7-Oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridines as Novel Inhibitors of Human Eosinophil Phosphodiesterase

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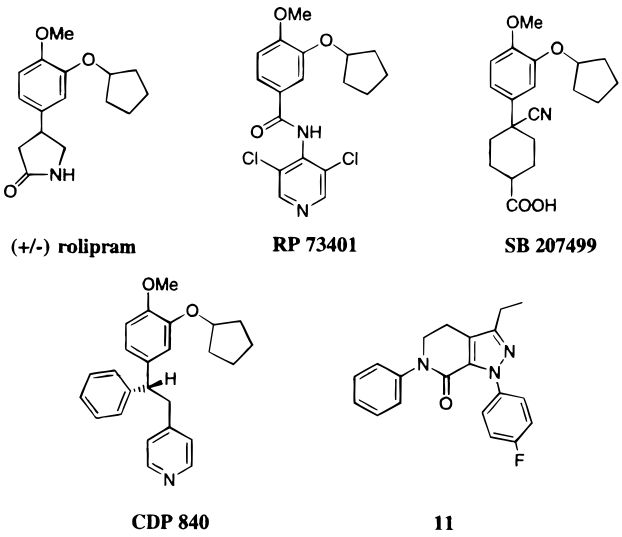
High-throughput file screening against inhibition of human lung PDE4 led to the discovery of 3-ethyl-1-(4-fluorophenyl)-6-phenyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (**11**) as a novel PDE4 inhibitor. Subsequent SAR development, using an eosinophil PDE assay, led to analogues up to 50-fold more potent than **11** with IC_{50} values of 0.03−1.6 μM. One such compound, CP-220,629 (**22**) (IC_{50} = 0.44 μM), was efficacious in the guinea pig aerosolized antigen induced airway obstruction assay (ED_{50} 2.0 mg/kg, po) and demonstrated a significant reduction in eosinophil (55%), neutrophil (65%), and IL-1β (82%) responses to antigen challenge in atopic monkeys (10 mg/kg, po).

Introduction

Phosphodiesterases (PDEs) have been classified into seven major families (PDE1−7) according to their substrate sensitivity, Ca^{2+}/calmodulin requirement, and inhibitor selectivity.[1−5] PDE4, a cAMP-specific and Ca^{2+}-independent enzyme, is a key isozyme in the hydrolysis of cAMP in mast cells, basophils, eosinophils, monocytes and lymphocytes.[1−5] As a result, inhibitors of PDE4 block the release of various mediators from these cells and may be useful antiinflammatory drugs. Recently, four human cDNA isoforms of PDE4 (PDE4-A, -B, -C, and -D) were identified; mRNA for all four isoforms was expressed in human lung; and PDE4-A, -B, and -D were expressed in eosinophils.[6]

Over the past decade, many pharmaceutical companies have focused on the inhibition of PDE4 for the treatment of asthma.[1−5] The biology of the PDE4 isozyme and the SAR of its reported inhibitors have recently been reviewed in the literature.[1−5] In general the therapeutic utility of selective PDE4 inhibitors, such as the prototypical agent rolipram, have been hampered by nausea and emesis limiting their therapeutic potential.[7,8] We and several other pharmaceutical companies have developed SAR around the structure of rolipram in efforts to decrease emetic potency and retain efficacy.[5,9] These efforts have led to second generation PDE4 inhibitors derived from rolipram, and some of these compounds have entered clinical trials for asthma (e.g., RP 73401,[10] SB 207499, and CDP 840[11]).[2,5] However, selective PDE4 inhibitors have yet to provide significant efficacy in the clinic.

As an alternative to designing analogues derived from the structure of rolipram, random file screening was conducted against human lung PDE4 in the hope of finding a novel PDE4 inhibitor. This exercise led to the discovery of the pyrazolopyridine, **11**, a selective PDE4 inhibitor that is structurally unrelated to rolipram. Despite this structural dissimilarity, SAR optimization of **11** led to the hypothesis that the ethyl and 4-fluorophenyl moieties of **11** could mimic the methoxy and cyclopentoxy moieties of rolipram, respectively (Figure 1), in binding to the active site of PDE4. In this paper we report the evolution of the SAR of **11** and its possible relationship to rolipram. Since eosinophils are believed to be a critical proinflammatory target cell for asthma,[1,2] the SAR developed around **11** was evaluated against human eosinophil PDE.



Chemistry

A procedure for the preparation of **11** has been reported in the patent literature.[12] We have modified the synthesis in order to optimize yields and provide for structural modifications in the latter steps of the synthesis at the 1-, 3-, and 6-positions of the pyrazolopyridine bicycle (Scheme 1). The substituent at the lactam nitrogen (6-position of the pyrazolopyridines) was introduced in the first step by a Goldberg reaction between an aryl iodide and 2-pyrrolidinone in the presence of copper and K_2CO_3 at 130−170 °C to form the *N*-arylpyrrolidinones **1a**−**g**. This substituent could also be incorporated in the last step of the synthesis as

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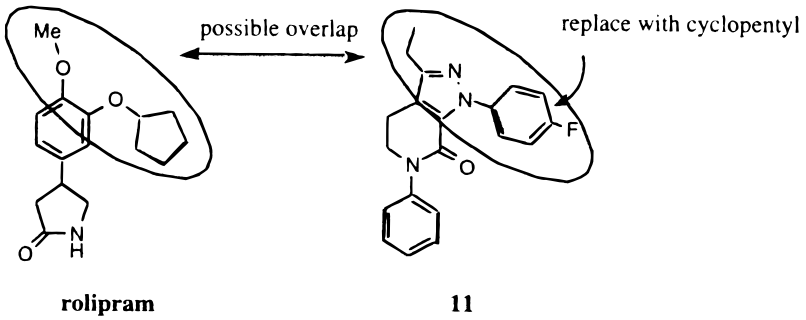
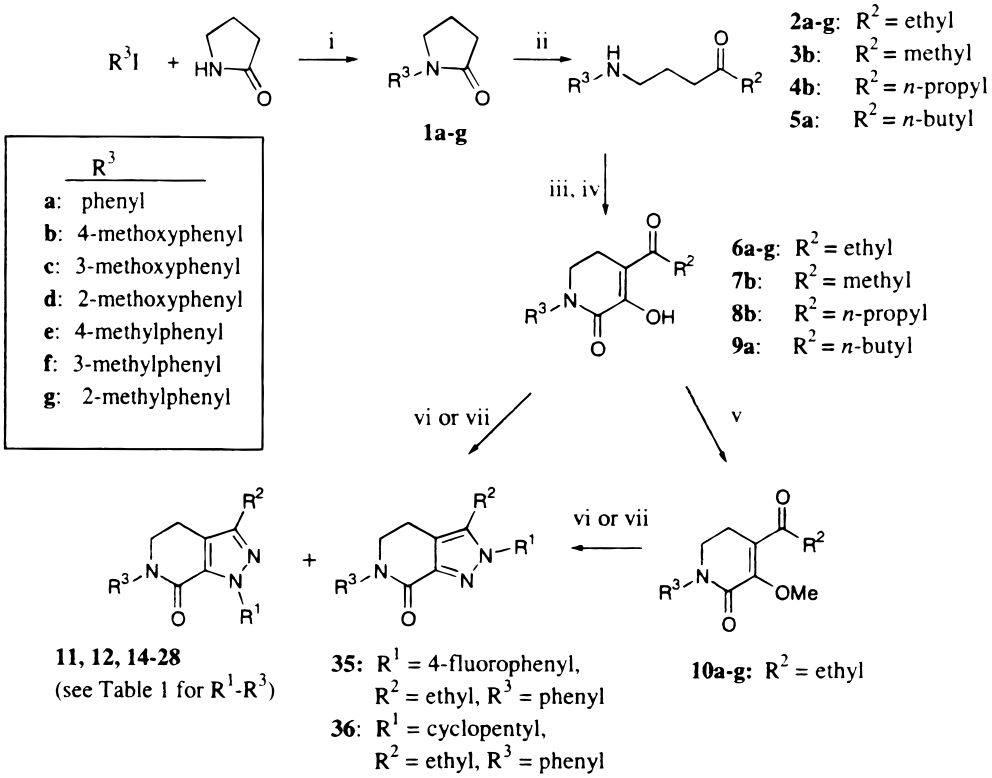


rolipram **11**

Figure 1. Hypothesis: the ethyl and 4-fluorophenyl moieties of **11** overlap with the methoxy and cyclopentoxy moieties of rolipram, respectively.

Scheme 1[a]



R3
a: phenyl
b: 4-methoxyphenyl
c: 3-methoxyphenyl
d: 2-methoxyphenyl
e: 4-methylphenyl
f: 3-methylphenyl
g: 2-methylphenyl

[a] Reagents and conditions: (i) Cu, K_2CO_3, 130−170 °C [general procedure A]; (ii) R_2MgBr, ether, 0 °C [general procedure B]; (iii) ethyl oxalyl chloride, NaOH, benzene [general procedure C]; (iv) NaOEt, EtOH; (v) methyl-4-tolyltriazine, dichloroethane, reflux [general procedure D]; (vi) R^1NHNH_2·HCl, NaOMe, EtOH, reflux [general procedure E]; (vii) R^1NHNH_2·HCl; 120 °C; N_2 stream [general procedure F].

