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Novel *N*-chlorinated derivatives of 2*H*-1-benzopyran-2-imines

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Abstract—A series of new *N*-chlorobenzopyran-2-imines were prepared in moderate to good yields, by reacting sodium hypochlorite in an acidic medium with benzopyran-2-imines obtained via the Knoevenagel condensation. Some of the *N*-chlorobenzopyran-2-imines obtained are potential antiproliferating agents.

Coumarins (2*H*-1-benzopyran-2-ones) have been extensively investigated regarding their synthesis, their isolation from natural sources, and their potential broad spectrum biological activity, e.g. antibacterial,[1] antiviral,[2] and antitumour[3] activity. They have also found application as photosensitisers, fluorescent and laser dyes.[4–6] The 2-imino analogues are less known, but comprise a very important class of protein tyrosine kinase (PTK) inhibitors. Thus they are most valuable for the treatment of diseases involving excess cell proliferation.[7] Antitumour activity against *Ehrlich ascites* tumour has also been shown.[8] Some 2-imino derivatives were also studied as potential laser dyes.[9]

N-Haloamino and *N*-haloimino functional groups have considerable value in synthetic organic chemistry and in analytical chemistry. *N*-Chloroimines can be used for the preparation of amines (e.g. vinylglycine,[10] α-aminoketones[11]), hardly accessible benzimidazoles[12] and imidazoles, and various antihistaminic compounds and fungicides.[13,14] 2,6-Dichlorobenzoquinone-*N*-chloroimine and 2,6-dibromobenzoquinone-*N*-chloroimine[15] are used for the colorimetric detection of phenols, thioketones, sulphur-containing pesticides, alkyl- and aryl-heterocyclic mercapto compounds, anilines, adrenalines and noradrenalines.[16]

N-Halo derivatives of amines and imines are commonly prepared by reacting the amine or imine with an active halogen reagent, e.g. sodium hypochlorite, *t*-BuOCl, *N*-chlorosuccinimide, hexachlorocyclohexadienone or via gas-phase reaction with molecular halogen.[13,17–19] *N*-Chloroimine derivatives can also be prepared by base-prompted elimination of hydrochloric acid from *N*,*N*-dichloroamines.[14]

In the present paper we describe the synthesis of several benzopyran-2-imines followed by their transformation into the *N*-chloro derivatives. To the best of our knowledge *N*-halo derivatives of 2*H*-1-benzopyran-2-imines were unknown. We expected that *N*-chlorination would enhance the reactivity of the imino group, thus facilitating covalent bond formation with a protein substrate. A conjugate with an imino spacer would be formed. If the new compounds show high fluorescence activity they might find use as protein labelling reagents.

Using the Knoevenagel protocol, benzopyran-2-imines **4** or **8** were obtained from a one-pot reaction from 2-hydroxybenzaldehyde derivatives **1** (1 mmol) and active methylene compounds **2** or **5** (1 mmol) (e.g. malononitrile **2** or ethyl cyanoacetate **5**). Catalytic amounts of piperidine at room temperature caused condensation to the styryl intermediates **3**, **6** and **7** (Scheme 1). Benzopyran-2-imines were formed by spontaneous cyclisation between the *ortho* hydroxy group and the side-chain cyano group in moderate to good yields (Table 1) (recrystallised from ethanol). An interesting result is that the reactions between the hydroxybenzaldehyde derivatives **1** and ethyl cyanoacetate **5** gave the benzopyran-2-imines **8** with only minor quantities of the benzopyran-2-ones **9**. The cyclisation in this case preferentially occurred by condensation of the *ortho* hydroxy group with the nitrile group. This sug-

Keywords: *N*-chlorobenzopyran-2-imines; benzopyran-2-imines; *N*-chlorination; sodium hypochlorite; Knoevenagel condensation; antiproliferating agents; fluorophores.

