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Novel 2,4-dichlorophenoxy acetic acid substituted thiazolidin-4-ones as anti-inflammatory agents: Design, synthesis and biological screening

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ABSTRACT

A library of fourteen 2-imino-4-thiazolidinone derivatives (**1a-1n**) has been synthesized and evaluated for *in vivo* anti-inflammatory activity and effect on *ex-vivo* COX-2 and TNF–α expression. Compounds **1k** (5-(2,4-dichloro-phenooxy)-acetic acid (3-benzyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) and **1m** (5-(2,4-dichloro-phenooxy)-acetic acid (3-cyclohexyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) exhibited *in vivo* inhibition of 81.14% and 78.80% respectively after 5 h in comparison to indomethacin which showed 76.36% inhibition of inflammation without causing any damage to the stomach. Compound **1k** showed a reduction of 68.32% in the level of COX-2 as compared to the indomethacin which exhibited 66.23% inhibition of COX-2. The selectivity index of compound **1k** was found to be 29.00 in comparison to indomethacin showing selectivity index of 0.476. Compounds **1k** and **1m** were also found to significantly suppress TNF-α concentration to 70.10% and 68.43% in comparison to indomethacin which exhibited 66.45% suppression.

Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[1] It is a protective attempt by the organism to remove the injurious stimuli there by initiating the healing process. Non-Steroidal anti-Inflammatory drugs (NSAIDs) such as indomethacin, ibuprofen, naproxen, and fenbufen are most commonly used for the treatment of inflammation, pain, fever and arthritis.[2] However NSAIDs are associated with adverse effects such as gastrointestinal disorders, ulceration and kidney damage.[3] Therefore the development of new drugs with potent anti-inflammatory activity and reduced side effects is still a great challenge.

4-Thiazolidinones are an important class of compounds which have been found to exhibit, anti-inflammatory,[4–8] analgesic,[9] anti-HIV,[10] anticancer,[11] anti-bacterial,[12] anti-fungal,[13] anti-tubercular,[14] anti-histaminic activities[15] and have also been found out to act as tumor necrosis factor-α antagonists.[16] Of particular importance in the anti-inflammatory potential of thiazolidinones containing compounds.[17] Sharma et al.[18] reported a series of thiazolidinone derivatives and found 2-hydroxyphenyl thiazolidinones as a potential anti-inflammatory and analgesic agent. Ali et al.[6] also reported a series of 4-thiazolidinone derivatives and found 2-imino-4-thiazolidinons as a potent anti-inflammatory as well as TNF-α antagonists. In addition, thiazolidinone has been considered as an effective lead scaffold for anti-inflammatory COX-2 inhibitors. Ottana et al.[19] evaluated 3,3-(1,2-ethanediyl) bis[2-(4-methoxyphenyl)-thiazolidin-4-one] as a new COX-2 inhibitor and showed its ability to attenuate the carrageenan-induced lung injury in experimental models. Considering the biological importance of 4-thiazolidinones as anti-inflammatory agents, we herein report the synthesis of novel 2,4-dichlorophenoxy acetic acid based 4-thiazolidinone derivatives. The synthesized molecules have been subjected to *in vivo* anti-inflammatory evaluation. In order to explore the mechanistic aspects of activity, the synthesized molecules were subjected to *in silico* molecular docking studies with respect to COX-2 as well as TNF-α target. All the synthesized compounds were also evaluated for their *ex-vivo* COX-2 and TNF-α inhibition. The synthesized compounds have been further screened for their anti-nociceptive potential and also evaluated for lipid peroxidation and gastric risk.

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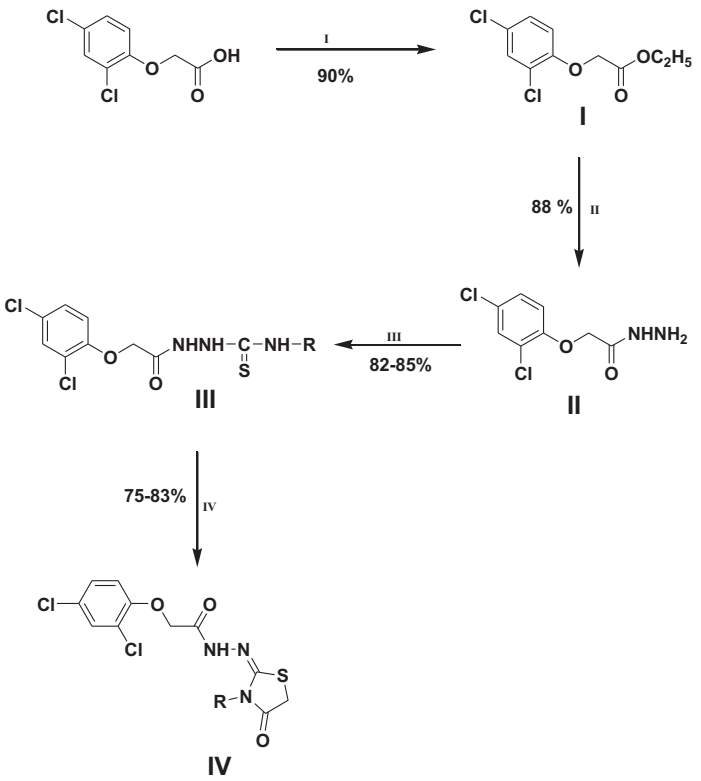
In the present study, the library of 5-(2,4-dichloro-phenoxy)-acetic acid 3-(4-substituted-phenyl-4-oxo-thiazolidin-2-ylidene)-hydrazides were prepared by the condensation of substituted thiosemicarbazides with ethyl chloroacetate in the presence of sodium acetate (Scheme 1).

