MedChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

Journal Name

ARTICLE

Synthesis of novel 1-substituted triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives as potent anti-inflammatory agents and inhibitors of monocyte-to-macrophage differentiation

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Malla Reddy Gannarapu[ab], Sathish Babu Vasamsetti[ac], Nagender Punna[ab], Srigiridhar Kotamraju[ac*], Narsaiah Banda[ab*]

A series of novel 1-substituted-triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives **8a-s** & **12a-l** were prepared from 1-substituted 1,2,3-triazol-4-aldehydes **6** & **11** with *N*-methyl-3-acetyl-4-hydroxy benzothiazin-1,1-dioxide **7** by condensation. Final compounds **8** and **12** were evaluated for the anti-inflammatory activity and their ability to inhibit monocyte-to-macrophage transformation, a process pivotal during the development and progression atherosclerosis. Among all the compounds **12e, 12g, 12i, 12j, 12k** and **12l** showed impressive anti-inflammatory activity against TNF-α, IL-1β and MCP-1 cytokines release in a dose-dependent manner. The most promising compounds **12g, 12i** and **12l** further significantly inhibited Phorbol 12-myristate 13-acetate (PMA)-induced MMP-9 activity and PMA-induced monocyte-to-macrophage differentiation.

Introduction

The Inflammation is a complex physiological response of multi-cellular organisms in response to various infections and tissue injuries as part of the host defense mechanism [1]. Under normal conditions, this process is auto regulated by limiting the expression levels of pro-inflammatory cytokines. However, in metabolic disorders like diabetes mellitus, obesity, hypertension, ageing etc., immune cells become hypersensitive and trigger continuous production of diverse repertoire of inflammatory mediators even in the absence of infections and tissue injuries [2]. This unrestrained production of low-grade chronic inflammation is the main cause and consequence of diverse clinical disease manifestation [3]. Interleukin (IL)-1β is a multi-potent, inflammatory cytokine of many acute, chronic non-infectious diseases and its prolonged release is implicated in impaired neurogenesis, atherosclerosis, rheumatoid arthritis (RA), Alzheimer's etc [4]. Tumor necrosis factor (TNF)-α has both immune-regulatory and a pro-inflammatory function as seen in several auto-immune disorders including systemic lupus erythematosus [5]. Monocyte chemoattractant protein-1 (MCP-1) is an important chemokine which recruits circulating leukocytes to the sites of inflammation, a process known to be enhanced in various disorders like RA, insulin-resistance, diabetes, atherosclerosis etc [6]. Substantial evidences implicate that macrophages act as a predominant source of inflammation responsible for all the aforementioned disorders [7]. Particularly in vascular disorders, macrophages ingest lipid moieties that have accrued in the surroundings of vessel wall by expressing scavenger receptors elaborating accumulation of fatty streaks which constitute the bulky atherosclerotic lesion [8]. Moreover, matrix metalloproteinase's (MMPs) secreted by macrophages contribute to disease progression by promoting atherosclerotic plaque rupture [9-10]. All these events lead to the narrowing of the lumen of the artery which ultimately leads to ischemia/stroke and myocardial infarction. Monocyte-to-macrophage differentiation plays a predominant role in the development of several vascular disorders including atherosclerosis by eliciting a plethora of inflammatory promoting events [11] and restrain of monocytes recruitment into the aortic wall attenuates the risk of atherosclerosis [12]. Hence, targeting bio-actives responsible for inducing inflammation and inflammatory-dependent signaling cascades is an important strategy to combat those disease pathologies wherein inflammation plays a key role.

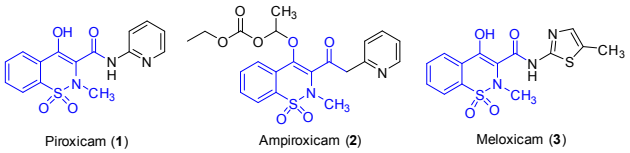


Figure 1: Structures of known 1,2-benzothiazine based NSAIDs

[a] Academy of Scientific and Innovative Research, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500607, India.
[b] Fluoroorganic division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500607, India.
[c] Centre for Chemical Biology, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500607, India.

† Electronic Supplementary Information (ESI) available: Experimental procedures, physico chemical properties and characterization data for all synthesized compounds are provided. See DOI: 10.1039/x0xx00000x

Benzothiazine derivatives are known to possess a versatile range of biological activities [13-17]. Among these, 1,2-benzothiazine-3-carboxamide-1,1-dioxide such as piroxicam (**1**), ampiroxicam (**2**) and meloxicam (**3**) which belong to the oxicam class of NSAIDs, are promising anti-inflammatory agents with proven therapeutic potential in various inflammatory and immune disorders. The reported candidates disrupt the biosynthesis of the prostaglandins and thromboxanes by inhibiting the enzyme cyclooxygenase (COX). Recent findings describe the role of 1,2-benzothiazine 1,1-dioxide derivatives as excellent anti-microbial [14,15], anti-oxidant [13], anti-hepatitis C virus (HCV) [16] and 11β-HSD1 inhibitors [17]. During the last few decades, synthetic modifications of 1,2-benzothiazine 1,1-dioxides have been intensively studied to enhance their bioactivity and to develop better anti-inflammatory candidates [18, 19].

