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Synthesis, crystal structures, Hirshfeld surface analysis and spectroscopic studies of two Schiff bases of anisaldehyde and their urease and acetylcholinesterase inhibitory and antioxidant properties

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ABSTRACT

The growing demand of pharmaceutical industry for more effective drugs requires new molecules with promising medicinal activities. In the present work, a natural product anisaldehyde was treated with hydrazine and 3,5-dichloroaniline to synthesize their Schiff bases, ASB1 and ASB2, which were assessed for various bioactivities. ASB1 was synthesized by conventional reflux method while ASB2 was synthesized by reflux as well as by mechanochemical grinding method which gave higher yield. The bases were recrystalised, and their structures were elucidated based on XRD and spectroscopic studies. Hirshfeld surface analysis was also carried out. They showed considerable urease inhibitory activity, almost comparable with the standard thiourea. The activity of ASB1 was much higher than ASB2. Acetylcholinesterase inhibitory activity of ASB1 was also higher than that of ASB2. The antioxidant activities were determined using DPPH, ABTS radical scavenging and total antioxidant capacity (TAC) assays. The bases were very poor scavengers of DPPH radical. However, they showed considerable anti-radical activity against ABTS radical, ASB2 being more active than ASB1, while ASB1 showed higher TAC than ASB2. In conclusion, the bases appeared to have good drugability as inhibitors of urease and acetylcholinesterase enzymes. They can be easily synthesized for possible large-scale applications. The grinding method proved to be more efficient than the reflux method.

1. Introduction

Anisaldehyde (4-methoxybenzaldehyde) is a naturally occurring organic compound found in the fennel and anise plants. It is a pale-yellow liquid with strong aniseed odour. It is an important intermediate for synthesis of other compounds in perfume and pharmaceutical industries [1]. At room temperature, it is a liquid with melting point -1 °C and boiling point 248 °C. It is insoluble in water and soluble in organic solvents, such as ethanol. The aim of the present work was to synthesize its Schiff bases and to determine some of their bioactivities. Schiff bases are azomethines having a carbon-nitrogen double bond. They are important organic compounds with a variety of applications. They are also used as starting material for synthesis of many bioactive heterocyclic compounds. Schiff bases have also been reported to have anti-HIV, anti-convulsion, antiviral, antibacterial, anti-inflammatory, and anthelmintic properties [2, 3]. They are well-known ligands with remarkable ability to make coordination complexes with metals [4]. Urease inhibitors are required for medicinal and agricultural applications. In medicine, they are required for the treatment of peptic ulcer caused by the bacterium *Helicobacter pylori.*, which produces, through urease activity, basic medium around it and, thus, survives in the acidic medium of the stomach [5]. The fertilizer urea is not fully available for the crops since it is hydrolysed by urease producing ammonia. This is a great economic loss. Secondly, the produced ammonia elevates the pH of the soil making it unconducive for plant growth. Moreover, the ammonia released into the atmosphere is hazardous for the environment [6]. Alzheimer's disease (AD) is a major type of neurodegenerative disorder. The disease is characterized by a loss of memory and thought, impaired speech comprehension, poor coordination, and diminished executive functions [7]. Several drugs are available, but they have serious side effects and none of them provides cure for the disease. It has been found to be associated with an overexpression of the enzymes cholinesterases

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acetylcholinesterase and butyrylcholinesterase [8]. Thus, more effective, safe and affordable inhibitors of these enzymes are required to treat AD effectively. Excessive production of reactive oxygen species (ROS) in our body causes a syndrome called oxidative stress which may lead to serious health issues such as cancer, cardiovascular disorders and neurodegenerative diseases [9]. Antioxidants are, thus, required to combat the oxidative stress. Another important application of antioxidants is to preserve food from rancidity [10].

Based on the rich literature review, the present project was planned as a part of our efforts to discover new candidates for drug development. New chemical compounds with appropriate structural features are important to meet the growing needs of the different sectors of industry. A knowledge of their crystal structure is important to understand their molecular structure as well as intermolecular interactions.

2. Materials and methods

2.1. Chemicals and reagents

Anisaldehyde, 3,5-dichloroaniline, and monohydrated hydrazine were purchased from Merck (Darmstadt, Germany), phenol, ascorbic acid, urease (from jack bean), DPPH (2,2-diphenyl-1-picrylhydrazyl), acetylthiocholine iodide (ATCI), acetylcholinesterase, and 5,5-ditiobis(2-nitrobenzoic) acid (DTNB) were from Sigma-Aldrich (Steinheim, Germany). Sodium hypochlorite, and sodium salicylate were from Daejung (Siheung City, Korea). Lithium chloride, sodium nitrite and sodium nitroprusside were from Riedel de Haën (Seelze, Germany). Urea, ethylenediaminetetraacetic acid and thiourea were from BDH Labs (Cambridge, England).

2.2. Synthesis of compounds

2.2.1. Schiff base ASB1

The Schiff base ASB1 was synthesized by the reaction of anisaldehyde with hydrazine (Fig. 1).

