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Synthesis, antimicrobial and anticancer activities of some naphthylthiazolylamine derivatives.

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Abstract

New 4-naphthyl-2-aminothiazole derivatives were synthesized. The newly synthesized compounds were screened for their *in vitro* antimicrobial and anticancer activities. The results indicated that compound 5b namely 2-(4-methylpiperidine-1-yl)-4-(naphthalene-2-yl) thiazole exhibited highest MIC value (62.5 µg/ml) against *P. aeruginosa* in the tested microorganisms. This compound showed equipotent antifungal effect on *C. albicans* and *C. glabrata* as compared with ketoconazole. Compounds 4c, 4d, 5a and 5f showed remarkably antifungal activity against *C. albicans*. In addition, anticancer activity and cytotoxicity studies were also carried out in Hep-G2 and A549 cell lines to examine the ability of inhibiting the cell growth for 4a-4f and 5a-5f compounds. The cell viability and anticancer activity were determined by MTT assay. 4a-4f compounds has led to an increase in cell proliferation in both cell lines (Hep-G2 and A549 cells), unlike 5a-5f series showed a weak anticancer activity.

Keywords: Thiazole, Naphthalene, Antimicrobial activity, Cytotoxicity, MTT assay.

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Introduction

Thiazole derivatives are important heterocyclic compounds because of their biological activities, such as antibacterial [1-5], antifungal [3-9], antitumor [3,10-16], anticonvulsant [17], antitubercular [18,19]. Also aminothiazoles as well as 2-amino-4-arylthiazole derivatives are shown antimicrobial [20-22] activities and amino thiazoles are known to be ligands of oestrogen receptor [23] as well as a novel class of adenosine receptors antagonists [24]. In addition, cyclic seconder amine derivatives (thiomorpholine, morpholine etc.) containing an azole nucleus or aryl group are exhibited significant antimicrobial [25-33], anti-inflammatory [34], cytotoxic [35], antidepressant [36], antileukemic [37], antibiotic [38], antiurease [31] activities.

Furthermore, naphthalene derivatives are found to be associated with various biological activities such as cytotoxic, antimicrobial [39-43], antioxidant [41], antiinflammatory [44].

Based on the above reports we were synthesized a series of new naphthyl thiazolylamine derivatives based on Scheme 1. We considered the synthesis of new thiazole derivatives from dialkylaminothiocarbamide and 2-bromo-(naphthalene-1/2-yl) ethanone and tested them for their *in vitro* antimicrobial and anticancer activity. The antimicrobial properties of these naphthylthiazolylamine compounds were evaluated against various selected bacterial and fungal strains using the Minimum Inhibitory Concentration (MIC) method. In addition, cytotoxicity studies were also carried out in Hep-G2 and A549 cell lines to examine the ability of these compounds to inhibit the cell growth.

Materials and Methods

Chemistry

All chemicals were used without further purification. All melting points were measured by using an Electrothermal 9300 digital melting points apparatus. Spectroscopic data were recorded on the following instruments: a Perkin Helmer FTIR 100 spectrophotometer; an ^{1}H NMR (nuclear magnetic resonance) Bruker DPX 500 NMR spectrophotometer. Chemical shift values are given in δ-scales; a Mass Spectrometry (MS) Agilent 1100 MSD spectrometer (Agilent Technologies, Palo Alto, CA); an elemental analysis were performed in a Thermo Finnigan Flash EA 1112 elemental analyser. The completion of reactions was checked by Thin Layer Chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) using a mixture of ethyl acetate and petroleum ether (1:1 v/v). N-benzoyl-dialkylamino-thiocarbamide (1a-1f) and dialkylaminothiocarbamide (2a-2f) series were prepared according to the methods described in the literature [45].

General procedure for the synthesis of the compounds

N-benzoyl-dialkylamino-thiocarbamides (1a-1f): Benzoyl chloride (0.1 mol, 11.6 ml) were reacted with ammonium thiocyanate (0.105 mol, 8 g) in acetone and the mixture was heated under reflux for 15 minutes. Heating was stopped and the appropriate seconder amine derivatives (0.105 mol) were stirred for 30 minutes at room temperature. Then, this mixture was refluxed for 5 minutes until boiling temperature and checked by TLC. After completing the reaction, it was poured onto excess cracked ice with vigorous stirring. The precipitate formed was filtered and washed with water and dried.

Dialkylaminothiocarbamides (2a-2f): The appropriate N-benzoyl-dialkylamino-thiocarbamide (1a-1f) was added to solution of 10% aq NaOH and this mixture was refluxed at 150°C for 10 minutes. The mixture was poured onto excess ice and to reaction media was added excess 37% HCl. After ammonia was added until pH 8. The mixture was kept in a cool place until pure product collapsed. The obtained precipitation was filtered and dried.