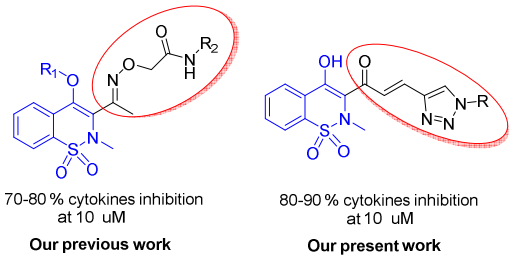


Figure 2: Our previous and present modifications on benzothiazine scaffold

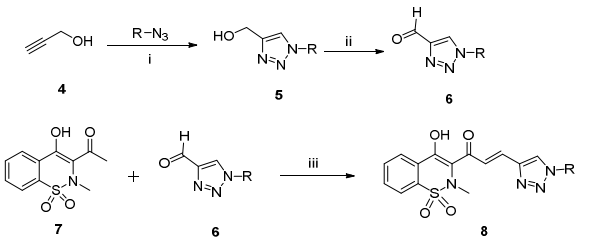
More recently, we have reported 1,2-benzothiazine 1,1-dioxide-3-ethanone oxime *N*-aryl acetamide ether derivatives as potent anti-inflammatory agents and inhibitors of monocyte-to-macrophage differentiation, where we have explored 3rd position of 1,2-benzothiazine 1,1-dioxide and prepared acetamide derivatives by using oxime ether link [20]. In continuation of our efforts to identify better anti-inflammatory agents, the present work aimed to functionalize the 3rd position of 1,2-benzothiazine 1,1-dioxide with 1,2,3-triazole moiety (Figure 2).

Thus, we have synthesized 1,2-benzothiazine 1,1-dioxide derivatives **8a-s** and **12a-l** and evaluated against TNF-*α,* IL-1β and MCP-1 production in Phorbol 12-myristate 13-acetate (PMA)-stimulated monocytes and discussed their structure versus activity relation. The most promising compounds **12e, 12g, 12i, 12j, 12k** and **12l** were selected for further assessment of their dose-dependent inhibitory effects against PMA-induced TNF-*α,* IL-1β and MCP-1 release, and also investigated their effects on MMP-9 activity. Finally, compounds **12g, 12i** and **12l** imposed a significant inhibition on PMA-induced monocyte-to-macrophage differentiation along with an inhibition of MMP-9 activity, thereby suggesting their potential to serve not only as new anti-inflammatory agents, but also as novel molecules affecting monocyte-to-macrophage differentiation.

Results and Discussion

Chemistry

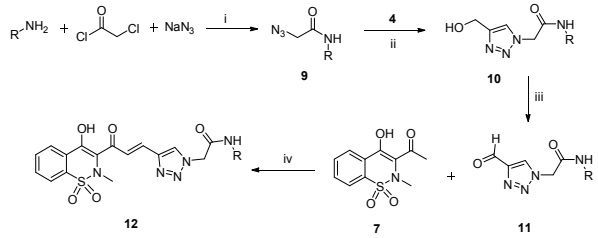
The *N*-methyl-3-acetyl-4-hydroxy benzothiazin-1,1-dioxide **7** was prepared in three steps starting from sodium salt of saccharin [13, 20]. In order to construct 1,2,3-triazole scaffold, the propargyl alcohol **4** was independently reacted with alkyl azide and alkyl amide azide **9** (which intern synthesized from the coupling of amines, chloroacetyl chloride and sodium azide in one pot) in t-butanol in the presence of $Cu(OAc)_2$ produced (1-alkyl substituted-1*H*-1,2,3-triazol-4-yl)methanol **5** and 2-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-*N*-substitutedacetamide **10** respectively. The alcohol **5** and **10** were further oxidized to aldehydes **6** and **11** by using Jones reagent. The aldehyde **6** and **11** were reacted with compound **7** in ethanol under reflux conditions and obtained propenone derivatives **8** and **12** respectively. The details of reactions outlined in Scheme 1 and 2. The purity of all the products was determined by HPLC and pure compounds (≥95%) were used for biological experiments.



