Recent Development in the Field of Dual COX / 5-LOX Inhibitors

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Abstract: Cyclooxygenases and lipoxygenase are key enzymes in the arachidonic acid metabolism. Dual inhibitors are drugs able to block both the COX and the 5-LOX metabolic pathways. Compared to COX or LOX pathways single inhibitors, dual inhibitors present at least two major advantages. First, dual inhibitors, by acting on the two major arachidonic acid metabolic pathways, possess a wide range of anti-inflammatory activities. Secondly, dual inhibitors appear to be almost exempt from gastric toxicity, which is the most troublesome side effect of non-selective COX inhibitors.

Keywords: Cancer, cyclooxygenases, dual inhibitors, inflammation, leukotrienes.

Inflammation is mediated by several families of mediators. Among these, the eicosanoids, a family of lipid mediators produced through the arachidonic acid (AA) metabolism represents one of the most investigated family [1]. Those mediators are formed mainly via the cyclooxygenase (COX) and the lipoxygenase (LOX) pathways. The COX pathway leads to the formation of the prostanoids (prostaglandins, prostacyclin and thromboxane) while the LOX pathway carries out the production of leukotrienes (LTs). Non-steroidal, anti-inflammatory drugs (NSAIDs) inhibit COX pathway [2]. The non-selective inhibition of the two isoforms of COX is thought to be responsible for the gastric sides effects associated with the use of NSAIDs. Recently, the research has focused on the development of COX-2 selective inhibitors, which are demonstrated to possess a significantly enhanced safety compared to non-selective COX inhibitors. Nevertheless, those gastric side effects are also induced by leukotrienes. Indeed, it appears that leukotrienes significantly contribute to gastric injury. Furthermore, these mediators are the major AA derivatives synthesized in the gastric mucosa under COX inhibition [3]. This aspect has encouraged the search for dual inhibitor both of COX-2 and 5-LOX, which should display an enhanced anti-inflammatory potency with less side effects. On the other hand, both cyclooxygenase and lipoxygenase products (prostanoids and leukotrienes) are also implicated in the cancer progression and may represent important targets for cancer chemoprevention or treatment [4, 5].

I. THE CYCLOOXYGENASE PATHWAY

I.1 The Metabolism of Arachidonic Acid

Cyclooxygenases catalyse the two step conversion of AA into PGH_2 (Fig. (**1**)). This transformation starts with the formation of the endoperoxide PGG_2 (prostaglandin G_2; cyclooxygenase activity), which is secondly metabolised into PGH_2 (prostaglandin H_2; peroxidase activity). Following this two steps conversion, PGH_2 is the substrate for different isomerases to form the prostanoids (PGD_2, PGE_2 and $PGF_{2\alpha}$), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) that is unstable and rapidly degraded to thromboxane B_2 (TXB_2).

I.2 Cyclooxygenase Enzymes

COX enzymes exist in two distinct isoforms. The first one, called COX-1, is a constitutive enzyme, which is responsible for the homeostatic production of prostaglandin, prostacyclin and thromboxane. The second one, called COX-2, is an inducible enzyme, which is manly expressed under pathological conditions such as inflammation or cancerogenesis. The main structural difference between these two enzymes is the presence of a larger active site in the COX-2 enzyme structure [6].

I.3 Implication of Prostanoids

Prostaglandins display effects on different systems such as cardiovascular, smooth muscle, kidney and gastric mucosa. In the cardiovascular system, PGs are potent vasodilatatory agents while TXA_2 exerts vasoconstrictor effects [7]. PGs also contract uterine smooth muscle and longitudinal smooth muscle of the gastrointestinal tract [8]. PGs and, especially, PGI_2 diminish pepsin secretion and enhance mucus production by the gastric mucosa and are therefore considered as key mediators in the gastric protection [9].

II.THE LIPOXYGENASE

The term "lipoxygenase" currently designates 5-lipoxygenase (5-LOX), 8-lipoxygenase (8-LOX), 12-lipoxygenase (12-LOX) and 15-lipoxygenase (15-LOX). These four enzymes catalyse the same reaction consisting in the regio- and enantioselective insertions of oxygen into different positions of arachidonic acid [10]. In this review, we will focus on the 5-LOX, which is the most common and important LOX enzyme.

