SAR of a Series of 5,6-Dihydro-(9*H*)-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridines as Potent Inhibitors of Human Eosinophil Phosphodiesterase

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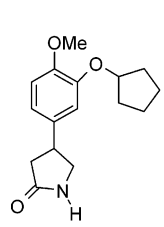
The potency and physical properties of a previously reported 7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]-pyridine series of human eosinophil phosphodiesterase inhibitors were improved by tying the lactam moiety into a triazolo ring. The resulting 5,6-dihydro-(9*H*)-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine series provided nonionizable analogs with melting point properties suitable for micronization. Substitution at the 3-position of the 5,6-dihydro-(9*H*)-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine tricycle led to a 2-thienyl analog, **19** (tofimilast), a potent PDE4 inhibitor with low oral bioavailability and no emesis-associated behaviors in ferrets at plasma concentrations up to 152 ng/mL.

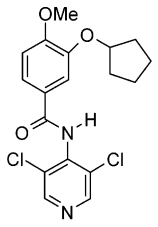
Introduction

Phosphodiesterases (PDEs) have been classified into at least 11 families (PDE1−11) according to their substrate sensitivity, inhibitor selectivity, Ca^{2+}/calmodulin requirement, and amino acid sequence.[1−3] PDE4, a cAMP-specific and Ca^{2+}-independent enzyme, exists in four different isoforms (PDE4-A, -B, -C, and -D) and is a key isozyme in the hydrolysis of cAMP in mast cells, basophils, eosinophils, monocytes, and lymphocytes as well as areas in the brain and airway smooth muscle.[1−4] Increasing the intracellular concentration of cAMP in the airway tissues and cells suppresses inflammatory cell function and thus should be beneficial for treatment of asthma and chronic obstructive pulmonary disease (COPD).[1,4−6] Over the last two decades pharmaceutical companies have placed numerous PDE4 inhibitors into clinical trials for asthma and/or COPD. While a small number of these drugs may have the potential to be approved for market (e.g., cilomilast and roflumilast), most were discontinued from development due to a narrow window between efficacy and the undesired side effects of nausea and emesis (e.g., rolipram, RP 73401).[1−3,5−8] It has been hypothesized that these side effects could be due to either binding at a high-affinity allosteric binding site of PDE4 (called the rolipram binding site), effecting gastric acid secreting cells in the gut, or activation of emetic centers within the CNS.[1,9−12] The latter may be dependent upon the inhibition of specific PDE4 isoforms (A−D) that have varying degrees of expression in the brain versus target inflammatory cells.[1−2,11−12]

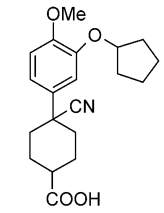
Another method for potentially maximizing the efficacy of a PDE4 inhibitor for treatment of an airway disease while minimizing the emetic liability is to administer the compound directly into the lung. Since inhaled delivery minimizes systemic exposure, we were not concerned with the inhibition of any particular PDE4 isozyme being an emetic liability, but rather our strategy was to equally inhibit all of the PDE4 isozymes in efforts to maximize efficacy. In this article we report our efforts toward the development of an inhaled (nonisoform selective) PDE4 inhibitor.



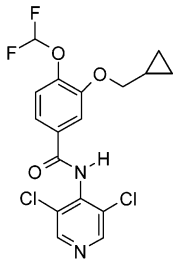
(+/-) rolipram



RP 73401



cilomilast



roflumilast

Chemistry

Preparation of tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine **1** has been reported in previous publications.[13,14] Thiolactam **2** was prepared from **1** by treatment with phosphorus pentasulfide in 1,4-dioxane at reflux.[15] Reaction of thiolactam **2** with diazomethane in the presence of neutral silica gel in ether at 0 °C provided methylsulfide **3**.[16] The 5,6-dihydro-(9*H*)-pyrazolo[3,4-c]-1,2,4-triazolo[4,3-α]pyridines **4**−**34** were prepared by one of two routes. The first route started with treatment of thiolactam **2** with hydrazine in pyridine at 70 °C and was followed by addition of the appropriate acid chloride. The solvent was changed to DMF in order to heat at a high enough temperature to close the triazolo ring. In the second route methylsulfide **3** was heated with the appropriate hydrazide in pyridine. In this procedure, in order to close the triazolo ring, pyridine was removed and the neat mixture was heated to 150 °C. The former route was developed due to the limitation of commercially available hydrazides, Scheme 1.

