

# Novel indolyl-pyrimidine derivatives: synthesis, antimicrobial, and antioxidant evaluations

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**Abstract** In the present study, a novel series of indolyl-pyrimidines (**1–13**) were synthesized starting from 4-hydrazinopyrimidine-5-carbonitrile **3**. Elemental analysis, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and mass spectral data elucidated structure of newly synthesized compounds. All compounds were screened for their in vitro antibacterial, antifungal, and some for antioxidant activities. Compounds **5**, **9g**, **9i**, and **9j** showed pronounced antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus flavus* compared to the reference drugs, while compounds **3** and **9g** displayed promising free radical scavenging activity and found to be more potent than standard, ascorbic acid (vitamin C). Further, some compounds were evaluated for cytotoxic activity by SRB assay method against human colon carcinoma (CaCo-2) and showed that compounds **4** and **9g** were found to be the highly active compared to the reference drug doxorubicin. Their structure and activity relationship were discussed.

**Keywords** Indolyl-pyrimidines · Antimicrobial · Antioxidant activity · Radical scavenging · SRB assay · Structure activity relationship

## Introduction

Pyrimidine derivatives and related fused heterocycles are important classes of heterocyclic compounds that exhibit a broad spectrum of biological activities such as anticancer (Cocco *et al.*, 2006; Ibrahim and El-Metwally, 2010; Le Brazidec *et al.*, 2012), antiviral (Martinez-Montero *et al.*, 2012), antibacterial (Kotaiah *et al.*, 2012), antioxidant (Abu-Hashem *et al.*, 2011), and anti-inflammatory (Hanna, 2012). Another important class of pyrimidine is 2-thiopyrimidine (2-TP) and its derivatives. Similarly, the related thiouracil derivatives are potential chemotherapeutics as antiviral, anticancer, and antimicrobial agents (Grigoryan *et al.*, 2005; Al-Masoudi *et al.*, 2011; Agbaje *et al.*, 2011). In particular, 6-*n*-propyl-2-thiouracil (6-PTU) is antithyroid drug (Cooper, 2005) where its *S*-alkylation and N3-alkylation products have been recently reported as novel antibacterial and cytotoxic agents (Prachayasittikul *et al.*, 2009). 6-Aryl-5-cyano-2-thiouracils possess antimicrobial and antitumor activities. 1-Alkyl-2-(alkylthio)-4-aryl-6-oxo-1,6-dihydropyrimidine-5-carbonitriles are displayed promising anticancer activity against leukemia, non-small cell lung, melanoma, and renal cancer (Taher and Helwa, 2012). 6-(4-Bromophenyl)-5-cyano-4-oxo-3,4-dihydropyrimidin-2-yl 2-(4-bromophenyl)-*N'*-(4-hydroxyphenyl)-2-oxo-ethane-hydrazonothioate possessed superior antibacterial activity against the gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* compared to amoxicillin (Taher and Abou-Seri, 2012). Moreover, 6-aryl-5-cyano-2-thiouracils possess inhibition of hepatitis C viral NS5B RNA dependent RNA polymerase (Ding *et al.*, 2006). Reports from our laboratory revealed that several hydrazinopyrimidine derivatives show significant biological activities (Mohamed *et al.*, 2011a, b, c, 2012a, b; Awad *et al.*, 2013). In particular, hydrazinopyrimidine-5-

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carbonitriles were utilized as a pharmacophoric tool for the development of more efficacious antimicrobial and anticancer agents (Ram *et al.*, 1987; Kadry *et al.*, 2008; Fathalla *et al.*, 2009; El-zahar *et al.*, 2011). This study was undertaken in view of the fact that hyrazone (Onnis *et al.*, 2009; Edrees *et al.*, 2010), triazole (Atta, 2011; Singh *et al.*, 2012), and pyrazole (Nitulescu *et al.*, 2010; Chaudhari *et al.*, 2011) moieties have been reported to possess significant chemotherapeutic activities. In the field of medicinal chemistry, azoles belong to a class of antimicrobial agents that are widely used and studied because of their safety profile and high therapeutic index. Ribavirin, riza-triptan, alprazolam, vorozole, letrozole, and anastrozole are the best examples of drugs containing 1,2,4-triazole moiety (Ashok *et al.*, 2007; Hancu *et al.*, 2007; Cai *et al.*, 2007). Among azole-based drugs, conazoles, such as itraconazole, fluconazole, voriconazole, and ravuconazole, constitute a major class being used for the treatment of fungal infections (Gupta *et al.*, 2007; Schiller and Fung, 2007). Moreover, indole and its derivatives play an important role as biologically active compounds. 2- and 3-aryl-indoles displayed noteworthy antimicrobial activity such as 3-phenylindole which is an inhibitor of brassinin glucosyltrans-ferase, a phytoalexin detoxifying enzyme from the fungus, *Sclerotinia sclerotiorum* (Leboho *et al.*, 2009).

Indole-3-carbinol (I3C) has anti-proliferative and anti-estrogenic activities in human breast cancer cells (Souli *et al.*, 2008). 1-Benzyl-I3C displayed enhanced potency in suppressing the growth of both estrogen responsive (MCF-7) and estrogen-independent (MDA-MB-231) human breast cancer cells (Nguyen *et al.*, 2010). Also, 2-indolylmethanones (Mahboobi *et al.*, 2005), camalexin (indolyl thiazole), 4-(3-indolyl)oxazoles, and 5-(3-indolyl)-1,3,4-oxadiazoles were reported as potential anticancer agents against many types of human cancer cell lines (Kumar *et al.*, 2010). Arbidol (ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivative) is a broad-spectrum antiviral agent that inhibits acute and chronic HCV infection (Sellitto *et al.*, 2010). Di-indolylmethane derivatives possess potential radical scavenging activity (Benabadji *et al.*, 2004). In view of these observations, herein we report the synthesis of some new indolyl-pyrimidines incorporating different potent pharmacophores as trial to develop a novel antimicrobial, antioxidant, and anticancer agents. Their structure and activity relationship were also examined.

## Experimental

All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a

Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK), and chemical shifts were expressed as ppm against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 70 eV EI MS-QP1000 EX (Shimadzu, Japan), Faculty of Science, Cairo University, Cairo, Egypt. Microanalyses were operated using a Vario, Elementar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt. Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm) using chloroform: methanol (3:1) solvent system. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within  $\pm 0.4\%$  of the theoretical values. Compound **1** was prepared as reported in the literature (Mohamed *et al.*, 2011b). Target compounds were synthesized as outlined in Schemes 1 and 2.

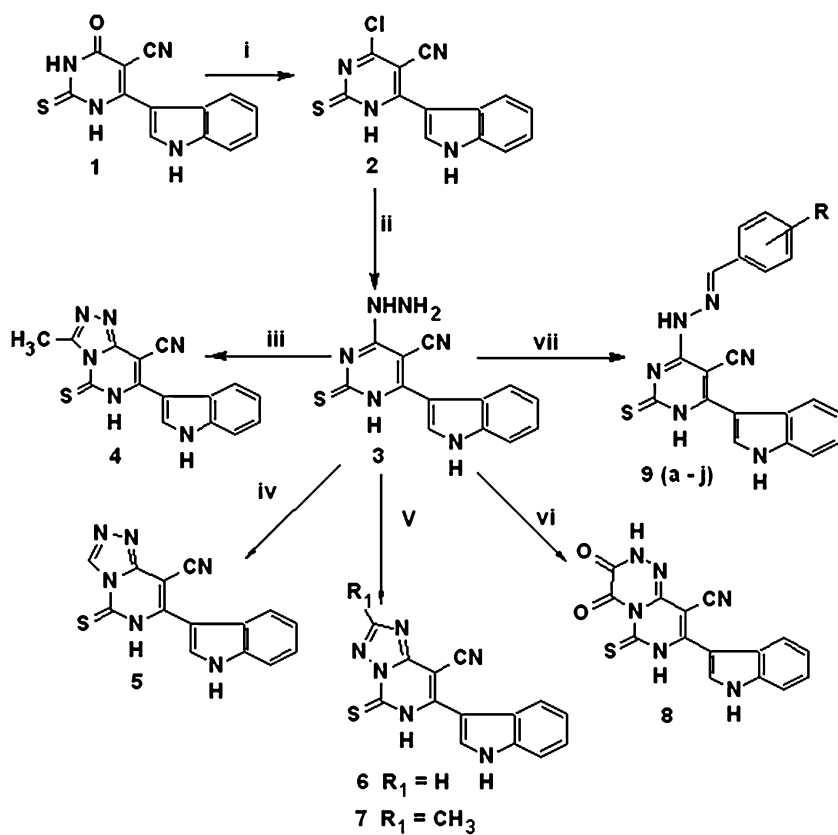
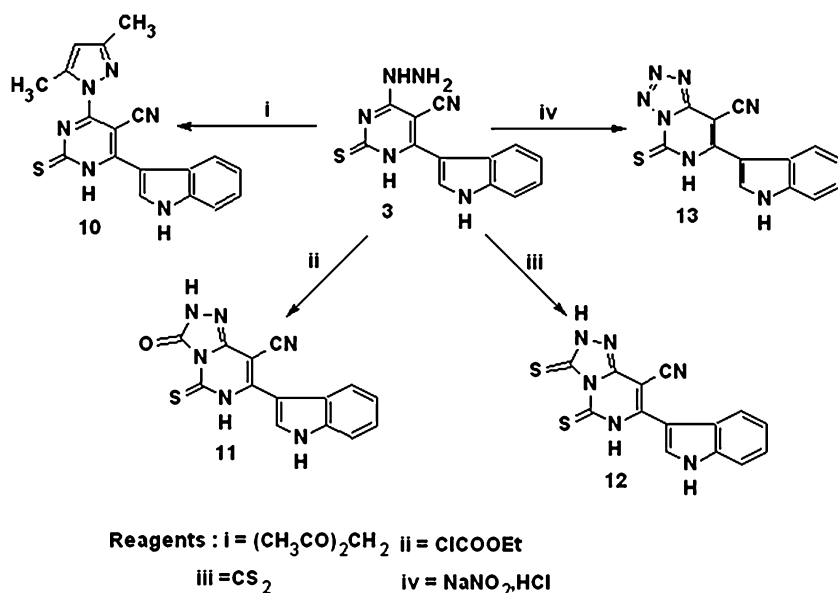
## Synthesis

