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T. Annavarapu[1], S. Kamepalli[1], S.K. Kondala[2], V. Kotra[3], S.R. Challa[4], M. Rudrapal[5*], A.R. Bendale[6]

[1]*Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India;*
[2]*School of Pharmaceutical Sciences, Vignan's Foundation for Science, Technology and Research, Guntur, Andhra Pradesh, India;*
[3]*Faculty of Pharmacy, Quest International University Perak (QIUP), Perak, Malaysia;*
[4]*Department of Cancer Biology and Pharmacology, College of Medicine, University of Illinois, Peoria, Illinois, USA;*
[5]*Rasiklal M. Dhariwal Institute of Pharmaceutical Education & Research, Pune, Maharashtra, India;*
[6]*Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India*
*(*Corresponding author's e-mail: rsmrpal@gmail.com)*

Anti-inflammatory and antioxidant activities of 4-allylpyrocatechol and its derivatives with molecular docking and ADMET investigations

Abnormal production of pro-inflammatory mediators and generation of reactive oxygen species (ROS) play a key role in the development and progression of various human disorders. The study aims to investigate the *in vitro* anti-inflammatory and antioxidant activity of 4-allyl pyrocatechol (4-APC) and its derivatives (APC-1 and APC-2) by albumin denaturation and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) methods, respectively. Also, the test compounds are studied *in silico* for their inhibitory potential against the pro-inflammatory and oxidative markers (calpain, FAAH, and TNF-α) via molecular docking. The compounds have exhibited appreciable *in vitro* anti-inflammatory and antioxidant activities. The APC-2 compound has demonstrated significant anti-inflammatory and antioxidant activity (percentage inhibition = 69±0.76 and 77.05±0.92, respectively, at 100 µg/ml) compared to the standard drugs, aspirin and ascorbic acid (percentage inhibition = 82±0.83 and 92.35±0.75, respectively, at 100 µg/ml). The docking study has showed that APC-2 significantly inhibited calpain (PDB ID: 2R9C), FAAH (2WJ1) and TNF-α (2AZ5) inflammatory markers. The drug-likeness, bioactivities, ADME profile (pharmacokinetic) and toxicity properties have also been determined using online tools (Molinspiration, pkCSM, SwissADME, PreADMET). The test compounds have showed acceptable drug-likeness, bioactivity score, ADME and toxicity properties. Finally, we conclude that the 4-allylpyrocatechol and its derivatives can be used as lead molecules for their further development as therapeutically useful anti-inflammatory agents.

Keywords: pyrocatechol, anti-inflammatory, antioxidant, lead molecule, inflammatory markers, calpain, FAAH, TNF-α, docking, ADMET.

Introduction

Chronic anti-inflammatory diseases, including rheumatoid arthritis, are still one of the major health problems of the world's population. Chronic inflammation may lead to considerable tissue damage in human diseases [1, 2]. Inflammation can cause damage to body tissues (endothelial cells, muscle cells, nerve cells) through the production of an array of pro-inflammatory and inflammatory mediators, such as prostaglandins (PGEs), interleukins (ILs), tumor necrosis factor-alpha (TNF), etc. [3]. The abnormal production of pro-inflammatory mediators and the generation of reactive oxygen species (ROS) play a key role in the development of inflammations and associated human illness [4, 5]. ROS are free oxygen radicals that can rapidly react with biological molecules (lipids, DNA, and proteins), resulting in oxidative stress (OS) and consequently cellular damage [6]. The production of free radicals and pro-inflammatory/ inflammatory mediators

is believed to be the underlying cause of inflammatory diseases [6–9]. The anti-inflammatory, antioxidant, antimicrobial, antifungal activities of 4-allylpyrocatechol (APC) have already been reported [10, 11]. As continuation of our work [12], this work aims to study the anti-inflammatory and antioxidant activity of previously synthesized 4-allylpyrocatechol and its derivatives by using *in vitro* methods. Further, molecular docking was carried out by *in silico* method to investigate the inhibitory potential of test compounds against oxidative (calpain), fatty acid amide hydrolase (FAAH), and pro-inflammatory (TNF-α) markers. To evaluate the overall drug-likeness, drug-likeness parameters, bioactivities, ADME profile (pharmacokinetic) and toxicity properties were also determined using online tools.

Experimental

Chemicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), DMSO, ethanol, ascorbic acid, bovine albumin were purchased from National Scientific Products, Guntur, A.P. India. All other chemical reagents and chemicals used were of analytical grade.

