RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 1 |501-507| January - March | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SPECTRAL AND BIOLOGICAL ACTIVITIES OF TiO_2 MEDIATED SYNTHESIS OF BENZOHYDRAZIDES

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ABSTRACT

The sol-gel method was used to synthesize TiO_2 nanoparticles, which were then characterized by powder XRD, SEM, EDS, and spectroscopy. The benzohydrazide derivatives (1-4) were synthesized utilizing sol-gel-produced TiO_2 (R) as a catalyst in a solvent environment and characterized by FT-IR, NMR, and mass spectrum analyses. Antimicrobial tests were conducted against bacterial strains such as *E. coli, S. typhi, S. aureus* and *P. aeruginosa*, as well as fungal strains such as *C. albicans, A. niger, Mucor* and *Rhizopus sp.*

Keywords: Benzohydrazides, TiO_2, XRD, SEM, EDS, Antimicrobial.

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INTRODUCTION

Hydrazones have been shown to have antibacterial, anti-inflammatory, anti-tubercular, anticancer, and antitumor properties.[1,2] Hydrazones surmount an azomethine proton and are a key family of chemicals for novel medication development. As a result, we synthesised these moieties as target structures and tested their biological activity.[3] These observations guided the development of novel hydrazones with various biological functions.[4,5] Benzohydrazide and its derivatives are efficient compounds with -CO, -NH-, and -NH2 functions in their structures.[6-8] As a result, we can imagine these moieties performing as obvious nucleophiles in various reactions; $-NH_2$ acts as a nucleophile.[9] Nitrogen-related heterocyclic compounds garnered a lot of attention because of their extensive use. A significant range of heterocyclic moieties having the 1,2,4-triazole are related by a variety of pharmacological features, including antibacterial activity.[10-15] are anticancer.[16-20] Hydrazines were synthesised to increase intracellular concentration and to try to eliminate resistance that had evolved as a result of the drug's decreased intracellular concentration. These synthesised moieties were done preliminary biological evaluation. In recent years, several chemical and medical researchers have been conducted on heterocyclic ring systems containing hydrazone. Hydrazone are also quite popular due to their diverse biological, pharmacological and anticancer effects. Titanium dioxide has several industrial applications[21,22] and has been expanded to the photo degradation of insecticides[23] and carcinogenic.[24,25] TiO_2 has been worn as a immature, low-cost, soft, and eco-friendly varied Lewis acid probable catalyst in definite organic transformation, such as Beckmann rearrangement[26], Fridel-Crafts acylation[27], Biginelli condensation[28], as well as the synthesis of dihydropyrazines[29], piperazines[30], quinoxalines[31] and photocatalytic amine oxidation.[32]

EXPERIMENTAL

Spectral Details

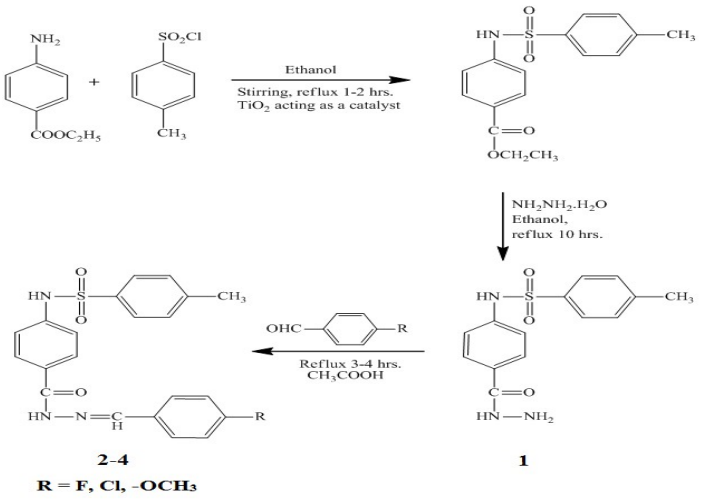
Bruker 400 MHz (Bruker, USA), ^1H and ^{13}C at 400 MHz, Using KBr pellets, the infrared spectra was obtained on a JASCO FT - IR Spectrometer in the 4000 - 400 cm^{-1}. XRD pattern were acquired for

http://doi.org/10.31788/RJC.2025.1819041

centrifuged and dehydrated sample by an X-ray Rigaku diffractometer with a Cu Kα resource (35 kV, 100 mA), at a scrutinize velocity of 2.0000 deg/min, thickness of 0.1000 deg, and in 2θ array of 20 - 100. The nanosemiconductors were obtained with a JEOL JSM-5610 SEM with a back electron detector. The theoretical computations were done using the B3LYP/6-31G (d,p) basis set.[33]

