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Indole-5-phenylcarbamate derivatives as human non-pancreatic secretory phospholipase A2 inhibitor

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Abstract—The synthesis of the human non-pancreatic secretory phospholipase A2 inhibitor (IC_{50} = 1.81 ± 0.59 μM) is reported.

1. Introduction

A study of rheumatoid arthritis (RA) patients found that serum secretory phospholipase A2 (PLA2) activity correlates with disease activity.[1] When purified PLA2 enzyme from synovial fluid and snake venom were injected into animal joints, an acute inflammatory response with swelling of synovial cells, hyperplasia, and edema have been aggravated.[2,3] High levels of PLA2 are observed in a variety of severe inflammatory diseases and have been considered to be accountable for some inflammatory reactions. Thus, the inhibitors of PLA2 would have great potential for the treatment of inflammatory diseases.

The first indole inhibitor of human non-pancreatic secretory (hnps) PLA2 was reported by Eli Lilly and Company.[4] Then many indole-3-acetamides have been synthesized and showed inhibition of PLA2.[5] Two doses of the indole inhibitors (LY315920Na/S-5920) have been tested for the efficacy and safety, from clinical pharmacology studies, in critically ill patients with sepsis.[6]

Based on the crystal structure of hnps PLA2 complexed with indole inhibitor[4] (PDB code: 1db4), we confirmed that ligation of the catalytic calcium, hydrogen bond, and hydrophobic interaction are the necessary conditions for sPLA2 inhibitors' potency, and the most important condition is the ligand having the right shape for interacting with the hnps PLA2 substrate binding pocket. Different types of fused heterotricyclic compounds were chosen to be candidates. In the present study, we prepared new 1-benzyl-3-(carbamoylmethyl)-2-methyl-1*H*-indol-5-yl-phenylcarbamate derivatives by multistep synthesis and tested their inhibitory activity. The compound had showed good inhibition as expected.

2. Design

All molecular modeling and docking calculations were performed on the Linux workstation by using SYBYL 6.91[7] molecule modeling software and Autodock3.05.[8] The crystal structure of hnps PLA2 complexed with indole inhibitor[4] (PDB code:1db4) was used as the starting complex structure. Compounds **1**, **3**, and **5** were built using SKETCH option in SYBYL. The atomic charges were calculated with the Gasteiger–Hückel method. The molecules were geometry-optimized in standard Tripos force field using the steepest descent and conjugate gradients methods.

3. Synthesis

Indomethacin **1** was used as starting material. Using mixed anhydride method, **1** reacted with ethyl chloroformate, followed by mixing with ammonia, NaOH, and then acidification of the mixture, 5-methoxy-2-methyl-1*H*-indole-3-acetamide **2** was obtained in high yields. Compound **2** was alkylated on the indole nitrogen by forming the sodium salt with sodium hydride and treated with benzyl chloride, 5-methoxy-2-methyl-1-(phenylmethyl)1*H*-indole-3-acetamide **3** was obtained. Boron

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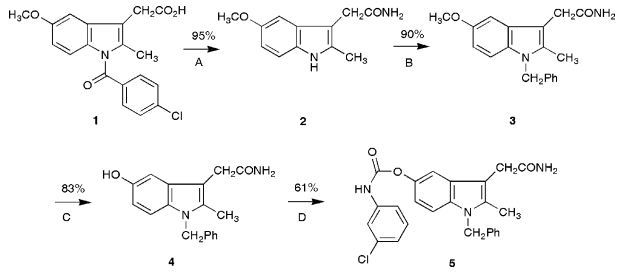

Scheme 1. Synthetic approach. Reagents: (A) $ClCO_2C_2H_5$, $NH_3{\cdot}H_2O$, NaOH; (B) NaH, $PhCH_2Cl$, DMF; (C) BBr_3, CH_2Cl_2; (D) 3-ClPhCNO, NEt_3, CH_3CN.

tribromide demethylation of **3** gave the hydroxyl indole **4**, which was treated with substituted phenylisocyanates and Et_3N in CH_3CN to give the title compounds **5**.

Our general synthetic approach to the title compounds is shown in Scheme 1.

