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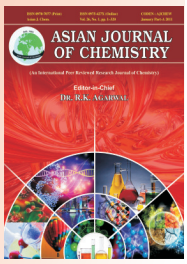

Synthesis, Spectroscopic and Biological Activities of Aromatic Schiff Base

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In this paper the preparation of 5-[(2-aminophenyl)imino]-3,3-dimethylcyclohexanone and 5-[(4-aminophenyl)imino]-3,3-dimethyl cyclohexanone in the ethanolic medium. The structures of these compounds were characterized by elemental analysis, FT-IR, ^{1}H-NMR and ^{13}C-NMR. The antibacterial and antifungal activity of these compounds has been investigated by agar disc diffusion method using Gram-negative and Gram-positive bacterial strain and four kinds of fungi.

Keywords: Schiff base, Dimedone, *o*-Phenylenediamine, *p*-Phenylenediamine.

INTRODUCTION

Schiff base compounds attracted many scientists because of their versatile application in the area of chemical transformation[1]. Schiff base having azomethine groups, generally synthesized from amino and carbonyl group by nucleophelic addition to generate imine. Schiff base compound are used as ligand in coordination chemistry[2,3], enzymatic intermediates in biochemistry[4] and others. Dimedone is an cyclic diketone compound having flanked dimethyl groups. It is an excellent precursor for partially hydrogenated fused hetrocycles[1]. Dimedone is used as synthetic reagent like multi component heterocyclization reaction[5,6], trivalent metal complexes[7], polyhydroacridine[8], tetrahydrobenzopyran[9,10], polyhydroquinolines[11], pyrrolo[2,3-d]pyrimidines[12], 1,4-dihydropyridines[13], 1,8-dioxo-octahydroxanthenes[14,15], indeno[1,2-b]quinolinedione[16], xanthenes derivatives[17] and spiro compounds[18]. Aromatic diamine is also used as synthetic reagent various type of organic compounds.

In this work, we report synthesis of schiff base compounds from dimedone using *o*- and *p*-diphenylamine. Synthesized compounds were characterized by elemental analysis, FT-IR, ^{1}H-NMR and ^{13}C-NMR. Antibacterial and antifungal activity also observed with various pathogens like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella*, *Salmonella*, *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *Candida* sp.

EXPERIMENTAL

Dimedone (5,5-dimethylcyclohexane-1-3-dione) (Riedel-Dehaen Ag Seelze-Hannover, Germany), *orthro*-phenylene diamine, *para*-phenylenediamine (Loba Chemie, India), Ethanol (Merck, India) and other chemicals were used as received.

FT-IR spectra were recorded on a Perkin Elmer, Spectrum 100 spectrophotometer using KBr discs. The ^{1}H and ^{13}C-NMR spectra were recorded using deuterated chloroform ($CDCl_3$) solvent on JeolDPX400 MHz and tetramethylsilane (TMS) as an internal standard. Thermal analysis was carried out by TGA/DSC1 (Mettler Toledo AG, Analytical CH-8603, Schwerzenbach, Switzerland), in nitrogen atmosphere at heating rate of 10 °C/min. Elemental analysis was done using 2400 Series II CHNS/O Elemental Analyzer, Perkin Elmer, USA.

in vitro Antimicrobial activities of the synthesized compounds were analysed against the following ATCC reference and clinical microbial strains. The tested compounds were solubilized in dimethyl sulfoxide and the stock solution was of 1000 µg/mL concentration.

Preparation of 5-[(2-aminophenyl)imino]-3,3-dimethyl cyclohexanone (a): 0.01 mol of dimedone and 0.01 mol of *o*-phenylene diamine dissolved in ethanol and were refluxed at 40 °C and for 4 h. Completion of reaction was monitored by TLC. The reaction mixture was cooled at room temperature then washed with water and methanol to remove any unreacted material from the product.

Pale yellow powder, yield: 86 %, m.p. 172-174 °C. FT-IR (KBr,ν_{max},cm^{-1}) 3374, 3203, 3076, 2956, 1622, 1567, 1501, 1461, 1410, 1370, 1304, 1277, 1247, 1211, 1151, 1078, 922, 890, 826. ^{1}H-NMR ($CDCl_3$ δ, ppm) 1.06 (s, 6H, $2CH_3$), 2.15 (s, 2H), 2.19 (s, 2H) 2.30 (s, 2H), 6.35 (s, 2H), 6.69 (d, 1H), 6.96 (d, 1H), 6.98 (t, 1H), 7.08 (t, 1H). ^{13}C-NMR ($CDCl_3$ δ, ppm) 28.38, 42.73, 52.73, 197.61, 116.74, 119.08, 123.47, 127.96, 128.51, 142.50. Anal. (%) Calc. for $C_{14}H_{18}N_2O$: C, 73, H, 8, N, 12.05. Found C, 74.96, H, 7.54, N, 17.48.

