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ORIGINAL PAPER

Synthesis, Characterization and Cytotoxic Activity of S-Benzyldithiocarbazate Schiff Bases Derived from 5-Fluoroisatin, 5-Chloroisatin, 5-Bromoisatin and Their Crystal Structures

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Abstract Schiff bases were prepared from S-benzyldithiocarbazate with 5-fluro-, 5-chloro- and 5-bromoisatin. All are potential tridentate nitrogen, oxygen, sulfur donors. They were found to be selectively active against MCF-7 cell line (Human non-metastatic mammary gland adenocarcinoma cell line). The bromide and fluoride compounds were the most active with IC_{50} values of 6.40 μM (2.6 μg/mL) and 9.26 μM (3.2 μg/mL) respectively while the chloride derivative was weakly active with an IC_{50} value of 38.69 μM (14.0 μg/mL). The cytotoxic activity of the halo substituted isatins against the breast cancer cell lines tested is in the order of Br > F > Cl. Planarity of the isatin ring in the Schiff bases can be arranged in the following order SB5FISA > SB5ClISA > SB5BrISA while the perpendicularity of the benzyl ring towards the dithiocarbazate plane can be ordered as follows, SB5FISA > SB5BrISA > SB5ClISA.

Keywords Dithiocarbazate · Schiff base · Isatin · MCF-7 · Structure

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Introduction

Dithiocarbazate, $NH_2NHCS_2^-$, is a chelating agents derived from S-alkyl esters of dithiocarbazic acid, the metal complexes of which have been extensively studied over the past two decades, not only because of their behaviour as ligands in transition metal complexes, but also have been shown to exhibit interesting physico-chemical and potentially chemotherapeutic properties [1–3].

Isatin is an endogenous compound isolated in 1988 and reported to possess a wide range of activities involving the central nervous system [4–7]. Schiff bases and Mannich bases derived from isatin have been reported to possess antibacterial [8–17], antifungal [11–13, 15, 17], antiviral [10, 14], anti-HIV [11–13, 15, 17], antiprotozoal [18, 19], and antihelminthic activities [20, 21].

Varieties of halogenated compounds particularly fluorine-containing aromatic compounds have drawn much attention because of their biological activities [22]. Many of them are used as pharmaceuticals and pesticides or have found use as precursors for the synthesis of biologically active compounds. In 2006, SU11248 (Sutent), a 5-fluoro-3-substituted-2-oxoindole, was approved by the US FDA for the treatment of gastrointestinal stromal tumors [23] and advanced renal-cell carcinoma [24].

Although much attention has been directed to study of Schiff base and Mannich bases derived from isatin [8, 9, 12, 13, 15–17] no investigations have appeared in the

literature to describe the Schiff bases derived from dithiocarbazate with 5-haloisatins. Furthermore, only limited studies have been done on dithiocarbazato Schiff bases having nitrogen, oxygen, sulfur (NOS) donor sequences. Therefore, as part of our ongoing study on Schiff bases derived from S-benzyldithiocarbazate, we report herein the synthesis, characterization and cytotoxic activity of Schiff bases formed from the condensation of S-benzyldithiocarbazate with 5-fluroisatin, 5-chloroisatin and 5-bromoisatin that are potential NOS donor ligands.

Experimental

Physical Measurements

All the chemicals, reagents and solvents for the synthesis of compounds were analytical grade and used without further purification. Microanalyses for carbon, hydrogen, nitrogen and sulfur were carried out using a LECO CHNS-932 instrument. The IR spectra, as KBr pellets were recorded using a Perkin–Elmer FT IR 1750X spectrophotometer (4000–400 cm^{-1}). NMR spectra were acquired on a JOEL-JNM ECA 400 MHz spectrometer, where proton (^1H) and carbon (^{13}C) spectra were obtained at 300 and 100 MHz respectively. Spectra were recorded in DMSO-d_6 with TMS as the internal standard. All chemical shift values were recorded in ppm (δ).

X-ray Structure Determination

Dark orange crystals of SB5FISA, brownish orange crystals of SB5ClISA and orange crystals of SB5BrISA formed after EtOH solutions were allowed to slowly evaporate over a few weeks. Data for the SB5FISA (Table 1) was collected at 93 K using a Rigaku MM007 High brilliance RA generator (Mo Kα radiation, confocal optics) and Mercury CCD system at Molecular Structure Laboratory, School of Chemistry, University of St. Andrews. At least a full hemisphere of data was collected using ω scans. Intensities were corrected for Lorentz-polarisation and for absorption. Selected crystals of SB5BrISA and SB5FISA were mounted on glass fiber using perfluropolyether oil and cooled rapidly to 150 K in a stream of cold N2 using Oxford Cryosystems CRYOSTREAM unit at the Chemistry Research Laboratory, University of Oxford. Diffraction data were measured using an Enraf–Nonius Kappa CCD diffractometer (graphite-monochromatic Mo Kα radiation, $\lambda = 0.71073$ Å). Intensity data were processed using the DENZO-SMN package [25]. The crystal structures of all three Schiff bases were solved using the direct-methods program SIR92 [26] which located all non-hydrogen atoms. Subsequent full-matrix least-squares refinement of F/F2 was carried out using CRYSTALS Program Suite [27] Coordinates and anisotropic thermal parameters of all non-hydrogen atoms were refined. Hydrogen atoms were positioned geometrically after each cycle of refinement. Refinement converged satisfactorily to give good R and R_w values with the best residual electron density minimum and maxima. Tables 1, 2 and 3 summarizes crystal data and structure refinement results of the Schiff bases.

