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Studies on Prodrugs. IV. Preparation and Characterization of *N*-(5-Substituted 2-oxo-1,3-dioxol-4-yl)methyl Norfloxacin

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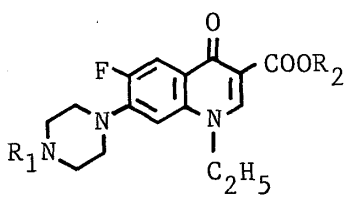
As a new type of norfloxacin (NFLX) prodrug, *N*-(5-substituted 2-oxo-1,3-dioxol-4-yl)methyl NFLXs were designed. These *N*-masked NFLXs were prepared and confirmed to produce higher NFLX levels in blood than NFLX itself after oral administration to mice. *N*-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX was found to be smoothly hydrolyzed in mouse blood *in vitro*, and when administered orally, gave about 5-fold higher blood levels of NFLX than NFLX itself. Thus, the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group was confirmed to function as an amine-type promoiety.

Keywords——(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group; prodrug; norfloxacin; oral absorption; promoiety

It was reported that 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-piperazinylquinoline-3-carboxylic acid (norfloxacin; NFLX, **1**) exhibited stronger antimicrobial activities and a broader spectrum than conventional nalidixic acid analogs, and showed excellent activities against clinically isolated strains such as *P. aeruginosa*.[1] It has been found, however, that the blood levels and the urinary recoveries after oral administration of NFLX to animals were not sufficient,[2] and the *in vivo* antimicrobial activity values (ED_{50}) of NFLX against experimentally induced infections of mice were not as good as expected from the *in vitro* data (MIC).[3]

We have been developing the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group as an ester-type promoiety, and the effectiveness of a novel ampicillin prodrug with this promoiety (KBT-1585) has been reported recently.[4] As a part of our studies of this promoiety we prepared NFLX (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**2**), and confirmed that the ester liberated NFLX in the presence of mouse blood. However, when we measured the blood level of



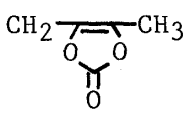
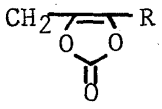
Compd.	R_1	R_2
NFLX **1**	H	H
NFLXester **2**	H	
pefloxacin **3**	CH_3	H
N-masked NFLX **4**		H

Chart 1

NFLX after oral administration of **2** to mice, it was rather lower than that after an equimolar dose of NFLX itself (about 1/2 in terms of C_{max}).

On the other hand, it was reported that although 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (pefloxacin, **3**) showed somewhat lower *in vitro* activity than NFLX, it exhibited 2.2—7-fold higher *in vivo* activities against experimentally induced infections of mice after oral administration.[5] This interesting result was considered to be due to the enhancement of oral absorbability of pefloxacin, and the enhancement seemed to be a result of masking of the secondary amine of NFLX.

Consequently, we examined the possibility of a prodrug approach on the secondary amine with our original promoiety, the (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl group. Because prodrug moieties applicable to amine are fewer than in the cases of carboxylic acids or alcohols,[6] the results of our studies on improving the oral absorbability are presented here.

Chemistry

NFLX (**1**) was synthesized in accordance with the method of Koga *et al.*[1a] The method for preparing the masking agent, 5-substituted 4-bromomethyl-1,3-dioxol-2-one (**5**), was reported previously,[4] and its derivative, 4-(1-bromoalkyl)-1,3-dioxol-2-one (**6**), was also

