Synthesis of Polycyclic Pyridazinediones as Chemiluminescent Compounds

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Reactions of 1,3-disubstituted 5-aminopyrazole-4-carbonitrile derivatives **3a-o** with dimethyl acetylenedicarboxylate in the presence of potassium carbonate in dimethyl sulfoxide gave the corresponding dimethyl 1,3-disubstituted pyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates **4a-o** which were allowed to react with excess hydrazine hydrate under ethanol refluxing conditions followed by heating at 250-300° to give 1,3-disubstituted 4-amino-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones **7a-s** in good yields. Similarly, 1,3-disubstituted 4-hydroxy-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones **10a-c** were obtained from alkyl 1,3-disubstituted 5-aminopyrazole-4-carboxylates **8a-c**. These tricyclic pyridazine derivatives were alternatively synthesized from 4-hydroxypyrrolo[3,4-*e*]pyrazolo[3,4-*b*]pyridine-5,7-diones **13a-c** prepared by reactions of 5-aminopyrazoles (**8e-g**) with methyl 1-methyl-4-methylthio-2,5-dioxo-1*H*-pyrrole-3-carboxylate (**11a**) followed by the Gould/Jacobs reaction.

1-Methyl-4-methylthio-2,5-dioxo-1*H*-pyrrole-3-carbonitrile smoothly reacted with 2-aminobenzimidazoles to give the corresponding 5-amino-3-methyl-1*H*-pyrrolo[3',4':4,5]pyrimido[1,2-*a*]benzimidazole-1,3(2*H*)-diones **16a-e**, which were readily converted to the desired 12-aminopyridazino[4',5':4,5]pyrimido-[1,2-*a*]benzimidazole-1,4(2*H*,3*H*)-diones **17a-e** in good yields. Other pyridazinopyrimidine derivatives were also obtained by the reaction of the corresponding 2-aminoheterocycles with the maleimide in good yields.

Substituted anilines reacted **11b** in refluxing methanol to give the corresponding methyl 4-phenylamino-1-methyl-2,5-dioxo-1*H*-pyrrole-3-carboxylates **25a-e** which were converted in good yields to 2-methylpyrrolo[3,4-*b*]quinoline derivatives **26a-e** by heating in diphenyl ether. Reaction of **26a-c** with hydrazine hydrate gave 10-hydroxypyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones **27a-e** in good yields. The desired 10-aminopyridazino[4,5-*b*]pyridazine-1,4(2*H*,3*H*)-diones **30a-e** were obtained in good yields by the chlorination of **4a-e** with phosphorus oxychloride followed by aminolysis with 28% ammonium hydroxide.

Some pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones **37a,b** as luminescent compounds were synthesized *via* several steps from indolizine derivatives. The key intermediates, dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylates **34**, **36**, were synthesized by the [8 + 2] cycloaddition reaction of the corresponding 7-dimethylaminoindolizines **33**, **35** with dimethyl acetylenedicarboxylate in the presence of Pd-C in refluxing toluene.

Some were found to be more efficient than luminol in light production. 4-Amino-3-methylsufonyl-1-phenyl-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione (**7r**), 10-hydroxypyridazino[4,5-*b*]-quinoline-1,4(2*H*,3*H*)-diones **27a-e**, and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones **30a-e** showed the greatest chemiluminescence intensity in the presence of hydrogen peroxide peroxidase in a solution of phosphate buffer at *p*H 8.0.

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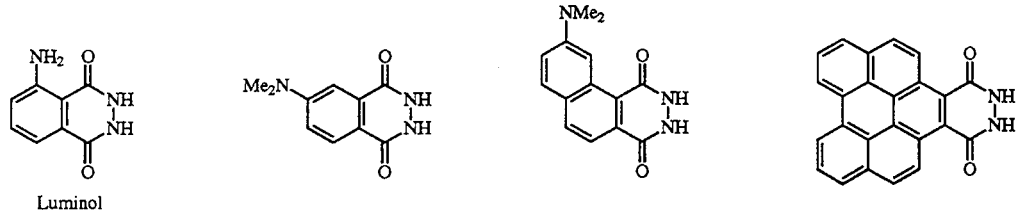
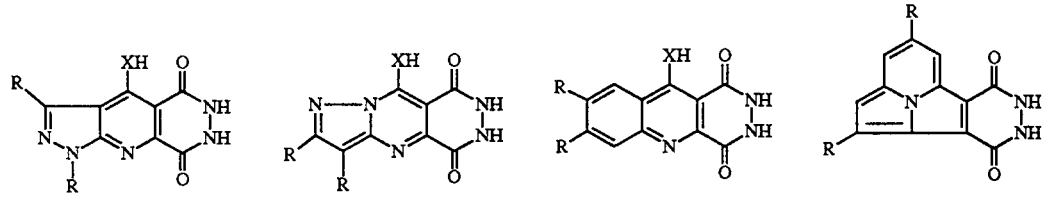
Polycyclic pyridazines have been extensively studied because they are of great importance in biological and medicinal chemistry [1]. We have considerable interest in this field, and have focused our attention on the synthesis and chemiluminescent properties of polycyclic fused pyridazine-1,4-dione derivatives [2]. Many polycyclic hydrazides have been synthesized in efforts to increase the efficiency of light production [3].

Since its discovery by Albrecht in 1928 [4], the analytical usefulness of the chemiluminescence of luminol has been the subject of extensive study [5]. The chemiluminescence assays are an attractive analytical method not only in the field of inorganic chemistry for determining trace metals but also in diagonostic medicine and biological sciences in general because of their high sensitivity, rapid reaction and wide dynamic range [6]. The synthetic development of luminol derivatives and their analogs has been pursued actively to meet the requests of clinical analyses for something more useful than luminol. We now describe here the synthesis of polycyclic pyridazinediones as chemiluminescent derivatives based on the results obtained in our own laboratory.



Luminol

Figure 1.



XH = NH_2, OH

Figure 2.

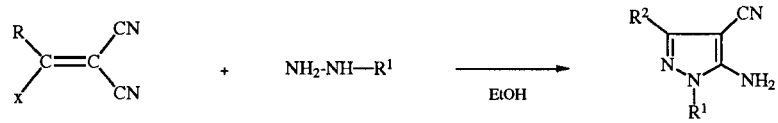
1. Pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione Derivatives.

In the course of our study on the synthesis of a tricyclic pyridazine-containing ring system from dimethyl pyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates [7], attention was directed to the chemiluminescence properties of 4-aminopyrazolo[4',3':5,6]pyrido[3,4-*b*]pyridazine-5,8(*6H,7H*)-diones containing the fundamental pyridopyridazine ring in an effort to assess chemiluminescence activity. No study on the chemiluminescence of pyrazolopyridopyridazines and their related compounds has been conducted to date [8]. We chose pyrazolo[4',3':5,6]pyrido[3,4-*b*]pyridazine-5,8(*6H,7H*)-diones in a tricyclic pyridazine-containing ring system as a key compound in the search for chemiluminescent compounds. The details in regard to chemiluminescence of 1,3-disubstituted 4-amino- or 4-hydroxypyrazolo[4',3':5,6]pyrido[3,4-*b*]pyridazine-5,8(*6H,7H*)-diones are presented in the following publications [2,7].

The starting materials **3a-o** were readily prepared in good yield by reactions of ketene dithioacetals and their derivatives and ethoxymethylene compounds with hydrazine derivatives as shown in Scheme 1 [9]. Reactions of 5-aminopyrazole-4-carbonitriles **3a-o** with dimethyl acetylenedicarboxylate in the presence of potassium carbonate as a base in dimethyl sulfoxide gave the corresponding dimethyl 4-aminopyrazolo[3,4-*d*]pyridine-5,6-dicarboxylates in the yields shown in Table 1.

The oxidation of **4h,l** with one equivalent *m*-chloroperbenzoic acid in dichloromethane at room temperature gave the desired sulfoxide products **5a,b** in good yields. Use of excess *m*-chloroperbenzoic acid in this reaction, of course, gave the corresponding sulfonyl compounds **6a,b** in 79 and 97% yields, respectively.

