DESIGN AND SYNTHESIS OF 5-OXOPYRROLIDINE-3-CARBOXYLIC ACID DERIVATIVES AS POTENT ANTI-INFLAMMATORY AGENTS

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ABSTRACT

To established novel 5-oxopyrrolidine-3-carboxylic acid derivatives with improved anti-inflammatory activity. We are reporting here the synthesis and *in-vitro* anti-inflammatory evaluation of a series of pyrrolidinone derivatives. A series of new 5-oxopyrrolidine-3-carboxylic acid derivatives were synthesized from the reaction of 2-(5-((5-benzoyl-1H-benzo[d][1,2,3] triazol-1-yl) methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) acetohydrazide with aromatic aldehydes and succinic anhydride reaction. Anti-inflammatory activity of all the compounds was screened against MMP-2 and MMP-9, all results are excellent, **3d**, **3e**, and **3f** compounds are quite promising against matrix metalloproteins (MMPs). All the isolated compounds were characterized by elemental analysis, ¹HNMR, ¹³CNMR, and mass spectral analysis.

Keywords: Synthesis, 5-Oxopyrrolidine-3-carboxylic Acid, Anti-inflammatory Activity.

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INTRODUCTION

Heterocyclic compounds are the important class of molecules for its biologicals function in which pyrrolidinone hold exclusive because of its lead use.¹⁻⁵ Pyrrolidinones are heterocyclic compounds which show a broad area of biological potency, for example, antibacterial and antifungal.^{6,7} Pyrrolidinones are a class of 5-membered lactams with a four-carbon ring structure with biological interest.⁸⁻¹⁰ Pyrrolidinones are substantial molecules that are constructed in many pharmaceutical drug syntheses and active natural products. One familiar biochemically substantial pyrrolidinone is cotinine and alkaloid discovered in tobacco that is the predominant metabolite of nicotine.¹¹ Various Famous illustrations of natural products have the pyrrolidinone subdivision. Pyrrolidinone-having natural products exhibit immensely interesting biological activities. Lactacystin, e.g., is an organic compound naturally produced by bacteria of the genus *Streptomyces*, as was first discovered in 1971.¹² One more interesting natural product is called Salinosporamide A, this aquatic natural product is created by the constraining aquatic bacteria *Salinispora tropica* and *Salinispora arenicola*, which are in ocean silt.¹³ Azaspirorene is a new angiogenesis inhibitor that is detached from the fungus *Neosartorya* sp.¹⁴ Substituted 3-pyrroline-2-ones with a 2-pyrrolidinone subdivision are also of merits in medicinal chemistry as abundant derivatives have demonstrated momentous pharmacological and biological potency, as, e.g., anti-cancer agents^{15,16}, antitumours¹⁷, HIV-1 integrase inhibitors^{18,19}, anti-microbial²⁰, antibacterial²¹, and anti-inflammatory²². The anticipated concern of substituted pyrrolidinones, different synthetic procedures has been disclosed²³⁻³¹.

Over the past decades, many attempts have been made to identify the structural features³² essential for anti-inflammatory activity. As a result, it is well established that one of the important core fragments is defined by a nitrogen heteroatomic system, usually cyclic imide, with at least one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system.³³⁻³⁵ The examples of the above-mentioned structures are pyrrolidine-2,5-dione, pyrrolidin-2-one or imidazolidine-2,4-dione. They are present in the molecules of both first-generation AEDs such as phenytoin, ethosuximide, and also in newer drugs like levetiracetam, brivaracetam, and seletacetam (Fig.-1).

Fig.-1: Structures of Model AEDs based on the 5-Membered Heterocyclic Rings

Cyclic oxopyrrolidine³⁶ compounds are building blocks and are versatile intermediates for the total synthesis of a wide variety of natural and unnatural compounds with interesting biological activities³⁷. Oxopyrrolidine has countless pharmaceutical properties e.g. antibacterial, anticancer, antimicrobial, antiviral, and antigenic activities³⁸. Rebecamycin, which has an oxopyrrolidine structure, is known topoisomerase I inhibitor and exhibits anti-tumor activity against L1210 leukemia, B16 melanoma implanted in mice, and P388 leukemia and also inhibits the growth of human lung adenocarcinoma cells (A549)^{39,40} (Fig.-2). Among the maleimides, 3,4-disubstituted maleimides have also found applications in material science as components of red light-emitting diodes (LEDs) because of their intense color.⁴¹ These compounds have also been used in the development of photocatalysts immobilized on surfaces.⁴²

