Hesham T. Y. Fahmy[a,b], Sherif A. F. Rostom[a], Manal N. Saudi[a], Jordan K. Zjawiony[b], David J. Robins[c]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt
[b] Department of Pharmacognosy, School of Pharmacy, University of Mississippi, USA
[c] Department of Chemistry, University of Glasgow, Scotland, UK

Synthesis and *in vitro* evaluation of the anticancer activity of novel fluorinated thiazolo[4,5-d]pyrimidines

The synthesis of several thiazolo[4,5-d]pyrimidines containing a fluorophenyl moiety substituted at different positions and through different bridges is described. Twenty new compounds were prepared and evaluated for their anticancer activity using the USA-NCI *in-vitro* screening program. Three compounds were found active and their anticancer activity against 60 human tumor cell lines are described in detail.

Keywords: Thaizole; Fluorinated; Thiazolo[4,5-d]pyrimidines; Anticancer activity

Received: October 15, 2002; Accepted: February 20, 2003 [FP734]
DOI 10.1002/ardp.200300734

Introduction

Thiazolo[4,5-d]pyrimidines can be considered as thia-analogues of the naturally occurring purine bases, adenine and guanine, due to the insertion of a sulphur atom in the place of the nitrogen atom at position 7 of the purine ring. These compounds have acquired a growing importance as anticancer immunotherapeutic agents [1], antiviral agents used in the treatment of human cytomegalovirus (HCMV) [2–6], antitumour [7] and antibacterial agents [8]. Recently they have been found to strongly antagonise the activity of the corticotropin releasing factor (CRF) and thus have the potential to be CNS active agents [9, 10]. Thiazolo[4,5-d]pyrimidines have also been reported to have antidepressive activity [11]. Many of these compounds are therefore of interest to major pharmaceutical companies [3–6, 9–11].

In recent decades, advances in organic fluorine chemistry have been successfully utilized in the field of medicinal chemistry for the development of new drugs [12, 13]. It is hoped that the incorporation of fluorine will result in drugs with increased therapeutic efficacy and improved pharmacological properties. As the second smallest substitute, fluorine most closely mimics hydrogen with respect to steric requirements at enzyme receptor sites. Furthermore, the presence of fluorine often increases the lipid solubility of a drug thereby enhancing its rate of *in vivo* absorption. The high electro negativity of fluorine frequently alters electronic effects, and thereby, chemical reactivity and physical properties of compounds. Fluorine imparts increased oxidative and thermal stability because the C-F bond is stronger than the C-H bond. The therapeutic benefits of different pharmacological classes of drugs, such as anticancer agents, have recently been enriched through the use of fluorinated drugs. Fluoropyrimidines and purine nucleotides such as 5-fluorouracil and Fludarabine are examples of such compounds. Other fluorinated anticancer compounds include Gemcitabine and the antitumor agents ME 2303, Flutamide, and Casodex [13].

Previously, we described methods developed to synthesize and modify the structure of the thiazolo[4,5-d] pyrimidine ring system to obtain biologically active compounds [14–18]. Here we describe structural modifications of the thiazolo[4,5-d] pyrimidine ring system to introduce a fluorophenyl moiety into different positions of the molecule using various bridges. The prepared compounds were then evaluated for their anticancer activity. Three compounds were found to possess anticancer activity using the NCI screening program. The full anticancer activity spectrum of the active compounds is described in detail.

