[UCI]I804:24011-200000241024

February 2011
Ph.D. Dissertation

Thiazolidinedione derivatives as novel mPGES-1 inhibitors

Graduate School of Chosun University

Department of Bio New Drug Development

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2011 February 25

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A DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL OF THE CHOSUN UNIVERSITY IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACY

October 2010

Graduate School of Chosun University

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December 2010

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ABBREVIATIONS

15-PGDH	15-Hydroxyprostaglandin dehydrogenase
AA	Arachidonic acid
BBB	Blood brain barrier
COX	Cyclooxygenase
cPGES	Cytosolic prostaglandin E synthase
$cPLA_2$	Cytosolic phospholipase A_2
CSF	Cerebrospinal fluid
DMF	Dimethylformamide
DP	Prostaglandin D receptor
EP	Prostaglandin E receptor
FLAP	Five lipoxygenase activating protein
FP	Prostaglandin F receptor
GSH	Reduced glutathione
IL-1ra	Interleukin 1 receptor antagonist
IL-1α	Interleukin 1 alpha

IL-1β	Interleukin 1 beta
IP	Prostacyclin I receptor
$iPLA_2$	Cytosolic Ca^{2+}-independent phospholipase A_2
LPS	Lipopolysaccharide
LTC_4S	Leukotriene C4 synthase
MAPEG	Membrane associated proteins in eicosanoid and glutathione metabolism
MGST	Microsomal glutathione transferase
mPGES	Microsomal prostaglandin E synthase
MRP4	Multidrug resistance-associated protein 4
NF-kB	Nuclear factor Kappa B
NSAIDs	Non steroidal anti-inflammatory drugs
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
PGI_2	Prostaglandin I_2 (Prostacyclin)
PGSH	Prostaglandin H synthase
PGT	Prostaglandin transporter
POA	Preoptic area
PPh_3	Triphenylphosphine
RA	Rheumatoid arthritis
RT-PCR	Real time polymer chain reaction
SAR	Structure activity relationship
SDS-PAGE	Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis
$sPLA_2$	Secretory phospholipase A_2
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLR-4	Toll like receptor 4
TNF-α	Tumor necrosis factor alpha

TX	Thromboxane
TZDs	Thiazolidinediones
VGEF	Vascular endothelial growth factor

ABSTRACT

Thiazolidinedione derivatives as novel mPGES-1 inhibitors

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Prostaglandins (PGs) have been implicated in an extensive variety of physiological and pathological processes. Among them, PGE_2 is believed to be a key player in the control of various physiological functions and a major mediator of inflammation. PGE_2 is formed by PG synthetase from the endoperoxide prostaglandin H_2 (PGH_2) which is derived from arachidonic acid (AA) through the cyclooxygenase (COX) pathway. Most non-steroidal anti-inflammatory drugs (NSAIDs) function by inhibiting biosynthesis of PGs through inhibition of COX-1 and/or COX-2. Since COX-1 has a protective function in the gastrointestinal tract, non-selective inhibition of both COX leads to moderate to severe gastrointestinal side effects. Selective COX-2 inhibitors such as celecoxib and rofecoxib could also causes sudden myocardial infarction and thrombosis. Therefore, there is a need to target more downstream enzyme such as microsomal prostaglandin E_2 synthase-1 (mPGES-1) that catalyzes the formation of PGE_2 from PGH_2.

Fifty eight thiazolidinediones (TZDs) derivatives were synthesized by using three schemes. The substituted intermediate benzaldehyde (SIB) was afforded by Mitsunobu coupling between starting material p-hydroxybenzaldehyde and

various substituents (alcohol group) in reproducible good yield. Knoevenagel condensation between the SIB and thiazolidine-2, 4-dione in refluxing toluene gives the final compound of TDZs. Each compound was assayed *in vitro* for the mPGES-1 inhibitory activity. PGE_2 concentration was measured by enzyme immunoassay kit and IC_{50} values were calculated.

In **Scheme 1,** structure activity relationship (SAR) suggested that the two methyl group between cyclohexane and ether linkage at 4 position in SIB ring showed good inhibitory activity for mPGES-1. When methyl group at R_1 position was replaced by methoxy group, the inhibitory activity decreased. **Scheme 2,** SAR suggested that the binding efficiency with mPGES-1 decreased with the ether linkage change from 4 position to 3 position in SIB ring. Finally in **scheme 3**, introduction of chlorine at 2 position and ether linkage at 4 position in SIB ring with different functional group at R position showed inhibitory activity was significantly changed. Of 58 TZDs, percentage cytotoxicity of top six mPGES-1inhibitors and rosiglitazone were checked by using HaCaT cell line. Cytotoxicity of top six mPGES-1 inhibitor showed that comparative cytotoxicity with that of rosiglitazone.

Top 6 mPGES-1 inhibitors included **5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 0.54 μM), **5-[4-(Thiophen-3-ylmethoxy) benzylidene]thiazolidine-2,4-dione** (IC_{50} 2.84 μM), **5-(4-(2-cyclopentylethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 3.70 μM), **5-(3-(benzyloxy)-2-chloro benzylidene)thiazolidine-2,4-dione** (IC_{50} 9.80 μM), **5- (2-chloro -3-(cyclohexyl propoxy)benzylidene)thiazolidine-2, 4-dione** (IC_{50} 11.10 μM) and **5-(2-chloro-4-(3- cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione** (IC_{50} 15.00 μM).

It is concluded that these novel TZDs could be ideal anti-inflammatory drugs to replace COX-1/COX-2 inhibitors.

국문초록

mPGES-1 억제제로서 신규한 thiazolidinedione 유도체 개발

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Prostaglandins (PGs)은 생리적, 병리학적 과정에 광범위하고 다양하게 관련되어 있다. 그 중 PGE_2 는 여러 생리적 기능의 억제와 염증의 주요한 매개 물질로 중요한 역할을 하는 것으로 여겨지고 있다. PGE_2 형성과정은, arachidonic acid (AA)가 cyclooxygenase (COX)경로를 통하여 endoperoxide prostaglandin H_2 (PGH_2)가 만들어지는데, 이물질로부터 PG synthetase 에 의하여 만들어진다. 대부분의 non-steroidal anti-inflammatory drugs (NSAIDs)은 COX-1 혹은 COX-2를 억제하여 PGE_2 의 생합성 억제하는 작용을 한다. 그러나 COX-1 은 위장기관을 보호하는 기능이 있기 때문에, NSAIDs 에 의한 두 COX 의 비선택적 억제는 심한 위장장애를 일으킨다. 따라서 COX-2 만을 타겟으로 하는 선택적 억제제로서 celecoxib와 rofecoxib 등이 개발되었으나 이러한 약들의 장기간 복용은 심근경색과 혈전증과 같은 심각한 부작용을 일으킨다. 따라서 PGH_2 에서 PGE_2 를 형성하는

microsomal prostaglandin E_2 synthase-1 (mPGES-1)와 같은 하위 signal 을 타겟으로 하여 COX-1 또는 COX-2 억제제로부터 야기되는 부작용을 방지할 필요성이 있다.

따라서, 본 실험에서는 mPGES-1 억제제 개발을 목적으로 하여, 58개 thiazolidinediones (TZDs) 유도체들을 크게 3가지 경로를 사용하여 합성하였다. 치환된 Benzaldehyde 중간체 (SIB) 는, 초기물질로 p-hydroxybenzaldehyde 에 여러가지 알코올치환기를 사용하여 Mitsunobu coupling 하여 만들었다. Refluxing toluene 존재하에 SIB와 thiazolidine-2,4-dione 을 Knoevenagel 농축시켜 최종산물 TDZs 를 얻었다. 얻어진 각각의 화합물에 대하여는 *in vitro* mPGES-1 억제 활성을 측정하였다. PGE_2의 농도는 enzyme immunoassay (EIA) kit을 이용하여 측정하였으며, 각각 화합물의 mPGES-1 에 대한 IC_{50} 값을 계산하였다.

Scheme 1 에 의해 형성된 화합물들의 structure activity relationship (SAR)을 보면, cyclohexane 과 SIB ring 의 4번 위치에 있는 ether 와의 두개의 methyl 기가 있는 물질이 mPGES-1 의 억제효과가 큰 것으로 나타났고 R_1 위치에 methyl 기가 methoxy 기로 치환되면 억제효과는 줄어들었다. **Scheme 2** 에 의해 형성된 화합물들은 SIB ring의 ether 결합이 4번 위치에서 3번 위치로 바뀌면 mPGES-1 에 대한 결합능력이 감소되었다. 마지막으로 **Scheme 3** 에서는 SIB ring 의 2번 위치에 chlorine, 4번 위치에 ether 결합이 있고 R 에 다른 기들이 있으면 억제효과 가 매우 달라졌다. 본 실험에서 합성한 58가지의 TZDs 중에서, 강한 mPGES-1 억제효과를 보이는 6개의 화합물을 대상으로

세포독성 실험을 HaCaT 세포주를 이용하여, 알려진 inhibitor rosiglitazone 과 비교 수행하였다. 비교군에 비해 독성의 정도는 약간 더 있었으나 그 이용 가능성을 보여주었다.

본 실험에서 합성한 가장 강한 6가지 mPGES-1 억제제는 다음과 같다. **5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 0.54 μM), **5-[4-(Thiophen-3-ylmethoxy) benzylidene]thiazolidine-2,4-dione** (IC_{50} 2.84 μM), **5-(4-(2-cyclopentylethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 3.70 μM), **5-(3-(benzyloxy)-2-chloro benzylidene)thiazolidine-2,4-dione** (IC_{50} 9.80 μM), **5- (2-chloro -3-(cyclohexyl propoxy)benzylidene)thiazolidine-2, 4-dione** (IC_{50} 11.10 μM) and **5-(2-chloro-4-(3- cyclohexylpropoxy) benzylidene) thiazolidine-2,4-dione** (IC_{50} 15.00 μM).

이러한 실험 결과를 볼 때, 본 실험에서 신규 합성된 TZDs는 COX-1/COX-2 억제제를 대신할, 유력한 항 염증제로 사용할 수 있다고 결론을 내릴 수 있다.

1. INTRODUCTION

1.1 BRIEF HISTORY

In 1930, Ulf Von Euler [1] of Sweden described prostaglandins (PGs) from human semen. Since prostatic gland was the organ from which the substance was isolated, Von Euler named the substance as PG. Prior to this identification, Battez and Boulet [2] in 1913 discovered that extracts from the human prostatic gland could lower blood pressure and contract the urinary bladder in dogs. Similar research led by two gynecologists, Kurzrok and Lieb [3] showed that human semen contracted and relaxed human uterus during artificial insemination. The research on PGs was continued and the E and F series of PGs were isolated by Bergström and Sjövall [4]. Soon after, the structures of these compounds were solved by mass spectrometry [5]. In 1964, Bergström *et al* [6] and van Dorp *et al* [7] independently observed that incubation of homogenates of the sheep seminal vesicular gland with [^{3}H]-labeled arachidonic acid (AA) resulted in an enzymatic conversion to PGE_2 and thus established that PGs originate from C20 polyunsaturated fatty acids. Subsequently, Hamberg *et al* [8-9] detected and isolated an endoperoxide by short-time incubations of AA with the microsomal fraction of homogenates of sheep seminal vesicular glands and the endoperoxide was later termed PGH_2.

In the 5th century BC, Hippocrates, a Greek physician found that a bitter powder extracted from willow bark could provide relief from pain and fever. The active-extract of the bark, called salicin, was isolated in its crystalline form in 1828 by Henri Leroux, a French pharmacist. Few years later, Raffaele Piria, an Italian chemist isolated the acid in the pure state. In 1971, Sir John Vane [10] discovered that the analgesic effect of aspirin was the result of its inhibition of PG biosynthesis. In honor to their discoveries in the field of PGs and related bioactive substances and the mechanism of action of aspirin, Sune Bergström, Bengt Samuelsson and Sir John Vane were awarded the 1982 Nobel Prize in physiology or medicine.

PGs are important mediators of various physiological processes such as regulation of gastrointestinal, renal and blood homeostasis. On the other hand, they also act as potent mediators of inflammation and fever. Cyclooxygenase (COX) converts AA to prostaglandin H_2 (PGH_2), the precursor of the series-2 prostanoids. The enzyme contains two active sites; heme with peroxidase activity, responsible for the reduction of PGG_2 to PGH_2, and a COX site, where AA is converted into the hydroperoxy endoperoxide prostaglandin G_2 (PGG_2). The reaction proceeds through H atom abstraction from arachidonic acid by a tyrosine radical generated by the peroxidase active site. Two O_2 molecules then react with the arachidonic acid radical, yielding PGG_2. Two distinct COX isoenzymes were discovered, COX-1, the constitutive form and COX-2, the inducible enzyme [11-15]. Nonspecific COX inhibitors such as aspirin caused various side effects including gastrointestinal bleeding, Reye's syndrome, hives and swelling. COX-2 specific inhibitors were developed in order to reduce their side effects. However, in 2004, Vioxx™, a COX-2 specific inhibitor was withdrawn by Merck from the market following several investigations showing increased risk of cardiovascular related deaths [16-17].

1.2 EICOSANOIDS

Eicosanoids are a class of polyunsaturated hydrophobic molecules that act as autocrine and paracrine mediators. The term 'eicosanoids' (evolved from Greek word eicosi meaning 20) is used collectively to denote fatty acid molecules with 20 carbon atoms. The eicosanoid family constitutes PGs, leukotrienes (LTs), thromboxanes (TXs), lipoxines (LXs) and other related compounds. Certain fatty acids such as linoleic acids (18:2 cis-Δ^9, Δ^{12}) and α-linolenic acids (18:3 cis-Δ^9, Δ^{12}, Δ^{15}) are essential in the human diet [18]. Humans can easily make monounsaturated fatty acids with a double bond at ω-9 positions, but do not have the enzyme machinery necessary to introduce a double bond beyond ω-9 position of the fatty acid chain. However, AA (20:4, ω6) and dihomo-γ-linolenic acids (20:3, ω6) can be synthesized

from linoleic acid [18]. **Figure 1** illustrates different precursors of eicosanoids.

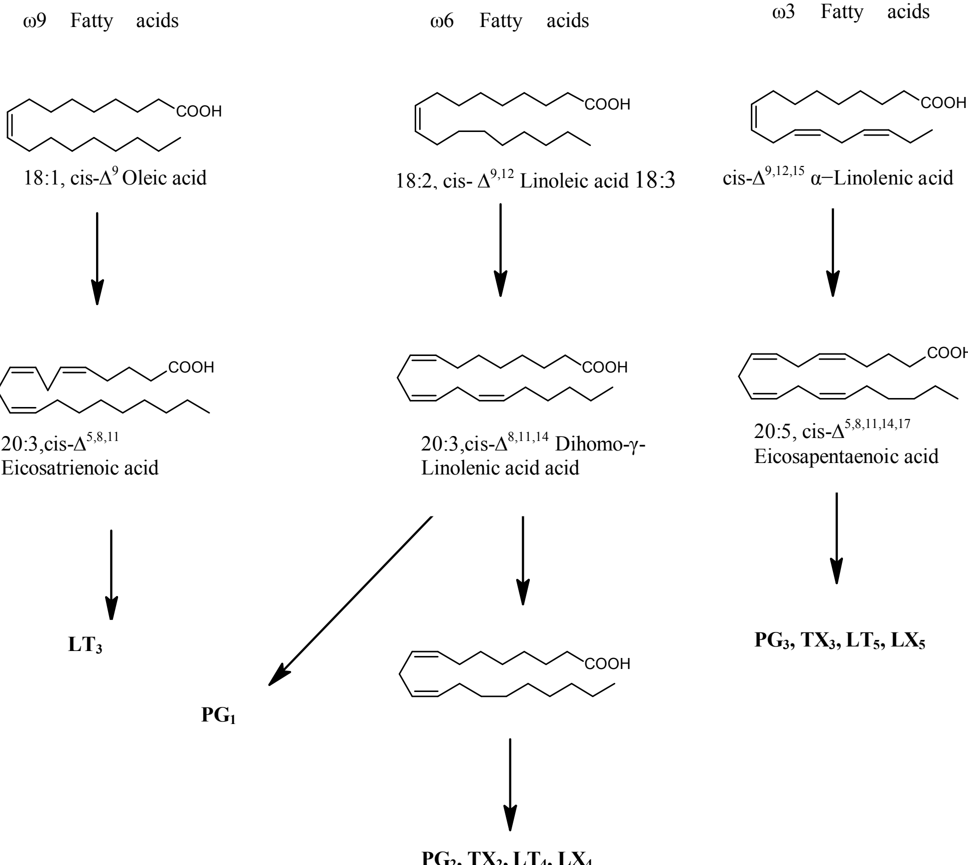

Figure 1. Precursors of eicosanoids.

1.3 PHOSPHOLIPASES RELEASE ARACHIDONIC ACID

Among the various fatty acids, AA is the most abundant in human cells. This C_{20} polyunsaturated fatty acid is predominantly bound to the *sn*-2 position of membrane phospholipids [19]. The level of free AA under normal physiological condition is very low. However, different stimulatory agents trigger the release of AA by phospholipids. Availability of free AA is essential for the biosynthesis of eicosanoids and it is a rate-limiting step in this process. phospholipids and acyl-CoA transferases determine the concentration of free AA through hydrolysis and re-

esterification of phospholipids [20]. A number of different enzymes have so far been identified with phospholipase activity [21-22] and present in different isoforms in different cell types [23]. The superfamily of the phospholipase A_2 (PLA_2) enzymes is divided into four classes: secretory ($sPLA_2$), cytosolic Ca^{2+}-dependent ($cPLA_2$), cytosolic Ca^{2+}-independent ($iPLA_2$) and platelet-activating factor (PAF) acetyl hydrolase [22].

1.3.1 Phospholipase (PLase) enzymes

1.3.1.1 $sPLA_2$s

$sPLA_2$ consists of 14-19 kDa secreted enzymes and comprise the largest group of PLase. These enzymes contain a highly conserved Ca^{2+} binding loop and a catalytic site. The presence of at least 6 conserved disulphide bonds contributes to the high degree of stability of these enzymes [22]. $sPLA_2$s hydrolyze the ester bond at the *sn*-2 position of glycerophospholipids in the presence of mM concentrations of Ca^{2+} with no strict fatty acid selectivity [22]. The genes for the different subgroups of $sPLA_2$s enzymes are clustered on the same chromosome locus and are often referred to as the group II subfamily. Several of the $sPLA_2$ have been reported to take part in a number of biological processes such as inflammation and host defense. $sPLA_2$-IIa is the most widely distributed isozyme in human. The expression of $sPLA_2$-IIA is markedly induced by pro-inflammatory stimuli [24] and downregulated by anti-inflammatory cytokines or glucocorticoids in a wide variety of cells and tissues [25]. Using $sPLA_2$-IIA transgenic mice, the anti-bacterial and atherosclerotic properties of $sPLA_2$ has been studied [26-27]. Indeed, $sPLA_2$-IIA possesses the strongest bactericidal activities among the $sPLA_2$ members and several lines of recent evidences suggest that the anti-bacterial function of $sPLA_2$-IIA appears to be the primary physiological function [28]. Transgenic over-expression of $sPLA_2$-IIA in mouse testis leads to infertility due to the impairment of spermatogenesis indicating abnormal expression of this enzyme in male genital organs may cause developmental defects [29].

$sPLA_2$-IID is structurally similar to $sPLA_2$-IIA and constitutively expressed in the immune and digestive organs and upregulated by pro-inflammatory stimuli in some restricted tissues [30]. $sPLA_2$-IIE, which is another $sPLA_2$-IIA related enzyme, is expressed constitutively in several tissues at low levels and also upregulated by proinflammatory stimuli [31]. Similarly, $sPLA_2$-IIF is expressed in the testes of adult mice and also detected in low levels in various human tissues. This enzyme is thought to be involved in the regulation of developmental process as a high level of expression is found in the mouse embryo [32].

$sPLA_2$-III is an unusually large protein (55 kDa) among the $sPLA_2$ family. It is expressed in the kidney, heart, liver and skeletal muscles. $sPLA_2$-V is mainly detected in the human heart and lungs [33] and its expression is also found to be induced by pro-inflammatory stimuli [34]. $sPLA_2$-X is synthesized as a zymogene and the removal of the N-terminal propeptide produces an active mature enzyme [35].

1.3.1.2 $cPLA_2$

The $cPLA_2$ family consists of three isozymes, $cPLA_2\alpha$, $cPLA_2\beta$ and $cPLA_2\gamma$. $cPLA_2\alpha$ is constitutively expressed in most cells and tissues and its expression is induced under certain conditions [36]. $cPLA_2\alpha$ shows remarkable selectivity toward phospholipids containing AA at the *sn*-2 position [37]. It also possesses *sn*-1 lysophospholipase activity and weak transacylase activity [38]. In contrast, $cPLA_2\beta$ and $cPLA_2\gamma$ possess less specificity towards the fatty acid selectivity and, in fact, the sn-1 hydrolysis by these enzymes is more potent compared to *sn*-2 hydrolysis [39]. Submicromolar concentration of Ca^{2+} is required for the translocation of $cPLA_{2\alpha}$ from the cytosol to the membrane which is necessary for the release of AA [40]. $cPLA_{2\alpha}$ has been reported to play a role in cellular proliferation, transformation and oncogenesis in certain cell types [41]. $cPLA_2\alpha$ deficient mice showed markedly reduced airway anaphylactic response [42], significantly reduced experimental Parkinsonian syndrome [43] and delayed onset of labor [44] demonstrating its role in maintaining both physiological functions and pathophysiological

reactions.

1.3.1.3 $iPLA_2$

$iPLA_2$ is classified as group VI PLA_2 and two enzymatically active forms namely $iPLA_2$-VIA and -VIB have been identified. $iPLA_2$ exists in several splice variants [45-46]. $iPLA_2$-VIA and -VIB are ubiquitously expressed in various tissues and are fully active in the absence of Ca^{2+}. The $iPLA_2$ protein is about 85 kDa in size and contains lipase consensus sequence and ATP binding motif [47]. Besides their roles as housekeeping genes, such as maintenance of phospholipids homeostasis, $iPLA_2$-VIA seems also involved in stimulus coupled AA release [48].

1.3.1.4 PAF

PAF-AH acetylhydrolase specifically catalyzes the hydrolysis of phospholipids containing an acetyl group at *sn-2* position and it degrades PAF, a potent inflammatory mediator. Two types of PAF-AH have been identified, a 45 kDa secreted form with potent anti-inflammatory properties [49] and an intracellular form containing three subunits of 29, 30 and 45 kDa [50]. These two forms show significant sequence identity (～ 41%) and classified as group VII enzymes. A high level of the plasma type enzyme is expressed in thymus and tonsil, while the intracellular enzyme is abundant in liver and kidney [49-50].

1.4 METABOLISM OF AA

In mammals, oxygenation of free AA is carried out by three different enzymatic pathways. COX pathway produces PGs and TXs via production of the intermediate PGG_2 and PGH_2. On the other hand, the lipoxygenase pathway produces LTs and certain hydroperoxy acids (HpETEs). The cytochrome P-450 pathway produces a series of epoxy and hydroxyl-acid-derivatives [51].

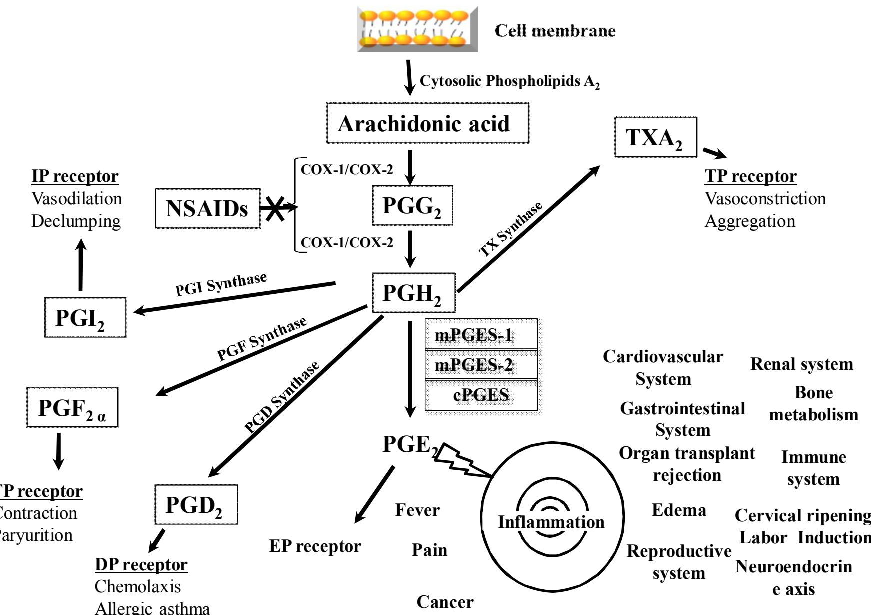

Figure 2. Synthesis of the different PGs and their actions. A "generic" cell when activated by mechanical trauma, cytokines, growth factors, or various inflammatory stimuli triggers signaling, including $cPLA_2$ translocation to ER and nuclear membranes, AA release from membrane lipids and metabolism by COX-1 or COX-2 to the intermediate PGH_2. Other PLA_2 subtypes could be involved in AA release for eicosanoid synthesis but are not shown here. *De novo* COX-2 enzyme synthesis can be induced by a host of factors to reinforce PGs formation. In a cell-type restricted fashion, a heterogeneous family of PGH_2 metabolizing enzymes can form PGE_2, PGD_2, PGF2α, PGI_2 and TxA_2. These PGs may undergo facilitated transport from the cell through a known PGT or other carrier to exert autocrine or paracrine actions on a family of PG receptors (EP, DP, FP, IP and TP) on the different cell types. Only a few of the many diverse activities of PGs are shown here. PGs could potentially enter the nucleus and activate nuclear hormone receptors such as PPARγ. X marks the site of inhibition by NSAIDs (aspirin, ibuprofen, indomethacin) and the coxibs celecoxib and rofecoxib.

1.5 BIOSYNTHESIS OF PGs

PGs are formed by most cells in our bodies and act as autocrine and paracrine lipid mediators (i.e., they signal at or immediately adjacent to their site of synthesis, respectively. They are not stored but are synthesized *de novo* from

membrane, released AA when cells are activated by mechanical trauma or by specific cytokine, growth factor, and other stimuli [e.g., collagen and adenosine diphosphate (ADP) in platelets, bradykinin and thrombin in endothelium] [52]. A host of enzymes exquisitely regulate cellular levels of AA, keeping it esterifies until mobilized by PLA_2. The control of AA release from membranes has undergone several paradigm shifts in recent years with the continuing identification of new PLA_2 members [53]. Despite this, type IV $cPLA_2$ remains the key player for eicosanoid production because cells lacking $cPLA_2$ are generally devoid of eicosanoid synthesis. Cell-specific and agonist-dependent events coordinate translocation of $cPLA_2$ to the nuclear envelope, endoplasmic reticulum (ER), and Golgi apparatus [54].

