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Anticancer agents for treatment of breast cancer established on Melphalan scaffolding

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ABSTRACT

Breast cancer is a type of cancer beginning in the inner lining of the milk ducts or lobules. This study presents 14 novel drug constructs for the treatment of breast cancer that are modeled from melphalan scaffolding. Melphalan is a nitrogen mustard agent which has been utilized successfully for the clinical treatment of metastatic breast cancer. Likewise each 14 new construct are nitrogen mustard agents with zero violations of the Rule of 5. Important pharmacological molecular properties were determined which showed a interesting range in Log P, molar volume, aqueous solubility, and formula weight. The numerical values of Log P range from 0.58 to 3.43, formula weight from 275.05 to 488.16, molar volume from 242.2 A^3 to 471.98 A^3, and aqueous solubility from 0.37 mg/Liter to 250.8 mg/Liter. Results of this study reveal the substantial range of effects that substituent substitution imputes on properties that are important for pharmaceutical activity. Cluster analysis of molecular properties revealed the most similar constructs to melphalan. Analysis of similarity (ANOSIM) indicated that all 14 constructs were related to melphalan. Pattern recognition methods reveal efficacious variations in pharmacodynamic activity among the constructs. In summation, an assemblage of 14 potentially beneficial anticancer agents were elucidated.

KEYWORDS

Breast cancer;
Melphalan;
Cancer.

INTRODUCTION

Breast cancer is a type of cancer that begins usually in the inner lining of the milk ducts or lobules. There are various cases of breast cancer that will have varying levels of proliferation, aggressiveness, and genetic constitution. Survival rates vary depending on factors of aggressiveness, genetic description, and rate of proliferation. The 10-year disease-free survival rate will vary from 10% o 98%. Clinical treatment includes surgery, drugs (hormone therapy and chemotherapy), with or without radiation. Breast cancer is the second most common type of cancer after lung cancer. Previous studies have shown that melphalan can be utilized separately, but as a second in a sequential ternary drug regimen (paciltaxel, melphalan, cyclosphos- phamide) for metastatic breast cancer treatment and providing a high response outcome[1]. A ternary drug regimen (busulfan,

melphalan, thiotepa) which includes melphalan has shown substantial benefit in the treatment in stage II and stage III operable tumors of the breast[2,3]. A well tolerated and beneficial outcome for treatment of metastatic breast carcinoma has been achieved by combination chemotherapy having mitomycin C, melphalan, and methotrexate[4].

Clinical treatment of breast cancer that includes melphalan with supplemental care show considerable potential in managing proliferated disease. The single application of an intensive combination alkylating agents (high dose cyclophosphamide, cisplatin, melphalan or carmustine) in addition to bone marrow support produces a more rapid and more frequent complete response compared to conventional chemotherapy[5]. A high dose regimen of melphalan treatment applied with surgery has demonstrated substantial benefits for cancer patients[6].

Melphalan is administered by intravenous injection rather than orally, which is an approach that allows greater manageable control of toxicity and greater predictability in blood levels[7]. The application of melphalan for the clinical treatment of multiple myeloma, and without autologous bone marrow transplant, results in favorable outcome[8]. Adjuvant treatment of ovarian carcinoma with use of melphalan but after surgery resulted in a very favorable absence of residual disease[9]. Encouraging evidence resulted after high dose melphalan was administered with noncryopreserved autologous bone marrow as a treatment for malignant melanoma and neuroblastoma[10]. When melphalan is included in a multidrug regimen for treatment of relapsed Hodgkin's disease a comparable result was achieved as that credited to other combination chemotherapy regimens currently in use[11].

Studies of tumor response pertaining to melphalan clinical use strongly supports the contention that perfusion and drug distribution are major factors in variable tumor response[12]. Multidrug resistance to important anticancer drugs such as melphalan in chemotherapy of breast cancer[13-15], is an alarming development that stresses the need for further elucidation and development of chemotherapeutic agents. This work presents 14 novel drug designs having modified structural features that alter the pharmaceutical properties to present a flexible and effective treatment regimen in the face of tumor to tumor variation.

