New Biologically Active Compounds from 1, 3-Diketones

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Abstract
The ready availability of cyclohexanones and the enhanced reactivity at their α-positions render them starting materials of choice in the present study. The synthesis of new compounds of antimicrobial activity was undertaken by the coupling of aromatic amines with 5,5-dimethyl cyclohexan-1,3-dione (dimedone). The products were refluxed with N–benzyl-N-phenylhydrazine in acetic acid. The structures of the products were elucidated using micro- and IR-spectral analyses. They were confirmed using ^{1}H NMR at 60MHz and TMS as internal standard. The diketone derivatives were tested for their biological activity against gram-positive Cocci and Bacilli, and gram-negative Bacilli. The study showed that the derivatives gave a wide range of activity from inactive to highly active, which proves it to be of fresh pharmaceutical interest.
Key words: Dimedone, Antimicrobial activity, Gram-positive *Cocci* and *Bacilli*, Gram-negative *Bacilli*

Introduction

Cyclohexanedione derivatives far exceed any other alicyclic system in number. In nature the preponderance of cyclohexanedione derivatives over those of other alicyclic systems is overwhelming. The introduction of the second carbonyl group into the cyclohexane ring has a profound effect on the enolisation of the first carbonyl[1,2]. Cyclohexan-1,3-diones are also completely enolised and possess an acidity comparable to that of carboxylic acids. Cyclohexan-1,3-diones are synthesised by several methods[3], among which are by reduction of benzenoid compounds, application of the Dieckmann[4], and by Michael condensation[5-7]. Cyclohexan-1,3-diones react with amino compounds in various ways, including reactions between simple amines to give enaminones, and with *o*-aminothiophenol to give phenothiazinones. They also undergo coupling reactions with diazonium salts to give the 2-arylazo-cyclohexan-1,3-dione.

The object of the present study was to synthesise new compounds of 1,3-diketones with diazonium salts and *N*-benzyl-*N*-phenyl hydrazine with a view to investigating their biological activity towards various micro-organisms.

Material and Methods

Materials and apparatus: All chemicals and solvents were of reagent grade (Merck, Fluka and Sigma – Aldrich) and used without further purification. All melting points (℃) were determined by the open tube capillary method and quoted uncorrected. The purity of the compounds was controlled by thin layer chromatography (TLC). IR spectra were recorded in KBr pellets on Mattson 5000 FTIR spectrophotometer (USA). The C, H, N, Cl data was estimated by using Perkin–Elmer Instrument (200B, USA). The ^{1}H NMR spectra of the compounds were measured in CDCl$_3$ and DMSO-d$_6$ solution on a DRX – 300MHz spectrometer (Bruker, UK) using TMS as internal standard.

Synthesis of 2-arylazo-1, 3-diketones (2): The appropriate aromatic amines (see Scheme, 15 m mol) in sodium acetate solution (1.23g, 15 m mol, 10 ml) and hydrochloric acid (15 m mol) (warmed where necessary) were diazotized with sodium nitrite solution (1.04g, 15 m mol) at 0–5℃. This solution was added to 5,5-dimethylcyclohexan-1,3-dione (**1**, 2.1g, 15 m mol) dissolved in ethanol (20 ml) in the cold (0-5°C) and left overnight at ambient temperature, filtered and left to dry in a desiccator. The obtained crystals were re-crystallised from ethanol and the melting points together with percentage yields determined.

Synthesis of 2-arylazo-1, 3-diketones derivatives (3): 2-arylazocyclohexan-1,3-dione (**2**, 0.002 mol) were dissolved in a mixture of acetic acid (25 ml) and *N*-benzyl-*N*-phenyl hydrazine (0.396g, 0.4 ml, 2 m mol), refluxed for 3 – 4 hours, left to cool, filtered and the product re-crystallised from suitable solvents. The coupling process was carried out at temperatures of 0–5℃ and the products isolated and re-crystallised from methanol and water (50–80% yields).

