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Practical synthesis of a chromene analog for use as a retinoic acid receptor *alpha* antagonist lead compound

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Abstract

Retinoic acid receptor *alpha* (RAR) selective compounds may guide the design of drugs that can be used in conjunction with hormonal adjuvant therapy in the treatment of breast cancer. Herein we report a modified synthesis of a known RAR antagonist, 2-fluoro-4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)chroman-6-yl]carbonyl]amino]benzoic acid and a synthesis of its unknown, desfluoro analog, 4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)chroman-6-yl]carbonyl]amino]benzoic acid. The modified route allows for facile reaction workups, increased yields, lower cost and incorporates a green alternative step. Structure–activity relationship studies determined through functional cell-based assays, demonstrated antagonism to RAR for both compounds. Molecular modeling within the RAR binding pocket was used to compare binding interactions of the desfluoro analog to a known RAR antagonist.

Keywords

Breast cancer; Hormone therapy; Retinoic acid receptor; Synthesis of antagonists

1. Introduction

Hormonal adjuvant therapy using estrogen antagonists, selective estrogen receptor modulators (e.g. tamoxifen) or inhibitors of estrogen synthesis (e.g. aromasin) are a mainstay in the treatment of breast cancer in the majority of patients [1,2]. However, in many cases hormonal adjuvants are only tumoristatic wherein the residual tumor cells can sustain a basal level of cell growth that may enable the generation and progression of genetic or epigenetic changes that eventually lead to the emergence of drug resistant tumors [2–5]. Recent evidence indicates that in estrogen-dependent breast cancer cells, the retinoic acid receptor alpha 1 (RAR 1) activates growth genes in a ligand-insensitive manner to support a basal level growth in the absence of hormone or in the presence of tamoxifen. RAR 1 is

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Appendix A. Supplementary data Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2013.02.012>.

genetically redundant in normal tissues. It is consistently expressed in breast tumors. Targeted inactivation or depletion of RAR α in cancer cells could potentially enhance current hormonal adjuvant therapies [2].

Teng et al. have identified an interesting series of antagonists that are selective for RAR α compared to RAR β and RAR γ [6]. These structures are shown in Fig. 1.

In functional assays for the three retinoic acid receptors, the des- and mono-fluoro compounds **1** and **2** exhibited nearly equal inhibitory potency toward RAR α that was approximately 10-fold greater than that of the di-fluoro compound **3**. Higher dosing was required to inhibit RAR β and RAR γ . Between the chromene pair, the mono-fluoro analog **4** was again about 10 times more potent than the di-fluoro analog **5** at RAR α . Both of the chromenes exhibited enhanced selectivity compared to series **1–3**, with **5** showing the highest overall [6]. Taking advantage of this preceding work, we wanted to further assess the possibility that a chromene type of compound might be able to serve as a lead for elaboration of antagonists at RAR α . For practical reasons we sought a compound that could be produced from readily available starting materials. The preceding structure–activity relationships suggest that the des-fluoro analog of **4** could potentially fulfill the desired biological profile while taking advantage of ethyl *p*-aminobenzoate as a common starting material amenable to considerable chemical modification. We report herein: (i) An improved synthesis of **4** along with the first-time synthesis of its des-fluoro analog; (ii) Comparison of their activities as antagonists of the classical transcriptional mechanism of RAR α using a cell-based promoter activity assay; and, (iii) A tentative binding mode for the des-fluoro analog within the RAR α pocket derived from X-ray data coupled with preliminary molecular modeling studies.

2. Results and discussion

2.1. Synthetic chemistry

The reported synthetic route [6] to **4** as deployed within our lab is depicted in Scheme 1. We were able to repeat this sequence in an uneventful manner, achieving similar yields at each step except for the bromination and Grignard reactions (steps **f** and **h**, respectively). While lower than the reported quantitative yield, we felt that 71% was still quite acceptable during bromination. Alternatively, we suspected that the Grignard reaction might be able to be enhanced by optimizing conditions conducive to its overall mechanism. We also noted that pyridinium *p*-toluenesulfonate (PPTS) was better for dehydration to **14**. Although reported at 25%, in our case we were able to achieve only a 10% yield of crude intermediate **14** (NMR), making this process the lowest yielding step in the overall sequence. In addition to addressing the Grignard reaction, we imagined that the initial sequence of reactions leading to intermediate **11** might be accomplished in fewer steps by taking advantage of a high-yielding approach to various chromenes that we had utilized previously within our labs [7]. Finally, we also felt that it would be worthwhile to substitute the reagents used in step (**j**) with a composite that reflects greener chemistry [8]. Our modified synthetic route is depicted in Scheme 2.