In vitro anti-inflammatory activity: The protein denaturation method was used to determine the anti-inflammatory activity of the synthesized molecules according to the previously reported technique [8] with some minor modifications. The final volume of the reaction mixture was 5 ml and comprised 0.2 ml bovine albumin (1 %), 0.70 ml phosphate buffer saline (PBS, pH 6.4), and 0.1 ml test compound (sample). The pH was adjusted to 1N HCl. The reaction mixture was incubated in water bath for 15 min at 37 °C, and then it was heated to 70 °C for 5 min. The absorbance of the turbid solution was determined at 660 nm by using a UV-Visible Spectrophotometer (Elico, India). The phosphate buffer was used as a control, and aspirin was used as a standard reference drug. The percentage inhibition of protein denature was calculated using the following formula:

$$\text{Percentage inhibition of denaturation} = ([1 - (A_s/A_c)] \times 100),$$

where A_c — the absorbance of a control; A_s — the absorbance of a sample.

In vitro antioxidant activity: The antioxidant activity was assessed by DPPH radical scavenging assay according to the previously published method [13] with some modifications. The 1 mg/ml stock solution was prepared by mixing the test compound using DMSO as a solvent. The DPPH radical solution (0.5 mM) was prepared using ethanol. The stock solution was used to prepare different concentrations (100 µg/ml, 50 µg/ml, and 25µg/ml) of the test compound with DMSO. The standard solution of ascorbic acid was also prepared in the same way as mentioned above. The different final test solutions consisted of 0.5 ml sample solution, 3 ml absolute ethanol and 0.3 ml 0.5 mM DPPH solution in ethanol. The blank consisted of 3.3 ml ethanol and 0.5 ml sample solution. The control solution was prepared by mixing 3.5 ml ethanol and 0.3 ml DPPH solution. Test solutions were incubated for 30 min at room temperature. The change in color (from deep violet to light yellow) was measured at 517 nm using a UV-Visible Spectrophotometer against the blank. The radical scavenging activity was measured in percentage using the following formula:

$$\text{Percentage of scavenging activity} = ([1 - (Ab_s/Ab_c)] \times 100),$$

where Ab_c — the absorbance of a control; Ab_s — the absorbance of a sample.

Statistical analysis: The statistical analysis was performed using GraphPad Prism 8.0, and values were expressed in mean ± SEM. The statistical comparison was made using ANOVA, where $p < 0.05$ was considered statistically significant.

Molecular docking study: The docking study was performed for 4-allyl pyrocatechol (4-APC) and its two derivatives, APC-1 and APC-2, against the oxidative and pro-inflammatory markers such as calpain, FAAH, and TNF-α. The protein-ligand docking was conducted in PyRx Virtual Screening software 0.8 [14–16].

The X-ray crystal structures of calpain (PDB ID: 2R9C), FAAH (PDB ID: 2WJ1, FAAH) and TNF-α (PDB ID: 2AZ5) were retrieved from Protein Data Bank [17–20]. The water molecules were removed, hydrogen atoms were added, and co-crystal ligands were extracted using PyMOL 2.3.4 and saved in .mol2 format. The mol2 file of protein was loaded converted to .pdbqt format through AutoDock module Macromolecule tool in PyRx Virtual Screening software 0.8.

The 2D structures of ligands (4-APC, APC-1, and APC-2), which were previously characterized by ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrophotometry [12], were drawn using ChemSketch v14.00 and saved as .sdf file. The ligand files were subjected to energy minimization (force filed-off) through the Open babel tool and then conformers for the selected ligands were generated through AutoDock .pdbqt files in PyRx Virtual screening software 0.8. The 2D structures of 4-APC and its derivatives (APC-1 and APC-2) are presented in Figure 1.

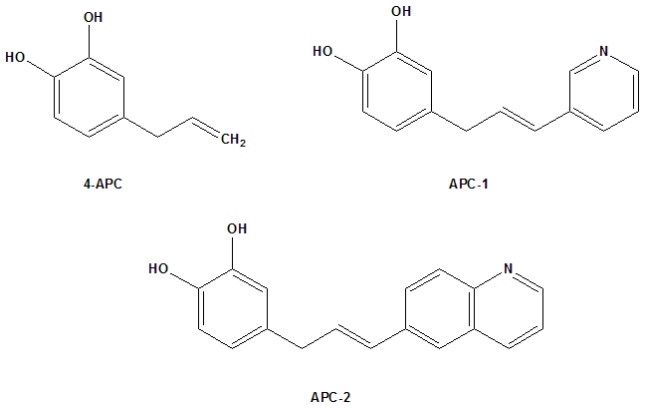


Figure 1. Structures of 4-APC and its derivatives (APC-1 and APC-2)

Drug-likeness, bioactivities, ADME and toxicity prediction: The 2D structures of 4-APC, APC-1, and APC-2 were used for assessing drug-likeness, bioactivities, ADME and toxicity properties [21–23]. Various online tools such as Molinspiration, pkCSM, SwissADME and PreADMET [24, 25] were used to calculate the abovementioned properties.

