HETEROCYCLES, Vol. 63, No. 5, 2004, pp. 1083 - 1091
Received, 22nd January, 2004, Accepted, 4th March, 2004, Published online, 5th March, 2004

SYNTHESIS OF FLUORESCENT DERIVATIZATION REAGENTS: REACTION OF ISATIN WITH 3-ARYL-7-DIETHYLAMINO-COUMARINS AND THEIR FLUORESCENT PROPERTIES[1]

Naozumi Nishizono,* Kazuaki Oda, Yutaka Kato, Kosei Ohno, Masaru Minami, and Minoru Machida

Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan
(E-mail: nishizon@hoku-iryo-u.ac.jp)

Abstract – Fluorescent derivatization reagents, 3-aryl-7-diethylaminocoumarin derivatives, were synthesized and reacted with isatin to give the adduct. Their fluorescent properties are discussed.

Since isatin plays physiologically important roles in various biological systems,[2] there has been a demand for practical methods to determine the concentration of isatin. In the previous paper, we reported the synthesis of 4-*tert*-butyloxycarbonylaminooxybutyl [2-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)-thiazol-4-yl]acetate (DACT-ONH) as a fluorescent derivatization reagent for isatin (Figure 1).[1] However, it has been difficult to use DACT-ONH for determination of isatin by HPLC because of the formation of *E* and *Z* stereoisomers, and because the sensitivity in measuring the concentration of isatin was reduced to a half of fluorescence intensity of the reagent itself. The decreased sensitivity seems to be based on some interactions through a long linkage of the reacting group in DACT-ONH. In order to resolve the problems of stereoisomer formation and sensitivity, the reacting group with a short linkage will be preferable.

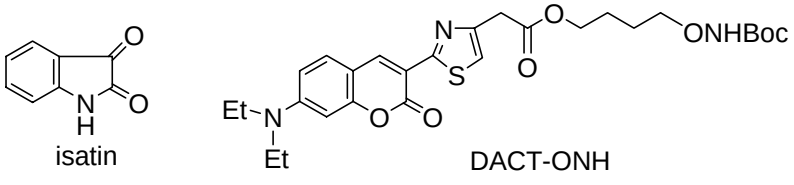
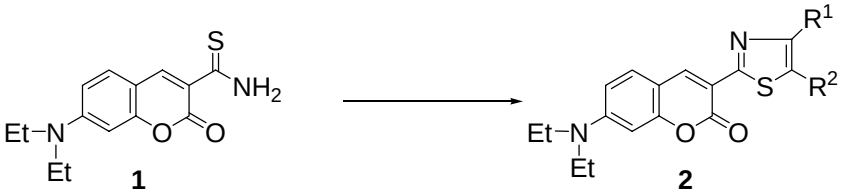


Figure 1. Structures of isatin and DACT-ONH.

Isatin has been used in various reactions, for example, reactions with diamines,[3] aldol reaction,[4] Friedel-Crafts-type reaction,[5] Grignard reaction,[6] Wittig reaction,[7] and so on. Since the aldol reaction of isatin-carbonyl (3-position) with acetone in the presence of diethylamine is known to easily give an aldol adduct, acetyl group was selected as a reacting group for the carbonyl of isatin. In general, the introduction of an electron-withdrawing group at the 5-position on the thiazole ring causes a bathchromic shift with increases in molar absorptivity and fluorescence intensity.[9] Therefore, thiazolylcoumarin possessing an acetyl group at the 5-position on the thiazole ring was designed.

The synthesis of 7-diethyamino-3-thiazolylcoumarin having an acetyl group at the 5-position and the fluorescent properties of related compounds are described in this paper.

First, coumarin-thiazolylmethyl chloride (**2a**) as an *N*-alkyl agent was synthesized from 7-diethylamino-2-oxo-2*H*-chromene-3-thiocarboamide (**1**) and 1,3-dichloropropan-2-one (Scheme 1). The reaction of **2a** with isatin in acetonitrile was carried out in the presence of potassium carbonate. The resulting *N*-labeled compound (**3**) was nonfluorescent. Quenching of fluorescence seems to result in intersystem crossing process to an excited triplet state or an energy transfer from the fluorophore to the isatin moiety. Such a nonfluorescenct species was also observed in the isatin-hydrazone derivative having a carbon-nitrogen double bond as reported in the previous paper.[1] However, compound (**3)** upon treatment with acetone in the presence of potassium carbonate gave an acetone adduct (**4**) that strongly fluoresced (Scheme 2).



