STUDIES IN ANTIFUNGAL ACTIVITY OF N-HETEROCYCLIC SUBSTITUTED HYDRAZONE SCHIFF'S BASES.

Vrushali T. Kale[1],Subodh A.Bhandarkar[2]

[1]Department of Chemistry, Government Institute of Science and Humanities, Amravati (M.S.), Pin 444604, India.

Archana S. Burghate[3] and Shrikant A. Wadhal[4]

[3]Department of chemistry, Shri Shivaji Science College, Amravati - 444603, M.S. India.

ABSTRACT

In the present study, we have reported the synthesis, spectral studies and biological evolution of substituted hydrazone schiff's bases derivatives of benzothiazolyl and benzimidazolyl for antifungal activity. In the present research series of 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol, 2-(Benzothiazole-2-yl hydrazono)-methyl)- phenol, N-Benzothiazole-2-yl-N-(1-4-chloro-phenyl)-ethylidene]-hydrazine ,2-[1-H-Benzimidazole-2-yl hydrazono)-methyl]-4-methyl phenol were synthesized by green chemistry technique. Structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H-NMR and CHN analysis. The antifungal activities of these synthesized compounds are done by cup plate method The fresh cultures of Candida albicans (NCIM 3103). Present work was based on the synthesis of substituted benzothiazole derivatives in order to find out the antifungal activity against *C. albicans* and *Pityrosporium ovalue*. since *Candida albicans* is mainly responsible for skin infection, especially in the epithelial cells of the vagina. Candida albicans is a yeast that commensally inhabits the human body and can cause opportunistic or pathogenic infections.

Keywords: Antifungal activity; Cup plate method, Skin infection, 2-substituted benzothiazole.

INTRODUCTION

Hydrazones constitute an important class of biologically active drug molecules[1].A number of hydrazone derivatives have been reported to exert notably as anti-bacterial [2], anti-microbial and cytotoxic[3], anti-diabetic [4], antitumor [5], anti- inflammatory [6], anthelmintic [7], antifungal [8] activities etc. Schiff bases play crucial role in inorganic chemistry, as they simply kind stable complexes with most transition metal ions the eventof the field of bioinorganic chemistry has enhanced the interest in Schiff base complexes, Candida species, particularly C. albicans has normally inhabited the human skin surface and cause infection. However, skin barrier level defence mechanisms area unit terribly economical. Therefore, the skin is Associate in Nursing effective barrier against fungal infection. C. albicans as the most common fungal pathogens since last two decades mainly due to increased devlopement of resistance to antifungal agents. We reported here a study on synthesis of some novel Schiff's base derivatives of benzothiazole and benzimidazole derivatives. These derivatives were screened for antifungal activity against the *Candida albicans*

MATERIALS AND METHOD

All reagents and solvents were purchased from spectrochem and merk used as received. The melting point of the synthesized compounds were determined in open capillary tube and are uncorrected.the IR spectra were record on a Perkin-Elmer model 2000 spectrophotometer using KBr phase. ^{1}H-NMR spectra were recorded on Brucker (400MHz) spectrophotometer using TMS as an internal standard. The purity of the synthesized compounds was checked by TLC on Silica gel in solvent system toluene and ethyl acetate (1:1) and the spot were visualized under UV chamber. Microwave synthesis was carried out in domestic microwave oven.

SYNTHESIS

Synthesis of 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol. (3a)

Method A: Conventional Method

A mixture 2-Hydrazinobenzothiazole (0.036 mole, 6 g) and 2-hydroxy-5methyl acetophenone (0.036 mole, 0.49g) was taken in a 100ml round bottom flask was shaken in 15-20 ml ethanol; it was refluxed for 7-8 hours. The progress of reaction was monitored by TLC, after completion of reaction; reaction mixture was cooled to room temperature and poured in ice cold water. The wet product was dried under vacuum for 10 min. and then dried at 40°C for 20 min under vacuum, to obtain 2-[1-(Benzothiazole-2-yl hyd

Method B:

Microwave Assisted Synthesis of 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol(3a)

