

7-Methyl-3-(2'-oxo-2H-benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one과 그 유도체의 합성

Vinata .V. Mulwad*, Hitesh .T. Parmar, and Abid Ali Mir

Department of Chemistry, The Institute of Science, 15-Madam Cama Road, Mumbai 400 032, India
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Synthesis of 7-Methyl-3-(2'-oxo-2H-benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one and Its Derivatives

Vinata .V. Mulwad*, Hitesh .T. Parmar, and Abid Ali Mir

Department of Chemistry, The Institute of Science, 15-Madam Cama Road, Mumbai 400 032, India

*E-mail: vinata_mulwad@hotmail.com

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INTRODUCTION

Coumarins are the important class of heterocyclic compounds and have gained considerable synthetic and pharmacological interest for a long time because of their various biological activities,¹⁻⁵ such as antihelminthic, anti-HIV activity, antioxidant. Several nitrogen mustards synthesized from 6-amino coumarin are reported as antiviral agents and especially effective against HIV.⁶ Several physiological activities of various thiazole derivatives have proved the efficacy in combating various diseases, and have good antibacterial and antifungal activities,^{7,8} it has been observed that thiazole analogues incorporated with different nuclei have shown variety of pharmacological profiles such as anticancer,⁹ antifungal¹⁰ activities. Although various thiazolopyrimidones are known in literature for their pharmacological activities¹¹ but only few multi step pathway for its preparation are known which tedious and time consuming. Furthermore thiazole and coumarin derivatives with heterocyclic system at position 3 and 6 of benzopyran moiety have shown some promising biological activity.¹² By observing the biological importance of the above heterocycles, we thought to incorporate thiazolopyrimidones moiety on coumarin nucleus and scan for their antimicrobial activity.

In continuation of our earlier work on synthesis of heterocyclic systems derived from 6-amino-coumarin. We here report an alternative synthetic pathway for synthesis of thiazolo-pyrimidinone derivatives under milder condition by cyclocondensation reaction on a pre-existing 6-(2-amino-1,3-thiazole-

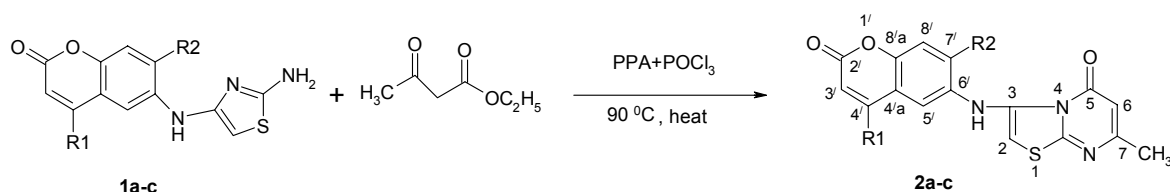
4-yl)amino-coumarin¹³ with EAA in a mixture of POCl₃ and PPA to give 7-methyl-3-(2'-oxo-2H-benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one **2a-c** in one step process Scheme 1.

However ring closure does not proceed in POCl₃ or PPA alone. This mixed reagent had also been used for the preparation several nitrogen bridged systems. Hence by seeing the biological activities of above heterocycles, we here report the synthesis of thiazolo-pyrimidinone derivatives using POCl₃ and PPA as mixed reagent and then screen for their antimicrobial activity.

