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Retinoic Acid Metabolism Inhibition by 3-Azolylmethyl-1*H*-indoles and 2, 3 or 5-(α-Azolylbenzyl)-1*H*-indoles

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Among a library of 70 azoles, 8 indole derivatives substituted in the 2-, 3- or 5- position with an azolylmethyl or α-azolylbenzyl chain were evaluated for retinoic acid (RA) metabolism inhibitory activity. The most active inhibitors identified in this study were 5-bromo-1-ethyl-3-methyl-2-[(phenyl)(1*H*-1,2,4-triazol-1-yl)methyl]-1*H*-indole (3) (68.9% inhibition) and 5-bromo-1-ethyl-2-[(4-fluorophenyl)(1*H*-1,2,4-triazol-1-yl)methyl]-3-methyl-1*H*-indole (6) (60.4% inhibition). At the same concentration (100 μM) ketoconazole exerted similar inhibitory effect (70% inhibition).

Keywords: Retinoic acid (RA); Retinoic acid metabolism blocking agents (RAMBAs); Ketoconazole; Azolylindoles

INTRODUCTION

All-*trans*-retinoic acid (ATRA), an endogenous active metabolite of retinol (vitamin A), plays a key role in many essential life processes including embryonic development, regulation of cell growth and differentiation.[1] The effects of ATRA are known to be mediated by its interaction with the two families of retinoic nuclear receptors each, subdivided into three subtypes, retinoic acid receptors (RARs α, β, and γ) and retinoid X receptors (RXRs α, β, and γ).[2] Particularly, because of its functions in proliferation and differentiation of both normal and malignant cells, ATRA and its derivatives (retinoids and arotinoids) have been extensively studied and show interesting effects in the treatment of skin disorders such as acne, psoriasis[3] and a variety of cancers including prostate cancer[4] and acute promyelocytic leukemia.[5] In cancers, retinoids have demonstrated their utility for both chemotherapeutic and chemopreventive applications.[6,7] However, the biological efficacy of ATRA and retinoids is greatly impaired by its rapid rate of metabolism and degradation.

The major pathway of ATRA metabolism consists in the 4-hydroxylation of the cyclohexenyl ring to form 4-hydroxy-retinoic acid (RA),[8] which is further transformed into more polar metabolites.[9,10] A large number of cytochromes P450 (CYPs) are implicated in this 4-hydroxylation, but the relative importance of individual CYPs is unclear. In humans, two CYPs are major contributors to ATRA 4-hydroxylation: CYP2C8[11] and P450RAI[12] (CYP26). In addition, other CYPs have also been implicated in this reaction, i.e., CYPs 3A7, 3A5, 3A4, 2C9 and 1A1.[13,14] (Figure 1)

Logically, ATRA became an attractive target for drug discovery in diseases characterized by aberrations in these cellular processes. A new approach to treat and to prevent cancer is the use of retinoic acid metabolism blocking agents (RAMBAs), which increase levels of endogenous ATRA within the tumor cells by blocking their metabolism. Azole-containing compounds (imidazole or triazole) are well known to interact with cytochrome P450-dependent enzymes and therefore constitute a logical choice of RAMBAs. Two different approaches are possible to design RAMBAs. The first one, developped by Njar and coworkers,[15] is to synthesize substrate-based agents of ATRA metabolism enzyme(s) such as compound (±)-4-(1*H*-imidazol-1-yl)RA. The second one is to focus on only the cytochrome part of the ATRA metabolism enzyme(s) and in this connection it may be possible to produce inhibitors interfering with

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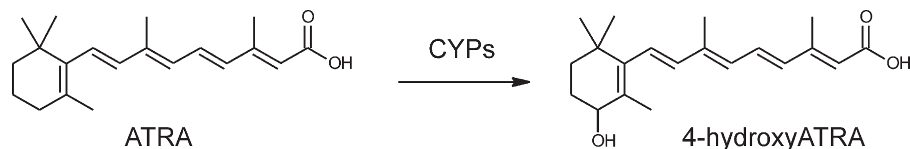


FIGURE 1 CYP-mediated 4-hydroxylation of ATRA.