2-Bromo-1-(naphthalene-1/2-yl) ethanone (3a and 3b): 1-(naphthalene-1/2-yl) ethanone (0.03 mol) was taken in 25 ml of acetic acid in a 100 ml two-necked round bottomed flask. A solution of bromine (0.01 mol) in acetic acid was prepared. The bromine solution was dropped into the flask for two hours and then this mixture was stirred for six hours. After TLC, the mixture was kept in a cool place. 2-bromo-1-(naphthalene-1-yl) ethanone (3a) was obtained as a liquid. 2-bromo-1-(naphthalene-2-yl) ethanone (3b) was obtained as a solid, the resulting solid mass was filtered and dried.

2-(Dialkylamino)-4-(naphthalene-1/2-yl) thiazoles (4a-4f and 5a-5f): A mixture of 2-bromo-1-(naphthalene-1/2-yl) ethanone (3a and 3b) (0.002 mol), appropriate dialkylaminothiocarbamide (2a-2f) (0.002 mol) in ethanol was refluxed for ten hours and checked by TLC. After completing the reaction, the cooled mixture to room temperature was filtered and dried. The physical properties and spectral data of the final compounds are given below.

2-(Piperidine-1-yl)-4-(naphthalene-1-yl) thiazole (4a): Yield 60%; mp. 153-154°C; FT-IR n_{max} (cm^{-1}): 3049 (aromatic CH), 2917-2848 (aliphatic CH), 1613 (C=N), 1592, 1515, 1476 (C=C condense system), 1150-1100 (aliphatic amin), 630 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.65 (^{6}H, s, piperidine-H), 3.7 (^{4}H, s, piperidine-H), 6.8 (^{1}H, t, J: 7.71 Hz, naphthalene C_3-H), 7.0 and 7.14 (^{1}H, two t, J: 7.45 Hz, J: 7.63 Hz, naphthalene C_6-H), 7.06 and 7.31 (^{2}H, two d, J: 6.99 Hz, J: 7.08 Hz, naphthalene C_2-H, naphthalene C_7-H), 7.49-7.55 (^{2}H, m, C_5-H, thiazole-H), 7.57 and 7.85 (^{1}H, two d, J: 6.8 Hz, J: 7.75 Hz, naphthalene C_4-H), 7.77 and 7.97 (^{1}H, two d, J: 8.19 Hz, J: 8.23 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 295; Analysed and Calculated for $C_{18}H_{18}N_2S$ (294.12): C, 73.43; H, 6.16; N, 9.51. Found: C, 73.88; H, 5.85; N, 9.46%.

2-(4-Methylpiperidine-1-yl)-4-(naphthalene-1-yl) thiazole (4b): Yield 35%; mp. 139-140°C; FT-IR n_{max} (cm^{-1}): 3050 (aromatic CH), 2955-2861 (aliphatic CH), 1606 (C=N), 1505, 1472, 1456 (C=C condense system), 1150-1100 (aliphatic amin), 631 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 0.95 (^{3}H, d, J: 6.44 Hz, CH_3), 1.2-1.8 (^{5}H, m, piperidine-H), 3.2 (^{2}H, t, J: 11.59 Hz, piperidine-H), 4.1 (^{2}H, s, piperidine-H), 6.8 (^{1}H, t, J: 7.68 Hz, naphthalene C_3-H), 7.0 and 7.14 (^{1}H, two t, J:7.44 Hz, J: 7.63Hz, naphthalene C_6-H), 7.06 and 7.31 (^{2}H, two d, J: 6.85 Hz, J: 7.15Hz, naphthalene C_2-H, naphthalene C_7-H), 7.49-7.55 (^{2}H, m, naphthalene C_5-H, thiazole-H), 7.57 and 7.85 (^{1}H, two d, J: 6.78 Hz, J: 7.82 Hz, naphthalene C_4-H), 7.77 and 7.97 (^{1}H, two d, J: 8.21 Hz, J: 8.25 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and Calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.06; H, 5.98; N, 8.85%.

