A Reappraisal of the Effect upon Thymidine Kinase of Thymidine Derivatives Carrying Large Groups at the 5'-Position

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Several thymidine derivatives with hydrophobic 5'-substituents, linked by chemically stable amide and ether links, were synthesized as potential thymidine kinase inhibitors. None of these was active nor were several derivatives of thymidine 5'-acetate, which were previously reported to be inhibitors. It was shown that the apparent inhibition by the latter compounds was due to their facile hydrolysis in aqueous solution with release of thymidine. These results must cast doubt on any conclusions drawn from biological studies with 5'-esters of thymidine.

Our interest in blocking nucleoside salvage pathways has led us to study inhibitors of thymidine kinase. Baker and Neenan[1] synthesized a series of thymidine derivatives with bulky 5'-substituents, which was tested against thymidine kinase from Walker 256 carcinoma cells. Only those derivatives with a 5'-(aryloxy)acetyl or 5'-(arylthio)acetyl group seemed to show significant inhibitory activity, the [4-(benzyloxy)phenoxy]acetate **1** and α-(naphthylthio)-acetate being quoted as the most effective. Harrap et al.[2] and Stringer[3] later reported that a similar compound, 5'-*O*-[[4-[(4-nitrobenzyl)oxy]phenoxy]acetyl]thymidine (**2**) inhibited Yoshida sarcoma thymidine kinase. However, the ester linkage in these compounds is likely to be hydrolyzed in biological systems.[4] We therefore prepared a number of analogues in which the ester link was replaced by an amide or ether link.

Chemistry

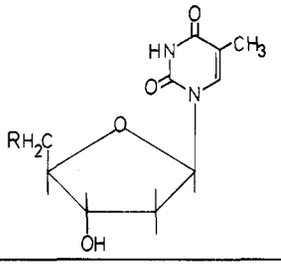
The two amides **3** and **4** were made by condensation of the corresponding acids **7** and **8** with 5'-amino-5'-deoxythymidine (**6**) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC). Buzas et al.[5] reported that, for strongly basic amines, much greater yields of amides, based on starting amine, were obtained when 2 equiv of acid were used rather than 1 equiv. This observation was confirmed by use of acid **7** and cyclohexylamine.

$RC_6H_4CH_2OC_6H_4OCH_2R'$
7, R = H; R' = CO_2H
8, R = NO_2; R' = CO_2H
9, R = H; R' = CH_2Br
10, R = H; R' = CH_2OH
11, R = H; R' = $CO_2NHC_6H_{11}$
all aromatic substitutions are para

Ether **5** was prepared by the reaction, using NaH as base, of 3'-*O*-tritylthymidine and 1-[4-(benzyloxy)phenoxy]-2-bromoethane (**9**), followed by detritylation with 80% acetic acid. The mass spectral fragmentation pattern demonstrated that alkylation had occurred on the sugar rather than the base. This was confirmed by the ultraviolet spectrum that showed the same change in extinction coefficient on addition of base as did that of thymidine. The halide **9** was made from acid **7** by reduction to alcohol **10** with diborane and subsequent bromination of **10** with (bromomethylene)dimethylammonium bromide.

We synthesized ester **1** by a modification of Baker and Neenan's route and improved the yield from 4% to 15%.

Table I. Inhibition of [^{3}H]TMP Production by L1210 Thymidine Kinase



no.	R[a]	concn tested, μM	% inhib[b]
1	$PhCH_2OC_6H_4OCH_2CO_2$	250[c]	33 ± 3 (6)
2	$O_2NC_6H_4CH_2OC_6H_4OCH_2CO_2$	200[c]	35 ± 3 (5)
3	$PhCH_2OC_6H_4OCH_2CONH$	125[c]	0 ± 1 (4)
4	$O_2NC_6H_4CH_2OC_6H_4OCH_2CONH$	125[c]	1 ± 2 (4)
5	$PhCH_2OC_6H_4OCH_2CH_2O$	200[c]	14 ± 5 (3)
6	NH_2	50	76 ± 3 (4)

[a] All aromatic substitutions are para. [b] Results are given are mean ± SE with the number of determinations in parentheses. Assay conditions were 5 μM [^{3}H]TdR, 2 mM ATP, 1 mM $MgCl_2$, and 10% Me_2SO in 45 mM Tris (pH 7.8). Compounds were added in Me_2SO solution, and the reaction was followed for 10 min. [c] Maximum concentration tested (because of the limiting solubility of the compound in 10% Me_2SO).

