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SHORT COMMUNICATION

# Synthesis and Fluorescence Properties of new Monastrol Analogs Conjugated Fluorescent Coumarin Scaffolds

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**Abstract** A mild and efficient method has been used for the synthesis of ethyl 4-(3-hydroxphenyl)-6-methyl-2-thioxo-1,3-dihydroprimidine-5-carboxylate (monastrol) (**2**), via Biginelli reaction. Alkylation of **2** with the fluorescent coumarin **3** afforded the new thioether analog **4**. Similarly, ethyl 4-(6,8-dichloro-2-oxo-2 H-chromen-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (**6**) was prepared. The synthesized compounds are fluorescent active and show wavelength of maximum absorption ($\lambda_{max}$) in UV or visible region in MeOH at room temperature.

**Keywords** Biginelli reaction · Coumarins · MPA supported on Y zeolite · Monastrol · Fluorescence properties

## Introduction

A common strategy for cancer therapy is the development of drugs that interrupt the cell cycle during the stage of mitosis. Compounds that perturb microtubule shortening (depolymerization) or lengthening (polymerization) cause arrest of the cell cycle in mitosis due to perturbation of the normal microtubule dynamics necessary for chromosome movement [1]. Therefore, microtubule dynamics is an important target for the developing anticancer drugs [2] but differ in their mode of action from other drugs because they target the mitiotic spindle and not the DNA. A variety of such drugs that bind to tubulin and thus inhibit spindle assembly are currently under investigation in cancer therapy, however, they have been classified on the basis of their mode of action and binding site [3–5]. Among these drug is monastrol (ethyl 4-(3-hydroxyphenyl)-6-methyl-2-sulfanylidene-3,4-dihydro-1 *H*-pyrimidine-5-carboxylate) (**2**) [6–9]. Coumarin core moieties have wide biological application, in particular for the imaging of living cells [10, 11], since exhibited spectral range and high emission quantum yields [12]. In this communication, two new monastrol analogs have been prepared with study of their fluorescence properties.

## Experimental

### Physical Measurements: See ref. [13]

*Ethyl 4-(3-hydroxphenyl) -6-methyl-2-thioxo-1,3-dihydroprimidine-5-carboxylate (Monastrol) (2)*

*Method A.* A mixture of **1** (122 mg, 1.0 mmol), ethyl acetoacetate (130 mg, 1.0 mmol), and thiourea (190 mg, 2.5 mmol) in MeCN (15 ml) was mixed with HPA supported on Y zeolite (8 wt% NaY + 0.5 mM HPA) and refluxed for 7 h. After cooling, the heteropoly acid (HPA) supported on HY filtered off and washed with hot water and ethanol to remove thiourea from the surface of the catalyst. Then, the catalyst dried and was maintained for new runs. The filtrate was evaporated to dryness and the residue was recrystallized

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from EtOH to afford **2** (254 mg, 87 %), m.p. 181–185 °C (Lit. [6, 7] 184–186 °C), $R_f$ = 0.71. $^1$H NMR (DMSO-$d_6$): δ 10.29 (s, 1 H, NH), 9.60 (s, 1 H, NH), 9.44 (s, 1 H, OH), 7.10 (t, $J$ = 7.8 Hz, H-5), 6.65 (d, 1 H, $J_{2',4'}$ = 2.4 Hz, $H_{arom.}$-2′), 6.64 (m, 2 H, $H_{arom.}$-4 + $H_{arom.}$-6), 5.09 (d, 1 H, $J_{NH,4}$ = 5.5 Hz, H-4), 4.02 (q, 2 H, $J$ = 7.8 Hz, $CH_2CH_3$), 2.28 (s, 3 H, $C_6$-Me), 1.12 (t, 3 H, $J$ = 7.8 Hz, $CH_2CH_3$). $^{13}$C NMR (DMSO-$d_6$): δ 174.6 (C = S), 165.7 ($CO_2$Et), 157.9 (C-6), 145.3 ($C_3$'-OH + $C_{arom.}$-1′), 130.0 ($C_{arom.}$-5′), 117.7 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 133.6 $C_{arom.}$-4′), 101.2 (C-5), 60.1 ($CH_2CH_3$), 54.5 (C-4), 17.7, 17.6 ($C_6$-Me), 14.5 ($CH_2CH_3$). EI-MS: $m/z$ (%) = 292 $[M]^+$. Anal. Calcd. for $C_{14}H_{16}N_2O_3S$ (292.35): C, 57.52; H, 5.52; N, 9.58. Found: C, 57.32; H, 5.39; N, 9.32 %.

