CYTOSTATIC TETRAZOLE–BUTENOLIDE CONJUGATES: LINKING TETRAZOLE AND BUTENOLIDE RINGS VIA STILLE COUPLING AND BIOLOGICAL ACTIVITY OF THE TARGET SUBSTANCES

Vojtěch BALŠÁNEK[a], Lucie TICHOTOVÁ[b1], Jiří KUNEŠ[b2], Marcel ŠPULÁK[b3], Milan POUR[b4,*], Ivan VOTRUBA[c] and Vladimír BUCHTA[d]

[a] *RE&D VUFB Ltd., Poděbradská 56/186, 180 66 Prague 9, Czech Republic; e-mail: balsanek@readvufb.cz*

[b] *Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 03 Hradec Králové, Czech Republic; e-mail: [1] lucie.tichotova@faf.cuni.cz, [2] jiri.kunes@faf.cuni.cz, [3] marcel.spulak@faf.cuni.cz, [4] milan.pour@faf.cuni.cz*

[c] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic; e-mail: votruba@uochb.cas.cz*

[d] *Department of Clinical Microbiology, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: buchta@fnhk.cz*

Received March 18, 2009
Accepted May 23, 2009
Published online July 24, 2009

Dedicated to Dr. Alfred Bader on the occasion of his 85th birthday.

A series of tetrazoles linked to the butenolide core via benzene rings were prepared by the Stille coupling reaction of α-(tributylstannyl)butenolides and 5-(alkylsulfanyl)-1-(4-iodophenyl)tetrazoles, and the compounds were tested for antifungal and cytostatic activity. Interesting antifungal activities against the filamentous strain *Absidia corymbifera*, and cytostatic activities against leukemic cells HL-60 and CCRF-CEM were found. The cytostatic activity requires the presence of both the butenolide ring and the alkylsulfanyl group bound to tetrazole ring. In addition, the feasibility of Pd-coupling reactions with 5-iodotetrazoles was evaluated.

Keywords: Pd catalysis; Cross-coupling reactions; Heterocycles; Tetrazoles; Butenolides; Dihydrofuranes; Antifungals; Biological activity.

Tetrazoles, a class of nitrogen-containing heterocycles, attract considerable attention of medicinal chemists. The tetrazole ring can serve as a metabolically stable surrogate for carboxylic group; tetrazole-containing antihypertensives of the sartan type (such as **1**) are good examples[1] of a successful employment of tetrazoles as parts of medicinal agents (Chart 1). Even

doi:10.1135/cccc2009040

though the tetrazole moiety as such is not associated with any specific biological activity, several recent reports have described antituberculous activity of 1-aryl-5-(benzylsulfanyl)tetrazoles[2,3]. Several years ago, we described the antifungal activity of a wide variety of 3,5-disubstituted-2,5-dihydrofuran-2-ones[4–7], including those bearing a heteroaryl substituent[8] at C3 of the furanone ring (**2**). In conjunction with these studies, we were also interested in substituting the 2,5-dihydrofuranone ring with a tetrazolyl moiety in position 3 (**3**), and evaluating antifungal properties of the compounds. Given the structure of the target compounds, a Pd-mediated coupling process was an obvious choice of strategy. Since the coupling reactions of tetrazoles have not been systematically investigated, preparation of tetrazolyl-substituted butenolides would also contribute to exploration of the potential of Pd-catalyzed procedures in the synthesis of 1,5-disubstituted tetrazoles. An alternative strategy for the preparation of compounds **3**, i.e. building the tetrazole ring via a 1,3-dipolar addition of azide anion across the cyano group appears less advantageous, since this would mean the necessity of preparation of α-cyanobutenolides and treatment of the compounds (good Michael acceptors) with a strongly nucleophilic azide anion. In this report, we wish to give an account of our work including the biological activities of the substances prepared.

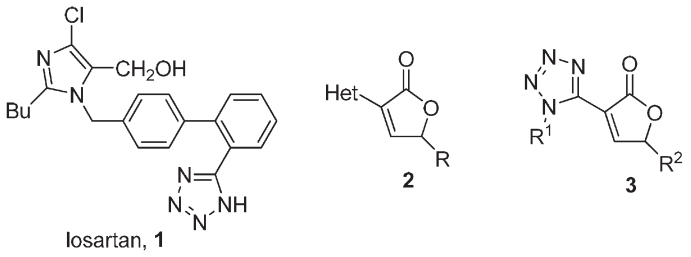
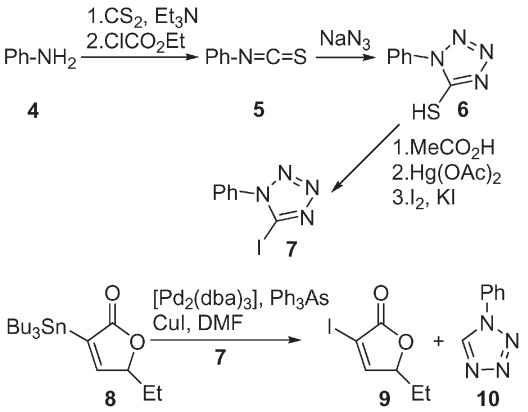


CHART 1

RESULTS AND DISCUSSION

An obvious process to be attempted first was the Stille coupling reaction of a 5-iodotetrazole derivative with the previously prepared[8] tributylstannyl lactone **8**. The choice of a 5-halotetrazole as coupling component was dictated by the instability of 5-lithiated tetrazoles[9], and by easy availability of α-(tributylstannyl)lactones in gram quantities. Thus, 1-phenyl-5-sulfanyltetrazole (**6**), easily available from aniline in two steps[10], was converted into 5-iodo-1-phenyltetrazole (**7**) via oxidative desulfurization followed by mercuration and decomposition of the organomercury intermediate with

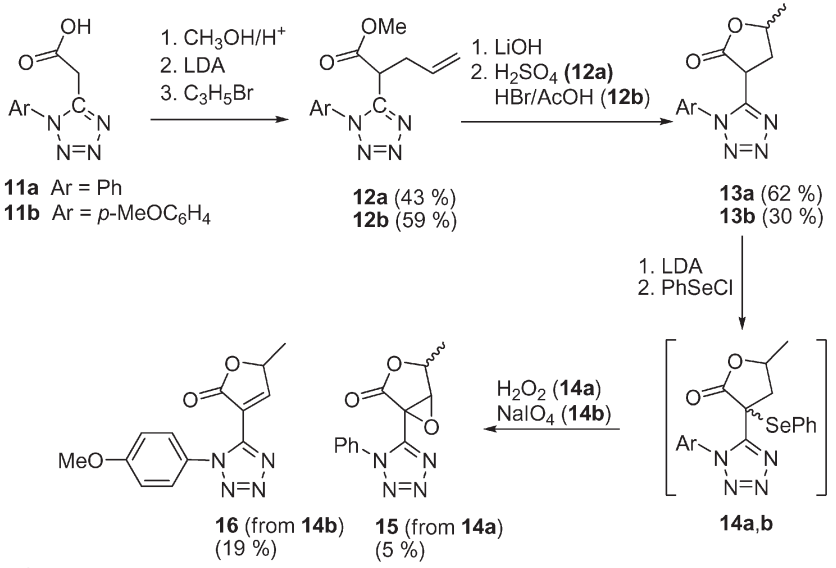
iodine[11]. Similarly to our previous results[8] on coupling α-(tributylstannyl)-butenolide **8** with 2-iodofuran, Pd-mediated coupling failed upon using a number of established procedures, including variation of catalysts, solvents and reaction conditions. A reaction was enforced employing the protocol proposed by Farina for difficult cases[12]; however, apart from a recovered starting material (26%), the reaction afforded iodolactone **9** and the product of deiodination of the tetrazole ring **10** [13] as the only isolable products (Scheme 1).



