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2016년 2월

석사학위 논문

세포 내의 PGE₂ 농도 조절을 위한
2,4-dioxothiazolidin-5-ylidene
유도체 합성 및 활성평가

조선대학교 대학원

신재생에너지융합학과

윤 은 정

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Synthesis and activity evaluation of
2,4-dioxothiazolidin-5-ylidene derivatives for
regulating intracellular concentration of PGE₂

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Abbreviations

PGs: Prostaglandins

AA: Arachidonic acid

PLA₂: Phospholipase A₂

cPLA₂: Cytosolic phospholipase A₂

cAMP: Cyclic adenosine monophosphate

COX: Cyclooxygenase

15-PGDH: 15-Hydroxyprostaglandin dehydrogenase

NAD⁺: nicotinamide-adenine dinucleotide

cDNA: Complementary DNA

DTT: Dithiothreitol

ECM: Extracellular matrix

EDTA: Ethylenediamine-N,N,N',N'-tetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

EP: Prostaglandin E receptor

IL: Interleukin

MAPEG: Membrane-associated proteins in eicosanoid and glutathione

PGE₂: Prostaglandin E₂

PGHS: Prostaglandin H synthase

mPGES: Microsomal PGE synthase

DCC: N,N'-Dicyclohexylcarbodiimide

DMAP: 4-Dimethylaminopyridine

SDS: Sodium dodecylsulfate

IPTG: isopropyl β -D-1-thiogalactopyranoside

FBS: Fetal bovine serum

TGF- β 1: Transforming growth factor β 1

TXs: Thromboxanes

TLC: Thin layer chromatography

TDs: Thiazolidinediones

VEGF: Vascular endothelial growth factor

초 록

Synthesis and activity evaluation of 2,4-dioxothiazolidin-5-ylidene derivatives for regulating intracellular concentration of PGE₂

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The prostaglandins (PGs) are a group of important lipid compound mediators and have been found in almost every cell in human body. They are synthesized from arachidonic acid (AA) released from the cell membrane when the cell is activated by a physical or chemical stimulation. The released AA is metabolized into PGH₂ and then to different derivatives by various enzymes.

The prostaglandin E₂ (PGE₂) among them is known to have an important role in body function as an important factor for reproduction, inflammatory regulation, wound healing, peptic ulcer, neuroendocrine and immune system regulation, contraction and expansion of blood vessel and regulation of blood pressure. However, as PGE₂ is quickly metabolized by 15-PGDH using NAD⁺ as the accessory factor, it has a very short half-life in the body. Therefore, 15-PGDH must be inhibited for the therapeutic management of diseases requiring elevated PGE₂ levels. In this study, I synthesized various 15-PGDH inhibitors based on

thiazolidinedione, which is known to inhibit 15-PGDH, and conducted activity evaluation.

The activity evaluation showed, while most synthesized compounds had good inhibiting effects, the best 15-PGDH inhibitor was the compound **29** (Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate with the IC_{50} value of 0.0145. To check whether the synthesized inhibitor increases the amount of PGE_2 inside a cell, PGE_2 concentration in the A549 cell was measured. The most effective compound for increasing the PGE_2 concentration was the compound **44** which increased the concentration to 579% level. A scratch wound healing assay using the HaCaT cell was conducted to check whether the compounds that showed outstanding inhibiting effect and PGE_2 concentration improvement could also treat the wound well. The assay confirmed that the compound promoted the cell proliferation after 24 hours and was effective in wound healing.

The results confirmed that the compounds inactivated 15-PGDH and increased the PGE_2 level to help wound healing and was expected to help therapeutic management of diseases requiring elevated prostaglandin E_2 levels.

1. 서 론

Prostaglandins (PGs)은 eicosanoid라고 알려진 중요한 지질 중재자의 부류 중 하나로 essential fatty acids (EFAs)로부터 효소적으로 유도되었으며 동물의 체내에서 중요한 기능을 가지고 있다. 모든 PGs는 5-탄소 고리를 포함한 20개의 탄소 원자를 가진 유도체로 Prostaglandin이라는 이름은 prostate gland에서 유래되었다. PGs는 전립선에서 발견되었는데 1935년 스웨덴의 생리학자 Ulf Von Euler와 독립적으로 M. W. Goldblatt에 의해 정액으로부터 최초로 분리되었다. 그들은 PGs를 전립선 분비물의 일부라고 여겼으나, 훗날 PGs가 우리 몸의 여러 조직에서 분비되며 다양한 기능을 한다는 것이 밝혀졌다 [1].

PGs는 우리 몸 속 대부분의 세포에서 형성되며, 자가 분비와 주변 분리의 지질 중재자로서 행동한다. PGs는 저장되지 않으며, 특정한 물리적 또는 화학적 자극에 의해 활성화 되었을 때 세포막으로부터 방출되는 arachidonic acid (AA)로부터 새로이 합성된다 [2]. 여기서 AA는 인간의 세포에서 가장 풍부한 지방산으로, 유리된 AA의 양은 특정 자극에 의해 작동되기 전까지 보통의 생리적 조건하에서는 매우 적은 양으로 존재하는데, 숙주효소(host enzyme)는 phospholipase A₂ (PLA₂)에 의해 기동되기 전까지 에스테르화된 상태로 AA를 존재하게 하여 유리된 AA의 양을 정교하게 조절한다 [3]. 세포막으로부터 AA의 방출 억제수단은 현재까지 몇몇 이론의 변화가 있었으나 그럼에도 불구하고 현재는 type IV cytosolic PLA₂ (cPLA₂)가 eicosanoid 생성을 위한 핵심 물질로 여겨지고 있다. 그 이유는 cPLA₂가 결핍된 세포들에서는 일반적으로 eicosanoid 합성이 전혀 없기 때문이다 [4]. 소포체(endoplasmic reticulum)와 핵막에서 cPLA₂에 의해 방출된 AA는 prostaglandin H 생성효소(PGHS 또는 COX)에 의해 불안정한 중간체인 prostaglandin H₂ (PGH₂)로 대사된다. 이 PGHS는 PGHS-1 (COX-1)과 PGHS-2 (COX-2)라고 불리는 두 가지 이성질체 형태로 존재하는데, 단순한 측면에서 COX-1은 기저가 되는 본질적인 PGs 합성에 대한 책임 효소이고, 반면에 COX-2는 다양한 염증성 및 유도 조절에 중요한 효소이다. COX-2는 전염증성의 사이토카인(pro-inflammatory cytokines), 성장인자(growth factors), 발암 촉진자(tumor promoters)에 의해 유도된다. COX-1과 COX-2는 COX-2에서의 기질접근에 대한 큰 'side-pocket' 으로 이어지는 하나의 주목할 만한 아미노산 차이를 제외하고는 두

결정 구조가 비슷하다 [5]. COX는 AA를 산화된 환화(cyclization)로 변형시키며 PGs, thromboxane, prostacyclin의 직접적인 전구체인 PG endoperoxide (PGH₂)로 대사한다. 그런 후 PGH₂는 각각의 효소에 의해 prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂), prostaglandin F_{2α} (PGF_{2α}), prostaglandin I₂ (PGI₂), thromboxane A₂ (TXA₂)의 하류물질로 대사 된다 (**Fig. 1**). 하류물질인 thromboxane 생성효소는 혈소판과 대식세포에서 발견되며, prostacyclin 생성효소는 내피세포, prostaglandin F 생성효소는 자궁에서, 두 가지 종류의 prostaglandin D 생성효소는 뇌와 비만세포에서 발견된다. 그리고 MAPEG (eicosanoid 및 glutathione 대사속의 막 관련 단백질)의 구성원인 microsomal PGE 생성효소(mPGES)는 PGE₂ 생성을 조절하는 물질이며 [6], 위 효소들은 prostanoid 경로 속에서 다양한 효소의 유도를 조절한다. 특히 mPGES와 COX-2 효소는 현재 염증성 환경에서 활발히 연구되고 있다 [7].

이렇게 대사산물로 생성된 PGE₂는 세포내 신호 전달 통로의 활성화로 4가지의 EP 수용체 아류형들(EP₁-EP₄)을 가지며 EP 수용체 아류형의 결합에 의하여 다양한 영향을 나타낸다. EP₁ 수용체는 Gq와 결합하여 세포내 칼슘농도 증가를 조절하며, 스트레스 조건하에서 높아진 도파민의 활성화 반응과 관련된 충동행위 제어 또한 EP₁을 통하여 작동된다. EP₂와 EP₄ 수용체는 Gs와 결합하며, cyclic adenosine monophosphate (cAMP) 농도 증가를 조절하며, 관절염의 critical mechanism과 연계되어 있다 [8,9]. 또한 EP₂와 EP₄의 활성화를 통하여 cytokine 생성물과 IL-6와 같은 성장인자, 혈관내피성장인자, 부갑상선호르몬-방출된 펩티드와 대식세포 증식자극 인자등도 조절된다. 그에 반해, EP₃ 수용체는 COOH-말단기 꼬리와 결합된 대체물질에 의해 다수의 결합 변종이 존재하며, Gi 단백질이 결합된 adenylylate cyclase의 억제와 세포내 cAMP의 감소를 촉진한다 (이차적인 중재자인 cAMP와 Ca²⁺의 일시적인 세포내 변형은 특정유도를 발생하게 한다.) (**Fig. 2**) [10]. 이러한 PGE₂는 재생(reproduction), 소화성 궤양, 신경내분비 및 면역시스템조절, 평활근의 수축과 이완, 혈관의 수축과 팽창 및 혈압조절, 염증조절 그리고 눈썹과 두발 형성 및 골 형성 등의 중요한 매개인자로 광범위한 신체 기능에 중요한 역할을 담당하고 있다 [11,12]. 최근, 임상연구 결과 PGE₂가 인간과 동물의 신체 털 성장을 촉진한다는 것이 입증되어 탈모치료제에 대한 가능성을 보여주고 있다 [13]. 실험은 인간의 두피에서 PGE₂가 모발 밀도를 증가시킬 수 있음을 보여주었다 [14]. 또한, PGE₂는 피부손상 시 상처치유 과정에 관여한다. 피부상처의 치유는 복잡하고 혈액응고, 염증반응, 육아조직의 축적, 세포 외 기질의 침착 및 형성을 포함한다 [15]. 상처 가장자리의 표피 근위에서 세포의 유사분열과 전환에 의해 달

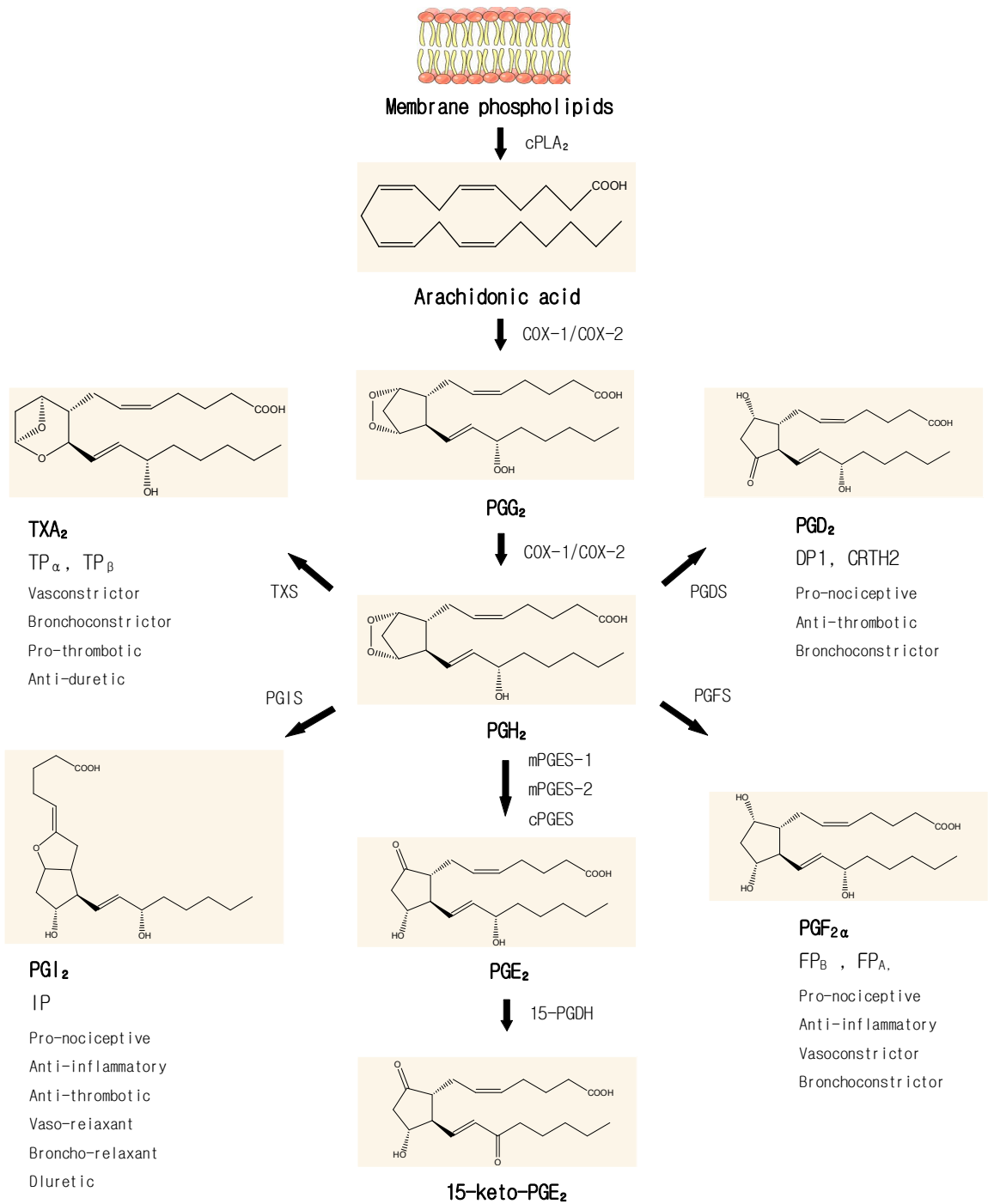


Fig. 1. Biosynthesis of prostaglandins

성된 케라틴 세포에 의한 재생피화는 상처치유의 중심적인 일이다 [16]. 특히 PGE₂는 EP₁과 EP₄을 통하여 상처 치유의 중요한 인자로 작용한다. 이러한 prostanoid 대사로 인하여 상처치유 자극은 상처를 받은 후 즉각적으로 발생하게된다 [17,18]. Prostanoids는 피부나 창자의 상피, 섬유아세포, 케라틴 생성 세포, 각막의 내피를 포함하는 다양한 조직에서의 상처 치료 과정에 관여하며 이러한 과정에서 COX-1의 기능도 작용한다 [19-21]. COX의 기능적 결합은 이미 쥐과 동물의 피부 재생에서 COX-1과 PGE₂/D₂사이의 기능적 결합이 입증 되었으며, 그 실험 내용은 COX-2의 발현이 상처의

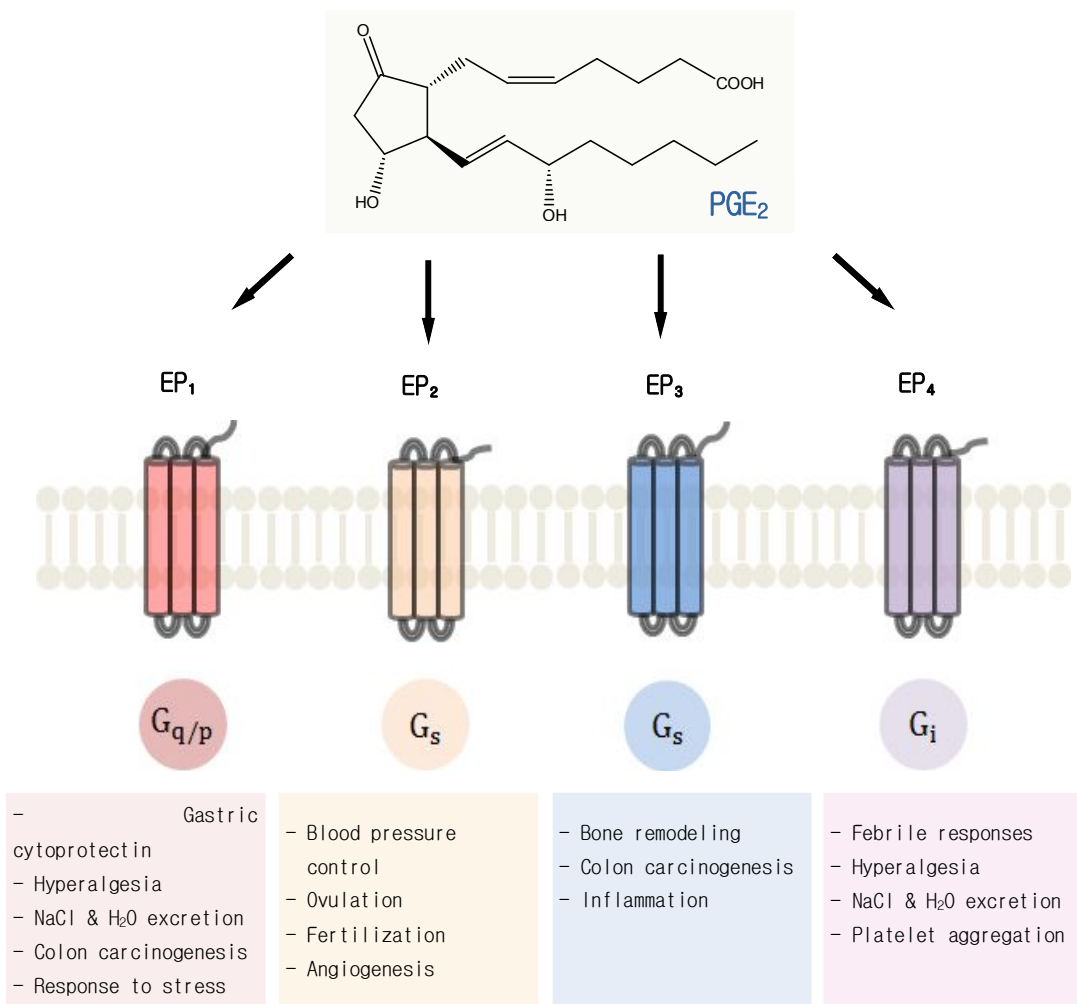


Fig. 2. Actions of PGE₂ with EP receptors.

육아조직에서 강하게 유도되어도 COX-2는 상처 속 높은 PGE₂/D₂ 농도에 관여하지 않는다는 것이다. 이 실험 결과는 COX-1과 결합된 PGE₂/D₂가 피부재생의 핵심역할을 한다는 것을 강력하게 제시하는 증거이기도 하다. 상처치유과정에서 PGE₂는 4가지 EP 수용체의 작용으로 케라틴 생성 세포(keratinocytes), 수지상 세포(dendritic cells), 섬유아세포(fibroblasts) 등을 포함하는 광범위하고 다양한 세포활동을 조절한다 [22-24]. 그리고 이밖에도 상처 치유과정에서 혈전 형성 후 손상된 부분은 침투된 호중구, 단핵백혈구, 대식세포의 도움에 의해 청소되며 여기서 대식세포는 transforming growth factor α (TGF- α), interleukin-1 (IL-1), transforming growth factor β (TGF- β), insulin-like growth factor 1 (IGF-1) 등을 포함하는 중요한 cytokine들을 많이 전달한다. cytokine들은 새로운 조직형성 시작에 필수적 역할을 하는 인자들이다. 또한, 상처 속에서 섬유아세포(fibroblasts) 이동과정을 통하여 세포외 기질(ECM; extracellular matrix)의 합성을 시작하게 된다 [25]. 그리고 서서히 TGF- β 1의 도움으로 교원 기질(collagenous matrix)로부터 섬유아세포를 대신하며, 이 과정에서 신혈관 형성(neovascularization), 상처 축소(wound contraction)를 하게 된다. 임상 데이터를 보면 TGF- β 의 과잉생산과 PGE₂의 억제가 정상 상처 치유와 비교해 과도한 흉터를 남긴다는 것을 살펴 볼 수 있다. 따라서 PGE₂는 과도한 흉터 예방에 중요한 역할을 할 수 있다.

이러한 eicosanoid은 특정 자극에 따라 세포의 효소활동의 파열에 의해 만들어진다. 뿐만 아니라 이들 분자는 바로 근처 지점에서 세포에 세포 활성화의 정보를 전달한다. 그러므로 이들의 대사과정을 신속하게 비활성화 하는 것은 각각의 행동조절을 위해 매우 중요하며, 주요 경로로는 불활성 물질로의 대사전환이고, 다수의 효소들이 이러한 불활성화 과정에 관여하고 있다. 이러한 불활성화 대사에 관여하는 효소들 중 하나가 바로 15-hydroxyprostaglandin dehydrogenase (15-PGDH)이다. cytosolic 효소인 15-PGDH는 두 가지 유형이 존재하는데, 첫 번째 유형은 활성을 위하여 산화제로 nicotinamide-adenine dinucleotide (NAD⁺)를 보조인자(cofactor)로 사용하는 경우와 두 번째 유형으로는 산화제로 nicotinamide-adenine dinucleotide phosphate (NADP⁺)을 보조인자로 사용하는 경우이다. 첫 번째 유형은 PGs에 더 기질특이성을 보이며, PGs에 대하여 더 낮은 K_m 값을 보인다. 반면에 두 번째 유형의 경우, 훨씬 광범위한 기질특이성을 가지며, PGs에 대해 더 높은 K_m 값을 보인다. 현재, 두 번째 유형은 carbonyl 환원효소(reductase)와 동일한 것으로 여겨지고 있으며, 첫 번째 유형은 PGs를 생리학적으로 불활성화 하는 주요효소로 여겨지고 있다 [26,27]. NAD⁺ 의존형

15-PGDH은 포유류의 조직에서 흔히 존재하며 가장 높은 활동은 폐, 신장 및 태아에서 발견된다 [28]. 이는 다양한 방법을 통해 정제되고 있으며 이 효소는 29 kDa의 분자 무게를 가진 동일한 기본단위로 구성된 이합체이며, 단합체인 경우도 활성화될 수 있다고 제안되고 있다 [29]. 15-PGDH-NAD⁺-PGE₂ 사이의 결합을 3D 구조로 Fig. 3에 나타내었다. 초록색 분자는 기질인 PGE₂이고, 빨강색 분자는 보조인자인 NAD⁺ 이다.

15-PGDH 효소의 cDNA는 복제되었으며 [31] 아미노산 서열 분석을 통해 60가지 이상의 다른 효소를 포함하는 짧은 사슬의 dehydrogenase(탈수소효소)종에 속한다는 것을 알 수 있다 [32]. 이 효소는 기질의 사용에 상당한 다양성을 보여준다. 다른 짧은 사슬의 dehydrogenase와 15-PGDH의 아미노산 서열의 비교는 엄격히 보존되어 온 몇몇의 아미노산 잔기와 20% 상동성을 드러낸다.

15-PGDH는 tyrosine 151, lysine 155와 serine 138이 촉매활동에 중요하며 이 3가지 잔기가 촉매 화음을 형성하는 것으로 나타났다 [33-35]. 이러한 연구는 serine과 tyrosine의 hydroxyl group이 생산성결합모드에서 산화를 겪는 기질의 hydroxyl group의 산소원자와 상호작용하는 것을 보여준다. lysine 잔기의 ε-amino group은 coenzyme의 nicotinamide ribose의 cis-diol에 수소 결합을 형성한다. 또한 인간 15-PGDH의 cysteine 182은 NAD⁺ 결합 도메인의 한 부분을 형성하는데 필수적이라는 것



Fig. 3. A stereoview of 3D structure of 15-PGDH-NAD⁺-PGE₂ complex [30].

을 발견했다 [36]. 최근에 인간의 15-PGDH의 threonine 188이 NAD^+ 와의 상호작용에 중요하며 serine 188 mutant처럼 NAD^+ 의 K_m 이 100배 증가했으며 반면 PGE_2 의 K_m 은 변화가 없었다 [37]. NAD^+ 결합을 포함하는 다른 잔기를 계속적으로 찾아보면 우리는 보존된 threonine 110이 NAD^+ 와 상호작용에 관여하는 것으로 예측하고 있다.

이 15-PGDH 효소는 prostaglandins의 C-15에서 가역적 산화, 환원을 촉매한다. 15-PGDH는 PGE_2 의 15-hydroxyl group을 짝지은 α, β -불포화 케톤으로 대사시킨다. 이 과정에서 15-PGDH의 아미노산 잔기와 NAD^+ 에 의해 PGE_2 의 대사를 촉매화하며, 대사산물은 15-keto- PGE_2 와 NADH 를 생성하게 된다. 이러한 15-PGDH- NAD^+ - PGE_2 의 반응 메커니즘을 Fig. 4에 나타내었다.

그러므로 현재까지 PGE_2 의 부족으로 유발되는 여러 가지 질병 치료에 사용하기 위하여 15-PGDH의 활성을 억제하려는 여러 물질들이 개발되어지고 있으며, 15-PGDH는

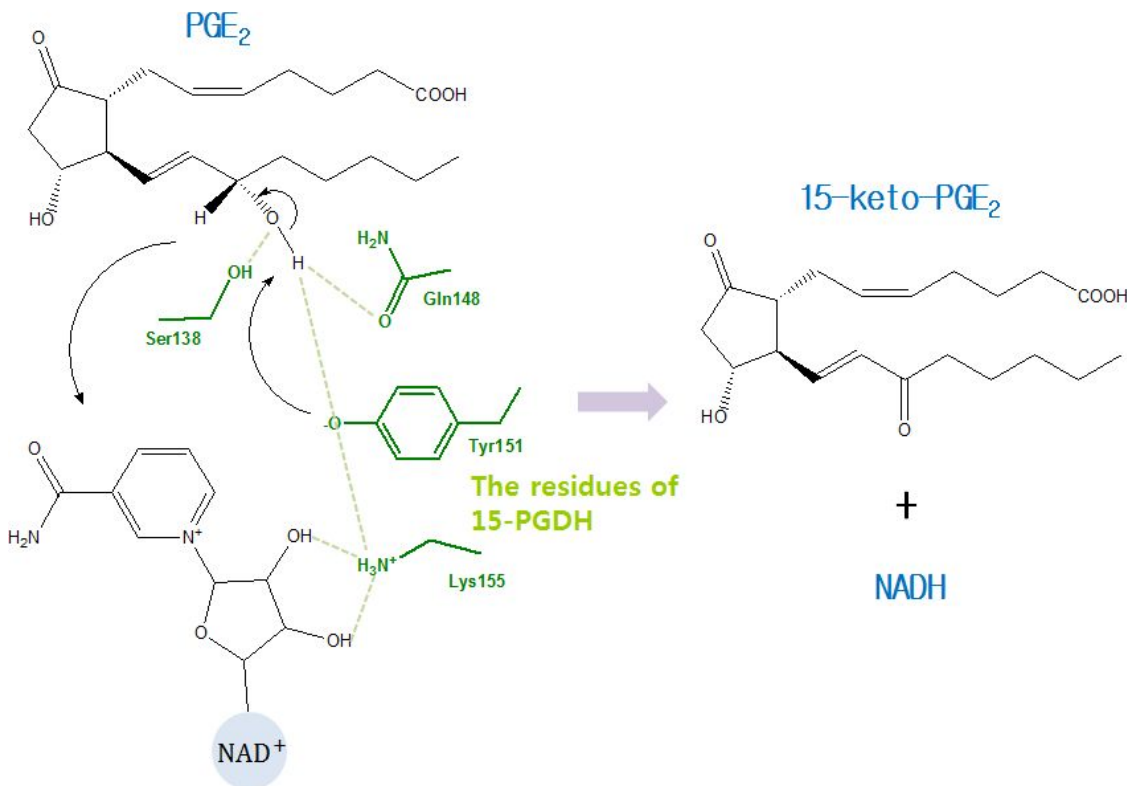


Fig. 4. Catalytic mechanism of 15-PGDH.

N-ethyl maleimide와 같은 sulfhydryl 억제제에 민감하며 cysteine 잔기에 나타나는 Cu^{2+} 와 같은 금속이온이 활성을 위해 필요한 것으로 잘 알려져 있다 [38,39]. 또한 이 효소는 indomethacin과 같은 비스테로이드성 소염제 [40], panaxynol과 같은 항혈소판제 [41], flavinoid baicalein과 같은 항알레르기제 [42], plaunotol과 같은 항케양제 [43], sulfasalazine 유도체와 같은 항대장염제, methylethergometrine maleate, acrolein, papaverine, 지방산 등과 같은 산과 약물을 포함하는 다양한 약물에 의해 억제된다. 이들 물질 중에 2-hydroxy-5-(3,5-dimethoxycarbonyl-benzoyl)-benzene acetic acid, 즉 sulfasalazine 유도체는 가장 강한 억제제로 발견되었다 [44].

Thiazolidinediones (TDs)는 다른 생리학적인 과정의 조절에 깊은 관련이 있기 때문에 폭넓은 연구의 대상이 되고 있다. TD 유도체는 면역력 증가, 항관절염 효과, 항암효과 등 여러 가지 효과로 인해 많은 약물로 이용되고 있다 [45].

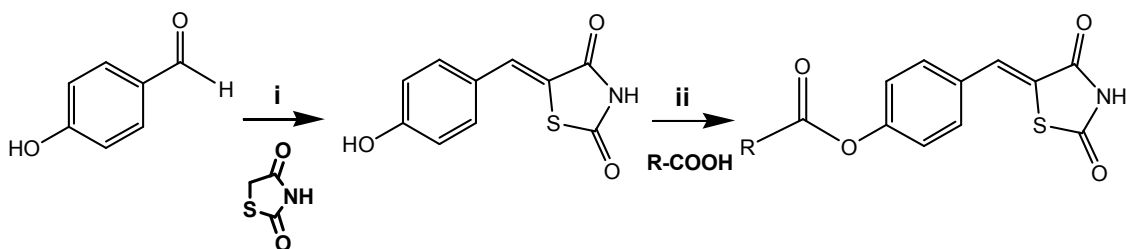
troglitazone, pioglitazone, rosiglitazone과 같은 TDs는 생체 내에서 혈장 glucose 단계의 강력한 환원제로 이용되고 있다. 초창기에 ciglitazone은 당뇨병 치료에 사용되었으며 그것은 15-PGDH 효소의 활성을 억제하는 길항제로 보고되었다 [46]. ciglitazone은 rosiglitazone과 troglitazone보다 더 좋은 억제 효과가 있다. 이들 구조를 보면 에테르 결합으로 연결된 benzylidenethiazolidine-2,4-dione 유도체들이 억제 효과에 중요한 역할을 하는 것으로 볼 수 있다 [47]. 게다가 TDs는 항당뇨 효과 이외에도 혈관 세포에 대한 항염증 효과를 발휘하는 것으로 알려졌다 [48]. 또한 염증성 cytokines의 생산 및 단핵구/대식세포 유도성 산화질소 합성이 발현을 억제하는 것으로 밝혀졌으며 [49,50] 결장, 유방, 전립선 등 여러 암세포주의 성장을 억제하는 것으로 밝혀졌다 [51-53].

따라서 본 실험에서는 이미 안정성이 검증되어 있으며 15-PGDH 억제 효과도 있는 TD를 기본골격으로 하여 활성억제의 구조에 기초하여 페닐 고리에 치환기를 갖는 다른 여러 가지 TD유도체를 일련의 반응을 사용해 합성하고 15-PGDH 억제 활성 평가를 진행하였다. 또 인간 폐암세포인 A549 cell을 모델 시스템으로 사용하여 15-PGDH 억제제가 PGE_2 의 수준을 상승시켜 질병의 치료에 도움이 될 수 있다는 것을 보여주었으며 더 강력한 화합물들은 HaCaT cell을 이용해 상처치유효과를 규명하기 위해 양성 대조군인 TGF- β 1과 함께 약물처리 하여 wound healing test를 진행하였다.

II. 재료 및 방법

1. 15-PGDH 억제제 합성

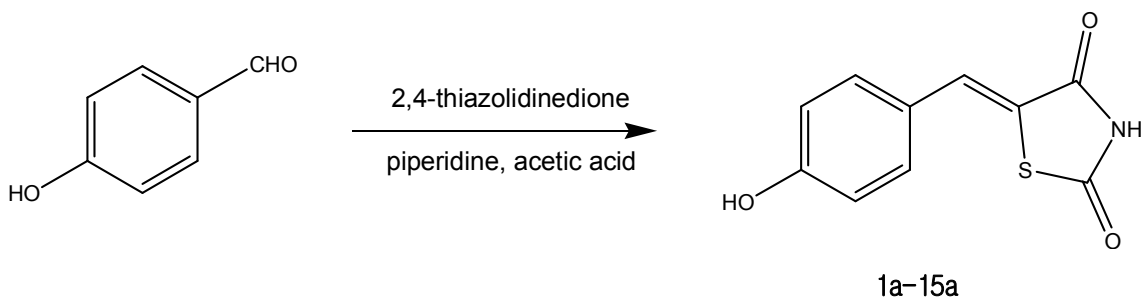
1.1. 화합물 (1-15) 합성



Scheme 1. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

Scheme 1은 1부터 15까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 아래의 (1a-15a)와 1에 나타내었으며, 이후의 실험방법은 모두 동일하므로 생략하였다.

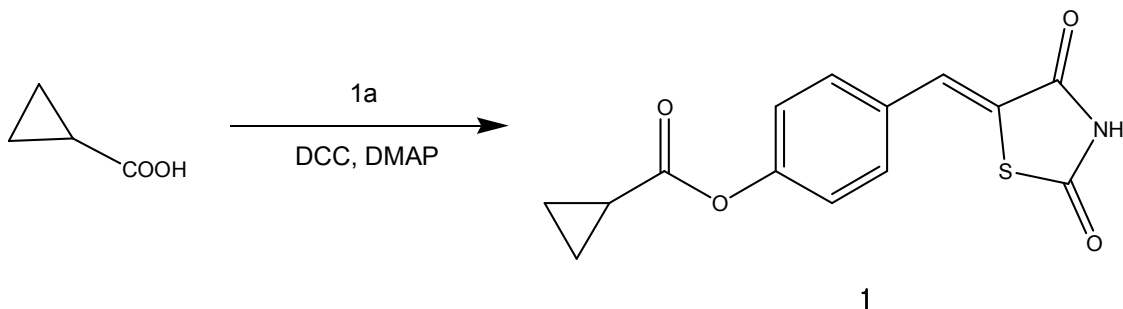
5-(4-Hydroxy-benzylidene)-thiazolidine-2,4-dione (1a-15a)



Dean-Stark trap이 장착된 둥근 플라스크에 4-Hydroxy-benzaldehyde 1 g (8.19 mmol)과 2,4-thiazolidinedione 0.959 g (8.19 mmol)을 넣은 후 반응 용매인 toluene 20 mL에 녹이고 여기에 piperidine 0.404 mL (4.09 mmol)과 acetic acid 0.234 mL (4.09 mmol)까지 넣어준 후 80 °C에서 18시간 이상 반응 시켰다. 반응의 완료는 TLC로 확인하였으며 생성된 침전물을 재결정한 후 감압 여과하여 순수한 고체를 얻었다.

Yield : 95.5 %; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.46 (s, 1H), δ 10.32 (s, 1H), δ 7.69 (s, 1H), δ 7.46 (d, $J=8.43$ Hz, 2H), δ 6.92 (d, $J=8.43$ Hz, 2H)

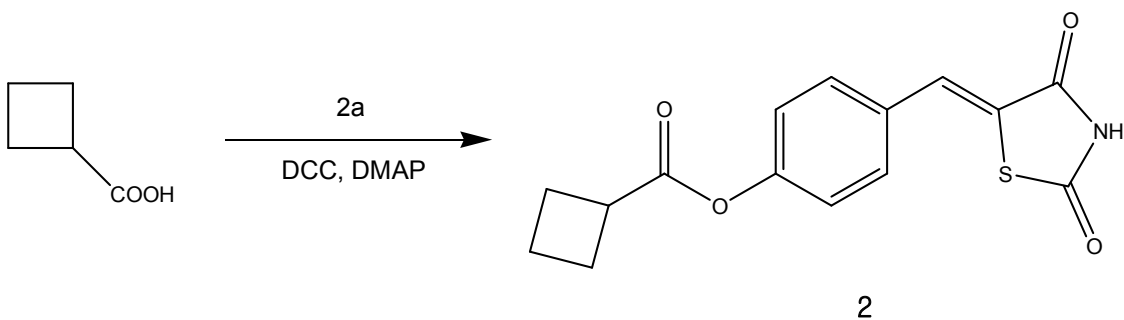
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropanecarboxylate
(1)



둥근 플라스크에 1a를 1 g (4.52 mmol)를 넣고 Cyclopropanecarboxylic acid 0.36 g (4.52 mmol), DMAP (4-(Dimethylamino)pyridine) 0.046 g (0.38 mmol)까지 넣어준 후 Dichloromethane (20 mL)에 녹여 ice bath에 넣고 교반시켰다. 5분 후 DCC (N,N'-Dicyclohexylcarbodiimide) 를 0.858 g (4.16 mmol)넣고 상온에서 반응시켰으며 TLC로 확인하여 반응이 종결되면 생성된 고체인 urea를 감압 여과하여 거른 후 여과액을 산·염기 추출과 재결정을 하여 순수한 고체를 얻었다.

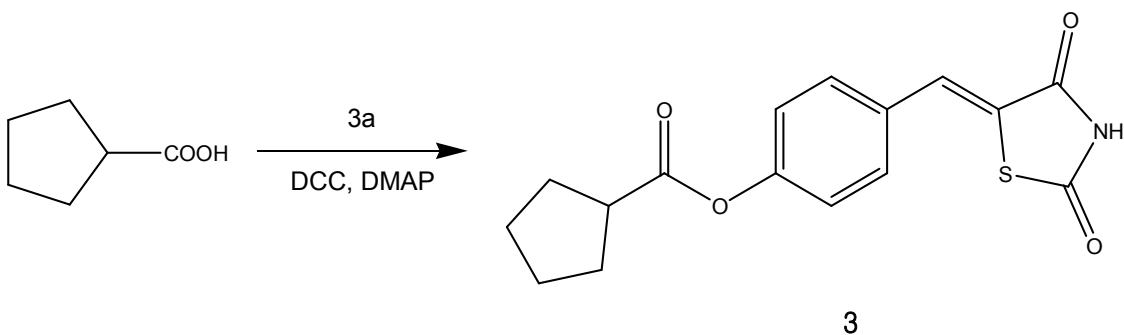
Yield : 81.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.43$ Hz, 2H), δ 7.32 (d, $J=8.43$ Hz, 2H), δ 1.95 (m, $J=4.41$ Hz, 1H), δ 1.15 (m, $J=4.41$ Hz, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutanecarboxylate (2)



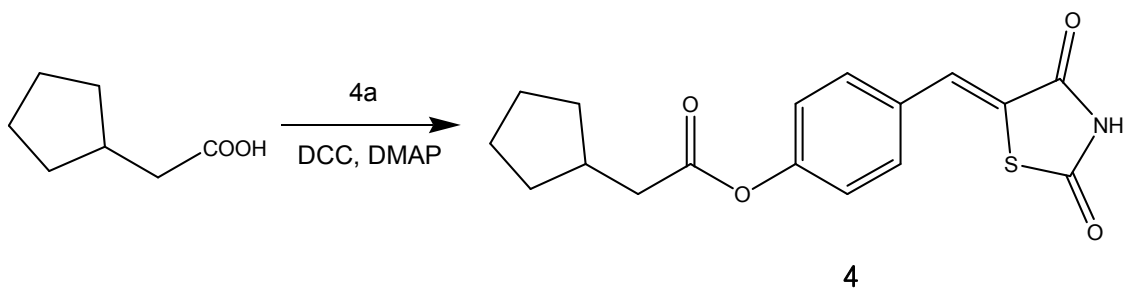
Yield : 83.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.69 (d, $J=8.43$ Hz, 2H), δ 7.31 (d, $J=8.43$ Hz, 2H), δ 3.52 (m, 1H), δ 2.39 (m, 4H), δ 2.07 (m, 1H), δ 1.94 (m, 1H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentanecarboxylate (3)



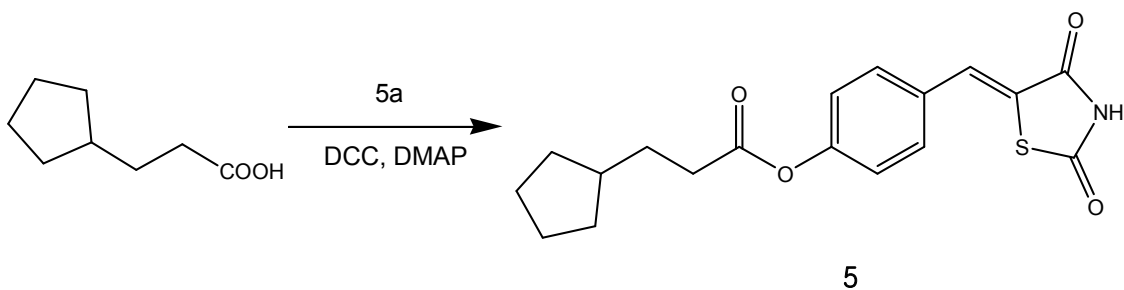
Yield : 91.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.81 (s, 1H), δ 7.66 (d, $J=8.43$ Hz, 2H), δ 7.31 (d, $J=8.43$ Hz, 2H), δ 3.11 (m, $J=7.32$ Hz, 1H), δ 2.00 (m, $J=7.32$ Hz, 4H), δ 1.67 (m, $J=7.32$ Hz, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (4)



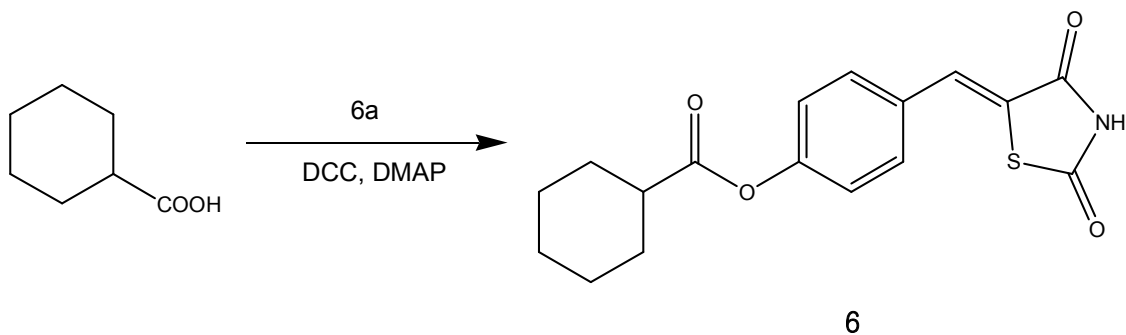
Yield : 88.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.70 (d, $J=8.79$ Hz, 2H), δ 7.30 (d, $J=8.79$ Hz, 2H), δ 2.62 (d, $J=7.32$ Hz, 2H), δ 2.32 (m, $J=7.32$ Hz, 1H), δ 1.88 (m, $J=6.96$ Hz, 2H), δ 1.68 (m, $J=6.96$ Hz, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (5)



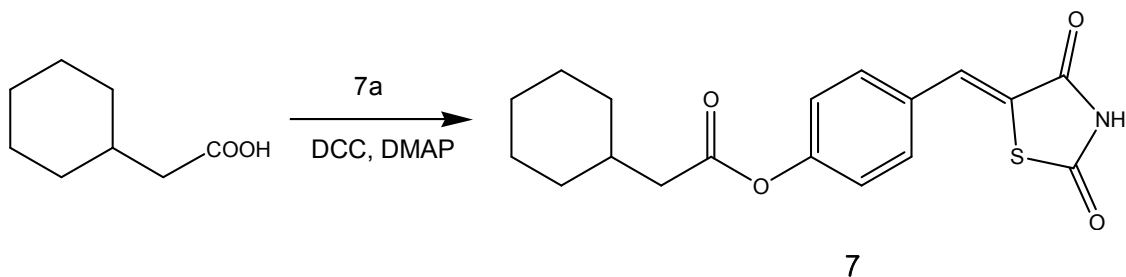
Yield : 92.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.4$ Hz, 2H), δ 7.31 (d, $J=8.4$ Hz, 2H), δ 2.63 (d, $J=7.68$ Hz, 2H), δ 1.87 (m, 3H), δ 1.69 (m, 6H), δ 1.14 (m, $J=7.68$ Hz, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexanecarboxylate (6)



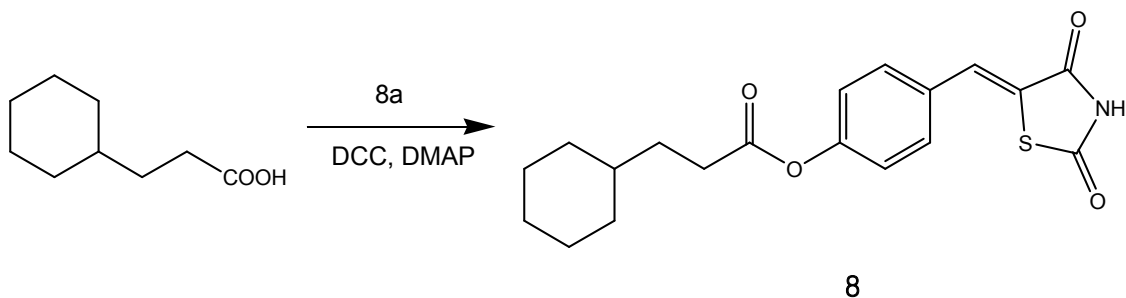
Yield : 86.6%, $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.63 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.4$ Hz, 2H), δ 7.29 (d, $J=8.4$ Hz, 2H), δ 2.62 (m, 1H), δ 2.00 (m, 2H), δ 1.73 (m, 2H), δ 1.64 (m, 3H), δ 1.36 (m, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate (7)



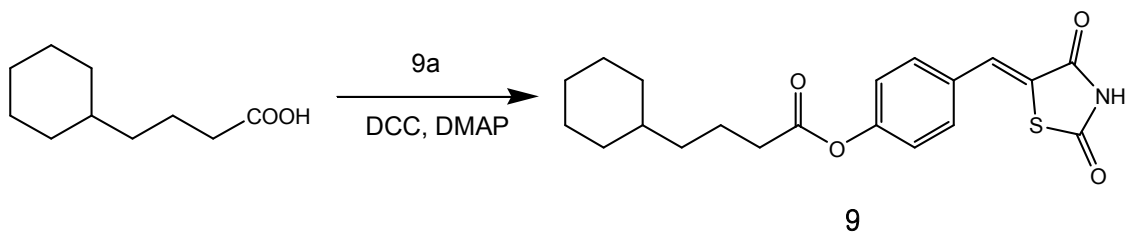
Yield : 89.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.79$ Hz, 2H), δ 7.30 (d, $J=8.79$ Hz, 2H), δ 2.49 (d, 2H), δ 1.87 (m, 6H), δ 1.32 (m, 5H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (8)



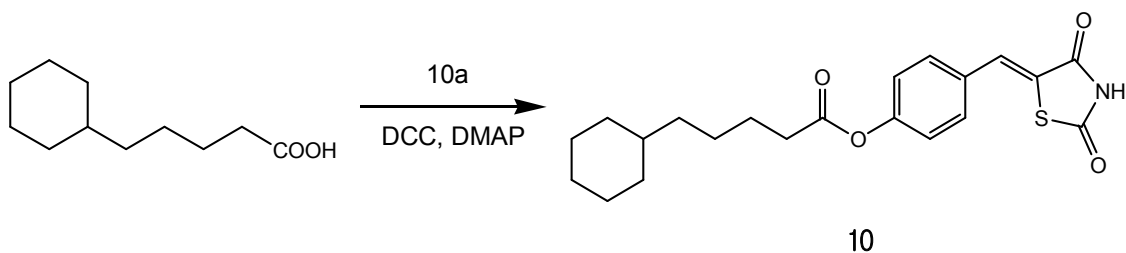
Yield : 90.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.63 (s, 1H), δ 7.80 (s, 1H), δ 7.65 (d, $J=8.79$ Hz, 2H), δ 7.29 (d, $J=8.79$ Hz, 2H), δ 2.62 (t, $J=7.71$ Hz, 2H), δ 1.73 (m, $J=7.71$ Hz, 7H), δ 1.34 (m, $J=7.71$ Hz, 4H), δ 0.95 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (9)



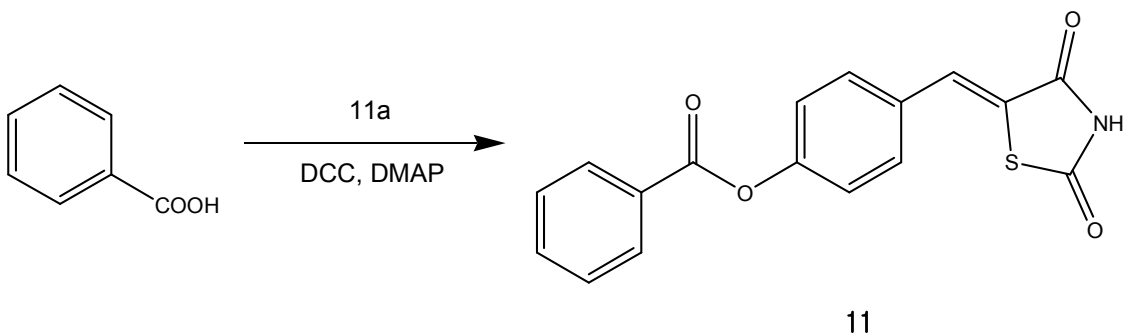
Yield : 90.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.4$ Hz, 2H), δ 7.30 (d, $J=8.4$ Hz, 2H), δ 2.60 (t, 2H), δ 1.71 (m, 7H), δ 1.26 (m, 6H), δ 0.92 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexylpentanoate (10)



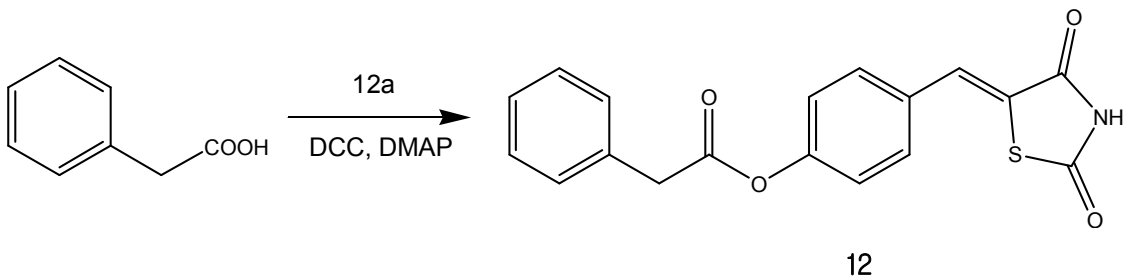
Yield : 84.5%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.43$ Hz, 2H), δ 7.30 (d, $J=8.43$ Hz, 2H), δ 2.62 (t, $J=7.32$ Hz, 2H), δ 1.69 (m, $J=7.32$ Hz, 7H), δ 1.40 (m, 2H), δ 1.21 (m, $J=7.32$ Hz, 6H), δ 0.89 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (11)



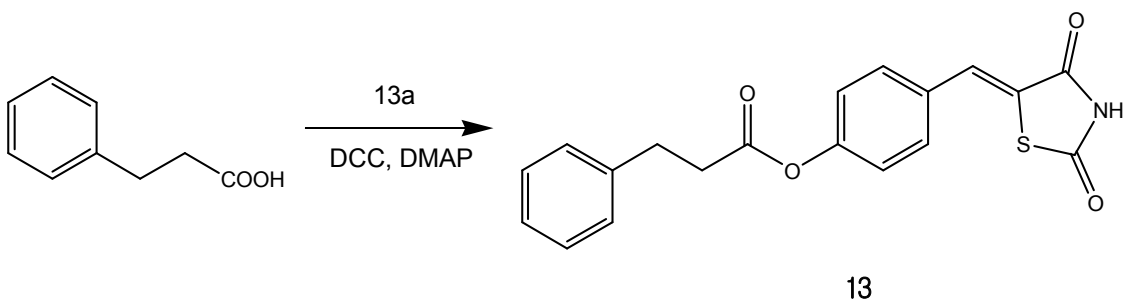
Yield : 72.2%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 8.16 (d, $J=7.32$ Hz, 2H), δ 7.84 (s, 1H), δ 7.80 (m, $J=7.32$ Hz, 3H), δ 7.65 (t, $J=7.32$ Hz, 2H), δ 7.50 (d, $J=7.32$ Hz, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (12)



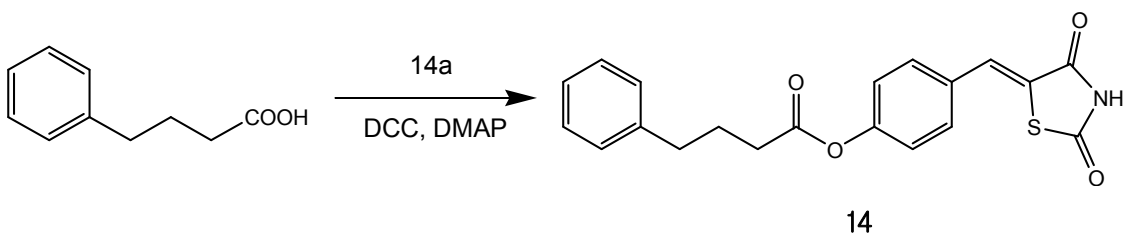
Yield : 88.8%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.68 (d, 2H), δ 7.40 (m, 7H), δ 4.00 (s, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (13)



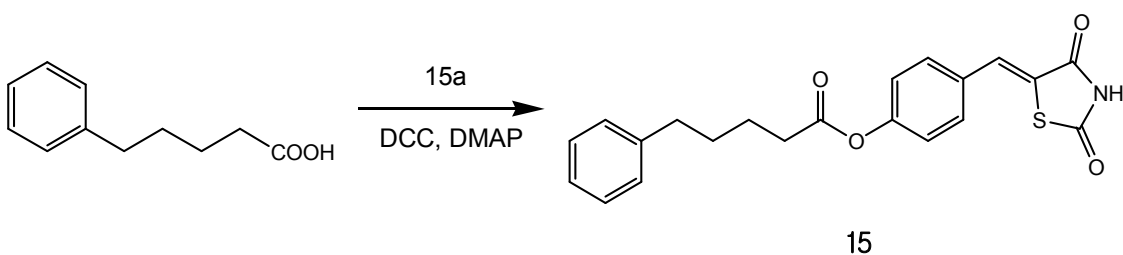
Yield : 83.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.79 (s, 1H), δ 7.65 (d, $J=8.43$ Hz, 2H), δ 7.35 (m, $J=8.43$ Hz, 4H), δ 7.26 (m, $J=8.43$ Hz, 3H), δ 2.99 (m, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (14)



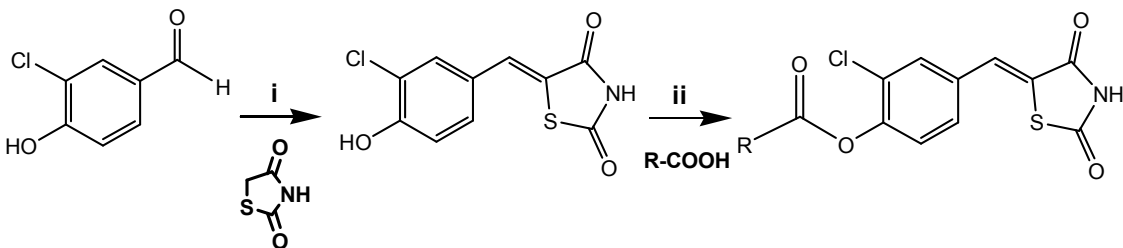
Yield : 80.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, 2H), δ 7.33 (m, 7H), δ 2.63 (t, 4H), δ 1.96 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (15)



Yield : 77.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.62 (s, 1H), δ 7.80 (s, 1H), δ 7.66 (d, $J=8.43$ Hz, 2H), δ 7.30 (m, $J=8.43$ Hz, 7H), δ 2.63 (t, 4H), δ 1.68 (m, 4H)

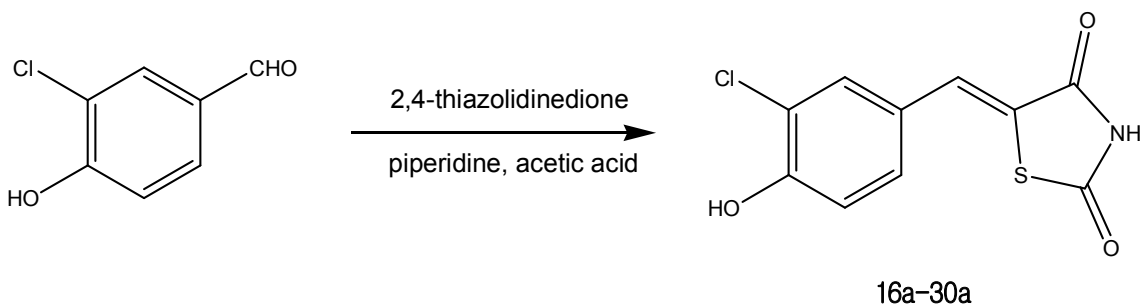
1.2. 화합물 (16-30) 합성



Scheme 2. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

Scheme 2은 16부터 30까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

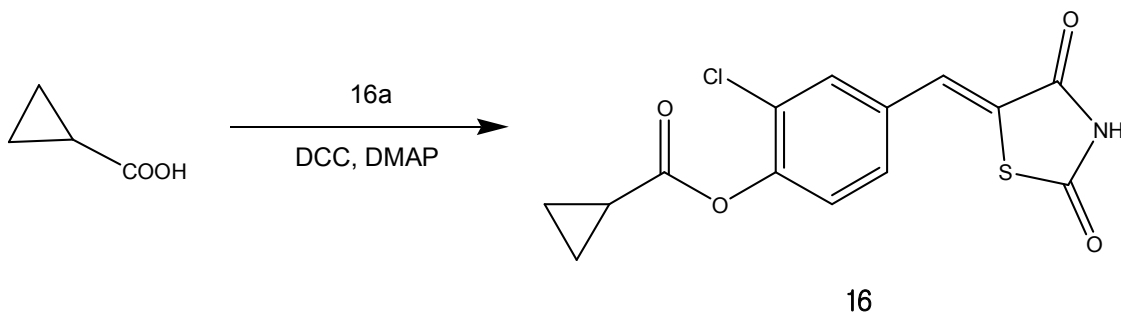
5-(3-Chloro-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (16a-30a)



Yield : 89.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.50 (s, 1H), δ 11.15 (s, 1H), δ 7.68 (s, 1H), δ 7.63 (d, $J=2.19$ Hz, 1H), δ 7.41 (dd, $J=8.43$ and 2.19 Hz,

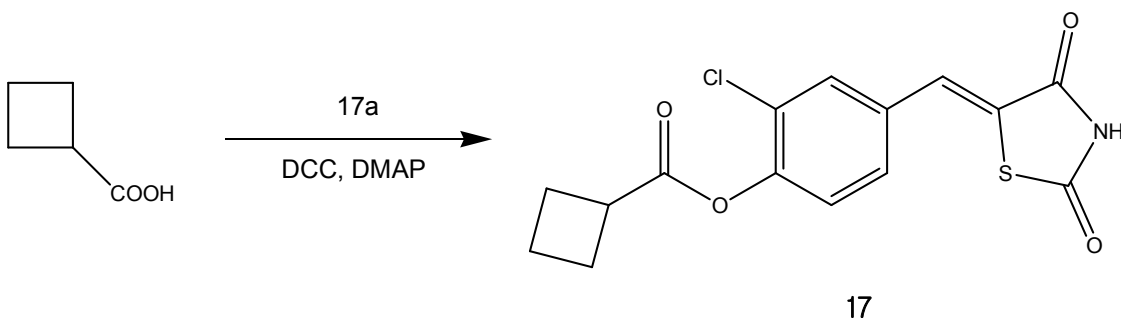
1H), δ 7.12 (d, $J=8.43$ Hz, 1H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropane
-carboxylate (16)



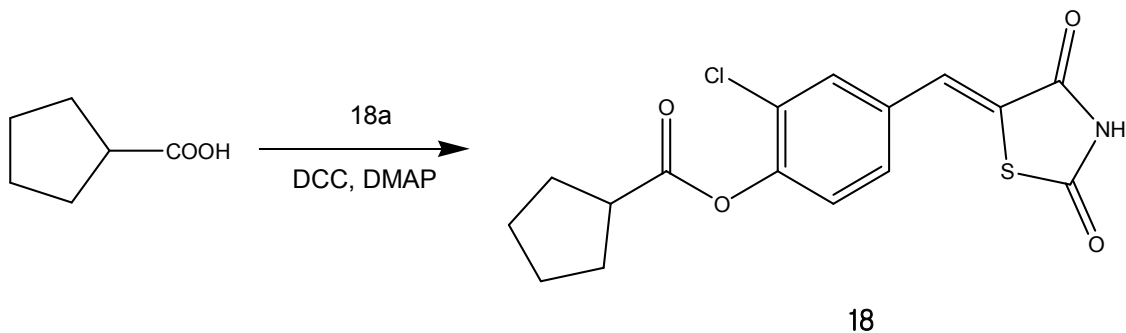
Yield : 82.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.60 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.50 (d, $J=8.43$ Hz, 1H), δ 2.02 (m, 1H), δ 1.16 (m, 4H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane
-carboxylate (17)



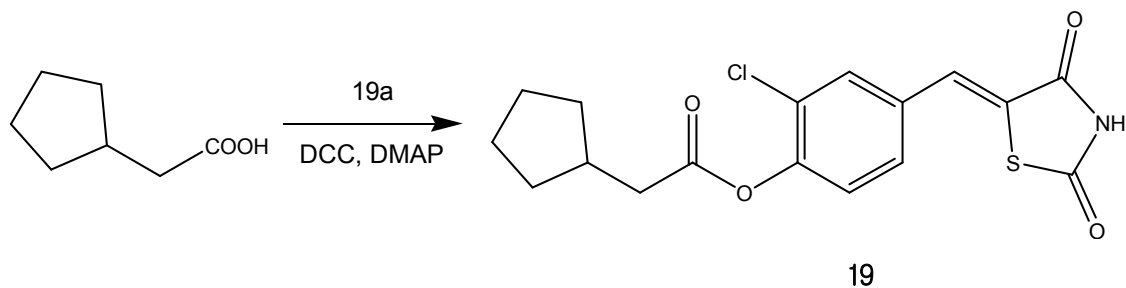
Yield : 83.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.83 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.60 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 7.49 (d, $J=8.4$ Hz, 1H), δ 3.58 (m, 1H), δ 2.42 (m, 4H), δ 2.09 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane
-carboxylate (18)