Biology

SAR was developed from a human eosinophil (HEOS) PDE assay that was reflective of a mixture of PDE4 isoforms.[13]

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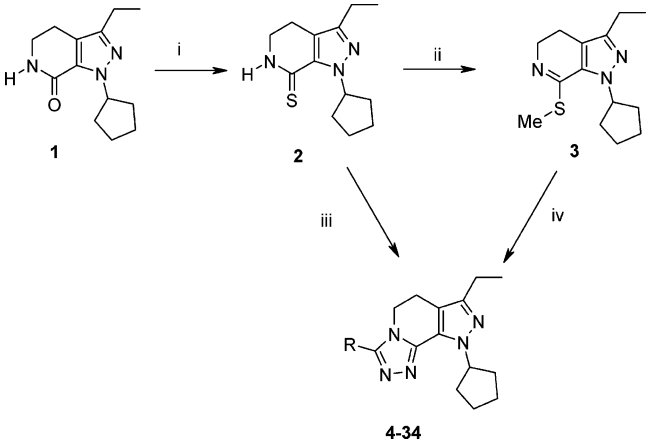
Scheme 1[a]



[a] Reagents and conditions: (i) P_4S_{10}, 1,4-dioxane, reflux, 12 h; (ii) diazomethane, neutral silica gel, ether, 0 °C, 1 h; (iii) hydrazine, pyridine, 70 °C; RC(O)Cl, pyridine, 2 h; DMF reflux, 2 h; (iv) $RC(O)NHNH_2$, pyridine, 135 °C, 4 h; 150 °C, 4 h.

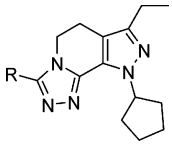
Selected compounds were evaluated in a human recombinant PDE4A, -B, -C, and -D assay, derived from a *baculovirus*-infected caterpillar cell line.[17] PDE family selectivity was evaluated against isolated soluble PDE1 (dog heart), PDE2 (dog heart), PDE3 (human heart), PDE5 (human platelets), and PDE7 (recombinant human enzyme).

Results and Discussion

One of the criteria we set for this aerosol approach was little or no oral bioavailability. Even though inhaled doses are relatively small (<2 mg) a large portion of inhaled agents are inadvertently swallowed and thus may contribute to potential adverse events such as nausea in the case of PDE4 inhibition. Since development of inhaled agents typically requires micronization, we set a melting point hurdle of 125 °C to prevent melting due to friction during this process.

As shown in Table 1, we varied the substituent at the 3-position of the 5,6-dihydro-(9*H*)-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine tricycle. Previous work on a bicyclic 7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine series (see structure of **1**)[13] suggested that the ethyl and cyclopentyl groups around the pyrazolo moiety were optimal, so these were kept constant. The phenyl analog **4** was potent in the HEOS PDE assay relative to rolipram (IC_{50} = 120 nM vs 3.34 μM) and was the starting point for this series. Substitution at the ortho position of the phenyl ring had little influence on HEOS PDE potency (**5**−**8**). However, meta substitution appeared to decrease potency by 10-fold (**9** and **10**) and para substitution by more than 10-fold (**11**−**14**). Replacement of the phenyl moiety of **4** with a 2- or 4-pyridyl group (**15** and **17**) had little effect, whereas the 3-pyridyl (**16**) showed a 10-fold decrease in potency. Other heterocycles such as 2-furanyl (**18**) and 2-thienyl (**19**) were equipotent to **4**. Substituents on the thienyl ring generally decreased potency (**20**) in an analogous manner to substitution around the phenyl ring of **4**. The aromatic ring of **4** could be homologated out and still retain similar potency (**21** and **22**). Alkyl groups were also evaluated. Small alkyl groups such as methyl, ethyl, and propyl (**23**−**25**) had no potency advantage over **4**. Larger alkyl groups such as butyl, cyclobutyl, cyclopentyl, cyclohexyl, and 3-pentyl (**26**, **27**, **28**, **29**, and **31**, respectively) were up to 4 times more potent than **4**, but the more potent of these compounds had low melting points and/or oily physical properties. The low melting point could be circumvented by incorporation of heteroatoms (**30**), a tertiary butyl group (**33**), or a more rigid bicyclic group (**34**). The 1-methylcyclohex-1-yl analog, **32**, had particularly good potency (IC_{50} = 40 nM), but we were unable to obtain compound **32** in crystalline (or even solid) form.

Table 1.



