

ANALOGS OF MK-886 AS POTENTIAL ANTI-INFLAMMATORY DRUGS WITH A NOVEL MECHANISM OF ACTION

C.Maugeri^a, M.A.Alisi^a, I.Coletta^a, E.Fioravanzo^b, G.Mangano^a, R.Ombrato^a, V.Rincicotti^a, M.Vitiello^a, and N.Cazzolla^a

^aAngelini Farmaceutici, A.C.R.A.F. SpA, P.le Stazione snc, 00040 S. Palomba, Rome, Italy (www.angelini.it)

^bS-IN Soluzioni Informatiche, via Salvemini 9, 36100 Vicenza, Italy (www.s-in.it)

Introduction

Microsomal Prostaglandin E Synthase-1 (mPGES-1) is an inducible PGES responsible for the production of PGE₂, the major prostanoid that contributes to inflammation, fever and pain¹. At least three distinct PGES isoforms have been identified including cPGES-1, mPGES-1 and mPGES-2. mPGES-1 is up-regulated by proinflammatory stimuli and is functionally coupled with COX-2. mPGES-1 is one of the member of the family of membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) which includes several mediators such as 5-lipoxygenase-activating protein (FLAP), leukotriene C₄ (LTC₄) synthase and others²; all members of the MAPEG family show common enzymatic activities, sequence motifs, and structural properties³. MK-886 is a potent FLAP inhibitor and one of the first mPGES-1 inhibitors known with an IC₅₀ of 3.2 μM⁴.

The aim of the present study was the identification of new potential inhibitors of mPGES-1 using MK-886 as starting reference compound.

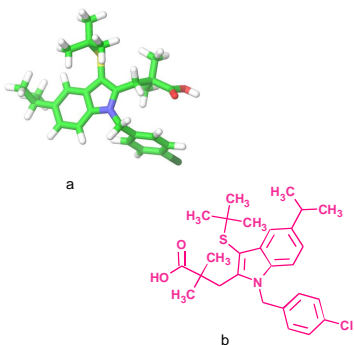


Figure1- a) 3D structure of MK-886; b) 2D structure of MK-886

Materials, Methods and Discussion

Starting point of the present study was the structure of MK-886 which is shown as 3D (figure 1a) and 2D (figure 1b). Several criteria were used to perform 2D and 3D similarity searches. In Chem-X pharmacophores may be deduced rapidly and automatically from the structure using the ChemDBS-3D module. 2D Chem-X recognises five types of potential interaction centres in a structure: H-bond donors, H-bond acceptors, positively-charged atoms, aromatic ring centroids and hydrophobic centres. ChemDBS-3D identifies pharmacophores by comparing the distance keys and formula keys for a set of structures, looking for common features. A single solution was found, consisting of three aromatic ring centres, an H-bond acceptor and an H-bond donor. We searched into our corporate database in order to identify compounds that possess these structural features.

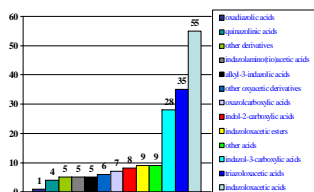


Figure2- Distribution of selected compounds in chemical classes

A conformational analysis of the reference compound MK-886 was then performed employing the MonteCarlo method implemented in the MacroModel software. Two different energy minima were identified and used to generate two different 3D pharmacophores with Chem-X. Both 2D and 3D pharmacophores were then used to search the corporate database for new potential hits.

We could select 200 compounds that were subdivided in 13 chemical classes as shown in figure 2.

EVA (Eigenvalue analysis) was used to describe all selected compounds using Tanimoto, Petke, Hodgkin and Carbo coefficients.

PCA is a Multivariate Data Analysis that describes the structural diversity of a set of molecules depending on the chemical space occupied by a single molecule. In figure 3 is reported how the set of 200 compounds is located into the corporate database of molecules.

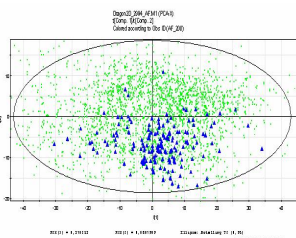


Figure3- PCA of 200 molecules (blue) over corporate database (green)

In figure 4 is shown the PCA of the 200 molecules to verify the presence of outliers. We can distinguish two chemical sets drawn in circles.

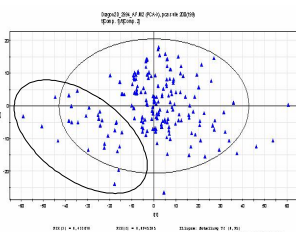


Figure 4- PCA of 200 selected compounds

Only 106 out of the total 200 compounds had the requested purity to perform the *in vitro* enzymatic test. In figure 5 the PCA of the 106 compounds over the initial 200 selected compounds is reported.

In vitro test was performed in order to verify enzymatic inhibitory activity against mPGES-1 at 50 μM. Results of more active compounds (compounds 1-5) are shown in table 1.

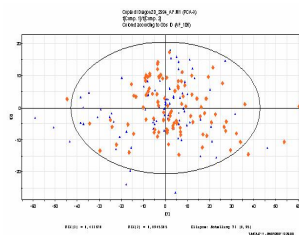


Figure5- PCA of 106 molecules (blue) over the total 200 molecules (orange).

Compound 2 was taken as our new reference molecule for a supplementary 2D and 3D similarity search using same criteria as above. 21 compounds were selected and tested *in vitro* against mPGES-1 at 50 μM. 4 compounds showed inhibitory activity >40%; (table 1, compounds 6-9).

Compound	%inhibition	standard deviation
1	42	2
2	73	33
3	41	6
4	48	0
5	52	43
6	57	12
7	63	3
8	51	6
9	49	4
MK-886	47	8

Table1 - Pharmacological results of active compounds

After excluding similar compounds or molecules belonging to the same chemical class we could chose 3 hit compounds (molecules 3, 4 and 5). Based on them we synthesised 43 new compounds that were tested at 50 μM. Only 3 compounds showed inhibitory activity >60%. Best compounds were chosen in order to investigate their effects on inducible PGES activity by using the human lung adeno carcinoma A549 cell line stimulated by IL-1^β.

The selectivity of inhibition was evaluated by a different inhibition of PGE₂ production respect to PGF_{2α} simultaneously produced.

Conclusions

2D and 3D similarity search using Chem-X on MK-886 led us to identify 200 potential mPGES-1 inhibitors into our corporate database. 106 plus 21 structures were considered and tested for enzymatic activity; 9 plus 3 compounds showed good inhibitory activity at 50 μM. Selectivity PGE₂/PGF_{2α} *in vitro* test on A549 cells was performed on more active compounds. Unfortunately, none of them showed good selectivity.

Our similarity search method proved to be quite robust in identifying new potential inhibitors against mPGES-1. Also, we gained good information about structures required for enzyme selectivity. More accurate SAR studies and new synthesis will allow us to select new structures in order to identify mPGES-1 inhibitors and PGE₂/PGF_{2α} selective compounds.

References

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