Synthesis of Novel 2,4-Diaminopyrrolo-[2,3-*d*]pyrimidines with Antioxidant, Neuroprotective, and Antiasthma Activity

Gordon L. Bundy,* Donald E. Ayer, Lee S. Banitt, Kenneth L. Belonga, Stephen A. Mizsak, John R. Palmer, James M. Tustin, Jia En Chin, Edward D. Hall, Kelly L. Linseman, Ivan M. Richards, Heidi M. Scherch, Frank F. Sun, Patricia A. Yonkers, Philip G. Larson, Jasmine M. Lin, Guy E. Padbury, C. S. Aaron, and Judy K. Mayo

Upjohn Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

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A large number of pathological conditions have been associated with elevated levels of reactive oxygen species[1-4] (superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen), although the often-implied cause and effect relationship has in many cases not been firmly established.[1,5] Since an excess of reactive oxygen species can result either from overproduction or from a breakdown of the body's endogenous antioxidant defense mechanisms,[6,7] it is not surprising that supplementation of the natural defense system by administration of reactive oxygen inhibitors and scavengers constitutes an increasingly popular experimental approach to the prevention and treatment of a wide variety of diseases.[8-10]

Several 21-aminosteroids have been reported[11] to be potent inhibitors of iron-dependent lipid peroxidation and to exhibit neuroprotective activity in several models of central nervous system (CNS) trauma. The most thoroughly studied member of this class, tirilazad mesylate (U-74006F; Figure 1), is currently undergoing clinical evaluation for the treatment of head injury, subarachnoid hemorrhage, and spinal cord trauma.[12] During chemical studies related to tirilazad mesylate, we discovered a series of novel 2,4-diaminopyrrolo[2,3-*d*]pyrimidines (U-87663 is prototypical), many of which exhibited a similarly promising pharmacological profile.

Treatment of triaminopyrimidine intermediate **1** (related to the heterocyclic portion of tirilazad mesylate) with phenacyl bromide under mildly basic conditions afforded not the expected α-amino ketone but, rather, 7-methyl-6-phenyl-2,4-di-1-pyrrolidinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (U-87663) (Scheme 1). If the alkylation/cyclization was performed at 25 °C, the crystalline hemiaminal **3** could be isolated in 83% yield by simple filtration of the reaction mixture. Dehydration of **3** to U-87663 subsequently took place in quantitative yield by exposure of **3** to silica gel, by warming solutions of **3** in organic solvents (i.e., attempted recrystallization), or by heating the hemiaminal to its melting point. Even more simply, the original reaction mixture could be heated to reflux and the pyrrolopyrimidine could be isolated directly by filtration in 85% yield.

The unambiguous assignment of pyrrolopyrimidine **4** as the 6-phenyl regioisomer, not the alternative 5-phenyl isomer, was based on NMR experiments. Irradiation of the vinyl hydrogen signal at 6.42 ppm led to NOE enhancement of the *o*-phenyl hydrogen and the down-

