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Synthesis, characterization and *in vitro* antimicrobial activity of novel 2-thioxo-4-thiazolidinones and 4,4′-bis (2-thioxo-4-thiazolidinone-3-yl)diphenylsulfones

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ABSTRACT

2-Thioxo-4-thiazolidinones (**3a**,**b**) were achieved by cyclocondensation of isothiocyanatosulfonamides (**1a**,**b**) with sulfanylacetic acid at reflux temperature in dioxane in the presence of triethylamine. Compound (**3a**) was exploited to synthesize the versatile hitherto unknown 2-thioxo-4-thiazolidinones (**5**–**10**) via its reaction with some electrophiles. Cyclization of 4,4′-diisothiocyanate diphenylsulfone (**11**) with sulfanylacetic acid furnished 4,4′-bis(2-thioxo-4-thiazolidinone-3-yl)diphenylsulfone (**12**) which on treatment with excess 4-methoxybenzaldehyde in refluxing dioxane in the presence of piperidine yielded the bisbenzylidene derivative (**13**). The novel synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral studies. All the synthesized compounds were screened *in vitro* for their antibacterial and antifungal activities.

1. Introduction

Derivatives of 4-thiazolidinone have been demonstrated to possess antibacterial [1], antifungal [2], anticonvulsant [3], anticancer [4], antituberculosis [5] and anti-human immunodeficiency virus type 1 (HIV-1) [6] activities. Peptidoglycan is an essential component of the cell wall of both Gram-positive and Gram-negative bacteria. 4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B which was a precursor acting during the biosynthesis of peptidoglycan [7]. In addition, antibacterial [8], antifungal [9], insulin releasing [10] and carbonic anhydrase inhibitory [11] activities of sulfonamides were reported. A literature survey revealed that many different protocols have been developed in a way that allows the synthesis of 4-thiazolidinone skeletons [12]. The method more used employs a two-step preparation: reaction of substituted isothiocyanates with sulfanylacetic acid or its ester followed by acid cyclization of the resulting (thiocarbamoyl)sulfanylacetic acids and acetate [12]. Based on these facts and in continuation of our studies on the synthesis and biological activity of heterocycles including 4-thiazolidinones [13–16], we report herein a simple and one-pot route to the synthesis of hitherto unknown 2-thioxo-4-thiazolidinone and 4,4′-bis(2-thioxo-4-thiazolidinone-3-yl)diphenylsulfone derivatives via the reaction of isothiocyanates with sulfanylacetic acid in order to evaluate their antimicrobial activity.

2. Results and discussion

2.1. Chemistry

Isothiocyanates are useful, widely-used building blocks in the synthesis of nitrogen, sulfur and oxygen heterocycles and organometallic compounds of academic, pharmaceutical and industrial interests [17,18]. Isothiocyanatosulfonamides **1a**,**b** were synthesized by thiophosgenation of sulfonamides in the presence of dilute HCl at room temperature [19], Scheme 1.

Cyclocondensation of isothiocyanates **1a**,**b** with sulfanylacetic acid in dioxane in the presence of triethylamine at reflux temperature yielded the 2-thioxo-4-thiazolidinones **3a**,**b**, Scheme 2. The molecular structure of compounds **3a**,**b** was confirmed on the basis of their elemental analyses and spectral data. The infrared spectrum of **3a** showed NH_2 stretching bands at 3348, 3258 cm^{-1}, in addition to stretching band at 1738 cm^{-1} attributed to the C=O functional group (4-thiazolidinone). The ^{1}H NMR spectrum of

