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Quinolinone-based agonists of $S1P_1$: Use of a N-scan SAR strategy to optimize in vitro and in vivo activity

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ABSTRACT

We reveal how a N-scan SAR strategy (systematic substitution of each CH group with a N atom) was employed for quinolinone-based $S1P_1$ agonist **5** to modulate physicochemical properties and optimize in vitro and in vivo activity. The diaza-analog **17** displays improved potency ($hS1P_1$ RI; **17**: EC_{50} = 0.020 μM, 120% efficacy; **5**: EC_{50} = 0.070 μM, 110% efficacy) and selectivity ($hS1P_3$ Ca^{2+} flux; **17**: EC_{50} >25 μM; **5**: EC_{50} = 1.5 μM, 92% efficacy), as well as enhanced pharmacokinetics (**17**: CL = 0.15 L/h/kg, V_{dss} = 5.1 L/kg, $T_{1/2}$ = 24 h, %*F* = 110; **5**: CL = 0.93 L/h/kg, V_{dss} = 11 L/kg, $T_{1/2}$ = 15 h, %*F* = 60) and pharmacodynamics (**17**: 1.0 mg/kg po, 24 h PLC POC = −67%; **5**: 3 mg/kg po, 24 h PLC POC = −51%) in rat.

Sphingosine-1-phosphate (**1**, S1P) is the endogenous ligand for the G-protein-coupled receptors $S1P_{1-5}$ and participates in modulating a wide variety of physiological processes including cell motility, pulmonary endothelium integrity, and cardiovascular function (Fig. 1).[1] S1P (**1**) and the sphingosine-1-phosphate-1 receptor ($S1P_1$) were discovered several years ago to regulate the trafficking of lymphocytes from the lymphatic system.[2] Exposure to high concentrations of **1** or synthetic agonists of $S1P_1$ induces receptor internalization (RI) of $S1P_1$ located on the cell surface of lymphocytes in lymphatic tissues and prevents lymphocyte egress to the systemic circulation and central nervous system (**1**: $hS1P_1$ RI EC_{50} = 0.018 μM, 91% efficacy).[3,4] Synthetic agonists of $S1P_1$ are being investigated intensely as potential immunosuppressive therapies for a variety of conditions, including multiple sclerosis (MS).[5]

FTY720-P (**2a**) is the first nonnatural agonist of $S1P_1$ to be reported and the bioactive metabolite of fingolimod (**2b**, FTY720),[6] the first oral therapy for relapsing forms of MS (Fig. 1).[7] The efficacy of fingolimod (**2b**) is believed to arise primarily from the agonism of $S1P_1$ by **2a** ($hS1P_1$ RI EC_{50} = 0.0020 μM, 115% efficacy)[3] and the ensuing peripheral lymphocyte depletion.[8]

Notably, fingolimod (**2b**) has also been shown to induce dose-dependent transient bradycardia in preclinical animal models and human subjects.[9] Until recently, the activation of $S1P_3$ by **2a**

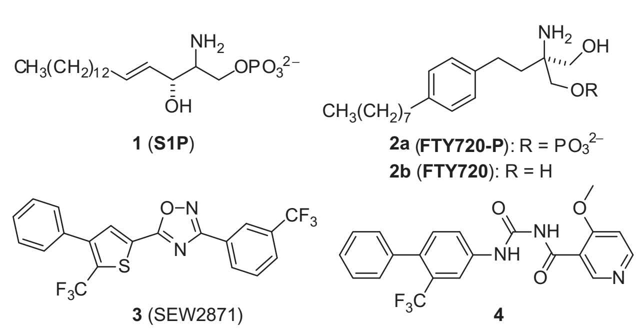

Figure 1. Natural and nonnatural agonists of $S1P_1$.