Synthesis of Benzohydrazide Derivatives

A solution of ethyl-4-aminobenzoate (1 mmol), tosyl chloride (1 mmol), $(CH_3CH_2)N$ (1 mmol), NH_2-NH_2. H_2O (1 mmol) in 70 mL of C_2H_5OH was refluxed for around 4 hours with TiO_2 performing as a catalyst. The result is being monitor by TLC. After the result finished, it was placed into H_2O and filtered. The mixed ether extort was thoroughly wash with a 10% $NaHCO_3$ solution and dehydrated over anhydrous $NaSO_4$. The obtained result was purifying via column technique with C_6H_6: $CHCl_3$ (9:1) as an eluent (Scheme-1).



Scheme-1: Synthetic Method for Benzohydrazide Compounds

Compound (1)

M.p. 278 ˚C, MF: $C_{14}H_{15}O_3N_3S$, IR (cm^{-1}): 3318.12 (N-H); 3151.83, 3045.61, 2941.34, 2875.11 (C-H vibrations); 1648.14 (C=O). ^1H NMR (δ, ppm): 10.60 (s, 1H, NH); 9.76 (s, 1H, SO_2 - NH); 2.29 (s, 3H); 7.11-7.85 (8H, aryl). ^{13}C: 20.76 (CH_3); 165.85 (C=O); 118.32-144.09 (aryl carbons). Calculated (m/z) value: 305.37 (M^+).

Compound (2)

M.p. 277 ˚C, MF: $C_{21}H_{18}O_3N_3SF$, IR (cm^{-1}): 3223.41 (N-H); 3188.25, 3079.34, 3046.81, 2938.32, 2876.11 (Aliphatic and aryl C-H); 1641.54 (C=O); 1605.93 (C=N). ^1H NMR (δ, ppm): 11.83 (s, 1H, NH); 10.80 (s, 1H, SO_2 - NH); 8.31 (s, 1H); 2.29 (s, 3H, CH_3); 7.18-7.76 (12H aryl group). ^{13}C: 20.81 (CH_3); 163.08 (C=O); 115.68-147.02 (aryl groups); 147.03 (N-CH). Calculated (m/z) value: 411.47 (M^+).

Compound (3)

M.p. 271 ˚C, MF: $C_{21}H_{18}O_3N_3SCl$, IR (cm^{-1}): 3236.35 (N-H); 3148.48, 3072.30, 3047.73, 2932.62, 2871.02 (C-H vibrations); 1643.48 (C=O); 1604.38 (C=N). ^1H NMR (δ, ppm): 11.85 (s, 1H, NH); 10.76 (s, 1H, SO_2 NH); 8.31 (s, 1H, N-CH); 2.28 (s, 3H, CH_3); 7.12-7.73 (12H aryl group). ^{13}C: 20.82 (CH_3); 163.07 (C=O); 118.14-146.77 (aryl 'C'); 146.75 (N-CH). Calculated (m/z) value: 427.91 (M^+).

Compound (4)

M.p. 279 ˚C, MF: $C_{22}H_{21}O_4N_3S$, IR (cm^{-1}): 3289.40 (N-H); 3075.64, 3041.72, 3007.39, 2932.94, 2837.37 (C-H vibrations); 1655.67 (C=O); 1608.29 (C=N). ^1H NMR (δ, ppm): 11.71 (s, 1H, NH); 8.26 (s, 1H, N-CH); 2.27 (s, 3H, CH_3); 6.94-7.75 (12H aryl group); 3.74 (s, 3H, methoxy). ^{13}C: 20.77 (CH_3); 163.02 (C=O); 114.18-148.23 (aryl 'C'); 55.32; 148.21 (N-CH). Calculated (m/z) value: 423.48 (M^+).

