Bioorganic & Medicinal Chemistry 21 (2013) 869–873

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of derivatives of the keto-pyrrolyl-difluorophenol scaffold: Some structural aspects for aldose reductase inhibitory activity and selectivity

Eleni Kotsampasakou, Vassilis J. Demopoulos *

Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 31 October 2012
Revised 11 December 2012
Accepted 13 December 2012
Available online 20 December 2012

Keywords:
ARI
Aldose reductase
Aldehyde reductase
Diabetes mellitus
Long-term diabetes complications
Friedel–Crafts acylation reaction

ABSTRACT

Seven novel ARIs (**3a–c**, **4a–c** and **5**) were synthesized with the implementation of an optimized and, partially, selective synthetic procedure, via a Friedel–Crafts acylation reaction. The synthesized ARIs have values of IC_{50}^{ALR2} ranging from 0.19 μM (in case of compound **3b**) to 2.3 μM (in case of compound **4a**), while the values of selectivity index towards ALR1 range from 1 (in case of compound **3b**) to 238 (in case of compound **3a**). Finally, we found out that the presence of an additional (secondary) aromatic area is not a prerequisite feature for ARI activity.

1. Introduction

Diabetes mellitus is a complex metabolic disorder that is characterized by abnormal metabolism of carbohydrates, proteins and lipids, as a result of lack of insulin production or insulin resistance.[1] During the 20th century diabetes has acquired an epidemic prevalence and from the beginning of the 21st century it is developing to a pandemic phenomenon. In 2011, 366 million people were affected from the disease, while by 2030 the number is estimated to reach 522 million patients, with frequency of prevalence 7.7%.[2] In addition, diabetes is not exclusively a disease of the developed world, since it appears in very high rates in some developing countries of Asia and Latin America.[3] Diabetes is closely associated with high morbidity and mortality due to its chronic complications that develop over the years because of insufficient glycemic control.[4] Among these chronic complications are angiopathy and cardiovascular diseases, neuropathy, nephropathy, retinopathy and cataract.[5]

The enzyme that is considered responsible for diabetes' chronic complications is aldose reductase (ALR2, AR, AKR1B1, EC 1.1.1.21), a cytosolic enzyme, member of the aldo-ketoreductase superfamily, which under normal conditions is responsible for a series of physiologic functions.[6] ALR2 is the first enzyme of the polyol pathway, which converts glucose into sorbitol using NADPH as a co-factor. Sorbitol is slowly converted into fructose by the second enzyme of the pathway, sorbitol dehydrogenase (SDH), which uses NAD^+ as a co-factor.[7]

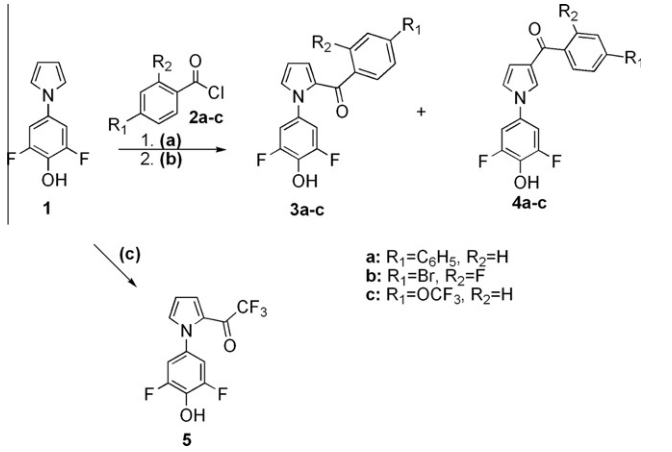
ALR2 has a low affinity to glucose and the polyol pathway is involved only to a minor degree in the metabolism of glucose. However, in case of hyperglycemia, glucose is rapidly converted by ALR2 to sorbitol, which cannot easily cross membranes and accumulates into cells, causing cell and tissue disfunction.[8,9] Additionally, the exhaustion of NADPH and the disturbance of NADH/NAD^+ ratio can cause oxidative stress, which is also related to diabetic chronic complications.[1,9–11] Furthermore, fructose, via oxidation, gives intermediates that can lead to advanced glycation end products (AGEs), which are also considered responsible for diabetes' chronic complications.[10,12] Finally, recent studies have shown that ALR2 is related to ischemic and inflammatory pathologic conditions,[5,13,14] as well as to some particular types of cancer.[5,15]

All the above evidence point out that ALR2 is an important pharmacological target and its inhibition could be the key for the treatment of many pathological conditions. Of course, this means that there is a continuously rising need for new and effective ALR2 inhibitors (ARIs), since today only epalrestat is on the market and only in Japan. Even though many active ARIs have been

Abbreviations: ARI(s), aldose reductase inhibitor(s); ALR2/AR, aldose reductase; ALR1, aldehyde reductase; SDH, sorbitol dehydrogenase; AGEs, advanced glycation end products; DBN, 1,5-diazabicyclo[4.3.0]non-5-ene; SI, selectivity index; TLC, thin layer chromatography; DMSO, dimethyl sulfoxide.

