Synthesis, Spectral Characterization, Antimicrobial and Theoretical Calculation of Some 4-(tosylamino)benzohydrazide Derivatives

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Abstract

The novel synthesis of benzohydrazide derivatives were synthesized by condensation reaction of 4-(tosylamino)benzohydrazide and substituted benzaldehyde. The synthesized compounds were characterized by IR, NMR and Mass spectral analysis. The benzohydrazide derivatives 1-4 were tested for antibacterial and antifungal activities. HOMO-LUMO analysis were carried out theoretically using Gaussian-03 package at DFT/B3LYP/6_31G(d, p) method. Dipole moment (D), polarisability (α), and hyperpolarisability (β) values show that the synthesized molecules possess electronic properties.

Keywords: Benzohydrazide derivatives; IR; NMR; HOMO-LUMO; Antibacterial studies

Introduction

The work of an organic and medicinal chemist is centered on the discovery of new lead compounds with specific medicinal properties. It includes the development of more effective and safer analogues of these new and existing lead compounds. Thus, the main interest of an organic chemist normally lies in conceiving an ideal structure for the needed drug with very minimal or negligible adverse effect usually based on theoretical consideration and in constructing a plausible way for the strategical synthesis towards that target drug.

N-((Pyridin-4-yl)methyl)phenothalein-2-amine was synthesized by means of a reaction in which stoichiometric amounts were used of the reagents, viz., 2-naphthylamine,4-pyridylcarboxyaldehyde, anhydrous ethanol to obtain the (Z)-N-((Pyridin-4-yl)methylene)phenothalein-1-amine and their later reduction with NaBH₄ produced the required compound [1]. 2-(N-naphthylamido)benzoic acid was synthesized by a solution of phthalic anhydride in acetic acid was added to a solution of naphthylamine in acetic acid and the mixture was stirred at room temperature overnight. The light purple precipitate was filtered, washed with a cold distilled water and air dried [2]. 2-[(4-N,N-dimethylamino)benz-2-ylideneamino]-5(6)-methylbenzimidazole was synthesized by using 2-amino-5(6)-methylbenzimidazole, 4-N-(dimethylamino) benzaldehyde in absolute ethanol and the mixture was stand for 20 hrs at room temperature. The yellow precipitate was filtered, washed with distilled water and air dried [3-5].

Obafemi and Akinpelu have synthesized 2-oxo-1,2-dihydroquinoxaline-6-sulfonyl azide derivatives and 2,3-oxido-1, 2, 3, 4-tetrahydroquinoline-6-sulfonyl azide derivatives and studied their antimicrobial activity against gram-positive and gram-negative bacterial strains [6]. Both the compounds showed broad spectral activity against the bacterial strains. A series of oxazolidinone derivatives carrying sulfonyl group was synthesized and their antibacterial activity was evaluated by Cui et al. and Topala et al. have prepared four new cholesteryl carbamates exhibiting liquid crystal properties and evaluated their thermodynamic properties by differential scanning calorimetry [7,8]. Reflux of tosyl chloride with p-tolylurea for about 30 minutes using pyridine as a base and poured the reaction mixture into 10 mL of cold water and stirred until the product crystallizes and filtered off the solid and wash several times with water to get the corresponding derivatives [9].

Ethyl-1-(R)-5-benzamido-1H-pyrazole-4-carboxylate was obtained by adding ethyl-1-R-5-aminopyrazole-4-carboxylate to a solution of benzoyl chloride in anhydrous acetonitrile [10]. By refluxing 4-phenylxazol-2-amine with cinnamyl chloride in toluene to get the corresponding product [11]. 1-Hydrazinocarbonylmethyl-3-ethoxycarbonyl-5-substituted-2-methylindoles is another hydrazone shown versatile bio-activity [12-14].

Hydrazine hydrate was added to ethyl-N-benzotriazolooacetate in ethanol and refluxed until the completion of the reaction. The solid was filtered and dried to get N-benzotriazololooacetyl hydrazine [15]. N-hydrazidomethyl-1,4-benzoilazine-3(1H)-one was synthesized from N-ethoxycarbonylmethyl-1,4-benzolazine-3(1H)-one with hydrazine hydrate [16]. Ethyl-4-amino benzole treated with hydrazine hydrate in ethanol and the reaction mixture was refluxed for 6 hrs, to get corresponding compound [17]. Benzfuran-2-carbohydrazides was synthesized from ethyl benzofuran-2-carboxylate [18].