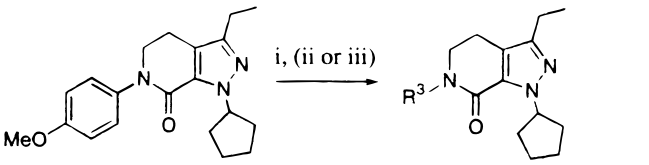
described below. The pyrazolopyridine 3-substituent was introduced by treating **1a**−**g** with a Grignard reagent to give amino ketones **2**−**5**. The amino ketone intermediates were found to be unstable and were therefore acylated with ethyl oxalyl chloride immediately after purification and subsequently cyclized to 3-hydroxy-2-oxo-1,2,5,6-tetrahydropyridines **6**−**9** by heating at reflux with NaOMe/EtOH. Esterification of **6a**−**g** with methyl-4-methylphenyltriazine[13] provided the corresponding vinylogous methyl esters, **10a**−**g**. The vinylogous acids **6**−**9** or esters **10a**−**g** were treated with an alkyl or aryl hydrazine hydrochloride and either fused at 120 °C or heated at reflux in NaOMe/EtOH to give a mixture of the desired 7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridines (**11**, **12**, **14**−**28**) and the corresponding regioisomers (e.g., **35** and **36**). The correct regiochemical assignment to **11** was confirmed by single-crystal X-ray diffraction.

To prepare a variety of substituents at the lactam nitrogen of the pyrazolopyridines, the 4-methoxyphenyl analogue, **12**, was treated with aqueous ceric ammonium nitrate in acetonitrile[14] to give the unsubstituted lactam **13** (Scheme 2). The lactam nitrogen of **13** was either coupled with an aryl- or heteroaryl halide in the presence of copper and K_2CO_3 at 130−170 °C to give compounds **29**−**33** or alkylated with benzyl bromide to give compound **34**.

Biology

For the purpose of high throughput screening, a PDE4 isozyme mixture was used, which was isolated from human lung tissue[15] with slight modifications to the published procedure.[9] Rolipram-sensitive PDE4 fractions had 2 mL of ethylene glycol added for storage at −25 °C. Subsequent SAR was developed from a human eosinophil PDE assay, also utilizing a PDE4 isozyme

Scheme 2[a]



12

13: R^3 = H
29-33: R^3 = aryl (defined in Table 1)
34: R^3 = benzyl

[a] Reagents and conditions: (i) ceric ammonium nitrate, CH_3CN/water; 0 °C [general procedure G]; (ii) aryl iodide or bromide, Cu, K_2CO_3, 130−170 °C; (iii) NaH, THF, benzylbromide [general procedure H].

mixture. Selected compounds were evaluated for PDE4 isozyme selectivity against isolated soluble PDE1 (human cardiac ventricle), PDE3 (human heart), and PDE5 (human corpus cavernosal tissue). Compounds were also evaluated for their ability to elevate intracellular levels of cAMP in human U-937 cells.

The anti-anaphylactic activity of PDE4 inhibitors was determined by their ability to block the airway obstructive response of anti-OA-IgG_1 passively sensitized guinea pigs challenged with aerosolized ovalbumin.[9] Selected active compounds were subsequently evaluated in monkeys using a crossover experimental design that allows each monkey to serve as its own negative control. Previous studies demonstrated that treatment with the PDE4 inhibitor, rolipram, prior to antigen challenge, could reduce the magnitude of leukocyte infiltration into the lungs of atopic monkeys and also reduce levels of TNF-α and IL-8 in their bronchoalveolar (BAL) lavage fluid.[16] Chronic treatment with rolipram also prevented the development of airway hyperresponsiveness and eosinophil influx after multiple antigen challenge in atopic monkeys.[16] Because the efficacy during chronic studies may reflect the acute efficacy of a compound, we evaluated selected PDE4 inhibitors for their ability to inhibit cellular and biochemical responses 4 h after aerosolized antigen challenge.

Structure−Activity Relationships

Since the evaluation of compound **11** (IC_{50} = 0.44 μM) and its regioisomer, **35** (7% inhibition, 100 μM), in the human lung PDE4 assay revealed that the 2-substituted pyrazolopyridines (**35**) were poor PDE4 inhibitors, only the 1-substituted isomers were subsequently evaluated in the human eosinophil PDE assay.

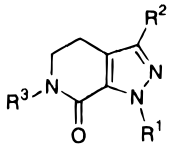
As shown in Table 1, replacement of the 4-fluorophenyl group (R^1) in compound **11** with cyclopentyl to give **14** improved the human eosinophil PDE inhibitory potency by 10-fold. Insertion of either a methyl or methoxy group at the 2- or 3-position of the phenyl ring of **11** (R^3) enhanced the eosinophil PDE inhibitory activity (**16**, **17**, **19**, and **20**), whereas placing these groups in the 4-position slightly reduced the activity (**15** and **18**). Combining the cyclopentyl group at R^1 with either the 3-methoxyphenyl or 2-methylphenyl group at R^3 (Table 1) led to compounds **21** (eosinophil PDE IC_{50} = 34 nM) and **22**, respectively. Further modifications of R^1 replacing the cyclopentyl group in compound **21** with cyclohexyl (**23**), cyclobutyl (**24**), or 3-sulfolanyl (**25**) offered no additional improvement. Modification of R^2 gave dramatic results as replacement of the ethyl group with methyl, *n*-propyl, or *n*-butyl significantly reduced the eosinophil PDE potency (**26**−**28**).

With the 1- and 3-positions of the pyrazolpyridine bicycle optimized as cyclopentyl and ethyl, respectively, we returned to modification of R^3, incorporating various heterocyclic and substituted phenyl groups. Replacement of the phenyl group of **14** with 2-thiophene (**29**) improved eosinophil PDE activity (IC_{50} = 0.063 vs 0.16 μM), replacement with either the 3-thiophene group (**30**) or the 2-pyridyl group (**32**) slightly decreased activity (IC_{50} = 0.66 and 1.64 μM, respectively), and replacement with the 3-pyridyl group (**33**) was equipotent. The 3-chlorophenyl analogue **31** was equipotent to the 3-methoxyphenyl analogue **21**. The benzyl analogue **34** (IC_{50} = 0.42 μM) showed evidence that an aryl group is not necessary at R^3. Notably, the eosinophil PDE potencies of compounds such as **11**, **14**, **16**−**25**, and **29**−**34** are comparable to that of PDE4 inhibitors which have progressed into clinical development (rolipram, RP 73401, SB 207499, and CDP 840).

With the above SAR in hand we considered whether **11** had any structural overlaps with either rolipram or cAMP. Initial studies using classical molecular models led to a possible similarity between the ethyl and 4-fluorophenyl moieties of **11** and the methoxy and cyclopentoxy moieties of rolipram, respectively (Figure 1). Subsequently, low-energy conformers for each compound were determined using the SYBYL package. Since the molecular shape and atom types of both rolipram and **11** allow for a number of possible overlaps, we chose to characterize the electrostatic field around both compounds by using the GRID suite of programs.[17] This method calculates the steric and electrostatic field of a compound by systematically moving a particle of defined volume and charge around the molecule of interest. These compounds were analyzed with several different GRID probes (CH_2, CO, NH, OH, CO^-, and NH_3). While the CH_2 probe was useful in defining the boundaries of the molecules, the remaining probes indicated areas predisposed to either accepting or donating a hydrogen bond. On the basis of the electrostatic map garnered through the use of the NH_3 probe, we found that the interaction potential around the pyrazolo nitrogens of **11** closely approximates the same potential around the ether oxygens of rolipram. This suggested an overlap of these two compounds based on their interaction potentials as shown in Figure 2. We reasoned that if this hypothesis were correct, it would explain the improved affinity of the cyclopentyl analogues such as compound **21** for PDE4. The overlap of compound **21** with rolipram based on their GRID interaction potential is shown in Figure 3.

