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Inhibitory effect of some new uracil and thiouracil derivatives on cercarial penetration enzymes

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Abstract Some uracil- and thiouracil-5-sulfonohydrazide derivatives have been synthesized to be evaluated as antischistosomal agents. *N*-[2-(1,5-Dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**3c**) was formulated in jojoba oil and used to paint mice tails before infection with *Schistosoma mansoni* cercariae. Using Boc-Val-Leu-Gly-Arg-$_{P}$NA, a specific substrate for trypsin-like serine proteinases, compound **3c** inhibited cercarial serine protease activity with 50 % inhibition concentration (IC_{50}) of 160 μg. Upon topical application on mice tails before infection with *S. mansoni* cercariae, it caused a 20 % reduction in worm burden compared with untreated infected mice. Using soluble crude cercarial antigen in enzyme-linked immunosorbent assay (ELISA), no significant changes were observed in the levels of immunoglobulin M (IgM) and IgG in sera from treated infected mice at 2, 4, and 6 weeks postinfection (WPI) compared with the level in sera from infected untreated mice. At 4 WPI, sera from treated infected mice showed significantly low ($P < 0.05$) IgM reactivity to crude soluble worm antigen compared with infected nontreated ones. IgG levels in sera from treated infected mice at 2 and 4 WPI were significantly lower ($P < 0.05$) than in sera from infected nontreated mice. At 6 WPI, the IgG response showed no significant differences in sera from both mice groups. Sera from treated infected mice at 2, 4, and 6 WPI had

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generally lower IgM reactivity to soluble egg antigen when compared with the level in sera from nontreated infected mice. At all time points postinfection, sera collected from treated infected mice showed significantly low IgG reactivity ($P < 0.05$) compared with infected nontreated mice.

Keywords Cercariae · Cercarial serine protease · IgM · IgG · 4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonohydrazide · *Schistosoma mansoni*

Introduction

Schistosomiasis (formerly called bilharziasis or bilharziosis) is a parasitic disease produced by a flatworm of the class trematoda, relatively common in developing countries, especially in Africa, called schistosoma (or esquistosoma). More than 207 million individuals are infected worldwide, and in addition, 700 million are at risk of infection [1]. Around 90 % of individuals who need preventive chemotherapy for schistosomiasis are in Africa, where the disease is endemic in 42 countries [1]. Chemotherapy is an important control strategy against this parasitic disease [2]; however, it has not reduced endemicity [3], and rapid reinfection demands frequent treatment [4]. Therefore, it is considered that an effective vaccine combined with chemotherapy would be an efficient control mechanism [5]. The initial step in the infection of humans by schistosomal parasites is penetration of cercariae, the infective stage of the parasite, into the host skin [6, 7]. Attempts to develop a topical formulation to prevent this step date back to Pellegrino [8]. Niclosamide was tested as a cercaricidal compound in the field for its ability to prevent such infections [9, 10]. Some efficacy of niclosamide in preventing infection by *Schistosoma mansoni* but not by *Schistosoma haematobium* was reported, but this was insufficient to warrant a recommendation for its widespread use. Penetration of cercariae into the host skin is facilitated by secretion of a potent cercarial serine protease from the preacetabular glands of cercariae [11–13]. Several uracil and thiouracil derivatives constitute an interesting group of compounds, many of which possess widespread pharmacological activities especially as antiviral [14], antibacterial [15], antitubercular [16], inhibition of serine protease of *S. mansoni* [12], antihypertensive [17], analgesic and anti-inflammatory [18], anticonvulsant [19], and anticancer agents [20]. As a continuation of our previous studies on uracil and thiouracil chemistry [12, 21–23], the present work deals with synthesis of new compounds carrying these important nuclei in order to study their inhibitory effects on cercarial penetration enzymes to block penetration of *S. mansoni* cercariae into mouse skin.

Experimental

All melting points were uncorrected and determined in capillary tubes by melting point microscope (Boetius; Great Britain Stuart Scientific Co. Ltd.). Infrared (IR) spectra were recorded on an infrared spectrophotometer (PU9712; Beckman, Madison, WI, USA) with KBr discs. ^{1}H and ^{13}C nuclear magnetic resonance (NMR)

spectra were measured on a Joel EX500-MHZ (Japan) spectrometer using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded by mass spectrometer (SSQ7000; California, USA) at 70 eV. Microanalyses were carried out at the Microanalytical Center, Cairo University. All reactions were followed and checked by thin-layer chromatography (TLC) using chloroform/methanol (3:1), and spots were examined by UV analysis lamp λ 254/366 nm.

Synthesis of the starting compound (**1**)

The starting material thiouracil-5-sulfonylhdrazide (**1**) was prepared by reaction of thiouracil-5-sulfonylchloride with hydrazine hydrate 99 % [12].

*General procedure for synthesis of the Schiff bases (**2a–d**)*

A mixture of thiouracil-5-sulfonylhdrazide (**1**, 2.3 g, 10 mmol) and the appropriate aldehyde, namely 4-bromobenzaldehyde, 5-methyl-2-furfural, 4-antipyrinecarboxaldehyde or salicylaldehyde (10 mmol), in absolute ethanol (25 ml) was stirred at room temperature for 2 days. The reaction mixture was cooled, and the formed precipitate was then filtered off and dried. The obtained product was crystallized from the proper solvent to give the title compounds **2a**–**d**, respectively.

*N′-(4-Bromobenzylidin)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**2a**)*

Yield: 64 %; m.p.: 251–253 °C; crystallized from dimethylformamide (DMF)/water; IR (KBr, cm^{-1}): 3325, 3225, 3122 (3NH), 1680 (CO of thiouracil), 1322, 1125 (SO_2NH); ^{1}H-NMR (dimethyl sulfoxide, DMSO-d_6, δ ppm): 7.30, 7.71 (d, d, 4H, aromatic H, $J = 6.5$ Hz), 8.42 (s, 1H, thiouracil-H6), 8.90 (s, 1H, –N=CH), 11.10, 11.33, 11.80 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.3 (C=S), 164.0 (C=O), 103.1, 125.1, 131.2, 132.4, 133.0, 143.7, 150.1 (aromatic-C; CH=N); MS *m/z*: 388 (M^+) (30 %), 390 ($M^+ + 2$) (29 %); Anal. calcd. for $C_{11}H_9BrN_4O_3S_2$ (389.25): C, 33.94; H, 2.33; N, 14.39. Found: C, 33.82; H, 2.31; N, 14.34.

*N′-[(5-Methyl-2-furyl)methylene]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**2b**)*

Yield: 72 %; m.p.: 263–265 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3318, 3217, 3198 (3NH), 1680 (CO of thiouracil), 1327, 1162 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.33 (s, 3H, CH_3), 6.31, 7.20 (d,d, 2H, furan ring, $J = 7.4$ Hz), 8.34 (s, 1H, thiouracil-H6), 8.72 (s, 1H, –N=CH), 10.32, 11.41, 11.72 (s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.1 (C=S), 164.6 (C=O), 103.2, 106.7, 110.2, 134.7, 147.3, 150, 152.5 (aromatic-C; CH=N), 15.1 (CH_3); MS *m/z*: 314 (M^+) (34 %); Anal. calcd. for $C_{10}H_{10}N_4O_4S_2$ (314.36): C, 38.20; H, 3.21; N, 17.82. Found: C, 38.17; H, 3.16; N, 17.78.

*N′-[(1,5-Dimethyl–3-oxo-2-phenylpyrazolidin-4-yl)methylene]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**2c**)*

Yield: 66 %; m.p.: 275–277 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3325, 3225, 3215 (3NH), 3080 (C–H aromatic), 1712 (CO of pyrazoline ring), 1683 (CO of thiouracil), 1345, 1127 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 3.71 (s, 3H, –N–CH_3), 6.43–7.33 (m, 5H, aromatic-H), 8.2 (s, 1H, thiouracil-H6), 9.10 (s, 1H, –N=CH), 10.11, 11.01, 11.42 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 176.1 (C=S), 165.2, 164.1 (2C=O), 103.2, 102.7, 113.2, 119.2, 129.1, 136.7, 147.5, 150, 154.2 (aromatic-C; CH=N), 15.3, 15.5 ($2CH_3$); MS *m/z*: 420 (M^+) (6 %); Anal. calcd. for $C_{16}H_{16}N_6O_4S_2$ (420.48): C, 45.70; H, 3.83; N 19.98; Found: C, 45.43; H, 3.71; N, 19.95.

*N′-(2-Hydroxybenzylidin)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**2d**)*

Yield: 71 %; m.p.: 281–283 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3450 (OH), 3315, 3225, 3115 (3NH), 1692 (CO of thiouracil), 1343, 1125 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.31–7.80 (m, 4H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 8.74 (s, 1H, –N=CH), 10.32, 10.99, 11.62, 12.21 (4s, 4H, 3NH, OH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.1 (C=S), 164.0 (C=O), 103.1, 116.7, 118.2, 130.3, 121.0, 143.2, 150.5, 161.4 (aromatic-C; CH=N); MS *m/z*: 326 (M^+) (17 %); Anal. calcd. for $C_{11}H_{10}N_4O_4S_2$ (326.38): C, 40.48; H, 3.09; N 17.17. Found: C, 40.39; H, 3.04; N, 17.11.

General procedure for preparation of thiazolidinone derivatives **3a–d**

A solution of thioglycolic acid (1 g, 10 mmol) in dry benzene (15 ml) was added to a solution of the compound **2a–d** (10 mmol) in dry benzene (20 ml), then the mixture was refluxed on a water bath for 8–12 h. The formed precipitate was filtered off and crystallized from the proper solvent to give **3a–d**.

*N-[2-(4-Bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**3a**)*

Yield: 70 %; m.p.: 271–273 °C; recrystallized from ethanol; IR (KBr, cm^{-1}): 3410, 3321, 3225 (3NH), 1725 (CO of thiazolidine), 1672 (CO of thiouracil), 1320, 1135 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 4.30 (s, 2H, CH_2), 4.52 (s, 1H, –N–CH), 6.3, 7.4 (d,d 4H, aromatic-H, $J = 8.4$ Hz), 8.23 (s, 1H, thiouracil-H6), 10.32, 10.52, 11.33 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.1 (C=S), 170.1, 164.0 (2C=O), 103.2, 121.2, 131.1, 132.0, 138.4 150.9 (aromatic-C), 51.5, 55.3 (thiazolidinone-C); MS *m/z*: 462 ($M^{+\cdot}$) (11 %), 464 ($M^+ + 2$) (10 %); Anal. calcd. for $C_{13}H_{11}BrN_4O_4S_3$ (463.36): C, 33.69; H, 2.39; N, 12.09. Found: C, 33.56; H, 2.16; N, 12.05.

*N-[2-(5-Methyl-2-furyl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**3b**)*

Yield: 72 %; m.p.: 280–282 °C; crystallized from ethanol; IR (KBr, cm^{-1}): 3340, 3225, 3210 (3NH), 1715 (CO of thiazolidine), 1695 (CO of thiouracil), 1323, 1120 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.43 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.41 (s, 1H, –N–CH, thiazolidine), 7.11, 7.59 (d, d, 2H, furan-H, $J = 7.0$ Hz), 8.10 (s, 1H, thiouracil-H6), 10.41, 11.23, 11.52 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.0 (C=S), 164.2, 169.3 (2C=O), 103.2, 107.2, 149.2, 150.0, 151.0, 152.5 (aromatic-C), 34.1, 58.2 (thiazolidinone-C), 15.4 (CH_3); MS *m/z*: 388 (M^+) (12 %); Anal. calcd. for $C_{12}H_{12}N_4O_5S_3$ (388.45): C, 37.10; H, 3.11; N, 14.42; Found: C, 37.03; H, 3.08; N, 14.39.

*N-[2-(1,5-Dimethyl-3-oxo-2-phenylpyrazolin-4–yl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**3c**)*

Yield: 65 %; m.p.: 290–292 °C; recrystallized from ethanol; IR (KBr, cm^{-1}): 3330, 3321, 3225 (3NH), 1721 (CO of thiazolidine), 1695 (CO of thiouracil), 1323, 1122 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.62 (s, 3H, CH_3), 3.80 (s, 3H, –N–CH_3), 4.52 (s, 2H, CH_2), 4.72 (s, 1H, –N–CH), 6.60–7.31 (m, 5H, aromatic-H), 8.22 (s, 1H, thiouracil-H6), 10.21, 10.40, 11.62 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.0 (C=S), 164.2, 169.3, 166.2 (3C=O), 103.2, 104.2, 121.5, 124.1, 129.1, 134.1, 150.1, 154.0 (aromatic-C), 33.5, 70.9 (thiazolidinone-C), 15.2, 15.8 ($2CH_3$); MS *m/z*: 494 (M^+) (4 %); Anal. calcd. for $C_{18}H_{18}N_6O_5S_3$ (494.58): C, 43.71; H, 3.66; N, 16.99; Found: C, 43.65; H, 3.44; N, 16.76.