### 6-(1*H*-Indol-3-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (**1**)

A mixture of ethylcyanoacetate (0.01 mol), thiourea (0.01 mol), indole-3-carboxaldehyde (0.01 mol), and 25 mL sodium ethoxide/ethanol was stirred for 1 h at room temperature. The reaction mixture poured onto ice and neutralized with 2 N HCl, the produced solid was filtered off, dried, and recrystallized from DMF/Water to give compound **1** as a yellow solid. Yield: 80 %; m.p.: 195–198 °C; IR (KBr)  $\text{cm}^{-1}$ : 3300, 2230, 1275; MS (EI)  $m/z$ : 268 ( $\text{M}^+$ , 60.5 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 6.6–7.5 (m, 5H, Ar-H), 10.0 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 10.5 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.2 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 80 (C, C-5), 117 (CN), 105 (C, C-3'), 115 (CH, C-7'), 120 (CH, C-4', C-6'), 122 (CH, C-5'), 124 (CH, C-2'), 128 (C, C-8'), 134 (C, C-9'), 160 (C=O), 168 (C, C-6), 180 (C=S); Anal. Calcd. for  $\text{C}_{13}\text{H}_8\text{N}_4\text{OS}$  (268.29): C, 58.20; H, 3.01; N, 20.88; S, 11.95 %. Found: C, 58.32; H, 3.20; N, 20.73; S, 12.01 %.

### 4-Chloro-6-(1*H*-indol-3-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**2**)

A mixture of indolyl-pyrimidine derivative **1** (0.01 mol) was heated under reflux in phosphorus oxychloride (25 mL) and phosphorus pentachloride (0.01 mol) for 8 h, cooled and poured onto ice to give precipitate, which was washed with petroleum ether, filtered off, and dried under vacuum to give compound **2** as a brown solid. Yield:

**Scheme 1** Synthesis of compounds 1–9**Scheme 2** Synthesis of compounds 10–13

50 %; m.p.: 150–152 °C; IR (KBr)  $\text{cm}^{-1}$ : 3200, 2220, 1275; MS (EI)  $m/z$ : 285 ( $\text{M}^+$ , 60.5 %), 287 ( $\text{M}+2$ , 20.4 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.7 (m, 5H, Ar-H), 10.2 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.2 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 88.5 (C, C-5), 102 (C, C-3'), 115.4 (CH, C-7'), 117 (CN), 122.2 (CH, C-4', C-6'), 124 (CH, C-5'), 128 (CH, C-2'), 130.5 (C, C-8'), 136 (C, C-9'), 159.2 (C=N), 160.1 (C, C-6), 180 (C=S); Anal. Calcd. for  $\text{C}_{13}\text{H}_7\text{ClN}_4\text{S}$  (286.73): C, 54.45; H, 2.46; N, 19.54; S, 11.18 %. Found: C, 54.60; H, 2.55; N, 19.56; S, 11.29 %.

**4-Hydrazino-6-(1H-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (3)**

A mixture of 4-chloropyrimidine **2** (0.01 mol) and hydrazine hydrate (20 mL, 99 %) was refluxed in methanol (30 mL) for 20 min, cooled, stirred for 24 h, and poured onto ice water. The solid obtained was filtered, dried, and recrystallized from DMF/water to yield compound **3** as a brown solid. Yield: 60 %; m.p.: 220–222 °C; IR (KBr)  $\text{cm}^{-1}$ : 3460, 3320, 2200, 1270; MS (EI)  $m/z$ : 282 ( $\text{M}^+$ , 40 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 5.1–5.7 (s, 3H, NH,  $\text{NH}_2$ ,  $\text{D}_2\text{O}$  exchangeable), 7.0–7.8 (m, 5H, Ar-H), 10.3 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.3 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 84.5 (C, C-5), 102 (C, C-3'), 111 (CH, C-7'), 117 (CN), 122.8 (CH, C-4', C-6'), 124.5 (CH, C-5'), 127.2 (CH, C-2'), 128 (C, C-8'), 136 (C, C-9'), 164 (C=N), 167.1 (C, C-6), 180 (C=S); Anal. Calcd. for  $\text{C}_{13}\text{H}_{10}\text{N}_6\text{S}$  (282.32): C, 55.30; H, 3.57; N, 29.77; S, 11.36 %. Found: C, 55.40; H, 3.66; N, 29.90; S, 11.45 %.

**7-(1H-Indol-3-yl)-3-methyl-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-c]pyrimidine-8-carbonitrile (4)**

A mixture of hydrazine derivative **3** (0.01 mol) and acetic anhydride (30 mL) was heated under reflux for 4 h. The solid obtained was filtered, dried, and recrystallized from acetic acid to yield compound **4** as a black crystal. Yield: 55 %; m.p.: 265–267 °C; IR (KBr)  $\text{cm}^{-1}$ : 3250, 2225, 1597, 1275; MS (EI)  $m/z$ : 306 ( $\text{M}^+$ , 45 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.3 (s, 3H,  $\text{CH}_3$ ), 6.5–7.8 (m, 5H, Ar-H), 10.1 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.5 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 20.6 ( $\text{CH}_3$ ), 80.2 (C, C-5), 102.9 (C, C-3'), 115.4 (CH, C-7'), 117 (CN), 122.4 (CH, C-4', C-6'), 124.5 (CH, C-5'), 127.1 (CH, C-2'), 130.5 (C, C-8'), 139.6 (C, C-9'), 151.1, 160 (2C=N), 162.6 (C, C-6), 175.2 (C=S); Anal. Calcd. for  $\text{C}_{15}\text{H}_{10}\text{N}_6\text{S}$  (306.34): C, 58.81; H, 3.29; N, 27.43; S, 10.47 %. Found: C, 58.90; H, 3.33; N, 27.55; S, 10.56 %.

**7-(1H-Indol-3-yl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-c]pyrimidine-8-carbonitrile (5)**

A mixture of hydrazine derivative **3** (0.01 mol) and triethyl orthoformate (30 mL) was heated under reflux for 5 h. The solid obtained was crystallized from acetic acid to yield compound **5** as dark brown crystals. Yield: 60 %; m.p.: 238–240 °C; IR (KBr)  $\text{cm}^{-1}$ : 3237, 2225, 1582; MS (EI)  $m/z$ : 292 ( $\text{M}^+$ , 42 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.9 (m, 5H, Ar-H), 8.3 (s, 1H, C5-H), 9.7 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.0 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 90 (C, C-5), 103 (C, C-3'), 116.6 (CH, C-7'), 117.9 (CN), 122.5 (CH, C-4', C-6'), 124.2 (CH, C-5'), 127.2 (CH, C-2'), 129 (C, C-8'), 137 (C, C-9'), 152 (2C=N), 167 (C, C-6), 179 (C=S); Anal. Calcd. for  $\text{C}_{14}\text{H}_8\text{N}_6\text{S}$  (292.31): C, 57.52; H, 2.76; N, 28.75; S, 10.97 %. Found: C, 57.67; H, 2.85; N, 28.90; S, 11.10 %.

