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SYNTHESIS OF NOVEL SPIROTHIAZOLIDINONE DERIVATIVES AND EVALUATION OF ANTI-INFLAMMATORY ACTION

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ABSTRACT: The objective of this investigation was to synthesize spirothiazolidinone-chalcone derivatives and evaluate them for anti-inflammatory action. Five 4-substituted benzaldehydes were utilized for the aldol condensation leading to chalcones of spirothiazolidinone. The compounds were obtained in yield of 64-72% and displayed varying solubility with all compounds soluble in chloroform and DMSO. The NMR spectra revealed protons of amine, imine, hydroxy and aromatic groups. The presence of the molecular ion peak of the isotopic peak was found in the mass spectra of the compounds confirming the formation of the compounds. The anti-inflammatory activity was determined using the albumin denaturation method and antiprotease method. All the compounds exhibited dose dependent inhibition of albumin denaturation with 7d having the highest capacity to cause the inhibition (61.56 ± 1.033 %) at the concentration of 500µg/mL. The antiprotease action was also dose dependent and 7d at 500µg/mL was able to inhibit (46.32±3.011 %) of protease activity. The type of substitution on the chalcone phenyl ring played a vital role in the activity of the compounds. The results led to the conclusion that newer chalcone based molecules with anti-inflammatory activity were obtained from the current work.

INTRODUCTION: Spirocyclic compounds isolated from plant and animal origins have important applications in medicinal chemistry [1]. Spiro compounds having cyclic structures fused at a central carbon are of recent interest because of their interesting conformational features and their structural implications for biological systems. The asymmetric characteristic of the molecule due to the chiral spiro carbon is one of the important criteria of biological activities.

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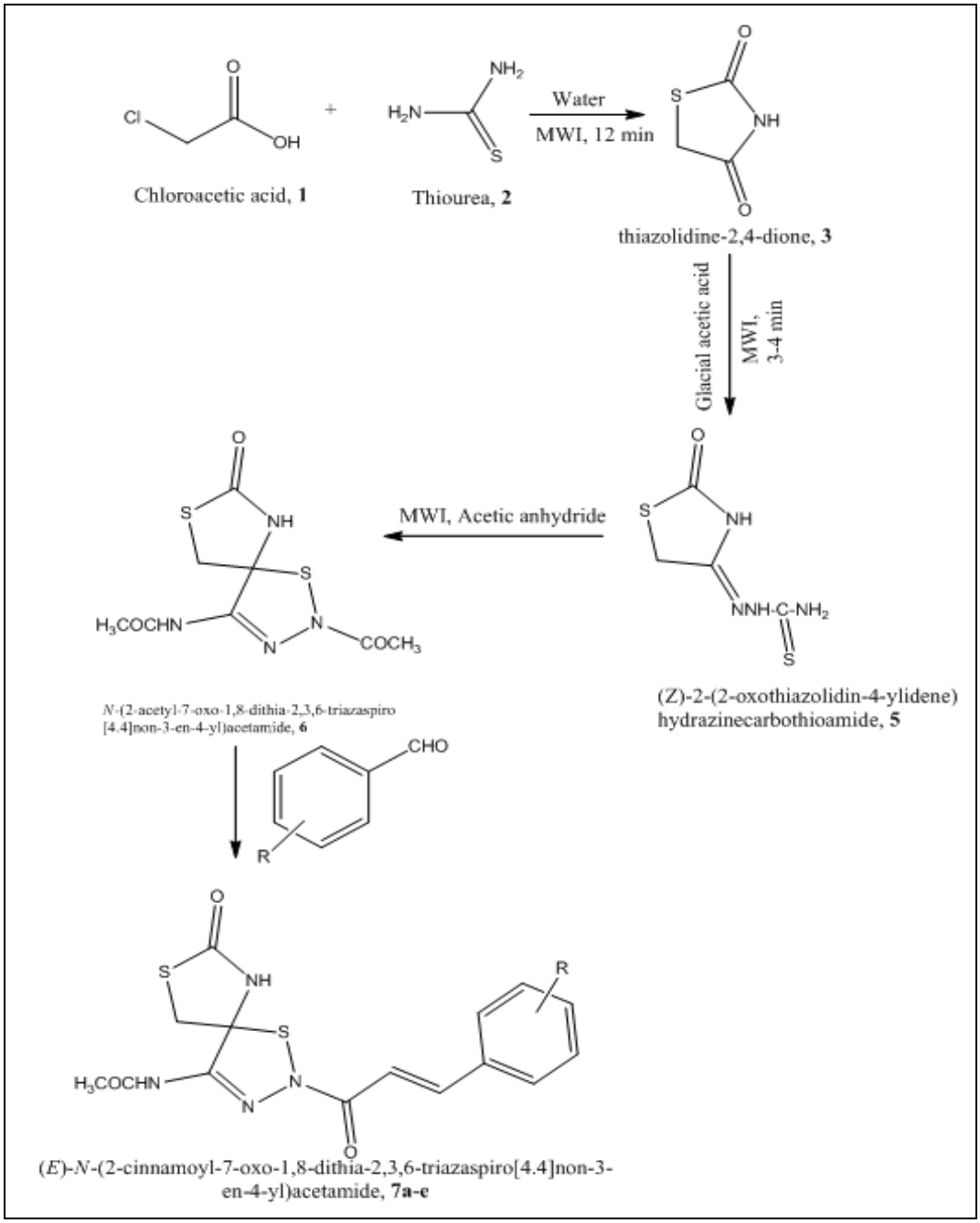
Spiro compounds represent an important class of naturally occurring substances characterized by their highly pronounced biological properties [2-6]. Although, a number of molecules have been developed as antimicrobial agents. But the demand for the effective and potent antimicrobial agent is always on high priority due to the development of resistant for the current drugs.

