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Synthesis, Characterization and Bioactivity Study of 5, 5-dimethylcyclohexane 1, 3-dione and its derivatives

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Abstract:
1, 3-diketones are known for their versatile co-ordination behavior and antimicrobial properties. Dimedone (5, 5-dimethyl cyclohexane -1, 3-dione) is one of the important 1, 3 dicarbonyl cyclohexane derivative. 1, 3 dicarbonyl compounds find versatile applications in making biomolecules, dyes, rubber, pharmaceuticals and pesticides. In this view 5, 5-dimethylcyclohexane 1, 3-dione and its derivatives are synthesized. The structural features of these compounds have been determined using, IR, UV, NMR and Mass spectroscopy. In order to evaluate the biological activity of the synthesized compounds, they have been screened for their antibacterial, antifungal and anthelmintic activity. The synthesized imines were screened for their antibacterial activity against four bacterial strains, *Escherichia coli* (ATCC 11246), *Salmonella abony* (ATCC 23564), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 6538P) and also tested for Antifungal activity (in-vitro) against yeast strains *Saccharomyces cereviseae* (ATCC 9763), *Aspergillus niger* (ATCC 16404). The anthelmintic assays were performed on adult Indian earthworm, *Eicinia foetida.*

Keywords: 5, 5-dimethylcyclohexanone 1, 3-dione, derivatives, Biological activity

Introduction:

1, 3-diketones exhibited various biological properties. Their strong absorbance in the UV range makes them important in cosmetic preparations. Thiosemicarbhazone derivatives of 1, 3- diketones exhibit biological, structural and electro chemical properties1. It was reported that marine organism such as sponges contain cyclic 1, 3–diketone unit which protect them from UV radiations. Dimedone and other 1, 3-dione derivatives are utilized as photo

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protective-sunscreen agents. Worm infections are also a major cause for concern in veterinary medicine, affecting domestic pets from animals. Helminthiasis is among the most important animal diseases inflicting heavy production losses. More than half of the population of the world suffers from various types of infection and majority of cattle's suffering from worm infections[1]. Albendazole is used as a standard study. The assays were performed on adult Indian earthworm, Eicinia foetida due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [2-6]. The lethal effect of Albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake [7].

Literature survey revealed that xanthene and acridine derivatives obtained from dimedone showed biological activity. The reports revealed that parasitic worms infect livestock and crops affecting food production. Despite this prevalence of parasitic infections the research on anthelmentic drug is poor. A number of medicinal plants have been used to treat parasitic infections in man and animals [8-9]. Researchers have tried and developed synthetically, some anthelmintic drugs. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozonide and bithionol are interfere with energy generation in helminthes parasites [10]. The current choices of drugs like mebendazole, albendazole, theabendazole, pyrental, diethylcarbamazile, piperazine, levamizole etc. for worm infections are common in Indian subcontinent [11]. The gastrointestinal helminthes become resistant to currently available anthelmintic drugs [12]. Literature survey illustrated that Schiff bases possess wide variety of applications in many fields e.g. biological, industrial and analytical. Schiff bases derived from acetyl acetone and p-anisidine showed great activity against some bacteria and Fungi [13]. In the present study all synthesized compounds were screened for their biological properties. Efforts were made to evaluate the bioactivity against bacteria and fungi. The experiments were performed to evaluate anthelmintic activity with Indian earthworms *Eicinia foetida.*

Materials and Method:

Preparation of 5, 5-dimethylcyclohexane 1, 3-dione and its derivatives -

The compounds were synthesized under anhydrous conditions by utilizing equimolar reactants (Dimedone **1** with aniline & substituted anilines **2, 4, 6, 8, 10 & 12**) using anhydrous $MgS0_4$ as dehydrating agent. The reaction mixture was refluxed for two hours in dry methanol. The progress of the reaction was monitored by TLC. The reaction mixture was separated from $MgS0_4$. The solvent was removed under vacuum to get crude solid. Repeated crystallization from ethanol gives crystalline product. Purity of the molecule was confirmed

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by TLC. The melting points of all synthesized compounds were matched with the standard. Various compounds were synthesized as reported in Scheme 1.

