Cytochrome P450 Retinoic Acid 4-Hydroxylase Inhibitors: Potential Agents for Cancer Therapy

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Abstract: Retinoids play a crucial role in cellular differentiation and proliferation of epithelial tissue and their utility in oncology and dermatology is well documented. This mini review focuses on the role of all-*trans*-retinoic acid (ATRA or RA), the principal endogenous retinoid and its metabolism in cancer therapy.

ATRA has been used successfully in differentiating therapy of acute promyelecytic leukemia and other types of cancers. However, its usefulness is limited by the rapid emergence of ATRA resistance due (in part) to ATRA - induced acceleration of ATRA metabolism. A novel strategy to subjugate the limitation associated with exogenous ATRA therapy has been to modulate and/or increase the levels of endogenous ATRA by inhibiting the cytochrome P450-dependent ATRA-4-hydroxylase enzyme(s) responsible for ATRA metabolism. These inhibitors are also referred to as retinoic acid metabolism blocking agents (RAMBAs). This review highlights development in the design, synthesis and evaluation of RAMBAs since 1987. Major emphasis is given to liarozole, the most studied and only RAMBA to undergo clinical investigation and also the recently developed novel and highly potent 4-azoly retinoids. The potential role of a new family of cytochrome P450 enzymes, CYP26, with specificity towards ATRA is also discussed.

INTRODUCTION

Retinoids (vitamin A and its natural metabolites and synthetic analogs) are currently the subject of intense biological interest stimulated by the discovery and characterization of retinoid receptor and the realization of these compounds as nonsteroidal small-molecule hormones [1]. All-*trans*-retinoic acid (ATRA), the biologically most active metabolite of vitamin A plays a major role in cellular differentiation and proliferation of epithelial tissue. Differentiating agents redirect cells towards their normal phenotype and therefore may reverse or suppress evolving malignant lesions or prevent cancer, and indeed represents an attractive target for medicinal intervention. ATRA is being used in differentiation therapy of cancer, in cancer chemoprevention and for the treatment of acne [2]. Recently, ATRA has proven useful in cancer chemotherapy [3]. One of the most impressive effects of ATRA is on acute promyelocytic leukaemia. Treatment of acute promyelocytic leukaemia patients with high dose of ATRA resulted in complete remission [4, 5]. Furthermore, several experiments in animals have demonstrated that ATRA inhibited the induction and caused the disappearance of prostate tumors [6]. In spite of these encouraging results, the effects of prolonged ATRA therapy on human cancers in the clinic has been scarce and disappointing [7]. It has been suggested that the therapeutic effects of ATRA are undermined by its rapid *in vivo* metabolism and catabolism by cytochrome P450 enzyme(s) [8]. An important consideration is that two cellular retinoic acid binding proteins (referred to as CRABP-I and CRABP-II) are believed to be involved in the presentation of ATRA to metabolizing CYP enzymes [9]. However, a discussion of CRABPs is outside the scope of this review. It should be stated that the two natural isomers of ATRA, 9-cis-retinoic acid (9-CRA) or 13-cis-retinoic acid (13-CRA) are also being investigated for cancer chemoprevention and/or therapy.

One of the strategies for preventing *in vivo* catabolism of ATRA is to inhibit the P450 enzyme(s) responsible for this process. Indeed, this seems to be an emerging approach that may yield effective agents for the chemoprevention and/or treatment of cancers [10]. This review highlights development in the design, synthesis and evaluation of RAMBAs since 1987. Major emphasis is given to liarozole, the most studied and only RAMBA to undergo clinical investigation and also the recently developed novel and potent 4-azoly retinoids. The potential role of a new family of cytochrome P450 enzymes, CYP26, with specificity towards ATRA is also discussed. For recent presentations of work in this field, the review by Miller [10] is recommended. To our knowledge, this represents the first comprehensive review of inhibitors of retinoic acid metabolism enzyme(s).

CYTOCHROME P450 ENZYMES INVOLVED IN ATRA METABOLISM

ATRA is rapidly metabolized by cytochrome P450 (CYP)-dependent enzymes via several routes leading to a

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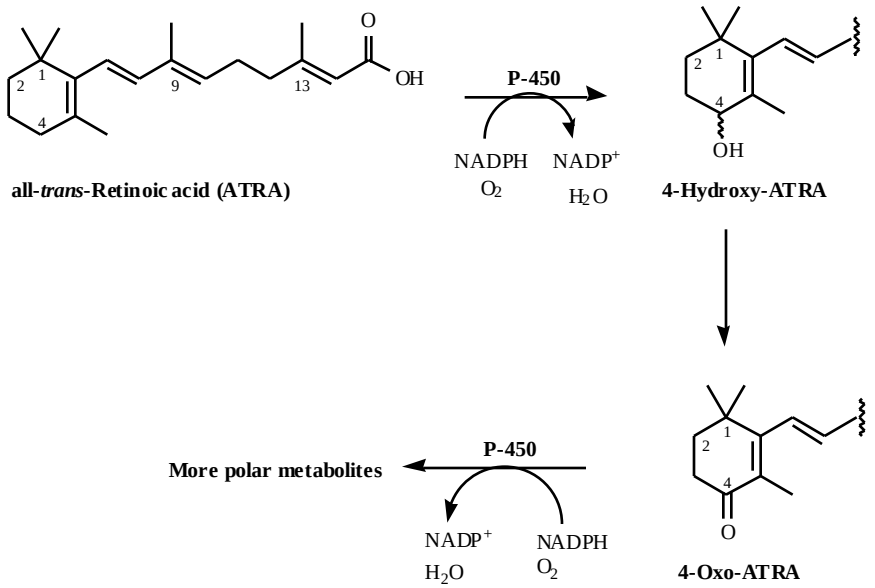


Scheme 1. Metabolic pathway of all-trans retinoic acid.

variety of polar metabolites [11]. However, it is believed that the physiologically most prominent pathway starts with the rate-limiting hydroxylation at C-4 position of the cyclohexenyl ring leading to formation of 4-hydroxy-ATRA. It should be stated that the stereochemistry at C-4 of 4-hydroxy-ATRA is yet to be determined. The latter compound is converted by a reductase enzyme into 4-oxo-ATRA that is then further transformed by CYP(s) into more polar metabolites "Scheme (**1**)" [11]. Although most of these ATRA metabolism studies have been conducted with rodent liver microsomes, similar results have also been obtained using human liver microsomes [12, 13]. Three independent groups have established that of the several human liver CYP isoforms capable of metabolizing ATRA via the 4-hydroxylation route, CYP2C8 is the major contributor, though CYP3A4 and, to a lesser extent CYP2C9, also make contributions [14-16].

