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Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: https://www.tandfonline.com/loi/lsyc20

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To cite this article: Alaa R. Sakr, Mohamed G. Assy & Youssra S. ELasaad (2020): Design and chemical behavior of novel pyrimidine derivatives and their evaluation of cytotoxicity, Synthetic Communications, DOI: 10.1080/00397911.2020.1735444

To link to this article: https://doi.org/10.1080/00397911.2020.1735444

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https://doi.org/10.1080/00397911.2020.1735444

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Design and chemical behavior of novel pyrimidine derivatives and their evaluation of cytotoxicity

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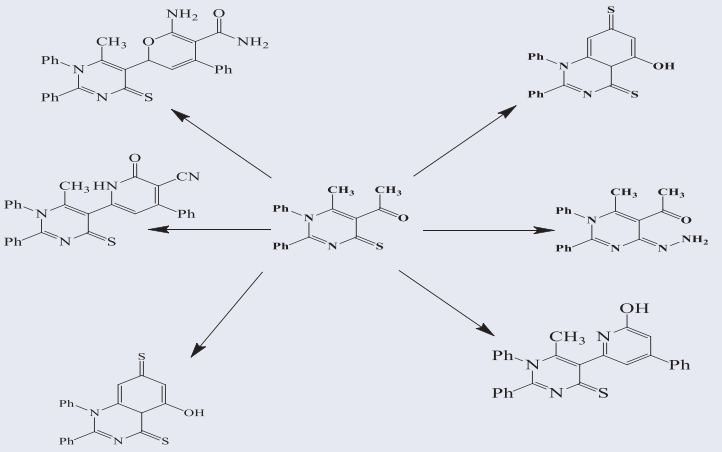
ABSTRACT

Diphenyl-4-thioxo-1,4-dihydropyrimidin-5-yl)ethan-1-one **4** was obtained upon the reaction of (phenylamino)but-2-enethioyl)benzamide **3** with sodium hydroxide. The reactions of **4** with several active species such as benzaldehyde, urea, malononitrile, ethyl cyanoacetate, hydrazine, and carbon disulfide were studied. The antitumor activities of some selective compounds were examined against two cell lines: Mammalian cell lines: A-549 cells (human lung cancer cell line) and MRC-5 cells (normal human lung fibroblast cell line); some of the compounds were highly efficient; compound **13** has the most effective anticancer activity.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 12 November 2019

KEYWORDS
Enamine; benzoyl isothiocyanate; pyrimidine; pyran; antitumor assay

Introduction

Enaminone derivatives are useful for synthetic precursors and their utilization in organic synthesis due to the presence of the nucleophilic character of the enamine moiety and the electrophilic character of the enone moiety.[1–3]

The synthesis of pyrimidine and condensed pyrimidines has attracted the attention of medicinal chemists because of their potential pharmacodynamic properties. Numerous

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publications describe the synthesis of condensed pyrimidines possessing a variety of pharmacological activities such as anticonvulsant[4] and antifertility activities.[5] In conjunction with the work on the synthesis of pyrimidinethione derivatives for biological evaluations,[6–9] A series involving synthesis and reaction pyrimidinthione moiety is described.

Acyl isothiocyanates[10] are bifunctional compounds containing an acyl group and thiocyanate group. Its reactivity determined by three active centers: the nucleophilic nitrogen atom, the electrophilic carbon atoms of the carbonyl and the thiocarbonyl groups, which make them capable of participating in diverse types of addition and cyclization reactions. Isothiocyanates are a key reagent in heterocyclic chemistry because they are very useful in the synthesis of such functionalized thiazoles, thiadiazoles, triazoles, imidazoles, oxazolines, triazines, oxazines, and pyrimidine compounds. They have been shown to exhibit anti-cancer activity in animals treated with chemical carcinogens due to their inhibition of carcinogenic metabolic activation.[11]