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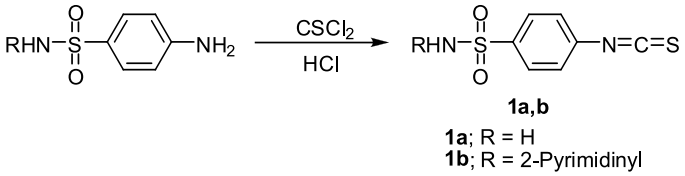
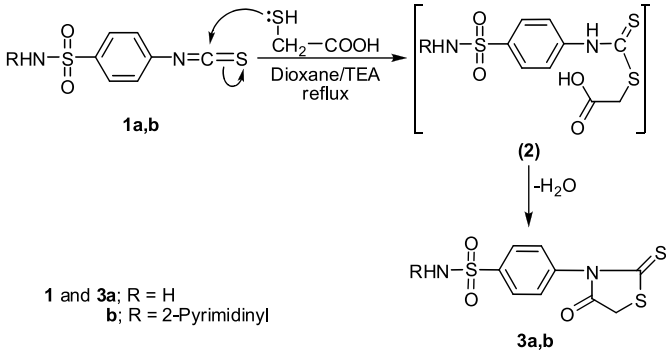


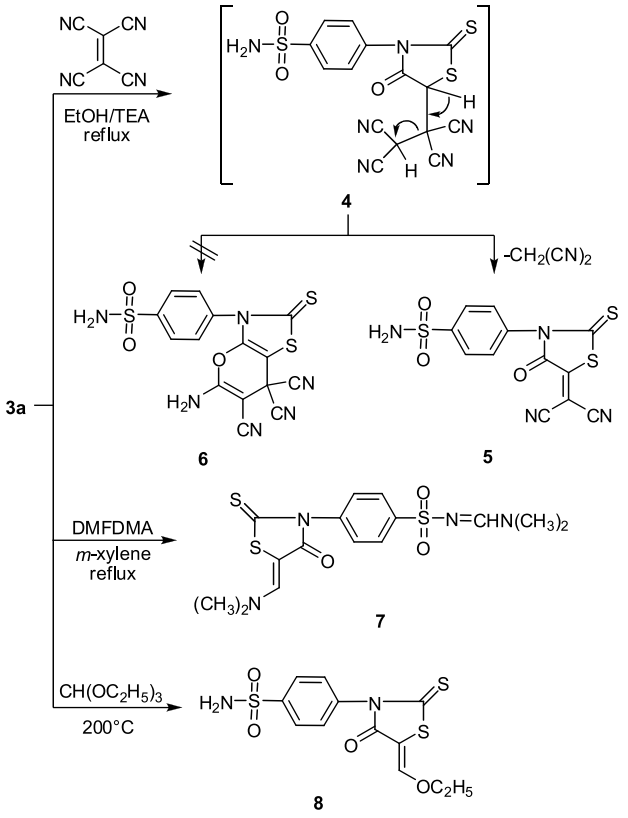
Scheme 1.

compound **3a** revealed the presence of a singlet signal at δ 4.21 ppm corresponding to methylene moiety of thiazolidinone, in addition to the presence of aromatic and NH_2 protons. Mass spectrum of **3a** exhibited a molecular ion peak at *m*/*z* 288 (98.8%) corresponding to the molecular formula $C_9H_8N_2O_3S_3$. The base peak was found in the spectrum at *m*/*z* 64 which is characteristic for SO_2 moiety. The formation of **3** is assumed to proceed through nucleophilic attack of mercapto functional group of sulfanylacetic acid to the thiocarbonyl moiety of isothiocyanate followed by intramolecular cyclization through dehydration of the non-isolable intermediate **2** [20] as depicted in Scheme 2.

