NJC

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Journal:	*New Journal of Chemistry*
Manuscript ID	NJ-ART-01-2015-000078.R4
Article Type:	Paper
Date Submitted by the Author:	14-Oct-2015

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Molecular modeling and synthesis of some new 2-imino-4-thiazolidinone derivatives with promising TNF-α inhibitory activity

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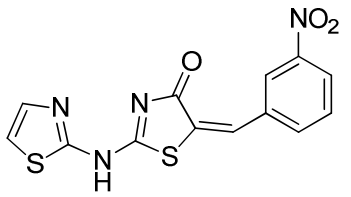
A new series of thirty two 2-imino-4-thiazolidinone derivatives were synthesized and the synthesized compounds were docked for *in silico* studies against TNF-α target. The predicted results were confirmed through *in vitro* TNF-α study which revealed that compounds **3f**and **3g** showed better TNF-α inhibition as compared to the standard drug indomethacin without causing any cytotoxicity. Fourteen compounds exhibiting significant *in vitro* TNF-α activity were further tested for *in vivo* anti-inflammatory activity by carrageenan induced method. Compounds **3f** and **3g** showed better inhibition of inflammation *in vivo* as compared to the standard drug without causing any damage to stomach. Furthermore, immunohistochemical study showed that the compounds **3f** and **3g** exhibited better reduction in protein expression of TNF-α as compared to the indomethacin. The *in silico*, *in vitro* and *in vivo* studies suggested that the compounds **3f** and **3g** might be considered as potent anti inflammatory agents.

Keywords: 2-imino-4-thiazolidinone, molecular docking, anti-inflammatory, TNF-α, immunohistochemistry

Introduction

Inflammation is a dynamic process with tumor necrosis factor TNF-α playing central role in pathogenesis. It is associated with many chronic diseases, including atherosclerosis, allergy, arthritis and auto-immune diseases. A large percentage of our population depends on NSAIDs for the treatment of inflammatory diseases. But the long term use of these NSAIDs leads to adverse side effects such as gastrointestinal ulceration, kidney damage and bleeding[1-3]. Thiazolidinones belong to an important group of heterocyclic compounds containing sulfur and nitrogen in a five membered ring. Thiazolidinones posses wide range of biological activities such antimicrobial,[4,5] anti-HIV[6], anti-cancer[7], antitubercular[8,9], anti-histaminic[10], amoebicidal[11] and anaesthetic[12] activities. Recently, 4-thiazolidinone derivatives have been found to show promising anti-inflammatory and TNF-α antagonist activity.[13-16] For example, Geronikaki *et al*[17] identified thiazolidinones such as **I** exhibiting potent anti-inflammatory activity viadual COX/LOX inhibition. Similarly, 5-Benzylidene-2-phenylthiazolinones (**II**) were found to possess promising anti-inflammatory potency through 5-LO inhibition.[18] More recently, thiazolidinone derivatives such as **III** and **IV** were synthesised which exhibited significant anti-inflammatory activities via the inhibition of pro-inflammatory cytokines such as TNF-α and IL6.[16] It was proposed that modulating the functions of pro-inflammatory cytokines (such as TNF-α) involved in systemic inflammation can provide a target for controlling inflammatory diseases.[16] On the other hand, structurally related furan-containing thiazolidinediones[19] (**V**) and rhodanines[20](**VI**) have also been found to possess significant activities against inflammation targets. Hence, considering the biological importance of 4-thiazolidinones, specifically towards the design of anti-inflammatory agents, and the importance of furan scaffold, we decided to combine these two structural features and herein report the synthesis of novel 5-furanyl-2-imino-4-thiazolidinone derivatives as anti-inflammatory TNF-α antagonist agent with better gastric tolerance.



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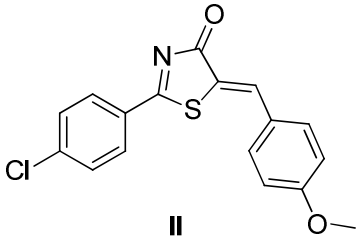

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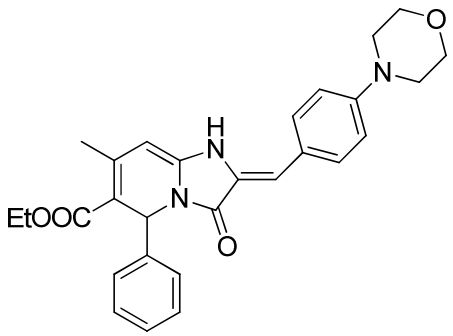

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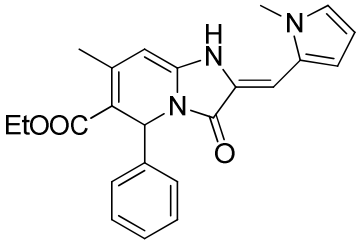

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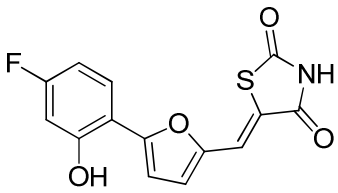
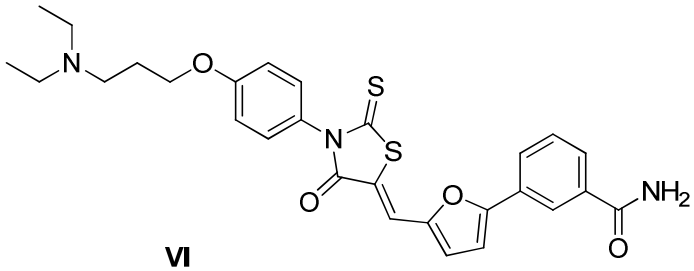


V



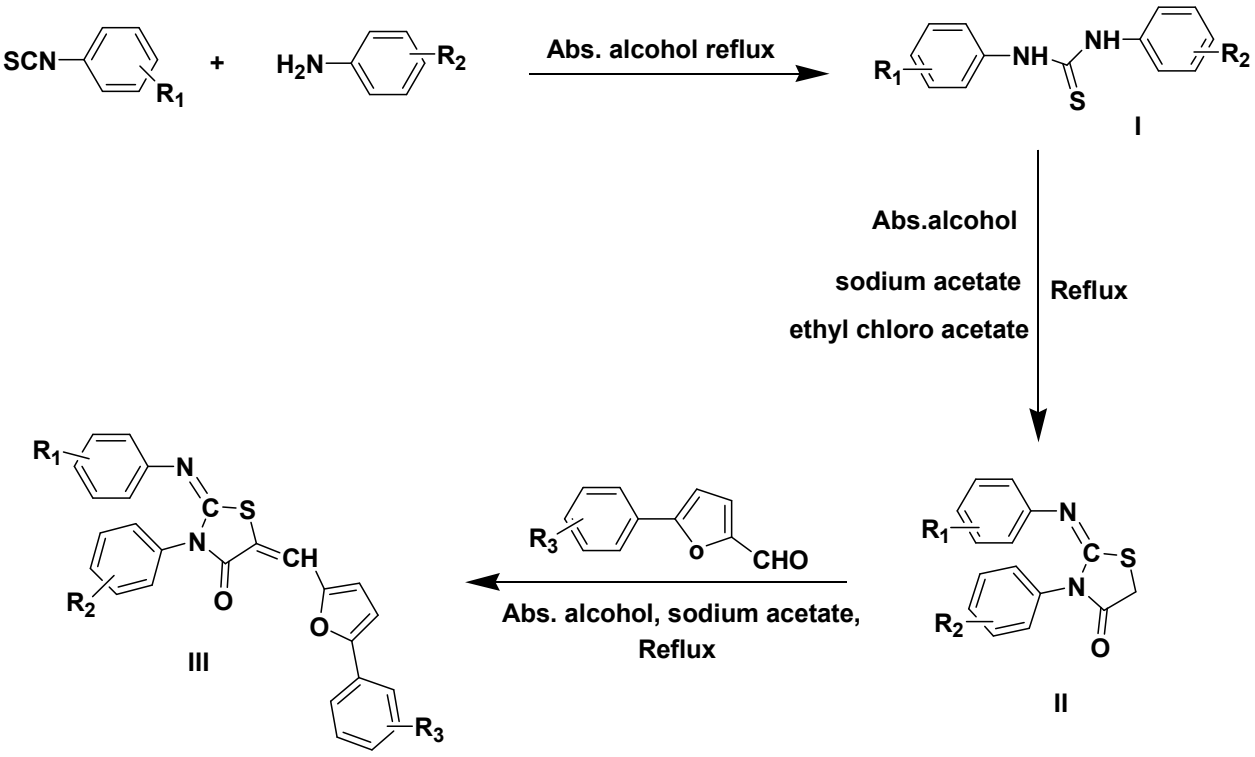
VI

Results and discussion

Chemistry

The synthetic route used to synthesize substituted-2-imino-4-thiazolidinone derivatives is outlined in **scheme 1**. Key starting material i.e different aromatic thiosemicarbazides **(I)** were prepared in good yield by condensation of different aromatic amines with appropriate aromatic isothiocynates in presence of absolute alcohol. Substituted-2-imino-4-thiazolidinones **(II)** were synthesized by the reaction of **I** in ethylchloroacetate and sodium acetate in presence of absolute alcohol. The final compounds were prepared by the reaction of **(II)** with different5-(substituted-phenyl)-furan-2-carbaldehydesin presence of absolute alcohol and sodium acetate. The structural confirmation of final compounds was done by ^{1}H NMR, ^{13}C NMR, IR and mass spectra. The formation of final compounds was confirmed by the appearance of singlets for the olefinic

hydrogen at δ7.43 to δ7.63 and by the disappearance of CH_2 proton signals of intermediate II of the thiazolidinones ring at δ4.2 to δ4.6 from the ^1H NMR spectra of the final compounds. The remaining protons appeared in aromatic region as expected. Mass spectra of all the compounds showed $[M]^+$, $[M+1]^+$ and $[M+2]^+$peaks with reasonable intensity confirming the structures. Crystallography data of the compound (3e) confirmed the *Z*-stereochemistry around both the exocyclic olefinic and the imino linkages (**fig. 2**). List of substituents are shown in **table 1.**



Scheme1 Synthesis of substituted-2-imino-4-thiazolidinone

Where

I = 1-(substituted-phenyl)-3-(4-substitiuted-phenyl)-thiourea

II = 3-(substituted-phenyl)-2-(4-substituted-phenyl imino)-thiazoli-4-one

III =5-[5-(substituted-phenyl)-furan-2-ylmethylene]-3-4-(substituted-phenyl)-2-(substituted phenyl imino)-thiazolidin-4-one