4. Biological evaluation

The fluorescence-based assay[9] method was used to evaluate bioactivity of the compounds. Human recombinant hnps PLA2 was used as the target enzyme. The expression and purification of the PLA2 followed a method similar to the one given in literature[10] with the synthesized gene inserted into pET 21a plasmid. The assay was carried out in a 96-well plate utilizing a multiwell fluorometer (SpectraMax GeminiXS, Molecular Devices). All solutions were prepared with high purity (18 MΩ conductivity) water. The buffer used in all the experiments was 0.25 M Tris, pH 9.0, 2 mM $CaCl_2$. The substrate was 0.25 mg/mL NBD-C12-PG buffer solution, which was sonicated for 1 min to get the micell suspension. All the samples were dissolved in DMSO. A 200 μL reaction solution contained 2.6 nM PLA2, 0.0125 mg/mL substrate, 0.1 mg/mL BSA, and 10 μL inhibitor solution. The reaction was monitored by excitation at 485 nm and emission at 535 nm. Fluorescent signals were monitored by kinetics mode program. Reported IC_{50} values, determined by plotting concentration–velocity curves are the mean of at least three separate experiments.

5. Discussion

The potential binding modes of compounds **1**, **3**, and **5** were obtained by molecular docking. When the ligands formed hydrogen bonds and liganded to the active site calcium in suitable distance, the pK_i were calculated to be 5.37, 6.13, and 7.09, respectively. It is clear that compound **5** will be a better candidate. It formed hydrogen bonds with Gly29, His47, and Asp48 with distances of 0.305, 0.290, and 0.257 nm, respectively. The calcium ion was liganded by the oxygen atom of indole-3-carbamoylmethyl group at a distance of 0.244 nm (Fig. 1).

Compound **3** was prepared through four steps by Nicholas J. Bach's group, and it has been shown to be a lead

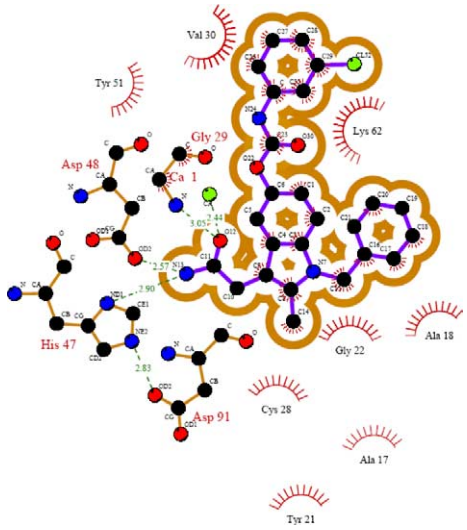

Figure 1. The binding mode of indole inhibitor to hnps-PLA2 (drawn by Ligplot[11]).

compound for potent and selective inhibitors of hnps PLA2.[5] First, 4-methoxy phenylhydrazine was converted by the classical Fischer-indole synthesis to give indole acetic acid esters; second, the indole-3-acetic acid ester was alkylated by forming the sodium salt and treating with an arylmethyl halide to give 5-methoxy-2-methyl-1-(phenylmethyl)1*H*-indole-3-acetic acid ester; third, by heating with hydrazine hydrate in ethanol, 1-(arylmethyl)indole-3-acetic acid hydrazide was got; finally, heating the hydrazides with Raney nickel in ethanol gave the corresponding 1-(arylmethyl)indole-3-acetamides. In this procedure, products from two out of four steps were of lower yield, and needed chromatography for purification. The overall yield is 5.88%. Our method here only contains two-step reactions at lower or room temperature with rare side reactions. The overall yield is 78.3%, which is 13.3 times as much as the reported method. In our experiment, compounds **4** and **5**[12] were obtained very easily with high yields.

