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Discovery of 2-(Cyclopentylamino)thieno[3,2-*d*]pyrimidin-4(3*H*)-one Derivatives as a New Series of Potent Phosphodiesterase 7 Inhibitors

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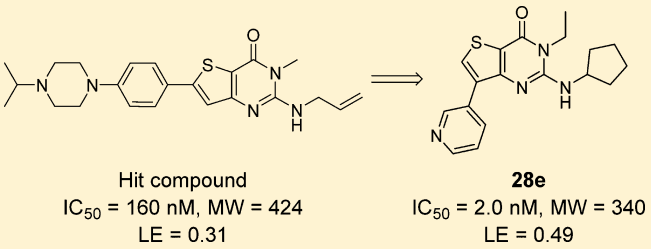
Supporting Information

ABSTRACT: The discovery of a new series of potent phosphodiesterase 7 (PDE7) inhibitors is described. Novel thieno[3,2-*d*]pyrimidin-4(3*H*)-one hit compounds were identified from our chemical library. Preliminary modifications of the hit compounds were performed, resulting in the discovery of a fragment-sized compound (**10**) with highly improved ligand efficiency. Compound design was guided by structure–activity relationships and computational modeling. The 6-substituted derivatives of the thienopyrimidinone showed diminished activity and enzyme selectivity. However, synthesis of the 7-substituted derivatives resulted in the discovery of **28e**, a desirable lead compound that selectively inhibits PDE7 with single-digit nanomolar potency while displaying potent cellular efficacy.



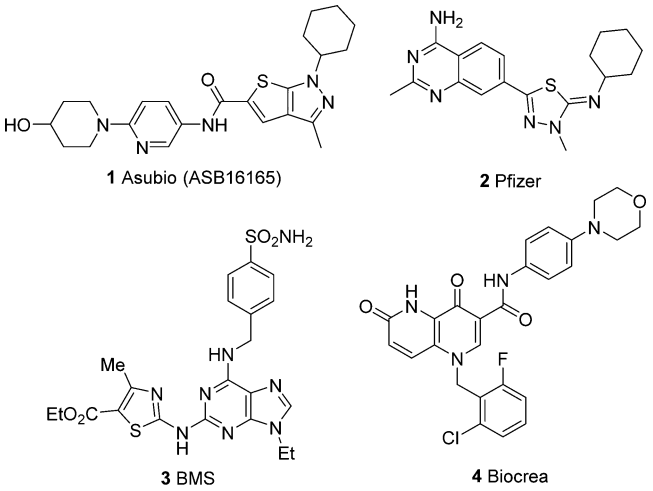
INTRODUCTION

Phosphodiesterases (PDEs) are enzymes that hydrolyze the second messenger molecules 3′,5′-cyclic adenosine monophosphate (cAMP) and 3′,5′-cyclic guanosine monophosphate (cGMP). Currently, 11 families of PDE enzymes and 21 human genes have been identified. Some PDE enzymes (PDE4, PDE7, and PDE8) selectively degrade cAMP; others (PDE5, PDE6, and PDE9) selectively degrade cGMP, and the remaining enzymes degrade both cAMP and cGMP. This substrate selectivity is regulated by the orientation of a conserved glutamine, which is anchored by the hydrogen-bonding network provided by the surrounding residues.[1]

PDE enzymes are recognized as promising targets to treat various diseases.[2,3] For example, inhibitors of PDE3, PDE4, and PDE5 have been approved for clinical use in the treatment of heart failure, respiratory diseases, and erectile dysfunction, respectively. Currently, PDE7 is considered to be a possible target for treating various diseases, including leukemias,[4] central nervous system (CNS) disorders,[5] and airway diseases.[6] To date, many PDE7 inhibitors have been discovered by a number of institutions, including CSIC,[7,8] Celltech,[9] Pfizer,[10,11] Biocrea,[12] BMS,[13] and others[14−16] (Chart 1). In addition, a PDE7 inhibitor from Asubio (ASB16165) was found to be effective in a mouse skin inflammation model, indicating that PDE7 is a potential target for treating inflammatory diseases.[16]

Aimed at the development of remedies to treat the aforementioned diseases, our objective is to find a lead compound with potency and structural novelty that selectively inhibits PDE7. In this paper we describe our use of a chemical library, bioassays, computational modeling, and medicinal chemistry efforts to discover and identify novel PDE7 inhibitors.

Chart 1. Examples of Published PDE7 Inhibitors



RESULTS AND DISCUSSION

Synthetic Strategy. Thienopyrimidinone hits, represented by compounds **5**−**7**, were discovered to be the most potent compound series from our chemical library (Figure 1). None of the three hits have the structures of pan assay interference compounds.[17]

The thienopyrimidinone hits not only exhibited strong affinity for PDE7 (IC_{50} range 160−1100 nM), but are also structurally different from known PDE7 inhibitors. Further-

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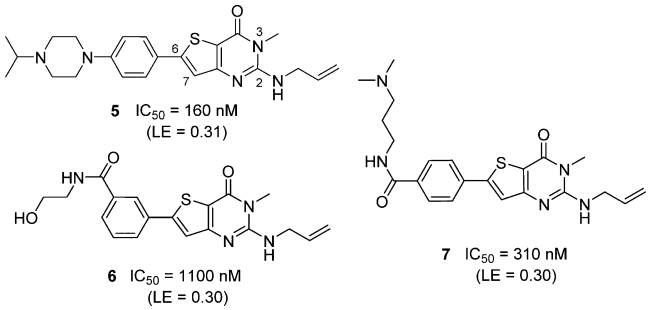

Figure 1. Structures of the thienopyrimidinone hits.

more, these hits exhibited good ligand efficiency[18,19] values of approximately 0.30. Ligand efficiency was proposed to be an important metric for the discovery of better lead compounds, in which good compounds show higher ligand efficiency values. In this study, ligand efficiency (kcal·mol^{-1}·HA^{-1}) was calculated by the formula $-RT[\ln(IC_{50})](HA)^{-1}$, where $R = 1.987 \times 10^{-3}$ kcal·mol^{-1}·K^{-1}, $T = 298$ K, and HA represents the heavy atom count.

To identify the molecular determinants responsible for the ligand binding to PDE7, computational modeling was performed for the hit compounds **5**–**7**. The Glide and Maestro[20] applications with a PDE7 cocrystal structure (PDB ID 1ZKL[21]) were used for this purpose. A proposed model of **5** in the PDE7 catalytic site is shown in Figure 2, while the

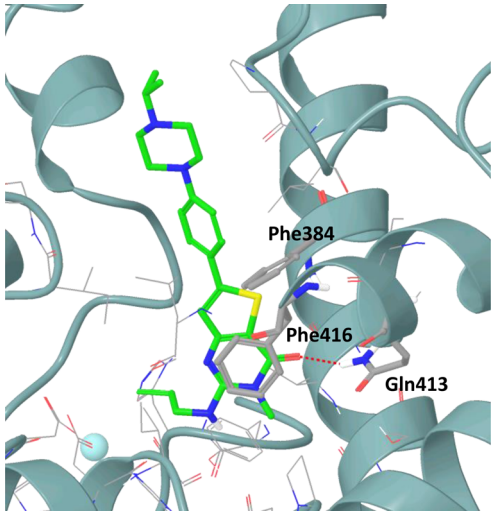

Figure 2. Proposed binding mode of **5**. A hydrogen bond is represented by a dashed line.

binding modes of **6** and **7** are shown in the Supporting Information, Figure S1. The binding model was determined to be unique, because **5** has a linear and rigid structure and contains a large substituent on the thienopyrimidinone scaffold. In this model, the thienopyrimidinone core was found to have two $\pi-\pi$ interactions and one hydrogen bond with PDE7 residues: a face-to-face orientation with the phenyl ring of Phe416, a T-shaped edge-to-face orientation with the phenyl ring of Phe384, and a hydrogen bond between the 4-carbonyl group of thienopyrimidinone and the carboxamide side chain of Gln413. Therefore, we decided to maintain these three key interactions in the drug design and syntheses for the discovery of potent PDE7 inhibitors.

This model was used to devise the synthetic strategies that would generate a series of compounds useful for the exploration of structure–activity relationships. Our synthetic strategies are summarized as follows: (1) reduce the molecular size to reveal the minimal structural requirement for demonstrating PDE7 activity; (2) re-establish the SAR of the 6-position because insufficient information was obtained around this position from the library screening data; (3) explore the SAR of the 7-position, where there is a large space in that region of the binding pocket. These three strategies were applied in the discovery of potent and selective PDE7 inhibitors.

Minimal Structural Requirements of the Thienopyrimidinone. As a starting point, we investigated the initial SAR of this series to reveal the minimal structural requirements for demonstrating activity against PDE7 (Figure 3). First, compound **8** was designed and synthesized with the maximum common substructure of the three hit compounds. The result was a substantial loss in activity, but with an almost identical ligand efficiency (**8**; IC_{50} = 6800 nM, LE = 0.34). Further removal of a phenyl ring slightly increased the activity and provided higher ligand efficiency than the hit compounds (**9**; IC_{50} = 5000 nM, LE = 0.48). Compound **9**, which is considered to have two $\pi-\pi$ interactions and one hydrogen bond, was successfully determined as the minimal structure displaying measurable enzymatic activity.

