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Synthesis, Antioxidant, Antinociceptive Activity of Novel Phenoxy acetyl carboxamides

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ABSTRACT

A series of novel phenoxy acetyl carboxamides (4a-4g) were synthesized by amidation using phenoxy acetyl hydrazide and various acid chlorides (benzoyl, adamantly carbonyl cinnamoyl, 4-chloro benzoyl chlorides) or bases (piperidine, morpholine & substituted piperidinone) and evaluated for antioxidant and antinociceptive activities. The title compounds were purified by recrystallization using ethanol and characterized by spectral (FTIR, ^{1}H NMR, and Mass) analysis. Compound 4a was effective in scavenging the DPPH radicals (57%) and nitric oxide (NO) radicals (52%) while compound 4e was able to significantly neutralize ABTS cation radicals (58%). However, the radical scavenging ability was lesser compared to the standard antioxidant agents. Among the tested compounds, 4f and 4g elicited good antinociceptive activity in the central and peripheral animal models (25 mg/kg body weight). Compounds 4b and 4f seem to open ATP-sensitive potassium channels (KATP channels), a possible mechanism for their peripheral effects. The carboxamides bind well with the monoglyceride lipase enzyme (MAGL) and established strong interactions at the active site.

Keywords: Phenoxy acetyl carboxamides, Antioxidant, Antinociceptive, MAGL, Acetic acid-induced writhing test, Tail immersion test, KATP channels

INTRODUCTION

Pain is more prevalent in women more than forty years of age and older. Approximately half of the world's population suffers from different types of pain affecting both physical and mental health. Narcotic analgesics such as pethidine, pentazocine, and non-steroidal

anti-inflammatory agents like aspirin, diclofenac, and celecoxib are still drugs of choice to treat pain conditions despite their adverse effects[1-3]. In the quest for safer novel antinociceptive agents, devoid of serious adverse effects, various compounds (synthetic, natural) were tested by researchers worldwide[4,5]. Among them, compounds having phenoxy acid scaffold in their structure were found to exhibit peripheral and central antinociceptive activity[6,7].

Phenoxy acid derivatives display interesting therapeutic activities such as anticancer, antiviral[8], antioxidant[9], antimicrobial, anticonvulsant, anti-inflammatory[10], and antihyperlipidemic activities[11]. Various phenoxy acid derivatives have been synthesized and reported to have potent antinociceptive activity in different animal models. Few phenoxy acetyl hydrazones were reported to exhibit potential antinociceptive activity[7]. High production and enhanced levels of reactive oxygen species (ROS) could be a leading cause of neuropathic pain and several efforts were directed toward finding the involvement of free radicals like hydroxyl, superoxide, and nitric oxide radicals in pain pathways[12]. Accordingly, global research demands the emergence of effective antioxidant therapies for pain that is associated with chronic diseases such as cancer, diabetes, and spinal injury[13].

Thus driven by the potentiality of phenoxy acetyl hydrazones as antinociceptive agents and taking into account the role of oxidative free radicals, their link to the pain disorders, novel phenoxy acetohydrazides were synthesized and screened for antioxidant and antinociceptive activities.

EXPERIMENTAL

Materials

Sigma melting point apparatus was used to determine the melting points and Infrared Spectra was obtained using KBr pellets on a Bruker FTIR spectrophotometer (cm^{-1}). The ^1H NMR spectra were taken in $CDCl_3$ on Bruker-400 MHz. Mass (m/z) spectra were obtained using Apex Mass spectrum (300800.D). All the chemicals used in the present work were obtained from the chemical suppliers.

Methods

General synthetic procedure for phenoxy acetyl methyl ester (2)

Simple esterification was carried out to synthesize phenoxy acetyl methyl ester from phenoxy acetic acid. For this, 0.01mol of phenoxy acetic acid (1), 20 mL of methanol, and 2 mL conc. H_2SO_4 were mixed thoroughly and refluxed for 7hr. The mixture was distilled, cooled, and then quenched by saturated $NaHCO_3$ solution. The product was obtained in the form of an oily layer.

General synthetic procedure for phenoxy acetyl hydrazide (3)

To obtain phenoxy acetyl hydrazide, ester was refluxed in hydrazine hydrate and methanol (1:2) mixture for 5hr. Upon completion, the mixture was distilled, and the hydrazide (3) was obtained in the solid form.

