Synthesis of Certain 6-(Arylthio)uracils and Related Derivatives as Potential Antiviral Agents

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New series of 6-(arylthio)uracils, 6-(4-substituted-1-piperazinyl)uracils, 2,4,5-trioxo-1*H*,3*H*-benzothiopyrano[2,3-*d*]pyrimidine and 5-aryl-2,4-dioxo-1*H*,3*H*-pyrimido[5,4-*f*]benzo[1,4]thiazepines have been prepared and screened for their *in vitro* activity against herpes simplex-1 virus (HSV-1) and human immunodeficiency virus-1 (HIV-1). The *in vitro* cytotoxic activity was also evaluated. The results of biological testing revealed that compound **5b** showed marginal activity against HSV-1, while compounds **5b** and **5f** exhibited marginal activity against HIV-1. The rest of the tested compounds were found devoid of antiviral activity against both HSV-1 and HIV-1.

Key Words : Substituted uracils, Benzothiopyrano[2,3-*d*]pyrimidine, Pyrimido[5,4-*f*]benzo[1,4]thiazepines, Antiviral activity, Cytotoxicity

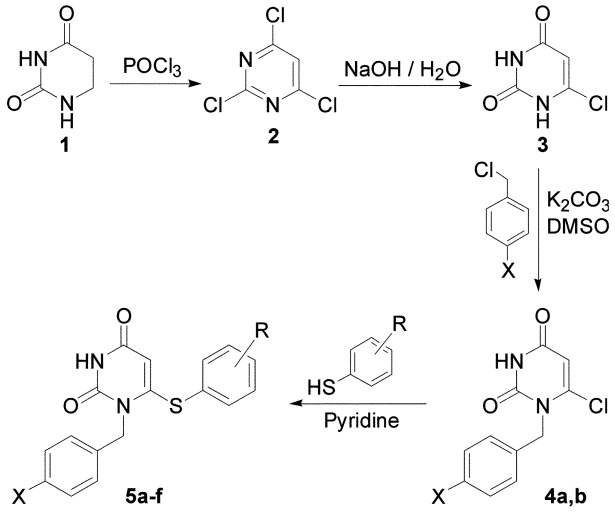
Introduction

Efforts have been directed towards the discovery of chemotherapeutic agents that have antiviral activity especially against human immunodeficiency virus HIV. A large number of pyrimidine nucleosides are clinically useful in the control of retroviral infections.[1-4] In the last decade, Non-nucleoside reverse transcriptase inhibitors appeared to be very promising therapies in the treatment of retroviral diseases. Among the non-nucleoside derivatives, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) and its derivatives which represent a new class of potent anti-HIV agents.[5-12] Potent antiviral activity was also reported among several tricyclic derivatives including dipyridodiazepinones,[13-18] pyridazinobenzodiazepinones,[19,20] dibenzoxazepinones and pyridobenzoxazepinones,[21] and pyrimidobenzothiazepines.[22] In addition, several bis(heteroaryl)piperazine derivatives were introduced as potent antiviral drugs.[23,24] In continuation to our researches in the field of nucleoside and non-nucleoside antiviral agents,[25-28] we rationalized to synthesize new series of 1-substituted-6-(arylthio)uracils, 6-(4-substituted-1-piperazinyl)uracils and the pyrimido tricyclic derivatives 2,4,5-trioxo-1*H*,3*H*-benzothiopyrano[2,3-*d*]pyrimidine and 5-aryl-2,4-dioxo-pyrimido[5,4-*f*]benzo[1,4]thiazepines as potential antiviral agents.