*N-[2-(2-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**3d**)*

Yield: 70 %; m.p.: >300 °C; recrystallized from ethanol; IR (KBr, cm^{-1}): 3343, 3225, 3219 (3NH), 1724 (CO of thiazolidine), 1675 (CO of thiouracil), 1325, 1120 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 4.23 (s, 2H, CH_2), 4.62 (s, 1H, –N–CH), 7.31–7.80 (m, 4H, aromatic-H), 8.12 (s, 1H, thiouracil-H6), 10.32, 11.23, 11.6, 12.01 (4s, 4H, 3NH, OH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.0 (C=S), 164.2, 169.3 (2C=O), 103.5, 114.2, 158.2, 130.1, 150.5, 154.0 (aromatic-C), 35.5, 56.9 (thiazolidinone-C), MS *m/z*: 400 (M^+) (13 %); Anal. calcd. for $C_{13}H_{12}N_4O_5S_3$ (400.46): C, 38.99; H, 3.01; N, 13.98; Found: C, 38.65; H, 3.24; N, 13.76.

General procedure for synthesis of Mannich bases **4a**, **b**, **5b**, **c**, and **6c**, **d**

A mixture of paraformaldehyde (0.5 g, 5 mmol) and the appropriate amine, namely *N*-methyl piperazine, diethylamine or morpholine (52 mmol), in ethanol (25 ml) was refluxed for 0.5 h till complete solubility of paraformaldehyde. A warmed solution of thiazolidinone derivative **3a**–**d** (20 mmol) in absolute ethanol (30 ml) was then added to the reaction mixture. The whole mixture was refluxed for 6–9 h

and then left at room temperature for 3 days. The volatile material was evaporated under vacuum, and the dry residue was extracted with chloroform to give **4a**, **b**, **5b**, **c**, and/or **6c**, **d**, respectively.

*4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonic acid [5-(4-methyl-piperazine-1-ylmethyl)-4-oxo-2-(5-methyl-2-furyl)-thiazolidin-3-yl]amide (**4a**)*

Yield: 65 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3335, 3230, 3210 (3NH), 1723 (CO of thiazolidine), 1697 (CO of thiouracil), 1322, 1125 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.11–3.50 (m, 7H, N–CH_3, CH_2–N–CH_2), 3.62–3.82 (m, 4H, CH_2–N–CH_2), 4.11 (d, 2H, CH_2, $J = 6.2$ Hz), 4.44 (s, 1H –N–CH), 6.11 (t, 1H, $J = 6.6$ Hz, S–CH), 6.53–7.40 (d,d, 4H, aromatic-H $J = 7.2$ Hz), 8.32 (s, 1H, thiouracil-H6), 10.5, 10.7, 11.2 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 179.1 (C=S), 171.1, 165.2 (2C=O), 103.1, 121.1, 131.0, 131.9, 138.1, 150.3 (aromatic-C), 52.3, 54.1, 56.3, 52.0, 55.2, 43.3 (thizolidinone-C; –CH_2–N); MS *m/z*: 574 (M^+) (7 %), 576 ($M^+ + 2$) (6.5 %); Anal. calcd. for $C_{19}H_{23}BrN_6O_4S_3$ (575.53): C, 39.65; H, 4.02; N, 14.60; Found: C, 39.61; H, 4.11; N, 14.56.

*4-Oxo-2-thioxo-1,2,3,4 3-tetrahydropyrimidine-5-sulfonic acid [5-(4-methyl-piprazin-1-ylmethyl)-4-oxo-2-(5-methyl-2-furyl)-thiazolidin-3-yl]amide (**4b**)*

Yield: 71 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3335, 3240, 3220 (3NH), 1723 (CO of thiazolidine), 1692 (CO of thiouracil), 1322, 1125 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.31 (s, 3H, CH_3), 3.13-3.5 (m, 7H, –N–CH_3, CH_2–N–CH_2), 3.60–3.81 (m, 4H, CH_2–N–CH_2), 4.12 (d, 2H, CH_2, $J = 6.5$ Hz), 4.43 (s, 1H, –N–CH), 6.14 (t, 1H, $J = 6.5$ Hz, S–CH), 7.20–7.41 (d, d, 2H, furan ring, $J = 7.4$ Hz), 8.42 (s, 1H, thiouracil-H6), 10.24, 10.91, 11.31 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 179.1 (C=S), 171.1, 165.2 (2C=O), 103.1, 107.2, 149.4, 150.1, 150.3, (aromatic-C), 52.3, 54.1, 56.3, 52.0 (thizolidinone-C; –CH_2–N), 43.2, 15.2 ($2CH_3$); MS *m/z*: 500 (M^+) (3 %); Anal. calcd. for $C_{18}H_{24}N_6O_5S_3$ (500.63): C, 43.18; H, 4.83; N, 16.78; Found: C, 43.15; H, 4.93; N, 16.63.

*4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonic acid (5-diethyl-amino-methyl-4-oxo-(5-methyl-2-furyl)–thiazolidin-3-yl)amide (**5b**)*

Yield: 67 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3335, 3246, 3229 (3NH), 1723 (CO of thiazolidine), 1692 (CO of thiouracil), 1322, 1130 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.11, 1.30 (2t, 6H, $2CH_3$ of ethyl gp), 2.31 (s, 3H, CH_3)), 4.22 (q, 4H, $J = 7.9$ Hz, $2CH_2$),), 4.47 (d, 2H, CH_2, $J = 5.9$ Hz), 4.56 (s, 1H, –N–CH), 6.10 (t, 1H, $J = 6.9$ Hz, S–CH), 7.12, 7.52 (d,d, 2H, furan ring, $J = 6.8$ Hz), 8.41 (s, 1H, thiouracil-H6), 10.22, 11.00, 11.53 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.0 (C=S), 164.2, 169.3 (2C=O), 103.1, 109.0, 143.2, 143.4, 150.1 (aromatic-C), 48.5, 50.2, 54.1, 55.6, (thiazolidinone-C; $3CH_2$), 13.4, 14.3 ($3CH_3$); MS *m/z*: 473 (M^+) (30 %). Anal.

calcd. for $C_{17}H_{23}N_5O_5S_3$ (473.60): C, 43.11; H, 4.89; N, 14.78; Found: C, 43.05; H, 4.83; N, 14.63.

*4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonic acid (5-diethyl-aminomethyl-2-(1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl)-4-oxo-(thiazolidin-3-yl)amide (**5c**)*

Yield: 65 %; m.p.: >300 °C; recrystallized from DMF/water. IR (KBr, cm^{-1}): 3325, 3219, 3210 (3NH), 1723 (CO of thiazolidine), 1687 (CO of thiouracil), 1322, 1115 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.11, 1.31 (2t, 6H, $J = 6.7$ Hz, $2CH_3$), 2.42 (s, 3H, CH_3), 3.80 (s, 3H, –N–CH_3), 4.20 (q, 4H, $J = 7.7$ Hz, $2CH_2$ of ethyl gp), 4.45 (d, 2H, $J = 5.7$ Hz, CH_2), 4.58 (s, 1H, –N–CH), 6.13 (t, 1H, $J = 6.7$ Hz, S–CH), 7.10–7.5 (m, 5H, aromatic-H), 8.30 (s, 1H, thiouracil-H6), 10.32, 11.01, 11.23 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.0 (C=S), 163.3, 164.2, 169.3 (3C=O), 103.1, 147.2, 119.1, 113.1, 129.4, 136.1, 150.1 (aromatic-C), 48.5, 50.2, 54.1, 55.6, (thiazolidinone-C; $3CH_2$), 13.4, 14.3 ($3CH_3$); MS *m/z*: 480 (M^+) (52 %); Anal. calcd. for $C_{23}H_{29}N_7O_5S_3$ (579.73): C, 47.65; H, 5.04; N, 16.91; Found: C, 47.52; H, 5.31; N, 16.81.

*4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonic acid (1,5-dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-5-[(morpholin-4-yl-)methyl]-4-oxo–(thiazolidin-3-yl)amide (**6c**)*

Yield: 61 %; m.p.: >300 °C; recrystallized from DMF/water; IR (KBr, cm^{-1}): 3350, 3220, 3210 (3NH), 1710 (CO of thiazolidine), 1691 (CO of thiouracil), 1320, 1118 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.21 (s, 3H,CH_3), 2.41, 3.31 (m, 4H, CH_2–N–CH_2), 3.82 (s, 3H, N–CH_3), 3.71, 4.21 (m, 4H, CH_2–O–CH_2), 4.63 (s, 1H, –N–CH), 4.82 (d, 2H, $J = 6.$ 1 Hz, CH_2), 6.22 (t, 1H, $J = 6.1$ Hz, S–CH), 6.69–7.50 (m, 5H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 10.32, 10.61, 11.30 (3s, 3H,3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 176.0 (C=S), 164.2, 165.2, 169.5 (3C=O), 103.1, 112.3, 115.2, 130.2, 131.1, 147.2, 150.2, 156.6 (aromatic-C), 52.3, 53.2, 66.8, 54.1, 56.3 (thiazolidinone-C; morpholine-C; –CH_2–N), 15.4 ($2CH_3$); MS *m/z*: 593 (M^+) (5 %); Anal. calcd. for $C_{23}H_{27}N_7O_6S_3$ (593.71): C, 46.52; H, 4.58; N, 16.51; Found: C, 46.48; H, 4.52; N, 16.47.

*4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonic acid -5[(morpholin-4-yl)methyl]-4-oxo-2-[(2-hydroxyphenyl)-thiazolidine-3-yl]amide (**6d**)*

Yield: 55 %; m.p.: >300 °C; recrystallized from DMF/water; IR (KBr, cm^{-1}): 3332, 3240, 3215 (3NH), 1723 (CO of thiazolidine), 1697 (CO of thiouracil), 1322, 1125 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.32, 3.12 (m. 4H, CH_2–N–CH_2), 3.92, 4.10 (m, 4H, CH_2–O–CH_2), 4.62 (s, 1H, –N–CH), 4.81 (d, 2H, $J = 6.2$ Hz CH_2), 6.21 (t, 1H, $J = 6.7$ Hz, –S–CH), 7.27–7.90 (m, 4H, aromatic-H), 8.13 (s, 1H, thiouracil), 10.30, 10.51, 11.60, 12.01 (4s, 4H, 3NH, OH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 176.0 (C=S), 165.2, 169.5 (2C=O), 103.1, 115.2, 130.2, 131.1, 150.2, 156.6 (aromatic-C), 52.3, 53.2, 66.8, 54.1, 56.3 9 (thiazolidinone-C;

morpholine-C; $–CH_2–N$); Ms *m/z*: 499 (M^+) (40 %); Anal. calcd. for $C_{18}H_{21}N_5O_6S_3$ (499.59): C, 43.27; H, 4.23; N, 14.02; Found: C, 43.21; H, 4.41; N, 14.12.

General procedure for synthesis of *N*-[4-(6-amino or oxo-5-cyano-4-substituted phenyl)-1,2-dihydropyridin-2-yl)-phenyl]-2,4-dioxo or 4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamides **8a–c**, **9a–c**, **10a–c**, and **11a–c**

A mixture of the compound **7a**, **b** (3 mmol), the appropriate aldehyde, namely 2-chloro-5-nitrobenzaldehyde, 2,5-dimethoxybenzaldehyde and 3,5-dimethoxybenzaldehyde (3 mmol), malononitrile or ethyl cyanoacetate (21 mmol), and ammonium acetate (24 mmol) in *n*-butanol (50 ml) was refluxed for 8–10 h. The reaction mixture was concentrated to half volume, then cooled and left overnight. The formed precipitate was filtered off, dried, then crystallized from the proper solvent.

