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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW HETEROCYCLIC AROMATIC RETINOIDS

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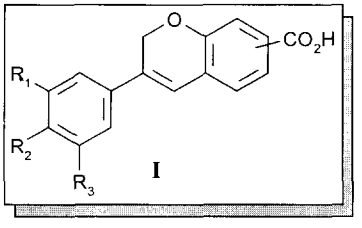
Abstract: A series of 3-aryl-2*H*-1-benzopyrancarboxylic acid derivatives was synthesized and evaluated as Retinoic Acid Receptor (RAR) agonists. By modifications of the 3-aryl group, we have obtained new retinoids exhibiting potent cellular differentiating activities and high affinities for RARs. Moreover, hydrogenation of the *2H*-1-benzopyran ring led to the 3-(5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-naphthalen-2-yl)-3,4-dihydro-*2H*-1-benzopyran-7-yl carboxylic acid, characterized by a RARβ binding profile.

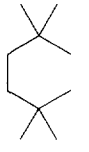
Retinoids, synthetic and natural analogues of *all-trans*-retinoic acid, exert profound effects on cell differentiation and proliferation[1]. These properties confer a high potential for the treatment of hyperproliferative disorders[2], for example psoriasis and certain forms of cancer. However, widespread use of retinoids in therapy is generally limited by side effects such as teratogenicity, muco-cutaneous irritation and the hypervitaminosis A syndrome.

Many biological effects of these compounds are mediated by activation of nuclear receptors. There are three known distinct RAR subtypes (RARα, -β and -γ) located in the cell nucleus. After docking to a ligand, RARs associate with their cognate response element as a heterodimer with Retinoid X Receptors (RXR), in order to activate gene transcription[3].



I

R_1=Ad, R_2=OH and R_3=H
R_1=Ad, R_2=OCH_3 and R_3=H
R_1 and R_3=tBu, R_2=OH
R_1=tBu, R_2=OH and R_3=H

R_1 and R_2=  R_3=H

General structure

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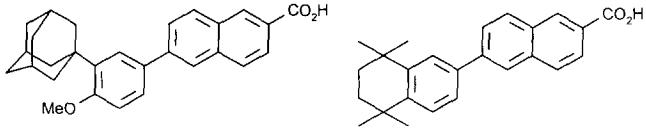
It is likely that receptor specific ligands will activate much more defined pathways involved in biological responses than will non-specific ones. Consequently, we sought to obtain new retinoids having specific interactions with their receptors. Compounds with specific affinities for RARα[4], RARβ and RARγ[5] subtypes have been described. Additionally, selective RXR ligands have been reported[6,7]. RARα and predominantly RARγ are the most highly expressed RARs in the skin[8]. The RARβ–γ binding profile of Adapalene (CD 271)[5], a new compound used in the topical treatment of acne led us to design a new series of related heterocyclic aromatic retinoids (general structure **I**).

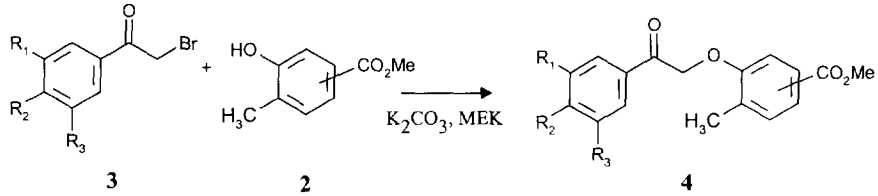


Adapalene (CD 271) **TTNN**[9]

Chemistry

Our first attempt was to prepare these compounds by bromination of compound **4** (Scheme 1).

