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Chemiluminescent properties of some luminol related compounds (II)

Hideyuki Yoshida, Kiyoshi Ureshino, Junichi Ishida, Hitoshi Nohta, Masatoshi Yamaguchi *

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma Johnan-ku, Fukuoka, 814-0180, Japan

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Abstract

Four new luminol related compounds having hydrazino or hydrazono group in the structure, viz. 6-hydrazinophthalazine-1,4(2*H*,3*H*)-dione (6-HP), 1,4-dioxo-2,3-dihydrophthalazine-6-carbaldehyde hydrazone (6-PCH), 6-hydrazinomethylphthalazine-1,4 (2*H*,3*H*)-dione (6-HMP) and 6-[*N*-(3-Propionohydrazino)thioureido]benzo[*g*]phthalazine-1,4(2*H*,3*H*)-dione (PROB), were newly synthesized. The chemiluminescent properties of the compounds were examined, and compared with other luminol analogues. All evaluated compounds produced chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in sodium hydroxide solution. Chemiluminescence intensities were affected by the concentrations of hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide. Chemiluminescence reaction conditions, and the time dependence of the chemiluminescence of the compounds and luminol as a reference, were studied. 6-HP, 6-PCH, 6-HMP and PROB were found to produce chemiluminescence 1.4, 0.2, 0.1 and 32.5% of luminol, respectively.

Keywords: Chemiluminescent property; Luminol related compound

1. Introduction

In recent years, chemiluminescence has been an attractive detection method for high-performance liquid chromatography (HPLC). In HPLC with chemiluminescence detection, luminol related compounds have been widely utilized as chemiluminescence derivatization reagents, because of their high sensitivity and selectivity.

We have already developed some luminol related compounds; viz. 6,7-diaminophthalazine-1,4(2*H*,3*H*)-dione as a chemiluminogenic derivatization reagent for α-keto acids [1] and α-dicarbonyl compounds [2], and 6-aminomethylphthalazine-1,4(2*H*,3*H*)-dione (6-AMP) for 5-hydroxyindoles [3]. Next, we will aim to develop a new luminol type chemiluminescence derivatization reagent for carboxylic acids and ketones, which has a cyclic phthalhydrazide group as a light emitter of the chemiluminescence, and a hydrazino or hydrazono group moiety as a reaction site.

In this work, we newly synthesized some luminol related compounds containing a hydrazino or hydrazono group in the structure; viz. 6-hydrazinophthalazine-1,4(2*H*,3*H*)-dione (6-HP), 1,4-dioxo-2,3-dihydrophthalazine-6-carbaldehyde hydrazone (6-PCH), 6-hydrazinomethylphthalazine-1,4(2*H*,3*H*)-dione (6-HMP) and 6-[*N*'-(3-propionohydr-

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* Corresponding author.

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azino)thioureido]benzo[*g*]phthalazine-1,4(2*H*,3*H*)-dione (PROB). Further, the chemiluminescent properties (relative intensity, optimum reaction conditions and the time dependence of the chemiluminescence) of the new compounds were studied, and compared with conventional luminol analogues.

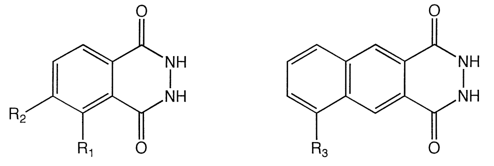
2. Experimental

2.1. General

Chemiluminescence intensities and reaction time dependences were measured using a Microluminoreader MLR-100 (Corona Electric Co., Ibaragi, Japan) equipped with a Corona DP-50 autopipetter. Uncorrected fluorescence excitation and emission spectra and its intensities were measured with a Hitachi 650-60 fluorescence spectrofluorimeter (Tokyo, Japan) in 10×10 mm-quartz cells; a spectral bandwidth of 5 nm was used in both the excitation and emission monochromators. Fast atom bombardment mass spectra (FAB-MS) were taken with a JEOL (Tokyo, Japan) DX-300 spectrometer. High resolution FAB-MS measurement was made on a JEOL JMS-HX110 instrument. Uncorrected melting points were obtained with a Gallenkamp (Loughborough, UK) melting point apparatus. Luminol was purchased from Nacalai Tesque (Tokyo, Japan). All other chemicals were of analytical reagent grade.

