SYNTHESIS AND BIOASSAY OF SOME BENZOPYRANO [2, 3-B] PYRIDINE DERIVATIVES

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Abstract

One 2-aminopyridine derivative 7 and three nitrogen analogues of xanthones (1-azaxanthones) 8-10 have been
synthesized from 3-formylchromone (2) and 3-cyanochromone (5), respectively. The prepared compounds
have been screened for activity against Biomphalaria glabrata, miracidia of Schistosoma haematobium and
cercariae of Shistosoma mansoni.

1. Introduction

The chemistry of 4-oxo-4H-benzopyrone, commonly
called chromone, continues to be of interest to a
number of investigators. Some of the attractions to
this class of heterocyclic compounds include its
natural occurrence (Ellis, 1977), wide range of
biological activities (Hutter and Dale, 1951; Devi et
al., 1988; Achaiah and Reddy, 1991; Lacova et al.,
1995; El-Shaer et al., 1998) and interesting
synthetic possibilities (Nohara et al., 1977; Fitton et
al., 1979; Kubicova et al., 2002; Lacova et al., 1998).
Khellin 1 (a furanochromone), an active ingredient
from the extract of the fruit of a Middle Eastern plant
of the Umbelliferae family, Ammi visnaga, has been
in use for centuries in folk medicine and has been
attributed with many therapeutic properties,
including its use as an antispasmodic in asthma and
a coronary vasodilator in Angina pectoris (Hutter
and Dale, 1951). The discovery by Nohara and co-
workers that a carbonyl group (C=O) at C-3 position
enhanced the anti-allergic activities of chromones
led to intensive investigations of 3-substituted
chromones (Nohara et al., 1977). 3-
Formylchromones 2 and/or some of their
condensation products with primary amines have
been reported to exhibit antibacterial and antifungal
activities (Achaiah and Reddy, 1991; El-Shaer et
al., 1998), antimycobacterial activity (Lacova et al.,
1995; El-Shaer et al., 1998), anti-inflammatory and
analgic properties (Devi et al., 1988, 1988),
antiallergic (Nohara et al., 1974, 1977 Achaiah et
al., 1991).

In general, 3-acylchromones 2 are versatile synths
in heterocyclic chemistry (Fitton et al., 1979; Lacova
et al., 1998; Kubicova et al., 2002). They react easily
with nucleophiles, with C-2 being the preferred site
of attack. The C-2, C-4 and HC=O positions are
electron-deficient, hence 3-formylchromones react
smoothly with nucleophiles under mild conditions
(Fitton et al., 1979; Lacova et al., 1998)
The initial product 3 may form ‘ring-opened’
products 4 which may react further to form new
heterocyclic compounds. With diamines, 2 gives
pyrimidines. On the other hand, 2 and its derivatives
react with active methylene compounds, like malonic
acid derivatives and enaminoketones, to give
condensation products and benzopyrano-pyridines
(Elden, 1981), commonly known as 1-azaxanthones
(e.g. 8). Such compounds have not been studied as
potential drugs against infestations with
schistosomes. Only lucanthone, a thioxanthone has
been studied as an agent against S. mansoni
infestations (Davis et al., 1965).

This paper describes the reaction of 3-
formylchromone 2 and 3-cyanochromone 5 with
some active methylene compounds and the
investigation of the activity of the reaction products
as molluscicidal and antischistosomal agents.

2. Materials and Methods

(a) Bioassay Procedures

Biomphalaria glabrata were reared as described by
Madsen (Madsen, 1984). Molluscicidal tests were
carried out, according to the WHO provisional plan
of 1961 (WHO 1961; Adewunmi et al., 1987). Eggs
of Schistosoma haematobium, recovered from urine of
infected school children by standard parasitological
techniques, were hatched by exposing them to bright light for 60 min. Five miracidia
recovered from hatched eggs were used for each
collection of the synthetic chemicals. The activity

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of the miracidia was monitored at 1 h and 2 h exposure to the chemicals. *Schistosoma mansoni* cercariae were harvested from *B. pfeifferi* by standard techniques. Groups of 5 cercariae were exposed to 100 ppm of the compounds for 2 h (Adewunmi and Furu, 1989).

**b) Synthesis**

All melting points were determined with open capillary tubes, on a Gallenkamp (variable heater) melting point apparatus and are uncorrected. UV spectra were run in methanol solution on an SP8-400 uv/vis spectrometer (λ max in nm (ε)). IR spectra were recorded on a Perkin-Elmer 727B machine (in KBr or nujol, ν max in cm⁻¹). ¹H- and ¹³C-NMR were recorded in CDCl₃ or DMSO-d₆ solutions on a Varian FT 80 NMR spectrometer (δ in ppm relative to Me₄Si) (at the Polytechnic of North London, UK).