The conventional stirring method was used for the synthesis. As both the reactants were liquid, they were directly mixed together in 250-mL round bottom flask without the use of any solvent. The reaction was carried out at room temperature by stirring with a magnetic stirrer. A few drops of glacial acetic acid were added as a catalyst. The amounts of anisaldehyde and monohydrated-hydrazine used were 20 mmoles and 10 mmoles, respectively. Yellow coloured precipitates were formed instantaneously. The mixture was stirred for 1 hour to complete the reaction, which was monitored through TLC. The yellow precipitates formed were filtered on a Whatman filter paper 1 and washed with ethanol to remove any unreacted reactants. The residue was dried at room temperature and transferred into a beaker. DCM was added to dissolve it. Proper dissolution required some warming at about 30 °C. The solution was filtered, and the filtrate was collected in a china dish and allowed to dry. Bright yellow crystals were obtained.

2.2.2. Schiff base ASB2

The reaction of anisaldehyde with 3,5-dichloroaniline yielded the corresponding Schiff base (Fig. 2).

Two methods were used for this synthesis, the conventional reflux method and mechanochemical green method. In Reflux method, 10 mmoles anisaldehyde dissolved in 10 mL methanol was taken in a 250-mL round bottom flask. 3,5-Dichloroaniline (10 mmole) dissolved in 10 mL methanol was added. Glacial acetic acid (1 mL) was used as a catalyst. The solution was refluxed for 8 hours at temperature 60 °C on a hot plate. Reaction was monitored through TLC. The content of the flask was transferred into a china dish, which was placed in a fume hood at room temperature to evaporate the solvent. The dried content was washed with ethanol on a filter paper and dissolved in DCM. The DCM solution was filtered, and the filtrate was collected in a china dish. The solvent was allowed to evaporate. As a result, grey coloured needle like crystals were obtained.

The mechanochemical reaction was carried out in a mortar [11, 12]. No solvent was used. In a mortar, 3,5-dichloroaniline (10 mmole) and anisaldehyde (10 mmole) were placed. They were mixed with the help of a pestle forming a paste. Glacial acetic acid (around 5 drops) was added to catalyse the reaction. The mixture was manually ground for about 6 hours, adding a few drops of acetic acid after about every 30 min. The reaction was monitored through TLC. The fine powder formed was transferred into a beaker and washed with ethanol. The product was recrystalised from DCM as described above.

2.3. X-ray crystallographic analysis

Single-crystal X-ray diffraction (XRD) studies of the Schiff bases ASB1 and ASB2 were conducted by mounting high-quality crystals of sizes (0.220 × 0.180 × 0.080 mm^3) and (0.300 × 0.130 × 0.040 mm^3), respectively. Data were acquired on a Bruker D8 Venture equipped with PHOTON-100 and Cu Kα radiation (λ = 1.54178 Å) using φ-ω scan mode at 100 K. After the data collection on each compound, SAINT program was used for data reduction and SHELXS-86 for structure solution. All the non-hydrogen atoms were refined anisotropically by using full-matrix least-squares refinement methods on F^2 through the program SHELXL-2014. Hydrogen atoms were constrained on parent atoms *via* HFIX commands, while the hydrogen on hetero atoms were positioned from Fourier-maps and refined isotropically with thermal parameter $U_{iso}(H)$ = 1.2. *Crystal Explorer* 3.0. was used for Hirshfeld surface analysis and 2-D finger print plot in order to understand the inter-molecular interactions toward crystal stability and their impact on the different properties [13].

2.4. Spectroscopic studies

The spectroscopic studies used for the structure elucidation of the synthesized compounds included IR, MS, and NMR.

2.5. Determination of enzyme inhibitory activities

2.5.1. Urease inhibitory activities

The urease inhibitory activity of the synthesized Schiff bases was determined as per a reported protocol based on the indophenol method described by Weatherburn [14]. The enzyme solution (5 unit/mL) was prepared by dissolving urease in double distilled water. A series of solutions of the Schiff bases (1 μg/mL- 20 μg/mL) was prepared in DMSO. Buffer solution (pH 8.2) was prepared by dissolving 0.01 M K_2HPO_4, 100 mM Urea, 1 mM EDTA, and 0.01 M LiCl in 1000 mL distilled water. For

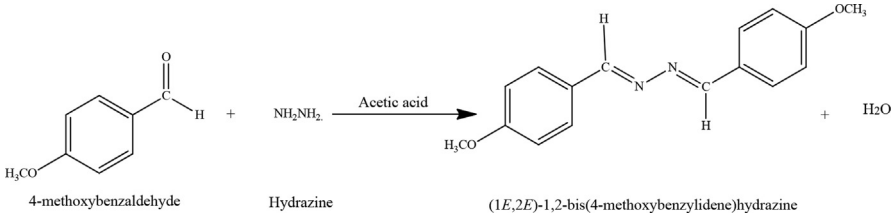

Fig. 1. Synthesis of Schiff base ASB1.