Although several CYPs have been shown to be involved in the catalysis of ATRA 4-hydroxylation, their specificity for ATRA is generally low [14-16]. Recently, a new family of cytochrome P450 enzymes, CYP26A1, has been cloned and characterized in zebra fish, human, and mouse tissues [17-22]. CYP26A1 is ATRA-inducible and appears to be the most dedicated ATRA 4-hydroxylase enzyme known. Interestingly, CYP26A1 does not hydroxylate the closely related 9-CRA or 13-CRA [22, 23]. The enzyme displays high specificity towards ATRA and it may function as an important regulator of differentiation and a possible modulator of disease states by controlling retinoid concentrations and homeostasis. Recent reviews on the cloning and characterization of CYP26A1 have appeared [24, 25]. It should be stated that a new subfamily of the CYP26 family, named CYP26B1, which is 44% identical to CYP26A1 from both mouse and humans has recently been found in humans and zebrafish [26]. Although substrate specificity studies on CYP26B1 are yet to be conducted, it is plausible that this enzyme may recognize 9-CRA and/or 13-CRA, complementing the CYP26A1 activity.

DISTRIBUTION AND ROLE OF CYP26A1

CYP26A1 is expressed in the liver, heart, pituitary gland, adrenal gland, testis and in specific regions of the brain and the placenta [reviewed in 26, 25, 27]. Based on recent studies [25], it is suggested that the major role of CYP26A1 is a protective one, that is, the regulation of intracellular ARTA steady-state levels, exhibiting a similar negative feedback as has been demonstrated for CYP24, which is involved in cholecalciferol catabolism [28]. Although the major retinoid products (4-hydroxy- and 4-oxo-ATRA) of CYP26A1 were originally considered to be inactive retinoids, there is compelling evidence which suggest that they are highly active modulators of positional specification in amphibian embryonic development and they bind and activate retinoic acid receptors (RAR) subtypes as efficiently as ATRA [29, 30]. Thus, in development CYP26A1 may fulfil functions distinct from metabolic inactivation of ATRA.

CYP26A1 is readily induced by ATRA in a variety of normal and cancer cells and the enzyme efficiently converts ATRA into its oxygenated derivatives. Although the therapeutic potential of ATRA has been demonstrated (reviewed in [6, 31]), a major draw back to its clinical application is the prompt emergence of resistance, attributed to the induction of oxidative catabolism through CYPs [8, 32-35], and CYP26A1 could be a major contributor. Because ATRA deficiency is associated with the progression of some cancers [36-38], it is possible that ATRA-induced CYP26A1 is involved in rapid metabolism of ATRA in cancer patients.

The cloning and characterization of CYP26A1 represents an important development in ATRA (retinoid) biochemistry and molecular biology. The enzyme's inducibility by ATRA, and its ATRA metabolic/catabolic activity defines a feedback loop, which may be critical in regulating both normal and therapeutic levels of ATRA. This emphasizes the importance of maintaining stable physiological levels of

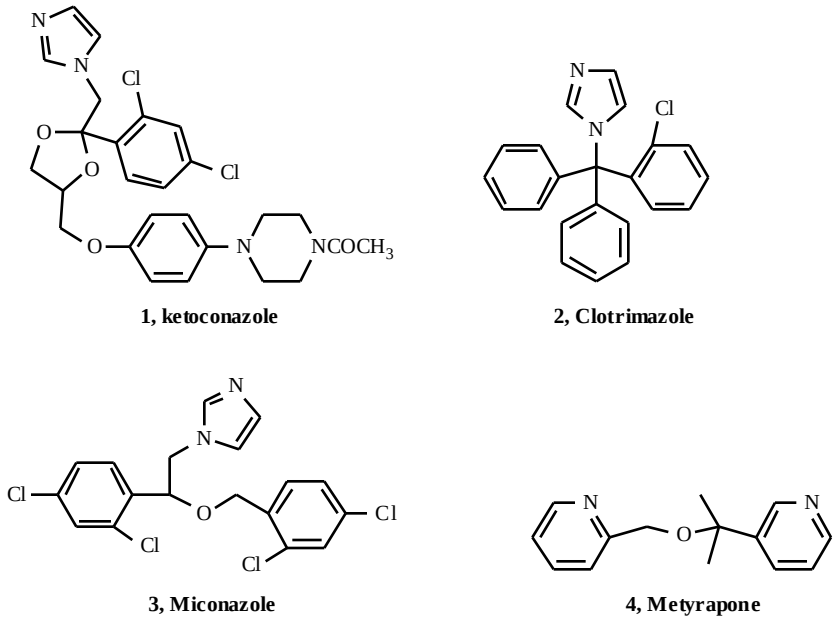


Fig. (1). Structure of antimycotics.

ATRA. Thus, compounds designed to inhibit CYP26A1 activity may be useful in elevating normal tissue ATRA levels or maintaining high therapeutic levels of ATRA. As stated earlier, since ATRA has proven useful in the treatment and/or chemoprevention of some cancers and skin disorders, it is now possible to investigate the contributions of the expression/activity of CYP26 (or lack thereof) in various disease. CYP26 has recently been mapped to human chromosome 10q23-q24 [39], a region where several suppressor gene loci have been described [40] as well as the split-hand-split foot syndrome (SHSF-3) [41]. Thus, it is possible that mutations in CYP26 may play a role in these diseases.

ATRA 4-HYDROXYLASE INHIBITION

The realization that the metabolism of ATRA may be responsible for its limited efficacy in the clinics provided the impetus behind the search for inhibitors of the CYP-mediated metabolism of ATRA. Although the pioneering paper by Napoli's group in 1987 which led the way in this field was greeted with enthusiasm [42], research in the area have been rather slow with only a few inhibitors described over the past 14 years.