Table 1 Summary of crystal data and structure refinement parameters for SB5FISA

Chemical formula	$C_{16}H_{12}F_1N_3O_1S_2$
Formula weight	345.42
Crystal class	Monoclinic
Space group	$P2_1$
a (Å)	4.576(3)
b (Å)	9.688(7)
c (Å)	17.119(12)
α (°)	90
β (°)	94.615(19)
γ (°)	90
V (Å^3)	756.4(9)
Z	2
Mo Kα (Å)	0.7107
T (K)	93
ρ_{calc} (mg m^{-3})	1.52
μ (mm^{-1})	0.370
Crystal dimension (mm)	0.01 × 0.02 × 0.20
F (000)	356
Theta range for data collection (°)	2–29
Limiting indices	$(-5 \le h \le 5)$
	$(-11 \le k \le 10)$
	$(-20 \le l \le 17)$
Reflections collected/unique	5038/2542
Maximum & minimum transmission	0.57, 1.00
Data/restrains/parameters	2536/1/209
Goodness-of-fit on F^2	0.87
Minimum & maximum residual electron density (e Å^{-3})	−0.58, 0.57
R, R_w (%)	6.5, 15.3

Cell Culture

Two types of cell lines were used: MCF-7 (Human non-metastatic mammary gland adenocarcinoma cell line.) and MDA-MB-231 (Human metastatic mammary gland adenocarcinoma cell line.). All the cell lines were obtained from the National Cancer Institute, USA. MCF-7 was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum while MDA-MB-231 was cultured in DMEM medium supplemented with 10% foetal bovine

Table 2 Summary of crystal data and structure refinement parameters for SB5ClISA

Chemical formula	$C_{16}H_{12}Cl_1N_3O_1S_2$
Formula weight	361.88
Crystal class	Triclinic
Space group	$P-1$
a (Å)	6.4447(2)
b (Å)	8.0844(3)
c (Å)	15.8506(6)
α (°)	94.3189(16)
β (°)	99.2169(17)
γ (°)	92.0591(17)
V (Å^3)	811.90(5)
Z	2
Mo Kα (Å)	0.7107
T (K)	150
ρ_{calc} (mg m^{-3})	1.48
μ (mm^{-1})	0.499
Crystal dimension (mm)	0.20 × 0.26 × 0.40
F (000)	372
Theta range for data collection (°)	5–28
Limiting indices	$(-8 \le h \le 7)$
	$(-10 \le k \le 10)$
	$(-20 \le l \le 19)$
Reflections collected/unique	6118/3793
Maximum & minimum transmission	0.88, 0.91
Data/restrains/parameters	3126/0/208
Goodness-of-fit on F^2	1.077
Minimum & maximum residual electron density (e Å^{-3})	−0.33, 0.39
R, R_w (%)	3.8, 3.7

Table 3 Summary of crystal data and structure refinement parameters for SB5BrISA

Chemical formula	$C_{16}H_{12}Br_1N_3O_1S_2$
Formula weight	406.33
Crystal class	Triclinic
Space group	$P-1$
a (Å)	6.5350(2)
b (Å)	8.0867(2)
c (Å)	15.9265(5)
α (°)	92.9504(12)
β (°)	99.2083(12)
γ (°)	92.0396(14)
V (Å^3)	828.89(4)
Z	2
Mo Kα (Å)	0.7107
T (K)	150
ρ_{calc} (mg m^{-3})	1.63
μ (mm^{-1})	2.738
Crystal dimension (mm)	0.20 × 0.30 × 0.40
F (000)	408
Theta range for data collection (°)	5 to 27
Limiting indices	$(-8 \le h \le 7)$
	$(-10 \le k \le 10)$
	$(-20 \le l \le 20)$
Reflections collected/unique	6556/3786
Maximum & minimum transmission	0.44, 0.58
Data/restrains/parameters	3184/0/208
Goodness-of-fit on F^2	1.082
Minimum & maximum residual electron density (e Å^{-3})	−0.56, 1.17
R, R_w (%)	3.2, 3.4

serum. Both cells were incubated in an atmosphere of 5% CO_2 and 100% relative humidity at 37 °C.

Treatments and Sample Dilutions

Cells from the cell lines were counted, diluted and inoculated onto sterile 96-well microtiter plates. Cells were inoculated in a volume of 100 µL per well at densities between 5000 and 7500 cells per well. The microtiter plates containing the cells were preincubated overnight at 37 °C in an atmosphere of 5% CO_2 and 100% relative humidity to allow stabilization prior to addition of compounds.

Stock solutions of the compounds (10 mg/mL) were prepared using DMSO. The compounds were then diluted with complete medium ranging from 100 to 0.3 µg/mL. Immediately after preparation of these intermediate dilutions, 100 µL aliquots of each dilution were added to the appropriate microtiter plate wells. The treated microtiter culture plates were incubated for 4 days in an atmosphere of 5% CO_2 and 100% relative humidity at 37 °C. The end point was measured using MTT assay as explained below.

3-[4,5-Dimethylthiazo-2-yl]-2,5-diphenyltetrazolium bromide (MTT) Assay

Cytotoxicity was evaluated using MTT (3-[4,5-dimethylthiazo-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma, USA) assay as reported by Mosmann [28]. The MTT assay is based on the metabolic reduction of (3-[4,5-dimethylthiazo-2-yl]-2,5-diphenyltetrazolium bromide) (MTT). A 40 µL aliquot of MTT solution (2.5 mg MTT in 1 mL of PBS solution) was added directly to all the appropriate microtiter plate wells containing cells, complete growth media and test agents as prepared above. The culture was then incubated for 4 h to allow for MTT to metabolise to formazan. The supernatant was pipetted out and 200 µL of DMSO was added to dissolve the formazan. The plates

were agitated on a plate shaker to ensure homogeneity of the solution and the optical densities were read on an automated spectrophotometric plate reader (model MRX II microplate Elisa reader) at a test wavelength of 570 nm and a reference wavelength of 630 nm. Cytotoxicity is shown as the minimum concentration required to reduce the absorbance of treated cells by 50% with reference to the untreated cells (IC_{50}). Tamoxifen, was the cytotoxic standard.

Chemistry

Preparation of S-Benzyldithiocarbazate (SBDTC)

This compound was synthesized as previously reported [29]. Potassium hydroxide (22.8 g, 0.4 mol) was dissolved completely in 90% ethanol (140 mL) and the mixture was cooled in ice bath. To the mixture, hydrazine hydrate (20 g, 0.4 mol) was added slowly with stirring. Carbon disulfide (30.4 g, 0.4 mol) was then added dropwise with vigorous stirring. The temperature of the reaction mixture was not allowed to rise above 6 °C during the period of addition of carbon disulfide. The resulting yellow oil was separated by means of a separatory funnel and dissolved in 40% ethanol (40 mL) and the solution was cooled in ice. Benzyl chloride (58 g, 0.4 mol) was added slowly with vigorous stirring. The white product was separated by filtration, washed with water and dried in air. The crude product was recrystallized from ethanol. Yield, 20 g (40%). The melting point was 124 °C (Lit. 124–125 °C) [30].