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(hS1P$_3$ Ca^{2+} flux EC$_{50}$ = 0.027 μM, 35% efficacy)[10] was believed to be the sole cause of these cardiovascular side effects in humans,[11] and the search for S1P$_3$-sparing synthetic agonists of S1P$_1$ has garnered significant attention.[12] SEW2871 (**3**) is the first example of an S1P$_1$-selective agonist lacking a polar head group that is typical of most synthetic agonists of this receptor (Fig. 1).[13] Although the potency of **3** is modest (hS1P$_1$ RI EC$_{50}$ = 0.27 μM, 86% efficacy),[3] its selectivity for S1P$_1$ versus S1P$_3$ is excellent (hS1P$_3$ Ca^{2+} flux EC$_{50}$ >25 μM).[10,14]

Our laboratory recently disclosed carbamoylnicotinamide **4** as a novel example of this atypical chemotype of S1P$_1$ agonist lacking a polar head group (Fig. 1).[15] Carbamoylnicotinamide **4** displays improved potency (hS1P$_1$ RI EC$_{50}$ = 0.035 μM, 96% efficacy)[3] compared to SEW2871 (**3**), while maintaining excellent selectivity against S1P$_3$ (hS1P$_3$ Ca^{2+} flux EC$_{50}$ >25 μM).[10] The greater cell potency of **4** versus **3** is recapitulated in vivo. Whereas carbamoylnicotinamide **4** effects dramatic reduction of circulating lymphocytes in rat 24 h after a 1 mg/kg oral dose (POC = −78%; [**4**]$_{plasma}$ = 63 ng/mL),[15,16] SEW2871 (**3**) requires a much larger dose (30 mg/kg po) to achieve similar levels of peripheral lymphocyte depletion at the 24 h time point (POC = −66%; [**3**]$_{plasma}$ = 1100 ng/mL).[16] As part of an effort to discover new and potentially improved atypical chemotypes of S1P$_1$ agonists lacking a polar head group, we set out to explore conformationally constrained analogs of **4**.

Because a large number of heterocyclic bioisosteres of the acyl urea moiety of carbamoylnicotinamide **4** have been reported in S1P$_1$ agonists (e.g., the oxadiazole of **3**),[5,13] we focused our attention on the right-hand side of **4** (Fig. 2). We envisioned replacing the intramolecular hydrogen bond between the carbamoyl NH group and the nicotinamide OMe substituent of **4** with a covalent linkage, and substituting the pyridyl N atom of **4** with a CH group to give quinolinone **5**. This cyclization strategy would simultaneously serve to impart a more rigid conformational constraint and remove a hydrogen bond donor.[17,18] Furthermore, we desired to systematically substitute each CH group in **5** with a N atom to probe the effect on potency and tune molecular and physicochemical properties with minimal impact on molecular weight. This homologous series of aza-substituted, quinolinone-based S1P$_1$ agonists was anticipated to reveal structure–activity relationships (SAR) that might lead to further improvement of in vitro and in vivo activity by the potentially additive effects of multiple N atom substitution. We expected that the synthesis of these aza- and poly-aza-analogs of **5** would challenge this N atom scan (N-scan) SAR strategy.

The results of this study are detailed in Table 1. Pleasingly, quinolinone **5** was found to exhibit similar potency toward S1P$_1$ (hS1P$_1$ RI EC$_{50}$ = 0.070 μM, 110% efficacy)[3] compared to carbamoylnicotinamide **4**. It is also notable that quinolinone **5** is the only compound in Table 1 that displays appreciable agonism of S1P$_3$ (hS1P$_3$ Ca^{2+} flux; **5**: EC$_{50}$ = 1.5 μM, 92% efficacy; **6**–**19**: EC$_{50}$ >2.5 or 25 μM).[10] Other salient features of this investigation are as follows. The data for aza-analogs **7**, **10**, **12**, **13**, **15**, and **16** revealed that N atom substitution at positions 5, 8, 5′, 6′, 3″, and 4″, respectively, has a deleterious effect on potency toward S1P$_1$ (hS1P$_1$ RI EC$_{50}$ = 4.1, 0.19, 1.1, 2.9, 0.31, and 0.45 μM; and 86%, 100%, 59%, 170%, 110%, and 110% efficacy, respectively)[3] compared to **5**. In contrast, the data for aza-analogs **6**, **8**, **9**, **11**, and **14** showed that N atom substitution at positions 2, 6, 7, 3′, and 2″, respectively, has either a beneficial or neutral effect on potency toward S1P$_1$ (hS1P$_1$ RI EC$_{50}$ = 0.041, 0.024, 0.029, 0.042, and 0.060 μM; and 130%, 100%, 120%, 120%, and 110% efficacy, respectively)[3] and, with the exception of the 3′ position (aza-analog **11**), imparts improved cell permeability (P_{app} = 2.3, 5.7, 3.4, <1.0, and 11 × 10^{-6} cm/s, respectively) versus **5**. In general, the effects of a single N atom substitution on solubility and microsomal stability were found to be modest; however, incorporation of a N atom at positions 2″, 3″, or 4″ (compounds **14**, **15**, and **16**, respectively) was observed to improve solubility in 0.010 M aq HCl (Sol = 12, 23, and 6.4 μg/mL, respectively) compared to **5**, and aza-analogs **15** and **16** were the only derivatives found to exhibit >14 μL/min/mg clearance in rat liver microsomes (CL$_{int}$ = 18 and 44 μL/min/mg, respectively).