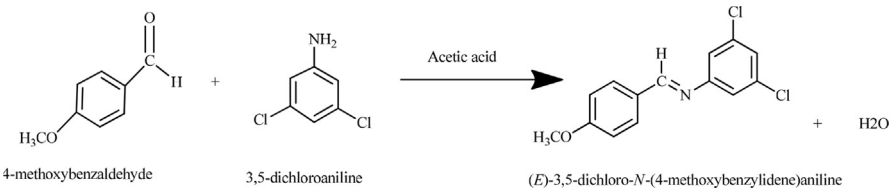

Fig. 2. Synthesis of Schiff base ASB2.

the preparation of phenol reagent, 1 % (w/v) phenol and 0.0005 % (w/v) sodium nitroprusside were dissolved in 100 mL distilled water. The alkali reagent used in the assay was prepared as follows. In a 150-mL volumetric flask, 5% (w/v) sodium hydroxide and 1% (w/v) sodium nitroprusside were dissolved in 100 mL distilled water in equal volumes. In a 96-well plate, 10 μL enzyme solution, 10 μL sample and 30 μL buffer soluion were mixed. The the mixture was incubated for 30 min at 37 °C. Afterward, 20 μL alkali reagnt and 20 μL phenol reagent were added. The resulting solution was incubated for 30 min at 37 °C. Then, absorbance was noted at 630 nm. The percent enzyme inhibitory activity was calculated accoridng to the following formula:

$$\% \text{ Activity} = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

where A_s and A_c are the absorbance of sample and control, respectively. The control 10 μL DMSO in place of sample. Thiourea was used as a standard.

2.5.2. Acetylcholinesterase inhibitory activities

Acetylcholinesterase inhibitory activity was determined on the basis of Elman's method [15, 16]. Sodium phosphate buffer (0.1 M, pH 8.0) was prepared by adding 12.208 g of disodium hydrogenphosphate and 2.168 g of sodium dihydrogenphosphate in 1 L deionised water. pH of solution was adjusted by using 0.1 M NaOH and 0.1 N phosphoric acid. Enzyme solution of 0.09 unit/mL was prepared by dissolving 0.1 mg enzyme in 272.2 mL buffer. Acetylthiocholine iodide (ATCI) solution (14 mM) was prepared by dissolving 100 mg ATCI in 46.29 mL sodium phosphate buffer. Dithiobis(2-nitrobenzoic) acid (DTNB) solution (10 mM) was prepared by dissolving 100 mg DTNB in 25.2 mL sodium phosphate buffer. A series of solutions with increasing concentration (1 μg/L, 2 μg/mL, ...) of each Schiff base were prepared in dimethyl sulfoxide (DMSO). 150 μL buffer, 10 μL tested compound and 20 μL enzyme solutions were taken in a test tube and incubated for 15 min at room temperature. After incubation, 10 μL substrate solution and 10 μL DTNB solution were added followed by an incubation of 15 min at room temperature. Absorbance was measured at 405 nm. Each determination was done in triplicate. The negative control had 10 μL DMSO in place of the sample, while rest of the procedure was the same. The enzyme inhibitory activity of a sample was calculated using Eq. (1). Where in, A_s and A_c are the absorbance of sample and control, respectively. Whereas, Neostigmine (trade name: Neostig) containing 2.5 mg Neostigmine Sulphate solution per mL was used as a positive control.

2.6. Determination of antioxidant activities

Antioxidant activities of the synthesised Schiff bases were determined according to three different methods, which were DPPH radical scavenging, ABTS radical scavenging and total antioxidant capacity.

2.6.1. DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was conducted according to a reported method [17]. The stock solution of DPPH solution was prepared by dissolving 24 mmole DPPH in 100 mL methanol. The working solution of DPPH was prepared by diluting 10 mL DPPH stock solution with 30 mL methanol so as to get absorbance of about 0.98 (($\pm$0.02) at 517 nm. Different dilutions of the synthesized Schiff bases (50–1000 μg/mL) were prepared in dimethylformamide (DMF). The blank contained 3.6 mL DPPH working solution in 400 μL DMF. This solution was also the negative control. To measure the anti-radical activity of a Schiff base, 3.6 mL DPPH working solution of DPPH was mixed with a 400 μL solution of the given Schiff base in a test tune. The mixture was incubated for 30 min at 37 °C, and its absorbance was measured at 517 nm. Percent anti-radical activity was calculated using Eq. (1) and Ascorbic acid was used as a standard.

2.6.2. $ABTS^{\cdot+}$ radical scavenging assay

The $ABTS^{\cdot+}$ decolourization assay was carried out as per the method by [18]. The ABTS stock solution was made by mixing equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate. The stock solution was kept in dark for 16 hours prior to use. The working solution of ABTS was made by mixing 5 ml ABTS stock solution in 100 mL methanol so as to have an absorbance of 0.770 $\pm$ 0.002 at 734 nm. Different dilutions of Schiff bases (50–1000 μg/mL) were prepared in DMSO. For the determination of activity, 300 μL sample was placed in a test tube and mixed with 3 mL $ABTS^{\cdot+}$ working solution. After passing 6 min, the absorbance was recorded at 734 nm. The anti-radical activity was determined using Eq. (1). Wherein, the control consisted of 3 mL ABTS working solution diluted with 300 μL DMSO. Ascorbic acid was used as a standard.