Table 1 List of substituents

Compounds	R_1	R_2	R_3
1a	*p*-OC_2H_5	H	*p*-Cl
1b	*p*-OC_2H_5	*p*-F	*p*-Cl
1c	*p*-OC_2H_5	*p*-Cl	*p*-Cl
1d	*p*-OC_2H_5	*p*-Br	*p*-Cl
1e	*p*-OC_2H_5	*p*-OCH_3	*p*-Cl
1f	*p*-OC_2H_5	*p*-OC_2H_5	*p*-Cl
1g	*p*-OC_2H_5	*o*- OCH_3	*p*-Cl
1h	*p*-OC_2H_5	*o*- CH_3	*p*-Cl
2a	*p*-OC_2H_5	H	*o*-Cl
2b	*p*-OC_2H_5	*p*-F	*o*-Cl
2c	*p*-OC_2H_5	*p*-Cl	*o*-Cl
2d	*p*-OC_2H_5	*p*-Br	*o*-Cl
2e	*p*-OC_2H_5	*p*-OCH_3	*o*-Cl
2f	*p*-OC_2H_5	*p*-OC_2H_5	*o*-Cl
2g	*p*-OC_2H_5	*o*- OCH_3	*o*-Cl
2h	*p*-OC_2H_5	*o*- CH_3	*o*-Cl
3a	*p*-OCH_3	H	*o*-Cl
3b	*p*-OCH_3	*p*-F	*o*-Cl
3c	*p*-OCH_3	*p*-Cl	*o*-Cl
3d	*p*-OCH_3	*p*-Br	*o*-Cl
3e	*o*-OCH_3	*p*-OCH_3	*o*-Cl
3f	*p*-OCH_3	*p*-OC_2H_5	*o*-Cl
3g	*p*-OCH_3	*o*- OCH_3	*o*-Cl
3h	*p*-OCH_3	*o*- CH_3	*o*-Cl
4a	*p*-OCH_3	H	*p*-Cl
4b	*p*-OCH_3	*p*-F	*p*-Cl
4c	*p*-OCH_3	*p*-Cl	*p*-Cl
4d	*p*-OCH_3	*p*-Br	*p*-Cl
4e	*p*-OCH_3	*p*-OCH_3	*p*-Cl
4f	*p*-OCH_3	*p*-OC_2H_5	*p*-Cl
4g	*p*-OCH_3	*o*- OCH_3	*p*-Cl
4h	*p*-OCH_3	*o*- CH_3	*p*-Cl

In silico molecular docking

The molecular docking study was performed by placing all the synthesized compounds inside the binding site of TNF-α (2AZ5 protein). All docking runs were carried out by maestro (Schrödinger). Molecular docking studies provided insights of molecular binding modes of molecules inside the pocket of TNF-α receptor. Crystallized structure of 2AZ5 protein was chosen from protein data bank and used as target for molecular docking studies with the reference ligand indomethacin.[21-23]The synthesized compounds docked against the optimized grid showed good binding energies ranging from **-30.17 to -49.26** kcal/ mol. Among all the synthesized molecules, some molecules showed better glide score as compared to the reference ligand (indomethacin) and other reference compound-V. The most promising molecules were **3f, 3g, 2f** and **2h** with the glide score **-6.27, -6.07, -6.06 and -6.05,** respectively. The binding modes of compounds **3f, 3g, 2f, 2h** and indomethacin with TNF-α are shown in **fig S1 (Supporting information)**. Compounds **3g, 2f** and **2h** were found to be aligned perfectly with the hydrophobic pocket and formed π-π stacking with TYR-A59, TYR-B59 and TYR-B119 of the target protein, respectively. However, the compound **3f** was found to form hydrogen bond with GLY-B121. On the other hand, indomethacin was found to be aligned perfectly with the hydrophobic pocket of the TNF-α protein showing glide score of **-5.02** and no other interactions were found. Compound-V which is structurally related to the synthesized derivatives was also used as a reference compound and docked in the protein binding site showing a π-π stacking with TYR-A59 and H-bonding interactions with GLN-A161 and GLY-A121. The glide score and binding energies of all the synthesized compounds are shown in **table 2.**

Table 2 Docking results of synthesized compounds

Ligands	Glide score	Glide energy	QPlog Po/w	QPlogS	PSA
1a	-4.01	-42.31	6.944	-7.526	50.138
1b	-5.73	-37.75	7.157	-7.792	50.146
1c	-5.65	-42.44	7.332	-7.857	50.175
1d	-5.47	-41.91	7.371	-7.902	50.388
1e	-4.72	-50.08	6.754	-6.754	58.566
1f	-5.08	-41.66	6.832	-7.048	55.173
1g	-5.22	-40.68	7.148	-7.214	58.18
1h	-3.86	-38.55	7.118	-7.662	47.441

2a	-5.77	-47.85	6.812	-7.211	50.603
2b	-5.68	-36.37	7.031	-7.486	50.609
2c	-5.21	-41.44	7.378	-8.388	50.639
2d	-5.18	-36.37	7.296	-7.54	50.651
2e	-5.24	-42.21	6.633	-6.454	59.034
2f	-6.06	-42.73	6.843	-7.776	58.425
2g	-5.78	-44.29	7.093	-7.119	58.443
2h	-6.05	-48.94	7.06	-7.613	47.591
3a	-5.73	-46.67	6.343	-6.509	51.142
3b	-5.67	-41.36	6.662	-7.053	50.956
3c	-5.34	-44.24	6.765	-6.985	51.172
3d	-5.50	-37.90	6.831	-7.051	51.179
3e	-4.55	-41.80	6.276	-6.171	59.518
3f	-6.27	-44.05	6.244	-6.089	56.117
3g	-6.07	-41.22	6.948	-7.044	58.969
3h	-4.32	-30.17	6.573	-6.793	49.645
4a	-4.95	-41.84	6.471	-6.816	50.677
4b	-5.30	-43.17	6.776	-7.408	50.697
4c	-5.13	-41.84	6.934	-7.371	50.69
4d	-5.05	-42.56	6.96	-7.357	50.716
4e	-5.05	-45.89	6.404	-6.493	59.056
4f	-4.97	-49.26	6.331	-6.279	56.294
4g	-5.76	-47.55	6.894	-7.205	58.536
4h	-3.47	-40.75	6.722	-7.136	49.189
Indomethacin	-5.02	-33.09	4.28	-5.31	84.32
Reference-compound-V	-5.2	-33.6	2.031	-3.556	99.29

***In vitro* TNF- α assay**

Twenty four compounds showing better glide score than indomethacin in molecular docking studies were further subjected to *in vitro* TNF-α level studies in order to confirm their mode of action. The results of *in vitro* TNF-α study are shown in **table 3.** From the data, it is clear that compounds **3f (75.85%), 3g (73.89%), 2f (72.68%), 2h (71.56%)** and **2b** (**71.01%**) showed significant inhibition of TNF-α level as compared to indomethacin (**69.89**%). Compounds **1b, 2g, 3b, 4b** and **4g** exhibited TNF-α inhibition comparable to the standard drug.

Compounds **3f, 3g, 2f, 2h** and **2b** showed better *in vitro* TNF-α activity as compared to the standard drug indomethacin. This is well supported by docking studies which show **3f** molecule forming a Hydrogen bond with GLY-121 residue, that is deeply buried into the hydrophobic binding pocket of TNF-α wherein the reference molecule indomethacin shows only hydrophobic and shape driven interactions. Similarly **3g**, **2f** & **2h** were found to show additional hydrophobic interactions with TYR-A59, TYR-B59 & TYR-B119 residues in the binding pocket of TNF-α. The molecules **1b, 2c, 2d, 2e, 2g, 3b, 3c, 3d, 4b, 4g** showed comparable glide score with respect to indomethacin but were found to show slightly less *in vitro* percent inhibition with respect to indomethacin (**59.65-67.51**%). The molecules **1c, 2d, 2f, 1g, 2a, 4e, 4d, 4c** and **3a** showed better glide score but were unable to show comparable percent inhibition *in vitro* (**46.98-58.51**%). These effects could be attributed to the docking methodology. All Docking programs currently in use exploit empirically based algorithms, avoiding systematic search of conformational space and scoring is done using simple equations, to speed up the process, therefore making it necessary to verify the results by subsequent *in vitro* studies. Our docking as well as *in vitro* studies are showing correlation although not exact. There are deviations in observed value of TNF-α inhibition and docking results because the hydrophobic pocket of TNF-α molecule is buried about 330 angstrom into protein surface. This gives rise to Y- shape binding pocket and makes difficult for the ligands to bind into the pocket if they are not flexible.

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Table 3 Effect of active compounds on LPS induced TNF-α cytokine level in the RAW 264.7 cell line.

Compounds	% inhibition
Standard (indomethacin)	69.89%
1b	63.58%
1c	58.10%
1d	56.15%
1f	52.74%
1g	57.98%
2a	42.67%
2b	71.01%
2c	64.10%
2d	60.35%
2e	64.51%
2f	72.68%
2g	66.18%
2h	71.56%
3a	46.98%
3b	67.51%
3c	62.50%
3d	59.65%
3f	75.85%
3g	73.89%
4b	64.53%
4c	57.95%
4d	54.46%
4e	55.45%
4g	60.97%

Cytotoxicity assay

In order to evaluate the cytotoxicity effect of the most active compounds **3f**, **3g**, **2f**, **2h** and **2b,** we carried out MTT proliferation assay. The results of cytotoxicity assay are shown in **table 4.** The results clearly indicate that the cell viability of compounds **3f**, **3g**, **2f**, **2h** and **2b** was more than 85% i.e. these compounds did not cause any abnormal cell death as compared to the standard drug indomethacin which has a cell viability of 60%.

Table 4 Cytotoxicity assay of active compounds

Compounds	% cytotoxicity
Standard drug (indomethacin)	42.41 %
2b	12.18%
2f	5.65 %
2h	9.73 %
3f	2.98 %
3g	4.18%

***In vivo* anti-inflammatory activity**

Fourteen compounds showing significant *in vitro* TNF-α suppression were further evaluated for their *in vivo* anti-inflammatory activity (**Table 5**). It was clear that compounds **3f** (**76.32% at 3h, 80.92% at 5h)** and **3g** (**74.51% at 3h , 78.42%** at 5h) showed better anti-inflammatory activity as compared to the standard drug indomethacin which showed an inhibition of **72.14% at 3h** and **78.11% at 5h**. Other compounds **2h** (**73.02%**), **2b (72.07%), 2f** (**70.27%**) and **3b** (**68.52%**) showed anti-inflammatory activity comparable to the standard drug at 5 h.[24-27]Compounds **3f** and **3g** showed better docking results as well as better *in-vitro* and *in-vivo* anti-inflammatory activity. Compounds **2h** and **2f** showed better docking results and *in vitro* TNF-α activity but these compounds showed less anti-inflammatory activity as compared to the standard drug indomethacin.

