# Design, Synthesis, and Structure−Activity Analysis of Isoform-Selective Retinoic Acid Receptor β Ligands

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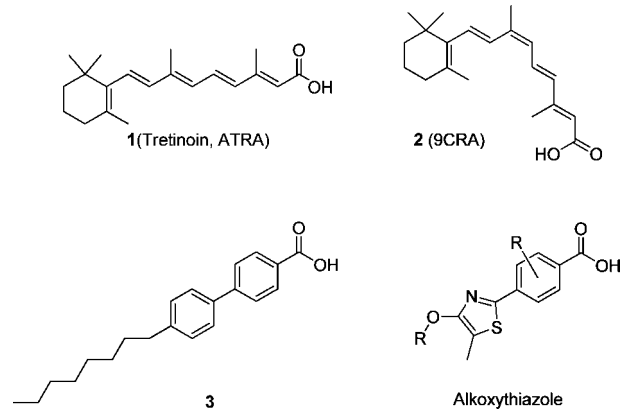
We recently discovered the isoform selective RARβ2 ligand 4′-octyl-4-biphenylcarboxylic acid (**3**, AC-55649). Although **3** is highly potent at RARβ2 and displays excellent selectivity, solubility issues make it unsuitable for drug development. Herein we describe the exploration of the SAR in a biphenyl and a phenylthiazole series of analogues of **3**. This ultimately led to the design of **28**, a novel, orally available ligand with excellent isoform selectivity for the RARβ2.

## Introduction

Retinoids, biologically active metabolites and synthetic analogues of vitamin A, e.g., tretinoin (*all-trans*-retinoic acid, ATRA,[a] **1**) and 9-*cis*-retinoic acid (9CRA, **2**) (Chart 1), have not yet reached their full potential in drug discovery mainly because of severe toxicity when administered systemically. In part, this toxicity is believed to originate from their nonselective activation of retinoic acid receptor subtypes (RARs, α, *β*, *γ*), retinoic X receptor subtypes (RXRs, α, *β*, *γ*), or both and further at the respective subtype isoforms denoted as α1, α2, *β*1−*β*5, *γ*1, and *γ*2.[1,2] Recently, it was reported that the isoforms have specific expression patterns and thus discrete pharmacology.[3] For example, the RARβ2 isoform, which is one of five discovered isoforms (*β*1−*β*5) for the RARβ subtype, has been associated with the induction of neural differentiation, motor axon outgrowth, and neural patterning displayed by ATRA (**1**).[3a,4] Consequently, the RARβ2 isoform might have a role in the treatment of neurodegenerative diseases, e.g., Alzheimer's disease and Parkinson's disease.[5] Thus, isoform selective ligands would be of interest as leads in drug discovery and as pharmacological tools in exploratory biology. Designing isoform selective RARβ ligands is, however, an intricate endeavor, as the ligand-binding domain (LBD) of RARβ subtype only differs by one residue from that of its paralogue RARα and by two residues from that of RAR*γ*.[6] Further complicating the design of RARβ2 isoform selective ligands is the fact that the structural difference between the RARβ1 and RARβ2 isoforms lies within the N-terminal domain that encompasses the ligand independent activation domain, and thus, the isoforms have identical LBDs.[5,7−11]

We recently reported on the discovery of the first agonists displaying isoform selectivity (RARβ2) using the high-throughput cell-proliferation assay, R-SAT.[12] Through a hit to lead optimization effort, the lipophilic and poorly soluble alkylbiphenyl hit **3** was transformed into a more potent alkoxythiazole series of RARβ2 agonists with retained isoform selectivity and improved physicochemical properties. Herein, we report on establishing the underlying structure−activity relationship (SAR) and the optimization of the drug properties of the initial hit compound.

**Chart 1.** Chemical structures




## Evaluation of Hits

Screening of an in-house chemical library using a cell-based functional assay, R-SAT, led to the discovery of the isoform selective RARβ2 agonist 4′-octylbiphenyl-4-carboxylic acid (**3**).[12,13] Compound **3** was originally developed in the context of liquid crystal research and consequently has low aqueous solubility (<0.001 mg/mL) and high lipophilicty (log $P = 7.9$). However, **3** displays 100-fold selectivity for RARβ2 versus the other retinoid receptors including RARβ1 and toward all of the other nuclear receptors tested.[14−17] The RARβ2 isoform is known to stimulate neurite outgrowth.[3,4] In line with this, the RARβ2 isoform selective agonist **3** induced neurite outgrowth of NTERA-2 cells, indicating that it mediates neuronal differentiation similar to that observed with retinoic acid.[13] The compound was further evaluated in a reporter gene assay, and again **3** was demonstrated to be a potent activator of RARβ2. The activity of **3** was further established in an assay of inhibition of proliferation of the breast cancer cell line MCF-7.[18−21]

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[a] Abbrevations: ATRA, *all-trans*-retinoic acid; BB, building block; cLogP, calculated log *P*; 9CRA, 9-*cis*-retinoic acid; LBD, ligand-binding domain; MW, microwave; RAR, retinoic acid receptor; R-SAT, receptor selection and amplification technology; RXR, retinoic X receptor; SAR, structure−activity relationship.

