Ifosfamide. Metabolic Studies, New Therapeutic Approaches and New Analogs

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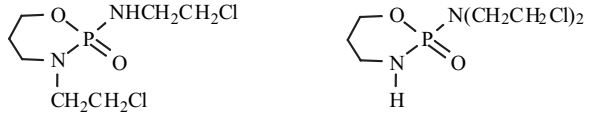
Abstract: Ifosfamide (IFO), an oxazaphosphorine-type anticancer alkylating agent, was found to be particularly useful in the treatment of a wide variety of neoplasm in adults and children. IFO is a positional isomer of cyclophosphamide (CPA) and was introduced into clinical practice in the '80s and has recently attracted much attention. Therapeutic application of high-dose IFO is limited by several side-effects; among them neurotoxicity and nephrotoxicity give the greatest concern. The presence of these side-effects is likely to be connected with the metabolism of this drug. In recent years there have been many studies aiming better understanding metabolism of this drug, employing new therapeutic approaches and preparing new analogs.

Keywords: Ifosfamide, Cyclophosphamide, Bromofosfamide, Oxazaphosphorine drugs.

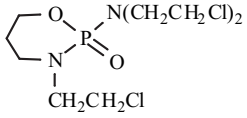
#This paper is dedicated to Professor Wojciech J. Stec on the occasion of his 65th birthday.

INTRODUCTION

Ifosfamide {[*N*,3-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-amine 2-oxide], IFO, CAS 3778-73-2} is an anticancer alkylating agent found to be particularly useful in the treatment of a wide variety of neoplasms in adults and children [1, 2]. It belongs to the family of oxazaphosphorine drugs along with cyclophosphamide (CPA) and trofosfamide (TRO) (Fig. **1**).



IFO CPA

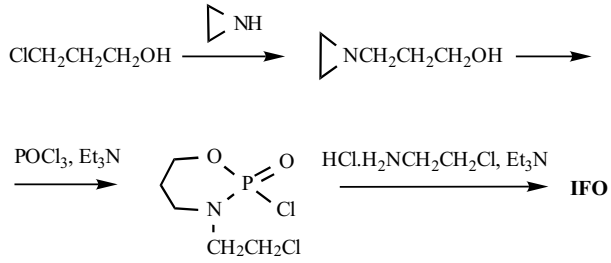


TRO

Fig. (1). Chemical structures of ifosfamide (IFO), cyclophosphamide (CPA), and trofosfamide (TRO).

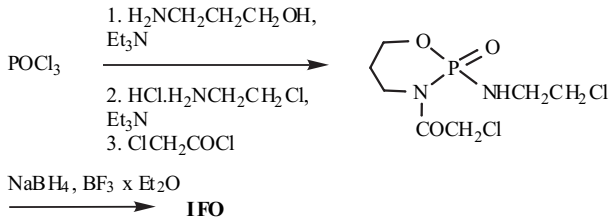
These three drugs, although having similar chemical structure, differ in their metabolism, pharmacokinetics, and spectrum of anticancer activity. Chemistry [3-5], pharmacology [6-9] and pharmacokinetics [10-12] of CPA have been extensively reviewed. The use of TRO in clinic is limited mostly to the palliative treatment of cancer patients [13]. In this review we will summarize the latest developments on ifosfamide.

After the introduction at the end of '50s, of CPA into clinic intensive structure-activity relationship studies were performed in the Asta-werke Laboratories in Germany [14]. One of these studies focused on different arrangement of 2-chlorethyl alkylating groups. IFO was synthesized as an effect of this program in three steps starting from aziridine and 3-chlorpropanol [15] (Scheme **1**).



Scheme 1.

The use of highly toxic and volatile aziridine is inconvenient for the industrial preparation of IFO and a new method was developed (Scheme **2**) [16, 17] based on a chloroacetylation-carbonyl reduction procedure [18] of introduction of 2-chlorethyl moiety on *N*3 atom of 1,3,2-oxazaphosphorine ring.



Scheme 2.

IFOSFAMIDE METABOLISM

The pharmacology [19] and pharmacokinetics [10-12] of IFO have been reviewed. Briefly, IFO is a pro-drug activated *in vivo* by cytochrome P450, mostly CYP3A4 an CYP2B6 subtypes, which take part in hydroxylation of C-4 atom of tetrahydro-2*H*-1,3,2-oxazaphosphorine ring (Fig. **2**).