Antimicrobial activity –

The synthesized imines (compound 3, 5, 7, 9, 11 & 13) were screened for their antibacterial activity against four bacterial strains, Escherichia coli (ATCC 11246), Salmonella abony (ATCC 23564), Pseudomonas aeruginosa (ATCC 27853) and Staphylococcus aureus (ATCC 6538P) and also tested for Antifungal activity (in-vitro) against yeast strains Saccharomyces cereviseae (ATCC 9763), Aspergillus niger (ATCC 16404).

The well diffusion method was used. Test samples of each compound (20 mg) were dissolved in ethyl alcohol (1 ml). Sterile 8.00 mm diameter wells were impregnated with 40 µL of these solutions. The bacterial strains were inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1 □C while yeast strain was inoculated on nutrient broth and incubated for 48 hours at 25 ± 0.1 □C. Adequate amount of Muller Hinton Agar and Chloramphenicol Yeast Glucose Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial strains and yeast strain was adjusted to yield 1 X 107 to 1 X 108 mL-1 and 1 X 105 to 1 X 106 mL-1 respectively. The test organisms (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with test organism were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37 ± 0.1 □C for 24 hours while the yeast plates at 25 ± 0.1 □C for 48 hours.After incubation all the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters. Streptomycin discs (10 µg/disc) and fluconazole discs (50 µg/disc) were used as positive controls.

Anthelmintic activity (in –vitro study) -

The anthelmintic activity was performed on adult Indian earthworm, *Eicinia foetida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Albendazol and normal saline were purchased from authorized pharmacist and Indian Earthworm species *Eicinia foetida* was collected from Mahatma Phule Agricultural University, Pune, Maharashtra, India. All earthworms were of equal in size (8 to 10 cm) were selected for the study. The experiments were carried out as per the standard method [14/53] with some minor changes and modifications. The concentration of compounds under investigation

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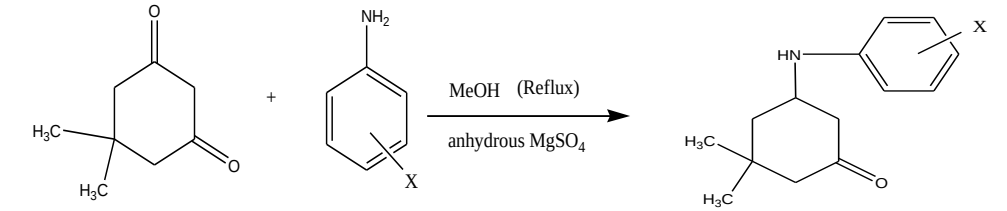
(5, & 10, mg / ml) in normal saline solution were tested for the study, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as a standard (5&10mg / ml) and normal saline served as control. Three groups of four earthworms were released into 10 ml of normal saline. The Albendazole and prepared solutions of compounds under investigations were added to their respective group. Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement was observed except when the worms were shaken vigorously. Death was concluded when the worms lost their movement when dipped in warm water followed by fading of their body colour.

Scheme 1-



5,5-dimethylcyclohexane-1,3-dione Aniline 3,3-dimethyl-5-(phenylamino)cyclohexanone

Here,	X =	H	(Substituent 2)	X = H (Compound 3 / L_1)
		= o-CH_3	(Substituent 4)	= o-CH_3 (Compound 5 / L_2)
		= m-CH_3	(Substituent 6)	= m-CH_3 (Compound 7 / L_3)
		= p-CH_3	(Substituent 8)	= p-CH_3 (Compound 9 / L_4)
		= m-Cl	(Substituent 10)	= m-Cl (Compound 11 / L_5)
		= *p*-Cl	(Substituent 12)	= *p*-Cl (Compound 13 / L_6)

