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ORIGINAL PAPER

Synthesis of novel 1,8-dioxo octahydroacridine functionalized thioureas and thiazolidinones and evaluation of their antimicrobial activities

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Abstract New series of thiourea and thiazolidinone derivatives containing a 1,8-dioxo octahydroacridinyl moiety have been synthesized through the reaction of 1-(4-Amino-phenyl)-3-methyl-thiourea with various aromatic aldehydes and dimedone. All the synthesized compounds were tested against several microbial pathogens *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*.

Keywords Thiourea · Thiazolidinone · 1,8-Dioxo octahydroacridine · Dialkyl acetylenedicarboxylate · Antimicrobial

Introduction

One of the important heterocyclic systems that exists in biologically active compounds is thiazole ring. Thiazoles are present in many biologically active molecules such as antimicrobial drugs (sulfathiazole and penicillin) [1].

Acridine derivatives are another important heterocyclic structures having antimalarial, antiviral and antiallergic properties [2–4]; acridines act as powerful drugs for anti-tumor activity both in vitro and in vivo against some of rat and human tumors [5]. Some of fluorinated acridones were announced to have anticancer activity [6, 7].

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Various 1, 3-disubstituted thiourea derivatives are exceptionally flexible building blocks for the synthesis of a collection of heterocyclic compounds and exhibit a wide range of biological activities. Many thiourea derivatives exhibited remarkable antimicrobial activities [8]; 1, 3-dialkyl or diaryl thiourea derivatives showed significant antifungal activity against plant pathogens [9].

Experimental

All chemicals purchased from Merck chemical company and used without further purification.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ultrashield-400 NMR spectrometer using DMSO-d_6 as a solvent. IR spectra were recorded on a Bruker Tensor-27 FT-IR spectrophotometer using KBr pellets. Melting points were measured on an Electrothermal-9100 apparatus. Elemental analyses were run on a LECO-932 series.

Synthesis of 1-(4-Amino-phenyl)-3-methyl-thiourea 1

A mixture of 1, 4-phenylene diamine (10 mmol) and methyl isothiocyanate (10 mmol) in 20 mL ethanol was heated at 60 °C. The promotion of the reaction was monitored by TLC. When the reaction was completed (90 min), the solvent was vaporized and remaining solid was washed with the mixture of hexane/ethyl acetate (9:1). The resultant precipitate was filtered and dried. After that, the precipitate was dissolved in 20% HCl solution and filtered. Then, 10% NaOH solution was added to the filtered solution until pH 7–8 was obtained and 1-(4-Amino-phenyl)-3-methyl-thiourea **1** as a white precipitate was separated.

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1-(4-Amino-phenyl)-3-methyl-thiourea (1)

White solid, mp 173–176 °C. IR (KBr): 1565, 1630, 2935, 3000, 3165, 3370 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 2.87 (3H, d, $J = 4.0$ Hz, CH_3), 5.06 (2H, s, NH_2), 6.54 (2H, d, $J = 8.4$ Hz, arom), 6.86 (2H, d, $J = 8.4$ Hz, arom), 7.12 (1H, s, NH, thiourea), 9.07 (1H, s, NH, thiourea). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 31.3, 114.0, 126.5, 146.7, 181.1. Anal. Calcd. For $C_8H_{11}N_3S$: C, 53.01; H, 6.12; N, 23.18%. Found. C, 53.18; H, 6.31; N, 22.97%.

General procedure for the synthesis of 3a–e

A mixture of an aldehyde (1 mmol), dimedone (2 mmol), 1-(4-Amino-phenyl)-3-methyl-thiourea (1.5 mmol) and one drops of HCl in 5 mL DMF was refluxed for the appropriate time. When the reaction was completed (indicated by TLC), the mixture was discharged into cold water. The resulting precipitate was purified by column chromatography ethyl acetate/hexane (9:1) to afford decahydroacridine-1,8-diones **3a–e**.

1-Methyl-3-(4-(3, 3, 6, 6-tetramethyl-1,8-dioxo-9-phenyl-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl) phenyl)thiourea (3a)

Pale goldenrod solid; mp 168-170 °C; IR (KBr): 1637, 2869, 2956, 3027, 3058, 3323 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.72 (6H, s, $2CH_3$), 0.89 (6H, s, $2CH_3$), 1.82–2.26 (8H, m, $4CH_2$), 2.98 (3H, d, $J = 4.0$ Hz, N–CH_3), 5.07 (1H, s, CH), 7.09 (1H, t, $J = 7.2$ Hz, arom), 7.24 (2H, t, $J = 7.6$ Hz, arom), 7.33 (2H, d, $J = 7.2$ Hz, arom), 7.78 (2H, d, $J = 8.4$ Hz, arom) 8.01 (1H, s, br, NH), 9.91 (1H, s, br, NH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.3, 31.0, 31.8, 31.9, 40.9, 49.5, 112.9, 122.3, 125.7, 127.4, 127.8, 129.0, 133.1, 140.4, 146.2, 150.6, 180.9, 195.0. Anal. Calcd. For $C_{31}H_{35}N_3O_2S$: C, 72.34; H, 6.87; N, 8.18%. Found. C, 72.18; H, 6.64; N, 8.40%.