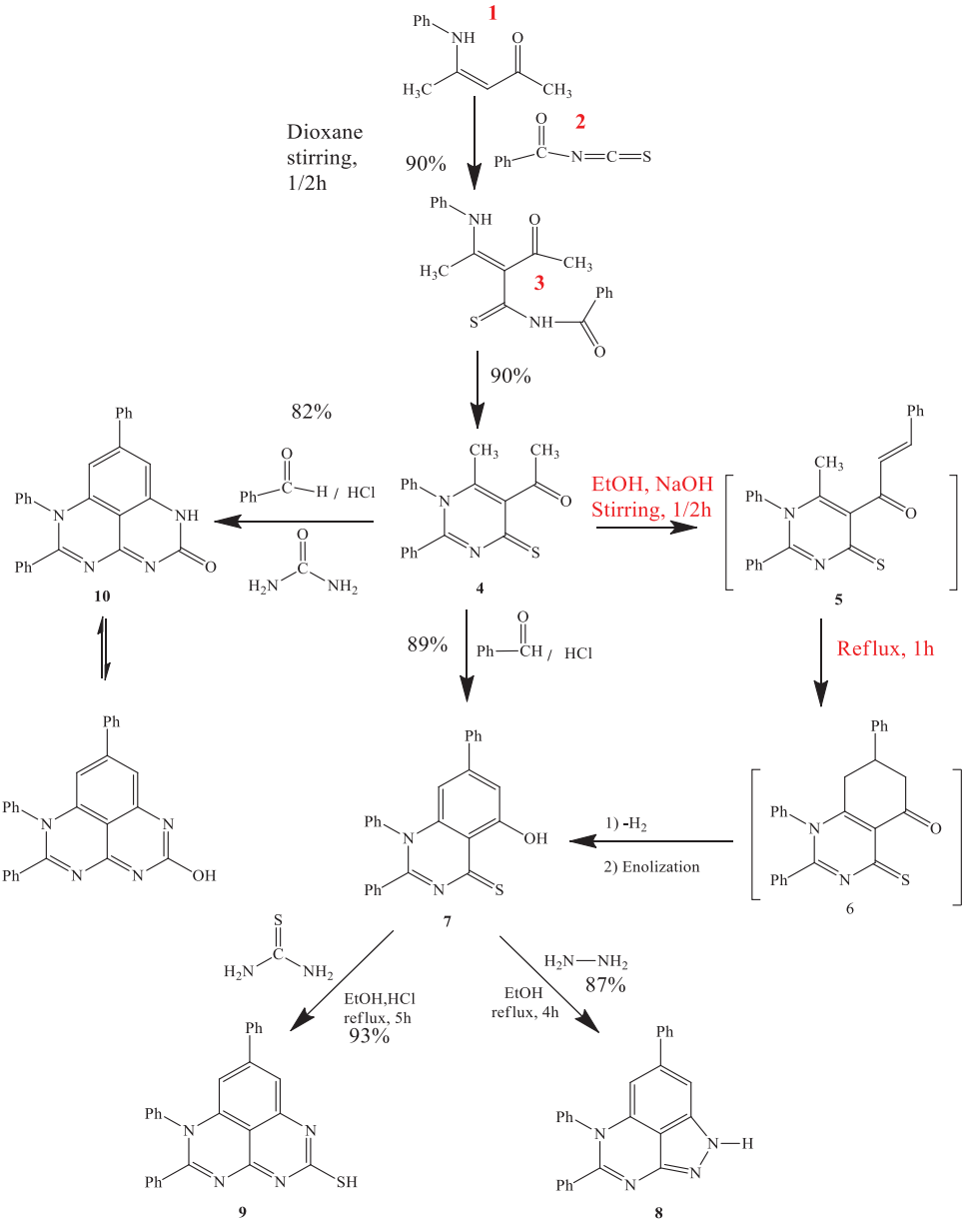
Result and discussion

In continuation of our work in the synthesis of pyrimidines, this paper describes the preparation of some pyrimidines from simply available laboratory reagents.[12,13]