General synthetic procedure for phenoxy acetyl carboxamides (4a-4c)

To the solution of phenoxy acetyl hydrazide (3) (0.01 mol) in dichloromethane (20 mL), triethylamine (3 drops) was added as a base. To this mixture, ethyl chloroformate (1:1) was added drop-wise at 0°C. After 2-3h of stirring, piperidine (2 mL) was added and again stirred for 2-3 h at ice-cold conditions. The solid was washed with saturated $NaHCO_3$, ^{1}N HCl, brine, distilled water, brine, and dichloromethane successively. After evaporating the organic layers, the final product (4a) was dried and stored at 20°C. For the synthesis of 4b and 4c, substituted piperidinone and morpholine were utilized and similar reaction conditions were applied (Scheme 1; Figure 1).

General synthetic procedure for 4d-4g

For synthesizing 4d-4g, the grinding technique was employed. Equimolar proportions of phenoxy acetyl hydrazide (3) and different acid chlorides (benzoyl, adamantly carbonyl cinnamoyl, 4-chloro benzoyl chlorides) (Fig. 1) were triturated until the mixture turned to a paste. Trituration was continued until the solid product was deposited on the mortar walls. After that, ice cubes were added and the mixture was kept aside. The product was filtered and recrystallized using ethanol[14].

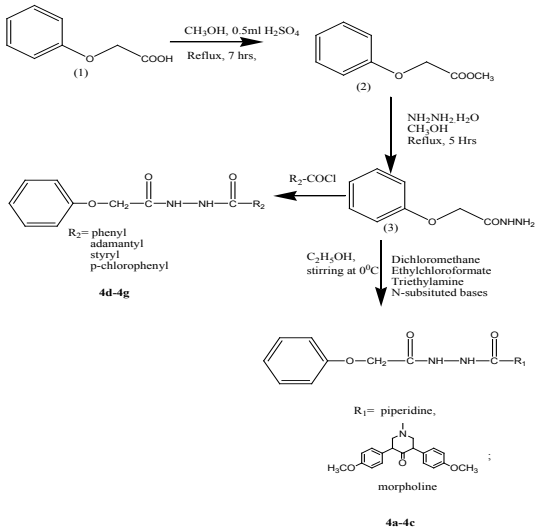


Fig. 1. Scheme for the synthesis of title compounds 1a-1g

In vitro antioxidant studies

Methods

The antioxidant ability of the synthesized phenoxy acetamides was determined using DPPH, NO radical scavenging, and ABTS assays. For the DPPH assay, the title compounds and ascorbic acid were prepared, each of 100μM concentrations using ethanol, and the antioxidant activity was investigated as described in the literature[15]. To the 2 mL of test solutions, 2 mL of DPPH ethanolic solution was added. After incubating for 20 min at ambiance the absorbance was measured (517nm). To prepare the negative control, the above procedure was followed without adding any test solutions and for the positive control, 2 mL ascorbic acid solution was used. All the experiments were performed in triplicates and to calculate % scavenging the below-given equation was used

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} x100$$

In NO scavenging assay, sodium nitroprusside combines with oxygen present in the buffered saline (pH-7.4) and generates nitrite ions. Griess reagent quantitatively reacts with the nitrite ions to produce a colored solution. The absorbance can be accurately determined at 546nm. The NO scavenging ability of the phenoxy acetamides was screened as the procedure given by Babu *et al.,* with slight modifications[16]. The test solutions (100μM concentration) were prepared with methanol. To 2 mL of test solution, 2 mL of sodium nitroprusside solution, and 0.5 mL of saline were added and incubated at 25°C for 5 horus. Then 2 mL of the reaction mixture was mixed with 2 mL of Griess reagent and the absorbance was measured after color development at 546nm. The same procedure was followed to prepare negative control by replacing 2ml test solution with methanol. For the positive control readings, curcumin 100μM concentrations was prepared in methanol and replaced with a 2 mL test solution. %scavenging was calculated from the given equation.

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} x100$$

(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations are produced when ABTS (2, 2'-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) is mixed with potassium persulfate. The reaction requires sufficient time after which these radicals absorb (blue-green color) light at a wavelength of 734nm. The maximum wavelength greatly depends on the pH of the solution. To determine ABTS scavenging ability, 1 mL working solution of ABTS solution was mixed with 1 mL of test solution (100μM). After 15 min, the absorbance values are measured at 734nm[17]. %scavenging was calculated from the given equation.