Results and Discussion:

3, 3-dimethyl-5-(phenylimino) cyclohexanone (compound 3 /L_1), having molecular formula C14H17NO, a pale yellow solid is crystallized from ethanol having melting point 186 ^{0}C. The absorption in the UV region is observed at 226 nm. Mass spectrum (Fig.1) indicates molecular ion peak at 215 amu and it is the base peak. IR spectrum (Fig.2) show absorption at 3237cm^{-1}& 3184 cm^{-1} for secondary amine, and 3063 cm^{-1}observed for the (=C - H) of olefinic stretching. A broad band at 1640 cm $^{-1}$ indicates presence of α, β unsaturated ketone,1597 cm $^{-1}$ for (-C=C-) and 1570 cm -1,1528cm^{-1},1447 cm^{-1}, for aromatic stretching. A characteristic strong and sharp absorption band at 710 cm $^{-1}$ exhibits presence of para di - substituted aromatic ring. ^{1}H NMR spectrum (Fig.3,) shows down field peaks at δ 7.29 (d, J =

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7.5 Hz, 2H) and at δ 7.13 (m, 3H). They are assigned for H-2', H-6' and H-3', H-4', & H-5' of aromatic protons. A sharp singlet is seen at δ 5.55 for olefinic proton at H-6 position. Two singlets appearing at δ 2.43 and δ 2.23 are assigned for H-2 and H-4 protons of cycloalkyl ring peak. A sharp and strong peak is observed at δ 1.06 (s, 6H) for two methyl groups present at C-3 position (H-3 methylene protons).

The detailed spectral analysis and results are discussed only for compound 3 / L_1. Physico chemical analysis for all synthesized compounds (L_1 to L_6) is given in Table 1 & 2. IR Frequencies have been quoted and compared in Table 3. ^{1}H NMR of the synthesized compounds has been mentioned and data for all compounds are in agreement with the standards as given in Table 4. The synthesized Schiff bases were screened in – vitro for their antibacterial activity against gram positive and gram negative bacteria, results are summarized in Table 5. The antimicrobial studies suggested that Schiff bases derived from dimedone are inactive against all strains.

The single crystal X-ray structure of compound 5 reveals its tautomeric form as enamino ketone, where lone pair of aromatic nitrogen is in conjugation with ketonic function, thus demonstrating planar nature. The above facts are in agreement with the experimental results for tested gram positive and gram negative antimicrobial strains. Though there is Nitrogen and Oxygen as donor atoms, the π electron delocalization does not occur and hence no activity of ligand is observed.

The anthelmentic activity study shows that the compounds get paralyzed after three hours and death of worms is observed after four hours. The results are summarized in Table 6. It is observed that as concentration increases paralysis and death time decreases. The mechanism of action for *Eicinia foetida* is not yet fully understood. The anthelmintic activity, as evident from the results of current study, could be attributed to its inhibition of tubulin polymerization and blocking glucose uptake due to its similarity in action with Albendazole. Moreover, the activity may be attributed to the constituents present in the compound. Presence of electronegative atoms, group of atoms of aromatic rings are known to have a positive contribution to the biological activities. However all synthesized compounds display negligible activity against *Eicinia foetida,* as the results come out after two to four hours.