**7-(1H-Indol-3-yl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]pyrimidine-8-carbonitrile (6) and 7-(1H-indol-3-yl)-2-methyl-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]pyrimidine-8-carbonitrile (7)**

A mixture of hydrazine derivative **3** (0.01 mol) and formic or acetic acid (30 mL) was heated under reflux for 8–10 h, then cooled, and poured onto ice water. The solid obtained was crystallized from acetic acid to yield compounds **6** and **7** as black crystals, respectively.

**6:** Yield: 52 %; m.p.: 230–232 °C; IR (KBr)  $\text{cm}^{-1}$ : 3300, 2229, 1608, 1270; MS (EI)  $m/z$ : 292 ( $\text{M}^+$ , 60 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.8 (m, 5H, Ar-H), 8.6 (s, 1H, C3-H), 10.3 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.0 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 88.2 (C, C-5), 102 (C, C-3'), 115.4 (CH, C-7'), 117.2 (CN), 122.2 (CH, C-4', C-6'), 124.2 (CH, C-5'), 128 (CH, C-2'), 130.5 (C, C-8'), 136 (C, C-9'), 155.2 (2C=N), 160 (C, C-6), 180 (C=S); Anal. Calcd. for  $\text{C}_{14}\text{H}_8\text{N}_6\text{S}$  (292.31): C, 57.52; H, 2.76; N, 28.75; S, 10.97 %. Found: C, 57.66; H, 2.78; N, 28.89; S, 11.17 %.

**7:** Yield: 55 %; m.p.: 243–245 °C; IR (KBr)  $\text{cm}^{-1}$ : 3310, 2216, 1606, 1270; MS (EI)  $m/z$ : 306 ( $\text{M}^+$ , 62 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.9 (m, 5H, Ar-H), 10.2 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable), 11.5 (s, 1H, NH,  $\text{D}_2\text{O}$  exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 20.2 ( $\text{CH}_3$ ), 75 (C, C-5), 105 (C, C-3'), 112.1 (CH, C-7'), 117 (CN), 124.5 (CH, C-4', C-6'), 127 (CH, C-5'), 128 (CH, C-2'), 130.5 (C, C-8'), 136 (C, C-9'), 148, 160 (2C=N), 169 (C, C-6), 178 (C=S); Anal. Calcd. for  $\text{C}_{15}\text{H}_{10}\text{N}_6\text{S}$  (306.34): C, 58.81; H, 3.29; N, 27.43; S, 10.47 %. Found: C, 58.90; H, 3.34; N, 27.55; S, 11.01 %.

8-(1*H*-Indol-3-yl)-3,4-dioxo-6-thioxo-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*c*][1,2,4]triazine-9-carbonitrile (**8**)

A solution of hydrazine derivative **3** (0.01 mol) and diethylxalate (0.01 mol) in absolute ethanol (40 mL) was heated under reflux for 12 h. The solid obtained was crystallized from the benzene to yield compound **8** as reddish brown crystals. Yield: 65 %; m.p.: 240–242 °C; IR (KBr)  $\text{cm}^{-1}$ : 3450, 3230, 2220, 1675, 1274; MS (EI)  $m/z$ : 336 ( $M^+$ , 10 %),  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.9 (m, 5H, Ar-H), 8.2 (s, 1H, NH, D<sub>2</sub>O exchangeable), 9.8 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 88.2 (C, C-5), 106 (C, C-3'), 118.8 (CH, C-7'), 117.2 (CN), 122.5 (CH, C-4', C-6'), 124.3 (CH, C-5'), 127.2 (CH, C-2'), 128 (C, C-8'), 136 (C, C-9'), 158.2 (C=N), 160 (C, C-6), 165.9 (2C=O), 180 (C=S); Anal. Calcd. for C<sub>15</sub>H<sub>8</sub>N<sub>6</sub>O<sub>2</sub>S (336.32): C, 53.57; H, 2.40; N, 24.99; S, 9.53 %. Found: C, 53.60; H, 2.49; N, 25.12; S, 10.14 %.

4-[(2*E*)-2-Substituted-(benzylidene)hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles (**9a–9j**)

A mixture of hydrazine derivative **3** (0.01 mol) and appropriate aldehyde (0.01 mol) in ethanol (30 mL) was heated under reflux for 4–7 h. The solid obtained was crystallized from benzene to yield compounds **9a–j**, respectively.

4-[(2*E*)-2-Benzylidene-hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9a**) Yield: 68 %; yellowish brown crystals; m.p.: 190–192 °C; IR (KBr)  $\text{cm}^{-1}$ : 3220, 2225, 1606; MS (EI)  $m/z$ : 70 ( $M^+$ , 15 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.4–7.8 (m, 10H, Ar-H), 8.6 (s, 1H, CH=N), 10.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 72 (C, C-5), 111 (C, C-3'), 117 (CN), 120.8 (CH, C-7'), 122.5 (CH, C-4', C-6'), 124 (CH, C-5'), 127 (CH, C-2'), 128 (C, C-8'), 129 (CH, C-3'', C-5''), 130 (CH, C-2'', C-6''), 132 (CH, C-4''), 135 (C, C-1''), 136 (C, C-9'), 154.3 (N=CH), 163 (C=N), 168.5 (C, C-6), 180 (C=S); Anal. Calcd. for C<sub>20</sub>H<sub>14</sub>N<sub>6</sub>S (370.43): C, 64.85; H, 3.81; N, 22.69; S, 8.66 %. Found: C, 64.95; H, 3.95; N, 22.77; S, 8.80 %.

4-[(2*E*)-2-(4-Fluorobenzylidene)-hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9b**) Yield: 62 %; yellow crystals; m.p.: 198–200 °C; IR (KBr)  $\text{cm}^{-1}$ : 3300, 2224, 1608; MS (EI)  $m/z$ : 388 ( $M^+$ , 13 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s,

1H, NH, D<sub>2</sub>O exchangeable), 6.5–7.8 (m, 9H, Ar-H), 8.4 (s, 1H, CH=N), 10.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.2 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 79 (C, C-5), 105 (C, C-3'), 111 (CH, C-7'), 115 (CH, C-3'', C-5''), 117 (CN), 120 (CH, C-4', C-6'), 122 (CH, C-5'), 124 (CH, C-2'), 126 (CH, C-2'', C-6''), 128 (C, C-8'), 128.7 (C, C-1''), 130 (C, C-4''), 138 (C, C-9'), 154.3 (N=CH), 164 (C=N), 166 (C, C-6), 180 (C=S); Anal. Calcd. for C<sub>20</sub>H<sub>13</sub>FN<sub>6</sub>S (388.42): C, 61.84; H, 3.37; N, 21.64; S, 8.26 %. Found: C, 61.96; H, 3.49; N, 21.69; S, 8.33 %.

4-[(2*E*)-2-(4-Bromobenzylidene)-hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9c**) Yield: 60 %; light brown crystals; m.p.: 170–172 °C; IR (KBr)  $\text{cm}^{-1}$ : 3230, 2225, 1608; MS (EI)  $m/z$ : 448 ( $M^+$ , 18 %), 450 ( $M+2$ , 18.6 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.6–7.9 (m, 9H, Ar-H), 8.4 (s, 1H, CH=N), 10.5 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 79.5 (C, C-5), 106 (C, C-3'), 111 (CH, C-7'), 117 (CN), 120 (CH, C-3'', C-5''), 122 (CH, C-4', C-6'), 124 (CH, C-5'), 125 (CH, C-2'', C-6''), 126 (CH, C-2'), 128 (C, C-8'), 130 (C, C-1''), 132 (C, C-4''), 136 (C, C-9'), 153 (N=CH), 164 (C=N), 167 (C, C-6), 182 (C=S); Anal. Calcd. for C<sub>20</sub>H<sub>13</sub>BrN<sub>6</sub>S (449.32): C, 53.46; H, 2.92; N, 18.70; S, 7.14 %. Found: C, 53.55; H, 3.02; N, 18.82; S, 7.28 %.

4-[(2*E*)-2-(4-Chlorobenzylidene)-hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9d**) Yield: 60 %; yellow crystals; m.p.: 195–197 °C; IR (KBr)  $\text{cm}^{-1}$ : 3360, 2222, 1605; MS (EI)  $m/z$ : 403 ( $M^+$ , 25 %), 405 ( $M+2$ , 8.3 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.1–7.8 (m, 9H, Ar-H), 8.5 (s, 1H, CH=N), 10.5 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.5 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 73.6 (C, C-5), 102 (C, C-3'), 110 (CH, C-7'), 117 (CN), 120 (CH, C-4', C-6'), 122 (CH, C-5'), 123 (CH, C-2'), 124 (CH, C-3'', C-5''), 126 (C, C-8'), 128 (CH, C-2'', C-6''), 130 (C, C-1''), 131.2 (C, C-4''), 136 (C, C-9'), 154.7 (N=CH), 164 (C=N), 171 (C, C-6), 180 (C=S); Anal. Calcd. for C<sub>20</sub>H<sub>13</sub>ClN<sub>6</sub>S (404.87): C, 59.33; H, 3.24; N, 20.76; S, 7.92 %. Found: C, 59.45; H, 3.37; N, 20.88; S, 8.08 %.

