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# Synthesis and Biological Evaluation of 2′-Oxo-2,3-dihydro-3′*H*- spiro[chromene-4,5′-[1,3]oxazolidin]-3′yl]acetic Acid Derivatives as Aldose Reductase Inhibitors

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Aldose reductase (ARL2) is the first enzyme in the polyol pathway which catalyzes the NADPH-dependent reduction of glucose to sorbitol. Its involvement on diabetic complications makes this enzyme a challenge therapeutic target widely investigated to limit and/or prevent them. On this basis, a limited series of 4-spiro-oxazolidinone-benzopyran derivatives (**1–7**) were synthesized to evaluate them as potential ARL2 inhibitors. The activity was determined spectrophotometrically by monitoring the oxidation of NADPH catalyzed by ALR2. Within the series of compounds, the 4-methoxy derivative **1b** showed to be the most active compound, exhibiting inhibitory levels in the submicromolar range. In addition, the activity against the aldehyde reductase isoform (ARL1) was also evaluated. Unlike sorbinil (reference drug) that lack of selectivity towards the two enzyme all the tested compounds resulted to be devoid of ARL1 inhibitory activity ($IC_{50} > 10$ μM), thus proving to be selective.

Aldose reductase / Aldose reductase inhibitors (ARI) / Benzopyran, Spiro-oxazolidinone

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## Introduction

Aldose reductase (ALR2), a member of the aldo-keto reductase superfamily (AKR), is the first enzyme in the polyol pathway which catalyzes the NADPH-dependent reduction of glucose to sorbitol. In the second step of this pathway, sorbitol is slowly converted into fructose by sorbitol dehydrogenase (SDH) with $NAD^+$ as cofactor. In this pathway both NADPH and $NAD^+$ are consumed as cofactors for the enzymes ALR2 and SDH. In pathological conditions, sorbitol, unable to cross cell membranes, accumulates inside the cell, resulting in oxidative stress due to changes in the ratio of NADPH/$NADP^+$ and reduced (NADH)/$NAD^+$ which are the major cause of various complications of secondary diabetes.

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**Abbreviations: ARL1,** aldehyde dehydrogenase; **ARL2,** aldose reductase; **IPC,** ischemic preconditioning.

This compromised ability of the cell to protect itself from oxidative stress seem to be linked to diverse metabolic changes related to the synthesis of nitric oxide and to the activation of protein kinases. In diabetes, many complications observed at microvascular (*i.e.*, retinopathy, neuropathy, and nephropathy) [1] or macrovascular levels such as coronary artery disease, peripheral vascular disease and cerebrovascular disease, are largely responsible for the morbidity and mortality [2]. Beyond the well-known roles played by ARL2 in the onset of diabetic complications, most recent studies suggest that it also contributes to the pathogenesis of oxidative stress-induced inflammation by affecting the expression of cytokines and chemokines. [3] The increased level of ROS correlated to the ARL2 activity play a key role also in myocardial ischemia. In recent years it was demonstrated that ARL2 contributes to the myocardial ischemia injury and that inhibitors of this enzyme protect the hearts from myocardial damages induced by I/R cycles [4]. One of the possible mechanism by which ARL2 pathway mediated ischemia/reperfusion injury is the modulation of the opening of mitochondrial membrane permeability transition pore (MPTP) [5].

The MPTP remains closed during ischemia but opens at the onset of reperfusion [6]. Suppression of the MPTP opening at

early reperfusion leads to cardioprotection [7]. The MPTP pore is redox, $Ca^{2+}$, voltage, and pH sensitive. In particular, increases in matrix $Ca^{2+}$ and oxidative stress induce MPT pore opening [8, 9].

These findings suggest that targeting ALR2 could be useful for therapeutic intervention against myocardial injury induced by I/R cycle.

As part of our research program devoted to the synthesis and the evaluation of new cardioprotective agents, we extensively studied several five- and six-membered spiroheterocycle-benzopyran compounds endowed of a significant antiischemic activity [10–13]. These compounds showed to be able to protect the heart against the ischemic damage targeting on mito-$K_{ATP}$ channel, thus suggesting the spiro-benzopyran core as the pharmacophore element for myocardial cells.

