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New Thiazole and Thiazolopyrimidine Derivatives: Synthesis, Antimicrobial, Antiquorum-Sensing and Antitumor Evaluation

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ABSTRACT

New thiazole and thiazolopyrimidine derivatives 2-13 were synthesized and estimated for antimicrobial efficacy on S. aureus, B. cereus, E. coli, C. albicans, A. fumigatus 293 and A. terreus. The obtained results proved that compounds 3 and 10 have significant activity toward S. aureus and B. cereus, whereas 2, 11 and 13 exhibited good activity over B. cereus. E. coli was significantly sensitive to compound 10. From another point of view, 11 and 13 exhibited promising efficacy over A. fumigatus 293 and A. terreus. Quorum-sensing inhibitory activity of the new compounds was esteemed over C. violaceum ATCC 12472, where 3, 4, 8, 9b, 9d, 10, 11 and 13 have acceptable efficacy. In vitro antitumor effectiveness of the new analogs against liver (HepG2), colon (HCT-116) and breast (MCF-7) cancer cells was also assessed. Compounds 2 and 7 showed outstanding effectiveness over the three cell lines, whereas 11 displayed eminent activity toward HCT-116 and MCF-7 cells. Moreover, 3 and 9d were found to have good activity against MCF-7 cells. The in vitro active antitumor analogs were assessed for in vivo antitumor effectiveness over EAC in mice, as well as cytotoxicity toward WI38 and WISH normal cell lines. The active antimicrobial and antitumor members were investigated for DNA-binding affinity, where 2, 7, 10 and 11 showed the strongest affinity. In silico studies proved that majority of the analyzed compounds meet the optimal requirements for good oral absorption.

Keywords: Thiazoles, Thiazolopyrimidines, Anti-microbial, Anti-quorum-sensing, Anti-tumor, Cytotoxicity, DNA-binding.

INTRODUCTION

Antibiotics represent one of the widely used therapies in medicine. The efficacy of antibiotics is decreased by a growing number of antibiotic-resistant pathogens. Antibiotic resistance, which is the reason for high morbidity and mortality rates, as well as raised treatment costs, is considered to be one of the global public health threats [1]. Improper use of antimicrobials results in the emergence of resistant microbial strains [2]. Therefore, recent antivirulence strategies will be valuable in recognition of virulence determinants for a number of pathogens, and hence they will put a selective pressure on pathogens. Quorum-sensing (QS) is one of the more promising antivirulence targets [3]. QS refers to the production and release of chemical signals termed autoinducers [4], gene expression of these compounds excites a variety of responses, such as the formation of biofilms [3]. Inhibition of QS activity prevents the liberation of signal molecules and debilitates bacterial pathogenicity; therefore, might be useful in treatment of bacterial resistance [5]. Because of the previous facts, many researchers gave great attention to QS inhibitors in order to discover new antipathogens. In addition, cancer now is the reason for more deaths than all coronary heart diseases [6].

Cancer results from the fast creation of abnormal cells that spread to adjacent parts of the body and extend to other organs, this process is called metastasis. Metastasis is the major reason for cancer deaths [7,8]. Chemotherapy is one of the most powerful cancer therapeutics. However, the non-selectivity of chemotherapeutic agents is one of the hurdles against the prosperity of cancer chemotherapy. Perfect anticancer agents must be targeted toward cancer cells with minimal or no effect on normal cells [9]. So that, there is a continuous need for developing new effective anticancer agents with high selectivity and minimal side effects.

Thiazole derivatives attract the attention of medicinal chemists owing to their diversified activities, such as antimicrobial [10-16] and antitumor [15,17-23] activities. Furthermore, thiazolopyrimidines were reported to have antimicrobial [24-31] and antitumor [25,32-37] activities. The 2- ((un) substituted phenylamino) thiazole compounds **A** [10], **B** [15] and **C** [16] were explored as efficient antimicrobial agents (Figure. 1), whereas **B** [15] and **D** [23] were described as antitumor agents (Figure 1). Literature highlighted the prominence of thiazolo[4,5-*d*]pyrimidines **E, F** [30] and **G** [31] as antimicrobial agents (Figure 2). On the other hand, thiazolo[4,5-*d*]pyrimidines **H** [34], **I** [36] and **J** [37] were described as antitumor agents (Figure 2). Moreover, literature revealed that thiazole derivatives have prominent DNA-binding affinity [38]. Also, the isosteric thiadiazole and thiadiazolopyrimidine derivatives were reported to have DNA-binding affinity [39-41]. Taking all these findings in consideration and in continuation of our previous work [39-43], new series of thiazoles (**2-10)** (Figure 3) and thiazolopyrimidines (**11-13)** (Figure. 4) were synthesized and estimated for antimicrobial, anti-QS and antitumor activities. In addition, the effective antitumor analogs were studied for *in vitro* cytotoxic effectiveness. DNA-binding affinity of the active compounds was evaluated to inspect their preliminary mode of action. Structure-activity relationship (SAR) based on the obtained results was discussed, and it will flatten the road for design and synthesis of new effective derivatives.

EXPERIMENTAL PROCEDURE

Chemistry

Stuart (SMP30) melting point apparatus was employed to record melting points (ºC). IR spectra (KBr) were recorded on Nicolet 10 spectrometer (υ in cm^{-1}). Bruker Avance 300 MHz spectrometer and Jeol Reasonance 11.7 tesla 500 MHz spectrometer were utilized to record ^{1}H and ^{13}C NMR spectra in DMSO-d_6. Mass spectra were recorded on JEOL JMS-600 H spectrometer (70 eV) and Thermoscientific dsqII mass spectrometer (70 eV). Microanalysis (C, H, N) were done and the attained data were in harmony with the expected structures within ± 0.4% of the calculated values, Microanalytical unit, Cairo University. TLC sheets (silica gel 60 F25) were applied for regulating reaction times. Spots were visualized by UV (366 nm), and chloroform/methanol (9:1) was utilized as eluent. *Ortho* aminonitrile **1** was synthesized employing the preceding procedure [44].

Preparation of ethyl N- (5-cyano-2- (phenylamino) thiazol-4-yl) formimidate (2)

Ortho aminonitrile **1** (0.216 g, 0.001 mol) in triethyl orthoformate (10 mL) was refluxed for 24 hrs. Excess triethyl orthoformate was evaporated and the formed precipitate was crystallized from ethanol/water (1:2).

