Supplementary data

Phenoxyacetic Acid Scaffold as a Platform for Dual Anticonvulsant and Anti-Inflammatory Drug Design

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Toxicity impact

Classification	Target	Shorthand	Prediction	Probability
Events	isoxazolepropionate receptor (AMPAR)			
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.85
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.83
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.88
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.77
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.91
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.83
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.80
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.69
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.69
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.66
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.99

ProTox-3.0 - Prediction of TOXicity of chemicals

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.56
Organ toxicity	Neurotoxicity	neuro	Active	0.51
Organ toxicity	Nephrotoxicity	nephro	Active	0.64
Organ toxicity	Respiratory toxicity	respi	Active	0.75
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.50
Toxicity end points	Carcinogenicity	carcino	Inactive	0.54
Toxicity end points	Immunotoxicity	immuno	Active	0.61
Toxicity end points	Mutagenicity	mutagen	Inactive	0.54
Toxicity end points	Cytotoxicity	cyto	Inactive	0.65
Toxicity end points	BBB-barrier	bbb	Inactive	0.67
Toxicity end points	Ecotoxicity	eco	Inactive	0.75
Toxicity end points	Clinical toxicity	clinical	Active	0.60
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.66
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.76
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.92
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.89
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.97
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.92
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.92
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.84
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.94
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.95
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Active	0.57
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.64
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.64
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.75
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.78
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.91
Molecular Initiating	alpha-amino-3-hydroxy-5-methyl-4-	mie_ampar	Inactive	0.96

Classification	Target	Shorthand	Prediction	Probability
Events	isoxazolepropionate receptor (AMPAR)			
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.98
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.87
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.84
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.92
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.77
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.92
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.82
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.78
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.63
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.68
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.69
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.99

ProTox-3.0 - Prediction of TOXicity of chemicals

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.58
Organ toxicity	Neurotoxicity	neuro	Inactive	0.5
Organ toxicity	Nephrotoxicity	nephro	Active	0.70
Organ toxicity	Respiratory toxicity	respi	Active	0.73
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.54
Toxicity end points	Carcinogenicity	carcino	Inactive	0.64
Toxicity end points	Immunotoxicity	immuno	Active	0.85
Toxicity end points	Mutagenicity	mutagen	Inactive	0.59
Toxicity end points	Cytotoxicity	cyto	Inactive	0.63
Toxicity end points	BBB-barrier	bbb	Inactive	0.52
Toxicity end points	Ecotoxicity	eco	Inactive	0.72
Toxicity end points	Clinical toxicity	clinical	Active	0.61
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.65
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.80
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.92
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.87
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.92
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.89
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.89
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.84
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.91
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.95
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Active	0.64
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.61
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.68
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.73
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.79
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.93
Molecular Initiating	alpha-amino-3-hydroxy-5-methyl-4-	mie_ampar	Inactive	0.97

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Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.91
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Toxicity end points	Carcinogenicity	carcino	Inactive	0.57
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Toxicity end points	Mutagenicity	mutagen	Inactive	0.52
Toxicity end points	Cytotoxicity	cyto	Inactive	0.62
Toxicity end points	BBB-barrier	bbb	Inactive	0.66
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Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.86
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.97
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Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Active	0.65
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.57
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.56
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.86
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.79
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.98
Molecular Initiating	alpha-amino-3-hydroxy-5-methyl-4-	mie_ampar	Inactive	0.99