The high structurally analogies of the basic scaffold of mito-$K_{ATP}$ openers to the core of sorbinil, a well-known ARL2 inhibitor, let us to afford slight modifications of the spirocycle-benzopyran core in order to design new potential inhibitor of ARL2 which could be useful tools to study the protection of the heart against ischemia. Firstly, the idantoin nucleus of sorbinil which seems to be responsible for its high toxicity [14] has been replaced by the oxazolidinone one (Fig. 2).

Moreover, bearing in mind the results of the plethora of well-resolved crystal structures of aldose reductase, which reveals a binding site composed of a fairly flexible specificity pocket and an anionic binding site [15–18], further modifications were afforded on the spirocycle-benzopyran scaffold. In particular, the nitrogen atom of the spirooxazolodinone-nucleus was linked to an acetic chain and the carbon in 2-position of benzopyran was substituted by an aromatic group. These two modifications may confer to the pharmacophoric spiro-benzopyran core the ability to selectively interact with both the binding pockets of ARL2. In effect the carboxylic function (*i.e.*, tolrestat, epalrestat) as well as the presence of additional hydrophobic substituents

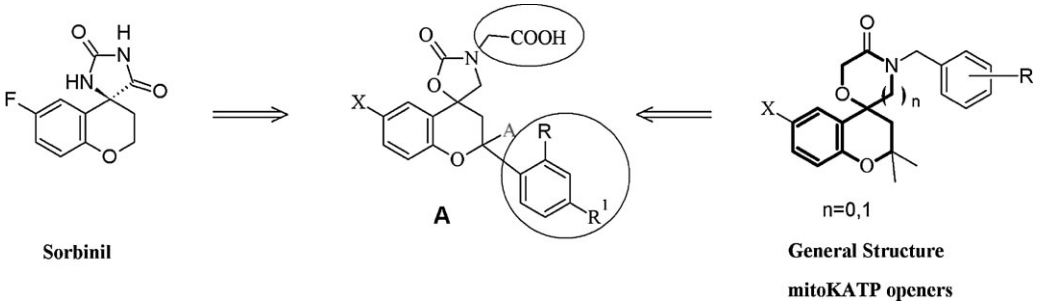



**Figure 1.** General structure **A** of the new compounds synthesized as potential ARL2 inhibitors.

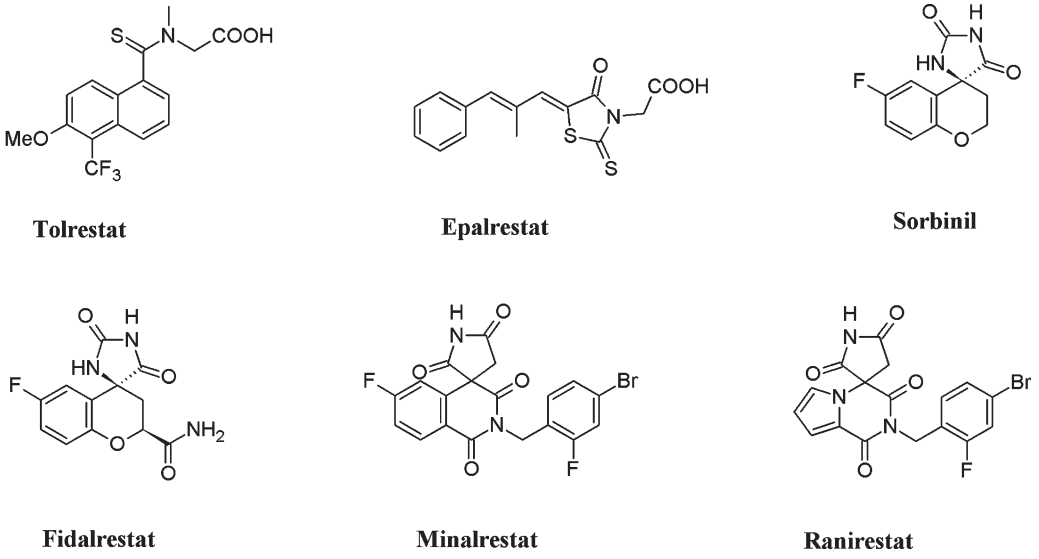



**Figure 2.** Structures of the most representative well-known aldose reductase inhibitors (ARIs).

(*i.e.*, minalrestat, ranirestat) have been widely investigated in order to improve the inhibitory activity of several ARIs. This work describes the synthesis of new spirobenzopyran derivatives (**1–7**) to evaluate them as potential inhibitor of ARL2 enzyme.

