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Me_3N-promoted synthesis of 2,3,4,4a-tetrahydroxanthen-1-one: Preparation of thiosemicarbazone derivatives, their solid state self-assembly and antimicrobial properties

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Abstract:

A new strategy has been used to synthesize 2,3,4,4a-tetrahydroxanthen-1-one (**3**) in high yield. Using Me_3N-promoted domino Baylis-Hillman/oxa-Michael reaction sequence at room temperature, the reaction of salisaldehyde (**1**) with cyclohexanone (**2**) provided **3** in 88% yield, which further undergo reaction with thiosemicarbazides (**4**) to afford a series of new xanthene-based thiosemicarbazones (**5a-5j**). All the synthesized compounds were characterized by their physical and spectral data. The solid state self-assembly studies for **5d** and **5i** were carried out by single crystal X-ray technique to investigate the prevalence of the thioamide dimer synthon and its role in molecular alignment in the solid state. Furthermore, the compound (**5a-5j**) were tested for their antibacterial activity. The compounds **5b**, **5c**, **5d** and **5i** showed excellent antibacterial activity against one or more of the tested bacterial strains. Notably, the antibacterial activity for the compound **5c** was found comparable to standard reference drug, ciprofloxacin against *Salmonella typhi*.

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1. Introduction

Xanthene scaffold is very important due to its presence in a number of natural products.[1] Apart from this, the synthetic organic molecules containing xanthene scaffold have attracted considerable attention in recent years due to their wide range of applications in medicinal and material chemistry.[2-9] In particular, (9S)-9-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)-3,3-dimethyl-2,3,4,9-tetrahydro-1H-xanthen-1-one has been found as selective and orally active neuropeptide Y Y5 receptor antagonist.[10] Some of these compounds showed anti-cancer activity,[11-13] while others have been found effective against fungal diseases of Tomato.[14] Such molecules have also been found as very efficient laser dyes and demonstrated outstanding photophysical properties as is evidenced by the presence of reviews on their aggregation phenomena,[15] their photochemistry,[16] and triplet absorption spectra.[17] Furthermore, they have been reported as pH sensitive fluorescent materials for the visualization of biomolecules,[18] charge control agents in electrophotographic toners (laser printers and photocopiers),[19, 20] and as markers or biological stains.[21] Rhodamines and rosamines, particularly, have been used in inks for ink-jet printers.[22]

Thiosemicarbazones or *N*-imino thioureas (a condensation product of aldehydes/ketones and thiosemicarbazides) in general, constitute an important class of compounds.[23-28] These are versatile molecules not only due to their broad profile in the medicinal chemistry but also due to their unique structural features.[29, 30] They are known to have antimicrobial, anticonvulsant, antiviral, antihypertensive, antioxidant, anti-inflammatory, hypoglycemic, antitubercular, cytotoxic, anticancer and enzymatic inhibition activities.[23-28] From the structural point of view, they are conformationally freezed due to intramolecular hydrogen bonding and adopt preferably S-*cis*/S-*trans* conformation as compared to their thiourea analogs which can adopt both the S-

cis/S-*trans* and S-*trans*/S-*trans* conformations depending on the substituents. Due to these structural features, they are more attractive than their thiourea analogs and have potential applications in the field of crystal engineering.[29, 30]

Keeping in view the tremendous importance of xanthene derivatives, and our recent interest in the applications of biologically important hetrocycles[31-38] and solid state structural features of thiosemicarbazones,[29, 30] we thought it worthwhile to synthesize some new xanthene-based thiosemicarbazones to see the effect of xanthene pendant on the solid state properties of thiosemicarbazones and their biological potential. So, herein, we report the Me_3N-promoted efficient synthesis of 2,3,4,4a-tetrahydroxanthen-1-one, its thiosemicarbazone derivatives, and the solid state self-assembly and antibacterial activity of the synthesized xanthene-based thiosemicarbazones.

2. Experimental

2.1 General

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (KBr discs) were run on Shimadzu Prestige-21 FT-IR spectrometer. The ^{1}H & ^{13}C NMR spectra were recorded in DMSO-*d*6 on Bruker (Rhenistetten-Forchheim, Germany) AM 300 spectrometer, operating at 300 MHz and using TMS as an internal standard. ^{1}H NMR chemical shifts are reported in δ/ppm and coupling constants in Hz. The progress of the reaction and purity of the products were checked on TLC plates coated with Merck silica gel 60 GF254, and the spots were visualized under ultraviolet light at 254/366 nm and/or spraying with iodine vapours. *In vitro* biological evaluation of the synthesized compounds (**5a-5j**) was done at Department of Chemistry, University of Sargodha, Sargodha.

2.2 Synthesis of 2, 3, 4, 4a-tetrahydro-1H-xanthen-1-one (3)

To a stirred mixture of salicyladehyde (5mmol, 0.5ml) and aqueous trimethylamine (5mmol, 0.3ml) in methanol (5ml) was added cyclohexenone (15mmol, 1.4ml). The reaction mixture was further stirred for another 24 hours, after which bright yellow crystalline precipitates were filtered and washed thoroughly with cold methanol to afford pure 2,3,4,4a-tetrahydro-1*H*-xanthen-1-one.

Yield, 88%; mp, 136-138℃ [135-136 ^{o}C][42]; IR (KBr), υ (cm^{-1}): 1729, 1605 (C=O). ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.39 (s, 1H, C_{10}-H CH=C), 7.11-7.20 (m, 2H, $C_{2,4}$-H *ArH*), 6.88 (dd, 1H, *J* = 7.8 Hz, 2.1 Hz, C_3-H *ArH*), 6.81 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 4.89 (s, 1H, C_8-H CH-O), 2.84 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.24-2.49 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.52-1.80 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.0, 154.5, 131.6, 129.6, 122.1, 122.0, 74.7, 39.2, 29.5, 18.4, HRMS calc. for $C_{13}H_{12}O_2$ 200.0837, found 200.0840.

2.3 Synthesis of xanthene-based thiosamicarbazones (5a-5j)

A solution of the appropriate thiosemicarbazide (1mmol) in ethanol (10 ml) was added dropwise to a stirred hot 10 ml ethanolic solution of 2, 3, 4, 4a-tetrahydro-1*H*-xanthen-1-one (**3**) (1mmol, 0.2g). *p*-Toluene sulphonic acid (0.1% by weight) was then added as catalyst and the reaction mixture was heated to reflux for 2 h, after which, the reaction mixture was cooled to room temperature. The yellowish crystalline precipitates thus appeared, were filtered, washed thoroughly with cold ethanol and dried to get pure products of **5a-5j**.

