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Original article

Synthesis, antiprotozoal and anticancer activity of substituted 2-trifluoromethyl- and 2-pentafluoroethylbenzimidazoles

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Abstract

The synthesis of several halogenated benzimidazoles substituted in position 2 with trifluoromethyl, pentafluoroethyl and 2-thioethylaminodimethyl group is reported. Antiprotozoal and anticancer activity of series of newly synthesized and previously obtained compounds was studied. All of tested bezimidazoles showed remarkable antiprotozoal activity against *Giardia intestinalis*, *Entamoeba histolytica* and *Trichomonas vaginalis*. Of the studied collection of halogenated benzimidazoles the most anticancer-active was the 5,6-dichloro-2-pentafluoroethyl compound, particularly against breast and prostate cancer cell lines.

Keywords: 2-Trifluoromethylbenzimidazoles; 2-Pentafluoroethylbenzimidazoles; Antiprotozoal activity; Anticancer activity

1. Introduction

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications [1]. This heterocyclic ring system is present in numerous antiparasitic, fungicidal, anthelmintic and antiinflammatory drugs (see Refs. [2–5]). Substituted 2-trifluorobenzimidazoles are potent decouplers of oxidative phosphorylation in mitochondria. These compounds also inhibit photosynthesis and therefore exhibit appreciable herbicidal activity [6]. Their antibacterial and antifungal activity was also observed [7,8]. Most recently, antiprotozoal activity of 2-trifluoromethylbenzimidazoles, in particular that of their chlorosubstituted derivatives, was reported [9], which is consistent with earlier observations of antigiardial activity of various benzimidazole derivatives [10,11]. These findings have inspired us to widen the list of 2-trifluoromethylbenzimidazoles and 2-pentafluorobenzimidazoles, especially with a series of bromine-substituted derivatives, and extend our study on the relationship between the substituted benzimidazoles' structure and antiprotozoal activity. Some of polyhalogenosubstituted benzotriazoles are inhibitors of regulatory enzymes, particularly casein kinases 1 and 2 [12], and helicases [13]. Because some of the previously described benzotriazoles, as well as the newly studied compounds shown below, reveal certain structural similarity to the above mentioned benzotriazoles and therefore may exert a number of effects on the metabolism of living cells, we decided to test also their anticancer activity. In addition to the previously described derivatives [6,14], we have also synthesized for this study a number of new 2-trifluoromethyl- and 2-pentafluoroethyl-benzimidazole derivatives.

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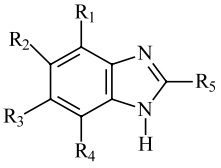
2. Chemistry

The structures of studied benzimidazoles **1a**–**k** and their 4-aza and 5-aza congeners **2a** and **2b** are presented in Fig. 1. The 2-trifluoromethylbenzimidazoles **1b**, **1i** and **1k** that were substituted in the benzene part of the benzimidazole ring system were obtained by condensation of the corresponding *o*-phenylenediamine **3** with trifluoroacetic acid or pentafluoropropionic acid in 4 N hydrochloric acid under the reflux conditions. In the case of trifluoroacetic acid the reaction was accomplished within 4–5 h, whereas the synthesis of 2-pentafluorethylbenzimidazoles using pentafluoropropionic acid required longer heating times. Several bromine-substituted derivatives were synthesized by Büchel [14] by direct bromination of 2-trifluoromethylbenzimidazole. We have observed that the preparation of 4,5,6-tribromo-2-trifluoromethyl- and 4,5,6,7-tetrabromo-2-trifluoromethylbenzimidazole using this procedure gives chromatographically and spectrally pure compounds. However, the lower brominated derivatives i.e. 5-bromo- and 5,6-dibromo-compounds resulted in the formation of some impurities that were difficult to purge away. For the synthesis of 5-bromo- 5,6-dibromo- and 4,6-dibromo-derivatives the use of the corresponding *o*-diamines for the condensation guaranteed pure compounds **1c** and **1d**. The exhaustive bromination of 2-pentafluoroethylbenzimidazole **4** in water provided the expected 4,5,6,7-tetrabromo-2-pentafluoroethylbenzimidazole **5**. Condensation of 3,5-dibromo-1,2-phenylenediamine **6** with carbon disulfide yielded 4,6-dibromo-2-thiobenzimidazole **7**. The latter gave the corresponding 2-dimethylaminoethylthio-compound **8** following alkylation with dimethylaminoethylchloride (Fig. 2). New compounds were characterized by elemental analysis, UV and H-NMR spectroscopy.



