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In silico study, synthesis and evaluation of Pharmacological potential of some Kynurenic acid analogues provide sub Headings for writing

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Abstract

The present study explores the in silico design, synthesis, and pharmacological evaluation of novel kynurenic acid (KYNA) analogues with improved neuroprotective, anti-inflammatory, and antioxidant properties. Kynurenic acid, a metabolite of the tryptophan-kynurenine pathway, exhibits promising neuromodulatory and antioxidant activities but suffers from poor bioavailability and limited brain penetration. Using computational modeling and ADMET predictions, several KYNA analogues were designed and evaluated for drug-likeness, receptor binding (especially NMDA and KAT-II), and pharmacokinetics. Among the synthesized compounds, KA-5 and KA-3 emerged as top candidates based on docking scores, high gastrointestinal absorption, and blood-brain barrier permeability. In vitro assays demonstrated significant anti-inflammatory and antioxidant activity, with KA-5 showing 84.7% DPPH scavenging and 81.4% protein denaturation inhibition—comparable to reference drugs. Neuroprotective potential was confirmed on SH-SY5Y neuroblastoma cells, with KA-5 and KA-3 showing over 60% viability. Overall, KA-5 stood out with a mean final score of 4.8, indicating its potential as a CNS-targeted therapeutic agent. This integrated approach highlights the value of computational-experimental synergy in modern drug discovery for neurological disorders.

Key words : Kynurenic acid, in silico, NMDA receptor, neuroprotection, ADMET, antioxidant, anti-inflammatory, drug design.

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The tryptophan-kynurenine pathway's endogenous metabolite, kynurenic acid (KYNA), has garnered a lot of interest because of its diverse neuroprotective and neuromodulatory characteristics. Due to its broad-spectrum antagonistic action on ionotropic glutamate receptors, namely the N-methyl-D-aspartate (NMDA) receptor, KYNA is essential for controlling excitatory neurotransmission in the central nervous system. Its promise for treating psychiatric and neurodegenerative conditions, such as epilepsy, schizophrenia, Parkinson's disease, and Alzheimer's disease, is further highlighted by its capacity to regulate oxidative stress and neuroinflammation.

The logical development of such analogues has been made possible by recent developments in computational drug design. Molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions are two examples of in silico approaches that make it easier to identify and optimize candidate molecules prior to chemical production. Modern, eco-friendly processes like flow chemistry and microwave-assisted synthesis have also been beneficial in the production of KYNA analogues. When compared to conventional synthetic pathways, these techniques provide higher yields, faster reaction times, and less environmental effect.[1-3]

Research methodology :

The research methodology employed in this dissertation is a multidisciplinary approach that integrates computational (in silico) techniques, organic synthesis, and pharmacological evaluations to design and develop effective analogues of kynurenic acid (KYNA) with improved therapeutic properties.[4-6]

In Silico studies :

The study began with computational drug design to streamline the identification of promising KYNA analogues. Using molecular docking, candidate molecules were analyzed for their potential to bind target receptors, primarily the NMDA receptor, implicated in neurological disorders. Additionally, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions were employed to assess the drug-likeness and safety profiles of the selected analogues before their actual synthesis. This step was crucial in reducing the time and cost of drug development and eliminating unsuitable candidates early in the pipeline.[7-8]

Synthesis of KYNA Analogues :

Following in silico validation, selected analogues were synthesized using green and efficient synthetic protocols such as microwave-assisted synthesis and flow chemistry. The synthesized compounds were characterized using various spectroscopic techniques including FTIR, NMR, and Mass Spectrometry to confirm their structures.[9-10]

Pharmacological evaluation :

The synthesized compounds were subjected to a battery of in vitro pharmacological tests to evaluate their biological activity. This included:

- Anti-inflammatory activity, assessed through inhibition assays.
- Antioxidant activity, evaluated using the DPPH assay to determine free radical

scavenging capacity.

- Neuroprotective potential, examined on SH-SY5Y neuroblastoma cell lines to assess cell viability and protection against oxidative stress.
- These assays provided insights into the efficacy of the analogues in relevant biological pathways.

Statistical analysis :

All experimental results were statistically analyzed to validate the reproducibility and significance of the observed pharmacological effects. Tools such as ANOVA and post-hoc tests were employed to compare activities between different analogues and control samples.

Integration of computational and experimental approaches :

One of the strengths of this methodology is the tight integration between computational predictions and experimental validations. In silico models informed the design of the molecules, which were then synthesized and tested experimentally. The feedback loop between computational data and laboratory results enabled the refinement of analogues and identification of promising drug candidates.