8a; R = -$(CH_2)_9$-CH_3
8b; R = -$(CH_2)_7$-CH_3
8c; R = -C_6H_5
8d; R = 4-$CH_3C_6H_4$
8e; R = 4-$CF_3C_6H_4$
8f; R = 4-$OCH_3C_6H_4$
8g; R = 4-$NO_2C_6H_4$
8h; R = 4-FC_6H_4
8i; R = 3-Cl,4-FC_6H_3
8j; R = 3-ClC_6H_4
8k; R = 3-$CF_3C_6H_4$
8l; R = 2-Cl,5-$NO_2C_6H_3$
8m;R = 2,4-$F_2C_6H_3$
8n; R = 2-BrC_6H_4
8o; R = -$(CH_2)_2$-C_6F_{13}
8p; R = 3-FC_6H_4
8q; R = 3-$OCH_3C_6H_4$
8r; R = 3-$NO_2C_6H_4$
8s; R = 2-FC_6H_4

Reagents and conditions: (i) $Cu(OAc)_2.5H_2O$, ^{t}BtOH, RT, 18 h; (ii) Jones Reagent, acetone, 0 °C, 15 min; (iii) Piperidine, EtOH, reflux, 2-4 h.

Scheme 1: Preparation of substituted-1-alkyl/aryl triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives (**8a-s**)



12a; R = C_6H_5
12b; R = 4-FC_6H_4
12c; R = Cyclo hexyl
12d; R = 2-Br,4-FC_6H_3
12e; R = 4-$CF_3C_6H_4$
12f; R = 4-$OCH_3C_6H_4$
12g; R = 3-ClC_6H_4
12h; R = 2,6-$F_2C_6H_3$
12i; R = 4-$CH_3C_6H_4$
12j; R = 3-FC_6H_4
12k; R = 3-CF_3,4-ClC_6H_3
12l; R = 3-$CF_3C_6H_4$

Reagents and conditions: (i) Et_3N, dry DMF, 0 °C for 1 h then RT, 12 h; (ii) $Cu(OAc)_2.5H_2O$, ^{t}BtOH, RT, 18 h; (iii) Jones Reagent, acetone, 0 °C, 15 min; (iv) Piperidine, EtOH, reflux, 2-4 h.

Scheme 2: Preparation of substituted N-alkyl/aryl-1-acetamide-triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives (12a-l)

Biological activity

Inhibition of TNF-α, IL-1β and MCP-1 production by* 1,2-benzothiazine 1,1-dioxide derivatives *in PMA stimulated THP-1 monocytes:

All the final compounds, 1,2-benzothiazine 1,1-dioxide derivatives **8a-s** and **12a-l** were screened for the anti-inflammatory activity in PMA-stimulated THP-1 monocytes by measuring TNF-α, IL-1β and MCP-1 levels with reference to piroxicam and celecoxib (Table 1). Among the tested compounds, compounds having aliphatic alkyl chain **8a** (R= $-(CH_2)_9$-CH_3) and perfluoro alkyl chain **8o** (R= $-(CH_2)_2$-C_6F_{13}) showed cytotoxicity at 10 μM whereas compound with eight carbons aliphatic chain **8b** (R = $-(CH_2)_7CH_3$) possessed moderate inhibitory effect on IL-1β and MCP-1 levels without affecting cell viability (Table 1). Remaining compounds however did not show significant effect on viability of cells even at 20 μM concentration. This clearly indicates that, the anti-inflammatory activities of these compounds are not due to the cytotoxic effect on cells.

Among the substituted-1-alkyl/aryl triazole linked benzothiazine derivatives **8a-s**, only one compound having

Table 1: Secretion inhibition efficacy of the benzothiazine derivatives (8a-s & 12a-l) for TNF-α, IL-1β and MCP-1a

