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Utility of 6-Amino-2-thiouracil as a Precursor for the Synthesis of Pyrido[2,3-*d*]Pyrimidines and their *in vitro* and *in vivo* Biological Evaluation.

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Abstract

The condensation of 6-amino-2-thiouracil **1** with aromatic aldehydes afforded azamethine derivatives **3a,b**. The formed azamethines underwent [4+2] cycloaddition with enaminones **4a-c** and enaminonitrile **9** to form the corresponding condensed pyrimidines **8a-f** and **11a,b** respectively. On the other hand the interaction of **3a,b** with acetylene derivatives **12a,b**, **14** afforded the corresponding pyrido[2,3-*d*]pyrimidine **13a-d** and **16a,b** respectively. The synthesized 2-azadiene **18** failed to add either electron-poor or push-pull dienophile. The *in vitro* antimicrobial activity of some of the newly synthesized compounds was examined. All the tested compounds proved to be active as antibacterial and antifungal agents. Also the *in vivo* antitumor activity of compounds **8a**, **11b**, **13a,d** and **16b** against lung (H460) and liver (HEPG2) carcinoma cells was examined. Compounds **8a**, **16b** showed moderate activity against lung carcinoma cell line (H460).

Keywords

6-Amino-2-thiouracil, 2-Azadiene, Pyrido[2,3-*d*]pyrimidine, Antimicrobial, Antitumor.

Introduction

Pyrido[2,3-*d*]pyrimidines are biologically interesting molecules that have established utility in the pharmaceutical and the agrochemical industries. Compounds with these ring systems have diverse pharmacological activity such as antitumor **[1,2]**, cardiotonic **[3,4]**, hepatoprotective **[3]**, antihypertensive **[3]**, antibronchitic **[5]**, antifungal **[6]**, antibacterial **[7]** and antifolate **[8]**. Therefore these fused heterocycles have been extensively investigated and their synthetic preparations are well documented **[9-11]**. As a result, a number of reports appeared in literature; however they usually require forcing conditions **[12]**, long reaction times **[13,14]** and complex synthetic pathways **[2]**. So new routes for the synthesis of these molecules have attracted a considerable attention as a rapid entry for the formation of these heterocycles **[15,16]**. This report explains a simple route for synthesis of pyrido[2,3-*d*] pyrimidine *via* [4+2] cycloaddition.

Experimental

All melting points are uncorrected. The IR spectra expressed in cm^{-1} and recorded in KBr pellets on a Pa-9721 IR spectrometer. ^{1}H-NMR, ^{13}C-NMR spectra were obtained on a Varian EM-390 300 MHz spectrometer in DMSO-d_6 as a solvent and TMS as a internal reference. Chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kartos (75 eV) MS equipment. Elemental analyses were carried out by the micro-analytical unit at the National Research Center, Giza, Egypt. Microbiological analyses were carried out by the Micro-analytical Center, Faculty of Science, Cairo University, Giza, Egypt. the antitumor activity was evaluated by the National Cancer Institute, Cancer Biology Department, Cairo University, Egypt. All starting materials used were commercially available by Aldrich company otherwise stated.

Synthesis of 2-azadiene 3a,b:

To a solution of 6-amino-2-thiouracil **1** (1.43 g, 0.01 mol) in DMF (30 ml), an equivalent amount of aromatic aldehyde (0.01 mol) and few drops of acetic acid

were added. The reaction mixture was heated under reflux for 4 h, then left to cool. The solid product formed after pouring into ice/water was filtered and crystallized from (ethanol/dioxane mixture).

***6-(Benzylidene-amino)-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one, 3a*: [17]**
yellow crystals, yield: 2.03 g (88%); mp: 278 ^{0}C; IR (KBr, cm^{-1}): 3386 (NH), 1654 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 5.26 (s, 1H, H-5 pyrimidine), 6.96-7.11 (m, 5H, aromatic protons), 7.84 (s, 1H, N=CH), 11.37 (s, 1H, NH), 11.84 (s, 1H, NH); MS (m/z)= 231 (M^+, 22%). Anal. Calcd. For $C_{11}H_9N_3OS$: C 57.13%, H 3.92%, N 18.17%, S 13.86%. Found: C 57.08%, H 3.86%, N 18.14%, S 13.78%.

***6-[(4-Methoxy-benzylidene)-amino]-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one, 3b*:** yellow crystals, yield: 2.38 g (91%); mp: 288 ^{0}C; IR (KBr, cm^{-1}): 3387 (NH), 1656 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.69 (s, 3H, OCH_3), 5.34 (s, 1H, H-5 pyrimidine), 6.77 (d, 2H, aromatic protons, J= 8.6 Hz), 6.96 (d, 2H, aromatic protons, J= 8.6 Hz), 7.93 (s, 1H, N=CH), 11.39 (s, 1H, NH), 11.93 (s, 1H, NH); MS (m/z)= 262 (M^+, 20%). Anal. Calcd. For $C_{12}H_{11}N_3O_2S$: C 55.16%, H 4.24%, N 16.08%, S 12.27%. Found: C 55.04%, H 4.18%, N 15.92%, S 12.13%.

