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CONDENSATION OF 2-(2-GUANIDINO-4-OXO-4, 5-DIHYDROTHIAZOL-5-YL) ACETIC ACID WITH DICARBONYL COMPOUNDS

Mohammad Y. Abu Shuheil

Department of Chemistry, Faculty of Arts and Science, Northern Border University, Turif , 833, KSA.

Abstract:

A hybrid pharmacophore approach was adopted to design and synthesize new series of pyrimidine-thiazole hybrid compounds via heterocyclization of 2-(2-guanidino-4-oxo-4,5-dihydrothiazol-5-yl)acetic acid with dicarbonyl compounds, such as diketones and derivatives of acetoacetic ester. The structures of the compounds were established by IR, ^{1}H NMR, ^{13}C NMR. The products were obtained in a good yield, short time and a simple experimental procedure. The synthesized compounds were evaluated for their anti-bacterial activity against Gram-positive and Gram-negative bacteria. The compounds exhibited excellent zone of inhibition against tested bacteria. The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition.

Keywords: *2-Guanidino-4-thiazolinone-5-yl acetic acid; heterocyclization; Pyrimidine-Thiazole Hybrids, Synthesis, Antimicrobial activity.*

Corresponding author:

Mohammad Y. Abu Shuheil,

Department of Chemistry,
Faculty of Arts and Science,
Northern Border University,
Turif , 833, KSA.

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INTRODUCTION:

Molecular hybridization is a valuable structural modification approach that comprises the incorporation of two or more pharmacophores into a single entity. In the last few years, hybrid drug design has emerged as a prime tool for the discovery of innovative antimicrobial therapies that can potentially overcome most of the pharmacokinetic drawbacks encountered when using conventional antimicrobial drugs.

The existence of a thiazolidinonone structural unit is a key to the pharmacological activities of many natural and synthetic drugs [1-4]. Heterocycles containing the thiazolidinone moiety are important targets in synthetic and medicinal chemistry because this fragment is a key moiety in a number of natural and synthetic biologically active agents [5,6]. A large number of compounds having pyrimidine and dihydropyrimidinone (DHPMs) entities have been reported to possess different kind of biological activities like antiviral, antitumor, antibacterial, anti-inflammatory activities [7,8], calcium channel blocking [9], and neuropeptide Y (NPY) antagonistic activity [10]. DHPMs are used as therapeutics agents in the clinical treatment of cardiovascular diseases [11] such as hypertension [12], cardiac arrhythmias and orangina pectoris [13]. The synthetic methodology used to generate DHPMs has been well documented and has typically involved variations of the original Biginelli reaction [14-16]. However, this reaction often requires harsh conditions and long reaction time and affords low yield, particularly when substituted aromatic and aliphatic aldehydes are employed. However, in spite of their potential utility, many of these reported methodologies still have certain limitations such as expensive and air sensitive nature of catalysts, toxicity of solvents, restrictions for large scale applications, critical product isolation procedures, difficulty in recovery of high boiling solvents, excessive amounts of catalysts and generation of large amounts of toxic wastes in scaling up for industrial applications leading to environmental issues.

In view of these reports and in continuation with the previous work, we therefore envisaged that integrating pyrimidine and thiazolidinone moieties in one molecular platform could potentially produce novel compounds with significant synergistic antimicrobial properties. These new pyrimidines analogues bearing thiazolidinone moiety **(5-9)** were prepared to verify their efficacy as antimicrobial agents.

MATERIALS AND METHODS:

All chemicals were purchased from Aldrich or Merck with high-grade quality, and used without further purification. Melting points are uncorrected. IR spectra (KBr) were measured on Shimadzu 440 spectrometer, ^{1}H NMR spectrum were obtained in DMSO-d_6 on a Bruker 500 MHz-Avance III spectrometer using TMS as internal standard, chemical shifts are reported as (ppm). The elemental analyses were carried out at the Chemistry department, Faculty of Science, the University of Jordan, Amman (Jordan).

Synthesis of 2-(2-guanidino-4-oxo-4,5-dihydrothiazol-5-yl)acetic acid 3.

