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Synthesis and Antifungal Activity of Halogen-Substituted 2,1-Benzothiazine-2,2-dioxide Derivatives

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A convenient synthesis of a series of new halogen-substituted 2,1-benzothiazine-2,2-dioxide derivatives has been described. The starting compound un/substituted methyl anthranilate is converted into 2,1-benzothiazine-2,2-dioxide heterocyclic ring system *via* sulfonamide formation, N-alkylation, cyclization and subsequent dihalogenation. The target halogen-substituted molecules are expected to serve as prodrugs. Structural elucidation was achieved by FT-IR, NMR, MS and single crystal X-ray diffraction analyses. Crystal structure data for compound **5f** has been reported here. The 3,3-dichloro- and dibromo-derivatives (**5b** and **5c**) have shown promising antifungal activities against *Acremonium chrysogenum*, *Aspergillus niger* and *Penicillium notatum*.

Key Words: 2,1-Benzothiazine-2,2-dioxide, Dihalogenation, Antifungal activity, Crystal structure.

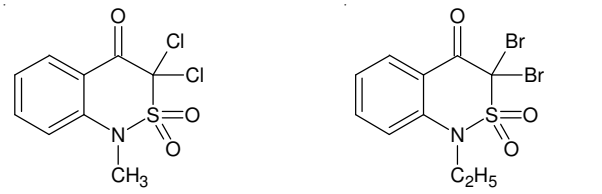
INTRODUCTION

The synthesis of heterocyclic ring systems is of continuing interest, as a large number of biologically active molecules are heterocyclic in nature[1]. In particular, 2,1-benzothiazine-2,2-dioxide derivatives have been reported to possess potent biological activities such as lipoxygenase inhibition, antituberculotic agent's precursors and as drugs for heart diseases[2,3]. The synthesis of a variety of 2,1-benzothiazines has recently been reported in literature for their applications in the preparation of medicinally important natural products, new chiral ligands for catalysis and molecular recognition[4,5].

The main focus of present research program is to synthesize antifungal, antibacterial and antimicrobial agents with better biological potential against the most resistant strains. In this reference we have already reported a few derivatives of this type of compounds[6]. Herein, we explore the antifungal potential of certain dichloro and dibromo-substituted 2,1-benzothiazine-2,2-dioxide derivatives with and without a bromo- moiety on the adjacent benzene ring in the main benzothiazine nuclei. The 3,3-dichloro-1-methyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (**5b**) and 3,3-dibromo-1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (**5c**) derivatives have shown excellent antifungal activities.

EXPERIMENTAL

Melting points were recorded on an electrothermal (Griffin 1090) apparatus and are reported as uncorrect. Analytical



