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Inhibition of Group IVA Cytosolic Phospholipase A_2 by Thiazolyl Ketones in Vitro, ex Vivo, and in Vivo

George Kokotos,*[,†] Astrid J. Feuerherm,[⊥,‡] Efrosini Barbayianni,[⊥,†] Ishita Shah,[§] Mari Sæther,[‡] Victoria Magrioti,[†] Thuy Nguyen,[‡] Violetta Constantinou-Kokotou,[∥] Edward A. Dennis,[§] and Berit Johansen*[,‡]

[†]Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece

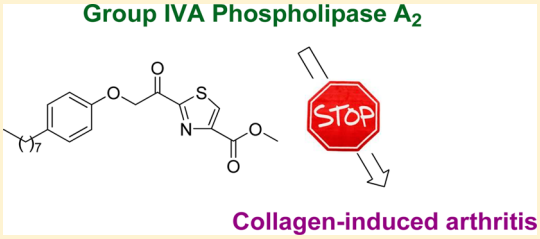
[‡]Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

[§]Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, University of California−San Diego, La Jolla, California 92093-0601, United States

[∥]Chemical Laboratories, Agricultural University of Athens, Athens 11855, Greece

Supporting Information

ABSTRACT: Group IVA cytosolic phospholipase A_2 (GIVA $cPLA_2$) is the rate-limiting provider of pro-inflammatory mediators in many tissues and is thus an attractive target for the development of novel anti-inflammatory agents. In this work, we present the synthesis of new thiazolyl ketones and the study of their activities in vitro, in cells, and in vivo. Within this series of compounds, methyl 2-(2-(4-octylphenoxy)-acetyl)thiazole-4-carboxylate (GK470) was found to be the most potent inhibitor of GIVA $cPLA_2$, exhibiting an $X_1(50)$ value of 0.011 mole fraction in a mixed micelle assay and an IC_{50} of 300 nM in a vesicle assay. In a cellular assay using SW982 fibroblast-like synoviocytes, it suppressed the release of arachidonic acid with an IC_{50} value of 0.6 μM. In a prophylactic collagen-induced arthritis model, it exhibited an anti-inflammatory effect comparable to the reference drug methotrexate, whereas in a therapeutic model, it showed results comparable to those of the reference drug Enbrel. In both models, it significantly reduced plasma PGE_2 levels.



INTRODUCTION

The purification, sequence, and cloning of the first human cytosolic phospholipase A_2 ($cPLA_2$) from the U937 macrophage cell line was reported in 1991.[1,2] Two years later, arachidonoyl trifluoromethyl ketone (**1**, Figure 1) was reported as the first synthetic inhibitor of this enzyme.[3] The enzyme has subsequently attracted special attention as a medicinal target, in particular after the reports that gene-targeted mice that lack $cPLA_2$ are much less prone to inflammatory pathological responses to disease, stresses, and physical injuries, which, in essence, protects them from cellular and systemic damage.[4,5] Wyeth has expended major efforts to develop novel $cPLA_2$ inhibitors as targeted therapeutics for inflammatory diseases.[6−9] Three indole-based inhibitors, Ecopladib (**2a**, Figure 1), Efipladib (**2b**, Figure 1), and Giripladib (**2c**, Figure 1), entered clinical trials. Giripladib was the most promising of this indole series, as it was advanced into a phase II clinical trial for osteoarthritis; however, in 2007, the trial was terminated.[10]

Phospholipases A_2 constitutes a superfamily of enzymes that currently includes 16 groups and many subgroups, most of which differ in their primary sequence and structure, but they also exhibit a variety of different mechanisms of action.[11,12] Human $cPLA_2$ has been designated as GIVA $cPLA_2$ and is the most well-studied enzyme of this group.[13] Several inhibitors of GIVA $cPLA_2$ have been reported in the literature and are summarized in various review articles.[11,14,15] Most recently, the inhibitors described in the patent literature have been highlighted.[15] Apart from the inhibitors developed by Wyeth, Shionogi has developed thiazolidinedione inhibitors based on a pyrrolidine template, including pyrrophenone (**3**, Figure 1).[16,17] Pyrrophenone strongly inhibited arachidonic acid release and prostaglandin E_2, thromboxane B_2, and leukotriene B_4 formation in human whole blood. Pyrroxyphene (**4**, Figure 1), which also belongs to this class of inhibitors, displayed anti-arthritic and anti-bone destructive action in a murine arthritis model.[18] Another important class of GIVA $cPLA_2$ inhibitors is indol-1-yl-propane-2-ones, for example, compound **5** (Figure 1).[19−24] One of them was reported to inhibit benzalkonium chloride-induced skin inflammation.[25] Moreover, heterocyclic derivatives have been claimed as GIVA $cPLA_2$ inhibitors.[26] Recently, inhibitors containing an α-methyl-2-ketothiazole as a metabolically stable serine trap have been developed, for example, compound **6** (Figure 1).[27]

During the past decade, we have designed, synthesized, and studied novel inhibitors targeting various PLA_2 groups. We have developed various 2-oxoamides,[28−32] for example, **7a** and **7b** (Figure 1), as inhibitors of GIVA $cPLA_2$, and fluoroke-

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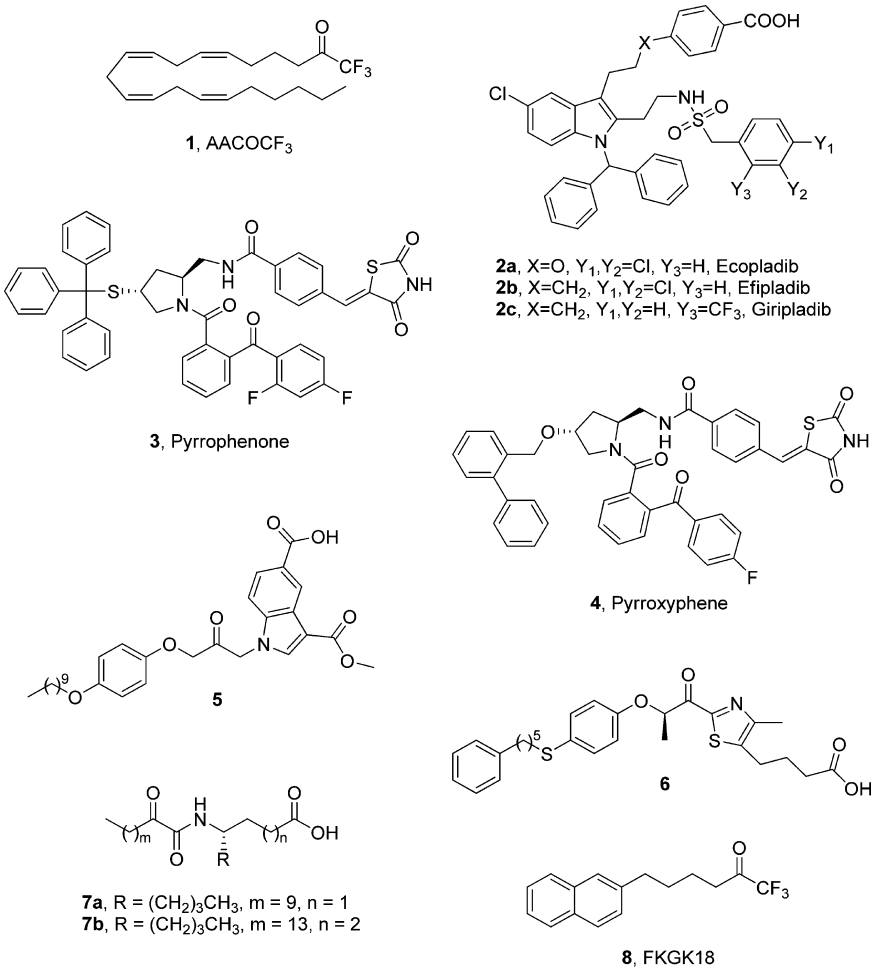

Figure 1. Common inhibitors of phospholipases A_2.

tones,[33−35] for example, FKGK18,[34] (**8**, Figure 1), as inhibitors of the other major intracellular cytosolic phospholipase A_2 (GVIA iPLA$_2$) enzyme. Some fluoroketones exhibit remarkable inhibition of GIVA cPLA$_2$.[33−35] Using a combination of molecular dynamics simulation and deuterium exchange mass spectrometry, the interactions of our synthetic inhibitors with the active site of GIVA cPLA$_2$ and GVIA iPLA$_2$ were studied.[36,37] In a continuation of our studies, we have now developed new inhibitors targeting GIVA cPLA$_2$ and tested their anti-inflammatory effect in vivo. Herein, we report the synthesis of several thiazolyl ketone derivatives, the study of their in vitro potency and specificity and their effect on the release of arachidonic acid (AA) and oleic acid (OA) ex vivo, and the first in vivo studies of the most potent inhibitor of this series in an animal model of collagen-induced arthritis (CIA).