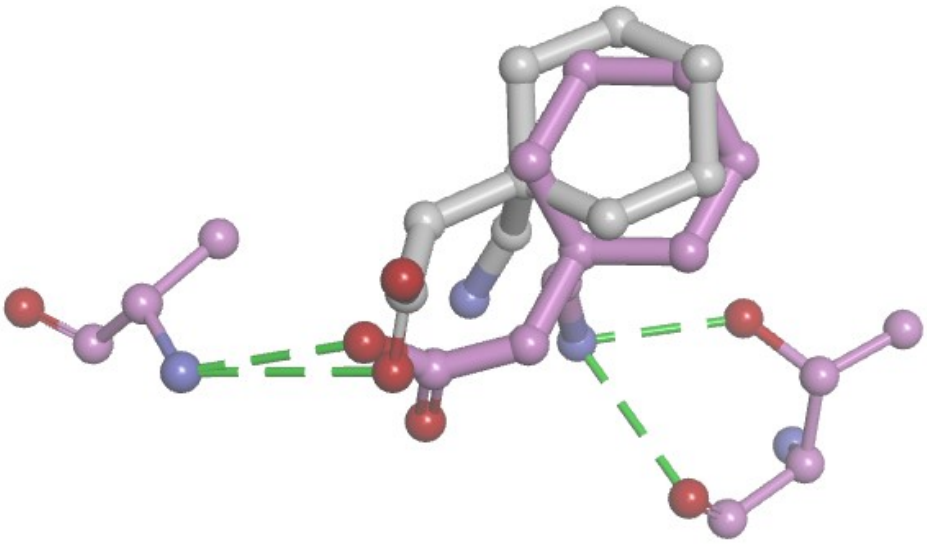
ProTox-3.0 - Prediction of TOXicity of chemicals

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.52
Organ toxicity	Neurotoxicity	neuro	Inactive	0.60
Organ toxicity	Nephrotoxicity	nephro	Active	0.69
Organ toxicity	Respiratory toxicity	respi	Active	0.57
Organ toxicity	Cardiotoxicity	cardio	Active	0.51
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Inactive	0.80
Toxicity end points	Mutagenicity	mutagen	Inactive	0.50
Toxicity end points	Cytotoxicity	cyto	Inactive	0.66
Toxicity end points	BBB-barrier	bbb	Inactive	0.55
Toxicity end points	Ecotoxicity	eco	Inactive	0.80
Toxicity end points	Clinical toxicity	clinical	Active	0.61
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.63
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.62
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.88
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.90
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.93
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.93
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.82
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.90
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.91
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Active	0.64
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.58
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.65
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.84
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.77
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.98
Molecular Initiating	alpha-amino-3-hydroxy-5-methyl-4-	mie_ampar	Inactive	0.99

Classification	Target	Shorthand	Prediction	Probability
Events	isoxazolepropionate receptor (AMPAR)			
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.93
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	1.0
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.84
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.88
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.82
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.93
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.82
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.74
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.57
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.73
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.67
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.99

Figure S1: *Toxicity impact of designed compounds* ***5d-f, 7b, 10d, e,*** *and* ***13b*** *using ProTox 3.0 program.*

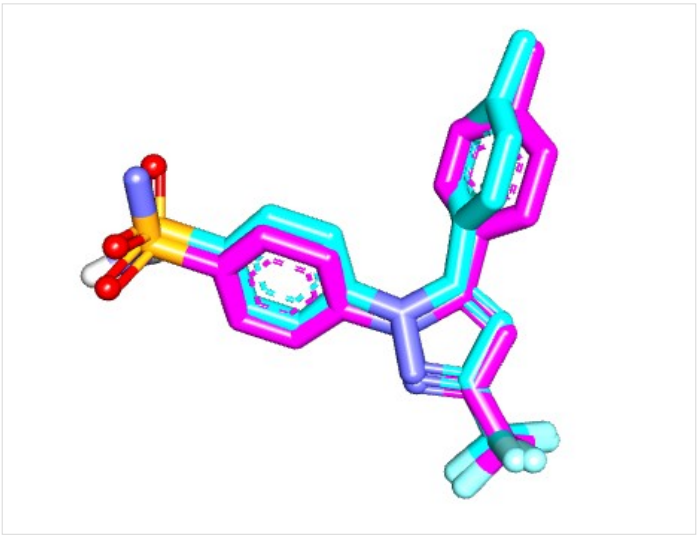
Molecular docking survey



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-5.5	0.000	0.000
2	-5.2	2.142	3.012
3	-5.0	11.938	13.072
4	-4.9	1.961	2.784
5	-4.9	2.377	3.152
6	-4.8	11.793	12.482
7	-4.8	1.952	2.452
8	-4.7	2.794	3.591
9	-4.7	8.714	10.064

Writing output ... done.

