Journal of Medicinal Chemistry

Article

Discovery of Potent and Orally Active Lipoprotein-Associated Phospholipase A (Lp-PLA) Inhibitors as a Potential Therapy for Diabetic Macular Edema

Xinde Chen, Kai Wang, Wenwei Xu, Quanxin Ma, Minli Chen, Lili Du, Mingguang Mo, Yi-Ping Wang, and Jianhua Shen

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01930 • Publication Date (Web): 29 Feb 2016

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Potent and Orally Active Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2) Inhibitors as a Potential Therapy for Diabetic Macular Edema

Xinde Chen[1†], Kai Wang[1†], Wenwei Xu[1], Quanxin Ma[2], Minli Chen[2], Lili Du[1], Mingguang Mo[1], Yiping Wang[1]*, Jianhua Shen[1]*

[1] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

[2] Laboratory Animal Research Center, Zhejiang Chinese Medical University, Hangzhou 310053, China

ABSTRACT:

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is considered to be a promising therapeutic target for several inflammation-associated diseases. Herein, we describe the discovery of a series of pyrimidone derivatives as Lp-PLA_2 inhibitors. Systematic structural modifications led to the identification of several pyrimidone compounds with promising *in vitro* inhibitory potency and pharmacokinetic properties. Compound **14c**, selected for *in vivo* evaluation, demonstrated decent pharmacokinetic profiles and robust inhibitory potency against Lp-PLA_2 in Sprague-Dawley (SD) rats. Furthermore, **14c** significantly inhibited retinal thickening in STZ-induced diabetic SD rats as a model of diabetic macular edema (DME) after oral dosing for 4 weeks. Taken together, these results suggested that **14c** can serve as a valuable lead in the search for new Lp-PLA_2 inhibitors for prevention and/or treatment of DME.

INTRODUCTION

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a vascular specific enzyme that circulates mainly with low-density lipoprotein in human plasma.[1] It is produced by inflammatory cells,[1-3] and is able to hydrolyze oxidized modified phosphatidylcholine to generate two products, oxidized free fatty acids and lysophosphatidylcholine (lyso-PC),[4] both products are pro-inflammatory mediators and are considered to promote inflammatory responses.[5, 6] Extensive studies have shown that Lp-PLA_2 plays a key role in the development and progression of atherosclerosis,[7-11] and Lp-PLA_2 has long been considered a promising therapeutic target for atherosclerosis. Additionally, it was reported that inhibition of Lp-PLA_2 had beneficial effects on the functional integrity of the blood−brain barrier (BBB) in a diabetic and hypercholesterolemia porcine model,[12] and another study demonstrated that inhibition of Lp-PLA_2 might be protective against the hyperglycemia-related compromise of the blood−retinal barrier (BRB) in Brown Norway rats.[13] As a result of these observations, inhibition of Lp-PLA_2 has been suggested to be a potential therapeutic target for neurodegenerative diseases and diabetic macular edema (DME).

Unlike atherosclerosis and well-known neurodegenerative diseases, DME receives less attention by researchers in the pharmaceutical industry. However, it is a severe diabetic eye complication, and is the leading cause of visual loss in diabetic patients.[14] It occurs as a direct consequence of disruption of the BRB, which leads to increased accumulation of fluid in the macular.[15] Currently available pharmacotherapies for DME

are anti-VEGF drugs and corticosteroids, both which are delivered by somewhat risky and inconvenient intravitreal injection. Additionally, both anti-VEGF drugs and corticosteroids therapies have a percentage of nonresponders, and are associated with risks of visual loss.[16] Therefore, it is obvious that there is an urgent need to further develop riskless and effective drug candidates for DME treatment. Most recently, a phase-II trial revealed that oral administration of darapladib (a Lp-PLA_2 inhibitor) was safe and well tolerated, and demonstrated modest improvements in macular edema and vision acuity in center-involved DME patients.[17] These results further indicated that Lp-PLA_2 inhibitors might serve as potential therapeutic agents for the treatment of DME.

Several Lp-PLA_2 inhibitors have been discovered over the past decade (Figure 1), and can be separated into two categories based on their structures: non-pyrimidone compounds, and pyrimidone derivatives. Non-pyrimidone compounds include β-lactams,[18] oximes,[19, 20] amides of xanthurenic acid,[21, 22] and carbamates,[23] however, none of these compounds were reported to have efficacy *in vivo*. Pyrimidone derivatives include the compound darapladib, which was developed by GSK.[24] Although darapladib is the most advanced Lp-PLA_2 inhibitor,[25] it suffers from rather high lipophilicity (clogP, 8.33) and molecular weight (MW, 666.78),[26] and it missed its `primary end points in two phase-III trials focusing on coronary heart disease.[27, 28] Examination of the patent literatures suggested that GSK's researchers have shifted their efforts away from darapladib analogues in favor of a series of pyrimidone compounds with simplified structures, as exemplified by pyrimidone derivatives 1–3.[29-36]

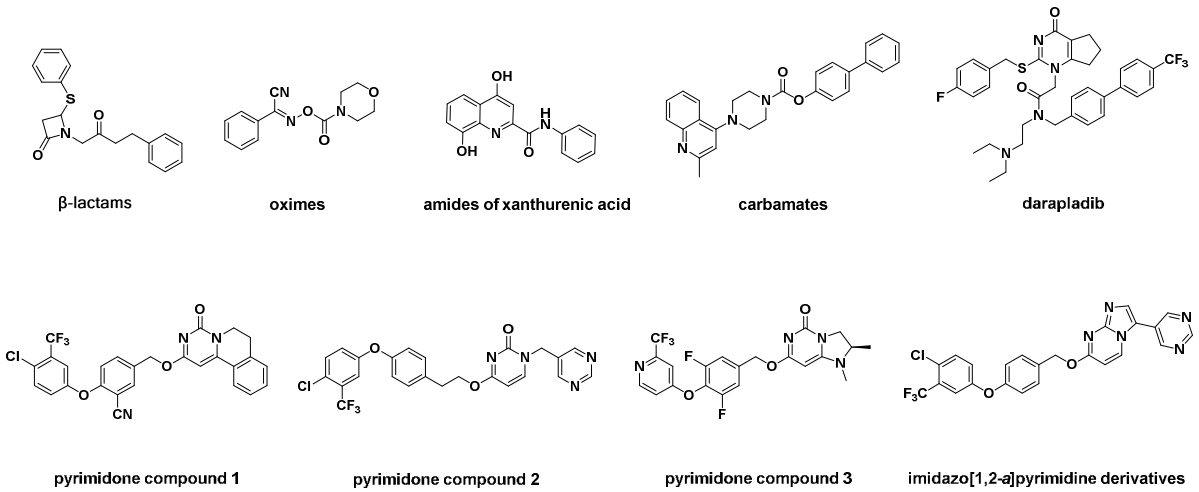


Figure 1. Representative structures of Lp-PLA_2 inhibitors

Our research group has focused on the discovery of Lp-PLA_2 inhibitors for many years. We previously reported a series of imidazole and triazole derivatives as analogues of darapladib.[37, 38] Very recently, we discovered a novel series of imidazo[1,2-*a*]pyrimidine derivatives (Figure 1), which have favorable pharmacokinetic properties and demonstrated robust inhibitory efficacy against Lp-PLA_2 *in vivo*.[39] However, given the high attrition rate of drug candidates, the discovery of additional candidates with different chemotypes is still needed.

Inspired by the excellent results of the imidazo[1,2-*a*]pyrimidine derivatives, we then concentrated our attention on the discovery of novel Lp-PLA_2 inhibitors with simplified structures. As part of our continued efforts, we describe here the design of a series of Lp-PLA_2 inhibitors based on a pyrimidone scaffold (Figure 2). On the basis of the structure−activity relationship (SAR) results of our previously reported imidazo[1,2-*a*]pyrimidine derivatives, and assuming that the left hand side of the newly designed pyrimidone compounds adopt a similar binding mode to that of the

imidazo[1,2-*a*]pyrimidine derivatives, we suggested that subtle changes in the left hand side of the pyrimidone compounds would also cause a large shift in potency similar to the imidazo[1,2-*a*]pyrimidine derivatives. Given the challenges of aligning potency and pharmacokinetic properties in a drug candidate, we expected to further define another structural region so that inhibitory potency and pharmacokinetic properties could be modulated. After careful analysis of the structure, we opted to focus our attention on the right hand side of the structure (R^1 and R^2).

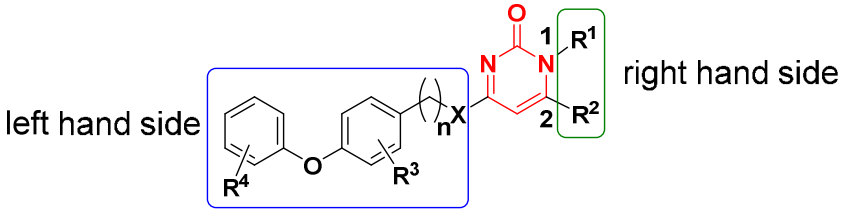


Figure 2. General structure of the pyrimidone derivatives

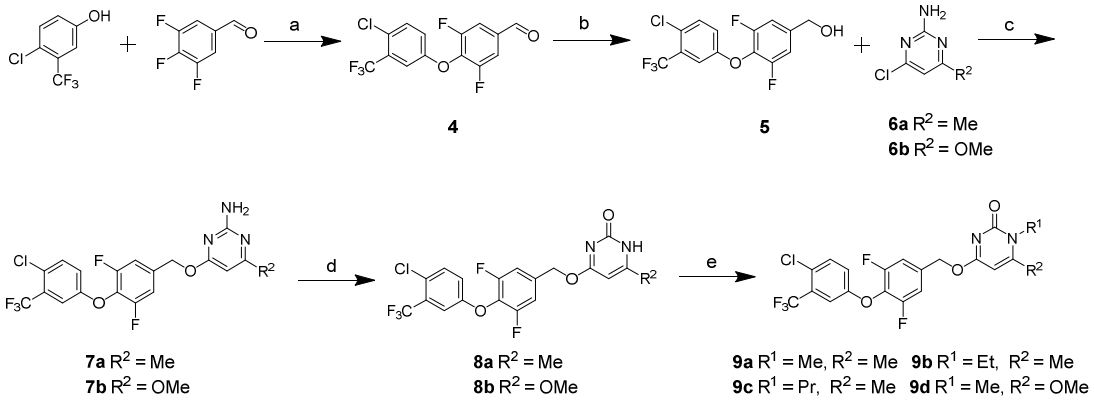
Herein, we describe the design, synthesis, and systematic structural modifications of the pyrimidone derivatives as Lp-PLA_2 inhibitors. These efforts resulted in the identification of compound **14c**, which displayed significant efficacy in a rat model of DME.

CHEMISTRY

The synthesis of derivatives **9a–d** is summarized in Scheme 1. A nucleophilic substitution reaction between 4-chloro-3-(trifluoromethyl)phenol and 3,4,5-trifluorobenzaldehyde in the presence of K_2CO_3 yielded intermediate **4**, and subsequent reduction of **4** using $NaBH_4$ provided intermediate **5**. A nucleophilic substitution reaction between **5** and **6a–b** in the presence of NaH gave intermediates **7a–**

b, which were converted to **8a–b** using $NaNO_2$. Compounds **9a–d** were obtained by substitution of **8a–b** with R^1I using Cs_2CO_3 as a base.

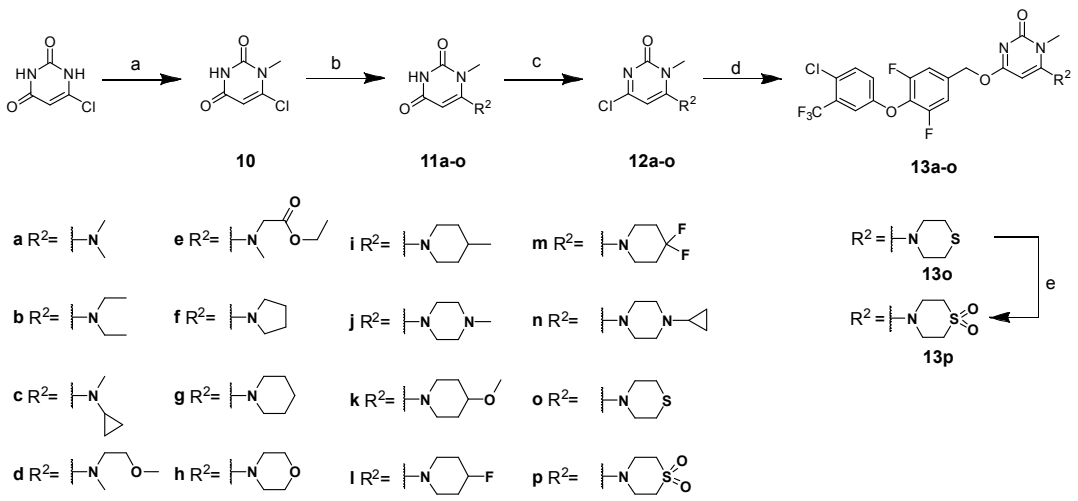
Scheme 1. **Synthesis of Compounds 9a−d**[a]



[a]Reagents and conditions: (a) K_2CO_3, DMF, 120 °C, 2 h; (b) $NaBH_4$, EtOH, rt; (c) NaH, THF; (d) $NaNO_2$, HAc,rt; (e)) R^1I, DMF, Cs_2CO_3.

