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[C002]

O-SUBSTITUTED DERIVATIVES OF 2,3-DIHYDRO-2,2-DIMETHYL-7-BENZOFURANOL - CYTOTOXIC ACTIVITY AND STRUCTURAL STUDIES

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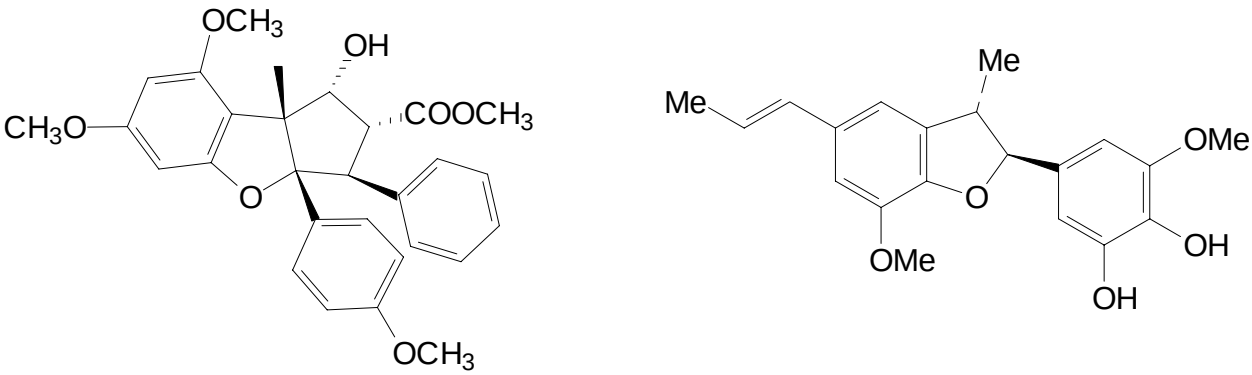
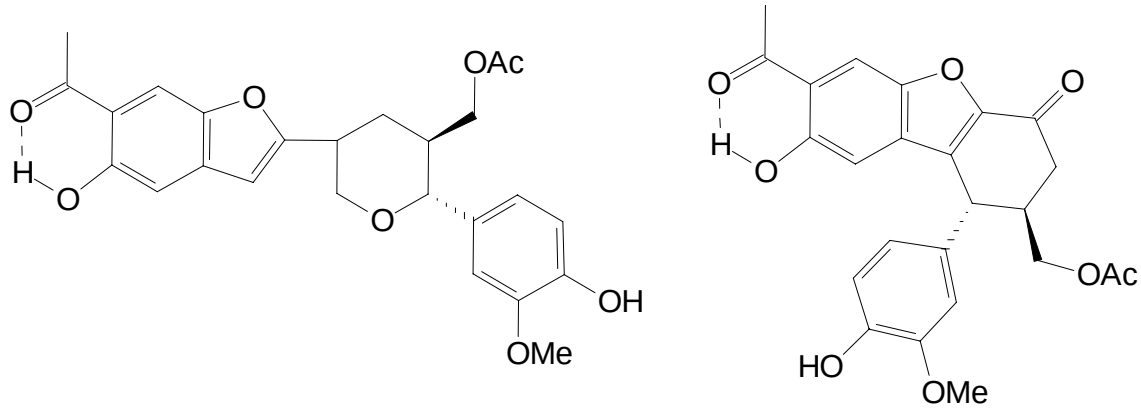
Several screening programs for novel anticancer agents employed investigation benzofuran derivatives isolated from natural sources as well as synthetic products. It was found [**1**-**3**] that flavaglines [comprising cyclopenta[b]tetrahydrobenzofurans (rocaglamides), cyclopenta[bc]benzopyrans and benzo[b]oxepines] obtained from several tropical and subtropical trees genus *Aglaia* show pronounced activity against different human tumor cell lines. Two examples of active dihydrobenzofuran derivatives are shown in Fig.1.



Methyl rocaglate Obovatifol

Fig.1. The structures of naturally occurring dihydrobenzofurans.

