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Novel Retinoic Acid Receptor α Agonists: Syntheses and Evaluation of Pyrazole Derivatives

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Abstract—We have designed and synthesized a series of pyrazole derivatives as candidate retinoic acid receptor (RAR) agonists. One of them, 4-[5-(1, 5-diisopropyl-1*H*-3-pyrazolyl)-1*H*-2-pyrrolyl]benzoic acid (**11b**), which possesses a 2,5-disubstituted pyrrole moiety, showed selective transactivation activity for the RARα receptor, and had highly potent cell-differentiating activity on HL-60 cells.

Introduction

Retinoids, natural and synthetic analogues of all-*trans* retinoic acid (ATRA), play important roles in many biological functions, including induction of cellular proliferation, differentiation and death, as well as developmental changes.[1] It has been shown that retinoids exert their function through nuclear receptors (RARα, β, and γ).[2]

Although retinoids are thought to have great therapeutic potential, the clinical use of retinoids is so far limited mainly to dermatological diseases[3] and also some cancers, for which retinoids may have both chemotherapeutic and chemopreventive applications.[4] For example, ATRA and Etretinate are prescribed for acute promyelocytic leukemia (APL) and psoriasis patients, respectively (Chart 1). The main reason for the limited use of retinoids so far is the wide range of toxic effects of retinoids.[5] Recent research has focused on the synthesis and development of subtype-selective retinoids in order to reduce the toxicity.[6]

Only a few of RARα agonists have been reported so far. These include Am80, Am580[6a] and AGN193836.[6b] Am80 is more potent than ATRA (all-*trans* retinoic acid) as an in vitro differentiation inducer, and clinically Am80 was effective in leukemia patients[7] and psoriasis patients.[8] Moreover, this compound inhibited rat CII arthritis.[9] Therefore, RARα agonists appear to be promising compounds for the treatment of cancer, dermatological diseases, and immunological disorders.

In the course of our studies aimed at synthesizing novel retinoids,[10] we have already reported that ER-34617, which possesses both a quinoxaline and a 2,5-disubstituted pyrrole moiety, showed selectivity for the RARα receptor, as well as highly potent cell-differentiating activity on HL-60 cells.[10c] Many synthetic retinoids, such as Am80 and ER-34617 (Chart 2), possess a bicyclic hydrophobic part, except for Etretinate and Barrero's monocyclic retinoids, in which the phenyl hydrophobic part of Etretinate was replaced with monocyclic pyrrole and imidazole.[11] We first introduced a monocyclic pyrazole moiety into the hydrophobic part of ER-34617. We hoped that such simple retinoid derivatives might be selective RARα agonists. Here we discuss the synthesis and structure–activity relationships (SAR) of retinoids which possess a pyrazole and a 2,5-disubstituted pyrrole as the hydrophobic part and linker, respectively.

Chemistry

A number of known active retinoids possess bulky structure at the hydrophobic part. Therefore, we introduced an isopropyl group at the 5-position of pyrazole in order to enhance the affinity for RARs. The synthesis of the pyrazole derivatives is shown in Scheme 1.

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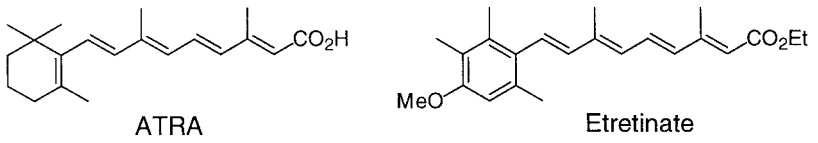


Chart 1.

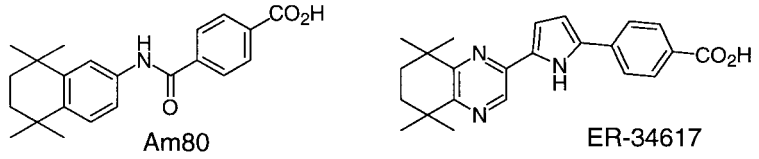
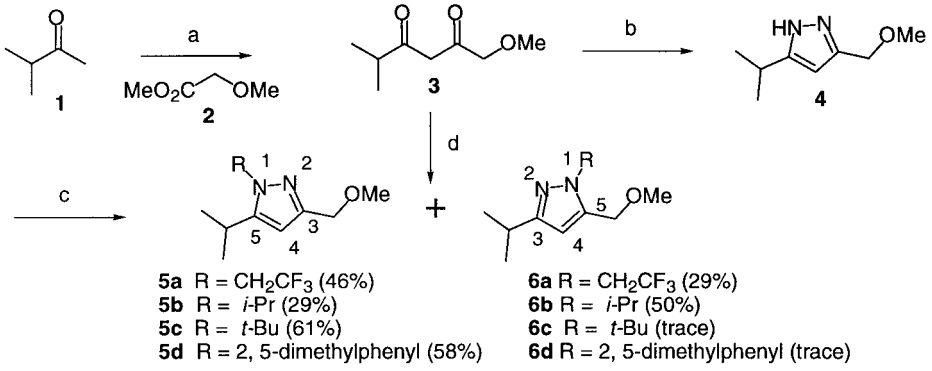


Chart 2.