EXPERIMENTAL

Molecular modeling and in silico search

Modeling was accomplished utilizing ACD/ ChemSketch modeling v. 10.00 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada). Numerical values of molecular properties such polar surface area, violations of Rule of 5, molecular volume, number of oxygens and nitrogens, amines (-NH_n) and hydroxyls (-OH), etc were determined using Molinspiration (Molinspiration Chemiformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). In silico structure search for substituent replacement was accomplished using Chemical substructure and similarity search with MolCart Chemical Data Base (Molsoft L.L.C. 3366 North Torrey Pines Court, Suite 300, La Jolla, CA 92037 U S A).

Pattern recognition and expatiation

To identify underlying associations/patterns within the properties numerical matrix required the use of various pattern recognition techniques. Included in the analysis is hierarchical cluster analysis accomplished by KyPlot v. 2.0 Beta 15 (copyright Koichi Yoshioka 1997-2001). ANOSIM (analysis of similarity analysis was performed by PAST v. 1.80 (copyright Oyvind Hammer, D.A.T. Harper 1999-2008).

Numerical analysis of multivariate data

Statistical analysis of all numerical data was performed by Microsoft EXCEL (EXCEL 2003, copyright 1985-2003). Correlation analysis by Pearson r was done for some descriptors and was accomplished by GraphPad Software (GraphPad Instat v. 3.00 for Windows 95 GraphPad Software, San Diego California USA).

RESULTS AND DISCUSSION

Melphalan (Alkeran) is a bifunctional alkylating nitrogen mustard agent that can utilized either orally or intravenously for the clinical treatment of breast cancer, multiple myeloma[7], and ovarian cancer[9]. The two principal types of breast cancer are the most common forms of ductal carcinoma and lobular carcinoma which be-