II.1 The Lipoxygenase Pathway

In this pathway, AA is first converted into 5-HPETE (5-hydroperoxyeicosatetraenoic acid) [11] and then into

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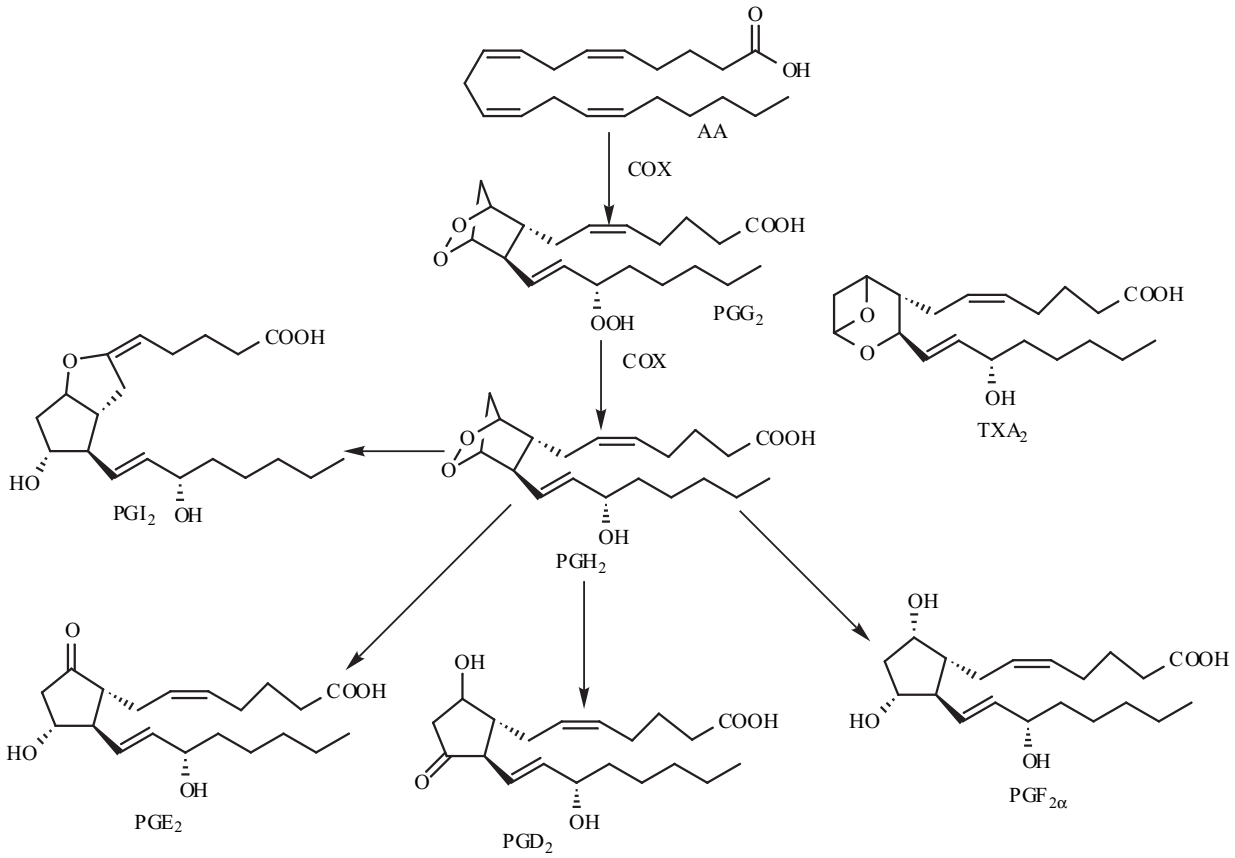

Fig. (1). Cyclooxygenase pathway, formation of prostanoids and thromboxane.

leukotriene A_4 (LTA_4) by 5-LOX (Fig. (**2**)). This leukotriene can both be the substrate of the LTA_4 hydrolase to form leukotriene B_4 (LTB_4) and be metabolised by the glutathione-transferase enzyme (LTC_4 synthase) to provide the peptido-lipid leukotriene C_4 (LTC_4) [12]. This peptido-leukotriene can be degraded by several specific enzymes in order to form , in turn, LTD_4 and LTE_4. LTC_4, LTD_4 and LTE_4 form the slow reacting substance of anaphylaxis (SRS-A).