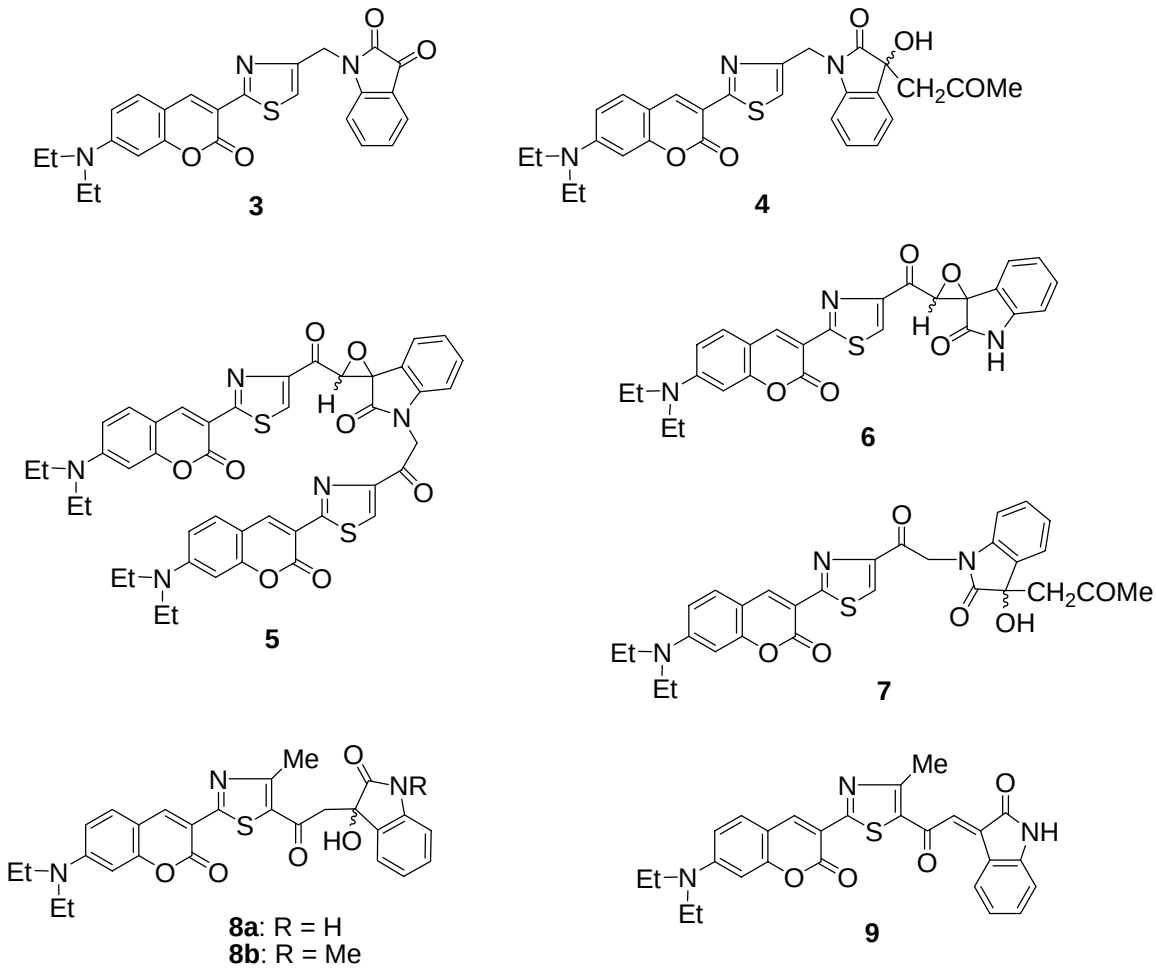
Reagents and conditions: **2a** (R^1 = CH_2Cl, R^2 = H): $ClCH_2COCH_2Cl$, DMF, reflux; **2b** (R^1 = $COCH_2Br$, R^2 = H): $BrCH_2COCOCH_2Br$, DMF, rt; **2c** (R^1 = Me, R^2 = COMe): MeCOCHBrCOMe, DMF, rt; **2d** (R^1 = R^2 = H)[8]: $BrCH_2CHO$, K_2CO_3, DMF, rt.