2-(2-Methylpiperidine-1-yl)-4-(naphthalene-1-yl) thiazole (4c): Yield 30%; mp. 148-149°C FT-IR n_{max} (cm^{-1}): 3058, 3023 (aromatic CH), 2973-2864 (aliphatic CH), 1586 (C=N), 1508, 1471, 1440 (C=C condense system), 1150-1100 (aliphatic amin), 626 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.27 (^{3}H, d, J: 6.8 Hz, CH_3), 1.5-1.8 (^{6}H, m, piperidine-H), 4.00 and 4.41 (^{1}H, two s, piperidine-H), 3.2 (^{2}H, t, J: 12.42 Hz, piperidine-H), 6.8 (^{1}H, t, J: 7.62 Hz, naphthalene C_3-H), 7.0 and 7.14 (^{1}H, two t, J: 7.40 Hz, J: 7.63Hz, naphthalene C_6-H), 7.06 and 7.31 (^{2}H, two d, J: 6.96 Hz, J: 7.61 Hz, naphthalene C_2-H, naphthalene C_7-H), 7.49-7.55 (^{2}H, m, naphthalene C_5-H, thiazole-H), 7.57 *vs.* 7.85 (^{1}H, two d, J: 6.83 Hz, J: 7.97 Hz, naphthalene C_4-H), 7.77 and 7.97 (^{1}H, two d, J: 8.17 Hz, J: 8.25 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and Calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.15; H, 5.96; N, 8.86%.

2-(Pyrrolidin-1-il)-4-(naphthalene-1-yl) thiazole (4d): Yield 45%; mp. 142-143°C FT-IR n_{max} (cm^{-1}): 3173, 3049 (aromatic CH), 2985-2820 (aliphatic CH), 1654 (C=N), 1589-1468 (C=C condense system), 1150-1100 (aliphatic amin), 624 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 2.1 (^{4}H, s, pyrrolidin-H), 3.6 (^{4}H, s, pyrrolidin-H), 6.8 (^{1}H, t, J: 7.71 Hz, naphthalene C_3-H), 6.9 and 7.15 (^{1}H, two t, J: 7.45 Hz, J: 8.03 Hz, naphthalene C_6-H), 7.04 and 7.29 (^{2}H, two d, J: 6.95 Hz, J: 7.04Hz, naphthalene C_2-H, naphthalene C_7-H), 7.48-7.54 (^{2}H, m, naphthalene C_5-H, thiazole-H), 7.56 and 7.85 (^{1}H, two d, J: 6.78 Hz, J: 7.63 Hz, naphthalene C_4-H), 7.76 and 7,95 (^{1}H, two d, J: 8.22 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 281; Analysed and Calculated for $C_{17}H_{16}N_2S$ (280.10): C, 72.82; H, 5.75; N, 9.99. Found: C, 73.06; H, 5.37; N, 9.61%.

2-(Hexamethyleneamin-1-yl)-4-(naphthalene-1-yl) thiazole (4e)

Yield 54%; mp. 119-120°C FT-IR n_{max} (cm^{-1}): 3072 (aromatic CH), 2972-2885 (aliphatic CH), 1685 (C=N), 1581-1460 (C=C condense system), 1158-1131 (aliphatic amin), 621 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.6 (^{4}H, s, hexamethyleneamin-H), 1.8 (^{4}H, s, hexamethyleneamin-H), 2.2 (^{4}H, s, hexamethyleneamin-H), 6.8 (^{1}H, t, J: 7.7 Hz, naphthalene C_3-H), 7.0 and 7.14 (^{1}H, two t, J:7.46 Hz, J: 7.63 Hz, naphthalene C_6-H), 7.06 and 7.3 (^{2}H, two d, J: 6.97 Hz, J: 7.12 Hz, naphthalene C_2-H, naphthalene C_7-H), 7.49-7.55 (^{2}H,

m, naphthalene C_5-H, thiazole-H), 7.57 and 7.85 (^{1}H, two d, J: 5.85 Hz, J: 7.67 Hz, naphthalene C_4-H), 7.77 and 7.97 (^{1}H, two d, J: 8.21 Hz, J: 8.23 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and Calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.34; H, 6.83; N, 8.98%.

2-(Thiomorpholine-4-yl)-4-(naphthalene-1-yl) thiazole (4f): Yield 40%; mp. 165-166°C FT-IR n_{max} (cm^{-1}): 3043 (aromatic CH), 2965-2803 (aliphatic CH), 1622 (C=N), 1600-1477 (C=C condense system), 1144-1126 (aliphatic amine), 623 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 2.1 and 2.8 (^{4}H, s, thiomorpholine-H), 3.9 (^{4}H, s, thiomorpholine-H), 6.8 (^{1}H, t, J: 7.62 Hz, naphthalene C_3-H), 7.02 and 7.16 (^{1}H, two t, J: 7.42 Hz, J: 7.63Hz, naphthalene C_6-H), 7.08 and 7.33 (^{2}H, two d, J: 6.94 Hz, J: 7.59 Hz, naphthalene C_2-H, naphthalene C_7-H), 7.5-7.54 (^{2}H, m, naphthalene C_5-H, thiazole-H), 7.57 and 7.86 (^{1}H, two d, J: 6.88 Hz, J: 95 Hz, naphthalene C_4-H), 7.78 and 7.98 (^{1}H, two d, J: 8.18 Hz, J: 8.23 Hz, naphthalene C_8-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 313; Analysed and Calculated for $C_{17}H_{16}N_2S_2$ (312.08): C, 65.35; H, 5.16; N, 8.97. Found: C, 66.9; H, 4.99; N, 8.7%.

