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Utility of 6-amino-2-thiouracil as a precursor for the synthesis of bioactive pyrimidine derivatives

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Abstract—The condensation of 6-amino-2-thiouracil **1** with aromatic aldehydes afforded azomethine derivatives **3a**,**b**. The formed azomethines 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*]pyrimidines **13a**–**d** and **16a**,**b**, respectively. The newly synthesized 2-azadiene **18** reacted with *ortho*-aminophenol and *ortho*-aminothiophenol **19a**,**b** to yield the amidines **21a**,**b**. 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).

1. 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] hepato-protective,[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 the 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.

2. Results and discussion

2.1. Chemistry

6-(Benzylidene-amino)-2-thiouracil **3a** was synthesized previously.[17] However, in this report a simple condensation reaction of 6-amino-2-thioxo-1*H*-pyrimidine-4-one **1**[18] 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 $\delta = 5.26$ ppm for pyrimidine H-5 and one proton signal at $\delta = 7.84$ ppm for azamethine proton (N=CH). That established structure **3** (Scheme 1).

We have looked at the activity of this synthesized diene system in cycloaddition reaction. It has been found that enaminones **4a**–**c**[19] 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 $\delta = 7.63$ ppm typical

Keywords: Diels–Alder cycloaddition; 2-Azadiene; 6-Amino-2-thiouracil; Enaminone; Pyrido[2,3-*d*]pyrimidine; Amidines; Antimicrobial; Antitumor activity.
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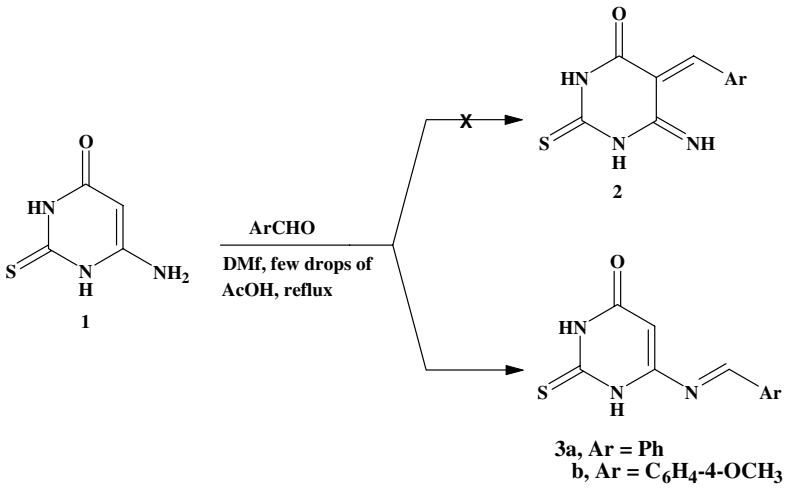
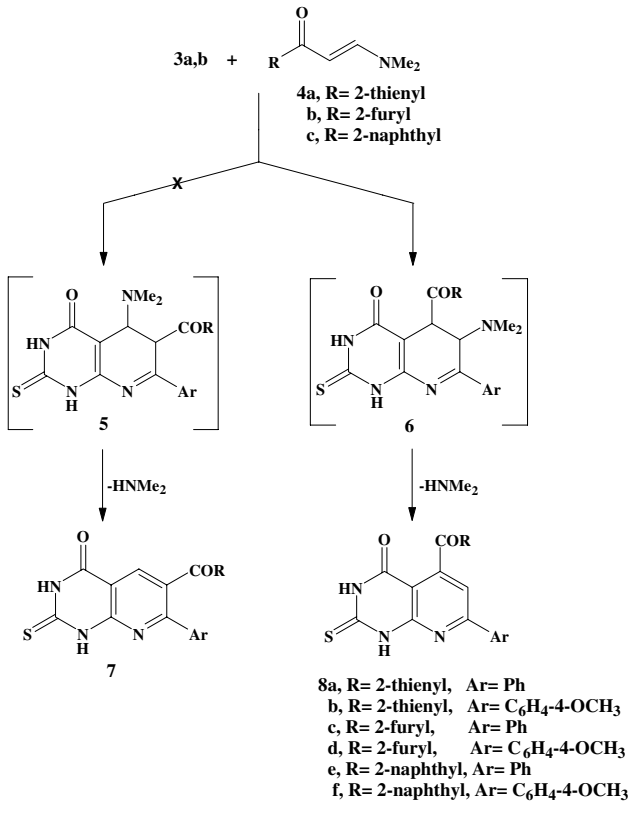