Novel cytotoxic benzofuran derivatives were also isolated from Brazilian propolis [4] (Fig.2).



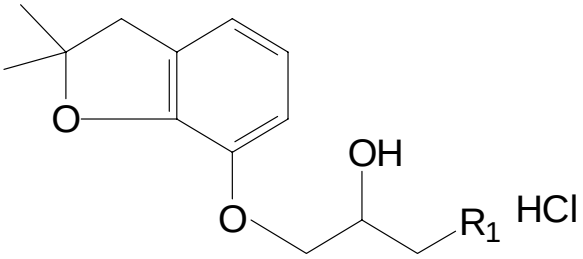
A B

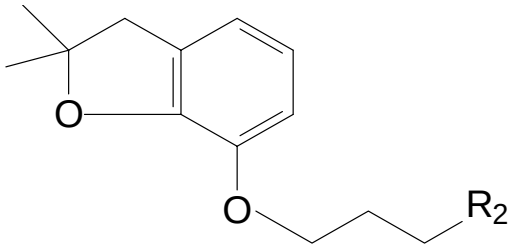
Fig. 2. Propolis-benzofurans **A** and **B.**

Both compounds exhibited cytotoxicity toward highly liver-metastatic murine colon carcinoma and human fibrosarcoma cells.

Hellesbeux et al. [5] reported the synthesis of 2-isopropenyl-2,3-dihydrobenzofuran enantiomers. *In vivo* antitumor activity against a non-small cell bronchopulmonary carcinoma line (NSCLC-N6) of these enantioisomers had been investigated.

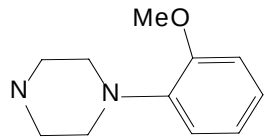
Inspired by these reports we submitted six O-substituted derivatives of 2,3-dihydro-2,2-dimethyl-7-benzofuranol [**6**] (see the structures in Fig.3) to anticancer assay to Division of Cancer Treatment and Diagnosis National Cancer Institute (Bethesda, USA).



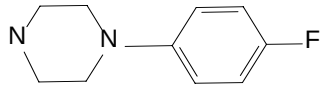


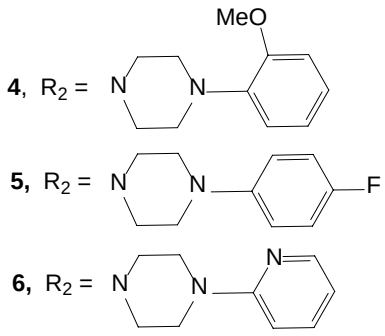
1HCl, R_1 = $NHC(CH_3)_3$

2HCl, R_1 =



3HCl, R_1 =





chemical names:

7-(3-*t*-Butylamino-2-hydroxypropoxy)-2,3-dihydro-2,2-dimethylbenzofuran hydrochloride (**1HCl**).

7-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]-2-hydroxypropoxy]-2,3-dihydro-2,2-dimethylbenzofuran hydrochloride (**2HCl**).

7-[3-[4-(4-Fluorophenyl-1-piperazinyl]-2-hydroxypropoxy]-2,3-dihydro-2,2-dimethylbenzofuran hydrochloride (**3HCl**).

7-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propoxy]-2,3-dihydro-2,2-dimethylbenzofuran (**4**).

7-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propoxy]-2,3-dihydro-2,2-dimethylbenzofuran (**5**).

7-[3-[4-(2-Pyridyl)-1-piperazinyl]propoxy]-2,3-dihydro-2,2-dimethylbenzofuran (**6**).

		Growth T/C		
Compound	Concentration	**Breast MCF 7**	**Non-Small Cell Lung NCI-H460**	**CNS SF-268**
1HCl	$1 \cdot 10^{-4}$ M	1.02	1.04	1.00
2HCl	$5 \cdot 10^{-5}$ M	0.83	0.90	0.97
3HCl	$5 \cdot 10^{-5}$ M	0.57	0.12	0.85
4	$5 \cdot 10^{-5}$ M	0.71	0.43	0.82
5	$5 \cdot 10^{-5}$ M	0.94	1.11	0.96
6	$5 \cdot 10^{-5}$ M	0.43	0.81	0.92

Fig.3. Structures and chemical names of compounds **1HCl** – **6**.