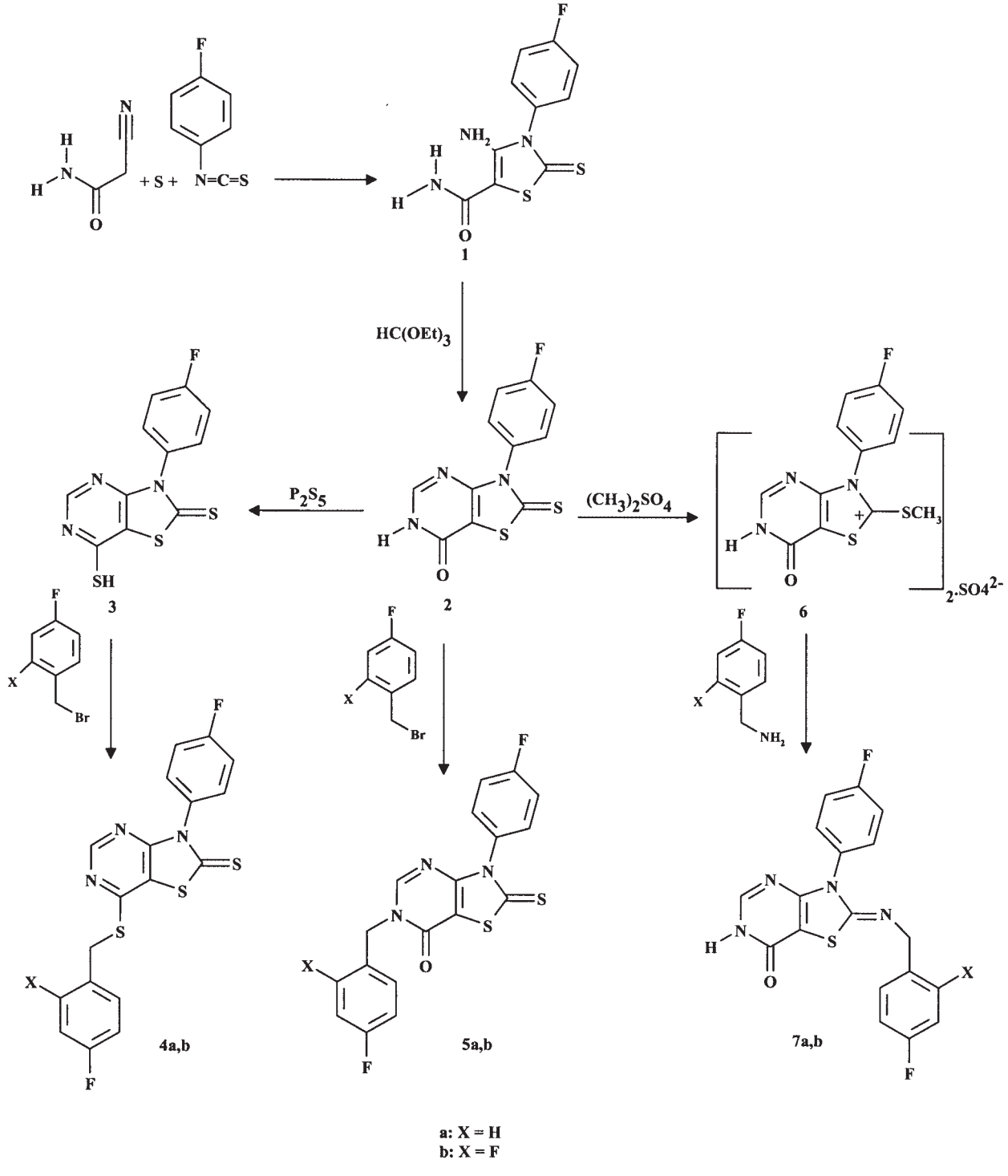
Chemistry

The synthetic pathways utilized to prepare the target compounds are illustrated in Schemes 1–4.

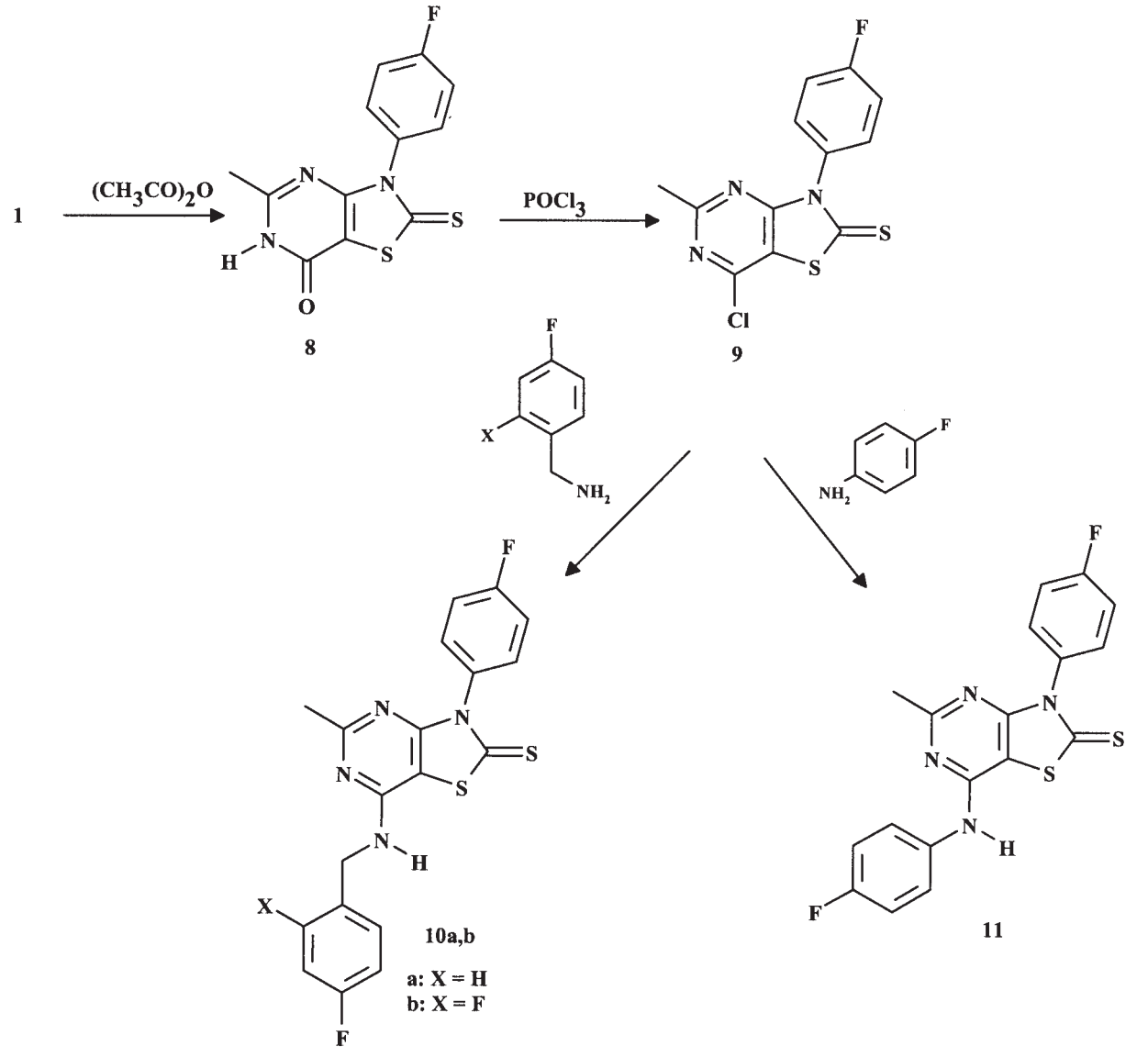
Correspondence: Hesham T. Y. Fahmy, Department of Pharmacognosy, School of Pharmacy, P. O. Box 3479, University of Mississippi, MS 38677, USA. Phone: +1 662 915-7290, Fax: +1 662 915-7026, e-mail: heshamfahmy@hotmail.com