Scheme 1

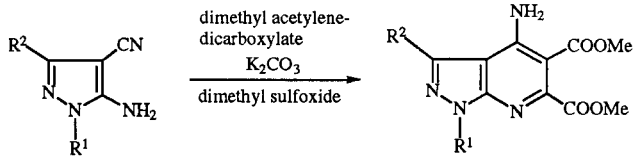


1a-h **2a-d** **3a-o**

1a: R = H, X = OEt
b: R = Me, X = OEt
c: R = C_6H_5, X = OEt
d: R = C_6H_4-NMe_2, X = CN
e: R = 2-thienyl, X = SMe
f: R = 2-benzothienyl, X = SMe
g: R = SMe, X = SMe
h: R = SC_6H_5, X = SC_6H_5
i: R = SC_6H_4-Me(4), X = SC_6H_4-Me(4)
j: R = SC_6H_4-Cl(4), X = SC_6H_4-Cl(4)

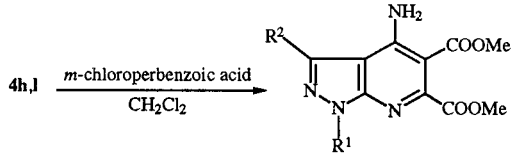
2a: $R^2 = C_6H_5$
b: $R^2 = SO_2C_6H_4$-Me(*p*)
c: $R^2 = C_6H_4$-NO_2(*p*)
d: R^2 = 2-benzothiazolyl

Scheme 2



3a-o **4a-o**

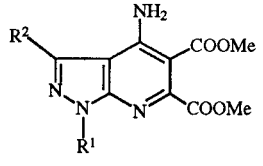
	R^1	R^2
a:	C_6H_5	H
b:	$SO_2C_6H_4$-Me(*p*)	H
c:	C_6H_5	Me
d:	C_6H_5	C_6H_5
e:	C_6H_5	C_6H_4-NMe_2(*p*)
f:	C_6H_5	2-thienyl
g:	C_6H_5	2-benzothienyl
h:	C_6H_5	SMe
i:	$SO_2C_6H_4$-Me(*p*)	SMe
j:	C_6H_4-NO_2(*p*)	SMe
k:	2-benzothiazolyl	SMe
l:	C_6H_5	SC_6H_5
m:	C_6H_5	SC_6H_4-Me(*m*)
n:	C_6H_5	SC_6H_4-Cl(*p*)
o:	2-benzothiazolyl	SC_6H_4-Me(*m*)



5a,b

5a: $R^1 = C_6H_5$, R^2 = SOMe
b: $R^1 = C_6H_5$, $R^2 = SOC_6H_5$

MCPA in CH_2Cl_2

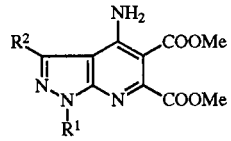


6a: $R^1 = C_6H_5$, $R^2 = SO_2Me$
b: $R^1 = C_6H_5$, $R^2 = SO_2C_6H_5$

6a,b

Table 1

Dimethyl 1,2-Disubstituted 4-Aminopyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates

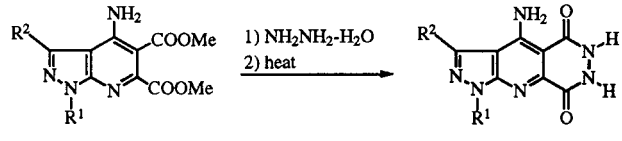


4a-o

No.	R^1	R^2	Yield (%)	mp (°C)	MS: *m/z* M^+ (%)
a	C_6H_5	H	31	178-180	326 (100)
b	$SO_2C_6H_4$-Me(*p*)	H	38	220-222	404 (46)
c	C_6H_5	Me	14	190-191	340 (100)
d	C_6H_5	C_6H_5	29	177-179	402 (100)
e	C_6H_5	C_6H_4-NMe_2(*p*)	21	207-208	445 (100)
f	C_6H_5	2-thienyl	17	171-172	408 (100)
g	C_6H_5	2-benzothienyl	20	218-220	458 (100)
h	C_6H_5	SMe	26	120-122	372 (100)
i	$SO_2C_6H_4$-Me(*p*)	SMe	45	163-165	450 (26)
j	C_6H_4-NO_2(*p*)	SMe	46	235-237	417 (100)
k	2-benzothiazolyl	SMe	53	213-215	429 (100)
l	C_6H_5	SC_6H_5	42	160-161	434 (100)
m	C_6H_5	SC_6H_4-Me(*m*)	28	138-139	448 (48)
n	C_6H_5	SC_6H_4-Cl(*p*)	42	189-190	470 (38), 468 (100)
o	2-benzothiazolyl	SC_6H_4-Me(*m*)	32	224-227	505 (100)

Dimethyl 4-amino-3-methylthio-1-phenylpyrazolo[3,4-*b*]-pyridine-5,6-dicarboxylate (**4h**) was refluxed with excess hydrazine hydrate in ethanol followed by removal of ethanol by distillation. The residue was heated at 250-300° for 30 minutes to afford 4-amino-3-methylthio-1-phenylpyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione (**7h**) in 92% yield. In a similar manner, other compounds such as **7a-g,i-s** were readily obtained from the corresponding dimethyl pyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates in good yields as shown in Table 2.

Scheme 3

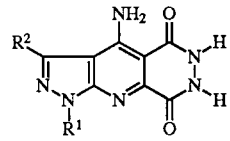


4a-o, 5a,b, 6a,b **7a-s**

No.	R^1	R^2
a	C_6H_5	H
b	H	H
c	C_6H_5	Me
d	C_6H_5	C_6H_5
e	C_6H_5	C_6H_4-NMe_2(*p*)
f	C_6H_5	2-thienyl
g	C_6H_5	2-benzothienyl
h	C_6H_5	SMe
i	H	SMe
j	C_6H_4-NO_2(*p*)	SMe
k	2-benzothiazolyl	SMe
l	C_6H_5	SC_6H_5
m	C_6H_5	SC_6H_4-Me(*m*)
n	C_6H_5	SC_6H_4-Cl(*p*)
o	2-benzothiazolyl	SC_6H_4-Me(*m*)
p	C_6H_5	SOMe (**5c**)
q	C_6H_5	SOC_6H_5 (**5b**)
r	C_6H_5	SO_2Me (**6a**)
s	C_6H_5	$SO_2C_6H_5$ (**6b**)

Table 2

1,2-Disubstituted 4-Amino-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones



7a-s

No.	R^1	R^2	Yield (%)	mp (°C)	MS: *m/z* M^+ (%)
a	C_6H_5	H	94	353-356	294 (100) [7]
b	H	H	76	>360	370 (100) [7]
c	C_6H_5	Me	85	340-342	308 (86)
d	C_6H_5	C_6H_5	85	358-368 dec	370 (100)
e	C_6H_5	C_6H_4-NMe_2(*p*)	92	355-368 dec	413 (100)
f	C_6H_5	2-thienyl	91	355-366 dec	376 (100)
g	C_6H_5	2-benzothienyl	95	350-361 dec	426 (8)
h	C_6H_5	SMe	92	353-358	340 (100)
i	H	SMe	91	>360	264 (100)
j	C_6H_4-NO_2(*p*)	SMe	72	>360	385 (100)
k	2-benzothiazolyl	SMe	88	320-325	397 (100)
l	C_6H_5	SC_6H_5	86	320-324	403 (27)
m	C_6H_5	SC_6H_4-Me(*m*)	86	288-295	416 (53)
n	C_6H_5	SC_6H_4-Cl(*p*)	97	>360	438 (40), 436 (100)
o	2-benzothiazolyl	SC_6H_4-Me(*m*)	94	>360	473 (100)
p	C_6H_5	SOMe (**5c**)	88	>360	356 (100)
q	C_6H_5	SOC_6H_5 (**5b**)	76	314-316	418 (100)
r	C_6H_5	SO_2Me (**6a**)	90	>360	372 (100)
s	C_6H_5	$SO_2C_6H_5$ (**6b**)	78	>360	434 (100)