The methylene moiety in compound **3a** was exploited to synthesize hitherto unknown thiazolidinone derivatives through its reaction with some electrophiles. Treatment of compound **3a** with tetracyanoethylene led to the formation of 5-dicyanomethylene-2-thioxo-4-thiazolidinone derivative **5** and the other possible structure pyranothiazole **6** was eliminated on the basis of analytical and spectral data. Its infrared spectrum showed the strong and sharp absorption band at 2200 cm^{-1} corresponding to the C≡N functional group. The ^1H NMR spectrum of compound **5** in DMSO-d_6 revealed the absence of methylene moiety. In addition, the structure of **5** was supported by its mass spectrum which revealed a molecular ion peak at *m*/*z* 350 (2.06%). Compound **5** was assumed to be formed via Michael addition of the active methylene group in **3a** to the activated double bond in tetracyanoethylene to form **4** followed by the elimination of malononitrile [21] to furnish **5**, Scheme 3. Condensation of compound **3a** with dimethylformamide–dimethylacetal (DMF–DMA) in refluxing *m*-xylene yielded the 5-dimethylaminomethylene-3-[4-(*N*-dimethylaminomethylenesulfamoyl)phenyl]-2-thioxo-4-thiazolidinone **7**. The infrared spectrum of compound **7** indicated the absence of the NH_2 absorption band and contains characteristic absorption band at 1690 cm^{-1} for the C=O functional group. The molecular structure of compound **7** was established by ^1H NMR spectrum which exhibited the presence of two signals at δ 2.98 and 3.18 corresponding to dimethyleneaminomethylene and *N*-dimethylaminomethylenesulfamoyl fragments, respectively. The molecular ion peak of compound **7** was found in the mass spectrum at *m*/*z* 398 (9.5%) corresponding to the molecular formula $C_{15}H_{18}N_4O_4S_3$. Also, in the mass spectrum the base peak was found at *m*/*z* = 101. 5-Ethoxymethylene derivative **8** was obtained in 87% yield by the



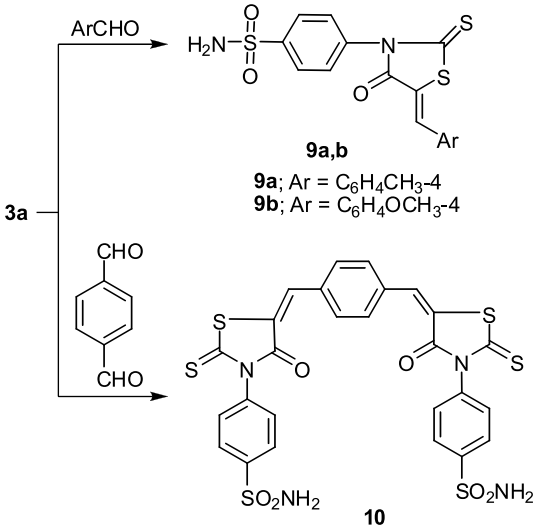
Scheme 2.



Scheme 3.

reaction of compound **3a** with triethyl orthoformate at 200 °C under solvent free condition, Scheme 3.

A Knoevenagel condensation of compound **3a** with aromatic aldehydes in refluxing dioxane in the presence of triethylamine furnished benzylidene derivatives **9a**,**b**. Mass spectrum of compound **9a** revealed a molecular ion peak at *m*/*z* 390 (4.85%) together with base peak at *m*/*z* 148. The reaction of compound **3a** with terephthalaldehyde in dioxane in the presence of a catalytic amount of triethylamine at reflux temperature led to the formation of bisbenzylidene derivative **10**, Scheme 4. ^1H NMR spectrum of compound **10**



Scheme 4.

indicated the absence of methylene moiety which was present in the parent compound and showed absorption at δ 7.78–8.05 ppm attributed to two amino, methylene-H, aromatic protons.

4,4′-Bis(2-thioxo-4-thiazolidinone-3-yl)diphenylsulfone **12** was achieved by cyclization of 4,4′-diisothiocyanate diphenylsulfone **11** [22] with sulfanylacetic acid in refluxing dioxane in the presence of triethylamine, Scheme 5. The mass spectrum of compound **12** showed a molecular ion peak at *m*/*z* 480 (45.7%) and the base peak was observed at *m*/*z* 64 (SO_2 moiety). Reaction of compound **12** with excess 4-methoxybenzaldehyde by refluxing in dioxane in the presence of a catalytic amount of piperidine afforded the 4,4′-bis[5-(4-methoxybenzylidene)-2-thioxo-4-thiazolidinone-3-yl]diphenylsulfone. Mass spectrum of compound **13** showed a molecular ion peak at *m*/*z* 390 (5.95%) with base peak at *m*/*z* 108 which is characteristic for anisole moiety.