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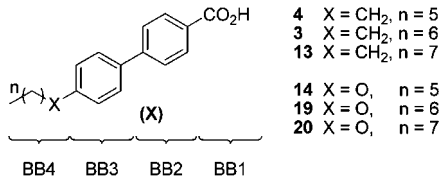
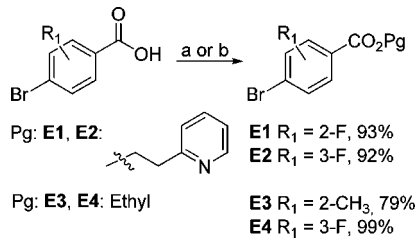



**Figure 1.** Compound **3** was divided into four building blocks BB1−BB4.

**Scheme 1.** Synthesis of Electrophiles for the Negishi Reactions[a]




[a] (a) **E1**, **E2**, and **E4**, PgOH, EDCI·HCl, DIPA, HOBt, $CH_3CN$, room temp, 16 h; (b) **E3**, EtOH, $H_2SO_4$, MW, 120 °C, 5 min.

In summary, it was concluded that the hit **3** exhibits retinoid properties and because of its unique selectivity profile, **3** was selected as a starting point for a hit-to-lead optimization effort.

## Chemistry

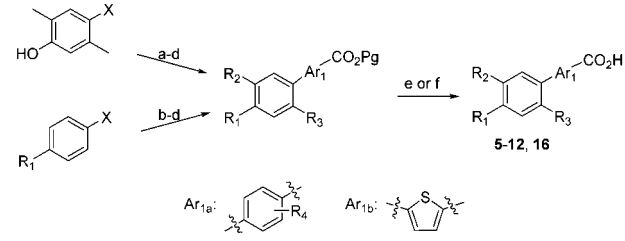
A number of synthetic strategies were developed in the hit-to-lead optimization effort. To facilitate the synthetic investigations and subsequent structure activity-relationship analysis, biphenyl **3** was divided into four building blocks BB1−BB4 (Figure 1), each of which could be modified independently one by one or several at a time. To the extent possible the reactions were conducted in parallel using a 48-well format or, as in the case of the microwave (MW) assisted reactions, in a sequence using an automated system. The libraries based on Negishi cross-coupling reactions were purified by a mass-triggered preparative LC−MS system collecting one fraction per sample (20 mL). The goal was to get 2 mg of each compound with >90% purity, enough to generate the initial activity profile. Carboxylic acids displaying interesting activities were resynthesized using the same protocol as for the library synthesis to confirm activity and in some cases to get a physicochemical profile. Around 200 compounds were synthesized with a success rate of 50%, meeting the above criteria.

To effectively explore the SAR around the biphenyl system, libraries of compounds based on Negishi cross-coupling reactions were synthesized. This provided compounds with different alkyl chains and biphenyl systems (BB2, BB3, and BB4). Electrophiles used in the Negishi reactions (**E1**−**E4**) that were not commercially available were synthesized from the corresponding carboxylic acids by two methods (Scheme 1).

The synthesis of **5**−**12** and **16** is outlined in Scheme 2. In the synthesis of **5**−**12**, a halogen−lithium exchange reaction followed by transmetalation gave the required zinc reagents. After the subsequent Negishi cross-coupling reactions, the crude products were subjected to a basic ester hydrolysis under MW irradiation conditions, giving the free acids (**5**−**12**, Scheme 2). The synthesis of **16** included an O-alkylation step prior to the Negishi reaction and the ester hydrolysis.

Another route to obtain biphenyl analogues of **3** is depicted in Scheme 3. The commercially available 4′-hydroxybiphenyl-4-carboxylic acid was transformed into the corresponding ethyl ester followed by O-alkylation with a set of different alkyl halides. The subsequent MW assisted hydrolysis gave the desired acids **15**, **17**, and **21**.

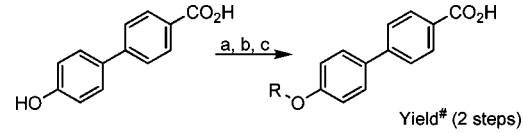
**Scheme 2.** Reaction Details for the Negishi Cross-Coupling Reactions and the Basic Hydrolysis[a]




| | | | Yield,# ester | Yield,# acid |
|---|---|---|---|---|
| **5** | $R_1 = CH_3(CH_2)_6$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 2-F | 67% | 49% |
| **6** | $R_1 = CH_3(CH_2)_6$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 3-Me | - | 3 mg* |
| **7** | $R_1 = CH_3(CH_2)_6$, | $R_2, R_3$ = H, | $Ar_{1b}$ | - | 3 mg* |
| **8** | $R_1 = CH_3(CH_2)_7$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 2-F | 83% | 85% |
| **9** | $R_1 = CH_3(CH_2)_7$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 2-Me | 73% | 87% |
| **10** | $R_1 = CH_3(CH_2)_7$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 3-F | 73% | 94% |
| **11** | $R_1 = CH_3(CH_2)_7$, | $R_2, R_3$ = H, | $Ar_{1a}$: $R_4$ = 3-Me | - | 6 mg* |
| **12** | $R_1 = CH_3(CH_2)_7$, | $R_2, R_3$ = H, | $Ar_{1b}$ | 75% | 98% |
| **16** | $R_1 = CH_3(CH_2)_3O(CH_2)_2O$, | $R_2, R_3 = CH_3$, | $Ar_{1a}$: $R_4$ = H | 84% | 4 mg* |