A mixture of 2-mercaptosuccinic acid (100 mmol), dicyandiamide (100 mmol) in glacial acetic acid (30 ml) was heated under reflux for 6 hours. The reaction mixture allowed cooling at room temperature to afford the crude product. The solid product has been recrystallized from ethanol to afford pure needles in 70% yield, mp 230-232°C; IR (cm^{-1}): 3393, 3316, 3220 (NH_2/NH), 2920, 2870 (CH-aliph.), 1720, 1680 (C=O), 1615 (C=N); ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.96 (d, 2H, CH_2), 4.11 (t, 1H, CH), 7.19, 8.17 (2s, 4H, $2NH_2$, exchangeable with D_2O), 12.47 (s, 1H, OH, exchangeable with D_2O); Anal. Calcd. For $C_6H_8N_4O_3S$ (216.22): C, 33.33; H, 3.73; N, 25.91. Found: C, 33.20; H, 3.52; N, 25.68.

General procedure for preparation of compounds (4-8). A mixture of compound **3** (50 mmol) and 10 ml of acetylacetone, ethyl acetoacetate, ethyl benzoylacetate, diethyl malonate or ethyl cyanoacetate, in the presence of a few drops of catalytic glacial acetic acid was heated under reflux for 8–24 h. The resulting solid product was collected by filtration, dried under vacuum, washed with cold ethanol and recrystallized from ethanol in excellent yield.

2-(2-((4,6-dimethylpyrimidin-2-yl)amino)-4-oxo-4,5-dihydro-thiazol-5-yl)acetic acid 4.

This compound was obtained from acetylacetone in 70% yield as yellow crystals reaction time 6 h; m.p.: 261–263°C; IR (cm^{-1}): 3178 (NH), 1718, 1686 (C=O), 1620 (C=N) ; ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.34 (s, 6H, CH_3), 2.90 (d, 2H, CH_2), 4.21 (t, 1H, CH), 6.44 (s, 1H, pyrimidine-H), 8.22 (1s, 1H, NH, exchangeable with D_2O), 12.35 (s, 1H, OH, exchangeable with D_2O); ^{13}CNMR: δ 20.8, 43.3, 55.8, 112.6, 155.1, 157.3, 164.4, 178.3, 188.2; Anal. Calcd. For $C_{11}H_{12}N_4O_3S$ (280.30): C, 47.14; H, 4.32; N, 19.99. Found: C, 46.94; H, 4.16; N, 19.72.

2-(2-((4-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)amino)-4-oxo-4,5-dihydro-thiazol-5-yl)acetic acid 5.

This compound was obtained from ethyl acetoacetate in 75% yield as yellow crystals reaction time 15 h; m.p.: 274–276 °C; IR (cm^{-1}): 3160 (NH), 1732, 1698, 1648 (C=O), 1618 (C=N); ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.09 (s, 6H, CH_3), 2.96 (d, 2H, CH_2), 4.26 (t, 1H, CH), 6.25 (s, 1H, pyrimidine-H), 7.90 (s, 1H, NH, exchangeable with D_2O), 10.43, 12.14 (2s, 2H, 2OH, exchangeable with D_2O); ^{13}CNMR: δ 21.2, 42.7, 56.5, 108.2, 153.6, 156.4, 165.3, 166.2, 177.1, 187.3; Anal. Calcd. For $C_{10}H_{10}N_4O_4S$ (282.27): C, 42.55; H, 3.57; N, 19.85. Found: C, 42.34; H, 3.36; N, 19.63.

2-(4-Oxo-2-((6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)amino)-4,5-dihydro-thiazol-5-yl)acetic acid 6.

