Research Article

Synthesis, X-Ray Crystal Structure Study, Hirshfeld Surface Analysis, and Biological Activity of N-(2-amino-phenyl)-2-methyl-benzamide

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The title compound crystallizes in monoclinic crystal system, with space group $P2_1/c$. The compound exhibits intermolecular interactions of the types $N-H\cdots N$, $C-H\cdots O$, and $C-H\cdots \pi$; intramolecular interactions of the type $N-H\cdots N$. The intercontacts are also studied using Hirshfeld surface analysis. The compound showed no remarkable antibacterial activity when screened against two gram-negative and two gram-positive bacteria.

1. Introduction

The title compound is a benzamide derivative. The compound consists of an amide group bridged to a benzene ring to which a methyl is attached on one side and a phenyl ring to which an amino group is attached on the other side. Benzamides are derived from benzoic acid, which are slightly soluble in water and soluble in many organic compounds.

Amides are pervasive in nature. Compounds containing amide groups show many biological activities, mainly antiproliferative, antiviral, antimalarial, general anesthetics, anti-inflammatory, and antimicrobial activities [1]. Literature reveals that the N-substituted benzamides have many pharmacologically important properties such as antifungal, antiallergic, antihypertensive, anti-inflammatory, antibacterial, analgesic, antirheumatic, antipyretic, and anti-HIV activities [2].

Hence, herein we report the synthesis, characterization, X-ray crystal structure study, *in vitro* antibacterial activity,

and Hirshfeld surface analysis of N-(2-amino-phenyl)-2-methyl-benzamide.

2. Experimental

2.1. Materials and Methods. Chemicals were purchased from Sigma Aldrich Chemical Corporation. ¹H NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer in DMSO- d_6 solvent and the chemical shifts were recorded in δ (ppm) downfield from tetramethylsilane. Elemental analysis was done using Perkin Elmer 2400 elemental analyzer and results are within 0.4% of the calculated value. Infrared spectra were recorded on a Perkin Elmer spectrophotometer in the range of 400–4000 cm^{-1} .

2.2. Synthesis of N-(2-amino-phenyl)-2-methyl-benzamide. The mixture of *o*-phenylenediamine (0.0037 mol) in dry dichloromethane (15 mL), 2,6-dimethyl pyridine, and 2-methyl-benzoic acid (0.0037 mol) was stirred for 30 minutes

SCHEME 1: Schematic diagram of the title compound.

at 25–30°C. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (0.0037 mol) was added to the mixture, whose temperature was maintained below 5°C. The reaction was monitored by Thin Layer Chromatography using chloroform:methanol (9:1). The reaction mass was diluted with 20 mL of dichloromethane. The organic layer was washed with water (25 mL), dried over anhydrous sodium sulfate, concentrated to syrupy liquid, and recrystallized twice by ethyl ether to afford N-(2-amino-phenyl)-2-methylbenzamide, in good yield of 90%. The melting point of the compound is 94–96°C. The obtained crystals were white in color (Scheme 1).

2.3. X-Ray Diffraction. A suitable white single crystal was selected to collect X-ray diffraction data. Data were collected on a Bruker Kappa Apex II single crystal X-ray diffractometer equipped with Cu K α radiation and CCD detector [3]. Crystal structure was solved by direct methods using *SHELXS-97*. After locating all nonhydrogen atoms, the structure was refined by full-matrix least-squares method using *SHELXL-97* [4]. The obtained model was refined by isotropic thermal parameters later by anisotropic thermal parameter. Hydrogens were placed at chemically acceptable positions. 156 parameters were refined with 1929 unique reflections which converged the residual (*R*) to 0.057.

2.4. In Vitro Antibacterial Activity. As N-substituted benzamides and their derivatives have numerous biological activities, it is worthwhile to investigate the antibacterial activity of the newly synthesized compound.

Antibacterial activity was examined against two gram-positive bacteria, namely, *Bacillus subtilis* (MTCC number 121) and *Staphylococcus aureus* (MTCC number 7443), and two gram-negative bacteria, namely, *Proteus vulgaris* (MTCC number 742) and *Escherichia coli* (MTCC number 730). The bacterial strains were inoculated in nutrient broth and kept for overnight culture at 37°C. Minimum inhibitory concentration (mic) is the lowest concentration at which blue color of the dye (indicator) turns to pink color [5]. MIC was determined by microbroth dilution method using resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) as an indicator. Resazurin is a blue nonfluorescent, nontoxic, oxidation-reduction indicator. This was performed on 96-well microtiter plates [6].