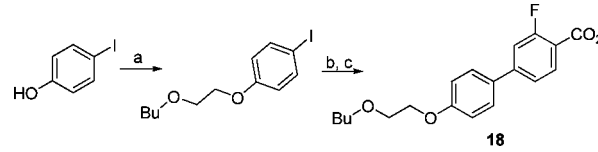
[a] (a) (i) KOH, $CH_3CN$, 60 °C, 2 h; (ii) RX, KI, 60 °C, 3 h, 34%; (b) *t*-BuLi, THF, −20 °C, 1 h; (c) $ZnBr_2$, THF, −20 °C to room temp, 0.5 h; (d) $XAr_1CO_2Pg$, $Pd_2(dba)_3$, tfp, THF/NMP 2:1, room temp, 16 h; (e) LiOH, $H_2O$, THF, 60 °C, 14 h; (f) LiOH, $H_2O$, THF, MW, 160 °C, 5 min. (#) Resynthesis. (*) Amount after purification of 20 mg of crude material by preparative LC/MS; X=halogen.

**Scheme 3.** O-Alkylation Procedure[a]




[a] (a) EtOH, $H_2SO_4$, MW, 170 °C, 1 min, 77%; (b) RX, $K_2CO_3$, KI, $CH_3CN$, MW, 180 °C, 25 min; (c) LiOH, $H_2O$, THF, MW, 160 °C, 5 min. (#) Resynthesis. (*) Yield after purification of 20 mg of crude material by preparative LC/MS; X=halogen.

**Scheme 4.** Synthesis of 4′-(2-Butoxyethoxy)-3-fluorobiphenyl-4-carboxylic Acid (**18**)[a]



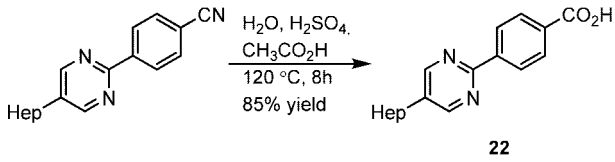

[a] (a) 1-(2-Bromoethoxy)butane, $Cs_2CO_3$, DMF, MW, 180 °C, 25 min, 95%. (b) (i) *t*-BuLi, THF, −20 °C, 1 h; (ii) $ZnBr_2$, THF, −20 °C to room temp, 0.5 h; (iii) 2-(pyridin-2-yl)ethyl 4-bromo-2-fluorobenzoate, $Pd_2(dba)_3$, tfp, THF/NMP 2:1, room temp, 16 h, 83%; (c) LiOH, $H_2O$, THF, MW, 160 °C, 5 min, 95%.

The synthesis of **18** was based on the procedures previously developed for the O-alkylations, Negishi cross-coupling reaction, and basic hydrolysis and yielded the product in 75% yield over the three steps (Scheme 4).

Conversion of commercially available heteroaromatic biaryls into analogues of **3** was further investigated. Disappointingly, only a limited number of compounds were available for the purpose; e.g., 4-(5-heptyl-2-pyrimidinyl)benzonitrile was converted into carboxylic acid **22**[21] in 85% yield by heating under acidic conditions (Scheme 5).

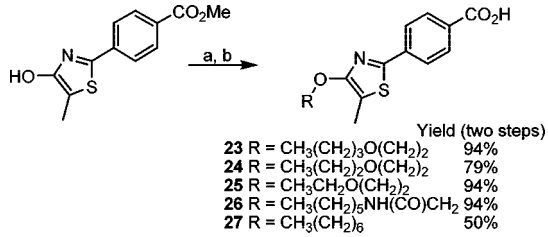
A small library of alkoxythiazoles was synthesized by a two-step protocol (Scheme 6). As previously observed for the O-alkylation of ethyl 4′-hydroxybiphenyl-4-carboxylate (Scheme

**Scheme 5.** Synthesis of 4-(5-Heptyl-2-pyrimidinyl)benzoic Acid (**22**)




**22**

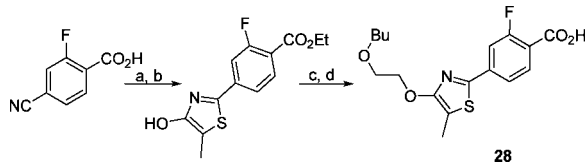
**Scheme 6.** Synthesis of Alkoxythiazoles[a]




| | Yield (two steps) |
|---|---|
| **23** R = $CH_3(CH_2)_3O(CH_2)_2$ | 94% |
| **24** R = $CH_3(CH_2)_2O(CH_2)_2$ | 79% |
| **25** R = $CH_3CH_2O(CH_2)_2$ | 94% |
| **26** R = $CH_3(CH_2)_5NH(CO)CH_2$ | 94% |
| **27** R = $CH_3(CH_2)_6$ | 50% |

[a] (a) RX, $K_2CO_3$, $CH_3CN$, MW, 180 °C, 25 min; (b) LiOH, $H_2O$, THF, MW, 160 °C, 5 min.

**Scheme 7.** Synthesis of a Fluorinated Alkoxythiazole **28**[a]




**28**

[a] (a) EtOH, EDCI·HCl, DIPEA, HOBt, $CH_3CN$, room temp, 16 h, 93%; (b) 2-mercaptopropanoic acid, pyridine, MW, 180 °C, 15 min, 67%; (c) 1-(2-bromoethoxy)butane, KI, $Cs_2CO_3$, $CH_3CN$, MW, 180 °C, 25 min; (d) LiOH, $H_2O$, THF, MW, 160 °C, 5 min, 68% (two steps).