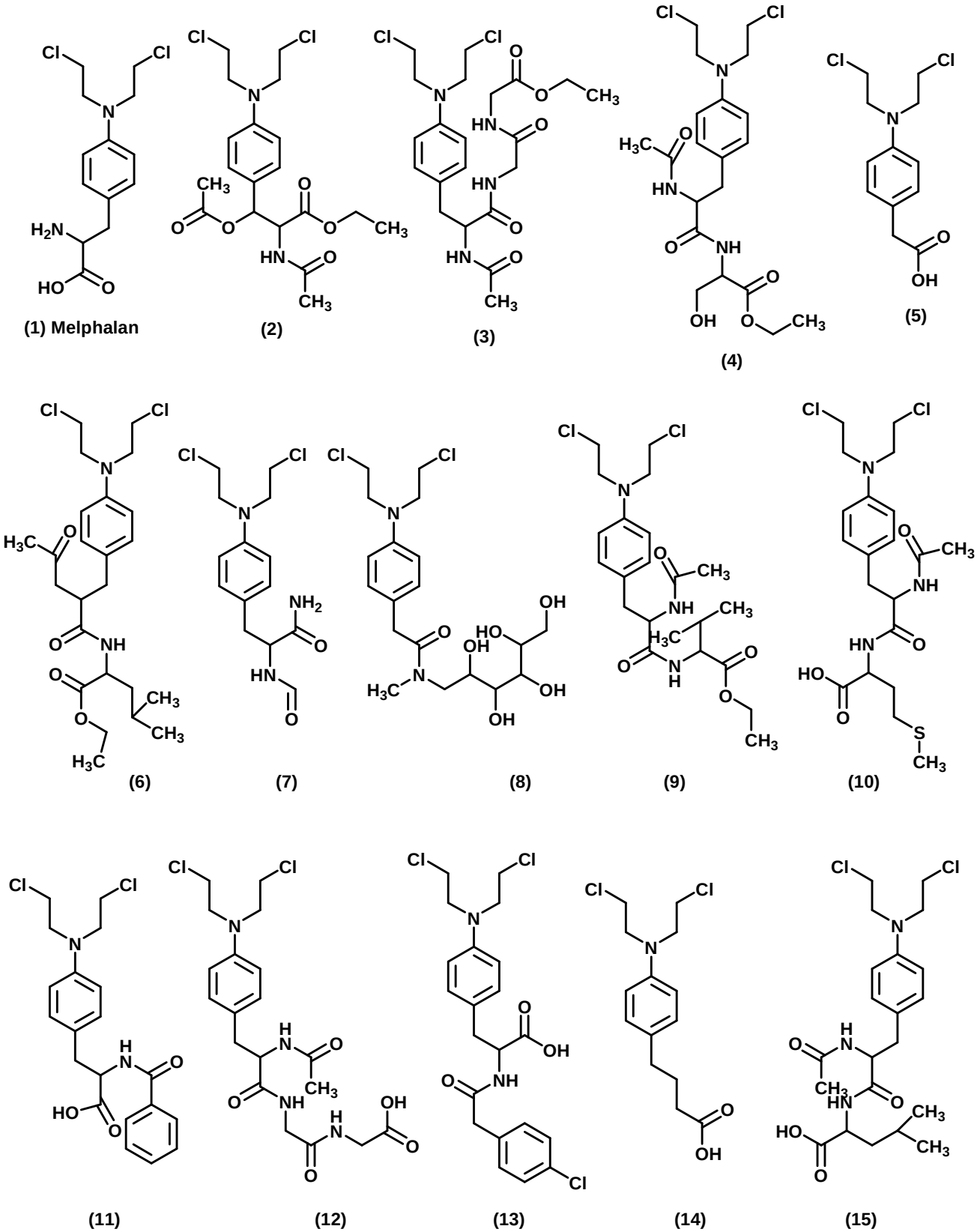

Figure 1 : Drug constructs (2) through (15) with the parent scaffold of melphalan. Note that each drug construct retains the N-mustard group that is covalently bonded to an aromatic ring similarly to melphalan. Variations in structure for constructs (2) through (15) occurs at the substituent that is in para position to the N-mustard group. All compounds have zero violations of the Rule of 5

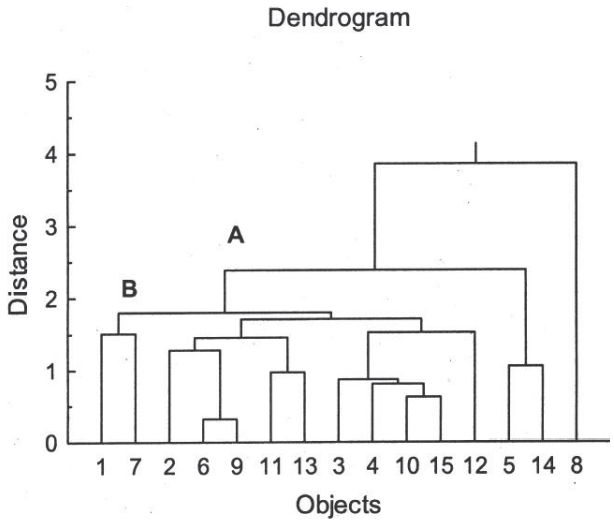

Figure 2 : Hierarchical cluster analysis of drugs (1) through (15) by molecular properties presented in TABLE 1 will arrange into clusters (nodes A, B) which members have highest levels of similarity. The 2-way dendrogram presents into Cluster A) Drugs 1(melphalan), (2), (3), (4), (6), (7), (9), (10), (11), (12), (13), (15); Cluster B) Drugs (1) (melphalan) and (7). Interestingly drug 8 is distinguished from all other constructs and placed into a distinct cluster

gins in the lobules or milk producing tissue. Melphalan has been shown previously to be valuable in the treatment of breast carcinoma individually[5], consorted with surgery[6], and as part of a multidrug chemotherapy regimen[11]. However issues have been identified associating melphalan to multidrug resistance in cancer treatment[13-15]. Novel drug constructs derived by substituent substitution and in silico optimization to melphalan are presented here to demonstrate the efficacy of modifying molecular properties to enhance pharmacodynamic action.