Results and Discussion

Anti-inflammatory activity: The results of *in vitro* anti-inflammatory activity of the test compounds are shown in Figure 2. The compound APC-2 showed maximum inhibitory activity of 69±0.76 % at 100 µg/ml. The standard anti-inflammatory drug aspirin exhibited 74±0.83 % inhibition of protein denaturation at 100 µg/ml. The other two compounds, 4-APC and APC-1 also exhibited anti-inflammatory activity with 47±0.96 % and 56±0.45 % inhibition of protein denaturation at 100 µg/ml, respectively.

Antioxidant activity: Figure 3 presents the results of *in vitro* antioxidant activity. Test compounds, 4-APC, APC-1, and APC-2 showed significant DPPH radical scavenging activity compared with the standard drug, ascorbic acid. The decrease in absorbance of DPPH radicals was observed with increase in the concentration of test compounds due to the scavenging of radicals by hydrogen donation. This was easily observed with change in color from purple to yellow. Among the test samples, APC-2 exhibited a significant percentage inhibition (77.05±0.92 %, 100 µg/ml) compared with standard ascorbic acid (92.35±0.75 %, 100 ug/ml). The other two compounds (4-APC and APC-1) showed considerable DPPH radical scavenging activity.

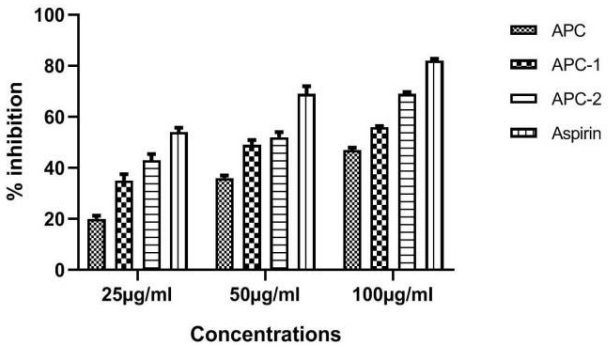


Figure 2. Protein denaturation activity of 4-APC and its derivatives (APC-1 and APC-2)

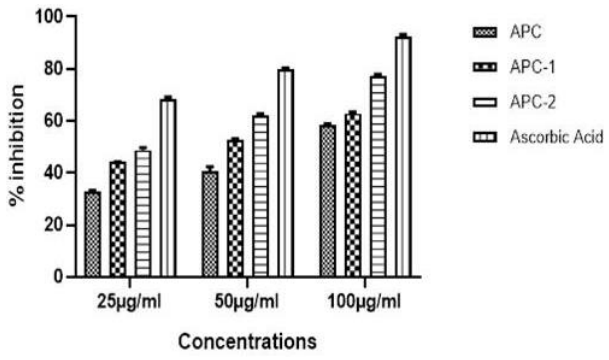


Figure 3. DPPH radical scavenging activity of 4-APC and its derivatives (APC-1 and APC-2)

Docking study: A molecular docking study substantiated the anti-inflammatory and antioxidant activities of 4-APC, APC-1, and APC-2. Three oxidative and pro-inflammatory markers, such as calpain, FAAH, and TNF-α, were used as target proteins in the docking study. Results of protein-ligand docking are expressed as binding energies (kcal/mol) of protein-ligand interaction and are presented in Tables 1, 2, 3. From docking results, the compound APC-2 showed the lowest binding energies against all three different proteins. The other two compounds (4-APC and APC-1) exhibited comparatively more binding energies than the compound APC-2. However, the binding energies of internal ligands (co-crystal ligands) were found over the test compounds. From binding energies, it is clear that APC-2 possesses more binding affinity than

the other two compounds (4-APC and APC-1) against all the three target proteins (calpain, FAAH, and TNF-α).