This compound was obtained from ethyl benzoylacetate in 68% yield as yellow crystals reaction time 12 h; m.p.: 282–284 °C; IR (cm^{-1}): 3198, 3145 cm^{-1} (NH), 3045 (CH-arom.), 1724, 1685, 1654 (C=O), 1610 (C=N); ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.97 (d, 2H, CH_2), 4.13 (t, 1H, CH), 6.56 (s, 1H, pyrimidine-H), 7.27-7.80 (m, 5H, Ar-H), 8.06 (1s, 1H, NH, exchangeable with D_2O), 12.11, 11.35 (2s, 2H, 2OH, exchangeable with D_2O); ^{13}CNMR: δ 41.6, 58.4, 106.1, 127.4, 128.9, 130.5, 137.0, 153.6, 156.4, 165.3, 168.2, 176.1, 186.7; Anal. Calcd. For $C_{15}H_{12}N_4O_4S$ (344.35): C, 52.32; H, 3.51; N, 16.27. Found: C, 52.21; H, 3.38; N, 16.14.

2-(2-((4,6-Dioxo-1,4,5,6-tetrahydropyrimidin-2-yl)amino)-4-oxo-4,5-dihydro-thiazol-5-yl)acetic acid 7.

This compound was obtained from ethyl acetoacetate in 61% yield as yellow crystals reaction time 15 h; m.p.: 255–257 °C; IR (cm^{-1}): 3420, 3170 cm^{-1} (OH/NH), 1710, 1670 (C=O), 1616 (C=N); ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.93 (d, 2H, CH_2), 4.20 (t, 1H, CH), 6.54 (s, 1H, pyrimidine-H), 8.70 (1s, 1H, NH, exchangeable with D_2O), 12.38, 11.24 (2s, 2H, 2OH, exchangeable with D_2O); ^{13}CNMR: δ 42.4, 55.8, 95.3, 154.4, 158.6, 167.5, 168.2, 176.1, 186.7, 192.3; Anal. Calcd. For $C_9H_8N_4O_5S$ (284.25): C, 38.03; H, 2.84; N, 19.71. Found: C, 37.87; H, 2.56; N, 19.62.

2-(2-((4-Amino-6-oxo-1,6-dihydropyrimidin-2-yl)amino)-4-oxo-4,5-dihydro-thiazol-5-yl)acetic acid 8.

This compound was obtained from ethyl acetoacetate in 69% yield as yellow crystals reaction time 8 h; m.p.: 284–286 °C; IR (cm^{-1}): 3420 (OH), 3345, 3292, 3199 (NH_2/NH), 1715, 1692, 1651 (C=O), 1608 (C=N); ^{1}HNMR (500 MHz, DMSO-d_6): δ 2.95 (d, 2H, CH_2), 4.12 (t, 1H, CH), 6.33 (s, 1H, pyrimidine-H), 6.79 (s, 2H, NH_2, exchangeable with D_2O), 8.23 (1s, 1H, NH, exchangeable with D_2O), 12.41, 11.82 (2s, 2H, 2OH, exchangeable with D_2O); ^{13}CNMR: δ 41.9, 53.5, 90.8, 153.5, 157.3, 160.0, 166.5, 177.2, 189.4; Anal. Calc. For $C_9H_9N_5O_4S$ (283.26): C, 38.16; H, 3.20; N, 24.72. Found: C, 37.95; H, 3.00; N, 24.58.

Antimicrobial activity:

All compounds were dissolved in DMSO. In order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations.

The inhibitory effect of compounds **3, 4-8** on the in vitro growth of broad spectrum of bacteria representing different types of Gram-positive and Gram-negative bacteria, namely *Bacillus subtilis* (ATCC 6635), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923) was evaluated using agar diffusion method (cup and plate method) [17]. DMSO was used as solvent control. All plates were incubated at 37±0.5ºC for 24hrs. The zone of inhibition of compounds was measured using cm scale. The results in Table 1 revealed that most compounds at high concentration (50.000 ppm) showed generally a good inhibitory effect against all types of bacteria. Antifungal activity was also studied by disk diffusion method. For assaying antifungal activity Candida albicans (ATCC 10231), Aspergillus fumigatus were inoculated in Sabouraud Dextrose broth medium (Hi-Media Mumbai) and incubated for 48–72 h at 35ºC, and subsequently, a suspension of about 1.6 X 104-6 X 104 c.f.u./mL was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatmann No. 1 filter paper and sterilized by dry heat at 140ºC for 1 h. Fluconazole (30 mg/mL) was used as positive control while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 48–72 h at 35ºC. The susceptibility was assessed on the basis of diameter of zone of inhibition against albicans and non-albicans strains of fungi. The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition. Compound exhibited the highest fungal inhibitory effect while **7, 8** showed the lowest. Inhibition zones were measured and compared with the controls.