Results and Discussion

Chemical Synthesis. 6-Chlorouracil **3**, the key starting material necessary for this study, was synthesized from barbituric acid **1** *via* reaction with phosphorus oxychloride and dimethylaniline to yield 2,4,6-trichloropyrimidine **2**,[29] which was subsequently hydrolysed with aqueous sodium hydroxide[30] to yield **3**. Interaction of 6-chlorouracil **3** with benzyl chloride or 4-chlorobenzyl chloride in dimethylsulphoxide, in the presence of potassium carbonate yielded the corresponding N^1-substituted derivatives **4a**[31] and **4b**. Interaction of compounds **4a**, **b** with thiophenol, *p*-thiocresol or thiosalicylic acid in pyridine afforded the corresponding 1-(benzyl or 4-chlorobenzyl)-6-(arylthio) uracils **5a-f** (Scheme 1, Table 1).

6-Chlorouracil **3** was similarly reacted with thiosalicylic acid to yield 6-(2-carboxyphenylthio)uracil **6**, which was cyclized to 2,4,5-trioxo-1*H*,3*H*-benzothiopyrano[2,3-*d*] pyrimidine **7** by the action of sulphuric acid at room temperature or *via* heating with polyphosphoric acid (PPA) at 100-120 °C. Treatment of 6-Chlorouracil **3** with certain monosubstituted piperazines in pyridine yielded the



Scheme 1

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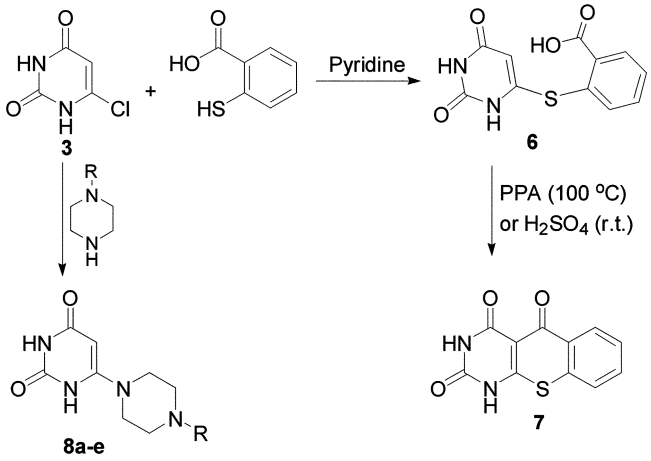
Table 1. Characterization data of compounds **5a-f**

Comp. No.	X	R	M.p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
5a	H	H	175-7	57	$C_{17}H_{14}N_2O_2S$ (310.08)
5b	Cl	H	245-7	52	$C_{17}H_{13}ClN_2O_2S$ (344.82)
5c	H	*p*-CH_3	150-2	43	$C_{18}H_{16}N_2O_2S$ (324.40)
5d	Cl	*p*-CH_3	205-8	42	$C_{18}H_{15}ClN_2O_2S$ (358.84)
5e	H	*o*-COOH	280-1	56	$C_{18}H_{14}N_2O_4S$ (354.38)
5f	Cl	*o*-COOH	240-2	52	$C_{18}H_{13}ClN_2O_4S$ (388.83)

corresponding 6-(4-substituted-1-piperazinyl)uracils **8a-e** (Scheme 2, Table 2).

The reaction of 6-chlorouracil **3** with 2-aminothiophenol in ethanolic potassium hydroxide solution afforded 6-(2-aminophenylthio)uracil **9**,[22] which upon heating with substituted benzaldehydes or 2-thienaldehyde in acetic acid, underwent intramolecular Mannich type cyclization,[32] to yield the corresponding 5-substituted-2,4-dioxo-1*H*,3*H*-pyrimido[5,4-*f*]benzo[1,4]thiazepines **10a-j** (Scheme 3, Table 3).