The formation of the synthesized compounds (**1a-1n**) was confirmed by IR, ^{1}H, ^{13}C NMR and mass spectrometry studies (Supplementary data). The ^{1}H NMR data showed the appearance of NH protons as singlets in the range of δ 8.72–10.65 along with CH_2 (thiazolidinone ring) protons appearing as singlets at δ 3.88–4.25 in the ^{1}H NMR spectra. In the ^{13}C NMR spectra, CH_2 carbon of thiazolidinone ring appeared at δ 31.89–32.98. Other peaks were observed at appropriate values. Further confirmatory evidence was obtained from their mass spectra.

All the synthesized compounds (1a-1n) were evaluated for their *in vivo* anti-inflammatory activity by carrageenan induced rat paw edema model. The results of anti-inflammatory activity are shown in Table 1. Standard drugs indomethacin and celecoxib are well known anti-inflammatory drugs which are already available in the market, so we used these two drugs as standard drugs for comparing anti-inflammatory activities of synthesized compounds. The compounds **1k** and **1m** showed 81.14% and 78.80% inhibition, respectively which was better than the standard drugs indomethacin and celecoxib (76.31% inhibition and 77.67%, respectively) after 5 h. The compounds **1d** (72.22%), **1h** (68.12%), **1j** (68.02%) and **1c** (64.00%) showed anti-inflammatory activity comparable to the standard drug indomethacin.

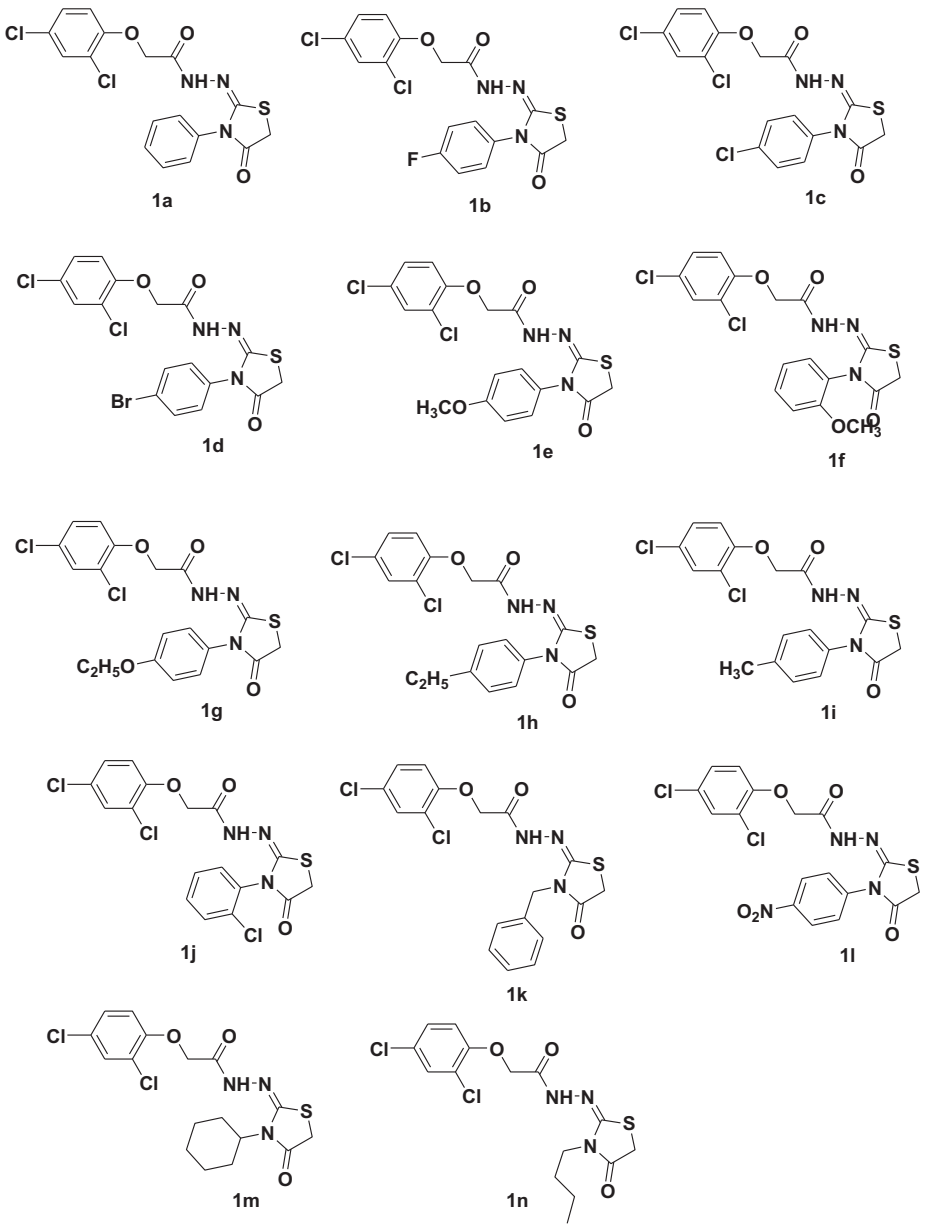
In order to determine their mode of action, all the synthesized compounds (**1a-1n**) were docked against COX-2 target (PDB No. 3LN1). All docking runs were carried out using Maestro (Schrodinger). However, only nine molecules (**1a**, **1c**, **1d**, **1e**, **1g**, **1h**, **1k**, **1l** and **1n**) could be docked against the target protein. The docking scores of ligands **1h** and **1k** were found to be −7.5 and −8.4 respectively. The molecular binding and interactions of ligands **1h** and **1k** in 3D and 2D space are shown in Fig. 1. Molecule **1h** interacted through H-bonding with the ARG-499 residue, whereas molecule **1k** was found to exhibit hydrophobic interactions and π-π stacking with TYR-341 and ARG-106 residues. The docking score of reference ligands indomethacin and celecoxib were found to be −7.06 and −11.57 respectively. Reference drug celecoxib binds into the binding pocket with specific interactions including H-bond formation with residues like LEU-338, PHE-504 and ARG-499, whereas indomethacin exhibits hydrophobic interactions with target protein as shown in Fig. 1. The glide score and binding energy of all the nine docked molecules have been summarized in Table 2. Although compound 1 m exhibited significant *in vivo* inhibition of inflammation but it could not be docked against COX-2 target. In 1 m molecule, cylcohexyl ring is directly attached to oxo-thiazolidin-ylidene ring, resulting in a rigid bulky structure having restricted rotation. All these factors hinder docking of the molecule into the binding pocket of COX-2 target.