Yield: 50%, m.p. 218-220°C. IR: 3282 (NH), 2208 (C≡N). ^{1}H NMR δ: 1.21 (t, 3H, $CH_2C\underline{H_3}$), 2.50-2.53 (q, 2H, $C\underline{H_2}CH_3$), 7.39-7.64 (m, 6H, Ar-H, CH=N), 11.31 (s, 1H, NH). ^{13}C NMR δ: 15.2, 63.1, 86.0, 112.4, 129.5, 129.7, 130.7, 137.1, 148.7, 165.0, 167.4. MS *m/z* (%): 273 (40.30, M^++1), 272 (44.78, M^+), 57 (100.00). Anal. Calc. (Found) for $C_{13}H_{12}N_4OS$ (272.33): C, 57.34 (57.56); H, 4.44 (4.15); N, 20.57 (20.77)%.

Preparation of 2-chloro-N- (5-cyano-2- (phenylamino) thiazol-4-yl) acetamide (3)

A mixture of *ortho* aminonitrile **1** (0.216 g, 0.001 mol), chloroacetyl chloride (0.226 g, 0.002 mol) and triethylamine (0.3 mL) in DMF (5 mL) was stirred at room temperature for 1 hr. The mixture was poured onto ice and the attained solid was filtered and crystallized from ethanol/water (1:2).

Yield: 85%, m.p. 208-210°C. IR: 3349 (2NH), 2214 (C≡N), 1698 (C=O). ^{1}H NMR δ: 4.15 (s, 2H, CH_2), 7.07-7.70 (m, 5H, Ar-H), 8.28 (s, 1H, NH), 8.70 (s, 1H, NH). MS *m/z* (%): 293 (15.58, M^+), 292 (7.11, M^+-1), 77 (100.00). Anal. Calc. (Found) for $C_{12}H_9ClN_4OS$ (292.74): C, 49.24 (48.98); H, 3.10 (3.35); N, 19.14 (19.39)%.

Preparation of N- (5-cyano-2- (phenylamino) thiazol-4-yl) acetamide (4)

A solution of *ortho* aminonitrile **1** (0.216 g, 0.001 mol) in acetic anhydride (5 mL) was refluxed for 4 hrs. The mixture was cooled and the formed solid was filtered and crystallized from methanol.

Yield: 60%, m.p. 270-272°C. IR: 3448, 3200 (2NH), 2209 (C≡N), 1679 (C=O). ^{1}H NMR δ: 2.00 (s, 3H, CH_3), 7.52-7.63 (m, 6H, Ar-H, NH) 10.89 (s, 1H, NH). ^{13}C NMR δ: 23.3, 84.0, 112.5, 128.8, 129.6, 130.0, 138.7, 148.7, 160.2, 167.9. MS *m/z* (%): 260 (3.76, M^++2), 259 (10.46, M^++1), 258 (61.52, M^+), 215 (100.00). Anal. Calc. (Found) for $C_{12}H_{10}N_4OS$ (258.30): C, 55.80 (55.56); H, 3.90 (3.92); N, 21.69 (21.89)%.

Preparation of ethyl (5-cyano-2- (phenylamino) thiazol-4-yl) carbamate (5)

A mixture of *ortho* aminonitrile **1** (0.216 g, 0.001 mol) and K_2CO_3 (0.207 g, 0.0015 mol) in ethyl chloroformate (5 mL) was refluxed for 1 hr. The mixture was filtered and excess solvent was evaporated. The produced precipitate was crystallized from ethanol/water (1:2).

Yield: 50%, m.p. 190-192°C. IR: 3223 (2NH), 2209 (C≡N), 1743 (C=O). ^{1}H NMR *δ*: 1.11 (t, 3H, $OCH_2C\underline{H_3}$), 4.08-4.17 (q, 2H, $OC\underline{H_2}CH_3$), 7.38-7.48 (m, 5H, Ar-H), 6.84 (s, 1H, NH), 10.48 (s, 1H, NH). ^{13}C NMR *δ*: 13.8, 61.2, 63.7, 115.4, 128.6, 128.7, 129.3, 137.8, 150.2, 153.5, 162.3. MS *m/z* (%): 290 (6.88, M^++2), 289 (21.08, M^++1), 288 (100.00, M^+). Anal. Calc. (Found) for $C_{13}H_{12}N_4O_2S$ (288.32): C, 54.16 (54.47); H, 4.20 (4.34); N, 19.43 (19.66)%.

Preparation of ethyl (5-cyano-2- (phenylamino) thiazol-4-yl) glycinate (6)

A mixture of *ortho* aminonitrile **1** (0.216 g, 0.001 mol) and K_2CO_3 (0.207 g, 0.0015 mol) in ethyl chloroacetate (5 mL) was refluxed for 3 hrs. The mixture was filtered and excess ethyl chloroacetate was evaporated. The remained solid was crystallized from methanol.

Yield: 70%, m.p. 180-182°C. IR: 3387, 3222 (2NH), 2176 (C≡N), 1744 (C=O). ^{1}H NMR *δ*: 1.29 (t, 3H, $OCH_2C\underline{H_3}$), 4.22-4.28 (q, 2H, $OC\underline{H_2}CH_3$), 4.56 (s, 2H, CH_2), 4.83 (s, 1H, NH), 7.28 (s, 1H, NH), 7.40-7.51 (m, 5H, Ar-H). ^{13}C NMR *δ*: 14.2, 53.9, 61.6, 63.4, 115.6, 127.0, 129.1, 130.4, 143.2, 163.5, 168.6, 171.0. MS *m/z* (%): 304 (4.55, M^++2), 303 (12.54, M^++1), 302 (74.09, M^+), 91 (100.00). Anal. Calc. (Found) for $C_{14}H_{14}N_4O_2S$ (302.35): C, 55.62 (55.40); H, 4.67 (4.82); N, 18.53 (18.67)%.

Preparation of N- (5-cyano-2- (phenylamino) thiazol-4-yl) acetohydrazonamide (7)

A mixture of acetamide **4** (0.258 g, 0.001 mol) and hydrazine hydrate (0.50 g, 0.01 mol) in THF (5 mL) was refluxed for 1 hr. The solvent was evaporated and the produced precipitate was triturated with ice, filtered and crystallized from ethanol/water (1:2).

