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Design, Synthesis, and Structure-Activity Relationship Study of Pyrazolones as Potent Inhibitors of Pancreatic Lipase

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Pancreatic lipase (PL), a key target for the prevention and treatment of obesity, plays crucial roles in the hydrolysis and absorption of in dietary fat. In this study, a series of pyrazolones was synthesized, and their inhibitory effects against PL were assayed by using 4-methylumbelliferyl oleate (4-MUO) as optical substrate for PL. Comprehensive structure–activity relationship analysis of these pyrazolones led us to design and synthesize a novel compound **P32** (5-(naphthalen-2-yl)-2-phenyl-4-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-pyrazol-3-one) as a potent mixed-competitive inhibitor of PL (IC_{50} = 0.30 μM). In addition, **P32** displayed some selectivity over other known serine hydrolases. A molecular docking study for **P32** demonstrated that the inhibitory activity of **P32** towards PL could be attributed to the π-π interactions of 2-naphthyl unit (R^1) and hydrophobic interactions of phenyl moiety (R^3) with the active site of PL. Thus, **P32** could serve as promising lead compound for the development of more efficacious and selective pyrazolones-type PL inhibitors for biomedical applications.

Obesity is a multifactorial disease defined as a long-term energy in take over consumption, resulting in excessive energy stored in the body in the form of fat (adipose tissue).[1] Being obese increases the risk of many diseases and health conditions, including type 2 diabetes,[2] coronary heart disease (CHD),[3] dyslipidemia,[4] hypertension,[5] nonalcoholic fatty liver,[6,7] sleep apnea.[8,9] As rates of obesity have soared over the past two decades, it is increasingly clear that the number of people who cannot achieve and maintain a healthy weight is not limited to adults, but also includes children.[10] Obesity has become a critical public health issue that is currently affecting billions of people worldwide, especially children and adolescents. Hence, prevention and treatment of obesity have become global task. Numerous methods are available for the prevention and treatment of obesity before serious clinical consequences become apparent, such as lifestyle changes and drug intervention.[11] Among these, pharmacotherapy is considered to be the most commonly used one for the treatment of obesity.

Pancreatic lipase (triacylglycerol acyl hydrolase, PL,E.C. 3.1.1.3), one of the most important serine hydrolases distributed in the gastrointestinal tract, catalyzes the hydrolysis of the ester bond of triacylglycerols to monoacylglycerols and fatty acids, and plays a key role in dietary triacylglycerol absorption (hydrolysis of 50–70% of total dietary fats).[12,13] The inhibition of PL could restrain the hydrolysis of dietary glycerides of fat, and thus reduce the subsequent intestinal absorption of the lipolysis products (free fatty acids and monoacylglycerols). Therefore, PL has become a promising target for the treatment of obesity.[14] The first PL inhibitor orlistat (tetrahydrolipstatin) had been approved by the Food and Drug Administration (FDA) for the treatment of obesity in 1999.[15] At present, orlistat remains the only PL inhibitor approved by FDA for obesity management in conjunction with a reduced caloric diet. However, orlistat can cause non-negligible adverse effects, including stomach pain, steatorrhea and incontinence, abdominal cramping and fat-soluble vitamin deficiencies, hepatotoxicity, and acute pancreatitis.[16,17] Thus, it is highly desirable to find more safe and effective PL inhibitors for the prevention and treatment of obesity.

Pyrazolone, which was reported more than one century ago, is a privileged five-membered nitrogen-containing pharmacophore in medicinal chemistry as its derivatives possessing many important biological properties.[18] The very first synthetic antipyretic and analgesic drug phenazone was synthesized in 1883.[19] In recent years, many efforts have been devoted to develop pyrazolone based lead compounds and drugs, such as neuroprotective drug edaravone, antibacterial reagent, antitumor reagent, p38 inhibitors, and our previously developed hCE1 inhibitor, and so on (Scheme 1).[20–22] In continue with our interest in the development of lead compounds with pyrazolone scaffold, the design of novel pancreatic lipase inhibitor featuring this simple and easily accessible scaffolds will be a promising candidate to overcome some of the side-effect of orlistat. Herein, a series of easily accessible pyrazolones (**P1**–**31**) were synthesized by a tow-step protocol and the inhibitory effects of these compounds on PL were determined by using 4-methylumbelliferyl oleate (4-MUO) as substrate probe. Comprehensive structure-activity relationship study brought novel