Biological Results

The compounds were tested for inhibitory activity against a thymidine kinase preparation from L1210 cells, and the results are shown in Table I. Most of these compounds have relatively low aqueous solubilities, and so the assay used contained a low concentration of thymidine, the substrate with which they might compete. Compounds **3** and **4** were inactive, while compounds **1**, **2**, and **5** appeared to show marginal activity. Compound **6** showed significant inhibitory activity, which was consistent with previous reports.[6] The results with **1** and **2** were unexpectedly low in view of the previous data of Baker and Neenan[1] and of Harrap et al.[2]

As mentioned earlier, Baker and Neenan[1] noted that only the 5'-(aryloxy)acetates and 5'-(arylthio)acetates appeared to be active in their enzyme preparation. The phenoxyacetyl group has been suggested as a labile protecting group for the 5'-position of nucleosides.[7] This

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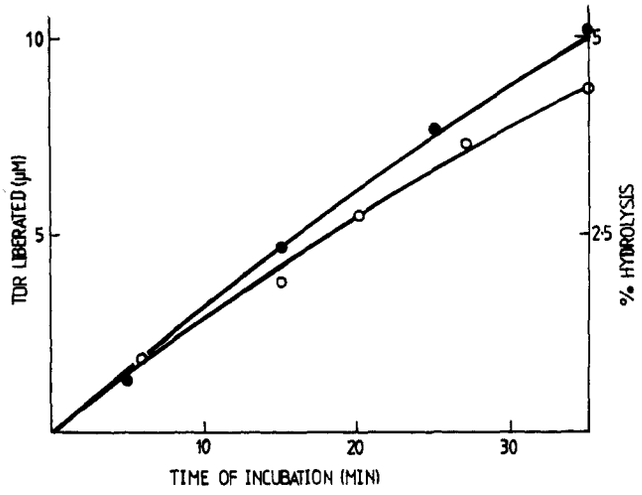

Figure 1. Aqueous hydrolysis of esters 1 (●) (250 μM) and 2 (○) (200 μM) in the assay buffer at 37 °C and the thymidine liberated therefrom. Each point was the mean of at least two determinations.

Table II. Thymidine Kinase Activity as Measured by the Radiochemical and Nonradiochemical Methods[a]

compd added	radiochem assay	nonradiochem assay, +TdR	nonradiochem assay, −TdR
none	100	100	0
1	65	152	118
2	62	144	110
6	26	27	0

[a] Concentrations of 1, 2, and 6 were the same as in Table I. Activity is expressed as a percentage of the control rate measured in the presence of 5 μM TdR. The results are the mean of at least two separate experiments. The nonradiochemical assay gave control rates of 104% that for radiochemical assay.

raised the possibility that 1 and 2 might undergo hydrolysis in aqueous solution either before or during an assay. The cold thymidine thereby released would dilute the radiolabeled substrate, and the consequent fall in labeled TMP production could be misinterpreted as being due to enzyme inhibition.

For the assays reported here, the compounds were prepared in Me_2SO and only exposed to an aqueous environment for the short preincubation and assay. To determine the extent of chemical breakdown that would occur during this period, compounds 1 and 2 were incubated at 37 °C in 10% Me_2SO, 45 mM Tris, pH 7.8 (the buffer used in the assay), and the thymidine liberated was determined by HPLC. The results are shown in Figure 1. Although the rates of hydrolysis were low, sufficient thymidine would be liberated during the course of the enzyme assay to produce considerable inhibition of [^{3}H]-TMP production. In addition, since the enzyme preparation used here was relatively crude, some thymidine may have been liberated enzymically, giving rise to a greater degree of apparent enzyme inhibition.