Cycloaddition reaction of 2-azadiene 3a,b with β-enaminones and enaminonitrile:

Equimolar amounts of each of **3a,b** (0.01 mol) and one of the β- enaminone **4a-c** (0.01 mol) or the enaminonitrile **9** (25 ml) were heated under reflux in dry dioxane for 16 h. The solvent evaporated under vacuum and the remaining residue was treated with petroleum ether 40-60 ^{0}C. The precipitate formed was collected by filtration and crystallized from dioxane.

***7-Phenyl-5-(thiophene-2-carbonyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-]pyrimidin-4-one, 8a*:** yellow crystals, yield: 2.90 g (79.4%); mp: 199 ^{0}C; IR (KBr, cm^{-1}): 3327 (NH), 1649 (CO), 1630 (CO); ^{1}H-NMR (DMSO-d_6): δ

(ppm)= 7.05-7.24 (m, 5H, aromatic protons), 7.63 (s, 1H, H-6), 7.67-7.75 (m, 2H, thiophene protons), 8.15 (d, 1H, thiophene protons, J= 3 Hz), 11.84 (s, 1H, NH), 12.07 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm)= 90.86, 91.32, 125.81, 127.01, 128.33, 128.41, 128.71, 128.93, 131.25 138.0, 146.0, 148.55, 153.98, 163.53 (C=O), 173.42 (C=O), 179.59 (C=S); MS (m/z)= 365 (M^+, 38%). Anal. Calcd. For $C_{18}H_{11}N_3O_2S_2$: C 59.16%, H 3.03%, N 11.50%, S 17.55%. Found: C 59.04%, H 2.95%, N 11.47%, S 17.43%.

7-(4-Methoxy-phenyl)-5-(thiophene-2-carbonyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, 8b**:** yellow crystals**,** yield 3.30 g (84%); mp: 226 ^{0}C; IR (KBr, cm^{-1}): 3406 (NH), 1646 (CO), 1631 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.84 (s, 3H, OCH_3), 6.75-7.34 (m, 4H, aromatic protons), 7.63 (s, 1H, H-6), 7.68-7.98 (m, 2H, thiophene protons), 8.15 (d, 1H, thiophene protons, J= 3 Hz), 11.84 (s, 1H, NH), 12.07 (s, 1H, NH); MS (m/z)= 395 (M^+, 72%). Anal. Calcd. For $C_{19}H_{13}N_3O_3S_2$: C 57.71%, H 3.31%, N 10.63%, S 16.22%. Found: C 57.67%, H 3.26%, N 10.52%, S 16.14%.

5-(Furan-2-carbonyl)-7-phenyl-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, 8c**:** brown crystals, yield: 2.86 g (82%); mp: 212 ^{0}C; IR (KBr, cm^{-1}): 3426 (NH), 1644 (CO), 1633 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 6.58-7.05 (m, 5H, aromatic protons), 7.18 (d, 1H, furan proton, J= 9 Hz), 7.35 (s, 1H, H-6), 7.48-7.88 (m, 2H, furan protons), 11.83 (s, 1H, NH), 12.06 (s, 1H, NH); MS (m/z)= 349 (M^+, 27%). Anal. Calcd. For $C_{18}H_{11}N_3O_3S$: C 61.88%, H 3.17%, N 12.03%, S 9.18%. Found: C 61.77%, H 3.08%, N 11.96%, S 9.03%.

5-(Furan-2-carbonyl)-7-(4-methoxy-phenyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, 8d**:** brown crystals**,** yield: 3.25 g (85%); mp: 228 ^{0}C; IR (KBr, cm^{-1}): 3330 (NH), 1640 (CO), 1631 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.85 (s, 3 H, OCH_3), 6.76 (d, 2H, aromatic protons, J= 7.5 Hz), 6.96 (d, 2H, aromatic protons, J= 7.5), 7.03-7.13 (m, 1H, furan proton), 7.35 (s, 1H, H-6), 7.67

(d, 1H, furan proton, J= 9 Hz), 7.86 (d, 1H, furan proton, J= 9 Hz), 11.83 (s, 1H, NH), 12.05 (s, 1H, NH); MS (m/z)= 379 (M^+, 25%). Anal. Calcd. For $C_{19}H_{13}N_3O_4S$: C 60.15%, H 3.45%, N 11.08%, S 8.45%. Found: C 60.04%, H 3.32%, N 10.93%, S 8.37%.