To test if the above compounds could inhibit PDE4 in whole cells, selected compounds were evaluated for their ability to elevate intracellular levels of cAMP in the human monocytic U-937 cell line (Table 1). Note that with the exception of the very weak eosinophil PDE inhibitors, **27** and **28**, all analogues were active. This assay was only used as a means to evaluate a compound's ability to penetrate cells and was not used as a tool to develop SAR. It is unclear why some compounds

Table 1. Structure−Activity Relationships of Close-in Analogues of **11** and Inhibition of Eosinophil PDE



compd	R^1	R^2	R^3	formula	analysis[a]	mp (°C)	HEOS PDE IC_{50} (μM) ± SE (n)	U-937 cells cAMP elev EC_{50} (μM) ± SE (n)
11	4-fluorophenyl	ethyl	phenyl	$C_{20}H_{18}FN_3O$	C, H, N	112−5	1.64 ± 0.86 (3)	0.88 ± 0.39 (3)
14	cyclopentyl	ethyl	phenyl	$C_{19}H_{23}N_3O$	C, H, N	68−9	0.16 ± 0.07 (3)	0.07 ± 0.04 (2)
15	4-fluorophenyl	ethyl	4-methoxyphenyl	$C_{21}H_{20}FN_3O_2$	C, H, N	108−9	6.04 ± 1.96 (3)	6.25 ± 1.31 (3)
16	4-fluorophenyl	ethyl	3-methoxyphenyl	$C_{21}H_{20}FN_3O_2$	C, H, N	139−40	0.49 ± 0.29 (4)	
17	4-fluorophenyl	ethyl	2-methoxyphenyl	$C_{21}H_{20}FN_3O_2$	C,[b] H, N	103−4	1.08 ± 0.58 (3)	3.21 (1)
18	4-fluorophenyl	ethyl	4-methylphenyl	$C_{21}H_{20}FN_3O$	C, H, N	134−6	2.24 ± 0.44 (3)	0.50 (1)
19	4-fluorophenyl	ethyl	3-methylphenyl	$C_{21}H_{20}FN_3O$	C, H, N[c]	94−5	0.27 ± 0.08 (3)	0.78 (1)
20	4-fluorophenyl	ethyl	2-methylphenyl	$C_{21}H_{20}FN_3O$	C, H, N	141−2	0.89 ± 0.49 (3)	
21	cyclopentyl	ethyl	3-methoxyphenyl	$C_{20}H_{25}N_3O_2$	C, H, N	63−4	0.034 ± 0.010 (3)	0.33 ± 0.07 (5)
22	cyclopentyl	ethyl	2-methylphenyl	$C_{20}H_{25}N_3O$	C, H, N	130−1	0.44 ± 0.23 (4)	0.33 ± 0.09 (4)
23	cyclohexyl	ethyl	3-methoxyphenyl	$C_{21}H_{27}N_3O_2$	C, H, N		0.048 ± 0.010 (3)	0.98 ± 0.66 (3)
24	cyclobutyl	ethyl	3-methoxyphenyl	$C_{19}H_{23}N_3O_2$	C, H, N[d]		0.15 ± 0.10 (4)	0.52 ± 0.36 (2)
25	3-sulfolanyl	ethyl	3-methoxyphenyl	$C_{19}H_{23}N_3O_4S$	C, H, N[e]		0.46 ± 0.25 (3)	5.27 (1)
26	4-fluorophenyl	methyl	4-methoxyphenyl	$C_{20}H_{18}FN_3O_2$	C, H, N	140−2	15.7 ± 1.9 (3)	
27	4-fluorophenyl	*n*-propyl	4-methoxyphenyl	$C_{22}H_{22}FN_3O_2$	C, H, N	97−8	84.9 ± 10.5 (2)	11% at 1.0 (1)
28	4-fluorophenyl	*n*-butyl	phenyl	$C_{22}H_{22}FN_3O$	C, H, N	95−6	84.7 (1)	13% at 0.1 (1)
29	cyclopentyl	ethyl	2-thiophene	$C_{17}H_{21}N_3OS$	C, H, N	59−60	0.063 ± 0.030 (5)	0.001 ± 0.001 (5)
30	cyclopentyl	ethyl	3-thiophene	$C_{17}H_{21}N_3OS$	(HRMS)[f]		0.66 ± 0.24 (4)	0.036 ± 0.020 (4)
31	cyclopentyl	ethyl	3-chlorophenyl	$C_{19}H_{22}ClN_3O$	C, H, N	72−3	0.039 ± 0.02 (3)	0.041 ± 0.02 (3)
32	cyclopentyl	ethyl	2-pyridyl	$C_{18}H_{22}N_4O$	(HRMS)[f]		1.64 ± 0.17 (3)	
33	cyclopentyl	ethyl	3-pyridyl	$C_{18}H_{22}N_4O$	(HRMS)[f]		0.16 ± 0.02 (3)	0.017 (1)
34	cyclopentyl	ethyl	benzyl	$C_{20}H_{25}N_3O$	C, H, N	35−6	0.42 ± 0.07 (3)	0.036 (1)
rolipram							3.34 ± 0.79 (16)	1.23 ± 0.22 (8)
RP 73401							0.009 ± 0.001 (8)	0.04 ± 0.01 (5)
SB 207499							0.17 ± 0.05 (5)	3.63 ± 2.35 (3)
CDP 840							0.67 ± 0.33 (3)	0.29 ± 0.12 (3)

[a] Unless indicated, all values were within 0.4%. [b] C: calcd, 69.03; found, 69.70. [c] N: calcd, 12.03; found, 11.52. [d] N: calcd, 12.91; found, 12.34. [e] N: calcd, 10.79; found, 9.82. [f] High-resolution mass spectra showed peak for [M + 1].

Figure 2. Overlap of rolipram with **11**. The interaction potential of the rolipram ether oxygens is shown in red, and the interaction potential of the pyrazol nitrogens for **11** is shown in blue. Rolipram and **11** are respectively colored magenta and green.

(**11**, **14**, **18**, **29**, **30**, **33**, and **34**) are more potent in the U-937 cell assay than in the human eosinophil assay.

Two compounds were chosen as representatives to evaluate PDE4 selectivity. As shown in Table 2, **11** and **22** were weak inhibitors of human PDE1 (cardiac ventricle), PDE3 (heart), and PDE5 (corpus cavernosal tissue).

In vivo, **11** and **22** were active in the guinea pig aerosolized antigen-induced airway obstruction assay (AAIAO) (ED_{50} = 4.3 and 2.0 mg/kg, po, respectively)

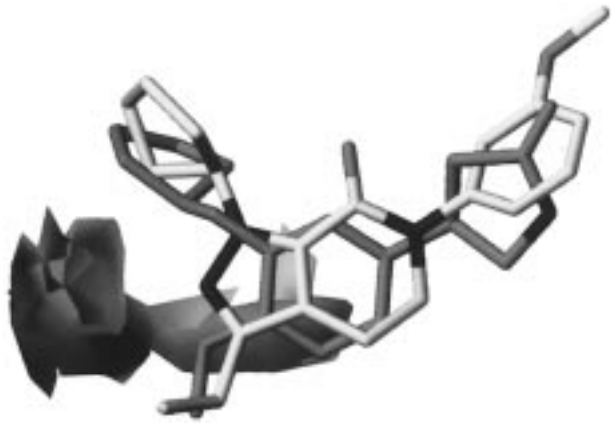


Figure 3. Overlap of rolipram with **21**. The interaction potential of the rolipram ether oxygens is shown in red, and the interaction potential of the pyrazol nitrogens for **21** is shown in orange. Rolipram and **21** are respectively colored magenta and yellow.

Table 2. Inhibitory Activity of **11** and **22** against Other Phoshodiesterases

compd	PDE1	PDE3	PDE5
11	2% at 10 μM	33% at 100 μM	50% at 10 μM 1% at 1 μM
22	8% at 10 μM	9% at 100 μM	21% at 10 μM 7% at 1 μM

at doses comparable to rolipram (ED_{50} 0.6 mg/kg, po) (Table 3). In atopic monkeys challenged with antigen, **22** (10 mg/kg, po) inhibited eosinophil increases by 55%, neutrophil increases by 65%, and reduced BAL levels

Table 3. Effects of **11**, **22**, and Rolipram in the Guinea Pig Aerosolized Antigen Induced Airway Obstruction (AAIAO) Assay and on Acute Cellular and Biochemical Responses to Antigen Challenge in Atopic Monkey

compd	AAIAO assay ED_{50}[a] (mg/kg, po)	monkeys dosed at 10 mg/kg, po		
		% eosinophil inh	% cytokine inh[b]	plasma levels[c]
11	4.3	not tested	not tested	not tested
22	2.0	55.2 ± 8.71	82.1 ± 12.6 (IL-1β)	0.28 ± 0.015 μM
rolipram	0.6	49.4 ± 17.6	84.0 ± 4.9 (IL-8)	0.91 ± 0.15 μM
			33.4 ± 3.3 (TNF)	

[a] ED_{50} based on log linear regression on a minimum of three point dose response curve. Each point is generated by $n = 5$ animals. [b] Data only reported for cytokine levels that were significantly lower after treatment compared to controls. [c] Plasma samples were taken at the time of antigen challenge and evaluated by HPLC.

of IL-1β by 82%; **22** had no statistically significant effect on IL-8 responses, and TNF-α could not be measured in this experiment. Rolipram (10 mg/kg, po) had a similar effect on eosinophil (49% inhibition) and neutrophil increase (62%). However, unlike **22**, rolipram reduced BAL levels of IL-8 and TNF by 84% and 33%, respectively, and had no statistically significant effect on IL-1β. Neither compound reduced IL-6 levels after antigen challenge. Although the implications of differential effects on cytokine responses are currently unclear, it is fair to say that both compounds are capable of inhibiting biochemical responses in addition to chemotactic responses of inflammatory leukocytes.

Finally, ferrets[9] dosed with **22** (1 mg/kg, sc, five animals) showed no adverse effects, whereas five out of five ferrets dosed with rolipram (1 mg/kg, sc) displayed a gagging behavior.

Conclusions

In conclusion, we have discovered a novel PDE4 inhibitor (**11**) by high-throughput file screening. Molecular modeling of **11** overlapped with rolipram provided hypothetical similarities that helped rationalize SAR results. Optimization of substituents at the 1-, 3-, and 6-position of the pyrazolopyridine bicycle of **11** led to CP-220,629 (**22**), which shows potent inhibition of PDE4 activity both in vitro and in vivo, and a reduced emetic liability compared to rolipram.

