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PREPARATION AND EVALUATION OF THE ANTIAGGREGATING AND VASODILATING ACTIVITIES OF THE NOVEL 3-NITRO-4(3*H*)-QUINAZOLINONE DERIVATIVES

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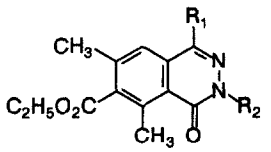
Abstract: The structures of the novel adducts (**9**), prepared from the reaction between powder of 4(3*H*)-quinazolinone derivatives (**8**) and acetyl nitrate at room temperature, were elucidated by chemical modification and spectroscopy. The final structure of **9a** (designated as AP3) was confirmed by X-ray crystallography as the 3-nitro-4(3*H*)-quinazolinone derivative. **9a** elevated the cyclic GMP level in cells showing antiaggregating activities and had a relaxing effect on rabbit vascular smooth muscle in a concentration dependent manner *in vitro*.

We previously reported[1)] that the 2-nitro-1(2*H*)-phthalazinone derivative (**1**) showed a potent hypotensive effect and dose dependently inhibited platelet aggregation. To examine their structure and activity relationship, various kinds of 2-nitro analogues of **1** were prepared. The 4-unsubstituted derivative (**2**) did not afford a corresponding 2-nitro-4-unsubstituted analogue (**3**) but gave a 4-acetoxy-2(1*H*)-phthalazinone (**4**) at 0°C or its 2-nitro derivative (**5**) at room temperature. The 1,2-addition of various kinds of chemical species to the azomethine group *via* ionic or concerted mechanisms has been reported,[2)] but the acetoxylation of the azomethine group has not been reported except for a potential intermediate (**6**) proposed by Dewar *et al.*[3)] On the other hand, Bordwell *et al.*[4)] suggested that acetyl nitrate added to various kinds of alkenes *via* cyclic addition mechanism gives a cis-adduct of β-nitro acetates. Because the distribution of the highest occupied molecular orbital (HOMO) was observed at the azomethine region of **2** and **7** as shown in Fig.2,[5)] we calculated the molecular orbital for 50 kinds of compounds such as heterocycles and the shiff bases in order to extend this "acetoxylation" to other types of compounds possessing the azomethine moiety in the molecule. Then we employed the 4(3*H*)-quinazolinone derivatives (**8**), which are the regioisomers of **7** and showed a similar distribution of the HOMO as shown in Fig.2. The present paper describes the preliminary results of the reaction on **8** and the pharmacological profiles of the novel products.

The reaction between acetyl nitrate (AN) and powder of **8** (10mmol) was allowed to proceed in the same



1, $R_1 = CH_2ONO_2$, $R_2 = NO_2$
2, $R_1 = H$, $R_2 = H$
3, $R_1 = H$, $R_2 = NO_2$
4, $R_1 = OCOCH_3$, $R_2 = H$
5, $R_1 = OCOCH_3$, $R_2 = NO_2$

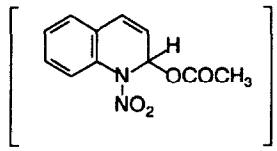


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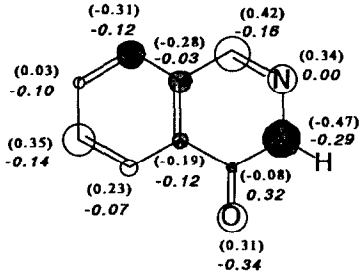
Fig. 1



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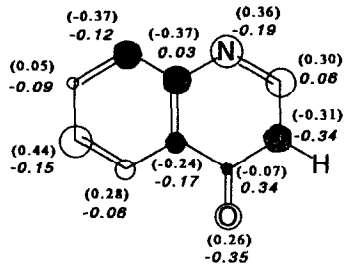

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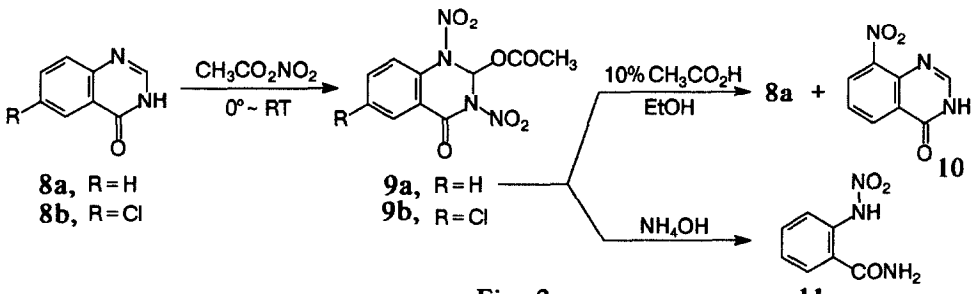
Italic : atomic charge
in parenthesis: pz value of the HOMO