Scheme 1.

for H-6 proton. This value is different than the expected value for H-5 proton that was expected to appear at $\delta = 8.40$ ppm[20] (Scheme 2).

Similarly, we examined the cycloaddition reaction of the azadiene **3a**,**b** with enaminonitrile **9**.[21] 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 $\delta = 7.38$ ppm for pyridopyrimidine H-6. If the reaction product was the isomeric **10**; then proton H-5 should appear at a much lower field[20] (Schemes 2 and 3).



Scheme 2.

In addition compounds **3a**,**b** could be successfully added to dimethyl acetylenedicarboxylate and diethyl acetylenedicarboxylate **12a**,**b** yielding **13a**–**d** in good yield (Scheme 4).

Again 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).

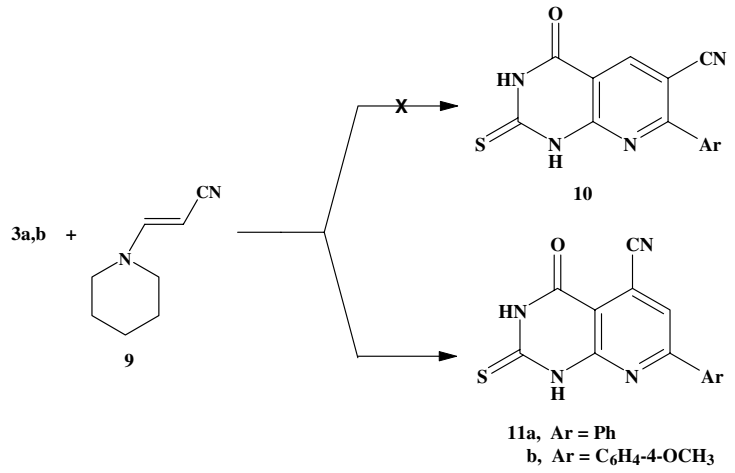
Structure **16** could be established based on ^{1}H NMR. For example, the ^{1}H NMR spectrum of **16a** showed the presence of H-6 proton as singlet at $\delta = 7.38$ ppm. In contrast H-5 proton should appear at lower field that expected at $\delta = 8.40$ ppm (cf. Section 4).

Finally, other new 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).

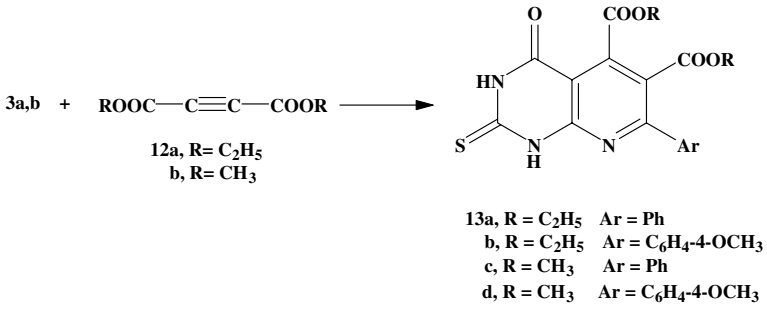
However, 2-azadiene **18** reacted with *ortho*-aminophenol and *ortho*-aminothiophenol (**19a**,**b**) to yield the amidines **21a**,**b**. These compounds can be formed through dinucleophilic substitution with elimination of methanethiol as a byproduct forming the intermediate **20**. Methylation of **20** by methanethiol yielded the final product **21a**,**b** (Scheme 7).