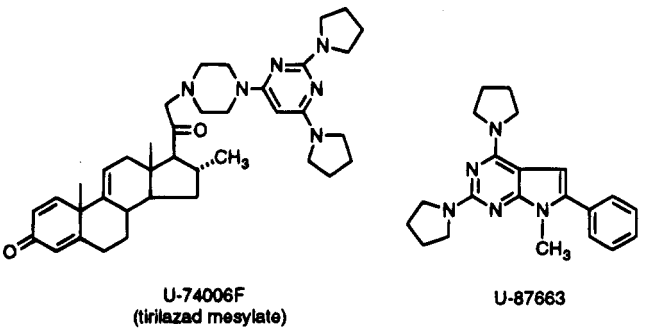
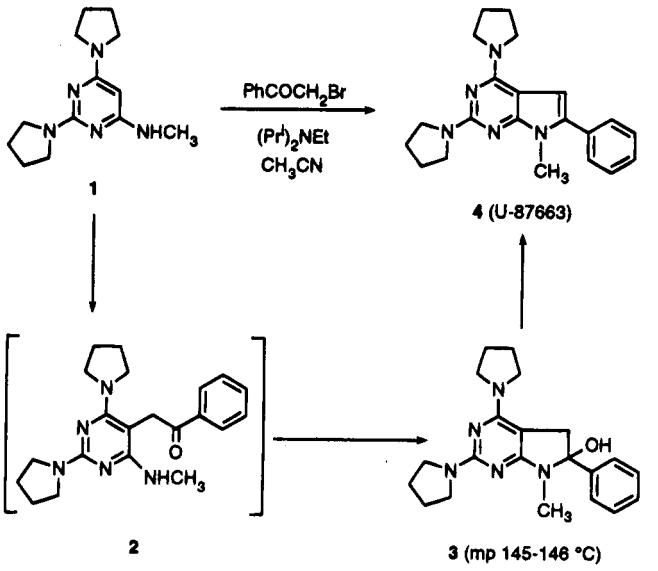
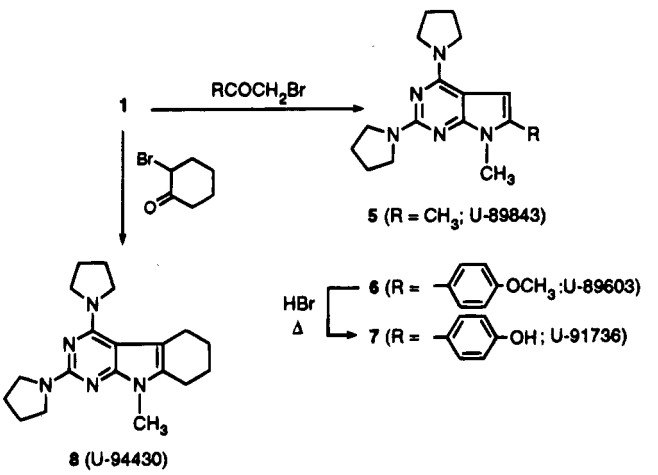


Figure 1.

Scheme 1



Scheme 2



field pyrrolidine $-NCH_2-$ signals but not of the N-7 methyl signal. Also, the N-7 methyl protons were coupled strongly to two quaternary carbons (C-6, C-7a) in HMBC (heteroatom multiple-bond coupling) experiments. The NMR spectrum of intermediate hemiaminal **3** was likewise consistent only with the regiochemical assignment indicated. Hence the initial alkylation step in this indolization likely occurred at the unusually nucleophilic C-5 position of pyrimidine intermediate **1**, not on the secondary amine nitrogen. This reaction is thus clearly distinct from the classical Bischler indole synthesis,[13] which proceeds under protonic or Lewis acid

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Table 1. Antioxidant, CNS Neuroprotection Pharmacology

compd	IC_{50} (μM) inhib of MDA form[a]	IC_{50} (μM) spinal neuron protect[b]	human astrocyte cytoprotect[c] EC_{50} (μM)	mouse head inj av incr in 1 h grip score[d] (%)	lowest obsd oxidation potential[e] (mV)
4·MsOH (U-87663E)	5	1.3	0.9	104	530
5·MsOH (U-89843E)	1	7.0	0.2	100	423
6·MsOH (U-89603E)	1	3.4	0.4	73	500
7·HBr (U-91736B)	1	0.13	0.2	138	410
8·MsOH (U-94430E)	2	0.34	0.2	276	369
11·2HCl (U-101033E)	nd[f]	1.1	0.3	163	408
12·HCl·0.5H_2O (U-104067F)	nd	10.6	3.0	nd	693 (shoulder) 894

[a] Reference 14. Malondialdehyde (MDA) is a minor (but consistent) byproduct of lipid peroxidation. Test compounds were assessed for their ability to inhibit Fe^{2+}-induced MDA formation in rat brain homogenate. Compounds with $IC_{50} \leq 10$ μM are generally considered worthy of further study. [b] Reference 15. Treatment of fetal mouse spinal neurons (cell culture) with ferrous ammonium sulfate leads, via oxidative processes, to cell damage and death. Aminoisobutyric acid (AIB) uptake is used as the measure of cell viability. Compounds worthy of further interest are those which inhibit the Fe^{2+}-induced damage with $IC_{50} \leq 10$ μM. [c] Reference 16. Cultured human astroglial cells were subjected to injury by iodoacetic acid, an injury thought to be mediated by oxidative species. Compounds active in this assay include both antioxidants and compounds capable of stabilizing membranes, with compounds exhibiting cytoprotective effects with $EC_{50} \leq 10$ μM being of interest. [d] Reference 17. Mice were treated intravenously with the test drug (1 mg/kg) 5 min following a concussive head injury. For the survivors (~70%), their neurological status was evaluated at 1 h postinjury using a simple grip test. Results are expressed as average percent improvement in grip score (s) relative to vehicle-treated controls. Scores of ≥100% indicated test compounds that warrant further attention. [e] Observed oxidation potentials were determined by cyclic voltammetry at a drug concentration of ca. 20 μM in 25 mM sodium acetate buffer adjusted to pH 5 with glacial acetic acid. Voltammetry data were obtained on a Bioanalytical Sciences EC100A electrochemical analyzer using a glassy carbon working electrode, platinum auxiliary electrode, and Ag/AgCl reference electrode at room temperature (21–23 °C). [f] Not determined.