Experimental Section

Biology. High-Throughput PDE4 Activity. The PDE4 assay[9] was adapted to a 96-well microtiter plate format for high throughput screening. This adaptation required reduced volumes and the use of Millipore MultiScreen plates to provide the equivalent of 96 miniature Affi-Gel 601 columns for separation of [^{3}H]cAMP from the product [^{3}H]AMP. Reaction components were added to 96-well microtiter plates, in sequence (using a Soken SigmaPet 96 liquid dispenser): 5 μL of compound (or 0.3% DMSO), 5 μL of 4X assay buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.5), 5 μL of [^{3}H]cAMP/cAMP (1 μM [^{3}H]cAMP/cAMP), 5 μL of PDE4 enzyme (for blank, enzyme was deactivated by boiling for 10 min). Components were mixed in microtiter plates. Plates were incubated at 37 °C for 20 min. The reaction was stopped by adding 100 μL of hot wash buffer per well (0.1 M *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES), 0.1 M NaCl, pH 8.5). From each well of the reaction plate, 75 μL of the reaction mix was applied to a 200 μL bed volume/well Affi-Gel 601 (boronate affinity gel from Bio-Rad, equilibrated with washing buffer) in a Millipore MultiScreen 0.65 μm membrane-bottomed plates. The unbound [^{3}H]cAMP was washed from the resin with 2.0 mL/well wash buffer, using the Millipore MultiScreen plates in conjuction with a Pall Biosupport Silent Monitor vaccum manifold and a custom 96-port elution buffer reservoir. [^{3}H]AMP was subsequently eluted with 0.25 M acetic acid. The first 300 μL of eluent was discarded. The next 150 μL of eluent was collected in a microtiter plate for quantitation. A 50 μL aliquot of each eluant was spotted on Mylar sheet, allowed to air-dry completely, and ^{3}H counted using a Packard Matrix 96 counter. This method provided the capacity of screening approximately 3000−6000 compounds per week. Each compound was tested at a final concentration of ~40 μM. Potential inhibitors identified in the primary HTS screen were subsequently confirmed using traditional test tube assays to determine IC_{50}'s.

Eosinophil Phosphodiesterase Activity. Human peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA), diluted 1:2 in piperazine-*N*, *N*′-bis-2-ethanesulfonic acid (PIPES) buffer, and then layered over 60% Percoll solution. Gradients were formed by centrifugation for 30 min at 2000 rpm at 4 °C. The remainder of the isolation procedure, which was based on the procedure of Kita et al.,[18] was carried out at 4 °C. The neutrophil/eosinophil layer was collected from the Percoll gradient, and the red blood cells were lysed. Remaining cells were washed in PIPES (1% fetal calf serum), incubated with anti-CD16 microbeads (MACS) for 1 h, and passed over a magnetic column to remove the neutrophils. Eosinophils were collected in the eluate and analyzed for viability by trypan blue and purity by Diff-Quick (Baxter) stain. Eosinophil purity was routinely >98% using this method.

Purified eosinophils were resuspended in 750 μL of PDE lysis buffer (20 mM triethylamine, 1 mM EDTA, 100 μg/mL bacitracin, 2 mM benzamidine, 50 μM leupeptin, 50 μM phenylmethyl sulfonyl fluoride, and 10 μg/mL soybean trypsin inhibitor) and quick frozen in liquid nitrogen. Cells were thawed slowly and sonicated, and disruption was confirmed by Trypan blue stain. Disrupted cells were centrifuged at 105000*g* for 30 min at 4 °C to isolate membranes. Cytosol was decanted, and the membrane was resuspended to 500 μg/mL for use as PDE source in the hydrolysis assay.

Compounds were dissolved in DMSO at 0.01 M and then diluted 1:25 in water to 0.40 mM. This suspension was serially diluted 1:10 in 4% DMSO, for a final DMSO concentration in the assay of 1%.

cAMP hydrolysis was assesed by adding equal volumes of Tris/$MgCl_2$ assay buffer, 4 nM cAMP, test compound, and PDE source to duplicate 12 × 75 mm glass tubes and incubating for 25−30 min in a 37 °C shaking water bath. Reaction was stopped by boiling samples 5 min. Samples were applied to Affi-gel columns (1 mL bed volume) previously equilibrated with 0.25 M acetic acid followed by 0.1 mM HEPES/0.1 mM NaCl wash buffer (pH 8.5). cAMP was washed off column with HEPES/NaCl, and 5′-AMP was eluted with 4 mL of 0.25 M actetic acid. One milliliter of eluate was counted in 3 mL of Ready-Safe for 1 min ([^{3}H]).

Substrate conversion = (cpm positive control × 4)/total activity. Conversion rate must be between 3 and 15% for experiment to be valid.

% inhibition = 1 − (eluted cpm − bkgd cpm/control cpm − bkgd cpm) × 100. IC_{50}s were generated by linear regression of inhibition titer curve (linear portion) and were expressed in μM.

Human U-937 cAMP Elevation Assay. Human U937 cells grown in continuous culture were obtained and centrifuged at 419*g* at 22 °C for 5 min. The supernatant was decanted, and the cell pellet was resuspended in RPMI 1640

cell culture medium plus 2% fetal bovine serum (FBS). Cells were counted and viability was checked using a hemocytometer. An appropriate volume of RPMI 1640 + 2% FBS was added to the cell suspension to make the cell concentration 10^6 cells/mL. A 500 μL aliquot (5×10^5 cells) of the suspension was added to a 12 × 75 mm glass tube containing 5 μL of either DMSO or compound, in duplicate or triplicate. This mixture was allowed to incubate at 37 °C for 15 min. PGE1 (1 μL) was added, and the incubation was allowed to proceed for another 15 min, at which time the tubes were placed in a boiling water bath for 10 min.

Tubes were centrifuged for 10 min at 2930*g*. The samples were either assayed fresh or stored at −20 °C. A cAMP radioimmunoassay kit was used to quantitate cAMP levels. The EC_{50} value of a compound is that concentration of compound which produced 50% of an arbitrary maximal response (e.g., that concentration of cAMP produced by 10 μM rolipram) after subtraction of a baseline cAMP level.

Aerosolized Antigen Induced Airway Obstruction Assay. This assay was used to evaluate the ability of a compound to block the airway obstruction resulting from an aerosolized antigen induced pulmonary anaphylaxis and has been described.[9]

Monkey Allergic Inflammation Model. Using a cross-over design, six atopic, cynomolgus monkeys were treated orally with vehicle (controls, $n = 3$) or drug ($n = 3$). One hour later, each monkey was anesthetized (10 mg/kg Ketamine, 1.0 mg/kg Rompun, intramuscularly) and intubated with a cuffed endotracheal tube. Bronchoalveolar lavage (BAL) was performed using one 15 mL wash of phosphate buffered saline (PBS) delivered through a pediatric fiberoptic bronchoscope (Olympus, model BF 3C20, Japan) inserted through the endotracheal tube and wedged into a third to fifth generation bronchus. Lavage fluid was gently aspirated and collected in a syringe. After BAL was complete, each animal received a 2 min exposure to a concentration of *Ascaris suum* aerosol which doubled respiratory system resistance (Rrs) in previous experiments. Each monkey was returned to its cage, and 4 h later, a second BAL was performed, using 15 mL of PBS, on the opposite side of the lung. One week after the first trial, control and treated monkeys were reversed and the experiment was repeated. This design yielded an $n = 6$ with each monkey serving as its own control. Compound **22** was dissolved in 2% Tween80 and delivered orally.

To determine the percent composition of each leukocyte type, two slides from each monkey BAL sample were obtained by centrifuging 2 × 150 μL of the lavage fluid for 2 min at 500 rpm in a Cytospin centrifuge (Shandon Instruments, Sewickly, PA). The slides were stained in Diff-Quick for differential cell count. Two hundred leukocytes were counted on each slide. The percent composition of each cell type was averaged for the two slides. Total leukocyte count per milliliter of BAL fluid was determined by diluting 20 μL of sample in 20 mL of Isoton (Coulter Diagnostics, Hialeah FL), adding 3 drops of Zapoglobin (Coulter Diagnostics) to lyse erythrocytes, and reading the sample using a Coulter Counter (Coulter Electronics, Hialeah, FL).

After total and differential leukocyte counts were determined, BAL samples were centrifuged at 1800 rpm for 20 min. The supernatant was collected in Centriprep-3 concentrator tubes (Amicon, Inc. Beverly, MA) and centrifuged at 3000 rpm until a 2 mL concentrate remained (approximately 95 min, then 45 min). Commercially available ELISA kits for human IL-1β, IL-6, IL-8, and TNFα (R & D Systems, Minneapolis, MN) were used to analyze the concentrated BAL sample for the levels of these cytokines.