Scheme 1

Furthermore, as an *N*-labeling reagent, bromoacetylthiazolylcoumarin (**2b**) was synthesized from **1** and 1,4-dibromobutane-2,3-dione. The reaction of **2b** with isatin in acetonitrile gave epoxides (**5**, **6**) that showed fluorescence. The compound (**2b**) also gave strongly fluorescent products (**5**, **7**) by the reaction in acetone. Thus, it was found that disappearance of the isatin-carbonyl (3-position) in fluorescent derivatization restored the fluorescence. These results suggest that quenching of the fluorescence results in some participation of a π bond in an isatin-carbonyl (3-position).

Next, a derivatization reagent (**2c**) was prepared from thioamide (**1)** and 3-bromopentane-2,4-dione. In order to determine effective derivatization for isatin, the reaction of **2c** with isatin was examined under

various conditions. When potassium carbonate as a base was used in acetonitrile, an aldol adduct (**8a**) was obtained in maximum yield of 60-70%. A satisfactory yield was not obtained because of the involvement of the retro-aldol reaction.



Scheme 2

Since both the aldol adducts (**8a** and **8b**[8]) showed strong fluorescence, it was suggested that fluorescence quenching results in participation of the carbonyl group at the 3-position in the isatin moiety. In order to obtain a clue for resolving this problem, some nonfluorescent species were required. To determine whether a carbon-carbon double bond at the benzylic position in the isatin moiety is also responsible for the fluorescence quenching, the formation of a carbon-carbon double bond by dehydration of **8a** was examined under various acid catalyst conditions. When **8a** was treated with thionyl chloride, a dehydrating product (**9**) that was nonfluorescent was obtained in maximum yield of 44%. As predicted, participation of a carbon-carbon double bond in **9** was again suggested.

The structures of coumarin-isatin adducts (**3** ~ **9**) were assigned on the basis of results of elemental analyses and spectral data.

For example, the FAB-MS of **8a** showed an ion peak (MH^+) at *m/z* 504 consistent with the adduct of **8a** to isatin. In the ^{1}H-NMR spectrum, signals due to the aromatic protons in **8a** showed ones analogous to the

reagent and isatin themselves. Characteristic peaks due to α-methylene, two doublets with coupling constants of J = 16.6 Hz, appeared at 3.31 and 3.55 ppm, respectively, and the peak due to acetyl protons disappeared. In the ^{1}H-^{13}C cosy spectrum, two doublets correlated with the signal at 44.4 ppm in the ^{13}C-NMR spectrum, indicating the presence of a newly formed methylene group instead of the methyl moiety in an acetyl group. Moreover, in the ^{13}C-NMR spectrum of **8a**, a peak due to newly formed quaternary carbon having a hydroxy group appeared at 72.8 ppm, but stereochemistry of the benzylic carbon was unclear. These results indicate that an aldol addition reaction occurred between the acetyl group and the carbonyl group in isatin.

Table 1. Absorption and fluorescence properties of thiazole-coumarin derivatives.

Compound	Absorption[a)]		Fluorescence[b)]		
	λ_{max} (nm)	ε	Ex (nm)	Fλ_{max} (nm)	Relative intensity
2a	451	48000	451	481	1.04
2b	455	30800	455	504	0.44
2c	472	60700	472	500	1.51
2d	433	46000	433	477	1.00
3	452	59500	-	-	-
4	436	41700	436	480	1.19
5	456	96700	456	482	1.61
6	457	34200	457	485	0.65
7	456	49500	456	483	1.19
8a	478	56200	478	508	1.29
8b	479	60600	479	509	1.45
9	494	51500	-	-	-

a) Concentration: 1.5 X 10^{-5} M. b) Concentration: 3.5 X 10^{-6} M.