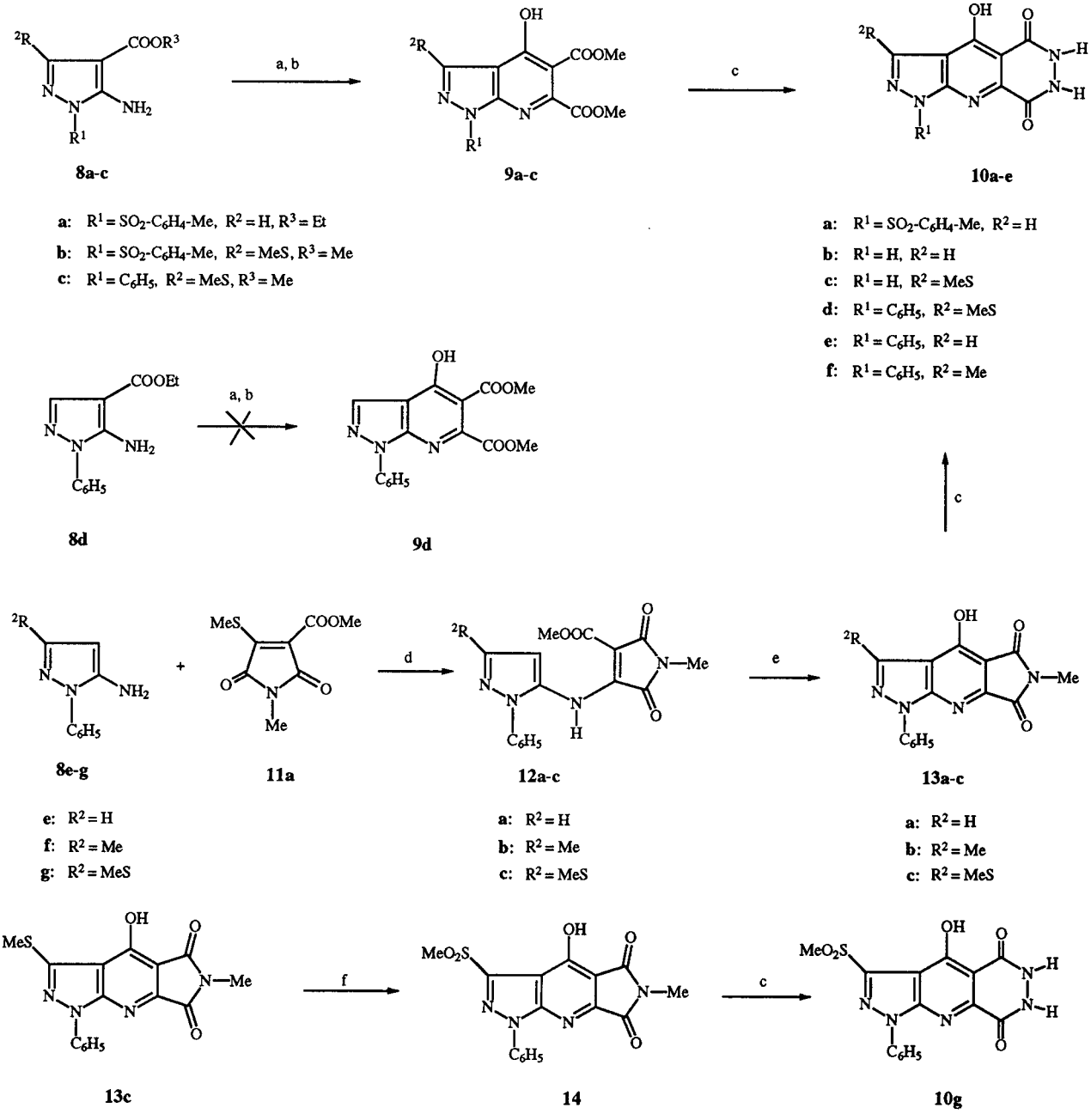
The authors directed their attention to the chemiluminescence of the compounds bearing a hydroxyl group instead of an amino group. In some cases, the hydroxyl group was shown to be capable of affecting fluorescence and light production [10]. No study on chemiluminescence of polycyclic pyridazinediones or related compounds bearing a hydroxy group has been conducted to date. Details regarding the chemiluminescence of 1,3-disubstituted 4-hydroxypyrazolo[4',3':5,6]pyrido[2,3-*d*]-pyridazine-5,8(6*H*,7*H*)-diones are also presented.

The synthesis of 4-aminopyrazolo[4',3':5,6]pyrido[2,3-*d*]-pyridazine-5,8(6*H*,7*H*)-diones was considered highly applicable to synthesis of the key compounds, dimethyl 4-hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates. The reactions of ethyl 5-amino-1-*p*-toluenesulfonylpyrazole-4-carboxylate (**8a**) with dimethyl acetylenedicarboxylate in the presence of potassium carbonate as a base in dimethyl sulfoxide gave the corresponding dimethyl 1-*p*-toluenesulfonyl-4-hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylate (**9a**) in 61% yield. Similarly, the 3-methylthio derivative **9b** was prepared by the reaction of **8b** [11] with dimethyl acetylenedicarboxylate in 48% yield. The reaction of **8c** [12] with dimethyl acetylenedicarboxylate gave the desired product **9c** in only 3% yield. Compound **9d** could not be obtained at all by the reaction of ethyl 5-amino-1-phenylpyrazole-4-carboxylate (**8d**) [13] with dimethyl acetylenedicarboxylate under the same conditions. To obtain the 3-unsubstituted 4-hydroxypyrazolopyridopyridazine-5,8-diones and increase the yield of **9c**, an alternative method of synthesis for the pyrazolopyridopyridazine derivatives had to be established. Reaction of 5-amino-1-phenylpyrazoles **8e-g** [14-16] with methyl 1-methyl-4-methylthio-2,5-dioxo-1*H*-pyrrole-3-carboxylate (**11a**) [15] by refluxing in methanol gave the corresponding displacement products **12a-c** of the methylthio group in **11a** in 82% yield. The cyclization of **12a-c** by heating in diphenyl ether afforded 4-hydroxy-3-methyl-1-phenylpyrrolo[3,4-*e*]pyrazolo[3,4-*b*]pyridine-5,7-diones **13a-c** in good yields.

Dimethyl 4-hydroxy-1-*p*-toluenesulfonylpyrazolo[3,4-*b*]-pyridine-5,6-dicarboxylate (**9a**) was refluxed with excess hydrazine hydrate in ethanol followed by removal of ethanol by distillation to give 4-hydroxy-1-*p*-toluenesulfonylpyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione (**10a**) in 87% yield. When hydrazine hydrate was used in large excess, desulfonylation simultaneously occurred to give **10b** in 67% yield. In a similar manner, 4-hydroxy-3-methylthiopyrazolo[4',3':5,6]pyrido-[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione (**10c**) was obtained from **9b** in 62% yield. 1,3-Disubstituted compound **10d** was obtained from **9c** in 91% yield. Compounds **10d, e** and **f** were synthesized by reactions of **13a-c** with

hydrazine hydrate under refluxing in methanol in 78, 82, and 76% yields, respectively. The oxidation of **13c** with excess *m*-chloroperbenzoic acid in chloroform at room temperature gave the desired sulfonyl product **14** in good yield. The 3-methylsulfonyl derivative **10g** was obtained from **14** in 86% yield [17].

Scheme 4



a: Dimethyl acetylenedicarboxylate, K_2CO_3 in dimethyl sulfoxide, room temperature, 20 hours; b: 10% HCl; c: $H_2NNH_2 \cdot H_2O$, reflux in MeOH; d: Reflux in MeOH, 30 minutes to 1 hour; e: Reflux in diphenyl ether, 30 minutes; f: *m*-Chloroperbenzoic acid in $CHCl_3$.

The 1,3-unsubstituted compound **7b** was found to be a weak chemiluminescent in spite of the strongly basic solution (*p*H 10), although the 1-phenyl derivative **7a** shows better chemiluminescence than **7b**. It may be said in this regard that the deaminated compound, pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione, failed to produce any light emission in the present system. The sulfur atom at the 3-position is very important for chemiluminescence production. The pyrazole ring may also be an essential component in the pyrazolopyridopyridazine series, since 4-amino-3-cyano-2-methylthiopyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione, an aza analog of luminol, was found not to emit light. Light emission from methylsulfenyl and methylsulfonyl compounds, **7p-s**, did not decrease and was best at *p*H 8.0. The chemiluminescent intensity in this pyrazolopyridopyridazine series decreased in the order **7r > 7p > 7h > 7q > 7i > 7l > 7c > 7a > 7g > 7f > 7b > 7e > 7d > luminol** as shown in Table 3 [22]. These compounds increased in chemiluminescence intensity with rise in *p*H, as also noted for luminol. The use of arthromyces ramosus peroxidase (ARP) or horseradish peroxidase as the peroxidase gives satisfactory results. Presented in Figures 3 and 4 is a comparison of the chemiluminescence intensity using luminol and pyrazolopyridopyridazine compounds for the sensitive detection of hydrogen peroxide and horseradish peroxidase. These compounds can be used to determine hydrogen peroxide and enzymatic activity with high sensitivity, exceeding that of luminol.

Table 3

Chemiluminescent Intensity of Compounds 7

Compounds	Chemiluminescence (c.p.m.) [a]
Luminol	4.5×10^5
7a	2.4×10^6
7b	1.9×10^6
7c	4.7×10^6
7d	8.9×10^6
7e	5.2×10^5
7f	1.7×10^6
7g	1.8×10^6
7h	2.8×10^7
7i	1.5×10^7
7l	7.4×10^6
7p	3.9×10^7
7q	2.8×10^7
7r	4.6×10^7

[a] Counts per minute.

Solutions (1 ml) containing 0.1 mg/ml of Luminol or the respective compound, 1U/ml horseradish peroxidase, 0.5 mg/ml Triton X-100 in 20 m*M* phosphate buffer *p*H 8.0 were prepared. 400 µl of each reaction solution was incubated in a glass tube at 37° for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer, maintaining the system at 37°. Photons were counted for 1 minute after addition of 10 µl of 90 µ*M* hydrogen proxide and 10 µl of 1.0 *M* sodium hydroxide.

