of one experiment, which has been duplicated.

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Supplementary Material Available: Tables of calculated H-atom coordinates, anisotropic temperature factors U_{ij}, full list of bond lengths, bond angles, and torsion angles (14 pages); observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

Notes

Synthesis and Biological Activity of 5-(2,2-Difluorovinyl)-2′-deoxyuridine†

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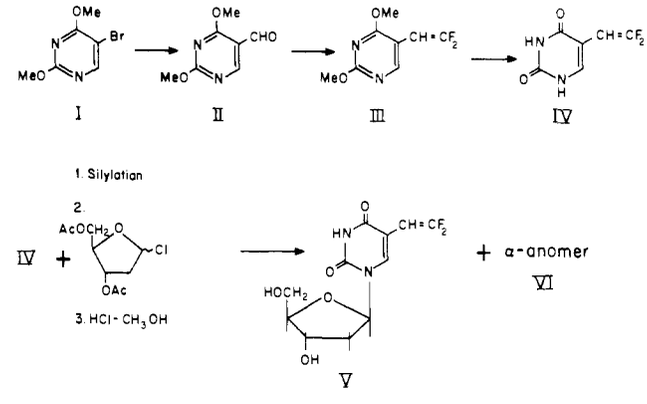
5-(2,2-Difluorovinyl)uracil (IV) was synthesized from 2,4-dimethoxy-5-bromopyrimidine by sequential formylation, difluoromethylenation, and removal of the 2- and 4-methyl groups. Condensation of the trimethylsilyl derivative of IV with protected D-*erythro*-pentofuranosyl chloride followed by separation of anomers and deblocking gave 5-(2,2-difluorovinyl)-2′-deoxyuridine (V). Compound V was active against herpes simplex virus type 1 (HSV-1) infection as well as tumor cells transformed by the HSV-1 thymidine kinase gene.

2′-Deoxy-5-vinyluridine and some related 5-halovinyl analogues exhibit potent activity against herpes simplex virus type I and type II.[1a] Among more than 100 5-vinylpyrimidine nucleosides reported so far in the literature,[1b] (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) has emerged as the most potent anti-HSV-1 agent. A fluoro analogue of BVDU, (*E*)-5-(2-fluorovinyl)-2′-deoxyuridine showed much reduced anti-HSV-1 activity[1c] and a dibromo analogue, 5-(2,2-dibromovinyl)-2′-deoxyuridine (DBVDU), was essentially inactive.[1b] A pronounced reduction in the antiviral activity[1b] also resulted from changing the *E* geometry of the halovinyl group to *Z*. While these results suggested that 5-(2,2-difluorovinyl)-2′-deoxyuridine (DFVDU) may lack strong anti-HSV-1 activity, other considerations, such as the strong electronegative nature and the chemical reactivity of the 2,2-difluoro group, raised the possibility that DFVDU may be capable of acting as an irreversible inhibitor of thymidylate synthase and/or of other enzymes in the DNA path. This paper reports the synthesis and antiherpes activity of 5-(2,2-difluorovinyl)-2′-deoxyuridine (V, Scheme I) and some reactions of the 5-(2,2-difluorovinyl) group in 2,4-dimethoxy-5-(2,2-difluorovinyl)pyrimidine.

Our initial attempts to prepare 5-(2,2-difluorovinyl)uracil (IV, Scheme I) by condensation of in situ generated (difluoromethylene)triphenylphosphorane with readily available 5-formyluracil failed to produce significant amounts of IV. We, therefore, used a protected form of 5-formyluracil, 2,4-dimethoxy-5-formylpyrimidine (II),[2] which was prepared from the corresponding 5-bromo derivative I by a modified procedure, employing *N*-formylpyperidine[3] in toluene in place of dimethylformamide in THF.[2] Reaction of II with the (difluoromethylene)-phosphorane generated from lithium chlorodifluoroacetate[4]

Scheme I



in the presence of triphenylphosphine gave 2,4-dimethoxy-5-(2,2-difluorovinyl)pyrimidine (III). Treatment of III with hexamethyldisilane in the presence of iodine[5] removed the protective methyl groups to give IV. For the preparation of the 5-(2,2-difluorovinyl)uracil nucleoside V (Scheme I), IV was sequentially silylated with a 1:1 mixture of chlorotrimethylsilane and hexamethyldisilazane in re-

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Table I. Biological Activity of Compounds IV and V

