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Toll-like receptor-8 agonistic activities in C2, C4, and C8 modified thiazolo[4,5-*c*]quinolines†

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Toll-like receptor (TLR)-8 agonists typified by the 2-alkylthiazolo[4,5-*c*]quinolin-4-amine (CL075) chemotype are uniquely potent in activating adaptive immune responses by inducing robust production of T helper 1-polarizing cytokines, suggesting that TLR8-active compounds could be promising candidate vaccine adjuvants, especially for neonatal vaccines. Alkylthiazoloquinolines with methyl, ethyl, propyl and butyl groups at C2 displayed comparable TLR8-agonistic potencies; activity diminished precipitously in the C2-pentyl compound, and higher homologues were inactive. The C2-butyl compound was unique in possessing substantial TLR7-agonistic activity. Analogues with branched alkyl groups at C2 displayed poor tolerance of terminal steric bulk. Virtually all modifications at C8 led to abrogation of agonistic activity. Alkylation on the C4-amine was not tolerated, whereas *N*-acyl analogues with short acyl groups (other than acetyl) retained TLR8 agonistic activity, but were substantially less water-soluble. Immunization in rabbits with a model subunit antigen adjuvanted with the lead C2-butyl thiazoloquinoline showed enhancements of antigen-specific antibody titers.

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Introduction

There can be no greater substantiation of Benjamin Franklin's adage, 'An ounce of prevention is better than a cure' than the resounding impact that vaccines have had in preventing morbidity and mortality due to infectious diseases.[1] Vaccines afford protection by the induction of immune responses, both humoral and cellular, specifically directed against the pathogen. Such adaptive immune responses are mobilized and amplified by engagement of the innate immune system, a phylogenetically and teleologically ancient system of host defense.[2–4] Unlike adaptive immunity, the initial innate immune responses rely on a limited number of germline-encoded pattern recognition receptors which recognize specific molecular patterns present in molecules that are broadly shared by pathogens but are sufficiently different so as to be distinguishable from host molecules.[5,6]

Innate immune afferent signals include those originating from Toll-like receptors (TLRs), as well as RIG-I-like receptors[7] and Nod-like receptors (NLRs).[8,9] There are 10 functional TLRs encoded in the human genome, which are trans-membrane proteins with an extracellular domain having leucine-rich repeats (LRR) and a cytosolic domain called the Toll/IL-1 receptor (TIR) domain.[6] The ligands for these receptors are highly conserved molecules such as lipopolysaccharides (LPS) (recognized by TLR4), lipopeptides (TLR2 in combination with TLR1 or TLR6), flagellin (TLR5), single stranded RNA (TLR7 and TLR8), double stranded RNA (TLR3), CpG motif-containing DNA (recognized by TLR9), and profilin present on uropathogenic bacteria (TLR11).[10] TLR1, -2, -4, -5, and -6 recognize extracellular stimuli, while TLR3, -7, -8 and -9 function within the endolysosomal compartment.[6]

The activation of TLRs by their cognate ligands leads to production of inflammatory cytokines, and up-regulation of MHC molecules and co-stimulatory signals in antigen-presenting cells as well as activating natural killer (NK) cells (innate immune response), which leads to the priming and amplification of T-, and B-cell effector functions (adaptive immune responses).[11–14]

In recognition that the initial innate immune responses serve to marshal, focus, and amplify subsequent adaptive immune responses,[15,16] there is considerable interest in utilizing TLR agonists as vaccine adjuvants.[17,18] In contradistinction to early vaccines which used killed whole organisms,[19–22] or attenuated live vaccines,[23–25] modern vaccines increasingly rely on subunit vaccines which have the distinct advantages of ease of production, quality control, and safety; however, such

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†Electronic supplementary information (ESI) available: Characterization of intermediates and final compounds (^{1}H, ^{13}C, mass spectra). See DOI: 10.1039/c2ob26705e

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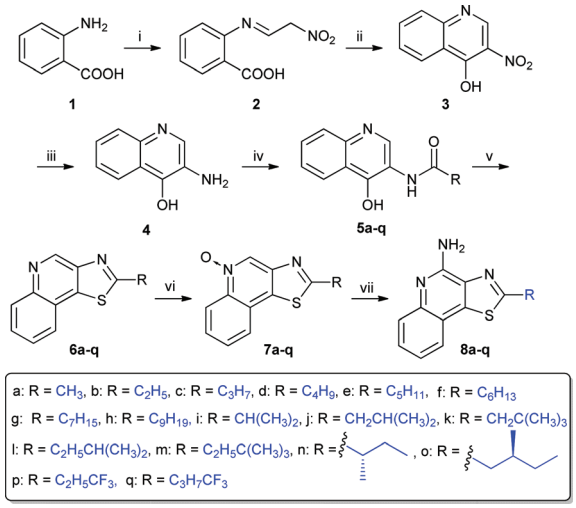
subunit antigens which are largely soluble proteins are poorly immunogenic, and require adjuvants to induce robust immune responses. Currently, the only TLR agonist approved by the FDA as an adjuvant is 3-*O*-desacyl-4′-monophosphoryl lipid A, (MPL), a TLR4 agonist derived from hydrolytic treatment of lipopolysaccharide isolated from *Salmonella minnesota* Re595.[26]

We have recently begun exploring[27,28] a variety of TLR agonists with a view to identifying safe and potent vaccine adjuvants. The chemotypes that we have explored thus far include agonists of TLR2,[29–31] TLR7,[32–36] nucleotide oligomerization domain 1 (Nod1),[37] as well as C–C chemokine receptor type 1 (CCR1).[38] We now extend our investigations toward delineating structure–activity relationships in small-molecule agonistic ligands of TLR8 (typified by the thiazolo[4,5-*c*]quinolines) for the following reasons:

First, the thiazoloquinolines are of interest because like the imidazoquinolines, these compounds were identified in antiviral assays long before the discovery of the endosomal TLR7 and TLR8 receptors,[39–43] and other than the original landmark studies performed by investigators at 3M Pharmaceuticals,[44,45] structure–activity relationships in the thiazoloquinoline chemotype remains poorly explored; qualitative assays for TNF-α and IFN-α induction in human blood were performed in initial studies by 3M as surrogate biomarkers of immunostimulation, and no data on TLR-7 and -8 specific agonistic activities exist in the literature.

Second, TLR8 agonists, both single-stranded RNA as well as small molecule imidazoquinoline ligands such as R-848[46] and thiazoloquinolines such as 3M-002 (CL075)[47,48] appear uniquely potent in activating co-stimulatory responses in neonatal antigen-presenting cells (APCs), inducing robust production of the T helper 1 (Th1)-polarizing cytokines TNF-α and IL-12, which are not observed upon stimulation by TLR-2, -4, or -7 agonists. Such Th1-biasing compounds are of particular interest as candidate vaccine adjuvants in the newborn.[49] During the first few weeks of life, newborns rely almost entirely on maternal IgG antibodies acquired by passive transplacental passage,[50] and remain susceptible to a wide range of pathogens until early infancy.[51] Neonates and infants, in whom vaccines could—and perhaps should—have the greatest impact do not mount adequate adaptive immune responses, and therefore are most vulnerable; consequently, even the most efficacious vaccines that confer excellent protection in adults may fail to elicit strong immune responses in them.[52,53] There is mounting evidence pointing to significant differences between adult and infant innate immune responses.[54] The neonatal immunophenotype is characterized by decreased production of both type I and type II interferons, Interleukin (IL)-12, IL-18, IL-23 and other proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), the preferential induction of memory B lymphocytes rather than immunoglobulin-secreting plasma cells, as well as a pronounced T-helper type 2 (Th2) skewing of T-cell responses.[51,52,55,56] TLR8 agonists induce the production of IL-12, IL-18 and IFN-γ, and may therefore be of value in developing vaccines for the neonate.



Scheme 1 Syntheses of C2-alkylthiazoloquinoline analogues. Reagents: (i) HCl, HON=CHCH$_2$NO$_2$, H$_2$O; (ii) (CH$_3$CO)$_2$O, CH$_3$COOK; (iii) Pt/C, H$_2$, DMF; (iv) RCOCl, Et$_3$N, CH$_2$Cl$_2$: DMF(10 : 1); (v) P$_2$S$_5$, pyridine; (vi) *m*-CPBA, CHCl$_3$; (vii) (a) benzoyl isocyanate, CH$_2$Cl$_2$, (b) NaOCH$_3$, MeOH.

Given the potential utility of TLR8 agonists as adjuvants for neonatal vaccines, it was of interest to explore SAR in the thiazoloquinolines (typified by CL075, **8c** in Scheme 1). Our studies began with examining the optimal alkyl chain length at the C2 position. We found that the C2-alkyl thiazoloquinolines exhibit mixed TLR8/7 agonistic activities in primary reporter gene assays with the optimal chain length being butyl. We observed an unexpectedly strict length dependence with only the C2-butyl, but none of the other analogues, inducing IFN-α in human peripheral blood mononuclear cells (hPBMCs). Examination of analogues with branched alkyl groups at C2 suggested poor tolerance of terminal steric bulk. We noted, however that certain C2-branched analogues were substantially more TLR8-selective than their corresponding straight-chain analogues. Virtually all modifications at C8 led to abrogation of agonistic activity. Alkylation on the C4-amine was not tolerated, whereas *N*-acyl analogues with short acyl groups (other than acetyl) retained activity.

Results and discussion

In our previous work on the TLR7-active imidazoquinolines, we had observed a distinct relationship between C2-alkyl chain length and TLR7-agonistic potency,[33] and we therefore thought it logical to begin our SAR studies on the thiazoloquinolines by examining analogues with C2-alkyl groups of varying chain lengths. These analogues (**8a–8h**, Scheme 1) were synthesized in parallel from the 3-aminoquinolin-4-ol precursor **4**, using the strategy described earlier.[33,44]

In primary screens using human TLR7 and TLR8-specific reporter gene assays, these analogues exhibited mixed TLR8/TLR7 agonism; the EC$_{50}$ values of **8c** (CL075) were found to be 1.32 μM and 5.48 μM, in TLR8 and TLR7 assays, respectively

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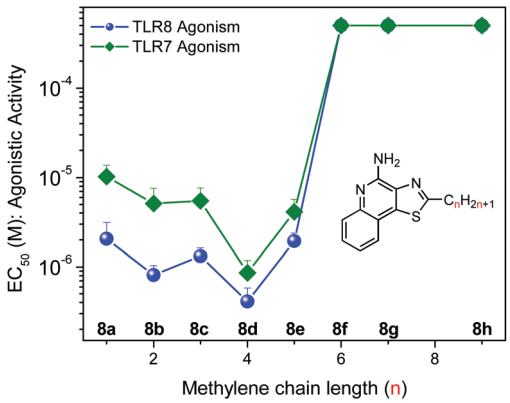

Fig. 1 TLR8 and TLR7 agonistic potencies of the C2-alkyl thiazoloquinoline homologues. Data points represent means and standard deviations of EC_{50} values derived from dose-response profiles and are computed on quadruplicates.

(Fig. 1, Table 1). As in our earlier SAR studies on the imidazoquinolines,[33] we observed a clear dependence of agonistic potency on the C2-alkyl chain length. The C2-methyl, -ethyl, and -propyl analogues (**8a–8c**) displayed comparable potencies in the hTLR8 and hTLR7 reporter gene assays (Fig. 1). Maximal TLR8-agonistic potency was observed in the butyl analogue (**8d**). Increasing the chain length to pentyl (**8e**) led to attenuation of potency, and higher homologues (**8f–8h**) showed abrogation of activity (Fig. 1, Table 1).

The C2-butyl analogue **8d** appeared to exhibit substantially higher TLR7-agonistic potency than **8c** (Fig. 1 and 3), suggesting that small variations at C2 could modulate TLR8 *versus* TLR7 specificity. The introduction of an isopropyl group at C2 (**8i**) led to an approximately ten-fold reduction in TLR8-agonistic potency (Table 1), while the attenuation of its activity in TLR7-specific reporter gene assays was more modest (about two-fold). These results warranted the syntheses of several additional analogues of both **8c** (C2-*n*-propyl) and **8d** (C2-*n*-butyl) with branched alkyl groups at C2 (**8j–8o**, Scheme 1, Fig. 2). Additional methyl substitutions at $\omega - 1$ as well as $\omega - 2$ positions were well tolerated in **8c** derivatives (**8j** and **8n**, respectively; Scheme 1, Fig. 2). On the other hand only the $\omega - 2$ methyl-substituted compound **8o** was active in the **8d** series, whereas the **8l** congener with a terminal methyl substitution was entirely inactive. Both **8k** and **8m** bearing terminal trimethyl groups were also inactive, suggesting poor tolerance of terminal steric bulk in these compounds.

We also evaluated bioisosteric analogues **8p** and **8q** (Scheme 1) with terminal trifluoromethyl groups. A decrease in potency at TLR8 was observed in the longer homologue (**8q**). We noted that, in general, analogues with branched C2-alkyl groups of optimal chain length and bulk (such as **8j**, **8n**, and **8o**) are substantially more TLR8-selective than their corresponding straight-chain analogues (Fig. 2).

However, in secondary cytokine induction screens using human PBMCs, the branched-chain analogues behave differently in that they were neither as potent as their straight-chain parent compounds (Fig. 3), nor did they show enhanced IL-12, IL-18, and IFN-γ production (data not shown) as would be expected for TLR8-selective compounds.[47,48] We do not yet understand the basis of attenuated activity of the C2-branched analogues, and we are currently exploring whether differential plasma protein binding behavior of these compounds could be contributory.[57]

Previous reports on the thiazoloquinolines[44,45,58] had been performed largely on the C7 position, and C8 and C4 analogues remain unexplored. We therefore first examined substituents on the quinoline at the C8 position of **8c** (CL075). Electrophilic substitution on the quinoline ring selectively afforded the 8-nitro analogue **9** (Scheme 2), and the 8-bromo analogue **14** (Scheme 4), both of which were inactive. The 8-amino (**10**) and 8-azido (**11**) analogues were obtained from **9** (Scheme 2). Compound **10** showed attenuated activity relative to **8c**, and **11** was inactive. The triazole derivatives **12a–g** were synthesized from **11** (Scheme 2) using conventional copper-catalyzed 'click' chemistry; of these analogues, the triazolo analogue **12e** displayed feeble, but selective TLR8 agonism (Table 1). Selective C8 *N*-alkylation and *N*-acylation of the 8-amino analogue **10** provided analogues **13a** and **13b** (Scheme 3), both of which were inactive (Table 1).

Next, the C4-amine of **8c** was alkylated or acylated, yielding analogues **15a–15l** (Scheme 5). The C4-*N*-alkylated compounds **15a** and **15b** were devoid of any agonistic activity, while some, but not all of the C4-*N*-acylated derivatives with short acyl groups were found to be active. Specifically, the formyl (**15d**), and butyryl (**15f**) analogues, but not the acetyl (**15e**) compounds were active.

Although weaker in potency than the parent compound **8c**, these C4-*N*-acyl derivatives exhibited a pronounced specificity for TLR8 (Table 1). We observed an unusual discontinuity in the activity profiles in this homologous series of compounds. The TLR8-agonistic potency of the formyl analogue **15d** was comparable to that of **8c**. The acetyl analogue **15e**, however, was virtually inactive, but activity was partially restored in the butyryl compound **15f** (Fig. 4A), while higher homologues (**15g** and **15h**) were inactive (Table 1).

Cytokine induction profiles in hPBMCs mirrored these findings with a near-complete loss of activity for the acetyl compound **15d** (Fig. 5A).