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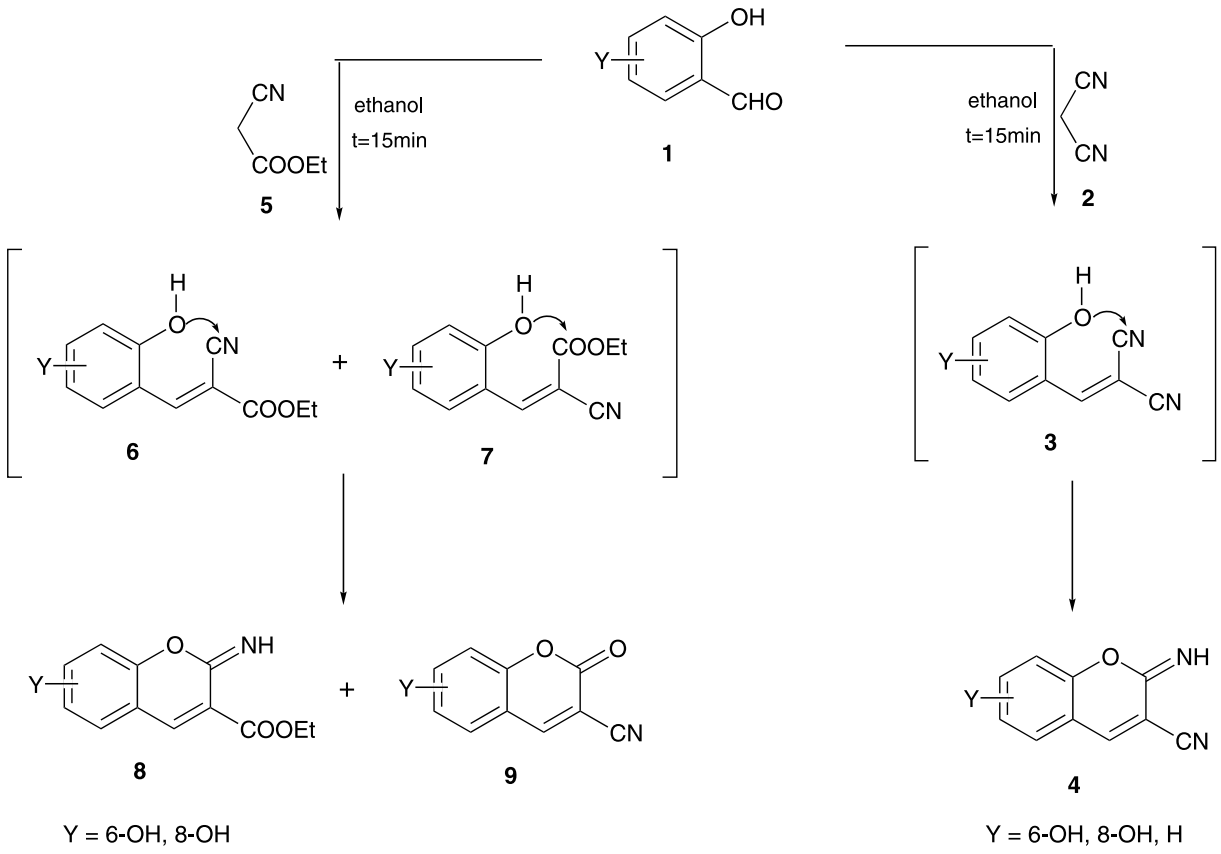


Scheme 1.

gests that the Knoevenagel reaction in these case is stereoselective yielding *E*-styryl derivatives **6** as the main intermediates in which the aromatic ring and the cyano group are in the *cis* configuration, thus facilitating selective cyclisation. Our results are in accordance with literature data.[20] The structure of the products was confirmed by spectroscopic methods and in one case **8b** also by X-ray analysis (Fig. 1).[21]

The 6-hydroxylated compounds **4a** and **8a** revealed a high 'Green-Yellow' fluorescence efficiency (absorption between 390–410 nm and emission between 480–500 nm). The 8-hydroxylated benzopyran-2-imines **4b** and **8b** and the nonhydroxylated iminocoumarin **4c** were photophysically inert.

The reaction of the benzopyran-2-imines **4** or **8** with sodium hypochlorite was performed at 0°C in acetonitrile under acidic reaction conditions (pH <6). Within a few minutes the *N*-chlorination reaction was complete (TLC) and the *N*-chlorobenzopyran-2-imines **10** were precipitated upon dropwise addition of cold water to the reaction mixture. Purification was accomplished by column chromatography (methanol/dichloromethane, 1:100). The average to low yields obtained, are partly due to competing electrophilic aromatic chlorination. Indeed, besides the expected products, aromatic chlorinated derivatives **11a** (X = CN, Y = 6-OH) and **11b** (X = COOEt, Y = 6-OH) were also isolated (Scheme 2, Table 2).

Table 1. Melting points and yields of benzopyran-2-imines **5**

Benzopyran-2-imine	X	Y	Mp (°C)	Yield (%)
4a	CN	6-OH	>250	98
4b	CN	8-OH	>250	47
4c	CN	H	140–141	61
8a	COOEt	6-OH	240–241	78
8b	COOEt	8-OH	225–226	81

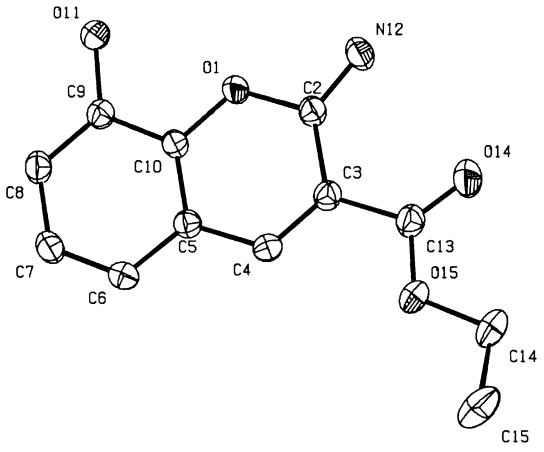
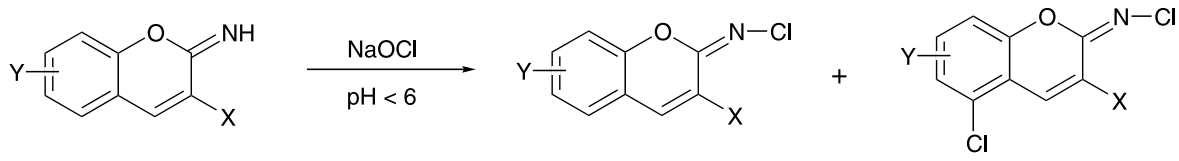


Figure 1. Molecular structure view of **8b** (30% probability).