We desired next to examine the effect of quinolinone **5** and its best aza-analogs **6**, **8**, **9**, **11**, and **14** on peripheral lymphocyte counts (PLC) in vivo (1 mg/kg po).[16] Notably, aza-analog **8** is the only compound that effects statistically significant (P <0.05 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test) reduction in circulating lymphocytes (POC = −64%; [**8**]$_{plasma}$ = 350 ng/mL). Given their comparable in vitro profiles, it is unclear why derivatives **5**, **6**, **9**, **11**, and **14** do not achieve higher plasma levels and realize statistically significant reduction of peripheral lymphocyte counts. Because N atom substitution at positions 3′ and 2″ was shown to have either a beneficial or neutral effect on potency toward S1P$_1$ (compounds **11** and **14**, respectively), diaza-analogs **17** and **18** and triaza-analog **19** were synthesized. The data for diaza-analog **17** showed that N atom substitution at both positions 6 and 3′ has a neutral effect on potency (hS1P$_1$ RI EC$_{50}$ = 0.020 μM, 120% efficacy)[3] and a positive effect on cell permeability (P_{app} = 6.7 × 10^{-6} cm/s) compared to aza-analogs **8** and **11**. In contrast, the data for diaza-analog **18** and triaza-analog **19** revealed that having N atom substitution at both the 6 and 2′ positions has a negative effect on potency (hS1P$_1$ RI EC$_{50}$ = 0.20, 0.21 μM and 100%, 130% efficacy, respectively)[3] but a positive effect on cell permeability (P_{app} = 16 and 15 × 10^{-6} cm/s, respectively) and solubility (Sol = 120, <1.0, 6.7 and >200, 3.0, 5.8 μg/mL, respectively) versus aza-analogs **8** and **14** (for **18**) and diaza-analog **17** (for **19**). Of these three poly-aza-substituted compounds, only diaza-analogs **17** and **18** effect statistically significant (P <0.05 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test) reduction in circulating lymphocytes (POC = −64% and −32%, respectively; [**17**]$_{plasma}$ = 130 ng/mL; [**18**]$_{plasma}$ = 150 ng/mL).[16] The data presented in Table 1 show that embedded N atoms are tolerated at specific positions of **5**, and the resulting aza-analogs generally have improved physicochemical properties that can result in markedly enhanced in vivo pharmacodynamics.[19]

Pharmacokinetic profiles of compounds **5**, **8**, and **17** were undertaken in preparation for more detailed studies in vivo (Table 2). In

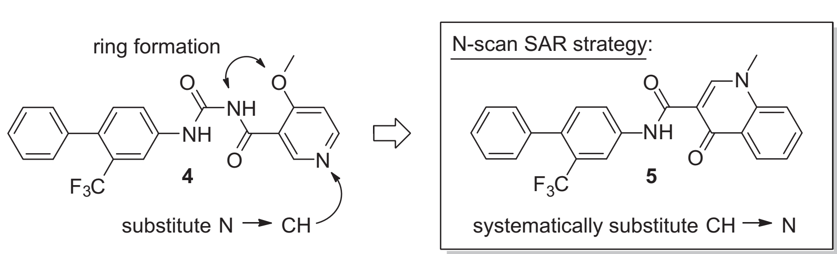
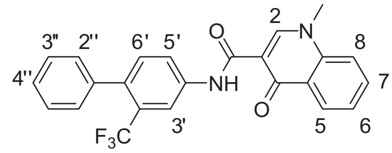


Figure 2. Transformation of **4** → **5** and design of the N-scan SAR strategy.