At the beginning we evaluated the influence of small electron-donor (OMe) and electron-withdrawal (Cl) groups around the aromatic ring in 2-position and the effect induced by an additional steric hindrance, thus introducing a methyl group, in the same position of benzopyran. Moreover, also the effects induced by the introduction of a bromine atom in 6-position of benzopyran has been assessed.

### Chemistry

#### *Synthesis*

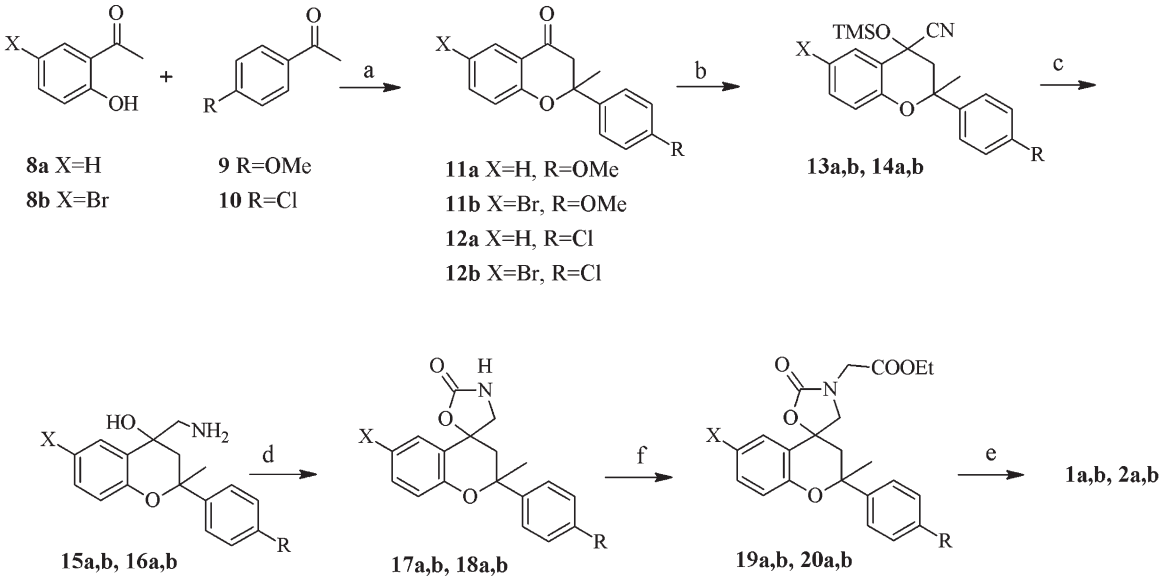
Compounds **1a,b** and **2a,b** were synthesized following the synthetic procedure illustrated in Scheme 1. Chromanones **11a,b** and **12a,b** were obtained from the appropriate 2-hydroxyacetophenone **8a,b** and 4-methoxy or 4-chloro-acetophenone respectively, and subjected to a nucleophilic addition with trimethylsilylcyanide (TMSCN) in the presence of $ZnI_2$ as the Lewis acid to afford the corresponding trimethylsilyl cyanohydrins. Compounds **13a,b** and **14a,b** were directly reduced to aminoalcohols with $LiAlH_4$ in accordance with the procedure of Amundsen and Nelson [19]. Spiro-oxazolidinones **17a,b** and **18a,b** were obtained by cyclization of the appropriate aminoalcohols in the presence of CDI. The subsequent reaction with ethyl bromoacetate in the presence of *n*-BuLi led to the corresponding *N*-ethylacetate derivatives. The cleavage of the esters with KOH in MeOH yielded the final compounds **1a,b–2a,b**.