Entry	Compounds	% of inhibition			Cell viability[c] (% control)
		TNF-α[a] secretion	IL-1β[a] secretion	MCP-1[a] secretion	
1	**8b**	2.9±1.2	64.7±5.4	40.9±6.5	92.32±1.20
2	**8c**	24.0±3.4	56.9±7.8	31.9±3.1	94.20±3.36
3	**8d**	NA	50.2±6.5	45.2±5.3	94.32±1.08
4	**8e**	NA	17.7±2.4	12.7±2.9	96.62±2.42
5	**8f**	NA	53.9±4.1	58.9±6.8	94.40±1.92
6	**8g**	NA	36.5±3.7	31.5±2.6	92.98±2.08
7	**8h**	NA	43.9±3.5	58.9±5.6	95.32±1.54
8	**8i**	NA	16.3±6.4	NA	94.26±2.60
9	**8j**	NA	37.3±8.6	5.4±1.2	96.03±1.52
10	**8k**	NA	23.9±4.5	NA	93.80±3.24
11	**8l**	52.5±6.3	61.8±2.8	67.1±4.5	94.26±2.06
12	**8m**	11.2±6.5	47.7±3.8	29.6±3.2	96.20±2.30
13	**8n**	13.7±2.9	45.9±4.3	16.2±3.9	94.40±1.54
14	**8p**	48.3±3.4	NA	NA	93.05±2.16
15	**8q**	NA	42.6±2.9	NA	94.15±1.29
16	**8r**	NA	25.6±4.7	30.7±5.3	92.60±2.20
17	**8s**	NA	40.2±4.5	NA	96.06±1.15
18	**12a**	33.3±4.5	49.3±3.2	76.7±6.2	96.23±1.02
19	**12b**	73.4±6.5	42.4±4.5	41.4±4.5	92.5±2.16
20	**12c**	39.7±2.8	47.7±5.3	56.1±5.5	95.18±1.19
21	**12d**	59.8±3.8	63.8±4.5	65.4±3.2	93.56±2.02
22	**12e**	94.2±4.5	91.1±3.2	81.1±6.8	95.08±1.19
23	**12f**	62.9±5.6	49.8±5.8	64.8±8.2	94.45±2.23
24	**12g**	91.1±4.5	80.4±6.2	84.0±4.4	98.23±1.45
25	**12h**	31.3±3.2	46.3±4.6	41.3±2.8	95.62±2.08
26	**12i**	84.7±6.2	74.9±7.2	87.2±5.3	97.03±1.25
27	**12j**	70.7±5.6	69.5±6.5	48.2±4.8	96.45±2.22
28	**12k**	93.2±2.8	93.6±4.8	88.4±3.6	94.28±2.04
29	**12l**	87.6±4.8	83.6±5.6	85.1±5.3	96.20±3.03
30	Celecoxib[b]	37.4±8.3	31.1±3.2	20.1±3.2	-
31	Piroxicam[b]	25.8±4.5	21.8±6.2	11.4±2.3	-

[a] THP-1 monocytes were pre-treated with 10 μM of the above mentioned benzothiazine derivatives (**8a-s &12a-l**) for 2 h and stimulated with 100 nM of PMA to induce inflammation for a period of 48 h. At the end of the treatment, conditioned media was collected and TNF-α, IL-1β and MCP-1 levels were measured by ELISA as described in the Materials and Methods. [b] Celecoxib and piroxicam were were used as positive control; NA: denotes no inhibition at observed concentration. [c] THP-1 cell viability with synthesized compounds (20 μM).

2-Cl, 5-NO_2 phenyl group on triazole ring (**8l**) suppressed the secretion of all the three pro-inflammatory cytokines with more than 50% inhibition. Among the substituted *N*-alkyl/aryl-1-acetamide-triazole linked benzothiazine derivatives **12a-l**, several compounds have shown more than 50% inhibition of all the three pro-inflammatory cytokines. Among these, compounds having trifluoromethyl substitutions *i.e.,* **12e** (4-CF_3), **12k** (3-CF_3, 4-Cl) and **12l** (3-CF_3) were found to potently inhibit the cytokines expressions (more than 90%), whereas three more compounds **12g** (3-Cl), **12i** (4-CH_3) and **12j** (3-F) showed 60-80% inhibition of all the three pro-inflammatory cytokines. Overall, the screening results indicated that substituted *N*-alkyl/aryl-1-acetamide-triazole linked benzothiazine derivatives (**12a-l)** showed a better inhibition of

proinflammatory cytokines production than the substituted-1-alkyl/aryl triazole linked benzothiazine derivatives (**8a-s)** in PMA stimulated monocytes.