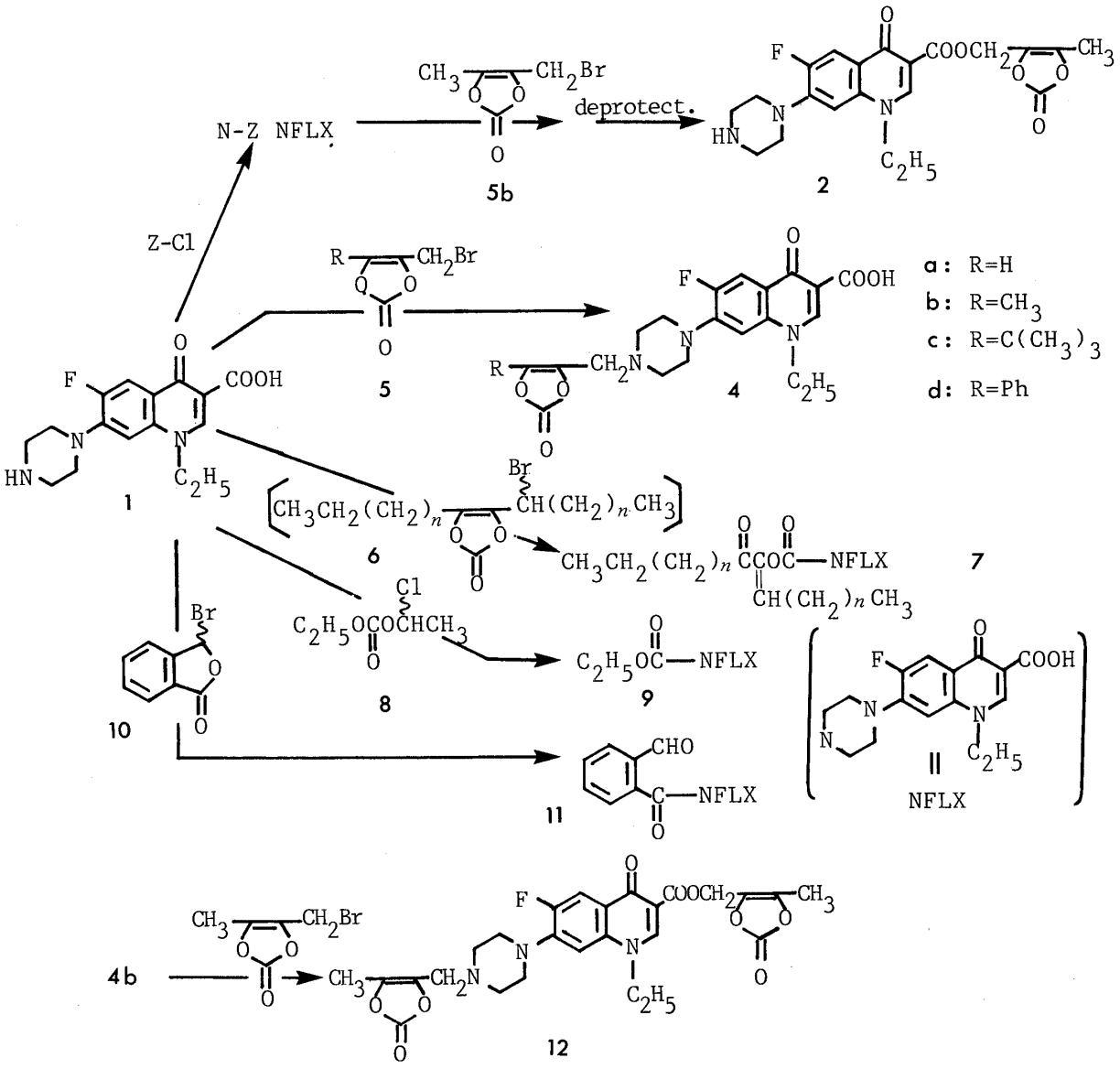


Chart 2

obtained by the same procedure, though **6** was not isolated.

For the preparation of the NFLX ester (**2**), the free amine of NFLX was protected with the benzyloxycarbonyl (Z) group, and the protected NFLX was esterified with **5b** and then deprotected to give the ester, using the method of Yajima, Kiso *et al.*[7] The ester thus obtained was the trifluoromethanesulfonate, but attempts to prepare the free base were unsuccessful because of its instability.

On the other hand, the preparation of *N*-masked NFLX (**4**) was achieved by a one-step reaction in good yield from NFLX without protection of the carboxylic acid. Thus, the secondary amine of NFLX attacked an α-carbon to the bromine atom in the case of the primary bromide **5**, and produced *N*-masked NFLX (**4**). However, in the reactions of secondary halides and NFLX, the amine preferentially attacked the carbonyl carbon of the halide. As a result, the undesired carbamates (**7** and **9**) and amide (**11**) were obtained in the reactions of **6**, **8** (α-chlorodiethyl-carbonate, a bacampicillin-synthesizing reagent) and **10** (3-bromophthalide, a talampicillin-synthesizing reagent), respectively, as shown in Chart 2. It was reported that *N*-phthalidyl NFLX was prepared from phthalaldehydic acid by dehydration at high temperature.[8]

The synthesis of the double-masked NFLX (**12**) was further investigated, and achieved from **4b** by esterification using the bromide (**5b**) and a stronger base under heating.