RESULTS AND DISCUSION

Design and Synthesis of Inhibitors. GIVA cPLA$_2$ consists of an N-terminal C2 domain and a C-terminal catalytic domain and utilizes an unusual catalytic dyad (Ser-228/Asp-549) located in the α/β hydrolase domain to catalyze the hydrolysis of the substrates.[38] We have previously studied derivatives containing the 2-oxoamide and the polyfluoroketone functionalities targeting the active site serine of PLA$_2$.[28−35] In both cases, it is proposed that the activated ketone interacts with the catalytic serine. Although these two functionalities markedly differ in the potency of their activated carbonyl group, derivatives containing either the oxoamide or the fluoroketone functionality have proven to be efficient inhibitors of GIVA cPLA$_2$. Apparently, not only the potency of the activated carbonyl group but also the presence of other groups able to present appropriate hydrophobic and/or hydrophilic interactions contributes to the overall binding of the inhibitor to the enzyme, determining the inhibitory potency. In the present work, we study derivatives containing the ketothiazolyl functionality (Figure 2). The presence of the two heteroatoms

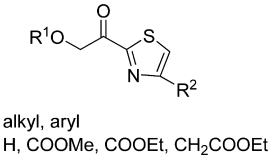

Figure 2. General structure of thiazolyl ketones.

on the heterocyclic ring ensures the activation of the carbonyl group. In addition, the presence of an oxygen atom at the β-position enforces the activation. The R^1 group may be either an aliphatic or an aromatic group, while a substituent R^2 may be present on the heterocyclic ring. In fact, a variety of α-ketoheterocycles have been reported as inhibitors of diverse serine and cysteine proteases as well as fatty acid amide hydrolase.[39] As outlined in the Introduction, some such heterocyclic derivatives have been claimed as agents for the

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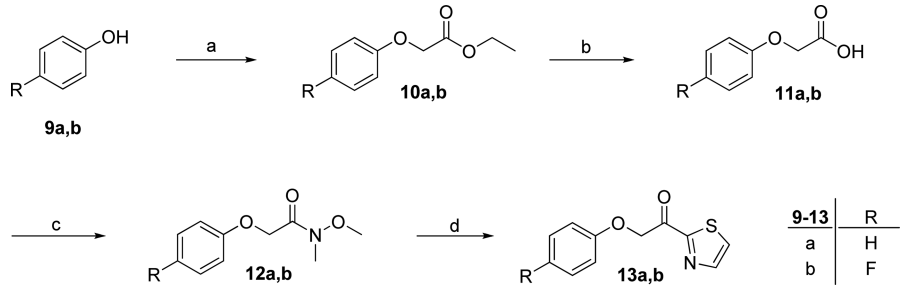
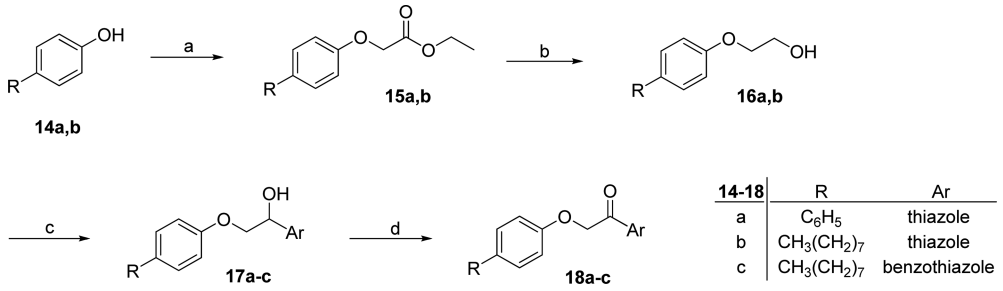
Scheme 1[a]



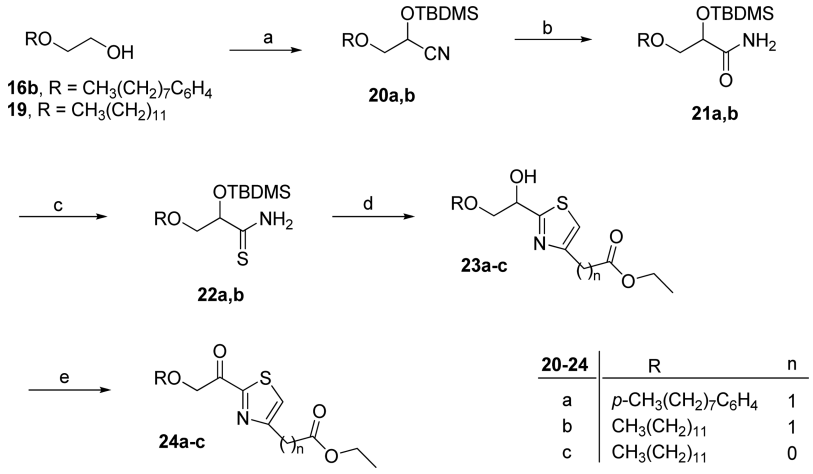
[a]Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, acetone; (b) 1 N NaOH(aq), EtOH; (c) HCl·HN(OMe)Me, NMM, DMAP, WSCI·HCl, CH_2Cl_2; (d) thiazole, *n*-BuLi, Et_2O.

Scheme 2[a]



[a]Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, acetone; (b) DIBALH, Et_2O; (c) (i) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C, (ii) thiazole for **16a,b** or benzothiazole for **16b**, *n*-BuLi, Et_2O; (d) Dess−Martin periodinane, CH_2Cl_2.

Scheme 3[a]



[a]Reagents and conditions: (a) (i) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C, (ii) TBDMSCN, 18-crown-6, KCN, CH_2Cl_2; (b) 30% H_2O_2(aq), Bu_4NHSO_4, 0.5 N NaOH(aq), CH_2Cl_2; (c) Lawesson's reagent, toluene; (d) $ClCH_2COCH_2COOEt$ for **22a,b** or $BrCH_2COCOOEt$ for **22b**, EtOH, conc. H_2SO_4; (e) Dess−Martin periodinane, CH_2Cl_2.

treatment of inflammatory diseases, however, without providing any in vivo data.[26] Inhibitors containing an α-methyl-2-ketothiazole as a metabolically stable serine trap have been presented.[27]

The synthesis of thiazole derivatives **13a,b** and **18a−c** is presented in Schemes 1 and 2. Phenols **9a,b** and **14a,b** were treated with ethyl bromoacetate. Esters **10a,b** were hydrolyzed and converted to their corresponding Weinreb amides. Treatment of Weinreb amides **12a,b** with lithium thiazole[40] led to the target derivatives **13a,b**. Thiazolyl ketones **18a−c** were prepared by another procedure. Alcohols **16a,b** were oxidized to aldehydes and treated with lithium thiazole or benzothiazole.[41] Compounds **17a−c** were then oxidized by Dess−Martin periodinane[42] to the final compounds.

Substituted thiazoles **24a−c** and **28** were synthesized as illustrated in Schemes 3 and 4. The key step for their synthesis was the formation of the substituted heterocyclic ring. Alcohols **16b** and **19** were oxidized to aldehydes and directly treated with *tert*-butyldimethylsilyl cyanide (TBDMSCN). Silyl-protected cyanohydrins **20a,b** were converted into amides and subsequently into thioamides by reaction with Lawesson's reagent. Treatment of **22a,b** with ethyl 4-chloroacetoacetate and of **22b** with ethyl bromopyruvate, according to previously published protocols,[43] in the presence of conc. H_2SO_4 led to

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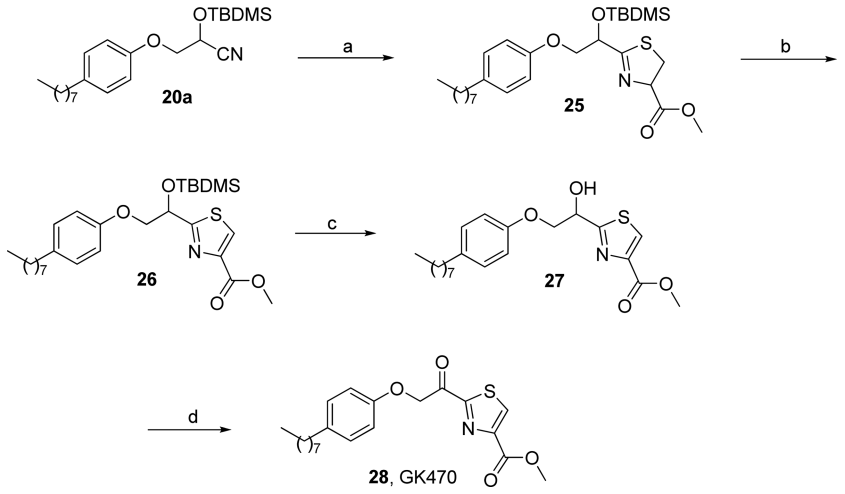
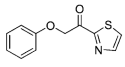
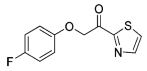
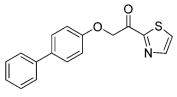
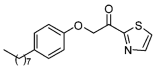
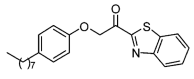
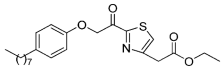
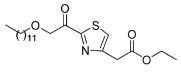
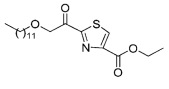
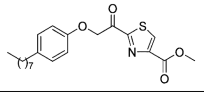
Scheme 4[a]



[a]Reagents and conditions: (a) HCl·H-L-Cys-OMe, $CH_3COO^-NH_4^+$, MeOH; (b) DBU, $BrCCl_3$, CH_2Cl_2; (c) 4 N HCl/MeOH; (d) Dess−Martin periodinane, CH_2Cl_2.

Table 1. In Vitro and ex Vivo Activities of Thiazolyl Ketones

Entry	No	Structure	*In vitro* assays						*Ex vivo* assays		
			GIVA cPLA$_2$		GVIA iPLA$_2$	GV sPLA$_2$	GIVA cPLA$_2$ vesicle assay		AA release		OA release
			% Inhibition[a]	X_I(50)	% Inhibition[a]	% Inhibition[a]	% Inhibition[b]	IC$_{50}$ (μM)[c]	% Inhibition[d]	IC$_{50}$ (μM)[e]	% Inhibition[d]
1	**13a**		40		13	0	26	NA	12	NA	20
2	**13b**		58		5	4	7	NA	No		25
3	**18a**		80		23	41	26	NA	73	2.8	25
4	**18b**		>90	0.02 ±0.007	67	46	43	1.2	>90	3.6	No
5	**18c**		>90	0.06 ±0.002	77	55	No		71	5.3	35
6	**24a**		64		76	50	25	NA	71	5.0	20
7	**24b**		49		91	55	No		No		No
8	**24c**		48		92	48	21	NA	9	NA	No
9	**28**		>90	0.011 ±0.005	86	41	74	0.3	>90	0.6	40

[a]Percent inhibition at 0.091 mole fraction of each inhibitor. [b]Percent inhibition at 1 μM concentration. [c]Inhibitors were tested in the 0−3 μM range. [d]Cellular assays using SW982 fibroblast-like synoviocytes: inhibitors were tested in the 0−20 μM range with 4h IL-1β stimulation; percent inhibition at 10 μM thiazolyl ketone is given. [e]Inhibitors were tested in the 0−20 μM range. "No" denotes no effect within the given concentration range, whereas "NA" denotes that IC$_{50}$ was not achieved within the given concentration range.

