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Short communication

Inhibition of the enzymes in the leukotriene and prostaglandin pathways in inflammation by 3-aryl isocoumarins

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ABSTRACT

The biosynthesis of leukotrienes in one of the arachidonic acid pathways and PGE_2 in the other by 5-LOX and mPGES1 respectively, play pivotal roles in augmenting inflammatory responses. PGE_2 is known to participate in cancer pathological processes as well. Isocoumarins are natural compounds with a wide range of biological activities. In this study, 3-aryl isocoumarin derivatives are synthesized and tested against 5-LOX enzyme *in vitro* and PGE_2 production in HeLa cells. Most of the compounds show high activity, and **1c** is identified as a dual inhibitor with an IC_{50} of 4.6 ± 0.26 μM and 6.3 ± 0.13 μM against 5-LOX and PGE_2 production respectively. Another compound **7f**, exhibits an IC_{50} of 12.4 ± 0.14 μM against 5-LOX. Further investigations reveal that the mechanism of action of **1c** and **7f** against 5-LOX is mixed and competitive modes of action respectively. Thunberginol (**7c**) exhibits IC_{50} of 15.8 ± 0.03 μM against PGE_2 production. **1c** and **7c** inhibit the mRNA expression of mPGES1 and COX-2. The study has identified a novel scaffold, **1c** with a dual inhibitory activity which can be further optimized to compete against Licofelone which is under clinical trials (with IC_{50} of 6.0 μM for mPGES1 & 0.2 μM for 5-LOX). To conclude, 3-aryl isocoumarin derivatives appears as promising tools to fight against inflammatory diseases as well as cancer.

1. Introduction

Inflammation is an important hallmark of many pathological diseases including rheumatoid arthritis, periodontitis, cancer etc [1]. It progresses through the prostaglandin (PG) and leukotriene (LT) pathways starting with the release of arachidonic acid (AA) from the membrane lipid bilayer [2]. Inhibitors designed to target selectively the cyclooxygenase-2 (COX-2) enzyme in an attempt to curb inflammation, resulted in serious side effects including cardiovascular complications and gastro-intestinal disturbances [3]. In this regard, 5-lipoxygenase (5-LOX) and microsomal prostaglandin E_2 synthase 1 (mPGES1), the two key downstream enzymes in the LT & PG pathways respectively, appear to be the best targets in combating inflammation. 5-LOX is a crucial non-heme iron containing enzyme in the biosynthesis of leukotrienes. It catalyzes AA to 5(*S*)-hydroperoxyeicosatetraenoic acid (5-HPETE) and further to LTA4 [4]. Microsomal prostaglandin E_2 synthase 1 (mPGES1) is the enzyme that converts PGH_2 to PGE_2. PGE_2 exerts its effect through its receptors, EP_1–EP_4, and is found to be involved in the etiology of tumorigenesis, cancer survival, Epithelial Mesenchymal Transition (EMT), regulation of vascular tone, fever and pain [5]. HIF1α which is an important trigger for tumor progression and angiogenesis is induced by the production of PGE_2 [6]. PGE_2 also participates in EMT by aiding the cancer cells to breach and cross the basement membrane thereby reaching the extracellular matrix [7]. Inhibition of its synthesis reduces Akt phosphorylation which leads to the activation of pro-apoptotic molecules and down-regulation of anti-apoptotic markers in leukaemia [8]. So, inhibition of PGE_2 synthesis is viewed as one of the approaches to limit the growth and spread of several types of cancers.

Several chemical derivatives and natural products are developed and tested as potential inhibitors against these enzymes. The identification of dual inhibitors has definite advantage over single target inhibitor in a complex scenario such as inflammation.

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Licofelone has been observed to act against multiple targets such as COX-2, mPGES1, 5-LOX and FLAP [9]. Aminothiazole [10], embelin [11], α-naphthylpirinixic acid scaffold [12] and lonazolac derivatives and indomethacin derivatives [13], have been shown to suppress the activity of both 5-LOX and mPGES1 effectively. Despite all these reported compounds, none of them could be developed as a drug due to their pharmacokinetic parameters. Hence, identifying a potential inhibitor that could act against multiple targets is still the need of the hour.

