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STRUCTURE-BASED DRUG DESIGN OF KYNURENINE 3-MONOOXYGENASE (KMO) INHIBITORS: USE OF THEORETICAL TOOLS

Chemistry

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ABSTRACT

Kynurenine pathway has prompted a burgeoning research domain functioning to explicate the mechanism to germinate pharmaceutical agents for Cancer, inflammation and neurodegeneration. Mammalian tryptophan degradation is extensively carried out by this pathway. Recognition of kynurenine pathway (KP) metabolites for their neuroactive profile has aged decades, yet its application to brain treatments is quite recent. Blocking of KMO bioactivity which is a crucial KP enzyme, seems an interesting therapeutic approach for various chronic and acute NDs. KMO inhibitors are proposed to eligibly increase levels of neuroprotective kynurenic acid: neurotoxic 3-hydroxykynurenine to ameliorate neurodegeneration. The present study aims to design small inhibitor molecule having capability to bind with KMO enzyme and block its path to catalyze neurotoxicity precursor protein. This (KMO) flavin dependent enzyme is well suited for designing mechanism based inhibitors. A series of new lead analogs were designed via implication of structure activity relationship properties. These were then energy minimized and docked against KMO using online software and tools. Analogs of lead molecules namely Ro 61-8048 and UPF 648 incorporated with variant chemical groups and cysteine as backbone structure visibly have high binding affinity. In this study we carried out molecular docking for pthalazinone based inhibitors present in this database (using Autodock 3.05). Analysis of resulting data demonstrates the specific conformations adopted by inhibitors and crucial H bonding and π-stacking interactions during active site binding. All of these helps to identify the main contributing factors whose absence decreases the activity many folds. Proposed hit molecules may pave the routes for treatment of NDs.

KEYWORDS

Neurodegeneration, Kynurenine pathway, Molecular docking, kynurenic acid and pthalazinone derivatives.

INTRODUCTION

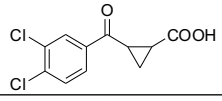
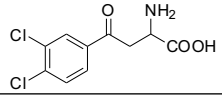
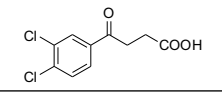
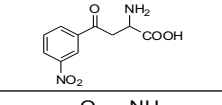
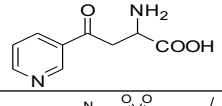
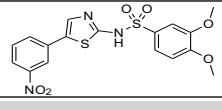
Neurodegenerative diseases (NDs) and mental disorders such as Schizophrenia, Huntington disease, depression and anxiety are dreadfully disenabling afflictions and relevant psychotropic drugs holds top rank in medicinal market[1]. However many among these still have no effective cure. Their rare palliative treatments available indicate the high degree of need and scope for exploring all possible mechanistic routes to address them. NDs are a result of intricate interaction of environmental and genetic factors that is poorly understood. Neurodegeneration is an umbrella term for all those conditions that leads to loss of nerve function and structure. They are frightful diseases because patient suffers inability, pain, loss of many physiological processes and dependency. In reference to toxic events causing neuronal death it is speculated that extensive formation of excitotoxic amino acid neurotransmitters, majorly glutamate from decaying neurons exacerbates the detriment to brain. Over limit exposure to L-Glu is involved in numerous ND pathogenesis. In fact a variety of psychiatric and neurological disorders implicates NMDAR antagonists as their therapeutic keys via this pathological cascade. Furthermore its intake could be responsible for severe side effects and toxicity [2]. KMO defines an interesting route for reducing NMDAR function, indirectly via elevation of kynurenine availability to produce kynurenic acid (KA), a neuroprotector. Excitotoxicity proposed by glutamate receptors together with free radicals genesis are speculated to lower the concentration of kynurenic acid in such diseases. KA at higher concentration behaves as glutamine NMDAR antagonist and has neuroprotective effect.

Prerequisite of the therapeutic approach is to regulate the kynurenine pathway (KP) in blood and other tissues in order to shift kynurenine more towards formation of kynurenic acid so as to promote glutamate blocking activity. Thus, disruption of KMO-Kynurenine interaction sounds to be a novel therapeutic strategy for dealing neurodegeneration [3,4,5] and a number of small inhibitor molecules have been reported to interact with KMO active site with hopeful results in ND models of yeast, bacteria and drosophila. These agents will reduce NMDA receptor function, elevate kynurenic acid via enhanced availability of kynurenine and decreases quinolinic acid. In simple words stimulations offered by NMDAR can be easily transformed via change in concentration of endogenous quinolinic acid versus KA.

