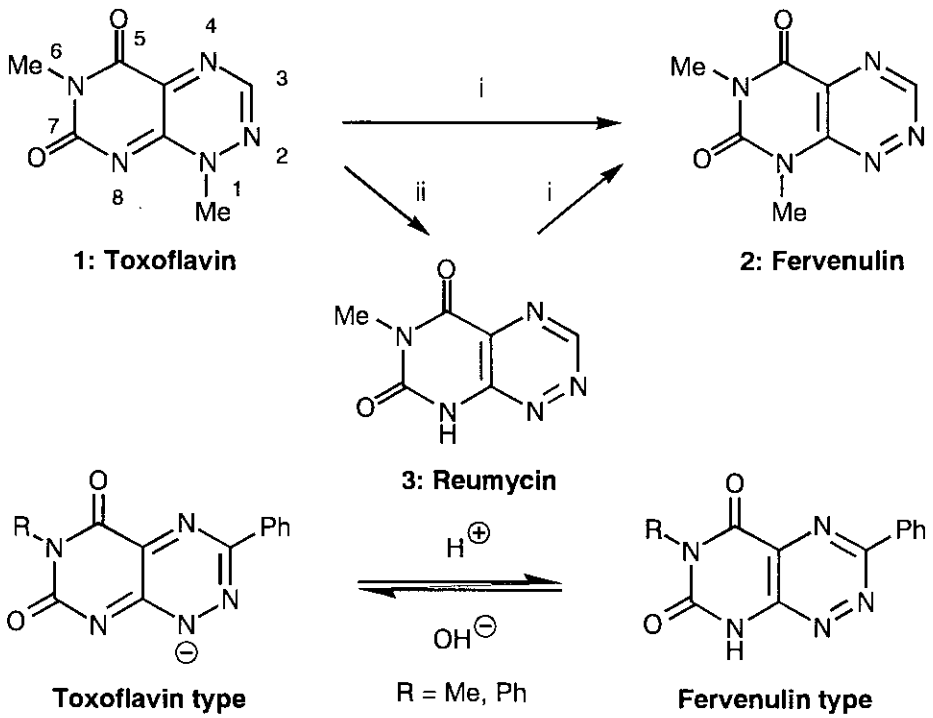
FACILE AND GENERAL SYNTHESES OF 1-ALKYLTOXOFLAVIN AND 8-ALKYLFERVENULIN DERIVATIVES OF BIOLOGICAL SIGNIFICANCE BY THE REGIOSPECIFIC ALKYLATION OF REUMYCIN (1-DEMETHYLTOXOFLAVIN, 8-DEMETHYL-FERVENULIN) DERIVATIVES