Isocoumarins are isomers of coumarins (1,2-benzopyrone) [14,15]. They are present widely throughout the plant kingdom, including bergenin (*Bergenia ciliate*, *Shorea leprosula*) [15], thunberginol (*Hydrangea macrophylla*) and cajanolactone (*Cajanus cajan*) [16,17]. The anti-inflammatory, anti-microbial and anti-cancer effects of these naturally occurring compounds have attracted the medicinal chemists to develop several structural derivatives with the aim of increasing their potencies [17].

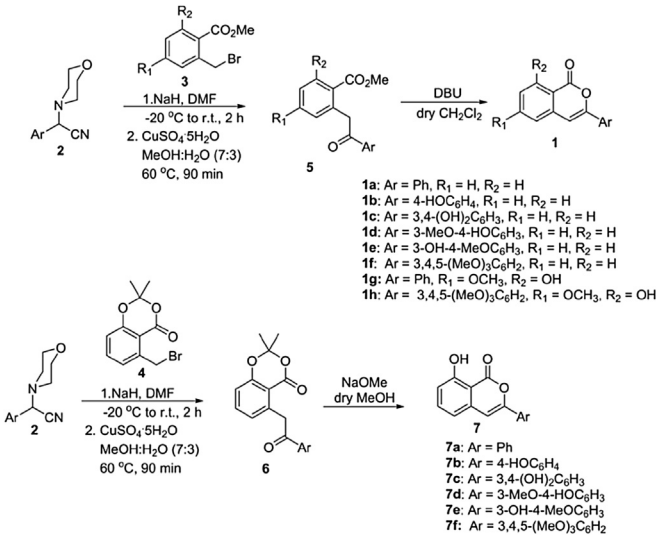
Isocoumarins are known to act as anti-inflammatory molecules and they also possess radical scavenging ability [18]. Paepalantine, an isocoumarin obtained from *Paepalanthus bromelioidesis* is demonstrated to have anti-inflammatory activity against colitis when tested in rat models [19]. Several fluorinated derivatives are found to exhibit anti-inflammatory activity [20]. Though significant anti-inflammatory activity of isocoumarins has been documented, no reports are available that elucidate their mechanism of action and their ability to act as dual inhibitors.

Isocoumarins have also been reported to exhibit anti-cancer activity. Cytogenin, an isocoumarin isolated from *Streptoverticillium eurocidium* exhibits significant anti-cancer activity in mice models of Ehrlich carcinoma [21]. Synthetically prepared ones (NM-3) when tested with Cytogenin resulted in synergistic inhibition of angiogenesis in mouse models [22]. Fluorinated and 3,4 dihydroisocoumarins has been found to prevent metastasis in MCF-7 breast cancer cell line [23].

In this paper, the inhibitory effect of chemically synthesized 3-aryl isocoumarin derivatives against the enzymes in the leukotriene and prostaglandin pathways are investigated including their mode of action.

2. Chemistry

All the screened 3-aryl Isocoumarins, **1** and **7**, were synthesized using a new method developed by us earlier [17], which involved use of α-aryl aminonitriles **2** as acyl anion equivalents. The α-aryl aminonitriles, **2,** on alkylation with bromides, **3** and **4,** followed by hydrolysis afforded the corresponding aryl ketones **5** and **6** respectively. These aryl ketones, on base promoted intramolecular cyclization, furnished 3-aryl Isocoumarins **1** and **7** respectively (Scheme 1). The route has been generalized and applied to the synthesis of thunberginol A (**7c**) and other 3-aryl isocoumarins used in the present study.



Scheme 1. Synthesis of 3-aryl isocoumarins.