Molecular Docking Study

To gain insights into substrate molecule binding of enzyme computer aided docking studies were performed employing broadly distributed molecular docking software namely, grid based AutoDock version 3.05 [6]. It was done using Marvin 5.3.7, 2010, ChemAxon (http://www.chemaxon.com). In this automated studies substrate has total docking energy in terms of Hydrogen bond, van der Waals, coulombic electrostatic and steric interaction energies [7]. For each compound from ligand library, docked conformations were produced. These were arranged as per their alpha HB score, which tells how well ligand geometrically fits the binging cavity along with H-bond effect. Selective ligands with a lower docking score conformation were analyzed further via interaction energies and free energy approximations (table 1).

Table 1: Ligand Library With Free Energy Value And Inhibition Constant

Ligand	Structure	ΔG (KMO)	Inhibition constant (Ki)	unit
DBCC (drug)		-5.12	177.99	µM
DERV (drug)		-5.41	108.23	µM
FCE (drug)		-5.22	149.09	µM
mNBA (drug)		-3.84	1.53	mM
NAL (drug)		-3.45	2.97	mM
Ro-61-8048 (drug)		-6.75	11.22	µM

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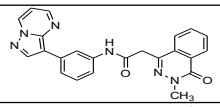
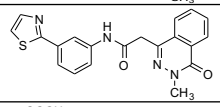
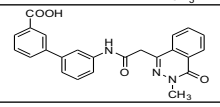
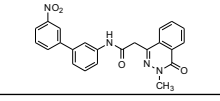
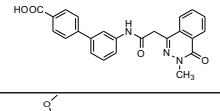
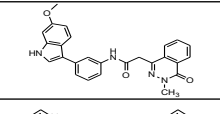
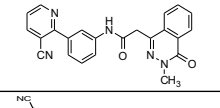
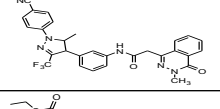
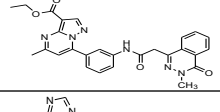
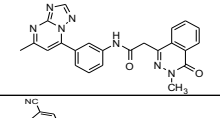
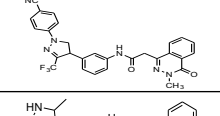
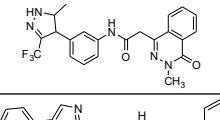
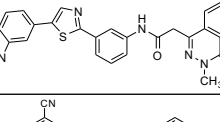
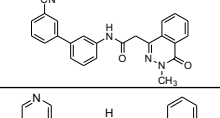
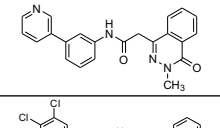
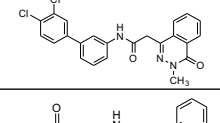
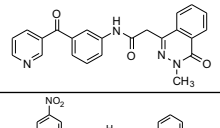
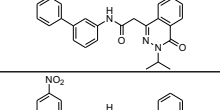
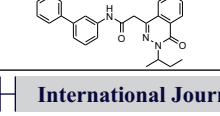
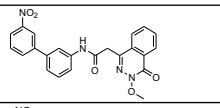
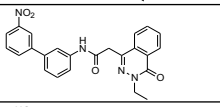
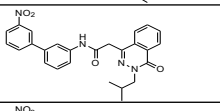
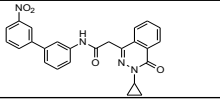
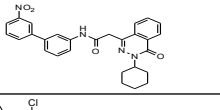
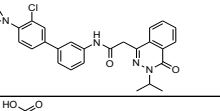
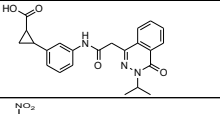
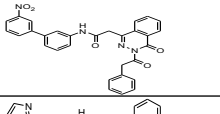
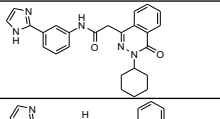
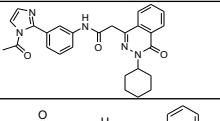
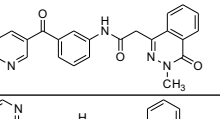
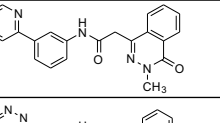
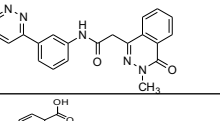
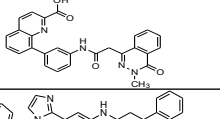
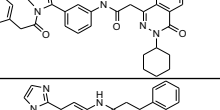
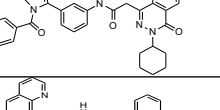
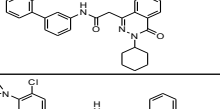
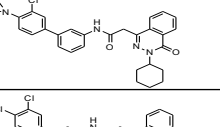
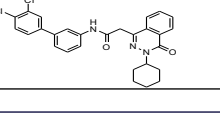
Apo1		-7.38	3.89	µM
Apo2		-7.14	5.86	µM
Apo3		-7.43	3.58	µM
Apo4		-8.55	536.73	nM
Apo5		-7.44	3.53	µM
Apo6		-7.51	3.15	µM
Apo7		-7.46	3.41	µM
Apo8		-5.88	49.16	µM
Apo9		-5.82	54.51	µM
Apo10		-6.89	8.94	µM
Apo11		-6.84	9.62	µM
Apo12		-4.94	237.68	µM
Apo13		-7.32	4.34	µM
Apo14		-8.5	592.47	nM
Apo15		-7.3	4.49	µM
Apo16		-8.26	880.14	nM
Apo17		-7.51	3.13	µM
Apo18		-8.68	432.34	nM
Apo19		-8.42	676.02	nM