(2*Z*)-*N*-phenyl-2-(2, 3, 4, 4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5a)

Yield, 78%; mp, 202℃; IR (KBr), υ (cm^{-1}): 3676, 3100, 2933 (NH), 1531(C=N), 1176 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.81 (brs, 1H, NH-N), 10.14 (brs, 1H, NH-CS), 7.36 (s, 1H, C_{10}-H

CH=C), 7.13-7.21 (m, 2H, $C_{2,4}$-H *ArH*), 6.88 (dd, 1H, *J* = 7.7 Hz, 2.0 Hz, C_3-H *ArH*), 6.81 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 6.45-6.78 (m, 3H, $C_{22\text{-}24}$-H *PhH*), 6.41 (dd, 2H, *J* = 7.44 Hz, 1.8 Hz $C_{21,25}$-H *PhH*), 4.90 (s, 1H, C_8-H CH-O), 2.83 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.26-2.50 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.50-1.78 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.0, 154.5, 153.5, 140.3, 138.8, 137.4, 134.7, 131.6, 130.0, 129.6, 128.2, 125.0, 122.1, 122.0, 74.7, 39.2, 29.5, 18.4, HRMS calc. for $C_{20}H_{19}N_3OS$ 349.1249, found 349.1252.

(2*Z*)-*N*-(2-fluorophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1 carbothioamide (5b)

Yield, 71%; mp, 222℃; IR (KBr), υ (cm^{-1}): 3676, 3100, 2933 (NH), 1531 (C=N), 1176 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.78 (brs, 1H, NH-N), 10.01 (brs, 1H, NH-CS), 7.91 (dd, 1H, *J* = 7.6 Hz, 2.1 Hz, C_{22}-H *PhH*), 7.38 (s, 1H, C_{10}-H CH=C), 7.13-7.21 (m, 2H, $C_{2,4}$-H *ArH*), 6.91-7.15 (m, 2H, $C_{23,24}$-H *PhH*), 6.88 (dd, 1H, *J* = 7.7 Hz, 2.0 Hz, C_3-H *ArH*), 6.81 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 6.78 (dd, 1H, *J* = 7.2 Hz, 1.9 Hz C_{25}-H *PhH*), 4.87 (s, 1H, C_8-H CH-O), 2.81 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.22-2.51 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.51-1.80 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.2, 154.5, 147.8, 146.1, 141.8, 140.1, 139.8, 139.2, 138.8, 137.5, 131.6, 129.6, 128.7, 128.2, 122.1, 122.0, 74.7, 39.2, 29.5, 18.4, HRMS calc. for $C_{20}H_{18}FN_3OS$ 367.1154, found 367.1157.

(2*Z*)-*N*-(4-fluorophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1 carbothioamide (5c)

Yield, 71%; mp, 222℃; IR (KBr), υ (cm^{-1}): 3679, 3107, 2931 (NH), 1529 (C=N), 1177 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.77 (brs, 1H, NH-N), 9.85 (brs, 1H, NH-CS), 7.61 (d, 2H, *J* = 7.9 Hz, $C_{22,24}$-H *PhH*), 7.48 (d, 2H, *J* = 7.9 Hz, $C_{21,25}$-H *PhH*), 7.37 (s, 1H, C_{10}-H CH=C), 7.12-7.19

(m, 2H, $C_{2,4}$-H *ArH*), 6.86 (dd, 1H, *J* = 7.7 Hz, 2.0 Hz, C_3-H *ArH*), 6.80 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 4.86 (s, 1H, C_8-H CH-O), 2.79 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.21-2.47 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.50-1.77 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.2, 154.5, 147.8, 141.0, 139.8, 139.5, 139.0, 138.8, 137.1, 131.2, 129.7, 128.7, 128.2, 122.0, 74.6, 39.5, 29.7, 18.5, HRMS calc. for $C_{20}H_{18}FN_3OS$ 367.1154, found 367.1158.

(2*Z*)-*N*-(2-methoxyphenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1 carbothioamide (5d)

Yield, 88%; mp, 245℃; IR (KBr), υ (cm^{-1}): 3675, 3101, 2930 (NH), 1529 (C=N), 1179 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.75 (brs, 1H, NH-N), 10.19 (brs, 1H, NH-CS), 8.4 (dd, 1H, *J* = 7.6 Hz, 2.1 Hz, C_{22}-H *PhH*), 7.37 (s, 1H, C_{10}-H CH=C), 7.08-7.19 (m, 4H, $C_{1\text{-}4}$-H *ArH*), 6.84-6.96 (m, 3H, $C_{23\text{-}25}$-H *PhH*), 4.88 (s, 1H, C_8-H CH-O), 3.89 (s, 3H, OCH_3), 2.84-2.86 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.25-2.50 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.50-1.81 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.0, 154.1, 147.3, 142.3, 141.7, 140.4, 139.7, 139.1, 138.6, 137.2, 131.9, 129.5, 128.1, 127.1, 122.1, 120.9, 74.6, 54.6, 39.5, 29.7, 18.9, HRMS calc. for $C_{21}H_{21}N_3O_2S$ 379.1355, found 379.1352.

(2*Z*)-*N*-(4-methoxyphenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5e)

Yield, 85%; mp, 231℃; IR (KBr), υ (cm^{-1}): 3678, 3106, 2928 (NH), 1530 (C=N), 1178 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.47 (brs, 1H, NH-N), 9.95 (brs, 1H, NH-CS), 7.63 (d, 2H, *J* = 7.9 Hz, $C_{22,24}$-H *PhH*), 7.38 (s, 1H, C_{10}-H CH=C), 7.10-7.18 (m, 2H, $C_{2,4}$-H *ArH*), 7.01 (d, *J* = 2H, 7.89 Hz, $C_{21,25}$-H *PhH*), 6.85 (dd, 1H, *J* = 7.66 Hz, 1.9 Hz, C_3-H *ArH*), 6.81 (d, 1H, *J* = 7.66 Hz, C_1-H *ArH*), 4.85 (s, 1H, C_8-H CH-O), 3.77 (s, 3H, OCH_3), 2.81 (brs, 1H, C_{13}-H cyclohexyl-

CH_2), 2.26-2.50 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.49-1.92 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.6, 154.1, 139.2, 139.4, 139.1, 138.3, 137.1, 136.7, 133.2, 131.2, 129.7, 128.7, 128.2, 122.1, 74.2, 39.5, 29.6, 18.7, HRMS calc. for $C_{21}H_{21}N_3O_2S$ 379.1355, found 379.1357.