2-(Piperidine-1-yl)-4-(naphthalene-2-yl) thiazole (5a): Yield 40%; mp. 149-150°C FT-IR n_{max} (cm^{-1}): 3051 (aromatic CH), 2955-2831(aliphatic CH), 1628 (C=N), 1583-1443 (C=C condense system), 1129 (aliphatic amine), 626 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.7 (^{6}H, s, piperidine-H), 3.7 (^{4}H, s, piperidine-H), 6.97 (^{2}H, t, J: 5.68 Hz, naphthalene $C_{6,7}$-H), 7.29 -7.56 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.71 -7.86 (^{2}H, m, naphthalene $C_{3,8}$-H), 7.98 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 295; Analysed and Calculated for $C_{18}H_{18}N_2S$ (294.12): C, 73.43; H, 6.16; N, 9.51. Found: C, 73.86; H, 5.91; N, 9.46%.

2-(4-methylpiperidine-1-yl)-4-(naphthalene-2-yl) thiazole (5b): Yield 25%; mp. 124-125°C FT-IR n_{max} (cm^{-1}): 3058-3040 (aromatic CH), 2955-2870 (aliphatic CH), 1606 (C=N), 1594-1442 (C=C condense system), 1155-1104 (aliphatic amine), 626 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 0.95 (^{3}H, d, J: 6.48 Hz, CH_3), 1.2-1.3 (^{2}H, m, piperidine-H), 1.75 (^{2}H, d, J: 12.75 Hz, piperidine-H), 3.2 (^{3}H, t, J: 9.36 Hz, piperidine-H), 4.07 (^{2}H, d, J: 11.07 Hz, piperidine-H), 6.97 (^{2}H, t, J: 6.11 Hz, naphthalene $C_{6,7}$-H), 7.29-7.56 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.71-7.86 (^{2}H, m, naphthalene $C_{3,8}$-H), 7.98 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.02; H, 6.01; N, 8.92%.

2-(2-Methylpiperidine-1-yl)-4-(naphthalene-2-yl) thiazole (5c): Yield 35% mp. 102-103°C FT-IR n_{max} (cm^{-1}): 3056 (aromatic CH), 2940-2863 (aliphatic CH), 1652 (C=N), 1595-1436 (C=C condense system), 1164-1128 (aliphatic amine), 627 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.3 (^{3}H, d, J: 6.81 Hz, CH_3), 1.48-1.7 (^{6}H, m, piperidine-H), 3.25 (^{1}H, t, J: 11.15 Hz, piperidine-H), 3.9 (^{1}H, d, J: 11.32 Hz, piperidine-H), 4.4 (^{1}H, s, piperidine-H), 6.97 (^{2}H, t, J: 5.65 Hz, naphthalene $C_{6,7}$-H), 7.29-7.56 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.71-7.86 (^{2}H, m, naphthalene $C_{3,8}$-H), 7.98 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and Calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.10; H, 5.99; N, 8.88%.

2-(Pyrrolidin-1-yl)-4-(naphthalene-2-yl) thiazole (5d): Yield 40% mp. 191-192°C; FT-IR n_{max} (cm^{-1}): 3058 (aromatic CH), 2953-2851 (aliphatic CH), 1628 (C=N), 1583-1443 (C=C condense system), 1128 (aliphatic amine), 627 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 2.1 (^{4}H, s, pyrrolidin-H), 3.55 (^{4}H, s, pyrrolidin-H), 6.97 (^{2}H, t, J: 5.12 Hz, naphthalene $C_{6,7}$-H), 7.29-7.55 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.71-7.86 (^{2}H, m, naphthalene $C_{3,8}$-H), 7.98 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 281; Analysed and Calculated for $C_{17}H_{16}N_2S$ (280.10): C, 72.82; H, 5.75; N, 9.99. Found: C, 73.10; H, 5.39; N, 9.64%.

2-(Hexamethyleneamin-1-yl)-4-(naphthalene-2-yl) thiazole (5e): Yield 35%; mp. 132-133°C; FT-IR n_{max} (cm^{-1}): 3062 (aromatic CH), 2939-2852 (aliphatic CH), 1606 (C=N), 1581-1442 (C=C condense system), 1148-1127 (aliphatic amine), 626 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 1.6 (^{4}H, s, hexamethyleneamin-H), 1.8 (^{4}H, s, hexamethyleneamin-H), 3, 7 (^{4}H, s, hexamethyleneamin-H), 6.75 (^{2}H, t, J: 6.19 Hz, naphthalene $C_{6,7}$-H), 7.29-7.55 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.71-7.85 (^{2}H, m, naphthalene $C_{3,8}$-H), 7.98 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 309; Analysed and Calculated for $C_{19}H_{20}N_2S$ (308.13): C, 73.99; H, 6.54; N, 9.08. Found: C, 74.32; H, 6.78; N, 8.96%.