Preparation of Schiff Bases

S-benzyldithiocarbazate, SBDTC (1.36 g, 0.01 mol) was dissolved in hot ethanol (35 mL) and to this solution was added an equimolar amount of 5-fluorosatin (1.65 g, 0.01 mol), 5-chloroisatin (1.81 g, 0.01 mol) or 5-bromoisatin (2.26 g, 0.01 mol). The mixtures were heated while being stirred for 15 min and later allowed to stand for ~20 min until product formed. Solids were filtered, washed with ethanol and recrystallized from ethanol. Yields were high, ca 80%.

S-Benzyl 2-(5-fluoro-2-oxoindolin-3-ylidene)hydrazinecarbodithioate (SB5FISA)

The compound was obtained as dark orange powder in a yield of 80%; mp 235–236 °C. FTIR (KBr, cm^{-1}) 3280 ν(N–H); 1686 ν(C=O); 1628 ν(C=N); 1056 ν(C=S), 1136 ν(N–N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.93 (s, 1H, $NH_{dithiocarbazate}$), 11.36 (s, 1H, NH_{isatin}), 7.30–7.44 (m, 8H, aromatic), 4.53 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 199.5 (C=S), 162.6 (C=O), 129.36 (C=N), 108.1–157.2 (aromatic), 38.2 (S-CH_2) ppm. Anal. Calcd. For C_{16} $H_{12}F_1N_3O_1S_2$: C, 55.63; H, 3.50; N, 12.17; S, 18.57. Found; C, 55.35; H, 3.44; N, 12.12; S, 18.38. (CCDC reference 696006).

S-Benzyl 2-(5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbodithioate (SB5ClISA)

The compound was obtained as brownish orange powder in a yield of 80%; mp 247–248 °C. FTIR (KBr, cm^{-1}) 3250 ν(N–H); 1694 ν(C=O); 1586 ν(C=N); 1076 ν(C=S), 1150 ν(N–N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.88 (s, 1H, $NH_{dithiocarbazate}$), 11.46 (s, 1H, NH_{isatin}), 7.35–7.50 (m, 8H, aromatic), 4.55 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 199.5 (C=S), 162.3 (C=O), 131.8 (C=N), 113.0–142.1 (aromatic), 38.2 (S-CH_2) ppm. Anal. Calcd. For C_{16} $H_{12}Cl_1N_3O_1S_2$: C, 53.11; H, 3.31; N, 11.61; S, 17.72. Found; C, 53.05; H, 3.32; N, 11.45; S, 17.67. (CCDC reference 696008).

S-Benzyl 2-(5-bromo-2-oxoindolin-3-ylidene)hydrazinecarbodithioate (SB5BrISA)

The compound was obtained as dark orange powder in a yield of 80%; mp 249–250 °C. FTIR (KBr, cm^{-1}) 3300 ν(N–H); 1696 ν(C=O); 1616 ν(C=N); 1066 ν(C=S), 1148 ν(N–N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.86 (s, 1H, $NH_{dithiocarbazate}$), 11.45 (s, 1H, NH_{isatin}), 7.32–7.49 (m, 8H, aromatic), 4.54 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 199.5 (C=S), 162.1 (C=O), 129.3 (C=N), 114.5–142.4 (aromatic), 38.2 (S-CH_2) ppm. Anal. Calcd. For C_{16} $H_{12}Br_1N_3O_1S_2$: C, 47.30; H, 2.98; N, 10.34; S, 15.78. Found; C, 47.10; H, 2.84; N, 10.29; S, 15.28. (CCDC reference 696007).

Result and Discussion

Chemistry

The results of elemental analyses (carbon, hydrogen, nitrogen, and sulfur) obtained are in good agreement with calculated values.

Fourier-Transform Infrared Data for the Schiff Bases

Bands in the region 3300–3250 cm^{-1} are attributable to NH stretching. The Schiff bases contain a thione group (C=S) with protons adjacent to it. Although it has been noted that the thione group (C=S) is relatively unstable in the monomeric form and tends to convert to a stable C–S single bond by enethiolization if there is at least one

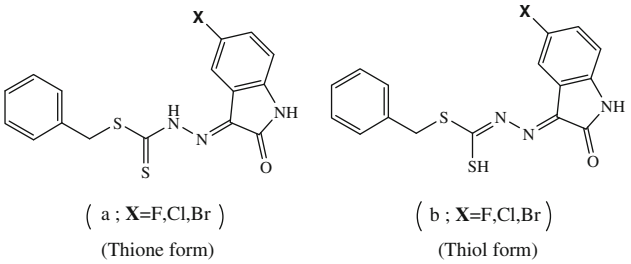


Fig. 1 Thione-thiol tautomerism of the Schiff bases

hydrogen atom adjacent to the C=S bond [30]. The IR spectra of the Schiff bases did not display ν(S–H) at ca. 2570 cm^{-1} indicating that in the solid-state they remain in the thione form (Fig. 1a). However, equilibrium with the thiolo tautomeric form (Fig. 1b) may be established in solution.

ν(C=S) bands were observed at 1056–1076 cm^{-1} while ν(C=N), ν(N–N) and ν(C=O) bands were observed at 1586–1628 cm^{-1}, 1136–1150 cm^{-1} and 1686–1694 cm^{-1}, as expected for these SBDTC Schiff bases.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The signal at 4.53–4.55 ppm was attributed to the methylene hydrogen (CH_2). The methylene hydrogen shift is generally in the range 1.2–1.4 ppm [31]. The downfield shift of methylene hydrogen in all the Schiff bases can be ascribed to the deshielding effect of electronegative sulfur and the aromatic ring. The signal at 13.86–13.93 ppm was assigned to the secondary proton from the dithiocarbazate and the singlet peak at 11.36–11.46 ppm can be assigned to NH in the pyrrole group of the isatin ring. The NH signal from the isatin ring appears upfield to NH from the dithiocarbazate indicating intermolecular hydrogen bonding [32]. The cluster of peaks obtained in the region of 7.30–7.50 ppm is associated with the aromatic hydrogen in the aromatic ring.

