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Quinoline-4-methyl esters as human nonpancreatic secretory phospholipase A_2 inhibitors

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ABSTRACT

A series of novel fused heterocycle methyl esters were designed and synthesized as human nonpancreatic secretory phospholipase A_2 (hnps-PLA_2) competitive inhibitors. Among the 22 synthesized compounds, 17 quinoline-4-methyl esters displayed hnps-PLA_2 inhibition activity in the in vitro bioassay. The IC_{50} value for the best compound **3o** was 1.5 μM. The structure-inhibition–activity relationships of the compounds were studied using molecular docking.

1. Introduction

The phospholipase A_2 superfamily consists of more than 100 enzymes, which are defined by their ability to catalyze hydrolysis of the sn-2 ester bond of phosphoglycerides to yield fatty acids and lysophospholipids.[1] The released fatty acids, such as arachidonic acid, may act as second messengers and precursors of eicosanoids, including prostaglandins and leukotrienes that exacerbate many diseases with an inflammatory component.

Secretory phospholipase A_2 (sPLA$_2$) is Ca^{2+}-dependent and considered to be one of the most important targets for anti-inflammation therapy. Human nonpancreatic secretory phospholipase A_2 (hnps-PLA_2) with high levels of activity is found in synovial fluid from arthritis patients.[2] Hnps-PLA_2 is also used as an independent predictor of cardiovascular events in relation to its hydrolysis of low density lipoprotein (LDL).[3] Although an hnps-PLA_2 inhibitor as a treatment for rheumatoid arthritis,[4] the same inhibitor succeeded in reducing hnps-PLA_2 concentration as well as oxidized LDL concentration and C-reactive protein (CRP) in patients with coronary heart disease.[5] Recently, PLA_2 have been paid more attention for its relationship with cardiovascular events and cancer.[6,7]

We previously reported that quinoline compounds from structural based drug design as hnps-PLA_2 inhibitors.[8] Molecular docking results gave hints about how to further develop hnps-PLA_2 inhibitors with 2,4-disubstitute quinoline: (i) the quinoline scaffold should be retained in the substrate binding site; (ii) the 2-substitute group is necessary to increase the hydrophobic interactions; and (iii) the 4-substitute group is also necessary and must possess an oxygen atom to form hydrogen bonds and interact with the calcium ion.

We recently studied quinoline-4-methyl esters in order to develop novel hnps-PLA_2 inhibitors. Here, we report the synthesis and bioassay of a series of novel fused heterocycle methyl esters. These compounds were docked to the hnps-PLA_2 substrate binding site and analyzed for their binding interactions. The structure–activity relationships of this series of compounds were also discussed.

2. Chemistry

The 4-quinoline carboxylic acid scaffold of these compounds was achieved by two methods. (1) Pfitzinger synthesis:[9] the condensation of isatin with acetophenone was carried in the presence of alkalies; and (2) Doebner–Miller synthesis:[10] the cyclocondensation of benzaldehyde with pyruvic acid and aniline was made. Then, by esterification of 4-quinoline carboxylic acid, 22 new quinoline-4-methyl esters were obtained. Their structures were confirmed by IR, ^{1}H NMR, MS and elemental analysis. The synthesis of quinoline-4-methyl esters **3a**–**o** is depicted in Scheme 1.

The starting materials were isatin or 5-substitute isatin (**1a**–**d**) and one kind of acetophenone. When catalyzed by alkalis, the

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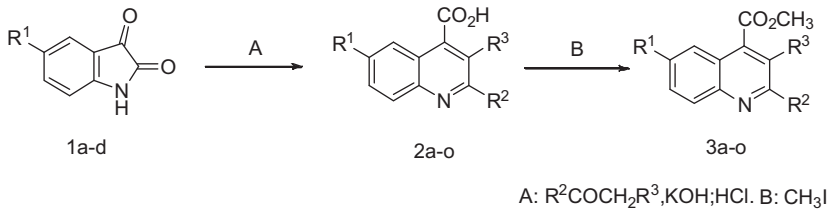
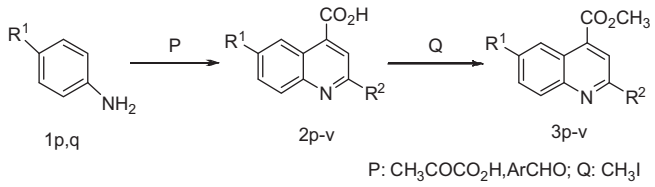


Scheme 1. Pfitzinger synthesis of quinoline-4-methyl esters **3a**–**o**.