II.2 Implications of Leukotrienes

LTB_4 promotes neutrophil chemotaxis and adhesion to vascular endothelium through specific integrin [1]. This leukotriene can also induce inflammatory reactions and acute airway obstruction [13]. LTC_4 and LTD_4 are potent bronchoconstrictors and also induce airway mucus secretion [14], contract the rat stomach and colon and at high concentration provoke acid secretion [15]. In cancer development, leukotrienes have been shown to stimulate cellular proliferation either directly or as intermediates in the mitogenic pathway mediated by growth factors [16]. Indeed overproduction of leukotrienes has been demonstrated in different types of cancer such as human prostate or colon cancer [17].

III. COX AND LOX DUAL INHIBITORS

Several approaches have been followed in order to develop dual COX / 5-LOX inhibitors. A first approach was the modification of older COX inhibitors by adding LOX pharmacophore with the aim to combine both inhibitory activities. Another approach was the screening of known compound for a lead, which presents the dual activity followed by optimisation.

III.1 Derivation of COX Inhibitors

The coupling of a 5-LOX inhibitor pharmacophore, such as hydroxamic acid or *N*-hydroxyurea moiety, with classical COX inhibitors, was widely investigated in order to obtain dual inhibitors. The derivation of classical COX inhibitors was achieved among others from indomethacin and fenamate derivatives.

III.1.1 Indomethacin Derivatives

Indomethacine (1) possesses a carboxylic acid moiety. This function can easily be substituted with an hydroxamic acid [18] or by *N*-hydroxyurea [19] (Fig. (**3**)). For example, compound 2 was 6.8 times more selective for the COX than for the 5-LOX when evaluated in an intact rat basophilic leukaemia (RBL-1) cell line for the inhibition of COX and 5-LOX (IC_{50} COX= 1.1 μM; IC_{50} 5-LOX= 7.5 μM). Nevertheless, this inhibitor can be classified as a prodrug of indomethacine due to the rapid *in vivo* hydrolysis of the hydroxamate to regenerate indomethacine [20]. Compound 3, an internal *N*-hydroxyurea, was reported to inhibit 5-LOX in a broken cells assay (IC_{50}= 0.34μM) and COX in Sf9 insect cells expressing recombinant human COX (COX-1= 9% at 10 μM and COX-2= 48% at 10μM) [19]. Compound