Table 1

Binding energies and interacting amino acids of 4-APC, APC-1, and APC-2 against calpain

Sl. No.	Compound	Binding energy (kcal/mol)	Interaction(s)	Interacting amino acids
1	4-APC	-4.7	H bond pi-alkyl	Gly110 Trp298, Val301
2	APC-1	-5.8	H bond	Ser251, Glu349
3	APC-2	-6.8	H bond pi-alkyl	Arg270, His272, Glu300 Ala262
4	Internal ligand (GRD)	-8.2	H bond	Glu109, Leu112, Cys115, Gly208, Thr210, Trp298, Glu349

Table 2

Binding energies and interacting amino acids of 4-APC, APC-1, and APC-2 against FAAH

Sl. No.	Compound	Binding energy (kcal/mol)	Interaction(s)	Interacting amino acids
1	4-APC	-6.5	pi-sigma Pi-alkyl	Phe192 Ser193
2	APC-1	-8.6	H bond pi-alkyl	Tyr194, Gly216, Ser241, Leu404, Val491
3	APC-2	-9.1	H bond pi-sigma pi-alkyl	Lys142, Thr236, Ser241, Gln273 Ile238, Met191 Val270, Leu278
4	Internal ligand (S99)	-8.6	H bond pi-sigma pi-alkyl	Cys269, Val270 Ile238 Leu278, Leu380

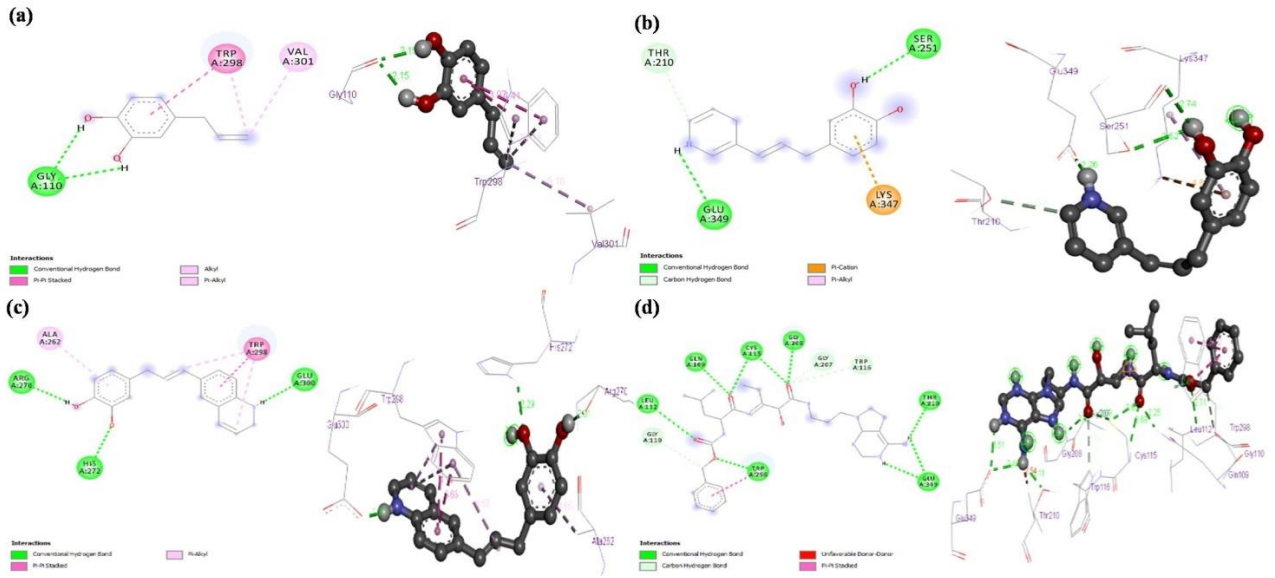
Table 3

Binding energies and interacting amino acids of 4-APC, APC-1, and APC-2 against TNF-α

Sl. No.	Compound	Binding energy (kcal/mol)	Interaction(s)	Interacting amino acids
1	4-APC	-4.7	H bond pi-alkyl pi-pi staking	Leu120, Gly121 Ile155 Tyr59
2	APC-1	-5.6	H bond pi-alkyl pi-pi staking	Ser60, Leu120 Leu57 Tyr59
3	APC-2	-6.6	H bond pi-alkyl pi-pi stacking	Gln61 His15, Tyr59, Tyr151 Tyr59, Tyr119
4	Internal ligand (307)	-6.8	H-bond pi-alkyl pi- pi staking	Lue120 Gln61, Tyr119 Tyr59, Tyr119, Tyr151