compd	R	mp(°C)	HEOS PDE IC_{50} (μM) ± SE (*n*)
(±)-rolipram			3.34 ± 0.79 (16)
cilomilast			0.17 ± 0.05 (5)
RP 73401			0.009 ± 0.001 (8)
4	phenyl		0.12 ± 0.02 (3)
5	2-methoxyphenyl	132-7	0.58 ± 0.18 (3)
6	2-methylphenyl	109-12	0.16 ± 0.04 (3)
7	2-chlorophenyl	132-4	0.29 ± 0.19 (4)
8	2-trifluoromethylphenyl	154-5	0.43 ± 0.07 (2)
9	3-methoxyphenyl	130-2	1.3 ± 0.8 (3)
10	3-chlorophenyl	158	1.4 ± 0.6 (3)
11	4-methoxyphenyl	156-9	30 ± 7 (3)
12	4-methylphenyl	142-5	22 (1)
13	4-chlorophenyl	160-2	36 ± 13 (3)
14	4-trifluoromethylphenyl	134-7	57 (1)
15	2-pyridyl	147	0.26 ± 0.04 (3)
16	3-pyridyl	153-5	1.2 ± 0.4 (3)
17	4-pyridyl	198-200	0.09 ± 0.02 (3)
18	2-furanyl	95-7	0.28 ± 0.01 (3)
19 (tofimilast)	2-thienyl	125-6	0.14 ± 0.05 (5)
20	3-chloro-4-methylthien-2-yl	136-8	1.0 ± 0.8 (2)
21	benzyl	116-7	0.4 ± 0.11 (3)
22	3-thenyl	134-5	0.12 ± 0.08 (2)
23	methyl	174-5	0.91 ± 0.43 (3)
24	ethyl	118-9	0.13 ± 0.04 (3)
25	propyl	88-92	0.18 ± 0.02 (4)
26	butyl	oil	0.04 ± 0.01 (2)
27	cyclobutyl	116-9	0.03 ± 0.03 (2)
28	cyclopentyl	oily solid	0.09 ± 0.03 (3)
29	cyclohexyl	138-9	0.14 ± 0.02 (3)
30	4-tetrahydropyranyl	165-6	0.23 ± 0.05 (3)
31	3-pentyl	81-2	0.03 ± 0.01 (2)
32	1-methylcyclohex-1-yl	oil	0.04 ± 0.01 (3)
33	*tert*-butyl	144-5	0.08 ± 0.01 (3)
34	bicyclo[2.2.2]octanyl	221-2	2.4 ± 0.8 (2)

Table 2. PDE4 Isoform Data for Selected Compounds [IC_{50} (nM), mean of $n = 3$]

compd	PDE4A	PDE4B	PDE4C	PDE4D
(−)-rolipram	162	231	3690	622
cilomilast	150	84	610	39
RP 73401	4	3	14	2
7	35	20	77	13
9	770	430	2440	180
16	250	210	700	70
19	23	13	>100	13
33	11	8	40	9

In order to confirm that this series of compounds was nonselective against the PDE4 isoforms, selected compounds were tested for human recombinant PDE4A, -B, -C, and -D isoform activity. As shown in Table 2, this series of compounds showed no significant selectivity (>5 fold) for any particular PDE4 isoform. In general, compounds profiled in the PDE4 isoform assay had the same rank order potency as in the HEOS PDE assay. Several potent compounds met our melting point criteria (**17**, **19**, **22**, **29**, and **33**), but compound **19** was the only one that had no emesis or emesis-associated behavior in ferrets at doses up to 10 mg/kg, po (**19**, C_{max} = 152 ng/mL).[18] Compound **19** was found to have a minimal effective dose (MED) in the ferret emesis model of 30 mg/kg, po (plasma C_{max} = 392 ng/mL, 1.25 h post dose). Difference in exposure is one possible explanation for the decreased emetic liability of **19**

Table 3. Pharmacokinetics (mean ± SD) of **19** Following Single-Dose Intravenous and Oral Administration to Rats and Dogs

species/dose[a]	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	AUC_{0-} (ng·h/mL)	C_{max} (ng/mL)	$t_{1/2}$ (h)	*F* (%)
			Sprague−Dawley rat			
5 mg/kg IV	29.5 ± 10.4	2.0 ± 0.6	3200 ± 1430		2.1 ± 0.7	
10 mg/kg PO			76 ± 59	42 ± 3		1.2
			beagle dog			
1 mg/kg IV	26.8 ± 9.8	2.4 ± 1.1	649 ± 215		1.7 ± 0.2	
1 mg/kg PO			23.2 ± 5.9	12 ± 4	1.2 ± 0.4	3.2
5 mg/kg PO			263 ± 78	98 ± 40	1.8 ± 0.2	8.1

[a] Results are from four male rats and two/gender for dogs. [b] AUC0-tlast.

compared to its close-in analogs. For example, we found that one of the more potent PDE4 inhibitors, **33**, had a MED in the ferret emesis model of only 1 mg/kg, po, but its C_{max} was 153 ng/mL, only ~2-fold lower than **19** when dosed at 30 mg/kg, po. Furthermore, at the identical C_{max} (152 ng/mL) in the ferret experiment, **33** was emetic whereas **19** was not. In rats compounds **19** and **33** had 1.2% and 12% oral bioavailability, respectively, and **19** had only 3% bioavailability in dogs (Table 3). Thus, since **19** was reasoned to have a decreased risk of causing nausea due to inadvertently swallowing drug from an inhalation device, the compound was chosen for additional studies. Compound **19** inhibited human monocyte PDE mediated cAMP catabolism with an IC_{50} of 67 nM and LPS stimulated human monocyte TNFα release with an IC_{50} of 59 nM (TNFα IC_{50} = 429 nM in the presence of whole human blood; human plasma protein binding, $f_u = 0.02$).[19] Compound **19** was inactive at concentrations > 5000 nM (1700 ng/mL) against PDE1, PDE2, PDE3, PDE5, PDE6, and PDE7 enzyme-mediated cAMP breakdown. Across a panel of over 60 receptors, **19** had <50% inhibition at 10 μM against all receptors except adenosine A_1 (IC_{50} = 1.3 μM), A_{2A} (IC_{50} = 4.4 μM), and A_3 (IC_{50} = 4.8 μM). The A_1 binding activity of **19** was followed up in a cell-based functional assay using CHO cells expressing the human adenosine A_1 receptor (A_1 antagonist IC_{50} = 5.2 μM). Providing a mechanistic link between PDE4 enzyme inhibition and its effects on cell function, **19** increased cAMP levels in PGE_1-stimulated U937 cells (human-derived monocytic cell line)[13] with an EC_{50} of 230 ± 120 nM (78 ± 41 ng/mL, *n* = 4). Compound **19** was progressed to clinical evaluation for its potential use to treat chronic pulmonary inflammatory diseases.