Fig.-2: Maleimides with Pharmaceutical Properties

A literature survey reveals that the pyrrolidine ring is important for anti-inflammatory activity. Also, many hetero-nitrogen derivatives exhibit a wide variety of biological activities, such as antimicrobial, antimycobacterial, antihistaminic, antihypertensive, hypnotic and anticonvulsant activity (Fig.-3). From previous work, Schiff bases are known to possess antimicrobial and anti-inflammatory activities. Also, thiosemicarbazide and triazole derivatives are of interest with potential activities including antimicrobial, analgesic, and anticonvulsant. In addition, we have reported that certain of our new heterocyclic compounds exhibited antibacterial and antifungal activities. Because of these observations and in continuation of our previous work in heterocyclic chemistry and the interest of Pyrrolidinone, in this

study, we have established a novel methodology for the preparation of substituted pyrrolidinone in the attendance of succinic anhydride as a reagent and toluene as a solvent (Fig.-4).

Fig.-3: Pyrrolidine Derivatives

EXPERIMENTAL

Materials

All the reactants were reagent grade, and purchased from Sigma Aldrich, and used without further purification unless otherwise stated. DCM (dichloromethane) was distilled from Calcium hydride (desiccant). All solvents were used without further drying or purification and were of ACS grade purchased from local suppliers. TLC plates (Silica Gel) were purchased from Sigma-Aldrich.

Instrumentation

Melting points were determined in open capillary tubes in a Buchi 510 circulating oil apparatus and are uncorrected (Table 1). Elemental analysis data were obtained with Perkin Elmer C, H, N analyzer model 2400. Nuclear Magnetic Spectroscopy (NMR) spectra were produced using the Varian 300 MHz spectrophotometer. The instrument was maintained at 25° C operating at 300 MHz for ¹H NMR, and 75 MHz for ¹³C NMR. The deuterated solvent (CDCl₃) used for each respective spectrum is referenced to the appropriate literature peak shift.

Procedure for the synthesis of (E)-N'-(substituted methylene)-2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetohydrazide ⁴³(2)

Take the compound **1** and Aromatic aldehyde (listed in scheme 1) in a molar ratio (1:1 or 1:2) and make soluble in the EtOH (100 cm³) and reflux in for about 4-5hrs at 79°C with a catalytic amount of glacial acetic acid (1-2 drops) on a water bath. The reaction was monitored by TLC. The mixture was evaporated under reduced pressure to give a residue. The residue was dissolved in DCM, and the organic layer was dried over Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel chromatography (PE/EtOAc = 1:1) and recrystallized from PE/EtOAc to afford products 3a-n.

General Procedure for the synthesis of 2-Substituted-1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-5-oxopyrrolidine-3-carboxylic acid ⁴⁴(3a-f)

Reactant **2** (0.135mol) and succinic anhydride (0.15mol) were dissolved in anhydrous toluene (150ml) and stirred under nitrogen during 24hr at 40 °C temperature. The progress of the reaction was measured by TLC (n-hexane: EtOH, 10:7). The mixture was cooled to room temperature and the solid was separated by filtration and purified by extraction with ethyl acetate after addition of sodium carbonate. The aqueous layer was treated with phosphoric acid. The white solid product separated by filtration was recrystallized in hot ethanol to obtain white crystal.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-5-oxo-2-phenylpyrrolidine-3-carboxylic acid (3a)