Compounds **3–7** were synthesized starting from the appropriate 2-hydroxyacetophenone and the appropriate benzaldehyde (Scheme 2). The isolated α-β unsaturated compounds **26** and **27** were directly cyclized with glacial AcOH to yield the corresponding chromanones **28–29**. On the contrary, chromanones **30–32** were directly obtained from the appropriate 2-hydroxyacetophenone and the corresponding benzaldehyde by refluxing in MeOH in the presence of KOH. The subsequent nucleophilic addition with TMSCN and $ZnI_2$ afforded to trimethylsilyl-cyanohydrin derivatives. The reduction with $LiAlH_4$ followed by cyclization in the presence of CDI gave compounds **43–47**. The same reactions of alkylation and cleavage of the ester described above, led to the final compounds **3–7**.

## Results and discussion

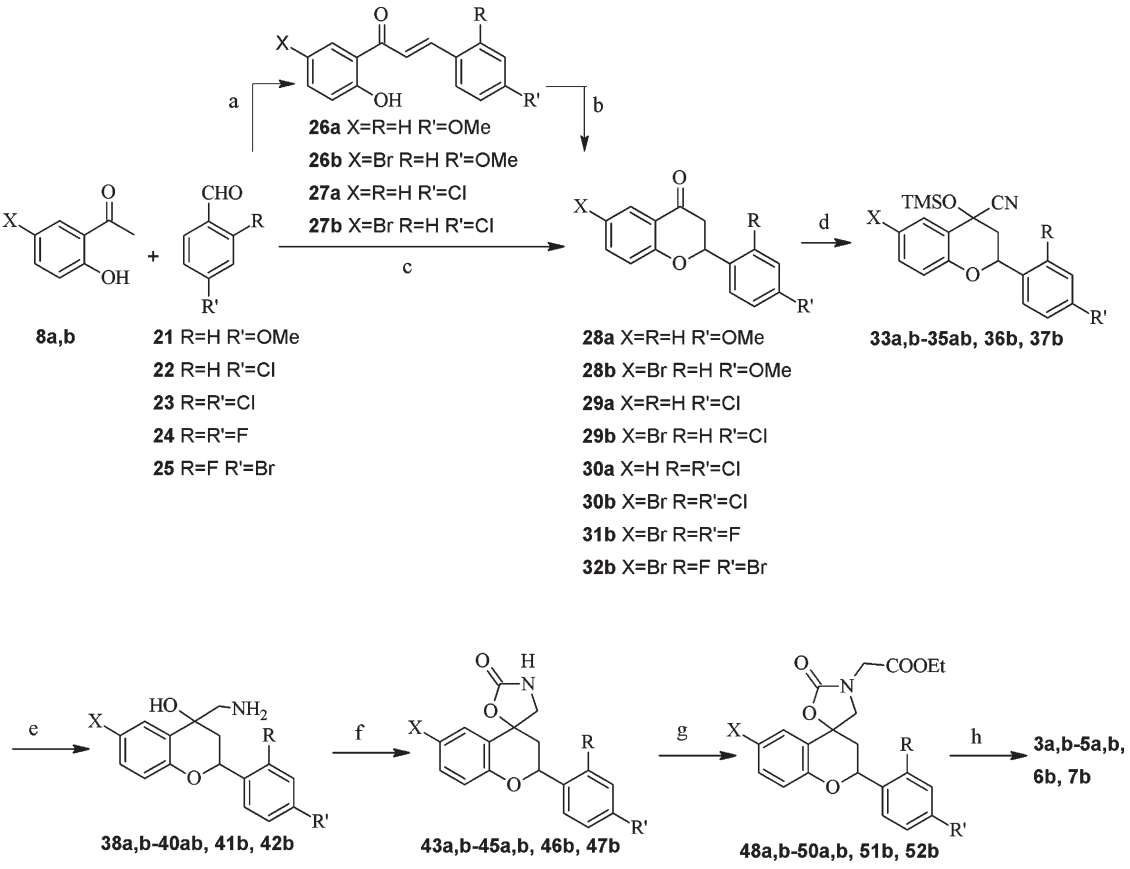
### Biological evaluation

This research project aimed at synthesizing a limited number of spirocycle-benzopyran derivatives (**1–7**) in order to evaluate them for their activity and selectivity against ALR2 and aldehyde dehydrogenase (ALR1), another enzyme belonging to the AKR superfamily that possessed the highest structural homology (65% identity in their amino acid sequences). As references drugs were used sorbinil and tolrestat. Although sorbinil lacks of selectivity against ARL1 and ARL2, it was used as reference drug because the compounds synthesised should be considered sorbinil-like. On the contrary, tolrestat was used as reference drug because of its selectivity and potency against ARL2.