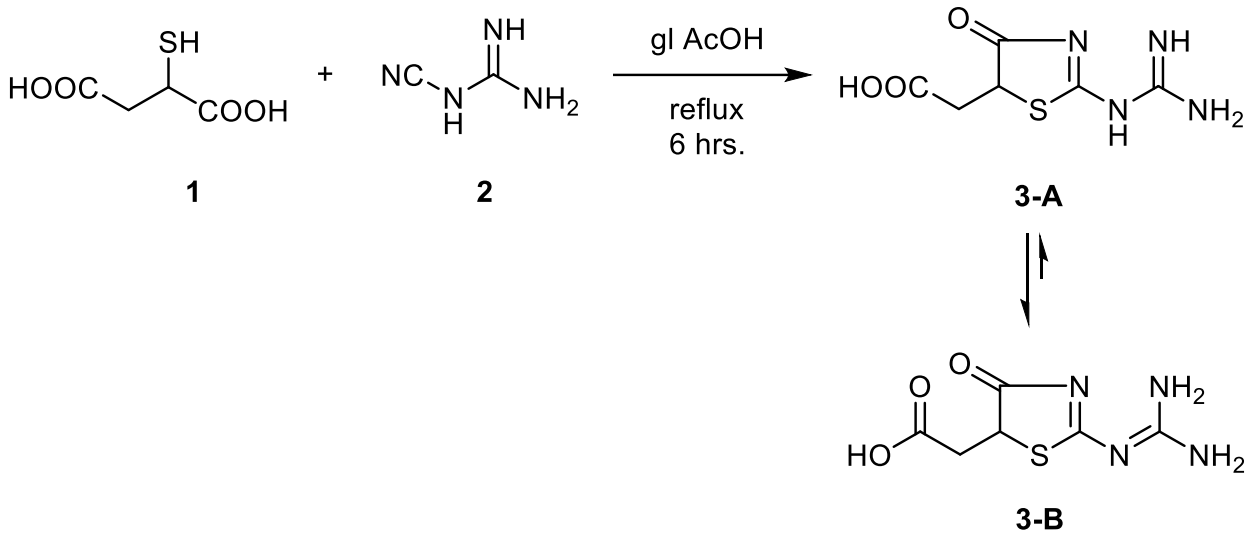
Table 1: Results of anti-bacterial and Anti-fungal evaluation of compounds 3, 5-9.

Types of Bacteria Or fungi	Compound 3			Compound 4			Compound 5		
	Concentrations			Concentrations			Concentrations		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm
Bacillus subtilis (ATCC 6635)	0.9 cm	1.3 cm	1.6 cm	0.5 cm	0.9 cm	1.4 cm	0.7 cm	1.0 cm	1.2 cm
Escherichia coli (ATCC 25922)	0.8 cm	0.9 cm	1.1 cm	0.2 cm	0.5 cm	1.0 cm	0.5 cm	0.6 cm	1.0 cm
Salmonella typhimurium (ATCC 14028)	0.4 cm	0.6 cm	0.9 cm	0.6 cm	1.0 cm	1.5 cm	0.4 cm	0.8 cm	1.3 cm
Staphylococcus aureus (ATCC 25923)	0.3 cm	0.7 cm	1.0 cm	0.1 cm	0.4 cm	0.7 cm	0.5 cm	0.9 cm	1.4 cm
Candida albicans (ATCC 10231)	+	++	+++	+	+	+	+	-	+
Aspergillus fumigatus	+	+	++	+	+	-	+	+	-
Types of Bacteria Or fungi	**Compound 6**			**Compound 7**			**Compound 8**		
	Concentrations			**Concentrations**			**Concentrations**		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm
Bacillus subtilis (ATCC 6635)	0.5 cm	0.8 cm	1.2 cm	0.6 cm	0.9 cm	1.5 cm	0.6 cm	1.0 cm	1.4 cm
Escherichia coli (ATCC 25922)	0.6 cm	0.8 cm	1.3 cm	0.3 cm	0.5 cm	1.2 cm	0.4 cm	0.8 cm	1.1 cm
Salmonella typhimurium (ATCC 14028)	0.5 cm	0.9 cm	1.4 cm	0.5 cm	1.0 cm	1.5 cm	0.3 cm	0.6 cm	1.2 cm
Staphylococcus aureus (ATCC 25923)	0.3 cm	0.7 cm	1.0 cm	0.1 cm	0.4 cm	0.7 cm	0.7 cm	1.1 cm	1.5 cm
Candida albicans (ATCC 10231)	-	-	+	-	-	+	+	-	+
Aspergillus fumigatus	-	+	-	-	+	+	+	-	+

