Synthesis and in vitro antimicrobial evaluation of 2,4-diamino-8,8-dimethyl-6-oxo-5-(heteroaryl)-6,7,8,9-tetrahydro-5H-chromeno[2,3-b] pyridine-3-carbonitrile

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ABSTRACT: Four-component one pot cyclocondensation of heteroaromatic aldehyde1, malononitrile2, 5,5-dimethyl-cyclohexane-1,3-dione (dimedone)3 and 1,4-diazabicyclo [2.2.2] octane4 in aqueous-ethanol after initial Knoevenagel condensation, subsequent Michael and final heterocyclization reactions gave substituted and functionalized pyrano(c)chromene derivatives. These pyrano(c)chromenes on reaction with malononitrile result in the formation of diversely substituted linear tricyclic chromenopyridine5, via cyclocondensation followed by heterocyclization. All the compounds are biologically active against various bacterial and fungal species.

KEYWORDS: Multicomponent reactions, dihydropyrano(c)chromenes DABCO, antimicrobial activity.

Introduction

Multi-component reactions (MCRs) have attracted considerable interest because of their exceptional synthetic and practical efficiency1,2. Polycyclic-ized pyran derivatives are some examples of multi-component synthesis and are found in variety of important natural products including alkaloids, pheromones, carbohydrates and antibiotics3,4. Dihydropyrano(c)chromene structures are of considerable interest as they possess a wide range of biological properties such as spasmylytic, diuretic, anticoagulant, antitumor, antiallergic, and potassium channel activators5-6. In addition, they can be used as cognitive enhancers, for the treatment of neurodegenerative diseases7. The pyridopyrimidines are very popular and widely known compounds as a consequence of their activity against a variety of pathogenic bacteria and potential application as antipyretic, bacteriostatic, sedative and coronary dilating agents5. The chemical transformations of the pyridopyrimidine ring system by the introduction and assemblage of different substituents and heterocyclic rings in fused form have allowed the expansion of research to the structure activity relationship to afford new straight into the molecular interaction at the receptor level. Condensed systems having 1,8-naphthyridine and a pyrimidine nucleus constitutes a group of important compounds because of their vital pharmacological properties8,10.

The above reports stimulated interest to synthesize a series of compounds containing chromeno [2,3-b]pyridine ring system associated with cyano and amino groups to evaluate their antimicrobial activity.

Results and discussion

Chemistry

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in Scheme 1. The key intermediates 5a-e used as starting materials in the synthetic Scheme-2 have been prepared in excellent yields by stirring heteroaryl aldehydes1, malononitrile2, dimedone3 and catalytic amount of DABCO in ethanol-water solvent mixture (1:1) (Scheme 1).

The IR spectrum of compound 6a showed strong absorption bands at 2202, 3355, 3442 cm-1 corresponding to nitrile and amino group respectively and a sharp singlet at δ 4.33 due to the presence of chiral CH in 1H NMR. The reactivity of this compound towards different active methylene compounds was investigated. The treatment of 5a-e with malononitrile in refluxing ethanol-water in the presence of catalytic amount of DABCO gave 2,4-diamino-8,8-dimethyl-5-(2-heteroaryl)-6-oxo-6,7,8,9-tetrahydro-5H-chromeno-[2,3-b]pyridine-3-carbonitrile 6a-e. The structures of the latter products have been established on the basis of their spectral data and elemental analysis. The 1H NMR spectrum of the product 6a shows signals at δ 3.85 and 7.98 for two amino groups. The aromatic protons at δ 6-8 indicated the presence of heteroaromatic ring. The IR spectrum of compound 6a showed strong absorption bands at 3350-3430 cm-1 (NH2), 2202 cm-1 (CN) and 1689 cm-1 (C=O) and the mass spectrum gave molecular ion peak at 350 m/z favoring the assigned structure as 2,4-diamino-8,8-dimethyl-5-(furan-2-yl)-6-oxo-6,7,8,9-tetrahydro-5H-chromeno[2,3-b]pyridine-3-carbonitrile for it.
Scheme 1 DABCO catalyzed synthesis of heteroaryl substituted fused heterocycles.

Table 1 Heteroaryl substituted heterocycles with m.p., yield, mol. formulae and elemental analysis.

