Chemistry of Heterocyclic Compounds, Vol. 42, No. 3, 2006

SYNTHESIS OF SOME NEW BIOLOGICALLY ACTIVE COUMARIN DERIVATIVES

N. Hamdi[1], C. Lidrissi[2], M. Saoud[2], A. Romerosa Nievas[2], and H. Zarrouk[1]

The reaction of 4-hydroxycoumarin in toluene with a variety of aromatic binucleophilic compounds has been studied. 3-(Dimethylaminomethylene)chromane-2,4-dione was used as a key intermediate for the preparation of bis[N-(4-oxocoumarinylmethylene)]-1,4-diamines. Alternative synthetic procedures and antibacterial activity data of some of the new compounds are given.

Keywords: benzodiazepin-2-one, 4-hydroxycoumarin, *o*-phenylenediamine, antibacterial activity.

Several coumarin derivatives have revealed pronounced medicinal value as antibacterial and antifungal agents [1-5]. Others have displayed antituber- culosis activity [6] and some have insecticidal properties [7]. This prompted us to investigate the preparation of a new series of compounds containing coumarin moieties with different side chains or fused rings.

We have studied the reaction of 4-hydroxycoumarin (**1**) and 3-(dimethylaminomethylene) chromane-2,4-dione (**2**) with aromatic binuclephilic compounds.

Compound **1** was found to react with *o*-phenylenediamine on refluxing in toluene to give product **3** (Scheme 1). According to the elemental analysis, and IR and NMR spectroscopy data, the structure of 4-(2-hydroxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepin-2-one can be ascribed to compound **3**.

A mass-spectrometric study was carried out to establish the structure of compound **3**. Its mass spectrum contains the molecular ion peak *m/z* 252 (16.98%) and a base peak (100%) at *m/z* 210, corresponding to 2-(2-hydroxyphenyl)benzimidazole (**4**). A tendency towards decreasing the heterocycle size is characteristic of the mass-spectrometric behavior of 1,5-benzodiazepin-2-ones [8] and consequently the mass spectra of these compounds contain intense peaks of the corresponding benzimidazoles. It is also known that the mass-spectrometric fragmentation of 1,5-benzodiazepines is similar to their thermal or acid decomposition [1, 8]. In fact, refluxing compound **3** in concentrated sulfuric acid yields benzimidazole **4** as the main product.

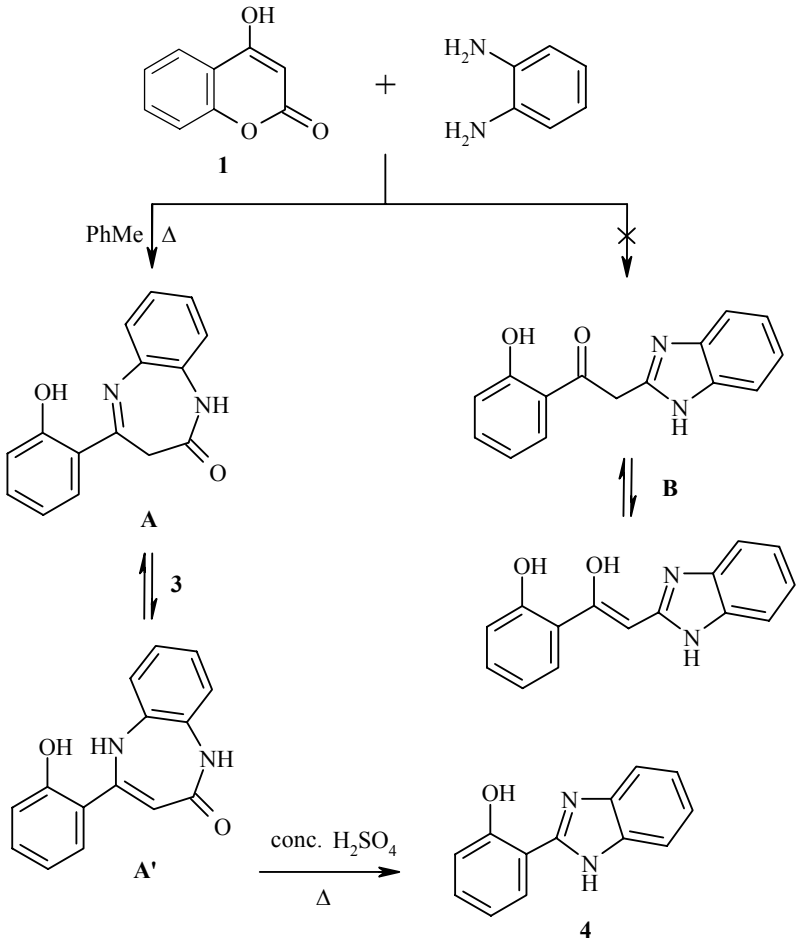
According to the ^{1}H NMR spectrum in DMSO-d_6 benzodiazepine **3** exists as a 4:1 mixture of tautomers **A** and **A'**. Benzodiazepin-2-one **3** is formed due to the substitution of the hydroxyl group of coumarin **1** by one of the amino groups of *o*-phenylenediamine and the C–O bond cleavage in the pyrone ring upon reaction with the second amino group.