2.6.3. Total antioxidant capacity by phosphomolybdate assay

Total antioxidant capacity (TAC) of synthesized compounds was determined as per the phosphomolybdate assay [19]. Equal volumes of these three reagents (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were taken in a beaker to obtain the phosphomolybdate reagent. Different dilutions of Schiff bases were prepared in DMSO (50–500 μg/mL). Ascorbic acid solution in DMSO was used as a standard. In a test tube, 300 μL sample was mixed with phosphomolybdate reagent. It was wrapped in an aluminium foil and incubated at 95 °C for 90 min in a water bath. After the incubation, the solution was allowed to cool to room temperature and absorbance was recorded at 765 nm. The blank contained DMSO in place of sample. The total antioxidant capacity was calculated in μg/mL as ascorbic acid equivalent (AAE) as per the equation obtained from its calibration curve.

3. Results and discussion

3.1. Synthesis and structure elucidation

In the present study, two Schiff bases of anisaldehyde were synthesized with different amines, hydrazine and 3,5-dichloroaniline. The Schiff base with hydrazine (ASB1) was prepared through conventional reflux method but the second Schiff base (ASB2) was synthesized through the reflux method as well as the mechanochemical green method by grinding (Table 1).

As Table 1 shows, the mechanochemical green method gave better yield than the reflux method. The reported crystalline structure of ASB1 was triclinic while our work showed it monoclinic [21]. The crystal structure of ASB2 is being reported here for the first time.

3.1.1. XRD analysis of ASB1and ASB2

Crystallographic data and experimental details for structural analyses of compounds ASB1and ASB2 are summarized in Table 2. The structural study of Schiff base ASB1 shows that it consists of two planer rings A(C1-

Table 1
Some physical parameters of the synthesized Schiff bases.

Schiff base	Reflux method yield (%)	Grinding method yield (%)	Appearance	Melting point (°C)
ASB1	95	Not conducted*	Yellow crystals	181
ASB2	82	90	Greyish needle like crystals	63

* Because both the reactants were liquid, grinding method could not be applied.

Table 2
Crystallographic data and structure refinement parameters for the Schiff bases (ASB1 and ASB2).

Parameters	ASB1	ASB2
Empirical formula	$C_{16}H_{16}N_2O_2$	$C_{14}H_{11}Cl_2NO$
Formula weight (g/mol)	268.31	280.14
Temperature	299(2) K	299(2) K
Wavelength	1.54178 Å	1.54178 Å
Crystal system	Monoclinic	Orthorhombic
Space group	Cc	$Pca2_1$
Crystal size (mm^3)	0.220 × 0.180 × 0.080	0.300 × 0.130 × 0.040
Unit cell dimensions	a (Å) = 17.4031(8), α = 90.000° b (Å) = 10.7012(5), β = 113.735(2)° c (Å) = 8.4151(4), γ = 90.000°.	a (Å) = 20.8600(14), α = 90.000° b (Å) = 3.9640(3), β = 90.000° c (Å) = 16.2314(10), γ = 90.000°
Volume ($Å^3$)	1434.62(12)	1342.16(16)
Z	4	4
Density (mg/m^3)	1.242	1.386
Absorption coefficient (mm^{-1})	0.670	4.239
F(000)	568	576
Theta range for data collection	4.978 to 66.653°	4.239 to 66.611°
Index ranges	-20<=h<=20, -12<=k<=12, -10<=l<=10	-20<=h<=24, -4<=k<=4, -14<=l<=19
Reflections collected	10615	4745
Completeness to theta = 67.679°	99.7 %	98.8 %
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Independent reflections	2480 [R(int) = 0.0550]	1750 [R(int) = 0.0672]
Data/restraints/ parameters	2480/2/184	1750/1/165
Goodness-of-fit on F2	1.006	1.023
Absolute structure parameter	0.0(2)	0.05(3)
Extinction coefficient	0.0026(7)	0.017(2)
Final R indices [I > 2sigma(I)]	$R_1 = 0.0452$, $wR_2 = 0.1096$	$R_1 = 0.0573$, $wR_2 = 0.1317$
R indices (all data)	$R_1 = 0.0584$, $wR_2 = 0.1192$	$R_1 = 0.0716$, $wR_2 = 0.1432$
Largest diff. peak and hole	0.149 and -0.160 e.$Å^{-3}$	0.321 and -0.383 e.$Å^{-3}$