At the ER and nuclear membrane, AA released by $cPLA_2$ is presented to PG H synthase (PGHS; referred to colloquially as COX) and is then metabolized to an intermediate PGH_2 (**Figure 2**). PGHS exists as two isoforms referred to as PGHS-1 (COX-1) and PGHS-2 (COX-2) [55]. In simplistic term, COX-1 is the enzyme responsible for basal, constitutive PG synthesis, whereas COX-2 is important in various inflammatory and “induced” settings. There are notable exceptions to this oversimplification, but in general this classification has aided the rapid advancement in this field since the discovery of COX-2 (20 years ago). The COX enzymes are monotopically inserted in the ER and nuclear membrane with the substrate binding pocket precisely orientated to take up released AA. The crystal structures of COX-1 and COX-2 are remarkably similar, with one notable amino acid difference that leads to a larger “side-pocket” for substrate access in COX-2 [55].

The coupling of PGH_2 synthesis to metabolism by downstream enzymes is intricately orchestrated in a cell-specific fashion. TX synthase is found in platelets and macrophages, prostacycline synthase is found in endothelial cells and PGF synthase in uterus, and two types of PGD synthase are found in brain and mast cells and PGE synthase in most cell [56]. Microsomal PGE synthase (mPGES), consists

of two isoenzymes, mPGES-1 and mPGES-2, are responsible for PGE_2 synthesis [57]. Coordinate induction of multiple enzymes in the prostanoid pathway, in particular mPGES-1 and COX-2, in inflammatory settings is a current concept being developed [58].

1.6 MECHANISM OF PG ACTION

PGs are released from cells predominantly by facilitated transport through a prostaglandin transporter (PGT), the organic anion transporter polypeptide family [59]. Due to the evanescent nature of TX and PGI (which have half-lives on the order of seconds to a few minutes) [60], these compounds must act near their sites of synthesis. There are at least 9 known PG receptor forms in mouse and man, as well as, several additional splice variants with divergent carboxy termini [61]. Four of the receptor subtypes bind PGE_2 (EP_1–EP_4), two bind PGD_2 (DP_1 and DP_2) [52, 62-63], and three (FP, IP, and TP) binds $PGF_{2\alpha}$, PGI_2, and TxA_2 respectively. The PG receptors belong to three clusters (on the basis of homology and signaling attributes rather than by ligand-binding properties) within a distinct subfamily of the G protein coupled receptors (GPCRs) superfamily of seven transmembrane spanning proteins with the exception is DP_2, a member of the chemoattractant receptor subgrouping. The “relaxant” receptors IP, DP_1, EP_2, and EP_4 form one cluster, signaling through Gs-mediated increases in intracellular cyclic adenosine monophosphate (cAMP); the “contractile” receptors EP_1, FP, and TP form a second group that signals through Gq-mediated increases in intracellular calcium. The EP_3 receptor is regarded as an “inhibitory” receptor that couples to Gi and decreases cAMP formation [52]. Although most of the PG GPCRs are localized at the plasma membrane, some are situated at the nuclear envelope [64].

1.6.1 COX and NSAID

COX-1 and COX-2 are the major targets for non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs (e.g., aspirin, indomethacin, ibuprofen),

known to block PGHS-derived PG synthesis, are well-established in the common man's practice of analgesics and anti inflammatories. Over the past years, NSAIDs have been widely used to treat various types of chronic and acute pain. The classical NSAIDs inhibit both COX-1 and COX-2 isoenzymes, producing therapeutic but adverse effects as presented in **Figure 2**. Although the mechanism of COX inhibition by NSAIDs is rarely disputed, some NSAIDs affect the transcription factors like nuclear factor kappa B (NF-kB) and peroxisome proliferator-activated receptor (PPAR) family members even though higher concentrations are required than those that effectively block COX activity [65-66]. Aspirin remains the sole member of this class of drugs with a unique mechanism of action on COX by covalently acetylating its serine residue. This blocks proper substrate access and orientation at the active site [52]. Therapeutic doses of classical NSAIDs often lead to gastrointestinal bleeding and inhibition of mucoprotective PGs [67].

In 1999, Warner *et al* [68] reported a full *in vitro* analysis of COX-1 & COX-2 selectivities for a wide range of NSAIDs and COX-2 selective compounds and discovered that inhibition of COX-1 underlies the gastrointestinal toxicity of NSAIDs which also reconfirmed previous observations [69]. Using a carrageenan-induced inflammatory rat model, Seibrt *et al* [70] have demonstrated that expression of COX-2 mRNA was upregulated in response to carrageenan and a selective COX-2 inhibitor blocked the resultant edema formation. The structural basis for the selective inhibition of COX-2 was explained from the structures of COX-2 and selective COX-2 inhibitors determined at 3.0-2.5Å resolution. This structure study also demonstrated some of the conformational changes associated with time-dependent inhibition of COX-2 [71]. In 1999, rofecoxib and celecoxib were the first two drugs in this new class of selective COX-2 inhibitors to be approved for use [72]. In 2001, valdecoxib was another addition to this class of selective COX-2 inhibitors [73]. These new generations of COX-2 inhibitors were widely used to treat inflammatory diseases such as rheumatoid arthritis [74-76], osteoarthritis [77-79], as well as neurodegenerative diseases including Alzheimer's disease [80] and Parkinson's disease [81-82]. Many

forms of cancers were shown to be associated with overexpression of COX-2 and COX-2 specific inhibitors were used in attempts to lower the progression of these diseases [83-86].

However, in addition to the anti-inflammatory effect, COX-2 specific inhibitors were found to trigger cardiovascular disease such as myocardial infarction and stroke [87] and in September 2004, Vioxx was withdrawn from the market due to the increased risk of myocardial infarction found among the group taking 25 mg/day rofecoxib [17]. These side effects caused by COX-2 specific inhibitors bring limitations to the use of these drugs in general and patients with less sensitivity to gastrointestinal side effects are today treated with non-specific COX inhibitors [88].

1.6.2 COX-3

A third isoform of COX enzyme was thought to exist with the observation that acetaminophen, a drug with potent anti-pyretic action but very-weak anti-inflammatory activity reduces the levels of PG metabolites in urine [89]. In 2003, Chandrasekharan *et al* [90] reported the identification of a splice variant of COX-1, named COX-3 and cloned into baculovirus. The COX-3 mRNA is transcribed from COX-1 gene and retains intron-1 and therefore the COX-3 protein is also called COX-1b. RT-PCR of canine cerebral cortex RNA, as well as analysis of Northern blots indicated that COX-3 mRNA is present in the brain region at about 5% of the level of COX-1 mRNA. In human, COX-3 mRNA is transcribed as 5.2 kb transcript and abundantly expressed in cerebral cortex and heart. Recently the rat COX-3 mRNA from cerebral endothelial cells was cloned and a vector containing the rat COX-3 cDNA was transfected to COS 7 cells [91]. Western blot analysis using an affinity-purified antibody against COX-3 protein demonstrated highest expression in heart, kidney and neuronal tissues. Notably, the COX-3 protein did not show any COX activity and thus the physiological significance for the existence of COX-3 is not known.

1.7 PROSTANOID SYNTHASES

At least three enzymes are known to convert PGH_2 to PGE_2 in humans microsomal PGES (mPGES)-1, mPGES-2 and cytosolic PGES (cPGES) [92]. mPGES-1 is an inducible, glutathione-dependent 16 kDa enzyme which belongs to the membrane associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) family [93], which also includes leukotriene C_4 (LTC_4) synthase and FLAP (5-lipoxygenase activating protein). Its closest homologue is microsomal glutathione S-transferase 1 (MGST-1), with which it shares 38% sequence identity [57]. Another isoform, termed mPGES-2, is ubiquitously expressed under basal conditions in many tissues and is activated by reducing agents [94]. The cytosolic form of PGES was first identified in 2000 by Tanioka *et al* [95] which is constitutive in nature. **Table 1** summarizes various properties of the three isoforms of PGES.

Table1. Isoforms of PGES and their properties [96]

	Chromosome	Regulation	Molecular mass	Localization	Tissue distribution	V_{max} (μmol/ min mg)	k_{cat} (s^{-1})	K_m (PGH_2) (mM)
mPGES-1	9q34.4	Inducible	15 – 16 kDa	Nuclear membrane	Testis, prostrate, placenta, mammary gland, bladder, oncogenic pulmonary fibroblasts	170	50	0.16
mPGES-2	9q33–q34	Constitutive	33 kDa	Golgi. Translocates to cytoplasm	Brain, heart, kidney, liver, striated muscle	3.3	1.8	0.028
cPGES	12q13.13	Constitutive	26 kDa	Cytoplasm. Translocates to nuclear membrane	Ubiquitous	~ 1.9	~ 0.8	0.014

1.7.1 mPGES-1

mPGES-1 has a V_{max} of 170 μmol/min/mg and a k_{cat}/K_m of 310/mM/S for the conversion of PGH_2 to PGE_2 at 37 °C, which is many orders of magnitude higher than other terminal PG synthases [97]. There exists 80% sequence homology between the human mPGES-1 and the mouse/rat/rabbit mPGES-1. Within cells, mPGES-1 localizes to the perinuclear membrane [98] and is found in the microsomal fraction on sub-cellular fractionation [57, 99]. The constitutive

expression of mPGES-1 is to a very low level, and has been reported at moderate levels in human seminal vesicles [58, 100]. The protein has been found to be expressed constitutively in murine brain and lung as well as in rabbit kidney. However, expression could be detected in liver, lungs and heart following infarcts/hepatitis or after exposure to various inflammatory stimuli [101-102]. A functional coupling of mPGES-1 to COX-2 has been proposed [103-104], though there are reports that such a functional coupling may not be present in all tissues [105-106]. mPGES-1 also catalyses the oxidoreduction of endocannabinoids into PG glycerol esters [107] and PGG_2 into 15-hydroperoxy-PGE_2 [97].

1.7.2 mPGES-2

mPGES-2 is a constitutively expressed 33 kDa protein [94] localized to the Golgi, which translocates to cytoplasm [108] and is reported to function efficiently with COX-1 as well as COX-2 [102-103]. The V_{max} for PGH_2 is 3.3 μmol/min/mg and K_m is 28 μM [94]. Though mPGES-2 is reported to function independently from glutathione [109], it is actively upregulated by reducing agents like dithiothreitol, 2-mercaptoethanol and glutathione [110]. Despite its constitutive nature, it has been found to be markedly over expressed in colorectal cancer [102]. Its structure is vastly different from that of mPGES-1 and resembles thioredoxin by virtue of having a conserved Cys110-x-x-Cys113 active site motif with Cys110 being essential for activity [109]. A recent report showed that mPGES –/– mice were healthy and showed no measurable decrease in PGE_2 levels across various tissues and cell types, suggesting that analysis of mPGES-2-deficient mouse lines does not substantiate the contention that mPGES-2 is a PGE_2 synthase [111].

1.7.3 cPGES

cPGES is constitutively expressed in a wide variety of mammalian cell lines and tissues, and is unaltered by pro-inflammatory stimuli, except that it is significantly increased in rat brain in response to bacterial LPS [95]. Peptide micro-sequencing of this 26 kDa protein revealed its identity to p23, a highly conserved

protein functioning as a co-chaperone for heat shock protein Hsp90 [112-113]. The activity of cPGES has been shown to be regulated Hsp90 and a client protein kinase, casein kinase II [114]. Recently, it was also shown that cPGES activity, but not levels are affected by inflammatory cytokines. Tyr9 present near the N terminus has been shown to be critical for its activity. It may exist within the cells as a homodimer and act as a putative chaperone for lipophilic steroid hormone receptors apart from its PGE_2 biosynthetic function [96]. Many groups have put in efforts to determine coupling of various PGES to either COX-1 or COX-2. It was also thought that cPGES couples exclusively with COX-1 to regulate the constitutive production of PGE_2 [95]. However, later studies showed that the coupling was not exclusive and cPGES showed differential coupling with COX-1 as well as COX-2 in brain, which depended upon cell compartment and chronological age of the species [104]. Though the *in vivo* role of cPGES remains unclear, there are at least two reports that cPGES deficiency causes prenatal lethality due to improper lung maturation [115-116]. Characterization of embryonic tissue and primary embryonic fibroblasts from these mice demonstrated that cPGES is not required for PGE_2 synthesis [115].

1.8. STRUCTURE, REGULATION AND PHYSIOLOGY OF mPGES-

1.8.1 Structure of mPGES-1

There is a high degree of homology between mPGES-1 sequences from various species. It also shows high similarity with other membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family proteins like 5-lipoxygenase activating protein (FLAP), microsomal glutathione s-transferase (MGST)-1, MGST-2 and MGST-3 [93, 117]. The crystal structure of mPGES-1 has recently been reported [118]. A 10 Å projection structure of mPGES-1 indicated that the enzyme is a trimer of four-helix bundles in which the hydrophobic helices traverse the membrane [97]. This was recently confirmed by Jegerschold *et al* [118] who have solved the crystal structure of mPGES-1 at a

resolution of 3.5 Å, shown in **Figure 3**. The first transmembrane (TM) helix was highly variable amongst all MAPEG family proteins and hence unlikely to be crucial for enzyme activity. Pro81 present in the second TM helix is highly conserved and the cytoplasmic face of this helix is crucial for GSH binding. Conserved residues Glu66, Arg67, Arg70 and Tyr117 are involved in this interaction. Non-catalytic FLAP has a Thr instead of Arg at position 70 and mutating Arg 67 to Ala completely abolishes mPGES-1 activity. Glu 77 and Arg110 are also essential for catalytic activity. An open–close dynamic structural model has been suggested wherein the enzyme exists in 'closed' conformation with helix 1 occluding the active site as it clashes with PGH_2, while the 'open' conformation, which allows access, is the conformation of the productive enzyme. This also follows that the 'closed' state could be a target to develop inhibitors stabilizing this conformation thereby preventing its 'opening', and hence inhibiting enzyme catalysis.

The residues Arg110 and Tyr117 are highly conserved in the MAPEG family and mutating these residues in human mPGES-1 results in abrogation of its catalytic function [97]. Chemical modification experiments using thiol-based reagents revealed the importance of cysteine residues for catalysis. It also indicated the importance of Arg110 and Thr114 in binding to PGH_2. A recent report showed the existence of inter-monomer salt bridge as well as inter-helical interaction within each monomer, which included polar H-bonds as well as hydrophobic π-stackings contributing to a stable homo-trimeric structure of mPGES-1. The study also revealed three potential inhibitor binding sites per mPGES-1 trimer [119].

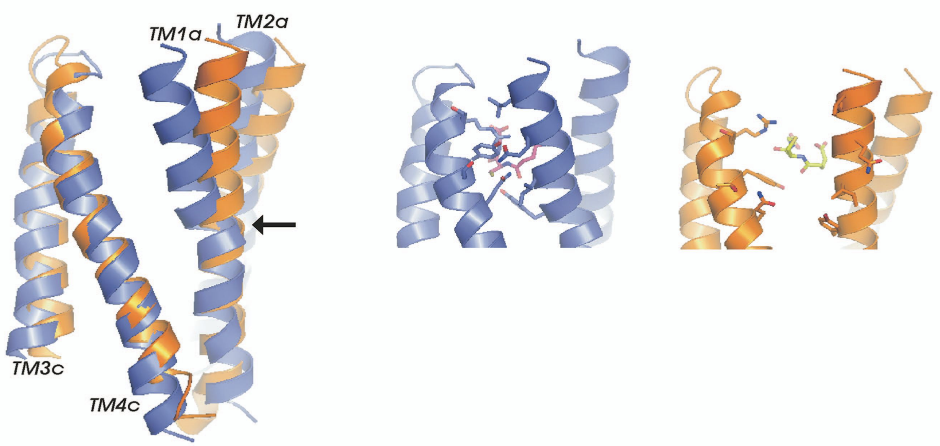

Figure 3. Crystal structure of mPGES-1. (*Left*) The largest difference between the closed and open conformation is a bending of the cytoplasmic half ofTM1about a hinge fixed by the Lys-26/Asp-75 salt bridge (arrow). (*Center*) The closed conformation with Ser-127, Tyr-130, Thr-131, and Gln-134 onTM4 pointing toward residues Ile-32, Gln-34, and Leu-39 in TM1 in the next subunit. Pointing inwards binding to the GSH in magenta are Arg-126 from TM4 and Tyr-28 from TM1. (*Right*) The open conformation model with the same residues shown and with GSH in yellow [118].

1.8.2 Regulation of mPGES-1

The transcriptional regulation of mPGES-1 is still under investigation. Forsberg *et al* [120] determined the gene structure of human mPGES-1 in 2000 and located it on chromosome 9q34.3 spanning 18.3 kilobases and containing three exons. Transcriptional elements present in other MAPEG family genes, such as barbie boxes and arylhydrocarbon regulatory element (ARE) could be detected. The promoter region of the gene was studied in an attempt to find similarities with COX-2, which is co-regulated with mPGES-1, but none of the transcriptional elements, like TATA boxes, present in COX-2 gene or other cytokine-stimulated genes could be detected [121]. This indicates that divergent transcriptional mechanisms are in operation for regulating the inducible expression of COX-2 and mPGES-1. Kojima *et al* [122] reported that selective COX-2 inhibitors could decrease mPGES-1 levels in IL-1β-stimulated rheumatoid arthritis (RA) synovial fibroblasts and chondrocytes, suggesting the existence of a positive feedback loop between COX-2 and mPGES-1. PGE_2 acting through EP_2 and EP_4 receptors recruits

cAMP as a second messenger to upregulate mPGES-1 gene expression, which was inhibited by selective COX-2 inhibitors. It was hence postulated that such an autoregulation of mPGES-1 expression by its own product, PGE_2, might be largely responsible for the vicious cycle of inflammation that is typical of arthritis [123]. Recently, it has been shown that mPGES-1 but not cPGES was significantly up regulated in COX-1 and COX-2 knockout mouse fibroblast cells, indicating its important role in PGE_2 synthesis even in COX-knockout cell lines [124].

1.8.3 Physiology of mPGES-1

mPGES-1 was initially found to be expressed only on induction by inflammatory agents. Later work revealed constitutive expression in urogenital organs [108, 125], spleen, gastric mucosa and macrophages of the peritoneum [126] and liver [127]. The specific physiological role of mPGES-1 in these tissues is not clear. Various studies over the past few years have thrown light on the role of mPGES-1 in multiple tissues and in multiple disease states, which includes inflammation, pain, fever and cancer.

1.8.3.1 Inflammation

Jakobsson *et al*, [57] reported mPGES-1 to be induced by IL-1β in A549 cells. A similar induction was then reported in synovial cells [128], macrophages and osteobalsts [129], along with a suppression by anti-inflammatory corticosteroids [121, 130], endothelial cells [131-132], fibroblasts [133], chondrocytes [134-135], smooth muscle cells and cardiomyocytes [136]. Expression of mPGES-1 was tested in various animal models of inflammatory disease. Induction of mPGES-1 in inflamed tissue was found in an LPS-induced pyresis model [58], an adjuvant-induced arthritis model [58, 130] and a carrageenan-induced rat paw inflammation model [137]. In patients with RA, there was marked upregulation of mPGES-1 in synovial tissue [102, 128], cartilage and chondrocytes of osteoarthritic patients [134-135]. These reports indicated an

unequivocal role for mPGES-1 in arthritis, osteoarthritis and inflammatory processes with it being implicated in pyresis and pain.

The induction of mPGES-1 as well as PGE_2 levels was sensitive to the MAPEG inhibitor MK-866 at an IC_{50} of 2.4 μM. A diminished role of PGI_2 in a rat adjuvant-induced arthritis model has also been shown [130]. In an acute model of carrageenan-induced arthritis in rat paw, expression of mPGES-1 was strongly upregulated in the CNS (brain and spinal cord) during peripheral inflammation, whereas no change was detected for the expression of cPGES, mPGES-2, COX-1, and terminal prostaglandin D, thromboxane or prostaglandin I synthases [137]. The results show that the carrageenan-induced edema in the paw elicits an early phase of COX-2 induction in the CNS leading to an increase synthesis in PGD_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 in addition to the major PGE_2 response.

1.8.3.2 Pain and fever

PGE_2 is one of the key mediators of pain and pyresis due to inflammation, by virtue of which inhibitors of PGE_2 synthesis like paracetamol and other NSAIDs give relief against fever and pain [96]. As mPGES-1 inhibition would also inhibit PGE_2 synthesis, it was expected that it would be associated with pyresis and pain. The first attempt to outline the role of mPGES-1 in inflammatory pain was made by Ek *et al* [138], who reported up-regulation of both COX-2 and mPGES-1, albeit with different kinetics, on administering a pro-inflammatory stimulus, IL-1β. They showed that COX-2 mRNA levels in cerebral blood vessels transiently peaked at 1 h whereas mPGES mRNA levels peaked at 3 h, which matched the time course and the order in which these two enzymes are engaged in PGE_2 biosynthesis. Later studies corroborated this finding and mPGES-1 was found at higher levels in brain in a rat model of adjuvant-induced arthritis [139] and carrageenan-induced paw edema [137], models of chronic and acute inflammation, respectively. mPGES-1 levels were also found to be upregulated after intra-parenchymal injection of IL-1β [140], in a model of peripheral burn injury [141] as well as pilocarpine induced neuro-degeneration [142], a model for seizures and epilepsy. In the brain,

endothelial cells, microglial cells [143] and B-amyloid treated astrocytes [144] have been shown to express mPGES-1, as also seen in spinal cord and dorsal root ganglia [145] in rats. Hence, mPGES-1 inhibition could present a novel target for controlling CNS-controlled acute phase reactions without the systemic side-effects of COX-2 inhibitors [138].

1.8.3.3 Cardiovascular system

Upregulation of mPGES-1, EPs and COX-2 has been reported in symptomatic atherosclerotic carotid plaques, with subsequent upregulation of MMP-2 and MMP-9 [146-147]. The study provided evidence of the link between COX-2 and mPGES-1 overexpression and plaque instability In another study carried out in diabetic versus non-diabetic patients, increased mPGES-1 levels were found in atherosclerotic plaques in diabetics [148], which was associated with renal tumor antigen (RAGE) overexpression and which contributed to plaque destabilization by inducing MMP-9. Induction of mPGES-1 has been found to downregulate PGE_2 levels in vascular smooth muscle cells. Hence under inflammatory conditions, these cells could significantly contribute to plaque instability [149]. These data hence indicate a pathophysiological role of mPGES-1 in atherosclerosis and its inhibition might hence be a way to bring about antiatherogenic effects and plaque stabilization.

1.8.3.4 Neoplasia

Tsujii *et al* [84] showed the involvement of COX-2 in angiogenesis [150]. It was also shown that inactivation of COX-2 by pharmacological or genetic means led to suppression of cell survival and growth. In a tumor situation, it corresponds to reduction in size and invasiveness of cancer cells and a reduction in metastasis [150-151], thus establishing the role of COX-2 in development of colorectal cancer and other types of cancer. As NSAIDs were effective inhibitors of this enzyme, their antineoplastic role was examined in greater detail, and NSAIDs were found to be effective in inducing regression of existing polyps in familial adenomatous

polyposis patients as well as reducing tumor load in animal colorectal cancer models. A further link to the involvement of PGE_2 in oncogenesis was obtained when disruption of EP_2 receptor resulted in reduced gastrointestinal polyps [152]. PGE_2 was shown to promote cancer by enhancing angiogenesis [150]. Between 2001 and 2003, multiple reports described the overexpression of mPGES-1 in various cancers, including NSCLC [153], squamous cell dysplasia [154], head and neck squamous cell carcinoma [155], gastric as well as colorectal adenocarcinoma [153, 156]. These observations have been supported by *in vitro* studies wherein HEK293 cells co-transfected with mPGES-1 and COX-2, but not COX-1, led to transformation and aggressive cell growth with aberrant morphology and high PGE_2 levels [103]. These co-transfected cells were tumorigenic when implanted in nude mice [157]. *Helicobacter pyroli*, which has been largely implicated in gastric tumors, is also known to upregulate gastric epithelial PGE_2 production, contributing to gastric cancer induced by *H pyroli* infection [158]. Though deletion of mPGES-1 has been found to reduce inducible and basal PGE_2 production and alter the gastric prostanoid profile [126], it remains to be seen if the mPGES-1-null mice would have lower incidence of gastric adenomas.

1.8.3.5 Alzheimer's disease

Saloh *et al* [144] showed for the first time, to our knowledge, the induction of PGES mRNA by treatment of rat astrocytes with β-amyloid, using a cDNA subtraction technique. This finding assumed significance as deposition of β-amyloid in the brain is critical for the onset of Alzheimer's disease. It was previously shown that COX-2 is also overexpressed in brains of patients with Alzheimer's disease and NSAIDs can slow its progress [159-160]. These findings were confirmed recently when mPGES-1 was shown to be normally expressed constitutively in human neurons, microglia, astrocytes and endothelial cells but was found to be upregulated in Alzheimer's disease patients [161]. These facts indicate an important role mPGES-1 and COX-2 may have in disease pathogenesis.

1.8.3.6 Kidney

mPGES-1 has been found to be constitutively expressed in epithelium of the distal tubules and medullary collecting ducts of the kidney as well as in the epithelial cells of the bladder and the ureter [125]. Cells of the macula densa, descending thin limb and the cortical collecting tubule are known to generate PGE_2 dependent on mPGES-1/COX-2, which regulates renal salt and water transport by an autocrine mechanism and medullary and glomerular vasculature by a paracrine mechanism [101]. Lack of mPGES-1 activity resulted in a 50% reduction in basal levels of PGE_2 in the kidney [126]. In support of these data is the report of increased expression of mPGES-1 in macula densa cells in patients with hyperprostaglandin E syndrome or classic Bartter syndrome that presents with an activated renin–angiotensin system due to salt and water losses [162].