Yield: 65%, m.p. 256-258°C. IR: 3460, 3297, 3157 (NH_2, 2NH), 2175 (C≡N). ^{1}H NMR *δ*: 2.10 (s, 3H, CH_3), 6.89-7.60 (m, 8H, Ar-H, NH_2, NH), 10.62 (s, 1H, NH). ^{13}C NMR *δ*: 16.2, 57.0, 116.3, 118.3, 122.9, 128.4, 139.7, 142.1, 164.5, 164.7. MS *m/z* (%): 274 (5.87, M^++2), 273 (8.31, M^++1), 272 (16.00, M^+), 55 (100.00). Anal. Calc. (Found) for $C_{12}H_{12}N_6S$ (272.33): C, 52.93 (53.17); H, 4.44 (4.19); N, 30.86 (30.53)%.

General method for preparation of 8 and 9a-d

A mixture of acetamide **4** (0.258 g, 0.001 mol), thiosemicarbazide (0.092 g, 0.001 mol) or aryl hydrazide (0.001 mol) and triethylamine (0.3 mL) in DMF (10 mL) was refluxed for 10 hrs. The mixture was cooled and poured onto ice. The produced solid was filtered and crystallized from methanol/water (1:2).

2- (1- ((5-Cyano-2- (phenylamino) thiazol-4-yl) amino) ethylidene) hydrazine-1-carbothioamide (8)

Yield: 55%, m.p. 250-252°C. IR: 3448, 3295, 3209, 3113 (NH_2, 3NH), 2215 (C≡N). ^{1}H NMR *δ*: 2.10 (s, 3H, CH_3), 7.06-7.63 (m, 8H, Ar-H, NH_2, NH), 10.73 (s, 1H, NH), 10.89 (s, 1H, NH). ^{13}C NMR *δ*: 23.4, 77.0, 113.5, 118.8, 123.7, 129.6, 140.0, 140.2, 151.7, 164.2, 168.3. MS *m/z* (%): 332 (14.00, M^++1), 331 (28.00, M^+), 216 (100.00). Anal. Calc. (Found) for $C_{13}H_{13}N_7S_2$ (331.42): C, 47.11 (46.93); H, 3.95 (3.82); N, 29.58 (29.21)%.

N'-Benzoyl-N- (5-cyano-2- (phenylamino) thiazol-4-yl) acetohydrazonamide (9a)

Yield: 62%, m.p. 232-234°C. ^{1}H NMR *δ*: 2.08 (s, 3H, CH_3), 7.07-7.93 (m, 10H, Ar-H), 10.51 (s, 1H, NH), 10.89 (s, 1H, NH), 10.93 (s, 1H, NH). ^{13}C NMR *δ*: 23.0, 76.4, 113.1, 118.3, 123.2, 127.5, 128.5, 129.2, 131.9, 132.6, 139.5, 151.2, 163.7, 165.8, 167.9. MS *m/z* (%): 378 (4.07, M^++2), 377 (11.84, M^++1), 376 (21.28, M^+), 55 (100.00). Anal. Calc. (Found) for $C_{19}H_{16}N_6OS$ (376.43): C, 60.62 (60.38); H, 4.28 (4.05); N, 22.33 (22.65)%.

N- (5-Cyano-2- (phenylamino) thiazol-4-yl) -N'- (2-hydroxybenzoyl) acetohydrazonamide (9b)

Yield: 58%, m.p. 243-245°C. IR: 3448, 3296, 3210 (3NH), 2215 (C≡N), 1670 (C=O). ^{1}H NMR *δ*: 2.08 (s, 3H, CH_3), 7.05-7.61 (m, 9H, Ar-H), 8.67 (s, 1H, NH), 10.89 (s, 1H, NH), 10.93 (s, 1H, NH), 11.19 (s, 1H, OH). ^{13}C NMR *δ*: 23.0, 76.4, 113.0, 117.6, 118.3, 123.2, 128.9, 129.2, 129.4, 133.5, 139.5, 149.4, 151.2, 159.2, 163.7, 165.8, 167.9. MS *m/z* (%): 394 (0.25, M^++2), 393 (19.13, M^++1), 392 (30.26, M^+), 55 (100.00). Anal. Calc. (Found) for $C_{19}H_{16}N_6O_2S$ (392.44): C, 58.15 (58.03); H, 4.11 (4.20); N, 21.42 (21.09)%.

N'- (2-Chlorobenzoyl) -N- (5-cyano-2- (phenylamino) thiazol-4-yl) acetohydrazonamide (9c)

Yield: 50%, m.p. 255-257°C. ^{1}H NMR *δ*: 2.07 (s, 3H, CH_3), 7.05-7.61 (m, 9H, Ar-H), 7.94 (s, 1H, NH), 10.89 (s, 1H, NH), 10.93 (s, 1H, NH). ^{13}C NMR *δ*: 23.0, 76.4, 113.1, 118.3, 123.2, 125.2, 127.9, 129.2, 130.1, 132.3, 133.1, 136.1, 139.5, 142.1, 151.2, 163.7, 167.9. MS *m/z* (%): 412 (13.80, M^++1), 411 (31.61, M^+), 216 (100.00). Anal. Calc. (Found) for $C_{19}H_{15}ClN_6OS$ (410.88): C, 55.54 (55.37); H, 3.68 (3.48); N, 20.45 (20.76)%.

N- (5-Cyano-2- (phenylamino) thiazol-4-yl) -N'- (isonicotinoyl) acetohydrazonamide (9d)

Yield: 65%, m.p. 247-249°C. ^{1}H NMR *δ*: 2.08 (s, 3H, CH_3), 7.05-7.61 (m, 9H, Ar-H), 10.64 (s, 1H, NH), 10.89 (s, 1H, NH), 10.93 (s, 1H, NH). ^{13}C NMR *δ*: 23.0, 76.4, 113.1, 118.3, 123.2, 128.9, 129.2, 130.1, 135.0, 139.5, 144.6, 151.2, 163.7, 167.9. MS *m/z* (%): 379 (9.50, M^++2), 378 (16.00, M^++1), 377 (30.00, M^+), 216 (100.00). Anal. Calc. (Found) for $C_{18}H_{15}N_7OS$ (377.42): C, 57.28 (57.39); H, 4.01 (4.33); N, 25.98 (25.67)%.