***5-(Naphthalene-2-carbonyl)-7-phenyl-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, 8e*:** yellow crystals**,** yield: 3.50 g (86%); mp: 233 ^{0}C; IR (KBr, cm^{-1}): 3399 (NH), 1643 (CO), 1630 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 6.70-7.25 (m, 5H, aromatic protons), 7.30 (s, 1H, H-6), 7.54-8.02 (m, 6H, naphthyl protons), 8.50 (s, 1H, naphthyl proton), 11.85 (s, 1H, NH), 12.10 (s, 1H, NH); MS (m/z)= 409 (M^+, 43%). Anal. Calcd. For $C_{24}H_{15}N_3O_2S$: C 70.40%, H 3.69%, N 10.26%, S 7.83%. Found: C 70.33%, H 3.57%, N 10.13%, S 7.79%.

***7-(4-Methoxy-phenyl)-5-(naphthalene-2-carbonyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, 8f*:** yellow crystals**,** yield: 3.80 g (87%); mp: 248 ^{0}C; IR (KBr, cm^{-1}): 3408 (NH), 1646 (CO), 1628 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.55 (s, 3H, OCH_3), 6.75 (d, 2H, aromatic protons, J= 7.5 Hz), 6.94 (d, 2H, aromatic protons, J= 7.5 Hz), 7.55 (s, 1H, H-6), 7.56-8.05 (m, 6H, naphthyl protons), 8.50 (s, 1H, naphthyl protons), 11.85 (s, 1H, NH), 12.04 (s, 1H, NH); MS (m/z)= 439 (M^+, 27%). Anal. Calcd. For $C_{25}H_{17}N_3O_3S$: C 68.32%, H 3.90%, N 9.56%, S 7.30%. Found: C 68.21%, H 3.85%, N 9.47%, S 7.23%.

***4-Oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carbonitrile, 11a*:** brown crystals**,** yield: 1.90 g (70%); mp: 213 ^{0}C; IR (KBr, cm^{-1}): 3398 (NH), 2203 (CN), 1643 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 6.80-7.23 (m, 5H, aromatic protons), 7.38 (s, 1H, H-6), 11.83 (s, 1H, NH), 12.06 (s, 1H, NH); MS (m/z)= 280 (M^+, 7%). Anal. Calcd. For $C_{14}H_8N_4OS$: C 59.99%, H 2.88%, N 19.99%, S 11.44%. Found: C 59.87%, H 2.75%, N 19.87%, S 11.38%.

7-(4-Methoxy-phenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidine-5-carbonitrile, 11b**:** brown crystals**,** yield: 2.30 g (76%); mp: 228 ^{0}C; IR (KBr, cm^{-1}): 3401 (NH), 2182 (CN), 1649 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.55 (s, 3H, OCH_3), 6.75 (d, 2H, aromatic protons, J= 7.5 Hz), 6.94 (d, 2H, aromatic protons, J= 7.5 Hz), 7.35 (s, 1H, H-6), 11.83 (s, 1H, NH), 12.06 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm)= 55.52 (OCH_3), 90.42, 91.12, 113.70, 113.84 (CN), 128.05, 128.82, 130.21, 142.0, 153.96, 157.69, 163.50 (C=O), 173.37 (C=S); MS (m/z)= 310 (M^+, 8%). Anal. Calcd. For $C_{15}H_{10}N_4O_2S$: C 58.06%, H 3.25%, N 18.05%, S 10.33%. Found: C 57.92%, H 3.12%, N 17.96%, S 10.24%.

Cycloaddition reaction of 2-azadiene 3a,b with acetylenes:

To a solution of each of 2-azadiene **3a,b** (0.01 mol) (in dry dioxane (30 ml)), equimolar amounts of one of the acetylenes **12a,b** or **14** was added. The reaction mixture was heated under reflux for 4 h. The solvent was evaporated under vacuum. After treaturation of the remaining residue with n-hexane; the solid product formed was collected by filtration and crystallized from dioxane.

Diethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5,6-dicarboxylate, 13a**:** orange crystals**,** yield: 3.50 g (87%); mp: 235 ^{0}C; IR (KBr, cm^{-1}): 3419 (NH), 1760 (CO), 1726 (CO), 1633 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 1.13-1.29 (m, 6H, $2CH_3$), 4.13-4.28 (m, 4H, $2CH_2$), 6.77-7.21 (m, 5H, aromatic protons), 11.85 (s, 1H, NH), 12.05 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm)= 14.02, 14.50 ($2CH_3$), 62.49, 66.87 ($2CH_2$), 109.40, 119.10, 126.89, 127.07, 127.90, 128.29, 142.0, 156.0, 161.53, 163.0 (C=O), 166.01 (C=O), 166.80 (C=O), 172.90 (C=S); MS (m/z)= 399 (M^+, 9%), 354 (M^+ -OEt, 9%). Anal. Calcd. for $C_{19}H_{17}N_3O_5S$: C 57.13%, H 4.29%, N 10.52%, S 8.03%. Found: C 57.04%, H 4.23%, N 10.43%, S 7.9%.

Diethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]-pyrimidine-5,6-dicarboxylate, 13b**:** orange crystals, yield: 3.80 g (88%); mp: 246 ^{0}C; IR (KBr, cm^{-1}): 3426 (NH), 1762 (CO), 1733 (CO), 1637 (CO); ^{1}H-NMR (DMSO-

d_6): δ (ppm)= 1.13-1.29 (m, 6H, $2CH_3$), 3.69 (s, 3H, OCH_3), 4.13-4.45 (m, 4H, $2CH_2$), 6.77-6.98 (m, 4H, aromatic protons), 11.82 (s, 1H, NH), 12.01 (s, 1H, NH); MS (m/z)= 429 (M^+, 3%), 384 (M^+ -OEt, 61%). Anal. Calcd. for $C_{20}H_{19}N_3O_6S$: C 55.94%, H 4.46%, N 9.78%, S 7.47%. Found: C 55.86%, H 4.34%, N 9.69%, S 7.36%.

***Dimethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidine-5,6-dicarboxylate, 13c*:** yellow crystals, yield: 3.20 g (86%); mp: 200 ^{0}C; IR (KBr, cm^{-1}) 3426 (NH), 1762 (CO), 1732 (CO), 1636 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.72 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 7.01-7.38 (m, 5H, aromatic protons), 11.80 (s, 1H, NH), 12.05 (s, 1H, NH); MS (m/z) = 371 (M^+, 2%), 356 (M^+ -Me, 3%). Anal. Calcd. for $C_{17}H_{13}N_3O_5S$: C 54.98%, H 3.53%, N 11.31%, S 8.63%. Found: C 54.87%, H 3.44%, N 11.27%, S 8.57%.

***Dimethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidine-5,6-dicarboxylate, 13d*:** yellow crystals, yield 3.60 g (89%); mp: 220 ^{0}C; IR (KBr, cm^{-1}): 3425 (NH), 1763 (CO), 1733 (CO), 1642 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 3.58 (s, 3H, OCH_3), 3.72 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 7.01-7.38 (m, 4H, aromatic protons), 11.85 (s, 1H, NH), 12.05 (s, 1H, NH); MS (m/z) = 401 (M^+, 2%), 386 (M^+ -Me, 20%). Anal. Calcd. for $C_{18}H_{15}N_3O_6S$: C 53.86%, H 3.77%, N 10.47%, S 7.99%. Found: C 53.79%, H 3.63%, N 10.34%, S 7.82%.

***Ethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylate, 16a*:** yellow crystals, yield: 2.65 g (81%); mp: 242 ^{0}C; IR (KBr, cm^{-1}): 3407 (NH), 1761 (CO), 1644 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 1.21 (t, 3H, CH_3, J= 7 Hz), 4.14 (q, 2H, CH_2, J= 7 Hz), 7.05-7.20 (m, 5H, aromatic protons), 7.38 (s, 1H, H-6), 11.85 (s, 1H, NH), 12.05 (s, 1H, NH); MS (m/z)= 327 (M^+, 2%), 254 (M^+ -COOEt, 6%). Anal. Calcd. for $C_{16}H_{13}N_3O_3S$: C 58.70%, H 4.00%, N 12.84%, S 9.79%. Found: C 58.61%, H 3.96%, N 12.76%, S 9.68%.

***Ethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidine-5-carboxylate, 16b*:** yellow crystals, yield: 3.0 g (84%); mp: 258 ^{0}C; IR (KBr, cm^{-1}) 3414 (NH), 1764 (CO), 1646 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 1.23 (t, 3H, CH_3, J= 7.2 Hz), 3.69 (s, 3H, OCH_3), 4.15 (q, 2H, CH_2, J= 7.2 Hz), 6.76 (d, 2H, aromatic protons, J= 7.5 Hz), 6.93 (d, 2H, aromatic protons, J= 7.5 Hz), 7.35 (s, 1H, H-6), 11.80 (s, 1H, NH), 11.99 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 14.08 (CH_3), 54.89 (OCH_3), 66.31 (CH_2), 90.48, 94.01, 113.23, 127.33, 127.46, 129.49, 153.30, 157.06, 160.60, 162.90 (C=O), 164.04 (C=O), 172.70 (C=S); MS (m/z) = 357 (M^+, 1%), 328 (M^+ -C_2H_5, 26%). Anal. Calcd. for $C_{17}H_{15}N_3O_4S$: C 57.13%, H 4.23%, N 11.76%, S 8.97%. Found: C 57.04%, H 4.17%, N 11.60%, S 8.89%.