* Corresponding author. Tel.: +30 2310 997626; fax: +30 2310 997852.
E-mail address: vdem@pharm.auth.gr (V.J. Demopoulos).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.12.015



Scheme 1. Synthesis of target compounds **3a–c**, **4a–c** and **5** from phenol **1**. Reagents: (a) **2a–c**/$AlCl_3$ (1:1.07 or 1:0.54), $ClCH_2CH_2Cl$; (b) H_2O/NaOH, 1,4-dioxane; (c) $(CF_3CO)_2O$, $ClCH_2CH_2Cl$.

synthesized over the years, most of them are derivatives of the carboxylic acid or the hydantoin moiety, which have proved to have either poor bioavailability or serious adverse effects.[16]

In order to overcome the problematic pharmacokinetic profile of carboxylic acids, novel bioisosteric approaches have been implemented, as for example with the trifluoromethyl ketone substituted ARIs.[17] In the present work, and in continuation of searching for new ARI chemotypes,[4] we have prepared and in vitro tested the keto-pyrrolyl-difluorophenol derivatives **3a–c**, **4a–c** and **5** (Scheme 1). In these molecules 2,6-difluorophenol was used as a non-classical bioisosteric replacement of the acetic acid moiety,[18] while the pyrrole ring was substituted by different keto-groups.

The biphenyl ring system, present in compounds **3a** and **4a**, is considered as a privileged scaffold, providing affinity, due to its rigid aromatic structure, which adapts well to protein hydrophobic pockets, where π-stacking with phenylalanine and tyrosine are commonly observed.[19]

The 4-bromo-2-fluoro-phenyl moiety, present in compounds **3b** and **4b**, has been shown to be correlated with ALR2 inhibition, since known ARIs contain this group (e.g., minalrestat[6] and ranirestat[20]). Our intention was to introduce it as a 4-bromo-2-fluorobenzoyl substructure, which could be easily inserted into the pyrrole ring via a Friedel–Crafts acylation reaction.

The trifluoromethoxy moiety, present in compounds **3c** and **4c**, is considered not just a bioisosteric replacement of a methoxy group, since it attains vertical spatial disposition, it has a special electronic distribution, and the C–O bond acquires a character between a single and a double. Overall, it has a unique hydrogen bonding ability, critical to putative ligand–enzyme interactions.[21,22]

The trifluoro-acetyl group, present in compound **5**, was selected in an effort to minimize lipophilicity, while enhancing metabolic stability and retaining the capacity of hydrogen bond formation.[23]

2. Results and discussion

2.1. Chemistry

In a previous study[4] in our laboratory we have utilized Friedel–Crafts aroylations on 2,6-difluoro-4-(1*H*-pyrrol-1-yl)phenyl arylcarboxylates in order to prepare C-α or C-β substituted keto-pyrrolyl-difluorophenol derivatives. In the present work we targeted to control the selectivity of the Friedel–Crafts aroylation on the phenol **1**, without esterifying it. Thus, we systematically altered (Scheme 1) the equivalents (in respect to **1**) of $AlCl_3$, the amount of solvent employed, and the reaction's time. In all cases, we utilized two equivalents (in respect to **1**) of the acylating chlorides, followed by a final hydrolytic step. The latter was based on the report[24] that a phenol could be esterified during the course of an $AlCl_3$ catalyzed acylation reaction. Although, a high degree of selectivity for the pyrrolyl C-β regioisomers **4a–c** was attained (**2a–2c**/$AlCl_3$ 1:1.07), the selectivity for the respective C-α compounds **3a–c** was poor (**2a–2c**/$AlCl_3$ 1:0.54). Furthermore, in the case of preparing **3b–c** it proved to be a sluggish reaction and additional reagent/catalyst was added, with a prolongation of the reactions' time. The C-α selectivity was not improved by replacing $AlCl_3$ with $BF_3{\cdot}Et_2O$, while the utilization of the DBN catalyzed Friedel–Crafts pyrrolyl C-α acylation methodology[25] to **1** was unsuccessful. Finally, an attempt to implement on **1** the reported[26] C-α aroylation procedure of *N*-tosylpyrroles by carboxylic acids and trifluoracetic anhydride, led to the exclusive formation of **5**. This compound, though, was conveniently prepared by reacting **1** with trifluoroacetic anhydride in refluxing 1,2-dichloroethane.