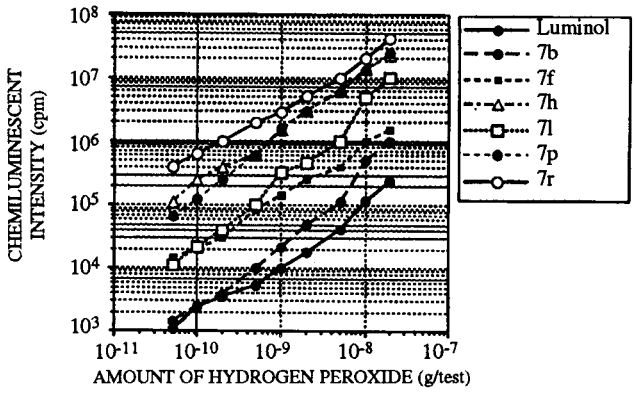


Figure 3. H_2O_2 Standard Curve

Conditions: Solutions (1 ml) containing 0.1 mg/ml of the respective compound, 1U/ml of horseradish peroxidase and 0.5 mg/ml of Trition X-100 in 20 m*M* phosphate buffer at *p*H 8.0 were prepared. 400 µl of each reaction solution was incubated in a glass tube at 37° for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer, maintianing the system at 37°. Photons were counted for 1 minute after addition of 10 µl of various concentrations of hydrogen peroxide and 10 µl of 1.0 *M* sodium hydroxide.

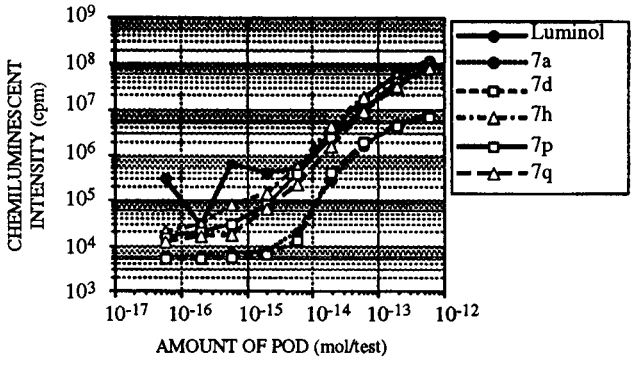


Figure 4. Horseradish Peroxidase Standard Curve

Conditions: Solutions (1 ml) containing 0.1 mg/ml of the respective compound, 1U/ml of horseradish peroxidase and 0.5 mg/ml of Triton X-100 in 20 m*M* phosphate buffer at *p*H 8.0 were prepared. 400 µl of each reaction solution was incubated in a glass tube at 37° for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer, maintaining the system at 37°. Photons were counted for 1 minute after addition of 10 µl of 300 m*M* hydrogen peroxide and 10 µl of various concentration horseradish peroxidase solution.

The chemiluminescent intensity in this pyrazolopyridopyridazine series is shown in Table 4 [23]. 4-Hydroxy-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones **10d-g** showed nearly the same or somewhat stronger light intensity than luminol at *p*H 8.0. The 1-unsubstituted compounds **10b,c** did not show chemiluminescence at *p*H 8.0. Compounds **10a-g** also showed chemiluminescence similar to luminol at *p*H 10.0. The aryl group at the 1-position is very important for chemiluminescent production. The light emission of methylsulfonyl compound **10g** did not decrease. These compounds showed increased light intensity with rise in *p*H, as noted also for luminol. The use of horseradish peroxidase as the peroxidase gave satisfactory results for chemiluminescent production.

Table 4

Chemiluminescent Intensity of Compounds **10**

Compound	Chemiluminescence (c.p. 10 sec) [a]	*p*H 10 *p*H 8
10a	1.0×10^5	
	9.6×10^2	
10b	1.7×10^5	
	0	
10c	7.2×10^4	
	0	
10d	9.5×10^6	
	2.6×10^3	
10e	1.6×10^6	
	9.0×10^4	
10f	1.2×10^5	
	8.5×10^3	
10g	9.9×10^4	
	1.5×10^4	
Luminol	1.5×10^7	
	8.5×10^3	

[a] Counts per 10 seconds. (Their values were subtracted from each background.)

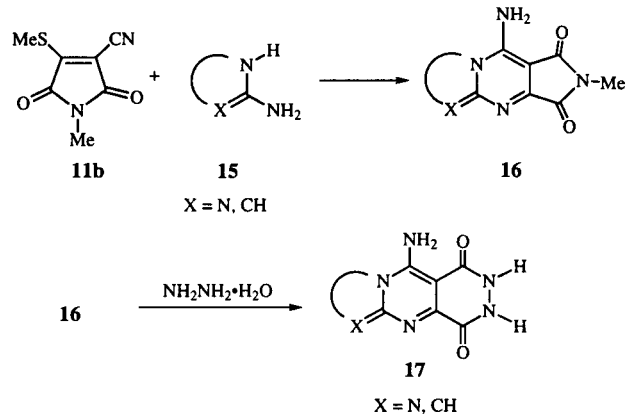
A reaction solution containes 10 mmol/l of phosphate buffer *p*H 8.0, 0.5 ml/l of Triton X-100, 2.5×10^{-7} mol/l test compound, and 2500 U/l horseradish peroxidase. (Each test compound was prepared to obtain concentration of 1.5×10^{-5} mol/l in dimethyl sulfoxide.) The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) and immediately placed in water bath (37°) for 10 minutes. At the end of incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 10 seconds after addition of 0.3 ml of 1.1×10^{-5} mol/l of hydrogen peroxide (1.0×10^{-6} mol/l as final concentration) and 0.3 ml of 0.2 mol/l of glycine buffer *p*H 8.0 or *p*H 10.

2. Synthesis of Fused Pyrimidopyridazinediones.

Next, we have considerable interest in the synthesis of fused pyrimidopyridazines which are aza analogs of the above fused pyridopyridazine derivatives. In some cases, the formation of a pyridazine ring from diester derivatives does not proceed as smoothly as from fused maleimide derivatives. In the development of a new efficient method for the synthesis of polycyclic pyridazinediones bearing an amino group, a convenient approach to the synthesis of an aminopolycyclic pyridazinedione nucleus is considered to be the pathway as illustrated in Scheme 5.

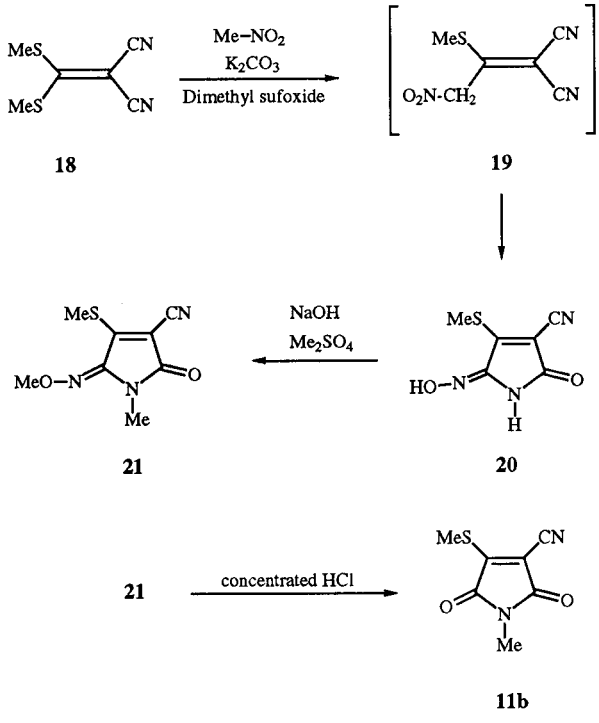
In order to obtain the fused pyrrolopyrimidine derivatives which are potential key intermidiates for the desired fused pyrimidopyridazine derivatives, we had to find a new reagent as a building block in the preparation of heterocycles [15]. The desired reagent is 1-methyl-4-methylthio-2,5-dioxo-1*H*-pyrrole-3-carbonitrile (**11b**) which will play a very important role not only as a reagent of 1,3-dipolarophiles in the 1,3-dipolar cycloadition reaction and of dienophiles in the Diels-Alder reaction but also as synthetically useful intermediates for the preparation of polycyclic and functionalized pyridazine derivatives [2a]. In an extension of our study on ketene dithioacetals for the synthesis of heterocycles [17], we fortunately succeeded in the preparation of new functionalized maleimides from ketene dithioacetal and their application to the synthesis of polycyclic aminopyridazinediones which are anticipated to possess chemiluminescent properties similar to luminol.

Scheme 5



In the course of our studies on nitro ketene dithioacetal, we found an efficient method for the synthesis of 5-hydroximino-4-methylthio-2-oxo-1*H*-pyrrole-3-carboxylate derivatives by the reaction of a nitro ketene dithioacetal with methyl cyanoacetate [18]. This method is useful for the synthesis of cyano-methylthio-maleimide derivatives. The reaction of bis(methylthio)methylenepropanedinitrile (**18**) with nitromethane in the presence of potassium carbonate in dimethyl sulfoxide followed by treatment with hydrochloric acid gave 5-hydroximino-4-methylthio-2-oxo-1*H*-pyrrole-3-carbonitrile (**20**) in 42% yield. The desired maleimide product **11b** [2a] was obtained by the methylation of **20** followed by hydrolysis with concentrated hydrochloric acid in 48% yield from **20**.