C7), and B (C8-C12). Both the rings are benzylic, bonded *via* azomethine bonds *i.e.* C7 = N1, C8 = N2 with bond lengths 1.264(5) Å and 1.277(6) Å, respectively (*ORTEP*). The azomethine bonds were in *trans*-mode, with the torsion angle 179.4(4)° (Fig. 1). The dihedral angle between the rings (C1-C7) and (C18-C14) was found to be 8.18(19)°. The crystal structure of ASB**1** showed the one inter-molecular interaction O1···H5 with donor-acceptor distance of 3.381(6) Å (Table 3) in the unit cell. This inter-molecular interaction in the crystal lattice results sheets of molecules parallel to *a-axis* and is responsible for conformational rigidity (Fig. 3). In addition, 3D Hirshfeld (Fig. 4) and 2D finger print plots (Fig. 5) were generated in support of quantitative study of all types of inter-molecular interactions such as H···H, C···H/H···C, O···H/H···O contributions 46.3%, 32.4%, 11.6% and 0.1%, respectively, toward crystal stability. The Hirshfeld surface analysis is a powerful technique to determine intermolecular interactions both qualitatively and quantitatively [13, 20].

Table 3
Hydrogen bond lengths (Å) and bond angles (°) of ASB1.

D-H...A	D-H (Å)	H...A (Å)	D...A (Å)	< D-H...A (°)
C(16)-H(16A)...O(2)[1]	0.96	2.63	3.381(6)	135.4

Symmetry transformations used to generate equivalent atoms: [1] x-1, y, z.

The crystal structure of ASB1 was reported by Jin et al (2007) as triclinic while our work showed it to be monoclinic [21]. The base, therefore, displays polymorphism and exists in these forms. Since the reported work used ethanol for recrystallization and we used dichloromethane as a solvent for this purpose, the change in crystal structure may be caused by this difference.

The structure of Schiff base ASB**2** was also established *via* single-crystal X-ray diffraction technique. It contained a phenyl ring A(C1-C6) substituted with chlorine at C2 and C4 positions, and a benzyl ring B (C7-C11) substituted with methoxy moiety on C-11. The bond lengths of C6-N1 and C7 = N1 bonds are 1.413 (6) Å and 1.2657 (7) Å evidently indicate on their bond length differences. The torsion angle of atoms C6-N1-C7-C8 between two aryl rings is 172.47° (Fig. 6). The dihedral angle between the two rings A and B was 38.0 (3)°. In the crystal lattice of ASB2, there are two intermolecular interactions *i.e.* C1…H1—O1 and C5-H5——CL1 with donor and acceptor distances 3.511(7) Å and 3.847(6) Å, respectively, which align the molecules in a sophisticated structure resulting in a three-dimensional network. The contact distance and angles of non-covalent interactions which engaged the crystal lattice in unit cell packing are summarized in Table 4. The contribution of different inter-molecular interactions is given as 28.9%, 28.3%, 14.7%, 8.9%, 6.2% 4.3%, and 3.5% for H···H, CL…H, C···H, C···C, O···H, N···H, and CL…CL respectively, which was calculated through Hirshfeld surface analysis as shown in Fig. 7. Diagrammatic representations of 2D finger plots of ASB2 are displayed in Fig. 8.

3.1.2. Spectroscopic studies

The mass spectrum of ASB1 showed the molecular ion peak $[M]^+$ at 268 m/z. The base peak was at m/z 161. Other important peaks were134, 77 and 92. The IR spectrum of ASBI showed an intense peak at 1603 cm^{-1} indicating the presence of azomethine group (N=C). The peaks for C=*O group* (at 1679 cm^{-1}) of the aldehyde and NH_2 group (at 3358 cm^{-1}) of amine were absent confirming the occurrence of the reaction. The ^{1}H-NMR spectrum of ASB1 had a singlet at 8.59 ppm which is a characteristic signal for azomethine proton (2H). The aromatic protons appeared at 6.87 (4H, doublet) and7.70 (4H, doublet) ppm. The OCH_3 protons appeared at 3.84 ppm as a singlet (6H).

The mass spectrum of ASB2 had the molecular ion peak $[M]^+$ at 279 m/z with a $[M+2]^+$ peak at m/z 281 indicating isotopes of chlorine. The base peak was at m/z 149. Another prominent peak in mass spectrum was at m/z 167. The IR spectrum of ASB2 had a peak at 1606 cm^{-1} for its azomethine group, white it too did not have peaks for C=O and NH_2 (at 3330 cm^{-1}) groups providing and an evidence of the conversion of reactant into the product. In the ^{1}H-NMR spectrum of ASB2, azomethine proton appeared at 8.30 ppm. The aromatic protons appeared at 6.89, 7.05 and 7.74 ppm. The methoxy protons appeared at 3.86 ppm.