2.2. In vitro results

2.2.1. ALR2 inhibitory activity

The evaluation of the ALR2 inhibitory activity was conducted on partially purified rat lenses ALR2. Human ALR2 and rat ALR2 exhibit 85% sequence homology, while the catalytic active site of both the enzymes is considered identical.[4] ALR2 inhibitory activity is expressed as IC_{50} values (Table 1). All the compounds exhibited submicromolar inhibitory activity, except for **4a**, which exhibited micromolar inhibitory activity.

According to Table 1, it was found that the most active compound is **3b** with an IC_{50} of 0.19 μM. Compound **4b** also shows quite strong ALR2 inhibition, which justifies our initial idea of inserting the 4-bromo-2-fluoro-benzoyl group into the pyrrole ring. Compounds **3a** and **3c** also show a quite strong inhibitory activity, which implies that, both the biphenyl and the trifluoromethoxy moieties are important for ARI activity, even though they proved weaker, than in case of 4-bromo-2-fluorobenzoyl group. Surprisingly, the privileged scaffold of biphenyl in case of C-β substitution at the pyrrole ring attributed much lower inhibitory activity, than in case of C-α substitution. Generally, in this series of compounds, C-α substitution at the pyrrole ring gives more active ALR2 inhibitors. Finally, the strong inhibitory potency of compound **5** indicates that the presence of an additional (secondary) aromatic area is not a prerequisite feature for ARI activity, as it was believed before.[27]

2.2.2. ALR1 inhibitory activity

Aldehyde reductase (EC 1.1.1.2, ALR1, AKR1A1) is a cytosolic enzyme, member of the aldoketoreductase superfamily, like aldose reductase (ALR2). It also catalyzes several physiological functions, among them, detoxification of toxic aldehydes.[28–30] Furthermore, the two enzymes share a high level of sequence (~65%) and structural homology,[6] as well as physical and chemical properties.[31] This has as a result the increased tendency of many substrates or ligands to bind with both ALR1 and ALR2.[28,32] The inhibition of both the enzymes is thought to lead to toxic phenomena,[6] due to the resulting insufficient detoxification capacity of the living cell. However, it has been indicated that there are other available enzymes for the aldehydes' detoxification, in case of parallel inhibition of ALR2 and ALR1,[7] but this matter needs further investigation.

In the present study, ALR1 inhibitory activity was evaluated on partially purified ALR1 obtained from rat's kidney. It is expressed as IC_{50} values accompanied with the selectivity index ($SI = IC_{50}^{ALR1}/IC_{50}^{ALR2}$) and the % inhibition of ALR1 at 10^{-5} M (Table 1). From the obtained results we can see that almost all of our compounds present a relatively high selectivity, except for

Table 1
The IC_{50} values for ALR2 and ALR1, the% inhibition of ALR1 at 10^{-5} M and the selectivity index for compounds **3a–c**, **4a–c** and **5**

Compound	IC_{50}^{ALR2} ± SEM[a] (μM)	IC_{50}^{ALR1} ± SEM[a] (μM)	% Inhibition ALR1 at 10^{-5} M	Selectivity index[b] (SI)
3a	0.51 ± 0.030	121.4[c]	5.85	238
4a	2.3 ± 0.026	5.63 ± 0.026	72.85	1.87
3b	0.19 ± 0.003	0.19 ± 0.0014	96.8	1
4b	0.40 ± 0.009	4.31 ± 0.305	75.3	14.08
3c	0.52 ± 0.005	16.2[d]	30.85	31.15
4c	0.75 ± 0.003	13.14[d]	38.05	17.52
5	0.93 ± 0.012	18.87[d]	26.5	20.3
Sorbinil	0.25 ± 0.01	—	—	—
Valproic acid	—	56.1 ± 1.56	—	—

[a] $n = 3$.
[b] Defined as $IC_{50}^{ALR1}/IC_{50}^{ALR2}$.
[c] Extrapolated from the % inhibition at 5×10^{-5} M (=20.6).
[d] Extrapolated from the % inhibition at 10^{-5} M.

3b and **4a**, which have none and low selectivity respective. The most selective compound proved to be **3a**, which implies that the biphenyl group attributes selectivity, but only in case of C-α substitution at the pyrrole ring as the C-β substituted analogue (**4a**) showed very low selectivity.