1.8.3.7 Gastrointestinal tract

It is already well known that the role of PGE_2 is critical in maintaining gastrointestinal mucosal homoeostasis. The gastric lesions caused by aspirin and other non-selective COX inhibitors are largely attributed to inhibition of GI tract PGE_2 [163]. It is also implicated in Crohn's Disease and ulcerative colitis. The PGE_2 receptor EP_4 has shown to be responsible for its GI tract effects [164-165]. Patients with inflamed GI tract show increased expression of mPGES-1 whereas mPGES-1-null mice have almost an 80% reduction in levels of induced as well as basal PGE_2 as compared with wild-type mice [126], indicating that the COX-1/mPGES-1 pathway of PGE_2 synthesis is active in gastric mucosa. Other PGs (thromboxane B_2, PGD_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$) were significantly elevated in stomach of mPGES-1-null mice but not in other tissues. Examination of mRNA for several terminal PG synthases did not reveal any changes, indicating shunting of COX products to other terminal synthases responsible for the altered gastric prostanoid profile [126].

1.8.3.8 Fertility

PGE_2 is known to be an important mediator of gonadotropins in the female reproductive tract. It has a critical role in mediating ovulation and acts in concert with COX-2 in the ovarian follicles [166-167]. Knockout studies in mice have established the importance of COX-2 and the PGE_2 receptor EP_2 in female fertility [168]. Deficiency of COX-2 led to multiple defects in ovulation, fertilization, implantation and decidualization and though mice deficient in EP_2 remain sterile, defects in cumulus expansion cause drastically reduced rate of fertilization and implantation of zygote. Thus the role of mPGES-1 in the biosynthesis of PGE_2 in the ovaries and its subsequent role in fertility were looked into. It was found that mPGES-1 and COX-2 were highly expressed in the granulosa tissue, just prior to ovulation [169]. mPGES-1 was also found to upregulated at luminal epithelial and decidual cells at the site of implantation in hamsters [170], and in extravillous trophoblasts, cytotrophoblasts and macrophages during early gestation in human placenta [171]. High levels of mPGES-1 have also been found in mouse oocytes, all stages of the pre-implantation embryo [172], in amniotic epithelial cells and chorionic trophoblasts of foetal membranes during labor [171]. mPGES-1 has also been detected in mouse leydig cells, epididymal epithelial cells, vas deferens and seminal vesicles in male mice. Orchidectomy results in higher mPGES-1 expression in male rats, indicating the possible role of testosterone in regulation of mPGES-1 [118]. Such a high involvement of mPGES-1 in the reproductive system might indicate a critical role in maintaining fertility. But surprisingly, mice deficient in mPGES-1 have been found to be viable and fertile with no reproductive abnormalities [109]. This might be due to redundancy amongst the various PGES for PGE_2 production. Such a redundancy can be successfully exploited to develop mPGES-1 inhibitors without any major adverse effects concerning fertility.

1.8.3.9 Neonatal role

At the onset of parturition, there is a transient increase in the levels of PGE_2, which is belied to have a cytoprotective role and help in maintenance of

supply of blood to the descending aorta through the ductus arteriosus (DA) during the strong uterine contractions of labor [173]. PGE_2 receptor EP_4 has shown to be involved in this process, and mice lacking EP_4 or COX-1/COX-2 die immediately following birth due to failure of DA closure [174]. Perinatal levels of PGE_2 are mediated by high expression of COX-2 and mPGES-1 and regulated by platelet activating factor. But mice with knockout of mPGES-1 are born normally [175], with no post-natal death, suggesting redundancy of multiple PGE synthases in the production of PGE_2 for DA closure in neonates.

1.9 PG TRANSPORT

PGH_2 is synthesized from AA by COX in the lumen of the stomach [176] and believed to diffuse through the ER membrane to the cytosol where it is converted to more polar prostanoids by terminal enzymes [177-178]. PGs efflux across the plasma membrane to the extracellular compartment is driven by pH and the membrane potential [179]. The first PG carrier characterized was the rat PG transporter (rPGT) which was identified as an 'organic anion transporter' (OATP) [180]. Subsequently, human PG transporter (hPGT) [38] and the mouse PG transporter (mPGT) transporters [181] were cloned and characterized. PGT exhibits a broad tissue mRNA expression in rat, human and mouse [70, 182]. The carrier-mediated epithelial transport of PG has been demonstrated by northern blot detection of PGT mRNA in epithelial tissues [180]. In a recent investigation, expression of two principal PG carriers, i.e. the PGT and MRP4 were examined both *in vitro* and *in vivo* in cells of blood brain barrier (BBB) and choroids epithelial cells in rat brain after LPS challenge [183]. Both PGT and MRP4 were found to express in cerebral epithelial cells (CEC) under basal conditions and the levels of expression in these cells were not influenced by LPS treatment. Strong evidence suggests that two cellular transporters of PGE_2, the transmembrane influx PGT (carrying into the cytoplasm) and efflux MRP4 (carrying out to the extracellular milieu) are deeply implicated in colorectal neoplasia. Although it is the first identified (in 1995) and

best-studied PGT [179-180, 184], PGT has not previously been studied in any cancer setting. Effective termination of PGE_2 may require both PGT, which has a high affinity and specificity for PGE_2, and 15-PGDH [184]. MRP4 in non-cancer model systems, MRP4 knockout or knockdown led to a pronounced reduction in extracellular PGE_2, and MRP4 was inhibited by certain NSAIDs [185] and it is overexpressed in colorectal and other cancers [186]. In rat brain, PGT was highly expressed in supraoptic and paraventricular nuclei of the hypothalamus and the expression was induced by LPS treatment. This indicates a potential role of PGT and MRP4 in transporting PGs through BBB. Recently a new class of PGT inhibitors was developed by screening a library of small molecules. This allowed to study the mechanism of PGE_2 influx and efflux and supported the hypothesis that PGE_2 efflux occurs by simple diffusion [187].

1.10 PG CATABOLISM

Biological inactivation of PGs and related eicosanoids is carried out mainly by 15-hydroxyprostaglandin dehydrogenases (15-PGDH) [188]. Two types of 15-PGDH have been identified. The type I enzyme is NAD^+-dependent and primarily utilizes PGs and related eicosanoids as substrates [189]. The type II enzyme uses both NAD^+ and $NADP^+$ as cofactors and exhibits broader substrate specificity [190]. In contrast to type I, the type II enzyme possesses a higher K_m towards PGs and hence is not believed to catabolize PGs. The type I 15-PGDH catalyzes the initial oxidation of the 15(S)-hydroxyl group followed by a reduction of the Δ^{13} double bond to 15-keto-13,14-dihydro PGs catalyzed by the 13-keto PG reductase [191-192]. In a recent investigation, the levels of type I 15-PGDH was compared in normal and tumor tissues. 15-PGDH was found to be present in high levels in human and mouse large intestine whereas the expression and activity of this enzyme was significantly downregulated in several colorectal carcinoma cell lines [21]. Moreover, genetic disruption of 15-PGDH completely blocked the production of urinary PGE_2 metabolite [21]. 15-PGDH was also found to be underexpressed in human lung

tumors [193]. In addition, mice injected with A549 cells expressing wild type 15-PGDH displayed a significant decrease in tumor growth compared to mice injected with control A549 cells. This study also demonstrated that overexpression of 15-PGDH induced apoptosis in A549 cells. These studies suggest a potential role of 15-PGDH in tumor suppression by decreasing the levels of proliferative PGE_2 [193]. Carbonyl reductase is another PG-inactivating enzyme that possesses 9-keto-reductase activity and thus inactivates PGE_2 by converting it to $PGF_{2\alpha}$ [194]. Both 15-PGDH and carbonyl reductase are widely distributed in peripheral tissues but are weakly expressed in mammalian brain [195-196]. A recent finding shows a negative correlation between the expression of carbonyl reductase and tumor progression and angiogenesis [197]. In non-small cell lung cancer (NSCL) high expression of carbonyl reductase was demonstrated to be a significant factor to predict a favorable prognosis.

1.11 THAIZOLIDINEDIONE

Thiazolidinediones (TZDs), also known as glitazones, are a class of medications used in the treatment of diabetes mellitus type 2. They were introduced in the late 1990s [198]. TZDs also increase the synthesis of certain proteins involved in fat and glucose metabolism, which reduces levels of certain types of lipids. TZDs generally decrease triglycerides and increase high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) [199]. Although the increase in LDL-C may be more focused on the larger LDL particles, which may be less atherogenic, the clinical significance of this is currently unknown.

Chemically, the members of this class are derivatives of the parent compound thiazolidinedione, and include:

- Rosiglitazone (Avandia), which was put under selling restrictions in the US and withdrawn from the market in Europe due to an increased risk of cardiovascular events.
- Pioglitazone (Actos)

- Troglitazone (Rezulin), which was withdrawn from the market due to an increased incidence of drug-induced hepatitis.

Experimental agents include MCC-555 (a powerful antidiabetic agent), rivoglitazone, and the early non-marketed thiazolidinedione ciglitazone. The only approved use of the thiazolidinediones is in diabetes mellitus type-2. It is being investigated experimentally in polycystic ovary syndrome (PCOS) [200], non-alcoholic steatohepatitis (NASH) [201], psoriasis [202], autistim [203], ovarian hyperstimulation syndrome by VEGF inhibition in granulosa cells [204]. It has been shown that TZDs suppress the growth of several cancer cell lines including colon, breast and prostate [205]. Recent published studies showed that thiazolidinedione derivatives are also promising inhibitors for the 15-PGDH [206-207]. Therefore, we tried to investigate therapeutic option for mPGES-1 by the synthesis of different thiazolidinedione derivatives.

O
N H
S
O

Figure 4. Structure of thiazolidinedione.

2. MATERIALS AND METHODS

2.1 MATERIALS

FreeStyle™ 293 F cell, MAX 293 Expression system, Expression medium, MAX Reagent and OptiPro™ SFM were purchased from Invitrogen™ (Carlsbad, CA, USA). 125 ml polycarbonate, disposable, Sterile Erlenmeyer flask with ventilated cap was purchased from VWR (West Chester PA, USA). PGH_2 was purchased from Cayman Chemical (Ann Arbor, MI, USA). PGE_2 enzyme immunoassay kit was purchased from Thermo Scientific (Rockford, IL, USA). Sodium dodecylsulfate (SDS), EDTA, reduced glutathione, $SnCl_2$ and rest of essential chemical and reagents were purchased from Sigma (St. Louis, MO, USA). All chemical reagents used for synthesis were commercially available. The TLC plates were prepared using Kieselgel 60 PF254. The NMR spectra were recorded on a JEOL JNM-LA 300 spectrometer (JOEL, Tokyo, Japan). The chemical shifts are reported in parts per million (d) and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiple). Various thiazolidinediones were synthesized using published procedures [208].

2.2 METHODS

2.2.1 Cell culture

2.2.1.1 HEK 293 F cell

FreeStyle™ 293 F cell line was cultured in 30 mL Expression medium in 125 mL polycarbonate, disposable, sterile Erlenmeyer flask with vented cap. The flask was incubated in a 37 ^{0}C incubator containing humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 135 rpm. Cells were maintained and subcultured.

2.2.1.2 HaCaT cell

HaCat cells, a human keratinocyte cell line, were cultured in Dulbecco's modified Eagle's media (DMEM). DMEM media were supplemented with 10% heat inactivated fetal bovine serum (Sigma) and 100 μg/mL penicillin, in 5% CO_2 at 37 ^{0}C. Cells were maintained and subcultured.

2.2.2 Cell viability assay

Cell viability was determined by the MTT assay [209]. HaCaT cells (1 x 10^4) cells were seeded in 96 well plates per 90 μL of DMEM medium. After the overnight of incubation, different TZDs samples were treated for 72 hours followed by 4 hours of incubation with 10 μl of MTT (5 mg/mL stock solution). Then medium was removed and followed by addition of 150 μL of DMSO to dissolve formazin crystals. Absorbance was measured at 540 nm using an ELISA microplate reader (Perkin-Elmer, Gly., USA).

2.2.3 Transfection of plasmid DNA (mPGES-1)

Plasmid DNA (pcDNA3-mPGES-1) was kindly gifted by Dr. H.H. Tai (University of Kentucky, USA). Approximately 24 hours before transfection, 7 x 10^5 cells/mL cells were seeded and flask was placed on the orbital shaker platform rotating at 135 rpm at 37 ^{0}C, 8% CO_2. On the day of transfection, cell was maintained at 1 x 10^6 cells/mL with more than 90% viability. 30 ml of cell suspension was maintained in each flask. 37.5 μg of plasmid DNA and 37.5 μl of MAX reagent were diluted in to total volume 600 μl of OptiPro™ SMF in separate tube followed by gentle mix by inverting. Both tubes were mixed to gather and incubate DNA-lipid mixture for 10 minutes at room temperature. 1.2 mL of DNA-lipid mixture was slowly added in to the 125 mL flask containing cells while slowly swirling the flask. Transfected cell cultures were incubate at 37 ^{0}C, 8% CO_2 on an orbital shaker platform rotating at 135 rpm for 7 days. There was no need to change the culture medium during the transfection time.

2.2.4 Collection of microsomal fraction

Cells were harvested (5000 x g for 2 minutes) and washed with 1x PBS for 2 times. Cells were resuspended in TSEGP buffer (15 mM Tris-HCl pH 8.0, 250 mM sucrose, 0.1 mM EDTA, 1 mM glutathione and 1 x Complete cocktail protease inhibitor) and sonicated (4 x 30 s at 4 ^{0}C) using Sonics Vibra~cells (Model: VCX 500), 70% duty cycle. Disrupted cells were centrifuged at 10,000 x g for 15 min and the supernatant was centrifuged further 170,000 x g for 1.5 h. The membrane pallets obtained was resuspended into resuspension buffer (100 mM Potassium phosphate buffer pH 7.4, 0.1 mM EDTA, 1 mM glutathione, 1X protease inhibitor and 10% glycerol). The concentration of enzyme was determined and protein band was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), protein band was observed around 17 kDa.

2.2.5 Coomassie blue staining

SDS-PAGE gel was fixed by fixing solution (50% methanol and 10% glacial acetic acid in water) for one hour with gentle agitation. The gel was stained by staining solution (0.1 % coomassie briliant blue R-250, 50% methanol and 10% glacial acetic acid in water) for 20 minutes with gentle agitation. The gel was destained by destaining solution (40% methanol, 10% glacial acetic acid in water) for several times until background of gel was fully destained. Finally destained gel was stored in storage solution (5% glacial acetic acid in water).

2.2.6 Protein quantification

Protein concentration was measured by Bio-Red protein assay based on the method of Bradford. The standard curve was prepared by using bovine serum albumin (BSA) at the concentration of 0, 1.25, 2.5, 5, and 10 mg/mL in water. Bio-Rad protein assay dye reagent was diluted in water at the ratio of 1:4. 2 μL of standards and sample were added in 1 mL of diluted dye reagent and absorbance

was measured at 595 nm. Sample protein concentration was determined from standard curve prepared from BSA.

2.2.7 mPGES-1 activity assay

mPGES-1 activity was measured by PGE_2 enzyme immunoassay kit. The protein sample was diluted with potassium phosphate buffer (100 mM, pH 7.4) containing 2.5 mM glutathione. The reactions were performed using a final volume of 100 μL in 1.5 mL polypropylene tubes at room temperature. Before addition of substrate 4 μL of PGH_2 (stock 100 μg/mL in acetone), the diluted enzyme with different concentration of mPGES-1 inhibitor was incubated 15 min at room temperature. Reaction was stopped at 30 s by 10 μL of $SnCl_2$ (40 mg/mL stock) after addition of PGH_2 and reaction mixture was kept in ice. Further reaction mixture was diluted 2,000 times with reagent diluent (Thermo Scientific) and PGE_2 concentration was measured with PGE_2 enzyme immunoassay kit according to manufacturer's protocol. Each concentration of inhibitors was assayed in triplicate.

2.2.8 Statistical data analysis

The experiments were carried out at least three times. The data is reported as mean value ± standard deviation (SD). Comparison of the effects of various treatments was performed by one way analysis of variance and two-tailed t-test. P values < 0.05 were considered as a statistically significance.

2.2.9 Synthesis of compounds

2.2.9.1 Scheme 1

Diethyl Azodicarboxylate (40% in toluene, 11 mmol) was added slowly to stirring solution of cyclohexaneethanol (10 mmol), *p*-hydroxybenzaldehyde (10 mmol) and triphenylphosphine (11 mmol) in 25 mL of tetrahydrofuran (THF) for 10 min at 0 ^{0}C. The solution was stirred for 18 h at room temperature. The resulting solution was concentrated under reduced pressure and purified by column

chromatography over silica gel (elution with hexane : ethyl acetate :: 20 : 1) to afford **4-(2-cyclohexylethoxy)benzaldehyde** (89%) as yellow oil.

Compound 1-37

Compound 38-39

a) DEAD, PPh3 and THF. b) piperidine, acetic acid and toluene. c) $CoCl_2.6H_20$, dimethylglyoxime, $NaBH_4$, 1N NaOH , 0 ^{0}C.

4-(2-cyclohexyl ethooxy)benzaldehyde (4.3 mmol), 2,4-thiazolidinedione (4.3 mmol), piperidine (2.15 mmol) and acetic acid (2.15 mmol) was added in 20 mL toluene and this mixture was boiled under reflux in a Dean-Stark water trap overnight. The solution was cooled and filtered, the precipitate washed with ether or hexane and dried in the oven to give **(4-(2-cyclohexylethoxy) benzylidene) thiazolidine-2,4-dione (Compound 1).**

2.2.8.2 Scheme 2

Compound 40-49

a) DEAD, PPh3 and THF. b) piperidine, acetic acid and toluene.

Diethyl Azodicarboxylate (40% in toluene, 11 mmol) was added slowly to stirring solution of cyclohexanol (10 mmol), 2-chloro-3-hydroxybenzaldehyde (10 mmol) and triphenylphosphine (11 mmol) in 25 mL of tetrahydrofuran (THF) for 10 min at 0 ^{0}C. The solution was stirred for 18 h at room temperature. The resulting solution was concentrated under reduced pressure and purified by column chromatography over silica gel (elution with hexane : ethyl acetate :: 20 : 1) to afford **2-chloro-3-(cyclohexyloxy)benzaldehyde** (90%) as yellow oil. **2-chloro-3-(cyclohexyloxy)benzaldehyde** (4.3 mmol), 2,4-thiazolidinedione (4.3 mmol), piperidine (2.15 mmol) and acetic acid (2.15 mmol) was added in 20 mL toluene and this mixture was boiled under reflux in a Dean-Stark water trap overnight. The solution was cooled and filtered, the precipitate washed with ether or hexane and dried in the oven to give **5-(2-chloro-3-(cyclohexyloxy)benzylidene)thiazolidine-2,4-dione** (**Compound 40).**

2.2.8.3 Scheme 3

R1 CHO F a R—OH R O R1 CHO b O NH S O R O R1 O NH S O

Compound 50-58

a) NaH and DMF. b) piperidine, acetic acid and toluene.

Cyclohexanol (10 mmol), sodium hydride (13 mmol, 60% dispersion in oil) and 2-chloro-4-fluorobenzaldehyde (10 mmol dissolve in DMF) were added in dry dimethylformamide (20 mL) under nitrogen at room temperature. The reaction solution was stirred for 18 h at room temperature until the disappearance of starting material (TLC analysis). The mixture was added ice water (20 mL) and extracted

with ethyl acetate and water. The organic layer was washed several times with water and dried using magnesium sulfate anhydrous, filtered and evaporated. The residual oil was purified by chromatography over silica gel (elution with hexane : ethyl acetate :: 20 : 1) to afford **2-chloro-4-(cyclohexyloxy)benzylidene** (1.64 g, 87%) as yellow oil. **2-chloro-4-(cyclohexyloxy)benzylidene** (4.3 mmol), 2,4-thiazolidine dione (4.3 mmol), piperidine (2.15 mmol) and acetic acid (2.15 mmol) was added in 20 mL toluene and mixture was boiled under reflux in a Dean-Stark water trap overnight. The solution was cooled and filtered, the precipitate washed with ether or hexane and dried in the oven to give **5-(2-chloro-4-(cyclohexyloxy) benzylidene)thiazolidine-2,4-dione (Compound 50).**

3. RESULTS AND DISCUSSION

PGs have been implicated in an extensive variety of physiological and pathological processes. Among them, PGE_2 is believed to be a key player in the control of various physiological functions and a major mediator of inflammation [210-211]. The production of PGE_2 by PGE_2 synthetase (PGES) from PGH_2 which is derived from AA through COX pathway is believed to play role in multiple physiological processes including reproduction [169], bone metabolism [103], and kidney functions [125] as well as a number of pathologies such as inflammation [58, 103, 212-213], pain [214], fever [215-216], tumorigenesis [153, 157, 217] and Alzheimer′s disease [144].

Most NSAIDs function by inhibiting biosynthesis of PGE_2 by inhibition of COX-1 and/or COX-2. COX-1 is mainly responsible for the 'housekeeping' functions of PGE_2 [51] has a protective function in the gastro-intestinal tract (GIT), non-selective inhibition of both COX leads to moderate to severe gastro-intestinal side effect. COX-2 is inducible and regulated by cytokines and mitogens and is the key player in PGE_2-derived inflammation [218] and cancer. Though deviations from this rule do exist [219-221], this was largely the pretext for development of selective inhibitors of COX-2, like celecoxib, rofecoxib and valdecoxib, which were designed to avoid the gastro-intestinal distress associated with non-specific COX-inhibiting NSAIDs.

mPGES-1 is the physiological substrate for conversion of PGH_2 to PGE_2. Therefore, the search for inhibitors of this enzyme began with fatty acids and PG analogues. Multiple fatty acids and PGH_2 analogues were found to exhibit weak mPGES-1-inhibitory activity. LTC_4 was found to inhibit the enzyme with a potency of 1.2 μM (rat enzyme) to 5 μM (human enzyme). Stable analogues of PGH_2 were also tested and U-51605 was potent, showing around 70% inhibition at 10 μM. Of

the various other PGs tested, the most potent molecule was 15-deoxy-$\Delta^{12,14}$-PGJ_2, with an IC_{50} of 0.3 μM. It was found that cycloxygenase inhibitors NS398 and sulindac weakly inhibited mPGES-1 and interestingly, paracetamol could not inhibit it at even very high concentrations [96].

The concomitant induction of mPGES-1 and COX-2, along with their functional coupling, accounts for the predominant production of PGE_2 during inflammation. Therefore, the inducible from of mPGES-1 provide a novel therapeutic target for inflammation and other PGE_2-mediated pathologies downstream of COX-2.

Fifty eight thiazolidinediones (TZDs) derivatives were synthesized by using three schemes (**scheme 1**, **2** and **3**). The substituted intermediate benzaldehyde (SIB) was afforded by Mitsunobu coupling between starting material (p-hydroxybenzaldehyde 2-chloro-3-hydroxybenzaldehyde and 2-chloro-4-fluoro benzaldehyde) with various substituents (alcohol group) in reproducible good yield. Knoevenagel condensation between the SIB and thiazolidine-2,4-dione in refluxing toluene gives the final compound of TDZs. All the synthesized compounds were assayed *in vitro* against mPGES-1. **Table 2**, **3** and **4** list their inhibitory activities (IC_{50} values) respectively for the schemes.

Table 2 summarizes the list of TZDs compounds and their inhibitory activity for mPGES-1. We introduce different functional group at the position of R, R_1, R_2 and R_3 respectively and inhibitory activity was determined by EIA kit. Structure activity relationship (SAR) suggested that the two methyl group between cyclohexane and ether linkage at 4 position in SIB ring showed good inhibitory activity for mPGES-1. When methyl group at R_1 position was replaced by methoxy group the inhibitory activity decreased. Introduction of one more methoxy group at R_2 position showed that inhibitory activity was significantly decreased. The chain length of carbon between cyclohexane and ether linkage was increased, the inhibitory activity was significantly decreased up to

two carbon chain and again significantly increased. The optimum activity was measured at 2 carbon chain length between cyclohexane ring and ether linkage.

When cyclohexyl ring (R_1 position) was replaced by the 2-thiomorpholin ring, the inhibitory activity was significantly increased. In SIB ring at R_1 position shows that it had inhibitor property for mPGES-1 but not stronger like cyclohexyl ring. Introduction of thiophen ring at R_1 position showed good inhibitory activity. The top three inhibitors for this series of TDZs were **compound 29 (5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione**), **compound 33** (**5-[4-(Thiophen-3-ylmethoxy) benzylidene]thiazolidine-2,4-dione**) and **compound 18 5-(4-(2-cyclopentylethoxy) benzylidene)thiazolidine-2,4-dione** with mPGES-1-inhibitory activity 0.54 µM, 2.84 µM and 3.70 µM respectively.

Table 2. Compound structures (**scheme 1**) and mPGES-1-inhibitory activity (IC_{50})

Compound	R	R_1	R_2	X	IC_{50} (µM)
1	Cyclohexylethyl	H	H	S	22.0
2	Cyclohexylethyl	CH_3	H	S	11.0
3	Cyclohexylethyl	OCH_3	H	S	27.5
4	Cyclohexylethyl	OCH_3	OCH_3	S	8.0
5	Cyclohexylethyl	Cl	H	S	46.0
6	Cyclohexylethyl	Br	H	S	14.0
7	Cyclohexylethyl	Cl	OCH_3	S	70.0
8	Cyclohexylethyl	H	H	CH_2	36.2
9	Cyclohexylpropyl	NO_2	H	S	17.8
10	Cyclohexylpropyl	OCH_3	H	S	37.0

11	Cyclohexylbutyl	OCH_3	H	S	85.0
12	cyclohexylacetaldehyde	Cl	H	S	16.2
13	2-(Cyclohexylamino)ethyl	H	H	S	40.1
14	2-(Piperidine-1-yl)ethyl	H	H	S	19.1
15	4-(Methylcyclohexyl)methyl	CF_3	H	S	78.0
16	2-Thiomorpholinoethyl	H	H	S	2.75
17	3-Thiomorpholine-1,1-dioxideethyl	H	H	S	93.0

18	Cyclopentylmethyl	H	H	S	3.70
19	Cyclopentylethyl	Cl	H	S	18.0
20	H_3C, O, H_3C 2-Isopropoxyethyl	H	H	S	30.0
21	2-Isopropoxyethyl	Cl	H	S	45.0
22	4-Methylbenzyl	H	H	S	23.0
23	O 4-Methoxylbenzyl	H	H	S	23.5
24	O_2N 4-Nitrobenzyl	Cl	H	S	18.0
25	N 2-(Pyridine-2-yl)ethyl	H	H	S	19.9

26	Biphenyl-4-ylmethyl	H	H	S	89.0
27	(2,3-Dihydrobenzo[b][1,4]dioxin-2yl)methyl	H	H	S	78.0
28	5-ethyl-1,3-benzodioxoleyl	H	H	S	33.1
29	2-(Thiophen-2yl)ethyl	H	H	S	0.54
30	2-(Thiophen-2yl)ethyl	Cl	H	S	10.0
31	2-(Thiophen-2yl)ethyl	OCH_3	H	S	16.0
32	Thiophen-2-ylformaldehyde	H	H	S	5.0
33	Thiophen-3-ylmethyl	H	H	S	2.84

34	Thiophen-3-ylethyl	H	H	S	22.1
35	Thiophene-3-ylformaldehyde	Cl	H	S	13.2
36	Furan-3-ylmethyl	H	H	S	10.4
37	(5-Nitrofuran-2-yl) ethyl	Cl	H	S	29.0
38	Cyclohexylpropyl	Cl	H	S	44.4
39	Phenyl	H	H	S	43.0

Table 3 summarizes the list of **scheme 2** compounds and their mPGES-1-inhibitory activity. In this group of compound, we linked ether linkage at the 3 position of SIB ring and investigated the mPGES-1 inhibitory activity. We try to investigate different functional group at the R position and observed that the inhibitory activity was significantly changed then the **scheme 1**. SAR suggested that the binding efficiency decreased with the ether linkage change from 4 position to 3 position. In this scheme, strongest inhibitors for mPGES-1 was **compound 49** (**5-(3-**

(benzyloxy)-2-chlorobenzylidene)thiazolidine-2,4-dione) and **compound 44** (**5-(2-chloro-3-(cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione**) with mPGES-1 inhibitory-activity activity 9.8 µM and 11.1 µM, respectively.