Spiro moiety is part of various natural products and medicinal agents. In literature, it has been reported that sharing of the indole-3 carbon atom during formation of spirothiazolidinone derivatives greatly enhances its biological activity. Thus, considering this fact, we planned to synthesize some spirothiazolidinone derivatives to improve antimicrobial efficacy.

MATERIAL AND METHODS:

Materials: All the reagents and chemicals were used as obtained. All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure [7]. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR). Melting point were determined on Biotechnics melting point apparatus using open capillary. The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates using hexane: ethylacetate in the ratio 8:2.

The synthesis of the desired compounds was achieved using scheme 1.



SCHEME 1: SYNTHETIC SCHEME

Preparation of 3,4-thiazolidinedione: To 0.6 mole of chloroacetic acid, **1** in 60 ml of water, followed by the addition of 0.6 mole of thiourea, **2**. The reaction mixture was stirred until the completion of the reaction (white precipitate was formed, approximately 15 min.) and refluxed under microwave irradiation at 240 Watt power until the completion of the reaction (approximately 12 min).

On cooling, a solid separated, which was recrystallized from ethyl alcohol to give the pure thiazolidine-2,4-dione, 3 [8].

Preparation of Hydrazine Carbothioamide Derivative, 5: Thiazolidine-3,4-dione, **3** (0.94 mmol) was taken in a mixture of thiosemicarbazide, 4 (0.95 mmol) and glacial acetic acid (3.5 mL) and the reaction mixture was irradiated under microwave for 50 sec pulses.

The progress of the reaction was monitored by TLC (EtOAc: Hexane, 1:4). On completion of reaction (3-4 min), the reaction mixture was cooled to room temperature. A yellow solid was obtained, which was filtered off and washed with hexane and recrystallized from methanol to give the hydrazinecarbothioamide compound as a pale yellow crystalline solid.

Preparation of Triazaspiro Derivative, 6: Compound 5 (0.53 mmol) and freshly distilled acetic anhydride (2.5 mL) were irradiated under microwave for 5 minutes. The progress of the reaction was monitored by TLC (EtOAc: $CHCl_3$, 1:3). The reaction mixture was cooled to room temperature. A yellow solid was obtained, which was filtered off and crude solid was recrystallized from MeOH to give compound 6.

General Method for Synthesis of Compounds 7a-e: In a fume hood was placed a two-necked flask, equipped with a magnetic stirring bar, and reflux condenser was added 0.2g of the compound 6 and 10 mL of dried THF. The round bottom flask is placed in an ice bath kept on a stirring plate and 2 equivalent of desired benzaldehyde was slowly added to it. The two-necked flask is then placed in the microwave and irradiate at constant power of 400 W for 15 minutes. After this period the reaction mixture was poured into a vessel containing ice (30 g) and hydrochloric acid was added to it to adjust pH to 2.5. The formed solid is filtered using a vacuum filtration apparatus.

Inhibition of Albumin Denaturation: The technique of inhibition of albumin denaturation reported previously [9, 10] was used with slight modifications. The synthesized molecules were individually dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration.

A solution of 1% BSA in deionized water was prepared for the test. The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solutions. Ibuprofen solution (1 µg/mL) was used in the positive control and distilled water was used in the negative control vessels instead of test solution. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

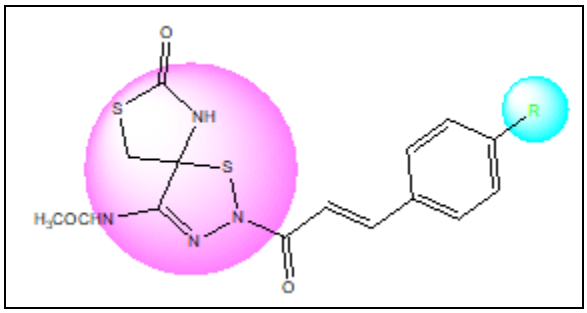
Antiprotease Action Method: The technique of antiprotease action reported by Oyedepo *et al* [11] and Sakat *et al* [12] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mMTris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL).