3), the O-alkylation of methyl 4-(4-hydroxy-5-methylthiazol-2-yl)benzoate did not go to completion unless the reaction was performed under forcing conditions using MW irradiation at 180 °C for 25 min. The second step consisted of a basic hydrolysis, and overall products were obtained in good to excellent yields (Scheme 6).

The synthesis of **28**[12] (Scheme 7) was based on a literature procedure where the benzonitrile, 2-mercaptopropionic acid, and pyridine were reacted to form a hydroxythiazole under conventional heating.[22] However, we found the reaction sluggish using the reported conditions, while MW irradiation gave a cleaner reaction. Subsequently, the hydroxythiazole was O-alkylated under microwave irradiation conditions. It soon became apparent that the purification of the alkyoxythiazoles was hampered by the instability of the compounds toward chromatography; however, purification by distillation, crystallization, or both gave pure compounds. Kugelrohr distillation of the crude reaction mixture was used to remove low boiling byproducts. The residue was then subjected to an ester hydrolysis, and carboxylic acid **28** was obtained in 42% yield over four steps.[12]

## Results and Discussion

As previously reported, we discovered that biphenyl analogues substituted with 4′-alkyl carbon chains displayed activity at the RAR$\beta$2 (Table 1). From the in vitro pharmacology of these analogues, which have 4′-alkyl chain lengths ranging from two to nine carbons, it was apparent that the aliphatic chain length had a profound effect on the activity and selectivity for RAR$\beta$2. While heptyl, octyl, and nonyl analogues (**4**, **3**, and **13**, respectively) displayed full agonist activity at the RAR$\beta$2 receptor, the nonyl analogue **13** also showed significant activity at the RAR$\beta$1 isoform. Furhermore, 4′-alkoxybiphenyl analogues (**14**, **19**, and **20**; see Figure 1) were also found to display activity at the RAR$\beta$2, and with regard to both activity and selectivity, the same trend was seen as for the 4′-alkylbiphenyl analogues with six, seven, and eight carbon chains, **14**, **19**, and **20**, respectively. Consequently, we decided to direct the medicinal chemistry effort to mainly synthesizing compounds having a 4′-alkyl or 4′-alkoxy chain length mimicking the chain length of the heptyl and octyl biphenyl analogues. The main focus of the medicinal chemistry effort was on the synthesis of compounds with improved physicochemical properties while retaining the isoform selectivity.

**Table 1.** Activities at RAR$\beta$2 and RAR$\beta$1[a]

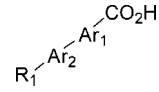
| | RAR$\beta$2 | | RAR$\beta$1 | |
|---|---|---|---|---|
| compd | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ |
| Am-580 | 100 | 7.7 ± 0.4 | 103 ± 11 | 7.3 ± 0.4 |
| **1** (ATRA) | 127 ± 10 | 8.2 ± 0.4 | 230 ± 13 | 7.6 ± 0.4 |
| **4** | 64 ± 19 | 7.6 ± 0.3 | na[b] | |
| **3** | 92 ± 24 | 6.9 ± 0.4 | 29 ± 13 | 5.7 ± 0.1 |
| **13** | 100 ± 10 | 7.8 ± 0.4 | 58 ± 11 | 6.5 ± 0.2 |
| **14** | 72 ± 44 | 7.5 ± 1.0 | na[b] | |
| **19** | 62 ± 24 | 7.2 ± 0.3 | na[b] | |
| **20** | 70 ± 21 | 7.0 ± 0.2 | 49 ± 15 | 6.0 ± 0.1 |

[a] AM-580[12] was used as reference and set to 100% Eff. $pEC_{50}$ and efficacy values are the mean values of at least three experiments ± SD. [b] na: no activity at $pEC_{50} > 5.0$. [c] Max efficacy at concentrations of <10 $\mu$M.

Attention was drawn to the low solubility of **3**. The crystal packing and the lipophilic properties of the biphenyl structure of **3** were likely to cause the low solubility of <0.001 mg/mL at physiological pH 7.4. Therefore, we speculated that the solubility would probably be improved by substituting at least one of the phenyl groups in the biphenyl system with a heteroaromatic ring. In addition, the introduction of heteroatoms, substituents, or both in the biphenyl system might alter the crystal packing through disruption of the symmetry, which also could lead to improved solubility. This hypothesis was supported by melting point changes indicative of solubility changes. Introducing a fluorine substituent in **3** gave a slight decrease of the melting point from 149−150 °C to 142−143 °C (**8**), and introduction of a heteroaryl in the form of a thiazole (**28**) gave a melting point of 109−110 °C. The modifications of the aromatic system were obtained through focused libraries based on Negishi cross-coupling reactions. In total, using all procedures described, more than 200 compounds were synthesized and tested in R-SAT for their activities at RAR$\beta$2, and the results for representative compounds are listed in Table 2.