Chemistry. General Methods. Anhydrous THF and ether were distilled over Na under a N_2 atmosphere. Other solvents and reagents were of reagent grade and were used as supplied by the manufacturer. All reactions were run under a N_2 atmosphere. Organic extracts were routinely dried over anhydrous Na_2SO_4. Concentration refers to rotory evaporation under reduced pressure. Chromatography refers to "flash chromatography" on EM Science silica gel (40−63 μm). Melting points were determined using a Mel-Temp II capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Schwarzkopf Microanalytical Lab., Woodside, NY. Reference compounds were prepared using modified literature procedures.[19]

General Procedure A. *N*-Arylation of 2-Pyrolidinone. 1-(2-Methoxyphenyl)-2-pyrrolidinone (1d). A mixture of 2-pyrrolidinone (15.0 g, 176 mmol), 2-iodoanisole (7.6 mL, 59 mmol), copper powder (7.5 g, 117 mmol), and K_2CO_3 (8.1 g, 59 mmol) was stirred under nitrogen at 150 °C. After 18 h the reaction mixture was allowed to cool to room temperature and was suspended in THF (50 mL). The mixture was filtered through Celite, concentrated to a brown oil, and chromatographed (1:1 ethyl acetate/hexane) to give 9.3 g (82%) of a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (pentet, 2H), 2.55 (t, $J = 8.0$ Hz, 2H), 3.75 (t, $J = 6.0$ Hz, 2H), 3.82 (s, 3H), 6.93−7.02 (m, 2H), 7.25−7.30 (m, 2H); MS *m*/*z* 191.

General Procedure B. Grignard Reaction with *N*-Aryl-2-pyrrolidinone. *N*-(3-Methylphenyl)-6-aminohexan-3-one (2f). To a stirred suspension of Mg turnings (1.9 g, 79 mmol) in 30 mL of anhydrous ether was added dropwise bromoethane (5.9 mL, 79 mmol). A mild reflux was initiated after ∼1 mL was added. After all of the Mg was consumed, the reaction mixture was cooled to 0 °C, and 1-(3-methylphenyl)-2-pyrrolidinone (8.7 g, 50 mmol) was added at once. After being warmed to room temperature and stirred for 2 h, the reaction mixture was poured over ice and extracted with ethyl acetate. The combined organics were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to afford 8.8 g (86%) of an unstable white solid which was used immediately in the next step without purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00−1.10 (m, 3H), 1.83−1.96 (m, 2H), 2.22 (s, 3H), 2.39−2.46 (m, 2H), 2.50−2.58 (m, 2H), 3.06−3.15 (m, 2H), 3.59 (broad s, 1H), 6.35−6.56 (m, 2H), 6.99−7.09 (m, 1H), 7.22−7.28 (m, 1H).

***N*-Phenyl-6-aminohexan-3-one (2a):** no purification; unstable pale yellow solid; 98% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, $J = 7.3$ Hz, 3H), 1.84−1.94 (m, 2H), 2.45 (q, $J = 7.3$ Hz, 2H), 2.56 (t, $J = 7.2$ Hz, 2H), 3.15 (t, $J = 6.8$ Hz, 2H), 3.66 (broad s, 1H), 6.58−6.72 (m, 3H), 7.16−7.23 (m, 2H).

***N*-(4-Methoxyphenyl)-6-aminohexan-3-one (2b):** recrystallized from isopropyl ether; unstable off-white solid; 90% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (t, $J = 7.3$ Hz, 3H), 1.84−1.95 (m, 2H), 2.38−2.46 (m, 2H), 2.54 (t, $J = 7.0$ Hz, 2H), 3.07 (t, $J = 6.0$ Hz, 2H), 3.74 (s, 3H), 6.58 (d, $J = 6.0$ Hz, 2H), 6.67 (d, $J = 6.0$ Hz, 2H).

***N*-(3-Methoxyphenyl)-6-aminohexan-3-one (2c):** no purification; unstable pale yellow solid; 93% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, $J = 7.3$ Hz, 3H), 1.84−1.93 (m, 2H), 2.40−2.49 (m, 2H), 2.55 (t, $J = 7.0$ Hz, 2H), 3.10 (t, $J = 7.0$ Hz, 2H), 3.75 (s, 3H), 6.12−6.27 (m, 3H), 7.02 (d, $J = 7.5$ Hz, 1H).

***N*-(2-Methoxyphenyl)-6-aminohexan-3-one (2d):** no purification; unstable yellow oil; 68% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, $J = 7.3$ Hz, 3H), 1.90−1.97 (m, 2H), 2.42 (q, $J = 7.3$ Hz, 2H), 2.53 (t, $J = 7.2$ Hz, 2H), 3.13 (t, $J = 6.8$ Hz, 2H), 3.83 (s, 3H), 4.19 (broad s, 1H), 6.58−6.88 (m, 3H), 7.23−7.29 (m, 1H).

***N*-(4-Methylphenyl)-6-aminohexan-3-one (2e):** no purification; unstable off-white solid; 96% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, $J = 7.3$ Hz, 3H), 1.84−1.96 (m, 2H), 2.25 (s, 3H), 2.49−2.58 (m, 2H), 2.55 (t, $J = 7.0$ Hz, 2H), 3.11 (t, $J = 7.0$ Hz, 2H), 3.53 (broad s, 1H), 6.54 (d, $J = 8.0$ Hz, 2H), 6.98 (d, $J = 8.0$ Hz, 2H).

***N*-(2-Methylphenyl)-6-aminohexan-3-one (2g):** no purification; unstable yellow oil; 53% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, $J = 7.3$ Hz, 3H), 1.91−2.03 (m, 2H), 2.14 (s, 3H), 2.42−2.50 (m, 2H), 2.59 (t, $J = 7.5$ Hz, 2H), 3.18 (t, $J = 8.0$ Hz, 2H), 6.56−6.68 (m, 2H), 7.03−7.18 (m, 2H).

***N*-(4-Methoxyphenyl)-5-aminopentan-2-one (3b):** no purification; unstable pale yellow oil; 99% yield; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.88 (m, 2H), 2.13 (s, 3H), 2.56 (t, $J = 8.0$ Hz, 2H), 3.07 (t, $J = 8.0$ Hz, 2H), 3.74 (s, 3H), 6.56 (d, $J = 9.0$ Hz, 2H), 6.78 (d, $J = 9.0$ Hz, 2H).

***N*-(4-Methoxyphenyl)-7-aminoheptan-4-one (4b):** no purification; unstable pale green oil; 71% yield; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H), 1.62 (m, 2H), 1.88 (m, 2H), 2.40 (t, J = 8.0 Hz, 2H), 2.53 (t, J = 8.0 Hz, 2H), 3.08 (t, J = 7.5 Hz, 2H), 3.74 (s, 3H), 6.58 (d, J = 7.5 Hz, 2H), 6.79 (d, J = 7.5 Hz, 2H).

***N*-Phenyl-8-aminooctan-5-one (5a):** recrystallized from isopropyl ether; unstable white crystals; 54% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.8 Hz, 3H), 1.25−1.38 (m, 2H), 1.52−1.64 (m, 2H), 1.88−1.96 (m, 2H), 2.42 (t, J = 7.0 Hz, 2H), 2.55 (t, J = 7.0 Hz, 2H), 3.14 (t, J = 7.0 Hz, 2H), 3.60 (broad s, 1H), 6.59−6.71 (m, 3H), 7.14−7.20 (m, 2H).

General Procedure C. Acylation with Ethyl Oxalyl Chloride Followed by Dieckmann Condensation. 3-Hydroxy-2-oxo-4-propionyl-1-(3-methylphenyl)-1,2,5,6-tetrahydropyridine (6f). *N*-(3-Methylphenyl)-6-aminohexan-3-one (**2f**) (8.8 g, 43 mmol) was dispersed in a mixture of 40 mL of benzene and 86 mL of 1 N NaOH, and with vigorous mechanical stirring ethyl oxalyl chloride (7.2 mL, 64 mmol) was added. After the mixture was stirred at reflux for 1.5 h, the layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organics were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give an amber oil. The oil was dissolved in 20 mL of anhydrous ethanol and treated with a solution of NaOMe in MeOH (prepared from the careful addition of Na (1.0 g) to 10 mL of anhydrous MeOH). After being stirred at reflux over 1.5 h, the mixture was concentrated and 100 mL of water was added. The resulting mixture was acidified to pH 3 with 6 N HCl, and the dull yellow precipitate was filtered and washed with water. Recrystallization from 75 mL of isopropyl ether afforded 6.8 g (61%) of pale yellow crystals: mp 115−116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, J = 7.2 Hz, 3H), 2.37 (s, 3H), 2.74−2.82 (m, 4H), 3.85 (t, J = 6.8 Hz, 2H), 7.08−7.14 (m, 3H), 7.30 (t, J = 7.7 Hz, 1H); MS m/z 259.

3-Hydroxy-2-oxo-1-phenyl-4-propionyl-1,2,5,6-tetrahydropyridine (6a): pale yellow solid; 72% yield from **2a**; mp 141−3 °C (lit.[12] 144−6 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.2 Hz, 3H), 2.74−2.81 (m, 4H), 3.82 (t, J = 6.81 Hz, 2H), 7.24−7.44 (m, 5H), 11.72 (broad s, 1H). Anal. ($C_{14}H_{15}NO_3$) C, H, N.