Apo20		-7.52	3.09	µM
Apo21		-7.28	4.63	µM
Apo22		-7.7	2.25	µM
Apo23		-8.06	1.23	µM
Apo24		-8.91	293.97	nM
Apo25		-8.01	1.34	µM
Apo26		-6.85	9.5	µM
Apo27		-7.93	1.53	µM
Apo28		-7.67	2.39	µM
Apo29		-7.99	1.39	µM
Apo30		-7.29	4.55	µM
Apo31		-7.25	4.83	µM
Apo32		-7.08	6.49	µM
Apo33		-6.66	13.04	µM
Apo34		-8.47	616.52	nM
Apo35		-8.1	1.16	µM
Apo36		-6.92	8.39	µM
Apo37		-9.33	143.97	nM
Apo38		-8.38	718.54	nM

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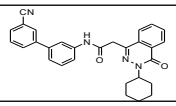
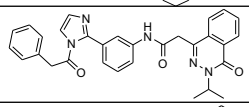
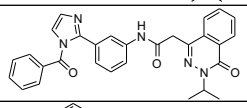
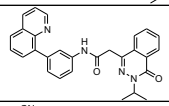
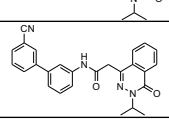
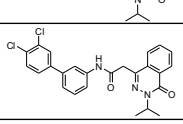
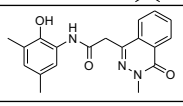
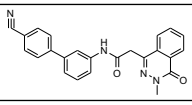
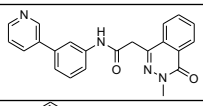
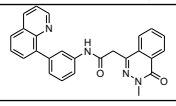
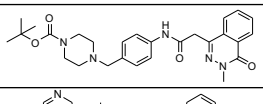
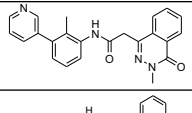
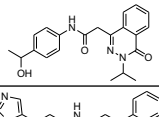
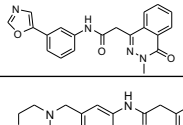
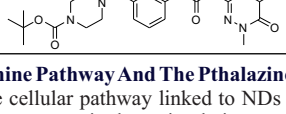
Apo39		-7.9	1.61	µM
Apo40		-7.55	2.91	µM
Apo41		-6.59	14.78	µM
Apo42		-7.07	6.54	µM
Apo43		-7.95	1.49	µM
Apo44		-7.56	2.88	µM
Apo45		-5.79	56.8	µM
Apo46		-7.61	2.64	µM
Apo47		-7.29	4.54	µM
Apo48		-8.2	975.89	nM
Apo49		-5.99	40.54	µM
Apo50		-6.8	10.38	µM
Apo51		-6.67	12.83	µM
Apo52		-7.27	4.72	µM
Apo53		-4.74	335.01	µM

Kynurenine Pathway And The Pthalazinone Ligands

A unique cellular pathway linked to NDs is the KP that facilitates a critical way to manipulate stimulative processes corresponding to L-glutamate.Kynurenine pathway defines prime source of catabolizing L-tryptophan, as a consequence of which plenty of metabolites are generated [8]. Being neuroactive these metabolites participates in immune tolerability [9] and neurodegeneration.

Molecule of compound Apo48 is stabilized within the active site of KMO via interactions between aromatic ring moiety of Apo48 and side chain residues Ile-110(figure 2). Trp- and His-290 stabilize Apo48 via π-stacking interaction against the side chain of His-978. Interestingly, Trp-254 also participates in cofactor binding through H-bonding.