Table 1
N-scan SAR study of **5**: effects on physicochemical properties, potency, microsomal stability, and lymphocyte count



Compd	CH → N	c log P[a]	ACD log P[b] ($ACDLogD_{7.4}$)	$hS1P_1$ RI EC_{50}[c] μM (% eff.)	Sol (μg/mL)[d] HCl, PBS, SIF	P_{app} (ER)[e] ($\times 10^6$ cm/s)	CL_{int}, RLM[f] (μL/min/mg)	PLC, POC[g] ($[C]_{plasma}$, ng/mL)
5	–	4.8	6.2 (6.2)	0.070 (110)	1.3, <1.0, 11	1.2 (0.40)	<14	−3 (20)[h]
6	2	4.9	5.5 (5.5)	0.041 (130)	<1.0, 1.6, 12	2.3 (1.2)	<14	+6 (9.7)[h]
7	5	3.5	4.7 (4.7)	4.1 (86)	ND[i]	16 (1.4)	<14	ND[i]
8	6	3.5	4.8 (4.8)	0.024 (100)	<1.0, <1.0, 4.9	5.7 (0.80)	<14	−64 (350)[j]
9	7	3.5	4.7 (4.7)	0.029 (120)	<1.0, 2.6, 6.1	3.4 (1.0)	<14	−36 (140)[h]
10	8	3.5	5.3 (5.3)	0.19 (100)	<1.0, <1.0, 5.9	2.2 (1.7)	<14	ND[i]
11	3′	3.8	5.4 (5.4)	0.042 (120)	<1.0, <1.0, 2.3	<1.0 (NR)[k]	<14	+7 (1.3)[h]
12	5′	3.8	5.0 (5.0)	1.1 (59)	ND[i]	<1.0 (NR)[k]	<14	ND[i]
13	6′	4.0	5.8 (5.8)	2.9 (170)	<1.0, <1.0, 13	11 (1.9)	<14	ND[i]
14	2″	3.5	4.8 (4.8)	0.060 (110)	12, <1.0, 5.2	11 (0.80)	<14	+25 (5.1)[h]
15	3″	3.3	4.9 (4.9)	0.31 (110)	23, <1.0, 6.7	18 (1.6)	18	ND[i]
16	4″	3.3	4.8 (4.8)	0.45 (110)	6.4, 7.9, 12	15 (1.5)	44	ND[i]
17	6, 3′	2.4	4.1 (4.0)	0.020 (120)	<1.0, 2.4, 10	6.7 (1.0)	<14	−64 (130)[j]
18	6, 2″	2.2	3.5 (3.5)	0.20 (100)	120, <1.0, 6.7	16 (1.0)	15	−32 (150)[j]
19	6, 3′, 2″	1.2	2.7 (2.7)	0.21 (130)	>200, 3.0, 5.8	15 (1.0)	<14	−32 (140)[h]

[a] Calculated using Daylight Chemical Information Systems software (web site: www.daylight.com).
[b] Calculated using Advanced Chemistry Development (ACD/Labs) software (web site: www.acdlabs.com).
[c] Data represents an average of at least two determinations; see Ref. 15 for experimental details.
[d] Solubility in 0.010 M aqueous hydrogen chloride (HCl), phosphate-buffered saline (PBS) at pH 7.4, or simulated intestinal fluid (SIF).
[e] Apparent permeability (P_{app}) through porcine proximal tubule cells (LLC-PK1 cell line) and efflux ratio (ER).
[f] Estimated intrinsic clearance (CL_{int}) determined by incubation of test compound with rat liver microsomes (RLM) for 30 min and measurement of % turnover.
[g] Percent-of-control (POC) reduction versus vehicle in peripheral lymphocyte count (PLC) 24 h after a single oral dose (1 mg/kg; vehicle = 20% Captisol, 1% HPMC, 1% Pluronic F68, pH 2.0 with MSA) administered to female Lewis rats (N = 5/group) and total compound concentration in plasma ($[C]_{plasma}$) at the 24 h time point.
[h] The measured POC reduction in PLC is not statistically significant (P >0.05 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test).
[i] ND, not determined.
[j] The measured POC reduction in PLC is statistically significant (P <0.05 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test).
[k] NR, not reportable.