Biological activity tests[8-9]: Requisite quantities of the liquid agar media were poured into sterile Petri dishes to a depth of 3-4 mm. After solidifying, the liquid media test organism was spread over the solidified agar media and incubated in the Petri dish at 37°C for 24 hours to allow the micro-organisms to grow. With the help of a sterile rod, a hole was made on the medium and poured on the known (10 mL of 100 or 1000 µg/mL concentrations) test solution in that hole. The biological activity of the derivatives was evaluated by determining the average diameter of the inhibition zone (figure-1).

Results and Discussion

Compound (**2a**) was obtained as yellow crystals in 69% yield, m.p. 143°C; IR, V$_{max}$ in cm^{-1}: 3069, 1636, 1498 (CH-aromatic), 2951, 2873, 1387 (CH$_3$), 1686 (>C=O). Compound (**2b**) was obtained as yellow crystals in 54.5% yield, m.p 157°C, C$_{14}$H$_{15}$N$_3$O$_4$ (RMM, 273.28). Compound (**2c**) was obtained as light brown crystals in 68%, m.p. 217°C,

C$_{14}$H$_{15}$N$_2$ClO$_2$ (RMM, 278.74). Compound (**2d**) was obtained as red crystals in 80% yield, m.p. 178°C, C$_{14}$H$_{14}$N$_3$O$_3$ (RMM, 272.27). Compound (**2e**) was obtained as yellow crystals in 50% yield, m.p. 183°C, C$_{14}$H$_{15}$N$_3$O$_4$ (RMM, 272.27). Compound (**2f**) was obtained as yellow crystals in 59% yield, m.p. 224℃, C$_{14}$H$_{15}$N$_3$O$_4$ (RMM, 273.28). Compound (**2g**) was obtained as red crystals in 79% yield, m.p. 224℃, C$_{14}$H$_{15}$N$_3$O$_4$ (RMM, 273.28); IR, V$_{max}$ in cm^{-1}: 3110 (aromatic), 2956, 2875, 1434, 1332 (-CH$_3$), 1697, (>C=O), 747, 774 (=CH). Compound (**2h**) was obtained as golden yellow crystals in 59% yield, C$_{14}$H$_{10}$N$_2$O$_4$ (RMM, 272.3).

Derivatives of 2-arylazo-1, 3-diketones were afforded in good yield. The products (**3a – 3h**) were isolated, re-crystallised (from mixtures of methanol and water) and obtained in 40 – 60% yields. Compound (**3a**) was obtained as red crystals in 54% yield, m.p. 81℃. Anal. calcd for C$_{22}$H$_{26}$ClO$_2$ (RMM, 465.41): C, 65.72; H, 5.31; N, 11.36; Cl, 14.37; Found: C, 65.35; H, 5.70; N, 11.21; Cl, 14.37%. Compound (**3b**) was obtained as dark brown crystals in 49% yield, m.p. 100 ℃; IR, V$_{max}$ in cm^{-1}: 3058, 3027, (CH, aromatic), 2953, 2877, 1455, 1358, (CH$_3$), 695, 739, (=CH), 792, (C-Cl), 1609, 1492, (CH, aromatic), 738, 693, (=CH). Anal. calcd for C$_{22}$H$_{27}$N$_5$O$_3$ (RMM, 469.53): C, 69.06; H, 5.80; N, 14.92; Found: C, 69.39; H, 5.50; N, 14.81%. Compound (**3c**) was obtained as orange crystals in 52% yield, m.p 115℃; IR, V$_{max}$ in cm^{-1}: 3539 – 3208, (NH), 3058, 1649, 1598 (CH, aromatic), 2957, 2857, 1456, 1347, (CH$_3$), 696 (=CH), 823 (C-Cl). ^{1}H NMR spectra at 60MHz (δ units ppm) showed; 1.033(q, 3H, CH$_3$), 0.933(t, 2H, CH$_2$), 7.653(s, 5H, CH$_{ar}$), 7.416(s, 1H, CH), 7.369(s, 1H, CH$_{ar}$), 6.234(d, 1H,=NH, -OH), 7.351(s, 5H, CH), 7.351(s, 5H, CH), and 7.149(s,5H, CH). Anal. calcd for C$_{27}$H$_{27}$N$_4$ClO (RMM, 458.98): C, 70.65; H, 5.93; N, 12.21; Cl, 7.73; Found: C, 70.55; H, 5.75; N, 12.21; Cl, 7.69%.