1-(4-(9-(4-chlorophenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl) phenyl)-3-methylthiourea (3b)

Pale goldenrod solid; mp 156–158 °C; IR (KBr): 1637, 2869, 2957, 3044, 3325 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.72 (6H, s, $2CH_3$), 0.89 (6H, s, $2CH_3$), 1.83–2.26 (8H, m, $4CH_2$), 2.98 (3H, d, $J = 4.0$ Hz, N–CH_3), 5.05 (1H, s, CH), 7.28–7.35 (6H, m, arom), 7.79 (2H, d, $J = 7.0$ Hz, arom), 8.02 (1H, s, br, NH), 9.92 (1H, s, br, NH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.2, 31.0, 31.6, 31.9, 40.8, 49.5, 112.5, 127.7, 127.8, 129.3, 129.8, 130.2, 132.9, 140.4, 145.1, 150.8, 180.9 (C=S), 195.0 (C=O). Anal. Calcd. For $C_{31}H_{34}ClN_3O_2S$: C, 67.93; H, 6.25; N, 7.67%. Found. C, 68.09; H, 6.15; N, 7.90%.

1-(4-(9-(4-methoxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)-3-methylthiourea (3c)

Pale goldenrod solid; mp 170–173 °C; IR (KBr): 1637, 2869, 2955, 3060, 3326 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.73 (6H, s, $2CH_3$), 0.89 (6H, s, $2CH_3$), 1.79–2.24 (8H, m, $4CH_2$), 2.94 (3H, d, $J = 3.6$ Hz, N–CH_3), 3.69 (3H, s, O-CH_3), 4.97 (1H, s, CH), 6.80 (2H, d, $J = 8.8$ Hz, arom), 7.21 (2H, d, $J = 8.8$ Hz, arom), 7.27–7.35 (2H, m, arom), 7.73 (2H, d, $J = 8.4$ Hz, arom), 7.97 (1H, s, br, NH), 9.88 (1H, s, br, NH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.3, 30.8, 31.1, 31.9, 40.8, 49.5, 54.8, 113.1, 113.2, 122.4, 128.3, 128.9, 133.1, 138.5, 140.2, 150.3, 157.2, 180.8 (C=S), 195.0 (C=O). Anal. Calcd. For $C_{32}H_{37}N_3O_3S$: C, 70.69; H, 6.86; N, 7.73%. Found. C, 70.88; H, 6.97; N, 7.95%.

1-methyl-3-(4-(3, 3, 6, 6-tetramethyl-9-(4-nitrophenyl)-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)thiourea (3d)

Saddle brown solid; mp 175–178 °C; IR (KBr): 1642, 2868, 2957, 3058, 3345, 3389 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.71 (6H, s, $2CH_3$), 0.90 (6H, s, $2CH_3$), 1.83–2.27 (8H, m, $4CH_2$), 2.98 (3H, d, $J = 4.0$ Hz, N–CH_3), 5.13 (1H, s, CH), 7.39 (2H, d, $J = 8.0$ Hz, arom), 7.58 (2H, d, $J = 8.8$ Hz, arom), 7.77 (2H, d, $J = 8.0$ Hz, arom), 8.02 (1H, s, br, NH), 8.15 (2H, d, $J = 8.4$ Hz, arom), 9.94 (1H, s, br, NH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.1, 31.0, 31.9, 32.7, 40.8, 49.3, 111.8, 121.9, 123.3, 128.8, 129.2, 140.4, 140.5, 145.6, 151.3, 153.6, 180.9, 195.0. Anal. Calcd. For $C_{31}H_{34}N_4O_4S$: C, 66.64; H, 6.13; N, 10.03%. Found. C, 66.82; H, 6.25; N, 10.27%.

1-(4-(9-(3, 4-dihydroxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)-3-methylthiourea (3e)

Pale goldenrod solid; mp 255–258 °C; IR (KBr): 1631, 2871, 2966, 3314 cm-1. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.75 (6H, s, $2CH_3$), 0.89 (6H, s, $2CH_3$), 1.78–2.22 (8H, m, $4CH_2$), 2.98 (3H, d, $J = 4.0$ Hz, N–CH_3), 4.88 (1H, s, CH), 6.53–6.58 (2H, m, arom), 6.74 (1H, d, $J = 1.6$ Hz, arom), 7.30–7.36 (2H, m, arom), 7.74 (2H, d, $J = 8.8$ Hz, arom), 7.95 (1H, s, br, NH), 8.46 (1H, s, br, OH), 7.70 (1H, s, br, OH), 9.83 (1H, s, br, NH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.2, 27.9, 28.6, 29.3, 30.6, 31.9,

40.8, 49.6, 113.3, 115.0, 115.1, 118.0, 135.3, 137.6, 140.2, 143.1, 144.4, 150.0, 160.2, 162.3, 180.8, 195.0. Anal. Calcd. For $C_{31}H_{35}N_3O_4S$: C, 68.23; H, 6.46; N, 7.70%. Found. C, 68.08; H, 6.58; N, 7.93%.

General procedure for the synthesis of 4a–j

To a magnetically stirred solution of **3** (1 mmol) in ethanol (10 mL) was added DAAD (1 mmol) at ambient temperature. The reaction mixture was then stirred for the appropriate time. The solvent was removed under reduced pressure and the residue recrystallized from 2:1 hexane–ethyl acetate affording products **4a–j**.