3-Hydroxy-1-(4-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (6b): recrystallized from 1:9 ethyl acetate/ether; pale yellow crystals; 65% yield from **2b**; mp 121−2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, J = 7.2 Hz, 3H), 2.75−2.82 (m, 4H), 3.82 (t, J = 6.8 Hz, 2H), 3.80 (s, 3H), 6.93 (d, J = 9.0 Hz, 2H), 7.22 (d, J = 9.0 Hz, 2H); MS m/z 276.

3-Hydroxy-1-(3-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (6c): recrystallized from isopropyl ether; white crystals; 90% yield from **2c**; mp 130−2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.2 Hz, 3H), 2.74−2.81 (m, 4H), 3.81 (s, 3H), 3.86 (t, J = 6.8 Hz, 2H), 6.81−6.90 (m, 3H), 7.29−7.34 (m, 1H); MS m/z 275. Anal. ($C_{15}H_{17}NO_4$) C, H, N.

3-Hydroxy-1-(2-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (6d): recrystallized from isopropyl ether; white crystals; 49% yield from **2d**; mp 119−20 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.2 Hz, 3H), 2.74−2.81 (m, 4H), 3.65−3.72 (m, 2H), 3.84 (s, 3H), 6.97−7.02 (m, 2H), 7.22−7.35 (m, 2H); MS m/z 275.

3-Hydroxy-2-oxo-4-propionyl-1-(4-methylphenyl)-1,2,5,6-tetrahydropyridine (6e): recrystallized from isopropyl ether; golden brown solid; 54% yield from **2e**; mp 110−12 °C (lit.[12] mp 116−8 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, J = 7.2 Hz, 3H), 2.35 (s, 3H), 2.75−2.82 (m, 4H), 3.84 (t, J = 6.9 Hz, 2H), 7.21 (m, 4H); MS m/z 260.

3-Hydroxy-2-oxo-4-propionyl-1-(2-methylphenyl)-1,2,5,6-tetrahydropyridine (6g): pale yellow amorphous solid; 65% yield from **2g**; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12−1.23 (m, 3H), 2.25 (s, 3H), 2.39−2.50 (m, 2H), 2.74−2.85 (m, 2H), 3.53−3.91 (m, 2H), 7.07−7.30 (m, 4H); MS m/z 259.

4-Acetyl-3-hydroxy-1-(4-methoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyridine (7b): yellow amorphous solid; 87% yield from **3b**; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.76 (t, J = 7.2 Hz, 2H), 3.78−3.84 (m, 5H), 6.93 (d, J = 8.9 Hz, 2H), 7.23 (d, J = 8.9 Hz, 2H); MS m/z 262.

4-Butyryl-3-hydroxy-1-(4-methoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyridine (8b): recrystallized from 1:9 ethyl acetate/ether; yellow needles; 13% yield from **4b** (not optimized); mp 125−6° C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3H), 1.71 (q, J = 7.2 Hz, 2H), 2.68−2.80 (m, 4H), 3.80 (s, 3H), 3.79−3.86 (m, 2H), 6.93 (d, J = 9.0 Hz, 2H), 7.24 (d, J = 9.0 Hz, 2H); MS m/z 290.

3-Hydroxy-2-oxo-1-phenyl-4-valeryl-1,2,5,6-tetrahydropyridine (9a): white solid; 44% yield from **5a**; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.3 Hz, 3H), 1.32−1.46 (m, 2H), 1.60−1.71 (m, 2H), 2.72−2.81 (m, 4H), 3.89 (t, J = 6.5 Hz, 2H), 7.22−7.45 (m, 5H), 11.97 (broad s, 1H); MS m/z 273.

General Procedure D. Esterification of Vinylogous Acids 6a−g. 3-Methoxy-1-(4-methylphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (10e). A solution of **6e** (5.9 g, 23 mmol) and 3-methyl-1-*p*-methylphenyltriazene (5.1 g, 34 mmol) in 1,2-dichloroethane (25 mL) was heated at reflux for 45 min. The mixture was allowed to cool to room temperature and was poured into water and acidified with 6 N HCl. The aqueous layer was extracted 3 times with CH_2Cl_2, and the combined organics were washed with 1 N HCl followed by water and brine, dried over $MgSO_4$, filtered, and concentrated to give 6.2 g (quantitative yield) of a brown oil. Note that the aqueous layer contained *p*-toluidine as a byproduct and was isolated for disposal. No impurities were detected by TLC or ^{1}H NMR and the product was used without purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (t, J = 7.2 Hz, 3H), 2.34 (s, 3H), 2.71 (t, J = 6.7 Hz, 2H), 2.93 (q, J = 7.2 Hz, 2H), 3.77 (t, J = 6.8 Hz, 2H), 3.94 (s, 3H), 7.20 (s, 4H); MS m/z 273.

3-Methoxy-2-oxo-1-phenyl-4-propionyl-1,2,5,6-tetrahydropyridine (10a): dark brown oil; quantitative yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, J = 7.2 Hz, 3H), 2.73 (t, J = 6.7 Hz, 2H), 2.93 (q, J = 7.2 Hz, 2H), 3.81 (t, J = 6.7 Hz, 2H), 3.94 (s, 3H), 7.23−7.62 (m, 5H); MS m/z 260.

3-Methoxy-1-(4-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (10b): brown solid; quantitative yield; mp 81−2° C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, J = 7.2 Hz, 3H), 2.71 (t, J = 6.8 Hz, 2H), 2.93 (q, J = 7.2 Hz, 2H), 3.75 (t, J = 6.8 Hz, 2H), 3.81 (s, 3H), 3.94 (s, 3H), 6.91 (d, J = 9.0 Hz, 2H), 7.23 (d, J = 9.0 Hz, 2H). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

3-Methoxy-1-(3-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (10c): red brown solid; 95% yield; mp 54−5° C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, J = 7.2 Hz, 3H), 2.72 (t, J = 6.7 Hz, 2H), 2.94 (q, J = 7.2 Hz, 2H), 3.79 (t, J = 6.7 Hz, 2H), 3.81 (s, 3H), 3.94 (s, 3H), 6.78−6.92 (m, 3H), 7.27−7.33 (m, 1H), MS m/z 290.

3-Methoxy-1-(2-methoxyphenyl)-2-oxo-4-propionyl-1,2,5,6-tetrahydropyridine (10d): brown oil; quantitative yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (t, J = 7.2 Hz, 3H), 2.74 (t, J = 6.7 Hz, 2H), 2.96 (q, J = 7.2 Hz, 2H), 3.59−3.67 (m, 2H), 3.89 (s, 3H), 3.97 (s, 3H), 6.96−7.02 (m, 2H), 7.20−7.33 (m, 2H); MS m/z 289.

3-Methoxy-2-oxo-4-propionyl-1-(3-methylphenyl)-1,2,5,6-tetrahydropyridine (10f): brown oil; 97% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, J = 7.2 Hz, 3H), 2.36 (s, 3H), 2.71 (t, J = 6.7 Hz, 2H), 2.93 (q, J = 7.2 Hz, 2H), 3.78 (t, J = 6.7 Hz, 2H), 3.94 (s, 3H), 7.09−7.37 (m, 4H); MS m/z 273.

3-Methoxy-2-oxo-4-propionyl-1-(2-methylphenyl)-1,2,5,6-tetrahydropyridine (10g): brown oil; 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (t, J = 7.2 Hz, 3H), 2.25 (s, 3H), 2.76 (t, J = 6.7 Hz, 2H), 2.95 (q, J = 7.2 Hz, 2H), 3.68−3.70 (m, 2H), 3.97 (s, 3H), 7.10−7.30 (m, 4H); MS m/z 273.

General Procedure E. Condensation of Vinylogous Acids 6−9 or Ester 10 with an Alkyl- or Arylhydrazine. 3-Ethyl-1-(4-fluorophenyl)-6-phenyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (11) and 3-Ethyl-2-(4-fluorophenyl)-6-phenyl-7-oxo-4,5,6,7-tetrahydro-2*H*-pyrazolo[3,4-*c*]pyridine (35). A solution of NaOMe in MeOH was prepared by adding Na (127 mg, 5.5 mmol) to anhydrous MeOH (3 mL). To this was added a solution of compound **6a**

(2.7 g, 11.0 mmol) in anhydrous EtOH (90 mL) followed by solid 4-fluorophenylhydrazine hydrochloride (1.95 g, 12.0 mmol). After being stirred at reflux for 20 h the mixture was cooled to room temperature, concentrated and purified by chromatography (2:3 ether/hexane) to provide 1.9 g (54%) of a white solid (**11**): mp 112−5 °C (lit.[12] mp 106−8 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, J = 7.6 Hz, 3H), 2.74 (q, J = 7.6 Hz, 2H), 2.97 (t, J = 6.7 Hz, 2H), 4.11 (t, J = 6.7 Hz, 2H), 7.02−7.57 (m, 9H). Anal. ($C_{20}H_{18}FN_3O$) C, H, N.