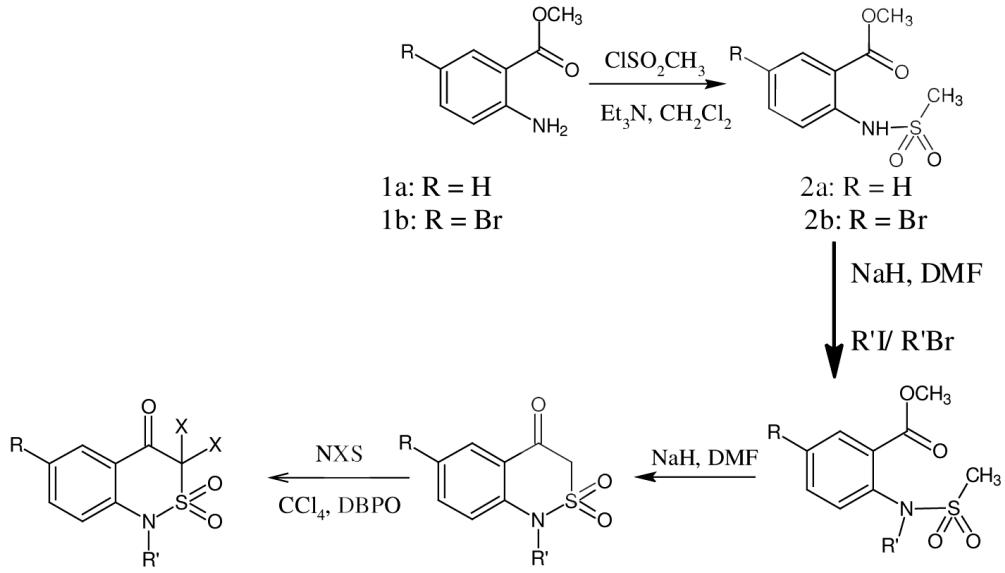
5b **5c**

Fig. 1. Chemical structures of **5b** and **5c**

thin-layer chromatography (TLC) was performed on commercial Merck plates coated with silica gel GF_{254} (0.25 mm thick). ^1H NMR (300 MHz) measurements were performed on a Bruker DPX-400 instrument. Chemical shifts are reported as δ in ppm using tetramethylsilane (TMS) as internal reference standard. IR spectra were recorded on a Perkin Elmer 1600-FT spectrometer. Mass spectra were taken on Agilent MSD 5890 instrument. All reagents and chemicals were commercially available and are used as such without further purification. Drying of the solvents by standard methods was employed where necessary.

Sulfonamide synthesis (general procedure): Precursor molecules **4a**, **4b**, **4c**, **4d** and **4e** are reported in literature[7] and are prepared as shown in **Scheme-I**.

A solution of methanesulfonyl chloride (4.8 mmol) in dichloromethane (10 mL) was added to **1a-1b** (4.0 mmol) over 15-20 min at room temperature. The reaction mixture was stirred for 3.5 h (room temperature) to 22 h (60-70 ºC) for **1a**



5a: R = H; R'= Me; X = Br **5b:** R = H; R'= Me; X = Cl
5c: R = H; R'= Et; X = Br **5d:** R = H; R'= Et; X = Cl
5e: R = H; R'= Pr; X = Br **5f:** R = H; R'= Pr; X = Cl
5g: R = H; R'= Bz; X = Br **5h:** R = H; R'= Bz; X = Cl
5i: R = Br; R'= Me; X = Br **5j:** R = Br; R'= Me; X = Cl

4a: R = H; R' = Me **4b:** R = H; R' = Et
4c: R = Br; R' = Pr **4d:** R = H; R' = Bz
4e: R = Br; R' = Me

3a: R = H; R' = Me **3b:** R = H; R'= Et
3c: R = H; R' = Pr **3d:** R = H; R'= Bz
3e: R = Br; R' = Me

Me = Methyl, Et = Ethyl, Pr = Propyl Bz = Benzyl

Scheme-I

and **1b**, respectively keeping the pH of the mixture alkaline with triethylamine. After completion, the reaction mixture was neutralized with dilute hydrochloric acid. The separated organic layer was dried ($MgSO_4$) and removed under vacuum (11 torr) to get yellow crude solids which gave white crystalline products **2a** and **2b** after recrystallization from ethanol.

Methyl-5-bromo-2-[(methylsulfonyl)amino]benzoate (2b): White crystalline solid. Yield 82.0 %, m.p. 92-93 ºC. ^{1}H NMR (MeOD) δ: 5.2 (1H, s, NH), 3.4 (3H, s, OCH_3), 7.7-7.9 (2H, m, Ar), 8.1 (1H, s, Ar). IR (KBr, ν_{max}, cm^{-1}): 3250 (NH), 1725 st. (CO), 1230 def (C-O), 1157, 1388 (SO_2), 2975 (CH, Ar). MS m/z (%): 309 (56.3), 307 (53.48), (M + 1, isotopic peaks), 275, 277(100), 228, 230 (12.15), 198 (31.26), 170 (27.48).

N-Alkylation (general procedure): 2a-2b (33 mmol) was dissolved in DMF (10 mL) and added to *n*-hexane washed suspension of NaH (66 mmol) in DMF (10 mL). After stirring the suspension for 45 min, alkyl iodide/bromide was added (66 mmol) and the resulting mixture was stirred at room temperature for 1.5 h for **3a**, **3b**, **3c** and **3d** while for **3e** it was stirred for 14-16 h at room temperature. The mixture was poured into cold 3 N hydrochloric acid and pH was neutralized with sodium carbonate. The resultant mixture was extracted with ethyl acetate and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to get compounds **3a-3e**[8].