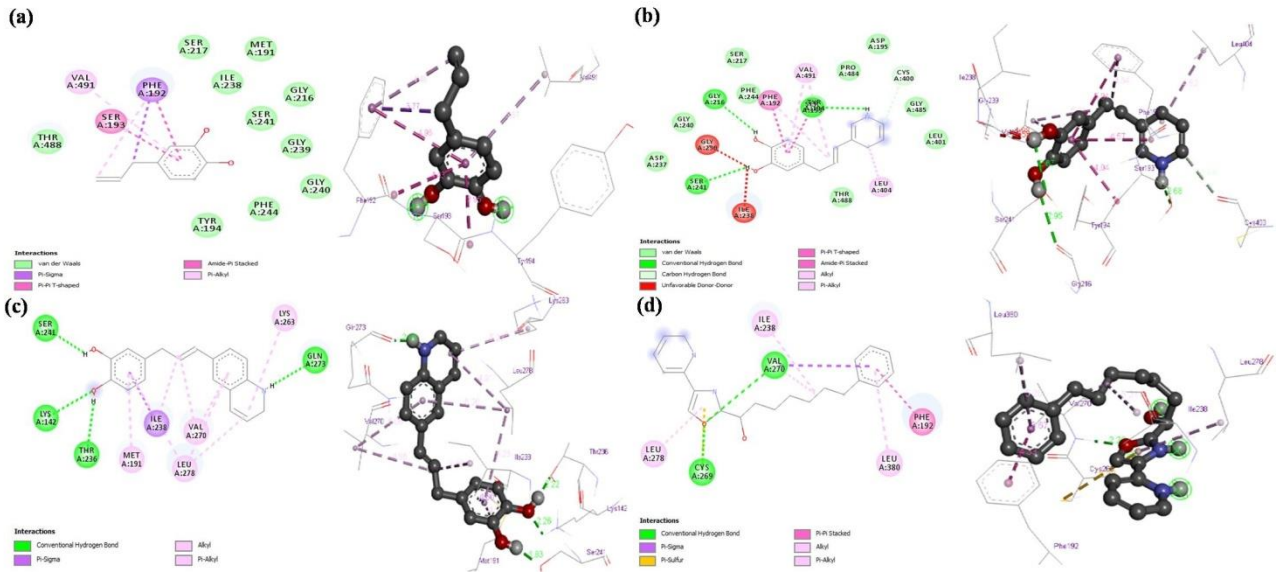
Upon analysis of protein-ligand interactions, various non-bonding interactions exist between ligands (4-APC, APC-1, and APC-2) and proteins (calpain, FAAH, and TNF-α). Non-bonding interactions like H bond, pi-alkyl and pi-pi staking are involved between ligand and protein molecules. Various amino acid residues, such as Tyr59, Ser60, Gln61, Tyr119, Leu120, Gly121, Ile155, Tyr194, Gly216, Ser241, Leu404, Val491, and so on, are involved in the interactions from the active binding sites of calpain, FAAH, and

TNF-α. Binding poses (3D) and protein-ligand interaction diagrams (2D) of protein-ligand are presented in Figures 4, 5, 6.



a — 4-APC; *b* — APC-1; *c* — APC-2; *d* — internal ligand (GRD)

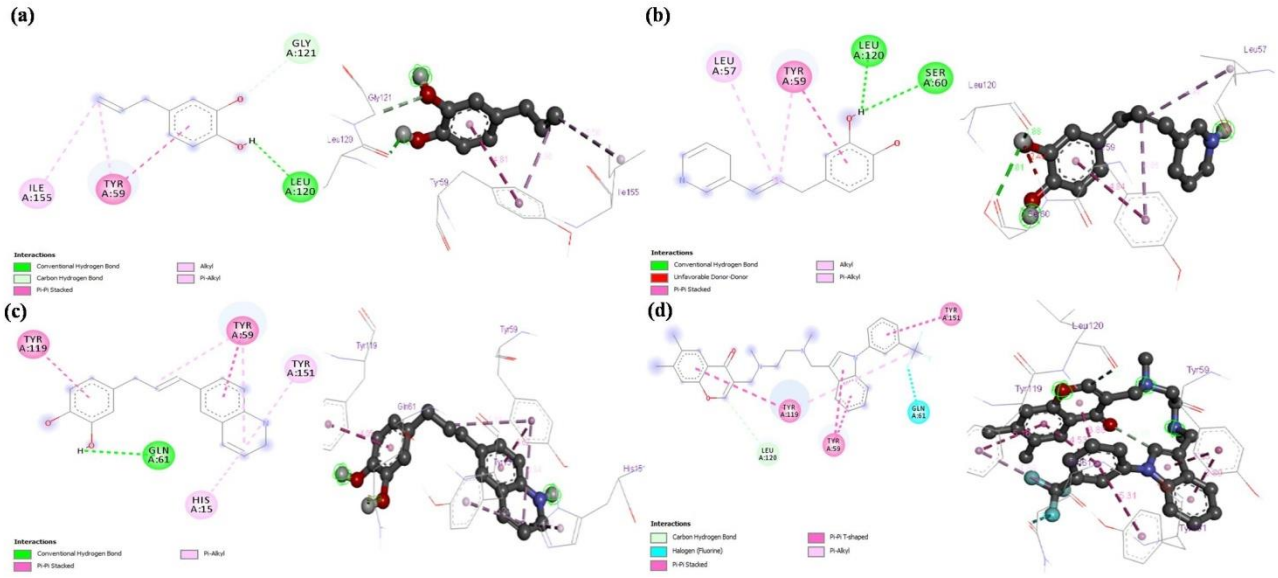
Figure 4. Binding poses (3D) and interaction diagrams (2D) of protein-ligand docking against calpain



a — 4-APC; *b* — APC-1; *c* — APC-2; *d* — internal ligand (S99)