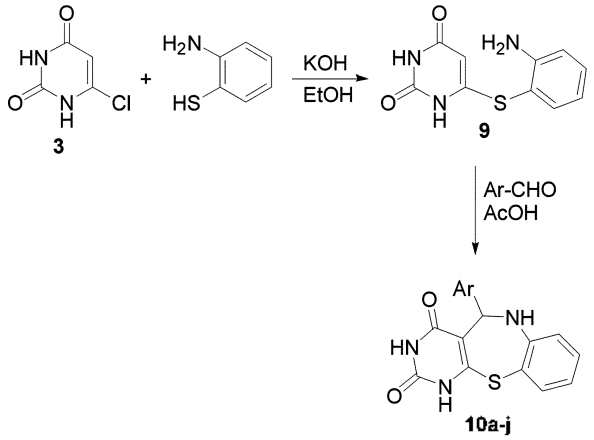


Scheme 2

Table 2. Characterization data of compounds **8a-e**

Comp. No.	R	Cryst. Solvent	M.p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
8a	C_6H_5	AcOH	280-2	43	$C_{14}H_{16}N_4O_2$ (272.30)
8b	$C_6H_5CH_2$	DMF/H_2O	285-8	45	$C_{15}H_{18}N_4O_2$ (286.33)
8c	4-FC_6H_4	EtOH	285-7	53	$C_{14}H_{15}FN_4O_2$ (290.29)
8d	2-$MeOC_6H_4$	EtOH/H_2O	283-5	50	$C_{15}H_{18}N_4O_3$ (302.23)
8e	4-$MeOC_6H_4$	EtOH	220-3	54	$C_{15}H_{18}N_4O_3$ (302.23)

***In vitro* Anti-Herpes Simplex-1 Virus (HSV-1).** The synthesized compounds were tested for their *in vitro* antiviral activity against Herpes simplex-1 virus (HSV-1) grown on Vero African green monkey kidney cells. The antiviral antimitotic antibiotic aphidicolin was used as a positive control.[33] Antiviral activity is defined as confluent, relatively unaltered monolayers of stained Vero cell treated with HSV-1. The cytotoxic activity of the tested compounds was performed using Vero cell culture.[33] Cytotoxicity was estimated as the concentration that caused approximately 50 % loss of the monolayer present around the plaques caused by HSV-1 (Table 4). An improved plaque reduction assay for antiviral activity was used to test the compounds. Plaque reduction assay typically used a monolayer of cultured host



Scheme 3

Table 3. Characterization data of compounds **10a-j**

Comp. No.	Ar	Cryst. Solvent	M.p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
10a	4-ClC_6H_4	Acetone/H_2O	180-3	52	$C_{17}H_{12}ClN_3O_2S$ (357.81)
10b	4-BrC_6H_4	EtOH/H_2O	165-7	45	$C_{17}H_{12}BrN_3O_2S$ (402.27)
10c	4-FC_6H_4	EtOH	155-8	53	$C_{17}H_{12}FN_3O_2S$ (341.36)
10d	2-ClC_6H_4	Acetone/H_2O	152-5	45	$C_{17}H_{12}ClN_3O_2S$ (357.81)
10e	2.6-$Cl_2C_6H_3$	EtOH	159-62	49	$C_{17}H_{11}Cl_2N_3O_2S$ (392.26)
10f	2-Cl.5-$NO_2C_6H_3$	Acetone/AcOEt	170-3	62	$C_{17}H_{11}ClN_4O_4S$ (402.81)
10g	2-HOC_6H_4	EtOH	162-5	34	$C_{17}H_{13}N_3O_3S$ (339.07)
10h	4-$CH_3OC_6H_4$	DMF/H_2O	180-3	55	$C_{18}H_{15}N_3O_3S$ (353.40)
10i	3.4-$(CH_3O)_2C_6H_3$	EtOH	190-2	38	$C_{19}H_{17}ClN_3O_4S$ (383.42)
10j	2-Thienyl	EtOH/H_2O	178-80	45	$C_{15}H_{11}N_3O_2S_2$ (329.40)

Table 4. The cytotoxic and anti-HSV-1 activities of compounds **5a-f**, **7**, **8a-e**, **10a-j** and the antiviral antibiotic aphidicolin