Enamine of type **1** added its reactive nucleophilic carbon to activated heteroallene carbon of benzoyl isothiocyanate to provide the acyclic thioamide derivative **3** (Scheme 1). Infrared (IR) spectrum of compound **3** absorption peaks at 3275, 1670, and 1257 cm^{-1} for NH, C=O, and C=S groups, respectively. ^{1}H NMR of the same compound showed downfield signals at 12.63 and 11.57 ppm for NH protons in addition to aromatic multiplet signal. ^{13}C NMR for the acyclic compound **3** contained sp2 carbon for C=S and 2 C=O at 201.43, 193.50, and 179.59 ppm in addition to aliphatic sp^3 carbon signal. Intramolecular cyclization of acyclic compound **3** was achieved by heating in base mediated alcoholic solution to furnish pyrimidinthione **4**, also absorption frequencies for C=S and C=O sp^2 carbon.

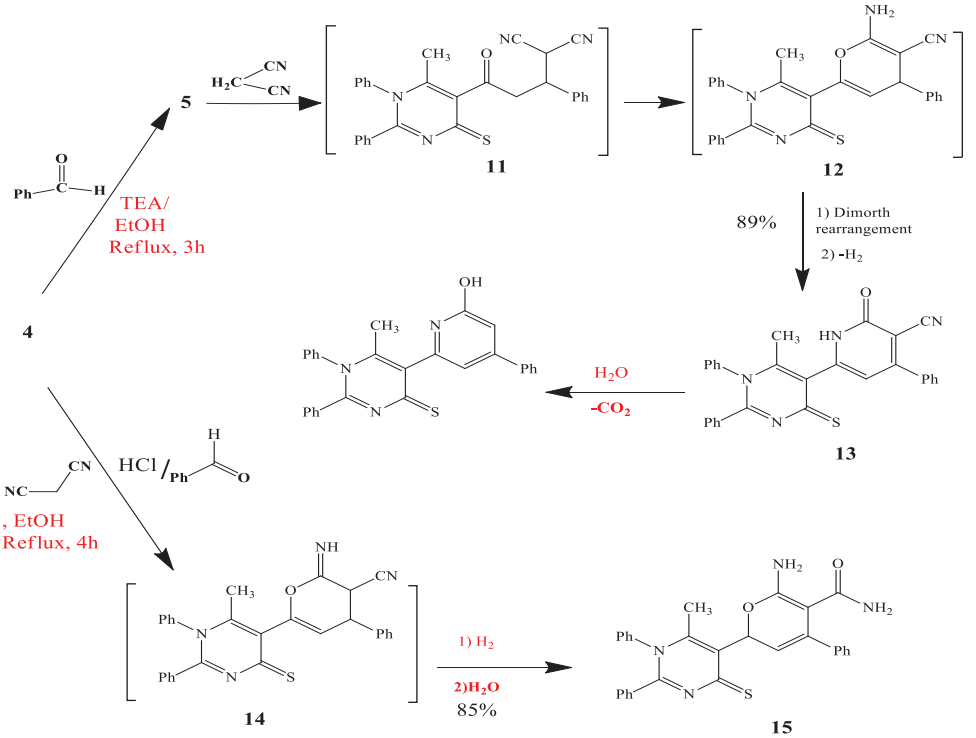
Acetylpyrimidinthione **4** undergoes cyclization with benzaldehyde to form qunazoline derivative **7**. The reaction may be started with the formation of cinnamoyl derivative **5** that cyclized intramolecularity via the addition of the activated methyl to α, β-unsaturated system, dehydrogenated and finally enolized to stable enolic form. IR spectrum of target condensed compound **7** showed OH peaks at 3344 cm^{-1} and C=S at 1261 cm^{-1}. ^{1}H NMR displayed of deshielded signal at δ10.24 together multiplet signal for aromatic protons. ^{13}C NMR provided C=N carbon at 166.12 ppm. When compound **7** was subjected to condensation with hydrazine, it resulted in the cyclization of pyrazole to provide polyheterocyclic compound **8**, compound **8** contained downfield signal for NH (Scheme 1).