4-[(2*E*)-2-(2-Methoxybenzylidene)-hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9e**) Yield: 62 %; brown crystals; m.p.: 184–186 °C; IR (KBr)  $\text{cm}^{-1}$ : 3350, 2223, 1606; MS (EI)  $m/z$ : 400 ( $M^+$ , 10 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 3.81 (s, 3H, OCH<sub>3</sub>), 6.5–7.8 (m, 9H, Ar-H), 8.5 (s, 1H, CH=N), 10.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.5 (s, 1H, NH, D<sub>2</sub>O

exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 56 (O-CH<sub>3</sub>), 78.5 (C, C-5), 111 (C, C-3'), 114.9 (CH, C-3''), 116 (C, C-1''), 117 (CN), 120 (CH, C-7'), 122.3 (CH, C-4', C-6'), 120.9 (C, C-5''), 124.5 (CH, C-5'), 127.7 (CH, C-2'), 128.5 (C, C-8'), 130 (CH, C-6''), 131 (CH, C-4''), 136 (C, C-9'), 152 (N=CH), 160 (C, C-2''), 163 (C=N), 169 (C, C-6), 182 (C=S); Anal. Calcd. for C<sub>21</sub>H<sub>16</sub>N<sub>6</sub>OS (400.45): C, 62.98; H, 4.03; N, 20.99; S, 8.01 %. Found: C, 63.11; H, 4.23; N, 21.15; S, 8.12 %.

4-[(2E)-2-(3,4-Dimethoxybenzylidene)-hydrazino]-6-(1H-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (**9f**) Yield: 63 %; brown crystals; m.p.: 178–180 °C; IR (KBr) cm<sup>-1</sup>: 3375, 2225, 1609; MS (EI) *m/z*: 430 (M<sup>+</sup>, 12 %);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 3.4, 3.81 (s, 6H, OCH<sub>3</sub>), 6.6–7.9 (m, 8H, Ar-H), 8.5 (s, 1H, CH=N), 10.5 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 56.3, 56.6 (O-2CH<sub>3</sub>), 73.6 (C, C-5), 105 (C, C-3'), 111.6 (CH, C-7'), 115 (CH, C-5''), 116 (CH, C-2''), 117 (CN), 120 (CH, C-6''), 122.6 (CH, C-4', C-6'), 124.3 (CH, C-5'), 126 (C, C-1''), 127.8 (CH, C-2'), 128.4 (C, C-8'), 136.9 (C, C-9'), 138 (C, C-3''), 140 (CH, C-4''), 154.5 (N=CH), 162 (C=N), 167.2 (C, C-6), 184 (C=S); Anal. Calcd. for C<sub>22</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S (430.48): C, 61.38; H, 4.21; N, 19.52; S, 7.45 %. Found: C, 61.45; H, 4.39; N, 19.69; S, 7.56 %.

4-[(2E)-2-(3,4,5-Trimethoxybenzylidene)-hydrazino]-6-(1H-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9g**) Yield: 65 %; yellow crystals; m.p.: 194–196 °C; IR (KBr) cm<sup>-1</sup>: 3380, 2220, 1606; MS (EI) *m/z*: 460 (M<sup>+</sup>, 11 %);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 3.2–3.8 (s, 9H, 3OCH<sub>3</sub>), 6.5–7.7 (m, 7H, Ar-H), 8.5 (s, 1H, CH=N), 10.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.3 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 56.3 (O-2CH<sub>3</sub>), 56.5 (O-CH<sub>3</sub>), 79.7 (C, C-5), 104 (C, C-3'), 107 (CH, C-2''), 111.6 (CH, C-7'), 117 (CN), 122.6 (CH, C-4', C-6'), 124.2 (CH, C-5'), 126 (C, C-1''), 127.8 (CH, C-2'), 128.4 (C, C-8'), 130 (C, C-4''), 136.9 (C, C-9'), 142 (C, C-3''), 154.2 (N=CH), 162 (C=N), 166 (C, C-6), 182 (C=S); Anal. Calcd. for C<sub>23</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub>S (460.50): C, 59.99; H, 4.38; N, 18.25; S, 6.96 %. Found: C, 60.30; H, 4.46; N, 18.35; S, 7.08 %.

4-[(2E)-2-(4-Dimethylamino)benzylidene)-hydrazino]-6-(1H-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9h**) Yield: 62 %; brown crystals; m.p.: 185–187 °C; IR (KBr) cm<sup>-1</sup>: 3300, 2222, 1608; MS (EI) *m/z*: 413 (M<sup>+</sup>, 18 %);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 3 (s, 6H,

N(CH<sub>3</sub>)<sub>2</sub>), 6.8–8.1 (m, 9H, Ar-H), 8.4 (s, 1H, CH=N), 10.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.4 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 40.3 (N-2CH<sub>3</sub>), 80 (C, C-5), 102 (C, C-3'), 111 (CH, C-7'), 113 (CH, C-3''), 117 (CN), 120 (CH, C-4', C-6'), 122.4 (CH, C-5'), 125 (C, C-1''), 126.5 (CH, C-2'), 128.6 (C, C-8'), 130 (CH, C-2''), 134.5 (C, C-9'), 140 (C, C-4''), 154.5 (N=CH), 162 (C=N), 169 (C, C-6), 184 (C=S); Anal. Calcd. for C<sub>22</sub>H<sub>19</sub>N<sub>7</sub>S (413.49): C, 63.90; H, 4.63; N, 23.71; S, 7.75 %. Found: C, 63.98; H, 4.78; N, 23.88; S, 7.88 %.

6-(1H-Indol-3-yl)-4-[(2E)-2-(4-nitrobenzylidene)-hydrazino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9i**) Yield: 64 %; reddish brown crystals; m.p.: 200–202 °C; IR (KBr) cm<sup>-1</sup>: 3380, 2220, 1609; MS (EI) *m/z*: 415 (M<sup>+</sup>, 15 %);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.8–8.2 (m, 9H, Ar-H), 8.4 (s, 1H, CH=N), 10.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 82 (C, C-5), 104 (C, C-3'), 112 (CH, C-7'), 117 (CN), 119 (CH, C-4', C-6'), 120 (CH, C-5'), 122.8 (CH, C-2'), 124.6 (C, C-8'), 128 (CH, C-3''), 129 (CH, C-2''), 132 (C, C-9'), 137 (C, C-1''), 145 (C, C-4''), 154.9 (N=CH), 164 (C=N), 169.2 (C, C-6), 180 (C=S); Anal. Calcd. for C<sub>20</sub>H<sub>13</sub>N<sub>7</sub>O<sub>2</sub>S (415.42): C, 57.82; H, 3.15; N, 23.60; S, 7.72 %. Found: C, 57.96; H, 3.27; N, 23.80; S, 7.82 %.

6-(1H-Indol-3-yl)-4-[(2E)-2-(4-methylbenzylidene)-hydrazino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9j**) Yield: 66 %; brown crystals; m.p.: 196–198 °C; IR (KBr) cm<sup>-1</sup>: 3356, 2223, 1609; MS (EI) *m/z*: 384 (M<sup>+</sup>, 19 %);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.2 (s, 3H, CH<sub>3</sub>), 2.4 (s, 1H, NH, D<sub>2</sub>O exchangeable), 6.5–7.9 (m, 9H, Ar-H), 8.4 (s, 1H, CH=N), 9.5 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 12.5 (CH<sub>3</sub>), 82 (C, C-5), 105 (C, C-3'), 114 (CH, C-7'), 117 (CN), 120 (CH, C-4', C-6'), 122 (CH, C-5'), 124.6 (CH, C-2'), 128.5 (C, C-8'), 129 (C, C-1''), 130 (CH, C-2''), 131 (CH, C-3''), 132 (C, C-4''), 134.5 (C, C-9'), 154.1 (N=CH), 164 (C=N), 168 (C, C-6), 182 (C=S); Anal. Calcd. for C<sub>21</sub>H<sub>16</sub>N<sub>6</sub>OS (384.45): C, 65.61; H, 4.19; N, 21.86; S, 8.34 %. Found: C, 65.77; H, 4.28; N, 21.95; S, 8.45 %.

4-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(1H-indol-3-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**10**)

A mixture of hydrazine derivative **3** (0.01 mol) and acetyl acetone (0.1 mL, 0.001 mol) in acetic acid (15 mL) was refluxed for 15 h. The reaction mixture was cooled and poured onto ice water. The solid obtained was crystallized from ethanol to yield compound **10** as brown crystals.