*N-[4-(6-Amino-5-cyano-4-(2-chloro-5-nitrophenyl)-1,2-dihydropyridin-2-yl)phenyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**8a**)*

Yield: 65 %; m.p.: >300 °C; recrystallized from DMF/water; IR (KBr, cm^{-1}): 3467 (NH_2), 3442, 3320, 3220 (3NH), 2212 (CN), 1735, 1691 (2 CO of uracil), 1563, 1350 (NO_2), 1327, 1129 (SO_2NH); ^{1}H-NMR (DMSO-d_6 δ ppm): 4.92 (s, 2H, NH_2 exchangeable with D_2O), 6.52 (s, 1H, pyridine-H3), 7.21–7.69 (m, 7H, aromatic-H), 8.40 (s, 1H, uracil-H6), 10.21, 10.80, 11.22 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.5, 164.2 (2C=O), 162.0 (C–NH_2), 150.0 (C–NO_2), 100.2, 107.2, 111.1, 116.8, 119.1, 120.2, 124.3, 129.3, 126.3, 128.4, 133.2, 136.4, 142.1, 145.2 158.2 (aromatic-C), 117.2 (CN); MS *m/z*: 540 (M^+) (40 %), 542 ($M^{+\cdot}$+2) (13 %); Anal. calcd. for $C_{22}H_{14}ClN_7O_6S$ (539.92): C, 48.94; H, 2.61; N, 18.16; Found: C, 48.92; H, 2.59; N, 18.11.

*N-[4-(6-Amino-5-cyano-4-(2,5-dimethoxyphenyl)-1,2-dihydropyridin-2-yl)phenyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**8b**)*

Yield: 68 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3553 (NH_2), 3440, 3321, 3210 (3NH), 2209 (CN), 1722, 1685 (2CO of uracil), 1327, 1129 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.62, 3.71 (2s, 6H, $2OCH_3$), 4.72 (s, 2H, NH_2), 6.62 (s, 1H, pyridine-H3), 7.41–7.82 (m, 7H, aromatic-H), 8.52 (s, 1H, uracil-H6), 10,00, 10.62, 11.21, (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 153.2, 164.2 (2CO), 162.0 (C–NH_2), 103.1, 118.3, 112.0, 124.2, 127.9, 127.4, 128.2, 129.3, 138.2, 139.2, 150.0, 152.3, 155.2, 159.1 (aromatic-C), 117.0 (CN), 55.9 ($2OCH_3$); MS *m/z*: 520 (M^+) (40 %); Anal. calcd. for $C_{24}H_{20}N_6O_6S$ (520.53): C, 55.37; H, 3.87; N, 16.14; Found: C, 55.46; H, 3.85; N 16.44.

*N-[4-(6-Amino-5-cyano-4-(3,5-dimethoxyphenyl)-1,2-dihydropyridin-2-yl)phenyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**8c**)*

Yield: 71 %; m.p.: 290–292 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3540 (NH_2), 3435, 3430, 3220 (3NH), 2211 (CN), 1720, 1689 (2CO of uracil),

1325, 1220 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.59, 3.80 (2s, 6H, $2OCH_3$), 4.82 (s, 2H, NH_2), 6.71 (s, 1H, pyridine-H3), 7.32–7.72 (m, 7H, aromatic-H), 8.32 (s, 1H, uracil-H6), 10,21, 10.52, 11.30 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.5, 164.2 (2C=O), 162.0 (C–NH2), 100.2, 100.9, 103.3, 107.2, 111.1, 116.8, 120.2, 126.3, 128.4, 136.4, 140.2, 142.1, 158.2, 162.2 (aromatic-C), 117.3 (CN), 55.9 ($2OCH_3$) MS *m*/*z*: 520 (M^+) (40 %); Anal. calcd. for $C_{24}H_{20}N_6O_6S$ (520.52): C, 55.37; H; 3.87; N, 16.14; Found: C, 55.35, H, 3.94; N, 16.00.

*N-[4-(6-Amino-5-cyano-4-(2-chloro,5-nitrophenyl)-1,2-dihydropyridin-2-yl)phenyl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**9a**)*

Yield: 66 %; m.p.: 295–297 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3520 (NH_2), 3420, 3360, 3240 (3NH), 2212 (CN), 1720 (CO of thiouracil), 1560, 1347 (NO_2), 1327, 1129 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 4.90 (s, 2H, NH_2 exchangeable with D_2O), 6.51 (s, 1H, pyridine-H3), 7.21, 7.61 (m, 7H, aromatic-H), 8.41 (s, 1H, uracil-H6), 10.21, 11.21, 11.63 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.9 (C=S), 164.2 (C=O), 162.4 (C–NH_2), 151.2 (C–NO_2), 101.2, 107.2, 111.2, 116.8, 119.1, 120.2, 124.3, 129.3, 126.3, 128.4, 133.2, 136.4, 142.1, 145.2, 158.2 (aromatic-C), 117.3 (CN); MS *m*/*z*: 540 (M^+) (40 %), 542 ($M^+ + 2$) (13 %); Anal. calcd. for $C_{22}H_{14}ClN_7O_5S_2$ (555.97): C, 47.94; H, 2.54; N, 17.64; Found: C, 48.22; H, 2.39; N, 18.01; MS *m*/*z*: 555 (M^+) (27 %), 557 ($M^+ + 2$).

*N-[4-(6-Amino-5-cyano-4-(2,5-dimethoxyphenyl)-1,2-dihydropyridin-2-yl)phenyl]4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**9b**)*

Yield: 68 %; m.p.: 288–290 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3510 (NH_2), 3439, 3346, 3250 (3NH), 2212 (CN), 1720 (CO of thiouracil), 1329, 1120 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.61, 3.80 (2s, 6H, $2OCH_3$), 4.90 (s, 2H, NH_2 exchangeable with D_2O), 6.51 (s, 1H, pyridine-H3), 7.21–7.62 (m, 7H, aromatic-H), 8.41 (s, 1H, thiouracil-H6), 10.21, 10.83, 11.24, (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.3 (C=S), 164.2 (CO), 162.2 (C–NH_2), 103.5, 118.3, 112.2, 124.2, 127.9, 127.4, 128.2, 129.3, 138.2, 139.2, 150.0, 152.3, 155.2, 156.1 (aromatic-C), 117.0 (CN), 54.8 ($2OCH_3$); MS *m*/*z*: 536 (M^+) (15 %); Anal. calcd. for $C_{24}H_{20}N_6O_5S_2$ (536.59): C, 53.72; H, 3.75; N, 15.66; Found: C, 53.70; H, 3.70; N, 15.59.

*N-[4-(6-Amino-5-cyano-4-(3,5-dimethoxyphenyl)-1,2-dihydropyridin-2-yl)phenyl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**9c**)*

Yield: 71 %; m.p.: 287–289 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3520 (NH_2), 3435, 3340, 3210 (3NH), 2212 (CN), 1720 (CO of thiouracil), 1327, 1129 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.61, 3.80 (2s, 6H, $2OCH_3$), 4.91 (s, 2H, NH_2 exchangeable with D_2O), 6.52 (s, 1H, pyridine-H3), 7.22–7.63 (m, 7H, aromatic-H), 8.42 (s, 1H, thiouracil-H6), 10.21, 10.83, 11.25 (3s, 3H, 3NH

exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 179.2 (C=S), 164.2 (C=O), 162.0 (C–NH2), 100.2, 100.9, 103.3, 107.2, 111.1, 116.8, 120.2, 126.3, 128.4, 136.4, 140.2, 142.1, 158.2, 162.2 (aromatic-C), 117.2 (CN), 55.9 ($2OCH_3$); MS *m/z* 536 (M^+) (17 %); Anal. calcd. for $C_{24}H_{20}N_6O_5S_2$ (536.59): C, 53.72; H, 3.75; N, 15.66; Found: C, 53.67; H, 3.65; N, 15.52.

*N-{4-[4-(2-Chloro-5-nitrophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**10a**)*

Yield: 68 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3532, 3219, 3160, 3157 (4NH), 2209 (CN), 1720, 1697, 1689 (3CO), 1550, 1347 (NO_2), 1322, 1127 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 6.42 (s, 1H, pyridone-H3), 7.11–7.80 (m, 7H, aromatic-H), 8.32 (s, 1H, uracil-H6), 9.82, 10.31, 11.23, 12.23 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.5, 162.0, 164.2 (3C=O), 150.3 (C–NO_2), 101.2, 108.2, 111.1, 116.8, 119.1, 121.2, 124.3, 129.3, 126.3, 128.4, 133.2, 136.4, 142.1, 145.2 158.2 (aromatic-C), 117.2 (CN); MS *m/z* 540.5 (M^+) (30 %), 542.5 (M^++**2**) (10 %); Anal. calcd. for $C_{22}H_{13}ClN_6O_7S$ (540.90): C, 48.85; H, 2.42; N, 15.53; Found: C, 48.83; H, 2.40; N, 15.32.

*N-{4-[5-Cyano-4-(3,5-dimethoxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**10b**)*

Yield: 62 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3442, 3260, 3160, 3157 (4NH), 2209 (CN), 1715, 1689, 1679 (3CO), 1322, 1126 (SO_2NH); ^{1}H-NMR (DMSO-δ, ppm): 3.71, 3.96 (2s, 6H, $2OCH_3$), 6.42 (s, 1H, pyridone-H3), 7.33–7.90 (m, 7H, aromatic-H), 8.32 (s, 1H, uracil-H6), 9.81–10.31, 11.20, 12.25 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 153.2, 162.3, 164.2 (3CO), 103.1, 118.3, 112.0, 124.2, 127.9, 127.4, 128.2, 129.3, 138.2, 139.2, 150.0, 152.3, 155.2, 159.1 (aromatic-C), 117.0 (CN), 55.9 ($2OCH_3$); MS *m/z* 521 (M^+) (25 %); Anal. calcd. for $C_{24}H_{19}N_5O_7S$ (521.51): C, 55.27; H, 3.67; N, 13.42; Found: C, 55.55; H, 3.85; N, 13.49.

*N-{4-[5-Cyano-4-(2,5-dimethoxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**10c**)*

Yield: 65 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3442, 3360, 3264, 3157 (4NH), 2209 (CN), 1725, 1685, 1676 (3CO), 1321, 1122 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.62, 3.90 (2s, 6H, $2OCH_3$), 6.64 (s, 1H, pyridone-H3), 7.21–8.13 (m, 7H, aromatic-H), 8.41 (s, 1H, thiouracil-H6), 9.92, 10.82, 11.52, 12.32 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.5, 163.0, 164.2 (3C=O), 100.2, 100.9, 103.3, 107.2, 111.1, 116.8, 120.2, 126.3, 128.4, 136.4, 140.2, 142.1, 158.2, 162.2 (aromatic-C), 117.2 (CN), 54.9 ($2OCH_3$); MS *m/z* 521.5 (M^+) (23 %); Anal. calcd. for $C_{24}H_{19}N_5O_7S$ (521.50): C, 55.27; H, 3.67; N, 13.42; Found: C, 55.34; H, 3.64; N, 13.60.

*N-{4-[4-(2-Chloro-5-nitrophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**11a**)*

Yield: 67 %; m.p.: 294–296 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3510, 3442, 3264, 3157 (4NH), 2205 (CN), 1720, 1685 (2CO), 1560, 1350 (NO_2), 1322, 1126 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 6.41 (s, 1H, pyridone-H3), 7.32–8.22 (m, 7H, aromatic-H), 8.52 (s, 1H, thiouracil-H6), 9.82, 10.51, 11.22, 11.52 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.9 (C=S), 164.4, 165.2 (2C=O), 150.1 (C–NO_2), 101.2, 107.2, 111.2, 116.8, 119.1, 120.2, 124.3, 129.3, 126.3, 128.4, 133.2, 136.4, 142.1, 145.2, 155.2 (aromatic-C), 117.1 (CN); MS *m/z* 556 (M^+) (27 %), 558 ($M^+ + 2$) (9 %); Anal. calcd. for $C_{22}H_{13}ClN_6O_6S_2$ (556.96): C, 47.44; H, 2.35; N, 15.08; Found: C, 47.64; H, 2.41; N, 15.34.

*N-{4-[5-Cyano-4-(3,5-dimethoxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**11b**)*

Yield: 71 %; m.p.: 286–288 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3490, 3360, 3265, 3224 (4NH), 2208 (CN), 1718, 1680 (2CO), 1320, 1120 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.71, 3.92 (2s, 6H, $2OCH_3$), 6.52 (s, 1H, pyridone-H3), 7.11–8.32 (m, 7H, aromatic-H), 8.42 (s, 1H, thiouracil-H6), 9.82, 10.51, 11.23, 11.53 (4s, 4H, 4 NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.3 (C=S), 162.2, 164.2 (2CO), 103.5, 119.3, 111.2, 125.2, 127.4, 127.9, 128.2, 129.3, 138.2, 139.2, 151.0, 152.3, 155.2, 156.1 (aromatic-C), 117.2 (CN), 55.8 ($2OCH_3$); MS *m/z* 537.5 (M^+) (22 %); Anal. calcd. for $C_{24}H_{19}N_5O_6S_2$ (537.57): C, 53.62; H, 3.56; N, 13.02; Found: C, 53.40, H, 3.54; N, 13.31.