Preparation of 5-[(4-aminophenyl)imino]-3,3-dimethylcyclohexanone (b): A mixture of 0.01 mol of dimedone and 0.01 mol *p*-phenylene diamine in ethanol. The reaction mixture was refluxed at 50 °C and progress of the reaction was monitored by TLC. The product was cooled at room temperature and washed with methanol to separate out the product from unreacted materials.

Dark brown powder, yield: 85 %, m.p. 198-200 °C. FT-IR (KBr, ν_{max}, cm^{-1}) 3415, 3213, 3040, 2951, 1632, 1566, 1512, 1412, 1311, 1243, 1266, 1147, 1126, 1073, 923, 890, 826. ^{1}H-NMR ($CDCl_3$ δ, ppm) 1.03 (s, 6H, $2CH_3$), 1.63 (s, 2H), 2.17 (s, 2H), 2.28 (s, 2H), 6.56 (s, 2H), 6.61 (d, 1H), 7.25 (d, 1H). ^{13}C-NMR ($CDCl_3$ δ, ppm) 28.45, 32.98, 50.40, 52.7, 163.90, 42.81, 196.45, 138.67, 122.2, 116.79, 145.8. Anal. (%) Calc. for $C_{14}H_{18}N_2O$: 73.00, 8, 12.05. Found C, 74.33, H, 7.65, N, 17.41.

Determination of antibacterial activity of a and b: Antibacterial activity of the synthesized compounds **a** and **b** was analyzed against the following bacterial strains: Gram-positive, *Staphylococcus aureus* ATCC 25923, 29213, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* and Gram-negative, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Shigella, Salmonella*. All isolates were grown on Mueller-Hinton agar media and were preserved at 4 °C till the use.

Antibacterial activity of compound **a** and **b** against above bacterial strain was evaluated by an adapted disk diffusion method. For qualitative analysis, Petri dishes with Mueller Hinton medium were seeded with bacterial inoculum as for the conventional antibiotic susceptibility testing (Kirby-Bauer method); filter paper disks of 5 mm diameter were located on the seeded medium. After that, the disks were soaked with 20 µL (1000 µg/mL) of the stock solution. The plates were kept at room temperature for 20-30 min and then incubated at 37 °C ± 2 °C for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the disks.

For qualitative assay, minimum inhibitory concentration (MICs) of a and b was determined by binary micro dilution method, in Mueller Hinton broth (MHB, Merck) distributed in 96 multi-well plates as per the protocol of CLSI[19]. Bacterial isolates as mentioned above were dispensed in ultrapure water with final concentration of 10^5 CFU/mL. Serial dilutions of the compounds ranging between 100 µg/mL to 0.02 µg/mL were performed in a 150 µL volume of MHB (Merck) and each well of microtiter plates (96 wells, flat bottom, Corning. USA) was seeded with 50 mL of bacterial inoculum. 200 mL pure MHB and bacterial cell inoculation without compound treatment were served as the negative control. Control tests were also carried out in the presence of known standard antibiotics (ofloxacin). The influence of the solvent (DMSO) was also quantified in a series of wells containing DMSO, diluted accordingly with the dilution scheme used for the complexes. After incubated in aerobic condition at 37 °C ± 2 °C for 24 h, the optical densities (OD) were measured in a microtiter plate (ELISA) reader (Labsystems, USA) in 570 nm wavelength. The experiment was repeated three times and results are showed as the mean values of three independent determinations.

Determination of antifungal activity of a and b: In order to determine the antifungal activities of synthesized compound **a** and **b**, *C. albicans* ATCC90028, 66027, *C. tropicalis* ATCC 66029 and *C. parapsilosis* 22019 were procured from American type culture collection. Moreover, other *Candida* isolates of high vaginal swabs [*Candida* sp. (HVS) 178], [*Candida* sp. (BLOOD) 12810] and urine [*Candida* sp. (URINE) 12485] were collected from Prince Salman Hospital (Riyadh, Saudi Arabia) and introduced in experiments. All isolates were grown on Sabouraud dextrose agar media (Merck) and were preserved at 4 °C till the use.

Antifungal activity of compound **a** and **b** for qualitative analysis against above mention *Candida* isolates were evaluated by modified agar disk diffusion method that recommended by the Clinical and Laboratory Standards Institute (CLSI)[20]. Approximately 10^6 colony forming units (CFUs) of each fungus were inoculated on sabouraud dextrose agar plates (Merck) and filter paper disks of 5 mm diameter were located on the seeded medium. After that, the disks were soaked with 20 µL (1000 µg/mL) of the stock solution. The plates were kept at room temperature for 20-30 min and incubated at 35 ± 2 °C for 24 h, the diameters of the inhibition zones were measured.