RESULTS AND DISCUSSION:

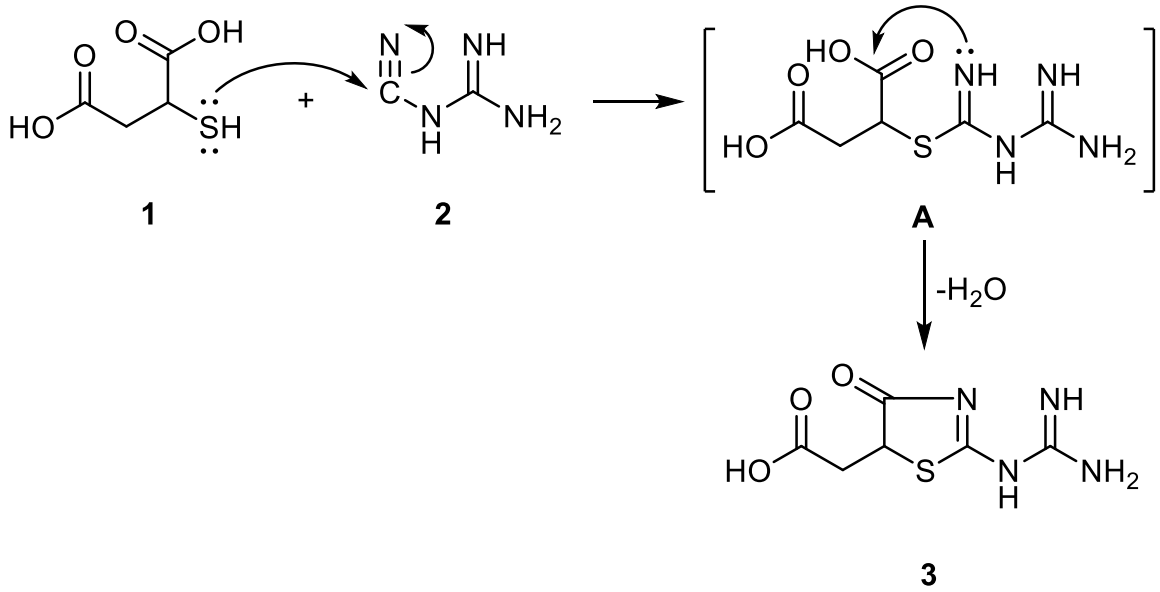
The reaction of mercaptosuccinic acid with cyanoguanidine in glacial acetic acid under reflux afforded the corresponding 2-guanidino-4-thiazolinone **3** (Scheme 1). The molecular structure of compound **3** was elucidated with the help of spectral and elemental analyses. In particular, the reaction product was found to be existing in a single tautomeric form **3-B** rather than **3-A**, as suggested by the ^{1}H-NMR spectral data.

The infrared spectrum of compound **3** exhibited absorption bands corresponding to amino and imino groups at 3393, 3316, 3220 cm^{-1}, aliphatic-CH at 2920, 2870 cm^{-1} and carbonyl groups at 1720, 1680 cm^{-1}. ^{1}H-NMR spectrum of compound **3** showed a singlet signal at 12.47 ppm corresponding to OH group, two singlets at 8.17, 7.19 ppm for two amino groups, triplet and doublet at 4.11 and 2.96 ppm corresponding to methine and methylene protons, respectively.