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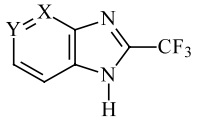

2

a $R_1, R_2, R_3, R_4 = H, R_5 = CF_3$ [14]
b $R_1, R_2, R_3 = F, R_4 = H, R_5 = CF_3$
c $R_1, R_3, R_4 = H, R_2 = Br, R_5 = CF_3$ [14]
d $R_1, R_4 = H, R_2, R_3 = Br, R_5 = CF_3$ [14]
e $R_1, R_3 = Br, R_2, R_4 = H, R_5 = CF_3$ [6]
f $R_1, R_2, R_3 = Br, R_4 = H, R_5 = CF_3$ [14]
g $R_1, R_2, R_3, R_4 = Br, R_5 = CF_3$ [14]
h $R_1, R_4 = H, R_2, R_3 = Cl, R_5 = CF_3$ [9,14]
i $R_1, R_4 = H, R_2, R_3 = Cl, R_5 = C_2F_5$
j $R_1, R_4 = H, R_2, R_3 = CH_3, R_5 = C_2F_5$
k $R_1, R_4 = H, R_2, R_3 = NO_2, R_5 = CF_3$ [14]

a X = N, Y = CH [18]
b X = CH, Y = N [18]

Fig. 1. Structure of investigated benzimidazole derivatives.

3. Biological results and discussion

3.1. Antiprotozoal activity studies

In the antiprotozoal activity assays, metronidazole and albendazole were used as reference compounds because they both are highly effective for giardiasis, and metronidazole is widely used also for the treatment of amoebiasis and trichomoniasis. Detailed results of the biological activity tests are shown in Table 1.

All the newly synthesized compounds were found to be considerably active against *Giardia intestinalis*. Notably, compounds **5** and **8** were as active as albendazole against this parasite and their IC_{50}s were about 20-fold lower on molar basis than that of metronidazole. All the newly synthesized compounds showed higher activity than albendazole against *Entamoeba histolytica*, and compound **1e** with IC_{50} sevenfold lower than that of metronidazole showed the highest activity against this protozoan. Compound **1g** was the one most active against *Trichomonas vaginalis*, and its IC_{50} was 110-fold lower than that of metronidazole; the efficacy of compounds **1d** and **1e** against the same species was similar to that of metronidazole.

Some of the new compounds tested, e.g. derivatives **1e** and **1d**, were similarly active against all three protozoan species used in this study. However, some compounds that were highly active against one parasite species showed considerably weaker action against the other two species used. For instance, derivatives **5** and **8** had the lowest IC_{50} against *G. intestinalis*, but were much less active against *E. histolytica* and *T. vaginalis* (yet, compound **5** had good activity against *E. histolytica* and compound **8** showed high activity against *T. vaginalis*), whereas compound **1g** was markedly more active against *T. vaginalis* than against *E. histolytica* and *G. intestinalis* (however, the activity against these two latter parasites was also very high).

The newly synthesized compounds **1d** and **1e** are trifluoromethyl derivatives substituted each with two bromine atoms in position 5 and 6, and 4 and 6, respectively, and thus are closely related to trifluoromethylbenzimidazoles the synthesis and biological activity of which against the same three protozoans were recently reported from this laboratory (9). One of those compounds, namely 5,6-dichloro-2-trifluoromethylbenzimidazole, was 15.6- and 32-fold more active than metronidazole against *G. intestinalis* and *E. histolytica*, respectively. It is noteworthy that the activity of compound **1d** against these two parasite species was, in relative terms (see Table 1), markedly lower than that of its 5,6-dichlorinated analog.

