A SIMPLE SYNTHESIS OF 2-AMINO-4-THIOPHENYL-4H,5H-[1]-BENZO PYRANO[4,3-B]-PYRAN-3-CARBONITRILES AND EVALUATION OF ANTIBACTERIAL ACTIVITY

Mohd. Basheer miya\textsuperscript{a}, Y. Jaya Prakash Rao\textsuperscript{b*} & G.L. David krupadanam\textsuperscript{a}

\textsuperscript{a} Department of Chemistry, Osmania University Hyderabad-500007
\textsuperscript{b} Department of Chemistry, PG College of Science, Saifabad, Osmania University, Hyderabad 500004

Abstract
Synthesis of novel densely functionalized 2-amino-4-thiophenyl-4H,5H-[1]-benzo pyrano[4,3-b]-pyran-3-carbonitriles 3a-e has been accomplished by the Michael addition of malononitrile to the \(\alpha,\beta\)-unsaturated -3-thiophenylidene-4-chromones 2a-e. The antibacterial activity of new compounds evaluated.

Key words: chromonones, Michael addition, 3-thiophenylidene-4-chromonones

Introduction
4-chromonones are versatile intermediates for the synthesis of many natural products such as brazilin, hematoxylin, ripariochromene, clausenin, calanolide(A) and nophyllum (B)\textsuperscript{1,2}. Eucomine, the first member of arylidene chromonone has been isolated from \textit{Eucomis bicolor}\textsuperscript{(3)}. Chromonone heterocycles have also drawn much attention because of their important pharmacological properties\textsuperscript{3}, high activity against anti-human immunodeficiency virus type-1(HIV-1)\textsuperscript{4}. The derivatives of 4-chromonone have activity against trichomands and fungi\textsuperscript{5,6}. 4H,5H-[1]-benzo thiopyrano[4,3-b]-pyran-4-one and 1,4 dihydro-5H-[1] benzothiopyrano[4,3-b]pyridine-4-one derivatives have anti allergic and antifungal activity\textsuperscript{7,8}. In view of pharmacological activity of the chromonone derivatives and arylidene chromon-4-ones, we have synthesized novel 2- amino-4-thiophenyl-4H,5H-[1]-benzopyrano[4,3-b]-pyran-3-carbonitriles by the cyclization of 3- thiophenylidene 4-chromones with malononitrile and all the newly synthesized compounds screened for antibacterial activity.

Results and Discussion
The \(\alpha,\beta\)-unsaturated -3-thiophenylidene-4-chromonones 2a-e containing exocyclic double bonds as dipolarophiles were prepared by the acid catalyzed reactions of various 4-chromonones 1a-e with thiophene-2-aldehyde and the products were assigned E-configuration based on the \(^1\text{H}-\text{nmr}\) data\textsuperscript{9}. In its IR(KBr) spectrum 2a showed the carbonyl peak at 1673 cm\(^{-1}\). In UV (\(\lambda_{\text{max}}\), \(\text{log}\varepsilon\)) peaks are at 208 (4.7), 275 (4.8), 335 (4.9), 372 (4.0) suggest enone chromophore. In the \(^1\text{H}-\text{nmr}\) of 2a the olefinic proton (H-3a) appeared at \(\delta\) 8.00 (d, \(J=1.0\text{Hz}\)) assignable to E-configuration where as Z isomer arylidene proton signal is reported to resonate at \(\delta\) 6.0\textsuperscript{9}. The 2-CH\textsubscript{2} appeared as doublet at \(\delta\) 5.46 (\(J=1.0\text{Hz}\)), the aromatic protons resonated at \(\delta\) 8.05 (d, \(J=9.0\text{ Hz}, \text{H}-5\)), \(\delta\)
6.65 (dd, J=9.0 Hz, 2.5 Hz, H-6), δ 6.40 (d, J=2.5Hz, H-8) the C-7 methoxyl protons appeared as singlet at δ 3.85. The thiophenyl protons appeared as multiplet at δ 7.15-7.60

**Synthesis of 2-amino-4-thiophenyl-4H,5H-[1]-benzopyrano[4,3-b]-pyran-3-carbonitriles 3a-e.**

Treatment of the 3-thiophenylidene-4-chromonone 2a-e with malononitrile in the presence of piperidine afforded 2-amino-4-thiophenyl 4H,5H-[1]-benzo pyrano[4,3-b]-pyran-3-carbonitriles 3a-e (Scheme-1). In its IR (KBr) of 3a showed the cyano and amino peaks at 2200 and 3400cm⁻¹. In the ¹Hnmr of 3a, the NH₂ resonated as broad singlet at δ 5.35, a two proton singlet at δ 4.80 assigned to H-5, while H-4 appeared as a singlet at δ 4.50, H-10 and the aromatic protons appeared at δ 7.52 (d, J=2.5Hz, C7- H ), δ 6.75(dd, J=9.0Hz, 2.5Hz, H9), δ 8.35(d, H, J=9.0Hz, H-10) , and the C-8 methoxy protons appeared at δ 3.85 as singlet. The thiophenyl protons resonated as a multiplet in the region δ 7.10-7.30.