SCHEME 1

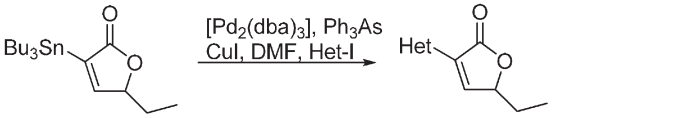
Since the above coupling conditions were rather harsh, we suspected that the low stability of the product may have been one of the reasons why the isolation of the desired product was unsuccessful. Thus, further elaboration of the acetic acid moiety in the tetrazole-substituted compounds **11a**[14] and **11b** was another strategy we employed (Scheme 2). The reaction sequence employing generally mild conditions has been used before by us[4–6] and others[15]. The acids were converted into the corresponding methyl esters, deprotonated and the enolates treated with allyl bromide to afford intermediates **12a** and **12b**. The carboxylic groups were then liberated by hydrolysis and cyclized onto the terminal double bond. Finally, the saturated lactones **13a** and **13b** were enolized with LDA and the enolates quenched with PhSeCl. The intermediate 3-(phenylselanyl) derivatives were, due to their limited stability, immediately oxidized to furnish the corresponding selenoxides, which underwent a spontaneous *syn*-elimination introducing the double bond. However, due to the electron-withdrawing properties of the tetrazolyl moiety, the double bond of the lactone ring was extremely prone to a nucleophilic attack. Hence, in case of the 1-phenyltetrazol-5-yl

derivative **14a**, only a negligible yield of epoxide **15** was isolated from a complex reaction mixture after oxidation of the intermediate phenylselanyl derivative with H_2O_2. In order to suppress or avoid epoxide formation, $NaIO_4$ was employed for the oxidation of **14b** instead of H_2O_2 and derivative **16** with the tetrazole ring linked directly to the butenolide ring was obtained in a low, 19% yield. The structure of compound **16** was evident from the ^1H NMR spectrum of the crude reaction mixture, which contained butenolide **16**, the signals of which corresponded very well to the spectra of a number of related compounds as the major product. However, this compound was quickly decomposing during flash chromatography, and its limited stability was apparent even at low temperatures.



SCHEME 2

We suspected that the electron-withdrawing properties of the tetrazole ring facilitating Michael addition to the β-carbon of the furanone could have been the major reason for the instability of the compounds. This assumption was easily verified by the preparation of analogous butenolides bearing pyridin-3-yl[6] and pyrimidin-5-yl moieties with different withdrawing powers in the α-position. The compounds were prepared via Stille coupling of the corresponding heteroaryl iodides with tributylstannyl lactone **8** (Scheme 3). As expected, while the pyridyl analogue **17** was sufficiently stable, the pyrimidinyl-substituted compound **18**, though obtained in a respectably high yield, was quickly decomposing.

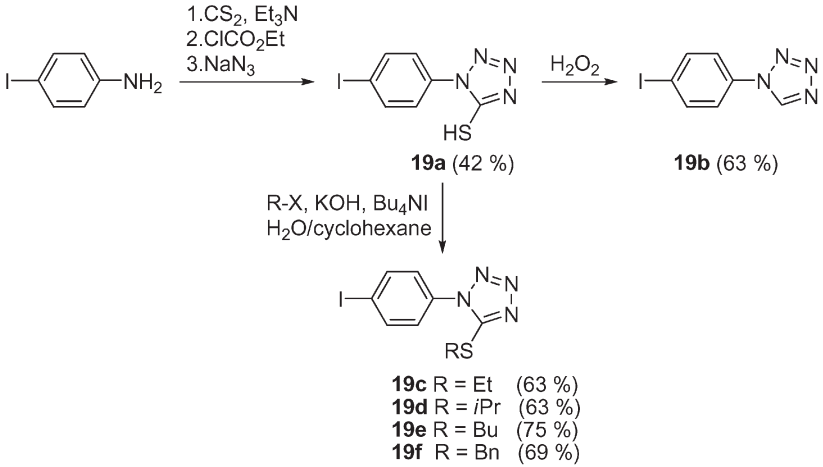


8

17 Het = pyridin-3-yl (85 %)
18 Het = pyrimidin-5-yl (80 %)

SCHEME 3

Since the target compounds were shown to be preparable, but inherently unstable, we made another attempt to synthesize compounds that would combine the tetrazole and butenolide pharmacophoric substructures. As the strong electron-withdrawing properties of the tetrazolyl moiety were clearly one of the major reasons for the instability of the directly conjugated compounds, we reasoned that more stable compounds could possibly be obtained if a suitable linker was inserted between both rings. Having in mind the planned exploration of their biological activity, we used the benzene ring to join the two units with limited flexibility and to preserve the possibility of transmission of electronic effects between them. Hence, 1-(4-iodophenyl)-5-sulfanyltetrazole (**19a**) was prepared from 4-iodoaniline via standard procedure[16] (Scheme 4) as a component for the Pd-coupling reaction with tributylstannyl lactone **8**. Compound **19a** was further converted into the product of oxidative desulfurization **19b** and 5-alkylsulfanyl derivatives **19c**–**19f**.



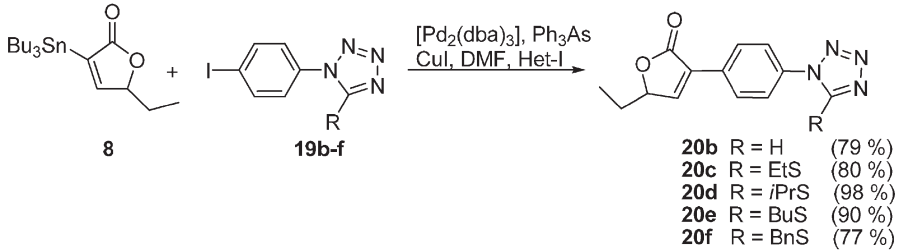
19a (42 %)

19b (63 %)

19c R = Et (63 %)
19d R = *i*Pr (63 %)
19e R = Bu (75 %)
19f R = Bn (69 %)

SCHEME 4

It should also be noted that another reason for the preparation of the alkylsulfanyl compounds was the finding of Waisser et al.[17] that an alkylsulfanyl group attached to an electron-deficient carbon atom is a pharmacophore of antitubercular activity. Hence, the inclusion of the alkylsulfanyl group would add another dimension of biological activity to the target compounds. All aryl iodides **19b**–**19f** smoothly coupled with lactone **8** under the above conditions to yield the desired products **20b**–**20f** as stable crystalline compounds in good to excellent yields (Scheme 5).



SCHEME 5

Antifungal activity screening of **20b**–**20f** against a panel of yeasts and filamentous fungi (Table I) revealed that the benzylsulfanyl derivative **20f** was practically inactive, and compound **20b** without a sulfanyl group was marginally active against *Absidia corymbifera*, *Trichophyton mentagrophytes* and *Microsporum gypseum*. Some activity against both yeasts and filamentous strains was detected only with the alkylsulfanyl derivatives **20c**–**20e**. Since the *i*-PrS-substituted derivative **20d** was the least active of the three substances, branching had a negative influence on the activity. While the activities of the other two compounds against yeasts can be described as moderate, their MICs (in particular those of the ethylsulfanyl derivative **20c**) against *Aspergillus fumigatus* and *Absidia corymbifera* are of special interest since both are dangerous pathogens whose attacks often lead to lethal infections. The MICs are roughly comparable to that of ketoconazole against *A. fumigates*, and lower than that of this standard azole drug against *A. corymbifera*. For the sake of fair comparison, however, it should be stated that even the relatively low MICs against *A. corymbifera* would not be lower than that of the polyene antibiotic amphotericin B.

The target compounds **20** (except **20d**) were also evaluated for cytostatic activity against three leukemic and one solid tumor line. Cell growth inhibition at 10 μmol/l showed that **20b** was inactive at this concentration, while the other derivatives exhibited significant growth inhibition. The results of the subsequent IC_{50} determination are summarized in Table II.