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} x100$$

Antinociceptive activity

The animal studies were authenticated by the ethical committee (CPCSEA No: 1677/PO/Re/S/2012/CPCSEA/18). Healthy Swiss albino mice were used to evaluate the antinociceptive activity of the synthesized compounds.

Tail immersion method

Procedure

The tail immersion method evaluates centrally acting analgesic agents. Morphine-like drugs can prolong the reaction time that is tail-withdrawal reflex time in mice induced by tail immersion in the water of 55°C[18]. After the oral administration of the title compounds (25 mg/kg) and standard drug tramadol (10 mg/kg), the tail immersion method was performed as mentioned in the literature. The time was noted (time taken for tail withdrawal) as the latency period (cut-off time:15 sec)[19].

Acetic acid-induced writhing test; Involvement of ATP-sensitive K^+channel (K_{ATP}) pathway

The glibenclamide, a KATP channel blocker at 10 mg/kg was used to study the effect of compounds on ATP-sensitive K^+channels an important mediator of peripheral nociception. The possible involvement of the KATP channel in the phenoxy acetyl carboxamide-mediated antinociceptive effect was evaluated as mentioned in the literature[20]. For this,

mice were treated with glibenclamide (10 mg/kg) 15 min before administering test compounds (25 mg/kg, p.o). After an hour, animals were treated with an intraperitoneal injection of 0.6% acetic acid and immediately placed in a chamber to observe writhings. The abdominal writhings were recorded for thirty min, five min after injection.

Molecular docking Studies

Molecular docking with MAGL protein (PDB ID: 3PE6) was performed using AutoDock 4.2 with flexible docking and regular precision modes. The protein preparation (refining by adding polar hydrogens & partial atomic charges) and ligand preparation (drawing the structures in ChemDraw Ultra 8.0 & energy minimization with MM2 force fields)steps were accomplished using AutodockTools-1.5.6 and the files were converted to pdbqt format with Open bable 3.1.1. The grid box was generated around the active site of the human MAGL protein with grid centre as: x=-17.924, y=21.077, z=-9.836 and grid box size: x=56, y=46, z=52. Ten docking conformations were generated as output by using the Lamarckian Genetic algorithm. Finally, binding-free energy is obtained which is based on different interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions. The results were analyzed by using Pymol 2.4.1[21].

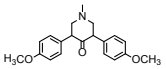
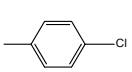
RESULTS AND DISCUSSION

Chemistry

Saturated sp^3-rich motifs are well documented for their less metabolic toxicities[22]. Owing to the pronounced biological properties of the piperidines viz., antioxidant, anticancer, antibacterial, antimalarial, antihypertensive, etc this synthetic approach of piperidines functionalized phenoxy acetic acid derivatives was initiated. Morpholine, a potential bioisostere for piperidine rings was utilized for the functionalization procedure. The pharmacologically active template of phenoxy acetyl hydrazide was further functionalized with phenyl and p-chlorophenyl rings. This could provide a view of the effect of the aromatic ring and the substituted aromatic ring, in comparison to the aliphatic, piperidines, and morpholine moieties. Previous studies have suggested that the phenyl ring could be a good replacement for the adamantyl group[23]. The view of our study was to design biologically active phenoxy acetyl carboxamides with varied functionalities.

A series of novel phenoxy acetyl carboxamides (4a-4g) were synthesized by amidation using phenoxy acetyl hydrazide and various acid chlorides or bases. Compounds 4a-4c were synthesized by coupling with phenoxy acetyl hydrazide and ethyl chloroformate followed by a reaction with piperidine, 1,4-diphenyl piperidine-4-one, and morpholine. Compounds 4d-4g were synthesized by reacting phenoxy hydrazide and different acid chlorides, including benzoyl chloride, adamantly carbonyl chloride, cinnamoyl chloride and 4-chloro benzoyl chloride in equimolar quantities using a grinding technique Table 1.