Inhibitors of ATRA 4-hydroxylase (also referred to as retinoic acid metabolism blocking agents [RAMBAs]) should delay *in vivo* ATRA catabolism resulting in increased endogenous levels. This effect should improve control of neoplastic differentiation and growth and possibly exhibit antitumor activity.

EARLY STUDIES OF ATRA 4-HYDROXYLASE INHIBITORS

The antimycotics, ketoconazole (keto), clotrimazole, miconazole and metyrapone "Fig. (**1**)" appear to be the first compounds evaluated as potential inhibitors of ATRA metabolism enzyme(s) [42]. As shown in "Table (**1**)" keto and clotrimazole were about equipotent, exhibiting potent inhibition of ATRA metabolism in F9 embryonal carcinoma cells. Miconazole had no more than 10% of the activity of clotrimazole, while metyrapone had about 1% of the activity of clotrimazole. The inhibition of ATRA metabolism by most of these compounds is not surprising since they are well known inhibitors of CYP-P450-mediated metabolism.

Following this initial study, Van Wauwe *et al.* [43], in 1988, examined the effects of keto on ATRA metabolism, *in vitro* using hamster liver microsomes and *in vivo* using normal rats treated with [^{3}H]-ATRA. *In vitro*, keto inhibited the CYP-P450-mediated metabolism of ATRA to the corresponding polar 4-hydroxy- and 4-oxo- derivatives. *In vivo*, keto suppressed the formation of polar ATRA metabolites by normal rats dosed intrajugularly with 200 mg of [^{3}H]-ATRA. These studies are significant as they demonstrated for the first time the feasibility to design CYP inhibitors that could prolong the half-life of exogenously administered ATRA to animals. In 1990, this group examined the effects of keto, R75251 (now known as liarozole or liazal ^{TM}) and five other CYP-P450 inhibitors on the *in vivo* metabolism of ATRA in normal rats [44]. Keto and R75251 were the only compounds found able to delay the metabolism of exogenously administered ATRA. In addition, liarozole enhanced the endogenous plasma levels of ATRA, exerting ATRA-mimetic effects *in vivo* [45]. It should be noted that the same group of Janssen researchers first reported that CYP-P450-dependent aromatase, 17-hydroxylase/17,20-lyase (CYP17), and 11-hydroxylase were the target enzymes for R75251 [46]. But in another related paper, they also reported that the antitumoral effects of R75251 on the growth of transplantable R3327 prostate adenocarcinoma in rats was independent of its inhibition of androgen biosynthesis [47]. Subsequent studies in this area were mostly conducted by researchers of Janssen Pharmaceutical, who eventually settled on liarozole for development as an anti-cancer agent and for the treatment of

Table 1. Inhibition of Retinoic Acid Metabolism in F9 Embryonal Carcinoma Cells by Antimycotics [42]

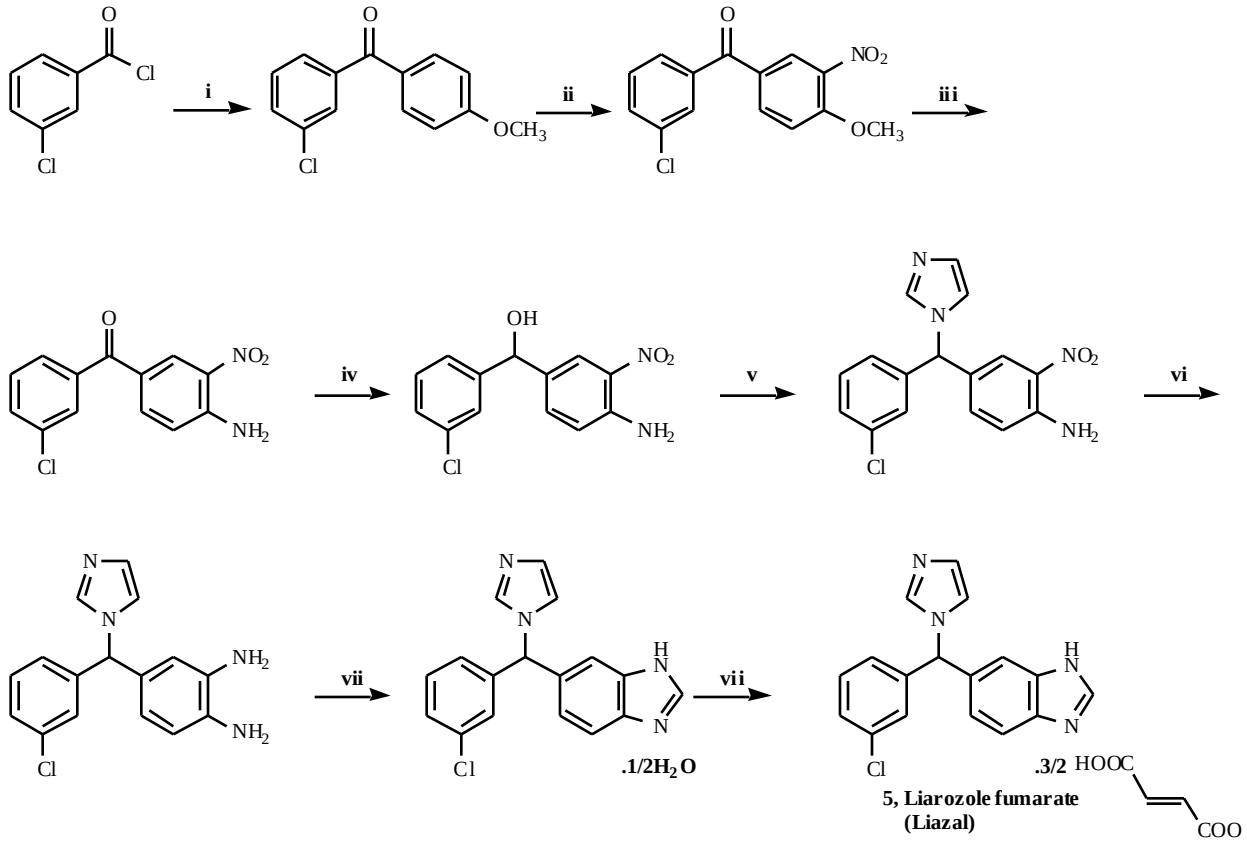
Inhibitor	% Inhibition Concentration (μM)		
	1	10	100
Ketoconazole	42	84	100
Clotrimazole	22	62	84
Miconazole	8	12	56
Metyrapone	0	2	26

dermatological diseases. Liarozole is the most studied inhibitors of ATRA 4-hydroxylase and remains to date the only RAMBA to have been evaluated clinically and, as such, is a standard against which future RAMBAs may be judged. The summary of studies on liarozole and the recent RAMBAs are discussed below.

