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Research Article

Design, Spectral and Biological Evaluation of Benzohydrazide Derivatives

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Abstract

TheTiO$_2$(R) Nanoparticles was synthesised by the sol–gel method and characterized by powder X-ray diffraction(XRD) scanning electron microscopy (SEM) and energy dispersive X-ray (EDS) spectra. The benzohydrazide derivatives (1-4) have been synthesized by using sol-gel prepared TiO$_2$ (R) acting as a catalyst under solvent condition and characterized by FT-IR, NMR and mass spectral studies. Antimicrobial studies were carried out against the bacterial strains *viz., Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonasaeruginosa* and fungal strains *viz., Candida albicans, Aspergillusniger, Mucor*and*Rhizopus sp*.

Keywords:*Benzohydrazide;TiO2(R);XRD;SEM;EDS;Antimicrobial*

Introduction

Hydrazones have established to possess antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular, anticancer and antitumor activities [Allen *et al.,* 1987, Bedia *et al.,]*. Hydrazones overcome an azomethine –NHN=CH– proton constitutes an important class of compounds for new drug development. Therefore synthesized these compounds as goal structures and evaluated their biological activities [Titov, *et al.,* 1968]. These observations had served as guides for the growth of new hydrazones that possess different biological activities [Byrkit, *et al.,* 1950- Feuer, *et al.,* 1959]. Benzohydrazide and their derivatives were efficient molecules bearing -CO, –NH– and –NH$_2$ functionalities in their structures [Kaymakcolu, *et al.,* 2006- sangwan, *et al.,* 1986]. Therefore, we can expect these compounds to behave as ambident nucleophiles in several reactions but in our case –NH$_2$ act as a nucleophile [Ramadan, *et al.,* 1983].The nitrogen related heterocyclic compounds received significant consideration due to their wide application. A large number of heterocyclic compounds containing 1, 2, 4-triazole moiety are associated with varied pharmacological properties such as antimicrobial [koplancikl *et al., 2008*- Saadeh et al., 2010] and anticancer [karakus *et al., 2010* - Al-soud *et al.,* 2003]. Hydrazines were synthesized so as to enlarge intracellular concentration and so as to try and reduce the resistant developed due to decrease intracellular concentration of the drug. These synthesized compounds were subjected to preliminary biological valuation. In recent years various chemical and medicinal studies are made on heterocyclic ring system having hydrazone derivatives. The hydrazone derivatives serve synthetic intermediate for the preparation of various compounds. Hydrazone derivatives are also attracted very much due to their wide range of biological, pharmacological and anticancer activities.

Experimental

Materials and measurements

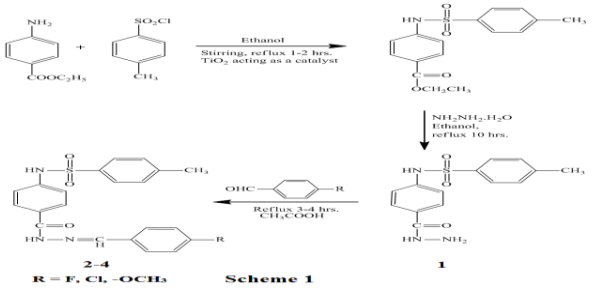
Titanium (IV) isopropoxide, ethyl-4-aminobenzoate, tosylchloride, triethylamine and hydrazine hydrate all other reagents have been purchased from Sigma-aldrich. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^{1}H NMR spectra were recorded on BRUKER AMX operating at 500 MHz and the ^{13}C NMR spectra were recorded in the same instrument and the operating frequency is 106 MHz. All NMR measurements were made on 5 mm NMR tubes. For recording ^{1}H NMR spectra, solutions were prepared by dissolving about 10 mg of the compound was dissolved in 0.5 mL of CDCl$_3$ (or) DMSO. While for recording ^{13}C NMR spectra, about 50 mg of the compound was dissolved in the same volume of the solvent. Here, tetramethylsilane (TMS) Spectrometer (EI mode). XRD patterns were was used as an internal standard. Mass spectra were recorded on VARIAN CP-3800 GC Mass recorded for the centrifuged and dried samples using X–ray Rigakudiffractometer with Cu K$_\alpha$ source (30 kV, 100 mA), at a scan speed of 3.0000 deg/min, step width of 0.1000 deg, in a 2θ range of 20-80°. The energy dispersive X-ray (EDS) spectra were recorded with a JEOL JSM-5610 scanning electron microscope (SEM) equipped with back electron (BE) detector and EDX. The sample was placed on an adhesive carbon slice supported on copper stubs and coated with 10 nm thick gold using JEOL JFC-1600 auto fine coater prior to measurement. .