**Scheme 1.** Synthetic routes for derivatives **1a,b** and **2a,b**. **Reagents**: a) Pyrrolidine, $CH_3CN$, reflux; b) TMSCN, $CH_2Cl_2$, rt; c) $LiAlH_4$, THF, rt; d) CDI, THF, rt; e) *n*-BuLi, ethylbromoacetate, THF, –78°C, rt, $N_2$; f) KOH, MeOH, reflux.




**Scheme 2.** Synthetic routes for derivatives **3a,b–5a,b**, **6b**, **7b**. **Reagents**: a) EtOH ass. NaOH, rt; b) AcOH, reflux; c) MeOH, KOH, reflux; d) TMSCN, $CH_2Cl_2$, rt; e) $LiAlH_4$, THF, rt; f) CDI, THF, rt; g) *n*-BuLi, ethylbromoacetate, THF, −78°C, rt, $N_2$; h) KOH, MeOH, reflux.

Aiming at a preliminary screening, we decided to evaluate all the compounds synthesized (**1–7**) as diasteromeric mixtures to select those one with the best inhibitory activity. Only later, the selected active diastereomeric mixtures will be further explore for the properties of each component.

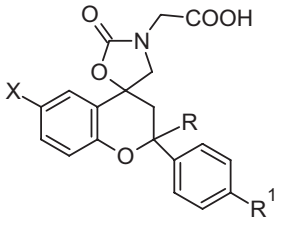
All the compounds were tested for their activity and selectivity against ARL1 and ARL2. Unlike sorbinil (the reference compound) that showed to be selective towards the ARL1 ($IC_{50}$ ARL2 = 0.65 μM, $IC_{50}$ ARL1 = 0.029 μM), none of the compounds synthesized, showed to have an appreciable activity against ARL1 ($IC_{50} > 10$ μM), proving to be completely selective towards ALR2 enzyme. Tables 1 and 2 report the activity against ALR2 expressed as $IC_{50}$ values of all the compounds synthesized. All of them proved to inhibit this enzyme, exhibiting potency levels in the micromolar/submicromolar range. The compound **1a**, substituted with a methoxy-group on the phenyl ring in 2-position of benzopyran, showed appreciable activity with $IC_{50}$ value of 2.25 μM. The insertion of an additional steric hindrance in the same position (**2a**) induced a decrease in the inhibitory potency with respect to the unsubstituted parent compound **1a** ($IC_{50}$ 43.8 μM *vs.* 2.25 μM, respectively). The 6-bromine-analogues of **1a** and **2a** (*i.e.*, **1b** and **2b)** showed an increased potency. In particular, **1b** proved to be most potent compound of this series showing an $IC_{50}$ values similar to the reference drug ($IC_{50}$ 0.58 μM for **1b** and 0.65 μM for sorbinil).

The replacement of the methoxy substituent of both **1a** and its superior homolog **2a** with the electronegative chlorine atom proved to contrasting results. In effect this kind of substitution was deleterious for compound **3a**, which showed $IC_{50}$ value of 5.69 μM, while the chlorine-substituted derivative **4a** proved to inhibit ARL2 with a potency 7-fold greater than the methoxy-substituted **2a** ($IC_{50}$ 6.11, 43.8, respectively). Conversely to the inhibitory activity trend of *p*-methoxy derivatives (**1a,b** and **2a,b**), the results obtained for p-chlorine substituted analogues **3a,b** and **4a,b** showed that both the insertion of a methyl group in 2-position as well as the presence of a bromine atom in 6-position affect only slightly the activity against ARL2.