LIAROZOLE (LIAZAL™, 5-[(3-CHLOROPHYNYL-1H-IMIDAZOLE-1-YL)METHYL]-1H-BENIMIDAZOLE)

Following discovery of the undesirable side-effects of keto (*vide supra*) in PCa patients, the quest for more potent non-steroidal CYP17 inhibitors ensued. Liarozole (Liazal™, 5-[(3-chlorophynyl-1H-imidazole-1-yl)methyl]-1H-benimidazole) is the result of extensive SAR studies on imidazole derivatives [48]. Very recently, a paper has appeared which describes a practical 8-step synthesis of Liazal™ "Scheme (**2**)" [49].

Liarozole is a good inhibitor of rat CYP17 (IC_{50} = 260 nM) with similar potency to keto (IC_{50} = 340 nM), but unlike keto, it was also a potent inhibitor of aromatase [46]. However, following extensive *in vitro* and *in vivo* studies, liarozole's biological effects are ascribed to inhibition of the P450-dependent 4-hydroxylase that catalyzes ATRA metabolism [50]. *In vitro*, liarozole (IC_{50}, 2.2 μM) suppressed the P-450-mediated conversion of ATRA to more polar metabolites by hamster liver microsomes. *In vivo*, it enhanced the plasma level of ATRA from mostly undectectable values (less than 0.5 ng/ml) in control rats to 1.4 ± 0.1 and 2.9 ± 0.1 ng/ml in animals treated p.o. with 5 and 20 mg/kg of liarozole [45]. Anti-tumoral action was detected in androgen-dependent and androgen-independent rat prostate carcinoma models [47, 51, 52]. Remarkable anti-tumor activity was observed against prostate cancer xenographs in immunodepressed mice [53, 54] and further studies revealed that the anti-tumor properties of liarozole correlates with an increase in tumor differentiation, following accumulation of ATRA [55]. These studies established that the anti-tumoral properties of the compound



Reagents and conditions: i, $PhOCH_3$, $AlCl_3$, CH_2Cl_2, 5-10°C; ii, HNO_3/H_2SO_4, CH_2Cl_2, 10-15°C, 1h; iii, NH_3*g,i.* PrOH, 100°C, 16h; iv, $NaBH_4$,i. PrOH, reflux, 1h; v) CDI, CH_2Cl_2, reflux, 1h; vi, H_2, Pt/C 5% thiophene sol., MeOH, rt.; HCOOH, 4N HCl, reflux; viii, EtOH, 50°C, fumaric acid.

Scheme 2. Synthesis of Liarozole.

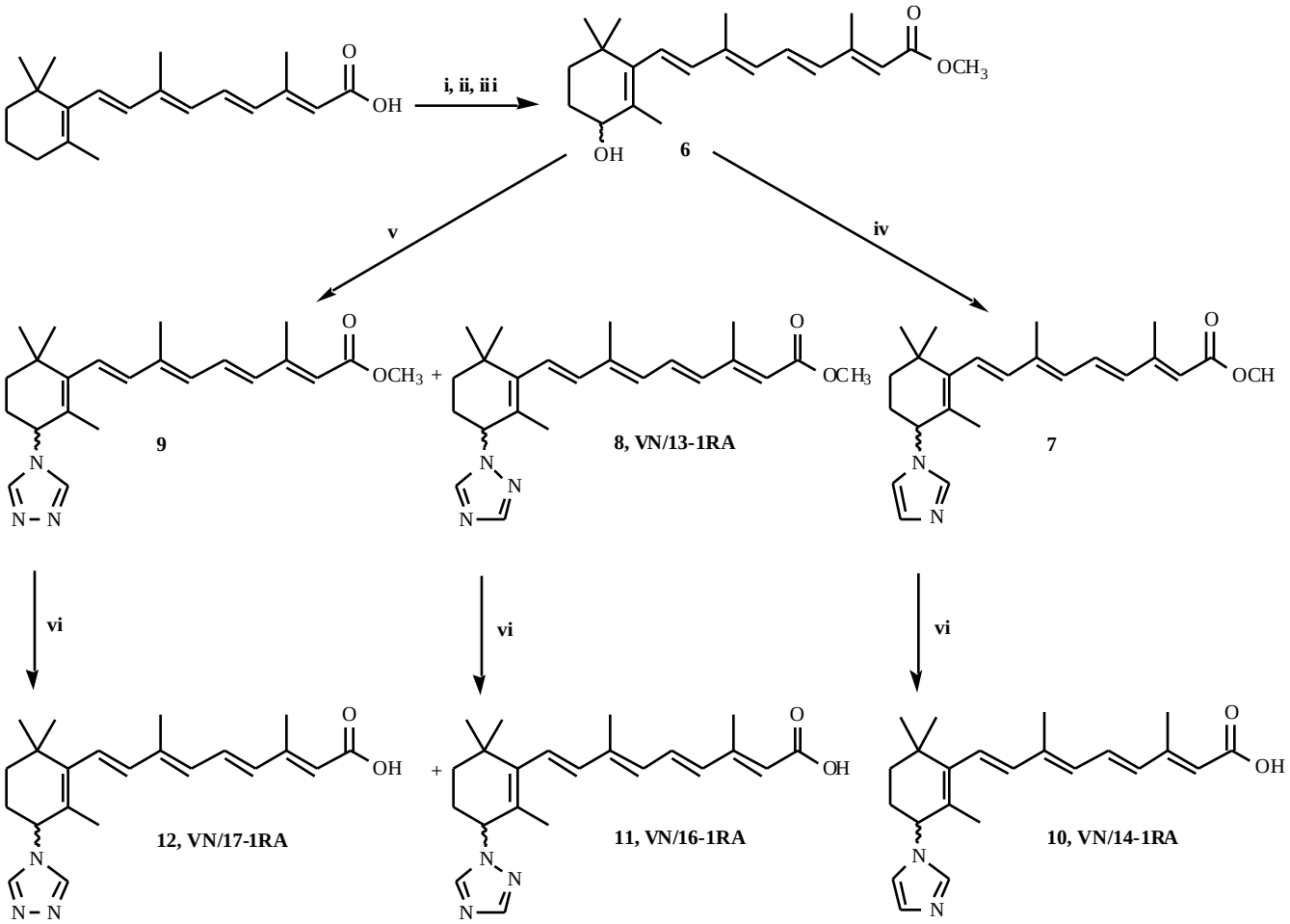
are related to its inhibition of ATRA metabolism and that the previously demonstrated inhibition of CYP17 (inhibition of androgen synthesis) is marginal *in vivo*.