Oral Absorption Test in Mice

In order to estimate the absorbability of NFLX derivatives, the antimicrobial activities in serum were measured in mice after oral administration of the derivatives.

Five fasted male ddY mice (about 22 g body weight) in each group were orally given a suspension of each NFLX derivative in a 0.5% aqueous solution of carboxymethyl cellulose at a dose equivalent to 100 mg/kg of NFLX. The mice were killed at 30, 60, 120 and 240 min after dosing, and blood taken from the cut axilla region was centrifuged to obtain serum samples. Serum specimens obtained at the same time were combined and assayed on the day of sampling.

Bioassay Method——The antimicrobial activities in serum were measured by microbioassay using *B. subtilis* ATCC 6633 as a test organism. Specimens were assayed against standard solutions of NFLX prepared in mouse serum. The assay plates were incubated

Table I. Antimicrobial Activities in Serum (Equivalent to NFLX) after Oral Administration of NFLX Derivatives to Mice ($n=5$)

Compd.	Eq. concentration to NFLX (μg/ml)			
	30	60	120	240 (min)
2	0.7	1.0	0.9	0.7
4a	3.7	1.8	0.9	0.6
4b	14.6	7.7	2.8	0.5
4c	9.4	10.0	9.1	4.5
4c[a]	(2.1, 8.1)	(3.2, 8.0)	(3.6, 7.3)	(2.8, 1.5)
4d	2.4	2.7	2.2	1.9
7 ($n=0$)	<0.5	<0.5	<0.5	<0.5
11	1.5	0.9	<0.5	<0.5
12	6.9	3.7	1.8	0.5
N-Phthalidyl NFLX	1.4	1.5	1.4	<0.5
NFLX (**1**)	1.8	2.0	1.7	0.8

a) Conc. of (NFLX, **4c**) by HPLC method.

overnight at 35 °C, the diameters of the inhibition zones were measured, and the NFLX concentrations of the test specimens were derived from standard plots constructed by the use of standard solutions. The results are summarized in Table I.

HPLC Method——It seemed to be difficult to determine the exact NFLX concentrations by the bioassay method after administration of **4c**, which liberated NFLX rather slowly in mouse blood as shown later. Thus, the concentrations of NFLX and **4c** were measured individually by high performance liquid chromatography (HPLC), after the serum specimens had been treated with trichloroacetic acid and centrifuged to give protein-free specimens. A Waters Assoc. HPLC machine equipped with a model 6000A pump, a model U6K universal injector, a Shimadzu model SPD-2A spectrophotometric detector (at 280 nm) and a μ-Bondapak C_{18} column (30 cm × 4 mm i.d.) was used. The mobile phase consisted of 0.1 M citrate buffer (pH 4.0)–methanol (55 : 45 for NFLX and 30 : 70 for **4c**, v/v) and the flow rate was 1.0 ml/min. The specimens were assayed against standard solutions of NFLX and **4c** prepared in mouse serum and then treated by the above-mentioned method. The NFLX and **4c** concentrations after oral administration of **4c** are shown in parentheses in Table I.

Stability Test

Their half-lives in artificial gastric juice (pH 1.2) and intestinal juice (pH 6.8)[9] were determined by the HPLC method to estimate the stabilities of the NFLX derivatives in the digestive tract. Each solution of the NFLX derivatives was shaken at 37 °C and the concentration of the remaining initial compound was measured periodically by HPLC, then the half-life of the degradation was calculated. The HPLC conditions were the same as mentioned above, except for the mobile phase content ratio (0.1 M citrate buffer (pH 4.0)–methanol(varied from 55 : 45 to 30 : 70, v/v). The results are summarized in Table II.

Liberation of NFLX

In order to confirm the effective liberation of NFLX from *N*-masked NFLX *in vivo*, the period required for complete hydrolysis of the *N*-masked NFLX was determined by bioautography.

Each of the *N*-masked NFLX was incubated at 37 °C in 40% mouse blood which had been heparinized and diluted with 1/15 M phosphate buffer. A test sample was taken periodically, spotted on a silica gel thin layer chromatography (TLC) plate (Merck silica gel plate No. 5715), and developed with chloroform–methanol (10 : 1, v/v). The dried TLC plate was sprayed with 30% aq. mouse blood and incubated at 37 °C for 30 min to convert the unchanged *N*-masked NFLX to NFLX. The bioautography was carried out by using nutrient agar plates with *B. subtilis* ATCC 6633 as a test organism. The results are summarized in Table II.