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cmdc.202000850

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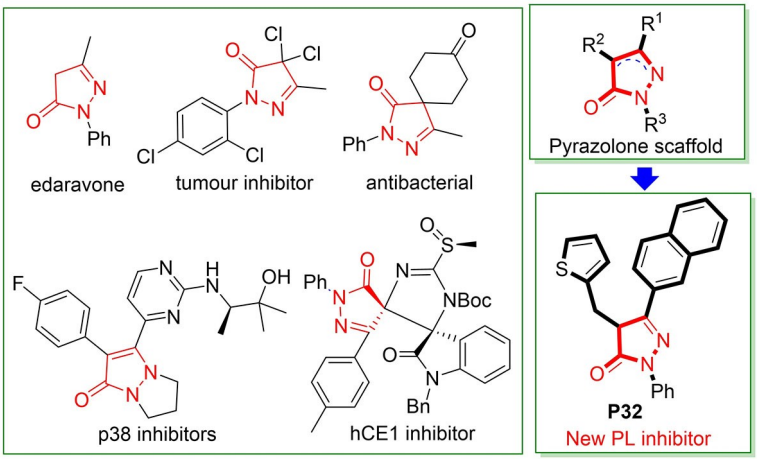
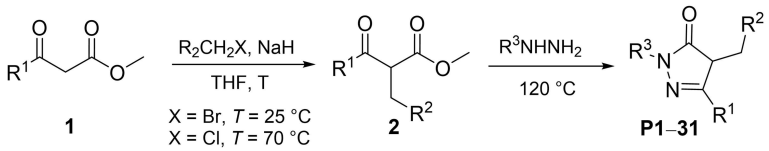


Scheme 1. Examples of pyrazolone-based biological active compounds and the newly designed PL inhibitor.

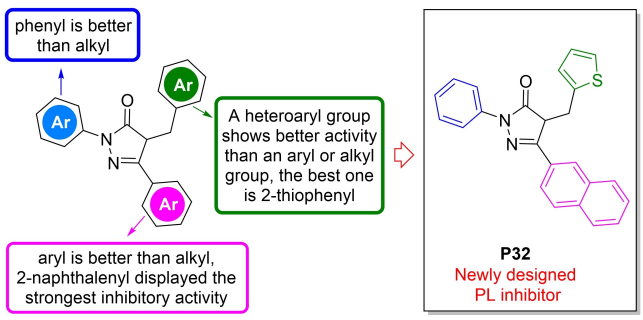


Scheme 2. Synthesis of pyrazolones **P1**–**31**.

insights into the structure modification. A new 4-mono-substituted pyrazolone **P32** was designed and developed as a powerful pancreatic lipase inhibitor (Scheme 1).

Our experiments were conducted with the preparation of a series of variously substituted pyrazolones (**P1**–**31**) by a two-step protocol (Scheme 2). Mono-alkylation of β-keto esters **1** with alkenyl halide in the presence of NaH in THF afforded the mono-substituted β-keto esters **2** in quantitative yield. The following reaction with substituted hydrazine at 120 °C gave the corresponding pyrazolone **P1**–**31** (see the Supporting Information for more details).