Experimental

Synthesis of ethyl-4-(tosylamino)benzoate

A mixture of ethyl-4-aminobenzoate (5 mmol), tosyl chloride (5 mmol) and triethylamine (5 mmol) in 50 mL of methanol was refluxed for about 2 hrs. The reaction is monitored by TLC. After completion of reaction, it was poured into water and extracted with three 50 mL portion of ether. 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. This upon evaporation and subsequent recrystallization furnished the

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product ethyl-4-(tosylamino)benzoate in pure form (m.p. 242-246; yield 95%).

**Synthesis of 4-(tosylamino)benzohydrazide**

A mixture of ethyl-4-(tosylamino)benzoate (5 mmol) and hydrazine hydrate (5 mmol) in methanol was refluxed for about 10 hrs (Scheme 1). The reaction was monitored by TLC. After completion of reaction, it was poured into ice cold water to remove the excess of hydrazine hydrate. The solid was filtered and dried. The synthesized compound was further purified by column chromatography using benzene: chloroform mixture (6:4) as an eluent. This upon evaporation and recrystallization furnished the product 4-(tosylamino)benzohydrazide in pure form.

**Synthesis of 4-(tosylamino)benzohydrazide derivatives**

A mixture of 4-(tosylamino)benzohydrazide (5 mmol), substituted benzaldehyde (5 mmol) and 3 drops of acetic acid in methanol was refluxed for about 2-3 hrs (Scheme 2). The reaction is monitored by TLC. After completion of reaction, excess of solvent was removed. The final mass was poured into ice cold water to remove the excess of the aldehyde. The residue obtained was purified by column chromatography using benzene: chloroform mixture (8:2) as an eluent. This upon evaporation and subsequent recrystallization furnished the product 4-(tosylamino)benzohydrazide derivatives in pure form.

**Spectral measurements**

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) was used as an internal standard. Mass spectra were recorded on VARIAN CP-3800 GC Mass Spectrometer (EI mode).

**Antimicrobial studies**

**Antibacterial studies:** The following Gram-positive and Gram-negative strains have been used for the study [19,20].

1. *Escherichia coli* (Gram-negative); 2. *Salmonella typhi* (Gram-negative); 3. *Pseudomonas aeruginosa* (Gram-negative); 4. *Staphylococcus aureus* (Gram-positive).

**Determination of antibacterial activity by disc-diffusion method:** Nutrient agar plates were prepared under sterilized conditions and incubated overnight to detect contamination. About 0.2 mL of working stock culture was transferred into separate nutrient agar plates and spreaded thoroughly using a glass spreader. Whatmann No. 1 discs (6 mm in diameter) were impregnated in the test compounds dissolved in DMSO (200 μg/mL) for about half an hour. Commercially available drug disc (Ciprofloxacin 10 μg/disc) was used as positive reference standard. Negative controls were also prepared by impregnating the disc of same size in DMSO solvent. The discs were placed on the inoculated agar plates and incubated at 37 ± 1°C for about 18-24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

**Antifungal studies:** The following fungal strains were used for the study. 1. *Candida albicans*; 2. *Aspergillus niger*; 3. *Mucor* and 4.
Rhizopus sp. Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud's dextrose broth (SDB) medium. The subculture and the viable count were carried out by the same procedure as done in antibacterial studies except the temperature which should be maintained at 28 ± 1°C for about 72 hours. Similarly, for disc diffusion method, the petridishes were incubated at 28 ± 1°C for about 72 hours. The same concentration of the test compound, solvent (DMSO) and Amphotericin B (standard) prepared previously were used for the antifungal studies.

Computational details

The quantum chemical calculations were performed using the Gaussian-03 package [21]. Computations of the vertical excitations, difference density plots and optimization of the ground and excited states were performed using density functional theory (DFT) and time-dependent DFT (TD-DFT) using B3LYP/6-31G (d,p) basis set, respectively. The ground and excited states HOMO and LUMO frontier orbitals of 4-(tosylamino) benzohydrazide derivatives were calculated by methods at the B3LYP/6-31G(d,p) level.