Figure 5. Binding poses (3D) and interaction diagrams (2D) of protein-ligand docking against FAAH

Drug-likeness, bioactivities, ADME and toxicity profile: The results of drug-likeness, bioactivities, ADME and toxicity profile are given in Tables 4, 5, 6 and 7. Three compounds, such as 4-APC, APC-1, and APC-2, possess acceptable drug-likeness parameters (molecular weight, LogP, H bond acceptors, H bond donors etc.), bioactivities (GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease or enzyme inhibitor etc.), pharmacokinetics (ADME), and toxic properties. All the compounds were found to be drug-like molecules, biologically active, water/lipid soluble, non-toxic or non-mutagenic.



a — 4-APC; *b* — APC-1; *c* — APC-2; *d* — internal ligand (307)

Figure 6. Binding poses (3D) and interaction diagrams (2D) of protein-ligand docking against TNF-α

Table 4

Predicted drug-likeliness properties of 4-APC, APC-1, and APC-2

Sl. No.	Descriptor	4-APC	APC-1	APC-2
1	Molecular weight	150.177	227.263	277.323
2	LogP	1.8263	2.7487	3.9019
3	H bond acceptors	2	3	3
4	H bond donors	2	2	3
5	Surface area	65.425	99.702	122.384

Table 5

Predicted Bioactivity of 4-APC, APC-1, and APC-2

Sl. No.	Bioactivity	Bioactivity score		
		4-APC	APC-1	APC-2
1	GPCR ligand	-0.88	0.11	0.28
2	Ion channel modulator	-0.28	0.33	0.25
3	Kinase inhibitor	-1.26	-0.04	0.18
4	Nuclear receptor ligand	-0.76	0.06	0.31
5	Protease inhibitor	-1.28	-0.48	-0.12
6	Enzyme inhibitor	-0.40	0.30	0.31

Table 6

Predicted ADME profile of 4-APC, APC-1, and APC-2

Parameter	ADME properties	4-APC	APC-1	APC-2
1	2	3	4	5
Absorption	Water solubility (log mol/L)	-0.974	-2.511	-4.428
	Caco2 permeability (log Papp in 10^{-6} cm/s)	1.596	1.298	1.342
	Intestinal absorption (human) (% Absorbed)	90.171	91.252	92.235
	Skin permeability (log Kp)	-2.278	-2.612	-2.766
	P-glycoprotein substrate (Yes/No)	No	Yes	Yes
	P-glycoprotein I inhibitor (Yes/No)	No	No	No

Continuation of Table 6

1	2	3	4	5
	P-glycoprotein II inhibitor (Yes/No)	No	No	No
Distribution	VDss (human) (log L/kg)	0.31	0.196	0.133
	Fraction unbound (human) (Fu)	0.448	0.198	0.033
	BBB permeability (log BB)	0.166	0.118	0.057
	CNS permeability (log PS)	-2.075	-2.164	-1.864
Metabolism	CYP2D6 substrate (Yes/No)	No	No	No
	CYP3A4 substrate (Yes/No)	No	Yes	Yes
	CYP1A2 inhibitor (Yes/No)	Yes	Yes	Yes
	CYP2C19 inhibition(Yes/No)	No	Yes	Yes
	CYP2C9 inhibitor (Yes/No)	No	No	Yes
	CYP2D6 inhibitor (Yes/No)	No	No	No
	CYP3A4 inhibitor (Yes/No)	No	No	Yes
Excretion	Total clearance (log ml/min/kg	0.214	0.175	0.164
	Renal OCT2 substrate (Yes/No)	No	No	No