Intrigued by the docking results, *ex-vivo* COX-2 activity of the nine compounds (**1a**, **1c**, **1d**, **1e**, **1g**, **1h**, **1k**, **1l** and **1n**) was determined. Compound **1k** was found to show a higher suppression 68.32% of COX-2 enzyme as compared to the standard drug indomethacin which exhibited 66.23% inhibition but the suppression was slightly less than the other reference drug *viz* celecoxib which showed 72.96% COX-2 suppression. Other compounds showed



Reagents and Conditions: (I) Absolute alcohol, sulphuric acid refluxes; (II) Hydrazine hydrate, absolute alcohol, refluxes; (III) Absolute alcohol, isothiaocyanates, reflux; (IV) ethyl chloroacetate, absolute alcohol, sodium acetate, reflux.

Scheme 1. Protocol for synthesis of title compounds.



Structure of the synthesized compounds (1a-1n)

Scheme 1 (*continued*)

Table 1
Anti-inflammatory activity of synthesized compounds.

Compounds	R	Dose (mg/kg po)	Change in paw volume (ml) mean ± (SEM)		% inhibition	
			3h	5h	3h	5h
Control	–	2 ml/kg	1.550 ± 0.020	1.590 ± 0.011		
Standard (indomethacin)	–	20 mg/kg	0.557 ± 0.017**	0.528 ± 0.013**	73.57	76.31
Standard (celecoxib)	–	20 mg/kg	0.555 ± 0.020**	0.524 ± 0.017**	74.56	77.67
1a	Phenyl	20 mg/kg	0.984 ± 0.013*	0.966 ± 0.015*	43.48	46.63
1b	p-Fluro phenyl	20 mg/kg	0.801 ± 0.018*	0.860 ± 0.026*	55.40	59.06
1c	p-chloro phenyl	20 mg/kg	0.720 ± 0.014**	0.702 ± 0.015**	62.00	64.00
1d	p-bromo phenyl	20 mg/kg	0.603 ± 0.015**	0.581 ± 0.017**	69.96	72.22
1e	p-methoxy phenyl	20 mg/kg	0.904 ± 0.020*	0.870 ± 0.023*	48.94	51.90
1f	O-methoxy phenyl	20 mg/kg	0.841 ± 0.014*	0.801 ± 0.024*	55.40	59.60
1g	p-ethoxy phenyl	20 mg/kg	0.950 ± 0.017*	0.920 ± 0.017*	45.94	50.00
1h	p-C_2H_5 phenyl	20 mg/kg	0.672 ± 0.019**	0.654 ± 0.017**	66.06	68.12

(*continued on next page*)

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Table 1 (*continued*)