Table 2. Lung Inflammation Pharmacology

compd	inhibition of lung eosinophilia (%) mouse, dose (mg/kg/day, po) 3	10	30	rat, dose (mg/kg/day, po) 3	10	30
5 (U-89843D)	0	0	0	0	27	28
8 (U-94430E)	0	31	41	72	NT	47[a]
11 (U-101033E)	37	56	72	13[a]	30	62[b]
12 (U-104067F)	53	62	71	NT	87	85[b]

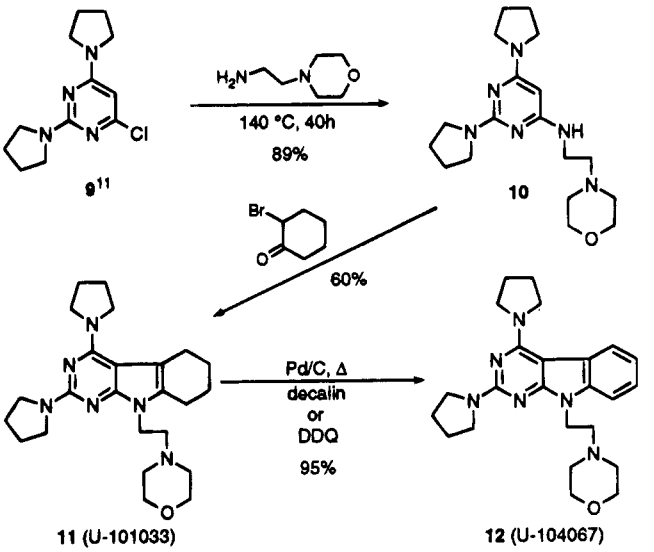
[a] Average of two experiments (10 animals/dose/experiment). [b] Average of three experiments.

catalysis, under considerably more vigorous conditions via initial alkylation of an aniline nitrogen.

This triaminopyrimidine/α-bromo ketone indolization proved to be a very versatile and reliable reaction, provided that neither component was excessively hindered, and a large number of pyrrolopyrimidines were thereby prepared. Several typical examples are recorded in Schemes 2 and 3.

While the in vitro pharmacological profiles of compounds **4**–**8** were attractive, options for preparing suitable pharmaceutical formulations were somewhat limited by their modest aqueous solubility. Hence

Scheme 3



compounds **11** and **12**, which incorporate a more hydrophilic indole nitrogen substituent, were synthesized (Scheme 3) to provide analogs with improved aqueous solubility. Alternative water-solubilizing moieties could

Table 3. Pharmacokinetic Parameters for Selected Pyrrolopyrimidines in the Male Sprague–Dawley Rat[a]

compd	systemic clearance (L/h/kg)	terminal half-life (h)	steady state volume of distribution (L/kg)	C_{max} (μg/mL)	t_{max} (h)	oral bioavailability (%)
4 (U-87663)	1.42 ± 0.47	22.1 ± 4.1	44.0 ± 11.0	1.42 ± 0.24	1–4	55.9 ± 7.7
5 (U-89843)	0.53 ± 0.13	10.9 ± 5.9	39.7 ± 9.5	5.71 ± 3.15	2–4	84.6 ± 33.8
6 (U-89603)	1.24 ± 0.43	24.3 ± 8.0	42.8 ± 20.1	0.60 ± 0.28	1–4	59.8 ± 20.6
7 (U-91736)	2.33 ± 0.41	6.1 ± 4.3	66.2 ± 48.6	0.72 ± 0.27	1–8	31.4 ± 8.8
8 (U-94430)	0.10 ± 0.15	8.9 ± 1.7	1.09 ± 1.52	40.3 ± 25.5	2–6	70.2 ± 5.4
11 (U-101033)	0.058 ± 0.002	11.2 ± 1.1	0.94 ± 0.06	19.4 ± 5.68	2	78.3 ± 18.9
12 (U-104067)	0.12 ± 0.032	15.7 ± 4.9	1.92 ± 0.76	14.7 ± 3.77	1–2	69.9 ± 24.6