3. Result and discussion

3.1. 5-LOX enzyme activity

The 5-LOX enzyme activity was determined in a cell free assay by measuring the conversion of AA to the product, 5(*S*)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE) which was measured in terms of OD at λ_{236} [24,25]. Most of the compounds (at 50 μM) exhibited 5-LOX inhibitory activity (Table 1). Compound **1c** was found to be the best with an IC_{50} of 4.6 ± 0.26 μM followed by **7f** with an IC_{50} of 12.4 ± 0.14 μM. Other compounds such as **1f** and **1d** in the series exhibited IC_{50} of 20.9 ± 0.03 μM and 22.8 ± 0.07 μM respectively. Compounds that had electron donating groups attached to the C3-aryl substitution (hydroxyl in **1c** and methoxy in **7f**) seemed to be responsible for the inhibitory activity. It can be seen from Table 1 that, compound **1c** having hydroxyl groups at R_1 and R_2 positions exhibits 84.1% inhibition. The presence of two hydroxyl groups in the ortho position might have resulted in additional resonance stabilization and *o*-quinone formation which is responsible for the high activity [26–28]. In contrast, one hydroxyl group at the R_4 position in compound **7c** lowers the activity by half. Compound **1d** substituted with one methoxy and one hydroxyl group at the R_1 and R_2 positions respectively shows 78.8% inhibition probably due to the formation of a methylene dioxy ring by these two groups [29,30]. Inhibition activity decreased drastically to almost by half (35.6%) when R4 is substituted with hydroxyl group, as seen in **7d**. In compound **7f** (80.3%), incorporation of hydroxyl at R_4 decreases activity marginally (**1f** = 72.0%). However, presence of one methoxy group at R_5 position in **1h** decreases inhibition to half (36.2%).

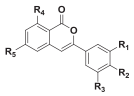
1d also exhibited appreciable inhibitory activity (78.8% at 50 μM concentration) because of the presence of OH and OCH_3 groups. But one of the naturally occurring plant isocoumarin synthesized here in the laboratory, thunberginol (**7c**), showed moderate 5-LOX inhibition (only 40.9% at 50 μM). Commercial drug, Zileuton and the known dual inhibitor Licofelone were tested as positive controls which exhibited an IC_{50} of ~1.0 μM and 0.5 μM respectively [31,32].

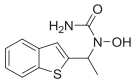
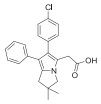
Thus, one can conclude that, for the first set of six compounds (Table 1) having H in the R_4 position, it is important to have OH at R_2 position and for the remaining set of eight compounds having OH in the R_4 position, presence of OMe group at R_1, R_2 and R_3 is necessary but not at R_5 position for high 5-LOX inhibitory activity.

3.2. Kinetics of 5-LOX inhibition

The mechanism of action of these two most active isocoumarins (**1c** and **7f**) was determined by measuring the initial rate of product formation for various concentrations of the substrate (AA) at two different concentrations of these inhibitors [33]. Lineweaver-Burke plot (Fig. 1a) indicates that in the presence of **1c,** *V*max (0.83 ± 0.06, 0.93 ± 0.11 and 0.64 ± 0.1 nmol/min) and Km values (2.2, 1.38 and 1.17 μM) are different for different concentrations of it (0, 10 and 50 μM) indicating mixed or non con-competitive inhibition. If the inhibitor has same affinity for both the enzyme and enzyme-substrate complex then it can be regarded as non-competitive

Table 1
Inhibition of 5-LOX & mPGES1, antioxidant activity & cellular toxicity of 3-aryl isocoumarins.