Compound	% Reduction in Number in plaques	Minimum Antiviral Conc (MAC) μM/L	Cytotoxicity (IC_{50}) μM/L[a]
Aphidicolin	100	0.02	0.58
5a	B	C	0.23
5b	30	0.29	0.09
5c	B	C	0.46
5d	B	C	0.61
5e	B	C	0.11
5f	B	C	1.39
7	B	C	0.13
8a	B	C	0.59
8b	B	C	1.05
8c	B	C	0.79
8d	B	C	0.17
8e	B	C	0.56
10a	B	C	0.07
10b	B	C	0.08
10c	B	C	0.06
10d	B	C	0.11
10e	B	C	0.64
10f	B	C	0.45
10g	B	C	0.38
10h	B	C	> 1.70
10i	B	C	> 1.56
10j	B	C	0.36

[a]IC_{50}: The concentration of drug that caused 50% loss of the monolayer present around the plaques. B: 0% Reduction in number of viral plaques. C: Inactive compound.

cells which were allowed to bind virus, then overlayed with a layer of medium thickened with agar or another thickener. Samples to be tested were either incorporated into the thickened layer or absorbed in a paper disc laid on the thickened layer. Abou-Karam and Shier[33] modified this approach to allow the production of acceptable HSV-1 plaques without the use of a thickening. A serial dilutions of samples in 96-plates were used to estimate the end-point concentration for antiviral agents.[34] In the same time, this assay retains the ability to estimate the cytotoxicity which is reflected as loss of the cell monolayer in which the plaques were normally formed. Among the 22 tested compound, compound **5b** showed marginal activity as it reduced the number of the plaques by 30% at a minimum antiviral concentration (MAC) of 0.29 μM/L with cytotoxicity (IC_{50} = 0.09 μM/L). The rest of the compounds did not show any inhibitory effect against HSV-1 (Table 4). The results also revealed that the synthesized compounds showed different levels of cytotoxicity. The highest cytotoxicity was observed in compounds **5b**, **10a**, **10b** and **10c** (IC_{50} < 0.1 μM/L), which are all halogen containing derivatives. On the other hand, the methoxy and dimethoxy derivatives **10h** and **10i** were found to possess the lowest cytotoxic effect (IC_{50} > 1.5 μM/L).

***In vitro* Anti-Human Immunodeficiency Virus-1 (HIV-1).** The procedure used to evaluate the anti-HIV-1 potency is designed to detect agents acting at any stage of the virus reproductive cycle.[35] The assay involves the killing of T4 lymphocytes by HIV-1; compounds that interfere with viral activities will protect cells from cytolysis. The median effective concentration (EC_{50}) of the tested compounds using infected cells was compared with their cytotoxic effect (IC_{50}) in uninfected cultures. Infected and uninfected cultures were incubated without test compounds to serve as controls. Zidovudine (AZT) treated cultures were also used as a positive controls. The screening results (Table 5) revealed that the 4-chlorobenzyl derivatives **5b** and **5f** exhibited marginal activity against HIV-1 with EC_{50} 6.5 and 5.25 μM/L, respectively. Although the effective concentrations of compounds **5b** and **5f** are greatly higher than that of AZT, they seemed to be less cytotoxic. The obtained results proved that N^1 substitution of the uracil nucleus is essential for antiviral activity. The weak activity of compounds **5b** and **5f** and the absence of activity in the other compounds may be attributed to the lack of alkyl substitution in position 5 of the uracil nucleus as compared with the active HEPT derivatives.

Table 5. The cytotoxic and anti-HIV-1 activities of compounds **5a-f**, **8a-e**, **10a** and the antiviral drug Zidovudine (AZT)

Comp.	IC_{50}(μM/L)	EC_{50} (μM/L)	TI_{50}(IC_{50} / EC_{50})
5a	> 100	> 100	> 1.00
5b	> 100	6.5	> 15.38
5c	> 100	> 100	> 1.00
5d	> 100	> 100	> 1.00
5e	> 100	> 100	> 1.00
5f	> 100	5.25	> 19.05
8a	> 100	> 100	> 1.00
8b	> 100	> 100	> 1.00
8c	> 100	> 100	> 1.00
8d	> 100	> 100	> 1.00
8e	> 100	> 100	> 1.00
10a	> 100	> 100	> 1.00
AZT	35.6	0.7	50.86

IC_{50}: 50% inhibitory concentration. (the molar concentration of drug that caused 50% inhibition of cell growth). EC_{50}: 50% effective concentration, (the molar concentration of drug that caused 50% protection against HIV cytopathic effect). TI_{50}: Therapeutic index.