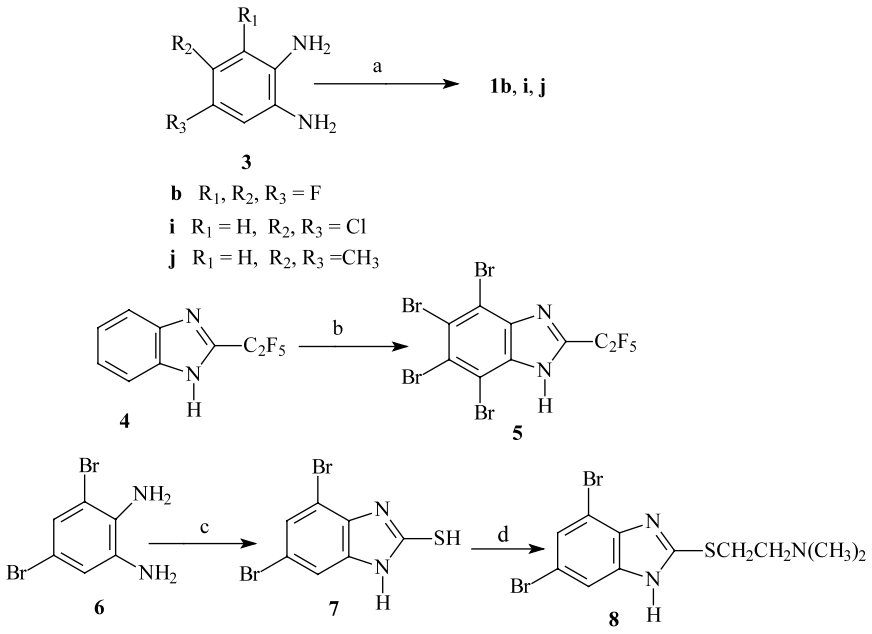

Fig. 2. Synthetic procedures for compounds **1b**, **1i**, **1j**, **5** and **6**–**8**. Reagents and conditions: (a) CF_3COOH or C_2F_5COOH, 4 N HCl, reflux; (b) bromine, water, reflux, 3 days; (c) CS_2, KOH, EtOH–water, reflux; (d) acetonitrile, DBU, $Cl(CH_2)_2N(CH_3)_2 \times HCl$, 70 °C, 3 h.

The presence of pentafluoroethyl group instead of trifluoromethyl group in position 2 of the benzimidazole nucleus did not substantially enhance the activity against *E. histolytica*, and resulted in a marked reduction of the activity against *T. vaginalis* (cf. the IC_{50}s for **1g** and **5** shown in Table 1). A similar relationship can be deduced from the respective data for 5,6-dichloro-2-trifluoromethylbenzimidazole (see Ref. [9]) and **1i** against *Giardia* and *Entamoeba* species. It is also noteworthy that compound **8** that carries solubility-enhancing 2-dimethyaminolethylthio group instead of 2-trifluoromethyl group was as active as albendazole against *G. intestinalis*, and as active as metronidazole against *T. vaginalis*; efforts to extend this particular series of halogenated and 2-substituted benzimidazoles are in progress in our laboratory.

Additionally, we have tested the antiprotozoal activity of compound **1k** that possesses two reducible nitro-groups and thus might act through a mechanism similar to that of metronidazole that is also a nitrocompound. This new benzimidazole derivative appeared slightly less potent than metronidazole in the present study.

There was a trend for correlation between the antigiardial and antitrichomonial activities of the brominated benzimidazoles tested and the number of bromine atoms introduced (cf. data for **1c**, **1d**, **1e**, **1f** and **1g**). However, this was not the case for the activity of these derivatives against *E. histolytica* (except of **1e** and **1d** which are almost equally active against all

Table 1
In vitro susceptibility of *Giardia intestinalis*, *Entamoeba histolytica* and *Trichomonas vaginalis* to benzimidazole derivatives