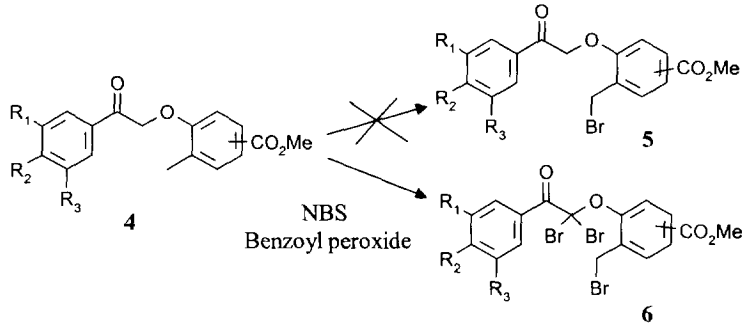
Scheme 1



3 **2** **4**

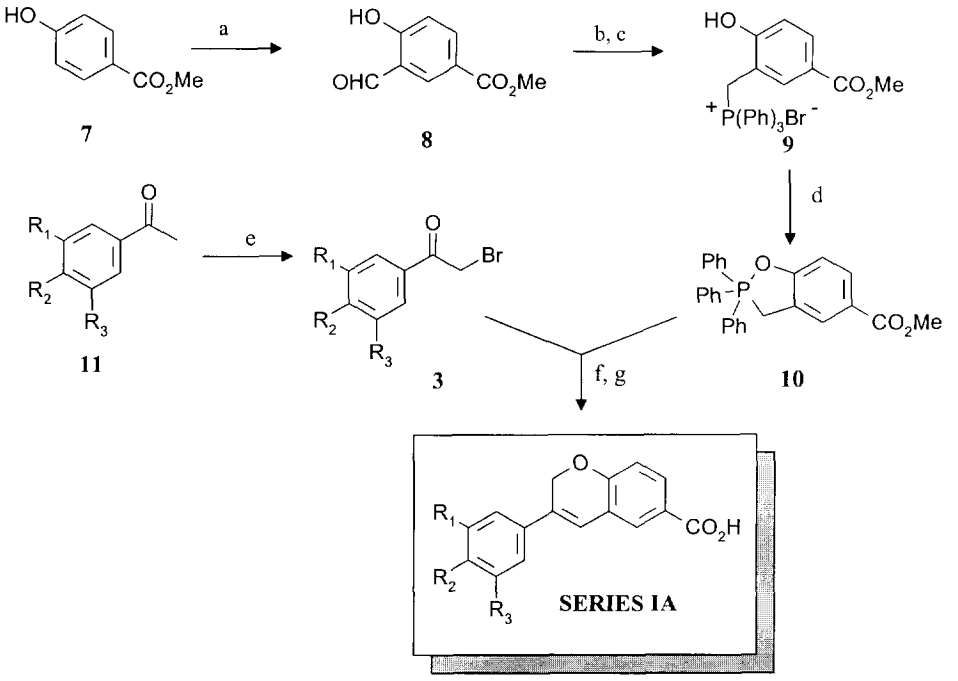
However, various attempts to halogenate compound **4** did not provide compound **5** but rather the tribrominated ester **6** (Scheme 2).

Scheme 2



We finally choose another pathway based on one-pot condensation-cyclization of a benzoxaphosphole derivative with the halogenocetone **3** . The syntheses are depicted in scheme 3 for series **IA** and in scheme 4 for **IB** . Phosphonium salt **9** was prepared in three steps *via* Duff formylation[10] of the appropriate phenol **7** (36%), followed by reduction with sodium borohydride (82%) and conversion of the resulting alcohol by the action of triphenylphosphine hydrobromide[11] (93%). The salt **9** was then converted to the 2,2,2-triphenyl-(3H)-benzoxaphosphole **10** by action of aqueous sodium hydroxide.