The allylamine group of **9** was replaced with a cycloalkylamino group. Pfizer's compounds include a cycloalkyl group on the thiadiazole scaffold, and ASB16165 also has a cycloalkyl group on its fused ring scaffold (Chart 1). We hence considered that the cycloalkylamino group could replace the allylamine group of **9**. The assay result was beyond our expectation, as introduction of a cyclopentylamino group at the 2-position resulted in a substantial increase in PDE7 inhibitory activity (**10**). The introduction of a cyclohexylamino group also resulted in an increase in the inhibitory activity (**11**). The bulkier cycloalkyl group was considered to have a better fit in the hydrophobic binding subpocket, resulting in an increase in activity. Because **10** demonstrated superior ligand efficiency (LE = 0.52), we decided to use **10** as a starting point to evaluate the SAR around the thienopyrimidinone scaffold.

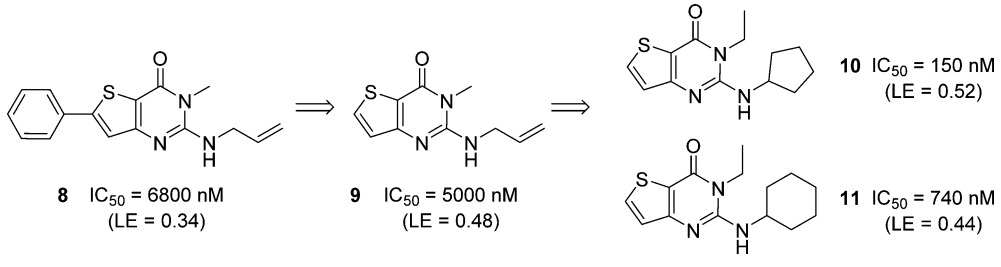

Figure 3. Results of the initial SAR study.

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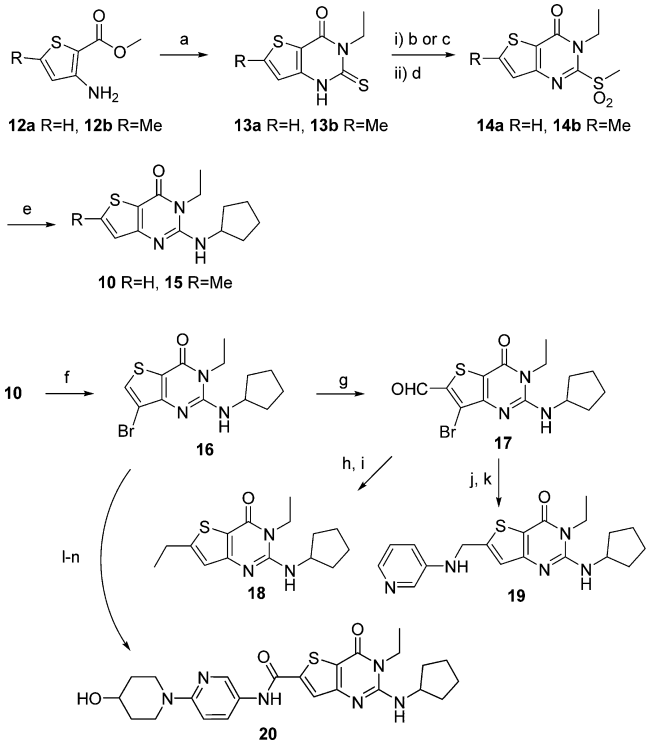
Design, Synthesis, and SAR at the 6-Position. As the hit compounds have substituents at the 6-position, various 6-substituted thienopyrimidinone derivatives were designed with substituents ranging in size. To focus on the critical interactions with the enzyme, small substituents such as methyl and ethyl groups were initially introduced. The design of **20** was inspired by the structure of ASB16165 (**1**); however, we did not perform structural overlays, because without cocrystal structures, we were uncertain whether our compounds and the known inhibitors would bind to PDE7 in the same manner. Derivatives were synthesized in four to eight steps from the starting materials **12a,b** (Scheme 1). Amines **12a,b** were treated with ethyl isothiocyanate to give the fused ring products **13a,b**, respectively. Methylation of **13a,b** with iodomethane, followed by oxidation of the thio group, yielded **14a,b**. The methylsulfonyl moiety of **14a,b** was then substituted by cyclopentylamine, yielding **10** and **15**. The more functionalized 6-substituted thienopyrimidinone derivatives **18**–**20** were prepared in four steps from **10**.

Scheme 1. Synthesis of 6-Substituted Compounds[a]

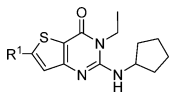


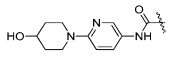
[a]Reaction conditions: (a) ethyl isothiocyanate, pyridine, reflux, 76–84%; (b) NaH, MeI, DMF, 0 °C, 98%; (c) K_2CO_3, MeI, CH_3CN, rt, 75%; (d) *m*-CPBA, CH_2Cl_2, rt, 66–87%; (e) cyclopentylamine, THF, reflux, 74–89%; (f) $PhNMe_3{\cdot}Br_3$, $CaCO_3$, CH_2Cl_2, MeOH, rt, 48%; (g) LDA, DMF, THF, 0 °C, 71%; (h) MeMgBr, THF, 0 °C, 84%; (i) $PdCl_2$, Et_3SiH, EtOH, rt, 54%; (j) 3-aminopyridine, toluene, reflux, then $NaBH_4$, MeOH, rt, 62%; (k) H_2, $Pd(OH)_2$, MeOH, rt, 47%; (l) LDA, CO_2, THF, 0 °C, 100%; (m) 1-(5-aminopyridin-2-yl)piperidin-4-ol,[22] WSC, HOBt, NMM, DMF, rt, 62%; (n) H_2, Pd/C, THF, rt, 5%.

Regioselective bromination of **10** was achieved with phenyltrimethylammonium tribromide. Treatment of **16** with LDA and DMF gave aldehyde **17** in 71% yield. A Grignard reaction on **17**, followed by hydrogenolysis using Et_3SiH and $PdCl_2$ in ethanol,[23] gave the ethyl-substituted compound **18**. Both the secondary alcohol and the bromine were removed by this reduction. The reductive amination of intermediate **17**, followed by reduction of the bromine, gave amine **19** in moderate yield. The introduction of a carboxylate group was achieved by treatment of **16** with LDA and CO_2 (dry ice). Finally, amidation followed by reduction of the bromine gave amide **20**.

The SAR at the 6-position of the thienopyrimidinone is shown in Table 1. To improve the inhibition against PDE7 and the selectivity profile of the compounds, the activity against PDE4, which is also a cAMP-specific enzyme and is most similar to PDE7, was measured at this lead discovery stage. PDE4 is an attractive target for treating a number of inflammatory diseases, including asthma and chronic obstructive pulmonary disease.[24] However, the clinical utility of PDE4 inhibitors has been limited due to the occurrence of side effects, including nausea and emesis.[25,26] Thus, the discovery of new inhibitors with good selectivity against PDE4 is desirable.

Table 1. Structure–Activity Relationship for the 6-Position



compd	R^1	IC_{50} (nM)		LE[c]
		PDE7A[a]	PDE4B[a]	
10	H	150	4900	0.52
15	Me	11000	ND[b]	0.36
18	Et	6500	3500	0.35
19		>1000	920	<0.31
20		2600	ND	0.22

[a]IC_{50} values are the mean of at least two experiments. [b]ND = not determined. [c]Ligand efficiency was calculated for PDE7A.

Surprisingly, the introduction of small substituents such as a methyl or ethyl group resulted in a 43–73-fold loss in potency and a decrease in ligand efficiency. The introduction of bulkier substituents slightly increased the potency, but the ligand efficiency was further decreased (**19**, **20**). In addition, **18** and **19** both showed stronger potency against PDE4 than PDE7, indicating an undesirable selectivity profile. We halted further exploration at the 6-position, including investigation of the original hits, because the assay results indicated that the introduction of substituents at this position led to decreased ligand efficiency and undesirable selectivity profiles.

In reviewing the SAR, the thienopyrimidinone scaffold was found to have a 32-fold selectivity for PDE7 over PDE4 (**10**). This result encouraged us to design and synthesize additional compounds in our efforts to discover lead compounds from this series. In consideration of the 6-position SAR, it is possible that obstructive factors, such as steric hindrance, prevented the formation of favorable interactions between the ligand and the PDE7 enzyme. In addition, the PDE7 catalytic site in the modeling structure suggested that there is an open space for substituents at the 7-position. We thus decided to synthesize compounds with substituents at the 7-position.