Compound	IC_{50} (μg ml^{-1}) [μM] [a], 95% conf. limits (μg ml^{-1})		
	Giardia intestinalis	*Entamoeba histolytica*	*Trichomonas vaginalis*
1c	0.168 [0.64] 0.167–0.168	0.347 [1.33] 0.341–0.352	0.408 [1.57] 0.405–0.408
1d	0.0405 [0.12] 0.034–0.047	0.15 [0.44] 0.148–0.151	0.073 [0.22] 0.072–0.073
1e	0.084 [0.25] 0.084–0.084	0.017 [0.050] 0.011–0.012	0.064 [0.19] 0.064–0.064
1f	0.059 [0.14] 0.059–0.059	5.80 [13.8] 5.67–5.93	0.111 [0.27] 0.110–0.113
1g	0.028 [0.056] 0.028–0.028	0.151 [0.30] 0.149–0.152	0.001 [0.002] 0.001–0.001
1i	0.165 [0.56] 0.163–0.166	0.624 [0.21] 0.621–0.627	0.899 [0.30] 0.885–0.912
1k	0.059 [0.24] 0.059–0.059	0.373 [1.54] 0.371–0.376	0.138 [0.57] 0.137–0.138
5	0.012 [0.023] 0.012–0.012	0.118 [0.23] 0.116–0.119	5.253 [10.1] 5.18–5.32
8	0.011 [0.025] 0.011–0.011	0.632 [1.45] 0.626–0.638	0.116 [0.37] 0.115–0.117
Albendazole	0.010 [0.038] 0.008–0.012	15.00 [56.3] 10.00–20.00	0.422 [1.6] 0.419–0.425
Metronidazole	0.210 [1.2] 0.150–.0270	0.060 [0.35] 0.029–0.103	0.037 [0.22] 0.037–0.037

IC_{50}, the concentrations required to inhibit growth by 50%.
[a] Values are means of two experiments with triplicates.

protozoa tested). We speculate that this distinction was due to the fact that the main cytoskeleton protein in *G. intestinalis* and *T. vaginalis* is tubulin, whereas the main protein of *E. histolytica* is actin. It is known that benzimidazole carbamates bind to tubulin and inhibit its polymerization. However, as recently demonstrated, benzimidazoles must bear a carbamate-residue in position 2 and a H-atom in position 1 in order to bind tubulin. Therefore, the differences in the susceptibility to our compounds among *G. intestinalis*, *T. vaginalis* and *E. histolytica* could be explained in terms of different metabolism or characteristics of the membranes [15].

In conclusion, the results of the activity tests indicated that introduction of either chlorine or bromine atom into the benzene ring of the benzimidazole nucleus conferred a considerable antiprotozoal activity onto the resulting trifluoromethylbenzimidazole derivatives.

3.2. Cytotoxicity against malignant and normal human cells

Cytotoxic activity of the substituted benzimidazoles was tested using three hematopoietic malignant cell lines (IM-9, MOLT-3, and U-937) and two cell lines representing two common forms of human cancer, i.e. breast cancer and prostate carcinoma (MCF-7 and PC-3, respectively). Because hematopoiesis and functioning of some normal blood cell types involves massive proliferation of cells that takes place over the entire life span of an individual, we included in the tests phytohemagglutinin-stimulated normal circulating T cells as a control. On the contrary, there is little or no proliferation in normal breast and prostate tissues, which makes them resistant to cytostatics and precludes long-term in vitro culture; therefore no good controls are available.

Ten out of 15 compounds tested exerted an 80% or stronger growth inhibition in at least one cell line at a concentration of less than 25 μg ml^{-1}. The results in Table 2 demonstrate the sensitivity of individual cell types. The most potent compound against all cell types was **1i**. As a rule, no prominent cell-type selectivity was found, although a few compounds, e.g. **1b 1d**, **1h**, **1i** and **5**, tended to be more active against epithelial malignancies (MCF-7 and PC-3) than against other tumour cell lines. Five compounds (**1a**, **1j**, **1k**, **2a**, **2b**) showed considerably weaker activity against tested cell lines ($IC_{80} > 25$ μg ml^{-1}) and the respective data were not included in the table. We have chosen IC_{80} index (instead of conventional IC_{50} used in microbiology) for anticancer activity measure in our in vitro study because it seemed more or less equivalent to ID_{80} (inhibitory dose resulting in 80% inhibition of tumour growth) that is customarily used for antitumour in vivo testing. After all, the main goal of this part of the study was not to compare antiprotozoal activity against anticancer activity of the drugs tested, but to provide a general information on their biological activities.