Preparation of 4-amino-2- (phenylamino) thiazole-5-carboxamide (10)

A solution of *ortho* aminonitrile **1** (0.216 g, 0.001 mol) in 70% sulfuric acid (7 mL) was heated at 60°C for 8 hrs. The mixture was cooled, diluted with water, and neutralized with 10% aqueous NaOH solution. The precipitated solid was filtered and crystallized from ethanol.

Yield: 56%, m.p. 158-160°C. IR: 3446, 3419 ($2NH_2$, NH), 1636 (C=O). ^{1}H NMR δ: 3.98 (s, 2H, NH_2), 7.00-7.82 (m, 7H, Ar-H, NH_2), 11.15 (s, 1H, NH). ^{13}C NMR δ: 120.2, 121.8, 129.2, 130.3, 138.7, 146.1, 166.7, 176.2. MS *m/z* (%): 234 (5.32, M^+), 118.1 (100.00). Anal. Calc. (Found) for $C_{10}H_{10}N_4OS$ (234.28): C, 51.27 (51.53); H, 4.30 (4.52); N, 23.92 (23.67)%.

Preparation of 5-methyl-2- (phenylamino) thiazolo[4,5-d]pyrimidin-7 (6H) -one (11)

A mixture of *ortho* aminocarboxamide **10** (0.234 g, 0.001 mol) and acetic anhydride (5 mL) was refluxed for 6 hrs. The mixture was cooled and excess acetic anhydride was evaporated. The attained residue was filtered and crystallized from ethanol/water (1:2).

Yield: 70%, m.p. 113-115°C. IR: 3448, 3341 (2NH), 1690 (C=O). ^{1}H NMR δ: 2.15 (s, 3H, CH_3), 6.85-7.55 (m, 5H, Ar-H), 9.65 (s, 1H, NH), 9.89 (s, 1H, NH). ^{13}C NMR δ: 20.7, 119.1, 123.0, 128.8, 129.9, 130.1, 139.6, 148.9, 157.5, 168.0. MS *m/z* (%): 260 (17.58, M^++2), 191.95 (100.00). Anal. Calc. (Found) for $C_{12}H_{10}N_4OS$ (258.30): C, 55.80 (55.56); H, 3.90 (3.70); N, 21.69 (21.91)%.

Preparation of 5-phenyl-2- (phenylamino) thiazolo[4,5-d]pyrimidin-7 (6H) -one (12)

A mixture of *ortho* aminocarboxamide **10** (0.234 g, 0.001 mol) and benzoyl chloride (5 mL) was refluxed for 1 hr. The mixture was cooled and treated with petroleum ether (60-80). The formed precipitate was filtered and crystallized from petroleum ether (60-80).

Yield: 30%, m.p. 98-100°C. IR: 3448, 3422 (2NH), 1687 (C=O). ^{1}H NMR δ: 7.29-7.90 (m, 12H, Ar-H, 2NH). MS *m/z* (%): 321 (6.13, M^++1), 320 (9.03, M^+), 105 (100.00). Anal. Calc. (Found) for $C_{17}H_{12}N_4OS$ (320.37): C, 63.73 (63.53); H, 3.78 (3.63); N, 17.49 (17.23)%.

Preparation of 2- (phenylamino) -5-thioxo-5,6-dihydrothiazolo[4,5-d]pyrimi-din-7 (4H) -one (13)

A mixture of *ortho* aminocarboxamide **10** (0.234 g, 0.001 mol), carbon disulfide (5 mL, excess) and KOH (0.085 g, 0.0015 mol) in ethanol (20 mL) was refluxed for 8 hrs. The mixture was cooled, diluted with water and acidified with concentrated HCl. The produced precipitate was filtered and crystallized from ethanol/water (1:2).

Yield: 60%, m.p. > 300°C. IR: 3448 (3NH), 1701 (C=O). ^{1}H NMR δ: 6.99-7.80 (m, 8H, Ar-H, 3NH). MS *m/z* (%): 277 (44.59, M^++1), 276 (35.06, M^+), 64 (100.00). Anal. Calc. (Found) for $C_{11}H_8N_4OS2$ (276.34): C, 47.81 (47.63); H, 2.92 (2.71); N, 20.28 (20.54)%.

Biology

The biological screening procedures are described in details in the supplementary data.

Antimicrobial and antiquorum-sensing evaluation

Antibacterial assay: The new members were esteemed for antibacterial activity [39,45,46].

Antifungal assay: The new derivatives were screened for antifungal effectiveness according to the published method [39,45,47,48].

Antiquorum-sensing assay: Antiquorum-sensing activity was assessed applying the previous procedure [39,45,49].

***In vitro* antitumor assay:** *In vitro* antitumor effectiveness was evaluated following the published method [50-52].

***In vivo* antitumor assay:** The previously reported method [53-55] was adopted for *in vivo* antitumor evaluation.

***In vitro* cytotoxicity assay:** Cytotoxicity screening of **2**, **3**, **7**, **9d** and **11** was performed in accord to the MTT assay [50-52].

DNA-binding assay: DNA-binding affinity of **2**, **3**, **7**, **9b**, **9d** and **10-13** was assessed adopting the preceding procedure [56].

RESULTS AND DISCUSSION

Chemistry

Preparation of compounds (**2-13)** was described in Figures 1-3. 4-Amino-2- (phenylamino) thiazole-5-carbonitrile (**1**) was prepared following the former method [44]. Interaction of *ortho* aminonitrile **1** with triethyl orthoformate afforded the ethyl formimidate analog **2** (Figure 1). Interaction of *ortho* aminonitrile **1** with chloroacetyl chloride in dimethylformamide (DMF) in presence of triethylamine (TEA) produced the chloroacetamide analog **3** in 85% yield (Figure 1).

Refluxing **1** in acetic anhydride produced the acetamide analog **4** in 56% yield (Figure 1). Compounds **5** and **6** were synthesized *via* heating **1** with ethyl chloroformate or ethyl chloroacetate, respectively using K_2CO_3 as a catalyst (Figure 1).