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heterocyclic derivatives **23a−c**, which were then oxidized to the final compounds **24a−c**. Following another method for the formation of the heterocyclic ring,[44] condensation of cysteine methyl ester with nitrile **20a** afforded a diastereomeric mixture of thiazoline **25**, which was transformed into thiazole **26** using $BrCCl_3$ and 1,8-diazabicycloundec-7-ene (DBU).[45] Subsequent removal of the silyl group and Dess−Martin oxidation led to the thiazolyl ketone **28**.

In Vitro Inhibition of GIVA cPLA$_2$, GVIA iPLA$_2$, and GV sPLA$_2$. All synthesized thiazolyl ketones were tested for their in vitro activity on recombinant human GIVA $cPLA_2$ using both mixed micelle and vesicle assays. In addition, their selectivity over human GVIA $iPLA_2$ and GV $sPLA_2$ was studied using mixed micelle assays.

The in vitro inhibition of human GIVA $cPLA_2$, GVIA $iPLA_2$, and GV $sPLA_2$ was carried out using previously described mixed micelle-based assays.[29−31] The inhibition results are presented in Table 1, either as percent inhibition or as $X_I(50)$ values. At first, the percent of inhibition for each PLA_2 enzyme at 0.091 mole fraction of each inhibitor was determined. Then, the $X_I(50)$ values were measured for compounds that displayed greater than 90% inhibition of GIVA $cPLA_2$. The $X_I(50)$ is the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme activity by 50%.

Thiazolyl ketone **13a** as well as its derivatives **13b** and **18a** containing a fluorine atom or a phenyl group at the para position did not present any significant inhibition of GIVA $cPLA_2$ (entries 1−3, Table 1). However, when a para-*n*-octyl chain was introduced, a significant inhibitory activity was observed for **18b** (entry 4, Table 1). The replacement of the thiazole ring by the benzothiazole resulted in reduced inhibitory potency (entry 5 vs entry 4, Table 1). Derivatives **24b** and **24c** containing an alkoxy group and a substituted thiazole group proved to be inactive (entries 7 and 8, Table 1). However, the introduction of a para-*n*-octyl-phenoxy group, together with an ester group on the thiazole ring, led to the potent inhibitor of GIVA $cPLA_2$ **28** (GK470) showing a $X_I(50)$ value of 0.011 (entry 9, Table 1). Interestingly, moving the ester group one carbon atom away from the heterocyclic ring resulted in a dramatic loss of the activity for **24a** (entry 6, Table 1).

The effect of the thiazolyl ketones synthesized on GIVA $cPLA_2$ was measured in vesicles as previously described[46,47] with modifications.[48] The results are presented in Table 1 and are in full agreement with those using the micellar assay. Compound **28** was found to be the most potent inhibitor of GIVA $cPLA_2$ within this series of thiazolyl ketones, with an IC_{50} value of 0.3 μM (entry 9, Table 1). Compound **18b** also presented an interesting inhibition in this vesicle assay, with an IC_{50} value of 1.2 μM (entry 4, Table 1). From both assays, it is clear that the introduction of the ethyl ester group directly on the heterocyclic ring substantially increases the potency of inhibition. This group presumably develops additional interactions within the enzyme's active site. By comparing the results obtained in mixed micelles and in vesicles, it is obvious that inhibitor **28** stands out in this series of thiazolyl ketones.

Ex Vivo Inhibition of AA and OA Release in Synoviocytes. The effect of the thiazolyl ketones synthesized on the release of AA and OA in synoviocytes was evaluated as previously described.[49] The percent inhibition of AA and OA was determined at a 10 μM inhibitor concentration, whereas for the determination of the IC_{50} value, the inhibitors were tested in a 0−20 μM range after interleukin-1β (IL-1β) stimulation for 4 h. No toxic effects were observed for any inhibitor in the range 0−20 μM (results not shown). A number of thiazolyl ketones (**18a**, **18b**, **18c**, and **24a**) exhibited significant inhibition of the AA release (Table 1). However, in accordance with the in vitro results, inhibitor **28** exhibited the most potent effect, inhibiting AA release with an IC_{50} value of 0.6 μM, without displaying equivalent potency on OA release (entry 9, Table 1). None of the thiazolyl ketones presented significant inhibition of OA release.

In Vivo Prophylactic Anti-inflammatory Effect of Inhibitor 28 in CIA. Inhibitor **28** clearly presented a potent inhibitory effect of the GIVA $cPLA_2$ activity in vitro and a potent suppression of the AA release in cells. Thus, studies of its anti-inflammatory properties in vivo were designed. First, a pilot study was performed to evaluate possible toxic effects of inhibitor **28**. No toxic or adverse effects were found at the doses 1, 5, 15, 30, or 60 mg/kg following daily intraperitoneal (ip) injections of the compound for 1 week (results not shown). Next, the CIA mouse model, a common autoimmune model of rheumatoid arthritis in which GIVA $cPLA_2$ activity is important,[18,50,51] was employed for the evaluation of the in vivo activity of inhibitor **28**.

The prophylactic effect of inhibitor **28** on the CIA model in male DBA/1 mice[52] following ip administration, with treatment starting 1 h prior to the last immunization, was studied. A study comparing naïve mice (healthy, non-CIA, nontreated), vehicle-treated mice (CIA with DMSO ip), and CIA mice treated daily with inhibitor **28** (7.5 mg/kg, ip) or methotrexate (MTX) (0.3 mg/kg, ip) was performed. In order to capture early disease progression and the effect of inhibitor **28** and MTX, additional groups of animals (histology groups) enabling comparison of naïve mice, vehicle-treated mice, and CIA mice treated with inhibitor **28** or MTX were designed. CIA developed rapidly in mice immunized with collagen type II (CII). In the prophylactic study, a 100% incidence of CIA was observed by day 29 in CII-immunized mice, with a maximum arthritis index (AI) of 8.55 observed at 41 days post-immunization. The AI and incidence of all groups increased in a time-dependent mode from day 25 to day 41.

The AI of the group dosed with inhibitor **28** at 7.5 mg/kg on days 32−41 was significantly reduced in comparison to that of the CIA control group ($p < 0.005$), similar to the effect of MTX (Figure 3). Within the smaller histology groups that were sacrificed at day 32, following 13 days of treatment, there was

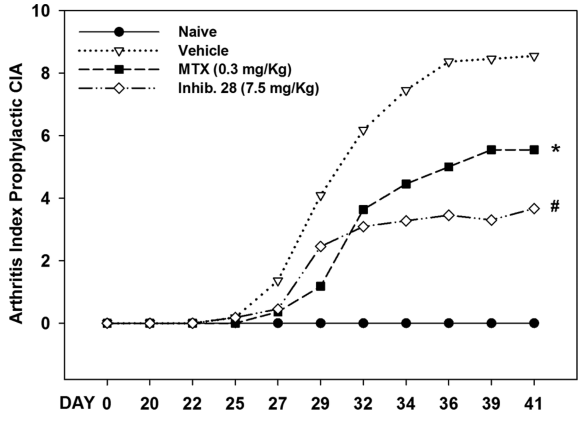

Figure 3. GIVA $cPLA_2$ inhibitor **28** inhibits arthritis progression more efficiently than MTX in the prophylactic CIA study design. *$p < 0.05$, # $p < 0.005$ vs vehicle at study termination.

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no significant difference in the AI value between the histology groups and main groups ($p > 0.05$). Following 13 days of treatment, one hind paw of mice in each histology group was collected for histopathology. Compared with that of the vehicle group, inhibitor **28** at 7.5 mg/kg significantly reduced articular cavity and peripheral tissue inflammatory cell infiltration ($p < 0.03$), as well as capillary and synovial hyperplasia ($p < 0.05$), but it had no significant effect on reducing cartilage damage (Figure 4A). This finding was supported by histopathological analyses of joints at end of the study that also documented reduced inflammatory cell infiltration and hyperplasia in inhibitor **28**-treated animals compared to that in the vehicle-treated group (Figure 4B). In contrast, MTX did not reduce any of these parameters of joint inflammation and joint damage ($p > 0.05$). These data suggest a disease-modifying property of inhibitor **28**.

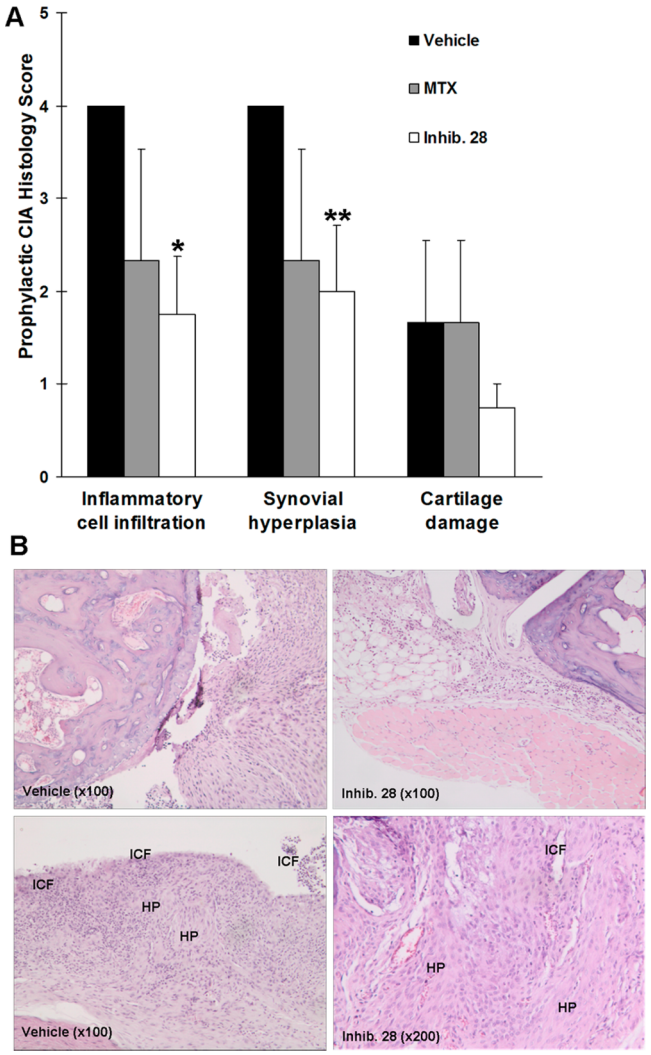

Figure 4. (A) Inhibitor **28** reduces parameters of joint inflammation and joint damage more efficiently than MTX in the prophylactic CIA study design. Histopathology analysis was performed on hind paws from mice sacrificed at day 32, following 13 days of treatment, as described in the Experimental Section, and were evaluated for articular cavity and peripheral tissue inflammatory cell infiltration, capillary and synovial hyperplasia, and articular damage (scores 0–5). *$p < 0.03$, **$p < 0.05$ vs vehicle; error bars denote standard error of mean (n = 3–10). (B) Representative histology depicting overall joint structure, capillary and synovial hyperplasia (HP), and inflammatory cell infiltration (ICF) in vehicle-treated and inhibitor **28**-treated mice at study termination, day 41.