The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of protease inhibitory activity was calculated by the following formula:

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

RESULTS: Five 4-substituted benzaldehydes were utilized for the aldol condensation leading to chalcones of spirothiazolidinone. The compounds were characterized for yield, melting point, solubility and retention factor (by TLC). The physicochemical properties of the compounds 7a-e using the various aromatic aldehydes is presented in **Table 1.**

TABLE 1: PHYSICOCHEMICAL FEATURES OF SPIROTHIAZOLIDINONE-CHALCONE COMPOUNDS, 7A-E



Compound	R	Color	Yield (%)	Melting Point (°C)
7a	H	White	72	210-212
7b	OH	White	75	211-214
7c	Cl	Pale White	67	199-203
7d	NO_2	Pale Yellow	64	185-190
7e	CH_3	Pale Yellow	67	205-208

The solubility of the synthesized compounds 7a-e was determined in water, methanol, chloroform and DMSO. The compounds displayed varying solubility with all compounds soluble in chloroform and DMSO.

The structure of the compounds was determined using proton NMR in $CDCl_3$ solvent, IR and Mass study.

(E)-N-(2-cinnamoyl-7-oxo-1, 8-dithia-2, 3, 6-triazaspiro [4.4] non-3-en-4-yl) acetamide, 7a: Molecular Formula: $C_{15}H_{14}N_4O_3S_2$; FT-IR (cm^{-1}): 3342 (N-H), 3907 (C-H aliphatic), 1690.78 (C=O); 1H-NMR (DMSO-d6) (δ ppm): 2.087 (N-H), 2.541 (CH methylene), 3.881 (CH methoxy), 6.962-7.980 (CH Aromatic); Mass (m/e): 365.1 (M^++2).

(E)-N-(2-(3-(4-hydroxyphenyl) acryloyl)-7-oxo-1, 8-dithia-2, 3, 6-triazaspiro[4.4] non-3-en-4-yl) acetamide. 7b: Molecular Formula: $C_{15}H_{14}N_4O_4S_2$; FT-IR (cm^{-1}): 2925 (C-H aliphatic), 1741 &1683.50 (C=O); 1H-NMR (DMSO-d6) (δ ppm): 2.087 (N-H), 2.750-2.541 (CH methylene), 3.881 (CH methoxy), 6.962-7.980 (CH Aromatic); Mass (m/e): 379.0 (M^+).

(E)-N-(2-(3-(4-chlorophenyl)acryloyl)-7-oxo-1,8-dithia-2, 3, 6-triazaspiro[4.4] non-3-en-4-yl) acetamide, 7c: Molecular Formula: $C_{15}H_{13}ClN_4O_3S_2$;FT-IR (cm^{-1}): 3340.46 (N-H), 2756.18 (C-H aliphatic), 1699.89 (C=O); 1H-NMR (DMSO-d6) (δ ppm): 2.082 (N-H), 2.539 (CH methylene), 3.884 (CH methoxy), 6.952-7.983 (CH Aromatic); Mass (m/e): 517.0 (M^+).

(E)-N-(2-(3-(4-nitrophenyl) acryloyl)-7-oxo-1, 8-dithia-2, 3, 6-triazaspiro[4.4] non-3-en-4-yl) acetamide, 7d: Molecular Formula: $C_{15}H_{13}N_5O_5S_2$; FT-IR (cm^{-1}): 2759.22 (C-H aliphatic), 1741.65 &1682.94 (C=O); 1H-NMR (DMSO-d6) (δ ppm): 2.085 (N-H), 2.576 (CH methylene), 3.980 (CH methoxy), 6.807-8.018 (CH Aromatic); Mass (m/e): 407.5 (M^+).

(E)-N-(7-oxo-2-(3-(p-tolyl)acryloyl)-1, 8-dithia-2, 3, 6-triazaspiro[4.4]non-3-en-4-yl)acetamide, 7e: Molecular Formula: $C_{16}H_{16}N_4O_3S_2$; FT-IR (cm^{-1}): 2756.14 (C-H aliphatic), 1748.01 &1690.93 (C=O); 1H-NMR (DMSO-d6) (δ ppm): 2.085 (N-H), 2.576 (CH methylene), 3.980 (CH methoxy), 6.807-8.018 (CH Aromatic); Mass (m/e): 377.0 (M^+).

Anti-inflammatory Action: The anti-inflammatory action of the synthesized compounds was evaluated using two of the well established *in-vitro* methods *viz.*, antiprotease activity and inhibition of albumin denaturation. The results are presented in table 2 and 3 respectively.