 0365-6233/04–05/0216 $ 17.50+.50/0

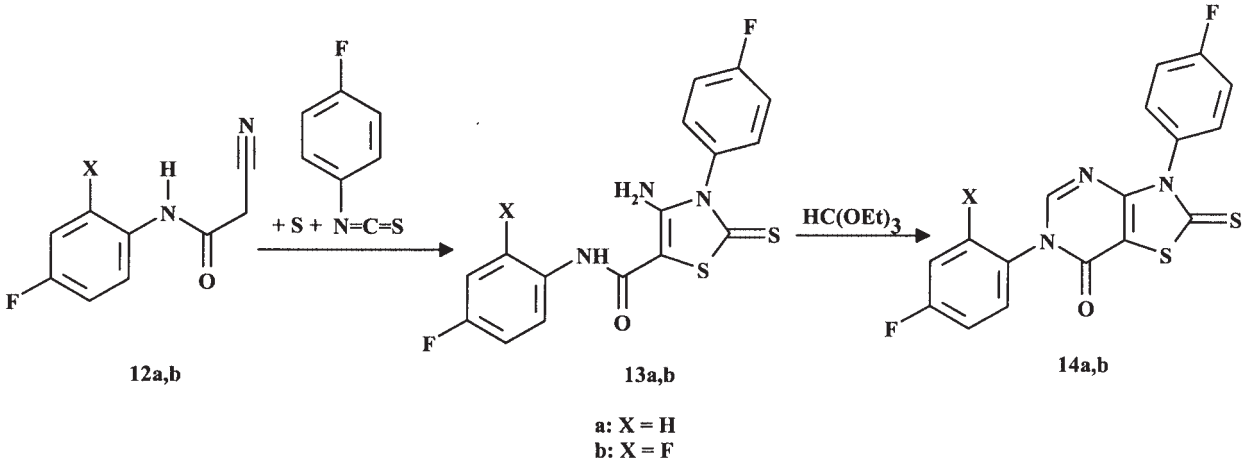
The starting 4-amino-5-carboxamido-2,3-dihydrothiazole-2-thione **1** was prepared form cyanoacetamide, sulphur and 4-fluorophenyl isothiocyanate according to the procedure reported by Gewald [19]. This compound was cyclized to the thiazolo[4,5-d]pyrimidine **2** using a triethylorthoformate/acetic anhydride mixture as described [19]. 7-mercaptothiazolo[4,5-d]pyrimidine **3** was obtained through reaction of **2** with phosphorus pentasulphide, following which S-alkylation was carried out to obtain the 7-fluorobenzylthio derivatives **4a**, **b**. N-



Scheme 1



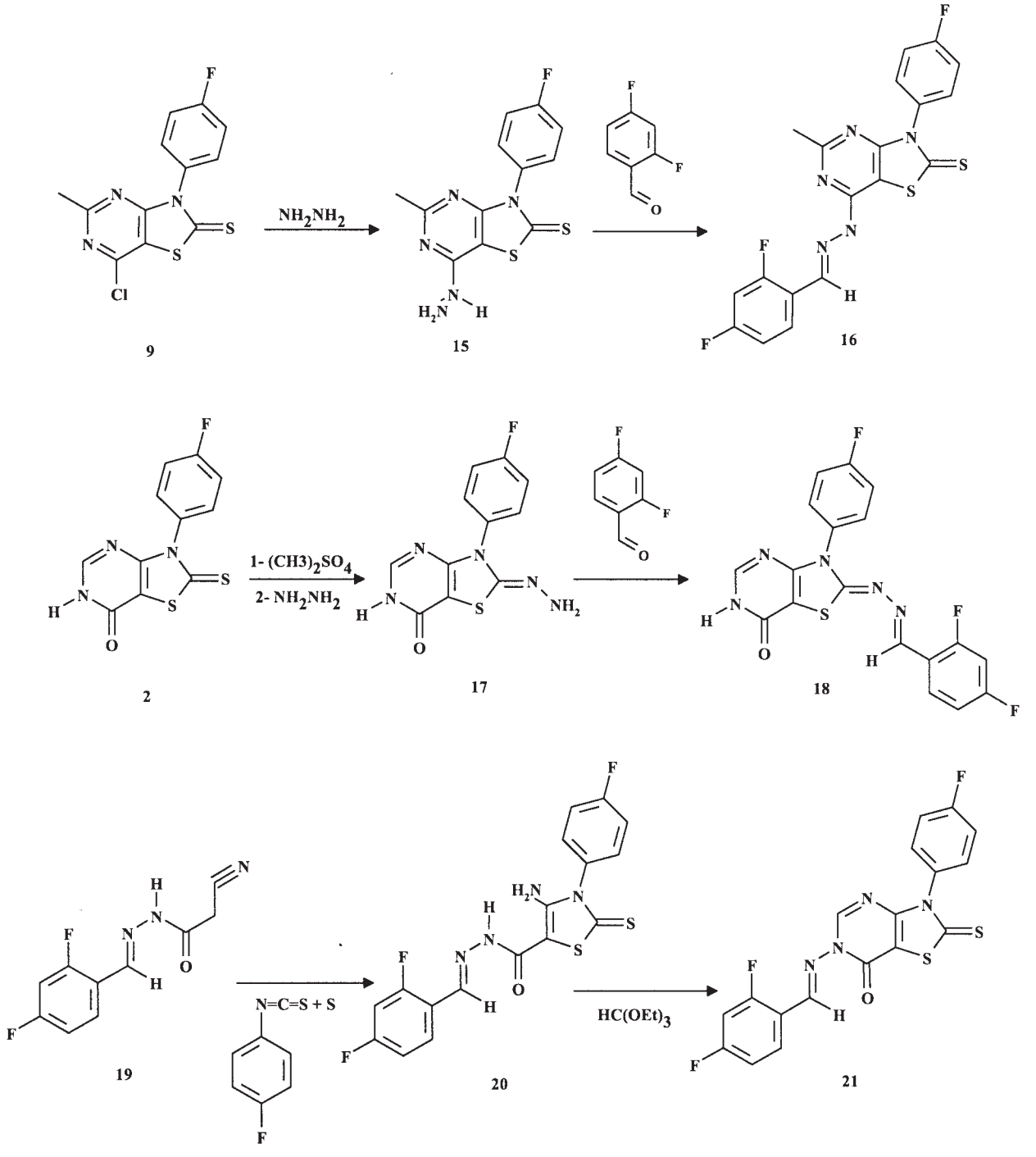
Scheme 2



Scheme 3

alkylation of **2** gave the 6-fluorobenzyl derivatives **5 a**, **b**. Reaction of **2** with dimethylsulphate followed by reaction of the produced 2-methylthiothiazolium salt **6** with fluorobenzyl amines as described [20] gave the 2-fluorobenzyl derivatives **7 a**, **b**.