Table 1: Physical characterization of phenoxy acetyl carboxamides (Ia-Ig)

Compound	R_1/R_2	Molecular formula	Melting point(°C)	% Yield
Ia		$C_{14}H_{19}N_3O_3$	72-74	56
Ib		$C_{28}H_{29}N_3O_6$	122-124	53
Ic		$C_{13}H_{17}N_3O_4$	68-60	65
Id		$C_{15}H_{14}N_2O_3$	122-124	60
Ie		$C_{19}H_{24}N_2O_3$	62-64	61
If		$C_{17}H_{16}N_2O_3$	104-106	59
Ig		$C_{15}H_{13}ClN_2O_3$	122-124	57

IR spectra of the title compounds displayed characteristic absorption bands (cm^{-1}) in the regions 3739.06-3244.28 cm^{-1} (N-H str) and 1707.09-1629.91 cm^{-1} (C=O of amide). Mass spectra of the compounds showed characteristic peaks.

4a)N'-(2-phenoxyacetyl)piperidine-1-carbohydrazide: FTIR (KBr) cm^{-1}: 3473.12 (N-H str), 1662.50 (C=O str of amide), 1594.44 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.31-4.30 (m, 10H, CH_2), 4.74 (s, 2H, O-CH_2), 6.75 (m, 5H, Ar-H), 8.63-8.66 (s, 2H, NH-NH of hydrazide); m/z 277.2$[M]^+$, 278.2 [M+1]

4b) 2,6-bis(4-methoxyphenyl)-4-oxo-N'-(2-phenoxyacetyl)piperidine-1-carbohydrazide: 300.80; FTIR (KBr) cm^{-1}: 3447.24 (N-H str), 1683.43 (C=O str of amide), 1596.97 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37-2.62[d, 4H,$(CH_2)_2$] 3.91(s ,6H, $(OCH_3)_2$] 4.51 (s, 2H, O-CH_2), 5.12 (m, 2H, CH morpholine), 6.92-7.60 (m, 13H, Ar-H), 8.98 (s, 1H, NH of hydrazide), 9.30 (s, 1H, NH of hydrazide); m/z503.2 $[M]^+$, 504.2 [M+1]

4c) N'-(2-phenoxyacetyl)morpholine-4-carbohydrazide: FTIR (KBr) cm^{-1}: 3419.39 (N-H str), 1668.97 (C=O str of amide); ^{1}H NMR($CDCl_3$, 400 MHz) δ 3.45-4.30 (m, 8H, CH_2), 4.60 (s, 2H, O-CH_2), 7.01-7.87 (m, 5H, Ar-H), 8.92 (s, 1H, NH of hydrazide), 9.23 (s,1H,NHof hydrazide); m/z279.1$[M]^+$, 278.1[M-1]

4d) N'-(2-phenoxyacetyl)benzohydrazide: FTIR (KBr) cm^{-1}: 3424.06 (N-H str), 1656.20 (C=O str of amide), 1497.45 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.63 (s, 2H, O-CH_2), 6.93-7.84 (m, 10H, Ar-H), 9.02-9.30 (d, 2H, NH-NH of hydrazide) ; m/z270.0 $[M]^+$, 269.0 [M-1]

4e) N'-(2-phenoxyacetyl)adamantane-1-carbohydrazide: FTIR (KBr) cm^{-1}: 3533.27 (N-H str), 1692.60 (C=O str of amide), 1501.11 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.68-2.07 (m, 15H, adamantyl), 4.62-4.66 (s, 2H, O-CH_2), 6.93-7.33 (m, 5H, Ar-H), 8.50 (d, 1H, NH of hydrazide), 9.30 (d, 1H, NH of hydrazide); m/z 328.2 $[M]^+$, 327.1[M-1]

4f) N'-(2-phenoxyacetyl)-3-phenylacrylohydrazide: FTIR (KBr) cm^{-1}: 3431.42 (N-H str), 1697.56 (C=C str), 1633.15 (C=O str of amide), 1497.39 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.65 (s, 2H, O-CH_2), 6.45-6.96 (d, 2H, HC=CH), 7.01-7.55 (m, 10H, Ar-H), 8.95 (s, 1H, NH of hydrazide), 9.51 (d, 1H, NH of hydrazide);m/z 295.0 [M-1]