Theoretical calculations were performed by using B3LYP/6-31G (d,p) basis set [Gaussian 03, *et al.,* 2004].

Synthesis of benzohydrazide derivatives

A mixture of ethyl-4-aminobenzoate (5 mmol), tosyl chloride (5 mmol), triethylamine (5 mmol) and hydrazine hydrate (5 mmol) in 50 mL of ethanol, was refluxed for about 2 hrs, TiO$_2$ (R) acting as a catalyst. The reaction is

monitored by TLC. After completion of reaction, it was poured into water and Filtered. The combined ether extract was then washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulphate. The afforded product was purified by column chromatography using benzene: chloroform (6:4) as an eluent (Scheme 1). The newly synthesized benzohydrazide derivatives have been characterised by FT-IR (Figure 1), ^{1H} (Figure 2) and ^{13}C (Figure 3) NMR and mass (MS) spectra (Figure 4).



Scheme 1. Synthetic route of benzohydrazide derivatives.

N-(4-(hydrazinecarbonyl)phenyl)-4-methylbenzenesulfonamide(1)

- Yield (%): 90, M.p. 277 ˚C, Molecular Formula: $C_{14}H_{15}O_3N_3S$
- IR (KBr) (cm^{-1}): 3319 (N-H stretching); 3150, 3046, 2940, 2876 (Aliphatic and aromatic C-H stretching); 1649 (C=O stretching).
- ^{1}H NMR (δ, ppm): 10.61 (s, 1H, CONH); 9.75 (s, 1H, SO_2 NH); 2.29 (s, 3H, CH_3 Protons); 7.10-7.86 (8H, aryl protons).
- ^{13}C NMR (δ, ppm): 20.77 (CH_3 carbon); 165.86 (C=O carbon); 118.33-144.08 (aromatic carbons). Mass (m/z): 305.36 (M^+).

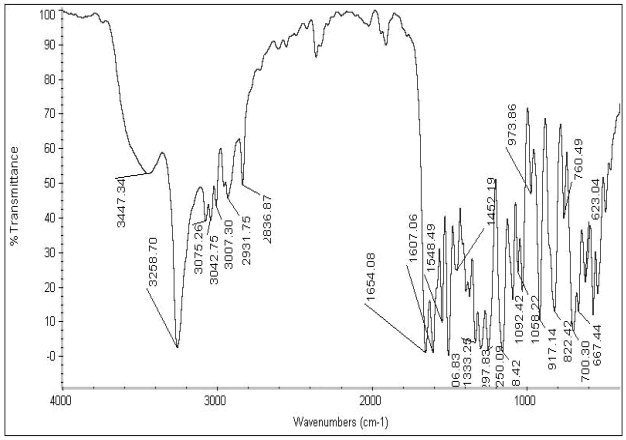


Fig1. IR Spectrum of Compound 1

N-(4-(2-(4-fluorobenzylidene)hydrazinecarbonyl)phenyl)-4-methylbenzene sulfonamide (2)

- Yield (%): 85, M.p. 275 ˚C, Molecular Formula: $C_{21}H_{18}O_3N_3SF$
- IR (KBr) (cm^{-1}): 3224 (N-H stretching); 3189, 3079, 3047, 2938, 2875 (Aliphatic and aromatic C-H stretching); 1641 (C=O stretching); 1606 (C=N stretching).
- ^{1}H NMR (δ, ppm): 11.82 (s, 1H, CONH); 10.81 (s, 1H, SO_2 NH); 8.31 (s, 1H, N=CH); 2.29 (s, 3H, CH_3); 7.18-7.77 (12H aryl protons).
- ^{13}C NMR (δ, ppm): 20.81 (CH_3 carbon); 163.07 (C=O carbon); 115.69-147.01 (aromatic carbons); 147.01 (N=CH). Mass (m/z): 411.49 (M^+).