Although a number of researcher have questioned liarozole's mechanism of action [56, 57], we are of the opinion that there is currently sufficient evidence (*vide supra*) to ascribe its mechanism of action to inhibition of ATRA metabolism. Although it has been suggested that its mechanism of action may also involve inhibition of aromatase, and of androgen biosynthesis; the fact that specific and potent aromatase inhibitors were without effects in clinical trials of patients with prostate cancer (PCa), and that androgen synthesis inhibitors are ineffective in androgen-independent prostate tumors makes a strong case for their irrelevance in this setting.

A large Phase III international study has very recently been completed comparing liarozole 300 mg twice daily with cyproterone acetate (CPA) 100 mg twice daily in a total of 321 patients with metastatic prostate cancer in relapse after first-line endocrine therapy [58]. The adjusted hazard ratio for survival was 0.74 in favor of liarozole (P = 0.039), indicating a 26 % lower risk of death than in patients treated with CPA. Liarozole was superior to CPA in terms of prostate-specific antigen (PSA) response, PSA progression, and survival, and was capable of maintaining patients' quality of life. The observed adverse events were relatively mild to moderate in nature. The results indicate that liarozole might be a possible treatment option for PCa following failure of first-line endocrine therapy.

Although most experiences of liarozole as an anti-cancer agent have been limited to prostate cancer, a few experiments with breast cancer have also been conducted. In cultured human breast cancer MCF-7 cells, liarozole potentiated the antiproliferative and differentiative effects of ATRA [59-62]. This enhancement of ATRA effects could be explained by the inhibition of enzymatic degradation of ATRA in these cells.. Liarozole has proven antitumor activity in steroid-insensitive TA3-mouse mammary carcinoma and in NUM-induced mammary carcinoma in rats [63].

Despite these encouraging preclinical and clinical results, the usefulness of liarozole therapy is considered limited due to adverse side effects that are attributed to its lack of CYP



Reagents and conditions: i, $TMSCHN_2$/benzene: MeOH, N_2, rt.; ii, Activated MnO_2/CH_2Cl_2, rt.; iii, $NaBH_4$/MeOH, rt.; iv, CDI/CH_3CN, rt.; v, CDT/CH_3CN, rt.; iv, 10% KOH/MeOH, Ar, reflux.

Scheme 3. Synthesis of 4-azolyl retinoids.

isozyme specificity and its moderate potency of ATRA 4-hydroxylase. Consequently, Janssen have since discontinued clinical development of liarozole [64].

NEW INHIBITORS OF ALL-TRANS-RETINOIC ACID CYTOCHROME P450 4-HYDROXYLASE

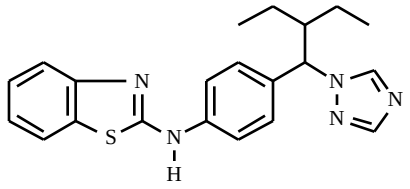
Azolyl Retinoids

The emerging role of RAMBAs as potential agents in the treatment of both hormone-dependent and hormone-independent cancers [10, 65, 66] has led to our interest in this area. Given the significance of azole grouping of many drugs which are P450 enzymes [67-69], we reasoned that introducing azole group at C-4 (the site of initial enzymatic hydroxylation) of ATRA should yield specific and potent inhibitors of ATRA 4-hydroxylase. Indeed, we very recently described the synthesis of a number of novel 4-azolyl ATRA derivatives, some of which are amongst the most potent inhibitors of this enzyme [70, 71]. The synthesis of these compounds is outlined in "Scheme (**3**)", with the key reaction being the near quantitative formation of the 4-azolyl retinoids (**7**-**9**) following the reaction of allylic alcohol (**6**) with N, N'- carbonyldiimidazole (CDI) or N, N'-carbonylditriazole (CDT) [71, 72].

The azolyl ester, 4ξ-(1*H*-1,2,4-triazol-1-yl)methyl retinoate (**8, VN/13-1RA**), and the free acids, 4ξ-(1*H*-imidazol-1-yl)retinoic acid (**10**, **VN/14-1RA**), 4ξ-(1*H*-1,2,4-triazol-1-yl)retinoic acid (**11**, **VN/16-1RA**) and 4ξ-(4*H*-1,2,4-triazol-1-yl)retinoic acid (**12**, **VN/17-1RA**) exhibited highly potent inhibition of hamster liver microsomal RA 4-hydroxylase with IC_{50s} of 680, 100, 880 and 1600 nM, respectively, being 4 - 60 times more potent than liarozole (IC_{50} = 6000 nM). Under this assay conditions, keto had an IC_{50} value of 34000 nM [70, 71]. Preliminary antiproliferative activity of these novel azolyl RAMBAs (**7**-**12**) have been tested against a human breast cancer cell line, MCF-7 and two prostate cancer cell lines, the androgen dependent LNCaP and androgen independent PC-3. Each of these compounds significantly enhanced the antiproliferative action of ATRA on these cell lines, and they were more effective than liarozole[1]. Further studies with these novel azolyl RAMBAs, and the identification of other new compounds are in progress. World and USA patent applications to protect these new compounds have been filed.

