Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 267–272

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF LIAZAL™, A RETINOIC ACID METABOLISM BLOCKING AGENT (RAMBA) WITH POTENTIAL CLINICAL APPLICATIONS IN ONCOLOGY AND DERMATOLOGY

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Received 18 September 1997; accepted 22 December 1997

Abstract : The synthesis of LIAZAL™ (compound **9**, R085246) is described. LIAZAL™ inhibits all-trans-retinoic acid metabolism and thereby exerts retinoid-like effects *in vivo*.

Introduction

Retinoic acid, an endogenous metabolite of retinol (vitamin A), plays a key role in cellular processes, regulating epithelial growth and differentiation. Therefore, it is an attractive target for medicinal intervention in diseases characterized by aberrations in these processes. As such, all-*trans*-retinoic acid (ATRA) is being used in differentiation therapy of cancer, in cancer chemoprevention and for the treatment of acne and skin photodamage [1-4]. Clinical successes were obtained with ATRA treatment but toxicity turned out to be a problem, especially with systemic administration. Moreover, resistance to treatment usually occurs due to induction by ATRA of its own metabolism [5].

ATRA can be metabolized *via* several routes. The physiologically most prominent pathway starts with hydroxylation at the 4-position of the cyclohexenyl ring, leading to formation of 4-hydroxy-ATRA that is converted into 4-oxo-ATRA. This metabolite is further transformed into more polar metabolites [6-7]. The first and rate-limiting step in this oxidative catabolic pathway is catalyzed by 4-hydroxylase, a cytochrome P450-dependent enzyme. The exact nature of this enzyme remains to be elucidated. Recently, a P450-enzyme that is ATRA-inducible and capable of 4-hydroxylating ATRA, has been cloned from zebra fish (P450 RAI) [8].

Initially, 4-hydroxylase activity was thought to mainly reside in the liver, but its presence has now been demonstrated in skin and tumor cells and tissues [9-11]. Theoretically, inhibitors of 4-hydroxylase should increase endogenous levels of ATRA (acting as 'ATRA-mimetics') and thus overcome ATRA-resistance. Azole-containing compounds are well known to interact with cytochrome P450-dependent enzymes and therefore constitute a logical first-choice source of potential 4-hydroxylase inhibitors. The antimycotic ketoconazole has indeed been shown to be an, albeit weak, inhibitor of ATRA 4-hydroxylase [12].

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0960-894X/98/$19.00
PII: S0960-894X(98)00004-3

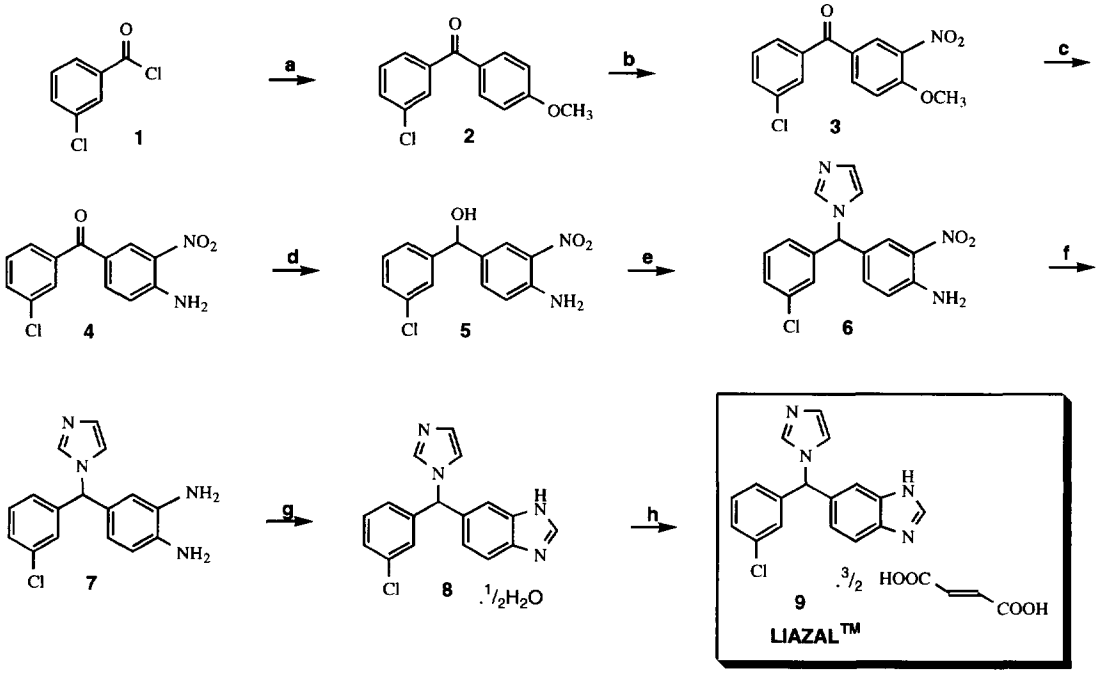
In the present paper we describe the synthesis and some relevant pharmacological data of LIAZAL™ (liarozole-fumarate), a new 4-hydroxylase inhibitor.