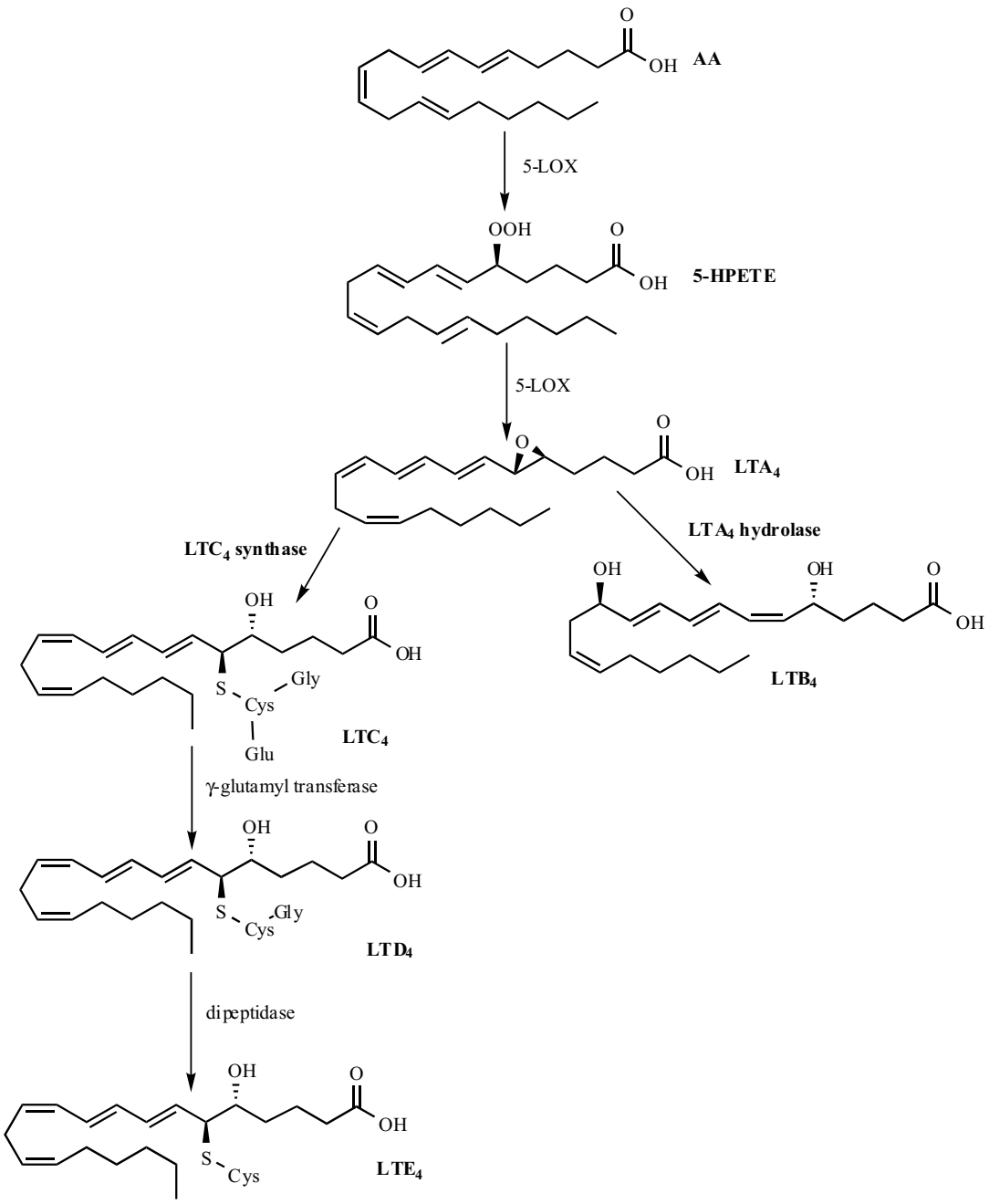

Fig. (2). The 5-lipoxygenase pathway; biosynthesis of leukotrienes.

4, a terminal *N*-hydroxyurea, is also inhibitor of both COX and LOX (IC_{50} 5-LOX= 0.2 μM; COX-1= 64% at 30μM; COX-2 88% at 30μM). Those two compounds possess a good chemical stability and do not undergo hydrolysis to form indomethacine.

III.1.2 Fenamates Derivatives

For this family, the replacement of the carboxylic acid function with an heterocycle not only retained the COX inhibitory activity of the parent compound but also introduced 5-LOX inhibitory activity. For example, the replacement of the carboxylic acid moiety of flufenamic acid (compound 5) by substituted 1,3,4-oxadiazole or by substituted 1,3,4-thiadiazole led to dual COX / LOX inhibitors (Fig. (**3**)) [21]. Those analogues were evaluated in an intact rat basophilic leukaemia (RBL-1) cell line for inhibition of the production of LTB_4 (5-LOX activity) and production of $PGF_{2\alpha}$ (COX activity). Table 1 shows us that compounds 6 and 8 are well balanced dual inhibitor.