In Vivo Therapeutic Anti-inflammatory Effect of Inhibitor 28 in CIA. The therapeutic effect of inhibitor **28** on the CIA model in male DBA/1 mice following ip administration with treatment starting 7 days after the last immunization was explored. CIA developed rapidly in mice immunized with CII, with a maximum AI of 10.2 observed at 39 days post-immunization. The AI and incidence of vehicle- and inhibitor **28**-treated groups increased in a time-dependent manner from day 29 to 41. The AI observed was significantly decreased in comparison to that of the CIA control group both in the inhibitor **28**-treated with 30 mg/kg group and in the Enbrel group on days 36–41 ($p < 0.05$) (Figure 5). Inhibitor **28** and Enbrel performed equally well, and there was no significant difference between these treatment groups.

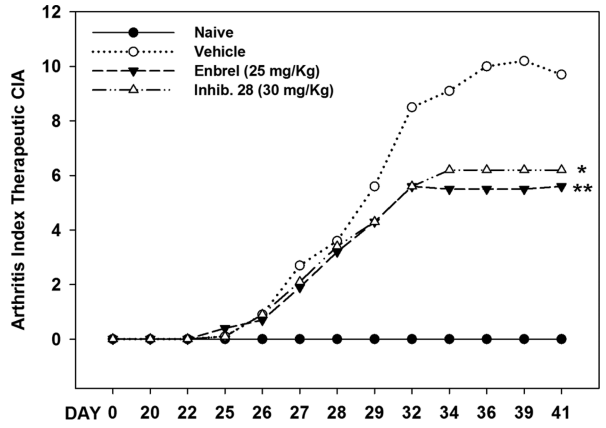

Figure 5. GIVA cPLA$_2$ inhibitor **28** reduces the arthritic index in a manner comparable to that of Enbrel in a therapeutic CIA study design. *$p < 0.05$, **$p < 0.01$ vs vehicle, at study termination.

Inhibitor 28 Efficiently Reduces Plasma Prostaglandin E$_2$ (PGE$_2$) Levels. PGE$_2$ is recognized as an important contributor to joint inflammation in rheumatoid arthritis,[53] and we investigated if plasma PGE$_2$ levels were changed in response to treatment. In the prophylactic study (n = 11), PGE$_2$ levels in the DMSO-treated vehicle group (223 ± 107 ng/mL) were significantly elevated by 3-fold ($p < 0.001$) compared to that in the nonarthritic healthy mice (70 ± 37 ng/mL) (Figure 6A). The elevated PGE$_2$ level was significantly reduced by inhibitor **28** (7.5 mg/mL, 140 ± 92 ng/mL, $p < 0.03$), comparable to that by MTX (0.3 mg/mL, 107 ± 62 ng/mL, $p < 0.004$). There were no significant differences between the treatment groups ($p > 0.05$). In the therapeutic study (n = 10), similar results were obtained: PGE$_2$ levels in the DMSO-treated vehicle group (231 ± 110 ng/mL) were significantly elevated by 3-fold ($p < 0.001$) compared to that in the nonarthritic healthy mice (71 ± 36 ng/mL) (Figure 6B). The elevated PGE$_2$ levels were significantly reduced with inhibitor **28** (30 mg/mL, 139 ± 55 ng/mL, $p < 0.03$) treatment, but not with Enbrel (25 mg/kg, 188 ± 74 ng/mL, nonsignificant $p > 0.05$). In summary, plasma PGE$_2$ levels in inhibitor **28**-treated animals were significantly reduced by about 40% in both prophylactic and therapeutic modes of the animal disease models. In the prophylactic model, inhibitor **28** produced PGE$_2$ plasma level reduction comparable to that of the reference drug MTX. Furthermore, in the therapeutic model, it appears that

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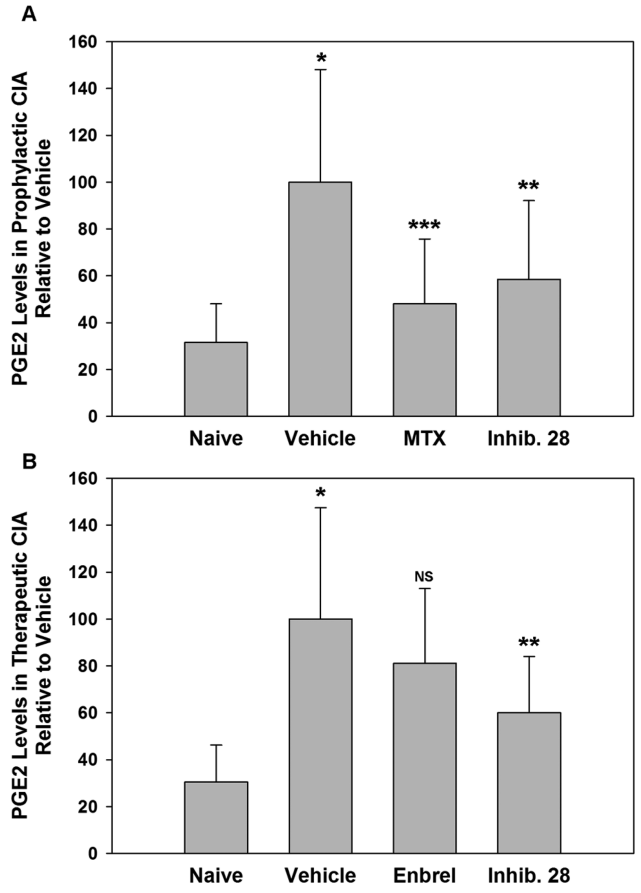

Figure 6. (A) In the prophylactic CIA study (n = 11), inhibitor **28** (7.5 mg/kg) significantly reduced plasma PGE_2 levels, comparable to the effect of MTX (0.3 mg/kg). (B) In the therapeutic CIA study (n = 10), **28** (30 mg/kg) significantly reduced plasma PGE_2 levels in the therapeutic CIA mice, whereas Enbrel (25 mg/kg) showed no significant reduction in PGE_2 levels. * $p < 0.001$ vs naïve; NS, not significant; ** $p < 0.03$ and *** $p < 0.004$ vs vehicle, error bars denote standard deviation.

inhibitor **28** caused more potent PGE_2 reduction in plasma than that of the reference drug Enbrel.

Pharmacokinetic Properties of Inhibitor 28. Initial in vitro metabolic studies of inhibitor **28** in human whole blood and mouse plasma established a $t_{1/2}$ of approximately 190 min for the compound (data not shown). To assess the pharmacokinetic profile of inhibitor **28** in vivo, we measured maximum compound concentration achieved after a single ip administration of 30 mg/kg in Swiss male mice. To compare with levels achieved after iv administration, inhibitor **28** was also injected iv at 5 mg/kg in a different group of mice (see Experimental Section). Overall, inhibitor **28** reached a C_{max} of 234.5 ng/mL 2 h after ip administration, with detectable levels persisting for 24 h after administration. In comparison, iv levels of inhibitor **28** peaked at 30 min after administration (2.7 μg/mL) rapidly declining to background levels at 24 h post-administration. Calculated AUC_{0-t} values over the same period were 1266.1 and 1861.9 ng/mL·h for ip and iv administration, respectively. These results collectively indicate that inhibitor **28** administered ip is bioavailable and justify use of a daily dosing scheme for the compound in further animal studies.

CONCLUSIONS

Various routes for the synthesis of thiazolyl ketones have been studied. The key step for the synthesis of substituted thiazoles was either the condensation of a thioamide with a halo ketoester or the condensation of a nitrile with cysteine methyl ester, followed by aromatization. The compounds synthesized were tested for their in vitro inhibitory activity toward GIVA $cPLA_2$ using both mixed micelle and vesicle assays. Among them, inhibitor **28** was found to be the most potent inhibitor, with an IC_{50} value of 0.6 μM in an AA release assay, exhibiting a $X_I(50)$ value of 0.011 mole fraction in a mixed micelle assay and an IC_{50} of 300 nM in a vesicle assay. This inhibitor was able to suppress the release of AA in fibroblast-like synoviocytes (IC_{50} 600 nM) without significantly effecting potency in OA release. The in vivo activity of this inhibitor was evaluated in the CIA mouse model. In the prophylactic model at a dose of 7.5 mg/kg, it exhibited an anti-inflammatory effect comparable to that of the reference drug methotrexate, whereas in the therapeutic model at a dose of 30 mg/kg, it showed results comparable to those of the reference drug Enbrel. In both the prophylactic and the therapeutic models, the 3-fold elevated PGE_2 plasma levels were significantly reduced by about 40% using inhibitor **28**. Thus, we have identified a new GIVA $cPLA_2$ inhibitor, which presents interesting anti-inflammatory and disease-modifying in vivo effects in the CIA model; inhibitor **28** may hence represent a new agent for the treatment of inflammatory diseases.

EXPERIMENTAL SECTION

General Methods. Chromatographic purification of products was accomplished using Merck Silica Gel 60 (70−230 or 230−400 mesh). Thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 aluminum plates. Spots were visualized with UV light and/or phosphomolybdic acid in EtOH. Melting points were determined using a Büchi 530 apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury (200 and 50 MHz, respectively) in $CDCl_3$ or as specified. ^1H and ^{13}C NMR spectra of inhibitor **28** were recorded on a Bruker AVANCE III (600 and 150 MHz, respectively) in $CDCl_3$. Chemical shifts are given in ppm, and coupling constants (J), in Hz. Peak multiplicities are described as follows: s, singlet; d, doublet; t, triplet; and m, multiplet. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan, Surveyor MSQ Plus spectrometer. Dichloromethane, diethyl ether, and toluene were dried by standard procedures and stored over molecular sieves. All other solvents and chemicals were reagent grade and used without further purification. The purity of all compounds subjected to biological tests was determined by analytical HPLC and was found to be ≥95%. HPLC analyses were carried out on an Agilent 1100 Series apparatus and a Thermo Scientific Hypersil Silica column, using 10% i-PrOH in hexane, at a flow rate of 1.0 mL/min. HRMS spectra were recorded on a Bruker Maxis Impact QTOF Spectrometer.