*Method B*. A mixture of **1** (244 mg, 2.0 mmol), ethyl acetoacetate (300 mg, 2.30 mmol), and thiourea (380 mg, 5.0 mmol) in EtOH (15 ml) in the presence conc. Hydrochloric acid (1 ml) was heated under reflux for 3 h. After cooling, the mixture was poured onto ice (20 g). The precipitate was filtered and dried and recrystallized from EtOH to give **2** (292 mg, 50 %), The NMR spectra, m.p. and mixed m.p. were almost similar for those of **2** prepared in method A.

*Ethyl 4-(3-hydroxphenyl) -2-(((7-methoxy-2-oxo-2 H-chromen-4-yl)methyl)thio) -6-methyl-1,4-dihydropyrimidine-5-carboxylate (**4**)*

A mixture of **2** (100 mg, 0.34 mmol) and 4-bromomethyl-7-methoxycoumarin (**3**) (97 mg, 0.36 mmol) in DMF (10 ml) containing $K_2CO_3$ (50 mg, 0.36 mmol) was heated 100 °C for 5 h. After cooling, the solution was evaporated to dryness and the residue was partitioned between $CHCl_3$ (20 ml) and water (3 × 20 ml). The combined organic extracts were dried ($Na_2SO_4$), filtered and evaporated to dryness. The residue was poured onto an $SiO_2$ column (10 g) and eluted, in gradient, with MeOH (0–5 %) and $CHCl_3$ as eluent to give **4** (60 mg, 35 %), m.p. 94–97 °C, $R_f$ = 0.84. $^1$H NMR (DMSO-$d_6$): δ 9.88 (d, 1 H, $J_{NH,4}$ = 4.5 Hz, NH), 7.93–7.84 (m, 8 H, $H_{arom.}$ + OH), 6.69 (s, 1 H, $H_{coum.}$-3′), 5.18 (d, 1 H, $J_{NH,4}$ = 4.5 Hz, $H_{pyrimid.}$-4), 4.06 (q, 2 H, $J$ = 7.2 Hz, $CH_2CH_3$), 2.23 (s, 3 H, $C_6$-Me), 1.16 (t, 3 H, $J$ = 7.2 Hz, $CH_2CH_3$). - $^{13}$C NMR (DMSO-$d_6$): δ = 166.7 ($CO_2$Et), 160.3 ($C_{pyrimid.}$-2 + $C_{coum.}$-2′), 160.2 ($C_{coum.}$-7′), 156.9 ($C_3$'-OH), 155.7 (C-4 + $C_{coum.}$-4′), 153.8 ($C_{coum.}$-8a'), 139.4 ($C_{arom.}$-1), 132.4 ($C_{arom.}$-5), 128.3 ($C_{coum.}$-5′), 116.9 ($C_{arom.}$-6), 115.5 ($C_{coum.}$-4a'), 114.3 ($C_{arom.}$-2), 112.8 ($C_{coum.}$-3′ + $C_{arom.}$-4), 112.0 ($C_{coum.}$-6′), 107.9 ($C_{coum.}$-8), 102.0 ($C_{arom.}$-6), 59.9 ($CH_2$-$CH_3$), 59.6 (OMe), 56.5 (C-4), 34.8 ($CH_2$S), 22.4 ($C_6$-Me), 14.6 ($CH_2CH_3$). EI-MS: $m/z$ (%) = 480 $[M]^+$. Anal. Calcd. for $C_{25}H_{24}N_2O_6S$ (480.53): C, 62.49; H, 5.03; N, 5.83. Found: C, 62.28; H, 4.89; N 5.59 %.