Tomohisa Nagamatsu* **and Hirofumi Yamasaki**

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

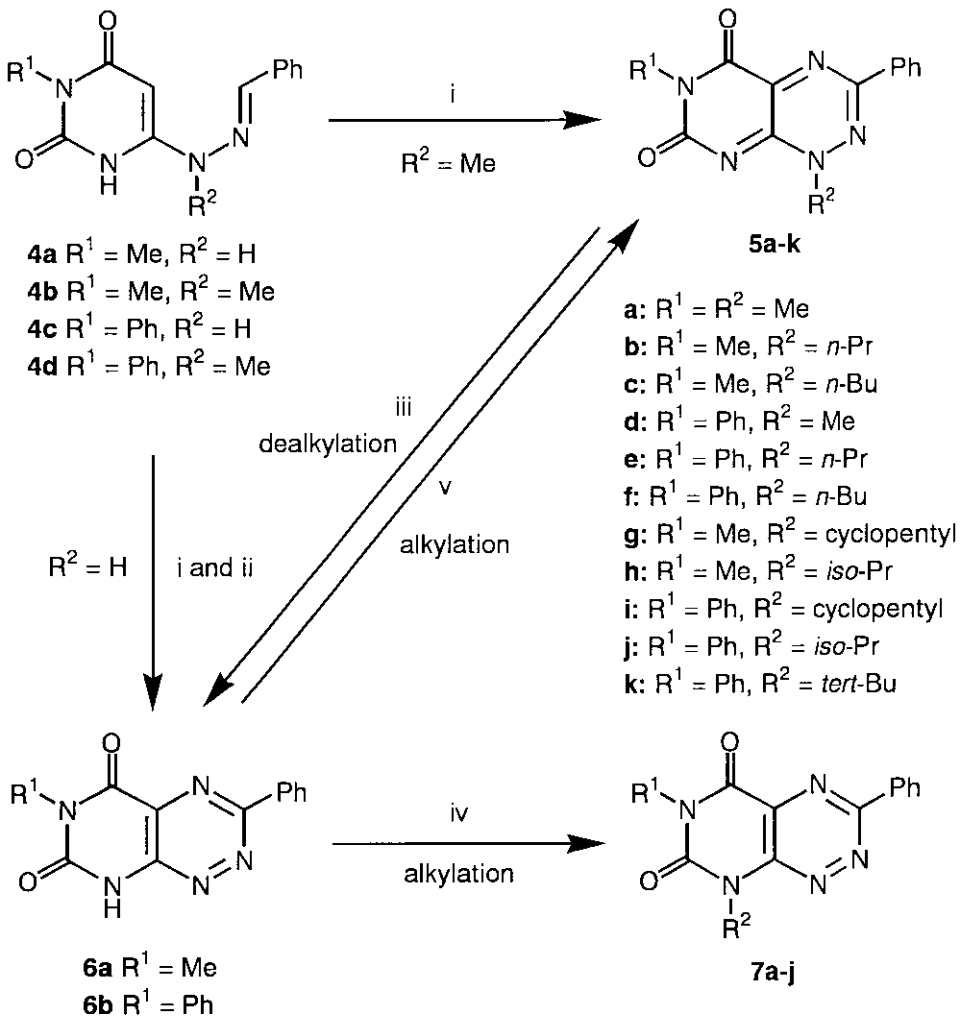
Abstract —— Regiospecific alkylation of reumycins (**6**) under alkaline conditions with a dialkyl sulfate or alkyl halide in dioxane and in DMF to provide 1-alkyltoxoflavins (**5**) of biological significance and 8-alkylfervenulins (**7**), respectively, is described.

Since the isolation and characterization of the natural occurring antibiotics of 7-azapteridines (pyrimido[5,4-*e*]-1,2,4-triazines) such as toxoflavin (**1**),[1] fervenulin (**2**)[2] and reumycin (**3**)[3] isolated from *Pseudomonas cocovenenans*, *Streptomyces fervens* n. sp. and *Actinomyces*, respectively, the 7-azapteridines have been the subjects of a great deal of synthetic study,[4] because of their marked biological activities[5] (**Scheme 1**). We have previously reported several convenient syntheses of toxoflavin (**1**) and its 3- and/or 6-substituted derivatives,[6] and evaluated their potent anti-viral[5] and anti-tumor activities.[7] These findings prompted us to explore synthetic methods to prepare 1-substituted toxoflavin type analogs and structure-activity relationships for such activities. However, we encountered difficulties when attempting to prepare the derivatives having a substituent of some kind at the 1-position of the toxoflavin skeleton (**1**) by a method of nitrosative or nitrative cyclization of the aldehyde hydrazones of 6-(1-alkylhydrazino)uracils (**4**).[5,6]



Scheme 1. *Reagents and conditions*: i, MeI, K_2CO_3, DMF, reflux; ii, DMF, reflux.

Because it was not easy to get monosubstituted hydrazines except for methylhydrazine, the preparation of several 6-(1-alkylhydrazino)uracils as intermediates of the hydrazones (**4**) was difficult. Previously, we have reported that toxoflavin (**1**) and its 3-substituted derivative (**5a**) readily underwent demethylation at the 1-position with several nucleophiles such as DMF and dimethylacetamide to give the corresponding 1-demethyltoxoflavins (**3**, **6a**), while the nucleophiles themselves were methylated by the methyl group eliminated, and during the reactions novel radical species were observed.[8] Moreover, the methylation of compounds (**3**, **6a**) under alkaline conditions with dimethyl sulfate or methyl iodide in DMF provided not toxoflavins (**1**, **5a**) but fervenulins (**2**, **7a**).[9] We have formerly investigated that the biological activities of toxoflavins (**1**) were stronger than those of fervenulins (**2**) and reumycins (**3**).[5,7] Despite several biological activities of the 1-substituted toxoflavin derivatives (**5**) are expected, no report on the synthesis except for compounds (**1**, **5a**, **5d**) is available so far. Here we report a convenient and general methodology for the preparation of 1-alkyltoxoflavin derivatives (**5a-k**) by the regiospecific alkylation of