The ^{13}C NMR spectra of the Schiff bases showed a signal at 199.5 ppm which is attributed to the thioamide carbon. The low intensity is due to the deshielding effect of the nitrogen and two sulfur atoms adjacent to the carbon. These electronegative elements produce a large downfield shift since they are directly attached to the carbon atom. Another downfield signal observed in each spectrum at 162.1–162.6 ppm is ascribed to the carbonyl group (C=O) of the isatin derivatives. The remaining signals observed in the region of 108.1–157.2 ppm may be assigned to carbons in the aromatic ring.

Single Crystal X-ray Analyses of SB5FISA, SB5ClISA and SB5BrISA

The crystal structures of the Schiff bases with their atom numbering schemes, intramolecular and intermolecular hydrogen bonds are shown in Figs. 2, 3, 4, 5, 6, 7. Selected bond lengths and bond angles of the Schiff bases are listed in Table 4. The structures of SB5ClISA and SB5BrISA feature centrosymmectric H-bonded dimeric motifs which are held by the strong intermolecular hydrogen bonds of (N–H–O). These hydrogen bond motifs are different to that observed in SB5FISA which is held by the weak intermolecular hydrogen bond of (C–H–O). Variations in these hydrogen bonding motifs would give a different packing of molecules in a crystal [33].

In SB5FISA (Fig. 2), the six- and five-membered rings of the isatin moieties which consist of C1–C8–C7–C6–C5–C4 and C2–C1–C4–N1–C3 are almost perfectly coplanar with dihedral angle of 0.78° between the two mean planes. The

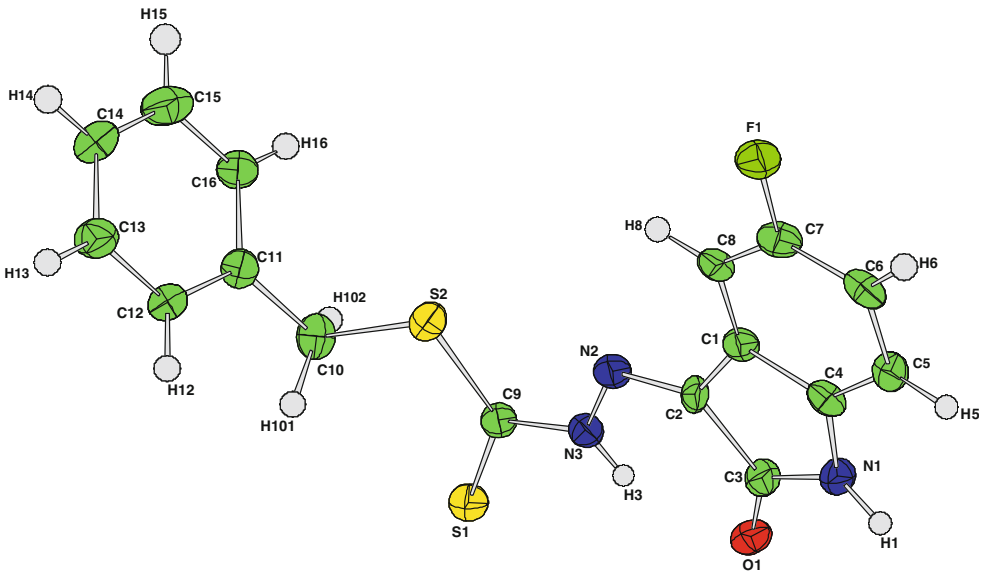
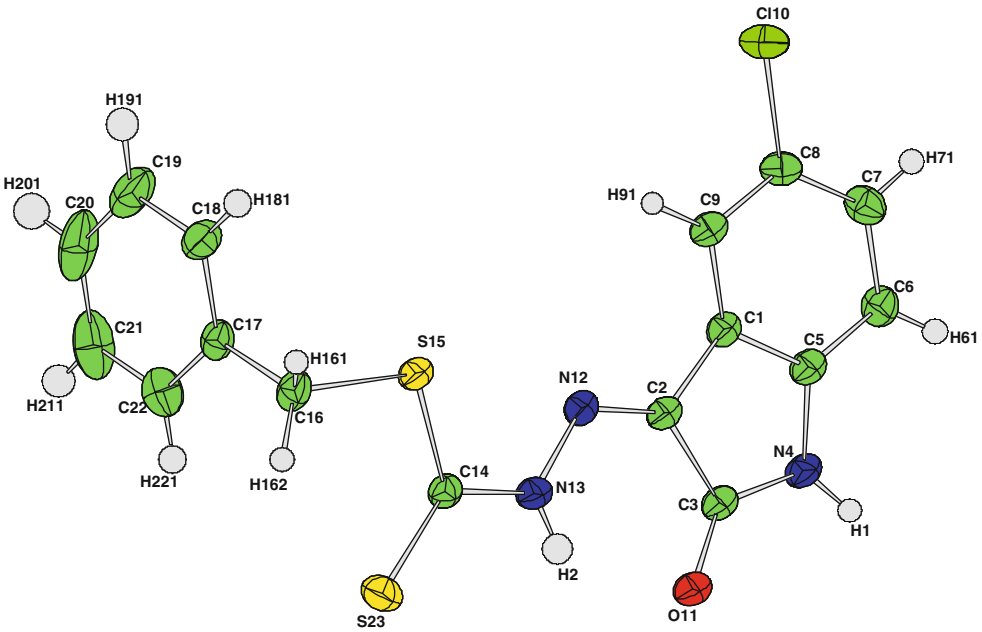