Results and Discussion

IR spectral analysis of compounds 1, 2, 3 and 4

The FT-IR spectra were recorded in the region of 4000-400 cm⁻¹. The vibrational assignments for the characteristic absorption of compounds 1, 2, 3 and 4 are shown Figure 1. A collection of medium bands observed in the region of 3189-2836 cm⁻¹ is attributed due to C-H stretching vibration of aromatic and aliphatic groups. Strong absorption bands in the region of 1641-1654 cm⁻¹ are characteristic for amide carbonyl group stretching vibrations. Absorption band at 1600-1607 cm⁻¹ are characteristic for C=N stretching vibrations of the synthesized compounds 1, 2, 3 and 4. A band at 1548-1559 cm⁻¹ can be easily assigned to C=C stretching mode of phenyl rings. All the observed IR bands are supporting evidences for the formation of compounds 1, 2, 3 and 4.

![Figure 1: Representative IR spectral analysis of N’-(4-fluorobenzylidene)-4-(tosylamino) benzohydrazide.](image)

NMR spectral analysis of N’-(4-fluorobenzylidene)-4-(tosylamino) benzohydrazide (2)

1H and 13C NMR spectra of compound 2 are shown in Figures 2a and 2b. The signals appeared at 7.18-8.31 ppm is assigned to aromatic protons. There are two singlets at 10.81 and 11.82 ppm with corresponding one proton integral values. These signals are due to sulfonyl group attached N-H proton and hydrazide N-H proton respectively. The methyl protons signal is appeared at 2.29 ppm.

![Figure 2a: Representative NMR spectral analysis of N’-(4-fluorobenzylidene)-4-(tosylamino) benzohydrazide.](image)

NMR spectral analysis of 4-(tosylamino)benzohydrazide (1)

1H NMR spectrum of compound 1, the signals observed in the region of 7.10-7.86 ppm with an expected integral value. Therefore, these signals are unambiguously assigned to aromatic protons. The methyl proton signal is observed in the region of 2.29 ppm as a singlet. A hydrazide N-H proton is observed at 10.61 ppm and sulfonyl amino protons signals are merged with DMSO solvent peak.
NMR spectral analysis of N’-(4-chlorobenzylidene)-4-(tosylamino) benzohydrazide (3)

$^1$H and $^{13}$C NMR spectra of compound 3 the signals appeared at 7.11-8.30 ppm is assigned to aromatic protons. There are two singlets appeared at 10.75 and 11.86 ppm with one proton integral values. These signals are due to sulfonyl group attached N-H proton and hydrazide N-H proton respectively. The methyl protons signal is appeared at 2.29 ppm. The $^{13}$C NMR spectrum the signals appeared in the region 118.14-146.76 ppm is due to the aromatic carbons. The phenyl ipso carbons C-1, C-4, C-8, C-11, C-16 and C-19 are observed at 127.60, 143.95, 135.99, 141.05, 130.46, and 163.12 ppm respectively. The signals appeared at 146.76, 163.06 ppm is assigned to aldehyde (N=CH) carbon and carbonyl carbon. The methyl carbon signal is appeared at 20.81 ppm.

NMR spectral analysis of N’-(4-methoxybenzylidene)-4-(tosylamino) benzohydrazide (4)

$^1$H and $^{13}$C NMR spectra of compound 4 the signals observed in the region of 6.95-8.26 ppm are assigned to aromatic protons. Further a singlet appeared at 11.70 ppm with one proton integral value. This signal is due to hydrazide N-H proton. There are two singlets appeared in the aliphatic region at 2.27 and 3.74 ppm. These signals are assigned to methyl and methoxy protons respectively.

The $^{13}$C NMR spectrum the signals appeared in the region 114.18-148.22 ppm is assigned to the aromatic carbons. All the phenyl ipso carbons C-1, C-4, C-8, C-11, C-16 and C-19 are observed at 127.70, 143.99, 135.87, 140.91, 126.39 and 160.83 ppm respectively. The signals appeared at 148.22 and 163.02 ppm, these signals are assigned to aldehyde (N=CH) carbon and carbonyl carbon respectively.

Mass spectral analysis of compounds 1, 2, 3 and 4

El Mass spectra were recorded on VARIAN CP-3800 Gas Chromatography for compounds 1, 2, 3 and 4 and it is displayed in Figure 3. The obtained molecular ion peak (m/z) of the compounds 1, 2, 3 and 4 at 305.3 (M$^+$), 427.9 (M$^+$), 411.5 (M$^+$), 423.5 (M$^+$), confirmed the formation of our synthesized compounds.