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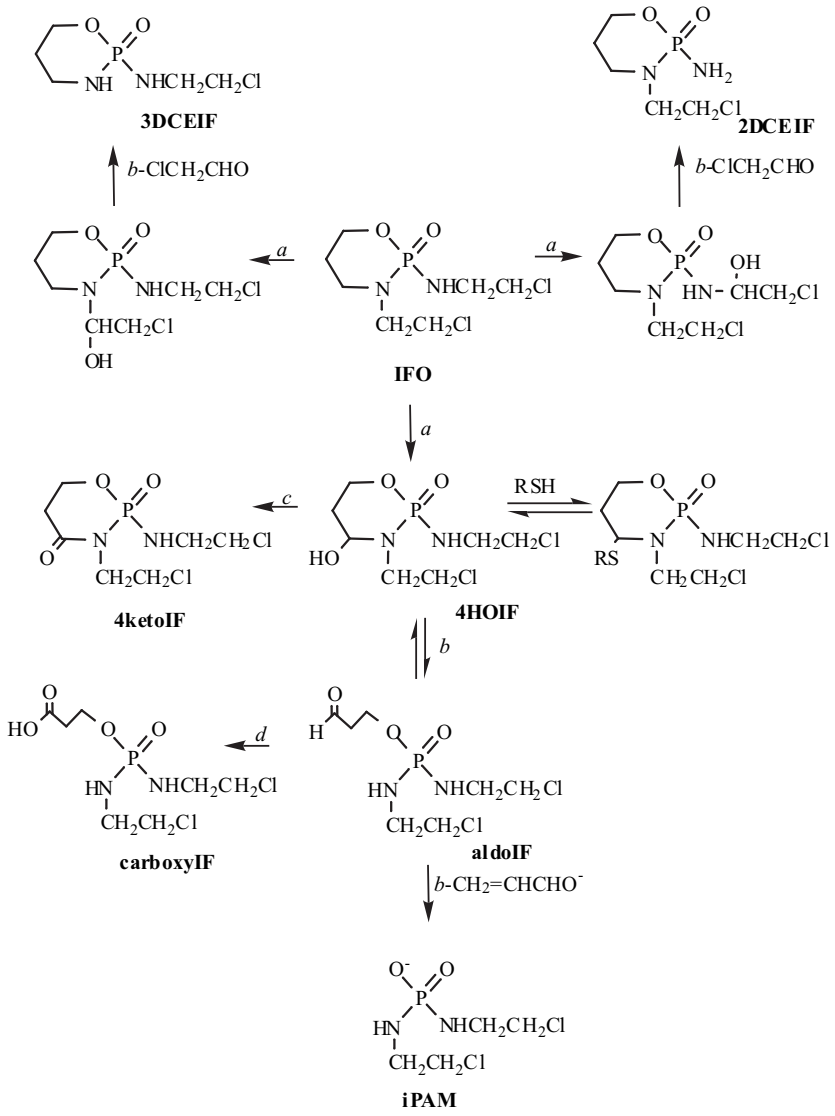


Fig. (2). Ifosfamide (IFO) metabolic pathways.

The resulting 4-hydroxyifosfamide (4HOIF) is in tautomeric equilibrium with aldoifosfamide (aldoIF), which spontaneously releases isophosphoramide mustard (iPAM), a final DNA bis-alkylating metabolite, and acrolein. This last metabolite is suspected of causing bladder toxicity. To avoid this side-effect, IFO therapy similarly as CPA one, is combined with the use of 2-mercaptoethylsulfonate (Mesna). At the same time 4HOIF and aldoIF are oxidized into 4-ketoifosfamide (4ketoIF) and carboxyifosfamide (carboxyIF), respectively. Both of these metabolites are biologically inactive and excreted in the urine. Contrary to CPA, IFO also undergoes metabolic hydroxylation of C-1 atoms of 2-chloroethyl chains leading to the formation of unstable hydroxy intermediates which spontaneously collapse into 2- and 3-dechloroethylated metabolites, 2DCEIF and 3DCEIF and chloracetaldehyde. This last compound is suspected to be responsible for neurotoxicity and nephrotoxicity of IFO, the major dose-limiting side-effects of this drug [20-22]. Undesired side-chain hydroxylation metabolic pathways consist up to 50% of IFO metabolism in adult patients [23, 11]. Metabolism of IFO was extensively examined with the use of various analytical techniques such as TLC, HPLC, GC-MS and ^{31}P NMR spectroscopy [24-30]. This last method is recently the most widely used since it enables quantification of unchanged IFO and all its stable metabolites excreted with the urine without further work-up or derivatization.

Recently, an attempts have been undertaken to correlate amounts of chloracetaldehyde formed during metabolism of IFO with neurotoxicity and nephrotoxicity of this drug in cancer children [31]. Study of IFO metabolism using ^{31}P NMR spectroscopy showed a great variation of the level of excreted unmetabolized drug and its metabolites 2DCEIF and 3DCEIF which are formed in equimolar amounts with chloracetaldehyde. However, no correlation between higher level of 2DCEIF and 3DCEIF and the occurrence of neurotoxicity and nephrotoxicity were found. Such a result suggests the presence some additional factors influencing

these side-effects, probably connected with a second phase of metabolism and activity of glutathione S-transferase which is able to detoxify chloracetaldehyde. Such hypothesis is now under examination in our Laboratory. However, other studies proved that expression of specific sub-types of cytochrome P450 can be linked to therapeutic effect of IFO [32] and its nephrotoxicity [33].