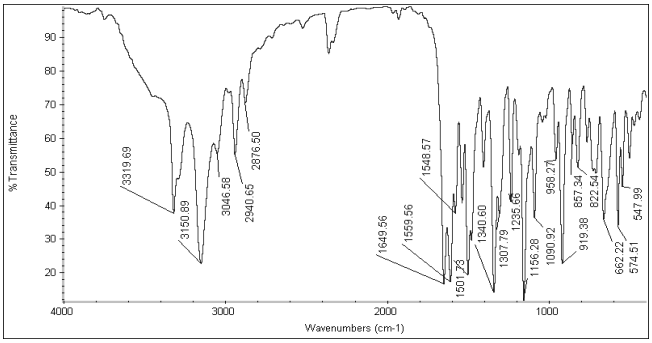


Fig1. IR Spectrum of Compound 2

N-(4-(2-(4chlorobenzylidene)hydrazinecarbonyl)phenyl)-4-methylbenzenesulfonamide (3)

- Yield (%): 85, M.p. 272 ˚C, Molecular Formula: $C_{21}H_{18}O_3N_3SCl$
- IR (KBr) (cm^{-1}): 3237 (N-H stretching); 3147, 3073, 3047, 2933, 2870 (Aliphatic and aromatic C-H stretching); 1643 (C=O stretching); 1604 (C=N stretching).
- ^{1}H NMR (δ, ppm): 11.86 (s, 1H, CONH); 10.75 (s, 1H, SO_2 NH); 8.30 (s, 1H, N=CH); 2.29 (s, 3H, CH_3); 7.11-7.74 (12H aryl protons).
- ^{13}C NMR (δ, ppm): 20.81 (CH_3 carbon); 163.06 (C=O carbon); 118.14-146.76 (aromatic carbons); 146.76 (N=CH). Mass (m/z): 427.91 (M^+).

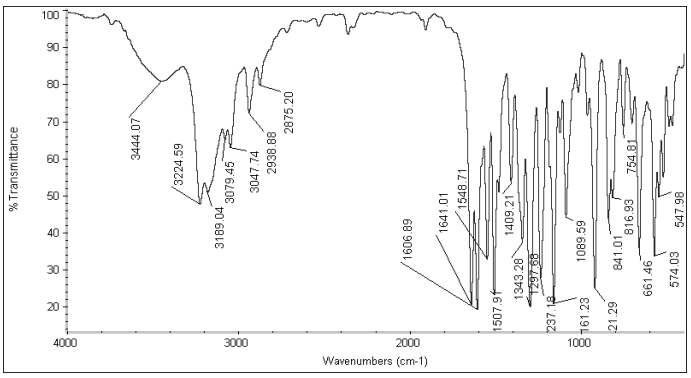


Fig1. IR Spectrum of Compound 3

N-(4-(2-(4-methoxybenzylidene)hydrazinecarbonyl)phenyl)-4-methylbenzenesulfonamide (4)

- Yield (%): 80, M.p. 278 ˚C, Molecular Formula: $C_{22}H_{21}O_4N_3S$
- IR (KBr) (cm^{-1}): 3288 (N-H stretching); 3075, 3042, 3007, 2931, 2836 (Aliphatic and aromatic C-H stretching); 1654 (C=O stretching);

  1607 (C=N stretching).

- ^{1}H NMR (δ, ppm): 11.70 (s, 1H, CONH); 8.25 (s, 1H, N=CH); 2.27 (s, 3H, CH_3); 6.95-7.74 (12H aryl protons); 3.74 (s, 3H, OCH_3).
- ^{13}C NMR (δ, ppm): 20.77 (CH_3 carbon); 163.02 (C=O carbon); 114.18-148.24 (aromatic carbons); 55.33 (OCH_3 carbon); 148.22 (N=CH). Mass (m/z): 423.49 (M^+).

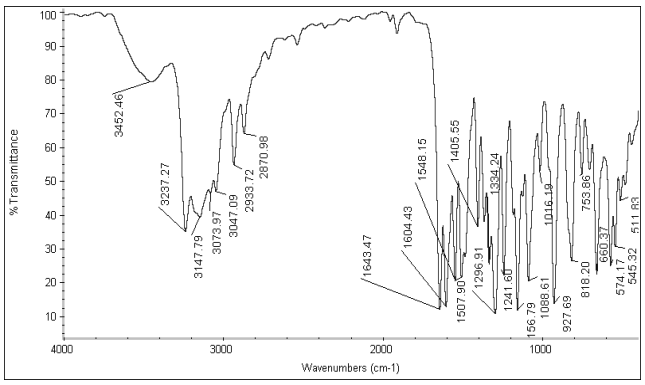


Fig 1. IR Spectrum of Compound 4

Synthesis of nocrystalline TiO_2 (R) by Sol- gel method

The TiO_2 (R) nanocrystal was prepared by sol-gel method of titanium (IV) isopropoxide, followed by calcination. About 1ml of titanium isopropoxide was dissolved in 20 ml isopropyl alcohol and the solution was dropped slowly into 10 ml of distilled water, pH 2-6 was adjusted by 1M HNO_3 for acidic condition and 1M NaOH for basic condition. The formed white sol-gel of hydrous oxide was stirred vigorously for 4 hours at room temperature and then allowed to age overnight. The solid was centrifuged and was redispersed in ethanol to minimize agglomeration. The resulting material was then dried and calcinated at 400 °C for 3 hrs, respectively.