Scheme 2. *Reagents and conditions*: i, $NaNO_2$, AcOH, 5-10 °C; ii, Ac_2O, reflux; iii, DMF, 140 °C or *n*-$BuNH_2$, dioxane, 100 °C; iv, $(R^2O)_2SO_2$, R^2I or R^2Br, K_2CO_3, DMF, 140 °C; v, $(R^2O)_2SO_2$ or R^2Br, K_2CO_3, dioxane, 120 °C.

reumycin derivatives (**6a**,**b**) under alkaline conditions with a dialkyl sulfate or alkyl halide in dioxane. 3-Phenyltoxoflavins (**5a**,**d**) were prepared by nitrosative cyclization of the corresponding 6-(2-benzylidene-1-methylhydrazino)-3-methyl (or phenyl)uracils (**4b**,**d**) according to the reported method[5,6] (**Scheme 2**). Compounds (**5a**,**d**) thus obtained were heated in DMF at 140 °C for 2 h to afford the desired key intermediates, 3-phenylreumycins (**6a**,**b**). Compounds (**6a**,**b**) were also prepared by nitrosation of 6-benzylidenehydrazino-3-methyl (or phenyl)uracils (**4a**,**c**), followed by refluxing the 5-nitroso intermediates

formed with acetic anhydride for 1 h.[10] As we reported previously, 3-phenyltoxoflavin (**5a**) was transformed into 3-phenylfervenulin (**7a**) *via* 3-phenylreumycin (**6a**) by usual alkylating conditions in DMF.[9] That is to say, the alkylation of **6a** in DMF proceeded predominantly into the direction of the 8-position to afford 3-phenylfervenulin (**7a**) due to the demethylation of 3-phenyltoxoflavin (**5a**) by DMF. Liao *et al.*[11] suggested that an equilibrium existed between 1-demethyltoxoflavin and 8-demethylfervenulin under different environmental conditions. We also found a similar fact in comparison of the ultraviolet absorption spectra of 3-phenyl derivatives of reumycin (**6a,b**) in different media as shown in **Scheme 1**. Namely, the spectra of **6a** in neutral and acidic media (pH 1) resembled those of fervenulin (**7a**) in dioxane.[12] In basic medium (pH 10) the spectrum of **6a** (as the anion) was strikingly similar to that of toxoflavin (**5a**) in dioxane. For the purpose of the regiospecific alkylation at the 1-position of reumycins (**3**, **6a,b**), it has been found that the choice of reaction solvent in the basic medium was the most important. Now we found dioxane as the most suitable solvent for the regiospecific alkylation.

First, the primary alkylation of reumycin derivatives (**6a,b**) is describe below. As can be seen from Runs 1, 4, 6, 8, 10 and 12 in **Table 1**, a mixture of **6a** or **6b** (2 mmol), dialkyl sulfate (6 mmol) and potassium carbonate (4 mmol) in dioxane (50 ml) was heated in a sealed vessel under atmosphere of argon at 120 °C for 2 h to afford the corresponding 1-alkyltoxoflavin type compounds (**5a-f**) in good yields. However, the use of methyl iodide as the alkylating agent in the reaction with **6a** gave not **5a** but fervenulin type compound (**7a**) in 80% yield (Run 3). When the same reaction was carried out in DMF (50 ml) instead of dioxane at 140 °C for 2 h, the corresponding 8-alkylfervenulin type compounds (**7a-f**) only formed in good yields (Runs 2, 5, 7, 9, 11 and 13). Next, the secondary alkylation is as follows. The reaction of **6a** or **6b** in dioxane with alkyl bromide (3 equi.) such as cyclopentyl bromide and *iso*-propyl bromide and potassium carbonate (2 equi.) in the same reaction conditions as mentioned above afforded 1-alkyltoxoflavins (**5g-j**) (Runs 14, 16, 18 and 20), whereas the reaction in DMF yielded 8-alkylfervenulins (**7g-j**) (Runs 15, 17, 19 and 21) in good yields. Finally, the reaction of **6b** in dioxane with the tertiary alkylating agent such as *tert*-butyl bromide (3 equi.) and potassium carbonate (2 equi.) in a similar manner afforded 1-*tert*-butyltoxoflavin (**5k**) in 85% yield (Run 22). When the same reaction was carried out in DMF, no alkylated product was obtained owing to steric hindrance between the carbonyl at the 7-position and the *tert*-butyl group at the 8-position to produce the expected 8-alkylfervenulin (**7k**) (Run 23). The structures of