Compound (**3d**) was obtained as dark brown crystals in 58% yield, m.p. 98°C; ^{1}H NMR spectra at 60 MHz (δ unit in ppm); 0.98(q, 3H, CH$_3$), 4.09(t, 2H, CH$_2$), 7.589(s, 5H, CH), 7.277(s, 5H, CH), 7.233(s,

5H, CH), 8.019(s, 5H, CH), 7.333(d, 1H, CH), 7.52(s, 5H, CH), 7.24(s, 5H, CH), and 6.712(s, 5H, CH).   Anal. calcd for $C_{28}H_{26}N_4ClO_3$ (RMM, 503.98): C, 64.34; H, 5.20; N, 13.90; Cl, 7.04; Found C, 64.61; H, 5.35; N, 13.65; Cl, 7.15%. Compound (**3e**) was obtained as brown crystals in 40% yield, m.p. 180^oC; IR, V_{max} in cm^{-1}: 3086, 3059, 1609, 1490 (CH, aromatic) 2957, 2929, 1339, (CH_3), 739, 697, (=CH), 892 (C-Cl). Anal. calcd for $C_{27}H_{26}N_5ClO_3$ (RMM, 503.98): C, 64.34; H, 5.20; N, 13.90; Cl, 7.04; Found C, 64.66; H, 5.29; N, 13.59; Cl, 7.10%. Compound (**3f**) was obtained as red crystals in 60% yield, m.p. 79^oC; IR, V_{max} in cm^{-1}; 3555 – 3253 (NH, OH), 3084 – 3003, 1585, 1497, (CH, aromatic) 2957, 2925, 1452, 1323, (CH_3), 737, 697 (C=H). Anal. calcd for $C_{27}H_{27}N_5O_3$ (RMM, 469.53): C, 69.06; H, 5.80; N, 14.92; Found: C, 69.39; H, 5.75; N, 14.69%.

Compound (**3g**) was obtained as dark brown crystals in 49.2% yield, m.p. 118^oC; IR, V_{max} in cm^{-1}: 3511 – 3404 (NH), 2956, 2929, 1453, 1348, (CH_3), 1659, 1529, (CH, aromatic), 735, 694 (=CH). Anal. calcd for $C_{27}H_{26}N_5O_3$ (RMM, 469.53): C, 69.21; H, 5.59; N, 14.95; Found C, 69.41; H, 5.65; N, 14.75%. Compound (**3h**) was obtained as red crystals in 51.2% yield m.p. 112^oC, RMM, 468.53. Anal.calcd for $C_{28}H_{28}N_4O_3$: C, 71.77; H, 6.02; N, 11.96; Found: C, 71.91; H, 6.11; N, 12.01%.

Biological screening: The 2-arylazo-1,3-diketone derivatives (**3**) were examined *in vitro* against bacterial species which included gram-positive *Cocci*, gram-positive *Bacilli* and gram-negative *Bacilli*. The photographs provided (figure 1) represent the situations that prevailed. Tables 1 and 2 show the spectral data of antimicrobial activity of the synthesised compounds (**3a – 3h**) at 100 and 1000 µg/mL concentration levels, respectively, against the micro-organisms.