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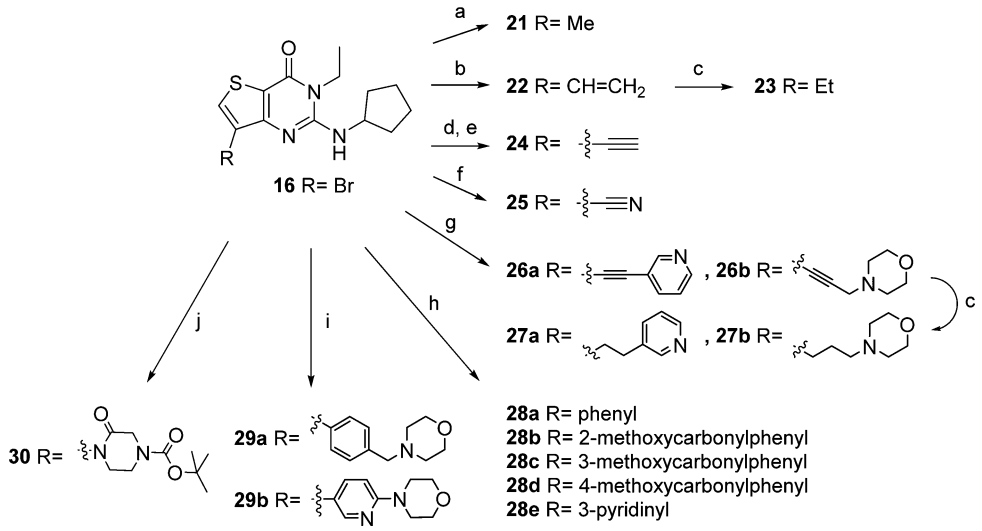
Scheme 2. Synthesis of 7-Substituted Compounds[a]



[a]Reaction conditions: (a) trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, H_2O, 1,4-dioxane, 100 °C, 83%; (b) potassium vinyltrifluoroborate, $PdCl_2(dppf)\cdot CH_2Cl_2$, TEA, EtOH, 80 °C, 71%; (c) H_2 (3 atm), 10% Pd/C, THF, rt, 85−99%; (d) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, TEA, CH_3CN, 80 °C, 19%; (e) TBAF, THF, rt, 92%; (f) CuCN, DMF, 150 °C, 24%; (g) alkyne, $PdCl_2(PPh_3)_2$, CuI, CH_3CN, 80 °C, 38−49%; (h) Ar−B(OH)$_2$, $Pd(OAc)_2$, PPh_3, saturated aq $NaHCO_3$, DMF, EtOH, 80 °C, 77−99%; (i) ArBPin, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane, 90 °C or reflux, 28−64%; (j) *tert*-butyl 3-oxopiperazine-1-carboxylate, CuI, *N*,*N*-dimethylethylenediamine, K_3PO_4, 1,4-dioxane, reflux, 59%.

Design, Synthesis, and SAR at the 7-Position. The 7-substituted thienopyrimidinone derivatives were designed and prepared starting from the key intermediate **16** (Scheme 2). Cross-coupling reactions such as the Suzuki−Miyaura reaction and the Sonogashira reaction were used to prepare **21**−**30**. Hydrogenation of **22** and **26a**,**b** was performed with a palladium catalyst to give **23** and **27a**,**b**, respectively, in 85−99% yield.

The inhibitory activities of **21**−**30** against PDE7 and PDE4, and the effects of these compounds on IL-2 production in activated mouse lymphocytes, are shown in Table 2. Li et al. reported that activation of human blood T cells is associated with induction of PDE7.[27] Although PDE4 is considered to be more important for cytokine production in T cells than PDE7,[28] a selective PDE7 inhibitor (ASB16165) was shown to block cytokine production in activated T cells.[29] In addition, another selective PDE7 inhibitor (PF 0332040) has also been shown to inhibit human T cell activation.[30] Therefore, our PDE7 inhibitors were tested to see whether they could inhibit IL-2 production in T cells. Ligand efficiency values are also specified. First, the effect of 7-substitution on **10** with aliphatic groups was examined (**21**−**24**). The introduction of methyl and ethyl groups (**21**, **23**) showed a positive effect of increasing PDE7 inhibitory activity while maintaining high ligand efficiency. In contrast to the 6-substituted compounds, these compounds showed good selectivity against PDE4. The PDE7 inhibitory activities for compounds containing unsaturated groups, such as vinyl, ethynyl, and cyano groups (**22**, **24**, **25**), were the same as those for compounds containing the methyl and ethyl groups. However, significantly increased affinities were observed for much bulkier substituents (**26a**, **27a**). The pyridine-containing compounds **26a** and **27a** exhibited especially high ligand efficiencies and single-digit nanomolar IC_{50} values. These two compounds also exhibited greater than 150-fold selectivity for PDE7 over PDE4.

The X-ray cocrystal structure of **24** bound to the PDE7 catalytic site is shown in Figure 4a (PDB ID 4PM0). The 2D interaction diagram in Figure 4b shows that the carbonyl oxygen of **24** forms a hydrogen bond with Gln413. The thiophene ring has two $\pi-\pi$ interactions, one each with the phenyl groups of Phe384 and Phe416. In addition, the NH group of **24** forms a hydrogen bond with a water molecule. This water molecule is stabilized by other hydrogen bonds formed with the side chains of Tyr211 and Asp362 (Figure 4a). The modeled structure of **5** (green) is overlaid with the cocrystal of **24** in Figure 4c. Although the interaction points of the thienopyrimidinone ring (i.e., two $\pi-\pi$ interactions and a hydrogen bond) were correctly estimated from the modeling, the position of the thieno region was slightly different from that in the cocrystal structure of **24**. This overlaid structure revealed that the region predicted to be occupied by the substituent at the 6-position was slightly different from the region predicted by the cocrystal structure.

The cocrystal structure of **24** and the SAR results could possibly explain the difference in activity between substitution at the 6-position and substitution at the 7-position. When a substituent was introduced at the 6-position, the PDE7 inhibitory activity decreased due to the steric hindrance between the ligand molecule and the surrounding residues, such as Leu401. In contrast to the 6-position, there is an open space in the direction of the 7-position; therefore, introducing substituents at the 7-position leads to increased inhibitory activity.

We think that the binding model and the X-ray structure perform a complementary role. The binding model, which was readily obtained at the hit identification stage, gave critical information to establish the strategy to introduce the substituent at the 7-position. As expected, we successfully increased the potency and selectivity by exploring substitution at the 7-position. On the other hand, this study revealed the limitation of the use of the binding model; we found it difficult to explain the SAR between the 6-position and 7-position. However, as mentioned, this SAR could successfully explain by

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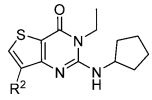
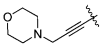
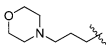
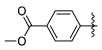
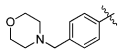
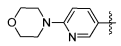
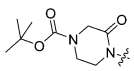
Table 2. Structure−Activity Relationship for the 7-Position



compd	R^2	IC_{50} (nM)			LE[c]
		PDE7A[a]	PDE4B[a]	IL-2[b]	
10	H	150	4900	5400	0.52
21	Me	98	3800	>3000	0.50
23	Et	42	5900	2900	0.50
22		30	3700	980	0.51
24		45	3000	560	0.50
25		67	3200	890	0.49
26a		4.2	1400	1300	0.44
26b		19	820	1700	0.39
27a		7.3	1100	1000	0.43
27b		39	3400	2000	0.37
28a		5.6	720	1800	0.47
28b		380	3800	9100	0.31
28c		5.0	480	290	0.40
28d		4.9	1000	1300	0.40
28e		2.0	380	89	0.49
29a		2.3	640	1000	0.38
29b		1.0	520	77	0.41
30		12	3300	3000	0.21

[a]IC_{50} values are the mean of at least two experiments. [b]IC_{50} values are the mean of at least four experiments. [c]Ligand efficiency was calculated for PDE7A.

using the X-ray structure, which was obtained at the lead discovery stage.

Further modifications were carried out to study the influence of substituents at the 7-position. Compounds with a phenyl ring (**28a−d** and **29a**) and compounds with a pyridyl ring (**28e**, **29b**) were synthesized. The introduction of a phenyl group resulted in a compound with single-digit nanomolar potency against PDE7 (**28a**), with a 27-fold enhancement of activity over that of **10**. Although both *meta*- and *para*-substituted methyl benzoate compounds (**28c,d**) exhibited almost identical inhibition of PDE7 activity, **28c** showed good cellular activity. The *ortho*-substituted methyl benzoate **28b** showed a 68-fold loss in potency. Among the methyl benzoates, **28c** showed both potent PDE7 inhibition, with 96-fold selectivity over PDE4, and strong cellular activity (IC_{50} = 290 nM). The oxopiperadine **30** showed good potency for PDE7 and 275-fold selectivity over PDE4.

With respect to the pyridyl derivatives, **28e** exhibited single-digit nanomolar potency, and the morpholine-substituted compound **29b** showed the strongest activity (IC_{50} = 1.0 nM) among the synthesized compounds given in Tables 1 and 2. In particular, **28e** exhibited very potent activity as well as good ligand efficiency. An aromatic substituent, such as a phenyl or pyridyl ring, provided greater positive effects on PDE7 enzyme inhibition than an aliphatic side chain. The pyridyl compound **28e** exhibited a selectivity of 190-fold for PDE7 over PDE4. In addition, **28e** showed high selectivity against other PDE enzymes, including a selectivity of 800-fold over PDE3 and 1650-fold over PDE5. We believe that selectivity, especially for the three enzymes PDE3, PDE4, and PDE5, is important to avoid side effects. Compound **28e** also has strong cellular activity (IC_{50} = 89 nM), indicating that this thienopyrimidinone derivative is a suitable lead compound.

Whereas the IC_{50} values for PDE3, PDE4, PDE5, and PDE7 were measured internally, the full PDE selectivity profiling assay for **30** was performed at Caliper Discovery Alliances & Services using the Caliper LabChip 3000 to reveal the selectivity profile of the thienopyrimidinone. The result is shown in the Supporting Information (Figure S2). The data show that **30** has a good selectivity profile. Although the selectivity profile of **28e** was not evaluated, considering structural similarity, **28e** is believed to exhibit selectivity for other PDE enzymes.