2.2. In vitro evaluation antimicrobial activity

All the synthesized compounds were evaluated *in vitro* for their antibacterial activity against *Staphylococcus aureus* (NCTC-7447), *Bacillus cereus* (ATCC-14579), *Serratia marcescens* (IMRU-70) and *Proteus mirabilis* (NCT-289). Also, the antifungal activity against *Aspergillus ochraceus* Wilhelm (AUCC-230) and *Penicillium chrysogenum* Thom (AUCC-530) was evaluated using the agar-diffusion technique [23]. A 1 mg mL^{-1} solution in dimethylformamide (DMF) was used. The bacteria and fungi were grown on nutrient agar and Czapek's–Dox agar media, respectively. DMF as a negative control did not show inhibition zones. The agar media were inoculated with different microorganism cultures tested. After 25 h of incubation at 30 °C for bacteria and 48 h for fungi, the diameter of inhibition zone (mm) was measured. Ampicillin (25 μg mL^{-1}) and Mycostatine (30 μg mL^{-1}) were used as reference drugs for antibacterial and antifungal activities, respectively.

Most of the synthesized compounds were found to possess various antimicrobial activities towards all the microorganisms used (Table 1). Thiazolidinones **5**, **9a** and **10** having biologically active sulfamoyl and thioxo moieties were found to possess highest antibacterial towards *B. cereus* with minimal inhibitory concentration (MIC) values <50 μg mL^{-1}. In addition thiazolidinone derivative **3b** bearing pyrimidine nucleus, sulfamoylphenyl and thioxo moieties revealed high activity against *P. chrysogenum* Thom MIC values <50 μg mL^{-1}. These compounds are nearly as active as reference drugs. However none of the test compounds show superior activity than the reference drugs.

In conclusion, we report herein a convenient route for the synthesis of some novel 2-thioxo-4-thiazolidinones starting from isothiocyanates and sulfanylacetic acid in order to investigate their antimicrobial activity.

3. Experimental

All melting points are uncorrected and were determined on a digital Gallen-Kamp MFB-595 instrument. IR spectra (KBr) were measured on a Shimadzu 440 spectrometer. ^{1}H NMR spectra were recorded in dimethyl-sulfoxide (DMSO-d_6) on a Varian Gemini 200 (200 MHz) spectrometer using TMS as an internal standard; chemical shifts are reported as δ units. Mass spectra were measured on GSMS-QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University.

3.1. General procedure for the reaction of isothiocyanate derivatives with sulfanylacetic acid

A mixture of sulfanylacetic acid (0.92 g, 100 mmol) and either of each **1** (100 mmol) or **11** (2.32 g, 100 mmol) and triethylamine (1.01 g, 100 mmol) in dioxane (20 mL) was refluxed for 3 h. The reaction mixture was left to cool at room temperature. The solid product, so formed, was collected by filtration and recrystallized from an appropriate solvent.

3.1.1. 3-(4-Sulfamoylphenyl)-2-thioxo-4-thiazolidinone (**3a**)