(B)-N-[4-[2-ethyl-1-(1H-1,2,4-triazol-1-yl)butyl]phenyl]-2-benzothiazolamine, R115866

Researchers at Janssen Research Foundation (JFR) have recently reported on a novel 1H-triazole derivative, R115866, **13** ("Fig. (**2**)") that is a potent inhibitors of human CYP26A1 (IC_{50}, 4 nM), being 750 times as potent as liarozole (IC_{50}, 3 μM) [73]. However, details of its synthesis are yet to be reported. R115866 is highly selective for CYP26 as it exhibited mediocre inhibitory effects on aromatase, CYP17, CYP211, CYP3A, and CYP2A1, respectively. *In vivo*, administration of R115866 (2.5 mg/kg. p.o) to rats induced significant and transient increase of endogenous ATRA levels in plasma, skin, fat, kidney, and testis. Consequently, the compound exerted retinoidal effects, for example, inhibition of vaginal keratinization in rats. Although these studies with R115866 seem to be focused on dermatological therapy [73, cited in 74], their potential as agents for the treatment of cancers are warranted. Because of the compound's high inhibitory potency and selectivity for CYP26A1, it should be considered less likely to produce unwanted side effects as those experienced with liarozle therapy.



13, R115866

Fig. (2). Structure of R115866.

R116010

Limited data on a novel RAMBA, R116010 (structure not available) have been presented at several scientific meetings in abstract forms by researchers at JFR[2]. R116010 is reported to be 100-fold more potent than liarozole, and selective for CYP26A versus other cytochrome P450-dependent metabolizing enzymes. Furthermore, R116010 exhibited strong antitumor effects against androgen-independent rat prostate adenocarcinoma R3327/PIF1 and also against estrogen-independent mouse TA3-Ha mammary tumors. These results suggest that R116010 is a suitable candidate for further development for the treatment of prostate and breast cancers.

CONCLUSION

Retinoid therapy is based on differetiation of premalignant and malignant cells with the potential of redirecting the cells towards their normal phenotype. However, exogenous retinoid therapy is yet to fulfil the expectations raised by *in vitro* and *in vivo* studies in cancer models in the clinics. Modulation of endogenous ATRA and possibly its natural sterioisomers with the use of new RAMBAs may present an addition cancer therapy strategy. It

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[1] Njar, V. C. O., Huynh, C. K., Brodie, A. M. H. *Unpublished results*, **2001**.

[2] (1). Van Ginckel, R., Floren, W., Moelans, P., Janssens, B., Molenberghs, K., van Dun, J., Venet, M., Mabire, D., Wouters, W. Abstract #2615. *Proc. 90th AACR*, **1999**, *40*, 395.
(2). Van Ginkel, R., Van der Leede, B., Smets, G., Wouters, W. Abstract #2339. *Proc. 91st AACR*, **2000**, *41*, 368.
(3). Van Heusden, J., Bruwiere, H., Van der Leede, B., Van Dun, J., Dillen, L., Van Hove, C., Willemsens, G., Sanz, G., Venet, M., Janicot, M., Wouters, W. Abstract #3821. *Proc. 91st AACR*, **2000**, *41*, 600

is clear from this mini review that only a few potent RAMBAs are currently available. We are presenting this review with the goal of stimulating interest in the area to enable more studies that may lead to new RAMBAs that would be directed towards their potential evaluation as therapeutics in oncology and dermatology.

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NOTE ADDED IN PROOF

J. Van Heusden and others have recently presented a detailed study that characterize R116010 (structure given) as a potent and selective inhibitor of all-*trans* retinoic acid metabolism, exhibiting antitumor (of murine estrogen-independent TA3-Ha mammary tumors) activity. (*Br. J. Cancer*, **2002**, *86*, 605-811).

REFERENCES

[1] (a) Evans, R. M. *Science* **1988**, *240*, 889-895. (b) Mangelsdorf D. A., Umesono, K., Evans, R. M. In *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed., Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds., Raven Press, Ltd.: New York, **1994**; pp 319-349. (c) Katzenellenbogen, J. A., Katzenellenbogen, B. S. *Chem. Biol.*, **1996**, *3*, 529-536.

[2] (a) De Luca, L. M. *FASEB J.* **1991**, *5*, 2924-2932. (b) Lotan, R. *FASEB J.* **1996**, *10*, 1031-1039. (c) Griffiths, C. E. M., Fischer, G. J., Finkel, L. J., Voorhees, J. J. *Br. J. Dermatol.*, **1992**, *127(Suppl)*, 21-24.

[3] (a) Lanitzki, I., Goodman, D. S. *Cancer Res.*, **1974**, *34*, 1567-1571. (b) Chopra, D. P., Wilkoff, L. J. *J. Natl. Cancer Inst.*, **1977**, *58*, 923-930. (c) Chytil, F. *Pharmacol. Rev.*, **1984**, *36*, 935-1005.

[4] Haung, M. E., Yu-Chen, Y., Shu-Rong, C. *et al. Blood*, **1988**, *72*, 567-572.

[5] Castigne, S., Chomienne, C., Daniel, M. T. *et al. Blood*, **1990**, *76*, 1704-1709.

[6] Hong, W. K., Itri, L. In *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed., Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds., Raven Press, Ltd.: New York, **1994**; pp 923-930.

[7] Trump, D. L., Smith, D., Stiff, D., Adedoyin, A., Bahnson, R., Day, R., Branch, R. *Proc. Am. Soc. Clin. Oncol.* **1994**, *751*, 241.

[8] (a) Mundi, J., Frankel S. R., Miller (Jr.) W. H., Jakubowski, A., Scheinberg, D. A., Young, C. W., Dmitrovsky, E., Warrell (Jr.), R. P. *Blood*, **1992**, *79*, 299-303. (b) Mundi, J., Frankel S. R., Miller (Jr.) W. H., Huselton, C., Degrazia, F., Garland, W. A., Young, C. W., Dmitrovsky, E., Warrell (Jr.), R. P. *Cancer Res.*, **1992**, *52*, 2138-2142.

[9] Giguere, V. *Endocrine Rev.*, **1994**, *15*, 61-79.

[10] Miller (Jr.), W. H. *Cancer*, **1998**, *83*, 1471-1482.

[11] Roberts, A. B., Nichols, M. D., Newton, D. C., Sporn, M. B. *J. Biol. Chem.*, **1979**, *254*, 6296-6302.