The methodical and mensurated substitution of substituents comprising the scaffolding of melphalan produces novel drug structures that have modified pharmaceutical properties which may enhance and expand the medicinal usefulness of this anticancer agent. In silico search by optimization (EXPERIMENTAL) produced 14 agents that are presented in figure 1. All 14 constructs retain a minimum of one aromatic ring and the nitrogen mustard bifunctional alkylating moiety. Other structure components appearing include carboxyl groups (-COOH), hydroxyl (-OH), amide (-CONHR), and aliphatic carbons. Numerical values of important molecular properties are presented in TABLE 1.

Various tools for profiling drug likeness have improved the efficiency of identifying potential clinical chemotherapeutic agents in the face of rising costs and complexity of synthesis and pharmacodynamics/pharmacokinetics. The Lipinski's Rule of 5 contends that orally active drugs have the following attributes[16]: 1) Not more than 5 hydrogen donors (-OH and $-NH_n$); 2) Not more than 10 hydrogen bond acceptors (oxygen and nitrogen); 3) A Log P less than 5; 4) Formula weight less than 500. Wholly all of these criteria are fulfilled by these 15 compounds (Figure 1). Compounds having violations are believed to have problems in drug absorption. Consequently, agents **(2)** through **(15)** would be anticipated to have favorable intestinal absorption and cellular permeation[16]. However alternate references for drug-likeness include those of Ghose[17], which have acceptable values for Log P and formula weight to be Log P -0.4 to 5.6, and formula weight 160 to 480, being somewhat different than those of Rule of 5.

Anticancer activity of these agents are not limited to breast cancer but could also include treatment of carcinomas of the central nervous system if using the parameters determined to be maximal for blood-brain barrier penetration[18,19]: 1) Log P from 1 to 4; 2) Formula weight less than 400; 3) Polar surface area less than 90 Angstroms^2. Following these criteria then the following analogues to melphalan are determined to have potential as brain cancer chemotherapeutics: **(5)**, **(7)**, **(11)**, and **(14)**. Treatment of brain tumors are highly problematic due to the limitations present in delivery caused by the blood-brain barrier. However, a dual application imputes higher efficacious potential for these particular drug constructs.

Criteria for favorable intestinal absorption have also been investigated with focus on polar surface area as the primal guiding descriptor[20]. Applying these criteria it can be determined that up to 50 % of orally administered drug would be absorbed in the intestinal tract for analogues **(3)**, **(4)**, **(8)**, **(10)**, **(12)**, **(15)**, **(16)**, and **(20)**. By polar surface area values then the following analogues would have greater than 50 % intestinal absorption: **(2)**, **(5)**, **(6)**, **(7)**, **(9)**, **(11)**, **(13)**, **(14)**, **(17)**, **(18)**, **(19)**, **(21)**, **(22)**, **(23)**, **(24)**, and **(25)**. Clearly these outcomes strongly support the potential of analogs of melphalan for administration as treatment for breast cancer and carcinoma of the central nervous system in the case of analogues **(5)**, **(7)**, **(11)**, **(14)**, **(17)**, and

TABLE 1 : Molecular properties of anti-cancer compounds

Compound	Formula wt.	log P	Polar surface area (Angstrom^2)	Molar volume (Angstrom^3)	nON	nOHnNH	Aqueous solubility (mg/L)	Violations of rule of 5
Melphalan 1	304.07	0.58	52.74	267.47	4	2	32.03	0
2	432.12	3.43	69.92	407.5	7	1	7.76	0
3	488.16	1.52	98.53	471.98	9	3	9.68	0
4	461.15	1.47	90.96	436.64	8	3	25.76	0
5	275.05	2.9	32.03	242.2	3	1	67.67	0
6	487.2	3.4	73.84	481.26	7	2	1.22	0
7	331.09	1.86	63.19	299.02	5	2	53.45	0
8	452.15	1.28	103.69	411.46	8	5	250.8	0
9	473.18	3.26	72.83	463.73	7	2	1.38	0
10	477.13	1.18	81.16	443.67	7	3	6.99	0
11	408.1	2.7	56.13	370.3	5	2	1.23	0
12	460.13	0.45	106.21	431.97	9	4	20.75	0
13	456.08	3.32	56.14	405.69	5	2	0.37	0
14	303.08	3.26	32.03	277.86	3	1	16.03	0
15	459.17	1.79	81.16	441.97	7	3	3.34	0

(18). Clearly, the design of anticancer drugs utilizing melphalan as the parent scaffold will yield many potentially advantageous constructs.

