Agents for the Treatment of Overactive Detrusor. I. Synthesis and Structure–Activity Relationships of 1,1′-Biphenyl Derivatives

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A series of 1,1′-biphenyl-2,6-dicarboxylic acid diesters were synthesized and examined for their inhibitory activity on guinea-pig detrusor muscle contraction at electrical field stimulation *in vitro*. Among them, 6-isopropyl 2-methyl 3-hydroxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate, FR75513 (8a) was one of the potent compounds ($IC_{50} = 3.3 \times 10^{-6}$ g/ml). This compound (8a) exhibited a strong inhibitory activity on detrusor contraction after intravenous administration in anesthetized rats ($ID_{50} = 0.04$ mg/kg).

Keywords overactive detrusor; inhibitory activity; electrical field stimulation; 1,1′-biphenyl-2,6-dicarboxylic acid diester

The overactive detrusor syndrome is a disease which makes the number of micturition extraordinarily large in a single day. Detrusor contraction is mainly mediated by muscarinic receptors in the detrusor and it is well known that anticholinergics alone are unable to completely suppress detrusor contraction (atropine resistance).[1] To overcome this atropine resistance, clinically available agents for the treatment of overactive detrusor have anticholinergic and other pharmacological activities.

For example, terodiline (Mictrol) apparently acts by combined competitive anticholinergic and calcium antagonistic activities.[2] Oxybutynin (Pollakisu), a potent anticholinergic agent, displays potent local anesthetic and spasmolytic activities as well.[3]

On the other hand, nifedipine, a typical calcium antagonist, is more potent in suppressing detrusor contraction at electrical field stimulation *in vitro* than terodiline or oxybutynin, and also suppresses detrusor contraction stimulated by carbachol *in vitro* (Table I). However nifedipine possesses high vascular selectivity.

Recently, attempts to replace the 1,4-dihydropyridine nucleus in nifedipine with other heterocyclic rings such as dihydropyran,[4a] dihydropyridazine,[4b] dihydrothiopyran,[4c] dihydropyrazine,[4d] and dihydropyrimidine[4e] have been carried out, and they also show calcium antagonistic activities. The selectivity of relaxant activity on detrusor muscle to artery muscle of nifedipine might be improved by replacing the 1,4-dihydropyridine nucleus with another skeleton, hence possibly leading to the creation of a new agent for treatment of an overactive detrusor without side effects based on anticholinergic activity.

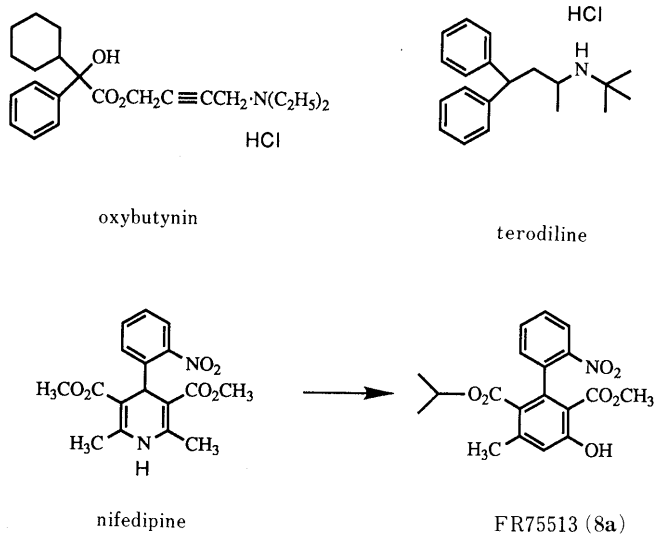


Fig. 1

Our design concepts were as follows; 1) A change in the basic skeleton of nifedipine, 1,4-dihydropyridine, to another skeleton (in this case benzene ring). 2) Introduction of a hydroxyl group to compensate for the decrease of the molecular hydrophilicity due to the replacement described above. 3) Since the unsymmetric esters in 1,4-dihydropyridine tended to show stronger relaxant activity than symmetric esters,[5] ester groups were made unsymmetrical. Using these concepts 6-isopropyl 2-methyl 3-hydroxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate, FR75513 (**8a**) was designed and synthesized (Fig. 1). This compound **8a** possessed weaker inhibitory activity on KCl induced contraction of rat aorta than nifedipine, but had a more potent relaxant activity on detrusor contraction at electrical field stimulation than terodiline or oxybutynin (Table I). In addition to this, the selectivity of the relaxant activity on the detrusor muscle to artery muscle of FR75513 was much improved in comparison to that of nifedipine. The desirable properties of FR75513 as an agent for the treatment of the overactive detrusor made us further investigate this compound. Herein, we report the synthesis of FR75513 and related compounds, pharmacological results *in vitro* and *in vivo* assay, and its structure–activity relationships.

Chemistry Charts 1—3 illustrate the synthetic routes to the present 1,1′-biphenyl related compounds **4**, **6**—**15**.

TABLE I. Effect on Electrically and Carbachol Induced Contractions of G-P Detrusor and KCl Induced Contraction in Rat Aorta[a]

Compd.	G-P detrusor		Rat aorta
	Electrically induced contraction IC_{50} (g/ml)	Carbachol induced contraction IC_{50} (g/ml)	KCl induced contraction IC_{50} (g/ml)
Terodiline	1.4×10^{-5}	9.8×10^{-6}	1.2×10^{-6}
Oxybutynin	2.1×10^{-5}	9.9×10^{-8}	5.4×10^{-7}
Nifedipine	4.7×10^{-7}	1.6×10^{-7}	2.1×10^{-9}
FR75513	3.4×10^{-6}	$>1.0 \times 10^{-5}$	1.1×10^{-6}

a) The *in vitro* test procedures were carried out as described in the experimental section.