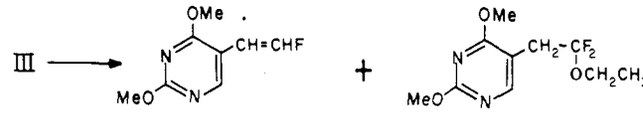
assay system		MIC_{50},[a] μg/mL					
virus	cell	IV	VI (α-anomer)	V (β-anomer)	DBVDU	IDU	BVDU
HSV-1 (KOS)	PRK	>200	20	0.2	20	0.15	0.02
HSV-1 (F)	PRK	>200	20	0.3	70	0.2	0.02
HSV-1 (McIntyre)	PRK	>200	20	0.3	20	0.3	0.02
HSV-2 (G)	PRK	>200	150	6	200	1.5	2
HSV-2 (196)	PRK	>200	100	10	150	3	7
HSV-2 (Lyons)	PRK	>200	70	3	200	1.5	7
Vaccinia	PRK	>200	150	15	100	0.5	20
Vesicular stomatitis	PRK	>200	>200	>200	>400	>200	>200
TK⁻HSV-1 (B2006)	PRK	>200	>200	>200	>150	>200	>200
	L1210	>100	>100	30	>100	ND	31
	FM3A/0	>100	>100	55	>100	ND	13
	FM3A/TK⁻	>100	>100	>100	>100	ND	0.4
	FM3A/TK⁻/HSV-1 TK⁺	>100	>100	0.25	7	ND	0.0004
	Raji/0	>100	>100	33	>100	ND	29
	Raji/TK⁻	>100	>100	>100	>100	ND	78
	Molt/4F	>100	>100	42	>100	ND	>100

[a] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity or cell growth by 50%. For abbreviations, see text. ND, not determined.

fluxing toluene and reacted with 3,5-di-*O*-acetyl-D-*erythro*-pentofuranosyl chloride in the presence of stannic chloride. The protected sugar chloride was prepared by acetylation of the crude methyl 2-deoxy-D-*erythro*-pentofuranoside followed by treatment with hydrogen chloride in acetic acid. Both the intermediate acetylated methyl glycoside as well as the sugar chloride were syrupy and were used without purification or separation from minor components such as pyranosyl derivatives. (Crystalline pentofuranosyl chlorides[6] protected with aroyl type groups are not suitable for the synthesis of V since deprotection requiring hydrolytic treatment in the presence of strongly basic catalysts results in the modification of the difluorovinyl group.[7] Condensation of the acetylated sugar chlorides with the silylated IV gave a reaction mixture that contained the 3,5-di-*O*-acetyl derivatives of V and its α-anomer VI (approximate 2:3 ratio) and two minor components, presumably the acetylated pyranosyl isomers of V and VI. The mixture was separated by multiple chromatographic runs on silica gel, and the syrupy anomers were deacetylated by treatment with methanolic HCl at room temperature to provide crystalline V and its α-anomer VI. In the ^1H NMR spectrum of V, the signal arising due to H-1′ appeared as a triplet while that due to H-1′ of the α-anomer VI showed as two doublets.[8]

Since the *gem*-difluorovinyl group can readily undergo a variety of chemical reactions,[9] it was of interest to examine the chemical reactivity of 2,4-dimethoxy-5-(2,2-difluorovinyl)pyrimidine (III). Compound III as well as acetylated V and VI was unstable at room temperature in chloroform, giving rise to more polar unidentified products, presumably as a result of polymerization of the difluorovinyl group. In contrast, in a methanolic solution both in the presence and absence of HCl, III did not show any change when kept at room temperature for several days. Treatment of III with sodium borohydride in ethanol

Scheme II



VII VIII

(Scheme II) gave a mixture of two products: a product of reduction, namely, (*E*)-2,4-dimethoxy-5-(2-fluorovinyl)-pyrimidine (VII), and a product of base-catalyzed addition of ethanol to III, namely, 2,4-dimethoxy-5-(2,2-difluoro-2-ethoxyethyl)pyrimidine (VIII). The configuration of compound VII and VIII was assigned on the basis of proton and fluorine NMR spectra. In the ^1H and ^{19}F NMR spectra of VII, the proton and fluorine resonances exhibited a geminal HCF coupling constant (85.5 Hz) consistent with the values reported previously for terminal fluoro olefins.[10] *E*-isomer assignment was made on the basis of the magnitude of the coupling of the terminal hydrogen and fluorine atoms with H on the vicinal carbon atom.[10]

The ^1H NMR spectrum of VIII exhibited a triplet for the 5-methylene group at δ 3.17 and the ethoxy group generated a quartet (δ 3.92) and a triplet (δ 1.23). Coupling (10.7 Hz) between 5-methylene and the 2′,2′-*gem*-difluoro group was also observed in the ^{19}F NMR spectrum of VIII where the fluorine resonsance appeared as a triplet.