The second parent thiazolo[4,5-d]pyrimidine **8** was prepared by cyclization of the starting thiazole **1** with acetic anhydride using our reported procedure [15]. Chlorination of **8** with phosphorus oxalyl cholride gave the 7 chlorothiazolo[4,5-d] pyrimdine **9** which upon treatment with fluorobenzyl amines gave the 7-fluorobenzylamines **10 a**, **b**. Treatment of compound **9** with 4-fluoroaniline gave the 7-(4-fluorophenyl)amino derivative **11**, but the reaction required the presence of a basic catalyst such



Scheme 4

as triethylamine to proceed. It is worth mentioning that the 2,4-difluorophenylamine failed to react even in the presence of strong bases. This may be attributed to its poor nucleophilicity due to the electron withdrawing effects of the 2 fluorine atoms.

Another starting thiazole derivative, the 4-amino-5-fluorophenylaminocarbonyl-2,3-dihydrothiazole-2-thione **13** was prepared from fluorophenylamino cyanoacetamides **12 a**, **b** following the same reaction procedure as described for **1**. When **12 a**, **b** were cyclized with orthoformate, they yielded the 3,6-fluoro phenylthiazolo[4,5-d]pyrimidines **14 a**, **b**.

Scheme 4 describes different approaches to prepare thiazolo[4,5-d]pyrimidines derivatives substituted with a 2,4-difluorobenzylideneamino moiety at positions 2, 6 or 7. To prepare 7-(2,4-difluorobenzylidenehydrazinothiazol[4,5-d]pyrimidine **16**, the chloro compound **9** was reacted with hydrazine hydrate to give the 7-hydrazino derivative **15** which was then condensated with 2,4-difluorobenzaldehyde to yield the target compound. The 2-(2,4-difluorobenzylidenehydrazonothiazolo[4,5-d] pyrimidine **18** was prepared from the thiazlo[4,5-d]pyrimidine **2** through its reaction with dimethylsulphate and then reacting the produced 2-methylthio thiazolium salt with hydrazine hydrate to give the 2-hydrazono compound **17** which then under went condensation with 2,4-difluorobenzaldehyde to yield compound **18**. The 6-(2,4-difluorobenzylidineaminothiazolo[4,5-d] pyrimidine **21** was prepared by first generating the 4-amino-5-[2,4-difluorobenzylidenehydrazinecarbonyl-2,3-dihydrothiazole-2-thione **20** which was then cyclized with triethyl orthoformate.

Results and discussion

The prepared compounds were evaluated for their anticancer activities using the NCI in-vitro anticancer screening assay [21–25].

a) Primary anticancer assay (3-cell line/one dose assay):

In this assay, a panel of 3 cell lines consisting of MCF-7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) was used. Each cell line was seeded and pre-incubated on micro titer plates. Test agents were then added at a single concentration and the culture incubated for an additional 48 hours. End point determinations were made with alamar blue. The results for each test agent were re-

Table 1. Anticancer activity of compounds in the 3 cell line/one dose assay.

Compound Number	Concentration	Growth percentage			Activity
		Lung NCI-H460	Breast MCF7	CNS SF-268	
2	100 µM	77	98	94	Inactive
3	100 µM	71	89	97	Inactive
4 a	100 µM	81	93	105	Inactive
4 b	100 µM	79	94	96	Inactive
5 a	100 µM	73	73	74	Inactive
5 b	100 µM	40	78	71	Inactive
7 a	100 µM	90	91	75	Inactive
7 b	100 µM	77	59	80	Inactive
8	100 µM	96	91	96	Inactive
9	**100 µM**	***71***	***1***	***2***	***Active***
10 a	100 µM	82	91	67	Inactive
10 b	100 µM	82	91	67	Inactive
11	**100 µM**	***0***	***0***	***0***	***Active***
14 a	100 µM	67	72	90	Inactive
14 b	100 µM	66	76	92	Inactive
15	100 µM	81	91	103	Inactive
16	100 µM	69	80	68	Inactive
17	100 µM	93	92	112	Inactive
18	100 µM	69	85	93	Inactive
21	**100 µM**	***6***	***2***	***14***	***Active***

ported as the percent of growth of treated cells compared to untreated control cells. Compounds which reduced the growth any of the cell lines to 32 % or less (negative numbers indicate cell kill) were then evaluated for their anticancer activity (over a 5-log dose range) using an extended panel of 60 cell lines.

b) Full anticancer assay:

The NCI's *in vitro* antitumor screen consists of 60 human cell lines derived from nine types of cancer. The anticancer activity of the novel fluorinated thiazolo[4,5-d]pyrimidines were tested at a minimum of five concentrations using 10-fold dilutions (0.01–100 μM) on these cell lines. Cells were exposed to the compounds for 48 hours. Cell viability and growth was then estimated using the sulforhodamine B (SRB) protein assay.

The five concentrations of each compound tested were used to create log concentration-% growth inhibition curves. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value corresponds to the concentration of compound which decreases net cell growth by 50 %. The TGI value is the concentration of compound which results in total growth inhibition and the LC_{50} value is the concentration of compound which causes a net 50 % loss of initial cells at the end of the incubation period (48 h). Sub-panel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the sub-panel or the full panel, respectively [21].

In the present study, the prepared compounds were evaluated for their antitumor activity using the NCI screening program [21–25]. Initially, they were evaluated using the 3-cell line/one dose assay, the results of which are presented in Table 1. Three compounds (**9**, **11**, and **20**) were considered active in the initial screen.

The anticancer activity of these compounds was then evaluated using an extensive panel of 60 human cell lines. The compounds showed variable antitumor activities against most of the tested sub-panel tumor cell lines as evaluated by the calculated GI_{50} values. The median growth inhibitory concentration (the average sensitivity of each sub-panel towards each of the test compounds) and the full panel mean graph mid point (MG-MID) (the average of all cell lines towards each of the test compounds) for the sub-panel tumor cell lines are reported in Table 2. Compound **9** displayed good antitumor activity against all of the cell lines used in the screen which GI_{50} values of 0.004 μM/mL (ovarian cancer) and 0.003 μM/mL (prostate cancer). Compounds **11** and **21** were less active with GI_{50} values of 0.054–0.144 μM/mL and 0.021–0.038 μM/mL, respectively.