Figure S2: *Superimposition of co-crystalized gabapentin (purple) and redocked gabapentin (white) for validation of docking protocol.*



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-11.0	0.000	0.000
2	-8.8	4.943	6.434
3	-8.5	3.875	5.950
4	-8.2	4.874	7.274

Writing output ... done.

Figure S3: *Superimposition of co-crystalized celecoxib* ***B*** *(purple) and redocked celecoxib (Cyan) for validation of docking protocol.*

Experimental procedures and instruments

1 Material and methods

1.1 Bioinformatics study

1.1.1 Identification of related targets for anti-inflammatory, anti-epilepsy and Prediction of compound targets

Targets associated with anti-inflammatory and anti-epilepsy were retrieved from GeneCards platform (https://www.genecards.org/) and DisGeNET (https://disgenet.com/). Then, these collected targets were merged, and duplicates were removed. In parallel, the predicted molecular targets of the active compounds **5d, 5e, 5f, 7b, 10d, 10e**, and compound **13d**, were obtained using Swiss target prediction (STP) (http://www.swisstargetprediction.ch/). specifying Homo sapiens as the target species. The intersection of anti-inflammatory and anti-epilepsy -related targets and compound-associated targets was determined and visualized using VENNY 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

1.1.2 Gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and genomes (KEGG) pathway analysis

ShinyGO (http://bioinformatics.sdstate.edu/go/) is a web-based tool designed for the analysis of gene expression data, focusing on GO terms, pathway analysis, and protein–protein interaction networks. GO enrichment analysis primarily focuses on the biological processes while, KEGG enrichment analysis is aimed at identifying the biological pathways and functions associated with those targets. It enables researchers to explore BP associated with gene lists, offering valuable insights into disease mechanisms and potential therapeutic targets.

1.2 Experimental animals

Experiments were performed on adult male Sprague-Dawley rats (weighing ~130–150 g) and male Swiss albino mice (weighing ~23–30 g). The rats were used for anti-inflammatory and analgesic assays, while the mice were used for the seizure models and subsequent toxicity assessments. All animals were housed under standard laboratory conditions (22 ± 2 °C, 50-60% relative humidity, 12 h light/dark cycle) with free access to standard chow and water. Animals were acclimatized for at least one week prior to experimentation to minimize stress. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Faculty Pharmacy Research Ethics Committee.

1.3 Carrageenan-induced paw edema (anti-inflammatory assay)

Acute anti-inflammatory activity was evaluated using the carrageenan-induced hind paw edema model. Sprague-Dawley rats were randomly divided into eleven groups (n = 6 per group): a normal control group (vehicle only, no carrageenan), a negative control group (carrageenan with vehicle pretreatment), two reference drug groups (indomethacin and celecoxib), and one group for each of the seven test compounds (**5d, 5e, 5f, 7b, 10d, 10e, 13b**). Test compounds and reference drugs were prepared as suspensions in 0.5% carboxymethylcellulose (CMC) and administered orally (p.o.) at a dose of 20 mg/kg. One hour after treatment, inflammation was induced in all groups except the normal control by a subplantar injection of 0.1 mL λ-carrageenan (1% w/v in sterile saline) into the right hind paw. The normal control group received an equal volume of saline in the paw instead. Paw thickness (swelling) was measured for each rat using a digital Vernier caliper at baseline (immediately before carrageenan injection) and then at hourly intervals for 5 hours post-injection. The degree of edema at each time point was calculated as the change in paw thickness (Δ thickness = thickness at time t minus baseline thickness). From these data, the percentage inhibition of edema in each treated group relative to the carrageenan control was determined using the formula: Inhibition (%) = [(Δ paw thickness of carrageenan control - Δ paw thickness of treated) / Δ paw thickness of carrageenan control] × 100. At the end of the 5 h observation period, the rats were humanely euthanized with an overdose of pentobarbital (100 mg/kg i.p.) followed by cervical dislocation. Both hind paws were then excised at the ankle joint and weighed. The edema was additionally quantified as the percentage increase in paw weight of the inflamed (right) paw compared to the non-inflamed (left) paw, calculated as: paw weight increase (%) = [(right paw weight - left paw weight) / left paw weight] × 100.

