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The quest for a predictive design of anticancer drugs

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Abstract - A predictive design is explored with nitroxyl labeled derivatives, and other congeners of alkylating drugs TEPA/Thio-TEPA, CCNU/MeCCNU, and Streptozotocin/Chlorozotocin. The design is based on correlations of lipophilicities of these drugs with their antineoplastic activities *in vivo* against the murine lymphocytic leukemia P388. Some results are also included of evaluations using the murine lymphoid leukemia L1210. Several of these compounds possess considerably higher therapeutic indices than those of the parent drugs which are in clinical use, and, hence, warrant further studies.

INTRODUCTION

Cancer diseases existed during the whole evolutionary process of multicellular organisms (ref. 1,2). This fact is not surprising, considering that all cancers, in spite of their diversities, have also common, unifying features, i.e., they involve intracellular events, more specifically, they involve the malfunctioning of the genetic mechanism which guaranties the hereditary pattern of proliferation and survival of individual species, the DNA (ref. 3). Hence, the development of new antineoplastic drugs should be aimed at a selective destruction of the DNA coding of the cancerous cells without affecting the DNA of the healthy cells. This task can only be achieved by a design of drugs which would contain components capable of a decisive interaction with the DNA coding mechanism, and, furthermore, would ensure a selective permeation of the drug through the cancerous cell membranes, and not the healthy cell membranes. It is surprising that while the former aspect has been extensively investigated, the latter aspect has not been widely considered in various designs of antineoplastic drugs.

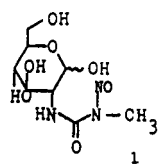
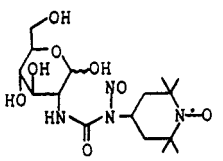
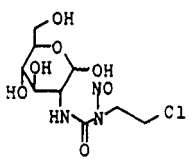
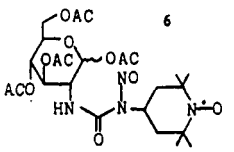
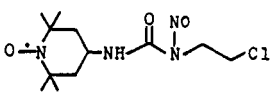
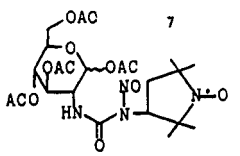
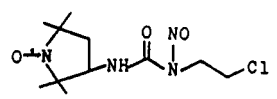
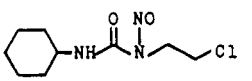
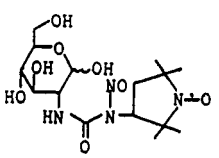
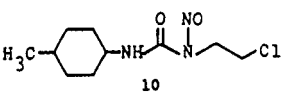
CORRELATION OF ANTINEOPLASTIC ACTIVITY WITH LIPOPHILICITY

The transformation of a healthy cell into a malignant cell is accompanied by at least a few dozen of known, and, probably, by an additional number of unknown changes (ref. 3,4,5). Therefore, it would be a futile undertaking to attempt to design an antitumor drug taking into account all the possible known and assumed parameters. In particular, since during the therapy the cancer cell is continually undergoing further recognizable and unrecognizable transformations. As a consequence, considering our present still highly inadequate scientific capabilities, it would be a more manageable task to select, if posssible, a dominant parameter which would permit an approximate correlation between the structure and anticancer activity. Over the years it has been well recognized (refs. 6,7,8,9) that the most important property of many biologically active compounds is their lipophilicity which can be conveniently measured by the partition coefficient using, e.g., the octanol/water solvent system. The reason for the dominant role of the partition coefficient in correlating the structure with the activity of a drug can be plausibly explained. Thus, since it can be considered as established (ref. 1,2,3) that all cancer diseases are diseases affecting the DNA, in combating any cancer disease it is essential to design a drug which would selectively reach the cancerous DNA, but not the DNA of the healthy cell. In order for a drug to reach the DNA, it will have to be compatible with aqueous extracellular and cell surface environment, independent of the mode of permeation through the cell membranes. In addition, in order to permeate through the highly hydrophobic bilayer of the membrane, the drug must also possess a certain degree of compatibility with this hydrophobic environment. A logarithmic plot of partition coefficients against the corresponding activities will result in a distorted bell-shaped curve (parabolic model, ref. 7) with the most efficient drug expected to be located at the apex of the curve. A bilinear model (ref. 10) is also feasible and theoretically justifiable. In the present study the correlation between the anticancer activities and lipophilicities assembled in Tables 1, 2 and 3 are graphically presented in a logarithmic plot (Fig. 1) indicating linear relationships. Systematic synthetic manipulations and correlations of log P with activities will lead to the development of such an optimum drug.