**4-Oxo-4H-chromene-3-carbonitrile: (3-cyanochromone), 5.**

Hydroxylamine hydrochloride (3.4 g, 48.9 mmol) and hydrochloric acid (8.0 mL) were added to a solution of 3-formylchromone (8.5 g, 48.9 mmol) in 90 % ethanol (40 mL) and then refluxed for 12 h. The reaction mixture was cooled and the resulting yellow precipitate filtered and recrystallised from ethanol to give 5, (8.1 g, 97 %), mp 158-60 °C. (Found: C, 70.10; H, 2.90; N, 8.28; C₁₀H₅NO₂ calculated: C, 70.18; H, 2.94; N, 8.18). IR (cm⁻¹): 3030, 1650 (C=O), 2210 (CN), 1140. λ max (nm), (ε): 286 (20,000), 240 (48,800). ¹H nmr (d): 9.10 (s, 1H, H-2), 8.25-7.50 (m, 4H, Ar).

Reaction of 3-Formylchromone with Malononitrile to give [(4-Oxo-4H-chromen-3-yl) methylene] malononitrile, 6

Malononitrile (0.19 g, 2.9 mmol), containing 2 drops of pyridine, was added to 3-formylchromone 2 (0.5 g, 2.9 mmol) in ethanol (20 mL) and the mixture refluxed for 4 h. Cooling of the reaction mixture gave a red precipitate which was filtered and recrystallised from ethanol to give 6 (0.3 g, 47 %), mp (dec) >290 °C. (Found: C, 70.27; H, 2.60; N, 12.68; C₁₂H₁₂N₂O₂ calculated: C, 70.27; H, 2.27; N, 12.61). IR (cm⁻¹): 3030, 2220 (CN), 1700 (C=O), 1602, 1070: Imax (nm) (ε): 340 (5000), 286 (5500), 240 (7000).

**2-Amino-5-(2-hydroxybenzoyl)nicotinonitrile, 7**

A mixture of malononitrile (0.38 g, 5.8 mmol) and ammonium acetate (0.44 g, 5.8 mmol) was added to a solution of 3-formylchromone (1.0 g, 5.8 mmol) in ethanol (20 mL), followed by refluxing for 4 h. The reaction mixture was cooled and the resulting solid filtered and then recrystallised from ethanol to give light brown crystals of 7 (0.75 g, 55 %), mp (dec.) 207-210 °C. Found: C, 65.30; H, 3.84; N, 17.50; C₁₅H₁₅N₂O₂ calculated: C, 65.27; H, 3.79; N,
17.56). IR (cm⁻¹): 3380, 3290, 3200 (OH, NH₂), 3030, 2220 (CN), 1660 (C=O), 1110. Imax (nm) (e): 328 (12200), 232 (13200). 1H nmr (d, DMSO-d6): 10.10 (br s, 1H, OH, D₂O exchangeable), 8.45 (d, 1H, H-4); 8.10 (d, 1H, H-6); 7.50 (br s, 2H, NH₂, D₂O exchangeable); 7.41-6.50 (m, 4H, Ar).

2-Amino-5-oxo-5H-chromeno[2, 3-b]pyridine-3-carboximide, 8
Compound 8 was prepared as described for compound 6, from the reaction of 3-cyanochromone with malononitrile (5.9 mmol each) containing 2 drops of pyridine. It was obtained as red crystals, yield = 0.47 g, 34%, mp (dec.) 225-227 °C (Found: C, 65.60; H, 2.90; N, 17.95; C₁₉H₁₁N₂O calculated: C, 65.82; H, 2.97; N, 17.71). IR (cm⁻¹): 3380, 3140 (NH₂), 3030, 2220 (CN), 1650 (C=O), 1120. Imax (nm) (e): 344 (4800), 310 (3000), 280 (3500), 240 (9400). 1H nmr (d, DMSO-d6): 7.71-7.20 (m, 5H, Ar), 6.90 (br s, 2H, NH₂, D₂O exchangeable).

3-Acetyl-2-methyl-5H-chromeno[2, 3-b]pyridin-3-one, 9
Compound 9 was obtained as brown crystals, from the reaction of 5 with acetylacetone using pyridine as base, as described above. It was recrystallized from chloroform-toluene (1:9) to give the pure crystals of 9, mp 210-212 °C. (Found: C, 71.10; H, 4.11; N, 5.72; C₁₀H₉NO calculated: C, 71.14; H, 4.38; N, 5.53). IR (cm⁻¹): 3030, 1670 (ArCO), 1650 (pyrone C=O), 1570, 1130. Imax (nm) (e): 322 (20000), 239 (70400). 1H nmr (d, DMSO-d6): 8.90 (s, 1H, H-4); 8.20-7.25 (m, 4H, Ar); 2.65 (s, 3H, CH₃); 2.64 (s, 3H, CH₃).