male Sprague–Dawley rats (N = 3) given a single intravenous dose (1 mg/kg), diaza-analog **17** displays a more favorable pharmacokinetic profile (CL = 0.15 L/h/kg, V_{dss} = 5.1 L/kg, $T_{1/2}$ = 24 h) compared to that for both aza-analog **8** (CL = 0.16 L/h/kg, V_{dss} = 9.2 L/kg, $T_{1/2}$ = 41 h) and quinolinone **5** (CL = 0.93 L/h/kg, V_{dss} = 11 L/kg, $T_{1/2}$ = 15 h). Additionally, diaza-analog **17** displays enhanced bioavailability (%F = 60, 95, and 110 for **5**, **8**, and **17**, respectively).[20] Although it is not perfectly linear, diaza-analog **17** also exhibits better dose-proportional exposure at high doses versus **5** and **8** ([$AUC_{0\rightarrow 24h}$ (100 mg/kg po)]/[$AUC_{0\rightarrow 24h}$ (3 mg/kg po)] = 4.7, 6.0, and 11 for **5**, **8**, and **17**, respectively). The comparable plasma protein binding of **5**, **8**, and **17** (%PPB = 97, 92, and 98, respectively) suggests that the improved pharmacokinetic profile of **17** is not merely the result of greater nonspecific binding in this compartment. Because **17** displays better cell permeability (P_{app} = 6.7 × 10^{-6} cm/s) than carbamoylnicotinamide **4** (P_{app} = 2.3 × 10^{-6} cm/s),[15] and comparable pharmacokinetics,[15] the diminished solubility of **17** (Sol = <1.0, 2.4, 10 μg/mL in HCl, PBS, and SIF, respectively) versus **4** (Sol = 17, 6.6, 16 μg/mL in HCl, PBS, and SIF, respectively)[15] may be responsible for its nonlinear exposure at high doses (⩾100 mg/kg po), a shortcoming that **4** does not suffer.[15]

We next sought to evaluate the pharmacodynamic effects of **5**, **8**, and **17** in more detail (Fig. 3).[16] Following a single oral dose in female Lewis rats (0.30, 1.0, or 3.0 mg/kg), compounds **5**, **8**, and **17** exhibit dose-proportional plasma exposure ($[\mathbf{5}]_{plasma}$ = 5.5, 18, and 48 ng/mL; $[\mathbf{8}]_{plasma}$ = 96, 380, and 1100 ng/mL; and $[\mathbf{17}]_{plasma}$ = 47, 180, and 640 ng/mL, respectively), and concomitant dose-dependent reduction in circulating lymphocytes at the 24 h time point. Statistically significant lymphocyte depletion is first realized for the 3.0 mg/kg dose for **5** and **8** and the 1.0 mg/kg dose for **17** (**5**: −51%, **8**: −64%, and **17**: −67%, respectively; P <0.05, <0.01, and <0.001 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test, respectively).[21] This dose-dependent decrease in circulating lymphocytes reaching a plateau at 76% maximal reduction (from the 3.0 mg/kg dose of **17**) is consistent with the $S1P_1$ agonist activity of **5**, **8**, and **17**.[13,14]

Compounds **5**, **8**, and **17** were prepared as detailed in Scheme 1, and their syntheses serve to exemplify the general manner in which all of the compounds discussed in Table 1 were synthesized. The preparation of quinolinone **5** was facile. Commercially available compounds 2-(trifluoromethyl)biphenyl-4-amine (**20**) and 1-methyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (**21**) were coupled using O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and Hünig's base to give quinolinone **5** in 62% yield. The synthesis of aza-analog **8** was more demanding. Methylation of commercially available ethyl 4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate (**22**) with Me_2SO_4/K_2CO_3 afforded ethyl 1-methyl-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate (**23**). Saponification of **23** ($LiOH/H_2O$) provided lithium carboxylate **24**, which was used in the next step of the synthesis without purification. The union of biphenylamine **20** and **24** was mediated by HATU/i-Pr_2NEt to afford aza-analog **8** in 30% yield. The synthesis of diaza-analog **17** was the most challenging of these three compounds. Commercially available 6-(trifluoromethyl)-2-pyridinamine (**26**) was regioselectively brominated using NBS in $CHCl_3$ to give 5-bromo-6-(trifluoromethyl)-2-pyridinamine (**27**) in moderate yield. A phenyl group was appended to **27** by palladium-mediated cross-coupling ($(Ph_3P)_4Pd/K_2CO_3$) with phenylboronic acid, affording 5-phenyl-6-(trifluoromethyl)-2-pyridinamine (**28**) in 84% yield. The lower nucleophilicity of pyridinamine **28** versus biphenylamine **20** demanded recourse to more forcing coupling conditions than those used for the synthesis of **5**