Scheme 3

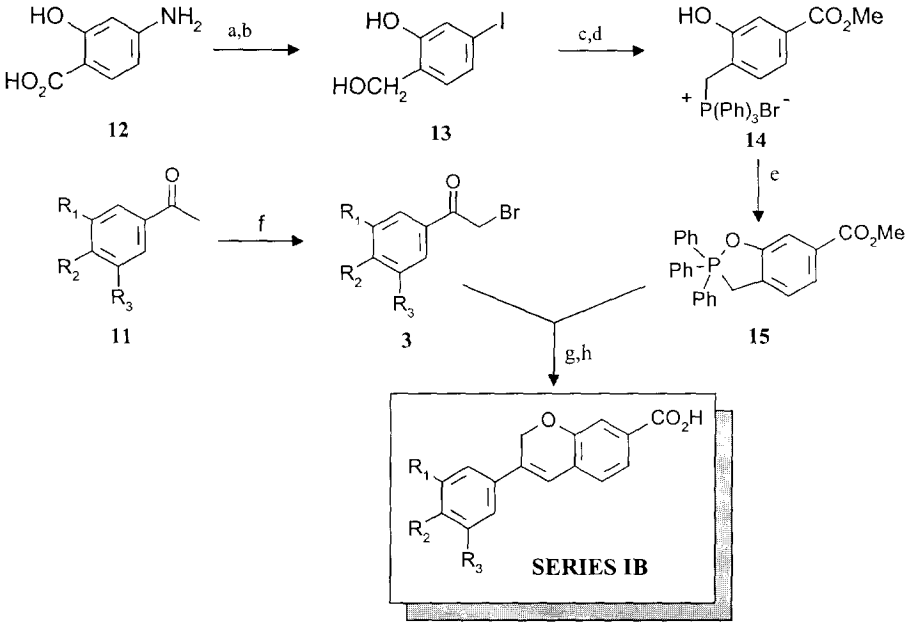


a/ Hexamethylenetetramine, TFA; b/ $NaBH_4$; c/ $P(Ph)_3HBr$, CH_3CN; d/ NaOH, H_2O; e/ Br_2, Et_2O , or $CuBr_2$, THF; f/ i)CH_2Cl_2, ii)MeONa, dioxane; g/ NaOH, H_2O, MeOH, THF

A different route was chosen for the series **1B** because in this case, Duff formylation did not afford the required isomer in good yield. Phosphonium salt **14** was synthesised in four steps. The commercially available aminosalicylic acid **12** was converted to the iodosalicylic acid *via* the diazonium intermediate (70%), then reduced to the benzylic alcohol **13** by borane-tetrahydrofuran complex (88%). Carbomethoxylation using the Heck procedure[12] (70%), followed by action of triphenylphosphine hydrobromide (91%) afforded the phosphine **14** which was then converted to 2,2,2-triphenyl-(3H) benzoxaphosphole **15**. Synthesis of the bromoacetophenone **3** was accomplished by bromination of acetophenone **11** (90%).

The one-pot coupling and cyclisation of 2,2,2-triphenyl-(3H)-benzoxaphosphole **10** or **15** with bromoacetophenone **3**[13] (42-50%) followed by hydrolysis (80-90%) yielded chromene derivatives of series **IA** or **IB**.

Scheme 4



a/ $NaNO_2$, KI, CuI, H_2SO_4; b/ BH_3, THF; c/ MeOH, CO, $Pd(OAc)_2$, Et_3N; d/ $P(Ph)_3HBr$, CH_3CN; e/ NaOH H_2O; f/Br_2, Et_2O or $CuBr_2$, THF; g/ i)CH_2Cl_2, ii)MeONa, dioxane; h/ NaOH, H_2O, MeOH, THF

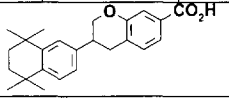
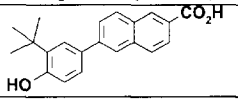
Biological Results

All the molecules were tested as cellular differentiation inducers using an F9 cell line[14], for their affinities to RAR subtypes[14](Table 1) and as transactivators on RARs[15] and RXR α[7]. Compounds of series **IA** are inactive in the F9 differentiation model, do not bind to RARs and are inactive in a RXRα transactivation assay[7].

Compound **II** has the highest affinities for the RARs in this series and displays higher affinities for RAR β and RAR γ than for RAR α. It is interesting to note that this mixed RARβ-γ binding profile is very similar to that of **TTNN** (Table1). The benzopyran moiety was then introduced in place of the naphthyl ring in the **CD 271** skeleton to give compound **III**. The methoxy adamantyl analog **III** also showed RARβ-γ selectivity similar to the **CD 271** binding profile. The replacement of the methoxy by an hydroxyl group in molecule **IV** dramatically reduced affinity for RARβ and was without effect on RARα and $-\gamma$ affinities. This confirms that RARβ is very sensitive to the presence of a polar function *ortho* to the adamantyl group and *para* to the biaryl link. Consequently, compound **IV** is partially RARγ selective. In order to further explore this effect we synthesised two phenol analogues, compounds **V** and **VI**. The replacement of the adamantyl ring by a *tert*-butyl group (Compound **V**) resulted in loss of both activity and affinity. The bulkiness and/or the lipophilicity in this part of the molecule seems to be particularly important for interaction with RARs. In the naphthoic acid series, the related analog exhibited a modest affinity for RARγ, suggesting that in this case the benzopyran heterocycle is weakly less well recognized, compared to the naphthoic group, by the RARγ (table 1). The introduction of a