Ifosfamide is a chiral molecule and exists in enantiomeric forms (Fig. **3**) but stereochemical aspects of its metabolism are largely uninvestigated.

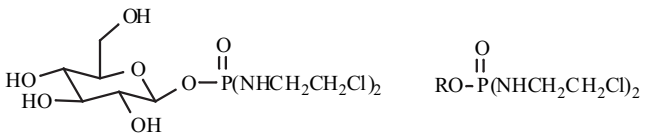
(S)-(-)-IFO **(R)-(+)-IFO**

Fig. (3). Chemical structures of ifosfamide (IFO) enantiomers.

We were the first to prove that metabolic hydroxylation of IFO side-chains is stereoselective [23] and this was later confirmed in other studies [34, 35]. It has consequences in the differences between antitumor activities of IFO enantiomers against several experimental tumors in mice [36]. In these studies we found that (*S*)-(-)-IFO was more active than either a racemate or the (*R*)-(+)-IFO. However, some very recent studies on enantioselective metabolism and cytotoxicity of (*R*)- and (*S*)-IFO [37, 38] suggest that tumors mostly expressing CYP3A enzymes should be more sensitive to the *R* enantiomer. The problem of stereoselectivity of IFO metabolism and antitumor activity clearly needs further investigation.

NEW THERAPEUTIC APPROACHES

The toxicity related to conventional anticancer chemotherapy is mostly caused by the lack of selectivity of cytostatics used against target tumor cells. The desired selectivity can be obtained by, *inter alia*, the use of gene therapy. One method of such anticancer experimental therapy is enzymatic activation of pro-drug in genetically modified tumor cells, so-called Gene-Directed Enzyme Pro-drug Therapy (GDEPT) [39, 40]. In recent years several enzyme/pro-drug systems were elaborated, some are now under clinical trial [41]. The possibility of the use of IFO and of cytochrome P450 isoforms was intensively examined in recent years and these studies have been summarized in some reviews [42-45]. Gene therapy employing IFO and retroviral delivery of CYP2B1 and CYP2B6 is now under phase II clinical trials.



GFO

1. R = $C_6H_5CH_2$
2. *p*-$CH_3C_6H_4CH_2$
3. *p*-$FC_6H_4CH_2$
4. *p*-$NO_2C_6H_4CH_2$
5. $CH_3COSCH_2CH_2$
6. $(CH_3)_3CCOSCH_2CH_2$
7. $C_6H_5COSCH_2CH_2$
8. $(C_6H_5CH_2O)_2P(O)OC_6H_4CH_2$
9. $(HO)_2P(O)OC_6H_4CH_2$

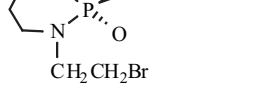
Fig. (4). Chemical structures glufosfamide (GFO) and newly obtained isophosphoramide mustard derivatives (**1**-**9**).

Recently, studies were undertaken to examine the possibility of the use in GDEPT derivatives of iPAM, the final IFO metabolite. [46, 47] (Fig. **4**). Such derivatives can have good, desirable pharmacokinetic properties, as already shown for the glycoside ester of iPAM, glufosfamide (GFO) [48].

Three groups of iPAM derivatives have been obtained: benzyl (**1**-**4**), which can be activated by cytochrome P450, acylthioethyl (**5**-**7**), possibly activated by hydrolases, and phosphate (**8**-**9**), activated by phosphatases. A correlation between the ability of these compounds to be activated by the appropriate enzymes and their cytotoxicity *in vitro* and antitumor activity *in vivo* has been found. Acylthioethyl compounds (**5**-**7**) possess strong antitumor activity. (However, the employment of iPAM derivatives in GDEPT should rather be based on the use of enzymes, which are not present in human cells; in this context the use of phosphotriesterases [49], enzymes now examined for detoxification of phosphoroorganic nerve gases, seems to be very promising). Such studies are now underway in this Laboratory. The use of differently substituted iPAM derivatives, e.g. with 2-bromoethyl group(s), gives a chance to modulate their alkylating properties. In other studies, the use of the phosphoramide mustard (PAM, active metabolite of cyclophosphamide) derivatives in GDEPT has been examined recently [50-53].

