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Discovery of a novel pyrazole series of group X secreted phospholipase A2 inhibitor ($sPLA_2X$) via fragment based virtual screening

Hongming Chen [a,*], Laurent Knerr [a], Tomas Åkerud [c], Kenth Hallberg [c,†], Linda Öster [c], Mattias Rohman [d], Krister Österlund [b], Hans-Georg Beisel [b], Thomas Olsson [b], Johan Brengdhal [b], Jenny Sandmark [c], Cristian Bodin [c]

[a] Chemistry Innovation Center, Discovery Sciences, AstraZeneca R&D Mölndal, SE-43183 Mölndal, Sweden
[b] CVMD Innovative Medicines, AstraZeneca R&D Mölndal, SE-43183 Mölndal, Sweden
[c] Structure and Biophysics, Discovery Sciences, AstraZeneca R&D Mölndal, SE-43183 Mölndal, Sweden
[d] Reagents and Assay Development, Discovery Sciences, AstraZeneca R&D Mölndal, SE-43183 Mölndal, Sweden

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ABSTRACT

The discovery of potent novel pyrazole containing group X secreted phospholipase A2 inhibitors via structure based virtual screening is reported. Docking was applied on a large set of in-house fragment collection and pharmacophore feature matching was used to filter docking poses. The selected virtual screening hits was run in NMR screening, a potent pyrazole containing fragment hit was identified and confirmed by its complex X-ray structure and the following biochemical assay result. Expansion on the fragment hit has led to further improvement of potency while maintaining high ligand efficiency, thus supporting the further development of this chemical series.

Secreted phospholipases A2 ($sPLA_2$) are a family of disulfide-rich, Ca^{2+}-dependent enzymes that hydrolyze the *sn*-2 position of glycero-phospholipids to release a fatty acid and a lysophospholipid.[1] There is now accumulating evidence that several $sPLA_2$ isoforms, namely group IIA, III, V, and X play significant, distinct, or overlapping roles in one or several steps of atherogenesis.[2–4] The $sPLA_2$s are believed to exert multiple proatherogenic effects in the arterial wall by generating proinflammatory bioactive lipid mediators and proatherogenic low-density lipoprotein (LDL) particles, and activating different inflammatory cells through catalytically dependent and independent mechanisms.[5]

Previously the group IIA $sPLA_2$ ($sPLA_2$IIa) has received the most attention among the $sPLA_2$s. Substituted indoles and indolizines first reported by researchers at Lilly and Shionogi are the most potent group II $sPLA_2$ inhibitors.[6–9] Clinical candidate Varespladib (compound **4** in Fig. 1) has moved into late stage clinical trial for acute coronary syndrome. Recently potent group X $sPLA_2$ ($sPLA_2X$) inhibitors has also been reported in literatures.[10,11] In the mid-1990s screening technologies have been developed to detect inhibitory activity sensitively in the mM–μM range.[12] This opened up the possibility of designing drugs by step-wise addition of functional groups to simpler low-molecular-weight chemical entities. Since then, fragment-based approaches have played an increasingly important role in lead generation of drug discovery projects.[13–15] In current study, a combination of fragment based proton NMR screening technology and in silico virtual screening approach was applied in developing potent $sPLA_2X$ inhibitors.

When the discovery project started, the only reported $sPLA_2X$ structure is an apo structure (1le6),[16] but both in-house and external $sPLA_2$IIa complex structures were available. Due to the high sequence similarity between $sPLA_2X$ and $sPLA_2$IIa, three public available and three in-house $sPLA_2$IIa complex structures were chosen as reference structures for pharmacophore analysis to $sPLA_2X$ inhibitors. The structures for these six inhibitors were shown in Figure 1. These $sPLA_2$IIa protein structures and the $sPLA_2X$ apo structure were superimposed as displayed in Figure 2. Figure 2a shows that the $sPLA_2X$ and $sPLA_2$IIa structures are well aligned and the $sPLA_2X$ binding site is slightly bigger than that of $sPLA_2$IIA structure due to the fact that both Phe2 and Phe98 in $sPLA_2$IIa were changed to ILEs in $sPLA_2X$. It seems that interaction

* Corresponding author. Tel.: +46 31 7065285.
E-mail address: Hongming.chen@astrazeneca.com (H. Chen).
† Current address: Sprint Biosciences AB, SE-11428 Stockholm, Sweden.