Our provisional interpretation that a strict length requirement exists for the acyl substituents on the C4-amine was borne out in compounds **15j–15l** (azidoacetamide, azidopropionamide and pentynamide analogues, respectively) which displayed weak, TLR8-selective agonistic activity (Table 1). Aromatic amides at this position appear not to be tolerated, since the benzamide analogue **15i** was entirely inactive.

A series of carbamates (**15m–15p**), and sulfonamides (**15r–15v**) were also synthesized (Scheme 5). The carbamate derivatives showed very weak activity in primary reporter gene assays, while the sulfonamide analogues were completely inactive (Table 1). Several other analogs including urea (**15q**; Scheme 5), phosphoramidate (**15w**; Scheme 5) and guanidine

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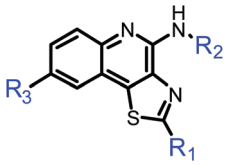
Table 1 EC_{50} values of thiazoloquinolines in human TLR8- and TLR7-specific reporter gene assays



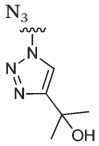
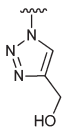
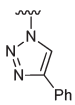
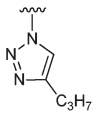
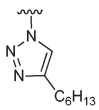
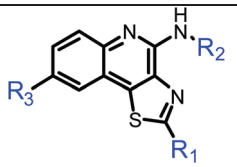
Compound number	R_1	R_2	R_3	Agonistic activity (μM) TLR8	TLR7
8a	CH_3	H	H	2.06	10.24
8b	C_2H_5	H	H	0.81	5.12
8c	C_3H_7	H	H	1.32	5.48
8d	C_4H_9	H	H	0.41	0.86
8e	C_5H_{11}	H	H	1.94	4.14
8f	C_6H_{13}	H	H	Inactive[a]	Inactive
8g	C_7H_{15}	H	H	Inactive	Inactive
8h	C_9H_{19}	H	H	Inactive	Inactive
8i	$CH(CH_3)_2$	H	H	10.85	9.67
8j	$CH_2CH(CH_3)_2$	H	H	1.55	6.96
8k	$CH_2C(CH_3)_3$	H	H	Inactive	Inactive
8l	$C_2H_5CH(CH_3)_2$	H	H	Inactive	Inactive
8m	$C_2H_5C(CH_3)_3$	H	H	Inactive	Inactive
8n	(*S*)-2-(*sec*-Butyl)	H	H	2.77	8.11
8o	(*S*)-2-(2-Methylbutyl)	H	H	2.51	4.74
8p	$C_2H_5CF_3$	H	H	1.61	3.63
8q	$C_3H_7CF_3$	H	H	6.29	2.25
9	C_3H_7	H	NO_2	Inactive	Inactive
10	C_3H_7	H	NH_2	3.88	8.19
11	C_3H_7	H	N_3	2.06	10.24
12a	C_3H_7	H		Inactive	Inactive
12b	C_3H_7	H		Inactive	Inactive
12c	C_3H_7	H		Inactive	Inactive
12d	C_3H_7	H		Inactive	Inactive
12e	C_3H_7	H		Inactive	Inactive

Table 1 *(Contd.)*



Compound number	R_1	R_2	R_3	Agonistic activity (μM) TLR8	TLR7
12f	C_3H_7	H		9.84	Inactive
12g	C_3H_7	H		Inactive	Inactive
13a	C_3H_7	H	C_6H_{13}	Inactive	Inactive
13b	C_3H_7	H	COC_3H_7	Inactive	Inactive
14	C_3H_7	H	Br	Inactive	Inactive
15a	C_3H_7	C_4H_9	H	Inactive	Inactive
15b	C_3H_7	C_6H_{13}	H	Inactive	Inactive
15c	C_3H_7	$C_{16}H_{33}$	H	Inactive	Inactive
15d	C_3H_7	CHO	H	Inactive	Inactive
15e	C_3H_7	$COCH_3$	H	0.88	Inactive
15f	C_3H_7	COC_3H_7	H	1.14	Inactive
15g	C_3H_7	COC_7H_{15}	H	Inactive	Inactive
15h	C_3H_7	$COC_{15}H_{31}$	H	Inactive	Inactive
15i	C_3H_7	COC_5H_5	H	Inactive	Inactive
15j	C_3H_7	$COCH_2N_3$	H	1.20	3.53
15k	C_3H_7	$COC_2H_4N_3$	H	2.23	Inactive
15l	C_3H_7	$COC_2H_4C{\equiv}C$	H	1.20	Inactive
15m	C_3H_7	CO_2CH_3	H	Weak	Inactive
15n	C_3H_7	$CO_2C_2H_5$	H	Inactive	Inactive
15o	C_3H_7	$CO_2C_4H_9$	H	Inactive	Inactive
15p	C_3H_7	$CO_2C_8H_{17}$	H	Inactive	Inactive
15q	C_3H_7	$CONH_2$	H	Inactive	Inactive
15r	C_3H_7	SO_2CH_3	H	10.56	Inactive
15s	C_3H_7	$SO_2C_2H_5$	H	Inactive	Inactive
15t	C_3H_7	$SO_2C_3H_7$	H	Inactive	Inactive
15u	C_3H_7	$SO_2C_4H_9$	H	Inactive	Inactive
15v	C_3H_7	$SO_2C_6H_4CH_3$	H	Inactive	Inactive
15w	C_3H_7	$PO(OEt)_2$	H	Inactive	Inactive
17	C_3H_7	$CNHNH_2$	H	Inactive	Inactive
18a	C_4H_9	CHO	H	2.31	2.54
18b	C_4H_9	$COCH_3$	H	Inactive	Inactive
18c	C_4H_9	COC_3H_7	H	0.42	0.49

[a] EC_{50} value is >100 μM.

(**17**; Scheme 6) functional groups at C4 were also examined, but were found to be inactive.

The peculiar SAR, with only the acetamide analogue (**15e**) showing loss of activity was unexpected and, desiring to confirm this pattern, we also synthesized select amide analogues of **8d** (**18a–18c**, formamide, acetamide, and butyramide derivatives, respectively; Scheme 7). As with the **8c** derivatives, a virtually identical pattern was observed, with a selective loss of activity for the acetamide analogue **18b** (Fig. 4B, 5B, Table 1).

Our studies aimed at delinating structure–activity relationships in the thiazoloquinolines have led to selecting **8d** as a lead thiazoloquinoline. This compound distinguishes itself from its closely-related congener, **8c** (CL075) in possessing a more balanced TLR8/TLR7-agonistic properties. Virtually all other analogues were either of lower potency, exhibited very poor aqueous solubility (such as the amide analogues **15d** and **18a**), or despite apparent greater TLR8 selectivity in primary

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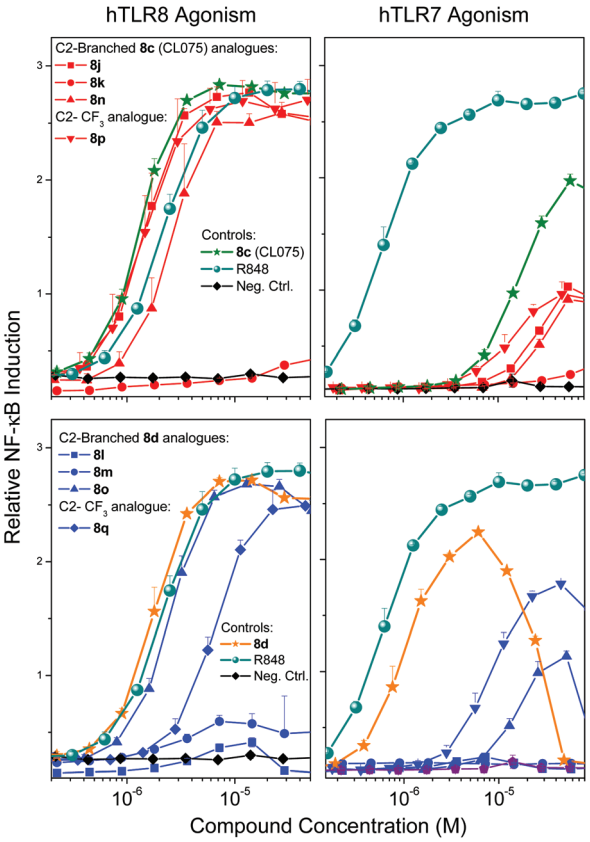


Fig. 2 TLR8- and TLR7-specific NF-κB induction profiles of branched-chain and trifluoromethyl analogues of **8c** and **8d**. Means and SD obtained from quadruplicate samples are shown.

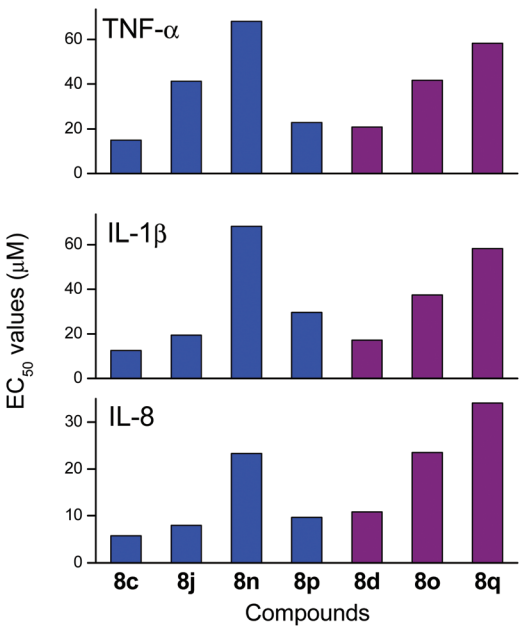
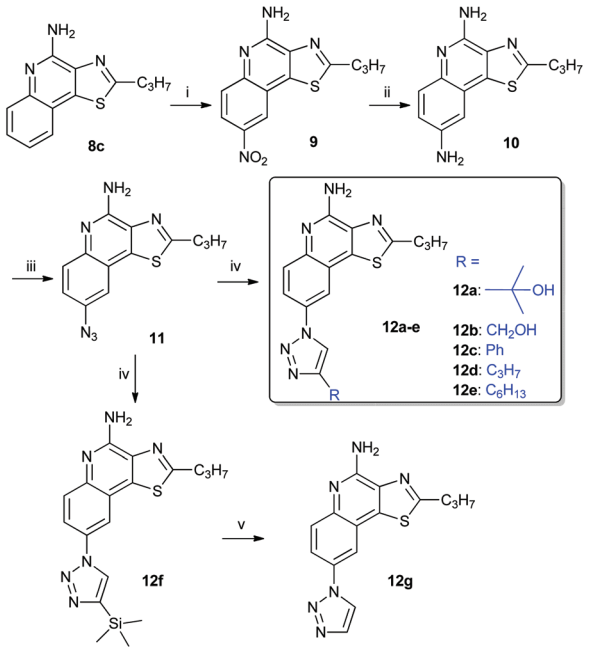
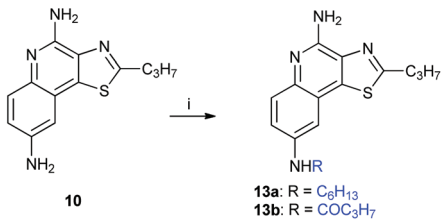


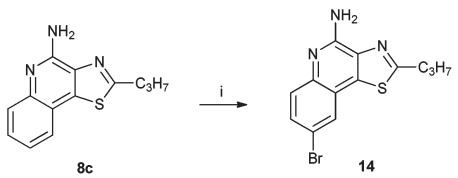
Fig. 3 EC_{50} values of proinflammatory cytokine induction in human PBMCs by analogues of **8c** and **8d**. Representative data from three independent experiments are shown.



Scheme 2 Modification at the C8 position. Reagents: (i) HNO_3, H_2SO_4; (ii) Zn, NH_4COOH, MeOH; (iii) $NaNO_2$, CH_3COOH, NaN_3; (iv) alkyne, $CuSO_4$, sodium ascorbate, THF, H_2O (v) TBAF, THF.



Scheme 3 Synthesis of C8 *N*-alkyl and *N*-acyl analogues. Reagents: (i) For **13a**, $C_6H_{13}I$, K_2CO_3, DMF; For **13b**, C_3H_7COCl, Et_3N, CH_2Cl_2.



Scheme 4 Synthesis of 8-bromothiazoloquinoline. Reagents: (i) NBS, NH_4OAc, CH_3CN.

screens, were either unremarkable in secondary screens employing human PBMCs, or inferior in their cytokine induction profiles (**8j**, **8n**, and **8o**, for instance). We therefore elected to directly evaluate the adjuvantic properties of **8d**, and benchmark it against **8c** (CL075), noting that adjuvantic properties for the thiazoloquinolines have not been previously reported in the literature. In light of the fact that murine TLR8 is thought to be functionally inactive,[59–61] we chose to examine the adjuvantic activities of **8c**, **8d** directly in a rabbit model using bovine α-lactalbumin as a model subunit vaccine

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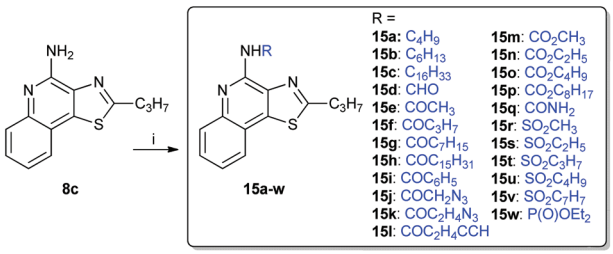


Scheme 5 Syntheses of C4 *N*-alkyl and *N*-acyl analogues. Reagents: (i) for **15a**, **15b** and **15c**, RX, NaH, THF; for **15d**, 2,2,2-trifluoroethylformate, Et_3N; for **15e–i**, RCOCl, Py; for **15j–l**, RCO_2H, HBTU, Et_3N; for **15m–p**, ROCOCl, Et_3N, CH_2Cl_2; for **15q**, $ClSO_2NCO$, $NaHCO_3$; for **15r–v**, RSO_2Cl, CH_2Cl_2; for **15w**, $ClP(O)OEt_2$, CH_2Cl_2.

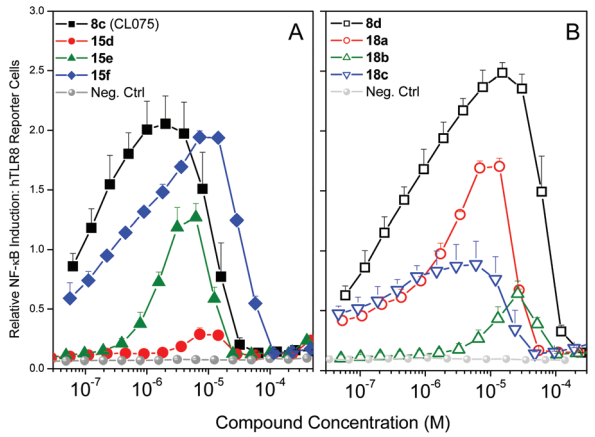


Fig. 4 TLR8 induction by C4-amides of **8c** (Panel A) and **8d** (Panel B). Data points represent means and standard deviations on quadruplicates.