For susceptibility testing the plates were prepared in duplicates. Nutrient broth of 50 μ L was distributed to all the wells. 50 μ L compound was added to third and fourth wells. Serial dilution was performed from the fourth well till the

TABLE 1: Elemental analysis for the title compound.

Element	Experimental (%)	Calculated (%)
Carbon	74.31	74.33
Hydrogen	6.24	6.25
Nitrogen	12.38	12.41

concentration reaches 0.39×10^{-2} mg/mL. Finally, 10 μ L of bacterial suspension was added to all the wells.

The concentrations of the prepared solutions were as follows: 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.625×10^{-1} mg/mL, 0.3125×10^{-1} mg/mL, 0.156×10^{-1} mg/mL, 0.78×10^{-2} mg/mL, and 0.39×10^{-2} mg/mL. Blue color indicates that the compound inhibits the growth of the bacteria, whereas pink color indicates the bacterial growth.

Inoculated plates were incubated at 37°C for 24 hours. One hour before the end of incubation 10 μ L of resazurin was added to all the wells. The plates were incubated for another hour. The change in color was assessed visually. The MIC was recorded.

3. Results and Discussions

3.1. Elemental Analysis. In order to confirm the chemical composition of the synthesized compound, carbon (C) and hydrogen (H) analysis was carried out. The experimental and calculated percentages of C and H are given in Table 1. The differences between experimental and calculated percentages of C and H were very small and are within the experimental errors. This confirms the formation of the product in the stoichiometric proportion.

3.2. FT-IR Spectral Analysis. In FT-IR spectra the peaks observed at 1660 cm^{-1} are assigned to C=O of carbonyl of the amide group. The peak at 1715 cm^{-1} is for N–H stretching vibrations.

3.3. ^1H NMR Spectral Analysis. The ^1H NMR spectra of the compound are shown in Figure 1. The NMR peak at δ 2.25 (s, 3H) clearly indicates that the three hydrogens of methyl group are attached to aromatic ring. The peak at δ 4.25 (bs, 2H) corresponds to the two hydrogens of the amino group. The peaks at δ 7.15–7.52 refer to eight aromatic hydrogens of the compound.

3.4. X-Ray Crystal Structure Determination. The compound $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}$ crystallizes in monoclinic crystal system, with space group $P2_1/c$. The unit cell parameters are $a = 11.471(4) \text{ \AA}$, $b = 8.269(3) \text{ \AA}$, $c = 12.518(4) \text{ \AA}$, and $\beta = 96.155(14)^\circ$. The details of the crystal data and structure refinement are given in Table 2. The geometrical calculations were carried out using the program *PLATON* [7]. The molecular and packing diagrams were generated using *Mercury* [8]. Figure 2 shows the ORTEP diagram of the molecule with thermal ellipsoids drawn at 50% probability. The bond

FIGURE 1: ^1H NMR spectra of the title compound.

FIGURE 2: ORTEP diagram of the molecule with thermal ellipsoids drawn at 50% probability.

distances and angles are listed in Table 3. Torsion angles are listed in Table 4.

The phenyl rings are planar. The r.m.s. deviation of the ring C2–C7 (ring-1/*Cg*(1)) from the mean plane is 0.011(2) Å. Atoms C5 and C6 deviate by 0.010(2) Å from the mean plane defined for the ring. The r.m.s. deviation of the ring C10–C15

(ring-2/*Cg*(2)) from the mean plane is 0.000(2) Å. This ring is highly planar. The O9=C8 is 1.240(2)°; this confirms the double bond character.

Ring-1 and ring-2 are *sp*² hybridized. They are described by the torsion angles 1.13° and 0.00°, respectively, which suggest that they adopt *+syn-periplanar* (*+sp*) conformation.

TABLE 2: The crystal data and structure refinement details.