In a similar manner, 4-hydroxycoumarin reacts with equimolar amounts of 4-aminothiophenol and 4-aminophenol to give the corresponding coumarin derivatives **5a,b** (Scheme 2).

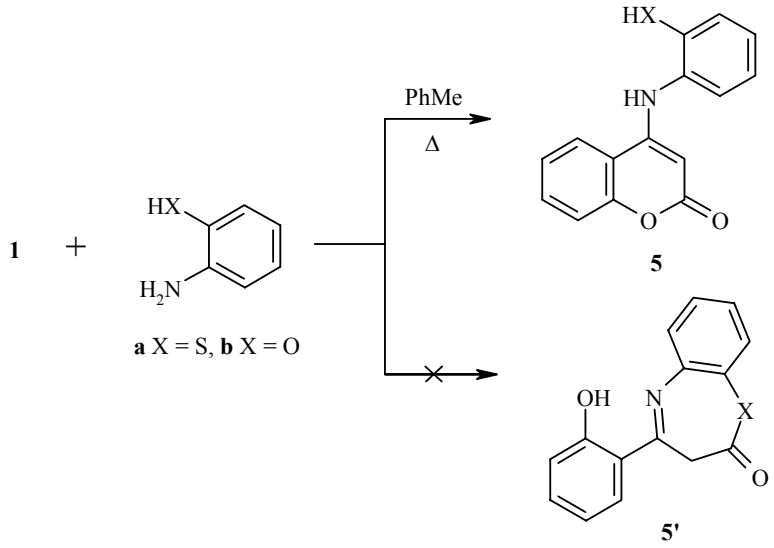
---

[1] Institut National de Recherche et d'Analyse physicochimique, Technopole de Sidi Thabet 2020, Tunisie; e-mail: hamdi_naceur@yahoo.fr. [2] Dpto. Quimica Fisica, Bioquimica y Quimica Inorganica, Universidad de Almeria, Almeria, Spain. Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 3, pp. 361-366, March, 2006. Original article submitted December 22, 2005.

Scheme 1



Scheme 2

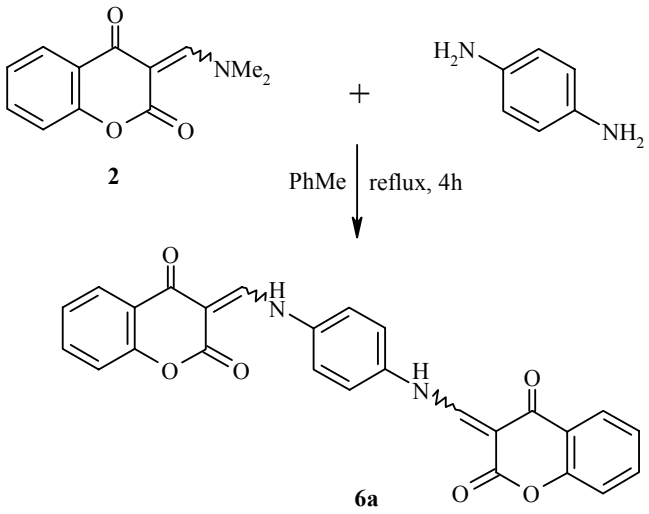


The ^{1}H NMR spectrum of compound **5a** in DMSO-d_6 showed the presence of a signal at 12.5 ppm corresponding to the exchangeable NH proton, the ethylenic proton as a singlet at δ 5.6 ppm, and the aromatic protons appear between 7.27 and 7.80 ppm. The elemental and spectral analysis was in agreement with the structures of these compounds.