Fig. 2 ORTEP diagram and numbering scheme of SB5FISA



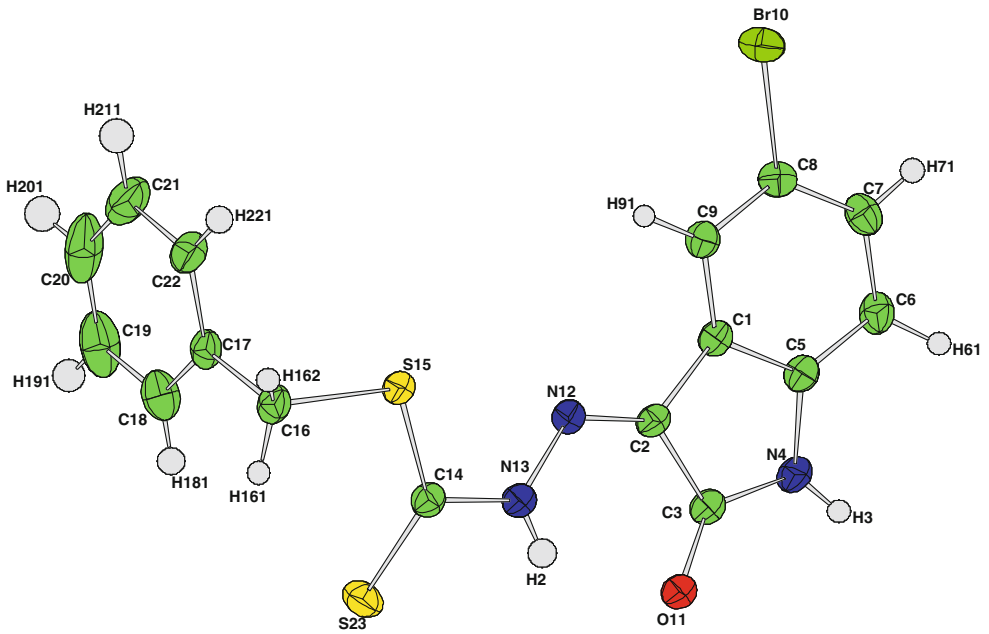


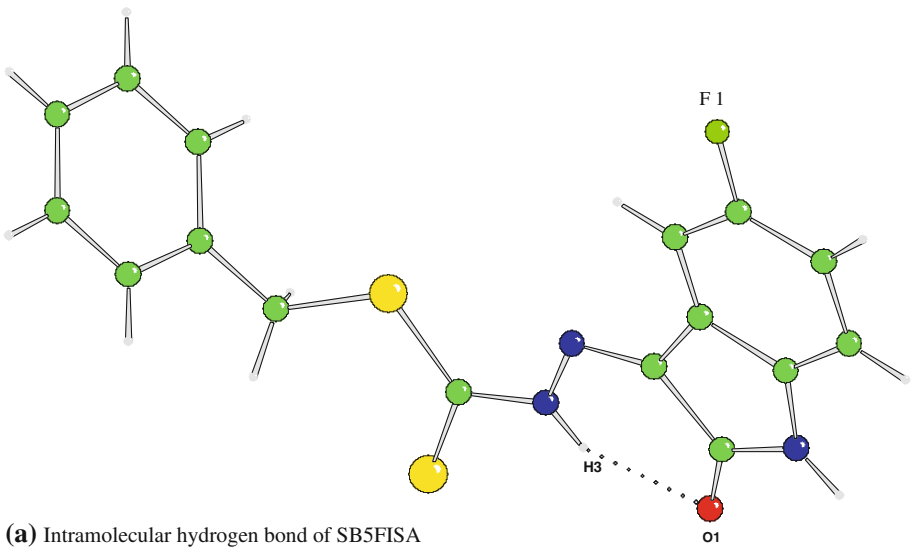
Fig. 3 ORTEP diagram and numbering scheme of SB5ClISA

Fig. 4 ORTEP diagram and numbering scheme of SB5BrISA

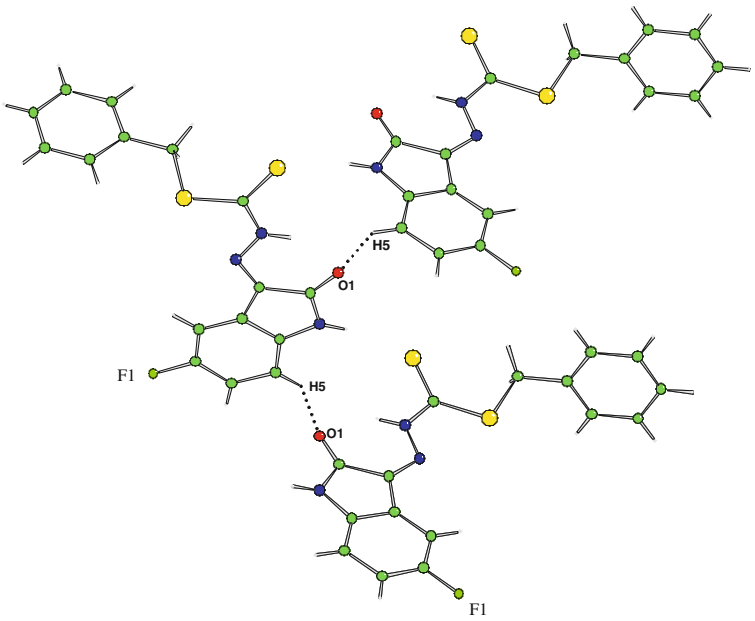
planarity of the molecules was stabilized by a strong intramolecular hydrogen bond N3–H3···O1 = 2.690(9) Å (Table 5) leading to the formation of the pseudo six-membered ring. The benzyl ring C10–C11–C12–C13–C14–C15–C16 is nearly perpendicular to the methylene-dithiocarbazate plane consisting of atoms N2–N3–C9–S1–S2 with the dihedral angle between the two mean planes of 83.12°. The isatin moiety is *trans* to the C9–N3 and C9–S1 bonds while the benzyl group is *cis* to the C9–N3 and C9–S2 bonds respectively.

The N2–N3–C9–S1 and N3–C9–S2–C10 chains adopt *trans* conformation with the torsion angles of 174.58° and −176.82° respectively whereas the S1–C9–S2–C10 chain adop *cis* conformation with the torsion angle of 2.70°. The S-benzyldithiocarbazate moiety is in the *Z* configuration with respect to C2=N2 double bond (torsion angle C2–C3–N2–N3 = 1.18°), as a consequence of formation of the intramolecular hydrogen bond N3–H3···O1 = 2.690(9) Å (Table 5) and intermolecular hydrogen bond C5–H5···O1 = 3.209(9) Å (Table 6).