The synthesis of compounds **13a–p** is outlined in Scheme 2. Methylation of 6-chlorouracil using CH_3I provided intermediate **10**. A nucleophilic substitution reaction between intermediate **10** and R^2H gave intermediates **11a–p**, which were converted to chlorine-substituted intermediates **12a–p** using $POCl_3$. Compounds 13**a–p** were obtained by treating **12a–p** with intermediate **4** through a nucleophilic substitution reaction. Morpholine derivatives **14a–e** were synthesized using a method similar to that described for compounds **13a**–**p** starting from intermediate **12h** and the corresponding benzyl alcohols (synthesis according to reference[39]).

Scheme 2. **Synthesis of Compounds 13a−p**[a]



[a]Reagents and conditions: (a) CH_3I, K_2CO_3, DMSO, rt; (b) R^2H, EtOH, 70 °C; (c) $POCl_3$, 70 °C; (d) NaH, THF, rt; (e) *m*-CPBA, DCM, rt.

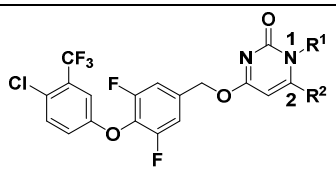
RESULT AND DISCUSSION

SAR, Metabolic Stability, and Caco-2 Permeability. Compounds were first evaluated for their inhibitory activity against recombinant human Lp-PLA_2 (rhLp-PLA_2) at two concentrations (100 nM and 10 nM) *in vitro*, and rhLp-PLA_2 activity was measured using 2-thio-PAF as the substrate. IC_{50} values against rhLp-PLA_2 for those compounds with an acceptable inhibitory activity (>50% at 10 nM) in initial assessments were tested to confirm their inhibitory potency. Compounds with acceptable potency were also evaluated for their metabolic stabilities in human and rat liver S9 fractions, and Caco-2 permeability tests were conducted to assess the penetration properties of those compounds with favorable potency and metabolic stabilities.

Our initial SAR efforts were directed toward the introduction of substituents at the

1-position and 2-position of the pyrimidone moiety to examine their tolerability for the potency (Table 1). Interestingly, the first prepared compound, **9a**, with methyl at the 1- and 2-positions displayed single digit nanomolar potency. Replacement of the methyl group at the 1-position with bulkier substituents, such as ethyl (**9b**) or propyl (**9c**), resulted in a decrease in potency, whereas introduction of a bulkier group to the 2-position, such as methoxy (**9d**), dimethylamine (**13a**) or diethylamine (**13b**), resulted in comparable potency with the methyl derivative **9a**. These results suggested that the 2-position of the pyrimidone moiety might possess great tolerability for a variety of substituents. Therefore we focused our structural modifications at this position. Given the excellent potency and synthetic accessibility of the amine derivatives **13a** and **13b**, we sought to further explore the inhibitory potency and pharmacokinetic properties of the amine series.

Table 1. Preliminary SAR of the R^1 and R^2 substituents



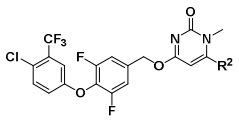
compd	R^1	R^2	% inhibition against rhLp-PLA_2 100 nM	% inhibition against rhLp-PLA_2 10 nM	IC_{50} （nM）
9a	Me	Me	98	81	2.6
9b	Et	Me	93	56	8.0
9c	Pr	Me	72	41	
9d	Me	OMe	97	75	3.7
13a	Me	$N(Me)_2$	90	88	3.9
13b	Me	$N(Et)_2$	88	77	4.2
darapladib					0.7[a]

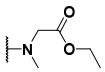
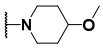
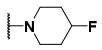
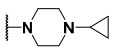
[a]Reported IC_{50} = 0.25 nM.

To obtain a preliminary understanding of the pharmacokinetic properties of this amine series, the metabolic stabilities of **13a** and **13b** were evaluated. As shown in Table 2, although **13a** and **13b** both displayed a low intrinsic clearance in human liver S9 fractions, a high and modest intrinsic clearance in rat liver S9 fractions was observed for **13a** and **13b**, respectively. Therefore, further structural modifications of these compounds were aimed at improving their metabolic stabilities in rat liver S9 fractions. We

postulated that the poor metabolic stabilities of **13a** and **13b** might have resulted from rapid N-dealkylation of the dimethylamine and diethylamine groups. Cyclopropyl was then introduced to replace one of the methyl groups of the dimethylamine in **13a**, the resulting compound **13c** displayed unexpected higher intrinsic clearance when compared with **13a**. More polar functional groups were also introduced to evaluate their effect on metabolic stabilities, the resulting methoxyethylamine **13d** and ethyl ester **13e** both maintained favorable potency, but displayed rather different metabolic stabilities in rat liver S9 fractions. Compound **13d** with a methoxyethylamine group displayed rather poor stability in rat liver S9 fractions, whereas ethyl ester **13e** was quite stable in both human and rat liver S9 fractions. Additionally, **13e** displayed modest permeability, with a P_{app} value of 0.32×10^{-6} cm/s (apical side to basolateral side, A to B) and an efflux ratio of 1.19 in a Caco-2 permeability assay.

Table 2. SAR, Metabolic Stability, and Caco-2 Permeability of Analogues 13a–n



compd	R^2	% inhibition against rhLp-PLA$_2$		IC_{50} (nM)	liver S9 stability (mL/min/kg)		Caco-2 permeability	
		100 nM	10 nM		human	rat	A to B (10^{-6} cm/s)	efflux ratio
13a		90	88	3.9	5.04	82.80	0.65	1.57
13b		88	77	4.2	9.87	41.90		
13c		100	88	4.0	0.00	123		
13d		100	93	1.2	25.5	107		
13e		100	96	1.1	9.68	13.8	0.32	1.19
13f		85	48		0.00	3.11	0.08	12.40
13g		94	57	8.3	5.69	21.80	0.01	32.30
13h		100	89	1.6	4.09	25.40	1.39	0.77
13i		15						
13j		100	77	3.5	65.5	1138		
13k		100	88	1.1	20.90	184		
13l		100	89	2.2	1.49	5.20	0.28	1.90
13m		96	76	4.6	0.00	23.6	0.09	0.61
13n		97	83	1.8	3.26	13.7	0.43	0.60
13o		90	83	1.8	49.1	1198		
13p		99	91	1.7	57.5	83.4		

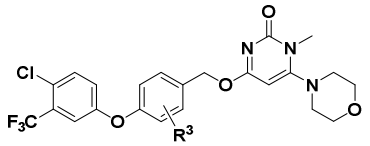
In a parallel strategy to improve the metabolic stabilities, several cyclic amines were introduced as cyclic substituents often display better metabolic stabilities when compared with noncyclic substituents. As shown in Table 2, both the pyrrolidine analogue **13f** and the piperidine analogue **13g** were rather stable in human and rat liver S9 fractions, indicating that cyclic substituents at the 2-position actually had beneficial effects on the metabolic stabilities of these pyrimidone analogues. However, **13f** and **13g** demonstrated somewhat reduced potency, and unexpected low permeability with a high efflux ratio of 12.4 and 32.3, respectively. A real breakthrough was made by the morpholine analogue **13h**, which showed increased inhibitory potency and similar metabolic stability, and gave a significant improvement in permeability (efflux ratio, 0.77) when compared with the piperidine analogue **13g**. Thus, we hypothesized that introduction of hydrogen-bond acceptor(s) to the cyclic substituents at the 2-position was beneficial for both inhibitory potency and permeability. The beneficial effect of hydrogen-bond acceptor(s) on potency was further verified by the distinctly different potency of compounds **13i** and **13j**. Compound **13j** with a 1-methylpiperazine group demonstrated comparable potency with the morpholine analogue **13h**, whereas the 4-methylpiperidine analogue **13i** was almost inactive. On the basis of these results, we therefore undertook a systematic exploration of a series of cyclic substituents containing hydrogen-bond acceptor(s).

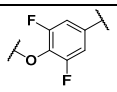
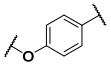
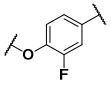
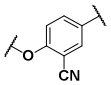
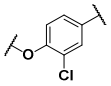
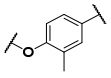
As shown in Table 2, replacement of the morpholine in **13h** with a 4-methoxypiperidine group (**13k**) resulted in compound that displayed a rather high intrinsic clearance in rat liver S9 fractions. Introduction of 4-fluoropiperidine (**13l**) or

4,4-difluoropiperidine (**13m**) resulted in compounds with excellent potency and metabolic stabilities, but the Caco-2 permeability of these two compounds was relatively low. The 1-methylpiperazine analogue **13j** was also rather unstable in both human and rat liver S9 fractions. Cyclopropyl was introduced to replace the methyl of 1-methylpiperazine in **13j** to block the possible N-demethylation metabolic pathway. The resulting compound **13n** turned out to be quite stable, and the Caco-2 permeability of this compound was modest. Replacement of the oxygen atom of the morpholine in **13h** with a sulfur atom resulted in thiomorpholine **13o**, although this compound also possessed excellent inhibitory potency, it was cleared quickly in both human and rat liver S9 fractions. Assuming that the sulfur atom can be oxidized to sulfoxide and sulphone, sulphone **13p** was then prepared in an effort to block this hypothetical metabolic pathway, however, **13p** also showed relatively high intrinsic clearance in both human and rat liver S9 fractions.

On the basis of its excellent inhibitory potency against rhLp-PLA_2, low intrinsic clearance, and favorable Caco-2 permeability, compound **13h** was selected for further optimization by investigating the left hand side of the structure. Similar to that described in our previously reported imidazo[1,2-*a*]pyrimidine derivatives, most of the structural modifications of the linker and the terminal benzene ring resulted in different degrees of reduction in potency (details in Supporting Information), therefore we quickly focused our attention on the exploration of the central benzene ring (Table 3). To our delight, compounds with the central benzene ring as benzene (**14a**) or monofluoro-benzene (**14b**) both displayed similar potency, metabolic stability, and Caco-2 permeability as compared with **13h**. The cyano-substituted analogue **14c**, which was somewhat unstable in rat liver S9 fractions, showed excellent inhibitory potency and the best permeability among these pyrimidone derivatives. The chlorine-substituted analogue **14d** and methyl-substituted analogue **14e** both showed a decrease in potency, indicating that a bulkier electron-withdrawing substituent or electron-donating substituent in the central benzene ring was detrimental for the inhibitory potency. These SAR results were similar to those described for our previously reported imidazo[1,2-*a*]pyrimidine series.[39]

Table 3. SAR, Metabolic Stability, and Caco-2 Permeability of Analogues 14a–e

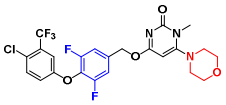
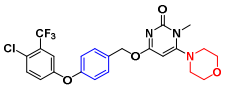
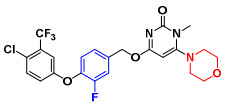
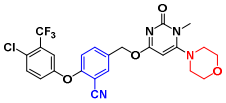
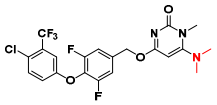
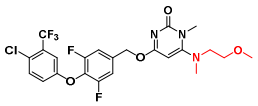
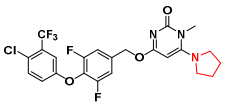
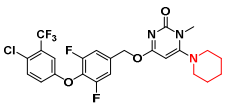


compd		% inhibition against rhLp-PLA$_2$		IC$_{50}$ （nM）	liver S9 stability (mL/min/kg)		Caco-2 permeability	
		100 nM	10 nM		human	rat	A to B (10^{-6} cm/s)	efflux ratio
13h		100	89	1.6	4.09	25.40	1.39	0.77
14a		100	81	1.9	3.89	13.3	2.05	1.09
14b		100	90	2.7	5.55	15.10	2.01	0.73
14c		100	92	2.2	19.7	94.8	6.54	1.03
14d		91	49					
14e		84	44					

On the basis of the results outlined in Tables 2 and 3, morpholine analogues **13h**, and **14a–c** were selected to further evaluate their potency against Lp-PLA$_2$ in human plasma. As shown in Table 4, these selected compounds demonstrated similar potency in human plasma when compared with their potency against rhLp-PLA$_2$. For example, at a concentration of 10 nM, compound **13h** displayed similar inhibition of 89% and 95% against rhLp-PLA$_2$ and Lp-PLA$_2$ in human plasma, respectively. These results illustrated that nonspecific binding had a negligible effect on the binding affinity between these

pyrimidone analogues and the Lp-PLA_2 enzyme in human plasma. The inhibitory potency against Lp-PLA_2 in rat plasma of these selected compounds was also evaluated before an *in vivo* assessment was conducted. As shown in Table 4, these morpholine analogues displayed somewhat different potency against Lp-PLA_2 in rat plasma. In particular, difluorine-substituted analogue **13h** (39% at 10 nM) had relatively lower potency in rat plasma when compared with analogues **14a–c** (**14a**, 72% at 10 nM; **14b**, 60% at 10 nM; **14c**, 94% at 10 nM). Among these morpholine analogues, cyano-substituted analogue **14c** displayed the best potency in rat plasma, and its inhibitory potency in rat plasma was further confirmed by determination of IC_{50} value as 1.0 nM. Driven by the different SAR results of the substituents on the central benzene ring in rat plasma, several compounds with different R^2 groups (**13a**, **13d**, **13f**, **13g**) were then selected to further investigate the SAR of R^2 in rat plasma (Table 4). Unlike the substituents on the central benzene ring, the SAR of R^2 in rat plasma was similar to that observed for rhLp-PLA_2, though the potencies of these compounds in rat plasma were relatively lower when compared with their potencies against rhLp-PLA_2.

