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In-vitro ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF NOVEL 1-SUBSTITUTED-3-SUBSTITUTED PROPANE-1,3-DIONES (β-DIKETONES) DERIVED FROM VANILLIN

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ABSTRACT

In synthetic chemistry,propane-1,3-diones or β-diketones act as significant intermediates for the synthesis of core heterocycles due to their specific chemoselectivity properties as well as provide convenient building blocks to biologically active substituents. In the present work, a new series of β-diketones namely 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl) propane-1,3-diones 4(a-e) have been synthesized from versatile 4-hydroxy-3-methoxybenzaldehyde viz. vanillin and studied their invitro anti-inflammatory and antioxidant potential by performing inhibition of protein denaturation assay and reducing power assay method respectively. All the newly synthesized compounds exhibit notable and satisfactory anti-inflammatory potential as well as potent antioxidant activity due to the presence of vanillin moiety in their molecular structures.

Keywords: β-diketones, Propane-1,3-diones, Vanillin, Anti-Inflammatory, Antioxidant.

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INTRODUCTION

The property of substances that reduce inflammation is termed anti-inflammatory and substances as anti-inflammatory drugs. Diseases or medical conditions that cause inflammation have a name ending in '-itis' such as Bronchitis (an inflammation of the bronchi), Cystitis (an inflammation of the bladder), Dermatitis (a disease where the skin is inflamed), Uveitis (an inflammation inside the eye), etc. Usually, non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most common therapeutic groups of agents used worldwide for the treatment of pain, inflammation, and fever.[1] Most frequently used NSAIDs drugs are salicylates (such as Aspirin), paraaminophenol derivatives (such as Paracetamol), pyrrole derivatives (such as Ketorolac), indole derivatives (such as Ibuprofen), propionic acid derivatives (such as ibuprofen, Ibuprofen and Paracetamol combination, Flurbiprofen, Ketoprofen, Naproxen, Fenamates, and Mefenamic acid), aryl acetic acid derivatives (such as Diclofenac sodium, Diclofenac potassium, Diclofenac and paracetamol combination, Diclofenac and Serratiopeptidase combination), pyrazolones (such as Phenylbutazone and Oxyphenbutazone), others (such as Celecoxib, Rofecoxib, Valdecoxib, and Nimesulide), etc. Nowadays, both steroidal and non-steroidal anti-inflammatory drugs are used extensively for the relief of inflammatory pain because inflammatory diseases are becoming very common in society throughout the world. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, their long-term use causes serious adverse effects. Prolonged use of NSAIDs is also associated with gastric irritation, nausea, vomiting, peptic ulcers, gastric ulcers, and gastric bleeding. Owing to a wide assortment of side effects of NSAIDs, an alternative anti-inflammatory drug with the least side effects is needed for an hour. In recent years, the population in rural areas uses many alternative drugs such as substances produced from medicinal plants (ayurveda) for the treatment of inflammation, because the management of inflammation-related diseases is a real and challenging issue in rural communities still today. Hence, the search for natural remedies and phytochemicals with anti-inflammatory properties has significantly increased. For example, curcumin, a bioactive compound

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containing bis-α, β-unsaturated 1,3-diketone moiety, is found mainly in the turmeric plant rhizome (*Curcuma longa*) has a wide range of beneficial activities such as anti-inflammatory, antitumor, antioxidative, cardiovascular protective effects.[2]A survey of the literature reveals that compounds containing β-diketones moiety unit exhibit good anti-inflammatory as well as anti-mitotic activities.[3] Ramaa and More reported the synthesis and anti-inflammatory activity of fluorinated propanedione derivatives (β-diketones) which possessed good anti-inflammatory activity against carrageenan-induced edema in the rat paw.[3]Also, β-diketone ligand prepared by Baker-Venkataraman transformation and its complexes shows antioxidant and anti-inflammatory action.[4-5]Antioxidants are also significant compounds that reduce or neutralize free radicals, thus protecting the cells from oxidative injury because the high level of free radicals can cause damage to biomolecules such as enzymes, proteins, lipids, nucleic acids in the cells membrane which may result in many diseases such as cancer, diabetes, cardiomuscular and autoimmune diseases, and neurodegenerative disorders.[6] Examples of common antioxidants include vitamins A, C, and E, selenium, and carotenoids such as β-carotene, lycopene, lutein, and zeaxanthin. Vegetables and fruit-rich diets which are good sources of antioxidants have been found to be healthy, however, research has not shown antioxidant dietary supplements to be beneficial in improving health in humans, or to be effective in preventing diseases. Hence, considerable research has been directed toward the identification of new antioxidant molecules to prevent radical-induced damage. Various methods are reported in the literature for determining antioxidant activity but DPPH (Diphenylpicryl hydrazine) free radical scavenging assay is one of the widely accepted methods for screening antioxidant activity.[7]Also, the protein denaturation inhibition assay suggested by Mizushima and Kobayashi and Sakat *et al.* is widely used for the determination of anti-inflammatory activities. A careful review of the literature indicates that very little work has been reported on the anti-inflammatory and antioxidant activities of β-diketones or their derivative. The literature survey also surprised that, no approach has been made to evaluate the in vitro anti-inflammatory and antioxidant potential of β-diketones comprising a moiety of phenolic aldehyde vanillin. In our previous work, we have synthesized 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-nitrophenyl) propane-1, 3-dione (4a), 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-methoxyphenyl) propane-1, 3-dione (4b), 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(2'-chlorophenyl) propane-1, 3-dione (4c), 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-chlorophenyl) propane-1, 3-dione (4d), 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(2',4'-dichlorophenyl) propane-1,3-dione (4e) from vanillin by using Fries rearrangement and Baker-Venkataraman transformation, characterized them by IR and 1H NMR spectra and studied their antimicrobial activities.[8]In continuation of our previous work, we have carried out the invitro anti-inflammatory and antioxidant activity of these novel compounds 4(a-e).