second *tert*-butyl substituent (**VI**) is sufficient to regain affinity for RARγ and moderately so in the case of RARα. These compounds in series **IB** are agonists in RAR transactivation assays[15] and their activities are in agreement with their RAR affinities (data not shown). Taken together, these data show that 3-aryl-2*H*-1-benzopyran-7-carboxylic acid is a bioisoster of 2-aryl-6-naphthoic acid in terms of *in vitro* retinoid-like biological activities. Thus it is possible to transpose the structure-activity relationships of the naphthyl ring system[5].

Table 1: Biological results for compounds in series IB.

Compound	R_1	R_2	R_3	F9 AC_{50} (nM)	Binding RAR K_i (nM)[b] RAR α	RAR β	RAR γ
II			**H**	58	487	36	19
III	**Ad**	**CH_3O**	**H**	34	764	71	174
IV	**Ad**	**HO**	**H**	107	821	>3000	148
V	**tBu**	**HO**	**H**	NA	>3000	>3000	>3000
VI	**tBu**	**HO**	**tBu**	305	1471	>3000	531
VII [a]				300	5787	90	1036
Adapalene (CD271)				37	1100	34	130
				NA	>3000	>3000	200
TTNN				15	580	13	40

[a] Product **VII** was obtained by hydrogenation of product **II** (H_2, Pd/C, AcOEt)

[b] In a RXRα functional transactivation assay[7], none of the compounds was active at a concentration of 1μM.

The hydrogenated compound **VII** in which conjugation between the two aryl groups is absent and in which the torsion angle between the two cycles is modified exhibits partial selectivity for RARβ. This compound contains an asymmetric center whose optical resolution may lead to a better selectivity.

Compounds of series **IB** were tested for their ability to induce differentiation of F9 cells as estimated by plasminogen activator secretion (PA). These cells express high basal levels of RARα and RARγ, whereas RARβ is not expressed but is inducible by retinoids. All compounds induced plasminogen activator secretion with half maximal induction potency (AC_{50}) in the same range as their K_i values for the RAR receptor subtypes.

In this work, new series of retinoids have been identified which exhibit valuable biological properties: potent cellular differentiating activities, high affinities for retinoic receptors (RARs) and partially selective binding profiles. RARβ selective derivatives may be potentially useful in lung cancer treatment[16]. Additionally, some of these compounds will provide a significant basis for molecular modeling studies. The pharmacokinetic

and toxicological studies of a few derivatives are continuing in order to provide further support for their therapeutic potential.

Acknowledgements

The pSG5-derived expression vectors for RAR α and β were generous gifts from Dr. P. Chambon. The pSG5 plasmid for RAR γ was kindly provided by Dr. M. Pfahl.

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14. Binding assays were performed as previously described[15]. Cos-7 cells were transfected with the different pSG-derived expression vectors of human RARs using the polybrene technique. Cells were lysed, and the nuclear extracts were recovered by centrifugation and submitted to DNase digestion, extractions and competition experiments with [3H]CD 367 (2 nM) as the radioligand[17]. Separation of free and bound ligand was performed by high-performance size exclusion chromatography.
    Cellular differentiating activity was assessed in F9 murine embryonal teratocarcinoma cells[18]. F9 cells were grown in Dubelcco's modified Eagle medium supplemented with 15% fetal bovine serum and treated for 3 days with retinoids. Cell differentiation was quantified by assaying plasminogen activator (PA) secretion. The retinoid concentration eliciting half-maximal PA secretion (AC_{50}) was calculated by means of nonlinear regression analysis. Results are the means of three separate experiments.
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