The test results presented in table 1 suggested that compounds (**3b**, **3f**, and **3g**) showed high antimicrobial activity (i.e. at 100µg/ml) against all the tested micro-organisms. This is attributed to the presence of the nitro (-NO_2) group. The reactivity might be strongly dependent on the electronic richness of the nitrogen atoms and on the steric hindrance of the substituent[10,11]. Compounds (**3a and 3c**) with a chlorine atom on the arylazo- group showed high antimicrobial activity against the gram-positive *Cocci* and gram-positive *Bacilli*.

On the contrary, both compounds (**3a and 3c**) were inactive against all the gram-negative *Bacilli*. Compounds (**3h**) with a carboxyl group on the arylazo moiety showed relative biological activity on all the micro-organisms tested. Compound (**3h**) was highly active against all the gram-positive *Cocci* and gram-positive *Bacilli*. The test results presented on gram-negative *Bacill*i by compound (**3h**) ranged from inactive to moderate activity against the tested micro-organisms. It showed moderate activity on *Aerobacterium klebsiella*, *Bacillus Arizona*, *Bacillus Proteus*, *Bacillus Pseudomonas*, *Escherichia Coli* and *Salmonella Paratyphi A*. Compound (**3h**) was inactive against *Salmonella Paratyphi B, Salmonella Paratyphi C, Shigella flexneri* and *Shigella sonnei.*

Compound (**3d**) was highly active against both gram-positive *Cocci* and gram-positive *Bacilli*. Compound (**3f**) was biologically inactive against *Aerobacterium klebsiella* and *Bacillus Arizona*, and moderately active against the rest of the gram-negative *Bacilli*. Compound (**3e**) was moderately active against all the tested gram-negative *Bacilli,* highly active on gram-positive *Bacilli.* The results show the effect of the nitro and chlorine substituents on the biological activity.

When the concentration was increased to 1000 µg/ml, there was a slight change in the antimicrobial activity of most of the products (**3a – 3h**) (table 2). It should be noted that with exception of product (**3a**) which was highly active against *Bacillus subtilis*, product (**3e**), became highly active against *Sarcina lutea*, *Bacillus permal* and *subtilis*. Products (**3e** and **3h**) were inactive against *Aerobacterium klebsiella, Bacillus Arizona, pseudomonas* and *proteus, Salmonella* and *proteus, Salmonella paratyphi* A, B and C, *Shigella flexneri* and *sonnei*, and *Escherichia coli* (table 2).

Conclusion

This study shows that 5,5-dimethylcyclohexan-1,3-dione represents an adaptable starting material for the preparation of new biologically active compounds that might prove to be of pharmaceutical interest. Some of the products however exhibited total inactivity towards the tested micro-organisms.

Acknowledgements

References

1. French H.S. and Holden M.E.T., Absorption Spectra of Certain α,β-Unsaturated Ketones, including Benzal Compounds, *J. Amer. Chem. Soc.,* **67,** 1239 (**1945**)

2. Schwarzenbach G. and Wittwer Ch., Über das Keto-Enol-Gleichgewicht bei cyclischen α-Diketonen, *Helv. Chim. Acta*, **30**, 663 (**1947**)

3. Conroy H., Picrotoxin. II., The Skeleton of Picrotoxinin. The Total Synthesis of *dl*-Picrotoxadiene, *J. Amer. Chem. Soc.*, **74,** 3046 (**1952**)

4. Meek E. G., Turnbull J. H. and Wilson **W.,** Alicyclic compounds. Part II. The preparation of *cyclo*hexane-1:3-diones and their enol ethers, *J. Chem. Soc*. 811 (**1953**)

5. Shriner R.L. and Todd H.R., 1,3-Cyclohexadione-5,5-dimethyl, *Org. Synth*., **II,** 200 (**1943**)

6. Frank R.L. and Hall H.K., Monocyclic Terpenes from Cyclic 1,3-Diketones, *J. Chem. Soc*. **72,** 1645 (**1950**)

7. Pal B.C., Dehydration of β-Phenylethylcyclohexanol-3, *J. Amer. Chem. Soc*., **11,** 3397 (**1955**)

8. Chitra M., Shyamala D.C.S. and Sukumar E., Antibacterial Activity of Embelin, *Filotropia*, **74**, 401 (**2003**)

9. Manjudar S.H., Chakra G.S. and Kulkarni K.S., Medicinal Potential of *Semecarpus anacardium Nut*., *J. Herb. Med. Toxicol.,* **2**, 9 (**2008**)