Conclusions

In conclusion, we developed SAR around the 3-position of the 5,6-dihydro-(9*H*)-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine tricycle leading to **19** (tofimilast), a novel potent PDE4 inhibitor with low systemic exposure, low emetic liability, and physical properties conducive to formulation development in an inhaler device.

Experimental Section

Biology. *Phosphodiesterase Assay.* PDE activity was measured as described[17] with the following modifications: a one-step assay was run using a 100-μL assay volume containing 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.1 unit of 5′ nucleotidase (from *Crotalus atrox* venom), [^{3}H]cAMP, and various concentrations of cold cAMP to give the final concentration range of 3−300 μM. The reaction was started by addition of 25 L of appropriately diluted enzyme supernatant. Reactions were run directly in mini Poly-Q scintillation vials (Beckman Instruments Inc., Fullerton, CA). Assays were incubated at 37 °C for a time period that would give less than 15% cAMP hydrolysis to avoid nonlinearity associated with product inhibition. The reaction was stopped by addition of 1 mL of Dowex AG1 × 8 (Cl form) resin (1:3 slurry). A 3 mL amount of Ready Safe scintillant (Beckman) was added, and the vials were well mixed. The vials were allowed to settle for 1 h before counting. When inhibitors were tested, all reactions contained 1% Me_2SO and were done at a cAMP substrate concentration of about 1 μM. The assay blank contained all reagents minus the enzyme aliquot. The level of PDE activity was so high in PDE4-infected insect cells that the amount of basal insect cell cAMP hydrolysis was considered negligible and ignored. At the dilutions used, the amount of cAMP hydrolysis by the mock infected insect cell lysate was not significantly different from the no enzyme assay blank.

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., 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, 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 105k*g* for 30 min at 4 °C to isolate membranes. Cytosol was decanted, and 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 assessed 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 the column with HEPES/NaCl; 5′-AMP was eluted with 4 mL of 0.25 M actetic acid. A 1 mL amount 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 micromolar.

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 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 rotary 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.

1-Cyclopentyl-3-ethyl-7-thio-4,5,6,7-tetrahydro-1*H*-pyrazolo-[3,4-*c*]pyridine (2). A solution of 1-cyclopentyl-3-ethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (10.0 g, 42.9 mmol) in anhydrous 1,4-dioxane was treated with phosphorus pentasulfide (3.9 g, 8.8 mmol). After stirring at reflux for 12 h, the mixture was cooled to ambient temperature and concentrated under reduced pressure. The resulting yellow oil was dissolved in methylene chloride and washed with water and brine, dried over sodium sulfate, and concentrated under reduced pressure. The orange residue was purified by chromatography on a silica gel column using a gradient mixture of hexanes in methylene chloride as eluent to give 9.3 g (87%) of a yellow solid: mp 152−3 C; MS *m*/*z* 250; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (t, J = 7.7 Hz, 3H), 1.56−1.68 (m, 2H), 1.87−1.91 (m, 2H), 1.98−2.03 (m, 2H), 2.08−2.15 (m, 2H), 2.62 (q, J = 7.7 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H), 3.48 (dt, J = 3.3 and 7.0 Hz, 2H), 6.37 (quintet, J = 7.7 Hz, 1H), 7.47 (br s, 1H). Anal. ($C_{13}H_{19}N_3S$) C, H, N.

1-Cyclopentyl-4,5-dihydro-3-ethyl-7-methylthio-1*H*-pyrazolo-[3,4-*c*]pyridine (3). A magnetically stirred mixture of 1-cyclopentyl-3-ethyl-7-thio-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine (0.322 g, 1.29 mmol), neutral silica gel (10 g), and ether (100 mL) in a 500 mL Erlenmeyer flask was cooled to 0 °C. To this mixture was slowly added an excess solution of diazomethane in ether. Evolution of gas occurred, and after 1 h the reaction was quenched with acetic acid (1 drop), filtered, and concentrated under reduced pressure to give a yellow oil. The oil was purified by chromatography on a silica gel column using 1:4 ethyl acetate/hexane as eluent to give 0.232 g (68%) of a yellow oil: MS *m*/*z* 264; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.19 (t, J = 7.6 Hz, 3H), 1.63−1.69 (m, 2H), 1.85−1.96 (m, 2H), 2.06−2.14 (m, 4H), 2.47 (s, 3H), 2.52 (t, J = 7.6 Hz, 2H), 2.61 (q, J = 7.6 Hz, 2H), 3.72 (t, J = 7.6 Hz, 2H), 5.28 (quintet, J = 7.5 H, 1H). Anal. ($C_{14}H_{21}N_3S$) C, H, N.