The results of the primary anticancer assay are presented in Table 1. Compound **3HCl** showed the highest level of ability to inhibit the growth of Non-small Cell Lung Cancer Cells NCI-H460 and was selected for 60 Cell Testing.

Table 1. Primary screening results.

We observe an exponential relation between the growth of cancer cells and calculated [7] lipophilicity log P (Fig.4).

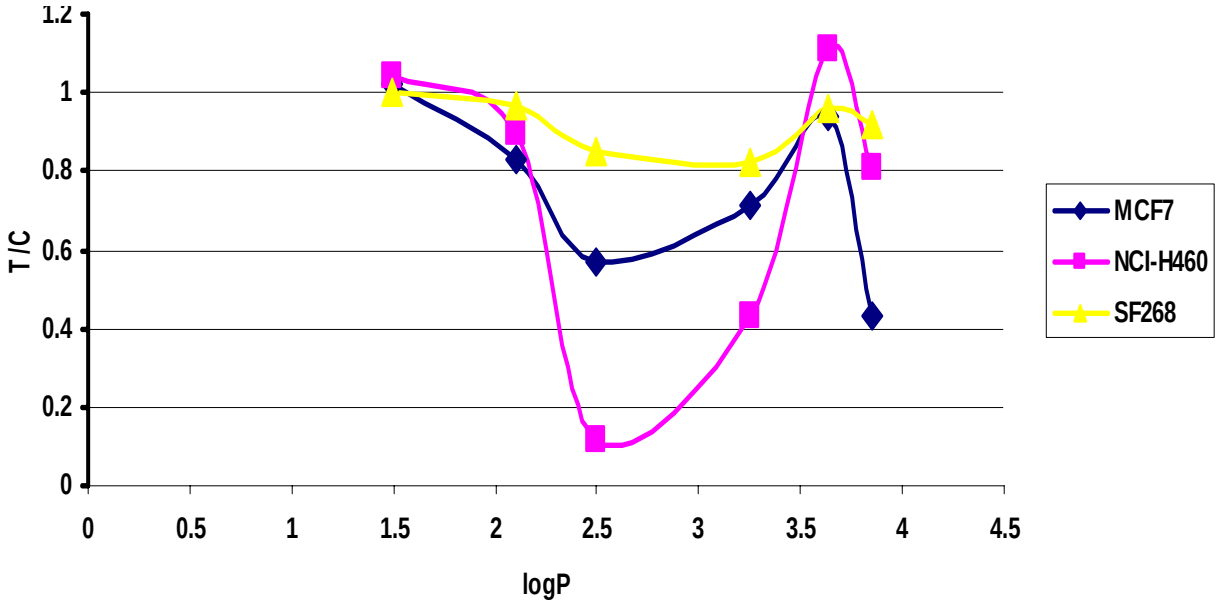


Fig. 4. Relation between the growth of cancer cells and lipophilicity.

The exemplary equation for NCI-H460 cell lines:

$$y = -1.56\log P^4 + 16.91\log P^3 - 66.04\log P^2 + 108.87\log P - 62.84$$

Leukemia RPMI-8226	**Lung** HOP-92	**Colon** HCT-116	**CNS** SF-295	**Melanoma** M14	**Renal** 786-0	**MGM**[b]
9.18	8.15	7.34	8.83	7.36	5.66	4.83

$R^2 = 0.988$

Compound **3HCl** was passed on for evaluation in the full panel of 60 different cell lines, representing human leukemia, non-small cell lung cancer, colon cancer, CNS (central nervous system) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cell lines. Results from representative cell lines are listed in Table 2, along with mean graph midpoint (MGM) values for all 60 lines.