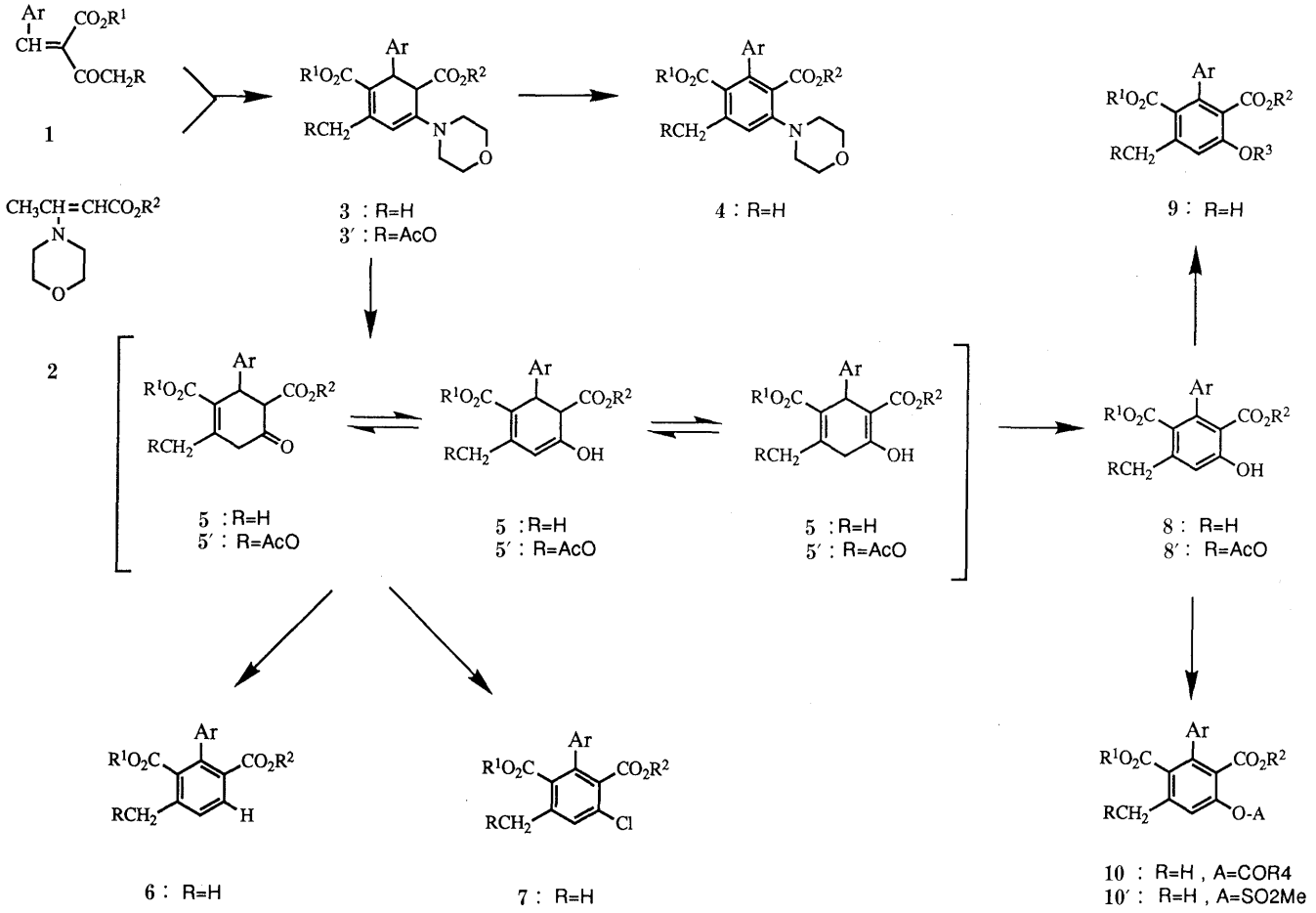


Chart 1

Benzylidene derivative **1**, available by the Knoevenagel condensation of alkyl acetoacetate with the appropriate arylaldehyde, was condensed with morpholinocrotonate **2** to afford the compound **3**, **3′**. Oxidation of **3** afforded morpholino derivative **4**. Hydrolysis of the obtained compound **3**, **3′** afforded a tautomeric mixture of 3-cyclohexenone **5**, **5′**. Oxidation of the tautomeric mixture **5**, **5′** by iodine/sodium acetate or bromine[6] afforded a new phenol derivative **8**, **8′** which has four other substituents in the nucleus. Conversion of the hydroxyl group in compound **8** to a hydrogen atom **6** was achieved by the following procedure. Reduction of **5** with sodium borohydride followed by dehydration of the resulting alcohol with $POCl_3$ and pyridine afforded 2,5-dihydrobenzene-1,3-dicarboxylic acid diester. Oxidation of the dihydrobenzene with iodine/sodium acetate afforded compound **6**. Phosphorous pentachloride treatment of 3-cyclohexenone **5** afforded chloro derivaive **7**. Alkylation of the phenol **8** afforded ether **9** and acylation afforded the corresponding carboxylate and carbonate **10**. Sulfonylation of the phenol **8** afforded sulfonate **10′** (Chart 1).

Deacetylation of the compound **8′** with *p*-toluenesulfonic acid in methanol afforded lactone **11**. Reaction of **8a** with methylamine in ethanol exclusively afforded amide **12**. Removal of the *tert*-butyl group in the ester moiety of **8** with trifluoroacetic acid (TFA) afforded monocarboxylic acid **13**. Dicarboxylic acid **14** was obtained by hydrolysis of diester **8** (Chart 2).

Hydrogenation of the nitro group **9** or hydrogenolysis of the benzyloxy group **8** on the aryl moiety afforded lactam **15** or lactone **15′** which were formed between the amino group or the hydroxy group and the smaller ester group (methyl group). In this case steric effects determined the direction of the cyclization (Chart 3).

Chart 4 illustrates the synthesis of methyl derivative **19**, **21**.

The Sandmeyer reaction of 2-amino-1, 3-dicyano-4, 6-dimethylbenzene (**16**) afforded 2-bromo-1, 3-dicyano-4, 6-dimethylbenzene (**17**). Hydrolysis of the nitrile groups followed by esterification afforded the symmetrical diester **18**, which was coupled with 1-iodo-2-nitrobenzene to afford the 1,1′-biphenyl derivative **19**. In this coupling reaction a dimeric compound was obtained as a minor product.[7] Partial hydrolysis of diester **19** and re-esterification afforded the unsymmetrical diester **21**.

Structure–Activity Relationships (Tables II—V) To study the structure–activity relationships, the parent structure of FR75513 was considered to be comprised of four active regions of importance: the nitrophenyl group (aryl moiety), the ester group, the phenolic hydroxyl group and the methyl group.

Modification of the aryl moiety revealed that substitution at the *ortho* position of the benzene ring in aryl moiety produced higher activity than substitution at the *para* and *meta* positions (**8a**,**b**,**c**, Table II). As for the substituents **8a**,**d**—**j**, electron withdrawing groups such as nitro and cyano groups **8a**,**j** had strong activity and electron releasing groups such as methoxy and benzyloxy groups **8f**,**g**

decreased the activity. In the case where an amino or a hydroxy group is at the ortho position, ring formation between the substituents and the ester group occurs to afford a lactam **15** or lactone **15′**, both of which showed weak activity compared with the parent compound, FR75513 (Table III). Replacement of the benzene ring with heterocycles such as thiophene and pyridine rings **8k—n** also showed a decrease in activity. In the case of pyridine substitution, substitution at the 2- position also showed relatively higher activity than substitution at the 4- and 3-positions.

Concerning modification of the ester groups at the 2- and 6-positions, the unsymmetrical esters **8a,o,q—s,u** exhibited stronger activity than the symmetrical ester **8p** except **8t**. Conversion of the ester function to the carboxylic acid or the amide **12—14** resulted in a nearly complete loss of activity.

Replacement of the phenolic hydroxyl group of the compound **8a** with an alkoxy group **9a—g** or a methanesulfonyloxy group **10′a** decreased the activity. Introduction of an ester **10a,b**, a methyl group **19**, or a chlorine atom **7** instead of the hydroxyl group almost maintained the activity (Table V). In the case of 3,5-dimethyl derivatives **19**, **21**, the symmetric ester **19** was stronger than the unsymmetric ester **21**. Replacement of the hydroxyl group with a hydrogen atom **6** or a carbonate group **10c** slightly decreased the activity.

Replacement of 5-methyl group of the compound **8a** with an acetoxymethyl group **8′a** dramatically decreased the activity. Conversion of **8′a** to the lactone **11** resulted in a complete loss of the activity as seen in 1,4-dihydropyridine lactone derivative[8] (Table IV).