Compounds	R	Dose (mg/kg po)	Change in paw volume (ml) mean ± (SEM)		% inhibition	
			3h	5h	3h	5h
1i	p-methyl phenyl	20 mg/kg	0.788 ± 0.023*	0.822 ± 0.016*	57.20	55.90
1j	O-methyl phenyl	20 mg/kg	0.636 ± 0.019**	0.670 ± 0.021**	64.71	68.02
1k	Benzyl	20 mg/kg	0.510 ± 0.015**	0.491 ± 0.021**	74.45	81.14
1L	p-nitro phenyl	20 mg/kg	0.920 ± 0.020*	0.964 ± 0.022**	47.47	45.90
1m	Cyclohexyl	20 mg/kg	0.516 ± 0.021**	0.490 ± 0.010**	76.57	78.80
1n	Butyl	20 mg/kg	1.040 ± 0.016*	1.080 ± 0.011*	37.23	35.96

Data is analyzed by one way ANOVA followed by Dunnett's '*t*' test and expressed as mean ± SEM from five observations.
* $p < 0.05$.
** $p < 0.01$.

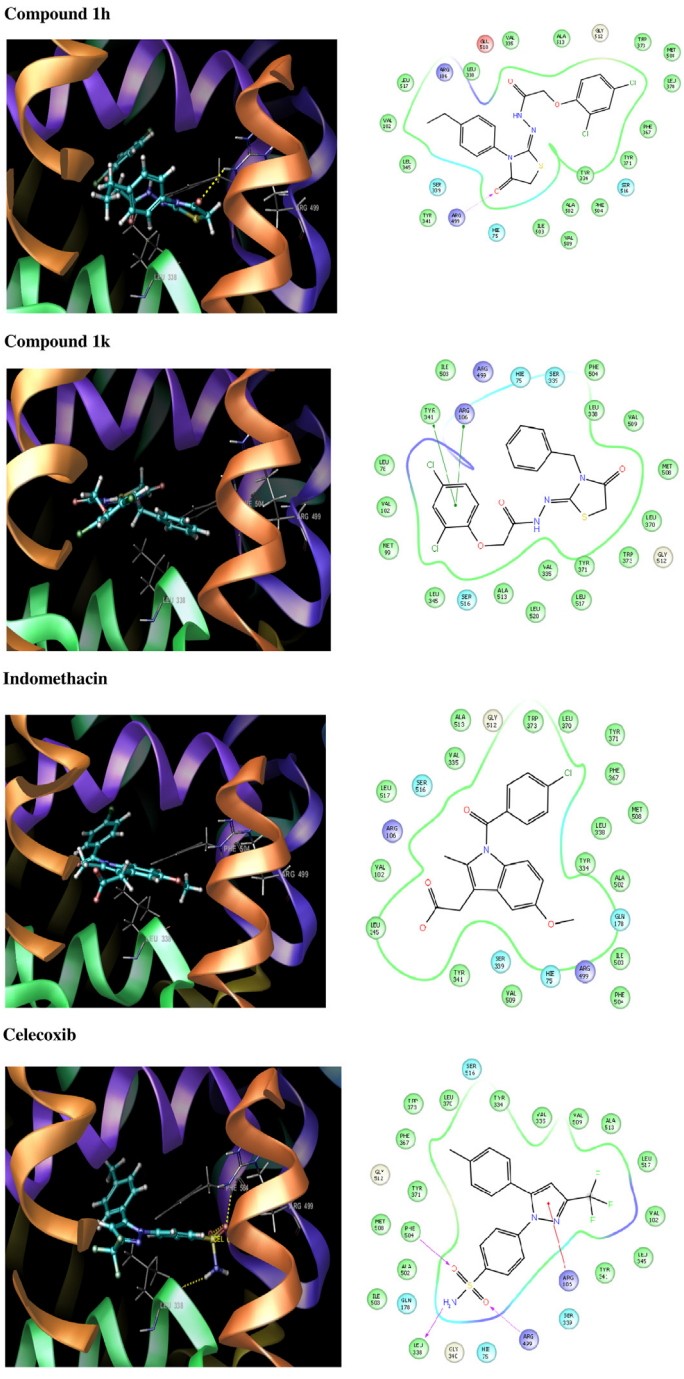

Fig. 1. Docking images of active compounds (**1h**, **1k**) and standard drugs indomethacin and celecoxib with respect to COX-2 target.

Table 2
Docking results of synthesized compounds with respect to COX-2 target.

Ligands	Glide score	Glide energy
1a	−4.968	−9.655
1c	−4.088	−0.638
1d	−4.94	−13.084
1e	−3.977	−14.584
1g	−5.623	−15.34
1h	−7.525	−12.868
1k	−8.402	−19.939
1L	−4.828	−11.697
1n	−6.549	−7.832
Standard (indomethacin)	−7.06	−26.165
Celecoxib	−11.574	−61.768

Table 3
Selectivity index of active compounds.