Cyclization (general procedure): A suspension of *n*-hexane washed sodium hydride (96 mmol) was prepared in dry dimethyl formamide (30 mL). To this suspension, a solution of **3a-3e** (48 mmol) in dry dimethyl formamide (70 mL) was added. The resulting reaction mixture was stirred at room temperature for 1.5 h for **3a**, **3b**, **3c** and **3d** while 3-4 h for **3e** and after completion, the reaction mixture was poured into cold 3N hydrochloric acid and pH was neutralized with sodium carbonate to get the precipitates which were dried at room temperature to get **4a-4e**[9].

Dihalogenation (general procedure): A mixture of the respective 2,1-benzothiazine-2,2-dioxide molecule (0.862 mmol), N-bromo/chloro succinimide (1.724 mmol) and dibenzoylperoxide (0.0495 mmol) in carbon tetrachloride (10 mL), was heated under reflux (80 ºC) for 1.0-1.5 h with the result of complete conversion of reactant molecule. Carbon tetrachloride solvent was removed under vacuum (11 torr) to get crude solid product which was washed with slightly warm water to remove the *in situ* formed succinimide and the resultant residue was re-crystallized in ethanol or ethanol:ethyl acetate mixture.

3,3-Dibromo-1-methyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5a): Yellow needle like crystals. Yield 74 %, m.p. 181-182 ºC. ^{1}H NMR (MeOD) δ: 8.1-8.2 (1H, m, Ar), 7.7-7.8 (1H, m, Ar), 7.3-7.5 (2H, m, Ar), 3.7 (3H, s, CH_3). IR (KBr, ν_{max}, cm^{-1}): 3045 (CH of CH_3), 1690 (CO), 2975 (CH

aromatic), 1160, 1380 (SO_2). MS (%) m/z: 370 (32.38), 369 (53.33), 368 (27.61), (M + 1, isotopic peaks), 289 (41.91), 291 (40.95), 223.8 (82.38), 226 (80), 211 (41.71), 77 (100).

3,3-Dichloro-1-methyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (5b): White crystalline powder. Yield 72 %, m.p. 166-168 ºC. ^{1}H NMR (MeOD) δ: 6.5-7.6 (m, 4H, Ar), 2.78 (s, 3H, CH_3). IR (KBr, ν_{max}, cm^{-1}): 3050 (C-H, Ar), 2935 (C-H, CH_3), 1703 (C=O). MS m/z (%): 281 (52), 279 (78) (M + 1, isotopic), 182 (22), 180 (100), 152 (64), 77 (20).

3,3-Dibromo-1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (5c): Red coloured prismatic crystals. Yield 69 %, m.p. 120-121 ºC. Crystal structure has been published[6].

3,3-Dichloro-1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5d): White prismatic crystals. Yield 68 %, m.p. 136-138 ºC. Crystal strructure has been reported[6].

3,3-Dibromo-1-propyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5e): Brown prismatic crystals. Yield 71 %, m.p. 135-138 ºC. ^{1}H NMR (MeOD) δ: 7.3-7.8 (m, 4H, Ar), 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 4.1 (t, 2H, N-CH_2). IR (KBr, ν_{max}, cm^{-1}): 3030 (C-H, Ar), 1695 (C=O), 1165, 1385 (SO_2).

3,3-Dichloro-1-propyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (5f): Needle-like light yellow crystals. Yield 68 %, m.p. 104-106 ºC. Crystal structure data is given in the crystallographic part of this paper.

3,3-Dibromo-1-benzyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (5g): Light brown solid, yield 65 %, m.p. 128-130 ºC. ^{1}H NMR ($CDCl_3$) δ: 6.51-7.10 (m, 4H, Ar), 7.12-7.30 (m, 5H, Ar), 3.69 (s, 2H, CH_2). IR (KBr, ν_{max}, cm^{-1}): 3060 (C-H, Ar), 2930 (C-H, CH_2), 1695 (C=O), 1165, 1385 (SO_2). MS m/z (%): 447 (17), 445 (30) (M + 1, isotopic), 285 (61), 194 (100), 76 (49).

3,3-Dichloro-1-benzyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5h): White solid, yield 67 %, m.p. 118-120 ºC. ^{1}H NMR ($CDCl_3$) δ: 7.26-7.30 (m, 5H, Ar), 6.91-7.15 (m, 4H, Ar), 3.75 (s, 2H, CH_2). IR (KBr, ν_{max}, cm^{-1}): 3050 (C-H, Ar), 2930 (C-H, CH_2), 1703 (C=O), 1170, 1390 (SO_2). MS m/z (%): 357 (53), 355 (65) (M + 1, isotopic), 284 (43), 193 (74), 77 (100).