Table 4. Inhibitory Activity against Lp-PLA$_2$ in Human and Rat Plasma

compd	structure	% inhibition in plasma			
		human		rat	
		10 nM	1 nM	100 nM	10 nM
13h		95	74	98	39
14a		92	61	100	72
14b		85	28	100	60
14c		92	67	100	94
13a		89	68	84	30
13d		90	56	92	28
13f		76	0	65	12
13g		68	0	54	4
darapladib		94	59	98	84

Pharmacokinetic Studies *in Vivo*. Compound **14c**, although somewhat unstable in rat liver S9 fractions, displayed the most promising inhibitory potency *in vitro*, and the best permeability in the Caco-2 assay, was selected to investigate its pharmacokinetic

properties in male SD rats. As shown in Table 5, after intravenous administration at a dose of 5 mg/kg, **14c** displayed a moderate clearance of 18.6 mL/min/kg, and a relatively large volume of distribution (9.1 L/kg). At an oral dose of 50 mg/kg, **14c** achieved good oral plasma exposure, with a peak plasma concentration (C_{max}) of 1879 ng/mL, an AUC value of 19.9 μg·h/mL, and a decent oral bioavailability of 45%. These PK values were all much higher than those of darapladib (C_{max}, 115 ng/mL; AUC, 1.3 μg·h/mL; F, 11%).

Table 5. *In Vivo* Pharmacokinetic Parameters in SD Rats of 14c and Darapladib[a]

compd	Pharmacokinetics (iv)				Pharmacokinetics (po)			
	$AUC_{0\text{-}24h}$ (μg·h/mL)	$t_{1/2}$ (h)	Cl (ml/min/kg)	V_{ss} (L/kg)	$AUC_{0\text{-}24h}$ (μg·h/mL)	C_{max} (ng/mL)	T_{max} (h)	F (%)
14c[b]	4.4	5.7	18.6	9.1	19.9	1879	4.6	45
darapladib[c]	1.2	3.3	121.0	34.4	1.3	115	4.2	11

[a]n = 5 animals/group, data are the mean values. [b]Dosed iv: administered at 5 mg/kg, vehicle was 5% DMSO/5% Tween-80 in saline; dosed po: administered at 50 mg/kg, vehicle was 0.5% carboxymethylcellulose sodium. [c]Dosed iv: administered at 10 mg/kg in tartrate salt form, vehicle was water; dosed po: administered at 50 mg/kg in tartrate salt form, vehicle was water.

Assessment of the Inhibitory Activity of Lp-PLA_2 *in Vivo*. In view of the promising inhibitory potency *in vitro* and decent pharmacokinetic profiles *in vivo*, it came as no surprise that compound **14c** demonstrated robust inhibitory potency against

Lp-PLA_2 in male SD rats after oral administration (Figure 3). And it was worth mentioning that **14c** could still efficiently inhibit Lp-PLA_2 activity even at a relatively low dose of 3 mg/kg, and this activity was relatively better than that of darapladib, which was administered at a single oral dose of 25 mg/kg in the tartrate salt form.

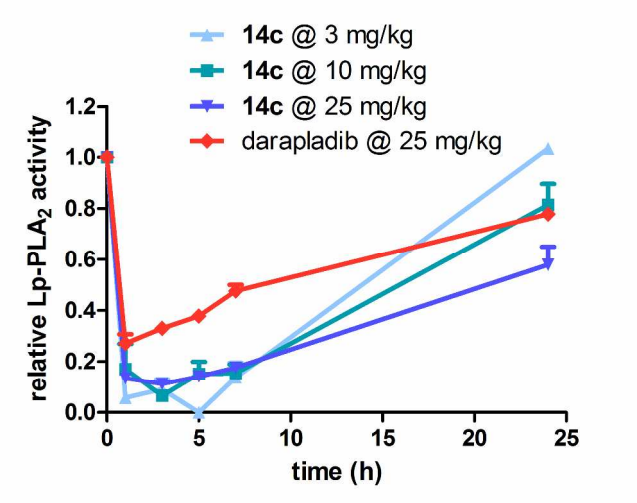


Figure 3. Relative activity of Lp-PLA_2 in the serum of SD rats after a single oral dose (n = 5). Error bars represent the SD. **14c** was formulated in 0.25% carboxymethylcellulose sodium, darapladib in the tartrate salt form was dissolved in water.

Effects of 14c on Retinal Thickening in STZ-induced Diabetic Rats. DME is characterized by retinal thickening, which is induced by the accumulation of fluid in the retinal layers, and it was reported that hyperglycemia would induce retinal thickening in STZ-induced diabetic rats.[40-42] To preliminary determine if these pyrimidone derivatives could display potential therapeutic effects for DME, compound **14c** was evaluated for its effects on retinal thickening in STZ-induced diabetic SD rats as a pathological model of DME with a relatively high oral dose of 25 mg/kg/day. As shown in Figure 4, diabetic

retinals were significantly thicker than control retinals, and a significant inhibitory effect was observed on retinal thickening in diabetic rats treated with **14c** after oral dosing for 4 weeks. Additionally, the inhibitory efficacy of **14c** on retinal thickening was achieved without significantly affect the metabolism of blood glucose and serum lipids (details in Supporting Information). Although the inhibitory efficacy on retinal thickening of **14c** was not differentiated from that of darapaldib in this animal model at a dose of 25 mg/kg/day, these preliminary positive results warrant comprehensive investigation of these pyrimidone derivatives in more animal studies including dose-response experiment to further understand their potential as therapeutic agents for DME.

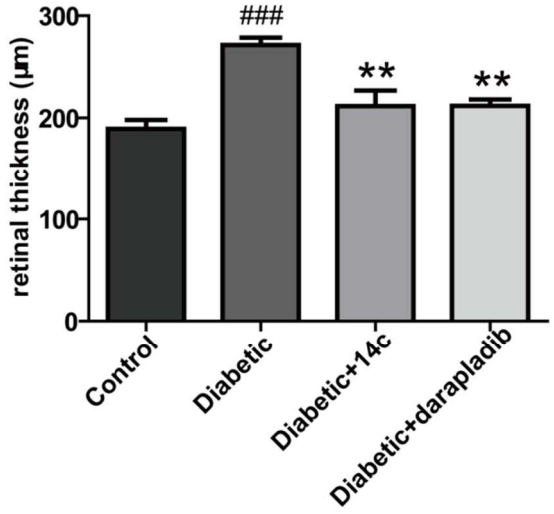


Figure 4. Effect of compound **14c** on retinal thickening in STZ-induced diabetic rats (n = 5, 25 mg/kg, po, 4 weeks). ###, $p<0.001$ vs normal control. **, $p<0.01$ vs diabetic control. Error bars represent SEM. One-way ANOVA statistical analysis was used. **14c** was formulated in 0.25% carboxymethylcellulose sodium, darapladib in the tartrate salt form was dissolved in water.

CONCLUSION

We have described the discovery of a series of pyrimidone derivatives as potent Lp-PLA_2 inhibitors. Our preliminary SAR explorations indicated that the 2-position of the pyrimidone moiety could tolerate a range of substitutions and thus suggested that inhibitory potency and pharmacokinetic properties might be modulated through structural modifications at this position. Our chemical efforts at this position first led to the identification of morpholine analogue **13h**, further structural modifications of the left hand side of the structure resulted in the identification of several morpholine derivatives with promising inhibitory potency and pharmacokinetic properties *in vitro*.

Compound **14c** selected for *in vivo* evaluation displayed decent pharmacokinetic profiles, and demonstrated robust inhibitory potency against Lp-PLA_2 in male SD rats after oral administration. Furthermore, **14c** significantly inhibited retinal thickening in STZ-induced diabetic SD rats as a model of DME after oral dosing for 4 weeks. Our preliminary results warrant further *in vivo* experiments with **14c** or other pyrimidone derivatives to fully understand their potential as therapeutic agents for DME.

EXPERIMENTAL SECTION

In Vitro **Assay to Measure the Inhibitory Activity of Lp-PLA_2.**[43] Activities against rhLp-PLA_2, human plasma, rat plasma, and mouse plasma Lp-PLA_2 were measured using 2-thio-PAF as the substrate. Briefly, 10 μL of the rhLp-PLA_2 enzyme (or plasma) and 10 μL of a DMSO solution of the compound were added to 0.1 mol/L Tris-HCl (pH 7.2)

containing 1 mmol/L EGTA, 50 μmol/L 2-thio-PAF, and 10 μL of 2 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid) in a total volume of 200 μL. The assay was carried out using a plate reader to obtain absorbance values at 414 nm every minute for 10 min. Percent inhibition was determined using the following equation:

$$\text{inhibition (\%)} = 1 - \frac{Vmax_{\text{compound}} - Vmax_{\text{blank}}}{Vmax_{\text{positive}} - Vmax_{\text{blank}}} \times 100\%$$

Vmax: slope of absorbance values for 10 min, calculated by MolecularDevice, SpectraMax M2e. The blank sample contained no rhLp-PLA_2 enzyme (or plasma) or test compound in assay buffer. The positive sample contained no test compound.

This study has been approved and supervised by Institutional Animal Care and Use Committee (IACUC), Shanghai Institute of Materia Medica, Chinese Academy of Sciences (IACUC Approval Number: SIMM-2014−08-WYP-18) and the Ethics Committee of Shanghai Xuhui Central Hospital.

Metabolic Stability Tests In human and Rat Liver S9 Fractions. The test compound was dissolved in DMSO and diluted to the desired concentration with an aqueous solution of 0.1% BSA. Liver S9 (0.33 mg/mL; pooled human liver S9 purchased from Celsis In Vitro Technologies; Wistar rat liver S9 purchased from Research Institute for Liver Diseases), test compounds (0.1 μM), $MgCl_2$ (5.0 mM), BSA (0.005%), and NADPH (1.0 mM) in Tris buffer (0.1 M, pH 7.4) were incubated in a 96-well plate at 37 °C. An aliquot was removed at each time point and the enzymatic reaction stopped by protein precipitation in cold methanol. Half-lives of the compounds in liver S9 fractions

were calculated based on the first-order rate constants that were measured. Intrinsic clearance was calculated using the following equation:

$$\mathrm{CL_{int}} = \frac{0.693}{in\ vitro\ \mathrm{t_{1/2}}} \times \frac{\text{mL of incubation}}{\text{mg of liver S9 protein}} \times \frac{\text{mg of liver S9 protein}}{\text{g of tissue}} \times \frac{\text{g of tissue}}{\text{kg of weight}}$$

Scaling factors for the human liver (145 mg of S9 protein/g liver and 24.3 g of liver/kg body weight) and rat liver (179 mg of S9 protein/g liver and 40 g of liver/kg body weight) were employed in this calculation.

Caco-2 Permeability Assay. Caco-2 cells were obtained from the ATCC (Cat#HTB-37) and maintained in Dulbecco's modified Eagle's Medium containing 10% fetal bovine serum, 1% glutamine, 1% nonessential amino acids, 100 μg/mL streptomycin, and 100 μg/mL penicillin. Caco-2 cells were cultured at 37 °C in a 5% CO_2 and 90% relative humidity environment. Caco-2 cells were passaged every 7 days at a ratio of 1:10. Cells were used between passages 30 and 40. After 21 days of culture, the integrity of the cell monolayer was verified by measuring the transepithelial electrical resistance (TEER). Drug transport from the apical side to the basolateral side (A-B) and from the basolateral side to the apical side was measured simultaneously under the same condition. Propranolol and Nadolol were used as the hypertonic and hypotonic control, respectively. Digoxin was used as the positive control for Pgp-mediated drug efflux. In brief, the method was as follows. After washing the monolayer with HBSS three times, the compounds were diluted and added to the appropriate well (pH 6.8 for apical side and pH 7.4 for basolateral side). The plate was incubated at 37 °C for 95 min. Samples were collected from the donor side at 5 and 95 min, and from the receiver side at 35 and 95

min post-incubation. The concentration of samples was measured by LC-MS/MS. The P_{app} was calculated from the following equation:

$$P_{app} = \frac{V_A}{S_A \times T} \times \frac{[drug]_{acceptor}}{[drug]_{initial\ donor}}$$

Where V_A is the volume of the acceptor well, S_A is the surface area of the membrane, T is the total transport time, $[drug]_{acceptor}$ is the drug level at the acceptor side, and $[drug]_{initial\ donor}$ is the drug level at the donor side at $T = 0$.

Animals. Male Sprague−Dawley (SD) rats were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Animal experiments were approved by Animal Care and Use Committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and Zhejiang Chinese Medicine University Animal Care Committee.