TABLE 2: INHIBITION OF ALBUMIN DENATURATION BY TEST COMPOUNDS

Treatment	Inhibition of albumin denaturation (%)					
	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	10 µg/mL
7a	5.2 ± 0.391	10.8 ± 1.246	20.6 ± 1.044	28.7 ± 2.217	38.2 ± 2.759	ND

7b	4.37 ± 1.163	9.16 ± 1.196	16.79 ± 1.695	21.13 ± 2.068	28.57 ± 3.162	ND
7c	8.69 ± 1.169	18.77 ± 2.016	31.03 ± 2.032	41.75 ± 2.116	50.87 ± 0.066	ND
7d	12.98 ± 2.105	22.68 ± 2.004	32.39 ± 3.036	51.13 ± 3.101	61.56 ± 1.033	ND
7e	8.22 ± 2.002	12.73 ± 2.139	23.15 ± 2.058	33.93 ± 2.189	40.84 ± 2.534	ND
Ibuprofen	ND	ND	ND	ND	ND	52.38 ± 2.516

ND-Not Determined; n=5; Values are Mean ± SD

TABLE 3: PERCENT INHIBITION OF PROTEASE ACTION BY TEST COMPOUNDS

Treatment	Inhibition of Protease Action (%)					
	10 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Ibuprofen	52.26 ± 1.066	ND	ND	ND	ND	ND
7a	ND	4.09 ± 0.066	7.22 ± 0.033	10.79 ± 1.033	19.01 ± 1.033	22.75 ± 3.011
7b	ND	3.53 ± 0.613	6.85 ± 0.869	10.01 ± 1.135	13.46 ± 1.135	18.96 ± 1.139
7c	ND	6.15 ± 1.039	13.33 ± 0.911	18.25 ± 2.136	22.08 ± 1.299	32.95 ± 3.113
7d	ND	8.38 ± 1.066	14.96 ± 1.038	20.73 ± 2.111	33.29 ± 2.036	46.32 ± 3.011
7e	ND	4.56 ± 0.925	8.23 ± 0.663	13.21 ± 0.966	17.93 ± 2.033	26.11 ± 2.123

ND-Not Determined; n=5; Values are Mean±SD

DISCUSSION: The synthesis of the spirothiazolidinone compounds was done via the reaction of thiazolidine-3,4-dione with thiosemicarbazide under MW-irradiation to yield Schiff bases which were subjected to intramolecular cyclization in presence of acetic anhydride resulting in the formation of the spiro compound. The spiro compound was reacted with various aromatic aldehydes using aldolcondenstation resulting in the formation of the desired chalcone derivatives. Previous study has shown that microwave assisted synthesis of spirothiazolidines using water as solvent resulted in 90-95% yield of the compound1 [13].

In the IR spectrum, the sharp absorption bands at 3340 corresponding to NH stretching and 1740 to 1680 corresponding to carbonyl groups were prominent along the fingerprint bands of the aromatic system. The NMR spectra revealed protons of amine, imine, hydroxy and aromatic groups. The presence of the molecular ion peak of the isotopic peak was found in the mass spectra of the compounds confirming the formation of the compounds.

The anti-inflammatory activity was determined using the albumin denaturation method and antiprotease method. Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. It has been said that tissue injury might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of the test compounds to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity. It has also been reported that leukocytes protease has an important role in the development of tissue damage during inflammatory reactions and significant level of protection could be provided by protease inhibitors. Hence the inhibition of protease action by test compounds signifies its role as anti-inflammatory molecules.

All the compounds exhibited dose dependent inhibition of albumin denaturation with 7d having the highest capacity to cause the inhibition (61.56 ± 1.033 %) at the concentration of 500µg/mL. The antiprotease action was also dose dependent and 7d at 500µg/mL was able to inhibit (46.32±3.011 %) of protease activity. The type of substitution on the chalcone phenyl ring played vital role in the activity of the compounds. It was evident from the results that the higher the electron withdrawing ability of the substitution attached on this ring, the better was the anti-inflammatory activity of the compound. The order of activity was 7d>7c>7e>7a>7b.

CONCLUSION: In the present study, spriothiazolidinone-chalcone compounds were synthesized using the reaction of thiazolidinedione and thiosemicarbazide to prepare the reactive intermediate which underwent intramolecular cyclization and finally aldol condensation. The compounds were found to be of good purity and yield. The compounds exhibited good antibacterial potential against gram negative bacterium tested. Two compounds 7d & 7c were found to be significantly potent. These compounds would be used for designing newer similar compounds with

the aid of computer aided drug design techniques like pharmacophore modeling or docking which might lead to generation of a new lead molecule for antiinflammatory activity.

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CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation.

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