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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†

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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-hydroxybenzothiazine-1,1-dioxide **7** by condensation. Final compounds **8** and **12** were evaluated for their anti-inflammatory activity and ability to inhibit monocyte-to-macrophage transformation, a process pivotal during the development and progression of 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 released 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

Inflammation is a complex physiological response of multicellular organisms to various infections and tissue injuries as part of the defense mechanism of a host.[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, aging *etc.*, immune cells become hypersensitive and trigger the continuous production of diverse range of inflammatory mediators even in the absence of infections and tissue injuries.[2] This unrestrained generation of low-grade chronic inflammation is the main cause and consequence of manifestation of diverse clinical diseases.[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 disease *etc.*[4] Tumor necrosis factor (TNF)-α has both immune-regulatory and pro-inflammatory functions 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 into the sites of inflammation, a process known to be enhanced in various disorders like RA, insulin-resistance, diabetes, atherosclerosis *etc.*[6] Substantial evidence implicates that macrophages act as predominant sources of inflammation responsible for all the aforementioned disorders.[7] Particularly in vascular disorders, macrophages ingest lipid moieties that have accrued in the surroundings of the vessel wall by expressing scavenger receptors elaborating accumulation of fatty streaks which constitute the bulky atherosclerotic lesion.[8] Moreover, matrix metalloproteinases (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 inflammation-promoting events[11] and restraining monocyte recruitment into the aortic wall attenuates the risk of atherosclerosis.[12] Hence, targeting bio-active compounds

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responsible for inducing inflammation and inflammation-dependent signaling cascades are important strategies to combat those disease pathologies wherein inflammation plays a key role.

Benzothiazine derivatives are known to possess a versatile range of biological activities.[13–17] Among these, 1,2-benzothiazine-3-carboxamide-1,1-dioxide derivatives, 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 for various inflammatory and immune disorders (Fig. 1). The reported candidates disrupt the biosynthesis of 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 past few decades, synthetic modifications of 1,2-benzothiazine 1,1-dioxides have been intensively studied to enhance their bioactivity and develop better anti-inflammatory candidates.[18,19]

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 the 3rd position of 1,2-benzothiazine 1,1-dioxide and prepared acetamide derivatives by using an 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 a 1,2,3-triazole moiety (Fig. 2).

Thus, we have synthesized 1,2-benzothiazine 1,1-dioxide derivatives **8a–s** and **12a–l**, evaluated them against TNF-α, IL-1β and MCP-1 production in phorbol 12-myristate 13-acetate (PMA)-stimulated monocytes and discussed their structure *versus* activity relationship. 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 their effects on MMP-9 activity were also investigated. Finally, compounds **12g**, **12i** and **12l** imposed significant inhibition on PMA-induced monocyte-to-macrophage differentiation along with 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.

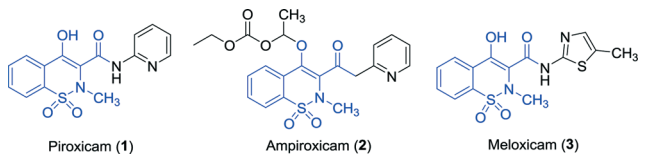


Fig. 1 Structures of known 1,2-benzothiazine based NSAIDs.

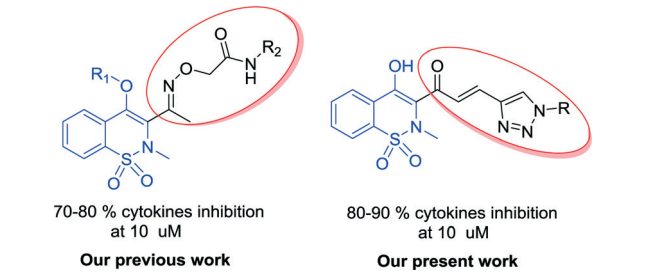


Fig. 2 Our previous and present modifications on the benzothiazine scaffold.