To test this hypothesis further, an additional assay procedure for thymidine kinase was developed that did not make use of radiochemicals. The assay conditions were the same as for the normal radiochemical assay, but at the end of the incubation the nucleotides and nucleosides were separated and the nucleotides hydrolyzed to nucleosides, which were quantitated using HPLC. Both the radiochemical and nonradiochemical assays were run in parallel, and the results are shown in Table II. The control rate

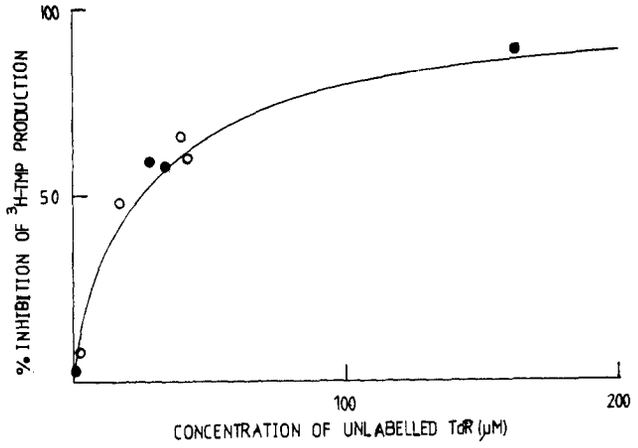

Figure 2. Inhibition of enzymic [^{3}H]TMP production caused by dilution of the ^{3}H substrate with unlabeled thymidine added to the assay mixture. The line represents the theoretical relationship for this assay, which contained 25 μM [^{3}H]TdR. The points represent the experimental data obtained with aqueous samples of esters 1 (●) and 2 (○), partially hydrolyzed during storage for up to 24 h at 37 °C. The degree of inhibition observed is plotted against the concentration of unlabeled thymidine contributed by these samples.

measured was the same for both assays, as was the degree of inhibition produced by compound 6. However, the presence of esters 1 or 2 inhibited the production of [^{3}H]TMP but stimulated the production of TMP. In addition, even without the addition of exogenous TdR, considerable TMP was formed during the incubation in the presence of compounds 1 or 2. Heat inactivation of the enzyme preparation before incubation resulted in no TMP being produced. These results indicate that esters 1 and 2 do not inhibit thymidine kinase but that they do break down during the assay period and that the thymidine liberated is phosphorylated to TMP. Thus the results obtained with the radiochemical assay are misleading.

Since test compounds may be prepared in aqueous solutions some time before use, the degree of hydrolysis could vary greatly and affect the results obtained. To test this, compounds 1 and 2 were prepared in 20% Me_2SO and left for up to 24 h at 37 °C. The extent of hydrolysis and consequent liberation of thymidine was considerable. When these samples were assayed for inhibitory activity, the inhibition of [^{3}H]TMP production observed was also considerable and corresponded well to that anticipated from the dilution of the radioactive substrate with cold thymidine from the samples (Figure 2).

Conclusion

Our results show that thymidine derivatives with bulky hydrophobic substituents, such as (aryloxy)acetyl, at the 5′-position are not good inhibitors of thymidine kinase, a conclusion in contrast to the report of Baker and Neenan. The finding that esters of this type undergo aqueous hydrolysis under neutral conditions casts doubt on the conclusions drawn from biological studies with these compounds.

Experimental Section

NMR spectra were determined with a Perkin-Elmer R12B 60-MHz spectrometer. Me_4Si was used as the internal standard in both $CDCl_3$ and CD_3CO_2D; either sodium 3-(trimethylsilyl)-propanesulfonate or sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ was the internal standard in Me_2SO. Ultraviolet spectra were measured on a Pye Unicam SP8-150 spectrometer. Chemical-ionization mass spectra were determined on a VG 7070H spectrometer at 50 eV with methane as reagent gas. N^3-Methylthymidine was a gift from Dr. M. Jarman. Thin-layer chroma-

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tograms were run on fluorescent silica (Merck 5735), with spot location by UV light and by the cysteine/sulfuric acid reagent[8] for 2′-deoxy nucleosides. Column chromatography, other than preparative HPLC, was carried out on Merck Kieselgel 60 (Art. 7734). Preparative HPLC was carried out on Merck Kieselgel 60 (Art. 15111), using a Jobin-Yvon Chromatospac Prep 10 coupled to a Cecil 212A UV monitor operating at 254 nm. Melting points were measured on a Koffler block and are uncorrected unless specified otherwise. DMF was dried by distillation from CaH_2 at atmospheric pressure; diglyme and dioxane were dried by distillation from $LiAlH_4$ at atmospheric pressure. Benzene was dried azeotropically.