In the present set of active inhibitors, we further evaluated the pharmacokinetic profile of **28e**, **29a**, and **29b**. The data are provided in Table S1 of the Supporting Information. Whereas these compounds showed good permeability, they are metabolically unstable in human and rat liver microsomes. The findings from this study will be useful for further optimization studies.

CONCLUSION

In conclusion, we have described the discovery of a series of 2-(cyclopentylamino)thieno[3,2-*d*]pyrimidin-4(3*H*)-one compounds as novel and potent PDE7 inhibitors. Screening of our chemical library led to the identification of hit thienopyrimidinone compounds with substituents at the 6-position. SAR studies around the hit compounds, and computational modeling, contributed to the discovery of 7-substituted derivatives as a series of novel and potent PDE7 inhibitors. As a result, we identified the lead compound **28e**, which demonstrated single-digit nanomolar potency for PDE7 with 190-fold selectivity over PDE4, as well as potent cellular efficacy (IL-2 IC_{50} < 100 nM). This lead compound also showed good selectivity against other PDE enzymes.

EXPERIMENTAL SECTION

General Methods. Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise indicated. Moisture-sensitive reactions were carried out under an argon atmosphere in dried glassware using anhydrous solvents from commercial suppliers. ^{1}H NMR spectra were recorded on a JEOL JNM-ECA400 spectrometer (400 MHz). Chemical shifts are reported in δ units (ppm) relative to TMS as an internal standard. Coupling constants (J) are reported in hertz. High-resolution mass spectrometry (HRMS) analyses were performed on a Thermo Scientific Exactive mass spectrometer in positive or negative ionization electrospray mode operating at 25 000 resolution. The purities of the

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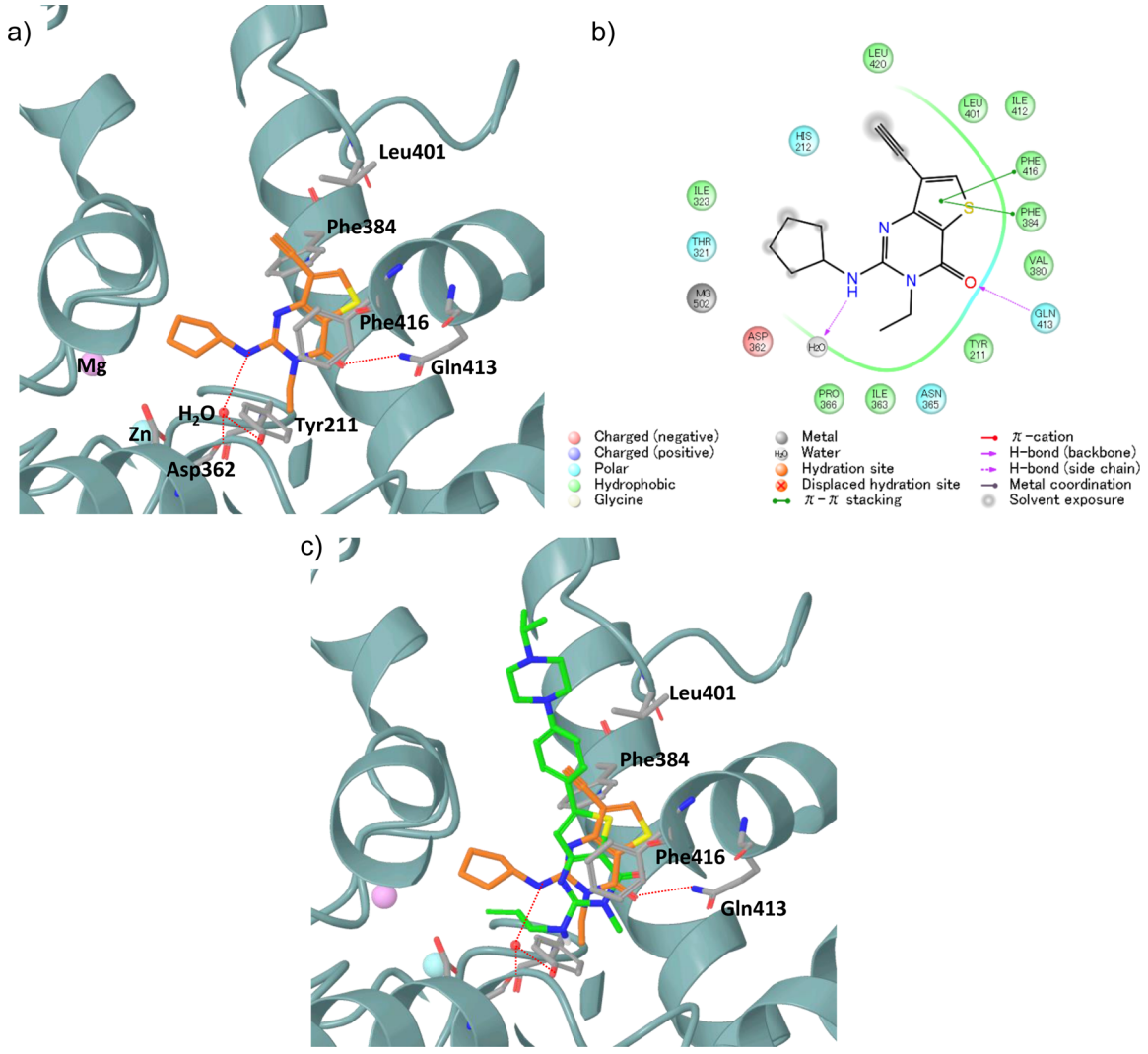

Figure 4. Cocrystal structure of **24** bound to the PDE7A catalytic site (PDB ID 4PM0, 2.1 Å resolution). (a) Complex of **24** (orange) with binding site residues. A hydrogen bond is represented by a dashed line, and only the important water molecule is shown. (b) 2D ligand interaction diagram of **24** generated with Maestro 9.3. (c) Complex of **24** overlaid with the modeled structure **5** (green).

final compounds were determined using HPLC (area percentage at 254 nm). HPLC analyses were performed using a Hitachi La Chrom Elite system. The HPLC purities were ≥95% for all of the compounds, with the exception of compounds **18** and **19**.

2-(Allylamino)-6-(4-(4-isopropylpiperazin-1-yl)phenyl)-3-methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (5). This compound was purchased from BioFocus DPI (purity 98%). ^{1}H NMR ($CDCl_3$): δ 1.11 (6H, d, J = 6.9 Hz), 2.71 (1H, m), 3.30 (8H, m), 3,52 (3H, s), 4.16 (2H, m), 4.64 (1H, m), 5.22–5.34 (2H, m), 5.99–6.09 (1H, m), 6.93 (2H, d, J = 8.7 Hz), 7.17 (1H, s), 7.58 (2H, d, J = 9.2 Hz). MS (ESI): m/z 424 $(M + H)^+$.

3-(2-(Allylamino)-3-methyl-4-oxo-3,4-dihydrothieno[3,2-*d*]-pyrimidin-6-yl)-*N*-(2-hydroxyethyl)benzamide (6). This compound was purchased from BioFocus DPI (purity 98%). MS (ESI): m/z 385 $(M + H)^+$.

4-(2-(Allylamino)-3-methyl-4-oxo-3,4-dihydrothieno[3,2-*d*]-pyrimidin-6-yl)-*N*-(3-(dimethylamino)propyl)benzamide (7). This compound was purchased from BioFocus DPI (purity 97%). MS (ESI): m/z 426 $(M + H)^+$.

3-Ethyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidin-4(1*H*)-one (13a). To a solution of **12a** (9.58 g, 60.9 mmol) in pyridine (122 mL) was added ethyl isothiocyanate (10.7 mL, 122 mmol). The reaction mixture was heated under reflux for 14 h. After the mixture was cooled to rt, the solvent was evaporated under reduced pressure. The residue was washed with chloroform to yield 10.8 g (84%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.22 (3H, t, J = 6.9 Hz), 4.44 (2H, q, J = 6.9 Hz), 7.01 (1H, d, J = 5.5 Hz), 8.16 (1H, d, J = 5.5 Hz).

3-Ethyl-2-(methylthio)thieno[3,2-*d*]pyrimidin-4(3*H*)-one. To a solution of **13a** (27.2 g, 0.128 mmol) in DMF (0.43 L) were added methyl iodide (16 mL, 0.26 mol) and sodium hydride (60% in oil, 7.6 g, 0.19 mol). The reaction mixture was stirred at 0 °C for 1 h. Saturated aq NH_4Cl solution and water were then sequentially added to the reaction mixture. The mixture was filtered, and the precipitate was washed with water and dried under reduced pressure to yield 28.6 g (98%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.38 (3H, t, J = 6.9 Hz), 2.63 (3H, s), 4.24 (2H, q, J = 7.3 Hz), 7.20 (1H, d, J = 5.0 Hz), 7.70 (1H, d, J = 5.5 Hz).