Scheme 6

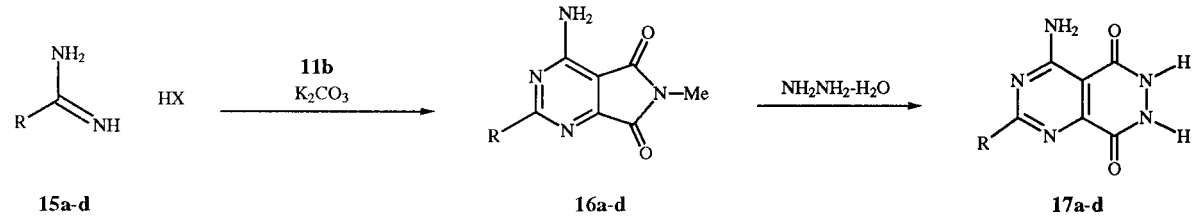


It is known that the reaction of ketene dithioacetal **18** with bifunctionalized nucleophiles such as hydrazine or amidine derivatives give the corresponding pyrazoles or pyrimidines in efficient yields [17,2c]. The reaction of **11b** with guanidine carbonate (**15a**) in the presence of triethylamine under ethanol refluxing conditions gave 1,3-diamino-6-methyl-5,7-dihydropyrrolo[3,4-*d*]pyrimidine-5,7-dione (**16a**) in 74% yield. Similarly, reaction of **11b** with acetamidine hydrochlroride (**15b**), benzamidine hydrochloride (**15c**), and *S*-benzylisothiourea (**15d**) gave the corresponding fused pyrimidine derivatives **16b-d** in 56, 84, and 75% yields, respectively. This method of preparation of pyrimidine derivatives is being applied to synthesis of polycyclic pyrrolopyrimidine derivatives. At first, the reaction of **11b** with 3-amino-5-methylpyrazole (**15e**) by refluxing in ethanol gave 4-amino-2-methyl-5,7-dihydropyrrolo[3,4-*d*]pyrazolo[1,5-*a*]pyrimidine-5,7-dione (**16e**) in 91% yield. In a similar manner, the 5-phenyl derivative **16f** was also obtained by the reaction of **11b** with 2-amino-5-phenylpyrazole (**15f**) in 91% yield. In a previous section on the study of chemiluminescence of polycyclic pyridazinediones, both methylthio and aryl groups were found to be very important substituted groups for increasing the chemiluminescent effects. 1-Aryl-2-methylthiopyrrolopyrazole derivatives **16g,h** are the key intermediates of the final desired 1-aryl-2-methlythiopyrazolopyridopyridazinediones **17g,h**. 5-Amino-3-methylthio-4-phenylpyrazole (**15g**), which was obtained by the reaction of 2-phenyl-3,3-bis(methylthio)acrylonitrile, reacted with **11b** under refluxing ethanol conditions to give **16g** in 92% yield. Compound **16h** was also synthesized in 86% yield from **15h** and **11b** in a manner similar to that described for the preparation of **16e**. 3-Aminotriazole compounds **15i**, and **j** were also smoothly reacted with **11b** under the same reaction conditions to yield pyrrolotriazopyrimidine derivatives **16i,j** in 86 and 94% yields, respectively. A four-ring system, pyrrolopyrimidobenzimidazole derivative **16k**, was also synthesized by the reaction of **11b** with 2-aminobenzimidazole (**15k**) in 93% yield. Similarly, 2,3-dimethyl derivative (**16l**) was synthesized from **11b** and **15l** in 93% yield. The reaction of **16a-l** with hydrazine hydrate in ethanol afforded the corresponding polycyclic aminopyridazinediones **17a-l** in good yields.

In a similar manner, 12-hydroxypyridazino[4',5':4,5]-pyrimido[1,2-*a*]benzimidazole-1,4(2*H*,3*H*)-diones **23a,b** were obtained in good yields by the reaction of *o*-phenylendiamines **15k,l** with **11a** followed by treatment with hydrazine hydrate.

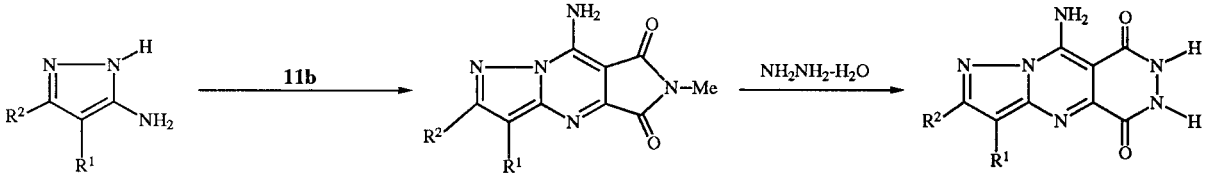
While fused pyrimidopyridazine derivatives **17a-j** were found to be weakly chemiluminescent in spite of the strongly basic solution, four-ring system compounds, pyridazino[4',5':4,5]pyrimido[1,2-*a*]benzimidazole-1,4(2*H*,3*H*)-diones **17k,l,23a,b** showed high chemiluminescent intensity like luminol (See Table 5 and Figure 8 [23]).

Scheme 7



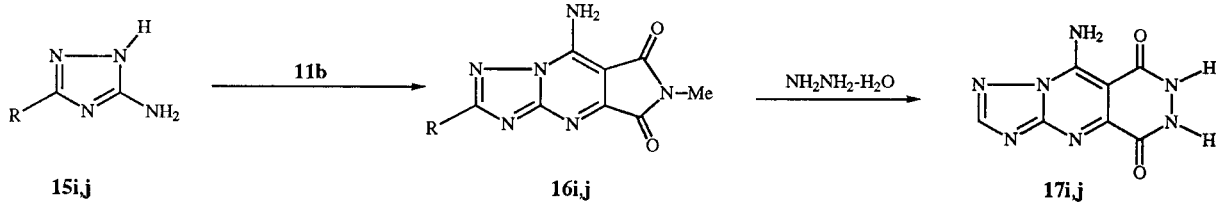
15a-d **16a-d** **17a-d**

a: R = NH_2
b: R = Me
c: R = C_6H_5
d: R = S-CH_2-C_6H_5



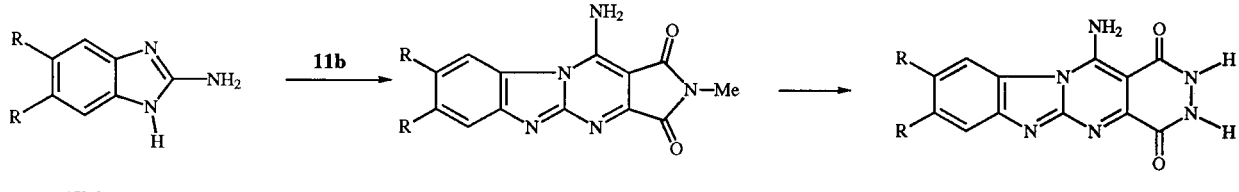
15e-h **16e-h** **17e-h**

e: R^1 = H, R_2 = Me
f: R^1 = H, R^2 = C_6H_5
g: R^1 = C_6H_5, R^2 = SMe
h: R^1 = C_6H_4-Cl(p), R^2 = SMe



15i,j **16i,j** **17i,j**

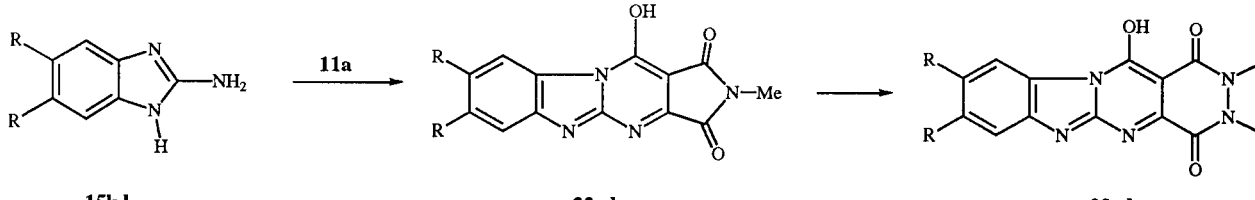
i: R = H
j: R = SMe



15k,l **16k,l** **17k,l**

k: R=H
l: R=Me

Scheme 8



15k,l **22a,b** **23a,b**

k: R = H
l: R = Me

Table 5

Chemiluminescent Intensity of Compounds **17** and **23**

Compounds	Chemiluminescence (c.p. 10 sec) [a]	*p*H 10 *p*H 8
17b	1.1×10^4	
	0	
17d	2.9×10^4	
	0	
17f	2.5×10^4	
	0	
17i	5.6×10^3	
	0	
17j	6.8×10^3	
	0	
17k	2.5×10^6	
	4.7×10^5	
17l	6.6×10^5	
	4.7×10^4	
23a	1.4×10^7	
	9.2×10^6	
23b	8.7×10^6	
	6.5×10^6	
Luminol	5.7×10^6	
	3.1×10^3	

[a] Counts per 10 seconds. (Their values were subtracted from each background.)