Antimicrobial studies

The preliminary antimicrobial activity (Figures 4 and 5) of the compounds 1-4 are examined using disc diffusion method. The bacterial strains viz., Escherichia coli, Salmonella typhi, Staphylococcus aureus and Pseudomonas aeruginosa and fungal strains viz., Candida albicans, Aspergillus niger, Mucor and Rhizopus sp., are used in this study. Dimethyl sulphoxide is used as a control while Ciprofloxacin and Amphotericin B are used as a reference for bacterial and fungal studies respectively.

The antibacterial studies revealed that the reported compounds 2, 3 and 4 against E. coli, S. typhi, P. aeruginosa and S. aureus shows considerable inhibition activity whereas compound 1 did not show any inhibition activity against the bacterial strains. The antifungal studies of compounds 1, 2, 3 and 4 indicates that all the compounds exhibit moderate to maximum activity 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. 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 orbital of all molecules 2, 3 and 4 contributed the entire molecule except the methyl substituted aryl ring and the corresponding energies are tabulated in Table 1. HOMO molecular orbital picture of all molecules show that the bonding character is more in the aryl ring whereas anti-bonding character is more in the LUMO orbital of 2, 3 and 4.

| Compounds | HOMO (eV) | LUMO (eV) | Eg values   |
|-----------|-----------|-----------|-------------|
| 14        | -9.4423   | -1.6871   | -11.1294    |
| 15        | -8.1634   | -5.358    | -13.5214    |
| 16        | -8.1961   | -5.377    | -13.5731    |

Table 1: HOMO-LUMO and Energy gap values of 2-4.

The energy gap values show that all the synthesized molecules are polar and intramolecular charge transfer takes place within the molecule. From this conclusion it was aimed to carry out NLO analysis by both experimental and theoretically. The molecule was sent to IISC Bangalore to measure the NLO property experimentally. The theoretical NLO analysis was carried out by Gaussian 03 package (DFT/B3LYP-6_31G (d, p)). The polarisability (α) and hyperpolarisability (β) values are tabulated Table 2 along with the dipole moment (D) values. These values strongly support that these molecules possess electronic properties.

| Parameter                          | p-F     | p-Cl    | p-OCH3   |
|------------------------------------|---------|---------|----------|
| Dipole moment $\mu_{\text{tot}}$   | 4.7222  | -9.0914 | -2.9336  |
| Polarisability $\alpha_{\text{tot}} \times 10^{21}$ | -232.97 | -156.478 | -148.6321 |
| Hyperpolarisability $\beta_{\text{tot}} \times 10^{32}$ | 489.483 | 885.28  | 587.1285 |

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

Optimized structures of 2-4: Optimized structures of various compounds are shown in Figure 6.
The benzohydrazide derivatives namely, 4-(tosylamino)benzohydrazide (1), N’-(4-methoxybenzylidene)-4-(tosylamino)benzohydrazide (2), N’-(4-chlorobenzylidene)-4-(tosylamino)benzohydrazide (3) and N’-(4-fluorobenzylidene)-4-(tosylamino)benzohydrazide (4) were synthesized by condensation reaction of 4-(tosylamino)benzohydrazide and substituted benzaldehyde. Synthesised compounds were characterized by IR, NMR and Mass spectral analysis. The antibacterial studies revealed that the reported compounds 2, 3 and 4 shows considerable inhibition activity whereas 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. HOMO-LUMO and NLO analysis were carried out theoretically using Gaussian-03 package at DFT/B3LYP/6-31G (d, p) method. The HOMO molecular orbital pictures of 2-4 show that the bonding character is more in the aryl ring, whereas antibonding character is more in the LUMO orbital. Energy gap values show that intramolecular charge transfer takes place within the molecule. Dipole moment (D), polarisability (a), and hyperpolarisability (β) values show that the synthesized molecules possess electronic properties.