Scheme 2. Doebner–Miller ring formation synthesis of quinoline-4-methyl esters **3p**–**v**.

isatin ring was opened and changed into a quinoline ring or other fused heterocycle, different 2- or 3- or 6-substitutes depending on the starting materials. After acidification, carboxylic acid **2a**–**o** was obtained. Then, **2a**–**o** reacted with iodomethane under the catalysis of potassium carbonate, **3a**–**o** was obtained in high yield, as expected.

The synthesis of other compounds is depicted in Scheme 2.

The starting material **1p**,**q** was 4-substitute aniline. When it reacted with pyruvic acid and one kind of benzaldehyde, a quinoline ring was formed. Generally, quinoline-4-carboxylic acid **2p**–**v** was obtained in medium or high yield. Then, **2p**–**v** reacted with iodomethane under the catalysis of potassium carbonate, to give **3p**–**v**.

3. Results and discussion

All synthesized quinoline-4-methyl esters were tested for their ability to inhibit hnps-PLA_2 (Table 1)

Most compounds showed significant inhibition to hnps-PLA_2. IC_{50} value of eight compounds (**3a**–**3d**, **3i**, **3j**, **3o** and **3q**) reached the micromolar range, and compound **3o** showed the strongest inhibition with an IC_{50} value of 1.5 μM. Another seven compounds (**3k**, **3l**, **3n**, **3p**, and **3r**–**t**) had IC_{50} values lower than 100 μM, ranging from 12 to 57 μM. Although compound **3e** and **3h** showed strong inhibition at 10 μM, the inhibition strength dropped sharply along with the decrease of compound concentration, thus their IC_{50} values could not be determined using the current assay system.

Among the 15 compounds with 2-aryl substituted quinoline backbones, 13 significantly inhibited the activity of hnps-PLA_2. Different combinations of the 2-aryl and 6-substitute group had complex effect on inhibition: when the 2-aryl was 4-methoxyphenyl, 6-chloro (**3a**) or 6-fluoro (**3j**) compound had stronger inhibition activity than 6-methoxy compound (**3r**); but for 2-phenethyl substituted compounds, the IC_{50} values of 6-chloro (**3b**) and 6-methoxy (**3o**) compounds were close but one magnitude smaller than that of the 6-fluoro substituted compound (**3l**). Compounds **3q**–**v** only differed in substitute group in the p-position on benzyl of quinoline. They showed decreasing inhibition activity with the increasing carbon chain length.

There were 4 compounds having 3-aryl substituted quinoline backbones. **3c** and **3d** with 6-chloro substitution had stronger inhibition activity than **3k** (6-fluoro) and **3n** (6-methoxy).

To prove whether the quinoline ring could be extended, compounds **3f**, **3g**, and **3m** were designed, but none of them had inhibition activity.

Molecular docking (performed with program AutoDock 4.0[11]) was used to study the structure and activity relationship of the compounds. All the synthesized quinoline-4-methyl esters were docked into the hnps-PLA_2 substrate binding site.

Docking results showed that the 2-aryl substituted compounds bound to hnps-PLA_2 in an orientation similar to LY311727 (Fig. 1). The strongest inhibitor **3o**, for example, had its quinoline ring located in the center of the pocket, and the phenethyl group was in the hydrophobic site close to the N-terminal end, corresponding with other docking studies.[12,13] The inhibitor interacted with the

Table 1
Hnps-PLA_2 inhibition activity of quinoline-4-methyl esters

Compound	R^1	R^2	R^3	IC_{50}[a] (μM)
Positive control[14]				1.0 ± 0.1
3a	Cl–	4-CH_3OPh–	H–	8.1 ± 0.1
3b	Cl–	$PhCH_2CH_2$–	H–	1.8 ± 0.1
3c	Cl–	CH_3–	Ph–	2.9 ± 0.3
3d	Cl–	CH_3–	$PhCH_2$–	2.8 ± 0.4
3e	Cl–	2-CH_3OPhCH_2–	H–	87%[b]
3f	Cl–	–$CH_2CH_2CH_2CH_2$–		Inactive
3g	H–	–$CH_2CH_2CH_2CH_2$–		Inactive
3h	CF_3–	4-CH_3OPh–	H–	71%[b]
3i	F–	2-CH_3OPhCH_2–	H–	2.3 ± 0.2
3j	F–	4-CH_3OPh–	H–	6.0 ± 0.1
3k	F–	CH_3–	$PhCH_2$–	16 ± 4
3l	F–	$PhCH_2CH_2$–	H–	12±1
3m	F–	–CH_2CH_2 CH_2CH_2–		Inactive
3n	CH_3O–	CH_3–	$PhCH_2$–	34 ± 2
3o	CH_3O–	$PhCH_2CH_2$–	H–	1.5 ± 0.1
3p	H–	4-C_4H_9OPh–	H–	44 ± 3
3q	CH_3O–	Ph–	H–	2.0±0.6
3r	CH_3O–	4-CH_3OPh–	H–	15 ± 2
3s	CH_3O–	4-C_3H_7OPh–	H–	27 ± 4
3t	CH_3O–	4-C_4H_9OPh–	H–	57 ± 3
3u	CH_3O–	4-$C_5H_{11}OPh$–	H–	Inactive
3v	CH_3O–	4-$C_6H_{13}OPh$–	H–	Inactive