Pharmacokinetic Studies. Test compounds were subjected to pharmacokinetic studies on male SD rats with five animals in each group. Compound **14c** was formulated for intravenous (5 mg/kg) and oral (50 mg/kg) administrations in 5% DMSO, 5% Tween-80 in saline and 0.5% carboxymethylcellulose sodium, respectively. Darapladib was formulated for intravenous (10 mg/kg) and oral (50 mg/kg) administrations in water. Serial specimens were collected at predose, 5, 15 and 30 min and 1, 3, 5, 7, and 24 h following intravenous administration and at predose, 0.5, 1, 2, 3, 5, 7, and 24 h following oral administration. The concentration of compounds in the plasma samples was determined with a liquid chromatography−mass spectrometry. Pharmacokinetic parameters were calculated from the mean serum concentration by noncompartmental

analyses using DAS software 2.1.1.

Assay to Measure the Inhibitory Activity of Lp-PLA$_2$ *in Vivo*. A group of five male SD rats were fasted overnight, and administered test compounds by gavage. **14c** were formulated in 0.25% carboxymethylcellulose sodium, darapladib in the tartrate salt form was dissolved in water. Blood samples were drawn predose as well as 1, 3, 5, 7 and 24 h after administration to measure Lp-PLA$_2$ activity in serum. Lp-PLA$_2$ activity in the serum of SD rats was measured according to the method described for measurement of Lp-PLA$_2$ inhibitory activity *in vitro*.

Effects of 14c on Retinal Thickening in STZ-Induced Diabetic Rats. Male SD rats were induced to diabetic by intraperitoneal injection of streptozotocin (STZ, Sigma) in 10 mmol/L sodium citrate (PH 4.6) at 50 mg/kg after fasting for 16 h. Blood glucose was measured from blood samples 1 week after injection of STZ. Rats with blood glucose values >10 mmol/L were considered diabetic. Test compounds were subjected to studies on STZ-induced diabetic SD rats with five animals in each group, and were administered at an oral dose of 25 mg/kg/day for 4 weeks starting 1 week after injection of STZ. Compound **14c** were formulated in 0.25% carboxymethylcellulose sodium, darapladib in the tartrate salt form was dissolved in water. To determine the retinal thickness, retinal cryosections (8 μM) were prepared and stained with hematoxylin/eosin. Digitized images of the retina were captured at 400× magnification (Eclipse 80i, Nikon). The retinal thickness was defined from the internal limiting membrane to the external membrane, and was measured using Image-Pro plus 6.0.

Materials and Methods. All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Yields were not optimized. Microwave reactions were performed in a Biotage Initiator. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC400 or a Bruker AC500 NMR spectrometer using tetramethylsilane as an internal reference. Low-resolution mass spectra were determined on an Agilent liquid-chromatography mass spectrometer system that consisted of an Agilent 1260 infinity LC coupled to Agilent 6120 Quadrupole mass spectrometer (electrospray positive ionization; ESI). High-resolution mass spectra were conducted on a triple TOF 5600^{+} MS/MS system (AB Sciex, Concord, Ontario, Canada) in the positive ESI mode. The purity of test compounds was determined by HPLC (Agilent ChemStation, Agilent Eclipse XDB-C18, 5 μM, 4.6×150 mm, 30 °C, UV 240 nM, flow rate = 1.0 mL/min) with aqueous CH_3CN (50−90%) containing ammonium formate (10 mmol/L) for 25 min. All the assayed compounds possess ≥95% purity. Column chromatography was performed on silica gel (200−300 mesh), and preparative TLC was performed on HSGF 254 (0.4−0.5 mm thickness; Yantai Jiangyou Company, Yantai, Shangdong, China).

4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzaldehyde (4). A solution of 4-chloro-3-(trifluoromethyl)phenol (5.00 g, 25.51 mmol), 3,4,5-trifluorobenzaldehyde (3.16 mL, 28.03 mmol), and K_2CO_3 (4.58 g, 33.19 mmol) in DMF (50 mL) was heated at 120 °C for 2 h under nitrogen. Then, the solution was cooled

down and poured into ice water (150 mL), and extracted with ethyl acetate (80 mL × 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 20:1) to yield intermediate **4** as a yellow solid (8.13 g, 94%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.95 (s, 1H), 7.65–7.56 (m, 2H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 3.0 Hz, 1H), 7.06 (dd, *J* = 8.8, 2.9 Hz, 1H). MS (ESI): *m/z* 337 $[M+H]^+$.

(4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorophenyl)methanol (5). To a solution of **4** (4.00 g, 11.83 mmol) in ethanol (100 mL) was added $NaBH_4$ (438 mg, 11.83 mmol) at 0 °C, and the reaction was stirred at rt for 0.5 h. After the reaction was complete, the solvent was evaporated under reduced pressure, water was added to the residue, then extracted with ethyl acetate (100 mL × 2), washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 4:1) to yield intermediate **5** as a white solid (4.00 g, 99%).^{1}H NMR (400 MHz, chloroform-d) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.28 (d, *J* = 2.9 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 2H), 7.02 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.73 (d, *J* = 4.2 Hz, 2H). MS (ESI): *m/z* 321 $[M–H_2O+H]^+$.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methylpyrimidin-2-amine (7a). To a solution of **5** (4.00 g, 11.76 mmol) in anhydrous THF (100 mL) was added sodium hydride (1.41 g, 35.25 mmol, 60% in mineral oil) at 0 °C, **6a** (1.52 g, 10.58 mmol) was added after the mixture was stirred at 0 °C for 0.5 h, and the

mixture was stirred at rt overnight. Then, the reaction was quenched with NH_4Cl solution, extracted with ethyl acetate (100 mL × 3), washed with brine, dried over $NaSO_4$, filtered, and concentrated, the residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 1:1) to yield intermediate **7a** as a white solid (1.70 g, 32%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 3.0 Hz, 1H), 7.12–7.06 (m, 2H), 7.02 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.05 (s, 1H), 5.32 (s, 2H), 2.29 (s, 3H). MS (ESI): *m/z* 446 $[M+H]^+$.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methoxy pyrimidin-2-amine (7b). To a solution of **5** (2.00 g, 5.88 mmol) in anhydrous THF (80 mL) was added sodium hydride (0.70 g, 17.64 mmol, 60% in mineral oil) at 0 °C, **6b** (0.84 g, 5.29 mmol) was added after the mixture was stirred at 0 °C for 0.5 h, and the mixture was stirred at 70 °C overnight. Then, the reaction was quenched with NH_4Cl solution, extracted with ethyl acetate (80 mL × 3), washed with brine, dried over $NaSO_4$, filtered, and concentrated, the residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 1:1) to yield intermediate **7b** as a white solid (0.70 g, 30%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 2.9 Hz, 1H), 7.13–7.05 (m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.56 (s, 1H), 5.29 (s, 2H), 3.86 (s, 3H). MS (ESI): *m/z* 462 $[M+H]^+$.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methylp yrimidin-2(1*H*)-one (8a). To a solution of **7a** (250 mg, 0.56 mmol) in acetic acid (15 mL)

was added $NaNO_2$ (78 mg, 1.13 mmol) potionwise, the mixture was stirred at rt overnight. Then, the solvent was evaporated under reduced pressure, DMC (100 mL) was added to the residue, then washed with saturated $NaHCO_3$ solution (80 mL × 2),, dried over Na_2SO_4, filtered, and concentrated, the residue obtained was purified with column chromatography (DCM/MeOH = 40:1) to yield intermediate **8a** as a white solid (130 mg, 52%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 12.73 (s, 1H), 7.42 (d, *J* = 8.9 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.14–7.06 (m, 2H), 7.02 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.87 (s, 1H), 5.44 (s, 2H), 2.37 (s, 3H). MS (ESI): *m/z* 447 $[M+H]^+$.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methoxy pyrimidin-2(1*H*)-one (8b). Intermediate **8b** was obtained as a brown solid from **7b** using a method similar to that described for intermediate **8a** in 25% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 12.12 (s, 1H), 7.42 (d, *J* = 8.9 Hz, 1H), 7.30 (d, *J* = 2.9 Hz, 1H), 7.14 – 7.07 (m, 2H), 7.02 (dd, *J* = 8.8, 2.8 Hz, 1H), 5.39 (s, 2H), 5.37 (s, 1H), 3.93 (s, 3H). MS (ESI): *m/z* 463 $[M+H]^+$.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1,6-dimeth ylpyrimidin-2(1*H*)-one (9a). To a solution of **8a** (35 mg, 0.08 mmol) in acetone (3 mL) was added K_2CO_3 (23 mg, 0.17 mmol), CH_3I (25 μL, 0.40 mmol), the mixture was stirred at rt overnight. Then, the solvent was evaporated under reduced pressure, ethyl acetate (20 mL) was added to the residue, then washed with saturated brine, dried over Na_2SO_4, filtered, and concentrated, the residue obtained was purified with preparative TLC

(DCM/MeOH = 30:1) to yield **9a** as a white solid (25 mg, 69%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.44 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 3.0 Hz, 1H), 7.15–7.08 (m, 2H), 7.03 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.87 (s, 1H), 5.42 (s, 2H), 3.53 (s, 3H), 2.37 (s, 3H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 169.68, 157.67, 157.54, 155.87, 155.64 (dd, *J* = 252.6, 4.5 Hz, 2C), 135.24 (t, *J* = 8.2 Hz), 132.59, 129.93 (t, *J* = 15.4 Hz), 129.51 (q, *J* = 32.2 Hz), 125.91, 122.29 (q, *J* = 273.6 Hz), 119.29, 115.11 (q, *J* = 5.6 Hz), 111.98 (dd, *J* = 18.7, 4.2 Hz, 2C), 95.18, 66.17, 32.06, 20.70. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{20}H_{15}ClF_5N_2O_3$, 461.0686; found, 461.0686.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-ethyl-6-methylpyrimidin-2(1*H*)-one (9b). To a solution of **8a** (40 mg, 0.09 mmol) in DMF (2 mL) was added Cs_2CO_3 (175 mg, 0.54 mmol), CH_3CH_2I (72 μL, 0.90 mmol), the mixture was stirred at rt overnight. Then, the solution was poured into water (10 mL), and extracted with ethyl acetate (10 mL × 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated, the residue obtained was purified with preparative TLC (DCM/MeOH = 30:1) to yield **9b** as a white solid (14 mg, 69%). ^{1}H NMR (400 MHz, chloroform-*d*) ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.44 (d, *J* = 8.7 Hz, 1H), 7.32 (d, *J* = 2.8 Hz, 1H), 7.15–7.07 (m, 2H), 7.06–7.00 (m, 1H), 5.84 (s, 1H), 5.41 (s, 2H), 4.06 (q, *J* = 7.0 Hz, 2H), 2.40 (s, 3H), 1.35 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 169.64, 157.08, 156.97, 155.87, 155.65 (dd, *J* = 252.6, 4.2 Hz, 2C), 135.26 (t, *J* = 8.2 Hz), 132.59, 129.93 (t, *J* = 14.8 Hz), 129.52 (q, *J* = 32.0 Hz),

125.91, 122.29 (q, J = 273.5 Hz), 119.29, 115.12 (q, J = 5.4 Hz), 111.97 (dd, J = 18.7, 4.4 Hz, 2C), 95.38, 66.13, 40.38, 19.98, 13.62. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{21}H_{17}ClF_5N_2O_3$, 475.0842; found, 475.0842.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methyl-1-propylpyrimidin-2(1*H*)-one (9c). The title compoud was obtained as a white solid from **8a** and bromopropane using a method similar to that described for **9b** in 21% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 3.0 Hz, 1H), 7.13–7.06 (m, 2H), 7.01 (dd, J = 8.8, 2.9 Hz, 1H), 5.81 (s, 1H), 5.39 (s, 2H), 3.94–3.87 (m, 2H), 2.36 (s, 3H), 1.76 (dt, J = 15.6, 7.6 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 169.61, 157.20, 157.16, 155.89, 155.66 (dd, J = 253.6, 4.4 Hz, 2C), 135.30 (t, J = 8.2 Hz), 132.60, 129.95 (t, J = 15.0 Hz), 129.54 (q, J = 32.1 Hz), 125.91, 122.31 (q, J = 274.6 Hz), 119.31, 115.14 (q, J = 5.6 Hz), 111.98 (dd, J = 17.3, 5.4 Hz, 2C), 95.31, 66.13, 46.84, 21.69, 20.20, 11.24. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{22}H_{19}ClF_5N_2O_3$, 489.0999; found, 489.1027.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-methoxy-1-methylpyrimidin-2(1*H*)-one (9d). The title compoud was obtained as a white solid from **8a** and CH_3I using a method similar to that described for **9a** in 69% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 2.9 Hz, 1H), 7.14–7.06 (m, 2H), 7.01 (dd, J = 8.9, 3.0 Hz, 1H), 5.41 (s, 2H), 5.38 (s, 1H), 3.96 (s, 3H), 3.42 (s, 3H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 170.99, 164.90, 156.51, 155.87, 155.65 (dd, J = 255.0, 6.9 Hz, 2C), 135.38 (t, J = 8.2 Hz), 132.60, 129.92 (t, J = 15.0 Hz), 129.51 (q, J

= 32.0 Hz), 125.91, 122.30 (q, *J* = 273.5 Hz), 119.31, 115.09 (q, *J* = 5.4 Hz), 112.00 (dd, *J* = 18.6, 4.2 Hz, 2c), 73.71, 66.43, 57.07, 28.94. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{20}H_{15}ClF_5N_2O_4$, 477.0635; found, 477.0627.