TABLE I
Antifungal activity of compounds **20b**–**20f** and ketoconazole (KET)

Strain	Time h	MIC[n], μmol/l					KET
		20b	**20c**	**20d**	**20e**	**20f**	
CA1[a]	24	62.5	7.81	31.25	7.81	>62.5	0.11
	48	>62.5	15.63	125	31.25	>62.5	0.11
CA2[b]	24	>62.5	7.81	125	7.81	>62.5	≤0.06
	48	>62.5	15.63	125	31.25	>62.5	≤0.06
CP[c]	24	>62.5	31.25	>125	62.5	>62.5	0.11
	48	>62.5	31.25	>125	>62.5	>62.5	0.11
CK1[d]	24	>62.5	7.81	125	15.63	>62.5	1.96
	48	>62.5	15.63	>125	31.25	>62.5	1.96
CK2[e]	24	>62.5	7.81	125	15.63	>62.5	3.91
	48	>62.5	15.63	>125	31.25	>62.5	3.91
CT[f]	24	>62.5	31.25	>125	62.5	>62.5	15.63
	48	>62.5	62.5	>125	>62.5	>62.5	15.63
CG[g]	24	>62.5	31.25	>125	62.5	>62.5	0.24
	48	>62.5	31.25	>125	>62.5	>62.5	0.49
CL[h]	24	>62.5	15.63	>125	31.25	>62.5	0.06
	48	>62.5	31.25	>125	>62.5	>62.5	0.11
TB[i]	24	>62.5	31.25	>125	>62.5	>62.5	0.11
	48	>62.5	125	>125	>62.5	>62.5	0.11
AF[j]	24	>62.5	15.63	>125	15.63	>62.5	15.63
	48	>62.5	32.25	>125	62.5	>62.5	15.63
AC[k]	24	31.25	7.81	62.5	15.63	>62.5	31.25
	48	31.25	7.81	125	15.63	>62.5	31.25
TM[l]	72	62.5	15.63	125	31.25	>62.5	0.98
	120	62.5	15.63	125	62.5	>62.5	1.96
MG[m]	72	31.25	7.81	31.25	15.63	>62.5	NT[o]
	120	62.5	15.63	62.5	31.25	>62.5	NT

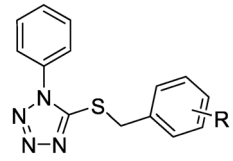
[a] *Candida albicans* ATCC44859, [b] *C. albicans* ATCC90028, [c] *C. parapsilosis* ATCC22019, [d] *C. krusei* ATCC 6258, [e] *C. krusei* E28, [f] *C. tropicalis* 156, [g] *C. glabrata* 20/I, [h] *C. lusitaniae* 2446/I, [i] *Trichosporon beigelii* 1188, [j] *Aspergillus fumigatus* 231, [k] *Absidia corymbifera* 272, [l] *Trichophyton mentagrophytes* 445, [m] *Microsporum gypseum*, [n] defined as 80% inhibition of the control growth, [o] not tested.

TABLE II
Cytostatic activity (cell growth inhibition – IC_{50}, μmol/l) of compounds **20b**, **20c**, **20e** and **20f**

Compound	L1210	HL-60	HeLa S3	CCRF-CEM
20b	NA[a]	NA	NA	NA
20c	4.6	2.6	8.4	2.3
20e	6.3	2.7	NA	3.6
20f	6.0	3.2	NA	3.2

[a] Not active at relevant concentrations.

The compounds manifested cytostatic effect especially in the leukemic lines HL-60 and CCRF-CEM; only compound **20c** showed a rather low activity in the HeLa S3 cells. Notably, the cytostatic activity does not parallel the antifungal action since the antifungally inactive benzylsulfanyl derivative **20f** possessed a cytostatic effect. It was thus interesting to speculate which part of the molecule was responsible for the cytostatic activity. In order to shed more light on this issue, we prepared a series of 1-phenyl-5-(substituted benzylsulfanyl)tetrazoles **21a**–**21f** by alkylation of compound **6** with variously substituted benzyl halides (H, 3-OMe, 4-OMe, 4-Cl, 4-F, 4-NO_2). All compounds were prepared under phase transfer catalysis as described in Scheme 4, with the exception of 4-nitrobenzyl compound **21f**, the preparation of which was unsuccessful under these conditions. Instead, formation of the sodium salt of **6** with MeONa and subsequent SN reaction with 4-nitrobenzyl chloride furnished this compound in a satisfactory yield of 40%. The overview of the 1,5-disubstituted tetrazoles is given in Chart 2.



21a R = H
21b R = 3-OMe
21c R = 4-OMe
21d R = 4-Cl
21e R = 4-F
21f R = 4-NO_2

CHART 2

Screening of cytostatic activity of tetrazoles **21** against the above cell lines showed a minimum, if any, growth inhibition at 10 μmol/l. These results rendered further IC_{50} determination pointless as the values would have been well above this concentration. Hence, the presence of the butenolide ring in **20c**, **20e** and **20f** is an absolute necessity for their cytostatic effect, even though its role must have been combined with that of sulfur (cf. inactivity of **20b**).

CONCLUSION

In summary, we have not only extended the previously published series of 5-alkyl-3-heteroarylfuranones with a series of novel compounds possessing interesting biological properties, but also explored the preparation of 1,5-disubstituted tetrazoles by cross coupling reactions. Notably, we confirmed that the substitution of the furanone ring in the α-position with strongly electron-withdrawing heteroaryl moieties leads to compounds with limited to low stability. This obstacle, however, can be circumvented by inserting a suitable linker between both heterocyclic rings. While the antifungal activity of the compounds against yeasts was rather low, the MIC values against *A. corymbifera* and *A. fumigatus* render compound **20c** interesting for further development, since both the alkylsulfanyl and the ethyl groups at C5 of the furanone can be further varied. Cytostatic activity requires the presence of both the furanone ring and alkylsulfanyl group, but the structure–cytostatic activity relationships are very likely different from the antifungal activity profile. Given the low IC_{50} values against HL-60 and CCRF-CEM lines, further development by varying the RS group in the tetrazole ring and the alkyl at C5 of the furanone might also bring useful results.

EXPERIMENTAL

General Experimental Procedures

THF was distilled from benzophenone ketyl and diisopropylamine from CaH_2. Substituted phenylacetic acids were obtained from Sigma–Aldrich and used as received. All anhydrous reactions were performed in flame-dried Schlenk tubes under Ar atmosphere. Analytical thin-layer chromatography (TLC) was conducted on Merck TLC plates (silica gel 60 F_{254}, aluminum back). Silica gel 60 (230–400 mesh) for column chromatography was purchased from Merck. Melting points were determined on a Kofler block and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^1H. Chemical shifts are given as δ values in ppm and are indirectly referenced to tetramethylsilane (TMS) via the solvent signal. Coupling constants (*J*) are

given in Hz. All assignments were made on the basis of gCOSY, gHSQC and gHMBC experiments. Mixtures of isomers are referred to as A and B. IR spectra (ν_{max}, cm^{-1}) were recorded on a Nicolet Impact 400 spectrophotometer. Low-resolution mass spectra were measured on a Magnum Finnigan Mat apparatus.

Antifungal Activity

In vitro antifungal activities of the prepared compounds and ketoconazole (Janssen–Cilag) were evaluated on a panel of four ATCC (*Candida albicans* ATCC 44859, *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) and nine clinical isolates of yeasts (*C. krusei* E28, *C. tropicalis* 156, *C. glabrata* 20/I, *C. lusitaniae* 2446/I, *Trichosporon beigelii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445, *Microsporum gypseum*) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three of the above ATCC strains (*C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) also served as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested.

Minimum inhibitory concentrations (MICs) were determined according to the microdilution format of the NCCLS M27-A guidelines[18]. Dimethyl sulfoxide (100%) served as a diluent for all compounds; their final concentration did not exceed 2%. RPMI 1640 (Sevapharma, Prague), a medium supplemented with L-glutamine and buffered with 0.165 M morpholine-1-propanesulfonic acid (Serva) to pH 7.0 by 10 M NaOH was used as the test medium. The wells of microdilution tray contained 100 µl of the RPMI 1640 medium with two-fold serial dilutions of the compounds (1000–0.24 µmol/l for new compounds) and 100 µl of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu ml^{-1}. The trays were incubated at 35 °C and MICs were read visually for filamentous fungi and photometrically for yeasts as an absorbance at 540 nm after 24 and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes* and *M. gypseum*) were determined after 72 and 120 h. The MICs were defined as 80% inhibition of the control growth. MICs were determined twice and in duplicate. The deviations from the usually obtained values given in Table I were no higher than the nearest concentration value up and down the dilution scale.