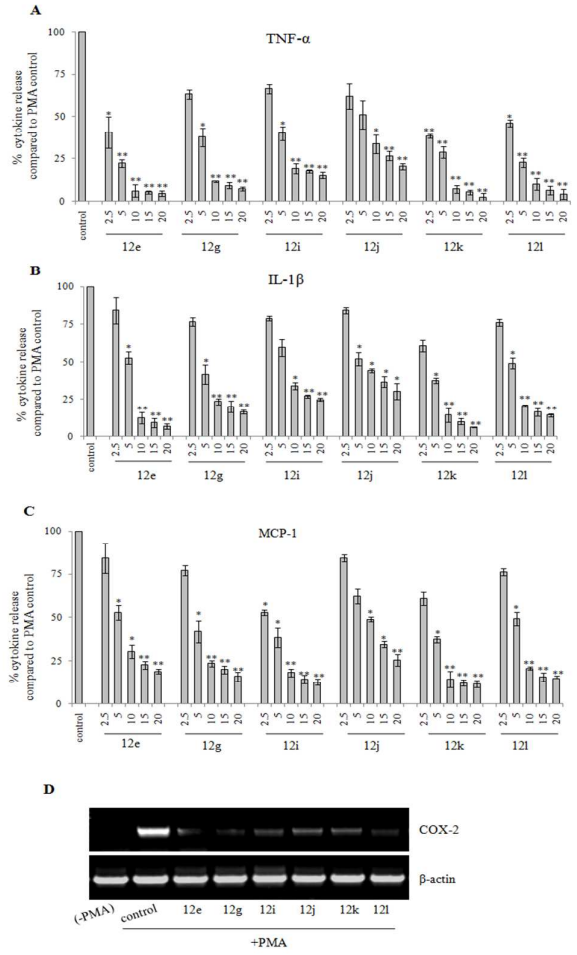


Figure 3: Compounds **12e**, **12g**, **12i**, **12j**, **12k** and **12l** inhibit PMA-induced TNF-α, IL-1β and MCP-1 release in a dose dependent manner.
THP-1 monocytes were pretreated with indicated concentrations of 12e, 12g, 12i, 12j, 12k and 12l compounds for 2 h, followed by stimulation with PMA (100 nM) for 48 h and analyzed (A) TNF-α (B) IL-1β and (C) MCP-1levels in the conditioned medium using ELISA. The results were expressed as percentage of inhibition compared to PMA control. D) Monocytes were pretreated with 10 μM concentration of compounds for 2 h, followed by stimulation with PMA (100 nM) for 48 h and analyzed the COX-2 mRNA levels using RT-PCR. Each bar represents the mean ± SD of three independent experiments. Statistical significance relative to the PMA groups is indicated: *, $p < 0.05$; **, $p < 0.01$.

Dose-dependent inhibitory effect of promising compounds on PMA-induced inflammation:

Based on the screening results, the most promising compounds **12e, 12g, 12i, 12j, 12k** and **12l** were selected for further assessment for their dose-dependent inhibitory effects on inflammation. For this, monocytes were pre-treated with compounds (2.5-20 μM) for 2 h followed by incubation with PMA (100 nM) for 48 h and subsequently analyzed TNF-α, IL-1β and MCP-1 levels in conditioned media by ELISA. All the promising compounds dose-dependently inhibited PMA-induced TNF-α, IL-1β and MCP-1 production (Figure 3A, Figure 3B and Figure 3C), thereby confirming the anti-inflammatory potential of these compounds.

Effect of promising compounds on PMA-induced COX-2 mRNA expression:

Besides TNF-α, IL-1β and MCP-1 as evaluated above, COX-2 has been demonstrated as an inflammatory mediator in various health disorders [21]. As compounds **12e, 12g, 12i, 12j, 12k** and **12l** significantly inhibited the levels of TNF-α, IL-1β and MCP-1, we were interested to study the effect of these compounds on COX-2 expression. To this context, we measured the COX-2 mRNA levels with PMA treatment in the presence or absence of compounds using RT-PCR after 48 h.
All these compounds significantly inhibited the transcript levels of PMA-induced COX-2 (Figure 3D). These results suggest that promising compounds along with the inhibitory efficacy on proinflammatory cytokines profile also affect the COX-2 signaling at transcription level.

Effect of promising compounds on PMA-induced monocyte-to-macrophage differentiation:

Macrophages are an inflammatory phenotype of monocytes, known to play a key role in the development and progression of several vascular disorders [8]. It is well known that PMA stimulation differentiate monocytes into macrophages as reflected by increased cell adherence and increase in cell size along with increased number of cellular organelles including mitochondria [22]. In tune with this, we next examined the effect of promising substituted-acetamide triazole linked benzothiazine derivatives on PMA-induced monocyte-to-macrophage differentiation process by treating monocytes with **12e**, **12g**, **12i**, **12j**, **12k** and **12l** compounds (5-20 μM) for 2 h followed by stimulation with PMA (100 nM) for 48 h. From the phase contrast images it was observed that, compounds **12g**, **12i** and **12l** at 15 μM concentration significantly inhibited the PMA-induced cell adherence (Figure 4A). However, **12e**, **12j** and **12k** failed to inhibit the PMA-induced cell adherence even at 20 μM concentration (data not shown). Also, **12g, 12i** and **12l** significantly inhibited the PMA-induced increase in cell size as reflected by a decrease in the mitochondrial content (Figure 4B)**.** In addition, these three compounds significantly inhibited the PMA-induced LOX-1 and CD-36 scavenger receptors (markers of monocyte differentiation) transcript levels by RT-PCR (Figure 4C). These results were further supported by lysotracker staining as shown in Figure 4D. It is known that, macrophages express significantly higher levels of lysosomal enzymes [22]. Treatment of monocytes with these three compounds greatly inhibited PMA-induced lysosomal activity (Figure 4D), thereby, indicating that these compounds inhibit PMA-mediated monocyte-to-macrophage differentiation. Taken together, these results confirmed that, compounds **12g, 12i** and **12l** inhibit PMA-induced monocyte-to-macrophage differentiation, a key step during progression of atherogenesis.