1.4 PTZ-induced seizure model (anticonvulsant screening)

The anticonvulsant activity of the test compounds was first screened in the acute pentylenetetrazol (PTZ)-induced seizure model. Male Swiss albino mice were randomly divided into nine groups (10 mice per group) for this experiment: (1) Vehicle control + PTZ, mice pretreated with vehicle (0.5% CMC p.o.) and then challenged with PTZ; (2) Valproate + PTZ, positive control group in which mice received the reference antiepileptic drug sodium valproate (300 mg/kg p.o.) 30 min before PTZ; (3-9) Test compound + PTZ, groups pretreated with compounds 5d, 5e, 5f, 7b, 10d, 10e, or 13b (each at 20 mg/kg p.o., 30 min before PTZ). The PTZ convulsive challenge was

administered to all groups as a single intraperitoneal injection of 85 mg/kg PTZ (in 0.9% saline) **(Du et al., 2025)**. Immediately after PTZ injection, mice were placed individually into observation chambers and monitored for seizure activity by an observer blinded to the treatments. Seizure manifestations were identified as per standard criteria (forelimb clonus and/or loss of righting reflex indicating a generalized tonic-clonic seizure). Each mouse was observed for at least 30 minutes post-PTZ, or until a generalized seizure followed by recovery or death had occurred. The occurrence of tonic-clonic seizures was recorded. The primary outcome measure for anticonvulsant efficacy was the protection rate, defined as the percentage of mice in each group that did not develop generalized seizures during the observation period and relative efficacy compared with the valproate-treated group. Additionally, mortality was recorded for 24 hours after PTZ injection as a secondary outcome.

1.5 Pilocarpine-induced seizure model (status epilepticus)

Compounds were further evaluated in the pilocarpine-induced seizure model. For this assay, male mice were assigned to the following groups (n = 10 per group): Normal control (vehicle only, no pilocarpine), Pilocarpine control (vehicle pretreatment, then pilocarpine), Valproate + pilocarpine (300 mg/kg valproate p.o.), and Test compound + pilocarpine groups for each compound (20 mg/kg p.o.). All mice received an intraperitoneal injection of scopolamine butylbromide (1 mg/kg, i.p.) 20 min prior to pilocarpine, to minimize peripheral cholinergic symptoms (salivation, convulsions in peripheral muscles) without affecting central seizure activity. Pilocarpine was then administered at 300 mg/kg i.p. to induce seizures. This dose is known to provoke an episode of status epilepticus (SE) in untreated mice, characterized by continuous or recurrent seizures lasting for hours. Behavioral seizure activity was monitored for 120 minutes after pilocarpine injection in each animal. Seizure severity was scored according to Racine's scale (stage 0 = no response; 1 = facial automatisms and drooling; 2 = head nodding; 3 = forelimb clonus; 4 = rearing with clonus; 5 = generalized tonic-clonic seizures with loss of posture) at 30 min intervals. The latency to seizure onset was defined as the time from pilocarpine injection to the first occurrence of a stage 3 seizure (onset of forelimb clonus). The number of animals that survived 24 hours after pilocarpine was recorded for each group. For humane reasons, any mouse still in status epilepticus at 2 h was given diazepam to halt seizures and counted as a survivor if it lived 24 h, though its seizure severity was maximal.