Fig. 5 Intramolecular and intermolecular hydrogen bonds of SB5FISA



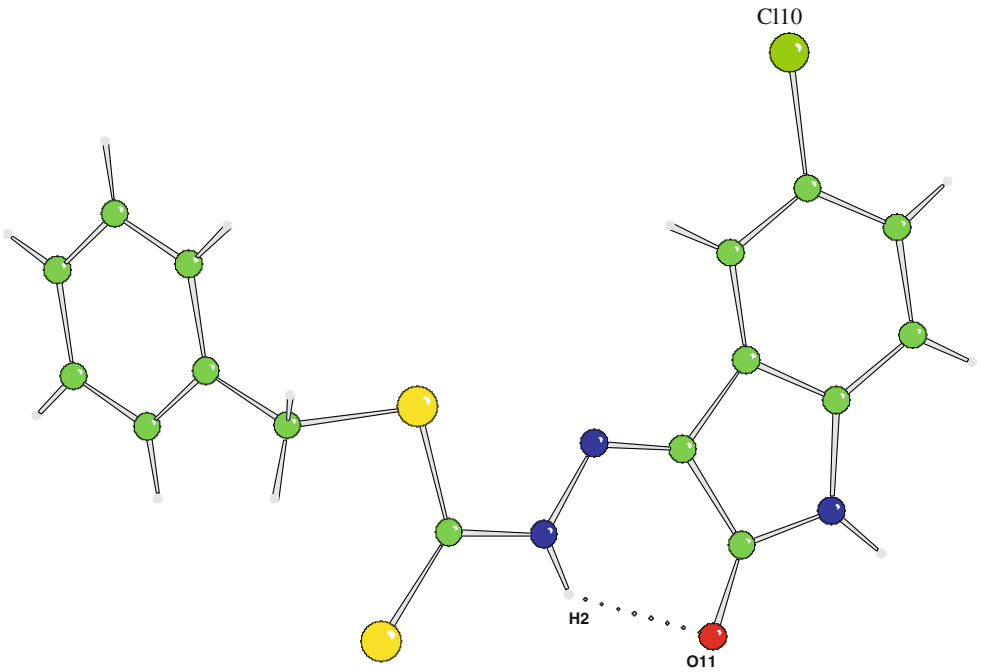
(a) Intramolecular hydrogen bond of SB5FISA



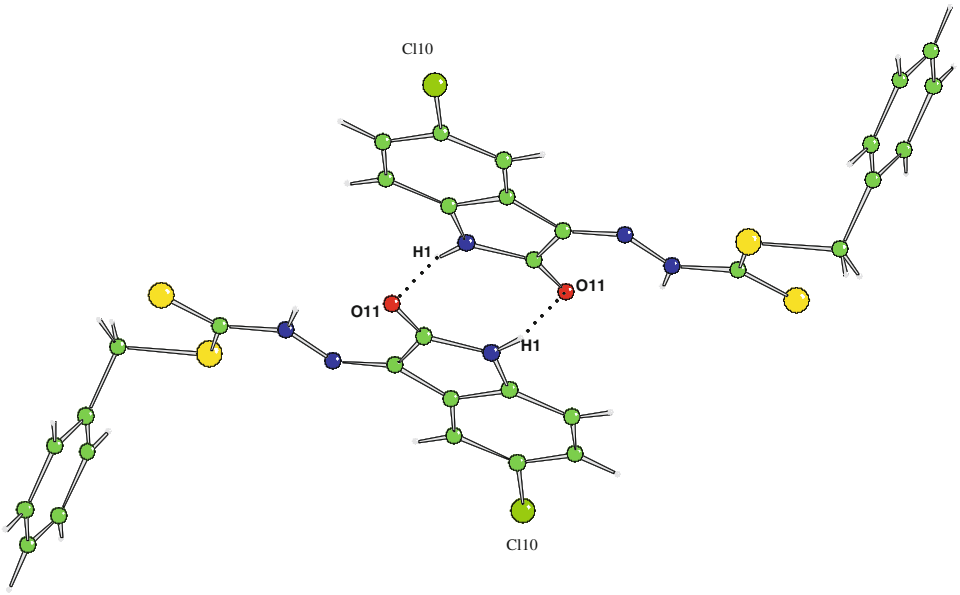
(b) Intermolecular hydrogen bond of SB5FISA

In SB5ClISA (Fig. 3) and SB5BrISA (Fig. 4), the six- and five-membered rings of the isatin moieties which consist of C1–C5–C6–C7–C8–C9 and C1–C2–C3–N4–C5 are not exactly planar with the dihedral angle of 1.50° and 1.70° respectively. The benzyl ring of SB5ClISA and SB5BrISA, C16–C17–C18–C19–C20–C21–C22 are nearly perpendicular to the methylene-dithiocarbazate plane consisting of atoms N12–N13–C14–S23–S15 with the dihedral angle between the two mean planes of 72.84° and 74.79° respectively. Both of the isatin moieties in SB5ClISA and SB5BrISA are *trans* to the C14–N13 and C14–S23 bonds while the benzyl groups are *cis* to the C14–N13 and C14–S15 bonds respectively. A similar configuration also was reported for SBDTC [34, 35].

In these compounds, the N12–N13–C14–S23 and N13–C14–S15–C16 chains adopt *trans* conformation with the torsion angles of (173.51° and 177.53° for SB5ClISA) and (172.81° and 176.91° for SB5BrISA) respectively. The S-23-C14-S15-C16 chains adop *cis* conformation with the torsion angle of −3.80° and −5.18° for SB5ClISA and SB5BrISA respectively. Both of the S-benzyldithiocarbazate moieties are in the *Z* configuration with respect to C2=N12 double bond (torsion angle C2–C3–N12–N13 = −1.57° for SB5ClISA and −3.04° for SB5BrISA),