<table>
<thead>
<tr>
<th>Product</th>
<th>Ar</th>
<th>Yield %</th>
<th>Mp °C [Ref]</th>
<th>Mol. Formula (Mol. Wt.)</th>
<th>Calc % (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>1a</td>
<td>95</td>
<td>216-218</td>
<td>C_{16}H_{16}N_{2}O_{3} (284)</td>
<td>C67.59(67.88), H5.67(5.56), N 9.85(9.63)</td>
</tr>
<tr>
<td>5b</td>
<td>1b</td>
<td>94</td>
<td>205-207</td>
<td>C_{17}H_{16}N_{2}O_{3} (298)</td>
<td>C68.44(68.40), H6.08(6.10), N9.39(9.41)</td>
</tr>
<tr>
<td>5c</td>
<td>1c</td>
<td>95</td>
<td>208-210</td>
<td>C_{16}H_{16}N_{2}O_{2}S (300)</td>
<td>C63.98(63.92), H5.37(5.34), N9.33(9.34), S10.67(10.69)</td>
</tr>
<tr>
<td>5d</td>
<td>1d</td>
<td>95</td>
<td>214-215</td>
<td>C_{17}H_{18}N_{2}O_{2}S (314)</td>
<td>C64.94(64.91), H5.77(5.74), N8.91(8.89), S10.20(10.24)</td>
</tr>
<tr>
<td>5e</td>
<td>1e</td>
<td>97</td>
<td>217-219</td>
<td>C_{17}H_{18}N_{2}O_{2}S (314)</td>
<td>C64.94(64.92), H5.77(5.75), N8.91(8.86), S10.20(10.26)</td>
</tr>
<tr>
<td>6a</td>
<td>1a</td>
<td>75</td>
<td>152-156</td>
<td>C_{19}H_{18}N_{4}O_{3} (350)</td>
<td>C65.13(65.10), H5.18(5.16), N15.99(5.95)</td>
</tr>
<tr>
<td>6b</td>
<td>1b</td>
<td>78</td>
<td>148-149</td>
<td>C_{20}H_{20}N_{4}O_{3} (364)</td>
<td>C65.92(65.87), H5.53(5.51), N15.38(5.33)</td>
</tr>
<tr>
<td>6c</td>
<td>1c</td>
<td>80</td>
<td>135-137</td>
<td>C_{19}H_{18}N_{4}O_{3} (366)</td>
<td>C62.28(62.25), H4.95(4.91), N15.29(15.34), S8.75(8.70)</td>
</tr>
<tr>
<td>6d</td>
<td>1d</td>
<td>82</td>
<td>144-146</td>
<td>C_{20}H_{20}N_{4}O_{3} (380)</td>
<td>C63.14(63.10), H5.30(5.27), N14.73(4.69), S8.43(8.39)</td>
</tr>
<tr>
<td>6e</td>
<td>1e</td>
<td>79</td>
<td>141-142</td>
<td>C_{20}H_{20}N_{4}O_{3} (380)</td>
<td>C63.14(63.10), H5.30(5.28), N14.73(14.69), S8.43(8.39)</td>
</tr>
</tbody>
</table>
### Table 2  Antimicrobial activity of some synthesized compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in mm (Antibacterial activity)</th>
<th>Compound</th>
<th>Zone of inhibition in mm (Antifungal activity)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>14</td>
<td>14</td>
<td>5a</td>
</tr>
<tr>
<td>5b</td>
<td>15</td>
<td>14</td>
<td>5b</td>
</tr>
<tr>
<td>6a</td>
<td>15</td>
<td>20</td>
<td>6a</td>
</tr>
<tr>
<td>6b</td>
<td>17</td>
<td>19</td>
<td>6b</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26</td>
<td>24</td>
<td>Amphotericin B</td>
</tr>
</tbody>
</table>

### Antimicrobial Evaluation

Most of the prepared compounds were tested for their antimicrobial activity against two types of bacteria, one Gram-positive *Staphylococcus aureus* and one Gram-negative bacterium *Escherichia coli*. The antifungal activity was tested using pathogenic yeast stain *Candida albicans* and *Aspergillus niger*. Compounds 5a, b, and 6a, b, exhibit moderate activity exhibit highest activity against *S. aureus*; the compounds 5a and b, exhibited moderate antibacterial activity and 6a and b 10a exhibited highest activity against *E. coli*. Concerning the antifungal activities, the compounds 5a, b and 6a, b, exhibited moderate activity and 7b, 8a and 10a exhibited highest activity against *A. niger*; the compounds 5a, b and 6a, b, exhibited moderate activity against *C. albicans*.

### Conclusion

In conclusion, we have developed a simple, efficient and improved protocol for the synthesis of biologically active fused heterocycles in presence of DABCO as the catalyst with excellent yields. The simplicity of the system, excellent yields of the products and ease of work-up fulfill the triple bottom line philosophy of green chemistry.

The work reported in this manuscript is yet another modest effort in the field of medicinal chemistry as well as synthetic chemistry and sincerely contribute to a healthier human beings.