Thirty-one pyrazolones (**P1**–**P31**) were assayed for their inhibitory effects against PL. As shown in Table 1 (entries 1–31), pyrazolone **P1** with methyl (R^1), benzyl (R^2) and phenyl (R^3) substitutes demonstrated poor inhibitory effects on PL. Then, we focused our attention on the variation of the R^2 moiety of pyrazolones (**P2**–**P8**). The phenyl unit (R^2) of pyrazolone with bromine substitute at the 4-position (**P2**) also displayed poor inhibition toward PL with IC_{50} value of 95 μM, but the phenyl unit with 4-chlorine (**P3**) and 4-methoxyl (**P4**) group showed good inhibitory effect against PL with IC_{50} values of 23 and 15 μM, respectively. Compound **P5** and **P6**, containing 2-nitrophenyl and 2-methylphenyl unit, respectively, demonstrated moderate inhibitory effects on PL, while the variation of the phenyl group with 2-furanyl (**P7**) or 2-thienyl group (**P8**) led to an increase of the inhibitory effects on PL. Compound **P9** with ethyl substitute (R^1) displayed stronger inhibition toward PL than compound **P1** (R^1, methyl), while the variation of the ethyl unit with phenyl unit compound **P10** finally resulted in a significant increase of potency (IC_{50}, 10 μM). Further characterizations of compounds **P11**–**P24** with the variation of R^2 unit demonstrated that compound **P22** with the 2-thiopheny substitute is more beneficial for compound inhibitory property toward PL with IC_{50} value of 2.1 μM. Compound **P27** with 2-naphthyl unit (R^1) afforded increased inhibitory effect against PL compared with compound **P10**, while the variation of the phenyl group (R^3) with and alkyl group (**P30** and **P31**) finally resulted in a loss of potency. Comprehensive SAR analysis of all tested pyrazolones (**P1**–**P31**) provide insights into the relationships linking between the inhibitory effects on PL and the substituent properties of these compounds. The 2-naphthyl unit (R^1), 2-thienyl (R^2), and phenyl (R^3) moieties were beneficial for pyrazolones inhibitory property toward PL, and led to a dramatic increase of the inhibitory effect on PL (Scheme 3).



Scheme 3. The structure–activity relationships and design of the novel potent inhibitor.

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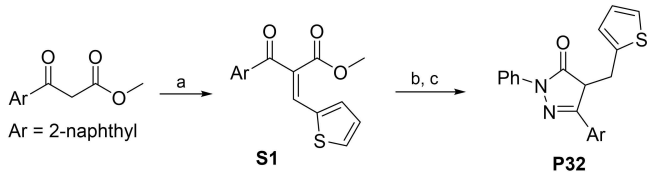
Table 1. The IC_{50} values of pyrazolones toward PL.[a]

	P	R^1	R^2	R^3	IC_{50} (PL) [μM]
1	**P1**	Me	Ph	Ph	>100
2	**P2**	Me	4-Br-C_6H_4	Ph	95±15
3	**P3**	Me	4-Cl-C_6H_4	Ph	23±2.7
4	**P4**	Me	4-OMe-C_6H_4	Ph	15±4.2
5	**P5**	Me	2-NO_2-C_6H_4	Ph	45±11
6	**P6**	Me	2-Me-C_6H_4	Ph	63±12
7	**P7**	Me	2-furanyl	Ph	16±2.6
8	**P8**	Me	2-thienyl	Ph	9.4±1.6
9	**P9**	Et	Ph	Ph	57±5.0
10	**P10**	Ph	Ph	Ph	10±1.9
11	**P11**	Ph	4-Me-C_6H_4	Ph	7.6±2.0
12	**P12**	Ph	3-Me-C_6H_4	Ph	19±3.5
13	**P13**	Ph	2-Me-C_6H_4	Ph	11±1.5
14	**P14**	Ph	4-OMe-C_6H_4	Ph	4.0±0.4
15	**P15**	Ph	4-F-C_6H_4	Ph	14±1.8
16	**P16**	Ph	2-Cl-C_6H_4	Ph	9.1±1.0
17	**P17**	Ph	4-Br-C_6H_4	Ph	8.9±1.2
18	**P18**	Ph	4-CF_3-C_6H_4	Ph	9.4±1.5
19	**P19**	Ph	3,5-$(CF_3)_2$-C_6H_3	Ph	9.0±0.8
20	**P20**	Ph	2-NO_2-C_6H_4	Ph	22±2.9
21	**P21**	Ph	1-naphthyl	Ph	3.9±0.40
22	**P22**	Ph	2-thienyl	Ph	2.1±0.32
23	**P23**	Ph	Et	Ph	32±3.5
24	**P24**	Ph	$CHCH_2$	Ph	22±3.6
25	**P25**	4-Me-C_6H_4	Ph	Ph	2.2±0.36
26	**P26**	3-Me-C_6H_4	Ph	Ph	1.0±0.13
27	**P27**	2-naphthyl	Ph	Ph	0.71±0.055
28	**P28**	2-F-C_6H_4	Ph	Ph	13±1.4
29	**P29**	2-thienyl	Ph	Ph	11±2.7
30	**P30**	Ph	Ph	Me	>100
31	**P31**	Ph	Ph	Cy	12±2.5
32	**P32**	2-naphthyl	2-thienyl	Ph	0.30±0.050
33	orlistat[b]				0.19±0.045

[a] All experimental data are averages of at least three independent experiments. [b] A positive inhibitor of PL.