General Procedure A. 9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-pyridyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (16). 1-Cyclopentyl-4,5-dihydro-3-ethyl-7-methylthio-1*H*-pyrazolo[3,4-*c*]pyridine (0.036 g, 0.14 mmol) and nicotinic acid hydrazide (0.021 g, 0.15 mmol) were dissolved in anhydrous pyridine (5 mL) in a flame-dried flask. An oven-dried condenser was added that was septa sealed and had an outlet to a bubbler. A long stainless steel needle was pierced through the septa and condenser center into the magnetically stirred solution. Nitrogen was bubbled through the long needle as the flask was heated to 135 °C over 4 h. The pyridine was then removed under nitrogen purge, and the resulting oil was heated to 150 °C for 4 h. The flask was cooled to ambient temperature and contained a white solid that was purified by column chromatography (silica gel, gradient mixture of ethyl acetate and hexanes) to give 45 mg (96%) of a white solid: mp 147 °C (sharp); MS *m*/*z* [M + 1] 335; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.68 (q, 2H), 2.98 (t, 2H), 4.25 (t, 2H), 5.60 (quintet, 1H), 7.47 (dd, 1H), 8.09 (d, 1H), 8.75 (dd, 1H), 8.9 (s, 1H). Anal. ($C_{19}H_{22}N_6$) C, H, N: calcd, 68.23, 6.63, 25.13; found, 68.65, 7.17, 23.80.

General Procedure B. 9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(thien-2-yl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (19). 1-Cyclopentyl-3-ethyl-7-thio-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]-pyridine (0.35 g, 1.4 mmol) was dissolved in 4 mL of anhydrous pyridine in a flame-dried flask under nitrogen. The flask was warmed to 70 °C, and 1.5 mL of anhydrous hydrazine was added. The yellow solution turned pink and was stirred for 5 min. The pyridine and excess hydrazine were then removed under reduced pressure to give a pink solid that turned light green after being placed under vacuum (0.1 mm) for 30 min. Next, anhydrous pyridine (4 mL) followed by 2-thiophene carbonyl chloride (0.69 g, 4.7 mmol) was added to the flask, and the mixture was stirred for 2 h. The pyridine was removed under reduced pressure, and the residue was dissolved in DMF (4 mL) and heated at reflux for 2 h. The mixture was then cooled to ambient temperature, diluted with water, and extracted with ethyl acetate. The aqueous layer was basified to pH 12 with 1 N sodium hydroxide and extracted with ethyl acetate three times. The combined organics were washed with 1 N sodium hydroxide, water, and brine, dried over sodium sulfate, and concentrated under reduced pressure. The resulting oil was purified by chromatography (1:1 ethyl acetate/hexanes) followed by recrystallization of the resulting solid from ether to give 219 mg (46%) of a white crystalline solid: mp 125−6 °C; MS *m*/*z* [M + 1] 340; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.70 (m, 2H) 1.94 (m, 2H), 2.15 (m, 4H), 2.66 (q, 2H), 3.0 (t, 2H), 4.32 (t, 2H), 5.58 (quintet, 1H), 7.18 (t, 1H), 7.50 (m, 2H). Anal. ($C_{18}H_{21}N_5S$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(phenyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (4). Procedure A; no purification; off-white amorphous solid; 60% yield; MS *m*/*z* [M + 1] 334; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.71 (m, 2H), 1.97 (m, 2H), 2.17 (m, 4H), 2.67 (q, 2H), 2.97 (t, 2H), 4.26 (m, 2H), 5.6 (m, 1H) 7.55 (m, 4H), 7.7 (m, 1H); HRMS calcd for $C_{20}H_{24}N_5$ [M + 1] 334.2032, found 334.2032.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-methoxyphenyl)-9*H*-pyrazolo[3,4-*c*] -1,2,4-triazolo[4,3-α]pyridine (5). Procedure A; purified by chromatography (ethyl acetate); white solid; 65% yield; mp 132−137 °C; MS *m*/*z* [M + 1] 364; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.71 (q, 2H), 2.98 (t, 2H), 3.80 (s, 3H), 4.00 (t, 2H), 5.63 (quintet, 1H), 7.01 (dd, 1H), 7.20 (m, 1H), 7.44 (m, 2H). Anal. ($C_{21}H_{25}N_5O$ + 0.33H_2O) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-methylphenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (6). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); yellowish solid; 45% yield; mp 109−112 °C; MS *m*/*z* [M + 1] 348; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.15 (m, 4H), 2.32 (s, 3H), 2.65 (q, 2H), 2.88 (t, 2H), 3.94 (t, 2H), 5.64 (quintet, 1H), 7.33 (m, 4H). Anal. ($C_{21}H_{25}N_5$ + 0.33H_2O) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-chlorophenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (7). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 85% yield; mp 132−134 °C; MS *m*/*z* [M + 1] 368; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.71 (q, 2H), 2.98 (t, 2H), 4.00 (t, 2H), 5.63 (quintet, 1H), 7.50 (m, 4H). Anal. ($C_{20}H_{22}N_5Cl$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-trifluoromethylphenyl)-9*H*-pyrazol o[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (8). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 61% yield; mp 154−155 °C; MS *m*/*z* [M + 1] 402; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.61 (q, 2H), 2.82 (t, 2H), 3.89 (t, 2H), 5.61 (quintet, 1H), 7.20 (dd, 1H), 7.70 (dd, 2H), 7.86(d, 1H). Anal. ($C_{21}H_{22}N_5F_3$ + 0.5H_2O) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-methoxyphenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (9). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); yellowish solid; 98% yield; mp 130−132 °C; MS *m*/*z* [M + 1] 364; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.71 (q, 2H), 2.98 (t, 2H), 3.87 (s, 3H), 4.24 (t, 2H), 5.63 (quintet, 1H), 7.01 (dd, 1H), 7.20 (dd, 2H), 7.44 (t, 1H). Anal. ($C_{21}H_{25}N_5O$) C, H, N: calcd, 19.27; found, 18.13.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-chlorophenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (10). Procedure B; purified by chromatography (ethyl acetate); white solid; 69% yield; mp 158 °C sharp; MS *m*/*z* [M + 1] 368; ^{1}H NMR (250 MHz,$CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.71 (q, 2H), 2.98 (t, 2H), 4.24 (t, 2H), 5.63 (quintet, 1H), 7.62 (m, 4H). Anal. ($C_{20}H_{22}N_5Cl$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-methoxyphenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (11). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); pale yellow solid; 69% yield; mp 156−159 °C; MS *m*/*z* [M + 1] 364; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H),