1.6 Assessment of inflammatory mediators in carrageenan-induced paw edema

Following the completion of the anti-inflammatory assay, only the most promising candidate, compound **7b**, was advanced for biomarker evaluation. Compound 7b was selected based on its superior inhibition of paw edema and favorable profile in preliminary assays. To explore its mechanistic effects on key inflammatory pathways, paw exudates were collected from carrageenan-injected rats treated with 7b, along with normal control, carrageenan control and reference drugs groups. Immediately after sacrifice, paw tissues from the site of injection were excised and paw exudate were analyzed for inflammatory markers. The expression levels of cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), and prostaglandin E_2 (PGE_2) were quantified using ELISA kits specific for each marker: COX-2 (MyBioSource, USA), TNF-α (Cloud-Clone Corp., USA), and PGE_2 (MyBioSource, USA). All assays were conducted in accordance with manufacturers' instructions. Protein content was determined using the biuret protein assay using bovine serum albumin as standard, and results were normalized to the protein concentration in each sample.

1.7 Hot plate test (analgesic assay)

To verify potential central analgesic effects, compound **7b** was evaluated in the hot plate latency test, as it had demonstrated the most robust anti-inflammatory profile in the carrageenan assay. Male Sprague-Dawley rats were divided into four groups (n = 6 per group): vehicle control, indomethacin, celecoxib, and **7b**. All treatments were administered orally at 20 mg/kg suspended in 0.5% CMC. Analgesic response was assessed at 30, 60, 90, and 120 minutes post-administration using a heated plate maintained at 50 ± 0.5 ℃. Animals were confined on the plate with a transparent acrylic cylinder, and the response latency (time to hind paw lick or jump) was recorded. A cut-off time of 30 seconds was enforced to exclude tissue injury. All measurements were conducted by blinded observers to minimize bias.

1.8 In vitro cyclooxygenase (COX-1/COX-2) inhibition assay:

The *in vitro* enzyme assays were performed at Biochemistry Department, Cairo General Hospital, Egypt. Cayman® colorimetric COX (ovine) inhibitor screening assay kit (Item No. 560131 – Michigan, USA) was used for monitoring the activity of COX-1 and COX-2 enzymes in presence of all newly compounds. Generally, this kit is dependent on the reductive reaction of COX. A specific antibody binds to PGH_2 produced by COX and further quantified by ELISA

technique as previously reported. After that, the selectivity index (SI) was calculated as the following equation:

$$Selectivity\ index\ (SI) = \frac{IC_{50} of\ COX-1}{IC_{50}\ of\ COX-2}$$

1.9 In vitro lipoxygenase (5-LOX) inhibition assay:

According to Cayman® (Michigan, USA) guidelines, 5-LOX inhibitor screening assay kit (Item No. 760700) was employed for detection the activity of 5-LOX against quercetin (QRC) in presence of all newly compounds.

1.10 Biomarker analysis in the hippocampus

To investigate neurochemical and inflammatory changes underlying seizure protection, brain tissue was collected from mice in the pilocarpine experiments for biochemical analyses. In particular, for the most effective compound (7b), as well as for comparison groups (untreated normal mice, pilocarpine-alone controls, and valproic acid treated group), hippocampal tissues were isolated at the end of the 24 h observation period. Mice were deeply anesthetized and decapitated; the brains were rapidly removed and the hippocampi dissected on ice. The tissue samples were frozen at -80 ℃ until assay. Hippocampal samples were homogenized in ice-cold phosphate-buffered saline (10% w/v homogenate), and the homogenates were centrifuged at 12,000 × g for 15 min at 4 ℃. The supernatants were collected for ELISA quantification of glutamate and inflammatory markers. Specifically, glutamate was (as an index of excitatory neurotransmitter release), TNF-α and IL-6 (pro-inflammatory cytokines), and the glial activation markers GFAP (astrocytic marker) and Iba-1 (microglial marker) in the hippocampal supernatants. Commercial ELISA kits were used for each analyte: glutamate (kit from MyBioSource, USA), TNF-α (Cusabio, USA), IL-6 (R&D Systems, USA), GFAP (MyBioSource, USA), and Iba-1 (MyBioSource, USA), according to the manufacturers' instructions. All measurements were normalized to the total protein content of the sample (pg or ng per mg of protein). Total protein was determined by the biuret protein assay using bovine serum albumin as standard.