A reaction solution contains 10 mmol/l of phosphate buffer *p*H 8.0, 0.5 ml/l of Triton X-100, 2.5×10^{-7} mol/l of test compound, and 2500 U/l horseradish peroxidase (Each test compound was prepared to obtain concentration of 1.5×10^{-5} mol/l in dimethyl sulfoxide). The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) and immediately placed in a water bath (37°) for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 10 seconds after addition of 0.3 ml of 1.1×10^{-5} mol/l of hydrogen peroxide (10×10^{-6} mol/l as final concentration) and 0.3 ml of 0.2 mol/l of glycine buffer *p*H 8.0 or *p*H 10.

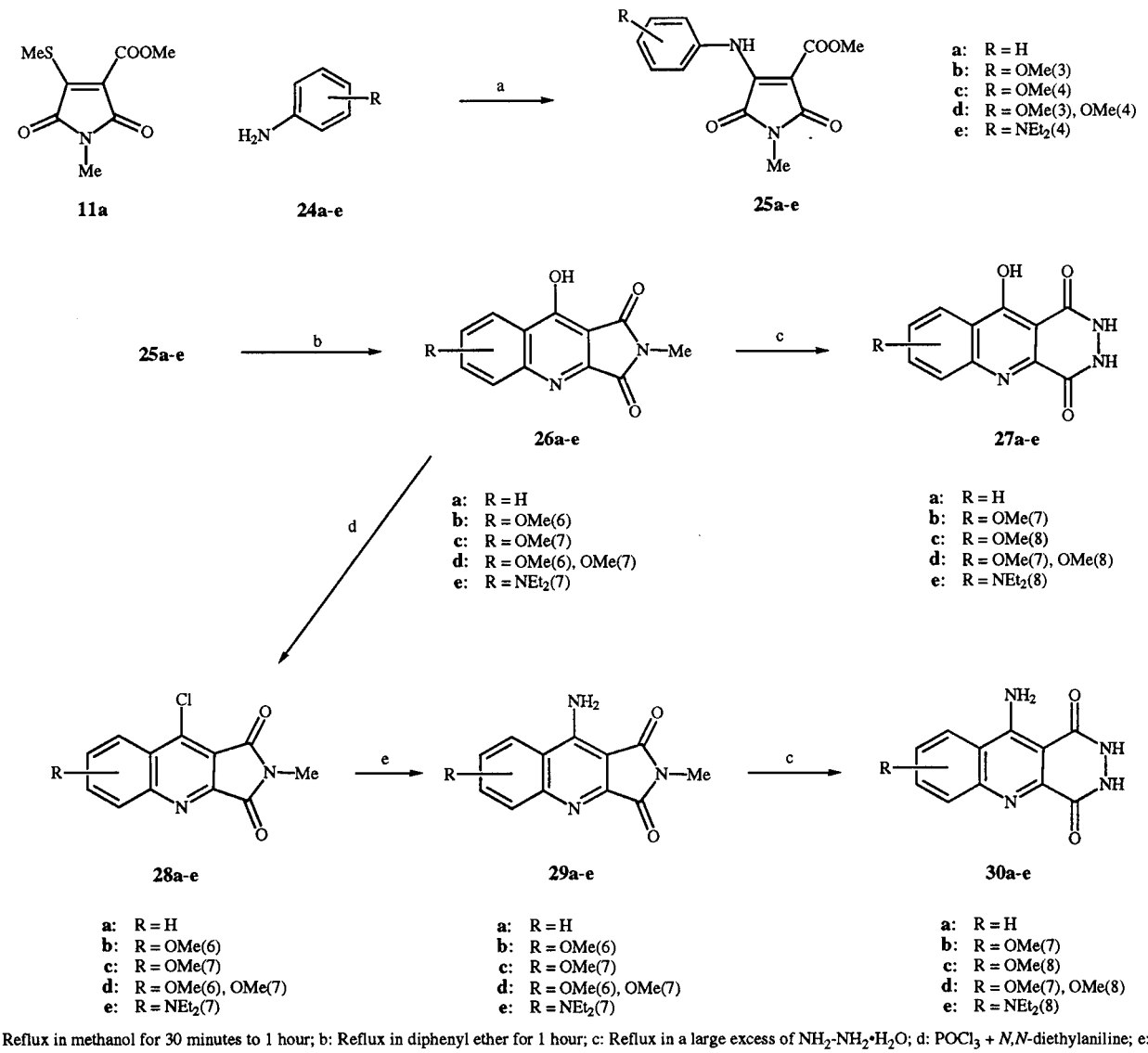
3. 10-Hydroxy- and 10-Aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones.

This section presents the synthesis of 10-hydroxypyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones and 10-aminopyridazino[4,5-*b*]pyridazine-1,4(2*H*,3*H*)-diones as chemiluminescent compounds by the use of **11a** [2b,15]. Compound **11b** reacted smoothly with various amino compounds to give the corresponding polycyclic aminopyrimidines containing a pyrroline ring, which are readily converted to the desired polycyclic pyridazine derivatives in good yields. A combination of the above process and the Gould/Jacobs reaction was considered highly applicable for the synthesis of the key compounds, 2-methylpyrrolo-[3,4-*b*]quinoline derivatives [20].

Reaction of **11a** with various aniline derivatives **24a-e** in refluxing methanol readily gave the corresponding 2,5-dioxo-4-phenylamino-1*H*-pyrrole derivatives **25a-e** which were smoothly converted in good yields to pyrrolo[3,4-*b*]quinolines **26a-e** by refluxing in diphenyl ether for 1 hour. The reaction of **26a-e** with a large excess of hydrazine hydrate afforded the corresponding polycyclic hydroxypyridazinediones, 10-hydroxypyridazino[4,5-*b*]-quinoline-1,4(2*H*,3*H*)-diones **27a-e** in good yields.

The chlorination of **26a-e** with phospholic oxychloride in the presence of diethylaniline was carried out to give the corresponding 9-chloro-2-methylpyrrolo[2,3-*d*]quinolines **28a-e** which were converted to the expected 9-amino-2-methylpyrrolo[2,3-*d*]quinolines **29a-e** by aminolysis with 28% ammonium hydroxide at 180° in a mini autoclave. The desired 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-dione derivatives **30a-e** were readily obtained in good yields, respectively, by the general reaction of compounds **7** with a large excess of hydrazine hydrate under refluxing conditions [2d].

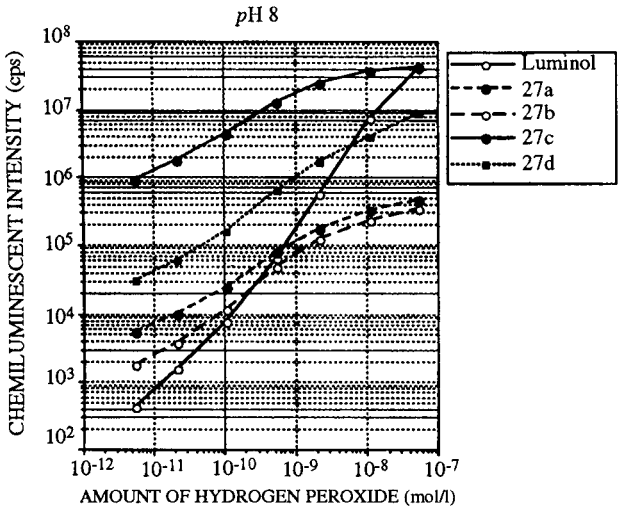
Scheme 9

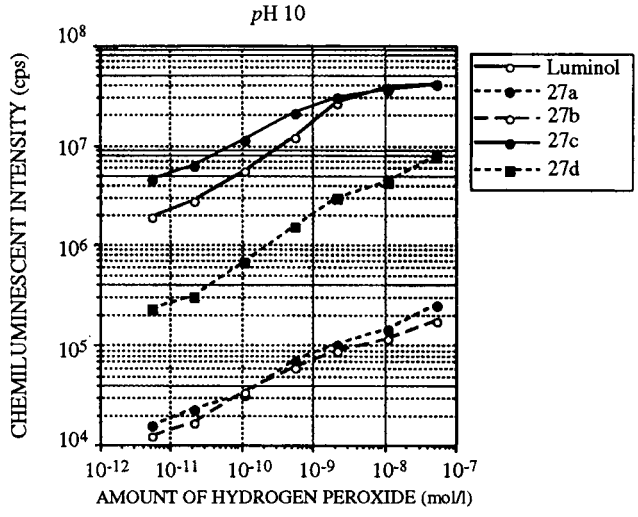


a: Reflux in methanol for 30 minutes to 1 hour; b: Reflux in diphenyl ether for 1 hour; c: Reflux in a large excess of NH_2-NH_2•H_2O; d: $POCl_3$ + *N,N*-diethylaniline; e: 28% NH_4OH, 180° in a mini autoclave.

