ice-water, filtered, dried, and recrystallized from a suitable solvent, such as CH_2Cl_2.

General Method 5 - acid-catalyzed cyclization of 5 with water-removal

A solution of p-TSA (5g) in toluene (100mL) was made up. To this was added **5** (5g), and the solution was heated to reflux, under N_2, with a Dean and Stark water separator. After heating for about 18h the mixture was cooled and poured into H_2O (200mL.) After stirring for 0.5h the mixture was filtered and the solid product was purified by recrystallization from a suitable solvent, such as IPA.

General Method 6 - base-promoted cyclizations (5; n = 3)

A compound **5**, in which n = 3, when treated according to **General Method** 1, is converted to the 4-bromobutyl derivative, not the tricyclic material directly. Treatment of this bromo-compound, in CH_2Cl_2 solution, with one equivalent of NEt_3, followed by isolation and purification by chromatography on silica gel, and recrystallization from a suitable solvent, yields the 7-membered ring ethers, **9** and **21**.

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References and Notes

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