However the intrinsic similarities of these constructs that uses melphalan as the parent scaffolding is reinforced by application of Analysis of Similarity (ANOSIM). ANOSIM tests for differences among groups of cases (drugs) within a multivariate data set[21]. This test acquires the distance measurements within the data set and converts these into ranks. In this analysis the R value closest to the numerical value of one suggests substantial dissimilarity among groups. ANOSIM analysis of TABLE 1 properties produces a result of R equal to 0.07117, a value acknowledging the high similarity of structures of constructs **(2)** to **(15)** to the parent melphalan (drug 1).

Furthermore, calculation of Pearson's correlation coefficient r for objects (drugs) produced a very high r for all drugs of greater than 0.8900 (an extremely high correlation). In addition the coefficient of determination (R^2) at 0.7921 indicates that these properties account for more than 79.21% of the variance observed this group of compounds. Clearly the examination and comparison of these descriptors from one drug to another is an effective approach to elucidating their capableness as anticancer agents for the treatment of breast cancer.

Hierarchical cluster analysis is a pattern recognition method for finding homogeneous clusters of drugs, by utilizing measured descriptors[21,22]. Outcome of cluster analysis is represented as a 2-way dendrogram that presents the drugs into clusters where each member is determined to be most similar to other members. Outcome for cluster analysis of TABLE 1 descriptors is shown in figure 2, utilizing single linkage (nearest neighbor) conditions and Euclidean distance (geometric distance in multidimensional space). The 2-way dendrogram representation clearly shows that at supernode (A) are drugs **(1)**, **(2)**, **(3)**, **(4)**, **(6)**, **(7)**, **(9)**, **(10)**, **(11)**, **(12)**, **(13)**, and **(15)**. Interestingly the cluster analysis shows drug 8 to be distinct from all other agents (including melphalan). Compound **(8)** possesses five hydroxyl groups (-OH) extending from an amide functional group that is in the para position relative to the nitrogen mustard alkylating moiety. The cluster analysis suggests the presence of five hydroxyl groups are highly differentiating and substantially impacts the molecular properties of this compound. At supernode (B) the drugs **(1)**, **(2)**, **(3)**, **(4)**, **(6)**, **(7)**, **(9)**, **(10)**, **(11)**, **(12)**, **(13)**, and **(15)** are further secernated into a multitude of subclusters to attain the highest description of similarity. The parent drug melphalan is determined to be most

similar to construct **(7)**. In terms of clinical expectations, drugs **(1)** (melphalan) and **(7)** would have the greatest level of analogous medicinal action. Drug **(8)** is unique in that it has a large amide group having five hydroxyl groups (-OH), which provides this drug with the highest water solubility at 250.8 milligrams/Liter in lieu of greater hydrogen bonding capability.

While some properties vary considerably, it is possible to determine the underlying relationships by applying pattern recognition methods of analysis. For many constructs the pivotal properties of molecular weight, polar surface area, partition coefficient Log P, etc strongly supports the contention that these novel constructs will have favorable bioavailability (by Rule of 5) in addition to delivering the bifunctional cytotoxic nitrogen mustard moiety that is the anticancer activity of this class of anticancer drugs. In this fashion novel drug designs are produced with supporting evidence of their substantial potential in the clinical treatment of breast cancer.

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REFERENCES

[1] L.T.Vahdat, C.Balmaceda, K.Papadopouos, K.Frederick, D.Donovan, E.Sharpte, E.Kaufman, D.Savage, A.Tiersten, G.Nichols, J.Haythe, A.Troxel, K.Antman, C.S.Hesdorffer; Bone Marrow Transplant, **30(3)**, 149 **(2002)**.