III.2 Combination of Pharmacophore

This second approach has led to tepoxalin (compound 10) [22] and compound 11 [19]. Those two compounds present the diaryl-substituted pyrazole structure of the COX-2 selective inhibitor celecoxib (12) (Fig. (**5**)). Tepoxalin possesses an hydroxamic acid and compound 11 the 4-(3-fluoro-5-oxy)phenyl-4-methoxytetrahydropyran group responsible for the 5-LOX inhibitory potency of ZD-2138, a

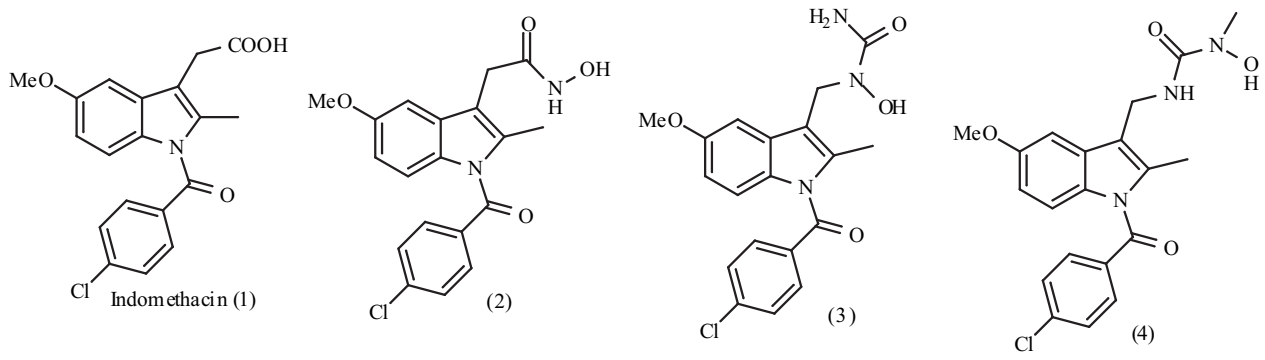

Fig. (3). Indomethacin and derivatives

5-LOX inhibitor developed by Zeneca. Tepoxalin, which has undergone clinical evaluation for the treatment of psoriasis [23], was able to inhibit the production of 5-HETE (LOX activity) and COX activity in intact rat basophilic leukaemia cell line with IC_{50} of 0.15 μM and 2.85 μM respectively. On the other hand, compound 11 was able to selectively inhibit the COX-2 over the COX-1 (IC_{50} = 0.05μM and >10 μM respectively) and strongly the 5-LOX (IC_{50} = 0.003μM).

this class are R-380 (13), CI-1004 (14) and NE-11740 (tebufelone, compound 15) Fig. (**6**).

R-380 inhibits PGE_2 production with an IC_{50} of 0.003 μM in stimulated rat synovial cells and significantly decreases the LTB_4 production by human neutrophil when stimulated by calcium ionophore [24]. CI-1004 is presented as a dual inhibitor when evaluated in rat basophilic leukaemia (RBL-1) cell line with IC_{50} of 0.39 μM and 0.77 μM towards the COX and 5-LOX respectively [25]. Finally,

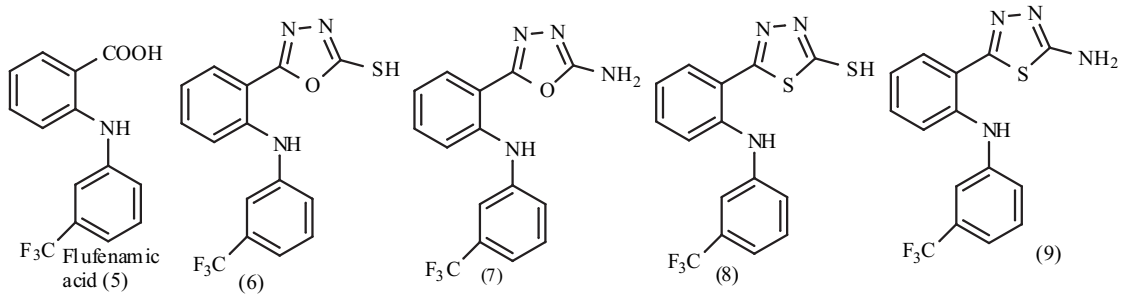

Fig. (4). Flufenamic acid and some analogues.