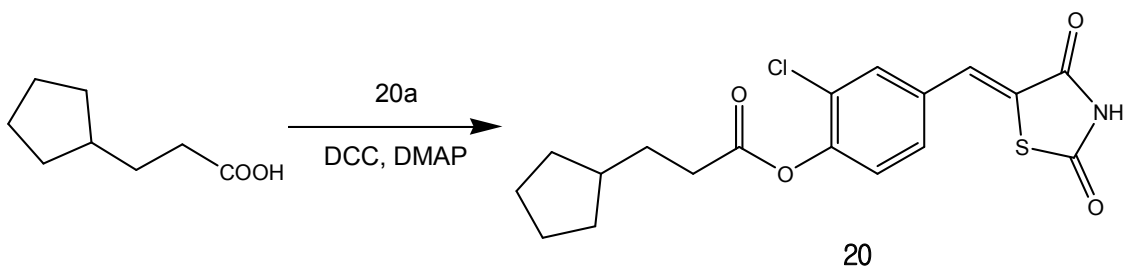
Yield : 89.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.73 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.50 (d, $J=8.43$ Hz, 1H), δ 3.18 (m, 1H), δ 2.08 (m, 4H), δ 1.70 (m, 4H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentyl
-acetate (19)



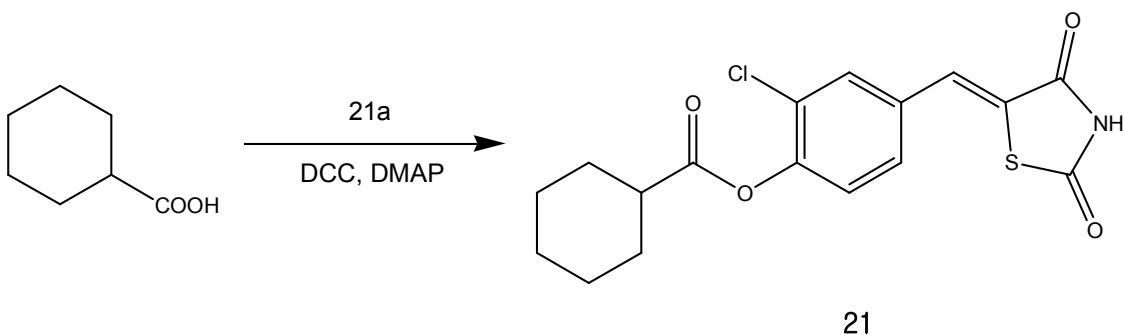
Yield : 89.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.72 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 2.68 (d, $J=7.32$ Hz, 2H), δ 2.33 (m, $J=7.32$ Hz, 1H), δ 1.90 (m, 2H), δ 1.66 (m, 4H), δ 1.30 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (20)



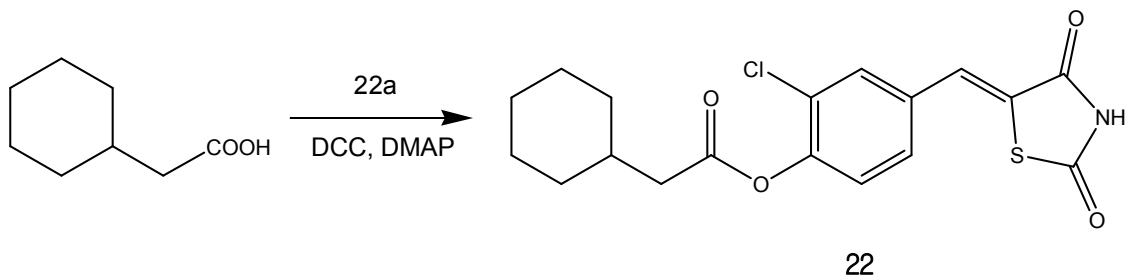
Yield : 89.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.73 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 7.49 (d, $J=8.4$ Hz, 1H), δ 2.07 (t, $J=7.32$ Hz, 2H), δ 1.90 (m, 4H), δ 1.62 (m, 4H), δ 1.19 (m, $J=7.32$ Hz, 3H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane-carboxylate (21)



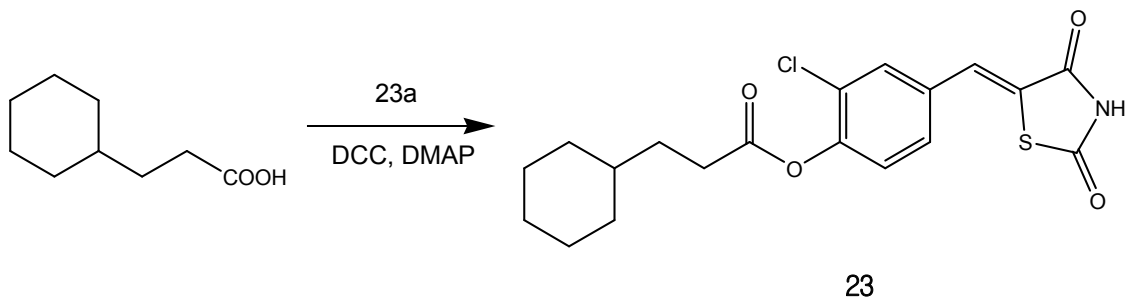
Yield : 92.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.74 (s, 1H), δ 7.84 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.47 (d, $J=8.43$ Hz, 1H), δ 2.73 (m, 1H), δ 2.08 (m, 2H), δ 1.76 (m, 2H), δ 1.65 (m, 2H), δ 1.41 (m, 4H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate (22)



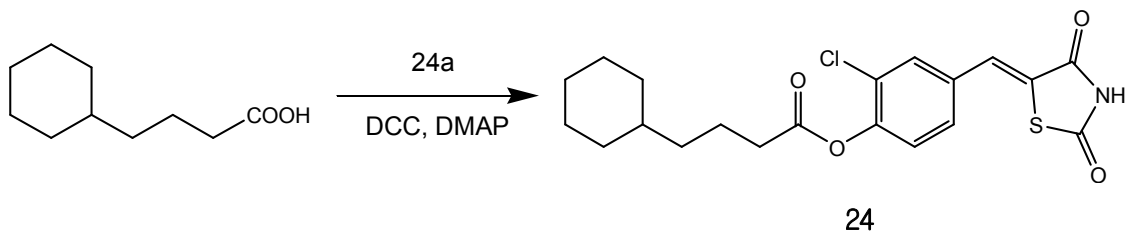
Yield : 86.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.86 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.4$ Hz, 1H), δ 2.51 (t, 2H), δ 1.86 (m, 1H), δ 1.71 (m, 4H), δ 1.32 (m, 6H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (23)



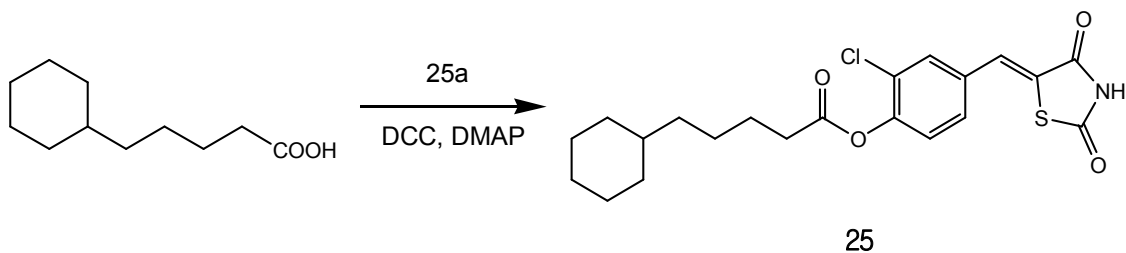
Yield : 93.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 2.69 (t, 2H), δ 1.75 (m, 8H), δ 1.33 (m, 3H), δ 0.95 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (24)



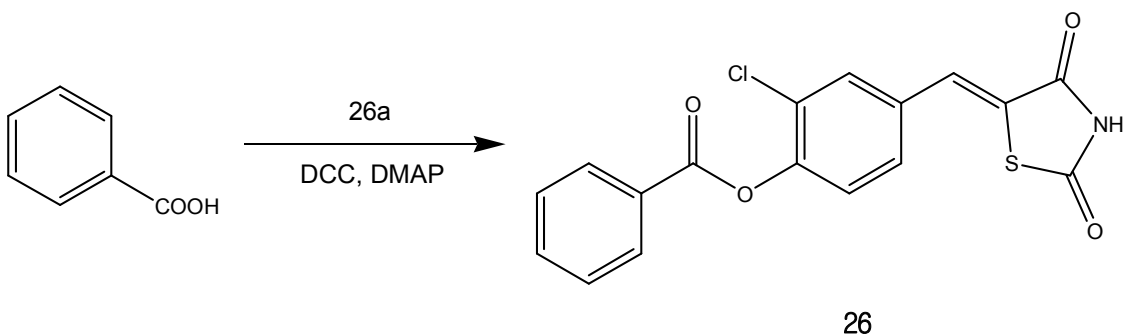
Yield : 89%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.85 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 2.66 (t, 2H), δ 1.70 (m, 7H), δ 1.30 (m, 6H), δ 0.92 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexylpentanoate (25)



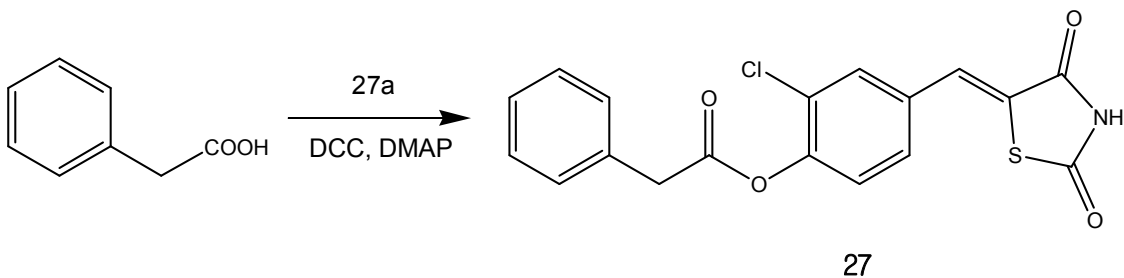
Yield : 91.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.84 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 2.67 (t, $J=7.32$ Hz, 2H), δ 1.69 (m, $J=7.32$ Hz, 7H), δ 1.43 (m, $J=7.32$ Hz, 2H), δ 1.21 (m, $J=7.32$ Hz, 6H), δ 0.90 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (26)



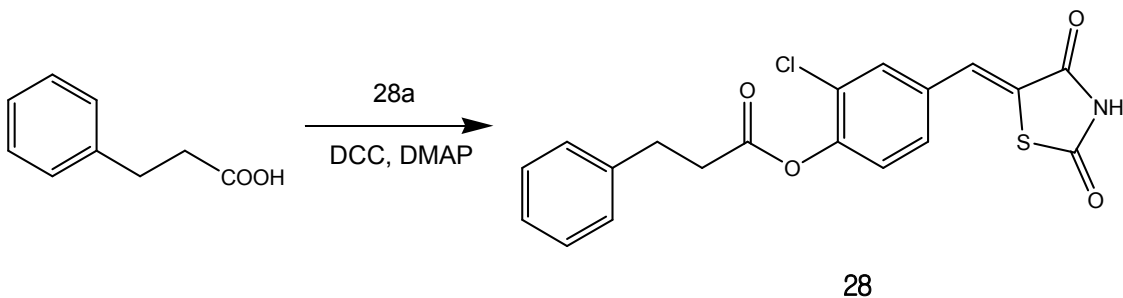
Yield : 71.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 8.18 (dd, $J=7.32$ and 1.47 Hz, 2H), δ 7.92 (d, $J=1.47$ Hz, 1H), δ 7.84 (t, $J=7.32$ Hz, 2H), δ 7.67 (m, 4H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (27)



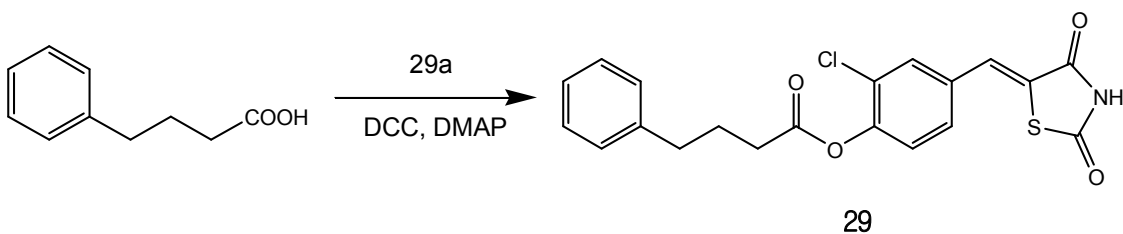
Yield : 90.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.85 (d, $J=2.19$ Hz, 1H), δ 7.79 (s, 1H), δ 7.61 (dd, $J=8.4$ and 2.19 Hz, 1H), δ 7.50 (d, $J=8.4$ Hz, 1H), δ 7.41 (t, 4H), δ 7.35 (m, 1H), δ 7.06 (s, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (28)



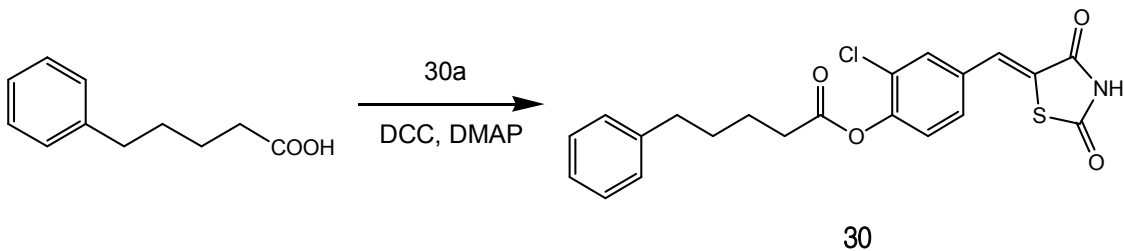
Yield : 92.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.72 (s, 1H), δ 7.84 (d, $J=2.19$ Hz, 1H), δ 7.79 (s, 1H), δ 7.60 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.41 (d, $J=8.43$ Hz, 1H), δ 7.32 (m, 4H), δ 7.25 (m, 1H), δ 3.00 (s, 4H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (29)



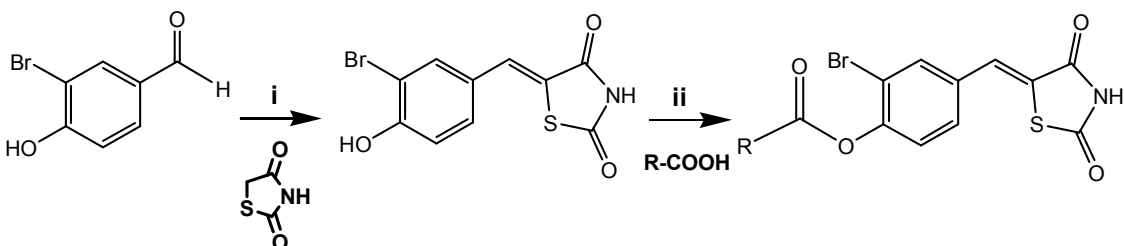
Yield : 87%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.72 (s, 1H), δ 7.86 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.61 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.5 (d, $J=8.43$ Hz, 1H), δ 7.33 (m, 5H), δ 2.72 (m, $J=7.68$ Hz, 4H), δ 2.02 (m, 2H)

(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (30)



Yield : 81.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.73 (s, 1H), δ 7.84 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.60 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 7.47 (d, $J=8.4$ Hz, 1H), δ 7.30 (t, $J=7.32$ Hz, 2H), δ 7.21 (m, $J=7.32$ Hz, 3H), δ 2.74 (t, 4H), δ 1.70 (m, 4H)

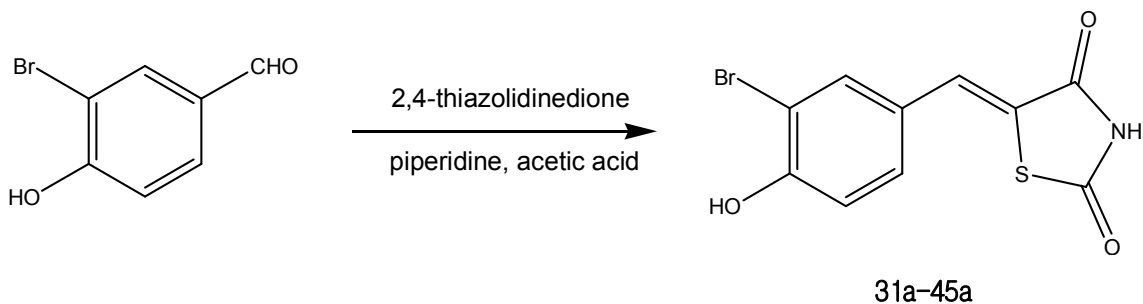
1.3. 화합물 (31-45) 합성



Scheme 3. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

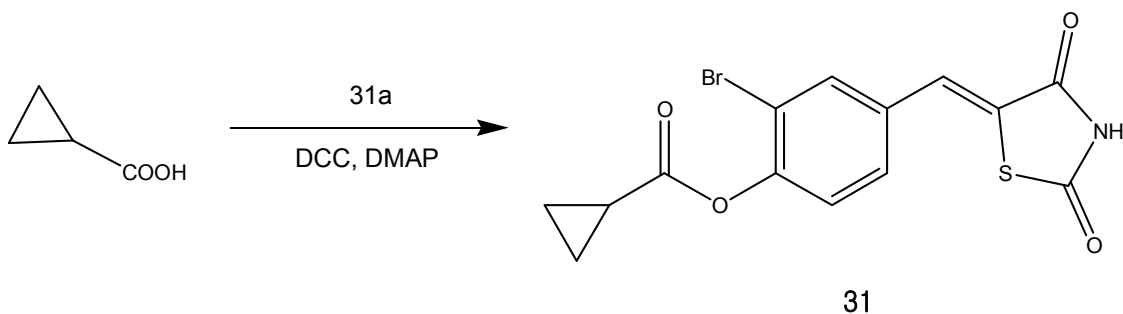
Scheme 3은 31부터 45까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

5-(3-Bromo-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (31a-45a)



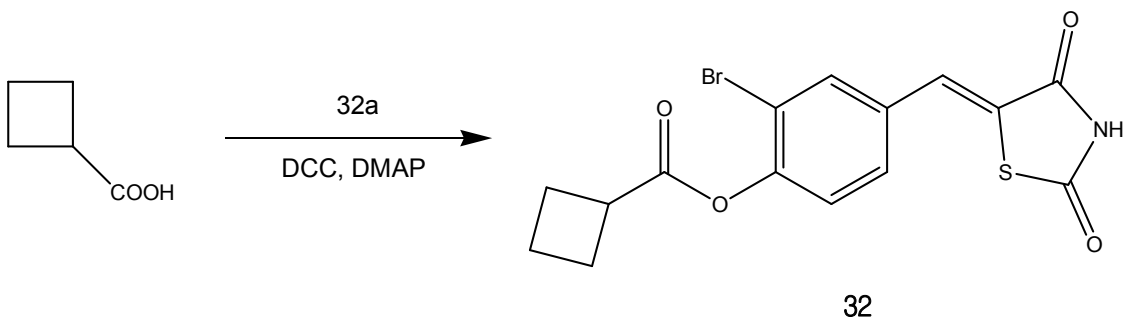
Yield : 94.1%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.54 (s, 1H), δ 11.16 (s, 1H), δ 7.78 (d, $J=2.19$ Hz, 1H), δ 7.69 (s, 1H), δ 7.45 (dd, $J=8.79$ and 2.19 Hz, 1H), δ 7.10 (d, $J=8.79$ Hz, 1H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropane-carboxylate (31)



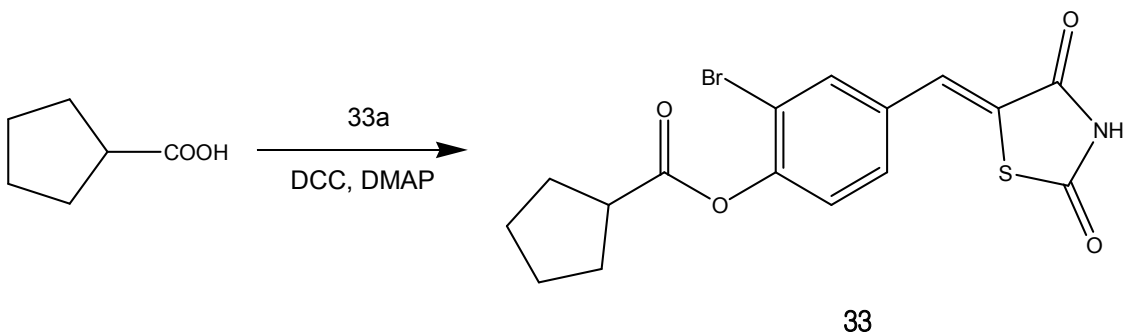
Yield : 83.1%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.97 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.63 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 2.01 (m, 1H), δ 1.16 (m, 4H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane-carboxylate (32)



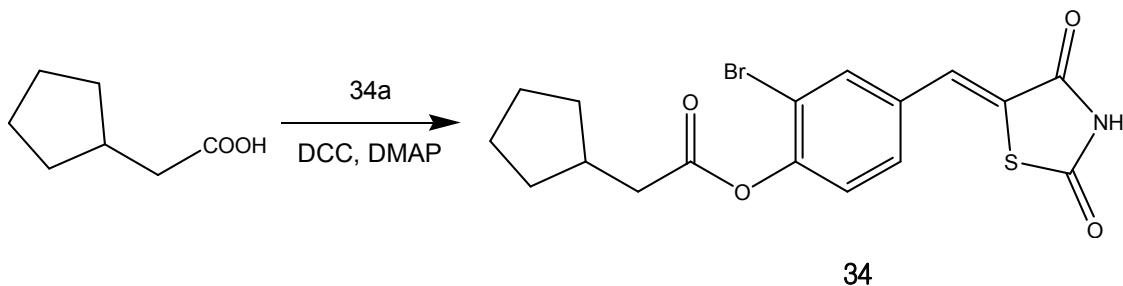
Yield : 81.3%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.69 (s, 1H), δ 7.98 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 7.47 (d, $J=8.4$ Hz, 1H), δ 3.58 (m, 1H), δ 2.42 (m, 4H), δ 2.10 (m, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-1-carboxylate (33)



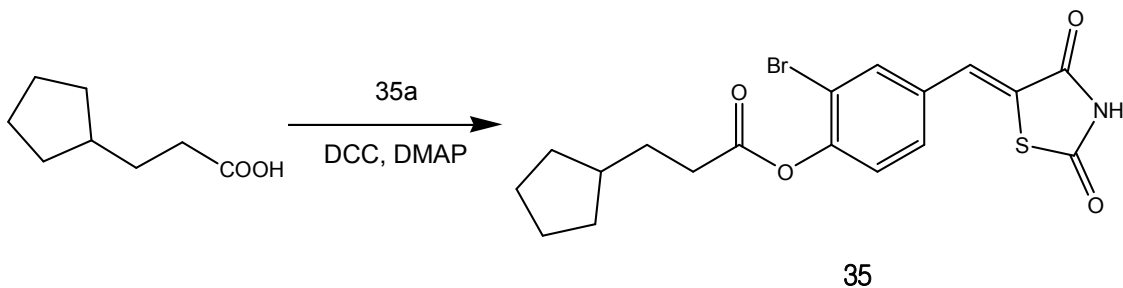
Yield : 84.5%; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 12.73 (s, 1H), δ 7.98 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.47 (d, $J=8.43$ Hz, 1H), δ 3.17 (m, $J=6.96$ Hz, 1H), δ 2.06 (m, $J=6.96$ Hz, 4H), δ 1.68 (m, $J=6.96$ Hz, 4H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (34)



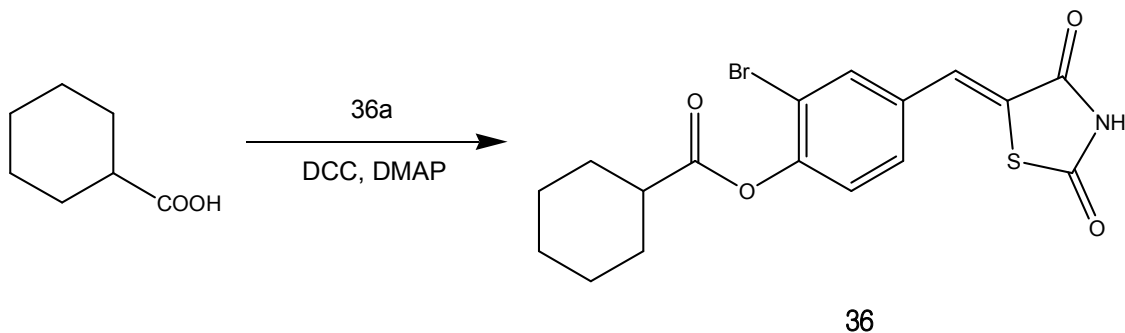
Yield : 91.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.73 (s, 1H), δ 7.99 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.45 (d, $J=8.43$ Hz, 1H), δ 2.67 (d, $J=7.32$ Hz, 2H), δ 2.34 (m, $J=7.32$ Hz, 1H), δ 1.91 (m, $J=6.96$ Hz, 2H), δ 1.66 (m, $J=6.96$ Hz, 4H), δ 1.30 (m, $J=6.96$ Hz, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (35)



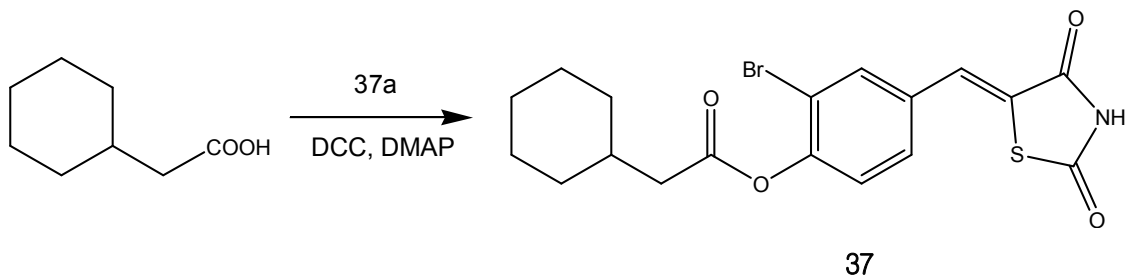
Yield : 93.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.72 (s, 1H), δ 7.98 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.46 (d, $J=8.43$ Hz, 1H), δ 2.68 (t, $J=7.32$ Hz, 2H), δ 1.90 (m, 9H), δ 1.15 (m, $J=7.32$ Hz, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane
-carboxylate (36)



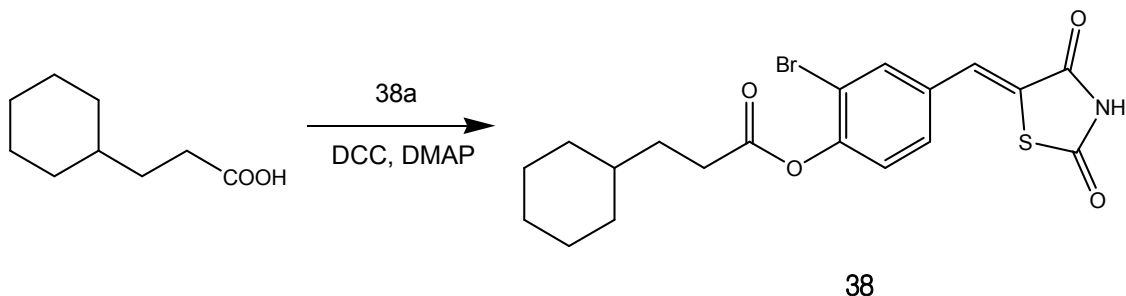
Yield : 83.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.98 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.4$ and 2.19 Hz, 1H), δ 7.45 (d, $J=8.4$ Hz, 1H), δ 2.72 (m, 1H), δ 2.08 (m, 2H), δ 1.78 (m, 8H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate
(37)



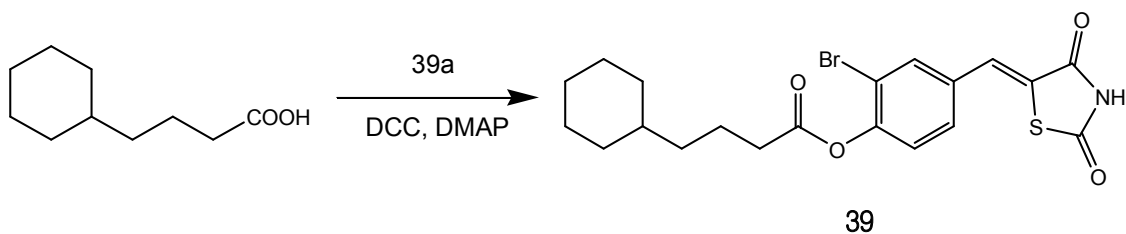
Yield : 88.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.99 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.45 (d, $J=8.43$ Hz, 1H), δ 2.54 (m, 1H), δ 1.90 (m, 2H), δ 1.71 (m, 4H), δ 1.32 (m, 6H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexyl
-propanoate (38)



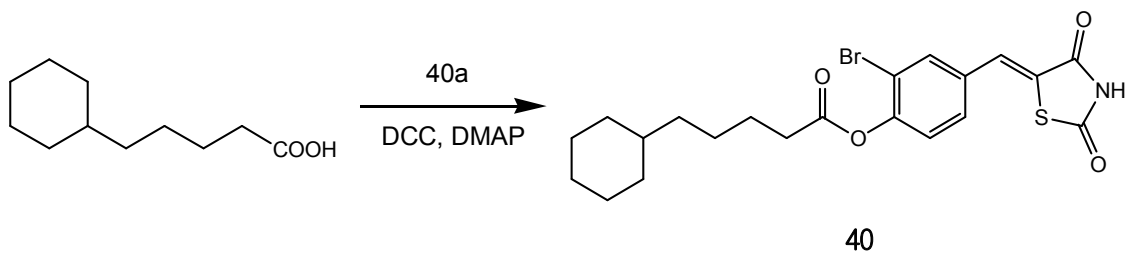
Yield : 91.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.71 (s, 1H), δ 7.98 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.4$ and 2.19 Hz, 1H), δ 7.45 (d, $J=8.4$ Hz, 1H), δ 2.68 (m, 2H), δ 1.75 (m, 7H), δ 1.38 (m, 4H), δ 0.96 (m, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexyl
-butanoate (39)



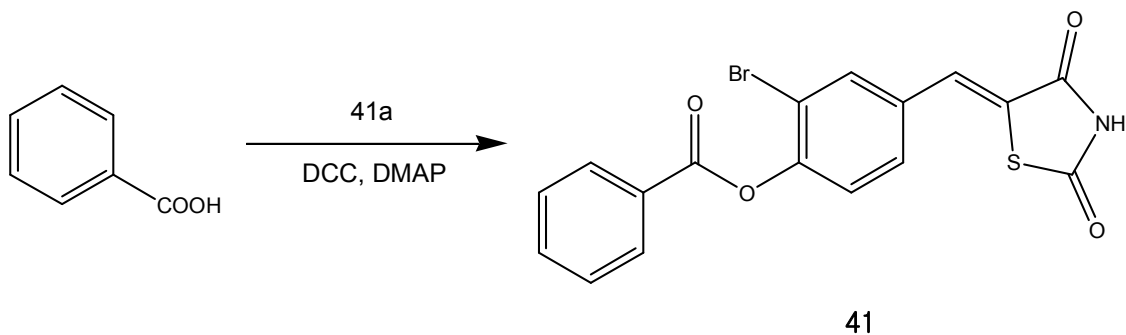
Yield : 92.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.71 (s, 1H), δ 7.98 (d, $J=2.19$ Hz, 1H), δ 7.80 (s, 1H), δ 7.65 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.46 (d, $J=8.43$ Hz, 1H), δ 2.65 (t, 2H), δ 1.71 (m, 7H), δ 1.31 (m, 6H), δ 0.92 (m, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl
-pentanoate (40)



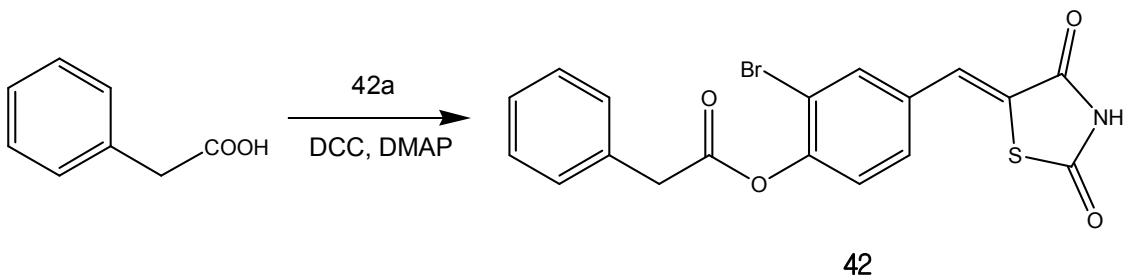
Yield : 81.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.70 (s, 1H), δ 7.97 (d, $J=1.83$ Hz, 1H), δ 7.79 (s, 1H), δ 7.64 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.45 (d, $J=8.43$ Hz, 1H), δ 2.66 (t, $J=7.32$ Hz, 2H), δ 1.69 (m, 7H), δ 1.43 (m, $J=7.32$ Hz, 2H), δ 1.21 (m, 6H), δ 0.90 (m, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (41)



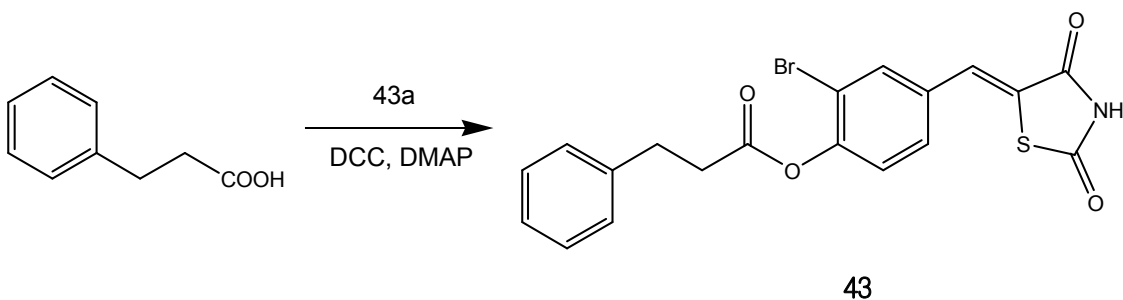
Yield : 70.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 8.18 (d, $J=7.32$ Hz, 2H), δ 8.04 (d, $J=1.83$ Hz, 1H), δ 7.83 (t, $J=7.32$ Hz, 2H), δ 7.71 (m, $J=7.32$ Hz, 4H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate
(42)



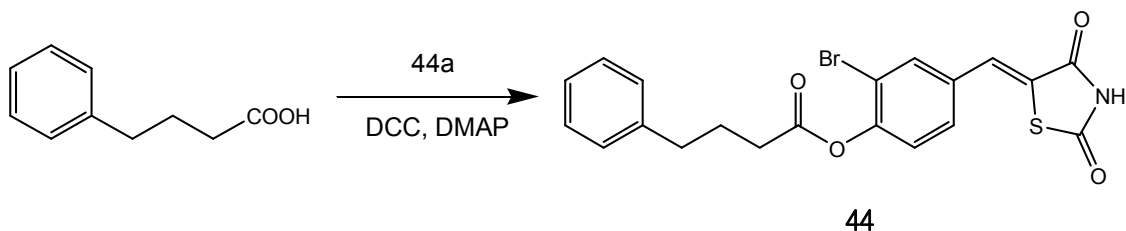
Yield : 89.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.97 (d, $J=2.19$ Hz, 1H), δ 7.79 (s, 1H), δ 7.66 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.48 (d, $J=8.43$ Hz, 1H), δ 7.42 (m, 5H), δ 4.06 (s, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate
(43)



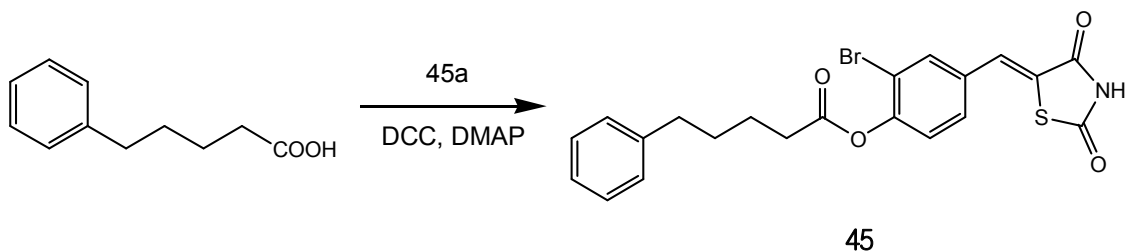
Yield : 88.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.72 (s, 1H), δ 7.98 (d, $J=2.19$ Hz, 1H), δ 7.79 (s, 1H), δ 7.64 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.39 (d, $J=8.43$ Hz, 1H), δ 7.32 (m, 4H), δ 7.26 (m, 1H), δ 3.00 (s, 4H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate
(44)



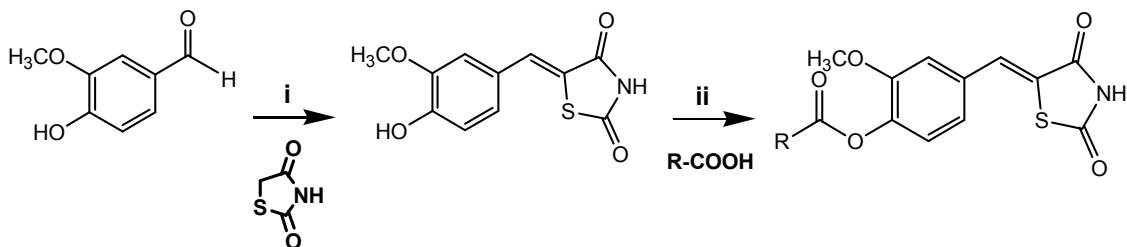
Yield : 82.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.70 (s, 1H), δ 7.98 (d, $J=1.83$ Hz, 1H), δ 7.80 (s, 1H), δ 7.64 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 7.46 (d, $J=8.43$ Hz, 1H), δ 7.33 (m, 5H), δ 2.73 (m, $J=7.32$ Hz, 4H), δ 2.03 (m, $J=7.32$ Hz, 2H)

(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (45)



Yield : 78.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.71 (s, 1H), δ 7.97 (d, $J=2.19$ Hz, 1H), δ 7.79 (s, 1H), δ 7.64 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 7.45 (d, $J=8.43$ Hz, 1H), δ 7.31 (m, 5H), δ 2.68 (m, 4H), δ 1.71 (m, 4H)

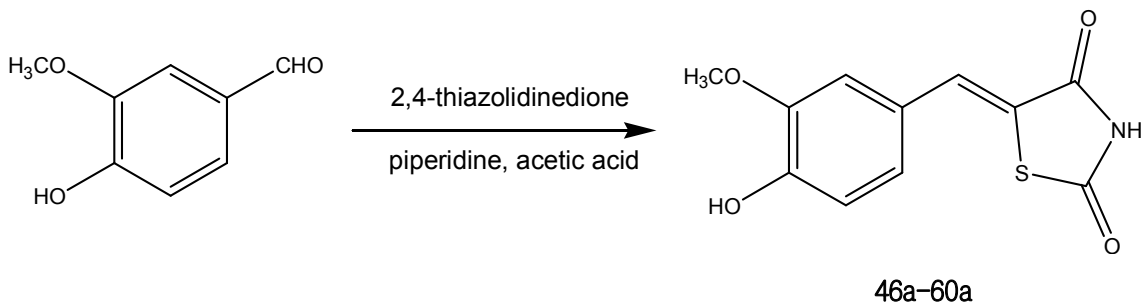
1.4. 화합물 (46-60) 합성



Scheme 4. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

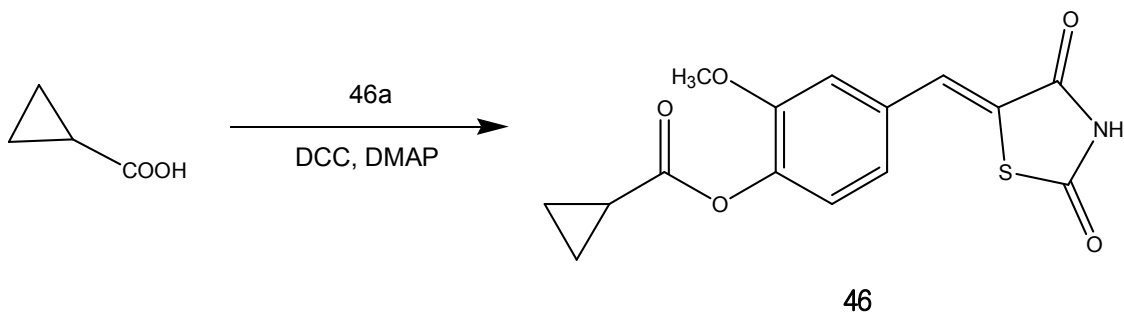
Scheme 4은 46부터 60까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

5-(4-Hydroxy-3-methoxy-benzylidene)-thiazolidine-2,4-dione (46a-60a)



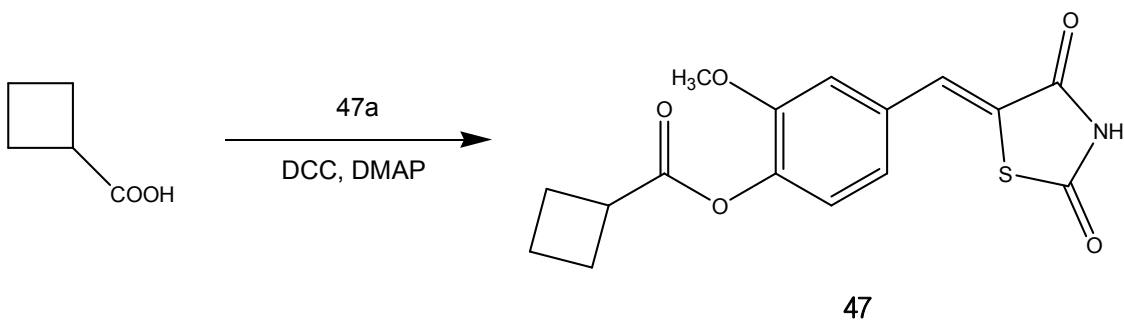
Yield : 94.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.47 (s, 1H), δ 9.96 (s, 1H), δ 7.71 (s, 1H), δ 7.17 (d, $J=1.83$ Hz, 1H), δ 7.08 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 6.93 (d, $J=8.04$ Hz, 1H), δ 3.86 (s, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclopropane-carboxylate (46)



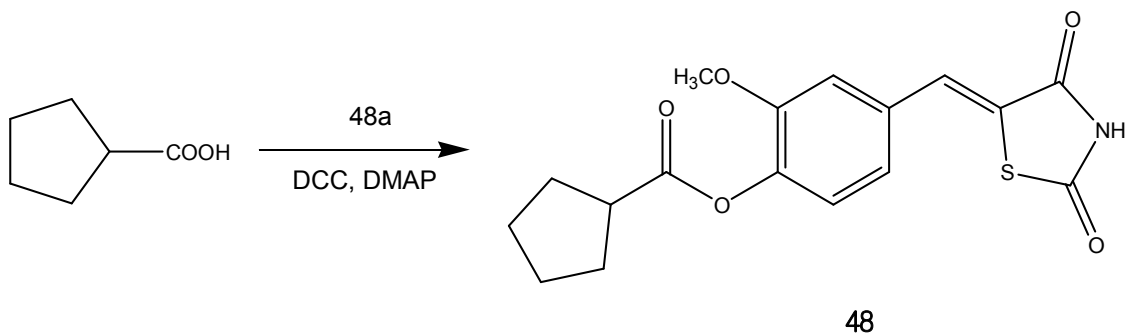
Yield : 76.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.28 (d, $J=8.04$ Hz, 1H), δ 7.19 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 3.82 (s, 3H), δ 1.95 (m, $J=3.3$ Hz, 1H), δ 1.15 (m, $J=3.3$ Hz, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclobutane-carboxylate (47)



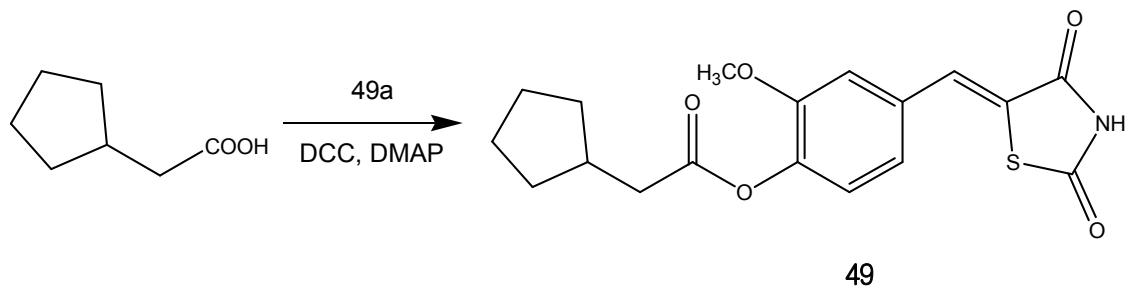
Yield : 81.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 7.81 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.27 (d, $J=8.43$ Hz, 1H), δ 7.19 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 3.81 (s, 3H), δ 3.51 (m, 1H), δ 2.35 (m, 4H), δ 2.07 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclopentane-carboxylate (48)



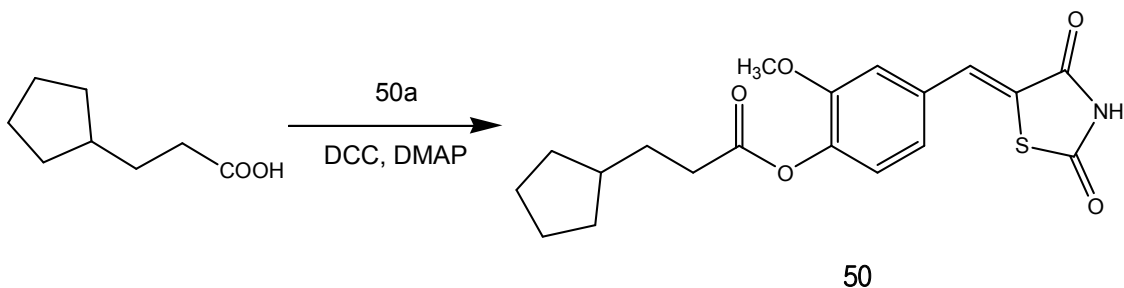
Yield : 83.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.27 (d, $J=8.43$ Hz, 1H), δ 7.19 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 3.81 (s, 3H), δ 3.10 (m, 1H), δ 1.99 (m, 4H), δ 1.69 (m, 4H)

Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-cyclopentylacetate (49)



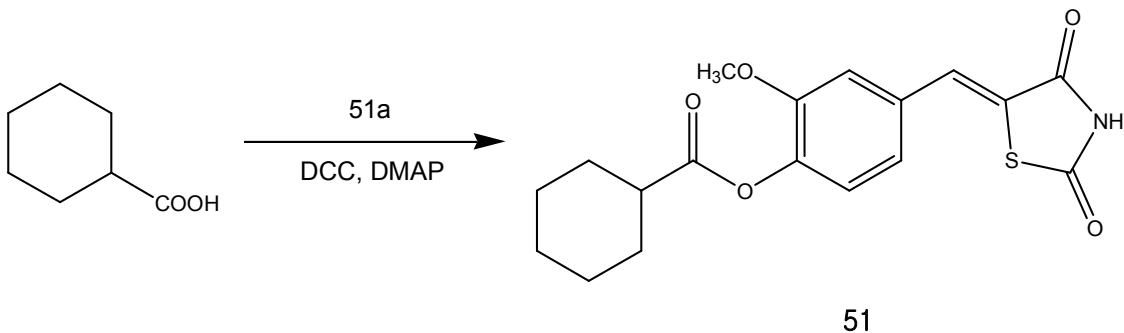
Yield : 81.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.26 (d, $J=8.07$ Hz, 1H), δ 7.19 (dd, $J=8.07$ and 1.83 Hz, 1H), δ 3.81 (s, 3H), δ 2.59 (d, $J=7.32$ Hz, 2H), δ 2.32 (m, $J=7.32$ Hz, 1H), δ 1.88 (m, $J=6.96$ Hz, 2H), δ 1.67 (m, 4H), δ 1.28 (m, $J=6.96$ Hz, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 3-cyclopentylpropanoate (50)



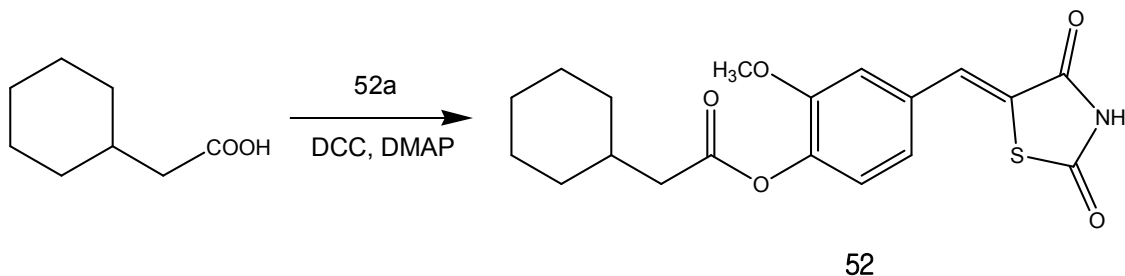
Yield : 88.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.47$ Hz, 1H), δ 7.26 (d, $J=8.4$ Hz, 1H), δ 7.19 (dd, $J=8.4$ and 1.47 Hz, 1H), δ 3.81 (s, 3H), δ 2.60 (d, $J=7.32$ Hz, 2H), δ 1.89 (m, $J=7.32$ Hz, 3H), δ 1.68 (m, $J=7.32$ Hz, 6H), δ 1.15 (m, $J=7.32$ Hz, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclohexane-carboxylate (51)



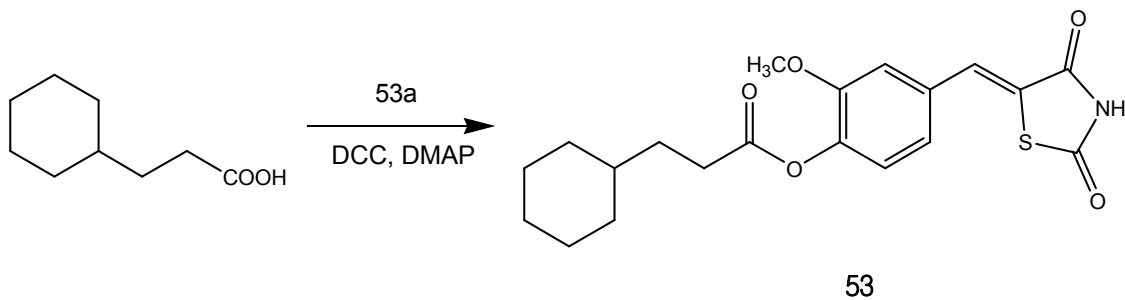
Yield : 85.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.07$ Hz, 1H), δ 7.19 (dd, $J=8.07$ and 1.83 Hz, 1H), δ 3.81 (s, 3H), δ 1.98 (m, 2H), δ 1.71 (m, 2H), δ 1.64 (m, 1H), δ 1.51 (m, 2H), δ 1.36 (m, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-cyclohexylacetate (52)



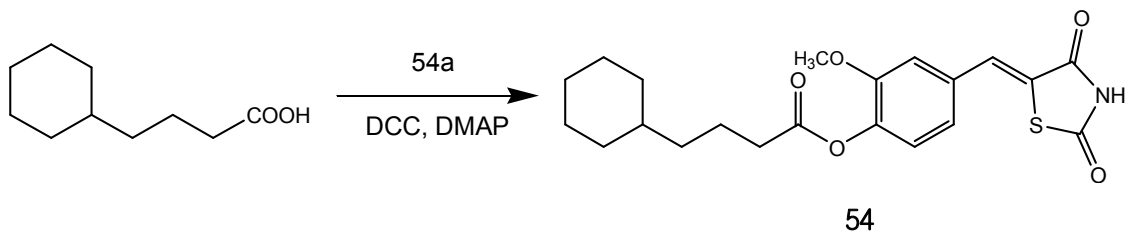
Yield : 90.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.04$ Hz, 1H), δ 7.19 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 3.80 (s, 3H), δ 2.46 (d, 2H), δ 1.85 (m, 6H), δ 1.32 (m, 5H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 3-cyclohexylpropanoate (53)



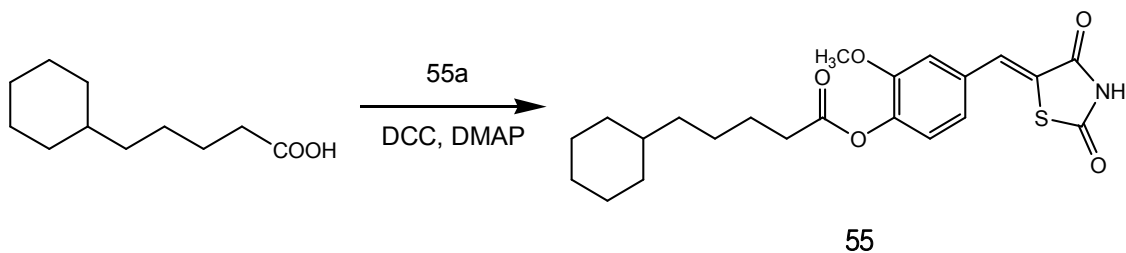
Yield : 93.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.47$ Hz, 1H), δ 7.26 (d, $J=8.43$ Hz, 1H), δ 7.19 (dd, $J=8.43$ and 1.47 Hz, 1H), δ 3.81 (s, 3H), δ 2.60 (t, 2H), δ 1.74 (m, 7H), δ 1.33 (m, 4H), δ 0.95 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-cyclohexylbutanoate (54)



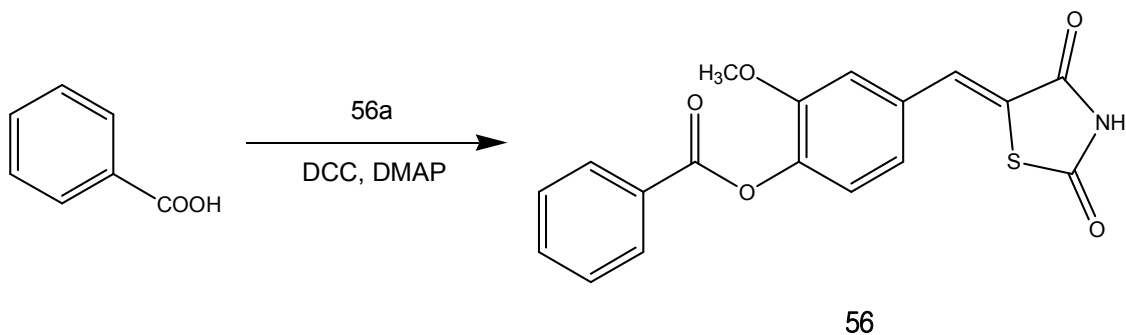
Yield : 90.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.28 (s, 1H), δ 7.43 (s, 1H), δ 7.00 (d, $J=1.83$ Hz, 1H), δ 6.89 (d, $J=8.4$ Hz, 1H), δ 6.82 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 3.44 (s, 3H), δ 2.14 (t, 2H), δ 1.34 (m, 7H), δ 0.90 (m, 6H), δ 0.55 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 5-cyclohexylpentanoate (55)



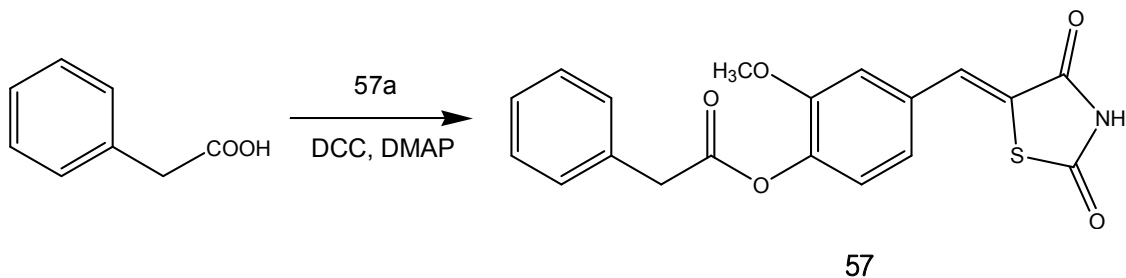
Yield : 79.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.07$ Hz, 1H), δ 7.19 (dd, $J=8.07$ and 1.83 Hz, 1H), δ 3.81 (s, 3H), δ 2.59 (t, 2H), δ 1.70 (m, 7H), δ 1.42 (m, 2H), δ 1.21 (m, 6H), δ 0.90 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl benzoate (56)



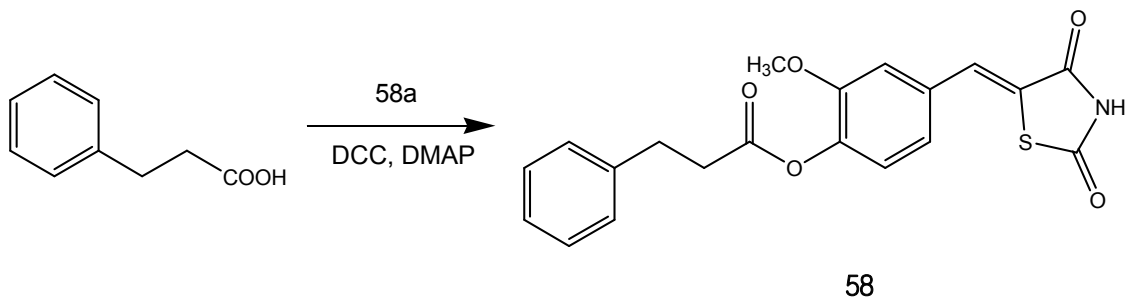
Yield : 73.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 8.13 (d, $J=7.32$ Hz, 2H), δ 7.84 (s, 1H), δ 7.79 (t, $J=7.32$ Hz, 1H), δ 7.64 (t, $J=7.32$ Hz, 2H), δ 7.44 (m, 2H), δ 7.26 (d, 1H), δ 3.82 (s, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-phenylacetate (57)



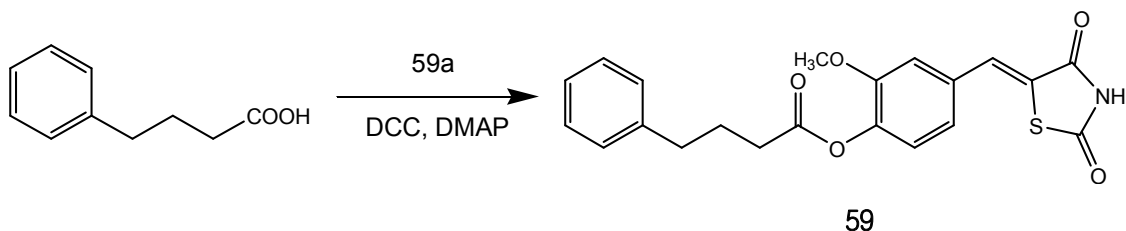
Yield : 81.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.38 (d, $J=4.38$ Hz, 5H), δ 7.34 (m, $J=4.38$ Hz, 2H), δ 7.19 (d, 1H), δ 3.98 (s, 2H), δ 3.79 (s, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 3-phenylpropanoate (58)



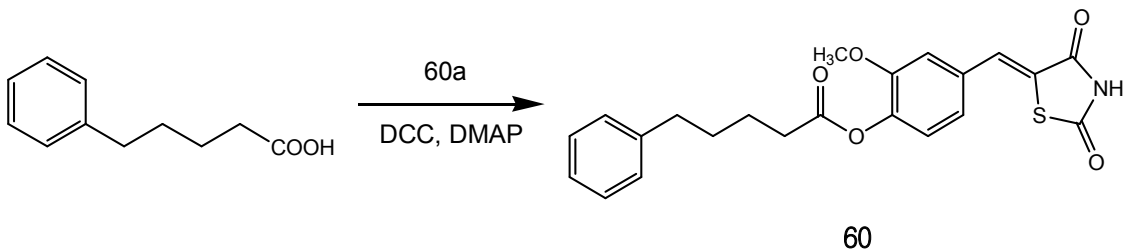
Yield : 85.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (m, 5H), δ 7.26 (m, 3H), δ 3.83 (s, 3H), δ 3.00 (m, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-phenylbutanoate (59)



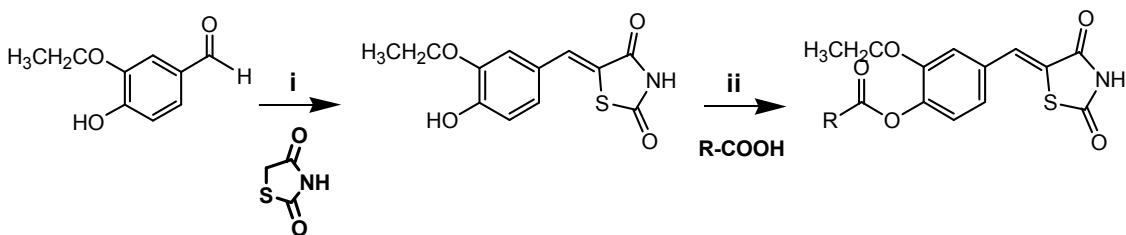
Yield : 92.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 7.81 (s, 1H), δ 7.38 (d, 1H), δ 7.33 (m, 7H), δ 3.82 (s, 3H), δ 2.68 (t, $J=7.32$ Hz, 2H), δ 2.58 (t, $J=7.32$ Hz, 2H), δ 1.98 (m, $J=7.32$ Hz, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 5-phenylpentanoate (60)



Yield : 80.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 7.80 (s, 1H), δ 7.37 (d, 1H), δ 7.31 (m, 7H), δ 3.78 (s, 3H), δ 2.62 (m, 4H), δ 1.67 (m, 4H)