CCDC deposit number	1418787
Empirical formula	C ₁₄ H ₁₄ N ₂ O
Formula weight	226.27
Temperature	293 K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Cell dimensions	$a = 11.471(4)$ Å
	$b = 8.269(3)$ Å
	$c = 12.518(4)$ Å
	$\beta = 96.155(14)^\circ$
Volume	1180.5(7) Å ³
Z	4
Density (calculated)	1.273 Mg m ⁻³
Absorption coefficient	0.619 mm ⁻¹
F_{000}	480
Crystal size	0.210 × 0.210 × 0.210 mm
θ range for data collection	3.88° to 64.69°
Index ranges	$-13 \leq h \leq 13$
	$-9 \leq k \leq 9$
	$-12 \leq l \leq 14$
Reflections collected	9047
Independent reflections	1929 [$R_{\text{int}} = 0.0384$]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1929/0/156
Goodness-of-fit on F^2	1.067
Final [$I > 2\sigma(I)$]	$R1 = 0.057$, $wR2 = 0.1627$
Largest diff. peak and hole	0.267 and -0.428 eÅ ⁻³

TABLE 3: Selected bond lengths and bond angles (Å, deg.).

Atoms	Distances	Atoms	Angles
O9–C8	1.240(2)	C8–N17–C10	125.86(14)
C1–C6	1.504(3)	N17–C10–C15	121.87(15)
N17–C8	1.346(2)	C11–C12–C13	119.14(19)
N17–C10	1.424(2)	N16–C15–C10	121.05(16)
C2–C3	1.370(3)	N16–C15–C14	120.69(17)
C14–C15	1.392(3)	C12–C13–C1	120.94(18)
C13–C14	1.375(3)	C10–C15–C14	118.06(16)
C11–C12	1.378(3)	O9–C8–N17	122.60(15)
C10–C11	1.383(3)	N17–C10–C11	117.23(16)
C12–C13	1.385(3)	C4–C5–C8	119.82(15)

Ring-1 and C=O of the amide group are *-anti-clinal* (*-ac*), this is confirmed by the torsion angle $-131.88(19)^\circ$ for C4–C5–C8–O9.

The torsion angle between two phenyl rings bridged *via* amide group is $-175.47(16)^\circ$ (C5–C8–N17–C10); this is greater when compared to the corresponding values

TABLE 4: Selected torsion angles (deg.).

Atoms	Angle	Atoms	Angle
C5–C8–N17–C10	$-175.47(16)$	C2–C3–C4–C5	$-0.6(3)$
N17–C10–C11–C12	$175.43(18)$	C11–C10–N17–C8	$124.64(19)$
N17–C10–C15–C14	$-175.19(16)$	C4–C5–C8–N17	$47.3(2)$
O9–C8–N17–C10	$3.7(3)$	C11–C10–C15–N16	$174.99(17)$
C7–C2–C3–C4	$1.5(3)$	C11–C12–C13–C14	$0.0(4)$
C3–C4–C5–C6	$-1.1(3)$	N17–C10–C15–N16	$-0.2(3)$
C4–C5–C8–O9	$-131.88(19)$	C1–C6–C7–C2	$176.30(19)$

FIGURE 3: Packing of molecules when viewed along *a*-axis.

of $-168.48(17)^\circ$ and $-173.38(16)^\circ$ for the isomeric benzamides 2-iodo-N-(2-nitrophenyl)benzamide (I) and N-(2-iodophenyl)-2-nitrobenzamide (II), respectively [9]. This may be due to the presence of methyl group attached to benzamide or the amino group attached to the phenyl ring or ring-2.

The packing views of the molecules down *a*- and *b*-axes are shown in Figures 3 and 4, respectively. The molecules are linked by the intermolecular interactions by three hydrogen bonds of types N–H $\cdots$ N, C–H $\cdots$ O, and C–H $\cdots$ π . The molecule is also stabilized by weak intermolecular interactions of type C–H $\cdots$ π . Atom C3 of ring-1 in a molecule acts as a donor to the C13–C14 (ring-2) atoms of another molecule. In addition, the molecule exhibits intramolecular interactions of types N–H $\cdots$ O and N–H $\cdots$ N. The hydrogen bond geometries are shown in Table 5.