Table 2 shows the inhibitory activity of benzopyran derivatives substituted on the phenyl ring in 2-position by more than one halogen atoms. The data showed that the insertion

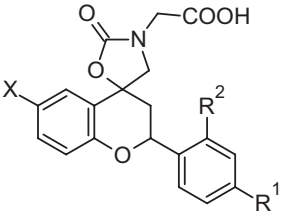
**Table 1.** ARL2 inhibitory activities of 4-spiro-oxazolidinone-benzopyrans **1–4**




| Compd. | X | R | $R^1$ | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|
| **1a** | H | H | OMe | 2.25 (1.85–2.71) |
| **1b** | Br | H | OMe | 0.58 (0.40–0.75) |
| **2a** | H | Me | OMe | 43.8 (35.5–51.1) |
| **2b** | Br | Me | OMe | 1.60 (1.22–2.08) |
| **3a** | H | H | Cl | 5.69 (4.27–7.01) |
| **3b** | Br | H | Cl | 8.25 (6.26–10.6) |
| **4a** | H | Me | Cl | 6.11 (4.40–7.51) |
| **4b** | Br | Me | Cl | 6.30 (4.48–7.72) |
| Tolrestat | – | – | – | 0.05 (0.03–0.06) |
| Sorbinil | – | – | – | 0.65 (0.49–0.82) |

[a] IC50 (95% CL) values represent the concentration required to produce 50% enzyme inhibition.

**Table 2.** ARL2 inhibitory activities of 2-aryl-4-spiro-oxazolidinone-benzopyrans **3–7**: Effect of the halide atoms on the phenyl ring in 2-position




| Compd. | X | $R^2$ | $R^1$ | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|
| **5a** | H | Cl | Cl | 3.43 (2.37–4.49) |
| **5b** | Br | Cl | Cl | 13.7 (10.1–16.3) |
| **6b** | Br | F | F | 1.97 (1.56–2.28) |
| **7b** | Br | F | Br | 6.19 (4.45–7.88) |
| Tolrestat | – | – | – | 0.05 (0.03–0.06) |
| Sorbinil | – | – | – | 0.65 (0.49–0.82) |

[a] $IC_{50}$ (95% CL) values represent the concentration required to produce 50% enzyme inhibition.

of an additional chlorine atom in 2′-position of the esocyclic aromatic group (**5a,b**) proved to modestly affect the inhibitory activity compared with their parent compounds ($IC_{50}$ 3.43 *vs.* 5.69 and 13.7 *vs.* 8.25, respectively). The replacement of electron-withdrawing chorine atoms with 2,4-fluorine (**6b**) or 2-fluorine, 4-bromine (**7b**) lead to divergent results. In fact, the substitution of chlorine with less bulkier electron-withdrawal atoms proved to improve the $IC_{50}$ value as for compound **6b** (1.97 *vs.* 13.7 of **5b**). The substitution of chlorine atoms with a bromine and a fluorine lead to compound **7b** which showed $IC_{50}$ values of 6.19 μM.

## Conclusion

The results of this study show that all the spirocycle-benzopyran compounds synthesized proved to be selective against ARL2 even if they showed to be weaker inhibitors than tolrestat. Within the series of compounds, the 4-methoxy derivative substituted with a bromine atom on the benzopyran nucleus results as the most active compound with $IC_{50}$ values in the submicromolar range and similar to that of sorbinil. This preliminary investigation led us to select **1b** as a potential new aldose reductase inhibitor that lack of activity against the isoform ARL1. Further investigations in *in-vitro* and *in-vivo* models of diabetes complications will be necessary to consider **1b** as a potential prototype of a new class of ARL inhibitors.

## Experimental procedures

### Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts ($\delta$) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced from solvent references. The elemental compositions of the compounds agreed to within 0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. Commercially available chemicals were purchased from Sigma-Aldrich.

*General procedure of the preparation of* ***1a,b–5a,b, 6b, 7b***

To a solution of the corresponding ester (0.76 mmol) in MeOH (2.8 mL) was added dropwise an aqueous solution of KOH 50% (0.13 mL). The resulting solution was refluxed for 2 h, then, after cooling, the solvent was evaporated. The residue was acidified to pH 3 with 1 N HCl and the aqueous phase was extracted with AcOEt. The organic phase was dried and the solvent was evaporated.