2.17 (m, 4H), 2.62 (q, 2H), 2.98 (t, 2H), 3.86 (s, 3H), 4.41 (t, 2H), 5.63 (quintet, 1H), 7.05 (d, 2H), 7.63 (d, 2H). Anal. ($C_{21}H_{25}N_5O$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-methylphenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (12). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); pale yellow solid; 94% yield; mp 142−145 °C; MS *m*/*z* [M + 1] 348; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.43 (s, 3H), 2.62 (q, 2H), 2.98 (t, 2H), 4.41 (t, 2H), 5.63 (quintet, 1H), 7.31 (d, 2H), 7.56 (d, 2H). Anal. ($C_{21}H_{25}N_5$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-chlorophenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (13). Procedure B; purified by chromatography (ethyl acetate); white solid; 72% yield; mp 160−162 °C; MS *m*/*z* [M + 1] 364; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.62 (q, 2H), 2.98 (t, 2H), 4.41 (t, 2H), 5.63 (quintet, 1H), 7.52 (d, 2H), 7.61 (d, 2H). Anal. ($C_{20}H_{22}N_5Cl$) C, H, N: calcd, 19.04; found, 18.20.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-trifluoromethylphenyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (14). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); pale yellow solid; 68% yield; mp 134−137 °C; MS *m*/*z* [M + 1] 402; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.69 (q, 2H), 2.99 (t, 2H), 4.27 (t, 2H), 5.61 (quintet, 1H), 7.85 (m, 4H). Anal. ($C_{21}H_{22}N_5F_3$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-pyridyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (15). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 74% yield; mp 153−155 °C; MS *m*/*z* [M + 1] 335; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.70 (q, 2H), 2.98 (t, 2H), 4.90 (t, 2H), 5.64 (quintet, 1H), 7.36 (dd, 1H), 7.84 (t, 1H), 8.37 (d, 1H), 8.66 (s, 1H).