The acetohydrazonamide **7** was synthesized *via* heating **4** with hydrazine hydrate in refluxing tetrahydrofuran (THF) (Figure 2). Heating **4** with thio-semicarbazide in DMF in presence of triethylamine gave the hydrazine-1-carbothioamide (**8)** in 55% yield (Figure 2). Reaction of acetamide **4** with the aryl hydrazide derivatives in refluxing DMF in presence of triethylamine yielded the acetohydrazonamides **9a-9d** in 60-70% yields (Figure 2).

Referring to Figure 3, hydrolysis of **(1)** *via* heating with 70% sulfuric acid yielded the corresponding *ortho* aminocarboxamide analog (**10)** in 56% yield. The thiazolopyrimidinone derivatives **(11)** and (**12)** were prepared *via* heating (**10)** in excess acetic anhydride or benzoyl chloride, respectively. Finally, heating (**10)** with carbon disulfide in ethanol in presence of KOH produced the thiazolopyrimidinone **(13).**

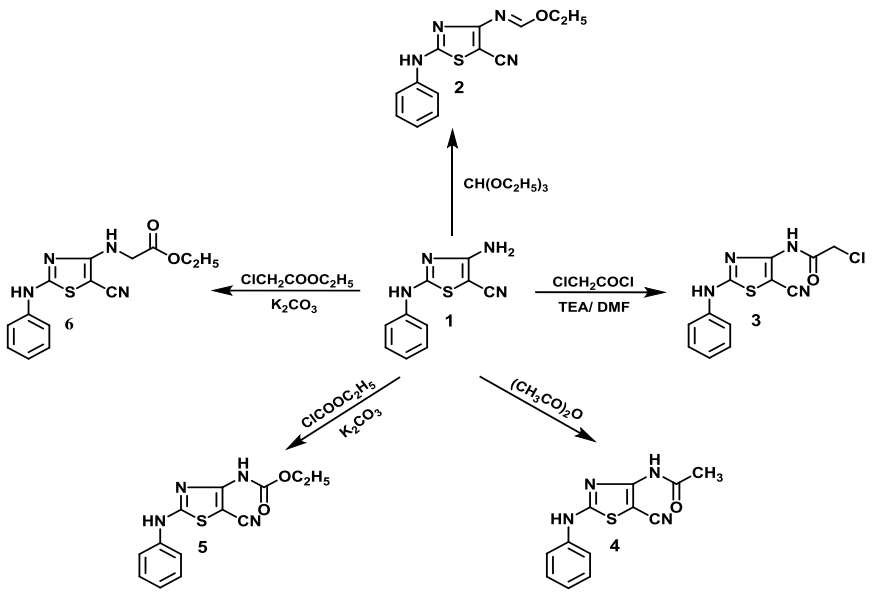

Figure 1: Reported 2-(un) substituted phenylamino) thiazoles with antimicrobial (A-C) and antitumor (B and D) activities

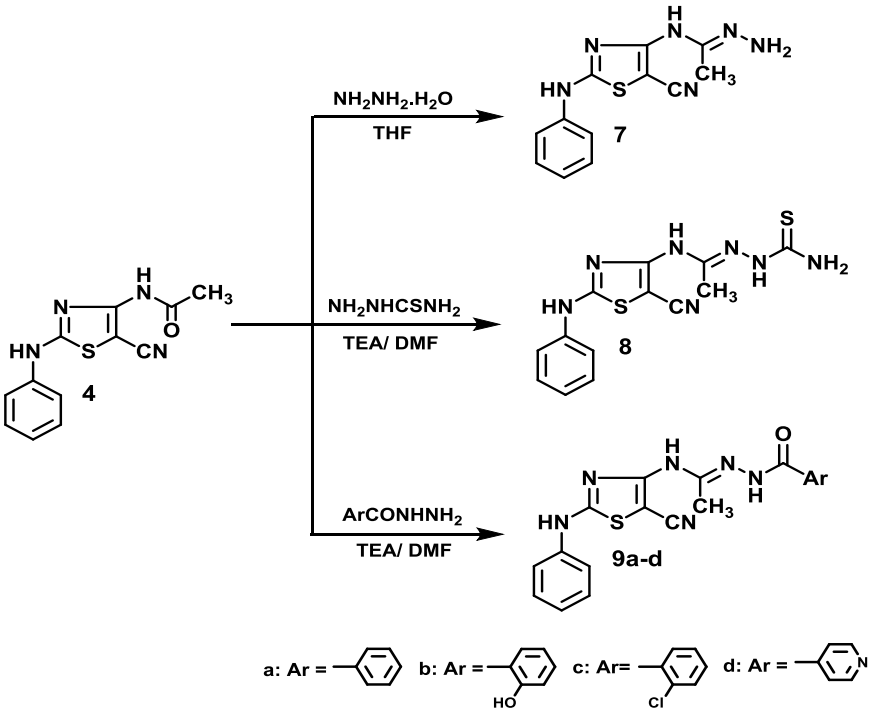

Figure 2: The designed 2-phenylaminothiazoles 2-10 with expected antimicrobial and antitumor activities

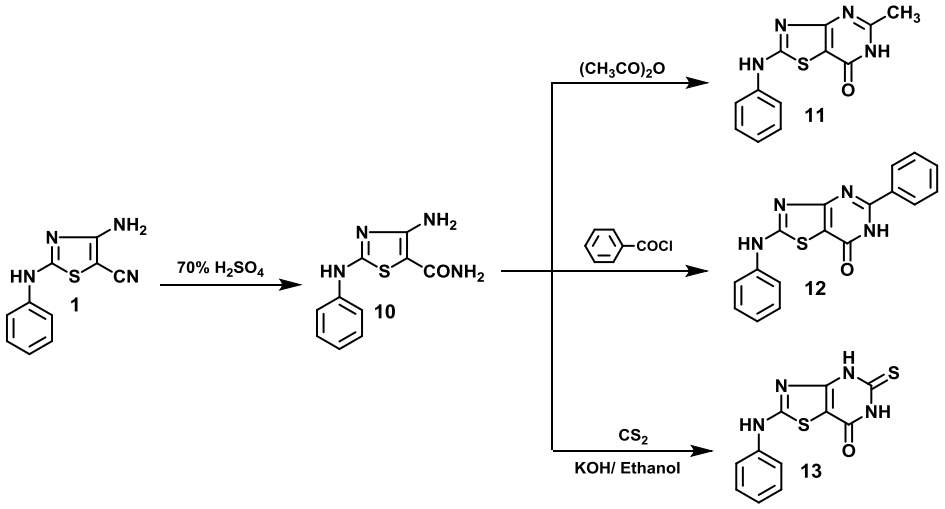

Figure 3: Reported thiazolo[4,5-d]pyrimidines with antimicrobial (E-G) and antitumor (H-J) activities

Biological screening

Anti-microbial and antiquorum-sensing screening: Antimicrobial efficacy of the new analogs was esteemed over Gram -ve bacterium (*Escherichia coli*), Gram +ve bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and pathogenic fungi (*Candida albicans*, *Aspergillus fumigatus* 293 and *Aspergillus terreus*).