Yield 58.2 %; white solid, mp 192 – 193 °C; ¹H NMR (300 MHz, Chloroform-*d*) δ(ppm) 12.24 (s, 1H), 8.46 – 8.40 (m, 1H), 8.25 – 8.17 (m, 1H), 7.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.60 – 7.52 (m, 1H), 7.49 – 7.41 (m, 2H), 7.38 – 7.23 (m, 6H), 5.00 (d, *J* = 7.9 Hz, 1H), 4.69 (d, *J* = 17.6 Hz, 1H), 4.46 (q, 2H), 4.23 (d, *J* = 13.6 Hz, 1H), 3.35 (td, *J* = 10.9, 7.9 Hz, 1H), 2.77 (dd, *J* = 12.4, 10.9 Hz, 1H), 2.52 (dd, *J* = 12.4, 10.9 Hz, 1H). ¹³C NMR (75 MHz, Chloroform-*d*) δ(ppm) 197.9, 181.4, 176.2, 175.9, 168.7, 143.3, 142.6, 137.6, 137.2, 133.6, 133.3, 131.9, 129.7, 128.9, 128.5, 128.5, 128.4, 128.2, 118.1, 110.1, 70.2, 48.1, 44.8, 40.7, 32.8.; HRMS (ESI): *m/z* calculated for C₂₉H₂₃N₇O₆S (M+H)⁺ 598.14 found 598.11, Anal. Calc. for C₂₉H₂₃N₇O₆S: C, 58.29; H, 3.88; N, 16.41%; found: C, 58.29; H, 3.87; N, 16.39%.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-5-oxo-2-(p-tolyl)pyrrolidine-3-carboxylic acid (3b)

Yield: 63.3 %; white solid, mp 187 – 188 °C; ¹H NMR (300 MHz, Chloroform-*d*) δ(ppm) 12.24 (s, 1H), 8.44 – 8.40 (m, 1H), 8.25 – 8.18 (m, 1H), 7.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.61 – 7.52 (m, 1H), 7.49 – 7.40 (m, 2H), 6.92 (q, 5H), 5.03 – 4.97 (m, 1H), 4.69 (d, *J* = 17.6 Hz, 1H), 4.46 (q, 2H), 4.23 (d, *J* = 13.6 Hz, 1H), 3.35 (td, *J* = 10.9, 7.9 Hz, 1H), 2.77 (dd, *J* = 12.4, 10.9 Hz, 1H), 2.52 (dd, *J* = 12.4, 10.9 Hz, 1H), 2.31 (s, 3H). ¹³C NMR (75 MHz, Chloroform-*d*) δ(ppm) 197.9, 181.4, 176.2, 175.9, 168.7, 143.3, 142.6, 137.6, 136.9, 136.8, 133.6, 133.3, 132.0, 129.7, 129.2, 129.0, 128.5, 126.4, 118.1, 110.1, 70.2, 48.1, 44.8, 40.7, 32.8, 19.0.; HRMS (ESI): *m/z* calculated for C₃₀H₂₅N₇O₆S (M+H)⁺ 612.16 found 612.1 Anal. Calc. C₃₀H₂₅N₇O₆S: C, 58.91; H, 4.12; N, 16.03%; found: C, 58.90; H, 4.12; N, 16.02%.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-2-(4-methoxyphenyl)-5-oxopyrrolidine-3-carboxylic acid (3c)

Yield: 54.7 %; white solid, mp 181 – 182 °C; ¹H NMR (300 MHz, Chloroform-*d*) δ(ppm) 12.24 (s, 1H), 8.43 – 8.41 (m, 1H), 8.24 – 8.19 (m, 1H), 7.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 7.85 – 7.77 (m, 2H), 7.61 – 7.52 (m, 1H), 7.50 – 7.41 (m, 2H), 7.15 – 7.06 (m, 2H), 6.79 – 6.71 (m, 2H), 5.00 (dt, *J* = 7.9, 0.6 Hz, 1H), 4.69 (d, *J* = 17.6 Hz, 1H), 4.46 (q, 2H), 4.23 (d, *J* = 13.6 Hz, 1H), 3.84 (s, 3H), 3.35 (td, *J* = 10.9, 7.9 Hz, 1H), 2.77 (dd, *J* = 12.4, 10.9 Hz, 1H), 2.52 (dd, *J* = 12.4, 10.9 Hz, 1H). ¹³C NMR (75 MHz, Chloroform-*d*) δ(ppm) 197.8, 181.4, 176.2, 176.0, 168.7, 158.2, 143.3, 142.6, 137.6, 133.6, 133.3, 133.3, 131.9, 129.7, 129.1, 129.0, 128.5, 118.1, 114.3, 110.1, 70.2, 55.4, 48.1, 44.8, 40.7, 32.8.; HRMS (ESI): *m/z* calculated for C₃₀H₂₅N₇O₇S (M+H)⁺ 628.15 found 528.0 Anal. Calc. for C₃₀H₂₅N₇O₇S: C, 57.41; H, 4.02; N, 15.62%; found: C, 57.40; H, 3.99; N, 15.62%.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-2-(4-chlorophenyl)-5-oxopyrrolidine-3-carboxylic acid (3d)