References
1. Camargo HA, Henau JA (2009) Power Diffrr 62: 72.
2. Shahid K, Ali S, Shahzadi S (2009) The chemistry, properties, and characterization of organotin (IV) complexes of 2-(N-naphthylamido)benzoic acid. J Coord Chem 62: 2919-2926.
3. Hranjec M, Starčević K, Pavelić SK, Lučin P, Pavelić K, et al. (2011) Synthesis, spectroscopic characterization and antiproliferative evaluation in vitro of novel Schiff bases related to benzimidazoles. Eur J Med Chem 46: 2274-2279.
4. Kushwaha UK, Yashovardhan, Bhati SK, Kumar A (2010) Int J Pharm Bio Sci 1: 3.
5. Mogliaiah K, Swamy TK, Chandra AV, Srivani N, Vidya K (2010) Claisen-Schmidt condensation under solvent-free conditions. Indian J Chem 49B: 382.
6. Obafemi CA, Akinpelu DA (2005) Synthesis and antimicrobial activity of some 2 (1H)-quinonolimine-6-sulfonyl derivatives. Phosphorus Sulfur Silicon Relat Elem 180: 1795-1807.
7. Cui Y, Yang Y, Chan K, Zhang S (2003) Bioorg Med Chem Lett 3: 2311.
8. Topală C, Căproul MT, Drăghici C (2005) Cholesteryl derivatives with a sulfonyl moiety. Arkivoc 10: 63-70.
9. Tyagi S, Kumar S, Kumar A, Singhla M (2010) Int J Pharm World Res 1: 2.
10. Daidone G, Raffa D, Plessia F, Maggio B, Roccaro A (2002) Synthesis of pyrazole-4-carboxyhydrazide derivatives of pharmaceutical interest. Arkivoc 11: 227-235.
11. Padmavathi V, Venkatesh BC, Padmaja A (2011) Synthesis and antimicrobial activity of amido linked pyrrolid and pyrazolyl-oxazoles, thiazoles and imidazoles. Eur J Med Chem 46: 5317-5326.
12. Bhovi MG, Gadaginamath GS (2005) Chemoselective reaction of indole 1, 3-dicarboxylates towards hydrazine hydrate: Bisbeterocycles: Synthesis and antimicrobial activity of some new 2-methyl-3-ethoxycarbonyl-1-oxadiazolyl/thiazolidinonyl/pyrrolaminocarbonylmethylindolines. Indian J Chem 44B: 1663-1668.
13. Ismail MM, Mohamed HM (2005) Synthesis and cyclization reactions with quinolinyl keto esters II. Synthesis of novel 3-diazolylquinolinones and their enzymic activity. Chem Papers 59: 127-138.
14. Mohamed SF, Youssef MM, Amr AE, Koh ER (2008) Antimicrobial Activities of some Synthesized Pyridines, Oxazines and Thiazoles from 3-Aryl-1-(2-naphthyl) prop-2-en-1-ones. Scientia Pharmaceutica 76: 279-304.
15. Shukla DK, Srivastava SD (2008) Synthesis of some new 5-[(2-[(1, 2, 3-benzotriazole)-1-yl-methyl]-1'-(4-substituted aryl-3'-chloro-2'-oxo azetidine)]-amino-1, 3, 4-thiadiazoles: Antifungal and antibacterial agents. Indian J Chem 47B: 463-469.
16. Deshmukh MB, Deshmukh SA, Jagtap SS, Mulik AR (2007) Synthesis and study of biological activity of some new 1, 4-benzothiazines. Indian J Chem 46B: 852-859.

17. Xavier JJ, Krishnasamy K, Sankar C (2012) Synthesis and antibacterial, antifungal activities of some 2r, 4c-diaryl-3-azabicyclo [3.3.1] nonan-9- one-4-aminobenzoyl hydrazones. Med Chem Res 21: 345-350.

18. Parekh S, Bhavsar D, Savant M, Thakrar S, Bavishi A, et al. (2011) Synthesis of some novel benzofuran-2-yl (4, 5-dihydro-3, 5-substituted diphenylpyrazol-1-yl) methanones and studies on the antiproliferative effects and reversal of multidrug resistance of human MDR1-gene transfected mouse lymphoma cells in vitro. Eur J Med Chem 46: 1942-1948.

19. Venkateswarlu P, Sundararani SB (2005) Indian J Chem 44B: 1257.

20. James G, Gappuccino, Sherman N (1992) In: Microbiology, A Laboratory Manual (3rd edn), The Benjamin/Cummings Publishing Company, California, US, p: 77.

21. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, et al. (2004) Gaussian 03, Revision C.02. Gaussian Inc., Wallingford, CT, USA.