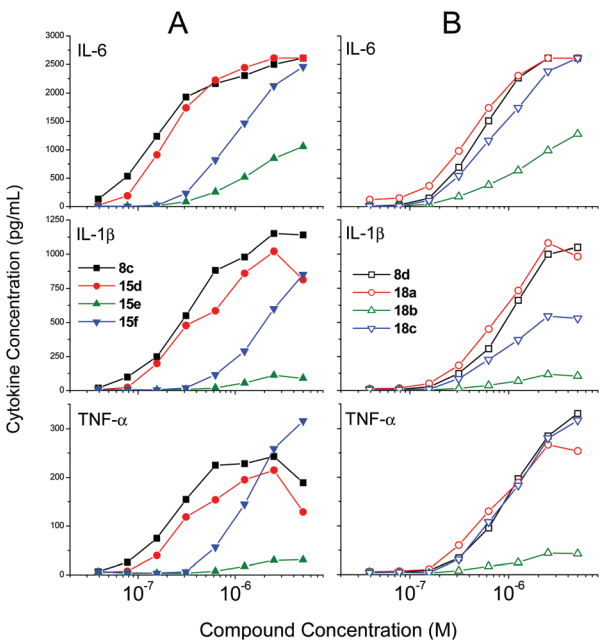
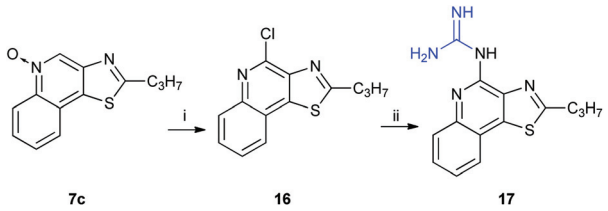
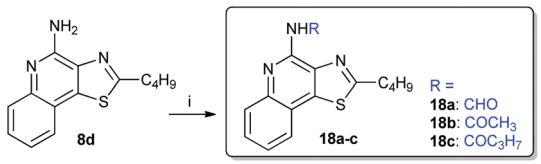


Fig. 5 Dose–response profiles of proinflammatory cytokine induction in hPBMCs by C4-amides of **8c** (Panel A) and **8d** (Panel B). Representative data from three independent experiments are presented. Vehicle controls (not shown) elicited undetectable levels of cytokines.



Scheme 6 Syntheses of C4 guanidino analog. Reagents: (i) $POCl_3$, 100 °C; (ii) guanidine hydrochloride, NaH, 1,4-dioxane.



Scheme 7 Syntheses of C4 *N*-alkyl and *N*-acyl analogues of **8d**. Reagents: (i) 2,2,2-trifluoroethylformate, Et_3N; For **18a**; RCOCl, pyridine for **18b** and **18c**.

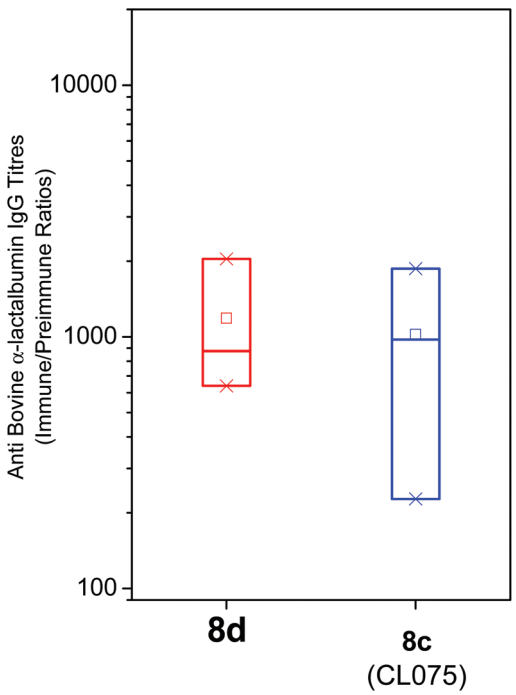


Fig. 6 Box-plots of anti-bovine α-lactalbumin IgG titers in cohorts of three rabbits immunized with α-lactalbumin adjuvanted with either **8c** or **8d**. Means and medians of titers are represented by □ and — symbols within the box, respectively, and the × symbols indicate the 1% and 99% percentile values.

antigen, which is a small (14 kDa), soluble protein which we have adopted as our test-antigen in ongoing humoral and cellular immune response assays.[35] We found that both **8c** and **8d** were adjuvantic in evoking high antigen-specific IgG titers (Fig. 6). Importantly, no evidence of local or systemic toxicity was apparent in any of the cohorts. The data suggests that **8d**, with its dual TLR8/TLR7-agonistic properties, elicits adjuvanticity with greater consistency and uniformity, as evidenced by narrower confidence intervals of antibody titers (Fig. 6).

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Conclusions

TLR8 agonists are thought to be uniquely potent in activating adaptive immune responses by inducing robust production of T helper 1-polarizing cytokines, and may be promising candidate vaccine adjuvants, especially for neonatal vaccines. Thiazoloquinoline analogues with methyl, ethyl, propyl and butyl groups at C2 displayed comparable TLR8-agonistic potencies; however, the C2-butyl compound **8d** was unique in possessing substantial TLR7-agonistic activity. Analogues with branched alkyl groups at C2 displayed poor tolerance of terminal steric bulk. C4-*N*-acyl analogues with short acyl groups (other than acetyl) retained TLR8 agonistic activity, but were substantially less water-soluble. Immunization in rabbits with a model subunit antigen adjuvanted with the most potent TLR8 agonist showed dramatic enhancements of antigen-specific antibody titers.

Experimental

Materials and equipment

All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or air-sensitive reactions were conducted under nitrogen atmosphere in oven-dried (120 °C) glass apparatus. The solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep Rf 'Gold' high performance silica columns on CombiFlash Rf instrument unless otherwise mentioned, while thin-layer chromatography was carried out on silica gel (200 μm) CCM pre-coated aluminum sheets. Purity for all final compounds was confirmed to be greater than 98% by LC-MS using a Zorbax Eclipse Plus 4.6 mm × 150 mm, 5 μm analytical reverse phase C18 column with H_2O–isopropanol or H_2O–CH_3CN gradients and either an Agilent ESI-TOF mass spectrometer (mass accuracy of 20 ppm) or an Agilent 6520 ESI-QTOF mass spectrometer (mass accuracy of <10 ppm) operating in the positive ion (or negative ion, as appropriate) acquisition mode.

Synthesis of compound 2: 2-(2-nitroethylideneamino)benzoic acid

Nitromethane (4.32 mL, 80 mmol) was added dropwise to a solution of NaOH (9.6 g, 250 mmol) in water (10 mL), at 0 °C. The mixture was then warmed to 40 °C and nitromethane (4.32 mL, 80 mmol) was again added slowly at 40–45 °C. The temperature was maintained until a clear solution was obtained. The reaction mixture was then heated to 55 °C for 2–5 minutes, cooled to 30 °C, poured onto crushed ice and acidified with conc. HCl (11 mL). The resultant solution of methazoic acid was added immediately to a filtered solution of anthranilic acid **1** (10 g, 73 mmol) and conc. HCl (3.3 mL) in water (75 mL). The reaction mixture was allowed to stand at room temperature for 12 h. After filtration, the residue obtained was washed with water, and dried to yield compound **2** (12.94 g, 85%). ^{1}H NMR (400 MHz, MeOD) δ 8.14 (d, J = 7.7 Hz, 1H), 7.83 (d, J = 6.1 Hz, 1H), 7.68–7.59 (m, 2H), 7.22 (t, J = 7.4 Hz, 1H), 6.73 (d, J = 6.3 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 168.6, 141.2, 136.6, 134.3, 132.0, 123.2, 116.7, 114.5, 100.0. MS (ESI) calculated for $C_9H_8N_2O_4$, m/z 208.05, found 209.06 $(M + H)^+$.

Synthesis of compound 3: 3-nitroquinolin-4-ol

A solution of compound **2** (12.94 g, 62.2 mmol) in acetic anhydride (50 mL) was placed in a 2-neck flask fitted with a reflux condenser. It was stirred and heated to 105 °C until a clear solution was obtained. Heating was then discontinued and potassium acetate (6.22 g, 63.5 mmol) was added. The mixture was then refluxed for 15 min with vigorous stirring, until a solid started to precipitate. The reaction mixture was then slowly cooled to room temperature. The residue was filtered, washed with glacial acetic acid until the washings were colorless, then suspended in water, filtered, washed with water and dried at 110 °C to get 3-nitroquinolin-4-ol **3** (4.68 g, 40%). ^{1}H NMR (500 MHz, DMSO) δ 13.04 (s, 1H), 9.21 (s, 1H), 8.25 (dd, J = 8.1, 1.1 Hz, 1H), 7.83–7.77 (m, 1H), 7.74–7.70 (m, 1H), 7.52 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 167.6, 142.5, 138.3, 133.2, 130.9, 128.1, 125.9, 125.8, 119.5. MS (ESI) calculated for $C_9H_6N_2O_3$, m/z 190.04, found 191.05 $(M + H)^+$.

Synthesis of compound 4: 3-aminoquinolin-4-ol

To a solution of compound **3** (1.89 g, 9.93 mmol) in DMF (25 mL), was added 5% Pt on carbon (20%, 0.38 g). The reaction mixture was allowed to react in a Parr hydrogenation apparatus at 60 psi H_2 pressure for 3.5 h with vigorous shaking. The reaction mixture was filtered through celite with several washes of methanol. The filtrate was concentrated by evaporation to get 4-amino-3-nitroquinoline (1.5 g, 94%). ^{1}H NMR (500 MHz, MeOD) δ 8.90 (d, J = 8.1 Hz, 1H), 8.33 (d, J = 5.8 Hz, 1H), 8.30–8.23 (m, 2H), 7.97 (ddd, J = 8.1, 6.0, 1.9 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 146.9, 140.8, 138.9, 134.2, 131.2, 130.6, 128.7, 127.5. MS (ESI) calculated for $C_9H_8N_2O$, m/z 160.06, found 161.07 $(M + H)^+$.

Synthesis of compound 5a: *N*-(4-hydroxyquinolin-3-yl)-acetamide

Compound **4** (200 mg, 1.25 mmol) was dissolved in a mixture of CH_2Cl_2 (20 mL) and DMF (2 mL) and stirred at room temperature for 5 min. Acetyl chloride (133 μL, 1.875 mmol) was added to the stirring reaction mixture at 0 °C and the solution was allowed to react for 1.5 h. Solvents were removed and the crude residue was purified using silica gel column chromatography (10% MeOH/CH_2Cl_2) to obtain the compound **5a** (106 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, J = 5.5 Hz, 1H), 8.86 (s, 1H), 8.46 (s, 1H), 8.41 (dd, J = 8.3, 1.0 Hz, 1H), 7.62 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.41–7.32 (m, 2H), 2.26 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$)δ 169.1, 137.9, 132.1, 126.6, 126.3, 123.6, 123.3, 122.9, 117.6, 24.6. MS (ESI) calculated for $C_{11}H_{10}N_2O_2$, m/z 202.07, found 203.08 $(M + H)^+$.

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Compounds **5b–5q** were synthesized similarly as compound **5a**.

5b: *N*-(4-Hydroxyquinolin-3-yl)propionamide

(132 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.71 (s, 1H), 8.42 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.4 Hz, 1H), 7.49 (s, 1H), 7.38 (s, 1H), 2.56 (dd, J = 14.2, 7.0 Hz, 2H), 1.31 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.9, 132.2, 126.1, 126.1, 124.5, 124.1, 124.0, 123.9, 118.0, 30.6, 9.9. MS (ESI) calculated for $C_{12}H_{12}N_2O_2$, m/z 216.09, found 217.10 $(M + H)^+$.

5c: *N*-(4-Hydroxyquinolin-3-yl)butyramide

^{1}H NMR (500 MHz, MeOD) δ 9.02 (s, 1H), 8.28 (dd, J = 8.3, 1.3 Hz, 1H), 7.68 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.42–7.35 (m, 1H), 2.46 (t, J = 7.5 Hz, 2H), 1.74 (dd, J = 14.9, 7.4 Hz, 2H), 1.01 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 174.6, 172.2, 139.7, 133.1, 131.3, 126.2, 124.8, 124.7, 122.8, 119.4, 39.6, 20.3, 14.0. MS (ESI) calculated for $C_{13}H_{14}N_2O_2$, m/z 230.11, found 231.11 $(M + H)^+$.

5d: *N*-(4-Hydroxyquinolin-3-yl)pentanamide

(155 mg, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.61 (s, 1H), 9.18 (s, 1H), 8.98 (s, 1H), 8.19 (d, J = 8.0 Hz, 1H), 7.72–7.62 (m, 2H), 7.37–7.30 (m, 1H), 2.44 (t, J = 7.4 Hz, 2H), 1.60–1.52 (m, 2H), 1.32 (dq, J = 14.7, 7.4 Hz, 2H), 0.89 (dd, J = 12.5, 5.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 168.9, 138.0, 131.4, 129.1, 124.0, 123.0, 122.9, 121.4, 118.5, 27.4, 21.9, 13.8. MS (ESI) calculated for $C_{14}H_{16}N_2O_2$, m/z 244.12, found 245.13 $(M + H)^+$.

5e: *N*-(4-Hydroxyquinolin-3-yl)hexanamide

(141 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.44 (d, J = 8.2 Hz, 1H), 8.31 (d, J = 7.2 Hz, 1H), 7.88 (t, J = 7.4 Hz, 1H), 7.68 (t, J = 7.3 Hz, 1H), 2.82 (t, J = 6.5 Hz, 2H), 1.88–1.76 (m, 2H), 1.48–1.35 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.9, 137.1, 133.7, 128.0, 124.8, 122.0, 120.7, 120.1, 36.1, 31.3, 25.6, 22.5, 14.1. MS (ESI) calculated for $C_{15}H_{18}N_2O_2$, m/z 258.14, found 259.15 $(M + H)^+$.

5f: *N*-(4-Hydroxyquinolin-3-yl)heptanamide

(105 mg, 31%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (d, J = 6.3 Hz, 1H), 8.88 (d, J = 1.6 Hz, 1H), 8.46 (s, 1H), 8.43–8.40 (m, 1H), 7.62 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.40–7.33 (m, 2H), 2.49–2.44 (m, 2H), 1.80–1.72 (m, 2H), 1.44–1.36 (m, 2H), 1.32 (td, J = 7.1, 3.5 Hz, 4H), 0.89 (dd, J = 9.7, 4.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 170.7, 138.0, 132.0, 126.5, 126.3, 123.5, 123.3, 122.8, 117.6, 37.7, 31.7, 29.1, 25.8, 22.6, 14.2. MS (ESI) calculated for $C_{16}H_{20}N_2O_2$, m/z 272.15, found 273.16 $(M + H)^+$.

5g: *N*-(4-Hydroxyquinolin-3-yl)octanamide

(263 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (d, J = 6.4 Hz, 1H), 8.99 (s, 1H), 8.46 (s, 1H), 8.43–8.40 (m, 1H), 7.62 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.40–7.33 (m, 2H), 2.49–2.44 (m, 2H), 1.81–1.73 (m, 2H), 1.41–1.24 (m, 8H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 170.8, 138.0, 132.0, 126.6, 126.3, 123.5, 123.3, 122.8, 117.6, 37.7, 31.9, 29.4, 29.2, 25.9, 22.8, 14.2. MS (ESI) calculated for $C_{17}H_{22}N_2O_2$, m/z 286.17, found 287.18 $(M + H)^+$.

5h: *N*-(4-Hydroxyquinolin-3-yl)decanamide

(231 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.47 (s, 1H), 8.41 (dd, J = 8.2, 1.0 Hz, 1H), 7.63–7.58 (m, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.37–7.32 (m, 1H), 2.50–2.43 (m, 2H), 1.80–1.72 (m, 2H), 1.43–1.20 (m, 12H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 170.8, 138.0, 132.0, 126.8, 126.2, 123.5, 123.3, 122.7, 117.7, 37.7, 32.0, 29.6, 29.5, 29.4, 29.4, 25.9, 22.8, 14.3. MS (ESI) calculated for $C_{19}H_{26}N_2O_2$, m/z 314.20, found 315.21 $(M + H)^+$.