Experimental Section

All melting points (°C) were determined on fisher john melting point apparatus and are uncorrected. Infra red spectra were recorded in KBr disc using a Unicam SP 1000 infrared spectrometer and expressed in wave number (cm^{-1}). ^1H NMR spectra were obtained on a Bruker AC 250 FT NMR spectrometer at 250 MHz, the chemical shifts are expressed in δ units using tetramethylsilan (TMS) as internal reference and DMSO-d_6 or $CDCl_3$ as solvents. Mass spectra were recorded on a Shimadzu GC-Ms-Qp 1000 Ex instrument at 70 eV. Microanalyses (C, H, N, S) were performed in microanalytical unit, Cairo University, and

were in agreement with the proposed structures within ± 0.4% of the theoretical values. The biological medium components were obtained from Sigma Chem. Co., St. Louis, MO, USA.

Chemical Synthesis.

1-(4-Chlorobenzyl)-6-chlorouracil (4b). A mixture of 6-chlorouracil **3** (2.93 g, 0.02 mol), 4-chlorobenzyl chloride (4.83 g, 0.03 mol) and potassium carbonate (1.38 g, 0.01 mol) in dimethyl sulphoxide (20 mL) was stirred at 60-70 °C for 1 hour and 0.1 *N* sodium hydroxide solution (20 mL) was then added to the hot reaction mixture with stirring. The mixture was extracted with benzene (2 × 10 mL) and the aqueous phase was adjusted to pH 2-3 with conc. HCl. The resulting aqueous mixture was refrigerated overnight and the precipitated product was filtered, washed with water, dried and crystallized from methanol/water. Yield 3.8 g (70%). M.p. 179-180 °C. ^{1}H NMR ($CDCl_3$): δ 5.1 (s, 2H, CH_2), 5.9 (s, 1H, C5-H), 7.45 (d, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 11.5 (br s, 1H, NH).

1-(Benzyl or 4-chlorobenzyl)-6-(arylthio)uracils (5a-d). A mixture of compound **4a** or **4b** (0.002 mol) and the appropriate thiophenol (0.002 mol) in dry pyridine (5 mL) was heated under reflux with stirring for 2.5 hours and allowed to stand overnight at room temperature. The mixture was then poured onto ice water (100 mL) and the separated solid product was filtered, washed with water, dried and crystallized from ethanol (Table 1).

5a: IR: 3310 (NH), 1705, 1640 (C=O), 1150 (C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.8 (s, 1H, C5-H), 5.2 (s, 2H, CH_2), 7.2-7.5 (m, 10H, Ar-H), 8.8 (br s, 1H, NH, D_2O-exchange).

5b: IR: 3317 (NH), 1695, 1640 (C=O), 1135 (C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.9 (s, 1H, C5-H), 5.3 (s, 2H, CH_2), 7.1-8.0 (m, 9H, Ar-H), 8.9 (br s, 1H, NH, D_2O-exchange). MS m/z (%): 346 (0.21, M^++2), 344 (0.5, M+), 221 (0.29), 220 (1.47), 219 (2.03), 125 (64.34), 111 (1.19), 109 (13.84), 69 (56.45), 56 (0.51), 45 (100).

5c: ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 4.85 (s, 1H, C5-H), 5.1 (s, 2H, CH_2), 6.8-7.3 (m, 9H, Ar-H), 9.6 (br s, 1H, NH, D_2O-exchange). MS m/z (%): 326 (1.13, M^+ +2), 325 (3.6, M^- +1), 324 (13.25, M^-), 181 (100).