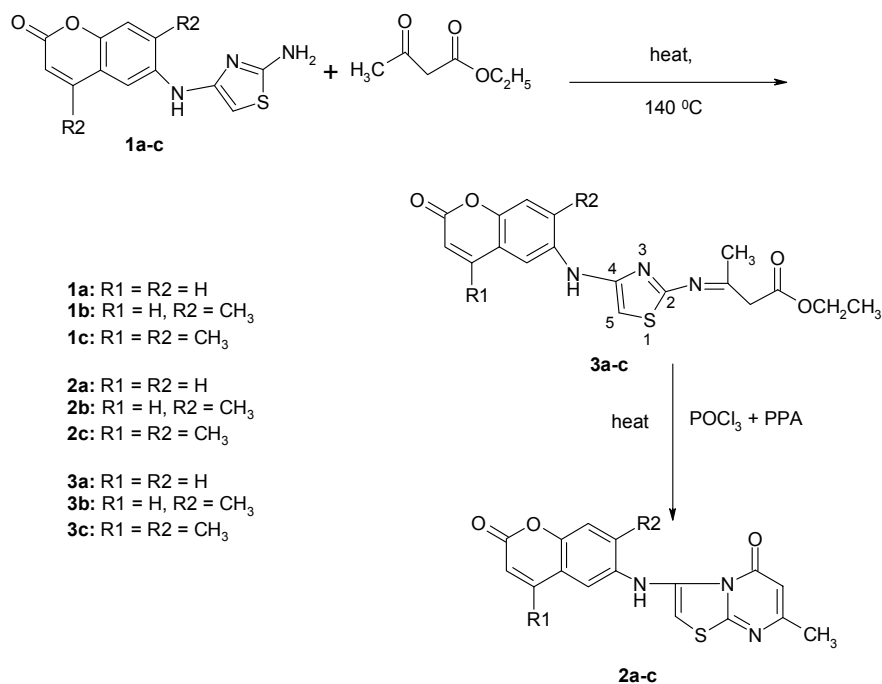
RESULTS AND DISCUSSION

Reaction of 6-(2-Chloro-acetyl)-amino-coumarin with thiourea resulted in the formation of 6-(2-amino-1,3-thiazole-4-yl)amino-coumarin¹³ **1a-c**. Condensation of **1a-c** with EAA gave the corresponding schiffs base 6-[2-(2'-oxo-2H-benzopyran-6'-yl)-1,3-thiazolo-2-yl] imino-butyric acid ethyl ester **3a-c**. The IR spectra of compound **3c** shows prominent peaks at 1618 cm⁻¹ (C=N), 1715 cm⁻¹ (C=O) and 1735 cm⁻¹ (COOEt). In its ¹H NMR it shows signals as triplet for CH₃ protons at δ 1.13 and a quartet at δ 4.20 for methylene protons. It also shows a singlet at δ 2.30 for three protons of -N=C-CH₃ along with the other signals. Compound **3a-c** on cyclization with a mixture of PPA and POCl₃ gave the 7-methyl-3-(2'-oxo-2H-Benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one **2a-c**. This is two step process Scheme 2.

IR spectrum in KBr of compound **2c** shows a peak at 1676



Scheme 1



Scheme 2

cm^{-1} for carbonyl of pyrimidine and a peak at 1721cm^{-1} for carbonyl carbon of coumarin stretch. In its ^1H NMR it shows a singlet at δ 6.28 for one protons of $-\text{CH}=\text{C}$. It also showed absence of signals for methyl protons and methylene protons which were observed in compound **3a-c** as a triplet and quartet indicating the formation of product.

Anti-microbial activity

All compounds have been screened for their antimicrobial activity and have found to exhibit significant biological activity (Table 1). The compounds **2a-c** and **3a-c** were screened for their antibacterial activity against *Bacillus subtilis*, *Escherichia coli*. and antifungal activity against *Candida albicans*, *Aspergillus Niger* by cup plate method at different concentrations (100 and 250 ppm) using DMSO as solvent. The zone of inhibition of the growth was measured in mm. The activity was compared with the standard drugs. A commercial antibacterial Streptomycin (100, 250 $\mu\text{g/mL}$) and antifungal Griseofulvin (100, 250 $\mu\text{g/mL}$) drug was also tested under similar conditions

for comparison.

From the antimicrobial screening of the compounds (**2a-c**), (**3a-c**) it could observe that the pyrimidinones derivatives **2a-c** show significant antimicrobial activity.