The evaluation was performed in accord to two-fold serial dilution procedure [39,45,46-48] employing ampicillin and fluconazole as reference drugs. Visual detection (no turbidity) is the method used for determination of the lowest concentrations of compounds that prohibit microbial growth (MICs, µg/mL and µM).

Results of Table 1 showed that *S. aureus* and *B. cereus* were significantly sensitive to **3** and **10**; in addition, **11** and **13** displayed eminent effectiveness over *B. cereus*. Compound **9b** exhibited good activity over *S. aureus* and *B. cereus*, also compound **10** is the only member that showed reasonable efficacy over *E. coli*. On the other hand, **11** and **13** exhibited prominent activity toward *A. fumigatus* 293 and *A. terreus*; moreover, **9b** and **12** showed reasonable efficacy over the same fungal strains, whereas **9d** displayed reasonable activity on *A. fumigatus*.

Table 1: Antibacterial and antifungal activities of 2-13

Comp. No.	MIC, µg/mL (µM) [a]					
	S. aureus	***B. cereus***	***E. coli***	***C. albicans***	***A. fumigatus***	***A. terreus***
2	312.5 (1148.90)	**156.25 (574.45)**	-	-	-	-
3	**156.25 (397.58)**	**156.25 (397.58)**	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-

8	-	-	-	-	-	-
9a	-	-	-	-	-	-
9b	312.5 (797.19)	312.5 (797.19)	-	-	312.5 (797.19)	312.5 (797.19)
9c	-	-	-	-	-	-
9d	-	312.5 (828.91)	-	-	312.5 (828.91)	-
10	**156.25 (667.735)**	**156.25 (667.735)**	312.5 (1335.47)	-	-	-
11	-	**156.25 (605.62)**	-	-	**156.25 (605.62)**	**156.25 (605.62)**
12	1250 (3906.25)	-	-	-	312.5 (976.56)	312.5 (976.56)
13	-	**156.25 (566.125)**	-	-	**156.25 (566.125)**	**156.25 (566.125)**
Ampicillin	2500 (7155.20)	1250 (3577.50)	19.53 (55.9)	NS[b]	NS[b]	NS[b]
Fluconazole	NS[b]	NS[b]	NS[b]	2500	-	-
Note: aMICs (µM) are illustrated between parentheses.-, MIC >2500 µg/mL. bNS, not set.Bold values show the favored data.						

Antiquorum-sensing efficacy of the new derivatives was rated over *Chromobacterium violaceum* ATCC 12472 utilizing catechin as a positive control [39,45,49]. Violacein (a violet pigment) was released by QS system of *C. violaceum* [57,58]. So that, drugs that hinder the efficacy of QS in *C. violaceum* will prevent the liberation of violacein. QS inhibition was set by subtraction of radius of bacterial growth inhibition (r_1) from total radius of growth and pigment inhibition (r_2), QS inhibition (mm) = (r_2-r_1). Compounds **3**, **4**, **8**, **9b**, **9d**, **10**, **11** and **13** displayed acceptable anti-QS effectiveness (Table 2).

Table 2: Quorum-sensing inhibitory activity of 2-13.

Comp. No.	**QSI diameter (mm)** [a]
	C. violaceum
2	-
3	8
4	4
5	-
6	-
7	-
8	5
9a	-
9b	2
9c	-
9d	3
10	10
11	8
12	-
13	9
Catechin	2
Note: [a] No efficacy (-, inhibition zone < 2 mm); weak efficacy (2-9 mm); moderate efficacy (10-15 mm); strong efficacy (>15 mm).	

Structure-activity relationship: The relation between the attained antimicrobial evaluation results of **2-13** and structural variations was studied.

Referring to compounds 2-6: Presence of ethoxymethyleneamino and chloroacetamido moieties at 4-position of thiazole ring led to augmented antibacterial activity over *S. aureus* and *B. cereus* (compounds **2** and **3**, respectively). On contrary, presence of acetamide, ethyl carbamate and ethyl glycinate counterparts at the same position diminished the activity over all chosen microbial strains (**4**, **5** and **6**, respectively).

Concerning compounds 7-9: The type of substituent at 4-position of thiazole nucleus influences the activity of thiazole derivatives **7-9**. Incorporation of acetohydrazonamide and (aminoethylidene) hydrazine-1-carbothioamide moieties at 4-position of thiazole nucleus resulted in diminished activity on all inspected microorganisms (compounds **7** and **8**). Furthermore, presence of 4- (*N'*- (benzoyl) acetohydrazonamide) and 4- (*N'*- (2-chlorobenzoyl) acetohydrazonamide) moieties abolished the activity against all tested microbial strains (compounds **9a** and **9c**). On contrary, presence of 4- (*N'*- (2-hydroxybenzoyl) acetohydrazonamide) moiety enhanced the efficacy over *S. aureus, B. cereus*, *A. fumigatus* and *A. terreus* (compound **9b**). In addition, incorporation of 4- (*N'*- (isonicotinyl) acetohydrazonamide) moiety resulted in outstanding efficacy over *B. cereus* and *A. fumigatus* (compound **9d**).