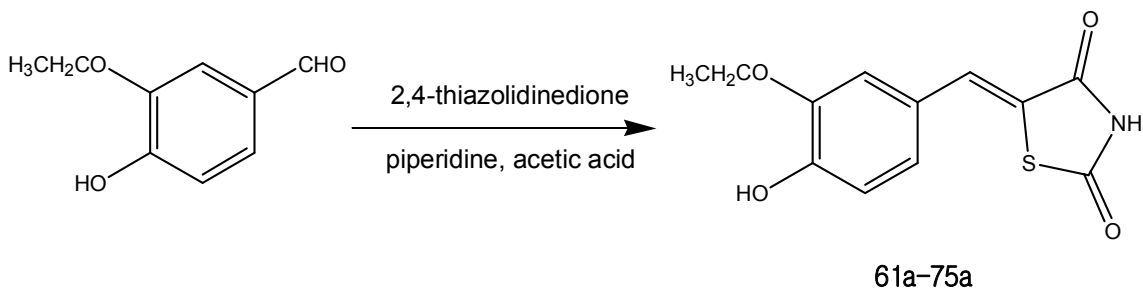
1.5. 화합물 (61-75) 합성



Scheme 5. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 $^{\circ}\text{C}$, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

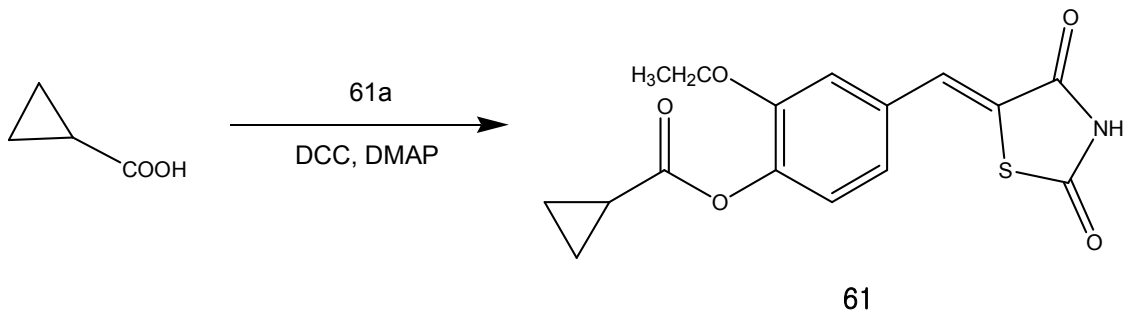
Scheme 5은 61부터 75까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

5-(3-Ethoxy-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (61a-75a)



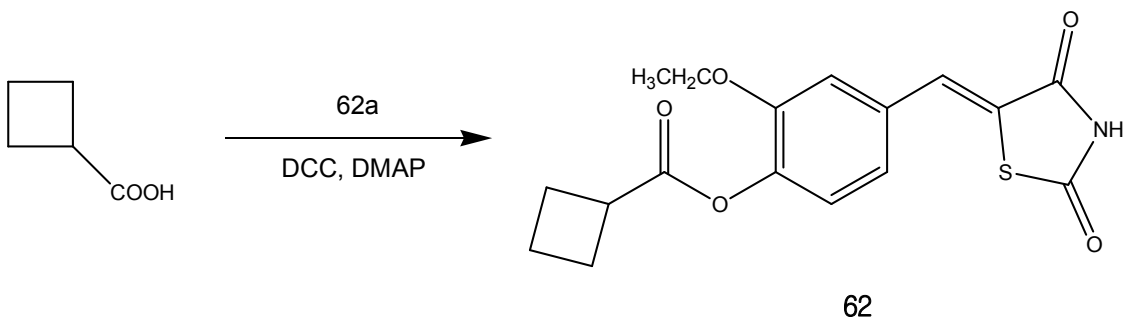
Yield : 90.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.46 (s, 1H), δ 9.88 (s, 1H), δ 7.69 (s, 1H), δ 7.15 (d, $J=1.83$ Hz, 1H), δ 7.07 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 6.94 (d, $J=8.43$ Hz, 1H), δ 4.10 (m, $J=6.96$ Hz, 2H), δ 1.37 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclopropane-carboxylate (61)



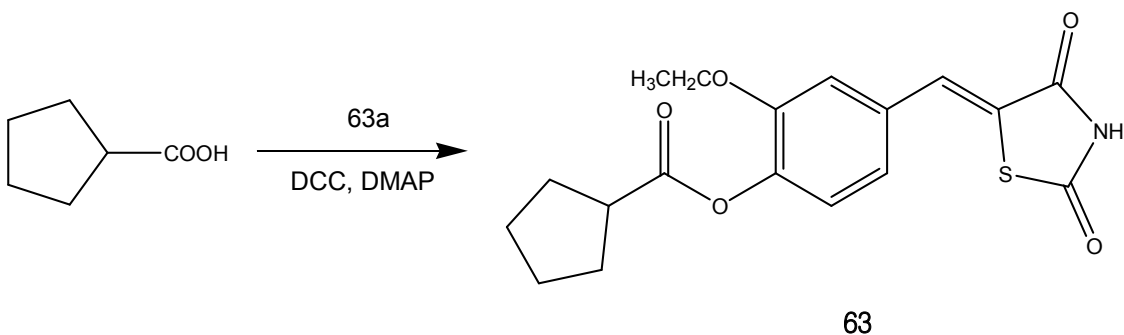
Yield : 83.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.78 (s, 1H), δ 7.33 (d, $J=1.83$ Hz, 1H), δ 7.27 (d, $J=8.43$ Hz, 1H), δ 7.17 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 4.10 (m, $J=6.96$ Hz, 2H), δ 1.92 (m, 1H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.07 (m, 4H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclobutane-carboxylate (62)



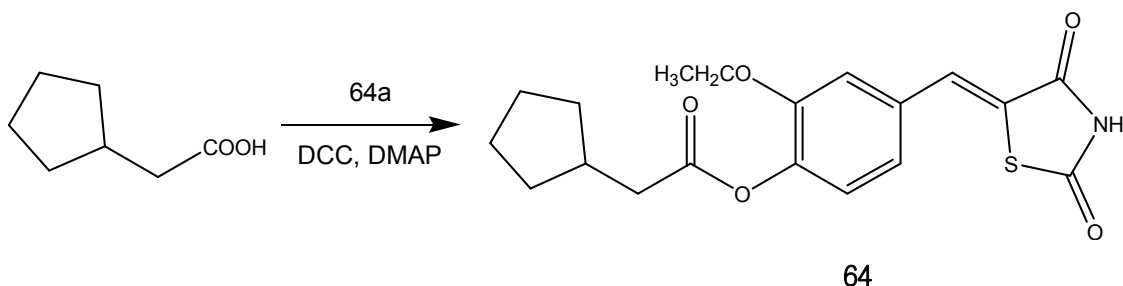
Yield : 81.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.63 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.83$ Hz, 1H), δ 7.26 (d, $J=8.07$ Hz, 1H), δ 7.18 (dd, $J=8.07$ and 1.83 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 3.50 (m, $J=8.4$ Hz, 1H), δ 2.35 (m, 4H), δ 2.08 (m, $J=8.4$ Hz, 2H), δ 1.35 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclopentane-carboxylate (63)



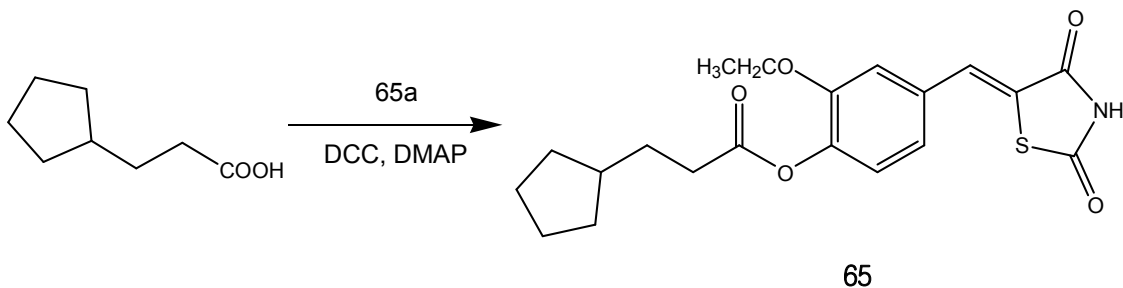
Yield : 79.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.63 (s, 1H), δ 7.78 (s, 1H), δ 7.33 (d, $J=1.83$ Hz, 1H), δ 7.26 (d, $J=8.04$ Hz, 1H), δ 7.18 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 4.09 (m, $J=6.96$ Hz, 2H), δ 3.09 (m, 1H), δ 1.96 (m, 4H), δ 1.68 (m, 4H), δ 1.32 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-cyclopentyl
-acetate (64)



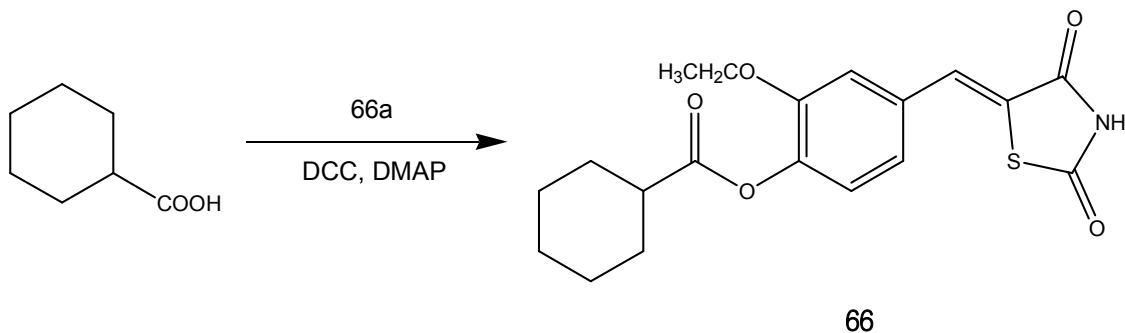
Yield : 76.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.47$ Hz, 1H), δ 7.24 (d, $J=8.43$ Hz, 1H), δ 7.17 (dd, $J=8.43$ and 1.47 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 2.57 (d, $J=7.32$ Hz, 2H), δ 2.32 (m, $J=7.32$ Hz, 1H), δ 1.89 (m, 2H), δ 1.67 (m, 4H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.24 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-cyclopentyl
-propanoate (65)



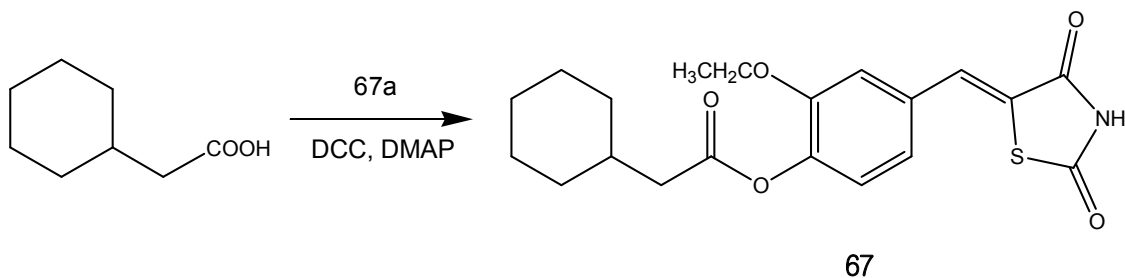
Yield : 90.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.4$ Hz, 1H), δ 7.18 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 2.59 (t, 2H), δ 1.92 (m, 9H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.15 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclohexane
-carboxylate (66)



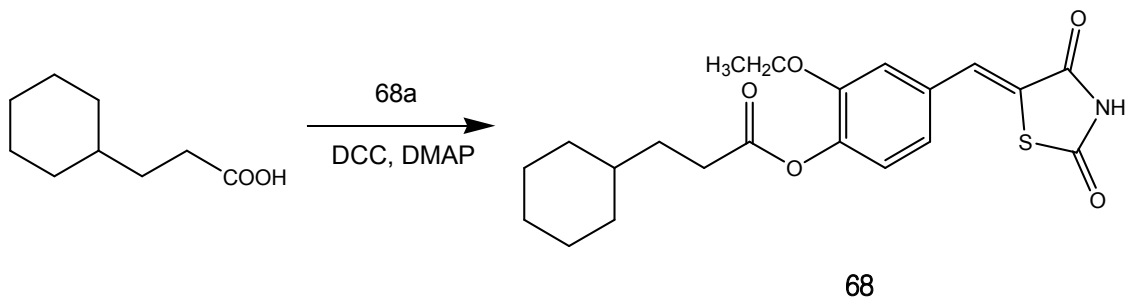
Yield : 81.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.33 (d, $J=1.83$ Hz, 1H), δ 7.24 (d, $J=8.4$ Hz, 1H), δ 7.18 (dd, $J=8.4$ and 1.83 Hz, 1H), δ 4.10 (m, $J=6.96$ Hz, 2H), δ 2.66 (m, 1H), δ 1.97 (m, 2H), δ 1.75 (m, 2H), δ 1.62 (m, 3H), δ 1.41 (m, 3H), δ 1.32 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-cyclohexyl
-acetate (67)



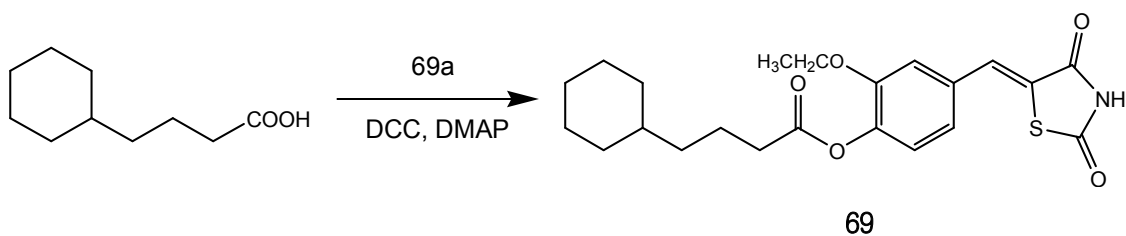
Yield : 81.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.44$ Hz, 1H), δ 7.24 (d, $J=8.43$ Hz, 1H), δ 7.18 (dd, $J=8.43$ and 1.44 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 2.45 (d, 2H), δ 1.84 (m, 3H), δ 1.70 (m, 2H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.23 (m, 6H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-cyclohexylpropanoate (68)



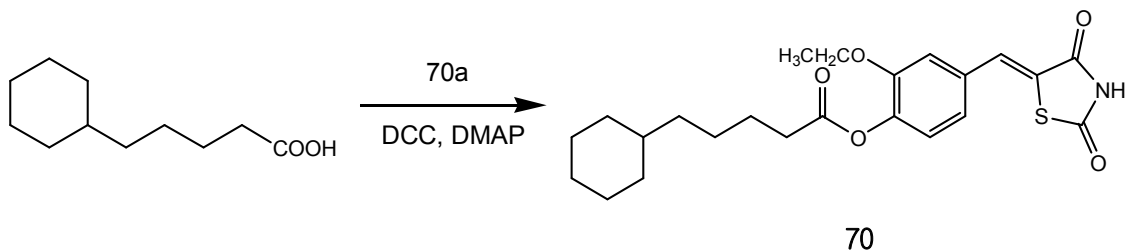
Yield : 90.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.35 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.43$ Hz, 1H), δ 7.18 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 2.60 (t, $J=7.32$ Hz, 2H), δ 1.75 (m, 8H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.22 (m, 3H), δ 0.95 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 4-cyclohexylbutanoate (69)



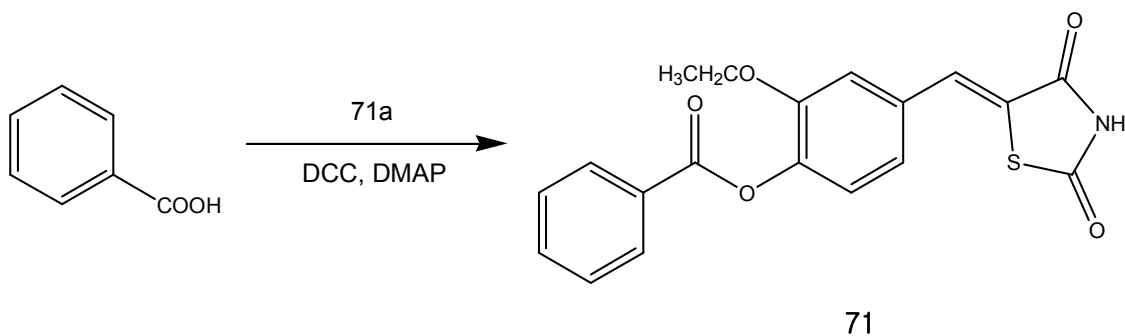
Yield : 93.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.83$ Hz, 1H), δ 7.25 (d, $J=8.43$ Hz, 1H), δ 7.18 (dd, $J=8.43$ and 1.83 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 1.66 (m, 8H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.18 (m, 7H), δ 0.88 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 5-cyclohexyl-pentanoate (70)



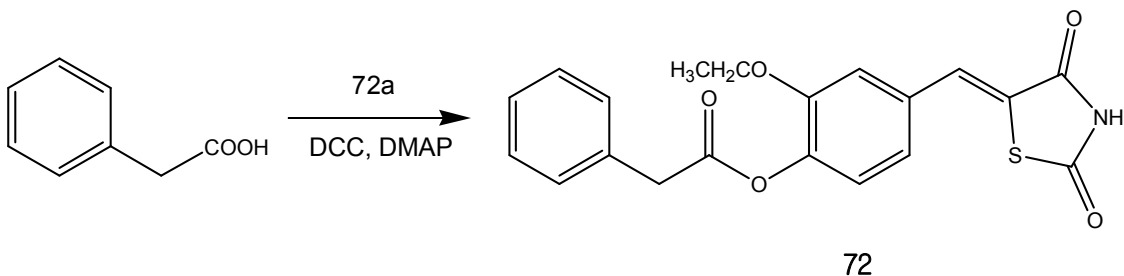
Yield : 89.5%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (d, $J=1.47$ Hz, 1H), δ 7.24 (d, $J=8.43$ Hz, 1H), δ 7.18 (dd, $J=8.43$ and 1.47 Hz, 1H), δ 4.11 (m, $J=6.96$ Hz, 2H), δ 2.58 (t, $J=6.96$ Hz, 2H), δ 1.69 (m, 8H), δ 1.42 (m, 2H), δ 1.32 (t, $J=6.96$ Hz, 3H), δ 1.21 (m, $J=6.96$ Hz, 5H), δ 0.89 (m, 2H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl benzoate (71)



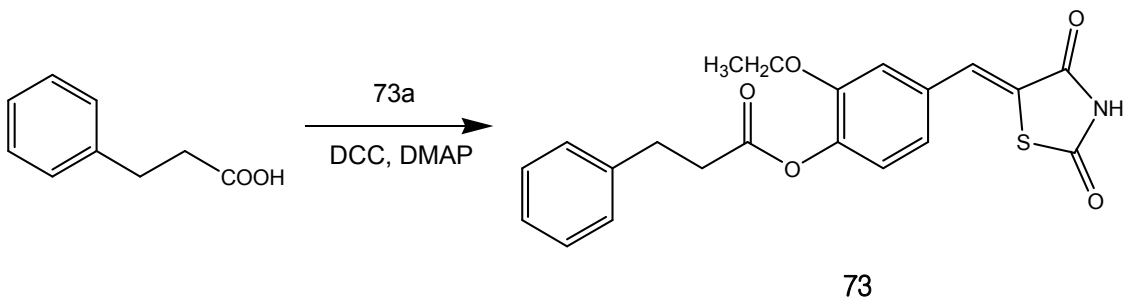
Yield : 73.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.66 (s, 1H), δ 8.13 (d, 2H), δ 7.83 (s, 1H), δ 7.78 (t, $J=7.32$ Hz, 1H), δ 7.64 (t, $J=7.32$ Hz, 2H), δ 7.43 (d, $J=8.43$ Hz, 1H), δ 7.40 (s, 1H), δ 7.24 (d, $J=8.43$ Hz, 1H), δ 4.14 (m, $J=6.96$ Hz, 2H), δ 1.23 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-phenylacetate
(72)



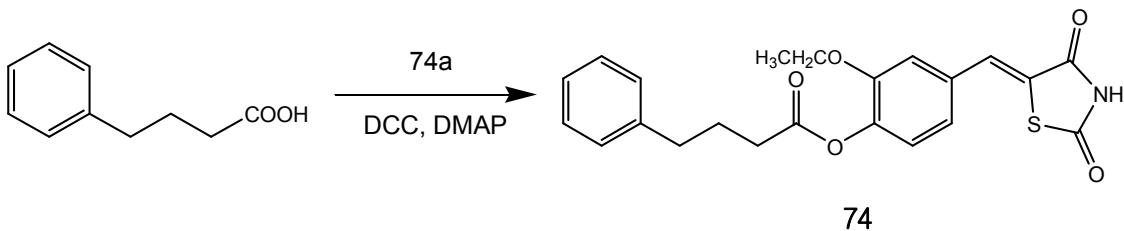
Yield : 89.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.78 (s, 1H), δ 7.39 (m, 7H), δ 7.18 (d, 1H), δ 4.07 (m, $J=6.96$ Hz, 2H), δ 3.96 (s, 2H), δ 1.25 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-phenylpropanoate
(73)



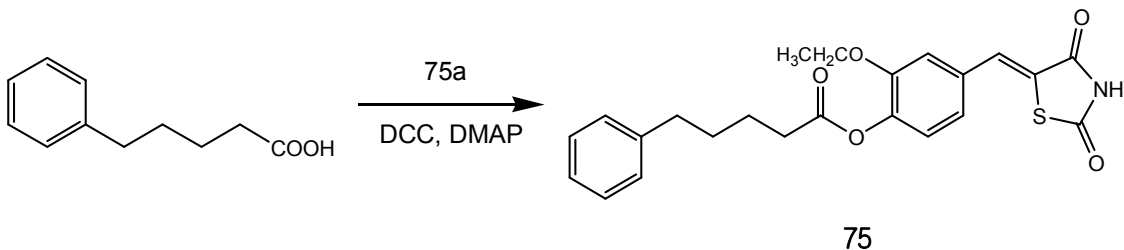
Yield : 89.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (m, 8H), δ 4.09 (m, $J=6.96$ Hz, 2H), δ 2.99 (m, 4H), δ 1.27 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 4-phenylbutanoate
(74)



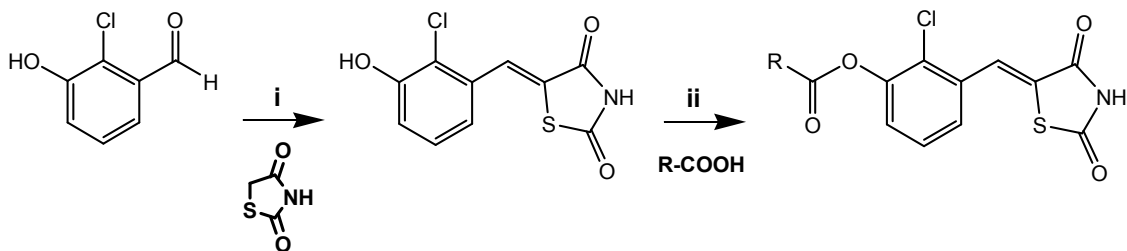
Yield : 84.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.65 (s, 1H), δ 7.79 (s, 1H), δ 7.35 (m, 8H), δ 4.12 (m, $J=6.96$ Hz, 2H), δ 2.72 (t, $J=7.32$ Hz, 2H), δ 2.60 (t, $J=7.32$ Hz, 2H), δ 1.99 (m, $J=7.32$ Hz, 2H), δ 1.30 (t, $J=6.96$ Hz, 3H)

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 5-phenylpentanoate (75)



Yield : 81.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.64 (s, 1H), δ 7.79 (s, 1H), δ 7.34 (m, 8H), δ 4.08 (m, $J=6.96$ Hz, 2H), δ 2.64 (m, $J=6.96$ Hz, 4H), δ 1.68 (m, $J=6.96$ Hz, 4H), δ 1.30 (t, $J=6.96$ Hz, 3H)

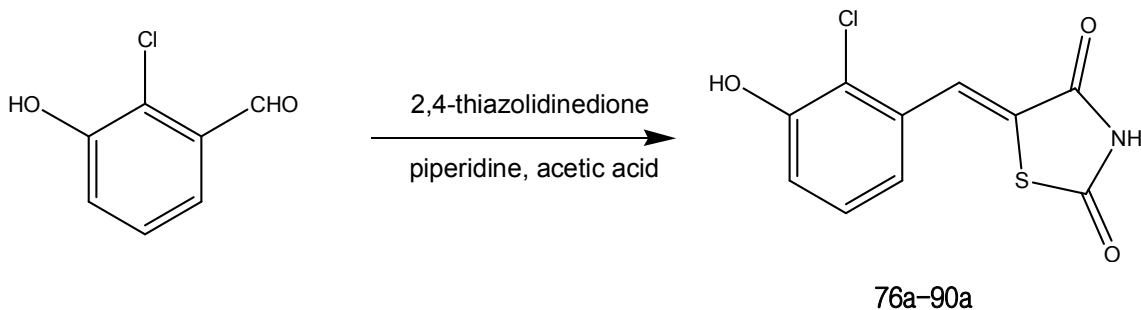
1.6. 화합물 (76-90) 합성



Scheme 6. Reagents and conditions; (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

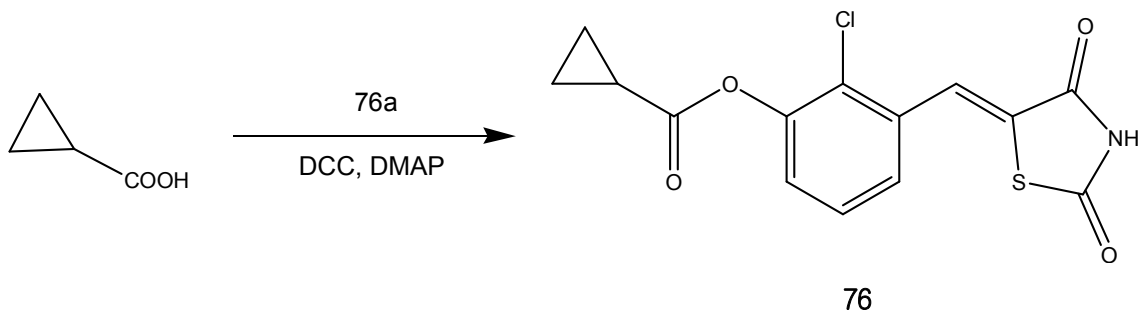
Scheme 6은 76부터 90까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

5-(2-Chloro-3-hydroxy-benzylidene)-thiazolidine-2,4-dione (76a-90a)



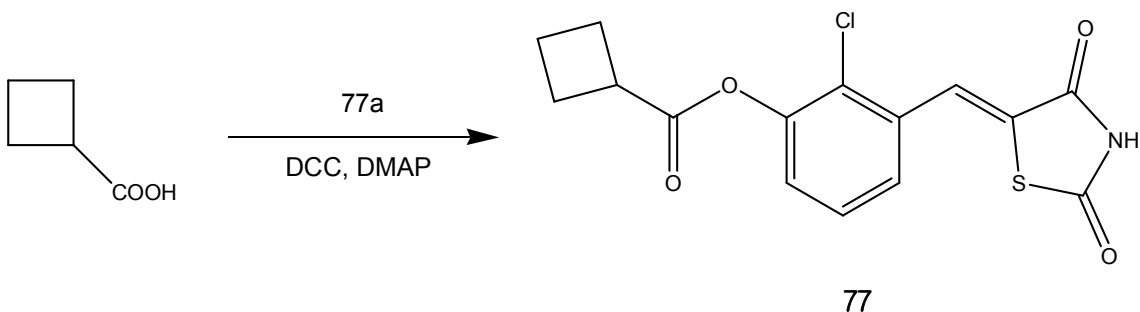
Yield : 71.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.71 (s, 1H), δ 10.60 (s, 1H), δ 7.93 (s, 1H), δ 7.33 (t, $J=8.04$ Hz, 1H), δ 7.09 (d, $J=8.04$ Hz, 1H), δ 7.02 (d, $J=8.04$ Hz, 1H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropane
-carboxylate (76)



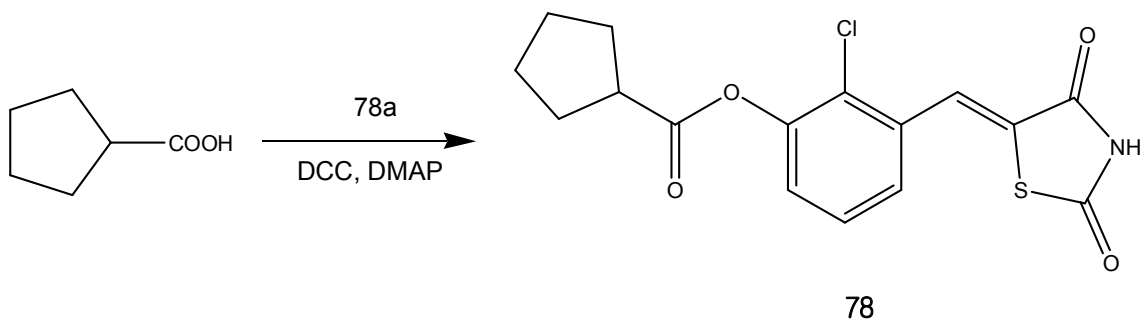
Yield : 71.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.80 (s, 1H), δ 7.88 (s, 1H), δ 7.58 (t, $J=8.07$ Hz, 1H), δ 7.49 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 7.44 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 2.02 (m, 1H), δ 1.16 (m, 4H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane-carboxylate (77)



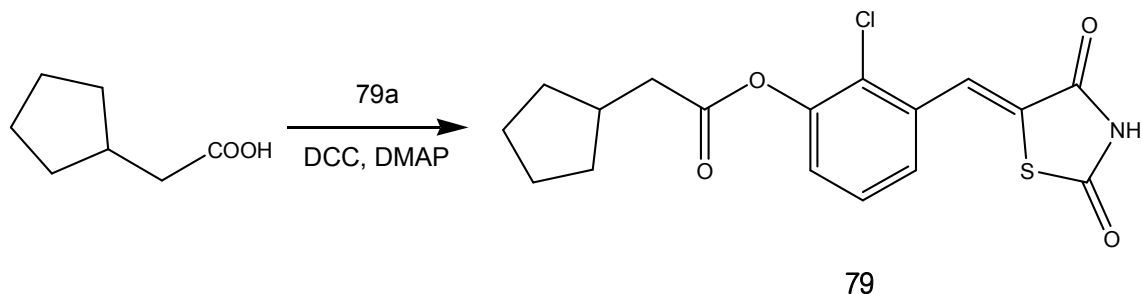
Yield : 73.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.79 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=8.07$ Hz, 1H), δ 7.50 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 7.46 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 3.59 (m, 1H), δ 2.40 (m, 4H), δ 2.10 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-carboxylate (78)



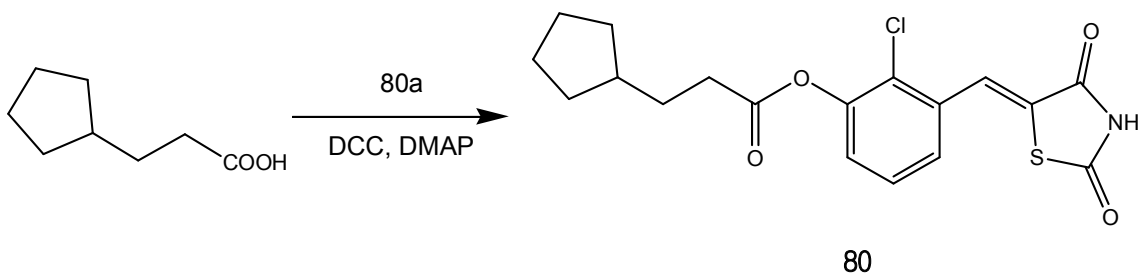
Yield : 69.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.80 (s, 1H), δ 7.89 (s, 1H), δ 7.59 (t, $J=7.68$ Hz, 1H), δ 7.50 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 7.45 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 3.18 (m, 1H), δ 2.01 (m, 4H), δ 1.71 (m, 4H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (79)



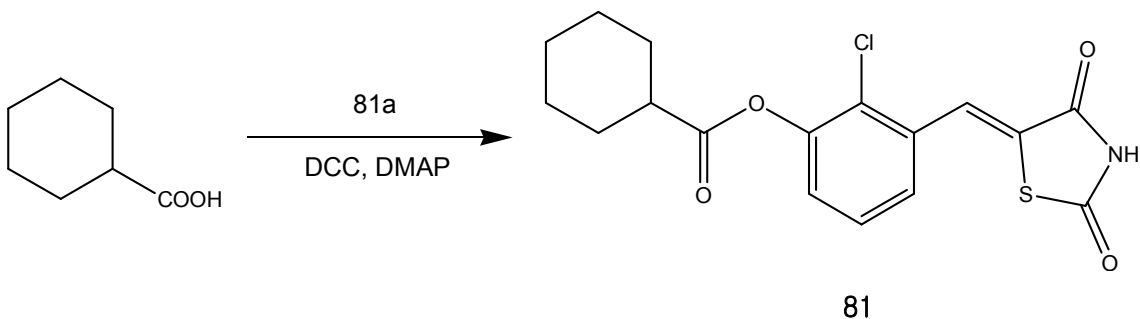
Yield : 75.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.81 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=8.07$ Hz, 1H), δ 7.50 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 7.44 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 2.68 (d, $J=7.32$ Hz, 2H), δ 2.36 (m, $J=7.32$ Hz, 1H), δ 1.90 (m, 2H), δ 1.68 (m, 4H), δ 1.30 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (80)



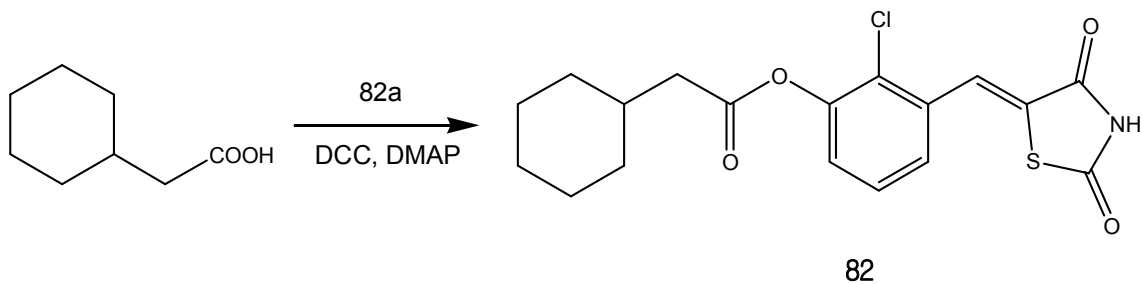
Yield : 76.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.80 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=8.04$ Hz, 1H), δ 7.50 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 7.45 (dd, $J=8.04$ and 1.83 Hz, 1H), δ 2.69 (t, 2H), δ 1.85 (m, 5H), δ 1.59 (m, 4H), δ 1.15 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane-carboxylate (81)



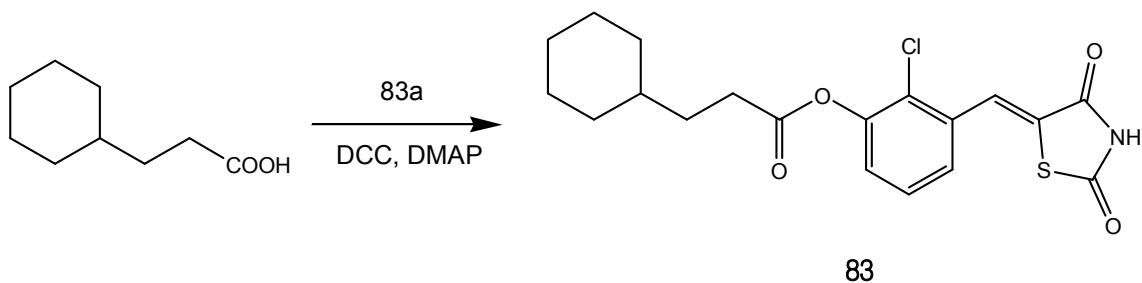
Yield : 71.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.82 (s, 1H), δ 7.88 (s, 1H), δ 7.59 (t, $J=7.68$ Hz, 1H), δ 7.50 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 7.44 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 2.75 (m, 1H), δ 2.08 (m, 2H), δ 1.76 (m, 2H), δ 1.64 (m, 6H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexyl-acetate (82)



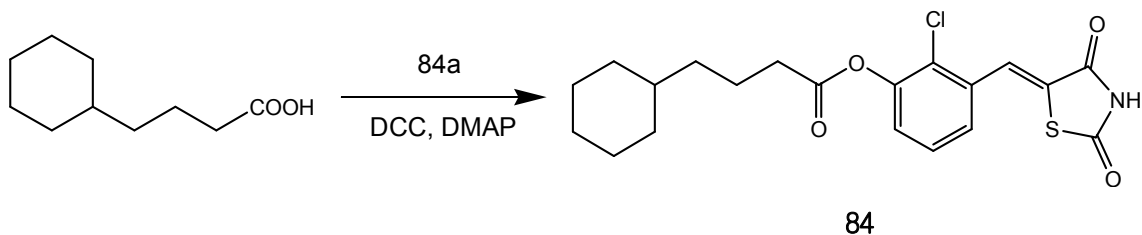
Yield : 68.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.79 (s, 1H), δ 7.89 (s, 1H), δ 7.59 (t, $J=7.68$ Hz, 1H), δ 7.50 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 7.43 (dd, $J=7.68$ and 1.47 Hz, 1H), δ 2.55 (d, 2H), δ 1.89 (m, 6H), δ 1.32 (m, 5H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (83)



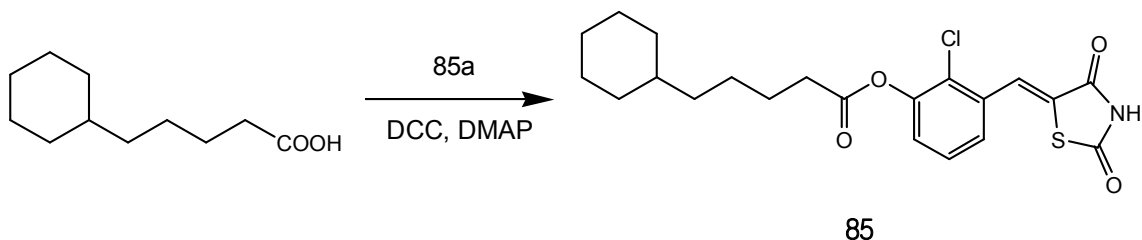
Yield : 79.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.79 (s, 1H), δ 7.89 (s, 1H), δ 7.59 (t, $J=7.68$ Hz, 1H), δ 7.50 (dd, $J=7.68$ and 1.83 Hz, 1H), δ 7.44 (dd, $J=7.68$ and 1.83 Hz, 1H), δ 2.69 (t, 2H), δ 1.75 (m, 7H), δ 1.38 (m, 6H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (84)



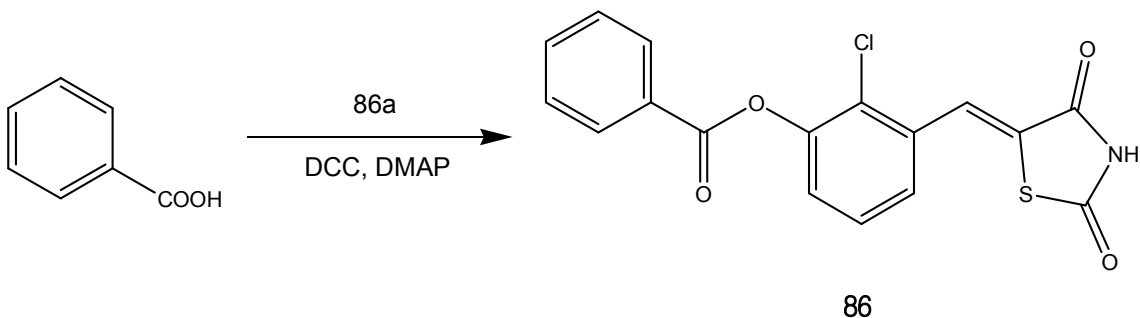
Yield : 71.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.79 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=8.04$ Hz, 1H), δ 7.50 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.44 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 2.66 (t, 2H), δ 1.71 (m, 7H), δ 1.31 (m, 6H), δ 0.92 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl-pentanoate (85)



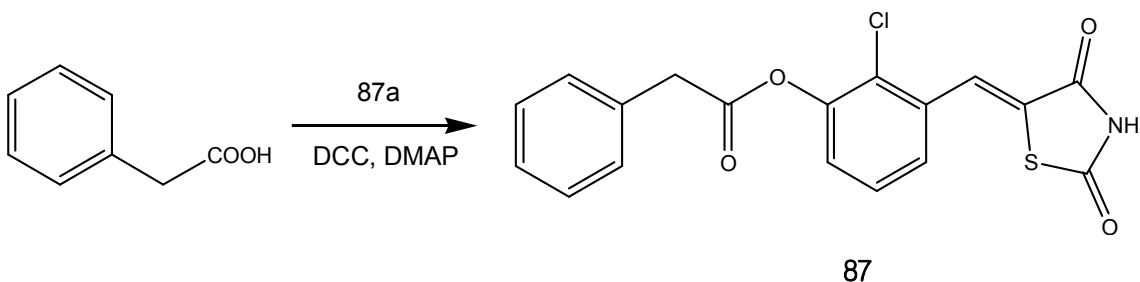
Yield : 69.2%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.80 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=8.07$ Hz, 1H), δ 7.51 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 7.45 (dd, $J=8.07$ and 1.47 Hz, 1H), δ 2.68 (t, 2H), δ 1.69 (m, 7H), δ 1.43 (m, 2H), δ 1.22 (m, 6H), δ 0.90 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (86)



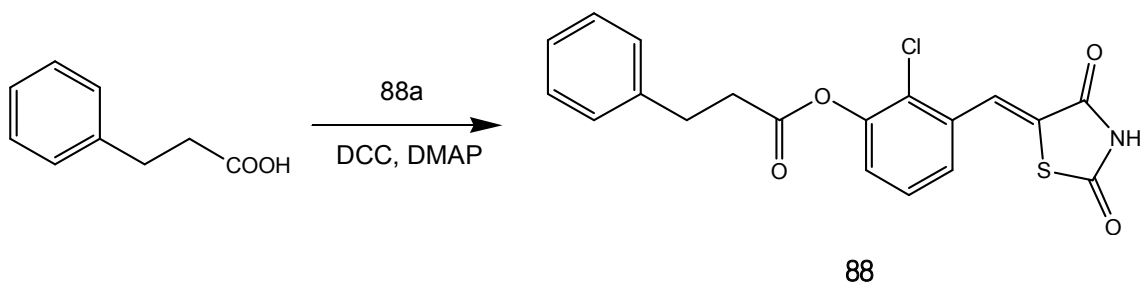
Yield : 76.6%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.83 (s, 1H), δ 8.19 (d, 2H), δ 7.92 (s, 1H), δ 7.83 (t, 1H), δ 7.67 (m, 4H), δ 7.59 (m, 1H), δ 1.69 (m, 7H), δ 1.43 (m, 2H), δ 1.22 (m, 6H), δ 0.90 (m, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (87)



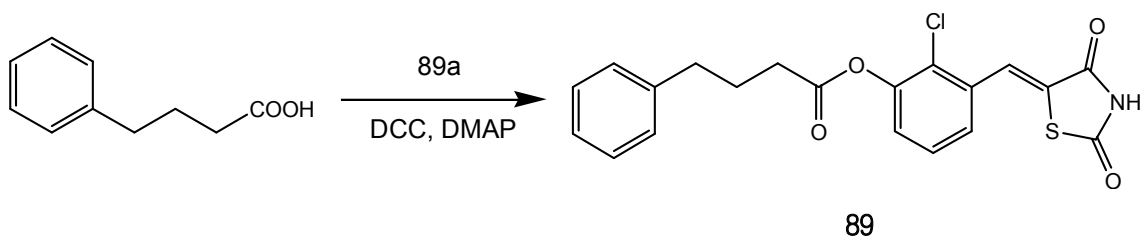
Yield : 77.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.81 (s, 1H), δ 7.87 (s, 1H), δ 7.60 (t, $J=8.04$ Hz, 1H), δ 7.51 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.47 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.42 (m, 5H), δ 4.07 (s, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (88)



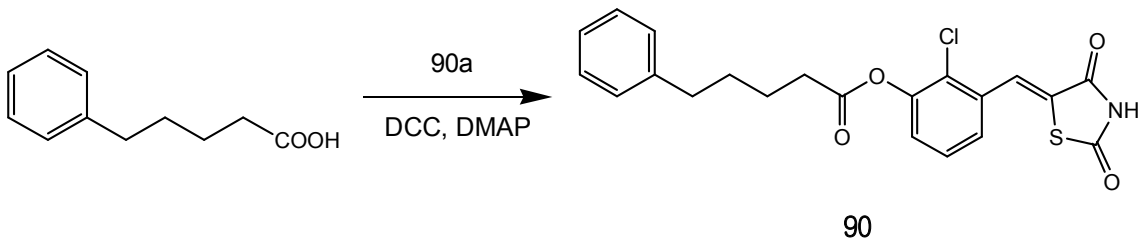
Yield : 71.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.86 (s, 1H), δ 7.86 (s, 1H), δ 7.59 (t, $J=8.04$ Hz, 1H), δ 7.50 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.36 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.32 (m, 5H), δ 3.01 (t, 4H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (89)



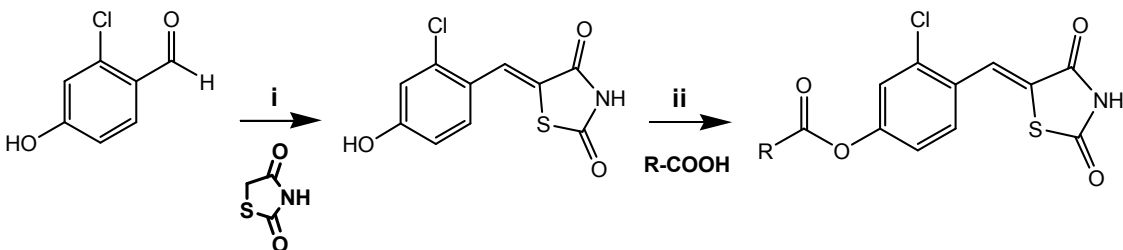
Yield : 80.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.82 (s, 1H), δ 7.89 (s, 1H), δ 7.60 (t, $J=7.68$ Hz, 1H), δ 7.51 (dd, $J=7.68$ and 1.44 Hz, 1H), δ 7.46 (dd, $J=7.68$ and 1.44 Hz, 1H), δ 7.34 (m, 5H), δ 2.72 (m, $J=7.32$ Hz, 4H), δ 2.02 (m, $J=7.32$ Hz, 2H)

(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (90)



Yield : 80.4%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.82 (s, 1H), δ 7.88 (s, 1H), δ 7.59 (t, $J=7.68$ Hz, 1H), δ 7.50 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.44 (dd, $J=8.04$ and 1.47 Hz, 1H), δ 7.30 (m, 5H), δ 2.70 (m, 4H), δ 1.69 (m, 4H)

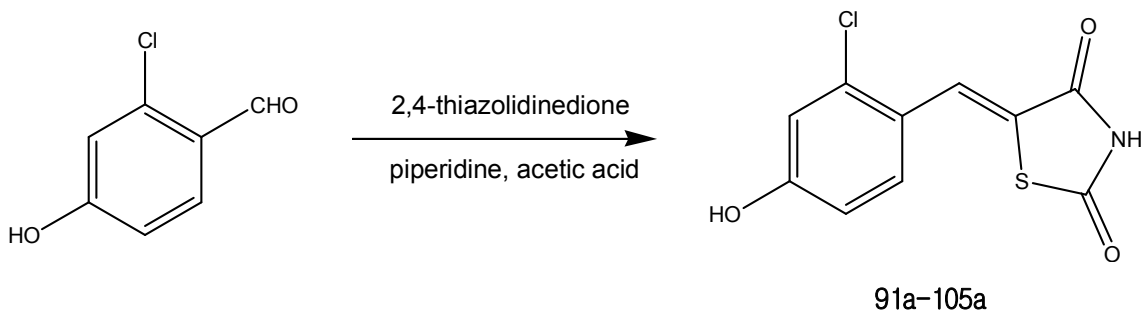
1.7. 화합물 (91-105) 합성



Scheme 7. Reagents and conditions: (i) piperidine, acetic acid, toluene, 80 °C, 18 h; (ii) DCC, DMAP, methylene chloride, reflux, 18 h

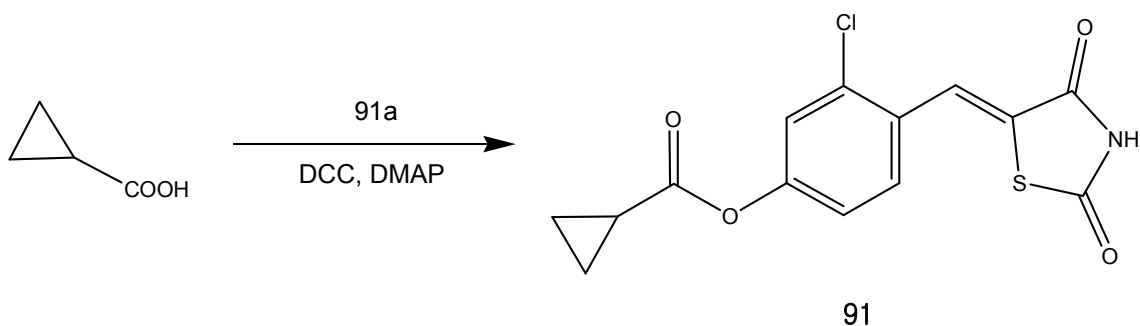
Scheme 7은 91부터 105까지의 화합물 합성방법을 도식화 하였다. 구체적인 실험 방법은 위와 모두 동일하므로 생략하였다.

5-(2-Chloro-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (91a-105a)



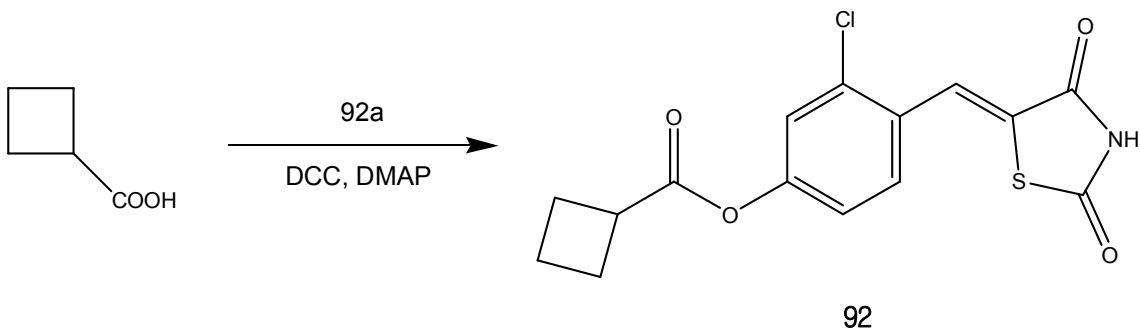
Yield : 95%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.63 (s, 1H), δ 10.76 (s, 1H), δ 7.88 (s, 1H), δ 7.44 (d, $J=8.79$ Hz, 1H), δ 7.01 (d, $J=2.58$ Hz, 1H), δ 6.95 (dd, $J=8.79$ and 2.58 Hz, 1H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropane
-carboxylate (91)



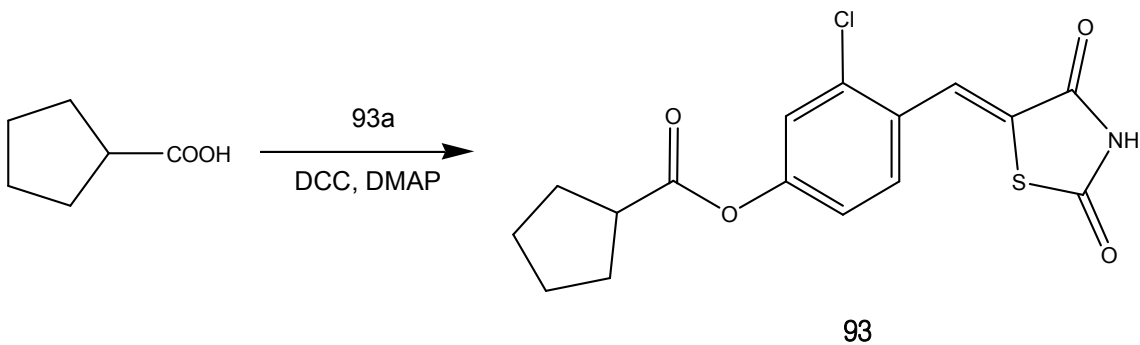
Yield : 92.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.79$ Hz, 1H), δ 7.59 (d, $J=2.58$ Hz, 1H), δ 7.36 (dd, $J=8.79$ and 2.58 Hz, 1H), δ 1.94 (m, 1H), δ 1.11 (m, 4H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane
-carboxylate (92)



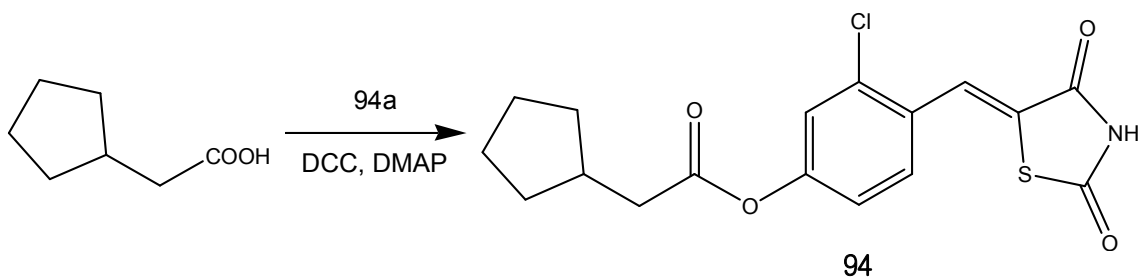
Yield : 89.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.78 (s, 1H), δ 7.88 (s, 1H), δ 7.62 (d, $J=8.79$ Hz, 1H), δ 7.58 (d, $J=2.19$ Hz, 1H), δ 7.35 (dd, $J=8.79$ and 2.19 Hz, 1H), δ 3.52 (m, 1H), δ 2.37 (m, 4H), δ 2.05 (m, 2H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-1-carboxylate (93)



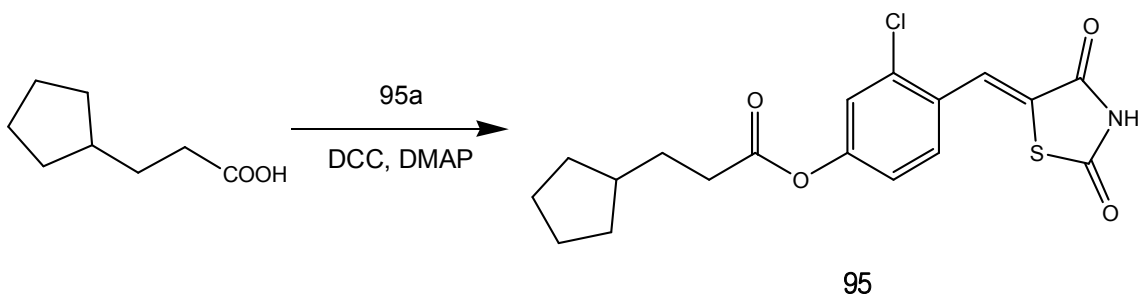
Yield : 92.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.62 (d, $J=8.79$ Hz, 1H), δ 7.57 (d, $J=2.19$ Hz, 1H), δ 7.34 (dd, $J=8.79$ and 2.19 Hz, 1H), δ 3.08 (m, 1H), δ 1.95 (m, 4H), δ 1.66 (m, 4H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentyl acetate (94)



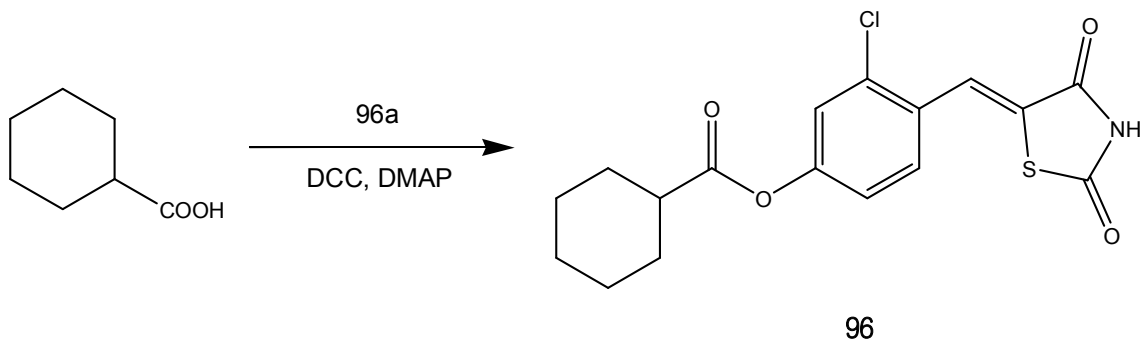
Yield : 88.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.78 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.4$ Hz, 1H), δ 7.54 (d, $J=2.19$ Hz, 1H), δ 7.34 (dd, $J=8.4$ and 2.19 Hz, 1H), δ 2.62 (m, $J=7.68$ Hz, 2H), δ 2.29 (m, $J=7.68$ Hz, 1H), δ 1.88 (m, 2H), δ 1.62 (m, 4H), δ 1.28 (m, 2H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (95)



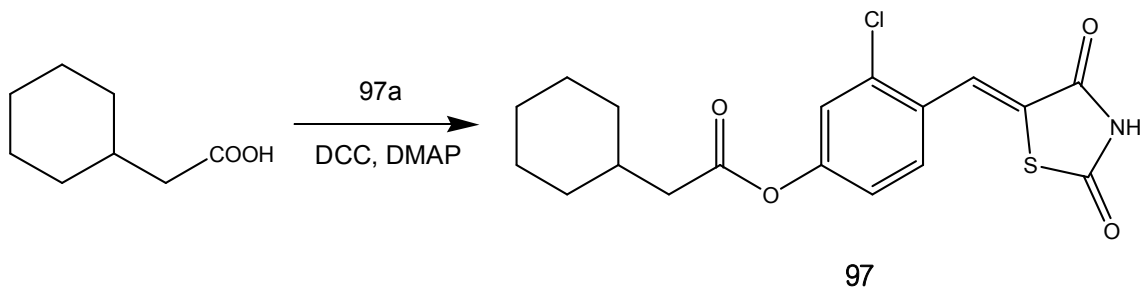
Yield : 93.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.78 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.43$ Hz, 1H), δ 7.56 (d, $J=2.19$ Hz, 1H), δ 7.34 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 2.61 (t, 2H), δ 1.80 (m, 10H), δ 1.14 (m, 1H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexanecarboxylate (96)



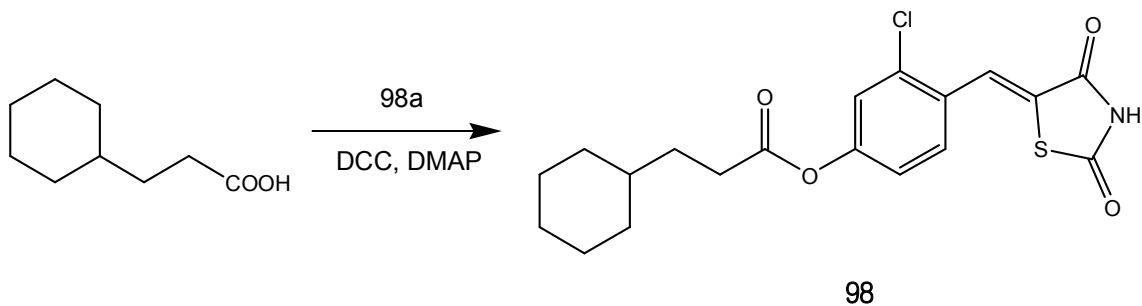
Yield : 91.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.43$ Hz, 1H), δ 7.55 (d, $J=2.22$ Hz, 1H), δ 7.33 (dd, $J=8.43$ and 2.22 Hz, 1H), δ 2.62 (m, 1H), δ 1.75 (m, 10H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate (97)



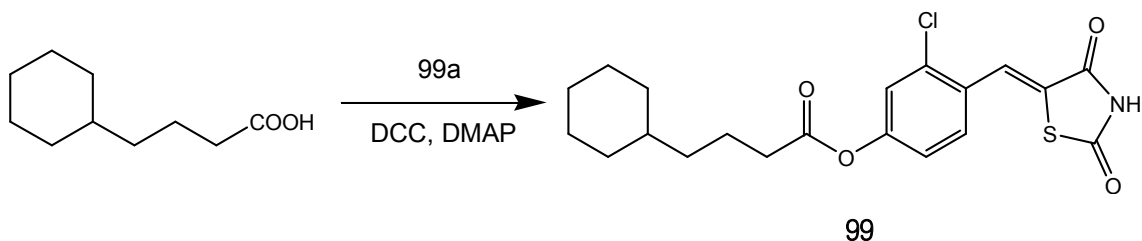
Yield : 87.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.78 (s, 1H), δ 7.89 (s, 1H), δ 7.63 (d, $J=8.43$ Hz, 1H), δ 7.56 (d, $J=2.19$ Hz, 1H), δ 7.33 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 1.86 (m, 7H), δ 1.32 (m, 6H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (98)



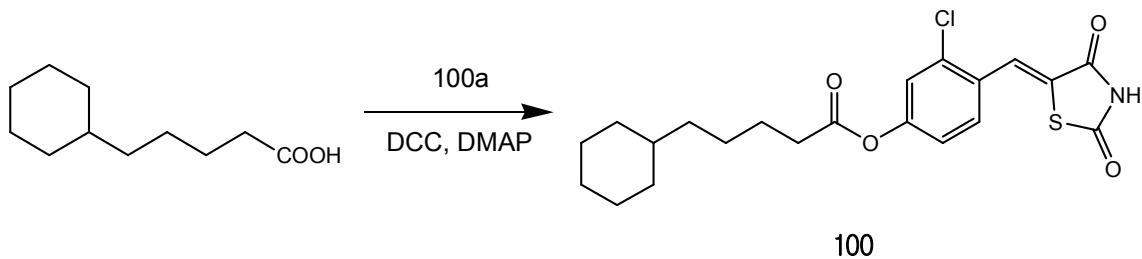
Yield : 90.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.4$ Hz, 1H), δ 7.55 (d, $J=2.19$ Hz, 1H), δ 7.34 (dd, $J=8.4$ and 2.19 Hz, 1H), δ 2.60 (t, 2H), δ 1.73 (m, 8H), δ 1.32 (m, 4H), δ 0.95 (m, 1H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (99)



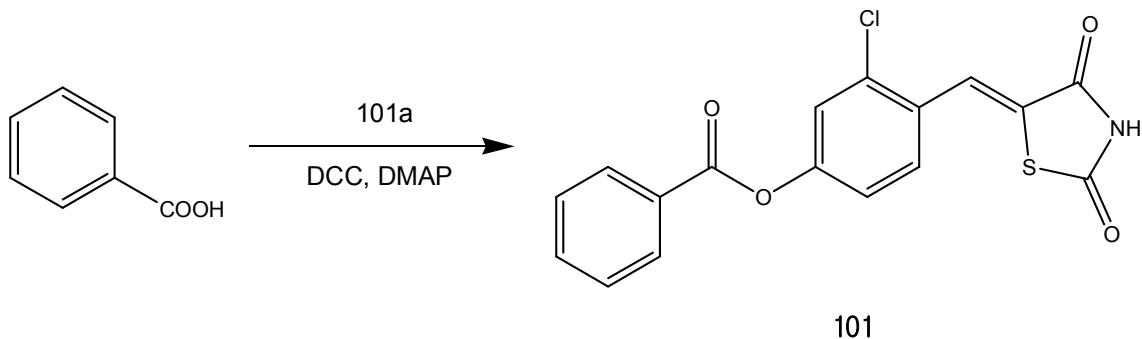
Yield : 91.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.78 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.79$ Hz, 1H), δ 7.55 (d, $J=2.19$ Hz, 1H), δ 7.34 (dd, $J=8.79$ and 2.19 Hz, 1H), δ 2.60 (t, 2H), δ 1.71 (m, 8H), δ 1.26 (m, 6H), δ 0.92 (m, 1H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl-pentanoate (100)



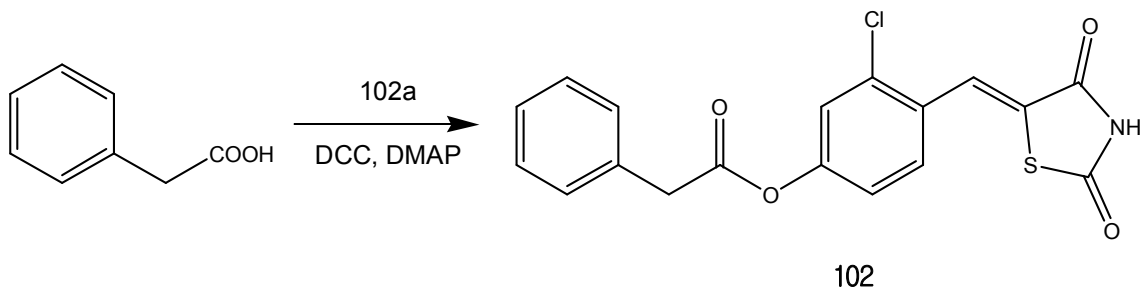
Yield : 90.7%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.43$ Hz, 1H), δ 7.54 (d, $J=2.19$ Hz, 1H), δ 7.33 (dd, $J=8.43$ and 2.19 Hz, 1H), δ 2.62 (t, 2H), δ 1.69 (m, 8H), δ 1.40 (m, 8H), δ 0.90 (m, 1H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (101)



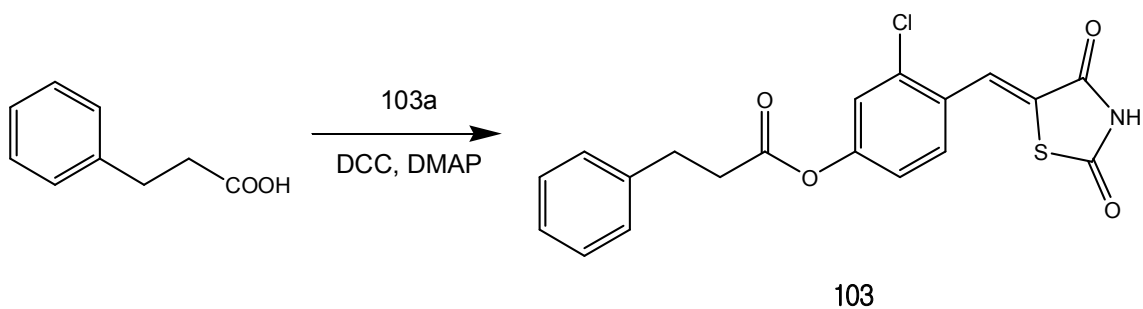
Yield : 87.1%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.79 (s, 1H), δ 8.15 (d, 2H), δ 7.92 (s, 1H), δ 7.80 (m, 6H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (102)



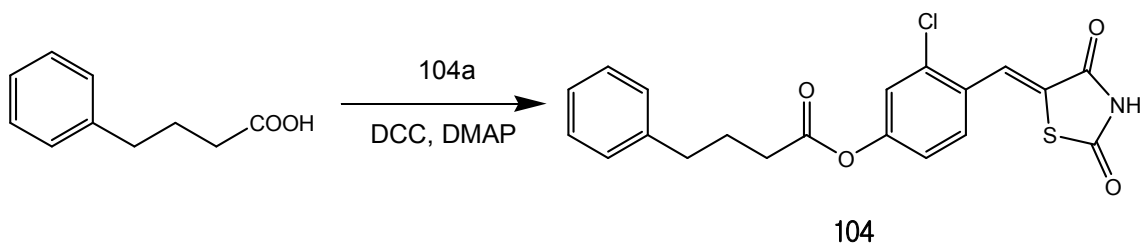
Yield : 88.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.63 (d, $J=8.79$ Hz, 1H), δ 7.57 (d, $J=2.19$ Hz, 1H), δ 7.40 (m, 6H), δ 4.00 (s, 2H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (103)



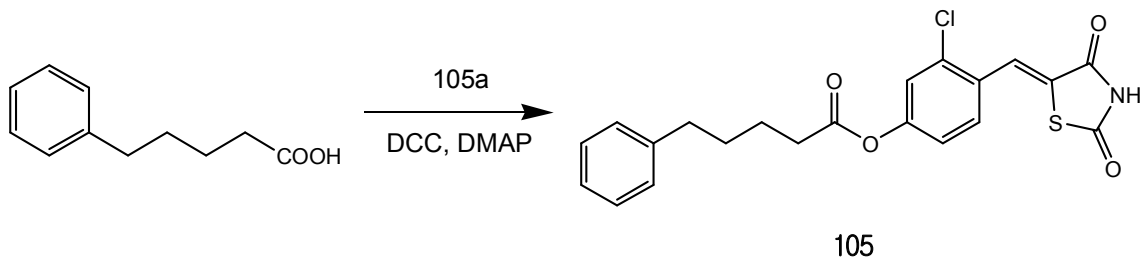
Yield : 93.3%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 12.77 (s, 1H), δ 7.87 (s, 1H), δ 7.62 (d, $J=8.4$ Hz, 1H), δ 7.45 (d, $J=2.19$ Hz, 1H), δ 7.34 (m, 6H), δ 2.99 (m, 4H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (104)



Yield : 88.8%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.62 (d, $J=8.79$ Hz, 1H), δ 7.54 (d, $J=2.19$ Hz, 1H), δ 7.34 (m, 6H), δ 2.70 (m, $J=7.32$ Hz, 4H), δ 1.99 (m, $J=7.32$ Hz, 2H)

(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (105)



Yield : 89.9%; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ 12.77 (s, 1H), δ 7.88 (s, 1H), δ 7.62 (d, $J=8.79$ Hz, 1H), δ 7.54 (d, $J=2.19$ Hz, 1H), δ 7.33 (m, 6H), δ 2.63 (m, 4H), δ 1.67 (m, 4H)

2. 15-PGDH 억제제 활성 측정

2.1. 15-PGDH 발현 및 정제

BamH I 과 EcoR I 사이트 사이에 재조합형 15-PGDH를 포함한 pGEX-2T expression vector를 *Escherichia coli* BL-21 DE₃ cell에 transformation 하였다 (fig. 5).

