Journal of Medicinal and Pharmaceutical Chemistry

VOL. 1, No. 6 (1959)

New Anti-viral Compounds with Considerable Activity *in vivo*—I. Biphenyl Derivatives

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Introduction

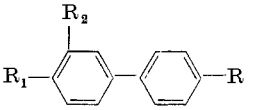
Previous publications[1,2] have reported encouraging results on the use of our concept of a so-called 'supporting moiety', namely that substances possessing pharmacological activity consist of two portions: (1) the 'supporting moiety' which confers on the substance a strong and selective affinity for biochemical substrates; and (2) the radical moiety which determines the type of pharmacological activity. It is necessary that the 'supporting moiety' show a close structural resemblance to the substrate. An analogy of this concept may be found in the union of a coenzyme with an appropriate apoenzyme which, although by a different type of bond, is a pre-requisite for specific enzymatic action.

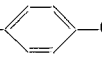
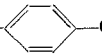
It has been observed recently[3-6] that some glyoxals display marked anti-viral activity against the influenza A–PR8 virus and the Newcastle (NJD) virus in the chick embryo. Although it has been reported[7] that this specific activity has not been confirmed *in vivo*, we believed that the glyoxal radical combined with a suitable 'supporting moiety' would lead to a molecule showing *in vivo* anti-viral activity. Our previous investigations had shown that biphenyl was one of the most suitable 'supporting moieties' for obtaining substances with pharmacodynamically interesting

properties in various areas.[8,9] As an explanation of these activities, one might suggest that biphenyl derivatives with suitable 'pharmacophoric' groups are especially suited to interfere with metabolic processes at different enzymatic levels.

Since all rational chemotherapeutic attempts to study a disruption of the virus–host cell relationship are based on selective interference with the synthetic or degradative processes of the

Table I.



R	R_1	R_2
—COCHO[a]	H	H
—COCHO[a]	—COCHO	H
—COCHO[a]	OCH_3	H
—COCHO[a]	OCH_3	Cl
—COCH=NNHCO— 	H	H
—COCHNH—  —COOH OH	H	H
—COCHNH—  —COOH[b] OC_2H_5	H	H

[a] Bisulphite derivatives of these ketoaldehydes were also prepared.
[b] The study on the structure of this product will form the subject of another paper.

virus, a combination of glyoxal groups and biphenyl moieties seemed especially adaptable to such an investigation. With this in mind, we synthesized a series of ketoaldehydes and some of their derivatives shown in Table I.

Chemical Synthesis

4,4′-Diacetylbiphenyl, 4-acetyl-4′-methoxybiphenyl, and 3′-chloro-4-acetyl-4′-methoxybiphenyl were prepared by Friedel–

Crafts reactions, according to methods reported in the literature.[10–12] 4-Biphenylglyoxal hydrate was prepared from 4-biphenyl methyl ketone by oxidation with selenium dioxide.[13] The other substances listed in Table I were synthesized by the same method. The biphenyl ketoaldehydes were isolated as hydrates; they reduced Tollens' reagent, but did not reduce Fehling's solution. All the ketoaldehydes were analysed according to Friedmann's procedure, which involves hydrogen peroxide oxidation of the substance and titration of the acids produced.[14]

The quinoxaline derivatives were obtained by condensation of *o*-phenylenediamine with the ketoaldehydes. 4-Biphenylglyoxal *iso*nicotinyl hydrazone was also prepared.

By heating *p*-aminobenzoic acid at 60° with 4-biphenylglyoxal in ethanol, we obtained *p*-(α-ethoxy-*p*-phenylphenacylamido) benzoic acid; whereas in aqueous dioxan *p*-(α-hydroxy-*p*-phenylphenacylamido)benzoic acid was formed.

Experimental*

4′-Methoxy-4-biphenylglyoxal hydrate. A solution of 1·55 g (0·014 mole) of selenium dioxide in 10 ml of 90 per cent aqueous dioxan was warmed to 70° and a solution of 2·26 g (0·01 mole) of 4′-methoxy-4-biphenyl methyl ketone in 12 ml of dioxan was added. The mixture was refluxed for 5 h. The selenium which separated was filtered off hot, the crystals which separated on cooling were collected and recrystallized from aqueous dioxan to give 4′-methoxy-4-biphenylglyoxal hydrate (1·9 g, 78 per cent); m.p. 138–139°.

Anal. Calcd. for $C_{15}H_{14}O_4$: C, 69·75; H, 5·46; CH_3O, 12·02. Found: C, 69·87; H, 5·70; CH_3O, 12·10.