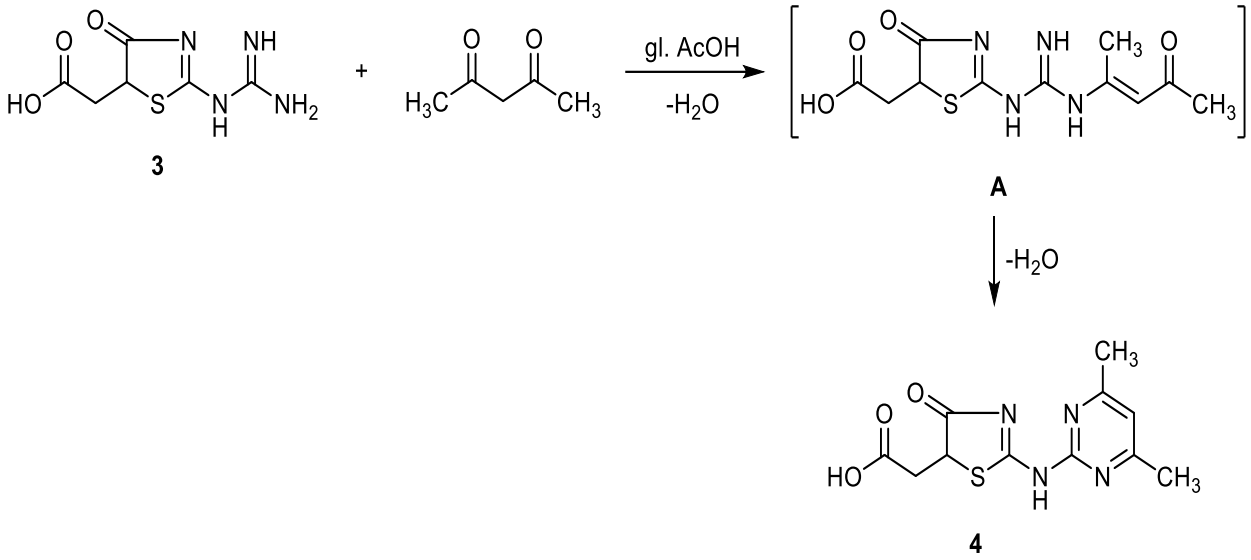
Scheme 1: Synthesizing of 2-(2-guanidino-4-oxo-4,5-dihydrothiazol-5-yl)acetic acid **3**

The formation of compound **3** was assumed to proceed through the formation of intermediacy **(A)** *via* nucleophilic addition of sulfanyl group of mercapto succinic acid **(1)** to the cyano group of dicyandiamide **(2)** followed by intramolecular cyclization *via* loss of water molecule to give the 4-thiazolinone derivative **3** (Scheme 2).



Scheme 2: Proposed Mechanistic route to the formation of 2-(2-guanidino-4-oxo-4,5-dihydrothiazol-5-yl)acetic acid **3**

The guanidine moiety in compound **3** was exploited to synthesize novel pyrimidines by reactions with electrophilic reagents. Thus, when compound **3** reacted with acetylacetone in presence of glacial acetic acid as a benign solvent afforded thiazolyl-pyrimidine derivative **4** (Scheme 3). IR spectrum of compound **4** showed disappearance of absorption bands corresponding to NH_2 groups. Its ^{1}HNMR spectrum showed new signals corresponding to NH group at 8.22 ppm, pyrimidine-H at 6.44 ppm, and two methyl's protons at 2.34 ppm respectively, in addition to protons of hydroxy, methine, and methylene protons. The formation of compound **4** is assumed to proceed via condensation reaction between amino group and acetyl group with elimination of water molecule followed by intramolecular heterocyclization of the non-isolable intermediate **(A)** by loss of water.