Table 7

Predicted drug toxicity for 4-APC, APC-1, and APC-2

Sl. No.	Toxicity parameter	4-APC	APC-1	APC-2
1	AMES toxicity	Yes	Yes	No
2	Max. tolerated dose (human) (log mg/kg/day)	0.696	0.484	0.049
3	hERG I inhibitor	No	No	No
4	hERG II inhibitor	No	No	Yes
5	Oral rat acute toxicity (LD_{50}) (mol/kg)	2.079	2.021	2.082
6	Oral rat chronic toxicity (log mg/kg_bw/day)	2.204	1.721	1.795
7	Hepatotoxicity	No	No	Yes
8	Skin sensitisation	Yes	No	No
9	Tetrahymena pyriformis toxicity (log ug/L)	0.166	1.211	1.067
10	Minnow toxicity (log mM)	1.724	1.062	-0.114
11	Acute algae toxicity	0.05680	0.06064	0.02052
12	2 years carcinogenicity bioassay in mouse	Positive	Negative	Negative
13	2 years carcinogenicity bioassay in rat	Negative	Negative	Negative
14	Acute daphnia toxicity	0.12609	0.16367	0.04595
15	*In vitro* Human ether-a-go-go related gene channel inhibition	Medium Risk	Medium Risk	Medium Risk
16	Acute fish toxicity (medaka)	0.02144	0.03946	0.00407
17	Acute fish toxicity (minnow)	0.01102	0.03670	0.00812
18	*In vitro* Ames test results in TA100 strain (Metabolic activation by rat liver homogenate)	Negative	Positive	Positive
19	*In vitro* Ames test results in TA100 strain (No metabolic activation)	Negative	Negative	Negative
20	*In vitro* Ames test results in TA1535 strain (Metabolic activation by rat liver homogenate)	Positive	Positive	Negative
21	*In vitro* Ames test results in TA1535 strain (No metabolic activation)	Positive	Positive	Negative

Conclusions

In conclusion, we conclude that the pyrocatechol and its derivatives possess appreciable *in vitro* anti-inflammatory and antioxidant activities. The pyrocatechol derivative 2 (APC-2) has a better activity profile among the three compounds. The *in silico* studies revealed a significant inhibitory potential of the compounds, especially the compound APC-2, against the oxidative and pro-inflammatory markers with an acceptable level of drug-likeness, ADME profile, and toxicities. Finally, it was suggested that the 4-allylpyrocatechol and its derivatives can be used as lead molecules for their further development as therapeutically useful anti-inflammatory agents.

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Т. Аннаварапу, С. Камепалли, С.К. Кондала, В. Котра,
С.Р. Чалла, М. Рудрапал, А.Р. Бендейл

4-Аллилпирокатехиннің қабынуға қарсы және антиоксиданттық белсенділігін және оның туындыларын молекулалық докинг пен ADMET арқылы зерттеу

Қабынуға қарсы медиаторлардың аномальды өнімдері және оттегінің белсенді түрлерінің генерациясы (ОБТ) адамның әртүрлі ауруларының дамуы мен өршуінде шешуші рөл атқарады. Осы зерттеудің мақсаты альбумин денатурациясы мен DPPH (1,1-дифенил-2-пикрилгидразил) әдісі арқылы 4-аллилпиратехиннің (4-APC) және оның туындыларының (APC-1 және APC-2) қабынуғақарсы және антиоксиданттық белсенділігін *in vitro* зерттеу. Сонымен қатар, сыналған қосылыстар *in silico*-да қабынуғақарсы және тотықтырғыш маркерлерге (кальпаин, FAAH және TNF-α) қатысты ингибиторлық потенциалы үшін молекулалық докинг әдісімен зерттелді. Қосылыстар *in vitro* жағдайында қабынуғақарсы және антиоксиданттық айқын белсенділікті көрсетті. APC-2 қосындысы аспирин және аскорбин қышқылы сияқты стандартты препараттармен салыстырғанда (пайыздық тежелу = 100 мкг/мл кезінде сәйкесінше 82±0,83 және 92,35±0,75) айтарлықтай қабынуғақарсы және антиоксиданттық белсенділікті көрсетті (пайыздық тежелу = 100 мкг/мл кезінде сәйкесінше 69 ± 0,76 және 77,05 ± 0,92). Молекулалық докинг әдісі APC-2 кальпаиннің қабыну белгілерін (PDB ID: 2R9C), FAAH (2WJ1) және TNF-α (2AZ5) айтарлықтай тежейтінін көрсетті. Онлайн құралдардың көмегімен (Molinspiration, pkCSM, Swiss ADME, PreADMET) дәрілік заттардың ұқсастығы, биологиялық белсенділігінің көрсеткіші, ADME профилі (фармакокинетикасы) және уыттылық қасиеттері сияқты параметрлер анықталды. Сонымен қатар, сыналған қосылыстар дәріге жақын ұқсастығын, биологиялық белсенділік көрсеткішін, ADME қасиеттерін және уыттылық қасиеттерін көрсеткен. Қорытындыда 4-аллилпирокатехин және оның туындыларын терапевтік пайдалы қабынуғақарсы құрал ретінде одан әрі дамыту үшін жетекші молекула ретінде пайдалануға болады деген тұжырым жасалған.