As anticipated from analogous literature methods [9] and previous experiments within our labs, coupling of the isoprene synthon **17** with phenol **18** was accompanied by intramolecular cyclization to afford chromene **19** in excellent yield [7]. Catalytic hydrogenation readily afforded **11**. This two-step sequence can be accomplished conveniently in 4 days to provide pure material in 70% yield after 1 flash column, whereas in our hands the published route across 5 steps required 7 days and 2 flash columns to provide pure **11** in 56% yield. Subsequent steps paralleled those of the published method in terms of conditions and reasonable yields, namely bromination followed by oxidation to

afford **13**. The next step involves the Grignard reaction and dehydration procedure that occurs in less than 10% yield, namely step (h) within Scheme 1. Consideration of the mechanism for this reaction led us to suspect that the first equivalent of Grignard reagent becomes spent by serving as a base to extract the acidic proton *alpha* to the carbonyl which, in turn, leads to ring opening. Our proposed mechanism is depicted in Fig. 2. Intentional production and isolation of the otherwise transient ring-opened intermediate **22** was confirmed by proton NMR (in the protonated form provided through an aqueous work-up). By encouraging its formation before adding the entire compliment of Grignard reagent followed by the dehydration and concomitant re-closure of the ring, we were able to significantly increase the yield of **14**. In the end, these steps can be accomplished within a single pot while using TLC to conveniently monitor the formation and then disappearance of **22**. While the prior process required 2 column chromatography purifications and resulted in a 10% yield, the modified procedure provides a 55% yield after crystallization without the need for a column.

Hydrolysis of ester **14** followed reported conditions. Activation of the exposed carboxyl group, as a prelude to coupling in step (g), utilized propanephosphonic acid anhydride (T3P®) rather than ethyl-dimethylaminopropyl-carbodiimide (EDC). T3P® is more environmentally friendly because it leads to easier workup with fewer side-products [8]. Yields were comparable when either reagent was tried with the same aryl amine partner. Significant differences were noted between the latter, however, with the fluorinated analog being less reactive and suffering from much lower overall yield (ca. 35%) than the des-fluoro analog (ca. 65%). This difference in reactivity is not surprising since the aryl amine becomes much less nucleophilic when the ring is substituted by an electron withdrawing fluorine atom. The accompanying improvement in overall yield for the des-fluoro analog, however, became quite fortuitous with our initial desire to deploy the latter as a coupling partner that would itself be highly amenable to chemical modification. Finally, hydrolysis of the penultimate ethyl esters, **16** and **20**, in step (h) followed the reported conditions to provide known product **4** and new product **21** in about 65% and 90% yields, respectively.

2.2. Biology

Target compounds **4** and **21** were tested for their antagonist effects on the classical, retinoid-dependent, mechanism of transcriptional activation in a promoter activity assay in MCF7 cancer cells. This assay uses all-*trans*-retinoic acid (AtRA) (10 nM) as the agonist. The degree of antagonism by the test compounds was measured by quantifying the decrease in the activity of the promoter-driven reporter luciferase. The results are depicted in Fig. 3. While the des-fluoro analog **21** is about 100 times less potent than the mono-fluoro compound **4**, it follows a stepped dose-response curve starting from 0.1 nM and is able to essentially abolish the agonist effects of AtRA at a dose of 10 nM. The latter represents a useful potency range for the future development of derivatives that can selectively attenuate the ligand-insensitive non-classical mechanism of action of RAR .