The absorption and fluorescence properties of all the compounds are listed in Table 1. The relative fluorescence intensity of the parent compound (**2d**) in chloroform is arbitrarily taken as 1.0.[9] The absorption and fluorescence maxima (λ_{max} and Fλ_{max}) of **8a** and **8b** appeared at longer wavelengths, 478-479 nm and 508-509 nm, respectively, than those of **2c**. The relative fluorescence intensities of **8a** and **8b** showed about the same values as that of **2c**. Thus, a methanol solution of a mixture of **2c**, **8a**, and **8b** was subjected to HPLC. As shown in Figure 2, peaks were clearly separated.

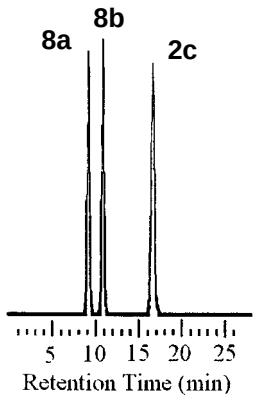


Figure 2. Chromatogram of **2c**, **8a**, and **8b**. Column: Inertsil ODS-2; Mobile Phase: 80% Methanol; Flow Rate: 1.0 mL/min; Detector: Fluorescence Spectrophotometer (Ex 480 nm Em 510 nm).

Although derivatization reagent (**2c)** is a promising candidate for use in fluorometric detection in combination with HPLC, an effective derivatization yield of isatin was not obtained because of the involvement of reversible retro-aldol reaction in this method. However, it was found that the fluorescence quenching of derivatized-isatin results in interactions of a π bond at the benzylic position in the isatin moiety and non-fluorescent species will result. The results of this study provide important information for the design of derivatization reagents to prevent the formation of non-fluorescent species by intramolecular quenching.

ACKNOWLEDGEMENTS

This study was partially supported by the Academic Science Frontier Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. We would like to thank Ms S. Oka, Ms M. Kikuchi, and Ms Y. Takihata for MS spectra measurements and Ms H. Matsumoto and Ms A. Maeda for elemental analysis (Center for Instrumental Analysis, Hokkaido University).

EXPERIMENTAL

All melting points were determined on a Yamato melting point apparatus (model MP-2) and are uncorrected. NMR spectra were recorded on a JEOL JNM-LA-300 and JEOL ECP-500 spectrometers. Chemical shifts are reported in ppm (δ) relative to TMS (0.0 ppm) as internal standard. Coupling constants, *J*, are given in Hz. MS spectra were obtained on a JEOL JMS-HX110 and JEOL JMS-700TZ. Column chromatography was conducted using silica gel (Merck, Silica gel 60, 70-230 mesh). Absorption and fluorescence spectra were measured with a Hitachi U-3310 and a Hitachi F-4100 fluorescence spectrophotometer, respectively. The HPLC system consisted of a Hitachi L-6200 pump, a Rheodyne Model 7125 injector valve, a Hitachi F-1040 fluorescence spectrometer, a Hitachi D-2500 chromato-integrator and a Gasukuro Kogyo/Model-545 degassing unit. The column was Inertsil ODS-2 (150 x 4.6 mm.; particle size 5 μm; Gasukuro Kogyo, Tokyo).

3-(4-Chloromethylthiazol-2-yl)-7-diethylamino-2*H*-chromen-2-one (2a)

A solution of 7-diethylamino-2-oxo-2*H*-chromene-3-thiocarboamide (**1**) (1.38 g, 5 mmol) and 1,3-dichloroacetone (726 mg, 6 mmol) in DMF (50 mL) was stirred at rt for 17 h. To the reaction mixture

was added brine, and the suspension was extracted with EtOAc. The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : $CHCl_3$ = 8 : 1) to give **2a** (1.30 g, 74%) as orange needles. mp 204-205 ºC ($CHCl_3$); EI-LRMS *m/z* 348 (M^+); ^{1}H-NMR ($CDCl_3$): δ 1.25 (t, 6 H, *J* = 7.3 Hz), 3.46 (q, 4 H, *J* = 7.3 Hz), 4.75 (s, 2 H), 6.55 (d, 1 H, *J* = 2.4 Hz), 6.66 (dd, 1 H, *J* = 2.4, 8.8 Hz), 7.35 (s, 1 H), 7.45 (d, 1 H, *J* = 8.8 Hz), 8.73 (s, 1 H); *Anal.* Calcd for $C_{17}H_{17}N_2O_2ClS$: C, 58.53; H, 4.91; N, 8.03. Found: C, 58.30; H, 4.96; N, 7.98.