Table 3. Compound structures **(scheme 2)** and mPGES-1-inhibitory activity (IC_{50})

S. No.	R	R_1	R_2	IC_{50}(µM)
40	Cyclohexyl	Cl	H	29.6
41	Cyclohexylmethyl	Cl	H	76.0
42	Cyclohexylethyl	H	NO_2	66.0
43	Cyclohexylethyl	Cl	OCH_3	17.5
44	Cyclohexylpropyl	Cl	H	11.1
45	Cyclohexylbutyl	Cl	H	25.0
46	Cyclopentyl	Cl	H	41.5
47	S	H	OCH_3	88.0

	Thiophene-2-ylethyl			
48		Cl	H	48.5
	Thiophene-2-ylformaldehyde			
49		Cl	H	9.8
	Benzylmethyl			

Table 4 summarizes the compounds of **scheme 3** and its mPGES-1-inhibitory activity. In this scheme we introduce chlorine at 2 position, ether linkage at 4 position in SIB ring with different functional group at R position and *in vitro* activity was measured for mPGES-1. SAR showed that these type of compound structure bind mPGES-1 protein weakly, so it has got higher IC_{50} then the **scheme 1**. In this series, **compound 53 (5-(2-chloro-4-(3-cyclohexylpropoxy) benzylidene) thiazolidine-2,4-dione**) was found to be strong inhibitor for mPGES-1 with IC_{50} of 15 μM.

Table 4. Compound structures (**scheme 3**) and mPGES-1-inhibitory activity (IC_{50})

S. No.	R	R_1	IC_{50}(μM)
50	Cyclohexyl	Cl	20.0
51	Cyclohexylmethyl	Cl	23.5
52		OCH_3	39.0

	Cyclohexylethyl		
53	Cyclohexylpropyl	Cl	15.0
54	Cyclohexylbutyl	Cl	16.0
55	2-(Cyclohexyloxy)ethyl	Cl	19.5
56	Benzylmethyl	Cl	36.0
57	Benzylethyl	Cl	58.0
58	Benzylpropyl	Cl	29.0

Of 58 TDZs, we selected top six potent mPGES-1 inhibitors (**Compound 29**, **33**, **18**, **49**, **44** and **53**) and rosiglitazone were investigated for the relative cytotoxicity by using HaCaT Cell line. Rosiglitazone and TDZs shares same backbone structure, so we tried to compare safety of our synthesized compound *in vitro* cell system. **Table 5** summarizes mPGES-1-inhibitory activity (IC_{50}) and relative cytotoxicity (IC_{50}) in HaCaT cell line. **Compound 29**, **44** and **53** have

similar cytotoxicity IC_{50} that of rosiglitazone (>400 μM) followed by **compound 18** (350 μM) and **compound 49** found to be more toxic in our selected top six inhibitors with that of rosiglitazone. In favor of pharmaceutical application, toxicity is very important. It is important to consider efficacy-cytotoxicity relation of synthesized compounds.

Table 5. mPGES-1 and cytotoxic activities of top six inhibitors and rosiglitazone

Compound	**IC_{50} (μM)**	
	mPGES-1	**Cytotoxicity**
18	3.70	350
29	0.54	>400
33	2.84	310
44	11.1	>400
49	9.8	278
53	15	>400
Rosiglitazone	ND	>400

ND: Not done

The mPGES-1 enzymatic reaction and the detection of PGE_2 in 96 well format were previously described [222] and protocol was slightly modified to detect in PGE_2 in EIA kit. The encumbering factor for the determination of mPGES-1 activity was the instability of the substrate. At room temperature and neutral pH, PGH_2 is highly unstable and predominantly degrades nonenzymatically in to PGE_2, the PG of interest [223]. The half-life of the PGH_2 at RT is 10 min but when kept at 0 ^{0}C, the half life is extended to 30 min [99, 224]. PGH_2 was always prepared fresh and kept on icebox. The mPGES-1 enzyme reaction was stopped the addition of $SnCl_2$ to convert remaining substrate to $PGF_{2\alpha}$ [9] which is marginally detected by EIA antibody (0.7% cross reactivity). In regard to the detection of PGE_2 by EIA kit, the first issue encountered in miniaturizing the assay was the transfer of the reaction sample into EIA plates. The samples require to 2000 fold dilution in reagent diluents provided by manufacturer.

Despite a good number of encouraging reports on the relevance of mPGES-1 as a target, limited information regarding its cardiovascular biology is a concern. mPGES-1 deletion leads to adverse left ventricular (LV) remodeling after myocardial infarction with animals showing more LV dilation, worse LV systolic and diastolic function and higher LV end-diastolic pressure. But despite these changes, cardiac mass, infarct size or mortality was unaltered, as compared with WT mice treated similarly [139]. A recent report also indicated the importance of mPGES-1 derived PGE_2 in buffering angiotensin II-induced vasoconstriction [140]. Amongst the few tissues where mPGES-1 is constitutively expressed in the urinary tract, with initial reports indicating its role in renal salt and water transport [96]. But mPGES-1-null mice do not apparently show any renal dysfunction, thereby leading one to speculate mPGES-1 is redundant and that the other two PGES can carry out PGE_2 production in the absence of mPGES-1 [96]. Further experiments need to be done to confirm this. Renal excretion of PGE_2 metabolites also indicates major differences in its biosynthetic pathway in males and females [225]. High levels of mPGES-1 are also found in the female reproductive tract [226], indicating its role therein. Hence, we hypothesize that TZDs compounds might have the above stated problems and safe for encounter above stated problems and use for human. We predicted that novel TZDs will be selective for mPGES-1.

4. CONCLUSION

Most inflammations have PGE_2 as a central player mediating disease pathology. Hence for the last few decades, inhibition of PGE_2 has been the mainstay of anti-inflammatory therapies. COX inhibitors had become the drug of choice for a host of anti-inflammatory therapies, including those for chronic illnesses like rheumatoid arthritis and osteoarthritis. Hence the fact that specific COX-2 inhibition led to increased incidences of thrombotic events and MI and the subsequent withdrawal of two blockbuster drugs from the market has left a major lacuna in the quest for a safe and efficacious therapy for inhibiting PGE_2 production as an anti-inflammatory strategy. The only other component of the PGE_2 synthesis pathway amenable to inhibition without any severe adverse effects is the terminal synthase mPGES-1. Targeting a terminal synthase, far more specific in inhibiting only PGE_2 and not the other downstream products of the eicosanoid pathway. Thus, it also does not detrimentally affect levels of other PGs.

We synthesized fifty eight TDZs derivatives and *in vitro* inhibitory activity for mPGES-1 was measured. IC_{50} data suggests that all the compounds were viable to bind with mPGES-1 protein. The mPGES-1 inhibitory activity (IC_{50}) ranges from nanomolar to micromolar range. As mentioned before, many mPGES-1 inhibitors were synthesized by different organizations with good inhibitory activity, unfortunately they had to stop their project due to toxicity. Our series of compounds are derivatives of thiazolidinedione, which were similar in backbone structure of ciglitazone, rosiglitazine and troglitazone. These compounds were established as a safe for human [227-229]. We investigate the relative cytotoxicity (IC_{50}) with rosiglitazone with top six TZDs. **Compound 29**, **44**, **53** and rosiglitazone have similar cytotoxicity (IC_{50}). We can hypothesize that our compound will not face

toxicological problem which is the main obstacle for the new drug development. Further clinical studies need to be conducted to evaluate safety and efficacy for humans. mPGES-1 is known to decrease the ability of vascular smooth muscle cells to produce prostacyclin, and hence mPGES-1 inhibition may be helpful in patients with cardiovascular liabilities.

Top six mPGES-1 inhibitors included **5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 0.54 μM), **5-[4-(Thiophen-3-ylmethoxy) benzylidene]thiazolidine-2,4-dione** (IC_{50} 2.84 μM), **5-(4-(2-cyclopentylethoxy) benzylidene)thiazolidine-2,4-dione** (IC_{50} 3.70 μM), **5-(3-(benzyloxy)-2-chloro benzylidene)thiazolidine-2,4-dione** (IC_{50} 9.80 μM), **5- (2-chloro -3-(cyclohexyl propoxy)benzylidene)thiazolidine-2, 4-dione** (IC_{50} 11.10 μM) and **5-(2-chloro-4-(3- cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione** (IC_{50} 15.00 μM).

It is concluded that these novel TZDs could be ideal anti-inflammatory drugs to replace COX-1/COX-2 inhibitors.

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ANNEX-I

COMPOUND STRUCTURE AND ^{1}HNMR DATA

Compound 1

5-(4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione: (1.2 g, 84% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.195 (s, 1H), 7.675 (s, 1H), 7.473 (d, J = 14.7 Hz, 2H), 6.895 (d, J = 14.7 Hz, 2H), 4.036(t, J = 11.7 Hz, 2H), 1.565-1.727 (m, 5H), 1.437-1.469 (m, 1H), 1.041-1.223 (m, 3H), 0.807-1.034 (m, 2H) [230].

Compound 2

5-(4-(2-cyclohexylethoxy)-3-methylbenzylidene)thiazolidine-2,4-dione: (1.25 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.20 (s, 1H), 7.783 (s, 1H), 7.087 (d, J = 8.7 Hz, 2H), 7.058 (s, 1H), 6.788 (d, J = 8.7 Hz, 2H), 4.023 (t, J = 14.7 Hz, 2H), 2.196 (s, 3H), 1.739 (t, J = 14.7 Hz, 2H), 1.577 (m, 1H), 1.213-1.284 (m, 2H), 0.965-1.044 (m, 4H), 0.826-0.880 (m, 4H) [230].

Compound 3

5-(4-(2-cyclohexylethoxy)-3-methoxybenzylidene)thiazolidine-2,4-dione: (1.3 g, 89% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.221 (s, 1H), 7.728 (s,

1H), 7.028 (d, J = 10.2 Hz, 2H), 6.924(d, J = 10.2 Hz, 2H), 6.895 (s, 1H), 4.074 (t, J = 14.4 Hz, 2H), 3.846 (s, 3H), 1.716 (t, J = 14.4 Hz, 2H), 1.434-1.515 (m, 4H), 1.078-1.214 (m, 4H), 0.782-0.976 (m, 3H) [231].

Compound 4

5-(4-(2-cyclohexylethoxy)-3,5-dimethoxybenzylidene)thiazolidine-2,4-dione : (1.25 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.623 (s, 1H), 7.781 (s, 1H), 6.768 (s, 2H), 4.10 (t, J = 13.5 Hz, 2H), 3.887 (s, 6H), 1.620 (t, J = 13.5 Hz, 2H), 1.505-1.738 (m, 6H), 1.096-1.325 (m, 3H), 0.884-0.951 (m, 2H) [230].

Compound 5

5-(3-chloro-4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione: (1.22 g, 84% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d6) δ8.19 (s, 1H), 7.738 (s, 1H), 7.522 (s, 1H), 7.386 (d, J = 10.5 Hz, 1H), 7.012 (d, J = 10.5 Hz, 1H)), 4.155 (t, J = 13.2 Hz, 2H), 1.800 (t, J = 13.2 Hz, 2H), 1.657-1.800 (m, 4H), 1.500-1.606 (m, 1H), 1.151-1.335 (m, 4H), 0.854-1.052 (m, 2H) [230].

Compound 6

5-(3-bromo-4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione : (1.24 g, 85% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.576 (s, 1H), 7.841 (s, 1H), 7.833 (s, 1H), 7.570 (d, J = 10.8 Hz, 1H), 7.284 (d, J = 10.8 Hz, 1H)), 4.174 (t, J = 12.6 Hz, 2H), 1.617-1.750 (m, 7H), 1.460-1.529 (m, 1H), 1.062-1.265 (m, 3H), 0.896-0.966 (m, 2H) [230].

Compound 7

5-(3-chloro-4-(2-cyclohexylethoxy)-5-methoxybenzylidene)thiazolidine-2,4-dione: (1.12 g, 84.2% yield) as yellow solid ; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.656 (s, 1H), 7.738 (s, 1H), 7.262 (s, 1H), 7.239 (s, 1H), 4.043 (t, J = 12.6 Hz, 2H), 3.872 (s, 3H), 1.557-1.754 (m, 8H), 1.144-1.239 (m, 3H), 0.891-0.964 (m, 2H) [230].

Compound 8

3-(4-(2-cyclohexylethoxy)benzylidene)pyrrolidine-2,5-dione: (1.2 g, 84% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.306 (s, 1H), 7.296 (s, 1H), 7.055 (d, J = 11.7 Hz, 2H), 6.838 (d, J = 11.7 Hz, 2H), 3.992 (t, J = 13.2 Hz, 2H), 3.854 (s, 2H), 1.627 (t, J = 13.2 Hz, 2H), 1.523-1.772 (m, 6H), 1.430-1.513 (m, 1H), 1.134-1.271 (m, 2H), 0.904-1.012 (m, 2H) [231].

Compound 9

5-(4-(3-cyclohexylpropoxy)-3-nitrobenzylidene)thiazolidine-2,4-dione: (1.2 g, 89% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.043 (s, 1H), 7.716 (s, 1H), 7.399 (d, J = 11.1 Hz, 1H), 7.262 (s, 1H)，7.041 (d, J = 8.7 Hz, 1H), 4.563 (d, J = 8.7 Hz, 1H), 4.048-4.087 (t, J = 14.1 Hz, 2H), 1.843-2.049 (m, 2H), 1.631-1.889 (m, 6H), 1.183-1.389 (m, 5H), 0.840-0.961 (m, 2H) [230].

Compound 10

5-(4-(2-cyclohexylpropoxy)-3-methoxybenzylidene)thiazolidine-2,4-dione: (1.13 g, 83.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.219 (s, 1H), 7.694 (s, 1H), 7.014 (d, J = 10.2 Hz, 2H), 6.893(d, J = 10.2 Hz, 2H), 6.764 (s, 1H), 4.123 (t, J = 14.4 Hz, 2H), 3.787 (s, 3H), 1.725 (t, J = 14.4 Hz, 2H), 1.444-1.563 (m, 4H), 1.102-1.224 (m, 6H), 0.796-1.103 (m, 3H) [231].

Compound 11

5-(4-(2-cyclohexylbutoxy)-3-methylbenzylidene)thiazolidine-2,4-dione: (1.19 g, 87.5% yield) as yellow solid ; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.787 (s, 1H), 7.349 (d, J = 8.7 Hz, 2H), 7.291 (s, 1H), 6.903(d, J = 8.7Hz, 2H), 4.045 (t, J = 12.9

Hz, 2H), 1.806-1.852 (m, 2H), 1.694-1.779 (m, 5H), 1.438-1.510 (m, 2H), 1.174-1.266 (m, 6H), 0.857-0.890 (m, 2H) [231].

Compound 12

2-chloro-4-[-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl cyclohexylacetate : (1.15g, 85.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.281 (s, 1H), 7.978 (s, 1H), 7.826 (d, J = 6.3 Hz, 1H), 7.559 (d, J = 6.0 Hz, 1H), 2.539 (d, J = 7.2 Hz, 2H), 1.662-1.777 (m, 6H), 1.124-1.389 (m, 5H).

Compound 13

5-(4-(2-cyclohexylamino)ethoxy)benzylidene)thiazolidine-2,4-dione: (1.26 g, 88% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.504 (d, J = 8.7 Hz, 2H), 7.310 (s, 1H), 7.069 (d, J = 8.7 Hz, 2H), 4.244 (t, J = 9.9 Hz, 2H), 3.302 (t, J = 9.9 Hz, 2H), 2.876-2.942 (m, 1H), 2.284 (s, 1H), 1.894-2.071 (m, 2H), 1.733-1.894 (m, 2H), 1.571-1.610 (m, 1H), 1.094-1.304 (m, 4H) [230].

Compound 14

5-[4-(2-(piperidin-1-yl)ethoxy)benzylidene]thiazolidine-2,4-dione: (1.26 g, 87%

yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.522 (s, 1H), 7.411 (d, J = 10.2 Hz, 2H), 6.967 (d, J = 10.2 Hz, 2H), 4.096 (t, J = 11.1 Hz, 2H), 2.814 (t, J = 11.1 Hz, 2H), 2.379 (m, 4H), 1.410-1.513 (m, 4H), 1.291-1.307 (m, 2H) [230].

Compound 15

5-[4-((4-metylcyclohexyl)methoxy)-3-(trifluromethyl)benzylidene]thiazolidine-2,4-dione: (1.02 g, 76.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.602 (s, 1H), 7.878 (s, 1H), 7.829(s, 1H), 7.805 (d, J = 8.4 Hz, 1H), 7.101 (d, J = 8.4 Hz, 1H), 4.117 (d, J = 6.6 Hz, 1H), 4.016 (d, J = 6.6 Hz, 1H), 1.685-1.907 (m, 4H), 1.454-1.523 (m, 4H), 1.083-1.122(m, 2H), 0.927 (d, J = 6.9 Hz, 3H) [230].

Compound 16

5-4-(2-morpholinoethoxy)benzylidene] thiazolidine-2,4-dione: (1.24 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.14 (s, 1H), 7.705 (s, 1H), 7.544 (d, J = 8.7 Hz, 2H), 7.104 (d, J = 8.7 Hz, 2H), 4.219 (t, J = 11.4 Hz, 2H), 3.808 (t, J = 9.6 Hz, 4H), 3.134 (t, J = 11.4 Hz, 2H), 2.764 (t, J = 9.6 Hz, 4H) [230].

Compound 17

5-(4-(2-Thiomorpholine 1,1-Dioxideethoxy)benzylidene)-2,4-thiazolidinedione: (1.21 g, 88% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.19 (s, 1H), 7.73 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 4.169 (t, J = 10.8 Hz, 2H), 3.086 (t, J = 10.2 Hz, 4H), 3.033 (t, J = 10.2 Hz, 4H), 2.945 (t, J = 10.8 Hz, 2H) [230].

Compound 18

5-(4-(2-cyclopentylethoxy)benzylidene)thiazolidine-2,4-dione: (1.16 g, 81% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.502 (s, 1H), 7.737 (s, 1H), 7.550 (d, J = 8.7 Hz, 2H), 7.093 (d, J = 8.7 Hz, 2H), 4.136 (t, J = 13.2 Hz, 2H), 1.807-1.976 (m, 1H), 1.700-1.769 (m, 4H), 1.455-1.611 (m, 4H), 1.102-1.185 (m, 2H) [230].

Compound 19

5-[3-chloro-4-(2-cyclopentylethoxy)benzylidene]-1,3-thiazolidine-2,4-dione: (1.18 g, 84.9% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.992 (s, 1H), 7.720 (s, 1H), 7.689 (s, 1H), 7.545 (d, J = 11.1 Hz, 1H), 7.317 (d, J = 8.7 Hz, 1H), 4.161 (t, J = 13.2 Hz, 2H), 1.189-1.921 (m, 9H), 1.053-1.212 (m, 2H).

Compound 20

5-[4-(2-isopropoxyethoxy)-benzylidene]thiazolidine-2,4-dione : (1.26 g, 87.5% yield) as yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ8.04 (s, 1H), 7.805 (s, 1H), 7.432 (d, J = 9.0 Hz, 2H), 7.022(d, J = 9.0 Hz, 2H), 4.19 (t, J = 9.6 Hz, 2H), 3.84 (t, J = 9.6 Hz, 2H), 3.65-3.76 (m, 1H), 1.24 (d, J = 6.0 Hz, 6H) [230].

Compound 21

5-(3-chloro-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione: (1.21 g, 88.3% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.585 (s, 1H), 7.725 (s, 1H), 7.708 (d, J = 2.4 Hz, 1H), 7.536 (d, J = 11.1 Hz, 1H), 7.315-7.363 (m, 2H), 7.017 (d, J = 3.3 Hz, 1H), 6.978 (d, J = 8.4 Hz, 1H), 4.354 (t, J = 12.6 Hz, 2H), 3.322 (t, J = 12.6 Hz, 2H).

Compound 22

5-(4-(4-metylbenzyloxy)benzylidene)thiazolidine-2,4-dione: (1.15 g, 80% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.514 (s, 1H), 7.800 (s, 1H), 7.560 (d, J = 8.7 Hz, 2H), 7.346 (d, J = 7.8 Hz, 2H), 7.206 (d, J = 7.8 Hz, 2H), 7.165 (d, J = 8.7 Hz, 2H), 5.125 (s, 2H), 2.295 (s, 3H) [230].

Compound 23

5-[4-(4-methoxybenzyloxy)benzylidene]thiazolidine-2,4-dione: (1.24 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.523 (s, 1H), 7.897 (s, 1H) 7.470 (d, J = 8.7 Hz, 2H), 7.249 (d, J = 8.7 Hz, 2H), 7.307 (d, J = 8.4 Hz, 2H), 7.165 (d, J = 8.4 Hz, 2H), 5.055 (s, 2H), 3,785 (s, 3H) [231].

Compound 24

5-(3-chloro-4-(4-nitrobenzyloxy)benzylidene)thiazolidine-2,4-dione: (1.09 g, 81.3% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.680 (s, 1H), 8.407 (s, 1H), 8.302 (d, , J = 8.7 Hz, 2H), 7.760 (d, J = 8.7 Hz, 2H), 7.730(s, 1H), 7.561 (d, J = 8.4 Hz, 1H), 7.451(d, J = 8.4 Hz, 1H), 5.473 (s, 2H).

Compound 25

5-((4-(2-piridin-2-yl) ethoxy)benzylidene)thiazolidine-2,4-dione: (1.25 g, 88% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ10.36 (s, 1H), 8.452 (d, J = 4.8 Hz, 1H), 7.783 (s, 1H), 7.719-7.277 (m, 1H), 7.488 (d, J = 8.4 Hz, 2H), 7.277 (d, J = 7.8 Hz, 1H), 7.190-7.232 (m, 1H), 6.924 (d, J = 8.4 Hz, 2H), 4.169 (t, J = 14.7 Hz, 2H), 3.048 (t, J = 14.7 Hz, 2H) [230].

Compound 26

5-(4-(2-cyclohexylethoxy)benzylidene)-4-thioxothiazolidindin-2-one: (1.15 g, 81% yield) as yellow oil; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.758 (s, 1H) 7.499 (d, J = 14.7 Hz, 2H), 6.970 (d, J = 14.7Hz, 2H), 4.036 (t, J =11.7 Hz, 2H), 3.575 (s, 1H), 1.704 (t, J = 11.7 Hz, 2H), 1.565-1.756 (m, 5H), 1.434-1.528 (m, 1H), 1.103-1.260 (m, 3H), 0.907-1.029 (m, 2H) [230].

Compound 27

4-(2,3-dihydro-1,4-benzodioxin-2-ylmethoxy)benzaldehyde ammoniate: (1.25g, 77.2% yield) as yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.904 (s, 1H), 7.880 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 6.843-6.941 (m, 4H), 4.556-4.643 (m, 1H), 4.386-4.433 (m, 1H), 4.311-4.361 (m, 1H), 4.061-4.279 (m, 2H) [230].

Compound 28

5-[4-(benzo[d][1,3]dioxol-5-ylmethoxy)benzylidene]thiazolidine-2,4-dione: (1.22 g, 84% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.498 (s,

1H), 7.722 (s, 1H), 7.558 (d, J = 9.0 Hz, 2H), 7.157 (d, J = 9.0 Hz, 2H), 7.014 (s, 1H), 6.962 (d, J = 8.1 Hz, 2H), 6.919 (d, J = 8.1 Hz, 1H), 6.011 (s, 2H), 5.059 (s, 2H) [230].

Compound 29

5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione: (1.25 g, 87% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.12 (s, 1H), 7.73 (s, 1H), 7.558 (d, J = 8.7 Hz, 2H), 7.354 (d, J = 6.0 Hz, 1H), 7.12 (d, J = 8.7 Hz, 2H), 6.942-6.973 (m, 2H), 4.28 (t, J = 12.6 Hz, 2H), 3.285 (t, J = 12.6 Hz, 2H) [230].

Compound 30

5-(3-chloro-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione: (1.21 g, 88.3% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.585 (s, 1H), 7.725 (s, 1H), 7.708 (d, J = 2.4 Hz, 1H), 7.536 (d, J = 11.1 Hz, 1H), 7.315-7.363 (m, 2H), 7.017 (d, J = 3.3 Hz, 1H), 6.978 (d, J = 8.4 Hz, 1H), 4.354 (t, J = 12.6 Hz, 2H), 3.322 (t, J = 12.6 Hz, 2H).

Compound 31

5-(3-methoxy-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione: (1.22 g, 88.4% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.438 (s, 1H), 7.722 (s, 1H), 7.357 (d, J = 6.6 Hz, 1H), 7.239 (s, 1H), 7.197 (d, J = 14.4 Hz, 2H), 6,946-6.994 (m, 2H), 4.265 (t, J = 6.6 Hz, 2H), 3.803 (s, 3H), 3.269 (t, J = 6.6 Hz, 2H).