### Experimental

#### Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers (Aldrich Chemical Co. and CDH Chemical Co.) and used without further purification. All reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel (60GF-254, Merck) plates and visualized with UV light. ¹H and ¹³C spectra were recorded on a BRUKER instrument 300 MHz and 75 MHz NMR spectrophotometer respectively on δ scale (ppm). LCMS analysis (EI, 70V) were performed on a Hewlett-Packard HP 5971 instrument. IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer. Melting points were determined on electro thermal apparatus by open capillary method and were uncorrected.

#### Synthesis of heteroaryl substituted dihydropyran(o) chromenes (5a-e)

5-Membered heteroaryl aldehyde¹ (10 mmol), malononitrile² (10 mmol), dimedone (10mmol)³ and 5 mole % DABCO⁴ were added to a R.B. flask containing 20 mL EtOH:H₂O (1:1). The reaction mixture was stirred for appropriate time at room temperature. (Table 1) The completion of the reaction was monitored by TLC. The solid obtained was washed with distilled water (3 x 10 mL) for removal of the catalyst. The product was extracted with dichloromethane and filtered; the solvent was evaporated under reduced pressure. The pure product⁵ was obtained by recrystallization from ethanol: water (4:1).

2-Amino-7,7-dimethyl-4-(furan-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5a: White solid, melting point 216°C-218°C, ¹H NMR (300 MHz, DMSO-d₆) : δ 0.99 (s, 3H, CH₃), 2.17 (m, 2H, CH₂), 2.48 (m, 2H, CH₂), 4.33 (s, 1H, chiral-CH), 6.05 (d, 1H, Ar-H), 6.32 (d, 1H, Ar-H), 7.07 (s, 2H), 7.48 (dd, 1H, Ar-H) ppm; ¹³C NMR (CDCl₃, 75 MHz): δ 186.3, 159.4, 155.8, 147.5, 142.5, 118.4, 114.6, 112.2, 63.2, 32.1, 28.5; EI-MS (m/z): 284 (M⁺).

2-Amino-7,7-dimethyl-4-(5-methyl-furan-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5b: Yellow solid, melting point 205°C-207°C, ¹H NMR (300 MHz, DMSO-d₆) : δ 2.17 (s, 3H, Ar-CH₃), 6.32-6.33 (dd, 1H, Ar-H), 6.05 (d, 1H, Ar-H), 7.07 (s, 2H), 7.48 (dd, 1H, Ar-H) ppm; ¹³C NMR (CDCl₃, 75 MHz): δ 186.3, 159.4, 155.8, 147.5, 142.5, 118.4, 114.6, 112.2, 63.2, 32.1, 28.5; EI-MS (m/z): 302 (M⁺).
2-Amino-7,7-dimethyl-4-(thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5c: Yellow solid, melting point 208°C-210°C (CBr): 3342, 3212 (NH2), 2977 (C-H), 2189 (CN), 1679 (C=O), 1661 (C=C) cm\(^{-1}\); \(^1\)HNMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 6.32 (dd, 1H, Ar-H), 6.15 (d, 1H, Ar-H), 6.05 (d, 1H, Ar-H), 7.08 (s, 2H, NH2), 4.33 (s, 1H, CH, chiral-CH), 2.50 (m, 2H, CH\(_2\)), 2.30 (m, 2H, CH\(_2\)), 1.04 (s, 3H, CH3), 0.99 (s, 3H, CH3); \(^13\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 181.9, 158.4, 153.2, 147.5, 142.5, 124.0, 118.9, 116.2, 63.2, 32.1, 28.5; EI-MS (m/z): 314(M\(^+\)).

2-Amino-7,7-dimethyl-4-(3-methyl-thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5d: Yellow solid, melting point 217°C-219°C. IR (KBr): 3386, 3219 cm\(^{-1}\); \(^1\)HNMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 6.32 (dd, 1H, Ar-H), 6.15 (d, 1H, Ar-H), 7.08 (s, 2H, NH2), 4.33 (s, 1H, CH, chiral-CH), 2.50 (s, 2H, CH\(_2\)), 2.35 (s, 2H, CH\(_2\)), 1.04 (s, 3H, CH3), 0.99 (s, 3H, CH3); \(^13\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 186.3, 159.4, 155.8, 149.5, 142.5, 129.4, 121.6, 118.2, 63.2, 32.1, 28.5; EI-MS (m/z): 300(M\(^+\)).