The chemiluminescent experiments reported here were performed in the presence of triton X-100, hydrogen peroxide, and horseradish peroxidase in a phosphate buffer solution at *p*H 8.0. The chemiluminescent intensity in the pyridazinoquinoline series is shown in Table 6 [23]. Both 10-hydroxy- and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-dione derivatives **27b-c**, **30b-c** showed nearly the same or somewhat stronger light intensity than luminol at *p*H 8.0. These compounds also showed increasing light intensity with an increase in *p*H, as noted also for luminol. The methoxy and diethylamino groups on the quinoline ring are very important groups for chemiluminescence production.

10-Hydroxy- and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones were found to be more efficient than luminol in light production. These pyridazinoquinoline-1,4-dione derivatives are the first example of chemiluminescent compounds in the pyridazinoquinoline derivatives (See Figures 3-8 [23]).

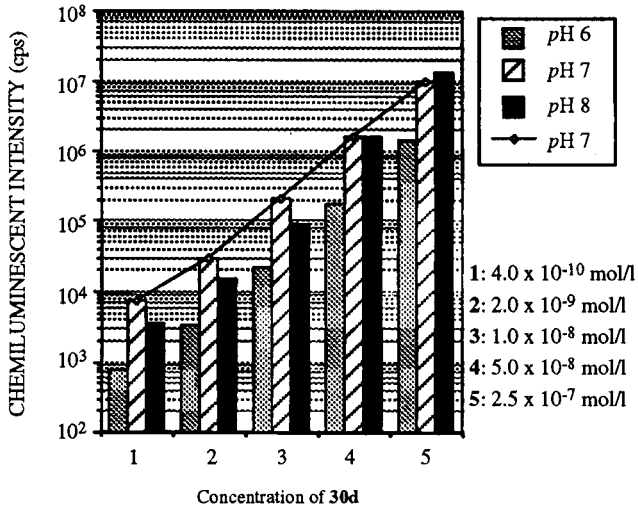




[a] Counts per 10 seconds (Their values were subtracted from each background.)

Figures 5 and 6. Chemiluminescence of 10-hydroxypyridzino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones.

A reaction solution contains 10 mmol/l of phosphate buffer *p*H 8.0, 0.5 ml/l of Triton X-100, 2.5 x 10^{-7} mol/l of test compound, and 2500 U/l of horseradish peroxide (Each test compound was prepared to obtain concentration of 1.5 x 10^{-5} mol/l in dimethyl sulfoxide). The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) and immediately placed in a water bath (37°) for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 10 seconds after addition of 0.3 ml of various concentration of hydrogen peroxide (Figures show the final concentration) and 0.3 ml of 0.2 mol/l glycine buffer *p*H 8.0 or *p*H 10.



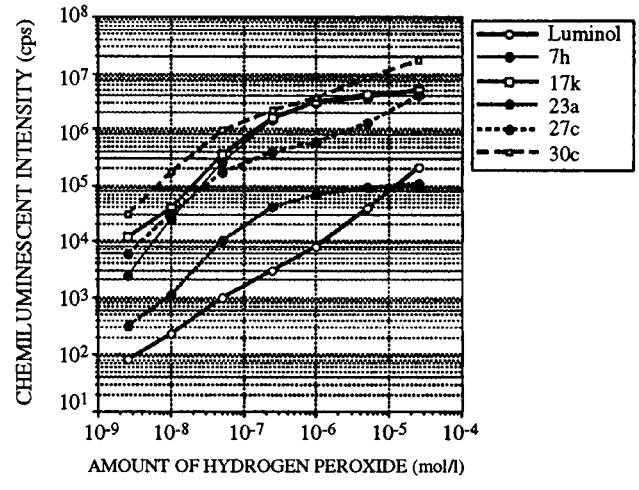


Figure 7. Chemiluminescence of Relative Concentration of **30d**

A reaction of solution contains 10 mmol/l of phosphate buffer at *p*H 8.0, 0.5 ml/l of Trition X-100, various concentration of test compound, and 2500 U/l horseradish peroxidase (Each test compound was prepared to obtain a concentration of 1.5 x 10^{-5} mol/l in dimethyl sulfoxide). The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) and immediately placed in a water bath (37°) for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 1.0 second after addition of 0.3 ml of 1.1 x 10^{-5} mol/l of hydrogen peroxide (1.0 x 10^{-6} mol/l as the final concentration).

Figure 8. Chemiluminescense of Pyridazinediones **7h**, **17k**, **23a**, **27c** and **30c**

A reaction solution contains 10 mmol/l phosphate buffer at *p*H 8.0, 0.5 ml/l Trition X-100, 2.5 x 10^{-7} mol/l test compound, and 2500 U/l of horseradish peroxidase (Each test compound was prepared to obtain concentration of 1.5 x 10^{-5} mol/l in dimethyl sulfoxide). The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) and immediately placed in a water bath (37°) for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 1.0 second after addition of 0.3 ml of various concentration of hydrogen peroxide (Figures show the final concentration).

Table 6

Compounds	Chemiluminescence (c.p.s.) [a]
Luminol	1.25×10^5
27a	4.54×10^4
27b	6.02×10^4
27c	1.65×10^7
27d	2.25×10^6
30a	4.58×10^5
30b	1.36×10^6
30c	1.90×10^7
30d	6.41×10^6
30e	1.75×10^4

[a] Counts per second.

A reaction solution was prepared with a 10 mol/l of phosphate buffer solution (*p*H 8) containing a 0.5 ml/l of Triton X-100, 2.5×10^{-7} mol/l of test compound, and 5000 units/l of arthromyces ramosus peroxidase (Each test compound was prepared to obtained concentration of 1.5×10^{-5} mol/l in dimethyl sulfoxide). The reaction solution (2.0 ml) was introduced into a Brosilicate glass tube and then incubated at 37° for 10 minutes. At the end of the incubation period, the sample tube to be counted was set into a chemiluminescence counter. The chemiluminescence reaction was initiated by injecting a 0.3 ml of 1.1×10^{-5} mol/l of hydrogen peroxide solution (1.0×10^{-6} mol/as final concentration). Photons were counted for 1 second from the start of the reaction. The chemiluminescence measurements were performed with a Magic analyzer (Corning Co. Ltd. USA); captions are the final values.

4. Pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones.

[2.2.3]Cyclazine, which are peripheral conjugate aromatic compounds with delocalized 10π-electrons, are interesting heteroaromatic compounds from both theoretical and practical standpoints [19]. We have considerable interest in the synthesis and chemiluminescence of cyclazine derivatives fused to a pyridazine ring [2e]. Our synthetic approach, which was achieved in a 4-step procedure, is outlined in Scheme 10. First, the desulfurization of **31** [21] with Raney-Ni in refluxing ethanol afforded ethyl 7-dimethylaminoindolizine-3-carboxylate (**32**) in 72% yield. Decarboxylation of **32** with polyphosphoric acid at 150° for 1 hour was smoothly carried out to give the expected 7-dimethylaminoindolizine **33** in 92% yield. The [8 + 2]cycloaddition reaction of **33** with dimethyl acetylenedicarboxylate in the presence of 5% Pd-C in refluxing toluene gave the expected product, dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylate (**34**) as orange needles, mp 125-126°, in 32% yield. Compound **34** was not obtained by the desulfurization of dimethyl 6-dimethylamino-3-methylthio[2.2.3]cyclazine-1,2-dicarboxylate (**36**) with Raney-Ni in refluxing methanol. Compound **36** was synthesized by the [8 + 2]cycloaddition reaction of 7-dimethylamino-2-methylthioindolizine (**35**) with dimethyl acetylenedicarboxylate in a similar manner to that described for the preparation of **34**.