Regarding compounds 10-13: The *ortho* aminocarboxamide analog **10** showed enhanced antibacterial activity over the three tested bacterial strains. The activity of thiazolopyrimidines **11-13** is relied on the type of substituent at 5-position of the thiazolopyrimidine nucleus, presence of 5-methyl and 5-thioxo substituents led to prominent activity over *B. cereus*, *A. fumigatus* and *A. terreus* (compounds **11** and **13**). On the other hand, presence of 5-phenyl substituent led to abolished activity toward *B. cereus* and decreased effectiveness on *A. fumigatus* and *A. terreus*; on contrary, it increased activity on *S. aureus* (compound **12** versus **11** and **13**).

***In vitro* antitumor screening:** Analogs **2-13** underwent *in vitro* antitumor assessment over liver (HepG2), colon (HCT-116) and breast (MCF-7) cancer cell lines applying MTT assay [50-52] and using 5-fluorouracil (5-FU) as a reference drug. Compound´s concentration that brings about 50% inhibition of cell viability (IC_{50}, µM) was established for all screened compounds. Results (Table 3) illustrated that **2** and **7** are the most active members over the three inspected cell lines, whereas **11** displayed good activity toward HCT-116 and MCF-7 cells. In addition, **3** and **9d** displayed interesting efficacy over MCF-7 cells.

Table 3: *In vitro* antitumor efficacy of 2-13

Comp. No.	IC_{50} (µM) [a,b]		
	HepG2	**HCT-116**	**MCF-7**
2	**8.45 ± 1.0**	**9.83 ± 1.1**	**8.34 ± 0.8**
3	40.21 ± 2.3	21.43 ± 1.7	**14.55 ± 1.4**
4	>100	88.20 ± 4.6	>100
5	>100	87.08 ± 4.5	88.28 ± 4.8
6	>100	77.23 ± 4.3	80.53 ± 4.4
7	**9.80 ± 1.1**	**10.46 ± 1.2**	**9.91 ± 1.3**
8	83.10 ± 4.2	55.24 ± 3.6	46.36 ± 3.3
9a	66.74 ± 3.7	48.38 ± 3.3	34.60 ± 2.7
9b	46.08 ± 2.5	39.01 ± 2.4	23.39 ± 1.8
9c	87.42 ± 3.9	56.25 ± 3.8	55.06 ± 3.5
9d	29.03 ± 1.9	26.54 ± 1.8	**17.87 ± 1.6**

10	81.70 ± 3.9	45.18 ± 2.8	33.03 ± 2.4
11	48.83 ± 2.7	**18.09 ± 1.4**	**11.13 ± 1.0**
12	>100	65.44 ± 3.9	64.60 ± 3.8
13	90.00 ± 4.5	61.16 ± 3.9	53.42 ± 3.4
5-FU	7.86 ± 0.5	5.35 ± 0.2	5.39 ± 0.3

Note: [a] IC_{50} values are the mean ± SD of three readings
[b] IC_{50} (µM): 1-10 (very strong activity); 11-20 (strong activity); 21-50 (moderate activity); 51-100 (weak activity); > 100 (no activity).
Bold values show the favored data.

Structure-activity relationship

Taking into account the structures of analogs 2-6: Incorporation of ethoxymethyleneamino substituent at 4-position of thiazole nucleus led to superb activity on all selected cell lines (analog **2**). Replacing 4-ethoxymethyleneamino substituent with 4-chloroacetamido, 4-acetamido, 4- (ethoxycarbonyl) amino and 4- (2-ethoxy-2-oxoethyl) amino counterparts led to diminished activity against all inspected cell lines (compound **2** versus **3-6**). On contrary, presence of 4-chloroacetamido substituent raised the activity against the three cell lines compared to 4-acetamido, 4- (ethoxycarbonyl) amino and 4- (2-ethoxy-2-oxoethyl) amino counterparts (compound **3** versus **4-6**).

Regarding compounds 7-9: Introduction of acetohydrazonamide moiety at 4-position of thiazole ring resulted in outstanding effectiveness on the three cell lines compared to the (aminoethylidene) hydrazine-1-carbothioamide counterpart (compound **7** versus **8**). Studying the activity of compounds **9a-d** showed that incorporation of *N'*- (isonicotinyl) acetohydrazonamide moiety at 4-position of thiazole nucleus reinforced the activity against all cell lines in comparison to *N'*- (benzoyl), *N'*- (2-hydroxybenzoyl) and *N'*- (2-chlorobenzoyl) counterparts (compound **9d** versus **9a-c**). In addition, the activity of **9a-c** is influenced by the type of substituent on the benzoyl moiety, whereas presence of electron-donating substituent increased the activity over the unsubstituted and the electron-withdrawing substituent, the activity order is **9b** > **9a** > **9c**.

Referring to compounds 10-13: The *ortho* aminocarboxamide analog **10** displayed reasonable activity toward HCT-116 and MCF-7 cells. The antitumor efficacy of thiazolopyrimidinone analogs **11-13** is influenced by the type of substituent at 5-position, the activity order is 5-methyl analog **11** > 5-thioxo analog **13** > 5-phenyl analog **12**.

***In vivo* antitumor screening:** The *in vitro* active antitumor compounds **2**, **3**, **7**, **9d** and **11** were estimated for *in vivo* antitumor effectiveness toward EAC in mice. Three substantial measurements were detected for estimation of antitumor effectiveness of the active analogs and 5-FU (reference agent) [53-55].% Increase in lifespan (% ILS) and MST (days of a mouse in a group/number of mice) are shown in Table 4, whereas compound **2** showed the highest% ILS of mice. Effects of these compounds on blood profile are displayed in Table 5, whereas **2** exhibited higher hemoglobin (Hb) and red blood cells (RBCs) levels and lower white blood cells (WBCs) count than 5-FU. Tumor volume and viable cell count were also measured and results illustrated that **2** has the lowest tumor volume and viable cell count (Table 6).

Table 4: Influence of 2, 3, 7, 9d and 11 on MST and% ILS of mice bearing EAC.

Group	MST (day) [a]	% ILS[a]
Normal Control	NS[b]	NS[b]
EAC only	14.5	NS[b]
2	**42.0**	**189.6**
3	19.0	31.0
7	27.0	86.2
9d	21.0	44.8
11	35.0	141.3
5-FU	53.0	265.5
Note: [a]Results are average of two readings. [b]NS: not set. Bold values show the favoreddata.		

Table 5: Influence of 2, 3, 7, 9d and 11 on blood profile of mice bearing EAC.