그 다음 LB 배지 15 mL에 생성된 colony와 100 µg/mL의 Ampicillin을 넣고 37 °C로 맞춰진 진탕배양기에 16시간 동안 배양하였다. 배양된 균을 LB 배지 1.5 L에 100분의 1의 용량으로 넣고 흡광도가 600 nm에서 0.6-0.8이 될 때까지 배양한 후 발현 유도제인 IPTG (isopropyl β-D-1-thiogalactopyranoside)를 1 mM 농도로 넣어주고 20 °C로 맞춰진 진탕배양기에 12시간 동안 배양하였다.

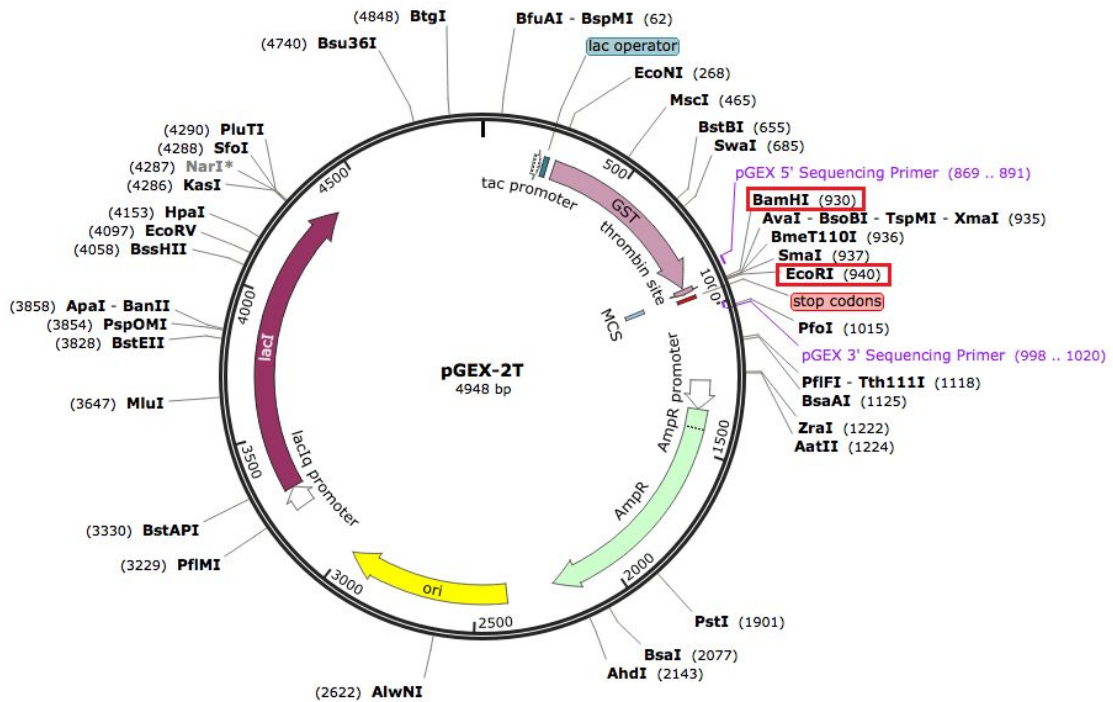


fig. 5. cDNA of 15-PGDH inserted into the pGEX-2T expression vector [54].

발현된 단백질을 10분 동안 4 °C, 6000 rpm에서 원심분리하고 상층액은 제거한다. 그 후 분리된 pellet에 Lysis buffer [1x PBS buffer (pH 7.4), 1 mM EDTA, 0.1 mM DTT]을 40 mL 정도 넣고 현탁시킨 후 4 °C에서 1회당 30초, 37% 강도에서 sonication 하는데 이를 4회 반복했으며 sonication에 의해 깨진 균을 20000 g-force에서 40분 동안 원심 분리하였다.

그 후 glutathione-sepharose 4B 비드를 column에 넣고 Lysis buffer로 평형화 시킨 후 원심분리 된 상층액을 넣어 binding 시켰으며 column에 넣어 washing buffer [50 mM Tris-HCl (pH 7.7)]로 씻어준 후 Elution buffer [50 mM Tris-HCl, 20 mM L-Glutathione reduced, (pH 8.0) 1 mM EDTA, 0.1 mM DTT]로 15-PGDH를 용출시켰으며 1x Bio-Rad protein Assay를 이용하여 눈으로 확인할 수 있었다. 용출된 15-PGDH는 10%의 양으로 glycerol을 첨가해 -80 °C에 보관하였다.

2.2. 15-PGDH 정량

15-PGDH의 농도는 Bradford assay 방법으로 검정곡선을 이용해 계산하였다. 우선 bovine serum albumin을 멸균 3차 증류수를 이용하여 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL, 0.156 mg/mL의 농도로 희석시켜 Bio-Rad protein assay 시약에 3 μ L씩 넣고 잘 섞어준 후 UV spectrophotometer을 이용하여 595 nm 파장에서 흡광도를 측정하여 검정곡선을 만들었다. 그리고 그 검정곡선을 이용하여 정제된 15-PGDH의 농도를 계산하였다.

2.3. SDS-PAGE

단백질의 분자량, 정제 정도, 단백질 정량의 정확성 등을 확인하기 위해 정량된 15-PGDH로 SDS-PAGE를 진행하였다. 12% resolving gel과 8% stacking gel을 사용하였으며 15-PGDH를 다양한 양으로 로딩하였다. gel에 있는 band를 더 선명하게 관찰하기 위해 0.1% Coomassie brilliant blue R-250, 50% methanol, 10% acetic acid를 혼합하여 염색제를 만들었으며 증류수에 10% methanol과 7% acetic acid를 혼합해 탈색제를 만들었다.

15-PGDH의 크기는 25 kDa이지만 15-PGDH가 붙어있는 GSH Vector의 크기가 29 kDa이므로 약 54 kDa의 크기로 15-PGDH를 확인할 수 있었다. SDS-PAGE 결과는 Fig. 6에 나타내었다. M은 protein mass marker이며, 1은 정제된 15-PGDH를 5 μ g 로딩한 결과이며 2는 4 μ g, 3은 3 μ g 로딩한 결과이다.

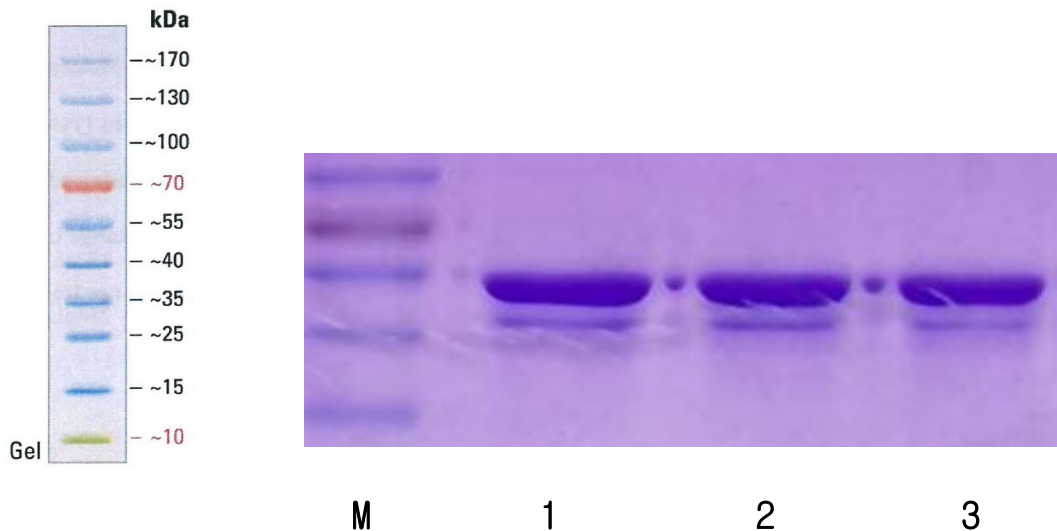


fig. 6. SDS-PAGE of the 15-PGDH.

2.4. 15-PGDH 억제제의 억제 활성 측정

합성한 억제제가 15-PGDH의 활성을 50% 억제하는 농도인 IC₅₀ 값을 계산하는 방법으로 50 mM Tris-HCl buffer (pH 7.4), 10 μ g의 정제된 15-PGDH protein, 0.25 mM NAD⁺, 0.1 mM DTT, 21 μ M PGE₂, 다양한 농도의 억제제를 네 면이 투명한 석영셀에 넣어 형광분광광도계(fluorescence spectrophotometer)를 이용하여 emission 468 nm, excitation 340 nm 파장에서 70초 동안 NADH의 흡광도를 측정하였다.

NADH를 농도별로 측정하여 검정곡선을 만든 후 검정곡선을 이용하여 15-PGDH 억제제의 IC₅₀ 값 [Blank 값을 기준으로 50% 억제된 농도]을 계산하였다.

3. PGE₂ 농도 측정

RPMI 1640 배지 [+8% FBS, AA]를 사용해 일주일 정도 계대 배양한 A549 cell을 6 well plate에 2.5×10^5 cells/mL (5×10^5 cells/well)만큼 깔아주고 37 °C, 5% CO₂ incubator에서 24시간 동안 배양하였다. 세포가 약 80% 이상 자라면 15-PGDH inhibitor를 5 μM 농도로 주입하였고 결과 비교를 위해 음성통제집단 (negative control)에는 1x PBS buffer를 동일한 양으로 주입하였다. 12시간 후 배지를 수거하였으며 수거된 배지는 ELISA Kit (회사; Abcam)를 이용하여 정해진 방법을 거쳐 PGE₂ 농도를 측정하였다. 농도는 배지에 standard를 농도 별로 희석한 후 흡광도 값으로 검정 곡선을 그려 정확한 값으로 나타낼 수 있다.

ELISA Kit을 이용한 PGE₂ 농도 측정 원리는 Fig.7 에 나타내었다. Goat polyclonal anti-mouse IgG로 코팅된 kit plate에 샘플 속 PGE₂와 binding하여 ELISA Kit Reader를 이용해 농도를 측정하는 것이다.

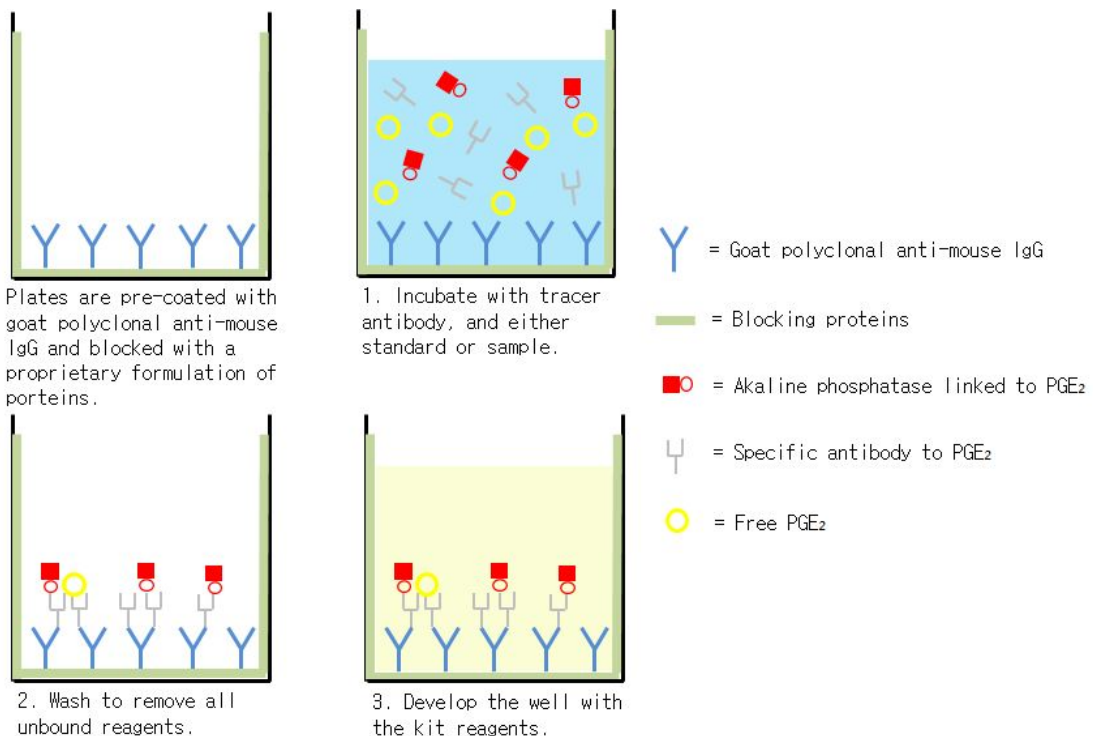


Fig. 7. Determination of PGE₂ using ELISA kit.

4. Wound healing assay

DMEM [+8% FBS, AA]를 이용해 일주일 정도 계대 배양한 HaCaT cell을 6well plate에 2×10^5 cells/mL (4×10^5 cells/well)만큼 깔아주고 37 °C, 5% CO₂ incubator에서 세포가 80% 자랄 때까지 약 24시간 동안 배양하였다. 배양 후에 1x PBS buffer로 cell을 씻어주고 FBS와 항생제를 넣지 않은 배지로 바꿔주었다. 그리고 mitomycin을 30 µg/mL 넣어준 후 다시 2시간 동안 배양하였다. 그 다음 1x PBS buffer로 씻어준 후 200 µL pipette tip을 이용하여 각 well에 scratch를 내주었다. scratch에 의해 떨어져 나간 세포들이 있으므로 1x PBS buffer로 반복해서 씻어준 후 FBS, AA 들어있는 배지를 채워주고 0시간일 때의 사진을 찍었다.

그런 후에 15-PGDH inhibitor를 5 µM 농도로 주입하고 결과 비교를 위해 음성통제집단 (negative control)에는 아무것도 넣지 않았으며 양성통제집단 (positive control)에는 TGF-β1을 1 ng/mL 농도로 주입한 후 48시간 동안 배양하였다. 48시간 후 사진을 다시 찍어 약물 효과로 세포가 얼마나 자랐는지를 확인하였다.

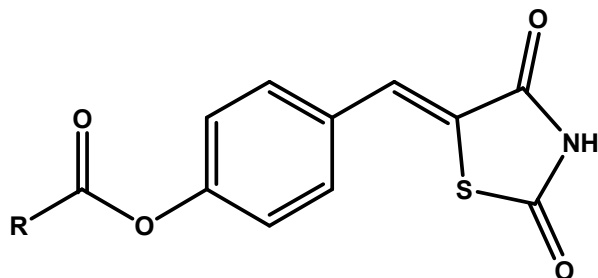
III. 결과 및 고찰

1. 15-PGDH 억제제의 활성 측정

위의 방법으로 합성한 여러 가지 15-PGDH 억제제들의 억제활성 측정을 진행하였다. PGE₂가 NAD⁺를 보조인자로 사용하는 15-PGDH에 의해 불활성화될 시 NADH를 생성하는데 NAD⁺는 형광을 띠지 않는 반면 emission 468 nm, excitation 340 nm 파장에서 형광을 띠는 NADH 농도를 측정하는 방법으로 15-PGDH 억제 활성을 측정하였고 이를 비교 분석하였다.

Table.1은 phenyl의 3번째 탄소가 -H 인 유도체들의 활성 측정 결과를 나타내었다. 가장 억제 효과가 높았던 유도체는 R0i cyclohexylethyl기인 화합물 **8**이었으며 가장 낮았던 화합물은 **10**이었다. -CH- 그룹이 없는 유도체들을 비교하였을 때 cyclohexyl > phenyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제 효과가 높았다. cyclophentyl ring 그룹을 도입하였을 때는 -CH- 그룹이 증가할수록 억제 효과가 증가함을 보였다. 또한 R0i cyclohexyl인 유도체들을 보면 -CH₂- 그룹의 개수가 2개 > 3개 > 1개 > 4개 > 0개 순으로 억제활성이 높았다. phenyl 유도체들을 보면 phenylpropyl 그룹이 가장 높은 억제 활성을 보였으며 -CH₂- 그룹의 개수가 3개 > 2개 > 4개 > 1개 > 0개 순으로 효과가 높았으며 4개를 제외하고는 대체적으로 개수가 줄어들수록 효과도 줄어든다는 것을 볼 수 있었다. 모든 ring 구조에서 -CH- 그룹이 있는 유도체들보다 없는 유도체들이 효과가 낮다는 일관성을 보였다.

Table 1. Inhibitory potency of compounds 1–15

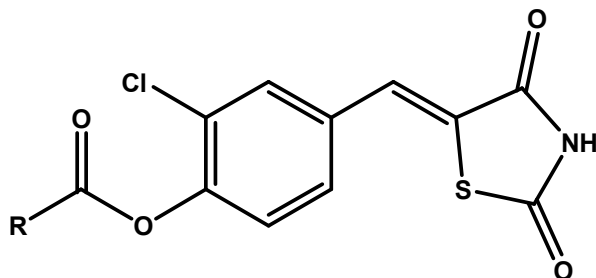


Compound	R	IC ₅₀ (μM)
1	Cyclopropyl	1.41
2	Cyclobutyl	0.4307
3	Cyclopentyl	0.1787
4	Cyclopentylmethyl	0.0521
5	Cyclopentylethyl	0.0232
6	Cyclohexyl	0.0888
7	Cyclohexylmethyl	0.0305
8	Cyclohexylethyl	0.0184
9	Cyclohexylpropyl	0.0261
10	Cyclohexylbutyl	0.0504
11	Phenyl	0.1236
12	Phenylmethyl	0.1083
13	Phenylethyl	0.0544
14	Phenylpropyl	0.0507
15	Phenylbutyl	0.0921

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 2는 phenyl의 3번째 탄소에 -Cl이 치환된 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 R이 cyclohexylethyl인 **23**이었으며 가장 낮았던 화합물은 R이 cyclopropyl인 **16**이었다. -CH- 그룹이 없는 유도체들을 비교하였을 때 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. cyclophentyl ring 그룹을 도입하였을 때 -CH- 그룹이 증가할수록 억제 효과가 증가하였으며 cyclohexyl ring 그룹을 도입하였을 때는 -CH- 그룹이 2개 > 3개 > 1개 > 4개 > 0개 순으로 억제효과가 높았고 또 phenyl ring일 때는 3개 > 0개 > 2개 > 4개 > 1개 순으로 높았다.

Table 2. Inhibitory potency of compounds 16–30

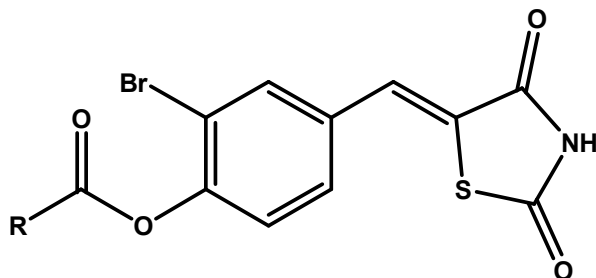


Compound	R	IC ₅₀ (μM)
16	Cyclopropyl	2.0904
17	Cyclobutyl	0.7822
18	Cyclopentyl	0.1958
19	Cyclopentylmethyl	0.0783
20	Cyclopentylethyl	0.0305
21	Cyclohexyl	0.1373
22	Cyclohexylmethyl	0.0238
23	Cyclohexylethyl	0.0153
24	Cyclohexylpropyl	0.0202
25	Cyclohexylbutyl	0.0317
26	Phenyl	0.0319
27	Phenylmethyl	0.0635
28	Phenylethyl	0.0418
29	Phenylpropyl	0.022
30	Phenylbutyl	0.044

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 3은 phenyl의 3번째 탄소에 -Br이 치환된 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 IC₅₀ 값이 0.0145 μM인 **44**이었고 가장 효과가 낮았던 화합물은 R이 cyclopropyl인 **31**이었다. 역시 -CH- 그룹이 없는 유도체들을 비교하였을 때 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. cyclophentyl ring 그룹을 도입하였을 때 -CH- 그룹이 증가할수록 억제 효과가 증가하였으며 cyclohexyl ring 그룹을 도입하였을 때는 -CH- 그룹이 3개 > 2개 > 1개 > 4개 > 0개 순으로 억제효과가 높아 대체적으로 개수가 줄어들수록 효과도 줄어드는 것을 확인할 수 있었다. phenyl ring을 도입하였을 때는 억제 효과가 -CH- 그룹이 3개 > 4개 > 0개 > 2개 > 1개 순으로 높았다.

Table 3. Inhibitory potency of compounds 31–45

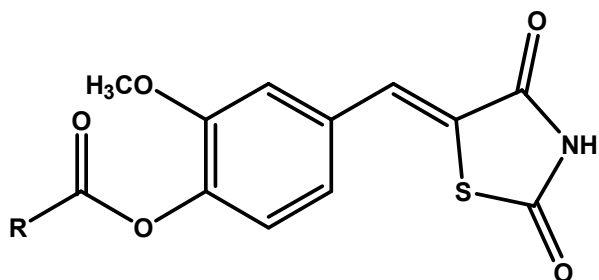


Compound	R	IC ₅₀ (μM)
31	Cyclopropyl	0.2201
32	Cyclobutyl	0.1057
33	Cyclopentyl	0.05
34	Cyclopentylmethyl	0.0198
35	Cyclopentylethyl	0.0158
36	Cyclohexyl	0.0376
37	Cyclohexylmethyl	0.0206
38	Cyclohexylethyl	0.0166
39	Cyclohexylpropyl	0.0154
40	Cyclohexylbutyl	0.0263
41	Phenyl	0.0254
42	Phenylmethyl	0.0406
43	Phenylethyl	0.0284
44	Phenylpropyl	0.0145
45	Phenylbutyl	0.0235

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 4는 phenyl의 3번째 탄소에 $-OCH_3$ 이 치환된 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 IC_{50} 값이 $0.0173 \mu M$ 인 **53**이었고 가장 효과가 낮았던 화합물은 IC_{50} 값이 $1.1239 \mu M$ 인 **46**이었다. 역시 $-CH-$ 그룹이 없는 유도체들을 비교하였을 때 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. 위의 유도체들과 마찬가지로 cyclophentyl ring 그룹을 도입하였을 때 $-CH-$ 그룹이 증가할수록 억제 효과가 증가하였으며 cyclohexyl ring 그룹을 도입하였을 때는 $-CH-$ 그룹이 2개 > 3개 > 1개 > 4개 > 0개 순으로 억제효과가 높았다. phenyl ring을 도입하였을 때는 억제 효과가 $-CH-$ 그룹이 3개 > 2개 > 4개 > 1개 > 0개 순으로 억제 효과가 높았으며 대체적으로 개수가 감소할수록 효과도 감소한다는 것을 확인할 수 있었다. phenyl ring을 도입했을 때보다 cyclohexyl ring을 도입했을 때 효과가 더 좋았으며 모든 ring 구조에서 $-CH-$ 그룹이 있는 유도체들보다 없는 유도체들이 효과가 낮다는 일관성을 보였다.

Table 4. Inhibitory potency of compounds 46-60

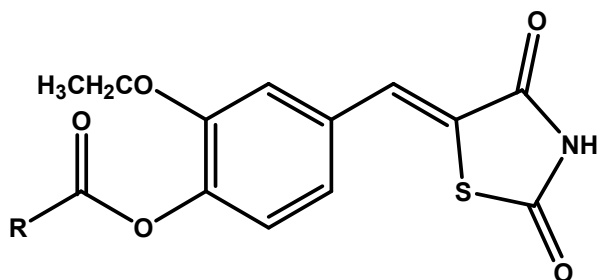


Compound	R	IC ₅₀ (μM)
46	Cyclopropyl	1.1239
47	Cyclobutyl	1.1186
48	Cyclopentyl	0.4936
49	Cyclopentylmethyl	0.0602
50	Cyclopentylethyl	0.0234
51	Cyclohexyl	0.2423
52	Cyclohexylmethyl	0.0278
53	Cyclohexylethyl	0.0173
54	Cyclohexylpropyl	0.0175
55	Cyclohexylbutyl	0.0282
56	Phenyl	0.1507
57	Phenylmethyl	0.0422
58	Phenylethyl	0.0286
59	Phenylpropyl	0.0239
60	Phenylbutyl	0.0389

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 5는 phenyl의 3번째 탄소에 $-OCH_2CH_3$ 이 치환된 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 IC_{50} 값이 $0.0238 \mu M$ 인 **69**이었고 가장 효과가 낮았던 화합물은 IC_{50} 값이 $2.1723 \mu M$ 인 **61**이었다. 역시 $-CH-$ 그룹이 없는 유도체들을 비교하였을 때 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. 위의 유도체들과 마찬가지로 cyclophentyl ring 그룹을 도입하였을 때 $-CH-$ 그룹이 증가할수록 억제 효과가 증가하였다. cyclohexyl ring 그룹을 도입하였을 때 억제 효과가 $-CH-$ 그룹이 3개 > 2개 > 1개 > 4개 > 0개 순으로 높았으며 phenyl ring을 도입하였을 때는 $-CH-$ 그룹이 3개 > 4개 > 2개 > 1개 > 0개 순으로 억제 효과가 높았다. cyclohexyl ring과 phenyl ring 두 그룹 모두 $-CH-$ 그룹이 4개일 때를 제외하고는 개수가 줄어들수록 억제 효과도 줄어든다는 것을 확인할 수 있었다. 또한 모든 ring 구조에서 $-CH-$ 그룹이 있는 유도체들보다 없는 유도체들이 효과가 낮다는 일관성을 보였다.

Table 5. Inhibitory potency of compounds 61–75

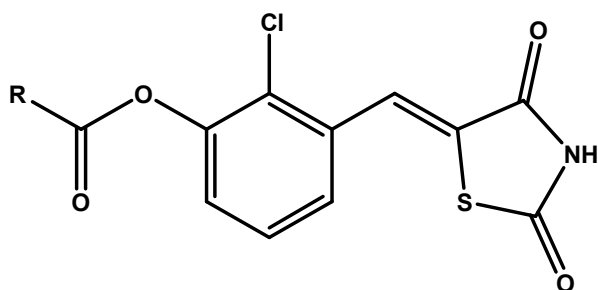


Compound	R	IC ₅₀ (μM)
61	Cyclopropyl	2.1723
62	Cyclobutyl	1.6214
63	Cyclopentyl	1.403
64	Cyclopentylmethyl	0.1285
65	Cyclopentylethyl	0.0290
66	Cyclohexyl	0.549
67	Cyclohexylmethyl	0.0328
68	Cyclohexylethyl	0.0245
69	Cyclohexylpropyl	0.0238
70	Cyclohexylbutyl	0.0344
71	Phenyl	0.298
72	Phenylmethyl	0.1185
73	Phenylethyl	0.0943
74	Phenylpropyl	0.0439
75	Phenylbutyl	0.0584

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 6은 phenyl의 2번째 탄소에 -Cl이 치환되어 있으며 다른 유도체들과 달리 3번째 탄소 위치에 ester 결합이 있는 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 IC₅₀ 값이 0.0225 μM인 **84**이었고 가장 효과가 낮았던 화합물은 IC₅₀ 값이 0.5424 μM인 **76**이었다. 역시 다른 유도체들과 마찬가지로 -CH- 그룹이 없는 유도체들을 비교하였을 때 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. 또한 다른 유도체들처럼 cyclophentyl ring 그룹을 도입하였을 때 -CH- 그룹이 증가할수록 억제 효과가 증가하였다. cyclohexyl ring 그룹을 도입하였을 때 억제 효과가 -CH- 그룹이 3개 > 2개 > 4개 > 1개 > 0개 순으로 높았으며 대체적으로 개수가 증가할수록 효과도 증가함을 보였다. phenyl ring을 도입하였을 때는 -CH- 그룹이 2개 > 3개 > 4개 > 0개 > 1개 순으로 억제 효과가 높았다.

Table 6. Inhibitory potency of compounds 76–90

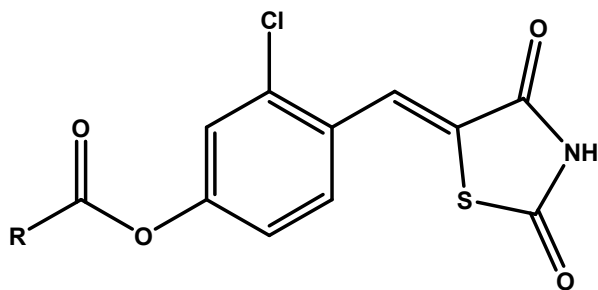


Compound	R	IC ₅₀ (μM)
76	Cyclopropyl	0.5424
77	Cyclobutyl	0.4818
78	Cyclopentyl	0.0562
79	Cyclopentylmethyl	0.0428
80	Cyclopentylethyl	0.0347
81	Cyclohexyl	0.0558
82	Cyclohexylmethyl	0.0327
83	Cyclohexylethyl	0.0228
84	Cyclohexylpropyl	0.0225
85	Cyclohexylbutyl	0.0256
86	Phenyl	0.0541
87	Phenylmethyl	0.1049
88	Phenylethyl	0.0225
89	Phenylpropyl	0.0251
90	Phenylbutyl	0.046

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

Table 7은 phenyl의 2번째 탄소에 -Cl이 치환되어 있으며 4번째 탄소 위치에 ester 결합이 있는 유도체들의 억제 활성 결과를 나타내었다. 가장 억제 효과가 높았던 화합물은 IC₅₀ 값이 0.0268 μ M인 **96**이었고 가장 효과가 낮았던 화합물은 IC₅₀ 값이 1.0833 μ M인 **91**이었다. -CH- 그룹이 없는 유도체들을 비교하였을 때 cyclohexyl > phenyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 억제효과가 높았으며 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. cyclophentyl ring 그룹을 도입하였을 때 다른 유도체들은 -CH- 그룹이 증가할수록 억제 효과가 증가한 반면 두 번째 탄소에 Cl이 있는 이 유도체들은 -CH- 그룹이 1개일 때가 가장 좋았으며 2개일 때가 그 다음으로 효과가 좋았다. cyclohexyl ring 그룹을 도입하였을 때도 다른 유도체와 달리 -CH- 그룹이 없는 유도체가 억제 효과가 가장 높았으며 0개 > 1개 > 2개 > 4개 > 3개 순으로 그룹이 늘어날수록 억제 효과가 줄어드는 것을 볼 수가 있었다. 이는 phenyl기를 도입하였을 때도 마찬가지로 결과를 얻었다.

Table 7. Inhibitory potency of compounds 91–105



Compound	R	IC ₅₀ (μM)
91	Cyclopropyl	1.0833
92	Cyclobutyl	0.2056
93	Cyclopentyl	0.0710
94	Cyclopentylmethyl	0.0327
95	Cyclopentylethyl	0.0508
96	Cyclohexyl	0.0268
97	Cyclohexylmethyl	0.0277
98	Cyclohexylethyl	0.0324
99	Cyclohexylpropyl	0.0392
100	Cyclohexylbutyl	0.0394
101	Phenyl	0.0451
102	Phenylmethyl	0.0753
103	Phenylethyl	0.0875
104	Phenylpropyl	0.0749
105	Phenylbutyl	0.1298

The enzyme was assayed fluorometrically. The IC₅₀ value was determined using NAD⁺ (250 μM) as coenzyme and PGE₂ (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector.

2. PGE₂ 농도 측정

PGE₂는 소화성 궤양, 신경내분비 및 면역시스템 조절, 혈관의 수축과 팽창 및 혈압 조절에 관여하는 물질로 특히 상처 치유 및 염증조절에 중요한 역할을 담당하고 있다.

그러므로 합성한 화합물이 *in vitro* 세포 시스템에서 15-PGDH를 억제하여 얼마만큼의 PGE₂를 높이는지 확인하기 위해 폐암세포인 A549 cell을 배양 후 5 μM 농도로 약물 주입하고 배지 층의 PGE₂를 ELISA kit를 이용하여 측정하였다.

Table. 8-14는 각각의 화합물들이 A549 세포내에서 얼마만큼의 PGE₂ 농도를 증가시켰는지 나타내고 있다.

PGE₂ 측정 결과, 화합물의 억제 활성인 IC₅₀ (μM) 값과 PGE₂ 농도 값이 항상 같은 양상을 나타내지 않았다. 억제효과가 높은 화합물이라도 PGE₂ 농도는 낮은 경우도 있었으며 억제효과가 낮았더라도 PGE₂ 농도는 높은 경우도 있었다. 이는 PGE₂ 값이 실제 세포에서 측정하는 실험이라 세포 속 많은 효소와 다른 요인으로 인한 이유라 생각된다. 가장 높은 PGE₂ 농도 증가를 보인 화합물은 579%까지 증가한 화합물 **59**인 (Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-phenylbutanoate 이었으며 두 번째로 높은 농도는 389.2% 증가한 화합물 **89**였으며 그다음으로 **14** > **29** 순으로 높은 농도 증가를 보였다.

Table 8. PGE₂ levels in A549 cells after administration of compounds **1-15**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
1	273.769	283.2688	3.47
2	273.769	301.0364	9.96
3	273.769	191.3645	-30.1
4	273.769	556.6271	103.32
5	273.769	501.1889	83.07
6	273.769	349.6304	27.71
7	273.769	329.5084	20.36
8	273.769	879.6746	221.32
9	273.769	792.1232	189.34
10	273.769	520.1337	89.99
11	119.6754	25.94563	-78.32
12	119.6754	77.92065	-34.89
13	119.6754	139.2902	16.39
14	119.6754	586.3975	389.99
15	119.6754	220.3344	84.11

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 9. PGE₂ levels in A549 cells after administration of compounds **16–30**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
16	253.0363	283.7043	12.12
17	253.0363	395.597	56.34
18	253.0363	198.1274	-21.7
19	253.0363	232.5151	-8.11
20	253.0363	828.2384	227.32
21	253.0363	411.1081	62.47
22	253.0363	480.187	89.77
23	253.0363	1040.03	311.02
24	253.0363	1010.425	299.32
25	253.0363	266.5484	5.34
26	197.2043	175.2752	-11.12
27	197.2043	393.7184	99.65
28	197.2043	572.5235	190.32
29	197.2043	922.5414	367.81
30	197.2043	280.7203	42.35

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 10. PGE₂ levels in A549 cells after administration of compounds **31-45**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
31	197.2043	199.4327	1.13
32	197.2043	316.7495	60.62
33	197.2043	192.6292	-2.32
34	197.2043	57.64282	-70.77
35	197.2043	455.1278	130.79
36	202.7598	408.196	101.32
37	202.7598	382.9119	88.85
38	202.7598	859.6813	323.99
39	202.7598	649.318	220.24
40	202.7598	218.7373	7.88
41	202.7598	23.64179	-88.34
42	202.7598	400.7345	97.64
43	202.7598	432.4258	113.27
44	202.7598	810.6337	299.80
45	202.7598	362.494	78.78

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 11. PGE₂ levels in A549 cells after administration of compounds **46–60**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
46	273.9787	278.8555	1.78
47	273.9787	185.1274	-32.43
48	273.9787	211.539	-22.79
49	273.9787	299.5683	9.34
50	273.9787	519.6554	89.67
51	273.9787	283.1296	3.34
52	273.9787	223.32	-18.49
53	273.9787	792.73	189.34
54	273.9787	828.9774	202.57
55	273.9787	295.349	7.8
56	159.1332	108.7675	-31.65
57	159.1332	131.3485	-17.46
58	159.1332	171.8161	7.97
59	159.1332	1081.055	579.34
60	159.1332	300.5549	88.87

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 12. PGE₂ levels in A549 cells after administration of compounds **61–75**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
61	159.1332	0.04774	-99.97
62	159.1332	178.4361	12.13
63	159.1332	237.6336	49.33
64	159.1332	199.4576	25.34
65	159.1332	284.8007	78.97
66	129.1737	132.3901	2.49
67	129.1737	139.9468	8.34
68	129.1737	255.0535	97.45
69	129.1737	302.8477	134.45
70	129.1737	219.5565	69.97
71	129.1737	53.76209	-58.38
72	129.1737	105.832	-18.07
73	129.1737	261.4347	102.39
74	129.1737	164.9677	27.71
75	129.1737	159.2841	23.31

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 13. PGE₂ levels in A549 cells after administration of compounds **76–90**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
76	119.6754	123.6726	3.34
77	119.6754	187.3399	56.54
78	119.6754	39.10992	-67.32
79	119.6754	116.875	-2.34
80	119.6754	236.9453	97.99
81	210.4123	232.1268	10.32
82	210.4123	610.1325	189.97
83	210.4123	634.0143	201.32
84	210.4123	245.488	16.67
85	210.4123	194.0212	-7.79
86	210.4123	184.9103	-12.12
87	210.4123	399.2994	89.77
88	210.4123	449.1671	113.47
89	210.4123	1048.274	398.2
90	210.4123	416.8478	98.11

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

Table 14. PGE₂ levels in A549 cells after administration of compounds **91–105**

Compound	Control (pg/mL)	PGE2 conc. (pg/mL)	Increment %
91	111.6575	106.5773	-4.5498
92	111.6575	47.25859	-57.6754
93	111.6575	114.9832	2.9785
94	111.6575	220.1299	97.1474
95	111.6575	238.3412	113.4574
96	111.6575	121.5809	8.8874
97	111.6575	120.703	8.1011
98	111.6575	266.5808	138.7487
99	111.6575	186.2131	66.7717
100	111.6575	107.4803	-3.7411
101	111.6575	200.4108	79.4871
102	111.6575	222.4242	99.2022
103	111.6575	290.2972	159.989
104	111.6575	349.9990	213.4577
105	111.6575	211.5461	89.4598

A549 cells were treated with 5 μ M of the synthesized compounds for 12 hours. Extracellular PGE₂ concentration was detected using ELISA kit.

3. Scratch wound healing assay

15-PGDH의 활성을 억제하면 생체내의 PGE₂ 농도가 증가하여 PGE₂가 관여하는 효과적인 상처치유에 중요한 역할을 한다. 그러므로 위의 PGE₂ 농도 측정실험에서 높은 증가를 보인 화합물 **14**, **29**, **59**, **89**를 이용하여 *in vitro* 세포 시스템으로 HaCaT cell에서 상처치유 효과를 실험하였다. pipette tip을 이용하여 scratch를 내었으며 결과 비교를 위해 음성통제집단 (negative control)에는 약물처리를 하지 않았고 양성통제집단(positive control)에는 상처 치유 관여인자인 TGF-β 1 (1 ng/mL)을 처리하였다.

Fig. 8과 **Fig. 9**는 약물처리 하기 전과 약물처리 후 24시간동안 세포를 배양한 후 scratch 낸 부분을 다시 찍은 사진이다.

우선 **Fig. 8**에 화합물 **14**, **29**의 실험 사진을 보면 아무 약물도 주입하지 않았던 Control의 세포와 비교하여 합성한 약물을 주입한 세포는 훨씬 더 잘 자란 것을 확인할 수 있었으며 TGF-β 1을 주입한 세포만큼 높은 scratch 회복을 보였다. 역시 **Fig. 9**를 보면 화합물 **59**, **89**가 Control과 비교하여 훨씬 잘 자랐으며 TGF-β 1을 처리한 세포만큼 자란 것을 확인할 수 있었다.

Fig. 10은 약물처리하기 전과 24시간동안 자란 세포의 wound closure rate를 퍼센트로 나타내는 그래프이다. Control이 24시간 동안 100% 회복률을 보였다면 **14**, **29**는 약 200%의 회복률을 보였다. 또 화합물 **59**, **89**를 보면 control과 비교하여 약 5배 이상 더 잘 자란 것을 확인할 수 있었다.

특히 눈에 띄게 높은 회복률을 보였던 **59**, **89**는 약물을 농도 별로 처리하여 5 μM보다 적은 농도에서도 scratch가 회복이 되는지를 알아보았다. **Fig. 11**, **12**를 보면 화합물 **59**와 **89** 모두 농도가 증가할수록 세포의 scratch 회복률이 증가하였으며 0.5 μM로 약물을 처리하였을 때도 Control과 비교하여 약 2배 이상 더 잘 자란 것을 확인하였다.

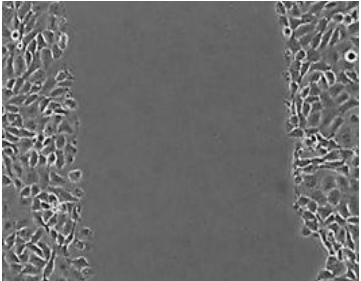
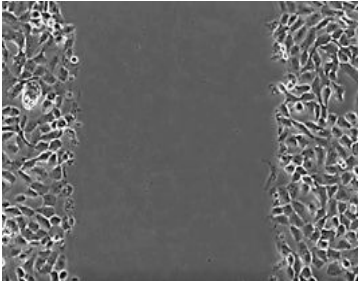
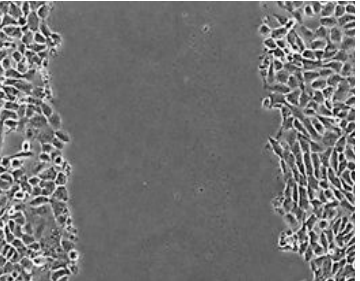
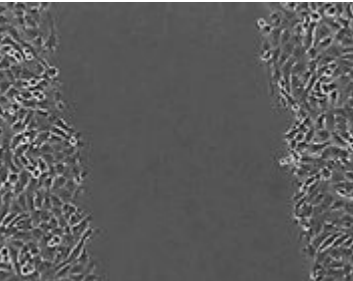
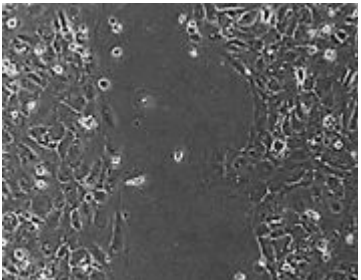
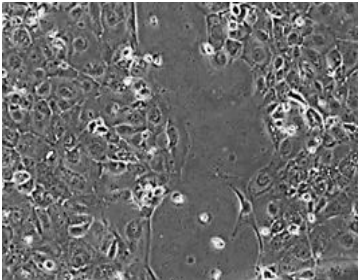
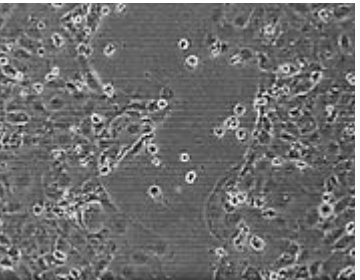
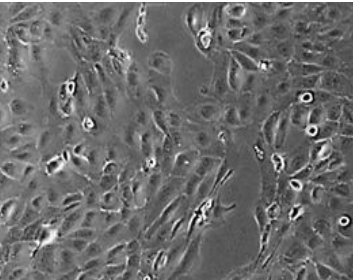
	Control	14	29	TGF-β 1
0 hour	 525 μm	 509 μm	 514 μm	 494 μm
24 hour	 350 μm	 179 μm	 210 μm	 122 μm

Fig. 8. Scratch wound healing of HaCaT confluent monolayers after administration of compounds 14 and 29.

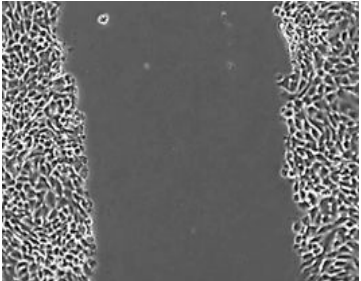
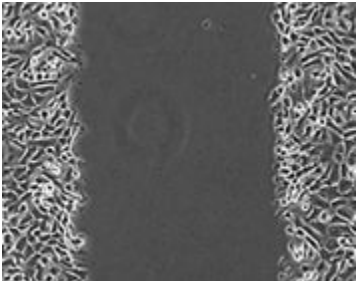
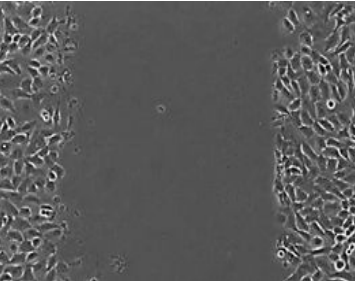
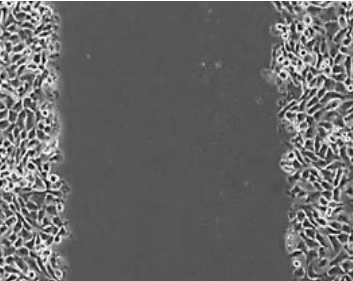
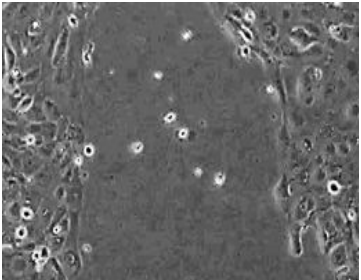
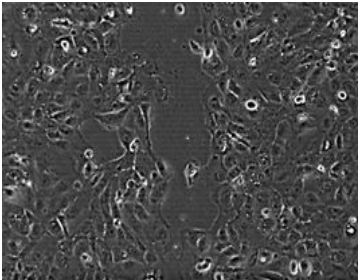
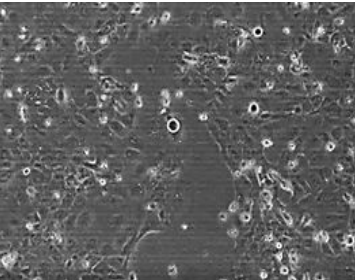
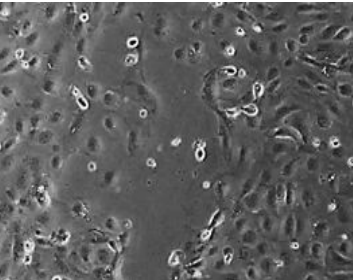
	Control	59	89	TGF-β 1
0 hour	 516 μm	 485 μm	 506 μm	 540 μm
24 hour	 444 μm	 133 μm	 159 μm	 189 μm

Fig. 9. Scratch wound healing of HaCaT confluent monolayers after administration of compounds 59 and 89.

Fig. 10. Wound closure rate of compounds 14, 29, 59 and 89 (* ; $P < 0.05$).

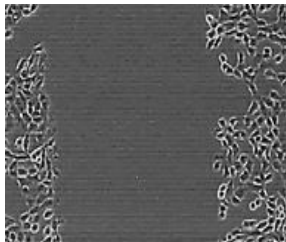
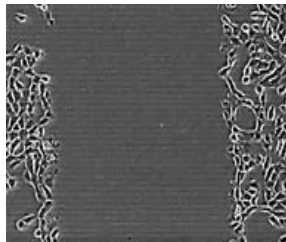
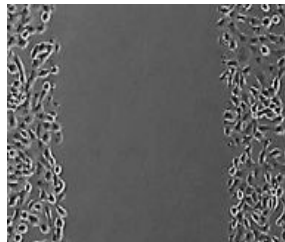
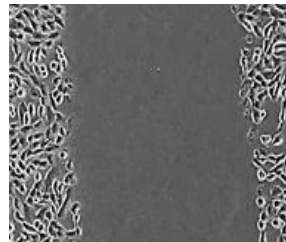
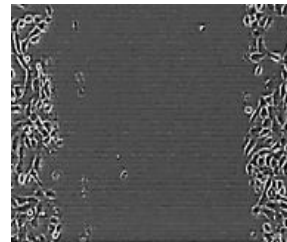
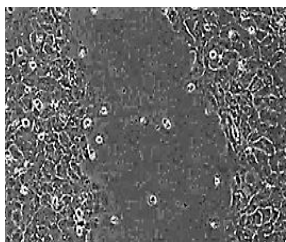
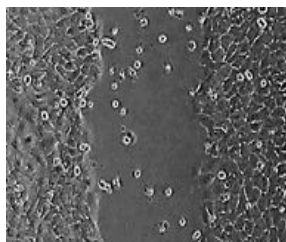
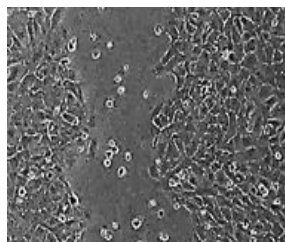
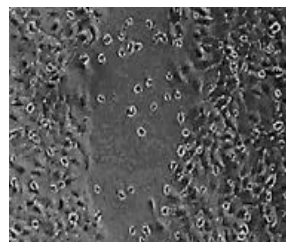
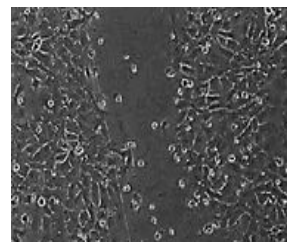
59	Control	0.5 μM	1 μM	3 μM	5 μM
0 hour	 530 μm	 579 μm	 538 μm	 601 μm	 608 μm
24 hour	 421 μm	 319 μm	 250 μm	 249 μm	 191 μm

Fig. 11. Scratch wound healing of HaCaT confluent monolayers after administration of compound 59 at different concentration (0.5 μM - 5 μM).

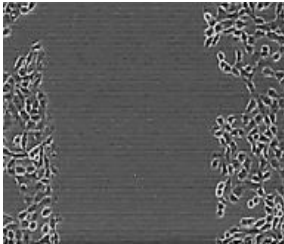
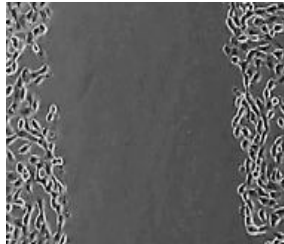
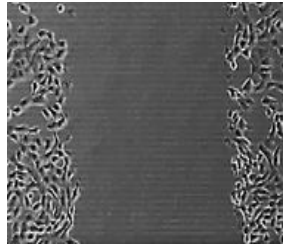
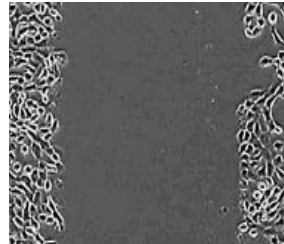
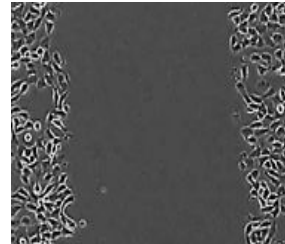
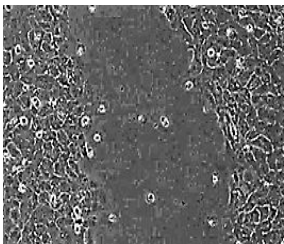
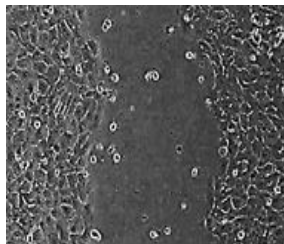
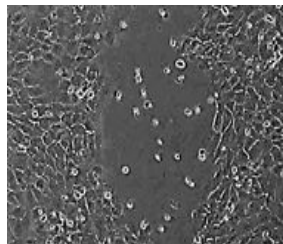
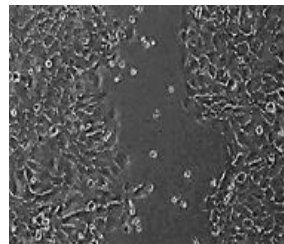
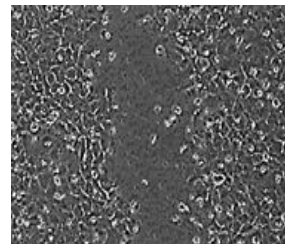
89	Control	0.5 μM	1 μM	3 μM	5 μM
0 hour	 530 μm	 583 μm	 528 μm	 582 μm	 581 μm
24 hour	 421 μm	 323 μm	 277 μm	 249 μm	 212 μm

Fig. 12. Scratch wound healing of HaCaT confluent monolayers after administration of compound 89 at different concentration (0.5 μM - 5 μM).

compound 59

compound 89

Fig. 13. Wound closure rate of compounds 59 and 89 at different concentration (0.5 μ M - 5 μ M). (* ; P < 0.05).

IV. 결론

Prostaglandins (PGs)는 중요한 지질 중재자의 부류 중 하나로 우리 몸 속 대부분의 세포에서 형성되며 특정한 물리적 또는 화학적 자극에 의해 활성화되었을 때 세포막으로부터 방출되는 arachidonic acid (AA)로부터 합성된다. 그 중에서 특히 PGE₂는 재생, 염증조절, 상처치유, 소화성 궤양, 신경내분비 및 면역시스템 조절, 혈관의 수축과 팽창 및 혈압조절 등 생체 내에서 중요한 역할을 담당하고 있다. 그러나 이러한 PGE₂는 NAD⁺를 보조인자로 사용하는 15-PGDH에 의해 빠르게 대사되기 때문에 생체 내에서 반감기가 매우 짧다. 따라서 본 연구에서는 PGE₂ 부족으로 유발되는 여러 가지 질병 치료를 위해 PGE₂를 불활성화 시키는 15-PGDH 활성을 억제하는 물질의 합성 및 활성평가를 진행하였다.

15-PGDH 억제제는 이미 안정성이 검증되어 있으며 15-PGDH 억제 효과도 있는 TD를 기본 골격으로 하여 다양한 유도체들을 합성하였다. 합성된 화합물이 15-PGDH를 얼마나 억제시키는지 알아보기 위해 억제 활성 측정 실험을 진행하였으며 그 결과, R이 cyclohexyl이나 cyclohexylpropyl일 때 효과가 가장 높았으며 R이 cyclopropyl일 때가 가장 효과가 낮았다. -CH- 그룹이 없는 유도체들을 비교하였을 때 대부분 phenyl > cyclohexyl > cyclopentyl > cyclobutyl > cyclopropyl 순으로 ring 그룹의 탄소 개수가 증가할수록 억제 효과가 높아짐을 보였다. 또 phenyl의 2, 4번째 탄소에 -Cl, ester 결합이 있는 유도체들을 제외한 모든 유도체들이 cyclopentyl ring 그룹을 도입하였을 때 -CH- 그룹이 증가할수록 억제 효과가 증가함을 보였으며 역시 phenyl의 2, 4번째 탄소에 -Cl, ester 결합이 있는 유도체들을 제외한 모든 유도체들이 cyclohexyl ring일 때는 -CH- 그룹이 2개 또는 3개일 때가 가장 억제 효과가 높았으며 phenyl ring일 때는 3개일 때가 가장 높았다. phenyl의 3번째 탄소에 전자 끄는 기(electron withdrawing group)인 Cl, Br가 왔을 때 전체적으로 효과가 좋았지만 -OCH₂CH₃ 기가 왔을 때는 다른 유도체들에 비해 효과가 낮았다. 또 ring 구조에서 -CH- 그룹이 있는 유도체들보다 없는 유도체들이 대부분 낮은 억제 효과를 보였다.

phenyl의 3,4위치에 -Cl, ester 결합이 있는 유도체들과 2,4위치에 -Cl, ester 결합이 있는 유도체, 2,3위치에 -Cl, ester 결합이 있는 유도체들을 비교한 결과 치환기의

위치에 따른 효과 차이는 크지 않았지만 2,3위치에 Cl, ester 결합이 있을 때가 억제 효과가 더 좋았다. 합성한 105개 화합물 중 가장 15-PGDH 억제 효과가 좋았던 것은 IC₅₀ 값이 0.0145 μM인 화합물 **44**이었으며 두 번째로 효과가 좋았던 화합물은 **23**이었다. 이 105개 화합물은 억제효과가 있는 thiazolidinedione을 기본 골격으로 한 유도체들로 대부분 기존에 합성된 억제제보다 억제 효과가 좋다는 것을 확인하였다.