*N-{4-[5-Cyano-4-(2,5-dimethoxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**11c**)*

Yield: 68 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3490, 3357, 3265, 3125 (4NH), 2208 (CN), 1718, 1680 (2CO), 1320, 1120 (SO_2NH); ^{1}H NMR (DMSO-d_6, δ ppm): 3.61, 3.82 (2s, 6H, $2OCH_3$), 6.32 (s, 1H, pyridone-H3), 7.21–8.42 (m, 7H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 9.82, 10.51, 11.02, 11.51 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 179.2 (C=S), 163.0, 164.2 (C=O), 100.2, 102.9, 103.3, 107.2, 111.1, 117.8, 120.2, 126.3, 128.4, 136.4, 140.2, 142.1, 158.2, 162.2 (aromatic-C), 117.2 (CN), 55.7 ($2OCH_3$); MS *m/z* 537.5 (M^+) (21 %); Anal. calcd. for $C_{24}H_{19}N_5O_6S_2$ (537.57): C, 53.62, H, 3.56; N, 13.02; Found: C, 53.59; H, 3.54; N, 13.22.

General procedure for synthesis of chalcones **12a–c** and **13a–c**

A mixture of **7a**, **b** (3 mol) and the appropriate aromatic aldehyde, namely 2-chloro-5-nitrobenzaldehyde, 2,5-dimethoxybenzaldehyde, and 3,5-dimethoxybenzaldehyde (3 mol), in 10 % ethanolic sodium hydroxide solution (50 ml) was stirred at room temperature for 24 h, then refluxed for 1 h. After reaction completion, the mixture was cooled and poured onto ice-cold water. The precipitate that appeared after

neutralization with diluted HCl was filtered off and crystallized from acetic acid to give the desired products.

*N-{4-[(2Z)-3-(2-Chloro-5-nitrophenyl)prop-2-enoyl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**12a**)*

Yield: 63 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^1): 3349, 3340, 3215 (3NH), 1724, 1715, 1685 (3CO), 1557, 1350 (NO_2), 1322, 1130 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 6.51, 6.80 (d,d, 2H, $J = 7.2$ Hz, –CH=CH–), 7.30–8.22 (m, 7H, aromatic-H), 8.32 (s, 1H, uracil-H6), 9.82, 10.61, 11.00 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.2, 170.2 (3C=O), 100.1, 116.8, 119.2, 121.4, 123.3, 127.1, 128.7, 130.2, 132.4, 139.5, 142.2, 143.3, 145.2, 149.3(aromatic-C; HC=CH); MS *m/z* 476.5 (M^+) (15 %), 478 ($M^+ + 2$) (5 %); Anal. calcd. for $C_{19}H_{13}ClN_4O_7S$ (476.86): C, 47.85; H, 2.74; N, 11.74; Found: C, 47.44; H, 2.73; N, 11.53.

*N-{4-[(2Z)-3-(2,5-Dimethoxyphenyl)prop-2-enoyl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**12b**)*

Yield: 65 %, m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3349, 3219, 3160 (3NH), 1720, 1710, 1683 (3CO), 1322, 1128 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.62, 3.81 (2s, 6H, 2 OCH_3), 6.32, 6.61 (d,d, 2H, $J = 7.2$ Hz – CH=CH–), 7.21–8.51 (m, 7H, aromatic-H), 8.43 (s, 1H, uracil-H6), 9.52, 10.42, 11.22 (s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.4, 170.6 (3C=O), 100.1, 101.5, 106.4, 107.3, 116.8, 121.4, 127.1, 128.4, 130.2, 142.2, 143.3, 145.2, 158.3, 158.5 (aromatic-C; HC=CH), 55.9 ($2OCH_3$); MS *m/z* 457.5 (M^+) (17 %); Anal. calcd. for $C_{21}H_{19}N_3O_7S$ (457.45): C, 55.13; H, 4.18; N, 9.18; Found: C, 55.12; H, 4.17; N, 9.07.

*N-{4-[(2Z)-3-(3,5-Dimethoxyphenyl)prop-2-enoyl]phenyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**12c**)*

Yield: 68 %, m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}) 3421, 3232, 3119 (3NH), 1715, 1697, 1685 (3CO), 1322, 1130 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.56, 3.81 (2s, 6H, $2OCH_3$), 6.22, 6.52 (d,d, 2H, $J = 7.4$ Hz –CH=CH–), 7.22–8.11 (m, 7H, aromatic-H), 8.32 (s, 1H, uracil-H6), 9.62, 10.22, 11.32 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.4, 170.6 (3C=O), 100.1, 101.5, 106.5, 107.3, 117.8, 121.4, 127.7, 128.4, 131.2, 142.2, 143.3, 145.2, 158.1, 158.8 (aromatic-C; HC=CH), 56.2 ($2OCH_3$); MS *m/z* 457.5 (M^+) (13 %); Anal. calcd. for $C_{21}H_{19}N_3O_7S$ (457.54): C, 55.13; H, 4.18; N, 9.18; Found: C, 55.11, H, 4.00; N, 9.36.

*N-{4-[(2Z)-3-(2-Chloro-5-nitrophenyl)prop-2-enoyl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**13a**)*

Yield: 66 %; m.p.: >300 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3232, 3219, 3157 (3NH), 1720, 1684 (2CO), 1560, 1346 (NO_2), 1321, 1127 (SO_2NH); ^{1}H-

NMR (DMSO-d_6, δ ppm): 6.52, 6.80 (d,d, 2H, $J = 7.2$ Hz, –CH=CH–), 7.32–8.21 (m, 7H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 9.82, 10.62, 11.00 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.4 (C=S), 164.2, 170.2 (2C=O), 100.5, 117.8, 119.2, 121.4, 124.3, 127.1, 128.7, 130.2, 132.4, 138.5, 142.2, 143.3, 145.2, 149.3 (aromatic-C; HC=CH); MS *m/z* 492 (M^+) (15 %), 494 ($M^+ + 2$) (5 %); Anal. calcd. for $C_{19}H_{13}ClN_4O_6S_2$ (492.92): C, 46.29; H, 2.65; N 11.36; Found: C, 46.26; H, 2.44; N, 11.15.

*N-{4-[(2Z)-3-(2,5-Dimethoxyphenyl)prop-2-enoyl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**13b**)*

Yield: 69 %; m.p. 292–294 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3352, 3220, 3150 (3NH), 1723, 1687 (2CO), 1325, 1127 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.51, 3.83 (3s, 6H, $2OCH_3$), 6.21, 6.54 (d, d, 2H, $J = 6.9$ Hz – CH=CH–), 7.22–8.11 (m, 7H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 9.62, 10.22, 11.32 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.4 (C=S), 164.4, 170.6 (2C=O), 100.1, 101.5, 106.4, 107.3, 116.8, 121.4, 127.1, 128.4, 130.2, 142.2, 143.3, 145.2, 158.3, 158.5 (aromatic-C; HC=CH), 55.9 ($2OCH_3$); MS *m/z* 473 (M^+) (21 %); Anal. calcd. for $C_{21}H_{19}N_3O_6S_2$ (473.52): C, 53.26; H, 4.04; N, 8.87; Found: C, 53.05; H, 4.00; N, 8.65.

*N-{4-[(2Z)-3-(3,5-Dimethoxyphenyl)prop-2-enoyl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**13c**)*

Yield: 63 %; m.p.: 286–288 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3232, 3220, 3119 (3NH), 1723, 1697 (2CO), 1322, 1215 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.61, 3.90 (2s, 6H, $2OCH_3$), 6.11, 6.42 (d,d, 2H, $J = 7.4$ Hz, – CH=CH–), 7.00–8.32 (m, 7H, aromatic-C), 8.32 (s, 1H, thiouracil-H6), 9.62, 10.50, 11.42 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.4 (C=S), 164.4, 170.6 (2C=O), 100.4, 101.5, 106.5, 107.3, 118.8, 121.4, 127.7, 129.4, 130.2, 142.2, 143.3, 144.2, 158.1, 158.8 (aromatic-C; HC=CH), 55.8 ($2OCH_3$); MS *m/z* 473.5 (M^+) (12 %); Anal. calcd. for $C_{21}H_{19}N_3O_6S_2$ (473.52): C, 53.26; H, 4.04; N, 8.87; Found: C, 53.24; H, 4.00; N, 8.64.

*(2Z)-2-[1-(4-{[(4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)sulfonyl]amino}phenyl)ethylidene]hydrazinecarbothioamide (**14**)*

A mixture of **7b** (3.25 g, 10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) in absolute ethanol (20 ml) containing a few drops of glacial acetic acid was refluxed for 3 h. The formed precipitate was filtered off and crystallized from DMF/water to give compound **14**. Yield: 68 %; m.p.: >300 °C; IR (KBr, cm^{-1}): 3490 (NH_2), 3330, 3265, 3220, 3215 (4NH), 1718 (CO of thiouracil), 1320, 1210 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.24 (s, 3H, CH_3), 6.52 (s, 2H, NH_2), 7.13–7.69 (m,4H, aromatic-H), 8.30 (s, 1H, thiouracil-H6), 9.81, 10.52, 11.21, 11.59 (4s, 4H, 4 NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.1, 180.1 (2C=S), 168 (C=N), 164.2(C=O), 103.2, 116.3, 124.9, 130.2, 140.2, 150.1(aromatic-C), 15.1

(CH_3); MS *m/z* 398 (M^+) (15 %); Anal. calcd. for $C_{13}H_{14}N_6O_3S_3$ (398.49): C, 39.18; H, 3.54; N, 21.08; Found: C, 39.16; H, 3.51; N, 21.27.

*(2Z)-2-[1-(4-{[(4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)sulfonyl]-amino}phenyl)ethylidene]-N-phenylhydrazinecarbothioamide (**15**)*

A mixture of **7b** (3.25 g, 10 mmol) and phenylthiosemicarbazide (1.70 g, 10 mmol) in absolute ethanol (20 ml) containing a few drops of hydrochloric acid was refluxed for 3 h. The formed precipitate was filtered off and crystallized from DMF/water to give the desired compound. Yield: 68 %; m.p.: >300 °C; IR (KBr, cm^{-1}): 3410, 3360, 3265, 3224, 3215 (5NH), 1718 (CO of thiouracil), 1320, 1130 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.23 (s, 3H, CH_3), 7.22–7.93 (m, 9H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 9.82, 10.50, 11.22, 11.51, 12.00 (5s, 5H, 5NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.1, 180.1 (2C=S), 168 (C=N), 164.2 (C=O), 103.2, 116.3, 124.3, 124.9, 126.5, 129.1, 130.2, 137.2, 140.2, 150.1 (aromatic-C), 15.1 (CH_3); MS *m/z* 474 (M^+) (21 %); Anal. calcd. for $C_{19}H_{18}N_6O_3S_3$ (474.59): C, 48.08; H, 3.82; N, 17.71; Found: C, 48.27; H, 3.80; N, 17.59.

*N-{4-[2-(Morpholin-4-yl)-2-thioxoethyl]phenyl}-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (**16**)*

A mixture of **7b** (3.25 g, 10 mmol), morpholine (0.86 g, 10 mmol), and sulfur (0.32 g, 10 mmol) was refluxed gently till the evolution of H_2S subsided, then more vigorously for a total period of 14 h. The hot reaction was poured onto warm ethanol (10 ml) and left to crystallize. The obtained crystals were filtered off and crystallized from ethanol. Yield: 68 %; m.p.: >300 °C; IR (KBr, cm^{-1}): 3490, 3350, 3222 (3NH), 1718 (CO of thiouracil), 1320, 1120 (SO_2NH), 1200 (SO_2); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.00 (m, 4H, $N(CH_2)_2$), 3.61 (m, 4H, $O(CH_2)_2$), 4.32 (s, 2H, CH_2), 7.20–7.94 (m, 4H, aromatic-H), 8.32 (s, 1H, thiouracil-H6), 9.81, 10.52, 11.21 (3s, 3H, 3NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 181.2, 178.3 (2C=S), 164.6 (C=O), 128.2, 129.3, 139.2, 148.2, 150.9 (aromatic-C), 46.2, 69.0 (morpholine-C), 37.4 ($\underline{C}H_2$–CS); MS *m/z* 426 (M^+) (21 %); Anal. calcd. for $C_{16}H_{18}N_4O_4S_3$ (426.55): C, 45.05; H 4.25; N 13.14; Found: C, 45.23; H, 4.23; N, 13.32.