3,3,6-Tribromo-1-methyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5i): Light-yellow needle like crystals. Yield 77 %, m.p. 149-150 ºC. ^{1}H NMR (MeOD) δ: 7.4-7.9 (m, 4H, Ar), 3.7 (s, 3H, NCH_3). IR (KBr, ν_{max}, cm^{-1}): 3025 (C-H, Ar), 1710 (C=O), 1160, 1390 (SO_2).

6-Bromo-3,3-dichloro-1-methyl-1*H*-2,1-benzothiazin-4(3*H*)-one-2,2-dioxide (5j): Yield 74 %, m.p. 118-120 ºC. Crystal structure and reaction procedure has been published[6].

X-Ray structural determination: X-ray data was collected at 296 K on a Bruker KAPPA APEX II diffractometer using MoK_α X-ray (0.71073 Å) source and a graphite monochromator. The unit cell dimensions were obtained from least-squares fit to setting angles of about 28 reflections. Multi-scan absorption corrections were applied. SAINT[10] was used for the cell refinement and data reduction, while, SHELXS-97[11] was used for structure solution and refinement. ORTEP-3 for Windows[12], PLATON[13] and WinGX[14] were used for molecular graphics. In the refinement procedure, all the non-hydrogen atoms were refined with anisotropic displacement parameters. The aromatic and aliphatic hydrogen atoms were positioned geometrically and treated as riding atom over their parent carbon atoms. Crystallographic data and refinement details are given in Table-1.

TABLE-1
CRYSTAL DATA AND STRUCTURE REFINEMENT DETAILS OF **5f**

Empirical formula	$C_{11}H_{11}NO_3SCl_2$
Formula weight	308.17
Temperature (K)	296(2)
Wavelength (Å)	0.71073
Crystal system, space group	Monoclinic, P2(1)/c
Unit cell dimensions	
a (Å)	8.8273(12)
α (º)	90.00
b (Å)	14.520(2)
β (º)	90.930(5)
c (Å)	10.2229(12)
γ (º)	90.00
Volume ($Å^3$)	1310.1(3)
Z, calculated density (g cm^{-3})	4, 1.562
Absorption coefficient (mm^{-1})	0.653
F(000)	632
Crystal size (mm)	0.31 × 0.19 × 0.06
Range for data collection (º)	2.30-28.52
Limiting indices	$-11 \le h \le 11$, $-19 \le k \le 19$, $-12 \le l \le 13$
Reflection collected/unique	14555/3301 [R(int) = 0.0861]
Completeness to θ	99.2 % (28.52)
Absorption correction	Multi-scan
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3301/0/164
Goodness-of-fit on F^2	0.976
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0508$, $wR_2 = 0.0928$
R indices (all data)	$R_1 = 0.1574$, $wR_2 = 0.1231$
Largest diff. peak and hole (e A^{-3})	0.23 and -0.30

Antifungal activity: Some of the dihalo-substituted 2,1-benzothiazine-2,2-dioxide derivatives have been evaluated for their antifungal activity against *Acremonium chrysogenum*, *Aspergillus niger* and *Penicillium notatum* using the Disc Diffusion method. The potato-dextrose agar (PDA) was used as the nutrient culture medium which was prepared by dissolving dehydrated PDA of calculated amount (27.3 g/700 mL water) in sterilized warm distilled water and the pH was adjusted to 3.5 with tartaric acid (10 %) to inhibit the growth of bacterial species and was sterilized by autoclaving at 121 ºC for 20 min.

Cultured *Acremonium chrysogenum*, *Aspergillus niger* and *Penicillium notatum* species were added to the warm PDA medium and it was poured into the petri dishes in laminar flow which were allowed to set in refrigerator for at least 1 h. After solidification, bores were made in the agar media containing fungal strains (four in each petri dish) with the help of an Agar borer. In each bore, the selected compound was added and these plates were then incubated at 30 ºC for 48 h allowing the fungi to grow where possible. Antifungal activity was measured as the average diameter of zone of inhibition in centimeters. Antifungal drug griseofulvin, was used as a standard. Solutions of both, the standard and test compounds were prepared at concentrations of 5 and 10 mg/mL in DMSO solvent. Standard at these concentrations showed no inhibition

activity against all the three fungal strains. Findings of the *in vitro* antifungal activity are summarized in Table-2.