*Ethyl 4-(6,8-dichloro-2-oxo-2 H-chromen-3-yl) -6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (**6**)*

*Method A*. This compound was prepared following the same procedure of preparation **2** (method B) using aldehyde **5** (243 mg, 1.0 mmol), ethyl acetoacetate (130 mg, 1.0 mmol), and thiourea (190 mg, 2.5 mmol), and HPA supported on Y zeolite (8 wt% NaY + 0.5 mM HPA) in MeCN (15 ml). The mixture was heated under reflux for 9 h to give, after working up, **6** (343 mg, 83 %); m.p. 256–257 °C, $R_f$ = 0.81. $^1$H NMR (DMSO-$d_6$): δ 10.36 (s, 1 H, NH-1), 9.91 (d, 1 H, $J_{NH,4}$ = 5.0 Hz, NH-3), 8.37 (s, 1 H, $H_{coum.}$-4'), 9.97 (d, 1 H, $J_{5',7'}$ = 2.3 Hz, $H_{coum.}$-7′), 8.18 (d, 1 H, $J_{5',7'}$ = 2.3 Hz, $H_{coum.}$-5′), 4.08, 3.98 (2xq, 2 H, $J$ = 7.5 Hz, $CH_2CH_3$ [$R$ + $S$]), 2.28 (s, 3 H, $C_6$-Me), 1.17, 1.08 (2xt, 3 H, $J$ = 7.5 Hz, $CH_2CH_3$ [$R$ + $S$]). $^{13}$C NMR (DMSO-$d_6$): δ 174.6 (C = S), 166.5 ($CO_2$Et), 163.5 ($C_{coum.}$-2′), 144.4 ($C_{coum.}$-8a'), 136.2 ($C_{coum.}$-4′), 130.1 ($C_{coum.}$-8′), 129.8 ($C_{coum.}$-6′ + $C_{coum.}$-7′), 126.4 (C-4a' + $C_{coum.}$-3′), 122.3 ($C_{coum.}$-5′), 104.6 (C-5), 60.1 ($CH_2CH_3$), 53.1 (C-4), 19.8 ($C_6$-Me), 14.2 ($CH_2CH_3$). EI-MS: $m/z$ (%) = 413 $[M]^+$. Anal. Calcd. for $C_{17}H_{14}Cl_2N_2O_4S$ (413.28): C, 49.41, H, 3.41; N, 6.78. Found: C, 49.20; H, 3.33; N, 6.52 %.

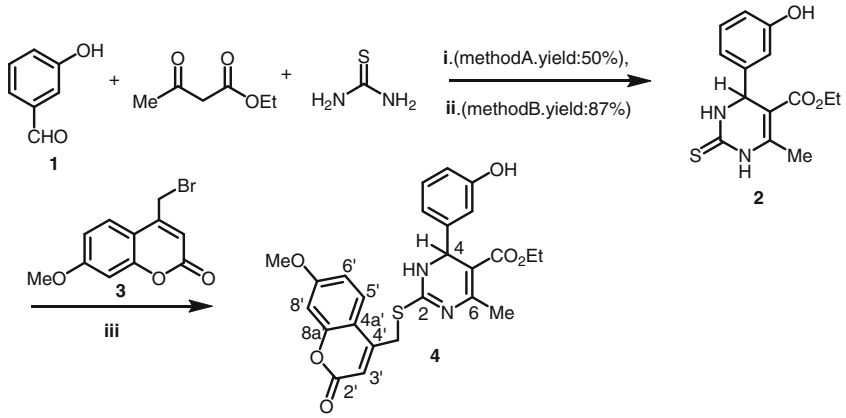
*Method B*. A mixture of ethyl acetoacetate (42 mg, 0.32 mmol), 6,8-dichloro-2-oxo-2 *H*-chromene-3-carbaldehyde (**5**) (100 mg, 0.29 mmol) and thiourea (55 mg, 0.73 mmol) in EtOH (10 ml) in the presence conc. Hydrochloric acid (0.5 ml) was heated under reflux for 3 h. After cooling, the mixture was poured onto ice (10 g). The precipitate was filtered and dried and recrystallized from EtOH to give **6** (50 mg, 42 %). The NMR spectra, m.p. and mixed m.p. were identical for those of **6** prepared in method A.