(a) Intramolecular hydrogen bond of SB5ClISA



(b) Intermolecular hydrogen bond of SB5ClISA

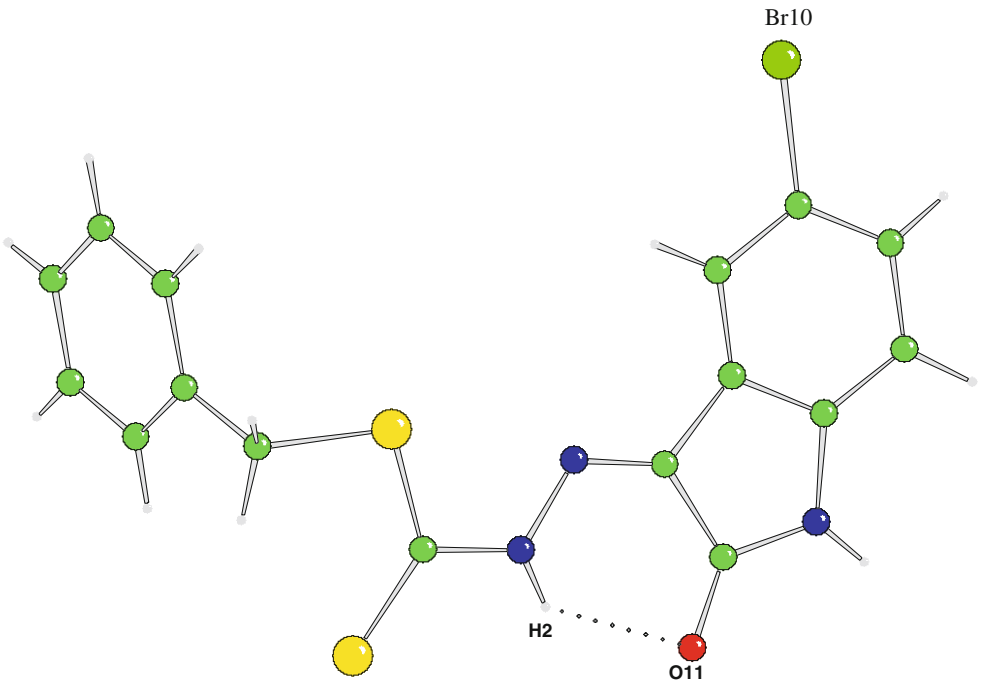
Fig. 6 Intramolecular and intermolecular hydrogen bonds of SB5ClISA

as a consequence of formation of the intramolecular hydrogen bonds N13–H2···O11 = 2.771(2) Å for SB5ClISA and 2.772(2) Å for SB5BrISA (Table 5), and intermolecular hydrogen bonds N4–H1···O11 = 2.825(2) Å for SB5ClISA and N4–H3···O11 = 2.826(2) Å for SB5BrISA (Table 6) respectively. Comparisons of the torsional angles of the Schiff bases are presented in Table 7.

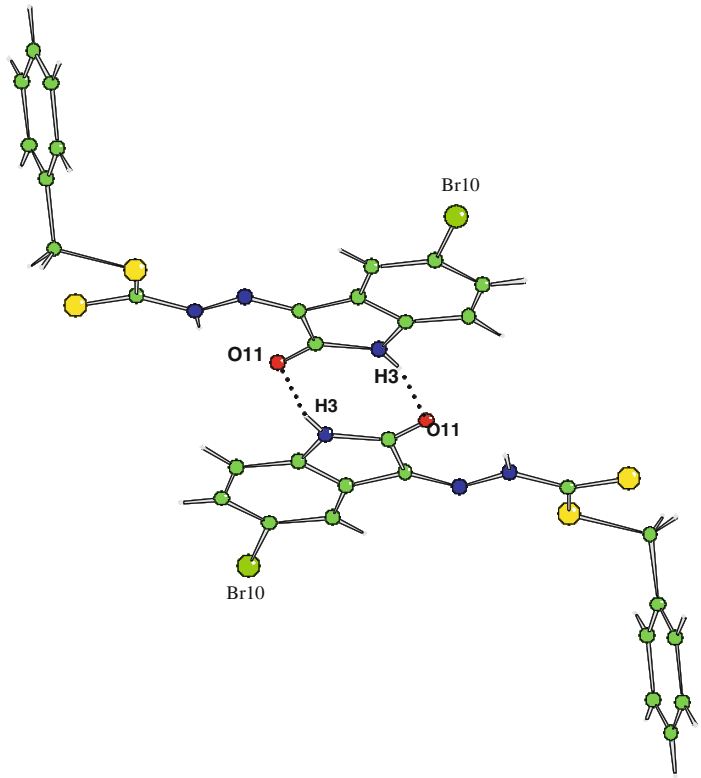
The C–Br bond length of 1.894 Å is slightly longer compared to C–Cl (1.744 Å) and C–F (1.374 Å) (Table 4). The bond length increased in the following order C–Br > C–Cl > C–F in order electronegativity as expected.

The C–S (Table 8) bond lengths in all the Schiff bases are intermediate between 1.82 Å for C–S single bond and 1.56 Å for C=S double bond [34] and are in agreement with the reported value in SBDTC [35]. Thus, the compound exists in the thioketo tautomeric form. Due to delocalization of electrons in the isatin ring, the N–N (Table 8) bond distances are slightly shorter than the corresponding bond length in unsubstituted SBDTC (1.406(3) Å) [35].

Fig. 7 Intramolecular and intermolecular hydrogen bonds of SB5BrISA



(a) Intramolecular hydrogen bond of SB5BrISA



(b) Intermolecular hydrogen bond of SB5BrISA

Table 9 shows the comparison of the dihedral angles between two selected planes. The isatin ring in SB5FISA is almost nearly planar with dihedral angles of 0.78° followed by SB5ClISA (1.50°) and SB5BrISA (1.70°). The planarity of the isatin ring in the Schiff bases can be arranged in the following order SB5FISA > SB5ClISA > SB5BrISA. The benzyl ring of SB5FISA is almost perpendicular to the dithiocarbazate plane with the dihedral angle of 83.12°

Table 4 Selected bond lengths (Å) and bond angles (°) for SB5FISA, SB5ClISA and SB5BrISA