2-(Thiomorpholine-4-yl)-4-(naphthalene-2-yl) thiazole (5f): Yield 40%; mp. 194-195°C; FT-IR n_{max} (cm^{-1}): 3049 (aromatic CH), 2960-2852 (aliphatic CH), 1628 (C=N), 1583-1474 (C=C condense system), 1174-1127 (aliphatic amine), 626 (C-S); ^{1}H NMR (500 MHz, DMSO-d6) δ: 2.8 (^{4}H, m, thiomorpholine-H), 3.9 (^{4}H, s, thiomorpholine-H), 6.97 (^{2}H, t, J: 5.29 Hz, naphthalene $C_{6,7}$-H), 7.29 -7.55 (^{3}H, m, naphthalene $C_{4,5}$-H, thiazole-H), 7.73-7.86 (^{2}H, m, naphthalene $C_{3,8}$-H), 8.1 (^{1}H, s, naphthalene C_1-H), ppm; MS (ESI) (m/z): $(M+H)^+$ 313; Analysed and Calculated for $C_{17}H_{16}N_2S_2$ (312.08): C, 65.35; H, 5.16; N, 8.97. Found: C, 66.5; H, 5.02; N, 8.74%.

Antimicrobial activity

The antimicrobial activity of the compounds was evaluated by micro-broth dilution method [46]. All compounds were tested for their *in vitro* antimicrobial activity against *Staphylococcus aureus* (ATCC-33862), *Bacillus cereus* (NRRL B-3711), *Listeria monocytogenes* (ATCC-7644), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853), *Salmonella typhimurium* (NRRL B-4420), *Candida albicans* (Clinical isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey) and *Candida glabrata* (Clinical isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey).

Stock solutions of tested compounds were prepared in Dimethyl Sulfoxide (DMSO). Two-fold serial dilutions of the solutions using sterile distilled water were prepared from 2 mg/ml to 0.0039 mg/ml in micro-test tubes that were transferred to 96 well microtiter plates. After overnight

incubation in double-strength Mueller-Hinton broth microbial suspensions were standardised to 10^8 CFU/ml using 0.5 McFarland solutions. One hundred microliter of each microorganism suspension was added the micro-plate wells containing of compound concentrations. The last two well on the micro-plates were used as controls. One was used as negative control containing uninoculated medium. The other well is containing sterile distilled water and inoculated medium as a positive growth control. The plates were incubated at 37°C for 18-24 h. After then, antimicrobial activity was detected by spraying of 0.5% TTC (Triphenyl Tetrazolium chloride, Merck) aqueous solution. Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the compounds that inhibited visible growth. Streptomycin was used as standard antibacterial agent and ketoconazole as an antifungal agent. All compounds tested were screened triplicate against each microorganism.

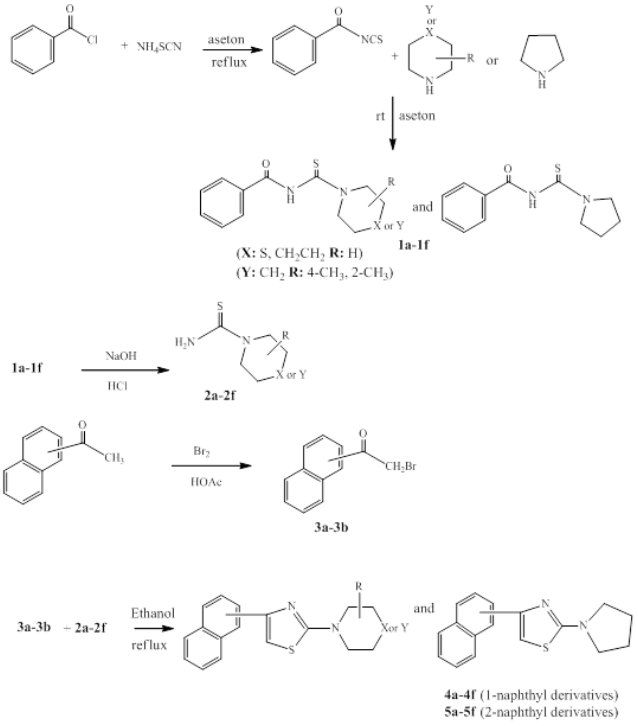
In-vitro cell viability and anti-tumoral activity

Cell line, culture conditions and preparation of compounds: Human hepatocarcinoma cells (Hep-G2) and human lung adenocarcinoma (A549) cells were used to determine the cytotoxic properties. Cell lines Hep-G2 and A549 were used from the cell line collections of Anadolu University (Eskisehir, Turkey). Hep-G2 cell lines were cultivated in Dulbecco's Modified Eagle's Medium (DMEM), while A549 cells were grown RPMI-1640 mediums. All of the growth media were supplemented with 10% Foetal Bovine Serum (FBS) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2. Confluent adherent cultures were detached using trypsin-EDTA and were propagated in subcultures [47]. All compounds and Doxorubicin as a positive control were dissolved in DMSO (Dimethylsulfoxide) and final concentrations were diluted in culture medium, maximum DMSO concentration was adjusted to 0.1% per well.