The ratio obtained by dividing a compound's full panel MG-MID (μM) by its individual sub-panel MG-MID (μM) is considered a measure of its selectivity. Ratios between 3 and 6 indicate a compound is moderately selective whereas ratios greater than 6 indicate high selectivity towards the corresponding cell line. Compounds with ratios below 3 are considered non-selective [24]. With the exception of compound **11**, which was highly selective against Colon cancer (selectivity ratio of 6), the active compounds in the present study proved to be non-selective against the nine tumor sub-panels tested.

Table 2. Median growth inhibitory concentration (GI_{50}) and full panel mean graph mid points (MG-MID) for sub-panel tumor cell lines.

Sub-panel Tumor cell line	Compound number **9**	**11**	**21**
Leukemia	0.014	0.042	0.133
Lung cancer	0.031	0.034	0.067
Colon Cancer	0.014	0.023	0.181
CNS cancer	0.014	0.036	0.050
Melanoma	0.018	0.031	0.078
Ovarian cancer	0.004	0.041	0.060
Renal cancer	0.014	0.037	0.050
Prostate cancer	0.034	0.043	0.060
Breast cancer	0.034	0.031	0.110
MG-MID	**0.013**	**0.145**	**0.073**

Acknowledgments

The authors are grateful to the staff of the Department of Health and Human Services, National Cancer Institute, Bethesda, Maryland, USA for the anticancer evaluation.

Experimental

Melting points were determined in open-glass capillaries using a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were determined with a Perkin-Elmer 1430 spectrophotometer using the KBr disc technique. The ^{1}H-NMR (δ-ppm) spectra were recorded on a Bruker (400 MHz) spectrometer using tetramethylsilane as the internal standard and $CDCl_3$ as the solvent. The elemental analyses were performed using a Perkin-Elmer RE 2400 C H N S Analyzer. All values for C, H, N and S are within ± 0.4 % of the calculated data.

4-Amino-5-carbamoyl-3-(4-fluorophenyl)thiazole-2(3H)thione (**1**)

4-Fluorophenyl isothiocyanate (7.65 g, 50 mmol) was added to a solution of 2-cyanoacetamide (4.2 g, 50 mmol), sulphur (1.6 g, 50 mmol) and triethylamine (6 mL) in ethanol (50 mL). The reaction mixture was heated under reflux for 1 h during which the product partially crystallized. After cooling, the product was filtered, washed with cold ethanol, dried and recrystal-

Table 3. Yields, melting points, molecular formulae and molecular weights of newly prepared thiazoles intermediates.

Compound No	Yield (%)	M.P. (C)	Mol. formula	Mol. Weight
1	85	270–272	$C_{10}H_8N_3FOS_2$	269.32
13 a	78	250–252	$C_{16}H_{11}N_3F_2OS_2$	363.41
13 b	72	260–262	$C_{16}H_{10}N_3F_3OS_2$	381.40
20	83	280–282	$C_{17}H_{11}N_4F_3OS_2$	408.43

Results for C, H, N, S are within ± 0.4 % of the theoretical values for the formulae given.

Table 4. Yields, melting points, molecular formulae and molecular weights of newly prepared thiazolo[4,5-d]pyrimidines.

Compound No	Yield (%)	M.P. (C)	Mol. formula	Mol. Weight
2	85	338–340	$C_{11}H_6N_3FOS_2$	279.32
3	80	325–327	$C_{11}H_6N_3FS_3$	263.32
4 a	87	237–239	$C_{18}H_{11}N_3F_2S_3$	403.50
4 b	82	239–241	$C_{18}H_{10}N_3F_3S_3$	421.49
5 a	77	220–222	$C_{18}H_{11}N_3F_2OS_2$	387.43
5 b	74	223–225	$C_{18}H_{10}N_3F_3OS_2$	405.42
7 a	72	312–314	$C_{18}H_{12}N_4F_2OS$	370.38
7 b	70	320–322	$C_{18}H_{11}N_4F_3OS$	388.37
8	88	385–387	$C_{12}H_8N_3FOS_2$	293.34
9	78	260–262	$C_{12}H_7N_3ClFS_2$	311.79
10 a	72	305–307	$C_{19}H_{14}N_4F_2S_2$	400.48
10 b	70	288–290	$C_{19}H_{13}N_4F_3S_2$	418.46
11	73	285–287	$C_{18}H_{12}N_4F_2S_2$	386.45
14 a	74	236–238	$C_{17}H_9N_3F_2OS_2$	373.41
14 b	72	212–214	$C_{17}H_8N_3F_3OS_2$	391.40
15	70	344–346	$C_{12}H_{10}N_5FS_2$	307.37
16	80	275–277	$C_{19}H_{12}N_5F_3S_2$	431.46
17	72	>400	$C_{11}H_8N_5FOS$	277.28
18	83	365–367	$C_{18}H_{10}N_5F_3OS$	401.37
21	85	245–247	$C_{18}H_9N_4F_3OS_2$	418.42

Results for C, H, N, S are within ± 0.4 % of the theoretical values for the formulae given.

lized from glacial acetic acid (Table 3). IR 3350, 3250 (N-H), 1660 (C=O), 1590, 1510 (C=C), 1570 (δNH), 1533, 1240 , 1085, 940 (N-C=S), 1215, 1020 (C-S-C) cm^{-1}.

3-(4-Fluorophenyl)-thiazolo[4,5-d]pyrimidin-7(6H)-one-2(3H)-thione (**2**)

A solution of compound **1** (2.96 g, 10 mmol) in a mixture of triethyl orthoformate and acetic anhydride (20 mL, 1:1) was heated under reflux for 1 h. After cooling, the crystalline product was filtered, washed with cold ethanol, dried and recrystallized from glacial acetic acid (Table 4). IR 3162 (N-H), 1681 (C=O), 1630 (CN), 1579, 1510 (C=C), 1533, 1240, 1085 (N-C=S), 1215 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.19–7.27 (m, 4 H, Ar-H), 7.92 (s, 1H, C_5-H).