3-Ethyl-2-(methylsulfonyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (14a). To a solution of 3-ethyl-2-(methylthio)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (28.6 g, 0.126 mol) in dichloromethane (0.63 L) was added 70% *m*-CPBA (79 g, 0.32 mol). The mixture was stirred at rt for 1 h. Saturated sodium bicarbonate and saturated sodium thiosulfate were added to the reaction mixture. The mixture was extracted with chloroform and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 28.5 g (87%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.48 (3H, t, J = 6.9 Hz), 3.54 (3H, s), 4.58 (2H, q, J = 6.9 Hz), 7.34 (1H, d, J = 5.5 Hz), 7.87 (1H, d, J = 5.0 Hz).

2-(Cyclopentylamino)-3-ethylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (10). To a solution of **14a** (11.0 g, 42.6 mmol) in THF (114 mL)

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was added cyclopentylamine (20 mL, 0.20 mol). The mixture was heated under reflux for 18 h. After the mixture was cooled to rt, the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 8.73 g (89%) of a white solid. HPLC purity: 97.2%. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.46−1.54 (2H, m), 1.66−1.79 (4H, m), 2.12−2.20 (2H, m), 4.09 (2H, q, J = 7.3 Hz), 4.37−4.45 (1H, m), 4.43 (1H, br s), 7.05 (1H, d, J = 5.5 Hz), 7.62 (1H, d, J = 5.5 Hz). HRMS (ESI): m/z calcd for $C_{13}H_{18}N_3OS$ $(M + H)^+$ 264.1165, found 264.1164.

3-Ethyl-6-methyl-2-thioxo-2,3-dihydrothieno[3,2-*d*]-pyrimidin-4(1*H*)-one (13b). According to the method described for derivative **13a**, transformation of **12b** (150 mg, 0.878 mmol) yielded 150 mg (76%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 6.9 Hz), 2.57 (3H, s), 4.56 (2H, q, J = 6.9 Hz), 6.64 (1H, s).

3-Ethyl-6-methyl-2-(methylthio)thieno[3,2-*d*]pyrimidin-4(3*H*)-one. To a solution of **13b** (150 mg, 0.663 mmol) in acetonitrile (2 mL) were added methyl iodide (50 μL, 0.795 mmol) and potassium carbonate (137 mg, 0.994 mmol). The reaction mixture was stirred at rt for 2 h. Then the solvent was evaporated under reduced pressure. Water was added to the reaction mixture. The mixture was extracted with chloroform and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 120 mg (75%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.37 (3H, t, J = 7.3 Hz), 2.59 (3H, s), 2.61 (3H, s), 4.21 (2H, q, J = 7.3 Hz), 6.87 (1H, s).

3-Ethyl-6-methyl-2-(methylsulfonyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (14b). To a solution of 3-ethyl-6-methyl-2-(methylthio)-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (120 mg, 0.499 mol) in dichloromethane (1 mL) was added 75% *m*-CPBA (267 mg, 1.25 mol). The mixture was stirred at rt for 2 h. Saturated sodium bicarbonate was added to the reaction mixture. The mixture was extracted with chloroform and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 90 mg (66%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.46 (3H, t, J = 7.2 Hz), 2.64 (3H, s), 3.51 (3H, s), 4.55 (2H, q, J = 7.2 Hz), 7.00 (1H, s).

2-(Cyclopentylamino)-3-ethyl-6-methylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (15). To a solution of **14b** (90 mg, 0.33 mmol) in THF (1 mL) was added cyclopentylamine (563 mg, 6.61 mmol). The mixture was heated under reflux for 4 h. After the mixture was cooled to rt, the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 68 mg (74%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.1 Hz), 1.42−1.54 (2H, m), 1.61−1.79 (4H, m), 2.09−2.19 (2H, m), 2.53 (3H, s), 4.06 (2H, q, J = 7.1 Hz), 4.34−4.41 (2H, m), 6.74 (1H, s). HRMS (ESI): m/z calcd for $C_{14}H_{20}N_3OS$ $(M + H)^+$ 278.1322, found 278.1319. HPLC purity: 98.0%.

7-Bromo-2-(cyclopentylamino)-3-ethylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (16). To a solution of **10** (8.73 g, 33.1 mmol) in a mixture of dichloromethane (55.2 mL) and methanol (55.2 mL) were added phenytrimethylammonium tribromide (21.5 g, 99.3 mmol) and calcium carbonate (13.2 g, 132 mmol). The reaction mixture was stirred at rt for 14 h. The residue was filtered, and the solvent was evaporated under reduced pressure. Then the residue was washed with water and extracted with ethyl acetate. The residue was purified by flash chromatography to yield 5.45 g (48%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.47−1.56 (2H, m), 1.67−1.78 (4H, m), 2.19−2.28 (2H, m), 4.10 (2H, q, J = 7.3 Hz), 4.44−4.58 (2H, m), 7.61 (1H, s).

7-Bromo-2-(cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-6-carbaldehyde (17). To a solution of **16** (1.49 g, 4.35 mmol) in THF (14.5 mL) was added a 2 M solution of LDA in THF (6.55 mL, 13.1 mmol) at 0 °C under an argon atmosphere. Anhydrous DMF (1.34 mL, 17.4 mmol) was then added to the solution and the resulting solution was stirred for 2 h at 0 °C. Saturated aq NH_4Cl solution was added, and the solution was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 1.15 g (71%) of a yellow solid. ^{1}H NMR (DMSO-d_6): δ 1.15 (3H, t, J = 6.9 Hz), 1.56−1.74 (6H, m), 2.03−2.07 (2H, m), 4.11 (2H, q, J = 6.9 Hz), 4.38−4.43 (1H, m), 7.06 (1H, d, J = 6.4 Hz), 10.08 (1H, s).

7-Bromo-2-(cyclopentylamino)-3-ethyl-6-(1-hydroxyethyl)-thieno[3,2-*d*]pyrimidin-4(3*H*)-one. To a solution of **17** (50 mg, 0.135 mmol) in THF (2.7 mL) was added a 1.1 M solution of methylmagnesium bromide in THF (0.184 mL, 0.203 mmol) at 0 °C. The solution was stirred for 5 h at 0 °C. Saturated aq NH_4Cl solution was added, and the mixture was extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 44 mg (84%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.2 Hz), 1.45−1.57 (2H, m), 1.59 (3H, d, J = 6.9 Hz), 1.64−1.78 (4H, m), 2.16−2.27 (2H, m), 2.42 (1H, d, J = 6.9 Hz), 4.06 (2H, q, J = 7.2 Hz), 4.40−4.55 (2H, m), 5.28−5.35 (1H, m).

2-(Cyclopentylamino)-3,6-diethylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (18). To a solution of 7-bromo-2-(cyclopentylamino)-3-ethyl-6-(1-hydroxyethyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (20 mg, 0.052 mmol) in ethanol (1.0 mL) were added palladium(II) chloride (9.2 mg, 0.052 mmol) and triethylsilane (83 μL, 0.52 mmol), and the mixture was stirred at rt for 6 h. The reaction solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 8.2 mg (54%) of a white solid. HPLC purity: 75.9%. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.3 Hz), 1,34 (3H, t, J = 7.6 Hz) 1.40−1.51 (2H, m), 1.60−1.78 (4H, m), 2.10−2.20 (2H, m), 2.88 (2H, q, J = 7.6 Hz), 4.06 (2H, q, J = 7.3 Hz), 4.30−4.41 (2H, m), 6.78 (1H, s). HRMS (ESI): m/z calcd for $C_{15}H_{22}N_3OS$ $(M + H)^+$ 292.1478, found 292.1473.

7-Bromo-2-(cyclopentylamino)-3-ethyl-6-[(pyridin-3-ylamino)methyl]thieno[3,2-*d*]pyrimidin-4(3*H*)-one. To a solution of **17** (40 mg, 0.11 mmol) in toluene (1.5 mL) was added 3-aminopyridine (32 mg, 0.33 mmol), and the reaction mixture was heated under reflux for 2 h. After the mixture was cooled to rt, the solvent was evaporated under reduced pressure. After the residue was dissolved in methanol (1.5 mL), sodium borohydride (12.3 mg, 0.33 mmol) was added, and the resulting solution was stirred at rt for 30 min. Saturated aq NH_4Cl solution was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. The residue was purified by flash chromatography to yield 25 mg (62%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.30 (3H, t, J = 7.2 Hz), 1.45−1.58 (2H, m), 1.65−1.81 (4H, m), 2.17−2.28 (2H, m), 4.04 (2H, q, J = 7.2 Hz), 4.38 (1H, t, J = 6.4 Hz), 4.43−4.56 (2H, m), 4.60 (2H, d, J = 6.4 Hz), 6.91 (1H, ddd, J = 1.5, 4.2, 8.2 Hz), 7.08 (1H, dd, J = 4.2, 8.2 Hz), 8.02 (1H, d, J = 4.2 Hz), 8.09 (1H, d, J = 1.5 Hz).

2-(Cyclopentylamino)-3-ethyl-6-[(pyridin-3-ylamino)-methyl]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (19). To a solution of 7-bromo-2-(cyclopentylamino)-3-ethyl-6-[(pyridin-3-ylamino)-methyl]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (10 mg, 0.022 mmol) in methanol (1.0 mL) was added 20% palladium hydroxide on carbon (3.2 mg, 32%, w/w), and the mixture was stirred at rt for 12 h under a hydrogen atmosphere. The reaction solution was filtered through a pad of Celite, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 6.2 mg (47%) of a pale yellow solid. HPLC purity: 67.7%. ^{1}H NMR ($CDCl_3$): δ 1.24 (3H, t, J = 6.9 Hz), 1.43−1.53 (2H, m), 1.64−1.80 (4H, m), 2.10−2.20 (2H, m), 4.06 (2H, q, J = 6.9 Hz), 4.33−4.41 (1H, m), 4.48 (1H, d. J = 5.5 Hz), 4.65 (2H, d, J = 5.5 Hz), 6.57 (1H, s), 7.02 (1H, d, J = 7.7 Hz), 7.39 (1H, dd, J = 5.0, 7.7 Hz), 7.91 (1H, d, J = 5.0 Hz), 8.44 (1H, s). HRMS (ESI): m/z calcd for $C_{19}H_{24}N_5OS$ $(M + H)^+$ 370.1696, found 370.1701.