Yield: 55 %; m.p.: 238–240 °C; IR (KBr)  $\text{cm}^{-1}$ : 3348, 2227, 1610; MS (EI)  $m/z$ : 346 ( $\text{M}^+$ , 27 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 2.3, 2.5 (s, 6H, 2CH<sub>3</sub>), 6.8 (s, 1H, C<sub>4</sub>-H), 7–7.9 (m, 5H, Ar-H), 10.0 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.0 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 10, 15.3 (2CH<sub>3</sub>), 92 (C, C-5), 102 (C, C-3'), 103 (C, C-4''), 117.7 (CH, C-7'), 117 (CN), 122.2 (CH, C-4', C-6'), 124 (CH, C-5'), 128.2 (CH, C-2'), 134.4 (C, C-8'), 138.3 (C, C-9'), 146 (C, C-5''), 148 (C=N), 164 (C=N), 169 (C, C-6), 174 (C=S); Anal. Calcd. for C<sub>18</sub>H<sub>14</sub>N<sub>6</sub>S (346.40): C, 62.41; H, 4.07; N, 24.26; S, 9.26 %. Found: C, 62.55; H, 4.19; N, 24.38; S, 9.33 %.

*7-(1H-Indol-3-yl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro[1,2,4]-triazolo[4,3-c]pyrimidine-8-carbonitrile (11)*

A mixture of hydrazine derivative **3** (0.01 mol) and ethylchloroformate (0.02 mol) in pyridine (30 mL) was heated under reflux for 12 h and poured on 2 N HCl. The solid obtained was crystallized from dimethyl formamide to yield compound **11** as black crystals. Yield: 60 %; m.p.: 230–232 °C; IR (KBr)  $\text{cm}^{-1}$ : 3210, 2229, 1665; MS (EI)  $m/z$ : 308 ( $\text{M}^+$ , 48 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.8 (m, 5H, Ar-H), 8.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 10.1 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.4 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 79.5 (C, C-5), 102.9 (C, C-3'), 112.2 (CH, C-7'), 115.7 (CN), 124.3 (CH, C-4', C-6'), 128 (CH, C-5'), 130.5 (CH, C-2'), 136 (C, C-8'), 147.8 (C, C-9'), 158 (C=N), 164 (C=O), 170.9 (C, C-6), 175 (C=S); Anal. Calcd. for C<sub>14</sub>H<sub>8</sub>N<sub>6</sub>OS (308.31): C, 54.54; H, 2.62; N, 27.26; S, 10.40 %. Found: C, 54.61; H, 2.77; N, 27.36; S, 10.54 %.

*7-(1H-Indol-3-yl)-3,5-dithioxo-2,3,5,6-tetrahydro[1,2,4]triazolo[4,3-c]pyrimidine-8-carbonitrile (12)*

To an ice-cold solution of hydrazine derivative **3** (0.01 mol) and KOH (0.01 mol) in ethanol (20 mL) was added dropwise with stirring CS<sub>2</sub> (10 mL), then the reaction mixture was refluxed on a water-bath for 5 h. The reaction mixture cooled, poured onto ice water, neutralized with 2 N HCl, filtered, and crystallized from ethanol to yield compound **12** as black crystals. Yield: 60 %; m.p.: 237–239 °C; IR (KBr)  $\text{cm}^{-1}$ : 3290, 2222, 1650, 1580; MS (EI)  $m/z$ : 324 ( $\text{M}^+$ , 30 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 6.5–7.9 (m, 5H, Ar-H), 9.5 (s, 1H, NH, D<sub>2</sub>O exchangeable), 10.3 (s, 1H, NH, D<sub>2</sub>O exchangeable), 11.5 (s, 1H, NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 75 (C, C-5), 104.2 (C, C-3'), 111.5 (CH, C-7'), 117 (CN),

124.2 (CH, C-4', C-6'), 128 (CH, C-5'), 130.5 (CH, C-2'), 136.2 (C, C-8'), 147.8 (C, C-9'), 159.9 (C=N), 169 (C, C-6), 178, 188.2 (2C=S); Anal. Calcd. for C<sub>14</sub>H<sub>8</sub>N<sub>6</sub>S<sub>2</sub> (324.38): C, 51.84; H, 2.49; N, 25.91; S, 19.77 %. Found: C, 51.95; H, 2.55; N, 26.10; S, 20.09 %.

*7-(1H-Indol-3-yl)-5-thioxo-5,6-dihydrotriazolo[1,5-c]pyrimidine-8-carbonitriles (13)*

A cold solution (0–5 °C) of sodium nitrite (1 g, 0.144 mol) in H<sub>2</sub>O (15 mL) was added gradually within 15 min to a cold stirred solution of hydrazine derivative **3** (0.01 mol) in 2 N HCl (15 mL). After addition, the reaction mixture was then further stirred for 4 h at the same temperature, and then it was diluted with cold H<sub>2</sub>O. The solid obtained was crystallized from ethanol to yield compound **13** as yellowish brown crystals. Yield: 62 %; m.p.: 210–212 °C; IR (KBr)  $\text{cm}^{-1}$ : 3345, 2227, 1507; MS (EI)  $m/z$ : 293 ( $\text{M}^+$ , 48 %);  $^1\text{H-NMR}$  (DMSO- $d_6$ , 300 MHz)  $\delta$  (ppm): 7.0–7.9 (m, 5H, Ar-H), 10, 11.2 (s, 2H, 2NH, D<sub>2</sub>O exchangeable);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 79 (C, C-5), 102.3 (C, C-3'), 116.5 (CH, C-7'), 117.5 (CN), 120.3 (CH, C-4', C-6'), 124.4 (CH, C-5'), 128.7 (CH, C-2'), 138.2 (C, C-8'), 140.2 (C, C-9'), 160 (C=N), 166 (C, C-6), 182 (C=S); Anal. Calcd. for C<sub>13</sub>H<sub>7</sub>N<sub>7</sub>S (293.30): C, 53.23; H, 2.41; N, 33.43; S, 10.93 %. Found: C, 53.38; H, 2.56; N, 33.57; S, 11.04 %.

*Antimicrobial activity*

All compounds were evaluated for antibacterial activity against several pathogenic representative Gram-positive bacteria (*S.aureus* ATCC12600, *B.cereus* ATCC14579), Gram-negative bacteria (*Escherichia coli* ATCC11775), and (*Candida albicans* ATCC26555, *Aspergillus flavus* ATCC 11495) as a representative for fungi using the disk diffusion method (Bauer *et al.*, 1996). All microorganisms used were obtained from the culture collection of the Department of Microbiology, Micro Analytical Centre, Faculty of Science, Cairo University, Cairo, Egypt. Media for disk sensitivity tests were the nutrient agar and Muller-Hinton agar (MHA) purchased from Difco (USA). Non-sterile powder of tested compounds was dissolved in sterile DMSO to yield 10 mg/mL solution, and passed through a 0.2  $\mu\text{m}$  membrane filter (MilliporeCorp, SA).

Penicillin (Bioanalyse, Turkey) and Fluconazole (Sigma-Aldrich, USA) were used as reference substances. Inhibition zones were measured in millimeters at the end of an incubation period (Table 1).

*Antibacterial evaluation*

All the newly synthesized compounds were screened for their antibacterial activity against Gram-positive bacteria



**Table 1** Inhibition zone diameters (mm) of test compounds

Compd. no <sup>a</sup>	Inhibition zone diameter (mm)				
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. flavus</i>
<b>1</b>	12	10	10	10	–
<b>2</b>	14	13	12	12	17
<b>3</b>	17	14	14	–	14
<b>4</b>	–	–	–	–	–
<b>5</b>	23	20	14	18	20
<b>6</b>	8	7	8	8	7
<b>7</b>	8	6	7	8	7
<b>8</b>	15	–	–	–	19
<b>9a</b>	17	15	12	15	15
<b>9b</b>	18	16	12	16	14
<b>9c</b>	19	17	12	17	18
<b>9d</b>	18	18	13	18	14
<b>9e</b>	19	19	15	16	18
<b>9f</b>	20	21	17	17	16
<b>9g</b>	24	22	19	19	17
<b>9h</b>	21	22	18	18	18
<b>9i</b>	27	23	19	19	19
<b>9j</b>	25	21	19	19	19
<b>10</b>	14	12	12	–	–
<b>11</b>	13	–	–	–	–
<b>12</b>	–	11	11	13	–
<b>13</b>	17	15	13	–	–
Penicillin <sup>b</sup>	16	13	12	–	–
Fluconazole <sup>b</sup>	–	–	–	20	21

– No inhibition

<sup>a</sup> 10 mg/mL<sup>b</sup> 50 µg/mL in DMSO, DMSO shows no activity

viz. *S. aureus* (ATCC12600) and *B. cereus* (ATCC14579), and Gram-negative bacteria viz. *E. coli* ATCC11775 by disk diffusion method (Bauer *et al.*, 1996).

