SYNTHESES AND ANTIMICROBIAL ACTIVITY OF NEW CYCLOHEXAPYRAZOLINE DERIVATIVE

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ABSTRAC

A new cyclohexapyrazoline compound was synthesized by reacting 2,6-bis(4-(dimethylamino)benzylidene) cyclohexanone (I) with hydrazine hydrate in methanol utilizing the microwave technique. The structure of the new cyclohexapyrazoline compound was confirmed by elemental and spectral analysis. Finally, the antimicrobial activity of the new cyclohexapyrazoline compound (II) was studied.

KEYWORDS: Michael addition; microwave irradiation; pyrazoline; antimicrobial activity.

INTRODUCTION

Pyrazolines are a form of heterocyclic compounds that have much attention, due to their biological activity[1]. Pyrazoline derivatives have antifungal[1-3], antiviral and antibacterial[4-6], and are also known for their chromosomal properties[7]. Optimizing synthetic procedures for pyrazolines consisting of different substituents an objective for of obtaining some new pyrazoline compounds with potentially enhanced properties. The development of new, fast and clean synthetic routes toward libraries of pyrazoline heterocycles is interest to the chemists. They have been reported in literature survey for the design and development of novel pyrazoline rings via multistep preparations[8-10]. The common synthesis of the pyrazoline is through Michael 1,4-addition of an *α, β*-enone with a hydrazine derivative[8]. The preparation of different pyrazoline derivatives, has been a developing field within the regality of heterocyclic chemistry for the past few years, because of their readily accessibility and the broad spectrum of biological activity of the products[11,12]. These results led us to synthesize diffrenet pyrazoline and discovering simple procedures such as irradiation chemistry[8,10,11], since it offers a new clean approach towards the synthesis of organic compounds. In the recent years, the ultrasonic microwave methods have become widely used in organic synthesis[10-14], This technique has been employed, not only to minimize reaction times, but also to improve yields in a large variety of heterocyclic compound, and ease of control[13]. Therefore, the development of these compounds for the use in different applications with improved characteristics is an important goal. In this present study, pyrazoline compound derived from dibenzylidene cyclohexanone with hydrazine hydrate was prepared. The effect of pyrazoline structure on the biological activities of this resin against bacteria and fungi have also been examined.

EXPERIMENTAL

Instrumentation:

Melting points were determined on a Perkin-Elmer 240ºC electro thermal melting point apparatus and are uncorrected. Elemental analyses were performed on Elementar Vario EL III instrument. Infrared spectra were recorded on a Shimadzu 2110 PC spectrophotometer with KBr pellets. The ^{1}H-NMR spectra were recorded on a GNM-LA 400 MH_z NMR spectrophotometer at room temperature in DMSO or $CHCl_3$ using TMS as the internal reference and the chemical shifts were recorded by ppm. Microwave irradiation carried out using a microwave closed system (GEEPAS. Model No: GMO1874.27LD,50Hz).

Reagents and solvents:

4-(dimethylamino)benzaldehyde and hydrazine hydrate were purchased from (Aldrich) and used without further purification. Methanol and all other solvents were of high purity and were further purified by standard methods[15].

Syntheses of pyrazoline compound:

Syntheses of 2,6-bis (4-(dimethylamino) benzylidene) cyclohexanone:(I)

The 2,6-bis (4-(dimethylamino) benzylidene) cyclohexanone (I) was prepared as described in the literature[16].

Syntheses of 4-((3-(4-(dimethylamino) phenyl)-,3,3a,4,5,6-hexahydroindazol-7-ylidene) methyl)-N,N-dimethylbenzenamine:(II)

Equimolar amounts 0.01 mole (3.60g) of compound (I), 0.05 mole (2.91 g) of hydrazine hydrate (98%) and dry Teflon vessels. In this case a minimum amount of methanol (10–15 ml) was used to dissolve the reaction mixture. This mixture was subjected to microwave irradiation at 400 W power for about 2 min with maximum heating of 60 °C. The completion of reaction was checked by TLC using mobile phase dichloromethane/ethyl acetate (7/2). The reaction mixture was allowed to cool to room temperature and the crude solid product was collected through vacuum filtration and washed several times with ethanol, and finally recrystallized from absolute ethanol to yield (85%). The pure product was conserved in a dark bottle in the refrigerator, mp. 270°C. Calculated composition of $C_{24}H_{30}N_4$: C, 77.00; H, 8.02; N, 14.97 % Found: C, 77.16; H, 8.11.; N, 14.73 %. IR(KBr): at 1610 cm^{-1} (s, C = C), at 1630 cm^{-1} (s, C = N),at 2850-2940 (C-H of cyclohexapyrazoline) and at 3220 cm^{-1} (br, N-H stretching).^{1}H-NMR (DMSO-d_6): at 8.00-7.00 (*m*,8H of Ar-H),at5.95 (*s*, H of CH=C), at 5.50 (*s*, H of N-H pyrazoline),at 4.3(*d*, H of CH-N pyrazoline),at3.9 (*d*, H of CH-C hexapyrazoline),at

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3.30 (*d*,12H of CH_3-N-CH_3), at2.70 (*t*, 4H of $2CH_2$ cyclohexapyrazoline), and at 1.20 (*t*,middle, 2H of CH cyclohexapyrazoline).