Fig. 2



8a, R=H
8b, R=Cl

9a, R=H
9b, R=Cl

10

11

Fig. 3

manner described before[1] and quick recrystallization of the crude product afforded colorless adducts (**9**)[6] over an 85% yield. The structures of **9** were elucidated by chemical modification and spectroscopy.[6b] Finally, the structure of **9a** was confirmed by X-ray crystallography[7] as the atomic features show in Fig.4. The negative charge on the nitrogen of the azomethine moiety of **8** as shown in Fig. 2 must be attributed to the stabilization of the N7-N11 bond of **9a** compared with the case of **5**. Thus, we succeeded in not only employing the "acetoxylation" to the azomethine moiety of 4(3*H*)-quinazolinone derivatives but also the nitration of both the lactam and the azomethine moiety, simultaneously. Furthermore, the isolation of the novel *N*-nitrolactam adducts such as **9a** and **9b** significantly support the contribution of the potential intermediate **6** on the nitration of

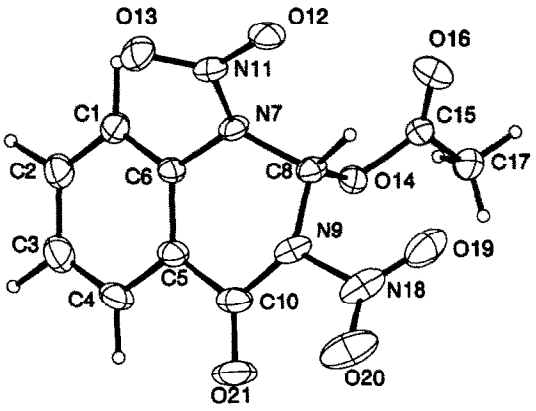


Fig. 4. ORTEP drawing of **9a** derived from the X-ray coordinates

quinoline.[3)]

The inhibitory activities of **9a** and **9b** on platelet aggregation were evaluated by a modification of the method of Born[8,1a)] using rabbit platelet rich plasma *in vitro* (Table 1). Both compounds **9a** and **9b** inhibited the aggregation induced by adenosine diphosphate (ADP), collagen, and arachidonic acid (AA) in a similar concentration dependent manner and inhibited the aggregation completely at a concentration of 100μM. Both compounds **9a** and **9b** also relaxed rabbit aortic strips in a concentration dependent manner and their pD_2 values were 5.36±0.72 and 5.40±0.65 compared with that of sodium nitropruside (SNP: 4.08±0.66), respectively. Since the vasodilation and the antiplatelet action of organic nitrates and thiol nitrates are mediated by cGMP,[9)] this mechanism was also studied. As shown in Fig.5, incubation of **9a** with the isolated aorta preparation elevated the cGMP level in the cell to 6.6-fold the basal level, but the pretreatment with methylene blue reduced it to 27.6%. Thus, our findings demonstrate that the *N*-nitrolactam derivatives such as **1**, **9a** and **9b** significantly increase the cGMP level in cells and are a new class of guanylate cyclase activator. The addition mechanism of AN to **8** and the pharmacological details of **9a** and **9b** will be reported in the future.

Table 1. Antiaggregating(IC_{50}, M) and vasodilating activities of **9a** and **9b**

	Inducers			
	ADP(30mM)	Collagen(10μg/ml)	AA(125μM)	pD_2
9a	4.8×10^{-5}	5.2×10^{-5}	5×10^{-5}	5.36 ± 0.72
9b	4.6×10^{-5}	5.2×10^{-5}	4×10^{-5}	5.40 ± 0.65
SNP	9.5×10^{-5}	10^{-4} <	1×10^{-4}	4.08 ± 0.66

Antiaggregating activities were measured by the turbidometric method[8)] and the test compounds were preincubated with PRP for 3min at 37°C, then each inducer was added to induce platelet aggregation. [1a)] Vasodilating activities were measured using a transverse strip preparation of rabbit thoracic aorta in oxygenated Kreb's buffer (pH 7.4) at 37°C. Each preparations were precontracted with KCl(30mM), then the test compounds were added to the organ chamber, cumulatively. Results are expressed as means ±SEM(n=3).