Development of TiO_2 Nanocrystal

The TiO_2 were formed by sol-gel synthesis of $C_{12}H_{28}O_4Ti$, follow by calcination. Approximately 2 ml of $C_{12}H_{28}O_4Ti$ was dissolve in 25 ml of C_3H_8O and result was gradually dripped into 15 ml deionized water,

with the pH accustomed to 2.5. Subsequent to adding H_2O to the alkoxide mixture, the resulting colorless hydrous oxide was energetically agitated at space hotness for 3 hours before being left to age overnight. To reduce agglomeration, the frozen was centrifuged and resuspended in ethanol. The ensuing substance was then dehydrated and calcinated at 700 °C for 3 hours. These results show that elevated acidity [pH3] promotes the development of rutile TiO_2.[34,35]

Studies on Antimicrobials

The agar well disc diffusion method will be used to test the benzohydrazide derivatives for antibacterial activity against standard *E. coli, S. typhi, P. aeruginosa* and *S. aureus* (Gram-positive), as well as antifungal activity against *C. albicans, A. niger, Mucor* and *Rhizopus sp.*[36,37] Suspensions of each microbe were made from 24-hour cultures, giving just about 106 colony form units (cfu) per ml for plating. The drug was dissolved at a concentration of 50 µg/0.05 ml in DMSO, which served as the control. It was aseptically transferred and useful to the cups generated on the dehydrated exterior of the infected plates, and then incubates at 37°C for the night (~18-20 hours). The mean widths of the clear inhibitory zones (mm) were assessed despite the presence of a particular colony or a faint haze cause by the inoculums. This experiment was done twice.

RESULTS AND DISCUSSION

Interpretation of TiO_2 Nanocrystal

Figure-1 the XRD pattern of rutile TiO_2 nanocrystal correspond to the JCPDS of tetragonal primitive (89-4921). The precious stone constants are 4 & 2.954 Å. The regular crystallite dimension (L) intentional as of the XRD consequences is 20.42 nm. This indicates crystalline dimension was considered by the Scherrer equation: $L = 0.9\ \lambda / \beta\cos\theta$. The nanocrystals precise exterior region (S) was designed with the connection $S = 6/\rho L$ (where ρ represents substance thickness). The predicted exterior region for rutile TiO_2 is 51.24 m_2/g.

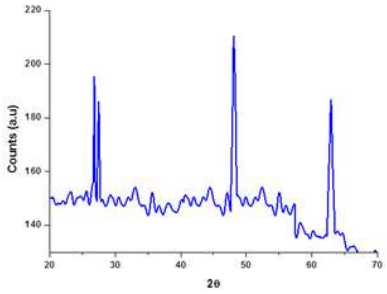


Fig.-1: XRD Pattern of TiO_2 Nanocrystal

The FE-SEM picture offers information about the particle character, topography and dimension. The synthesised TiO_2 (Rutile) nanoparticles were found to be consistent, agglomerated and devoid of the further overriding phases. This is owing to the high surface energy of each TiO_2 (Rutile) nanoparticle. **Figure-2** shows FE-SEM images of TiO_2 (Rutile) nanoparticles with an uneven shape. The dimension and configuration of the TiO_2 (Rutile) nanoparticles are generally consistent with XRD results, with minor variations.

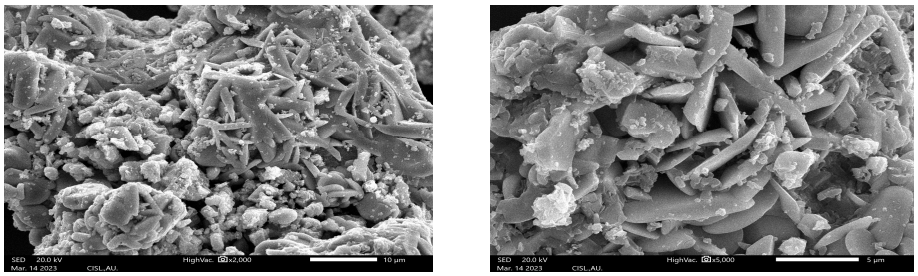


Fig.-2: SEM Picture of TiO_2 Nanocrystal

The Catalytic Activity of TiO_2

Increasing the reaction temperature to 180 ºC did not significantly boost the yield of the intended product. We discovered that the attendance of a catalytic quantity of TiO_2 under solvent conditions is optimal for