4g) 4-chloro-N'-(2-phenoxyacetyl)benzohydrazide: FTIR (KBr) cm^{-1}: 3444.09 (N-H str), 1688.17 (C=O str of amide), 1424.62 (C=C aromatic str); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.54 (s, 2H, O-CH_2), 7.02-7.98 (m, 9H, Ar-H), 9.04-9.26 (d, 2H, NH-NH of hydrazide); m/z 304.0 [M]+

Results of in vitro antioxidant studies

Among the tested compounds, strong DPPH inhibitory activity was observed for compounds possessing piperidine ring (4a and 4b with 57.12±0.2 and 58.68±0.1 respectively). The results are following previous observations[23]. The significant DPPH radical scavenging of 4a and 4b might be attributed to their hydrogen donating ability [24]. However, the potency is lower than ascorbic acid (69.07±0.3). Moderate inhibitory activity was shown by phenyl ring-containing compounds (4d, 4g, 30.17%, 34.21±0.1). In contrast, poor activity was observed for morpholine (4c, 22.67±0.5), and adamantyl (4e, 13.27±0.5) substituted compounds Table 2.

Table 2: *In vitro* antioxidant studies of phenoxy acetyl carboxamides (Ia-Ig**)**

Compound	%Inhibition of DPPH at 100 μM	%Inhibition of Nitric oxide at 100 μM	%Inhibition of ABTS at 100 μM
4a	57.12±0.2	52.16±0.3	24.41±0.2
4b	58.68±0.1	55.28±0.1	53.13±0.1
4c	22.67±0.5	33.73±0.2	37.67±0.2
4d	30.17±0.4	21.05±0.4	55.11±0.3
4e	13.27±0.5	36.11±0.3	58.13±0.1
4f	24.63±0.2	62.29±0.6	50.81±0.4
4g	34.21±0.1	20.55±0.5	47.32±0.2
Standard (Positive control)	Ascorbic acid 69.07±0.3	Curcumin 90.21±0.2	Butylatedhydroxy toluene 80.34±0.4

Results of the NO scavenging assay showed that the compound possessing cinnamoyl moiety (4f) exhibited potential antioxidant activity (62.29±0.6). These findings are in good agreement with earlier reports indicating that the amide derivatives and acyl hydrazones of the cinnamoyl scaffold afford good antioxidant activities[25,26]. The strong activity of 4f could be attributed to its styryl moiety, a component of the curcumins[27]. Moderate activity was observed with compounds 4a (52.16%), 4b (55.28%), 4c (33.73%) and 4e (36.11%) and poor activity was displayed by compounds 4d (21.05%) and 4g (20.55%) Table 2.

The compound 4e (adamantyl) showed poor DPPH free radical scavenging activity but exhibited moderate activity in the ABTS assay (58.13±0.1). However, the scavenging ability was lesser than BHT which had significant ability in the ABTS assay (80.34±0.4). Compound 4a showed low inhibitory activity i.e., (24.41±0.2), while other compounds (4b-4g) demonstrated moderate to good activity in this assay Table 2.

Effect of title compounds in tail immersion test

The results suggested an increase in latency of the tail withdrawal reflex for the test compounds (10 mg/kg) when compared to the control group. There was a significant increase in reaction time for all the test compounds (4a, 4c, 4e-4g) compared to disease control indicating that these compounds act by the central pain pathway. Among all, compounds containing cinnamoyl and 4-chlorophenyl moiety (4f, 4g) were found to be effective in this model, indicating that this substitution might favor the antinociceptive effects Table 3.

Table 3: Effect of phenoxy acetyl carboxamides in tail immersion test

S. no	Groups	Tail withdrawal reflex (in sec)
1	Control	04.40±1.14
2	4a	09.20±0.83*
3	4b	06.80±0.83*
4	4c	07.45±0.65*
5	4d	07.89±0.32*
6	4e	10.00±1.58*
7	4f	12.00±1.22*
8	4g	12.60±1.14*
9	Tramadol	14.20±0.83*

Values were expressed as Mean ± SD (n=6);*= p<0.05, considered statistically significant when compared to the disease control. Ia-Ig were administered at a dose of 25 mg/kg, p.o)

Acetic acid-induced writhing test (Involvement of KATP channel pathway)

The title compounds were evaluated for their involvement in the KATP-channel pathway. To identify the participation of these channels, the compounds were screened for effect on writhings both in the presence and absence of glibenclamide (K+ channel blocker). The data obtained showed no significant difference in the number of writhings in the case of the majority of the compounds except 4b and 4f Table 4. The effect shown by 4b and 4f indicated that when glibenclamide was administered together with 4b and 4f, it significantly ($p<0.05$) reversed the antinociceptive effects demonstrating the involvement of KATP-channels.