(2*Z*)-*N*-(4-bromophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5f)

Yield, 77%; mp, 212℃; IR (KBr), υ (cm^{-1}): 3678, 3108, 2931 (NH), 1530 (C=N), 1176 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.76 (brs, 1H, NH-N), 9.88 (brs, 1H, NH-CS), 7.51 (d, 2H, *J* = 7.7 Hz, $C_{22,24}$-H *PhH*), 7.35 (s, 1H, C_{10}-H CH=C), 7.28 (d, 2H, *J* = 7.7 Hz, $C_{21,25}$-H *PhH*), 7.11-7.20 (m, 2H, $C_{2,4}$-H *ArH*), 6.83 (dd, 1H, *J* = 7.78 Hz, 1.96 Hz, C_3-H *ArH*), 6.78 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 4.88 (s, 1H, C_8-H CH-O), 2.76 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.19-2.38 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.53-1.71 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.1, 154.0, 147.5, 141.3, 139.5, 139.3, 139.0, 138.1, 137.2, 131.1, 129.3, 128.2, 128.0, 121.8, 74.3, 39.2, 29.5, 18.6, HRMS calc. for $C_{20}H_{18}BrN_3OS$ 427.0354, found 427.0357.

(2*Z*)-*N*-(4-methylphenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5g)

Yield, 83%; mp, 241℃; IR (KBr), υ (cm^{-1}): 3677, 3107, 2930 (NH), 1530 (C=N), 1179 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.51 (brs, 1H, NH-N), 9.88 (brs, 1H, NH-CS), 7.53 (d, 2H, *J* = 7.6 Hz, $C_{22,24}$-H *PhH*), 7.39 (s, 1H, C_{10}-H CH=C), 7.11-7.20 (m, 2H, $C_{2,4}$-H *ArH*), 7.02 (d, 2H, *J* = 7.6 Hz, $C_{21,25}$-H *PhH*), 6.89 (dd, 1H, *J* = 7.8 Hz, 1.89 Hz, C_3-H *ArH*), 6.80 (d, 1H, *J* = 7.8 Hz, C_1-H *ArH*), 4.84 (s, 1H, C_8-H CH-O), 2.37 (s, 3H, CH_3), 2.83 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.21-2.47 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.51-1.87 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C

NMR (δppm) 198.1, 154.1, 141.1, 139.4, 139.1, 138.1, 137.0, 136.2, 133.2, 131.1, 129.5, 128.4, 128.2, 122.0, 74.2, 39.7, 29.2, 18.8, HRMS calc. for $C_{21}H_{21}N_3OS$ 363.1405, found 363.1407.

(2*Z*)-*N*-(2,4-difluorophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5h)

Yield, 75%; mp, 215℃; IR (KBr), υ (cm^{-1}): 3679, 3111, 2930 (NH), 1527 (C=N), 1178 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.79 (brs, 1H, NH-N), 9.87 (brs, 1H, NH-CS), 7.63 (d, 1H, *J* = 2.1 Hz, C_{22}-H *PhH*), 7.46-7.50 (m, 2H, $C_{24,25}$-H *PhH*), 7.36 (s, 1H, C_{10}-H CH=C), 6.86-7.17 (m, 4H, $C_{1\text{-}4}$-H *ArH*), 4.85 (s, 1H, C_8-H CH-O), 2.86 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.26-2.50 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.52-1.92 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.2, 154.5, 147.8, 141.6, 139.7, 139.0, 138.6, 138.1, 137.2, 136.7, 135.2, 131.2, 129.5, 128.1, 127.7, 122.5, 74.8, 39.9, 29.8, 18.7, HRMS calc. for $C_{20}H_{17}F_2N_3OS$ 385.1060, found 385.1064.

(2*Z*)-*N*-(2,4-dichlorophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5i)

Yield, 74%; mp, 195℃; IR (KBr), υ (cm^{-1}): 3678, 3110, 2932 (NH), 1525 (C=N), 1181 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.75 (brs, 1H, NH-N), 9.81 (brs, 1H, NH-CS), 7.62 (d, 1H, *J* = 1.8 Hz, C_{22}-H *PhH*), 7.45-7.49 (m, 2H, $C_{24,25}$-H *PhH*), 7.38 (s, 1H, C_{10}-H CH=C), 6.83-7.15 (m, 4H, $C_{1\text{-}4}$-H *ArH*), 4.84 (s, 1H, C_8-H CH-O), 2.85 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.20-2.45 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.51-1.90 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 197.9, 154.1, 147.3, 141.9, 139.4, 139.1, 138.3, 138.0, 137.7, 136.2, 135.5, 131.8, 129.1, 128.0, 127.6, 122.4, 74.7, 39.8, 29.5, 18.5, HRMS calc. for $C_{20}H_{17}Cl_2N_3OS$ 417.0469, found 417.0466.

(2*Z*)-*N*-(2,5-dichlorophenyl)-2-(2,3,4,4a-tetrahydro-1*H*-xanthen-1-ylidene)hydrazine-1-carbothioamide (5j)

Yield, 73%; mp, 188℃; IR (KBr), υ (cm^{-1}): 3677, 3111, 2931 (NH), 1526 (C=N), 1180 (C=S). ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.76 (brs, 1H, NH-N), 9.84 (brs, 1H, NH-CS), 7.60 (d, 1H, *J* = 7.8 Hz, C_{22}-H *PhH*), 7.45 (dd, 1H, *J* = 7.8 Hz, 1.9 Hz, C_{23}-H *PhH*), 7.38 (s, 1H, C_{10}-H CH=C), 7.31 (d, 1H, *J* = 1.9 Hz, C_{25}-H *PhH*), 7.13-7.21 (m, 2H, $C_{2,4}$-H *ArH*), 6.87 (dd, 1H, *J* = 7.9 Hz, 1.9 Hz, C_3-H *ArH*), 6.78 (d, 1H, *J* = 7.9 Hz, C_1-H *ArH*), 4.87 (s, 1H, C_8-H CH-O), 2.83 (brs, 1H, C_{13}-H cyclohexyl-CH_2), 2.21-2.45 (m, 2H, C_{12}-H cyclohexyl-CH_2), 1.50-1.85 (m, 3H, $C_{11,13}$-H cyclohexyl-CH_2),^{13}C NMR (δppm) 198.0, 154.2, 147.5, 141.7, 139.6, 139.4, 138.2, 138.0, 137.6, 136.2, 135.3, 131.9, 129.0, 128.0, 127.5, 122.1, 74.6, 39.7, 29.6, 18.7, HRMS calc. for $C_{20}H_{17}Cl_2N_3OS$ 417.0469, found 417.0471.