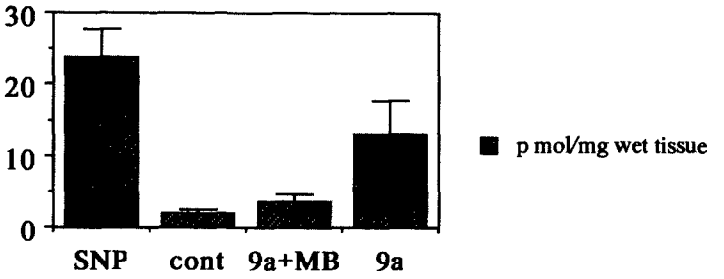


Fig. 5. Effects of **9a** (100mM) and SNP(30mM) on production of intracellular cGMP levels

Rat aortic transverse strip with intact endothelium was preincubated with 3-isobutyl-1-methyl xanthine(IBMX: 100μM; to inhibit phosphodiesterase) and indomethacin(30μM) for 30min at 37°C in the presence or absence of methylene blue(MB: 100μM, n=3). The test compound was treated with the medium for 1min at 37°C, then terminated with TCA. cGMP assay were performed with a radioimmunoassay kit(Amersham) in duplicate. Results are expressed as means ± SEM (n=3).

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5 a) The molecular orbital calculation was performed by the semiempirical molecular orbital calculation using AM1 method (MOPAC ver. 4.0): Dewar, M.J.S.; Zeobisch, E.G.; Healy, E.G.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, 107, 3902; Stewart, J.J.P. QCPE #455.
b) The distribution of the HOMO on the rings of **2** and **7** was almost identical.

6 a) The adducts turned slightly yellow under light and decomposed within a day in $CDCl_3$ or DMSO-d_6 at room temperature.
b) **9a**: colorless prisms, mp 98~99°C(acetone/n-hexane). MS(EI in beam) *m/z*: 296(M^+), 281, 250, 236, 206, 145. IR(KBr): 1783(2-OCOCH3), 1746(4-CO), 1605(NO_2), 1572cm^{-1}. UV(EtOH) λmax(log ε): 246(4.16), 303(3.66)nm. ^{1}H-NMR($CDCl_3$, 270MHz) δ: 2.08(3H, s, CH_3), 7.64(1H, dt, J=8.0 & 1.5Hz, H-6), 7.75(1H, dd, J=8.0 & 1.5Hz, H-8), 7.85(1H, dt, J=8.0 & 1.5Hz, H-7), 8.31(1H, dd, J=8.0 & 1.5Hz, H-5), 9.22(1H, s, H-2). ^{13}C-NMR(67.8MHz) δ: 20.1(2-OCOCH3), 85.2(C-2), 119.8(C-4a), 128.1(C-8), 129.6(C-6), 130.0(C-5), 133.7(C-8a), 135.5(C-7), 155.2(C-4), 166.9(2-OCOCH3). *Anal.* Calc for $C_{10}H_8N_4O_7$: C, 40.55; H, 2.72; N, 18.92. Found: C, 40.65; H, 2.83; N, 18.63.
9b: pale yellowish prisms, mp 97.5~99.5°C(acetone/n-hexane). MS(EI in beam) *m/z*: 270, 240, 225, 195. IR(KBr): 1779(2-OCOCH3), 1744(4-CO), 1620(NO_2)cm^{-1}. UV(EtOH) λ max(log ε): 220(4.80), 312.6(4.00)nm. ^{1}H-NMR($CDCl_3$, 270MHz) δ: 2.09(3H, s, CH_3), 7.70(1H, d, J=8.6Hz, H-8), 7.79(1H, dd, J=8.6 & 2.4Hz, H-7), 8.25(1H, d, J=2.4Hz, H-5), 9.21(1H, s, H-2). ^{13}C-NMR(67.8MHz) δ: 20.1(2-OCOCH3), 85.1(C-2), 121.2(C-4a), 129.5(C-8), 129.6(C-5), 132.0(C-8a), 135.6(C-7), 136.1(C-6), 154.3(C-4), 166.8(2-OCOCH3).

7 Crystallography of **9a**: clear prism(0.45 x 0.20 x 0.50mm), formula $C_{10}H_8N_4O_7$, molecular weight 296.20, space group P1(triclinic), Z=2, a=9.182(1), b=9.735(1), c=8.357(1)Å, α=120.74(1), β=90.40(6), γ=103.95(1)°, V=615.5(2)Å^3, Dc=1.598gcm^{-3}, λ(CuKα$_1$)=1.5405Å, μ=1.157mm^{-1}, F(000)=304, T=295K. Data were collected on a Rigaku AFC-5 diffractometer using graphite monochromated CuKα$_1$ radiation by ω-2θ scan method. The scan speed was 16°min^{-1}. The data were corrected for Lorents and Polarization factors, but no absorption correction was applied. A total of 1829 independent reflexions were measured within the 2θ angle of 130°. The structure was determined by the direct method and refined by the fullmatrix least-squares. The final R value was 0.058(wR=0.062) for 1596 reflexions above 3σ(*F*) including anisotropic thermal factors for nonhydrogen atoms and isotropic ones for hydrogen atoms.

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