Guided by these SAR results, we next designed and synthesized a new 4-mono-substituted pyrazolone **P32** that might exhibit a more potent inhibitory effect on PL (Scheme 3). The synthetic route is described in Scheme 4. Gratifyingly, pyrazolones **P32** was prepared from the methyl 3-(naphthalen-2-yl)-3-oxopropanoate according a three-step procedure to harvest the target compound. The novel pyrazolone **P32** showed unusually potent inhibitory activity against PL with much lower IC_{50} value of 0.30 μM (Table 1, entry 32). For comparison, the known drug orlistat was tested under identical conditions as a positive control (entry 33). The result indicated that compound **P32** showed similar inhibitory effect compared with orlistat.



Scheme 4. Synthesis of pyrazolone **P32**. a) 2-Thenaldehyde, pyrrolidine (10 mol%), AcOH (10 mol%), toluene, reflux; b) $NaBH_4$, pyridine, RT; c) $PhNHNH_2$, 120 °C.

In order to further prove the specificity of compound **P32** on PL, the selectivity of **P32** inhibitory effect was then carried out by a panel of most known serine hydrolase including human carboxylesterase 1 (hCES1A), human carboxylesterase 2 (hCES2A), dipeptidyl peptidase IV (DPP-IV), butyrylcholinesterase (BChE) and thrombin according to the known assays.[23–28] As shown in Table 2, compound **P32** was found with lower inhibitory effect on hCES1 A (IC_{50}, 0.77 μM) and hCES2 A (IC_{50}, 6.3 μM) compared with PL (IC_{50}, 0.30 μM), and a poor inhibitory effect on BChE, DPP-IV and thrombin with IC_{50} values greater than 100. These results indicate that compound **P32** is a good inhibitor of PL with some selectivity over other serine hydrolases.

The strong inhibition potency of compound **P32** against PL encouraged us to further investigate the inhibition types and the inhibition constants (K_i) of this compound toward PL, which would facilitate to deeply understand the interactions between **P32** and PL. As shown in Figure 1, the inhibitory behavior of **P32** against PL-mediated 4-MUO hydrolysis was performed. The dose-dependent inhibition curve demonstrated that **P32** potent inhibitory activity against PL. When the **P32** concentration is greater than 5 μM, the residual activity of PL is less than 20%. In addition, the double reciprocal of Lineweaver-Burk plot clearly demonstrated that a family of straight lines were intersected at the first quadrant of the *x*,*y*-plane. Thus, compound **P32** could

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Table 2. IC_{50} values of compound P32 towards serine hydrolases.[a]

	Serine hydrolase	IC_{50} [μM]
1	PL	0.30 ± 0.050
2	hCES1A	0.77 ± 0.066
3	hCES2A	6.3 ± 1.0
4	BChE	> 100
5	DPP-IV	> 100
6	thrombin	> 100

[a] All experimental data are averages of at least three independent experiments.

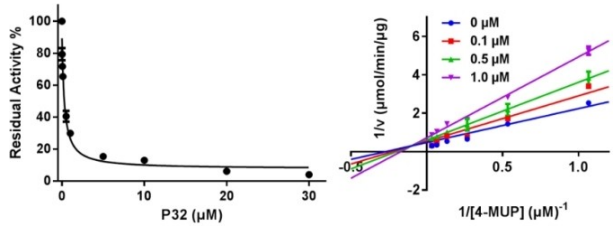

Figure 1. Inhibition behaviors of **P32** against PL mediated 4-MUO hydrolysis. Left: the dose-dependent inhibition curve. Right: the Lineweaver–Burk plot. All data represent the mean of triplicate determinations.

inhibit PL in a mixed-inhibition manner, with the K_i value of 0.77 μM.[29] We could infer from the results that the inhibitor **P32** may be not directly bind the hydrolysis active site (Ser153) of the PL to against the hydrolysis of substrate 4-MUO.