Compounds **10b**,[54] **12a**,[55] **12b**,[56] and **15a**[57] have been described elsewhere, and their analytical data are in accordance with literature.

Ethyl 2-(4-Octylphenoxy)acetate (15b). To a stirred solution of 4-n-octylphenol (1.0 mmol, 206 mg) in acetone (10 mL) were added K_2CO_3 (3 mmol, 415 mg) and ethyl bromoacetate (1.1 mmol, 215 mg), and the reaction mixture was refluxed for 5 h. Subsequently, the mixture was filtrated over Celite, and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C), 1:9]. Yield 98%; white oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.10 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 4.60 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 2.55 (t, J = 7.8 Hz, 2H), 1.69−1.46 (m, 2H), 1.45−1.12 (m, 13H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.1, 155.8, 136.1, 129.3, 114.4, 65.5, 61.3, 35.0, 31.8, 31.6, 29.4, 29.2, 22.6, 14.1; MS (ESI) m/z (%): 293 (90) $[M + H]^+$.

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Synthesis of Alcohols 16a,b. To a stirred solution of esters **15a,b** (1 mmol) in dry Et_2O (10 mL) was added DIBALH (2.5 mL, 2.5 mmol, 1.0 M in hexane) at 0 °C under an Ar atmosphere, and the reaction mixture was stirred for 2 h at room temperature. Water was then added (5 mL), and the mixture was stirred for 30 more minutes and filtrated over Celite. The organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C), 3:7].

2-(Biphenyl-4-yloxy)ethanol (16a). Yield 94%; white solid; mp 120−122 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63−7.22 (m, 7H), 7.07−6.94 (m, 2H), 4.19−4.07 (m, 2H), 4.05−3.94 (m, 2H), 1.91 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.1, 140.6, 134.2, 128.7, 128.2, 126.7, 114.8, 69.2, 61.4.

2-(4-Octylphenoxy)ethanol (16b). Yield 82%; white solid; mp 40−42 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.11 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 4.13−4.02 (m, 2H), 4.01−3.91 (m, 2H), 2.56 (t, J = 7.8 Hz, 2H), 2.19 (br, 1H), 1.70−1.48 (m, 2H), 1.45−1.14 (m, 13H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 156.5, 135.5, 129.3, 114.2, 69.1, 61.5, 35.0, 31.9, 31.7, 29.5, 29.2, 22.7, 14.1.

Synthesis of Ketones 13a,b. To a stirred solution of thiazole (3 equiv) in dry Et_2O (20 mL) at −78 °C under a dry argon atmosphere was added a solution of *n*-BuLi (1.6 M in hexanes, 3 equiv) dropwise over a period of 10 min. The resulting orange solution was stirred for 45 min. Then, a solution of amides **12a,b** (1 mmol) in dry Et_2O (2 mL) was slowly added, giving the mixture a dark brown color. After stirring for 30 min at −78 °C, the mixture was allowed to warm to room temperature over a period of 2 h. Then, saturated aqueous ammonium chloride solution was added, and the mixture was extracted with ether (2 × 10 mL). The combined extracts were washed with brine and then dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography eluting with the appropriate mixture of [EtOAc/petroleum ether (bp 40−60 °C)] afforded the product.

2-Phenoxy-1-(thiazol-2-yl)ethanone (13a). Yield 54%; white solid; mp 77−79 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08−8.02 (m, 1H), 7.79−7.74 (m, 1H), 7.39−7.22 (m, 2H), 7.07−6.93 (m, 2H), 5.55 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.4, 164.0, 157.8, 144.9, 129.5, 126.7, 121.6, 114.8, 70.0; HRMS (ESI) calcd for $C_{11}H_9NNaO_2S$ $[M + Na]^+$, 242.0246; found, 242.0256.

2-(4-Fluorophenoxy)-1-(thiazol-2-yl)ethanone (13b). Yield 61%; white solid; mp 74−77 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08−8.03 (m, 1H), 7.81−7.76 (m, 1H), 7.09−6.86 (m, 4H), 5.51 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.4, 164.0, 157.8 (d, J = 239.4 Hz), 154.1, 145.0, 126.8, 116.2, 115.9 (d, J = 15.8 Hz), 70.8; HRMS (ESI) calcd for $C_{11}H_8FNNaO_2S$ $[M + Na]^+$, 260.0152; found, 260.0159.

Synthesis of Compounds 17a−c. To a solution of alcohols **16a,b** (1.0 mmol) in a mixture of toluene (3 mL) and EtOAc (3 mL) was added a solution of NaBr (0.11 g, 1.1 mmol) in water (0.5 mL) followed by 2,2,6,6-tetramethylpiperidine-1-yloxy free radical (TEMPO) (2.2 mg, 0.01 mmol). To the resulting biphasic system, which was cooled at 0 °C, an aqueous solution of 0.35 M NaOCl (3.1 mL, 1.1 mmol) containing $NaHCO_3$ (0.25 g, 3 mmol) was added dropwise under vigorous stirring at 0 °C over a period of 1 h. After the mixture had been stirred for a further 15 min at 0 °C, EtOAc (10 mL) and H_2O (10 mL) were added. The aqueous layer was separated and washed with EtOAc (2 × 10 mL). The combined organic layers were washed consecutively with 5% aqueous citric acid (10 mL) containing KI (0.04 g), 10% aqueous $Na_2S_2O_3$ (10 mL), and brine and dried over Na_2SO_4. The solvents were evaporated under reduced pressure, and the residue was used without any further purification.

To a stirred solution of thiazole or benzothiazole (3 equiv) in dry Et_2O (20 mL) at −78 °C under a dry argon atmosphere was added a solution of *n*-BuLi (1.6 M in hexanes, 3 equiv) dropwise over a period of 10 min. The resulting orange solution was stirred for 45 min. Then, a solution of the above prepared aldehyde (1 mmol) in dry Et_2O (2 mL) was slowly added, giving the mixture a dark brown color. After stirring for 30 min at −78 °C, the mixture was allowed to warm to room temperature over a period of 2 h. Then, saturated aqueous ammonium chloride solution was added, and the mixture was extracted with ether (2 × 10 mL). The combined extracts were washed with brine and then dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography eluting with the appropriate mixture of EtOAc/petroleum ether (bp 40−60 °C) afforded the product.

2-(Biphenyl-4-yloxy)-1-(thiazol-2-yl)ethanol (17a). Yield 48%; pale yellow solid; mp 93−95 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.79 (d, J = 3.2 Hz, 1H), 7.67−7.22 (m, 8H), 7.09−6.93 (m, 2H), 4.18−3.90 (br, 1H), 5.44 (dd, J_1 = 3.9 Hz, J_2 = 7.0 Hz, 1H), 4.49 (dd, J_1 = 3.9 Hz, J_2 = 9.7 Hz, 1H), 4.30 (dd, J_1 = 7.0 Hz, J_2 = 9.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.9, 157.7, 142.4, 140.5, 134.5, 128.7, 128.2, 126.8, 126.7, 119.6, 115.0, 71.6, 70.6; MS (ESI) m/z (%): 298 (100) $[M + H]^+$.

2-(4-Octylphenoxy)-1-(thiazol-2-yl)ethanol (17b). Yield 34%; yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (d, J = 3.2 Hz, 1H), 7.34 (d, J = 3.2 Hz, 1H), 7.09 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 5.40 (dd, J_1 = 3.9 Hz, J_2 = 7.0 Hz, 1H), 4.42 (dd, J_1 = 3.9 Hz, J_2 = 9.7 Hz, 1H), 4.22 (dd, J_1 = 7.0 Hz, J_2 = 9.7 Hz, 1H), 2.54 (t, J = 7.8 Hz, 2H), 1.69−1.45 (m, 2H), 1.43−1.10 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.0, 156.1, 142.4, 135.9, 129.3, 119.5, 114.5, 71.6, 70.7, 35.0, 31.8, 31.7, 29.4, 29.2, 22.6, 14.1; MS (ESI) m/z (%): 334.2 (100) $[M + H]^+$.

1-(Benzo[*d*]thiazol-2-yl)-2-(4-octylphenoxy)ethanol (17c). Yield 42%; yellow solid; mp 93−95 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10−7.97 (m, 1H), 7.96−7.82 (m, 1H), 7.56−7.32 (m, 2H), 7.09 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 5.50 (dd, J_1 = 4.0 Hz, J_2 = 6.8 Hz, 1H), 4.52 (dd, J_1 = 4.0 Hz, J_2 = 9.7 Hz, 1H), 4.34 (dd, J_1 = 6.8 Hz, J_2 = 9.7 Hz, 1H), 2.55 (t, J = 7.8 Hz, 2H), 1.70−1.47 (m, 2H), 1.45−1.12 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.2, 156.0, 152.8, 136.0, 134.9, 129.3, 126.2, 126.1, 125.0, 122.9, 114.5, 71.4, 71.0, 35.0, 31.8, 31.7, 29.4, 29.2, 22.7, 22.6, 14.1; MS (ESI) m/z (%): 384 (100) $[M + H]^+$.

Synthesis of Nitriles 20a,b. To a mixture of TBDMSCN (1.0 mmol, 141 mg), potassium cyanide (0.2 mmol, 13 mg), and 18-crown-6 (0.4 mmol, 106 mg) was added dropwise a solution of the aldehyde derived from alcohol **16b** or **19**, according to the NaBr/TEMPO protocol mentioned above, (1.0 mmol) in CH_2Cl_2 at room temperature under nitrogen over 30 min. After addition was complete, the mixture was stirred overnight at room temperature. The organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C), 1:9].