7-Bromo-2-(cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-6-carboxylic Acid. To a solution of **16** (1.50 g, 4.38 mmol) in THF (14.6 mL) was added a 2.0 M solution of LDA in THF (6.55 mL, 13.1 mmol), and the mixture was stirred at 0 °C for 30 min. Dry ice (one piece) was added and the resulting mixture was stirred at 0 °C for 30 min. Water was added, and the mixture was washed with chloroform. Aqueous hydrochloride was

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then added to weakly acidify the mixture, which was then extracted with chloroform. The solvent was evaporated under reduced pressure to yield 1.70 g (100%) of a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.45−1.58 (2H, m), 1.72−1.76 (4H, m), 2.20−2.28 (2H, m), 4.07 (2H, q, J = 7.3 Hz), 4.45−4.50 (2H, m), 9.58 (1H, br s).

7-Bromo-2-(cyclopentylamino)-3-ethyl-*N*-[6-(4-hydroxypiperidin-1-yl)pyridin-3-yl]-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-6-carboxamide. To a solution of 7-bromo-2-(cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-6-carboxylic acid (151 mg, 0.391 mmol) in DMF (1.96 mL) were added NMM (154 μL, 1.17 mmol), HOBt (68.6 mg, 0.508 mmol), and WSC (97.5 mg, 0.508 mmol). 1-(5-Aminopyridin-2-yl)piperidin-4-ol[21] (90.6 mg, 0.469 mmol) was then added, and the reaction was stirred at rt for 14 h. The residue was filtered and purified by flash chromatography to yield 132.3 mg (62%) of a yellow amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, t, J = 7.3 Hz), 1.48−1.64 (4H, m), 1.68−1.80 (4H, m), 1.98−2.02 (2H, m), 2.22−2.28 (2H, m), 3.14−3.21 (2H, m), 3.90−3.97 (1H, m), 4.04−4.15 (4H, m), 4.47 (1H, sext, J = 6.4 Hz), 4.61 (1H, d, J = 6.0 Hz), 6.72 (1H, d, J = 9.2 Hz), 8.02 (1H, dd, J = 2.7, 9.2 Hz), 8.29 (1H, d, J = 2.7 Hz), 8.91 (1H, s).

2-(Cyclopentylamino)-3-ethyl-*N*-[6-(4-hydroxypiperidin-1-yl)pyridin-3-yl]-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-6-carboxamide (20). To a solution of 10% Pd/C (11.7 mg) in THF (1.07 mL) was added 7-bromo-2-(cyclopentylamino)-3-ethyl-*N*-[6-(4-hydroxypiperidin-1-yl)pyridin-3-yl]-4-oxo-3,4-dihydrothieno[3,2-*d*]-pyrimidine-6-carboxamide (117 mg, 0.214 mmol) under argon. The reaction mixture was stirred at rt for 48 h under a hydrogen atmosphere. The mixture was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 5.2 mg (5%) of a yellow solid. HPLC purity: 95.1%. ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, t, J = 7.3 Hz), 1.46−1.80 (8H, m), 1.96−2.02 (2H, m), 2.10−2.20 (2H, m), 3.13−3.20 (2H, m), 3.88−3.95 (1H, m), 4.50 (1H, d, J = 6.4 Hz), 6.71 (1H, d, J = 9.2 Hz), 7.54 (1H, s), 7.73 (1H, s), 7.94−7.98 (1H, m), 8.21 (1H, d, J = 2.7 Hz). HRMS (ESI): m/z calcd for $C_{24}H_{31}N_6O_3S$ $(M + H)^+$ 483.2173, found 483.2172.

2-(Cyclopentylamino)-3-ethyl-7-methylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (21). To a solution of **16** (100 mg, 0.29 mmol) in a mixture of 1,4-dioxane (0.9 mL) and water (0.1 mL) were added trimethylboroxine (0.1 mL, 0.72 mmol), potassium carbonate (120 mg, 0.87 mmol), and tetrakis(triphenylphosphine)palladium (35 mg, 0.03 mmol) under an argon atmosphere, and the mixture was stirred at 100 °C for 18 h. After the mixture was cooled to rt, water was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. The residue was purified by flash chromatography to yield 67 mg (83%) of a white solid. HPLC purity: 99.4%. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.3 Hz), 1.49−1.53 (2H, m), 1.65−1.80 (4H, m), 2.16−2.21 (2H, m), 2.28 (3H, d, J = 1.4 Hz), 4.08 (2H, q, J = 7.3 Hz), 4.40−4.47 (2H, m), 7.26−7.27 (1H, m). HRMS (ESI): m/z calcd for $C_{14}H_{20}N_3OS$ $(M + H)^+$ 278.1322, found 278.1320.

2-(Cyclopentylamino)-3-ethyl-7-vinylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (22). To a solution of **16** (100 mg, 0.29 mmol) in a mixture of potassium vinyltrifluoroborate (80 mg, 0.60 mmol) and triethylamine (0.1 mL, 0.72 mmol) in ethanol (3 mL) was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (25 mg, 0.031 mmol) under an argon atmosphere. The reaction mixture was stirred at 80 °C for 18 h. After the mixture was cooled to rt, water was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 60 mg (71%) of a white solid. HPLC purity: 96.7%. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.52−1.55 (2H, m), 1.66−1.79 (4H, m), 2.14−2.20 (2H, m), 4.09 (2H, q, J = 7.3 Hz), 4.37−4.47 (2H, m), 5.34 (1H, dd, J = 1.8, 11.5 Hz), 6.32 (1H, dd, J = 1.8, 17.9 Hz), 6.85 (1H, dd, J = 11.5, 17.9 Hz), 7.54 (1H, s). HRMS (ESI): m/z calcd for $C_{15}H_{20}N_3OS$ $(M + H)^+$ 290.1322, found 290.1323.

2-(Cyclopentylamino)-3,7-diethylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (23). To a solution of **22** (55 mg, 0.19 mmol) in THF (10 mL) was added 10% Pd/C (30 mg, 54.5%, w/w). The reaction mixture was stirred at rt for 18 h under a hydrogen atmosphere. The reaction solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure to yield 55 mg (99%) of a white solid. HPLC purity: 98.3%. ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, t, J = 7.3 Hz), 1.33 (3H, t, J = 7.3 Hz), 1.49−1.55 (2H, m), 1.60−1.80 (4H, m), 2.15−2.21 (2H, m), 2.73 (2H, dq, J = 1.4, 7.3 Hz), 4.08 (2H, q, J = 7.3 Hz), 4.36−4.41 (2H, m), 7.27 (1H, t, J = 1.4 Hz). HRMS (ESI): m/z calcd for $C_{15}H_{22}N_3OS$ $(M + H)^+$ 292.1478, found 292.1476.

2-(Cyclopentylamino)-3-ethyl-7-[(trimethylsilyl)ethynyl]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one. To a solution of **16** (300 mg, 0.88 mmol) in acetonitrile (9 mL) were added triethylamine (3 mL, 21.5 mmol) and ethynyltrimethylsilane (0.3 mL, 2.12 mmol). Under an argon atmosphere, bis(triphenylphosphine)palladium(II) dichloride (60 mg, 0.085 mmol) and copper(I) iodide (20 mg, 0.11 mmol) were added, and the mixture was stirred at 80 °C for 18 h. After being cooled to rt, the solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 60 mg (19%) of a white solid. ^{1}H NMR ($CDCl_3$): δ 0.27 (9H, s), 1.31 (3H, t, J = 7.3 Hz), 1.47−1.56 (2H, m), 1.65−1.79 (4H, m), 2.20−2.28 (2H, m), 4.07 (2H, q, J = 7.3 Hz), 4.40−4.48 (1H, m), 4.52 (1H, d, J = 5.5 Hz), 7.75 (1H, s).

2-(Cyclopentylamino)-3-ethyl-7-ethynylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (24). To a solution of 2-(cyclopentylamino)-3-ethyl-7-[(trimethylsilyl)ethynyl]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (55 mg, 0.15 mmol) in THF (1 mL) was added a 1 M solution of TBAF in THF (0.3 mL, 0.3 mmol), and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 40 mg (92%) of a white solid. HPLC purity: 97.2%. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.47−1.54 (2H, m), 1.65−1.78 (4H, m), 2.17−2.23 (2H, m), 3.23 (1H, s), 4.08 (2H, q, J = 7.3 Hz), 4.45−4.51 (2H, m), 7.81 (1H, s). HRMS (ESI): m/z calcd for $C_{15}H_{18}N_3OS$ $(M + H)^+$ 288.1165, found 288.1163.