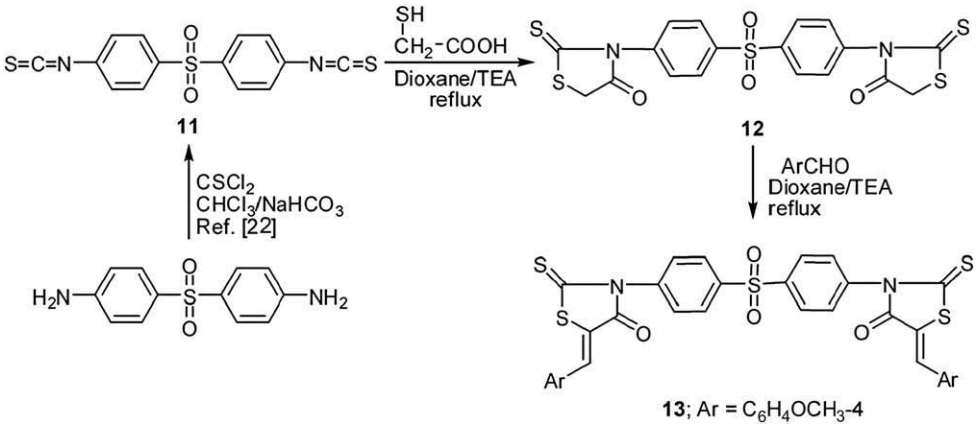
White crystals (dioxane), Yield: 80%, m.p. 265–267 °C; IR (KBr, cm^{-1}): (NH_2) 3348, 3258, (CO) 1738; ^{1}H NMR(DMSO-d_6, ppm): δ 4.21 (s, 2H, CH_2), 7.52–7.91 ppm (m, 6H, Ar and NH_2 protons); MS: *m*/*z* 288 (M^+), 246, 214, 202, 198, 156, 150, 135, 118, 90, 76. Anal. Calcd. for $C_9H_8N_2O_3S_3$: C, 37.50; H, 2.77; N, 9.72. Found: C, 37.40; H, 2.70; N, 9.50.

3.1.2. 3-[4-(N-Pyrimidin-2-yl)sulfamoyl]phenyl-2-thioxo-4-thiazolidinone (**3b**)

White crystals (dioxane), Yield: 63%, m.p. 284–285 °C; IR (KBr, cm^{-1}): (NH) 3100, (CO) 1742; ^{1}H NMR (DMSO-d_6, ppm): δ 4.14 (s, 2H, CH_2), 7.34–7.61 ppm (m, 4H, Ar-protons), 6.65 (t, $J = 4.7$ Hz, 1H, CH-pyrimidine), 8.62 (d, $J = 4.8$ Hz, 2H, two CH-pyrimidine), 11.01 (br, 1H, NH); MS: *m*/*z* 367 (M + 1). Anal. Calcd. for $C_{13}H_{10}N_4O_3S_3$: C, 42.62; H, 2.73; N, 15.30. Found: C, 42.50; H, 2.60; N, 15.40.

3.1.3. 4,4′-Bis(2-thioxo-4-thiazolidinone-3-yl)diphenylsulfone (**12**)

Yellow crystals (dioxane), Yield: 70%, m.p. 250–251 °C; IR (KBr, cm^{-1}): (CO) 1748 cm^{-1}; ^{1}H NMR (DMSO-d_6, ppm): δ 4.41 (s, 4H, $2CH_2$), 7.59–8.20 ppm (m, 8H, Ar-protons); MS: *m*/*z* 480 (M^+), 406, 332, 198, 140, 134, 118, 90, 77, 76. Anal. Calcd. for $C_{18}H_{12}N_2O_4S_5$: C, 45.00; H, 2.50; N, 5.83. Found: C, 44.90; H, 2.40; N, 5.70.



Scheme 5.

Table 1
Antimicrobial activity of the synthesized compounds (diameter zones in mm).