3-[4-(2-Bromoacetyl)thiazol-2-yl]-7-diethylamino-2*H*-chromen-2-one (2b)

A solution of 7-diethylamino-2-oxo-2*H*-chromene-3-thiocarboamide (**1**) (2.76 g, 10 mmol) and 1,4-dibromo-2,3-butanedione (2.93 g, 12 mmol) in DMF (50 mL) was stirred at rt for 15 h. To the reaction mixture was added brine, and the suspension was extracted with EtOAc. The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : $CHCl_3$ = 10 : 1) to give **2b** (1.86 g, 44%) as orange prisms. mp 222-224 ºC ($CHCl_3$-AcOEt); EI-LRMS *m/z* 420 (M^+); ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 6 H, *J* = 7.2 Hz), 3.49 (q, 4 H, *J* = 7.2 Hz), 4.77 (s, 2 H), 6.56 (d, 1 H, *J* = 2.4 Hz), 6.69 (dd, 1 H, *J* = 2.4, 8.8 Hz), 7.49 (d, 1 H, *J* = 8.8 Hz), 8.30 (s, 1 H), 8.74 (s, 1 H); *Anal.* Calcd for $C_{18}H_{17}N_2O_3BrS$: C, 51.31; H, 4.07; N, 6.65. Found: C, 51.57; H, 4.00; N, 6.74.

3-(5-Acetyl-4-methylthiazol-2-yl)-7-diethylamino-2*H*-chromen-2-one (2c)

A solution of 7-diethylamino-2-oxo-2*H*-chromene-3-thiocarboamide (**1**) (1.38 g, 5 mmol), K_2CO_3 (2.07 g, 15 mmol), and 3-bromopentane-2,4-dione (3.58 g, 20 mmol) in DMF (50 mL) was stirred at rt for 15 h. To the reaction mixture was added brine, and the suspension was extracted with EtOAc. The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was then dissolved into $CHCl_3$ (50 mL) and PPTs (250 mg, 1 mmol) was added. After the mixture was stirred under reflux for 11 h, the solvent was removed *in vacuo*. The residue was purified by column chromatography (benzene : AcOEt = 10 : 1) to give **2c** (576 mg, 32%) as a yellow solid. mp 227-229 ºC (hexane-AcOEt); EI-LRMS *m/z* 356 (M^+); ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 6 H, *J* = 7.3 Hz), 2.58 (s, 3 H), 2.78 (s, 3 H), 3.47 (q, 4 H, *J* = 7.3 Hz), 6.56 (d, 1 H, *J* = 2.4 Hz), 6.68 (dd, 1 H, *J* = 2.4, 8.8 Hz), 7.46 (d, 1 H, *J* = 8.8 Hz), 8.78 (s, 1 H); *Anal.* Calcd for $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86. Found: C, 64.14; H, 5.76; N, 7.78; EI-HRMS *m/z* 356.1181 (Calcd for $C_{19}H_{20}N_2O_3S$: 356.1195).

Reaction of 2a with Isatin in MeCN

To a solution of isatin (147 mg, 1 mmol) and **2a** (419 mg, 1.2 mmol) in MeCN (140 mL) was added K_2CO_3 (691 mg, 5 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 20 : 1) to give **3** (294 mg, 65%) as orange needles. mp 254-255 ºC ($CHCl_3$-AcOEt); FAB-LRMS *m/z* 460 (MH^+); ^{1}H-NMR

($CDCl_3$): δ 1.25 (t, 6 H, *J* = 7.2 Hz), 3.46 (q, 4 H, *J* = 7.2 Hz), 5.10 (s, 2 H), 6.54 (d, 1 H, *J* = 2.4 Hz), 6.66 (dd, 1 H, *J* = 2.4, 8.8 Hz), 7.04 (d, 1 H, *J* = 8.1 Hz), 7.19 (t, 1 H, *J* = 8.1 Hz), 7.25 (s,1 H), 7.45 (d, 1 H, *J* = 8.8 Hz), 7.54 (t, 1 H, *J* = 8.1 Hz), 7.62 (d, 1 H, *J* = 8.1 Hz), 8.63 (s,1 H); FAB-HRMS *m/z* 460.1320 (Calcd for $C_{25}H_{22}N_3O_4S$: 460.1331).