Cytostatic Activity Assays

Inhibition of the cell growth was estimated in mouse lymphocytic leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (human acute lymphoblastic leukemia, ATCC CCL 119), human promyelocytic leukemia HL-60 cells (ATCC CCL 240) and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2)[19]. L1210 cells, CCRF-CEM cells and HL-60 cells were cultivated in RPMI 1640 medium supplemented with calf foetal serum using 24-well tissue culture plates. The endpoint of the cell growth was 72 h following the drug addition. HeLa S3 cells were seeded to 24-well dishes in RPMI 1640 HEPES modification with foetal calf serum. 48 h following the drug addition the cultivation was stopped and the cell growth was evaluated. In parallel, cell viability was quantified using MTT standard spectrophotometric assay[20]. The inhibitory potency of the compounds tested was expressed as IC_{50} values.

5-Ethyl-3-iodo-2,5-dihydrofuran-2-one (**9**)

A solution of **7** (0.030 g, 0.11 mmol) in dry DMF (0.5 ml) was added to a stirred suspension of $[Pd_2(dba)_3]\cdot CHCl_3$ (3 mg, 0.003 mmol), Ph_3As (7 mg, 0.023 mmol) and CuI (3 mg, 0.016 mmol) in dry DMF (3 ml) under Ar. The resultant mixture was heated up to 50 °C and a solution of **8** (0.040 g, 0.10 mmol) in dry DMF (0.5 ml) was added dropwise. The reaction mixture was stirred at 50 °C for 6 h, then saturated aqueous potassium fluoride solution (1 ml) was added and the resultant suspension was stirred at room temperature for 30 min. Ethyl acetate (20 ml) was added, the mixture was filtered and the filtrate washed with a saturated sodium chloride solution (20 ml). Organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the mixture was purified by column chromatography (gradient elution, hexane–ethyl acetate 95:5–7:3) to afford compound **9** (colorless oil, 8 mg, yield 33%) and **10** [13] (white crystals, 6 mg, yield 38%). For **9:** ^{1}H NMR (300 MHz, $CDCl_3$): 7.72 (1 H, d, *J* = 1.7, H4), 5.00–4.92 (1 H, m, H5), 1.90–1.65 (2 H, m, -CH_2-), 1.00 (3 H, t, *J* = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 170.06, 160.97, 86.33, 85.39, 26.24, 8.85. IR ($CDCl_3$) ν_{max}: 2974, 2938, 1763, 1595, 1463, 1332, 1279. LRMS: 239 ($M^{\bullet+}$ + H, 100), 221 (4), 209 (10), 111 (7), 83 (4), 57 (18), 53 (7).

2-[1-(4-Methoxyphenyl)-1*H*-tetrazol-5-yl]acetic Acid (**11b**)

Butyllithium (1.6 M solution in hexanes, 10.35 ml, 16.56 mmol) was added to a stirred solution of 1-(4-methoxyphenyl)-5-methyl-1*H*-tetrazole (3.000 g, 15.77 mmol) in dry THF (40 ml) at 0 °C under Ar. The resultant solution was stirred at 50 °C for 30 min, then dry ice (100 g) was added, and the mixture was allowed to warm up to room temperature. Then 5% aqueous sodium hydroxide solution (50 ml) was added, and the reaction mixture washed with ethyl acetate (50 ml). The aqueous layer was acidified with aqueous hydrochloric acid to pH ~ 2, the resultant solution washed three times with ethyl acetate (50 ml), the organic layer dried (Na_2SO_4) and the solvents were removed under reduced pressure. Yield 70%. Brownish oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.40–7.35 (2 H, m, AA′BB′), 7.06–7.02 (2 H, m, AA′BB′), 4.03 (2 H, s, -CH_2-), 3.86 (3 H, s, -OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 177.48, 161.28, 149.44, 126.59, 125.56, 115.09, 55.67, 29.52. IR ($CDCl_3$), ν_{max}: 3012, 2938, 2841, 1716, 1610, 1514, 1408, 1291, 1258.

Preparation of Compounds **12a** and **12b**. General Procedure

Dowex 50® (2.4 g) was added to a stirred solution of **11** (12.00 mmol) in methanol (50 ml) at 0 °C under Ar. The resultant mixture was stirred at room temperature for 20 h, the ion exchanger filtered off, washed with ethyl acetate (50 ml), and the solvents removed under reduced pressure. The resultant colorless oil was dissolved in dry THF (5 ml) and added to a stirred solution of dry diisopropylamine (12.60 mmol) and butyllithium (1.6 M solution in hexanes, 13.20 mmol) in dry THF (60 ml) at –60 °C under Ar. After 30-min stirring, allyl bromide (12.6 mmol) was added, and the reaction mixture allowed to gradually warm to room temperature over 2 h. The mixture was then diluted with ethyl acetate (100 ml), washed with a saturated ammonium chloride solution (50 ml), the organic layer was dried (Na_2SO_4) and the solvents removed under reduced pressure. The crude product was purified by column chromatography (hexane–diethyl ether 8:2) to afford compound **12**.

Methyl 2-(1-phenyl-1H-tetrazol-5-yl)pent-4-enoate (**12a**): Yield 43%. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.62–7.56 (3 H, m, Ar), 7.45–7.40 (2 H, m, Ar), 5.71–5.53 (1 H, m, H4),

5.09–4.94 (2 H, m, H5), 3.97 (1 H, dd, J_1 = 9.1, J_2 = 6.3, H2), 3.66 (3 H, s, -CH_3), 3.04–2.82 (2 H, m, H3). ^{13}C NMR (75 MHz, $CDCl_3$): 168.84, 152.73, 133.28, 132.94, 130.87, 129.95, 125.60, 118.93, 52.95, 40.91, 34.39. IR ($CDCl_3$), ν_{max}: 2956, 1747, 1598, 1497, 1437, 1268. LRMS: 258 ($M^{\bullet+}$, 1), 239 (2), 201 (30), 171 (17), 158 (4), 144 (17), 131 (100), 117 (25), 104 (7), 91 (19), 77 (56), 64 (15), 51 (34).

Methyl 2-[1-(4-methoxyphenyl)-1H-tetrazol-5-yl]pent-4-enoate (**12b**): Yield 59%. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.36–7.30 (2 H, m, AA′BB′), 7.07–7.03 (2 H, m, AA′BB′), 5.71–5.52 (1 H, m, H4), 5.09–4.96 (2 H, m, H5), 3.93 (1 H, dd, J_1 = 9.3, J_2 = 6.3, H2), 3.87 (3 H, s, Ar-OCH_3), 3.67 (3 H, s, -CH_3), 3.01–2.82 (2 H, m, H3). ^{13}C NMR (75 MHz, $CDCl_3$): 168.93, 161.24, 153.00, 133.02, 126.18, 125.78, 118.86, 114.97, 55.65, 52.94, 41.00, 34.34. IR ($CDCl_3$), ν_{max}: 3084, 2984, 2955, 2841, 1743, 1609, 1518, 1444, 1303, 1258. LRMS: 289 ($M^{\bullet+}$ – H, 84), 259 (41), 245 (10), 228 (13), 219 (100), 201 (71), 186 (36), 174 (68), 161 (16), 149 (82), 133 (25), 121 (53), 106 (25), 92 (28), 78 (40), 63 (38), 53 (39).