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-pyridyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (17). Procedure A; purified by chromatography (9:1 ethyl acetate/methanol); white solid; 75% yield; mp 198−200 °C; MS *m*/*z* [M + 1] 335; ^{1}H NMR (250 MHz,-$CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.70 (q, 2H), 3.02 (t, 2H), 4.34 (t, 2H), 5.64 (quintet, 1H), 7.79 (d, 2H), 8.84 (d, 2H). Anal. ($C_{19}H_{22}N_6 + 0.14H_2O$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(2-furanyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (18). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); yellowish solid; 63% yield; mp 95−97 °C; MS *m*/*z* [M + 1] 324; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.68 (q, 2H), 2.98 (t, 2H), 4.25 (t, 2H), 5.60 (quintet, 1H), 6.6 (d, 1H), 7.14 (d, 1H), 7.61 (d, 1H). Anal. ($C_{18}H_{21}N_5O$) C, H, N: calcd, 66.85, 6.55, 21.67; found, 67.29, 7.13, 19.56.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-chloro-4-methylthien-2-yl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (20). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); off-white solid; 78% yield; mp 136−138 °C; MS *m*/*z* [M + 1] 388; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H), 1.70 (m, 2H) 1.94 (m, 2H), 2.15 (m, 4H), 2.28 (s, 3H), 2.66 (q, 2H), 3.00 (t, 2H), 4.32 (t, 2H), 5.58 (quintet, 1H), 7.25 (s, 1H). Anal. ($C_{19}H_{22}N_5$-Cl S) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(benzyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (21). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 65% yield; mp 116−117 °C; MS *m*/*z* [M + 1] 348; ^{1}H NMR (250 MHz,-$CDCl_3$) δ 1.19 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.60 (q, 2H), 2.83 (t, 2H), 3.84 (t, 2H), 4.25 (s, 2H), 5.56 (quintet, 1H), 7.26 (m, 5H). HRMS calcd for $C_{21}H_{25}N_5$ [M] 347.2110, found 347.2109.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-thenyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (22). Procedure A; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 32% yield; mp 134−135 °C; MS *m*/*z* [M + 1] 354; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.19 (t, 3H), 1.72 (m, 2H), 1.95 (m, 2H), 2.17 (m, 4H), 2.60 (q, 2H), 2.83 (t, 2H), 3.84 (t, 2H), 4.25 (s, 2H), 5.56 (quintet, 1H), 6.96(d, 1H), 7.03 (s, 1H), 7.29 (d, 1H). Anal. ($C_{19}H_{23}N_5S$) C, H, N: calcd, 19.81; found, 18.57.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-methyl-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (23). Procedure B; purified by chromatography (5% methanol/CH_2Cl_2); off-white solid; 55% yield; mp 174−175 °C; MS *m*/*z* [M + 1] 272; ^{1}H NMR (250 MHz, $CDCl_3$) 1.23 (t, 3H), 1.69 (m, 2H), 1.91 (m, 2H), 2.13 (m, 4H), 2.49 (s, 3H), 2.66 (q, 2H), 2.96 (t, 2H), 4.02 (t, 2H), 5.56 (quintet, 1H). Anal. ($C_{15}H_{21}N_5$) C, H, N.

9-Cyclopentyl-5,6-dihydro-3,7-diethyl-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (24). Procedure B; purified by chromatography (ethyl acetate); white solid; 14% yield; mp 118−19 °C; MS *m*/*z* [M + 1] 286; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.7 Hz, 3H), 1.37 (t, *J* = 7.7 Hz, 3H), 1.66−1.73 (m, 2H), 1.89−2.03 (m, 2H), 2.05−2.18 (m, 4H), 2.65 (q, *J* = 7.7 Hz, 2H), 2.81 (q, *J* = 7.7 Hz, 2H), 2.94 (t, *J* = 7.0 Hz, 2H), 4.01 (t, *J* = 7.0 Hz, 2H), 5.54 (quintet, *J* = 7.9 Hz, 1H). HRMS calcd for $C_{16}H_{24}N_5$ [M + 1] 286.2032, found 286.2048. Anal. ($C_{16}H_{23}N_5$) C, H, N: calcd, 67.34, 8.12, 24.54; found, 67.84, 8.47, 23.16.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-propyl-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (25). Procedure B; purified by chromatography (ethyl acetate); white solid; 28% yield; mp 88−92 °C; MS *m*/*z* [M + 1] 300; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.05 (t, *J* = 7.4 Hz, 3H), 1.24 (t, *J* = 7.6 Hz, 3H), 1.67−2.27 (m, 10 H), 2.66 (q, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 7.7 Hz, 2H), 2.95 (t, *J* = 7.0 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 5.56 (quintet, 1H). HRMS calcd for $C_{17}H_{26}N_5$ [M + 1] 300.2188, found 300.2188. Anal. ($C_{17}H_{25}N_5 + 0.2H_2O$) C, H, N: calcd, 23.11; found, 22.19.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-butyl-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (26). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); colorless oil; 49% yield; MS *m*/*z* [M + 1] 314; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.22 (t, *J* = 7.7 Hz, 3H), 1.44 (sextet, *J* = 7.5 Hz, 2H), 1.65−1.79 (m, 4H), 1.87−1.97 (m, 2H), 2.04−2.20 (m, 4H), 2.65 (q, *J* = 7.7 Hz, 2H), 2.78 (t, *J* = 7.7 Hz, 2H), 2.93 (t, *J* = 7.0 Hz, 2H), 4.01 (t, *J* = 7.0 Hz, 2H), 5.55 (quintet, *J* = 7.9 Hz, 1H). HRMS calcd for $C_{18}H_{28}N_5$ [M + 1] 314.2345, found 314.2333. Anal. ($C_{18}H_{27}N_5$) C, H, N: calcd, 22.34; found, 19.39.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-cyclobutyl-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (27). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); waxy solid; 28% yield; mp 116−118 °C; MS *m*/*z* [M + 1] 312; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7.7 Hz, 3H), 1.65−1,69 (m, 2H), 1.89−1.93 (m, 2H), 2.01−2.15 (m, 6H), 2.38−2.44 (m, 2H), 2.49−2.56 (m, 2H), 2.63 (q, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.0 Hz, 2H), 3.52−3.57 (m, 1H), 3.92 (t, *J* = 7.0 Hz, 2H), 5.53 (quintet, *J* = 7.8 Hz, 1H). Anal. ($C_{18}H_{25}N_5$) C, H, N: calcd, 22.49; found, 20.15.