NITROSOUREAS

Compounds of structures 1-10 (Table 1) containing either the nitrosourea or the chloroethylnitrosourea moieties possess potent anticancer activities (refs. 2,3,11). Some of these drugs, such as, CCNU (9), MeCCNU (10), and Streptozotocin (1), a naturally occurring product of the organism streptomyces acromogenes, have been extensively used (refs. 2,3,11) against a variety of cancer diseases. This class of compounds belongs to the so-called alkylating anticancer drugs (refs. 1,2,3,11,12), i.e., under physiological conditions, they are transformed into electrophilic species, which undergo alkylation and/or interstrand crosslinking reactions with DNA and biological proteins (refs. 3,12). The nitrosoureas are an attractive class of anticancer drugs not only because of their wide spectra of activities, but also because some of their members, e.g., CCNU (9) and MeCCNU (10) are capable of penetrating the blood-brain barrier, and, hence, can be applied for the chemotherapy of brain tumors (refs. 3,11) which have, in general, a dismal prognosis, independent of the mode of treatment. Unfortunately, the nitrosoureas also exhibit, as most anticancer drugs, a range of substantial toxic side effects including a cumulative bone marrow toxicity (refs. 3,11). However, it was found (refs. 3,13) that nitroso derivatives containing carbohydrate moieties possess a much lower bone marrow toxicity. Apparently, the carbohydrate moieties while ameliorating the toxic properties, also impart highly hydrophilic character to Streptozotocin (1) and Chlorozotocin (2) as compared to CCNU (9) and MeCCNU (10). We hypothesized that a more effective drug(s) could be designed by either increasing the hydrophobicity of Streptozotocin/Chlorozotocin type drugs or decreasing the hydrophobicity of CCNU/MeCCNU type drugs.

TABLE 1. Anticancer activity-lipophilicity correlation of nitrosoureas.

Compound	Activity[a] [%ILS]	Lipophilicity [Log P]	Compound	Activity[a] [%ILS]	Lipophilicity [Log P]
 1	178	-1.52	 6	437	1.87
 2	194	-0.84	 7	174	2.27
 3	542	1.58	 8	156	2.25
 4	514	1.67	 9	182	2.55
 5	437	1.81	 10	145	3.25

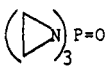
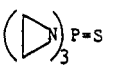
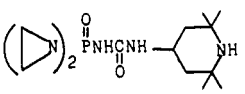
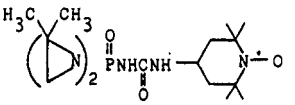
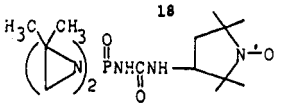
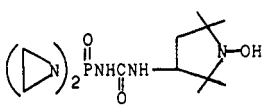
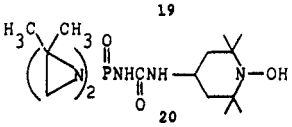
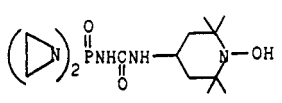
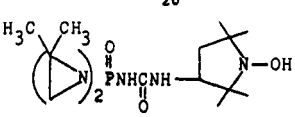
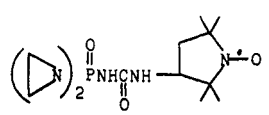
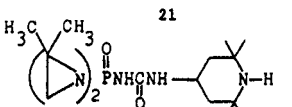
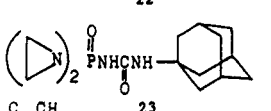
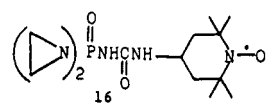
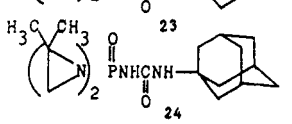
[a]Anticancer Activity against P388 lymphocytic leukemia in CD_2F_1 male mice. National Cancer Institute protocol followed exactly.