Table 2. Cytotoxicity (GI_{50} in μM)[a] of **3HCl**

[a]The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.

[b]Mean graph midpoint for all human cancer cell lines (ca. 60) tested.

The panels and cells employed included the following:

leukemia K-562, MOLT-4, RPMI-8226, SR;

non-small cell lung cancer: A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522;

colon cancer: COLO 205, HCT-116, HCT-15, HT-29, KM12, SW-620;
CNS cancer: SF-268, SF-295, SF-539, SNB-19, SNB-75, U251;
melanoma: LOX IMVI, M14, SK-MEL-2, SK-MEL-5, SK-MEL-28, UACC-257, UACC-62;
ovarian cancer: IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3;
renal cancer: 786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31;
prostate cancer: PC-3, DU-145;
breast cancer: MCF-7, NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, BT-549.

The NMR spectroscopy

Analyzing the ^{1}H NMR spectrum of **1HCl** (Fig.5) we have observed nonequivalence of both methyl groups at C17 in the dihydrofuran ring at ambient temperature. The coalescence point was found at 330 K. The energy of ring motions was roughly estimated on the basis of Eyring equation at coalescence temperature [**8**]. The value is 75 kJ/mol (Fig.5b). There is a stereogenicity centre at C-8 in the compound **1HCl.** This is a reason for non-equivalence of protons of the adjacent methylene groups.

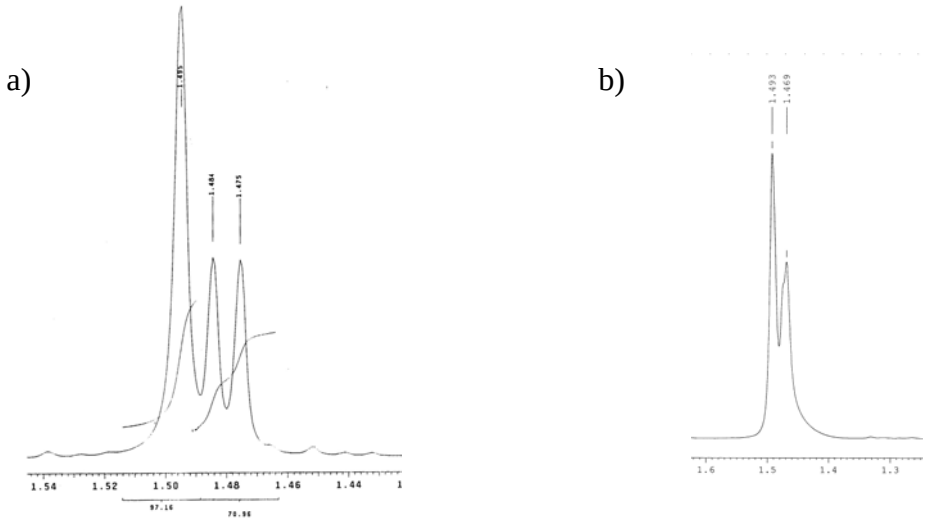


Fig. 5. Coalescence of the methyl groups resonances in ^{1}H NMR spectrum of **1HCl.**

To fully assign the resonances in solution we have used HMBC and HSQC NMR techniques. Exemplary spectrum is presented in Fig.6.