Compounds	COX-1 (IC_{50} μM)	COX-2 (IC_{50} μM)	Selectivity index (SI) COX-1/COX-2
Indomethacin	3.87	8.13	0.476
Celecoxib	25.39	0.64	39.67
1d	134.78	9.58	14.06
1h	113.12	6.93	16.32
1k	95.44	3.29	29.00

COX-2 suppression comparable to indomethacin (Fig. 2). The safer gastric profile of the compounds (**1d**, **1h** and **1k**) and may be related to their COX-2 selectivity (Table 3). Compound **1k** (COX-1 Ic_{50} = 95.44 μM; COX-2 Ic_{50} = 3.29 μM; SI = 29.00), **1h** (COX-1 Ic_{50} = 113.12 μM; COX-2 Ic_{50} = 6.93 μM; SI = 16.32) and **1d** (COX-1 Ic_{50} = 134.78 μM; COX-2 Ic_{50} = 9.58 μM; SI = 14.06) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 Ic_{50} = 3.87 μM, COX-2 Ic_{50} = 8.13 μm; SI = 0.476). The COX-1/COX-2 Selective Index (SI value) of the compounds **1d**, **1h** and **1k** shows the selective nature of these compounds towards COX-2 inhibition as compared to indomethacin.

The synthesized molecules (**1a-1n**) were also subjected to *in silico* molecular docking against the TNF-α target. Molecular docking studies were done to provide an insight into the molecular binding modes of the molecules inside the large pocket of TNF alpha. Crystallized structure of 2AZ5 was chosen from protein data bank and was used for molecular docking studies with the specific ligand indomethacin[20–22] which inhibits it. In order to determine the binding pattern and the energies of new ligands; they were docked individually against the generated grid. All the ligands docked against the grid showed good glide score as well as binding energies. The compounds **1k**, **1m**, **1j**, **1f**, **1d** and **1a**, exhibited better glide score of −6.18, −5.98, −5.77, −5.32, −5.28, and −5.24 respectively in comparison to indomethacin whose glide score was found to be −5.02. The standard drug indomethacin was found to align perfectly with the hydrophobic pocket of the TNF-α protein. Compounds **1k** and **1m** (Fig. 3) were found to form hydrogen bonds with GLY-121 residue of the target protein and π-π stacking with TRY-119, TRY-59 respectively. The glide score and binding energy of all the synthesized compounds are shown in Table 4.

All the synthesized compounds were then screened for *ex-vivo* TNF-α activity (Fig. 4). Compounds **1k** and **1m** suppressed the TNF-α concentration by 70.20% and 68.43%, respectively in comparison to indomethacin which suppressed the concentration by 66.45%. Whereas the compounds **1h**, **1d** and **1f** showed significant decrease in TNF-α concentration of 61.92%, 60.38% and 59.60% as compared to the standard drug. Other compounds showed moderate decrease in TNF-α level. Thus of all the compounds, **1k** and **1m** showed better *in vivo* anti-inflammatory activity with significant *ex-vivo* suppression in the levels of TNF-α. This result was also supported by docking studies.

Compounds showing significant *in vivo* anti-inflammatory activity were further tested for the anti-nociceptive activity by acetic acid induced writing test (Table 5). The compounds **1k** and **1m** showed 51.57% and 49.26% inhibition respectively in comparison to standard drug indomethacin which showed 54.37% inhibition.

Compounds **1k**, **1m**, **1d** and **1h** showing the most potent anti-inflammatory activity were further checked for ulcerogenic effect. The compounds **1k**, **1m** and **1d** did not cause any gastric ulceration (Fig. 5) and epithelial tissue damage as compared to indomethacin which caused some epithelial layer damage and gastric ulceration in the stomach wall of rats. Although the compound **1h** caused mild epithelial damage, it was found to be lesser than indomethacin.

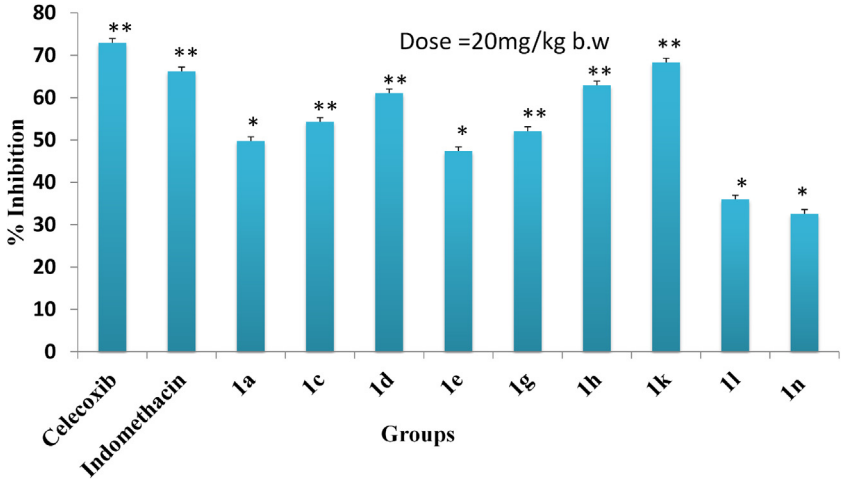

Fig. 2. Effect of synthesized compounds on carrageenan induced COX-2 cytokine level in the rat paw tissue. Data is analyzed by one way ANOVA followed by Dunnett's '*t*' test and expressed as mean ± SEM from five observations where $^{*}p < 0.05$, $^{**}p < 0.01$.