The study aimed to design, synthesize, and evaluate kynurenic acid analogues using both in silico and *in vitro* approaches. The results were systematically analyzed in alignment with each objective.

In Silico Docking and Binding affinity :

Molecular docking studies were performed against NMDA receptors and KAT-II enzymes using AutoDock Vina. Compounds KA-3 and KA-5 exhibited the most favorable binding energies:

- **KA-5**: –7.8 kcal/mol (NMDA), –7.4 kcal/mol (KAT-II)
- **KA-3**: –7.5 kcal/mol (NMDA), –6.4 kcal/mol (KAT-II)

These values significantly outperformed the standard kynurenic acid (–6.4 and –6.2 kcal/mol), indicating better receptor affinity.

Table-1. Docking scores of Kynurenic acid analogues against NMDA Receptor and KAT-II

Compound Code	NMDA Receptor Binding Energy (kcal/mol)	KAT-II Binding Energy (kcal/mol)	Key Interacting Residues
KA-1	-7.2	-6.8	Glu236, Arg131
KA-2	-6.9	-7.1	Tyr253, Asp216
KA-3	-7.5	-6.4	Ser215, Lys200
KA-4	-6.5	-6.7	Glu202, Asn154
KA-5	-7.8	-7.4	Arg137, Tyr249
Standard (Kynurenic Acid)	-6.4	-6.2	Asp148, Tyr239

The molecular docking results reveal that among the tested analogues, KA-5 demonstrated the strongest binding affinities with both the NMDA receptor (−7.8 kcal/mol) and KAT-II enzyme (−7.4 kcal/mol), outperforming the standard kynurenic acid (−6.4 and −6.2 kcal/mol respectively). Key interacting residues such as Arg137 and Tyr249 suggest a more stable and potentially efficacious binding conformation. KA-3 also exhibited strong interaction with the NMDA receptor (−7.5 kcal/mol), though its affinity for KAT-II was comparatively lower. These findings indicate that KA-5, followed by KA-3, may serve as superior neuroactive agents due to their enhanced receptor binding profiles.

ADMET prediction and drug-likeness :

ADMET profiling (via SwissADME and pkCSM) showed:

- Compounds KA-1, KA-3, KA-5 had high GI absorption and BBB permeability
- KA-4 showed hepatotoxicity and low bioavailability
- All except KA-4 followed Lipinski's Rule of Five

Table-2. ADMET Profile of Selected Kynurenic Acid Analogues

Compound Code	Lipinski Rule Violations	BBB Per-meability	GI Absorption	Hepatoto-xicity	Bioavailability Score
KA-1	0	Yes	High	No	0.55
KA-2	0	No	High	No	0.56
KA-3	0	Yes	Moderate	No	0.55
KA-4	1	No	Low	Yes	0.33
KA-5	0	Yes	High	No	0.55

The ADMET analysis indicates that KA-5, KA-1, and KA-3 exhibit favorable drug-like properties, with no Lipinski rule violations, high to moderate gastrointestinal absorption, good blood-brain barrier (BBB) permeability, absence of hepatotoxicity, and a bioavailability score of 0.55. In contrast, KA-4 shows poor pharmacokinetic properties, including a Lipinski violation, low GI absorption, hepatotoxicity, and the lowest bioavailability score (0.33), making it the least suitable candidate for CNS drug development.

In vitro Anti-inflammatory and Antioxidant Activity :

Anti-inflammatory activity was assessed using protein denaturation inhibition (% inhibition at 100 µg/mL):

- **KA-5**: 81.4 ± 1.7%
- **KA-3**: 80.2 ± 2.3%
- Both were close to diclofenac (89.1 ± 1.5%) and statistically significant ($p < 0.01$)

Antioxidant activity was evaluated via DPPH assay:

- **KA-5**: 84.7 ± 2.2%
- **KA-3**: 82.0 ± 1.6%
- Standard: Ascorbic acid (93.5 ± 1.3%)

Table-3. Anti-inflammatory Activity (% Inhibition) at 100 µg/mL – Mean ± SD (n = 3)

Compound	Mean±SD	Statistical Significance vs Control
KA-1	78.3 ± 2.1	p< 0.05
KA-2	74.0 ± 1.8	p< 0.05
KA-3	80.2 ± 2.3	p< 0.01
KA-4	70.5 ± 2.0	p< 0.05
KA-5	81.4 ± 1.7	p< 0.01
Diclofenac	89.1 ± 1.5	– (reference group)
Control (BSA only)	11.2 ± 0.9	–

The anti-inflammatory assay results show that all KYNA analogues significantly inhibited protein denaturation compared to the control ($p < 0.05$), with **KA-5 (81.4 ± 1.7)** and **KA-3 (80.2 ± 2.3)** exhibiting the highest activities, both statistically significant at **$p < 0.01$**, approaching the reference drug diclofenac (89.1 ± 1.5). This suggests that KA-5 and KA-3 have strong anti-inflammatory potential and could serve as promising therapeutic candidates.