the iron atom of the porphyrin of the cytochrome P450 moiety of ATRA metaboling enzyme(s). Among CYP inhibitors, ketoconazole has been largely studied for inhibiting several cytochrome P450 enzymes such as P450 arom (CYP19),[16] P450 17α or 17α-hydroxylase-17,20-lyase (CYP17),[16] lanosterol 14α-demethylase (CYP51).[17] Nevertheless, ketoconazole is a weak inhibitor of ATRA 4-hydroxylase.[18,19] Liarozole,[20,21] appears to be a more promising drug in the treatment of prostate cancer by inhibiting ATRA metabolism.[22] Fluconazole, a well-known triazole antifungal drug, can reverse the decline in ATRA plasma levels in leukemia patients.[23] A recent paper[24] reported on the pharmacological data for R115866 as an orally active inhibitor of RA metabolism, capable of enhancing RA levels and exerting retinoidal activities (Figure 2).

In this communication, we wish to report the biological activity of some potential inhibitors of ATRA metabolism enzyme(s); from among a library of 70 azoles, we selected eight compounds for potency testing (Figure 3).

MATERIALS AND METHODS

Chemicals

[11,12-^{3}H]All *trans*-retinoic acid was purchased from Dupont Ltd (Stevenage, Herts, UK). All *trans*-retinoic acid, NADPH, butylated hydroxyanisole, ketoconazole and protein standards were obtained from Sigma Chemical Company (Poole, Dorset, UK). Formic acid, ammonium acetate and Hisafe III scintillation fluid (optiphase III) were obtained from Fisons Ltd (Leicestershire, UK). All solvents used for chromatography were HPLC grade from Rathburn Chemicals Ltd (Walkerburn, UK). All other laboratory reagents were of analytical reagent grade and were obtained from British Drug House (Poole, Dorset, UK). The eight compounds tested in this communication have been previously described by us: compound (**1**),[25] compound (**2**),[25] compound (**3**),[26] compound (**4**),[27] compound (**5**),[27] compound (**6**),[26] compound (**7**)[25] and compound (**8**)[28].

Preparation of Rat Liver Microsomes

Rat liver microsomes were prepared by the method previously described[29] and stored at −80°C.

Determination of Retinoic Acid-metabolising Activity of Rat Liver Microsomes

The assay performed here was based on the general procedure described previously[30–32] and was briefly as follows. Hepatic microsomes (10 μL, 0.12 mg protein mL^{-1} final concentration) were incubated, in triplicate, with [11,12-^{3}H]all *trans*-RA (3 μM, 10 μL), NADPH (2 mM, 50 μL) and phosphate buffer (50 mM, pH 7.4, 320 μL) for 15 min at 37°C and then the reaction was terminated by adding formic acid (1% v/v, 100 μL). [11,12-^{3}H]All *trans*-RA and its (oxidative) metabolites were extracted into ethyl

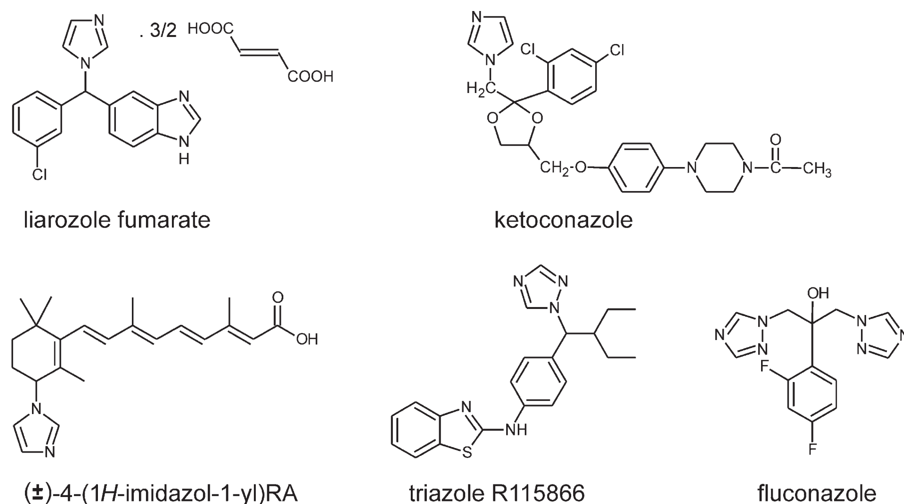
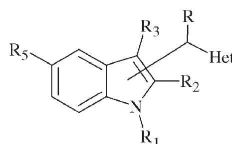


FIGURE 2 Structures of diverse RAMBAs.