Keeping a mixture of benzaldehyde, compound **4** provided the polycyclic compound **9** presumably via formation **7** that condensed with thiourea to give pyrimidine ring **9** (Scheme 1). IR spectrum was in agreement with the expected structure **9**. ^{1}H NMR revealed signal at 10.25 ppm in addition to aromatic multiplet. ^{13}C NMR showed sp^2 carbon of C=S at 166.11 ppm.



Scheme 1. Synthesis and reaction of pyrimidinethione 4 with amine derivatives and benzaldehyde.

Urea was also condensed with pyrimidinethione **4** under similar above reaction condition to provide compound **10** (Scheme 1). IR spectrum of compound **10** provided stretching frequencies at 3275 and 1670 cm^{-1} for OH and C=O groups, respectively. ^{1}H NMR revealed that the compound **10** present in equimolar amounts of keto/enol form at δ12.44 ppm for OH and NH at 11.57 ppm, respectively. ^{13}C provided C=O carbon signal at δ174; compound **4** undergoes base cyclization with benzaldehyde and malononitrile to provide pyridine **13**. The reaction may proceed via the formation of nonisolable cinnamoyl derivative **5**, then forming nonisolable adduct **11** via Michael addition of malononitrile with **5**,

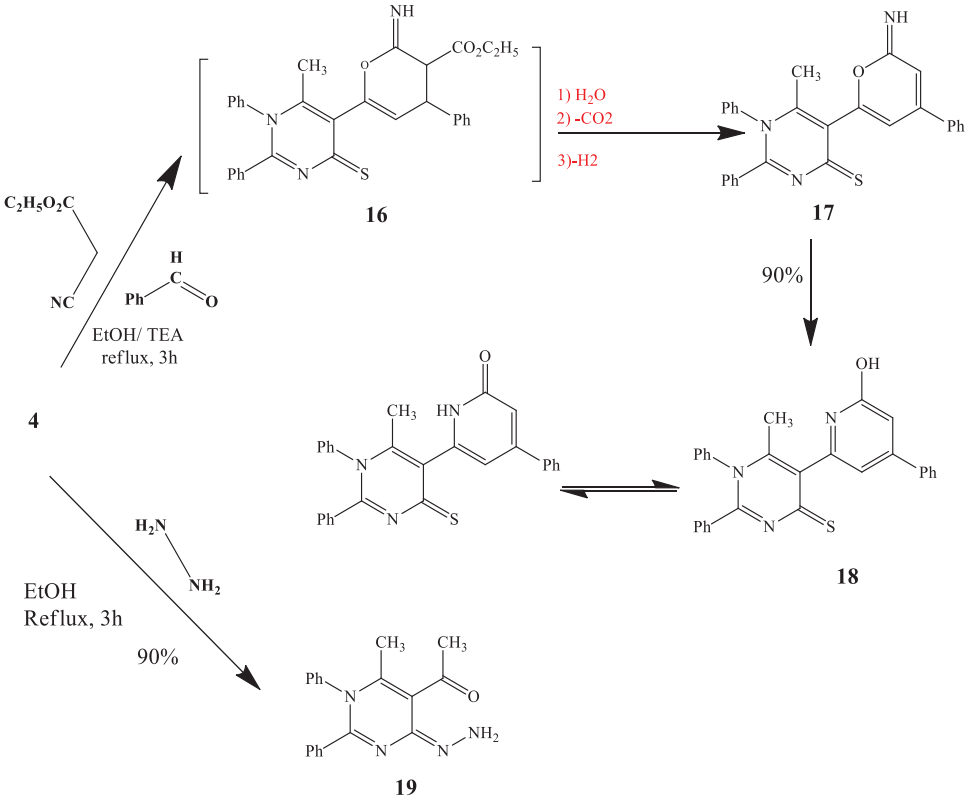


Scheme 2. Reaction of pyrimidinethione 4 with malononitrile.