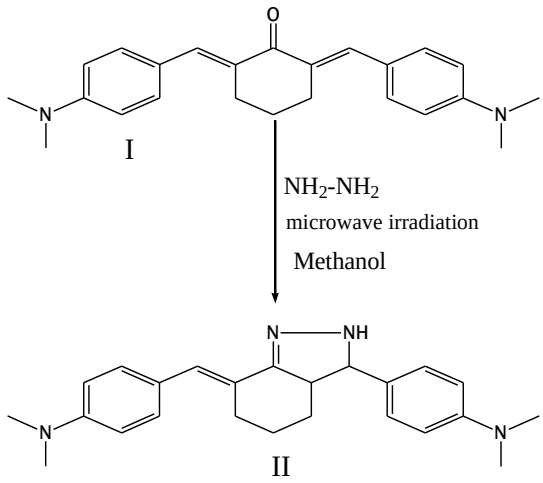
Antimicrobial activity screening of pyrazoline compound II:

The antibacterial activity of the synthesized pyrazoline compound was tested against Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli using nutrient agar medium. The antifungal activity of the compounds was tested against Candida albicans and Aspergillus niger using sabouraud dextrose agar medium. Both activities were performed by paper disc diffusion method[17]. The sterilized (autoclaved at 120°C for 30 min) medium (40–50°C) was inoculated (1 mL/100 mL of medium) with the suspension (105 cfu/mL) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petri dish to give a depth of 3–4 mm. The paper impregnated with the test compounds (50 µg/mL in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37°C for 24 and 48 h for antibacterial and antifungal activity respectively. Ciprofloxacin and ketoconazole were used as standard for antibacterial and antifungal activity respectively.

RESULTS AND DISCUSSION

Syntheses of pyrazoline compound:

In 1964 and by using Michael condensation, Sammour *et.al* [18] have reported some substituted cycloalkanones with phenyl hydrazine. In another study [19], doubly unsaturated ketones such as dibenzylideneacetone showed the two double bonds in dibenzylideneacetone underwent Michael condensation independent on each other In this work, a new heterocyclic compound based on cyclohexapyrazoline moiety was prepared by a condensation reaction between of 2,6-bis(4-(dimethylamino) benzylidene) cyclohexanone (I) and hydrazine (figure 1).



(Figure 1) Syntheses of pyrazoline compound II.

The IR data for the compound II showed the disappearance of the C=O group of starting material and the absorption bandappearance of the C=N group appeared at 1630 cm^{-1}. The N-H group of the cyclohexapyrazoline was also seen at 3220 cm^{-1}. This indicated that the compound II was formed. The^{1}H-NMR data confirmed the structure formula of the resulting pyrazoline derivativeII.

Antimicrobial activity:

The *in vitro* antimicrobial activity of the synthesized pyrazoline compound was performed using the disk diffusion method with different Gram positive (*Streptococcus pyogenes*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria as well as fungal strains (*Candida albicans*, *Aspergillus niger*). The antimicrobial activity potentials were qualitatively assessed by the inhibition zone diameters. Ciprofloxacin and ketoconazole were used as positive controls for bacteria and fungi respectively. In a preliminary assay, the tested pyrazoline compound showed activity against all tested microbial strains, and the obtained results were presented in table 1. The tested pyrazoline compound displayed a variable level of antimicrobial activity on all the tested strains. It can be clarified from table 1 that the highest antibacterial activity was observed against *Streptococcus pyogenes* with zone of inhibition of 18 mm followed by *Staphylococcus aureus* and Escherichia coli exhibiting a maximum zone of inhibition of 16 mm. It was interesting to observe that these activities were comparable with the standard drugs ciprofloxacin and ketoconazole demonstrating a zone of inhibition of 25 mm and 21 mm respectively.

(**Table 1)** Antimicrobial activity of the tested pyrazoline compound (expressed as the diameter of the inhibition zone[a])

Name of compound	Candida albicans	Aspergillus niger	E. coli	Streptococcus pyogenes	Staphylococcus aureus
Pyrazoline II	05	07	14	18	16
Ciprofloxacin	13	10	20	25	22
Ketoconazole	11	14	19	20	21

[a] *Average of three observations;*
Inhibition zone in mm (Disc diameter 6 mm)

The moderate antibacterial activity was noted with the pyrazoline moiety based on dibenzylidene cyclohexanone which may be attributed to the conformation of the heterocyclic pyrazoline ring II. The antifungal activity of the pyrazoline compound II was studied for the two pathogenic fungi. It was observed that pyrazoline compound II had the lowest activity against *C. albicans* and *A. niger*

exhibiting a maximum zone of inhibition of 7 mm. Moreover, the tested pyrazoline compound exhibited moderate antibacterial activity against all three strains of bacteria, but the activity was less against the two pathogenic fungi (*C. albicans* and *A. niger*).

CONCLUSION

New compound of dibenzylidene cyclohexanone con-

taining pyrazoline moiety was synthesized *via* ultrasonic microwave technique. The structure of the new pyrazoline compound was confirmed by spectral analysis. The synthesized pyrazoline compound could be considered as a moderate to good antimicrobial agents.

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