Compound no.	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Staphylococcus aureus (NCTC-7447)	*Bacillus cereus* (ATCC-14579)	*Serratia marcescens* (IMRU-70)	*Proteus mirabilis* (NTC-289)	*Aspergillus ochraceus* Wilhelm (AUCE-230)	*Penicillium chrysogenum* Thom (AUCC-530)
3a	28	13	20	24	18	16
3b	18	16	22	19	15	35
5	15	32	24	18	14	14
7	16	15	12	20	16	16
8	25	12	23	23	12	18
9a	14	34	18	17	14	10
9b	27	10	15	19	19	17
10	13	33	19	22	16	15
12	15	13	10	24	26	12
13	17	16	13	25	11	11
Ampicillin	40	43	40	40	10	16
Mycostatine	–	–	–	–	40	43

Less active 1–1.5 cm; moderately active: 1.5–2 cm; highly active 2–3 cm; very highly active 3–4.5 cm^{-1}.

*3.1.4. 5-Dicyanomethylene-3-(4-sulfamoylphenyl)-2-thioxo-4-thiazolidinone (**5**)*

A mixture of **3a** (2.88 g, 100 mmol), tetracyanoethylene (1.28 g, 100 mmol) and triethylamine (1.01 g, 100 mmol) in ethanol (10 mL) was refluxed for 30 min. After cooling, the resulting solid product was collected by filtration, washed with water, and the crude product recrystallized. Brown crystals (dioxane), m.p. >300 °C; IR (KBr, cm^{-1}): (NH_2) 3353, 3261, (CH-arom.) 3066, (C≡N) 2200, (C═O) 1690; ^{1}H NMR (DMSO-d_6, ppm): δ 7.54 (s, 2H, NH_2), 7.7–8.01 ppm (m, 4H, Ar-protons); MS: *m*/*z* 350 (M^+), 274, 222, 214, 136, 135, 134, 76. Anal. Calcd. for $C_{12}H_6N_4O_3S_3$: C, 41.14; H, 1.71; N, 16.00. Found: C, 41.10; H, 1.60; N, 16.20.

*3.1.5. 5-Dimethylaminomethylene-3-[4-(N-dimethylaminomethylensulfamoyl)phenyl]-2-thioxo-4-thiazolidinone (**7**)*

A mixture of **3a** (2.88 g, 100 mmol) and dimethylformamide–dimethylacetal (DMF–DMA) (2.38 g, 200 mmol) was refluxed in dry *m*-xylene (10 mL) for 1 h. The solvent was removed by evaporation under reduce pressure and the remainder was left to cool. The solid product so formed was collected by filtration, washed with petroleum ether (b.p. 40–60 °C) and the crude product recrystallized. Brown crystals (ethanol), Yield: 60%, m.p. 230–232 °C; IR (KBr, cm^{-1}): (CH-aliphatic) 2921, (CO) 1690; ^{1}H NMR (DMSO-d_6, ppm): δ 2.98, 3.18 (2s, 12H, 2N(CH_3)$_2$), 7.45–8.01 (m, 4H, Ar-protons), 8.10, 8.39 (2s, 2H, two methine-H); MS: *m*/*z* 398 (M^+), 399 (M + 1), 269, 263, 135, 134, 101, 100, 76. Anal. Calcd. for $C_{15}H_{18}N_4O_3S_3$: C, 45.22; H, 4.52; N, 14.07. Found: C, 45.10; H, 4.60; N, 14.00.

*3.1.6. 5-Ethoxymethylene-3-(4-sulfamoylphenyl)-2-thioxo-4-thiazolidinone (**8**)*

A mixture of **3a** (2.88 g, 100 mmol) and triethyl orthoformate (1.06 g, 100 mmol) was heated at 200 °C for 1 h. The reaction mixture was cooled. The resulting solid product was collected by filtration and recrystallized. Yellow crystals (ethanol), Yield: 87%, m.p. 170–172 °C; IR (KBr, cm^{-1}): (NH_2) 3342, 3252, (CH-aliphatic) 2992, (CO) 1711; ^{1}H NMR (DMSO-d_6, ppm): δ 1.20 (t, J = 7.3 Hz, 3H, CH_3), 4.36 (q, J = 7.3 Hz, 2H, CH_2), 7.37–8.19 (m, 6H, Ar and NH_2 protons), 8.72 (s, 1H, methine-H). Anal. Calcd. for $C_{12}H_{12}N_2O_4S_3$: C, 41.86; H, 3.48; N, 8.13. Found: C, 41.80; H, 3.40; N, 8.00.