EXPERIMENTAL

Melting points were taken in open capillaries and were uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded on Perkin Elmer FTIR and NMR (^1H and ^{13}C) was recorded on Jeol 300 MHz using TMS as standard. Mass spectra (GC-MS) on a Shimadzu GC-MS QP-2010. All products are purified by recrystallisation. The reaction are followed up and purity of the products is carried out on pre-coated TLC plates (Silica gel 60 F₂₅₄, Merck), visualizing the spots in ultraviolet light. All the compounds gave satisfactory elemental analysis.

Preparation of 7-methyl-3-(2'-oxo-2H-Benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one **2a-c** (Scheme 1)

Table 1. Antimicrobial Activities of compound **2a-c** & **3a-c**

Compounds	<i>S. aureus</i>		<i>E. coli</i>		<i>C. albicans</i>		<i>A. niger</i>	
	100 ppm	250 ppm	100 ppm	250 ppm	100 ppm	250 ppm	100 ppm	250 ppm
2a	-	+	-	++	-	+	-	++
2b	+	++	+	++	+	++	+	++
2c	++	+++	+++	+++	+	++	++	+++
3a	-	++	++	++	+	++	+	++
3b	+	++	+	++	+	++	+	++
3c	+	++	++	+++	++	+++	+	+++
sm	+++	+++	+++	+++				
Gf					+++	++++	+++	++++

Sm = Streptomycin, Zone of inhibition diameter in mm: (-) < 8, (+) 8-10, (++) 10-16, (+++) 16-22, (+++++) 22-27. Gf = Griseofulvin, Zone of inhibition diameter in mm: (-) < 7, (+) 7-10, (++) 12-18, (+++) 18-22, (+++++) 22-28.

A mixture 6-(2-amino-1,3-thiazole-4-yl)amino-coumarin **1a-c** (0.01 mol) and ethyl acetoacetate (0.01 mol) was suspended in POCl₃ (0.03 mol) at r.t. To this mixture freshly prepared polyphosphoric acid (0.003 mol) was added. The reaction mixture was refluxed on an oil bath at 90 °C for 3 to 4 hr till HCl evolution subsides. The reaction mixture was cooled and poured into crushed ice, the solid obtained was washed with 5% aqueous NaHCO₃ solution and with water and recrystallized from methanol to give **2a-c**.

(2a): Mol. Formula: C₁₆H₁₁O₃N₃S, m.p 223 °C, Yield 62%, IR (cm⁻¹) 3250 (-NH), 3050 (CH-arom), 1718 (C=O), 1686 (C=O, pyrimidone), 1170 (C-N). ¹H-NMR (CDCl₃): 2.39 (s, 3H, C₇-CH₃), 6.25 (d, 1H, *J* = 9 Hz, C₃'-H), 6.29 (s, 1H, C₆-H), 6.68 (s, 1H, CH-thiazole), 6.92 (d, 1H, *J* = 9.0 Hz, C₇'-H), 7.05 (d, 1H, *J* = 9.0 Hz, C₈'-H), 7.10 (s, 1H, C₅'-H), 7.84 (d, 1H, *J* = 9.0 Hz, C₄'-H), 9.40 (s, 1H, NH, D₂O-exchangable). Elemental analysis [Cal. (Obs.)]: C; 59.07% (59.23%), H; 3.41% (3.48%), N; 12.92% (12.99%), S; 9.86% (9.90%).

(2b): Mol. Formula: C₁₇H₁₃O₃N₃S, m.p 220 °C, Yield 58%, IR (cm⁻¹) 3192 (-NH), 2950 (CH-arom), 1718 (C=O), 1680 (C=O, pyrimidone), 1175 (C-N). ¹H-NMR (CDCl₃): 2.28 (s, 3H, C₇'-CH₃), 2.40 (s, 3H, C₇-CH₃), 6.20 (d, 1H, *J* = 9.3 Hz, C₃'-H), 6.33 (s, 1H, C₆-H), 6.70 (s, 1H, CH-thiazole), 7.08 (s, 1H, C₈-H), 7.15 (s, 1H, C₅-H), 7.80 (d, 1H, *J* = 9.0 Hz, C₄'-H), 9.40 (s, 1H, NH, D₂O-exchangable). Elemental analysis [Cal. (Obs.)]: C; 60.17% (59.33%), H; 3.86% (3.89%), N; 12.38% (12.45%), S; 9.45% (9.37%).

