Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 1, pp. 33-38, 1996

0960-894X/96 $15.00 + 0.00

0960-894X(95)00547-1

NEW REAGENTS FOR CONTROLLED RELEASE OF NITRIC OXIDE. STRUCTURE–STABILITY RELATIONSHIPS

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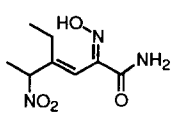
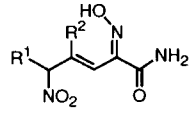
Abstract: The synthesis and structure-stability relationships of a series of novel FK409 derivatives (**1**, **2**, and **3**) are described. The rates of decomposition in aqueous solution (pH 8.0, 30 ℃) were parallel with those of spontaneous NO release measured by ESR spectroscopy using carboxy-PTIO. The compounds can cover a wide range of NO releasing rates by appropriate modification of the molecule.

FK409 (**1c**) is a new vasodilator isolated from the fermentation product of *Streptmyces griseosporeus* with a unique structure and potent antiplatelet activity.[1] FK409 has been shown to spontaneously decompose and release NO without metabolic activation in contrast to organic nitrates such as isosorbide dinitrate.[2] NO activates soluble guanylate cyclase, increasing the intracellular cyclic GMP. The potent pharmacological actions of FK409 are attributed to the elevation of intracellular cyclic GMP levels. Although there have been several reports on the pharmacological actions of FK409,[3] the mechanism of NO release has not been elucidated yet.

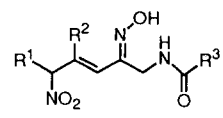
We have been studying the pharmacological and physicochemical properties of FK409 in order to investigate its potential utility as a cardiovascular drug.[4] FK409 is stable in acidic conditions but rapidly decomposes in aqueous alkaline solution at 37 ℃ (pH 2.0, $k = 9.28 \times 10^{-5}$ min^{-1}; pH 8.0, $k = 2.93 \times 10^{-2}$ min^{-1}).[5] We measured the amount of NO released from FK409 by various methods; chemiluminescence analysis, ESR spectroscopy using carboxy-PTIO, and nitrite analysis.[2,5] The rate of spontaneous NO release from FK409 was approximately parallel with that of the decomposition in aqueous solution. As a part of our program for designing



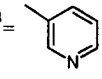
1c
FK409



(*E*)-**1**



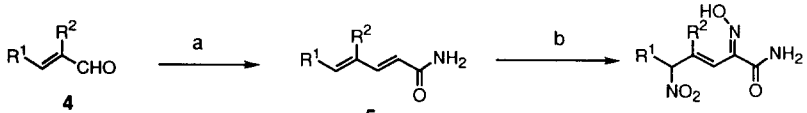
(*Z*)-**2**: R^3= –CH_3

(*Z*)-**3**: R^3= 

new NO donors, we have prepared a novel series of FK409 derivatives (**1**, **2**, and **3**) and investigated the structural factors affecting their stabilities and NO releasing rates in aqueous solution.

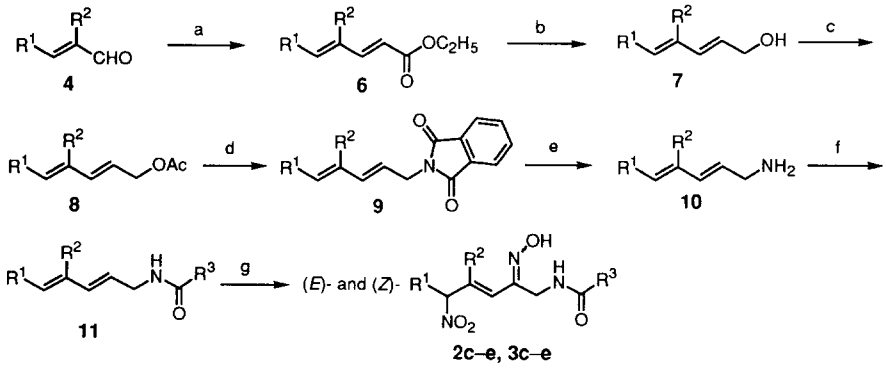
Synthesis. 2-Hydroxyimino-5-nitro-3-alkenecarboxamides (**1**) and 1-acylamino-2-hydroxyimino-5-nitro-3-alkenes (**2** and **3**) were prepared by the routes shown in Schemes 1, 2, and 3. The pivotal step in the synthesis was a novel "nitro-nitrosation" reaction which introduces a nitro and a hydroxyimino groups into the diene moiety of the molecule in one step. The "nitro-nitrosation" reaction was conducted by dropwise addition of hydrochloric acid to an aqueous solution of the substrate and $NaNO_2$. It is of interest that the reaction proceeds not only with the 2,4-dienecarboxamides (**5**) but also with the 2,4-dienes (**11**) which have no electron-withdrawing carbonyl group. However, yields of **1** were higher than those of **2** and **3**. The regioisomers of **2** and **3** were not isolated. Compound **1** was isolated as one isomer, whereas compounds **2** and **3** consisted of two components which were assumed to be stereoisomers at the hydroxyimino group by ^1H NMR spectroscopy. The stereochemistry of the hydroxyimino group was determined on the basis of NOE in the NOESY spectrum of **1c** and the two isomers of **3c**, and by X-ray crystallography of compounds **1c** and (*E*)-**3c**. Compounds **1** had the *E*-configuration and compounds **2** and **3** were a mixture of *E*- and *Z*-isomers which were readily isolated by silica gel column chromatography. Since *Z*-isomers of **2** and **3**, with higher melting points than *E*-isomers, were easily purified, *Z*-isomers were used in the decomposition experiments.