For the antibacterial assay, standard inoculums ( $1-2 \times 10^7$  c.f.u./MI 0.5 McFarland standards) were introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in the nutrient agar medium. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the standard drug Penicillin. The data are presented in Table 1.

Minimum inhibitory concentration (MIC) was determined by the broth micro-dilution procedures (NCCLS, 1982; Rostom *et al.*, 2009). Bacterial colonies of the test

**Table 2** Antimicrobial activity results of newly synthesized compounds, expressed as MIC (µg/mL) with the standard drugs

Compds. & Stander	MIC of tested compounds (µg/mL)				
	<i>S. aureus</i> ATCC 12600	<i>B. cereus</i> ATCC 14579	<i>E. coli</i> ATCC 11775	<i>C. albicans</i> ATCC 26555	<i>A. flavus</i> ATCC 1495
<b>1</b>	32	32	32	32	NT
<b>2</b>	32	32	32	32	16
<b>3</b>	16	64	32	NT	32
<b>5</b>	16	16	16	16	16
<b>6</b>	128	128	128	128	256
<b>7</b>	128	128	128	128	256
<b>8</b>	32	NT	NT	NT	32
<b>9a</b>	32	32	32	32	32
<b>9b</b>	32	32	32	32	32
<b>9c</b>	32	32	32	32	32
<b>9d</b>	32	32	32	32	32
<b>9e</b>	32	32	32	32	32
<b>9f</b>	32	32	32	32	32
<b>9g</b>	16	16	16	16	16
<b>9h</b>	32	32	32	32	32
<b>9i</b>	16	16	16	16	16
<b>9j</b>	16	16	16	16	16
<b>10</b>	32	32	32	NT	NT
<b>11</b>	32	NT	NT	NT	NT
<b>12</b>	NT	64	32	32	NT
<b>13</b>	16	32	32	NT	NT
Penicillin <sup>a</sup>	16	32	32	NT	NT
Fluconazole <sup>b</sup>	NT	NT	NT	16	16

All tested compounds = 10 mg/disk in DMSO, DMSO shows no activity  
NT not tested NB: **4** was inactive toward all tested organisms in both used concentration

<sup>a</sup> Penicillin = 10 mg/mL<sup>b</sup> Fluconazole = 50 µg/mL

organisms were suspended directly into a small volume of 0.9 % saline and further diluted until turbidity matched the Mc Farland Standard no: 0.5 Petri dishes containing Mueller-Hinton agar were impregnated with these microbial suspensions. The stock solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO), which had no effect on the organisms in the concentrations studied. All of the dilutions were done with distilled water. The solution of the newly synthesized compounds and standard drug were prepared at 1024, 512, 256, 128, 64, 32, and 16 µg/mL. DMSO was used as negative control. Penicillin was used as reference drug for antibacterial activity. All the inoculated plates were incubated at 37 °C, and results were evaluated after 24 h for bacteria. The lowest concentration of the compounds that prevented visible growth was considered minimal inhibitor concentrations (MICs) (Table 2).



### Antifungal evaluation

**Preliminary in vitro anti-fungal testing using diffusion disk method** All compounds **1–13** were also evaluated for in vitro antifungal activity against two fungi viz. *C. albicans* (ATCC26555) and *A. flavus* (ATCC 11495), by agar diffusion method (NCCLS, 1982). For the antifungal assay, Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri-dish, excess of suspension was decanted, and the plates were dried by placing in an incubator at 37 °C for 1 h. Using agar punch, wells were made, and each well was labeled. A control was also prepared and maintained at 37 °C for 3–4 days. The *C. albicans* was grown for 48 h at 28 °C in YPD broth (1 % yeast extract, 2 % peptone, and 2 % dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. flavus* was plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for 48 h. Spores were washed three times with sterile-distilled water and resuspended in distilled water to obtain an initial inoculum size of 10<sup>5</sup> spores/mL. Each plate contained three paper disks of the compound tested, standard drug, and solvent (DMSO). The plates were kept undisturbed for at least 2 h at room temperature. After incubation of the plates, the diameter of the inhibition zone was measured and compared with the standard drug Fluconazole (Table 1).

The in vitro antifungal activity of the compounds was tested in Sabouraud's dextrose broth (Difco) by micro-dilution procedures (NCCLS, 1982; Rostom *et al.*, 2009). Fungal colonies of the test organisms were suspended directly into a small volume of 0.9 % saline and further diluted until turbidity matched the Mc Farland Standard no: 0.5 Petri dishes containing Sabouraud Dextrose agar were impregnated with these microbial suspensions. The stock solutions of the synthesized compounds were prepared in DMSO, which had no effect on the organisms in the concentrations studied. All of the dilutions were done with distilled water. The solution of the newly synthesized compounds and standard drug were prepared at 1024, 512, 256, 128, 64, 32, and 16 µg/mL. DMSO was used as negative control. Fluconazole was used as reference drug for antifungal activity. All the inoculated plates were incubated at 28 °C, and results were evaluated after 48 h for fungi. The lowest concentration of the compounds that prevented visible growth was considered minimal inhibitor concentrations (MICs) (Table 2).

### Antioxidant activity

Determination of radical scavenging activity using DPPH assay: indolyl-pyrimidine derivatives **3**, **4**, **8**, **9g**, and **11** were evaluated for their antioxidative potential through in vitro DPPH radical scavenging model. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to reported methods (Blois, 1958; Brand-Williams *et al.*, 1995). Compounds of different concentrations were prepared in DMSO; 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200, and 300 µg/mL) were taken in different test tubes; 4 mL of 0.1 mg/mL DMSO solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and DMSO was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV–Visible spectrophotometer (Shimadzu 160A). Scavenging of DPPH free radicals was calculated from the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_c - A_t)/A_c] \times 100,$$

where  $A_c$  is absorbance of control,  $A_t$  is absorbance of compound.

### Results

The relation between DPPH scavenging percentage against compound concentrations is plotted to get compound concentration providing a 50 % decrease in absorbance of DPPH radical (IC<sub>50</sub> %). The radical scavenging activities were expressed as IC<sub>50</sub>. Vitamin C served as reference compound. The results have been given in Table 3 and Fig. 3.

**Table 3** IC<sub>50</sub> values<sup>a</sup> (in µg/mL) for DPPH scavenging ability of the compounds

Compounds	IC <sub>50</sub> (µg/mL)
<b>3</b>	7.52 ± 1.5
<b>4</b>	52.49 ± 5.2
<b>8</b>	28.36 ± 2.03
<b>9g</b>	6.21 ± 0.02
<b>11</b>	49.49 ± 4.6
Ascorbic acid	10 ± 0.043

<sup>a</sup> IC<sub>50</sub> values (in µg/mL), which the concentration required for a 50 % decrease in absorbance of DPPH radical. Results are presented as a mean ± SEM ( $n = 3$ ), eight replica each

### In vitro cytotoxicity activity

Indolyl-pyrimidines **3**, **4**, **8**, **9g**, and **11** were subjected to a screening system for evaluation of their anticancer activity against cell line of human cancer, namely colon (CaCo-2) cancer obtained from pharmacology screening unit of the National Cancer Institute (NCI), Cairo University, Egypt, following the Sulfo Rhod-amine-B-stain (SRB) assay method (Skehan *et al.*, 1990) in comparison to the known anticancer drugs: Doxorubicin. The SRB assay, which was developed in 1990, is one of the most widely used methods where it relies on the ability of SRB to bind to protein components of the cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

### Materials, methods, and reagents

Fetal calf serum (FCS) was from Invitrogen Co. (Carlsbad, CA). DMEM medium was from Cambrex (New Jersey, USA). DMSO, doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were from Sigma Chemical Co. (St. Louis, USA). Samples: Stock solutions of compounds were prepared in DMSO and kept at 20 °C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays. Final concentrations of DMSO did not interfere with the cell growth.

### SRB cytotoxic assay

The cultured colon carcinoma cell CaCo-2 from the National Cancer Institute (NCI, Cairo, Egypt) is routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum (FCS), antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). Cells were plated in 96-multiwell plate (10<sup>4</sup> cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5 % CO<sub>2</sub>. After 48 h, cells were fixed, washed, and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity is measured in an ELISA reader at a wavelength of 570 nm. Results are expressed as means of at least three independent experiments performed in duplicate. The results are

**Table 4** IC<sub>50</sub> values<sup>a</sup> (in µg/mL) for cytotoxic activity of the compounds against CaCo-2 cell by SRB assay

Compounds	IC <sub>50</sub> (µg/mL)
<b>3</b>	28.59 ± 2.10
<b>4</b>	8.28 ± 0.49
<b>8</b>	38.55 ± 4.49
<b>9g</b>	8.84 ± 0.11
<b>11</b>	15.97 ± 1.99
Doxorubicin	12 ± 0.043

<sup>a</sup> IC<sub>50</sub> values (in µg/mL), which the concentration required for a 50 % of cell growth inhibition. Results are presented as a mean ± SEM of three independent experiments performed in duplicate

expressed as growth inhibition of 50 % (IC<sub>50</sub>) of cells (Table 4).