5d: ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 4.7 (s, 1H, C5-H), 5.2 (s, 2H, CH_2), 6.8-7.8 (m, 8H, Ar-H), 9.6 (br s, 1H, NH, D_2O-exchange). MS m/z (%): 360 (2.23, M^+ +2), 359 (1.64, M^+ +1), 358 (5.76, M^-), 125 (100).

1-(Benzyl or 4-chlorobenzyl)-6-(2-carboxyphenylthio)-uracils (5e, f). A mixture of compound **4a** or **4b** (0.002 mol) and thiosalicylic acid (0.31 g, 0.002 mol) in dry pyridine (5 mL) was stirred and heated under reflux for 1 hour. The mixture was poured onto water (50 mL) and acidified with conc. HCl. The separated reddish brown precipitate was filtered, washed with water, dried and crystallized from methanol (Table 1).

5e: ^{1}H NMR (DMSO-d_6): δ 4.95 (s, 1H, C5-H), 5.4 (s, 2H, CH_2), 7.1-7.8 (m, 9H, Ar-H), 8.9 (br s, 1H, NH, D_2O-exchange), 11.3 (s, 1H, COOH, D_2O-exchange).

5f: ^{1}H NMR (DMSO-d_6): δ 4.9 (s, 1H, C5-H), 5.4 (s, 2H, CH_2), 7.2-7.9 (m, 8H, Ar-H), 9.8 (br s, 1H, NH, D_2O-exchange), 11.2 (s, 1H, COOH, D_2O-exchange). MS m/z (%): 390 (0.29, M^- +2), 389 (0.23, M^+ +1), 388 (1.03, M^-), 125 (100).

6-(2-Carboxyphenylthio)uracil (6). A mixture of 6-chlorouracil **3** (2.93 g, 0.02 mol) and thiosalicylic acid (3.1 g, 0.02 mol) in dry pyridine (5 mL) was heated under reflux for 2 hours. The mixture was poured onto cold water (50 mL) and the separated blue precipitate was filtered, washed with water, dried and crystallized from ethanol to yield 4.2 g (80%) of (**6**). M.p. 279-281 °C. IR: 3455 (CO*OH*), 3320 (NH), 1670, 1640 (C=O), 1125 (C-S-C) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 4.9 (s, 1H, C5-H), 7.6-8.1 (m, 4H, Ar-H), 9.8 (br s, 2H, 2NH, D_2O-exchange), 11.5 (s, 1H, COOH, D_2O-exchange).

2,4,5-Trioxo-1*H*,3*H*-benzothiopyrano[2,3-d]pyrimidine (7). *Method A:* 6-(2-Carboxyphenylthio)uracil **6** (1.1 g, 0.004 mol) was suspended in polyphosphoric acid (3 mL) and heated at 100-120 °C for 2 hours. On cooling, the syrupy mixture was treated with ice-water (10 mL) and the separated solid product was filtered, washed with water, dried and crystallized from ethanol to yield 0.54 g (55%) of (**7**). *Method B:* 6-(2-Carboxyphenylthio)uracil **6** (1.1 g, 0.004 mol) was added to concentrated sulphuric acid (5 mL) and allowed to stand at room temperature for 48 hours. The mixture was poured onto crushed ice (50 mL) and stirred for few minutes. The resulted yellowish orange precipitate was filtered, washed with cold water, dried and crystallized from ethanol to yield 0.42 g (43%) of compound (**7**). M.p. 216-218 °C. IR: 3445 (NH), 1676, 1585 (C=O), 1125 (C-S-C) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.8-8.2 (m, 4H, Ar-H), 10.2 (br s, 2H, 2NH, D_2O-exchange).