Yield: 51.4%; white solid, mp 170 – 172 °C; ¹H NMR (300 MHz, Chloroform-*d*) δ(ppm) 12.24 (s, 1H), 8.44 – 8.40 (m, 1H), 8.25 – 8.18 (m, 1H), 7.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.60 – 7.52 (m, 1H), 7.49 – 7.41 (m, 5H), 7.38 – 7.31 (m, 2H), 5.00 (dt, *J* = 7.9, 0.6 Hz, 1H), 4.69 (d, *J* = 17.6 Hz, 1H), 4.46 (q, 2H), 4.23 (d, *J* = 13.6 Hz, 1H), 3.35 (td, *J* = 10.9, 7.9 Hz, 1H), 2.77 (dd, *J* = 12.4, 10.9 Hz, 1H), 2.52 (dd, *J* = 12.4, 10.9 Hz, 1H). ¹³C NMR (75 MHz, Chloroform-*d*) δ(ppm) 197.9, 181.4, 176.2, 175.9, 168.7, 143.3, 142.6, 137.6, 136.9, 133.6, 133.3, 132.1, 131.9, 129.7, 129.1, 128.9, 128.5, 127.5, 118.1, 110.1, 70.2, 48.1, 44.8, 40.7, 32.8.; HRMS (ESI): *m/z* calculated for C₂₉H₂₂ClN₇O₆S₂ (M+H)⁺ 632.10 found 632.07 Anal. Calc. for C₂₉H₂₂ClN₇O₆S₂: C, 55.11; H, 3.51; N, 15.51%; found: C, 55.10; H, 3.50; N, 15.51%.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-5-oxo-2-(thiophen-2-yl)pyrrolidine-3-carboxylic acid (3e)

Yield: 60.1 %; white solid, mp 187 – 188 °C; ¹H NMR (300 MHz, Chloroform-*d*) δ(ppm) 12.24 (s, 1H), 8.44 – 8.39 (m, 1H), 8.25 – 8.17 (m, 1H), 7.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.62

– 7.52 (m, 1H), 7.49 – 7.40 (m, 2H), 7.18 (dd, $J = 5.4, 1.8$ Hz, 1H), 6.94 (dd, $J = 6.5, 5.5$ Hz, 1H), 6.75 (ddd, $J = 6.6, 1.7, 0.6$ Hz, 1H), 5.00 (dd, $J = 7.5, 0.6$ Hz, 1H), 4.69 (d, $J = 17.6$ Hz, 1H), 4.46 (q, 2H), 4.23 (d, $J = 13.6$ Hz, 1H), 3.35 (td, $J = 10.5, 7.5$ Hz, 1H), 2.77 (dd, $J = 12.4, 10.5$ Hz, 1H), 2.52 (dd, $J = 12.4, 10.6$ Hz, 1H). ^{13}C NMR (75 MHz, Chloroform- d) δ (ppm) 197.9, 181.4, 175.3, 173.5, 168.7, 143.3, 142.6, 137.6, 137.1, 133.6, 133.3, 131.9, 129.7, 128.9, 128.5, 125.7, 124.8, 124.8, 118.1, 110.1, 66.8, 48.1, 44.8, 38.4, 32.4.; HRMS (ESI): m/z calculated for $\text{C}_{27}\text{H}_{21}\text{N}_7\text{O}_6\text{S}_2$ ($\text{M}+\text{H}$) $^+$ 604.10 found 604.06 Anal. Calc. for $\text{C}_{27}\text{H}_{21}\text{N}_7\text{O}_6\text{S}_2$: C, 53.72; H, 3.51; N, 16.24%; found: C, 53.70; H, 3.51; N, 16.23%.