***N*-Cyclohexyl-2-[4-(benzyloxy)phenoxy]acetamide (11).** A freshly prepared mixture of acid **7**[9] (0.40 g, 1.56 mmol), DCC (0.18 g, 0.86 mmol), and redistilled DMF (2 mL) was cooled, with stirring, in ice. Thirty minutes later cyclohexylamine (77 mg, 0.77 mmol) was added and the mixture stirred overnight at 4 °C. Two drops of AcOH were then added and 30 min later the solution was filtered and taken to dryness under high vacuum. The residue was dissolved in EtAc (35 mL) and washed successively with dilute HCl, saturated $NaHCO_3$ solution, and water. The organic layer was dried ($MgSO_4$) and evaporated and the residue allowed to crystallize from ethanol, yielding white plates (160 mg, 61%), mp 120–130 °C, homogeneous on TLC (Et_2O). A sample recrystallized for analysis had mp 126–129 °C. Anal. ($C_{21}H_{25}NO_3$) C, H, N. When this reaction was repeated with half the proportion (0.20 g) of acid **7**, only a 30% yield of amide was obtained.

5′-[[4-(Benzyloxy)phenoxy]acetamido]-5′-deoxythymidine (3). The procedure was essentially the same as for compound **11**, acid **7** (0.52 g, 2.0 mmol), DCC (0.31 g, 1.5 mmol), amine **6**[10] (0.24 g, 1.0 mmol), and DMF (10 mL) being used. The delay in adding the amine was reduced to 2 min to minimize *N*-acylurea formation.[11] The residue from the acidification was extracted with EtAc (100 mL), the insoluble material being collected. The filtrate was extracted with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. The residue, together with the material insoluble in EtAc, was applied as a solution in $CHCl_3$–MeOH–AcOH (17:2:1) to a silica column (125 g, 2.7 × 3.8 cm), and the product was eluted with the same solvent mixture. The amide crystallized from AcOH–H_2O (3:7, 100 mL) as white plates (0.24 g, 49%); mp 219–220.5 °C (corr), homogeneous on TLC (EtAc–EtOH, 9:1). NMR (Me_2SO-d_6) δ 1.85 (3 H, s, 5-CH_3), 2.1 (2 H, q, 2′-CH_2), 3.5 (2 H, m, 5′-CH_2), 3.9 (1 H, m, 4′-CH), 4.3 (1 H, m, 3′-CH), 4.50 (2 H, s, CH_2CONH), 5.08 (2 H, s, benzyl CH_2), 5.4 (1 H, m, 3′-OH), 6.2 (1 H, t, 1′-CH), 6.98 (4 H, s, Ar), 7.45 (5 H, s, Ar), 7.56 (1 H, s, 6-CH), 8.25 (1 H, t, amide NH), 10.35 (1 H, s, 3-NH). Anal. ($C_{25}H_{27}N_3O_7$) C, H, N.

5′-Deoxy-5′-[[4-[(4-nitrobenzyl)oxy]phenoxy]acetamido]-thymidine (4). The procedure was essentially the same as for **3**, acid **8**[2] (1.21 g, 4.0 mmol), DCC (0.61 g, 3.0 mmol), and amine **6** (0.48 g, 2.0 mmol) being used. The residue from the acidified reaction mixture was applied as a solution in $CHCl_3$–MeOH–AcOH (18:1:1) to a silica column (400 g, 4.7 × 49 cm), and the product was eluted with the same solvent mixture. The amide crystallized from AcOH–H_2O (3:7, 100 mL) as colorless needles (0.57 g, 54%): mp 187–191 °C; homogeneous on TLC (EtAc–EtOH, 9:1); NMR (Me_2SO-d_6) δ 1.84 (3 H, s, 5-CH_3), 2.15 (2 H, t, 2′-CH_2), 3.3–3.6 (5 H, c, 5′-CH_2, 3′-OH, 3-NH, amide NH), 3.85 (1 H, m, 4′-CH), 4.2 (1 H, m, 3′-CH), 4.50 (2 H, s, CH_2CONH), 5.28 (2 H, s, benzyl CH_2), 6.2 (1 H, t, 1′-CH), 7.00 (4 H, s, Ar), 7.57 (1 H, s, 6-CH), 7.8 (2 H, d, Ar), 8.35 (2 H, d, Ar). Anal. ($C_{25}H_{26}N_4O_9$) C, H, N.