The mechanism for the formation of 3a-e is shown in (Scheme -2), the reaction of malononitrile with 3-thiophenylidene-4-chromonones 2a-e proceed by the Michael addition. Attack of the malononitrile anion at the activated double bond of 2a-e is followed by tandem cyclisation gives the intermediate 4 which tautomeres to substituted 2-amino-4-thiophenyl-4H,5H-[1]-benzo pyrano[4,3-b]-pyran-3-carbonitriles 3a-e.
Antibacterial activity

compounds 2a-e, 3a-e were tested in vitro for their antibacterial activity against Gram-positive bacteria Bacillus subtilis (MTCC 121), Staphylococcus aureus (MTCC 96), and Gram-negative bacteria Escherichia coli (MTCC 739), Pseudomonas aeruginosa (MTCC 2453) by using Dimethylsulphoxide (DMSO) as solvent control. All the compounds were screened at 1mg/mL concentration. Compounds 2a and 3d showed good activity against Gram-negative bacteria only i.e., E. coli (14.4-15.0 mm) and P. aeruginosa (13.5-15.0 mm) respectively. The remaining compounds exhibited moderate to low antibacterial activity against both Gram-positive (8.1-13.1 mm) and Gram-negative (8.6-14.8 mm) bacteria respectively. The results are recorded in Table 1. Streptomycin(16.4-19.6 mm) used as standard for comparison of antibacterial activity.

Table 1. Antibacterial activity of compounds (2a-e & 3a-e).

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>12.2</td>
<td>10.7</td>
<td>14.8</td>
<td>13.6</td>
</tr>
<tr>
<td>2b</td>
<td>10.2</td>
<td>9.6</td>
<td>12.6</td>
<td>9.8</td>
</tr>
<tr>
<td>2c</td>
<td>9.0</td>
<td>8.8</td>
<td>11.1</td>
<td>9.7</td>
</tr>
<tr>
<td>2d</td>
<td>10.7</td>
<td>12.8</td>
<td>11.9</td>
<td>10.6</td>
</tr>
<tr>
<td>2e</td>
<td>11.3</td>
<td>10.9</td>
<td>10.8</td>
<td>9.6</td>
</tr>
<tr>
<td>3a</td>
<td>8.1</td>
<td>-</td>
<td>9.7</td>
<td>11.9</td>
</tr>
<tr>
<td>3b</td>
<td>8.1</td>
<td>9.2</td>
<td>9.6</td>
<td>9.9</td>
</tr>
<tr>
<td>3c</td>
<td>8.8</td>
<td>9.1</td>
<td>12.5</td>
<td>10.3</td>
</tr>
<tr>
<td>3d</td>
<td>-</td>
<td>12.2</td>
<td>10.9</td>
<td>14.4</td>
</tr>
<tr>
<td>3e</td>
<td>9.5</td>
<td>13.1</td>
<td>9.1</td>
<td>8.9</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>16.9</td>
<td>19.6</td>
<td>16.4</td>
<td>16.9</td>
</tr>
</tbody>
</table>

Conclusion

The reaction of 3-thiophenylidene-4-chromonones 2a-e with malononitrile is a new one pot and facile route to the densely functionalized 2-amino-4-thiophenyl-4H,5H-[1]-benzo pyrano[4,3-b]-pyran-3-carbonitriles 3a-e. All the compounds are purified without lengthy chromatographic methods. Except 2a & 3d, all the other compounds have shown low to moderate antibacterial activity.
Experimental Section
All melting points were determined on a Polmon digital melting point apparatus (Model No. MP-96) and are uncorrected. IR spectra were recorded on a Schimadzu 435 instrument using KBr and UV spectra were recorded on a Schimadzu UV-VIS 1601 spectrophotometer. The $^1$H NMR (200 MHz) were recorded on a Bruker Spectrometer in CDCl$_3$ using TMS as internal standard (chemical shifts in δ ppm). EIMS spectra were obtained at 70ev.

Antibacterial study
The antibacterial activity was determined by disc diffusion method. The compounds dissolved in DMSO (1 mg/mL) in which the Watman paper No. 1 paper disks (6 mm diameter) impregnated, dried under sterile flow box and put on an agar plates inoculated on nutrient agar plates previously seeded with test bacteria. The plates were incubated at 37°C for bacteria (18-24 hours) and examined for the zones of inhibition. Streptomycin used as positive and negative control. The organisms B. subtilis MTCC 121, S. aureus MTCC 96, E. coli MTCC 739, P. aeruginosa MTCC 2453 used for antimicrobial assays. The experiment was performed in triplicates.