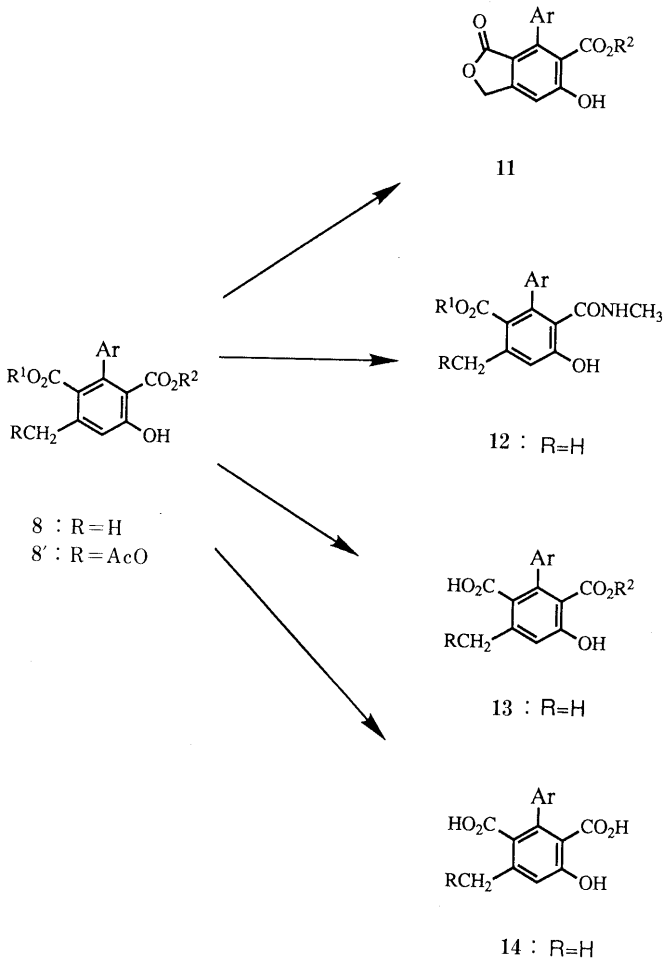


Chart 2

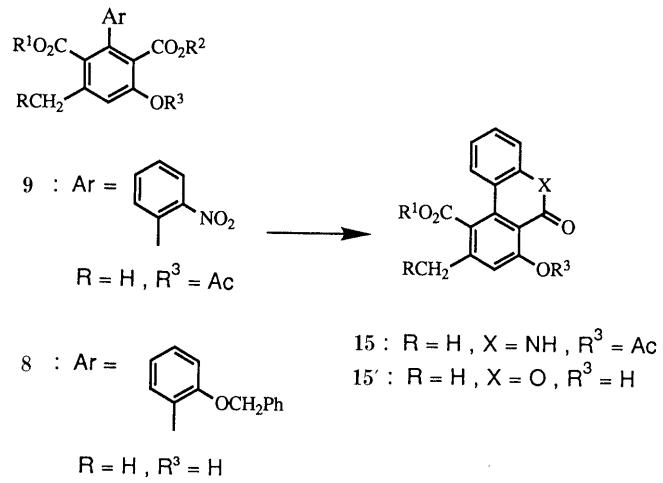


Chart 3

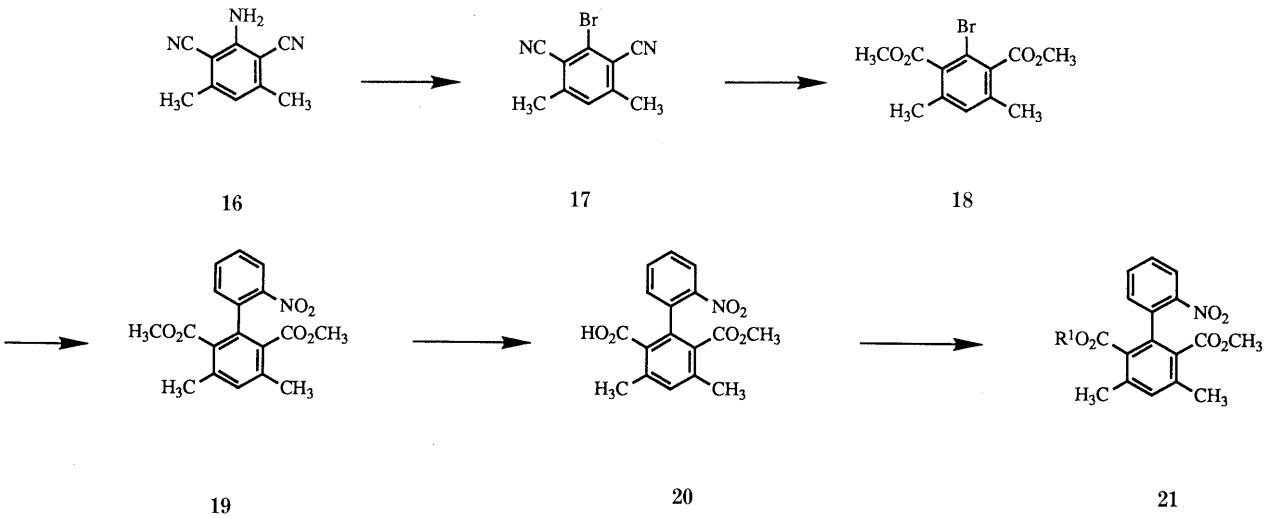
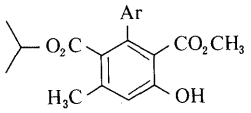


Chart 4

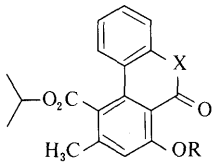
TABLE II. Physical and Pharmacological Properties of 1,1′-Biphenyl-2,6-dicarboxylic Acid Derivatives and Related Compounds



Compd. No.	Ar	mp (°C) Recryst. solv.[a]	Yield (%)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	*In vitro*[b] % inhibition at 10^{-5} g/ml
8a	2-NO_2-Ph	69—71 P.ether	34.4	$C_{19}H_{19}NO_7$	61.12	5.13	3.75	60.76	5.13	4.04	76.2[f]
8b	3-NO_2-Ph	74—76 P.ether	29.4	$C_{19}H_{19}NO_7$	61.12	5.13	3.75	60.71	5.09	3.52	11.8
8c	4-NO_2-Ph	117—119 IPE	18.2	$C_{19}H_{19}NO_7$	61.12	5.13	3.75	60.73	5.19	3.68	−4.3
8d	Ph	71—73[c]	31.3	$C_{19}H_{20}O_5$	69.50	6.14		69.43	6.15		40.0
8e	2-Me-Ph	Oil[d]	46.5	$C_{20}H_{22}O_5$							26.0
8f	2-MeO-Ph	102—104 IPE	5.4	$C_{20}H_{22}O_6$	67.03	6.19		66.61	5.99		10.3
8g	2-$PhCH_2O$-Ph	110.5—104 P.ether	25.1	$C_{26}H_{26}O_6$	71.87	6.03		72.14	6.04		11.6
8h	2-F-Ph	Oil[e]	27.3	$C_{19}H_{19}FO_5$							25.0
8i	2-Cl-Ph	53—55[c]	20.1	$C_{19}H_{19}ClO_5$	62.90	5.28		62.82	5.27		19.4
8j	2-CN-Ph	90—91 IPE	59.8	$C_{20}H_{19}NO_5$	67.98	5.42	3.96	67.87	5.61	4.02	66.1[g]
8k	2-Thienyl	101—102 c-H	17.2	$C_{17}H_{18}O_5S$	61.10	5.43		61.08	5.43		18.1
8l	2-Py·HCl	164 (dec.)[c]	27.5	$C_{18}H_{19}NO_5 \cdot HCl \cdot 0.25H_2O$	58.38	5.58	3.78	58.69	5.32	3.65	38.5
8m	3-Py	106—108 IPE	8.8	$C_{18}H_{19}NO_5 \cdot 0.25H_2O$	64.76	5.89	4.20	64.84	5.68	4.15	7.1
8n	4-Py	175—176 C-IPE	30.8	$C_{18}H_{19}NO_5$	65.64	5.81	4.25	65.39	5.62	4.12	24.0

a) P. ether, petroleum ether; IPE, diisopropyl ether; c-H, cyclohexane; C, chloroform. *b*) The *in vitro* tests (field stimulation) were carried out as described in the experimental section. *c*) Complete evaporation gave analytical sample. *d*) MS *m/z*: 342 (M^+), 310 ($M^+ - OMe$), 283 ($M^+ - O$-iso-Pr). *e*) MS *m/z*: 346 (M^+), 314 ($M^+ - OMe$). *f*) $IC_{50} = 3.3 \times 10^{-6}$ g/ml. *g*) $IC_{50} = 5.3 \times 10^{-6}$ g/ml.

TABLE III. Physical and Pharmacological Properties of 1,1′-Biphenyl-2,6-dicarboxylic Acid Derivatives



Compd. No.	X	R	mp (°C) Recryst.[a] Solv.	Yield (%)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	*In vitro*[b] % inhibition at 10^{-5} g/ml
15	NH	Ac	238—239 IPE–A	52.7	$C_{20}H_{19}NO_5 \cdot 0.25H_2O$	67.12	5.49	3.91	67.21	5.34	3.86	37.5
15′	O	H	142 P.ether	60.0	$C_{18}H_{16}O_5$	69.22	5.16		68.76	4.98		23.8

a) IPE, diisopropyl ether; A, ethyl acetate; P.ether, petroleum ether. *b*) The *in vitro* test were carried out as described in the experimental section.

Pharmacological Activity *in Vivo* and Discussion Among 12 compounds which showed more than 50% inhibition in the *in vitro* test, FR75513 (**8a**) showed the strongest activity and was selected for *in vivo* testing. The results are shown in Table VI.

FR75513 had strong inhibitory activity in i.v. administration ($IC_{50} = 0.04$ mg/kg), but had less activity in i.d. administration. The reason for its poor activity in i.d. administration was considered to be poor i.d. absorption, since this compound administered as polyethyleneglycol suspension exists as a white solid in duodenal lumen 2 h later. The results from FR75513 in that it did not have an anticholinergic property but suppressed the detrusor contraction at electrical field stimulation is different from those of agents on the market for an overactive detrusor. Since it is hoped it will become a new type of agent for the disease, attempts to increase its i.d. absorption were carried out. The results of such experimentation will be published in the following paper.

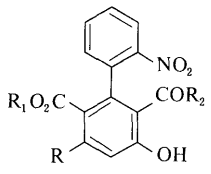
Experimental

All melting points were determined in open glass capillaries on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-10 infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Hitachi R-90H NMR spectrometer with tetramethylsilane as an internal standard (δ value, ppm). Mass (MS) spectra were recorded on JEOL JMS D-300 mass spectrometer. Elemental analyses were carried out on Perkin-Elmer 2400CHN Elemental Analyzer. Yields are not optimized.

3-(1-Methylethyl) 1-Methyl 4-Methyl-6-morpholino-2-(2-nitrophenyl)-1,2-dihydrobenzene-1,3-dicarboxylate (3a) A typical example is given to illustrate the general procedure.