Scheme 1. Reagents and conditions: (a) methyl 2-methoxyacetate (**2**), Na, toluene, 70 °C, 2 h; (b) NH_2NH_2, EtOH, reflux, 2 h; (c) *i*-PrI, NaH, DMF, RT, 30 min; (d-i) $CF_3CH_2NHNH_2$, EtOH; (d-ii) *t*-BuNHNH$_2$·HCl, EtOH, reflux, 2 h; (d-iii) 2,5-dimethylphenylhydrazine hydrochloride, EtOH, reflux, 2 h.

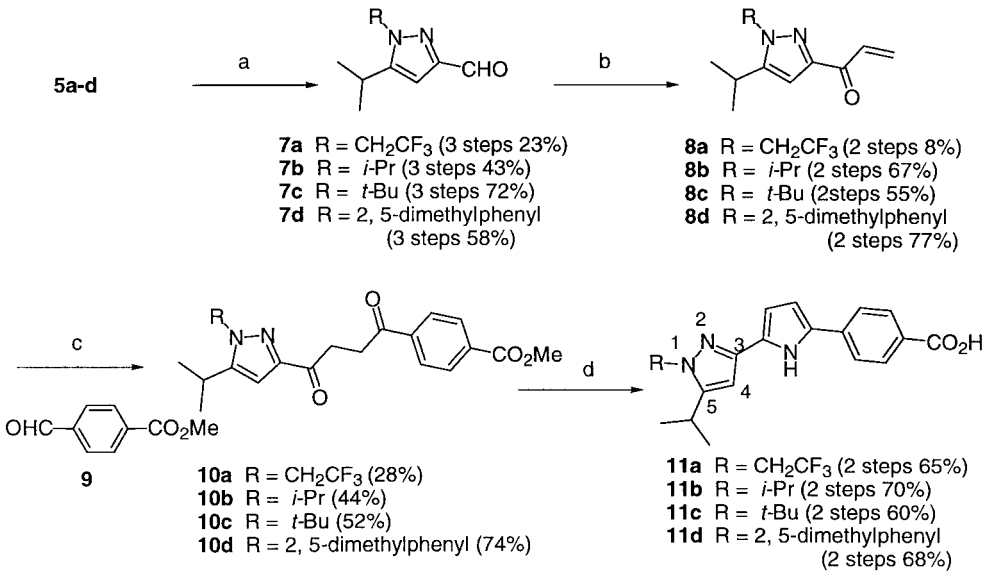
Condensation of 3-methyl-2-butanone (**1**) with methyl 2-methoxyacetate (**2**) in the presence of sodium gave the diketone (**3**). The pyrazole derivatives (**5a**–**d**) were prepared by two methods. Treatment of the diketone with hydrazine, followed by alkylation of the pyrazole derivative (**4**) with isopropyl iodide in the presence of sodium hydride afforded the 1,5-diisopropylpyrazole (**5b**) as a minor isomer.[12] A more direct method to synthesize the *N*-alkylated pyrazole was performed by treatment of the diketone (**3**) with substituted hydrazine. In this procedure, we obtained the pyrazole derivatives (**5a**, **5c**, **5d**) as the main product isomer (Scheme 1).

The formyl derivatives were obtained in three steps. Treatment of the methyl ethers (**5**) with boron tribromide followed by hydrolysis gave the alcohol, which was oxidized with manganese dioxide to afford the formyl derivatives (**7a**–**d**). These were treated with vinyl Grignard reagent followed by manganese dioxide oxidation to give the enones (**8a**–**d**). Synthesis of the 1,4-diketones (**10a**–**d**) was achieved by means of a thiazolium salt-catalyzed benzoin condensation type reaction. The enones (**8a**–**d**) were treated with a commercially available benzaldehyde derivative in the presence of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride and triethylamine to afford the 1,4-diketones (**10a**–**d**). These were treated with ammonium acetate followed by hydrolysis to afford the pyrrole derivatives (**11a**–**d**) (Scheme 2).