The structure of **21a** taken as example is assigned from its molecular ion peak ($m/z = 274$, M^+). In addition, ^{1}H NMR spectrum showed a singlet at $\delta = 3.56$ ppm characteristic of N—CH_3 group (cf. Section 4).

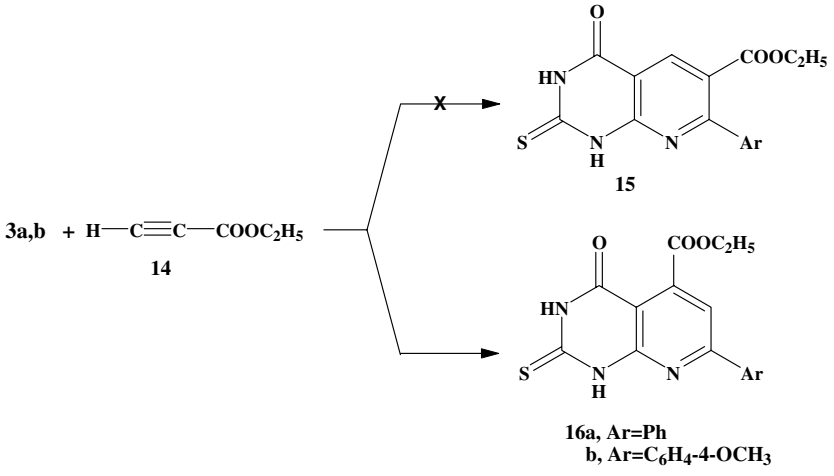
In conclusion, a simple route to azadiene could be developed and the reactivity of these azadiene systems in Diels–Alder cycloaddition has been investigated.



Scheme 3.



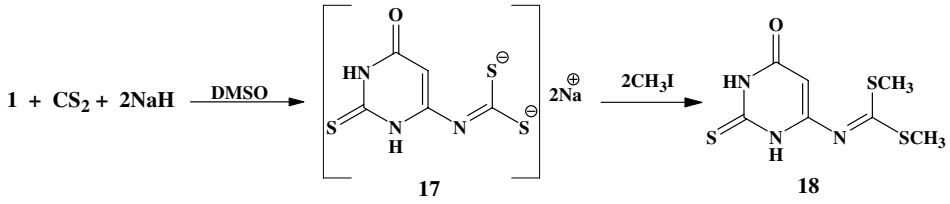
Scheme 4.



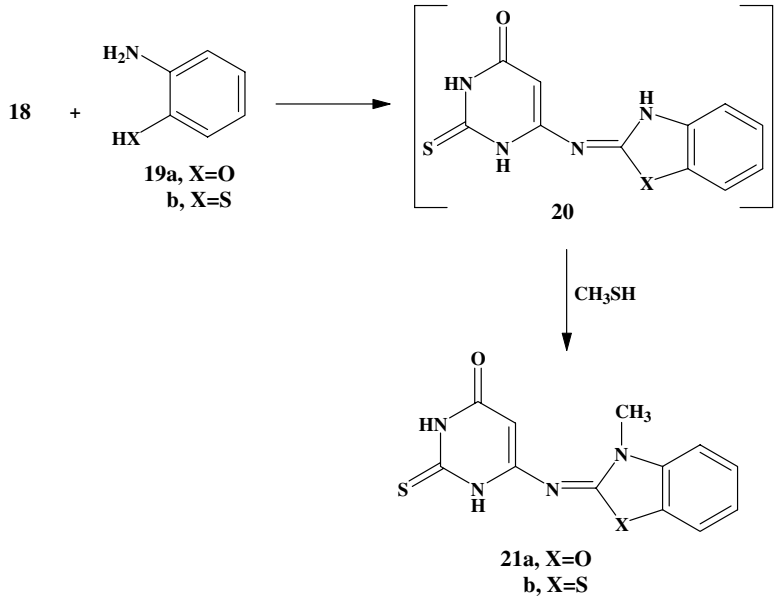
Scheme 5.