2-Hydrazinobenzothiazole (0.0036 mole, 0.6 g) was taken in 25 ml beaker to this added 2-hydroxy-5methyl acetophenone (0.036 mole, 0.49g) in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven covered with watch glass and irradiated with microwave irradiation for 5 minutes at 180 watt after completion of reaction beaker was removed and the granular solid was crystallized from hot Ethanol to give 80-85 % yields. Yield – 80-85%, M.Pt-200-220^{0}C, M.Wt -297.38, Formula-C16H15N3SO, IR-(KBr

cm^{-1}):-3244 (N-H), 3650 (0-H); -^{1}H-NMR:2.5 (S, 3H, CH3), 6.9-7.8 (m, 8H, Ar-H) , 11.8-12.00 (b.S, 1H, 0H),12.3-12.5 (S, 1H, NH) Elemental analysis: C16H15N3SO, calculated-C,64.62; H, 5.08; N, 14.13; found: C, 64.60; H 3.44; N, 20.40.razono)-ethyl]-5-methyl phenol with 55 to 60% yield.

Synthesis of - 2-(Benzothiazole-2-yl hydrazono)-methyl)- phenol (3b)

2-Hydrazinobenzothiazole (0.0036 mole, 0.6 g) was taken in 25 ml beaker to this added **2**-hydroxybenzaldehyde (0.0036 mole, 0.43g) in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven covered with watch glass and irradiated with microwave irradiation for 5minute at 200 watt after completion of reaction beaker was removed and the granular solid was crystallized from hot Ethanol to give 80-82 % yields. Yield – 80-82%, M.Pt-300-320^{0}C, M.Wt -269.33, Formula-C14H11N3OS, IR-(KBr cm^{-1}):- 3100 (N-H), 3900(O-H); ^{1}H-NMR: 6.8-7.6 (m, 8H, Ar-H), 8.5 (S, 1H, H), 10.4-10.5 (b.S, 1H, OH), 12.1-12.2(b.s, 1H, NH). Elemental analysis: C14H11N3OS, calculated- C, 62.43; H, 4.12; N, 15.6. Found: C, 62.50; H, 4.14; N, 15.8.

Synthesis of - N-Benzothiazole-2-yl-N-(1-4-chloro-phenyl-ethylidene]-hydrazine (3c)

2-Hydrazinobenzothiazole (0.0036 mole, 0.6 g) was taken in 25 ml beaker to this added P-chloroacetophenone (0.0036 mole, 0.556g) in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven covered with watch glass and irradiated with microwave irradiation for 4minute at 400 watt after completion of reaction beaker was removed and the granular solid was crystallized from hot Ethanol to give 75-80 % yields. Yield – 75-80%, M.Pt-280-300^{0}C, M.Wt -301.80, Formula-C15H12ClN3S, IR-(KBr cm^{-1}):- 3100(N-H),3857(O-H); ^{1}H-NMR : 2.4 (S, 3H, CH3), 7.00-7.9 (m, 8H, Ar-H), 11.6-11.7 (b.S, 1H, NH). Elemental analysis: C15H12ClN3S, calculated- C,59.7; H,4.01; N,13.92. Found: C,59.9; H,4.05; N,13.94 .

Synthesis of 2-[1-H-Benzoimidazole-2-yl hydrazono)-methyl]-4-methyl phenol (3d)

Microwave Assisted Synthesis of 2-[1-H-Benzoimidazole-2-yl hydrazono)-methyl]-4-methyl phenol (3d)

2-Hydrazino1-H-benzimidazole (0.005 mole, 0.6 g) was taken in 25 ml beaker to this added O-hydroxyacetophenone (0.005 mole, 0.69 g) in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven covered with watch glass and irradiated with microwave irradiation for 5 minutes at 300 watt after completion of reaction beaker was removed add to it was added cold water when the granular solid was isolated it was filtered and was crystallized from hot Ethanol to give 85-90 % yields. Yield – 85-90%, M.Pt-230--250^{0}C, M.Wt -266.31, Formula-C15H14N4O, IR-(KBr cm^{-1}):- 3280 (NH), 3757(OH); ^{1}H-NMR : 2.05(S, 3H, CH3), 6.8-7.7 (m, 8H, Ar-H), 10.9 (b.S, 1H, OH),12.3(S,1H,NH),12.9(S,1H,NH). Elemental analysis: C15H14N4O, calculated- : C, 67.65; H, 5.3: N, 21.04 Found: C, 67.67; H, 5.6: N, 21.07.