*(4-{[(4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)sulfonyl]amino}phenyl)acetic acid (**17**)*

A mixture of **16** (4.26 g, 10 mmol), glacial acetic acid (5 ml), conc. H_2SO_4 (1 ml), and water (2 ml) was refluxed for 5 h. Then the mixture was poured onto water (50 ml) and kept overnight. The formed solid was filtered off and washed with cold water and digested with a solution of 5 % NaOH. Then the solution was filtered off and the filtrate was acidified with HCl. The formed precipitate was filtered off and crystallized from DMF/water to give the desired product. Yield: 68 %; m.p. >300 °C; IR (KBr, cm^{-1}): 3543 (OH), 3460, 3353, 3222 (3NH), 1718, 1710 (2CO),

1320, 1130 (SO_2NH), 1200 (SO_2); ^{1}H-NMR (DMSO-d_6, δ ppm): 4.53 (s, 2H, CH_2), 7.20–7.81 (m, 4H, aromatic-H), 8.31 (s, 1H, thiouracil-H6), 9.81, 10.50, 11.21, 11.52 (4s, 4H, 3NH and OH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 179.2 (C=S), 164.1, 176.1 (2C=O), 128.2, 129.3, 139.2, 148.2, 150.9 (aromatic-C), 48.2 (CH_2); MS *m/z* 341.63 (M^+) (30 %); Anal. calcd. for $C_{12}H_{11}N_3O_5S_2$ (341.63): C, 42.22; H, 3.24; N, 12.30; Found: C, 42.29; H, 3.53; N, 12.50.

General procedure for synthesis of compounds **18a–f**

A mixture of uracil-5-sulfonylhydrazide (2.0 gm, 0.02 mol) and the appropriate sulfonyl chloride, namely benzenesulfonyl chloride, 4-bromobenzene sulfonyl chloride, 4-methylbenzenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, uracil-5-sulfonyl chloride, 2-thiouracil-5-sulfonyl chloride (0.02 mol), in methanol (30 ml) containing a few drops of pyridine was refluxed for 8–12 h. The precipitate obtained after cooling was filtered off, dried, and crystallized to give compounds **18a–f**, respectively.

*N-[(2,4-Dioxo-1,2,3,4-tetrahydropyrimidine-5-yl)sulfonodithioyl]benzenesulfonylhydrazide (**18a**)*

Yield: 85 %; m.p.: 230–232 °C; crystallized from DMF/water; IR (KBr, cm^{-1}): 3426–3257 (4NH), 1710, 1685 (2 CO of uracil), 1320, 1123 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.11–7.90 (m, 5H, Ar–H), 8.11 (s, 1H, uracil-H6) and 10.43, 10.62, 10.91, 11.45 (4s, 4H, 4 NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.2 (2C=O), 100.1, 128.6, 129.7, 130.2, 142.2 (aromatic-C); MS *m/z* 346 (M^+) (4 %); Anal. calcd. for $C_{10}H_{10}N_4O_6S_2$ (346.35): C, 34.67; H, 2.91; N, 16.18; Found: C, 34.43, H, 2.87; N, 16.24.

*4-Bromo-N-[(2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-yl)sulfonodithioyl]benzenesulfonylhydrazide (**18b**)*

Yield: 82 %; m.p.: 262–264 °C; IR (KBr, cm^{-1}): 3430–3208 (4NH), 1713, 1682 (2CO of uracil), 1322, 1213 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.41, 7.62 (d,d, 4H, $J = 7.6$ Hz, Ar–H), 8.42 (s, 1H, uracil-H6) and 10.23, 10.51, 10.75, 11.32 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.3, 164.7 (2C=O), 100.1, 123.4, 128.6, 131.7, 132.2, 142.2 (aromatic-C); MS *m/z* 424 (M^+) (16 %), 426 ($M^+ + 2$) (14 %); Anal. calc. for $C_{10}H_9BrN_4O_6S_2$ (425.24): C, 28.24; H, 2.13; N, 13.17; Found: C, 28.41; H, 2.09; N, 13.00.

*N-[(4-Methylphenyl)sulfonyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhdrazide (**18c**)*

Yield: 80 %; m.p.: 233–235 °C; IR (KBr, cm^{-1}): 3520–3278 (4NH), 1731, 1679 (2CO of uracil), 1324, 1124 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.71 (s, 3H, CH_3), 7.23, 7.80 (d,d, 4H, $J = 7.4$ Hz, Ar–H), 8.2 (s, 1H, uracil-H6) and 10.00,

10.48, 10.91, 11.43 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.7 (2C=O);, 101.2, 128.0, 129.1, 135.2, 135.6, 143.3 (aromatic-C), 24.2 (CH_3); MS *m/z* 360 (M^+) (8 %); Anal. calc. for $C_{11}H_{12}N_4O_6S_2$ (360.37): C, 36.66; H, 3.36; N, 15.54; Found: C, 36.41; H, 3.02; N, 15.50.

*N-[(2-Nitrophenyl)sulfonyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**18d**)*

Yield: 76 %; m.p.: 245–247 °C; IR (KBr, cm^{-1}): 3480–3259 (4NH), 1722, 1684 (2CO of uracil), 1320, 1123 (SO_2NH); ^{1}H-NMR(DMSO-d_6, δ ppm): 7.01, 7.53 (d,d, 4H, $J = 8.0$ Hz, Ar–H), 8.42 (s, 1H, uracil-H6), 10.41, 10.72, 10.92, 11.45 (4s, 4H, 4NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.2 (2C=O), 100.1, 121.4, 124.2, 129.6, 131.7, 134.2, 142.3, 149.2 (aromatic-C); MS *m/z* 391(M^+) (6 %); Anal. calcd. for $C_{10}H_9N_5O_8S_2$ (391.34): C, 30.69; H, 2.32; N, 17.90; Found: C, 30.63; H 2.20; N, 17.63.

*N-[(2,4-Dioxo-1,2,3,4-tetrahydropyrimidine-5-yl)sulfonoyl]2-4-dioxo-1,2,3,4-tetrahydropyramidine-5-sulfonylhydrazide (**18e**)*

Yield: 80 %; m.p.: >300 °C; IR (KBr, cm^{-1}): 3450–3108 (6NH), 1720, 1681 (4CO of 2 uracil rings), 1317, 1213 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 8.41, 8.62 (s, 2H, 2 uracil-H6), 10.21–11.75 (6s, 6H, 6NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.5 (4C=O), 100.1, 142.5 (aromatic-C); MS *m/z* 380 (M^+) (9 %); Anal. calc. for $C_8H_8N_6O_8S_2$ (380.32): C, 25.26; H, 2.11; N, 22.09; Found: C, 25.03; H, 2.28; N, 22.27.

*2,4-Dioxo-N-[(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl)sulfonyl]-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide (**18f**)*

Yield: 85 %; m.p.: >300 °C; IR (KBr, cm^{-1}): 3470–3110 (6NH), 1709, 1681 (3CO of uracil), 1321, 1119 (SO_2NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 8.11, 840 (2s, 2H, 2 uracil-H6) and 10.43–11.89 (6s, 6H, 6NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, δ ppm): 150.1, 164.5 (3C=O), 173.2 (C=S), 100.1, 114.2, 138.1, 142.5 (aromatic-C); MS *m/z* 396 (M^+) (11 %); Anal. calc. for $C_8H_8N_6O_7S_3$ (396.39): C, 24.24; H, 2.03; N 21.20; found: C, 24.11; H, 2.31; N, 21.15.

Treatment and infection

Inhibitory effect of the different pyrimidine derivatives (prepared in the present work) on *S. mansoni* cercarial serine protease activity was investigated.

Pyrimidine derivative **3c**, which showed in vitro inhibitory effect on cercarial penetration enzymes, was further tested at its most potent inhibitory concentration activity to block invasion of mice skin by cercariae of *S. mansoni*. Compound **3c** was dissolved in DMSO/ethanol (1/1), mixed with jojoba oil, and used to paint the tails of five mice. A control group of five mice was included, whose tails were painted with the oil mixed with the solvents without the tested compound. Each

mouse was infected with 80 cercariae of *S. mansoni* strain by tail immersion method [12].

Separation of mouse sera

Sera were collected from the treated infected and untreated infected mice at 2, 4, and 6 weeks postinfection and from uninfected mice to be used as negative control. For different mice groups (treated infected, control infected, and control uninfected) available sera for IgG and IgM measurements at the three time points were from at least three individual mice. Sera missing at one or more time points were excluded for either being hemolyzed or insufficient to be included or the bleeding was not successful at one or more time point because of some technical problem.

Assessments of worm recovery

Seven weeks postinfection, treated and untreated infected mice were perfused. Recovery of adult worms was performed through hepatic portal [24]. Total worm counts in intestines and livers were determined. Protection was assessed as percentage reduction in worm counts according to the following formula: $P = C - T/C \times 100$, where P is the percentage reduction in worm counts in liver and intestine, C is the mean worm count in control infected mice (tail painted with oil containing the solvent without compound **3c**), and T is the mean worm count in pre-tail painted mice with the oil containing compound **3c** before infection with *S. mansoni* cercariae.

Enzyme-linked immunosorbent assay (ELISA)

The assay was performed according to the method of Bahgat et al. [11] to determine levels of IgG and IgM in sera of different mice groups against antigens derived from early and late stages of the *S. mansoni* parasite. Plates were coated with 100 µl/well of three different schistosome antigens: cercarial antigenic preparation (CAP, early-stage antigen) at concentration 25 µg/ml, soluble worm antigen preparation (SWAP, late-stage antigen) at concentration 50 µg/ml, and soluble egg antigen (SEA, late-stage antigen) at concentration 25 µg/ml; then plates were incubated at room temperature overnight. Plates were washed three times using phosphate-buffered saline (PBS) containing 0.05 % 20 (PBST), and antigen-free sites were blocked against nonspecific binding using 200 µl/well PBST containing 1 % bovine serum albumin (BSA) (PBST-BSA), then left for 1 h at 37 °C. After three washes, 100 µl/well diluted sera (1:100 in PBST-BSA) was added and incubated at 37 °C for 2 h. Peroxidase-labeled anti-mouse IgG or IgM conjugates were added at dilution of 1:500 and 1:5,000, respectively, in PBST-BSA and incubated for 1 h at 37 °C. *O*-Phenylenediamine-dihydrochloride (OPD) substrate was used as substrate for visualization of the antigen antibody binding in the presence of H_2O_2. To avoid the increase in the background of the enzyme substrate reaction, HCl (2 N) was used as stopper, and changes in optical densities were measured at λ_{max} of 490 nm using a microwell plate ELISA reader (Tecan; Sunrise Remote Control, Groedig, Austria).

Preparation of cercarial secretions (CSs)

The secretions of schistosome cercariae were prepared following the previously described procedure [11] and were used as source for the cercarial serine protease activity. To obtain cercariae, infected snails were exposed to light for 1 h and were then gently poured into Petri dishes (15 cm diameter) that were previously painted with linoleic acid (0.9 g/ml) and air dried. A total of 10,000 cercariae were used per dish. The plates were incubated at 37 °C for 30 min. During this incubation, cercariae release the contents of acetabular glands while trying to penetrate into the linoleic acid and transforming into schistosomula. All the water containing cercarial materials was collected in 15-ml Falcon tubes, and larvae were sedimented on ice. The supernatant containing CSs was spun down for 2 min at $3{,}000 \times g$ to eliminate cercarial debris, then concentrated by lyophilization and reconstituted with water to one-tenth of its original volume. Protein content was measured by bicinchoninic acid (BCA) test.

Quantitative assay of serine proteinase activity using chromogenic substrate

The serine protease activity in CSs was quantitatively assessed by using Boc-Val-Leu-Gly-Arg-$_P$NA, a specific substrate for trypsin-like serine proteinases [25]. The release of yellow color was quantified by measuring the absorbance at 405 nm using microtiter plate reader (Tecan, Sunrise Remote Control, Groedig, Austria).

The intensity of the yellow color is directly proportional to the enzyme activity. Stock solution of the substrate at concentration of 10 mg/ml was prepared in dimethyl sulfoxide (DMSO) and was then diluted with substrate buffer (30 mM Tris containing 60 mM NaCl and 0.05 % NaN_3) to the final concentration of optimal pH.

Inhibition of cercarial serine protease activity

Inhibitory effect of the different pyrimidine derivatives (prepared in the present work) on *S. mansoni* cercarial serine protease activity was investigated.

All derivatives were found to be completely soluble in DMSO/ethanol mixture (1/1). Stock solutions of the pyrimidine derivatives of interest (10 mg/ml) were prepared. For performing inhibition assays, 50 µl CSs were preincubated with 10 µl of each of the serial dilutions of different pyrimidine derivatives for 10 min before adding the substrate L-1195. Similar volumes of the solvents (DMSO/ethanol) were mixed with the CSs as negative controls.