2.2. Bioactivity

2.2.1. 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 as an antibacterial standard agent and Nystatine as an antifungal standard agent. In general all tested compounds were capable of inhibiting the growth of all the tested strains. Compounds **8a** and **13c**,**d** are active as antibacterial and antifungal activity agents. The other compounds showed a moderate activity toward all the tested strains. Table 1 shows the results of the bioassay.



Scheme 6.



Scheme 7.

In conclusion, it is possible to report here the importance of these novel compounds as antibacterial and antifungal agents. Further studies should be made to elucidate their mechanism of action.

2.2.2. Antitumor activity. Evaluation of 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 lung carcinoma cell line (H460) of human in comparison with a cisplatine as a positive control. Different concentrations of the tested compounds were added to the cell monolayer of tumor. A 48 h continuous newly synthesized compound exposure is used to estimate all availability or growth.[22] The cytotoxic activity of each compound is deduced from dose–response curves. Tables 2 and 3 represent the cytotoxic activity for each concentration of the tested compounds.

3. Conclusion

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

We can assume that the kind of substituents in positions 5 and 6 of the tested compounds is effective with respect to the cytotoxic activity. The presence of thiophene-2-carbonyl 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.

4. Experimental

All melting points are uncorrected. The IR spectra are 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 and *JEOL* JNM-EX 500 MHz spectrometer in DMSO-d_6 as a solvent and TMS as an internal reference. Chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kartos (75 eV) MS equipment. Elemental analysis was carried out by the Microanalytical unit at the National Research Centre, Giza, Egypt. Microbiological analysis was carried out by the Microanalytical Centre,

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

Microorganism	Compound										
	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 2. Cytotoxic activity against HEPG2

Compound	Conc. % μg	HEPG2	
		Y	SEM
Cisplatine	0.0	1.000	±0.066
	1.0	0.511	±0.060
	2.5	0.494	±0.077
	5.0	0.314	±0.076
	10.0	0.253	±0.039
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 error mean.

Table 3. Cytotoxic activity against H460

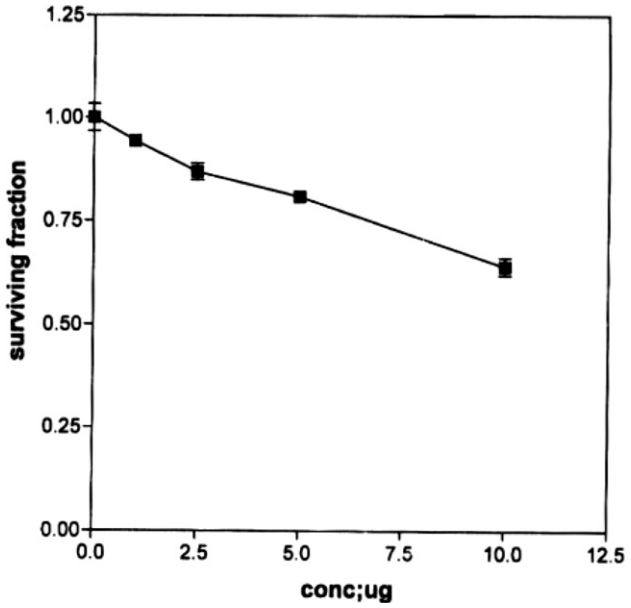
Compound	Conc. % μg	H460	
		Y	SEM
Cisplatine	0.0	1.000	±0.072
	1.0	0.796	±0.020
	2.5	0.665	±0.050
	5.0	0.484	±0.026
	10.0	0.401	±0.007
8a	0.0	1.000	±0.033
	1.0	0.943	±0.012
	2.5	0.869	±0.020
	5.0	0.808	±0.008
	10.0	0.641	±0.021
11b	0.0	1.000	±0.033
	1.0	0.948	±0.008
	2.5	0.897	±0.017
	5.0	0.836	±0.008
	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

Faculty of Science, Cairo University, Giza, Egypt. Antitumor activity was evaluated by the National Cancer Institute, Cancer Biology Department, Cairo University, Egypt. All starting materials used were commercially available from Aldrich company unless otherwise stated.