EXPERIMENTAL

Material and Methods

All the reagents used for the analysis of anti-inflammatory and antioxidant studies were of higher analytical grade. The anti-inflammatory activity was carried out in vitro by inhibition of protein denaturation assay reported by Mizushima and Kobayashi[9] and Sakat *et al.*[10-11]In-vitro antioxidant activity was performed by reducing power assay method.[12-13]

General Procedure

Determination of Anti-Inflammatory Activity

Initially, 500 μL of 1% albumin was added to 100 μL of the test sample. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Standard Diclofenac was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = 100-((A1 - A2)/A0) \times 100) \quad (1)$$

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control and A0 is the absorbance of the positive control.

Determination of Antioxidant Activity

Different concentrations of the drug (10-50µg/mL) were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using a vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). The colored solution was read at 700 nm against the blank with reference to the standard using a UV spectrophotometer. Here, Ascorbic acid (Vitamin C) was used as a reference standard or positive control at the same selected concentrations and in the same operating conditions as the sample, and reducing the power of the sample was compared with the reference standard.

RESULTS AND DISCUSSION

The results on anti-inflammatory activities of 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substituted phenyl) propane-1,3-diones compounds 4(a-e) are depicted in Table-1 to 6.As well the results of antioxidant studies of these compounds are depicted in Table-7 to 8 and shown in Fig.s-1 to 3.

Table-1: Anti-Inflammatory Activity of Standard Diclofenac

Concentration (µg/mL)	% Inhibition of Denaturation of Protein					
	4a	4b	4c	4d	4e	Standard Diclofenac
50 µg/mL	42.85	28.57	39.63	60.71	64.28	71
100 µg/mL	46.42	39.28	50.00	60.71	60.71	78
200 µg/mL	50.00	42.85	53.57	64.28	64.71	85
400 µg/mL	53.57	46.42	57.14	67.85	67.85	89

Table-2: Anti-Inflammatory Activity of Newly Synthesized Compound (4a)

Concentration (µg/mL)	The absorbance of the test sample	% of protein inhibition	Absorbance of Std drug (Diclofenac)	% ofprotein inhibition
50 µg/mL	0.016	42.85	0.008	71.42
100 µg/mL	0.015	46.42	0.006	78.57
200 µg/mL	0.014	50.00	0.004	85.71
400 µg/mL	0.013	53.57	0.003	89.28

Table-3: Anti-Inflammatory Activity of the Newly Synthesized Compound (4b)

Concentration (µg/mL)	The absorbance of the test sample	% of protein inhibition	Absorbance of Std drug (Diclofenac)	% ofprotein inhibition
50 µg/mL	0.020	28.57	0.008	71.42
100 µg/mL	0.017	39.28	0.006	78.57
200 µg/mL	0.016	42.85	0.004	85.71
400 µg/mL	0.015	46.42	0.003	89.28

Table-4: Anti-Inflammatory Activity of the Newly Synthesized Compound (4c)

Concentration (µg/mL)	The absorbance of the test sample	% of protein inhibition	Absorbance of Std drug (Diclofenac)	% ofprotein inhibition
50 µg/mL	0.017	39.63	0.008	71.42
100 µg/mL	0.014	50.00	0.006	78.57

200 µg/mL	0.013	53.57	0.004	85.71
400 µg/mL	0.012	57.14	0.003	89.28

Table-5: Anti-Inflammatory Activity of Newly Synthesized Compound (4d)