Synthesis of compound 6a: 2-methylthiazolo[4,5-*c*]quinoline

To a solution of compound **5a** (100 mg, 0.49 mmol) in pyridine (10 mL), was added phosphorous pentasulfide (109 mg, 0.49 mmol) and reaction mixture was refluxed for 2 h. The resulting solution was cooled to room temperature and pyridine was removed under reduced pressure. The residue was dissolved in water and pH was adjusted to 8 with saturated sodium bicarbonate solution and extracted in ethyl acetate. The organic layer was dried over sodium sulfate and concentrated to afford compound **6a** (64 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (s, 1H), 8.23 (d, J = 8.3 Hz, 1H), 7.94 (dd, J = 8.1, 0.9 Hz, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.62 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 2.94 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.5, 148.0, 145.6, 144.3, 141.1, 130.6, 128.8, 127.6, 125.0, 123.5, 20.3. MS (ESI) calculated for $C_{11}H_8N_2S$, m/z 200.04, found 201.05 $(M + H)^+$.

Compounds **6b–6q** were synthesized similarly as compound **6a**.

6b: 2-Ethylthiazolo[4,5-*c*]quinoline

(90 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.99–7.94 (m, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.64 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.26 (q, J = 7.6 Hz, 2H), 1.54 (dd, J = 10.6, 4.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.2, 147.9, 145.8, 144.2, 140.6, 130.6, 128.8, 127.6, 125.0, 123.6, 28.0, 14.0. MS (ESI) calculated for $C_{12}H_{10}N_2S$, m/z 214.06, found 215.08 $(M + H)^+$.

6c: 2-Propylthiazolo[4,5-*c*]quinoline

(950 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.97 (dd, J = 8.1, 0.9 Hz, 1H), 7.74 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.69–7.59 (m, 1H), 3.26–3.11 (m, 2H), 2.04–1.92 (m, 2H), 1.10 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8, 147.9, 145.8, 144.3, 140.7, 130.6, 128.8, 127.6, 125.0, 123.6, 36.4, 23.3, 13.8. MS (ESI) calculated for $C_{13}H_{12}N_2S$, m/z 228.07, found 229.08 $(M + H)^+$.

6d: 2-Butylthiazolo[4,5-*c*]quinoline

(108 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.96 (dd, J = 8.1, 0.9 Hz, 1H), 7.73 (ddd, J = 8.4, 5.4, 1.4 Hz, 1H), 7.63 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.25–3.19 (m, 2H), 1.97–1.89 (m, 2H), 1.55–1.46 (m, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 147.9,

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145.8, 144.2, 140.6, 130.6, 128.8, 128.8, 127.6, 125.0, 123.6, 34.2, 32.0, 22.4, 13.9. MS (ESI) calculated for $C_{14}H_{14}N_2S$, *m/z* 242.09, found 243.10 $(M + H)^+$.

6e: 2-Pentylthiazolo[4,5-*c*]quinoline

(104 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.96 (dd, J = 8.1, 0.9 Hz, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.63 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.24–3.17 (m, 2H), 1.95 (dt, J = 15.3, 7.7 Hz, 2H), 1.50–1.36 (m, 4H), 0.93 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 147.9, 145.8, 144.2, 140.7, 130.6, 128.8, 127.6, 125.0, 123.6, 34.4, 31.4, 29.6, 22.5, 14.1. MS (ESI) calculated for $C_{15}H_{16}N_2S$, *m/z* 256.10, found 257.14 $(M + H)^+$.

6f: 2-Hexylthiazolo[4,5-*c*]quinoline

(93 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.97 (dd, J = 8.1, 0.9 Hz, 1H), 7.74 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.66–7.62 (m, 1H), 3.25–3.19 (m, 2H), 1.98–1.90 (m, 2H), 1.48 (dd, J = 10.5, 4.5 Hz, 2H), 1.36 (ddd, J = 16.3, 7.0, 4.9 Hz, 4H), 0.90 (dd, J = 9.4, 4.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 147.9, 145.8, 144.3, 140.7, 130.6, 128.8, 127.6, 125.0, 123.6, 34.5, 31.6, 29.9, 28.9, 22.6, 14.2. MS (ESI) calculated for $C_{16}H_{18}N_2S$, *m/z* 270.12, found 271.14 $(M + H)^+$.

6g: 2-Heptylthiazolo[4,5-*c*]quinoline

(108 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 7.96 (dd, J = 8.1, 0.9 Hz, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.65–7.60 (m, 1H), 3.22–3.17 (m, 2H), 1.94 (dt, J = 15.3, 7.6 Hz, 3H), 1.50–1.43 (m, 3H), 1.40–1.35 (m, 2H), 1.31–1.27 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 147.9, 145.8, 144.2, 140.6, 130.6, 128.8, 127.6, 125.0, 123.6, 34.5, 31.8, 29.9, 29.2, 29.1, 22.7, 14.2. MS (ESI) calculated for $C_{17}H_{20}N_2S$, *m/z* 284.13, found 285.17 $(M + H)^+$.

6h: 2-Nonylthiazolo[4,5-*c*]quinoline

(157 mg, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.73 (ddd, J = 8.4, 5.3, 1.4 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H), 3.23–3.18 (m, 2H), 1.94 (dt, J = 15.4, 7.7 Hz, 2H), 1.51–1.43 (m, 2H), 1.40–1.34 (m, 2H), 1.31–1.24 (m, 8H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 147.9, 145.8, 144.2, 130.6, 128.8, 128.7, 127.6, 125.0, 123.6, 120.3, 34.5, 32.0, 29.9, 29.5, 29.4, 29.4, 29.2, 22.8, 14.2. MS (ESI) calculated for $C_{19}H_{24}N_2S$, *m/z* 312.17, found 313.19 $(M + H)^+$.

6i: 2-Isopropylthiazolo[4,5-*c*]quinoline

164 mg, 83%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.46 (s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.01–7.96 (m, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.64 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 3.58–3.48 (m, 1H), 1.56 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.3, 147.8, 145.9, 144.2, 140.2, 130.6, 128.8, 127.6, 125.0, 123.7, 34.3, 23.1. MS (ESI) calculated for $C_{13}H_{12}N_2S$, *m/z* 228.10, found 229.08 $(M + H)^+$.

6j: 2-Isobutylthiazolo[4,5-*c*]quinoline

150 mg, 72%. ^{1}H NMR (500 MHz, MeOD) δ 9.31 (s, 1H), 8.18 (d, J = 8.3 Hz, 1H), 8.11 (dd, J = 8.1, 0.9 Hz, 1H), 7.81 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.73 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 2.30 (m, J = 13.7, 6.8 Hz, 1H), 1.08 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.5, 148.7, 146.0, 144.7, 142.6, 130.5, 130.4, 129.3, 126.2, 124.7, 43.7, 31.1, 22.6. MS (ESI) calculated for $C_{14}H_{14}N_2S$, *m/z* 242.09, found 243.09 $(M + H)^+$.

6l: 2-Isopentylthiazolo[4,5-*c*]quinoline

165 mg 75%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.26–8.22 (m, 1H), 7.98–7.95 (m, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.64 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 3.26–3.20 (m, 2H), 1.88–1.82 (m, 2H), 1.75 (dd, J = 13.3, 6.7 Hz, 1H), 1.01 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.2, 147.9, 145.8, 144.3, 140.6, 130.6, 128.8, 127.6, 125.0, 123.6, 38.8, 32.5, 27.9, 22.5. MS (ESI) calculated for $C_{15}H_{16}N_2S$, *m/z* 256.10.10, found 257.10 $(M + H)^+$.

6o: (*S*)-2-(2-Methylbutyl)thiazolo[4,5-*c*]quinoline

170 mg 77%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.96 (dd, J = 8.1, 0.9 Hz, 1H), 7.73 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.63 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.21 (dd, J = 14.6, 6.2 Hz, 1H), 3.01 (dd, J = 14.6, 8.2 Hz, 1H), 2.12–2.02 (m, 1H), 1.58–1.48 (m, 1H), 1.40–1.30 (m, 1H), 1.03 (d, J = 6.7 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 147.9, 145.8, 144.2, 140.8, 130.6, 128.8, 127.6, 125.0, 123.6, 41.4, 36.3, 29.4, 19.2, 11.5. MS (ESI) calculated for $C_{15}H_{16}N_2S$, *m/z* 256.10, found 257.10 $(M + H)^+$.

6p: 2-(3,3,3-Trifluoropropyl)thiazolo[4,5-*c*]quinoline

180 mg 74%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.99–7.95 (m, 1H), 7.76 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.66 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 3.51–3.45 (m, 2H), 2.91–2.80 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 147.7, 145.8, 144.4, 140.8, 130.7, 129.2, 127.8, 126.4 (q, $^1J_{CF}$ = 276.5 Hz), 125.0, 123.4, 33.0 (q, $^2J_{CF}$ = 29.8 Hz), 27.0 (q, $^3J_{CF}$ = 3.5 Hz). MS (ESI) calculated for $C_{13}H_9F_3N_2S$, *m/z* 282.04, found 283.04 $(M + H)^+$.

6q: 2-(4,4,4-Trifluorobutyl)thiazolo[4,5-*c*]quinoline

190 mg 75%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.46 (s, 1H), 8.26 (d, J = 8.2 Hz, 1H), 8.00–7.95 (m, 1H), 7.76 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.66 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 3.32 (t, J = 7.2 Hz, 2H), 2.37–2.22 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 147.9, 145.8, 144.4, 140.7, 130.7, 129.1, 127.8, 127.0 (q, $^1J_{CF}$ = 276.4 Hz), 125.0, 123.5, 33.9 (q, $^2J_{CF}$ = 28.9 Hz), 33.0, 21.1 (q, $^3J_{CF}$ = 3.0 Hz). MS (ESI) calculated for $C_{14}H_{11}F_3N_2S$, *m/z* 296.05, found 297.06 $(M + H)^+$.

Synthesis of compound 7a: 2-methylthiazolo[4,5-*c*]quinoline 5-oxide

To a solution of compound **6a** (57 mg, 0.28 mmol) in $CHCl_3$ (10 mL), was added *m*-chloroperoxybenzoic acid (95 mg, 0.43 mmol) and the reaction mixture was stirred at room

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temperature for 18 h. The solvent was then removed under reduced pressure and the crude residue was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain the compound **7a** (57 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.89 (d, J = 8.9 Hz, 1H), 7.94 (dd, J = 7.9, 1.1 Hz, 1H), 7.81–7.72 (m, 2H), 2.93 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7, 147.2, 139.0, 131.5, 131.1, 129.9, 129.7, 125.6, 123.8, 121.6, 20.4. MS (ESI) calculated for $C_{11}H_8N_2OS$, m/z 216.04, found 217.05 $(M + H)^+$.

Compounds **7b–7q** were synthesized similarly as compound **7a**.

7b: 2-Ethylthiazolo[4,5-*c*]quinoline 5-oxide

(81 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.90 (dd, J = 8.6, 0.8 Hz, 1H), 7.95 (dd, J = 8.0, 1.0 Hz, 1H), 7.82–7.72 (m, 2H), 3.23 (q, J = 7.6 Hz, 2H), 1.53 (d, J = 15.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.4, 147.1, 139.0, 131.6, 130.7, 129.9, 129.6, 125.6, 123.9, 121.6, 28.0, 13.9. MS (ESI) calculated for $C_{12}H_{10}N_2OS$, m/z 230.05, found 231.06 $(M + H)^+$.

7c: 2-Propylthiazolo[4,5-*c*]quinoline 5-oxide

(950 mg 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.90 (d, J = 8.7 Hz, 1H), 7.95 (dd, J = 7.9, 1.1 Hz, 1H), 7.82–7.71 (m, 2H), 3.17 (t, J = 7.6 Hz, 2H), 1.95 (dt, J = 14.9, 7.4 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.0, 147.1, 139.0, 131.6, 130.7, 129.9, 129.6, 123.9, 121.6, 36.4, 23.2, 13.8. MS (ESI) calculated for $C_{13}H_{12}N_2OS$, m/z 244.07, found 245.07 $(M + H)^+$.

7d: 2-Butylthiazolo[4,5-*c*]quinoline 5-oxide

(82 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.90–8.86 (m, 1H), 7.94 (dd, J = 7.9, 1.1 Hz, 1H), 7.80–7.71 (m, 2H), 3.21–3.16 (m, 2H), 1.94–1.86 (m, 2H), 1.53–1.45 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.2, 147.1, 139.0, 131.6, 130.6, 129.9, 129.6, 125.6, 123.9, 121.6, 34.2, 31.8, 22.4, 13.9. MS (ESI) calculated for $C_{14}H_{14}N_2OS$, m/z 258.08, found 259.09 $(M + H)^+$.

7e: 2-Pentylthiazolo[4,5-*c*]quinoline 5-oxide

(90 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.88 (d, J = 8.1 Hz, 1H), 7.94 (dd, J = 8.0, 1.2 Hz, 1H), 7.81–7.70 (m, 2H), 3.22–3.13 (m, 2H), 1.92 (dt, J = 15.4, 7.7 Hz, 2H), 1.48–1.36 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.3, 147.0, 138.9, 131.6, 130.7, 129.9, 129.6, 125.6, 123.9, 121.6, 34.5, 31.3, 29.5, 22.4, 14.0. MS (ESI) calculated for $C_{15}H_{16}N_2OS$, m/z 272.10, found 273.11 $(M + H)^+$.

7f: 2-Hexylthiazolo[4,5-*c*]quinoline 5-oxide

(65 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.90 (d, J = 8.5 Hz, 1H), 7.96 (dd, J = 8.0, 1.0 Hz, 1H), 7.82–7.72 (m, 2H), 3.22–3.17 (m, 2H), 1.92 (dd, J = 15.3, 7.7 Hz, 2H), 1.51–1.44 (m, 2H), 1.40–1.30 (m, 4H), 0.90 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.3, 147.1, 139.0, 131.7, 130.8, 129.9, 129.6, 125.6, 123.9, 121.6, 34.5, 31.6, 29.8, 28.9, 22.6, 14.2. MS (ESI) calculated for $C_{16}H_{18}N_2OS$, m/z 286.11, found 287.12 $(M + H)^+$.

7g: 2-Heptylthiazolo[4,5-*c*]quinoline 5-oxide

(81 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.88 (dd, J = 8.7, 0.8 Hz, 1H), 7.94 (dd, J = 7.9, 1.1 Hz, 1H), 7.80–7.71 (m, 2H), 3.21–3.15 (m, 2H), 1.91 (dt, J = 15.3, 7.6 Hz, 2H), 1.49–1.42 (m, 2H), 1.41–1.35 (m, 2H), 1.32–1.26 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.3, 147.0, 138.9, 131.6, 130.7, 129.9, 129.6, 125.6, 123.9, 121.6, 34.5, 31.7, 29.8, 29.2, 29.0, 22.7, 14.2. MS (ESI) calculated for $C_{17}H_{20}N_2OS$, m/z 300.13, found 301.14 $(M + H)^+$.

7h: 2-Nonylthiazolo[4,5-*c*]quinoline 5-oxide

(105 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.88 (d, J = 8.5 Hz, 1H), 7.93 (dd, J = 7.9, 1.1 Hz, 1H), 7.81–7.69 (m, 2H), 3.19–3.14 (m, 2H), 1.91 (dt, J = 15.3, 7.6 Hz, 2H), 1.45 (dt, J = 14.9, 7.0 Hz, 2H), 1.35 (dd, J = 14.2, 6.9 Hz, 2H), 1.29–1.21 (m, 8H), 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.3, 147.0, 138.9, 131.6, 130.7, 129.8, 129.6, 125.6, 123.9, 121.5, 34.5, 31.9, 29.8, 29.5, 29.3, 29.2, 22.8, 14.2. MS (ESI) calculated for $C_{19}H_{24}N_2OS$, m/z 328.16, found 329.17 $(M + H)^+$.