3-(4-Fluorophenyl)-7-mercaptothiazolo[4,5-d]pyrimidine-2(3H)-thione (**3**)

A mixture of **2** (2.97 g, 10 mmol) and phosphorous pentasulphide (4.44 g, 10 mmol) in dry xylene (20 mL) was heated under reflux for 5 h and then cooled. The product obtained after addition of petroleum ether was filtered, washed with ethanol, dried and recrystallized from DMF (Table 4). IR 3119 (N-H), 1635 (CN), 1584, 1507 (C=C), 1552 (δ N-H), 1427, 1263, 1165 (N-C=S), 1234 (C-S-C) cm^{-1}. H^1-NMR ($CDCl_3$): 7.28–7.39 (m, 4H, Ar-H), 8.88 (s, 1H, C_5-H).

3-(4-Fluorphenyl)-7-(4-fluorobenzyl or 2,4-difluorobenzyl)-mercaptothiazolo[4,5-d] pyrimidine-2(3H)- thiones (**4 a**, **b**)

Fluorobenzyl bromide (10 mmol) was added to a mixture of **3** (2.63 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) in dry acetone (20 mL). The reaction mixture was heated under reflux for 3 h and then cooled. The crystalline product was filtered, washed with cold ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 1650 (C=N), 1604, 1511 (C=C), 1557, 1264, 1090, 841 (N-C=S), 1232, 1038 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 4 a: 4.63 (s, 2 H, Benzyl-CH_2), 7.00–7.44 (m, 8 H, Ar-H), 8.70 (s, 1 H, C_5-H). 4 b: 4.56 (s, 2 H, Benzyl-CH_2), 6.73–7.44 (m, 7 H, Ar-H), 8.65 (s, 1 H, C_5-H).

3-(4-Fluorophenyl)-6-(4-Fluorobenzyl or 2,4-difluorobenzyl)-thiazolo[4,5-d] pyrimidine-2(3H)thiones (**5 a**, **b**)

Fluorobenzyl bromide (10 mmol) was added to a solution of **2** (2.79 g, 10 mmol) and an equimolar amount of potassium hydroxide (0.6 g) in ethanol (20 mL). The reaction mixture was heated under reflux for 5 h and then cooled. Water (10 mL) was added and the product obtained was filtered, washed with cold ethanol, dried and recrystallized from acetone (Table 4). IR 1697–1692 (C=O), 1640 (CN), 1604, 1512-1509 (C=C), 1252–1248, 1101, 852 (N-C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 5 a: 5.07 (s, 2 H, Benzyl-CH_2), 6.92–7.30 (m, 8 H, Ar-H), 7.94 (s, 1 H, C_5-H). 5 b: 5.17 (s, 2 H, Benzyl-CH_2), 6.84–7.64 (m, 7 H, Ar-H), 8.20 (s, 1 H, C_5-H).

3-(4-Fluorophenyl)-2-(4-fluorobenzylidine or 2,4-difluorobenzylidene)thiazolo [4,5-d]pyrimidine-2(3H)-thiones (**7 a**, **b**)

Dimethyl sulfate (1.89 g, 1.42 mL, 15 mmol) was added to a solution of **2** (2.79 g, 10 mmol) in acetonitrile (20 mL). The reaction mixture was heated under reflux for 1 h and then cooled. The selected fluorobenzyl amine (10 mmol) was then added. The reaction mixture was heated under reflux for 1 h and cooled. The separated white crystalline product was filtered, washed with cold ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3500 (N-H), 1694–1680 (C=O), 1656–1648 (C=N), 1610–1604, 1514–1512 (C=C), 1535, 1256, 1159, 825 (N-C=S), 1234 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7 a: 4.51 (s, 2 H, Benzyl-CH_2), 7.03–7.55 (m, 8 H, Ar-H), 8.25 (s, 1 H, C_5-H). 7 b: 4.56 (s, 2 H, Benzyl-CH_2), 7.02–7.49 (m, 7 H Ar-H), 8.00 (s, 1 H, C_5-H).

3-(4-Fluorophenyl)-5-methyl-thiazolo[4,5-d]pyrimidin-7(6H)-one-2(3H)-thione (**8**)

A solution of **1** (2.69 g, 10 mmol) in acetic anhydride (20 mL) was heated under reflux for 3 h during which the product partially crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3100–3000 (N-H), 1676 (C=O), 1640 (CN), 1584, 1510 (C=C), 1554 (δ N-H), 1254, 1090 (N-C=S), 1230, 1048 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.4 (s, 3 H, CH_3), 7.18–7.27 (m, 4 H, Ar-H).

7-Chloro-3-(4-fluorophenyl)-5-methylthiazolo[4,5-d]pyrimidine-2(3H)-thione (**9**)

A solution of **8** (2.93 g, 10 mmol) in phosphorus oxalyl chloride (20 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was poured into ice-water (100 mL). The obtained product was filtered, washed with aqueous ethanol , dried and recrystallized from ethanol (Table 4). IR 1650 (CN), 1590, 1510 (C=C), 1552, 1245, 1090, 920 (N-C=S), 1230, 1032, 1036 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.5 (s, 3 H, CH_3), 7.18–7.28 (m, 4 H, Ar-H).

3-(4-Fluorophenyl)-7-(4-fluorobenzyl or 2,4-difluorobenzyl)-5-methylthiazolo[4,5-d]pyrimidines (**10 a**, **b**)

The selected fluorobenzyl amine (20 mmol) was added to a solution of **9** (3.11 g, 10 mmol) in ethanol (20 mL). The reaction mixture was heated under reflux for 3 h during which the product partially crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR) 1645 (CN), 1602, 1510 (C=C), 1535, 1260, 1100 (N-C=S), 1224, 1041 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 10 a: 2.40 (s, 3 H, CH_3), 3.60 (t, 3 H, NH), 4.72 (d, 2 H, Benzyl-CH_2), 6.76–7.26 (m, 8 H, Ar-H). 10 b: 2.41 (s, 3 H, CH_3), 3.55 (t, 1 H, NH), 4.67 (d, 2 H, Benzyl-CH_2), 6.98–7.30 (m, 7 H, Ar-H).