Table 5 Anti-inflammatory activity of active compounds

Compounds	Change in paw volume(ml) mean±(SEM)		% inhibition	
	3h	5h	3h	5h
Control	1.67±0.021	1.70±0.019	-	-
Indomethacin	0.60±0.023**	0.51±0.023**	72.14	78.11
1b	1.03±0.020*	1.01±0.021*	42.06	44.55
2b	0.65±0.013*	0.66±0.014**	67.96	72.07
2c	0.91±0.022*	0.87±0.016*	49.58	53.47
2d	0.98±0.018*	1.04±0.024*	47.03	43.59
2e	0.92±0.017*	0.95±0.024*	49.16	48.09
2f	0.68±0.021**	0.64±0.014**	66.57	70.27
2g	0.96±0.017*	1.03±0.018*	46.51	42.77
2h	0.66±0.018**	0.61±0.020**	69.08	73.02
3b	0.70±0.019*	0.65±0.011*	64.48	68.52
3c	0.70±0.014*	0.76±0.016*	64.00	61.58
3f	0.54±0.020**	0.48±0.013**	76.32	80.92
3g	0.55±0.015**	0.50±0.016**	74.51	78.42
4b	0.80±0.022**	0.74±0.017**	58.99	63.62
4g	0.81±0.022*	0.79±0.016*	56.82	58.99

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

Immunohistochemistry

The protein expression of pro-inflammatory mediator TNF-α in the presence of active compounds **3f** and **3g** was checked using immnohistochemistry. The modulation of cellular signaling network involving induction and activation of pro-inflammatory cytokine like TNF-α, has been considered a paradigm for preventing inflammation.[28]Therefore in the present study the potential of the active compounds in suppressing TNF-α cytokine was studied. The results of protein expression study of active compounds **3f** and **3g** in paw tissue of animals are shown in **fig.1**. For immunohistochemical analyses, brown colour indicates specific immunostaining of TNF-α expression. The intensity of brown colour in the animals treated with carrageenan only (group II) clearly indicates more number of cells having TNF- α expression as compared to that

of control group (I). Administration of active compounds **3f, 3g** and standard drug indomethacin reduced the expression of TNF-α significantly as compared to carrageenan induced group. However the reduction of TNF-α expression was more in the presence of **3f** and **3g** in comparison to the standard drug.

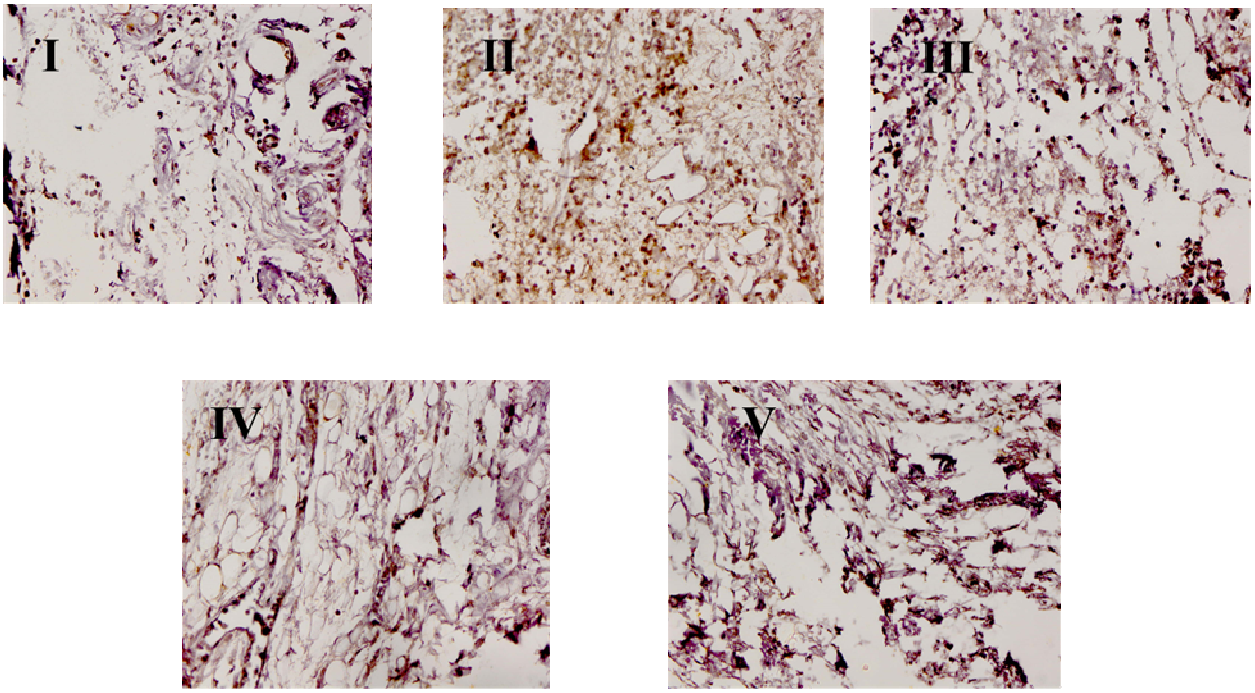

Fig. 1 Representative photomicrographs of immunohistochemistry (magnification 40X): Group I (only control), Group II (carrageenan only), Group III (carrageenan + Indomethacin), Group IV (carrageenan +3f), GroupV(carrageenan+3g)

Analgesic activity

The compounds **2b, 2f, 2h, 3f** and **3g** showing most potent anti-inflammatory activity (*in vivo*) were further tested for their analgesic activity. From the **table 6,** it is clear that compound **3f** showed significant analgesic activity **(54.58 %)** as compared to indomethacin which showed **57.91%.** Compound **3g** also showed activity comparable to the standard drug.

Table 6 Analgesic activity of active compounds

Group	Number of writhes in 10 min	% Protection
Control	96.0±2.20	-
Standard	40.4±1.63**	57.91
2b	49.2±`1.24**	48.95
2f	57.0±1.37*	40.62
2h	53.0±2.12*	44.79
3f	43.6±1.60**	54.58
3g	45.8±1.98**	52.29

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

Ulcerogenic activity

Compounds **2b, 2f, 2h, 3f** and **3g** showing potent anti-inflammatory activity were further checked for their ulcerogenic risk (**figure S2)**. Compounds **3f, 3g** and **2f** did not cause any damage to the stomach as compared to the standard drug indomethacin. Whereas, the compounds **2b** and **2h** caused a slight damage to the epithelium tissue which was lesser as compared to the standard drug causing significant epithelial mucosal damage to the stomach of tested animals.

Structure activity relationship

On the basis of docking score and biological activity, following SAR could be determined

- The presence of hydrogen at R_2 position in the synthesized compounds resulted in poor *in-vitro* and *iv-vivo* activity regardless of the glide scores.
- Halogen substitution at R_2 position showed better glide score as well as *in-vitro* and *in – vivo* activity in order of F>Cl>Br regardless of any substitution at R_1 and R_3.
- With R_2 as *o*-OCH_3 and R_1 =*p*-OC_2H_5 the in-vitro and in-vivo activity decreased with para substitution of chloro group in comparison to ortho substitution at R_3 position. Whereas the pattern reversed when R_1 was substituted with *p*-OCH_3.
- If R_2= *p*-OC_2H_5 and R_3 =*o*-Cl the glide score, *in-vitro* and *iv-vivo* activity are high regardless of substitution at R_1 as *p*-OC_2H_5or *p*-OCH_3.

Crystallographic study

Intensity data were collected at 183(2) K an Oxford Xcalibur Sapphire 3 diffractometer (a single wavelength Enhance X-ray source with MoK_α radiation, λ = 0.71073 Å).[29] The selected suitable single crystals were mounted using paratone oil on the top of a glass fiber fixed on a goniometer head and immediately transferred to the diffractometer. Pre-experiment, data collection, data reduction and analytical absorption corrections[30] were performed with the Oxford program suite *CrysAlisPro*.[31]The crystal structures were solved with SHELXS-97[32] using direct methods. The structure refinements were performed by full-matrix least-squares on F^2 with SHELXL-97.[32] All programs used during the crystal structure determination process are included in the WINGX software.[33]

The chemical formula and ring labeling system is shown in **Fig.2.** Crystal data for compound **3e**: $C_{28}H_{21}ClN_2O_4S$, Mr, 516.98; system, monoclinic; space group, P 21/c; unit cell dimensions, a = 17.4889(14)Å; b = 17.3814(11)Å; c = 8.2889(6)Å; β =99.779(8)°; V =2483.1(3)$Å^3$; Z = 4; T = 298 K; R_{int}, 0.0948; R(all), 0.2044;*Gof* = 0.988; $\Delta_{\rho max}$= 0.23 e $Å^3$; $\Delta_{\rho min}$= -0.23 e $Å^3$.The resolution obtained for the structure of the compounds was limited by the poor quality of the available crystals.

All hydrogen atoms were calculated after each cycle of refinement using a riding model, with C-H = 0.93 Å + U_{iso}(H) = 1.2U_{eq}(C) for aromatic H atoms, with C-H = 0.97 Å + U_{iso}(H) = 1.2U_{eq}(C) for methylene H atoms.

Crystallographic data for the structure **3e** have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number CCDC-1058214. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or atwww.ccdc.cam.ac.uk].