10. Cousinité S., Gressier M., Alphonse P. and Menu M.J., Silica-Based Nanohybrids containing Dipyridine, Urethan, or Urea Derivatives, *Chem. Matters,* **19**, 6492 (**2007**)

11. Bares J., Richard P., Meunier P., Pirio N., Padelkova Z., Cernoisck Z., Cysarova I. and Ruzicka, A., Reactions of C,N-chelated Tin(II) and Lead(II) Compounds with Zirconocene Dichloride Derivatives, *Organometallics,* **28**, 3105 (**2009**)

SCHEME
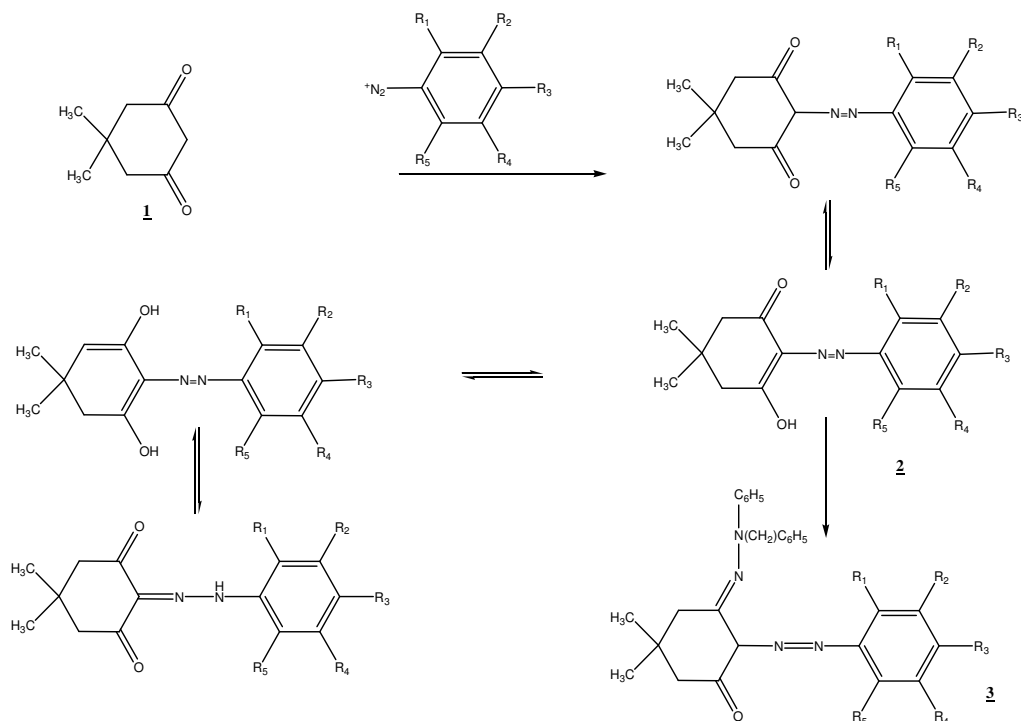
An illustrated reaction pathway for the synthesis of antimicrobial products using dimedone (**1**), aromatic amines and *N*-benzyl-*N*-phenyl hydrazine