기기를 통해 화합물의 15-PGDH 억제 활성을 측정하였으며 살아있는 세포에서 PGE₂를 증가시키는지 알아보려고 *in vitro* 세포 시스템에서 A549 cell을 이용하여 PGE₂ 농도 측정 실험을 진행하였다. 측정 결과, 화합물의 억제 활성인 IC₅₀ 값과 PGE₂ 농도 값이 항상 같은 양상을 나타내지 않았다. 이는 PGE₂ 값이 실제 세포에서 측정하는 실험이라 세포 속 많은 효소와 다른 요인으로 인한 이유라 생각된다. 가장 높은 PGE₂ 농도 증가를 보인 화합물은 579%까지 증가한 **59**인 (Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-phenylbutanoate 이었으며 두 번째로는 389.2% 증가한 화합물 **89**였으며 그 다음으로 **14** > **29** 순으로 높은 농도 증가를 보였다.

PGE₂는 특히 세포 재생과 상처치유에 중요하게 관여하고 있는 인자 중 하나이다. 그래서 높은 15-PGDH 억제 효과와 높은 PGE₂ 농도 증가를 보인 화합물들은 *in vitro* 세포 시스템에서 HaCaT cell을 이용하여 상처 치유 효과를 실험하였다. 결과 비교를 위해 negative control에는 약물처리를 하지 않았고 positive control에는 상처 치유 관여인자인 TGF-β 1을 처리하였다. 효과가 좋았던 **59**, **89**, **14**, **29** 화합물들을 약물로 처리 하였으며 24시간 후 약물처리를 하지 않은 control에 비해 눈에 띄게 세포 재생이 빨랐으며 화합물을 처리한 세포가 TGF-β 1을 처리한 세포만큼 자란 것을 확인하였다.

모든 활성 측정 결과, 합성한 15-PGDH 억제제가 15-PGDH를 효율적으로 억제하여 PGE₂를 증가시켰으며 scratch wound healing assay를 통해 PGE₂ 증가가 세포 재생을 촉진시켜 상처치유를 촉진시키는 효과를 보여주었다. 이번 연구를 통해 기존에 합성된 15-PGDH 억제제들보다 더 높은 효과를 가지는 화합물을 개발함으로써 PGE₂ 부족으로 유발되는 여러 가지 질병 치료에 도움이 될 것으로 판단된다.

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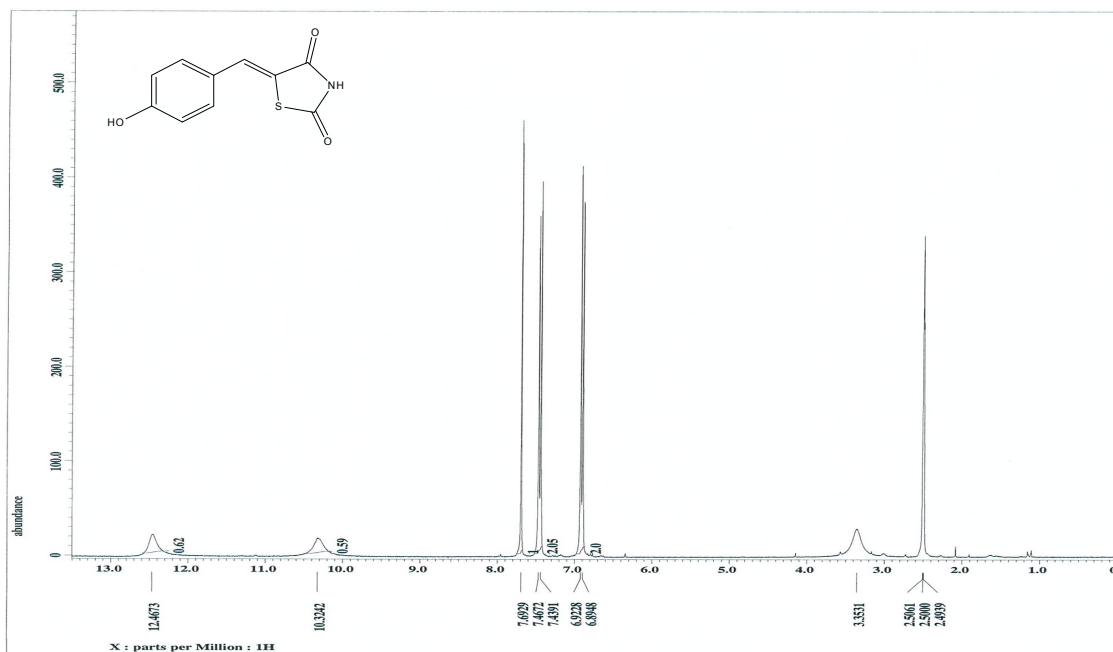
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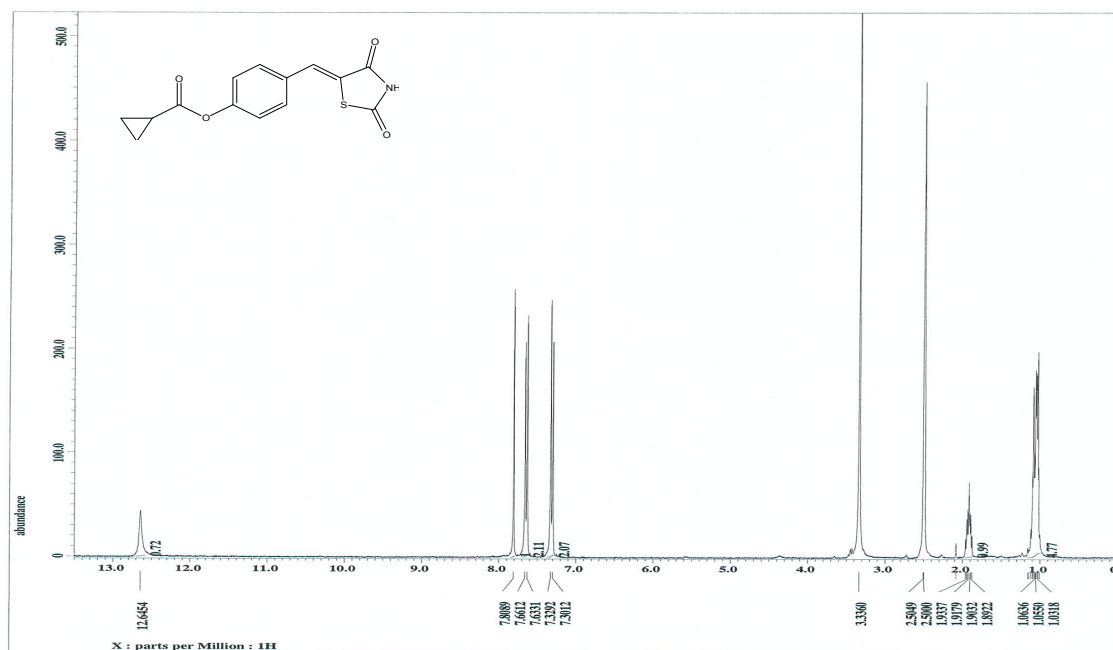
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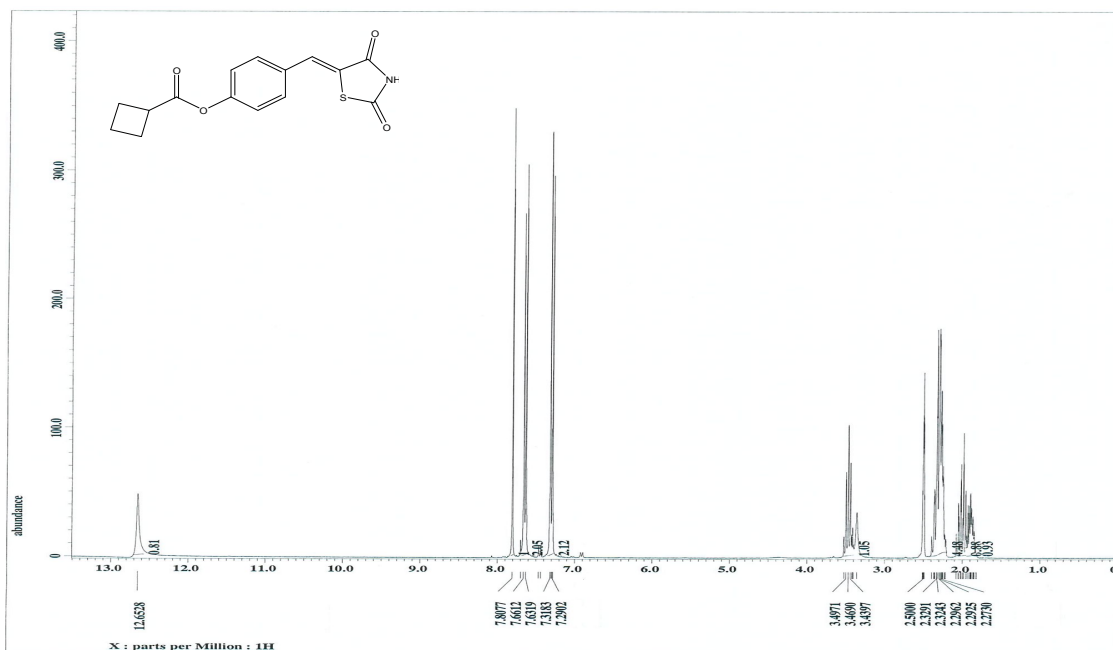
^1H NMR Spectra



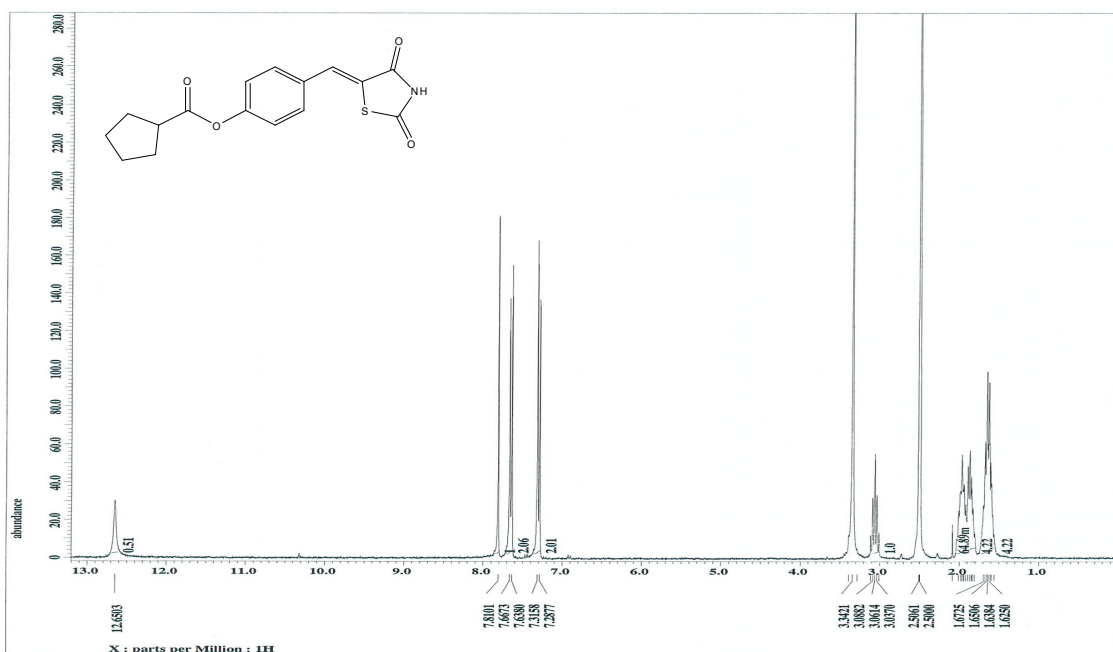
5-(4-Hydroxy-benzylidene)-thiazolidine-2,4-dione (1a-15a)



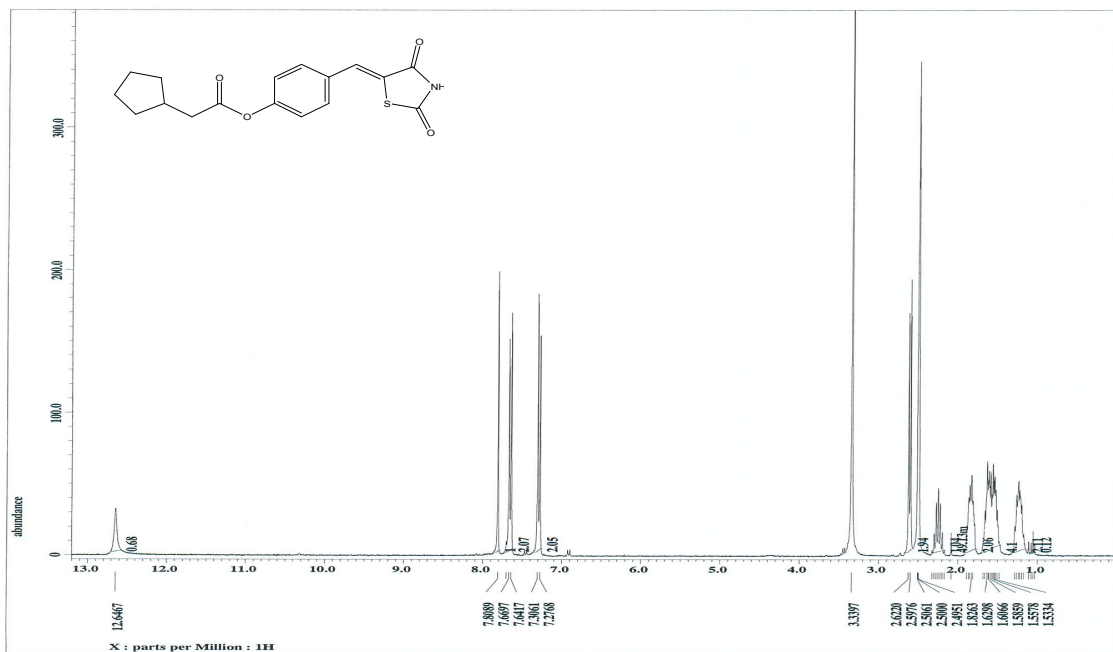
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropanecarboxylate (1)



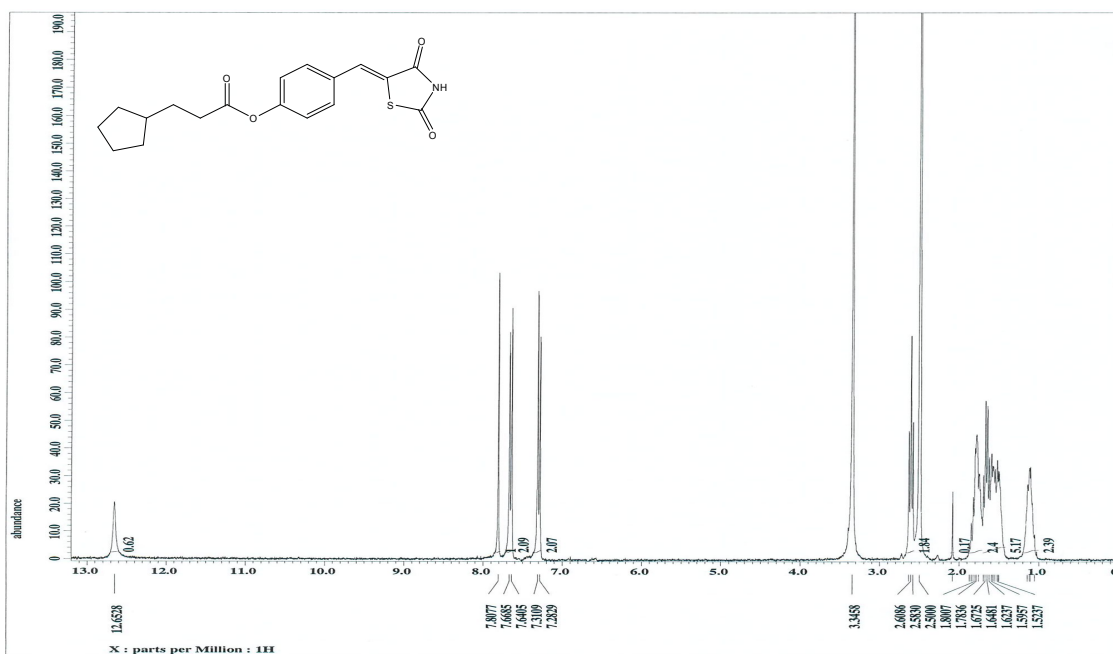
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutancarboxylate (2)



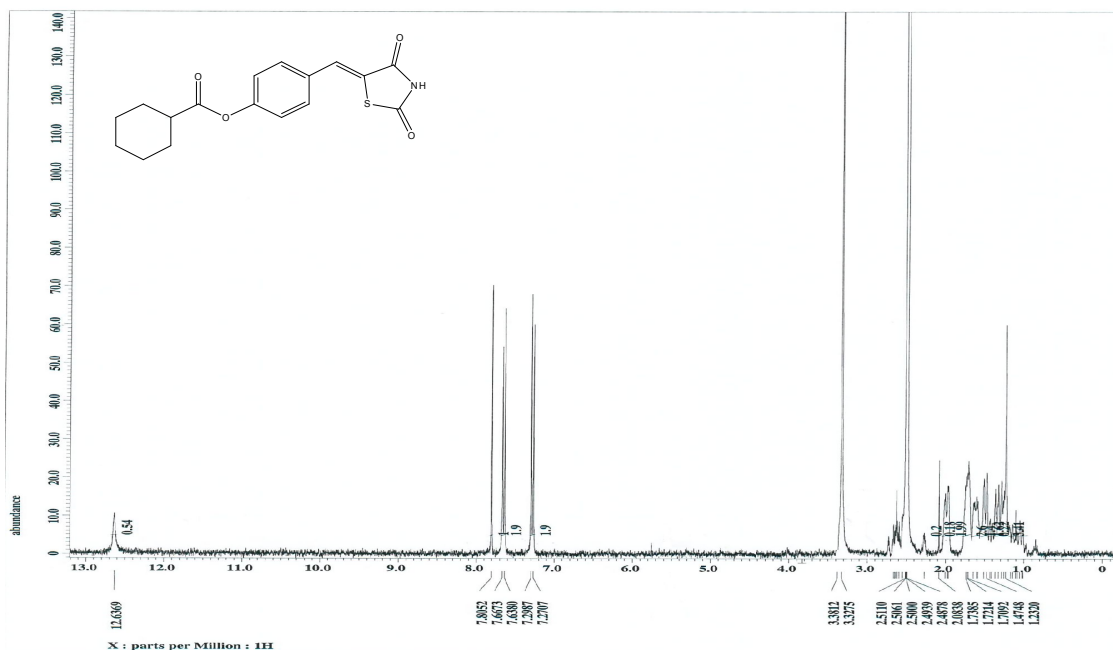
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentancarboxylate (3)



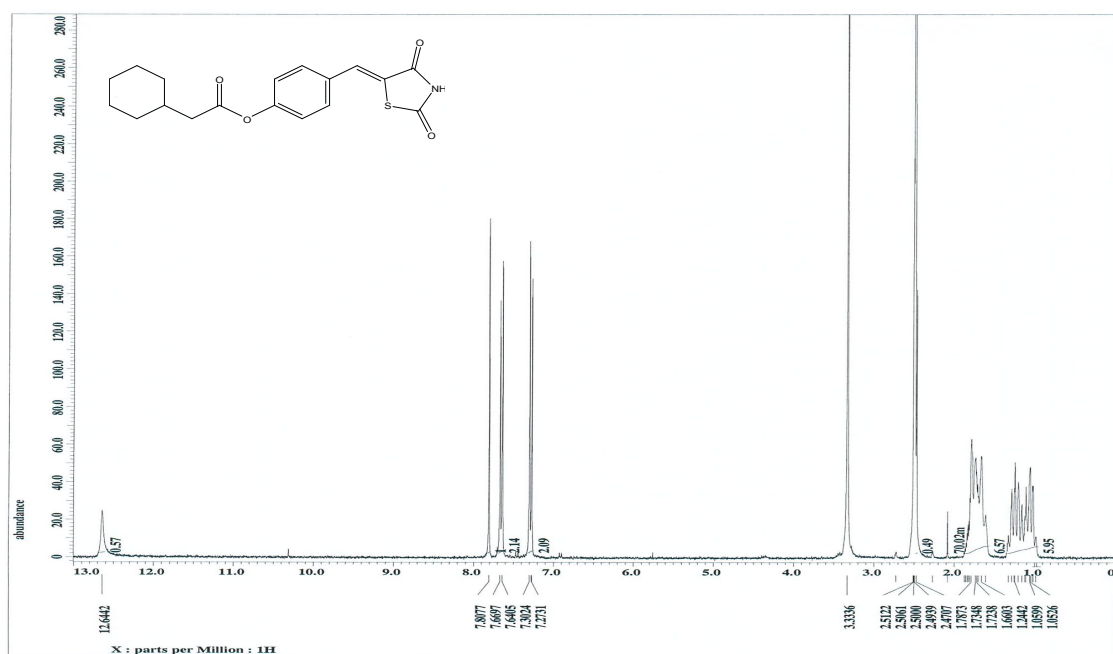
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (4)



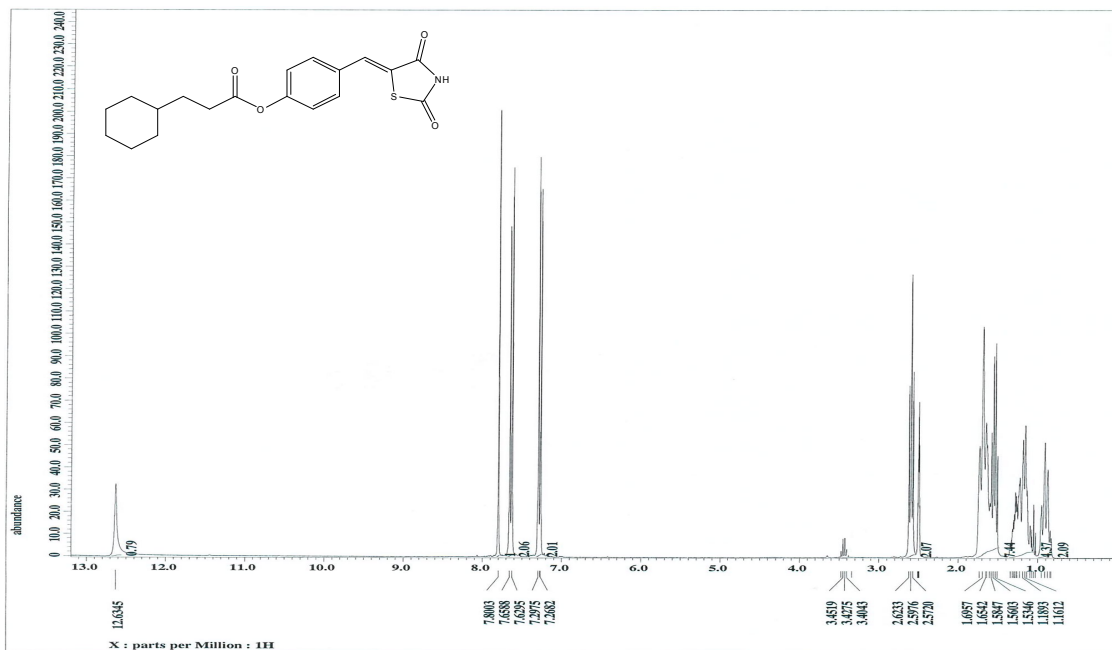
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (5)



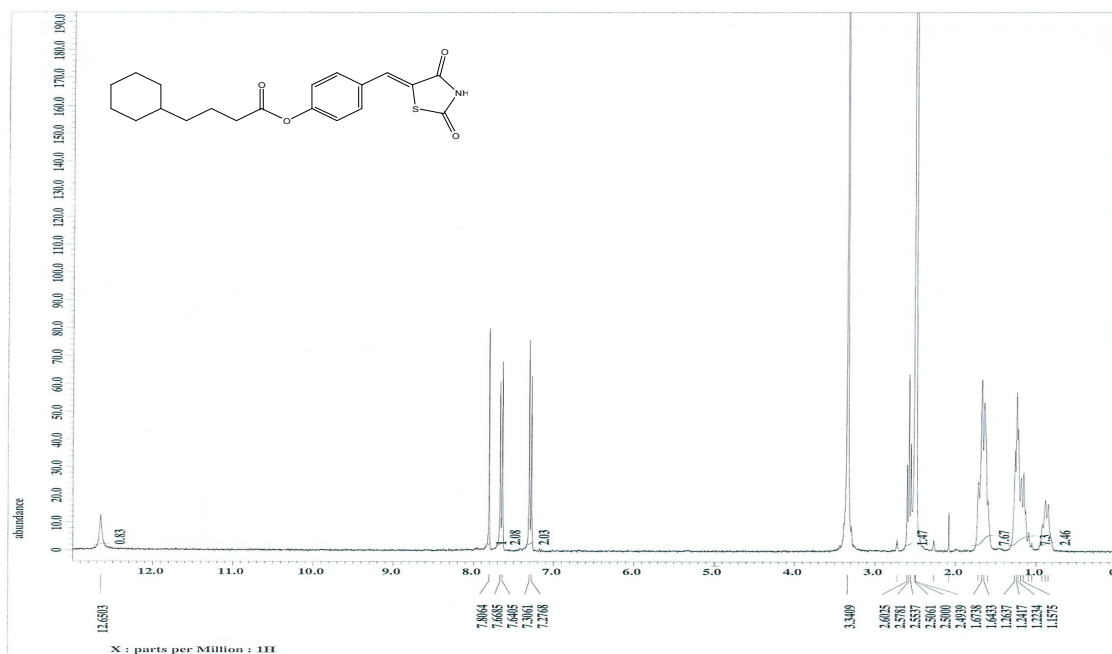
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexanecarboxylate (6)



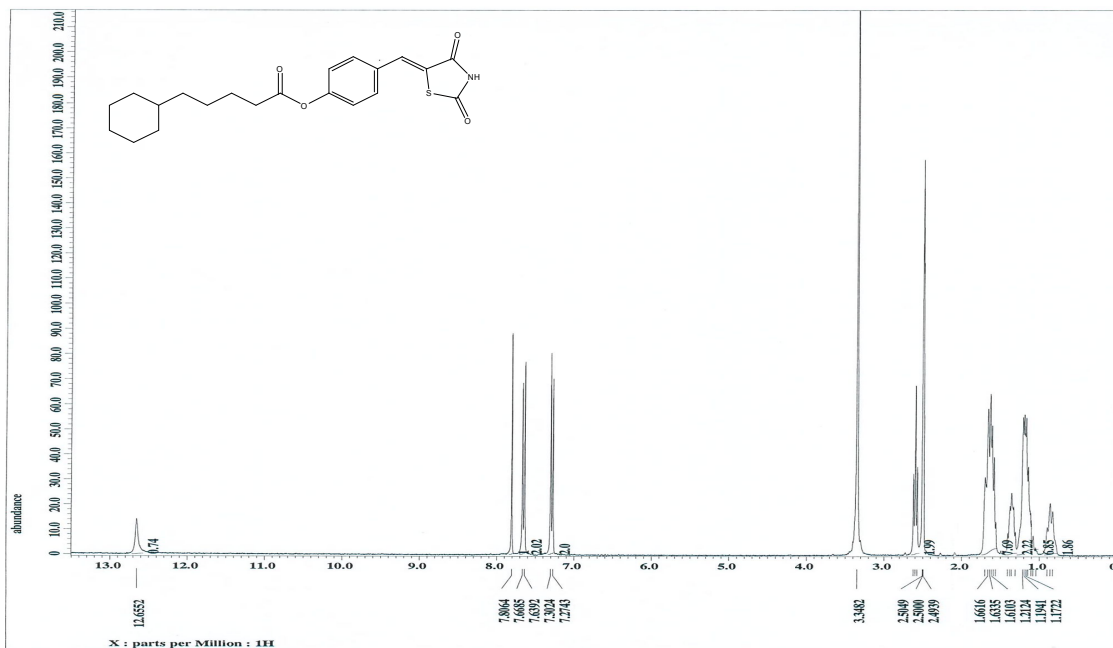
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate (7)



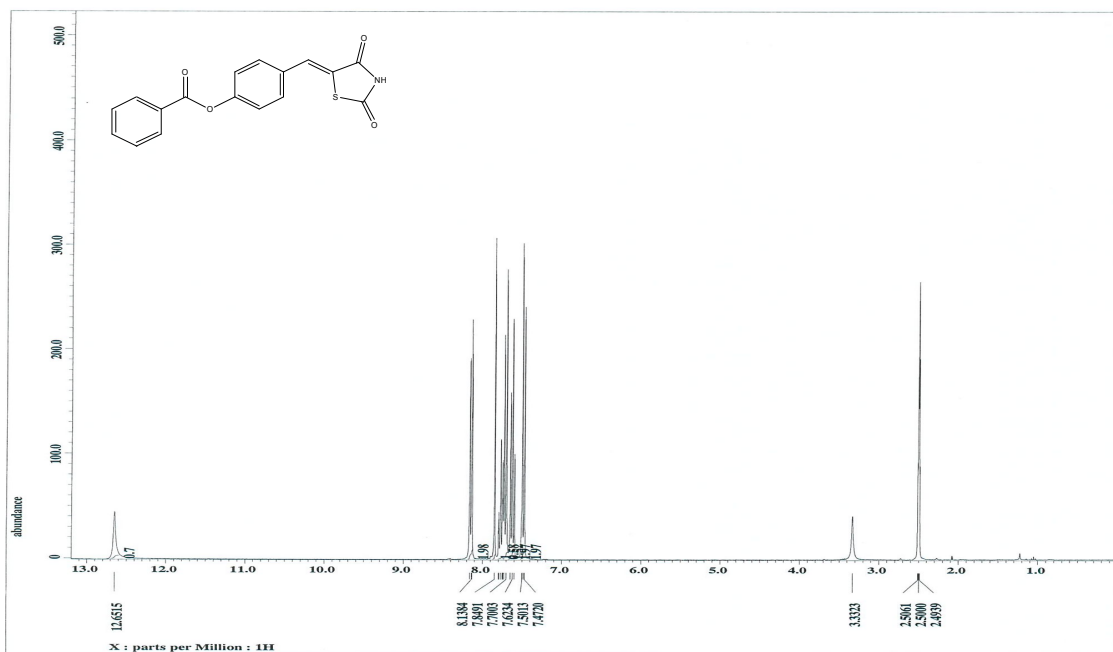
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (8)



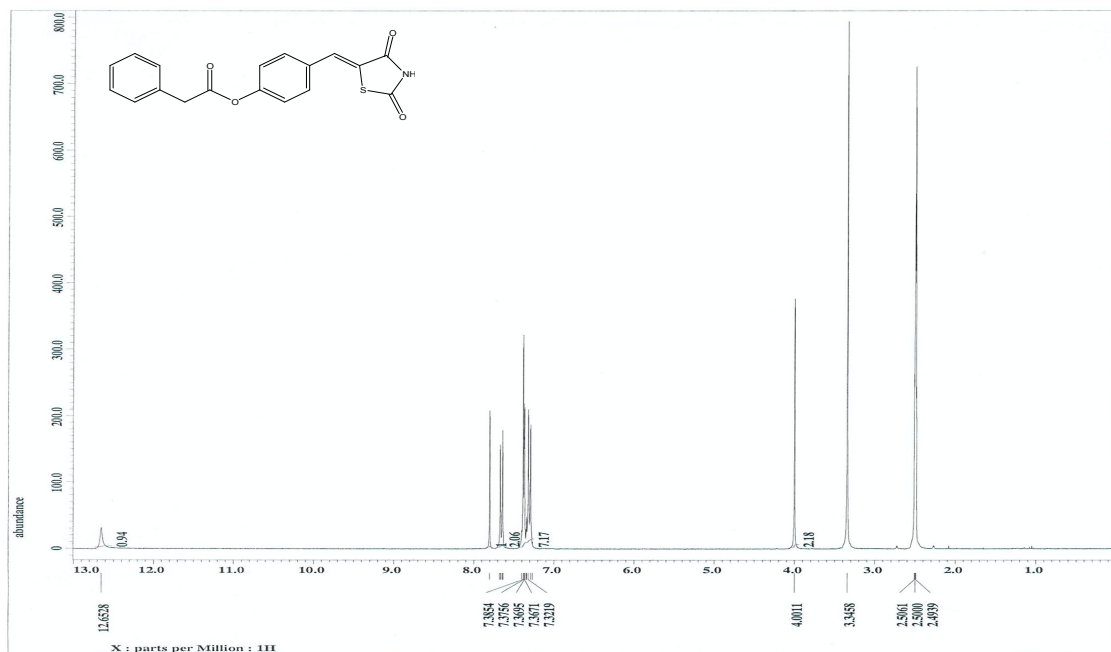
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (9)



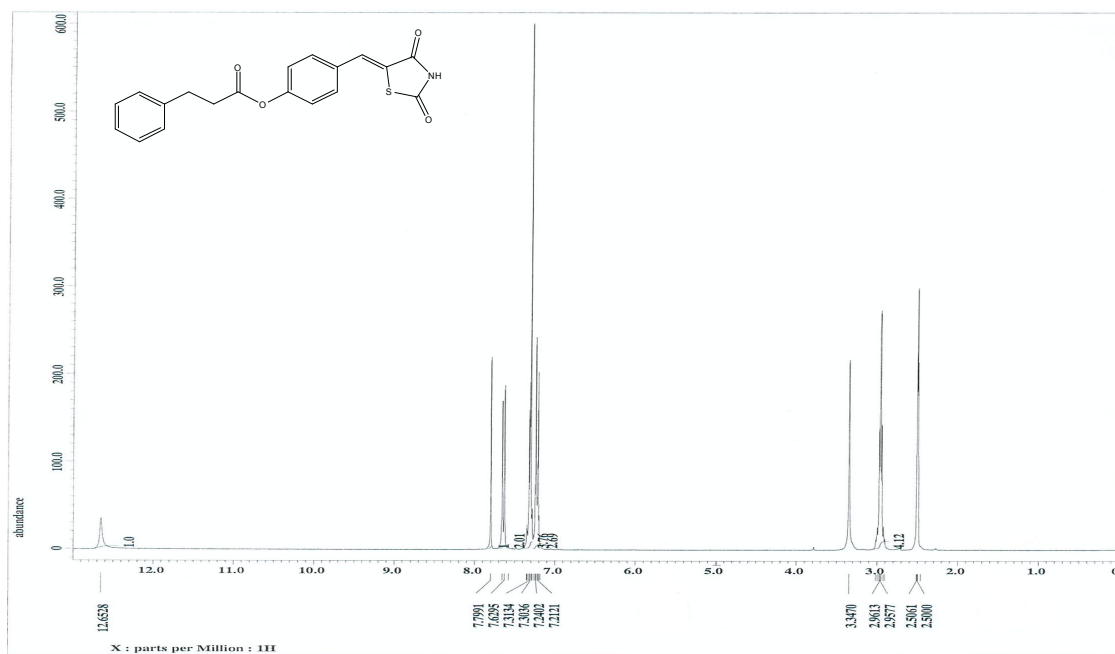
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexylpentanoate (10)



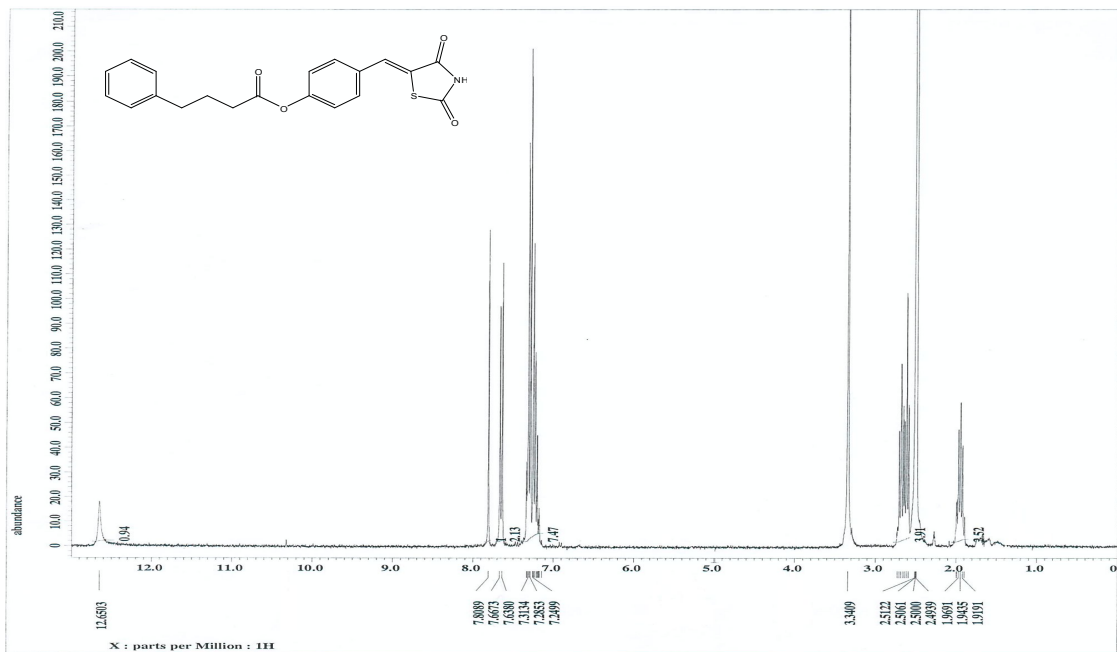
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (11)



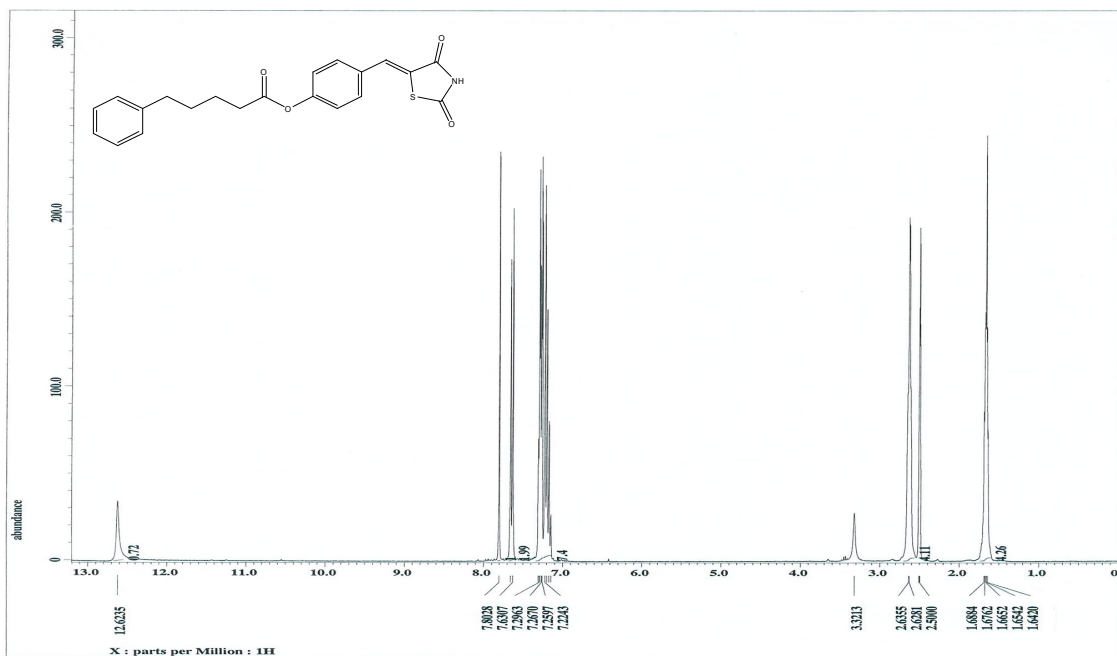
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (12)



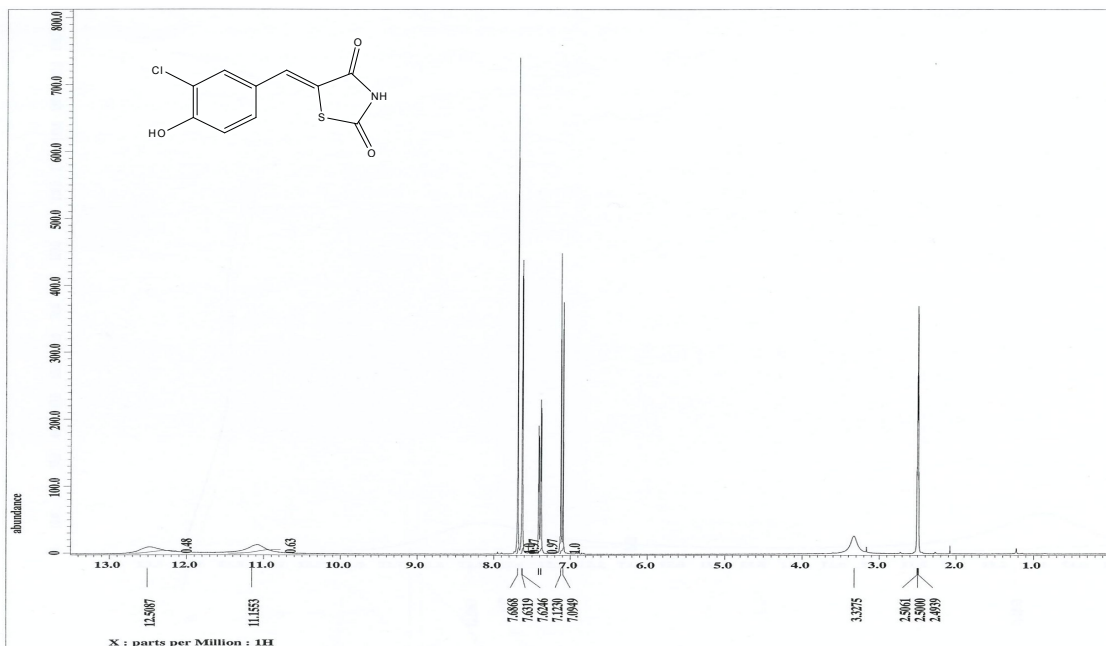
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (13)



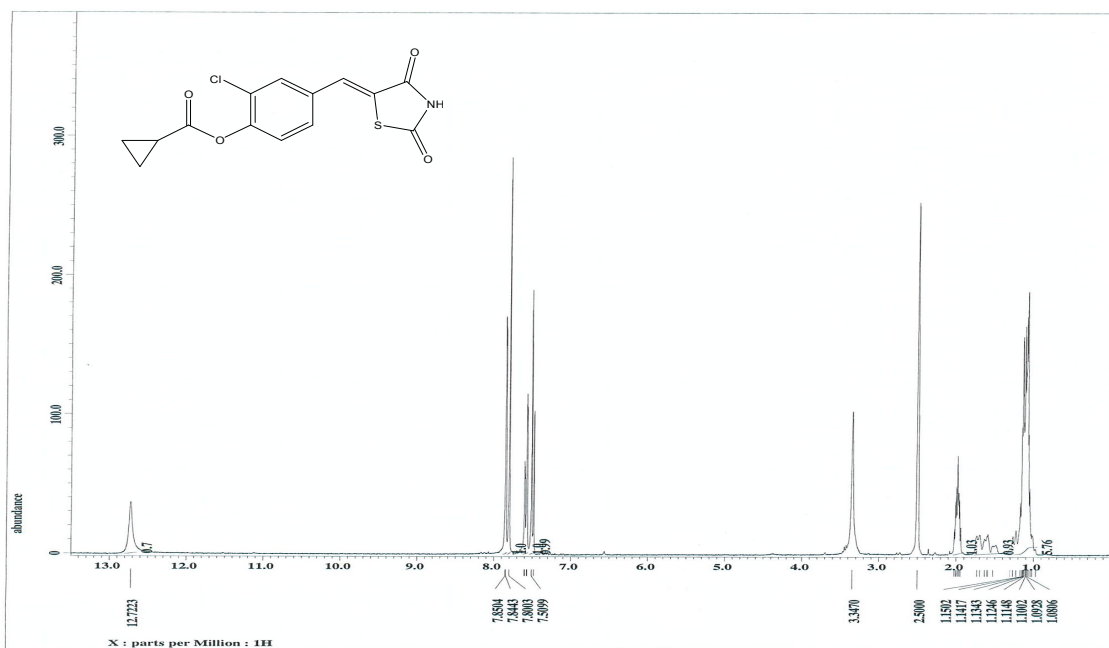
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (14)



(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (15)



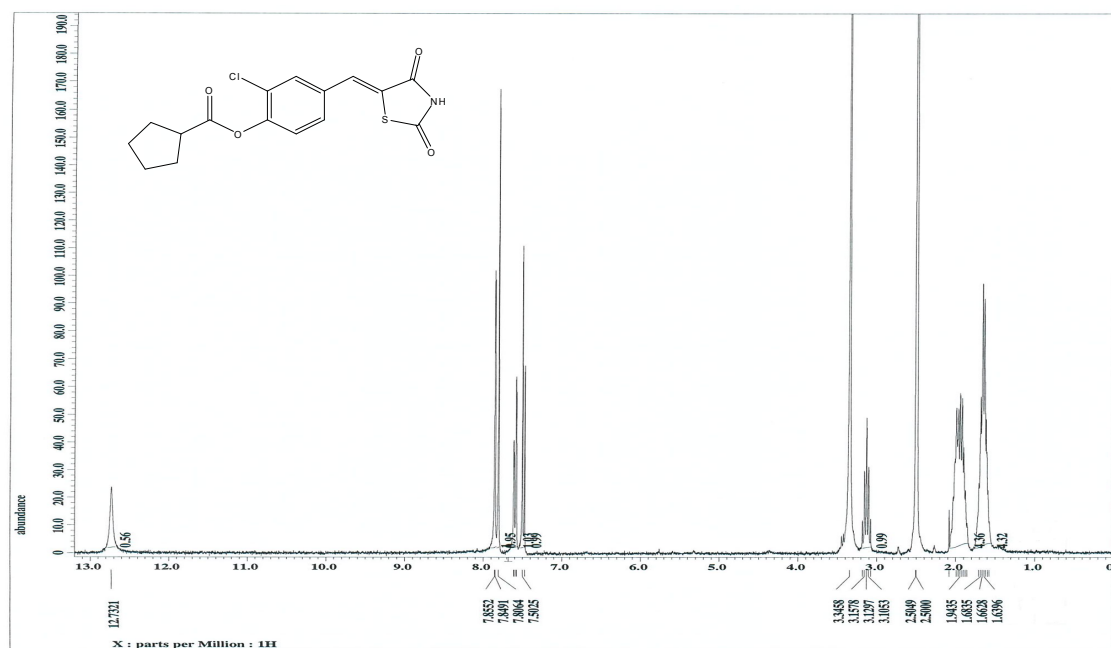
5-(3-Chloro-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (16a-30a)



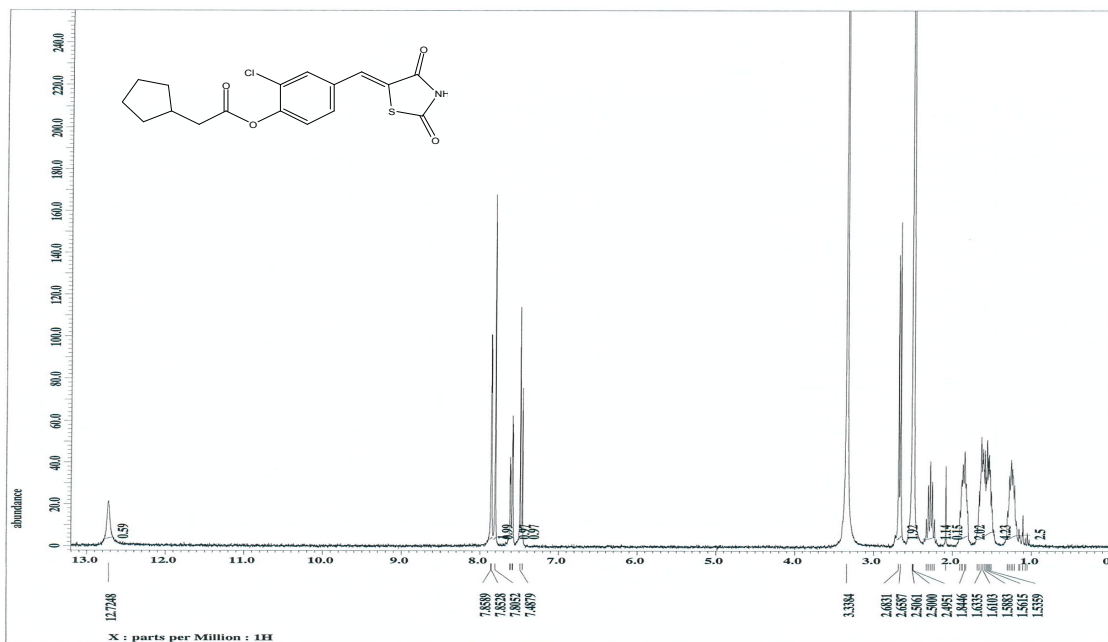
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopropane-carboxylate (16)



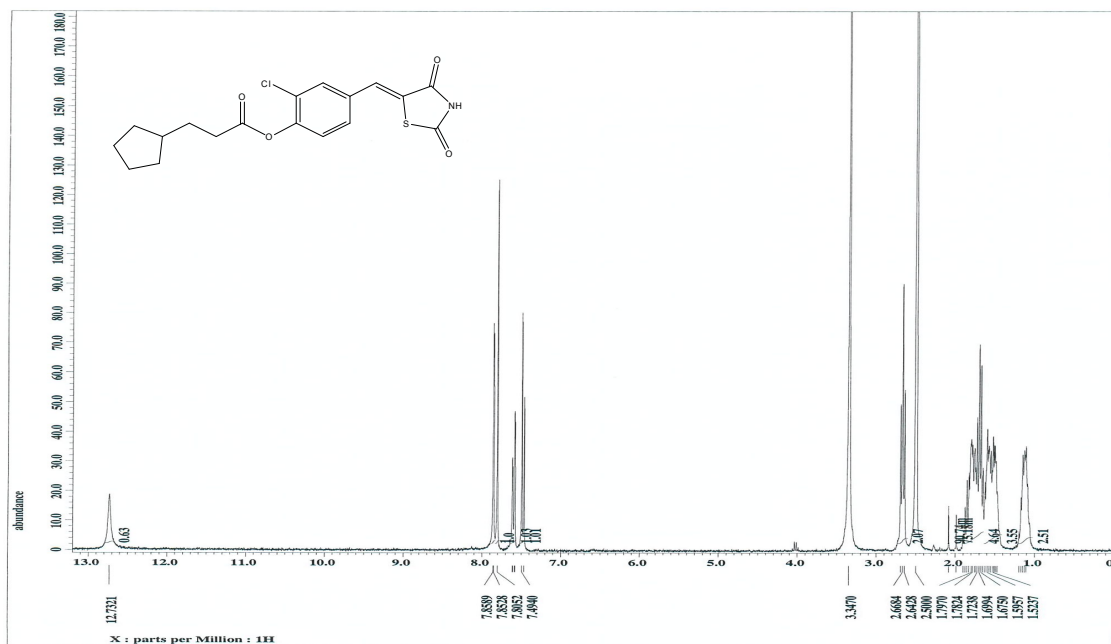
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane-carboxylate (17)



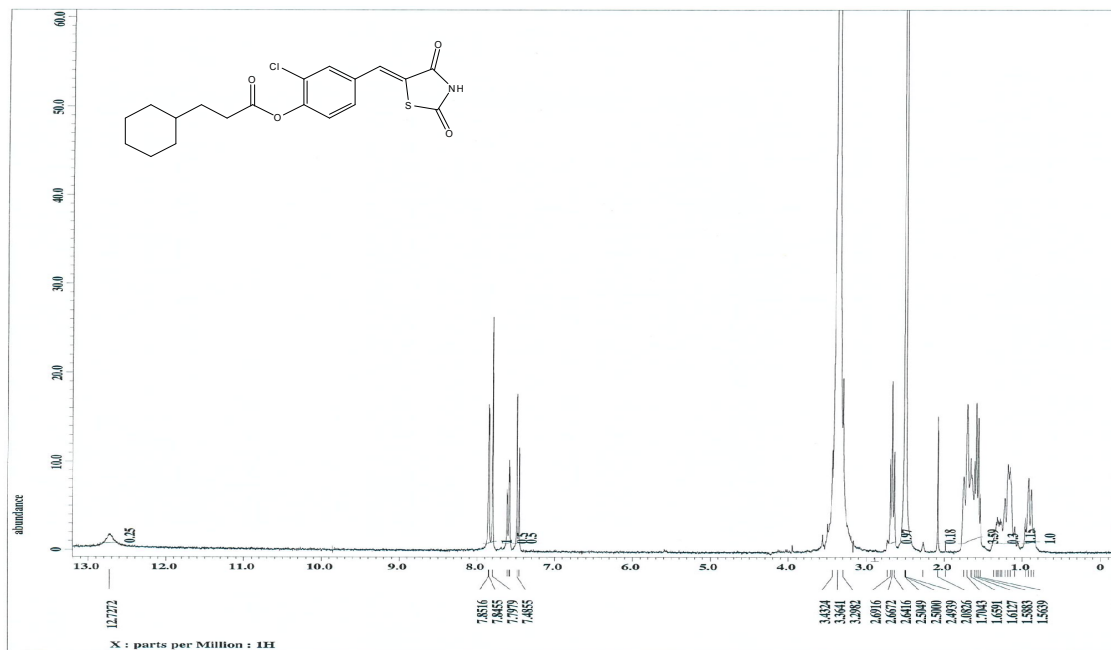
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-carboxylate (18)



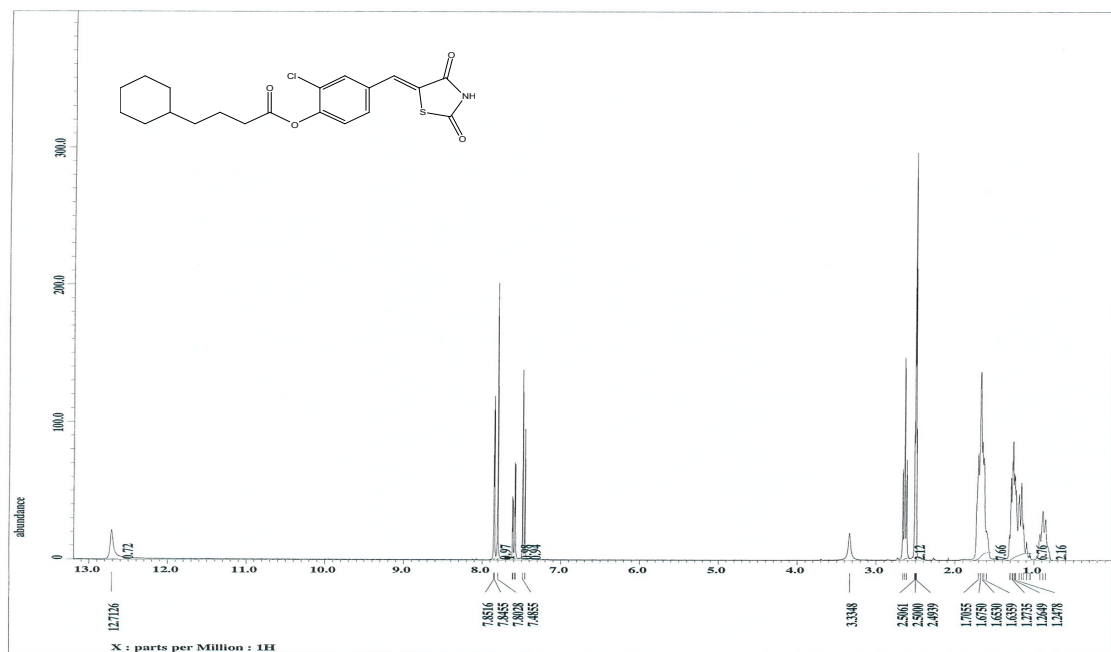
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (19)



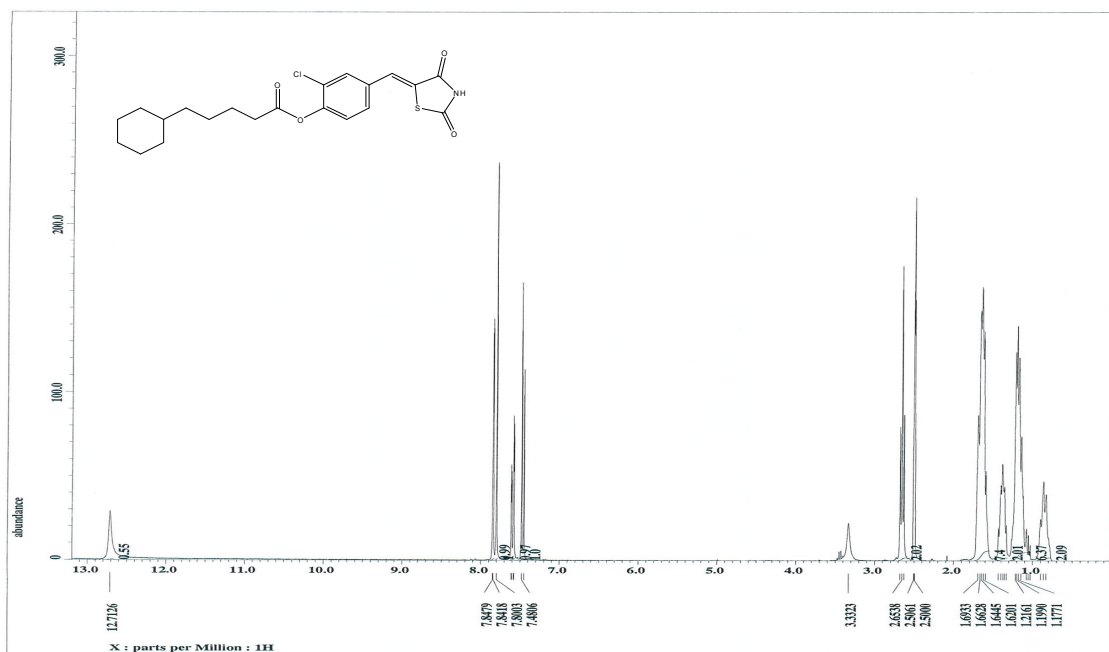
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (20)



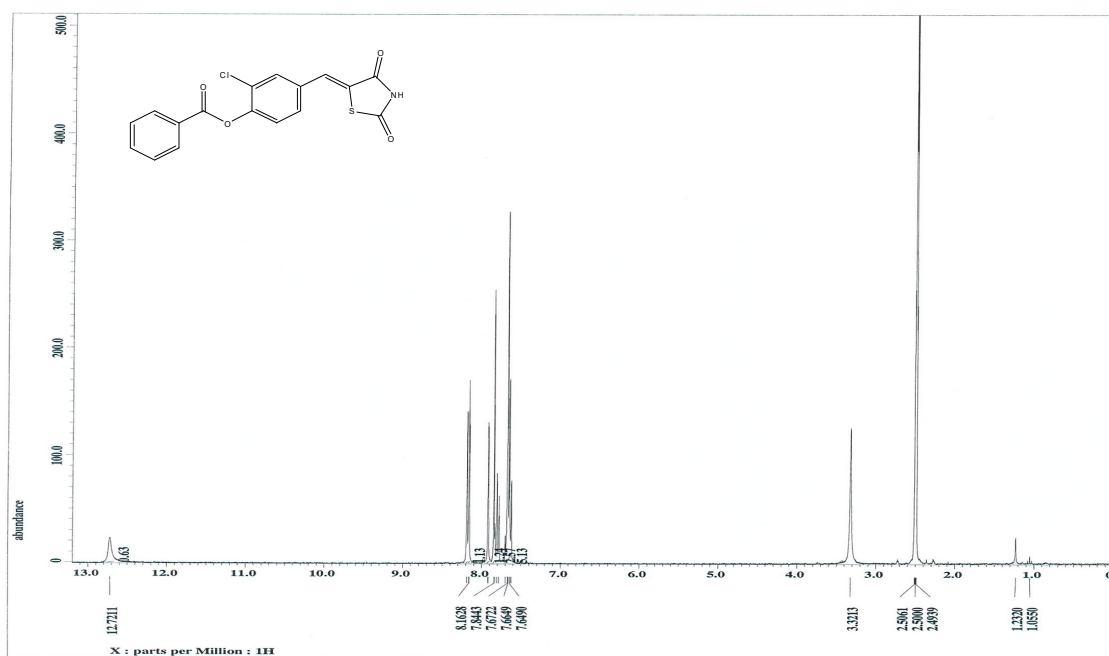
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (23)



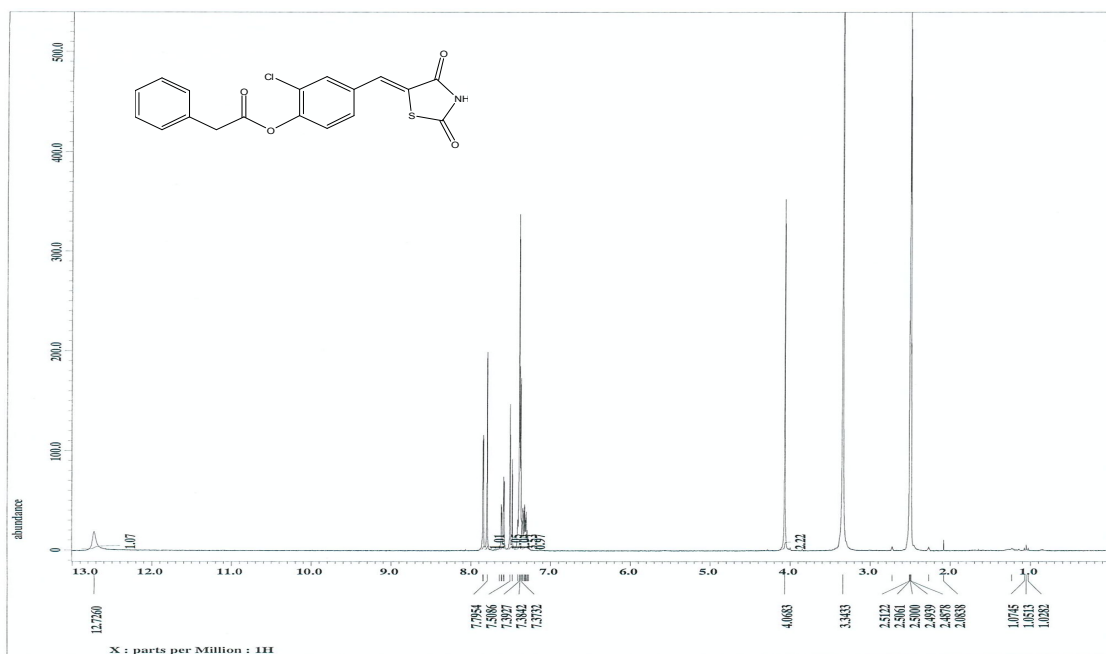
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (24)