Results and Discussion

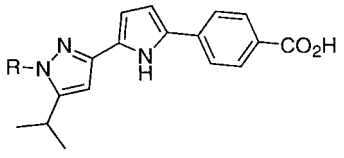
The above retinoids were synthesized and evaluated in vitro for the ability to bind to the individual RARs and to induce gene transcription in a co-transfection assay. Co-transfection assays were performed as reported,[10c,13] and results are reported as relative EC_{30} values (see footnotes to Table 1). Binding assays for RAR receptor subtypes were performed in a similar manner to that described by Boehm et al.[14] using [^{3}H] ATRA. HL-60 differentiation activity was measured using CD11b as a marker of differentiation. The results are summarized in the table. RXRα transactivation was also studied, but none of these compounds activated RXR (data not shown).

These four pyrazole derivatives showed weak affinity for RARs, and even the affinity at RARα was 10- to 100-fold less potent than that of ATRA. However, in the co-transfection assay, the 1-trifluoroethyl-5-isopropylpyrazole derivative (**11a**) possessed comparable activity and maximum response to ATRA at RARα, but was less potent at RARβ and RARγ, respectively. Moreover, **11a** showed comparable HL-60 differentiation-inducing activity to ATRA. Introduction of a bulkier



Scheme 2. Reagents and conditions: (a-i) BBr_3, CH_2Cl_2, 0 °C, 1 h; (a-ii) Na_2CO_3, dioxane, H_2O reflux, 3 h; (a-iii) MnO_2, CH_2Cl_2, RT, 13–16 h; (b-i) vinylmagnesium bromide, THF, 0 °C, 10 min; (b-ii) MnO_2, CH_2Cl_2, RT, 13–16 h; (c) methyl 4-formylbenzoate (**9**), 3-benzyl-5-(2-hydroxyethyl)-4-methylthiozolium chloride, Et_3N, DMF, 80 °C, 1–2 h; (d-i) $AcONH_4$, MeOH, reflux, 3–5 h; (d-ii) NaOH, aqeous MeOH, reflux, 1 h.

Table 1. Competitive binding, transactivation and induction of HL-60 differentiation by the pyrazole derivatives



		Binding affinity[a]			Subtype-specific transactivation[e]			HL-60 differentiation-inducing activity
		Relative IC_{50}[b]			Relative EC_{30}[f]			
No.	R	RARα	RARβ	RARγ	RARα	RARβ	RARγ	ED_{30} (nM)
11a	CF_3CH_2	98	—[c]	—[c]	1.0	15	88	0.62
11b	*i*-Pr	13	660	—[c]	0.19	1.8	14	0.27
11c	*t*-Bu	7.6	110	70	0.22	0.63	2.3	0.050
11d	2,5-dimethylphenyl	39	—[c]	—[c]	—[g]	—[g]	—[g]	>10000
ATRA	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5
		0.78 nM[d]	0.90 nM[d]	0.50 nM[d]	0.99 nM[h]	0.93 nM[h]	0.22 nM[h]	

[a]Specific binding affinity was defined as the total binding minus the nonspecific binding, and the 50% inhibitory dose (IC_{50}) values were obtained from logarithmic plots. In some cases, Scatchard plot analysis was performed. The selectivity of test compounds for each receptor is indicated as relative IC_{50}, where the IC_{50} value for each receptor was divided by that of the natural ligand (ATRA).
[b]IC_{50}/ATRA IC_{50}.
[c]—: not detectable (relative IC_{50} >1000).
[d]ATRA IC_{50}.
[e]EC_{30} values were determined from full dose-response curves ranging from 0.1 nM to 3 mM. Retinoid activity is expressed in terms of relative EC_{30}, which is the concentration of retinoid required to produce 30% of the maximal observed response, normalized relative to that of ATRA.
[f]EC_{30}/ATRA EC_{30}.
[g]—: not detectable (relative EC_{30} >10000).
[h]ATRA EC_{30}.

substituent at the 1-*N*-position of the pyrazole (**11b**,**c**) resulted in significant activation. Compound **11b** activated RARα, having a transcription activity more potent than that of ATRA. Although the 1-*N*-*tert*-butyl-5-isopropylpyrazole derivative (**11c**) possesses low selectivity for RARs, this compound is 30-fold more potent in HL-60 differentiation-inducing activity than ATRA. Introduction of a large aromatic substituent at the 1-*N*-position of the pyrazole (**11d**) did not activate any RARs, and this compound did not show HL-60 differentiation-inducing activity.

In conclusion, we have described the synthesis and evaluation of a new series of pyrazolylpyrrole derivatives.

We found that the pyrazole derivatives have strong transactivation activities. One of them, 4-[5-(1,5-di-isopropyl-1*H*-3-pyrazolyl)-1*H*-2-pyrrolyl]benzoic acid (**11b**; ER-38930) has selectivity for the RARα receptor, and shows potent cell differentiation-inducing activity. Thus, ER-38930 should be useful as a tool to study the physiological role of RARα. It may also be useful as a lead compound for RARα-selective retinoids, which might have fewer toxic effects and better therapeutic indices than non selective retinoid drugs.

Acknowledgements

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