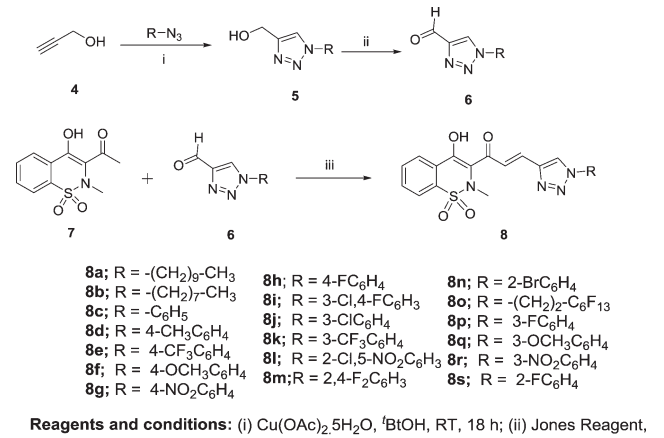
Results and discussion

Chemistry

N-Methyl-3-acetyl-4-hydroxybenzothiazine-1,1-dioxide **7** was prepared in three steps starting from the sodium salt of saccharin.[13,20] In order to construct a 1,2,3-triazole scaffold, propargyl alcohol **4** was independently reacted with alkyl azide and alkyl amide azide **9** (which were, in turn, 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*-substituted acetamide **10**, respectively. Alcohols **5** and **10** were further oxidized to aldehydes **6** and **11** by using the Jones reagent. Aldehydes **6** and **11** were reacted with compound **7** in ethanol under reflux conditions and propenone derivatives **8** and **12** were obtained, respectively. The details of the reactions are outlined in Schemes 1 and 2. The purity of all the products was determined by HPLC and pure compounds (≥95%) were used for biological experiments.

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 their



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

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

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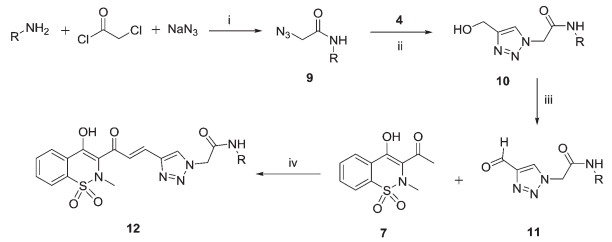

12a; R = C_6H_5 **12g;** R = 3-ClC_6H_4
12b; R = 4-FC_6H_4 **12h;** R = 2,6-$F_2C_6H_3$
12c; R = Cyclo hexyl **12i;** R = 4-$CH_3C_6H_4$
12d; R = 2-Br,4-FC_6H_3 **12j;** R = 3-FC_6H_4
12e; R = 4-$CF_3C_6H_4$ **12k;** R = 3-CF_3,4-ClC_6H_3
12f; R = 4-$OCH_3C_6H_4$ **12l;** R = 3-$CF_3C_6H_4$

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**).

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 an aliphatic alkyl chain (**8a**, R = $-(CH_2)_9-CH_3$) and a perfluoro alkyl chain (**8o**, R = $-(CH_2)_2-C_6F_{13}$) showed cytotoxicity at 10 μM, whereas the compound with an eight-carbon aliphatic chain (**8b**, R = $-(CH_2)_7CH_3$) possessed a moderate inhibitory effect on IL-1β and MCP-1 levels without affecting cell viability (Table 1). The remaining compounds however did not show significant effects on the viability of cells even at a 20 μM concentration. This clearly indicates that the anti-inflammatory activities of these compounds are not due to their cytotoxic effect on cells.

Among the 1-alkyl/aryl-substituted triazole linked benzothiazine derivatives **8a–s**, only the compound having a 2-Cl-5-NO_2 phenyl group on the 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 exhibited 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 cytokine expressions (more than 90%), whereas three

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

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

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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 better inhibition of pro-inflammatory cytokine production than 1-alkyl/aryl-substituted triazole linked benzothiazine derivatives (**8a–s**) in PMA-stimulated monocytes.

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 of their dose-dependent inhibitory effects on inflammation. For this, monocytes were pre-treated with the compounds (2.5–20 μM) for 2 h followed by incubation with PMA (100 nM) for 48 h and subsequently, TNF-α, IL-1β and MCP-1 levels in conditioned media were analyzed by ELISA. All the promising compounds dose-dependently inhibited PMA-induced TNF-α, IL-1β and MCP-1 production (Fig. 3A, B and C), 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, this triggered our interest to study the effect of these compounds on COX-2 expression. In this context, we measured COX-2 mRNA levels with PMA treatment in the presence or absence of the compounds using RT-PCR after 48 h.

All these compounds significantly inhibited the transcript levels of PMA-induced COX-2 (Fig. 3D). These results suggest that promising compounds with inhibitory effects on pro-inflammatory cytokine profiles also affect COX-2 signaling at the transcription level.