## Results and Discussion

### Chemistry

Our attention was focused on the synthesis of a new fluorescent coumarin conjugated monastrol via alkylation of thio group of the later scaffold, aiming to study their application as a tracer for detection of hypermetabolic circulating tumor cells (CTC) by fluorescence imaging. Thus, **2** has been prepared (50 %), via Biginelli reaction [14] in a one-pot three-component cyclocondensation reaction of 3-hydroxybenzaldehyde **1**, ethyl acetoacetate and thiourea in the presence of catalytic amount of HCl. The literatures reported various Lewis acids as a catalyst in an attempt to optimize the yield percentage due to the formation of monastrol in *R* and *S* isomers. However, we have optimized the yield of **2** (87 %) by using molybdophosphoric acid (MPA) supported on Y zeolite [15] as an efficient and reusable catalyst in boiling MeCN.

**Scheme 1** Reagents and condition: (i) method A: HCI, Et0H, reflux, 3 h; (ii) method B: molybdophosphoric acid (MPA) supported on Y zeolite, MeCN, reflux 7 h; (iii) 3, DMF, $K_2CO_3$, 100 °C, 5h




Alkylation of **2** with the commercially available coumarin fluorescent, 4-(bromomethyl)-7-methoxy-2 *H*-chromon-2-one (**3**) in DMF as a solvent and $K_2CO_3$ as a catalyst at 100 °C to give, after chromatographic purification, **4** (35 %) (Scheme 1).

In the $^1$H NMR spectrum of **4**, NH-3 and H-4 appeared as doublets at δ 9.88 and 5.18 ppm ($J_{NH,4}$ = 4.5 Hz), respectively, whereas the aromatic protons and OH resonated as a multiplet at the region δ 7.93–7.84 ppm. In the $^{13}$C NMR spectrum of **6**, the lower field signals at δ 166.7 ppm were assigned to C-6 and $CO_2$Et of the pyrimidine ring, while the resonance at δ 160.3 ppm was attributed to C-S ($C_{pyrimidin.}$-2) together with C-2 of coumarin ring. The resonances at δ 160.2 and 155.7 ppm were assigned to $C_{coum.}$-7' and $C_{pyrimid.}$-4 together with C_4' of coumarin, respectively, meanwhile carbon atoms 4a', 5', 6' and 8' of coumarin appeared at δ 115.5, 128.3, 112.0 and 107.9 ppm, respectively. Carbon atom of the same moiety 3' appeared together with $C_{arom.}$-4 at δ 112.8 ppm, while $CH_2$S and C-4 of pyrimidine resonated at δ 34.8 and 56.5 ppm, respectively. The difference in the chemical shifts value (~ 14 ppm) between $C_{pyrimidin.}$-2 (C-S) of **4** and $C_{pyrimid.}$-2 (C = S) of **2**, is indicative for the alkylation of **2** by the fluorescent coumarin **3**.

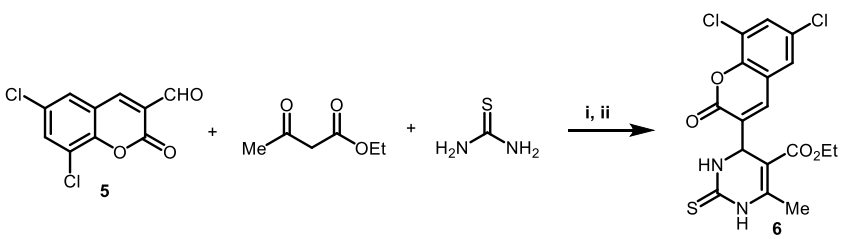
By following the same procedure of preparation of **2**, via Biginelli reaction, **6** was obtained in 42 % yield via a one-pot three-component cyclocondensation reaction of 6,8-dichlorocoumarin 3-carboaldehyde (**5**), ethyl acetoacetate and thiourea in the presence of catalytic amount of hydrochloric acid. Alternatively, **6** was obtained in 83 % yield, using MPA in boiling MeCN (Scheme 2).