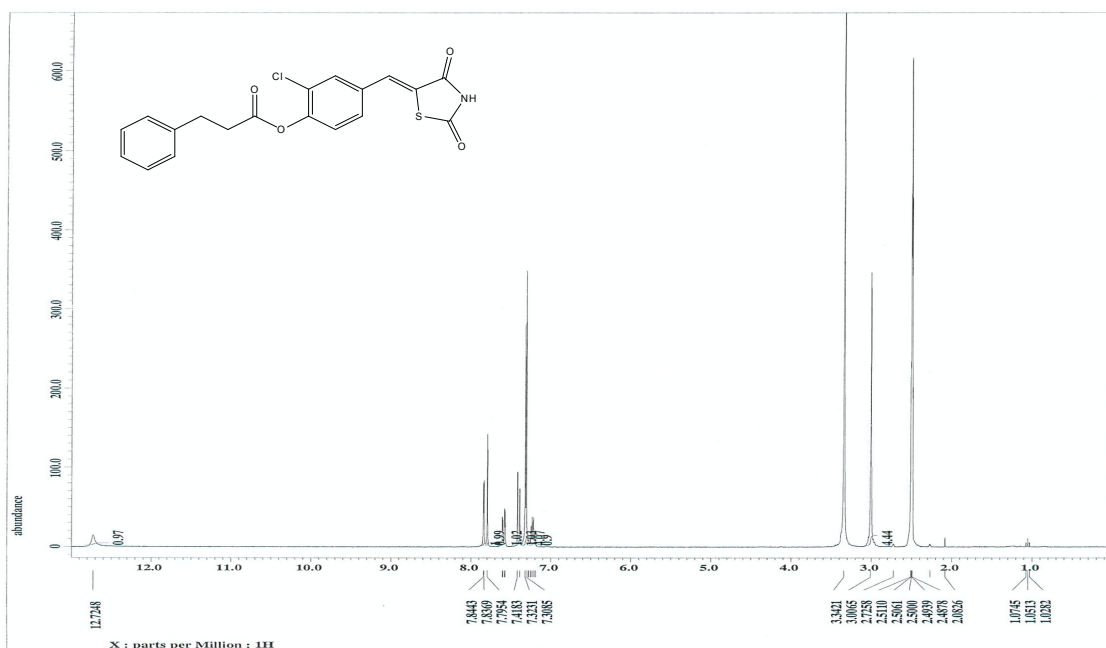
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl-pentanoate (25)



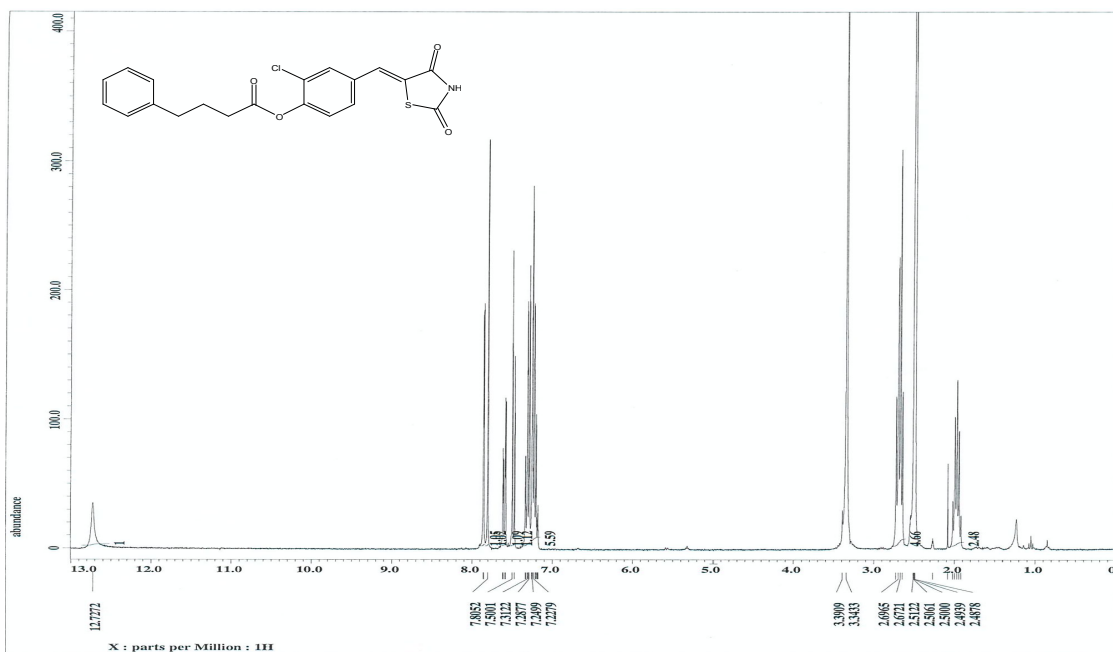
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (26)



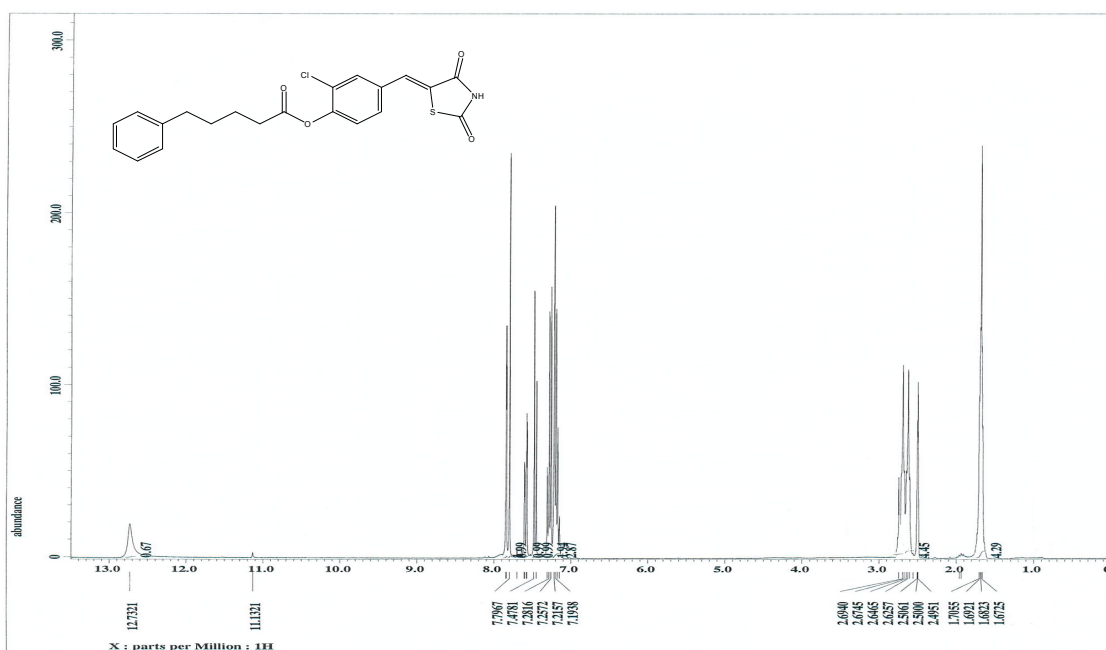
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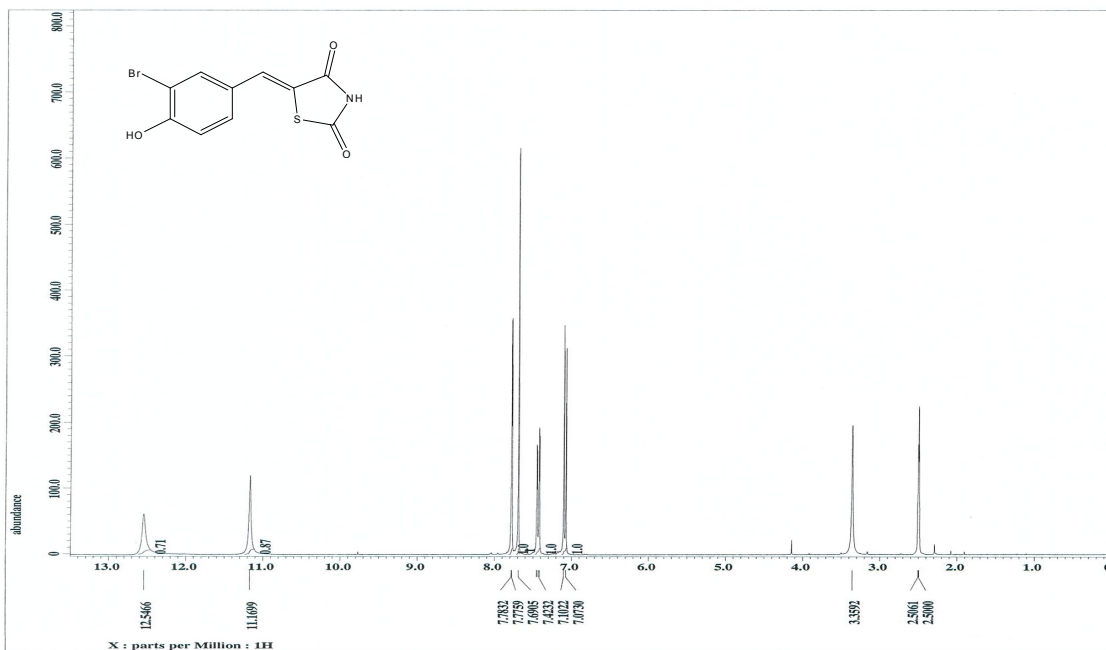
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (28)



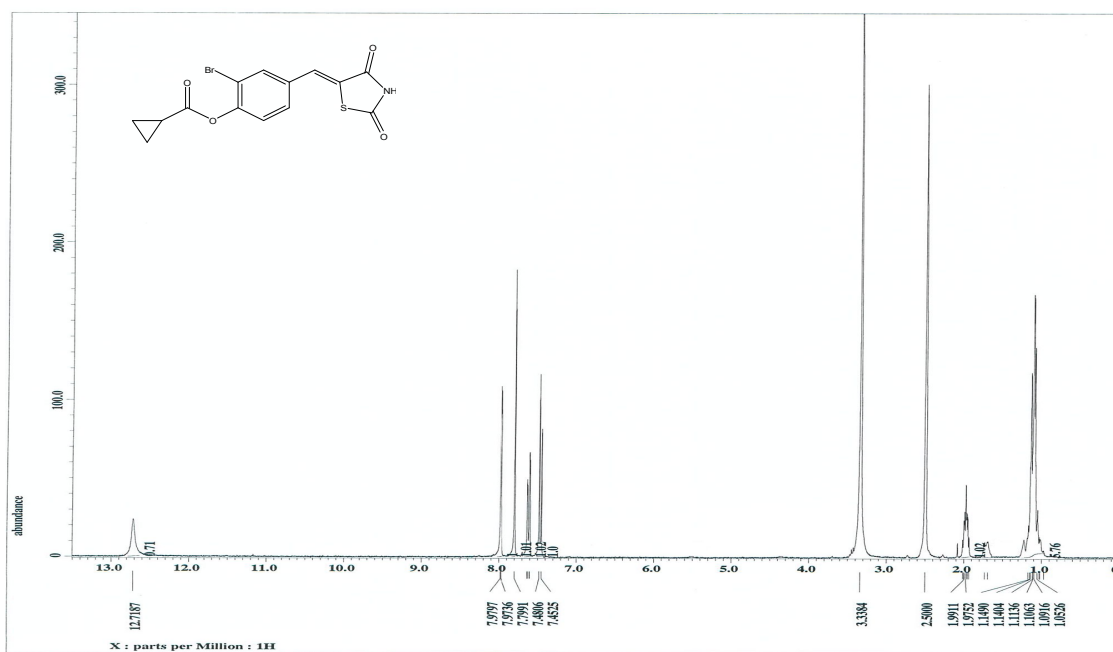
(Z)-2-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (29)



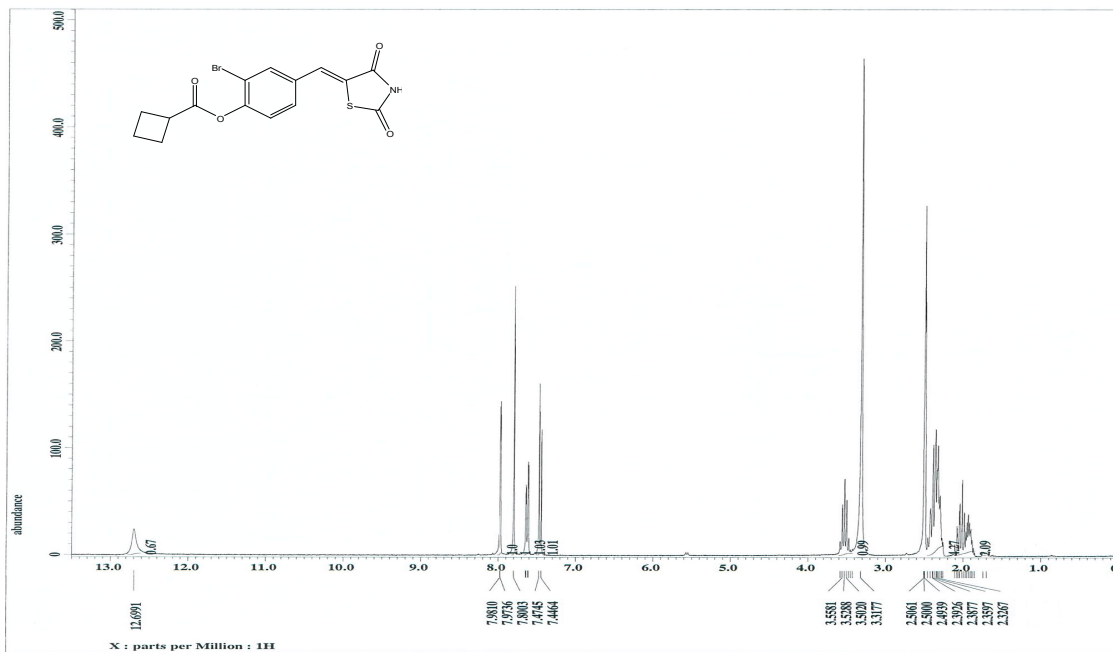
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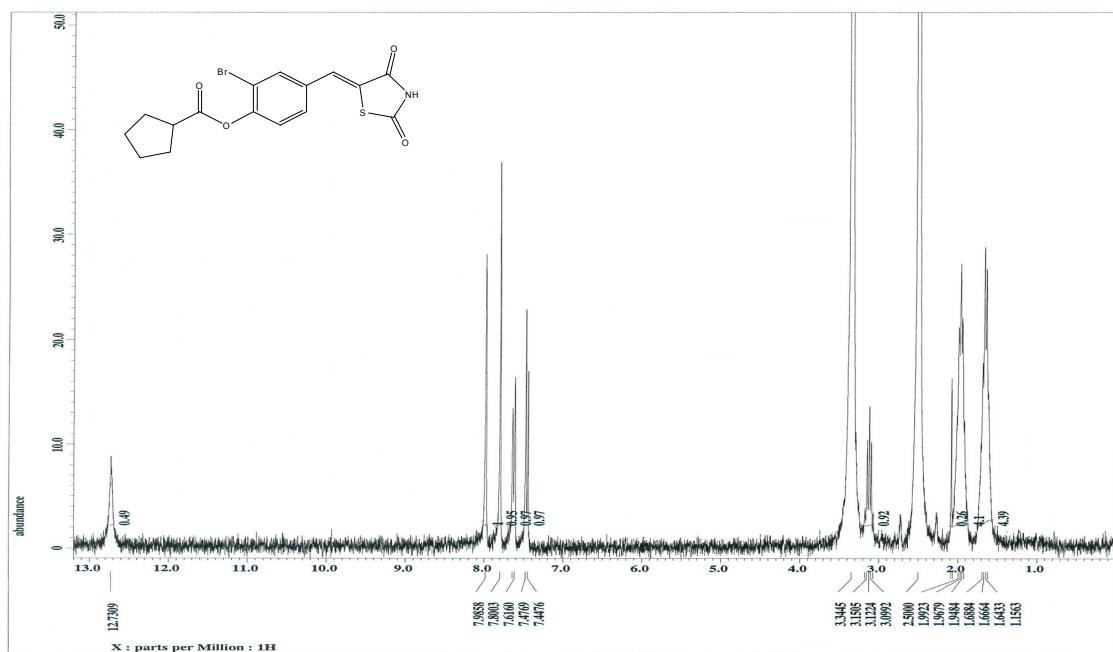
5-(3-Bromo-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (31a-45a)



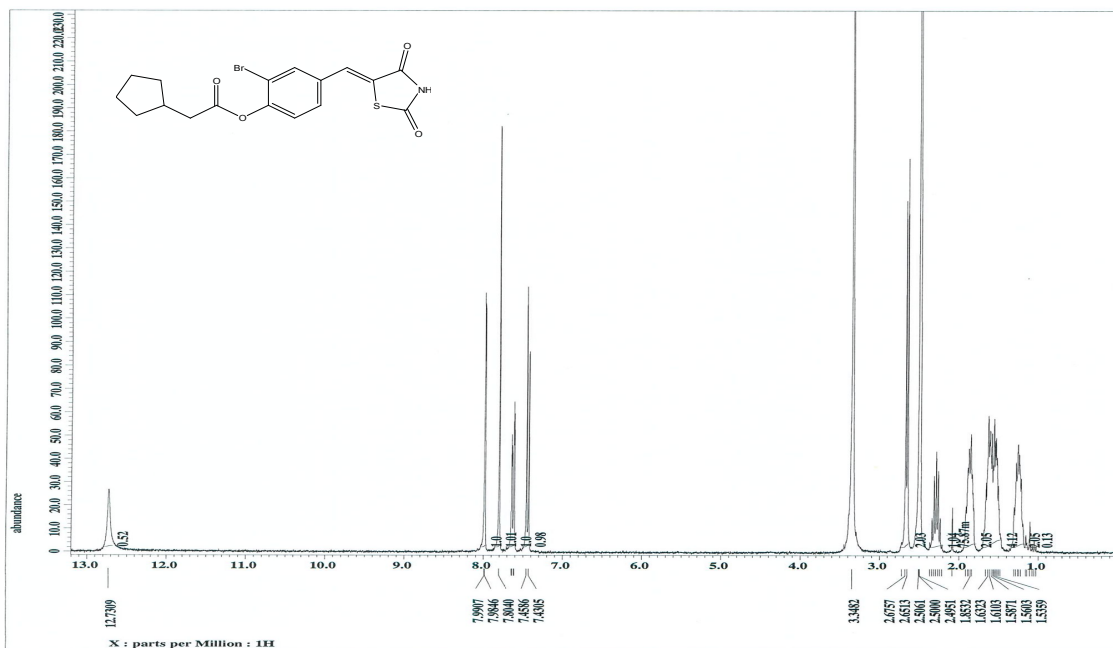
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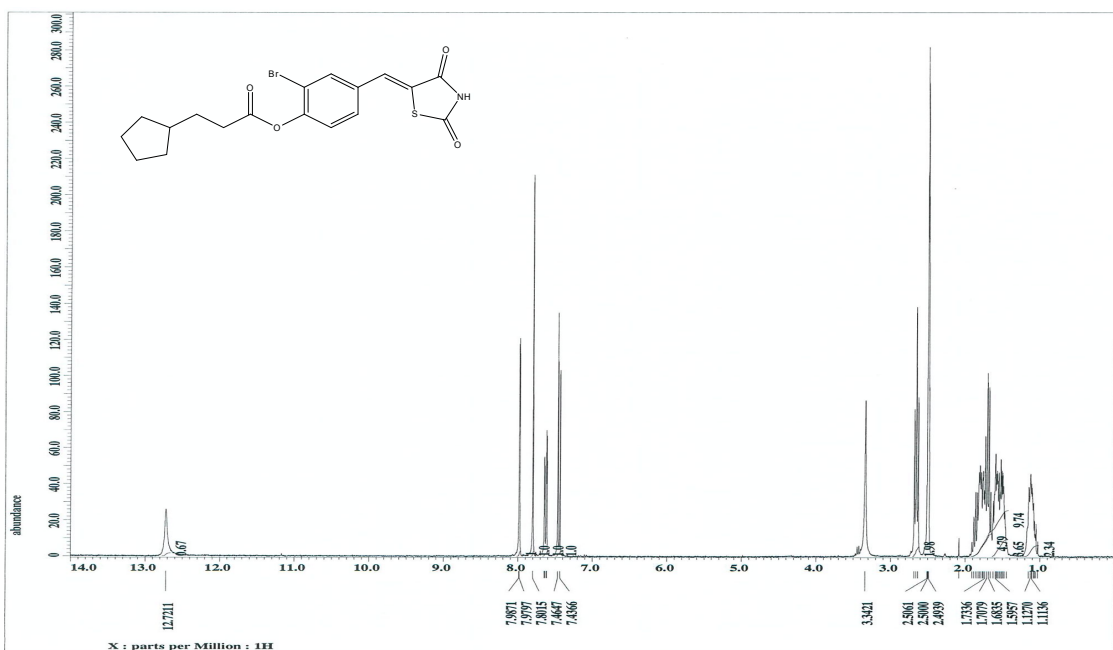
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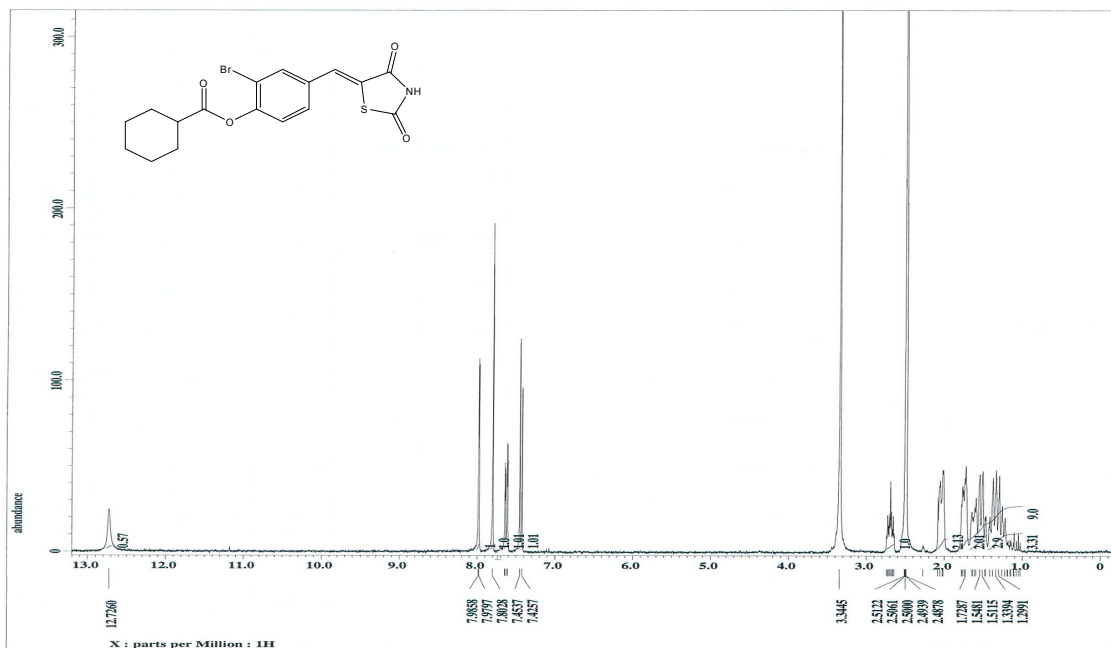
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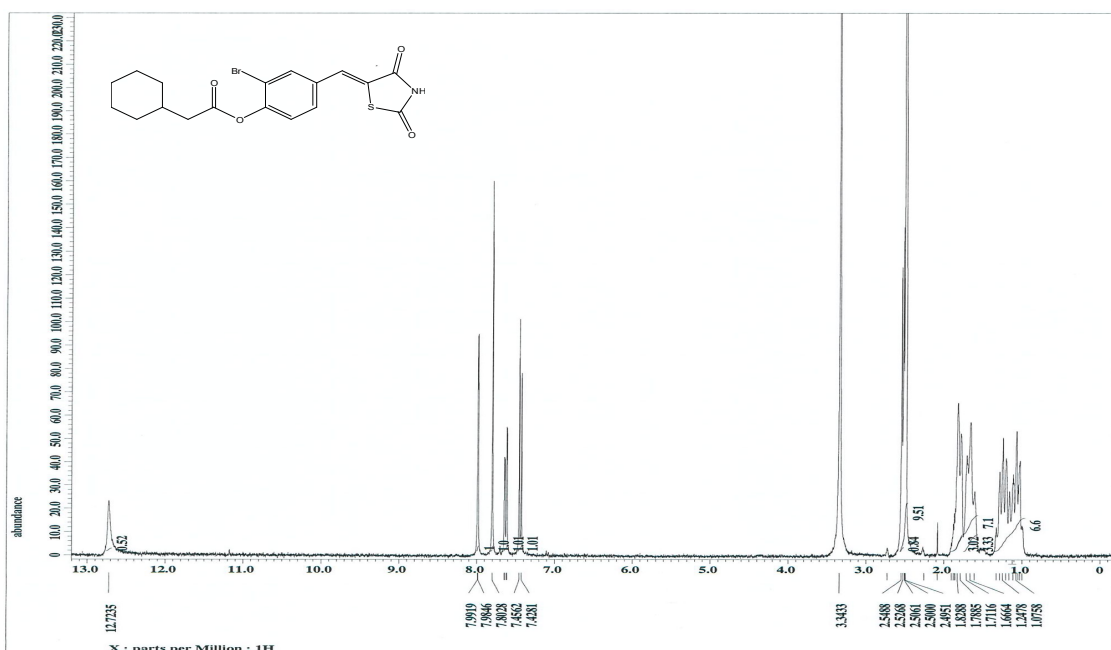
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (34)



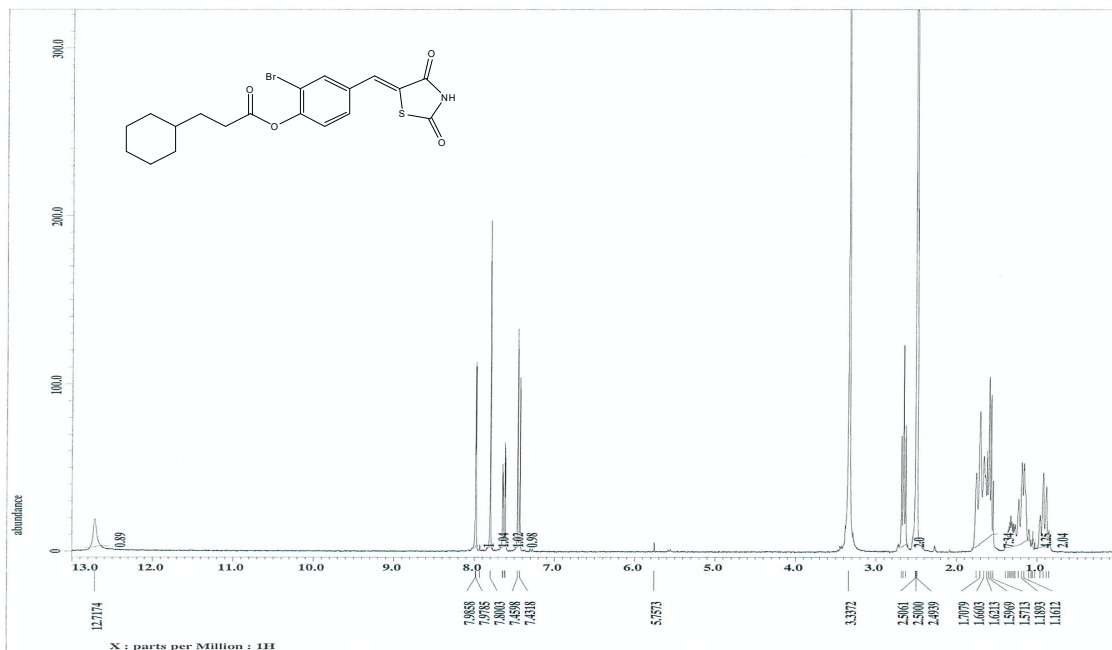
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (35)



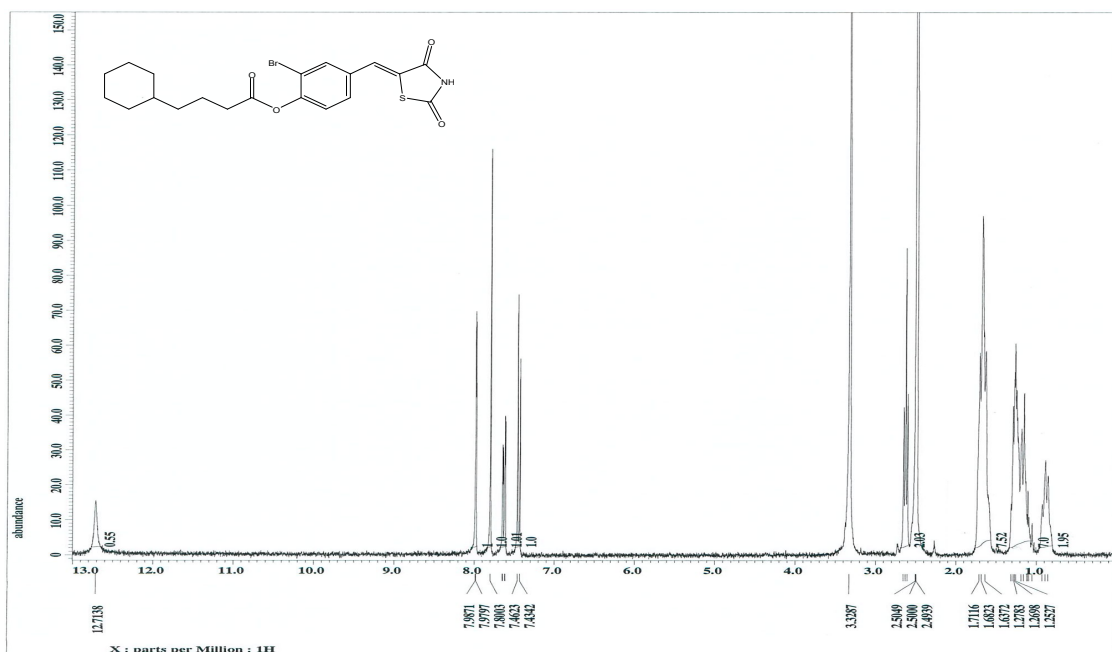
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane-carboxylate (36)



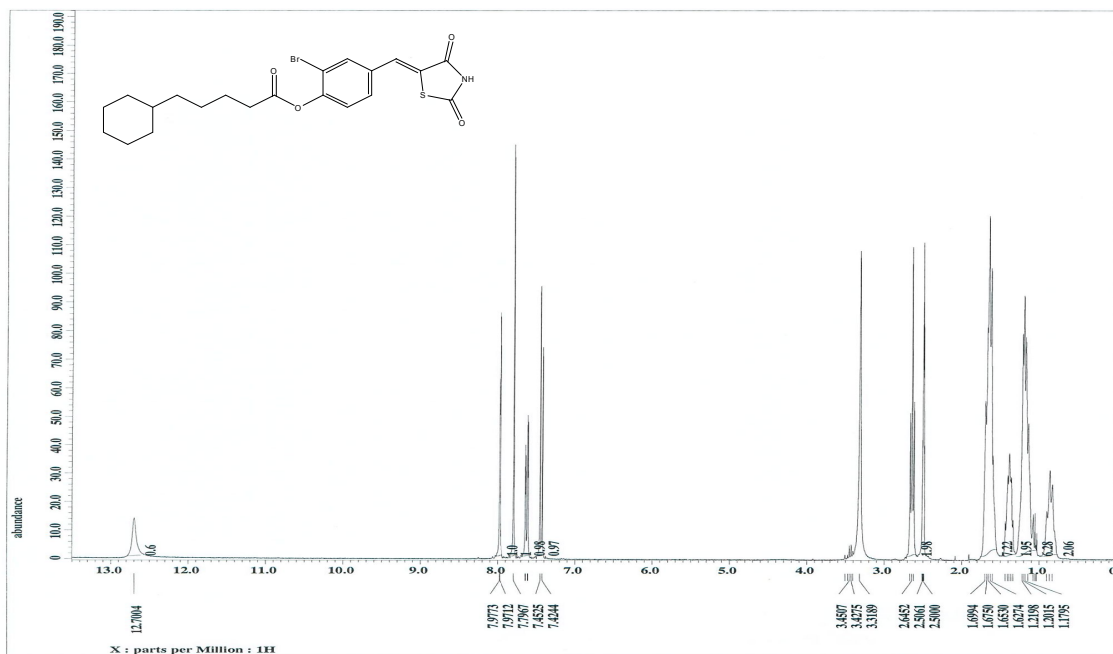
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexylacetate (37)



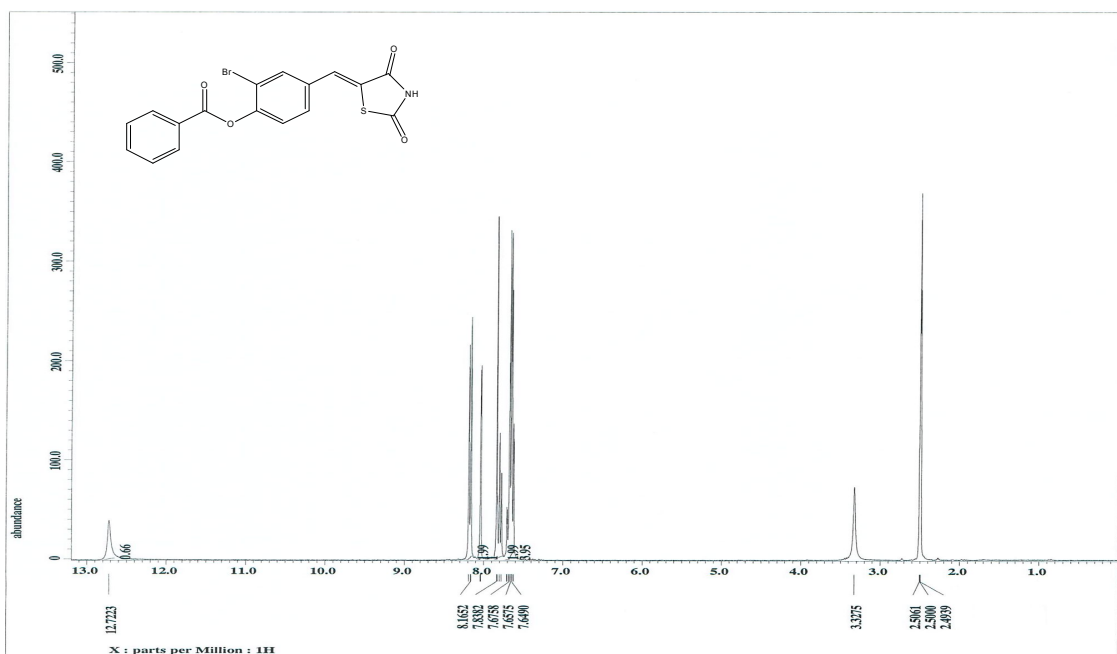
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexyl
 -propanoate (38)



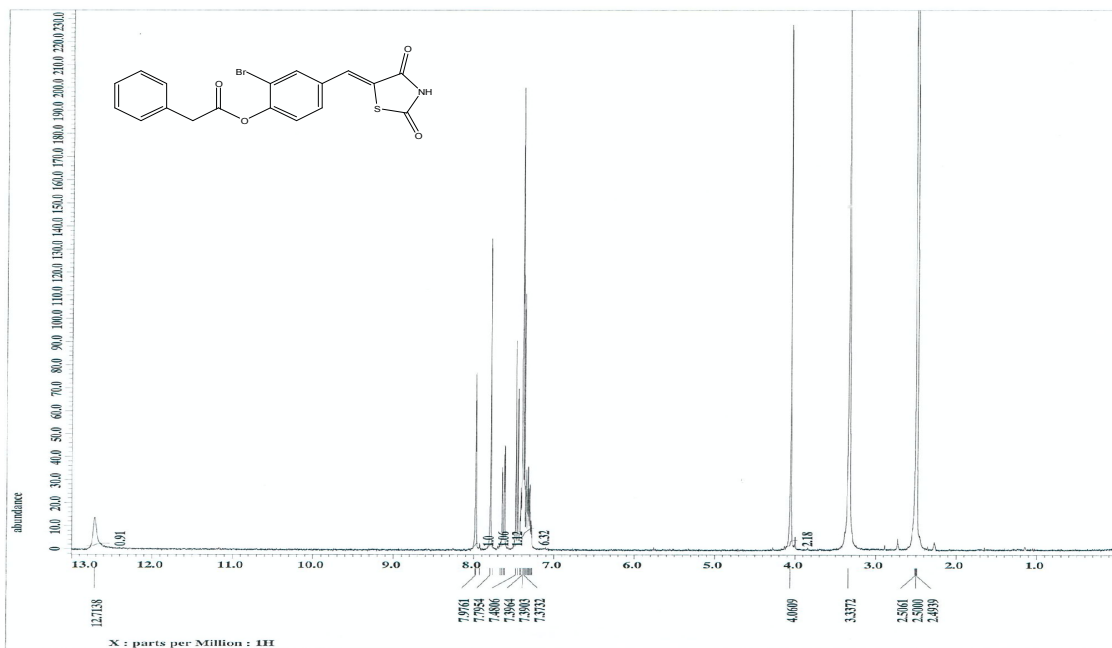
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexyl
 -butanoate (39)



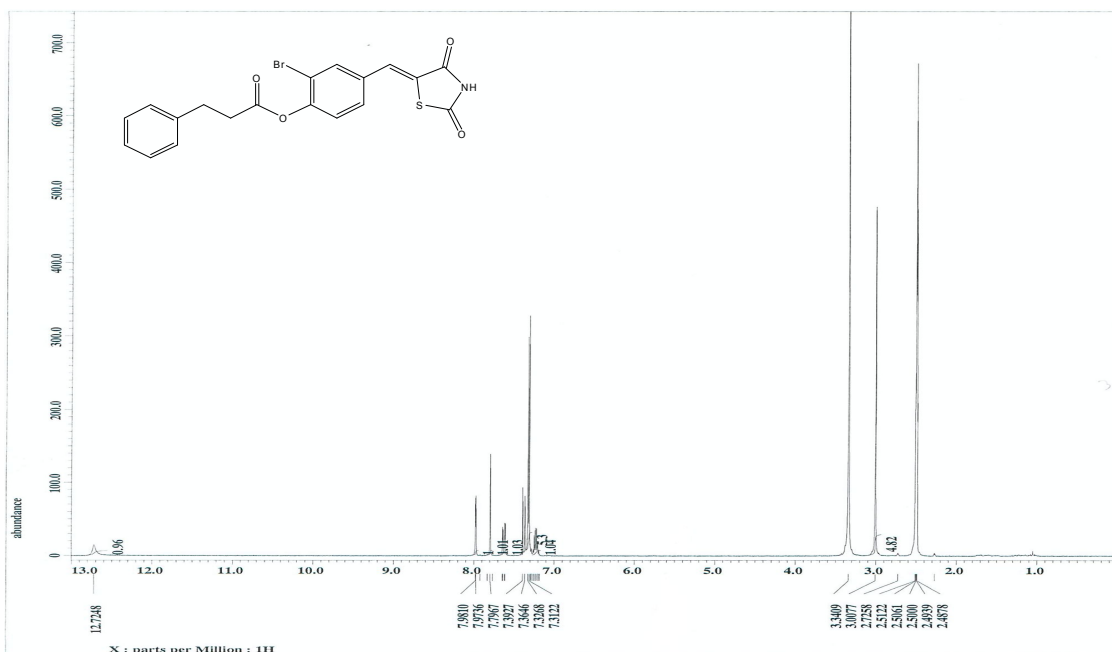
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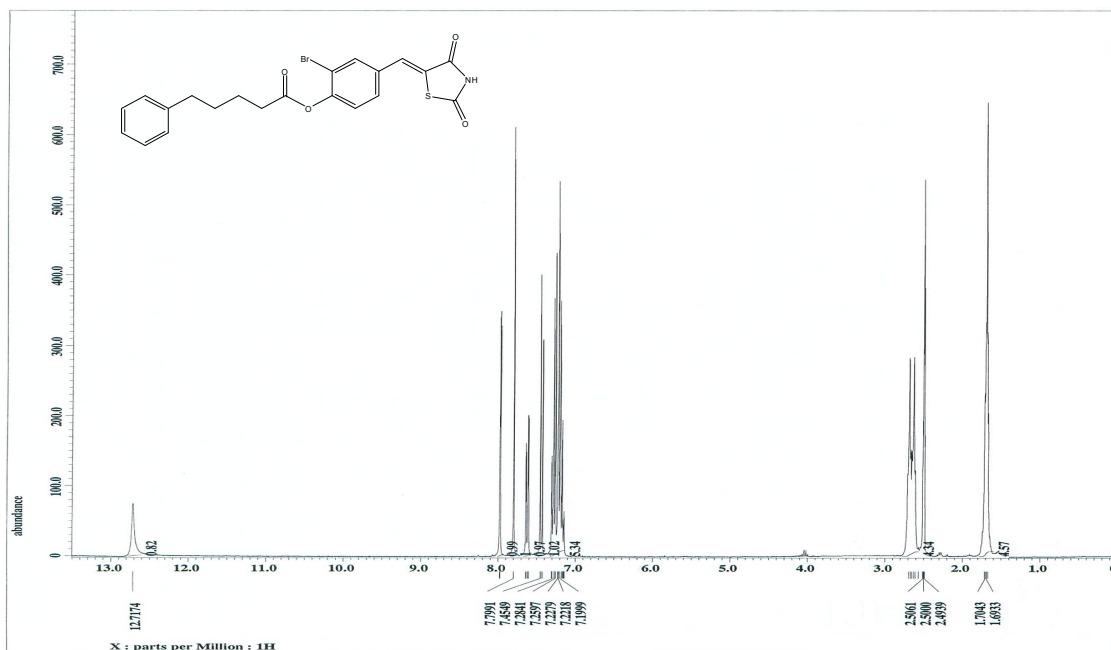
(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (41)

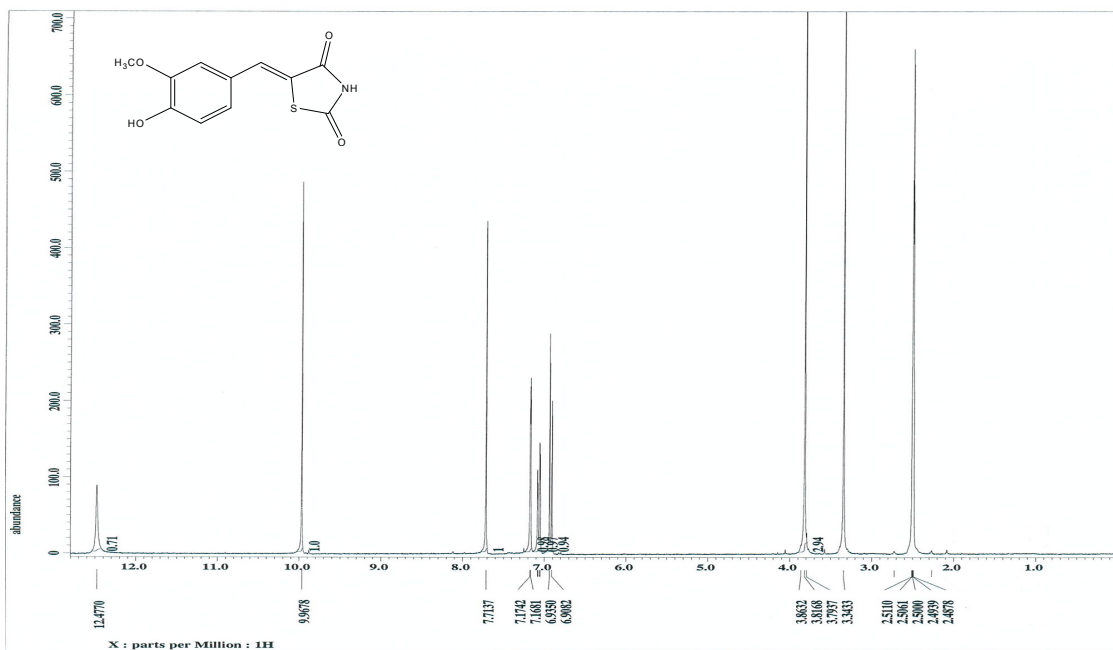


(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate
(42)

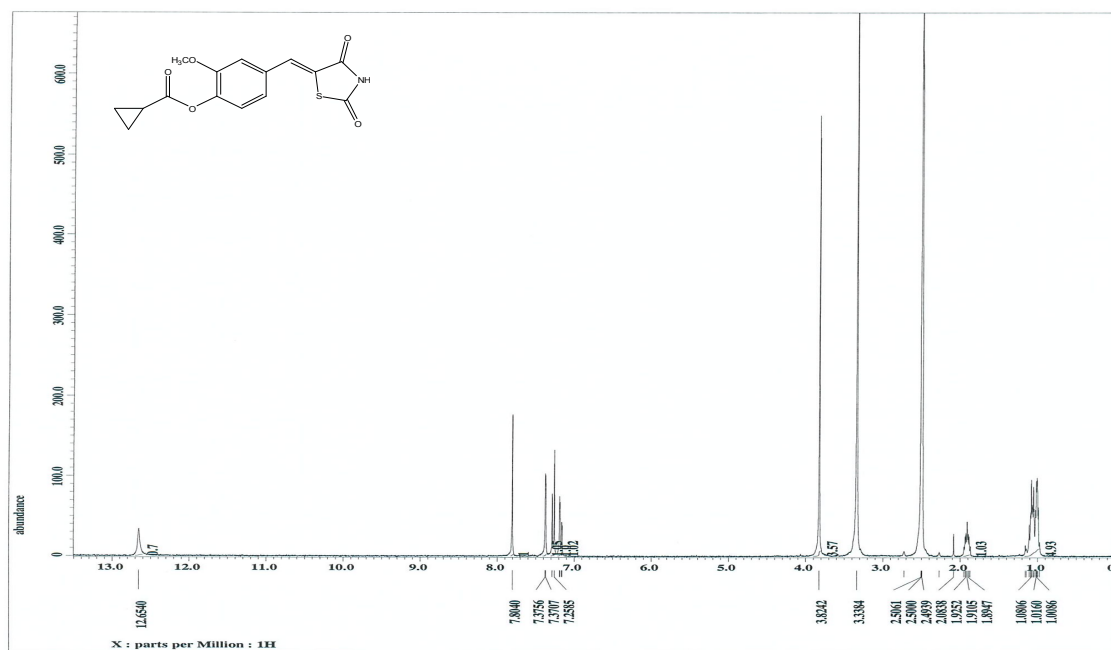


(Z)-2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate
(43)

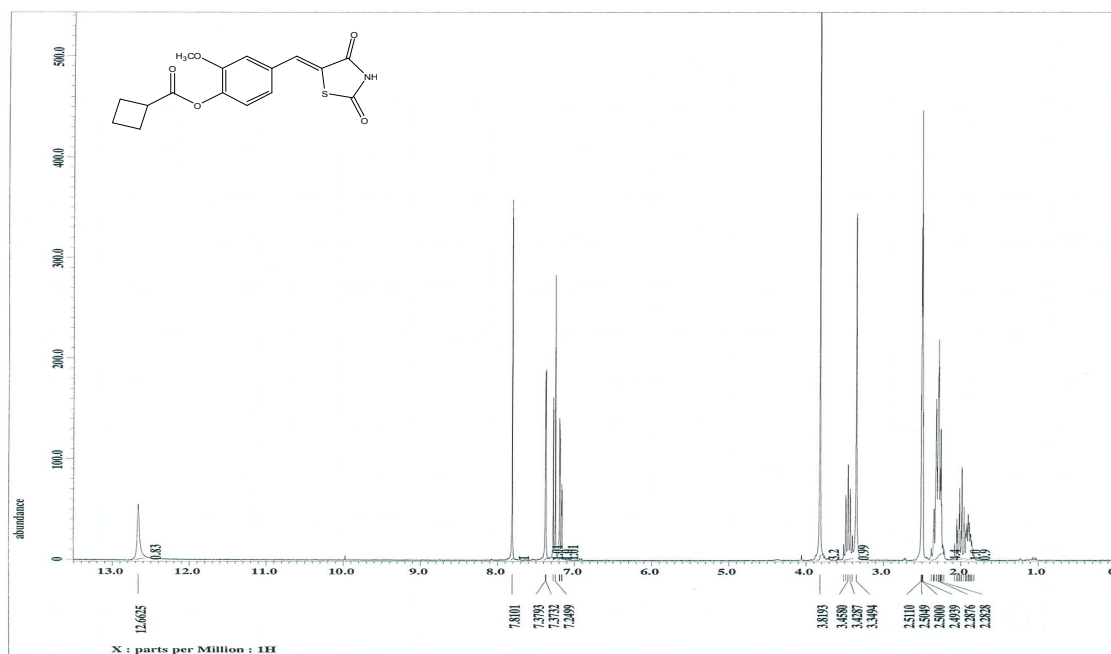




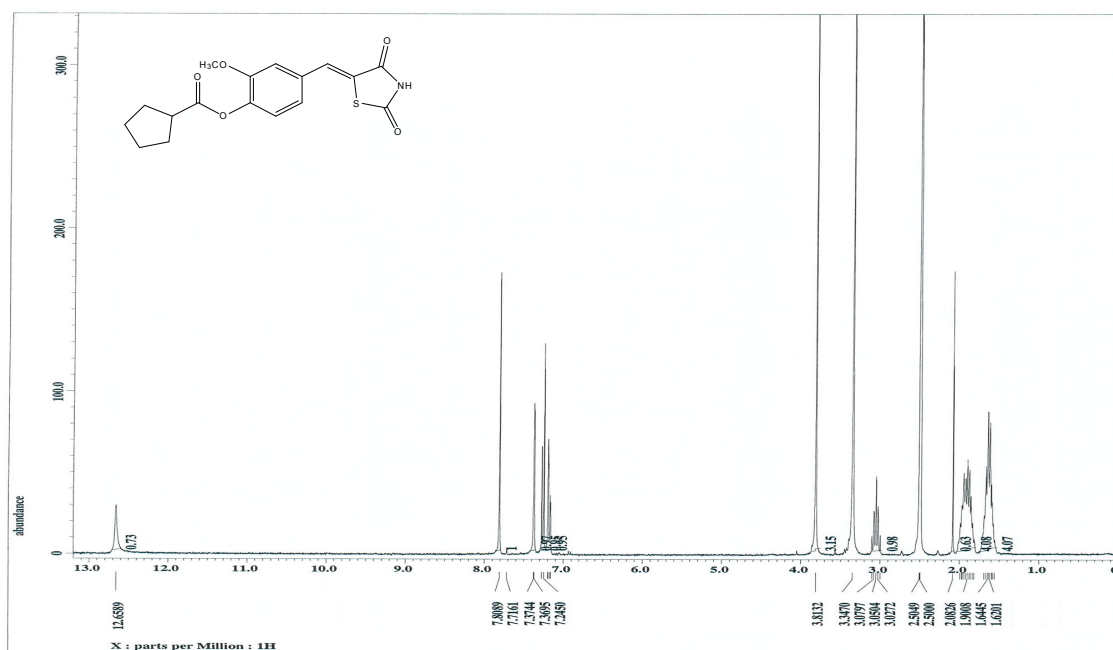
5-(4-Hydroxy-3-methoxy-benzylidene)-thiazolidine-2,4-dione (46a-60a)



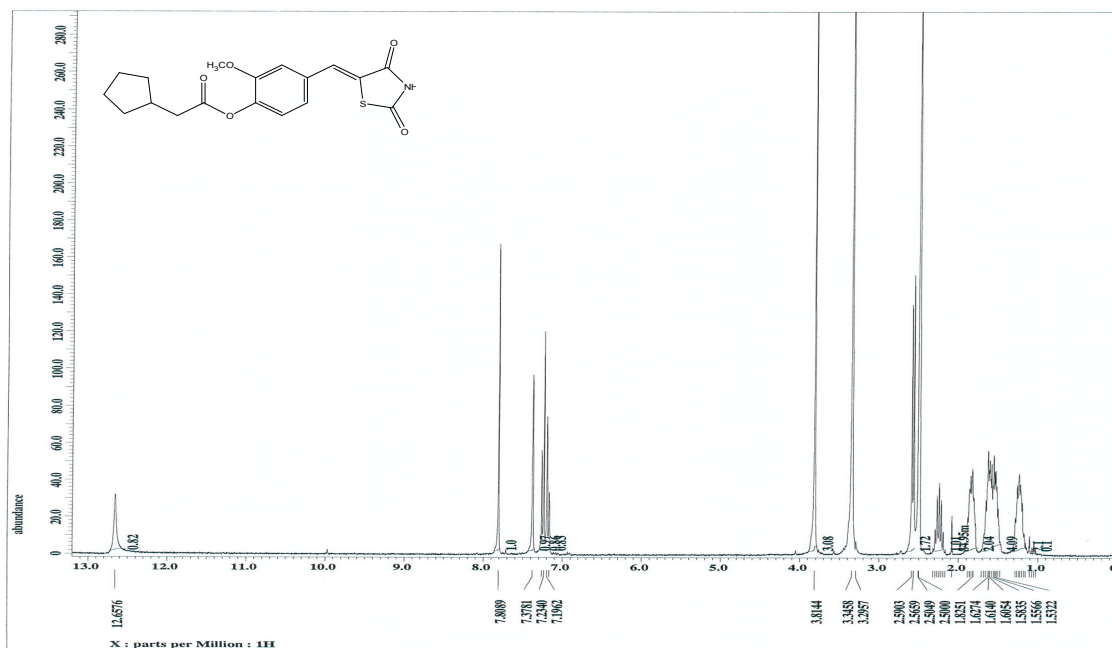
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclopropane-carboxylate (46)



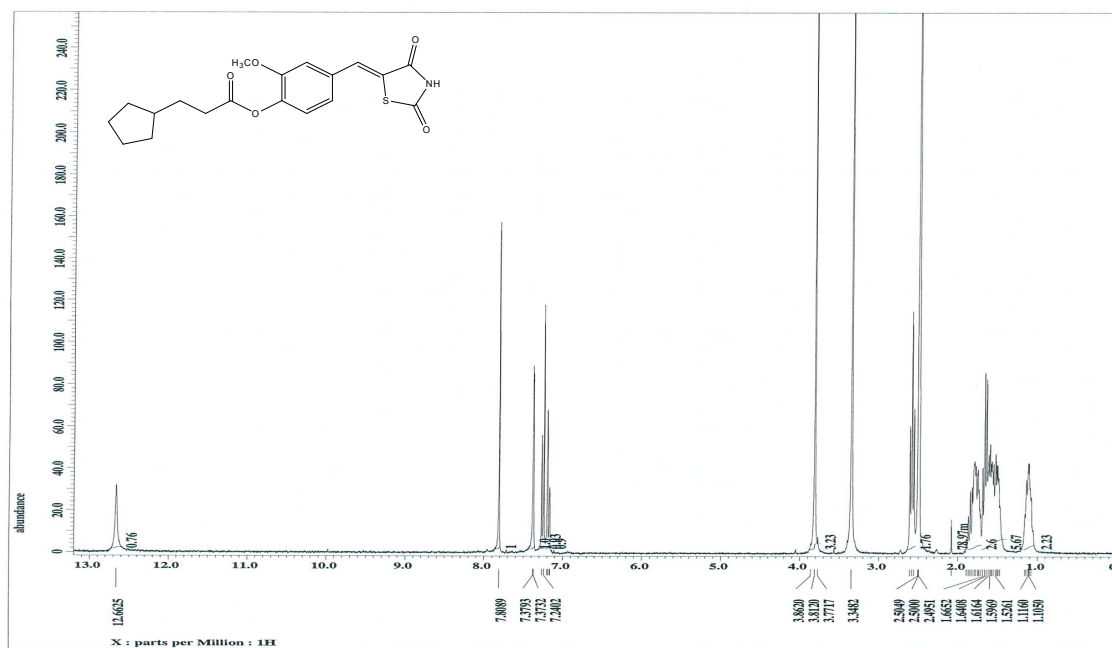
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclobutane-carboxylate (47)



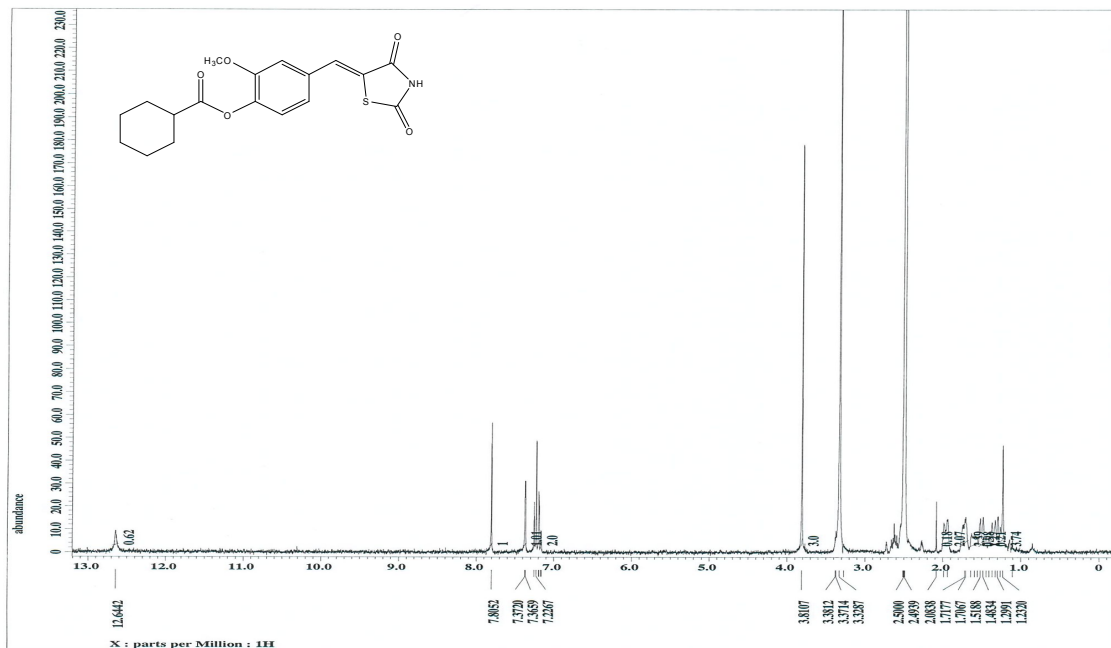
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclopentane-carboxylate (48)



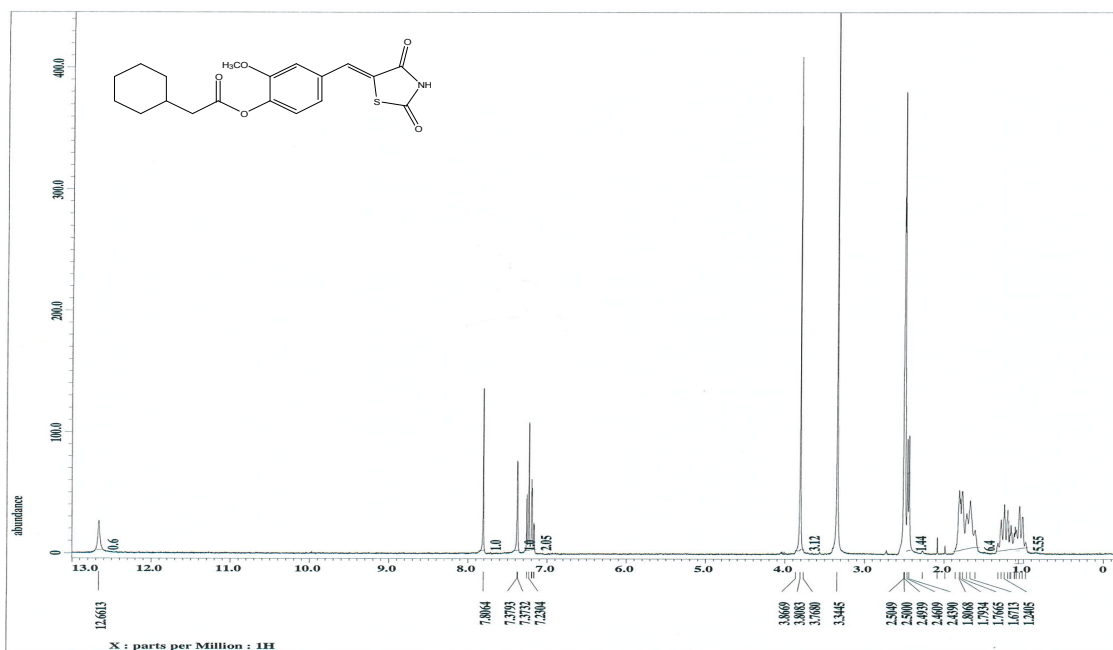
Z)-4-((2,4-dioxthiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-cyclopentyl -acetate (49)