No.	Azolyl grouping position on indole nucleus	R_1	R_2	R_3	R_5	R	Het
1	3	2-Cl-C_6H_4-CH_2	H	-	H	H	1,2,4-triaz
2	3	2-Cl-C_6H_4-CH_2	H	-	H	H	1,3,4-triaz
3	2	Et	-	CH_3	Br	C_6H_5	1,2,4-triaz
4	2	Et	-	CH_3	Br	3-Cl-C_6H_4	1,2,4-triaz
5	2	Et	-	CH_3	Br	2,4-diCl-C_6H_4	1,2,4-triaz
6	2	Et	-	CH_3	Br	4-F-C_6H_4	1,2,4-triaz
7	3	2-Cl-C_6H_4-CH_2	H	-	Br	4-F-C_6H_4	1,2,4-triaz
8	5	Et	H	H	-	4-F-C_6H_4	imid

FIGURE 3 Structures of the studied azolyl compounds **1–8.**

acetate containing 0.05% (v/v) butylated hydroxyanisole (2 × 2 ml). The extract was taken to dryness in-vacuo at room temperature and the residue dissolved in acetonitrile–water–formic acid (75:25: 0.05 v/v) containing ammonium acetate (10 mM) and the [^{3}H]metabolites separated and quantitatively determined by reversed-phase HPLC on a C_{18} µBondapak column (3.9 × 300 mm, Millipore) with a Reeve model 970 detector. The percentage metabolism was calculated from the areas under the curves: 100[(metabolites)/(metabolites + retinoic acid)].

Determination of Percentage Inhibition Values

The assay was conducted in the presence of the potential RAMBAs and ketoconazole at 100 µM final concentration in DMSO (10 µL). A control incubation was conducted with DMSO (10 µL) alone. The percentage inhibition was calculated from the areas under the curves: 100[(metabolites (control) − metabolites (inhibitor))/metabolites (control)] (Table I).

TABLE I Effect of ketoconazole and target compounds on RA metabolism

Compound	% inhibition[a] (100 µM)
1	40.7 ± 2.2
2	05.0 ± 1.1
3	68.9 ± 3.9
4	25.0 ± 3.1
5	47.8 ± 3.3
6	60.4 ± 4.5
7	16.0 ± 2.1
8	23.0 ± 3.2
Ketoconazole	70.0 ± 5.0

[a] Retinoic acid concentrations = 3 µM. Values are the means of three determinations ± s.d.

RESULTS AND DISCUSSION

Under these HPLC conditions used, the retention time for [11,12-^{3}H]-RA was 10 min. Several oxidative metabolites of [11,12-^{3}H]-RA were detected eluting between 3 and 7 min. The peaks of 4-keto RA and 4-hydroxy RA were identified at retention times of 3 and 7 min, respectively, by running standard samples and detecting by the on-line UV detector.

Compounds **1–8**, with rat liver microsomes, partially inhibited retinoic acid metabolism enzyme(s), the percentage inhibition values being between 5 and 69%. Ketoconazole was employed as a reference compound and inhibited RA metabolism by 65–75%. Regarding the percent inhibition values for compounds **1** (0.5%) and **2** (40.7%), the 1,3,4-triazole moiety seemed to be less powerful than the 1,2,4-triazole one. Among compounds **3–6** belonging to the series of 2-(α-azolylbenzyl)indoles, the most potent RAMBA was compound **3** (68.9%). Substitution on the benzyl moiety by 3-chloro (compound **4**) and 2,4-dichloro (compound **5**) groups induced a decrease in activity: the % inhibition values were 25 and 47.8%, respectively. The decrease in activity became less pronounced with the introduction of a 4-fluoro group as in compound **6** (60.4%). Compound **7**, a 3-(α-azolylbenzyl)indole derivative, was a weak inhibitor (16%). Although the only tested imidazole derivative, compound **8** (23% inhibition), could not be compared to its triazole congener, the imidazole moiety does not seem to represent an efficient pharmacophoric fragment.

In conclusion, this preliminary work indicates that two triazole derivatives, compounds **3** and **6**, exert similar inhibitory effects to ketoconazole.

Both of them are potentially valuable RAMBAs; to confirm their ability to increase endogenous levels of ATRA, we intend to determine, after oral administration in rats, ATRA concentrations in plasma and other tissues such as skin, liver, spleen, kidney, lung and testis.

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