NEW IFOSFAMIDE ANALOGS

In late '80s, based on the differences in alkylation ability between iPAM and PAM, we set up a working hypothesis that antitumor activity among these group of compounds strongly depends on the kind of leaving group present in the mustard moiety. Formation of aziridinium ions from the mustard 2-chlorethyl group is a key process in the DNA bis-alkylation and any interference in it should have a profound effect on anticancer activity [54]. Based on this hypothesis, a series of ifosfamide analogs possessing modified exocyclic 2-chloroethyl moiety have been synthesized. Among these compounds a bromo analog possessed the highest antitumor activity against L1210 Leukemia. Further studies on the antitumor [55], pharmacokinetics [56, 57], and pharmacological properties [58] of stereoisomers of bromo analogs of IFO led to the selection of the most active compound (*S*)-(-)-bromofosfamide (SBF) (Fig. **5**).

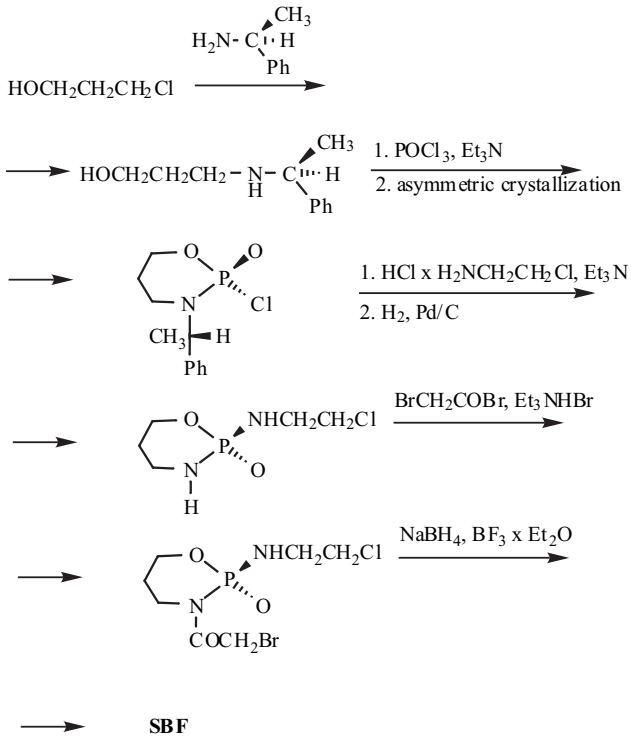


SBF

Fig. (5). Chemical structure of (*S*)-(-)-bromofosfamide (SBF).

In the studies on mice, this agent possessed similar toxicity against urinary tract as IFO, particularly when it was used together with Mesna [59]. At the same time it was found that a synergistic effect on antitumor activity of cells releasing interleukine 2 and SBF [60]. Efficient synthesis of

SBF was elaborated [61] (Scheme **3**) and now this compound can be prepared, starting from 3-chlorpropanol and (-)-α-methylbenzylamine, in a six steps with 25% total yield.



Scheme 3.

A comparison of antitumor activities of SBF and IFO using murine model tumors (Leukemia L1210, Lewis Lang carcinoma, and melanoma B16) showed an increase of therapeutic indices in the range 14-127%. Based on these encouraging results, SBF recently became a subject of phase I clinical studies in Poland in which maximal tolerated dose was elaborated to be 1.89 g/m^2 [62].

In the first stage of study of SBF metabolism, the synthesis of its potential metabolites (**10**-**14**) has been performed [63] (Fig. **6**).

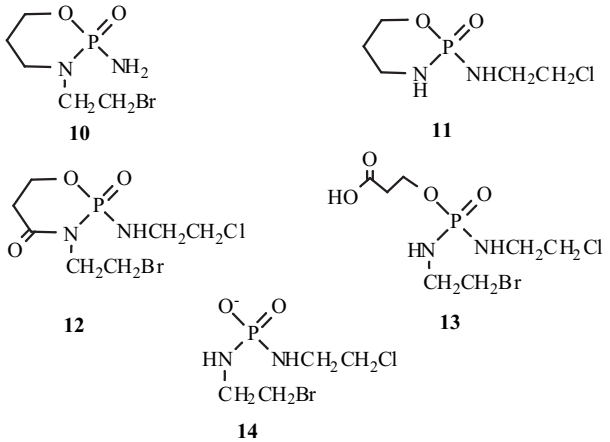


Fig. (6). Chemical Structures of Potential Metabolites (**10**-**14**) of (*S*)-(-)-Bromofosfamide.

Compounds (**10**-**14**) will be used as standards for quantitative analysis of SBF metabolism. These studies, in a combination of analysis of SBF anticancer mode of action, should lead to an explanation of high activity and stereoselectivity of this experimental drug. Its final metabolite (**14**) is prochiral and the most probably biological stereodifferentiation regulate the earlier stages of metabolism of SBF. It is noteworthy that SBF belongs to a small group of anticancer alkylating agents with a 2-bromoethyl group. Another such compound is tallimustine congener PNU 15977 [64].