Table 1. IC_{50} of Inhibition for 5-LOX and COX for Flufenamic Acid Analogues

Compound	IC_{50} 5-LOX (μM)	IC_{50} COX (μM)
6	0.77	0.27
7	0.68	7.1
8	0.87	0.85
9	0.69	11

III.3 Di-*tert*-butylphenol and Dihydrobenzofuran

The di-*tert*-butylphenol class is an important source of dual COX / 5-LOX inhibitors. Well-knwon compounds of tebufelone, a non-selective COX inhibitor (IC_{50} COX-1= 0.25 μM; IC_{50} COX-2= 0.1 μM), inhibits 5-LOX with an IC_{50} of 3 μM [26]. Moreover, the metabolite of tebufelone (compound 16) is active as a dual inhibitor and has been modulated to obtain new compounds. The variation of the 5-keto substituent [26] or the dihydrobenzofuran ring [27] has led to compounds that are potent inhibitors of COX and 5-LOX with a moderate selectivity for the COX-2.

III.4 Pyrrolizine Derivative

This class includes non-antioxydant, dual inhibitors. For these compounds, the balance between the activity against

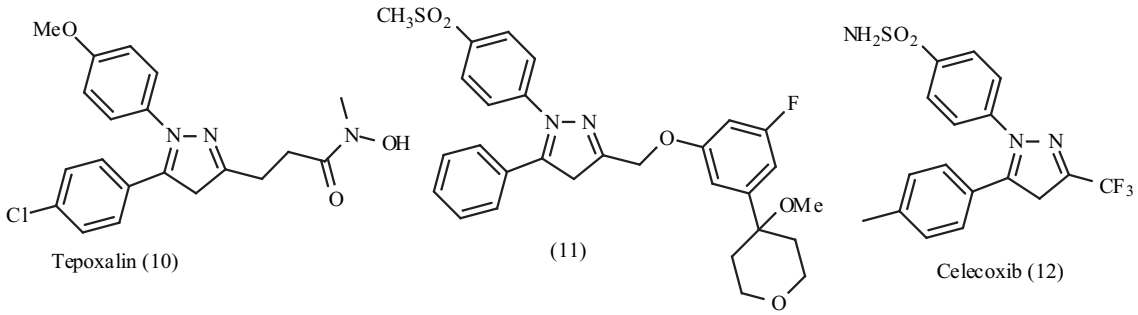

Fig. (5). Chemical structure of tepoxalin, compound 11 and celecoxib.

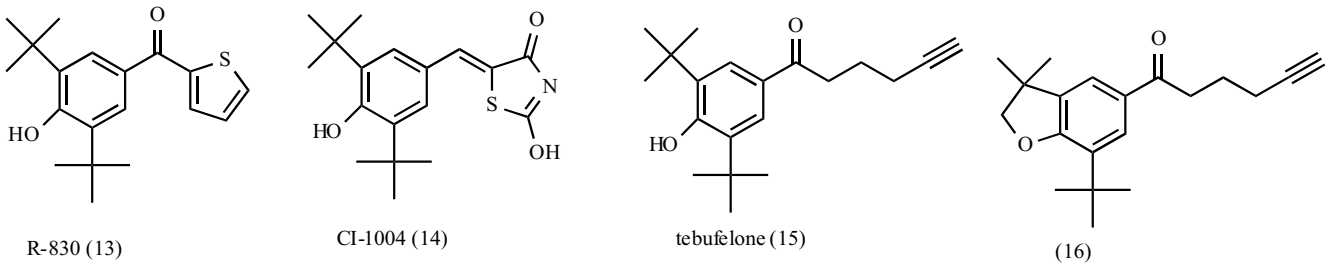

Fig. (6). Examples of di-*t*-butylephenol and dihydrobenzofuran.