3. Conclusions

In the present study seven novel ARIs (**3a–c**, **4a–c** and **5**) were synthesized with the implementation of an optimized and, partially, selective synthetic procedure. The synthesized ARIs were biologically evaluated for ALR2, as well as ALR1 inhibitory activities and SIs were calculated. The estimated values of IC_{50}^{ALR2} ranged from 0.19 μM (in case of compound **3b**) to 2.3 μM (in case of compound **4a**), while the calculated values of SI ranged from 1 (in case of compound **3b**) to 238 (in case of compound **3a**). From the used substituents, the 4-bromo-2-fluorobenzoyl moiety proved to attribute the strongest ALR2 inhibitory activity, since compounds **3b** (IC_{50}^{ALR2} = 0.19 μM) and **4b** (IC_{50}^{ALR2} = 0.40 μM) are the most active ARIs synthesized in this study. In case of SI, we cannot derive to a definite conclusion, since the 4-bromo-2-fluorobenzoyl moiety in case of C-α substitution at the pyrrole ring provides no selectivity, while in case of C-β substitution the SI is good (14.1). On the contrary, the 4-biphenylcarbonyl moiety provides a very high SI in case of C-α substitution (238), while in case of C-β substitution the SI is low (1.87). Finally, we found out that the presence of an additional (secondary) aromatic area is not a prerequisite feature for ARI activity, since compound **5** has an IC_{50}^{ALR2} value of 0.93 μM.

4. Experimental section

4.1. General notes

All reagents were purchased from Sigma–Aldrich and used without further purification, except for the solvents for flash chromatography and recrystallization, which were distilled. [1,1′-Biphenyl]-4-carbonyl chloride (**2a**) was obtained from Aldrich (product number: 161144) and 4-(trifluoromethoxy)benzoyl chloride (**2c**) was obtained from Fluka (product number: 91754). IR spectra were taken with a Perkin–Elmer FT–IR System Spectrum BX. ^{1}H NMR spectra were recorded on a Bruker AM 300 at 300 MHz, using TMS as the internal standard. UV–vis measurements were taken with UV/vis Spectrometer Lamda 20. Melting points are uncorrected and were determined in open glass capillaries using a Mel-Temp II apparatus. Flash column chromatography was carried out with Merck Silica Gel 9385. TLC was run with Fluka Silica gel/TLC-cards. Elemental analyses were performed by Perkin–Elmer 2400 CHN analyzer (in the Department of Organic Chemistry, School of Chemistry, Aristotle University of Thessaloniki).

4.2. Chemistry

4.2.1. Preparation of 4-bromo-2-fluorobenzoyl chloride (2b)

In a mixture of 4-bromo-2-fluorobenzoic acid (944 mg, 4.31 mmol) in CH_2Cl_2 (3.8 mL), oxalyl chloride (1095 mg, 8.62 mmol) in CH_2Cl_2 (3.7 mL) was added and the mixture was stirred at room temperature and under a nitrogen atmosphere for 1 h. Then DMF (14 μL) was added to the mixture and stirring was continued at room temperature, under a nitrogen atmosphere for 24 h. The mixture was evaporated under reduced pressure and the product [IR (neat) 1773, 1734 cm^{-1}] was used as an acylating agent in the following reactions without further purification, considering it being 80% pure.

4.2.2. General procedure for selective Friedel–Crafts aroylation of phenol 1 with $AlCl_3$ as Lewis acid in C-β position of the pyrrole ring. Preparation of (1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)(aroyl)methanones (4a–c)

To a mixture of anhydrous $AlCl_3$ (164 mg, 1.231 mmol) in ethylene dichloride (10 mL) the appropriate arroyl chloride **2a–c** (1.149 mmol) was added and stirred at room temperature and under a nitrogen atmosphere. After 10 min, a solution of the 2, 6-difluoro-4-(1*H*-pyrrol-1-yl)phenol (**1**)[18] (100 mg, 0.5128 mmol) in ethylene dichloride (5 mL) was added and the resulting mixture was stirred for 48 h under a nitrogen atmosphere. The reaction was quenched with ice and water and the product was extracted with $CHCl_3$ (+10% EtOH) (3 × 20 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in 1,4-dioxane (14 mL) and to this a solution of aqueous 5% NaOH solution (14 mL) was added. The mixture was stirred at room temperature and under nitrogen atmosphere for 24 h.

Then, the mixture was concentrated to half of its volume, water (30 mL) was added and the mixture was acidified under cooling (ice bath) with aqueous solution of HCl (10%). Afterwards, the mixture was extracted with EtOAc (3 × 20 mL) and the combined organic extracts were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in a mixture of petroleum ether/EtOAc (1:1 in case of **4a–b** and 2:1 in case of **4c**) and was washed with a phosphate buffer (Na_2HPO_4–KH_2PO_4) pH 8. After it was ascertained with TLC that the acid of the used aroyl chloride has been fully removed, the combined organic extracts were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and

evaporated under reduced pressure. The residue was flash chromatographed with a mixture of petroleum ether/EtOAc 6:1. Analytical samples were obtained by recrystallization from CH_2Cl_2/petroleum ether (**4a** and **4c**), or CH_2Cl_2 (**4b**).