Scheme 1. Synthesis of 2-Hydroxyimino-5-nitro-3-alkenecarboxamide Derivatives



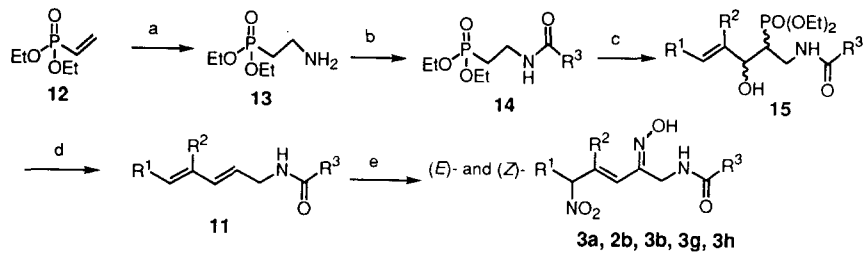
a) $(EtO)_2P(O)CH_2CONH_2$, *t*-BuOK; b) HCl, $NaNO_2$, H_2O

Scheme 2. Synthesis of 1-Acylamino-2-hydroxyimino-5-nitro-3-alkene Derivatives



a) NaH, $(EtO)_2P(O)CH_2COOEt$; b) $LiAlH_4$, THF; c) Ac_2O, Py; d) $(Ph_3P)_4Pd$, potassium phtalimide, DMF; e) NH_2NH_2, EtOH; f) 3-PyCOCl or Ac_2O, Py; g) HCl, $NaNO_2$, H_2O

Scheme 3. Synthesis of 1-Acylamino-2-hydroxyimino-5-nitro-3-alkene Derivatives



a) NH_3, EtOH; b) 3-PyCOCl or Ac_2O; c) LDA, **4**, THF; d) DBU, 100 °C, toluene; e) HCl, $NaNO_2$, H_2O

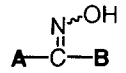
Decomposition Kinetics.[6] The compounds were incubated in pH 8.0 phosphate buffer (0.1 M) at 30 ℃ and the concentration of compounds recovered was monitored by HPLC at appropriate time intervals after quenching the aliquots by 0.1 N HCl. In each case, first-order kinetics was observed and the rate constant (k) was obtained via a standard linear regression analysis. The half-life data were calculated using the formula $t_{1/2} = 0.6931/k$. The rate constants (k) and half-lives ($t_{1/2}$) are summarized in Table 1.

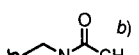
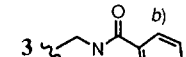
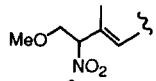
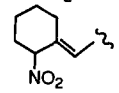
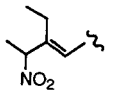
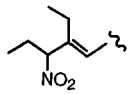
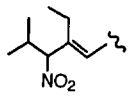
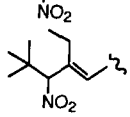
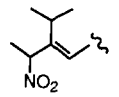
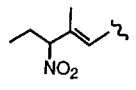
NO-Release Kinetics.[6] 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (Carboxy-PTIO) was reported to react with NO in a molar ratio of 1:1 to form carboxy-PTI.[7] The concentration of NO was calculated from the decrease in the peak height of the ESR signal of carboxy-PTIO in the lowest magnetic field. Compounds tested (0.5 mM) were incubated in pH 7.4 phosphate buffer containing carboxy-PTIO (0.51 mM) at 37 ℃. Aliquats were withdrawn at appropriate time intervals and their ESR spectra were recorded using an X-band ESR spectrometer, JES–RE3X (JEOL, Tokyo, Japan). The concentration of NO released versus time is shown in Figure 1.