Chemistry

The synthesis of LIAZAL™ (R083642, liarozole fumarate), the most promising candidate from a series of (1H-imidazol-1-ylmethyl)-1H- benzimidazole derivatives [13] is outlined in ***scheme 1***.

Compound **2** (4-methoxyphenyl, 3-chlorophenylmethanone) synthesized using Friedel-Crafts acylation as described [14], was nitrated in dichloromethane at 10°C to **3** (87% yields). Replacement of methoxy by amino upon treatment of **3** with ammonia-isopropanol (pressure vessel) at 100°C yielded **4**. Sodium borohydride reduction in isopropanol gave alcohol **5** (75% yield), which was converted to the imidazolyl derivative **6** (69% yield). Catalytic hydrogenation of **6**, followed by cyclisation of diamine **7** in a mixture of formic acid and HCl 4N at reflux, afforded benzimidazole **8**, which was finally isolated as the fumarate **9** (60% yield).

Scheme 1



a) $PhOCH_3$, $AlCl_3$, CH_2Cl_2, 5-10°C; **b)** HNO_3/H_2SO_4, CH_2Cl_2, 10-15°C, 1h; **c)** NH_3g, *i*.PrOH 100°C, 16h; **d)** $NaBH_4$, *i*.PrOH, reflux, 1h; **e)** CDI, CH_2Cl_2, reflux 1h; **f)** H_2, Pt/C 5%, thiophene sol., MeOH, RT; **g)** HCOOH, 4N HCl, reflux; **h)** EtOH, 50°C, fumaric acid

Pharmacology

LIAZAL™ inhibits several cytochrome P450-dependent enzymes such as aromatase (estrogen biosynthesis) and, at least in some species, 17,20-lyase (androgen biosynthesis). However its main biological effects can be ascribed to inhibition of ATRA metabolism.

In vitro, micromolar concentrations of LIAZAL™ inhibited ATRA metabolism by hamster and rat liver microsomes [15,16], neonatal rat skin epidermal microsomes [9], human skin epidermis [17] and in MCF-7 human breast cancer cells [18]. In the latter case, this inhibition resulted in a potentiation of ATRA effects on cell proliferation and on induction of differentiation (as measured by an increase in E-cadherin expression) [19].

In vivo, oral administration of LIAZAL™ to rats transiently increased the plasma, vaginal and tumor ATRA levels [15,20]. To further document the ability of LIAZAL™ to augment endogenous levels of ATRA in rat tissues, animals were orally treated with 20% PEG (vehicle) or LIAZAL™(10 mg/kg). Four hours later, ATRA concentrations were determined in skin, epididymal fat, kidney, lung and testis. As shown in Figure 1, mean ATRA concentrations (ng ± S.E. per g of tissue) were significantly ($p < 0.05$) increased from 2.1 ± 0.5 to 6.8 ± 0.9 in skin and from 4.3 ± 0.3 to 7.5 ± 0.5 in testis. No significant differences were observed in fat (3.7 ± 0.6 vs. 6.8 ± 1.4), kidney (4.3 ± 0.7 vs. 4.6 ± 0.3) and lung (5.1 ± 0.8 vs. 4.7 ± 0.4). Taken together, these data indicate that LIAZAL™ in rats enhanced basal ATRA levels in a tissue-dependent manner. Because of this property, LIAZAL™ in rats can exert ATRA-like effects, such as suppression of the process of vaginal keratinisation [15], inhibition of tumor growth in prostate tumor models [21] and modulation of the differentiation of tumor cells in situ [20].

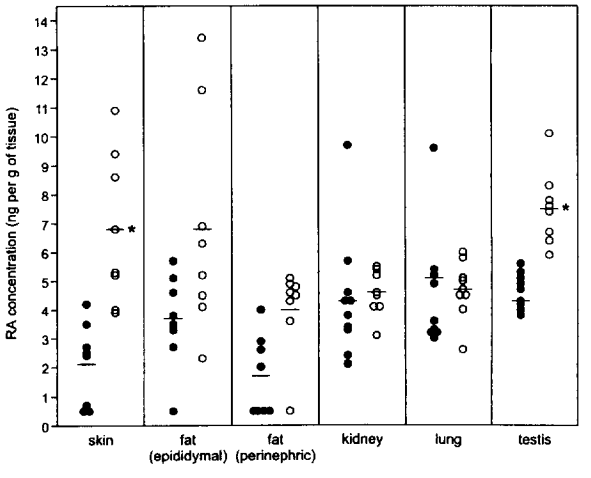


Figure 1. **RA levels in rat tissues (skin, fat, kidney, lung and testis) after oral treatment (-4h) with 20% PEG 200 (•), or LIAZAL™ (10 mg/kg) (o). The values for individual rats (n = 8-10 per treatment group) are shown. Horizontal bars indicate mean values. *indicates statistical difference ($P < 0.05$) from their respective vehicle value.**