[a] Average ± SD of triplicate individual results.
[b] Inhibition at 10 μM.

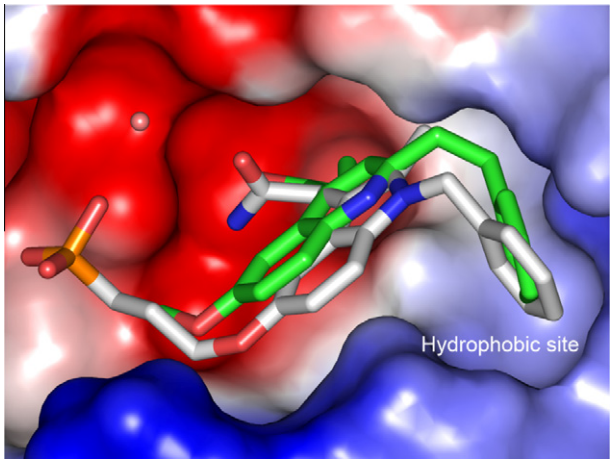


Figure 1. Comparison of **3o** (green) and LY311727 (white)[17,18] binding to hnps-PLA_2.

calcium ion using the ester carbonyl oxygen atom. This interaction is an important driving force for the binding of hnps-PLA_2 to the well-known indole inhibitors (e.g., LY311727) and the quinoline inhibitors we previously reported.[8] The ester groups went deep into the pocket.

The conformation of other 2-aryl substituted compounds changed slightly to maintain these contacts. In compound **3q** (Fig. 2A), the benzyl group almost fully occupies the hydrophobic site, so for **3r–v**, substitute group on the benzene ring would collide with the protein and reduce the binding affinity. But when the 6-substitute group was small as a halogen atom, the compound can slightly move away from the hydrophobic site to avoid the collision, so the inhibition activities of **3a** and **3j** were stronger than **3r**. In this binding conformation, there was little space around the 3-position of quinoline ring. This can explain compounds **3f**, **3g**, and **3m** were inactive, but conflicted with the strong inhibition of **3c** and **3d** to hnps-PLA_2. Molecular docking figured that 3-aryl substituted compounds bound to the enzyme in a reverse orientation (Fig. 2B). The quinoline ring moved toward the hydrophobic site, and the phenyl ring was close to the calcium ion. The ester group also pointed toward a side of the pocket. So far no protein-inhibitor complex structure similar to this conformation has been recorded in PDB, but it accorded with all our enzyme inhibition data: if compounds **3f**, **3g**, or **3m** bound to hnps-PLA_2 in this orientation, they would still collapse with the protein. To validate the reliability of this binding mode, molecular docking of **3c** and **3d** are also performed using programs DOCK 6.0[15] and Glide[16]: both programs gave the same orientation as AutoDock did for **3c**; for **3d**, Glide program provides a different result while there is no different orientation between DOCK and AutoDock results.