6-Chloro-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (10). To a solution of 6-chlorouracil (7.00 g, 47.94 mmol) in DMSO (30 mL) was added K_2CO_3 (3.36 mg, 24.35 mmol), CH_3I (9.6 mL, 154.14 mmol), the mixture was stirred at rt for 3 h. Then, water (38 mL) was added to the mixture, the resulting solution was stirred at 0 °C for 3 h. The solid formed was collected by filtration to give **10** as a white solid (5.80g, 76%). ^1H NMR (400 MHz, chloroform-*d*) δ 9.17 (s, 1H), 5.92 (s, 1H), 3.55 (s, 3H). MS (ESI): *m/z* 161 $[M+H]^+$.

6-(Dimethylamino)-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (11a). To a solution of **10** (120 mg, 0.75 mmol) in EtOH (20 mL) was added $NaHCO_3$ (380 mg, 4.52 mmol), dimethylamine hydrochloride (370 mg, 4.54 mmol), the mixture was stirred at 70 °C for 0.5 h. Then, the solution was filtered, and concentrated, the residue obtained was purified with column chromatography (DCM/MeOH = 30:1) to yield intermediate **11a** as a white solid (100 mg, 79%). ^1H NMR (400 MHz, chloroform-*d*) δ 9.40 (s, 1H), 5.14 (s, 1H), 3.37 (s, 3H), 2.75 (s, 6H). MS (ESI): *m/z* 170 $[M+H]^+$.

6-(Diethylamino)-4-hydroxy-1-methylpyrimidin-2(1*H*)-one (11b). Intermediate **11b** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 89% yield. ^1H NMR (400 MHz, chloroform-*d*) δ 9.03 (s, 1H), 5.19 (s, 1H), 3.35 (s, 3H), 3.04 (q, *J* = 7.1 Hz, 4H), 1.13 (t, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 198 $[M+H]^+$.

6-(Cyclopropyl(methyl)amino)-4-hydroxy-1-methylpyrimidin-2(1*H*)-one (11c). Intermediate **11c** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 78% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.48 (s, 1H), 5.32 (s, 1H), 3.31 (s, 3H), 2.82 (s, 3H), 2.46 (tt, *J* = 6.8, 3.7 Hz, 1H), 0.80 (q, *J* = 6.6 Hz, 2H), 0.55 (dt, *J* = 6.7, 3.6 Hz, 2H). MS (ESI): *m/z* 196 $[M+H]^+$.

4-Hydroxy-6-((2-methoxyethyl)(methyl)amino)-1-methylpyrimidin-2(1*H*)-one (11d). Intermediate **11d** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 76% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.03 (s, 1H), 5.19 (s, 1H), 3.57 (t, *J* = 5.3 Hz, 2H), 3.37 (s, 3H), 3.34 (s, 3H), 3.18 (t, *J* = 5.3 Hz, 2H), 2.79 (s, 3H). MS (ESI): *m/z* 214 $[M+H]^+$.

Ethyl N-(6-hydroxy-3-methyl-2-oxo-2,3-dihydropyrimidin-4-yl)-N-methylglycinate (11e). Intermediate **11b** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 73% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.38 (s, 1H), 5.19 (s, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.74 (s, 2H), 3.38 (s, 3H), 2.85 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). MS (ESI): *m/z* 242 $[M+H]^+$.

4-Hydroxy-1-methyl-6-(pyrrolidin-1-yl)pyrimidin-2(1*H*)-one (11f). Intermediate **11f** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 91% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.36 (s, 1H), 5.04 (s, 1H), 3.39 (s, 3H), 3.31–3.25 (m, 4H), 2.00–1.96 (m, 4H). MS (ESI): *m/z* 196 $[M+H]^+$.

4-Hydroxy-1-methyl-6-(piperidin-1-yl)pyrimidin-2(1*H*)-one (11g). Intermediate

11g was obtained as a white solid in a manner similar to that described for intermediate **11a** in 88% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.24 (s, 1H), 5.15 (s, 1H), 3.35 (s, 3H), 2.90 (s, 4H), 1.76–1.58 (m, 6H). MS (ESI): *m/z* 210 $[M+H]^+$.

4-Hydroxy-1-methyl-6-morpholinopyrimidin-2(1*H*)-one (11h). Intermediate **11h** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 91% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.65 (s, 1H), 5.19 (s, 1H), 3.89–3.80 (m, 4H), 3.38 (s, 3H), 3.01–2.93 (m, 4H). MS (ESI): *m/z* 212 $[M+H]^+$.

4-Hydroxy-1-methyl-6-(4-methylpiperidin-1-yl)pyrimidin-2(1*H*)-one (11i). Intermediate **11i** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 85% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.11 (s, 1H), 5.16 (s, 1H), 3.34 (s, 3H), 3.19 (d, J = 12.4 Hz, 2H), 2.63 (td, J = 12.2, 1.9 Hz, 2H), 1.77 (d, J = 13.0 Hz, 2H), 1.58 (ddh, J = 14.8, 6.7, 4.0 Hz, 1H), 1.32 (qd, J = 12.9, 3.7 Hz, 2H), 1.01 (d, J = 6.5 Hz, 3H). MS (ESI): *m/z* 224 $[M+H]^+$.

4-Hydroxy-1-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2(1*H*)-one (11j). Intermediate **11j** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 81% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.58 (s, 1H), 5.19 (s, 1H), 3.36 (s, 3H), 3.00 (t, J = 4.6 Hz, 4H), 2.58 (s, 4H), 2.36 (s, 3H). MS (ESI): *m/z* 225 $[M+H]^+$.

4-Hydroxy-6-(4-methoxypiperidin-1-yl)-1-methylpyrimidin-2(1*H*)-one (11k). Intermediate **11k** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 56% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.28 (s, 1H), 5.17 (s,

1H), 3.47–3.40 (m, 1H), 3.38 (d, *J* = 1.8 Hz, 3H), 3.35 (d, *J* = 1.7 Hz, 3H), 3.18–3.08 (m, 2H), 2.84–2.70 (m, 2H), 1.98 (s, 2H), 1.82–1.65 (m, 2H). MS (ESI): *m/z* 240 $[M+H]^+$.

6-(4-Fluoropiperidin-1-yl)-4-hydroxy-1-methylpyrimidin-2(1*H*)-one (11l). Intermediate **11l** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 73% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 5.49 (s, 1H), 4.94–4.74 (m, H), 3.35 (s, 3H), 3.17–3.07 (m, 2H), 3.03–2.95 (m, 2H), 2.13–1.89 (m, 4H). MS (ESI): *m/z* 228 $[M+H]^+$.

6-(4,4-Difluoropiperidin-1-yl)-4-hydroxy-1-methylpyrimidin-2(1*H*)-one (11m). Intermediate **11m** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 85% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.17 (s, 1H), 5.20 (s, 1H), 3.37 (s, 3H), 3.13–3.03 (m, 4H), 2.24–2.09 (m, , 4H). MS (ESI): *m/z* 246 $[M+H]^+$.

6-(4-Cyclopropylpiperazin-1-yl)-4-hydroxy-1-methylpyrimidin-2(1*H*)-one (11n). Intermediate **11n** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 81% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.35 (s, 1H), 5.16 (s, 1H), 3.37 (s, 3H), 2.94 (s, 4H), 2.76 (s, 4H), 1.70 (tt, *J* = 6.6, 3.7 Hz, 1H), 0.55–0.38 (m, 4H). MS (ESI): *m/z* 251 $[M+H]^+$.

4-Hydroxy-1-methyl-6-thiomorpholinopyrimidin-2(1*H*)-one (11o). Intermediate **11o** was obtained as a white solid in a manner similar to that described for intermediate **11a** in 88% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.33 (s, 1H), 5.18 (s, 1H), 3.35 (s, 3H), 3.21 (s, 4H), 2.83–2.74 (m, 4H). MS (ESI): *m/z* 228 $[M+H]^+$.

General Synthetic Procedure for 12a−o. To a solution of corresponding **11a−o** (0.50 mmol) and $POCl_3$ (2 mL) was stirred at 70 °C for 2.5 h, then, the solution was evaporated under reduced pressure, saturated $NaHCO_3$ aqueous solution was added to the resulting residue, the solution was extracted with DCM (20 mL × 3), washed with brine, dried over $MgSO_4$, filtered, and concentrated, the residue obtained was used in next step without further purification.

4-Chloro-6-(dimethylamino)-1-methylpyrimidin-2(1H)-one (12a). Intermediate **12a** was obtained as a white solid from **11a** according to the general procedure in 91% yield. MS (ESI): *m/z* 188 $[M+H]^+$.

4-Chloro-6-(diethylamino)-1-methylpyrimidin-2(1H)-one (12b). Intermediate **12b** was obtained as a white solid from **11b** according to the general procedure in 93% yield. MS (ESI): *m/z* 216 $[M+H]^+$.

4-Chloro-6-(cyclopropyl(methyl)amino)-1-methylpyrimidin-2(1H)-one (12c). Intermediate **12c** was obtained as a yellow solid from **11c** according to the general procedure in 86% yield. MS (ESI): *m/z* 214 $[M+H]^+$.

4-Chloro-6-((2-methoxyethyl)(methyl)amino)-1-methylpyrimidin-2(1H)-one (12d). Intermediate **12d** was obtained as a white solid from **11d** according to the general procedure in 88% yield. MS (ESI): *m/z* 232 $[M+H]^+$.

Ethyl

N-(6-chloro-3-methyl-2-oxo-2,3-dihydropyrimidin-4-yl)-N-methylglycinate (12e). Intermediate **12e** was obtained as an orange solid from **11e** according to the general procedure in 85% yield. MS (ESI): *m/z* 260 $[M+H]^+$.

4-Chloro-1-methyl-6-(pyrrolidin-1-yl)pyrimidin-2(1H)-one (12f). Intermediate **12f** was obtained as a white solid from **11f** according to the general procedure in 96% yield. MS (ESI): *m/z* 214 $[M+H]^+$.

4-Chloro-1-methyl-6-(piperidin-1-yl)pyrimidin-2(1H)-one (12g). Intermediate **12g** was obtained as a white solid from **11g** according to the general procedure in 92% yield. MS (ESI): *m/z* 228 $[M+H]^+$.

4-Chloro-1-methyl-6-morpholinopyrimidin-2(1H)-one (12h). Intermediate **12h** was obtained as a white solid from **11h** according to the general procedure in 87% yield. MS (ESI): *m/z* 230 $[M+H]^+$.

4-Chloro-1-methyl-6-(4-methylpiperidin-1-yl)pyrimidin-2(1H)-one (12i). Intermediate **12i** was obtained as a white solid from **11i** according to the general procedure in 85% yield. MS (ESI): *m/z* 242 $[M+H]^+$.

4-Chloro-1-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2(1H)-one (12j). Intermediate **12j** was obtained as a white solid from **11j** according to the general procedure in 83% yield. MS (ESI): *m/z* 243 $[M+H]^+$.

4-Chloro-6-(4-methoxypiperidin-1-yl)-1-methylpyrimidin-2(1H)-one (12k).

Intermediate **12k** was obtained as a white solid from **11k** according to the general procedure in 85% yield. MS (ESI): *m/z* 258 $[M+H]^+$.

4-Chloro-6-(4-fluoropiperidin-1-yl)-1-methylpyrimidin-2(1H)-one (12l). Intermediate **12l** was obtained as a white solid from **11l** according to the general procedure in 80% yield. MS (ESI): *m/z* 246 $[M+H]^+$.

4-Chloro-6-(4,4-difluoropiperidin-1-yl)-1-methylpyrimidin-2(1H)-one (12m). Intermediate **12m** was obtained as a white solid from **11m** according to the general procedure in 88% yield. MS (ESI): *m/z* 264 $[M+H]^+$.

4-Chloro-6-(4-cyclopropylpiperazin-1-yl)-1-methylpyrimidin-2(1H)-one (12n). Intermediate **12n** was obtained as a white solid from **11n** according to the general procedure in 87% yield. MS (ESI): *m/z* 269 $[M+H]^+$.

4-Chloro-1-methyl-6-thiomorpholinopyrimidin-2(1H)-one (12o). Intermediate **12o** was obtained as a yellow solid from **11o** according to the general procedure in 86% yield. MS (ESI): *m/z* 246 $[M+H]^+$.