The structure of **6** was assigned from the $^1$H and $^{13}$C NMR spectra, where the singlets at δ 10.36 and 8.37 ppm were assigned to NH-1 and H-4' of the coumarin moiety. The doublets at δ 9.91 and 8.81 ppm ($J_{NH,4}$ = 5.0 Hz, $J_{5',7'}$ = 2.3 Hz) were attributed to NH-3, and H-5′ of the coumarin, whereas the doublet and singlet at δ 9.97 and 2.28 ppm were assigned to H-7 of coumarin and methyl protons at C-6 of pyrimidine ring, respectively. The methylene and methyl protons of $CO_2$Et group were appeared as two doublets and triplets (as *R* + *S* isomers) at δ 4.08, 3.98 ppm and δ 1.17, 1.08 ppm ($J$ = 7.5 Hz), respectively. In the $^{13}$C NMR spectrum of **6**, the resonance at δ 174.6 ppm was attributed to C = S ($C_{pyrimid.}$-2), meanwhile the signals at δ 163.5, 136.2, 126.4 ppm were assigned to carbon atoms 2', 4', 4'a and 3' of coumarin scaffold. The carbon atoms 5'-8a' of coumarin were appeared at δ 122.3, 129.8, 130.1 and 144.4 ppm, respectively, in addition to resonance of carbonyl carbon atom ($CO_2$Et) at δ 166.5 ppm. C-5 and C-4 of pyrimidine ring together with methylene and methyl carbon atoms of ester group were oriented at δ 104.6, 60.1, 53.1 and 14.2 ppm, respectively. The structures of **4** and **6** were further identified from the HSQC [16] and HMBC [17] NMR experiments.

## Fluorescence and Photophysical Properties

Derivatization procedures in fluorescence enhancement are especially attractive because they introduce an additional

**Scheme 2** Reagents and condition: (i) method A: HCI, Et0H, reflux, 3h, 42 % yield; (ii) method B: molybdophosphoric acid (MPA) supported on Y zeolite, MeCN, reflux, 9 h, 83 % yield



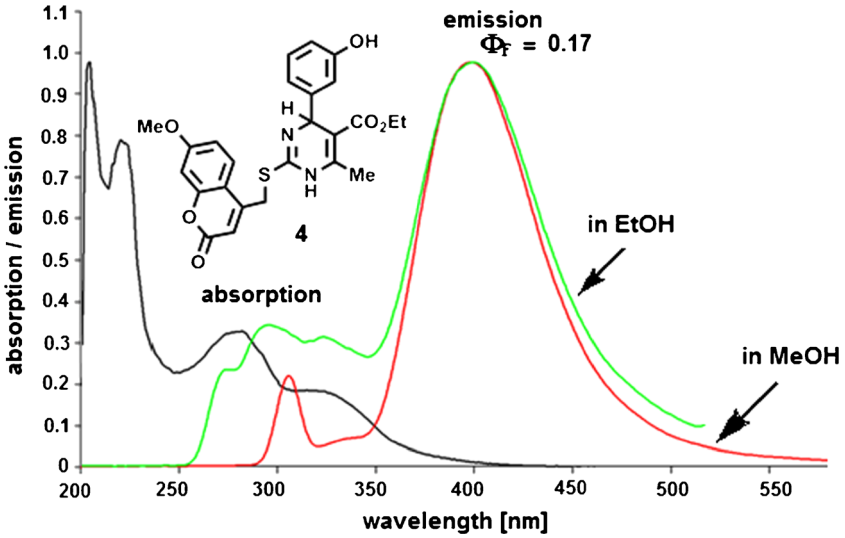



**Fig. 1** UV-visible and fluorescence spectra of compounds **4** in MeOH and EtOH

dimension of selectivity than can simplify analyses in a predictable and reproducible manner. The importance of **3** as an active fluorescent lable in biological system, especially for fatty acids [18–21] as well as alkylating agent prompted us for measurement the fluorescence property of comarinyl-monatrol **4**, with the aim to develop a new tracer for detection of hypermetabolic circulating tumor cells (CTC) by fluorescence imaging. An effective fluorescent agent for biological application has to present a good fluorescent intensity, high quantum yield and high photostability.