TABLE-2
ANTIFUNGAL ACTIVITIES OF SOME HALOGEN-SUBSTITUTED 2,1-BENZOTHIAZINE-2,2-DIOXIDE DERIVATIVES

Sample No.	*Acremonium chrysogenum*		*Aspergillus niger*		*Penicillium notatum*	
	Zone of inhibition (cm)		Zone of inhibition (cm)		Zone of inhibition (cm)	
	A*	B*	A	B	A	B
(5b)	3.5	4.0	1.3	1.6	2.30	3.0
(5c)	2.0	2.5	1.0	1.7	1.65	2.4
(5i)	1.5	2.0	–	–	1.35	1.4
(5j)	1.8	2.1	1.0	1.2	1.60	1.7

RESULTS AND DISCUSSION

2,1-Benzothiazine-2,2-dioxide precursor molecules with four different N-alkyl moities have been synthesized following literature procedures. For dihalogenation step, the presence of a carbonyl and a sulfamoyl group on either sides of CH_2 group results in a more labile 2,1-benzothiazine-2,2-dioxide nucleus. Hence the hydrogen atom at position three are more susceptible to free radical reactions. This facilitates the formation of dihalo target molecules instead of mono halo-substituted derivatives, in a single convenient step with good yields wherein dibenzoyl peroxide has served as an initiator. Mainly X-ray crystallographic analysis has been employed to identify the presence of two halogen atoms in the final products. Further, fragmentation pattern in mass spectra are characteristic of the presence of two halogen atoms with different relative abundance data for the bromo and chloro groups. Spectroscopic techniques like FT-IR, ^{1}H NMR were also observed to identify various intermediates and final products.

In order to observe geometrical behaviour of the molecules we crystallized a compound **5f** and selected a suitable crystal for crystallographic studies. The crystal structure shows that the subjected compound has a planar benzene ring which is fused with an envelope shaped thiazine nucleus (Fig. 2). The nitrogen atom is alkylated with a propyl group. No suitable hydrogen bonding interaction has been observed in the molecule. The dihedral angles between the phenyl and thiazine ring mean planes is 10.15 (19)º.

The 2,1-benzothiazine-2,2-dioxide derivatives with various N-substituted alkyl groups have shown no antifungal activity against all the three above mentioned fungal strains. The trihalo substituted benzothiazine derivatives (**5i** and **5j**) have shown some good antifungal results. The most promising results were obtained in case of 3,3-dihalo-substituted benzothiazines where the dichloro substituted derivative (**5b**) has shown best antifungal action against *A. chrysogenum*, better against *P. notatum* and reasonably mediocre inhibitory action against *A. niger*. This reflects the contribution of halogen atoms towards antifungal action. The overall results reveal that presence of halogen atoms inducts good degree of antifungal activity into the previously non-antifungal 2,1-benzothiazine nuclei. Further, the data explored that position three on the thiazine ring is more susceptible for antifungal action than position 5 on the benzene ring as presence of Br on position 5 of the benzene ring has shown no enhanced antifungal effect (**5i** and **5j** in comparison to **5b** and **5c**). Moreover, the 3,3-dichloro (**5b**) derivative has been observed to be more potent fungicide than the corresponding 3,3-dibromo benzothiazine (**5c**).

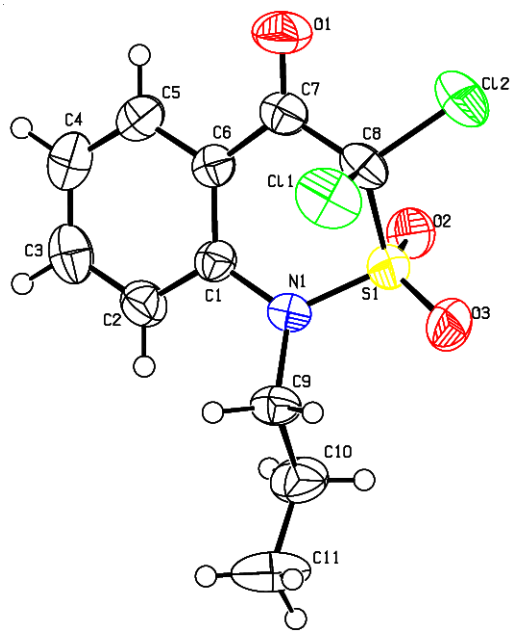


Fig. 2. ORTEP diagram for **5f** with the 50 % probability level of the thermal ellipsoids