2-(*tert*-Butyldimethylsilyloxy)-3-(4-octylphenoxy)-propanenitrile (20a). Yield 93%; white oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.12 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 4.80 (t, J = 5.4 Hz, 1H), 4.21−3.98 (m, 2H), 2.56 (t, J = 7.8 Hz, 2H), 1.65−1.42 (m, 2H), 1.40−1.13 (br, 10H), 1.04−0.75 (m, 12H), 0.24 (s, 3H), 0.19 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.7, 136.2, 129.4, 118.1, 114.4, 69.6, 61.5, 35.0, 31.9, 31.7, 29.4, 29.2, 25.4, 22.6, 18.1, 14.1, −5.3; MS (ESI) m/z (%): 407 (100) $[M + NH_4]^+$.

2-(*tert*-Butyldimethylsilyloxy)-3-(dodecyloxy)propanenitrile (20b). Yield 72%; white oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.55 (t, J = 6.4 Hz, 1H), 3.62 (d, J = 6.6 Hz, 2H), 3.52 (t, J = 6.6 Hz, 2H), 1.67−1.48 (m, 2H), 1.26 (br, 18H), 0.98−1.82 (m, 12H), 0.19 (s, 3H), 0.17 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 118.7, 72.6, 72.2, 62.0, 31.9, 29.6, 29.5, 29.4, 29.3, 25.9, 25.5, 22.7, 18.1, 14.1, −5.3; MS (ESI) m/z (%): 387 (100) $[M + NH_4]^+$.

Synthesis of Amides 21a,b. To a solution of nitriles **20a,b** (1 mmol) and Bu_4NHSO_4 (0.2 mmol, 68 mg) in CH_2Cl_2 (10 mL) were added dropwise a solution of 0.5 N NaOH(aq) (2.5 mL) and 30% H_2O_2 (4 mmol, 3.5 mL) at 0 °C. The biphasic reaction mixture was stirred overnight at room temperature. The organic layer was separated, washed with water (2 × 10 mL), and dried over Na_2SO_4. Organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C)].

2-(*tert*-Butyldimethylsilyloxy)-3-(4-octylphenoxy)-propanamide (21a). Yield 68%; white solid; mp 56−58 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.08 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 6.77 (br s, 1H), 6.08 (br s, 1H), 4.51 (dd, J_1 = 7.2 Hz, J_2 =

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2.2 Hz, 1H), 4.32 (dd, J_1 = 10.0 Hz, J_2 = 2.2 Hz, 1H), 4.03 (dd, J_1 = 10.0 Hz, J_2 = 7.2 Hz, 1H), 2.53 (t, J = 7.8 Hz, 2H), 1.63−1.42 (m, 2H), 1.39−1.07 (br, 10H), 0.99−0.73 (m, 12H), 0.17 (s, 3H), 0.16 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.2, 156.4, 135.4, 129.2, 114.2, 73.3, 70.7, 35.0, 31.9, 31.7, 29.5, 29.4, 25.8, 22.7, 18.1, 14.1, −4.50, −5.33; MS (ESI) m/z (%): 408 (100) $[M + H]^+$.

2-(*tert*-Butyldimethylsilyloxy)-3-(dodecyloxy)propanamide (21b). Yield 79%; white oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.66 (br s, 1H), 6.60 (br s, 1H), 4.24 (dd, J_1 = 6.2 Hz, J_2 = 2.2, 1H), 3.67 (dd, J_1 = 10.0 Hz, J_2 = 2.2, 1H), 3.52 (dd, J_1 = 10.0 Hz, J_2 = 6.2 Hz, 1H), 3.41 (t, J = 6.2 Hz, 2H), 1.63−1.45 (m, 2H), 1.24 (br, 18H), 1.02−1.79 (m, 12H), 0.11 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.0, 74.0, 73.5, 71.6, 31.9, 29.6, 29.5, 29.4, 29.3, 26.0, 25.7, 22.6, 18.1, 14.1, −4.7, −5.4; MS (ESI) m/z (%): 388 (100) $[M + H]^+$.

Synthesis of Thioamides 22a,b. Lawesson's reagent (0.6 mmol, 243 mg) was added to a solution of amides **21a,b** (1 mmol) in dry toluene (10 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography eluting with the appropriate mixture of EtOAc/petroleum ether (bp 40−60 °C).

2-(*tert*-Butyldimethylsilyloxy)-3-(4-octylphenoxy)-propanethioamide (22a). Yield 40%; pale yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.22 (br s, 1H), 7.83 (br s, 1H), 7.09 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 4.89 (dd, J_1 = 7.2 Hz, J_2 = 2.2 Hz, 1H), 4.55 (dd, J_1 = 9.9 Hz, J_2 = 2.2 Hz, 1H), 4.03 (dd, J_1 = 9.9 Hz, J_2 = 7.2 Hz, 1H), 2.54 (t, J = 7.4 Hz, 2H), 1.63−1.41 (m, 2H), 1.39−1.05 (m, 10H), 1.02−0.69 (m, 12H), 0.16 (s, 6H) ; ^{13}C NMR (50 MHz, $CDCl_3$): δ 205.4, 156.3, 135.5, 129.2, 114.3, 79.2, 72.6, 35.0, 31.9, 31.7, 29.5, 29.3, 25.8, 25.3, 22.6, 18.2, 14.1, −4.6, −5.2; MS (ESI) m/z (%): 424 (100) $[M + H]^+$.

2-(*tert*-Butyldimethylsilyloxy)-3-(dodecyloxy)-propanethioamide (22b). Yield 39%; pale yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.12 (br s, 1H), 7.97 (br s, 1H), 4.65 (dd, J_1 = 6.2 Hz, J_2 = 2.6, 1H), 3.88 (dd, J_1 = 10.0 Hz, J_2 = 2.6, 1H), 3.58 (dd, J_1 = 10.0 Hz, J_2 = 6.2 Hz, 1H), 3.51−3.38 (m, 2H), 1.63−1.45 (m, 2H), 1.25 (br, 18H), 1.05−1.82 (m, 12H,), 0.14 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 206.3, 80.1, 75.4, 71.7, 31.9, 29.6, 29.5, 29.4, 29.3, 26.0, 25.7, 22.6, 18.2, 14.1, −4.7, −5.3; MS (ESI) m/z (%): 404 (100) $[M + H]^+$.

Synthesis of Thiazoles 23a−c. To a stirred solution of thioamides **22a,b** (1.0 mmol) in EtOH (5 mL) was added ethyl bromopyruvate (1.2 mmol, 0.15 mL) or ethyl 4-chloroacetoacetate (1.0 mmol, 0.14 mL) and conc. H_2SO_4 (0.04 mL), and the reaction mixture was refluxed overnight. Organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C)].

Ethyl 2-(2-(1-Hydroxy-2-(4-octylphenoxy)ethyl)thiazol-4-yl)-acetate (23a). Yield 17%; yellowish oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.20 (s, 1H), 7.09 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.35 (dd, J_1 = 7.0 Hz, J_2 = 4.0, 1H), 4.38 (dd, J_1 = 9.6 Hz, J_2 = 4.0, 1H), 4.31−4.09 (m, 3H), 3.82 (s, 2H), 2.54 (t, J = 7.4 Hz, 2H), 1.69−1.45 (m, 2H), 1.43−1.18 (m, 13H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.5, 170.3, 156.1, 148.5, 136.0, 129.3, 116.7, 114.5, 71.5, 70.6, 61.1, 36.9, 35.0, 31.8, 31.7, 29.4, 29.2, 22.6, 14.1; MS (ESI) m/z (%): 420 (100) $[M + H]^+$.

Ethyl 2-(2-(2-(Dodecyloxy)-1-hydroxyethyl)thiazol-4-yl)-acetate (23b). Yield 26%; low mp yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.16 (s, 1H), 5.12 (dd, J_1 = 6.8 Hz, J_2 = 3.8 Hz, 1H), 4.18 (q, J = 7.4 Hz, 2H), 3.83 (dd, J_1 = 9.8 Hz, J_2 = 3.8 Hz, 1H), 3.80 (s, 2H), 3.70−3.42 (m, 4H), 1.65−1.46 (m, 2H), 1.40−1.12 (m, 21H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3, 170.3, 148.4, 116.3, 74.1, 71.7, 70.9, 61.0, 37.0, 31.9, 29.6, 29.5, 29.4, 29.3, 26.0, 22.6, 14.1; MS (ESI) m/z (%): 400 (100) $[M + H]^+$.

Ethyl 2-(2-(Dodecyloxy)-1-hydroxyethyl)thiazole-4-carboxylate (23c). Yield 68%; low mp off-white solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.14 (s, 1H), 5.20 (dd, J_1 = 7.0 Hz, J_2 = 3.8 Hz, 1H), 4.41 (q, J = 7.4 Hz, 2H), 3.92 (dd, J_1 = 9.8 Hz, J_2 = 3.8 Hz, 1H), 3.66 (dd, J_1 = 9.8 Hz, J_2 = 7.0 Hz, 1H), 3.60−3.42 (m, 3H), 1.68−1.49 (m, 2H), 1.39 (t, J = 7.2 Hz, 3H), 1.33−1.19 (m, 18H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 161.4, 147.0, 127.7, 73.7, 71.7, 70.9, 61.4, 31.9, 29.6, 29.5, 29.4, 29.3, 26.0, 22.6, 14.1; MS (ESI) m/z (%): 386 (100) $[M + H]^+$.

Methyl 2-(1-(*tert*-Butyldimethylsilyloxy)-2-(4-octylphenoxy)-ethyl)-4,5-dihydrothiazole-4-carboxylate (Mixture of Diastereomers) (25). To a stirred solution of **20a** (1.0 mmol, 390 mg) and $CH_3COO^-NH_4^+$ (3.6 mmol, 277 mg) in MeOH (4 mL) was added HCl·H-L-Cys-OMe (3.0 mmol, 515 mg), and the mixture was stirred overnight at room temperature. The organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C), 1:9]. Yield 67%; white oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 5.25−5.07 (m, 1H), 5.06−4.90 (m, 1H), 4.38−4.15 (m, 1H), 4.14−3.94(m, 1H), 3.82 (s, 3H), 3.63−3.33 (m, 2H), 2.54 (t, J = 7.8 Hz, 2H), 1.70−1.45 (m, 2H), 1.43−1.16 (br, 10H), 1.05−0.80 (m, 12H), 0.22−0.10 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.6 (178.0), 171.1, 156.4, 135.3, 129.1, 114.3, 78.3 (78.2), 72.5 (72.4), 71.5, 52.7 (52.6), 35.0, 33.7, 31.8, 31.7, 29.4, 29.2, 25.7, 22.6, 18.2, 14.1, −4.7, −5.2; MS (ESI) m/z (%): 508 (100) $[M + H]^+$.