Compounds (50 μM conc.)	R_1	R_2	R_3	R_4	R_5	% 5-Lox inhibition ± SD[a]	% PGE2 inhibition ± SD[b]	% Scavenging of DPPH ± SD	MTT assay (% cell proliferation)
1a	H	H	H	H	H	50.0 ± 8.6	75 ± 5.6	14.0 ± 0.6	76.7 ± 3.2
1b	H	OH	H	H	H	34.1 ± 1.1	65 ± 7.2	17.0 ± 0.3	81.4 ± 2.0
1c	OH	OH	H	H	H	84.1 ± 3.2	78 ± 2.9	53.2 ± 3.4	78.7 ± 8.1
1d	OCH_3	OH	H	H	H	78.8 ± 2.1	73 ± 5.7	47.8 ± 1.4	65.0 ± 7.3
1e	OH	OCH_3	H	H	H	65.9 ± 5.3	18 ± 7.9	20.0 ± 0.6	70.5 ± 2.8
1f	OCH_3	OCH_3	OCH_3	H	H	72.0 ± 1.1	73 ± 2.6	15.8 ± 2.5	75.7 ± 5.6
7a	H	H	H	OH	H	55.3 ± 1.1	60 ± 7.42	13.4 ± 0.3	75.6 ± 1.8
7b	H	OH	H	OH	H	36.4 ± 6.4	74 ± 3.5	12.6 ± 0.3	70.6 ± 2.0
7c	OH	OH	H	OH	H	40.9 ± 10.7	74 ± 3.7	49.0 ± 3.1	68.7 ± 6.5
7d	OCH_3	OH	H	OH	H	35.6 ± 5.3	75 ± 9.14	36.2 ± 0.8	79.8 ± 3.2
7e	OH	OCH_3	H	OH	H	59.1 ± 6.4	44 ± 9.9	17.8 ± 0.3	77.4 ± 4.9
7f	OCH_3	OCH_3	OCH_3	OH	H	80.3 ± 4.3	68 ± 6.6	12.4 ± 0.6	80.2 ± 1.1
1g	H	H	H	OH	OCH_3	49.2 ± 1.1	54 ± 5.29	16.6 ± 0.3	78.1 ± 3.7
1h	OCH_3	OCH_3	OCH_3	OH	OCH_3	36.2 ± 12.8	14 ± 3.37	15.4 ± 0.3	60.5 ± 9.0
Zileuton						97.0 ± 4.3	50.0 ± 4.8	33.6 ± 4.5	78.0 ± 2.2
Licofelone						71.0 ± 3.3	48.0 ± 9.1[c]	–	85.0 ± 4.1
Ascorbic acid						–	–	82.2 ± 0.3	–

[a] Cell free assay at 50 μM compounds concentration.
[b] Cell based assay at 50 μM compounds concentration in HeLa cells.
[c] Licofelone tested for mPGES1 inhibition at 5 μM concentration.

inhibition and if the affinities are different then it is termed as a mixed inhibition. Lineweaver-Burke plot for the compound **7f** (Fig. 1b**)** indicates that its mechanism of inhibition is competitive since Vmax values remain constant (i.e 0.87 ± 0.04 nmol/min for control, and 0.83 ± 0.02, 0.93 ± 0.1 nmol/min at 10 and 30 μM of **7f** respectively) while Km values increases with increasing concentration of the inhibitor (0.09, 1.2 and 1.45 μM for 0, 10 and 30 μM of **7f** respectively).

3.3. Pseudoperoxidase activity

5-LOX enzyme in its activated ferric form consumes lipid peroxides. In the presence of redox inhibitor, ferric ion of the activated enzyme gets converted to ferrous form (inactive enzyme). The ferrous iron consumes lipid peroxides to become ferric thereby reactivating the enzyme. This is measured in terms of the decrease in the absorbance at 234 nm wavelength due to the consumption of 13(*S*)-hydroperoxyoctadecadienoic acid (13(*S*) HpODE) [34]. The pseudoperoxidase activity of 5-LOX in the presence of the inhibitors, **1c** and **7f** and standard drug Zilueton are determined (Fig. 2). In the presence of compound **1c,** absorbance decreased by ~35% (indicating considerable degradation of the hydroperoxide product) suggesting that the inhibition follows the redox mechanism. Positive control Zilueton which is also a redox inhibitor consumes ~50% of the hydroperoxide substrate. Whereas with compound **7f,** no degradation of the hydroperoxide product is observed (Fig. 2) indicating a non-redox inhibitory mechanism These results also corroborate with the findings from L-B plot.

3.4. Radical scavenging activity

Radical scavenging activity of all the compounds is also determined with ascorbic acid as the positive control [35]. Most of the compounds possess poor antioxidant activity (Table 1). **1c** shows the highest radical scavenging activity (53%) which also supports the result that the mechanism of inhibition of 5-LOX by it is through the disruption of redox cycle and hence may be non-competitive or mixed inhibition. Compound **7f** possesses very low antioxidant activity (12%) which suggests that inhibition of 5-LOX by it is through non-redox process, may be competitive type, which is again supported from the two assays described above.