		2	**3**
i)	$R_1=R_3=R_5=H$, $R_1=R_4=Cl$	(2a)	(3a)
ii)	$R_1=R_2=R_4=R_5=H$, $R_3=NO_2$	(2b)	(3b)
iii)	$R_1=R_2=R_4=R_5=H$, $R_3=Cl$	(2c)	(3c)
iv)	$R_3=R_4=R_5=H$, $R_1=NO_2$, $R_3=Cl$	(2d)	(3d)
v)	$R_2=R_4=R_5=H$, $R_1=Cl$, $R_3=NO_2$	(2e)	(3e)
vi)	$R_1=R_3=R_4=R_5=H$, $R_2=NO_2$	(2f)	(3f)
vii)	$R_2=R_3=R_4=R_5=H$, $R_1=NO_2$	(2g)	(3g)
viii)	$R_1=R_2=R_4=R_5=H$, $R_3=COOH$	(2h)	(3h)

Figure 1

Table-1: Collective data showing the spectra of antimicrobial activity of the compounds (3a – 3h) at 100µg/ml concentration level against micro-organisms used

Test strains of micro-organisms	Test Compounds							
	3a	3b	3c	3d	3e	3f	3g	3h
A) Gram-Positive *Cocci*								
1. *Staphylococcus aureus*	+	+	+	+	±	+	+	+
2. *Staphylococcus epidermis*	+	+	+	+	±	+	+	+
3. *Sarcina lutea*	+	+	+	+	+	+	+	+
B) Gram- Positive *Bacilli*								
1. *Bacillus permal*	+	+	+	+	+	+	+	+
2. *Bacillus subtilis*	+	+	+	+	+	+	+	+
C) Gram-Negative *Bacilli*								
1. *Aerobacterium klebsiella*	-	+	-	-	±	+	+	±
2. *Bacillus Arizona*	-	+	-	-	±	+	+	±
3. *Bacillus proteus*	-	+	-	±	±	+	+	±
4. *Bacillus pseudomonas*	-	+	-	±	±	+	+	±
5. *Escherichia coli*	-	+	-	±	±	+	+	±
6. *Salmonella paratyphi* A	-	+	-	±	±	+	+	±
7. *Salmonella paratyphi* B	-	+	-	±	±	+	+	-
8. *Salmonella paratyphi* C	-	+	-	±	±	+	+	-
9. *Shigella flexneri*	-	+	-	±	±	+	+	-
10. *Shigella sonnei*	-	+	-	±	±	+	+	-

N.B. (+) High growth (inactive), (±) Moderate growth (moderate activity), (-) No growth (Highly active)

Table-2: Collective data showing the spectra of antimicrobial activity of the compounds (3a – 3h) at 1000µg/ml concentration level against micro-organisms used

Test strains of micro-organisms	Test Compounds							
	3a	3b	3c	3d	3e	3f	3g	3h
A) Gram-Positive *Cocci*								
1. *Staphylococcus aureus*	±	±	+	+	±	+	+	±
2. *Staphylococcus epidermis*	±	±	+	±	±	+	+	±
3. *Sarcina lutea*	±	±	+	+	-	+	+	±
B) Gram- Positive *Bacilli*								
1. *Bacillus permal*	+	±	±	±	-	+	+	+
2. *Bacillus subtilis*	+	±	+	+	-	+	+	+
C) Gram-Negative *Bacilli*								
1. *Aerobacterium klebsiella*	-	+	-	-	-	+	+	-
2. *Bacillus Arizona*	-	+	-	-	-	+	+	-
3. *Bacillus proteus*	-	+	-	-	-	+	+	-
4. *Bacillus pseudomonas*	-	+	-	-	-	+	+	-
5. *Escherichia coli*	-	+	-	-	-	+	+	-
6. *Salmonella paratyphi* A	-	+	-	-	-	+	+	-
7. *Salmonella paratyphi* B	-	+	-	±	-	+	+	-
8. *Salmonella paratyphi* C	-	+	-	±	-	+	+	-
9. *Shigella flexneri*	-	+	-	-	-	+	+	-
10. *Shigella sonnei*	-	+	-	-	-	+	+	-

N.B. (+) High growth (inactive), (±) Moderate growth (moderate activity), (-) No growth (Highly active)