Compound 32

O
O
NH
S
O
O
S

4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl thiophene-2-carboxylate: (1.15g, 85.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.529 (s, 1H), 10.300 (s, 1H), 7.706 (t, J = 8.4 Hz, 2H), 7.298 (t, J = 4.8 Hz, 2H), 6.913 (d, J = 8.4 Hz, 2H).

Compound 33

O
NH
S
O
O
S

5-[4-(Thiophen-3-ylmethoxy)benzylidene]thiazolidine-2,4-dione: (1.24 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.504 (s, 1H), 8.590 (s, 1H), 7.864 (d, J = 7.8 Hz, 1H), 7.733 (s, 1H), 7.573 (d, J = 8.7 Hz, 2H), 7.521 (d, J = 7.8 Hz, 1H), 7.197 (d, J = 8.7 Hz, 2H), 5.252 (s, 2H).

Compound 34

5-(4-(2-Thiophen-3-yl)ethoxy)benzylidene)thiazolidine-2,4-dione: (1.24 g, 86% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.12 (s, 1H), 7.69 (s, 1H), 7.556 (d, *J* = 11.7 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.305 (d, *J* = 7.8Hz, 1H), 7.11 (d, *J* = 11.7 Hz, 2H), 7.088(s, 1H), 4.28(t, *J* = 13.8 Hz, 2H), 3.07 (t, *J* = 13.8 Hz, 2H).

Compound 35

2-chloro-4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl thiophene-2-carboxylate: (1.15g, 85.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.695 (s, 1H), 8.173 (s, 1H), 7.730 (s, 1H), 7.636 (s, 1H), 7.374 (d, *J* = 5.7 Hz, 2H), 7.121 (d, *J* = 8.4 Hz, 1H), 6.968 (d, *J* = 7.8 Hz, 1H).

Compound 36

5-[4-(furan-2-ylmethoxy) benzylidene] thiazolidine-2,4-dione: (1.26 g, 87% yiel d) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.518 (s, 1H), 7.747 (s, 1H), 7.705 (t, *J* = 1.8 Hz, 1H), 7.572 (d, *J* = 8.4 Hz, 1H), 7.200 (d, *J* = 8.4 Hz, 2H), 6.630 (d, *J* = 3.0 Hz, 1H), 6.481 (d, *J* = 1.8 Hz, 1H), 5.142 (s, 2H) [230].

Compound 37

5-{3-chloro-4-[2-(5-nitrofuran-2-yl)ethoxy]benzylidene}-1,3-thiazolidine-2,4-dione: (1.15g, 79.2% yield) as yellow solid;^{1}H NMR (300 MHz, DMSO): δ11.099 (s, 1H), 8.978 (s, 1H), 7.634 (d, *J*=2.1, 1H), 7.406 (s, 1H), 7.116 (d, *J*=8.4, 1H), 4.007 (d, *J*=6.9, 4H), 1.096-1.185 (m, 4H).

Compound 38

5-(4-(2-cyclohexylethoxy)-3-ethoxybenzylidene)thiazolidine-2,4-dione: (1.12 g, 83.0% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.582 (s, 1H), 7.7 86 (s, 1H), 7.116 (d, *J* = 10.8 Hz, 1H), 6.959 (d, *J* = 10.8 Hz, 1H), 7.000 (s, 1H), 4.089 (m, 4H), 1.653-1.795 (m, 7H), 1.459-1.577 (m, 4H), 1.146-1.282 (m, 3H), 0.941-1.05 (m, 2H) [230].

Compound 39

5-(4-phenoxybenzyl)-1,3-thiazolidine-2,4-dione: (1.2 g, 83% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.498 (s, 1H), 7.736 (s, 1H) 7.566 (d, *J* = 8.7 Hz, 2H), 7.306-7.468 (m, 5H), 7.18 (d, *J* = 8.7 Hz, 2H), 5.176 (s, 2H) [230].

Compound 40

5-(2-chloro-3-(cyclohexyloxy)benzylidene)thiazolidine-2,4-dione: (1.12 g, 78.9% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.587 (s, 1H), 7.724 (s, 1H), 7.524 (d, J = 9.3 Hz, 1H), 7.315 (t, 1H), 7.195 (d, J = 9.3 Hz, 1H), 4.310-4.389 (m, 1H), 1.933-2.044 (m, 2H), 1.734-1.822 (m, 2H), 1.491-1.674 (m, 2H), 1.208-1.446 (m, 2H), 0.860-0.946 (m, 2H).

Compound 41

5-(2-chloro-3-(cyclohexylmethoxy)benzylidene)thiazolidine-2,4-dione: (1.06 g, 76.3% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.721 (s, 1H), 7.514 (d, J = 9.3 Hz, 1H), 7.138-7.327(m, 1H), 7.142 (d, J = 9.3 Hz, 1H), 3.862 (d, J = 6.0 Hz, 2H), 1.7.3-1.933 (m, 6H), 1.088-1.579 (m, 5H).

Compound 42

5-(3-(2-cyclohexylethoxy)-4-nitrobenzylidene)thiazolidine-2,4-dione: (1.24 g, 87% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.644 (s, 1H), 8.003 (d, J = 8.4 Hz, 1H), 7.799 (s, 1H), 7.611 (s, 1H), 7.273 (s, J = 8.4 Hz, 1H), 4.255 (t,

J = 13.5 Hz, 2H), 1.618-1.746 (m, 7H), 1.401-1.546 (m, 1H), 1.144-1.323 (m, 3H), 0.928-0.1.006 (m, 2H) [230].

Compound 43

5-(2-chloro-3-(2-cyclohexylethoxy)-4-methoxybenzylidene)thiazolidine-2,4-dione : (1.04 g, 78.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.743 (s, 1H), 7.319 (s, J = 8.7 Hz, 1H), 7.247 (s, J = 8.7 Hz, 1H), 4.007 (t, J = 12.6 Hz, 2H), 3.890 (s, 3H), 1.516-1.764 (m, 8H), 1.109-1.245 (m, 3H), 0.894-0.970 (m, 2H) [230].

Compound 44

5-(2-chloro-3-(cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione: (1.00 g, 74.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.943 (s, 1H), 7.521 (d, J = 9.3 Hz, 1H), 7.261-7.333 (m, 1H), 7.152 (d, J = 9.3 Hz, 1H), 4.045 (t, J = 13.2 Hz, 2H), 1.829-1.925 (m, 2H), 1.578-1.731 (m, 5H), 1.091-1.426 (m, 6H), 0.859-0.883 (m, 2H).

Compound 45

5-(2-chloro-3-(cyclohexylbutoxy)benzylidene)thiazolidine-2,4-dione: (1.12 g, 83.6% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.894 (s, 1H), 7.496 (d, J = 9.3 Hz, 1H), 7.264-7.342 (m, 1H), 7.203 (d, J = 9.3 Hz, 1H), 4.103 (t, J = 13.2 Hz, 2H), 1.839-1.913 (m, 2H), 1.569-1.726 (m, 5H), 1.089-1.436 (m, 8H), 0.912-0.998 (m, 2H).

Compound 46

5-[2-chloro-3-(cyclopentyloxy)benzylidene]-1,3-thiazolidine-2,4-dione: (1.15g, 79.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO): δ8.232 (s, 1H), 7.582 (d, J=6.0, 1H), 7.748 (t, J=4.5, 1H), 7.169 (d, J=6.6, 1H), 4.842 (s, 1H), 2.175 (s, 3H), 1.590-1.642 (s, 5H).

Compound 47

5-(3-(2-cyclohexylethoxy)-4-methoxybenzylidene)thiazolidine-2,4-dione: (1.26 g, 91.3% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.706 (s, 1H), 7.361 (d, J = 6.3 Hz, 1H), 7.232 (d, J = 1.8 Hz, 1H), 7.171 (d, J = 1.8 Hz, 1H), 7.112 (s, 1H), 6,952-6.994 (m, 2H), 4.237 (t, J = 13.2 Hz, 2H), 3.822 (s, 3H),

3.294 (t, J = 13.2 Hz, 2H).

Compound 48

2-chloro-3-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl thiophene-2-carboxylate: (1.15g, 85.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.781 (s, 1H), 10.614 (s, 1H), 7.915(s, 1H), 7.876 (s, 1H), 7.625 (d, J = 4.5 Hz, 2H), 7.550 (d, J = 4.8 Hz, 1H), 7.344(t, J = 3.0 Hz, 1H), 7.085(d, J = 7.2 Hz, 1H).

Compound 49

5-(3-(benzyloxy)-2-chlorobenzylidene)thiazolidine-2,4-dione: (1.05 g, 75% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.753 (s, 1H), 7.498 (d, J = 7.8 Hz, 1H), 7.339-7.413 (m, 6H), 7.241 (d, J = 7.8 Hz, 1H), 5.243 (s, 2H).

Compound 50

5-(2-chloro-4-(cyclohexyloxy)benzylidene)thiazolidine-2,4-dione: (1.01 g, 71.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ10.581 (s, 1H), 7.658 (s, 1H), 7.529 (s, 1H), 7.398 (d, J = 8.4 Hz, 1H), 7.273 (d, J = 8.4 Hz, 1H), 1.451-

1.465 (m, 11H).

Compound 51

5-(2-chloro-4-(3-cyclohexylmethoxy)benzylidene)thiazolidine-2,4-dione: (1.05 g, 75.5% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.637 (s, 1H), 7.838 (s, 1H), 7.656 (d, J = 8.7 Hz, 1H), 7.223 (s, 1H), 7.110 (d, J = 8.7 Hz, 1H), 3.881 (d, J = 6.0 Hz, 2H), 1.641-1.797 (m, 6H), 1.194-1.263 (m, 3H), 1.002-1.039 (m, 2H).

Compound 52

5-(4-(2-cyclohexylethoxy)-2-methoxybenzylidene)thiazolidine-2,4-dione: (1.15 g, 83.9% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.446 (s, 1H), 7.915 (s, 1H), 7.907 (d, J = 8.4 Hz, 1H), 6.709 (d, J = 8.4 Hz, 1H), 6.667 (s, 1H), 4.102 (t, J = 5.1 Hz, 2H), 3.878 (s, 3H), 1.587-1.750 (m, 7H), 1.451-1.586 (m, 1H), 1.104-1.271 (m, 3H), 0.884-0.994 (m, 2H) [230].

Compound 53

5-(2-chloro-4-(3-cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione: (0.98 g, 72.6% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.785 (s, 1H), 7.439 (s, 1H), 7.654 (d, J = 11.7 Hz, 1H), 7.515 (d, J = 11.7 Hz, 1H), 3.014 (t, J = 7.2 Hz, 2H), 1.135-1.642 (m, 4H), 1.029-1.222 (m, 7H), 0.822 - 1.127 (m, 4H).

Compound 54

5-(2-chloro-4-(3-cyclohexylbutoxy)benzylidene)thiazolidine-2,4-dione: (1.02 g, 76% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.649(s, 1H), 7.880 (s, 1H), 7.497 (s, 1H), 7.497 (d, J = 9.3 Hz, 1H), 7.515 (d, J = 9.3 Hz, 1H), 3.014 (t, J = 7.2 Hz, 2H), 1.653-1.724 (m, 4H), 1.315-1.642 (m, 4H), 1.069-1.215 (m, 7H), 0.820 – 0.852 (m, 2H).

Compound 55

5-{2-chloro-4-[2-(cyclohexyloxy)ethoxy]benzylidene}-1,3-thiazolidine-2,4-dione: (1.15g, 79.2% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ8.176 (s, 1H), 7.211 (d, J=9, 1H), 7.026 (d, J=2.4, 1H), 6.920 (d, J=10.5, 1H), 4.122 (t, J=6.9, 2H), 3.843 (t, J=9, 2H), 1.930 (s, 3H), 1.764(d, J=5.7, 3H), 1.253-1.311 (m, 4H).

Compound 56

5-(4-(benzyloxy)-2-chlorobenzylidene)thiazolidine-2,4-dione: (0.98 g, 70.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ7.850 (s, 1H), 7.561 (d, J = 9.9 Hz, 1H), 7.332-7.499 (m, 6H), 7.221 (d, J = 9.9 Hz, 1H), 5.224 (s, 2H).

Compound 57

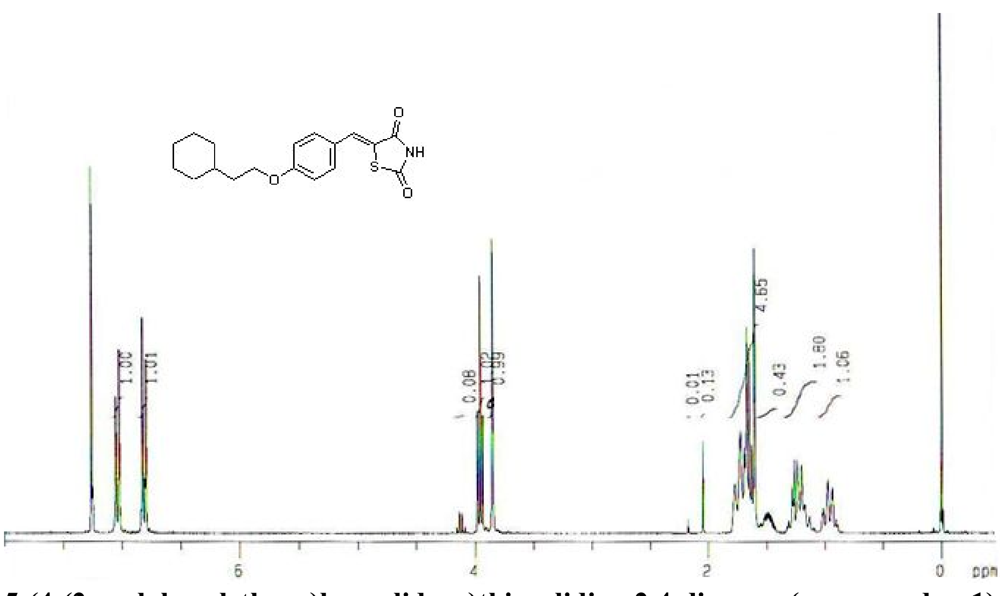
5-(2-chloro-4-phenethoxybenzylidene)thiazolidine-2,4-dione: (0.96 g, 69.6% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.645 (s, 1H), 7.863 (s, 1H), 7.500 (d, J = 7.8 Hz, 1H), 7.188-7.274 (m, 6H), 7.127 (d, J = 7.8 Hz, 1H), 4.323 (t, J = 13.5 Hz, 2H), 3.064 (t, J = 13.8 Hz, 2H).

Compound 58

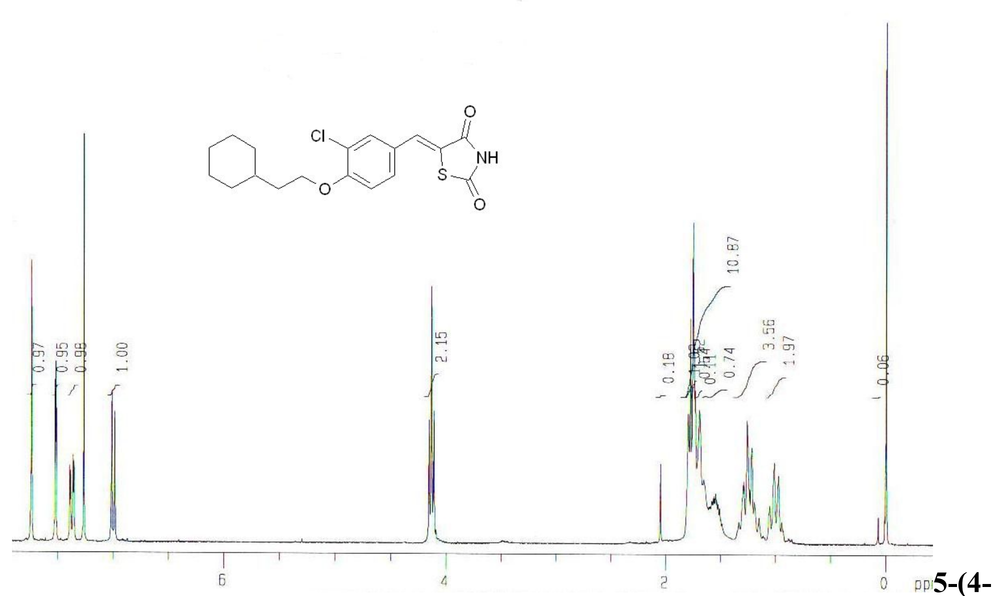
5-(2-chloro-4-phenbutoxybenzylidene)thiazolidine-2,4-dione: (0.98 g, 73.1% yield) as yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ12.64 (s, 1H), 7.81 (s, 1H), 7.511 (d, J = 9.3 Hz, 1H), 7.161-7.296(m, 6H), 7.105 (d, J = 9.3 Hz, 1H), 4.084 (t, J = 12.9 Hz, 2H), 2.654 (t, J = 13.8 Hz, 2H), 1.653-1.724 (m, 4H).

ANNEX-II

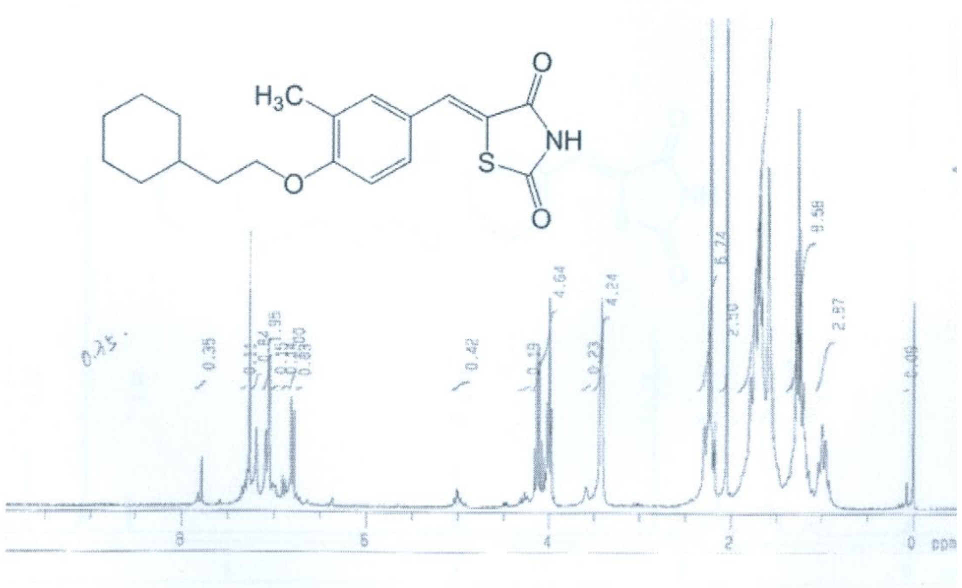
^{1}HNMR SPECTRA



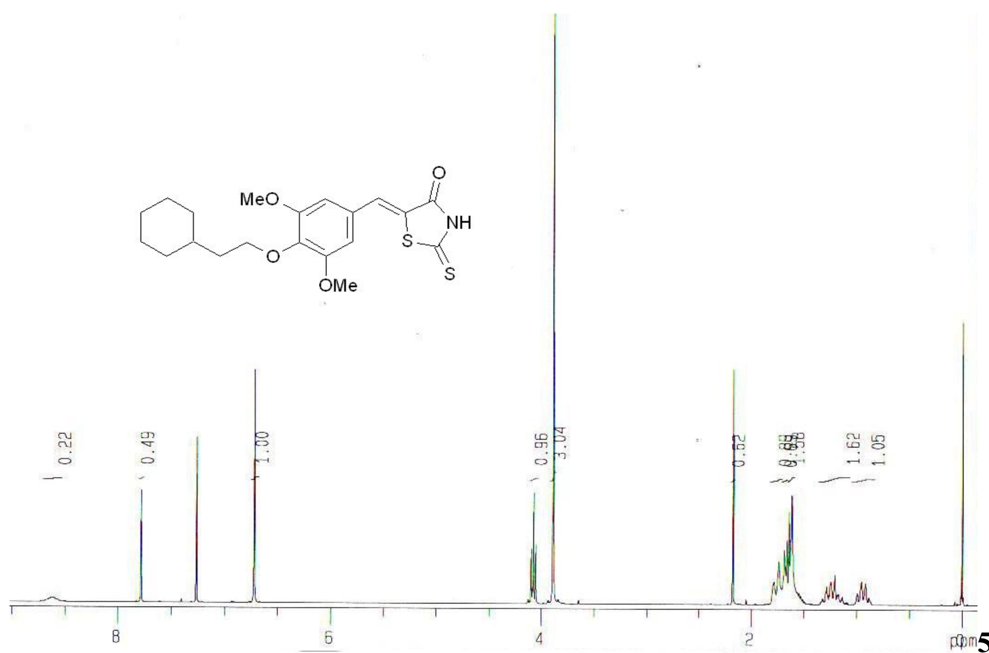
5-(4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione (compound 1) [230]



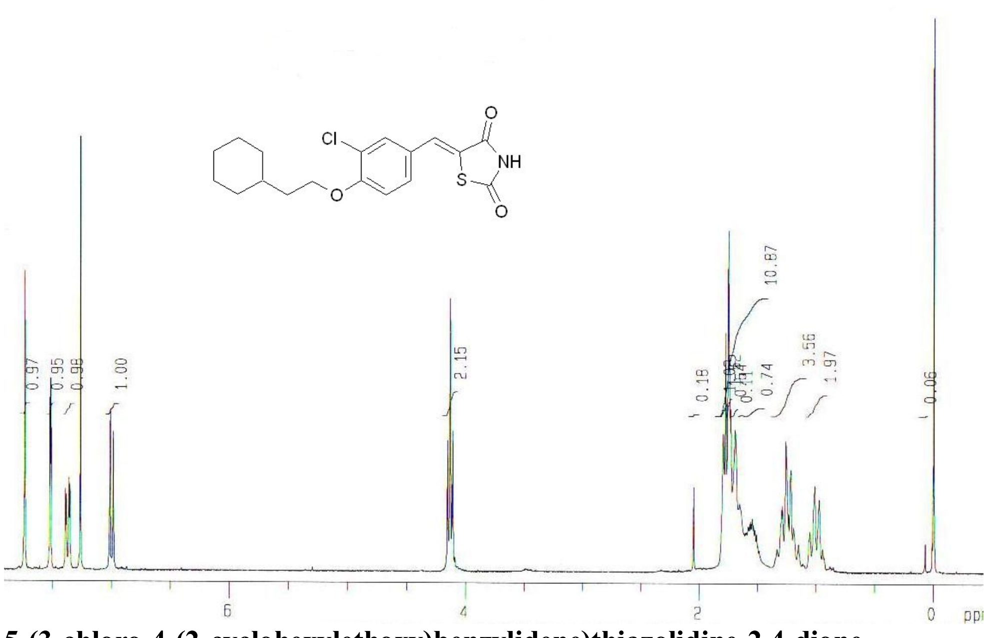
5-(4-(2-cyclohexylethoxy)-3-methylbenzylidene)thiazolidine-2,4-dione (compound 2) [230]



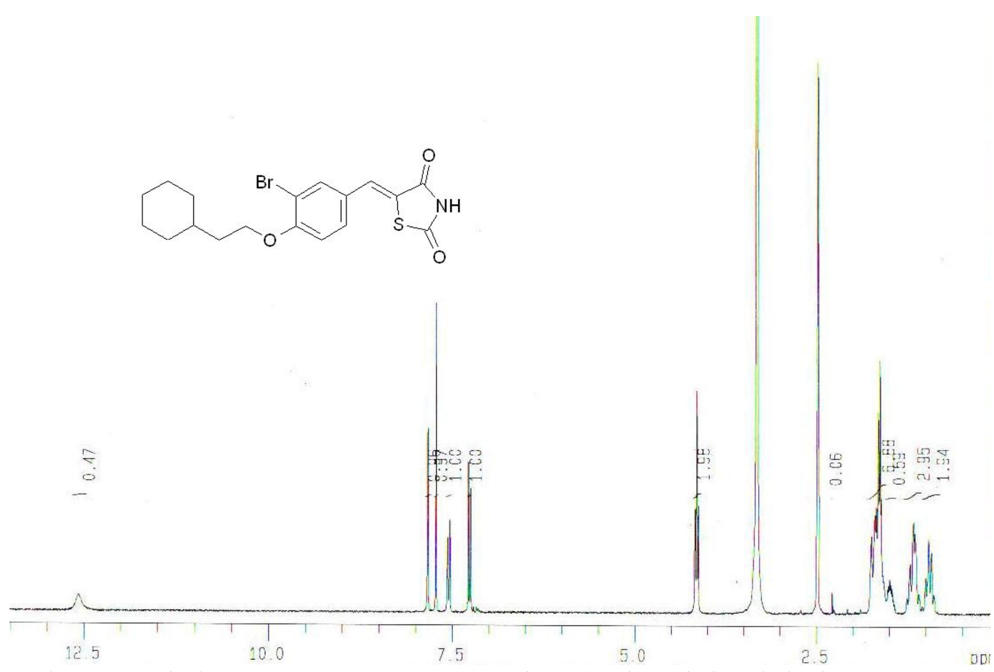
5-(4-(2-cyclohexylethoxy)-3-methoxybenzylidene)thiazolidine-2,4-dione (Compound 3) [231]



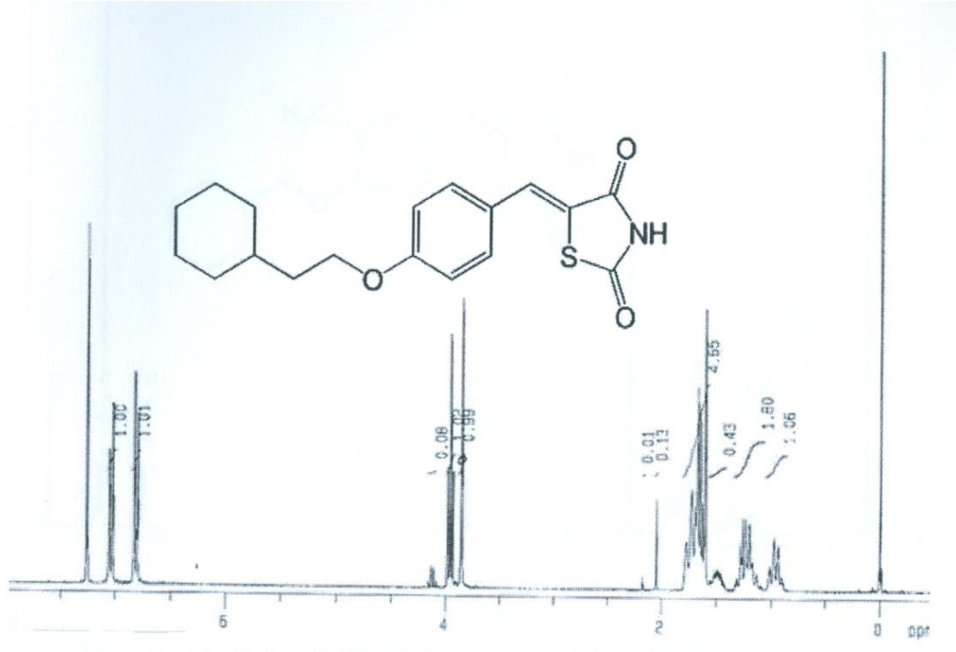
5-(4-(2-cyclohexylethoxy)-3,5-dimethoxybenzylidene)thiazolidine-2,4-dione (compound 4) [230]