7i: 2-Isopropylthiazolo[4,5-*c*]quinoline 5-oxide

150 mg, 62%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.90 (dd, J = 8.7, 1.0 Hz, 1H), 7.99–7.95 (m, 1H), 7.79 (ddd, J = 8.6, 7.0, 1.5 Hz, 1H), 7.77–7.72 (m, 1H), 3.54–3.45 (m, 1H), 1.54 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.5, 147.0, 138.9, 131.7, 130.3, 129.9, 129.6, 125.6, 124.0, 121.6, 34.4, 23.0. MS (ESI) calculated for $C_{13}H_{12}N_2OS$, m/z 244.07.10, found 245.07 $(M + H)^+$.

7j: 2-Isobutylthiazolo[4,5-*c*]quinoline 5-oxide

80 mg, 75%. ^{1}H NMR (500 MHz, MeOD) δ 9.26 (s, 1H), 8.79 (dd, J = 8.6, 0.9 Hz, 1H), 8.25–8.21 (m, 1H), 7.95 (ddd, J = 8.7, 7.1, 1.5 Hz, 1H), 7.90 (ddd, J = 8.2, 7.1, 1.4 Hz, 1H), 3.14 (d, J = 7.2 Hz, 2H), 2.35–2.25 (m, 1H), 1.09 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 176.8, 147.8, 139.0, 135.3, 133.5, 131.7, 131.6, 127.2, 125.2, 121.5, 43.7, 31.0, 22.6. MS (ESI) calculated for $C_{14}H_{14}N_2OS$, m/z 258.08.10, found 259.08 $(M + H)^+$.

7k: 2-Neopentylthiazolo[4,5-*c*]quinoline 5-oxide

80 mg, 75%. ^{1}H NMR (500 MHz, MeOD) δ 9.31 (s, 1H), 8.79 (dd, J = 8.7, 0.9 Hz, 1H), 8.26–8.22 (m, 1H), 7.99–7.88 (m, 2H), 3.16 (s, 2H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ 174.7, 147.8, 138.8, 135.9, 133.8, 131.8, 131.7, 127.3, 125.1, 121.4, 48.3, 33.2, 29.9. MS (ESI) calculated for $C_{14}H_{16}N_2OS$, m/z 272.10, found 273.10 $(M + H)^+$.

7l: 2-Isopentylthiazolo[4,5-*c*]quinoline 5-oxide

110 mg, 69%. ^{1}H NMR (500 MHz, MeOD) δ 9.23 (s, 1H), 8.77 (dd, J = 8.6, 0.9 Hz, 1H), 8.21–8.18 (m, 1H), 7.93 (ddd, J = 8.7, 7.0, 1.5 Hz, 1H), 7.89 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H), 3.27 (dd, J = 8.4, 7.4 Hz, 2H), 1.88–1.82 (m, 2H), 1.78–1.69 (m, 1H), 1.02 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 178.1, 147.7, 138.9, 135.1, 133.5, 131.6, 131.6, 127.1, 125.1, 121.5, 39.6, 33.1, 28.8, 22.6. MS (ESI) calculated for $C_{15}H_{16}N_2OS$, m/z 272.10, found 273.10 $(M + H)^+$.

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7m: 2-(3,3-Dimethylbutyl)thiazolo[4,5-*c*]quinoline 5-oxide

80 mg, 76%. ^{1}H NMR (500 MHz, MeOD) δ 9.22 (s, 1H), 8.77 (dd, J = 8.7, 0.9 Hz, 1H), 8.21–8.18 (m, 1H), 7.93 (ddd, J = 8.7, 7.1, 1.5 Hz, 1H), 7.89 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H), 3.27–3.22 (m, 2H), 1.89–1.84 (m, 2H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ 178.7, 147.7, 138.9, 135.1, 133.4, 131.6, 131.6, 127.1, 125.1, 121.4, 44.7, 31.5, 30.9, 29.5. MS (ESI) calculated for $C_{16}H_{18}N_2OS$, m/z 286.11, found 287.11 $(M + H)^+$.

7n: (*S*)-2-(*sec*-Butyl)thiazolo[4,5-*c*]quinoline 5-oxide

117 mg, 78%. ^{1}H NMR (500 MHz, MeOD) δ 9.23 (s, 1H), 8.77 (dd, J = 8.6, 1.0 Hz, 1H), 8.22–8.18 (m, 1H), 7.93 (ddd, J = 8.7, 7.1, 1.5 Hz, 1H), 7.88 (ddd, J = 8.2, 7.1, 1.4 Hz, 1H), 3.35 (dd, J = 13.9, 6.9 Hz, 1H), 2.04–1.94 (m, 1H), 1.92–1.82 (m, 1H), 1.52 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 183.1, 147.7, 138.9, 134.7, 133.5, 131.6, 131.6, 127.1, 125.2, 121.5, 42.3, 31.6, 20.9, 12.0. MS (ESI) calculated for $C_{14}H_{14}N_2OS$, m/z 258.09, found 259.08 $(M + H)^+$.

7o: (*S*)-2-(2-Methylbutyl)thiazolo[4,5-*c*]quinoline 5-oxide

103 mg, 74%. ^{1}H NMR (500 MHz, MeOD) δ 9.24 (s, 1H), 8.78 (dd, J = 8.7, 0.8 Hz, 1H), 8.23–8.19 (m, 1H), 7.94 (ddd, J = 8.7, 7.1, 1.5 Hz, 1H), 7.89 (dd, 1H), 3.26 (dd, J = 14.7, 6.2 Hz, 1H), 3.06 (dd, J = 14.7, 8.0 Hz, 1H), 2.14–2.03 (m, 1H), 1.60–1.50 (m, 1H), 1.44–1.30 (m, 1H), 1.04 (d, J = 6.7 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 177.0, 147.8, 139.0, 135.3, 133.5, 131.7, 131.6, 127.1, 125.1, 121.5, 41.8, 37.4, 30.2, 19.4, 11.6. MS (ESI) calculated for $C_{15}H_{16}N_2OS$, m/z 272.10, found 273.10 $(M + H)^+$.

7p: 2-(3,3,3-Trifluoropropyl)thiazolo[4,5-*c*]quinoline 5-oxide

120 mg, 75%. ^{1}H NMR (500 MHz, MeOD) δ 9.24 (s, 1H), 8.76 (dd, J = 8.8, 0.7 Hz, 1H), 8.17 (dd, 1H), 7.93 (ddd, J = 8.6, 7.0, 1.4 Hz, 1H), 7.88 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H), 3.54 (t, 2H), 2.99–2.87 (m, 2H). ^{13}C NMR (126 MHz, MeOD) δ 173.8, 147.6, 139.1, 135.2, 133.6, 131.8, 131.6, 128.1 (q, $^1J_{CF}$ = 275.6 Hz), 127.1, 125.0, 121.5, 33.2 (q, $^2J_{CF}$ = 29.7 Hz), 27.7 (q, $^3J_{CF}$ = 3.5 Hz). MS (ESI) calculated for $C_{13}H_9F_3N_2OS$, m/z 298.04, found 299.04 $(M + H)^+$.

7q: 2-(4,4,4-Trifluorobutyl)thiazolo[4,5-*c*]quinoline 5-oxide

128 mg, 81%. ^{1}H NMR (500 MHz, MeOD) δ 9.26 (s, 1H), 8.80–8.74 (m, 1H), 8.22–8.18 (m, 1H), 7.94 (ddd, J = 8.6, 7.1, 1.5 Hz, 1H), 7.89 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H), 3.36 (t, J = 7.5 Hz, 2H), 2.48–2.34 (m, 2H), 2.27–2.17 (m, 2H). ^{13}C NMR (126 MHz, MeOD) δ 176.0, 147.8, 139.0, 135.2, 133.6, 131.7, 131.6, 128.6 (q, $^1J_{CF}$ = 275.4 Hz), 127.1, 125.1, 121.5, 33.6 (q, $^2J_{CF}$ = 29.0 Hz), 33.5, 22.6 (q, $^3J_{CF}$ = 3.2 Hz). MS (ESI) calculated for $C_{14}H_{11}F_3N_2OS$, m/z 312.05, found 313.05 $(M + H)^+$.

Synthesis of compound 8a: 2-methylthiazolo[4,5-*c*]quinolin-4-amine

Compound **7a** (50 mg, 0.23 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL). Benzoyl isocyanate (68 mg, 0.46 mmol) was added to the reaction mixture and refluxed for 30 min. The solvent was then removed under vacuum and the residue was dissolved in anhydrous methanol (5 mL). Excess of sodium methoxide was added and the reaction mixture was refluxed for 2 h. After evaporating solvents under reduced pressure, the crude residue was purified using silica gel column chromatography (7% MeOH/CH_2Cl_2) to obtain compound **8a** as a white solid (41 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.72 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.61 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 151.5, 144.8, 141.0, 138.1, 129.1, 126.8, 124.8, 123.2, 120.1, 20.1. MS (ESI) calculated for $C_{11}H_9N_3S$, m/z 215.05, found 216.06 $(M + H)^+$.

Compounds **8b–8q** were synthesized similarly as compound **8a**.

8b: 2-Ethylthiazolo[4,5-*c*]quinolin-4-amine

White solid (36 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.74 (dd, J = 8.0, 1.1 Hz, 1H), 7.56 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.58 (s, 2H), 3.20 (q, J = 7.6 Hz, 2H), 1.51 (t, J = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 151.6, 144.8, 140.4, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 27.8, 14.1. MS (ESI) calculated for $C_{12}H_{11}N_3S$, m/z 229.07, found 230.08 $(M + H)^+$.

8c: 2-Propylthiazolo[4,5-*c*]quinolin-4-amine

White solid (450 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.4 Hz, 1H), 7.73 (dd, J = 8.0, 1.3 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.34–7.27 (m, 1H), 5.67 (s, 2H), 3.13 (t, J = 7.6 Hz, 2H), 1.94 (dd, J = 15.0, 7.4 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 151.7, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.1, 120.2, 36.2, 23.4, 13.8. MS (ESI) calculated for $C_{13}H_{13}N_3S$, m/z 243.08, found 244.09 $(M + H)^+$.

8d: 2-Butylthiazolo[4,5-*c*]quinolin-4-amine

White solid (25 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.6 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.56 (ddd, J = 8.5, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.56 (s, 2H), 3.20–3.13 (m, 2H), 1.90 (dt, J = 15.3, 7.6 Hz, 2H), 1.50 (dq, J = 14.7, 7.4 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 151.6, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 34.0, 32.0, 22.4, 13.9. MS (ESI) calculated for $C_{14}H_{15}N_3S$, m/z 257.10, found 258.11 $(M + H)^+$.

8e: 2-Pentylthiazolo[4,5-*c*]quinolin-4-amine

White solid (38 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.1 Hz, 1H), 7.73 (dd, J = 8.0, 1.1 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.34–7.29 (m, 1H), 5.59 (s, 2H), 3.18–3.12 (m, 2H), 1.96–1.86 (m, 2H), 1.50–1.35 (m, 4H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 151.6, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 34.3, 31.4, 29.7, 22.5, 14.1 MS (ESI) calculated for $C_{15}H_{17}N_3S$, m/z 271.11, found 272.12 $(M + H)^+$.

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8f: 2-Hexylthiazolo[4,5-*c*]quinolin-4-amine

White solid (25 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.56 (s, 2H), 3.21–3.11 (m, 2H), 1.91 (dt, J = 15.3, 7.5 Hz, 2H), 1.46 (dt, J = 9.2, 7.0 Hz, 2H), 1.39–1.30 (m, 4H), 0.91 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 151.6, 144.7, 140.51, 138.0, 129.1, 126.8, 124.8, 123.2, 120.2, 34.3, 31.6, 30.0, 29.0, 22.6, 14.2. MS (ESI) calculated for $C_{16}H_{19}N_3S$, m/z 285.13, found 286.14 $(M + H)^+$.

8g: 2-Heptylthiazolo[4,5-*c*]quinolin-4-amine

White solid (34 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.56 (s, 2H), 3.20–3.08 (m, 2H), 1.91 (dt, J = 15.3, 7.6 Hz, 2H), 1.50–1.42 (m, 2H), 1.38 (ddd, J = 14.0, 8.2, 5.2 Hz, 2H), 1.30 (dt, J = 11.5, 3.9 Hz, 4H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 151.6, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 34.3, 31.8, 30.0, 29.2, 29.1, 22.8, 14.2. MS (ESI) calculated for $C_{17}H_{21}N_3S$, m/z 299.15, found 300.16 $(M + H)^+$.

8h: 2-Nonylthiazolo[4,5-*c*]quinolin-4-amine

(26 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.5, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.57 (s, 2H), 3.21–3.09 (m, 2H), 1.91 (dt, J = 15.3, 7.6 Hz, 2H), 1.46 (ddd, J = 14.9, 8.7, 6.5 Hz, 2H), 1.36 (dd, J = 13.7, 6.7 Hz, 2H), 1.28 (s, 8H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 151.6, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 34.3, 32.0, 30.0, 29.6, 29.4, 29.4, 29.2, 22.8, 14.3. MS (ESI) calculated for $C_{19}H_{25}N_3S$, m/z 327.18, found 328.19 $(M + H)^+$.

8i: 2-Isopropylthiazolo[4,5-*c*]quinolin-4-amine

White solid (40 mg, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.75–7.71 (m, 1H), 7.55 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.66 (s, 2H), 3.51–3.41 (m, 1H), 1.52 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.7, 151.8, 144.7, 140.0, 137.9, 129.0, 126.8, 124.8, 123.1, 120.3, 34.2, 23.2. MS (ESI) calculated for $C_{13}H_{13}N_3S$, m/z 243.09.10, found 244.09 $(M + H)^+$.

8j: 2-Isobutylthiazolo[4,5-*c*]quinolin-4-amine

White solid (38 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.63 (s, 2H), 3.02 (d, J = 7.2 Hz, 2H), 2.30–2.20 (m, 1H), 1.06 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3, 151.7, 144.8, 140.6, 138.1, 129.1, 126.8, 124.9, 123.1, 120.2, 43.1, 30.0, 22.5. MS (ESI) calculated for $C_{14}H_{15}N_3S$, m/z 257.10, found 258.10 $(M + H)^+$.

8k: 2-Neopentylthiazolo[4,5-*c*]quinolin-4-amine

White solid (45 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.74 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 5.5, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.60 (s, 2H), 3.04 (s, 2H), 1.10 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 151.7, 144.8, 140.7, 138.2, 129.1, 126.8, 124.9, 123.2, 120.1, 47.8, 32.3, 29.7. MS (ESI) calculated for $C_{14}H_{17}N_3S$, m/z 271.11, found 272.11 $(M + H)^+$.

8l: 2-Isopentylthiazolo[4,5-*c*]quinolin-4-amine

White solid (40 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.61 (s, 2H), 3.20–3.13 (m, 2H), 1.84–1.78 (m, 2H), 1.77–1.69 (m, 1H), 1.00 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7, 151.6, 144.7, 140.5, 138.0, 129.0, 126.8, 124.8, 123.2, 120.2, 38.9, 32.3, 27.9, 22.5. MS (ESI) calculated for $C_{15}H_{16}N_2OS$, m/z 271.11, found 272.11 $(M + H)^+$.