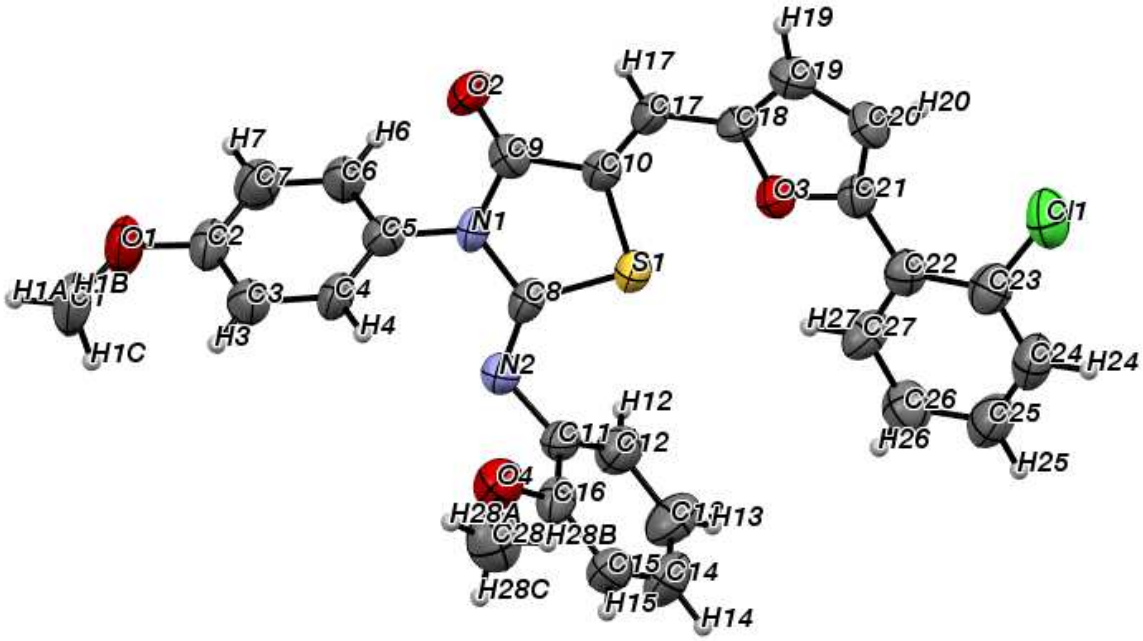

Fig. 2 Crystallographic structure of compound 3e

Conclusion

Thirty two 2-imino 4-thiazolidinones derivatives were subjected to i*n silico* molecular docking studies and evaluated for the effect on *in vitro* TNF-α target. The preliminary *in vitro* TNF-α activity on these synthesized derivatives suggested that the compounds **3f** and **3g** showed significant inhibition of TNF-α levels. These compounds (**3f**and **3g)** also showed significant *in vivo* anti-inflammatory activity as compared to indomethacin without causing cytotoxicity and

damage to the stomach. Moreover, compounds **3f** and **3g**also significantly suppressed the protein expression of TNF-α in carrageenan induced paw tissue of animals. Therefore, these compounds may be considered as potential candidates for the development of new anti-inflammatory agents.

Experimental

Materials and methods

All the reagents and starting materials were purchased from Sigma Aldrich and were used as such. Progress of the reaction was monitored by TLC (E.Merck Kieselgel 60 F254) and visualization was accomplished using UV light and iodine. The structural confirmation of the synthesized compounds was done by ^{1}H NMR (BruckerAvance II 300 NMR Spectrometer), ^{13}C NMR (BruckerAvance II 400 NMR Spectrometer), IR (Bio RAD. FTS 135) and mass spectral (ES-MS, LCQ Fleet,) data as well as elemental analysis (ElementarGmBH). Melting points were recorded by Veego (model VHP-DS) melting point apparatus and are uncorrected.

Preparation of 1-(substituted-phenyl)-3-(4-substitiuted-phenyl)-thiourea (I)

Different aromatic isothiocynates were reacted with different aromatic amines in the presence of absolute alcohol to get substituted thiourea. After completing the reaction, the solid part poured into crushed ice, filtered and crystallized with methanol.

Preparation of 3-(substituted-phenyl)-2-(4-substituted-phenyl imino)-thiazoli-4-one (II)

Different substituted thiourea reacted with ethylchloroacetate in the presence of absolute alcohol and sodium acetate to get substituted 2-imino-4-thiazolidinone. After completing the reaction, the obtained solid was washed with water and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated the solid part so obtained was crystallized by methanol.

Preparation of 5-[(substituted-phenyl)]-furan-2-carbaldehyde

5-[(substituted-phenyl)]-furan-2-carbaldehyde was prepared by reported method.[34]A mixture of para and ortho chlorine aniline (8.0 g, 0.1 M) dilHCl (15%, 30 ml) and water (40 ml) was heated on water bath to get a clear solution. The solution was cooled to 0°C to 5°C and diazotized with $NaNO_2$ solution (15%, 12 ml). After diazotization, a freshly distilled furfural aldehyde (6.0 ml, 0.1 M) and aqueous cupric chloride (1.2 g in 5 ml of water) were added with stirring. The stirring

was continued for 8 h and kept overnight. After completing the reaction, the solid was filtration and washed with water and crystallized from ethyl acetate.

Preparation of 5-[5-(substituted-phenyl)-furan-2-ylmethylene]-3-4-(substituted-phenyl)-2-(substituted phenyl imino)-thiazolidin-4-one (III)

Substituted 2-imino 4-thiazolidinone reacted with different aromatic aldehydes (5-substituted-phenyl)-furan-2-carbaldehyde in the presence of absolute alcohol and sodium acetate to get final product. After completing the reaction, the reaction mixture poured into crushed ice, filtered and crystallized with methanol.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-phenyl-thiazolidin-4-one (1a):light yellow solid; yield: 71%; M.p: 161-162^{o}C; M.W: 501.00;R_f: 0.56; FT-IR (υ_{max}; cm^{-1}KBr): 1578 (C=N), 1695 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$-d6, TMS): δ: 1.42 (t, 3H, $OCH_2\underline{CH_3}$,*J*= 7.1 Hz), 4.05 (q, 2H, $\underline{OCH_2}CH_3$ *J*=7.2 Hz),6.73-7.03 (m, 7H, Ar-H), 7.19-7.54 (m, 8H, Ar-H), 7.55 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.96, 63.52, 114.29, 114.07, 114.67, 114.90, 115.13, 116.16, 118.12, 120.12, 122.52, 127.80,127.89, 128.12, 129.10, 129.15, 130.16, 130.70, 141.96, 149.48, 153.19, 156.37, 156.99, 159.10, 166.66.Mass: m/z: 503.6 $[M+2]^+$.Elemental analysis for $C_{28}H_{21}ClN_2O_3S$: Calculated: C, 67.13; H, 4.22; N, 5.59; S, 6.40.found:C, 67.89; H, 4.31; N, 5.62; S, 6.47.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(4-fluoro-phenyl)-thiazolidin-4-one (1b):yellow solid ; yield: 69%; M.p: 134-135^{o}C; M.W: 518.99; R_f: 0.55; FT-IR (υ_{max}; cm^{-1}KBr): 1582 (C=N), 1688 (C=O); ^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.52 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 7.2 Hz), 4.09 (q, 2H, $\underline{OCH_2}CH_3$ *J*= 5.1 Hz) 6.80-7.13 (m, 7H, Ar-H), 7.34-7.39 (m, 4H, Ar-H), 7.46- 7.62 (m, 4H, Ar-H);^{13}C-NMR (120MHz, $CDCl_3$):δ:14.52, 63.72, 114.29, 114.35, 114.67, 114.90, 115.13, 116.61, 117.12, 120.10, 122.15, 127.60, 127.96, 128.70, 129.99, 130.29, 141.09, 149.12, 153.19, 156.37, 156.66, 159.44, 159.99, 166.10. Mass: m/z: 520.8 $[M+1]^+$.Elemental analysis for $C_{28}H_{20}ClFN_2O_3S$: Calculated: C, 64.80; H, 3.88; N, 5.40; S, 6.18.found: C, 64.94; H, 4.12; N, 5.52; S, 6.29.

3-(4-Chloro-phenyl)-5-[5-(4-chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-thiazolidin-4-one (1c):yellow solid; yield: 78%; M.p: 156-157^{o}C; M.W: 535.44; R_f: 0.55; FT-IR

(υ_{max}; cm^{-1}KBr); 1567 (C=N), 1681 (C=O);^{1}H-NMR (400 MHz, $CDCl_3$-d6, TMS,): δ: 1.44 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 6.0 Hz), 4.09 (q, 2H, $\underline{OCH_2}CH_3$ *J*= 6.9 Hz), 6.80-6.99 (m, 6H, Ar-H), 7.02-7.08 (m, 2H, Ar-H), 7.28-7.56 (m, 6H, Ar-H), 7.59 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$):δ:14.18, 63.57, 114.02, 114.03, 114.23, 115.74, 116.74, 118.65, 120.15, 122.15, 127.25, 127.35, 129.51, 129.51, 130.22, 130.52, 141.22, 149.22, 153.36, 156.31, 156.41, 159.41, 159.41, 159.51, 166.57. Mass: m/z: 536.20 $[M+1]^+$.Elemental analysis for$C_{28}H_{20}Cl_2N_2O_3S$: Calculated: 62.81; H, 3.76; N, 5.23; S, 5.99. found: C, 62.89; H, 3.86; N, 5.34; S, 6.12.

3-(4-Bromo-phenyl)-5-[5-(4-chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-thiazolidin-4-one (1d): yellow solid ; yield: 78%; M.p: 190-191^{o}C; M.W: 579.89;R_f: 0.53; FT-IR (υ_{max}; cm^{-1}KBr): 1589 (C=N), 1691 (C=O);^{1}H-NMR (300 MHz, $CDCl_3$-d6, TMS,): δ: 1.50(t, 3H, $OCH_2\underline{CH_3}$ *J*= 6.6 Hz), 4.09(q, 2H, $\underline{OCH_2}CH_3$ *J*= 5.4 Hz), 6.32-6.63 (m,5H, Ar-H), 6.87-7.04 (m, 2H, Ar-H), 7.28-7.81 (m, 8H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ:14.22, 63.72, 114.22, 114.22, 114.32, 114.33, 115.43, 116.44, 118.54, 120.55, 127.55, 127.55, 128.60, 129.16, 129.18, 130.20, 130.22, 130.22, 141.41, 149.27, 153.66, 156.14, 156.14, 159.14, 159.14, 166.15. Mass: m/z: 580.9 $[M+1]^+$.Elemental analysis for$C_{28}H_{20}BrClN_2O_3S$: Calculated: C, 57.99; H, 3.48; N, 4.83; S, 5.53. found: C, 58.13; H, 3.88; N, 4.89; S, 5.61.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(4-methoxy-phenyl)-thiazolidin-4-one (1e):light yellow solid; yield: 81%; M.p: 146-147^{o}C; M.W: 531.03; R_f: 0.55; FT-IR (υ_{max}; cm^{-1}KBr): 1549 (C=N), 1696 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ:1.47 (t,3H, $OCH_2\underline{CH_3}$ *J*=6.9 Hz), 3.83 (s, 3H, OCH_3), 4.09 (q, 2H, $\underline{OCH_2}CH_3$ *J*=6.9 Hz), 6.78(s, 2H, Ar-H), 6.85-7.34 (m, 8H, Ar-H), 7.36-7.55 (m, 4H, Ar-H), 7.58 (s, 1H, olefinic proton); ^{13}C-NMR (120 MHz, $CDCl_3$):δ:14.18, 55.22, 63.15, 114.26, 114.28, 114.30, 114.32, 11s5.47, 116.47, 118.56, 120.54, 122.54, 127.54, 128.62, 129.15, 130.20, 130.22, 141.22, 149.22, 153.13, 156.14, 156.63, 159.14, 159.15, 166.75.Mass: m/z: 532.3 $[M+1]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_4S$: Calculated: C, 65.59; H, 4.37; N, 5.28; S, 6.04. found: C, 65.83; H, 4.57; N, 5.45; S, 6.24.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-ethoxy-phenyl)-2-(4-ethoxy-phenylimino)-thiazolidin-4-one (1f): light yellow solid; yield: 79%; M.p: 201-202^{o}C; M.W: 545.05;R_f: 0.56; FT-IR (υ_{max}; cm^{-1}KBr): 1540 (C=N), 1624 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.42 (t, 6H, 2-$OCH_2\underline{CH_3}$ *J*= 6.88 Hz), 4.05 (q, 4H, 2-$\underline{OCH_2}CH_3$*J*= 6.88 Hz), 6.81 (d, 1H Ar-H, J=3.72