Biological Activity

Compounds IV–VI were evaluated for their antiviral activity in comparison with 5-(2,2-dibromovinyl)-2′-deoxyuridine[11] (DBVDU) and with the reference compounds[1b] 5-iodo-2′-deoxyuridine (IDU) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). The test system consisted of primary rabbit kidney (PRK) cells infected with herpes simplex virus type 1 (HSV-1; strains KOS, F or McIntyre), herpes simplex virus type 2 (HSV-2; strains G, 196 or Lyons), vaccinia virus, vesicular stomatitis virus, or thymidine kinase (TK) deficient HSV-1 (strain B2006). The antitumor activity of the agents was evaluated against murine L1210 leukemia cells, murine FM3A/0 mammary carcinoma cells, TK-deficient FM3A (FM3A/TK⁻) cells, HSV-1 TK gene-transformed TK-deficient FM3A

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(FM3A/TK$^-$/HSV-1 TK$^+$) cells, human B-lymphoblast Raji/0 cells, TK-deficient Raji (Raji/TK$^-$) cells and human T-lymphoblast Molt/4F cells.

The results presented in Table I show that compound IV did not exhibit any antiviral or antitumor effect. The β-anomer V had marked activity against HSV-1, a 10- to 30-fold lesser activity against HSV-2 and vaccinia virus, and no activity against TK$^-$HSV-1, vesicular stomatitis virus and several other RNA viruses (Coxsackie B4, polio type 1, parainfluenza type 3, reo type 1, Sindbis, Semliki forest: data not shown). The α-anomer VI showed much lower antiviral activity, particularly against HSV-1, than did V. 5-(2,2-Dibromovinyl)-2′-deoxyuridine (DBVDU) showed marginal activity against HSV-1 and against FM3A/TK$^-$HSV-1 TK$^+$ cells and was inactive against the remaining cell lines. The β-anomer V was rather effective against FM3A/TK$^-$HSV-1 TK$^+$ cells but had little activity against various other tumor cells lines. In all these effects, V (β-anomer) resembled BVDU, which is strongly inhibitory to HSV-1, less inhibitory to HSV-2 and vaccinia virus,[12] not inhibitory to TK$^-$HSV-1. BVDU is also weakly active against tumor cells,[13,14] except for FM3A cells transformed with the HSV-1 TK gene, which are exquisitely sensitive to the compound.[15,16] DFVDU (V, β-anomer) and BVDU differed in their behavior toward FM3A/TK$^-$ cells, which were more sensitive to BVDU[17] and less sensitive to DFVDU than was the parental cell line FM3A/0.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. NMR spectra were recorded on a Varian XL-100 spectrometer using Me_4Si and $CFCl_3$ as internal standards. Mass spectra were measured in the electron-impact mode on a Finnegan 4000 spectrometer. Silica gel TLC was performed on 60F-254 precoated sheets (E. Merck) and column chromatography was conducted on silica gel (60–250 mesh; J. T. Baker No. 3405). Elemental analyses were performed by Galbraith Laboratory, Knoxville, TN, and were within ±0.4% of the calculated values.

2,4-Dimethoxy-5-formylpyrimidine (II). 5-Bromo-2,4-dimethoxypyrimidine (I) (66 g, 0.301 mol) was dissolved in 1.6 L of dry toluene and cooled to −78 °C. To this solution were sequentially added 215 mL of *n*-BuLi in hexane (0.344 mol) and, after 10 min, 51 mL (0.46 mol) of *N*-formylpiperidine. The temperature of the reaction mixture was allowed to slowly (2–3 h) rise to 20 °C. The mixture was washed with 2 N hydrochloric acid (150 mL) and the aqueous phase was extracted with ether (200 mL). The combined organic solution was dried (Na_2SO_4) and evaporated to a crystalline residue. The residue, which was homogeneous by TLC ($CHCl_3$ or benzene-EtOAc, 9:1), was triturated with petroleum ether and filtered: yield 32 g (63%). The ^1H NMR ($CDCl_3$) spectrum was in agreement with the literature data.[2]