Reaction of 2a with Isatin in Acetone

To a solution of isatin (147 mg, 1 mmol) and **2a** (419 mg, 1.2 mmol) in acetone (140 mL) was added K_2CO_3 (691 mg, 5 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 20 : 1) to give **4** (294 mg, 57%) as orange needles. mp 203-205 ºC ($CHCl_3$-hexane); FAB-LRMS *m/z* 518 (MH^+); ^{1}H-NMR ($CDCl_3$): δ 1.24 (t, 6 H, *J* = 7.2 Hz), 3.17 (d, 1 H, *J* = 17.1 Hz), 3.34 (d, 1 H, *J* = 17.1 Hz), 3.45 (q, 4 H, *J* = 7.2 Hz), 4.99 (d, 1 H, *J* = 16.1 Hz), 5.18 (d, 1 H, *J* = 16.1 Hz), 6.53 (d, 1 H, *J* = 2.4 Hz), 6.64 (dd, 1 H, *J* = 2.4, 9.0 Hz), 6.86 (d, 1 H, *J* = 8.1 Hz), 7.03 (t, 1 H, *J* = 8.1 Hz), 7.24 (t, 1 H, *J* = 8.1 Hz), 7.25 (s,1 H), 7.36 (d, 1 H, *J* = 8.1 Hz), 7.42 (d, 1 H, *J* = 9.0 Hz), 8.66 (s, 1 H); FAB-HRMS *m/z* 518.1742 (Calcd for $C_{28}H_{28}N_3O_5S$: 518.1750).

Reaction of 2b with Isatin in MeCN

To a solution of isatin (147 mg, 1 mmol) and **2b** (506 mg, 1.2 mmol) in MeCN (140 mL) was added K_2CO_3 (691 mg, 5 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 8 : 1) to give **5** (154 mg, 19%) as yellow needles and **6** (112 mg, 23%) as orange needles: **5**: mp 240-241 ºC ($CHCl_3$-hexane); FAB-LRMS *m/z* 828 (MH^+); ^{1}H-NMR ($CDCl_3$): δ 1.26 (m, 12 H), 3.47 (m, 8 H), 5.27 (d, 1 H, *J* = 18.4 Hz), 5.45 (s, 1 H), 5.63 (d, 1 H, *J* = 18.4 Hz), 6.5-6.8 (m, 6 H), 7.00 (t, 1 H, *J* = 7.9 Hz), 7.30 (t, 1 H, *J* = 7.9 Hz), 7.49 (m, 2 H), 8.18 (s, 1 H), 8.31 (s, 1 H), 8.70 (s, 1 H), 8.81 (s, 1 H); FAB-HRMS *m/z* 828.2167 (Calcd for $C_{44}H_{38}N_5O_8S_2$: 828.2162). **6**: mp 194-195 ºC ($CHCl_3$-hexane); FAB-LRMS *m/z* 488 (MH^+); ^{1}H-NMR ($CDCl_3$): δ 1.25 (t, 6 H, *J* = 7.3 Hz), 3.41 (q, 4 H, *J* = 7.3 Hz), 5.45 (s, 1 H), 6.39 (dd, 1 H, *J* = 2.5, 8.8 Hz), 6.47 (d, 1 H, *J* = 2.5 Hz), 6.93 (d, 1 H, *J* = 7.4 Hz), 7.02 (t, 1 H, *J* = 7.4 Hz), 7.20 (d, 1 H, *J* = 7.4 Hz), 7.30 (t, 1 H, *J* = 7.4 Hz), 7.38 (d, 1 H, *J* = 8.8 Hz), 8.30 (s, 1 H), 8.51 (br s, 1 H), 8.56 (s, 1 H); FAB-HRMS *m/z* 488.1288 (Calcd for $C_{26}H_{22}N_3O_5S$: 488.1280).