4. Experimental

4.1. Chemistry

All chemicals and solvents were purchased from Sigma–Aldrich. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp-5 m.p. apparatus. Ultraviolet absorption spectra were recorded in a Kontron Uvikon 940 spectrophotometer. ^{1}H-NMR spectra (in ppm) were measured with a Varian Gemini 200 MHz (or a Varian UNITY plus 500 MHz) spectrometer at 298 K in D_6(DMSO) using Me_4Si as internal standard. Flash chromatography was performed with

Table 2
Inhibition of cell growth by benzimidazole derivatives

Compound	IC_{80} [a] (μg ml^{-1}) [μM]					
	Phytohemagglutinin-stimulated normal human lymphocytes	IM-9 (Multiple myeloma)	MOLT 3 (T-cell acute lymphoblastic leukemia)	U-937 (Monocytic leukemia)	MCF-7 (Breast carcinoma)	PC-3 (Prostate carcinoma)
1b	2.2 [9.2]	3.5 [14.5]	9 [37.5]	2.9 [12.0]	0.82 [3.4]	0.70 [2.9]
1c	3.9 [14.7]	4.6 [17.4]	13 [49.1]	4.5 [17.0]	2.6 [9.8]	3.3 [12.5]
1d	3.0 [8.7]	3.8 [11.0]	10 [29.1]	3.2 [9.3]	0.92 [2.7]	0.68 [2.0]
1e	12 [34.8]	9.6 [27.9]	16 [46.5]	11 [31.9]	4.5 [13.1]	4.0 [11.6]
1f	3.3 [7.8]	2.8 [6.6]	6.5 [15.3]	2.6 [6.1]	2.1 [5.0]	1.1 [2.6]
1g	4.6 [9.1]	3.2 [6.4]	5.0 [9.9]	2.7 [5.4]	2.8 [5.6]	1.0 [2.0]
1h	2.3 [9.0]	3.0 [11.8]	4.4 [17.3]	2.0 [7.8]	0.77 [3.0]	0.68 [2.7]
1i	0.74 [2.4]	1.6 [5.2]	3.9 [12.7]	0.87 [2.9]	0.50 [1.6]	0.39 [1.3]
5	3.2 [5.7]	3.9 [7.1]	13 [23.5]	2.2 [4.0]	0.82 [1.5]	0.68 [1.2]
8	18 [43.4]	18 [43.4]	17 [40.9]	18 [43.4]	18 [43.4]	> 25 [> 60]

[a] IC_{80} is the concentration of compound which caused an 80% decrease in [^{14}C]L-leucine incorporation by the cultured cells. Values were calculated from dose–response curves done in duplicate for each compound.

Merck silica gel 60 (200–400 mesh). Analytical TLC was carried out on precoated silica gel F_{254} (Merck) plates (0.25 mm thickness). Analyses of the new compounds, indicated by the symbols of the elements, were within ± 0.4% of the theoretical values.

4.1.1. General procedure for the synthesis *1b,i and j*

To a solution of respective *o*-phenylene diamine (5 mmol) in 4 N HCl (20 mL), trifluoroactic or pentafluoropropionic acids, respectively (10 mmol), was added. The stirred reaction mixture was stirred under reflux for 5 or 24 h, respectively, diluted with water (30 mL), treated with charcoal and brought to pH 4–5 with aq. ammonia. The precipitate formed was filtered off and crystallized from EtOH–H_2O.

4.1.1.1. 4,5,6-Trifluoro-2-trifluoromethylbenzimidazole (*1b*).

Yield: 45%; m.p. 169–171 °C; ^{1}H-NMR (D_6-DMSO): 7.67 (s, 1H, H-7); UV: (pH 6) 250 (4600), 271 (4800); (pH 12) 273 (5800); TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.51. Anal. Calc. for $C_8H_2F_6N_2$: C, 40.02; H, 0.84; N, 11.67. Found: C, 39.89; H, 1.01; N, 11.55%.