Conclusion

Synthesis of 3,3-dichloro and 3,3-dibromo-2,1-benzothiazine-2,2-dioxide derivatives has been reported in a straight forward four step strategy with over all good yields of the target dihalo-substituted molecules. Role of the halogen atoms onto the benzothiazine ring system has been elabotated with respect to its fungicidal activity against the three famous fungal strains namely *A. chrysogenum*, *A. niger* and *P. notatum*. Induction of halogen atoms at position 3 has developed antifungal activity in the target dihalo 2,1-benzothiazine-2,2-dioxide derivatives which had never been reported antifungal for this characteristic ring system in literature previously.

Supplementary material: Single crystal X-ray data for **5f** has been deposited with CCDC (deposition number 745879).

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REFERENCES

1. R.G. Franzen, *J. Comb.*, **2**, 195 (2000).
2. Y. Misu and H. Togo, *Org. Biomol. Chem.*, **1**, 1342 (2003).
3. M. Harmata, X. Hong and C. L. Barnes, *Org. Lett.*, **6**, 2201 (2004).
4. M. Harmata and X. Hong, *Org. Lett.*, **7**, 3581 (2005).
5. M. Harmata, N.L. Calkins, R.G. Baughman and C.L. Barnes, *J. Org. Chem.*, **71**, 3650 (2006).

6. (a) M. Shafiq, M.N. Tahir, I.U. Khan, S. Ahmad, W.A. Siddiqui, *Acta Cryst. E*, **64**, o1270 (2008); (b) M. Shafiq, M.N. Tahir, I.U. Khan, S. Ahmad and M.N. Arshad, *Acta Cryst. E*, **65**, o430 (2009); (c) M. Shafiq, M.N. Tahir, I.U. Khan, M.N. Arshad and Z. Haider, *Acta Cryst. E*, **65**, o1413 (2009).
7. (a) J.G. Lombardino, *J. Heterocycl. Chem*., 315 (1971); (b) Y. Volovenko, T. Volovnenko and K. Popov, *J. Heterocycl. Chem*., **44**, 1413 (2007); (c) B. Loev and K.M. Sander, *J. Heterocycl. Chem*., **4**, 403 (1967); (d) J.G. Lombardino and N.W. Treadway Jr., *Org. Preparat. Proced. Int*., **3**, 33 (1971).
8. (a) M. Shafiq, M.N. Tahir, I.U. Khan, R. Kia, M.N. Arshad and M. Aslam, *Acta Cryst. E*, **65**, o2588 (2009); (b) M. Shafiq, M.N. Tahir, I.U. Khan, W.A. Siddiqui, M.N. Arshad, *Acta Cryst. E*, **64**, o389 (2008); (c) M. Shafiq, M.N. Tahir, I.U. Khan, M.N. Arshad and Z. Haider, *Acta Cryst. E*, **66**, o248 (2010); (d) M. Shafiq, M.N. Tahir, I.U. Khan, M.N. Arshad and M.H. Khan, *Acta Cryst. E*, **65**, o955 (2009).
9. (a) M.N. Tahir, M. Shafiq, I.U. Khan, W.A. Siddiqui and M.N. Arshad, *Acta Cryst. E*, **64**, o557 (2008); (b) M. Shafiq, I.U. Khan, M.N. Tahir and W.A. Siddiqui, *Acta Cryst. E*, **64**, o558 (2008); (c) M. Shafiq, M.N. Tahir, I.U. Khan, M.N. Arshad and M.N. Asghar, *Acta Cryst. E*, **65**, o1182 (2009).
10. Bruker SADABS and Bruker AXS Inc., Madison, Wisconsin, USA (2007).
11. G.M. Sheldrick, *Acta Cryst. A*, **64**, 112 (2008).
12. L.J. Farrugia, *J. Appl. Cryst*., **30**, 565 (1997).
13. A.L. Spek, *Acta Cryst. D*, **65**, 148 (2009).
14. L.J. Farrugia, *J. Appl. Cryst*., **30**, 565 (1999).