Reaction of 2b with Isatin in Acetone

To a solution of isatin (147 mg, 1 mmol) and **2b** (506 mg, 1.2 mmol) in acetone (140 mL) was added K_2CO_3 (691 mg, 5 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 8 : 1) to give **5** (136 mg, 16%) as yellow needles and **7** (112 mg, 23%) as orange needles. **7**: mp 251-253 ºC ($CHCl_3$-hexane); FAB-LRMS *m/z* 546 (MH^+); ^{1}H-NMR (DMSO-d_6): δ 1.09 (t, 6 H, *J* = 7.2 Hz), 1.98 (s, 3H), 2.98 (d, 1 H, *J* = 16.1 Hz), 3.26 (d, 1 H, *J* = 16.1 Hz), 3.43 (q, 4 H, *J* = 7.2 Hz), 5.19 (d, 1 H, *J* = 18.5 Hz), 5.36 (d, 1

H, *J* = 18.5 Hz), 6.50 (d, 1 H, *J* = 2.0 Hz), 6.78 (dd, 1 H, *J* = 2.0, 8.8 Hz), 6.90 (d, 1 H, *J* = 7.3 Hz), 6.96 (t, 1 H, *J* = 7.3 Hz), 7.17 (t, 1 H, *J* = 7.3 Hz), 7.29 (d, 1 H, *J* = 7.3 Hz), 7.71 (d, 1 H, *J* = 8.8 Hz), 8.57 (s, 1 H), 8.87 (s, 1 H); FAB-HRMS *m/z* 546.1722 (Calcd for $C_{29}H_{28}N_3O_6S$: 546.1699).

3-{2-[2-(7-Diethylamino-2-oxo-2*H*-chromen-3-yl)-4-methylthiazol-5-yl]-2-oxoethyl}-3-hydroxyindolin-2-one (8a)

To a solution of isatin (14 mg, 0.09 mmol) and **2c** (45 mg, 0.14 mmol) in MeCN (4 mL) was added K_2CO_3 (200 mg, 1.4 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 5 : 1) to give **8a** (32 mg, 71%) as red prisms. mp 229-230 ºC ($CHCl_3$-EtOH); FAB-LRMS *m/z* 504 (MH^+); ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 6 H, *J* = 6.9 Hz), 2.75 (s, 3 H), 3.31 (d, 1 H, *J* = 16.6 Hz), 3.49 (q, 4 H, J = 6.9 Hz), 3.55 (d, 1 H, *J* = 16.6 Hz), 5.57 (s, 1 H), 6.56 (d, 1 H, *J* = 2.3 Hz), 6.69 (dd, 1 H, *J* = 2.4, 9.2 Hz), 6.89 (d, 1 H, *J* = 7.8 Hz), 7.01 (t, 1 H, *J* = 7.8 Hz), 7.30 (t, 1 H, *J* = 7.8 Hz), 7.45 (d, 1 H, *J* = 7.8 Hz), 7.49 (d, 1 H, *J* = 9.2 Hz), 8.77 (s, 1 H), 9.82 (s, 1 H); ^{13}C-NMR (DMSO-d_6): δ 12.4 (q), 44.4 (t), 72.8 (s), 96.2 (d), 108.0 (s), 109.4 (d), 110.0 (s), 110.5 (d), 121.2 (d), 123.8 (d), 129.0 (d), 130.0 (s), 131.7 (d), 142.0 (d), 152.4 (s9, 156.6 (s), 157.8 (s), 160.0 (s), 161.8 (s), 189.3 (s); FAB-HRMS *m/z* 504.1596 (Calcd for $C_{27}H_{26}N_3O_5S$: 504.1599); *Anal.* Calcd for $C_{27}H_{25}N_3O_5S$: C, 64.40; H, 5.00; N, 8.34. Found: C, 64.32; H, 5.01; N, 8.30.

3-{2-[2-(7-Diethylamino-2-oxo-2*H*-chromen-3-yl)-4-methylthiazol-5-yl]-2-oxoethyl}-3-hydroxy-1-methylindolin-2-one (8b)