In conclusion, LIAZAL™ is a new inhibitor of ATRA 4-hydroxylase that may find important application as a therapeutic agent in oncology and dermatology. Phase III clinical studies in prostate cancer and in psoriasis are ongoing.

Experimental section

Melting points were determined on a Mettler FP80 apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a Brüker AM-360 spectrometer using tetramethylsilane (TMS) as internal reference.
To dry the organic solutions, anhydrous $MgSO_4$ was used. For column chromatography Merck silicagel 60 (63-200µm; 70-230 mesh) was applied.

(4-methoxy-3-nitrophenyl)-3-chlorophenylmethanone (3). To a solution of ketone **2** (49.2g, 200mmol), cooled to 10-15°C, HNO_3 (25ml, 250mmol) was added dropwise. The mixture was stirred for 45min. at 10°C. Ice water was added, the organic layer was separated, dried, filtered and evaporated. The residual solid was crystallized from isopropanol (500ml) to afford **3** (50.7g, 87%) : mp 110.3°C; ^{1}H-NMR ($CDCl_3$) δ arom H at 8.30 (1H, d), 8.06 (1H, dd), 7.75 (1H, t), 7.6 (2H, m), 7.46 (1H, t) and 7.23 (1H, d), 4.07 (3H, s, OCH_3); Anal. $C_{14}H_{10}ClNO_4$, Calculated : C(57.64), H(3.46), N(4.80); Found : C(57.79), H(3.50), N(4.78).

(4-amino-3-nitrophenyl)-3-chloro-phenylmethanone (4). A mixture of ketone **3** (45.7g, 157mmol) in ammonia-isopropanol (350ml) was heated in a pressure vessel for 16h at 100°C. Water (150ml) was added and the compound was crystallized at room temperature and recrystallized from CH_3CN (350ml) to provide **4** (33.1g, 76%) : mp 171.7°C; ^{1}H-NMR ($CDCl_3$) δ arom H at 8.60 (1H, d), 7.95 (1H, dd), 7.73 (1H, t), 7.6 (2H, m), 7.45 (1H, t) and 6.92 (1H, d); 6.55 (2H, broad s, $N\underline{H}_2$); Anal. $C_{13}H_9ClN_2O_3$, Calculated : C(56.43), H(3.28), N(10.13); Found : C(56.41), H(3.24), N(10.01).

(4-amino-3-nitrophenyl)-3-chlorophenylmethanol (5). To a mixture of **4** (15.9g, 57mmol) in isopropanol (150ml) was added $NaBH_4$ (3.8g, 100mmol). The mixture was heated at reflux for 1h. The reaction was quenched with CH_3COOH (20ml) at ambient temperature. The solvent was removed in vacuo and the residue was partitioned between water and diisopropylether. The organic layer was dried, filtered and evaporated. The residue was crystallized from CH_2Cl_2 to give the desired alcohol **5** (11.9g, 75%) : mp. 113.9°C; ^{1}H-NMR ($CDCl_3$) δ arom H at 8.13 (1H, d), 7.37 (1H, t), 7.20-7.35 (4H,m) and 6.78 (1H), 6.10 (2H, broad s, $N\underline{H}_2$), 5.73 (1H, s, C$\underline{H}$); 2.3 (1H, broad s, O$\underline{H}$); Anal. $C_{13}H_{11}ClN_2O_3$, Calculated : C(56.03), H(3.98), N(10.05); Found : C(55.86), H(3.76), N(10.01).

4-[(3-chlorophenyl)(1H-imidazol-1-yl)methyl]-2-nitrobenzenamine (6). To a solution of alcohol **5** (15.9g, 57mmol) in CH_2Cl_2 (180ml) was added 1,1'-carbonyldiimidazole (11.2g, 69mmol) at ambient temperature. The mixture was refluxed for 1h, then washed with H_2O, dried, filtered and evaporated. The residue was purified on silica gel with ethylacetate-methanol (90:10) and the obtained imidazole derivative was crystallized from toluene to afford **6** (13g, 69%): mp. 119.6°C; ^{1}H-NMR ($CDCl_3$) δ 7.91 (1H, d), 7.43 (1H, t, Im.H-2), 7.35 (1H,dt), 7.32 (1H, t); 7.13 (1H, t, Im.H-4), 7.11 (1H, dd), 7.06 (1H, t), 6.96 (1H, dt), 6.86 (1H, d), 6.85 (1H, t, Im.H-5), 6.41 (1H, s, C$\underline{H}$), 6.39 (2H, broad s, $N\underline{H}_2$); Anal. $C_{16}H_{13}ClN_4O_2$, Calculated : C(58.46), H(3.99), N(17.04); Found : C(58.12), H(3.84), N(17.01).