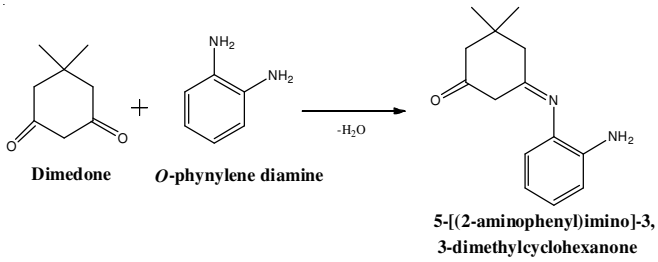
Minimum inhibitory concentration (MICs) of compund **a** and **b** for quantitative analysis was determined by broth microdilution method as per the protocol of CLSI. Cells of above mentioned *Candia* isolates were dispensed in ultrapure water with final concentration of 10^5 CFU/mL. Serial dilutions of the compounds ranging between 100 µg/mL to 0.02 µg/mL were performed in a 150 µL volume of Sabouraud's broth (Merck) and each well of microtiter plates (96 wells, flat bottom, Corning. USA) was seeded with 50 mL *Candida* cells inoculum. 200 mL pure broth and yeast cell inoculation without compound treatment were served as the negative control. The influence of the solvent (DMSO) was also quantified in a series of wells containing DMSO, diluted accordingly with the dilution scheme used for the complexes. Itraconazole was used as a positive control. After incubated in aerobic condition at 37 ± 2 °C for 24 h, the optical densities (OD) were measured in a microtiter plate (ELISA) reader (Labsystem, USA) in 570 nm wavelength. The test was repeated three times.

RESULTS AND DISCUSSION

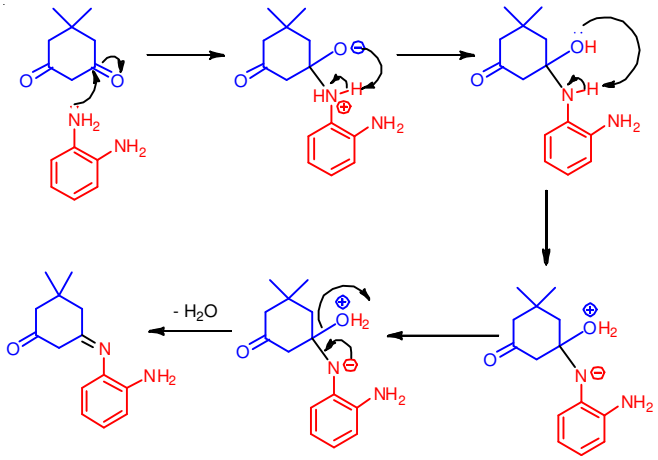
A single step reaction of dimedone and *o*/*p* phenylene diamine to give 5-[(2-aminophenyl)imino]-3,3-dimethylcyclohexanone[**a**] and 5-[(4-aminophenyl)imino]-3,3-dimethylcyclohexanone[**b**] in ethanolic medium at 40 and 50 °C, respectively. Progress of the reaction was monitored by thin layer chromatography (TLC). The structure of the compounds were confirmed by FT-IR, ^{1}H-NMR and ^{13}C-NMR. Band at 1622, 1632 cm^{-1} belong to C=N in FT-IR spectra and characteristics peaks of ^{1}H-NMR and ^{13}C-NMR spectra confirmed

the structure of compound **a** and **b**. **Scheme-I** and **II** shows the preparation of compound **a** and **b**. Compound **a** and **b** are soluble in tetrahydrofuron, acetone, chloroform and not soluble in distilled water, benzene and diethyl ether.

Mechanism: The detailed mechanism of these reactions as follows, amine as a nucleophilic attack on carbonyl carbon followed by H rearrangement leading to the formation alcohol intermediates. This lone pair of alcohol extracts the second proton of secondary amine this is followed by elimination of water molecule and the final product imine is formed.