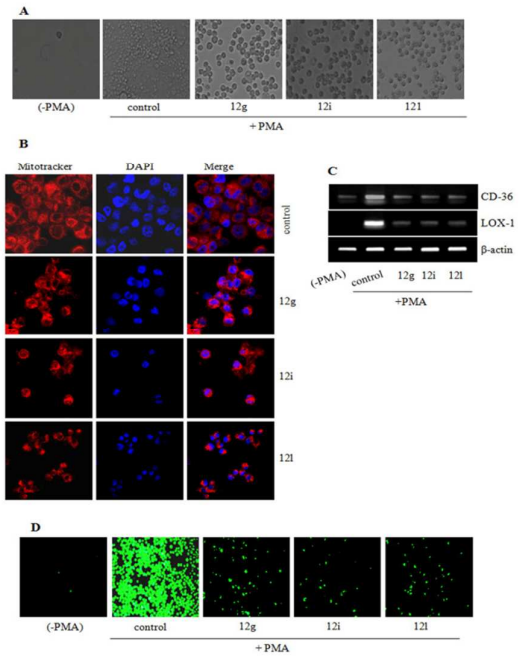


Figure 4: Compounds **12g**, **12i** and **12l** inhibit PMA-induced monocyte-to-macrophage differentiation.

THP-1 monocytes were pretreated with 12g, 12i and 12l compounds (15 μM) for 2 h, followed by stimulation with PMA (100 nM). A) Phase contrast images indicating the inhibition of PMA-induced monocyte-to-macrophage differentiation by compounds at 48 h. B) Same as *A,* except that mitochondrial staining was performed using mitotracker dye by confocal microscopy. C) Same as *A*, except that differentiation markers CD-36 and LOX-1 transcript levels were measured by RT-PCR at 24 h. D) Same as *A,* except that lysosomal staining was performed to measure lysosomal activity using lysotracker dye by fluorescence microscopy.

Inhibitory effect of* 12g, 12i *and* 12l *compounds on PMA-induced MMP-9 activity:

Increased secretion of matrix metalloproteases (MMPs) by macrophages destabilizes the plaque and enhances the plaque rupture during atherosclerosis. Differentiation of human peripheral blood mononuclear cell (HPBM) to macrophages with M-CSF or the differentiation of THP-1 monocytes by PMA has been shown to increase the levels of MMP-1,-7, and -9 [23]. As compounds **12g, 12i** and **12l** showed an excellent inhibitory effect on the differentiation of monocytes, we further examined the beneficial effects of these compounds by measuring the PMA-induced MMP-1 and 9 transcript levels using RT-PCR. It was found that these three compounds significantly inhibited the transcript levels of both MMP-1 and MMP-9 (Figure 5A). Next, we measured the MMP-9 activity in conditioned medium using gelatin loaded gels. In concurrence with the transcript levels, these compounds significantly inhibited PMA-induced MMP-9 enzyme activity, as studied by gelatin zymography (Figure 5B). However, under these conditions MMP-2 activity was not altered (Figure 5B). To further confirm these results, we next studied the effect of compounds **12g, 12i** and **12l** on PMA-induced monocyte/macrophage invasion using collagen coated Boyden-invasion chambers. Increased secretion of MMP's promote macrophage invasion by degrading the extracellular matrix components which often accentuates the migration of macrophages, cytokine signaling and leukocyte activation during the disease processes. In agreement with the inhibitory activity of these compounds on MMP-9 activity, these compounds also greatly inhibited the PMA-induced invasive capacity of monocyte/macrophage (Figure 5C). Taken together, these results suggest that compounds **12g, 12i** and **12l** regulate PMA-induced alterations during monocyte differentiation.

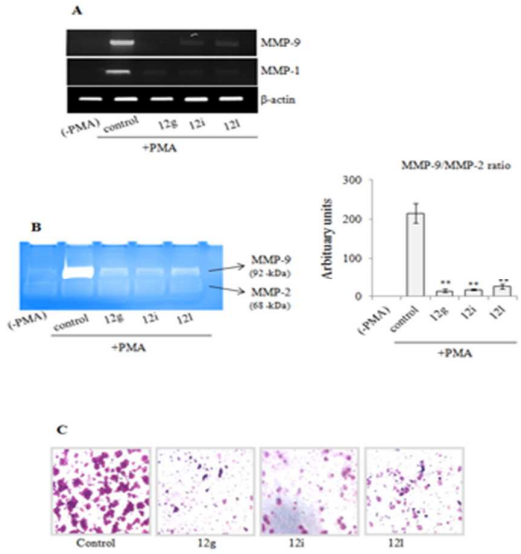


Figure 5: Compounds **12g, 12i** and **12l** inhibit PMA induced MMP-9 gelatinase activity and monocyte/macrophage invasion.