RESULTS AND DISCUSSION

As shown in fig-1 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol. (3a)was prepared from 2-Hydrazinobenzothiazole and 2-hydroxy-5methyl acetophenone 1:1 ratio, the mixture was moistened with ethanol and irradiated with microwave irradiation for 5minutes at 180 watt, obtained 85% yield. The present methods describe mild, efficient, convenient, ecofriendly and high yielding process. The structures of the synthesized compounds were characterized with the help of IR, ^{1}HNMR and elemental analysis. The compound 3a showed IR absorption band at 3244 and 3650 cm^{-1} attributed to the presence of OH and NH groups. The 1HNMR spectrum of this compound showed the presence of CH3 , The appearance of broad singlet at δ 12.00 and 12.3 NH and OH respectively. The appearance of multiple peaks in the region of δ7.9-7.8 showed ArH

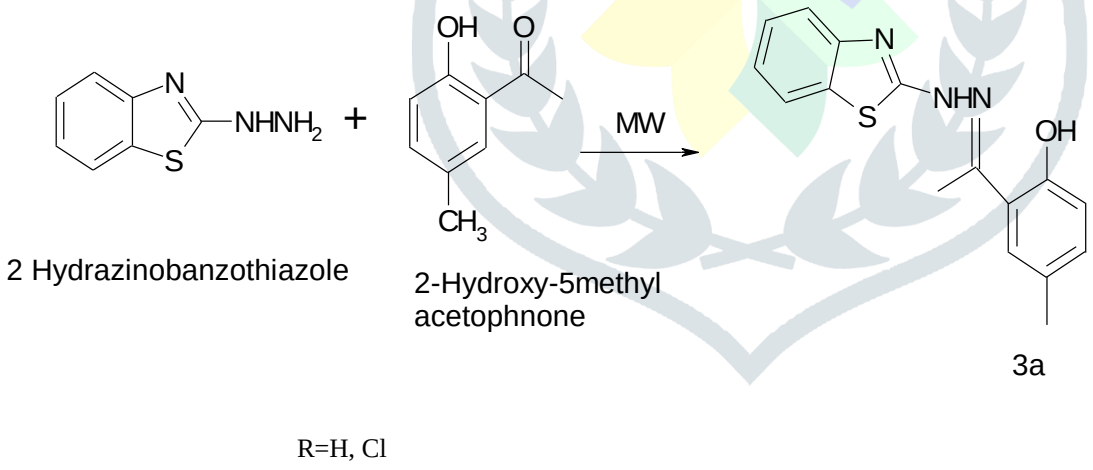


Fig 1: 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol. (3a)

As shown in fig-2 - 2-(Benzothiazole-2-yl hydrazono)-methyl)- phenol (3b) was prepared from 2-Hydrazinobenzothiazole and 2-hydroxybenzaldehyde 1:1 ratio, the mixture was moistened with ethanol and irradiated with microwave irradiation for 5minutes at 200 watt, obtained 82% yield. The present methods describe mild, efficient, convenient, ecofriendly and high yielding process. The structures of the synthesized compounds were characterized with the help of IR, ^{1}HNMR and elemental analysis. The compound 3b showed IR absorption band at 3100 and 3900cm^{-1} attributed to the presence of OH and NH groups. The 1HNMR spectrum of this compound showed the presence of CH3 , The appearance of of broad singlet at δ 10.5 and 12.2exchangeable with D2O showed NH and OH respectively. The appearance of singlet at δ 8.5 showed presence of Hx. The appearance of multiple peaks in the region of δ6.8-7.6 showed ArH