Carboxylic group in molecule Apo41 imparts a triad of key hydrogen bonds to Arg83 and Tyr97. The much needed ketonic spacers provides the right placement of phenyl functionality with respect to carboxylic group so as to achieve the H bond interactions. Particularly the dichloro aryl ring in molecules Apo32 and Apo45 facilitates various lipophilic interactions. Leu221, Ile 232 and Leu234 form lipophilic pocket at an optimal distance with aromatic ring. X substituent makes a favourable contact with flavin (3.4 Å) and Y substituent makes interaction with Phe322 at 3.7 Å.

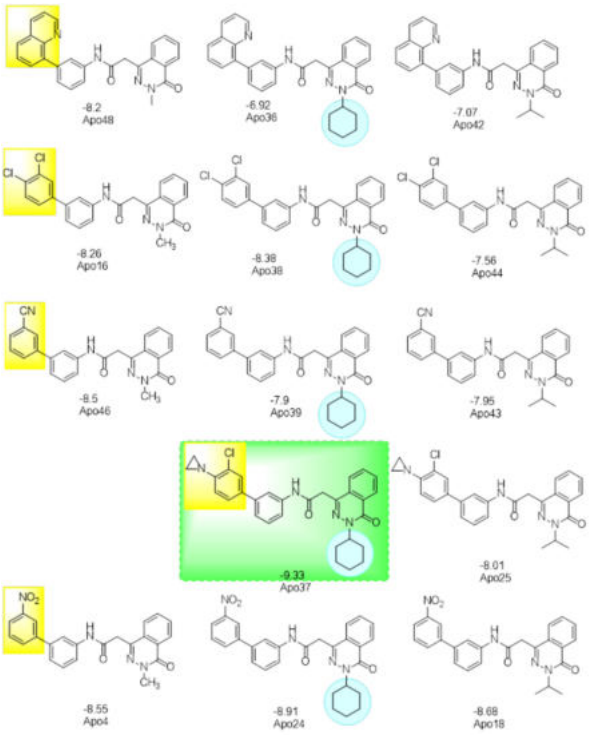


Figure1: Figure Of Best Free Energy Values (in nM range) Obtained For Pthalazinone Inhibitors Designed.

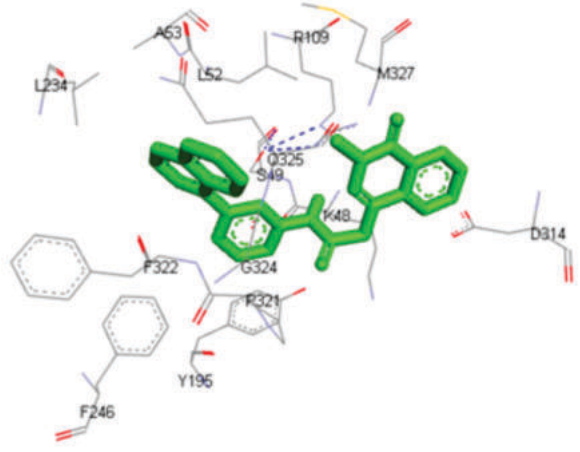


Figure 2: Figure Of Ligand Apo48 In Complex Structure With KMO

The best free energy values were obtained for the pthalazinone inhibitors containing certain functional groups as given in figure 1. It was seen that the presence of quinoline, 3,4-dichlorophenyl, 3-cyanophenyl, 3-nitrophenyl or 4-aziridine(3-chlorophenyl) substituent on the aromatic ring attached to pthalazinone by amide linkage gives better free energy values. Furthermore the substitution of methyl, cyclohexyl or isopropyl group at the pthalazinone nitrogen also helps to gain better free energy values.

CONCLUSION

We herein search for the compound that could provide regulation of KP metabolites in CNS as well as in periphery. To interrogate therapeutic potentials of inhibitors that may undertake kynurenine pathway manipulation we archived to design KMO inhibitors. Targeting KMO within KP could have therapeutic relevance in neurodegenerative diseases. We report the docking results of series of cysteine derivatives and describe their interactions with KMO showing its selectivity and potency for KMO. On limiting the selection criterion of ligand molecules such as H-bond acceptors and low molar mass, outlaid

molecules with pharmacokinetic adequacies which provide a hint for accessing correct receptor inhibitor design. The best of the designed compounds can be carried forward to MD studies for advanced knowledge of KMO-inhibitor binding. Later synthesis and *in vitro* analysis can be carried out for the best compounds so found. This will add to the validity of rational used for designing pharmacophore for inhibition of KMO.

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