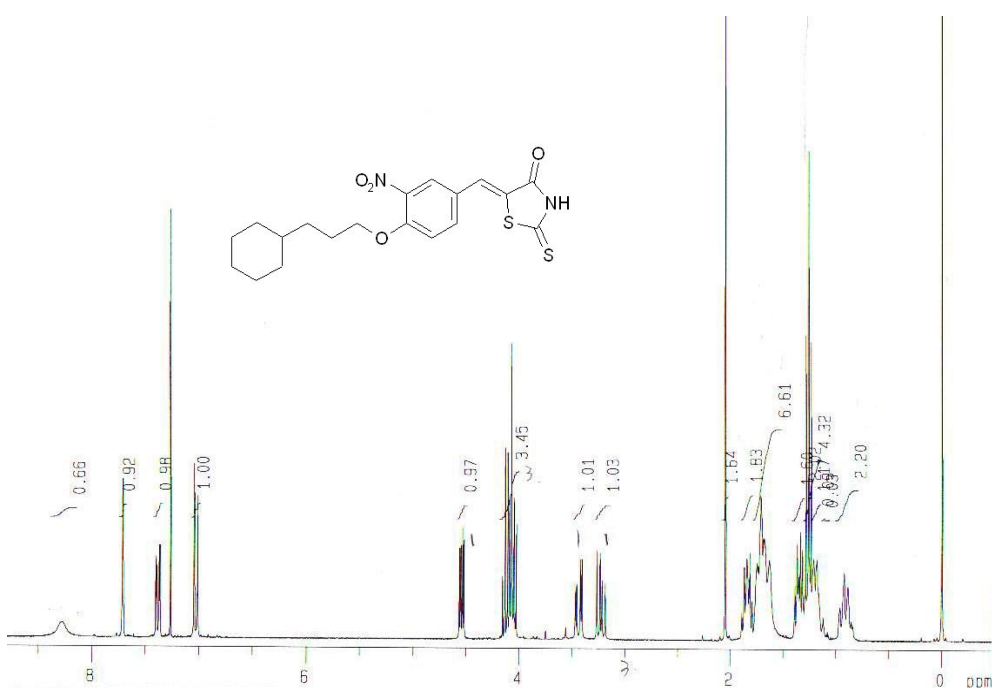
5-(3-chloro-4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione (compound 5) [230]



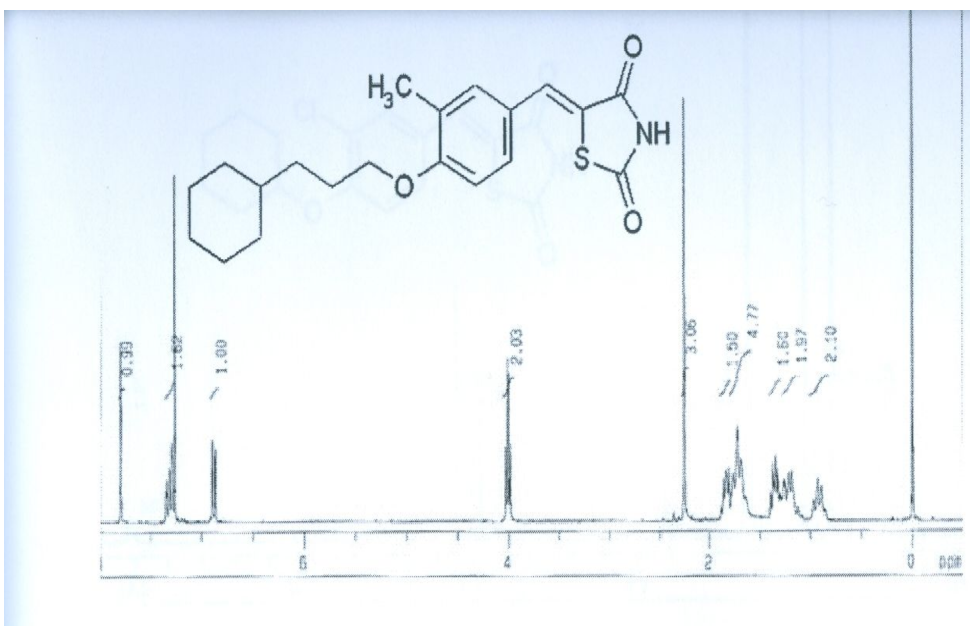
5-(3-bromo-4-(2-cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione (compound 6) [230]



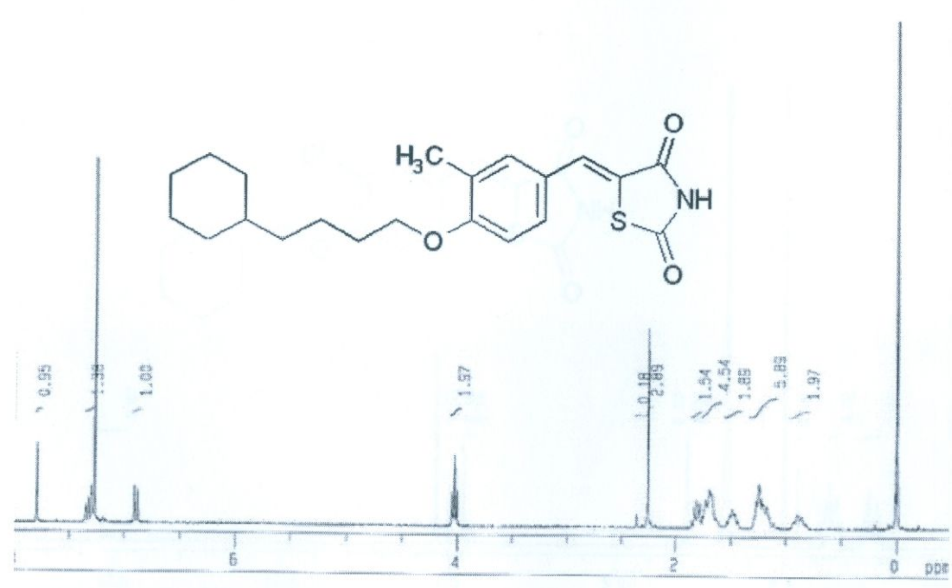
3-(4-(2-cyclohexylethoxy)benzylidene)pyrrolidine-2,5-dione (compound 8) [231]



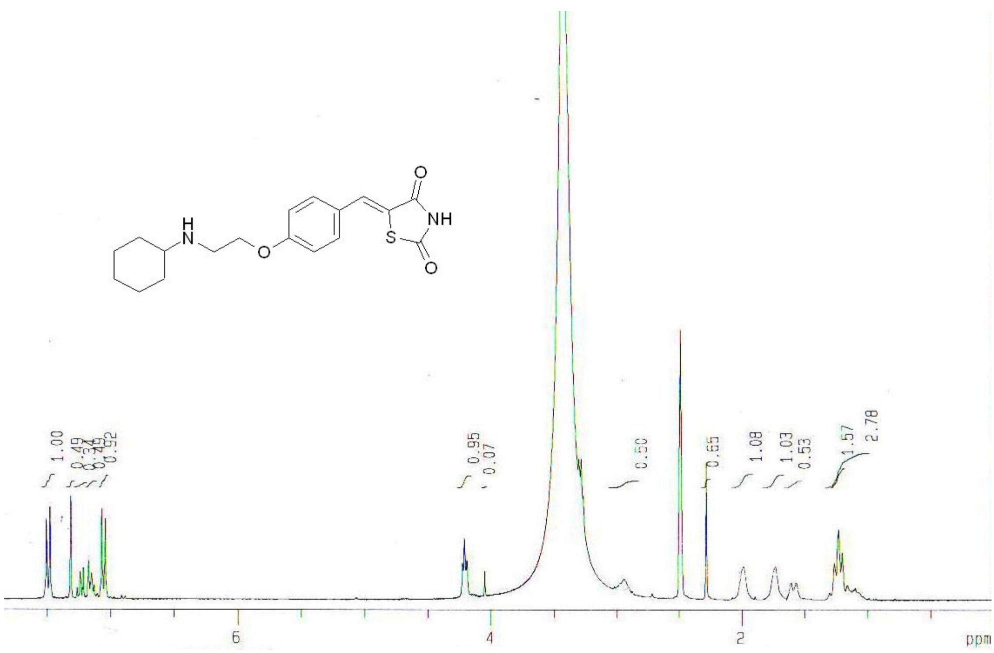
5-(4-(3-cyclohexylpropoxy)-3-nitrobenzylidene)thiazolidine-2,4-dione (compound 9) [230]



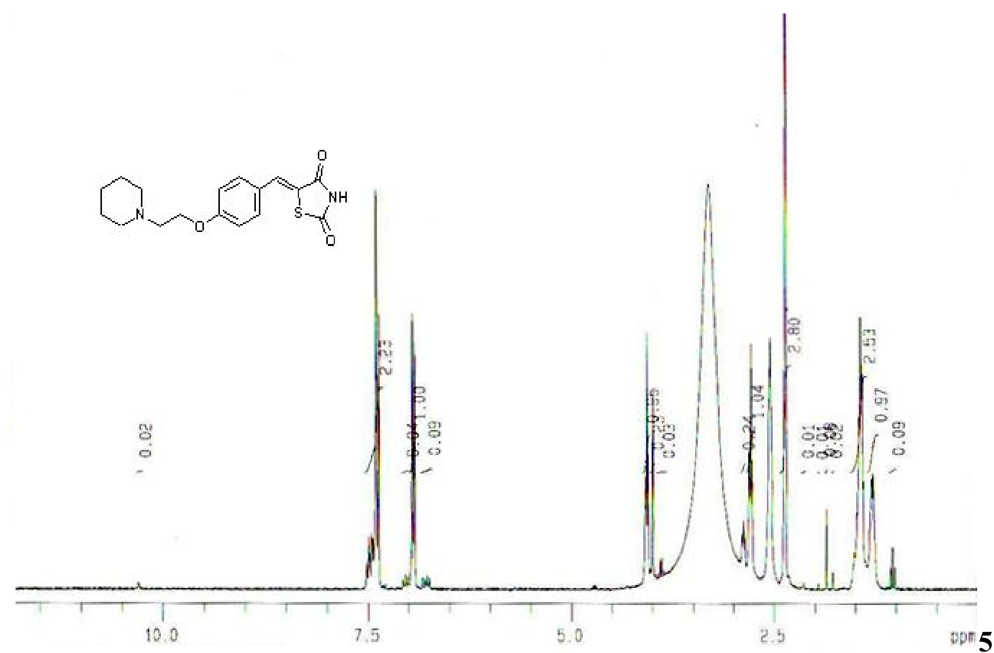
5-(4-(2-cyclohexylpropoxy)-3-methoxybenzylidene)thiazolidine-2,4-dione (compound 10) [231]



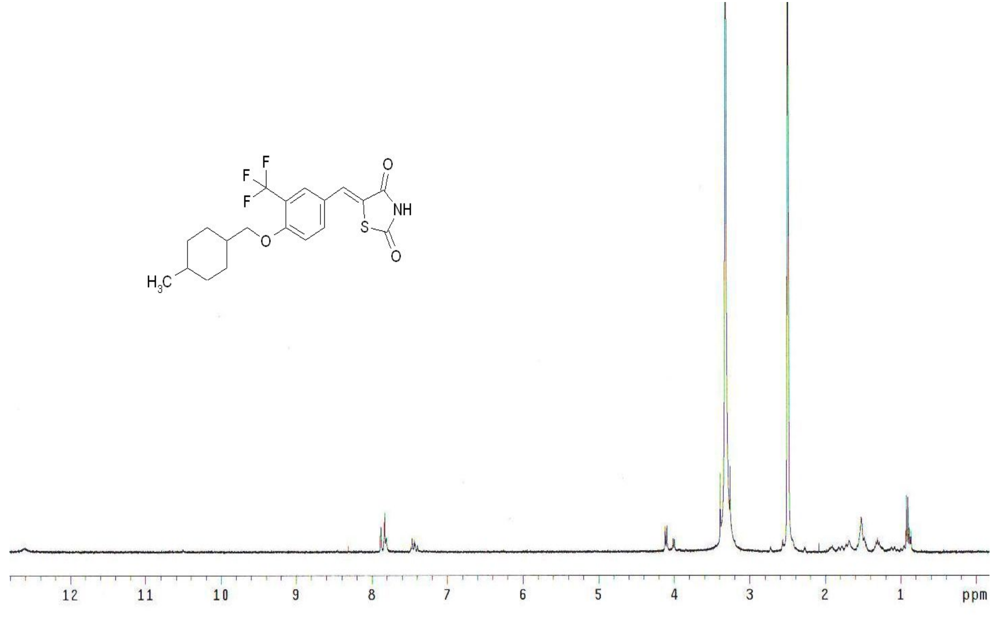
5-(4-(2-cyclohexylbutoxy)-3-methylbenzylidene)thiazolidine-2,4-dione (compound 11) [231]



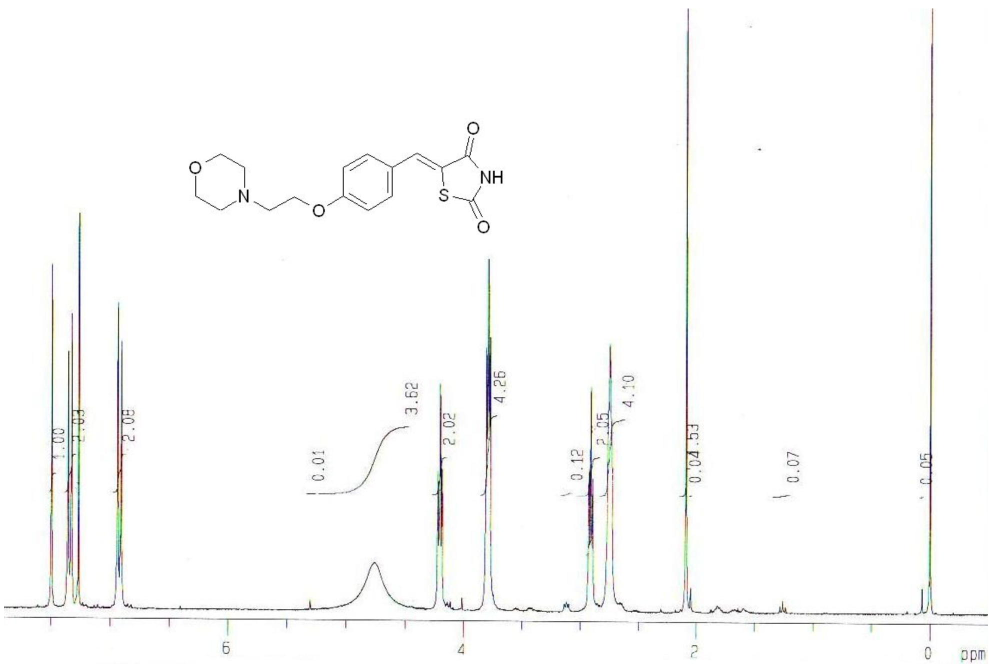
5-(4-(2-cyclohexylamino)ethoxy)benzylidene)thiazolidine-2,4-dione (compound 13) [230]



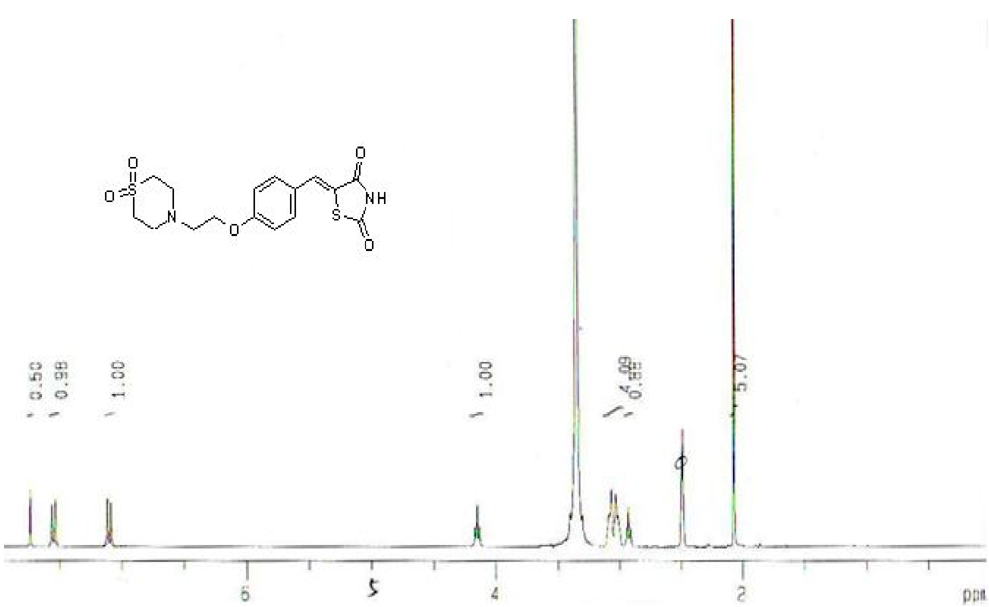
5-[4-(2-(piperidin-1-yl)ethoxy)benzylidene]thiazolidine-2,4-dione (compound 14) [230]



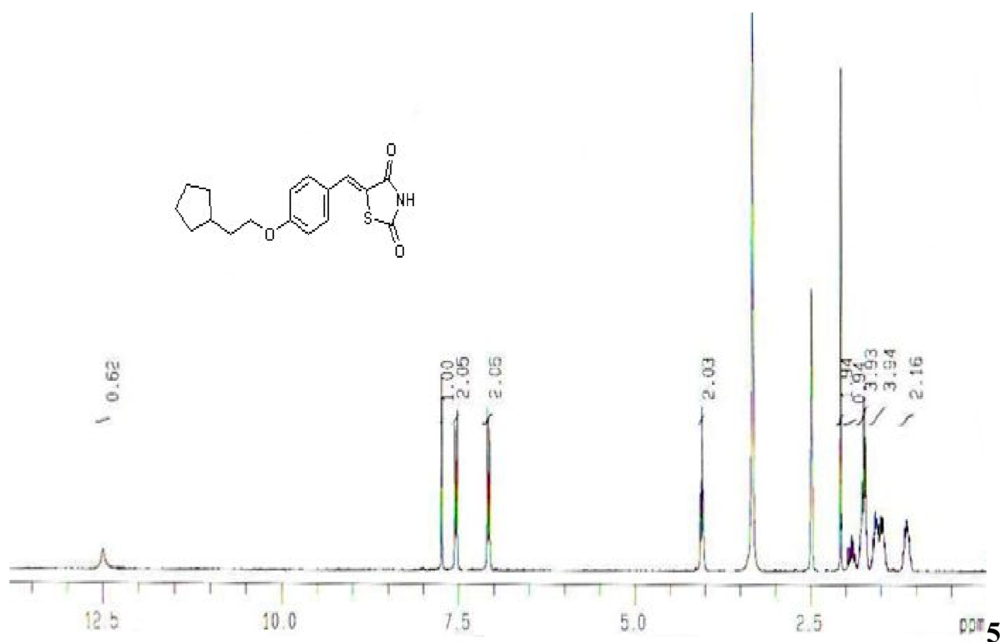
5-[4-((4-metylcyclohexyl)methoxy)-3-(triflurometyl)benzylidene]thiazolidine-2,4-dione (compound 15) [230]



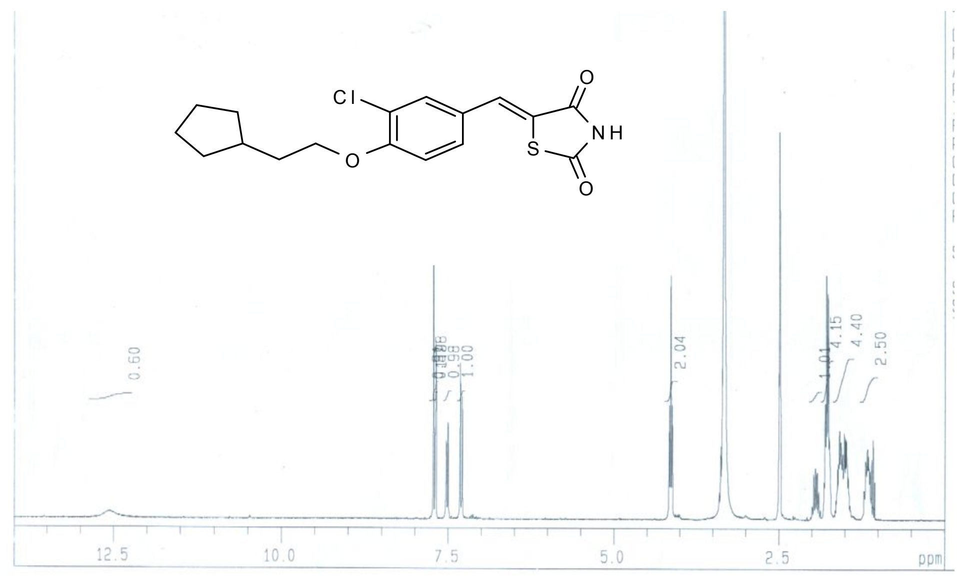
5-4-(2-morpholinoethoxy)benzylidene] thiazolidine-2,4-dione (compound 16) [230]



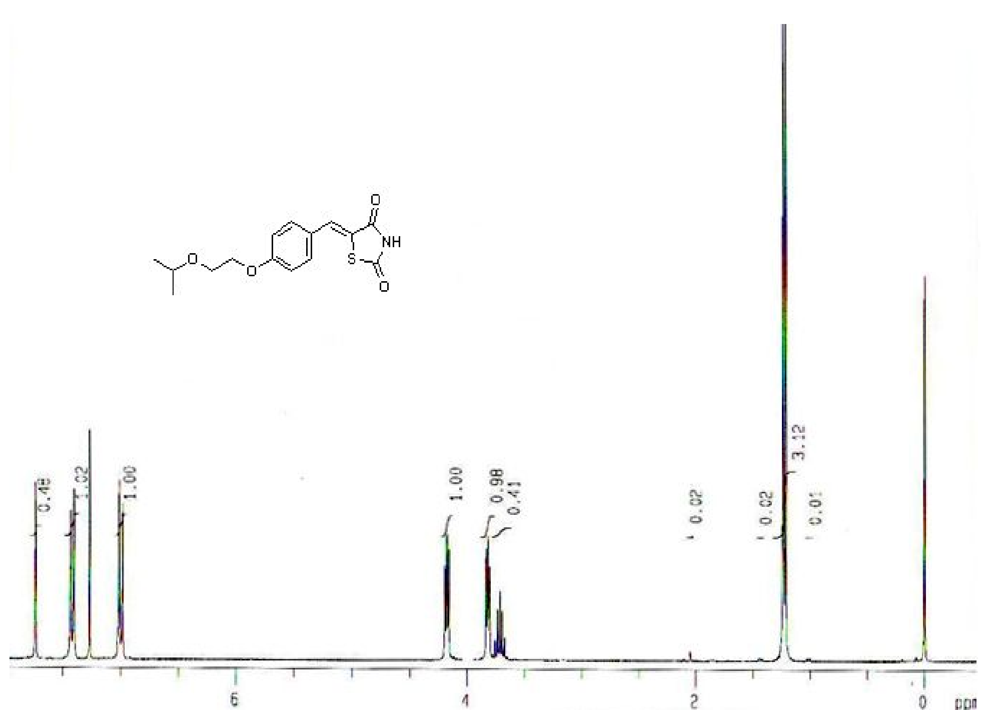
5-(4-(2-Thiomorpholine 1,1-Dioxideethoxy)benzylidene)-2,4-thiazolidinedione (compound 17) [230]



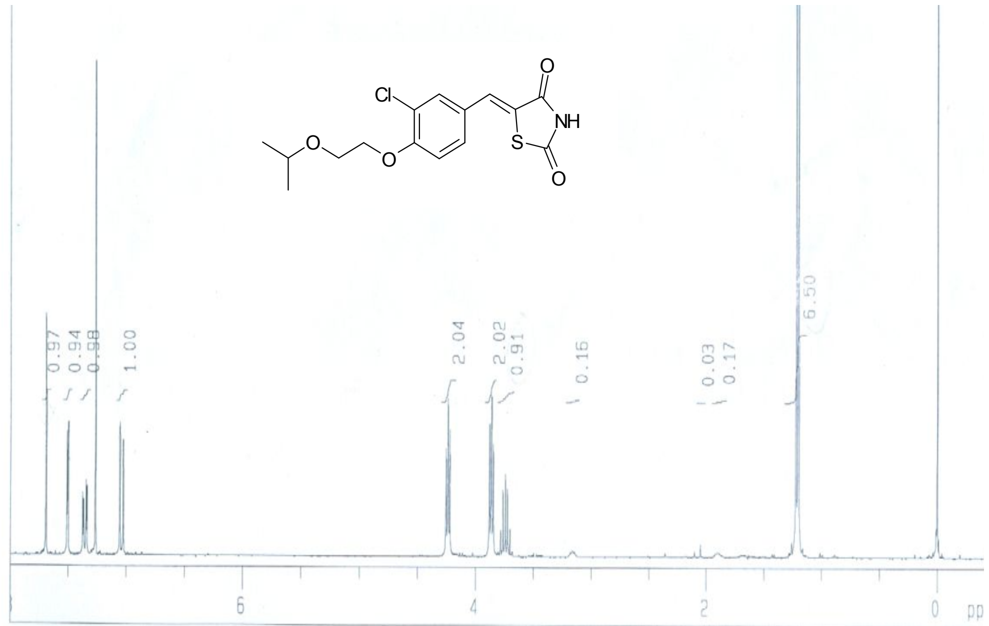
5-(4-(2-cyclopentylethoxy)benzylidene)thiazolidine-2,4-dione (compound 18)
[230]