Table 2
Pharmacokinetic profiles of **5**, **8**, and **17**[a]

Compd	PPB (%)[b] rat	CL (L/h/kg)[c] 1 mg/kg iv	V_{dss} (L/kg)[c] 1 mg/kg iv	$T_{1/2}$ (h)[c] 1 mg/kg iv	$AUC_{0\to24h}$ (ng h/mL)[d] 3 mg/kg po	F[e] (%)	$AUC_{0\to24h}$ (ng h/mL)[f] 100 mg/kg po
5	97	0.93	11	15	1200	60	5800
8	92	0.16	9.2	41	5100	95	31000
17	98	0.15	5.1	24	12000	110	130000

[a] In vivo experiments were conducted using male Sprague–Dawley rats (N = 3/group).
[b] Percent rat plasma protein binding (PPB) measured in vitro following separation by ultracentrifugation.
[c] Clearance (CL), volume of distribution (V_{dss}), and half-life ($T_{1/2}$) determined following a single intravenous dose (1 mg/kg; vehicle = DMSO).
[d] Area under the plasma concentration–time curve ($AUC_{0\to24\ h}$) determined following a single oral dose (3 mg/kg; vehicle = 20% Captisol, 1% HPMC, 1% pluronic F68, pH 2.0, with MSA).
[e] Percent bioavailability (F) calculated using $AUC_{0\to\infty}$ values determined from the 1 mg/kg iv and 3 mg/kg po doses.
[f] Area under the plasma concentration–time curve ($AUC_{0\to24\ h}$) determined following a single oral dose (100 mg/kg; vehicle = 20% Captisol, 1% HPMC, 1% pluronic F68, pH 2.0, with MSA).

and **8**. Lithium carboxylate **24** was treated with $SOCl_2$ in toluene at reflux to provide acid chloride **25** (quantitative yield, crude). Finally, exposure of a solution of **25** and **28** in dichloroethane to DMAP/*i*-Pr_2NEt afforded diaza-analog **17** in 63% yield. Comparison of the synthesis of compounds **5**, **8**, and **17** illustrates that incorporating an increasing number of specifically placed N atoms into a scaffold often results in progressively more lengthy and complex synthetic sequences.

In conclusion, a set of conformationally constrained, atypical chemotypes of $S1P_1$ agonists lacking a polar head group was discovered. This homologous series of aza-substituted, quinolinone-based agonists of $S1P_1$ provided insight into SAR trends that allowed improvement of both in vitro and in vivo activity. This study illustrates that the conceptually simple N-scan SAR strategy of systematically incorporating N atoms into a scaffold to tune molecular and physicochemical properties can lead to significant improvement in potency, selectivity, pharmacokinetics, and pharmacodynamics (e.g., quinolinone **5** → aza-analog **8** → diaza-analog **17**). However, this approach can be rendered challenging by the progressively more complex synthesis of these aza-substituted compounds. Finally, although diaza-analog **17** displays improved pharmacokinetics and pharmacodynamics compared to quinolinone **5** and aza-analog **8**, it exhibits nonlinear exposure at high doses required to establish a safety margin (⩾100 mg/kg po), limiting the prospects for further development of this compound.