3.2. Inhibitory and antioxidant activities

3.2.1. Urease inhibitory activity

Both the synthesized compounds displayed considerable urease inhibitory activity, which was dose dependant (Table 5). ASB1 was much more potent than ASB2, which was, however, less active than the standard thiourea. This indicates that ASB1 has a greater ability to bind with the urease enzyme thereby inhibiting its catalytic activity. This might be because of two nitrogen atoms in ASB1 which have lone pairs of electrons

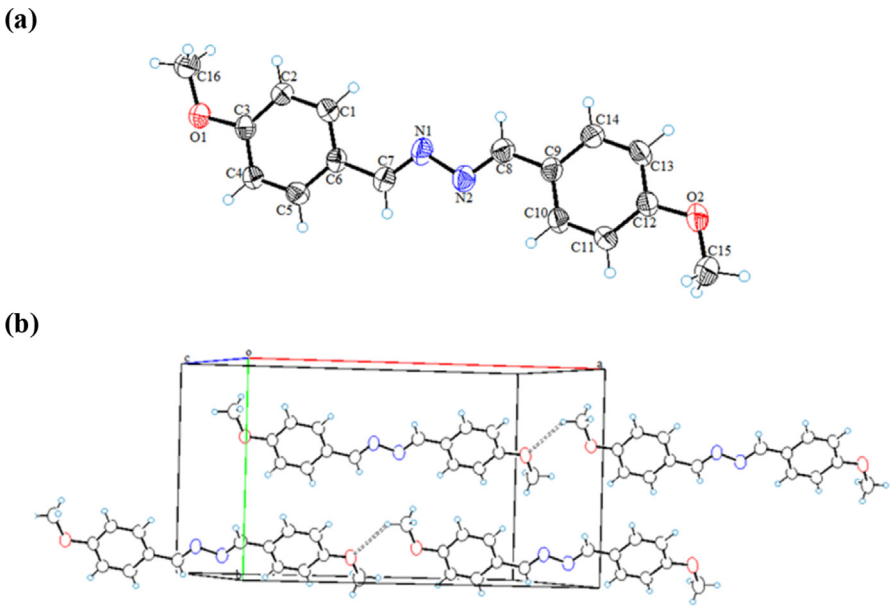

Fig. 3. (a) Molecular structure of ASB**1** with 40% ellipsoid probability and (b) is the unit cell packing diagram of ASB**1** with C16-H16A…O2 interaction.

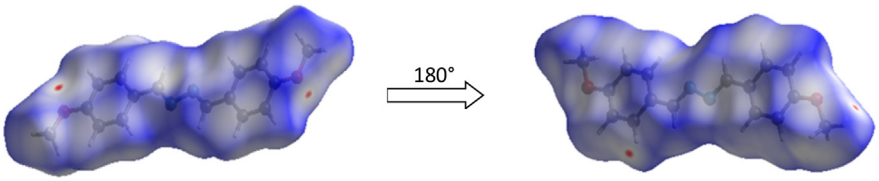

Fig. 4. 3D Hirshfeld surface of ASB**1**.

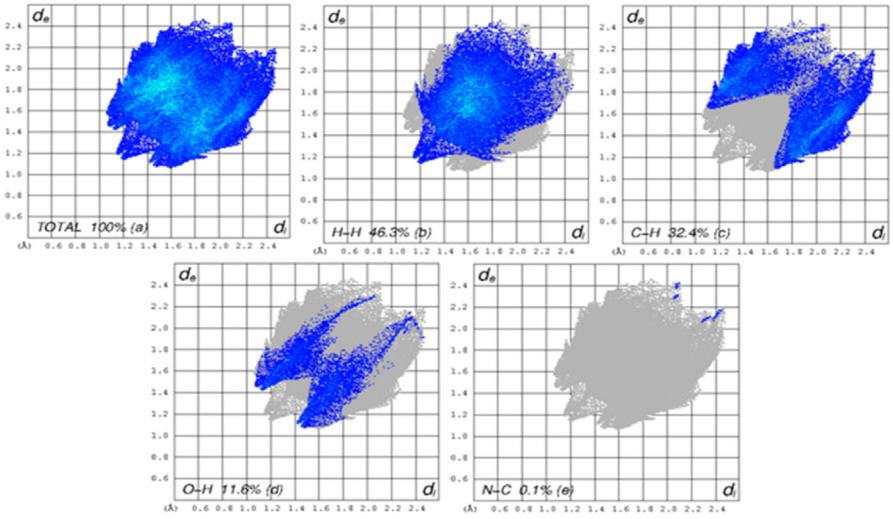

Fig. 5. 2D Finger print plots of ASB**1**.

which may be donated for bonding with the enzyme. ASB1 has a longer chain connecting the two aryl rings as compared to two in ASB2. Thus, ASB1 has greater flexibility which might favour a stronger binding between it and the enzyme. For a more comprehensive understanding of the nature of binding, however, computational and kinetic studies are required which are a part of our future work.

As the Schiff bases are practically susceptible to hydrolysis in water, any of their bioactivities determined in a medium containing water would depend on the extent of their stability in such a medium. In the determination of urease inhibitory activities, though the solutions of the Schiff bases were prepared in DMSO, but the solutions of other reagents contained water. Therefore, there is a chance that the stability of the Schiff bases may have been affected by water during the determination of the activity.