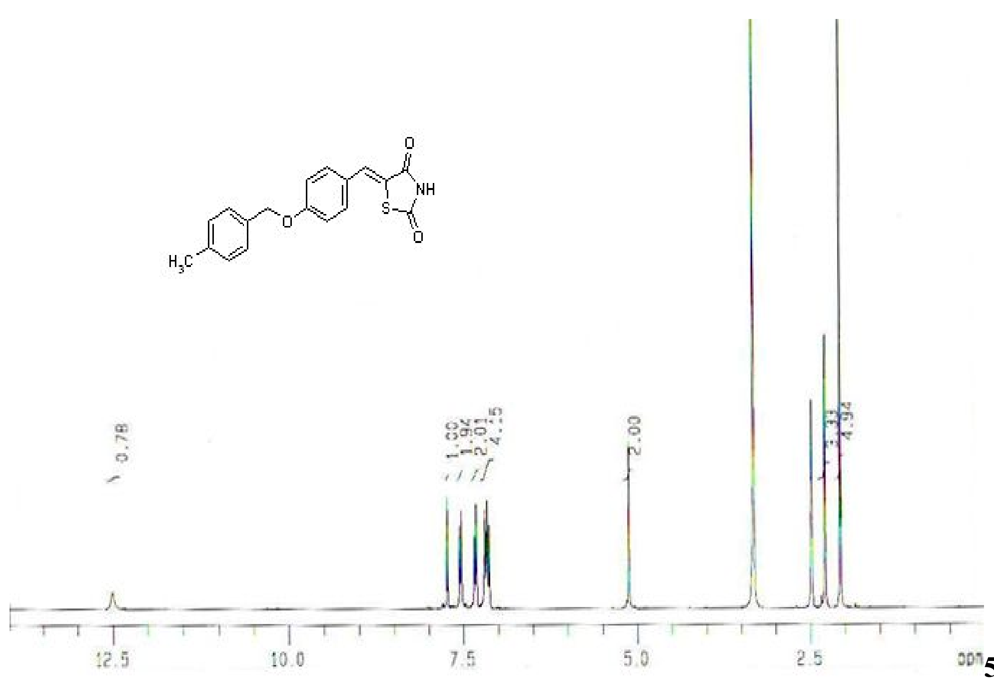
5-[3-chloro-4-(2-cyclopentylethoxy)benzylidene]-1,3-thiazolidine-2,4-dione (compound 19)



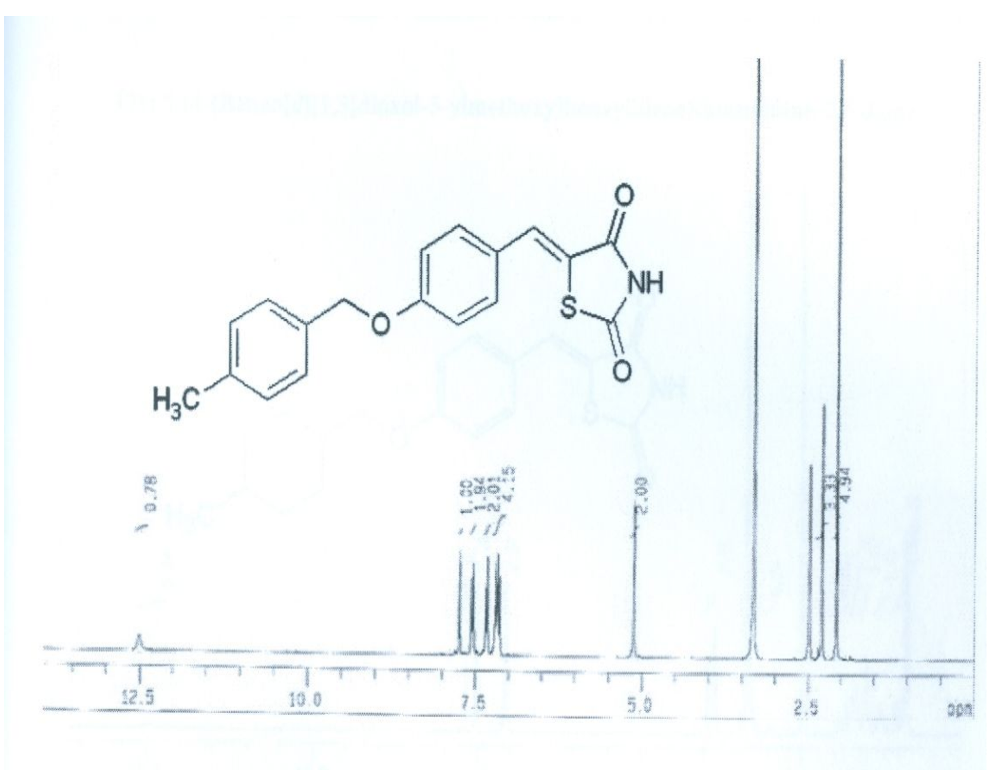
5-[4-(2-isopropoxyethoxy)-benzylidene]thiazolidine-2,4-dione (compound 20)
[230]



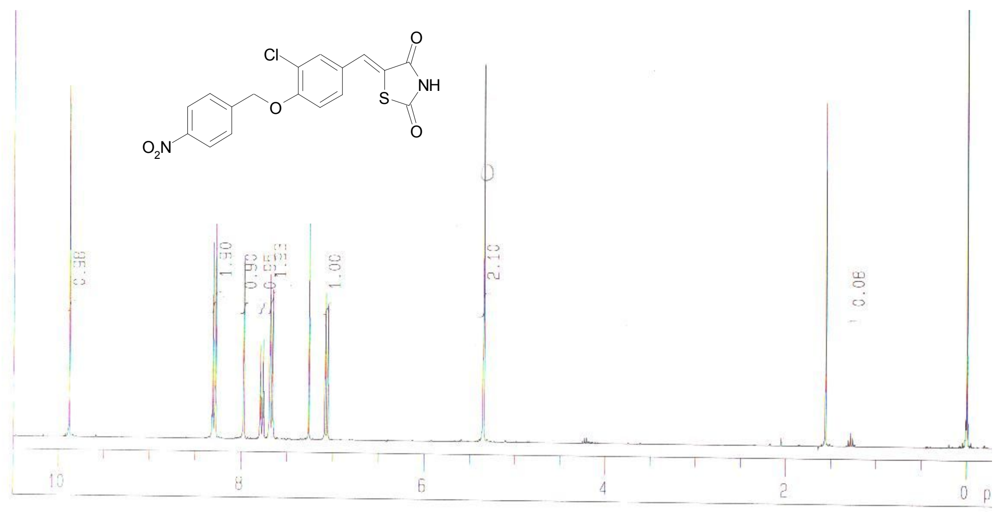
5-(3-chloro-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (compound 21)



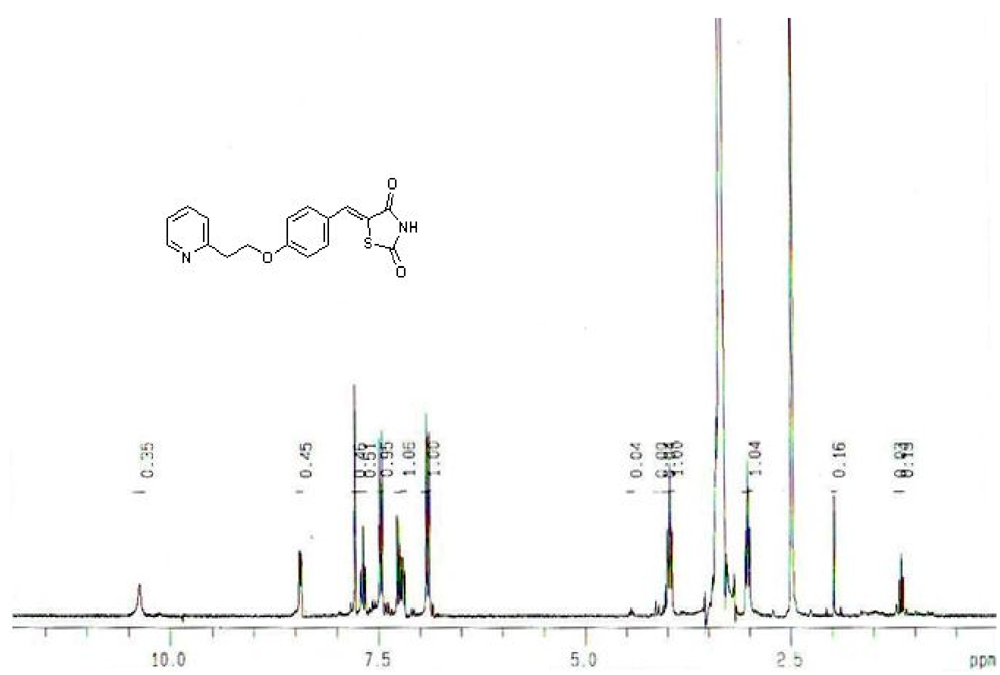
5-(4-(4-metylbenzyloxy)benzylidene)thiazolidine-2,4-dione (compound 22) [230]



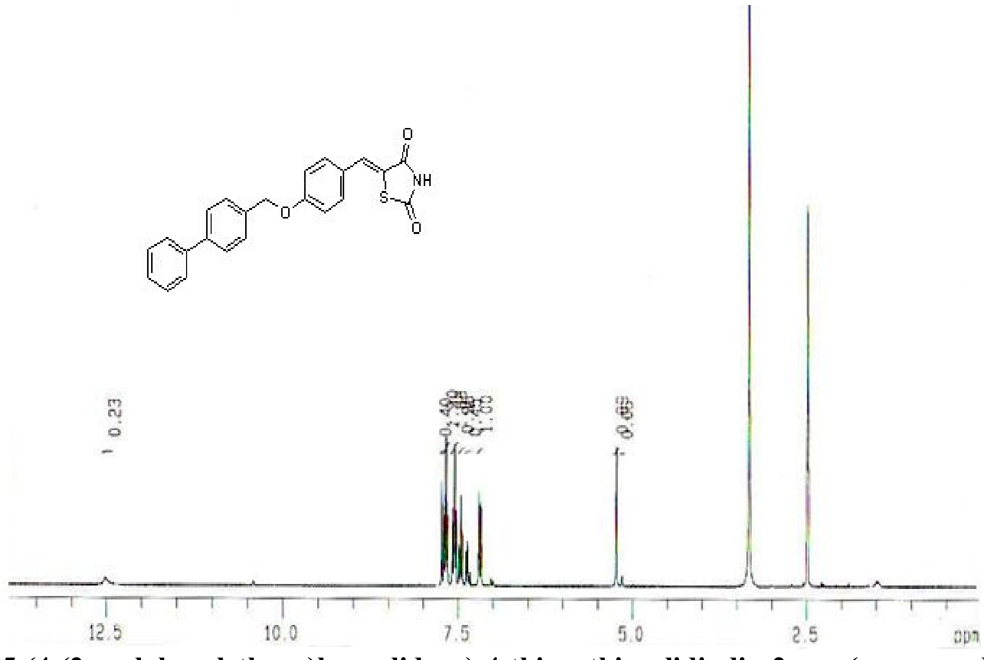
5-[4-(4-methoxybenzyloxy)benzylidene]thiazolidine-2,4-dione (compound 23) [231]



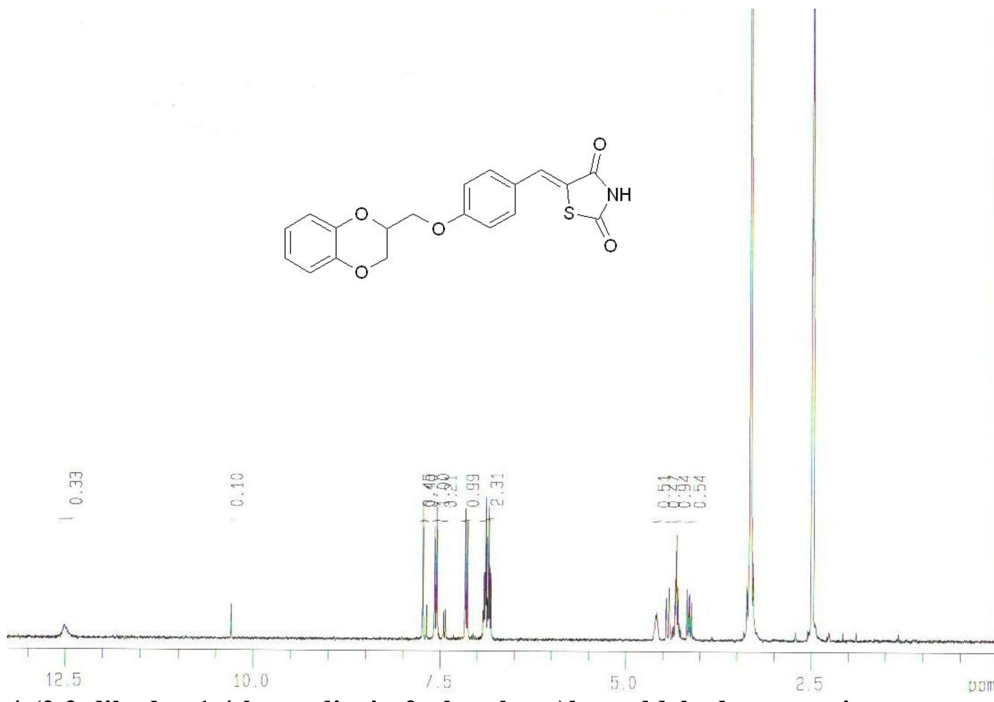
5-(3-chloro-4-(4-nitrobenzyloxy)benzylidene)thiazolidine-2,4-dione (compound 24) [230]



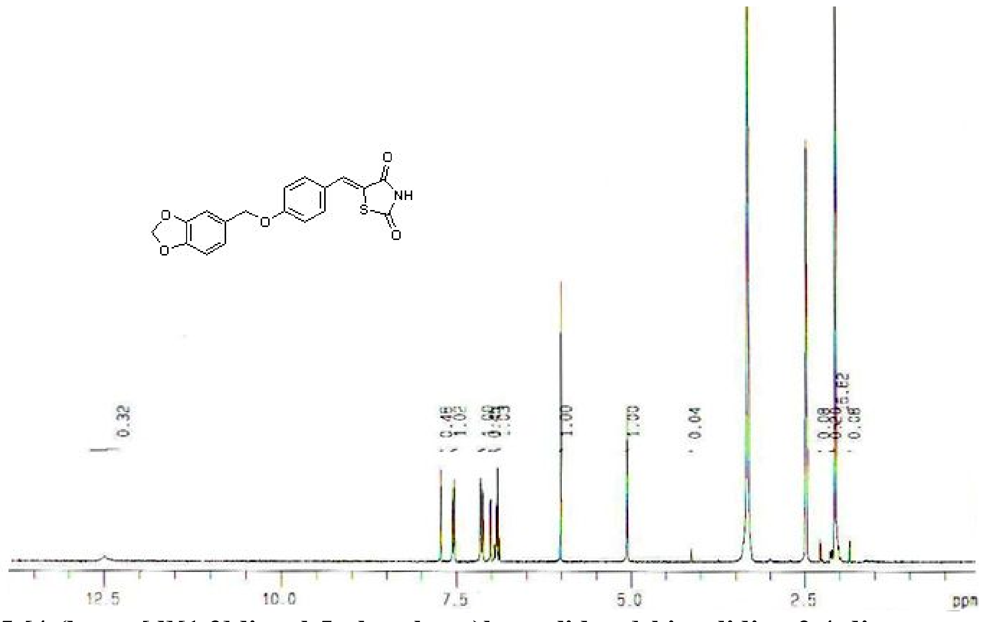
5-((4-(2-piridin-2-yl) ethoxy)benzylidene)thiazolidine-2,4-dione (compound 25) [230]



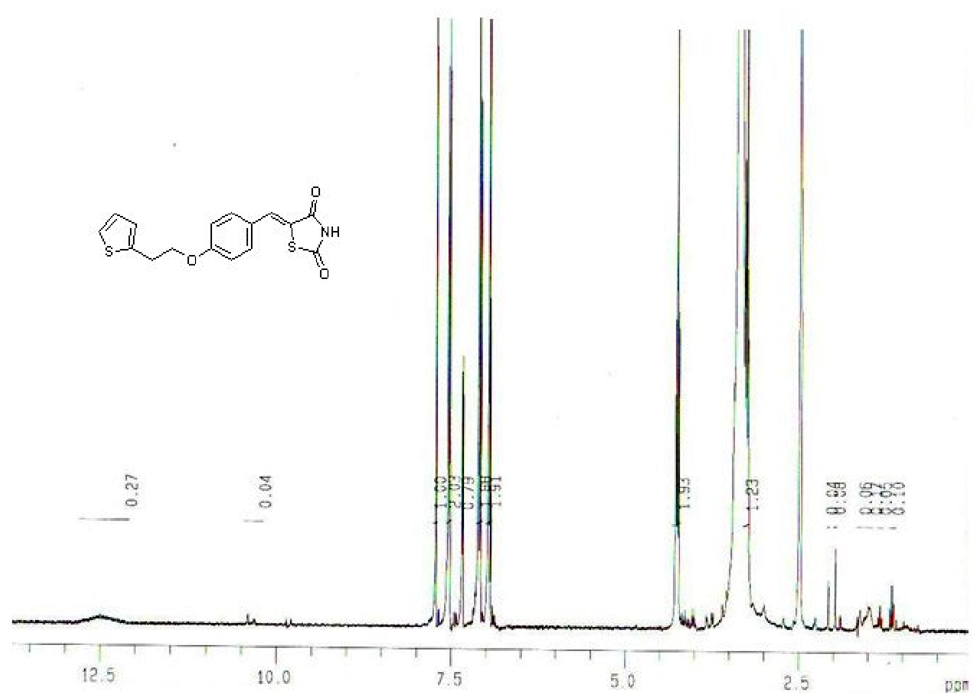
5-(4-(2-cyclohexylethoxy)benzylidene)-4-thioxothiazolidindin-2-one (compound 26) [230]



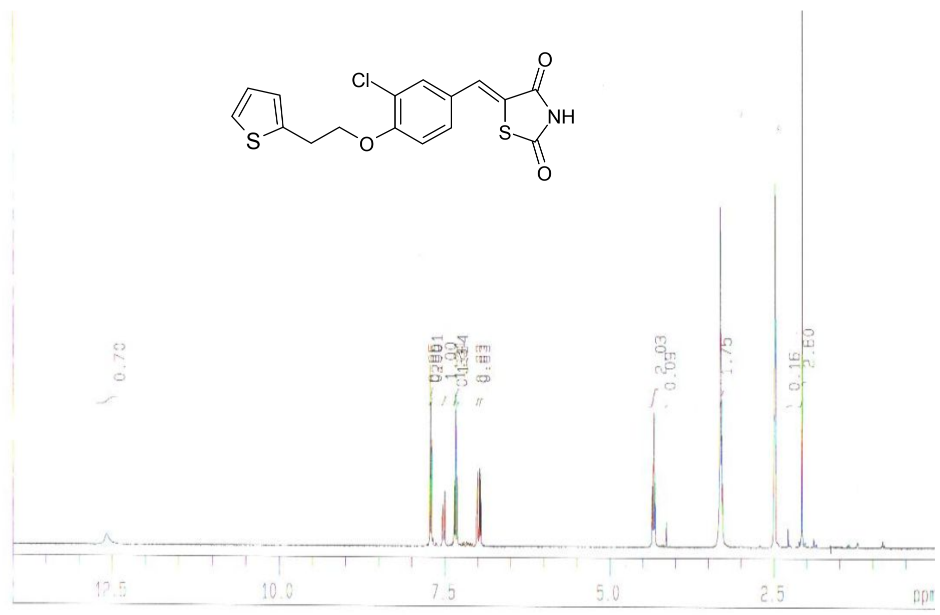
4-(2,3-dihydro-1,4-benzodioxin-2-ylmethoxy)benzaldehyde ammoniate (compound 27) [230]



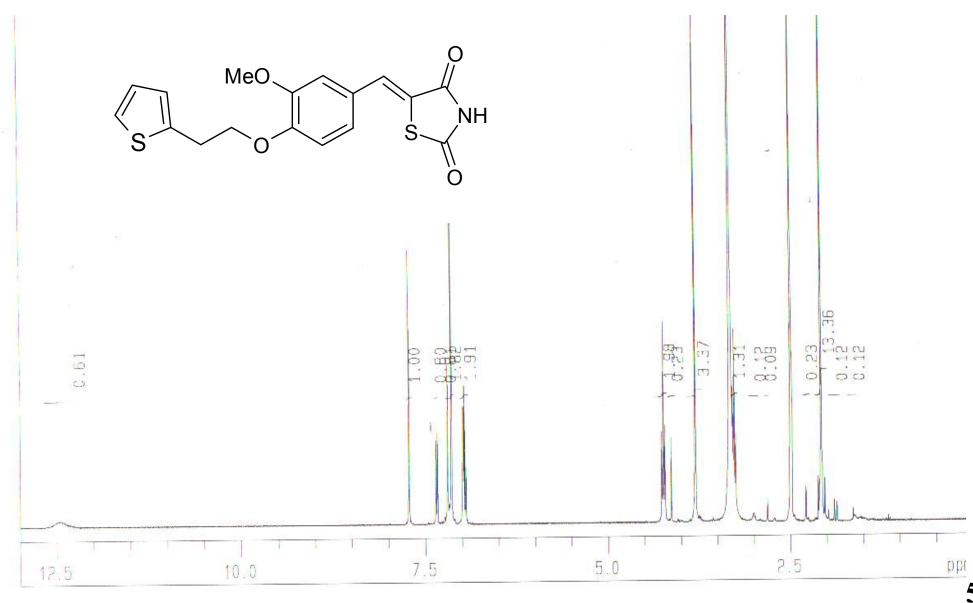
5-[4-(benzo[d][1,3]dioxol-5-ylmethoxy)benzylidene]thiazolidine-2,4-dione (compound 28) [230]



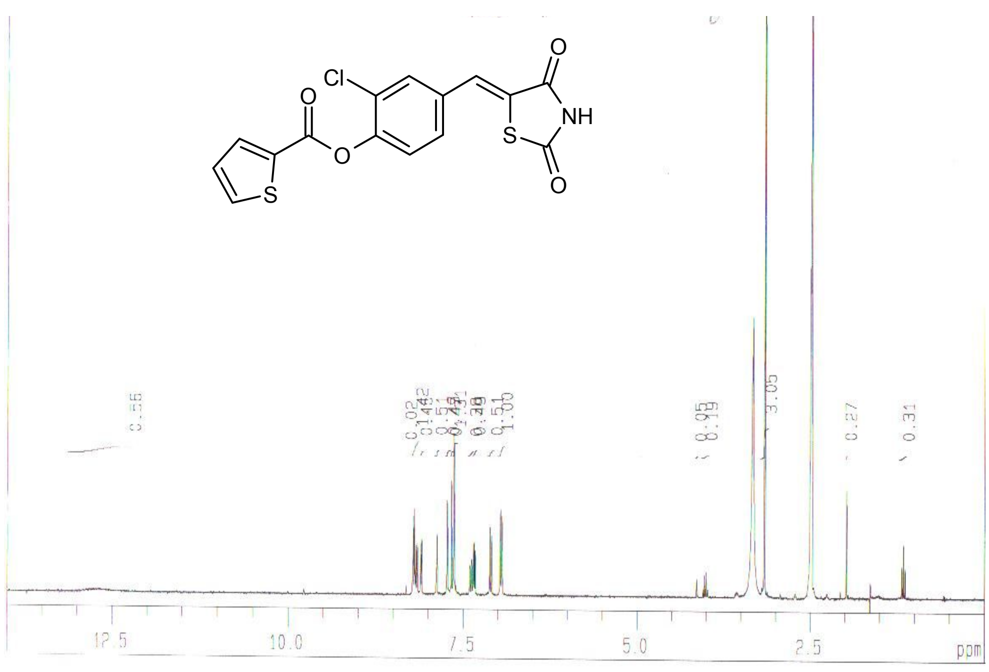
5-(4-(2-Thiophen-2-yl)ethoxy) benzylidene)thiazolidine-2,4-dione (compound 29) [230]



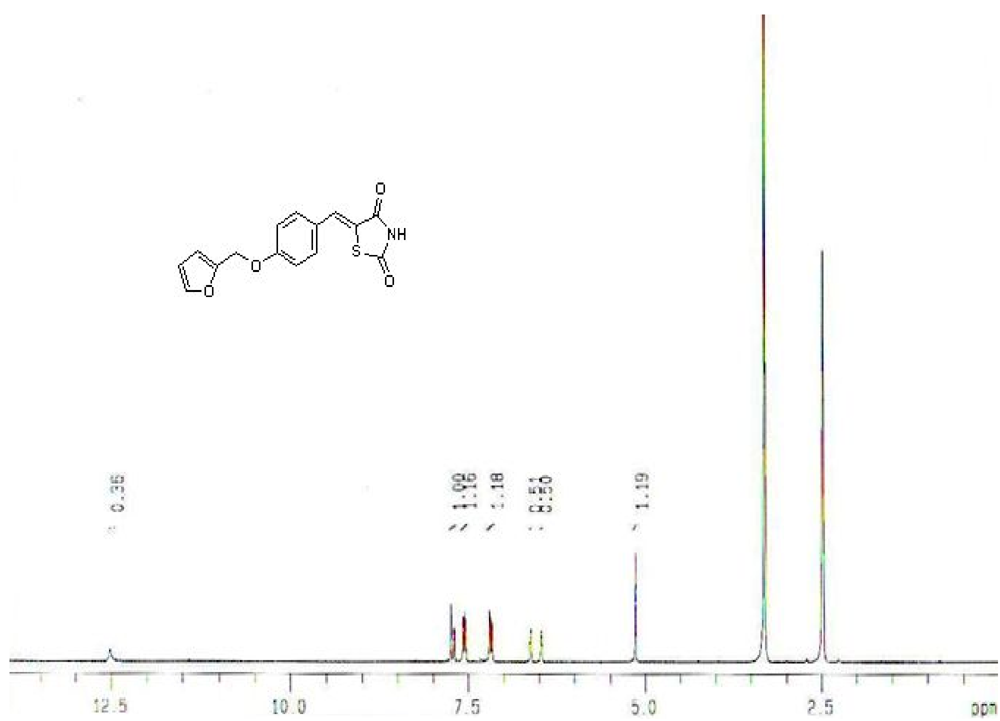
5-(3-chloro-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (compound 30) [230]