We also investigated the reaction of 4-hydroxycoumarin with an excess of N,N-dimethylformamide dimethyl acetal (DMFDMA) which afforded the corresponding 3-(dimethylaminomethylene)chromane-2,4-dione derivative **2**. The structure was confirmed by IR, NMR, and MS analyses.

The possibility that diamines might further react inter- or intramolecularly [9-11] at either of the two carbonyl groups prompted us to examine the interaction of aromatic diamines with compound **2**. The reactions of equimolar amounts of dione **2** and 4-phenylenediamine or 4-aminothiophenol were carried out by refluxing in toluene for 4 h (Scheme 3). Thin layer chromatography showed the formation of a single product in both cases. The ^{1}H NMR spectra of compound **6a** showed the presence of two doublets of the same intensity at δ 10-12, corresponding to two exchangeable protons, the ethylenic protons of the *Z*- and *E*-isomers [12] as two doublets at δ 7-8, and signals corresponding to the expected Ar protons.

Scheme 3



These observations indicated that an intermolecular double condensation has occurred to give a bis[N-(4-oxocoumarinylmethylene)]-1,4-disubstituted aromatic diamine. Data from the elemental analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%) and in conformity with the assigned structure. In the addition of molar equivalents of 1,4-aromatic binucleophilic compounds to compound **6** we did not observe any heterocyclic compound resulting from the further intermolecular nucleophilic attack on the single condensation product. Since the condensation of 3-(dimethylaminomethylene)chromane-2,4-dione with aromatic binucleophile is the only route to the new coumarinic compounds, this represents a useful synthetic method. The reactivity of compound **2** is currently under investigation.

Some representative examples of the new compounds were tested against a pathogenic bacterial strain and have shown activity against *Staphylococcus aureus* ATCC 25923. The benzodiazepin-2-one **3** exhibited the strongest antibacterial activity. The results are summarized in Table 1.

TABLE 1. Antibacterial Screening of Compounds **3** and **5**

Compound	Concentration, mg/disk	Inhibition zone, mm
3	1	50
	2	58
	4	58
5a	1	30
	2	33
	4	33
5b	1	39
	2	41
	4	40

EXPERIMENTAL

NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at room temperature on a Bruker AC instrument (300 MHz for ^1H, 75.47 MHz for ^{13}C). Chemical shifts are expressed with positive values downfield from Me_4Si. The IR spectra were recorded on a Bruker FT-IR IFS 28 in the region between 4000 and 400 cm^{-1}. Mass spectra were obtained with a Hewlett–Packard 5880a spectrometer. In this case electron impact techniques were employed. Elemental analyses were performed at the National Institute of Research and Physicochemical Analysis in Tunisia. Melting points were determined on a Buchi apparatus and are uncorrected. TLC was performed on aluminum-backed plates coated with silica gel 60 with F254 indicator. Column chromatography was carried out on a silica gel 60.

4-(2-Hydroxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepin-2-one (3). 1,2-Phenylenediamine (1.62 g, 15 mmol) was added to a solution of coumarin **1** (1 g, 6.17 mmol) in toluene (30 ml). The mixture was refluxed for 6 h. The precipitate that formed was filtered off and recrystallized from the corresponding solvent to give compound **3** as yellow needles, mp 160°C (EtOH); yield 80%. IR spectrum, ν, cm^{-1} (KBr): 3293 (NH), 1599 (C=C). ^1H NMR spectrum (DMSO-d_6), δ, ppm: 3.62 (2H, s, CH_2); 6.97-7.94 (8H, m, H_{arom}); 10.75 (1H, s, NH); 14.09 (1H, s, OH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 90.9 (CH_2); 115.7-154.1 (C_{arom}); 167.3 (C=O); 166.5 (C=N). Mass spectrum, *m/z* (*I*, %): 252 $[M]^+$ (16.98). Found, %: C 71.3; H 4.7; N 5.4. $C_{12}H_{12}N_2O_2$. Calculated, %: C 71.42; H 4.76; N 5.55.