COX and 5-LOX can be shifted by modifying the substitution pattern of the phenyl moiety at the 6-position of the pyrrolizine ring [28]. ML-3000 (17) Fig. (**7**), which has undergone clinical trial, inhibits the COX and 5-LOX activities in isolated human platelets with IC_{50} of 0.22μM and 0.37 μM respectively [29]. Due to its well-balanced, dual inhibition, this compound is a promising compound in clinical trials. Pharmacomodulation of ML-3000 has led to compound 18 [30]. This compound is a COX inhibitor (IC_{50} COX-1= 0.7 μM; IC_{50} COX-2= 0.005μM) and 5-LOX inhibitor (IC_{50}= 10 μM) when tested in intact cells. In this class, the replacement of the phenyl residue at C-6 by a thiophene or a furan moiety produced poor COX or LOX inhibitors.

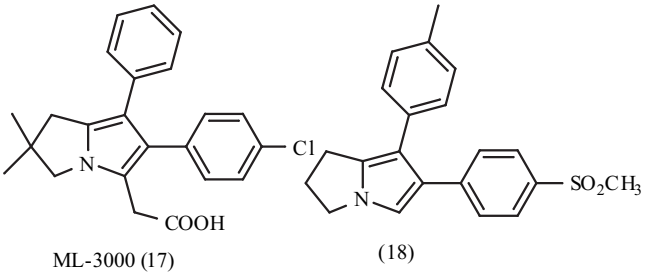

Fig. (7). Chemical structure of ML-3000.

III.5 The Sulfonamide Class

The development of *N*-(5-substitued) thiophene-2-alkylsulfonamides as 5-lox inhibitors has led to RWJ 63556 (compound 19) [31] Fig. (**8)**. This compound is able to inhibit COX-2 using a whole cell assay (human endothelial vein umbilical cells) with an IC_{50} equal to 1.86 μM. In rat basophilic leukaemia cells, this compound inhibits 5-LOX (IC_{50} : 0.13 μM) but not COX-1 (IC_{50}>100μM) [32]. RWJ 63556 has also been evaluated in pre-clinical trials but was discontinued at this stage [33].

IV. CONCLUSION

Dual inhibitors of both COX and LOX attract interest due to their ability to inhibit two key enzymes involved in the AA metabolism. The use of dual inhibitors warrants the simultaneous inhibition both of COX and LOX pathways, which is not achieved with the combination of two specific inhibitors, since they don't share the same pharmacokinetic and biodistribution pattern. The combination of COX and LOX inhibition could offer opportunities to treat inflammatory disease with more efficient and better tolerated drugs. At present time, only two promising compounds have entered clinical trials: ML-3000 and tepoxalin; and the results of these trials were especially expected by pharmacologists. This class of compound would certainly be of great interest for the treatment of inflammation but also others pathologies such as cancerogenesis and angiogenesis.

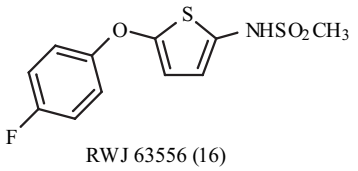

Fig. (8). Chemical structure of RWJ 63556.

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ABBREVIATIONS

5-HETE	=	5-hydroxyeicosatetreaenoic acid
5-HPETE	=	5-hydroperoxyeicosatetraenoic acid
AA	=	Arachidonic acid
COX	=	Cyclooxygenase
IC50	=	Inhibitting concentration
LOX	=	Lipoxygease
LT	=	Leukotriene
LTA_4	=	Leukotriene A_4
LTB_4	=	Leukotriene B_4
LTC_4	=	Leukotriene C_4
LTD_4	=	Leukotriee D_4
NSAID	=	Non-steroidal anti-inflammatory drugs
PG	=	Prostaglandin
PGD_2	=	Prostaglandin D_2
PGE_2	=	Prostaglandin E_2
$PGF_{2\alpha}$	=	Prostaglandin $F_{2\alpha}$
PGG_2	=	Prostaglandin G_2
PGH_2	=	Prostaglandin H_2
RBL	=	Rat basophilic leukaemia
SRS-A	=	Slow reacting substance of anaphylaxis
TXA_2	=	Thromboxane A_2
TXB_2	=	Thromboxane B_2

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