Methyl (Z)-2-((E)-3-methyl-4-oxo-2-((4-(3, 3, 6, 6-tetramethyl-1,8-dioxo-9-phenyl-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)imino) thiazolidin-5-ylidene)acetate (4a)

Pale yellow solid; mp 152–154 °C; IR (KBr): 1637, 1728, 2869, 2954, 3028, 3059 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.83 (6H, s, $2CH_3$), 1.00 (6H, s, $2CH_3$), 1.90–2.38 (8H, m, $4CH_2$), 3.43 (3H, s, N–CH_3), 3.82 (3H, s, O-CH_3), 5.14 (1H, s, CH), 6.93 (1H-vinyl, s, CH), 7.18 (1H, t, $J = 7.6$ Hz, arom), 7.30–7.35 (4H, m, arom), 7.41 (2H, d, $J = 6.8$ Hz, arom), 7.53–7.54 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.0, 29.2, 29.3, 31.9, 32.0, 40.9, 49.5, 52.5, 113.0, 115.5, 122.6, 125.7, 127.5, 127.8, 135.1, 140.9, 145.7, 146.2, 147.8, 150.4, 152.5, 164.3, 165.6, 195.0. Anal. Calcd. For $C_{36}H_{37}N_3O_5S$: C, 69.32; H, 5.98; N, 6.74%. Found. C, 69.36; H, 6.17; N, 6.49%.

Ethyl (Z)-2-((E)-3-methyl-4-oxo-2-((4-(3, 3, 6, 6-tetramethyl-1,8-dioxo-9-phenyl-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)imino) thiazolidin-5-ylidene)acetate (4b)

Pale yellow solid; mp 121–123 °C; IR (KBr): 1637, 1698, 1726, 2870, 2957, 3059 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.72 (6H, s, $2CH_3$), 0.89 (6H, s, $2CH_3$), 1.19 (3H, t, $J = 7.2$ Hz, CH_3), 1.80–2.27 (8H, m, $4CH_2$), 3.31 (3H, s, N–CH_3), 4.17 (2H, q, $J = 7.2$ Hz, O-CH_2), 5.05 (1H, s, CH), 6.78 (1H-vinyl, s, CH), 7.07 (1H, t, $J = 7.2$ Hz, arom), 7.19–7.24 (4H, m, arom), 7.31 (2H, d, $J = 8.0$ Hz arom), 7.42–7.43 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 13.8, 26.0, 28.6, 29.2, 31.8, 31.9, 40.9, 49.5, 61.4, 113.0, 115.8, 125.7, 127.5, 127.7, 127.8, 128.0, 135.1, 140.7, 146.2, 147.8, 150.3, 152.5, 164.3, 165.1, 194.9. Anal. Calcd. For $C_{37}H_{39}N_3O_5S$: C, 69.68; H, 6.16; N, 6.59%. Found. C, 69.79; H, 6.29; N, 6.81%.

Methyl (Z)-2-((E)-2-((4-(9-(4-chlorophenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4c)

Pale yellow solid; mp 154–156 °C; IR (KBr): 1637, 1728, 2870, 2955, 3063 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.75 (6H, s, $2CH_3$), 0.92 (6H, s, $2CH_3$), 1.83–2.30 (8H, m, $4CH_2$), 3.35 (3H, s, N–CH_3), 3.74 (3H, s, O-CH_3), 5.05 (1H. s, CH), 6.85 (1H-vinyl, s, CH), 7.23 (2H, d, $J = 8.8$ Hz, arom), 7.30 (2H, d, $J = 8.4$ Hz, arom), 7.35 (2H, d, $J = 8.4$ Hz, arom), 7.48 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.2, 29.3, 31.7, 31.9, 40.9, 49.4, 52.5, 112.6, 115.5, 122.5, 127.8, 129.4, 129.8, 130.2, 135.0, 140.9, 145.1, 147.8, 150.6, 152.4, 164.3, 165.6, 194.9. Anal. Calcd. For $C_{36}H_{36}ClN_3O_5S$: C, 65.69; H, 5.51; N, 6.38%. Found. C, 65.86; H, 5.70; N, 6.15%.

Ethyl (Z)-2-((E)-2-((4-(9-(4-chlorophenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4d)

Pale yellow solid; mp 151–153 °C; IR (KBr): 1637, 1697, 1726, 2870, 2958 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.82 (6H, s, $2CH_3$), 0.99 (6H, s, $2CH_3$), 1.30 (3H, t, $J = 7.2$ Hz, CH_3), 1.89–2.37 (8H, m, $4CH_2$), 3.40 (3H, s, N–CH_3), 4.27 (2H, q, $J = 7.2$ Hz, O-CH_2), 5.11 (1H, s, CH), 6.89 (1H-vinyl, s, CH), 7.30 (2H, d, $J = 8.4$ Hz, arom), 7.37–7.43 (4H, m, arom), 7.55 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 13.9, 26.1, 29.2, 29.3, 31.7, 31.9, 40.9, 49.4, 61.4, 112.6, 115.8, 122.4, 122.5, 127.8, 129.4, 130.2, 135.0, 140.7, 145.1, 147.8, 150.6, 152.5, 164.3, 165.1, 195.0. Anal. Calcd. For $C_{37}H_{38}ClN_3O_5S$: C, 66.11; H, 5.70; N, 6.25%. Found. C, 66.27; H, 5.82; N, 6.46%.