2.4 Crystallographic data collection and structural refinement

Single crystals of **5d** and **5i** were mounted on a thin glass fiber at room temperature and the reflection data were collected on a Bruker kappa APE XII CCD diffractometer equipped with graphite mono-chromated MoKα radiation (λ = 0.71073 Å). The data were also corrected to Lorentz and polarization effect. The structure was solved using SHELXS-97. Final refinement on F^2 was carried out by full-matrix least-squares techniques using SHELXL-97.[39] The crystal data of **5d** and **5i**, and refinement values are summarized in Table 1.

Table 1. Crystallographic data for compound 1

Crystal data	**5d**	**5i**
CCDC	1411063	1411064
Chemical formula	$C_{21}H_{21}N_3O_2S$	$C_{20}H_{17}Cl_2N_3OS$
M_r	379.47	418.32
Crystal system, space group	Triclinic, $P\bar{1}$	Triclinic, $P\bar{1}$
Temperature (K)	296	296
a, *b*, *c* (Å)	8.1682 (11), 10.1121 (12), 12.2265 (15)	9.6988 (10), 9.8693 (9), 10.7502 (12)

α, β, γ (°)	106.817 (5), 96.610 (5), 94.955 (5)	101.697 (6), 101.627 (6), 101.570 (6)
V (Å^3)	952.6 (2)	955.19 (17)
Z	2	2
Radiation type	Mo $K\alpha$	Mo $K\alpha$
μ (mm^{-1})	0.19	0.47
Crystal size (mm)	0.40 × 0.33 × 0.30	0.40 × 0.32 × 0.25
Data collection		
Diffractometer	Bruker Kappa APEXII CCD diffractometer	Bruker Kappa APEXII CCD diffractometer
Absorption correction	Multi-scan (*SADABS*; Bruker, 2005)	Multi-scan (*SADABS*; Bruker, 2005)
T_{min}, T_{max}	0.930, 0.943	0.835, 0.895
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	13685, 3973, 2180	13033, 3619, 2684
R_{int}	0.042	0.029
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.639	0.617
Refinement		
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.064, 0.204, 1.04	0.061, 0.171, 1.08
No. of reflections	3973	3619
No. of parameters	245	239
H-atom treatment	H-atom parameters constrained	H-atom parameters constrained
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.28, -0.24	0.62, -0.44

2.5 Antibacterial activity (in vitro)

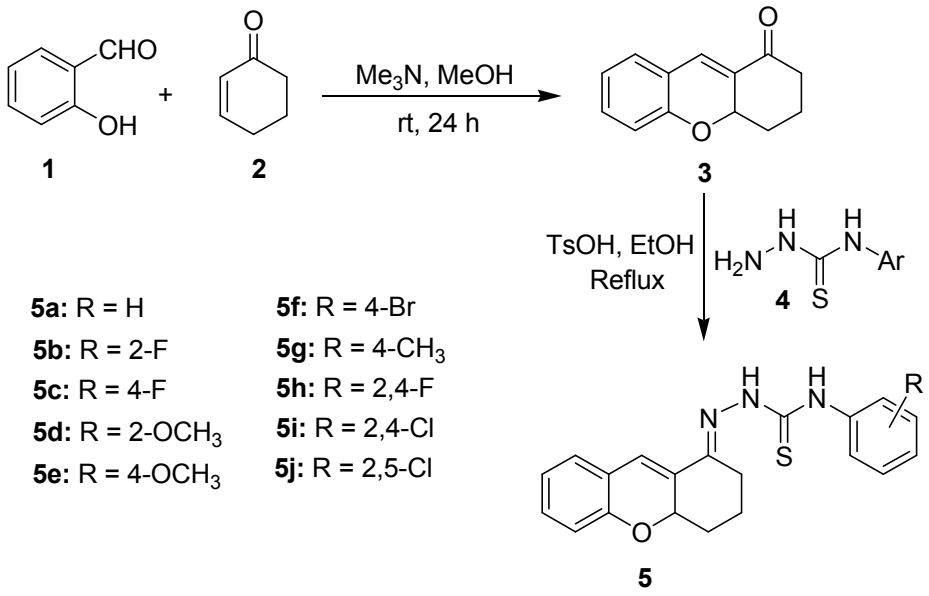
All the synthesized xanthene-based thiosemicarbazones (**5a-5j**) were screened for their *in vitro* antibacterial activity against two Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and two Gram-negative (*Escherichia coli, Salmonella typhi*) bacterial strains using the agar well diffusion method.[40] Two- to eight hour old bacterial inocula containing approximately 10^4-10^6 colony forming units (cfu) ml^{-1} were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centres at least 24 mm. The recommended concentration (100 μl) of the test sample (1 mg ml^{-1} in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and the reference antibacterial drug, ciprofloxacin served as

negative and positive controls, respectively. The plates were incubated immediately at 37 ^{o}C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared[41] with the standard drug ciprofloxacin. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone and they showed no activity against any bacterial strains.