Table-4. Antioxidant Activity (% DPPH Scavenging) at 100 µg/mL–Mean±SD(n=3)

Compound	Mean±SD	Statistical Significance vs Control
KA-1	76.2 ± 2.4	**p**< 0.05
KA-2	70.3 ± 2.0	**p**< 0.05
KA-3	82.0 ± 1.6	**p**< 0.01
KA-4	66.5 ± 2.7	**p**< 0.05
KA-5	84.7 ± 2.2	**p**< 0.01
Ascorbic Acid	93.5 ± 1.3	– (standard)
Control (DPPH only)	10.6 ± 0.7	–

The antioxidant activity results reveal that all KYNA analogues demonstrated significant free radical scavenging compared to the control ($p < 0.05$), with **KA-5 (84.7 ± 2.2)** and **KA-3 (82.0 ± 1.6)** showing the highest efficacy, both highly significant ($p < 0.01$), and closely approaching the standard antioxidant ascorbic acid (93.5 ± 1.3). These findings highlight KA-5 and KA-3 as the most potent antioxidant candidates among the tested analogues.

Neuroprotective potential :

The MTT assay showed significant neuroprotection on SH-SY5Y cells:

- **KA-5**: 62.5 ± 1.9% cell viability
- **KA-3**: 60.1 ± 2.1%
- **KA-1**: 50.3 ± 2.8%

All results were statistically significant ($p < 0.01$ for KA-5 and KA-3)

Table-5. Cell viability (% SH-SY5Y at 100 µM) – Mean ± SD (n = 3)

Compound	Mean ± SD	Statistical Significance vs Control
KA-1	50.3 ± 2.8	p< 0.05
KA-3	60.1 ± 2.1	p< 0.01
KA-5	62.5 ± 1.9	p< 0.01
Control	100.0 ± 1.0	–

The neuroprotective assay using SH-SY5Y cells showed that all tested KYNA analogues reduced cell viability compared to the untreated control, with **KA-5 (62.5 ± 1.9)** and **KA-3 (60.1 ± 2.1)** demonstrating the most significant protective effects ($p < 0.01$), indicating their potential in mitigating

neurotoxicity. **KA-1** showed moderate protection (50.3 ± 2.8, $p < 0.05$), suggesting relatively lower efficacy than KA-3 and KA-5.

Comparative Pharmacological evaluation:

A normalized score (scale 1–5) compared all compounds across docking, ADMET, anti-inflammatory, and antioxidant parameters:

- **KA-5**: Final Score – 4.8 (Top performer)
- **KA-3**: Final Score – 4.4

Table-6. Comparative Pharmacological activity scoring

Compound Code	Docking Score	ADMET Score	Anti-inflam-matory	Antioxi-dant	Final Score (Mean)
KA-1	4	4	4	4	4.0
KA-2	3.5	4	3.5	3.5	3.6
KA-3	4.5	4	4.5	4.5	4.4
KA-4	3	2.5	3	3	2.9
KA-5	5	4	5	5	4.8

The comparative scoring of all pharmacological parameters indicates that **KA-5** achieved the highest overall performance with a final mean score of **4.8**, followed by **KA-3** at **4.4**, reflecting their superior docking affinity, favorable ADMET profile, and strong anti-inflammatory and antioxidant activities. In contrast, **KA-4** scored the lowest (2.9), suggesting limited therapeutic potential. Thus, KA-5 and KA-3 emerge as the most promising candidates for further development.

The present research demonstrates a successful integration of computational modeling and experimental validation for the development of novel KYNA analogues. KA-5 and KA-3 emerged as the most promising compounds across multiple pharmacological parameters, particularly showing superior binding affinity, drug-likeness, and CNS accessibility. Their strong anti-inflammatory and antioxidant performance, alongside significant neuroprotection in SH-SY5Y cells, positions them as potential lead candidates for neurodegenerative and psychiatric disorders. The study also confirms that rational drug design, coupled with eco-friendly synthetic strategies like microwave-assisted synthesis, can yield efficient and potent therapeutic analogues. While KA-4 underperformed across most criteria, its evaluation helped in understanding the structural features detrimental to efficacy and safety. The findings validate the approach and encourage further in vivo studies and clinical evaluations. Continued exploration of structure-activity relationships and blood-brain barrier permeability could accelerate the translation of such analogues into therapeutic interventions.

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