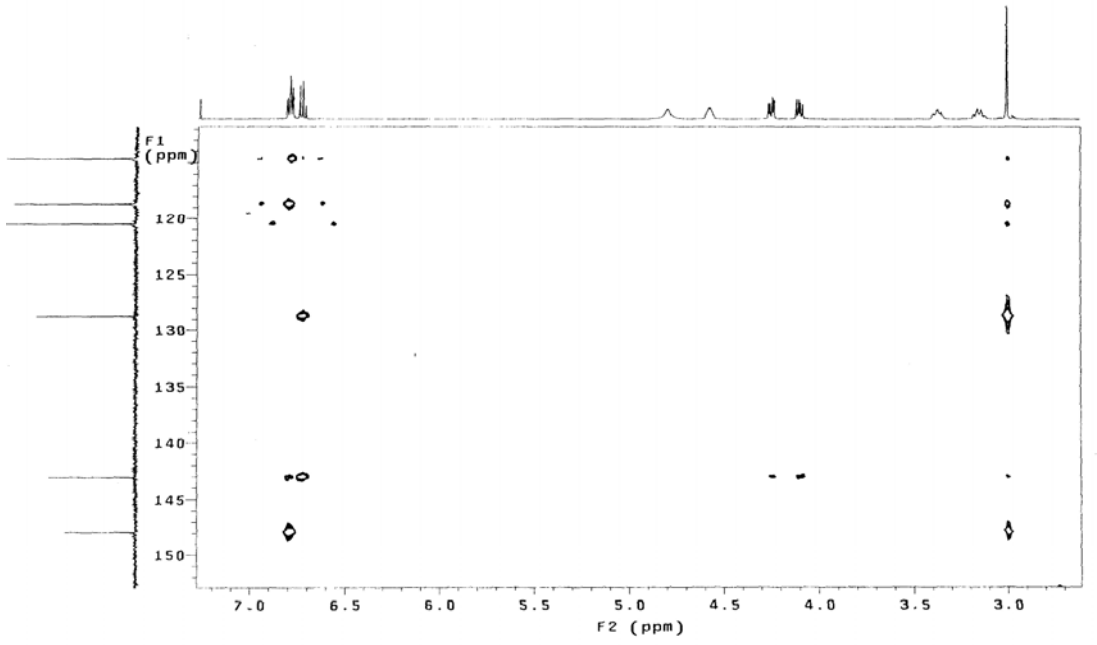
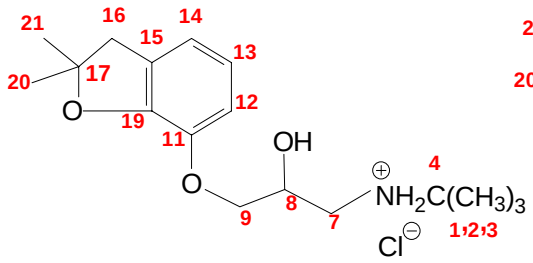
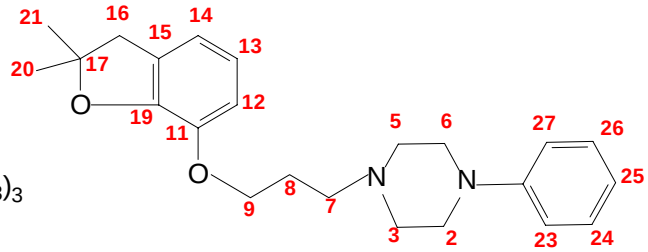


Fig. 6. The HMBC NMR spectrum of **1HCl**

Until now, the ^{13}C NMR data for reported derivatives of dihydrobenzofuran were not published. We have recorded the ^{13}C NMR spectra in solution of compounds **1HCl** and **7** (see Fig.7 for atoms numbering).



1 HCl



7

7-(3-*t*-Butylamino-2-hydroxypropoxy)-2,3-dihydro-2,2-dimethylbenzofuran hydrochloride (**1HCl**)

7-[3-(4-Phenyl-1-piperazinyl)propoxy]-2,3-dihydro-2,2-dimethylbenzofuran (**7**).

Fig.7. The structures, chemical names and atoms numbering of **1HCl** and **7**.

The most probably assignment of resonances is given in Table 3

Table 3. ^{13}C NMR chemical shift values [ppm] in $CDCl_3$ referenced to TMS.