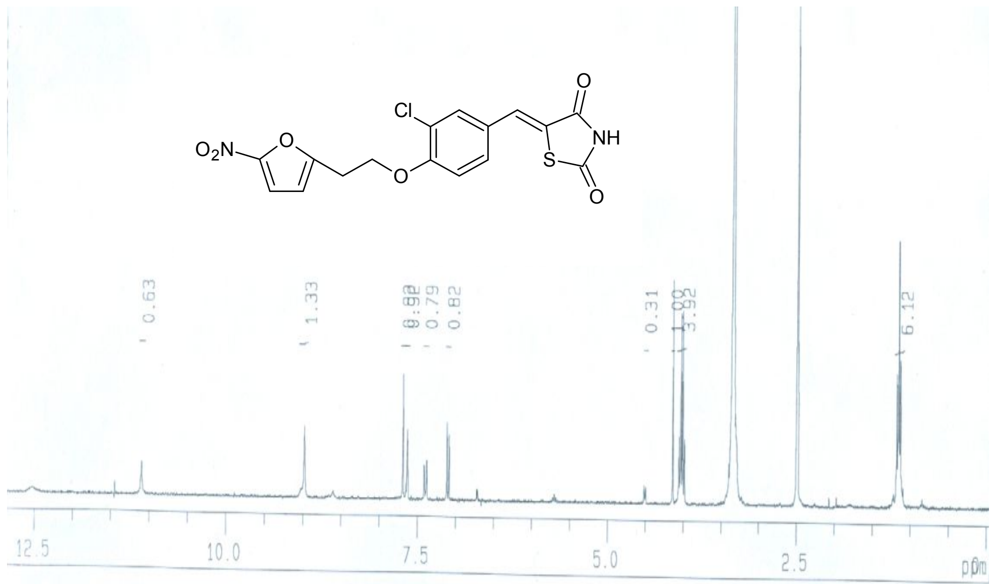
5
-(3-methoxy-4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (compound 31)



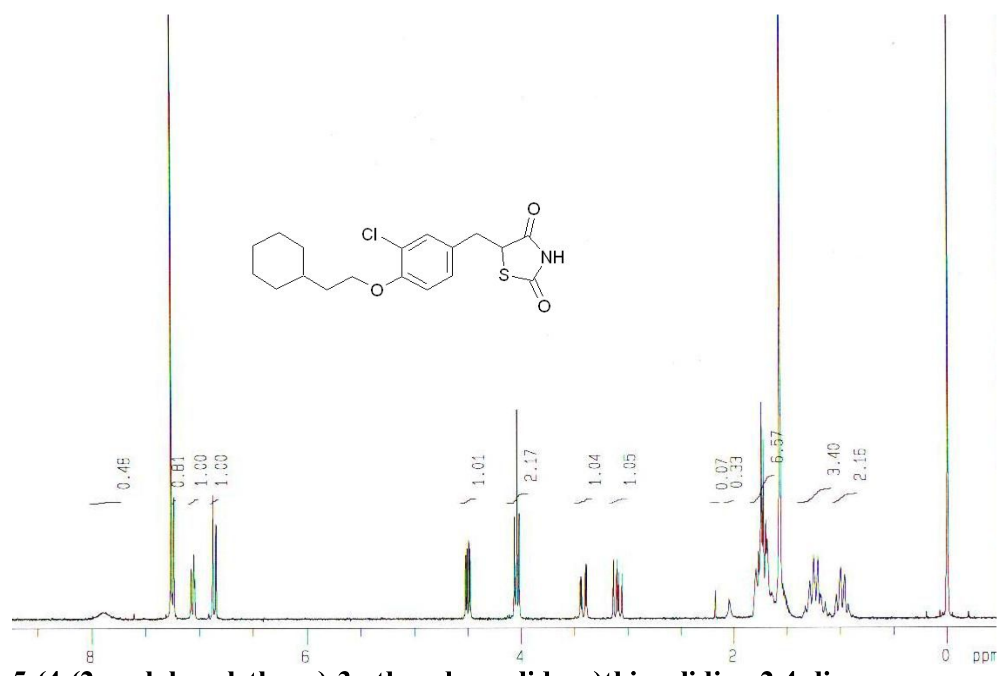
2-chloro-4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl thiophene-2-carboxylate (compound 35)



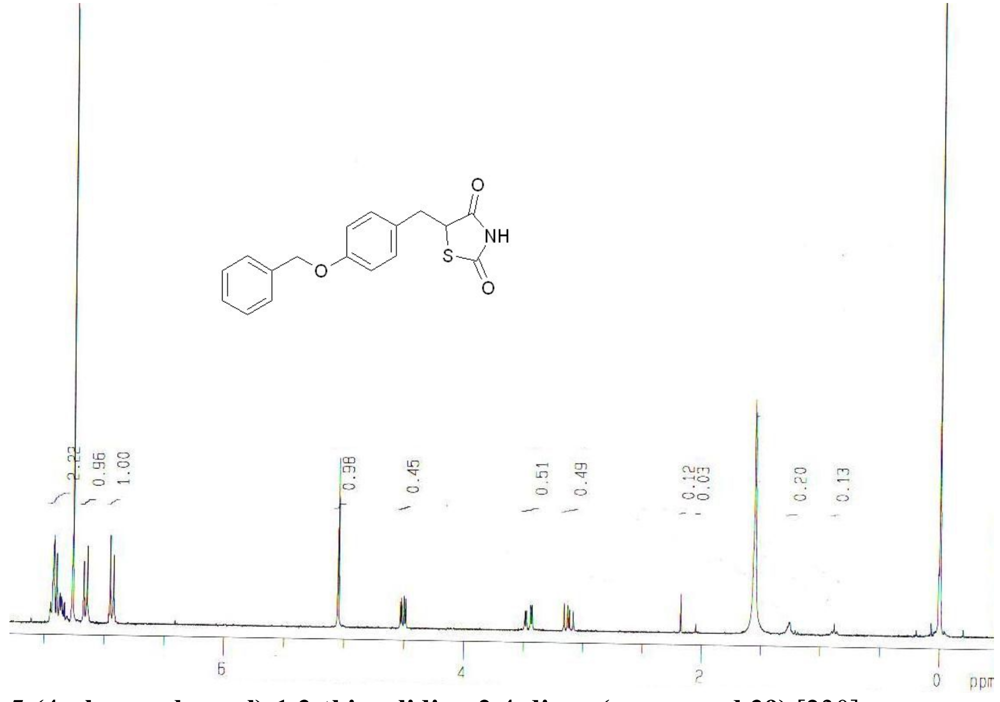
5-[4-(furan-2-ylmethoxy) benzylidene] thiazolidine-2,4-dione (compound 36)
[230]



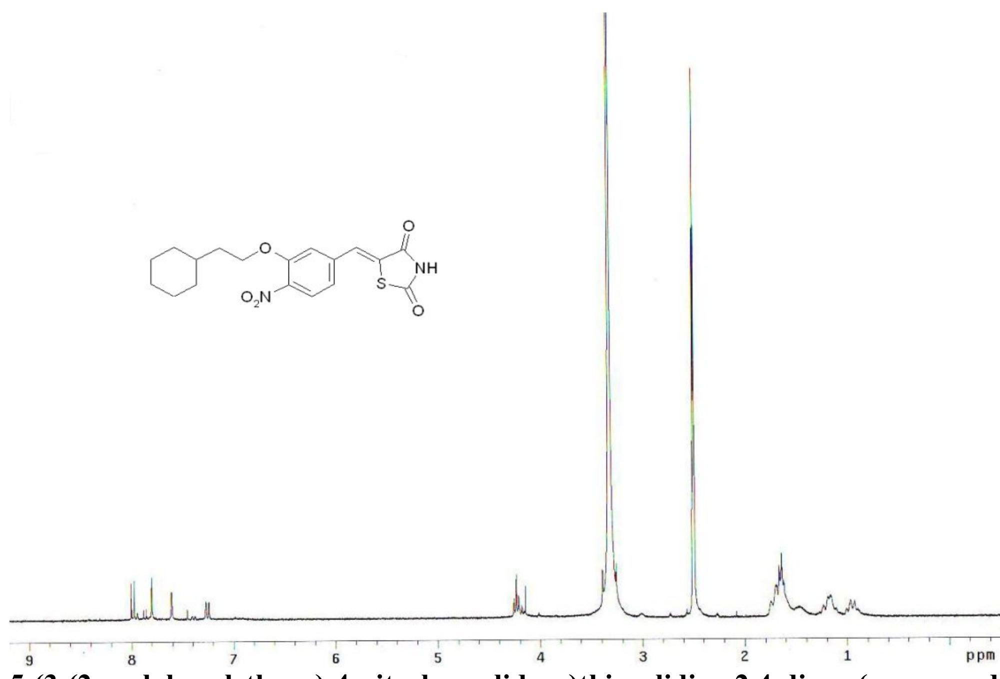
5-{3-chloro-4-[2-(5-nitrofuran-2-yl)ethoxy]benzylidene}-1,3-thiazolidine-2,4-dione (compound 37)



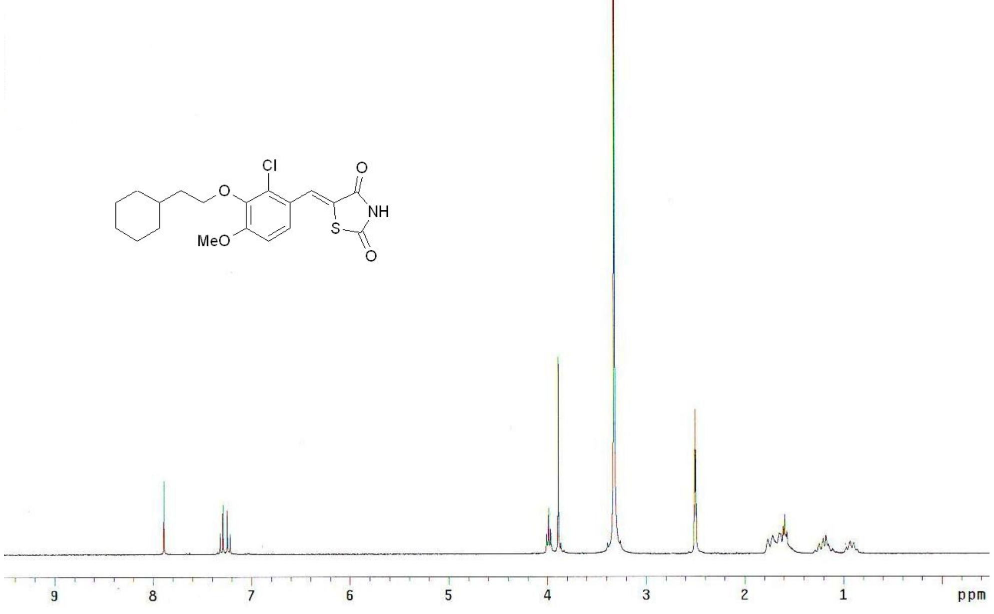
5-(4-(2-cyclohexylethoxy)-3-ethoxybenzylidene)thiazolidine-2,4-dione (compound 38) [230]



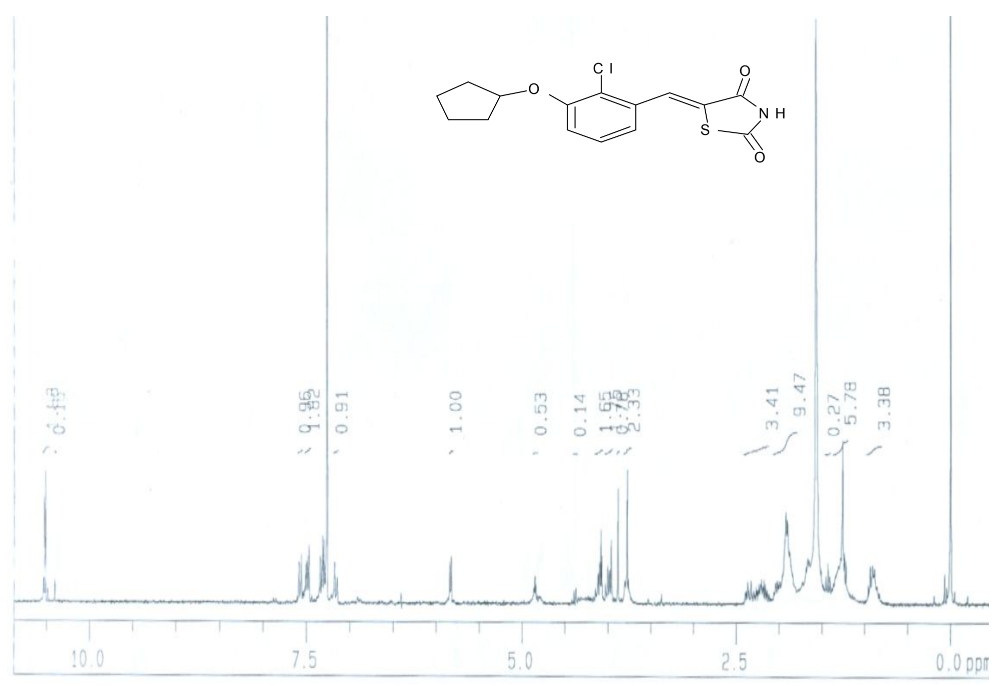
5-(4-phenoxybenzyl)-1,3-thiazolidine-2,4-dione (compound 39) [230]



5-(3-(2-cyclohexylethoxy)-4-nitrobenzylidene)thiazolidine-2,4-dione (compound 42) [230]

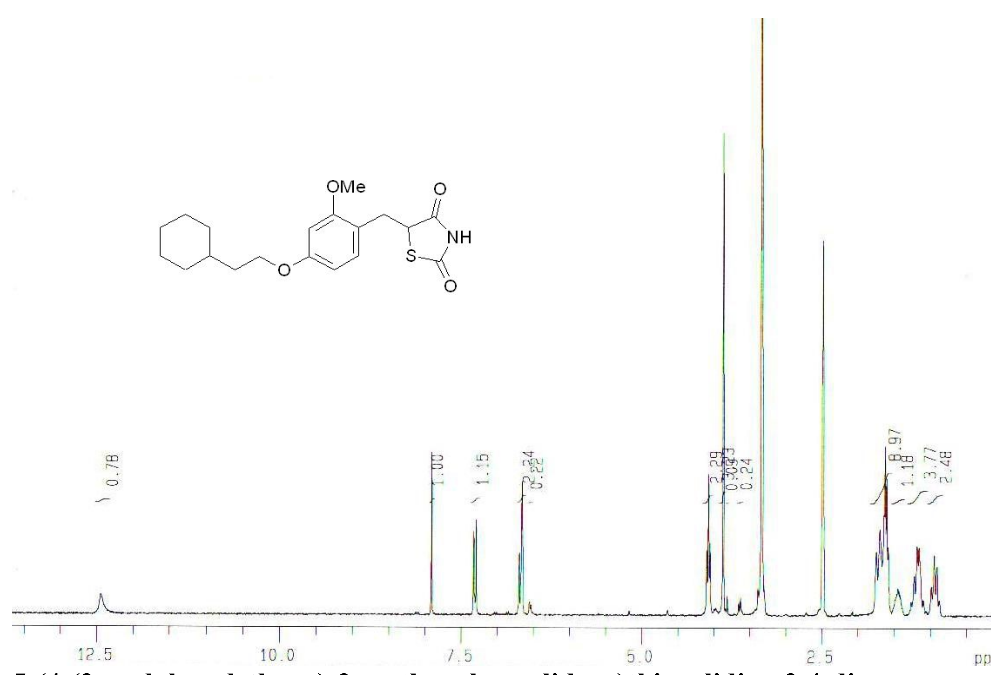


5-(2-chloro-3-(2-cyclohexylethoxy)-4-methoxybenzylidene)thiazolidine-2,4-dione (compound 43) [230]



5-[2-chloro-3-(cyclopentyloxy)benzylidene]-1,3-thiazolidine-2,4-dione (compound 46)

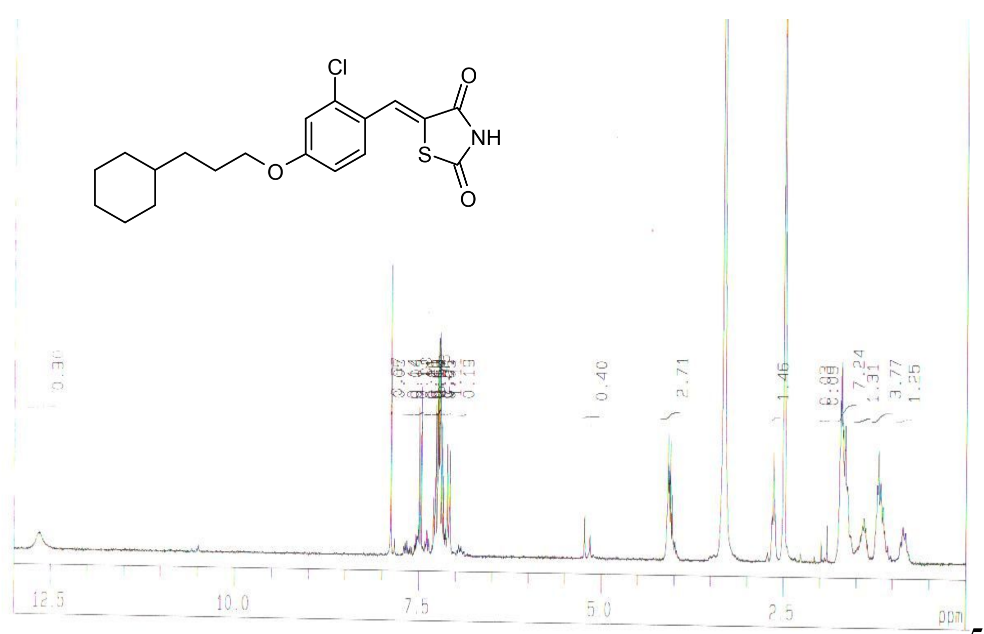
5-(3-(benzyloxy)-2-chlorobenzylidene)thiazolidine-2,4-dione (compound 49)



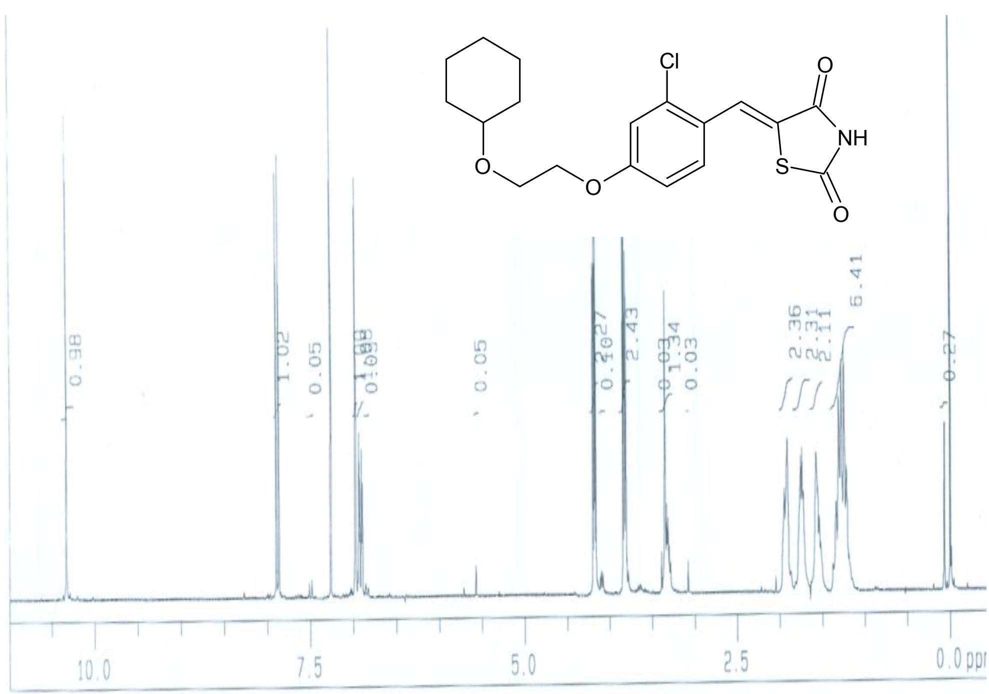
5-(4-(2-cyclohexylethoxy)-2-methoxybenzylidene)thiazolidine-2,4-dione (compound 52) [230]

5

-(2-chloro-4-(3-cyclohexylpropoxy)benzylidene)thiazolidine-2,4-dione (compound 53)

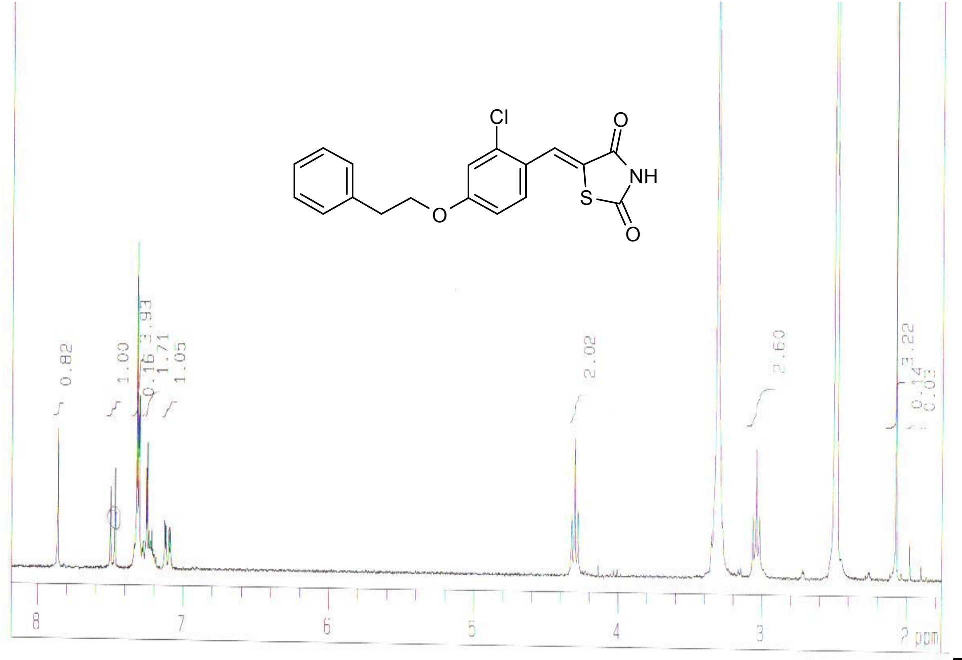


5-(2-chloro-4-(3-cyclohexylbutoxy)benzylidene)thiazolidine-2,4-dione (compound 54)

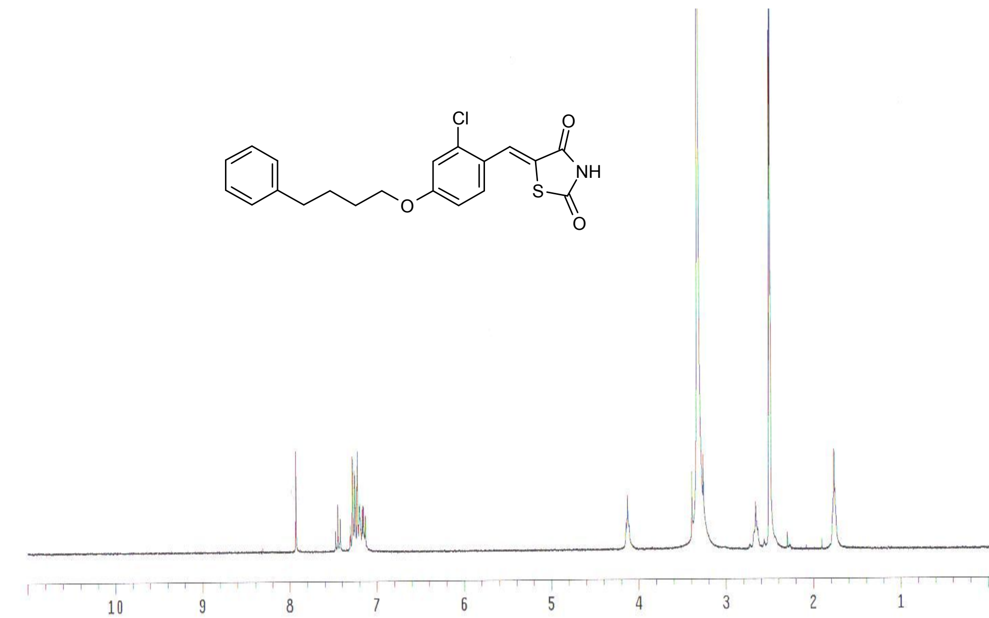


5-{2-chloro-4-[2-(cyclohexyloxy)ethoxy]benzylidene}-1,3-thiazolidine-2,4-dione (compound 55)

5-(4-(benzyloxy)-2-chlorobenzylidene)thiazolidine-2,4-dione (compound 56)



5-(2-chloro-4-phenethoxybenzylidene)thiazolidine-2,4-dione (compound 57)



5-(2-chloro-4-phenbutoxybenzylidene)thiazolidine-2,4-dione (compound 58)

ACKNOWLEDGEMENT

I would like to express my heartily gratitude to Prof. Cheol-Hee Choi my co-advisor and Prof. Hoon Cho my advisor, for their valuable support, whole-hearted teaching, creative ideas, outstanding scientific knowledge, guidance, advice, encouragement to the point of discussion, patient for going through all my research work to accomplish my academic goal.

I extended my special thanks to Prof. Sung-Chul Lim, Prof. Kyung Jong Kim, Prof. Ji Kang Yoo and Prof. Young Lea Moon to give their valuable time and patient to evaluate my dissertation and suggestions for the improvement in the contents.

My sincere thanks to Prof. Tae-Bum Lee, Mrs. Young-Sook Moon, Miss Yu-Lan Piao and all the colleagues for their cordial support during my laboratory work and valuable suggestions for my research work.

Finally, with deep sense of reverence I would like to express my whole hearted, thanks and deep gratitude to our parents and my wife Mrs. Darshana Karna who have always been a source of inspiration for me. Their everlasting co-operation and smiling affection inspired me to rise up to what I am today.

Thank you all.

Sandeep Karna

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본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

\- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의복제,기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만,저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의( ) 반대(○)

2010 년 12 월 17

저작자: 카나 센딥 (인)

조선대학교 총장 귀하