TABLE II. Hydrolyses of NFLX Derivatives in Artificial Gastric Juice (AGJ), Artificial Intestinal Juice (AIJ) and Mouse Blood

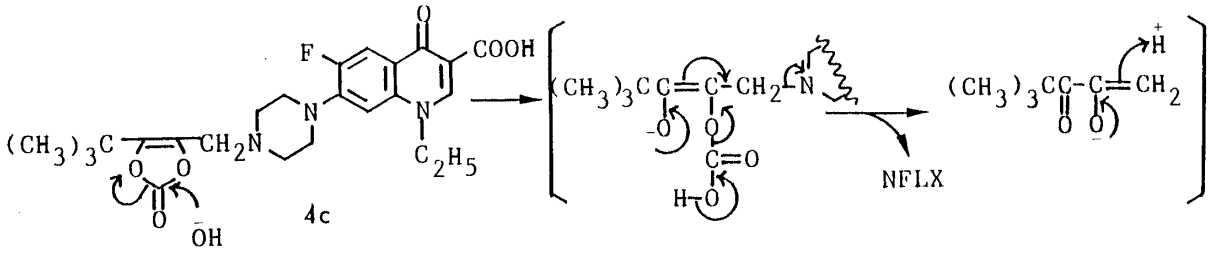
Compd.	Half-life (h)		Time (min) taken for complete hydrolysis in 40% mouse blood
	In AGJ	In AIJ	
4a	0.7	1.5	10
4b	12	8.5	15
4c	>20	18	120
4d	>20	>20	150
7 ($n=0$)	>20	>20	>200
N-Phthalidyl NFLX	≦0.1	≦0.1	≦5
12	18	11	20

The double-masked NFLX (**12**) was observed to change first to **4b** and then to NFLX on the bioautogram.

Alkaline Hydrolysis of *N*-Masked NFLX

If these *N*-masked NFLX are hydrolyzed through electron transfer-initiated cleavage of the cyclic carbonate like ampicillin (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl ester,[4] the hydrolysis products should be NFLX, α-dicarbonyl compound and carbon dioxide, as shown in Chart 3. In order to confirm this, the degradation products of *N*-(5-*tert*-butyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (**4c**) in alkali were investigated by the following method using TLC and gas chromatography (GC). The reason why **4c** was selected as a test compound is that the hydrolysis product (diketone) is more easily extracted into organic solvent and more clearly detectable by GC than the others.

A solution of **4c** (5 mg/ml in 0.01 N potassium hydroxide) was stirred at room temperature for 10 min followed by extraction with ethyl ether. NFLX in aqueous layer was identified by TLC (Merck silica gel No 5715) with ethyl acetate– acetone– acetic acid –water (5 : 2 : 1 : 1, v/v) as a developing solvent. GC was carried out at 70 °C on a glass column packed with 10% PEG 20 M (1 m × 3 mm i.d.). The main peak of the ethyl ether layer, having a retention time of 2.0 min coincided with authentic 4,4-dimethyl-2,3-pentandione (**13**).[10]



NFLX

CO_2

$(CH_3)_3CC(=O)C(=O)CH_3$

13

Chart 3

Discussion

Some of the *N*-masked NFLX (**4**) functioned effectively as NFLX prodrugs. In particular, *N*-(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (**4b**) was found to be smoothly hydrolyzed in mouse blood *in vitro* (within 15 min), and was confirmed to liberate the parent NFLX, producing about 5-fold higher serum levels of NFLX than NFLX itself in the oral absorption test in mice. This compound appears to be an interesting candidate NFLX prodrug for a clinical study.

N-(5-Substituted 2-oxo-1,3-dioxol-4-yl)methyl NFLXs (**4**) were generally well absorbed as compared with NFLX itself in mice. The improvement of the oral absorption may be due to enhancement of the lipophilicity of the drug by blocking the secondary amine of the amphoteric NFLX at physiological pH.

On the other hand, although the NFLX ester (**2**) should also show increased lipophilicity, like the *N*-masked compounds, the ester was poorly absorbed orally. The reason for this was

not investigated in the present work, but it is possible that if the free NFLX ester were stable, it would be absorbed well.