Кілт сөздер: пирокатехин, қабынуғақарсы әрекет, антиоксидант, қорғасын молекуласы, қабыну маркерлері, калпайн, FAAH, TNF-α, докинг, ADMET.

Т. Аннаварапу, С. Камепалли, С.К. Кондала, В. Котра,
С.Р. Чалла, М. Рудрапал, А.Р. Бендейл

Исследование противовоспалительной и антиоксидантной активности 4-аллилпирокатехина и его производных методами молекулярного докинга и ADMET

Аномальная продукция провоспалительных медиаторов и генерация активных форм кислорода (АФК) играют ключевую роль в развитии и прогрессировании различных заболеваний человека. Целью настоящего исследования было изучение *in vitro* противовоспалительной и антиоксидантной активности 4-аллилпирокатехина (4-APC) и его производных (APC-1 и APC-2) путем денатурации альбумина и DPPH (1,1-дифенил-2-пикрилгидразил) методом. Тестируемые соединения также были изучены *in silico* на предмет их ингибирующего потенциала в отношении провоспалительных и окислительных маркеров (кальпаин, FAAH и TNF-α) методом молекулярного докинга. Соединения показали *in vitro* заметную противовоспалительную и антиоксидантную активность. Соединение APC-2 продемонстрировало значительную противовоспалительную и антиоксидантную активность (процентное ингибирование = 69±0,76 и 77,05±0,92 соответственно при 100 мкг/мл) по сравнению с такими стандартными препаратами, как аспирин и аскорбиновая кислота (процентное ингибирование = 82±0,83 и 92,35±0,75 соответственно при 100 мкг/мл). Методом молекулярного докинга было показано, что APC-2 значительно ингибирует воспалительные маркеры кальпаина (PDB ID: 2R9C), FAAH (2WJ1) и TNF-α (2AZ5). С помощью онлайн-инструментов (Molinspiration, pkCSM, Swiss ADME, PreADMET) также

были определены такие параметры, как сходство с лекарством, биологическая активность, ADME профиль (фармакокинетика) и свойства токсичности. Испытываемые соединения показали приемлемое сходство с лекарством, показатель биологической активности, ADME свойства и свойства токсичности. В заключение был сделан вывод, что 4-аллилпирокатехин и его производные могут быть использованы в качестве ведущих молекул для их дальнейшего развития в качестве терапевтически полезных противовоспалительных средств.

Ключевые слова: пирокатехин, противовоспалительное действие, антиоксидант, молекула свинца, маркеры воспаления, кальпаин, FAAH, TNF-α, докинг, ADMET.

Information about authors

Annavarapu, Tirupathirao — Research Scholar, Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur-522508, Andhra Pradesh, India; e-mail: tirupathionline@gmail.com; https://orcid.org/0000-0001-5311-689X;

Kamepalli, Sujana — Assistant Professor, Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur-522508, Andhra Pradesh, India; e-mail: drksujana36@gmail.com;

Kondala, Sathish Kumar — Assistant Professor, School of Pharmaceutical Sciences, Vignan's Foundation for Science, Technology and Research, Guntur-522213, Andhra Pradesh, India; e-mail: sathishkonidala@gmail.com; https://orcid.org/0000-0002-8250-3570;

Kotra, Vijay — Associate Professor, Faculty of Pharmacy, Quest International University Perak (QIUP), Perak, Malaysia; e-mail: vijai.kotra@gmail.com; https://orcid.org/0000-0002-2043-4928;

Challa, Siva Reddy — Research Collaborator, Department of Cancer Biology and Pharmacology, College of Medicine, University of Illinois, Peoria-61605, Illinois, USA; e-mail: sivareddypharma@gmail.com; https://orcid.org/0000-0003-0487-2757;

Rudrapal, Mithun (corresponding author) — Associate Professor and HoD, Department of Pharmaceutical Chemistry, Rasiklal M. Dhariwal Institute of Pharmaceutical Education & Research, Pune-411019, Maharashtra, India; email: rsmrpal@gmail.com; https://orcid.org/0000-0002-8172-6633;

Bendale, Atul R. — Associate Professor, Sandip Institute of Pharmaceutical Sciences, Nashik-422213, Maharashtra, India; e-mail: atulbendale123@gmail.com; https://orcid.org/0000-0002-3219-0377