Synthesis of dithioimidocarbmate 18:

A mixture of equimolar amounts of 6-amino-2-thiouracil **1** (1.43 g, 0.01 mol), CS_2 (0.01 mol) and sodium hydride (0.48 g, 0.02 mol) in DMSO (50 ml) were stirred in ice bath for 3 h. The non-isolable disodium salt **17** was treated with MeI (2.84 g, 0.02 mol). The reaction mixture was stirred for another 3 h at room temperature then poured over ice/water. The solid product formed was collected by filtration and crystallized from (ethanol/dioxane mixture).

***Dimethyl 6-oxo-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yldithioimido-carbonate, 18*:** yellow crystals, yield: 2.10 g (85%); mp: 230 ^{0}C; IR (KBr, cm^{-1}): 3463 (NH), 1640 (CO); ^{1}H-NMR (DMSO-d_6): δ (ppm)= 2.49 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 6.39 (s, 1H, H-5 pyrimidine), 11.80 (brs, 2H, 2NH); MS (m/z)= 247 (M^+, 45%). Anal. Calcd. for $C_7H_9N_3OS_3$: C 33.99%, H 3.67%, N 16.99%, S 38.89%. Found: C 33.83%, H 3.55%, N 16.82%, S 38.76%.

Bioassay

1. Antimicrobial activity: A filter paper sterilized disc saturated with measured quantity of the sample is placed on plate containing solid bacterial medium (nutrient

agar broth) or fungal medium (Dox's medium) which has been heavily seeded with spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism.**[18-20]**

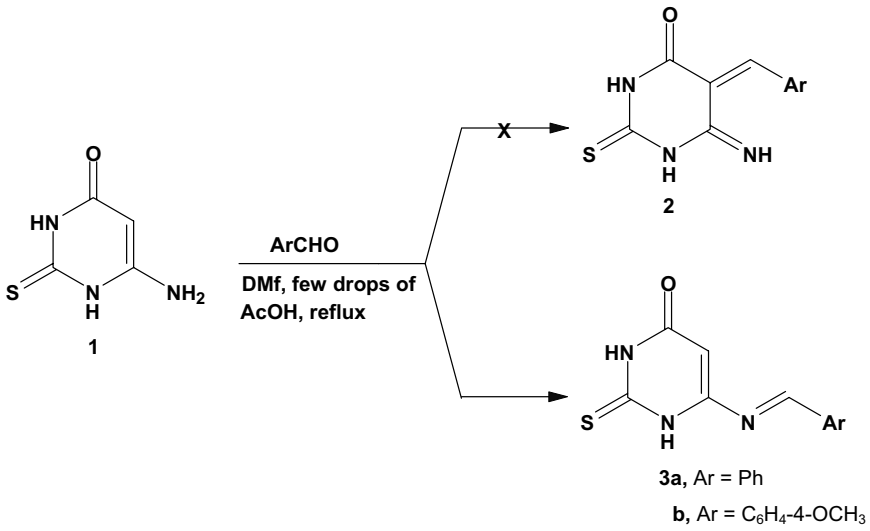
2. Antitumor activity

Potential cytotoxicity of the compounds was tested using the method of Skehan et al.**[21]** Cells were plated in 96 multiwell plate (104 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0.0, 1.0, 2.5, 5.0 and 10.0 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 hours at 37^0C and in atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentrations is plotted to get the survival curve of each tumor cell line after specified compound. The efficiency of the cytotoxic activity is expressed as IC_{50}.

Results and Discussion

1. Chemistry

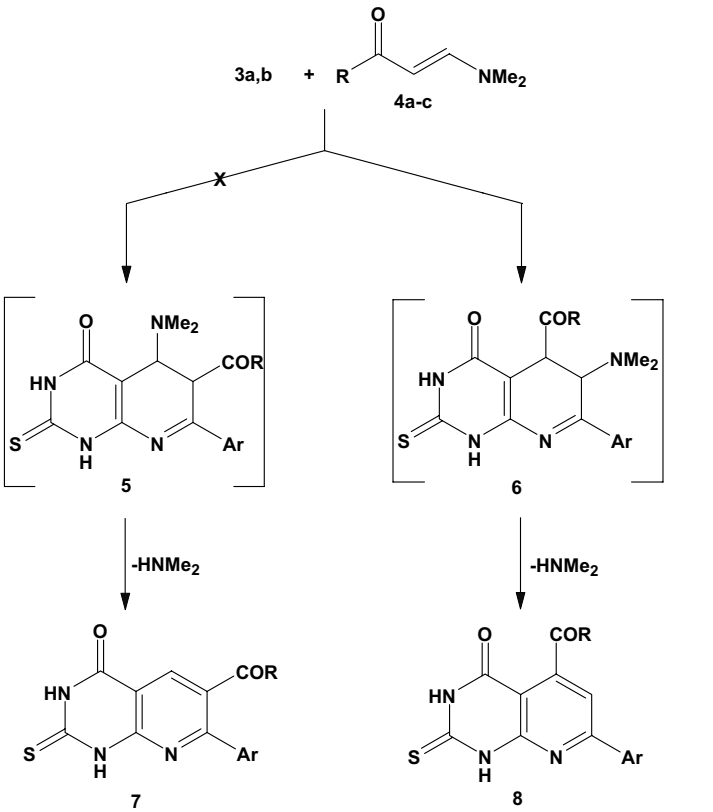
6-(Benzylidene-amino)-2-thiouracil **3a** was synthesized previously **[17]**. However, in this report a simple condensation reaction of 6-amino-2-thioxo-1H-pyrimidine-4-one **1 [22]** with aromatic aldehydes in DMF with few drops of acetic acid afforded the corresponding condensation products **2** or **3**. The formation of the isomeric imino compound **2** is not plausible because the imino group is much more nucleophilic than the CH in position 5 of thiouracil **1**. In addition the ^{1}H-NMR showed one proton signal at δ= 5.26 ppm for pyrimidine H-5 and one proton signal at δ= 7.84 ppm for azamethine proton (N=CH). That established structure **3** (Scheme 1).