3 A 4

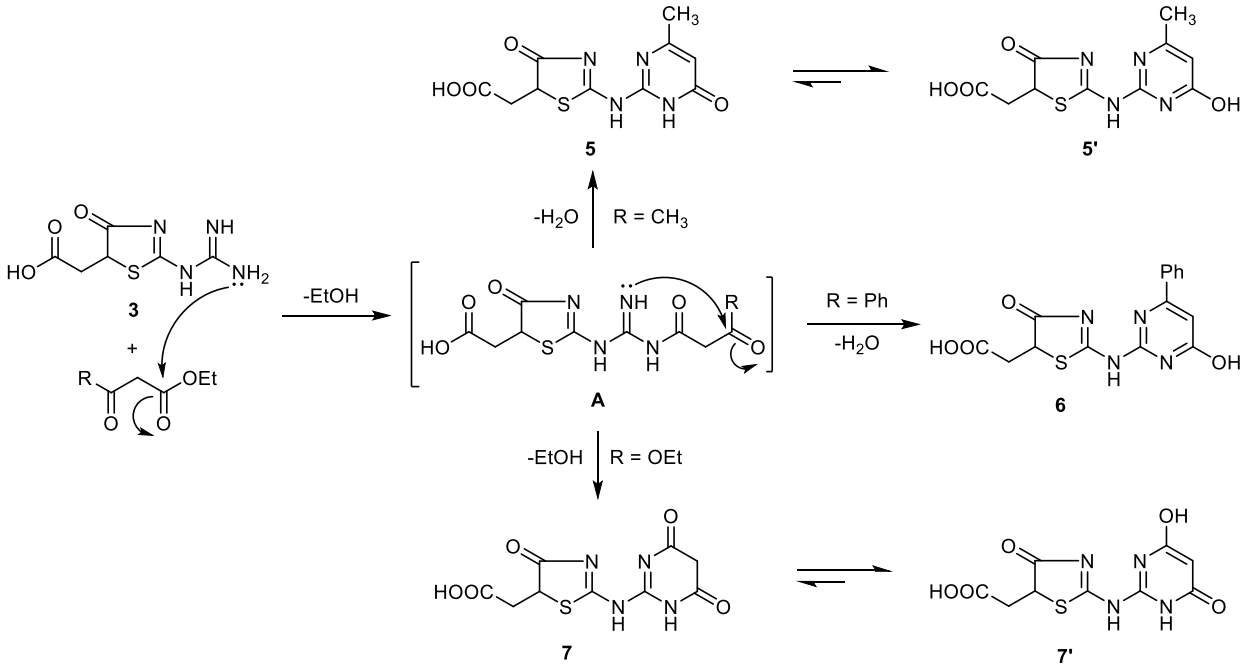
Scheme 3

6-Methyl-2,3-dihydropyrimidin-4(1H)-one derivative **5** was synthesized via reaction of **3** with ethyl acetoacetate in glacial acetic acid at reflux temperature (Scheme 4). IR spectrum of compound **5** showed absorption bands corresponding to NH groups at 3160 cm^{-1} and C=O groups at 1732, 1698, 1648 cm^{-1}. Its ^{1}HNMR spectrum showed five singlet signals at δ 2.09, 6.25, 7.90, 10.43 and 12.14 ppm, attributed to the presence of CH_3, CH (pyrimidine), NH, and hydroxy groups, respectively, in addition to the presence of a triplet signal at δ 4.26, doublet at δ 2.96 ppm for methine and methylene protons.

The formation of compound **5** is assumed to proceed via condensation reaction between amino group and ester group with elimination of ethanol molecule followed by nucleophilic addition of the other amino group on the second acetyl group, followed by loss of water molecule.