5-Methyl-3-(1-phenyl-1*H*-tetrazol-5-yl)tetrahydrofuran-2-one (**13a**)

Ester **12a** (1.175 g, 4.55 mmol) was dissolved in a mixture of methanol–water 3:1 (40 ml) and lithium hydroxide (0.267 g, 5.92 mmol) was added. The reaction mixture was stirred at room temperature for 20 h, methanol was removed under reduced pressure, and the resultant solution was diluted with water (15 ml) and acidified with aqueous hydrochloric acid to pH ~ 3. The mixture was washed three times with ethyl acetate (50 ml), the organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The product was cooled down to 0 °C and concentrated sulfuric acid (5 ml) was added dropwise under constant stirring. The reaction mixture was stirred at 0 °C for 20 min, then cautiously poured into a saturated sodium chloride solution (50 ml) and pH of the mixture was adjusted with a saturated aqueous sodium carbonate solution to ~8. The mixture was then washed with ethyl acetate (50 ml), the organic layer dried (Na_2SO_4) and the solvent removed under reduced pressure. The crude product was purified by column chromatography (hexane–diethyl ether 7:3) to afford lactone **13a** as a mixture of two diastereomers. Yield 62%. White crystals, m.p. 111–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$): A: 7.74–7.68 (2 H, m, Ar), 7.67–7.54 (3 H, m, Ar), 5.29–5.16 (1 H, m, H5), 4.11 (1 H, dd, J_1 = 9.2, J_2 = 3.4, H3), 3.09–2.97 (1 H, m, H4A), 2.38–2.26 (1 H, m, H4B), 1.48 (3 H, d, J = 6.3, -CH_3); B: 7.74–7.68 (2 H, m, Ar), 7.67–7.54 (3 H, m, Ar), 4.75–4.61 (1 H, m, H5), 4.15 (1 H, t, J = 10.4, H3), 2.84–2.71 (2 H, m, H4), 1.56 (3 H, d, J = 6.0, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): A: 172.30, 151.68, 132.92, 130.84, 129.93, 125.34, 77.20, 36.33, 35.63, 20.91; B: 171.79, 151.18, 132.99, 130.88, 129.90, 125.41, 76.51, 38.02, 36.12, 20.62. IR ($CDCl_3$), ν_{max}: 2984, 2933, 1773, 1598, 1500, 1457, 1387, 1349. LRMS: 245 ($M^{\bullet+}$ + H, 23), 228 (3), 216 (14), 207 (8), 197 (9), 185 (11), 171 (56), 157 (25), 145 (37), 130 (100), 117 (20), 103 (17), 91 (54), 77 (62), 64 (33), 51 (53).

3-[1-(4-Methoxyphenyl)-1*H*-tetrazol-5-yl]-5-methyltetrahydrofuran-2-one (**13b**)

Ester **12b** (1.156 g, 5.41 mmol) was dissolved in a mixture of methanol–water 3:1 (40 ml) and lithium hydroxide (0.318 g, 7.03 mmol) was added. The reaction mixture was stirred at 50 °C for 11 h, methanol was removed under reduced pressure, the resultant solution diluted with water (15 ml) and acidified with aqueous hydrochloric acid to pH ~ 3. The mixture was washed three times with ethyl acetate (50 ml), the organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. A 30% solution of hydrobromic acid in AcOH (10 ml) was slowly added to the product under Ar at room temperature, and the reac-

tion mixture was stirred for 6 h. The remaining reagent was removed under reduced pressure, the crude product was dissolved in methanol (15 ml) and sodium carbonate (1.232 g, 11.63 mmol) was added. After 45 min of stirring at room temperature, the mixture was diluted with ethyl acetate (50 ml), washed with a saturated aqueous sodium chloride solution (50 ml), the organic layer was dried (Na_2SO_4) and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (hexane–diethyl ether 7:3) to afford lactone **13b** as a mixture of two diastereomers. Yield 30%. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): A: 7.67–7.50 (2 H, m, AA′BB′), 7.12–6.98 (3 H, m, Ar), 5.31–5.15 (1 H, m, H5), 4.12 (1 H, dd, J_1 = 9.0, J_2 = 3.5, H3), 3.88 (3 H, s, -OCH_3), 3.09–2.96 (1 H, m, H4A), 2.36–2.21 (1 H, m, H4B), 1.48 (3 H, d, J = 6.3, -CH_3); B: 7.67–7.50 (2 H, m, Ar), 7.12–6.98 (3 H, m, Ar), 4.72–4.62 (1 H, m, H5), 4.14 (1 H, t, J = 10.6, H3), 3.88 (3 H, s, -OCH_3), 2.80–2.69 (2 H, m, H4), 1.54 (3 H, d, J = 6.0, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): A: 172.00, 151.32, 127.11, 122.94, 115.01, 77.19, 55.71, 36.21, 35.50, 20.83; B: 171.69, 160.69, 151.11, 127.03, 122.94, 115.16, 76.50, 55.71, 37.91, 35.97, 20.54.

3,4-Epoxy-5-methyl-3-(1-phenyl-1*H*-tetrazol-5-yl)tetrahydrofuran-2-one (**15**)

A solution of dry diisopropylamine (0.30 ml, 2.15 mmol) and butyllithium (1.6 M solution in hexanes, 2.26 mmol) in dry THF (5 ml) was stirred at 0 °C under Ar for 10 min. The LDA solution was then cooled to –60 °C, and a solution of lactone **13a** (0.500 g, 2.05 mmol) in dry THF (2 ml) was added dropwise. After 30-min stirring at –60 °C, a solution of phenylselanyl chloride (0.589 g, 3.08 mmol) in dry THF (5 ml) was added, and the resultant mixture was allowed to warm to room temperature over 2 h. The mixture was then diluted with ethyl acetate (50 ml), washed with a saturated ammonium chloride solution (50 ml), the organic layer was dried (Na_2SO_4) and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (hexane–diethyl ether 8:2) to afford phenylselanyl derivative **14a**, which was immediately used in the next step.

Lactone **14a** (0.267 g, 0.67 mmol) was dissolved in a mixture of ethyl acetate–THF (2:1, 3 ml), the reaction mixture was cooled down to 0 °C, and sodium hydrogencarbonate (0.150 g) and a 30% aqueous hydrogen peroxide solution (1 ml) were added. The resultant mixture was stirred at room temperature for 1 h, then diluted with ethyl acetate (50 ml), and washed with a saturated sodium chloride solution (50 ml). The organic layer was dried (Na_2SO_4) and the solvents removed under reduced pressure. The crude product was purified by column chromatography (hexane–diethyl ether 7:3) to afford lactone **15**. Yield 5%. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.70–7.65 (2 H, m, Ar), 7.63–7.58 (3 H, m, Ar), 4.81 (1 H, q, J = 6.9, H5), 4.57 (1 H, s, H4), 1.56 (3 H, d, J = 6.9, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 166.06, 145.14, 133.39, 130.91, 129.78, 124.38, 75.77, 64.84, 52.24, 17.84.

3-[1-(4-Methoxyphenyl)-5-methyl-1*H*-tetrazol-5-yl]-2,5-dihydrofuran-2-one (**16**)

A solution of dry diisopropylamine (0.22 ml, 1.57 mmol) and butyllithium (1.6 M solution in hexanes, 1.65 mmol) in dry THF (5 ml) was stirred at 0 °C under Ar for 10 min, then cooled down to –60 °C and a solution of lactone **13b** (0.411 g, 1.50 mmol) in dry THF (2 ml) was added dropwise. After 30 min of stirring at –60 °C, a solution of phenylselanyl chloride (0.430 g, 2.24 mmol) in dry THF (5 ml) was added and the resultant mixture was allowed to warm to room temperature over 2 h. The mixture was then diluted with ethyl acetate (50 ml), washed with a saturated ammonium chloride solution (50 ml), the organic

layer was dried (Na_2SO_4) and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (hexane–ethyl acetate 95:5) to afford phenylselanyl derivative **14b**, which was immediately used in the next step.