A mixture of 1-methylethyl 2-acetyl-3-(2-nitrophenyl)-2-propenoate (31.0 g) and methyl 3-morpholino-2-butenoate (20.71 g) in benzene (50 ml) was refluxed for 48 h with continuous azeotropic removal of water using a Dean-Stark apparatus. After cooling, the mixture was evaporated and the residue was triturated with IPE to afford a powder **3a**, (21.70 g). Recrystallization from a mixture of EtOAc and *n*-hexane afforded an analytical sample of the title compound, mp 133—134 °C. *Anal.* Calcd for

TABLE IV. Physical and Pharmacological Properties of 1,1′-Biphenyl-2,6-dicarboxylic Acid Derivatives



Compd. No.	R	R_1	R_2	mp (°C) Recryst. solv.[a]	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	*In vitro*[b] % inhibition at 10^{-5} g/ml
8o	Me	Me	O-iso-Pr	112.5—113.5 IPE	23.4	$C_{19}H_{19}NO_7$	61.12 (61.42)	5.13 5.22	3.75 3.66)	50.0[e]
8p	Me	Me	OMe	125—126 H–IPE	26.5	$C_{17}H_{15}NO_7$	59.13 (59.07)	4.38 4.47	4.06 4.05)	42.1
8q	Me	Cl-$(CH_2)_2$–	OMe	124—125 IPE	43.4	$C_{18}H_{16}ClNO_7 \cdot 0.25H_2O$	54.28 (54.41)	4.18 3.87	3.52 3.68)	69.0[f]
8r	Me	Et	OMe	139—141 H–A	32.1	$C_{18}H_{17}NO_7$	60.17 (60.24)	4.77 4.73	3.90 3.89)	69.4[g]
8s	Me	$PhCH_2$	OMe	123—125 IPE	10.7	$C_{23}H_{19}NO_7$	65.56 (65.84)	4.54 4.49	3.32 3.15)	65.4[h]
8t	Me	*tert*-Bu	OMe	134—136 IPE–P.ether	45.6	$C_{20}H_{21}NO_7$	62.01 (61.65)	5.46 5.34	3.62 3.36)	33.8
8u	Me	Cyclopentyl	OMe	101—102 IPE	23.9	$C_{21}H_{21}NO_7$	63.15 (63.30)	5.30 5.31	3.51 3.30)	56.1[i]
8′a	$AcOCH_2$	iso-Pr	OMe	Oil[c]	34.5	$C_{21}H_{21}NO_9$				6.1
11	–CH_2OCO–		OMe	208—210 MeOH	60.9	$C_{16}H_{11}NO_7 \cdot 0.25H_2O$	57.58 (57.83)	3.47 3.23	4.20 4.06)	−4.9
12	Me	iso-Pr	NHMe	189—190 C–IPE–A	75.5	$C_{19}H_{20}N_2O_6$	61.28 (60.96)	5.41 5.16	7.52 7.75)	12.9
13	Me	H	OMe	179—182[d]	97.0	$C_{16}H_{13}NO_7$	58.01 (57.86)	3.96 3.90	4.23 4.12)	−4.2
14	Me	H	OH	216—219[d]	52.9	$C_{15}H_{11}NO_7 \cdot 0.25H_2O$	55.99 (55.75)	3.60 3.45	4.35 4.15)	13.6

a) IPE, diisopropyl ether; H, *n*-hexane; A, ethyl acetate; P.ether, petroleum ether; C, chloroform. *b*) The *in vitro* tests (field stimulation) were carried out as described in the experimental section. *c*) MS *m/z*: 431 (M^+). *d*) Purification by changing the PH of the alkaline solution to 2 gave analytical sample. *e*) $IC_{50} = 1.0 \times 10^{-5}$ g/ml. *f*) $IC_{50} = 4.0 \times 10^{-6}$ g/ml. *g*) $IC_{50} = 3.6 \times 10^{-6}$ g/ml. *h*) $IC_{50} = 4.0 \times 10^{-6}$ g/ml. *i*) $IC_{50} = 1.9 \times 10^{-6}$ g/ml.

$C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 62.59; H, 6.46; N, 6.27. IR (Nujol) cm^{-1}: 1736, 1690. NMR ($CDCl_3$): 0.80 (3H, d, $J=6$ Hz), 1.12 (3H, d, $J=6$ Hz), 2.39 (3H, s), 2.95—3.30 (4H, m), 3.40—3.90 (5H, m), 3.74 (3H, s), 4.80 (1H, septet, $J=6$ Hz), 4.97 (1H, br s), 5.04 (1H, s) 7.15—8.90 (4H, m).

Other compounds listed in Tables VII and VIII were similarly prepared in accordance with the procedure as described above.

2-(1-Methylethyl) 6-Methyl 3-Methyl-5-morpholino-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (4) To a mixture of **3a** (1.07 g), NaOAc (0.84 g) in MeOH (10 ml) was added I_2 (1.36 g), and refluxed for 30 h. After cooling, the mixture was evaporated. The residue was partitioned between EtOAc and water. The organic layer was separated, washed with aq. $Na_2S_2O_3$, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography on silica gel with a mixture of $CHCl_3$ and MeOH as eluent to afford **4** (0.79 g), mp 122—124 °C (recrystallized from IPE). IR (Nujol) cm^{-1}: 1715. NMR ($CDCl_3$): 0.78 (3H, d, $J=9$ Hz), 1.00 (3H, d, $J=9$ Hz), 2.44 (3H, s), 2.95—3.25 (4H, m), 3.42 (3H, s), 3.65—3.93 (4H, m), 4.84 (1H, septet, $J=9$ Hz), 6.96 (1H, s), 7.16—7.75 (3H, m), 8.05—8.33 (1H, m).

1-Methyl 3-(1-Methylethyl) 2-(2-Nitrophenyl)-4-methyl-6-oxo-3-cyclohexene-1,3-dicarboxylate (5a) To a solution of **3a** (22.22 g) in tetrahydrofuran (THF) (110 ml) was added 1 N HCl (55 ml), the mixture was stirred for 3 h at room temperature and then saturated with NaCl. The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated. The residue was triturated with IPE to afford **5a** (12.08 g). Twice recrystallization from a mixture of IPE and EtOAc gave an analytical sample, mp 128—130 °C. *Anal.* Calcd for $C_{19}H_{21}NO_7$: C, 60.79; H, 5.69; N, 3.73. Found: C, 60.91; H, 5.53; N, 3.73. IR (Nujol) cm^{-1}: 1740, 1710. NMR ($CDCl_3$): 0.82 (3H, d, $J=6$ Hz), 1.12 (3H, d, $J=6$ Hz), 2.25 (3H, s), 3.07 (1H, d, $J=22$ Hz), 3.47 (1H, d, $J=22$ Hz), 3.77 (4H, s), 4.85 (1H, septet, $J=6$ Hz), 5.37 (1H, br s), 6.98—7.98 (4H, m).

Compounds **5a**,**c**,**f**,**j**,**m**,**n**,**p** listed in Table IX were obtained as crystals. Other compounds **5**, **5′** were used in the next reaction without purification.

Method A. 6-(1-Methylethyl) 2-Methyl 3-Hydroxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (8a) A typical example is given to illustrate the general procedure.

To a solution of **5a** (27.6 g) in MeOH (100 ml) were added NaOAc (15.19 g) and I_2 (20.14 g), and stirred for 5 h at room temperature. After removal of MeOH, EtOAc and water were added to the residue. The organic layer was separated, washed with aq. $Na_2S_2O_3$, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with a mixture of $CHCl_3$ and *n*-hexane as eluent, and crystallization from petroleum ether afforded **8a** (14.65 g), mp 69—71 °C. *Anal.* Calcd for $C_{19}H_{19}NO_7$: C, 61.12; H, 5.13; N, 3.75. Found: C, 60.76; H, 5.13; N, 4.04. IR (Nujol) cm^{-1}: 1720, 1660. NMR ($CDCl_3$): 0.77 (3H, d, $J=6$ Hz), 1.00 (3H, d, $J=6$ Hz), 2.33 (3H, s), 3.40 (3H, s), 4.77 (1H, septet, $J=6$ Hz), 6.93 (1H, s), 7.10—8.30 (4H, m), 11.40 (1H, s).

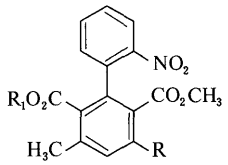
Other compounds listed in Table II and IV (**8a—q**, **i—m**, **o—u**, **8′a**), and compound **4** were simiarly prepared.

Method B. 6-(2-Chloroethyl) 2-Methyl 5-Methyl-3-hydroxy-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (8q) A typical example is given to illustrate the general procedure.