2.2. Examination of the chemiluminescent property

The ten examined compounds are listed in Fig. 1; viz, phthalazine-1,4(2*H*,3*H*)-dione (PD), luminol, 5-hydrazinophthalazine-1,4(2*H*,3*H*)-dione (5-HP), 6-AMP, 6-HP, 6-PCH, 6-HMP, benzo[*g*]phthalazine-1,4 (2*H*,3*H*)-dione (BP), 6-aminobenzo[*g*]-phthalazine-1,4(2H,3H)-dione (6-ABP) and PROB.



	R_1	R_2
PD	-H	-H
luminol	$-NH_2$	-H
5-HP	$-NHNH_2$	-H
6-AMP	-H	$-CH_2NH_2$
6-HP	-H	$-NHNH_2$
6-PCH	-H	$-CH=NNH_2$
6-HMP	-H	$-CH_2NHNH_2$

	R_3
BP	-H
6-ABP	$-NH_2$
PROB	$-NHCSNH(CH_2)_2CONHNH_2$

Fig. 1. Structures of chemiluminescence compounds examined.

Solutions (1×10^{-6} M) of the compounds examined were prepared as follows: PD in dimethyl sulfoxide (DMSO); luminol in 1% DMSO; 5-HP in 20% methanol; 6-AMP in 60% DMSO; 6-HP in 60% DMSO; 6-PCH in 80% DMSO; 6-HMP in DMSO; BP in DMSO; 6-ABP in DMSO; PROB in 80% *N*,*N*-dimethylformamide (DMF). Hydrogen peroxide (0.1–100 mM) and potassium hexacyanoferrate(III) (0.1–25 mM) solutions were prepared in water and sodium hydroxide (0.01–4 M) solutions, respectively

A 40 µl portion of the individual compound solution was placed into the well of a greiner (German) black FIA-plate and the chemiluminescence reaction was initiated by simultaneous injectons of 35 µl of the hydrogen peroxide solution and 75 µl of the alkaline potassium hexacyanoferrate(III) solution, automatically.

Chemiluminescence intensities were monitored immediately after the injection of the oxidizing reagent solutions.

2.3. Synthesis of chemiluminescence compounds

PD, 5-HP, 6-AMP, BP and 6-ABP were prepared according to the literature [3–6].

2.3.1. 6-Hydrazinophthalazine-1,4(2H,3H)-dione (6-HP)

6-HP was synthesized from 4-aminophthalimide (compound **Ia**) via compounds **Ib** and **Ic** by the following method, as illustrated in Fig. 2(a).

To a stirred suspension of compound **Ia** (1.2 g) in 1 M hydrochloric acid (62.5 ml) in ice-water bath was added 8 M sodium nitrite (1 ml). After stirring for 5 min, sodium sulfite (5.5 g) and sodium carbonate (10 g) in water (80 ml) was added dropwise to the reaction solution at 0°C, and the mixture was then stirred for 10 min at room temperature. The resulting orange solution was treated with

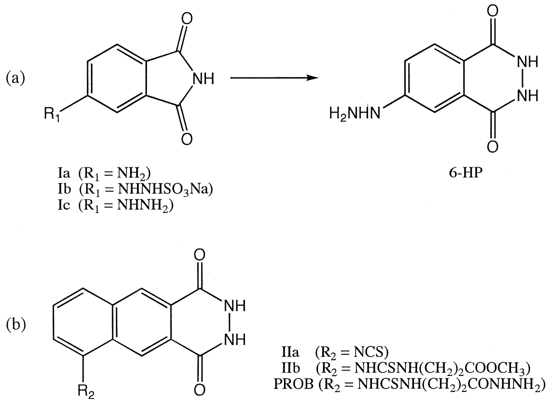


Fig. 2. Synthesis of (a) 6-HP and (b) PROB.

conc. hydrochloric acid (15 ml), and heated at 100°C for 30 min. After standing overnight at 4°C, the resulting liquor was filtered to give compound **Ib** (1.17 g, yield 57%) as pale yellow plates. FAB-MS showed $m/z = 278$ $[M-1]^-$, 256 [M−Na], (base peak).