3. Results and discussion

3.1 Synthesis

Kim *et al*[42] reported the synthesis of 2,3,4,4a-tetrahydroxanthen-1-one (**3**) for the first time by reacting salicylaldehyde (**1**) and cyclohexenone (**2**) in aqueous THF in the presence of DMAP (0.2-1.2 equiv.) at room temperature in 53% yield. More recently, Yu *et al*[43] synthesized the compound **3** by reacting **1** and **2** in the presence of catalytic amount of BSA (α-amylase from B. subtilis) in 48% yield. The major problem associated with these methods for the synthesis of 2,3,4,4a-tetrahydroxanthen-1-ones is their relatively slow reaction rate and low/moderate reaction yield. In view of this situation and inspired by the work of Tang *et al*[44] (the work in which dramatic rate acceleration in Baylis-Hillman Reaction was observed in homogeneous reaction medium in the presence of water), it was thought worthwhile to use domino Baylis-Hillman/oxa-Michael reaction sequence to approach 2,3,4,4a-tetrahydroxanthen-1-one (**3**). Pleasingly, when we used aqueous Me_3N in methanol solvent in the reaction between **1** and **2** at room temperature, the excellent yield (88%) of **3** was obtained (Scheme 1). It is interesting to mention here that use of other bases, solvents and higher temperatures either gave low yield of **3** or led to the complex reaction mixtures.



Scheme 1. Synthesis of 2,3,4,4a-tetrahydroxanthen-1-one (**3**) and its thiosemicarbazone derivatives (**5a-5j**)

After efficient synthesis of 2,3,4,4a-tetrahydroxanthen-1-one (**3**), it was further reacted with a series of thiosemicarbazides (**4**) in ethanol solvent containing a catalytic amount of *p*-toluene sulphonic acid to afford the corresponding thiosemicarbazone derivatives (**5a-5j)** in good to excellent (70-93%) yields (Scheme 1).

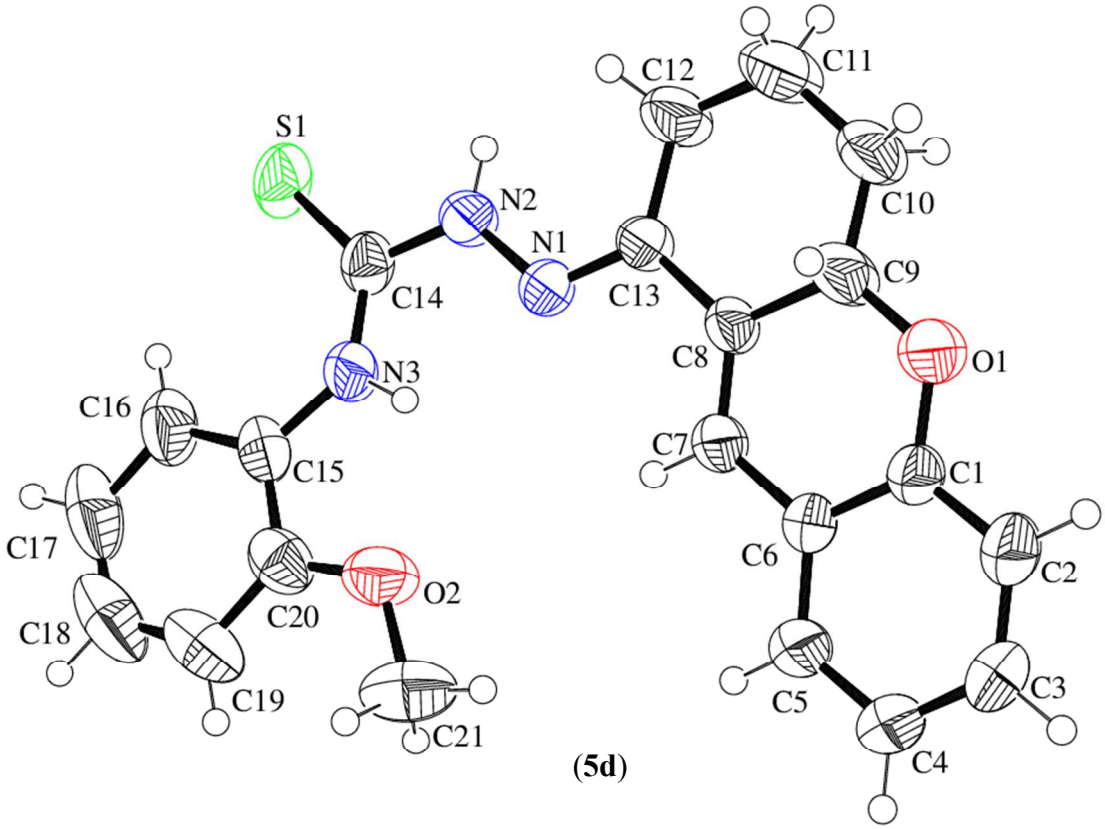
3.2 Solid state self-assembly

As the outstanding bulk properties of solids are generally associated with their molecular packing, therefore, the understanding of molecular packing in the crystal structures is very necessary to explore new functional solids and is currently the main objective of the crystal engineers.[45-48] In continuation of our attempt to highlight the potential of thiosemicarbazones in crystal engineering,[29, 30] the solid state self-assembly studies of xanthene-based thiosemicarbazones (**5a-5j**) were attempted using single crystal X-ray analysis in order to identify the dominant structural features that involve in govering the solid state alignment of

molecules. However, good quality single crystals for only two compounds **5d** and **5i** could be obtained. All our attempts to cultivate single crystals for other compounds of the series were unsuccessful. Selected crystal data and structural refinement parameters of compound **5d** and **5i** are presented in Table 1.

Both of the compounds **5d** and **5i** crystalized in the same solvent (1,4-dioxane) by its slow evaporation in order to provide good quality single crystal for X-ray analysis and showed triclinic crystal lattice with the $P\bar{1}$ space group. Their molecular structures along with crystallographic numbering scheme are presented in Figure 1. The central *N*-iminothiourea moiety is nearly planar in both the compounds **5d** and **5i** and is present in S-*cis*/S-*trans* conformation.[29, 30] It is important to mention here that this conformation is more stable than S-*trans*/S-*trans* conformation in these compounds due to the formation of intramolecular hydrogen bond (Figure 1). The dihedral angles between S(1)-C(14)-N(2)-H(2A) and S(1)-C(14)-N(3)-H(3A) are -1.59° and 175.24° in **5d**, and 6.31° and 174.45°, respectively in **5i**. This planarity around *N*-iminothiourea moiety can be credited to the significant amount of delocalization of nitrogen lone pairs onto the thiocarbonyl, which can clearly be demonstrated by the shorter N-C bond lengths [N(2)-C(14) 1.360(4) Å and N(3)-C(14) 1.335(4) Å] in **5d**, and [N(2)-C(14) 1.359(4) Å and N(3)-C(14) 1.339(4) Å] in **5i**, indicating the partial double bond character of N-C bonds. The slightly longer bond length in case of N-C bond that is directly attached to the imino (-N=C-) moeity signifies less delocalization of nitrogen lone pair towards thiocarbonyl, most probably due to electron withdrawing nature of neighbouring sp^2-hybridized nitrogen atom. Although, this planarity may lead to two different conformations around thiourea moiety; one in which both the hydrogens are trans to the sulfur (S-*trans*, S-*trans* conformation) and the other in which a hydrogen is present on both sides of the sulfur (S-*trans*, S-*cis* conformation), but as