Effect of promising compounds on PMA-induced monocyte-to-macrophage differentiation. Macrophages are inflammatory phenotypes 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 differentiates monocytes into macrophages as reflected by increased cell adherence and an increase in cell size along with an increased number of cellular organelles including mitochondria.[22] In tune with this, we next examined the effect of promising acetamide-substituted triazole linked benzothiazine derivatives on the PMA-induced monocyte-to-macrophage differentiation process by treating monocytes with compounds **12e**, **12g**, **12i**, **12j**, **12k** and **12l** (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 PMA-induced cell adherence (Fig. 4A). However, **12e**, **12j** and **12k** failed to inhibit PMA-induced cell adherence even at 20 μM concentration (data not shown). Also, **12g**, **12i** and **12l** significantly inhibited the PMA-induced increase in

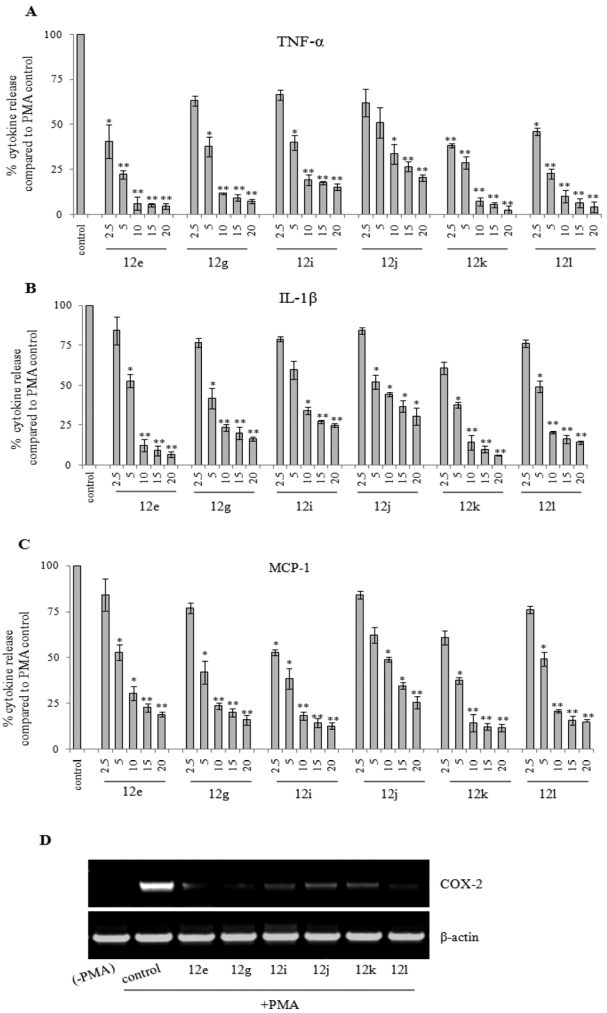

Fig. 3 Compounds **12e**, **12g**, **12i**, **12j**, **12k** and **12l** inhibit PMA-induced TNF-α, IL-1β and MCP-1 release in a dose-dependent manner. THP-1 monocytes were pretreated with indicated concentrations of compounds **12e**, **12g**, **12i**, **12j**, **12k** and **12l** for 2 h, followed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

cell size as reflected by a decrease in the mitochondrial content (Fig. 4B). In addition, these three compounds significantly inhibited the transcript levels of PMA-induced LOX-1 and CD-36 scavenger receptors (markers of monocyte differentiation) by RT-PCR (Fig. 4C). These results were further supported by lysotracker staining as shown in Fig. 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 (Fig. 4D), thereby indicating that these compounds inhibit PMA-mediated monocyte-to-macrophage

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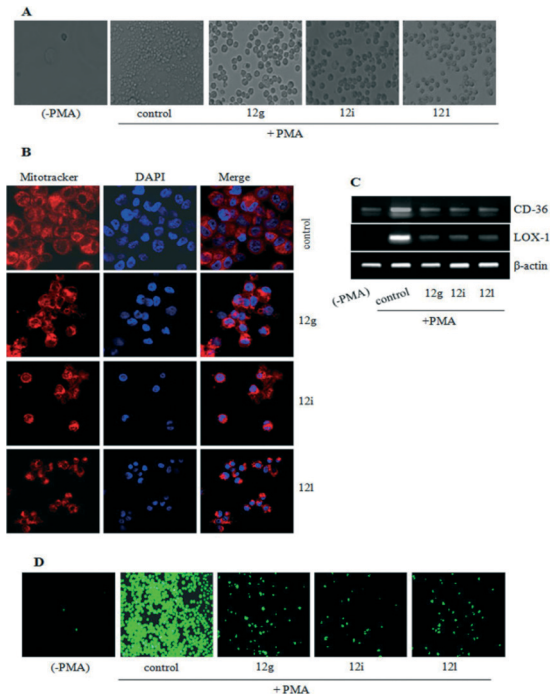