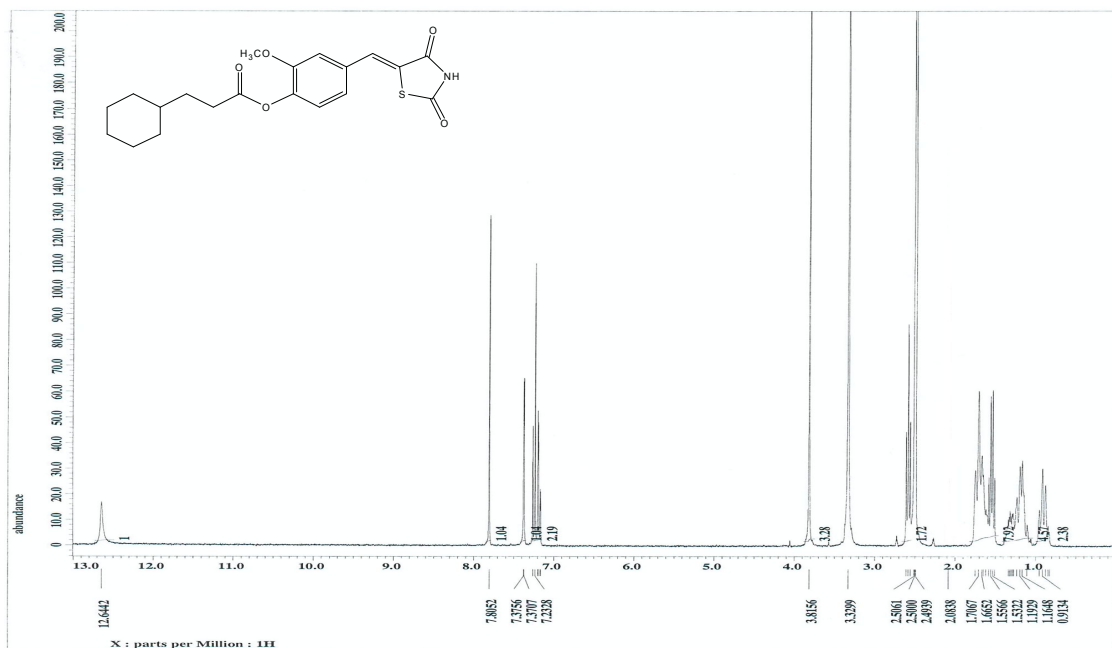
(Z)-4-((2,4-dioxthiazolidin-5-ylidene)methyl)-2-methoxyphenyl 3-cyclopentyl -propanoate (50)



(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl cyclohexane-carboxylate (51)



(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-cyclohexylacetate (52)



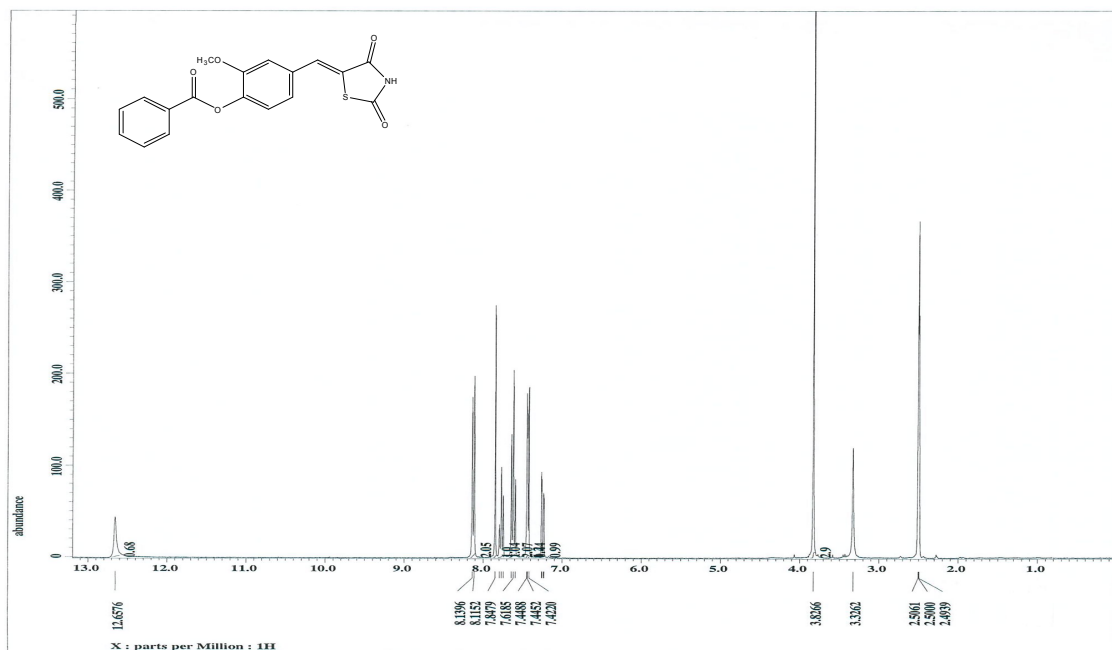
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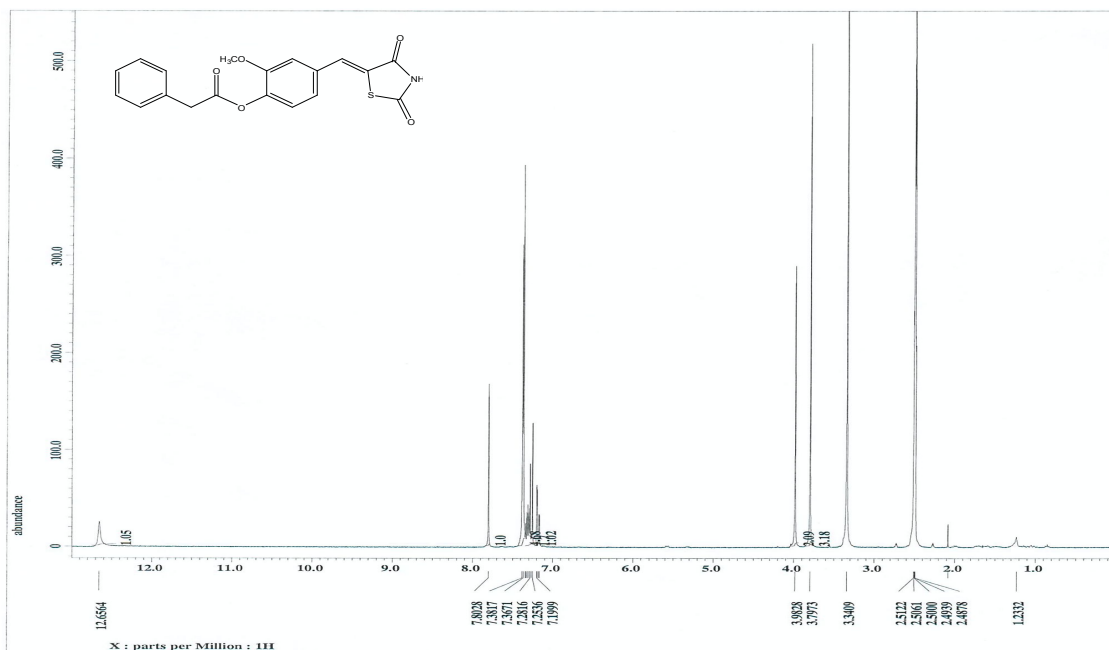
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-cyclohexylbutanoate (54)



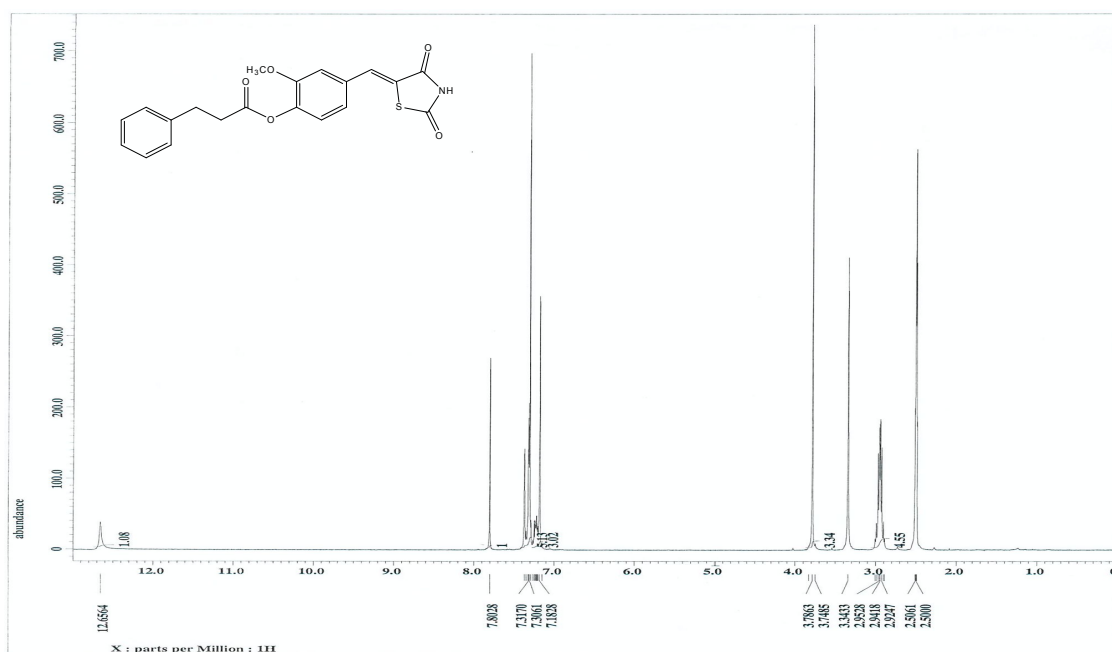
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 5-cyclohexyl-pentanoate (55)



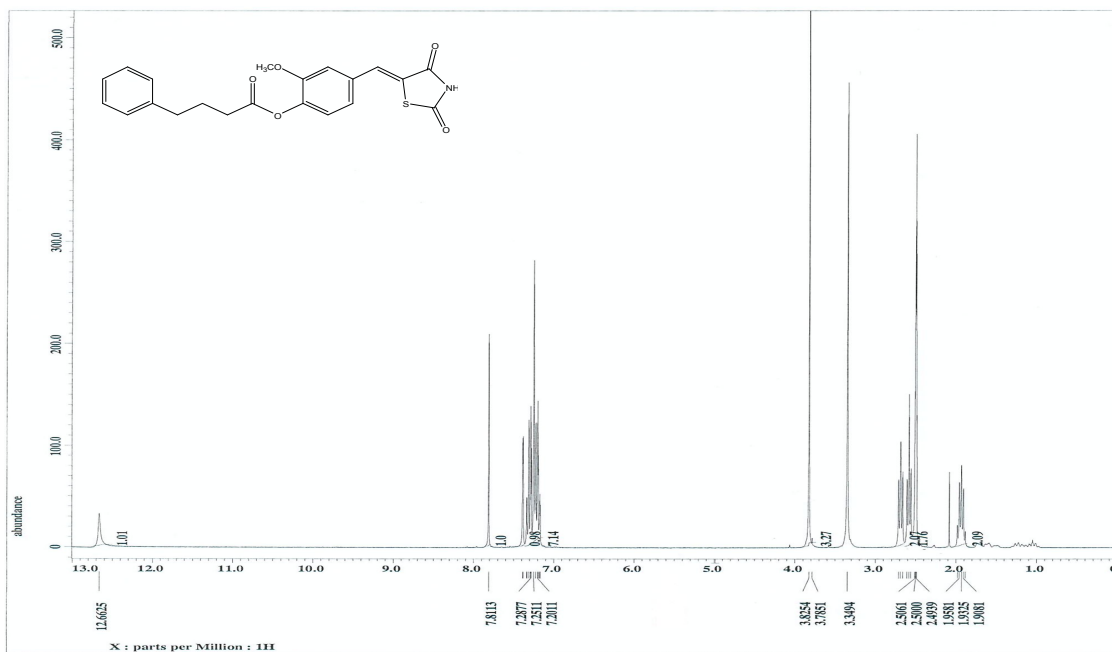
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl benzoate (56)



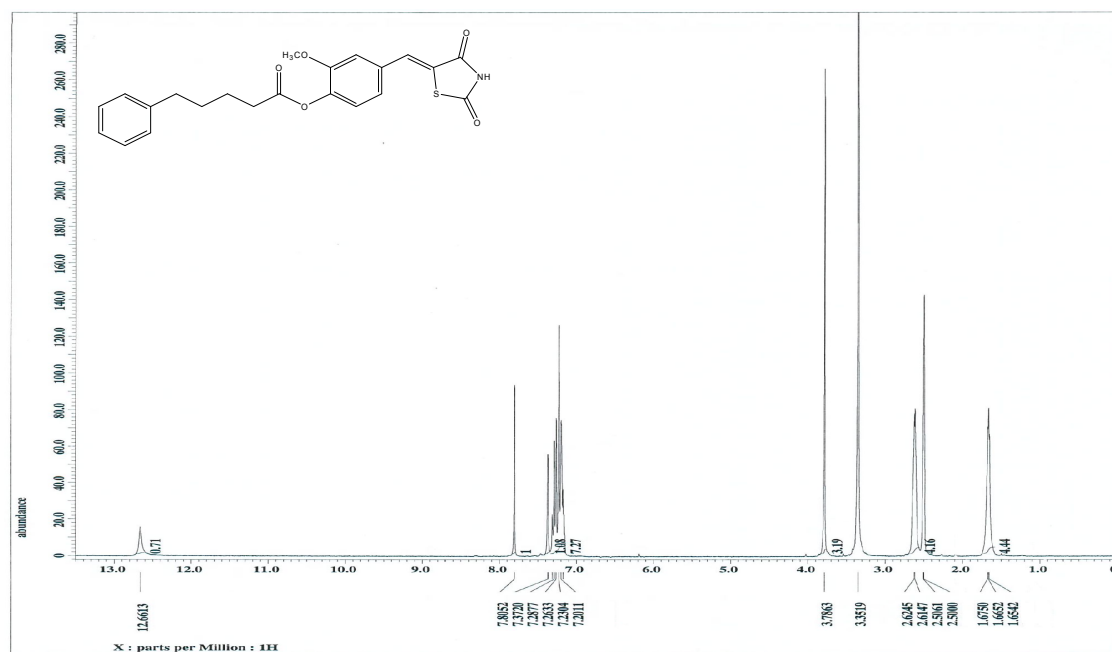
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 2-phenylacetate (57)



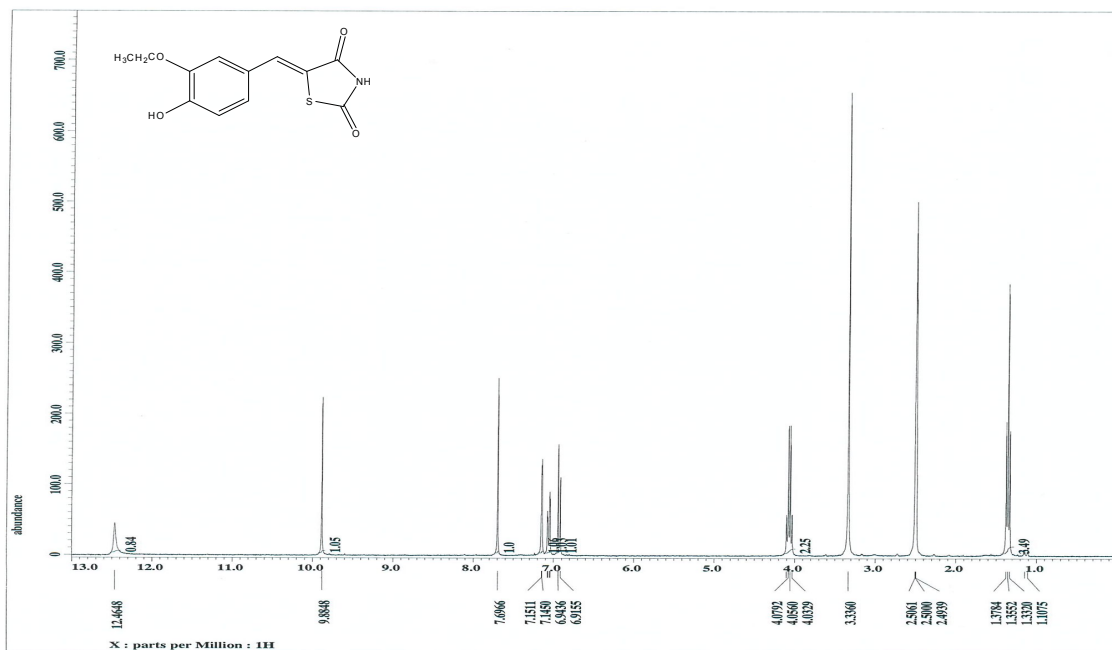
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 3-phenylpropanoate (58)



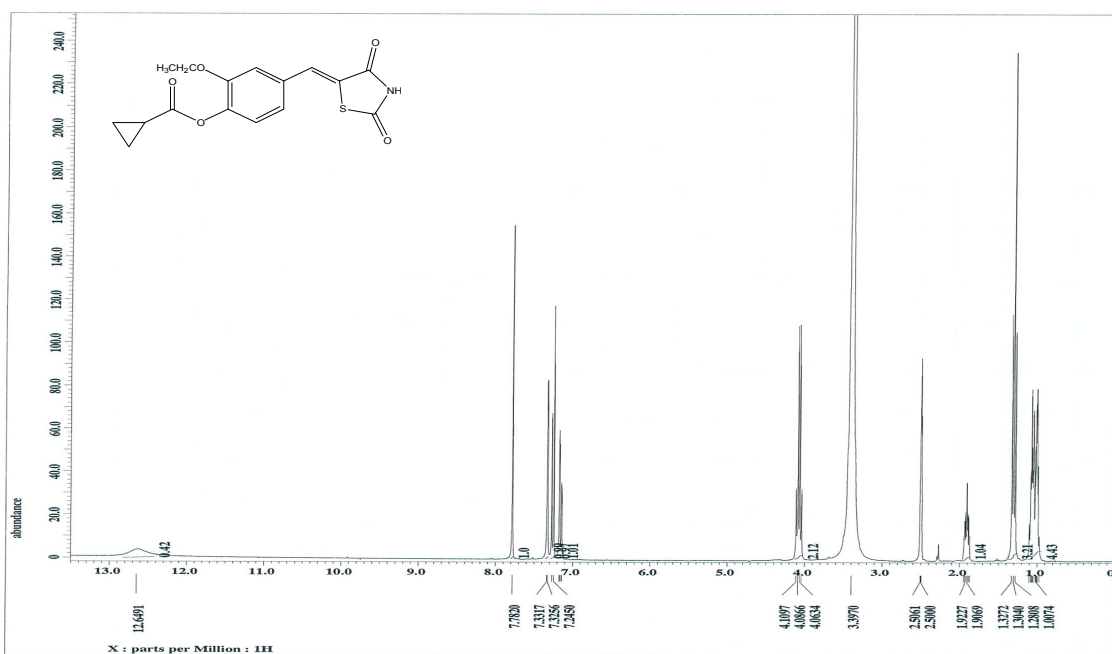
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 4-phenylbutanoate (59)



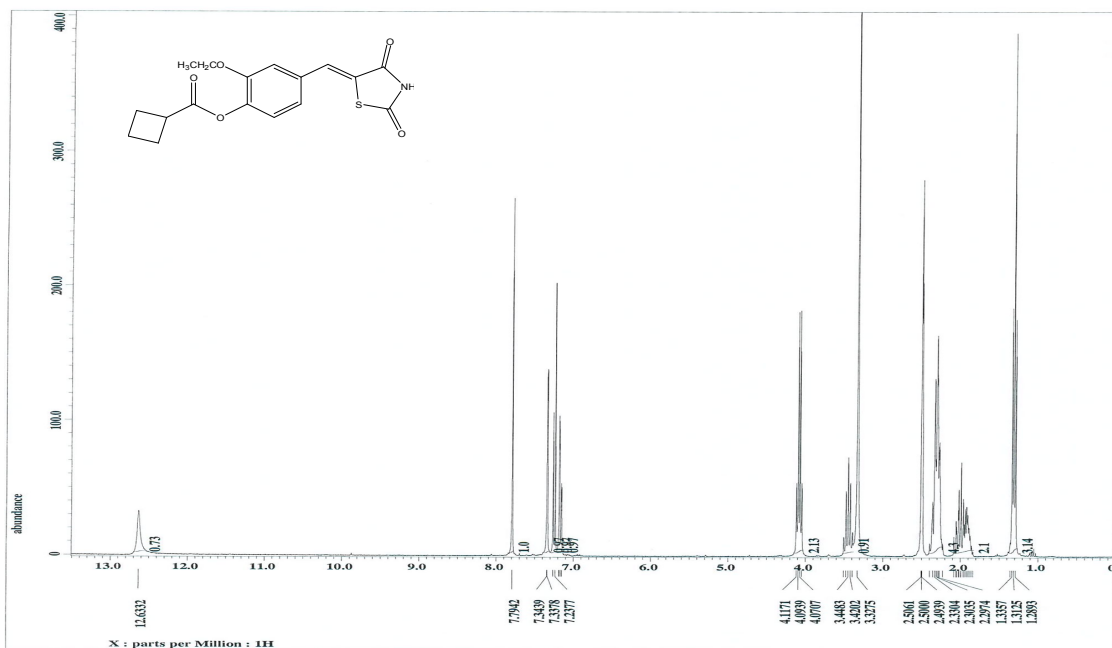
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenyl 5-phenylpentanoate (60)



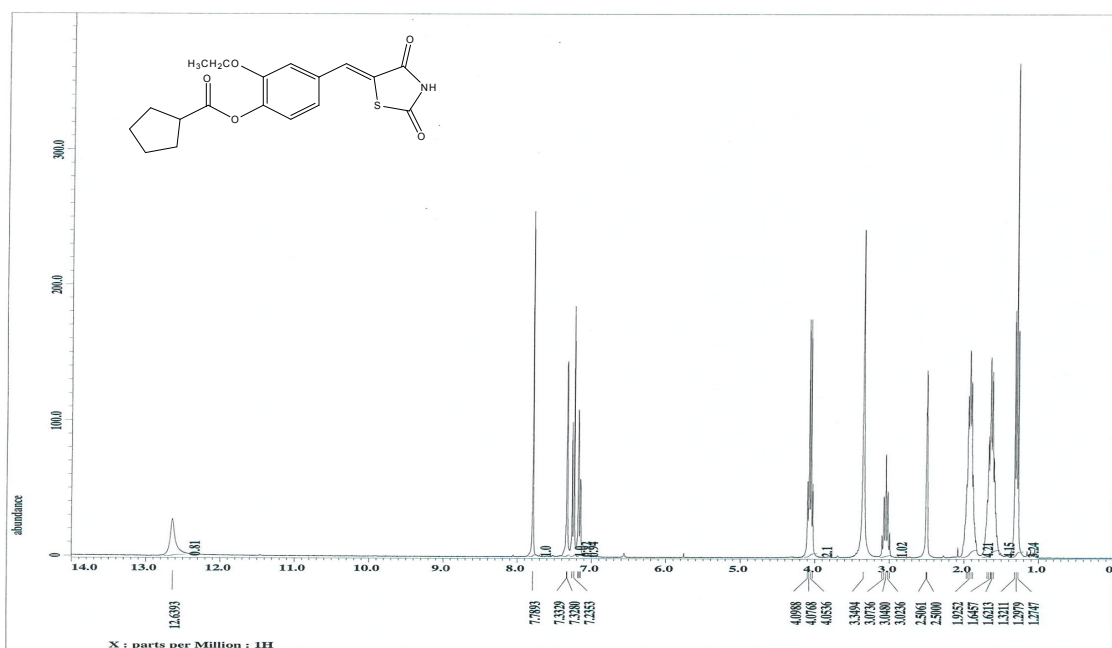
5-(3-Ethoxy-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (61a-75a)



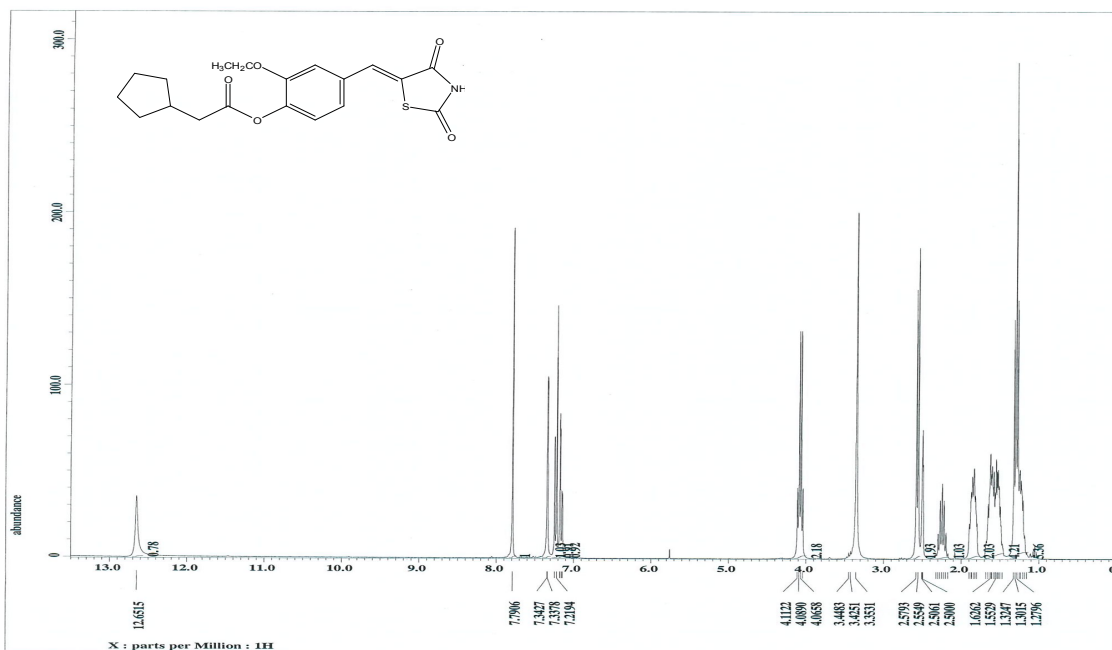
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclopropane-carboxylate (61)



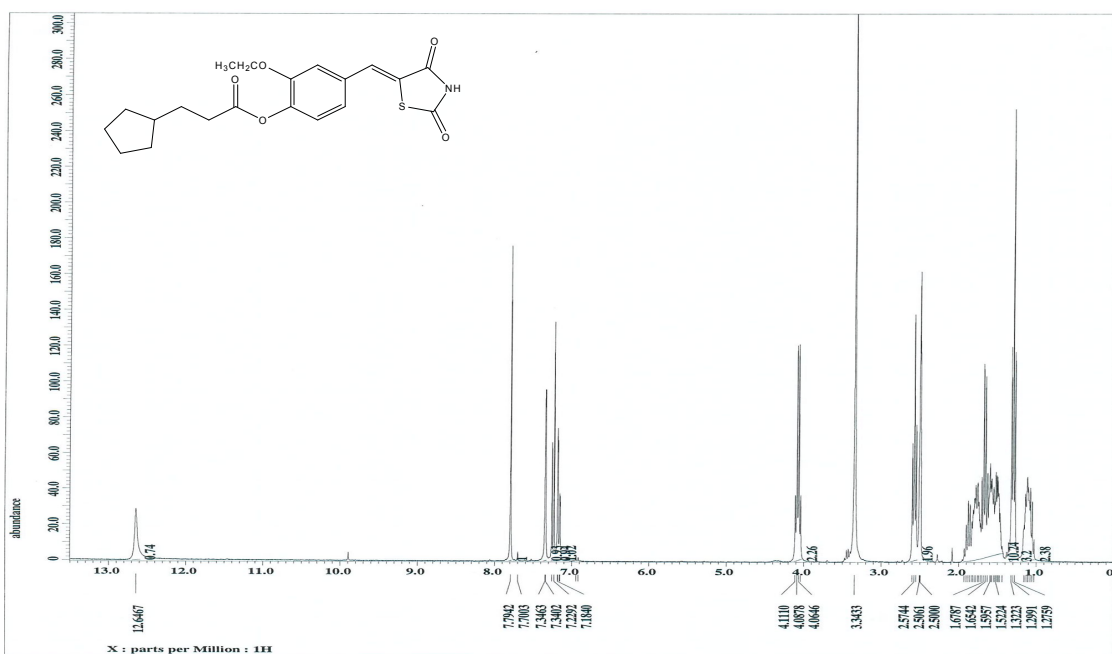
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclobutane-carboxylate (62)



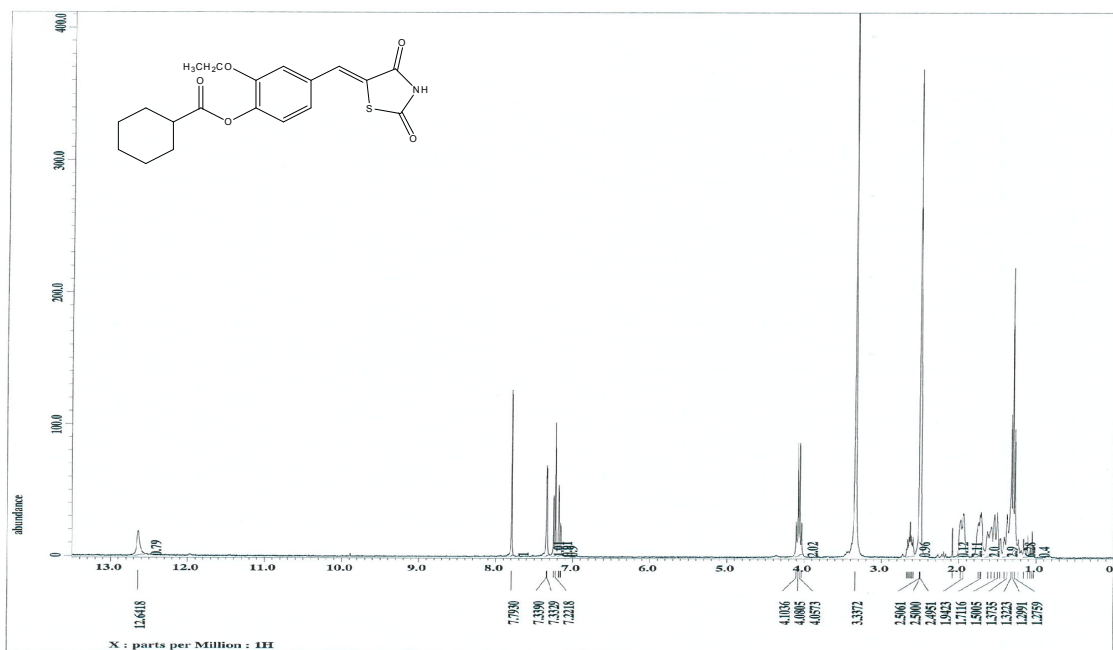
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclopentane-carboxylate (63)



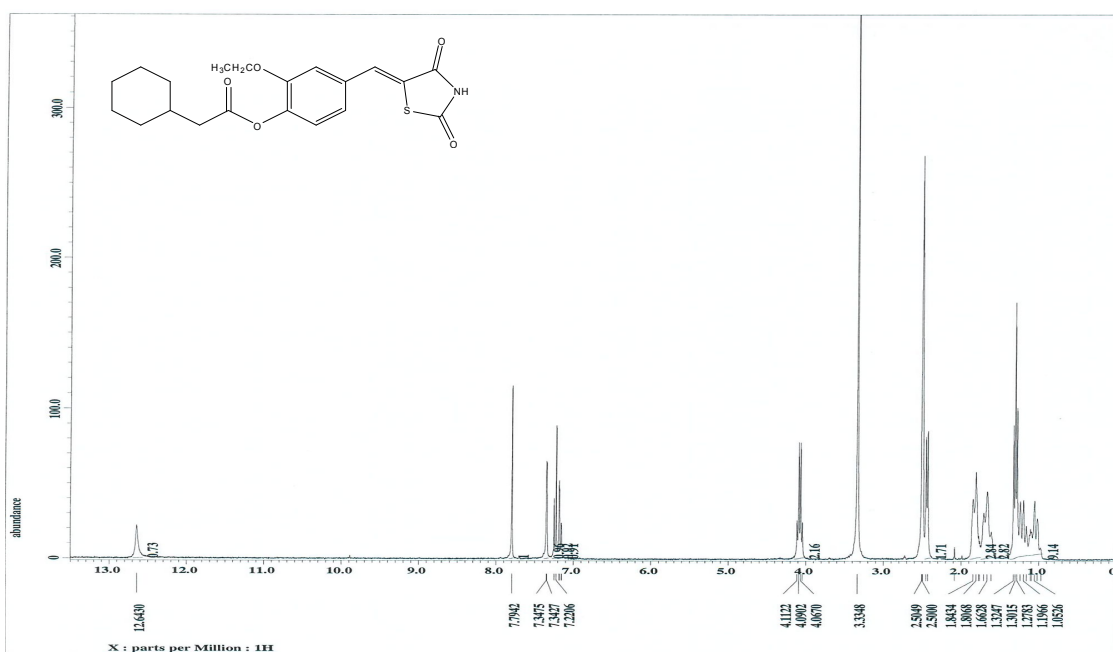
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-cyclopentylacetate (64)



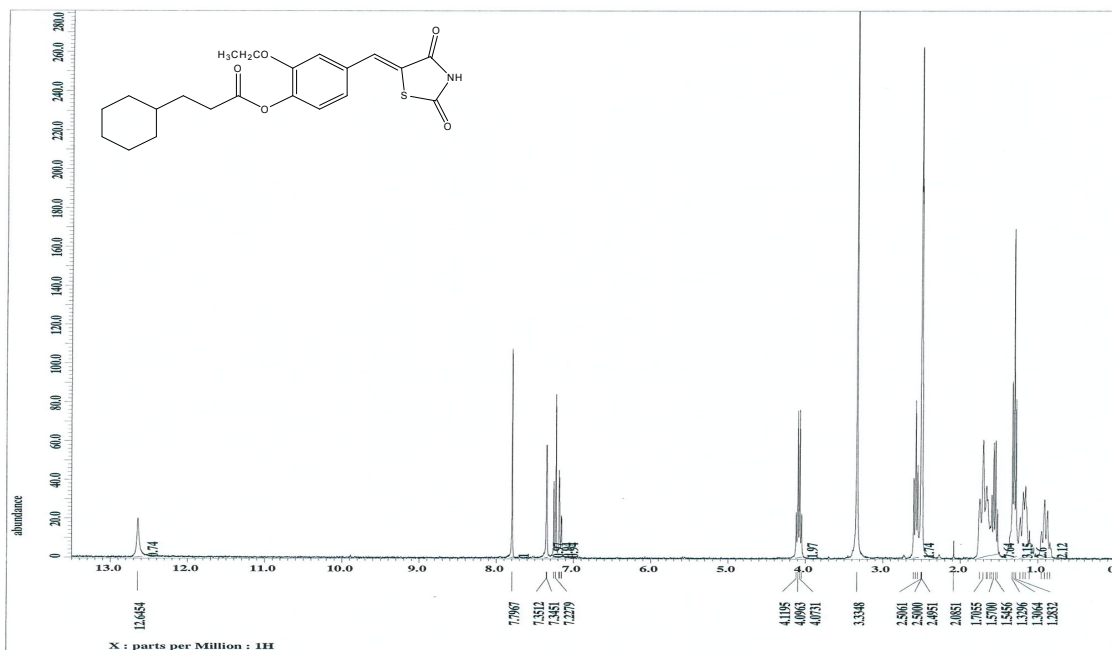
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-cyclopentylpropanoate (65)



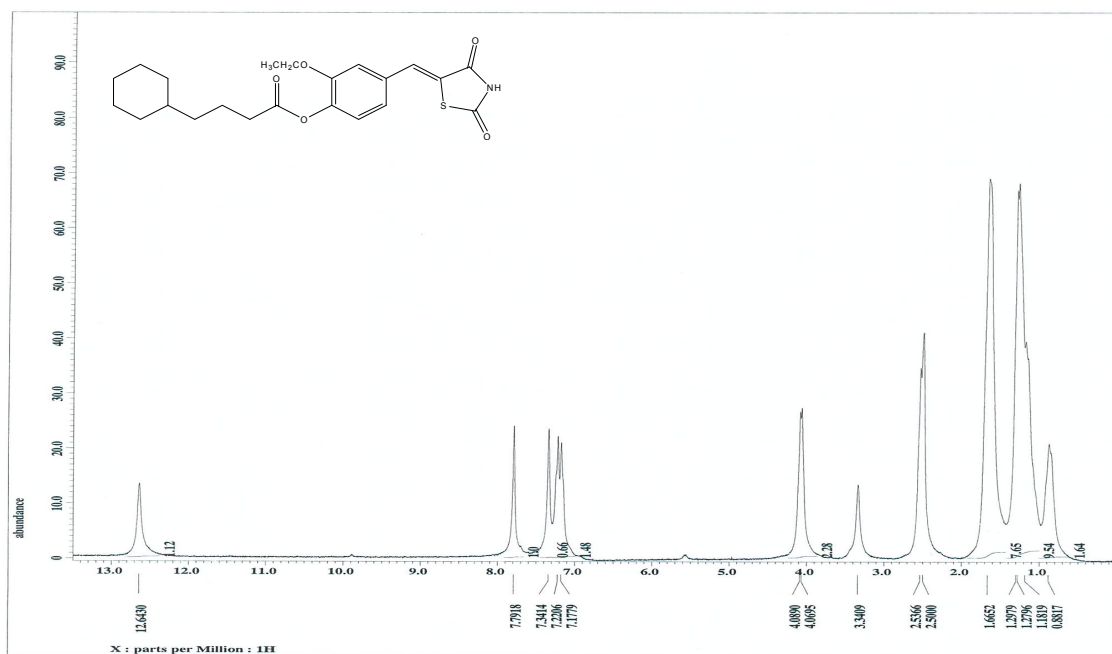
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl cyclohexane-carboxylate (66)



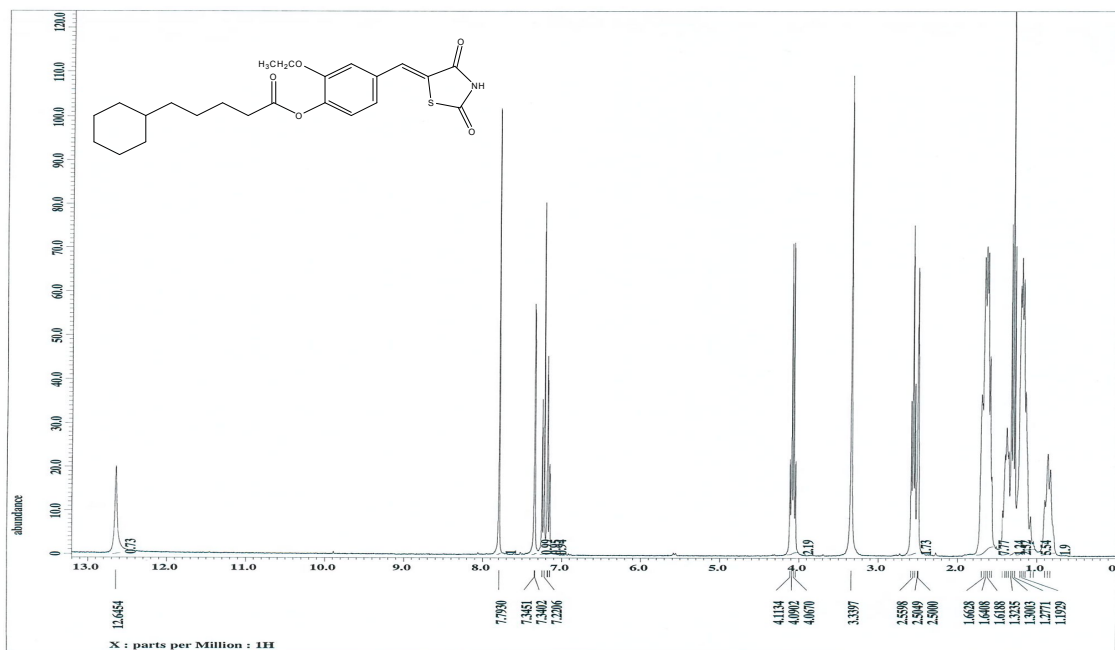
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-cyclohexyl-acetate (67)



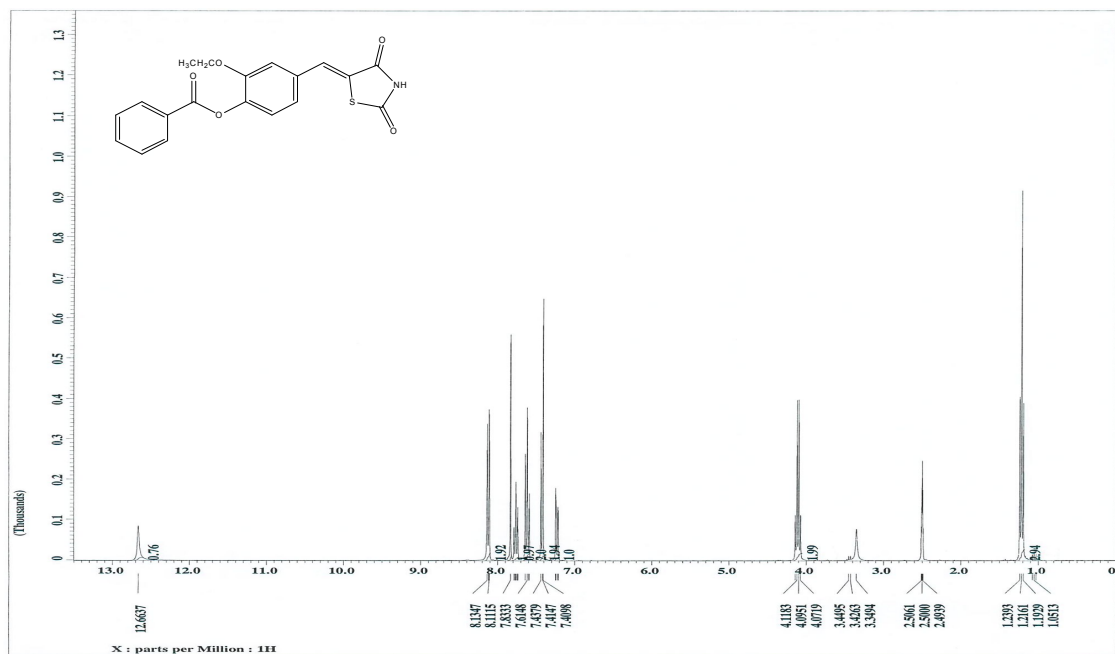
(Z)-4-((2,4-dioxthiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-cyclohexylpropanoate (68)



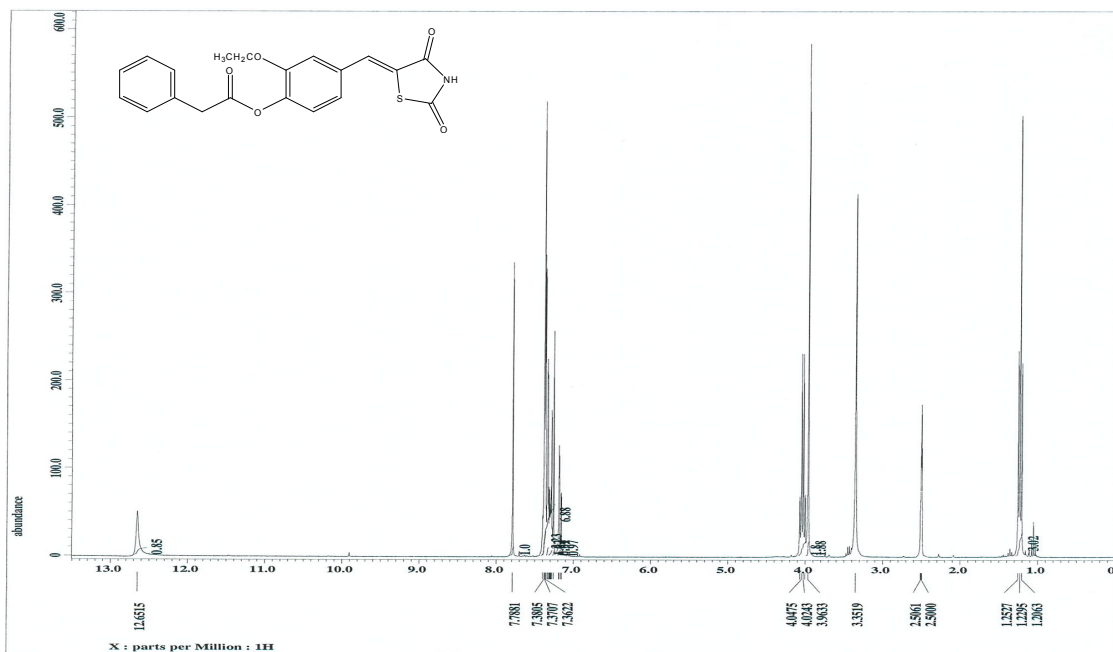
(Z)-4-((2,4-dioxthiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 4-cyclohexylbutanoate (69)



(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 5-cyclohexyl-pentanoate (70)



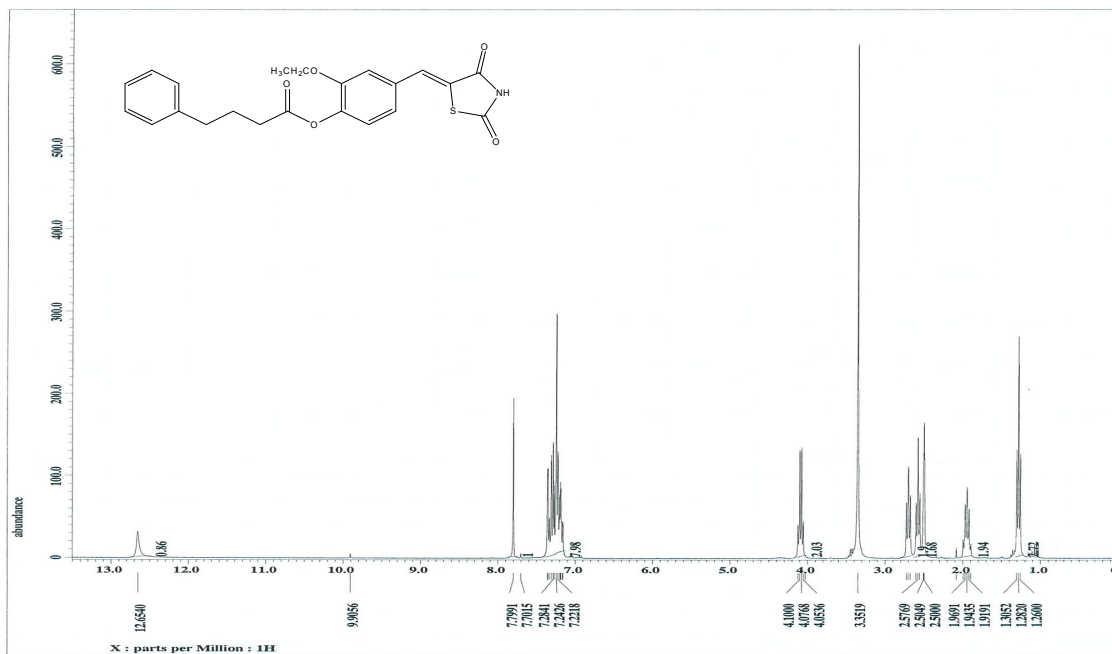
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl benzoate (71)



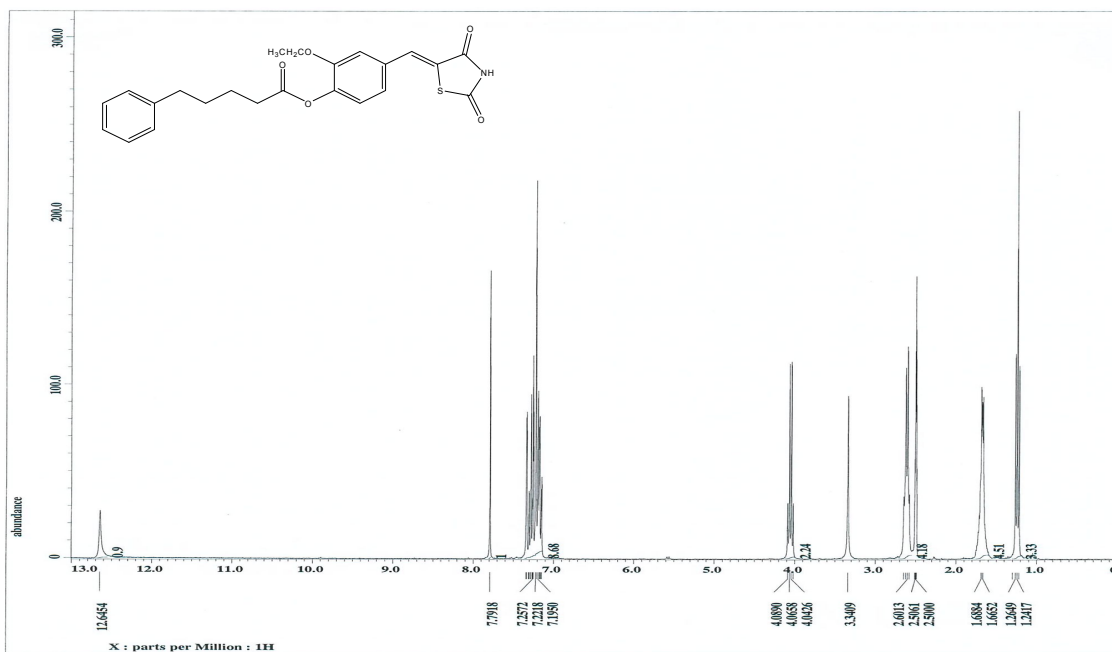
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 2-phenylacetate (72)



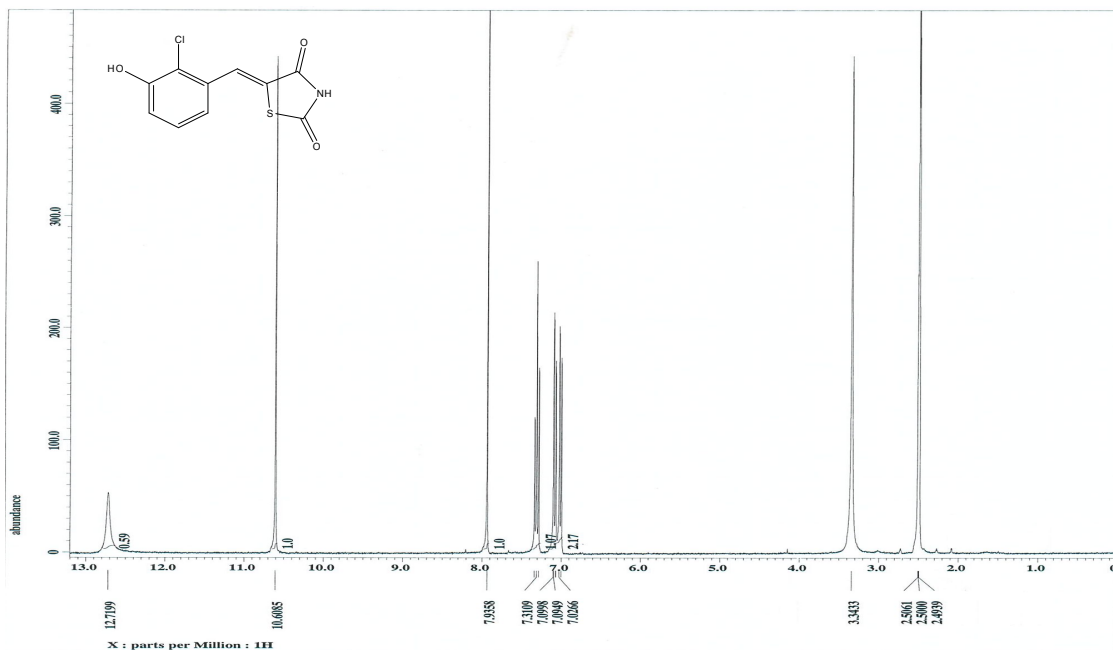
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 3-phenylpropanoate (73)



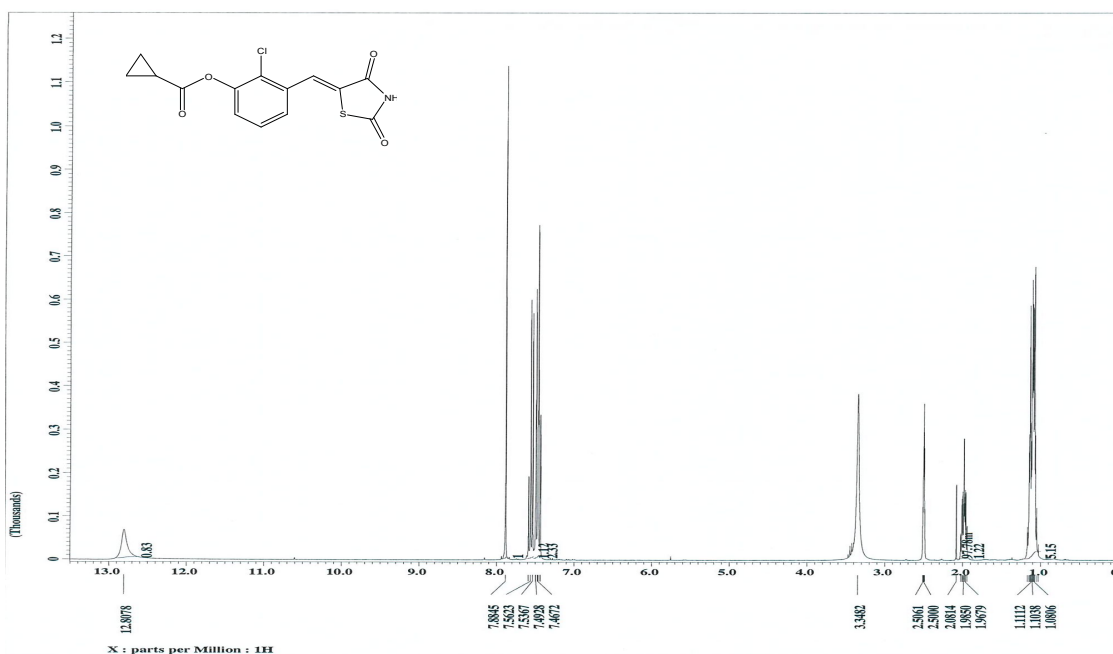
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 4-phenylbutanoate (74)



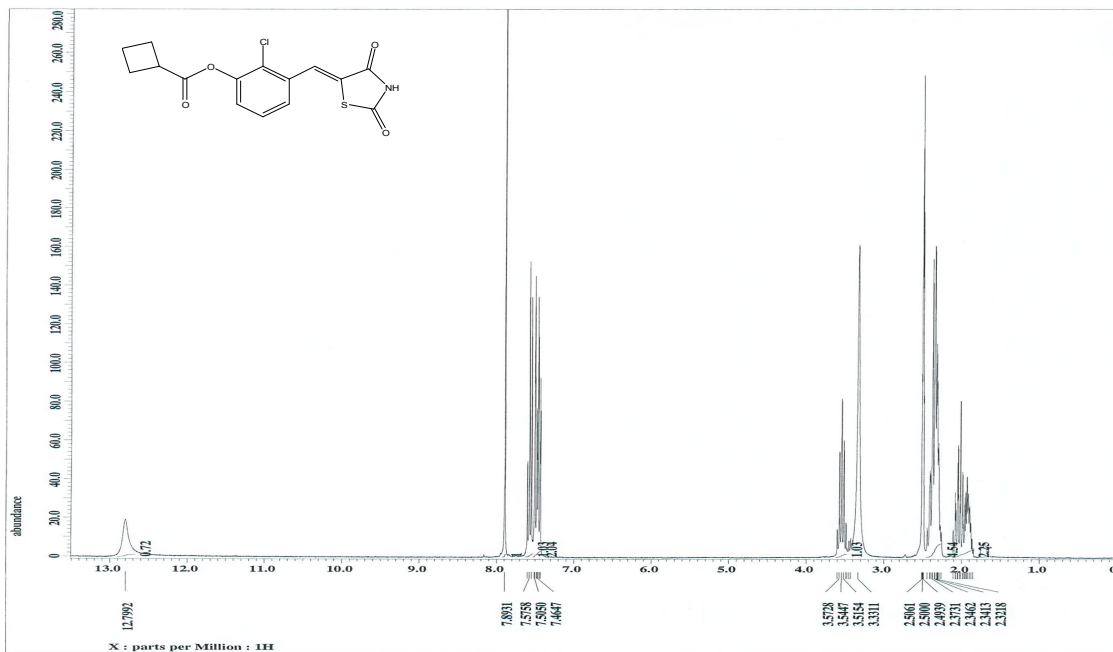
(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenyl 5-phenylpentanoate (75)



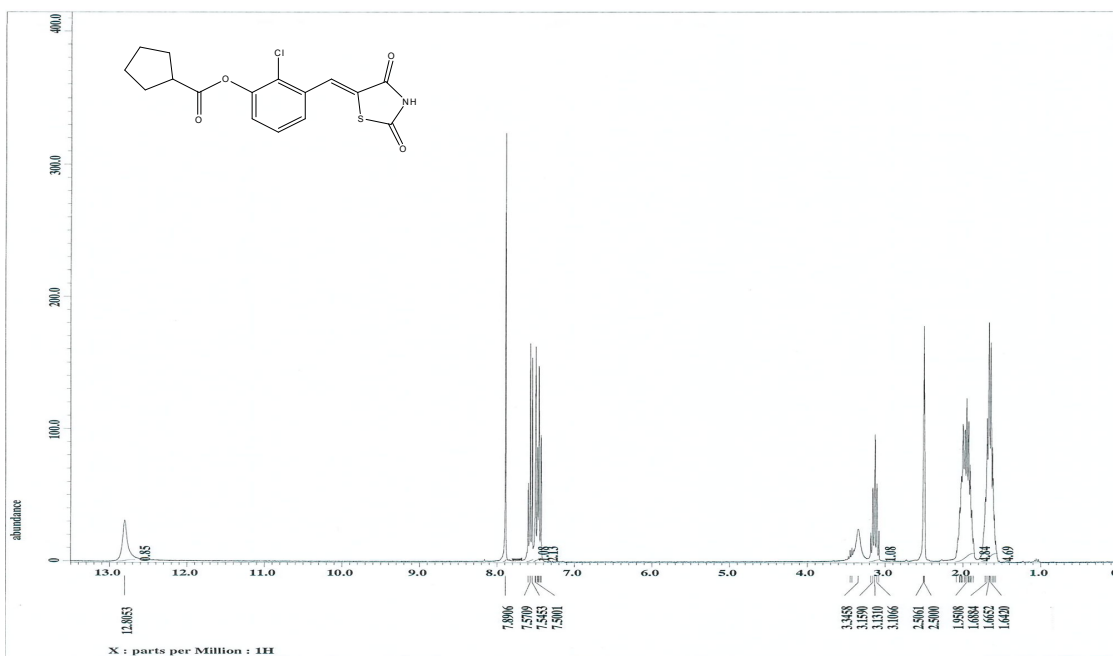
5-(2-Chloro-3-hydroxy-benzylidene)-thiazolidine-2,4-dione (76a-90a)



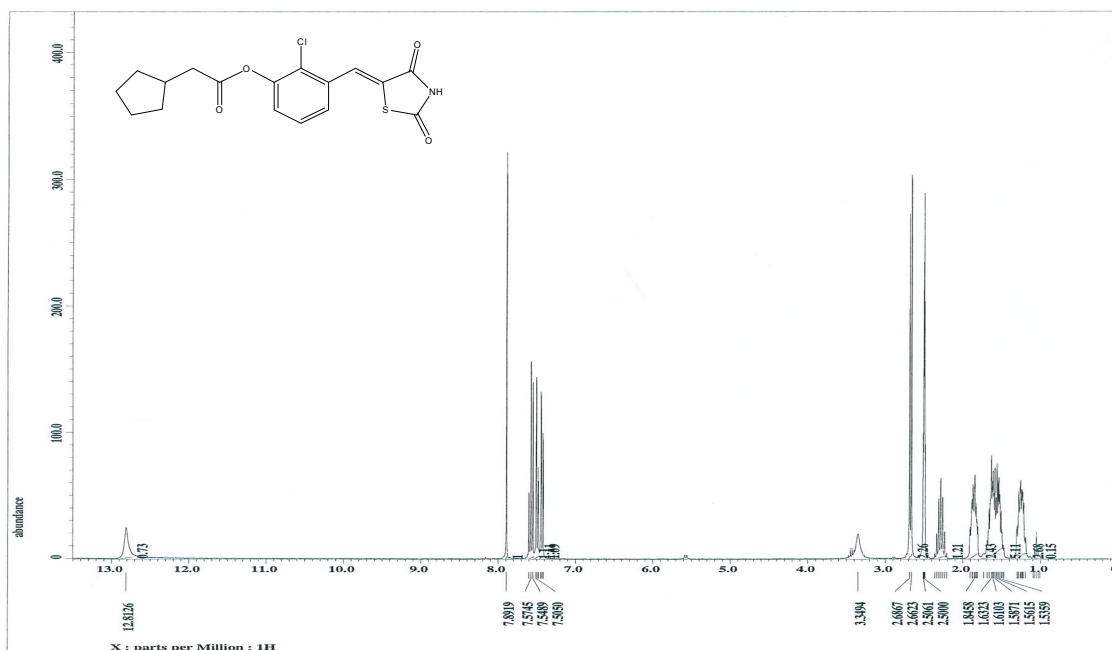
(Z)-2-chloro-3-((2,4-dioxthiazolidin-5-ylidene)methyl)phenyl cyclopropane-carboxylate (76)



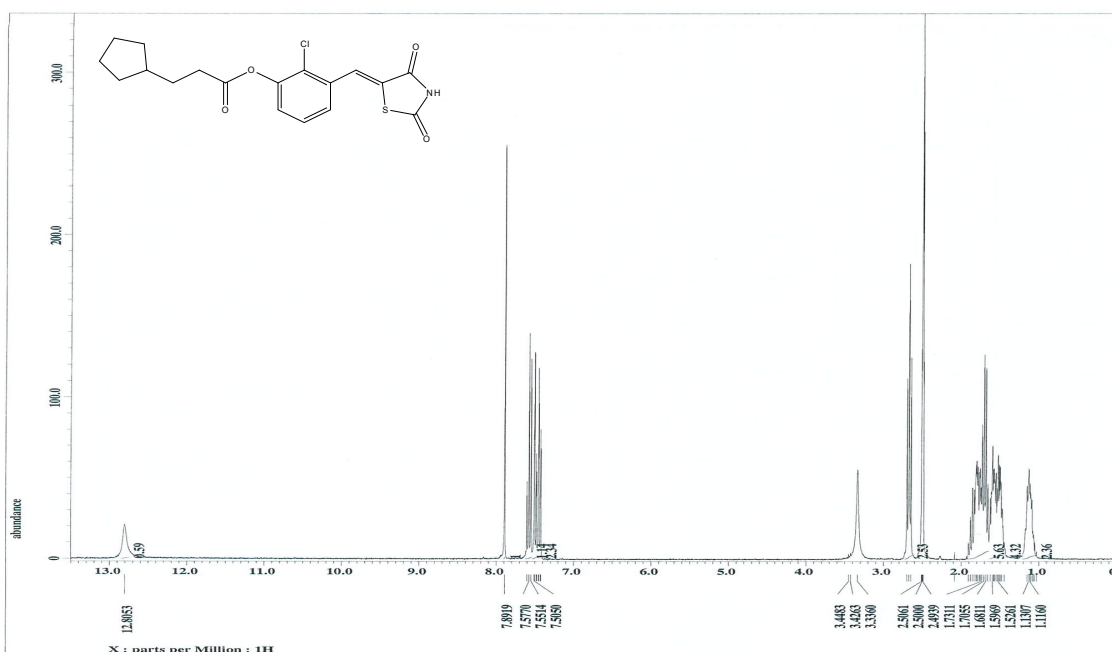
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane-carboxylate (77)