Methyl 2-(1-(*tert*-Butyldimethylsilyloxy)-2-(4-octylphenoxy)-ethyl)thiazole-4-carboxylate (26). To a solution of **25** (1 mmol, 508 mg) in CH_2Cl_2 (20 mL) were added $BrCCl_3$ (6.0 mmol, 0.59 mL) and DBU (6.0 mmol, 0.90 mL) at 0 °C. The reaction was stirred for 2 h at 0 °C and overnight at room temperature. The organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40−60 °C), 1:9]. Yield 82%; white oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.07 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.53−5.40 (m, 1H), 4.51−4.37 (m, 1H), 4.12−3.90 (m, 4H), 2.54 (t, J = 7.8 Hz, 2H), 1.70−1.45 (m, 2H), 1.44−1.14 (br, 10H), 1.07−0.79 (m, 12H), 0.17 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.5, 161.9, 156.5, 146.8, 135.4, 129.2, 128.0, 114.3, 72.7, 72.6, 52.4, 35.0, 31.9, 31.7, 29.5, 29.3, 25.7, 22.7, 18.3, 14.1, −4.5, −5.2; MS (ESI) m/z (%): 506 (100) $[M + H]^+$.

Methyl 2-(1-Hydroxy-2-(4-octylphenoxy)ethyl)thiazole-4-carboxylate (27). Compound **26** (1.0 mmol, 505 mg) was treated with a solution of 4 N HCl in MeOH. The organic solvent was evaporated under reduced pressure, and the residue was recrystallized from ether/petroleum ether (bp 40−60 °C). Yield 95%; white solid; mp 84−86 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.0 Hz, 2H), 5.60−5.28 (m, 1H), 4.57−4.36 (m, 1H), 4.31−4.12 (m, 1H), 4.08−3.82 (m, 4H), 2.52 (t, J = 7.8 Hz, 2H), 1.69−1.43 (m, 2H), 1.42−1.11 (br, 10H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 161.7, 155.8, 146.5, 136.0, 129.2, 128.2, 114.4, 71.2, 70.6, 52.5, 34.9, 31.8, 31.6, 29.4, 29.2, 22.6, 14.0; MS (ESI) m/z (%): 392 (100) $[M + H]^+$.

Synthesis of Thiazoles 18a−c, 24a−c, and 28. To a solution of compounds **17a−c**, **23a−c**, and **27** (1 mmol) in dry CH_2Cl_2 (10 mL) was added Dess−Martin periodinane (1.5 mmol, 637 mg), and the mixture was stirred for 1 h at room temperature. The organic solvent was evaporated under reduce pressure, and Et_2O (30 mL) was added. The organic phase was washed with saturated aqueous $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (1.5 g, 9.5 mmol) and then H_2O (20 mL) and dried over Na_2SO_4, and the organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether (bp 40−60 °C)/EtOAc as eluent.

2-(Biphenyl-4-yloxy)-1-(thiazol-2-yl)ethanone (18a). Yield 82%; white solid; mp 130−133 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.09−8.03 (m, 1H), 7.80−7.74 (m, 1H), 7.63−7.23 (m, 7H), 7.12−7.00 (m, 2H), 5.57 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.4, 164.4, 157.5, 145.0, 140.6, 134.8, 128.7, 128.2, 126.8, 115.1, 70.2; HRMS (ESI) calcd for $C_{17}H_{13}NNaO_2S$ $[M + Na]^+$, 318.0559; found, 318.0568.

2-(4-Octylphenoxy)-1-(thiazol-2-yl)ethanone (18b). Yield 79%; white solid; mp 65−67 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.06 (d, J = 3.0 Hz, 1H), 7.76 (d, J = 3.0 Hz, 1H), 7.11 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 5.52 (s, 2H), 2.55 (t, J = 7.8 Hz, 2H), 1.71−1.46 (m, 2H), 1.42−1.10 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.6, 164.2, 156.0, 145.0, 136.2, 129.3,

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126.6, 114.7, 70.3, 35.0, 31.9, 31.6, 29.4, 29.2, 22.6, 14.1; HRMS (ESI) calcd for $C_{19}H_{25}NNaO_2S$ $[M + Na]^+$, 354.1498; found, 354.1507.

1-(Benzo[d]thiazol-2-yl)-2-(4-octylphenoxy)ethanone (18c). Yield 75%; white solid; mp 80−82 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.26−8.17 (m, 1H), 8.08−7.96 (m, 1H), 7.69−7.51 (m, 2H), 7.13 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 5.64 (s, 2H), 2.56 (t, J = 7.8 Hz, 2H), 1.72−1.46 (m, 2H), 1.43−1.14 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 189.2, 163.5, 155.9, 153.4, 136.9, 136.3, 129.4, 128.0, 127.3, 125.5, 122.5, 114.7, 70.5, 35.1, 31.9, 31.6, 29.5, 29.3, 22.7, 14.1; HRMS (ESI) calcd for $C_{23}H_{27}NNaO_2S$ $[M + Na]^+$, 404.1655; found, 404.1664.

Ethyl 2-(2-(2-(4-Octylphenoxy)acetyl)thiazol-4-yl)acetate (24a). Yield 78%; low mp white solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.10 (d, J = 8.2 Hz, 2H), 6.90 (d, J = 8.0 Hz, 2H), 5.48 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 3.93 (s, 2H), 2.54 (t, J = 7.6 Hz, 2H), 1.66−1.44 (m, 2H), 1.40−1.14 (m, 13H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.5, 169.8, 163.2, 155.9, 151.5, 136.2, 129.3, 124.3, 114.7, 70.3, 61.3, 36.8, 35.0, 31.8, 31.6, 29.4, 29.2, 22.6, 14.1; HRMS (ESI) calcd for $C_{23}H_{31}NNaO_4S$ $[M + Na]^+$, 440.1866; found, 440.1883.

Ethyl 2-(2-(2-(Dodecyloxy)acetyl)thiazol-4-yl)acetate (24b). Yield 49%; low mp white solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.59 (s, 1H), 4.94 (s, 2H), 4.21 (q, J = 7.0 Hz, 2H), 3.89 (s, 2H), 3.60 (t, J = 6.6 Hz, 2H), 1.75−1.59 (m, 2H), 1.45−1.18 (m, 21H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 189.6, 169.8, 163.8, 151.3, 123.7, 73.1, 72.2, 61.3, 36.9, 31.9, 29.6, 29.5, 29.4, 29.3, 25.9, 22.7, 14.1; HRMS (ESI) calcd for $C_{21}H_{35}NNaO_4S$ $[M + Na]^+$, 420.2179; found, 420.2211.

Ethyl 2-(2-(Dodecyloxy)acetyl)thiazole-4-carboxylate (24c). Yield 84%; pale yellow solid; mp 58−61 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.44 (s, 1H), 5.04 (s, 2H), 4.44 (q, J = 7.2 Hz, 2H), 3.60 (t, J = 6.6 Hz, 2H), 1.76−1.58 (m, 2H), 1.46−1.17 (m, 21H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 189.7, 164.9, 160.6, 148.8, 132.9, 73.1, 72.2, 61.9, 31.9, 29.6, 29.5, 29.4, 29.3, 25.9, 22.6, 14.1; HRMS (ESI) calcd for $C_{20}H_{33}NNaO_4S$ $[M + Na]^+$, 406.2023; found, 406.2040.

Methyl 2-(2-(4-Octylphenoxy)acetyl)thiazole-4-carboxylate (28). Yield 93%; off-white solid; mp 69−71 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.10 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 5.57 (s, 2H), 4.00 (s, 3H), 2.54 (t, J = 7.8 Hz, 2H), 1.61−1.53 (m, 2H), 1.35−1.21 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 187.6, 164.5, 161.0, 155.8, 148.6, 136.3, 133.5, 129.3, 114.8, 70.4, 52.7, 35.0, 31.8, 31.6, 29.4, 29.2, 22.6, 14.1; HRMS (ESI) calcd for $C_{21}H_{27}NNaO_4S$ $[M + Na]^+$, 412.1553; found, 412.1556.

Biology. Recombinant human IL-1β was from Roche (UK). Phosphate-buffered saline solution (PBS) was from Oxoid (UK). Labeled ^{3}H-AA ([5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (specific activity 180−240 Ci/mmol)), ^{14}C-OA ([1-^{14}C]-oleic acid (specific activity 40−60 Ci/mmol)), L-α-1-palmitoyl-2-arachidonyl-[arachidonyl-1-^{14}C]-phosphatidylcholine (specific activity 40−60 Ci/mmol), and liquid scintillation cocktail Ultima Gold were from NEN PerkinElmer (USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), fatty acid-free bovine serum albumin (fBSA), dimethyl sulfoxide (DMSO), gentamicin, and L-glutamine were from Sigma-Aldrich (USA). Enzyme immunoassay (EIA) kit for PGE_2 analysis was from Cayman Chemicals (USA).

In Vitro Mixed Micelle Assay. The activity of GIVA $cPLA_2$, GVIA $iPLA_2$, and GV $sPLA_2$ was determined using a modified Dole assay.[29−31] The buffer and substrate conditions were optimized for each enzyme assay as follows: (i) GIVA $cPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 97 μM PAPC, 1.8 μM ^{14}C-labeled PAPC, and 3 μM PIP_2 in 100 mM HEPES buffer, pH 7.5, with 90 μM $CaCl_2$, 2 mM DTT, and 0.1 mg/mL BSA; (ii) GVIA $iPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM ^{14}C-labeled PAPC in buffer containing 100 mM HEPES, pH 7.5, 2 mM ATP, and 4 mM DTT; and (iii) GV $sPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM ^{14}C-labeled PAPC in buffer containing 50 mMTris, pH 8.0, and 5 mM 550 $CaCl_2$.