To explore the interactions between **P32** and PL at the atomic level, molecular docking was carried out. As depicted in Figure 2A, compound **P32** could be well-docked into the hydrophobic catalytic cavity of PL. As shown in Figure 2B, the 2-naphthyl ring of **P32** could create π-π interactions with Phe78, Phe216, and Tyr115 of PL, suggesting that the 2-naphthyl unit (R^1) was important for PL inhibition. Meanwhile, we also found that a phenyl (R^3) moiety could form hydrophobic interactions with Arg257, Val260, Ala261, and Leu265, thus suggesting that the phenyl ring was also beneficial for PL inhibition. In addition, the docking of compound **P32** and 4-MUO with the active site of PL (Figure S2) showed that the distance between the ester linkages of the substrate 4-MUO and catalytic residue Ser153

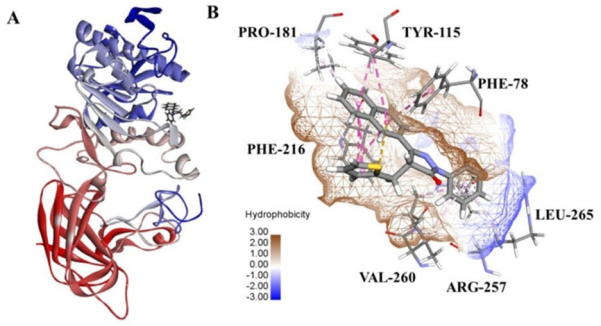

Figure 2. A) Overview of compound **P32** docked into the activity pocket of PL. B) Detailed view of the interacting residues of compound **P32**.

Oγ is 3.2 Å. however, there is no obvious interaction between non-covalently inhibitor **P32** and catalytic residue Ser153.[30] These observations were highly consistent with the structure-PL inhibition relationship studies above, as well as explained why compound **P32** exhibited the most potent PL inhibition activity in a mixed-inhibition manner against PL-mediated 4-MUO hydrolysis.

In summary, a novel pancreatic lipase (PL) inhibitor **P32** based on pyrazolone scaffold featuring 2-naphthyl (R^1), 2-thienyl (R^2), and phenyl (R^3) substituents was developed through a comprehensive structure-activity relationship analysis. The pyrazolone **P32**, a mixed-competitive inhibitor, showed strong activity to inhibit PL (IC_{50} 0.30 μM) and displayed some selectivity over other known serine hydrolase (hCES1A, hCES2A, DPP-IV, BChE and thrombin). In addition, a molecular docking study demonstrated that the π-π interactions of 2-naphthyl unit (R^1) and hydrophobic interactions of phenyl moiety (R^3) with the active site of PL should be responsible for the potent inhibitory activity of **P32** towards PL. This novel and promising lead compound could enhance the development of pyrazolone-type PL inhibitors for the prevention and treatment of obesity.

Acknowledgements

Authors are grateful for the support of Zhejiang University of Technology (2019414820029), the National Science and Technology Major Project of China (2018ZX09731016-004), and the National Key Research and Development Program of China (2017YFC1702000).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: inhibitors · pancreatic lipases · pyrazolones · structure-activity relationships

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[30] See Figure S3 in the Supporting Information for more details.

Manuscript received: October 28, 2020
Revised manuscript received: January 30, 2021
Accepted manuscript online: February 2, 2021
Version of record online: ■■, ■■■

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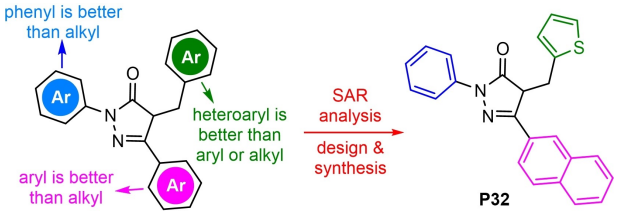

Weighty matters: A new, potent, mixed-competitive pancreatic lipase (PL) inhibitor **P32** has been developed after comprehensive structure-activity relationship analysis. It could serve as promising lead compound for the development of more efficacious and selective pyrazolones-type PL inhibitors for the prevention and treatment of obesity.