3-(4-Fluorphenyl)-7-(4-fluorophenylamino)-5-methylthiazolo[4,5-d]pyrimidine-2(3H)-thione (**11**)

4-Fluoroaniline (1.11 g, 10 mmol) and triethylamine (0.5 mL) were added to a solution of **9** (3.11 g, 10 mmol) in ethanol (20 mL). The reaction mixture was heated under reflux for 3 h during which the product partially crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3178 (N-H), 1645 (CN), 1580, 1510 (C=C), 1563 (δ N-H), 1539, 1244, 1157, 920 (N-C=S), 1220, 1049 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.55 (s, 3 H, CH_3), 7.17–7.43 (m, 8 H, Ar-H).

4-Amino-3-(4-fluorophenyl)65-(4-fluorophenyl, or 2,4-fluorophenylaminocarbonyl thiazol-2(3H)-thiones (**13 a**, **b**)

Compounds 13 a and b were prepared from N(fluorophenyl)cyanoacetamides **12 a**, **b** (10 mmol), finely divided sulphur (0.32 g, 10 mmol), 4-fluorophenyl isothiocyanate (1.53 g, 10 mmole) as described for compound 1. The product obtained was filtered, washed with cold ethanol, dried and recrystallized from ethanol (Table 3). IR: 3350, 3250 (N-H), 1670 (C=O), 1635 (C=N), 1602, 1509 (C=C), 1560 (δNH), 1550, 1295, 1080, 890 (N-C=S), 1234, 1025 (C-S-C).

3,6-Di(4-fluorophenyl)-thiazolo[4,5-d]pyrimidin-7(6H)-one-2(3H)-thione (**14 a**) *and 3-(4-fluorophenyl)-6-(2,4-difluorophenyl)-thiazolo[4,5-d]pyrimidin-7(6H)-one-2(3H)-thione* (**14 b**)

A solution of **13 a**, **b** (10 mmol) in a mixture of triethylorthofortate (10 mL) and acetic anhydride (10 mL) was heated under reflux for 3 h and then cooled. The product obtained was filtered, washed with cold ethanol, dried and recrystallized from ethanol (Table 4). IR 1692-1681 (C=O), 1650 (C=N), 1613–1604, 1510 (C=C), 1256, 844 (N-C=S), 1222, 1048 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 14 a: 7.25–7.54 (m, 8 H, Ar-H), 8.1 (s, 1 H, C_5-H), 14 b: 7.08–7.45 (m, 7 H, Ar-H), 7.95 (s, 1 H, C_5-H).

3-(4-Fluorophenyl)-7-hydrazino-5-methylthiazolo[4,5-d]pyrimidine-2(3H)-thione (**15**)

Hydrazine hydrate (1.0 g, 20 mmol) was added to a solution of **9** (3.11 g, 10 mmol) in ethanol (20 mL),. The reaction mixture was heated under reflux for 1 h during which the product crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3300, 3250 (N-H), 1640(CN), 1603, 1513 (C=C), 1570 (δ N-H), 1534, 1304, 1091, 940 (N-C=S), 1047 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.39 (s, 3 H, CH_3), 4.31 (bs, 2 H, NH_2), 7.19–7.26 (m, 4 H, Ar-H), 10.4 (bs, 1 H, NH).

3-(4-Fluorophenyl)-7-(2,4-difluorobenzylidenehydrazino-5-methylthiazolo[4,5-d] pyrimidine-2(3H)-thione (**16**)

2,4-Difluorobenzaldehyde (1.42 g, 10 mmol) was added to a solution of **15** (3.07 g, 10 mmol) in glacial acetic acid,. The reaction mixture was heated under reflux for 2 h during which the product crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF(1:9) (Table 4). IR 1640 (CN), 1598, 1512 (C=C), 1575, 1281, 1090, 850 (N-C=S), 1238, 1050 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.41 (s, 3 H, CH_3), 6.7–7.9 (m, 7 H, Ar-), 8.20 (s, 1 H, H-C=N).

3-(4-Fluorophenl)-2-hydrazonothiazolo[4,5-d]pyrimidine-7(6H)-one (**17**)

Compound **2** (2.79 g, 10 mmol) was reacted with dimethyl sulphate to form the 2-methylthiazolium salt 6 as described for compounds 7 a, b. Methanol (5 mL) was added to the thiazolium salt suspension in acetonitrile. The reaction mixture was poured into a solution of hydrazine hydrate (0.5 g, 10 mmole) in methanol (10 mL) while stirring. It was stirred for 2 h following which the obtained product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3337, 3199 (N-H), 1680 (C=O), 1648 (C=N), 1590, 1511 (C=C), 1535, 1261, 1160, 830 (N-C=S), 1224, 1099 (C-S-C) cm^{-1}.

3-(4-Fluorophenyl)-2-(2,4-difluorobenzylidenehydrazono)thiazolo[4,5d] pyrimidine -7(6H)-thione (**18**)

2,4-Difluorobenzaldehyde (1.42 g, 10 mmol) was added to a solution of **17** (2.77 g, 10 mmol) in glacial acetic acid (20 mL). The reaction mixture was heated under reflux for 2 h during which the product crystallized. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from aqueous DMF (1:9) (Table 4). IR 3077 (N-H), 1678 (C=O), 1620 (C=N), 1596, 1511 (C=C), 1554 (δ N-H), 1532, 1270, 1097, 828 (N-C=S), 1232, 1097 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 6.94–8.20 (m, 7 H, Ar-H), 8.3 (s, 1 H, C_5-H), 8.5 (s, 1 H, H-C=N).