The absorption and emission spectra of **4** (Fig. 1) and **6** were measured in MeOH and EtOH. Table 1 illustrated the absorption ($\lambda_{ex}$), emission ($\lambda_{em}$) and quantum yields ($\Phi_F$) of these analogs, using Rhodamin 6G as standard. Compounds **4** and **6** exhibited $\lambda_{em}$ = 398 and 339 nm, with quantum yields ($\Phi_F$) = 0.17 and 0.09, respectively, and remarkable stoke's effects (76 and 28, respectively) in MeOH. Unexpectedly, **4** showed a low quantum yield although **3** possesses a spectral property of ($\lambda_{ex}$ 322 nm; $\lambda_{em}$ = 395 nm, stoke's effect 73) [21] on derivatization. It is well known that the electron donating groups substituted on coumarin will increase the intermolecular electron transfer and thus enhance the fluorescence of coumarin derivatives, especially at position 7 [22]. Although **4** having coumarin moiety with methoxy group at C-7, but exhibited a low quantum yield. Such data may due to two factors: distortion of molecule in its own plane or twisted out of plane on intramolecular charge transfer caused by solvent, whereas the other factor may due to collosional quenching occurs when the excited state fluorophore (coumarin-methylthio moiety) is deactivated upon joining with other molecule (monastrol) via the thioether linkage in solution [23].

Regarding compound **6**, the presence of two electron with drawing groups (3,5-dichloro residues) at coumarin moiety might decrease the conjugation therefore would reduced the fluorescence property of such molecules instead of its optimization. The other reason of low quantum yield of **6** may due to distortion of the planarity of the molecule as in compound **4**, as well as the decrease in π−conjugation because of the saturated carbon atom (C-4) of monastrol. Furthermore, the emission spectrum of **4** has been measured in EtOH which revealed almost a similar pattern of emission as in MeOH (Fig. 1). The fluorescence of compounds **4** and **6** in $CH_2Cl_2$ showed a red chromic shift ($\lambda_{em}$ = 386 and 322 nm, respectively), therefore, it is obvious that $\lambda_{em}$ of both compounds are shifted to longer wavelengths with increasing solvent polarity. Such result indicated that solvent polarity had obvious effect on the emission spectrum [24, 25].

The quantum yield ($\Phi_F$) has been calculated according to the equation reported by C. Vielsack, h.D. Thesis, University of Konstanz, 1999.

**Table 1** Absorption and emission data of some new monastrol analogs

| Compd. | Mass [μg] | Molar Mass [μg/μmol] | $\lambda_{em}$[nm][a] | $\lambda_{em}$[nm][b] | $\Phi_F$[a] | $\lambda_{ex}$[nm][a] | $\lambda_{ex}$[nm][a] |
|---|---|---|---|---|---|---|---|
| **4** | 176 | 480.45 | 398 | 386 | 0.17 | 278 | 322 |
| **6** | 271 | 413.28 | 339 | 322 | 0.09 | 224 | 311 |
| **3**[c] | – | 269.09 | 395 | – | – | 322 | – |

[a] in MeOH; [b] in $CH_2Cl_2$; [c] after derivatization [21]; Volume: 25 ml; Ref.-Subst. Rh6G in EtOH ($\Phi_F$: 0.95) at RT; $n^2{}_D$ (MeOH): 1.77; $n^2{}_D$ (EtOH): 1.86/ Equipment and software: Perkin Elmer LS 50, Cary UV/vis 50; SpekWin 1.71.3, FL WinLab.

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