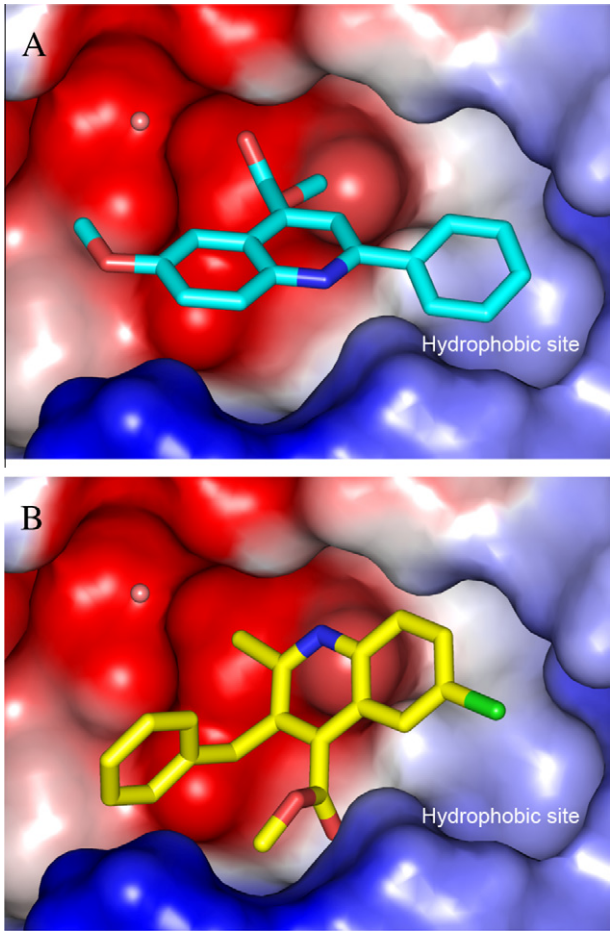


Figure 2. Different binding modes of inhibitors with hnps-PLA_2 substrate binding site. (A) **3q** (cyan), representing the 2-substituted quinoline ester binding mode; (B) **3d** (yellow), representing the 3-substituted quinoline ester binding mode (predicted by molecular docking).

Molecular docking results gave clues on the further optimization of the hnps-PLA_2 inhibitors with a quinoline-4-methyl ester backbone. Both 2- and 3-aryl substitution of quinoline ring provided good starting point for modification. For 2-aryl substituted compounds, not much space around the phenyl ring was available. However, the quinoline part was close to a larger pocket, so substitution at the 6- or 7-position may improve the binding affinity. For 3-aryl substituted compounds, more functional groups could be added to the phenyl ring and 6- or 7-position of the quinoline ring. Substitution at 5- and 8-position of the quinoline ring may decrease the inhibition activity of the compound for both 2-aryl and 3-aryl substituted compounds.

4. Conclusions

We have designed and synthesized a series of quinoline-4-methyl esters and tested their inhibitory activity against hnps-PLA_2.

In all the 22 synthesized compounds, 17 showed hnps-PLA_2 inhibition activity, and eight had IC_{50}s less than 10 μM. The best one, **3o**, showed significant inhibition with an IC_{50} of 1.5 μM.

Based on the structure–activity relationship analysis of this series of compounds, we concluded that both 2- and 3-aryl substitute quinoline-4-methyl esters were good backbones to develop hnps-PLA_2 inhibitors. The current study provides insights into the design of more effective hnps-PLA_2 inhibitors.

5. Experimental section

5.1. Chemistry

5.1.1. General

Melting points were obtained using an X6 apparatus, which were uncorrected. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer with a Nic-Plan IR microscope (54183). Mass spectra were recorded on a VG-ZAB-HS spectrometer. ^{1}H NMR spectra were measured on a Brucker APX 400 spectrometer using TMS as internal standard.

All compounds were routinely checked by TLC and ^{1}H NMR. TLC was performed with aluminum-baked silica gel plates. Developed plates were visualized by UV light. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Solutions were concentrated after reactions and extractions with a rotary evaporator operating at reduced pressure. The reagents and solvents were available commercially and purified according to conventional methods. The newly synthesized compounds were analyzed for C, H, N, and, when present, Cl and F. Analytical results agreed to within 0.4% of the theoretical values.

5.1.2. General procedure for the preparation of compounds 3a–o; example: methyl 6-chloro-2-(4-methoxyphenyl) quinoline-4-carboxylate (3a)

5.1.2.1. 6-Chloro-2-(4-methoxyphenyl) quinoline-4-carboxylic acid (2a). 5-Chloroindoline-2, 3-dione (0.908 g, 5 mmol) was weighed into a round-bottom flask and 4 mL, 33% potassium hydroxide solution was added. The reaction mixture was refluxed with magnetic stirring for 0.5 h and cooled to 80 °C. Then 1-(4-methoxyphenyl) ethanone (0.9 g, 65 mmol) was added, refluxed for 20 h, and cooled to room temperature. Then 200 mL water was added to the mixture, and extracted twice with ether; the water layer was acidified by slowly adding 6 M hydrochloric acid to pH 3. A light yellow solid formed. The product was collected

by suction filtration and air-dried. The crude product (**2a**) was obtained at 66% yield.