(2c): Mol. Formula: C₁₈H₁₅O₃N₃S, m.p 225 °C, Yield 52%, IR (cm⁻¹) 3230 (-NH), 2945 (CH-arom), 1721 (C=O), 1676 (C=O, pyrimidine), 1172 (C-N). ¹H-NMR (CDCl₃): 2.30 (s, 3H, C₇'-CH₃), 2.36 (s, 3H, C₇-CH₃), 2.45 (s, 3H, C₄'-CH₃), 6.20 (s, 1H, C₃'-H), 6.28 (s, 1H, C₆-H), 6.76 (s, 1H, CH-thiazole), 7.10 (s, 1H, C₈-H), 7.29 (s, 1H, C₅-H), 9.35 (s, 1H, NH, D₂O-exchangable). ¹³C NMR (300 MHz, CDCl₃): δ 16.5 (C₇'-CH₃), 18.9 (C₄'-CH₃), 22.3 (CH₃), 104.2 (C₂), 107.2 (C₆), 112.2-140.5 (7-aromatic-C), 150.3 (C₈'a), 153.0 (C₄'), 155 (C₇) 158.0(CO), 160.8

(C₂'-C=O), 161.0 (S-C=N). MS *m/z* (%) : M⁺ 353, 325, 310, 282, 277, 252 (100), 251, 245, 188, 179, 165, 159, 145, 137, 120, 117, 116, 97, 91, 87, 72, 45. Elemental analysis [Cal. (Obs.)]: C; 61.18% (61.42%), H; 4.28% (4.30%), N; 11.89% (11.75%), S; 9.07% (9.14%).

Preparation of 6-[2-(2'-oxo-2H-benzopyran-6'-yl)-1,3-thiazolo-2-yl]imino-butyrac acid ethyl ester **3a-c**

A mixture 6-(2-amino-1,3-thiazole-4-yl)amino-coumarin **1a-c** (1 mmol) and EAA (5 mL) was taken. The reaction mixture was refluxed on an oil bath for 6 - 8 hr, at 140 °C. The reaction mixture was cooled to r.t, the solid separated was filtered, dried and recrystallised from methanol to give **3a-c**.

(3a): Mol. Formula: C₁₈H₁₇O₄N₃S, m.p 188 °C, Yield 60%, IR (cm⁻¹) 3236 (-NH), 3050 (CH-arom.), 1735 (COOEt), 1718 (CO), 1554 (-C=C), 1610 (-C=N). ¹H-NMR (CDCl₃): 1.14 (t, 3H, CH₂-CH₃), 2.21 (s, 3H, -N=C-CH₃), 4.19 (q, 2H, CH₂-CH₃), 3.50 (s, 2H, CH₂), 6.21 (d, 1H, *J* = 9 Hz, C₃'-H), 6.89-7.20 (m, 4H, Arom-H), 7.80 (d, 1H, *J* = 9 Hz, C₄'-H), 9.50 (s, 1H, NH, D₂O-exchangable). Elemental analysis [Cal. (Obs.)]: C; 58.21% (58.48%), H; 4.61% (4.65%), N; 11.31% (11.39%), S; 8.63% (8.54%).