In the case of another amphoteric drug, ampicillin, the ester-type prodrugs show improved oral absorbability (*e.g.*, Talampicillin, Bacampicillin and KBT-1585), but *N*-masked ampicillins such as hetacillin or enamine-type prodrugs are not absorbed well, because they are so unstable to acid that they are hydrolyzed to the parent ampicillin before absorption.[11] Thus, the (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl group showed sufficient instability to function as an effective amine-type promoiety.

There were two types of ineffective derivatives among these *N*-masked NFLXs. One was too unstable in the gastrointestinal tract (such as **4a** or *N*-phthalidyl NFLX), and the other was too stable to liberate the parent NFLX smoothly after absorption (such as **4d**, **7** or **9**). The latter compounds were not completely converted to NFLX within 120 min in 40% mouse blood. Compound **4c** showed a long duration of antimicrobial activity in serum, but NFLX was released rather slowly *in vitro*. HPLC determination showed that unchanged **4c** stayed in the blood for a long time, and **4c** itself showed a moderate activity against the test organism, *B. subtilis*.

The *N*-masked NFLX with a conventional ester-type promoiety such as 1-ethoxycarbonyloxyethyl or phthalidyl could function as an amine prodrug, so we attempted to prepare such derivatives using the halides. The reaction, however, did not give the desired product but the undesired carbamate or amide. Since the same situation occurred with the other secondary halides (**6**), the secondary amine of NFLX seems to attack the sterically less-hindered carbonyl carbon of the secondary halides. These products were too stable in the blood and did not function as NFLX prodrugs. *N*-Phthalidyl NFLX, which was obtained by another method,[8] certainly liberated NFLX smoothly *in vitro*, but was found to be too labile in the artificial digestive juice.

The mechanism of liberation of NFLX from *N*-masked NFLX was not elucidated in the present study. It is well known that dealkylation of an alkylamine is initiated by oxidation of the α-carbon atom of the nitrogen atom or the nitrogen atom itself,[12] but **4b** was found to produce NFLX smoothly in the presence of mouse blood, so it was considered that the cyclic carbonate was initially attacked by esterase and then the carbon–nitrogen bond was cleaved by electron transfer as in the case of ampicillin (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl ester.[4] Compound **4c** was hydrolyzed by alkaline hydrolysis, the mechanism of which was considered to be similar to that of esterase-catalyzed hydrolysis,[6] to produce NFLX and diketone. The mechanism of the hydrolysis and the metabolites *in vivo* are under investigation.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, model MR-21, and all melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined at 100 MHz on a Nihon Denshi PS-100 NMR spectrometer using tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded with a Shimadzu IR-440 machine. Final compounds were analyzed for C, H, N, and the values were within 0.4% of the calculated theoretical ones. No attempts were made to maximize the yields.

***N*-Benzyloxycarbonyl NFLX (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester**——NFLX (synthesized in accordance with the lit.,[1a] 3.2 g) was dissolved in 2 N sodium hydroxide (7.5 ml) at 0 °C, and benzyloxycarbonyl chloride (2 ml) was added slowly. The mixture was stirred at room temperature for 1 h to give a white solid. The solid was collected by filtration, washed with water, dried and recrystallized from chloroform–ethanol to give 4.2 g (93%) of colorless needles. mp 223—224 °C. IR ν_{max}^{KBr} cm^{-1}: 1720, 1700, 1625 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.43 (3H, t, $NCH_2\underline{CH_3}$), 3.35 (4H, m, piperazine ring H), 3.65 (4H, m, piperazine ring H), 4.58 (2H, q, $N\underline{CH_2}CH_3$), 5.13 (2H, s, $\underline{CH_2}Ph$), 7.40 (5H, s, arom. H), 7.21 (1H, d, J=7 Hz, 8-H), 7.91 (1H, d, J=13 Hz, 5-H), 8.92 (1H, s, 2-H). *Anal.* Calcd for $C_{24}H_{24}FN_3O_5$: C, 63.57; H, 5.33; N, 9.27. Found: C, 63.66; H, 5.16; N, 8.93. Compound **5b** (synthesized in accordance with the lit.,[4] 2.6 g) and potassium carbonate were added to a solution of *N*-benzyloxy NFLX (4.5 g) in acetonitrile (60 ml), and the whole was stirred at 60 °C overnight. The solvent was removed *in vacuo*, then 150 ml of chloroform was added to the residue. The solution was washed with water, dried over anhydrous magnesium sulfate,