2,4-Dimethoxy-5-(2,2-difluorovinyl)pyrimidine (III). Lithium chlorodifluoroacetate (25 g, 0.18 mol) was added with stirring and under N_2 to a hot (60–70 °C) solution of II (17 g, 0.101 mol) and triphenylphosphine (40 g, 0.15 mol) in dry DMF (45 mL). The reaction mixture was then heated at 107–110 °C for 35 min, cooled to room temperature, and diluted with toluene (1 L). The mixture was extracted with water (3 × 200 mL) and the water phase was extracted with toluene (2 × 100 mL). The combined organic solution was dried (Na_2SO_4), treated with ether to precipitate a portion of triphenylphosphine oxide, filtered, and evaporated to an oily residue, which was placed on a silica gel column (3 × 150 cm). The column was washed with toluene to remove triphenylphosphine followed by a mixture of cyclohexane–ether (6:1). The appropriate fractions were combined and concentrated to a crystalline residue, which melted at about room temperature: yield 10.7 g (52%); ^1H NMR ($CDCl_3$) δ 8.28 (s, 1, H-6), 5.28 (q, 1, $J_{H1',Fa}$ = 4.5 Hz, $H_{H1',Fb}$ = 26 Hz, H-1′), 4.0 (2 s, 6, OCH_3); ^{19}F NMR ($CDCl_3$) δ −82.547 (octet, 1, $J_{Fa,b}$ = 29.83 Hz, $J_{H1',Fb}$ = 25.87 Hz, $J_{Fb,H-6}$ = 1.4 Hz, Fb), −83.283 (q, 1, $J_{Fa,b}$ = 29.83 Hz, $J_{Fa,H1'}$ = 3.85 Hz, Fa). Anal. ($C_8H_8F_2N_2O_2$) C, H, N.

5-(2,2-Difluorovinyl)uracil (IV). A solution of III (10.1 g, 0.05 mol), hexamethyldisilane (14 mL, 0.067 mol), and iodine (16.75 g, 0.066 mol) in 200 mL of $CHCl_3$ was heated at the reflux temperature for 26 h. The reaction mixture was cooled to room temperature and filtered. The filtered product was washed with $CHCl_3$ and ether to give 2.7 g of white crystalline material. The combined filtrate and washings deposited more precipitate, which was again filtered and washed with ether, yielding 1.09 g of brownish material. Concentration of the filtrate gave a precipitate, which was triturated with acetone and filtered to give 1.8 g of product. The second and third crops were combined and recrystallized from a methanol–ethanol solution to provide 2.3 g of pure IV: total yield 5.0 g (57%); mp 288–292 °C; ^1H NMR (Me_2SO-d_6) δ 11.25 and 7.95 (2 br s, 2 H, NH-3, NH-1), 7.44 (s, 1, H-6), 5.3 (q, 1, $J_{H-1',Fa}$ = 3.0 Hz, $J_{H-1',Fb}$ = 27 Hz, H-1′). Anal. ($C_6H_4F_2N_2O_2$) C, H, N, F.

3,5-Di-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranosyl Chloride. 2-Deoxy-D-ribose (13.415 g, 0.1 mol) was added to a 0.5% methanolic HCl solution (200 mL) and the mixture was stirred at room temperature for 1 h. The mixture was neutralized with Et_3N and evaporated to a syrupy residue, which was dissolved in dry pyridine (100 mL). Acetic anhydride (40 mL) was added slowly to this solution with stirring and cooling. The mixture was kept at room temperature overnight and evaporated to dryness, and the residue was dissolved in xylene (100 mL). The solvent was evaporated and this step was repeated two times. The residue was dissolved in CH_2Cl_2 (100 mL) and extracted with 2% cold (0 °C) aqueous H_2SO_4 (7 mL) and H_2O (7 mL), dried (Na_2SO_4), and evaporated. The syrupy residue showed only one spot by TLC on silica gel (EtOAc). For the preparation of the 1-chloro derivative, the residue was dissolved in AcOH (~200 mL) to give a total volume of 250 mL of a stock solution. An aliquot (66 mL) of this solution was diluted with CH_2Cl_2 (60 mL), cooled in an ice–water bath, and saturated with HCl. The mixture was kept at 0–4 °C for 2 h (TLC, C_6H_6–EtOAc, 4:1), evaporated (bath temeprature >35 °C) to a syrupy residue, which was coevaporated with dry xylene (3 × 50 mL). The residue was dissolved in 1,2-dichloroethane (150 mL) and used in the condensation reaction with the silylated IV.