8m: 2-(3,3-Dimethylbutyl)thiazolo[4,5-*c*]quinolin-4-amine

White solid (45 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.74–7.70 (m, 1H), 7.55 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.64 (s, 2H), 3.17–3.09 (m, 2H), 1.86–1.79 (m, 2H), 1.02 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 151.6, 144.7, 140.4, 138.0, 129.0, 126.8, 124.8, 123.1, 121.0, 44.1, 30.8, 30.1, 29.3. MS (ESI) calculated for $C_{16}H_{19}N_3S$, m/z 285.13, found 286.14 $(M + H)^+$.

8n: (*S*)-2-(*sec*-Butyl)thiazolo[4,5-*c*]quinolin-4-amine

White solid (45 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79–7.75 (m, 1H), 7.75–7.72 (m, 1H), 7.55 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 5.63 (s, 2H), 3.30–3.21 (m, 1H), 2.00–1.90 (m, 1H), 1.88–1.76 (m, 1H), 1.49 (d, J = 6.9 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.0, 151.7, 144.7, 140.0, 137.9, 129.0, 126.8, 124.8, 123.1, 120.3, 41.0, 30.9, 21.0, 11.9. MS (ESI) calculated for $C_{14}H_{15}N_3S$, m/z 257.10, found 258.10 $(M + H)^+$.

8o: (*S*)-2-(2-Methylbutyl)thiazolo[4,5-*c*]quinolin-4-amine

White solid (46 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, J = 8.4, 0.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.55 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.31 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.66 (s, 2H), 3.14 (dd, J = 14.6, 6.2 Hz, 1H), 2.96 (dd, J = 14.6, 8.1 Hz, 1H), 2.08–1.97 (m, 1H), 1.57–1.47 (m, 1H), 1.38–1.28 (m, 1H), 1.02 (d, J = 6.7 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 151.7, 144.7, 140.6, 138.1, 129.0, 126.8, 124.8, 123.1, 120.2, 41.2, 36.3, 29.3, 19.2, 11.5. MS (ESI) calculated for $C_{15}H_{17}N_3S$, m/z 271.11, found 272.11 $(M + H)^+$.

8p: 2-(3,3,3-Trifluoropropyl)thiazolo[4,5-*c*]quinolin-4-amine

White solid (70 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80–7.76 (m, 1H), 7.73 (dd, J = 8.0, 1.0 Hz, 1H), 7.58 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.35–7.30 (m, 1H), 5.63 (s, 2H), 3.45–3.39 (m, 2H), 2.87–2.76 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ

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166.5, 151.6, 145.0, 140.8, 137.9, 129.4, 126.9, 126.4 (q, $^1J_{CF}$ = 276.5 Hz), 124.9, 123.4, 119.9, 33.1 (q, $^2J_{CF}$ = 29.8 Hz), 26.8 (q, $^3J_{CF}$ = 3.4 Hz). MS (ESI) calculated for $C_{13}H_{10}F_3N_3S$, *m*/*z* 297.05, found 298.05 $(M + H)^+$.

8q: 2-(4,4,4-Trifluorobutyl)thiazolo[4,5-*c*]quinolin-4-amine

White solid (50 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 8.4, 0.4 Hz, 1H), 7.73 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.57 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.32 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 5.62 (s, 2H), 3.25 (t, *J* = 7.2 Hz, 2H), 2.36–2.26 (m, 2H), 2.26–2.18 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8, 151.6, 144.9, 140.6, 138.1, 129.3, 127.0 (q, $^1J_{CF}$ = 276.4 Hz), 126.9, 124.9, 123.3, 120.0, 33.1 (q, $^2J_{CF}$ = 29.7 Hz), 32.7, 21.9 (q, $^3J_{CF}$ = 3.0 Hz). MS (ESI) calculated for $C_{14}H_{12}F_3N_3S$, *m*/*z* 311.07, found 312.07 $(M + H)^+$.

Synthesis of compound 9: 8-nitro-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

To a stirred solution of compound **8c** (200 mg, 0.82 mmol) in H_2SO_4 (0.5 mL), was added HNO_3 (0.5 mL) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with 1 N NaOH and extracted with EtOAc (3 × 15 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **9** as yellow solid (171 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 2.5 Hz, 1H), 8.35 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.80 (d, *J* = 9.2 Hz, 1H), 6.09 (s, 2H), 3.17 (dd, *J* = 8.7, 6.4 Hz, 2H), 1.97 (dd, *J* = 15.0, 7.5 Hz, 2H), 1.11 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 153.6, 148.3, 142.5, 141.2, 138.7, 127.3, 123.2, 121.5, 119.1, 36.2, 23.3, 13.8. MS (ESI) calculated for $C_{13}H_{12}N_4O_2S$, *m*/*z* 288.07, found 289.08 $(M + H)^+$.

Synthesis of compound 10: 2-propylthiazolo[4,5-*c*]quinoline-4,8-diamine

To a stirred solution of compound **9** (200 mg, 0.69 mmol) in methanol (5 mL), were added zinc dust (453 mg, 6.94 mmol) and ammonium formate (437 mg, 6.94 mmol), and the reaction mixture stirred at room temperature for 30 min. The reaction mixture was then filtered through celite, the filtrate concentrated under reduced pressure, and purified over silica gel column chromatography (10% MeOH/CH_2Cl_2) to afford compound **10** as a yellow solid (185 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.46 (s, 2H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.00 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 3.17–3.09 (m, 2H), 1.99–1.88 (m, 2H), 1.08 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 170.3, 149.2, 143.9, 142.1, 137.1, 129.5, 121.9, 120.7, 118.7, 107.4, 36.1, 23.2, 13.8. MS (ESI) calculated for $C_{13}H_{14}N_4S$, *m*/*z* 258.09, found 259.10 $(M + H)^+$.

Synthesis of compound 11: 8-azido-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

To a stirred solution of compound **10** (25 mg, 0.096 mmol) in a 1 : 1 mixture of acetic acid and water (2 mL), was added sodium nitrite (20 mg, 0.29 mmol) and the reaction mixture was stirred at room temperature for 1 h. Sodium azide (18.8 mg, 0.29 mmol) was then added to the reaction mixture and stirred for additional 1 h. The reaction mixture was neutralized with 1 N NaOH and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified on silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **11** as a brown solid (18 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.9 Hz, 1H), 7.31 (d, *J* = 2.5 Hz, 1H), 7.26–7.23 (m, 1H), 5.60 (s, 2H), 3.17–3.12 (m, 2H), 2.01–1.90 (m, 2H), 1.09 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 151.3, 139.5, 138.5, 134.8, 128.4, 121.0, 120.8, 113.5, 36.2, 23.4, 13.8. MS (ESI) calculated for $C_{13}H_{12}N_6S$, *m*/*z* 284.08, found 285.09 $(M + H)^+$.

Synthesis of compound 12a: 2-(1-(4-amino-2-propylthiazolo-[4,5-*c*]quinolin-8-yl)-1*H*-1,2,3-triazol-4-yl)propan-2-ol

To a stirred solution of compound **11** (12 mg, 0.042 mmol) in THF (1 mL), were added $CuSO_4{\cdot}5H_2O$ (1.3 mg, 0.005 mmol, in 0.5 mL water), sodium ascorbate (2.1 mg, 0.010 mmol, in 0.5 mL water), 2-methylbut-3-yn-2-ol (8.4 μL, 0.084 mmol), and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was diluted with water and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified using silica gel column chromatography (10% MeOH/CH_2Cl_2) to obtain compound **11** as a white solid (14 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 2.0 Hz, 1H), 7.97 (s, 1H), 7.91–7.81 (m, 2H), 5.74 (s, 2H), 3.49 (s, 1H), 3.19–3.13 (m, 2H), 1.96 (dd, *J* = 15.0, 7.5 Hz, 2H), 1.74 (s, 6H), 1.10 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.5, 156.5, 152.2, 144.5, 140.2, 138.7, 132.0, 128.3, 121.5, 120.3, 117.8, 116.4, 68.9, 36.2, 30.7, 23.4, 13.8. MS (ESI) calculated for $C_{18}H_{20}N_6OS$, *m*/*z* 368.14, found 369.15 $(M + H)^+$.

Compounds **12b–12f** were synthesized similarly as compound **12a**.

12b: (1-(4-Amino-2-propylthiazolo[4,5-*c*]quinolin-8-yl)-1*H*-1,2,3-triazol-4-yl)methanol

White solid (15 mg, 83%). ^{1}H NMR (500 MHz, DMSO) δ 8.84 (s, 1H), 8.33 (d, *J* = 2.5 Hz, 1H), 8.06 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.13 (s, 2H), 5.37 (t, *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 5.9 Hz, 2H), 3.19 (t, *J* = 7.5 Hz, 2H), 1.88 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.8, 152.7, 149.2, 144.5, 139.1, 138.2, 130.8, 127.3, 121.2, 120.9, 118.9, 115.2, 55.1, 35.2, 22.8, 13.5. MS (ESI) calculated for $C_{16}H_{16}N_6OS$, *m*/*z* 340.11, found 341.11 $(M + H)^+$.

12c: 8-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

Solid (19 mg, 93%). ^{1}H NMR (500 MHz, DMSO) δ 9.44 (s, 1H), 8.36 (d, *J* = 2.5 Hz, 1H), 8.12 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.97 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.53 (dd, *J* = 10.6, 4.8 Hz, 2H), 7.44–7.37 (m, 1H), 7.17 (s, 2H), 3.21 (t, *J* = 7.5 Hz, 2H), 1.90 (dd, *J* = 14.9, 7.4 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C

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NMR (126 MHz, DMSO) δ 171.8, 152.8, 147.3, 144.7, 139.0, 138.2, 130.6, 130.4, 129.1, 128.3, 127.4, 125.3, 120.9, 119.8, 118.9, 115.3, 35.2, 22.8, 13.5. MS (ESI) calculated for $C_{21}H_{18}N_6S$, *m*/*z* 386.13, found 387.14 $(M + H)^+$.

12d: 2-Propyl-8-(4-propyl-1*H*-1,2,3-triazol-1-yl)thiazolo[4,5-*c*]-quinolin-4-amine

Yellow solid (16 mg, 86%). ^{1}H NMR (500 MHz, DMSO) δ 8.72 (s, 1H), 8.28 (d, J = 2.4 Hz, 1H), 8.04 (dd, J = 9.0, 2.5 Hz, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.12 (s, 2H), 3.19 (t, J = 7.5 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 1.89 (dd, J = 14.9, 7.4 Hz, 2H), 1.72 (dd, J = 14.9, 7.4 Hz, 2H), 1.04 (t, J = 7.4 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.8, 152.7, 148.0, 144.4, 139.0, 138.2, 130.9, 127.3, 120.8, 120.3, 118.9, 115.0, 35.2, 27.1, 22.8, 22.1, 13.7, 13.5. MS (ESI) calculated for $C_{18}H_{20}N_6S$, *m*/*z* 352.15, found 353.15 $(M + H)^+$.

12e: 8-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

Solid (19 mg, 91%). ^{1}H NMR (500 MHz, DMSO) δ 8.71 (s, 1H), 8.27 (d, J = 2.5 Hz, 1H), 8.04 (dd, J = 9.0, 2.5 Hz, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.12 (s, 2H), 3.19 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 1.89 (dd, J = 14.9, 7.4 Hz, 2H), 1.69 (dt, J = 15.3, 7.6 Hz, 2H), 1.42–1.26 (m, 6H), 1.04 (t, J = 7.4 Hz, 3H), 0.87 (dd, J = 9.2, 4.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.8, 152.7, 148.2, 144.4, 139.0, 138.2, 130.9, 127.3, 120.8, 120.2, 118.9, 114.9, 35.2, 31.1, 28.8, 28.3, 25.1, 22.8, 22.1, 14.0, 13.5. MS (ESI) calculated for $C_{21}H_{26}N_6S$, *m*/*z* 394.19, found 395.20 $(M + H)^+$.

12f: 2-Propyl-8-(4-(trimethylsilyl)-1*H*-1,2,3-triazol-1-yl)thiazolo-[4,5-*c*]quinolin-4-amine

White solid (18 mg, 90%). ^{1}H NMR (500 MHz, DMSO) δ 8.98 (s, 1H), 8.31 (d, J = 2.4 Hz, 1H), 8.07 (dd, J = 9.0, 2.5 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.13 (s, 2H), 3.19 (t, J = 7.5 Hz, 2H), 1.89 (dd, J = 14.8, 7.4 Hz, 2H), 1.04 (t, J = 7.4 Hz, 3H), 0.36–0.33 (m, 9H). ^{13}C NMR (126 MHz, DMSO) δ 171.8, 152.7, 146.1, 144.4, 139.1, 138.2, 130.7, 128.9, 127.3, 121.3, 118.9, 115.3, 35.2, 22.8, 13.5, −0.9. MS (ESI) calculated for $C_{18}H_{22}N_6SSi$, *m*/*z* 382.14, found 383.14 $(M + H)^+$.

Synthesis of compound 12g: 2-propyl-8-(1*H*-1,2,3-triazol-1-yl)-thiazolo[4,5-*c*]quinolin-4-amine

To a stirred solution of compound **12f** (20 mg, 0.0.52 mmol) in THF (1 mL) was added TBAF (20 mg, 0.078 mmol), and the reaction mixture was stirred at room temperature for 10 h. The reaction mixture was then diluted with water and extracted with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **12g** as a white solid (14 mg, 87%). ^{1}H NMR (500 MHz, DMSO) δ 8.94 (d, J = 1.1 Hz, 1H), 8.29 (d, J = 2.4 Hz, 1H), 8.04 (dd, J = 9.0, 2.5 Hz, 1H), 7.98 (d, J = 1.1 Hz, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.12 (s, 2H), 3.17 (t, J = 7.5 Hz, 2H), 1.91–1.83 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.7, 152.8, 144.6, 139.1, 138.2, 134.4, 130.7, 127.4, 123.4, 121.2, 118.9, 115.5, 35.23 22.8, 13.5. MS (ESI) calculated for $C_{15}H_{14}N_6S$, *m*/*z* 310.10, found 311.10 $(M + H)^+$.

Synthesis of compound 13a

N^8-Hexyl-2-propylthiazolo[4,5-*c*]quinoline-4,8-diamine. To a stirred solution of compound **10** (25 mg, 0.096 mmol) in DMF (2 mL) were added 1-iodohexane (20.5 mg, 0.096 mmol) and potassium carbonate (26.7 mg, 0.19 mmol), and the reaction mixture stirred at room temperature for 4 h. The reaction mixture was diluted with water and extracted with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain **13a** as a solid (16 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, J = 8.9 Hz, 1H), 6.96 (dd, J = 8.9, 2.6 Hz, 1H), 6.67 (d, J = 2.5 Hz, 1H), 6.15 (s, 2H), 3.87 (s, 1H), 3.18 (t, J = 7.1 Hz, 2H), 3.15–3.11 (m, 2H), 1.99–1.89 (m, 2H), 1.68 (dt, J = 14.7, 7.2 Hz, 2H), 1.49–1.41 (m, 2H), 1.35 (td, J = 7.2, 3.7 Hz, 4H), 1.09 (t, J = 7.4 Hz, 3H), 0.92 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 148.2, 145.5, 141.2, 137.4, 124.8, 120.3, 119.8, 102.9, 44.3, 36.2, 31.8, 29.4, 27.0, 23.3, 22.8, 14.2, 13.8. MS (ESI) calculated for $C_{19}H_{26}N_4S$, *m*/*z* 342.19, found 343.20 $(M + H)^+$.