3.5. Inhibition of PGE_2 production

Over expression of COX-2 and mPGES1 results in the increased levels of PGE_2 which is observed in cervical (HeLa) [2], non small cell lung (A549) [2] and prostate (DU145) cancers [36]. Hence HeLa cells are selected in this study to test the effectiveness of isocoumarins at a concentration of 50 μM. The sequential action of COX and PGES leads to the production of PGE_2, which is released into the cell culture supernatant and is monitored using an ELISA kit. Most of the compounds tested at 50 μM showed varying levels of inhibition with **1c** being the best of all (78%) (Table 1). Licofelone, has an IC_{50} of 6 μM ± 0.17. **7f** which is seen to inhibit 5-LOX efficiently (80%) exhibits 68% inhibitory potential towards the production of PGE_2. Bio-mimetically prepared natural product thunberginol (**7c**), is observed to reduce the PGE_2 levels by 74%.

The presence of hydroxyl group in **7c** at the R_4 position decreases the inhibitory potential marginally when compared to **1c**. As discussed earlier, the presence of two hydroxyl groups may

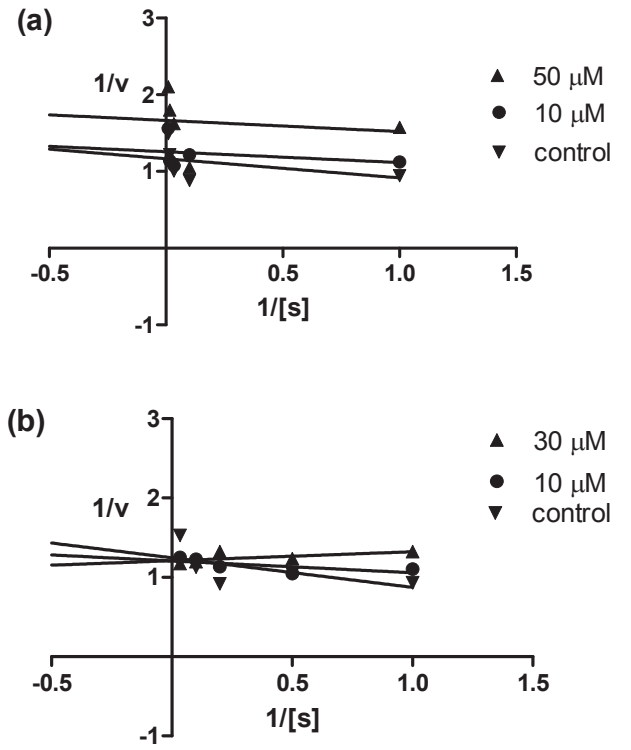


Fig. 1. (a) Lineweaver-Burk plot for compounds **1c** (at 10 and 50 μM concentrations) and (b) **7f** tested at concentrations (10 and 30 μM). [S] = Substrate (AA) concentration, V = Velocity of reaction. Control (without inhibitor).

stabilize the compound resulting in improved activity against mPGES1. Activity of **1d** (73%) is comparable to that of **7d** (75%). The presence of OMe and OH substituents in these compounds could result in the formation of a methylene dioxy ring which may be responsible for the efficient inhibition of PGE_2 production [29]. The IC_{50} values for both these compounds when tested with HeLa cells were 6.3 ± 0.13 and 15.84 ± 0.03 μM respectively. The IC_{50} values of other compounds which exhibited more than 70% inhibition were also determined and found to be 9.3 ± 0.07 μM (**1f**), 9.5 ± 0.11 μM (**1a**), 7.1 ± 0.20 μM (**7b**), 9.7 ± 0.10 μM (**1e**) and 11.3 ± 0.03 μM (**7e**). HeLa cells express both the pro-inflammatory enzymes, COX-2 and mPGES1, and hence if these compounds are tested with pure enzymes, one would be able to determine the exact IC_{50}.

The structure activity relation for the first set of six compounds (Table 1) having H in the R_4 position indicates that, the presence of OH in R_1 and R_2 is beneficiary. For the remaining set of eight compounds which have OH in the R_4 position, the presence of OH in the R_2 position is necessary for high activity.

3.6. Inhibition of PGE_2 production upon addition of arachidonic acid

AA is the direct substrate for COX enzymes [37]. To determine the effect of exogenously added substrate, AA, the HeLa cells were challenged with it as per a previously reported protocol [38]. There is a drastic increase in the levels of PGE_2 produced in AA (15 μM) supplemented cells when compared to the cells which did not receive it. Addition of AA externally is will be taken up by COX enzyme resulting in excess turnover of PGH_2 which is used as the substrate by the mPGES1 enzyme resulting in increased production of PGE_2.