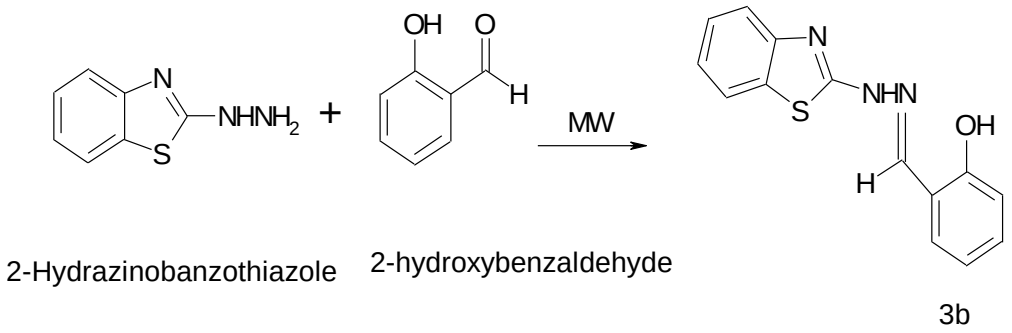


Fig 2: - 2-(Benzothiazole-2-yl hydrazono)-methyl)- phenol (3b)

As shown in fig-3 N-Benzothiazole-2-yl-N-(1-4-chloro-phenyl-ethylidine]-hydrazine (3c) was prepared from 2-Hydrazinobenzothiazole and P-chloroacetophenone 1:1 ratio, the mixture was moistened with ethanol and irradiated with microwave irradiation for 4minutes at 400 watt, obtained 80% yield. The present methods describe mild, efficient, convenient, ecofriendly and high yielding process. The structures of the synthesized compounds were characterized with the help of IR, ^{1}HNMR and elemental analysis. The compound 3c showed IR absorption band at 3100 and 3857 cm^{-1} attributed to the presence of OH and NH groups. The ^{1}HNMR spectrum of this compound showed the presence of CH3 , The appearance of of broad singlet at δ 11.6 and 11.7, the appearance of singlet at 2.4. The appearance of multiple peaks in the region of δ7.00-7.9showed ArH

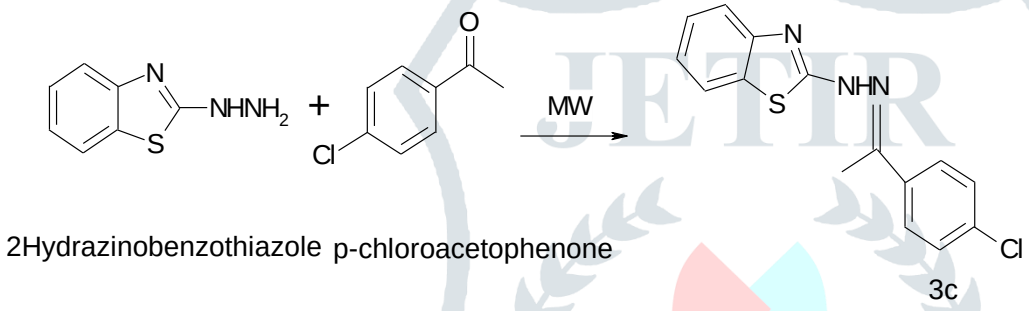


Fig 3: N-Benzothiazole-2-yl-N-(1-4-chloro-phenyl)-ethylidine]-hydrazine (3c)

In fig-4,2-[1-H-Benzoimidazole-2-yl hydrazono)-methyl]-4-methyl phenol(3d)was prepared from 2-Hydrazino1-H-benzimidazole and O-hydroxyacetophenone in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven and irradiated with microwave irradiation for 5 minutes at 300 watt. The structure was further confirmed by IR and ^{1}H-NMR spectroscopic method. The structures of the synthesized compounds were characterized with the help of IR, ^{1}H-NMR spectroscopy methods and elemental analysis. The compound 3d showed IR absorption band at 3280 cm^{-1} due to NH group. The ^{1}H-NMR spectrum of this compound showed the presence of CH3, the appearance of a singlet peak in the region of δ 12.3and peak in the region of δ 12.9 for N-H.