Hz), 6.88-7.04 (m, 6H, Ar-H), 7.21-7.44 (m, 6H Ar-H), 7.57(s, 1H, olefinic proton), 7.76-7.79 (m, 1H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.85, 14.97, 63.70, 114.07, 114.90, 115.12, 116.56, 118.09, 120.07, 122.51, 127.05, 127.16, 128.11, 129.02, 129.11, 130.58, 130.96, 141.60, 149.49, 152.23, 153.15, 156.36, 159.08, 166.42. Maas: m/z: 545.10 $[M+]^+$.Elemental analysis for$C_{30}H_{25}ClN_2O_4S$: Calculated: C, 66.11; H, 4.62; N, 5.14; S, 5.88.found: C, 66.21; H, 4.69; N, 5.34; S, 5.91.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(2-methoxy-phenyl)-thiazolidin-4-one (1g): light brownish solid; yield: 75%; M.p: 181-182^{o}C; M.W: 531.02; R_f: 0.55; FT-IR (υ_{max}; cm^{-1}KBr): 1549 (C=N), 1656 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.43 (t, 3H, $OCH_2\underline{CH_3}$ *J*=6.96 Hz), 3.86 (s, 3H, OCH_3), 4.04 (q, 2H, $\underline{OCH_2}CH_3$, *J*= 6.96Hz), 6.74 (d 2H, Ar-H, *J*= 3.08 Hz) 6.89-7.10(m, 6H, Ar-H), 7.17-7.36 (m, 3H, Ar-H), 7.41-7.54 (m, 3H, Ar-H), 7.55 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.11, 55.18, 63.22, 114.62, 114.82, 114.03, 114.23, 115.74, 116.74, 118.65, 120.15, 122.15, 127.15, 127.15, 128.56, 129.51, 129.51, 130.52, 141.22, 149.22, 153.36, 156.31, 156.41, 159.71, 166. 57. Mass: m/z: 533.2 $[M+2]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_4S$: Calculated: C, 65.59; H, 4.37; N, 5.28; S, 6.04. found: C, 65.82; H, 4.41; N, 5.56; S, 6.14.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-o-tolyl-thiazolidin-4-one(1h): light brownish solid; yield: 71%; M.p: 151-152^{o}C; M.W: 515.04; R_f: 0.55; FT-IR (υ_{max}; cm^{-1}KBr): 1556 (C=N), 1674 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.41 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 6.9 Hz), 2.41 (s, 3H, CH_3), 4.06 (q, 2H, $\underline{OCH_2}CH_3$ *J*= 6.90 Hz), 6.77-6.79(s, 2H, Ar-H), 6.91-7.15 (m, 5H, Ar-H), 7.15-7.31 (m, 3H, Ar-H), 7.41- 7.53 (m, 4H, Ar-H), 7.57 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.22, 63.11, 114.12, 114.12, 114.15, 114.23, 115.14, 116.14, 118.15, 120.12, 122.12, 127.13, 128.24, 129.55, 130.52, 130.76, 141.22, 149.22, 153.36, 156.33, 156.44, 159.44, 159.55. 166.52.Mass: m/z: 517.10 $[M+2]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_3S$: Calculated: C, 67.63; H, 4.50; N, 5.44; S, 6.23. found: C, 67.83; H, 4.59; N, 5.49; S, 6.56.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-phenyl-thiazolidin-4-one(2a):light yellow solid ; yield: 78%; M.p: 115-116^{o}C; M.W: 501.00; R_f: 0.57; FT-IR (υ_{max}; cm^{-1}KBr): 1559 (C=N), 1678 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.50 (t, 3H, $OCH_2\underline{CH_3}$ *J*=6.0 Hz), 4.10 (q, 2H, $\underline{OCH_2}CH_3$ *J*=6.9 Hz), 6.80 (s, 2H, Ar-H), 6.92-7.08 (m, 4H,

Ar-H), 7.22-7.59 (m, 9H.Ar-H), 7.60 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 14.85, 63.72, 108.67, 114.90, 115.14, 116.57, 116.79, 118.21, 118.39, 118.50, 119.40, 119.57, 119.66, 121.39, 122.51, 124.88, 125.51, 125.55, 127.04, 127.14, 127.81, 127.85, 128.09, 128.87, 128.93, 129.12, 129.14, 129.31, 134.31, 144.72, 148.57, 149.85, 149.89, 149.93, 152.39, 152.50, 155.66, 155.76, 156.42, 159.08, 159.12, 166.23, 166.39, 166.44.Mass: m/z: 502.8 $[M+1]^+$.Elemental analysis for$C_{28}H_{21}ClN_2O_3S$: Calculated: C, 67.13; H, 4.22; N, 5.59; S, 6.40. Found: 67.34; H, 4.31; N, 5.65; S, 6.48.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(4-fluoro-phenyl)-thiazolidin-4-one (2b):light yellow solid; yield: 71%; M.p: 126-127^{o}C; M.W: 518.99;R_f: 0.56; FT-IR (υ_{max}; cm^{-1}KBr): 1579 (C=N), 1671 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.47 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 4.8 Hz), 4.08 (q, 2H, $\underline{OCH_2}CH_3$*J*= 6.9 Hz), 6.84-7.12 (m, 5H, Ar-H), 7.25-7.49 (m, 8H, Ar-H), 7.63 (s, 1H, olefinic proton), 7.75-7.83 (m, 1H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ:14.72, 63.96, 114.12, 114.14, 114.87, 114.93, 115.31, 116.31, 116.57, 116.72, 117.85, 117.89, 117.93, 122.66, 123.76, 124.08, 124.42, 127.12, 127.23, 127.39, 127.44, 128.67, 128.90, 128.93, 129.14, 129.57, 129.69, 134.79, 141.21, 148.39, 149.50, 149.57, 152.39, 152.66, 155.51, 155.88, 156.51, 159.04, 159.55, 166.41, 166.81, 166.85. Mass: m/z: 519.8 $[M+]^+$.Elemental analysis for$C_{28}H_{20}ClFN_2O_3S$: Calculated: C, 64.80; H, 3.88; N, 5.40; S, 6.18. found: C, 64.97; H, 3.91; N, 5.49; S, 6.56.