2-Amino-7,7-dimethyl-4-(5-methyl-thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5e: Yellow solid, melting point 214°C-215°C. IR (KBr): 3396, 3209 (NH2), 2966 (C-H), 2196 (CN), 1680 (C=O), 1660 (C=C) cm\(^{-1}\); \(^1\)HNMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 6.29 (s, 3H,CH3), 6.32-6.33 (dd, 1H, Ar-H), 6.05 (d, 1H, Ar-H), 7.08 (s, 2H, NH2), 4.33 (s, 1H, chiral-CH), 2.50 (s, 2H, CH\(_2\)), 2.35 (s, 2H, CH\(_2\)), 1.04 (s, 3H, CH3), 0.99 (s, 3H, CH3); \(^13\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 190.4, 154.9, 152.8, 145.9, 141.7, 125.8, 118.4, 114.6, 64.6, 31.9, 28.1; EI-MS (m/z): 314(M\(^+\)).

Amino-7,7-dimethyl-4-(5-methyl-thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile: 5f: Yellow solid, melting point 217°C-219°C. IR (KBr): 3386, 3219 (NH2), 2976 (C-H), 2189 (CN), 1679 (C=O), 1660 (C=C) cm\(^{-1}\); \(^1\)HNMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 6.29 (s, 3H,CH3), 6.23 (dd, Ar-H), 6.05 (d, Ar-H), 7.08 (s, 2H, NH2), 4.33 (s, chiral-CH), 2.50 (s, 2H, CH\(_2\)), 2.30 (d, 2H, CH\(_2\)), 1.04 (s, 3H, CH3), 0.99 (s, 3H, CH3); \(^13\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 181.9, 158.4, 153.2, 147.5, 142.5, 128.5, 114.9, 112.2, 105.6, 60.8, 28.1; EI-MS (m/z): 366(M\(^+\)).

Synthesis of 2,4-diamino-8,8-dimethyl-6-oxo-5-(heteroaryl)-6,7,8,9-tetrahydro-5H-chromeno[2,3-b]pyridine-3-carbonitrile: 6a-e: A mixture of 5 (2 mmol), malononitrile (2 mmol) in ethanol containing DABCO (5 mole %) was refluxed on water bath for 2 hours. After the reaction was over as monitored on TLC, the reaction mixture was concentrated and left to cool at RT and then poured into ice-cold water. The solid residue was separated by filtration, washed with water, dried and purified by recrystallization from ethanol to give the products 6a-e (Table 1).

2,4-diamino-8,8-dimethyl-6-oxo-5-(furan-2-yl)-6,7,8,9-tetrahydro-5H-chromeno[2,3-b]pyridine-3-carbonitrile: 6a: This compound was obtained from 5a. IR (KBr): 3325-3430 cm\(^{-1}\) (2NH2), 3047 cm\(^{-1}\) (C-H, Aromatic), 2935 cm\(^{-1}\) (C-H, Aliphatic), 2194 cm\(^{-1}\) (C=O), 1674 cm\(^{-1}\) (C=O), 1078 cm\(^{-1}\) (C-O-C), 1605 cm\(^{-1}\) (C=O); \(^1\)HNMR (DMSO-d\(_6\)): \(\delta\) 4.74 (s, 1H, chiral CH), 2.38 (s, 2H, CH\(_2\)), 2.16 (s, 2H, CH\(_2\)), 1.08 (s, 3H, CH3), 0.99 (s, 3H, CH3), 6.05 (d, 1H, Ar-H), 7.48 (dd, 1H, Ar-H), 6.32 (d, 1H, Ar-H), 4.58 (s, 2H, NH2), 7.96 (s, 2H, NH2); \(^13\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 195.3, 169.5, 161.3, 156.3, 151.4, 146.1, 128.5, 114.9, 112.2, 54.2, 26.8; EI-MS (m/z): 350(M\(^+\)).

Antimicrobial Screening

The antimicrobial activity was studied by using the agar plate disc-diffusion method\(^{11,12}\) to assess the activity of the chosen compounds. Sterilized filter paper discs (6 mm in diameter) were wetted with 10 \(\mu\)L each of a solution of the tested compounds (10 mg/mL of the compound in DMSO). The discs were then allowed to dry and placed on the surface of agar plates seeded with the test organism. Nutrient agar was used for bacterial plating and Sabouraud’s dextrose agar for fungi. For conditions of cultivation, petridishes were poured with 15 mL of agar medium and then incubated at 37°C overnight. The test organism were grown in liquid medium for approximately 18 to 24 hours for bacteria and fungi respectively and then poured on preincubated petridishes containing medium. The test organism was evenly spread over the surface with the help of a sterilized glass spreader. After 15 minutes the filter paper disc presoaked with test compounds (10 mg/mL of the compound in DMSO) were tested on the agar plates in mm. The screening results are summarized in Table 2. The compounds showed mild inhibition against Gram-positive and Gram-negative bacteria. Ciprofloxacin and Amphotericin B were used as standard antibiotics in this study.
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References