THP-1 monocytes were pretreated with **12g**, **12i** and **12l** compounds (15 μM) for 2 h, followed by stimulation with PMA (100 nM). A) Transcript levels of MMP-1 and MMP-9 were quantified by RT-PCR at 24 h. B) Same as *A,* except that MMP-9 activity was performed in conditioned media using gelatin loaded gels at 48 h. C) Monocytes were treated with compounds **12g, 12i** and **12l** (15 μM) for 2 h, transferred to matrigel coated Boyden chambers followed by stimulation with PMA (100 nM) for 48 h. Results presented are mean ± SD of three independent experiments.*p< 0.01 vs. no treatment. ** p< 0.01 vs PMA control.

Conclusion

In summary, the triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives were prepared, screened for anti-inflammatory activity *in vitro*. The promising compounds **12e, 12g, 12i, 12j, 12k** and **12l** dose-dependently inhibited pro-inflammatory cytokine production and COX-2 expression during monocyte differentiation. In addition, the structure versus activity data revealed that, the presence of acetamide moiety on triazole ring further increase the inhibition of pro-inflammatory cytokine production. Finally, we conclude that, compounds **12g**, **12i** and **12l** by inhibiting PMA-induced monocyte-to-macrophage differentiation, modulate the pro-inflammatory cytokines production and may have beneficial effects in mitigating inflammation associated-disorders upon further validation.

Acknowledgments

Authors are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing financial assistance through XII five year plan projects DITSF code: CSC-0204 and "Small Molecules in Lead Exploration" (SMiLE). Authors (GMR, PN and SBV) are also thankful to CSIR, India for the financial assistance in the form of Research Fellowship and contingency grant.