Lactone **14b** (0.286 g, 0.67 mmol) was dissolved in a mixture of methanol–THF–water (5:5:1, 15 ml), and sodium hydrogencarbonate (0.068 g, 0.80 mmol) and sodium periodate (0.330 g, 1.54 mmol) were added. The resultant mixture was stirred at room temperature for 2.5 h, then diluted with water (30 ml), and washed with ethyl acetate (50 ml). The organic layer was dried (Na_2SO_4) and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (hexane–ethyl acetate 9:1) to afford lactone **16**. Yield 19%. Colorless oil (when freshly prepared). ^{1}H NMR (300 MHz, $CDCl_3$): 7.58–7.51 (3 H, m, AA′BB′ + H4), 7.12–7.01 (2 H, m, AA′BB′), 5.22 (1 H, qd, J_1 = 6.9, J_2 = 1.6, H5), 3.88 (3 H, s, -OCH_3), 1.55 (3 H, d, J = 6.9, -CH_3).

5-Ethyl-3-(pyrimidin-5-yl)-2,5-dihydrofuran-2-one (**18**)

A solution of 5-iodopyrimidine (0.227 g, 1.10 mmol) in dry DMF (1 ml) was added to a stirred suspension of $[Pd_2(dba)_3]\cdot CHCl_3$ (21 mg, 0.02 mmol), Ph_3As (49 mg, 0.16 mmol) and CuI (15 mg, 0.08 mmol) in dry DMF (4 ml) under Ar. The resultant mixture was heated up to 50 °C, and a solution of **8** (0.401 g, 1.00 mmol) in dry DMF (1 ml) was added dropwise. The reaction mixture was stirred at 50 °C for 6 h, a saturated aqueous potassium fluoride solution (1 ml) was then added, and the resultant suspension was stirred at room temperature for 30 min. Ethyl acetate (50 ml) was added, the mixture was filtered, and the filtrate washed with a saturated sodium chloride solution (50 ml). The organic layer was dried (Na_2SO_4), the solvents were removed under reduced pressure, and the crude product was purified by column chromatography (gradient elution, hexane–ethyl acetate 95:5–7:3) to afford lactone **18**. Yield 80%. Colorless oil, quickly decomposing. ^{1}H NMR (300 MHz, $CDCl_3$): 9.22 (1 H, s, Ar2), 9.20 (2 H, s, Ar4,6), 7.78 (1 H, d, J = 1.9, H4), 5.15–5.07 (1 H, m, H5), 2.01–1.75 (2 H, m, -CH_2-), 1.08 (3 H, t, J = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 170.58, 158.79, 154.96, 150.13, 127.03, 123.95, 82.57, 26.57, 9.22.

1-(4-Iodophenyl)-1*H*-tetrazole (**19b**)

Tetrazole **19a** (0.250 g, 0.82 mmol) was dissolved in acetic acid (3.5 ml), a 30% aqueous hydrogen peroxide solution (7.5 ml) was added, and the resultant mixture heated under reflux for 1.5 h. After cooling down to room temperature, sodium carbonate was slowly added (to pH ~ 8), and the mixture washed with ethyl acetate (60 ml). The organic layer was dried (Na_2SO_4), the solvents removed under reduced pressure, and the crude product purified by column chromatography (hexane–ethyl acetate 8:2) to afford tetrazole **19b**. Yield 63%. White crystals, m.p. 189–191 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.10 (1 H, s, Het), 8.04–7.98 (1 H, m, AA′BB′), 7.76–7.69 (1 H, m, AA′BB′). ^{13}C NMR (75 MHz, $CDCl_3$): 142.47, 139.01, 133.67, 123.18, 95.97. IR (KBr), ν_{max}: 3455, 3132, 1503, 1495, 1385, 1212, 996, 819. LRMS: 271 ($M^{\bullet+}$ – H, 1), 244 (100), 234 (3), 207 (3), 191 (2), 165 (2), 149 (4), 127 (20), 117 (35), 90 (66), 74 (11), 63 (47), 50 (24).

Preparation of Compounds **19c**–**19f**. General Procedure

Tetrazole **19a** (0.800 g, 2.63 mmol) was dissolved in cyclohexane (20 ml), and a 10 M aqueous sodium hydroxide solution (10 ml), and alkyl halide (2.63 mmol) and tetrabutylammonium

iodide (0.100 g, 0.27 mmol) were added. The reaction mixture was heated under reflux with rapid stirring for 30 min. After cooling down to room temperature, the resultant mixture was filtered and the filtrate washed with water (30 ml). The organic layer was dried (Na_2SO_4), the solvents were removed under reduced pressure and the crude product purified by recrystallization from ethanol.

5-(Ethylsulfanyl)-1-(4-iodophenyl)-1H-tetrazole (**19c**): R–X = ethyl bromide. Yield 63%. White crystals, m.p. 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.94–7.86 (2 H, m, AA′BB′), 7.39–7.31 (2 H, m, AA′BB′), 3.42 (2 H, q, J = 7.4, -SCH_2-), 1.50 (3 H, t, J = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 154.22, 138.97, 133.36, 125.26, 95.63, 27.88, 14.54. IR (KBr), ν_{max}: 3432, 3088, 2958, 2928, 1489, 1384, 1246, 1056, 1005, 829. LRMS: 316 (1), 244 (100), 207 (2), 127 (9), 117 (18), 90 (33), 74 (5), 63 (24), 50 (12).

1-(4-Iodophenyl)-5-isopropylsulfanyl-1H-tetrazole (**19d**): R–X = isopropyl iodide. Yield 63%. White crystals, m.p. 112–113 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.93–7.85 (2 H, m, AA′BB′), 7.37–7.29 (2 H, m, AA′BB′), 4.22–4.07 (1 H, m, -SCH), 1.51 (6 H, d, J = 6.9, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 153.98, 138.89, 133.37, 125.40, 95.61, 39.98, 23.22. IR (KBr), ν_{max}: 2965, 1492, 1385, 1240, 1059, 1006, 829. LRMS: 347 ($M^{\bullet+}$ + H, 1), 318 (100), 276 (10), 249 (42), 219 (1), 191 (2), 150 (5), 127 (4), 116 (8), 108 (2), 90 (13), 76 (5), 63 (23), 50 (10).

5-(Butylsulfanyl)-1-(4-iodophenyl)-1H-tetrazole (**19e**): R–X = butyl bromide. Yield 75%. White crystals, m.p. 110–111 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.93–7.87 (2 H, m, AA′BB′), 7.38–7.32 (2 H, m, AA′BB′), 3.41 (2 H, t, J = 7.3, -SCH_2-), 1.87–1.74 (2 H, m, -CH_2-), 1.54–1.41 (2 H, m, -CH_2-), 0.95 (3 H, t, J = 7.3, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 154.46, 138.96, 133.39, 125.28, 95.61, 33.17, 31.01, 21.76, 13.48. IR (KBr), ν_{max}: 2961, 2931, 1491, 1423, 1398, 1379, 1247, 1006, 827. LRMS: 361 ($M^{\bullet+}$ + H, 1), 244 (100), 234 (2), 207 (2), 127 (6), 117 (12), 90 (20), 75 (3), 63 (20), 50 (8).

5-(Benzylsulfanyl)-1-(4-iodophenyl)-1H-tetrazole (**19f**): R–X = benzyl chloride. Yield 69%. White crystals, m.p. 115–116 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.89–7.83 (2 H, m, AA′BB′), 7.45–7.38 (2 H, m, Ar), 7.36–7.25 (5 H, m, AA′BB′ + Ar), 4.63 (2 H, s, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$): 153.78, 138.91, 135.01, 133.21, 129.20, 128.84, 128.25, 125.21, 95.68, 37.74. IR (KBr), ν_{max}: 3059, 1495, 1402, 1382, 1248, 1060, 1005, 828, 699. LRMS: 244 (100), 234 (1), 207 (1), 127 (9), 117 (17), 90 (33), 74 (4), 63 (22), 50 (12).