4.1.1.2. 5,6-Dichloro-2-pentafluoroethylbenzimidazole (*1i*).

Yield: 38%; m.p. 207 °C; ^{1}H-NMR (D_6-DMSO): 8.05 (s, 2H, H-4 and H-7). UV: (pH 6) 260 (3500), 291 (5600), 301 (4900); TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.60. Anal. Calc. for $C_9H_3Cl_2F_5N_2$: C, 35.44; H, 0.99; N, 9.18. Found: C, 35.31; H, 1.11; N, 9.29%.

4.1.1.3. 5,6-Dimethyl-2-pentafluoroethylbenzimidazole (*1j*).

Yield: 63%; m.p. 204–206 °C; ^{1}H-NMR (D_6-DMSO): 2.32 (s, 6H, $2CH_3$), 7.46 (s, 2H, H-4 and H-7); UV: (pH 6) 262 (3600), 284 (5000), 291 (4800); (pH 12) 282 (7200). TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.71. Anal. Calc. for $C_{11}H_9F_5N_2$: C, 50.01; H, 3.43; N, 10.60. Found: C, 50.17; H, 3.45; N, 10.47%.

4.1.2. 4,5,6,7-Tetrabromo-2-pentafluoroethylbenzimidazole (*5*)

To a stirred and refluxed suspension of 2-pentafluoroethylbenzimidazole (1.4 g, 5.9 mmol) in water (70 mL), bromine (7.5 g, 47 mmol) was added portionwise within 8 h. The reflux was continued for 3 days. The reaction mixture was cooled and the pale yellow precipitate formed was filtered off, washed with cold water and crystallized from EtOH–H_2O (1:1) to give (1.59 g, 49%) of colourless crystals of m.p. 235–237 °C. ^{1}H-NMR (D_6-DMSO): 14.54 (bs, 1H, N–H); UV (MeOH–H_2O, 1:1): 233 (4300), 304.5 (2700); TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.77. Anal. Calc. for $C_9HBr_4F_5N_2$: C, 19.59; H, 0.18; N, 5.08. Found: C, 19. 43; H, 0.28; N, 4.95%.

4.1.3. 4,6-Dibromo-2-thiobenzimidazole (*7*)

To a stirred suspension of 3,5-dibromo-1,2-diaminobenzene dihydrochloride (4.6 g, 1.5 mmol) in EtOH–H_2O (70 mL, 9:1), carbon disulfide (3.8 g, 49.5 mmol) and KOH (2.4 g, 43 mmol) were added. The reaction mixture was stirred and refluxed for 3 h. The mixture was cooled and acidified to pH 2 with HCl. The precipitate formed was filtered off and purified by twice crystallization from EtOH–H_2O (1:1) to give white powder (2.5 g, 60%) of m.p. > 350 °C. ^{1}H-NMR (D_6-DMSO): 7.79 and 7.91 (2 × d, 2H, H-5 and H-7); UV: (pH 2) 251 (18 900), 314 (21 300); (pH 12) 242 (11 100), 314 (10 500); TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.58. Anal. Calc. for $C_7H_4Br_2N_2S$: C, 27.30; H, 1.30; N, 9.10. Found: C, 27.14; H, 1.42; N, 8.97%.

4.1.4. 4,6-Dibromo-2-dimethylaminoethylthiobenzimidazole (*8*)

To a solution of **7** (0.92 g, 3 mmol) in dry MeCN (15 mL) were added DBU (1.02 g, 6.7 mmol) and dimethylamineethylchloride hydrochloride (0.57 g, 4 mmol). The reaction mixture was stirred at 70 °C (bath temperature) for 30 min. The mixture was adsorbed on silica gel and chromatographed on silica gel column (3 × 15 cm) with $CHCl_3$–MeOH (9:1). The oily product was transformed in colourless crystalline hydrochloride by treating it with HCl-saturated MeOH (0.24 g, 19%). M.p. 230–232 °C. ^{1}H-NMR (D_6-DMSO): 2.84 (s, 6H, 2 × CH_3), 3.46 and 3.68 (2t, 4H, 2 × CH_2), 7.54 and 7.67 (2d, H-5 and H-7); UV: (pH 6) 301 (13 500), (pH 12) 307 (1500); TLC ($CHCl_3$–MeOH, 9:1, v/v): Rf 0.21. Anal. Calc. for $C_{11}H_{13}Br_2N_3S \times HCl$: C, 31.79; H, 3.40; N, 10.11. Found: C, 31.91, H, 3.53; N, 10.00%.