The in vitro RAR$\beta$2 data indicated that when BB2 was a phenyl group, fluorine was the only reasonably tolerated substituent ortho to the carboxylic acid functionality (see **5** and **8**). The *o*-fluorine substituent led to increased activity with the octyl 4′-chain (compare **3** and **8**, $pEC_{50}$ of 6.9 and 7.4, respectively), whereas in contrast, the potency dropped with the heptyl 4′-chain (compare **4** and **5**, $pEC_{50}$ of 7.6 and 6.9, respectively). In addition with an *o*-methyl substituent (**9**) the activity was considerably reduced. One might hypothesize that the increase in potency in **8** could be due to the inductive properties of fluorine which would lower the $pK_a$ (calculated $pK_a$ of **3** was 4.2 and that of **8** was 3.3) of **8**, thereby stabilizing the ligand−receptor interaction by reinforcing the salt bridge. However, inspection of several RAR cocrystallized complexes shows that crystal water is situated about 2.8 Å from where the fluorine atom of **8** would be positioned.[23] This water molecule is involved in the H-bonding network, which includes the

**Table 2.** Activities of RAR$\beta$2 Selective Agonists[a]




| | | | | RARβ2 | |
|---|---|---|---|---|---|
| compd | $R_1$ | $Ar_2$ | $Ar_1$ | Eff (%) | $pEC_{50}$ |
| **4** | $CH_3(CH_2)_6$ | Ph | Ph | 64 ± 19 | 7.6 ± 0.3 |
| **5** | $CH_3(CH_2)_6$ | Ph | 2-F-$C_6H_3$ | 50 ± 18 | 6.9 ± 0.2 |
| **6** | $CH_3(CH_2)_6$ | Ph | 3-$CH_3$-$C_6H_3$ | na[b] | |
| **7** | $CH_3(CH_2)_6$ | Ph | 2,5-thiophene | na[b] | |
| **3** | $CH_3(CH_2)_7$ | Ph | Ph | 92 ± 24 | 6.9 ± 0.4 |
| **8** | $CH_3(CH_2)_7$ | Ph | 2-F-$C_6H_3$ | 114 ± 33 | 7.4 ± 0.2 |
| **9** | $CH_3(CH_2)_7$ | Ph | 2-$CH_3$-$C_6H_3$ | 84 ± 15 | 6.2 ± 0.7 |
| **10** | $CH_3(CH_2)_7$ | Ph | 3-F-$C_6H_3$ | 85 ± 23 | 7.0 ± 0.4 |
| **11** | $CH_3(CH_2)_7$ | Ph | 3-$CH_3$-$C_6H_3$ | 37 ± 22 | 7.0 ± 0.2 |
| **12** | $CH_3(CH_2)_7$ | Ph | 2,5-thiophene | na[b] | |
| **13** | $CH_3(CH_2)_8$ | Ph | Ph | 100 ± 10 | 7.8 ± 0.4 |
| **14** | $CH_3(CH_2)_5O$ | Ph | Ph | 72 ± 44 | 7.5 ± 1.0 |
| **15** | $(CH_3)_2CH(CH_2)_4O$ | Ph | Ph | 75 ± 29 | 7.2 ± 0.2 |
| **16** | $CH_3(CH_2)_3O(CH_2)_2O$ | 2,5-$(CH_3)_2$-$C_6H_2$ | Ph | 41 ± 9 | 6.6 ± 0.3 |
| **17** | $CH_3(CH_2)_3O(CH_2)_2O$ | Ph | Ph | 78 ± 19 | 7.2 ± 0.2 |
| **18** | $CH_3(CH_2)_3O(CH_2)_2O$ | Ph | 2-F-$C_6H_3$ | 108 ± 5 | 7.7 ± 0.1 |
| **19** | $CH_3(CH_2)_6O$ | Ph | Ph | 62 ± 24 | 7.2 ± 0.3 |
| **20** | $CH_3(CH_2)_7O$ | Ph | Ph | 70 ± 21 | 7.0 ± 0.2 |
| **21** | $CH_3(CH_2)_5NH(CO)CH_2O$ | Ph | Ph | na[b] | |
| **22** | $CH_3(CH_2)_6$ | pyrimidinyl | Ph | 93 ± 21 | 6.0 ± 0.2 |

[a] AM-580 was used as reference and set to 100% Eff. $pEC_{50}$ and efficacy values are the mean values of at least three experiments ± SD. [b] na: no activity at $pEC_{50} > 5.0$.

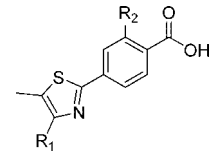
ligand's carboxylate, Ser280, and the backbone carbonyl group of Leu224 and other water molecules. The fluorine would occupy a very favorable position, which allows for H-bond formation with the water, and hence does not require its displacement. In the case of reduced potency incorporating an *o*-fluorine in the heptyl 4′-chain analogue (**5**), **4** and **5** show partial agonist efficacies of 64% and 50%, respectively. The efficacy has been shown to be dependent on the length of the 4′-chain (Table 1). Therefore, a tighter interaction in the carboxylic acid pharmacophore part would decrease an already suboptimal interaction of the 4′-chain with the receptor α-helix 12.[12] However, the presented rationale needs further clarification, preferable in the form of crystal data.

When BB2 is a phenyl, substituents in the meta-position were not as well tolerated as the *o*-fluoro substituent. This was indicated by the reduced activity of compounds with fluorine and methyl as meta-substituents (**6**, **10**, and **11**, respectively). Surprisingly, when a thienyl was introduced as BB2 (**7** and **12**), no activity was observed; in fact none of the compounds containing a heteroaromatic group as BB2 were active.[24]

With regard to modifications in BB3, it appeared that the activity at the RAR$\beta$2 was sensitive to steric hindrance as seen when $R_1$ is butoxyethoxy. This characteristic was observed for **16** having the 2,5-dimethyl substituted benzene ring (BB3), as potency and efficacy dropped, compared to the unsubstituted BB3 analogue **17**. Although **22**, pyrimidine (BB3), diplays full agonist activity, the potency was reduced 50-fold compared with **4** at RAR$\beta$2.