Reactions of 2-Aminothiophenol and 2-Aminophenol with 4-Hydroxycoumarin (1). 2-Aminothiophenol (1.62 g, 12.96 mmol) or 2-aminophenol (1.62 g, 14.86 mmol) was added to a solution of coumarin **1** (1 g, 6.17 mmol) in toluene (30 ml). The mixture was refluxed for 6 h. The precipitate that formed was filtered off and recrystallized from the corresponding solvent.

4-(2-Mercaptophenylamino)coumarin (5a). Yield 75%; black needles; mp 190°C (CH_2Cl_2). IR spectrum, ν, cm^{-1} (KBr): 3302 (NH), 1620 (C=C). ^1H NMR spectrum (DMSO-d_6), δ, ppm: 4.95 (1H, s, =CH); 7.02-7.26 (8H, m, H_{arom}); 4.9 (1H, s, NH); 5.8 (1H, s, OH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 160.2 ($C_{(2)}$); 84.2 ($C_{(3)}$); 121.5-150.2 (C_{arom}); 154.1 ($C_{(4)}$). Mass spectrum, *m/z* (*I*, %): 269 $[M]^+$ (90.50). Found, %: C 66.8; H 3.9; N 5.1; S 12.4. $C_{15}H_{11}NO_2S$. Calculated, %: C 66.91; H 4.08; N 5.20; S 11.89.

4-(2-Hydroxyphenylamino)coumarin (5b). Yield 85%; red needles; mp 170°C (CH_2Cl_2). IR spectrum, ν, cm^{-1} (KBr): 3345 (NH), 1610 (C=C). ^1H NMR spectrum (DMSO-d_6), δ, ppm: 4.86 (1H, s, =CH); 6.73-7.27 (8H, m, H_{arom}); 4.8 (1H, s, NH); 5.2 (1H, s, OH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 160.9 ($C_{(2)}$); 82.6 ($C_{(3)}$); 115.9-158.5 (C_{arom}); 153.2 ($C_{(4)}$). Mass spectrum, *m/z* (*I*, %): 253 $[M]^+$ (96.50). Found, %: C 71.1; H 4.2; N 5.5. $C_{15}H_{11}NO_3$. Calculated, %: C 71.14; H 4.34; N 5.53.

2-(2-Hydroxyphenyl)benzimidazole (4). A solution of benzodiazepin-2-one **3** (0.5 g, 1.6 mmol) in conc. H_2SO_4 (20 ml) was heated at 100ºC for 3 h. The reaction mixture was poured into water (50 ml). The precipitate that formed was filtered off and dried. After recrystallization from ethanol, benzimidazole **4** was obtained in 63% yield as white needles; mp 130°C (CH_2Cl_2). IR spectrum, ν, cm^{-1} (KBr): 3250 (OH); 1625 (C=C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 5.2 (1H, s, OH); 6.73-7.70 (8H, m, H_{arom}); 6.2 (1H, s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 152.9 (C=N); 115.3-158.5 (C_{arom}). Mass spectrum, *m/z* (*I*, %): 210 $[M]^+$ (90.50).

Synthesis of 3-(Dimethylaminomethylene)chromane-2,4-dione (2). A solution of coumarin **1** (0.7 g, 0.004 mol) and DMFDMA (5 ml) in toluene (20 ml) was refluxed for 4 h. The solid product formed was filtered, washed with petroleum ether, and recrystallized from a mixture of dichloromethane and petroleum ether to give red needles. Yield 70%; mp 145°C (CH_2Cl_2). IR spectrum, ν, cm^{-1} (KBr): 1714 (C=O); 2995 (C–H). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 8.00 (1H, d, *J* = 13.6, H-5); 7.53 (1H, d, *J* = 13.5, H-8); 7.20-7.30 (2H, m, H_{arom}); 8.40 (1H, s, CH); 3.42 (3H, s, CH_3); 3.52 (3H, s, CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 178.4 ($C_{(4)}$); 96.8 ($C_{(3)}$); 164.9 ($C_{(2)}$); 163.4 (=CH); 117.6-134.2 (C_{arom}); CH_3 (45.3); CH_3 (49.1). Mass spectrum, *m/z* (*I*, %): 217 $[M]^+$ (100).