Methyl (Z)-2-((E)-2-((4-(9-(4-methoxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4e)

Pale yellow solid; mp 139–142 °C; IR (KBr): 1637, 1728, 2834, 2954, 3060 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.90 (6H, s, $2CH_3$), 1.06 (6H, s, $2CH_3$), 1.94–2.43 (8H, m, $4CH_2$), 3.49 (3H, s, N–CH_3), 3.84 (3H, s, O-CH3), 3.88 (3H, s, O-CH_3), 5.13 (1H, s, CH), 6.96 (2H, d, $J = 8.4$ Hz, arom), 6.99 (1H-vinyl, s, CH), 7.37 (4H, m, $J = 8.4$ Hz, arom), 7.59 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 28.6, 29.3, 30.9, 31.9, 40.9, 49.5, 52.5, 54.8, 113.2, 113.3, 115.4, 122.5, 128.4, 128.9, 135.2, 138.5, 140.9, 147.7, 150.1, 152.5, 157.2, 164.3,

165.7, 195.0. Anal. Calcd. For $C_{37}H_{39}N_3O_6S$: C, 67.97; H, 6.01; N, 6.43%. Found. C, 67.79; H, 5.90; N, 6.62%.

Ethyl (Z)-2-((E)-2-((4-(9-(4-methoxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4f)

Pale yellow solid; mp 122–125 °C; IR (KBr): 1636, 1726, 2834, 2957, 3060 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.75 (6H, s, $2CH_3$), 0.91 (6H, s, $2CH_3$), 1.22 (3H, t, $J = 7.2$ Hz, CH_3), 1.80–2.28 (8H, m, $4CH_2$), 3.34 (3H, s, N–CH_3), 3.69 (3H, s, O-CH_3), 4.19 (2H, q, $J = 7.2$ Hz, O-CH_2), 4.98 (1H, s, CH), 6.81 (2H, d, $J = 8.8$ Hz, arom), 6.82 (1H-vinyl, s, CH), 7.23 (4H, d, $J = 8.8$ Hz, arom), 7.43–7.45 (2H, m, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 13.9, 26.1, 28.6, 29.3, 30.9, 31.9, 40.9, 49.5, 54.8, 61.5, 113.2, 113.3, 115.7, 122.3, 128.4, 128.9, 135.2, 138.5, 140.8, 147.8, 150.1, 152.5, 157.2, 164.3, 165.1, 195.0. Anal. Calcd. For $C_{38}H_{41}N_3O_6S$: C, 68.34; H, 6.19; N, 6.29%. Found. C, 68.49; H, 6.26; N, 6.08%.

Methyl (Z)-2-((E)-3-methyl-4-oxo-2-((4-(3, 3, 6, 6-tetramethyl-9-(4-nitrophenyl)-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)imino) thiazolidin-5-ylidene)acetate (4g)

Brown solid; mp 174–177 °C; IR (KBr): 1637, 1727, 2870, 2955, 3036, 3062 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.74 (6H, s, $2CH_3$), 0.92 (6H, s, $2CH_3$), 1.84–2.31 (8H, m, $4CH_2$), 3.35 (3H, s, N–CH_3), 3.74 (3H, s, O-CH_3), 5.14 (1H. s, CH), 6.85 (1H-vinyl, s, CH), 7.24 (2H, d, $J = 7.6$ Hz, arom), 7.55 (2H, d, $J = 8.4$ Hz, arom), 7.61 (2H, d, $J = 8.8$ Hz, arom), 8.16 (2H, d, $J = 8.8$ Hz, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.1, 29.3, 31.9, 32.8, 40.9, 49.3, 52.5, 111.9, 115.4, 122.2, 123.3, 128.9, 134.9, 140.9, 142.4, 145.6, 147.9, 151.1, 152.5, 153.6, 164.3, 165.7, 195.0. Anal. Calcd. For $C_{36}H_{36}N_4O_7S$: C, 64.66; H, 5.43; N, 8.38%. Found. C, 64.79; H, 5.52; N, 8.60%.

Ethyl (Z)-2-((E)-3-methyl-4-oxo-2-((4-(3, 3, 6, 6-tetramethyl-9-(4-nitrophenyl)-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl)imino) thiazolidin-5-ylidene)acetate (4h)

Brown solid; mp 169–171 °C; IR (KBr): 1639, 1697, 1724, 2958, 3074 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.74 (6H, s, $2CH_3$), 0.92 (6H, s, $2CH_3$), 1.22 (3H, t, $J = 7.2$ Hz, CH_3), 1.84–2.31 (8H, m, $4CH_2$), 3.35 (3H, s, N–CH_3), 4.19 (2H, q, $J = 7.2$ Hz, O-CH_2), 5.14 (1H, s, CH), 6.81 (1H-vinyl, s, CH), 7.24 (2H, d, $J = 7.6$ Hz, arom), 7.54 (2H, d, $J = 7.6$ Hz, arom) 7.61 (2H, d, $J = 8.8$ Hz, arom), 8.15 (2H, d, $J = 8.8$ Hz, arom). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 13.9, 26.1, 29.1, 29.2, 31.9, 32.8, 40.9, 49.3, 61.5, 111.9, 113.8, 115.7, 122.3, 123.2, 127.9, 128.9, 134.9, 140.7, 145.6, 147.9, 151.1, 152.6, 153.6, 164.3, 165.1, 195.3. Anal. Calcd. For $C_{37}H_{38}N_4O_7S$: C, 65.09; H, 5.61; N, 8.21%. Found. C, 65.26; H, 5.73; N, 8.42%.