and concentrated, and the residue was chromatographed on silica gel (Merck Silica gel 60 No. 7734). Elution with chloroform–methanol (100 : 1, v/v) and recrystallization from ethyl acetate gave 3.9 g (69%) of colorless needles. mp 143—145 °C. IR ν_{max}^{KBr} cm^{-1}: 1815, 1725, 1690, 1620 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.56 (3H, t, $NCH_2\underline{CH_3}$), 2.27 (3H, s, $C=C\underline{CH_3}$), 3.25 (4H, m, piperazine ring H), 3.75 (4H, m, piperazine ring H), 4.24 (2H, q, $N\underline{CH_2}CH_3$), 5.05 (2H, s, $\underline{CH_2}Ph$), 5.18 (2H, s, $C=C\underline{CH_2}$), 6.73 (1H, d, J=7 Hz, 8-H), 7.35 (5H, s, arom. H), 7.97 (1H, d, J=13 Hz, 5-H), 8.35 (1H, s, 2-H).

NFLX (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester (2)——Thioanisole (0.6 g) and trifluoromethanesulfonic acid (0.75 g) were added to a solution of the previous compound (1.7 g) in dioxane (30 ml), and the mixture was stirred at room temperature for 8 h, then the solvent was removed *in vacuo*. The residue was dissolved in methanol (3 ml), chloroform (10 ml) was added, and the mixture was stored at 5 °C overnight. The resultant crystals were collected, and recrystallized from acetone–ethyl ether to give 0.55 g of pale yellow needles of the ester as the trifluoromethanesulfonate. (Yield 32%) mp 198—202 °C. IR ν_{max}^{KBr} cm^{-1}: 3100—2550, 1830, 1820, 1720, 1280, 1170, 1075, 1035, 640 (SO_3H). ^{1}H-NMR (in DMSO-d_6) δ: 1.41 (3H, t, $NCH_2\underline{CH_3}$), 2.26 (3H, s, $C=C\underline{CH_3}$). 3.2—3.6 (8H, m, piperazine ring H), 4.48 (2H, q, $N\underline{CH_2}CH_3$), 5.13 (2H, s, $C=C\underline{CH_2}$), 7.14 (1H, d, J=7 Hz, 8-H), 7.85 (1H, d, J=13 Hz, 5-H), 8.67 (1H, s, 2-H), 8.7 (2H, br, NH_2). *Anal.* Calcd for $C_{22}H_{23}F_4N_3O_9S$: C, 45.44; H, 3.99; N, 7.23. Found: C, 45.31; H, 4.09; N, 7.07.

***N*-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (4b)**——NFLX (3.2 g), **5b** (2.3 g) and potassium bicarbonate (1.2 g) were suspended in 50 ml of *N,N*-dimethylformamide, and stirred for 5 h under ice cooling. The solvent was removed *in vacuo*, and the resultant residue was extracted with chloroform. The extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated, then the residue was recrystallized from chloroform–ether to give 2.8 g (66%) of pale yellow crystalline powder. mp 184—190 °C. IR ν_{max}^{KBr} cm^{-1}: 1815, 1725 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.43 (3H, t, $NCH_2\underline{CH_3}$), 2.15 (3H, s, $C=C\underline{CH_3}$), 2.70 (4H, m, piperazine ring H), 3.35 (4H, m, piperazine ring H), 3.50 (2H, s, $C=C\underline{CH_2}$), 4.60 (2H, q, $N\underline{CH_2}CH_3$), 7.20 (1H, d, J=7 Hz, 8-H), 7.90 (1H, d, J=13 Hz, 5-H), 8.92 (1H, s, 2-H). *Anal.* Calcd for $C_{21}H_{22}FN_3O_6$: C, 58.46; H, 5.14; N, 9.74. Found: C, 58.20; H, 5.14; N, 9.73.

Other *N*-masked NFLXs were obtained by the same procedure as described above and their physical properties were as follows.

***N*-(2-Oxo-1,3-dioxol-4-yl)methyl NFLX (4a)**——Yield 47%. mp 165—170 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1825, 1725, 1630 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.60 (3H, t, $NCH_2\underline{CH_3}$), 2.80 (4H, m, piperazine ring H), 3.40 (4H, m, piperazine ring H), 3.50 (2H, s, $C=C\underline{CH_2}$), 4.33 (2H, q, $N\underline{CH_2}CH_3$), 6.85 (1H, d, J=7 Hz, 8-H), 7.04 (1H, s, C=CH), 8.03 (1H, d, J=13 Hz, 5-H), 8.64 (1H, s, 2-H). *Anal.* Clacd for $C_{20}H_{20}FN_3O_6$: C, 57.55; H, 4.83; N, 10.07. Found: C, 57.20; H, 5.02; N, 9.81.