This idea was realized by replacing the hydrophobic cyclohexyl/4-methylcyclohexyl groups in CCNU/ MeCCNU with a nitroxyl moiety, thereby shifting the lipophilicity of the new drug to the more hydrophilic side (Table 1, Fig. 1). As a result, the new drug which we call SLCNU (3) (ref. 14,15,16,17) is less toxic (ref. 17) and more active than the clinical drugs CCNU (9) and MeCCNU (10), i.e., exhibiting a much higher therapeutic index (40) as compared to that of CCNU (5.7) (ref. 17). This compound (3) also possessed the highest ILS of 713% using the L1210 leukemia (ref. 17). Against the P388 leukemia the drug exhibited an ILS of 542%, while ILS for the clinical CCNU (9) and MeCCNU (10)was 182% and 145%, respectively (ref. 17).

Analogously, the replacing of the methyl and 2-chloroethyl groups in Streptozotocin (1) and Chlorozotocin (2), respectively, with a nitroxyl moiety resulted in a more hydrophobic and a more active congener (ref. 18). The new drug which we call SLGNU (6) elicited (ref. 18) an increase in life span (ILS) of 437% against the P388 lymphocytic leukemia at 20 mg/kg/day, whereby all mice were alive after 60 days, whereas the clinical drug Streptozotocin and the clinically tested drug Chlorozotocin at optimun doses resulted in ILS of 178% and 194%, respectively, and all mice were dead after 30 days. Similarly, against the L1210 lymphoid leukemia, SLGNU (6) elicited (ref. 18) at 50mg/kg/day a 557% ILS, and all mice were alive after 60 days, whereas Streptozotocin at an optimum dose of 100 mg/kg/day gave an ILS of 55%, and all mice were dead after 30 days.

The synthetic methodologies, analytical details and biological evaluations have been described in previous communications (ref. 17,18). The activities and lipophilicities of

TABLE 2. Anticancer activity-lipophilicity correlation of urea analogs of TEPA

Compound	Activity[a] [%ILS]	Lipophilicity [Log P]	Compound	Activity[a] [%ILS]	Lipophilicity [Log P]
 11	136	-0.62	 17	141	0.41
 12	224	-0.80	 18	62	0.40
			 19	56	0.45
 13	232	-0.60	 20	44	0.48
 14	292	-0.52	 21	40	0.51
 15	387	-0.48	 22	38	0.55
			 23	35	1.81
 16	439	-0.43	 24	32	1.98

[a]Anticancer activity against P388 lymphocytic leukemia in CD_2F_1 male mice. National Cancer Institute protocol followed exactly.

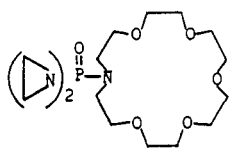
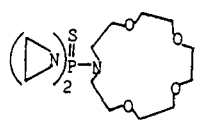
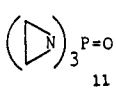
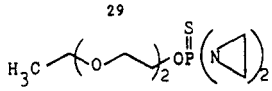
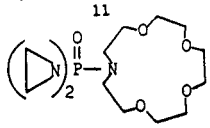
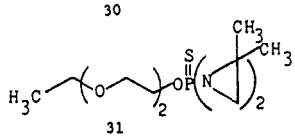
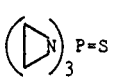
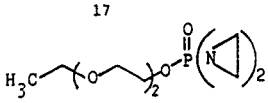
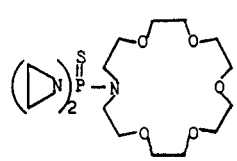
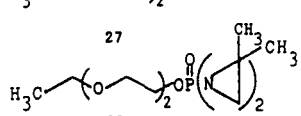
these compounds and of several additional congeners 1-10 are shown in Table 1, and the correlations are graphically presented in Fig. 1. It will be noticed, that while for a linear correlation at the hydrophobic side a sufficient number of points could be obtained, at the hydrophilic side, to date, only two values are available. Consequently, only for the hydrophobic side a mathematical expression is rendered. According to this presentation the most effective compound in this series is the nitroxyl labeled analog 3 of CCNU.