1-(2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamido)-2-(2-chloropyridin-4-yl)-5-oxopyrrolidine-3-carboxylic acid (3f)

Yield: 66 %; white solid, mp 198 – 199 °C; ^1H NMR (300 MHz, Chloroform- d) δ (ppm) 12.24 (s, 1H), 8.73 (d, $J = 4.7$ Hz, 1H), 8.45 – 8.40 (m, 1H), 8.25 – 8.17 (m, 1H), 7.94 – 7.78 (m, 5H), 7.61 – 7.52 (m, 1H), 7.50 – 7.41 (m, 2H), 7.35 (dd, $J = 2.2, 0.6$ Hz, 1H), 5.00 (dt, $J = 7.9, 0.6$ Hz, 1H), 4.69 (d, $J = 17.6$ Hz, 1H), 4.46 (q, 2H), 4.23 (d, $J = 13.6$ Hz, 1H), 3.35 (td, $J = 10.9, 7.9$ Hz, 1H), 2.77 (dd, $J = 12.4, 10.9$ Hz, 1H), 2.52 (dd, $J = 12.4, 10.9$ Hz, 1H). ^{13}C NMR (75 MHz, Chloroform- d) δ (ppm) 197.9, 181.4, 176.2, 175.9, 168.7, 148.9, 145.9, 144.4, 143.3, 142.6, 137.6, 133.6, 133.3, 131.9, 129.7, 128.9, 128.5, 126.2, 124.2, 118.1, 110.1, 70.2, 48.1, 44.8, 40.7, 32.8. HRMS (ESI): m/z calculated for $\text{C}_{28}\text{H}_{21}\text{ClN}_8\text{O}_6\text{S}$ ($\text{M}+\text{H}$) $^+$ 633.10 found 633.06 Anal. Calc. for $\text{C}_{28}\text{H}_{21}\text{ClN}_8\text{O}_6\text{S}$: C, 53.13; H, 3.34; N, 17.70%; found: C, 53.12; H, 3.33; N, 17.68%.

Table 1: Physiochemical Properties of the Synthesized Compounds (3a-f)

Compound	R	Colour	Yield (%)	Melting Point (°C)
3a	C_6H_5 -	White solid	58.2	192-194
3b	$p\text{-CH}_3\text{C}_6\text{H}_5$ -	White solid	63.3	187-189
3c	$p\text{-OCH}_3\text{C}_6\text{H}_5$ -	White solid	54.7	181-183
3d	$p\text{-ClC}_6\text{H}_5$ -	White solid	51.4	170-172
3e	2-thienyl	White solid	60.1	187-189
3f	2-ChloroPyridine	White solid	66	198-200

Table 2: *In vitro* Anti-inflammatory Results of Compounds 3a-f with % Bands and % Inhibition of MMP-2 and MMP-9

Entry	% Bands of MMP		% Inhibition of MMP	
	MMP-9	MMP-2	MMP-9	MMP-2
3a	85	65	15	35
3b	90	85	10	15
3c	40	55	60	45
3d	100	18	Nil	82
3e	100	15	Nil	85
3f	70	20	30	80
Tetracycline	00	00	100	100
DMSO control	-	-	-	-

Anti-inflammatory Screening

Inflamed tissue samples were collected from clinically diagnosed patients of chronic periodontitis and chopped it completely. The patients had no background in dental treatment or antibiotic or anti-inflammatory therapy for at least 3 months before the study. Tris buffer (5 mL) was centrifuged along with the inflamed tissue samples at 3000 rpm for 15 min and stored at 20 °C for additional use. It was named as MMP extract. 10 mg of each target compound was dissolved in 1 mL of DMSO separately. Hence, each mL contains 10 μg of the compound. For the initial evaluation, three trials were carried out with nonidentical concentrations, namely 100 $\mu\text{g}/\text{mL}$, 250 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ and found 500 $\mu\text{g}/\text{mL}$ with good inhibition. So, 0.05 mL of each sample which contained 500 $\mu\text{g}/\text{m}^3$ was prepared for all samples and used. 0.05 mL of MMP extract and 0.05 mL of the synthesized compounds were mixed separately and then incubated for 15–30 min. 29 non-reducing buffer in equal volume was added, mixed