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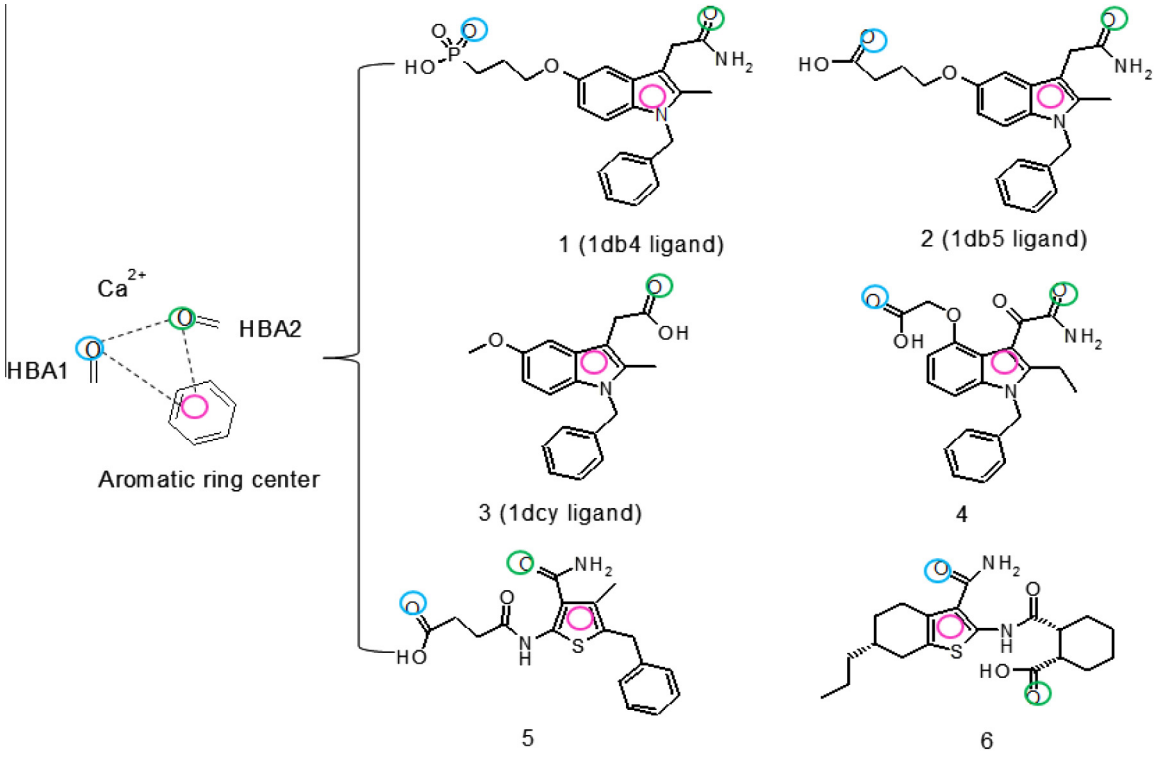

Figure 1. The $sPLA_2$IIa inhibitors used as references and the proposed pharmacophore model of $sPLA_2$X inhibitor. The circles on the structures refer to the pharmacophore elements in the model.

with calcium ion is crucial for ligand binding. For example, in crystal structure 1db4 and 1db5, the calcium ion forms bidentate interaction with oxygen atoms in phosphor acid/carboxylate acid and the amide function group. In structure 1dcy, although no bidentate interaction exists for the calcium ion, it still forms coordination interaction with the carboxylate acid of the ligand. Alignment of these six complex structures shows that the thiophene ring of compounds **5** and **6** can be well aligned with the indole ring in compounds **1**–**4**. Based on these observations, a pharmacophore hypothesis was proposed in Figure 1, which is composed by two hydrogen bond acceptors forming interaction with the calcium ion and an aromatic ring center forming hydrophobic interaction with ILE94 at the bottom of $sPLA_2$X binding site. The mapped pharmacophore elements in each reference compound are highlighted in Figure 1.

A structure based virtual screening protocol was utilized for selecting fragments (shown in Fig. 3). Firstly a fragment library was compiled by subsetting the whole in-house compound collection with molecular weight (MW) and *C*log*P*[17] filters: MW < 300 and *C*log*P* < 3.0. Altogether around 300 K compounds fulfilled the criteria and were further processed in following procedures to prepare 3D conformers for docking: the filtered subset compounds were initially run through OEleatherface[18] to generate possible tautomers, protonation states and canonical smiles, then Corina program[19] was used for generating 3D conformations for these standardized structures with default setting and these 3D conformations were further optimized by using Szybki[20] program from Openeye. Glide[21] docking program from Schrodinger was used to dock those 3D conformers into 1le6 $sPLA_2$X apo structure and for each docked structure maximal three docking poses were saved for analysis. The aforementioned $sPLA_2$X pharmacophore model was used to select interesting poses in the following way: firstly, three dummy atoms were created in the $sPLA_2$X apo structure to represent the three pharmacophore elements for $sPLA_2$X inhibitors and their coordinates were calculated as the average coordinates of those mapped atoms in six reference compounds (Fig. 1), whereas the aromatic ring centers were created based on those of the five member ring of reference compounds (labeled in Fig. 1). An in-house C++ program was written to search for the pharmacophore elements in docking poses which fit with three dummy pharmacophore elements by checking their distance in 3D space. If the distance between searched pharmacophoric atom of docking pose and one of the three dummy atoms was less than 0.6 Å, then the docking pose will be regarded as fulfilling one pharmacophore element. For each docking pose, the fitness with all three pharmacophore elements will be checked. After all docking poses were checked, 4000 top scored ones which satisfy at least two of the three pharmacophore elements are selected for further consideration. They correspond to 1275 unique compounds. After manual inspection and checking of stock availability, 198 compounds were checked for competitive binding to the $sPLA_2$X active site in an proton NMR screen.

Altogether 20 active compounds were identified in NMR screening and estimated binding potency for four example compounds were shown in Table 1. $sPLA_2$X complex structure for compound **7** was obtained (PDB code: 4UY1) and its bioactive conformation and the predicted docking pose are shown in Figure 4. It seems that the predicted docking pose is very similar to the experimental X-ray results. The terminal amide group interacts with the calcium ion and the pyrazole ring fit with the aromatic center of the pharmacophore model in Figure 2b. Given the availability of $sPLA_2$X complex structure and the attractiveness of the chemical structure, compound **7** was then selected for further development and a series of analogs were synthesized to explore the SAR of the pyrazole series.

The synthetic pathway for the preparation of compounds series 11–20 was very similar. As a representative example, the synthesis of compound **11** was illustrated in Scheme 1. Starting from ketone