The unsubstituted biphenyl analogue **17** with an oxygen atom as a methylene substitute ($R_1$ = butoxyethoxy) was potent and efficacious, which shows that the receptor tolerates heteroatoms in the aliphatic chain part (BB4). In addition, branching of the terminal end of the 4′-alkyl chain (**15**) provided a potent derivative. When the activities of the heptyl (**4**−**6**) and the octyl (**3**, **8**, and **11**) chain analogues are compared, it is apparent that compounds with the octyl chain are more efficacious than the heptyl analogues.

**Table 3.** Activities of RAR$\beta$2 Selective Alkoxythiazoles[a]




| | | | RARβ2 | |
|---|---|---|---|---|
| compd | $R_1$ | $R_2$ | Eff (%) | $pEC_{50}$ |
| **23** | $CH_3(CH_2)_3O(CH_2)_2O$ | H | 99 ± 30 | 7.1 ± 0.3 |
| **24** | $CH_3(CH_2)_2O(CH_2)_2O$ | H | 100 ± 41 | 6.9 ± 0.2 |
| **25** | $CH_3CH_2O(CH_2)_2O$ | H | 78 ± 5 | 7.2 ± 0.2 |
| **26** | $CH_3(CH_2)_5NH(CO)CH_2O$ | H | 85 ± 22 | 7.4 ± 0.3 |
| **27** | $CH_3(CH_2)_6O$ | H | na[b] | |
| **28** | $CH_3(CH_2)_3O(CH_2)_2O$ | F | 106 ± 26 | 8.1 ± 0.4 |

[a] AM-580 was used as reference and set to 100% Eff. $pEC_{50}$ and efficacy values are the mean values of at least three experiments ± SD. [b] na: no activity at $pEC_{50} > 5.0$.

The encouraging RAR$\beta$2 agonist activity of **8** prompted us to design and synthesize **18** which would combine the activity enhancing property of **8** (2-F substituent) with the less lipophilic chain of **17**. Compound **18** did indeed show an increased potency compared to **8** and **17**, and the solubility in phosphate buffer at pH 7.4 was significantly higher than that for **3** (**18**, 0.02 mg/mL; **3**, <0.001 mg/mL).

However, the solubility data suggested that to synthesize analogues of biphenyl **3** with dramatically altered physicochemical properties, we had to modify the biphenyl moiety. A 5-methylthiazole unit was identified as an interesting BB3 building block, which could easily be synthesized from commercially available starting materials (Schemes 6 and 7). The in vitro activity at RAR$\beta$2 of the thiazole analogues **23**−**28** showed some interesting results (Table 3). Most surprisingly, the alkoxythiazole containing a heptyloxy chain **27** displayed no agonist activity which is in contrast to the corresponding compound **19** ($pEC_{50}$ = 7.2, Eff = 58%) in the alkoxybiphenyl

**Table 4.** Biological Activities at the RAR Subtypes and Isoforms for Selected Compounds[a]

| | RARα | | RARβ1 | | RARβ2 | | RARγ | |
|---|---|---|---|---|---|---|---|---|
| compd | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ |
| AM-580 | 100 ± 4 | 7.1 ± 0.4 | 103 ± 11 | 7.3 ± 0.3 | 100 ± 3 | 7.7 ± 0.4 | 99 ± 5 | 7.6 ± 0.6 |
| **1** (ATRA) | 121 ± 11 | 8.2 ± 0.4 | 230 ± 13 | 7.6 ± 0.4 | 127 ± 10 | 8.2 ± 0.4 | 116 ± 5 | 7.3 ± 0.3 |
| **3** | 32 ± 18 | 5.6 ± 0.0 | 29 ± 13 | 5.7 ± 0.1 | 92 ± 24 | 6.9 ± 0.4 | 31 ± 14[b] | |
| **8** | 65[c] | 5.7[c] | 54 ± 20 | 6.4 ± 0.1 | 114 ± 33 | 7.4 ± 0.2 | 84[c] | 6.8[c] |
| **15** | na[d] | | 28 ± 30[b] | | 75 ± 29 | 7.2 ± 0.2 | 31 ± 25[b] | |
| **17** | na[d] | | na[d] | | 78 ± 19 | 7.2 ± 0.2 | na[d] | |
| **18** | 55[c] | 5.8[c] | 36 ± 10[b] | | 108 ± 5 | 7.7 ± 0.1 | 37 ± 22[b] | |
| **19** | na[d] | | na[d] | | 62 ± 24 | 7.2 ± 0.3 | na[d] | |
| **23** | na[d] | | 45 ± 17 | 5.9 ± 0.2 | 99 ± 30 | 7.1 ± 0.3 | 54 ± 16[b] | |
| **26** | 35 ± 25[b] | | 42 ± 20[b] | | 85 ± 22 | 7.4 ± 0.3 | 63 ± 23 | 6.2 ± 0.3 |
| **28** | 46 ± 25 | 6.2 ± 0.3 | 52 ± 16 | 6.4 ± 0.3 | 106 ± 26 | 8.1 ± 0.4 | 48 ± 23 | 6.3 ± 0.6 |