*[2-(4-Methoxyphenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′yl]acetic acid* ***1a***
Yield: 72%; m.p.: 84–86°C; $^1$H-NMR ($CDCl_3$) $\delta$ 2.45–2.65 (m, 2H, $CH_2$); 3.70–3.93 (m, 5H, $CH_2$, OMe); 4.12 (s, 2H, $CH_2$); 4.98–5.04 (m, 1H, CH); 6.88–7.03 (m, 4H, Ar); 7.22–7.27 (m, 1H, Ar); 7.37 (d, 2H, $J$ = 8.0 Hz, Ar); 7.46–7.49 (m, 1H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.52; 161.23; 159.83; 156.28; 133.14; 131.30; 128.64; 127.88; 124.27; 122.41; 117.99; 115.00; 78.52; 76.49; 60.51; 55.81; 45.76; 41.99. Anal. calcd. for $C_{20}H_{19}NO_6$: C 65.03; H 5.18; N 3.79; Found: C 64.83; H 5.16; N 3.77.

*[6-Bromo-2-(4-methoxyphenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***1b***
Yield: 60%; m.p.: 75–77°C; $^1$H-NMR ($CD_3OD$) $\delta$ 2.30–2.60 (m, 2H, $CH_2$); 3.81 (s, 3H, OMe); 3.90 (d, 1H, $J$ = 9.5 Hz, $CH_2$); 3.99 (d, 1H, $J$ = 9.5 Hz, $CH_2$); 4.04 (d, 1H, $J$ = 18.0 Hz, $CH_2$); 4.14 (d, 1H, $J$ = 18.0 Hz, $CH_2$); 5.15 (dd, 1H, $J$ = 2.2, 11.7 Hz, CH); 6.82 (d, 1H, $J$ = 8.8 Hz, Ar); 6.96 (d, 2H, $J$ = 8.8 Hz, Ar); 7.36–7.44 (m, 3H, Ar); 7.63 (d, 1H, $J$ = 2.4 Hz, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.76; 160.11; 159.39; 154.50; 134.61; 130.80; 129.64; 128.33; 127.65; 126.77; 120.41; 114.46; 78.32; 76.16; 60.58; 55.41; 45.70; 42.02. Anal. calcd. for $C_{20}H_{18}BrNO_6$: C 53.59; H 4.05; N 3.12; Found: C 53.37; H 4.03; N 3.10.

*[2-(4-Methoxyphenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***2a***
Yield: 60%; m.p.: 84–86°C; $^1$H-NMR ($CD_3OD$) $\delta$ 1.68 (s, 3H, Me); 2.25 (d, 1H, $J$ = 12.6 Hz, $CH_2$); 2.41 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.83 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.96 (d, 1H, $J$ = 12.6 Hz, $CH_2$); 3.11–3.31 (m, 2H, $CH_2$); 3.76 (s, 3H, OMe); 6.82–7.04 (m, 4H, Ar); 7.26–7.40 (m, 3H, Ar); 7.46–7.51 (m, 1H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 172.52; 160.48; 154.89; 137.22; 134.46; 132.06; 127.60; 124.53; 122.80; 118.93; 114.77; 114.38; 80.21; 76.50; 58.98; 55.60; 46.30; 46.15; 29.73. Anal. calcd. for $C_{21}H_{21}NO_6$: C 65.79; H 5.52; N 3.65; Found: C 65.92; H 5.53; N 3.65.

*[6-Bromo-2-(4-methoxyphenyl)-2-methyl-2′-oxo-2,3-dihydro-3′Hspiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***2b***
Yield: 45%; m.p.: 78–80°C; $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.63 (s, 3H, Me); 2.69 (d, 2H, $J$ = 17.0 Hz, $CH_2$); 3.30–3.40 (m, 2H, $CH_2$); 3.73 (s, 3H, OMe); 3.86 (d, 1H, $J$ = 17.9 Hz, $CH_2$); 4.00 (d, 1H, $J$ = 17.9 Hz, $CH_2$); 6.90 (d, 2H, $J$ = 8.7 Hz, Ar); 6.99–7.06 (m, 1H, Ar); 7.26–7.38 (m, 2H, Ar); 7.50 (dd, 1H, $J$ = 2.4, 8.7 Hz, Ar); 7.76 (d, 1H, $J$ = 2.4 Hz, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 172.05; 160.36; 159.30; 154.17; 137.04; 134.80; 130.86; 127.22; 126.77; 120.60; 114.90; 114.09; 80.08; 76.20; 58.72; 55.75; 46.32; 46.21; 29.53. Anal. calcd. for $C_{21}H_{20}BrNO_6$: C 54.56; H 4.36; N 3.03; Found: C 54.04; H 3.93; N 2.63.