3,9-Dicyclopentyl-5,6-dihydro-7-ethyl-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (28). Procedure B; purified by chromatography (5% methanol/CH_2Cl_2); oily white solid; 30% yield; MS *m*/*z* [M + 1] 326; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3H), 1.65−2.16 (m, 16H), 2.64 (q, 2H), 2.95 (t, 2H), 3.17 (quintet, 1H), 3.98 (t, 2H), 5.54 (quintet, 1H). HRMS calcd for $C_{19}H_{28}N_5$ [M + 1] 326.2345, found 326.2345.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-cyclohexyl-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (29). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes) followed by recrystallization from a 10:1 mixture of pentane and ether; white solid; 20% yield; mp 138−9 °C; MS *m*/*z* [M + 1] 340; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.7 Hz, 3H), 1.30−1.43 (m, 2H), 1.63−1.80 (m, 6H), 1.87−1.98 (m, 6H), 2.03−2.19 (m, 4H), 2.62−2.73 (m, including q at 2.65, *J* = 7.7 Hz, 3H), 2.92 (t, *J* = 6.9 Hz, 2H), 4.03 (t, *J* = 6.9 Hz, 2H), 5.55 (quintet, *J* = 7.9 Hz, 1H). HRMS calcd for $C_{20}H_{29}N_5$ [M + 1] 340.2501, found 340.2524. Anal. ($C_{20}H_{29}N_5$) C, H, N: calcd, 70.76, 8.61, 20.63; found, 71.28, 9.09, 18.79.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(4-tetrahydropyranyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (30). Procedure B; purified by chromatography (9:1 CH_2Cl_2/methanol) followed by recrystallization from a mixture of isopropylether and hexanes; light yellow crystalline solid; 26% yield; mp 165−166 °C; MS *m*/*z* [M

+ 1] 341; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, 3H), 1.6−2.2 (m, 12H), 2.64 (q, 2H), 2.95 (t, 2H), 2.99 (tt, 1H), 3.56 (td, 2H), 4.06 (t, 2H), 4.12 (dt, 2H), 5.54 (quintet, 1H). Anal. ($C_{19}H_{27}N_5O$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(3-pentyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (31). Procedure B; purified by chromatography (1:3 ethyl acetate/hexanes); white solid; 56% yield; mp 81−82 °C; MS *m*/*z* [M + 1] 328; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 7.4 Hz, 6H), 1.22 (t, J = 7.7 Hz, 3H), 1.64−2.20 (m, 12 H), 2.62−2.70 (m, including q at 2.65, J = 7.7 Hz, 3H), 2.93 (t, J = 6.9 Hz, 2H), 4.04 (t, J = 6.9 Hz, 2H), 5.56 (quintet, J = 7.9 Hz, 1H). Anal. ($C_{19}H_{29}N_5$) C, H, N: calcd, 21.39; found, 20.66.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(1-methylcyclohex-1-yl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (32). Procedure B; purified by chromatography (ether); colorless oil; 57% yield; MS *m*/*z* [M + 1] 354; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3H), 1.40−2.4 (m, 21H), 2.64 (q, 2H), 2.95 (t, 2H), 4.22 (t, 2H), 5.54 (quintet, 1H). HRMS calcd for $C_{21}H_{32}N_5$ [M + 1] 354.2658, found 326.2658. Anal. ($C_{21}H_{31}N_5 + 0.2H_2O$) C, H, N: calcd, 19.32; found, 18.14.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(*tert*-butyl)-9*H*-pyrazolo-[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (33). Procedure B; purified by chromatography (1:1 ethyl acetate/hexanes); white solid; 53% yield; mp 144−145 °C; MS *m*/*z* [M + 1] 314; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3H), 1.48 (s, 9H), 1.69 (m, 2H), 1.91 (m, 2H), 2.03 (m, 4H), 2.65 (q, 2H), 2.88 (t, 2H), 4.23 (t, 2H), 5.54 (quintet, 1H). Anal. ($C_{18}H_{27}N_5$) C, H, N.

9-Cyclopentyl-5,6-dihydro-7-ethyl-3-(bicyclo[2.2.2]octanyl)-9*H*-pyrazolo[3,4-*c*]-1,2,4-triazolo[4,3-α]pyridine (34). Procedure B; purified by chromatography (1:3 ethyl acetate/hexanes); white solid; 30% yield; mp 221−222 °C; MS *m*/*z* [M + 1] 410; ^{1}H NMR (400 MHz,$CDCl_3$) δ 1.19 (t, 3H), 1.45−2.2 (21H, m), 2.64 (q, 2H), 2.95 (t, 2H), 4.21 (t, 2H), 5.54 (quintet, 1H). Anal. ($C_{22}H_{31}N_5$) C, H, N.

Supporting Information Available: Results from combustion analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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