this mixture; highest yield (86%) was observed after 40 minutes after load with 0.1mol% TiO_2 at 120 °C (Table-1). Furthermore, TiO_2 can be retrieved and reuse multiple times lacking losing important movement (Fig.-3). This process is straightforward and convenient due to its elevated product, faster response, little catalyst loading, and facile work-up practice. Our method could provide a valuable giving to the accessible processes for benzohydrazide derivatives.

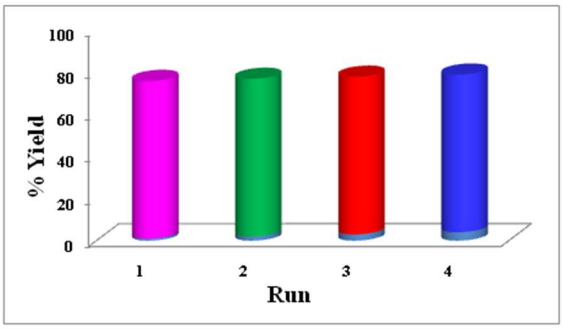


Fig.-3: Reusability of TiO_2 for Catalytic Production of Benzohydrazides

Table-1: The Impact of Catalyst and Temperature on the Synthesis of Benzohydrazide Derivatives

S.No.	Temp. (°C)	Solvent	Time (minutes)	Yield percentage	TiO_2 (mol%)
1	120	CH_3OH	70	44	1
2	120	C_2H_5OH	50	86	0.1
3	120	$CHCl_3$	145	43	1
4	120	CH_3CN	90	55	1

Antimicrobial Studies

An antibacterial examination exposed that moieties 2, 3, and 4 inhibited *E. coli, S.typhi, P. aeruginosa,* and *S. aureus* significantly, whereas compound 1 inhibited none of the bacterial strains (Fig.-4). Compounds 1, 2, 3, and 4 have all been shown to have moderate to high antifungal movement against the identified fungal strains. An antimicrobial study demonstrated that moieties 1, 2, 3, and 4 have higher antifungal embarrassment movement than antibacterial inhibition movement. Moiety 4 also possesses effective antimicrobial property (Fig.-5). This could be owing to the electric phenomena caused by the $–OCH_3$ group.

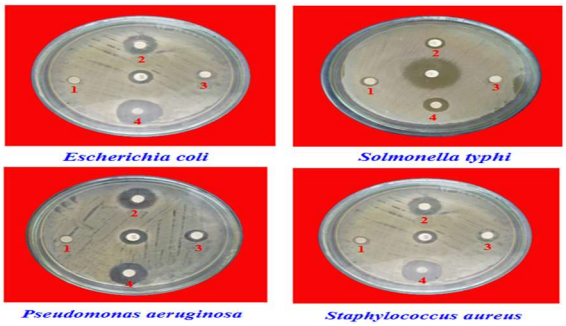


Fig.-4: Compounds 1-4 inhibit *E. coli, S. typhi, S. aureus,* and *P. aeruginosa*

Molecular Orbital Study between HOMO and LUMO

Table-2 shows the electron mass of the HOMO-LUMO orbitals of all moieties 2, 3, and 4 with the exception of the $–OCH_3$ substitute phenyl ring, and their associated energies. HOMO molecular orbital diagrams of all compounds demonstrate that bonding occurs extra in the phenyl ring, while anti-bonding occurs extra in the LUMO orbitals of 2, 3, and 4 (Fig.-6). The power slit values demonstrate that the synthesized compounds are polar and intramolecular energy shift occurs within the moiety. Based on this result, it was decided to conduct both experimental and theoretical NLO analyses. The molecule was delivered to IISC Bangalore for experimental measurements of the NLO property. The Gaussian 03 package was used to do the theoretical NLO analysis (DFT/B3LYP-6_31G (d, p). Table-3 includes the α, β, and D values. These values provide strong evidence that these compounds have electronic characteristics.