Group	Hb (g/dl) [a]	RBC Count (10^6/mm^3) [a]	Total WBC (10^6/mm^3) [a]
Normal Control	13.97	6.03	6.37
EAC only	8.11	3.69	21.93
2	**13.66**	**5.80**	**8.92**
3	9.21	4.04	19.54
7	10.79	4.42	18.31
9d	9.73	4.30	20.51
11	12.30	5.34	12.66
5-FU	13.00	5.67	9.67
Note: [a]Results are average of two readings. Bold values show the favored data.			

Table 6: Influence of 2, 3, 7, 9d and 11 on tumor volume and viable cell count in mice bearing EAC.

Group	Tumor volume (mL) [a]	Viable cell count (10^6/mL) [a]
Normal Control	NS[b]	NS[b]
EAC only	8.00	80.23
2	**1.25**	**34.34**
3	6.10	68.62
7	5.05	56.93
9d	6.95	72.23
11	1.95	34.46
5-FU	0.70	19.07
Note: aResults are average of two readings. bNS: not set. Bold values show the favored data.		

***In vitro* cytotoxicity screening:** Compounds **2**, **3**, **7**, **9d** and **11** were tested for cytotoxicity over lung fibroblast (WI38) and amnion epithelial (WISH) normal cells employing MTT assay [50-52] and using 5-FU as a positive control. IC_{50} values (µM) were calculated. The five investigated compounds exhibited lower cytotoxicity than 5-FU against both normal cell lines (Table 7).

DNA-binding assay: Numerous antimicrobial and antitumor drugs act *via* binding to DNA. So that, methyl green/DNA displacement assay [56] was followed for estimation of DNA-binding affinity of the effective members.

Methyl green/DNA displacement assay: Methyl green/DNA displacement assay is a colorimetric assay employed to assess the displacement of methyl green from DNA by compounds capable of binding to DNA [56]. The degree of displacement was assessed by measuring the change in the absorbance of methyl green/DNA complex. DNA-binding affinity (IC_{50}, µM) of **2**, **3**, **7**, **9b**, **9d**, **10-13** and doxorubicin (DNA intercalating agent) was established. Results (Table 8) illustrated that **2**, **7**, **10** and **11** have strong affinity, while **9b** and **9d** have moderate affinity. On the other hand, **3**, **12** and **13** displayed weak affinity.

Table 7: *In vitro* cytotoxicity of 2, 3, 7, 9d and 11 against WI38 and WISH normal cell lines.

Comp. No.	**IC_{50} (µM) [a,b]**	
	WI38	**WISH**
2	45.35 ± 2.9	68.29 ± 3.6
3	11.12 ± 1.4	10.48 ± 1.3
7	38.77 ± 2.6	50.87 ± 3.1
9d	15.39 ± 1.7	17.39 ± 1.5
11	28.40 ± 2.3	43.81 ± 2.8
5-FU	5.70 ± 0.6	6.57 ± 0.6
Note: [a]IC_{50} values are the mean ± SD of three readings [b]IC_{50} (µM): 1-10 (very strong activity); 11-20 (strong activity); 21-50 (moderate activity); 51-100 (weak activity); > 100 (non- cytotoxic).		

Table 8: DNA-binding affinity of 2, 3, 7, 9b, 9d and 10-13.

Comp. No.	**DNA-binding affinity IC_{50} (µM)**
2	**32.93 ± 2.0**
3	**78.02 ±** 4.2
7	**40.65 ± 2.4**
9b	**64.84 ±** 4.7
9d	**67.49 ±** 3.9
10	**43.71 ± 3.5**
11	**43.87 ± 2.8**
12	**75.18 ±** 4.9
13	**86.05 ±** 5.3
Doxorubicin	31.27 ± 1.8
Note: Bold values show the favored data.	

***In silico* studies:** Physicochemical properties of drugs, like partition coefficient, solubility and molecular size affect absorption, distribution and bioavailability of drugs [59]. So, studying these properties is very important in drug design. Molinspiration software was used for calculation of Veber's parameters [60] and Lipinski's rule [61]. Also, toxicity hazards, drug-likeness and drug score were determined for the new analogs using Osiris software [62]. Results are described in the supplementary data.

CONCLUSION

New thiazoles and thiazolopyrimidines with potent antimicrobial and/or antitumor activities were discovered in the present work. Results of antimicrobial assay revealed that **2** and **3** have eminent antibacterial activity on *S. aureus* and *B. cereus*; in addition, **3** have reasonable anti-QS activity. Also, **9b** showed good activity over *S. aureus*, *B. cereus*, *A. fumigatus* 293 and *A. terreus*, and reasonable anti-QS activity, whereas **10** exhibited prominent antibacterial effectiveness toward the three tested microorganisms, as well as eminent anti-QS efficacy. In addition, **11** and **13** displayed excellent efficacy over *B. cereus*, *A. fumigatus* 293 and *A. terreus*; moreover, **3**, **10**, **11** and **13** exhibited considerable anti-QS efficacy. Accordingly, **3**, **10**, **11** and **13** might be utilized as potent antibacterial agents with reduced hazard of microbial drug-resistance. Switching to results of *in vitro* antitumor assay, **2** and **7** are potent and broad spectrum antitumor agents with IC_{50} values a little higher than that of 5-FU toward the three cell lines; moreover, **2** showed the highest *in vivo* antitumor efficacy over EAC cells.

Furthermore, the same compound showed lower cytotoxicity than 5-FU on both WI38 and WISH normal cells; thus, it could be utilized as efficacious and selective antitumor agent. Collectively referred, **2** might be utilized as effective and promising antitumor agent with reduced hazard of G +ve bacterial infections and reduced toxicity over normal cells. Results of DNA-binding assay revealed that **2**, **7**, **10** and **11** have strong affinity; thus, they are awaited to act *via* binding to DNA. *In silico* studies indicated that all analyzed compounds are in accord with Lipinski's rule and Veber's norms and they are contemplated to display high oral bioavailability. The above data affirmed that the rational design of the new thiazole and thiazolopyrimidine analogs as antimicrobial and/ or antitumor agents was adequate; accordingly, the effective analogs in this research will undergo extra structural variations hoping to attain new more efficacious derivatives.

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