[12] Blanner, W. S., Olson, J. A. In *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed., Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds., Raven Press, Ltd.: New York, **1994**; pp 229-256.

[13] Napoli, J. L. *FASEB J.*, **1996**, *10*, 993-1001.

[14] Leo, M. A., Lasker, J. M., Raucy, J. L., Kim, C-I., Black, M., Lieber, C. S. *Arch. Biochem. Biophys.*, **1989**, *269*, 305-312.

[15] Nadin, L., Murray, M. *Biochem. Pharmacol.*, **1999**, *58*, 1201-1208.

[16] McSorley, L. C., Daly, A. K. *Biochem. Pharmacol.*, **2000**, *60*, 517-526.

[17] White, J. A., Guo, Y. D., Baetz, K., Beckett-Jones, B., Bonasoro, J., Hsu, K. E., Dilworth, F. J., Jones, G., Petkovich, M. *J. Biol. Chem.*, **1996**, *271*, 29922-29927.

[18] White, J. A., Beckett-Jones, B., Guo, Y. D., Dilworth, F. J., Bonasoro, J., Jones, G., Petkovich, M. *J. Biol. Chem.*, **1997**, *272*, 18538-18541.

[19] Ray, W. J., Bain, G., Yao, M., Gottilieb, D. I. *J. Biol. Chem.*, **1997**, *272*, 18702-18708.

[20] Fujii, H., Sato, T., Konako, S., Gotoh, O., Fujji-kuriyama, Y., Osawa, K., Kato, S., Hamada, H. *EMBO J.*, **1997**, *16*, 4163-4173.

[21] Abu-Abed, S. S., Beckett, B. R., Chiba, H., Chithalen, J. N., Jones, G., Metzger, D., Chambon, P., Petkovich, M. *J. Biol. Chem.*, **1998**, *273*, 2409-2415.

[22] Sonneveld, E., Van den Brink, C. E., van der Leede, B. J. M., Schulkes, R. K. A. M., Petkovich, M., van der Burg, B., van der Saag, P. T. *Cell Growth Differ.*, **1998**, *9*, 629-637.

[23] Marikar, Y., Wang, Z., Duell, E. A., Petkovich, M., Voorhees, J. J., Fisher, G. J. *J. Invest. Dermatol.*, **1998**, *111*, 434-439.

[24] Haque, M., Anreola, F. *Nutr. Rev.*, **1998**, *56*, 84-85.

[25] Sonneveld, E., Van der Saag, P. T. *Inter. J. Vit. Nutr. Res.*, **1998**, *68*, 404-410.

[26] Nelson, D. R. *Arch. Biochem. Biophys.*, **1999**, *371*, 345-347.

[27] Trofimova-Griffin, M. E., Juchau, M. R. *Biochem. Biophys. Res. Commun.*, **1998**, *252*, 287-291.

[28] Makin, G., Lohnes, D., Byford, V., Ray, R., Jones, G. *Biochem. J.*, **1989**, *262*, 173-180.

[29] Pijnappel, W. M. M., Hendriks, H. F. J., Folkers, G. E., van den Brink, C. E., Dekker, E. J., Edelenbosch, C., van der Saag, P. T., Durston, A. J. *Nature*, **1993**, *366*, 340-344.

[30] de Roos, K., Sonneveld, E., Compaan, B., ten Berge, D., Dunston, A. J., van der Saag, P. T. *Mech. Dev.*, **1999**, *82*, 205-211.

[31] Moon, R. C., Mehta, R. G., Rao, K. V. N. In *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed., Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds., Raven Press, Ltd. New York, **1994**; pp 573-595.

[32] Smith, M. A., Adamson, P. C., Bails, F. M., Feusner, J., Aronson, L., Murphy, R. F., Horowitz, M. E., Reaman, G., Hammond, G. D., Hittelman, W. N., Poplack, D. G. *J. Clin. Oncol.*, **1992**, *10*, 1666-1673.

[33] Warrell, R. P. Jr. *Blood*, **1993**, *82*, 1949-1953.

[34] Warrell, R. P. Jr., de The, H., Wang, Z. Y., Degos, L. *N. Engl. J. Med.*, **1993**, *329*, 177-189.

[35] Kizaki, M., Ueno, H., Yamazeo, Y., Shimada, M., Takayama, N., Muto, A., Matsushita, H., Nakajima, H., Marikawa, M., Koeffler, H. P., Ikeda, Y. *Blood*, **1996**, *87*, 725-733.

[36] Reichman, M. E., Hayes, R. B., Ziegler, R. G., Schatzkin, A., Taylor, P. R., Kahel, L. L., Fraumein, J. F. (Jr.). *Cancer Res*., **1990**, *50*, 2311-2315.

[37] Peehl, D. M., Wong, S. T., Stamey, T. A. *Prostate*, **1993**, *32*, 69-78.

[38] Pasquali, D., Rossi, V., Prezioso, D., Gentile, V., Colantuani, V., Lotti, T., Bellastella, A., Sinisi, A. A. *J. Clin. Endocrinol. Metab.*, **1999**, *84*, 1463-1469.

[39] White, J. A., Beckett, B., Scherer, S. W., Herbrick, J., Petkovich, M. *Genomics*, **1998**, *48*, 270-272.

[40] Gray, I. C., Phillips, S. M., Lee, S. J.,Neoptolemos, J. P., Weissenbach, J., Spurr, N. K. *Cancer Res.*, **1995**, *55*, 4800-4803.

[41] Gurrieri, F., Prinos, P., Tackels, D., Kilpatrick, M. W., Allanson, J., Genuardi, M., Vuckov, A., Nanni, L., Sangiorgi, E., Gorofola, G., Nunes, M. E., Neri, G., Schwartz, C., Tsipouras, P. *Am. J. Med. Genet.*, **1996**, *62*, 427-436.

[42] Williams, J. B., Napoli, J. L. *Biochem. Pharmacol.*, **1987**, *36*, 1386-1388.

[43] van Wauwe, J. P., Coene, M-C., Goossens, J., Van Nijen, G., Cools, W., Lauwers, W. *J. Pharmacol. Exp. Ther.*, **1988**, *245*, 718-722.