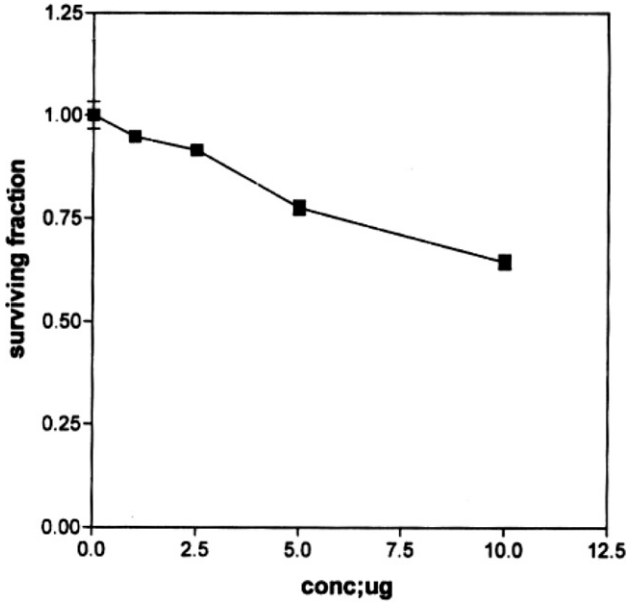
4.1. Synthesis of 2-azadienes (3a,b)

4.1.1. General procedure. To a solution of 6-amino-2-thiouracil **1** (1.43 g, 0.01 mol) in DMF (30 ml), 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.

4.1.1.1. 6-(Benzylidene-amino)-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (3a).[17] Yield 2.03 g (88%); yellow crystals, mp 278 °C; IR (KBr, cm^{-1}): 3386 (NH), 1654 (C=O), 1182 (C=S); ^1H NMR: 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^+, 22.4%). Anal. Calcd for $C_{11}H_9N_3OS$: C, 57.13%; H, 3.92%; N, 18.17%; S,



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



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

13.86%. Found: C, 57.08%; H, 3.86%; N, 18.14%; S, 13.78%.

4.1.1.2. 6-[(4-Methoxy-benzylidene)-amino]-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (3b). Yield 2.38 g (91%); yellow crystals; mp 288 °C; IR (KBr, cm^{-1}): 3387 (NH), 1656 (C=O), 1172 (C=S); ^1H NMR: 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%.

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

4.2.1. General procedure. 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** in dry dioxane (25 ml) were heated under reflux for 16 h. The solvent evaporated under vacuum and the remaining residue was treated with petroleum ether at 40–60 °C. The precipitate formed was collected by filtration and crystallized from dioxane.

4.2.1.1. 7-Phenyl-5-(thiophene-2-carbonyl)-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8a). Yield 2.9 g (79.4%); yellow crystals; mp 199 °C; IR (KBr, cm^{-1}): 3327 (NH), 1649 (C=O), 1630 (C=O), 1175 (C=S); ^1H NMR: 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: 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^+, 38.82%). 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%.

4.2.1.2. 7-(4-Methoxy-phenyl)-5-(thiophene-2-carbonyl)-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8b). Yield 3.3 g (84%); yellow crystals; mp 226 °C; IR (KBr, cm^{-1}): 3406 (NH), 1646 (C=O), 1631 (C=O), 1177 (C=S); ^1H NMR: 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%.

4.2.1.3. 5-(Furan-2-carbonyl)-7-phenyl-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8c). Yield 2.86 g (82%); brown crystals; mp 212 °C; IR (KBr, cm^{-1}): 3426 (NH), 1644 (C=O), 1633 (C=O), 1177 (C=S); ^1H NMR: 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^+, 27.72%). 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%.

4.2.1.4. 5-(Furan-2-carbonyl)-7-(4-methoxy-phenyl)-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8d). Yield 3.25 g (85%); brown crystals; mp 228 °C; IR (KBr, cm^{-1}): 3330 (NH), 1640 (C=O), 1631 (C=O), 1176 (C=S); ^1H NMR: δ (ppm) = 3.85 (s, 3H, OCH_3), 6.76 (d, 2H, aromatic protons, J = 7.5 Hz), 6.96 (d, 2H, aromatic protons, J = 7.5 Hz), 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^+, 25.9%). 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%.