Further elution of the column with 1:3 ethyl acetate/hexane provided a white solid which was recrystallized from isopropyl ether to give 0.5 g (14%) of a white crystalline solid (**35**): mp 199−200 °C (lit.[12] 208−210 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (t, J = 7.6 Hz, 3H), 2.69 (q, J = 7.6 Hz, 2H), 2.97 (t, J = 6.5 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 7.14−7.47 (m, 9H). Anal. ($C_{20}H_{18}FN_3O$) C, H, N.

1-Cyclopentyl-3-ethyl-7-oxo-6-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (14) and 2-Cyclopentyl-3-ethyl-7-oxo-6-phenyl-4,5,6,7-tetrahydro-2*H*-pyrazolo[3,4-*c*]pyridine (36): using general procedure E, upon cooling a precipitate formed from the reaction mixture and was filtered and washed with hexane to give a white crystalline solid (**36**); 28% yield; mp 145−7 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (t, J = 7.7 Hz, 3H), 1.63−2.30 (m, 8H), 2.69 (q, J = 7.6 Hz, 2H), 2.86 (t, J = 6.5 Hz, 2H), 3.98 (t, J = 6.5 Hz, 2H), 4.59 (pentet, J = 7.9 Hz, 1H), 7.22−7.44 (m, 5H).

The filtrate was purified by chromatography (1:1 ethyl acetate/hexane); white crystalline solid (**14**); 52% yield; mp 68−9 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (t, J = 7.7 Hz, 3H), 1.59−2.17 (m, 8H), 2.69 (q, J = 7.6 Hz, 2H), 2.89 (t, J = 6.6 Hz, 2H), 4.00 (t, J = 6.7 Hz, 2H), 5.69 (pentet, J = 7.9 Hz, 1H), 7.22−7.44 (m, 5H). Anal. ($C_{19}H_{23}N_3O$) C, H, N.

3-Ethyl-1-(4-fluorophenyl)-6-(3-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (16): purified by chromatography (1:1 ethyl acetate/hexane) followed by recrystallization from ethyl acetate/pentane; white crystals; 68% yield; mp 139−40 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, 7.8 Hz, 3H), 2.74 (q, J = 7.6 Hz, 2H), 2.96 (t, J = 6.7 Hz, 2H), 3.78 (s, 3H), 4.09 (t, J = 6.7 Hz, 2H), 6.76−7.56 (m, 8H). Anal. ($C_{21}H_{20}FN_3O_2$) C, H, N.

3-Ethyl-1-(4-fluorophenyl)-6-(2-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (17): purified by chromatography (1:3 ethyl acetate/hexane) followed by recrystallization from ethyl acetate/pentane; pale yellow crystalline solid; 31% yield; mp 103−4 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, J = 7.7 Hz, 3H), 2.74 (q, J = 7.6 Hz, 2H), 2.95 (t, J = 6.7 Hz, 2H), 3.83 (s, 3H), 3.80−4.25 (m, 2H), 6.94−7.59 (m, 8H). Anal. ($C_{21}H_{20}FN_3O_2$) H, N; C: calcd, 69.03; found, 69.70.

3-Ethyl-1-(4-fluorophenyl)-6-(4-methylphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (18): purified by chromatography (1:3 ethyl acetate/hexane); yellow solid; 23% yield; mp 134−6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, J = 7.6 Hz, 3H), 2.33 (s, 3H), 2.73 (q, J = 7.6 Hz, 2H), 2.95 (t, J = 6.7 Hz, 2H), 4.07 (t, J = 6.7 Hz, 2H), 7.02−7.08 (m, 2H), 7.18 (s, 4H), 7.50−7.55 (m, 2H). Anal. ($C_{21}H_{20}FN_3O$) C, H, N.

3-Ethyl-1-(4-fluorophenyl)-6-(3-methylphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (19): purified by chromatography (1:3 ether/hexane) followed by recrystallization from ether/pentane; yellow solid; 52% yield; mp 94−5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, J = 7.6 Hz, 3H), 2.34 (s, 3H), 2.74 (q, J = 7.6 Hz, 2H), 2.96 (t, J = 6.7 Hz, 2H), 4.08 (t, J = 6.7 Hz, 2H), 7.03−7.57 (m, 8H). Anal. ($C_{21}H_{20}FN_3O$) C, H; N: calcd, 12.02; found, 11.52.

3-Ethyl-1-(4-fluorophenyl)-6-(2-methylphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (20): purified by chromatography (1:4 ethyl acetate/hexane) followed by recrystallization from isopropyl ether; pale orange crystals; 33% yield; mp 141−2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, J = 7.7 Hz, 3H), 2.26 (s, 3H), 2.75 (q, J = 7.6 Hz, 2H), 2.88−3.08 (m, 2H), 3.80−3.86 (m, 1H), 4.02−4.11 (m, 1H), 7.02−7.59 (m, 8H). Anal. ($C_{21}H_{20}FN_3O$) C, H, N.

1-(4-Fluorophenyl)-6-(4-methoxyphenyl)-3-methyl-7-oxo-4,5,6, 7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (26): purified by chromatography (1:3 ethyl acetate/hexane) followed by recrystallization from ethyl acetate/petroleum ether (bp range 35−60 °C); light brown solid; 29% yield; mp 140−2° C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.90 (t, J = 6.7 Hz, 2H), 3.79 (s, 3H), 4.04 (t, J = 6.7 Hz, 2H), 6.85−7.55 (m, 8H). Anal. ($C_{20}H_{18}FN_3O_2$) C, H, N.

1-(4-Fluorophenyl)-6-(4-methoxyphenyl)-7-oxo-3-propyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (27): purified by chromatography (1:1 ethyl acetate/hexane) followed by recrystallization from ethyl acetate/pet ether (bp range 35−60 °C); off-white solid; 30% yield; mp 97−8 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.01 (t, J = 7.3 Hz, 3H), 1.66−1.81 (m, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.94 (t, J = 6.7 Hz, 2H), 3.79 (s, 3H), 4.04 (t, J = 6.7 Hz, 2H), 6.86−7.57 (m, 8H). Anal. ($C_{22}H_{22}FN_3O_2$) C, H, N.

3-Butyl-1-(4-fluorophenyl)-7-oxo-6-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (28): purified by chromatography (1:3 ether/hexane) followed by recrystallization from petroleum ether; white needles; 44% yield; mp 95−6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.3 Hz, 3H), 1.39−1.47 (m, 2H), 1.64−1.74 (m, 2H), 2.70 (t, J = 7.7 Hz, 2H), 2.96 (t, J = 6.6 Hz, 2H), 4.10 (t, J = 6.6 Hz, 2H), 7.02−7.09 (m, 2H), 7.20−7.40 (m, 5H), 7.50−7.55 (m, 2H). Anal. ($C_{22}H_{22}FN_3O$) C, H, N.

General Procedure F. Fusion of 10 with Alkyl or Aryl Hydrazines. 1-Cyclopentyl-3-ethyl-6-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (12). A 500 mL round-bottom flask was charged with compound **10b** (35.51 g, 122.7 mmol), anhydrous THF (200 mL), and cyclopentylhydrazine hydrochloride (20.13 g, 147.3 mmol). The mixture was stirred with heating until homogeneous, and the THF was then removed by distillation. The mixture was then purged with N_2 to blow off HCl gas (as evidenced by holding moistened pH paper in fume trail). The resulting dark orange oil was fused at 120 °C. After 2 h the oil was allowed to cool to ambient temperature at which time it solidified to a brown solid lump. Purification by chromatography (1:3 ethyl acetate/hexane) provided 38.9 g (93%) of a white solid: mp 103−4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.7 Hz, 3H), 1.59−1.63 (m, 2H), 1.85−1.91 (m, 2H), 2.01−2.07 (m, 4H), 2.64 (q, J = 7.7 Hz, 2H), 2.85 (t, J = 6.7 Hz, 2H), 3.80 (s, 3H), 3.92 (t, J = 6.7 Hz, 2H), 5.65 (pentet, J = 7.9 Hz, 1H), 6.92 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H). Anal. ($C_{20}H_{25}N_3O_2$) C, H, N.

3-Ethyl-1-(4-fluorophenyl)-6-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (15): purified by chromatography (1:4 ethyl acetate/hexane) followed by recrystallization from ether/pentane; white needles; 80% yield; mp 108−9° C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, J = 7.6 Hz, 3H), 2.75 (q, J = 7.6 Hz, 2H), 2.96 (t, J = 6.7 Hz, 2H), 3.79 (s, 3H), 4.05 (t, J = 6.7 Hz, 2H), 6.89 (d, J = 9.0 Hz, 2H), 7.06 (t, J = 8.7 Hz, 2H), 7.21 (d, J = 9.0 Hz, 2H), 7.53 (dd, J = 4.9 and 9.0 Hz, 2H). Anal. ($C_{21}H_{20}FN_3O_2$) C, H, N.

1-Cyclopentyl-3-ethyl-6-(3-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (21): purified by chromatography (1:3 ethyl acetate/hexane) followed by recrystallization from ether/pentane; white crystals; 71% yield; mp 63−4 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J = 7.6 Hz, 3H), 1.61−2.11 (m, 8H), 2.67 (q, J = 7.6 Hz, 2H), 2.87 (t, J = 6.7 Hz, 2H), 3.82 (s, 3H), 3.97 (t, J = 6.7 Hz, 2H), 5.61−5.71 (pentet, 1H), 6.79−7.34 (m, 4H). Anal. ($C_{20}H_{25}N_3O_2$) C, H, N.