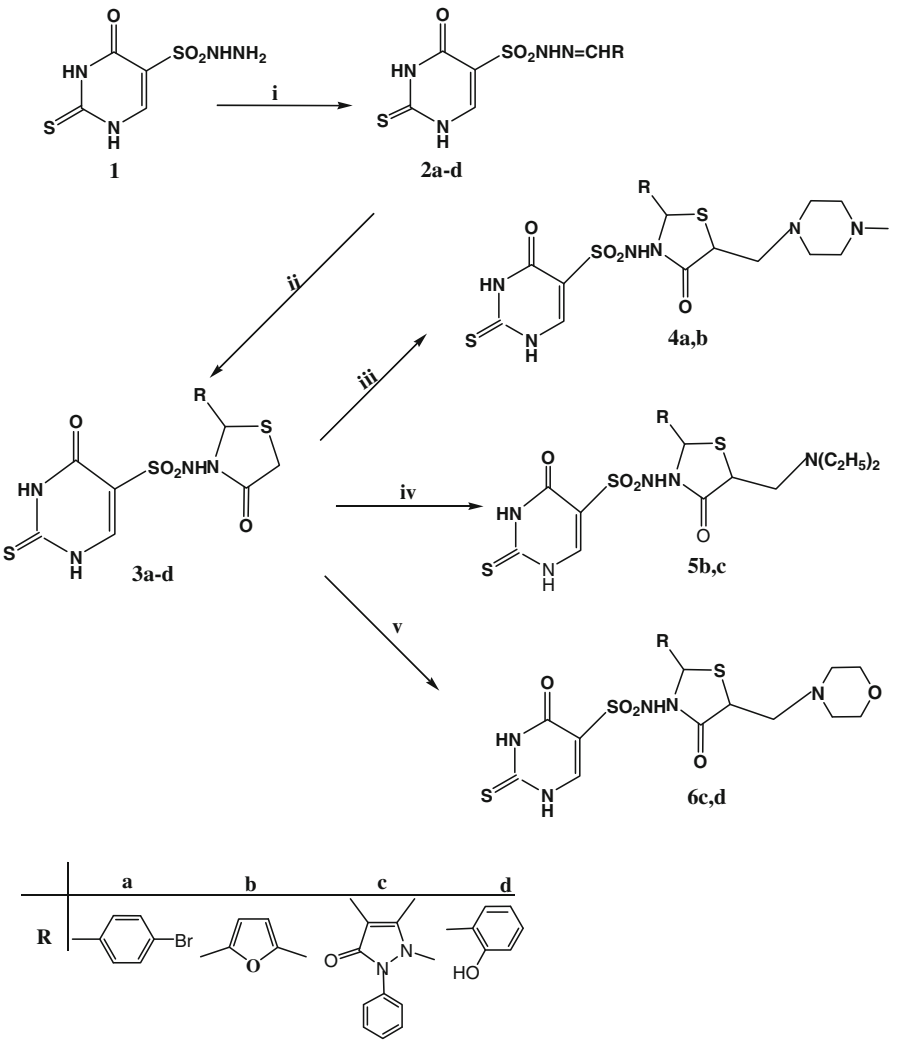
Results and discussion

Chemical studies

The key starting material thiouracil-5-sulfonylhydrazide prepared according to the reported method [12] was allowed to react with different aromatic aldehydes, namely 4-bromobenzaldehyde, 5-methylfurfural, 4-antipyrinecarboxaldehyde,

and/or 2-salicylaldehyde, to give the corresponding Schiff bases **2a–d**, respectively. The structures of the obtained derivatives were confirmed by microanalyses and spectral data. IR spectra exhibited different absorption bands at the region 3,325–3,122 cm^{-1} corresponding to NH groups, in addition to the presence of absorption bands at the region 1,692–1,680 cm^{-1} due to C=O of thiouracil ring. IR spectrum of **2c** showed another band at 1,712 cm^{-1} due to C=O of pyrazoline ring. ^1H NMR spectra of the compounds confirmed their chemical structures; e.g., the spectrum of **2b** showed a singlet signal at δ 2.33 ppm due the methyl group of furan ring and two doublets at δ 6.31, 7.20 ppm due to the two protons of the furan moiety, in addition to the other expected protons of the compound. Also, the spectrum of **2c** exhibited two singlet signals at δ 2.50, 3.71 ppm referring to the two methyl groups of the pyrazoline ring, in addition to the other aromatic protons and those of NH groups appearing in their expected regions. MS spectra of the compounds showed the molecular ion peaks of the compounds. Since Schiff bases are important intermediate precursors for synthesis of different heterocyclic ring systems, the derivatives **2a–d** were allowed to react with thioglycolic acid in dry benzene to furnish the new thiazolidinone derivatives **3a–d**, and their structures were confirmed using microanalytical and spectral data. IR spectra revealed the appearance of an absorption band at the range 1,715–1,725 cm^{-1} corresponding to C=O of thiazolidinone ring in addition to the absorption band at 1,680–1,692 cm^{-1} that is relevant to the carbonyl group of thiouracil ring. ^1H NMR spectra of **3a–d** exhibited the presence of two singlets at the range δ 4.10–4.50 and 4.51–4.61 ppm due to the protons of SCH_2 and N–CH–S groups of thiazolidinone ring. MS spectra showed the molecular ion peaks of the compounds. The Mannich bases **4a**, **b**, **5b**, **c**, and **6c**, **d** were obtained by reaction of the thiazolidinone derivatives **3a–d** with different secondary amines, namely *N*-methylpiperazine, diethylamine, and/or morpholine in refluxing ethanol in presence of paraformaldehyde (Scheme 1). The structures of the novel derivatives were established by microanalytical and spectral data. IR spectra appeared in agreement with their structures. ^1H NMR spectra of the compounds exhibited a doublet signal at the range δ 4.10–4.89 ppm corresponding to the two protons of CH_2 bridge that connects the amine moiety with the thiazolidinone ring. Also, a triplet signal appeared at the range δ 6.11–6.14 ppm due to the methine proton of S–CH of thiazolidinone ring, in addition to other signals corresponding to the expected protons of each derivative. MS spectra showed their molecular ion peaks.

The respective iminopyridine and pyridone derivatives **8a–c**, **9a–c**, **10a–c**, and **11a–c** were synthesized by one-pot reaction of the compounds **7a**, **b** [15] with the appropriate aldehydes, namely 2-chloro-5-nitrobenzaldehyde, 2,5-dimethoxybenzaldehyde and 3,5-dimethoxybenzaldehyde, malononitrile, and/or ethyl cyanoacetate and ammonium acetate in refluxing *n*-butanol for 8–10 h (Scheme 2). Microanalyses and spectral data confirmed the chemical structures of the novel derivatives. IR spectra of the compounds displayed the characteristic absorption bands of CN groups at the range 2,205–2,212 cm^{-1}, and those of CO groups of the pyrimidine rings appeared at the range 1,685–1,720 cm^{-1}. Compounds **10a–c** and **11a–c** showed additional bands around the same range corresponding to the pyridinyl CO group. ^1H NMR spectra of the compounds appeared in agreement with their

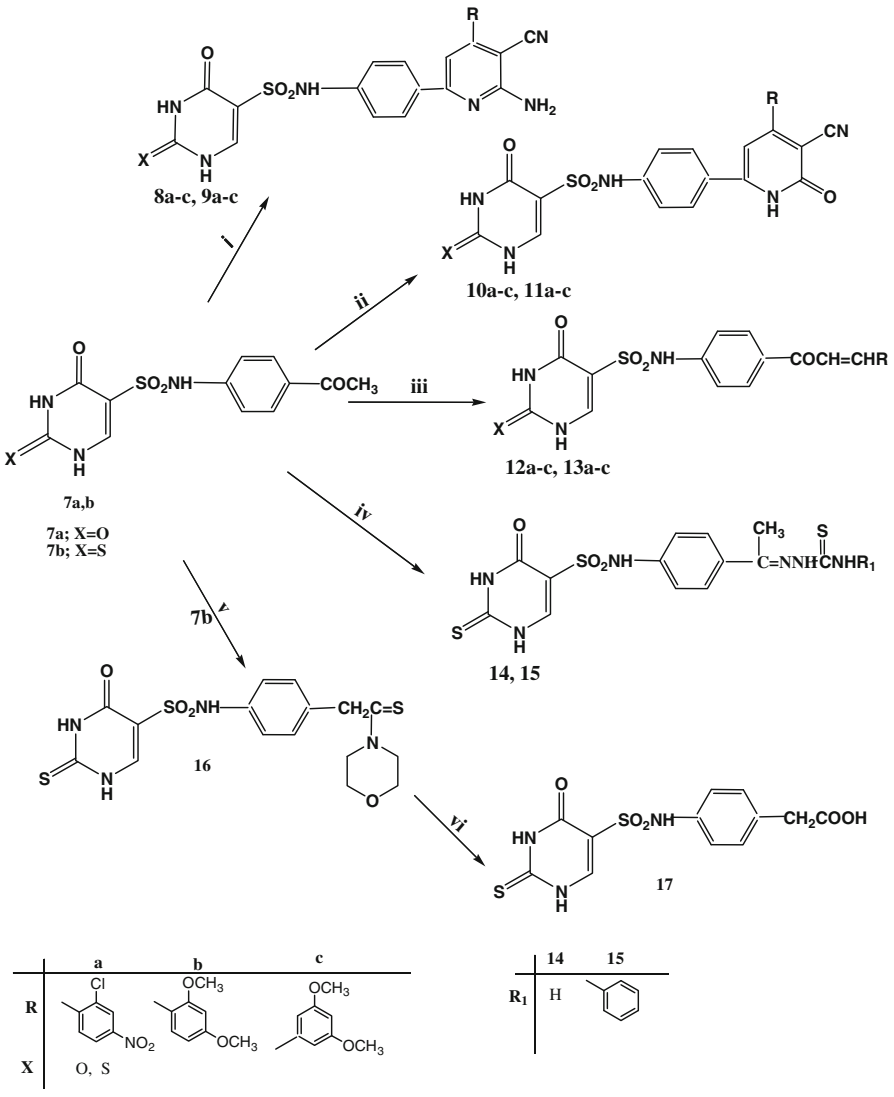


Reaction conditions:i: appropriate aldehyde, absolute ethanol, stirr at rm. temp., two days; **ii:**thioglyclic acid, dry benzene, reflux, water bath, 8-12 h; **iii, iv, v:** paraformaldehyde, appropriate amine, absolute ethanol, reflux, 6-9 h.

Scheme 1 Preparation of Schiff bases **2a–d**, thiazolidinone derivatives **3a–d**, and Mannich bases **4a**, **b**, **5b**, **c**, and **6c**, **d** starting from thiouracil-5-sulfonylhydrazide (**1**)

expected chemical structures. The molecular ion peaks of the compounds are exhibited in their MS spectra.

Treatment of compounds **7a**, **b** with the above-mentioned aldehydes in 10 % ethanolic NaOH solution furnished the chalcones **12a–c** and **13a–c**. IR spectra of the compounds showed the absorption bands of the chalcone CO groups in addition to those of the pyrimidine rings at the range 1,680–1,720 cm^{-1}. ^{1}H NMR spectra



Reaction conditions: i: Ar-CHO, malononitrile, ammonium acetate, reflux for 8-10h; **ii:**Ar-CHO, ethylcyanoactate, ammonium acetate, 8-10h; **iii:** Ar-CHO, ethanolic NaOH 10%, stirr at rm.temp.; **iv:** substituted thiosemicarbazide, ethanol, reflux for 3h; **v:** S, morpholine, reflux for 14h; **vi:** glacial acetic acid, conc. H_2SO_4, reflux for 5h.

Scheme 2 Preparation of 6-iminopyridines **8a–c**, **9a–c**, 6-oxopyridines **10a–c**, **11a–c**, chalcone derivatives **12a–c**, **13a–c**, hydrazinecarbothioamide derivatives **14**, **15**, thiomorpholide **16**, and acetic acid derivative **17** starting from compounds **7a**, **b**

exhibited two doublet signals at δ 6.10–6.81 ppm referring to the two protons of –CH=CH– group. MS spectra exhibited the molecular ion peaks of the compounds (Scheme 2).

Hydrazinecarbothioamide derivatives **14**, **15** were gained upon reaction of compounds **7a**, **b** with (phenyl)thiosemicarbazide in acidified ethanol. Microanalyses and spectral data confirmed the chemical structures of the compounds. ^{1}H NMR spectra exhibited a singlet signal around δ 2.23 ppm corresponding to the ethylidene CH_3 group.