Antimicrobial studies

The benzohydrazide derivatives will be evaluated for antibacterial activity against standard Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus (Gram-positive) and antifungal activity against Candida albicans, Aspergillusniger, Mucor, Rhizopussp. By agar well disc-diffusion method [Balaji *et al., 2013*, Balaji *et al., 2013*]. Suspensions of each microorganism were prepared from their 24 h cultures to obtain approximately 10^6 colony forming units (cfu) per ml for plating. DMSO was chosen as control and the compound under test were dissolved in DMSO at a concentration of 50 µg/0.05 ml. It were aseptically transferred and applied into the cups created on the dry surface of the inoculated plates and then incubated at 37 °C for overnight (~18-20h). This assay was performed in duplicates and the mean diameters of the clear inhibition zones (mm) were recorded disregard a single colony or a faint haze caused by the inoculums.

Results and discussion

Characterization of nano TiO_2 (R) The nano TiO_2 (R) obtained by sol–gel method has been characterized by XRD, SEM and energy dispersive X-ray. Figure 5 displays the X-ray diffraction pattern (XRD) of shows that the XRD pattern of TiO2 (R) phase. The diffraction pattern of TiO_2(R) match with the JCPDS pattern of tetragonal (89-4920). The crystal structures of TiO_2(R) with crystal constants a and b as 4.584A° and c as 2.953 A°. They have been obtained from the full width at half maximum (FWHM) of the most intense peaks of the respective crystals using the Scherrer equation, $L = 0.9\ \lambda/\beta\cos\theta$, where λ is the wavelength of the X-rays used, θ is the diffraction angle and β is the full width at half maximum of the peak. The specific surface area of the nanocrystals have been deduced by employing the relationship $S = 6/\rho D$, where S is the specific against the reported fungal strains.

The antimicrobial studies concluded that the prepared compound 1, 2, 3 and 4 shows more antifungal inhibition activity than the antibacterial inhibition activity. Further the compound 4 shows excellent antifungal and antibacterial inhibition activity (Figure 8). This may be due to electronic effect (+ I effect) exerted by methoxy group.

HOMO-LUMO molecular orbital studies

The electron density of HOMO-LUMO molecular surface area and ρ is the material density. The calculated, average crystalline size (20.6 nm) and surface area (51.9m^2/g). The SEM image (Figure 5) reveals agglomeration of the synthesized nanoparticles.

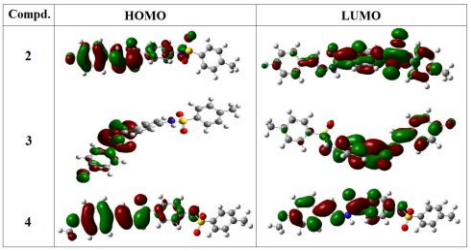


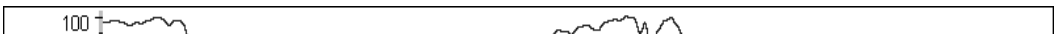
Fig 5 HOMO-LUMO orbital picture of compounds 2-4.

Catalytic activity of TiO_2 (R) semiconductor

To enhance the yield of the desired product, the temperature of the reaction was increased to 180 ºC, but no appreciable increment in the product yield was observed. We found that presence of a catalytic amount of TiO_2(R) under solvent-free condition is the best for this synthesis; maximum yield (85%) was obtained at 40 min on loading with 0.1mol% of TiO_2 (R) at 120 °C (Table 1). Moreover, TiO_2 can be recovered and reused several times without significant loss of activity (Figure 6). High product yield, shorter reaction time, low catalyst loading and easy work-up procedure, make this procedure quite simple and more convenient. Our methodology could be a valid contribution to the existing processes to synthesis of 4-(tosylamino)benzohydrazide derivatives.