The crude product **5a** (1 g) was dissolved in $CHCl_3$, and a solution of Br_2 (0.13 ml) in $CHCl_3$ (2 ml) was added thereto at 25—27 °C. After stirring for 2 h, ice water was added to the mixture. The organic layer was separated, washed with aq. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to afford **8q** (0.91 g). Crystallization from IPE afforded 0.43 g of **8q**, mp 125—125 °C. *Anal.* Calcd for $C_{18}H_{16}ClNO_7$: C, 54.90; H, 4.10; N, 3.56. Found: C, 54.41; H, 3.87; N, 3.68. IR (Nujol) cm^{-1}: 1710, 1670. NMR ($CDCl_3$): 2.35 (3H, s), 3.30 (2H, t, $J=6$ Hz), 3.40 (3H, s), 3.80—4.30 (2H, m), 6.90 (1H, s), 7.05—7.15 (1H, m), 7.40—7.60 (2H, m), 8.00—8.20 (1H, m), 11.33 (1H, s).

Other compounds listed in Table II (**8h**,**n**) were similarly prepared.

TABLE V. Physical and Pharmacological Properties of 1,1′-Biphenyl-2,6-dicarboxylic Acid Derivatives



Compd. No.	R_1	R	mp (°C) Recryst. solv.[a]	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	*In vitro*[b] % inhibition at 10^{-5} g/ml
4	iso-Pr		122—124 IPE	74.2	$C_{23}H_{26}N_2O_7 \cdot 0.25H_2O$	61.81 (61.93)	5.98 6.13	6.27 6.30)	13.9
6	iso-Pr	H	Oil[c]	18.1	$C_{19}H_{19}NO_6$				54.2[g]
7	Cyclopentyl	Cl	Oil[d]	20.1	$C_{21}H_{20}ClNO_6$				60.6[h]
9a	iso-Pr	OMe	120—122 IPE	67.9	$C_{20}H_{21}NO_7$	62.01 (62.26	5.46 5.73	3.62 3.71)	26.1
9b	iso-Pr	$O-(CH_2)_2-OH$	128—130 IPE	65.9	$C_{21}H_{23}NO_8$	60.43 (60.09	5.55 5.68	3.36 3.00)	25.1
9c	iso-Pr	$O-(CH_2)_2-OEt$	129—133 IPE	71.8	$C_{23}H_{27}NO_8$	62.01 (61.90	6.11 6.25	3.14 2.74)	29.6
9d	iso-Pr	OCH_2CO_2Me	116—118 EtOH	75.1	$C_{22}H_{23}NO_9$	59.32 (68.91	5.20 4.99	3.14 3.40)	7.7
9e	iso-Pr	OCH_2Ph	120—121 A–IPE	55.5	$C_{26}H_{25}NO_7$	67.38 (67.48	5.44 5.29	3.02 3.09)	8.7
9f	iso-Pr	$O-(CH_2)_3-OH$	133—138 IPE	22.6	$C_{22}H_{25}NO_8 \cdot 0.25H_2O$	60.61 (60.45	5.90 5.77	3.21 3.10)	13.8
9g	iso-Pr	$O-(CH_2)_2-NEt_2 \cdot HCl$	146—147 A	38.7	$C_{25}H_{32}N_2O_7 \cdot H_2O$	56.98 (56.90	6.69 6.18	5.32 5.48)	6.1
10a	iso-Pr	OAc	111—112 IPE–Tol	56.2	$C_{21}H_{21}NO_8$	60.72 (60.56	5.10 5.26	3.37 3.29)	70.6[i]
10b	iso-Pr	OCO-*tert*-Bu	Oil[e]	47.4	$C_{25}H_{29}NO_9$				60.0[j]
10c	iso-Pr	OCO_2-iso-Pr	89—90 IPE	49.5	$C_{24}H_{27}NO_9$	60.88 (60.77	5.75 5.61	2.96 2.87)	46.4
10′a	iso-Pr	OMs	Oil[f]	68.2	$C_{20}H_{21}NO_9S$				15.2
19	Me	Me	132—134 H–A	35.0	$C_{18}H_{17}NO_6$	62.97 (62.88	4.99 4.83	4.08 4.11)	70.0[k]
21	Ph-$(CH_2)_2$–	Me	72—75 H–ether	70.2	$C_{25}H_{23}NO_6$	69.27 (69.42	5.35 5.26	3.23 3.23)	44.0

a) A, ethyl acetate; Tol, toluene; H, *n*-hexane. *b*) The *in vitro* tests (field stimulation) were carried out as described in the experimental section. *c*) MS *m/z*: 326, 310. *d*) MS *m/z*: 386, 371. *e*) MS *m/z*: 426, 410. *f*) MS *m/z*: 420, 405. *g*) $IC_{50} = 5.8 \times 10^{-6}$ g/ml. *h*) $IC_{50} = 4.2 \times 10^{-6}$ g/ml. *i*) $IC_{50} = 3.1 \times 10^{-6}$ g/ml. *j*) $IC_{50} = 3.3 \times 10^{-6}$ g/ml. *k*) $IC_{50} = 2.8 \times 10^{-6}$ g/ml.

TABLE VI. Effect of FR75513 on the Urinary Bladder Rhythmic Contractions in Rat Cystometry

Dose (mg/kg)	Inhibition (%)	Duration (min)
0.01 (i.v.)	38	10
0.1 (i.v.)	45	10
1.0 (i.v.)	100	30
100 (i.d.)	14	—

a) The *in vivo* test procedures were carried out as described in the experimental section.

6-(1-Methylethyl) 2-Methyl 5-Methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (6) To a solution of **5a** (3.0 g) in a mixture of THF (15 ml) and MeOH (4 ml) was added a solution of $NaBH_4$ (0.30 g) in water (4 ml) at room temperature. The mixture was stirred for 3 h, and then AcOH (0.6 ml) was added thereto. The resulting mixture was evaporated, and the residue was dissolved in EtOAc. The solution was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with a mixture of toluene and EtOAc as eluent to afford 3-(1-methylethyl) 1-methyl 2-(2-nitrophenyl)-6-hydroxy-4-methyl-3-cyclohexene-1,3-dicarboxylate (0.60 g) as an oil. IR (neat) cm^{-1}: 3450, 1705. NMR ($CDCl_3$): 0.65 (3H, d, $J=6$ Hz), 1.03 (3H, d, $J=6$ Hz), 2.14 (3H, s), 2.20—2.90 (2H, m), 2.90—3.10 (1H, m), 3.30—3.65 (1H, m), 3.65 (3H, s), 4.10—4.36 (1H, m), 4.73 (1H, septet, $J=6$ Hz), 4.83—5.06 (1H, m), 7.20—7.96 (4H, m).

This product was dissolved in pyridine (2 ml), and then $POCl_3$ (0.12 ml) was added thereto with ice-bath cooling. The mixture was stirred for 1 h at room temperature, and then evaporated. EtOAc and water were added to the residue. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and evaporated.The residue was purified by column chromatography on silica gel with a mixture of toluene and EtOAc as eluent to afford pale yellow crystals. The crystals were recrystallized from MeOH to afford 3-(1-methylethyl) 1-methyl 4-methyl-2-(2-nitrophenyl)-2,5-dihydrobenzene-1,3-dicarboxylate (0.17 g), mp 103—104 °C. *Anal.* Calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.89; H, 5.74; N, 3.92. IR (Nujol) cm^{-1}: 1700. NMR ($CDCl_3$): 1.03 (3H, d, $J=6$ Hz), 1.10 (3H, d, $J=6$ Hz), 2.20 (3H, s), 3.02 (1H, ddd, $J=3.5$, 6, 24 Hz), 3.22 (1H, ddd, $J=3.5$, 6, 24 Hz), 3.57 (3H, s), 4.93 (1H, septet, $J=6$ Hz), 5.66 (1H, br triplet, $J=6$ Hz), 7.06 (1H, t, $J=3.5$ Hz), 7.20—8.00 (4H, m).

A mixture of 3-(1-methylethyl) 1-methyl 4-methyl-2-(2-nitrophenyl)-2,5-dihydrobenzene-1, 3-dicarboxylate (0.60 g), NaOAc (0.24 g), and I_2 (0.50 g) in MeOH (3 ml) was stirred for 3 h at room temperature, and then refluxed for 9 h. After cooling, EtOAc and water were added to the mixture. The organic layer was separated, washed with aq. $Na_2S_2O_3$, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as an eluent to afford **6** (0.36 g). IR (neat) cm^{-1}: 1710. NMR ($CDCl_3$): 1.23—1.90 (8H, m), 2.40 (3H, s), 3.63 (3H, s), 4.84—5.10 (1H, m), 7.17—7.37 (2H, m), 7.39—7.57 (2H, m), 8.06 (1H, d, $J=8$ Hz), 8.15—8.34 (1H, m). MS *m/z*: 326 ($M^+ - OMe$), 310 ($M^+ - NO_2$).