Willgerodt reaction [26] was carried out by the reaction of **7b** with morpholine and sulfur to get thiomorpholide derivative **16**, which was converted easily to the corresponding acetic acid derivative **17** (Scheme 2). Microanalyses and spectral data confirmed the chemical structures of the derivatives. IR spectrum of **16** revealed absorption bands at 1,718–1,710 cm^{-1} referring to 2CO of thiouracil ring and the carboxylic group. ^{1}H NMR spectrum of **16** presented two multiplets at δ 3.00 and 3.61 ppm assigned to the 8H of morpholine ring, in addition to a singlet at δ 4.31 ppm due to 2H of thioxoethyl group. ^{1}H NMR spectrum of **17** showed the disappearance of the multiplets of morpholine ring and the presence of a singlet due to 2H of acetic acid group at δ 4.53 ppm. The other expected protons of the derivatives were apparent in the spectra. MS spectra showed the molecular ion peaks of the compounds.

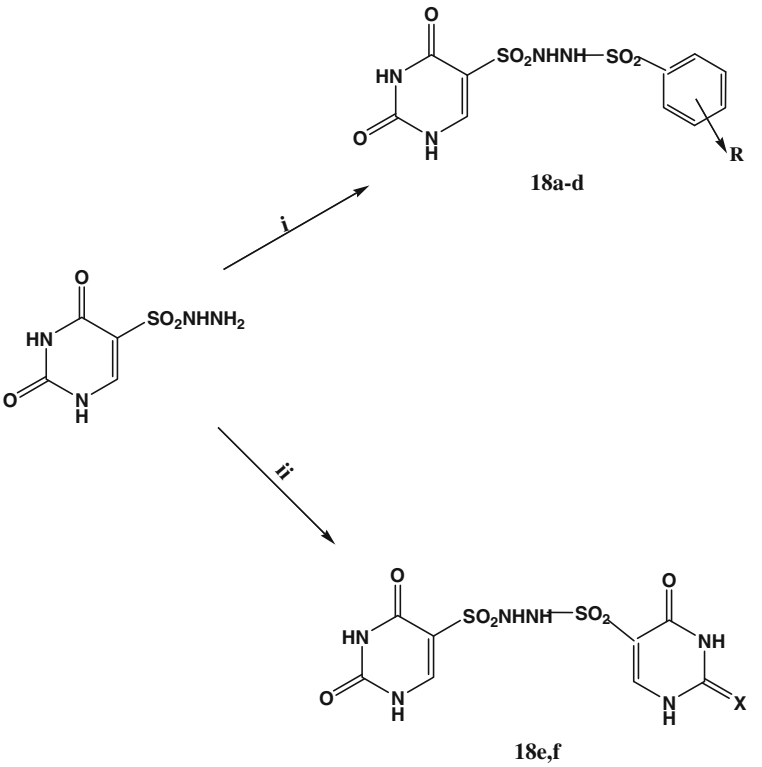
Further reactions were carried out by refluxing uracil-5-sulfonylhydrazide with equimolar amounts of different substituted benzenesulfonyl chloride in alkaline methanol to give benzenesulfonohydrazide derivatives **18a–f** (Scheme 3). Microanalytical and spectral data complied with the chemical structures of the novel derivatives.

Pharmacological studies

In this report, we describe a new serine protease inhibitor. We employed a cercarial serine protease that could be inhibited by known serine protease inhibitors [25]. The protease inhibitory effect of our new pyrimidine derivative is not surprising in the light of previous reports demonstrating that previously characterized pyrimidine derivatives can block several serine proteases, such as nifedipine, alkyl [sulfonyl(oxy)] uracils, 1,2-dihydrouracil, and hexahydroimidazo[1,2-*c*]pyridine derivative that could block leukocyte enzyme [27], and (5-amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-acetyl-Phe-CF_3 and pyrimidinone derivatives that showed chymase inhibitory effect [28]. Although the serine protease inhibitor described in this report and its effect to block cercarial penetration are new, the approach is a continuation of earlier trials carried out to develop protease inhibitory formulations that can successfully block cercarial penetration both in vitro and in vivo [29].

When our compound **3c** was formulated in jojoba oil, it slightly blocked cercarial penetration as indicated by 20 % reduction in worm burden. Although this reduction is not a high degree of lowering the worm burden, it may contribute to decreasing the pathology of the disease since reduction of worm count will certainly lead to reduction of egg count, which is the major cause of the severe pathology.

Cruthers et al. [30] reported that topical application of a 2-hydroxybenzoic anilide provided prophylactic protection against penetration by infective cercariae of the parasitic worms. Salafsky et al. found that praziquantel (PZQ) was moderately effective in blocking cercarial penetration when used as a topical paint.



	a	b	c	d	e	f
R	H	4-Br	4-CH_3	2-NO_2		
X					O	S

Reaction conditions: i, ii: appropriate sulfonylchloride**,** methanol, few drops of pyridine, reflux for 8-12h

Scheme 3 Preparation of benzenesulfonohydrazide derivatives **18a–f** starting from uracil-5-sulfonylhydrazide

Cremophor EL, 50 % propylene glycol, vegetable oil, and cod liver oil were used as PZQ vehicles. When Cremophor EL (ethoxylated castor oil) was used to administer PZQ, a 93 % reduction in cercarial penetration was seen at 0 h and a 98 %+ reduction rate was seen from 4 to 24 h postexposure. However, Cremophor EL alone had an essentially equivalent effect on cercarial penetration from 8 to 92 h after administration. Castor oil gave protection rate ranging from 90 to almost 100 %. They suggested that chemoprophylaxis may be possible by dietary supplementation with lipids having antipenetration activity or by molecules that resemble these lipids [31].

Cooper et al. [32] showed a dimethicone formulation to be effective at preventing *S. cercariae* from infecting skin. Current treatment depends on praziquantel, raising concerns of emergence of resistant parasites. Oxadiazole-2-

oxide was identified as an inhibitor of thioredoxin glutathione reductase (TGR), a selenocysteine-containing flavoenzyme required by the parasite to maintain proper cellular redox balance.

Bahgat et al. [12] found that *N*′-(4-methylbenzylidine)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonylhydrazide has potent inhibitory effect against *S. mansoni* cercariae; upon its use as a paint on mice tails before infection with *S. mansoni* cercariae, the compound formulated in jojoba oil caused a significant reduction (93 %) in worm burden. Rai et al. [33] studied the utility of oxadiazole-2-oxides as novel inhibitors and as efficacious antischistosomal agents.

The humoral, both primary and secondary, immune responses against several antigens derived from different stages of *S. mansoni* lifecycles were measured in sera from treated then infected mice, infected untreated mice, and uninfected untreated control mice. The antigens used were soluble cercarial antigenic preparation (CAP) that represents larval early-stage antigen and, as late-stage derived antigens, SWAP and SEA.

Comparing the IgM reactivity to CAP in sera collected from treated infected mice at regular time intervals postinfection with its reactivity in sera collected from infected untreated mice at the same time points showed that sera collected from treated infected mice at two weeks treated infection (2WTI) had 0.59-fold lower IgM reactivity when compared with sera collected from treated infected mice at two weeks treated infection (2WI). This could be due to the fact that treatment retarded penetration of cercariae and as a result lowered the primary immune response, particularly at such an early phase postinfection.

At 4 weeks postinfection, treated infected mice (4 WTI) showed no change to CAP compared with infected untreated (4 WI) ones. This could be due to the beginning of an enhanced primary immune response due to chemical attenuation of some of the cercariae by treatment. Those cercariae may have penetrated the mice skin and stimulated the increase in the IgM immune response among the treated infected mice when compared with the untreated infected ones.

At 6 weeks, the IgM response to CAP in sera from treated infected mice was 0.47-fold lower than in those from untreated mice. This may reflect that, although treatment caused attenuation of some cercariae and may have caused enhancement of the IgM response at 4 weeks postinfection, this effect was very transient and the duration of this IgM response was so short that it was lower at 6 weeks in the treated infected mice than in the untreated infected mice. The IgG level in the sera collected from treated infected mice at 2 and 4 weeks postinfection generally showed lower IgG reactivity when compared with the IgG level in sera collected from infected untreated mice at the same time point. This confirms our conclusion that treatment with our new compound caused attenuation of some of the penetrating cercariae. Comparison of both IgM and IgG responses to SWAP in sera collected from treated infected mice at regular time intervals postinfection versus the reactivity in sera collected from infected untreated mice at the same time points revealed that sera collected from treated infected mice had lower IgM level (0.84-fold) at 2 and 4 WTI and IgG at 2, 4, and 6 WTI than IgM level in sera from the infected untreated ones at the same time points. This could be due to the fact that attenuation of the cercariae caused by skin treatment may have caused retardation in the development of

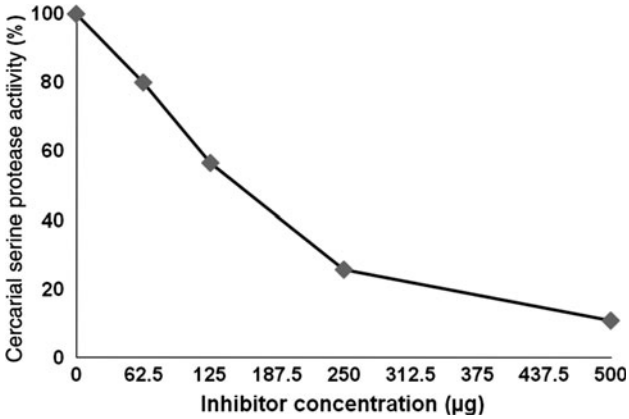


Fig. 1 Inhibition of serine protease activity of *Schistosoma mansoni* cercariae by compound **3c**

cercariae into schistosomula and juvenile worms and as a result caused retardation of the IgM and IgG responses developed to the adult worm antigens among sera from treated mice when compared with those of untreated ones.

Both IgM and IgG responses to soluble egg antigen were generally lower in sera collected at different time intervals from treated infected mice when compared with in sera collected from infected untreated mice at the same time points. We concluded that compound **3c** has an immunosuppressive response, and our results are in agreement with Bahgat et al. [34], who reported evaluation of a cyclohexanecarboxamide derivative, *N*-phenyl-*N*-[1-(piperidine-1-carbonyl)cyclohexyl]benzamide, for its inhibitory effects on *S. mansoni* cercarial serine protease activity and cercarial penetration. In addition, the IgM and IgG responses to crude *S. mansoni* cercarial, worm, and egg antigens were generally lower in sera from treated infected mice than untreated infected mice.

Inhibition of cercarial serine protease activity of *S. mansoni* cercariae

The prepared pyrimidine derivatives were tested in vitro for their inhibitory effects. Compound **3c** exerted an inhibitory effect on cercarial serine protease when tested at serial concentrations (Fig. 1). The IC_{50} (inhibitor concentration that abolishes 50 % of enzyme activity) of the compound was observed to be 160 µg.

Antischistosomal activity

The perfusion results of mice treated with compound **3c** are presented in Fig. 2. It caused a reduction (20 %) in the worm burden when compared with control infected mice, although the worm burden results of treated, tail-painted, mice did not differ significantly ($P = 0.2920$) from the control mice.

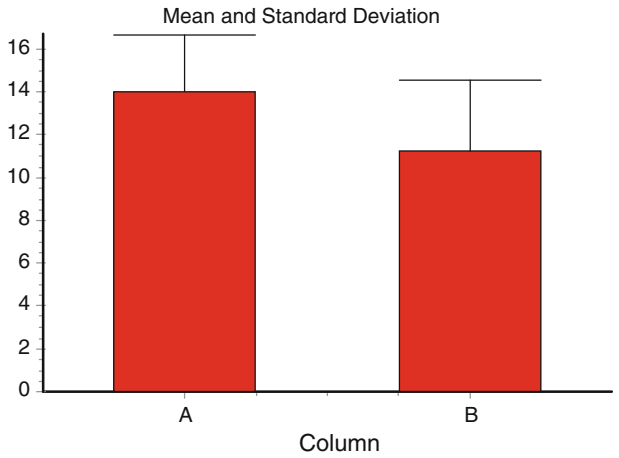


Fig. 2 Reduction in worm burden in mice treated with **3c** (column B) when compared with untreated control mice (column A). The results indicate 20 % reduction in worms recovered from treated mice compared with control mice

Detection of IgM and IgG levels posttreatment and infection with *S. mansoni*

Measurements of both IgM and IgG levels were carried out in sera collected at 2, 4, and 6 weeks postinfection from both treated and untreated mice.

Using CAP as an antigen in ELISA, the level of IgM or IgG showed nonsignificant change in sera from treated infected as compared with infected untreated mice at different time points (Table 1).