5.1.2.2. Methyl 6-chloro-2-(4-methoxyphenyl) quinoline-4-carboxylate (3a). 6-Chloro-2-(4-methoxyphenyl) quinoline-4-carboxylic acid (**2a**) (0.98 g, 3 mmol) and potassium carbonate (2.07 g, 15 mmol) were weighed into a round-bottom flask. Methyl iodide (0.95 mL, 15 mmol) and acetone (4 mL) were added. The reaction mixture was refluxed with magnetic stirring. The progress of the reaction was monitored by TLC. The reaction was completed after 5 h. The solvent was evaporated in vacuo and water was added to the remaining mixture. The product was collected by suction filtration and air-dried. The crude product was separated on a silica gel column to obtain pure **3a** at 90% yield. mp: 169–170 °C; IR (neat): ν = 1721 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.90 (s, 3H, OCH_3), 4.08 (s, 3H, CO_2CH_3), 7.03–8.79 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{18}H_{14}ClNO_3$: C, 65.96; H, 4.305, N, 4.273. Found: C, 65.89; H, 4.304, N, 4.239.

Compounds **3b**–**o** were synthesized by a similar method. The data are listed below:

5.1.2.3. Methyl 6-chloro-2-phenethylquinoline-4-carboxylate (3b). White powder; 92%, 90–91 °C; IR (neat): ν = 1721 cm^{-1}(C=O); ^1H NMR ($CDCl_3$): δ = 3.18 (t, 2H, CH_2), 3.32 (t, 2H, CH_2), 4.03 (s, 3H, CO_2CH_3), 7.20–8.79 (m, 9H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}ClNO_2$: C, 70.04; H, 4.949, N, 4.299. Found: C, 70.04; H, 4.922; N, 4.315.

5.1.2.4. Methyl 6-chloro-3-phenyl -2- methylquinoline-4-carboxylate (3c). White powder; 91%, 141–142 °C; IR (neat): ν = 1731 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 2.40 (s, 3H, 3-CH_3), 4.11 (s, 3H, CO_2CH_3), 7.26–8.10 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{18}H_{14}ClNO_2$: C, 69.35; H, 4.526; N, 4.493. Found: C, 69.03; H, 4.557; N, 4.433.

5.1.2.5. Methyl 3-benzyl-6-chloro-2-methylquinoline-4-carboxylate (3d). White powder; 87%, 141–142 °C; IR (neat): ν = 1731 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 2.58 (s, 3H, 3-CH_3), 3.98 (s, 3H, CO_2CH_3), 4.16 (s, 2H, CH_2), 7.08–8.04 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}ClNO_2$: C, 70.04; H, 4.949; N, 4.299. Found: C, 70.01; H, 4.876; N, 4.196.

5.1.2.6. Methyl 6-chloro-2-(2-methoxybenzyl)quinoline-4-carboxylate (3e). White powder; 85%,113–114 °C;IR (neat): ν = 1730 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.82 (s, 3H, OCH_3), 3.98 (s, 3H, CO_2CH_3), 4.38 (s, 2H, CH_2), 6.90–8.74 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}ClNO_3$: C, 66.76; H, 4.718; N, 4.097. Found: C, 66.82; H, 4.603; N, 4.027.

5.1.2.7. Methyl 7-chloro-1,2,3,4-tetrahydroacridine-9-carboxylate (3f). White powder; 70%, 110–111 °C; ^1H NMR ($CDCl_3$): δ = 1.89 (m, 2H, CH_2), 1.98 (m, 2H, CH_2), 2.93 (t, 2H, CH_2), 3.14 (t, 2H, CH_2), 4.07 (s, 3H, CO_2CH_3), 7.27–7.94 (m, 3H, acridine C–H). Anal. Calcd for $C_{15}H_{14}ClNO_2$: C, 65.33; H, 5.117; N, 5.079. Found: C, 65.45; H, 5.252; N, 4.754.

5.1.2.8. Methyl 1,2,3,4-tetrahydroacridine-9-carboxylate (3g). White powder; 72%, 77–78 °C;1H NMR ($CDCl_3$): δ = 1.90 (m, 2H, CH_2), 1.99 (m, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.16 (t, 2H, CH_2), 4.05 (s, 3H, CO_2CH_3), 7.27–8.02 (m, 3H, acridine C–H). Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.266; N, 5.805. Found: C, 74.66; H, 6.116; N, 5.810.