3-(4-Chloro-phenyl)-5-[5-(2-chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-thiazolidin-4-one (2c):light yellow solid; yield: 68 %; M.p: 158-159^{o}C; M.W: 535.44; R_f: 0.55; FT-IR (υ_{max}; cm^{-1}KBr): 1589 (C=N), 1678 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.50 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 5.7 Hz), 4.09 (q, 2H, $\underline{OCH_2}CH_3$*J*= 6.9 Hz), 6.80-7.07 (m, 7H, Ar-H), 7.28-7.47 (m, 5H, Ar-H), 7.51- 7.56 (m, 2H, Ar-H), 7.58 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 15.51, 63.96, 114.12, 114.14, 114.72, 114.87, 114.93, 115.31, 115.39, 115.66, 115.72, 116.51, 116.88, 121.51, 124.04, 124.14, 127.85, 127.89, 127.93, 128.39, 128.50, 128.66, 129.08, 129.76, 129.42, 134.12, 141.23, 148.39, 149.44, 149.67, 149.90, 152.14, 152.93, 155.57, 155.69, 156.79, 159.21, 159.39, 166.40, 166.50, 166.57. Mass: m/z: 536.11 $[M+1]^+$.Elemental analysis for$C_{28}H_{20}Cl_2N_2O_3S$: Calculated: C, 62.81; H, 3.76; N, 5.23; S, 5.99. found: C, 62.89; H, 3.86; N, 5.45; S, 6.10.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(4-bromo-phenyl)-thiazolidin-4-one (2d):light brownsoild; yield: 63 %; M.p: 166-167^{o}C; M.W: 579.89;R_f: 0.54; FT-IR (υ_{max}; cm^{-1}KBr): 1579 (C=N), 1694 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.45 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 6.9 Hz), 4.09 (q, 2H, $\underline{OCH_2}CH_3$,*J*= 6.9 Hz) 6.80-7.07 (m, 7H, Ar-H), 7.35-7.57 (m, 6H, Ar-H), 7.59 (s, 1H, olefinic proton), 7.60-7.71 (m, 1H, Ar-H);^{13}C-NMR (120 MHz,):δ: 15.39, 114.81, 114.85, 114.89, 114.93, 115.31, 115.66, 115.72, 116.51, 116.88, 121.51, 124.08, 124.14, 124.55, 124.81, 127.85, 127.89, 127.93, 128.39, 128.50, 128.66, 129.08, 129.76, 134.14, 141.23, 148.39, 149.44, 149.67, 149.90, 152.14, 152.93, 155.57, 155.69, 156.79, 159.12, 159.21, 166.14, 166.72, 166.87. Mass: m/z: 580.2 $[M+1]^+$.Elemental analysis for$C_{28}H_{20}BrClN_2O_3S$: Calculated: C, 57.99; H, 3.48; N, 4.83; S, 5.53. Found: C, 58.13; H, 3.54; N, 4.89; S, 5.58.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(4-methoxy-phenyl)-thiazolidin-4-one (2e): light yellow solid; yield: 69 %; M.p: 171-172^{o}C; M.W: 531.04;R_f: 0.56; FT-IR (υ_{max}; cm^{-1}KBr): 1592 (C=N), 1698 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.45 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 4.5 Hz), 3.87 (s, 3H, OCH_3), 4.07 (q, 2H, $\underline{OCH_2}CH_3$ *J*=6.9 HZ), 6.85-7.05 (m, 6H, Ar-H), 7.25-7.48 (m, 8H, Ar-H), 7.61 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 14.64, 46.28, 66.75, 114.81, 119.65, 121.17, 124.87, 127.43, 127.88, 128.51, 128.99, 129.37, 130.34, 131.85, 133.32, 136.06, 138.37, 148.14, 150.04, 151.64, 158.74, 166.79. Mass: m/z: 532.4 $[M+1]^+$.Elemental analysis for $C_{29}H_{23}ClN_2O_4S$: Calculated: C, 65.59; H, 4.37; N, 5.28; S, 6.04. found: C, 65.78; H, 4.43; N, 5.34; S, 6.17.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-ethoxy-phenyl)-2-(4-ethoxy-phenylimino)-thiazolidin-4-one (2f): light yellow solid; yield: 67 %; M.p: 175-176^{o}C; M.W: 545.05; R_f: 0.54; FT-IR (υ_{max}; cm^{-1}KBr): 1598 (C=N), 1656 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.47 (t, 6H, 2-$OCH_2\underline{CH_3}$ *J*= 4.8 Hz), 4.10 (q, 4H, 2-$\underline{OCH_2}CH_3$ *J*= 6.9 Hz), 6.84 (d, 1H Ar-H, *J*=3.7 Hz), 6.84-7.06 (m, 6H, Ar-H), 7.24-7.48 (m, 7H Ar-H), 7.61 (s, 1H, olefinic proton), 7.80-7.83 (m, 1H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$):δ:14.97, 63.97, 114.06, 114.74, 115.38, 116.58, 118.96, 120.64, 122.49, 127.23, 127.15, 127.36, 128.08, 129.07, 130.12, 130.90, 141.56, 149.09, 152.07, 153.51, 156.05, 159.15, 166.88. Mass: m/z: 547.06 $[M+2]^+$.Elemental analysis for$C_{30}H_{25}ClN_2O_4S$: Calculated: C, 66.11; H, 4.62; N, 5.14; S, 5.88. found: C, 66.41; H, 4.72; N, 5.34; S, 5.94.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-(2-methoxy-phenyl)-thiazolidin-4-one (2g):light yellow solid; yield: 69 %; M.p: 120-121^{o}C; M.W: 531.02;R_f: 0.56; FT-IR (υ_{max}; cm^{-1}KBr); 1567 (C=N), 1696 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.44 (t, 3H, $OCH_2\underline{CH_3}$ *J*= 6.9 Hz), 3.96 (s, 3H, OCH_3), 4.06 (q, 2H, $\underline{OCH_2}CH_3$*J*= 6.9 Hz), 6.74 (d, 2HAr-H, *J*=3.08 Hz) 6.80-7.17 (m, 5H, Ar-H), 7.12-7.37 (m, 4H, Ar-H), 7.43- 7.52 (m, 3H, Ar-H), 7.56 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.95, 55.88, 63.74, 108.64, 112.04, 112.48, 112.86, 115.13, 115.21, 116.43, 118.02, 118.18, 119.86, 120.09, 121.08, 122.01, 122.54, 123.62, 125.50, 125.53, 125.81, 127.07, 127.89, 128.97, 129.04, 129.09, 129.12, 130.02, 130.86, 134.23, 137.77, 141.91, 149.95, 150.01, 151.59, 153.26, 155.54, 155.63, 156.34, 159.03, 166.06, 166.48.Mass: m/z: 533.4$[M+2]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_4S$: Calculated:C, 65.59; H, 4.37; N, 5.28; S, 6.04. found: C, 65.72; H, 4.47; N, 5.45; S, 6.20.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-ethoxy-phenylimino)-3-o-tolyl-thiazolidin-4-one(2h): light yellowsoild; yield: 86 %; M.p: 129-130^{o}C;R_f: 0.57; M.W: 515.02; FT-IR (υ_{max}; cm^{-1}KBr): 1587 (C=N), 1686 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 1.48 (t, 3H, $OCH_2\underline{CH_3}$*J*= 4.5 Hz), 2.32 (s, 3H, CH_3), 4.08 (q, 2H, $\underline{OCH_2}CH_3$*J*= 6.9 Hz), 6.84-7.03 (m, 5H Ar-H), 7.06-7.37 (m, 5H, Ar-H), 7.40-7.76 (m, 5H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ: 14.97, 23.28, 63.14, 114.28, 114.29, 115.29, 116.30, 118.30, 120.41, 122.49, 127.52, 127.53, 128.59, 129.11, 129.66, 130.58, 141.64, 149.49, 152.23, 153.15, 156.36, 159.08, 166.42.Mass: m/z: 517.10 $[M+2]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_3S$: Calculated: C, 67.63; H, 4.50; N, 5.44; S, 6.23. found: C, 67.78; H, 4.56; N, 5.49; S, 6.45.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-3-phenyl-thiazolidin-4-one (3a*)*: light yellow solid; yield: 67 %; M.p: 124-125^{o}C; R_f: 0.58; M.W: 486.97; FT-IR (υ_{max}; cm^{-1}KBr): 1543 (C=N), 1646 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.88 (s, 3H, OCH_3), 6.84-7.13 (m, 6H, Ar-H), 7.15-7.62 (m, 8H, Ar-H), 7.73 (s, 1H, olefinic proton), 7.80-7.83 (m, 1H, Ar-H), ^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.64, 114.34, 119.85, 121.32, 124.05, 127.14, 127.37, 128.05, 128.64, 129.74, 130.79, 131.81, 133.65, 136.11, 138.81, 148.42, 150.82, 151.52, 158.92, 166.32. Mass: m/z: 487.1 $[M+1]^+$.Elemental analysis for$C_{27}H_{19}ClN_2O_3S$: Calculated: C, 66.59; H, 3.93; N, 5.75; S, 6.58. found: C, 66.81; H, 3.99; N, 5.81; S, 6.68.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-fluoro-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (3b*)*: yellow solid; yield: 73 %; M.p: 147-149^{o}C; R_f: 0.54; M.W: 504.96; FT-

IR (υ_{max}; cm^{-1}KBr): 1567 (C=N), 1649 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.89 (s, 3H, OCH_3), 6.80-7.13(m, 6H, Ar-H), 7.21-7.42 (m, 5H, Ar-H), 7.46 (s, 1H, olefinic proton), 7.48-761 (m, 3H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$):δ:55.73, 108.53, 114.05, 114.73, 115.25, 116.24, 116.47, 116.98, 117.12, 118.58, 118.66, 118.90, 119.99, 122.38, 122.43, 122.50, 125.57, 125.60, 127.76, 127.78, 127.82, 129.07, 129.17, 129.76, 129.90, 129.96, 134.16, 134.33, 141.92, 144.11, 144.45, 149.76, 149.84, 151.51, 153.58, 155.15, 155.76, 157.81, 159.13, 166.17, 166.19. Mass: m/z: 504.9 $[M+]^+$.Elemental analysis for$C_{27}H_{18}ClFN_2O_3S$:Calculated: C, 64.22; H, 3.59; N, 5.55; S, 6.35. found: C, 64.32; H, 3.71; N, 5.65; S, 6.56.

3-(4-Chloro-phenyl)-5-[5-(2-chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-thiazolidin-4-one(3c*)*: light yellow solid; yield: 62 %; M.p: 154-156^{o}C; R_f: 0.53; M.W: 521.41 FT-IR (υ_{max}; cm^{-1}KBr): 1567 (C=N), 1649 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.86 (s, 3H, OCH_3), 6.84-7.08 (m, 6H, Ar-H), 7.28-7.48 (m, 7H, Ar-H), 7.61 (s, 1H, olefinic proton), 7.80-7.83 (m, 1H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$): δ: 55.95, 114.34, 114.74, 116.26, 116.46, 118.55, 118.55, 120.17, 120.77, 122.03, 124.19, 126.12, 127.21, 127.23, 127.27, 127.29, 127.33, 127.75, 128.95, 128.98, 129.19, 129.42, 129.72, 130.00, 130.14, 130.12, 130.25, 130.26, 141.21, 147.28, 149.25, 149.26, 151.15, 152.17, 153.14, 153.17, 157.18, 159.12, 159.18, 166.16. Mass: m/z: 521.9 $[M+]^+$.Elemental analysis for $C_{27}H_{18}Cl_2N_2O_3S$: Calculated: C, 62.19; H, 3.48; N, 5.37; S, 6.15. found: C, 62.34; H, 3.58; N, 5.89; S, 6.25.

3-(4-Bromo-phenyl)-5-[5-(2-chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-thiazolidin-4-one (3d*)*: light yellow solid; yield: 64 %; M.p: 185-186^{o}C; R_f: 0.51; M.W: 565.87; FT-IR (υ_{max}; cm^{-1}KBr): 1581 (C=N), 1688 (C=O); ^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 3.89 (s, 3H, OCH_3),6.81-7.01 (m, 6H, Ar-H), 7.39-7.60 (m, 7H, Ar-H), 7.61 (s, 1H, olefinic proton), 7.71 (s, 1H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.08, 108.73, 114.76, 115.08, 115.39, 116.08, 118.18, 119.18, 119.19, 120.29, 122.20, 124.22, 125.24, 125.25, 126.26, 127.21, 127.22, 127.25, 127.29, 129.29, 129.41, 130.29, 130.30, 134.30, 141.34, 147.41, 149.47, 149.49, 151.49, 152.51, 155.52, 155.55, 156.59, 159.56, 159.59, 166.66.Mass: m/z: 567.5 $[M+1]^+$.Elemental analysis for $C_{27}H_{18}BrClN_2O_3S$: Calculated: C, 57.31; H, 3.21; N, 4.95; S, 5.67. found: C, 57.39; H, 3.41; N, 5.10; S, 5.87.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-methoxy-phenyl)-2-(2-methoxy-phenylimino)-thiazolidin-4-one (3e*)*: light yellow solid; yield: 69 %; M.p: 191-192^{o}C; R_f: 0.56;