The percentage inhibition of PGE_2 which is released into the cell culture supernatant by those treated with **1c** & **7c** (50 μM) for 15 min is 76 & 75% respectively. However, when the cells were stimulated with 15 μM AA for 15 min, following the compound treatment, the percentage inhibition dropped down to 44 & 17% respectively. The amount of PGE_2 produced is normalized by subtracting the amount produced by AA treatment alone (no compound treatment). Similarly, for Licofelone (5 μM) the percentage inhibition in the absence and in the presence of AA is 81% and 54.7% respectively. Even though exogenously added AA is shared by both, COX-1 as well as COX-2 isoenzymes, it is predominantly taken up by the latter as its expression is considerably induced by TNFα [37]. Moreover, we do not observe any change in the mRNA expression of COX-1 in **1c** and **7c** treated cells (Fig. 4a). Hence, the reduction of PGE_2 levels observed would be mainly due to the inhibition of COX-2 and mPGES1. A specific assay to determine the individual activity of mPGES1 and COX-2 would give the exact percentage of inhibition of each of these enzymes by these compounds. (see Fig. 3)

3.7. Inhibition of mRNA expression of PG enzymes

The reduction in PGE_2 levels could be due to, either the inhibition of mPGES1 alone or the upstream enzymes namely COX-2/COX-1. Previous studies have shown that compounds that target FLAP such as MK886 is able to inhibit mPGES1 also, as both of them belongs to the same MAPEG family [39]. Hence real time PCR analysis was carried out to determine the mRNA expression of

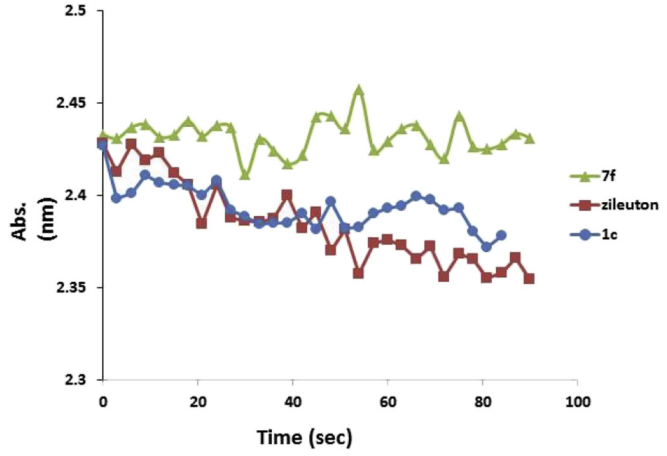


Fig. 2. Pseudoperoxidase activity plot (shown as absorbance) for the consumption of 13(*S*)-HpODE by compounds **1c, 7f** and Zileuton.

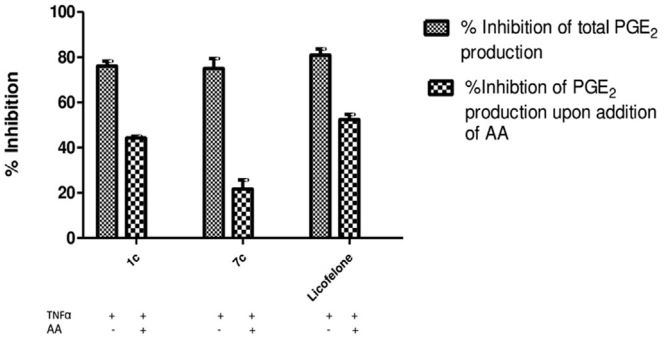


Fig. 3. Effect of compounds on PGE_2 production in the presence of exogenous AA. HeLa cells were treated with 50 ng/ml of TNFα to induce the production of COX-2 and mPGES1, followed by addition of 50 μM of compounds for 15 min and then AA (15 μM) for another 15 min. The amount of PGE_2 in the cell culture supernatant is estimated using EIA kit. The data is normalized with that of TNFα and AA induced cells without the treatment of compounds.