Methyl (Z)-2-((E)-2-((4-(9-(3, 4-dihydroxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4i)

Pale yellow solid; mp 244–247 °C; IR (KBr): 1634, 1727, 2870, 2955, 3063, 3446 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.78 (6H, s, $2CH_3$), 0.92 (6H, s, $2CH_3$), 1.79–2.28 (8H, m, $4CH_2$), 3.39 (3H, s, N–CH_3), 3.74 (3H, s, O-CH_3), 4.91 (1H, s, CH), 6.59 (2H, s, arom), 6.78 (1H, s, arom), 6.84 (1H-vinyl, s, CH), 7.24 (2H, d, $J = 5.6$ Hz, arom), 7.45 (2H, s, arom), 8.48 (1H, s, br, OH), 8.73 (1H, s, br, OH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 26.1, 29.2, 29.3, 30.7, 31.9, 40.9, 49.6, 52.5, 113.5, 115.0, 115.1, 115.5, 118.1, 121.8, 123.8, 135.3, 137.6, 140.8, 143.1, 144.4, 147.7, 149.8, 152.4, 164.3, 165.6, 195.0. Anal. Calcd. For $C_{36}H_{37}N_3O_7S$: C, 65.94; H, 5.69; N, 6.41%. Found. C, 65.76; H, 5.82; N, 6.22%.

Ethyl (Z)-2-((E)-2-((4-(9-(3, 4-dihydroxyphenyl)-3, 3, 6, 6-tetramethyl-1,8-dioxo-2, 3, 4, 5, 6, 7, 8, 9-octahydroacridin-10(1H)-yl)phenyl) imino)-3-methyl-4-oxothiazolidin-5-ylidene)acetate (4j)

Pale yellow solid; mp 235–238 °C; IR (KBr): 1634, 1726, 2870, 2957, 3438 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz), δ (ppm): 0.77 (6H, s, $2CH_3$), 0.91 (6H, s, $2CH_3$), 1.21 (3H, t, $J = 7.2$ Hz, CH_3), 1.78–2.27 (8H, m, $4CH_2$), 3.34 (3H, s, N–CH_3), 4.19 (2H, q, $J = 7.2$ Hz, O-CH_2), 4.90 (1H, s, CH), 6.58 (2H, s, arom), 6.77 (2H, s, arom), 6.80 (1H-vinyl, s, CH), 7.23 (2H, d, $J = 7.2$ Hz, arom), 7.44 (2H, s, arom), 8.50 (1H, s, br, OH), 8.75 (1H, s, br, OH). ^{13}C NMR (DMSO-d_6 100 MHz), δ (ppm): 13.8, 26.1, 27.9, 29.3, 30.7, 31.9, 40.9, 49.6, 61.5, 113.4, 115.0, 115.1, 115.7, 118.1, 121.8, 123.9, 135.3, 137.5, 140.7, 143.1, 144.4, 147.7, 149.8, 152.5, 164.3, 165.1, 195.0. Anal. Calcd. For $C_{36}H_{37}N_3O_7S$: C, 66.35; H, 5.87; N, 6.27%. Found. C, 66.16; H, 5.99; N, 6.45%.

Determination of antimicrobial activity

Initial antibacterial screening was carried out by the disk diffusion method using paper disks. The sterilized (autoclaved at 120 °C for 30 min), liquified Mueller–Hinton agar (40–50 °C) was inoculated (1 mL/100 mL of medium) with

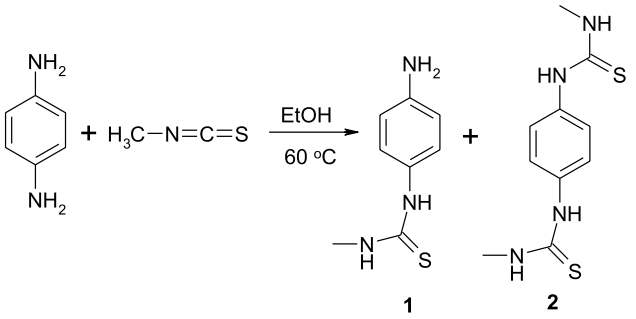
the suspension of the microorganism (turbidity was standardized to 0.5 McFarland) and discharged into a Petri dish to give a depth of 3–4 mm. The paper disks impregnated with the test compounds (100 mg/mL in dimethylsulfoxide) were placed on the solidified medium. The plates were incubated at 37 °C for 24 h.