TEPA AND THIO-TEPA DERIVATIVES

A more complete series of correlations of activities with lipophilicities (Fig. 1) was obtained with congeners 11-32 of TEPA (11) and Thio-TEPA (17) shown in Tables 2 and 3. Thio-TEPA (17) has been used clinically in the chemotherapy of Hodgkin disease, metastatic carcinoma of the breast, superficial papillary carcinoma of the bladder, carcinomatous meningitis, and ovarian cancer (refs. 3,11,19). TEPA (11) which is probably the first metabolite of 17 (refs. 19,20) was not introduced into oncology, although the therapeutic indicies (refs. 21,22) and %ILS parameters (Table 2) are very similar for both compounds (refs. 21,22). In contrast, their lipophilicity characteristics are entirely different (ref. 22). Thus, while 11 is fairly hydrophilic (log P = -0.62), compound 17 is relatively hydrophobic (log P = +0.41) as shown in Table 2. As mentioned earlier, the more effective drug(s) would be expected to possess a partition coefficient somewhere between those of 11 and 17. In the search for such a drug a series of congeners of 11 and 17 have been synthesized (refs. 22,23,24) and their activity and lipophilicity parameters determined (Tables 2 and 3). The synthetic methodologies, analytical details and biological evaluations have been adequately reported elsewhere (refs. 22,23,24).

Similar to 11 and 17, all compounds in Tables 2 and 3 are believed to be alkylating agents because of the presence of aziridine moieties. In support, it was shown in a flow cytometry study (ref. 25) that nitroxyl labeled analogs of 11 and 17 retard progression of cells through the S phase. Although the nitroxyl radicals are rapidly reduced intracellularly (ref. 26), the reduced forms containing either the amino (>NH) or hydroxylamine (>NOH) group

TABLE 3. Anticancer activity-lipophilicity correlation of polyether analogs of TEPA and Thio-TEPA.

Compound	Activity[a] [%ILS]	Lipophilicity [Log P]	Compound	Activity[a] [%ILS]	Lipophilicity [Log P]
 25	88	-1.36	 29	80	0.41
 11	136	-0.62	 30	77	0.38
 26	525	0.12	 31	42	0.47
 17	141	0.40			
 27	131	0.10	 32	20	0.74
 28	88	0.22			

[a]Anticancer activity against P388 lymphocytic leukemia in CD_2F_1 male mice. National Cancer Institute Protocol followed exactly.

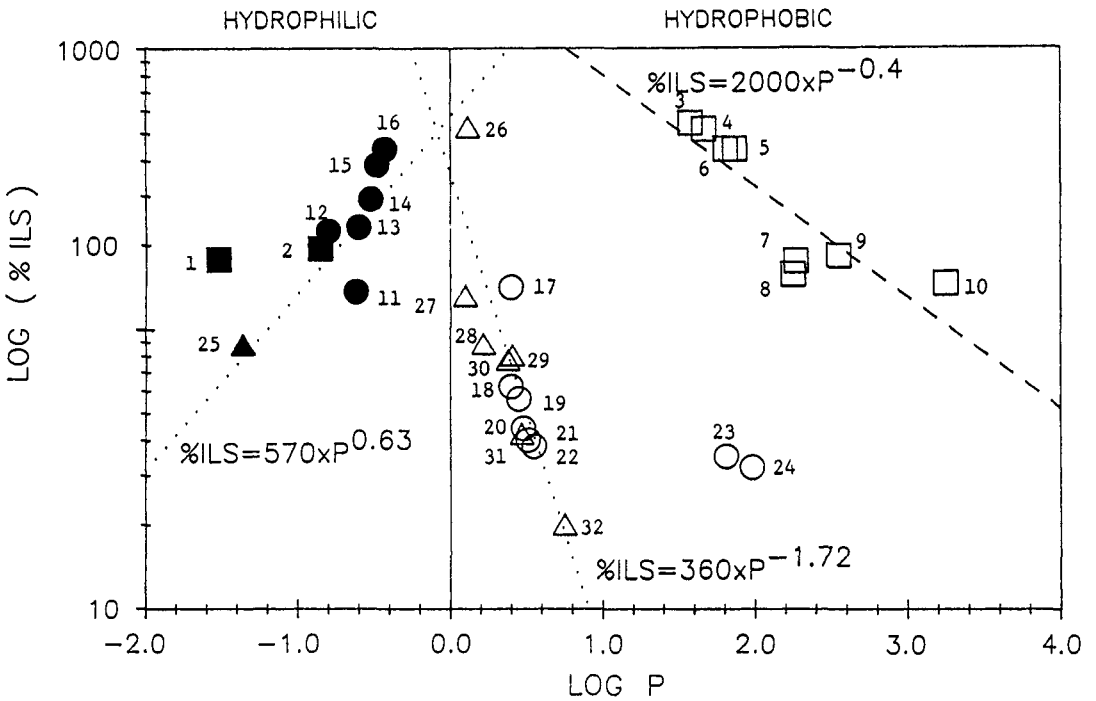


Fig. 1. Anticancer activity-lipophilicity correlations of urea analogs of TEPA (● ○), polyether analogs of TEPA and THIO-TEPA (▲ △) and nitrosoureas (■ □), closed symbols-hydrophilic, open symbols-hydrophobic.