## Results and discussion

### Chemistry

The synthetic strategy to synthesize the target indolyl-pyrimidine compounds **1–13** is depicted in Schemes 1 and 2. The synthesis of 4-chloro-2-thiopyrimidine **2** has been achieved via heating under reflux a mixture of 4-oxo-2-thiopyrimidine analog **1** with phosphorus oxychloride and phosphorus pentachloride in boiling water bath (Mohamed *et al.*, 2011c), then converted the latter to 4-hydrazino derivative **3** by reaction with hydrazine hydrate (99 %) in methanol. This hydrazino derivative was the key compound for preparation of all the rest indolyl-pyrimidine derivatives. The condensation of 4-hydrazino derivative **3** with acetic anhydride, triethyl orthoformate (TEOF), and formic or acetic acid (Mohamed *et al.*, 2005; Mohamed *et al.*, 2011a; Taher and Helwa, 2012) afforded 7-(1*H*-indol-3-yl)-5-thioxo-[1,2,4]triazolo[4,3-*c*]pyrimidine-8-carbonitriles **4**, **5**, and 7-(1*H*-indol-3-yl)-5-thioxo-[1,2,4]triazolo[1,5-*c*]pyrimidine-8-carbonitriles **6**, **7**, while reaction of compound **3** with diethyl oxalate in refluxing ethanol yielded carbonitrile **8**. Refluxing of **3** with appropriate aldehydes in absolute ethanol (Cocco *et al.*, 2006) gave 4-[(2*E*)-2-benzylidene]hydrazino]-6-(1*H*-indol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles **9a–j** as revealed in Scheme 1. The reflux of **3** with acetylacetone in acetic acid afforded compound **10** (El-zahar *et al.*, 2011). Further, cyclocondensation of **3** with ethyl chloroformate or CS<sub>2</sub>/KOH afforded carbonitriles **11** and **12**, respectively (Fathalla *et al.*, 2012; Mohamed *et al.*, 2012a). Finally, 7-(1*H*-indol-3-yl)-5-thioxo-5,6-dihydro-tetrazolo[1,5-*c*]pyrimidine-8-carbonitrile **13** was produced by stirring with sodium nitrite in HCl (El-Sawy *et al.*, 2010) as revealed in Scheme 2. The structures of new compounds were confirmed by MS, IR,

$^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , as well as elemental analysis. The structure of **2** was established on the basis of IR, which showed CN band at  $2220\text{ cm}^{-1}$  and absorbance band at  $3200\text{ cm}^{-1}$  corresponding NH, while  $^1\text{H-NMR}$  spectrum revealed signals for two NH protons. IR spectrum of compound **3** showed two absorbance bands at  $3460\text{--}3200\text{ cm}^{-1}$  corresponding  $\text{NHNH}_2$ .  $^1\text{H-NMR}$  spectrum showed three singlets of  $\text{NHNH}_2$  around 5.1 and 5.7 ppm. The IR spectrum of **4** showed the absence of absorbance bands at  $3460\text{--}3200\text{ cm}^{-1}$  for  $(\text{NHNH}_2)$ . Its  $^1\text{H-NMR}$  spectrum showed a singlet signal at  $\delta$  2.3 ppm ( $\text{CH}_3$ ) and two singlets at  $\delta$  10.1 and 11.5 ppm (2NH). The  $^1\text{H-NMR}$  for **5** showed singlet signals for  $\text{C}_5\text{-H}$  and two NH. Also, MS spectra gave their molecular ion peaks. The  $^1\text{H-NMR}$  spectrum for **6** revealed singlet signals for  $\text{C}_3\text{-H}$  and two NH, for **7** signals for  $\text{CH}_3$  and two NH. Compounds **8** and **9** were confirmed by spectral data, and the mass spectrum studies of these compounds gave additional evidence for the proposed structures. The  $^1\text{H-NMR}$  of **10** showed two singlet signals at 2.3, 2.5 ppm corresponding to two  $\text{CH}_3$  group and only two singlets corresponding to NH. The IR spectrum of product **10** was compatible with the proposed structure. The IR spectrum of compound **11** displayed absorbance band at  $1665\text{ cm}^{-1}$  for  $\text{C=O}$ . The  $^1\text{H-NMR}$  and mass spectra supported the structure. The IR spectrum of **12** showed additional absorbance band corresponding to the new  $\text{C=S}$ , and  $^1\text{H-NMR}$  showed three singlet signals for three NH protons. The absence of absorbance bands at  $3460\text{--}3200\text{ cm}^{-1}$  ( $\text{NHNH}_2$ ) in IR spectrum was characteristic for compound **13**. All structures were assigned by their mass spectra,  $^{13}\text{C-NMR}$  and elemental analysis.

#### Antimicrobial studies

##### Antibacterial activity

The antibacterial data (Tables 1, 2) revealed that all tested compounds have moderate to high antibacterial activity, except compounds **4**, **6**, and **7** were either inactive or weakly active against the tested microorganism. As compared to the standard drug Penicillin, compounds **5** and **9g, i, and j** showed very promising activity against *S. aureus*, *B. cereus*, *E. coli* (MIC = 16–32  $\mu\text{g/mL}$ ). Compound **3** shows potent antibacterial activity against *S. aureus* and *E. coli* (MIC = 16 and 32  $\mu\text{g/mL}$ , respectively), compound **10** against *B. cereus* and *E. coli* (MIC = 32  $\mu\text{g/mL}$ ). Also, compound **13** shows potent activity against *S. aureus* (MIC = 16  $\mu\text{g/mL}$ ), *B. cereus*, and *E. coli* (MIC = 32  $\mu\text{g/mL}$ ).

##### Antifungal activity

The screening data of antifungal activity of these compounds show moderate to good activity against the tested fungi except

compounds **4**, **10**, **11**, and **13** were inactive against *C. albicans* and *A. flavus*. Compounds **5** and **9g, i, and j** exhibited potent in vitro antifungal activity against *C. albicans* and *A. flavus* (MIC = 16  $\mu\text{g/mL}$ ) compared to the standard drug Fluconazole (MIC = 16  $\mu\text{g/mL}$ ). Also compound **2** showed pronounced antifungal activity against *A. flavus* (MIC = 16  $\mu\text{g/mL}$ ).

##### Structure–activity relationship (SAR)

The SAR of newly synthesized compounds based on the observed results explored the importance of the nature of the heterocycles nucleus and the nature of the substituent on position 4 of pyrimidine.

First, regarding the influence of the nature of the heterocycles nucleus, it is observed that triazolo[4,3-*c*]pyrimidine **5** acquired significant antimicrobial activity against Gram-positive (*S. aureus*, *B. cereus*), Gram-negative (*E. coli*) bacteria and fungi (*C. albicans* and *A. flavus*). On the other hand, increased antibacterial activity was achieved by cyclization to pyrazolopyrimidine **10** or tetrazolopyrimidine **13** against *S. aureus*, *B. cereus*, and *E. coli*, while cyclization to pyrimido[6,1-*c*][1,2,4]triazine **8** or triazolo[4,3-*c*]pyrimidine **11** increased activity only against *S. aureus* or *A. flavus*. However, cyclization to triazolo[1,5-*c*]pyrimidines **6** and **7** or triazolo[4,3-*c*] pyrimidines as in **4** and **12** either decreased or diminished antimicrobial activity (Fig. 1).

Regarding the nature of the substituent, 4-chloropyrimidine **2** shows significant activity against *B. cereus*, *E. coli* (MIC = 32  $\mu\text{g/mL}$ ), and *A. flavus* (MIC = 16  $\mu\text{g/mL}$ ) and moderate activity against *S. aureus* and *C. albicans* (MIC = 32  $\mu\text{g/mL}$ ). Compound **3**, in which the chlorine in the 4-position is replaced by hydrazine hydrate, shows significant activity against *S. aureus* (MIC = 16  $\mu\text{g/mL}$ ), *E. coli* (MIC = 32  $\mu\text{g/mL}$ ), and moderate activity against *B. cereus* (MIC = 64  $\mu\text{g/mL}$ ) and *A. flavus* (MIC = 32  $\mu\text{g/mL}$ ). Conversion of 4-hydrazine derivative **3** to hydrazones (**9a–j**) caused a pronounced inhibition effect against *S. aureus*, *B. cereus*, *E. coli*, *C. Albicans*, and *A. flavus* (MIC = 16–32  $\mu\text{g/mL}$ ).