Table1: Effect of catalyst and temperature in the synthesis of benzohydrazide derivatives

Entery	Temp (°C)	solvent	Time (min)	Yield (%)	TiO2 (mol%)
1	120	Methanol	60	43	1
2	120	Ethanol	40	85	0.1
3	120	Methanol	50	45	1
4	120	Chloroform	140	42	1
5	120	Acetonitrile	95	50	1



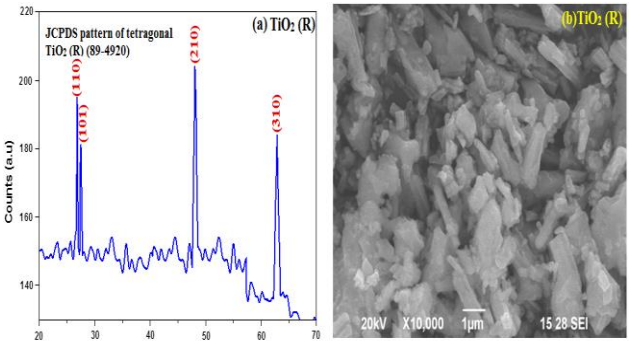


Fig 6. (a) X-ray diffraction pattern (XRD) of TiO_2 (R); (b) SEM image of TiO_2 (R)

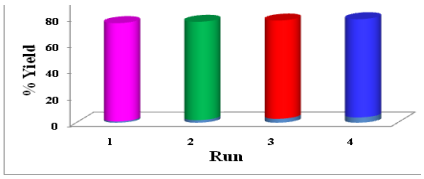


Figure 6. Reusability of TiO_2 (R) for catalytic synthesis of benzohydrazide derivatives

Antimicrobial Studies

The preliminary antimicrobial activity of the compounds 1-4 are examined using disc diffusion method. The bacterial strains *viz.,Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonasaeruginosa* and fungal strains *viz., Candida albicans, Aspergillusniger, Mucor*and*Rhizopus sp*.are used in this study. Dimethylsulphoxide is used as a control while Ciprofloxacin and Amphotericin B are used as a reference for bacterial and fungal studies respectively.(B3LYP-6_31G (d, p)). The polarisability (α) and hyperpolarisability (β) values are tabulated Table 3along with the dipole moment (D) values. These values strongly support that these molecules possess electronic properties.

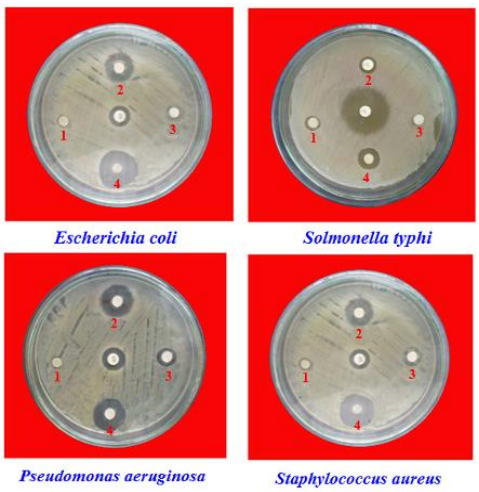


Figure 7. Zone of inhibition of compounds 1-4against E. coli, S. typhi, S. aureus and Pseudomonas aeruginosa

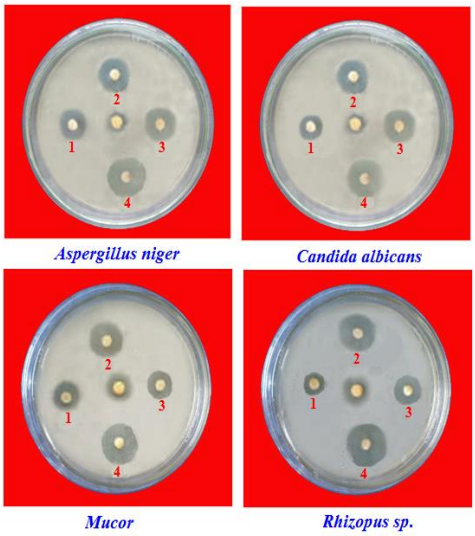


Figure 8. Zone of inhibition of compounds 1-4against C. albicans, A. niger, MucorandRhizopus sp

Conclusion

The facile synthesis of benzohydrazide derivatives using sol-gel synthesized TiO_2 (R) nanocrystal as catalyst. The synthesized TiO_2 (R) was characterized by XRD, SEM, EDS. Synthesized benzohydrazide derivatives characterised by IR, ^1H &^{13}C NMRand mass spectral analysis. The antibacterial studies revealed that the reported compounds 2, 3 and 4 show considerable inhibition activity where as the compound 1 did not exhibit any inhibition activity against the tested bacterial strains. The antifungal studies of compounds 1, 2, 3 and 4 indicated that all the compounds exhibit moderate to maximum activity against the reported fungal strains. The HOMO molecular orbital pictures of 2-4 show that the bonding character is more in the aryl ring, whereas antibonding characters more in the LUMO orbital.

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