these inflammatory enzymes. Licofelone, **1c** and **7c** inhibit the mRNA expression of COX-2 and mPGES1 (Fig. 4a) while that of COX-1 is not altered by any of them. However, the extent of inhibition of mPGES1 by **1c** is statistically significant when compared to that of COX-2. Hence, it is presumed that these isocoumarins could be acting both by inhibiting the expression of these enzymes as well as by inhibiting the activity of these enzymes at varying levels. Moderate inhibition of mRNA expression of COX-2 and no interference with COX-1 also indicates reasonable selectivity for mPGES1 by these 3-aryl isocoumarins.

The results are normalized to that of the untreated cells and β-Actin (****$p < 0.0001$). Statistical significance was calculated by 2-way ANOVA method.

3.8. Inhibition of mRNA expression of inflammatory cytokines

To elucidate the mechanism further, real time PCR analysis was carried out for various inflammatory cytokines and transcription factors (TF). A significant increase is observed in the expression of IFNγ in both **1c** (5.6 fold) and **7c** (9.6 fold) treated HeLa cells when compared to the untreated cells (Fig. 4b). Though IFNγ is predominantly identified as pro-inflammatory cytokine, its anti-inflammatory effects are also reported [40]. IFNγ has been shown to protect arthritis induced by streptococcus in rats by acting as anti-inflammatory molecule [41]. It functions mainly by inhibiting the other pro-inflammatory cytokines and activate cytokine antagonists [40]. IFNγ also triggers apoptotic pathway in leukocytes [42]. The expressions of IL-6, TGFβ3 and NFκb are found to be approximately 1 fold higher in **1c** and **7c** treated cells in comparison to the untreated control cells. NFκb have contradicting roles of being both apoptotic and anti-apoptotic in nature [43]. TGF family of proteins, are mainly reported to induce the cells to undergo apoptosis [44]. IL6 is predominantly a pro-inflammatory cytokine [45]. From these results it appears that the anti-inflammatory effects of these two isocoumarins are routed mainly through IFNγ and TGFβ pathways. Further studies to detect the apoptotic status of the compounds treated cells could reveal the exact function of these above said factors.

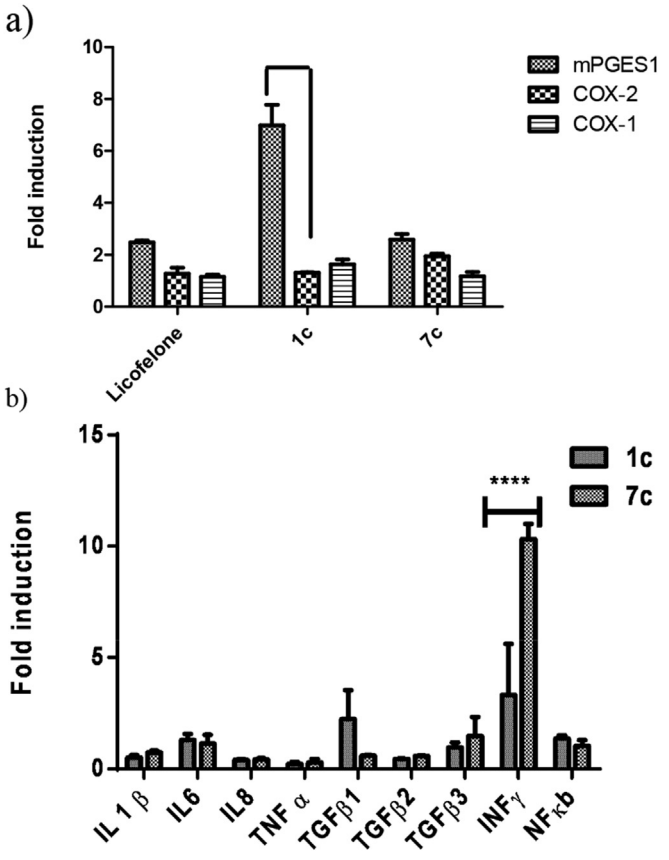


Fig. 4. a) Real time PCR analysis showing the fold decrease in the mRNA expression of mPGES1, COX-2 and COX-1 treated with Licofelone (5 μM), **1c** and **7c** (50 μM). b) Real Time PCR analysis showing mRNA expression of pro and anti-inflammatory cytokines in HeLa cells treated with **1c** and **7c** at 50 μM concentration.