Viability assay and antitumor activity

Viability of the test compounds-treated cells was evaluated by MTT (3-(4, 5-dimethylthiazol-2-il)-2, 5-diphenyltetrazolium bromide) assay as previously described by Mossman et al. [48]. The principle of the MTT method is based on the conversion of the yellow coloured tetrazolium salt (MTT dye) to purple formazan crystals by the enzymes in living cells and results are evaluated by spectrophometrically measurement of the intensity of the purple [49].

The cells were grown on to 96-well plates at the 1×10^4 for Hep-G2 and 4×10^3 for A549 cells per well and it was incubated for 24 h in culture conditions. Culture medium was replaced with fresh medium containing test compounds (4a-4f, 5a-5f) at different concentrations (12.5-200 μM). After 24 and 48 h incubations, cells were treated with fresh medium supplemented MTT (1 mg/ml) for 3 h at 37°C in incubator containing 5% CO_2. Following the 4 h, medium was discarded and formazan crystals were dissolved in 200 μL DMSO per well for 20 min on a shaker at 1600 rpm and room temperature in dark. The viability of the cell was determined by measuring the Optical Density (OD) of each well as an absorbance at 570 nm using a micro-plate reader (ELX 808IU, Biotek Ins. Inc., Winooski, VT, USA). IC_{50} value was calculated [50]. Obtained absorbance value was evaluated by comparing the solvent control (0.1% DMSO treatment cells) and negative control (non-treatment cells). Doxorubicin was uses as reference standard at different concentrations (0.1-100 μM). Percent of cell viability compared to the negative control was shown graphically in Microsoft Office Professional Plus 2013 Excel program and it is given in Figures1-3.



Scheme 1. *The synthesis of naphthylthiazolylamine derivatives.*

Results and Discussion

Chemistry

4-naphthyl-2-aminothiazole derivatives were synthesized as indicated in Scheme 1. The compounds N-benzoyl-dialkylamino-thiocarbamide (1a-1f), dialkylamino-thiocarbamide (2a-2f) were synthesized, in accordance with the method described in literature [45]. The synthesis of N-benzoyl-dialkylamino-thiocarbamide (1a-1f) was performed from the reaction of benzoyl chloride with ammonium thiocyanate in acetone. The obtained N-benzoyl-dialkylamino-thiocarbamide was reacted with secondary amines (morpholine, thiomorpholine, piperidine, pyrrolidine, hexamethyleneamine) and then it was converted to the corresponding dialkylamino thiocarbamide (2a-2f) by hydrolysis.

2-bromo-(1- or 2-naphthyl) ethanone compounds (3a and 3b) were prepared by reacting 1- or 2-acetyl naphthalene and bromine in acetic acid.

4-naphthyl-2-aminothiazole derivatives (4a-4f and 5a-5f) were synthesized by reacting dialkyl thiocarbamide (2a-2f) and 2-bromo-(1- or 2-naphthyl) ethanone (3a and 3b) in ethanol.

The structures of the final compounds (4a-4f and 5a-5f) obtained were elucidated using spectral data. In the IR spectra of the compounds, characteristic absorbtion bands in the range of 1586-1685 cm^{-1}, 1436-1600 cm^{-1}, 1100-1174 cm^{-1} corresponding to-C=N-stretching of thiazole, -C=C- condense system stretching of naphthalene ring and –C-N-C- stretching of aliphatic aminee respectively. ^{1}H NMR spectra of all the synthesized 4-naphthyl-2-aminothiazoles (4a-4f and 5a-5f), observed at δ 6.75-7.98 of naphthalene ring protons and δ 7.49-7.56 of thiazole ring proton in similar range. The secondary amine protons linked to thiazole were observed at δ 0.95-4.41.