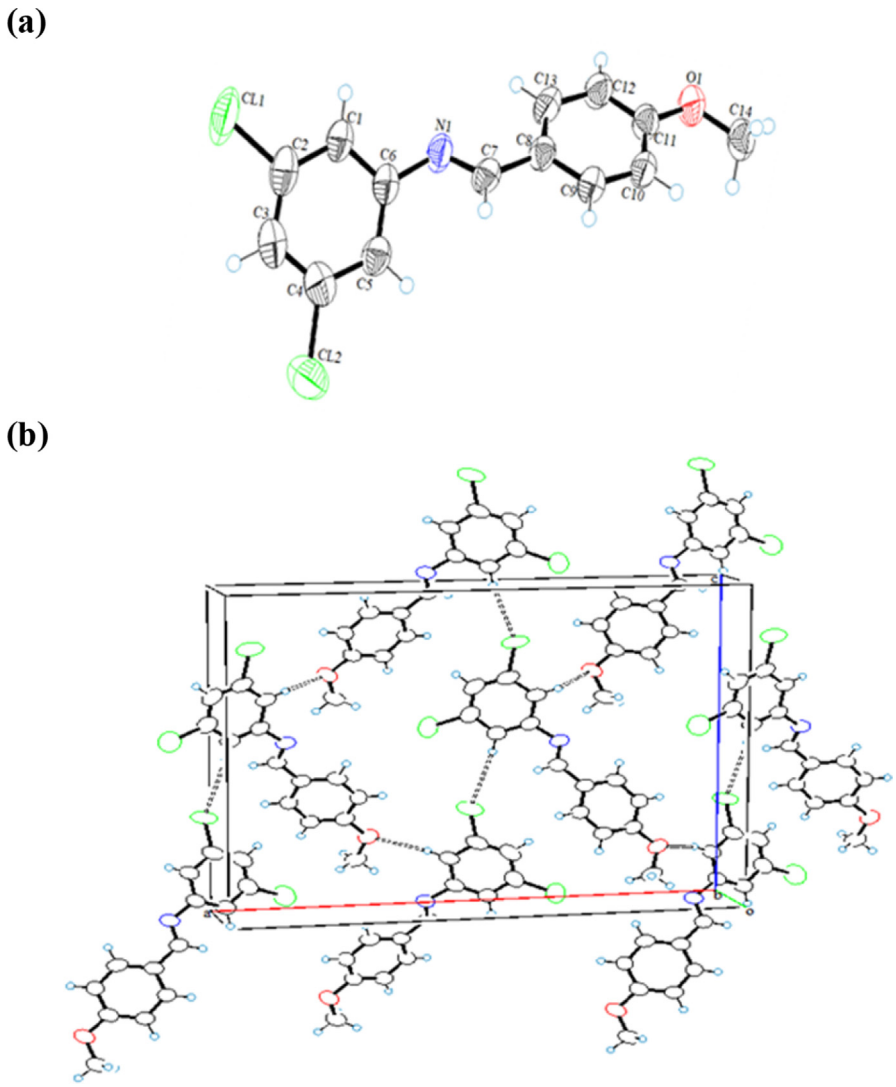

Fig. 6. (a) ORTEP diagram of ASB**2** with 40% ellipsoid probability and (b) is the representation of packing diagram of Schiff base ASB**2** with C1-H1...O1, C5-H5...CL1 interactions.

Table 4
Hydrogen bond lengths (Å) and bond angles (°) of ASB1.

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(1)-H(1)...O(1)[1]	0.93	2.64	3.511(7)	156.0
C(5)-H(5)...Cl(1)[2]	0.93	2.92	3.847(6)	172.6

Symmetry transformations used to generate equivalent atoms: [1]-x+1/2, y, z+1/2, [2]-x+1, -y, z-1/2.

3.2.2. *Acetyl cholinesterase inhibitory activity*

The synthesized bases exhibited considerable concentration dependent acetyl cholinesterase enzyme inhibitory activity (Table 5). The base ASB1 was almost twice as active as ASB2. The activities were, however, much less as compared to the standard drug neostigmine. The greater activity of ASB1 might be due to the same reasons as discussed above, i.e., two nitrogen atoms with donatable lone pairs and a greater flexibility.

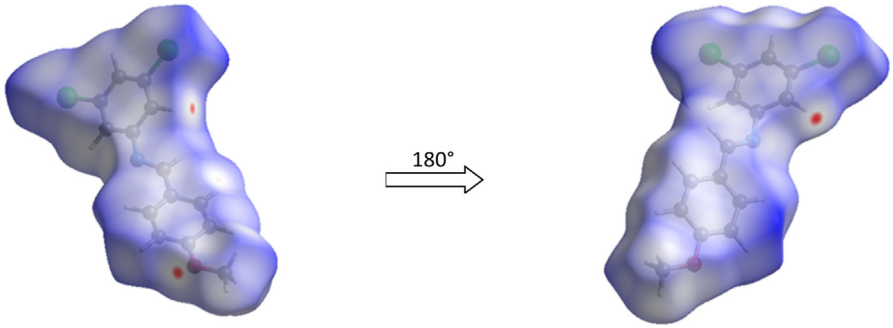

Fig. 7. 3D Hirshfeld surface analysis of ASB2.