5.1.2.9. Methyl 2-(4-methoxyphenyl)-6-(trifluoromethyl)quinoline-4-carboxylate (3h). White powder; 80%, 117–118 °C; IR (neat): ν = 1723 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.88 (s, 3H, OCH_3), 4.10 (s, 3H, CO_2CH_3), 7.06–9.13 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{14}F_3NO_3$: C, 63.16; H, 3.905; N, 3.876. Found: C, 62.95; H, 4.175; N, 3.974.

5.1.2.10. Methyl 6-fluoro-2-(2-methoxybenzyl)quinoline-4-carboxylate (3i). White powder; 89%, 108–109 °C; IR (neat): ν = 1723 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.86 (s, 3H, OCH_3), 4.01 (s, 3H, CO_2CH_3), 4.41 (s, 2H, CH_2), 6.89–8.43 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}FNO_3$: C, 70.14; H, 4.957; N, 4.305. Found: C, 70.26; H, 4.953; N, 4.332.

5.1.2.11. Methyl 6-fluoro-2-(4-methoxyphenyl)quinoline-4-carboxylate (3j). White powder; 86%, 119–120 °C; IR (neat): ν = 1722 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.89 (s, 3H, OCH_3), 4.06 (s, 3H, CO_2CH_3), 7.03–8.42 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{18}H_{14}FNO_3$: C, 69.45; H, 4.533; N, 4.499. Found: C, 69.63; H, 4.476; N, 4.445.

5.1.2.12. Methyl 3-benzyl-6-fluoro-2-methylquinoline-4-carboxylate (3k). White powder; 80%, 122–123 °C; IR (neat): ν = 1730 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 2.58 (s, 3H, 3-CH_3), 3.99 (s, 3H, CO_2CH_3), 4.17 (s,2H, CH_2), 7.09–8.06 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}FNO_2$: C, 73.77; H, 5.213; N, 4.528. Found: C, 73.88; H, 5.140; N, 4.524.

5.1.2.13. Methyl 6-fluoro-2-phenethylquinoline-4-carboxylate (3l). White powder; 87%, 64–65 °C; IR (neat): ν = 1723 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.18 (t, 2H, CH_2), 3.31 (t, 2H, CH_2), 4.02 (s, 3H, CO_2CH_3), 7.20–8.50 (m, 9H, benzene and quinoline C–H). Anal. Calcd for $C_{19}H_{16}FNO_2$: C, 73.77; H, 5.213; N, 4.528. Found: C, 73.95; H, 5.148; N, 4.511.

5.1.2.14. Methyl 7-fluoro-1,2,3,4-tetrahydroacridine-9-carboxylate; white powder (3m). 80%, 91–92 °C; ^1H NMR ($CDCl_3$): δ = 1.90 (m, 2H, CH_2), 1.98 (m, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.15 (t, 2H, CH_2), 4.06 (s, 3H, CO_2CH_3), 7.26–8.00 (m, 3H, acridine C–H). Anal. Calcd for $C_{15}H_{14}FNO_2$: C, 69.49; H, 5.442; N, 5.402. Found: C, 69.69; H, 5.431; N, 5.396.

5.1.2.15. Methyl 3-benzyl-6-methoxy-2-methylquinoline-4-carboxylate (3n). White powder; 80%, 122–123 °C; ^1H NMR ($CDCl_3$): δ = 2.54 (s, 3H, 2-CH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, CO_2CH_3), 4.15(s, 2H, CH_2), 6.95–7.97 (m, 8H, benzene and quinoline C–H). Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.959; N, 4.358. Found: C, 74.78; H, 5.957; N, 4.275.

5.1.2.16. Methyl 6-methoxy-2-phenethylquinoline-4-carboxylate (3o). White powder; 87%, 121–122 °C; ^1H NMR ($CDCl_3$): δ = 3.15 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.01 (s, 3H, CO_2CH_3), 7.20–8.19 (m, 9H, benzene and quinoline C–H). Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.959; N, 4.358. Found: C, 74.91; H, 5.937; N, 4.329.