A preliminary bioassay study in vitro showed that compound **5** displayed inhibition to synovial-fluid PLA2 with the average IC_{50} of 1.81 ± 0.59 μM, which is better to the reference compound's average IC_{50}

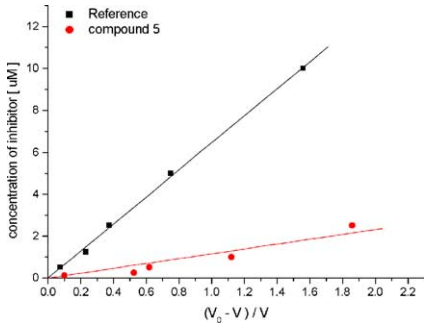

Figure 2. The concentration–velocity curve. (The curves were induced by equation $I = IC_{50}(V_0 - V)/V$. I is the concentration of the inhibitor. V_0 is the max-velocity of the enzyme without inhibitor. V is the max-velocity of the enzyme with certain concentration of inhibitor. The slope of the curve is the value of IC_{50}.)

(4.26 ± 0.72 μM) in the system we used. The concentration–velocity curve of compound **5** and reference are shown in Figure 2.

6. Conclusion

We have developed a new type of indole compounds as hnps PLA2 inhibitor. The compound was shown to be active for human recombinant hnps PLA2, which is comparable to the reported indole compounds from Eli Lilly and Company. The reported compounds here contain more cyclic component and can be easily synthesized.

Acknowledgment

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References and notes

1. Lin, M. K. S.; Farewell, V.; Vadas, P.; Bookman, A. A. M.; Keystone, E. C.; Pruzanski, W. *J. Rheumatol.* **1996**, *23*, 1162.
2. Vishwanath, B. S.; Fawzy, A. A.; Franson, R. C. *Inflammation* **1988**, *12*, 549.
3. Bomalaski, J. S.; Lawton, P.; Browining, J. L. *J. Immunol.* **1991**, *146*, 3904.
4. Schevitz, R. W.; Bach, N. J.; Carlson, D. G.; Chirgadze, N. Y.; Clawson, D. K.; Dillard, R. D.; Draheim, S. E.; Hartley, L. W.; Jones, N. D.; Mihellch, E. D.; Olkowski, J. L.; Snyder, D. W.; Sommers, C.; Wery, J. P. *Nat. Struct. Biol.* **1995**, *2*, 458.
5. Dillard, R. D.; Bach, N. J.; Draheim, S. E.; Berry, D. R.; Carlson, D. G.; Chirgadze, N. Y.; Clawson, D. K.; Hartley, L. W.; Johnson, L. M.; Jones, N. D.; McKinney, E. R.; Mihelich, E. D.; Olkowski, J. L.; Schevitz, R. W.; Smith, A. C.; Snyder, D. W.; Sommers, C. D.; Wery, J. P. *J. Med. Chem.* **1996**, *39*, 5119.
6. Abraham, E.; Naum, C.; Bandi, V.; Gervich, D.; Lowry, S. F.; Wunderink, R.; Schein, R. M.; Macias, W.; Skerjanec, S.; Dmitrienko, A.; Farid, N.; Forgue, S. T.; Jiang, F. *Crit. Care Med.* **2003**, *31*, 718.
7. *Sybyl*, version 6.91; Tripos Associates: St. Louis, MO, **1998**.
8. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
9. Blanchard, S. G.; Harris, C. O.; Parks, D. J. *Anal. Biochem.* **1994**, *222*, 435.
10. Othman, R.; Baker, S.; Li, Y.; Worrall, A. F.; Wilton, D. C. *Biochim. Biophys. Acta* **1996**, *1303*, 92.
11. Wallace, A. C.; Laskowski, R. A.; Thomton, J. M. *Protein Eng.* **1995**, *8*, 127.
12. All compounds gave satisfactory structural data. The physical data and spectrum data of compound **5** are as follows: mp: 181–182 °C; yield: 74%; IR: 1655 (NH_2), 1716 (NH), 3458, 3410, 3327, 3196 (NH and NH_2); ^1H NMR (400 MHz, DMSO): 2.34 (s, 3H, 2-CH_3), 3.34 (s, 2H, 3-CH_2CONH_2), 5.42 (s, 2H, 1-CH_2Ph), 6.84–7.81 (m, 12H, Ar and NH_2), 10.51 (s, 1H, NH_2); MS: *m/z* 482 (M+) calcd for $C_{25}H_{21}N_2O_3Cl_2$ 482.4; Anal (calcd) %: C, 62.04 (62.25); H, 4.269 (4.388); N, 8.405 (8.711).