*3.2. General procedure for preparation of **9a**,**b***

A mixture of **3a** (2.88 g, 100 mmol), aromatic aldehyde (100 mmol) and piperidine (100 mmol) in dioxane (20 mL) was refluxed for 4 h. The reaction mixture was left to cool and then poured into ice water. The solid product was collected by filtration, washed with water and recrystallized from an appropriate solvent.

*3.2.1. 5-(4-Methylbenzylidene)-3-(4-sulfamoylphenyl)-2-thioxo-4-thiazolidinone (**9a**)*

Yellow crystals (acetic acid), Yield: 67%, m.p. >300 °C; IR (KBr, cm^{-1}): (NH_2) 3340, 3250, (CH-arom). 3012, (CH-aliph). 2924, (CO) 1712; MS: *m*/*z* 390 (M^+), 391 (M + 1), 392 (M + 2), 148. Anal. Calcd. for $C_{17}H_{14}N_2O_3S_3$: C, 52.30; H, 3.58; N, 7.17. Found: C, 52.20; H, 3.50; N, 7.20.

*3.2.2. 5-(4-Methoxybenzylidene)-3-(4-sulfamoylphenyl)-2-thioxo-4-thiazolidinone (**9b**)*

Orange crystals (acetic acid), Yield: 74%, m.p. 296–298 °C; IR (KBr, cm^{-1}): (NH_2) 3350, 3266, (CH-aliph). 2930, (CO) 1712 cm^{-1}; ^{1}H NMR (DMSO-d_6, ppm): δ 3.88 (s, 3H, OCH_3), 7.15–7.80 (m, 8H, Ar-protons), 7.91 (s, 1H, benzylidene-H), 8.10 (s, 2H, NH_2). Anal. Calcd. for $C_{17}H_{14}N_2O_4S_3$: C, 50.24; H, 3.44; N, 6.89. Found: C, 50.10; H, 3.30; N, 6.80.

*3.3. Preparation of 4,4′-bis[3-(4-sulfamoylphenyl)-5-methylene-2-thioxo-4-thiazolidinone-5-yl]benzene (**10**)*

This compound was synthesized from **3a** (5.76 g, 200 mmol) and terephthalaldehyde (2.10 g, 100 mmol) in a manner similar to that described for the preparation of **9**. Orange crystals (acetic acid), yield: 80%, m.p. >300 °C; IR (KBr, cm^{-1}): (NH_2) 3360, 3268, (CO) 1721; ^{1}H NMR (DMSO-d_6, ppm): δ 7.78–8.05 (m, 18H, Ar, methylidene and two amino protons). Anal. Calcd. for $C_{26}H_{18}N_4O_6S_6$: C, 46.29; H, 2.67; N, 8.30. Found: C, 46.20; H, 2.60; N, 8.20.

*3.4. Formation of 4,4′-bis[5(4-methoxybenzylidene)-2-thioxo-4-thiazolidinone-3-yl]diphenylsulfone (**13**)*

This compound was synthesized from compound **12** (4.80 g, 100 mmol) and 4-methoxybenzaldehyde (2.72 g, 200 mmol) in a manner similar to that described for the preparation of **9**. Orange crystals (dimethylformamide), yield: 76%, m.p. >300 °C; IR (KBr, cm^{-1}): (CH-arom). 3010, (CH-aliph). 2925, CO 1692 cm^{-1}; MS: *m*/*z* 390 (M^+), 250, 192, 154, 140, 90, 85. Anal. Calcd. for $C_{34}H_{24}N_2O_6S_5$: C, 56.98; H, 3.35; N, 3.91. Found: C, 56.80; H, 3.30; N, 3.80.

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