As described above, therapeutic use of IFO and SBF in necessary high doses is limited mostly by nephrotoxicity and neurotoxicity [20-22]. The most probable explanation of these side-effects is the release of chlor(brom)acetaldehyde in a process of metabolic hydroxylation of C-1 atoms in 2-chloro(bromo)ethyl groups. Any inhibition of this reaction should diminish these side-effects. A hypothesis which was formulated by exchange of hydrogen atoms connected with C-1 atom by deuterium should lead to retardation of such undesired side-chain hydroxylation [65]. To this end, a series of deutero-substituted analogs of IFO, e.g. α,α,α',α'-tetradeuterated derivative of ifosfamide (IFO-d$_4$) (Fig. **7**), and SBF were obtained in a form of racemic and enantiomeric compounds. Halogenoacetylation and subsequent reduction of carbonyl group were used to introduce deuterium atoms.

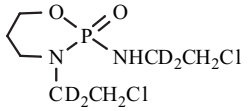


Fig. (7). Chemical Structure of α,α,α',α'-Tetradeuterated Derivative of Ifosfamide (IFO-d4).

IFO-d$_4$ was used for microsomal metabolic studies in which isotope effects in side-chain hydroxylation reactions have been established. Antitumor activity studies of IFO-d$_4$ and SBF-d$_4$ showed that these compounds were more active than IFO and SBF against of 1210 Leukemia in mice. It was also found that the levorotatory enantiomer of IFO-d$_4$ and SBF-d$_4$ were more active than a appropriate racemic compounds. Similar stereodifferentiation of biological activities had already been found for CPA and IFO [36] and bromo analogs of IFO [55]. Observed differences in biological activities among deutero-substituted compounds and unlabelled ones, confirm the hypothesis that IFO metabolic pathway leading to the chloracetaldehyde is undesired from a point of view of antitumor properties. However, the role of chloracetaldehyde formation during anticancer therapy with IFO is still controversial and other studies suggested a positive effect of this metabolite for anticancer activity of this drug [66].

To avoid undesired metabolic side-chain hydroxylation of IFO, a tetramethyl IFO analog {[*N*,3-bis(2-chloro-1,1-methylethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-amine 2-oxide], was designed [67]. However, many attempts to synthesize this compound have failed. The major problem is the introduction of the 2-chloro-1,1-methylethyl group at *N*3 of the 1,3,2-oxazaphosphorine ring. It was only possible to make a dimethyl IFO analog, *N*-(2-chloro-1,1-dimethylethyl)-3-(2-chlorethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-amine 2-oxide] (**15**) (Fig. **8**) in a

reaction involving ring-opening of *gem*-dimethylaziridinyl compound with hydrogen chloride.

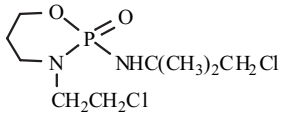


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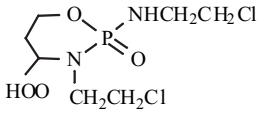
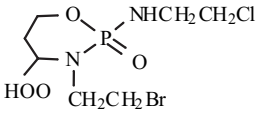
Fig. (8). Chemical Structure of *N*-(2-chloro-1,1-dimethylethyl)-3-(2-chlorethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-amine 2-oxide [**15**].

Microsomal activation of dimethyl IFO analog was examined and it was found to proceed in a similar rate as for IFO. However, this IFO congener was much less active against L1210 Leukemia than the parent drug. This result suggests that introducing methyl substituents into 2-chlorethyl groups of IFO diminishes the effectiveness of DNA alkylation by an appropriate final metabolite and this direction of IFO modification is not promising. Another study on the synthesis of dimethyl IFO analogs has been reported but no biological activities of obtained compounds were described [68].

Among oxazaphosphorine drugs, levorotatory enantiomers possess better antitumor activity than clinically used racemates [36, 55]. To assess the influence of stereochemistry of phosphorus on antitumor activity, the synthesis of pre-activated 4-hydroperoxy derivatives of IFO and SBF, 4HOOIFO and 4HOOSBF (Fig. **9**), were elaborated in a form of racemic and levorotatory enantiomers [69] .



4HOOIF



4HOOBF

Fig. (9). Chemical Structures of 4-Hydroperoksyifosfamide (4HOOIF) and 4-Hydroperoksy-bromofosfamide (4HOOBF).