[a] AM-580[12] was used as reference and set to 100% Eff. $pEC_{50}$ and efficacy values are the mean values of at least three experiments ± SD. [b] Max efficacy at concentrations of <10 μM. [c] Result of one experiment. [d] na: no activity at $pEC_{50} > 5.0$.

series. Likewise the alkoxythiazole analogue of **15** showed 100 times less activity compared with **15** at the RARβ2.[24] Beneficially, by use of the parallel synthesis strategy, a number of alkoxythiazole analogues had been prepared simultaneously; otherwise, the negative result with the obvious analogue **27** would probably have discouraged us from further work on this series. In contrast, while the biphenyl **21** showed no activity, the thiazole analogue **26** is quite potent and selective at RARβ2. Although the biphenyls and the thiazoles look similar, it is apparent from the molecular modeling that the 4′-chains of the two scaffolds occupy different areas in the receptor but converge in the interaction with α-helix 12;[12] therefore, it is not given that the 4′-chains are interchangeable between the two scaffolds. Four of the alkoxythiazoles **23**−**26** displayed comparable activity to biphenyl **3**. In these compounds the third $sp^3$ hybridized methylene in the alkyl chain of **27** has been exchanged with a heteroatom (O in **23**−**25** and NH in **26**) which slightly alters the preferred low energy conformations of the 4′-chain. This again clearly demonstrates the influence of this part of the molecule on the activity. These compounds showed that a substantial reduction of the lipophilicity from that of **3** (cLogP = 7.9) could be achieved (**23**, **24**, and **26** had cLogP values of 5.0, 4.5, and 4.8, respectively). This prompted us to further focus on the alkoxythiazoles; however, before we embarked on the synthesis of additional alkoxythiazole derivatives, the selectivity profile of the most active compounds was investigated.

The compounds were tested on all of the RARs revealing a good to excellent selectivity for the RARβ2 over RARβ1, RARα, and RARγ (Table 4). The biphenyl analogues **8**, **15**, **17**, and **18** were equally selective as **3**. In addition, alkoxythiazoles **23** and **26** showed good selectivity profiles at all the tested RARs. The selectivity profile of butoxyethoxy derivative **23** was slightly superior to that of acetamide **26**. On the basis of these findings and the indication that a fluorine ortho to the carboxylic acid on the phenyl ring in BB2 would enhance the activity (as observed for **8** and **18**), we synthesized **28** (Scheme 7). When tested, the *o*-fluoro compound **28** displayed a significantly improved activity at RARβ2 (Eff = 106%, $pEC_{50}$ = 8.1) compared to all synthesized alkoxythiazole analogues. The increase in potency of *o*-fluoro substituted compounds was more pronounced in the thiazole series as compared to the phenyl series, about 10 and 3 times, respectively, also correlating with the activity of the 2-F analogue of **24** ($pEC_{50}$ = 7.8).[24] The selectivity of **28** was also tested, and as expected, it was comparable to that of **3**, [12] both with regard to the RAR and RXR subtypes.[12]

The microsomal clearance and solubility of **3**, **8**, **18**, and **28** were measured (Table 5). The in vitro clearance of **28** in rat and human microsomes was determined to be 10 and 36 μL/(min·mg), respectively. In vitro clearance values of less than 75 and 50 μL/(min·mg) represent moderate to low clearance compounds (less than 30% liver blood flow) in rat and human, respectively. The solubility in phosphate buffer at pH 7.4 was increased almost 250 times from 0.02 mg/mL for biphenyl **18** to 4.8 mg/mL for **28** by changing the BB3 from a phenyl to a thiazole moiety (Table 5). Compound **28** showed good oral bioavailability (*F*) of 52% with an in vivo clearance of 41 mL/(min·kg) in rats.

**Table 5.** Metabolic Stability and Solubility of Selected Compounds

| compd | $Cl_{Int,human}$[a] (μL/(min·mg)) | $Cl_{Int,rat}$[a] (μL/(min·mg)) | solubility[b] (mg/mL) | $cLogD_{7.4}$ | cLogP |
|---|---|---|---|---|---|
| **3** | 5 | 5 | <0.001 | 6.1 (4.5)[c] | 7.7 (7.9)[c] |
| **18** | 8 | 0 | 0.02 | 0.8 | 4.6 |
| **28** | 36 | 10 | 4.8 | 0.7 (1.4)[c] | 5.2 (5.1)[c] |

[a] The hepatic extraction was calculated without taking the fraction of unbound drug in plasma into account. [b] The solubility was measured in phosphate buffer, pH 7.4. [c] Experimental value.

## Conclusion

A synthetic strategy involving parallel synthesis was successfully applied to rapidly establish a SAR starting from a unique isoform-selective RARβ2 agonist **3**. An alkoxythiazole series that retained the selectivity profile with improved physicochemical properties was designed and synthesized. An orally available potent RARβ2 agonist **28** was ultimately designed, which exhibits promising druglike properties and represents an excellent starting point for further lead optimization efforts. In addition, the discovered retinoid ligands are potential research tools that may shed light on the functions and therapeutic potential of the RARβ2 isoform.