(3b): Mol. Formula: C₁₉H₁₉O₄N₃S, m.p 198 °C, Yield 56%, IR (cm⁻¹) 3231 (-NH), 3042 (CH-arom.), 1730 (COOEt), 1720 (CO), 1550 (-C=C), 1611 (-C=N). ¹H-NMR (CDCl₃): 1.13 (t, 3H, CH₂-CH₃), 2.28 (s, 3H, -N=C-CH₃), 2.32 (s, 3H, C₇'-CH₃), 4.18 (q, 2H, CH₂-CH₃), 3.45 (s, 2H, CH₂), 6.21 (d, 1H, *J* = 9 Hz, C₃'-H), 6.92-7.21 (m, 3H, Arom-H), 7.82 (d, 1H, *J* = 9 Hz, C₄-H), 9.45 (s, 1H, NH, D₂O-exchangable). Elemental analysis [Cal. (Obs.)]: C; 59.21% (59.50%), H; 4.97% (5.02%), N; 10.90% (10.98%), S; 8.32% (8.25%).

(3c): Mol. Formula: C₂₀H₁₉O₄N₃S, m.p 194 °C, Yield 50%, IR (cm⁻¹) 3231 (-NH), 3042 (CH-arom.), 1735 (COOEt), 1715 (CO), 1550 (-C=C), 1618 (-C=N). ¹H-NMR (CDCl₃): 1.14 (t, 3H, CH₂-CH₃), 2.21 (s, 3H, C₇'-CH₃), 2.30 (s, 3H, -N=C-CH₃), 2.40 (s, 3H, C₄'-CH₃), 4.20 (q, 2H, CH₂-CH₃), 3.45 (s, 2H, CH₂),

6.21 (s, 1H, C₃'-H), 7.02-7.30 (m, 3H, Arom-H), 9.50 (s, 1H, NH, D₂O-exchangable). ¹³C NMR (300 MHz, CDCl₃): δ 14.8 (CH₃ of ethoxy), 17.0 (C₇'-CH₃), 19.0 (C₄'-CH₃), 21.0 (CH₃), 43.0 (CH₂), 60.20 (CH₂ of ethoxy), 104.4 (C₅), 112.2-140.2 (7-aromatic-C), 152.0 (C_{8a}'), 154.0 (C₄'), 160.0 (C₂'-C=O), 161.0 (C=O of ester), 165.0 (CH₃-C=N), 168.0 (C₂). MS *m/z* (%): M⁺ 399, 368, 367, 340, 326, 311(100), 285, 283, 215, 210, 185, 184, 181, 175, 174, 153, 114, 101, 96, 91, 89, 77, 72, 65. Elemental analysis [Cal. (Obs.)]: C; 60.15% (60.34%), H; 5.26% (5.30%), N; 10.52% (10.61%), S; 8.02% (8.15%).

Preparation of 7-methyl-3-(2'-oxo-2H-Benzopyran-6'-yl)-5H-1,4-thiazolo-[3,2-a]pyrimidin-5-one. 2a-c (Scheme 2)

A mixture 6-[2-(2'-oxo-2H-benzopyran-6'-yl)-1,3-thiazolo-2-yl]imino-butyric acid ethyl ester **3a-c** (0.01 mol) was suspended in POCl₃ (0.03 mol) at r.t. To this mixture freshly prepared polyphosphoric acid (0.003 mol) was added. The reaction mixture was refluxed on an oil bath at 90 °C for 3 hr till HCl evolution subsides. The reaction mixture was cooled and poured into crushed ice, the solid obtained was washed with 5% aqueous NaHCO₃ solution and with water and recrystallized from methanol to give **2a-c**.

Antimicrobial activity

The Cup plate method using Hi-Media agar medium was employed to study the antibacterial activity of compounds **2a-c** & **3a-c** against *Bacillus subtilis*, *Escherichia coli*. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure.¹⁴ Sample size for all the compounds was fixed at 0.1 mL. Using a sterilized cork borer cups were scooped out of agar medium contained in a petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cups and the petri dishes were subsequently incubated at 37 °C for 24 h. Streptomycin were used as reference drugs and DMSO as control. Zones of inhibition produced by each compound was measured in mm, and the results are listed in Table 1.

The antifungal activities of compounds **2a-c** and **3a-c** were tested against *Candida albicans*, *Aspergillus niger* by agar diffusion method.¹⁵

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