2-(Cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carbonitrile (25). To a solution of **16** (100 mg, 0.29 mmol) in DMF (2 mL) was added copper(I) cyanide (130 mg, 1.45 mmol). The reaction mixture was stirred at 150 °C for 6 h. After the mixture was cooled to rt, aqueous ammonia was added. The residue was filtered and purified by flash chromatography to yield 20 mg (24%) of a white solid. HPLC purity: 98.2%. ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, t, J = 7.3 Hz), 1.47−1.52 (2H, m), 1.70−1.80 (4H, m), 2.18−2.24 (2H, m), 4.07 (2H, q, J = 7.3 Hz), 4.48−4.53 (1H, m), 4.60 (1H, br s), 8.16 (1H, s). HRMS (ESI): m/z calcd for $C_{14}H_{17}N_4OS$ $(M + H)^+$ 289.1118, found 289.1111.

2-(Cyclopentylamino)-3-ethyl-7-(pyridin-3-ylethynyl)thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (26a). To a mixture of **16** (100 mg, 0.29 mmol), 3-ethynylpyridine (60 mg, 0.58 mmol), and triethylamine (1.0 mL, 7.15 mmol) in acetonitrile (3 mL) were added $PdCl_2(dppf)\cdot CH_2Cl_2$ (20 mg, 0.028 mmol) and copper(I) iodide (5.0 mg, 0.027 mmol) under an argon atmosphere. The reaction mixture was stirred at 80 °C for 18 h. After being cooled to rt, the reaction solution was filtered through a Celite pad. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 40 mg (38%) of a white solid. HPLC purity: 99.0%. ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, t, J = 7.3 Hz), 1.50−1.56 (2H, m), 1.67−1.81 (4H, m), 2.21−2.27 (2H,m), 4.09 (2H, q, J = 7.3 Hz), 4.45−4.52 (1H, m), 4.55 (1H, br s), 7.30 (1H, ddd, J = 0.9, 4.6, 7.8 Hz), 7.82 (1H, ddd, J = 1.8, 1.8, 7.8 Hz), 7.84 (1H, s), 8.56 (1H, dd, J = 1.8, 4.6 Hz), 8.79 (1H, dd, J = 0.9, 1.8 Hz). HRMS (ESI): m/z calcd for $C_{20}H_{21}N_4OS$ $(M + H)^+$ 365.1431, found 365.1428.

2-(Cyclopentylamino)-3-ethyl-7-(3-morpholinoprop-1-yn-1-yl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (26b). To a mixture of **16** (100 mg, 0.29 mmol), 3-morpholinopropyne (200 mg, 1.60 mmol), and triethylamine (1.0 mL, 7.15 mmol) in acetonitrile (3 mL) were added $PdCl_2(dppf)\cdot CH_2Cl_2$ (40 mg, 0.057 mmol) and copper(I) iodide (10 mg, 0.053 mmol) under an argon atmosphere. The reaction mixture was stirred at 80 °C for 18 h. After being cooled to rt, the reaction solution was filtered through a Celite pad. The solvent was

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evaporated under reduced pressure. The residue was purified by flash chromatography to yield 55 mg (49%) of a white solid. HPLC purity: 99.3%. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.3 Hz), 1.49−1.56 (2H, m), 1.64−1.80 (4H, m), 2.16−2.21 (2H, m), 2.71 (4H, t, J = 5.0 Hz), 3.59 (2H, s), 3.77 (4H, t, J = 5.0 Hz), 4.07 (2H, q, J = 7.3 Hz), 4.45−4.52 (2H, m), 7.70 (1H, s). HRMS (ESI): m/z calcd for $C_{20}H_{27}N_4O_2S$ $(M + H)^+$ 387.1849, found 387.1845.

2-(Cyclopentylamino)-3-ethyl-7-(2-pyridin-3-ylethyl)thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (27a). To a solution of **26a** (35 mg, 0.095 mmol) in THF (10 mL) was added 10% Pd/C (30 mg). The reaction mixture was stirred at rt for 24 h under a hydrogen atmosphere (3 atm). The reaction solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure to yield 30 mg (85%) of a white solid. HPLC purity: 95.0%. ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, t, J = 7.3 Hz), 1.53−1.58 (2H, m), 1.68−1.80 (4H, m), 2.12−2.21 (2H, m), 2.97−3.06 (4H, m), 4.09 (2H, q, J = 7.3 Hz), 4.39−4.43 (1H,m), 4.45 (1H, br s), 7.20 (1H, dd, J = 4.6, 7.8 Hz), 7.22 (1H, s), 7.47 (1H, ddd, J = 1.8, 1.8, 7.8 Hz), 8.45 (1H, dd, J = 1.8, 4.6 Hz), 8.49 (1H, d, J = 1.8 Hz). HRMS (ESI): m/z calcd for $C_{20}H_{25}N_4OS$ $(M + H)^+$ 369.1744, found 369.1739.

2-(Cyclopentylamino)-3-ethyl-7-(3-morpholinopropyl)-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (27b). To a solution of **26b** (50 mg, 0.13 mmol) in THF (10 mL) was added 10% Pd/C (30 mg, 60%, w/w). The reaction mixture was stirred at rt for 24 h under a hydrogen atmosphere (3 atm). The reaction solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure to yield 49 mg (96%) of a colorless oil. HPLC purity: 98.1%. ^{1}H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.3 Hz), 1.49−1.55 (2H, m), 1.64−1.78 (4H, m), 1.86−1.93 (2H, m), 2.11−2.19 (2H, m), 2.41 (2H, t, J = 7.3 Hz), 2.45 (4H, t, J = 4.6 Hz), 2.73 (2H, t, J = 7.3 Hz), 3.72 (4H, t, J = 4.6 Hz), 4.08 (2H, q, J = 7.3 Hz), 4.34−4.45 (2H, m), 7.28 (1H, s). HRMS (ESI): m/z calcd for $C_{20}H_{31}N_4O_2S$ $(M + H)^+$ 391.2162, found 391.2158.

2-(Cyclopentylamino)-3-ethyl-7-phenylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (28a). To a DMF (0.3 mL) solution of diacetoxypalladium (3.28 mg, 0.015 mmol) and triphenylphosphine (15.3 mg, 0.058 mmol) was added dropwise a DMF (1.5 mL) solution of **16** (100 mg, 0.292 mmol). After the resulting solution was stirred at rt for 5 min, an ethanol (0.3 mL) solution of phenylboronic acid (71.3 mg, 0.58 mmol) and saturated sodium bicarbonate (0.3 mL) were added. The reaction mixture was stirred at 80 °C for 2 h. After the mixture was cooled to rt, water was added, and the mixture was extracted with diethyl ether. The organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 76 mg (77%) of a white solid. HPLC purity: 98.9%. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 7.3 Hz), 1.50−1.61 (2H, m), 1.63−1.80 (4H, m), 2.11−2.21 (2H, m), 4.11 (2H, q, J = 7.3 Hz), 4.33−4.41 (1H, m), 4.47 (1H, d, J = 5.5 Hz), 7.30−7.36 (1H, m), 7.40−7.46 (2H, m), 7.77 (1H, s), 7.96−8.01 (2H, m). HRMS (ESI): m/z calcd for $C_{19}H_{22}N_3OS$ $(M + H)^+$ 340.1478, found 340.1474.

Methyl 2-[2-(Cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-7-yl]benzoate (28b). According to the method described for derivative **28a**, transformation of **16** (20 mg, 0.06 mmol) yielded 23 mg (99%) of a white solid. HPLC purity: 98.5%. ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, t, J = 7.3 Hz), 1.29−1.42 (2H, m), 1.55−1.71 (4H, m), 1.95−2.06 (2H, m), 3.56 (3H, s), 4.07 (2H, q, J = 7.3 Hz), 4.20−4.30 (1H, m), 4.37 (1H, d, J = 6.4 Hz), 7.41−7.47 (2H,m), 7.53−7.58 (1H, m), 7.61 (1H, s), 7.93−7.98 (1H, m). HRMS (ESI): m/z calcd for $C_{21}H_{24}N_3O_3S$ $(M + H)^+$ 398.1533, found 398.1531.

Methyl 3-[2-(Cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-7-yl]benzoate (28c). According to the method described for derivative **28a**, transformation of **16** (20 mg, 0.06 mmol) yielded 23 mg (99%) of a white solid. HPLC purity: 95.6%. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 7.3 Hz), 1.48−1.61 (2H, m), 1.63−1.80 (4H, m), 2.11−2.25 (2H, m), 3.93 (3H, s), 4.12 (2H, q, J = 7.3 Hz), 4.37−4.47 (1H, m), 4.51 (1H, d, J = 5.9 Hz), 7.50 (1H, t, J = 7.7 Hz), 7.84 (1H, s), 8.01 (1H, dt, J = 1.3, 7.7 Hz), 8.15 (1H, dt, J = 1.3, 7.7 Hz), 8.78 (1H, t, J = 1.3 Hz). HRMS (ESI): m/z calcd for $C_{21}H_{24}N_3O_3S$ $(M + H)^+$ 398.1533, found 398.1526.