Synthesis of compound 13b: *N*-(4-amino-2-propylthiazolo[4,5-*c*]-quinolin-8-yl)butyramide

To a stirred solution of compound **10** (20 mg, 0.077 mmol) in CH_2Cl_2 (2 mL) were added triethylamine (30 μL, 0.164 mmol) and butyryl chloride (8.7 μL, 0.082); the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with water and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified using silica gel column chromatography (10% MeOH/CH_2Cl_2) to furnish compound **13b** as a white solid (22 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 2.2 Hz, 1H), 7.71 (d, J = 8.9 Hz, 1H), 7.37 (dd, J = 8.9, 2.4 Hz, 2H), 5.67 (d, J = 1.2 Hz, 2H), 3.13 (t, J = 7.4 Hz, 2H), 2.41 (t, J = 7.4 Hz, 2H), 1.94 (dd, J = 15.0, 7.5 Hz, 2H), 1.85–1.74 (m, 2H), 1.11–1.02 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 171.5, 150.9, 140.6, 138.2, 133.4, 126.8, 122.1, 120.2, 114.6, 39.8, 36.2, 23.3, 19.2, 13.9, 13.8. MS (ESI) calculated for $C_{17}H_{20}N_4OS$, *m*/*z* 328.14, found 329.15 $(M + H)^+$.

Synthesis of compound 14: 8-bromo-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

To a stirred solution of compound **8c** (50 mg, 0.205 mmol) in CH_3CN (1 mL) were added *N*-bromosuccinimide (44 mg, 0.246 mmol), ammonium acetate (1.6 mg, 0.02 mmol) and the reaction mixture stirred at room temperature for 12 h. The reaction mixture was then diluted with water and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **14** as a white

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solid (40 mg, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (dd, J = 2.0, 0.6 Hz, 1H), 7.61 (dd, J = 3.9, 1.3 Hz, 2H), 5.68 (s, 2H), 3.16–3.11 (m, 2H), 1.95 (dt, J = 15.0, 7.4 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 151.8, 143.4, 139.3, 138.4, 132.1, 128.4, 126.9, 121.5, 115.9, 36.2, 23.4, 13.8. MS (ESI) calculated for $C_{13}H_{12}BrN_3S$, m/z 320.99, found 322.00 $(M + H)^+$.

Synthesis of compound 15a: *N*-butyl-2-propylthiazolo[4,5-*c*]-quinolin-4-amine

To a stirred solution of compound **8c** (50 mg, 0.205 mmol) in THF (1 mL) were added sodium hydride (10 mg, 0.41 mmol) and 1-iodobutane (35 μL, 0.308 mmol), the resulting mixture was stirred at room temperature for 6 h. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15a** (45 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (dd, J = 8.3, 0.5 Hz, 1H), 7.70–7.66 (m, 1H), 7.51 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 7.23 (ddd, J = 8.0, 7.1, 1.1 Hz, 1H), 5.96 (t, J = 4.8 Hz, 1H), 3.77–3.69 (m, 2H), 3.15–3.08 (m, 2H), 1.99–1.89 (m, 2H), 1.75 (ddd, J = 14.9, 11.1, 7.5 Hz, 2H), 1.52 (dq, J = 14.7, 7.4 Hz, 2H), 1.08 (t, J = 7.4 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 151.2, 145.3, 138.9, 138.3, 128.6, 126.9, 124.5, 122.1, 119.3, 40.6, 36.0, 31.9, 23.3, 20.3, 14.0, 13.7. MS (ESI) calculated for $C_{17}H_{21}N_3S$, m/z 299.15, found 300.14 $(M + H)^+$.

Compound **15b–c** was synthesized similarly as compound **15a**.

15b: *N*-Hexyl-2-propylthiazolo[4,5-*c*]quinolin-4-amine

White solid (9.9 mg, 72%, based on starting material recovery). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 1H), 7.68 (dd, J = 7.9, 1.1 Hz, 1H), 7.51 (ddd, J = 8.5, 7.1, 1.5 Hz, 1H), 7.23 (ddd, J = 8.1, 7.2, 1.1 Hz, 1H), 5.96 (t, J = 4.6 Hz, 1H), 3.71 (dt, J = 7.2, 5.8 Hz, 2H), 3.11 (dd, J = 8.7, 6.5 Hz, 2H), 1.94 (dd, J = 15.0, 7.5 Hz, 2H), 1.76 (t, J = 7.3 Hz, 2H), 1.53–1.44 (m, 2H), 1.36 (ddd, J = 6.9, 6.2, 3.3 Hz, 4H), 1.08 (t, J = 7.4 Hz, 3H), 0.91 (dd, J = 9.1, 5.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 151.3, 145.5, 139.1, 138.5, 128.8, 127.1, 124.6, 122.3, 119.5, 41.1, 36.2, 31.8, 29.9, 27.1, 23.5, 22.8, 14.2, 13.8. MS (ESI) calculated for $C_{19}H_{25}N_3S$, m/z 327.18, found 328.18 $(M + H)^+$.

15c: *N*-Hexadecyl-2-propylthiazolo[4,5-*c*]quinolin-4-amine

White solid (26 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.9 Hz, 1H), 7.68 (dd, J = 7.9, 1.1 Hz, 1H), 7.51 (ddd, J = 8.5, 7.1, 1.5 Hz, 1H), 7.23 (td, J = 7.1, 3.6 Hz, 1H), 5.96 (s, 1H), 3.71 (dd, J = 12.9, 7.1 Hz, 2H), 3.11 (dd, J = 8.7, 6.5 Hz, 2H), 1.98–1.88 (m, 2H), 1.76 (dt, J = 14.8, 7.4 Hz, 2H), 1.51–1.43 (m, 2H), 1.30–1.21 (m, 24H), 1.08 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 151.4, 145.5, 139.1, 138.5, 128.7, 127.1, 124.6, 122.3, 119.5, 41.1, 36.8, 36.1, 32.1, 29.9, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 27.3, 24.8, 23.5, 22.8, 14.3, 13.9. MS (ESI) calculated for $C_{29}H_{45}N_3S$, m/z 467.33, found 468.35 $(M + H)^+$.

Synthesis of compound 15d: *N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)formamide

To a stirred solution of compound **8c** (50 mg, 0.205 mmol) in 1,4-dioxane (1 mL) were added 2,2,2-trifluoroethylformate (60 μL, 0.616 mmol) and triethylamine (86 μL, 0.616 mmol). The resulting mixture was stirred at 80 °C for 12 h. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified using silica gel column chromatography (3% MeOH/CH_2Cl_2) to furnish compound **15c** as a white solid (26 mg, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (d, J = 10.8 Hz, 1H), 8.86 (d, J = 10.3 Hz, 1H), 8.03–7.94 (m, 1H), 7.84 (ddd, J = 8.0, 1.4, 0.5 Hz, 1H), 7.67 (ddd, J = 8.5, 7.0, 1.4 Hz, 1H), 7.50 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 3.24–3.08 (m, 2H), 2.05–1.87 (m, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 162.0, 143.8, 143.2, 141.7, 137.5, 129.5, 128.8, 125.8, 124.8, 121.9, 36.2, 23.1, 13.8. MS (ESI) calculated for $C_{14}H_{13}N_3OS$, m/z 271.08, found 272.09 $(M + H)^+$.

Synthesis of compound 15e: *N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)acetamide

To a stirred solution of compound **8c** (20 mg, 0.082 mmol) in pyridine (1 mL) was added acetyl chloride (5.8 μL, 0.082 mmol), the resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude compound was purified using silica gel column chromatography (5% MeOH/CH_2Cl_2) to furnish compound **15d** as a white solid (18 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 8.3 Hz, 1H), 7.88 (dd, J = 8.1, 0.9 Hz, 1H), 7.72 (ddd, J = 8.4, 7.2, 1.3 Hz, 1H), 7.59–7.54 (m, 1H), 3.17 (td, J = 7.6, 2.3 Hz, 2H), 2.77 (s, 3H), 1.96 (dd, J = 15.0, 7.5 Hz, 2H), 1.09 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 144.7, 138.3, 131.6, 130.0, 127.2, 126.5, 125.9, 125.3, 125.0, 121.2, 36.2, 25.7, 23.2, 13.8. MS (ESI) calculated for $C_{15}H_{15}N_3OS$, m/z 285.09, found 286.11 $(M + H)^+$.

Compounds **15f–15i** were synthesized similarly as compound **15e**.

15f: *N*-(2-Propylthiazolo[4,5-*c*]quinolin-4-yl)butyramide

White solid (20 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.82 (dd, J = 8.0, 1.0 Hz, 1H), 7.65 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.49 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.21–3.04 (m, 4H), 2.01–1.92 (m, 2H), 1.92–1.82 (m, 2H), 1.10 (td, J = 7.4, 6.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 144.5, 143.5, 140.8, 138.6, 129.2, 129.2, 125.7, 124.6, 121.4, 39.9, 36.2, 23.2, 18.6, 14.1, 13.8. MS (ESI) calculated for $C_{17}H_{19}N_3OS$, m/z 313.12, found 314.13 $(M + H)^+$.

15g: *N*-(2-Propylthiazolo[4,5-*c*]quinolin-4-yl)octanamide

White solid (25 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.82 (dd, J = 8.0, 0.9 Hz, 1H), 7.65 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.52–7.44 (m, 1H), 3.15 (t, J = 7.5

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Hz, 4H), 1.96 (dd, J = 14.9, 7.4 Hz, 2H), 1.83 (dd, J = 15.2, 7.7 Hz, 2H), 1.54–1.45 (m, 2H), 1.39–1.23 (m, 6H), 1.09 (t, J = 7.4 Hz, 3H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 144.5, 143.5, 140.8, 138.6, 129.3, 129.2, 125.7, 124.7, 121.4, 38.1, 36.2, 31.9, 29.8, 29.6, 29.3, 25.2, 23.2, 22.8, 14.2, 13.8. MS (ESI) calculated for $C_{21}H_{27}N_3OS$, m/z 369.19, found 370.22 $(M + H)^+$.

15h: *N*-(2-Propylthiazolo[4,5-*c*]quinolin-4-yl)palmitamide

Yellow solid (33 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.83 (dd, J = 8.0, 1.0 Hz, 1H), 7.65 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.49 (ddd, J = 10.4, 5.8, 2.2 Hz, 1H), 3.20–3.10 (m, 4H), 2.01–1.91 (m, 2H), 1.84 (dt, J = 15.2, 7.6 Hz, 2H), 1.53–1.44 (m, 2H), 1.43–1.34 (m, 2H), 1.34–1.19 (m, 20H), 1.09 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 144.5, 143.6, 140.8, 138.6, 129.3, 129.2, 125.7, 124.7, 121.5, 38.1, 36.2, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 25.2, 23.2, 22.8, 14.3, 13.8. MS (ESI) calculated for $C_{29}H_{43}N_3OS$, m/z 481.31, found 482.33 $(M + H)^+$.

15i: *N*-(2-Propylthiazolo[4,5-*c*]quinolin-4-yl)benzamide

White solid (24 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H), 8.07 (d, J = 7.4 Hz, 2H), 7.86 (d, J = 7.6 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.62–7.51 (m, 4H), 3.19 (t, J = 7.6 Hz, 2H), 1.98 (dt, J = 14.9, 7.5 Hz, 2H), 1.12 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 164.7, 144.7, 143.8, 140.9, 139.3, 135.0, 132.4, 130.1, 129.2, 129.0, 127.7, 126.1, 124.5, 121.8, 36.2, 23.4, 13.9. MS (ESI) calculated for $C_{20}H_{17}N_3OS$, m/z 347.11, found 348.12 $(M + H)^+$.

Synthesis of compound 15j: 2-azido-*N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)acetamide

To a stirred solution of 2-azidoacetic acid (18.6 mg, 0.123 mmol) in DMF (1 mL) were added triethylamine (23 μL, 0.164 mmol) and HBTU (46.6 mg, 0.123 mmol), and the resulting mixture was stirred at room temperature for 15 min. Compound **8c** (20 mg, 0.082 mmol) was then added, and stirring continued for 4 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15j** as a white solid (50 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (d, J = 0.6 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.84 (dd, J = 8.0, 1.0 Hz, 1H), 7.68 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.52 (ddd, J = 8.1, 7.2, 1.1 Hz, 1H), 4.86 (s, 2H), 3.17 (t, J = 7.5 Hz, 2H), 2.01–1.92 (m, 2H), 1.10 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 143.6, 143.1, 141.4, 138.2, 129.5, 129.1, 126.2, 124.8, 121.6, 54.7, 36.2, 23.2, 13.8. MS (ESI) calculated for $C_{15}H_{14}N_6OS$, m/z 326.10, found 327.11 $(M + H)^+$.

Compounds **15k–15l** were synthesized similarly as compound **15j**.

15k: 3-Azido-*N*-(2-propylthiazolo[4,5-*c*]quinolin-4-yl)propan-amide

White solid (59 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.84 (dd, J = 8.0, 0.9 Hz, 1H), 7.67 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.53–7.48 (m, 1H), 3.82 (t, J = 6.6 Hz, 2H), 3.59 (s, 2H), 3.16 (t, J = 7.5 Hz, 2H), 2.01–1.91 (m, 2H), 1.09 (dd, J = 9.3, 5.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 144.1, 143.3, 141.1, 138.3, 129.4, 129.1, 125.9, 124.7, 121.5, 46.9, 37.6, 36.2, 23.2, 13.8. MS (ESI) calculated for $C_{16}H_{16}N_6OS$, m/z 340.11, found 341.12 $(M + H)^+$.

15l: *N*-(2-Propylthiazolo[4,5-*c*]quinolin-4-yl)pent-4-ynamide

White solid (60 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.82 (dd, J = 8.0, 0.9 Hz, 1H), 7.66 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.49 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.49 (s, 2H), 3.15 (dd, J = 8.6, 6.5 Hz, 2H), 2.75 (ddd, J = 9.0, 6.9, 2.7 Hz, 2H), 2.03 (t, J = 2.7 Hz, 1H), 2.00–1.90 (m, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 144.2, 143.4, 141.0, 138.4, 129.3, 129.2, 125.8, 124.7, 121.5, 83.6, 68.9, 37.2, 36.2, 23.2, 14.3, 13.8. MS (ESI) calculated for $C_{18}H_{17}N_3OS$, m/z 323.11, found 324.13 $(M + H)^+$.

Synthesis of compound 15m: methyl (2-propylthiazolo[4,5-*c*]-quinolin-4-yl)carbamate

To a stirred solution of **8c** (50 mg, 0.205 mmol) in CH_2Cl_2 (2 mL) were added triethylamine (57 μL, 0.410 mmol) and methyl chloroformate (32 μL, 0.3075 mmol), and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15m** (58 mg, 94%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.82 (dd, J = 8.0, 0.9 Hz, 1H), 7.69–7.63 (m, 1H), 7.52–7.46 (m, 1H), 3.91 (s, 3H), 3.17–3.11 (m, 2H), 1.95 (dt, J = 14.8, 7.4 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 152.2, 144.3, 143.8, 140.8, 138.6, 129.7, 129.2, 125.7, 124.5, 121.4, 52.9, 36.2, 23.3, 13.8. MS (ESI) calculated for $C_{15}H_{15}N_3O_2S$, m/z 301.09, found 302.17 $(M + H)^+$.

Compounds **15n–15p** were synthesized similarly as compound **15m**.