[a] Compounds were administered at doses of 10 mg/kg intravenously and 25 mg/kg orally as 5–10 mg/mL solutions in propylene glycol USP. Doses were administered to fasted animals ($N = 3$/compound) in a crossover design with a 1 week washout period between treatments.

be installed by using ethanolamine in the initial alkylation step and then adjusting the 2-hydroxyethyl functionality, as desired, following indolization.

Several hundred pyrrolo[2,3-*d*]pyrimidines and pyrimido[4,5-*b*]indoles were prepared by the general routes outlined in Schemes 1–3. These compounds were evaluated in a variety of well-documented pharmacological test systems as antioxidants, CNS neuroprotective agents, and antiasthma agents. While a report of detailed structure–activity relationships must be deferred to a full paper, preliminary biological data for several of the more promising members of the pyrrolopyrimidine series are summarized in Tables 1 and 2. Although these results are preliminary, it is clear that the pyrrolopyrimidines reported herein are electrochemically active, i.e., good electron donors/antioxidants (**12**, U-104067F, less so than the others). This antioxidant capability is also manifested in their ability to act as inhibitors of iron-induced lipid peroxidation and their activity in protecting mouse spinal neurons and human astrocytes from oxidative injury. It is also consistent with their ability to minimize neurological damage in the mouse head injury model.[17] Although the 21-aminosteroids and pyrrolopyrimidines have not been subjected to direct head-to-head comparisons, the activities reported in Table 1 fall into the same range as those reported earlier for the more active 21-aminosteroids.[11] Tirilazad mesylate exhibited an IC_{50} of 18 μM in the MDA lipid peroxidation inhibition assay.[11]

In addition to the antioxidant and neuroprotective activities noted above, several of the new pyrrolopyrimidines have also proven to be effective inhibitors of antigen-induced lung eosinophilia in rodents. (Eosinophils have been implicated as important proinflammatory mediators in the pathogenesis of asthma.[18]) Table 2 shows the effect of selected pyrrolopyrimidines on antigen-induced bronchoalveolar eosinophilia in ovalbumin (OA)-sensitized rats[19] and mice.[20] The compounds were given po once daily for 7 days prior to provocation with aerosolized OA. The lung eosinophilia was assessed at 24 h following antigen challenge. For comparison, dexamethasone (1.0 mg/kg, po) produced full efficacy (100% inhibition of eosinophil influx) in both the rat and mouse models of lung inflammation.[19,20] Both **11** (U-101033E) and **12** (U-104067F) exhibited statistically significant activity ($p < 0.05$) in both models at only slightly higher doses. The mechanistic basis for the observed lung antiinflammatory activity of **12** is currently under investigation; mechanisms in addition to antioxidant/lipid peroxidation inhibition may well be involved.

In order to facilitate the choice between the various pyrrolopyrimidines for further development, they were evaluated with respect to their pharmacokinetic/oral bioavailability behavior in the male Sprague–Dawley rat. These data, summarized in Table 3, show that with the exception of the phenolic analog **7** (U-91736), all of the pyrrolopyrimidines reported herein exhibited oral bioavailabilities of >50%. Of the compounds with the most attractive pharmacokinetic profiles (**5**, **11**, and **12**), **5** was not a contender for further development because of genotoxicity problems, while **11** exhibited intrahepatic biliary tract and gallbladder toxicity at doses unacceptably close to the anticipated therapeutic doses.

On the basis of more in-depth pharmacological studies, thorough safety assessment, bioavailability considerations, and pharmaceutical/physicochemical suitability (all to be reported in detail later), 9-[2-(4-morpholinyl)-ethyl]-2,4-di-1-pyrrolidinyl-9*H*-pyrimido[4,5-*b*]indole, monohydrochloride, hemihydrate (**12**, U-104067F) has been selected for clinical evaluation for the treatment of asthma and several reactive oxygen-related chronic neurodegenerative disorders. Phase I safety/tolerance trials are currently in progress.

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