4.2.2.1. [1,1′-Biphenyl]-4-yl(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)methanone (4a). Prepared according to the above general procedure (104 mg, 54%). Mp 205–207 °C; IR (KBr) 3064, 1592 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.31–7.37 (m, 1H), 7.40–7.45 (m, 2H), 7.48–7.50 (m, 1H), 7.59–7.68 (m, 4H), 7.9 (d, J = 8.3 Hz, 2H), 9.55 (br s, 1H). Anal. Calcd for $C_{23}H_{15}F_2NO_2$: C, 73.59, H, 4.03, N, 3.73. Found: C, 73.39, H, 4.09, N, 3.63.

4.2.2.2. (4-Bromo-2-fluorophenyl)(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)methanone (4b). Prepared according to the above general procedure (39 mg, 49%). Mp 206–207 °C; IR (KBr) 3165, 1620 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.70–6.80 (m, 1H), 6.87–7.04 (m, 3H), 7.32–7.47 (m, 4H), 9.34 (br s, 1H). Anal. Calcd for $C_{17}H_9BrF_3NO_2$: C, 51.54, H, 2.29, N, 3.54. Found: C, 51.14, H, 2.34, N, 3.43.

4.2.2.3. (1-(3,5-Difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)(4-(trifluoromethoxy) phenyl)methanone (4c). Prepared according to the above general procedure (109 mg, 55%). Mp 133–134 °C; IR (KBr) 3143, 1603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.04 (br s, 1H), 6.83–6.89 (m, 1H), 7.00–7.10 (m, 3H), 7.35 (d, J = 8.4 Hz, 2H), 7.50–7.56 (m, 1H), 7.95 (d, J = 8.4 Hz, 2H). Anal. Calcd for $C_{18}H_{10}F_5NO_3$. 0.2 CH_2Cl_2: C, 54.61, H, 2.62, N, 3.50. Found: C, 54.73, H, 2.55, N, 3.47.

4.2.3. Friedel–Crafts arroylation of phenol 1 with $AlCl_3$ as Lewis acid in positions C-α and C-β of the pyrrole ring. Preparation of (1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-yl)(aroyl)methanones 3a and 4a

To a mixture of anhydrous $AlCl_3$ (82 mg, 0.615 mmol) in ethylene dichloride (5 mL), biphenyl]-4-carbonyl chloride (**2a**) (249 mg, 1.149 mmol) was added and stirred at room temperature and under a nitrogen atmosphere. After 10 min, a solution of the 2,6-difluoro-4-(1*H*-pyrrol-1-yl)phenol (**1**)[18] (100 mg, 0.5128 mmol) in ethylene dichloride (2.5 mL) was added and the resulting mixture was stirred for 11 h under a nitrogen atmosphere. Afterwards, the mixture was processed as in Section 4.2.2. The residue was flash chromatographed with a mixture of petroleum ether/EtOAc 9:1–7:1. Analytical samples were obtained by recrystallization from CH_2Cl_2/petroleum ether.

4.2.3.1. [1,1′-Biphenyl]-4-yl(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-2-yl)methanone (3a). Prepared according to the above procedure (65 mg, 34%). Mp 214–216 °C; IR (KBr) 3132, 1597 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.24–6.33 (m, 1H), 6.80–6.93 (m, 3H), 6.97–7.05 (m, 1H), 7.32–7.45 (m, 3H), 7.56–7.68 (m, 4H), 7.9 (d, J = 8.1 Hz, 2H), 9.31 (br s, 1H). Anal. Calcd for $C_{23}H_{15}F_2NO_2$: C, 73.59, H, 4.03, N, 3.73. Found: C, 73.04, H, 4.11, N, 3.64.

4.2.3.2. [1,1′-Biphenyl]-4-yl(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)methanone (4a). Prepared according to the above procedure (39 mg, 20%).

4.2.4. Friedel–Crafts aroylation of phenol 1 with $AlCl_3$ as Lewis acid in positions C-α and C-β of the pyrrole ring. Preparation of (1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-yl)(aroyl)methanones 3b and 4b

To a solution of crude 4-bromo-2-fluorobenzoyl chloride **(2b)** (estimated as being 546 mg, 2.298 mmol, Section 4.2.1.) in ethylene dichloride (10 mL), anhydrous $AlCl_3$ (164 mg, 1.231 mmol) was added and the mixture was stirred at room temperature and under a nitrogen atmosphere. After 10 min, a solution of the 2,6-difluoro-4-(1*H*-pyrrol-1-yl)phenol (**1**)[17] (200 mg, 1.024 mmol) in ethylene dichloride (5 mL) was added and the resulted mixture was stirred for 24 h under a nitrogen atmosphere. At this point, TLC analysis showed that the reaction was not completed. Thus, a solution of crude chloride **2b**, (estimated as being 273 mg, 1.149 mmol) in ethylene dichloride (7.5 mL) was added, followed by anhydrous $AlCl_3$ (82 mg, 0.615 mmol). The resulting mixture was stirred at room temperature for another 24 h under a nitrogen atmosphere. Afterwards, the mixture was processed as in Section 4.2.2. The residue was flash chromatographed with a mixture of petroleum ether/EtOAc 10:1–8:1. Analytical samples were obtained by recrystallization from CH_2Cl_2/petroleum ether in case of **3b** and from CH_2Cl_2 in case of **4b**.