Reaction of 1,4-Binucleophilic Compounds with Compound 2. A solution of compound **2** (1 g) and the 1,4-binucleophilic compounds (1 equivalent) in toluene was heated at reflux for 4 h with stirring. The progress of the reaction was monitored by thin layer chromatography (eluent dichloromethane–diethyl ether, 10:1). The precipitate formed upon cooling to room temperature or after evaporation of solvents was washed with methanol and recrystallized.

Bis[N-(4-oxocoumarinylmethylene)]-1,4-phenylenediamine (6a). Yield 65%; yellow needles; mp 220°C (EtOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 8.46 and 8.50 (1H, dd, *J* = 13.6, CH, *Z*- and *E*-), 10.38 (1H, m, NH, *Z*- and *E*-); 11.58 (1H, m, NH, *Z*- and *E*-); 7.23-7.94 (8H, m, Ar). Found, %: C 69.1; H 3.5; N 6.2. $C_{26}H_{16}N_2O_6$. Calculated, %: C 69.02; H 3.53; N 6.19.

Bis[N-(4-oxocoumarinylmethylene)]-4-aminothiophenol (6b). Yield 85%; white needles; mp 210°C (MeOH). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 8.52 and 8.62 (1H, dd, *J* = 13.5, CH, *Z*- and *E*-); 10.38 (1H, m, NH, *Z*- and *E*-); 11.58 (1H, m, NH, *Z*- and *E*-); 7.33-7.84 (8H, m, Ar). Found, %: C 66.20; H 3.40; N 2.8; S 7.3. $C_{26}H_{16}NO_6S$. Calculated, %: C 66.38; H 3.40; N 2.97; S 6.80.

Biological Assays. The antibacterial activity tests of the coumarin derivatives have been carried out using the disc diffusion (Bauer) method [13]. This consists in introducing the germ (in our case *Staphylococcus aureus* ATCC 25923) at a concentration of 10^6 CFC/ml on the surface of a Mueller-Hinton gelose plate. Then one applies sterile filter paper discs (diameter 6 mm) impregnated with the product that one wants to test. The samples are kept at a temperature of 40ºC during 2 h to permit the diffusion of the product on the gelose. After 24 h of incubation at 37ºC, the diameters of the inhibition zones around the discs are measured (in our case, we deposited on the same plates 2 to 3 discs containing the same product but with increasing doses of 1, 2, and 4 mg, to see if the inhibition of the bacterial growth is dose dependent or not).

Acknowledgements

We are very grateful to the Tunisian Ministry of Scientific Research, Technology, and Development of Competences and the Spanish Agency of International Cooperation for generous support of this work through the project A/2903/05.

REFERENCES

1. R. M. Kelkar, U. K Joshi, and M. V. Paradkar, *Synthesis,* 214 **(**1986**).**
2. K. S. R. Krisha-Rao and N. V. Subba-Rao, *Curr. Sci*., **33**, 614 (1964).
3. R. B. Moppet, *J. Med. Chem*., **7**, 446 (1964).
4. G. Redighiero and C. Antonello, *Bull. Chim. Farm*., **97**, 592 (1985).
5. A. M. El-Naggar, F. S. M. Ahmed, A. M. Abd El-Salam, M. A. Radi, and M. S. A. Latif, *J. Heterocyclic Chem*., **18**, 1208 (1981).
6. C. R. Merchant, A. S. Gupita, P. J. Shah, and S. S. Shirali, *Chem. Ind*., 351 (1979).
7. R. S. Mali, S. N. Yeola, and B. K. Kulkarn, *Ind. J. Chem*., **22B**, 352 (1983).
8. P. B. Terentiev *and* A. P. Stankevicius, *Mass-spectrometry of Biologically Active Nitrogenous Bases* [in Russian], Mokslas, Vilnius (1987).
9. V. R. Barchet and K. W. Merz, *Tetrahedron Lett*., **5**, 2239 (1964).
10. M. El Abbassi, E. M. Essassi, and J. Fifani, *Tetrahedron Lett.*, **28**, 1389 (1987).
11. T. Steinfurhrer, A. Hantschmann, M. Pietsch, and M. Weibenfels, *Liebigs Ann. Chem.*, 23 (1992).
12. O. S. Wolfbeis and E. Ziegler, *Z. Naturforsch.*, **31b**, 1519 (1976).
13. S. W. Bauer, W. M. Kirby, J. C. Sherris, and M. Thurck, *Am. J. Pathol.,* **45**, 408 (1966).