compound **11** cyclized to pyran derivative **12** which rearranged to give compound **13** (Scheme 2). IR spectrum of **13** showed CN in addition to the carbonyl group. Also ^{1}H NMR showed the lactam lactim equilibrium. Contrary to the above reaction conditions, when the reaction was carried out in acidic medium, it showed the pyran derivative **14**, which dehydrogenated and hydrolyzed yielding pyran derivative **15** (Scheme 2). The absence of CN group in IR spectrum of **15** potentiates the structure. ^{1}H NMR contained two deshielded signals at 12.62 and 11.57 ppm for NH_2 and $CONH_2$ protons, respectively. ^{13}C NMR provided *sp*2 carbon signal for C=S and C=O at 179.69 and 169.79 ppm, respectively.

A ternary mixture of **4**, benzaldehyde, ethyl cyanoacetate, and TEA resulted in pyran ring followed by transformation affording compound **18**. Compound **18** revealed stretching frequencies at 3271, 1670, 1604, and 1246 cm^{-1} for NH, C=O, C=N, and C=S, respectively. It also showed keto-enol form equilibrium in ^{1}H NMR as two downfield signals at 12.63 and 11.57 for OH and NH protons. Pyrimidinthione **4** was condensed with hydrazine in ethanol to produce the hydrazone derivative **19.** IR spectrum showed the presence of NH and C=O at 3342 $cm^{-1}$and 1658 cm^{-1}, respectively. ^{1}H NMR displayed downfield signal at 10.25, while ^{13}C NMR provide signal at 166.11 ppm for carbonyl carbon (Scheme 3).

Acetyl pyrimidinethione **4** added its activated methyl ketone group to carbon disulfide in the presence of alcoholic KOH to produce bicyclic compound **22** presumably through the nonisolable compound **20** which cyclized via loss of H_2S to give **21**, which dehydrogenated to give the final product **22** (Scheme 4).



Scheme 3. Reaction of pyrimidinethione 4 with hydrazine and benzaldehyde, ethyl cyanoacetate and TEA.

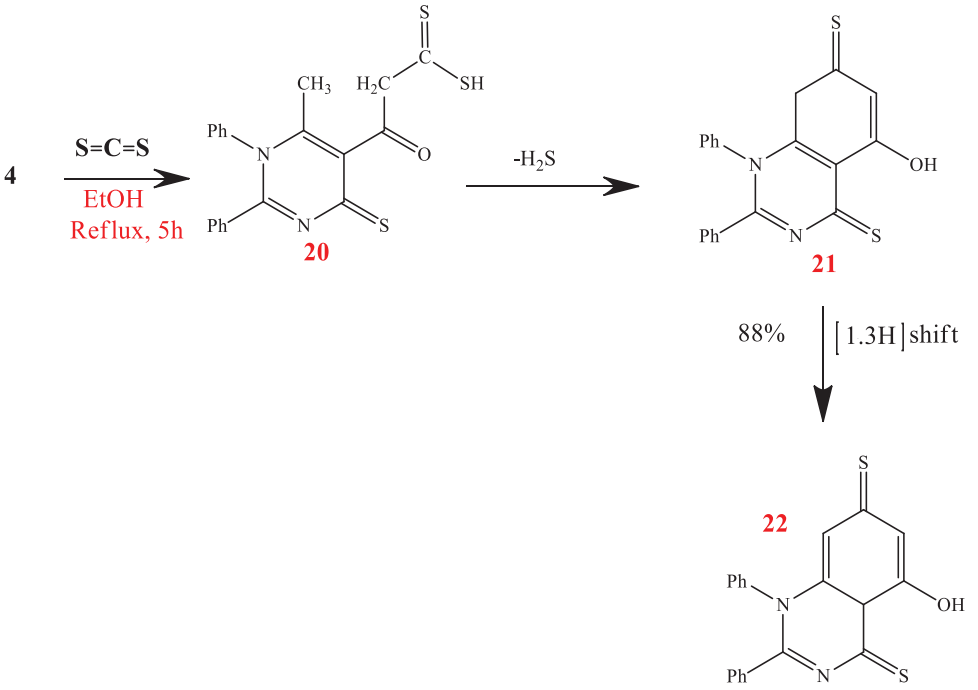
Compound **22** shows stretching frequency at ν3344, 1654 and 1261 cm^{-1} for OH, C=N, and C=S groups, respectively. ^{1}H NMR for the same target compound contains a deshielded signal at δ 10.24 ppm due to OH proton in addition to aromatic multiplet signal. ^{13}C NMR showed C=S carbon signal at δ 166 ppm and another C=S signal at 139 ppm (due to intramolecular hydrogen bond).