Scheme 1

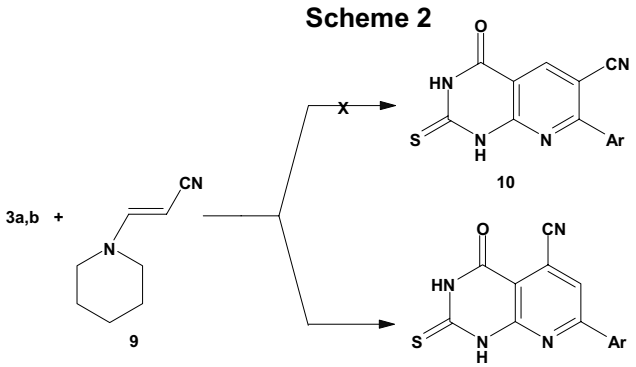
We looked at the activity of this synthesized diene system in cycloaddition reaction. It has been found that enaminones **4a-c [23]** readily condensed with **3a,b** to yield pyrido[2,3-*d*]pyrimidine *via* dimethylamine elimination. One can assume that a [4+2] cycloaddition initially occurred that was followed by secondary amine elimination. This would lead to **7** or isomeric **8**. Structure **8** could be established based on ^{1}H-NMR. For example, the ^{1}H-NMR of compound **8a** revealed a singlet at δ=7.63 ppm typical for H-6 proton. This value is different than the expected value for H-5 proton that expected to appear at δ= 8.40 ppm **[24]** (Scheme 2).

Similarly, we examined the cycloaddition reaction of the azadiene **3a,b** with enaminonitrile **9 [25]**. The condensed products were formed *via* piperidine elimination to yield the corresponding **10** or **11**. As an example the structure of **11a** was established depending on ^{1}H-NMR spectrum that revealed a singlet at δ= 7.38 ppm for pyridopyrimidine H-6. If the reaction product was the isomeric **10**; then proton H-5 should appear at a much lower field **[24]** (Scheme 3).



4,8a, R= 2-thienyl Ar = Ph
b, R= 2-thienyl Ar = C_6H_4-4-OCH_3
c, R= 2-furyl Ar = Ph
d, R= 2-furyl Ar = C_6H_4-4-OCH_3
e, R= 2-naphthyl Ar = Ph
f, R= 2-naphthyl Ar = C_6H_4-4-OCH_3

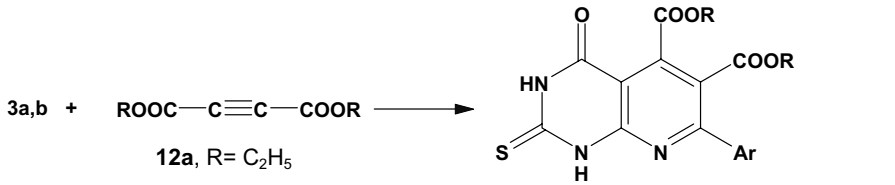
Scheme 2



11a, Ar = Ph
b, Ar = C_6H_4-4-OCH_3

Scheme 3

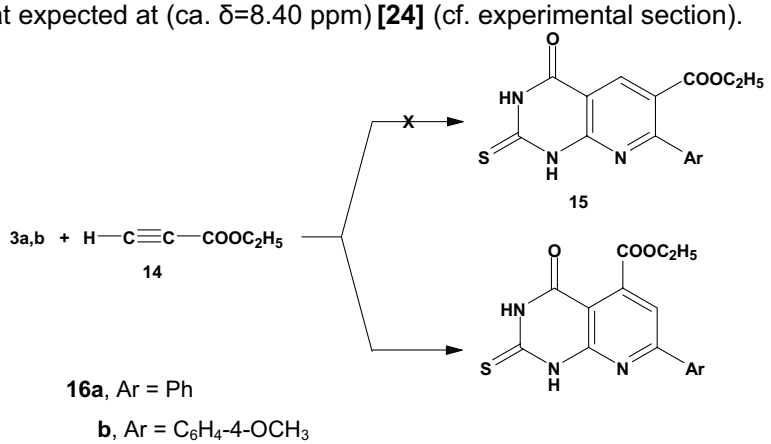
In addition compounds **3a,b** could be successfully added to dimethyl acetylenedicarboxylate and diethyl acetylenedicarboxylate **12a,b** in dioxane under reflux for 4 hours to produce **13a-d** in good yield (Scheme 4).