4-Amino-3-(4-fluorophenyl)-6-(2,4-difluorobenzylidenehydrazinocarbonyl)thiazole-2(3H)-thione (**20**)

Compound **20** was prepared from 2-(2,4-difluorobenzylidene-hydrazinocarbonyl) acetonitrile **19** (2.23 g, 10 mmol), sulphur (0.32 g, 10 mmol) and 4-fluorophenyl isothiocyanate (1.53, 10 mmol) as described for compound 1 and was recrystallized from glacial acetic acid (Table 3). IR: 3350, 3250 (N-H), 1660 (C=O), 1630 (C=N), 1600, 1510 (C=C), 1570 (δNH), 1550, 1290, 1075, 870 (N-C=S), 1230, 1020 (C-S-C). ^{1}H-NMR ($CDCl_3$): 7.20–7.80 (m, 4H, Ar-H), 7.9 (s, 1 H, H-C=N).

6-(2,4-Difluorobenzylideneamino)-3-(4-fluorophenyl)-thiazolo[4,5-d]pyrimidin-7(6H)-one-2(3H)-thione (**21**)

Compound **21** was prepared from 20 (4.08 g, 10 mmol) and triethyl orthoformate/acetic anhydride mixture as described for compound **2**. The product was recrystallized from aqueous DMF (1:9) (Table 4). IR 1695 (C=O), 1646 (C=N), 1613, 1516 (C=C), 1554, 1288, 1092, 856 (N-C=S), 12737, 1047 (C-S-C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 6.92–8.1 (m, 7 H, Ar-H), 8.40 (s, 1 H, C_5-H), 9.70 (s, 1 H, H-C=N).

References

[1] K. Nagahara, J. D. Anderson, G. D. Kini, N. K. Dalley, S. B. Larson, D. F. Smee, A. Jin, B. S. Sharma, W. B. Jolley, R. K. Robins, *J. Med. Chem.* **1990**, *33*, 407–415.

[2] G. D. Kini, J. D. Anderson, Y. S. Sanghvi, A. F. Lewis, D. F. Smee, G. R. Revankar, R. K. Robins, H. B. Cottam, *J. Med. Chem.* **1991**, *34*, 3006–3010.

[3] A. F. Lewis, G. R. Revankar, S. M. Fennewald, J. H. Huffman, R. F. Rando, *J. Hetrocycl. Chem.* **1995**, *32*, 547–556.

[4] B. K. Bhattacharya, T. S. Rao, A. F. Lewis, G. R. revankar, Y. S. Sanghvi, R. K. Robins, *J. Heterocycl. Chem.* **1993**, *30*, 1341–1349.

[5] G. R. Revankar, J. O. Ojwang, S. D. Mustain, R. F. Rando, E. De Clercq, J. H. Huffman, J. C. Drach, J. P. Sommadossi, A. F. Lewis, *Antiviral Chemistry and Chemotherapy* **1998**, *9*, 53–63.

[6] A. F. Lewis, J. C. Drach, S. M. Fennewald, J. H. Huffman, R. G. Ptak, J. P. Sommadossi, G. R. Revankar, R. F. Rando, *Antiviral Chemistry and Chemotherapy* **1994**, *38*, 2889–2895.

[7] R. K. Robins, H. B. Cottam, PCT Int. Appl. WO 8905649 **1989** [Chem. Abstr. **1990**, *112*, P56584s].

[8] H. Urgun, A. Balkan, M. Ozalp, *Arzneimittel-Forschung* **2000**, *50*, 1115–1119.

[9] J. P. Beck, M. A. Curry, R. J. Chorvat, L. W. Fitzgerald, P. J. Gillian, R. Zaczek, G. L. Trainer, *Bioorganic and Medicinal Chemisty Letters* **1999**, *9*, 1185–1188.

[10] J. P. Beck, PCT Int. Appl. WO 9951608 **1999** [Chem. Abstr. **1999**, *131*, P286529z].

[11] A. Berger, E. E. Borgeas, Ger. Offen. DE 2038922 **1971** [Chem. Abstr. **1971**, *74*, P125722w].

[12] H. Y. Aboul-Enein, *Toxicological and Environmental Chemistry* **1991**, *29*, 235–249.

[13] W. Hertel, R. J. Ternansky, **1993**, *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications, Filler, R., Yagupolskii, L. M., Elsevier Science Publishers, Amsterdam,* P1–5 and 23–71.

[14] E.A. Badawey, S. M. Rida, A. A. Hazza, H. T. Fahmy, Y. M. Gohar, Eur. *J. Med.Chem.* **1993**, *28*, 91–96.

[15] E.A. Badawey, S. M. Rida, A. A. Hazza, H. T. Fahmy, Y. M. Gohar, *Eur. J. Med.Chem.* **1993**, *28*, 97–101.

[16] N. S. Habib, S. M. Rida, E.A. Badawey, H. T. Fahmy, H. A. Ghozlan, *Eur. J. Med.Chem.* **1997**, *32*, 759–762.

[17] N. S. Habib, S. M. Rida, E. A. Badawey, H. T. Fahmy, *Monatshefte für Chemie* **1996**, *127*, 1203–1207.

[18] N. S. Habib, S. M. Rida, E. A. Badawey, H. T. Fahmy, *Monatshefte für Chemie* **1996**, *127*, 1209–1214.

[19] K. Gewald, *J. Prakt. Chem.* **1966**, *32*, 26–30.

[20] K. Gewald, U. Hain, P. Hartung, *Monatshefte für Chemie* **1981**, *112*, 1393–1399.

[21] M. R. Grever, S. A. Schepartz, B. A. Chabner, *Seminars Oncology,* **1992**, *19*, 622–638.

[22] M. R. Boyd, K. D. Paull, *Drug Dev. Res.* **1995**, *34*, 91–109.

[23] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Jangley, P. Cronisie, A. Viagro-Wolf, M. Gray-Goodrich, H. Campell, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

[24] E. M. Acton, V. L. Narayanan, P. A. Risbood, R. H. Shoemaker, D. T. Vistica, M. R. Boyed, *J. Med. Chem.* **1994**, *37*, 2185–2189.

[25] K. D. Paull, R. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, M. R. Boyd, *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.