General Synthetic Procedure for 13a−o. To a solution of intermediate **4** (0.10 mmol) and corresponding intermediates **12a−o** (0.10 mmol) in THF (2 mL) was added sodium hydride (0.25 mmol) at 0 °C. The reaction was stirred at rt for 0.5 h and then quenched with NH_4Cl solution, extracted with ethyl acetate (5 mL × 3), washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue obtained was purified

with preparative TLC (DCM/MeOH = 30:1) to yield the desired compound.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(dimethylamino)-1-methylpyrimidin-2(1*H*)-one (13a). The title compound was obtained as a white solid from **4** and **12a** according to the general procedure in 80% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.13–7.06 (m, 2H), 7.01 (dd, *J* = 8.8, 3.0 Hz, 1H), 5.43 (s, 1H), 5.40 (s, 2H), 3.47 (s, 3H), 2.83 (s, 6H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.59, 164.87, 158.52, 155.91, 155.63 (dd, *J* = 252.9, 4.4 Hz, 2C), 135.70 (t, *J* = 8.2 Hz), 132.59, 129.83 (t, *J* = 15.2 Hz), 129.49 (q, *J* = 31.9 Hz), 125.87, 122.31 (q, *J* = 274.0 Hz), 119.29, 115.12 (q, *J* = 5.4 Hz), 111.94 (dd, *J* = 18.3, 4.7 Hz, 2C), 81.25, 66.10, 41.88(2C), 34.46. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{21}H_{18}ClF_5N_3O_3$, 490.0951; found, 490.0958.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(diethylamino)-1-methylpyrimidin-2(1*H*)-one (13b). The title compound was obtained as a white solid from **4** and **12b** according to the general procedure in 80% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 2.9 Hz, 1H), 7.18–7.04 (m, 2H), 7.02 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.49 (s, 1H), 5.40 (s, 2H), 3.46 (s, 3H), 3.12 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.52, 163.28, 158.65, 155.91, 155.64 (dd, *J* = 252.8, 4.3 Hz, 2C), 135.62 (t, *J* = 8.3 Hz), 132.60, 129.87 (t, *J* = 15.2 Hz), 129.51 (q, *J* = 32.1 Hz), 125.88, 122.31 (q, *J* = 274.0 Hz), 119.31, 115.12 (q, *J* = 5.3 Hz), 112.01 (dd, *J* = 18.4, 4.7 Hz, 2C), 84.40, 66.22, 44.97 (2C), 33.89,

11.86 (2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{22}ClF_5N_3O_3$, 518.1264; found, 518.1257.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(cyclopropyl(methyl)amino)-1-methylpyrimidin-2(1*H*)-one (13c). The title compound was obtained as a white solid from **4** and **12c** according to the general procedure in 85% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 2.9 Hz, 1H), 7.17–7.04 (m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.60 (s, 1H), 5.39 (s, 2H), 3.41 (s, 3H), 2.88 (s, 3H), 2.54 (tt, *J* = 6.7, 3.7 Hz, 1H), 0.82 (q, *J* = 6.7 Hz, 2H), 0.60–0.52 (m, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.46, 164.25, 158.40, 155.91, 155.64 (dd, *J* = 252.8, 4.4 Hz, 2C), 135.69 (t, *J* = 8.3 Hz), 132.59, 129.84 (t, *J* = 15.1 Hz), 129.51 (q, *J* = 32.0 Hz), 125.88, 122.31 (q, *J* = 273.9 Hz), 119.30, 115.12 (q, *J* = 5.4 Hz), 111.99 (dd, *J* = 18.1, 4.8 Hz, 2C), 82.64, 66.16, 40.99, 34.66, 34.39, 8.32 (2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{20}ClF_5N_3O_3$, 516.1108; found, 516.1113.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-((2-methoxyethyl)(methyl)amino)-1-methylpyrimidin-2(1*H*)-one (13d). The title compound was obtained as a white solid from **4** and **12d** according to the general procedure in 65% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.13–7.07 (m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.49 (s, 1H), 5.40 (s, 2H), 3.58 (t, *J* = 5.3 Hz, 2H), 3.47 (s, 3H), 3.34 (s, 3H), 3.26 (t, *J* = 5.3 Hz, 2H), 2.86 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.58, 164.56, 158.53, 155.90, 155.64 (dd, *J* = 252.9,

4.4 Hz, 2C), 135.64 (t, *J* = 8.2 Hz), 132.59, 129.79 (d, *J* = 14.7 Hz), 129.51 (q, *J* = 32.0 Hz), 125.89, 122.30 (q, *J* = 274.0 Hz), 119.29, 115.12 (q, *J* = 5.4 Hz), 111.95 (dd, *J* = 18.3, 4.8 Hz, 2C), 82.68, 69.43, 66.16, 58.96, 53.25, 39.62, 34.34. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{22}ClF_5N_3O_4$, 534.1214; found, 534.1208.

Ethyl N-(6-((4-(4-chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-3-methyl-2-oxo-2,3-dihydropyrimidin-4-yl)-N-methylglycinate (13e). The title compound was obtained as a white solid from **4** and **12e** according to the general procedure in 75% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.44–7.39 (m, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 2H), 7.01 (dd, *J* = 8.7, 2.8 Hz, 1H), 5.50 (s, 1H), 5.39 (s, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.83 (s, 2H), 3.48 (s, 3H), 2.93 (s, 3H), 1.31 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.62, 168.38, 163.34, 158.35, 155.89, 155.64 (dd, *J* = 252.8, 4.2 Hz, 2C), 135.47 (t, *J* = 8.3 Hz), 132.59, 129.90 (t, *J* = 15.1 Hz), 129.52 (q, *J* = 32.0 Hz), 125.89, 122.30 (q, *J* = 273.9 Hz), 119.29, 115.13 (q, *J* = 5.4 Hz), 112.02 (dd, *J* = 18.4, 4.7 Hz, 2C), 82.90, 66.29, 61.76, 54.57, 40.08, 34.30, 14.17. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{22}ClF_5N_3O_5$, 562.1163; found, 562.1168.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-(pyrrolidin-1-yl)pyrimidin-2(1*H*)-one (13f). The title compound was obtained as a white solid from **4** and **12f** according to the general procedure in 77% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.13–7.06

(m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.40 (s, 2H), 5.33 (s, 1H), 3.49 (s, 3H), 3.39–3.32 (m, 4H), 2.05–1.96 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.35, 161.29, 158.68, 155.92, 155.62 (dd, *J* = 252.8, 4.4 Hz, 2C), 136.03 (t, *J* = 8.2 Hz), 132.57, 129.72 (t, *J* = 15.2 Hz), 129.50 (q, *J* = 31.9 Hz), 125.84, 122.30 (q, *J* = 274.0 Hz), 119.26, 115.13 (q, *J* = 5.4 Hz), 111.84 (dd, *J* = 18.3, 4.7 Hz, 2C), 77.78, 65.85, 51.12 (2C), 35.72, 25.60 (2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{20}ClF_5N_3O_3$, 516.1108; found, 516.1110.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-(piperidin-1-yl)pyrimidin-2(1*H*)-one (13g). The title compound was obtained as a white solid from **4** and **12g** according to the general procedure in 73% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.43 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 2.9 Hz, 1H), 7.15–7.08 (m, 2H), 7.03 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.47 (s, 1H), 5.42 (s, 2H), 3.47 (s, 3H), 3.03–2.94 (m, 4H), 1.79–1.72 (m, 4H), 1.71–1.65 (m, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.72, 164.86, 158.42, 155.90, 155.64 (dd, *J* = 252.8, 4.4 Hz, 2C), 135.62 (t, *J* = 8.3 Hz), 132.58, 129.85 (t, *J* = 14.9 Hz), 129.51 (q, *J* = 31.9 Hz), 125.88, 122.30 (q, *J* = 274.0 Hz), 119.29, 115.12 (q, *J* = 5.3 Hz), 111.95 (dd, *J* = 18.3, 4.8 Hz, 2C), 82.50, 66.16, 51.46 (2C), 33.49, 25.28 (2C), 23.83. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{22}ClF_5N_3O_3$, 530.1264; found, 530.1270.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-morpholinopyrimidin-2(1*H*)-one (13h). The title compound was obtained as a white

solid from **4** and **12h** according to the general procedure in 81% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 3.0 Hz, 1H), 7.14–7.06 (m, 2H), 7.01 (dd, *J* = 9.0, 3.1 Hz, 1H), 5.48 (s, 1H), 5.40 (s, 2H), 3.89–3.83 (m, 4H), 3.48 (s, 3H), 3.06–3.01 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.79, 163.77, 158.14, 155.87, 155.65 (dd, *J* = 257.4, 4.5 Hz, 2C), 135.33 (t, *J* = 8.2 Hz), 132.60, 129.95 (t, 15.2 Hz), 129.52 (q, *J* = 32.0 Hz), 125.91, 122.30 (q, *J* = 273.9 Hz), 119.33, 115.09 (q, *J* = 5.4 Hz), 112.03 (dd, *J* = 18.3, 4.8 Hz, 2C), 82.84, 66.36, 66.08 (2C), 50.48 (2C), 33.36. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{20}ClF_5N_3O_4$, 532.1057; found, 532.1068.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-(4-methylpiperidin-1-yl)pyrimidin-2(1*H*)-one (13i). The title compound was obtained as a white solid from **4** and **12i** according to the general procedure in 85% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.12–7.06 (m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.45 (s, 1H), 5.39 (s, 2H), 3.45 (s, 3H), 3.28 (d, *J* = 12.4 Hz, 2H), 2.70 (td, *J* = 12.1, 2.1 Hz, 2H), 1.83–1.75 (m, 2H), 1.41–1.30 (m, 3H), 1.02 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.71, 164.73, 158.46, 155.90, 155.63 (dd, *J* = 252.9, 4.4 Hz, 2C), 135.63 (t, *J* = 8.3 Hz), 132.59, 129.85 (t, *J* = 15.0 Hz), 129.50 (q, *J* = 32.0 Hz), 125.87, 122.31 (q, *J* = 274.0 Hz), 119.29, 115.12 (q, *J* = 5.3 Hz), 111.96 (dd, *J* = 18.4, 4.7 Hz, 2C), 82.60, 66.16, 50.80 (2C), 33.52, 33.50 (2C), 30.51, 21.62. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{25}H_{24}ClF_5N_3O_3$, 544.1421; found, 544.1423.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2(1*H*)-one (13j). The title compound was obtained as a white solid from **4** and **12j** according to the general procedure in 20% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.13–7.06 (m, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.53 (s, 1H), 5.40 (s, 2H), 3.46 (s, 3H), 3.28 (s, 4H), 2.92 (s, 4H), 2.61 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 170.73, 164.02, 158.25, 155.90, 155.65 (dd, *J* = 253.1, 4.3 Hz, 2C), 135.46 (t, *J* = 8.3 Hz), 132.59, 129.92 (t, *J* = 14.9 Hz), 129.52 (q, *J* = 32.3 Hz), 125.90, 122.31 (q, *J* = 274.4 Hz), 119.31, 115.13 (q, *J* = 5.4 Hz), 112.02 (dd, *J* = 17.2, 5.4 Hz, 2C), 82.79, 66.29, 54.18 (2C), 50.06 (2C), 45.92, 33.45. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{23}ClF_5N_4O_3$, 545.1373; found, 545.1379.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(4-methoxypiperidin-1-yl)-1-methylpyrimidin-2(1*H*)-one (13k). The title compound was obtained as a white solid from **4** and **12k** according to the general procedure in 82% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.09 (d, *J* = 8.1 Hz, 2H), 7.01 (dd, *J* = 8.6, 2.8 Hz, 1H), 5.47 (s, 1H), 5.40 (s, 2H), 3.48 (s, 1H), 3.46 (s, 3H), 3.38 (s, 3H), 3.26–3.14 (m, 2H), 2.87 (dt, *J* = 8.3, 4.1 Hz, 2H), 2.04–1.94 (m, 2H), 1.85–1.74 (m, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.71, 164.39, 158.33, 155.89, 155.64 (dd, *J* = 252.9, 4.4 Hz, 2C), 135.54 (t, *J* = 8.2 Hz), 132.59, 129.88 (t, *J* = 15.5 Hz), 129.50 (q, *J* = 31.9 Hz), 125.88, 122.30 (q, *J* = 273.9 Hz), 119.30, 115.12

(q, *J* = 5.4 Hz), 111.97 (dd, *J* = 18.3, 4.8 Hz, 2C), 82.72, 74.24, 66.21, 55.86 (2C), 47.42, 33.40, 30.01 (2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{25}H_{24}ClF_5N_3O_4$, 560.1370; found, 560.1381.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(4-fluoro piperidin-1-yl)-1-methylpyrimidin-2(1*H*)-one (13l). The title compound was obtained as a white solid from **4** and **12l** according to the general procedure in 68% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 2.9 Hz, 1H), 7.15–7.05 (m, 2H), 7.01 (dd, *J* = 8.8, 2.8 Hz, 1H), 5.50 (s, 1H), 5.40 (s, 2H), 5.02–4.81 (m, 1H), 3.47 (s, 3H), 3.12–3.11 (m, 2H), 3.07–2.97 (m, 2H), 2.10–1.92 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.74, 164.20, 158.19, 155.88, 155.15 (dd, *J* = 253.0, 4.5 Hz, 2C), 135.43 (t, *J* = 8.3 Hz), 132.59, 129.92 (t, *J* = 15.0 Hz), 129.51 (q, *J* = 32.1 Hz), 125.90, 122.30 (q, *J* = 274.0 Hz), 119.31, 115.11 (q, *J* = 5.3 Hz), 112.00 (dd, *J* = 18.3, 4.8 Hz, 2C), 86.33 (d, *J* = 172.4 Hz), 82.94, 66.29, 46.23 (d, *J* = 4.0 Hz, 2C), 33.22, 30.68 (d, *J* = 20.4 Hz, 2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{21}ClF_6N_3O_3$, 548.1170; found, 548.1182.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(4,4-diflu oropiperidin-1-yl)-1-methylpyrimidin-2(1*H*)-one (13m). The title compound was obtained as a white solid from **4** and **12m** according to the general procedure in 76% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 3.0 Hz, 1H), 7.13–7.06 (m, 2H), 7.02 (dd, *J* = 8.9, 3.0 Hz, 1H), 5.51 (s, 1H), 5.40 (s, 2H), 3.48 (s,