## Experimental Section

Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Spectroscopic data were recorded on a Varian XL 400 MHz spectrometer. Purities were determined by liquid chromatography/mass spectrometry performed on a Waters/Micromass ZQ2000 LC/MS instrument consisting of a ZQ single quadropole mass spectrometer equipped with an electrospray ionization interface and on a Waters Alliance HT with a 2795 separation module and 996 photodiode array detector (190−450nm). Elemental analyses were done in the microanalytical laboraties of Fakultät für Chemie, Universität Wien, Austria, and all compounds had a purity of >95%. High-resolution mass spectrometry was performed at the University of Lund, Sweden.

**General Negishi Cross-Coupling Method: 2-(2-Pyridinyl)ethyl 4′-(2-Butoxyethoxy)-3-fluorobiphenyl-4-carboxylate.** In a dry and argon flushed vial 1-(2-butoxyethoxy)-4-iodobenzene (1.5 mmol, 480 mg) was taken up in dry THF (2.5 mL). The vial was cooled

to −20 °C before slow addition of *t*-BuLi in hexane (1.6 M, 3.00 mmol, 1.88 mL). After 1 h at −20 °C $ZnBr_2$ (1.5 M, 1.65 mmol, 1.10 mL) was added and the cooling stopped. In another dry and argon flushed vial $Pd_2dba_3$ (0.075 mmol, 70 mg) and tfp (1.0 mmol, 70 mg) were taken up in dry NMP (2.5 mL). The activated catalyst was added to the zinc reagent via a syringe. Finally, 2-(2-pyridinyl)ethyl 4-bromo-2-fluorobenzoate (1.0 mmol, 324 mg) dissolved in dry THF (2.5 mL) was added to the mixture. The mixture was left at room temperature for 16 h, then quenched with $NH_4Cl$ (aq) and poured onto a HM-N SPE column, then extracted with EtOAc. The extract was concentrated in vacuo. Purification was by flash chromatography (heptane/EtOAc 4:1 to 3:1). Yield: 370 mg (84%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.60−8.54 (m, 1H), 7.94−7.85 (m, 1H), 7.68−7.58 (m, 1H), 7.58−7.47 (m, 2H), 7.40−7.22 (m, 3H), 7.21−7.12 (m, 2H), 4.73 (t, $J$ = 6.7 Hz, 2H), 4.17 (t, $J$ = 4.2 Hz, 2H), 3.82 (t, $J$ = 4.7 Hz, 2H), 3.56 (t, $J$ = 6.6 Hz, 2H), 3.28 (t, $J$ = 6.4 Hz, 2H), 1.70−1.56 (m, 2H), 1.49−1.32 (m, 2H), 0.94 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 165.4 (d, $J_{CF}$ = 257.0 Hz), 161.2, 159.3, 149.9 (2C), 149.0 (d, $J_{CF}$ = 9.0 Hz), 138.7 (2C), 133.5, 132.1 (d, $J_{CF}$ = 1.9 Hz), 129.4, 125.4, 123.4, 122.9 (d, $J_{CF}$ = 3.3 Hz), 117.2 (d, $J_{CF}$ = 9.9 Hz), 116.2, 115.3 (d, $J_{CF}$ = 23.3 Hz), 72.2, 70.3, 68.7, 65.4, 37.9, 32.8, 20.3, 14.2. Anal. Calcd for $C_{26}H_{28}FNO_4$: C, 71.4; H, 6.5; N, 3.2. Found: C, 70.8; H, 6.4; N, 3.1.

**4′-(2-Butoxyethoxy)-3-fluoro[1,1′-biphenyl]-4-carboxylic Acid (18).** A MW vial was charged with 2-(2-pyridinyl)ethyl 4′-(2-butoxyethoxy)-3-fluorobiphenyl-4-carboxylate (0.3 mmol, 131 mg), THF (1.5 mL), and water (0.8 mL). Then $LiOH \cdot H_2O$ (0.9 mmol, 38 mg) was added and the mixture was microwave irradiated at 160 °C for 5 min. After cooling, the mixture was transferred to a separation funnel with EtOAc and was washed with NaOH (1 M). The aqueous phase was acidified with HCl (1 M) and extracted with EtOAc. The organic phase was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo, yielding the desired product. Yield: 95 mg (95%). Mp 122.7−123.3 °C. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 10.59 (s, 1H), 8.10−7.99 (m, 1H), 7.60−7.49 (m, 2H), 7.46−7.38 (m, 1H), 7.38−7.28 (m, 1H), 7.07−6.96 (m, 2H), 4.19 (t, $J$ = 4.3 Hz, 2H), 3.84 (t, $J$ = 4.6 Hz, 2H), 3.58 (t, $J$ = 6.7 Hz, 2H), 1.71−1.56 (m, 2H), 1.50−1.33 (m, 2H), 0.95 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.3 (d, $J_{CF}$ = 3.5 Hz), 163.0 (d, $J_{CF}$ = 261.0 Hz), 159.7, 148.4 (d, $J_{CF}$ = 9.2 Hz), 133.2, 130.9 (d, $J_{CF}$ = 1.6 Hz), 128.3 (2C), 122.0 (d, $J_{CF}$ = 3.3 Hz), 115.2 (2C), 115.2 (d, $J_{CF}$ = 9.2 Hz), 114.7 (d, $J_{CF}$ = 23.1 Hz), 71.6, 69.2, 67.7, 31.8, 19.5, 14.2. Anal. Calcd for $C_{19}H_{21}FO_4$: C, 68.7; H, 6.4. Found: C, 68.4; H, 6.4.

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**Supporting Information Available:** General experimental methods, biological assays, the synthesis of **5**−**27**, their analytical data, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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