***N*-(5-*tert*-Butyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (4c)**——Yield 64%. mp 242—245 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1825, 1810, 1720, 1630 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.41 (9H, s, $C=CC(\underline{CH_3})_3$), 1.60 (3H, t, $NCH_2\underline{CH_3}$), 2.77 (4H, m, piperazine ring H), 3.38 (4H, m, piperazine ring H), 3.53 (2H, s, $C=C\underline{CH_2}$), 4.35 (2H, q, $N\underline{CH_2}CH_3$), 6.84 (1H, d, J=7 Hz, 8-H), 7.95 (1H, d, J=13 Hz, 5-H), 8.60 (1H, s, 2-H). *Anal.* Calcd for $C_{24}H_{28}FN_3O_6$: C, 60.88; H, 5.96; N, 8.87. Found: C, 60.69; H, 5.91; N, 8.72.

***N*-(5-Phenyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (4d)**——Yield 67%. mp 205—207 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1840, 1730, 1630 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.42 (3H, t, $NCH_2\underline{CH_3}$), 2.80 (4H, m, piperazine ring H), 3.30 (4H, m, piperazine ring H), 3.74 (2H, s, $C=C\underline{CH_2}$), 4.58 (2H, q, $N\underline{CH_2}CH_3$), 7.18 (1H, d, J=7 Hz, 8-H), 7.4—7.7 (5H, m, arom. H), 7.90 (1H, d, J=13 Hz, 5-H), 8.92 (1H, s, 2-H). *Anal.* Calcd for $C_{26}H_{24}FN_3O_6$: C, 63.28; H, 4.90; N, 8.52. Found: C, 63.06; H, 4.98; N, 8.40.

***N*-(4-Oxo-2-hexen-3-yloxy)carbonyl NFLX (7, n=0)**——A suspension of 4,5-diethyl-1,3-dioxol-2-one (synthesized in accordance with the lit.,[13] 0.36 g), *N*-bromosuccinimide (0.45 g) and a catalytic amount of benzoylperoxide in carbon tetrachloride (100 ml) was refluxed for 30 min, and then concentrated to half the initial volume. Insoluble materials were filtered off and the solution was concentrated to give a syrup. The syrup was dissolved in *N,N*-dimethylformamide (10 ml), and then NFLX (0.64 g) and potassium bicarbonate (0.24 g) were added. The mixture was stirred at room temperature for 5 h, then the solvent was removed *in vacuo*. The resultant residue was extracted with chloroform, and the extract was washed with water, dried over anhydrous magnesium sulfate, then concentrated to give a residue. The residue was chromatographed on silica gel (Merck Silica gel 60 No. 7734). Elution with chloroform–methanol (60 : 1, v/v) and recrystallization from ethanol–ethyl ether gave 0.41 g (45%) of colorless needles. mp 217—219 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1720, 1690, 1630 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.14 (3H, t, $COCH_2\underline{CH_3}$), 1.62 (3H, t, $NCH_2\underline{CH_3}$), 1.97 (3H, d, $C=CH\underline{CH_3}$), 2.71 (2H, q, $CO\underline{CH_2}CH_3$), 3.4 (4H, m, piperazine ring H), 3.8 (4H, m, piperazine ring H), 4.33 (2H, q, $N\underline{CH_2}CH_3$), 6.51 (1H, q, $C=C\underline{H}CH_3$), 6.85 (1H, d, J=7 Hz, 8-H), 7.98 (1H, d, J=12 Hz, 5-H), 8.57 (1H, s, 2-H). *Anal.* Calcd for $C_{23}H_{26}FN_3O_6$: C, 60.12; H, 5.70; N, 9.15. Found: C, 60.01; H, 5.77; N, 9.03.