6-Cyclopentyl 2-Methyl 3-Chloro-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (7) To a solution of PCl_5 (4.32 g) in $CHCl_3$ (50 ml) was added the crude product [3-cyclopentyl 1-methyl 2-(2-nitrophenyl)-4-methyl-6-

oxo-3-cyclohexene-1,3-dicarboxylate] (3.06 g) with ice-bath cooling. After stirring for 6 d at room temperarure, PCl_5 (1.4 g) was added to the mixture and refluxed for 5 h. After cooling, water and EtOAc were added to the solution. The organic layer was separated, washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and EtOAc as eluent to afford 3-chloro-6-cyclopentyloxycarbonyl-5-methyl-2′-nitro-(1,1′-biphenyl)-2-carbonyl chloride (1.17 g, higher *Rf*), and 6-cyclopentyl 2-methyl 3-chloro-5-methyl-2′-nitro-(1,1′-biphenyl)-2, 6-dicarboxylate (**7**) (0.64 g, lower *Rf*) as oils.

3-Chloro-6-cyclopentyloxycarbonyl-5-methyl-2′-nitro-(1,1′-biphenyl)-2-carbonyl Chloride: IR (neat) cm^{-1}: 1770, 1710. NMR ($CDCl_3$): 0.93—1.89 (8H, m), 2.42 (3H, s), 4.89—5.14 (1H, m), 7.26—7.51 (1H, m), 7.41 (1H, s), 7.50—7.79 (2H, m), 8.23—8.38 (1H, m). MS *m/z*: 422 (M^+), 386 ($M^+ - Cl$).

7: IR (neat) cm^{-1}: 1710. NMR ($CDCl_3$): 1.25—1.90 (8H, m), 2.43 (3H, s), 3.53 (3H, s), 4.90-5.17 (1H, m), 7.27—7.54 (1H, m), 7.39 (1H, s), 7.54—7.77 (2H, m), 8.16—8.36 (1H, m). MS *m/z*: 386 ($M^+ - OMe$), 3.71 ($M^+ - NO_2$).

6-(1-Methylethyl) 2-Methyl 3-Benzyloxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (9e) A typical example is given to illustrate the general procedure.

A mixture of **8a** (0.45 g), benzylchloride (0.17 g), K_2CO_3 (0.11 g), and KI (0.08 g) in dimethylformamide (DMF) (2.2 ml) was stirred at room temperature overnight. After partition of the reaction mixture between EtOAc and water, the organic layer was separated, washed with water and brine, dried over $MgSO_4$ and evaporated. The crude product was recrystallized from a mixture of EtOAc and IPE to afford pure **9e** (0.31 g), mp 120—121 °C. *Anal.* Calcd for $C_{26}H_{25}NO_7$: C, 67.38; H, 5.44; N, 3.02. Found: C, 67.48; H, 5.29; N, 3.09. IR (Nujol) cm^{-1}: 1720. NMR ($CDCl_3$): 0.77 (3H, d, J=9 Hz), 0.98 (3H, d, J=9 Hz), 2.93 (3H, s), 3.47 (3H, s), 4.83 (1H, septet, J=9 Hz), 5.17 (2H, s), 6.88 (1H, s), 7.10—7.73 (8H, m), 8.00—8.36 (1H, m).

Other compounds **9a—f** listed in Table V were similarly prepared.

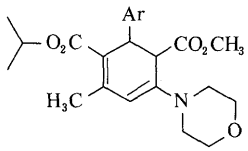
6-(1-Methylethyl) 2-Methyl 5-Methyl-3-(1-methylethoxycarbonyloxy)-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (10c) A typical example is given to illustrate the general procedure.

To a solution of **8a** (0.78 g) and Et_3N (1.5 ml) in $CHCl_3$ (6 ml) was added chloro 1-methylethylcarbonate (0.54 g) in an ice bath, and stirred for 1 h at room temperature. The mixture was washed with aq. $NaHCO_3$ and dil. HCl, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with a mixture of benzene and EtOAc as eluent and recrystallization from IPE afforded 0.49 g of pure **10c**, mp 89—90 °C. *Anal.* Calcd for $C_{24}H_{27}NO_9$: C, 60.88; H, 5.48; N, 3.05. Found: C, 60.77; H, 5.61; N, 2.87. IR (Nujol) cm^{-1}: 1755, 1715, 1705. NMR ($CDCl_3$): 0.83 (3H, d, J=6 Hz), 0.90—1.15 (9H, m), 2.06 (1H, octet, J=6 Hz), 2.44 (3H, s), 3.51 (3H, s), 4.06 (2H, d, J=6 Hz), 4.85 (1H, septet, J=6 Hz), 7.20 (1H, s), 7.16—7.79 (3H, m), 8.10—8.30 (1H, m).

Other compounds **10a, b, 10′a** listed in Table V were similarly prepared.

6-Methyl 5-Hydroxy-7-(2-nitrophenyl)-1(3*H*)-isobenzofuranone-6-carboxylate (11) A mixture of **8′a** (0.63 g) and *p*-TsOH (0.14 g) in MeOH (15 ml) was refluxed for 9 h and cooled. To the solution were added EtOAc and aq. $NaHCO_3$, and the organic layer was separated, and washed with water. The solvent was evaporated, and the residue was washed with toluene. The crude crystals were dissolved in $CHCl_3$–MeOH and the

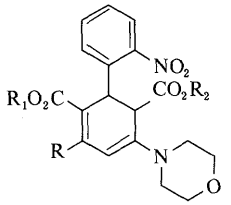
TABLE VII. Physical Properties of 1,2-Dihydrobenzene-1,3-dicarboxylic Acid Derivatives



Compd. No.	Ar	mp (°C) Recryst. solv.[a]	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N
3a	2-NO_2-Ph	133—134 IPE–A	43.7	$C_{23}H_{28}N_2O_7$	62.15 (62.59	6.35 6.46	6.30 6.27)
3b	3-NO_2-Ph	148—149 H–A	60.1	$C_{23}H_{28}N_2O_7$	62.15 (62.49	6.35 6.39	6.30 6.27)
3c	4-NO_2-Ph	118—122 IPE	54.2	$C_{23}H_{28}N_2O_7$	62.15 (62.13	6.35 6.41	6.30 5.93)
3d	Ph	125 IPE	29.9	$C_{23}H_{29}NO_5$	69.15 (69.09	7.32 6.85	3.51 3.50)
3e	2-Me-Ph	97—99 H–IPE	21.0	$C_{24}H_{31}NO_5$	69.71 (69.51	7.56 7.26	3.39 3.34)
3f	2-MeO-Ph	122.5—123.5 IPE	14.0	$C_{24}H_{31}NO_6$	67.10 (67.20	7.27 7.20	3.26 2.91)
3g	2-$PhCH_2O$-Ph	108—110 H–A	20.9	$C_{30}H_{35}NO_6$	71.27 (71.35	6.98 6.64	2.77 2.73)
3h	2-F-Ph	Oil[b]	26.3	$C_{23}H_{28}FNO_5$			
3i	2-Cl-Ph	117—118 IPE	38.8	$C_{23}H_{28}ClNO_5$	63.66 (63.95	6.50 6.64	3.23 3.14)
3j	2-CN-Ph	161—164 IPE–A	50.8	$C_{24}H_{28}N_2O_5$	67.91 (67.83	6.65 6.26	6.60 6.44)
3k	2-Thienyl	Oil[c]	30.6	$C_{21}H_{27}NO_5S$			
3l	2-Py	122—124 IPE	41.2	$C_{22}H_{28}N_2O_5$	65.98 (65.81	7.05 6.90	7.00 6.99)
3m	3-Py	126—128 IPE	36.0	$C_{22}H_{28}N_2O_5$	65.98 (65.81	7.05 7.06	7.00 6.98)
3n	4-Py	104—106 IPE	14.3	$C_{22}H_{28}N_2O_5 \cdot 0.25H_2O$	65.25 (65.54	7.09 6.88	6.92 7.00)

a) A, ethyl acetate; H, *n*-hexane. *b*) MS *m/z*: 417 (M^+), 358 ($M^+ - O$-iso-Pr). *c*) MS *m/z*: 405 (M^+), 346 ($M^+ - O$-iso-Pr).