Synthesis of compound 15n: ethyl (2-propylthiazolo[4,5-*c*] quinolin-4-yl)carbamate

Yellow solid (50 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.17 (t, J = 8.3 Hz, 1H), 8.17 (t, J = 8.3 Hz, 1H), 7.81 (dd, J = 8.0, 1.0 Hz, 1H), 7.81 (dd, J = 8.0, 1.0 Hz, 1H), 7.69–7.61 (m, 1H), 7.51–7.45 (m, 1H), 4.36 (q, J = 7.1 Hz, 2H), 3.18–3.12 (m, 2H), 2.01–1.90 (m, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 151.6, 144.5, 143.8, 140.8, 138.6, 129.8, 129.1, 125.7, 124.5, 121.3, 61.8, 36.2, 23.3, 14.7, 13.8. MS (ESI) calculated for $C_{16}H_{17}N_3O_2S$, m/z 315.10, found 316.19 $(M + H)^+$.

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Synthesis of compound 15o: butyl (2-propylthiazolo[4,5-*c*]-quinolin-4-yl)carbamate

White solid (52 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.18–8.13 (m, 1H), 7.81 (dd, J = 8.0, 0.9 Hz, 1H), 7.66 (qd, J = 8.5, 2.6 Hz, 1H), 7.52–7.45 (m, 1H), 4.31 (t, J = 6.7 Hz, 2H), 3.19–3.11 (m, 2H), 2.02–1.91 (m, 2H), 1.80–1.70 (m, 2H), 1.54–1.44 (m, 2H), 1.13–1.06 (m, 3H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 151.8, 144.5, 143.7, 140.8, 138.6, 129.8, 129.1, 125.7, 124.5, 121.3, 65.7, 36.2, 31.0, 23.3, 19.2, 13.9, 13.8. MS (ESI) calculated for $C_{18}H_{21}N_3O_2S$, m/z 343.14, found 344.14 $(M + H)^+$.

Synthesis of compound 15p: octyl (2-propylthiazolo[4,5-*c*]-quinolin-4-yl)carbamate

White solid (60 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.17–8.13 (m, 1H), 7.81 (dd, J = 8.0, 1.0 Hz, 1H), 7.68–7.61 (m, 1H), 7.47 (ddd, J = 8.1, 7.2, 1.1 Hz, 1H), 4.30 (t, J = 6.7 Hz, 2H), 3.15 (dd, J = 9.7, 5.5 Hz, 2H), 2.02–1.90 (m, 3H), 1.80–1.71 (m, 2H), 1.51–1.39 (m, 3H), 1.38–1.22 (m, 10H), 1.09 (dd, J = 9.0, 5.8 Hz, 5H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 151.8, 144.5, 143.9, 140.8, 138.6, 129.7, 129.1, 125.6, 124.5, 121.3, 66.0, 36.2, 31.9, 29.4, 29.4, 29.0, 26.0, 23.3, 22.8, 14.2, 13.8. MS (ESI) calculated for $C_{22}H_{29}N_3O_2S$, m/z 399.20, found 400.21 $(M + H)^+$.

Synthesis of compound 15q: 1-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)urea

To a stirred solution of **8c** (50 mg, 0.205 mmol) in acetonitrile (2 mL) were added $NaHCO_3$ (26 mg, 0.307 mmol) and chlorosulfonyl isocyanate (27 μL, 0.307 mmol), and the resulting mixture was stirred at room temperature for 1 h. Then, one more equivalent of chlorosulfonyl isocyanate (27 μL, 0.307 mmol) was added and the reaction mixture was stirred at room temperature for additional 1 h. The reaction mixture was diluted with water and extracted with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15q** as a white solid (26 mg, 45% starting material was recovered). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (d, J = 10.8 Hz, 1H), 8.86 (d, J = 10.3 Hz, 1H), 8.03–7.94 (m, 1H), 7.84 (ddd, J = 8.0, 1.4, 0.5 Hz, 1H), 7.67 (ddd, J = 8.5, 7.0, 1.4 Hz, 1H), 7.50 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 3.24–3.08 (m, 2H), 2.05–1.87 (m, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 162.0, 143.8, 143.2, 141.7, 137.5, 129.5, 128.8, 125.8, 124.8, 121.9, 36.2, 23.1, 13.8. MS (ESI) calculated for $C_{14}H_{14}N_4OS$, m/z 286.09, found 287.09 $(M + H)^+$.

Synthesis of compound 15r: *N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)methanesulfonamide

To a stirred solution of **8c** (40 mg, 0.146 mmol) in dichloromethane (2 mL) was added methyl methanesulfonyl chloride (26 μL, 0.292 mmol), and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15r** as a white solid (34 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.91 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.6 Hz, 2H), 3.29 (s, 3H), 3.20–3.15 (m, 2H), 1.97–1.87 (m, 2H), 1.07 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 148.8, 143.4, 141.6, 133.5, 130.6, 125.4, 125.2, 117.5, 116.9, 43.2, 36.3, 23.7, 13.8. MS (ESI) calculated for $C_{14}H_{15}N_3O_2S_2$, m/z 321.06, found 322.06 $(M + H)^+$.

Compounds **15s–15v** were synthesized similarly as compound **15r**.

Synthesis of compound 15s: *N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)ethanesulfonamide

White solid (35 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.72 (d, J = 6.7 Hz, 1H), 7.58 (t, J = 7.1 Hz, 1H), 7.40 (d, J = 7.3 Hz, 2H), 3.34 (d, J = 5.0 Hz, 2H), 3.18 (t, J = 7.7 Hz, 2H), 1.99–1.87 (m, 2H), 1.46 (t, J = 7.4 Hz, 3H), 1.07 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 149.9, 143.3, 141.9, 133.5, 130.7, 125.5, 125.0, 117.7, 116.8, 49.4, 36.3, 23.8, 13.8, 8.3. MS (ESI) calculated for $C_{15}H_{17}N_3O_2S_2$, m/z 335.08, found 336.08 $(M + H)^+$.

Synthesis of compound 15t: *N*-(2-propylthiazolo[4,5-*c*]quinolin-4-yl)propane-1-sulfonamide

White solid (35 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.72 (d, J = 6.7 Hz, 1H), 7.58 (t, J = 7.1 Hz, 1H), 7.40 (d, J = 7.3 Hz, 2H), 3.34 (d, J = 5.0 Hz, 2H), 3.18 (t, J = 7.7 Hz, 2H), 1.99–1.87 (m, 2H), 1.46 (t, J = 7.4 Hz, 3H), 1.07 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 149.9, 143.3, 141.9, 133.5, 130.7, 125.5, 125.0, 117.7, 116.8, 49.4, 36.3, 23.8, 13.8, 8.3. MS (ESI) calculated for $C_{16}H_{19}N_3O_2S_2$, m/z 349.09, found 350.09 $(M + H)^+$.

Synthesis of compound 15u: *N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)butane-1-sulfonamide

White solid (37 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.04 (s, 1H), 7.71 (d, J = 6.7 Hz, 1H), 7.57 (t, J = 7.0 Hz, 1H), 7.39 (d, J = 6.2 Hz, 2H), 3.32 (s, 2H), 3.18 (t, J = 7.7 Hz, 2H), 2.00–1.82 (m, 4H), 1.55–1.40 (m, 2H), 1.07 (t, J = 7.4 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8, 149.7, 143.3, 141.9, 133.5, 130.7, 125.5, 125.0, 117.6, 116.8, 54.8, 36.3, 25.5, 23.8, 21.7, 13.8, 13.8. MS (ESI) calculated for $C_{17}H_{21}N_3O_2S_2$, m/z 363.11, found 364.11 $(M + H)^+$.

Synthesis of compound 15v: 4-methyl-*N*-(2-propylthiazolo[4,5-*c*]-quinolin-4-yl)benzenesulfonamide

White solid (40 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.04 (s, 1H), 7.71 (d, J = 6.7 Hz, 1H), 7.57 (t, J = 7.0 Hz, 1H), 7.39 (d, J = 6.2 Hz, 2H), 3.32 (s, 2H), 3.18 (t, J = 7.7 Hz, 2H), 2.00–1.82 (m, 4H), 1.55–1.40 (m, 2H), 1.07 (t, J = 7.4 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H). 13C NMR (126 MHz, CDCl3) δ 172.8, 149.0, 143.4, 143.0, 142.1, 139.9, 133.5, 130.7, 129.4, 126.7, 125.5, 125.1, 117.7, 117.0, 36.2, 23.67, 21.7, 13.8. MS (ESI) calculated for $C_{20}H_{19}N_3O_2S_2$, m/z 397.09, found 398.10 $(M + H)^+$.

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Synthesis of compound 15w: diethyl (2-propylthiazolo[4,5-*c*]quinolin-4-yl)phosphoramidate

To a stirred solution of **8c** (50 mg, 0.205 mmol) in CH_2Cl_2 (2 mL) was added diethyl chlorophosphate (60 μL, 0.810 mmol), and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain compound **15w** as a white solid (45 mg, 56%). ^{1}H NMR (500 MHz, DMSO) δ 8.41 (d, J = 10.1 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.68 (dd, J = 11.2, 4.0 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 4.24 (p, J = 7.2 Hz, 4H), 3.20 (t, J = 7.5 Hz, 2H), 1.94–1.85 (m, 2H), 1.27 (t, J = 7.0 Hz, 6H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.9, 147.0, 143.0, 140.3, 138.0 (d, J = 10.4 Hz), 131.2, 129.2 (d, J = 207.2 Hz), 127.8 (d, J = 94.8 Hz), 124.9 (d, J = 12.8 Hz), 120.2, 63.1 (d, J = 5.6 Hz), 35.2, 63.1 (d, J = 5.6 Hz), 16.2 (d, J = 6.8 Hz) 13.5. MS (ESI) calculated for $C_{17}H_{22}N_3O_3PS$, m/z 379.11, found 380.12 $(M + H)^+$.

Synthesis of compound 17: 1-(2-propylthiazolo[4,5-*c*]quinolin-4-yl)guanidine

Compound **7c** (100 mg, 0.411 mmol) was dissolved in $POCl_3$ (3 mL) and stirred at 100 °C for 1 h. $POCl_3$ was evaporated under reduced pressure, ice cold water was added and the residue was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure to give crude **16** (100 mg, 93%) which was used further without purification. To a stirred solution of guanidine (62 mg, 0.615 mmol) in 1,4 dioxane (2 mL) was added NaH (12 mg, 0.492 mmol), the reaction mixtures was stirred at 60 °C for 30 min. Compound **16** (30 mg, 0.123 mmol) in DMF (2 mL) was added and heating was continued at 90 °C for 12 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (10% MeOH/CH_2Cl_2) to obtain compound **17** as a yellow solid (25 mg, 71%). ^{1}H NMR (500 MHz, MeOD) δ 8.05 (dd, J = 8.4, 0.5 Hz, 1H), 8.00–7.96 (m, 1H), 7.75 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.62 (ddd, J = 8.2, 7.1, 1.2 Hz, 1H), 3.25 (dd, J = 8.7, 6.4 Hz, 2H), 2.06–1.97 (m, 2H), 1.11 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 175.2, 157.5, 146.0, 143.5, 142.7, 138.9, 131.0, 129.3, 128.0, 125.9, 122.5, 36.9, 24.2, 14.0. MS (ESI) calculated for $C_{14}H_{15}N_5S$, m/z 285.10, found 286.11 $(M + H)^+$.

Compounds **18a** were synthesized similarly as compound **15c**.

Synthesis of compound 18a: *N*-(2-butylthiazolo[4,5-*c*]quinolin-4-yl)formamide

White solid (32 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (d, J = 10.8 Hz, 1H), 8.86 (d, J = 10.3 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.85 (dd, J = 8.0, 0.9 Hz, 1H), 7.67 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.50 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 3.20–3.16 (m, 2H), 1.91 (dt, J = 15.2, 7.6 Hz, 2H), 1.55–1.45 (m, 2H), 1.01 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8, 161.9, 143.7, 143.2, 141.6, 137.4, 129.4, 128.7, 125.7, 124.7, 121.8, 33.8, 31.6, 22.2, 13.8. MS (ESI) calculated for $C_{15}H_{15}N_3OS$, m/z 285.09, found 286.10 $(M + H)^+$.

Compounds **18b–c** were synthesized similarly as compound **15d**.

Synthesis of compound 18b: *N*-(2-butylthiazolo[4,5-*c*]quinolin-4-yl)acetamide

White solid (50 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.02 (d, J = 8.3 Hz, 1H), 7.82 (ddd, J = 8.0, 1.4, 0.5 Hz, 1H), 7.65 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.49 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 3.21–3.13 (m, 2H), 2.82 (s, 3H), 1.96–1.85 (m, 2H), 1.55–1.44 (m, 2H), 1.04–0.96 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 144.4, 143.4, 140.9, 138.5, 129.2, 129.1, 125.7, 124.7, 121.5, 34.0, 31.8, 25.9, 22.3, 13.9. MS (ESI) calculated for $C_{16}H_{17}N_3OS$, m/z 299.11, found 300.11 $(M + H)^+$.

Synthesis of compound 18c: *N*-(2-butylthiazolo[4,5-*c*]quinolin-4-yl)butyramide

White solid (55 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.82 (ddd, J = 8.0, 1.4, 0.4 Hz, 1H), 7.65 (ddd, J = 8.4, 7.1, 1.4 Hz, 1H), 7.49 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 3.22–3.06 (m, 4H), 1.95–1.83 (m, 4H), 1.54–1.45 (m, 2H), 1.10 (t, J = 7.4 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 144.3, 143.4, 140.7, 138.4, 129.1, 129.0, 125.6, 124.5, 121.3, 39.8, 33.8, 31.7, 22.2, 18.5, 14.0, 13.8. MS (ESI) calculated for $C_{18}H_{21}N_3OS$, m/z 327.14, found 328.14 $(M + H)^+$.

Human TLR-7/-8 reporter gene assays (NF-κB induction)

The induction of NF-κB was quantified using HEK-Blue-7 (hTLR7-specific) and HEK-Blue-8 (hTLR8-specific) cells as previously described by us.[27,32,33] HEK293 cells stably co-transfected with human TLR7 or human TLR8 and secreted alkaline phosphatase (sAP), were maintained in HEK-Blue™ Selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by appropriate TLR agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue cells were incubated at a density of ~10^5 cells per ml in a volume of 80 μL per well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently stimulated with graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.

Immunoassays for Interferon (IFN)-α, IFN-γ, Interleukin (IL)-12, IL-18, and cytokines

Fresh human peripheral blood mononuclear cells (hPBMC) were isolated from human blood obtained by venipuncture with informed consent and as per institutional guidelines on Ficoll-Hypaque gradients as described elsewhere.[62] Aliquots of

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PBMCs (10^5 cells in 100 μL per well) were stimulated for 12 h with graded concentrations of test compounds. Supernatants were isolated by centrifugation, and were assayed in triplicates using either high-sensitivity analyte-specific ELISA kits (PBL Interferon Source, Piscataway, NJ and R&D Systems, Inc., Minneapolis, MN), or analyte-specific multiplexed cytokine/chemokine bead array assays as reported by us previously.[63]

Rabbit immunization and antigen-specific ELISA

All experiments were performed at Harlan Laboratories (Indianapolis, IN) in accordance with institutional guidelines (University of Kansas IACUC permit # 119-06). Cohorts of adult female New Zealand White rabbits (n = 3 per cohort) were immunized intramuscularly in the flank region with (a) 100 μg of bovine α-lactalbumin in 0.2 mL saline, or (b) 100 μg of bovine α-lactalbumin plus 100 μg of either **8c** or **8d** in 0.2 mL saline. Pre-immune test-bleeds were first obtained on Day 1 *via* venipuncture of the marginal vein of the ear. Animals were immunized on Days 1 and 15. A final test-bleed was performed *via* the marginal vein of the ear on Day 28. Sera were stored at −80 °C until used. Bovine α-lactalbumin-specific ELISAs were performed in 384-well format using automated liquid handling methods as described by us.[35]

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