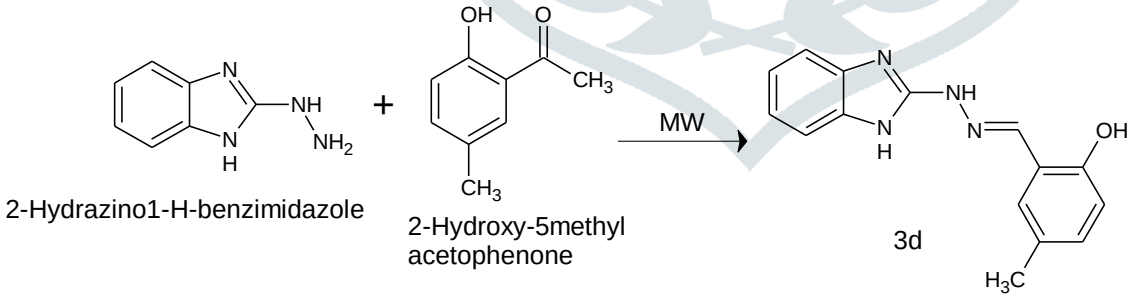


Fig 4: 2-[1-H-Benzoimidazole-2-yl hydrazono)-methyl]-4-methyl phenol (3d)

Table 1: Physical data of the compounds.

S. No.	Entry	R	M.P. (^{0}C)	Yield (%)	M.Wt.	Formula
1	3a	-	200-220	84	297.38	C16H15N3 OS
2	3I	H	210-230	85	283.35	C15H13N3 OS
3	3II	Cl	200-230	80	287.77	C14H10ClN3 S
4	3b	-	300-320	75	269.33	C14H11N3OS