To a solution of *N*-methylisatin (147 mg, 1 mmol) and **2c** (428 mg, 1.2 mmol) in MeCN (140 mL) was added K_2CO_3 (691 mg, 5 mmol), and the mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$: AcOEt = 5 : 1) to give **8b** (90 mg, 17%) as brown prisms. mp 145-147 ºC ($CHCl_3$-EtOH); FAB-LRMS *m/z* 518 (MH^+); ^{1}H-NMR ($CDCl_3$): δ 1.25 (t, 6 H, *J* = 6.8 Hz), 2.78 (s, 3 H), 3.23 (s, 3 H), 3.35 (d, 1 H, *J* = 16.6 Hz), 3.47 (q, 4 H, *J* = 6.8 Hz), 3.55 (d, 1 H, *J* = 16.6 Hz), 5.05 (s, 1 H), 6.54 (d, 1 H, *J* = 2.4 Hz), 6.67 (dd, 1 H, *J* = 2.4, 9.2 Hz), 6.85 (d, 1 H, *J* = 7.8 Hz), 7.04 (t, 1 H, *J* = 7.8 Hz), 7.32 (t, 1 H, *J* = 7.8 Hz), 7.42 (d, 1 H, *J* = 7.8 Hz), 7.45 (d, 1 H, *J* = 9.2 Hz), 8.77 (s, 1 H); *Anal.* Calcd for $C_{28}H_{27}N_3O_5S$: C, 64.97; H, 5.26; N, 8.12. Found: C, 65.07; H, 5.28; N, 8.11.

3-{2-[2-(7-Diethylamino-2-oxo-2*H*-chromen-3-yl)-4-methylthiazol-5-yl]-2-oxoethylidene}indolin-2-one (9)

A solution of **8a** (59 mg, 0.15 mmol) and $SOCl_2$ (60 μL, 0.82 mmol) in CH_2Cl_2 (10 mL) was stirred under reflux for 4 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (hexane: AcOEt = 1 : 1) to give **9** (36 mg, 49%) as a red solid. mp >300 ºC (MeOH); FAB-LRMS *m/z* 486 (MH^+); ^{1}H-NMR (DMSO-d_6): δ 1.16 (q, 6 H, *J* = 6.9 Hz), 2.78 (s, 3 H), 3.50 (q, 4 H, *J* = 6.9 Hz), 6.66 (d, 1 H, *J* = 2.3 Hz), 6.89-6.83 (m, 3 H), 6.99 (t, 1 H, *J* = 7.5 Hz), 7.36 (t, 1 H, *J* = 7.5

Hz), 7.43 (s, 1 H), 7.76 (d, 1 H, *J* = 9.2 Hz), 8.21 (d, 1 H, *J* = 7.5 Hz), 8.88 (s, 1 H), 10.82 (br s, 1 H); FAB-HRMS *m/z* 486.1479 (Calcd for $C_{27}H_{24}N_3O_4S$: 486.1487); *Anal.* Calcd for $C_{27}H_{23}N_3O_4S \cdot 0.4H_2O$: C, 65.81; H, 4.87; N, 8.53. Found: C, 65.79; H, 4.91; N, 8.34.

REFERENCES AND NOTE

1. Fluorescent Labeling Reagents. Part 6. Part 5. N. Nishizono, K. Oda, K. Ohno, M. Minami, and M. Machida, *Heterocycles*, 2001, **55**, 1897.
2. a) N. Hamaue, M. Minami, M. Hirafuji, M. Terado, M. Machida, N. Yamazaki, M. Yoshioka, A. Ogata, and K. Tashiro, *CNS Drug Reviews*, 1999, **5**, 331.; b) N. Hamaue, M. Minami, M. Terado, M. Hirafuji, T. Endo, M. Machida, T. Hiroshige, A. Ogata, K. Tashiro, H. Saito, and S. H. Parvez, *Neurotoxicology*, 2004, **25**, 205.
3. K. Niume, S. Kurosawa, F. Toda, M. Hasegawa, and Y. Iwakura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2293.
4. E. M. Beccalli and A. Marchesini, *Tetrahedron*, 1993, **49**, 4741.
5. H. N. Song, H. J. Lee, H. R. Kim, E. K. Ryu, and J. N. Kim, *Syn. Commun.*, 1999, **29**, 3303.
6. P. Hewawasam and M. Erway, *Tetrahedron Lett.*, 1998, **39**, 3981.
7. F. H. Osman and F. A. El-Samahy, *Tetrahedron*, 2000, **56**, 1863.
8. The compound (**8b**) as an internal standard for HPLC was prepared by the coupling of **2c** with *N*-methylisatin.
9. H. Takechi, Y. Oda, N. Nishizono, K. Oda, and M. Machida, *Chem. Pharm. Bull*., 2000, **48**, 1702.