Minimum inhibitory concentration (MIC)

MIC was ascertained for the compounds showing antimicrobial activity. Broth microdilution method was used for the determination of MIC values. The compounds were dissolved in DMSO to make 100 mg/mL final concentration. Then the compounds added to broth media in 96 wells of microtiter plates using two-fold serial dilution (from 100 mg/mL to 49 µg/mL). Thereafter, 100 µL inoculum of standard size was added to each well. Bacterial suspensions were used as positive control. The microtiter plates were incubated at 37 °C for 24 h. In wells where there was no visible growth, 10 µL of suspension was subcultured to media agar plates, and the agar plates were incubated at 37 °C for colony count. MBC was defined as the lowest dilution showing *P* value 99.9% kill after 24 h of incubation.

Anti-biofilm assessment (ABF)

Anti-biofilm effect of the synthesized compounds was studied by microtiter plate adhesion assessment. Biofilm formation in this assay was determined by a literature method [10]. In this study, a culture of the bacteria and fungi was grown over night in the broth media. Then, the cultures were diluted 1:100 into fresh medium for biofilm assessments. 100 µL of the dilution was added on well in a 96-well dish. For quantitative assays, we typically use 4 reproduced wells for each treatment. The microtiter plate was incubated for 24 h at 37 °C. After incubation, cells were unloaded by turning the plate over and shaking out the liquid. Finally, the biofilms were discolored by the 0.1% crystal violet. The microtiter plate was incubated at room temperature for 10–15 min. After that, the plate was washed 3–4 times with water and dried. Absorbance at 540 nm was read after dissolution of the dye with 95% ethanol and in an enzyme-linked immunosorbent assay (ELISA) plate reader. Values of biofilm formation of all strains were compared with the data for the negative control.



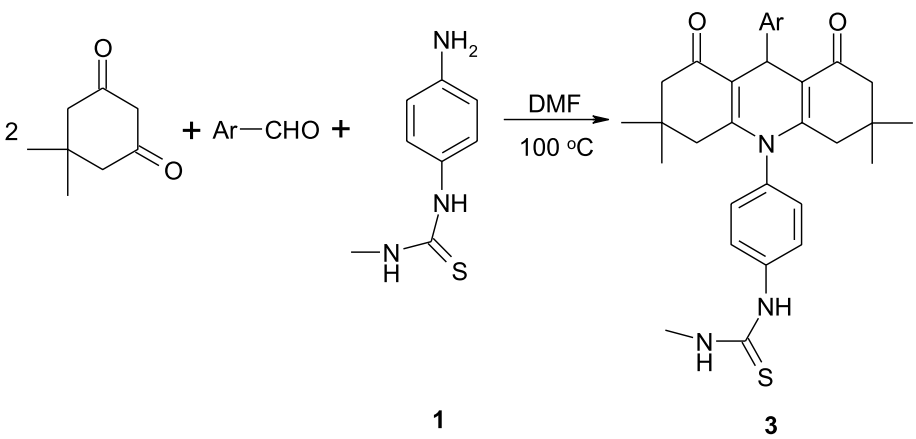
Scheme 1 Synthesis of 1-(4-Amino-phenyl)-3-methyl-thiourea

Results and discussion

As a part of our research program to synthesis of novel 1,8-acridindinones and thiazolidinones [11, 12], we have used 1-(4-Amino-phenyl)-3-methyl-thiourea **1** to synthesize various 1,8- acridinyl thiourea derivatives **3** with the goal to have antimicrobial activities. The synthesized acridinones were then reacted with dialkyl acetylenedicarboxylates (DAAD) to form thiazolidine-4-ones **4**.

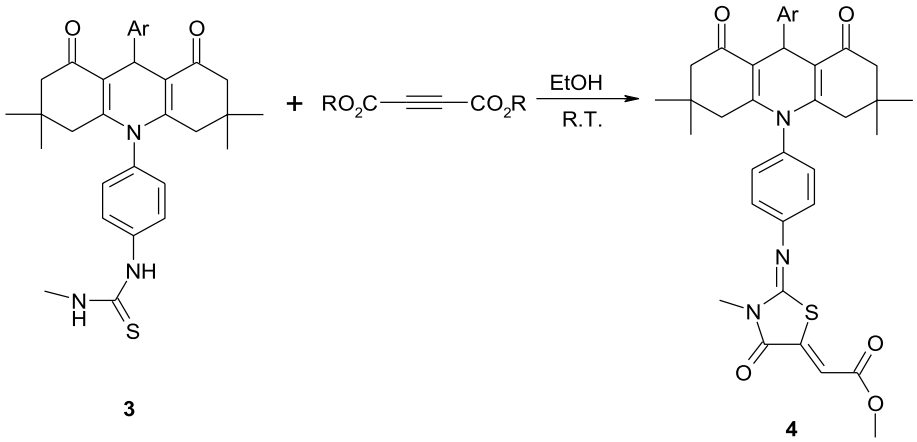
In the first stage, amino thiourea **1** was synthesized from the reaction of *p*-phenylene diamine and methyl isothiocyanate in ethanol as solvent. The 1:1 ratio of reactants was used to reduce the formation of 1, 1′-(1, 4-phenylene)bis(3-methylthiourea) **2**. However, bis thiourea **2** was formed and removed by extraction with HCl (Scheme 1).