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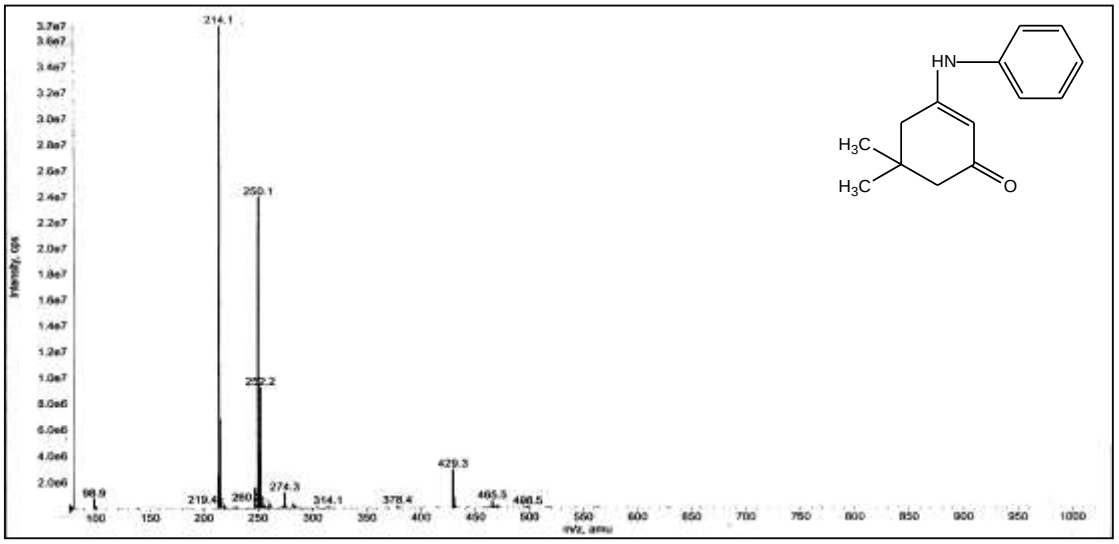