5.1.3. General procedure for the preparation of compounds 2p–v; example

5.1.3.1. 2-(4-Pentyloxyphenyl)quinoline-4-carboxylic acid (2u). 4-hydroxybenzaldehyde (1.22 g, 10 mmol) dissolved in DMF (10 mL), potassium hydroxide (0.731 g, 12 mmol) was added. After the reaction mixture was stirred at 120 °C for about 1 h, 1-bromopentane 1.87 mL (15 mmol) was added, then stirred at 120 °C (monitored by TLC). The reaction was completed about 5 h. The solution was cooled, poured into water, and extracted with $CHCl_3$ three times. The combined organic extracts, dried over Na_2SO_4, and concentrated in vacuo. 4-(pentyloxy)benzaldehyde crude product was used to next step without purfication.Under

the room temperature, 4-(pentyloxy)benzaldehyde was slowly dropped into the solution of4-methoxyaniline (0.861 g, 7 mmol) and ethanol (7 mL), strong stirring is necessary. A plenty of white solid was formed, after filtered, washed with water, dried, 1.77 g 4-methoxy-N-(4-(pentyloxy)benzylidene)aniline was got. The yield is 59.8%. Then, 1.77 g 4-methoxy-N-(4-(pentyloxy) benzylidene)aniline was dissolved in ethanol (25 mL), the reaction mixture was refluxed with magnetic stirring, pyruvic acid (9 mL) was dropped and reacted for 6 h. The solution was cooled, and concentrated in vacuo. The crude product was got and recrystallized by ethanol, 0.6229 g 2-(4-pentyloxyphenyl)quinoline-4-carboxylic acid (**2u**) was got, yield 28.5%, mp: 200.4–201.4 °C; IR (neat): ν = 1690 cm^{-1}, (C=O); ^1H NMR (DMSO): 0.91 (t, 3H, –C–CH_3), 1.38–1.45 (m, 4H, –CH_2–), 1.75 (m, 2H, –CH_2–), 3.92 (s, 3H, –OCH_3), 4.04 (t, 2H, –OCH_2–), 7.06–8.41 (m, 8H, Ar–H).

5.1.3.2. Methyl 6-methoxy-2-(4-(pentyloxy)phenyl)quinoline-4-carboxylate (3u). 0.548 g of compound 2u was dissolved in acetone (15 mL), 0.242 g potassium carbonate, methyl iodide (0.95 mL, 15 mmol) were added. The reaction mixture was refluxed with magnetic stirring. The progress of the reaction was monitored by TLC. The reaction was completed after 4 h. The solvent was evaporated in vacuo and water was added to the remaining mixture. Collected the product and dried. The crude product was recrystallized by ethanol, obtain pure **3u** 0.411 g, in 72% yield. mp: 101–102 °C.

IR (neat): ν = 1726 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 0.95 (t, 3H, –C–CH_3), 1.36–1.47 (m, 4H, –CH_2–), 1,81 (m, 2H, –CH_2–), 3.98(s, 3H, –OCH_3), 4.04 (t, 2H, –OCH_2–C), 4.06 (s, 3H, –OCH_3), 7.01–8.38 (m, 8H, Ar–H). Anal. Calcd for $C_{23}H_{25}NO_4$: C, 72.81; H, 6.642; N, 3.692. Found: C, 72.62; H, 6.582; N, 4.004.

Compounds **3p**–**t** and **3v** were synthesized as similar method. The data are listed for each of them:

5.1.3.3. Methyl 2-(4-butoxyphenyl)quinoline-4-carboxylate (3p). White powder; 82%, 119–120 °C; IR (neat): ν = 1720 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 1.00 (t, 3H, –C–CH_3), 1.47–1.59 (m, 2H, –CH_2–), 1.75–1.85 (m, 2H, –CH_2–), 4.06 (t, 2H, –OCH_2–), 4.07 (s, 3H, –OCH_3), 7.03–8.73 (m, 9H, Ar–H). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.311; N, 4.177. Found: C, 75.29; H, 6.451; N, 4.010.

5.1.3.4. Methyl 6-methoxy-2-phenylquinoline-4-carboxylate (3q). White powder; 82%, 116–118 °C; IR (neat): ν = 1720 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.99 (s, 3H, –OCH_3), 4.07 (s, 3H, –$COOCH_3$), 7.40–8.43 (m, 9H, Ar–H). Anal. Calcd for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.155; N, 4.777. Found: C, 73.65; H, 5.166; N, 4.617.

5.1.3.5. Methyl 6-methoxy-2-(4-methoxyphenyl)quinoline-4-carboxylate (3r). White powder; 90%, 113–114 °C; IR (neat): ν = 1719 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 3.89 (s, 3H, –OCH_3), 3.98 (s, 3H, –OCH_3), 4.06 (s, 3H, –$COOCH_3$), 7.03–8.38 (m, 8H, Ar–H). Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.300; N, 4.333. Found: C, 70.40; H, 5.298; N, 4.148.