Results and Discussions. Table 1 shows that the decomposition rate varies significantly with the modification of substituents R^1 and R^2. 3-Alkenecarboxamides (**1**) have approximately two-fold higher rate constants than the corresponding 1-acylamino-3-alkenes (**2** and **3**) (**1a** vs. **3a** ; **1c** vs. **2c** and **3c**). Introduction of a branched alkyl group on R^1 and R^2 positions decreased the rate constants. Steric hindrance seems to be a predominant factor in controlling the reaction. On the other hand, a methoxymethyl group at R^1 position increased drastically the decomposition rate in spite of the increased bulkiness (**3a** vs. **3h**), suggesting that the electronic properties of the substituents R^1 and R^2 are also an important factor in determining the decomposition rate. Quantitaive structure-stability relationships were investigated for 15 compounds using kinetic constants (k), electronic parameters (σ), and steric parameters (ES). Cyclohexane derivatives (**1b**, **2b**, and **3b**) were excluded in order to avoid the conformational influence of the cyclohexane ring. The regression analysis yielded the following equation with a good correlation coefficient ($r = 0.979$).

$$\log k = 0.676(ES^1 + ES^2) + 2.55(\sigma^{*1} + \sigma^{*2}) - 0.422I$$

Table 1. Rate Constants for Decomposition in pH 8.0 Phosphate Buffer at 30 ℃



A \ B	1  a)	2  b)	3  b)
a 	270×10^{-3} *c)* (2.6 min)		130×10^{-3} *d)* (5.3 min)
b 	20×10^{-3} (35 min)	8.0×10^{-3} (87 min)	8.3×10^{-3} (84 min)
c 	15×10^{-3} (46 min)	8.1×10^{-3} (86 min)	6.5×10^{-3} (107 min)
d 	4.5×10^{-3} (150 min)	2.6×10^{-3} (270 min)	2.0×10^{-3} (350 min)
e 	1.0×10^{-3} (690 min)	0.46×10^{-3} (1500 min)	0.38×10^{-3} (1800 min)
f 	0.24×10^{-3} (2900 min)		
g 	3.7×10^{-3} (190 min)		1.2×10^{-3} (580 min)
h 			2.0×10^{-3} (350 min)

a) *E* configuration at the hydroxyimino group. *b)* *Z* configuration at the hydroxyimino group. *c)* The rate constant for decomposition (k, min^{-1}). The values in parentheses are half-life data. *d)* **3a** has a pyridine-4-carbonyl group in place of a pyridine-3-carbonyl.

$$n = 15;\ r = 0.979;\ s = 0.196$$

In this equation, n is the number of data points upon which the correlation equation is based. r is the correlation coefficient and s is the standard deviation. k is the observed rate constant (min^{-1}) for decomposition in pH 8.0 phosphate buffer at 30 ℃. *ES* is Taft steric constant.[8] σ^* is Taft electronic constant for aliphatic system.[9] The numbers 1 and 2 attached to *ES* and σ^* denote substituents R^1 and R^2. I is a indicator variable taking the value of 0 for the compounds **1** and 1 for compounds **2** and **3**. Parameters used and the results of observed and calculated

log k are summarized in Table 2. This equation shows that both steric and electronic factors of R^1 and R^2 substiutents are important for controlling the decomposition rates. Due to the same contribution of R^1 and R^2 on the decomposition rate, the proton abstraction from the α position of the nitro group is assumed to play the critical role in the decomposition reaction. This result is in accord with our previous results on FK409 decomposition experiments, in which we reported that deprotonation at the 5-position of FK409 is the initial step of the decomposition and subsequent NO release in aqueous solution.[5]

Table 2. Kinetic Constants and Quantitative Structure-Stability Relationships Parameters