1.11 Toxicological evaluation

A preliminary safety assessment of compound **7b** was performed by examining vital organ function indicators. A separate cohort of Swiss albino mice was used for this purpose. Mice were

divided into two groups (n = 6 per group): a vehicle control group and a high-dose **7b** group. The treated group received a single oral dose of 7b at 100 mg/kg p.o. (suspended in 0.5% CMC), which is five times the dose used in efficacy tests, to challenge the safety margin. Control mice received an equivalent volume of vehicle. After dosing, all animals were closely observed for 48 hours for any signs of acute toxicity or distress. Observational parameters included general behavior, locomotor activity, posture and gait, grooming and fur appearance, food and water intake, and the occurrence of any neurological symptoms (tremors, convulsions, lethargy) or mortality. At the end of the 48-h observation period, mice were anesthetized and blood samples were collected via retro-orbital sinus puncture. Blood was allowed to clot and then centrifuged at 3000 rpm for 10 min to obtain serum. Biochemical markers of organ function were measured in serum using standard diagnostic kits according to the manufacturers' protocols. Hepatic function was evaluated by assaying serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using kits from Spectrum Diagnostics (Cairo, Egypt). Renal function was assessed by measuring blood urea nitrogen (BUN) and creatinine levels using kits from Spectrum Diagnostics. Cardiac injury markers were also examined: creatine kinase-MB (CK-MB) activity was measured as an indicator of myocardial enzyme release, and troponin T levels were determined by ELISA kits from LSBio (Cat# LS-F5746) and MyBioSource (Cat# MBS730382), respectively. These parameters (liver enzymes, renal indices, cardiac markers) were chosen to detect any subclinical organ toxicity from compound 7b. All measurements were compared to the vehicle control values to identify significant deviations.

1.12 Statistical analysis

Data were expressed as mean ± standard deviation (SD) for each experimental group. Statistical analysis was performed using GraphPad Prism software (v9.5.1, Demo). For paw edema, seizure severity, and analgesic activity, a two-way analysis of variance (ANOVA) was used, followed by Tukey's multiple-comparison post hoc test at each time point. Single-time-point comparisons were done using one-way ANOVA, followed by Tukey's post hoc test for pairwise group comparisons. Comparisons related to the toxicological evaluation were carried out using the unpaired Student's t-test. A P value < 0.05 was considered statistically significant for all analyses.

1.13 *In-silico* ADME study

The physicochemical descriptors, along with the predicted pharmacokinetic and drug-likeness profiles of the synthesized compounds, were assessed using the SwissADME and ProTox 3.0 web servers. In the early stages of drug development, it is imperative to evaluate these properties, as they play a critical role in determining the success of a candidate compound. Beyond potency, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics, as well as overall drug-likeness, are essential considerations in optimizing lead compounds for further development. These parameters help predict the compound's behavior in biological systems, thereby facilitating the selection of candidates with favorable pharmacological profiles, in addition the target prediction survey was conducted with SwissTargetPrediction

1.14 Molecular Docking Studies

Molecular docking was employed to investigate the interactions between the synthesized ligands and the target protein, in comparison with a standard drug. Docking simulations were performed using the crystal structure of reduced human cytosolic branched-chain aminotransferase (VGSCs; PDB ID: 2COJ), obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format. Molecular docking was carried out using AutoDock 4.2 software. The resulting protein–ligand interaction complexes were visualized, and 2D interaction diagrams were generated using BIOVIA Discovery Studio Visualizer to analyze binding modes and key interacting residues