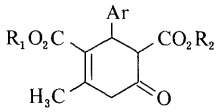
TABLE VIII. Physical Properties of 1,2-Dihydrobenzene-1,3-dicarboxylic Acid Derivatives



Compd. No.	R	R_1	R_2	mp (°C) Recryst. solv.[a]	Yield (%)	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
3o	Me	Me	iso-Pr	Oil[b]	76.2	$C_{23}H_{28}N_2O_7$						
3p	Me	Me	Me	151—152 IPE–A	47.6	$C_{21}H_{24}N_2O_7$	60.57	5.81	6.73	60.50	5.76	6.57
3q	Me	Cl-$(CH_2)_2$–	Me	152.5—154 EtOH	70.2	$C_{22}H_{25}ClN_2O_7$	56.83	5.42	6.03	56.93	5.29	6.03
3r	Me	Et	Me	159—161 H–A	71.2	$C_{22}H_{26}N_2O_7$	61.39	6.09	6.51	61.44	6.01	6.47
3s	Me	$PhCH_2$–	Me	165—167 H–A	53.4	$C_{27}H_{28}N_2O_7$	65.84	5.73	5.69	66.31	5.68	5.66
3t	Me	*tert*-Bu	Me	127—130 H–A	24.2	$C_{24}H_{30}N_2O_7$	62.87	6.59	6.11	62.82	6.79	6.14
3u	Me	Cyclopentyl	Me	168—169 H–A	54.3	$C_{25}H_{30}N_2O_7$	63.82	6.43	5.95	64.12	6.30	5.88
3′a	$AcOCH_2$	iso-Pr	Me	152—156 THF	34.5	$C_{25}H_{30}N_2O_9$	59.75	6.02	5.57	59.91	6.06	5.50

a) A, ethyl acetate; H, *n*-hexane. *b*) MS *m/z*: 444 (M^+), 385 ($M^+ - O$-iso-Pr).

TABLE IX. Physical Properties of 6-Oxo-3-cyclohexene-1,3-dicarboxylic Acid Diesters



Compd. No.	Ar	R_1	R_2	mp (°C) Crystallizing solv.[a]	Yield (%)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
5a	2-NO_2-Ph	iso-Pr	Me	127—130 A–IPE	72.7	$C_{19}H_{21}NO_7$	60.79	5.64	3.73	60.91	5.53	3.73
5c	4-NO_2-Ph	iso-Pr	Me	157—161 IPE	71.9	$C_{19}H_{21}NO_7$	60.79	5.64	3.73	60.76	5.56	3.73
5f	2-MeO-Ph	iso-Pr	Me	82—84 IPE	54.8	$C_{20}H_{24}O_6$	66.65	6.71		66.33	6.36	
5j	2-CN-Ph	iso-Pr	Me	92—97 IPE	25.4	$C_{20}H_{21}NO_5$	67.59	5.96	3.94	67.72	5.62	4.09
5m	3-Py	iso-Pr	Me	108—111 IPE	46.9	$C_{18}H_{21}NO_5$	65.24	6.39	4.23	64.93	6.36	4.20
5n	4-Py	iso-Pr	Me	122—123 IPE	67.4	$C_{18}H_{21}NO_5$	65.24	6.39	4.23	64.83	6.30	4.21
5p	2-NO_2-Ph	Me	Me	131—134 A–IPE	48.0	$C_{21}H_{25}NO_7$	58.79	4.93	4.03	59.04	4.96	4.06

a) A, ethyl acetate.

insoluble material was removed off by filtration. The filtrate was evaporated and the resulting crystals were washed with EtOAc and MeOH to afford **11** (0.29 g), mp 208—210 °C. *Anal.* Calcd for $C_{16}H_{11}NO_7 \cdot 0.25H_2O$: C, 57.58; H, 3.47; N, 4.20. Found: C, 57.83; H, 3.23; N, 4.06. IR (Nujol) cm^{-1}: 1755, 1660. NMR (DMSO-d_6): 3.40 (3H, s), 5.34 (2H, s), 7.15 (1H, s), 7.20—7.44 (1H, m), 7.63—7.87 (2H, m), 8.10—8.34 (1H, m).

1-Methylethyl 3-Hydroxy-5-methyl-2-methylcarbamoyl-2′-nitro-(1,1′-biphenyl)-6-carboxylate (12) A mixture of **8a** (0.77 g) and $MeNH_2$ (35% in EtOH, 10 ml) was stirred for 10 d at room temperature and diluted with IPE. The resulting precipitates were collected by filtration and recrystallized from $CHCl_3$–EtOAc to afford **12** (0.58 g), mp 189—190 °C. *Anal.* Calcd for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found: C, 60.96; H, 5.16; N, 7.75. IR (Nujol) cm^{-1}: 1715, 1635, 1625. Conformer A: NMR ($CDCl_3$): 0.94 (3H, d, J=6Hz), 0.98 (3H, d, J=6 Hz), 2.30 (3H, s), 2.55 (3H, s), 4.82 (1H, septet, J=6 Hz), 6.05 (1H, br s), 6.90 (1H, s), 7.23—7.43 (1H, m), 7.43—7.83 (2H, m), 8.00—8.33 (1H, m), 10.06 (1H, br s). Conformer B: NMR ($CDCl_3$): 0.94 (3H, d, J=6 Hz), 0.98 (3H, d, J=6 Hz), 2.30 (3H, s), 2.60 (3H, s), 4.82 (1H, septet, J=6 Hz), 6.05 (1H, br s), 6.90 (1H, s), 7.23—7.43 (1H, m), 7.43—7.83 (2H, m), 8.00—8.33 (1H, m), 10.06 (1H, br s).

3-Hydroxy-2-methoxycarbonyl-5-methyl-2′-nitro-(1,1′-biphenyl)-6-carboxylic Acid (13) A solution of **8t** (0.21 g) in TFA (0.5 ml) was stirred at room temperature overnight, made alkaline with aq. NaOH and washed with ether. The solution was acidifide with 2 N HCl and stirred for 0.5 h in an ice bath. The resulting precipitates were collected by filtration, washed with cold water and dried to afford **13** (0.14 g) as a powder, mp 170—172 °C. *Anal.* Calcd for $C_{16}H_{13}NO_7$: C, 58.01; H, 3.96; N, 4.23. Found: C, 57.86; H, 3.90; N, 4.12. IR (Nujol) cm^{-1}: 2600—1800, 1720, 1690, 1660. NMR ($CDCl_3$): 2.36 (3H, s), 3.40 (3H, s), 4.50—5.50 (1H, br m), 6.95 (1H, s), 7.10—7.73 (3H, m), 8.05—8.25 (1H, m), 10.3—11.3 (1H, br m).

3-Hydroxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylic Acid (14) To a solution of 2-(1-methylethyl) 6-methyl 3-hydroxy-5-methyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (0.2 g) in acetone (3 ml), 10% NaOH (1 ml) was added and refluxed overnight. After cooling the solution was washed with ether, acidified with 2 N HCl and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was triturated with a mixture of ether and petroleum ether to afford **14** (0.09 g), mp 216—219 °C. *Anal.* Calcd for $C_{15}H_{11}NO_7 \cdot 0.25H_2O$: C, 55.99: H, 3.60; N, 4.35. Found: C, 55.75; H, 3.45; N, 4.15. IR (Nujol) cm^{-1}: 1650, 1595, 1560, 1520. NMR (DMSO-d_6): 2.33 (3H, s), 3.00—4.00 (2H, br m), 6.90 (1H, s), 7.25 (1H, dd, J=7, 3 Hz), 7.50—7.90 (2H, m), 8.25 (1H, dd, J=7, 3 Hz), 11.0—12.5 (1H, br m).

6-(1-Methylethyl) 8-Hydroxy-6-methyl-9*H*-dibenzo[*b*,*d*]pyran-9-one-6-carboxylate (15′) A solution of **8a** (0.21 g) in MeOH (20 ml) was treated with 10% Pd/C (0.06 g) and stirred under a hydrogen atmosphere for 4 h. The mixture was filtered and the solvent was removed under reduced pressure. The residue was recrystallized from petroleum ether (27 ml) to afford **15′** (0.09 g), mp 142 °C. *Anal.* Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.76; H, 4.98. IR (Nujol) cm^{-1}: 1720, 1680. NMR ($CDCl_3$): 1.36 (6H, d, J=6 Hz), 2.40 (3H, s), 5.35 (1H, septet, J=6 Hz), 6.93 (1H, s), 7.1—7.6 (3H, m), 7.86 (1H, dd, J=1, 7 Hz).

Compound **15** was similarly prepared.