A solution of compound Ib (1.45 g) in conc. hydrochloric acid (10 ml) was refluxed at 100°C for 3 min. After cooling to room temperature, the solution was neutralized with 5.5 M ammonia solution in an ice-water bath. The resulting precipitate was collected, washed with water, and dried to give compound **Ic** (0.71 g, yield 77%) as a deep yellow powder, m.p. 267°C. FAB-MS, $m/z = 178$ $[M+1]^+$,(base peak).

Compound **Ic** (0.1 g) was suspended in hydrazine monohydrate (1 ml), and the suspension was heated under reflux at 100°C for 2 h. After cooling to room temperature, the reaction solution was evaporated under a nitrogen stream to give 6-HP (0.10 g, yield 91%) as a white powder, m.p. 318°C (decomp.). FAB-MS, $m/z = 191$ $[M-1]^-$,(base peak). High-resolution FAB-MS, $m/z = 191.0569$ (as calcd. for $C_8H_7O_2N_4$).

The total yield of the 6-HP synthesis from 4-aminophthalimide was ca. 40%.

2.3.2. 1,4-Dioxo-2,3-dlhydrophthalazine-6-carbaldehyde hydrazone (6-PCH)

4-Formyl-*N*-phenylphthalimide [7] (0.05 g) and hydrazine monohydrate (0.1 ml) were dissolved in ethanol (5 ml), and the solution was refluxed for 30 min. After cooling to 0°C, the resulting precipitate was filtered, washed with ethanol, dried to give 6-PCH (0.03 g, yield 74%) as a white crystalline material, m.p. 383°C (decomp.). FAB-MS, $m/z = 203$ $[M-1]^-$, (base peak). High-resolution FAB-MS, $m/z = 203.0577$ (as calcd. for $C_9H_7O_2N_4$).

*2.3.3. 6-Hydrazinomethylphthalazine-1,4(2*H*,3*H*)-dione (6-HMP)*

4-Bromomethyl-*N*-phenylphthalimide [3] (0.05 g) and hydrazine monohydrate (0.1 ml) were suspended in ethanol (5 ml), and the suspension was heated under reflux at 100°C for 30 min. After cooling in an ice-water bath, the resulting precipitate was filtered, washed with ethanol and dried in vacuo to give 6-HMP (0.02 g, yield 61%) as a pale yellow crystalline material, m.p. 273°C (decomp.). FAB-MS, m/z 205 $[M-1]^-$, (base peak). High-resolution FAB-MS, $m/z = 205.0723$ (as calcd. for $C_9H_9O_2N_4$)

2.3.4. 6-[N'-(3-Propionohydrazino)thiouveido]-benzo[g]phthalazine-1,4(2H,3H)-dione (PROB)

PROB was synthesized from 6-isothiocyanatobenzo[*g*]phthalazine-1,4(2*H*,3*H*)-dione [8] (compound IIa) via compound **IIb** by the following method, as illustrated in Fig. 2(b).

Compound **IIa** (0.10 g) and *β*-alanine methyl ester (0.08 g) were dissolved in DMSO (5 ml) and the solution was incubated at 80°C for 20 min. After cooling to room temperature, the reaction solution was dried under a nitrogen stream to give compound **IIb** (0.13 yield 93%).

Compound **IIb** (0.13 g) and hydrazine monohydrate (0.1 ml) were suspended in ethanol (10 ml) and the suspension was refluxed for 2 h. After cooling in an ice-water bath, the resulting precipitate was filtered to give PROB (0.1 g, yield 77%) as a pale yellow crystalline, m.p. 318°C (decomp.). FAB-MS, $m/z = 373$ $[M+1]^+$,(base peak). High-resolution FAB-MS, $m/z = 373.1091$ (as calcd. for $C_{16}H_{17}O_3N_6S$).