Figure 1

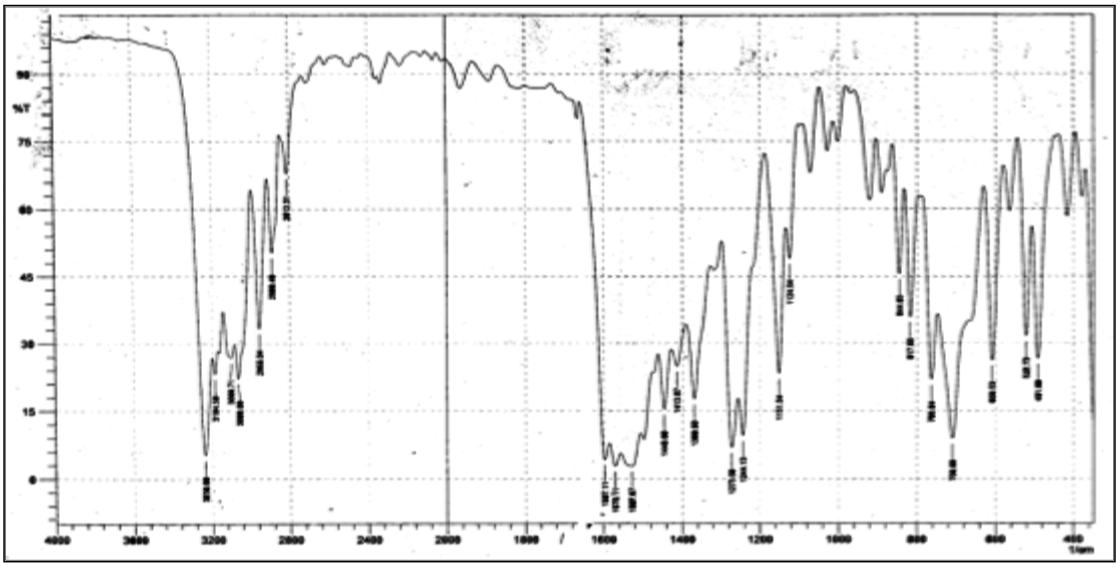

Figure 2

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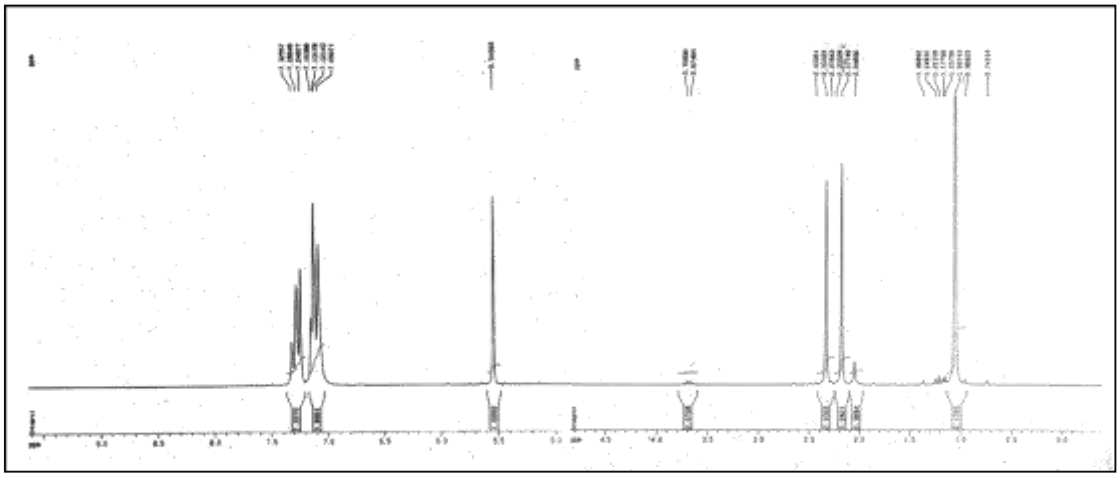

Figure 3

Table 1 Physicochemical Parameters of compounds

Compound	Molecular Formula	Colour	M .P. ^{0}C	Yield (%)
3 (L_1)	$C_{14}H_{17}NO$	Pale Yellow	186	47.86
5 (L_2)	$C_{15}H_{19}NO$	Pale Yellow	132	55.86
7 (L_3)	$C_{15}H_{19}NO$	Orange	156	59.21
9 (L_4)	$C_{15}H_{19}NO$	Pale Yellow	202	66.23
11 (L_5)	$C_{14}H_{16}ClNO$	Orange	155	41.24
13 (L_6)	$C_{14}H_{16}ClNO$	Pale Yellow	205	53.31

Table 2 Mass spectra and Elemental analysis of Compounds

Comp.	**Molecular Formula**	**UV Nm**	**Elemental analysis**			**M^+ ion peak**	**Base peak**
			Observed(Calculated)				
			%C	**%H**	**%N**	***m/z***	***m/z***
3 **(L_1)**	$C_{14}H_{17}NO$	226	77.76 (78.09)	8.27 (7.90)	6.80 (6.50)	215	215
5 **(L_2)**	$C_{15}H_{19}NO$	210	77.72 (78.49)	8.80 (8.28)	6.28 (6.10)	229	230
7 **(L_3)**	$C_{15}H_{19}NO$	304	79.35 (78.49)	8.80 (8.28)	5.27 (6.10)	229	173
9 **(L_4)**	$C_{15}H_{19}NO$	230	79.11 (78.49)	8.76 (8.28)	8.06 (6.10)	229	173
11 **(L_5)**	$C_{14}H_{16}ClNO$	208	-	-	-	249	193
13 **(L_6)**	$C_{14}H_{16}ClNO$	229	-	-	-	249	249

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Table 3 IR Frequencies (cm^{-1}) for Synthesized Compounds

Functional groups	**Compound**					
	3 (L_1)	**5 (L_2)**	**7 (L_3)**	**9 (L_4)**	**11 (L_5)**	**13 (L_6)**
- NH-	3236, 3184	3354, 3190	3211	3244, 3185	3246, 3203	3252, 3167
=C-H Stretching	3063	3005	3017	3061	3009	3055
α-β unsaturated ketone	1640	1640	1680	1660	1640	1660
>C=C<	1597	1560	1584	1578	1587	1597
Aromatic stretching	1570, 1528	1537, 1493	1524, 1489	1551, 1518	1566, 1477	1562, 1496
Substituted Ar-ring	710 (*unsub*.)	820 (*o*-di)	827,706 (*m*-di)	810 (p-di)	829,721 (*m*-di)	824 (*p*-di)