1-Cyclopentyl-3-ethyl-6-(2-methylphenyl)-7-oxo-4,5,6,7- tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (22): purified by chromatography (1:9 ethyl acetate/hexane) followed by recrystallization from ether/pentane; white crystals; 41% yield; mp 134−5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.6 Hz, 3H), 1.61−2.10 (m, 8H), 2.28 (s, 3H), 2.67 (q, J = 7.6 Hz, 2H), 2.79−3.00 (m, 2H), 3.69−3.77 (m, 1H), 3.91−4.00 (m, 1H), 5.59−5.70 (pentet, 1H), 7.17−7.31 (m, 4H). Anal. ($C_{20}H_{25}N_3O$) C, H, N.

1-Cyclohexyl-3-ethyl-6-(3-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (23): purified by

chromatography (1:1 ethyl acetate/hexane); 48% yield; colorless oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (t, J = 7.8 Hz, 3H), 1.25−2.05 (m, 10H), 2.70 (q, J = 7.6 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H), 3.80 (s, 3H), 3.95 (t, J = 7.2 Hz, 2H), 5.05−5.20 (m, 1H), 6.75−7.33 (m, 4H). Anal. ($C_{21}H_{27}N_3O_2$) C, H, N.

1-Cyclobutyl-3-ethyl-6-(3-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (24): purified by chromatography (1:4 ethyl acetate/hexane); 47% yield; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.7 Hz, 3H), 1.69−2.04 (m, 2H), 2.33−2.44 (m, 2H), 2.69 (q, J = 7.6 Hz, 2H), 2.60−2.73 (m, 2H), 2.87 (t, J = 6.7 Hz, 2H), 3.82 (s, 3H), 3.96 (t, J = 6.7 Hz, 2H), 5.73−5.79 (m, 1H), 6.79−7.33 (m, 4H). Anal. ($C_{19}H_{23}N_3O_2$) C, H; N: calcd, 12.91; found, 12.34.

3-Ethyl-6-(3-methoxyphenyl)-7-oxo-1-(3-sulfolanyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (25): purified by chromatography (1:3 ethyl acetate/hexane); 40% yield; pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.6 Hz, 3H), 2.60−2.77 (m, 4H), 2.89 (t, J = 6.7 Hz, 2H), 3.07−3.18 (m, 1H), 3.46−3.74 (m, 3H), 3.82 (s, 3H), 4.00 (t, J = 6.6 Hz, 2H), 6.07−6.18 (m, 1H), 6.70−6.91 (m, 3H), 7.30−7.37 (m, 1H). Anal. ($C_{19}H_{23}N_3O_4S$) C, H; N: calcd, 10.79; found, 9.82.

General Procedure G. Deprotection of the Lactam Nitrogen. 1-Cyclopentyl-3-ethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (13). A 2 L flask was charged with compound **12** (30.85 g, 90.0 mmol) and CH_3CN (750 mL). The pale yellow solution was cooled to 0 °C, and a solution of ammonium cerium nitrate (150 g) in water (500 mL) was slowly added over 15 min. The solution turned red/brown during the addition. After 1 h of stirring, the solution was diluted with cold water (500 mL) and extracted 4× with cold ethyl acetate. The combined organics were washed 2× with cold 50% saturated $NaHCO_3$ at which time an emulsion formed. The emulsion was broken up by adding solid NaCl and was then washed with 10% $NaSO_3$ and brine, dried over Na_2SO_4, and concentrated to a dark brown oil. Purification by chromatography (gradient of 1:1 ethyl acetate/hexane to neat ethyl acetate) provided 11.6 g (55%) of a yellow crystalline solid: mp 145−6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, J = 7.6 Hz, 3H), 1.62−1.71 (m, 2H), 1.83−1.87 (m, 2H), 1.88−2.12 (m, 4H), 2.62 (q, J = 7.6 Hz, 2H), 2.72 (t, J = 6.8 Hz, 2H), 3.51 (dt, J = 2.8 and 6.8 Hz, 2H), 5.61 (pentet, J = 7.8 Hz, 1H), 5.66 (broad s, 1H). Anal. ($C_{13}H_{19}N_3O$) C, H, N.

General Procedure H. Arylation of 13. 1-Cyclopentyl-3-ethyl-7-oxo-6-(2-thiophene)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (29). A mixture of compound **13** (0.15 g, 0.64 mmol), copper powder (82 mg, 1.29 mmol), 2-iodothiophene (0.135 mg, 0.64 mmol), and K_2CO_3 (89 mg, 0.64 mmol) were combined in a vial and stirred at 150 °C. After 18 h the mixture was allowed to cool to ambient temperature and was purified by chromatography (1:3 ether/hexane) followed by recrystallization from pentane to give 157 mg (49%) of white needles: mp 59−60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J = 7.6 Hz, 3H), 1.66−2.11 (m, 8H), 2.66 (q, J = 7.6 Hz, 2H), 2.92 (t, J = 6.8 Hz, 2H), 4.14 (t, J = 6.8 Hz, 2H), 5.68 (pentet, J = 7.8 Hz, 1H), 6.77 (dd, J = 1.4 and 4.0 Hz, 1H), 6.93 (dd, J = 3.8 and 5.6 Hz, 1H), 7.02 (dd, J = 1.3 and 5.6 Hz, 1H). Anal. ($C_{17}H_{21}N_3OS$) C, H, N.

1-Cyclopentyl-3-ethyl-7-oxo-6-(3-thiophene)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (30): purified by chromatography (1:9 ethyl acetate/hexane); pale yellow oil; 50% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.6 Hz, 3H), 1.59−2.11 (m, 8H), 2.66 (q, J = 7.6 Hz, 2H), 2.87 (t, J = 6.7 Hz, 2H), 4.03 (t, J = 6.7 Hz, 2H), 5.67 (pentet, J = 7.8 Hz, 1H), 7.17 (dd, J = 1.6 and 3.0 Hz, 1H), 7.26−7.31 (m, 2H); HRMS calcd for $C_{17}H_{22}N_3OS$ 316.1483, found 316.1475.

6-(3-Chlorophenyl)-1-cyclopentyl-3-ethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (31): purified by chromatography (1:19 ethyl acetate/hexane) followed by recrystallization from ether/pentane; colorless crystals; 52% yield; mp 71−2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J = 7.6 Hz, 3H), 1.55−2.10 (m, 8H), 2.65 (q, J = 7.6 Hz, 2H), 2.88 (t, J = 6.6 Hz, 2H), 3.97 (t, J = 6.6 Hz, 2H), 5.62 (pentet, J = 7.8 Hz, 1H), 7.21−7.35 (m, 4H). Anal. ($C_{19}H_{22}ClN_3O$) C, H, N.

1-Cyclopentyl-3-ethyl-7-oxo-6-(2-pyridyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (32): purified by chromatography (1:9 ethyl acetate/hexane); gold oil; 79% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.6 Hz, 3H), 1.61−2.11 (m, 8H), 2.66 (q, J = 7.6 Hz, 2H), 2.84 (t, J = 6.6 Hz, 2H), 4.29 (t, J = 6.6 Hz, 2H), 5.64 (pentet, J = 7.8 Hz, 1H), 7.09 (t, J = 6.1 Hz, 1H), 7.66−7.80 (m, 2H), 8.44 (broad s, 1H); HRMS calcd for $C_{18}H_{23}N_4O$ 311.187 17, found 311.187 52.

1-Cyclopentyl-3-ethyl-7-oxo-6-(3-pyridyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (33): purified by chromatography (1:1 ethyl acetate/hexane); pale yellow oil; 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.6 Hz, 3H), 1.60−2.09 (m, 8H), 2.65 (q, J = 7.6 Hz, 2H), 2.90 (t, J = 6.7 Hz, 2H), 4.02 (t, J = 6.6 Hz, 2H), 5.60 (pentet, J = 7.8 Hz, 1H), 7.33−7.38 (m, 1H), 7.73−7.76 (m, 1H), 8.47 (broad s, 1H), 8.62 (broad s, 1H); HRMS calcd for $C_{18}H_{23}N_4O$ 311.18717, found 311.1872.

6-(3-Benzyl)-1-cyclopentyl-3-ethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (34). To a stirred solution of compound **13** (0.30 g, 1.29 mmol) in anhydrous THF (5 mL) was added 60% NaH in mineral oil (77 mg, 1.93 mmol). The mixture was heated at reflux for 1h and then cooled to room temperature. Benzyl bromide was added and the resulting mixture was heated at reflux for 2 h, then cooled to room temperature and MeOH (few drops) was added. Concentration followed by chromatography (1:4 ethyl acetate/hexane) provided 385 mg (92%) of a colorless opaque oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (t, J = 7.5 Hz, 3H), 1.60−2.15 (m, 8H), 2.55−2.70 (m, 4H), 3.50 (t, J = 7.2 Hz, 2H), 4.70 (s, 2H), 5.70 (pentet, J = 7.8 Hz, 1H), 7.25−7.40 (m, 5H). Anal. ($C_{20}H_{25}N_3O$) C, H, N.

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