The synthesis of acridinyl thiourea **3** was performed by the three-component reaction of dimedone, aromatic



Scheme 2 Synthesis of acridinyl thiourea derivatives

Scheme 3 Synthesis of thiazolidin-4-ones from acridinyl thioureas **3**



aldehydes and 1-(4-Amino-phenyl)-3-methyl-thiourea **1** in DMF as solvent. One drop of HCl was used as a cheap and easy available catalyst (Scheme 2).

To obtain the optimized temperature, the reaction was performed at 60, 80, 100 and 120 °C. Best results were obtained at 100 °C.

The scope and generality of this reaction was checked by performing the reaction with aromatic aldehydes having electron-donating and electron-withdrawing groups. As Table 1 shows, electron-donating groups decrease the reaction rate.

The structures of compounds **3a–e** were designated on the basis of their spectral data and elemental analysis. Compound **3a** displayed a broad peak in the IR spectrum for NH protons at 3323 cm^{-1}. The ^{1}H NMR spectrum of **3a** shows two singlet for methyl protons at 0.72 and 0.82 ppm. A distinct peak was seen in 5.07 ppm for aliphatic CH proton.

In the ^{13}C NMR spectrum of **3a**, signals corresponding to thiourea and carbonyl group were observed at 180.9 and 195.0 ppm.

The cycloaddition of these stable acridinyl thiourea derivatives **3**, with DAAD resulted in the formation of thiazolidin-4-ones **4a–j** with good yields (Scheme 3).

The reaction was performed at room temperature without any catalyst, the results are summarized in Table 2.

The chemical structure of thiazolidinones **4a–j** was clarified from their IR, ^{1}H NMR and ^{13}C NMR spectra. The IR spectrum of **4a** showed strong bands at 1637 and 1728 cm^{-1} related to carbonyl groups. The ^{1}H NMR spectrum of **4a** showed clearly the presence of OCH_3 at 3.82 ppm. The singlet sharp peak at 6.93 ppm was attributed to the vinyl CH proton.

The ^{13}C NMR spectrum of **4a** supported the presence of ester and amide carbonyl groups at the 164.3 and 165.6 ppm.

Antimicrobial activities

The antibacterial and antifungal activity of the synthesized compounds was tested in vitro against the following bacterial strains: two Gram-positive bacteria, including *Staphylococcus aureus* PTCC1112, *Micrococcus luteus* PTCC1110, and three Gram-negative bacteria, *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and one fungi strain, *Candida albicans* PTCC5011. To determine the antimicrobial activity of the target compounds, the disk diffusion test was employed according to NCCLS guidelines [13] (Table 3).

Control positive tests were accomplished under similar conditions by using trimethoprim/sulfamethoxazole (SXT), ciprofloxacin for antibacterial activity and fluconazole for antifungal activity as standard drugs. Sterile disks impregnated with DMSO were employed as a negative control.

The results of the antibacterial and antifungal activities are presented in Table 3.

In this study, acridinyl thioureas **3** (except **3b**) were found to exhibit antibacterial activity only against Gram-positive strains *Staphylococcus aureus* or *Micrococcus luteus*, while some of the thiazolidinones **4** showed broader antibacterial spectrum and were effective on Gram-positive and Gram-negative bacteria. It is also noteworthy to mention that the compounds **4b** and **4d** showed antifungal activity whereas related thioureas showed no antifungal activity.

Minimum inhibitory concentration (MIC) was determined for the compounds showing antimicrobial activity. The lowest concentration showing no growth was taken as the MIC.

Anti-biofilm effect was another factor which was investigated for the active compounds.

Table 1 Synthesis of 1,8-dioxo octahydroacridine functionalized thioureas **3**

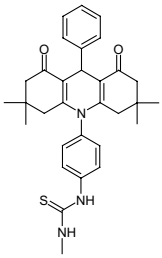
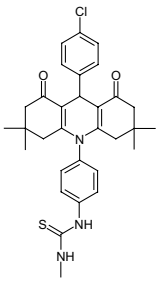
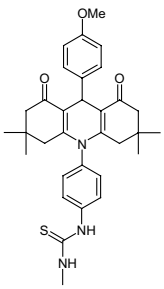
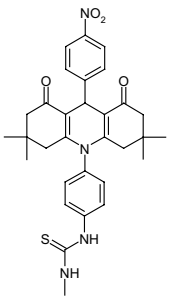
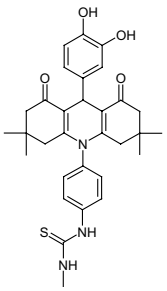
Compounds	Structure	Time (min)	Yield (%)
3a		30	61
3b		30	70
3c		75	90
3d		30	80
3e		60	62

Table 2 Thiazolidin-4-one derivatives **4** from 1,8-dioxo octahydroacridinyl thioureas **3** and DAAD