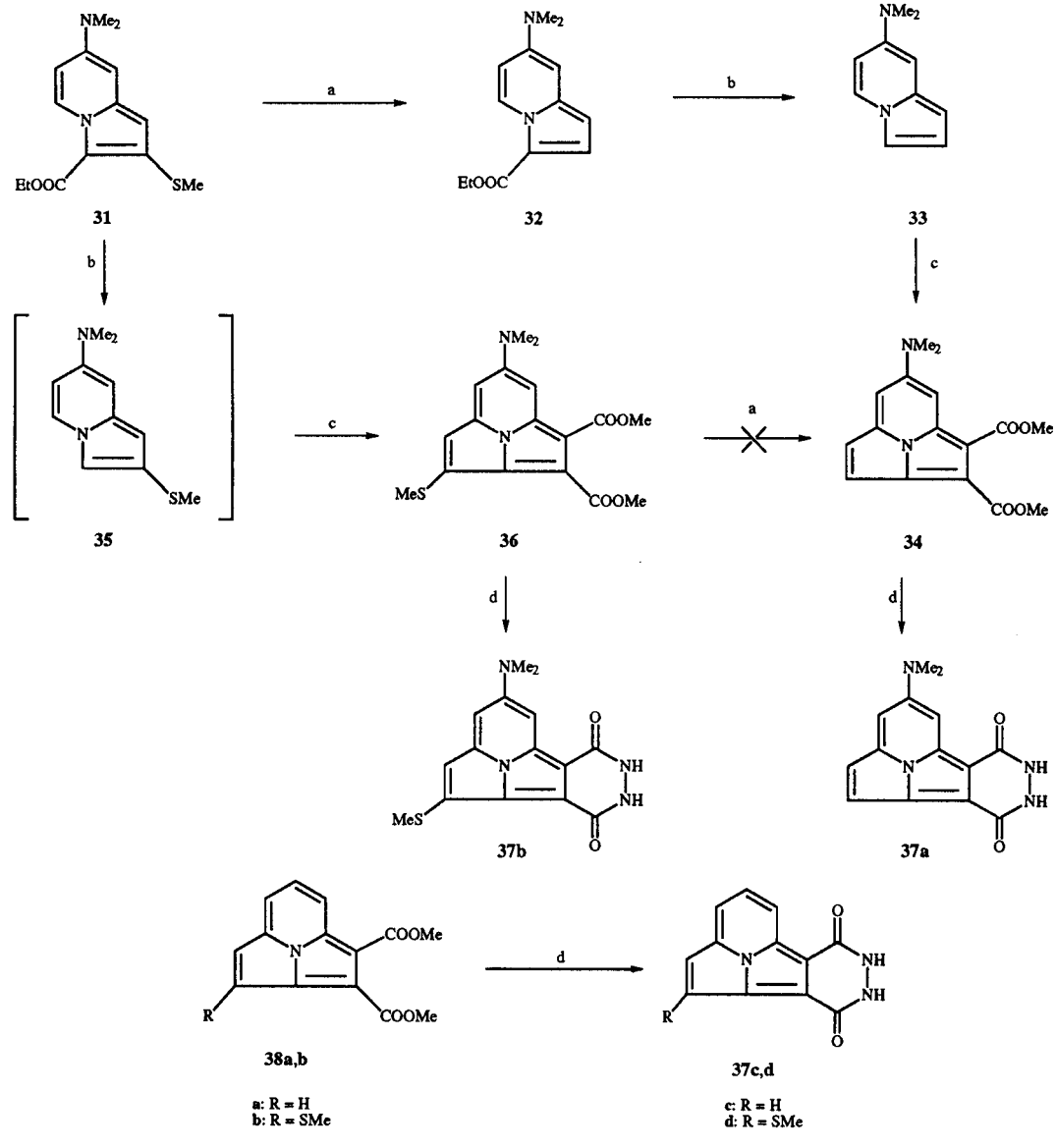
The expected 6-dimethylaminopyridazino[4,5-*a*]-[2.2.3]cyclazine-1,4(2*H*,3*H*)-diones **37a,b** were obtained by the reaction of **34** and **36** with a large excess of hydrazine hydrate in good yields and could be purified by recrystallization from dimethyl sulfoxide to give orange-red crystals. Similarly, pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones **37c,d** were also readily prepared from the corresponding dimethyl [2.2.3]cyclazine-1,2-dicarboxylate (**38a**) and the 3-methylthio derivative **38b** in good yields, respectively [2e].

Compounds **37a-d** were found to be efficiently chemiluminescent similarly to luminol in the presence of hydrogen peroxide and horseradish peroxidase in a solution of a phosphate buffer at *p*H 8.0 (See Figure 9 [23]).

Conclusions.

It has been proven that reaction of 1,3-disubstituted 5-aminopyrazole-4-carbonitrile derivatives with dimethyl acetylenedicarboxylate gave dimethyl 1,3-disubstituted 4-aminopyrazolo[3,4-*d*]pyridine-5,6-dicarboxylates which are key intermediates for the preparation of luminophores, 1,3-disubstituted 4-amino-1*H*-pyrazolo[4',3':5,6]pyrido-[2,3-*d*]pyridazine-5,8(6*H*,7H)-diones. This addition-cyclization of *o*-aminonitrile compounds with dimethyl acetylenedicarboxylate is an important and general method for the simultaneous direct introduction of both the amino and dicarboxylate groups. This type of process

Scheme 10



a: Raney-Ni, reflux for 5 hours in ethanol; b: Polyphosphoric acid at 150° for 1.5 hours, 10% NaOH; c: Dimethyl acetylenedicarboxylate reflux for 5 hours in toluene; d: Excess $NH_2NH_2 \cdot H_2O$.

has been applied to the synthesis of 4-hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates which are the key compounds in the synthesis of 1,3-disubstituted 4-hydroxy-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7H)-diones.

It is evident from the present data that the new maleimide derivatives, **11a,b,** are very useful and convenient reagents to react with various amines for the synthesis of polycyclic amino heterocycles, especially, for the synthesis of polycyclic pyrimidopyridazine and pyridazino[4,5-*b*]quinoline derivatives.

Pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones as chemiluminescent compounds were readily obtained from dimethyl [2.2.3]cyclazine-1,2-dicarboxylates which are prepared by the [8 + 2]cycloaddition reaction of dimethylamino-indolizines with dimethyl acetylenedicarboxylate.

Many polycyclic pyridazinedione derivatives reported in this paper were evaluated for chemiluminescence.

Some compounds were found to be more efficient than luminol in light production. Chemiluminescent assays using the above polycyclic pyridazinediones will prove quite useful for clinical analysis in consideration of the high sensitivity available.

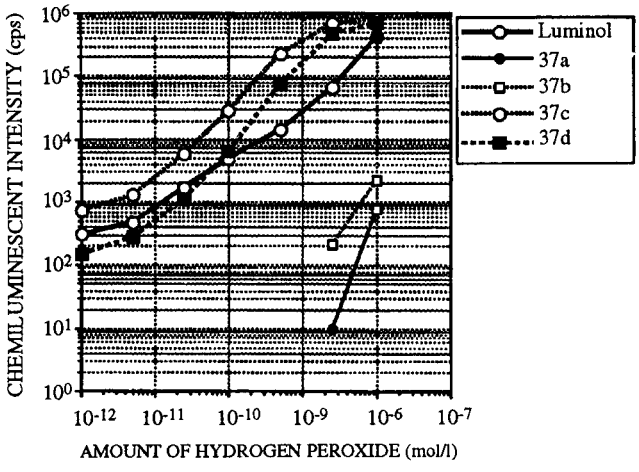


Figure 9. Chemiluminescense of Pyridazino[2.2.3]cyclazine-1,4(2*H*,3*H*)-diones

A reaction solution contains 10 mmol/l of phosphate buffer *p*H 8.0, 0.5 ml/l Triton X-100, 1.5 x 10^7 mol/l test compound, and 2500 U/l horseradish peroxidase (Each test compound was prepared to obtain concentration of 1.5 x 10^{-5} mol/l in dimethyl sulfoxide). The solution (3 ml) was transferred to a Borosilicate glass tube (12 x 75 mm) an immediately placed in a water bath (37°) for 10 minutes. At the end of the incubation period, the sample tube to be couunted was incorporated into a luminometer. Photons were counted for 1.0 seconds after additon of 0.3 ml of various concentration of hydrogen peroxide (Figures show the final concentration).

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[11] **8b**: This compound was prepared from methyl bis(methylthio)methylenecyanoacetate and *p*-toluenesulfonylhydrazide in the same manner as the preparation for **8a** in 73% yield, mp 155-156°, colorless prisms.

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[15] Compound **11a** was obtained by the hydrolysis of methyl 5-methoxyimino-1-methyl-4-methylthio-5-oxo-1*H*-pyrrole-3-carboxylate with concentrated hydrochloric acid in 67% yield, mp 120-121°; ir (potassium bromide): ν cm^{-1}: 1745, 1710, 1685 (CO), 1530, 1440, 1200; ^{1}H-nmr (deuteriochloroform): δ 2.88 (3H, *s*, SMe), 3.05 (6H, *s*, NMe), 3.90 (6H, *s*, OMe); *cf.* M. Sone, Y. Tominaga, R. Natsuki, Y. Matsuda and G. Kobayashi, *Chem. Pharm. Bull.*, **21**, 1667 (1973).

[16] Compound **8g** was prepared by the hydrolysis of **8c** with 10% methanolic sodium hydroxide solution followed by decarboxylation by heating at *ca.* 300°; colorless prisms, mp 114-115°; ^{1}H-nmr (deuteriochlorofrm) δ 2.51 (3H, *s*, SMe), 3.78 (2H, br *s*, NH_2), 5.60 (1H, *s*, 4-H), 7.30-7.56 (5H, m, phenyl-H).

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