4.2.1.5. 5-(Naphthalene-2-carbonyl)-7-phenyl-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8e). Yield 3.5 g (86%); yellow crystals; mp 233 °C; IR (KBr, cm^{-1}): 3399 (NH), 1643 (C=O), 1630 (C=O), 1178 (C=S); ^1H NMR: 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^+, 43.3%). 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%.

4.2.1.6. 7-(4-Methoxy-phenyl)-5-(naphthalene-2-carbonyl)-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (8f). Yield 3.8 g (87%); yellow crystals; mp 248 °C; IR (KBr, cm^{-1}): 3408 (NH), 1646 (C=O), 1628 (C=O), 1175 (C=S); ^1H NMR: 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^+, 27.9%). 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%.

4.2.1.7. 4-Oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5-carbonitrile (11a). Yield 1.9 g (70%); brown crystals; mp 213 °C; IR (KBr, cm^{-1}): 3398 (NH), 2203 (CN), 1643 (C=O), 1178 (C=S); ^1H NMR: δ (ppm) = 6.80–7.40 (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^+, 7.0%). 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%.

4.2.1.8. 7-(4-Methoxy-phenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5-carbonitrile (11b). Yield 2.3 g (76%); brown crystals; mp 228 °C; IR (KBr, cm^{-1}): 3401 (NH), 2182 (CN), 1649 (C=O), 1174 (C=S); ^1H NMR: 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^+, 8.8%). 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%.

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

4.3.1. General procedure. 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. The solid product formed after washing the remaining residue with *n*-hexane was collected by filtration and crystallized from dioxane.

4.3.1.1. Diethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5,6-dicarboxylate (13a). Yield 3.5 g (87%); orange crystals; mp 235 °C; IR (KBr, cm^{-1}): 3419 (NH), 1760 (C=O), 1726 (C=O), 1633 (C=O), 1182 (C=S); ^1H NMR: 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: 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^+, 9.6%), 354 (M^+-OEt, 9.6%). 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%.

4.3.1.2. Diethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5,6-dicarboxylate (13b). Yield 3.8 g (88%); orange crystals; mp 246 °C; IR (KBr, cm^{-1}): 3426 (NH), 1762 (C=O), 1733 (C=O), 1637 (C=O), 1180 (C=S); ^1H NMR: 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^+, 3.0%), 384 (M^+-OEt, 61.0%). 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%.

4.3.1.3. Dimethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5,6-dicarboxylate (13c). Yield 3.2 g (86%); yellow crystals; mp 200 °C; IR (KBr, cm^{-1}) 3426 (NH), 1762 (C=O), 1732 (C=O), 1636 (C=O), 1184 (C=S); ^1H NMR: 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^+, 2.5%), 356 (M^+-Me, 3.4%). 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%.

4.3.1.4. Dimethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5,6-dicarboxylate (13d). Yield 3.6 g (89%); yellow crystals; mp 220 °C; IR (KBr, cm^{-1}): 3425 (NH), 1763 (C=O), 1733 (C=O), 1642 (C=O), 1181 (C=S); ^1H NMR: 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^+, 2.1%), 386 (M^+-Me, 20.8%). 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%.

4.3.1.5. Ethyl 4-oxo-7-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5-carboxylate (16a). Yield 2.65 g (81%); yellow crystals; mp 242 °C; IR (KBr, cm^{-1}): 3407 (NH), 1761 (C=O), 1644 (C=O), 1173

(C=S); ^{1}H NMR: δ (ppm) = 1.21–123 (t, 3H, CH_3), 4.13–4.37 (q, 2H, CH_2), 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^+, 1.8%), 254 ($M^+-COOEt$, 5.6%). Anal. Calcd for $C_{16}H_{13}N_3O_3S$: C, 58.70%; H, 4.0%; N, 12.84%; S, 9.79%. Found: C, 58.61%; H, 3.96%; N, 12.76%; S, 9.68%.