	SB5FISA		SB5ClISA	SB5BrISA
Bond length				
C(10)–S(2)	1.828(6)	C(16)–S(15)	1.802(17)	1.824(2)
C(9)–S(2)	1.734(6)	C(14)–S(15)	1.748(17)	1.748(2)
C(9)–S(1)	1.676(6)	C(14)–S(23)	1.644(17)	1.649(2)
C(3)–O(1)	1.223(8)	C(3)–O(11)	1.234(2)	1.239(3)
C(2)–N(2)	1.295(7)	C(2)–N(12)	1.290(2)	1.290(3)
N(2)–N(3)	1.374(7)	N(12)–N(13)	1.349(2)	1.353(3)
C(9)–N(3)	1.347(7)	C(14)–N(13)	1.366(2)	1.365(3)
C(7)–F(1)	1.374(7)	C(8)–Cl(10)	1.744(17)	–
		C(8)–Br(10)	–	1.894(2)
Bond angles				
C(9)–S(2)–C(10)	102.2(3)	C(16)–S(15)–C(14)	102.86(8)	103.20(11)
C(9)–N(3)–N(2)	121.9(4)	C(14)–N(13)–N(12)	119.43(14)	119.40(18)
N(3)–C(9)–S(1)	118.4(4)	N(13)–C(14)–S(23)	119.62(13)	119.54(17)
N(3)–C(9)–S(2)	115.3(4)	N(13)–C(14)–S(15)	111.92(12)	112.06(16)
S(1)–C(9)–S(2)	126.3(3)	S(23)–C(14)–S(15)	128.45(10)	128.37(14)
C(3)–N(1)–C(4)	110.8(4)	C(3)–N(4)–C(5)	111.20(13)	111.18(18)
C(2)–C(3)–N(1)	105.2(5)	C(2)–C(3)–N(4)	106.31(14)	106.46(19)
N(1)–C(3)–O(1)	126.6(5)	N(4)–C(3)–O(11)	127.96(15)	127.20(2)
C(2)–C(3)–O(1)	128.1(5)	C(2)–C(3)–O(11)	125.73(15)	126.30(2)

Table 5 Intramolecular hydrogen bonds (Å, °)

Compound	D–H···A	D–H	H···A	D···A	D–H···A
SB5FISA	N3–H3···O1	0.85	2.00	2.690(9)	138
SB5ClISA	N13–H2···O11	0.86	2.16	2.771(2)	129
SB5BrISA	N13–H2···O11	0.85	2.12	2.772(2)	133

D donor, *A* acceptor, *H* hydrogen

Table 6 Intermolecular hydrogen bonds (Å, °)

Compound	D–H···A	D–H	H···A	D···A	D–H···A
SB5FISA	C5–H5···O1	0.92	2.46	3.209(9)	138
SB5ClISA	N4–H1···O11	0.89	1.95	2.825(2)	166
SB5BrISA	N4–H3···O11	0.87	1.98	2.826(2)	166

D donor, *A* acceptor, *H* hydrogen

Table 7 Comparison of the torsional angles (°) of the Schiff bases

Plane	SB5FISA	SB5ClISA	SB5BrISA
Torsional angles			
N2–N3–C9–S1	174.58	–	–
N3–C9–S2–C10	−176.82	–	–
S1–C9–S2–C10	2.70	–	–
C2–C3–N2–N3	1.18		
N12–N13–C14–S23		173.51	172.81
N13–C14–S–15–C16		177.53	176.91
S23–C14–S15–C16		−3.80	−5.80
C2–C3–N12–N13		−1.57	−3.04

Table 8 C–S and N–N bond lengths (Å) for SB5FISA, SB5ClISA and SB5BrISA

	SB5FISA	SB5ClISA	SB5BrISA
C–S Bond length			
C(9)–S(1)	1.676(6)	–	–
C(14)–S(23)	–	1.644(17)	1.649(2)
N–N Bond length			
N(2)–N(3)	1.374(7)	–	–
N(12)–N(13)	–	1.349(2)	1.353(3)

Table 9 Comparison of the dihedral angles (°) between two selected planes

Plane	SB5FISA	SB5ClISA	SB5BrISA
Dihedral angles			
C1–C8–C7–C6–C5–C4 and C2–C1–C4–N1–C3 (isatin ring)	0.78	–	–
C10–C11–C12–C13–C14–C15–C16 (benzyl ring) and N2–N3–C9–S1–S2 (dithiocarbazate)	83.12	–	–
C1–C5–C6–C7–C8–C9 and C1–C2–C3–N4–C5 (isatin ring)	–	1.50	1.70
C16–C17–C18–C19–C20–C21–C22 (benzyl ring) and N12–N13–C14–S23–S15 (dithiocarbazate)	–	72.84	74.79

Table 10 Cytotoxicity data (μg/mL)

Sample	MW	MCF-7	IC_{50} (μM)	MDA-MB-231	IC_{50} (μM)
SB5FISA	345.42	3.2	9.26	Inact	Inact
SB5ClISA	361.88	14.0	38.69	Inact	Inact
SB5BrISA	406.33	2.6	6.40	Inact	Inact
Tamoxifen	371.51	5.0	13.46	8.0	21.53

Values are shown as IC_{50} (concentration required to inhibit a 50% of the cell growth) in μg/mL. *Inact* inactive, $IC_{50} < 5.0$ μg/mL (Strongly active), $IC_{50} = 5.0$–10.0 μg/mL (moderately active), $IC_{50} = 10$–25.0 μg/mL (weakly active), $IC_{50} > 25.0$ μg/mL (inactive), *MCF-7* Human non-metastatic mammary gland adenocarcinoma cell line, *MDA-MB-231* Human metastatic mammary gland adenocarcinoma cell line

followed by SB5BrISA (74.79°) and SB5ClISA (72.84°). The perpendicularity of the benzyl ring towards the dithiocarbazate plane can be ordered as follows, SB5FISA > SB5BrISA > SB5ClISA.

Cytotoxic Activity

The Schiff bases were evaluated in vitro using MDA-MB-231 (Human metastatic mammary gland adenocarcinoma cell line) and MCF-7 Human non-metastatic mammary gland adenocarcinoma cell line. Measurement for cytotoxicity was in IC_{50}, where IC_{50} corresponds to the concentration required to inhibit a 50% of the cell growth [36]. The IC_{50} of the Schiff bases are shown in Table 10. The Schiff bases were found to be selectively active towards the MCF-7 cell lines with the IC_{50} values of 6.40 μM, for SB5BrISA, 9.26 μM for SB5FISA and 38.69 μM for SB5ClISA. The cytotoxic activity of the halo substituted isatins against the MCF-7 breast cancer cell lines tested is in the order of Br > F > Cl.

Supplementary Material

CCDC 696006, 696007 and 696008 contain the supplementary crystallography data for SB5FISA, SB5BrISA and SB5ClISA Schiff bases. This data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or email:deposit@ccdc.cam.ac.uk.

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