In Vitro Vesicle Assay. GIVA $cPLA_2$ was measured as described[46,47] with modifications.[48] In short, recombinant human GIVA $cPLA_2$ enzyme was preincubated with DMSO (1%) with or without inhibitor in assay buffer (80 s at 37 °C, 10 min at 25 °C). Lipid vesicles of L-α-1-palmitoyl-2-arachidonyl-[arachidonyl-1-^{14}C]-phosphatidylcholine (4.3 nmol) were dried under a stream of N_2(g). The dried lipid was resuspended in 2 mL assay buffer and sonicated twice (7 min, output 3.5 and 50% duty cycles, on ice) in a Branson Sonifier 250 (Branson Ultrasonic Corporation, Danbury, CT). Sonicated lipid (0.2 μM) was added to the reaction and incubated for 1 h at 37 °C followed by the addition of a chloroform/methanol buffer to terminate the enzymatic reaction. The reaction mixture was separated by centrifugation (5 min, 1640g). The lower phase was transferred to a glass tube and dried under a stream of N_2(g), resuspended in chloroform/methanol (9:1, by volume), and applied to a silica gel column. Free [1-^{14}C]arachidonic acid and, L-α-1-palmitoyl-2-arachidonyl-[arachidonyl-1-^{14}C]-phosphatidylcholine were separated by thin-layer chromatography and analyzed as previously described.[49]

Cell Culture. The human synovial sarcoma cell line SW982 was from ATCC (UK) and was used as a model system to monitor AA/OA release and activation of GIVA $cPLA_2$. SW982 cells were passaged biweekly by routine trypsin detachment and maintained in a subconfluent state as previously described.[58] Experiments were performed at 3 days postconfluency following overnight serum deprivation in serum-free DMEM to ensure differentiation and synchronization of the cells.

Ex Vivo Cellular AA and OA Release Assay. AA and OA release was analyzed as previously described.[49] At 2 days postconfluency, SW982 cells were serum-starved and labeled overnight with ^{3}H-AA (0.4 μCi/mL) and ^{14}C-OA (0.067 μCi/mL) in serum-free DMEM. Prior to the addition of the thiazolyl ketone inhibitors, the cells were washed twice with PBS containing fBSA (2 mg/mL) and PBS in order to remove unincorporated radioactivity. Cells were pretreated (1 h) with thiazolyl ketones prior to IL-1β stimulation (10 ng/mL, 4 h). Following IL-1β stimulation, the supernatants were cleared of detached cells by centrifugation (13 000 rpm, 5 min). The release of ^{3}H-AA and ^{14}C-OA from the cells was assessed by liquid scintillation counting (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Inc., USA). Adherent cells were dissolved in 1 N NaOH in order to determine incorporated ^{3}H-AA and ^{14}C-OA in the cells by liquid scintillation counting. In all experiments, DMSO was included for vehicle control (>0.05%). Following treatments, cells were routinely observed by microscopy to ensure unaltered cell morphology, integrity, and viability. The results are given as inhibition of released ^{3}H-AA and ^{14}C-OA in the supernatants relative to total ^{3}H-AA and ^{14}C-OA incorporated into the cells, from at least three independent experiments performed in triplicate.

PGE_2 Analysis. PGE_2 EIA analysis of blood plasma from the prophylactic and therapeutic CIA studies was performed according to the kit protocol. Plasma samples were diluted 1:1000−1:6000 in EIA buffer and allowed to hybridize overnight (18 h, 4 °C). The plate was read using a Multiscan plate reader (Ascent Labsystems) (OD550 nm). The corresponding Ascent software for Multiscan, version 2.4.1, was used to obtain the data. PGE_2 levels for all treatments are shown relative to the DMSO-treated vehicle arthritic mice (n = 10−11 mice in each category ± SD).

In Vivo Studies of Inhibitor 28. All in vivo studies were conducted in accordance with standard operating procedures (SOP) and generally accepted procedures for the testing of pharmaceutical compounds.

Separate prophylactic and therapeutic efficacy studies of inhibitor **28** were performed. MTX (Jiangsu Hengrui Medicine Co, no. 11041411), Enbrel (Boehringer Ingelheim Pharma KG, no. F39487), and vehicle (DMSO 100%, Sigma-Aldrich, no. D2650) were administered to all groups via ip injection once daily at a dose volume of 2 mL/kg. Clinical observations were conducted daily during the study. Body weights were measured and recorded prior to randomization and then once daily during the study. Food consumption was measured and recorded daily. Biopsies for midterm histology analysis were obtained on day 13 in the prophylactic study.

Necropsy examinations were performed at study termination in both prophylactic and therapeutic study modes; plasma samples were collected, organ weights were recorded, and hind paws were collected for histopathology analyses.

Induction of CIA. For the prophylactic and the therapeutic studies, CIA was induced in male DBA/1 mice (except naïve mice) by immunization with a 0.1 mL emulsion containing an equal volume of bovine type II collagen solution (2 mg/mL) and Freuds complete adjuvant at the tail base. The first injection was given on day 0, and the second injection as booster was given on day 21.[50−52] Inhibitor **28**, vehicle (DMSO), and MTX (0.3 mg/kg) were administered daily; Enbrel (25 mg/kg) was administrated twice a week. For the prophylactic study, treatment started 1 h before the second collagen injection and continued for 21 days except for the histology groups that were sacrificed at day 13 (33 days after immunization). For the therapeutic study, treatment started at day 28 and continued for 14 days.

CIA Assessment and Treatment. CIA was assessed in mice by two blinded observers to measure paw swelling with a capacity measurement method. The occurrence of arthritis was observed by scoring all paws for severity of erythema and swelling, using a clinical score ranging from 0 (no swelling) to 4 (severe swelling and erythema), i.e., yielding a maximum AI score of 16.[59,60]

Measurement of the Histopathology and Clinical Observations. At the end of the studies, one hind foot from mice in each group was collected for histopathology. The foot including the ankle was fixed in 10% neutral formalin. The ankle joints were decalcified, dehydrated, embedded in paraffin, sectioned, and stained with routine hematoxylin−eosin. Arthritis damage (histological damage score) was evaluated by light microscopy and scored by an investigator blinded for the treatment regimen. The following histopathology parameters were evaluated: (1) articular cavity and peripheral tissue inflammatory cell infiltration, (2) capillary and synovial hyperplasia, and (3) articular cartilage surface damage, each using a 0−5 grading system: 0, none; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe damage.

Terminal Studies. All animals completed the scheduled test periods and were disposed with carbon dioxide and subjected to necropsy, supervised by a pathologist. The sacrifices were performed at approximately 5 h after the last ip injection. A macroscopic examination of the animal was performed on all sacrificed animals, and any abnormality was recorded.

Statistical Analysis. Data of groups was examined by one-way analysis of variance, and individual groups were then compared with Student's unpaired t test. Data was given as mean ± SD, if no particular indication was made. $p < 0.05$ was considered significant.

Pharmacokinetic Studies. Compound **28** was tested in pilot pharmacokinetic studies in 5 week old Swiss male mice (around 30 g). Two groups of 12 mice were injected with 5 or 30 mg/kg of compound **28** via iv (bolus injection via the lateral tail vein) or ip route, respectively (6 mice per route, 2 sampling times per mouse, and 2 mice per time point). For iv administration, an aqueous-based solution of compound **28** (1 mg/mL) was administered to mice at a final volume of 5 mL/kg. All ip administrations were done in formulations containing compound **28** at 15 mg/mL in 100% DMSO at a final volume of 2 mL/kg. Four additional mice injected with vehicle controls (2 mice for each administration route) were also included in the study.

Blood samples (~200−300 μL/sample) were collected via sinus retro-orbital collection using capillary tubes containing lithium heparin and were stored on ice until they were centrifuged for 5 min at 2500 rpm at around 2−8 °C. The resulting plasma was separated, placed into labeled polypropylene tubes, and stored frozen at −80 °C prior to analysis. Overall, 6 time points were chosen: 30 min and 1, 2, 4, 8, and 24 h post-dose. For vehicle controls, all samples were collected 2 h after vehicle administration.

Isolated plasma samples were analyzed by standard LC−-MS/MS; the method had the lowest level of detection (LLOQ) at 2 ng/mL. Each time point analyzed comprised individual measurements from samples collected from 2 different mice per time point. Two main parameters were calculated: maximum plasma concentration (C_{max}) and area under the curve from the time of dosing to the last measurable concentration (AUC_{0-t}) using noncompartmental analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Code numbers of tested compounds; elemental analyses of synthesized compounds; and ^{1}H and ^{13}C NMR spectra and HPLC chromatogram of inhibitor **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(G.K.) Phone: +30 210 7274462; Fax: +30 210 7274761; E-mail: gkokotos@chem.uoa.gr.

*(B.J.) Phone: +47 73598691; E-mail: berit.johansen@ntnu.no.

Author Contributions

⊥A.J.F. and E.B. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): B.J. is a stockholder of Avexxin AS.

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■ ABBREVIATIONS USED

AA, arachidonic acid; AI, arthritis index; ATP, adenosine triphosphate; CIA, collagen-induced arthritis; CII, collagen type II; DBA/1, inbred mouse strain; DBU, 1,8-diazabicycloundec-7-ene; DIBALH, diisobutylaluminum hydride; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; DMEM, Dulbecco's modified Eagle's medium; EIA, enzyme immunoassay; fBSA, fatty acid-free bovine serum albumin; FBS, fetal bovine serum; GIVA cPLA$_2$, Group IVA cytosolic phospholipase A$_2$; GVIA iPLA$_2$, Group VIA calcium-independent phospholipase A$_2$; GV sPLA$_2$, Group V secreted phospholipase A$_2$; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IL-1β, interleukin-1β; ip, intraperitoneal; MTD, maximum tolerated dose; MTX, methotrexate; OA, oleic acid; PGE$_2$, prostaglandin E$_2$; PAPC, 1-palmitoyl-2-arachidonylphosphatidylcholine; PIP$_2$, phosphatidyl inositol (4,5)-bisphosphate; TBDMSCN, *tert*-butyldimethylsilyl cyanide; TEMPO, 2,2,6,6-tetramethylpiperidine-1-yloxy free radical

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