[44] van Wauwe, J. P., Coene, M-C., Goossens, J., Cools, W., Manbaliu, J. *J. Pharmacol. Exp. Ther.*, **1990**, *252*, 365-369.

[45] Van Weuwe, J., Van Nyen, G., Coene, M-C., Stoppie, P., Cools, W., Goossens, J, Borghgraef, P., Janssen, PAJ. *J. Pharmacol. Expt. Ther.* **1992**, *261*, 773-779.

[46] Bruynseels, J., De-Coster, R., van Rooy, P., Wouters, W., Coene, M-C., Snoeck, E., Raeymaekers, A., Freyne, E., Sanz, G., Vanden-Bussche, G., Vanden-Bossche, H., Willemsens, G., Janssen, P. A. J. *Prostate*, **1990**, *16*, 345-357.

[47] van Ginckel, R., DeCoster, R., Wouters, W., Vanherck, W., van DerVeer, R., Goeminne, N., Jagers, E., van Canteren, H., Wouters, L., Distelmans, W., Janssen, P. A. J. *Prostate*, **1990**, *16*, 313-323.

[48] Raeymaekers, A. H. M., Freyne, E. J. E., Sanz, G. C. *European Patent* 0,260,744.

[49] Freyne, E., Raeymaekers, A., Venet, M., Sanz, G., Wouters, W., De Coster, R., Van Wauwe, J. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 267-272.

[50] De Coster, R., Wouters, W., Van Ginckel., R., End, D., Krekels, M., Coene. M-C., Bowden, C. *J. Steroid Biochem. Molec. Biol.*, **1992**, *43*, 197-201.

[51] Smets, G., van Ginckel, R., van Wauwe, J., Coene, M-C., Raemaekers, F. C. S., Borgers, M., De Coster, R. *Urol. Res.*, **1992**, *20*, 439, Abstract 34.

[52] Steams, M. E., Wang, M., Fudge, K. *Cancer Res.*, **1993**, *53*, 3073-3077.

[53] Smets, G., van Ginckel, R., Xhonnenx, B., van Heusden, J., Janssen, B., Callens, M., Borgers, M., De Coster, R. *Eur. Soc. Urol. Oncol. Endocrinol.*, **1994**, Abs. 96.

[54] Smets, G., van Ginckel, R., Daneels, G., Moeremans, M., van Wauwe, J., Coene, M-C., Romarehers, F. C., Schalken, J. A., Borgers, M., De Coster, R. *Prostate*, **1995**, *27*, 129.

[55] Acevedo, P., Bertram, J. S. *Carcinogenesis*, **1995**, *16*, 2215-2222.

[56] Barrie, S. E., Jarman, M. *Endocr. Relat. Cancer*, **1996**, *3*, 25-29.

[57] Kelloff, G. J., Lubert, R. A., Lieberman, R., Eisenhauer, K., Steele, V. E., Crowell, J. A., Hawk, E. T., Boone, C. W., Sigman, C. C. *Cancer Epidemiol. Biom. Prev.*, **1998**, *7*, 65-78.

[58] Deberuyne, F. J. M., Murray, R., Fradet, Y., Johansson, J. E., Tyrrell, C., Boccardo, F., Denis, L., Marberger, J. M., Brune, D., Rasswiler, J., Vangeneugden, T., Bruynseels, J., Janssens, M., De Porre. P., (for the liarozole study group). *Urology*, **1998**, *52*, 72-81.

[59] Wouters, W., Van Dun, J., Dillen, A., Coene, M.-C., Cools, W., De Coster, R. *Cancer Res.*, **1992**, *52*, 2841-2846.

[60] van Heusden, J., Xhonneux, B., Wouters, W., Borgers, M., Ramaekers, F. C. S., De Coster, R., Smets, G. *Eur. J. Cell Biol*., **1995**, *5 (Suppl.)*, 95.

[61] Krekels, M. D. W. G., Verhoeven, A., van Dun, J., Cools, W., van Hove, C., Dillen, L., Coene, M-C., Wouters, W. *Br. J. Cancer*, **1997**, *75*, 1098-1104.

[62] van Heusden, J., Wouters, W., Ramaekers, F. C. S., Krekels, M. D. W. G., Dillen, L., Borgers, M., Smets, G. *Br. J. Cancer*, **1998**, *77*, 1229-1235.

[63] Wouters, W., De Coster, R., van Ginckel, R. *Proc. Am. Assoc. Cancer Res.*, **1990**, *414*, abstract 31.

[64] Janssen Pharmaceutica NV. *Company Communication*, **1999**, September 2.

[65] van Wauwe, J. P., Janssen, P. A. J., *J. Med. Chem.*, **1989**, *32*, 2231-2239.

[66] De Coster, R., Wouters, W., Bruynseels, J. *J. Steroid Biochem. Molec. Biol.*, **1996**, *56*, 133-143.

[67] Bossche, H. V. *J. Steroid Biochem. Molec. Biol.*, **1992**, *43*, 1003-1021.

[68] Njar, V. C. O., Brodie, A. M. H. *Curr. Phar. Design*, **1999**, *5*, 163-180.

[69] Njar, V. C. O., Brodie, A. M. H., *Investigational Drugs*, **1999**, *1*, 495-506.

[70] Njar, V. C. O., Nnane, I. P., Brodie, A. M. H. *218th ACS National Meeting, New Orleans, USA*, **1999**, MEDI 166.

[71] Njar, V. C. O., Nnane, I. P., Brodie, A. M. H. *Bioorg. Med. Chem. Lett*., **2000**, *10*, 1905-1908.

[72] Njar, V. C. O. *Synthesis*, **2000**, *14*, 2019-2028.

[73] Stoppie, P., Borgers, M., Borghraef, P., Dillen, L., Goossens, J., Sanz, G., Szel, H., van Hove, C., van Nyen, G., Nobles, G., Vanden Bossche, H., Venet, M., Willemsens, G., van Wauwe, J. *J. Pharmacol. Expt. Thera.*, **2000**, *293*, 304-312.

[74] Thacher, S. M., Vasudevan, J., Tsang, K-Y., Nagpal, S., Chandraratna, R. A. S. *J. Med. Chem.*, **2001**, *44*, 281-297.