pointed above, one conformation i-e S-*cis*/S-*trans* is more stable in this class of compounds due to the presence of additional imino moiety and its apparent ability to make intramolecular hydrogen bond [(N(1)-H(3A)···N(1) 2.098 Å in **5d**, (N(1)-H(3A)···N(1) 2.122 Å in **5i**. An expected consequence of this conformational preference is the facile formation of a centrosymmetric thioamide dimer $R_2^2(8)$ $\{\cdots H–N–C=S\}_2$ synthon; a key interaction involved in the molecular packing of compound **5d** and **5i**.



(**5d**)

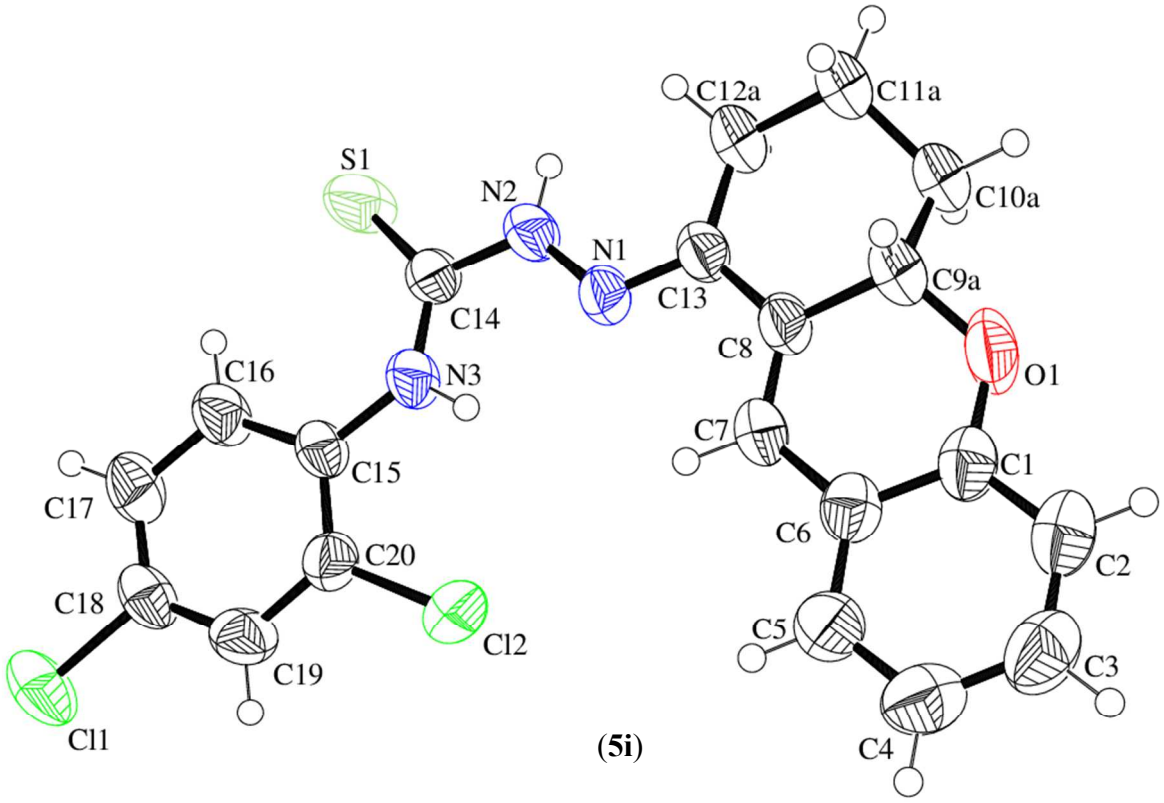
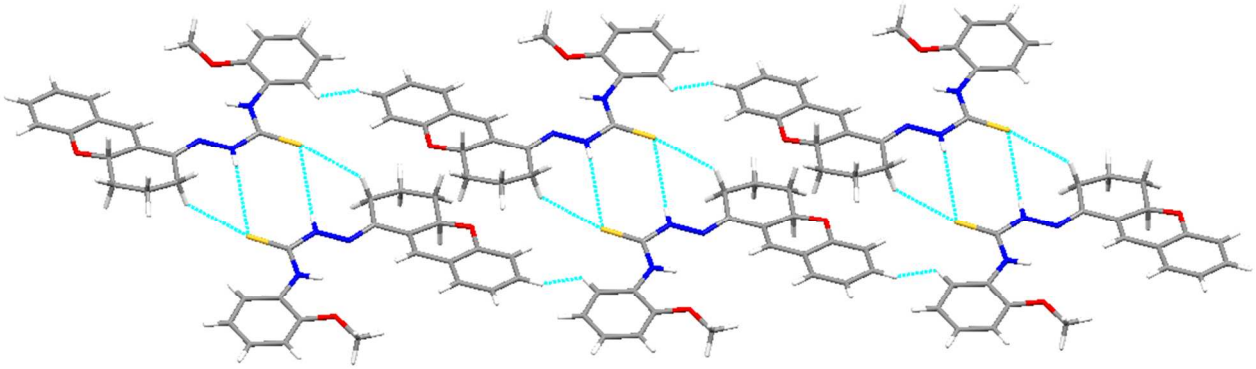