Table 1. Conditions and Yields for Selected Preparations of Toxoflavin (**5a-k**) and Fervenulin Derivatives (**7a-j**) by Alkylation of Reumycin Derivatives (**6a,b**)

Run	Substrate	Substituents		Alkylating agent	Rection conditions[a]		Yields (%) and mp (°C) of isolated products[b]	
		R^1	R^2		Solvent	Temp. (°C)	Toxoflavin-type[c]	Fervenulin-type[c]
1	**6a**	Me	Me	$(R^2O)_2SO_2$	dioxane	120	**5a**: 91, 228 (decomp)[d]	N.I.
2	**6a**	Me	Me	$(R^2O)_2SO_2$	DMF	140	N.I.	**7a**: 89, 277-279[e]
3	**6a**	Me	Me	R^2I	dioxane	120	N.I.	**7a**: 80, 277-279[e]
4	**6a**	Me	*n*-Pr	$(R^2O)_2SO_2$	dioxane	120	**5b**: 80, 169 (decomp)	N.I.
5	**6a**	Me	*n*-Pr	$(R^2O)_2SO_2$	DMF	140	N.I.	**7b**: 78, 212-214[e]
6	**6a**	Me	*n*-Bu	$(R^2O)_2SO_2$	dioxane	120	**5c**: 82, 172 (decomp)	N.I.
7	**6a**	Me	*n*-Bu	$(R^2O)_2SO_2$	DMF	140	N.I.	**7c**: 81, 179-181
8	**6b**	Ph	Me	$(R^2O)_2SO_2$	dioxane	120	**5d**: 86, 245 (decomp)[f]	N.I.
9	**6b**	Ph	Me	$(R^2O)_2SO_2$	DMF	140	N.I.	**7d**: 85, 269-271
10	**6b**	Ph	*n*-Pr	$(R^2O)_2SO_2$	dioxane	120	**5e**: 88, 135 (decomp)	N.I.
11	**6b**	Ph	*n*-Pr	$(R^2O)_2SO_2$	DMF	140	N.I.	**7e**: 84, 237-239
12	**6b**	Ph	*n*-Bu	$(R^2O)_2SO_2$	dioxane	120	**5f**: 82, 178 (decomp)	N.I.
13	**6b**	Ph	*n*-Bu	$(R^2O)_2SO_2$	DMF	140	N.I.	**7f**: 80, 220-222
14	**6a**	Me	cyclopentyl	R^2Br	dioxane	120	**5g**: 81, 228-230	N.I.
15	**6a**	Me	cyclopentyl	R^2Br	DMF	140	N.I.	**7g**: 65, 211-213
16	**6a**	Me	*iso*-Pr	R^2Br	dioxane	120	**5h**: 85, 252-254	N.I.
17	**6a**	Me	*iso*-Pr	R^2Br	DMF	140	N.I.	**7h**: 67, 224-226[e]
18	**6b**	Ph	cyclopentyl	R^2Br	dioxane	120	**5i**: 85, 224-226	N.I.
19	**6b**	Ph	cyclopentyl	R^2Br	DMF	140	N.I.	**7i**: 72, 247-249
20	**6b**	Ph	*iso*-Pr	R^2Br	dioxane	120	**5j**: 78, 237-239	N.I.
21	**6b**	Ph	*iso*-Pr	R^2Br	DMF	140	N.I.	**7j**: 82, 244-246
22	**6b**	Ph	*tert*-Bu	R^2Br	dioxane	120	**5k**: 85, 275-277	N.I.
23	**6b**	Ph	*tert*-Bu	R^2Br	DMF	140	N.I.	N.I.

[a]All reactions under atmosphere of argon in a sealed vessel were heated in the presence of potassium carbonate as a base for 2 h. [b]All products were recrystallised from 40% aqueous dioxane and were obtained as yellow or orange needles. N.I. means not isolated. [c]Satisfactory elemental combustion analyses and MS, IR, and ^{1}H-NMR spectral data were obtained for all new compounds. [d]See reference 6. [e]See reference 9. [f]See reference 5.