4.2.4.1. (4-Bromo-2-fluorophenyl)(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-2-yl)methanone (3b). Prepared according to the above procedure (56 mg, 14%). Mp 184–186 °C; IR (KBr) 3087, 1600 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.19–6.33 (m, 1H), 6.66–6.78 (m, 1H), 6.80–6.95 (m, 2H), 6.96–7.06 (m, 1H), 7.25–7.42 (m, 3H), 9.43 (br s, 1H). Anal. Calcd for $C_{17}H_9BrF_3NO_2$: C, 51.54, H, 2.29, N, 3.54. Found: C, 51.18, H, 2.37, N, 3.43.

4.2.4.2. (4-Bromo-2-fluorophenyl)(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)methanone (4b). Prepared according to the above procedure (64 mg, 16%).

4.2.5. Friedel-Crafts aroylation of phenol 1 with $AlCl_3$ as Lewis acid in positions C-a and C-b of the pyrrole ring. Preparation of (1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-yl)(aroyl)-methanones 3c and 4c

In a mixture of anhydrous $AlCl_3$ (164 mg, 1.231 mmol) in ethylene dichloride (10 mL), 4-(trifluoromethoxy)benzoyl chloride (**2c**) (546 mg, 0.38 mL, 2.298 mmol) was added and stirred at room temperature and under a nitrogen atmosphere. After 10 min, a solution of the 2,6-difluoro-4-(1*H*-pyrrol-1-yl)phenol (**1**)[18] (200 mg, 1.024 mmol) in ethylene dichloride (5 mL) was added and the resulted mixture was stirred for 24 h under a nitrogen atmosphere. At this point, TLC analysis showed that the reaction was not completed. Thus, **2c** (273 mg, 0.19 mL, 1.149 mmol), anhydrous $AlCl_3$ (82 mg, 0.615 mmol) and ethylene dichloride (7.5 mL) were added. The resulted mixture was stirred in room temperature for another 24 h under a nitrogen atmosphere. Afterwards, the mixture was processed as in Section 4.2.2. The residue was flash chromatographed with a mixture of petroleum ether/EtOAc 9:1–7:1. Analytical samples were obtained by recrystallization from CH_2Cl_2/petroleum ether.

4.2.5.1. (1-(3,5-Difluoro-4-hydroxyphenyl)-1*H*-pyrrol-2-yl)(4-(trifluoromethoxy)phenyl)methanone (3c). Prepared according to the above procedure (66 mg, 17%). Mp 158.5-160.5 °C; IR (KBr) 3132, 1608 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.18 (br s, 1H), 6.34–6.43 (m, 1H), 6.86–7.02 (m, 3H), 7.08–7.13 (m, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 8.5 Hz 2H). Anal. Calcd for $C_{18}H_{10}F_5NO_3$. 0.1 CH_2Cl_2: C, 55.49, H, 2.62, N, 3.57. Found: C, 55.63, H, 2.62, N, 3.56.

4.2.5.2. (1-(3,5-Difluoro-4-hydroxyphenyl)-1*H*-pyrrol-3-yl)(4-(trifluoromethoxy)phenyl)methanone (4c). Prepared according to the above procedure (84 mg, 21%).

4.2.6. Preparation of 1-(1-(3,5-difluoro-4-hydroxyphenyl)-1*H*-pyrrol-2-yl)-2,2,2-trifluoroethanone (5)

In a mixture of ethylene dichloride (2.4 mL) and trifluoroacetic anhydrite (2.379 g, 1.6 mL, 11.33 mmol), 2,6-difluoro-4-(1*H*-pyrrol-1-yl)phenol (**1**)[18] (100 mg, 0.513 mmol) was added and the mixture was stirred under reflux (bath temperature 65–70 °C)

and nitrogen atmosphere for 4 h. The reaction was poured into ice and water and the product was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was flash chromatographed with a mixture of petroleum ether/EtOAc 6:1 (87 mg, 58%).

Analytical sample was obtained by recrystallization from CH_2Cl_2/petroleum ether. Mp 100–102 °C; IR (KBr) 3345, 1664 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.7 (br s, 1H), 6.43–6.51 (m, 1H), 6.86–6.98 (m, 2H), 7.11–7.18 (m, 1H), 7.35–7.42 (m, 1H). Anal. Calcd for $C_{12}H_6F_5NO_2$: C, 49.50, H, 2.08, N, 4.81. Found: C, 49.19, H, 2.06, N, .4.68.