IgM reactivity to SWAP level in sera collected from treated infected mice at 2 (2 WTI) and (6 WTI) weeks postinfection showed nonsignificant differences when compared with sera collected from infected treated mice at the same time point (2 WI and 6 WI, respectively). At 4 weeks postinfection, the treated infected mice (4 WTI) showed significant decrease ($P = 0.004$) in IgM reactivity to SWAP as compared with infected untreated (4 WI) ones (Table 1).

The same comparison for IgG level is demonstrated in Table 1. The level of IgG in sera from treated infected mice at 2 and 4 weeks postinfection (2 WTI, 4 WTI) against SWAP showed significant decrease ($P = 0.04$ and 0.004, respectively) when compared with sera collected from infected treated mice at the same time point (2 WI and 4 WI, respectively). At 6 weeks, the IgG response showed nonsignificant differences in sera from treated infected mice compared with those from untreated mice (Table 1).

The IgM reactivity to SEA in sera collected from treated infected mice at 2, 4, and 6 WTI weeks postinfection showed nonsignificant differences when compared with the IgM level in sera from infected treated mice at the same time point (2, 4, and 6 WI). The IgG reactivity in sera from treated infected mice at 2, 4, and 6 WTI showed significant decrease ($P < 0.05$) when compared with the IgG level in sera from infected treated mice at the same time point (2, 4, and 6 WI) (Table 1).

Table 1 Detection of IgM and IgG against *Schistosoma mansoni* cercarial antigen preparation, soluble worm antigen preparation, and soluble egg antigen levels by ELISA in sera of mice whose tail was painted with compound **3c** at different time points postinfection

Experimental group	Weeks postinfection	Antigen used in ELISA					
		Cercarial antigen preparation		Soluble worm antigen preparation		Soluble egg antigen	
		Measured antibody class (mean ± SD)					
		IgM	IgG	IgM	IgG	IgM	IgG
Treated	2	0.28 ± 0.05, N.S.	0.28 ± 0.05, N.S.	0.56 ± 0.2, N.S.	0.39 ± 0.08, $P = 0.04$	0.84 ± 0.4, N.S.	0.48 ± 0.11, $P = 0.005$
	4	0.31 ± 0.05, N.S.	0.29 ± 0.02, N.S.	0.41 ± 0.06, $P = 0.004$	0.41 ± 0.06, $P = 0.004$	0.90 ± 0.5, N.S.	0.50 ± 0.04, $P = 0.004$
	6	0.46 ± 0.08, N.S.	0.39 ± 0.07, N.S.	0.68 ± 0.13, N.S.	0.56 ± 0.12, N.S.	1.38 ± 0.76, N.S.	0.16 ± 0.15, $P = 0.0008$
Control	2	0.47 ± 0.15	0.47 ± 0.2	0.66 ± 0.25	0.63 ± 0.15	0.93 ± 0.15	0.93 ± 0.15
	4	0.33 ± 0.05	0.33 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.26 ± 0.5	1.03 ± 0.21
	6	0.97 ± 0.5	0.37 ± 0.2	0.57 ± 0.2	0.7 ± 0.17	1.19 ± 0.64	1.39 ± 0.2

N.S. non significant, *SD* standard deviation

Conclusions

A new series of uracil- and thiouracil-5-sulfonohydrazide derivatives were synthesized to test their antischistosomal activity. It was found that compound **3c**, *N*-[2-(1,5-dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-4-oxo-1,3-thiazolidin-3-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide, inhibited cercarial serine protease activity with $IC_{50} = 160$ μg. Upon topical application on mice tails before infection with *S. mansoni* cercariae, it caused a reduction in worm burden when compared with untreated infected mice. Thus, further modifications must be performed on the synthesized derivatives to obtain more potent cercarial serine protease inhibition, greater reduction of cercarial penetration, and more potent antischistosomal efficacy.

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References

1. L.A.L. Quezada, J.H. McKerrow, Schistosome serine protease inhibitors: parasite defense or homeostasis? An. Acad. Bras. Cienc. **83**, 663–672 (2011)
2. A. Harder, Chemotherapeutic approaches to schistosomes: current knowledge and outlook. Parasitol. Res. **88**, 395–397 (2002)
3. P. Steinmann, J. Keiser, R. Bos, M. Tanner, J. Utzinger, Schistosomiasis and water resources development: systematic review, meta-analysis and estimates of people at risk. Lancet Infect. Dis. **6**, 411–425 (2006)
4. M. Ismail, S. Botros, A. Metwally, S. William, A. Farghally, Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am. J. Trop. Med. Hyg. **60**, 932–935 (1999)
5. N.R. Bergquist, Schistosomiasis: from risk assessment to control. Trends Parasitol. **18**, 309–314 (2002)
6. M.A. Stirewalt, C.H. Dorsey, *Schistosoma mansoni*: cercarial penetration of host epidermis at the ultrastructural level. Exp. Parasitol. **35**, 1–15 (1974)
7. J.H. McKerrow, G. Newport, Z. Fishelson, Recent insights into the structure and function of a larval proteinase involved in host infection by a multicellular parasite. Proc. Soc. Exp. Biol. Med. **197**, 119–124 (1991)
8. J. Pellegrino, Protection against human schistosome cercariae. Exp. Parasitol. **21**, 112–131 (1967)
9. R.R. Abu-Elyazeed, J.K. Podgore, N.S. Mansour, M.E. Kilpatrick, Field trial of 1 % niclosamide as a topical antipenetrant to *Schistosoma mansoni* cercariae. Am. J. Trop. Med. Hyg. **49**, 403–409 (1993)
10. J.K. Podgore, R.R. Abu-Elyazeed, N.S. Mansour, F.G. Youssef, R.G. Hibbs, J.A. Gere, Evaluation of a twice-a week application of 1 % niclosamide lotion in preventing *Schistosoma haematobium* reinfection. Am. J. Trop. Med. Hyg. **51**, 875–879 (1994)
11. M. Bahgat, K. Francklow, M.J. Doenhoff, Y.L. Li, R.M. Ramzy, C. Kirsten, A. Ruppel, Infection induces antibodies against the cercarial secretions, but not against the cercarial elastases of *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, and *Trichobilharzia ocellata*. Parasite Immunol. **23**, 557–565 (2001)
12. M. Bahgat, A.S. Maghraby, M.E. Heiba, A. Ruppe, O.A. Fathalla, Synthesis of new 4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine derivatives with an incorporated thiazolidinone moiety and testing their possible serine protease and cercarial elastase inhibitory effects with a possible prospective to block penetration of *Schistosoma mansoni* cercariae into the mice skin. Arch Pharm Res. **28**, 1002–1012 (2005)
13. A. Ruppel, K. Chlichlia, M. Bahgat, Invasion by schistosome cercariae: neglected aspects in *Schistosoma japonicum*. Trends Parasitol. **20**, 397–400 (2004)

14. H. Mitsuya (ed.), *Anti-HIV nucleosides: past, present and future* (Chapman and Hall, New York, 1997)
15. F. Ahmad Eweas, S. A. Swelam, O.A. Fathalla, N.M. Fawzy, Sh.I. Abdel-Moez, Synthesis, antimicrobial evaluation, and molecular modeling of new pyrazolo[3,4-d]pyrimidine derivatives. Med. Chem. Res. (2011). doi:10.1007/s00044-011-9911
16. T.S. Chitre, K.G. Bothara, S.M. Patil, K.D. Asgaonkar, S. Nagappa, M.K. Kathiravan, Design, synthesis, docking and anti-mycobacterial activity of some novel thiouracil derivatives as thymidine monophoshate kinase (TMPKmt) inhibitors. Int. J. Res. Pharm. Biomed. Sci. **2**, 616–623 (2011)
17. N.R. Mohamed, M.M. El-saidi, Y.M. Ali, M.H. Elnagdi, Utility of 6-amino-2-thiouracil as precursors for the synthesis of pyrido[2,3-*d*]pyrimidines and their in vitro and in vivo biological evaluation. Sci. Pharm. **75**, 9–28 (2007)
18. V. Alagarsamy, S. Vijayakumar, R.V. Solomon, Synthesis of 2-mercapto3-substituted-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-ones as new analgesic, anti-inflammatory agents. Biomed. Pharmacother. **61**, 285–291 (2007)
19. E. Nicola, M. Kirchner, F. Caussade, A. Cloarec, In vivo pharmacological characterization of UP 269–6, a novel non-peptide angiotensin II receptor antagonist. Eur. J. Med. Chem. **284**, 157–170 (1995)
20. O.A. Fathalla, M.A.H. Ismail, M.M. Anwar, K. A.M. Abouzid, A.A. K. Ramadan, Novel 2-thiopyrimidine derivatives as CDK2 inhibitors: molecular modeling, synthesis, and anti-tumor activity evaluation. Med. Chem. Res. (2012). doi:10.1007/s00044-012-0051-9
21. O.A. Fathalla, H.S.M. Gad, A.S. Maghraby, Synthesis of some new uracil-5-sulphonamide-p-phenyl derivatives and their effect on *Biomphalaria alexandrina* snail's nucleoproteins. Arch. Pharm. Res. **23**, 128–138 (2000)
22. O.A. Fathalla, M.E. Haiba, A.S. Maghraby, Synthesis of new uracil-5-sulfonamide derivatives and immuno-stimulatory effect of a chemically modified hemolymph of bio-mphalaia alexandrina on *Schistosoma mansoni* infected mice. Arch. Pharm. Res. **26**, 358–366 (2003)
23. O.A. Fathalla, A.S. Maghraby, Some pyrimidine-2,4 (1H,3H)-dione derivatives: immunization of mice with *Biomphalaria alexandrina* snail's nuceoproteines treated with some pyrimidine-2,4(1H,3H)-dione derivatives and prazaquantel induced mesentric lymph nodes and splenic lymphocytes. Bull. Fac. Pharm. Cairo Univ. **43**, 183–193 (2005)
24. O.A. Fathalla, Synthesis of some new thiouracil derivatives with expected biological activity. Ind. J. Chem. **40B**, 37–42 (2001)
25. L. Oliver, M.A. Stirewalt, An effective method for the exposure of mice to cercariae of *Schistosoma mansoni*. J. Parasitol. **38**, 1923 (1952)
26. A.I. Vogel, *Text book of organic chemistry*, 3rd edn. (Lowe and Brydone, London, 1972)
27. R.H. Duvall, W.B. Dewitt, An improved perfusion technique for recovering adult Schistosomes from laboratory animals. Am. J. Trop. Med. Hyg. **16**, 483–486 (1967)
28. M. Bahgat, A. Ruppel, Biochemical comparison of the serine protease (elastase) activities in cercarial secretions from *Trichobilharzia ocellata* and *Schistosoma mansoni*. Parasitol. Res. **288**, 495–500 (2002)
29. W. He, B. Hanney, M.R. Myers, A.P. Spada, K. Brown, D. Colussi, V. Chu, Non-benzamidine compounds as selective factor Xa inhibitors. Bioorg. Med. Chem. Lett. **10**, 1737–1739 (2000)
30. Cruthers et al., Topical prophylaxis against schistosomal infections, United States Patent 4659738–4173632. Nov 1979
31. B. Salafsk, A.C. Fusco, L.H. Li, J. Mueller, B. Ellenberger, *Schistosoma mansoni*: experimental chemoprophylaxis in mice using oral anti-penetration agents. Parasitology **69**, 263–271 (1989)
32. E. Cooper, A. Iqbal, A. Bartlett, C. Marriott, P.J. Whitfield, M.B.A. Brown, Comparison of topical formulations for the prevention of human schistosomiasis. J. Pharm. Pharmacol. **56**, 957–962 (2004)
33. G. Rai, A.A. Sayed, W.A. Lea, H.F. Luecke, H. Chakrapani, S.P. Chakrapani, A. Jadhav, W. Leister, M. Shen, J. Inglese, C.P. Austin, L. Keefer, S.J. Elias, A. Simeonov, D.J. Maloney, D.L. Williams, C.J. Thomas, Structure mechanism insights and the role of nitric oxide donation guide the development of oxadiazole-2-oxides as therapeutic agents against schistosomiasis. J. Med. Chem. **52**, 6474–6483 (2009)
34. M. Bahgat, M.N. Aboul-Enein, A.A. El Azzouny, A.S. Maghraby, A. Ruppel, W.M.A. Soliman, Cyclohexanecarboxamide derivatives with inhibitory effects on *Schistosoma mansoni* cercarial serine protease and penetration of mice skin by the parasite. Acta Pol. Pharm. **66**, 333–340 (2009)