A previous study by Turan-Zitouni G *et al.,* reported that the presence of free carboxylic acid moiety at the 4th position of the phenyl ring decreases the antinociceptive activity of aryloxyhydrazones at the central level which might be due to the impermeability of free carboxylic acid moiety into CNS[7]. In the present study, good central antinociceptive activity was observed for phenoxy acetyl carboxamides, which may be due to the absence of free carboxylic moiety and enhanced CNS permeability. Previous research emphasizes the contribution of the cinnamoyl group to the significant antinociceptive activity of several chemicals[28,29]. In our results, 4f displayed promising antinociception having a cinnamoyl moiety in its structure. Compound 4f was potent enough to scavenge NO (62.3%) and ABTS (50.8 %) free radicals, suggesting a relation with antinociception.

Molecular docking studies

A molecular docking study provides a detailed understanding of protein-ligand interactions. The literature revealed that phenoxy carboxamides exhibit potent MAGL/FAAH inhibitory activity[29]. Though the specific compounds were not evaluated for antinociceptive activity, MAGL inhibitors are potential compounds to develop antinociceptive and anti-inflammatory agents[30-31]. Considering the synthesized compounds possess phenoxy moiety and amide functionalities similar to that of known MAGL inhibitors, the compounds were docked with MAGL protein with a PDB ID of 3PE6. The binding energies, interacting amino acids, and type of interactions observed from the docking output are shown in Table 5. The active site of MAGL consisted ALA-51, HIS-121, SER-122, MET-123, ALA-151, SER-155, GLY-177, ILE-179, TYR-194, LEU-213, LEU-241, HIS-269 and LYS-273 residues.

Table 4: Involvement of ATP -sensitive K+ channel pathway on title compounds

S. no	Groups	No. of writhingsoccurred in presence of Glibenclamide	No. of writhingsoccurred in absence of Glibenclamide
1	Disease control	148.0±07.90	148.0±07.90*
2	Glibenclamide	104.6±11.15*	-
3	Ibuprofen	116.0±07.93*	066.2±05.16*
4	4a	112.6±07.09*	123.0±06.59*
5	4b	121.6±06.95*,	087.6±07.50*,
6	4c	126.2±05.67*	117.2±05.40*
7	4d	117.0±07.96*	116.4±06.22*
8	4e	125.2±09.65*	132.4±06.22*
9	4f	107.4±09.71*	080.4±06.06*
10	4g	118.8±08.70*	128.6±10.45*

Values were expressed as Mean ± SD (n=6); * =p<0.05 on comparison with disease control Ia-Ig were administered at a dose of 25 mg/kg, p.o)

Table 5: Binding affinities of the molecules into the active site of the MAGL enzyme

S. no	Molecule	Binding Affinity(in Kcal/mol)	Interacted amino acids and type of interaction
1	4a	-9.6	TYP-194, SER-122, HIS-269, ALA-51, MET-123(H-Bond), VAL-270 (Pi-Sigma), TYR-194 (Pi-Pi), LEU-148, LEU-213, LEU-241, ALA-51(Alkyl)
2	4b	-9.4	TYP-194, HIS-121, SER-122, HIS-269, ALA-51, MET-123(H-Bond), VAL-270 (Pi-Sigma), TYR-194 (Pi-Pi), LEU-148, LEU-213, LEU-241, ALA-51(Alkyl)
3	4c	-8.9	SER-155(Carbon-Hydrogen Bond), ALA-51, ILE-179, LEU-213, LEU-214(Pi-Alkyl)
4	4d	-9.0	ALA-51, MET-123 (H-Bond), SER-122 (Carbon-Hydrogen Bond), VAL-270 (Pi-Sigma), TYR-194(Pi-Pi)
5	4e	-10.4	ALA-51, ME-123(H-Bond), VAL-270, LEU-241 (Pi-Sigma), TYR-194 (Pi-Pi), ALA-51, ILU-179 (Pi-Alkyl)
6	4f	-11.0	ALA-51, HIS-121(H-Bond), LEU-213, VAL-270(Pi-Sigma), TYR-194(Pi-Pi), ALA-51, LEU-148, LEU-241, LYS-273(Pi-Alkyl)
7	4g	-11.1	ALA-51, HIS-121, MET-123(H-Bond), LEU-241, VAL-270 (Pi-Sigma), TYR-194 (Pi-Pi), LEU-205, LEU-213, LEU-241(Alkyl), ALA-51, LEU-213(Pi-Alkyl)
8	ZYH	-9.9	GLY-177 (H-Bond) SER-175 (Carbon-Hydrogen Interaction), SER-155 (Carbon-Hydrogen Bond), PHE-159(Pi-Pi), LEU-162, LEU-214(Pi-Alkyl), LEU-214, LEU-213, ALA-156, ALA-151(Alkyl)