1-(3,5-Di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(2,2-difluorovinyl)uracil and Its α-Anomer. A suspension of IV (3.84 g, 0.022 mol) in a mixture of dry toluene (200 mL), hexamethyldisilazane (20 mL, 0.094 mol), and chlorotrimethylsilane (10 mL, 0.078 mol) was stirred at the reflux temperature for 1 h. The resulting solution was cooled and evaporated to an oily residue, which was coevaporated with toluene (3 × 80 mL). The oil was dissolved in 150 mL of dry 1,2-dichloroethane and added to a solution of crude 3,5-di-*O*-acetyl-D-*erythro*-pentofuranosyl chloride, prepared from 66 mL (~20% excess) of the stock solution of methyl 3,5-di-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranoside, in 150 mL of 1,2-dichloromethane. To this solution, cooled to 0 °C, was added 0.5 mL of stannic chloride and the reaction mixture was stirred at 4 °C for 16 h. The mixture was diluted with 1 L of CH_2Cl_2 and neutralized with a cold saturated $NaHCO_3$ solution (200 mL). The separated CH_2Cl_2 solution was washed with cold water (200 mL), filtered through a layer of Celite, and dried (Na_2SO_4). Evaporation of the solvent gave 12.6 g of an oily residue, which was fractionated

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by chromatography on silica gel (2 × 120 cm) in $CHCl_3$–ether (3:2) to give, after evaporation of the solvent, four homogeneous (TLC) fractions. The fraction (0.15 g) that was eluted first was discarded. The second fraction (0.63 g) was the 3,5-di-*O*-acetyl derivative of V: ^1H NMR ($CDCl_3$) δ 9.7 (br s, 1, NH), 7.18 (s, 1, H-6), 6.58 (t, 1, $J_{1',2'}$ = 7.8 Hz, H-1'), 5.23 (q, 1, J = 27 and 3 Hz, C*H*=CF_2), 2.0 (2 s, 6, CH_3CO).

The third fraction (0.88 g) was the acetylated α-anomer VI: ^1H NMR ($CDCl_3$) δ 9.35 (br s, 1, NH), 7.60 (s, 1, H-6), 6.24 (d of d, 1, J = 5.4 and 8.1 Hz, H-1'), 5.31 (q, 1, J = 3 and 27 Hz, C*H* = CF_2), 2.05 (2 s, 6, CH_3CO). The fraction (0.02 g) that was eluted last was also discarded.

5-(2,2-Difluorovinyl)-2'-deoxyuridine (V) and Its α-Anomer VI. The acetylated V (1.1 g) was disolved in 75 mL of 0.75 N methanolic HCl and kept 4 h at room temperature. The solution was neutralized with weekly basic Amberlite IRA45 resin. The resin was filtered and washed with methanol (~50 mL). The filtrate that contained some decomposed material (base line, TLC, $CHCl_3$–MeOH, 9.1) was evaporated to a syrupy residue, which was coevaporated several times with ethanol and purified by chromatography on partially deactivated (8% H_2O) silica gel (2 × 100 cm), with ethyl acetate–acetone (1.1) as the eluant. The fraction collected from the column was free of decomposed material, but contained some partially deacetylated product. It was evaporated to a syrup, which was coevaporated with ethanol and crystallized from ethanol–acetone (9:1) to give two crops, 166 mg and 81 mg, respectively, of TLC (EtOAc) pure V. The combined filtrate was evaporated and deacetylated in 70 mL of 1 N methanolic HCl to give, after workup, 73 mg of pure V: yield 322 mg (40%); mp 152 °C; ^1H NMR (Me_2SO-d_6) δ 11.45 (br s, 1, NH), 8.04 (s, 1, H-6), 6.27 (t, J = 6.9 Hz, H-1'), 5.30 (q, 1, J = 3 and 27 Hz, C*H*=CF_2), 4.96 (t, 1, $J \sim$ 4.5 Hz, CH_2OH). Anal. ($C_{11}H_{12}F_2N_2O_5$) C, H, N, F.