Comp	C20,21	C17	C16	C15	C14	C13	C12	C11	C19
1HCl	28.32	87.57	43.22	128.73	118.70	128.54	114.70	143.07	147.91
7	28.26	87.20	43.32	128.45	129.12	117.70	113.16	151.22	147.85

Comp	C9	C8	C7	C4	*C1,2,3 C22	C3,5	C2,6	C24, 26	C23,27
1HCl	71.93	65.49	45.85	57.65	*25.92	----	----	----	----
7	67.36	26.33	55.08	----	143.63	53.08	48.85	129.12	116.14

We were not succesful in obtaining suitable crystals for X-ray analysis. Moreover, we have analyzed the solid state structure by ^{13}C CPMAS NMR technique. The results are presented in Fig.8 and Table 4. The ^{13}C CPMAS spectrum of the most active compound **3HCl** shows a simple pattern of signals. That is an evidence of lack of polymorphism.

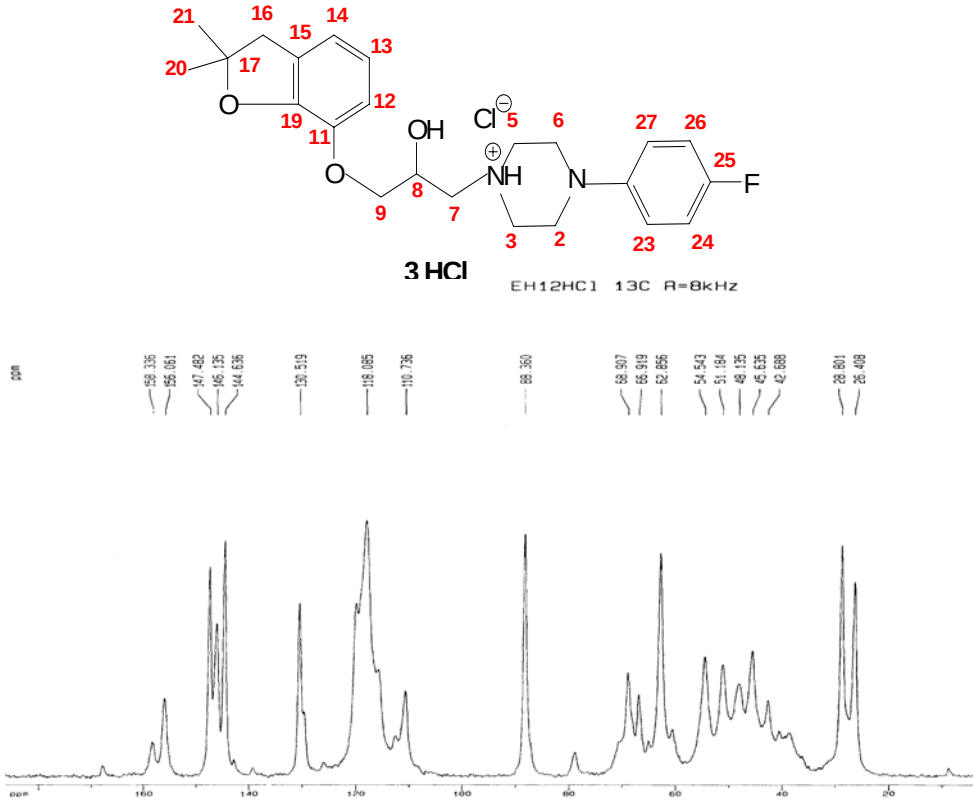


Fig.8. ^{13}C CPMAS NMR spectrum of **3HCl**

C20,21	C17	C16	C15	C12, 13,14	C11	C19
26.80 26.41	88.36	42.69	130.52	118.09	156.06	147.48

C9	C8	C7	C22	C3,5	C25	C2,6	C24, 26
62.86	66.92	40.00	146.14	51.18 54.54	158.34	48.14 48.64	110.74

CONCLUSIONS

60 lines anticancer assay shows that 7-[3-[4-(4-fluorophenyl-1-piperazinyl]-2-hydroxypropoxy]-2,3-dihydro-2,2-dimethylbenzofuran hydrochloride (**3HCl**) exhibits mild cytotoxicity. Structural examination by means of NMR spectroscopy revealed dynamics of the dihydrofuran ring in solution and lack of polymorphism for the compound **3HCl** in solid state.

Acknowledgment

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