2-[4-(Benzyloxy)phenoxy]ethanol (10). A solution of acid **7**[9] (2.58 g, 10 mmol) in pure, dry diglyme (20 mL) was added dropwise over several minutes to a stirred and cooled solution of $NaBH_4$ (1.13 g, 30 mmol) in the same solvent (25 mL). A solution of redistilled BF_3–Et_2O[12] (1.9 mL, 15 mmol) in pure dry diglyme (5 mL) was then added dropwise with stirring during several minutes. The mixture clarified and 45 min later the solution was poured into well-stirred AcOH–H_2O (1:9, 400 mL). When frothing had subsided, the mixture was heated to 70 °C and then allowed to cool. The solvent was evaporated and the residue partitioned between EtAc and H_2O, and the organic layer was washed successively with saturated $NaHCO_3$ solution, dilute HCl and H_2O, dried ($MgSO_4$), and evaporated. The alcohol crystallized from EtAc (25 mL) as white plates (1.81 g): mp 103.5–104.5 °C (corr); homogeneous on TLC (Et_2O); total yield (second crop, 0.30 g), 2.11 g (86%). Anal. ($C_{15}H_{16}O_3$) C, H.

1-[4-(Benzyloxy)phenoxy]-2-bromoethane (9). (Bromomethylene)dimethylammonium bromide[13] (0.25 g, 1.2 mmol) was dissolved in dry DMF (10 mL) in a dry flask equipped with a $CaCl_2$ tube. The solution was heated with stirring in an oil bath at 100 °C for several minutes prior to the addition of alcohol **10** (0.20 g, 0.82 mmol). Ten minutes later the solution was cooled and a few drops of water added. The solution was partitioned between $CHCl_3$ and H_2O and the organic layer dried ($MgSO_4$) and evaporated to dryness. The residue was applied as a solution in $CHCl_3$ to a small (pasteur pipet) column of silica, and the product was eluted with $CHCl_3$. Bromide **9** crystallized from MeOH (4 mL) as white plates (0.17 g): mp 80–82 °C; homogeneous on TLC ($CHCl_3$); total yield (second crop, 44 mg), 0.21 g (83%). A sample recrystallized for analysis had mp 82–82.5 °C (corr). Anal. ($C_{15}H_{15}O_2Br$) C, H, Br. Druey[14] records the melting point as 84–84.5 °C but gives no evidence for structure.