Concentration (µg/mL)	The absorbance of the test sample	% of protein inhibition	Absorbanceof Std drug (Diclofenac)	% ofprotein inhibition
50 µg/mL	0.011	60.71	0.008	71.42
100 µg/mL	0.011	60.71	0.006	78.57
200 µg/mL	0.010	64.28	0.004	85.71
400 µg/mL	0.009	67.85	0.003	89.28

Table-6: Anti-Inflammatory Activity of the Newly Synthesized Compound (4e)

Concentration (µg/mL)	The absorbance of the test sample	% of protein inhibition	Absorbanceof Std drug (Diclofenac)	% ofprotein inhibition
50 µg/mL	0.010	64.28	0.008	71.42
100 µg/mL	0.011	60.71	0.006	78.57
200 µg/mL	0.010	64.71	0.004	85.71
400 µg/mL	0.009	67.85	0.003	89.28

Table-7: Antioxidant Activity of Standard Ascorbic Acid

Sr. No.	Concentration (µg/mL)	Absorbance (A)
1	10	0.251
2	20	0.499
3	30	0.775
4	40	0.943
5	50	1.240

Table-8: Antioxidant Activity of Newly Synthesized Compounds 4(a-e)

Sr. No.	Sample	Concentration (µg/mL)	Absorbance (A)	Sr. No.	Sample	Concentration (µg/mL)	Absorbance (A)
1	4a	30	0.689	6	4c	50	0.874
2	4a	50	0.755	7	4d	30	0.806
3	4b	30	0.507	8	4d	50	0.831
4	4b	50	0.667	9	4e	30	0.721
5	4c	30	0.852	10	4e	50	0.794

The results on anti-inflammatory activity reveal that all the newly synthesized compounds 4(a-e) has shown good inhibition of denaturation of protein at all the tested concentration, however as compared to other compounds, compound 4d and 4e exhibit notable inhibition at all the tested concentrations when compared with standard Diclofenac. From the results on antioxidant activity, it was observed that all the samples showed 4c > 4d > 4e > 4a > 4b order of reducing power and their reducing strength was increased with increasing concentration. The high value of absorbance of the reaction mixture clearly indicated greater reducing power. By comparing their reducing strength with standard reference, it was observed that the reducing power of all the synthesized compounds was found to be in good agreement with the standard ascorbic acid as well as their values were found to be comparable with it.

Fig.-1:1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substituted phenyl) propane-1,3-diones Compounds 4(a-e)Before Centrifugation

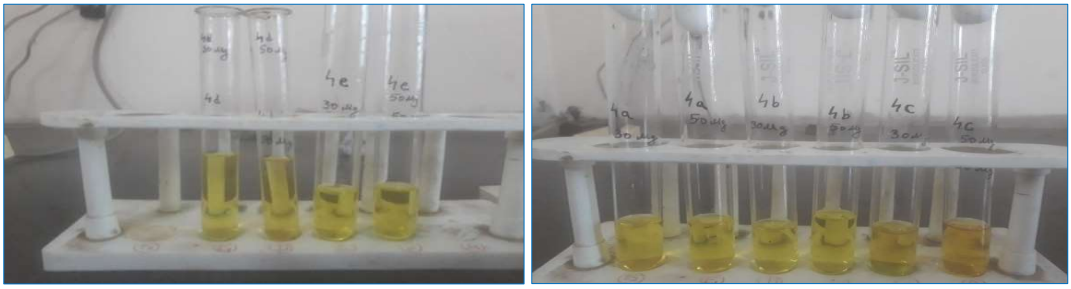

Fig.-2: 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl) propane-1,3-diones Compounds 4(a-e) After Centrifugation

Fig.-3:1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substituted phenyl) propane-1,3-diones compounds 4(a-e) With Colour Changes After Addition of $FeCl_3$Solution

CONCLUSION

In conclusion, from the results on anti-inflammatory activities presented in Table-1 to 6, it is concluded that all these newly synthesized β-diketones 4(a-e) comprising moiety of vanillin were found to possess satisfactory anti-inflammatory potential when equated with the reference Diclofenac. The screening results on antioxidant studies (Table-7 to 8) indicate that all five compounds exhibited potent antioxidant activities due to the presence of vanillin moiety in the molecular structure of synthesized compounds.

After comparing the results of antioxidant activities with standard Ascorbic acid, it is confirmed that the reducing power (as indicated by absorbance at 700 nm) of all samples increased with increasing their concentrations. As well, experimental findings on anti-inflammatory and antioxidant studies may be implicated as an informative resource for pharmaceutical industries engaged in the manufacturing of drugs and medicines not only for human beings but also for veterinary sciences.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication.The research profile of the authors can be verified from their ORCID ids, given below:

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