Compounds 1a-e were prepared by reported procedure.

General Procedure for the synthesis of 3-thiophenylidene -4-chromonones 2a-e: An ice cold solution of 4-chromone 1a-e (10mmol) and thiophene-2-carbaldehyde (10mmol) are taken in acetic acid(30ml) and treated with conc.HCl and set aside for 24hrs at 0°C. The products 2a-e separated from solution were collected and decomposed with cold water and recrystallized from aq.ethanol.

2a: m.p-206°C, yield-92%, IR(KBr) 1673cm$^{-1}$(CO), UV-λmax (logε) 208(4.7), 275(4.8), 335(4.9), 372(4.0); $^1$H NMR(200MHz- CDCl$_3$) δ 3.85(s, OCH$_3$), 5.46, (d, $J=1.0$Hz, 2-OCH$_2$), 6.40(d, $J=2.5$Hz, H-8), 6.65(dd, $J=2.5$Hz, 9.0Hz, H-6), 7.15-7.60(thiophenyl protons), 8.00(d, $J=1.0$Hz, H-3a), IR(KBr) 1673cm$^{-1}$(CO), UV-λmax (logε) 208(4.7), 275(4.8), 335(4.9), 372(4.0); $^1$H NMR(200MHz- CDCl$_3$) δ 3.85(s, OCH$_3$), 5.46, (d, $J=1.0$Hz, 2-OCH$_2$), 6.40(d, $J=2.5$Hz, H-8), 6.65(dd, $J=2.5$Hz, 9.0Hz, H-6), 7.15-7.60(thiophenyl protons), 8.00(d, $J=1.0$Hz, H-3a), 8.05(d, $J=9.0$Hz, H-5); MS: m/z M$^+$ 272 Anal, calcd for C$_{15}$H$_{12}$O$_3$S, C-66.20%, H-4.42% found C-66.17%, H-4.45%.

2b: m.p-150°C, yield-72%, IR(KBr)1675cm$^{-1}$(CO), UV-λmax (logε) 209(4.7), 275(4.8), 336(4.9), 372(4.0); $^1$H NMR (200 MHz CDCl$_3$) δ 5.45 (d, $J=1.0$Hz, 2-OCH$_2$), 6.93(d, $J=9.0$Hz, H-8), 7.40(dd, $J=2.0$Hz, 9.0Hz, H-7), 7.15-7.60(m, thiophenyl protons), 8.00(d, $J=1.0$Hz, H-3a), MS: m/z M$^+$ 276. Anal, calcd for C$_{14}$H$_9$O$_2$SCl, C-68.70%, H-3.70% found C-68.69%, H-3.69%.

2c: m.p-215°C, yield-77% IR(KBr)1674cm$^{-1}$(CO), UV-λmax (logε) 208(4.8), 275(4.9), 335(4.0), 371(4.0); $^1$H NMR (200 MHz CDCl$_3$) δ 5.43 (d, $J=1.0$Hz, 2-OCH$_2$), 6.60(dd, $J=2.5$Hz, 9.0Hz, H-8), 6.75(dd, $J=2.5$Hz, H-7), 7.20-7.50(m, thiophenyl protons) 7.80(d, $J=2.5$Hz, H-5 ), 8.00(d, $J=1.0$Hz, H-3a); MS: m/z M$^+$ 320. Anal, calcd for C$_{14}$H$_9$O$_2$BrS, C-52.34%, H-2.84%, found C-52.33%, H-2.83%.

2d: m.p-250°C, yield-72.5%, IR(KBr)1673cm$^{-1}$(CO), UV-λmax (logε) 207(4.7), 276(4.8), 356(4.9), 372(4.0); $^1$H NMR (200MHz CDCl$_3$) δ 5.43 (d, $J=1.0$ Hz, 2-OCH$_2$), 6.80(d, $J=2.5$Hz, H-8), 6.90(dd, $J=9.0$, 2.5Hz, H-6), 7.10-7.60(m, thiophenyl protons), 7.80( d, $J=1.0$Hz, H- 3a), 7.90(d, $J=9.0$Hz, H-5). MS: m/z M$^+$ 276. Anal,calcd for C$_{14}$H$_9$O$_2$SCl, C-68.69%, H-3.27% found C-68.68%, H-3.26%.
2e: m.p-180°C, yield-70%, IR(KBr) 1674 cm⁻¹(CO), UV-λmax (logs) 210(4.7), 280(4.8), 335(4.7), 374(4.1); ¹H NMR(200 MHz CDCl₃) δ 5.43 (d, J=1.0 Hz, 2-CH₂), 6.91(d, J=2.0 Hz, H-8), 7.41(dd, J=9.0, 2.0 Hz, H-6), 7.15-7.50(m, thiophenyl protons), 7.94(d, J=9.0 Hz, H-5), 7.97 (d, J=1.0 Hz, H-3a). MS: m/z M⁺ 320. Anal, calcd for C₁₄H₉O₂BrS, C-52.34%, H-2.84%, found C-52.33%, H-2.83%