13a, R = C_2H_5 Ar = Ph
b, R = C_2H_5 Ar = C_6H_4-4-OCH_3
c, R = CH_3 Ar = Ph
d, R = CH_3 Ar = C_6H_4-4-OCH_3

Scheme 4

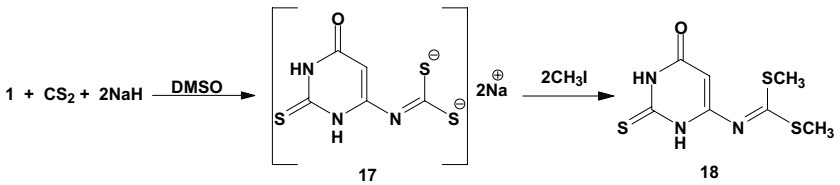
Similarly, the reaction of **3a,b** with ethyl propynoate **14** produced pyrido [2,3-*d*]pyrimidine **15** or its isomeric **16**, that revealed the aryl and ester functions not to be especially proximal (Scheme 5). Structure **16** could be established based on ^{1}H-NMR. For example, the ^{1}H-NMR spectrum of **16a** showed the presence of H-6 proton as singlet at δ= 7.38 ppm. In contrast H-5 proton should appear at a lower field that expected at (ca. δ=8.40 ppm) **[24]** (cf. experimental section).



16a, Ar = Ph
b, Ar = C_6H_4-4-OCH_3

Scheme 5

Finally, other azadiene system could also be prepared *via* reacting **1** with CS_2 in DMSO in the presence of NaH forming the non-isolable disodium salt **17**, which reacted with methyl iodide to produce the diene system **18**. This system failed to add either electron-poor or push-pull dienophile (Scheme 6).



Scheme 6

In conclusion a simple route to azadiene could be developed and the reactivity of these azadiene systems in Diels-Alder cycloaddition has been investigated.

2. Bioactivity

2.1. Antimicrobial activity

The *in vitro* antimicrobial activity of the newly synthesized compounds **8a,b,d,e**, **11a**, **13a,c,d** and **16a** against of three strains of Gram positive bacteria, three strains of Gram negative bacteria and two strains of fungi was investigated in comparison with Ampicillin and Nystatine. In general all tested compounds were capable of inhibiting the growth of the all tested strains. Compounds **8a** and **13c,d** showed a relatively high activity as antibacterial and antifungal activity agents. The other compounds showed a relatively moderate activity toward the all tested strains. Table (1) shows the results of the bioassay.

Microorganism	Compounds										
	8a	**8b**	**8d**	**8e**	**11a**	**13a**	**13c**	**13d**	**16a**	**Amp.**	**Nys.**
Bacillus Subtilis (G^+)	13	11	11	10	11	12	16	14	11	18	-
Staphylococcus Aureus (G^+)	12	11	11	11	11	12	16	15	11	20	-
Streptococcus Faecalis (G^+)	12	12	11	11	12	12	15	14	11	30	-
Escherichia Coli (G^-)	13	12	11	10	12	11	16	14	11	11	-
Neisseria gonorrhea (G^-)	13	14	12	12	12	13	15	14	12	13	-
Pseudomonas Aeroginosa (G^-)	12	11	11	11	11	13	16	14	11	19	-
Candida Albicans (Fungus)	13	12	11	10	12	12	15	14	11	-	12
Saccharomyces Cereviseae (Fungus)	12	12	12	12	12	11	16	14	12	-	11

G^+ = Gram positive G^- = Gram negative
Amp. = Ampicillin Nys. = Nystatine

Table 1. Antimicrobial potentialities of the tested compounds expressed as size (mm/mg sample) of inhibition zone.

2.2. Antitumor activity

Evaluation of the anticancer activity of compounds **8a**, **11b**, **13a,d** and **16b** was performed at the National Cancer Institute (NCI). The tested compounds were evaluated for cytotoxicity against the liver carcinoma cell line (HEPG2) and the lung carcinoma cell line (H460) of human. Different concentrations of the tested compounds were added to the cell monolayer of tumor. A 48 hours continuous drug

exposure is used to estimate all availability or growth.**[21]** The cytotoxic activity of each compound is deduced from the dose response curves. Table (2) and (3) represent the cytotoxic activity for each concentration of the tested compounds.