4.3. Biological evaluation

4.3.1. ALR2 preparation

Lenses were quickly removed from Fischer-344 rats of both sexes following euthanasia. The experiments conform to the law for the protection of experimental animals (Republic of Greece) and are registered at the Veterinary Administration of the Republic of Greece. ALR2 from rat lens was partially purified according to the reported procedure[4,33] as follows: lenses were quickly removed from rats following euthanasia and stored at −20 °C until used. The lenses were homogenized in 5 vol of cold distilled water. The homogenate was centrifuged at 10,000 g at 0–4 °C for 15 min. The supernatant was precipitated with saturated ammonium sulfate at 40% salt saturation and this solution was centrifuged at 10,000*g* at 0–4 °C for 15 min. The latter supernatant was either used directly or stored for maximum 24 h at −80 °C.

4.3.2. ALR1 preparation

Kidneys were quickly removed from Fischer-344 rats of both sexes following euthanasia. The experiments conform to the law for the protection of experimental animals (Republic of Greece) and are registered at the Veterinary Administration of the Republic of Greece. ALR1 from rat kidney was partially purified according to the reported procedure[4,34] as follows: kidneys were homogenized in a knife homogenizer followed by processing in a glass homogenizer with a teflon pestle in 3 vol of 10 mM sodium phosphate buffer, pH 7.2, containing 0.25 M sucrose, 2.0 mM EDTA dipotassium salt, and 2.5 mM b-mercaptoethanol. The homogenate was centrifuged at 10,000*g* at 0–4 °C for 30 min and the supernatant was subjected to ammonium sulfate fractional precipitation at 40%, 50%, and 75% salt saturation. The pellet obtained from the last step, possessing ALR1 activity, was redissolved in 10 mM sodium phosphate buffer, pH 7.2, containing 2.0 mM EDTA dipotassium salt and 2.0 mM b-mercaptoethanol to achieve total protein concentration of approx. 20 mg/mL. DEAE DE 52 resin was added to the solution (33 mg/mL) and after gentle mixing for 15 min removed by centrifugation. The supernatant containing ALR1 was then stored in smaller aliquots at −80 °C. No appreciable contamination by ALR2 in ALR1 preparations was detected since no activity in terms of NADPH consumption was observed in the presence of glucose substrate up to 150 mM.