Deacetylation of the blocked α-anomer (1.58 g) in 70 mL of 0.75 M methanolic HCl for 18 h followed by neutralization with Amberlite IRA 45, chromatography, and crystallization gave 0.487 g (39%) of the α-anomer VI: mp 173–174 °C; ^1H NMR (Me_2SO-d_6) δ 11.5 (br s, 1, NH), 8.10 (s, 1, H-6), 6.13 (d of d, 1, J = 2.8 and 7.5 Hz, H-1'), 5.33 (q, 1, J = 3 and 27 Hz, C*H*=CF_2), 4.8 (t, 1, CH_2OH). Anal. ($C_{11}H_{12}F_2N_2O_5$) C, H, N, F.

(*E*)-2,4-Dimethoxy-5-(2-fluorovinyl)pyrimidine (VII) and 2,4-Dimethoxy-5-(2,2-difluoro-2-ethoxyethyl)pyrimidine (VIII). To a solution of III (121 mg, 0.6 mmol) in 10 mL of absolute EtOH was added $NaBH_4$ (42 mg, 1.11 mmol) and the reaction mixture was stirred for 4 h at 50–55 °C and at room temperature overnight. The solution was neutralized with a 1% ethanolic H_2SO_4 and evaporated. The residue was coevaporated with benzene, dissolved in CH_2Cl_2 (40 mL), and filtered, and the solvent was evaporated. Separation of the residue by chromatography on silica gel, using petroleum ether–ether (6:1, v/v) as the eluent, gave three fractions. In the order they were eluted from the column (1 × 100 cm): Fraction 1 (4 mg) was the starting material (III). Fraction 2 (48 mg), compound VIII: ^1H NMR ($CDCl_3$) δ 8.20 (s, 1, H-6), 4.03, 4.02 (2 s, 6, OCH_3), 3.92 (q, 2 H, J = 7.1 Hz, OCH_2CH_3), 3.17 (t, 2 H, J_{HF} = 10.7 Hz, CH_2CF_2), 1.23 (t, 3 H, J = 7.1 Hz, OCH_2CH_3); ^{19}F NMR ($CDCl_3$) 74.666 (t, $J_{F,H}$ = 10.75 Hz). Fraction 3 (60 mg), compound VII: ^1H NMR ($CDCl_3$) δ 8.03 (s, 1, H-6), 7.26 (q, 1, $J_{F,H2'}$ = 85.5 Hz, $J_{H1',2'}$ = 11 Hz, H-2'), 6.15 (q, 1, $J_{F,H1'}$ = 21 Hz, $J_{1',2'}$ = 11 Hz, H-1'), 4.04, 3.97 (2 s, 6, OCH_3).

Biological Assays. The assay systems for measuring antiviral and antitumor activity, the source of the virus strains, and the growth characteristics of the tumor cell lines, including the FM3A cell line transformed with the HSV-1 TK gene,[16] have been described previously.[12-17]

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Synthesis and Antifungal Activity of a Series of Novel 1,2-Disubstituted Propenones

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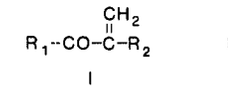
To find an antifungal agent other than those of the imidazole and triazole series, a new class of 1,2-disubstituted propenones I and II was prepared and tested for antifungal activity. Comparison of the structure–activity relationships showed that the conjugated structure of carbonyl and exomethylene groups in I and II plays an important role in potent antifungal activity. However, it is noteworthy that compounds **53**, **54**, and **56**, which have a hydroxymethyl or methoxymethyl group instead of an *exo*-methylene group in I, also showed potent activity. Although many compounds exhibited strong antifungal activity in vitro, none showed activity in vivo of oral efficacy against subacute systemic candidiasis in mice.

Previous papers[1-3] from our group reported the synthesis and biological evaluation of compounds from the imidazole and triazole series as a novel type antifungal agent. In continuing our study, we found a new class of 1,2-disubstituted propenones I and II that differ from imidazole and triazole compounds. These new compounds were prepared and screened for potential antifungal activity.



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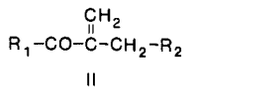

II

Chemistry

The general synthetic routes (methods A–F) for the preparation of I and II outlined in Scheme I. Various aryl or alkyl methyl ketones III were treated with bromine to obtain the bromo ketone IV and followed by treatment

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