The corresponding quinoxaline derivative was prepared by heating a mixture of the ketoaldehyde with the equivalent amount of *o*-phenylenediamine in ethanol. It crystallized from aqueous ethanol; m.p. 169–171°.

Anal. Calcd. for $C_{21}H_{16}N_2O$: C, 80·75; H, 5·16; N, 8·97. Found: C, 81·53; H, 5·13; N, 8·94.

3′-Chloro-4′-methoxy-4-biphenylglyoxal hydrate. Oxidation of 3′-chloro-4′-methoxy-4-acetylbiphenyl with selenium dioxide by

* All melting points are uncorrected.

the method described above gave 70 per cent of a product which was recrystallized from ethyl acetate–benzene; m.p. 141–142° (220° dec).

Anal. Calcd. for $C_{15}H_{13}ClO_4$: C, 61·54; H, 4·47; Cl, 12·11; CH_3O, 10·60. Found: C, 61·64; H, 4·41; Cl, 12·40; CH_3O, 10·30.

The quinoxaline derivative was crystallized from aqueous ethanol; m.p. 165–167°.

Anal. Calcd. for $C_{21}H_{15}ClN_2O$: Cl, 10·22; N, 8·07. Found: Cl, 10·30; N, 7·87.

4,4'-Bis-biphenylglyoxal dihydrate. Oxidation of 4,4'diacetylbiphenyl with selenium dioxide gave 50 per cent of a product which, when recrystallized from dilute dioxan, melted at 160–162°.

Anal. Calcd. for $C_{16}H_{14}O_6$: C, 63·57; H, 4·67. Found: C, 63·82; H, 4·81.

The quinoxaline derivative crystallized from dioxan, m.p. 260–262°.

Anal. Calcd. for $C_{28}H_{18}N_4$: C, 81·93; H, 4·42; N, 13·65. Found: C, 81·52; H, 4·63; N, 13·90.

4-Biphenylglyoxal, monosodium bisulphite addition product. A solution of 2·28 g (0·01 mole) of 4-biphenylglyoxal hydrate and 100 ml of ethanol was poured into 1·5 per cent aqueous sodium bisulphite (200 ml). After standing overnight the crystals which separated were collected and washed with water to give 3 g (95 per cent) of monosodium bisulphite addition product.

Anal. Calcd. for $C_{14}H_{11}NaO_5S$: C, 53·50; H, 3·52; S, 10·13. Found: C, 53·04; H, 3·89; S, 10·20.

4-Methoxy-4-biphenylglyoxal, monosodium bisulphite addition product. Prepared by the same procedure as above in theoretical yield.

Anal. Calcd. for $C_{15}H_{13}NaO_6S$: S, 9·31. Found: S, 9·80.

3'-Chloro-4'-methoxy-4-biphenylglyoxal monosodium bisulphite addition product. Prepared as above in theoretical yield.

Anal. Calcd. for $C_{15}H_{12}ClNaO_6S$: S, 8·45. Found: S, 8·80.

4,4'-Bis-biphenylglyoxal disodium bisulphite addition product.

Anal. Calcd. for $C_{16}H_{12}Na_2O_{10}S_2$: S, 13·53. Found: S, 13·50.

4-Biphenylglyoxal iso*nicotinyl hydrazone.* A mixture of 2·28 g (0·01 mole) of 4-biphenylglyoxal hydrate, 1·37 g (0·01 mole) of

*iso*nicotinyl hydrazide and 75 ml of ethanol was refluxed for 2 h. After cooling the crystals were filtered and recrystallized from ethanol. Yield, 2·8 g (81 per cent); m.p. 218–220°.

Anal. Calcd. for $C_{20}H_{15}N_3O_3$: C, 72·93; H, 4·59; N, 12·76. Found: C, 72·56; H, 4·67; N, 12·54.

p-(*α-Ethoxy*-p-*phenylphenacylamido*)*benzoic acid.* A mixture of 2·10 g (0·01 mole) of biphenylglyoxal, 1·37 g (0·01 mole) of *p*-aminobenzoic acid, and 40 ml of ethanol was heated at 60° for 4 h. After cooling the crystals were filtered. Yield, 2·8 g (80 per cent); m.p. 192–194° dec.

