

RESEARCH ARTICLE

STRUCTURE-ACTIVITY RELATIONSHIP: STUDY OF LEI–401 AS INHIBITOR OF NAPE-PLD BY PHARMACOPHORE MODEL

Nilesh S. Kadu

Department of Chemistry, Bharatiya Mahavidyalaya, Amravati, Maharashtra, India.

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Manuscript Info

Abstract

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..... N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) is considered to be the principal enzyme that produces N-acylethanolamines (NAEs), a family of signaling lipids. NAEs are involved in numerous physiological processes such as appetite, satiety, pain, inflammation, fertility, stress, and anxiety. Furthermore, aberrant NAE levels are associated with metabolic syndrome and non-alcoholic steatohepatitis (NASH). Several inhibitors for NAPE-PLD have been reported. But most of the inhibitors showed poor to moderate potency for NAPE-PLD in vitro. Recently, Mario van der Stelt et al describe the SAR of NAPE-PLD inhibitors that afforded LEI-401 in vitro. However, no attempt was instigated to produce a consensus pharmacophore model of LEI-401 as inhibitors of NAPE-PLD. Pharmacophore modeling is an efficient and useful approach to identify important patterns in a series of molecules for optimizations. The consensus pharmacophore model revealed the importance of structural features and their correlation with the biological activity.

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Introduction:-

N-acyl-phosphatidylethanolaminephospholipaseD (NAPE-PLD) hydrolyses N-acyl-phosphatidylethanolamines (NAPEs) to produce N-acylethanolamines (NAEs) (Merkel, O. et al, 2005). It is responsible for the generation of the NAE anandamide, the ligand of cannabinoid and vanilloid receptors (Okamoto, Y. et al, 2004). Although predominantly bacterial, a mitochondrial form exists (Merkel, O. et al, 2005). NAPE-PLD belongs to the metallobeta-lactamase family (Wang, J. et al, 2006). The NAE lipids exert their biological activity through the activation of various G-protein-coupled receptors (canna-binode receptors CB1 and CB2, GPR55, GPR110, and GPR119), ion channels (transient receptor potential vanilloid 1, TRPV1), and nuclear receptors (peroxisome proliferator-activated receptor α , PPAR- α) (Tsuboi, K. et al, 2018). Accordingly, NAEs are involved in numerous physiological processes such as appetite, satiety, pain, inflammation, fertility, stress, and anxiety (Maccarrone, M., 2017). Furthermore, aberrant NAE levels are associated with metabolic syndrome and non-alcoholic steatohepatitis (Mazier, W. et al, 2015; Kimberly, W. T. et al, 2017; Fanelli, F. et al, 2018).

Several inhibitors for NAPE–PLD have been reported. But most of the inhibitors showed poor to moderate potency for NAPE–PLD in vitro. Recently, Mario van der Stelt, et al(2020) reported LEI–401 as an inhibitor of NAPE–PLD, which exhibited nanomolar potency ($pIC_{50} = 7.14 \pm 0.04 \mu M$).LEI–401 reduced NAE levels including anandamide in Neuro–2a cells as well as in the brains of freely moving mice. In addition, LEI–401 elicited a marked effect on emotional behaviour in mice by activating the hypothalamus–pituitary–adrenal (HPA) axis and reducing fear

Corresponding Author:- Nilesh S. Kadu Address:- Assistant Professor, Department of Chemistry, Bharatiya Mahavidyalaya, Amravati - 444605Maharashtra, India. extinction of an aversive memory. Mario van der Stelt, et al(2021) also describe the structure–activity relationship (SAR) of a library of NAPE–PLD inhibitors that afforded LEI–401.

Even though, Mario van der Stelt, et al (2021) discussed SAR (Structure Activity Relationship), however, no attempt was instigated to produce a consensus pharmacophore model of LEI–401 as inhibitors of NAPE–PLD. Pharmacophore modeling is an efficient and useful approach to identify important patterns in a series of molecules for optimizations (Kadu, N.S., 2019; Masand, V.H. and Rastija, V., 2017; Kadu, N.S. et al, 2020). Thus, this is first ever effort to develop a consensus pharmacophore model of LEI–401 as inhibitors of NAPE–PLD using alignment approach. The outcomes could be advantageous to chemists when developing a new drug.

Materials and Methods:-

Selection of Dataset

The dataset consists of one hundred and sevenpyrimidine and pyridine scaffold derivatives exhibiting the inhibitor activity (pIC_{50}) in μ M range. The pyrimidine and pyridine scaffold derivatives possess good variation in substation pattern like the presence of different heterocyclic, aliphatic and aromatic ringsand change in linkers (Mario van der Stelt, et al, 2021). Therefore, the selected dataset is wide enough to develop a consensus pharmacophore model. Out of these the dataset of most active six compounds has been tabulated in table 1.