M.W: 517.00; FT-IR (υ_{max}; cm^{-1}KBr): 1588 (C=N), 1698 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS):δ: 3.89 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.82-7.09 (m 6H, Ar-H), 7.17-7.49 (m 7H, Ar-H), 7.61 (s, IH, olefinic proton), 7.73-7.82 (m, 1H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 55.82, 55.94, 108.65, 112.02, 114.26, 114.67, 116.58, 118.22, 119.81, 120.83, 121.08, 122.00, 122.54, 125.51, 125.83, 127.27, 127.85, 129.09, 134.24, 137.72, 149.93, 150.88, 153.27, 155.66, 159.61, 166.49. Mass: m/z: 518.4 $[M+1]^+$.Elemental analysis for $C_{28}H_{21}ClN_2O_4S$: Calculated: C, 65.05; H, 4.09; N, 5.42. S, 6.20.found: C, 65.88; H, 4.16; N, 5.49; S, 6.30.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-ethoxy-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (3f*)*: light yellow solid; yield: 69 %; M.p: 176-177^{o}C; R_f: 0.56; M.W: 531.02; FT-IR (υ_{max}; cm^{-1}KBr): 1589 (C=N), 1681(C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS):δ: 1.43 (t, 3H, $OCH_2\underline{CH_3}$, *J*=6.6 Hz), 3.84 (s, 3H, OCH_3),4.05(q, 2H, <u>OCH2</u>CH3, *J*=7.1 Hz), 6.82-7.05 (m, 7H, Ar-H), 7.22-7.45 (m, 6H, Ar-H), 7.59(s, 1H, olefinic proton), 7.78 (s, 1H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$): δ: 14.96 , 55.52, 63.71, 114.07, 114.29, 114.67, 114.90, 115.13, 116.61, 118.12, 120.02, 122.52, 127.89, 128.12, 129.10, 129.15, 130.60, 141.70, 149.48, 153.19, 156.37, 156.99, 159.10, 159.66, 166.44. Mass: m/z: 531.12 $[M+]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_4S$: Calculated: C, 65.59; H, 4.37; N, 5.28; S, 6.04.found: C, 65.91; H, 4.43; N, 5.58; S, 6.49.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-3-(2-methoxy-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (3g*)*: light brown solid; yield: 61 %; M.p: 179-180 ^{o}C; R_f: 0.57; M.W: 517; FT-IR (υ_{max}; cm^{-1}KBr); 1595 (C=N), 1688(C=O); ^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 3.83 (s, 6H, $2OCH_3$), 6.79(d 1H, Ar-H, *J* = 3.72 Hz), 6.90 (d, 2H, Ar-H, *J*= 8.88 Hz),6.92 (d, 2H, Ar-H, *J*=6.92 Hz), 7.04 (d, 2H, Ar-H, *J*=8.88), 7.19-7.24 (m, 2H, Ar-H), 7.28 (d, 1H, Ar-H, *J*=3.72), 7.37-7.43 (m, 3H, Ar-H), 7.56 (s, 1H, olefinic proton), 7.74-7.76 (m, 1H, Ar-H); ^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.51, 55.52, 114.09, 114.31, 114.67, 116.61, 118.16, 119.99, 122.54, 127.02, 128.09, 129.07, 129.17, 130.58, 130.97, 141.77, 149.96, 152.36, 153.17, 157.02, 159.67, 166.39.Mass:m/z: 517.21 $[M+]^+$.Elemental analysis for$C_{28}H_{21}ClN_2O_4S$: Calculated: C, 65.05; H, 4.09; N, 5.42; S, 6.20.found: C, 65.42; H, 4.69; N, 5.82; S, 6.85.

5-[5-(2-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-3-o-tolyl-thiazolidin-4-one (3h*)*: light yellow solid; yield: 55 %; M.p: 164-165^{o}C; R_f: 0.58; M.W:501.00; FT-IR (υ_{max}; cm^{-1}KBr): 1570 (C=N), 1675 (C=O); ^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 2.19 (s,

3H, CH_3), 3.87 (s, 3H, OCH_3), 6.79(s 2H, Ar-H), 6.91-7.07 (m, 6H, Ar-H), 7.32-7.41 (m, 4H, Ar-H), 7.54 (s, 1H, olefinic proton),7.57-7.58 (m, 2H,Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 17.94, 55.53, 114.08, 114.10, 114.31, 114.68, 114.72, 116.60, 116.73, 118.17, 118.22, 120.00, 120.03, 120.17, 122.56, 124.90, 126.38, 127.03, 128.04, 129.07, 129.13, 129.18, 130.12, 130.52, 130.70, 130.93, 13097, 141.78, 147.28, 149.36, 149.45, 151.98, 152.37, 153.16, 153.20, 157.03, 159.67, 159.74, 166.39.Mass: m/z: 502.8 $[M+1]^+$.Elemental analysis for $C_{28}H_{21}ClN_2O_3S$: Calculated: C, 67.13; H, 4.22; N, 5.59. S, 6.40; found: C, 67.59; H, 4.30; N, 5.96; S, 6.59.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-3-phenyl-thiazolidin-4-one(4a): light yellow solid; yield: 59 %; M.p: 118-119 ^oC; R_f: 0.59; M.W: 486.97; FT-IR (υ_{max}; cm^{-1}KBr): 1562 (C=N), 1671 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.88 (s, 3H, OCH_3), 6.80-7.08 (m, 9H, Ar-H), 7.34-7.42 (m, 4H, Ar-H), 7.56 (s, 1H, olefinic proton),7.59-7.7.69 (m, 2H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.64, 114.81, 119.65, 121.17, 124.87, 127.43, 127.88, 128.99, 129.37, 130.34, 131.85, 133.32, 136.05, 138.37, 148.14, 150.05, 151.64, 158.74, 166.79. Mass: m/z: 487.6 $[M+1]^+$.Elemental analysis for $C_{27}H_{19}ClN_2O_3S$:Calculated: C, 66.59; H, 3.93; N, 5.75; S, 6.58.found: C, 66.98; H, 3.53; N, 5.65; S, 6.91.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-fluoro-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4b): dark yellow solid; yield: 65 %; M.p: 137-138^oC; R_f: 0.58; M.W: 504.96; FT-IR (υ_{max}; cm^{-1}KBr): 1568 (C=N), 1678 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 3.86 (s, 3H, OCH_3), 6.77-6.80 (m, 2H, Ar-H), 6.92-7.10 (m, 5H, Ar-H), 7.20-7.25 (m, 1H, Ar-H), 7.32-7.39 (m, 2H, Ar-H), 7.43 (s, 1H, olefinic proton), 7.44-7.59 (m, 4H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.51 108.39, 114.73, 114.72, 115.75, 115.97, 116.24, 116.47, 116.98, 117.12, 118.58, 118.92, 119.11, 122.45, 122.76, 122.84, 125.51, 125.58, 127.51, 127.76, 127.81, 129.13, 129.17, 129.19, 129.90, 129.99, 134.43, 141.38, 144.57, 144.60, 149.78, 149.82, 151.76, 153.07, 155.90, 155.96, 157.17, 159.76, 166.16, 166.33. Mass: m/z: 504.6 $[M+]^+$.Elemental analysis for $C_{27}H_{18}ClFN_2O_3S$:Calculated: C, 64.22; H, 3.59; N, 5.55; S, 6.35.found: C, 64.78; H, 3.68; N, 5.89; S, 6.93.

3-(4-Chloro-phenyl)-5-[5-(4-chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4c): light yellow solid; yield: 62 %; M.p: 165-166 ^oC; R_f: 0.57; M.W: 521.41 FT-IR (υ_{max}; cm^{-1}KBr): 1592 (C=N), 1693 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.87 (s, 3H, OCH_3), 6.80 (s, 2H, Ar-H), 6.93-7.09 (m, 5H, Ar-H), 7.31-7.44 (m, 5H, Ar-H), 7.51 (s,

1H, olefinic proton),7.54-7.59 (m, 2H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 55.15, 108.74, 114.34, 114.74, 115.75, 115.95, 116.76, 116.86, 116.96, 118.58, 118.68, 118.98, 119.15, 122.42, 122.77, 122.87, 125.55, 127.17, 127.77, 127.87, 129.15, 129.16, 129.19, 129.97, 134.44, 141.38, 144.54, 144.64, 147.79, 147.89, 151.17, 153.83, 155.93, 157.17, 159.79, 166.16. 166.39.Mass: m/z: 521.4 $[M+]^+$.Elemental analysis for $C_{27}H_{18}Cl_2N_2O_3S$: Calculated: C, 62.19; H, 3.48; N, 5.37; S, 6.15. found: C, 62.69; H, 3.89; N, 5.77; S, 6.69.

3-(4-Bromo-phenyl)-5-[5-(4-chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4d): light yellow solid; yield: 69 %; M.p: 166-167^{o}C; *R_f*: 0.57; M.W: 565.86; FT-IR (υ_{max}; cm^{-1}KBr): 1599 (C=N), 1673 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.87 (s, 3H, OCH_3), 6.81-7.09 (m, 6H, Ar-H), 7.35-7.60 (m, 7H, Ar-H), 7.61 (s, 1H, olefinic proton), 7.67-7.71 (m, 1H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.73, 108.86, 114.06, 115.11, 116.08, 116.70, 118.30, 118.72, 119.21, 120.94, 122.81, 124.39, 125.66, 125.75, 126.42, 127.08, 127.15, 127.42, 127.65, 129.16, 129.10, 130.57, 130.77, 134.21, 141.33, 147.57, 149. 10, 149.67,151.51, 152.93, 155.51, 155.55, 156.39, 159.12, 159.14, 166.80.Mass: m/z: 567.1 $[M+2]^+$.Elemental analysis for$C_{27}H_{18}BrClN_2O_3S$: Calculated: C, 57.31; H, 3.21; N, 4.95; S, 5.67.found: C, 57.67; H, 3.56; N, 5.08; S, 5.56.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-methoxy-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4e): light brown solid; yield: 71 %; M.p: 150-151^{o}C; *R_f*: 0.57; M.W: 517; FT-IR (υ_{max}; cm^{-1}KBr): 1589 (C=N), 1683 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.86 (s, 3H, OCH_3) 3.87 (s, 3H, OCH_3), 6.85 -7.08 (m, 7H, Ar-H), 7.24-7.48 (m, 7H, Ar-H), 7.61 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 55.87, 55.94, 108.65, 112.02, 114.26, 114.67, 116.58, 118.22, 119.81, 120.83, 121.08, 122.00, 122.54, 125.51, 125.83, 127.27, 127.85, 129.09, 134.24, 137.72, 149.93, 150.88, 153.27, 155.66, 159.61, 166.49. Mas: m/z: 518.9 $[M+1]^+$.Elemental analysis for $C_{28}H_{21}ClN_2O_4S$: Calculated: C, 65.05; H, 4.09; N, 5.42; S, 6.20.found: C, 65.89; H, 4.30; N, 5.92; S, 6.45.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-3-(4-ethoxy-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one (4f): light yellow solid; yield: 71 %; M.p: 160-161^{o}C; *R_f*: 0.56; M.W:531.02; FT-IR (υ_{max}; cm^{-1}KBr): 1591 (C=N), 1688 (C=O);^{1}H-NMR (400 MHz,$CDCl_3$-d6, TMS): δ: 1.43(s, 3H,$OCH_2\underline{CH_3}$ *J*= 6.96 Hz), 3.86 (s, 3H, OCH_3), 4.06 (q 2H, $\underline{OCH_2}$ CH_3 *J*=7.1 Hz), 6.77(s, 2H,Ar-H), 6.90-7.05 (m, 6H, Ar-H), 7.31-7.39 (m, 4H, Ar-H), 7.52 (s,1H, olefinic