and 0.02 mL was loaded into each well using gel loading tips and 10 mm³ molecular weight marker was added to last well. 0.05 mL of a tissue sample with 0.9% normal saline used as the control. Electrodes were coupled and the tank was closed with cover and cables were stippled into the power supply. Initially, the gel electrophoresis apparatus was run at about 50V for 15 min and then at 100V until the bromophenol blue reached the bottom of the plates. After electrophoresis, the equipment was disassembled and the gel was gently removed and the equipment was washed with zymogram renaturing buffer, i.e., 2.5% Triton X-100 for 1 h to remove SDS from the gel and let the proteins denature. The zymogram renaturing buffer was removed and the gel was incubated in zymogram incubation buffer at 37°C overnight. The gel was smeared with Coomassie blue R-250 for 1 h. Gels should be smeared with an appropriate Coomassie R-250 smearing solution for about 2 h. The background stains blue with Coomassie stain where the gelatin is degraded. An appeared white band indicates the presence of gelatinases. The upper bands are gelatinase B (MMP-9) while the lower bands are gelatinase A (MMP-2) which are examined and using multi-image gel documentation systems the percentage of inhibition was assigned.

RESULTS AND DISCUSSION

Our research was focused on the verification of pyrrolidinones synthesis via a convenient and easy method. To the solution of (1H-benzo[d][1,2,3]triazol-5-yl)(phenyl)methanone in absolute Methanol, methyl chloroacetate, hydrazine monohydrate and anhydrous K₂CO₃ were heated under reflux to give 2-(5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (m.p. 145-147 °C) as a product which than reacted with CSCI₂ in presence of potassium hydroxide to obtained phenyl(1-((5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo [d] [1,2,3]triazol-5-yl) methanone (m.p. 177-179°C) as a second product which than again reacted with hydrazine monohydrate, methyl chloroacetate in presence of anhydrous K₂CO₃ to give 2-(5-((5-benzoyl-1H-benzo[d][1,2,3]triazol-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)aceto hydrazide (mp 186-188 °C) as a product (**1**). The detailed procedure for the above mention products was previously published⁴⁵ (Scheme-1).

Scheme-1: Reagents and Conditions (a) Various Aromatic Aldehyde, EtOH, Glacial Acetic Acid, Reflux, 4-5 hrs.
(b) Anhydrous Toluene, Succinic Anhydride, R.T., under N₂, 24h, ~50-60%.

Anti-inflammatory Activity

The anti-inflammatory activity of all newly synthesized compounds 3a–3f was screened using the gelatin zymography method; this method can detect degrading gelatin which is generating by proteolytic enzymes. The two key matrix metalloproteinases such as 72 kDa called gelatinase A (MMP-2) and 92 kDa gelatinase B (MMP-9), these two proteins have potent gelatin-degrading properties.^{46,47} In the physiological system, various gelatinases are present, and they play a key role in inflammation and autoimmunity stats⁴⁸. Thus, the activated inflammatory cells can express several proteinases designated as matrix metalloproteinases (MMPs) these can degrade all connective tissue macromolecules. Among these gelatinases, MMP-2 and MMP-9 together forming collagenase play an important role in connective tissue remodeling⁴⁹. MMP-2 and MMP-9 remain inactive, while they are with pro-domain, they get activates by denaturation, then it could be perceived on gelatin zymograms after staining with Coomassie

blue as one or two bands which are designated as a pro and activated forms. In vitro, anti-inflammatory activity results obtained are summarized in Table-2 and the inhibition percentage of MMP-2 and MMP-9 for each compound was calculated by subtracting from 100 with % of bands obtained. The listed results notice that all the synthesized hybrids (3a–3f) are active against both MMPs (MMP-2 and MMP-9). Interestingly, the series, compounds 3a–3f are more potential and promising against both MMPs and the high potentiality might be the hydrophilic nature of the compounds. The obtained results are also presented in Fig.-5.

Fig.-4: Synthesized 5-Oxopyrrolidine-3-carboxylic Acid Derivatives.

Fig. 5: Anti-inflammatory Activity of Synthesized Compounds 3a–f.

CONCLUSION

Few new heterocyclic compounds that contain 5-oxopyrrolidine-3-carboxylic acid moiety were synthesized using simple methods, their structures were proven by spectral characterization and they were tested for their anti-inflammatory activities. Most of the compounds showed promising activities against MMP-2 and MMP-9. These results are encouraging for the synthesis of similar compounds shortly.

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