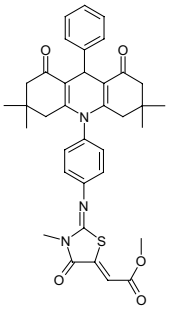
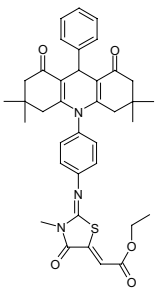
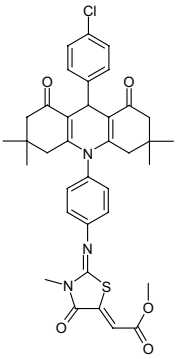
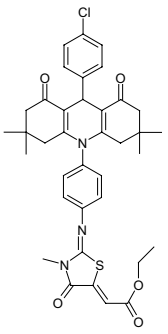
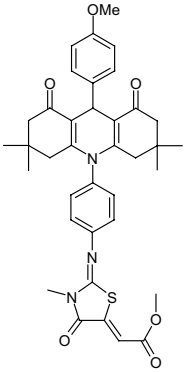
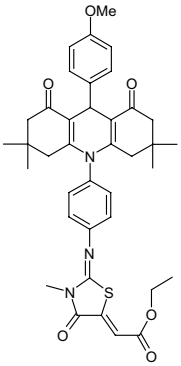
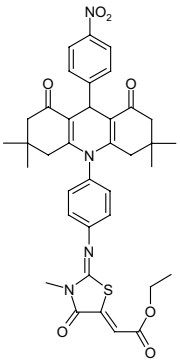
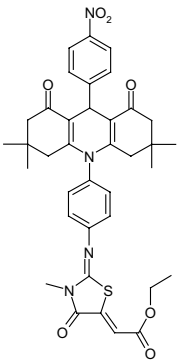
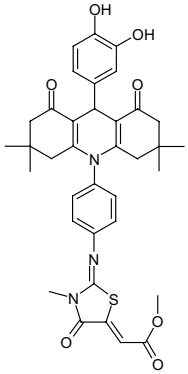
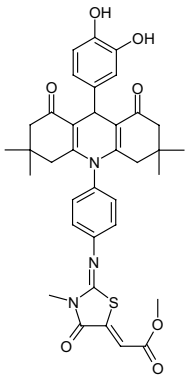
Compound	Structure	Time (h)	Yield (%)
4a		4	70
4b		4	68
4c		4	80
4d		4	85

Table 2 continued

Compound	Structure	Time (h)	Yield (%)
4e		4	65
4f		4	60
4 g		6	70
4 h		6	75
4i		6	88
4j		6	82

A biofilm is any group of microorganisms in which cells stick to each other and often these cells stick to a surface.

Anti-biofilm effect of the synthesized compounds was investigated by microtiter plate adhesion assay. A literature method was used for the determination of anti-biofilm formation [10].

The results of MIC and anti-biofilm formation (ABF) are reported in Table 4.

Conclusion

In conclusion, a series of novel 1,8-dioxo octahydroacridine functionalized thiourea and thiazolidinone derivatives were synthesized and their antimicrobial activities have been evaluated. Some of these compounds (**4d** and **4j**) showed potent inhibition against clinical strains such as *Pseudomonas aeruginosa* and *Klebsiella pneumonia* that are resistant to SXT and ciprofloxacin.

Table 3 Antimicrobial effect of acridinyl thioureas **3** and thiazolidinones **4**

Compound	Inhibition zone diameter (mm)					
	S. aureus PTCC1112	*M. luteus* PTCC1110	*E. coli* PTCC1330	*P. aeruginosa**	*K. pneumoniae**	*C. albicans* PTCC5011
3a	12	0	0	0	0	0
3b	0	0	0	0	0	0
3c	0	22	0	0	0	0
3d	0	19	0	0	0	0
3e	0	21	0	0	0	0
4a	12	0	0	0	0	0
4b	0	0	0	0	0	21
4c	16	0	0	0	0	0
4d	21	0	11	0	19	19
4e	0	0	0	0	0	0
4f	0	0	0	0	0	0
4 g	16	0	0	0	0	0
4 h	0	0	0	0	0	0
4i	16	0	0	0	0	0
4j	0	0	0	12	0	0
SXT	18	30	22.5	0	0	0
Ciprofloxacin	25	31	27	0	0	0
FL(25 µg)	–	–	–	–	–	32

* Isolated clinical samples

Table 4 Comparison of ABF (mg/mL) and MIC (mg/mL) of the acridinyl thioureas **2** and thiazolidinones **3**

Compounds	*S. aureus* PTCC1112		*M. luteus* PTCC1110		*E. coli* PTCC1330		*P. aeruginosa**		*K. pneumoniae**		*C. albicans* PTCC5011	
	ABF	MIC	ABF	MIC	ABF	MIC	ABF	MIC	ABF	MIC	ABF	MIC
3a	1.56	3.125	–	–	–	–	–	–	–	–	–	–
3c	–	–	0.097	6.25	–	–	–	–	–	–	–	–
3d	–	–	0.049	1.56	–	–	–	–	–	–	–	–
3e	–	–	0.097	1.56	–	–	–	–	–	–	–	–
4c	0.39	6.25	–	–	–	–	–	–	–	–	–	–
4d	1.56	6.25	–	–	0.049	6.25	–	–	6.25	12.5	ND**	6.25
4a	–	6.25	–	–	–	–	–	–	–	–	–	–
4b	–	–	–	–	0.78	3.125	–	–	–	–	ND	6.25
4 g	–	3.125	–	–	–	–	–	–	–	–	–	–
4i	0.195	6.25	–	–	–	–	–	–	–	–	–	–
4j	–	–	–	–	–	–	ND	6.25	–	–	–	–

* Isolated clinical samples

** Not Determined

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