5′-*O*-[2-[4-(Benzyloxy)phenoxy]ethyl]thymidine (5). A solution of 3′-*O*-tritylthymidine[15] (242 mg, 0.50 mmol) in dry C_6H_6–dioxane (3:1, 5 mL) was added to a suspension of NaH (24 mg, 1.0 mmol) in the same solvent (5 mL). The mixture was stirred overnight ($CaCl_2$ guard tube). The next day a solution of bromide **9** (154 mg, 0.50 mmol) in dry solvent (5 mL) was added in three separate lots at 15-min intervals. No product appeared to form (TLC, Et_2O) overnight at room temperature or after several hours of reflux. More NaH (20 mg) was added and reflux continued. The next day TLC (Et_2O) indicated some product formation. A few drops of MeOH were added and the solution was partitioned between C_6H_6 and H_2O. The organic layer was dried ($MgSO_4$) and evaporated, the residue dissolved in AcOH–H_2O (4:1, 25 mL), and the solution heated under reflux for 30 min. The solution was evaporated and the residue partitioned between EtAc and H_2O. The organic layer was dried ($MgSO_4$) and evaporated. Cyclohexane (10 mL) was added to the residue, the mixture boiled for several minutes, and the liquid decanted. The residue was applied, in EtAc solution, to a silica column (125 g, 2.8 × 39 cm), and the product was eluted with EtAc. The ether crystallized from EtAc as white plates (23 mg): mp 173–176 °C; homogeneous on TLC (EtAc). A second crop was obtained from methanol (7 mg): total yield 30 mg (13%); NMR (CD_3CO_2D) δ 1.85 (3 H, d, 5-CH_3), 2.2–2.55 (2 H, m, 2′-CH_2), 3.75–4.3 (8 H, c, 3′-CH, 4′-CH, 5′-CH_2, OCH_2CH_2O), 4.35–4.75 (1 H, m, 3′-OH), 5.04 (2 H, s, benzyl CH_2), 6.35 (1 H, t, 1′-CH), 6.88 (4 H, s, Ar), 7.35 (5 H, s, Ar), 7.8 (1 H, d, 6-CH); UV max 270: (ϵ in EtOH)/(ϵ in EtOH + KOEt) = 0.90; for thymidine UV max 267: (ϵ in EtOH)/(ϵ in EtOH + KOEt)= 0.89; for N^3-methylthymidine UV max 268: (ϵ in EtOH)/(ϵ in EtOH + KOEt) = 0.99; mass spectrum, *m/e* 468 $(M)^+$, 342 $(M - base)^+$, 324 $(M - base - H_2O)^+$, 127 $(base + 1)^+$. Anal. ($C_{25}H_{28}N_2O_7$) C, H, N.

5′-*O*-[[4-(Benzyloxy)phenoxy]acetyl]thymidine (1). A flask containing thymidine (1.94 g, 8.0 mmol), acid **7** (2.06 g, 8.0 mmol), and DCC (4.94 g, 24 mmol) was dried in vacuo. Dry DMF (30 mL) was then added and the reaction stirred for 20 h. Aqueous acetic acid (50%, 2.5 mL) was added and 15 min later the solution was filtered and taken to dryness under high vacuum. The residual gum was stirred with $CHCl_3$–EtOH (95:5; 50 mL) and the mixture filtered. The solid was washed with 50% ethanolic $CHCl_3$ until the only UV-absorbing material left was thymidine. The liquids were taken to dryness under high vacuum. The residue was applied in solvent ($CHCl_3$–EtOH, 97:3) to an HPLC column (220 g of silica) and the product eluted with the same solvent.

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The ester crystallized from ethanol (25 mL) as white plates (0.56 g, 15%): mp 160.5–164.5 °C (lit.[1] mp 162–163 °C); homogeneous on TLC (C_6H_6–EtOH, 9:1). NMR (Me_2SO-d_6) δ 1.82 (3 H, s, 5-CH_3), 2.2 (2 H, t, 2′-CH_2), 4.05 (1 H, t, 4′-CH), 4.15–4.55 (4 H, c, 3′-CH and -OH, 5′-CH_2), 4.80 (2 H, s, CH_2CO_2), 5.08 (2 H, s, benzyl CH_2), 6.3 (1 H, t, 1′-CH), 6.95 (4 H, s, Ar), 7.45 (6 H, s, Ar and 6-CH). Anal. ($C_{25}H_{26}N_2O_8$) C, H, N.

Enzyme Preparation and Assay. L1210 ascites cells were suspended at 7×10^7 cells/mL in 50 mM Tris-HCl (pH 7.8), containing 150 mM KCl, and sonicated for two bursts of 15 s each. The sonicate was spun at 100 000*g* for 30 min, and the supernatant was brought to 30% saturation with a saturated ammonium sulfate solution. The resulting precipitate was resuspended in 50 mM Tris-HCl (pH 7.8) and used directly. All procedures were carried out at 4 °C. The heat-inactivated sample was prepared by incubation at 100 °C for 5 min. The enzyme preparation routinely had a V_m of 90–100 nmol h^{-1} (mg of protein)$^{-1}$.