proton),7.53-7.56 (m, 2H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ:14.96, 55.12, 63.52, 114.04, 114.35, 114.72, 114.90, 115.13, 116.61, 118.12, 120.04, 122.54, 127.84, 128.15, 129.16, 129.18, 130.60, 130.96, 141.78, 149.49, 153.53, 156.54, 156.55, 159.56, 159.59, 166.66. Mass: m/z: 532.9 $[M+1]^+$.Elemental analysis for$C_{29}H_{23}ClN_2O_4S$: Calculated: C, 65.59; H, 4.37; N, 5.28; S, 6.04.found: C, 65.78; H, 4.57; N, 5.45; S, 6.56.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-3-(2-methoxy-phenyl)-2-(4-methoxy-phenylimino)-thiazolidin-4-one(4g): dark yellow solid; yield: 76 %; M.p: 166-167^{o}C; R_f: 0.56; M.W: 517.00; FT-IR (υ_{max}; cm^{-1}KBr): 1581 (C=N), 1678 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.79 (s, 2H, Ar-H), 6.95-7.08 (m, 5H, Ar-H), 7.19-7.37 (m, 3H, Ar-H), 7.46-7.55 (m, 4H, Ar-H), 7.59 (s, 1H, olefinic proton);^{13}C-NMR (120 MHz, $CDCl_3$):δ: 55.51, 55.52, 114.09, 114.31, 114.67, 116.61, 118.16, 119.99, 122.54, 127.02, 128.09, 129.07, 129.17, 130.58, 130.97, 141.77, 149.96, 152.36, 153.17, 157.02, 159.67, 166.39.Mass: m/z: 518.9 $[M+]^+$.Elemental analysis for$C_{28}H_{21}ClN_2O_4S$: Calculated: C, 65.05; H, 4.09; N, 5.42; S, 6.20. found: C, 65.34; H, 4.69; N, 5.92; S, 6.80.

5-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(4-methoxy-phenylimino)-3-o-tolyl-thiazolidin-4-one (4h): light yellow solid; yield: 58 %; M.p: 185-186 ^{o}C; R_f: 0.56; M.W: 501.00; FT-IR (υ_{max}; cm^{-1}KBr): 1588 (C=N), 1692 (C=O);^{1}H-NMR (300 MHz,$CDCl_3$-d6, TMS): δ: 2.19 (s, 3H, CH_3) 3.87(s, 3H, OCH_3) 6.84-7.10(m, 6H, Ar-H), 7.13-7.48 (m, 7H, Ar-H), 7.62 (s, 1H, olefinic proton), 7.73-7.82 (m, 1H, Ar-H);^{13}C-NMR (120 MHz, $CDCl_3$): δ: 17.53, 55.94, 114.00, 114.03, 114.17, 114.68, 114.72, 116.60, 116.73, 118.17, 120.08, 120.10, 120.31, 122.56, 124.90, 126.38, 127.07, 127.12, 127.52, 127.57, 127.70, 127.84, 128.04, 128.08, 129.07, 129.13, 130.93, 130.97, 141.78, 147.28, 149.36, 149.45, 151.98, 152.37, 153.16, 153.20, 157.03, 159.67, 159.74, 166.39. Mass: m/z: 501.2 $[M+]^+$.Elemental analysis for$C_{28}H_{21}ClN_2O_3S$: Calculated: C, 67.13; H, 4.22; N, 5.59; S, 6.40. found: C, 67.89; H, 4.67; N, 5.69; S, 6.49.

In silico molecular docking

Crystallized structure of 2AZ5 was chosen from Protein Data Bank and used as target for molecular docking studies. 2AZ5 structure was imported in Schrodinger using Protein Preparation Wizard. Missing hydrogen and atoms were added using prime interface. Undesired water molecules were removed. The protein was then optimized and minimized to give low energy and structurally correct target protein. As the target protein already had the site for

reference ligand, a grid was generated by selecting the ligand as the reference ligand. Finally the grid was validated and was used for further docking with new unknown ligands to predict their docking score. Chemical structures were drawn in maestro (schrodinger software) and geometrically refined by LigPrep module.[35] In this module 2-D structures were converted into 3-D structures, which were further subjected to OPLS-2005 force field to generate single low energy 3-D structure for each input structure. During this step chiralities were maintained. Docking was carried using Schrodinger Glide software with Extra precision and XP descriptor information. This generates favourable ligand poses which are further screened through filters to examine spatial fit of the ligand in the active site. Ligand poses which pass through initial screening are subjected to evaluation and minimization of grid approximation. Scoring is then carried on energy minimized poses to generate Glide score.

Drugs and chemicals

Indomethacin, carrageenan, carboxymethylcellulose, were purchased Chemicals Pvt. Limited (Bangalore, India), and ELISA kits of TNF-α were purchased from eBioscience (San Diego, CA, USA).

TNF-α Assay

RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100m g/ml) in a humidified atmosphere of 5% CO_2. The cells were harvested with trypsin-EDTA and diluted to a suspension in fresh medium. These cells were seeded in 96-well plates with 2.1×10^5 cells/well, and allowed to adhere for 1 h. Then the medium was induced with 100 μg/ml LPS (lipopolysaccharide), test samples (20μM), and incubated for 24 h. The supernatant (50 μL) was transferred into a 96-well ELISA plate and TNF-α level were quantified by ELISA kits according to the manufacturer's instructions.[36]

Cytotoxicity

Cell culture was carried out in triplicate following same protocol as that for TNF-α assay. RAW 264.7 cells (2×10^5) were cultured in 96-well plate containing DMEM supplemented with 10% FBS to get required confluency. These cells were stimulated with 20 μM test compounds in the presence of 100 μg/ml LPS for 24 h. After that, the cells were washed twice with DPBS and

incubated with 100 µl of 0.5 mg/ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) for 2 h at 37^{o}C for testing cell viability. The medium was then discarded and 100 µl dimethyl sulfoxide (DMSO) was added. After 30-min incubation, absorbance at 570 nm was recorded using a microplate reader.[36]

Anti-inflammatory activity

The anti-inflammatory activity was carried out by the reported method.[37]Animals were divided into 16 groups of five animals each. One group kept as a positive control group was orally administered with standard indomethacin (20mg/kg) and other group kept as a negative control group was administered with 0.5% carboxymethyl cellulose solution. The remaining groups were test groups and administered orally with synthesized compounds at dose of 20mg/kg b.w. A freshly prepared solution of carrageenan (1.0% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the subplantar region of the right hind paw after 1 h of administration of the test sample. Right hind paw volume was measured at 3 h and 5 h after carrageenan injection with the help of digital plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

$$\% \text{ Anti-inflammatory Activity} = [V_C - V_t/V_C] \times 100$$

Where, V_t represents the mean increase in paw volume in rats treated with test samples and V_C represents the mean increase in paw volume of rats in control group.

Immunohistochemistry

The paw tissues were fixed in formalin and embedded in paraffin. Sections of 5 µm thickness were cut onto poly-lysine coated glass slides. Sections were deparafinized three times (5 min) in xylene followed by dehydration in graded ethanol and finally rehydrated in running tap water. For antigen retrieval, sections were boiled in 10mM citrate buffer (pH 6.0) for 5-7 min. Sections were incubated with hydrogen peroxide for 15 min to minimize non-specific staining and then rinsed three times (5 min each) with 1X PBST (0.05% Tween-20). Blocking solution was applied for 10 min then sections were incubated with diluted (1:100) primary anti-bodies, purified rabbit polyclonal anti-TNF-α antibody (BioLegend) overnight at 4^{o}C in humid chamber. Further processing was done according to the instructions of Ultra Vision plus Detection System Anti-

Polyvalent, HRP/DAB (Ready-To-Use) staining kit (Thermo scientific system). The peroxidase complex was visualized with 3,3'-diaminobenzidine (DAB). Lastly the slides were counterstained with haematoxylin, cleaned in xylene, dehydrated with ethanol and after DPX mounting microscopic (BX 51 Olympus) analysis was done at 40X magnification.[38]

Analgesic activity

The analgesic activity was carried out by writhing test method using the previously reported method.[39] Swiss albino mice (35–40 g) of either sex were divided into seven different groups and each group contained five animals. Group I was taken as control and received CMC suspension only, group II received standard drug indomethacin and rest of the groups were orally administered with synthesized compounds at a dose of 20mg/kg. After 30 min of test samples administration, 0.1% acetic acid solution was given to mice intraperitoneally. The number of muscular contractions was counted over a period of 10 min after acetic acid injection. The data represents the total number of writhes observed during 10 min and is expressed as writhing numbers.

Ulcerogenic activity

The ulcerogenic study was carried out by earlier reported method.[40]The compounds which showed significant anti-inflammatory activity were further evaluated for their ulcerogenic effects. Control group rats were orally administered suspension of 1% carboxymethylcellulose only. The standard group and test samples were orally administration at a dose of 60mg/kg b.w which is three times of the dose used for anti-inflammatory activity. Animals were sacrificed after 5 h dosing of standard group and test samples.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements

The authors wish to express their thanks to Dr G N Qazi, Vice Chancellor, Jamia Hamdard for providing necessary research facilities. The authors are thankful to Dr. Javed Iqbal, former Director, Institute of Life Sciences (ILS), Hyderabad, for providing necessary *in vitro* facilities

and Dr. Parimal Misra for his help in performing *in vitro* studies. One of the authors, YA is also thankful to Hamdard National Foundation for providing financial assistance.

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Molecular modeling and synthesis of some new 2-imino-4-thiazolidinonederivatives with promising TNF-α inhibitory activity

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Out of 32 novel 2-imino-4-thiazolidinones, compounds **3f** & **3g** showed potent anti-inflammatory activity without causing any damage to the stomach.