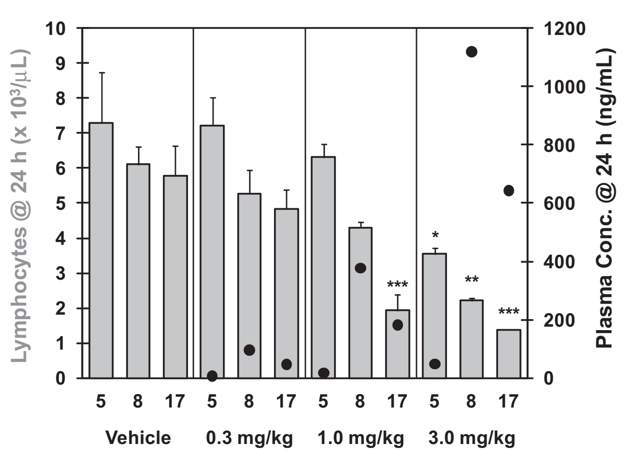
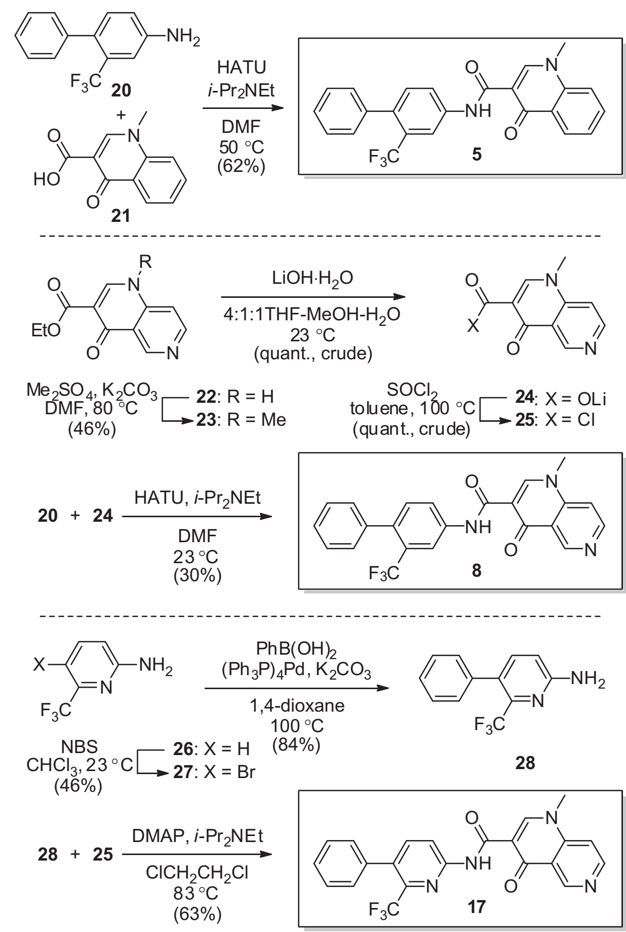


Figure 3. Female Lewis rats (N = 5/group; vehicle = 20% Captisol or HPBCD, 1% HPMC, 1% Pluronic F68, pH 2.0 with MSA) administered a single oral dose of **5**, **8**, or **17** (0.30, 1.0, or 3.0 mg/kg) showed dose-proportional plasma exposure (black circles represent average plasma concentration ± SE) and dose-dependent reduction in circulating lymphocytes (gray bars represent average blood lymphocyte counts ± SE) at the 24 h time point (statistical significance: $^{*}P$ <0.05, $^{**}P$ <0.01, or $^{***}P$ <0.001 versus vehicle by the ANOVA/Dunnett's Multiple Comparison Test); see Ref. 15 for experimental details.



Scheme 1. Chemical synthesis of compounds **5**, **8**, and **17**.

Efforts to modify the aza-quinolinone scaffold to overcome this challenge are the subject of a future disclosure.

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16. Female Lewis rats ($N = 5$/group) were administered a single oral dose of each compound; after 24 h, lymphocyte counts in blood and compound concentrations in plasma were measured (the 24 h time point was selected for these studies, as it was found to mitigate the effects of dosing on lymphocyte counts; see Ref. 15 for experimental details).
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19. Comparison of the ACDLogP and $ACDLogD_{7.4}$ values for each compound in Table 1 indicates that all are calculated to be neutral at physiological pH, and analysis of the ACD $\log P$ and $c\log P$ values reveals that each CH → N substitution typically effects ~1 unit reduction in calculated $\log P$.
20. Calculated using the $AUC_{0\rightarrow\infty}$ values determined from the 1 mg/kg iv and 3 mg/kg po doses.
21. For aza-analog **8**, the higher dose required to achieve statistically significant reduction in circulating lymphocytes in the dose–response study (3.0 mg/kg po; Fig. 3) versus the single dose experiment (1 mg/kg po; Table 1) may arise from the lower peripheral lymphocyte counts and greater variability in the vehicle group for this study ($6.1 \pm 2.5 \times 10^3$/μL vs $8.4 \pm 1.5 \times 10^3$/μL, respectively), as the peripheral lymphocyte counts and variability ($4.3 \pm 0.84 \times 10^3$/μL vs $3.1 \pm 1.2 \times 10^3$/μL, respectively), as well as total compound concentration in plasma and variability (380 ± 55 ng/mL vs 350 ± 23 ng/mL) were similar for the 1.0 mg/kg po dose in both studies.