3H), 3.19–3.11 (m, 4H), 2.25–2.12 (m, 4H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 170.66, 163.35, 157.94, 156.86, 155.65 (dd, *J* = 252.6, 4.4 Hz, 2C), 135.24 (t, *J* = 8.3 Hz), 132.60, 129.97 (t, *J* = 15.1 Hz), 129.51 (q, *J* = 31.7 Hz), 125.94, 122.29 (q, *J* = 273.6 Hz), 120.42 (t, *J* = 242.5 Hz), 119.32, 115.07 (q, *J* = 5.6 Hz), 112.02 (dd, *J* = 18.7, 4.2 Hz, 2C), 83.48, 66.40, 47.36 (t, *J* = 5.4 Hz, 2C), 33.693 (t, *J* = 23.8 Hz, 2C), 33.03. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{20}ClF_7N_3O_3$, 566.1076; found, 566.1088.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(4-cyclopropylpiperazin-1-yl)-1-methylpyrimidin-2(1*H*)-one (13n). The title compound was obtained as a white solid from **4** and **12n** according to the general procedure in 56% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.15–7.05 (m, 2H), 7.01 (dd, *J* = 8.9, 2.9 Hz, 1H), 5.45 (s, 1H), 5.39 (s, 2H), 3.47 (s, 3H), 3.01 (s, 4H), 2.78 (s, 4H), 1.71 (s, 1H), 0.58–0.38 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.72, 164.13, 158.29, 155.88, 155.64 (dd, *J* = 252.9, 4.5 Hz. 2C), 135.46 (t, *J* = 8.3 Hz), 132.58, 129.91 (t, *J* = 15.1 Hz), 129.52 (q, *J* = 32.2 Hz), 125.89, 122.30 (q, *J* = 273.7 Hz), 119.29, 115.12 (q, *J* = 5.4 Hz), 112.02 (dd, *J* = 18.3, 4.8 Hz, 2C), 82.66, 66.29, 52.39 (2C), 50.17 (2C), 38.24, 33.49, 29.71, 5.95 (2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{26}H_{25}ClF_5N_4O_3$, 571.1530; found, 571.1541.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-1-methyl-6-thiomorpholinopyrimidin-2(1*H*)-one (13o). The title compound was obtained as a white solid from **4** and **12o** according to the general procedure in 81% yield. ^{1}H NMR

(400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 2.8 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 2H), 7.01 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.48 (s, 1H), 5.40 (s, 2H), 3.45 (s, 3H), 3.33–3.22 (m, 4H), 2.86–2.75 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.72, 164.49, 158.06, 155.87, 155.65 (dd, *J* = 252.6, 3.9 Hz, 2C), 135.31 (t, *J* = 8.1 Hz), 132.59, 129.96 (t, *J* = 15.2 Hz), 129.51 (q, *J* = 31.8 Hz), 125.92, 122.29 (q, *J* = 274.0 Hz), 119.32, 115.09 (q, *J* = 5.3 Hz), 112.02 (dd, *J* = 18.4, 4.7 Hz, 2C), 83.70, 66.37, 52.68 (2C), 33.17, 27.17(2C). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{20}ClF_5N_3O_3S$, 548.0829; found, 548.0841.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3,5-difluorobenzyl)oxy)-6-(1,1-dioxidothiomorpholino)-1-methylpyrimidin-2(1*H*)-one (13p). To a solution of **12o** (50 mg, 0.09 mmol) in DCM (2 mL) was added *m*-CPBA (67 mg, 0.27 mmol, 70% purity) at 0 °C, and the mixture was stirred at rt for 1 h. Then, the reaction was quenched with $NaHCO_3$ saturated aqueous solution, and the organic layer was separated and washed with $NaHCO_3$ saturated aqueous solution, dried over Na_2SO_4, filtered, and concentrated. The residue obtained was purified with preparative TLC (DCM/MeOH = 20:1) to yield the desired compound as a white solid (38 mg, 72%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.28 (d, *J* = 3.0 Hz, 1H), 7.13–7.06 (m, 2H), 7.02 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.56 (s, 1H), 5.41 (s, 2H), 3.58–3.51 (m, 4H), 3.50 (s, 3H), 3.29–3.21 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.57, 162.41, 157.49, 155.83, 155.67 (dd, *J* = 253.1, 4.4 Hz, 2C), 134.88 (t, *J* = 8.2 Hz), 132.62, 130.12 (t, *J* = 15.2 Hz), 129.51 (q, *J*

= 32.0 Hz), 125.99, 122.29 (q, *J* = 273.9 Hz), 119.39, 115.04 (q, *J* = 5.4 Hz), 112.14 (dd, *J* = 18.4, 4.9 Hz, 2C), 84.75, 66.67, 51.28 (2C), 49.00 (2C), 32.79. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{20}ClF_5N_3O_5S$, 580.0727; found, 580.0740.

4-((4-(4-chloro-3-(trifluoromethyl)phenoxy)benzyl)oxy)-1-methyl-6-morpholino pyrimidin-2(1*H*)-one (14a). The title compound was obtained as a white solid from intermediate **12h** and corresponding alcohol using a method similar to that described for compounds **13a**−**o** in 58% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.47–7.40 (m, 3H), 7.33 (d, *J* = 2.5 Hz, 1H), 7.08 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 2H), 5.45 (s, 1H), 5.40 (s, 2H), 3.88–3.82 (m, 4H), 3.48 (s, 3H), 3.04–2.98 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 171.18, 163.43, 158.34, 155.89, 132.71, 132.21, 130.46 (2C), 129.58 (q, *J* = 31.8 Hz), 125.89, 122.43, 122.36 (q, *J* = 275.3 Hz), 119.34 (2C), 117.79 (d, *J* = 4.7 Hz), 115.46 (d, *J* = 20.5 Hz), 83.17, 67.78, 66.10 (2C), 50.47 (2C), 33.18. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{22}ClF_3N_3O_4$, 496.1245; found, 496.1246.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3-fluorobenzyl)oxy)-1-methyl-6-mo rpholinopyrimidin-2(1*H*)-one (14b). The title compound was obtained as a white solid from intermediate **12h** and corresponding alcohol using a method similar to that described for compounds **13a**−**o** in 65% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.32–7.27 (m, 2H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.10 (t, *J* = 8.2 Hz, 1H), 7.03 (dd, *J* = 8.8, 3.0 Hz, 1H), 5.47 (s, 1H), 5.40 (s, 2H), 3.87–3.83 (m, 4H), 3.48 (s, 3H), 3.05–3.00 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.99, 163.61, 158.25, 155.97,

154.08 (d, *J* = 251.1 Hz), 141.99 (d, *J* = 11.8 Hz), 134.76 (d, J = 6.4 Hz), 132.65, 129.52 (q, *J* = 31.9 Hz), 125.80, 124.74 (d, *J* = 3.4 Hz), 122.37, 122.34 (q, *J* = 274.0 Hz), 120.70, 117.20 (d, *J* = 18.9 Hz), 116.27 (q, *J* = 5.4 Hz), 83.01, 66.96, 66.09 (2C), 50.48 (2C), 33.28. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{21}ClF_4N_3O_4$, 514.1151; found, 514.1167.

2-(4-Chloro-3-(trifluoromethyl)phenoxy)-5-(((1-methyl-6-morpholino-2-oxo-1,2-dihydropyrimidin-4-yl)oxy)methyl)benzonitrile (14c). The title compound was obtained as a white solid from intermediate **12h** and corresponding alcohol using a method similar to that described for compounds **13a**−**o** in 73% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.76 (d, *J* = 2.1 Hz, 1H), 7.60 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.41 (d, *J* = 2.8 Hz, 1H), 7.18 (dd, *J* = 8.7, 2.8 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 5.46 (s, 1H), 5.41 (s, 2H), 3.88–3.82 (m, 4H), 3.48 (s, 3H), 3.06–3.00 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.80, 163.75, 158.13, 157.80, 153.82, 134.42, 133.65, 133.21, 132.89, 130.12 (q, *J* = 32.2 Hz), 128.06, 123.60, 122.13 (q, *J* = 267.6 Hz), 118.87 (q, *J* = 5.3 Hz), 117.86, 115.13, 104.76, 82.86, 66.19, 66.07 (2C), 50.46 (2C), 33.36. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{21}ClF_3N_4O_4$, 521.1198; found, 521.1212.

4-((3-Chloro-4-(4-chloro-3-(trifluoromethyl)phenoxy)benzyl)oxy)-1-methyl-6-morpholinopyrimidin-2(1*H*)-one (14d). The title compound was obtained as a white solid from intermediate **12h** and corresponding alcohol using a method similar to that described for compounds **13a**−**o** in 68% yield. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.56 (s, 1H), 7.43

(d, J = 8.8 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.28 (d, J = 2.6 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 6.99 (dd, J = 8.7, 2.6 Hz, 1H), 5.47 (s, 1H), 5.39 (s, 2H), 3.89–3.82 (m, 4H), 3.48 (s, 3H), 3.05–2.99 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 170.98, 163.60, 158.25, 155.61, 150.73, 134.44, 132.70, 130.81, 129.64 (q, J = 31.9 Hz), 128.21, 126.52, 125.92, 122.33 (q, J = 274.0 Hz), 121.61, 121.10, 116.70 (q, J = 5.4 Hz), 83.02, 66.88, 66.10 (2C), 50.48 (2C), 33.28. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{23}H_{21}Cl_2F_3N_3O_4$, 530.0856; found, 530.0866.

4-((4-(4-Chloro-3-(trifluoromethyl)phenoxy)-3-methylbenzyl)oxy)-1-methyl-6-morpholinopyrimidin-2(1*H*)-one (14e). The title compound was obtained as a white solid from intermediate **12h** and corresponding alcohol using a method similar to that described for compounds **13a**−**o** in 63% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.56 (d, J = 8.8 Hz, 1H), 7.46–7.43 (m, 1H), 7.36 (dd, J = 8.2, 1.8 Hz, 1H), 7.26 (d, J = 2.9 Hz, 1H), 7.08 (dd, J = 8.9, 2.9 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 5.70 (s, 1H), 5.37 (s, 2H), 3.87–3.82 (m, 4H), 3.48 (s, 3H), 3.12–3.07 (m, 4H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 171.22, 163.42, 158.38, 156.37, 153.24, 132.77, 132.61, 132.04, 130.34, 129.53 (q, J = 31.8 Hz), 127.77, 125.05, 122.42 (q, J = 274.0 Hz), 120.82, 120.05, 116.45 (q, J = 5.5 Hz), 83.21, 67.94, 66.11 (2C), 50.48 (2C), 33.18, 16.10. HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{24}H_{24}ClF_3N_3O_4$, 510.1402; found, 510.1413.

ASSOCIATED CONTENT

Supporting information

SAR of the linker and terminal benzene ring, blood glucose and serum lipids of STZ-induced diabetic rats treated with **14c**, and HPLC for the final compounds.

AUTHER INFORMATION

Corresponding Authors

*Tel: 86-21-50806733 E-mail: ypwang@mail.shcnc.ac.cn.

*Tel: 86-21-20231969 E-mail: jhshen@mail.shcnc.ac.cn.

Author Contributions

†These authors contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by grant from the National Science and Technology Major Project “Key New Drug Creation and Manufacturing program”（NOS. 2012ZX09103101-008）.

ABBREVIATIONS USED

MW, molecular weight; cLogP, calculated logP; rt, room temperature; *m*-CPBA, 3-chloroperbenzoic acid; NBS, bromosuccinimide; TMSOTf, trimethylsilyl trifluoromethanesulfonate; TEA, triethylamine; DMA, N,N-dimethylacetamide; THF,

tetrahydrofuran; DCM, dichloromethane; SD rats, Sprague-Dawley rats; TLC, thin-layer chromatography.

REFERENCES

1. Stafforini, D. M.; Elstad, M. R.; Mcintyre, T. M.; Zimmerman, G. A.; Prescott, S. M. Human macrophages secrete platelet-activating factor acetylhydrolase. *J. Biol. Chem.* **1990**, *265*, 9682-9687.

2. Nakajima, K.; Murakami, M.; Yanoshita, R.; Samejima, Y.; Karasawa, K.; Setaka, M.; Nojima, S.; Kudo, I. Activated mast cells release extracellular type platelet-activating factor acetylhydrolase that contributes to autocrine inactivation of platelet-activating factor. *J. Biol. Chem.* **1997**, *272*, 19708-19713.

3. Asano, K.; Okamoto, S.; Fukunaga, K.; Shiomi, T.; Mori, T.; Iwata, M.; Ikeda, Y.; Yamaguchi, K. Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 511-514.