4HOOIFO and 4HOOSBF were obtained from racemic and (-)-enantiomers of IFO and SBF by their oxidation by means of ozone and hydrogen peroxide. The *cis* and *trans* isomers formed in these reactions were separated from each other and their *in vitro* antitumor activity against SW707 (rectal adenocarcinoma), MCF-7 (breast carcinoma), KB (cervical carcinoma), and HCV29T (bladder cancer) was examined. It was found that 4HOOIFO species are more active than 4HOOSBF counterparts which was a bit unexpected in the context of higher activity of SBF than IFO. must probably, bromo analogs are chemically less stable than chloro ones. It was also found that there are no major difference in activities between *cis* and *trans* isomers and between racemic and levorotatory enantiomers. These results suggest that differences in antitumor activities between stereoisomers of IFO and SBF rely on stereoselectivity of side-chain hydroxylation and not on hydroxylation of 1,3,2-oxazaphosphorine ring. Such a hypothesis is also confirmed by the fact that large stereodifferentiation is observed for IFO and SBF, where metabolic hydroxylation of 2-chlor(brom)ethyl groups has a major role compared to CPA, where this metabolic pathway is only marginal.

4-Hydroperoxy compounds synthesized are probably too labile to use as therapeutics. However, other studies on pre-activated IFO analogs have shown high activity of 4-methoxyifosfamide [70] and sulfonyl derivatives of aldoifosfamide (aldoIF) [71] specially on tumors resistant for IFO treatment.

SUMMARY AND OUTLOOK

Recent studies on ifosfamide metabolism, new therapeutic approaches, and new analogs has led to a better understanding of the factors important for high antitumor activity of this drug. (*S*)-(-)-Bromofosfamide was evaluated to be a new IFO analog possessing promising anticancer activity. However, its metabolism and a mode of action are still poorly understood. A search for ifosfamide analogs devoid of neurotoxic and nephrotoxic side-effects is still largely unsuccessful. It can be expected that a more promising approach to realize this goal could be the supplementation of ifosfamide therapy with another agent which can inhibit the cytochrome P450 subtypes responsible for side-chain hydroxylation. The success of the use of ifosfamide in Gene-Directed Enzyme Pro-drug Therapy and preliminary studies presented above suggest that derivatives of the final, active metabolite isophosphoramide mustard can be used for this experimental gene therapy.

REFERENCES

[1] O'Byrne, K.; Steward, W.P. *Oncology,* **1999**, *56*, 13-23.
[2] Carli, M.; Passone, E.; Perilongo, G.; Bisogno, G. *Oncology,* **2003**, *65 (Suppl 2)*, 99-104.
[3] Rajski, S.R.; Williams, R.M. *Chem. Rev.,* **1998**, *98*, 2723-2795.
[4] Colvin, E.M.; Sasaki, J.C.; Tran, N.L. *Curr. Pharm. Design,* **1999**, *5*, 645-663.
[5] Ludeman, S.M. *Curr. Pharm. Design,* **1999**, *5*, 627-643.
[6] Colvin, O. M. *Curr. Pharm. Design,* **1999**, *5*, 555-560.
[7] Malet-Martino, M.; Gilard, V.; Martino, R. *Curr. Pharm. Design,* **1999**, *5*, 561-586.
[8] Gamcsik, M.P.; Dolan, M.E.; Andersson, B.S.; Murray D. *Curr. Pharm. Design,* **1999**, *5*, 587-605.
[9] Sladek, N.E. *Curr. Pharm. Design,* **1999**, *5*, 607-625.
[10] Boddy, A.V.; Yule, S.M. *Clin. Pharmacokinet.,* **2000,** *38*, 291-304.
[11] Kerbusch, T.; de Kraker, J.; Keizer, H.J.; van Putten, J.W.; Groen, H.J.; Jansen, R.L.; Schellens, J.H.; Beijnen, J.H. *Clin. Pharmacokinet.,* **2001,** *40*, 41-62.
[12] Baumann, F.; Preiss, R.J. *J. Chromatogr. B,* **2001**, *764*, 173-192.
[13] Latz, D.; Nassar, N.; Frank, R. *Onkologie,* **2004**, *27*, 572-576.
[14] Arnold, H.; Bourseaux, F.; Brock, N. *Arzneim.-Forsch*., ***1961***, *11*, 143-158.
[15] Brit. Patent 1 188 159.
[16] Pol. Patent 149 593.
[17] Pol. Patent 150 330.
[18] Pankiewicz, K.; Kinas, R.W.; Stec, W.J.; Foster, A.B.; Jarman, M.; Van Maanen, J.M.S. *J. Am. Chem. Soc.*, **1979**, *101*, 7712-7718.
[19] Williams. M.L.; Wainer, I.W. *Curr. Pharm. Design,* **1999**, *5*, 665-672.
[20] Furlanut, M.; Franceschi, L. *Oncology,* **2003**, *65 (Suppl 2)*, 2-6.
[21] Nicolao, P.; Giometto, B. *Oncology,* **2003**, *65 (Suppl 2)*,11-16.
[22] Skinner, R. *Med. Pediatr. Oncol.,* **2003**, *41*, 190-197.
[23] Misiura, K.; Okruszek, A.; Pankiewicz, K.; Stec, W.J.; Czownicki, Z.; Utracka, B. *J. Med. Chem.,* **1983**, *26*, 674-679.
[24] Norpoth, K.; Addiks, H.W.; Witting, U.; Muller, G.; Raidt, H. *Arzneimforsch.,* **1975**, *25*, 1331-1336.
[25] Goren, M.P. *J. Chromatogr.,* **1991**, *570*, 351-359.
[26] Wang, J. J.; Chan, K.K. *J. Chromatogr. B, Biomed. Appl.,* **1995**, *674*, 205-217.