The total yield of the PROB synthesis from 6-isothiocyanatobenzo[*g*]phthalazine-1,4(2*H*,3*H*)-dione was ca. 72%.

The newly synthesized compounds (6-HP, 6-PCH, 6-HMP and PROB) were stable in the crystalline state at room temperature for at least 6 months in a light-protected desiccator containing silica gel.

3. Results and discussion

The chemiluminescent properties of 6-HP, 6-PCH, 6-HMP, PROB and conventional luminol analogues (PD, luminol, 5-HP, 6-AMP, BP and 6ABP) were examined in order to evaluate the use of the compounds for highly sensitive analysis. The ten compounds synthesised produce chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in an alkaline medium (Fig. 3). The concentrations of hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide had a great influence on the chemiluminescence intensity (Fig. 4); 1–5 mM hydrogen peroxide, 2.5–10 mM potassium hexacyanoferrate(III) and 1.5–2 M sodium hydroxide gave almost maximum intensity for all the compounds. Various organic solvents were examined to dissolve these chemiluminophores. The solvents and their concentrations in the compound solution affected to the chemiluminescence efficiency, and the results differed from each compound. Conditions shown in the experimental section were selected to prepare the chemiluminescence compound solutions.

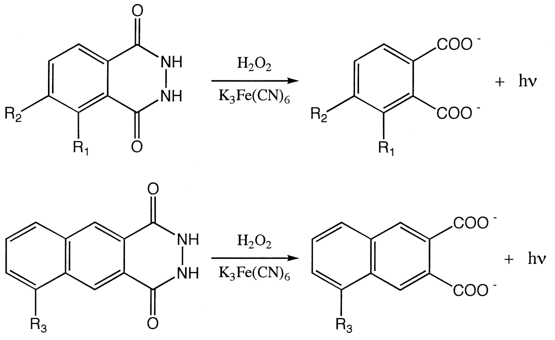


Fig. 3. Chemiluminescence reaction of luminol related compounds.

Under these optimum reaction conditions, the chemiluminescence intensities of the ten compounds were determined (Table 1). Four of the new compounds (6-HP, 6-PCH, 6-HMP and PROB) were found to generate chemiluminescence approximately 1/3–1/1000 times of luminol. Fig. 5 illustrates the time dependence of the chemiluminescence reaction of the new compounds and luminol. The generation of chemiluminescence was initiated by addition of hydrogen peroxide and alkaline potassium hexacyanoferrate(III) solutions to the tested solution. The chemiluminescence intensity was at its maximum at < 1.5 s after the addition of oxidizing reagents, and then decreased rapidly.

In the chemiluminescence of luminol, the 3-aminophthalate ion produced during the oxidizing reaction has been shown to be the light emitter. Therefore, in the case of the new luminol-type compounds, the corresponding dicarboxylate ions (Fig. 3) could be expected to be light emitting species. The fluorescent properties (excitation and emission maxima of the fluorescence and its relative