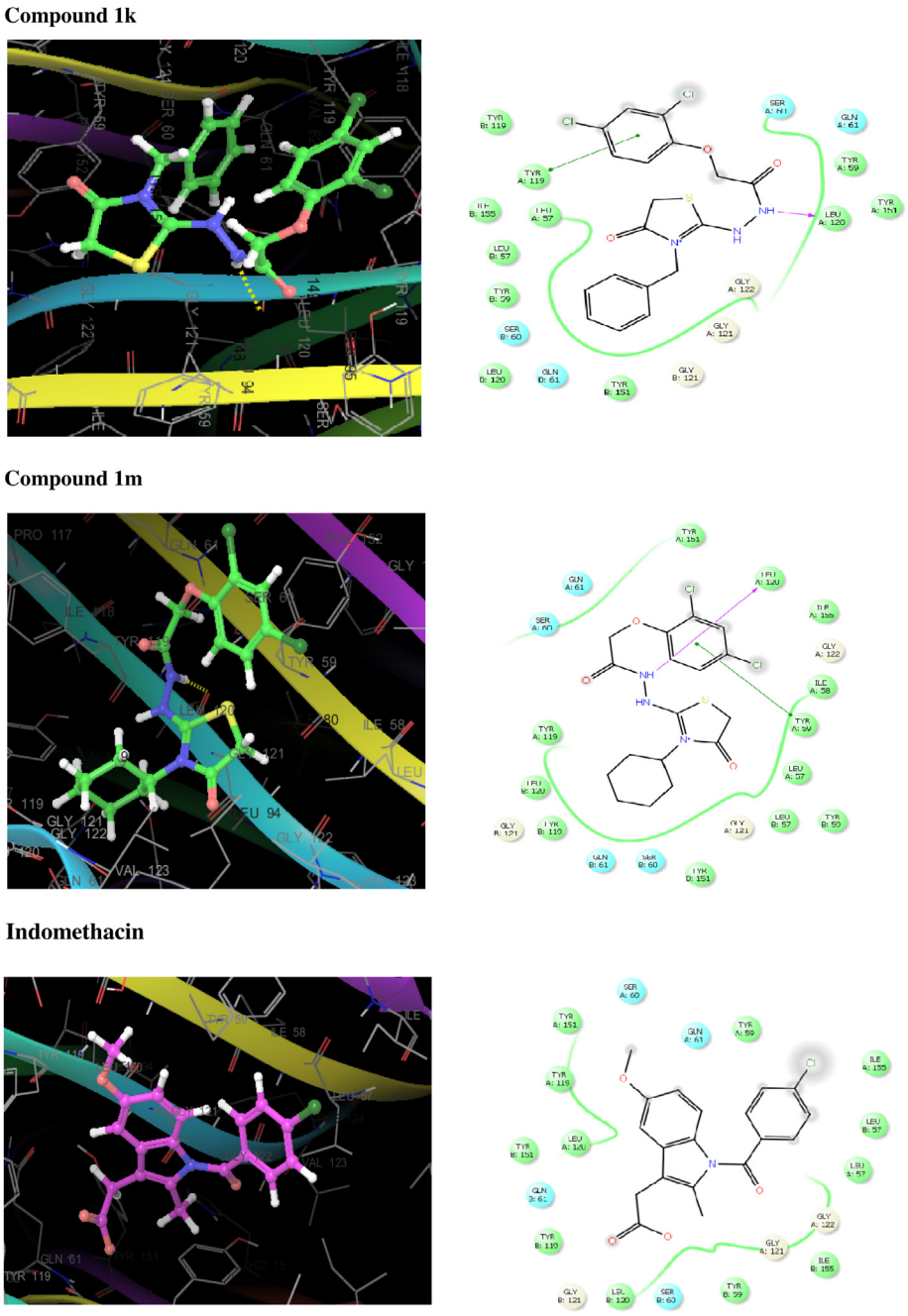

Fig. 3. Docking images of active compounds (**1k**, **1m**) and standard drug indomethacin with respect to TNF-α target.

Table 4
Docking results of synthesized compounds with respect to TNF-α target.

Ligands	Glide score	Glide energy
1a	−5.24	−40.06
1b	−4.68	−37.63
1c	−5.13	−42.41
1d	−5.28	−41.12
1e	−4.85	−42.50
1f	−5.32	−41.00
1g	−4.31	−38.18
1h	−4.71	−39.88
1i	−4.78	−39.21
1j	−5.77	−40.40
1k	−6.18	−39.58
1L	−5.67	−37.46
1m	−5.98	−37.50
1n	−4.80	−34.24
Standard (indomethacin)	−5.02	−33.09

Compounds **1k**, **1m**, **1d** and **1h** were also evaluated for their lipid peroxidation activity which is measured as nmol of MDA per 100 mg of gastric mucosa tissue. Gastric ulceration is also associated with cellular membrane disruption and destabilizing via lipid peroxidation. It has already been reported that the process of lipid peroxidation proceeds by free radical chain reaction which is scavenged through enzymatic and non enzymatic antioxidants. It has been observed that carrageenan increases the level of LPO in animals. The level of lipid peroxidation in the carrageenan induced animals was found to be significantly increased. However the level of LPO was found to decrease significantly in the presence of standard drug and the test compounds, **1k**, **1m**, **1d** and **1h**. The active compounds **1k**, **1m**, **1d**, caused better reduction in lipid peroxidation as compared to the standard drug (Fig. 6) whereas the compound **1h** caused lesser reduction as compared to the standard drug.