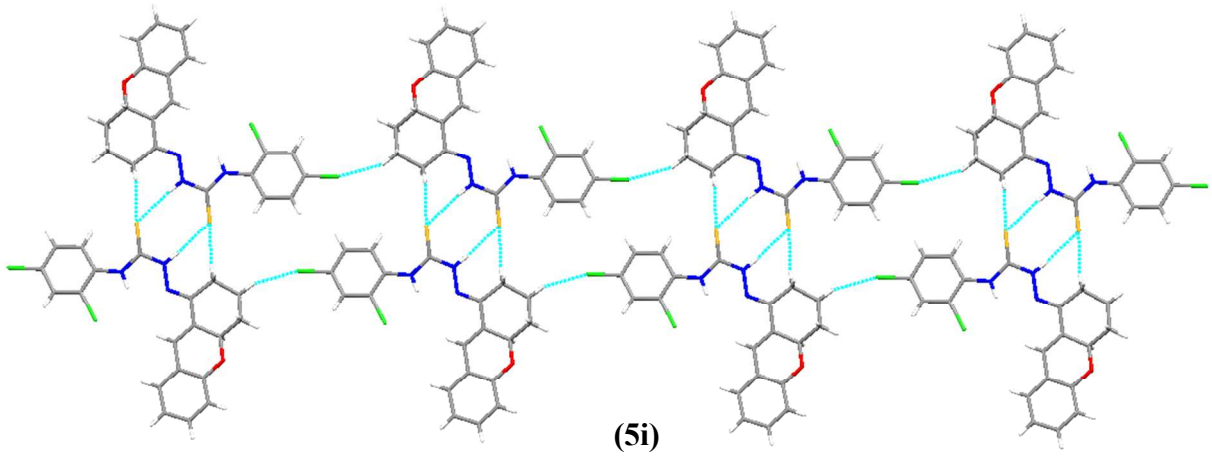
Figure 1. The molecular structures (ORTEP) of compound **5d** and **5i**

The 3D-network of both **5d** and **5i** consists of 1D-tapes (Figure 2). The tapes in compound **5d** have H···H interactions[49] [H(3)···H(16) 2.486 Å] in addition to centrosymmetric thioamide $R_2^2(8)$ dimer synthon [N(2)-H(2A)···S(1) 2.864 Å], whereas the tapes in compound **5i** are composed of CH-Cl interactions [C(11A)-H(11A)···Cl(1) 2.778 Å] along with centrosymmetric thioamide $R_2^2(8)$ dimer synthon [N(2)-H(2A)···S(1) 2.859 Å]. It is important to mention here that in both **5d** and **5i**, thioamide dimer synthon is further stabilized by CH-S [C(12)-H(12B)···S(1) 2.821 Å in **5d** and C(12A)-H(12A)···S(1) 2.637 Å in **5i**] interactions (Figure 2). These tapes are further connected to the neighbouring tapes by multiple CH-S [C(21)-H(21B)···S(1) 3.042 Å] and CH-π [C(11)-H(11A)···C(14) 2.963 Å] interactions in **5d** and CH-π [C(9A)-H(9A)···C(16) 2.707 Å, C(12A)-H(12B)···C(4) 2.854 Å] interactions[50, 51] in **5i** to form 3D-network structure (Figure 3). It is interesting to mention here that chloro at 2-position did not

participate in any kind of interaction in the network. Furthermore, it can be anticipated that although packing of both **5d** and **5i** are greatly affected by the nature of two substituents, but thioamide dimer synthon remain the key supramolecular moiety in both of these structures and is consistant with our previous observations.[29, 30]

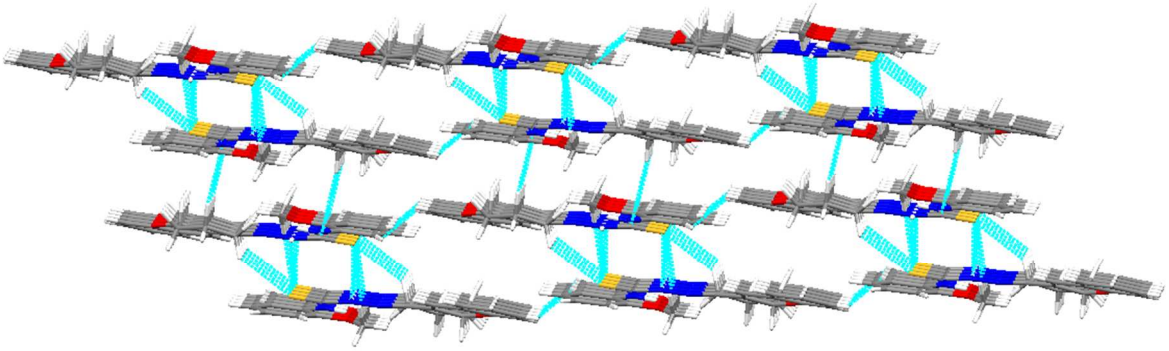


(5d)

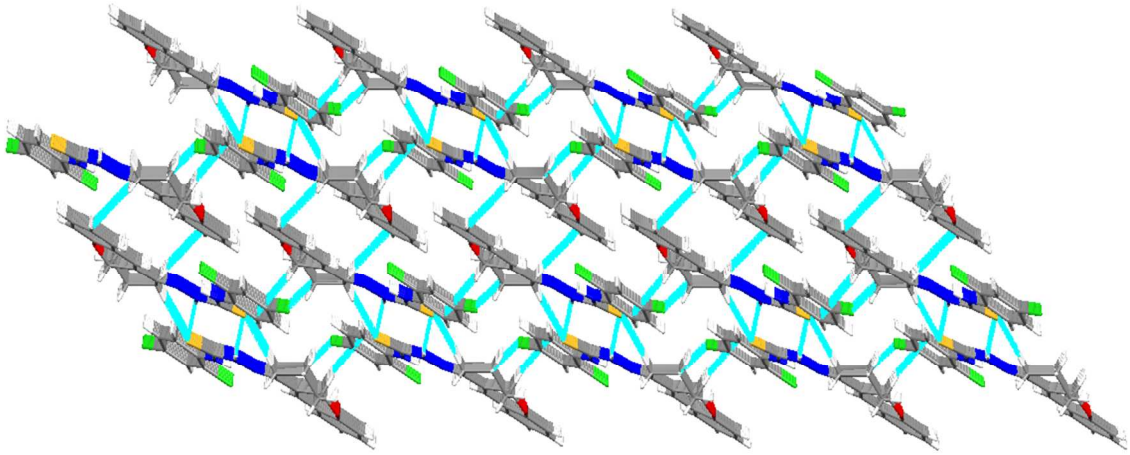


(5i)