2-Bromo-1,3-dicyano-4,6-dimethylbenzene (17) To a mixture of conc. H_2SO_4 (12 ml) and AcOH (3 ml), $NaNO_2$ (1.04 g) was added portionwise with cooling from an ice bath. The mixture was stirred for 1.5 h at room temperature. Compound **16** (2.57 g) was added portionwise to the reaction mixture, and stirred for 2.5 h with ice bath cooling. The above solution was added to a solution of CuBr (2.2 g) in 48% HBr (15 ml) in an ice bath. After stirring for 0.5 h, ice water was added to the mixture and the resulting precipitates were collected by filtration and recrystallized from a mixture of *n*-hexane and EtOAc to afford 2.32 g of **17**, mp 157—161 °C. *Anal.* Calcd for $C_{10}H_7BrN_2$: C, 51.09; H, 3.00; N, 11.92. Found: C, 51.23; H, 2.85; N, 11.96. IR (Nujol) cm^{-1}: 2210. NMR (DMSO-d_6): 2.56 (6H, s), 7.60 (1H, s).

Dimethyl 2-Bromo-4,6-dimethylbenzene-1,3-dicarboxylate (18) A suspension of **17** (301 mg) in conc. H_2SO_4 (3.2 ml) and water (0.7 ml) was refluxed for 7 h and cooled. To the solution was added a solution of $NaNO_2$ (283 mg) in water (1.5 ml) and heated for 2 h at 80 °C. After cooling, ice water was added to the solution and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was co-evaporated three times with toluene, dissolved in DMF (7 ml), and K_2CO_3 (1.42 g) and MeI (0.8 ml) were added thereto. The mixture was heated at 40 °C for 5 h. After cooling, ice water was added to the mixture, and extracted with CH_2Cl_2. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and CH_2Cl_2 as an eluent to afforded **18** (153 mg), mp 54—56 °C (recrystallized from a mixture of *n*-hexane and EtOAc). *Anal.* Calcd for $C_{12}H_{13}BrO_4$: C, 47.86; H, 4.35. Found: C, 48.13: H, 4.25. IR (Nujol) cm^{-1}: 1720. NMR ($CDCl_3$): 2.30 (6H, s), 3.92 (6H, s), 6.98 (1H, s).

Dimethyl 3,5-Dimethyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (19) To a melt of 1-iodo-2-nitrobenzene (1.90 g) and **18** (6.29 g) was added copper powder (6.42 g). The mixture was heated at 200 °C for 1 h and cooled. To the mixture was added CH_2Cl_2 and the insoluble material was removed by filtration. The filtrate was evaporated and the residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and CH_2Cl_2 as an eluent to afford 0.76 g of **19**, mp 132—134 °C (recrystallized from a mixture of *n*-hexane and EtOAc). *Anal.* Calcd for $C_{18}H_{17}NO_6$: C, 62.97; H, 4.99; N, 4.08. Found: C, 62.88; H, 4.83; N, 4.11. IR (Nujol) cm^{-1}: 1720. NMR ($CDCl_3$): 2.37 (6H, s), 3.43 (6H, s), 7.03—7.30 (2H, m), 7.32—7.63 (2H, m), 8.03—8.16 (1H, m).

0.30 g of 2,2′,6,6′-tetramethyl 3,3′,5,5′-tetramethyl-(1,1′-biphenyl)-2,2′,6,6′-tetracarboxylate was obtained as a minor product, mp 140—143 °C (recrystallized from a mixture of *n*-hexane and EtOAc). *Anal.* Calcd for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found: C, 65.23; H, 5.79. IR (Nujol) cm^{-1}: 1715. NMR ($CDCl_3$): 2.38 (12H, s), 3.48 (12H, s), 7.01 (2H, s).

3,5-Dimethyl-2-methoxycarbonyl-2′-nitro-(1,1′-biphenyl)-6-carboxylic Acid (20) A mixture of **19** (100 mg) and $Ba(OH)_2 \cdot 8H_2O$ (0.37 g) in dioxane (3.6 ml) and water (3.5 ml) was refluxed at 70 °C for 3 h. After cooling, the solution was neutralized with 5% HCl and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and MeOH as eluent to afford **20** (72 mg), mp 164—168 °C. *Anal.* Calcd for $C_{17}H_{15}NO_6 \cdot 0.5H_2O$: C, 60.35; H, 4.77; N, 4.14. Found: C, 60.53; H, 4.51; N, 4.07. IR (Nujol) cm^{-1}:

2800—2100, 1720, 1685. NMR ($CDCl_3$): 2.35 (3H, s), 2.38 (3H, s), 3.40 (3H, s), 7.03—7.25 (2H, m), 7.30—7.75 (3H, m), 7.95—8.10 (1H, m).

2-Methyl 6-(2-Phenylethyl) 3,5-Dimethyl-2′-nitro-(1,1′-biphenyl)-2,6-dicarboxylate (21) To a suspension of **20** (65 mg) in CH_2Cl_2 (1.5 ml) was added PCl_5 (46 mg) and stirred for 30 min with ice bath cooling. After being stirred for 2.5 h at room temperature, the solution was evaporated and then co-evaporated with benzene. The resulting powder was soon dissolved in CH_2Cl_2 and 2-phenylethylalcohol (0.6 ml) and pyridine (0.5 ml) were added thereto at 0 °C. After stirring at 70 °C for 3 h, the solution was acidified with 5% HCl and extracted with CH_2Cl_2. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and MeOH as eluent to afford **21** (60 mg), mp 72—75 °C (recrystallized from a mixture of ether and *n*-hexane). *Anal.* Calcd for $C_{25}H_{23}NO_6$: C, 69.27; H, 5.35; N, 3.23. Found: C, 69.42; H, 5.26; N, 3.23. IR (Nujol) cm^{-1}: 1720. NMR ($CDCl_3$): 2.30 (3H, s), 2.36 (3H, s), 2.57 (2H, t, $J=7$ Hz), 3.42 (3H, s), 4.02 (1H, t, $J=7$ Hz), 4.06 (1H, t, $J=7$ Hz), 6.93—7.60 (9H, m), 7.93—8.13 (1H, m).

***In Vitro* Studies** Guinea-pigs weighing 320—650 g were killed by blood-letting. The lower abdomen was opened and longitudinally oriented strips of the urinary bladder, 15—20 mm long and 5 mm wide, were excised. The strips were suspended in tissue baths containing 25 ml of Krebs solution. Throughout the experiment, the bathing solution was maintained at 37 °C and continuously aerated with a 95% oxygen and 5% carbon dioxide gas mixture. Bladder strip contactions were recorded isometrically with an electromechanical displacement transducer and a polygraph. All muscle strips were stretched initially to 1 g of tension and allowed to accommodate to this length, and to the bath milieu, for at least 30 min before any durg additions were made. In each instance 15 min intervals were allowed between drug additions. Single strips were exposed only to a single agonist or electrical stimulation and a drug.

Fixed doses of carbacol (10 μM), KCl (30 mM) were used as agonists. To stimulate the bladder strips electrically, two platinum electrodes were placed parallel to each other and 15 mm apart both sides of the tissue preparation. The intensity of square wave stimuli was adjusted to obtain submaximal contractions at a constant frequency of 10 Hz and duration of 1 ms. Usually the electrical intensity was around 10 V, and stimulation was given to the detrusor strips for 5 s every 5 min. The effects of the agonists or the electrical stimuli were examined with variation in concentration of the drugs added to the bath 10 min prior to the administration of the agonists or electrical stimuli.

Male S. D. strain rats, weighing 200—300 g, were killed by bleeding and the thoracic aorta were removed. The helical strips (2.0 × 15 mm) were suspended in an organ bath filled with 25 ml of Tyrode's solution. The strips were connected to a strain gauge and the tension was measured isometrically. The bath solution was bubbled with a mixture of 95% O_2 and 5% CO_2 and was maintained at 37 °C. After the resting tension was adjusted to 0.5 g, the arterial strips were contracted by 30 mM KCl. The test drugs were added in the organ bath cumulatively. At the end of each test, 10^{-4} M of papaverine was added to the organ bath to obtain the maximum relaxation. This effect was taken as a standard for 100% relaxation.

***In Vivo* Studies** Sprague Dawley rats, weighing 220—320 g, were anesthetized with a subcutaneous dose of 1.0 g/kg of urethane and fixed in a supine position. The lower abdomen was opened along the midline to fully expose the urinary bladder. A rubber balloon was inserted into the bladder through a small incision of the wall around the apex, and was connected with a pressure transducer through a polyethylene tube. The bladder was carefully packed with a cotton-wool pad soaked in warm saline and kept warm. The balloon was filled with approximately 1 ml of water, and then pressured. Rhythmic contractions of the urinary bladder became constant at a threshold intravesical pressure between 5 and 15 mmHg, and reached a maximum contraction 50 to 70 mmHg with an amplitude of contraction of 40 to 60 mmHg. After this control period, the drugs were administered intravenously or intraduodenally, and the inhibitory effects were estimated by the reduction in amplitude of the bladder contractions.

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