Compd.	ES^1	ES^2	σ^{*1}	σ^{*2}	$ES^1 + ES^2$	$\sigma^{*1} + \sigma^{*2}$	I	log k	
								Obsd.	Calcd.
1a	−1.43	−1.24	0.52	0.00	−2.67	0.52	0	−0.569	−0.478
1c	−1.24	−1.31	0.00	−0.10	−2.55	−0.10	0	−1.824	−1.980
1d	−1.31	−1.31	−0.10	−0.10	−2.62	−0.20	0	−2.347	−2.282
1e	−1.71	−1.31	−0.19	−0.10	−3.02	−0.29	0	−3.000	−2.784
1f	−2.78	−1.31	−0.30	−0.10	−4.09	−0.40	0	−3.620	−3.788
1g	−1.24	−1.71	0.00	−0.19	−2.95	−0.19	0	−2.432	−2.480
2c	−1.24	−1.31	0.00	−0.10	−2.55	−0.10	1	−2.092	−2.402
2d	−1.31	−1.31	−0.10	−0.10	−2.62	−0.20	1	−2.585	−2.705
2e	−1.71	−1.31	−0.19	−0.10	−3.02	−0.29	1	−3.337	−3.205
3a	−1.43	−1.24	0.52	0.00	−2.67	0.52	1	−0.886	−0.902
3c	−1.24	−1.31	0.00	−0.10	−2.55	−0.10	1	−2.187	−2.147
3d	−1.31	−1.31	−0.10	−0.10	−2.62	−0.20	1	−2.699	−2.705
3e	−1.71	−1.31	−0.19	−0.10	−3.02	−0.29	1	−3.420	−3.205
3g	−1.24	−1.71	0.00	−0.19	−2.95	−0.19	1	−2.921	−2.903
3h	−1.31	−1.24	−0.10	0.00	−2.55	−0.10	1	−2.699	−2.402

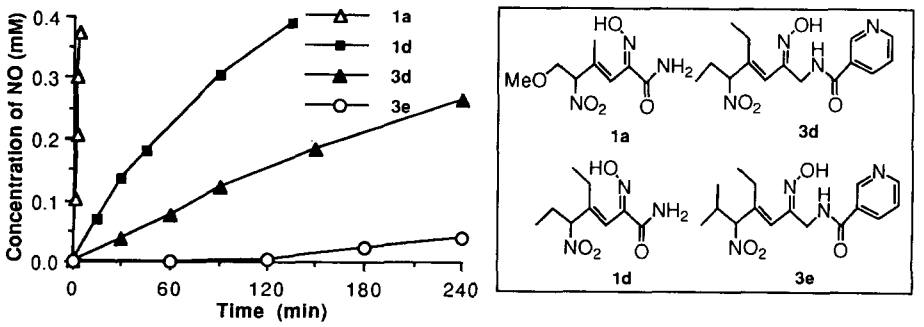


Figure 1. Concentration of NO Formed in Phosphate Buffer Solution (pH 7.4) at 37 °C.
Concentration of NO formed from the substrate (0.5 mM) was measured by X-band ESR spectrometry using carboxy-PTIO (0.51 mM).

Next, we measured NO formation from representative compounds by means of ESR spectroscopy using carboxy-PTIO as a NO trapping agent (Figure 1). These results show that the rate of spontaneous NO release was parallel with that of the decomposition in aqueous solution.

The present investigation showed that novel FK409 derivatives were new spontaneous NO releasers which had a wide range of NO-release rates. The half-life at pH 8.0 and 30 ℃ ranges from 2.6 min for **1a** to 48 h for **1f**. They are relatively stable in acidic conditions and unstable in aqueous alkaline solutions, in marked contrast with the chemical properties of amine/NO complex ions which were recently reported as spontaneous controllable NO releasers.[10] The FK409 derivatives studied here are stable solids and release NO spontaneously at a predictable rate by appropriate choice of substituents. We also found a strong correlation between NO releasing rate and the *in vitro* antiplatelet activity of FK409 derivatives.[11] NO has been shown to play an important role in the regulation of the cardiovascular system, central and peripheral nervous systems, and immune system responses. FK409 derivatives with a predictable NO-release rate should be valuable as research tools and potential drugs.

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11. Studies on the correlation between the *in vitro* antiplatelet activity and NO-releasing rate of FK409 derivatives will be reported elsewhere.

(*Received in Japan* 2 *October* 1995; *accepted* 20 *November* 1995)