Anal. Calcd. for $C_{23}H_{21}NO_4$: C, 73·58; H, 5·64; N, 3·75; C_2H_5O, 12·04. Found: C, 73·00; H, 5·68; N, 3·81; C_2H_5O, 12·50.

p-(*α-Hydroxy*-p-*phenylphenacylamido*)*benzoic acid.* A mixture of 2·73 g (0·012 mole) of 4-biphenylglyoxal hydrate, 1·37 g (0·01 mole) of *p*-aminobenzoic acid and 50 ml of 50 per cent aqueous dioxan was heated with stirring at 60° for 4 h, cooled and filtered. Yield, 2·8 g (80 per cent); m.p. 192–194° dec.

Anal. Calcd. for $C_{21}H_{17}NO_4$: C, 72·61; H, 4·93; N, 4·03. Found: C, 72·84; H, 5·05; N, 4·01.

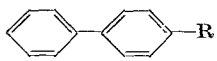
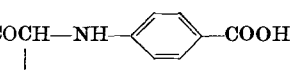
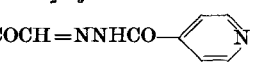
Pharmacology

These compounds were tested for anti-viral activity. The drugs were administered to mice infected with two different types of virus—influenza A–PR8 virus inoculated by the nasal route and the hepatitic MHV_3 virus inoculated by the subcutaneous route.

Tables II and III list the screening results with the most active compounds. The compounds were administered in a 3 per cent aqueous gum arabic suspension to 15 Swiss strain mice, weighing 15–20 g, daily for 11 days. The suspension for the oral and subcutaneous route was prepared so that 0·10 ml contained the dose of the compound. The viruses were inoculated 24 h after the beginning of the treatment. In order to determine the LD50 the compounds were administered orally in a 3 per cent aqueous gum arabic suspension.

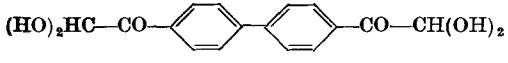
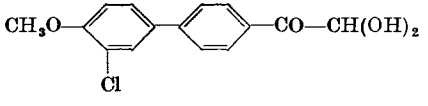
It is of interest to note that some of the compounds display a specific activity against the two viruses. Details of these findings will be published by Magrassi *et al.*

Table II. Influenza virus A–PR8—nasal inoculation 5 LD50 ($LD50=10^{-2\cdot13}$).

	Oral route			Subcutaneous route			LD50[a] mg/kg
	No. of animals	Dose, mg/kg	No. of survival animals 11 days after inoculation	No. of animals	Dose, mg/kg	No. of survival animals 11 days after inoculation	
Controls	30	—	0	30	—	0	
—CO—CHOHSO$_3$Na	15	177[b]	8	15	177[b]	8	1,200 (980–1,460)
	15	187[b]	10	15	187[b]	11	>1,500
	15	93[c]	7	15	93[c]	8	
	15	173[b]	9	15	173[b]	10	>1,500

[a] Calculated by the method of J. T. Litchfield and J. R. Wilcoxon.[15]
[b] Corresponding to 0·0005 mole.
[c] Corresponding to 0·00025 mole.

Table III. Hepatitic virus MHV_3—subcutaneous inoculation 60 LD50 ($LD50=10^{-3\cdot4}$).

	Oral route			Subcutaneous route			LD_{50} mg/kg
	No. of animals	Dose, mg/kg	No. of survival animals 11 days after inoculation	No. of animals	Dose, mg/kg	No. of survival animals 11 days after inoculation	
Controls	30	—	0	30	—	0	
	15	76[a]	10	15	76[a]	12	> 1,500
	15	38[b]	4	15	38[b]	8	
	—	—	—	15	19[c]	7	
	15	292[d]	7	15	292[d]	8	> 1,500

[a] Corresponding to 0·00025 mole.
[b] Corresponding to 0·00012 mole.
[c] Corresponding to 0·00006 mole.
[d] Corresponding to 0·001 mole.

Summary. A series of biphenyl ketoaldehydes derivatives has been synthesized and their anti-viral activity in mice has been investigated. Three of these compounds, namely 4-biphenylglyoxal monosodium bisulphite, *p*-(α-ethoxy-*p*-phenylphenacylamido)benzoic acid and 4-biphenylglyoxal *iso*nicotinyl hydrazone showed considerable anti-viral activity against influenza virus A–PR8 strain; two other of these series, 4′-methoxy-3′-chloro-4-biphenylglyoxal hydrate and 4,4′-bis-biphenylglyoxal dihydrate display an anti-viral activity against hepatitic virus MHV_3 strain.

(*Received 23rd September, 1959*)

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