Table 4 ^{1} H NMR of the Compounds ($CDCl_3$, 200* /300*MHz*).**

Protons	**Compounds**					
	1*	**2****	**3****	**4****	**5****	**6****
H 2 δ	2.43 (*s*, 2H)	2.48(*s*, 2H)	2.34(*s*, 2H)	2.31(*s*, 2H)	2.34(*s*, 2H)	2.34 (*s*, 2H)
H 3 δ	1.06(*s*, 6H)	1.23(*s*, 6H)	1.11(*s*, 6H)	1.09(*s*, 6H)	1.10 (*s*, 6H)	1.14 (*s*, 6H)
H 4 δ	2.23(*s*, 2H)	2.33(*s*, 2H)	2.32(*s*, 2H)	2.20(*s*, 2H)	2.32 (*s*, 2H)	2.30(*s*, 2H)
H 5 (NH)	7.09 (br *s*, 1H)	6.16(br *s*, 1H)	6.60(br *s*, 1H)	6.60(br *s*, 1H)	6.35 (br *s*, 1H)	5. 85 (br *s*, 1H)
H 6 δ	5.55(*s*,1H)	5.15(*s*, 1H)	5.60.(*s*, 1H)	5.51 (*s*, 1H)	5.5 9(*s*, 1H)	5. 64 (*s*, 1H)
H 2'&6'δ	7.29(d, *J*=7.5 *Hz*2H)	--	----	-----	------	--------
H 3'- 5' δ	7.13(m,3H)					
H 3'- 6 'δ	---------	7.30(*m*,4H)	------	---------	--------	------
H2' δ	------	------	7.22(dd,*J*=7.2 2.4*Hz*,1H)	----	7.21	-----
H 4'- 6' δ	-------	------	6.99(m,3H)	---	6.98	----
H2'&6'	-------	------	------	7.20, (d,*J*=8.1 *Hz*,2 H)	----	7.30 (d,*J*=9.0 Hz,2H)
H3'&5'	------	--------	--------	7.07(d,*J*=8. 1	------	7.20 (d,*J*=9.0

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				Hz ,2 H)		Hz,2H)

Table 5. Antimicrobial activities of synthesized compounds

Compound	Zone of inhibition * (mm)					
	Microorganism					
	E. coli	Salmonelll abony	P. aeruginosa	S. aureus	S. cereviseae	A. niger
3 (L_1)	-	-	-	-	-	-
5 (L_2)	-	-	-	-	-	-
7 (L_3)	-	-	-	-	-	-
9 (L_4)	-	-	-	-	-	-
11 (L_5)	-	-	-	-	-	-
13 (L_6)	-	-	-	-	-	-
Standard	20	29	25	25	-	
Fluconazole					**40**	**20**

Table 6. Anthelmentic Activity

Compound	Conc.(mg/ml)	Eicinia foeitida (Earthworms)	
		Paralysis time (min)	Death time (min.)
3 (L_1)	5 10	197 184	247 239
5 (L_2)	5 10	189 182	226 216
7 (L_3)	5 10	190 183	222 215
9 (L_4)	5 10	184 120	261 197
11 (L_5)	5 10	181 177	241 237
13 (L_6)	5 10	180 176	241 237
Albendazole	10	60	85

Conclusions:

All synthesized compounds are found to be inactive against tested antimicrobial strains. It is well known that there is change in structural, chemical and biological properties of organic molecules binding to metals. This work is related to synthesis, characterization and

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antimicrobial activities of 1, 3-diketone derivates. Many useful properties and applications of dimedone derivatives and their transition metal complexes in different fields can be expected from this work.

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