6H-Chromeno[3', 2': 5, 6]pyrido[2, 3-d]pyrimidine-2, 4, 6(1H, 3H)-trione, 10
3-Cyanochrome 5 (10.0 g, 58 mmole) was dissolved in ethanol (20 mL). Barbituric acid (5.90 g, 46 mmole) and pyridine (3 drops) were added and the resulting mixture refluxed for 6 h. Cooling gave orange precipitate of 10. This was recrystallised from ethanol to give 8.5 g (66%). Mp > 320 °C. (Found: C, 59.58; H, 2.69; N, 14.75. C₁₂H₁₁N₂O calculated: C, 59.80; H, 2.51; N, 14.94). IR (cm⁻¹): 3400, 3030, 1680, 1650, 1590, 1110. Imax (nm) (e): 342 (16800), 241 (44000). 1H nmr (CF, COOD/DMSO-d6): 11.40, 11.31 (br, s, 2 NH, D₂O exchangeable), 8.68 (s, 1H), 7.90-7.28 (m, 4H, Ar).

3. Results and Discussion
(a) Chemistry
3-Formylchromone (2) was prepared according to a literature procedure, via Vielsmeier-Haack double acylation (Nohara et al., 1974). The reactions of 2 with malononitrile in the presence of few drops of pyridine in ethanol gave the dicyanomethylidene derivative 6 as red crystals (scheme 2). When the same reaction was carried out using ammonium acetate (one mole equivalent) as base instead of pyridine, a 'ring-opened' pyridine derivative 7 was formed, similar to the observation of Nohara et al. (Nohara et al., 1974). The reaction, most probably, initially takes place by condensation of malononitrile with the formyl group, followed by attack of NH₃ (from NH₃OCHO) on the C-2 atom of the pyrone ring with opening of the ring (Scheme 3).

The 1H nmr spectrum of 7 showed the OH proton as broad singlet at δ 10.10 ppm and the NH₂ protons at δ 7.50 ppm, both exchangeable with D₂O. The 13C nmr spectrum is readily assigned and agree well with the assigned structure.

Compound 2 was converted to the corresponding 3-cyanochrome 5 following a literature procedure, by refluxing 2 with hydroxylamine-hydrochloride in ethanol containing HCl (Nohara et al., 1977). 5 condenses with the appropriate active methylene compounds (malononitrile, acetylaceton and barbituric acid) in ethanol, in the presence of pyridine or pyridine, to give 5-oxo-5H[1]benzopyran[2,3-b]pyridine derivatives (5-oxo-5H-chromeno[2,3-b]pyridines) 8, 9 and 10, respectively. The products are presumably formed via a derivative of 2-amino-3-formylchromone 11. Compound 5 is known to react in an aqueous or slightly basic medium in the form of 11 (Ishiguro et al., 1981). The reactive methylene compounds (e.g. acetylaceton) undergo Michael addition to the a,b-unsaturated ketone with concomitant ring-opening of the pyrone heterocycle to give the intermediate 2-hydroxybenzoyl alkene derivative 12, followed by a double cyclization to give the benzopyranopyridine derivatives, as shown in scheme 4.

The structures of the synthesized compounds were assigned by their spectral and elemental analyses. The infrared spectra of the compounds showed absorption bands for the CN, NH₂, CONH and CO groups at the expected regions.

Table 1: Molluscidal action of 7, 8, 9, & 10 on Biomphalaria glabrata at a concentration of 100 ppm

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Activity (%) Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: Effect of compounds 7, 8, 9, and 10 on miracidia of S. haematobium and cercariae of S. mansoni at a concentration of 100 ppm

<table>
<thead>
<tr>
<th>Compound No</th>
<th>S. haematobium miracidia (%) Mortality</th>
<th>S. mansoni cercariae (% mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>1h</td>
<td>1h</td>
</tr>
<tr>
<td>7</td>
<td>60%</td>
<td>80%</td>
</tr>
<tr>
<td>8</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>9</td>
<td>0%</td>
<td>40%</td>
</tr>
<tr>
<td>10</td>
<td>0%</td>
<td>80%</td>
</tr>
</tbody>
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Scheme 2

Scheme 3
(b) Biological activity
The results of the molluscidal assays are shown in Table 1 while the schistosomicidal results on the miracidia of *S. haematobium* and cercariae of *S. mansoni* are shown in Table 2. Compound 10 showed weak activity while the others, 7, 8, and 9, have no activity. In contrast to their weak or non-molluscidal potential, compounds 7 and 10 exhibited strong miracidial activity, while the cercarialic activity of the compounds are very impressive.

REFERENCES
Obafemi et al.: Synthesis and bioassay of some pyridine derivatives