4a : X = CN, Y = 6-OH
4b : X = CN, Y = 8-OH
4c : X = CN, Y = H
8a : X = COOEt, Y = 6-OH
8b : X = COOEt, Y = 8-OH

10a : X = CN, Y = 6-OH
10b : X = CN, Y = 8-OH
10c : X = CN, Y = H
10d : X = COOEt, Y = 6-OH
10e : X = COOEt, Y = 8-OH

11a : X = CN, Y = 6-OH
11b : X = COOEt, Y = 6-OH

Scheme 2.

Table 2. Melting points and yields of *N*-chlorobenzopyran-2-imines **7** and **8**

N-Chloro-benzopyran-2-imine	X	Y	Mp (°C)	Yield (%)
10a	CN	6-OH	209–210	41
10b	CN	8-OH	210–211	52
10c	CN	H	191–192	40
10d	COOEt	6-OH	176–177	26
10e	COOEt	8-OH	80–81	48
11a	CN	6-OH	204–205	11
11b	COOEt	6-OH	152–153	13

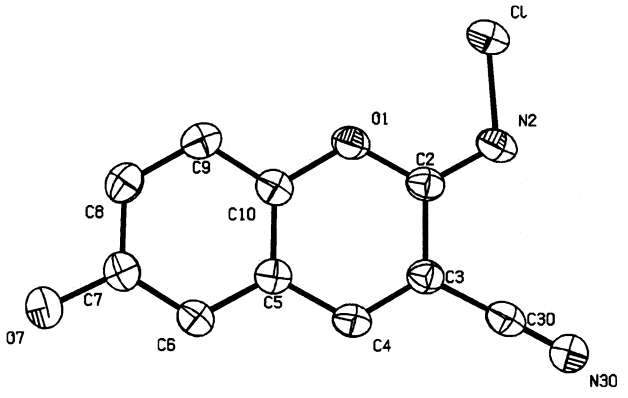


Figure 2. Molecular structure view of **10a** (30% probability).

All the *N*-chlorobenzopyran-2-imines **10** and **11** were characterised by spectroscopic methods (e.g. IR, ^{1}H, ^{13}C NMR, mass spectra) and elemental analysis. The structure of product **10a** (X = CN, Y = 6-OH) was also confirmed by X-ray analysis (Fig. 2).[22]

In conclusion, we have developed an easy route to *N*-chlorinated 2*H*-1-benzopyran-2-imines. Availability of these compounds will allow a new mode of covalent bonding of the fluorophoric benzopyran-2-imines to various substrates. They might be applied as potential labels for proteins. Indeed, preliminary labelling studies, proved a covalent bond formation between human serum albumin (HSA) and **10a**. Antiproliferative activity of compounds **10a** on MCF-7 cells in vitro was also found to be very promising.

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21. Selected data for **8b**: green crystals; mp 225–226°C (from ethanol); δ_H (300 MHz; DMSO-d_6) 1.32 (t, 3H, $J=7.1$ Hz, CH_2CH_3); 4.29 (q, 2H, $J=7.1$ Hz, CH_2CH_3); 6.96–7.16 (m, 3H, arom. H_5, H_6, H_7); 8.24 (s, 1H, H_4). Anal. calcd for $C_{12}H_{11}N_1O_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.96; H, 4.45; N, 5.09. MS (EI): m/z (rel. intensity) 76 (25), 103 (18), 134 (25), 161 (48), 187 (100), 233 (50). Crystallographic data for the structure **8b** have been deposited within the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 200889.
22. Selected data for **10a**: yellow crystals; mp 209–211°C (from ethyl acetate); δ_H (300 MHz; DMSO-d_6) 7.04 (d, 1H, $J_{H5H7}=3.0$ Hz, H_5); 7.10 (dd, 1H, $J_{H7H8}=9.0$ Hz, $J_{H5H7}=3.0$ Hz, H_7); 7.31 (d, 1H, $J_{H7H8}=9.0$ Hz, H_8); 8.44 (s, 1H, H_4); 10.06 (s, 1H, OH). Anal. calcd for $C_{10}H_5N_2O_2Cl$: C, 54.44; H, 2.28; N, 12.70. Found: C, 54.82; H, 2.30; N, 12.57. MS (EI): m/z (rel. intensity) 61 (62), 76 (22), 102 (34), 130 (22), 158 (31), 185 (100), 220 (90). Crystallographic data for the structure **10a** have been deposited within the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 200890.