The in vitro antimicrobial activity of compounds **9a–j** is shown to be increased when groups such as 3,4,5-( $\text{OCH}_3$ )<sub>3</sub>,  $\text{-NO}_2$ , and  $\text{-CH}_3$  are present as in **9g, i** and **j**, respectively (MIC = 16  $\mu\text{g/mL}$ ).

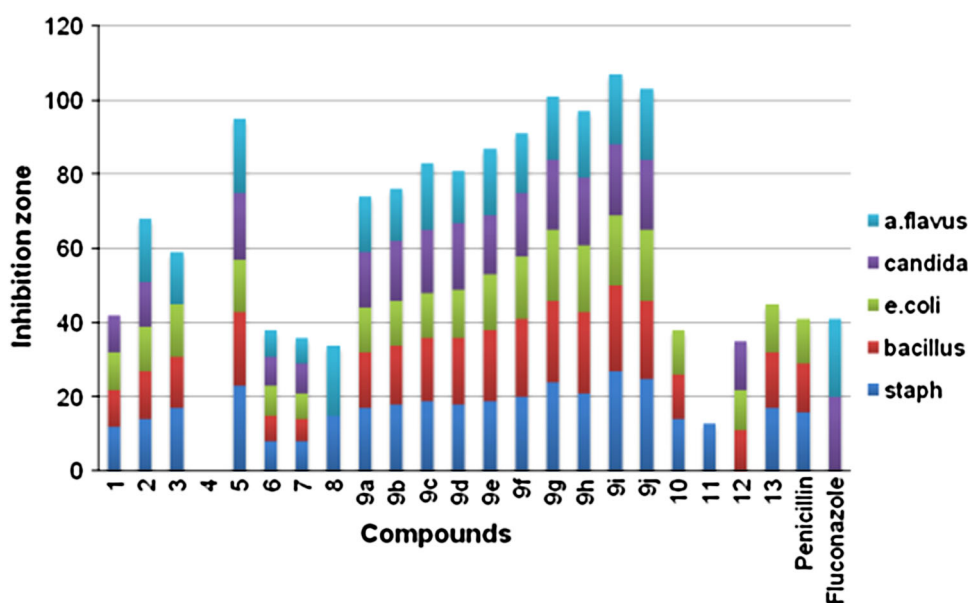
The SAR suggested that conversion of indolyl-pyrimidine to hydrazones **9g, i, and j** or triazolo [4,3-*c*] pyrimidine **5** showed higher antibacterial and antifungal activities than other derivatives.

##### Antioxidant evaluation

##### DPPH free radical scavenging assay

Recent evidence suggests that free radicals, which are generated in any bioorganic redox processes, may induce

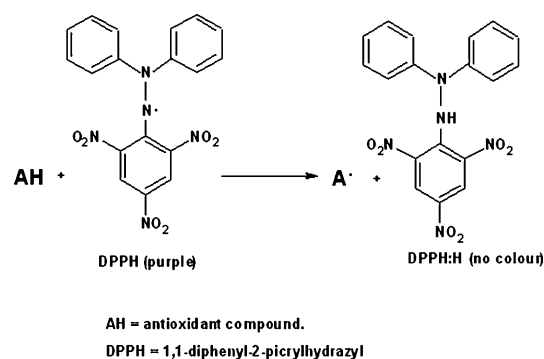
**Fig. 1** Antimicrobial activity (Gram +ve, -ve, Fungi) of synthesized compounds



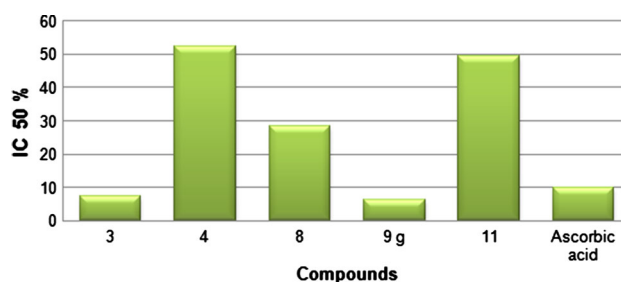
oxidative damage in various components of human body (e.g., lipids, proteins, and nucleic acids). Antioxidants are very interesting, particularly in terms of prevention of the presumed deleterious effects of free radicals in the human body and in fats or other constituents of food stuffs. There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Carocho and Ferreira, 2013). One such method that is currently popular is based on the use of the stable, free radical diphenyl picryl hydrazyl (DPPH). A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals, by providing hydrogen atoms (Fig. 2) or by electron donation via a free radical attack on the DPPH molecule, and convert them to colorless product (Amarowicz *et al.*, 2004; Siddhuraju and Becker, 2007).

Free radical scavenging activity of compounds **3**, **4**, **8**, **9g**, and **11** was evaluated by DPPH assay and compared to those of the well-known antioxidant vitamin C (Table 3). We can conclude that the studied compounds were able to reduce DPPH in a concentration-dependent manner. The tested samples were statistically different ( $P < 0.05$ , Kruskal–Wallis test) over the dose range used. Compounds **3** and **9g** showed more potent-free radical scavenging activity ( $IC_{50} = 7.52$  and  $6.21 \mu\text{g/mL}$ , respectively) than the reference drug, vitamin C ( $IC_{50} = 10 \mu\text{g/mL}$ ) (Fig. 3).

On critical overview of synthesized compounds, it has been found that compounds with electron donating group ( $-\text{OCH}_3$ ) on phenyl ring exhibited potent antioxidant activity.



**Fig. 2** The DPPH radical scavenging assay



**Fig. 3** Screening of antioxidant activity by the DPPH assay shows that **3** and **9g** have highest activity (more potent than the reference drug (RF). Each value represents a mean  $\pm$  SEM ( $n = 3$ ))

#### Structure activity relationship (SAR)

Interpretation of the obtained results and considering the SAR of the tested compounds showed that, 4-hydrazinopyrimidine **3** and hydrazone derivative with substituent ( $R = 3,4,5-\text{OCH}_3$ ) **9g** possess potent antioxidant activity. While, triazolo[4,3-*c*]pyrimidines as in **4** and **11** and



pyrimido[6,1-*c*][1,2,4]triazine **8** showed moderate antioxidant activity.

Therefore, more active compounds (**3** and **9g**) because it quenches DPPH free radical by providing hydrogen atoms or by electron donation via a free radical attack on the DPPH molecule.

#### Cytotoxic activity

Five of the newly synthesized compounds (**3**, **4**, **8**, **9g**, **11**) were primary screened for their in vitro cytotoxicity against (CaCo-2) cell line (Table 4). Compounds **3** (with hydrazine hydrate group in molecule) and **8** (with triazine moiety) showed moderate cytotoxic activity against CaCo-2 cell line ( $IC_{50} = 28.59$  and  $38.55$   $\mu\text{g/mL}$ , respectively). Compound **11** (with triazole moiety) showed significant cytotoxic activity ( $IC_{50} = 15.97$   $\mu\text{g/mL}$ ). Compounds **4** (with 3-methyl triazole moiety) and **9g** (with phenyl hydrazono moiety) showed more potent activity ( $IC_{50} = 8.28$  and  $8.84$   $\mu\text{g/mL}$ , respectively) than the reference drug doxorubicin ( $IC_{50} = 12$   $\mu\text{g/mL}$ ).

Compounds **4** and **9g** that contain 3-methyl triazole and phenyl hydrazono moieties attached to indolyl-pyrimidine moiety are associated with remarkable cytotoxicity against CaCo-2 cancer cell line.

#### Structural–activity relationship (SAR)

From the above obtained results (Table 1), we can conclude that the anticancer activity is due to:

- (i) The presence of triazole, phenylhydrazono moieties.
- (ii) The presence of nitrile generally enhancing the activity.
- (iii) The presence of 2-thiouracil, indole moieties is essential for activity.

#### Summary

We have synthesized a series of novel indolyl-pyrimidine derivatives and have been screened as antimicrobial, antioxidant, and anticancer agents. Interpretation of the results with considering the SAR proved that the most promising antimicrobial compounds are 7-(1*H*-indol-3-yl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-*c*]pyrimidine-8-carbonitrile (**5**), 6-(1*H*-indol-3-yl)-4-[(2*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9g**), 6-(1*H*-indol-3-yl)-4-[(2*E*)-2-(4-nitrobenzylidene)hydrazino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9i**) and 6-(1*H*-indol-3-yl)-4-[(2*E*)-2-(4-methylbenzylidene)hydrazino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**9j**).

Compounds **3** and **9g** were found to possess promising antioxidant activity when compared with standard ascorbic acid (vitamin C). Compounds **4** and **9g** were found to be the highly active compounds against CaCo-2 cell line compared to the reference drug doxorubicin. In conclusion, the preliminary biological studies lead to the identification of novel antimicrobials, antioxidant, and cytotoxic agents. The findings demonstrate indolyl-pyrimidines as novel leads for further development as medicinal agents.

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