Figure 2. 1D-tapes of **5d** and **5i** in the solid state, involving dimeric pairs associated *via* the $\{\cdots H–N–C=S\}_2$ synthon



(5d)



(5i)

Figure 3. View of the 3D-networks of **5d** and **5i**

3.3 Anti-bacterial activity

All the synthesized xanthene-based thiosemicarbazones **5a-5j** were evaluated for their anti-bacterial properties against two Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and two Gram-negative (*Escherichia coli, Salmonella typhi*) bacterial strains, using the agar well diffusion method.[40, 41] The ciprofloxacin (a commercially available antibiotic) was used as the standard reference (SR) drug in this study. Interestingly, the compounds **5b**, **5c**, **5d** and **5i** showed excellent antibacterial activity against one or more of the tested strains (Table 2, Figure 4). It is important to stress here that the compound **5c** (against *Salmonella typhi*) having fluoro substitutions on 4-position of the aryl ring have shown comparable activity to the standard drug

(Table 2, Figure 4)**.** This is most probably due to more lipophilic character of the fluorinated compounds as compared to the others, which is required to cross the cell membrane of the micro-organisms, and the better interactions of the fluorine atoms with the receptor sites. One compound i-e **5d** of the series with 2-methoxy substitution exhibited good to excellent activity against all the tested bacterial strains. All other compounds of the series showed low to moderate antibacterial activity against the tested strains (Table 2, Figure 4).

Table 1. Antibacterial activity (*in vitro*) of xanthene-based thiosemicarbazones (5a-5j)

Compound	R	*B. subtilis*	*S. aureus*	*E. coli*	*S. typhi*
5a	H	10	-	0	14
5b	2-F	24	-	10	10
5c	4-F	10	-	12	20
5d	2-OCH_3	20	18	14	18
5e	4-OCH_3	10	10	8	12
5f	4-Br	12	12	12	14
5g	4-CH_3	12	12	4	4
5h	2,4-F	10	-	6	12
5i	2,4-Cl	20	18	12	10
5j	2,5-Cl	10	12	14	6
Ciprofloxacin	-	**26**	**20**	**30**	**20**

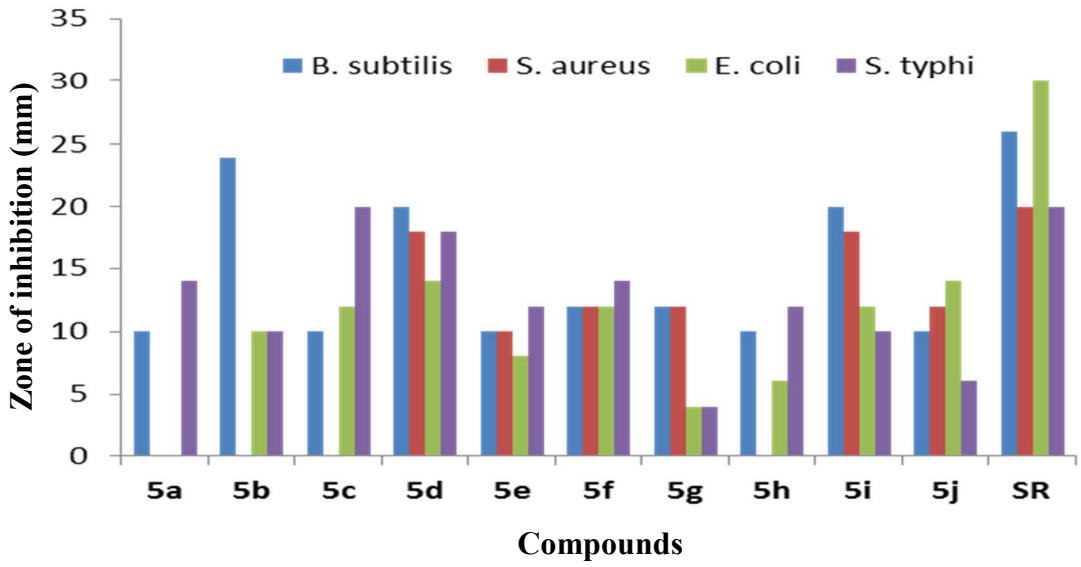


Figure 4. Comparison of anbacterial activity of xanthene-based thiosemicarbazones (**5a-5j**)

4. Conclusions

In conclusion, we have used Me_3N-promoted domino Baylis-Hillman/oxa-Michael reaction sequence for the synthesis of 2,3,4,4a-tetrahydroxanthen-1-one (**3**) in excellent yield from the reaction of salisaldehyde (**1**) with cyclohexanone (**2**). The reaction of 2,3,4,4a-tetrahydroxanthen-1-one (**3**) with various thiosemicarbazides also underwent smoothly to provide a series of new xanthene-based thiosemicarbazones (**5a-5j**), which were characterized by their physical and spectral data. The componds **5d** and **5i** were analysed by X-ray crystallographic technique to study their solid state self-assembly. The results showed that thioamide dimer synthon is the key supramolecular moiety in the packing of both **5d** and **5i,** despite of some influence of substituents. Furthermore, the compounds (**5a-5j**) were also screened for their antibacterial properties. The compounds **5b**, **5c**, **5d** and **5i** were found to have excellent antibacterial potential against one or more of the tested bacterial strains, especially compound **5c** with activity comparable to standard ciprofloxacin against *Salmonella typhi*. The

study with constructive hints about the synthesis of xanthene derivatives in high yield under mild conditions, the solid state structural features of xanthene-based thiosemicarbazones and their antibacterial potential will open up an avenue towards the applications of such compounds in various fields.

Acknowledgments

We are highly grateful to the Higher Education Commission (HEC), Govt. of Pakistan for financial support.

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Graphical Abstract

Me_3N-promoted synthesis of 2,3,4,4a-tetrahydroxanthen-1-one: Preparation of thiosemicarbazone derivatives, their solid state self-assembly and antimicrobial properties

A. Hameed, Z. Shafiq, M. Yaqub, M. Hussain, M. A. Hussain, M. Afzal, M. N. Tahir and M. M. Naseer

Thiosemicarbazones (**5a-5j**) have been synthesized from 2,3,4,4a-tetrahydroxanthen-1-one, obtained in high yield through Me_3N-promoted domino Baylis-Hillman/oxa-Michael reaction. Their solid-state self-assembly and antimicrobial properties are studied.