4-[(3-chlorophenyl)(1H-imidazol-1-yl]-1,2-benzenediamine (7). Compound **6** (6.56g, 20mmol) was hydrogenated in a mixture of MeOH (150ml), ammonia-MeOH (100ml) and thiophene solution (1ml, 1% in MeOH) over Pt/C (5%) catalyst (2g). After filtration of the reaction mixture through decalite, the filtrate was evaporated. The residue was purified on silica gel with chloroform-methanol (95:5) as eluent and the obtained

compound was stirred in diisopropylether to give 1,2-benzene diamine derivative **7** (7.4g, 54.3%) : mp 130.3°C; ^{1}H-NMR ($CDCl_3$) δ 7.40 (1H, t, Im.H-2), 7.30 (1H, dt), 7.27 (1H, t), 7.09 (1H, t, Im.H-4), 7.06 (1H, t), 6.95 (1H, dt), 6.83 (1H, t, Im.H5), 6.67 (1H, d), 6.45 (1H, dd), 6.43 (1H, d), 6.33 (1H, s, CH), 3.4 (4H, broad s, $2NH_2$); Anal. $C_{16}H_{15}ClN_4$, Calculated : C(64.32), H(5.06), N(18.75); Found : C(64.11), H(5.05), N(18.56).

5-[(3-chlorophenyl) (1H-imidazol-1-yl)methyl]-1H benzimidazole hemihydrate (8). To a mixture of 4N HCl (75ml) and formic acid (3.8ml) was added compound **7** (10g, 3.35 mmol) at ambient temperature. After an additional heating for 3h at 50°C, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between water and CH_2Cl_2 and made alkaline with aqueous ammonia. The organic layer was washed with water, dried, filtered and evaporated. The residue was dissolved in water, acidified with HCl 6N, treated with norite and filtered. The filtrate was made alkaline with aqueous ammonia and the product was extracted with CH_2Cl_2. The solvent was removed under reduced pressure to provide benzimidazole derivative **8** (8.93, 86%) : mp 114.7°C; ^{1}H-NMR (DMSO-d6) δ 12.53 (1H, broad s, Benz.NH), 8.27 (1H, s, Benz.H-2), 7.69 (1H, s, Im.H-2), 7.61 (1H, d, Benz.H-4), 7.40-7.47 (2H, m, Ph.H-4, and Ph.H-5), 7.33 (1H, broad s, Benz.H-7), 7.17 (2H, s, Ph.H-2 and Im.H-5), 7.12 (1H, m, Ph.H-2), 7.06 (1H, d, Benz.H-5), 7.03 (1H, s, CH), 6.98 (1H, s, Im.H-4); Anal. $C_{17}H_{13}ClN_4.0.5H_2O$, Calculated : C(66.13), H(4.24), N(18.15); Found C(65.49), H(4.47), N(17.93).

5-[(3-chlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-benzimidazole (E)-2-butenedioate (2:3) (9). To a solution of (**8**) (5.2g, 16.8mmol) in 65ml of ethanol, heated at 45°C, was added 2.82g (24.3mmol) of fumaric acid, and the mixture was allowed to cool. After additional stirring at room temperature for 2 days, the target compound **9** was obtained as white crystals; (4.86g, 60%) : mp 155°C (dec) : ^{1}H NMR (DMSO) δ 8.28 (1H, s, Benz.H-2), 7.79 (1H, t, Im.H-2), 7.62 (1H, d, Benz.H-4), 7.43 (2H, m, Ph.H-5, Ph.H-4), 7.34 (1H, m, Benz.H-7), 7.20 (1H, t, Im.H-5), 7.17 (1H, m, Ph.H-2), 7.11 (1H, m, Ph.H-6), 7.06 (1H, dd, Benz.H-5), 7.05 (1H, s, CH), 7.04 (1H, t, Im.H-4), 6.63 (3H, s, HOOC-CH); Anal. $C_{17}H_{13}ClN_4.{}^3/_2C_4H_4O_4$, Calculated : C(57.21), H(3.97), N(11.60), O(19.88); Found : C(57.10), H(3.90), N(11.54), O(19.79).

Acknowledgment

Microanalysis and ^{1}H-NMR data were kindly provided by the JRF Analytical Department .
Special thanks are due to M. Vanderhallen for secretarial assistance in the preparation of this manuscript.

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