4.3.3. Enzyme assays

ALR2 and ALR1 activities, according to the reported procedure,[4] were assayed spectrophotometrically by determining NADPH consumption at 340 nm. In order to determine ALR2 inhibitory activity, D,L-glyceraldehyde was used as a substrate and the measurements took place at 30 °C, whereas ALR1 inhibitory activity was determined with D-glycuronate as a substrate and the measurements took place at 37 °C. Compounds **3a–c**, **4a–c** and **5** were dissolved in DMSO. The final concentration of DMSO in all incubations was 0.3%. The compounds were tested at five concentrations, the log (dose)–response curves were then constructed from the inhibitory data, and the IC_{50} values were calculated by least-square analysis of the linear portion of the log (dose) versus response curves ($0.912 < r^2 < 0.996$). The experiments were performed in triplicate.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.12.015. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Tang, W. H.; Martin, K. A.; Hwa, J. *Front. Pharmacol.* **2012**, *3*, Article 87.
2. Lam, D. W.; LeRoith, D. *Curr. Opin. Endocr. Diabetes Obes.* **2012**, *19*, 93.
3. Danaei, G.; Finucane, M. M.; Lu, Y.; Singh, G. M.; Cowan, M. J.; Paciorek, C. J.; Lin, J. K.; Farzadfar, F.; Khang, Y. H.; Stevens, G. A.; Rao, M.; Ali, M. K.; Riley, L. M.; Robinson, C. A.; Ezzati, M. *Lancet* **2011**, *378*, 31.
4. Chatzopoulou, M.; Mamadou, E.; Juskova, M.; Koukoulitsa, C.; Ioannis Nicolaou, I.; Stefek, M.; Demopoulos, V. J. *Bioorg. Med. Chem.* **2011**, *19*, 1426.
5. Alexiou, P.; Pegklidou, K.; Chatzopoulou, M.; Nicolaou, I.; Demopoulos, V. J. *Curr. Med. Chem.* **2009**, *16*, 734.
6. El-Kabbani, O.; Carbone, V.; Darmanin, C.; Oka, M.; Mitschler, A.; Podjarny, A.; Schulze-Briese, C.; Chung, R. P. T. *J. Med. Chem.* **2005**, *48*, 5536.
7. Alexiou, P.; Demopoulos, V. J. *J. Med. Chem.* **2010**, *53*, 7756.
8. Minehira, D.; Takeda, D.; Urata, H.; Kato, A.; Adachi, I.; Wang, X.; Matsuya, Y.; Sugimoto, K.; Takemura, M.; Endo, S.; Matsunaga, T.; Hara, A.; Koseki, J.; Narukawa, K.; Hirono, S.; Toyooka, N. *Bioorg. Med. Chem.* **2012**, *20*, 356.
9. Darmanin, C.; Chevreux, G.; Potier, N.; Van, D. A.; Hazemann, I.; Podjarny, A.; El-Kabbani, O. *Bioorg. Med. Chem.* **2004**, *12*, 3797.
10. Rapposelli, S.; Da Settimo, F.; Digiacomo, M.; La Motta, C.; Lapucci, A.; Sartini, S.; Vanni, M. *Arch. Pharm.* **2011**, *344*, 372.
11. Ola, M. S.; Nawaz, M. I.; Siddiquei, M. M.; Al-Amro, S.; Abu El-Asrar, A. M. *J. Diabetes Complications* **2012**, *26*, 56.
12. El-Kabbani, O.; Ruiz, F.; Darmanin, C.; Chung, R. P. *Cell. Mol. Life Sci.* **2004**, *61*, 750.
13. Pandey, S.; Srivastava, S. K.; Ramana, K. V. *Expert Opin. Investig. Drug* **2012**, *21*, 329.
14. Bresson, E.; Lacroix-Péppin, N.; Boucher-Kovalik, S.; Chapdelaine, P.; Fortier, M. A. *Front. Pharmacol.* **2012**, *3*, Article 98.
15. Laffin, B.; Petrash, J. M. *Front. Pharmacol.* **2012**, *3*, 104.
16. Chatzopoulou, M.; Alexiou, P.; Kotsampasakou, E.; Demopoulos, V. J. *Expert Opin. Ther. Pat.* **2012**, *22*, 1303.
17. Maccari, R.; Ciurleo, R.; Giglio, M.; Cappiello, M.; Moschini, R.; Del Corso, A.; Mura, U.; Ottanú, R. *Bioorg. Med. Chem.* **2010**, *18*, 4049.
18. Nicolaou, I.; Zika, C.; Demopoulos, V. J. *J. Med. Chem.* **2004**, *47*, 2706.
19. Barelier, S.; Pons, J.; Gehring, K.; Lancelin, J.-M.; Krimm, I. *J. Med. Chem.* **2010**, *53*, 5256.
20. Chen, X.; Zhang, S.; Yang, Y.; Hussain, S.; He, M.; Gui, D.; Ma, B.; Jing, C.; Qiao, Z.; Zhu, C.; Yu, Q. *Bioorg. Med. Chem.* **2011**, *19*, 7262.
21. Böhm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. *ChemBioChem* **2004**, *5*, 637.
22. Ismail, F. M. D.; Drew, M. G. B.; Dascombe, M. J. *Chim. Oggi.* **2009**, *27*, 18.
23. Yang, E.; Reese, M. R.; Humphrey, J. M. *Org. Lett.* **2012**, *14*, 3944.
24. Adogla, E. A.; Janser, R. F. J.; Fairbanks, S. S.; Vortolomei, C. M.; Meka, R. K.; Janser, I. *Tetrahedron Lett.* **2012**, *53*, 11.
25. Taylor, J. E.; Jones, M. D.; Williams, J. M. J.; Bull, S. D. *Org. Lett.* **2010**, *12*, 5740.
26. Song, C.; Knight, D. W.; Whatton, M. A. *Tetrahedron Lett.* **2004**, *45*, 9573.
27. Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
28. Carbone, V.; Giglio, M.; Chung, R.; Huyton, T.; Adams, J.; Maccari, R.; Ottana, R.; Hara, A.; El-Kabbani, O. *Eur. J. Med. Chem.* **2010**, *45*, 1140.
29. Barski, O. A.; Gabbay, K. H.; Bohren, K. M. *Genomics* **1999**, *60*, 188.
30. Barski, O. A.; Tipparaju, S. M.; Bhatnagar, A. *Drug Metab. Rev.* **2008**, *40*, 553.
31. El-Kabbani, O.; Sthanam, L.; Narayana, V. L.; Moore, K. M.; Green, N. C.; Flynn, T. G.; Delucas, L. J. *Acta Crystallogr., Sect. D* **1993**, *49*, 490.
32. El-Kabbani, O.; Ramsland, P.; Darmanin, C.; Chung, R. P. T.; Podjarny, A. *Proteins* **2003**, *50*, 230.
33. Stefek, M.; Snirc, V.; Djoubissie, P.-O.; Majekova, M.; Demopoulos, V.; Rackova, L.; Bezakova, Z.; Karasu, C.; Carbone, V.; El-Kabbani, O. *Bioorg. Med. Chem.* **2008**, *16*, 4908.
34. Pegklidou, K.; Koukoulitsa, C.; Nicolaou, I.; Demopoulos, V. J. *Bioorg. Med. Chem.* **2010**, *18*, 2107.