4. MacPhee, C. H.; Moores, K. E.; Boyd, H. F.; Dhanak, D.; Ife, R. J.; Leach, C. A.; Leake, D. S.; Milliner, K. J.; Patterson, R. A.; Suckling, K. E.; Tew, D. G.; Hickey, D. M. B. Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem. J.* **1999**, *338*, 479-487.

5. Shi, Y.; Zhang, P.; Zhang, L.; Osman, H.; Mohler, E. R.; Macphee, C.; Zalewski, A.; Postle, A.; Wilensky, R. L. Role of lipoprotein-associated phospholipase A_2 in leukocyte

activation and inflammatory responses. *Atherosclerosis* **2007**, *191*, 54-62.

6. Quinn, M. T.; Parthasarathy, S.; Steinberg, D. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc. Natl. Acad. Sci.U.S.A.* **1988**, *85*, 2805-2809.

7. Caslake, M. J.; Packard, C. J.; Suckling, K. E.; Holmes, S. D.; Chamberlain, P.; Macphee, C. H. Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. *Atherosclerosis* **2000**, *150*, 413-419.

8. Anderson, J. L. Lipoprotein-associated phospholipase A_2: an independent predictor of coronary artery disease events in primary and secondary prevention. *Am. J. Cardiol.* **2008**, *101*, 23F-33F.

9. Wilensky, R. L.; Macphee, C. H. Lipoprotein-associated phospholipase A_2 and atherosclerosis. *Curr. Opin. Lipidol.* **2009**, *20*, 415-420.

10. Casas, J. P.; Ninio, E.; Panayiotou, A.; Palmen, J.; Cooper, J. A.; Ricketts, S. L.; Sofat, R.; Nicolaides, A. N.; Corsetti, J. P.; Fowkes, F. G. R.; Tzoulaki, I.; Kumari, M.; Brunner, E. J.; Kivimaki, M.; Marmot, M. G.; Hoffmann, M. M.; Winkler, K.; Marz, W.; Ye, S.; Stirnadel, H. A.; Boekholdt, S. M.; Khaw, K. T.; Humphries, S. E.; Sandhu, M. S.; Hingorani, A. D.; Talmud, P. J. PLA2G7 genotype, lipoprotein-associated phospholipase A_2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of European ancestry. *Circulation* **2010**, *121*, 2284-2293.

11. Thompson, A.; Gao, P.; Orfei, L.; Watson, S.; Di Angelantonio, E.; Kaptoge, S.;

Ballantyne, C.; Cannon, C. P.; Criqui, M.; Cushman, M.; Hofman, A.; Packard, C.; Thompson, S. G.; Collins, R.; Danesh, J. Lipoprotein-associated phospholipase A_2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet* **2010**, *375*, 1536-1544.

12. Acharya, N. K.; Levin, E. C.; Clifford, P. M.; Han, M.; Tourtellotte, R.; Chamberlain, D.; Pollaro, M.; Coretti, N. J.; Kosciuk, M. C.; Nagele, E. P. Diabetes and hypercholesterolemia increase blood-brain barrier permeability and brain amyloid deposition: beneficial effects of the LpPLA2 inhibitor darapladib. *J. Alzheimer's Dis.* **2013**, *35*, 179-198.

13. Canning, P.; Glenn, J.; Prise, V.; Gale, D.; Stitt, A.; Adamson, P. Lp-PLA_2 is a potential therapeutic target in diabetic macula edema. *Invest. Ophthalmol. Visual Sci.* **2013**, *54*, 4613-4613.

14. Bandello, F.; Parodi, M. B.; Lanzetta, P.; Loewenstein, A.; Massin, P.; Menchini, F.; Veritti, D. Diabetic macular edema. *Dev. Ophthalmol.* **2010**, *47*, 73-110.

15. Antcliff, R. J.; Marshall, J. The pathogenesis of edema in diabetic maculopathy. *Semin. Ophthalmol.* **1999**, *14*, 223-232.

16. Schwartz, S. G.; Flynn, H. W.; Scott, I. U. Emerging drugs for diabetic macular edema. *Expert Opin. Emerging Drugs* **2014**, *19*, 397-405.

17. Staurenghi, G.; Ye, L.; Magee, M. H.; Danis, R. P.; Wurzelmann, J.; Adamson, P.; McLaughlin, M. M. Darapladib, a lipoprotein-associated phospholipase A_2 Inhibitor, in diabetic macular edema: a 3-month placebo-controlled study. *Ophthalmology* **2015**, *122*,

990-996.

18. Tew, D. G.; Boyd, H. F.; Ashman, S.; Theobald, C.; Leach, C. A. Mechanism of inhibition of LDL phospholipase A_2 by monocyclic-beta-lactams. Burst kinetics and the effect of stereochemistry. *Biochemistry* **1998**, *37*, 10087-10093.

19. Jeong, T. S.; Kim, M. J.; Yu, H.; Kim, K. S.; Choi, J. K.; Kim, S. S.; Lee, W. S. (*E*)-phenyl- and -heteroaryl-substituted O-benzoyl- (or acyl)oximes as lipoprotein-associated phospholipase A_2 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1525-1527.

20. Jeong, H. J.; Park, Y. D.; Park, H. Y.; Jeong, I. Y.; Jeong, T. S.; Lee, W. S. Potent inhibitors of lipoprotein-associated phospholipase A_2: benzaldehyde O-heterocycle-4-carbonyloxime. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5576-5579.

21. Lin, E. C. K.; Hu, Y.; Amantea, C. M.; Pham, L. M.; Cajica, J.; Okerberg, E.; Brown, H. E.; Fraser, A.; Du, L.; Kohno, Y.; Ishiyama, J.; Kozarich, J. W.; Shreder, K. R. Amides of xanthurenic acid as zinc-dependent inhibitors of Lp-PLA_2. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 868-871.

22. Hu, Y.; Lin, E. C. K.; Pham, L. M.; Cajica, J.; Amantea, C. M.; Okerberg, E.; Brown, H. E.; Fraser, A.; Du, L.; Kohno, Y.; Ishiyama, J.; Kozarich, J. W.; Shreder, K. R. Amides of 4-hydroxy-8-methanesulfonylamino-quinoline-2-carboxylic acid as zinc-dependent inhibitors of Lp-PLA_2. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1553-1556.

23. Nagano, J. M. G.; Hsu, K. L.; Whitby, L. R.; Niphakis, M. J.; Speers, A. E.; Brown, S. J.; Spicer, T.; Fernandez-Vega, V.; Ferguson, J.; Hodder, P.; Srinivasan, P.; Gonzalez, T.

D.; Rosen, H.; Bahnson, B. J.; Cravatt, B. F. Selective inhibitors and tailored activity probes for lipoprotein-associated phospholipase A_2. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 839-843.

24. Blackie, J. A.; Bloomer, J. C.; Brown, M. J. B.; Cheng, H. Y.; Hammond, B.; Hickey, D. M. B.; Ife, R. J.; Leach, C. A.; Lewis, V. A.; Macphee, C. H.; Milliner, K. J.; Moores, K. E.; Pinto, I. L.; Smith, S. A.; Stansfield, I. G.; Stanway, S. J.; Taylor, M. A.; Theobald, C. J. The identification of clinical candidate SB-480848: a potent inhibitor of lipoprotein-associated phospholipase A_2. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1067-1070.

25. Magrioti, V.; Kokotos, G. Phospholipase A_2 inhibitors for the treatment of inflammatory diseases: a patent review (2010-present). *Expert Opin. Ther. Pat.* **2013**, *23*, 333-344.

26. Molecular weight and cLogP value were calculated by ChemDraw 14.

27. White, H. D.; Held, C.; Stewart, R.; Tarka, E.; Brown, R.; Davies, R. Y.; Budaj, A.; Harrington, R. A.; Steg, P. G.; Ardis-Sino, D.; Armstrong, P. W.; Avezum, A.; Aylward, P. E.; Bryce, A.; Chen, H.; Chen, M. F.; Corbalan, R.; Dalby, A. J.; Danchin, N.; De Winter, R. J.; Denchev, S.; Diaz, R.; Elisaf, M.; Flather, M. D.; Goudev, A. R.; Granger, C. B.; Grinfeld, L.; Hochman, J. S.; Husted, S.; Kim, H. S.; Koenig, W.; Linhart, A.; Lonn, E.; Lopez-Sendon, J.; Manolis, A. J.; Mohler, E. R.; Nicolau, J. C.; Pais, P.; Parkhomenko, A.; Pedersen, T. R.; Pella, D.; Ramos-Corrales, M. A.; Ruda, M.; Sereg, M.; Siddique, S.; Sinnaeve, P.; Smith, P.; Sritara, P.; Swart, H. P.; Sy, R. G.; Teramoto, T.; Tse, H. F.; Watson, D.; Weaver, W. D.; Weiss, R.; Viigimaa, M.; Vinereanu, D.; Zhu, J. R.; Cannon,

C. P.; Wallentin, L. Darapladib for preventing ischemic events in stable coronary heart disease. *N. Engl. J. Med.* **2014**, *370*, 1702-1711.

28. Mullard, A. GSK's darapladib failures dim hopes for anti-inflammatory heart drugs. *Nat. Rev. Drug Discovery* **2014**, *13*, 481-482.

29. Jin, Y.; Wan, Z.; Zhang, Q. Compounds. US2012 / 0142717 A1, 2012.

30. Wan, Z.; Zhang, X.; Wang, J.; Peng, C.; Jin, Y.; Hu, Y. Compounds. WO2012 / 075917 A1, 2012.

31. Wan, Z.; Zhang, X.; Tong, Z.; Long, K.; Dowdell, S. E.; Manas, E. S.; Goodman, K. B. Tricyclic compounds, preparation methods, and their uses. WO2012 / 037782 A1, 2012.

32. Wan, Z.; Long, K.; Sang, Y.; Su, X. 2,3-Dihydroimidazo[1,2-c]pyrimidin-5(1H)-one compounds use as Lp-PLA_2 inhibitors. WO2013 / 013503 A1, 2013.

33. Wan, Z.; Long, K.; Zhang, X.; Yu, H. Bicyclic pyrimidone compounds. WO2013 / 014185 A1, 2013.

34. Wan, Z.; Zhang, X. Compounds. WO2014 / 114248 A1, 2014.

35. Wan, Z.; Zhang, X. Bicyclic pyrimidone compounds as inhibitors of Lp-PLA_2. WO2014 / 114249 A1, 2014.

36. Sang, Y.; Wan, Z.; Zhang, Q. 2,3-Dihydroimidazol[1,2-c]pyrimidin-5(1H)-one based lipoprotein-associated phospholipase A2 (Lp-PLA_2) inhibitors. WO2014 / 114694 A1, 2014.

37. Wang, K.; Xu, W.; Liu, Y.; Zhang, W.; Wang, W.; Shen, J.; Wang, Y. Design and

synthesis of imidazole and triazole derivatives as Lp-PLA_2 inhibitors and the unexpected discovery of highly potent quaternary ammonium salts. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1187-1192.

38. Wang, K.; Xu, W.; Zhang, W.; Mo, M.; Wang, Y.; Shen, J. Triazole derivatives: A series of Darapladib analogues as orally active Lp-PLA_2 inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2897-2901.

39. Chen, X.; Xu, W.; Wang, K.; Mo, M.; Zhang, W.; Du, L.; Yuan, X.; Xu, Y.; Wang, Y.; Shen, J. Discovery of a novel series of imidazo[1, 2-*a*]pyrimidine derivatives as potent and orally bioavailable lipoprotein-associated phospholipase A_2 inhibitors. *J. Med. Chem.* **2015**, 58, 8529-8541.

40. Zhang, X.; Bao, S.; Lai, D.; Rapkins, R. W.; Gillies, M. C. Intravitreal triamcinolone acetonide inhibits breakdown of the blood-retinal barrier through differential regulation of VEGF-A and its receptors in early diabetic rat retinas. *Diabetes* **2008**, *57*, 1026-1033.

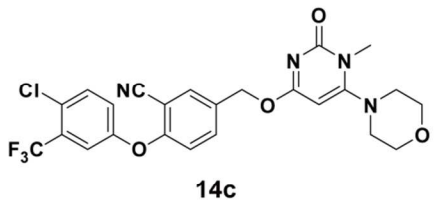
41. Leal, E. C.; Martins, J.; Voabil, P.; Liberal, J.; Chiavaroli, C.; Bauer, J.; Cunha-Vaz, J.; Ambrosio, A. F. Calcium dobesilate inhibits the alterations in tight junction proteins and leukocyte adhesion to retinal endothelial cells induced by diabetes. *Diabetes* **2010**, *59*, 2637-2645.

42. Berkowitz, B. A.; Bissig, D.; Ye, Y.; Valsadia, P.; Kern, T. S.; Roberts, R. Evidence for diffuse central retinal edema *in vivo* in diabetic male Sprague Dawley rats. *Plos One* **2012**, *7*, e29619.

43. Benitez, S.; Sanchez-Quesada, J. L.; Ribas, V.; Jorba, O.; Blanco-Vaca, F.;

Gonzalez-Sastre, F.; Ordonez-Llanos, J. Platelet-activating factor acetylhydrolase is mainly associated with electronegative low-density lipoprotein subfraction. *Circulation* **2003**, *108*, 92-96.

Table of contents graphic



14c

rhLp-PLA_2 IC_{50} = 2.2 nM
oral bioavailability= 45%