Table 1. *Antimicrobial activity of the compounds (4a-4f and 5a-5f) (µg/ml).*

	Microorganisms							
Compounds	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
4a	500	500	500	500	500	500	1000	1000
4b	500	500	500	500	500	500	1000	1000
4c	500	500	500	500	500	500	500	500
4d	500	500	500	500	500	500	500	500
4e	500	500	500	500	500	500	1000	1000
4f	500	500	500	500	500	500	1000	1000
5a	500	500	500	500	500	500	500	1000
5b	125	125	250	250	62.5	250	250	250
5c	500	500	500	500	500	500	1000	1000
5d	500	500	500	500	500	500	1000	1000
5e	500	500	500	500	500	500	1000	1000
5f	500	500	500	500	500	500	500	1000
Streptomycin	15.625	31.25	7.81	31.25	31.25	31.25	—	—
Ketoconazole	—	—	—	—	—	—	250	250

A: *Staphylococcus aureus* (ATCC-33862); B: *Bacillus cereus* (NRRL B-3711); C: *Listeria monocytogenes* (ATCC-7644); D: *Escherichia coli* (ATCC-25922); E: *Pseudomonas aeruginosa* (ATCC-27853); F: *Salmonella typhimurium* (NRRL B-4420); G: *Candida albicans* (Clinical isolate); H: *Candida glabrata* (Clinical isolate).

Antimicrobial activity

All the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activities against *S. aureus*, *B. cereus*, *L. monocytogenes* (as examples Gram positive bacteria*), E. coli, P. aeruginosa, S. typhimurium* (as examples Gram negative bacteria), *C. albicans* and *C. glabrata* (as examples yeasts) using streptomycin and ketoconazole as control drugs. The results are presented in Table 1.

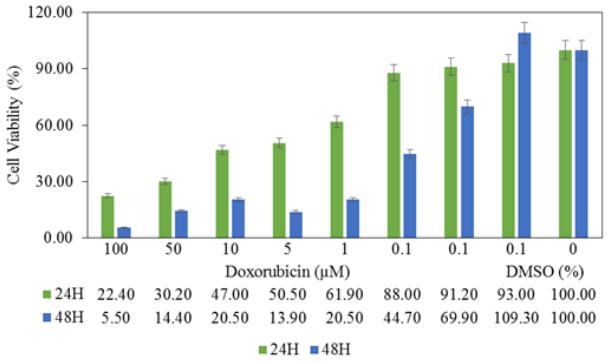


Figure 1. *Percentage of the cell viability of Doxorubicin against the Hep-G2 cell lines.*

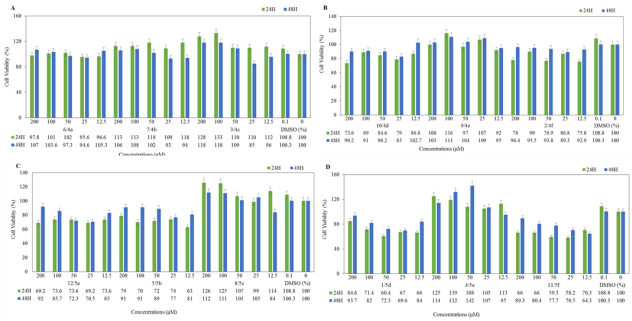


Figure 2. *Dose-response profiles for test compounds against the Hep-G2 cell lines. A. (1-5). Lines for 6/4a, (6-10) lines for 7/4b, (11-15). Lines for 3/4c, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. B. (1-5). Lines for 10/4d, 6-10 lines for 9/4e, (11-15). Lines for 2/4f, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. C. (1-5). Lines for 12/5a, (6-10) lines for 5/5b, (11-15). Lines for 8/5c, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. D. (1-5). Lines for 1/5d, (6-10) lines for 4/5e, (11-15). Lines for 11/5f, (16). Line for 0.1% DMSO and (17). Line non-treatment cells.*

In general, all compounds demonstrated better antibacterial activity than antifungal activity. The results indicated that the compound 5b exhibited the highest MIC value (62.5 µg/ml) against *P. aeruginosa* in the tested microorganisms. Compound 5b showed equipotent antifungal effect on *C. albicans* and *C. glabrata* as compared with ketoconazole with a MIC value of 250 µg/ml. All compounds (4a-4f and 5a-5f) showed less antimicrobial activity than streptomycin.

All compounds except compound 5b are equally active (MIC=500 µg/ml) against all of the tested bacteria. Compounds 4c, 4d, 5a and 5f showed significant antifungal activity against *C. albicans* with a MIC value of 500 µg/ml. Among the compounds, 5b was the most susceptible compound against the all of the tested microorganisms.

In-vitro cell viability and anticancer activity

In vitro cell culture studies were performed in the Cell Culture Laboratory, Faculty of Sciences, Department of Biology in Anadolu University, Eskisehir, Turkey. Compounds 4a-4f and 5a-5f were analysed in terms of cytotoxicity and antitumor

activity against human hepatocellular carcinoma (Hep-G2) and human lung adenocarcinoma (A549) cells. Doxorubicin was tested as a reference chemotherapeutic drugs and it showed IC_{50} values 5 µM for 24 h and 0.5 µM for 48 h in Hep-G2 cells (Figure 1).