2.3. Molecular modeling

Molecular modeling studies were conducted to dock **21** into a model of the RAR 1 pocket that was derived from an X-ray structure of the protein bound with a known antagonist **23** [10]. The latter's chemical structure is depicted within Panel A of Fig. 4. Its bound arrangement is shown within Panel B. Panel C shows a super-position of both **21** and **23** docked into this pocket while Panel D shows just **21**. Both compounds orient their carboxylic acid moiety so as to be aligned for interactions with Arg-276 and Ser-287 located in the Northwest corner of the receptor pocket. Both compounds likewise take advantage of their amide N-H to form a strong (<3 Å) hydrogen bond with Ser-232 which is centrally located within the pocket. Interestingly, moving to the Eastern edge of the pocket each

compound then displays its more lipophilic aryl moiety in either a Northerly (**23**) or a Southerly (**21**) direction, perhaps the bromine on the latter sufficing to capture any hydrophobic bonding advantage gained from a Northern orientation. While intended as only a preliminary examination of the binding process, these studies further demonstrate the utility of deploying **21** as a prototype for the rational design of antagonists that can interact with RAR α within its receptor pocket.

3. Conclusions

In conclusion, we devised a modified synthesis for **4** and implemented it for the preparation of the novel des-fluoro analog **21**. In lieu of the previous reported synthesis we were able to modify the procedure by: reducing the number of steps to form the key chromene intermediate; mechanistically reevaluating the Grignard reaction so as to prompt a change in conditions that significantly enhanced this low yielding step; and, adopting a greener reagent for the amide coupling reaction. Furthermore, by synthesizing the non-fluorinated analog we were able to reduce reagent cost as well as increase reaction yields. Biological studies in MCF7 breast cancer cells showed that **4** is a potent antagonist of ligand-dependent promoter activation by RAR α at sub-nanomolar potency while **21** is effective at low nanomolar potency. The latter is within a range that is useful as a parent compound for the synthesis of appropriate analogs to target the non-classical (ligand-insensitive) mechanism of action of RAR α .

Molecular modeling suggests how the des-fluorinated analog may be able to associate at the pertinent binding domains of the RAR α receptor binding pocket in a manner that is similar to the known antagonist **23**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Selective RAR-1 antagonists developed by Teng et al. [6].

Fig. 2. Proposed mechanism for the Grignard reaction shown in step (e) of Scheme 2.

Fig. 3. Antagonist effects of **4** and **21** on the classical ligand-sensitive promoter activation by RAR in MCF7 breast cancer cells. AtRA is all trans-retinoic acid; veh is vehicle control. Error bars reflect 1 standard deviation from the mean derived from triplicate experiments.

Fig. 4.
Panel A: RAR antagonist **23**. Panel B: **23** docked within a model of RAR 1. Panel C: **21** and **23** superimposed in the RAR 1 binding pocket model. Panel D: **21** docked within a model of RAR 1.

Scheme 1.

Synthesis of **4** [6]. Reagents, conditions and [yields]: (a) MeMgCl, THF, overnight, [100%]; (b) 15% H₂SO₄, toluene, 2 h, [95%]; (c) CH₃COCl, AlCl₃, DCM, 30 min, [65%]; (d) 5% NaOCl, dioxanes, NaOH, 85 °C, 4 days [86%]; (e) H₂SO₄, EtOH, 95 °C, overnight, [95%]; (f) AcOH, Br₂, overnight, [71%]; (g) CrO₃, AcOH, Ac₂O, benzene, 0 °C, 4 h, [70%]; (h) (1) *p*-tolylMgBr, THF, -78 °C to rt; (2) PPTS, MeOH, re flux, 4 h, [<10% over 2 steps]; (i) 20% NaOH, EtOH, THF, [95%]; (j) EDC, DMAP, DCM, rt, overnight, [39%]; (k) 2 M NaOH, EtOH, [65%].

Scheme 2.

Modified synthesis of **4** and first synthesis of **21**. Reagents, conditions and [yields]: (a) 3-Picoline, anhyd. xylene, 125 °C, 70 h, [86%]; (b) 35 psi H₂, Pd/C, rt, overnight, EtOAc, [82%]; (c) AcOH, Br₂, overnight, [71%]; (d) CrO₃, AcOH, Ac₂O, benzene, 0 °C, 4 h, [70%]; (e) (1) *p*-tolylMgBr, THF, -78 °C; (2) *p*-tolylMgBr, THF, -78 °C; (3) PPTS, MeOH, re flux, 4 h, [55% over 3 steps]; (f) EtOH, THF, NaOH, 45 °C, 2 h, [95%]; (g) T3P/EtOAc, reflux, overnight, [35%, 65%]; (h) 2 M NaOH, EtOH, [65%, 90%].