N-(5-Oxo-3-octen-4-yloxy)carbonyl NFLX (**7**, n=1) was also prepared by the same procedure from 4,5-dipropyl-1,3-dioxol-2-one. Yield 41%. mp 180—183 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1725, 1690, 1630 (C=O). ^{1}H-NMR (in $CDCl_3$) δ:0.97 (3H, t, $COCH_2CH_2\underline{CH_3}$), 1.12 (3H, t, $C=CHCH_2\underline{CH_3}$), 1.5—1.8 (5H, m, $NCH_2\underline{CH_3}$ and $COCH_2\underline{CH_2}CH_3$), 2.31 (2H, m, $C=CHCH_2\underline{CH_3}$), 2.66 (2H, t, $CO\underline{CH_2}CH_2CH_3$), 3.4 (4H, m, piperazine ring H),

3.8 (4H, m, piperazine ring H), 4.33 (2H, q, NCH_2CH_3), 6.40 (1H, t, $C=CHCH_2$), 6.84 (1H, d, $J=7$ Hz, 8-H), 7.97 (1H, d, $J=12$ Hz, 5-H), 8.57 (1H, s, 2-H). *Anal.* Calcd for $C_{25}H_{30}FN_3O_6$: C, 61.59; H, 6.20; N, 8.62. Found: C, 61.37; H, 6.07; N, 8.53.

N-Ethoxycarbonyl NFLX (**9**) or *N*-(2-formylbenzoyl) NFLX (**11**) was obtained by the same procedure from α-chlorodiethylcarbonate or 3-bromophthalide. The physical properties were as follows.

***N*-Ethoxycarbonyl NFLX (9)**——Yield 41%. mp 230—234 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1710, 1620 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.22 (3H, t, OCH_2CH_3), 1.44 (3H, t, NCH_2CH_3), 3.30 (4H, m, piperazine ring H), 3.60 (4H, m, piperazine ring H), 4.11 (2H, q, OCH_2CH_3), 4.60 (2H, q, NCH_2CH_3), 7.22 (1H, d, $J=7$ Hz, 8-H), 7.93 (1H, d, $J=14$ Hz, 5-H), 8.93 (1H, s, 2-H). *Anal.* Calcd for $C_{19}H_{22}FN_3O_5$: C, 58.30; H, 5.67; N, 10.74. Found: C, 58.12; H, 5.82; N, 10.51.

***N*-(2-Formylbenzoyl) NFLX (11)**——Yield 42%. mp 260—264 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1710, 1620 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.43 (3H, t, NCH_2CH_3), 3.50 (4H, m, piperazine ring H), 3.90 (4H, m, piperazine ring H), 4.58 (2H, q, NCH_2CH_3), 7.21 (1H, d, $J=7$ Hz, 8-H), 7.40—8.10 (4H, m, arom. H), 7.93 (1H, d, $J=13$ Hz, 5-H), 8.93 (1H, s, 2-H), 10.03 (1H, s, CHO). *Anal.* Calcd for $C_{24}H_{22}FN_3O_5$: C, 63.85; H, 4.91; N, 9.31. Found: C, 63.81; H, 5.02; N, 9.16.

***N*-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl NFLX (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester (12)**——Compound **5b** (0.87 g) was added to a suspension of **4b** (1.3 g) and potassium carbonate (0.62 g) in *N*,*N*-dimethylformamide (20 ml), and the mixture was stirred at 40 °C for 10 h, then poured into ice-water and extracted with 100 ml of chloroform. The extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated to give a residue, which was chromatographed on silica gel (Merck Silica gel 60, No. 7734). Elution with chloroform–methanol (100 : 1, v/v) and recrystallization from ethanol–ethyl ether gave 0.6 g (37%) of colorless needles. mp 168—172 °C. IR ν_{max}^{KBr} cm^{-1}: 1840, 1735, 1695, 1625 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.56 (3H, t, NCH_2CH_3), 2.16 (3H, s, $NCH_2C=CCH_3$), 2.25 (3H, s, $OCH_2C=CCH_3$), 2.74 (4H, m, piperazine ring H), 3.30 (4H, m, piperazine ring H), 3.42 (2H, s, $C=CCH_2N$), 4.21 (2H, q, NCH_2CH_3), 5.04 (2H, s, $C=CCH_2O$), 6.72 (1H, d, $J=6$ Hz, 8-H), 7.98 (1H, d, $J=13$ Hz, 5-H), 8.34 (1H, s, 2-H). *Anal.* Calcd for $C_{26}H_{26}FN_3O_9$: C, 57.46; H, 4.82; N, 7.73. Found: C, 57.31; H, 4.77; N, 7.71.

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