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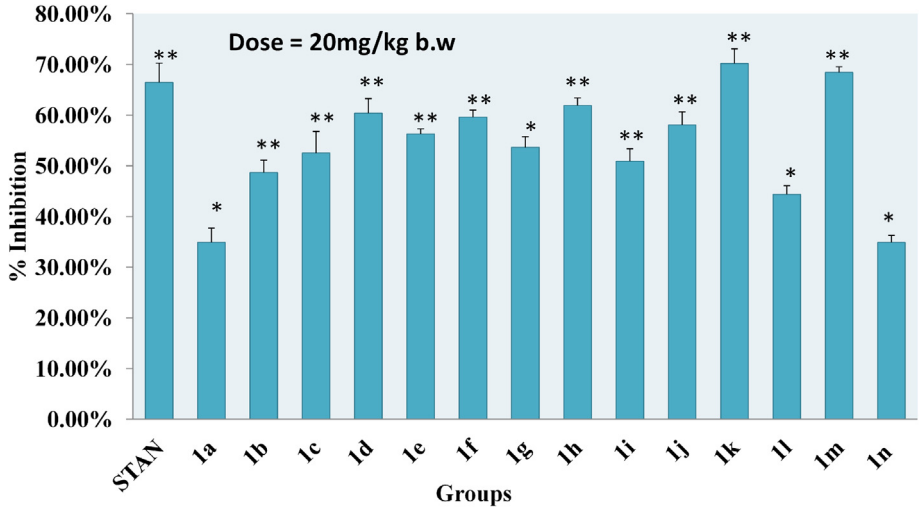

Fig. 4. Effect of synthesized compounds on carrageenan en induced TNF-α cytokine level in the rat paw tissue. Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 5
Analgesic activity of active compounds.

Groups	Dose (mg/kg po)	Number of writhes in 10 min	% Protection
Control	2 ml/kg	95 ± 1.51	–
Indomethacin	20 mg/kg	43 ± 1.84**	54.37
1d	20 mg/kg	55.2 ± 1.85**	41.81
1h	20 mg/kg	57.6 ± 1.60*	39.36
1j	20 mg/kg	51.6 ± 1.24*	46.10
1k	20 mg/kg	46.0 ± 1.64**	51.57
1m	20 mg/kg	51.2 ± 1.24**	49.26

Data is analyzed by one way ANOVA followed by Dunnett's '*t*' test and expressed as mean ± SEM from five observations.
* $p < 0.05$.
** $p < 0.01$.