3.5. Hirshfeld Surface Analysis. *CrystalExplorer 3.1* [10] program was used for understanding the interactions and the connectivity among the molecules efficiently. The crystallographic information file (.cif) was imported to the *CrystalExplorer* to generate the Hirshfeld surfaces. The Hirshfeld surface is the region around the molecule in the crystal space which can be considered as the boundary separating two regions—the interior (the reference molecule) and the

FIGURE 4: Packing of molecules when viewed along *b*-axis. FIGURE 5: d_{norm} mapped on Hirshfeld surface for the visualization of the intercontacts of the title compound.

exterior (neighboring molecules) [11]. The Hirshfeld surface of the title compound is shown in Figure 5. The red region indicates the hydrogen bond acceptors of $\text{N-H} \cdots \text{N}$ and $\text{C-H} \cdots \text{O}$ interactions ($\text{N17-H17} \cdots \text{N16}$ and $\text{C4-H4} \cdots \text{O9}$).

The fingerprint plot shows the percentage contributions to the total Hirshfeld surface area. The fingerprint plot data is shown in Table 6. Figures 6(a), 6(b), 6(c), 6(d), 6(e), and 6(f) show the fingerprint plots of the title compound. The major contribution (56.0%) is from $\text{H} \cdots \text{H}$ contacts. In Figure 6(a) the wings are due to the $\text{C-H} \cdots \pi$ interactions.

3.6. In Vitro Antimicrobial Activity. The results of biological activity of the title compound are given in Table 7. The resazurin assay showed that the compound has lower-to-average activity against various tested bacterial strains. The results of the compound with tested bacterial strains were compared with streptomycin. Streptomycin was used as standard in the experiment. The compound showed better/average activity against gram-negative bacteria *Proteus vulgaris* than any other bacteria, though it never outperforms streptomycin.

4. Conclusion

In this research work we have discussed the synthesis of the compound *N*-(2-amino-phenyl)-2-methyl-benzamide. The preliminary characterizations like elemental analysis, FT-IR,

TABLE 5: Hydrogen bond geometry ($\text{\AA}$, deg.).

D-H $\cdots$ A	D-H	H $\cdots$ A	D $\cdots$ A	D-H $\cdots$ A
$\text{N(17)-H(17)} \cdots \text{N(16)}^{(a)}$	0.86	2.31	3.123(2)	158
$\text{C(4)-H(4)} \cdots \text{O(9)}^{(b)}$	0.93	2.53	3.462(3)	175
$\text{N(16)-H(16B)} \cdots \text{O(9)}^*$	0.86	2.47	2.948(2)	116
$\text{N(16)-H(16B)} \cdots \text{N(17)}^*$	0.86	2.57	2.874(2)	102

* Intramolecular hydrogen bond interactions.

Symmetry codes: (a) $1 - x, 1 - y, 1 - z$; (b) $1 - x, 1/2 + y, 3/2 - z$.

TABLE 6: Percentage of various intermolecular contacts contributing to Hirshfeld surface.

Intercontacts	Contribution (%)	Intercontacts	Contribution (%)
$\text{H} \cdots \text{H}$	56.0	C-C	3.4
$\text{C-H/H} \cdots \text{C}$	27.7	$\text{N-H/H} \cdots \text{N}$	2.6
$\text{O-H/H} \cdots \text{O}$	9.8	N-O/O-N	0.4

TABLE 7: MIC of the title compound against various bacterial strains.

Bacterial strains	MIC (mg/mL)	
	Streptomycin (std.)	Compound
<i>Bacillus subtilis</i>	$0.3125 \cdot 10^{-1}$	0.125
<i>Staphylococcus aureus</i>	$0.3125 \cdot 10^{-1}$	0.125
<i>Proteus vulgaris</i>	$0.156 \cdot 10^{-1}$	$0.625 \cdot 10^{-1}$
<i>Escherichia coli</i>	$0.78 \cdot 10^{-2}$	0.25

and NMR confirms the chemical compositions of the compound. The structure was confirmed by the single crystal X-ray diffraction. The intermolecular interactions were studied by Hirshfeld surface analysis. Screening for biological activity showed that the compound shows better antibacterial activity against gram-negative bacteria *Proteus vulgaris*.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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FIGURE 6: Fingerprint plot of the title compound. (a) Highlighting full two-dimensional map. (b) Two-dimensional map resolved into $H \cdots H$ contacts. (c) Two-dimensional map resolved into $C-H/H \cdots C$ contacts. (d) All contacts. (e) Major $H \cdots H$ contacts. (f) Major $C-H$ contacts.

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