Table 1:- Six most active pyrimidine and pyridine scaffol	ld derivatives (SMILES notation) along with reported pIC ₅₀
(in μ M) used in the present work.	

Compound ID	SMILES	pIC ₅₀ in
LEI-401	O=C(NCC1CC1)C2=CC(N3CC[C@H](O)C3)=NC(N4CCC[C@@H](C5=CC=C5)C 4)=N2	μι νι 7.14
2	O=C(NCC1CC1)C2=CC(N3CCOCC3)=NC(N(C)CCC4=CC=CC=C4)=N2	6.15
3	O=C(NCC1CC1)C2=NC(N(C)CCC3=CC=CC3)=CC(N4CCOCC4)=C2	4.98
4	O=C(NCC1CC1)C2=CC(N(C)CCC3=CC=CC=C3)=NC(N4CCOCC4)=C2	5.84
5	CN(CCC1=CC=CC=C1)C2=CC(C(NCC3CC3)=O)=NC(N4CCOCC4)=N2	5.39
6	O=C(NCC1CC1)C2=NC(N3CCOCC3)=NC(N(C)CCC4=CC=CC=C4)=N2	<4.3

Generation of Pharmacophore Model

The standard procedure used in the present work for developing consensus pharmacophoremodeling involves recommended steps in the literature (Masand, V.H. and Rastija, V., 2017; Kadu, N.S. and Ingle, A.V., 2019). The four main steps are:

a) The structures were drawn using ChemSketch 2010 freeware.

b) Optimization using PM3 semi-empirical method using MOPAC 2012.

c) Alignment of molecules using Open3dAlign software.

d) Using the default settings, consensus pharmacophore model was created using PyMOI 2.0.

Result and Discussion:-

From fig. 1 it is seen that, the consensus pharmacophoric pattern of different inhibitors of NAPE–PLD is highlighted by three contour fields. The yellow colour field represented hydrophobic/lipophilic nature, blue colour field represented negative charge nature and red colour field represented positive charge nature of the compound. The present pharmacophore–oriented analysis unveils that the activity of different inhibitors of NAPE–PLD has good correlation with these three contour fields.



derivatives.

From fig. 1 it is observed that, all compounds show two types of hydrophobic/lipophilic field (yellow colour region), one is due to benzene moiety and second is due to cyclopropane ring. Thus, all compounds have similar activity towards hydrophobic/lipophilic nature. One remarkable observation shown by all compounds isexcept LEI–401, Compound–2,3,4,5 and 6 show two types of negative field (blue colour region), it is due to carbonyl group in amideandoxygen atom of morpholine. LEI–401 shows only one type of negative field and it is due to carbonyl group in amide.It is also noted that, allcompounds show variation in activity of positive field (red colour region).Except LEI–401, Compound–2,3,4,5 and 6 show single type of positive field and it is only due to –NH group. Among these, Compound–2and5 show negligible activity ofpositive fieldue to –NH group in amide moiety attached to pyrimidine ring.On the other hand, in Compound–6 activity of positive fields slightlyincreased as compared to Compound–2 and 5, this is because of–NH group in amide moiety attached to s–triazine ring.

Although, remarkable increase in the activity of positive field is observed in Compound–4 compared to Compound–2,5 and 6, as a result of –NH group in amide moiety attached to pyridine ring. The most notable observation regarding structural feature related to Compound–3 and 4 is that, Compound–3 shows positive field over secondary –NH group and not over –NH group in amide moiety even though it is attached to pyridine ring while Compound–4 though it is attached to pyridine ring shows positive field over –NH group. This highlights the importance of attachment of skeleton to heterocyclic ring (Pyridine)andactivity of positive field observed in compound–3 and 4.LEI–401 shows two types of activity positive field as compared to Compound–2,3,4,5 and 6 (show single positive field). These two types of positive field in LEI–401 has two types of positive field and only one type of negative field while Compound–2,3,4,5 and 6 have negligible or single type of positive field and two types of negative field.

Conclusion:-

The present work reveals important pharmacophoric patterns of LEI–401 derivative as inhibitors of NAPE–PLD. It unveils the importance of attachment of –NH group in amide moiety to pyrimidine, pyridine and s–triazine ring. It also highlights the importance of –OH group, secondary –NH group, carbonyl group in amide, oxygen atom of morpholine, cyclopropane ring and aromatic moiety and their correlation with the biological activity.Hence, such a combination of these moieties must be useful in future optimization. The outcomes of this present work could be advantageous to chemists while developing a new drug design.

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