5a,d and **7a,b,h** were determined by comparison of ir and ^{1}H-NMR spectral data of the authentic samples, and other new compounds (**5b,c, e-k** and **7c-g, i,j**) were assigned on the basis of elemental analyses and satisfactory spectral data. The ultraviolet spectra of the toxoflavin type compounds (**5a-k**) in dioxane showed two maximum absorption bands at *ca.* 295 nm and 435 nm, while those of the fervenulin type compounds (**7a-j**) showed at *ca.* 280 nm and 375 nm.[13]

It is necessary to evaluate the extent of dealkylation of 1-alkyltoxoflavin type derivatives (**5**) to examine their chemical stabilities. **Table 2** shows the transalkylation from toxoflavin derivative (**5**) to nucleophiles such as DMF and *n*-butylamine. The reaction was carried out by heating compounds (**5a, c-k**) (3 mmol) with DMF (10 ml) at 140 °C for 3 h and by heating compounds (**5g-k**) (3 mmol) with a mixture of *n*-butylamine (1 ml) and dioxane (10 ml) at 100 °C for 1 h. The rate of dealkylation in DMF was apparently retarded with increasing the alkyl chain length at the 1-position of the toxoflavin derivatives (**5d-f**) (see run

Table 2. Transalkylation from Toxoflavin Derivatives (**5**) to Nucleophiles such as DMF and *n*-Butylamine to produce Reumycin Derivatives (**6**)

Run	Starting material	R^1	R^2	Work-up procedure[a]	Proportion of isolated products **5** : **6**
1	**5a**	Me	Me	A	0 : 100
2	**5c**	Me	*n*-Bu	A	0 : 100
3	**5d**	Ph	Me	A	0 : 100
4	**5e**	Ph	*n*-Pr	A	10 : 90
5	**5f**	Ph	Bu	A	50 : 50
6	**5g**	Me	cyclopentyl	A	100 : 0
7	**5h**	Me	*iso*-Pr	A	100 : 0
8	**5i**	Ph	cyclopentyl	A	100 : 0
9	**5j**	Ph	*iso*-Pr	A	100 : 0
10	**5k**	Ph	*tert*-Bu	A	100 : 0
11	**5g**	Me	cyclopentyl	B	0 : 100
12	**5h**	Me	*iso*-Pr	B	0 : 100
13	**5i**	Ph	cyclopentyl	B	0 : 100
14	**5j**	Ph	*iso*-Pr	B	0 : 100
15	**5k**	Ph	*tert*-Bu	B	50 : 50

[a]Reaction conditions A: in DMF at 140 °C for 3 h; reaction conditions B: in *n*-butylamine / dioxane (1 / 10) at 100 °C for 1 h.

3-5). On the other hand, the dealkylation of **5g-k** having a secondary or tertiary alkyl group was not observed in this conditions (see Runs 6-10). However, the dealkylation of **5g-k** by *n*-butylamine took place easily (see Runs 11-15).

Thus, we have described the first successful synthesis of 1-substituted toxoflavin derivatives (**5**) by the regiospecific alkylation of reumycins (**6**), and this simple methodology provided a facile and convenient route to the preparation of 1-alkyltoxoflavins (**5**) which are biologically more active than 8-alkylfervenulins (**7**). Further detailed study for the regiospecific alkylation along with elucidation of the alkylation mechanism is in progress. Besides, these compounds could be of use as anti-tumor agent[14] and studies are also in progress towards that direction.