When the cytotoxicity and anticancer properties of compounds were evaluated towards to Hep-G2 hepatocarcinoma cells, some compounds showed quite variable effects (Figure 2).

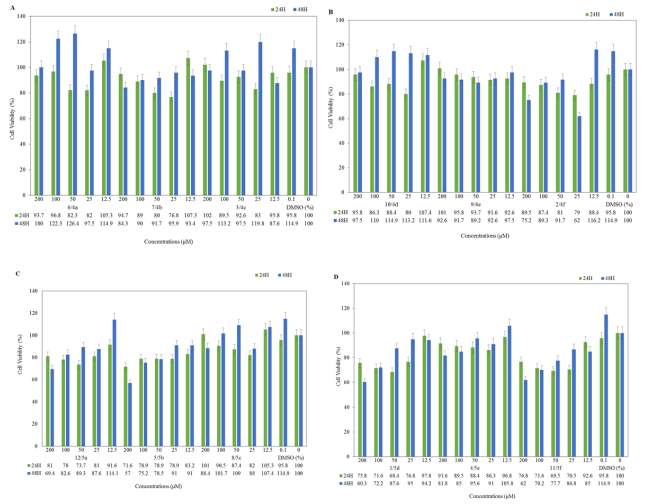


Figure 3. *Dose-response profiles for test compounds against the A549 cell line. A. (1-5). Lines for 6/4a, (6-10) lines for 7/4b, (11-15). Lines for 3/4c, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. B. (1-5). Lines for 10/4d, (6-10) Lines for 9/4e, (11-15). Lines for 2/4f, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. C. (1-5). Lines for 12/5a, (6-10) lines for 5/5b, (11-15). Lines for 8/5c, (16). Line for 0.1% DMSO and (17). Line non-treatment cells. D. (1-5). Lines for 1/5d, (6-10) lines for 4/5e, (11-15). Lines for 11/5f, (16). Line for 0.1% DMSO and (17). Line non-treatment cells.*

IC_{50} values could not found in any compound. However, compounds 5f and 5d were determined the most cytotoxic and anticancer potential compounds amongst others. After the 24 h incubation, when the treatment 200 µM compound 5f, cell viability was determined 66%. On the other hand, in 25 µM-treated cells was observed around 58% viability and after 24 and 48 hours, in 12.5 µM-treated cells showed 70% and 65% respectively. Similarly, compound 5d showed equipotent anticancer activity against hepatocarcinoma cells, 50 µM compound 5d treated cells exhibited 60% viability, in applied to 12.5 µM, viability was seen about 67% (Figure 2D). In addition, viability was about 70% in cells exposed to compound 4f and 5a (Figures 2B and 2C). Other compounds (4a-4d and 5c) were not effective on anticancer properties against hepatocarcinoma cell. However, compounds 4b, 4c and 5e slightly increased the cell proliferation at 24 h in Hep-G2 cells (Figures 2A-2C).

Considering the human lung adenocarcinoma cell lines (A549), only compound 5b with anticancer activity are noteworthy with 57% viability in 200 µM for 48 h, other concentrations of 5b were not cytotoxic (Figure 3C). Other compounds of the series 5a-5f; compounds 5a, 5d and 5f have led to 60, 62 and 69 % viability at 48 hour, respectively (Figure 3C and 3D). The compound 5c also did not cause an effect on cell proliferation. Further; almost all compounds of the series 4a-4f (excluding 4f) has no cytotoxic effects. In contrast, 4c and 4d compounds slightly increased the cell proliferation at 48 hours in lung cancer cells. The other hand, cells were exposed to 25 µM compound 4f showed 62% viability at 48 h (Figure 3A and 3B).

Conclusion

The synthesis of new 4-naphthyl-2-aminothiazole derivatives and evaluating their antimicrobial and anticancer activity has been investigated, in this study. The antimicrobial screening has revealed that compound 5b has remarkable antimicrobial activity, especially against *P. aeruginosa*. In addition, anticancer activities were evaluated against Hep-G2 and A549 tumor cell lines. When 4a-4f and 5a-5f anticancer profiles were compared, derivatives of series 5a-5f (excluding 5c) showed a weak anticancer activity. In contrast, the results indicate that; series 4a-4f has increased cell proliferation in both hepatocarcinoma and lung adenocarcinoma cells. When all data are compared with doxorubicin as standard chemotherapeutic drug (IC_{50} 0.5 µM at 48 h) (Figure 1), it has been seen that compounds of 5a-5f have poor chemotherapeutic potential.

Conflict of Interests

The authors have declared no conflict of interests

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