Preparation of Compounds **20b**–**20f**. General procedure

A solution of an aryl iodide **19** (1.10 mmol) in dry DMF (1 ml) was added to a stirred suspension of $[Pd_2(dba)_3]\cdot CHCl_3$ (21 mg, 0.02 mmol), Ph_3As (49 mg, 0.16 mmol) and CuI (15 mg, 0.08 mmol) in dry DMF (4 ml) under Ar. The resultant mixture was heated up to 50 °C and a solution of **8** (0.401 g, 1.00 mmol) in dry DMF (1 ml) was added dropwise. The reaction mixture was stirred at 50 °C for 6 h, a saturated aqueous potassium fluoride solution (1 ml) was added, and the resultant suspension stirred at room temperature for 30 min. Ethyl acetate (50 ml) was added, the mixture was filtered and the filtrate was washed with a saturated sodium chloride solution (50 ml). The organic layer was dried (Na_2SO_4), the solvents were removed under reduced pressure, and the crude product purified by column chromatography (gradient elution, hexane–ethyl acetate 99:1–8:2) to afford lactones **20b**–**20f**.

5-Ethyl-3-[4-(1H-tetrazol-1-yl)phenyl]-2,5-dihydrofuran-2-one (**20b**): Yield 79%. White crystals, m.p. 158–160 °C. ^{1}H NMR (300 MHz, $(CD_3)_2CO$): 9.79 (1 H, s, Het), 8.26–8.20 (2 H, m, AA′BB′), 8.19 (1 H, d, J = 1.7, H4), 8.04–7.96 (2 H, m, AA′BB′), 5.20–5.13 (1 H, m, H5), 1.98–1.72 (2 H, m, -CH_2-), 1.04 (3 H, t, J = 7.4, -CH_3). ^{13}C NMR (75 MHz, $(CD_3)_2CO$):

171.80, 151.94, 142.37, 135.24, 132.27, 129.99, 129.37, 121.93, 82.46, 27.08, 9.31. IR (KBr), ν_{max}: 3448, 3135, 2975, 1736, 1522, 1461, 1209, 1148, 1131, 975, 838, 534. LRMS: 255 ($M^{\bullet+}$ – H, 6), 221 (6), 207 (23), 193 (7), 172 (16), 157 (6), 144 (22), 131 (100), 117 (20), 104 (12), 91 (35), 77 (78), 65 (16), 51 (34). For $C_{13}H_{12}N_4O_2$ calculated: 60.93% C, 4.72% H, 21.86% N; found: 60.63% C, 4.92% H, 21.71% N.

5-Ethyl-3-{4-[5-(ethylsulfanyl)-1H-tetrazol-1-yl]phenyl}-2,5-dihydrofuran-2-one (**20c**): Yield 80%. White crystals, m.p. 64–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.10–8.05 (2 H, m, AA′BB′), 7.71 (1 H, d, *J* = 1.9, H4), 7.68–7.62 (2 H, m, AA′BB′), 5.11–5.02 (1 H, m, H5), 3.42 (2 H, q, *J* = 7.4, -SCH_2-), 2.01–1.76 (2 H, m, -CH_2-), 1.49 (3 H, t, *J* = 7.4, -SCH_2CH_3), 1.08 (3 H, t, *J* = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.18, 154.29, 149.42, 134.08, 131.19, 130.34, 128.39, 123.78, 81.71, 27.82, 26.61, 14.52, 9.21. IR (KBr), ν_{max}: 2927, 1750, 1636, 1515, 1385, 1120, 968, 845. LRMS: 315 ($M^{\bullet+}$ – H, 1), 291 (13), 269 (100), 229 (4), 208 (13), 167 (8), 155 (17), 138 (4), 119 (8), 91 (2), 69 (3), 57 (23). For $C_{15}H_{16}N_4O_2S$ calculated: 56.94% C, 5.10% H, 17.71% N, 10.14% S; found: 57.38% C, 5.41% H, 17.33% N, 9.80% S.

5-Ethyl-3-{4-[5-(isopropylsulfanyl)-1H-tetrazol-1-yl]phenyl}-2,5-dihydrofuran-2-one (**20d**): Yield 98%. White crystals, m.p. 122–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.10–8.02 (2 H, m, AA′BB′), 7.71 (1 H, d, *J* = 1.7, H4), 7.66–7.58 (2 H, m, AA′BB′), 5.10–5.03 (1 H, m, H5), 4.21–4.05 (1 H, m, -SCH), 2.04–1.72 (2 H, m, -CH_2-), 1.50 (6 H, d, *J* = 6.9, -CH_3), 1.07 (3 H, t, *J* = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.19, 154.04, 149.50, 134.06, 131.17, 130.27, 128.31, 123.92, 81.70, 39.87, 26.56, 23.17, 9.17. IR ($CDCl_3$), ν_{max}: 2973, 2930, 1758, 1607, 1517, 1462, 1390, 1338. LRMS: 330 ($M^{\bullet+}$, 7), 327 (16), 315 (13), 281 (8), 267 (4), 255 (5), 245 (22), 219 (10), 202 (18), 193 (6), 160 (8), 149 (14), 135 (18), 127 (24), 119 (10), 91 (56), 70 (46), 61 (100). For $C_{16}H_{18}N_4O_2S$ calculated: 58.16% C, 5.49% H, 16.96% N, 9.71% S; found: 58.51% C, 5.81% H, 16.68% N, 9.40% S.

3-{4-[5-(Butylsulfanyl)-1H-tetrazol-1-yl]phenyl}-5-ethyl-2,5-dihydrofuran-2-one (**20e**): Yield 90%. White crystals, m.p. 62–64 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.10–8.04 (2 H, m, AA′BB′), 7.71 (1 H, d, *J* = 1.7, H4), 7.67–7.62 (2 H, m, AA′BB′), 5.10–5.03 (1 H, m, H5), 3.40 (2 H, t, *J* = 7.3, -SCH_2-), 1.99–1.73 (4 H, m, -CH_2-), 1.54–1.39 (2 H, m, -CH_2-), 1.08 (3 H, t, *J* = 7.4, -CH_3), 0.94 (3 H, t, *J* = 7.3, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.19, 154.53, 149.44, 134.09, 131.18, 130.32, 128.38, 123.78, 81.71, 33.10, 30.97, 26.59, 21.72, 13.45, 9.19. IR (KBr), ν_{max}: 3087, 2956, 2931, 2873, 1747, 1515, 1381, 1340, 1121, 837. LRMS: 344 ($M^{\bullet+}$, 1), 281 (2), 246 (10), 207 (2), 181 (11), 147 (2), 121 (3), 91 (100), 77 (5), 65 (17), 51 (7). For $C_{17}H_{20}N_4O_2S$ calculated: 59.28% C, 5.85% H, 16.27% N, 9.29% S; found: 59.60% C, 6.10% H, 15.96% N, 8.98% S.

3-{4-[5-(Benzylsulfanyl)-1H-tetrazol-1-yl]phenyl}-5-ethyl-2,5-dihydrofuran-2-one (**20f**): Yield 77%. White crystals, m.p. 119–121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.07–8.01 (2 H, m, AA′BB′), 7.70 (1 H, d, *J* = 1.6, H4), 7.60–7.54 (2 H, m, AA′BB′), 7.44–7.38 (2 H, m, Ar), 7.35–7.26 (3 H, m, Ar), 5.08–5.02 (1 H, m, H5), 4.61 (2 H, s, -SCH_2-), 2.01–1.72 (2 H, m, -CH_2-), 1.06 (3 H, t, *J* = 7.4, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 171.13, 153.83, 149.62, 134.99, 133.81, 131.20, 130.08, 129.12, 128.73, 128.30, 128.12, 123.67, 81.67, 37.59, 26.48, 9.12. IR ($CDCl_3$), ν_{max}: 2974, 2939, 2881, 1755, 1606, 1516, 1455, 1387, 1338. LRMS: 377 ($M^{\bullet+}$ – H, 1), 246 (13), 181 (13), 121 (3), 91 (100), 77 (5), 65 (17), 51 (6). For $C_{20}H_{18}N_4O_2S$ calculated: 63.47% C, 4.79% H, 14.80% N, 8.47% S; found: 63.14% C, 4.89% H, 14.70% N, 8.28% S.