Notes and references

1 Citati J. Quintáns, Immunity and inflammation: the cosmic view, *Immunol. Cell Biol.,* 1994, **72**, 262.
2 G. R. Romeo, J. Lee and S. E. Shoelson, Metabolic syndrome, insulin resistance, and roles of inflammation-mechanisms and therapeutic targets, *Arterioscler Thromb. Vasc. Biol.,* 2012, **32**, 1771.
3 A. Kalogeropoulos, V. Georgiopoulou, B. Psaty, N. Rodondi, A. Smith, D. G. Harrison, Y. Liu, U. Hoffmann, D. C. Bauer, A. B. Newman, S. B. Kritchevsky, T. B. Harris and J. Butler, Inflammatory markers and incident heart failure risk in older adults: the Health ABC (Health, Aging, and Body Composition) study, *J. Am. Coll. Cardiol.*, 2010, **55**, 2129.
4 C. A. Dinarello, A clinical perspective of IL-1β as the gatekeeper of inflammation, *Eur. J. Immunol*., 2011, **41**, 1203.
5 M. Postal and S. Appenzeller, The role of Tumor Necrosis Factor-alpha (TNF-α) in the pathogenesis of systemic lupus erythematosus, *Cytokine.*, 2011, **56**, 537.
6 S. L. Deshmane, S. Kremlev, S. Amini and B. E. Sawaya, Monocyte chemoattractant protein-1 (MCP-1): an overview, *J. Interferon Cytokine Res*., 2009, **29**, 313.
7 M. F. Linton and S. Fazio, Macrophages, inflammation and atherosclerosis, *Int. J. Obes. Relat. Metab. Disord.*, 2003, **3**, 35.
8 A. C. Li and C. K. Glass, The macrophage foam cell as a target for therapeutic intervention, *Nat Med.*, 2002, **8**, 1235.
9 C. Gialeli, A.D. Theocharis and N. K. Karamanos, Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J.*, 2011, **278**, 16.
10 C. B. Jones, D. C. Sane and D. M. Herrington, Matrix metalloproteinases: a review of their structure and role in acute coronary syndrome, *Cardiovasc Res.*, 2003, **59**, 812.
11 K. J. Moore and I. Tabas, Macrophages in the pathogenesis of atherosclerosis, *Cell.*, 2011, **145**, 341.
12 S. B. Vasamsetti, S. Karnewar, A. K. Kanugula, A. T. Raj, J. M. Kumar and S. Kotamraju, Metformin inhibits monocyte-to-macrophage differentiation via AMPK mediated inhibition of STAT3 activation: Potential role in atherosclerosis, *Diabetes*, 2015, **64**, 2028.
13 (a) M. Zia-ur-Rehman, J. A. Choudary, M. R. Elsegood, H. L. Siddiqui and K. M. Khan, A facile synthesis of novel biologically active 4-hydroxy-N'-(benzylidene)-2H-benzo[e][1,2]thiazine-3-carbohydrazide 1,1-dioxides, *Eur. J. Med. Chem.*, 2009, **44**, 1311. (b) M. Ahmad, H. L. Siddiqui, M. Zia-ur-Rehman and M. Parvez, Anti-oxidant and anti-bacterial activities of novel N'-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2] benzothiazin-2(4H)-yl) acetohydrazides, *Eur. J. Med. Chem*., 2010, **45**, 698.
14 S. Sabatini, F. Gosetto, S. Serritella, G. Manfroni, O. Tabarrini, N. Iraci, J. P. Brincat, E. Carosati, M. Villarini, G. W. Kaatz and V. Cecchetti, Pyrazolo[4,3-c][1,2] benzo thiazines 5,5-dioxide:a promising new class of Staphylococcus aureus NorA efflux pump inhibitors, *J. Med. Chem.*, 2012, **55**, 3568.
15 N. Ahmad, M. Zia-ur-Rehman, H. L. Siddiqui, M. F. Ullah and M. Parvez, Microwave assisted synthesis and structure-activity relationship of 4-hydroxy-N'-[1-phenylethylidene]-2H/2-methyl-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides as anti-microbial agents, *Eur. J. Med. Chem.*, 2011, **46**, 2368.
16 (a) M. L. Barreca, G. Manfroni, P. Leyssen, J. Winquist, N. Kaushik-Basu, J. Paeshuyse, R. Krishnan, N. Iraci, S. Sabatini, O. Tabarrini, A. Basu, U. H. Danielson, J. Neyts and V. Cecchetti, Structure based discovery of pyrazolobenzothiazine derivatives as inhibitors of hepatitis C virus replication, *J. Med. Chem.*, 2013, **56**, 2270. (b) G. Manfroni, D. Manvar, M. L. Barreca, N. Kaushik-Basu, P. Leyssen, J. Paeshuyse, R. Cannalire, N. Iraci, A. Basu, M. Chudaev, C. Zamperini, E. Dreassi, S. Sabatini, O. Tabarrini, J. Neyts and V. Cecchetti, New Pyrazolo benzothiazine Derivatives as Hepatitis C Virus NS5B Polymerase Palm Site I Inhibitors, *J. Med. Chem.*, 2014, **57**, 3247.
17 (a) S. H. Kim, R. Ramu, S. W. Kwon, S. H. Lee, C. H. Kim, S. K. Kang, S. D. Rhee, M. A. Bae, S. H. Ahn, D. C. Ha, H. G. Cheon, K. Y. Kim and J. H. Ahn, Discovery of cyclicsulfonamide derivatives as 11 beta-hydroxysteroid dehydrogenase 1 inhibitors, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1065. (b) S. H. Kim, S. W. Kwon, S. Y. Chu, J. H. Lee, B. Narsaiah, C. H. Kim, S. K. Kang, N. S. Kang, S. D. Rhee, M. A. Bae, S. H. Ahn, D. C. Ha, K. Y. Kim and J. H. Ahn, Identification of cyclicsulfonamide derivatives with an acetamide group as 11β-hydroxysteroid dehydrogenase 1 inhibitors, *Chemical and Pharmaceutical Bulletin.*, 2011, **59**, 46.
18 E. S. Lazer, C. K. Miao, C. L. Cywin, R. Sorcek, H. C. Wong, Z. Meng, I. Potocki, M. Hoermann, R. J. Snow, M. A. Tschantz, T. A. Kelly, D. W. McNeil, S. J. Coutts, L. Churchill, A. G. Graham, E. David, P. M. Grob, W. Engel, H. Meier and G. Trummlitz, Effect of structural modification of enol-carboxamide-type nonsteroidal antiinflammatory drugs on COX-2/COX-1 selectivity. *J. Med. Chem.*, 1997, **40**, 980.
19 J. Wang, D. Limburg, J. Carter, G. Mbalaviele, J. Gierse and M. Vazquez, Selective inducible microsomal prostaglandin E2 synthase-1 (mPGES-1) inhibitors derived from an oxicam template, Bioorg. Med. Chem. Letters., 2010, **20**, 1604.
20 M. R. Gannarapu, S. B. Vasamsetti, N. Punna, N. K. Royya, S. R. Pamulaparthy, J. B. Nanubolu, S. Kotamraju and N. Banda, Synthesis of novel 1,2-benzothiazine 1,1-dioxide-3-ethanone oxime N-aryl acetamide ether derivatives as potent anti-inflammatory agents and inhibitors of monocyte-to-macrophage transformation, *Eur. J. Med. Chem.*, 2014, **75**, 143.
21 R. N. Dubois, S. B. Abramson, L. Crofford, R. A. Gupta, L. S. Simon, L. B. Van De Putte and P. E. Lipsky, Cyclooxygenase in biology and disease. *FASEB J*., 1998, **12**, 1063.
22 M. Daigneault, J. A. Preston, H. M. Marriott, M. K. Whyte and D. H. Dockrell, The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages, *PLoS One.*, 2010, **5**, e8668.
23 G. Corea, E. Fattorusso, V. Lanzotti, P. Di Meglio, P. Maffia, G. Grassia, A. Ialenti and A. Ianaro, Discovery and biological evaluation of the novel naturally occurring diterpene pepluanone as antiinflammatory agent, *J. Med. Chem.,* 2005, **48**, 7055.