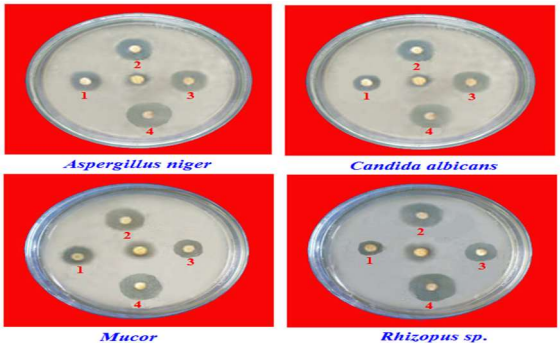


Fig.-5: Compounds 1–4 inhibit *C. albicans, A. niger, Mucor,* and *Rhizopus sp*

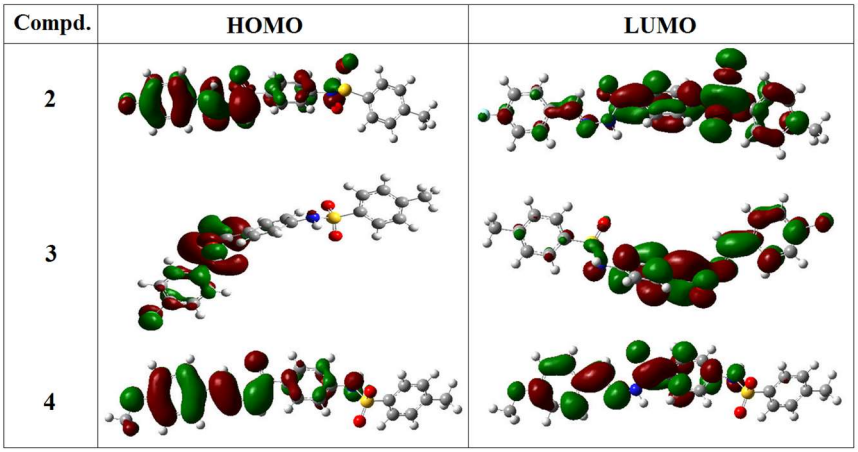


Fig.-6: HOMO-LUMO Image of Moieties 2-4

Table-2: HOMO-LUMO & Energy Gap Value of Moieties 2 - 4

Compds.	HOMO (eV)	LUMO (eV)	Eg values
2	-9.442	-1.687	7.755
3	-8.163	-5.358	2.805
4	-8.196	-5.377	2.819

Table-3: Electric Dipole Moment (μ), Polarisability (α), and Hyperpolarisability (β) Values of 2-4.

Parameter	*p*-F	*p*- Cl	*p*-OCH_3
Polarisability α_{tot} χ 10^{-21}	-233.971	-157.4781	-147.6322
Dipole moment μ_{tot}	5.7223	-8.0915	-3.9337
Hyperpolarisability β_{tot} χ 10^{-32}	488.4831	884.29	586.1286

CONCLUSION

The synthetic TiO_2 was analysed using XRD, SEM and EDS. Characterisation of synthesised benzohydrazide compounds was carried out using IR, ^1H and ^{13}C NMR and mass. Antibacterial study exposed that the confirmed moieties 2, 3, and 4 have significant embarrassment activity; however, compound 1 showed no inhibition activity beside the weathered bacterial strains. Compounds 1, 2, 3, and 4 demonstrated moderate to high antifungal efficacy beside the identified fungal strains. The HOMO orbital pictures of 2-4 prove that bonding occurs extra in the aryl ring, whereas anti-bonding occurs additional in the LUMO orbitals.

ACKNOWLEDGMENTS

Special thanks to Mr. M. Santhosh Kumar, Research Scholar, Department of Microbiology, Thiruvalluvar University, Serkadu, Vellore, for helping the biological activities. Mr. S. Sivaraj, Research Scholar, Department of Chemistry, Annamalai University, for helping the IR and NMR facilities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9041/2024]