Preparation of Compounds **21a**–**21e**. General Procedure

Tetrazole **6** (1.000 g, 5.61 mmol) was dissolved in toluene (20 ml), and a 1 M aqueous sodium hydroxide solution (20 ml), and alkyl halide R–X (15.00 mmol) and tetrabutylammonium bromide (0.160 g, 0.31 mmol) were added. The reaction mixture was heated under reflux with rapid stirring for 3 h. After cooling to room temperature, the layers were separated and the organic layer was washed with water (3 × 30 ml), dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane–ethyl acetate 9:1).

5-(Benzylsulfanyl)-1-phenyl-1H-tetrazole (**21a**)[21]: R–X = benzyl bromide. Yield 84%. White crystals, m.p. 68–69 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.56–7.50 (5 H, m, H2, H3, H4, H5, H6), 7.47–7.40 (2 H, m, H2′, H6′), 7.38–7.27 (3 H, m, H3′, H4′, H5′), 4.62 (2 H, s, CH_2).

5-[(3-Methoxybenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**21b**): R–X = 3-methoxybenzyl bromide. Yield 74%. White crystals, m.p. 79–81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.56–7.49 (5 H, m, H2, H3, H4, H5, H6), 7.22 (1 H, d, J = 8.0, H5′), 7.02–6.93 (2 H, m, H2′, H6′), 6.83 (1 H, dd, J = 8.0, J = 2.5, H4′), 4.67 (2 H, s, CH_2), 3.78 (s, 3 H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 159.8, 153.9, 136.6, 133.5, 130.1, 129.8, 129.7, 123.8, 121.5, 114.6, 113.8, 55.2, 37.6.

5-[(4-Methoxybenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**21c**)[22]: R–X = 4-methoxybenzyl chloride. Yield 55%. White crystals, m.p. 51–53 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.55–7.50 (5 H, m, H2, H3, H4, H5, H6), 7.38–7.31 (2 H, m, AA′BB′, H3′, H5′), 6.87–6.82 (2 H, m, AA′BB′, H2′, H6′), 4.59 (2 H, s, CH_2), 3.79 (3 H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 159.5, 154.0, 133.6, 130.5, 130.0, 129.7, 127.0, 123.8, 114.2, 55.3, 37.3.

5-[(4-Chlorobenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**21d**)[22]: R–X = 4-chlorobenzyl chloride. Yield 25%. White crystals, m.p. 98–101 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.55–7.50 (5 H, m, H2, H3, H4, H5, H6), 7.40–7.34 (2 H, m, AA′BB′, H3′, H5′), 7.31–7.26 (2 H, m, AA′BB′, H2′, H6′), 4.58 (2 H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 153.5, 134.1, 133.9, 133.5, 130.6, 130.2, 129.8, 129.0, 123.7, 36.7.

5-[(4-Fluorobenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**21e**)[22]: R–X = 4-fluorobenzyl chloride. Yield 94%. White crystals, m.p. 87–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.55–7.50 (5 H, m, H2, H3, H4, H5, H6), 7.45–7.37 (2 H, m, H3′, H5′), 7.05–6.96 (2 H, m, H2′, H6′), 4.59 (2 H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 162.5 (d, J = 247.4), 153.7, 133.5, 131.1, 131.0 (d, J = 8.3), 130.2, 129.8, 123.8, 115.7 (d, J = 21.8), 36.8.

5-[(4-Nitrobenzyl)sulfanyl]-1-phenyl-1*H*-tetrazole (**21f**)[21]

Tetrazole **6** (1.000 g, 5.61 mmol) and freshly prepared sodium methoxide (7.30 mmol) were dissolved in dry DMF (6 ml) and, after 10-min stirring at room temperature, 4-nitrobenzyl chloride (1.440 g, 8.41 mmol) was added. The reaction mixture was stirred at 100 °C for 48 h, then cautiously poured into water (30 ml), and the resultant mixture was washed with ethyl acetate (50 ml). The organic layer was washed with a 5% aqueous hydrochloric acid solution (30 ml), water (30 ml) and a 5% aqueous sodium hydrogencarbonate solution (30 ml). The organic layer was then dried (Na_2SO_4), the solvents removed under reduced pressure, and the crude product purified by column chromatography (hexane–ethyl acetate 75:25) to afford tetrazole **21f**. Yield 40%. Yellowish crystals, m.p. 151–153 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.21–8.14 (2 H, m, AA′BB′, H3′, H5′), 7.69–7.61 (2 H, m, AA′BB′, H2′, H6′), 7.58–7.48 (5 H, m, H2, H3, H4, H5, H6), 4.67 (2 H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 153.0, 147.6, 143.1, 133.3, 130.4, 130.2, 129.9, 123.9, 123.7, 36.3.

This work was supported by the Centre for New Antivirals and Antineoplastics funded by the Ministry of Education, Youth and Sports of the Czech Republic (1M0508) and by the Czech Science Foundation (project No. 203/07/1302). It is also a part of the research project MSM0021620822 of the Ministry of Education, Youth and Sports of the Czech Republic.

REFERENCES AND NOTES

1. Wermuth C. G.: *The Practice of Medicinal Chemistry*, 2nd ed., Part IV. Academic Press, London 2003.
2. Kuneš J., Hrabálek A., Pour M., Pilař M., Waisser K., Odlerová Ž.: *Zh. Org. Khim.* **1998**, *34*, 786.
3. Adamec J., Beckert R., Weiss D., Klimešová V., Waisser K., Möllmann U., Kaustová J., Buchta V.: *Bioorg. Med. Chem.* **2007**, *15*, 2898.
4. Pour M., Špulák M., Balšánek V., Kuneš J., Buchta V., Waisser K.: *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1893.
5. Pour M., Špulák M., Buchta V., Kubanová P., Vopršalová M., Wsól V., Fáková H., Koudelka P., Pourová H., Schiller R.: *J. Med. Chem.* **2001**, *44*, 2701.
6. Pour M., Špulák M., Balšánek V., Kuneš J., Kubanová P., Buchta V.: *Bioorg. Med. Chem.* **2003**, *11*, 2843.
7. Buchta V., Pour M., Kubanová P., Silva L., Votruba I., Vopršálová M., Schiller R., Fáková H., Špulák M.: *Antimicrob. Agents Chemother.* **2004**, *48*, 873.
8. Kuneš J., Balšánek V., Pour M., Buchta V.: *Collect. Czech. Chem. Commun.* **2001**, *66*, 1809.
9. Raap R.: *Can. J. Chem.* **1971**, *49*, 2139.
10. Hrabálek A., Puš L., Baránek V., Kuneš J., Palát K.: *Chem. Heterocycl. Comp.* **2002**, *38*, 183.
11. Stollé R., Henke-Stark F. J.: *Prakt. Chem.* **1929/1930**, *124*, 261.
12. Farina V., Kapadia S., Krishnan B., Wang C., Liebeskind L. S.: *J. Org. Chem.* **1994**, *59*, 5905.
13. Kauer J. C., Sheppard W. A.: *J. Org. Chem.* **1967**, *32*, 3580.
14. Jacobson C. R., Amstutz E. D.: *J. Org. Chem.* **1953**, *18*, 1183.
15. Yajima A., Mori K.: *Liebigs Ann.* **1996**, 1091.
16. Dubenko R. G., Panchenko V. D.: *Khim. Geterotsikl. Soedin. Sb.: Azotsoderzhashchie Geterotsikly* **1967**, 199.
17. Stachel H.-D., Odlerová Ž., Waisser K.: *Collect. Czech. Chem. Commun.* **1997**, *62*, 510.
18. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard, NCCLS Document M27-A, 771 E. Lancaster Avenue, Villanova, PA 19085, 1997.
19. Hocek M., Holý A., Votruba I., Dvořáková H.: *J. Med. Chem.* **2000**, *43*, 1817.
20. Carmichael J., DeGraff W. G., Gazdar A. F., Minna J. D., Mitchell J. B.: *Cancer Res.* **1987**, *47*, 936.
21. Goltsberg M. A., Koldobskii G. I.: *Russ. J. Org. Chem.* **1996**, *32*, 1194.
22. Adamec J., Waisser K., Kuneš J., Kaustová J.: *Arch. Pharm.* **2005**, *338*, 385.