[2] F.Gutierrez-Delgado, L.A.Holmberg, H.Hooper, F.R.Appelbaum, R.B.Livingston, R.T.Maziarz, P.Weiden, S.Rivkin, P.Montgovery, K.Kawahara, W.Bensinger; Bone Marrow Transplant, **26(1)**, 51, **(2000)**.

[3] W.I.Bensinger, K.S.Schiffman, L.Holmberg, F.R.Applebaum, R.Maziarz, P.Montgomery, E.Ellis, S.Rivkin, P.Weiden, K.Lilleby, S.Rowley, S.Petersdorf; Bone Marrow Transplantation, **19(12)**, 1183 **(1997)**.

[4] D.J.Perez, T.J.Powles, J.C.Gazet, H.T.Ford, R.C.Coombes; Cancer Chemotherapy and Pharmacology, **13(1)**, 36 **(2004)**.

[5] W.P.Peters, E.J.Shpall, R.B.Jones, G.A.Olsen, R.C.Bast, J.P.Gockerman, J.O.Moore; Journal of Clinical Oncology, **6(9)**, 1368 **(1988)**.

[6] H.J.Stemmler, H.Menzel, C.Salat, H.Lindhofer, S.Kahlert, V.Heinemann, J.H.Kolb. Anti-Cancer Drugs, **16(10)**, 1135 **(2005)**.

[7] G.Sarosy, B.Leyland-Jones, P.Soochan, B.D.Cheson; American Society of Clinical Oncology, **6(11)**, 1768 **(1988)**.

[8] D.Cunningham, L.Paz-Ares, M.E.Gore, J.Malpas, T.Hickish, M.Nicolson, M.Meldrum, C.Viner, S.Milan, P.J.Selby; Journal of Clinical Oncology, **12**, 764 **(1994)**.

[9] G.Spatti, M.Regazzoni, R.Koronel, A.M.Zecchini, G.De Palo; Tumori, **73(2)**, 157 **(1987)**.

[10] T.J.McElwain, D.W.Hedley, M.Y.Gordon, M.Jarman, J.L.Millar, J.Pritchard; Experimental Hematology, **7 Suppl 5**, 360 **(1979)**.

[11] D.J.Straus, J.Myers, B.Koziner, B.J.Lee, B.D.Clarkson; Cancer Chemotherapy and Pharmacology, **11(2)**, 80 **(1983)**.

[12] L.Simpson-Herren, P.E.Noker; Cancer Chemotherapy and Pharmacology, **22(2)**, 131 **(1988)**.

[13] T.Saeki, T.Tsuruo, W.Sato, K.Nishikawsa; Cancer Chemother. Pharmacol., **56 Suppl 7**, 84 **(2005)**.

[14] J.A.Moscow, P.G.Johnston, D.Cole, D.G.Poplack, K.H.Cowan; Biochem.Pharmacol., **49(8)**, 1069 **(1995)**.

[15] J.A.Moscow, C.A.Swanson, K.K.Cowan. British Journal of Cancer, **68(4)**, 732 **(1993)**.

[16] C.A.Lipinski, F.Lombardo, B.W.Dominy, P.J.Feeney; Adv.Drug Del. Rev., **16**, 3 **(2001)**.

[17] A.K.Ghose, V.N.Viswanadhan, J.J.Wendoloski; J.Combin.Chem., **1**, 55 **(1999)**.

[18] H.van de Waterbeemd, W.Kansy; Chimia, **46**, 299 **(1992)**.

[19] H.van de Waterbeemd, G.Camenisch, G.Folkers, J.R.Chretien, O.R.Raevsky; Journal of Drug Targeting, **6**, 161 **(1998)**.

[20] K.Palm, P.Stenberg, K.Luthman, P.Artursson; Pharm.Res., **14(5)**, 568 **(1997)**.

[21] S.T.Bow; 'Pattern Recognition', Marcel Dekker; New York, **(1984)**.

[22] M.R.Anderberg; 'Cluster Analysis for Applications', Academic Press; San Diego, **(1973)**.