4.2. Biology

4.2.1. Parasites

E. histolytica strain HM1-IMSS, *T. vaginalis* strain GT3 and *G. intestinalis* isolate IMSS:1090:1 were used in all experiments. Trophozoites of *E. histolytica* and *T. vaginalis* were maintained in TYI-S-33 medium supplemented with 10% bovine serum. *Giardia* trophozoites were cultured in TYI-S-33 modified medium supplemented with 10% calf serum and bovine bile [16]17.

4.2.2. Protozoa susceptibility assays

In vitro susceptibility assays were performed using a method previously described [16,17]. Briefly: 4×10^4 *G. intestinalis*, 6×10^3 *E. histolytica* or 4×10^4 *T. vaginalis* trophozoites were incubated for 48 h at 37 °C with different concentrations of compounds **1c**, **1d**, **1e**, **1f**, **1g**, **1i**, **1k**, **5**, **8**, albendazole, or metronidazole, each added as a solution in dimethyl sulfoxide (DMSO). As the negative control, trophozoites were incubated with DMSO. At the end of the treatment period, the cells were washed and subcultured for another 48 h in fresh medium to which no drug was added. The trophozoites were then counted with a haemocytometer and the 50% (IC_{50}) inhibitory concentrations, together with the

respective 95% confidence limits were calculated by probit analysis. Experiments were carried out using triplicate tubes and were repeated three times.

4.2.3. In vitro cytotoxicity tests

Toxicity of the compounds was determined by their effects on protein synthesis ([^{14}C]L-leucine incorporation). The cell lines IM-9, MOLT-3 and U-937 were obtained from the American Type Culture Collection. MCF-7 and PC-3 lines were a generous gift from Dr Jorma Isola (Department of Cancer Biology, University of Tampere). Mononuclear cells for phytohemagglutinin stimulation were obtained from healthy donors. These cells represent mainly activated normal human polyclonal T lymphocytes. Test compounds were added to duplicate suspension cultures in 96-well microplates containing 2×10^4 cells (IM-9, MOLT-3, U-937) or 10^5 peripheral blood mononuclear cells (for phytohemagglutinin stimulation) per a 200 μL-well. These cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, in humidified atmosphere containing 5% CO_2 at 37°. The adherent cell cultures (MCF-7 and PC-3) were initiated by splitting 1/5 of the corresponding confluent master culture. The cells were grown in microplates in the presence of the test compounds. MCF-7 cells were grown in Eagle's MEM medium and PC-3 cells in Ham's F-12 medium. After 3 days of culture, [^{14}C]L-leucine (0.5 μCi ml^{-1}, specific activity 1.3 mCi mmol^{-1}) was added and the cells were incubated for another 24 h. After incubation, the proteins were precipitated with 0.2 N $HClO_4$, and collected on glass fibre filters using a multiple cell harvester (Wallac, Turku, Finland). The radioactivity incorporated into proteins was measured in a scintillation counter (LKB-Wallac 1410, Turku, Finland). The growth inhibition was determined from dose–response curves representing five different concentrations of the test compounds. Some compounds were first screened with a single dose of 25 μg ml^{-1} by using only U-937 cells. If an 80% growth inhibition was not achieved, other cell types were not tested (see Section 3). We have shown in a number of experiments that there is a good correlation between the leucine incorporation and the number of living cells in 4-day cultures like this (see I. Kivekäs, L. Vilpo, J. Vilpo, Relationship of in vitro sensitivities tested with nine drugs and two types of irradiation in chronic lymphocytic leukemia, Leukemia Res. (2002) in press).

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