Compound	Conc. % µg	HEPG2	
		Y	SEM
8a	0.0	1.000	±0.033
	1.0	1.050	±0.008
	2.5	1.005	±0.012
	5.0	0.905	±0.012
	10.0	0.850	±0.020
11b	0.0	1.000	±0.033
	1.0	1.046	±0.012
	2.5	0.968	±0.008
	5.0	0.941	±0.008
	10.0	0.914	±0.008
13a	0.0	1.000	±0.033
	1.0	0.968	±0.008
	2.5	0.914	±0.014
	5.0	0.841	±0.012
	10.0	0.805	±0.008
13d	0.0	1.000	±0.033
	1.0	0.895	±0.024
	2.5	0.823	±0.012
	5.0	0.786	±0.012
	10.0	0.709	±0.008
16b	0.0	1.000	±0.033
	1.0	1.018	±0.020
	2.5	1.000	±0.009
	5.0	0.977	±0.012
	10.0	0.927	±0.008

Y= Surviving fraction, SEM= Standard deviation.

Table 2. Cytotoxic activity against HEPG2.

Compound	Conc. % µg	H460	
		Y	SEM
8a	0.0	1.000	±0.033
	1.0	0.943	±0.012
	2.5	0.869	±0.020
	5.0	0.808	±0.008
	10.0	0.641	±0.021
11b	0.0	1.000	±0.033
	1.0	0.948	±0.008
	2.5	0.897	±0.017
	5.0	0.836	±0.008
	10.0	0.808	±0.008
13a	0.0	1.000	±0.033
	1.0	0.855	±0.009
	2.5	0.827	±0.012
	5.0	0.771	±0.012
	10.0	0.720	±0.005
13d	0.0	1.000	±0.033
	1.0	0.962	±0.008
	2.5	0.887	±0.012
	5.0	0.808	±0.008
	10.0	0.743	±0.012
16b	0.0	1.000	±0.033
	1.0	0.948	±0.020
	2.5	0.915	±0.009
	5.0	0.776	±0.012
	10.0	0.646	±0.008

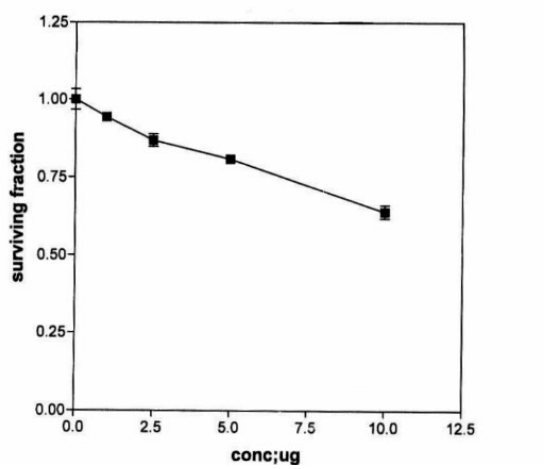
Y= Surviving fraction, SEM= Standard deviation.

Table 3. Cytotoxic activity against H460.

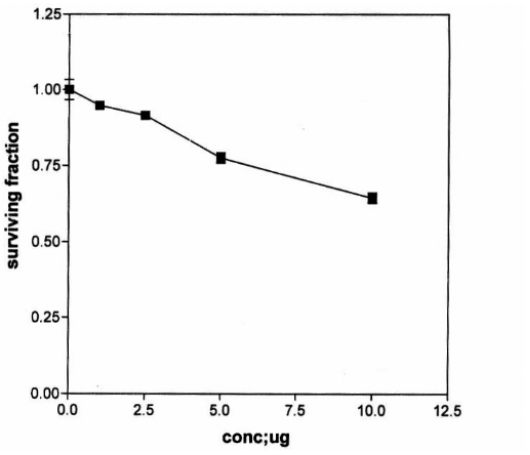
Conclusion

All the tested compounds showed limited cytotoxic activity against (HEPG2). Their efficiency is ranged from 10-30% only. On the other hand, the cytotoxic activity against (H460) is more localized and ranged from 20-36%. Compounds **8a** and **16b** are the relatively more active cytotoxic agents against (H460) tumor cells. (Curve 1, Curve 2).

We can assume that the kind of substituents in position 5 and 6 of the tested compounds are effective with respect to the cytotoxic activity. The presence of thionyl or ethyl carboxylate at position 5 and Hydrogen proton at position 6 increases the cytotoxic activity. In contrast, the presence of nitrile group or two carboxylate groups at positions 5 and 6 decreases the cytotoxic activity.



Curve 1. Cytotoxic activity of compound **8a** against H460.



Curve 2. Cytotoxic activity of compound **16b** against H460.

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