6-(4-Substituted-1-piperazinyl)uracils (8a-e). A mixture of 6-chlorouracil **3** (0.29 g, 0.002 mol) and the appropriate 4-substituted piperazine (0.002 mol), in dry pyridine (5 mL), was heated under reflux with stirring for 3 hours. On cooling, the mixture was poured onto water (20 mL) and the resulted reddish brown precipitate was filtered, washed with water, dried and crystallized from the appropriate solvent (Table 2).

8a: ^{1}H NMR (DMSO-d_6): δ 3.1-3.3 (m, 8H, 4 CH_2), 4.7 (s, 1H, C5-H), 6.8-7.2 (m, 5H, Ar-H), 10.4 (br s, 2H, 2 NH, D_2O-exchange). MS m/z (%): 273 (3.76, M^- +1), 272 (21.19, M^-), 132 (100).

8b: ^{1}H NMR (DMSO-d_6): δ 3.2-3.35 (m, 8H, 4 CH_2), 4.55 (s, 2H, CH_2), 4.7 (s, 1H, C5-H), 7.2-7.3 (m, 5H, Ar-H), 10.4 (br s, 2H, 2 NH, D_2O-exchange). MS m/z (%): 287 (1.72, M^- +1), 286 (7.66, M^+), 91 (100).

8c: ^{1}H NMR (DMSO-d_6): δ 3.1-3.3 (m, 8H, 4 CH_2), 4.7 (s, 1H, C5-H), 6.9-7.5 (m, 5H, Ar-H), 10.5 (br s, 2H, 2 NH, D_2O-exchange). MS m/z (%): 291 (3.54, M^- +1), 290 (18.89, M^-), 150 (100).

8d: ^{1}H NMR (DMSO-d_6): δ 3.1-3.25 (m, 8H, 4 CH_2), 3.8 (s, 3H, OCH_3), 4.8 (s, 1H, C5-H), 6.9-7.3 (m, 4H, Ar-H), 10.6 (br s, 2H, 2 NH, D_2O-exchange).

8e: ^{1}H NMR (DMSO-d_6): δ 3.1-3.35 (m, 8H, 4 CH_2), 3.8 (s, 3H, OCH_3), 4.75 (s, 1H, C5-H), 6.9-7.3 (m, 4H, Ar-H), 10.5 (br s, 2H, 2 NH, D_2O-exchange).

5-Aryl-2,4-dioxo-1*H*,3*H*-pyrimido[5,4-*f*]benzo[1,4]thi-

azepines (10a-j). A mixture of 6-(2-aminophenylthio)uracil **9** (0.47 g. 0.002 mol) and the appropriate aromatic aldehyde (0.002 mol) in glacial acetic acid (20 mL) was heated under reflux for 24 hours. The mixture was concentrated under reduced pressure. and water (20 mL) was then added to the mixture. The separated solid product was filtered. washed with water. dried and crystallized from the appropriate solvents (Table 3).

10a: ^{1}H NMR (DMSO-d_6): δ 5.7 (d, 1H. C*H*NH), 6.7 (d. 1H, CHN*H*, D_2O-exchange). 7.3-8.81 (m, 8H, Ar-H). 10.9 (br s. 2H. NH, D_2O-exchange). MS m/z (%): 357 (3.63. M^+ -1). 123 (100).

10b: ^{1}H NMR (DMSO-d_6): δ 5.6 (d, 1H, C*H*NH). 6.9 (d. 1H, CHN*H*. D_2O-exchange). 7.2-8.8 (m, 8H. Ar-H). 11.2 (br s. 2H. NH. D_2O-exchange).

10c: ^{1}H NMR (DMSO-d_6): δ 5.6 (d, 1H, C*H*NH), 6.8 (d. 1H, CHN*H*. D_2O-exchange). 7.4-8.5 (m, 8H. Ar-H). 11.4 (br s. 2H. NH, D_2O-exchange). MS m/z (%): 341 (4.34, M^+). 69 (100).

10d: ^{1}H NMR (DMSO-d_6): δ 5.6 (d. 1H. C*H*NH). 6.85 (d. 1H, CHN*H*. D_2O-exchange). 7.3-8.7 (m, 8H. Ar-H). 10.9 (br s. 2H. NH. D_2O-exchange).