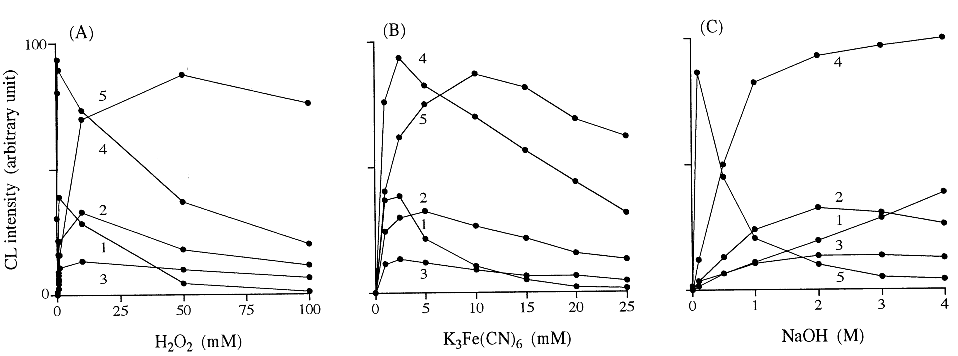


Fig. 4. Effects of concentrations of (A) hydrogen peroxide, (B) potassium hexacyanoferrate(III) and (C) sodium hydroxide on integrated chemiluminescence intensities (a run time of 60 s). Curves: 1, 6-HP(1×10^{-5} M); 2, 6-PCH(1×10^{-4} M); 3, 6-HMP(1×10^{-4} M); 4, PROB(1×10^{-6} M); 5, luminol(3×10^{-7} M).

Table 1
Relative chemiluminescence intensities (RCI) of luminol related compounds examined

Compound	RCI[a]
PD	0.1
luminol	100.0
5-HP	8.8
6-AMP	0.1
6-HP	1.4
6-PCH	0.2
6-HMP	0.1
BP	1.8
6-ABP	3.6
PROB	32.5

[a] Integrated chemiluminescence intensity (a run time of 60 s) of luminol was taken as 100.

Table 2
Fluorescence excitation (Ex) and emission (Em) maxima and relative intensities (RFI) of the compounds after the chemiluminescence reaction

Compound	Ex (nm)	Em (run)	RFI[a]
PD	355	405	14.6
luminof	315	450	100.0
5-HP	315	415	44.6
6-AMP	320	475	0.4
6-HP	300	435	22.8
6-PCH	320	490	16.8
6-HMP	325	480	0.7
BP	360	415	269.0
6-ABP	295	370	42.3
PROB	280	510	107.0

[a] Fluorescence intensity of luminol was taken as 100.

intensity) of the species, after the chemiluminescence reaction was complete, were measured, and compared with the former conventional luminol analogues (Table 2). The results suggest that the efficiency of the chemiluminescence compound is dependent on the fluorescence intensities of the light-emitting species produced during the reaction. Otherwise, it is known that the chemiluminescence wavelength of luminol related compound is based on the fluorescence emission of the corresponding phthalate ion [9]. Thus, PROB was expected to generate chemiluminescence at 510 nm, red-shifted with respect to luminol, which means that the use of PROB as the chemiluminescence labelling reagent might be profitable.

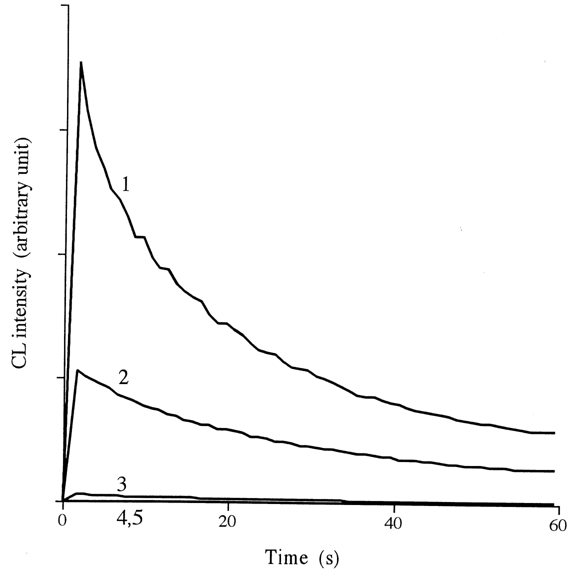


Fig. 5. Time dependence of the chemiluminescence reaction of the compounds. Curves:1, luminol; 2, PROB; 3, 6-HP; 4, 6-PCH; 5, 6-HMP.

4. Conclusion

The newly synthesized luminol-type compounds (6-HP, 6-PCH, 6-HMP and PROB) were found to produce chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in alkaline medium, and might be useful to utilize as chemiluminescence labelling reagents for carboxylic acid or ketone moiety. The application of the compounds as the labelling reagents for the acids or ketones is now in progress.

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