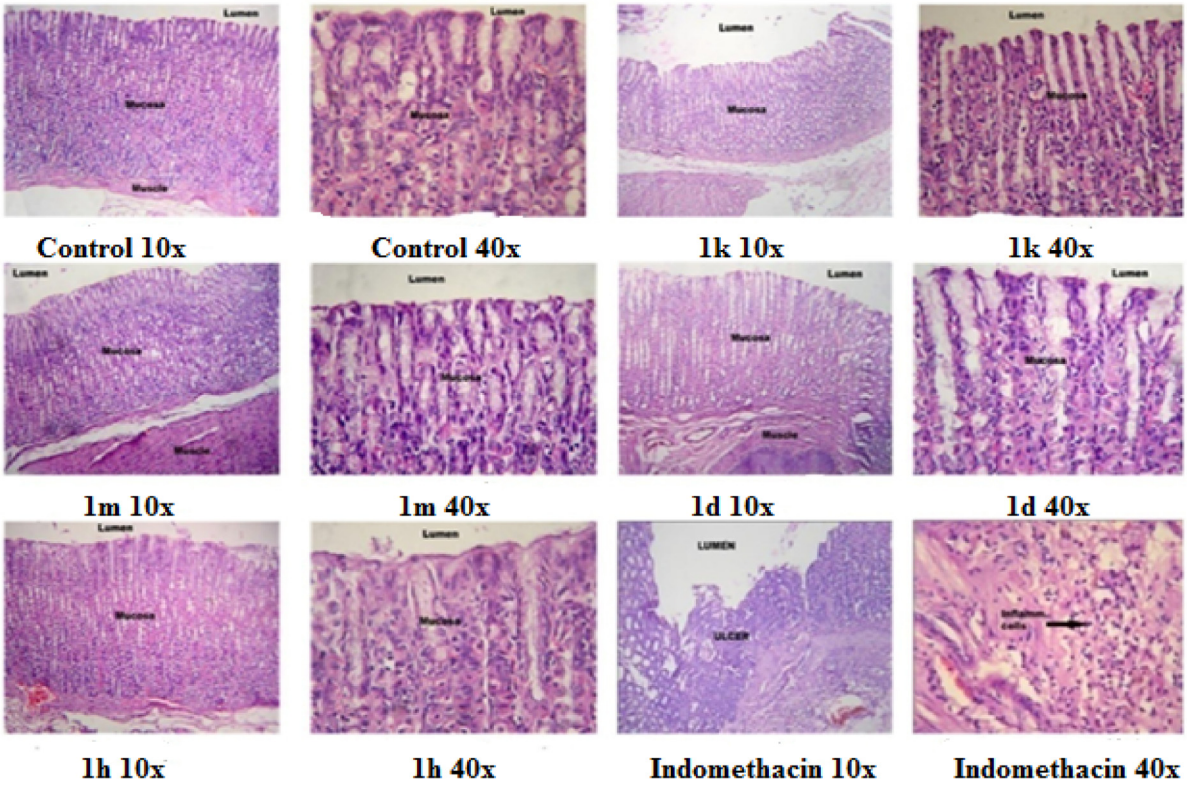

Fig. 5. Histopathology of rat stomach in Albino Wistar rats. Low power (10x) and high power photomicrographs (40x) of stomach wall of the animal groups administered with control group 1k, 1m, 1d, 1h, (90 mg/kg b.w) and standard drug, indomethacin, respectively.

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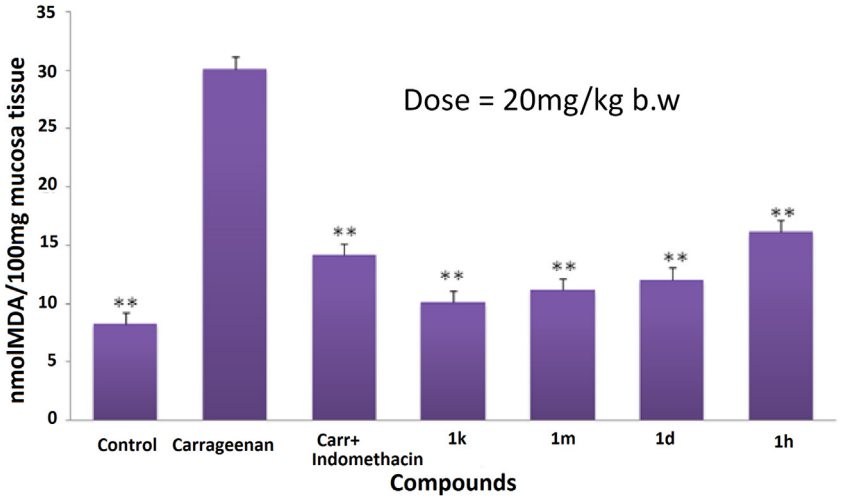
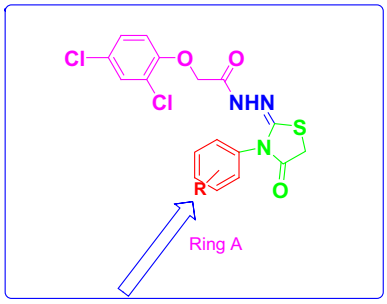


Fig. 6. Lipid peroxidation assay of active compounds.

Structure activity relationship

The structure activity relationship of the synthesized compounds has been analyzed as follows.



- Bullet Compounds having halogen atom on ring (**A**) showed better anti-inflammatory activity in the increasing order of the size of halogen atoms i.e. the order of anti-inflammatory activity was found to be **1b < 1c < 1d**.
- Bullet The presence of *para* ethyl group at ring (**A**) showed better activity as compared to methyl being present at *para* position i.e. the compound **1h** showed better activity than the compound **1i**. While no significant difference in activity was seen for *p*-methoxy and *p*-ethoxy group substitution at ring A.
- Bullet Compound having benzyl substitution (**1k**) in place of ring A showed maximum *in vivo* anti-inflammatory activity and suppression in COX-2 and TNF-α level.
- Bullet Cyclohexyl substitution (**1m**) in place of ring A showed significant activity as compared to any other substitution on ring A.
- Bullet Replacing ring A with butyl group (**1n**) significantly lowered *in vivo* as well as *ex-vivo* activity.
- Bullet *Ortho* substitution was found to be more active than *para* substitution when comparison of same substitution at different position on ring A was made.

In summary, a library of fourteen compounds has been synthesized out of which two compounds **1k** (5-(2,4-dichloro-phenooxy)-acetic acid (3-benzyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) and **1m** (5-(2,4-dichloro-phenooxy)-acetic acid (3-cyclohexyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) exhibited significant anti-inflammatory and analgesic activities. Compound **1k** reduced COX-2 level significantly and showed potent COX-2 selectivity. The level of TNF-α was also significantly reduced by compounds **1k** and **1m**. Furthermore these two compounds caused reduction in LPO concentration and did not cause any damage to the stomach lining. Compounds **1k** and **1m** may thus be considered as promising candidates for development of new and safer anti-inflammatory agents.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.069.

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