Antitumor evaluation of tested compounds

Compounds were tested for their antitumor activity against mammalian cell lines: A-549 cells (human lung cancer cell line) and MRC-5 cells (normal human lung fibroblast cell line) using viability assay method (Table 1).[13,14]

No significant difference was observed between IC_{50} of compound **13** and doxorubicin; therefore, compound **13** showed the lowest IC_{50} as compared to other test compounds. Hence, compound **13** has the most effective anticancer activity.

The compound **13** showed the best result more than the doxorubicin. Compound **13** inhibitory is 98.26% with a concentration of 250 µM/ml higher than doxorubicin inhibitory which is 98.02% with a concentration of 1000 µM/ml.



Scheme 4. Reaction of pyrimidinethione 4 with carbon disulfide.

Table 1. Evaluation of cytotoxic effects of tested compounds.[14]

Compound	IC_{50} μM/mL	CC_{50} μM/mL
7	288 ± 9.8	210 ± 5.4
9	215 ± 4.7	124 ± 3.1
13	6.86 ± 0.2	45.8 ± 1.9
18	116 ± 2.9	382 ± 8.7
22	328 ± 9.2	214 ± 5.2
Doxorubicin	0.89 ± 0.05	

Experimental

Melting points were measured using an Electrothermal IA 9100 apparatus with an open capillary tube and are uncorrected. The IR spectra (KBr disk) were recorded on a PyeUnicam Sp-3-300 or a Shimadzu FT-IR 8101 PC IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL-JNM-LA 400 MHz spectrometer using DMSO-d6 as a solvent. All chemical shifts were expressed on the δ (ppm) scale using tetramethylsilane (TMS) as an internal standard reference. The coupling constant (J) values are given in Hz. Analytical data were obtained from the Microanalysis Center at Cairo University, Giza, Egypt.

General experimental procedure for the preparation of compound: (E)-N-(2-methyl-3-(phenylamino)but-2-enethioyl)benzamide [3]

A mixture of enamine **1** (0.01 mol, 1.75 g) and benzoyl isothiocyanate **2**[15](0.01 mol, 1.63 g) in dioxane (15 mL) was stirred for 30 min. The red precipitate obtained was

filtered off and crystallized from ethanol to provide red crystals of compound **3** (3.22 g), 90% yield. MP 158 °C, IR: 1670 (C=O), 1604 (C=N), 1257 (C=S); ^{1}H NMR: 12.36 (s, 1H, NH), 11.75 (s, 1H, NH), 8.00 − 7.26 (m, 9H, ArH's + enaminon proton), 2.5 (s, 3H, CH_3):^{13}C NMR; 179.59 (C=S), 168.78 (C=O), 138.46, 133.60, 132.62, 129.16, 128.92, 126.80, 124.80, 30.93, 17.99, Anal. Calcd for $C_{18}H_{18}N_2OS$ (310.41): C, 69.58%; H, 5.79%; N, 9.02%;S, 10.30, found: C, 69.65%; H, 5.84%;N, 9.02%;S,10.33.

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