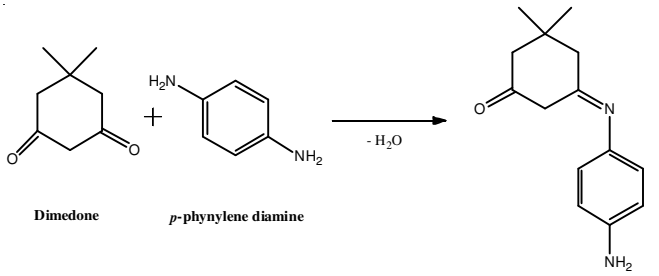


Scheme-I

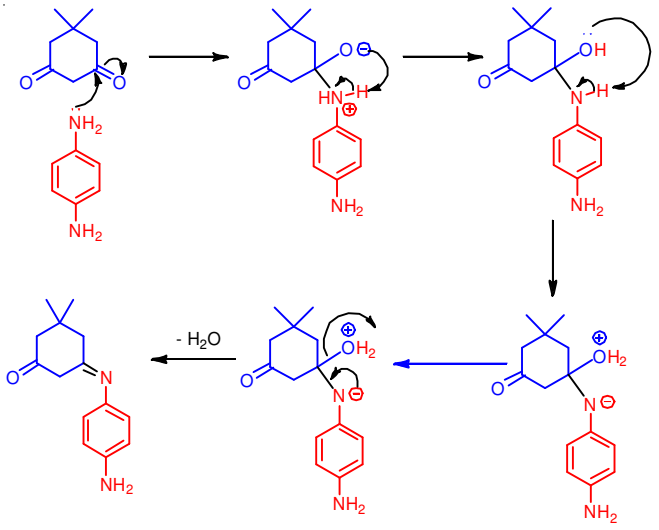


Mechanism

Antibacterial activity of compounds a and b: The results of the test compound **a** and **b** for qualitative and quantitative assay of the antibacterial activity, expressed by zone of inhibition (ZOI) in mm and minimum inhibition concentration (MIC) in µg/mL, values are summarized in Table-1. It is also to be mentioned that DMSO did not exhibit any noticeable antibacterial activity at the studied concentrations, thus the solvent did not influence the biological activity of the tested compound.



Scheme-II



Mechanism

Antifungal activity of compounds a and b: The results of the test compound **a** and **b** for qualitative and quantitative assay of the antifungal activity, expressed through zone of inhibition (ZOI) in mm and minimum inhibition concentration (MIC) in µg/mL, values are summarized in Table-2. It is also to be mentioned that DMSO did not exhibit any traceable anti-fungal activity at the studied concentrations, thus the solvent did not influence the biological activity of the tested compounds.

Conclusion

In conclusion, we have achieved a facile and efficient method for the synthesis of a variety of Schiff-base from aromatic amine and dimedone *via* condensation reaction. As

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUND **a** AND **b** AGAINST SELECTED GRAM POSITIVE AND GRAM NEGATIVE BACTERIAL STRAIN. OC: OFLOXACIN, DMSO: DIMETHYL SULFOXIDE; CM+BC: CULTURE MEDIA + BACTERIAL CULTURE (NEGATIVE CONTROL)

Bacterial isolates	Zone of Inhibition (ZOI in mm)				Minimum inhibitory concentration (MIC in µg/mL)			
	a	**b**	OC	DMSO	**a**	**b**	OC	CM + BC
Staphylococcus aureus ATCC 25923	14	12	20	NA	50	100	0.04	NA
Staphylococcus aureus (ATCC 29213	11	13	19	NA	25	50	0.04	NA
Bacillus subtilis ATCC 6633	12	12	19	NA	50	50	0.02	NA
Staphylococcus epidermidis	11	13	20	NA	50	100	0.04	NA
Escherichia coli ATCC 25922	13	15	21	NA	25	50	0.02	NA
Pseudomonas aeruginosa ATCC 27853	12	14	20	NA	25	50	0.09	NA
Shigella	12	15	21	NA	25	50	0.04	NA
Salmonella	11	11	19	NA	50	50	0.09	NA

TABLE-2
ANTIFUNGAL ACTIVITY OF COMPOUND AANDB AGAINST SELECTED CANDIDA STRAIN. IT: ITRACONAZOLE; DMSO: DIMETHYL SULFOXIDE; CM + CC: CULTURE MEDIA + CANDIDA CULTURE (NEGATIVE CONTROL)

Candida Isolates	Zone of Inhibition (ZOI in mm)				Minimum inhibitory concentration (MIC in µg/mL)			
	a	**b**	IT	DMSO	**a**	**b**	IT	CM + CC
C. albicans ATCC 90028	13	14	20	NA	50	50	0.09	NA
C. albicans ATCC 66027	15	16	23	NA	50	25	0.04	NA
C. tropicalis ATCC 66029	14	15	22	NA	50	50	0.19	NA
C. parapsilosis ATCC 22019	16	16	24	NA	25	25	0.04	NA
Candida sp. [HVS] 178	14	17	23	NA	50	25	0.19	NA
Candida sp. [BLOOD] 12810	14	15	23	NA	50	50	0.19	NA
Candida sp. [URINE] 12485	15	16	23	NA	50	50	0.04	NA

per the tables the result showed that **b** have more antimicrobial activity than except one bacterial strain (*Staphylococcus aureus*).

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