REFERENCES AND NOTES

1. A. G. van Veen and J. K. Baars, *Proc. Akad. Wetenschappen Amsterdam*, 1937, **40**, 498; R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell, and J. Charney, *Antibiotics and Chemotherapy,* 1954, **4**, 259.
2. C. DeBoer, A. Dietz, J. S. Evans, and R. M. Michael, *Antibiotics Annual,* 1959-1960, 220; K. Tanabe, Y. Asahi, M. Nishikawa, T. Shima, Y. Kuwada, T. Kanzawa, and K. Ogata, *Takeda Kenkyusho Nempo.,* 1963, **22**, 133 (*Chem. Abstr.,* 1964, **60**, 13242).
3. S. E. Esipov, M. N. Kolosov, and L. A. Saburova, *J. Antibiot.,* 1973, **26**, 537.
4. G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Am. Chem. Soc.,* 1961, **83**, 3904; G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Org. Chem.,* 1961, **26**, 5256; G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Am. Chem. Soc.,* 1962, **84**, 1724; F. Yoneda, K. Shinomura, and S. Nishigaki, *Tetrahydron Lett.,* 1971, 851; E. C. Taylor and F. Sowinsky, *J. Org. Chem.,* 1975, **40**, 2321; T. Nagamatsu, H. Yamasaki, and F. Yoneda, *Heterocycles*, 1994, **37**, 1147.
5. T. Nagamatsu, H. Yamasaki, T. Hirota, M. Yamato, Y. Kido, M. Shibata, and F. Yoneda, *Chem. Pharm. Bull.,* 1993, **41**, 362 and references cited therein.
6. F. Yoneda and T. Nagamatsu, *Chem. Pharm. Bull.,* 1975, **23**, 2001; F. Yoneda and T. Nagamatsu, *Synthesis,* 1975, 177; F. Yoneda and T. Nagamatsu, *Chem. Pharm. Bull.,* 1975, **23**, 1885; F. Yoneda, T. Nagamatsu, and K. Shinomura, *J. Chem. Soc., Perkin Trans. 1,* 1976,

713; F. Yoneda, Y. Sakuma, T. Nagamatsu, and S. Mizumoto, *J. Chem.Soc., Perkin Trans. 1,* 1976, 2398.

7. T. Nagamatsu, Y. Yamagishi, F. Yoneda, and A. Uehara, submitted to Japanese patent (application No.: H8-67814), March 25, 1996 (received No.: 59600136046).
8. F. Yoneda and T. Nagamatsu, *J. Am. Chem. Soc.,* 1973, **95**, 5735; F. Yoneda, T. Nagamatsu, and M. Ichiba, *J. Heterocycl. Chem.,* 1974, **11**, 83.
9. F. Yoneda and T. Nagamatsu, *Tetrahedron Lett.,* 1973, 1577; F. Yoneda and T. Nagamatsu, *J. Heterocycl. Chem.*, 1974, **11**, 271; F. Yoneda and T. Nagamatsu, *Bull. Chem. Soc. Jpn.,* 1975, **48**, 2884.
10. F. Yoneda, M. Kawamura, T. Nagamatsu, K. Kuretani, A. Hoshi, and M. Iigo, *Heterocycles,* 1976, **4**, 1503.
11. T. K. Liao, F. Baiocchi, and C. C. Cheng, *J. Org. Chem.,* 1966, **31**, 900.
12. *Ultraviolet spectral data* : **compound**: λ_{max} (log e) (solvent). **5a**: 293 (4.29), 434 (3.30) (dioxane); **6a**: 271 (4.26), 367 (3.42) (dioxane); 270 (4.21), 367 (3.11) (pH 1 buffer); 298 (4.19), 430 (3.39) (pH 10 buffer); **7a**: 277 (4.30), 375 (3.41) (dioxane).
13. *Ultraviolet spectral data* : **compound**: λ_{max} (log e) in dioxane. **5b**: 294 (4.33), 436 (3.35); **5c**: 294 (4.41), 435 (3.47); **5d**: 296 (4.37), 434 (3.38); **5e**: 295 (4.41), 436 (3.36); **5f**: 294 (4.48), 436 (3.47); **5g**: 295 (4.38), 436 (3.45); **5h**: 294 (4.30), 436 (3.33); **5i**: 299 (4.48), 436 (3.51); **5j**: 297 (4.41), 435 (3.41); **5k**: 299 (4.48), 430 (3.55); **7b**: 276 (4.34), 375 (3.33); **7c**: 281 (4.36), 377 (3.46); **7d**: 280 (4.38), 374 (3.44); **7e**: 281 (4.29), 376 (3.55); **7f**: 280 (4.41), 376 (3.49); **7g**: 280 (4.33), 376 (3.43); **7h**: 276 (4.33), 375 (3.32); **7i**: 282 (4.47), 374 (3.53); **7j**: 281 (4.41), 374 (3.44).
14. From preliminary studies compounds (**5**) were found to be active against CCRF-HSB-2 human lymphoblastic leukemia (T-cell) *in vitro* with IC_{50} being **5a**: 0.52, **5b**: 0.69, **5g**: 0.58 and **5e**: 1.02 μg/ml, and against KB-cell *in vitro* with IC_{50} being **5b**: 0.61, **5g**: 0.35, **5h**: 0.51 and **5k**: 0.73 μg/ml etc.

Received, 24th January, 1997