5.1.3.6. Methyl 6-methoxy-2-(4-propoxyphenyl)quinoline-4-carboxylate (3s). White powder; 87%, 114–115 °C; IR (neat): ν = 1726 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 1.07 (t, 3H, –C–CH_3), 1.85 (m, 2H, –CH_2–), 3.98 (s, 3H, –OCH_3), 4.04 (t, 2H, –OCH_2–), 4.06 (s, 3H, –$COOCH_3$), 7.02–8.38 (m, 8H, Ar–H). Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.77; H, 6.023; N, 3.987. Found: C, 71.43; H, 6.082; N, 4.297.

5.1.3.7. Methyl 2-(4-butoxyphenyl)-6-methoxyquinoline-4-carboxylate (3t). White powder; 82%, 106–107 °C; IR (neat): ν = 1722 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 1.01 (t, 3H, –C–CH_3), 1.52 (m, 2H, –CH_2–), 1.80 (m, 2H, –CH_2–), 3.99 (s, 3H, –OCH_3), 4.06 (t, 2H, –OCH_2), 4.07 (s, 3H, –$COOCH_3$), 7.02–8.39 (m, 8H, Ar–H). Anal. Calcd for $C_{22}H_{23}NO_4$: C, 72.30; H, 6.345; N, 3.824. Found: C, 72.15; H, 6.400; N, 4.104.

5.1.3.8. Methyl 2-(4-(hexyloxy)phenyl)-6-methoxyquinoline-4-carboxylate (3v). White powder; 83%, 109–110 °C; IR (neat): ν = 1714 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ = 0.92 (t, 3H, –C–CH_3), 1.34–1.39 (m, 4H, –CH_2–), 1.49 (m, 2H, –CH_2–), 1.81 (m, 2H, –CH_2–), 3.98 (s, 3H, –OCH_3), 4.40 (t, 2H, –OCH_2), 4.06 (s, 3H, –$COOCH_3$), 7.03–8.38 (m, 8H, Ar–H). Anal. Calcd for $C_{24}H_{27}NO_3$: C, 73.38; H, 6.906; N, 3.581. Found: C, 73.26; H, 6.916; N, 3.560.

5.2. Anti-hnps-PLA_2 activity in vitro

The assay for testing the inhibition activity of the synthesized compounds on hnps-PLA_2 was carried out in a 96-well plate, based on a continuous fluorescence assay using 1-anilinonaphthalene-8-sulfonate (ANS) as an interfacial probe,[19] with 2-(1-benzyl-5-methoxy-2-methyl-1*H*-indol-3-yl) acetamide, a reported hnps-PLA_2 inhibitor[14] as positive control.

The substrate solution was prepared by mixing 50 μL of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, 40 mM, methanol) and 50 μL of deoxycholic acid solution (40 mM, methanol) in dd-H_2O (1 mL), stirring for 1 min and sonicating in a water bath for 5 min. The assay buffer (150 μL), containing 50 mM Tris–HCl (pH 8.0), 100 mM NaCl, 2 mM NaN_3, 5 μg/mL bovine serum albumin (BSA) and 10 μM ANS, was added with 10 μL of the inhibitor stock solution in DMSO, 10 μL of $CaCl_2$ solution (0.1 M) and 20 μL of substrate solution. After incubation at room temperature for 5 min, 10 μL of hnps-PLA_2 solution (3.2 μg/mL) was added for the initiation of the enzymatic reaction and monitored under the wavelengths of excitation 377 nm and emission 470 nm.

The initial velocity of different inhibitor concentrations was used for fitting the IC_{50} with equation: $V_0/V = 1 + [I]/IC_{50}$. All reported IC_{50} values were the results of averages of independent experiments conducted at least three times.

5.3. Molecular docking

The 3D structures of the compounds were built in Schrödinger software using the LigPrep command.[20] The hnps-PLA_2 coordinates was retrieved from the X-ray crystal structure of hnps-PLA_2 in complex with an indole inhibitor (LY311727, PDB entry code: 1DB4).[17,18] Molecular docking with rigid receptors and flexible ligands for all compounds was performed using AutoDock 4.0.[11] Lamarckian genetic algorithm was used for sampling with the following parameters: the number of GA runs, 50; population size, 150; maximum number of energy evaluations, 25,000,000; and maximum number of generations 27,000. Docking results of all 50 runs were clustered with a RMS tolerance of 2.0 Å. The lowest energy conformation in the largest cluster was taken for further analysis. Molecular docking for **3c** and **3d** was also performed using programs DOCK 6.0[15] and Glide.[16] For DOCK 6.0, anchor and grow docking method with default parameters was used. Glide was run Schrödinger software, extra precision, default parameters).

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