General procedure for the synthesis of 2- amino-4-thiophenyl-4H, 5H-[1]-benzo pyrano[4,3-b]pyran-3-carbonitriles 3a-e
3-thiophenylidene-4-chromonones 2a-e(10 mmol) and malononitrile(10 mmol) were dissolved in ethanol(20 ml) and piperidine(3 ml) was added and the reaction mixture was stirred at room temperature for 24 hrs. The solvent was removed and the products 3a-e were filtered and dried, recrystallized from aq ethanol as yellow crystals.
3a: m.p-225°C, yield-53.2% IR-2200 cm⁻¹(CN), 3400 cm⁻¹ (NH₂); ¹H NMR(200 MHz CDCl₃) δ 3.85(s, OCH₃), 4.50(bs, H-4), 4.80(s, 5-CH₂), 5.35(bs, NH₂), 6.75(dd, J=9.0 Hz, 2.5 Hz, H-9), 7.10-7.30(m, thiophenyl protons), 7.52(d, J=2.5 Hz, H-7), 8.35(d, J=9.0 Hz, H-10). MS: m/z M⁺ 338, Anal, calcd for C₁₈H₁₄O₃N₂S, C-63.92%, H-4.15%, found C-63.90%, H-4.14%. N-8.28%.
3b: m.p-203°C, yield-52% IR-2200 cm⁻¹(CN), 3400 cm⁻¹ (NH₂); ¹H NMR δ 4.30(bs, H-4), 4.76(s, 5-CH₂), 5.25(bs, NH₂), 7.02(d, J=10.0 Hz, H-7), 7.10-7.30(m, thiophenyl protons), 7.64(dd, J=10.0 Hz, 2.5 Hz, H-8), 8.34(d, J=2.5 Hz, H-10). MS: m/z M⁺ 342, Anal calcd for C₁₇H₁₁O₂N₂SCl, C-59.57%, H-3.22%, N-8.18%, found C-59.56%, H-3.21% N-8.17%.
3c: m.p-227°C, yield-64.5% IR-2210 cm⁻¹(CN), 3405 cm⁻¹ (NH₂); ¹H NMR δ 4.50(bs, H-4), 4.85(s, 5-CH₂), 5.34(bs, NH₂), 6.85(dd, J=10.0 Hz, 2.5 Hz, H-8), 7.10-7.30(m, thiophenyl protons) 7.65(d, J=10.0 Hz, H-7), 8.47(d, J=2.5 Hz, H-10). MS: m/z M⁺ 354, Anal calcd for C₁₇H₁₁O₂N₂SBr, C-63.32%, H-3.10%, N-8.12%, found C-63.21%, H-3.09% N-8.10%.
3d: m.p-239°C, yield-55% IR-2200 cm⁻¹(CN), 3400 cm⁻¹ (NH₂); ¹H NMR δ 4.50(bs, H-4), 4.90(s, 5-CH₂), 5.25(bs, NH₂), 6.89(dd, J=10.0 Hz, 2.5 Hz, H-9), 7.10-7.30(m, thiophenyl protons), 7.67(d, J=2.5 Hz, H-7), 8.25(d, J=10.0 Hz, H-10). MS: m/z M⁺ 342, Anal calcd for C₁₇H₁₁O₂N₂SCl, C-59.57%, H-3.23%, N-8.13%, found C-59.56%, H-3.22% N-8.12%.
3e: m.p-211°C, yield-60% IR-2200 cm⁻¹(CN), 3380 cm⁻¹ (NH₂); ¹H NMR δ 4.31(s, H-4), 4.75(s, 5-CH₂), 5.25(bs, NH₂), 6.85(dd, J=10.0 Hz, 2.5 Hz, H-9), 7.10-7.30(m, thiophenyl protons), 7.61(d, J=2.5 Hz, H-7), 8.29(d, J=10.0 Hz, H-10). MS: m/z M⁺ 354, Anal calcd for C₁₇H₁₁O₂N₂SBr, C-63.31%, H-3.9%, N-8.11%, found C-63.22%, H-3.1% N-8.11%

Acknowledgements
One of the authors Basheer miya is thankful to the UGC New Delhi for the award of Junior research fellowship. Authors thank to Munnaswamy, Department of Biochemistry, Osmania University for his cooperation in screening the compounds for antibacterial activity.
References
2. B Chenera, M L West, J A Kinkelestein & G B Dreyer,. *JOC* 1993, **58**, 5605

Received on January 8, 2012.