Assay Procedure. 1. Radiochemical Assay. The assay mixture consisted of 2 mM ATP, 1 mM $MgCl_2$, 5 or 25 μM [^{3}H]TdR (100 μCi/μmol), 10% Me_2SO, 45 mM Tris-HCl (pH 7.8). The time course of the reaction at 37 °C was followed. Samples were taken at different times, and the reaction was stopped by boiling for 5 min. Aliquots of the reaction supernatant were spotted onto polyethylenimine cellulose squares (cut from Polygram cel 300 PEI plates), which were then washed three times in 5 mM Tris-HCl (pH 7.8) and once in 95% EtOH before drying. [^{3}H]TMP was eluted from the squares with 1 mL of 1 N HCl and counted with an efficiency of 30%.

2. Nonradiochemical Assay. The assay mixture was the same as for the radiochemical assay except that 0 or 5 μM unlabeled TdR was used. After 10 min of incubation at 37 °C, the reaction was stopped by dilution 20-fold with ice-cold water. This mixture was then diluted 10-fold with CH_3CN and passed through silica Seppaks (Waters Associates Ltd.) previously washed with CH_3CN–H_2O (9:1). The reaction tubes were washed with CH_3CN–H_2O (19:1) and the washings applied to the Seppaks, which were then washed with more CH_3CN–H_2O (19:1). This procedure washed through 99.9% of the applied thymidine but none of the TMP. The nucleotides were eluted with 10 mL of H_2O directly into round-bottom flasks, freeze-dried, redissolved in 1 mL of 25 mM $NaHCO_3$, and digested with alkaline phosphatase (4 units/mL) overnight. The nucleoside mixture was acidified with glacial acetic acid to about pH 5.5 and subjected to HPLC analysis, using a 23-cm 5μ C18 Apex column fitted with 2μ in-line filter, with a mixture (1:9) of MeOH and 50 mM succinic acid made to pH 5.7 with ammonia as solvent running at 1 mL/min. The absorbance at 254 and 280 nm was monitored, and the traces were analyzed by using a Trilab (Trivector, Sandy, Beds). To test recovery and reproducibility, control tubes were spiked with 1 μM TMP and processed with the assay tubes. Recovery was 102 ± 4% (mean ± SE for 14 determinations).

Thymidine Estimation. The thymidine in the samples was separated and quantitated by HPLC, using a 20 × 0.46 cm Spherisorb 5μ hexyl column running isocratically in 10% MeOH, 90% 25 mM acetic acid adjusted to pH 5.0 with ammonia, and fitted with 254- and 280-nm detectors.

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Structure–Activity, Theoretical, and X-ray Studies on the Intramolecular Interactions in a Series of Novel Histamine H_2 Receptor Antagonists

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The furan ring of the histamine H_2 receptor antagonist 3-amino-4-[[2-[[[5-[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]amino]-1,2,5-thiadiazole 1-oxide (**1a**) was replaced by thiophene, pyridine, benzene, and pyrrole. The relative receptor affinities of these analogues were estimated by in vitro and in vivo techniques. A theoretical model for the stacking interaction, observed by single crystal X-ray analysis of **1a**, was developed, and the ability to enter into this type of interaction was estimated. The X-ray analysis of the pyridine analogue of **1a** revealed no intramolecular stacking interaction. The theoretical studies were evaluated in light of the observed receptor affinities, and the relevance of the solid-state geometry of **1a** to the receptor-bound geometry was assessed. It is suggested that the stacked geometry found in the X-ray structure of **1a** does not represent a conformation that is relevant to that bound at the histamine H_2 receptor.

In a recent publication,[1a] the X-ray structure (Figure 1) of a novel, potent, and selective histamine H_2 antagonist, 3-amino-4-[[2-[[[5-[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]amino]-1,2,5-thiadiazole 1-oxide (**1a**), was described.[1a,b] This report delineates attempts to optimize potency via an understanding of the intramolecular interactions found in the diaminothiadiazole class of histamine H_2 antagonists.

In the solid state, cimetidine, a clinically useful histamine H_2 receptor antagonist, adopts an intramolecularly folded structure stabilized by a hydrogen bond.[2] From

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