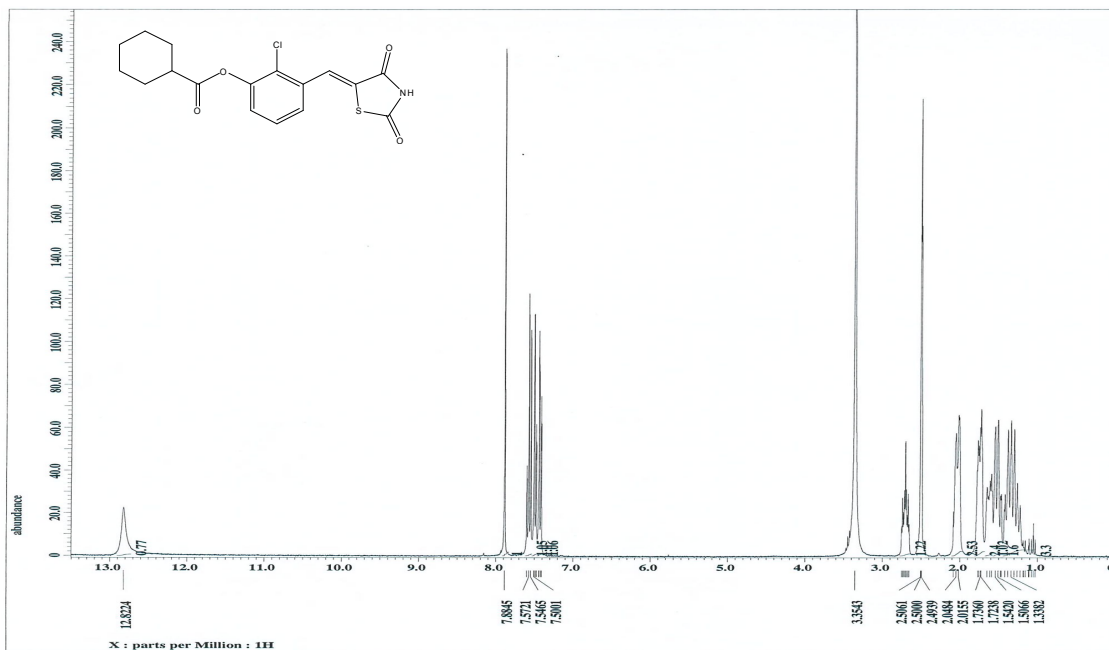
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-carboxylate (78)



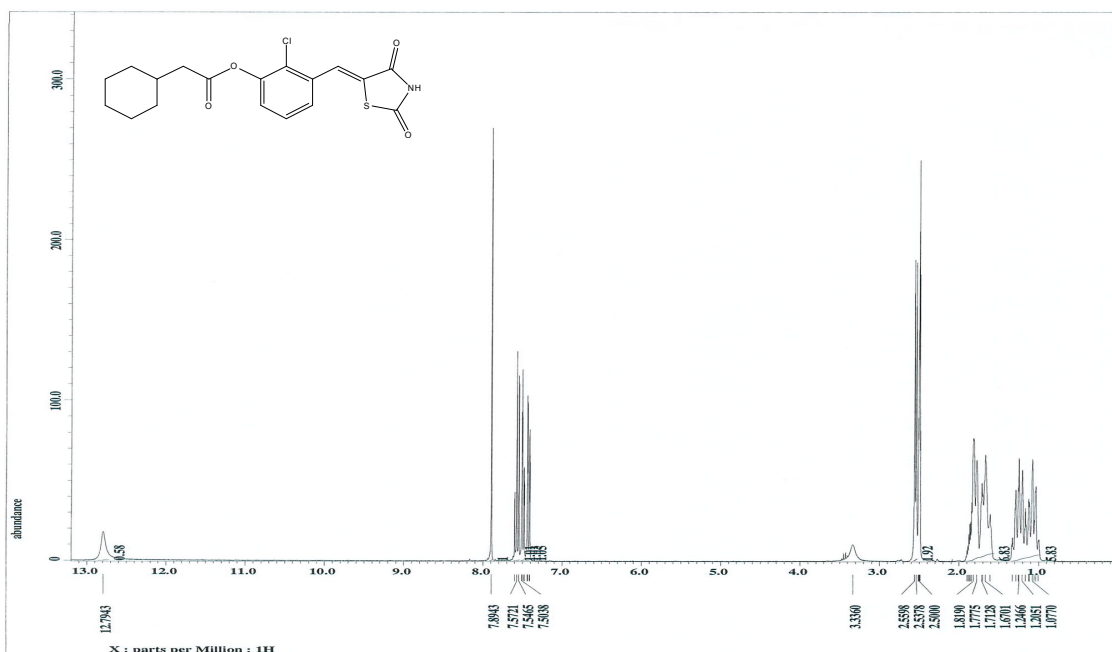
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (79)



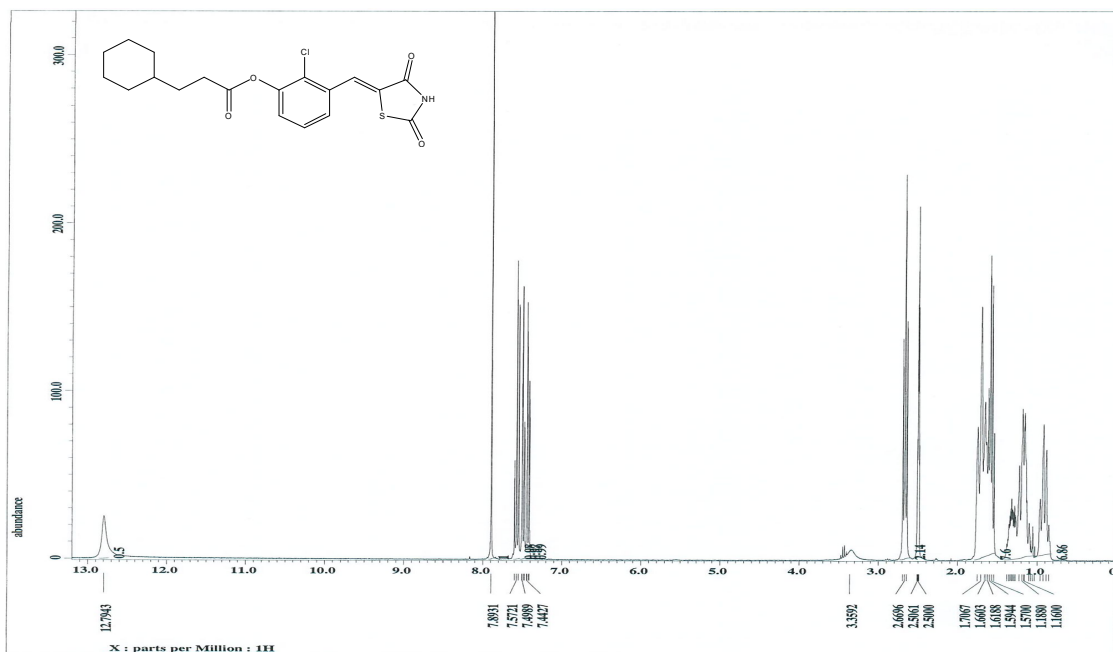
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (80)



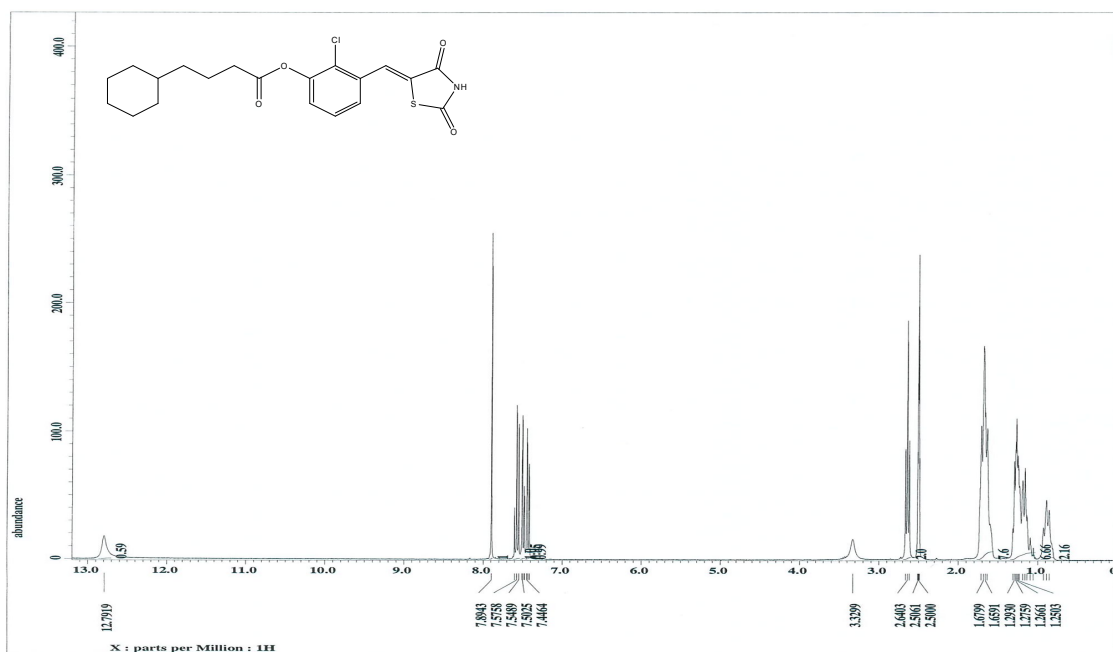
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane-carboxylate (81)



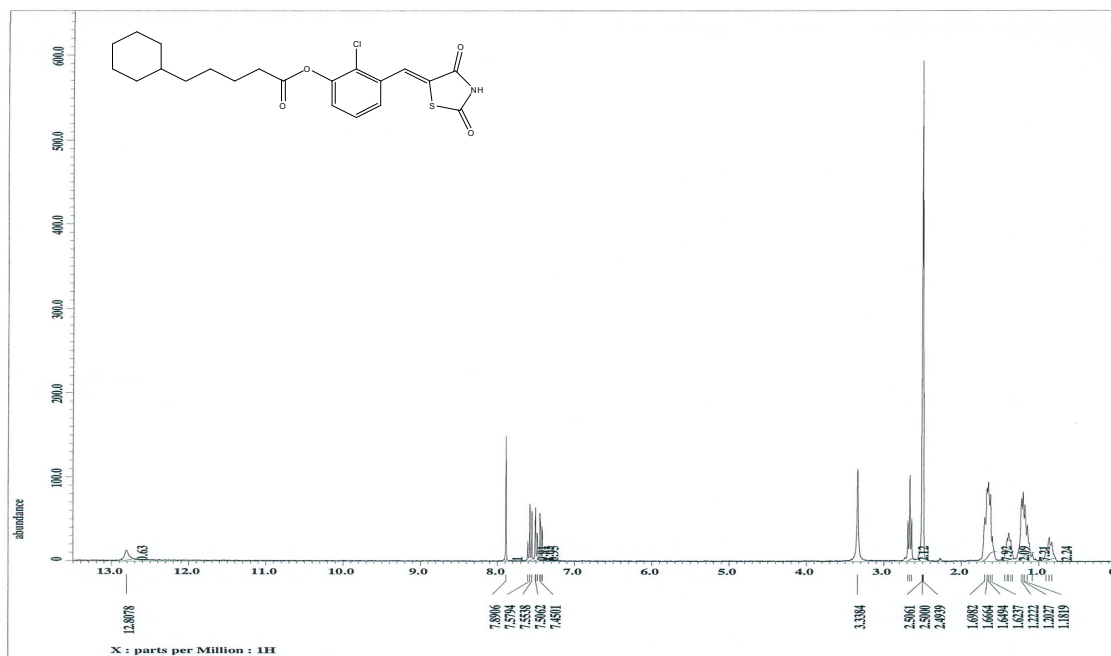
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexyl-acetate (82)



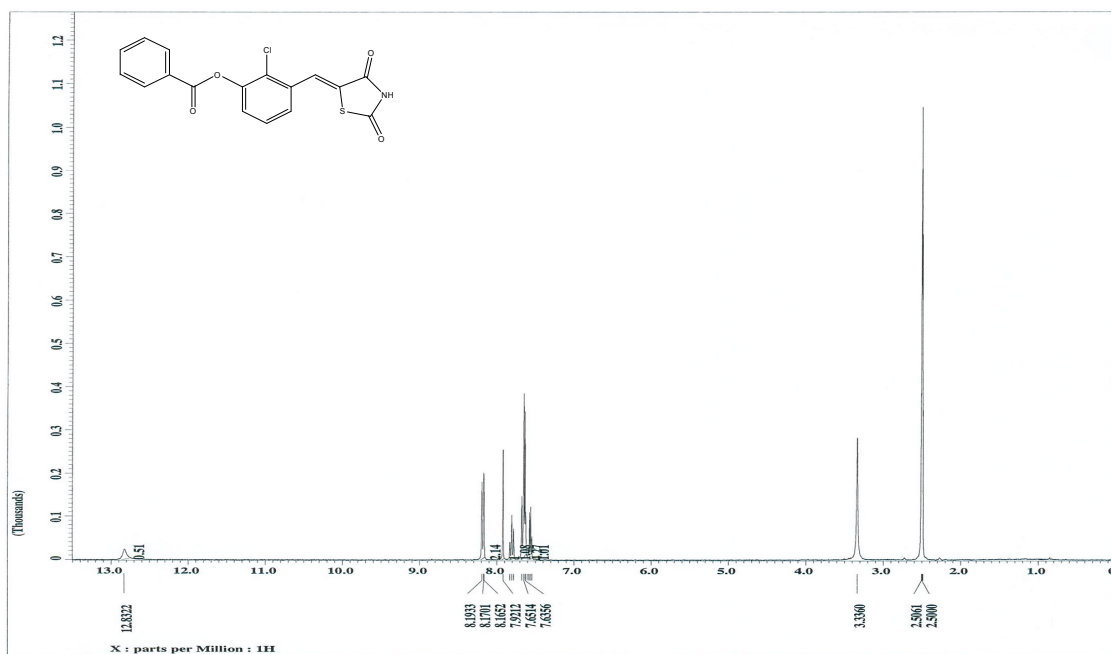
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (83)



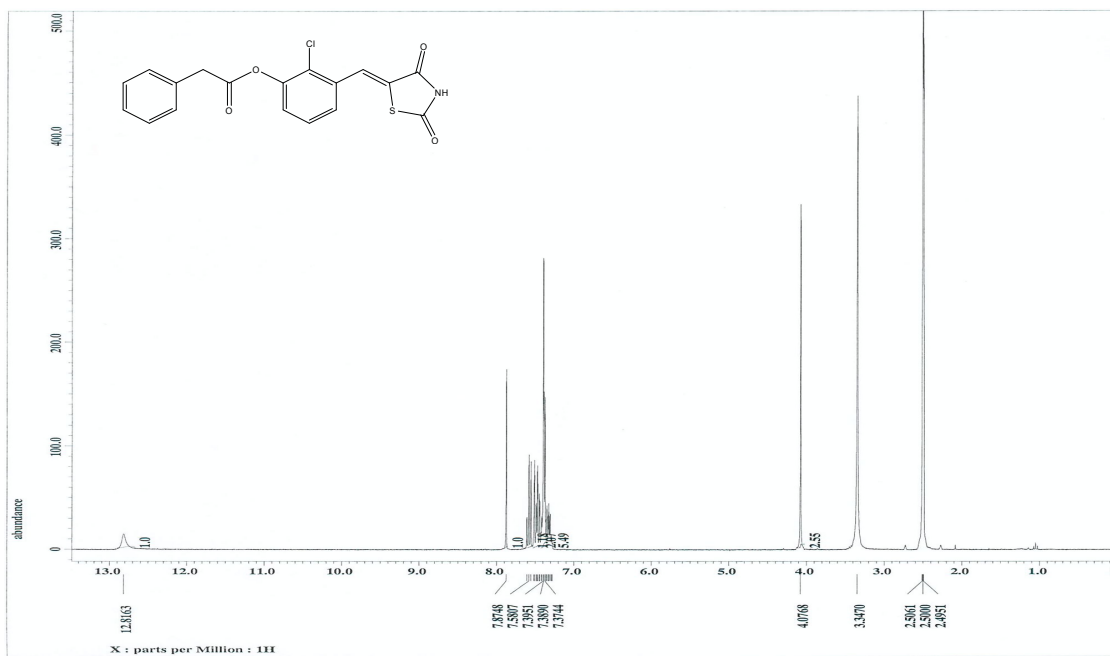
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (84)



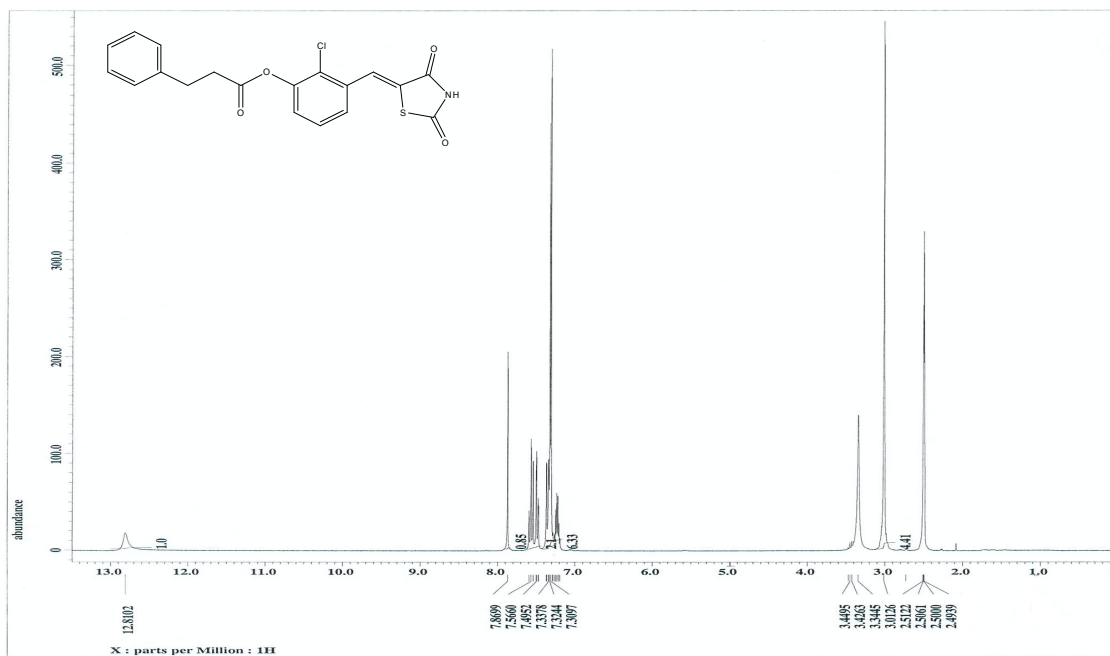
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl-pentanoate (85)



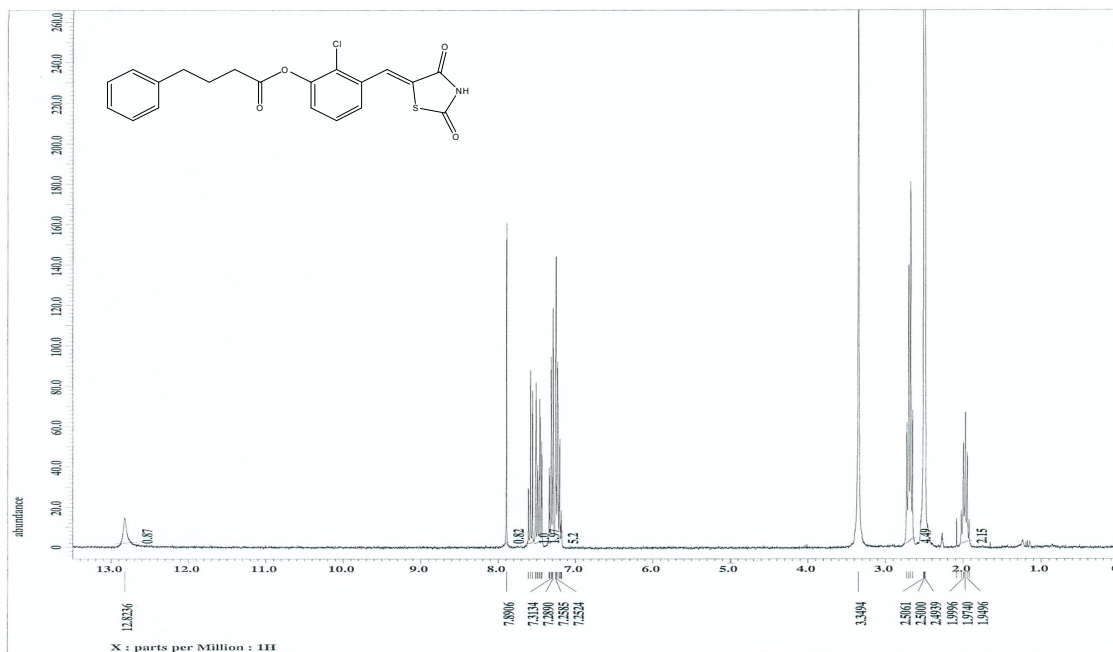
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (86)



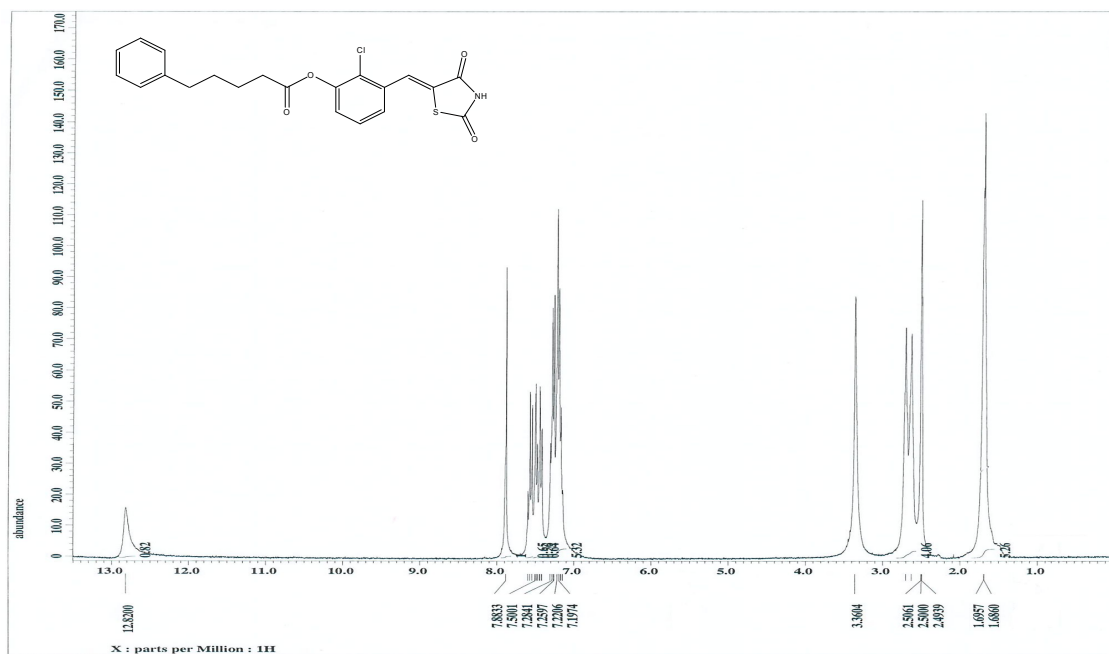
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate
(87)



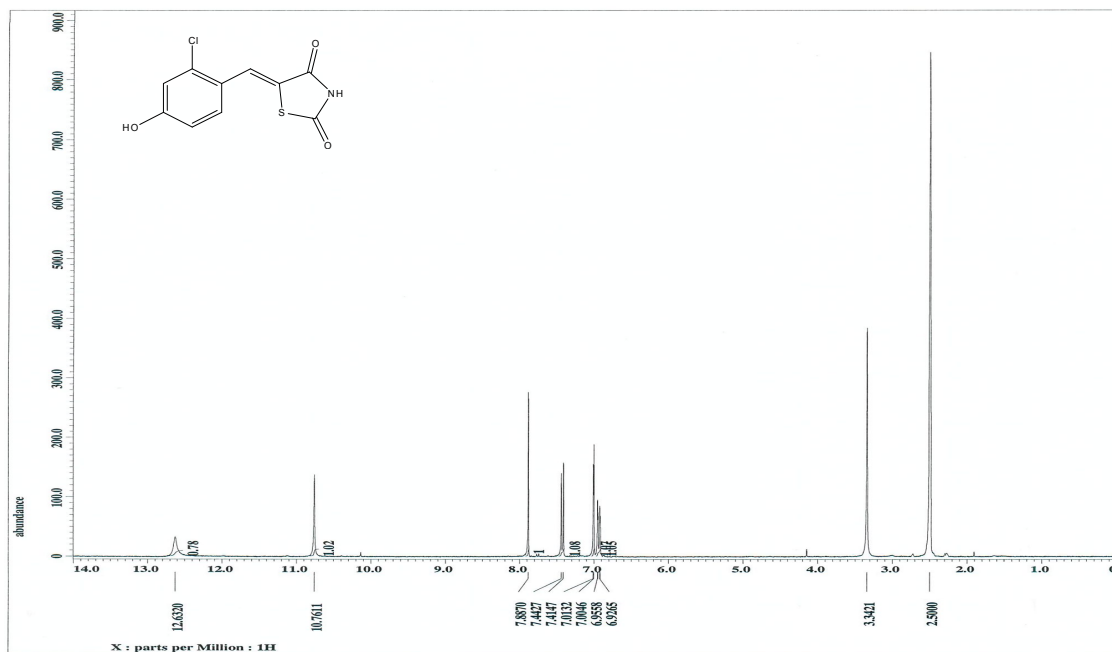
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate
(88)



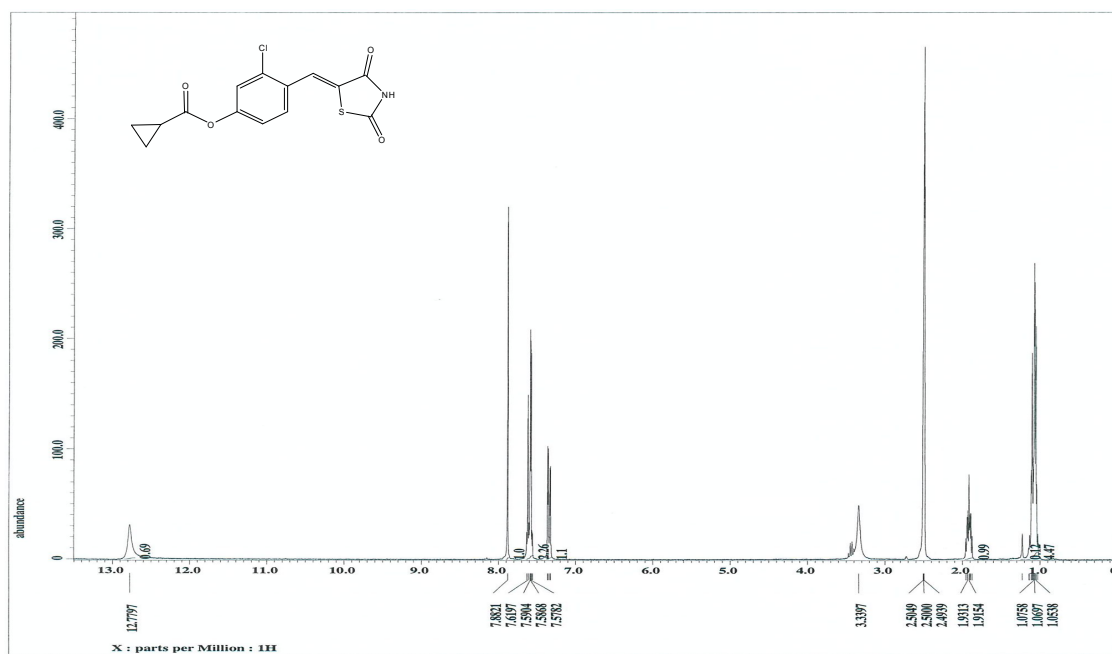
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (89)



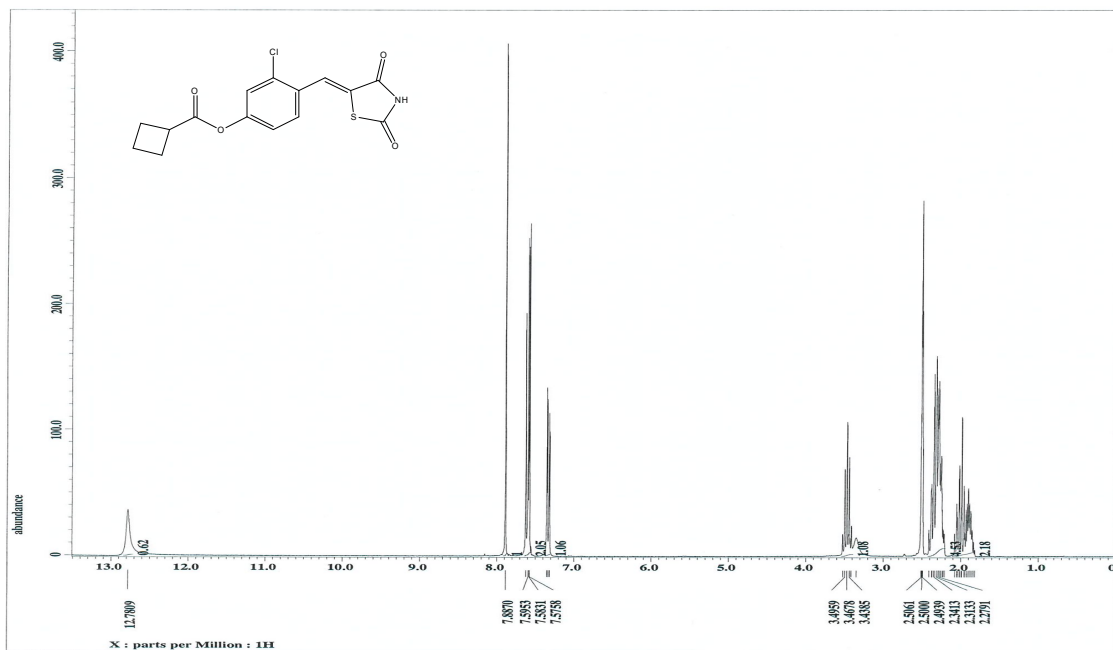
(Z)-2-chloro-3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (90)



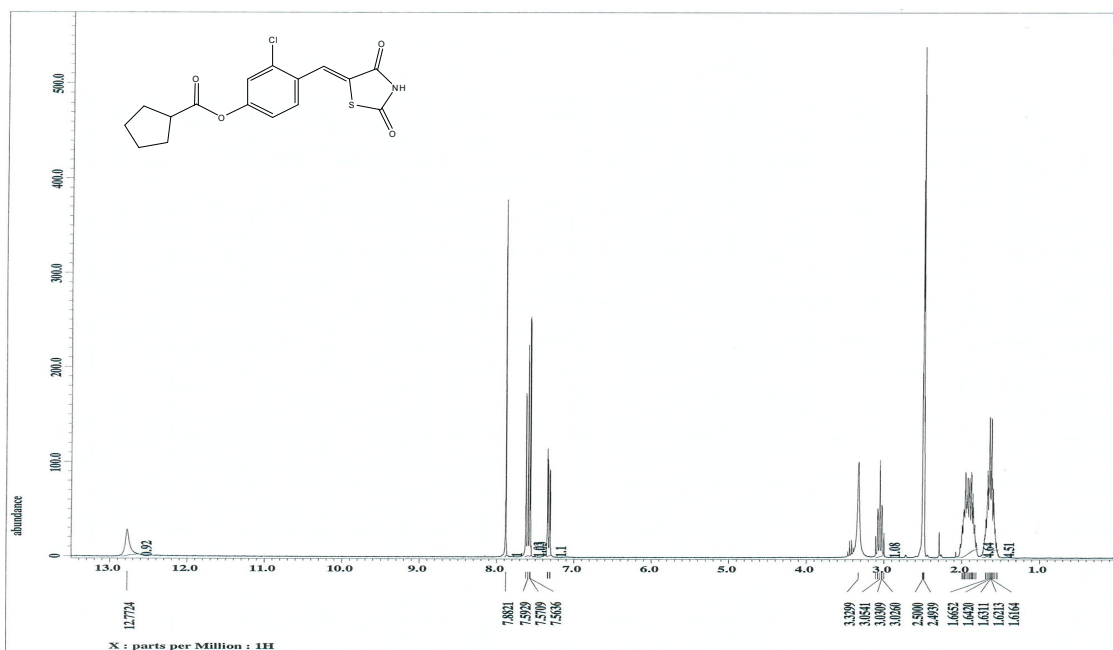
5-(2-Chloro-4-hydroxy-benzylidene)-thiazolidine-2,4-dione (91a-105a)



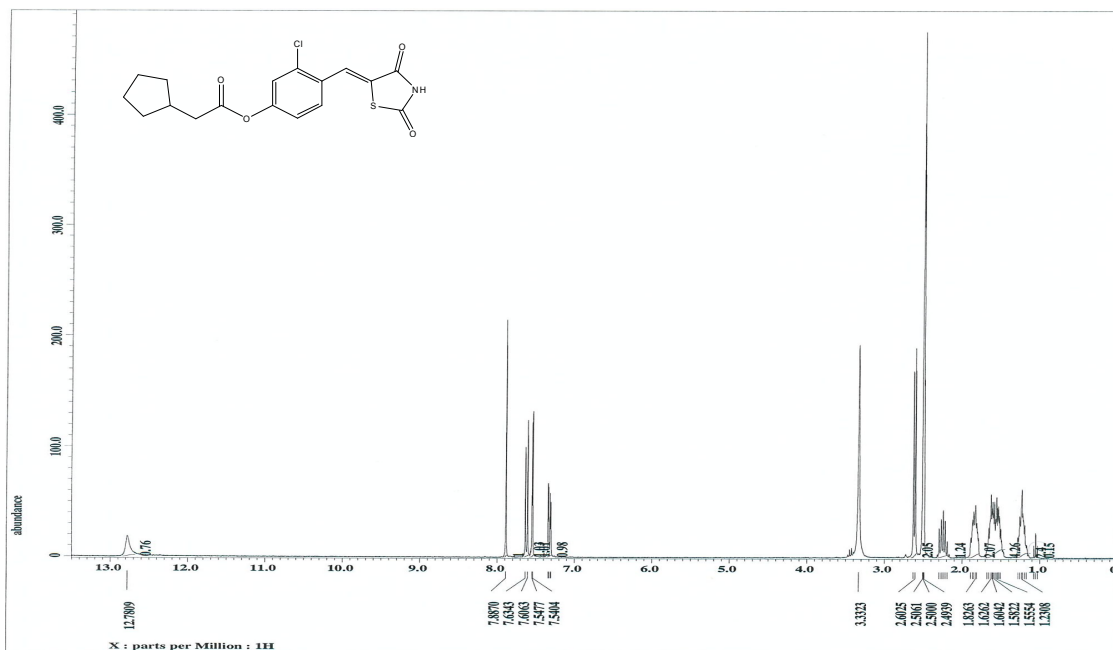
(Z)-3-chloro-4-((2,4-dioxthiazolidin-5-ylidene)methyl)phenyl cyclopropane-carboxylate (91)



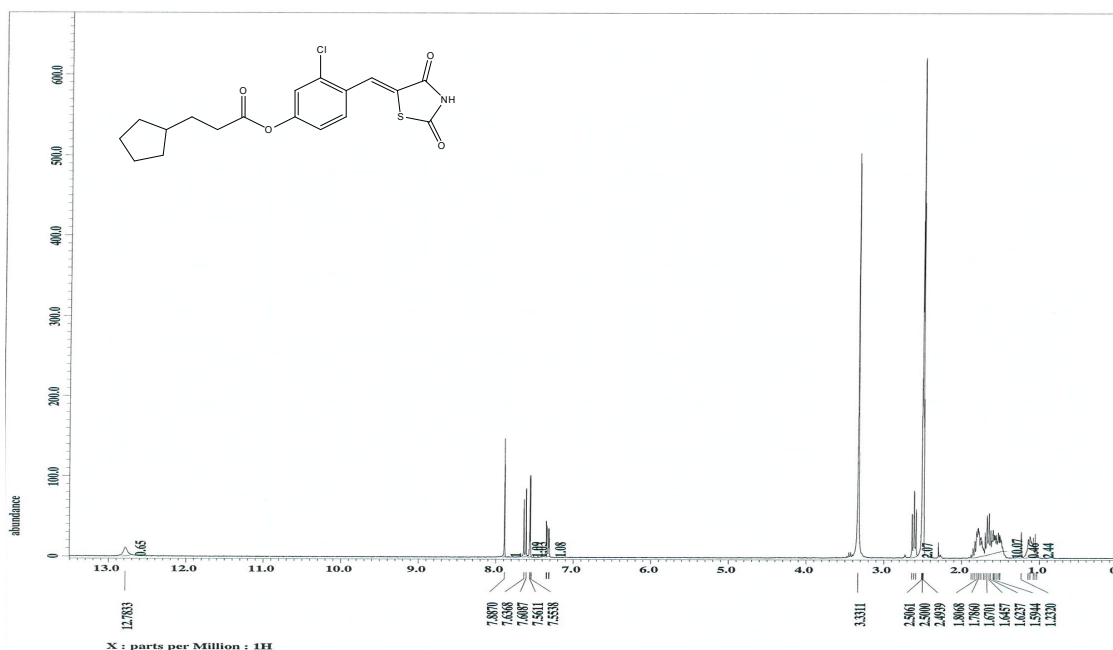
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclobutane-carboxylate (92)



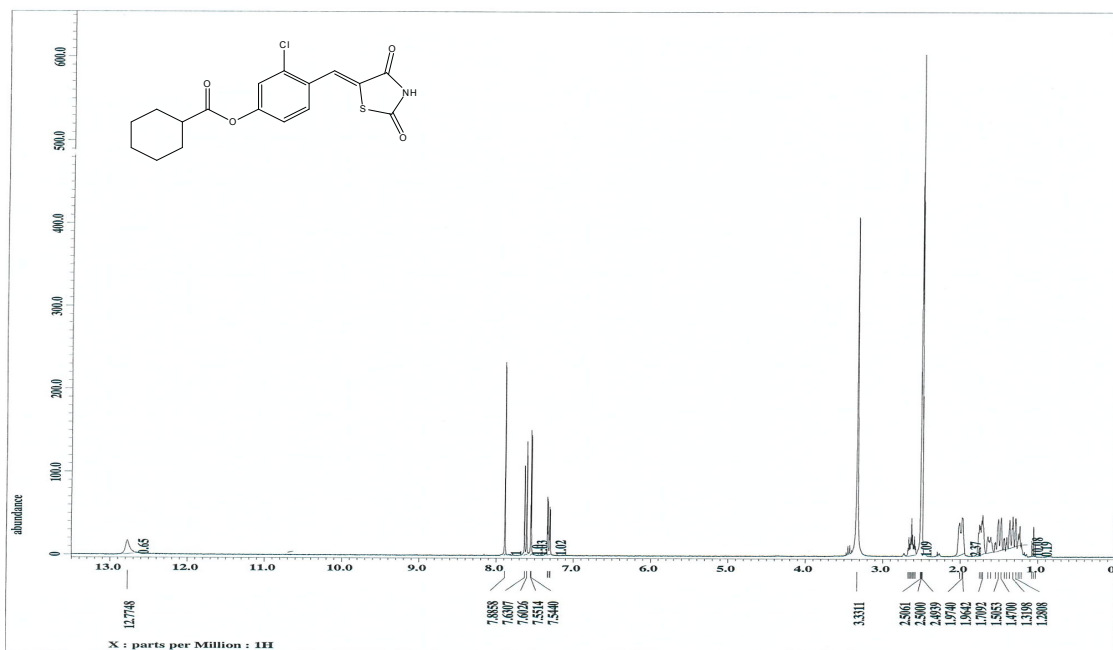
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclopentane-carboxylate (93)



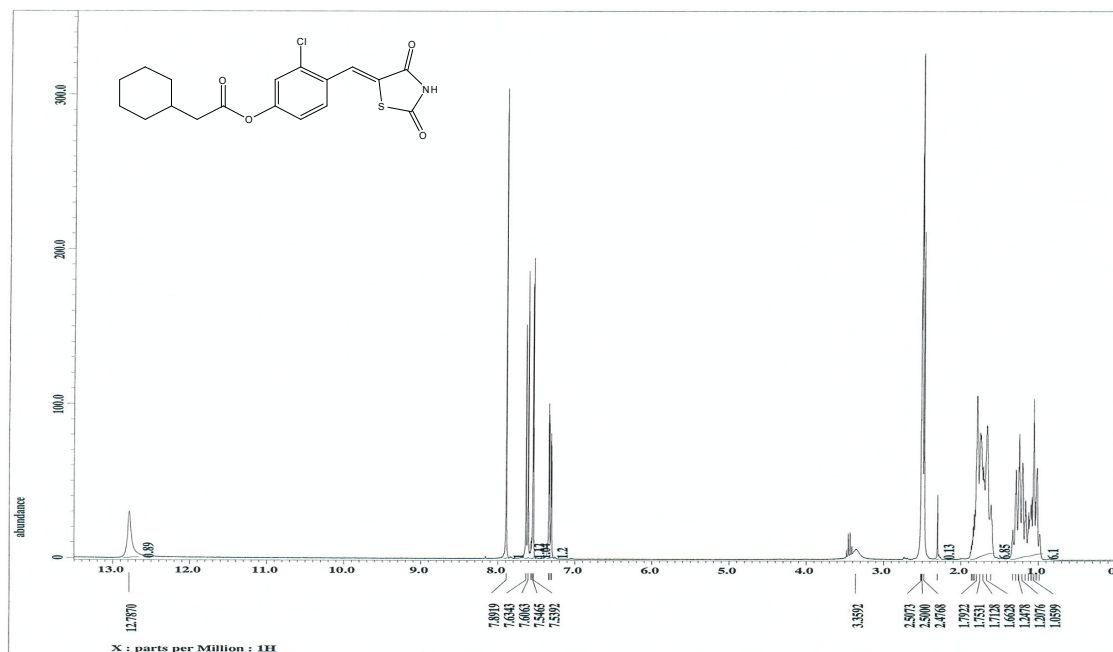
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclopentylacetate (94)



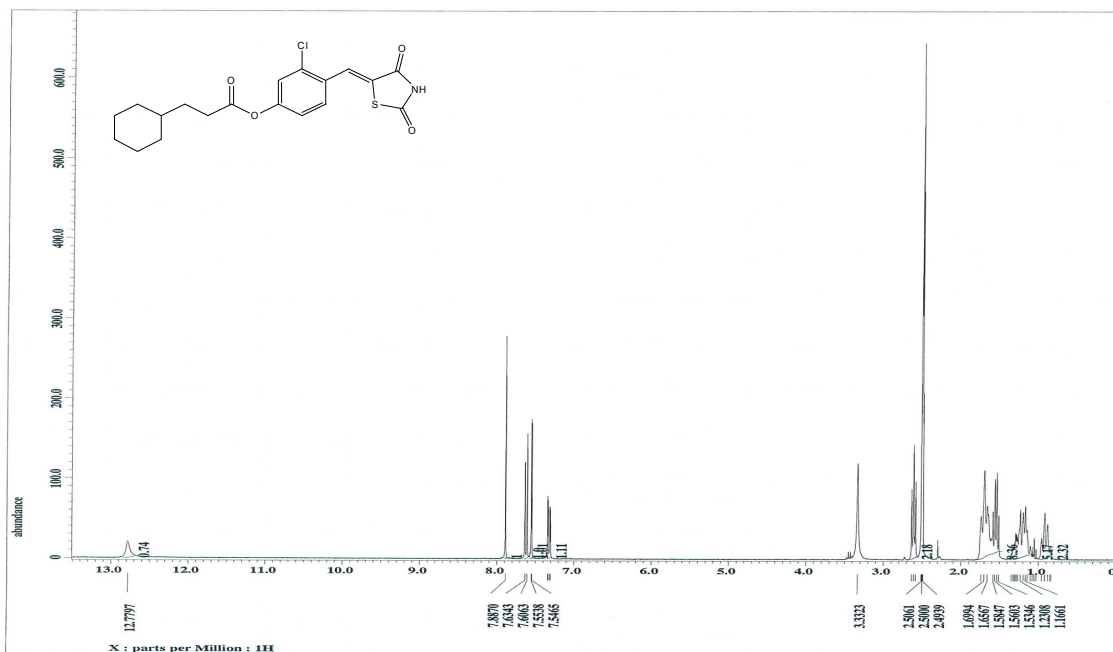
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclopentylpropanoate (95)



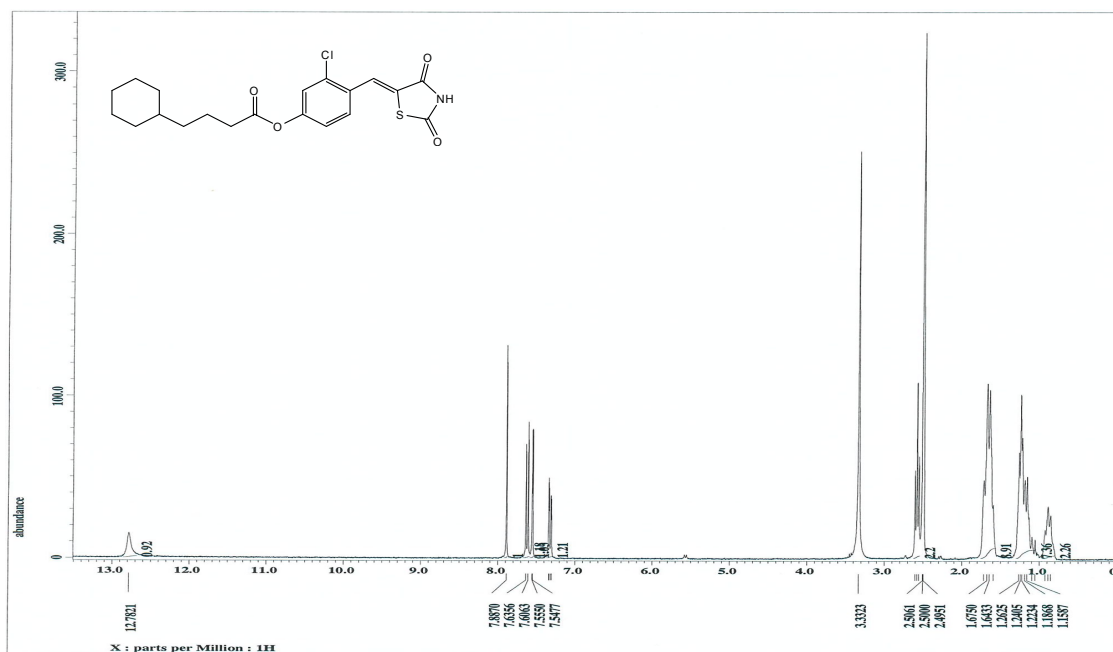
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl cyclohexane-carboxylate (96)



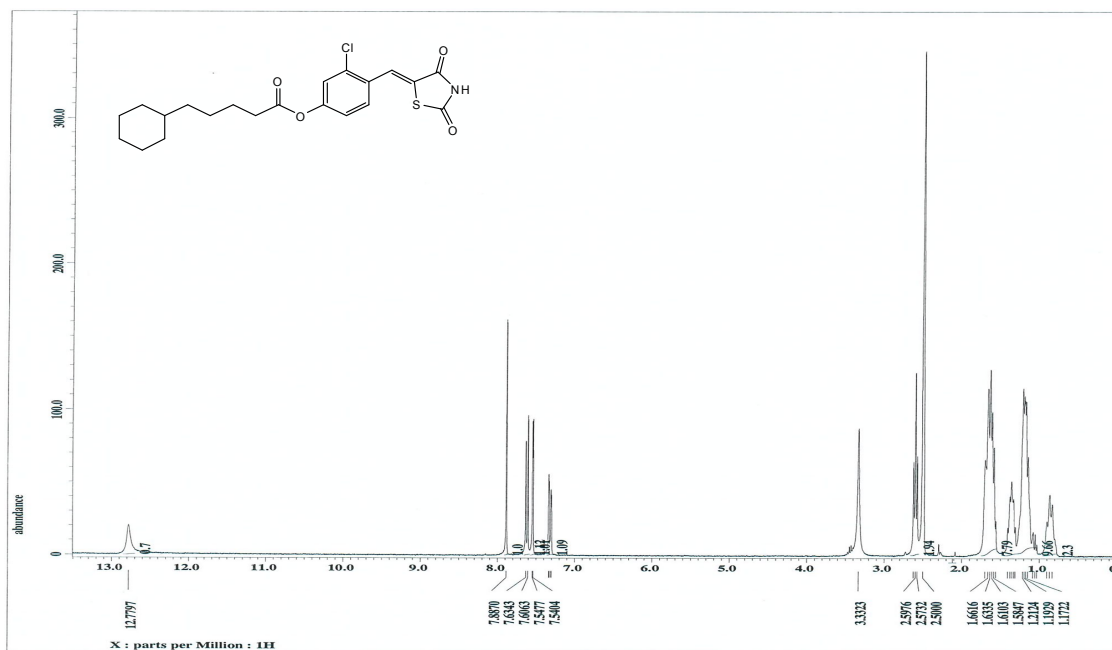
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-cyclohexyl-acetate (97)



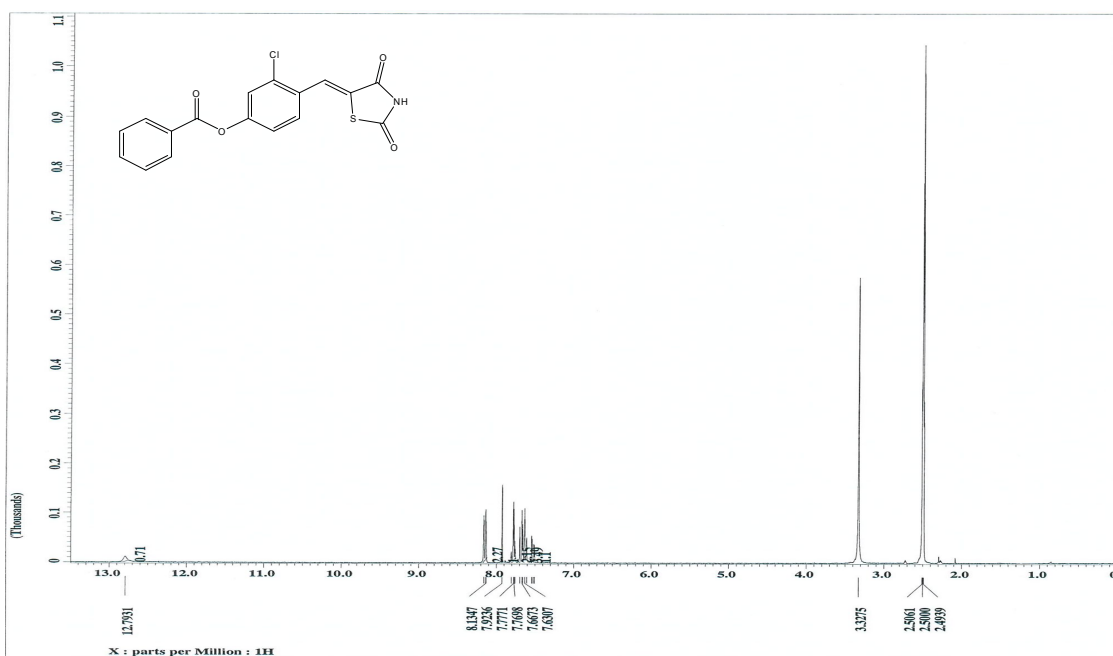
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-cyclohexylpropanoate (98)



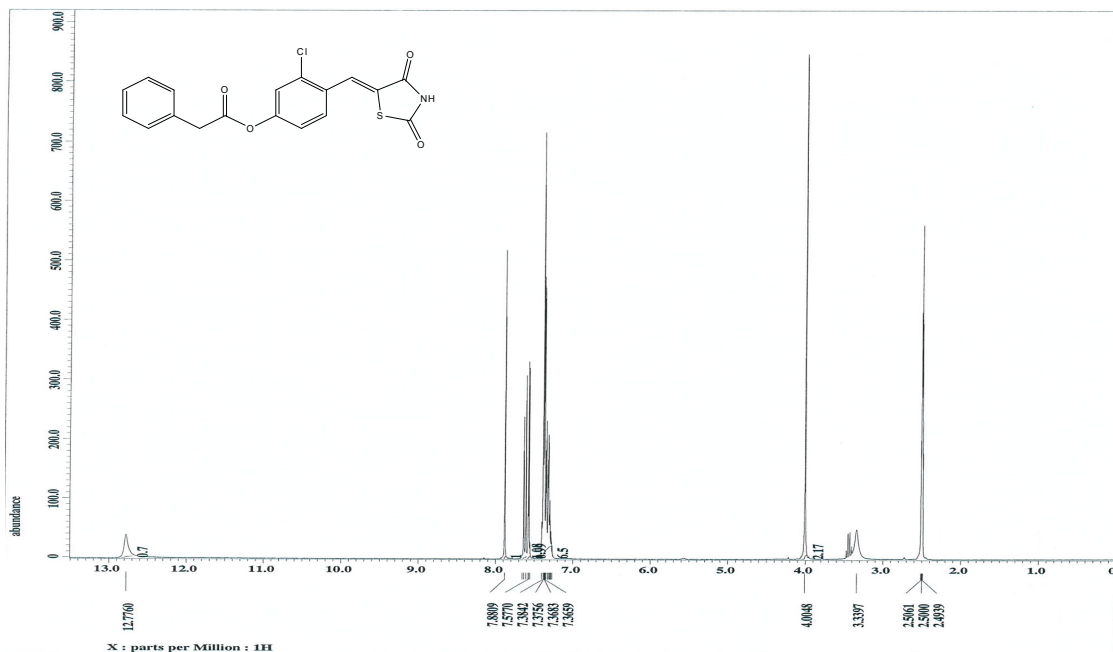
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-cyclohexylbutanoate (99)



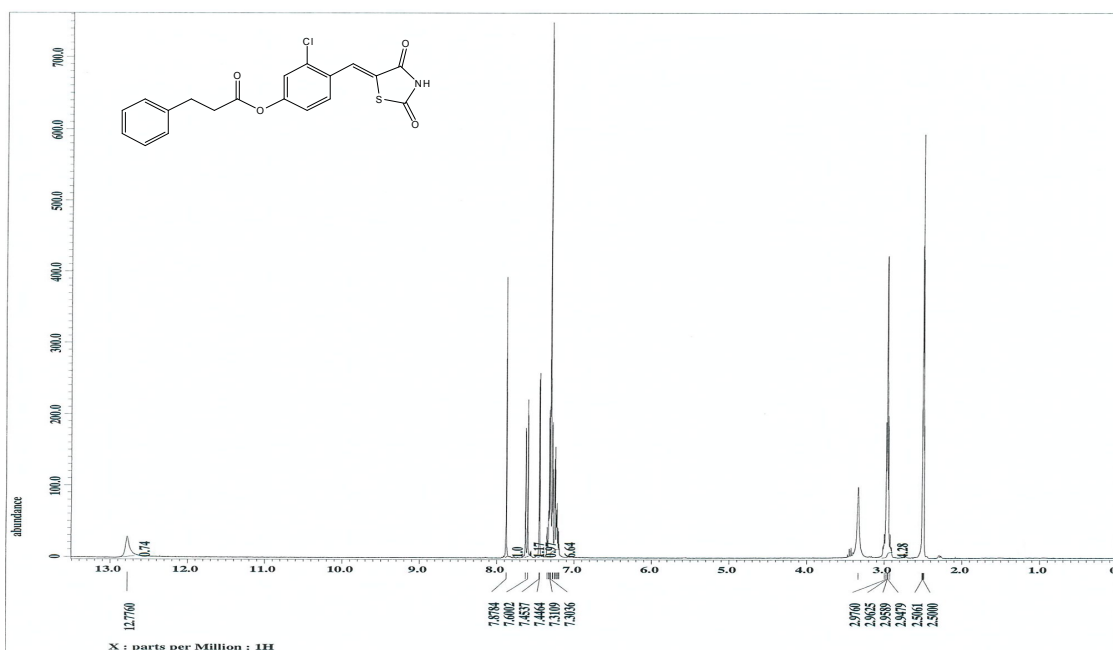
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-cyclohexyl-pentanoate (100)



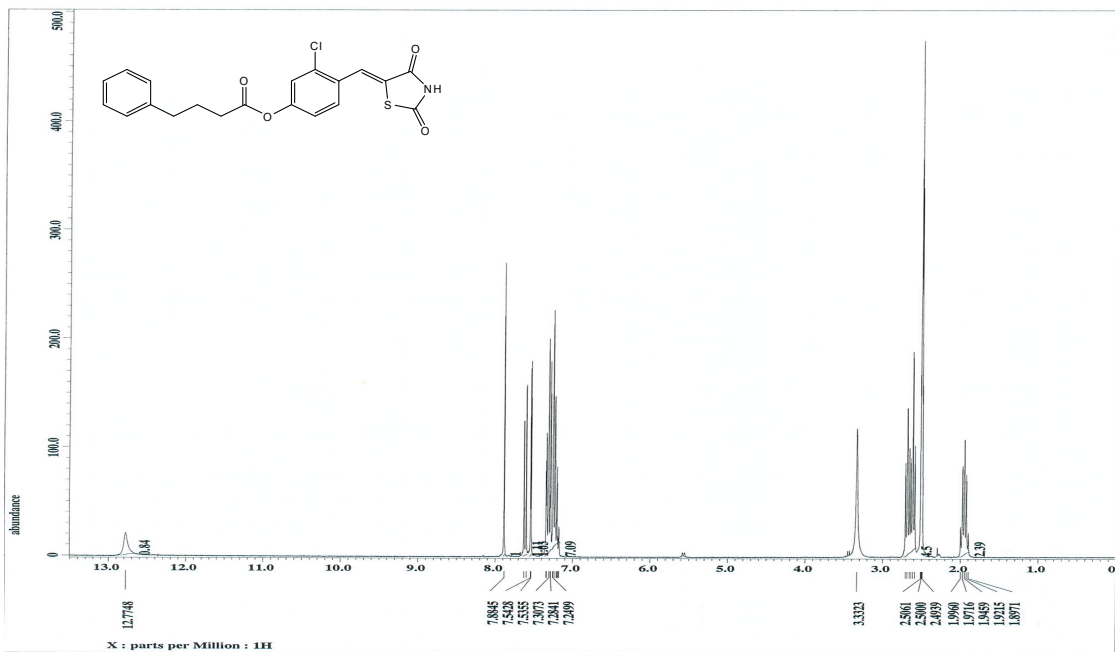
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzoate (101)



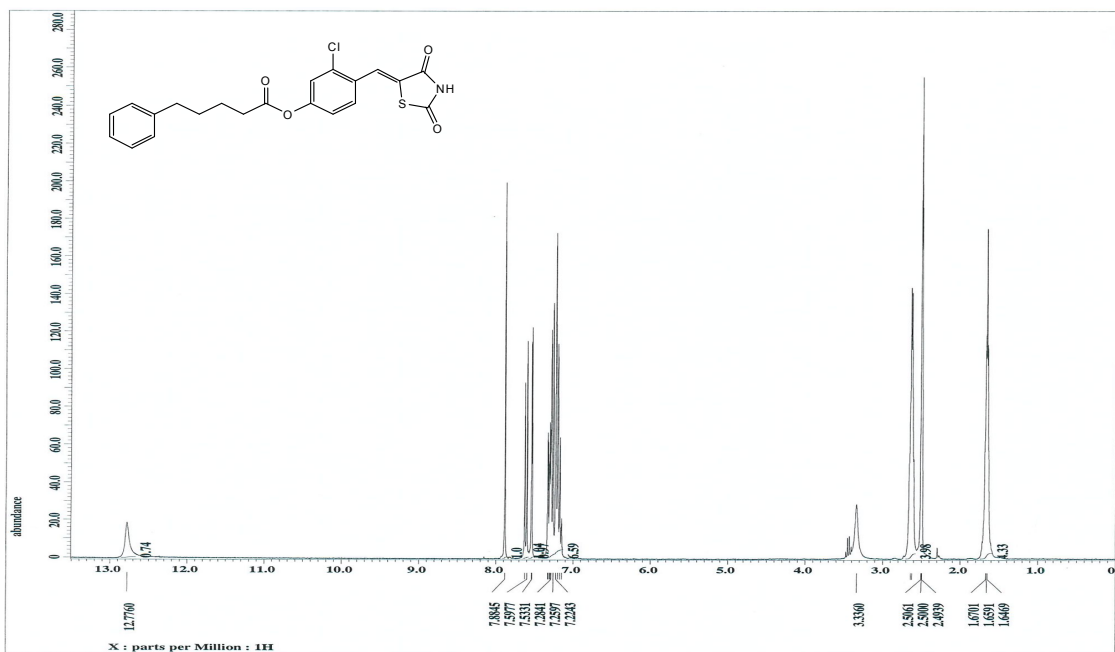
(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-phenylacetate (102)



(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 3-phenylpropanoate (103)



(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 4-phenylbutanoate (104)



(Z)-3-chloro-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 5-phenylpentanoate (105)