were found (refs. 22) to be less active than the corresponding nitroxyl derivatives (Table 2). The nitroxyl labeled TEPA analog SLDU (16) also possessed a higher therapeutic index (26.5) than those of TEPA (11), Thio-TEPA (17), 12 and 14 which were 2.2, 2.75, 8.0 and 6.2, respectively (refs. 21,22). Nevertheless, it was shown (ref. 21) that nitroxyl radicals by themselves have no antineoplastic effect even at high doses, and also no synergetic influence (ref. 27) on Thio-TEPA (11).

The most active compound of this series (11-32) appears to be the azacrown TEPA (26) with a therapeutic index of 18 and ILS of 525% (ref. 23,24). The highly hydrophobic adamantyl derivatives (23 and 24) were marginally active and outside the linear correlation in this series (Table 3). It is believed that the nitroxyl radical moieties and polyether moieties contribute to a decrease in toxicity of alkylating anticancer drugs, and facilitate a selective permeation of such drugs through cell membranes (refs. 22,23,26). A more detailed rationale in support of this contention was previously delineated (refs. 22,23).

Recapitulating, the effectiveness of an alkylating anticancer drug depends on its alkylating potential, and its capability to permeate selectively through cell membranes. The latter property is linked to the lipophilicity of the drug. Synthetic manipulations based on lipophilicity and anticancer activity correlations can lead to a predictive design of new anticancer drugs. Replacement of certain components in the clinical alkylating drugs of nitrosourea and TEPA/Thio-TEPA type with nitroxyl, nitroxyl labeled urea, polyether and carbohydrate moieties can substantially alter the lipophilicity of the parent drugs with concomitant lowering of toxicity and often, but not always, increase in activity which will depend on the degree of attained lipophilicity. Other components in the molecule, such as, alkyl, cycloalkyl and sulfur groups increase the hydrophobicity of the drugs (Tables 1-3). Thus, the carbohydrate moiety in Streptozotocin/Chlorozotocin imparts a fairly hydrophilic character to these drugs. A replacement of the carbohydrate moiety with cyclohexyl moieties results in the rather hydrophobic drugs CCNU/MeCCNU without significant change in activities (Table 1). The nitroxyl moiety can be used to provide a finer shift of lipophilicity, either to the hydrophilic or hydrophobic region, depending on the lipophilicity of the parent compound. Thus, the nitroxyl labeled analogs 5 and SLGNU (6) of Streptozotocin/ Chlorozotocin are more hydrophobic and more active than the parent compounds, but not as hydrophobic as CCNU/MECCNU (Table 1, Fig. 1). In contrast, the replacement of the cyclohexyl groups in CCNU/MeCCNU by a nitroxyl moiety results in more hydrophilic drugs SLCNU (3) and 4 with higher activity than the parent drugs, 9 and 10. Acylation (Ref. 28) of 5 and 6 results in more hydrophobic and less active derivatives 7 and 8, respectively (Table 1, Fig. 1). The replacement of one aziridine group in TEPA (11) with either a nitroxyl labeled urea or a suitable azacrown moiety results in an increased hydrophobicity and activity of analog SLDU (15) and azacrown TEPA (26), respectively.

All these results, obtained with P388 leukemia, can not be automatically used to predict the effectiveness of these drugs in other than leukemia tumors (ref. 29). It is believed that for each cancer the design of the most effective drug must be based on a new set of activity-lipophilicity correlations.

Finally, the drugs containing the paramagnetic nitroxyl moieties can be used in pharmacokinetics studies by paramagnetic resonance spectroscopy (ref.20), and should be further amenable to diagnostic monitoring during the therapy employing magnetic resonance imaging, in particular, if proton-electron double magnetic resonance imaging can be adapted to clinical practice (ref. 30).

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