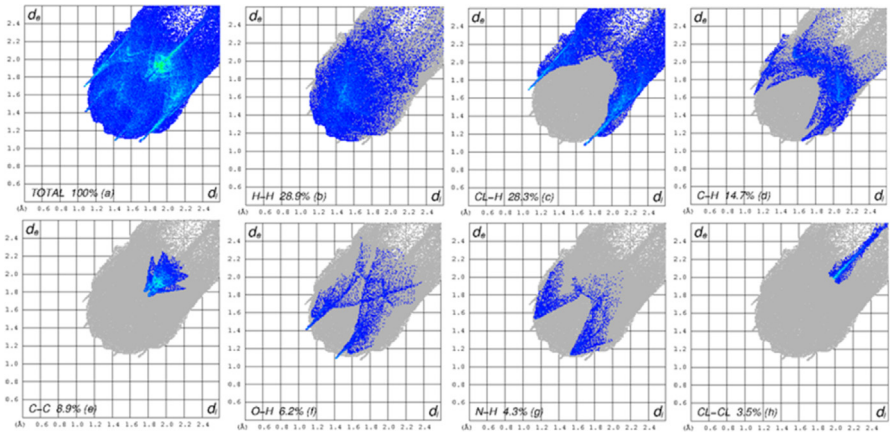

Fig. 8. Diagrammatic representation of 2D finger plots of ASB2.

Table 5
Urease and acetylcholinesterase (AChE) inhibitory activities IC_{50} (μg/mL) of the synthesized Schiff bases ASB1 and ASB2.

Enzyme	ASB1	ASB2	Standard
Urease	10.29	51.60	4.97*
AChe	12.06	22.38	1.933

*Thiourea; Neostigmine.

Table 6
Antioxidant activity (EC_{50} μg/mL) of the synthesized Schiff bases.

Assay	ASB1	ASB2	Standard Ascorbic acid
DPPH	2421.00	2448.00	6.48
ABTS	11.59	1.86	8.65

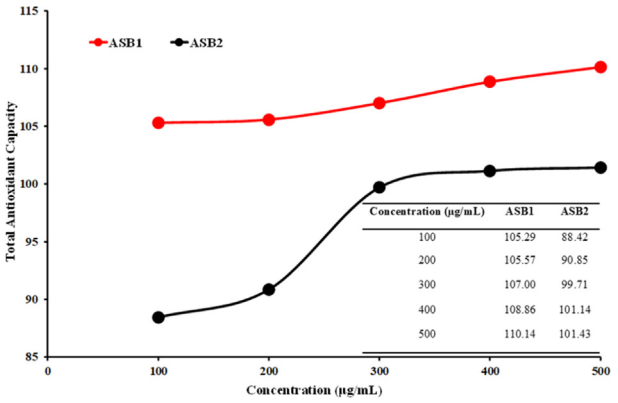

Fig. 9. The comparison of total antioxidant capacity of ASB1 and ASB2.

3.2.3. Antioxidant activity

Both the Schiff bases exhibited poor antioxidant activity as compared to the standard ascorbic acid in the DPPH assay. In the DPPH radical scavenging assay, ASB1 and ASB2 had almost equal potency as is shown by their EC_{50} values (Table 6). In the ABTS radical scavenging assay, ASB2 proved to be more potent antioxidant than ASB1, having EC_{50} values, 1.86 and 11.59 μg/mL, respectively (Table 6).

The bases were very poor scavengers of DPPH radical. However, they showed considerable anti-radical activity against ABTS radical. The difference may be due to the different mode of action of the two assays [22]. The ABTS is a radical cation and in the assay, it is generated and made to act in an aqueous medium. On the other hand, DPPH is a stable radical and, in the assay, it works in a hydrophobic environment.

Total antioxidant capacity (TAC) of the bases as determined according to the phosphomolybdate assay was expressed as μg/mL of ascorbic acid equivalents (AAE) and the results are shown in Fig. 9 as a function of concentration. ASB1 had slightly higher activity than ASB2.

As the different antioxidant assays follow different mechanisms, the difference in their activities is understandable [23]. The poor antioxidant potency of these Schiff bases alludes to a poor ability of these bases to scavenge free radicals by HAT (Hydrogen Atom Transfer) or SET (Single Electron Transfer) mechanisms [24].

4. Conclusions

Schiff bases of anisaldehyde were successfully synthesized and crystallised. They showed considerable urease and acetylcholinesterase inhibitory activities presenting themselves as potential candidates for new drugs for disorders associated with overexpression of these enzymes.

Declarations

Author contribution statement

Dildar Ahmed, Sammer Yousuf: Conceived and designed the experiments; Wrote the paper.

Amber Jan Muhammad: Performed the experiments.

Nida Tabassum: Analyzed and interpreted the data.

Muhammad Tariq Qamar: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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