5	3c	-	280-300	80	301.80	C15H12ClN3S
6	3d	-	230-250	85	266.31	C15H14N4O

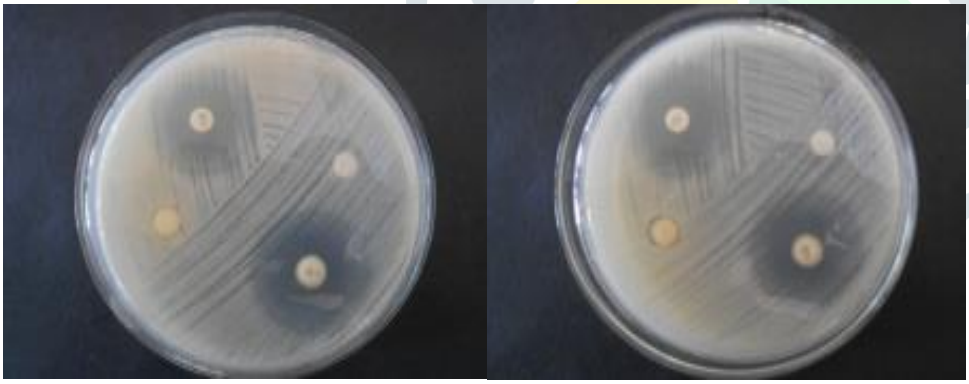
ANTIFUNGAL STUDY

Methods for the determination of antifungal activity

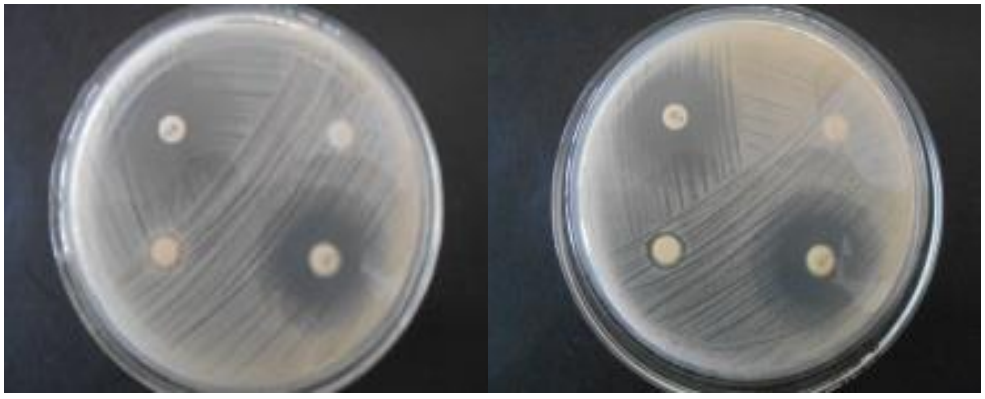
The antifungal activities of these synthesized compounds are done by cup plate method The fresh cultures of Candida albicans (NCIM 3103). Nutrient agar, while hot was poured to the sterilised petri dishes (20 ml in each dish) and allowed to attain room temperature. The seed layer medium was melted and cooled to 45~50°C5 with gentle shaking, overnight grown liquid culture was added asceptically to the base layer medium and mixed thoroughly to get the uniform distribution.Immediately it was poured into petridishes containing base layer and then allowed to attain room temperature.Thereafter the cups were made by punching into the set agar with a sterile cork borer and scooping out the punched part.The diameter of each cup was 10 mm. To these cups, 0.1ml(100 Mg) of drug solution was added. These plates were allowed to cool for an hour to facilitate the diffusion.Then the plates were incubated at 37°d for 24 hours. The zone of inhibition was measured in millimeters.

Table 2. antibacterial activity of some benzothiazolyl and benzimidazolyl substituted derivatives.

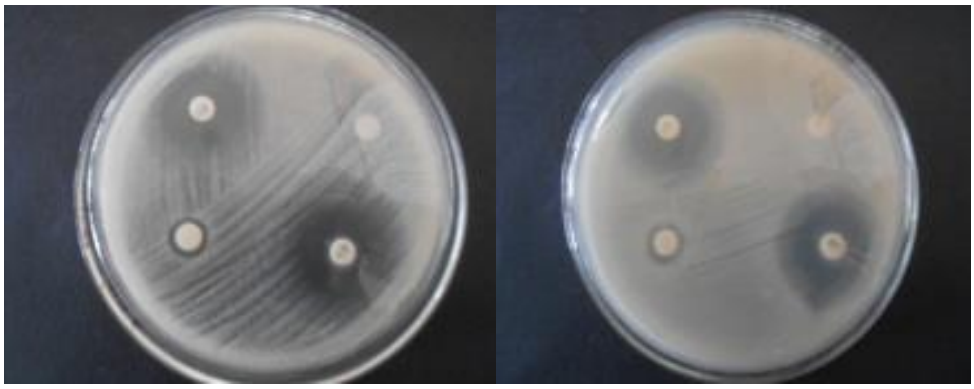
Entry	Zone of inhibition in mm	
	c.albicans	*P. ovalue.*
3a	13	13
3I	14	14
3II	15	12
3b	13	14
3c	13	13
3d	14	14



c.albicans ***P. ovalue***



c.albicans ***P. ovalue***



c.albicans *P. ovalue*

CONCLUSION

In the current study, totally a series of 2-[1-(Benzothiazole-2-yl hydrazono)-ethyl]-5-methyl phenol, 2-(Benzothiazole-2-yl hydrazono)-methyl)- phenol, N-Benzothiazole-2-yl-N-(1-4-chloro-phenyl)-ethylidene]-hydrazine and 2-[1-H-Benzoimidazole-2-yl hydrazono)-ethyl]-5-methyl phenol was synthesized and evaluated their antibacterial activity . We have developed convenient and eco-friendly method for the synthesis of benzothiazolyl and benzimidazolyl substituted compounds by green chemistry technique i.e. microwave synthesis. The excellent yield, easy work-up and simple reaction procedure is highlighted in the present work. The antifungal activities of ligands and their complexes reveal that the complexes are relatively better antifungal agents than the ligands. Almost all the complexes are moderately active against C.albicans. For future research the synthesized compounds will be implemented for physical parameter determination in binary solvent mixtures.

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