Fig. 4 Compounds **12g**, **12i** and **12l** inhibit PMA-induced monocyte-to-macrophage differentiation. THP-1 monocytes were pretreated with compounds **12g**, **12i** and **12l** (15 μM) for 2 h, followed by stimulation with PMA (100 nM). (A) Phase contrast images indicating the inhibition of PMA-induced monocyte-to-macrophage differentiation by the 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 the transcript levels of differentiation markers CD-36 and LOX-1 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.

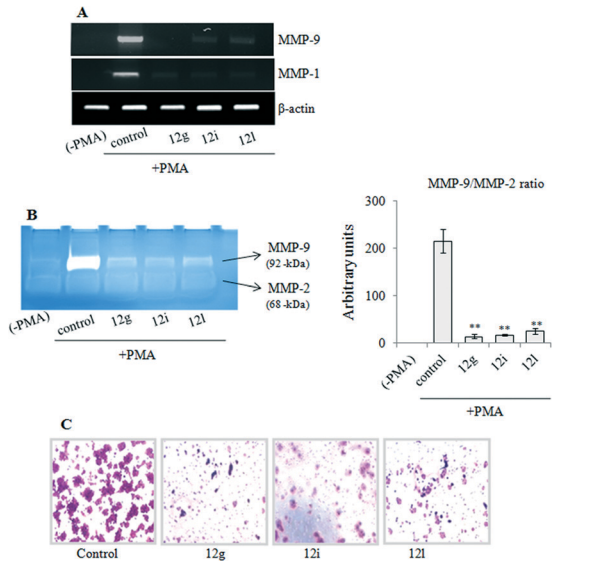

Fig. 5 Compounds **12g**, **12i** and **12l** inhibit PMA-induced MMP-9 gelatinase activity and monocyte/macrophage invasion. THP-1 monocytes were pretreated with compounds **12g**, **12i** and **12l** (15 μM) for 2 h, followed by stimulation with PMA (100 nM). (A) Transcript levels of MMP-1 and MMP-9 were quantified by RT-PCR at 24 h. (B) Same as A, except that MMP-9 activity was observed 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.

differentiation. Taken together, these results confirmed that compounds **12g**, **12i** and **12l** inhibit PMA-induced monocyte-to-macrophage differentiation, a key step during the progression of atherogenesis.

Inhibitory effect of compounds 12g, 12i and 12l on PMA-induced MMP-9 activity. Increased secretion of matrix metalloproteinases (MMPs) induced by macrophages destabilizes the plaque and enhances plaque rupture during atherosclerosis. Differentiation of human peripheral blood mononuclear cells (HPBM) to macrophages with M-CSF or differentiation of THP-1 monocytes by PMA has been shown to increase the levels of MMP-1, MMP-7, and MMP-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 PMA-induced MMP-1 and MMP-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 (Fig. 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 (Fig. 5B). However, under these conditions, MMP-2 activity was not altered (Fig. 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 MMPs promoted 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 effect of these compounds on MMP-9 activity, these compounds also greatly inhibited the PMA-induced invasive capacity of monocytes/macrophages (Fig. 5C). Taken together, these results suggest that compounds **12g**, **12i** and **12l** regulate PMA-induced alterations during monocyte differentiation.

Conclusion

In summary, triazole linked 1,2-benzothiazine 1,1-dioxido propenone derivatives were prepared and screened for their 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 the acetamide moiety on the triazole ring further increased the inhibition of pro-inflammatory cytokine production. Finally, we

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conclude that compounds **12g**, **12i** and **12l** modulate pro-inflammatory cytokine production by inhibiting PMA-induced monocyte-to-macrophage differentiation and may have beneficial effects in mitigating inflammation-associated disorders upon further validation.

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Notes and references

1 C. 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 cyclic sulfonamide 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 cyclic sulfonamide derivatives with an acetamide group as 11β-hydroxysteroid dehydrogenase 1 inhibitors, *Chem. Pharm. Bull.*, 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. Lett.*, 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.

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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.