Methyl 4-[2-(Cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-7-yl]benzoate (28d). According to the method described for derivative **28a**, transformation of **16** (20 mg, 0.06 mmol) yielded 22 mg (95%) of a white solid. HPLC purity: 96.9%. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 7.3 Hz), 1.50−1.62 (2H, m), 1.65−1.83 (4H, m), 2.10−2.22 (2H, m), 3.95 (3H, s), 4.11 (2H, q, J = 7.3 Hz), 4.30−4.41 (1H, m), 4.50 (1H, d, J = 6.0 Hz), 7.83 (1H, s), 8.07−8.13 (4H, m). HRMS (ESI): m/z calcd for $C_{21}H_{24}N_3O_3S$ $(M + H)^+$ 398.1533, found 398.1529.

2-(Cyclopentylamino)-3-ethyl-7-pyridin-3-ylthieno[3,2-*d*]-pyrimidin-4(3*H*)-one (28e). According to the method described for derivative **28a**, transformation of **16** (20 mg, 0.06 mmol) yielded 18 mg (90%) of a white solid. HPLC purity: 99.3%. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 7.3 Hz), 1.50−1.62 (2H, m), 1.64−1.80 (4H, m), 2.10−2.21 (2H, m), 4.12 (2H, q, J = 7.3 Hz), 4.34−4.42 (1H, m), 4.51 (1H, d, J = 5.5 Hz), 7.36 (1H, dd, J = 5.0, 7.8 Hz), 7.83 (1H, s), 8.27 (1H, ddd, J = 2.0, 2.0, 7.8 Hz), 8.57 (1H, dd, J = 2.0, 5.0 Hz), 8.78 (1H, d, J = 2.0 Hz). HRMS (ESI): m/z calcd for $C_{18}H_{21}N_4OS$ $(M + H)^+$ 341.1431, found 341.1429.

2-(Cyclopentylamino)-3-ethyl-7-[4-(morpholinomethyl)-phenyl]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (29a). To a solution of **16** (300 mg, 0.88 mmol) in 1,4-dioxane (3 mL) were added 4-([4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]morpholine (266 mg, 0.88 mmol) and tripotassium phosphate (186 mg, 0.88 mmol). Tetrakis(triphenylphosphine)palladium (1013 mg, 0.88 mmol) was added under an argon atmosphere and the resulting solution was stirred at reflux for 14 h. The solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 245 mg (64%) of a white solid. HPLC purity: 95.1%. ^{1}H NMR ($CDCl_3$): δ 1.33−1.37 (3H, m), 1.56−1.76 (6H, m), 2.14−2.20 (2H, m), 2.49 (4H, br s), 3.54 (2H, s), 3.73 (4H, t, J = 4.6 Hz), 4.11 (2H, q, J = 6.0 Hz), 4.38 (1H, m), 4.51 (1H, br d, J = 5.0 Hz), 7.38 (2H, d, J = 7.8 Hz), 7.76 (1H, s), 7.97 (2H, d, J = 7.8 Hz). HRMS (ESI): m/z calcd for $C_{24}H_{31}N_4O_2S$ $(M + H)^+$ 439.2162, found 439.2164.

2-(Cyclopentylamino)-3-ethyl-7-(6-morpholinopyridin-3-yl)-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (29b). To a solution of **16** (63 mg, 0.184 mmol) in 1,4-dioxane (0.61 mL) were added 4-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridinyl]morpholine (64.1 mg, 0.221 mmol) and tripotassium phosphate (78.1 mg, 0.368 mmol). Tetrakis(triphenylphosphine)palladium (10.6 mg, 0.0072 mmol) was added under an argon atmosphere and the resulting solution was stirred at 90 °C for 14 h. The solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 21.9 mg (28%) of a white solid. HPLC purity: 97.8%. ^{1}H NMR (DMSO-d_6): δ 1.15 (3H, t, J = 6.9 Hz), 1.56−1.73 (6H, m), 1.96−2.00 (2H, m), 3.49 (4H, t, J = 5.0 Hz), 3.71 (4H, t, J = 5.0 Hz), 4.14 (2H, q, J = 6.9 Hz), 4.30 (1H, m), 6.75 (1H, d, J = 6.0 Hz), 6.90 (1H, d, J = 8.7 Hz), 8.28 (1H, dd, J = 2.3, 8.7 Hz), 8.85 (1H, d, J = 2.3 Hz). HRMS (ESI): m/z calcd for $C_{22}H_{28}N_5O_2S$ $(M + H)^+$ 426.1958, found 426.1959.

***tert*-Butyl 4-(2-(Cyclopentylamino)-3-ethyl-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-7-yl)-3-oxopiperazine-1-carboxylate (30).** To a solution of **16** (995 mg, 2.91 mmol) in 1,4-dioxane (7.28 mL) were added tripotassium phosphate (1.85 g, 8.73 mmol), *N*,*N*-dimethylethylenediamine (0.25 mL, 2.33 mmol), and *tert*-butyl 3-oxopiperazine-1-carboxylate (640 mg, 3.20 mmol). Copper(I) iodide (333 mg, 1.75 mmol) was added under an argon atmosphere and the resulting solution was refluxed for 14 h. The solution was filtered through a Celite pad, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield 788 mg (59%) of a yellow solid. HPLC purity: 98.8%. ^{1}H NMR ($CDCl_3$): 1.32 (3H, t, J = 7.3 Hz), 1.50 (9H, s), 1.47−1.56 (2H, m), 1.63−1.77 (4H, m), 2.05−2.12 (2H, m), 3.80−3.83 (2H, m), 3.94 (2H, br s), 4.06 (2H, q, J = 7.3 Hz), 4.23−4.31 (1H, m), 4.28 (2H, s), 4.49 (1H, d, J = 5.5 Hz), 7.69 (1H, s). HRMS (ESI): m/z calcd for $C_{22}H_{32}N_5O_4S$ $(M + H)^+$ 462.2097, found 462.2162.

Modeling of the Phosphodiesterase 7 Protein−5 Structure. The substructure of **5** (2-amino-3-methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one) was docked into the PDE7A catalytic site using the Glide SP docking protocol. An appropriate model with spaces for the substituents was obtained. The substituents were then manually added to the model structure. The torsion angles were adjusted by visual inspection to place the substituents in a reasonable orientation.

PDE Activity Assay. Human PDE7A1 and PDE4B1 were overexpressed in insect cells. The enzymatic activity of PDE7A1 was monitored by measuring the hydrolysis of [^{3}H]cAMP to [^{3}H]AMP. The reaction mixture (100 μL final volume) contained 10 nM [^{3}H]cAMP and 40 nM cAMP in the assay buffer (20 mM Tris, pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA). In the case of PDE4B1, 20 nM [^{3}H]cAMP and 480 nM cAMP were added to the assay buffer.

The enzymatic reactions were initiated by adding a solution containing the phosphodiesterase protein. The mixtures were incubated for 30 min at room temperature. The reactions were stopped by the addition of 50 μL of 25 mM $ZnCl_2$, followed by 50 μL of 10 mM $Ba(OH)_2$. The precipitates were filtered through GF-B glass fiber filters (Millipore), and the filters were washed several times with tap water. The filters were dried and counted in TopCount NTX (PerkinElmer).

IL-2 Assay. Freshly isolated mouse lymphocytes were resuspended in culture media composed of RPMI1640 medium supplemented with 10% FBS, 10 mM HEPES, 1 mM sodium pyruvate, 0.5 mM monothioglycerol, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were stimulated with anti-CD3 and anti-CD28 antibodies and incubated for 3 days at 37 °C and 5% CO_2. The supernatants were collected, and the IL-2 concentration was measured by ELISA.

Crystallography. The PDE7A1 coding region of amino acids 130−482 was subcloned into vector pET32a. The plasmid pET32-PDE7A1-130−482 was transformed into *Escherichia coli* strain BL21 (codonplus) for overexpression. *E. coli* cells were grown in LB medium at 37 °C to absorption A_{600} = 0.6, and then 0.1 mM isopropyl β-D-thiogalactopyranoside was added for further growth at 15 °C for 24 h. The catalytic domain of PDE7A1 was purified using three chromatographic columns of nickel−nitrilotriacetic acid, Q-Sepharose, and Superdex 200. The PDE7A1−**24** complex was crystallized by the hanging drop method at 4 °C. The protein drops contained 2 μL of PDE7A1−**24** complex and 2 μL of well buffer containing 0.7−0.9 M $(NH_4)_2SO_4$, 3 mM β-mercaptoethanol, 10 mM EDTA, 10 mM $MgCl_2$, and 0.1 M Tris·HCl at pH 7.5. The well buffer plus 20% glycerol was used as the cryosolvent for freezing the crystals in liquid nitrogen. Data were collected at 100 K using a Micromax 007 generator and an R-AXIS VII image plate detector (Rigaku MSC). The data were integrated and reduced with HKL2000. Structures were determined by molecular replacement using the structure of the PDE7A1 catalytic domain (PDB ID 1ZKL[21]) as a starting model for rigid body refinement, with REFMAC as implemented in CCP4. The model was rebuilt manually with Coot and completed using iterative rounds of refinement and rebuilding. The coordinates and structural factors have been deposited into the Protein Data Bank with the accession code 4PM0.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Proposed binding models of **6** and **7**, full PDE selectivity profile of **30**, and physicochemical and pharmacokinetic profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

dppf, 1,1′-bis(diphenylphosphino)ferrocene; FBS, fetal bovine serum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; IL-2, interleukin 2; LE, ligand efficiency; NMM, *N*-methylmorpholine; Pin, pinacolate; PDE, phosphodiesterase; SAR, structure−activity relationship; WSC, water-soluble carbodiimide

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