[27] Kerbusch, T.; Jeuken, M.J.; Derraz, J.; van Putten, J.W.; Huitema, A.D.; Beijnen, J.H. *Ther. Drug Monitor.,* **2000**, *22*, 613-620.
[28] Gilard, V.; Malet-Martino, M. C.; de Forni, M.; Niemeyer, U.; Ader, J.C.; Martino, R. *Cancer Chemother. Pharmacol.,* **1997**, *31*, 387-394.
[29] Mancini, L.; Payne, G.S.; Dzik-Jurasz, A.S.; Leach, M.O. *Magn. Reson. Med.,* **2003**, *50*, 249-255.
[30] Payne, G.S.; Dzik-Jurasz, A.S.; Mancini L.; Nutley, B.; Raynaud, F.; Leach, M.O. *Cancer Chemother. Pharmacol.,* **2005**, *56*, 409-414.
[31] Misiura, K.; Zielinska, E.; Zubowska, M. *Arzneim. Forsch. Drug Res.,* **2003**, *53*: 372-377.
[32] Schmidt, R.; Baumann, F.; Knupfer, H.; Brauckhoff, M.; Horn, L.C.; Schonfelder, M.; Kohler, U.; Preiss, R. *Br. J. Cancer,* **2004**, *90*, 911-916.
[33] Aleksa, K.; Matsell, D.; Krausz, K.; Gelboin, H.; Ito, S.; Koren, G. *Pediatr. Nephrol*., **2005**, *20*, 872-885.
[34] Wainer, I.W.; Durchane, J.; Granvil, C.P.; Trudeau, M.; Leyland-Jones, B. *Lancet,* **1994**, *343*, 982-983.
[35] Granvil, C.P.; Durchane, J.; Leyland-Jones, B.; Trudeau, M.; Wainer, I.W. *Cancer Chemother. Pharmacol.,* **1996**, *37*, 451-456.
[36] Kusnierczyk, H.; Radzikowski, C.; Paprocka, M.; Budzynski, W.; Rak, J.; Kinas, R.W.; Misiura, K.; Stec, W. J. *J. Immunopharmacol.,* **1986**, *8*, 455-480.
[37] Schmidt, R.; Baumann, F.; Brauckoff, M.; Horn, L.C.; Schonfelder, M.; Kohler, U.; Preiss, R. *Br. J. Cancer,* **2004**, *90*, 911-916.
[38] Chen, C.S.; Jounaidi, Y.; Waxman, D.J. *Drug Metab. Dispos.,* **2005**, *33*, 1261-1267.
[39] Nicullescu-Duvaz, I.; Spooner, R.; Marais, R.; Springer, C.J. *Bioconjugate Chem.,* **1998**, *9*, 4-22.
[40] Nicullescu-Duvaz, I.; Friedlos, F.; Nicullescu-Duvaz, D.; Davies, L.; Springer, C.J. *Anti-Cancer Drug Design,* **1999**, *14*, 517-538.
[41] Fillat, C.; Carrio, M.; Cascante, A.; Sangro, B. *Curr. Gene Ther.,* **2003**, *3*, 13-26.
[42] Waxman, D.J.; Chen, L.; Hecht, J.E.D.; Jounaidi, Y. *Drug Met. Rev.,* **1999**, *31*, 503-522.
[43] Chen, L.; Waxman, D.J. *Curr. Pharm. Design,* **2002**, *8*, 1405-1416.
[44] Kan, O.; Kingsman, S.; Naylor, S. *Expert. Opin. Biol. Ther.,* **2002**, *2*, 857-868.
[45] Patterson, A.V.; Saunders, M.P.; Greco O. *Curr. Pharm. Design,* **2003**, *9*, 2131-2154.
[46] Misiura, K.; Szymanowicz, D.; Kusnierczyk, H.; Wietrzyk, J.; Opolski, A. *Acta Biochim. Pol.,* **2002**, *49*, 169-176.
[47] Misiura, K.; Szymanowicz, D.; Wietrzyk, J.; Opolski, A. *Acta Pol. Pharm. Drug Res.,* **2003**, *60*, 109-112.
[48] Giaccone, G.; Smit, E.F.; de Jonge, M.; Dansin, E.; Briasoulis, E.; Ardizzoni, A.; Douillard, J.Y.; Spaeth, D.; Lacombe, D.; Baron, B.; Bachmann, P.; Fumoleau, P. *Eur. J. Cancer,* **2004**, *40*, 667-672.