Results showed that title compounds could establish H-bonding, Pi-Sigma, and alkyl interactions with the active site amino acids similar to ZYH (crystal ligand). For ZYH, hydrogen bonding was observed with GLY-177, carbon-hydrogen bond with SER-155 and SER-175, Pi-alkyl interactions with LEU-162, and LEU-241 (Fig. 3). Among all the compounds, 4e, 4g, and 4f showed good binding affinity (-10.4 kcal/mol, -11.0 kcal/mol & -11.1 kcal/mol) with the enzyme compared to the others Table 5. These compounds displayed H-bond, Pi-Sigma, and Pi-alkyl interactions. Phenoxy acetamides containing p-chloro phenyl, adamantyl or cinnamoyl ring formed energetically favourable interactions at the active site in contrast to the aliphatic counterparts. Fig. 2 and 3 represents the binding pose of compound 4f and ZYH and various interactions formed with the enzyme.

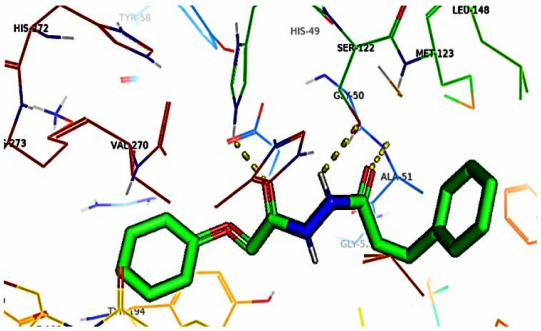


Fig. 2. Molecular docking of If into the active site of Human MAGL

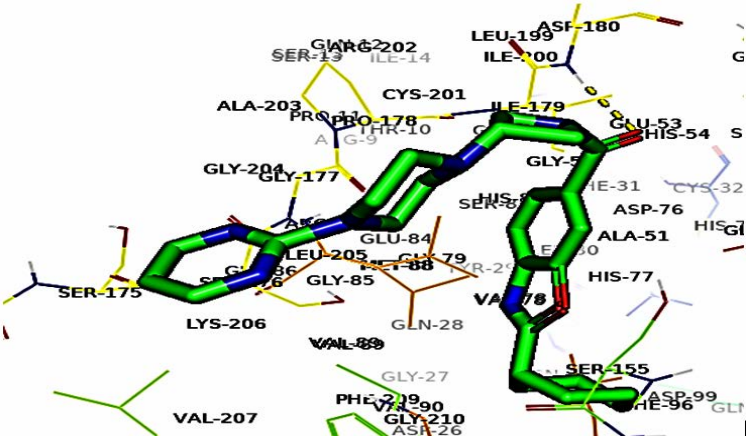


Fig. 3. Molecular docking of reference compound ZYH into the active site of MAGL

CONCLUSION

A series of phenoxy acetyl carboxamides (4a-4g) were synthesized and their structures were confirmed by spectral data. Compounds bearing piperidine and substituted piperidinone exhibited significant antioxidant activity. Compounds, 4f, and 4g exhibited good antinociceptive activity in central and peripheral models of nociception. In the case of 4f, there is a correlation between antinociceptive potentiality and antioxidant activity. Compounds 4b and 4f seem to open ATP-sensitive potassium channels (KATP channels), a possible mechanism for their peripheral nociception. The title compounds formed strong interactions with the MAGL enzyme, an emerging target in the field of nociception.

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