10e: ^{1}H NMR (DMSO-d_6): δ 5.7 (d, 1H, C*H*NH), 6.9 (d. 1H, CHN*H*. D_2O-exchange). 7.3-8.8 (m, 7H. Ar-H). 11.3 (br s. 2H. NH. D_2O-exchange).

10f: ^{1}H NMR (DMSO-d_6): δ 5.7 (d, 1H, C*H*NH). 6.95 (d. 1H, CHN*H*. D_2O-exchange). 7.5-8.8 (m, 7H. Ar-H). 11.2 (br s. 2H. NH. D_2O-exchange).

10g: ^{1}H NMR (DMSO-d_6): δ 5.75 (d. 1H. C*H*NH). 6.8 (d. 1H, CHN*H*. D_2O-exchange). 7.4-8.5 (m, 8H. Ar-H). 10.8 (br s. 2H, NH. D_2O-exchange), 11.5 (s. 1H, OH. D_2O-exchange).

10h: ^{1}H NMR (DMSO-d_6): δ 3.8 (s, 3H, OCH_3), 5.65 (d. 1H, C*H*NH). 6.8 (d. 1H, CHN*H*. D_2O-exchange), 7.2-8.1 (m. 8H. Ar-H). 10.6 (br s. 2H, NH. D_2O-exchange).

10i: ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 3H, OCH_3), 3.95 (s. 3H, OCH_3), 5.6 (d, 1H, C*H*NH), 6.8 (d, 1H. CHN*H*. D_2O-exchange). 7.2-8.3 (m, 7H. Ar-H). 10.6 (br s. 2H, NH. D_2O-exchange).

10j: ^{1}H NMR ($CDCl_3$): δ 5.6 (d, 1H, C*H*NH). 6.8 (d, 1H. CHN*H*, D_2O-exchange). 7.0-7.9 (m, 7H, thiophene & Ar-H). 10.8 (br s, 2H, NH. D_2O-exchange).

***In vitro* Anti-Herpes Simplex-1 Virus (HSV-1).** Samples were prepared by dissolving in DMSO and diluting aliquots into sterile culture medium before preparing serial dilution and placed in microtiter trays. Microtiter trays with confluent monolayer cultures of Vero cells were inverted. the medium shaken out. and replaced with serial dilutions of sterile extracts in triplicate in 100 μL medium followed by tittered virus in 100 μL medium containing 10% (v/v) calf serum in each well. In each tray. the last row of wells was reserved for controls that were not treated with compounds or not treated with virus. The tray were cultured and incubated at 37 °C in 5% CO_2 atmosphere for 6 hours. The trays were inverted onto a pad of paper towels. the remaining cells rinsed carefully with medium. and fixed with 3.7% (v/v) formaldehyde in saline for 20 minutes. The fine cells were rinsed with water. and examined visually. Antiviral activity is identified as confluent. relatively unaltered monolayers of stained *Vero* cells treated with HSV-1. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HSV-1 (Table 4).

***In vitro* Anti-Human Immunodeficiency Virus-1 (HIV-1).** Compounds were prepared for assay by dissolving in DMSO then diluted 1 : 100 in cell culture medium before preparing serial dilution and placed in microtiter trays. T4 lymphocytes (CEM cell line) were added and after a brief interval (1 min or more) HIV-1 was added resulting in a 1 : 200 final dilution of each of the tested compounds. Cultures were incubated at 37 °C in 5% CO_2 atmosphere for 6 days. Tetrazolium salt XTT was added to all cells and cultures were incubated to allow formazan color development by virally infected cells. Individual wells were analyzed spectrophotometrically to quantitative formazan production and. in addition, were viewed microscopically for detection of viable cells. Results were compared with controls and zidovudine (AZT) treated wells as a positive control and a determination about activity was made as a percentage protection of T4 cells against HIV-1 cytopathic effect (Table 5).

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