4.3.1.6. Ethyl 7-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylate (16b). Yield 3.0 g (84%); yellow crystals; mp 258 °C; IR (KBr, cm^{-1}) 3414 (NH), 1764 (C=O), 1646 (C=O), 1174 (C=S); ^{1}H NMR: δ (ppm) = 1.20–125 (t, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.14–4.17 (q, 2H, CH_2), 6.76 (d, 2H, aromatic protons, *J* = 7.5), 6.93 (d, 2H, aromatic protons, *J* = 7.5), 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^+, 1.2%), 328 ($M^+-C_2H_5$, 26.2%). 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, 11.6%; S, 8.89%.

4.4. Synthesis of the 2-azadiene (18)

4.4.1. General procedure. 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) was stirred in ice bath for 3 h. The non-isolable disodium salt **17** was treated with MeI (2.84, 0.02 mol). The reaction mixture was stirred for another 3 h at room temperature and then poured over ice/water. The solid product formed was collected by filtration and crystallized from (ethanol/dioxane mixture).

4.4.1.1. Dimethyl 6-oxo-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yldithioimidocarbonate (18). Yield 2.1 g (85%); yellow crystals; mp 230 °C; IR (KBr, cm^{-1}): 3463 (NH), 1640 (C=O), 1182 (C=S); ^{1}H NMR: δ (ppm) = 2.49 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 6.39 (s, 1H, H-5 pyrimidine), 11.70–11.90 (br s, 2H, 2NH); MS (*m*/*z*) = 247 (M^+, 45.0%). 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%.

4.5. Reaction of 2-azadiene 18 with *ortho*-aminophenols (19a,b)

4.5.1. General procedure. To a solution of 2-azadiene **18** (2.47 g, 0.01 mol) in dry dioxane (30 ml), *ortho*-aminophenols **19a**,**b** (0.01 mol) were added. The reaction mixture was heated under reflux for 15 h. The solvent was evaporated under vacuum and the remaining residue was treated with *n*-hexane. The solid products formed were collected by filtration and crystallized from ethanol.

4.5.1.1. 6-[3-Methyl-3*H*-1,3-benzoxazol-2-ylideneamino]-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (21a). Yield 2.3 g (84%); mp 257 °C, yellow crystals; IR (KBr, cm^{-1}): 3363 (NH), 1642 (C=O), 1181 (C=S); ^{1}H NMR: δ (ppm) = 3.56 (s, 3H, CH_3), 6.77–6.83 (m, 2H, aromatic protons), 6.87 (s, 1H, H-5 pyrimidine), 6.89–6.93 (m, 2H, aromatic protons), 11.77 (s, 1H, NH), 12.39 (s, 1H, NH); MS (*m*/*z*) = 274 (M^+, 32.1%). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.55%; H, 3.67%; N, 20.43%; S, 11.69%. Found: C, 52.47%; H, 3.5%; N, 20.31%; S, 11.52%.

4.5.1.2. 6-[3-Methyl-3*H*-1,3-benzothiazol-2-ylideneamino]-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (21b). Yield 2.1 g (72.4%); mp 260 °C; yellow crystals; IR (KBr, cm^{-1}): 3352 (NH), 1646 (C=O), 1182 (C=S); ^{1}H NMR: δ (ppm) = 3.44 (s, 3H, CH_3), 6.50 (s, 1H, H-5 pyrimidine), 7.28–7.44 (m, 4H, aromatic protons), 11.79 (s, 1H, NH), 12.30 (s, 1H, NH); MS (*m*/*z*) = 290 (M^+, 5.3%). Anal. Calcd for $C_{12}H_{10}N_4OS_2$: C, 49.64%; H, 3.47%; N, 19.30%; S, 22.09%. Found: C, 49.57%; H, 3.32%; N, 19.24%; S, 21.97%.

4.6. Bioassay

4.6.1. Antimicrobial activity. A filter paper sterilized disk 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.[23–25]

4.6.2. Antitumor activity. Potential cytotoxicity of the compounds was tested using the method of Skehan and Storeng.[22] Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, 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}.

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