4. Conclusion

To fight against multifaceted diseases such as inflammation or cancer, targeting more than one enzyme becomes a superior strategy than designing an inhibitor selectively for one target. Such dual or multi-targeted inhibitors with good pharmacokinetic properties could be a valuable aid in the management of certain cancers. Many natural products that are anti-inflammatory such as curcumin, resveratrol, garcinol, epigallocatechingallate (EGCG) are all identified to be acting on multiple targets [46]. Here in this study, isocoumarins which are also natural product derivatives have been synthesized in our lab and tested for multi-targeted actions against the enzymes involved in the leukotriene and prostaglandin synthesis.

Zileuton is the only marketed drug against 5-LOX and hence there is a need for finding out more inhibitors against this enzyme to treat anti-inflammatory disorders including asthma, allergy etc [47]. Here in this present work, **1c** and **7f** are identified as potent inhibitors of the 5-LOX enzyme. Also, compound **7f** has been identified as a promising inhibitor with non-redox mechanism whereas **1c** exhibits redox type of behaviour. In addition, compound **1c** is found to be superior with better antioxidant activity (53%) which supported the above observation of redox mechanism of action by it.

The *in vivo* efficacy of many potential drug candidates is not as appreciable as much as the *in vitro* activity. MK886, for instance, has an IC_{50} of 1.6 μM *in vitro* against mPGES1 but could not demonstrate similar potential when tested in whole blood (~20% inhibition for 100 μM) and gingival fibroblasts (no significant reduction at 2–4 μM) [48–50]. In contrast, in human cell lines such as Caco-2 and HT-29, PGE_2 production is found to be increased when tested at 10 μM concentration [51]. Licofelone on the other hand is a potent suppressor of PGE2 production *in vivo* (EC_{50} 0.1 μM) in A549 cells. The problem with this drug is that it simultaneously inhibits COX-1 (IC_{50} 0.8 μM). [52] Inhibiting the latter might shunt the normal production of other prostaglandins such as PGI_2 resulting in the disturbances of physiological processes.

From these observations it is heartening to note that, **1c** and **7c**, with their potential to suppress the PGE_2 production in the cancer cell line, HeLa, appears to be very promising for further improvement. Testing their efficacies in other *in vivo* models could help in authenticating the proficiency of these compounds.

To summarise, a few 3-aryl isocoumarins synthesized and tested here exhibit excellent inhibition of 5-LOX *in vitro* and PGE_2 *in vivo* in HeLa cells. Current study has identified a novel scaffold (**1c**) with dihydroxyl groups at C3-aryl substitution as a dual inhibitor of both 5-LOX and PGE_2 which could be further optimized for reducing its IC_{50} value. The molecular mechanism of action could be due to the activation of several anti-inflammatory and apoptotic cytokines and transcription factors. Structural optimization and further studies are warranted to decrease their IC_{50} to nanomolar range before they could be considered for treatment against inflammation and cancer.

Author contributions

SS carried out the 5-LOX experiments and antioxidant studies; MR performed the PGE_2 analysis and real time PCR experiments and KS synthesized the 3-aryl isocoumarin derivatives. MD and ISA designed the experiments, analyzed the data and corrected the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.08.066.

Abbreviations

AA	arachidonic acid
COX-2	cyclooxygenase-2
EGCG	epigallocatechingallate
EP receptor	prostaglandin E2 receptor
FLAP	5-LOX activating protein
HIF1α	hypoxia inducible factor 1 alpha
5-HPETE	5(*S*) hydroperoxyeicosatetraenoic acid
13(*S*) HpODE	13(*S*)-hydroperoxyoctadecadienoic acid
IFNγ	interferon gamma
IL6	interleukin 6
5-LOX	5-lipoxygenase
LT	leukotriene
mPGES1	microsomal prostaglandin E2 synthase
NF-kB	nuclear factor-κ-light chain enhancer of activated B cells
PG	prostaglandin
TGFβ	transforming growth factor beta

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