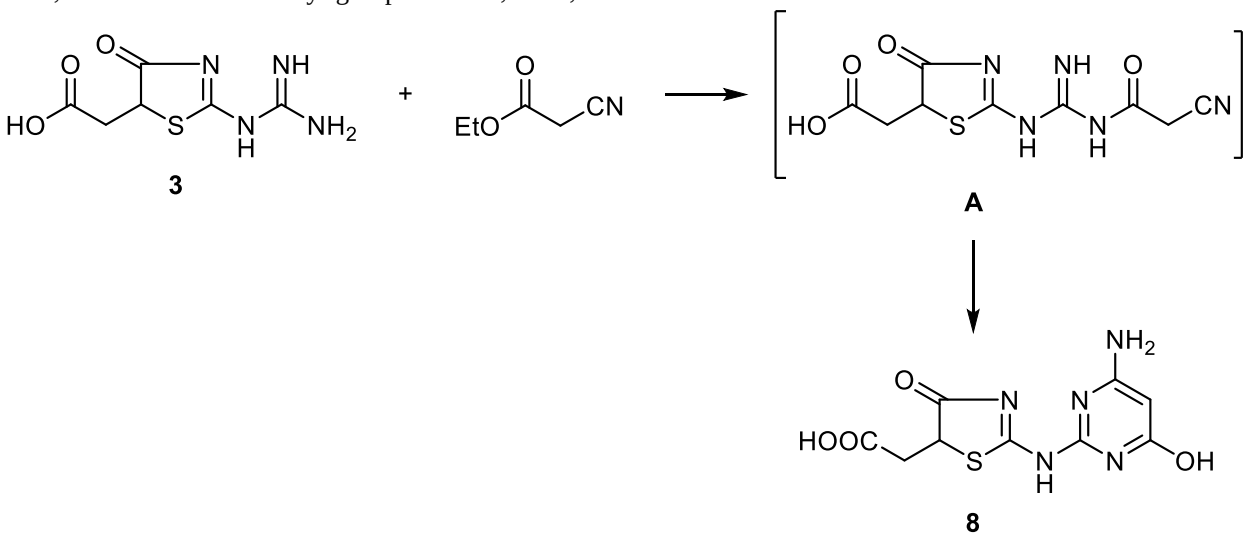
6-Phenyl-2,3-dihydropyrimidin-4(1H)-one derivative **6** was synthesized via reaction of **3** with ethyl benzoylacetate in glacial acetic acid at reflux temperature (Scheme 4). IR spectrum of compound **6** showed absorption bands corresponding to NH groups at 3198, 3145 cm^{-1} and C=O groups at 1724, 1685, 1654 cm^{-1}. The ^{1}HNMR spectrum of this compound revealed a four singlet signals at 12.11, 11.35, 8.06, 6.56 ppm corresponding to two hydroxy, NH groups, pyrimidine-H, in addition to the presence of aromatic protons at 7.27-7.80 ppm, triplet and doublet signals for methine and methylene protons at 4.13 and 2.97 ppm, respectively.

Furthermore, reaction of 2-guanidino-4-thiazole-5-ylacetic acid **3** with diethylmalonate in glacial acetic acid at reflux temperature gave 6-hydroxy-2,3-dihydropyrimidin-4(1H)-one derivative **7** (Scheme 4). IR spectrum of compound **7** showed new absorption bands at 3420, 3170 cm^{-1} for hydroxy and NH groups, 1710, 1670 cm^{-1} corresponding to C=O groups. Their ^{1}HNMR spectrum showed new signals at 12.38, 11.24 ppm corresponding to two hydroxyl groups, 8.70 ppm for NH, 6.54 ppm for pyrimidine-H, in addition to the presence of two signals at 4.20, 2.93 ppm corresponding to methine and methylene protons.



Scheme 4

Finally, reaction of compound **3** with ethyl cyanoacetate gave 2,3-dihydropyrimidin-4(1H)-one derivative **8** (Scheme 5). The infrared spectrum showed no absorption band due to the cyano function group, in addition to the presence of absorption bands for hydroxy, amino and imino groups at 3425, 3345, 3292, 3199 cm^{-1} and carbonyl groups at 1715, 1692, 1651 cm^{-1}. The ^{1}HNMR spectrum of compound **8** displayed two singlet signals at 12.41, 11.82 ppm for two hydroxy groups, 8.23 ppm for NH, 6.79 ppm for amino group, 6.33 ppm for pyrimidine-H, in addition to the presence of signals attributed to methine and methylene protons.



Scheme 5

The formation of compound **8** is assumed to proceed via condensation reaction between amino group and ester group with elimination of ethanol molecule to give the non-isolable intermediate **(A)** followed by nucleophilic addition of the amino group onto the cyano group.

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Conflicts of Interest
The author declares no conflicts of interest.

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