[49] Aubert, S.D.; Li, Y.; Raushel, F.M. *Biochemistry,* **2004**, *43*, 5707-5715.
[50] Hernick, M.; Flader, C.; Borch, R.F. *J. Med. Chem.,* **2002**, *45*, 3540-3548.
[51] Niculescu-Duvax, D.; Niculescu-Duvax, I.; Friedlos, F.; Martin, J.; Lehouritis, P.; Marais, R.; Springer, J.C. *J. Med. Chem.,* **2003**, *46*, 1690-1705.
[52] Hu, L.; Yu, C.; Jiang, Y.; Han, J.; Li, Z.; Browne, P.; Race, P.R.; Knox, R.J.; Searle, P.F.; Hyde, E.J. *J. Med. Chem.,* **2003**, *46*, 4818-4821.
[53] Jain, M.; Kwon, C.H. *J. Med. Chem.,* **2003**, *46*, 5428-5436.
[54] Misiura, K.; Kinas, R.W.; Stec, W.J.; Kusnierczyk, H.; Radzikowski, C.; Sonoda, A. *J. Med. Chem.,* **1988**, *31*, 226-230.
[55] Glazman-Kunierczyk, H.; Matuszyk, J.; Radzikowski, C. *Immunopharmacol. Immunotoxicol.,* **1992**; *14*, 883-911.
[56] Sloderbach, A.; Hladon, B.; Sochacki, M.; Kinas, R.W.; Kusnierczyk, H.; Laskowska, H. *Pol. J. Pharmacol.,* **1997**; *49*, 463-469.
[57] Kobylinska, K.; Kobylinska, M.; Sobik, B. *Arzneim. Forsch. Drug Res.,* **2001**, *51*, 596-599.
[58] Juszkiewicz, M.; Kleinrok, Z.; Sawiniec, Z. *Arch. Immunol. Ther. Exp., **1994**, 42*, 405-413.
[59] Kusnierczyk, H.; Konarski, L.; Kowalski, P.; Radzikowski, C. *Arch. Immunol. Ther. Exp., **1997**, 45*, 79-85.
[60] Kusnierczyk, H.; Pajtasz-Piasecka, E.; Radzikowski, C. *Med. Oncol.,* **1999**, *16*, 267-278.
[61] Misiura, K.; Kinas, R.W.; Kusnierczyk, H.; Radzikowski, C.; Stec, W.J. *Anti-Cancer Drugs,* **2001**, *12*, 453-458.
[62] Kobylinska, K.; Koralewski, P.; Sobik, B., Gasiorek, M.; Kobylinska, M. *Arzneim. Forsch. Drug Res.,* **2001**, *51*, 600-603.
[63] Misiura, K. *Pharmazie*, **2004**, *59*, 668-672.
[64] Baraldi, P.G.; Balboni, G.; Romagnoli, R.; Spalluto, G.; Cozzi, P.; Geroni, C.; Mongelli, N.; Rutigliano, C.; Bianchi, N.; Gambari, R. *Anticancer Drug Des.,* **1999**, *14*, 71-76.
[65] Misiura, K.; Kinas, R.W.; Kusnierczyk, H. *Bioorg. Med. Chem. Lett.,* **2002**, *12*, 156-161.
[66] Borner, K.; Kisro, J.; Bruggemann, S.K.; Hagenah, W.; Peters, S.O.; Wagner, T. Drug *Metab. Dispos.,* **2000**, *28*, 573-576.
[67] Misiura, K.; Kardacka, K.; Kusnierczyk H. *Arch. Pharm. Pharm. Med.Chem.,* **2001**, *334*, 291-294.
[68] Paci, A.; Guillaume, D.; Husson, H.P. *J. Heterocyclic Chem.,* **2001**, *38*, 1131-1134.
[69] Misiura, K.; Szymanowicz, D.; Kusnierczyk, H.; Wietrzyk, J.; Opolski, A. *Farmaco,* **2002**, *57*, 315-319.
[70] Paci, A.; Martens, T.; Royer, J. *Bioorg. Med. Chem. Lett.,* **2001**, *11*, 1347-1349.
[71] Jain, M.; Fan, J.Y.; Baturay, N.Z.; Kwon, C.H. *J. Med. Chem.,* **2004**, *47*, 3843-3852.

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