*[2-(4-Chlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***3a***
Yield: 99%; m.p.: 110–112°C; $^1$H-NMR ($CD_3OD$) $\delta$ 2.40–2.65 (m, 2H, $CH_2$); 3.90–4.23 (m, 2H, $CH_2$); 4.88 (s, 2H, $CH_2$); 5.19–5.25 (m, 1H, CH); 6.89–7.08 (m, 2H, Ar); 7.24–7.33 (m, 1H, Ar); 7.40–7.54 (m, 5H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.00; 159.76; 155.92; 139.88; 134.96; 131.38; 129.63; 128.83; 127.95; 124.13; 122.62; 117.96; 78.21; 75.91; 60.29; 41.90; 15.35. Anal. calcd. for $C_{19}H_{16}ClNO_5$: C 61.05; H 4.31; N 3.75; Found: C 60.80; H 4.29; N 3.73.

*[6-Bromo-2-(4-chlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***3b***
The crude product was purified by trituration with hexane. Yield: 34%; m.p.: 153–155°C; $^1$H-NMR ($CDCl_3$) $\delta$ 2.45–2.52 (m, 2H, $CH_2$); 3.79–3.92 (m, 2H, $CH_2$); 4.11 (s, 2H, $CH_2$); 4.98–5.08 (m, 1H, CH); 6.79 (d, 1H, $J$ = 9.0 Hz, Ar); 6.89–7.05 (m, 1H, Ar); 7.30–7.49 (m, 4H, Ar); 7.59 (d, 1H, $J$ = 2.2 Hz, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.10; 159.41; 155.57; 139.15; 135.00; 131.41; 129.66; 128.88; 128.02; 122.65; 120.18; 114.39; 78.24; 75.95; 60.34; 45.80; 41.95. Anal. calcd. for $C_{19}H_{15}BrClNO_5$: C 50.41; H 3.34; N 3.09; Found: C 50.67; H 3.15; N 3.22.

*[2-(4-Chlorophenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***4a***
The crude product was purified by precipitation from AcOEt/hexane. Yield: 55%; m.p.: 85–87°C; $^1$H-NMR ($CDCl_3$) $\delta$ 1.72 (s, 3H, Me); 2.58 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 2.72 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 3.26 (d, 1H, $J$ = 8.8 Hz, $CH_2$); 3.50 (d, 1H, $J$ = 8.8 Hz, $CH_2$); 4.05 (s, 2H, $CH_2$); 6.96–7.05 (m, 2H, Ar); 7.27–7.51 (m, 6H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): δ 171.50; 159.47; 154.54; 144.81; 134.20; 131.98; 129.52; 128.33; 127.73; 124.31; 122.71; 118.63; 79.21; 76.55; 58.91; 46.72; 45.67; 28.73. Anal. calcd. for $C_{20}H_{18}ClNO_5$: C 61.94; H 4.68; N 3.61; Found: C 62.18; H 4.71; N 3.63.

*[6-Bromo-2-(4-chlorophenyl)-2-methyl-2′-oxo-2,3-dihydro-3′Hspiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***4b***
The crude product was purified by precipitation from AcOEt/hexane. Yield: 62%; m.p.: 103–106°C. $^1$H-NMR ($CDCl_3$) $\delta$ 1.71 (s, 3H, Me); 2.56 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 2.70 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 3.18–3.52 (m, 2H, $CH_2$); 3.95–4.17 (m, 2H, $CH_2$); 6.89–7.04 (m, 1H, Ar); 7.26–7.52 (m, 5H, Ar); 7.69 (d, 1H, $J$ = 2.0 Hz, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.80; 159.67; 159.41; 154.67; 144.32; 135.00; 131.06; 129.61; 128.42; 127.68; 126.53; 120.81; 79.72; 75.93; 58.89; 46.16; 45.79; 28.59. Anal. calcd. for $C_{20}H_{17}BrClNO_5$: C 51.47; H 3.76; N 3.00; Found: C 51.12; H 3.57; N 2.76.

*[2-(2,4-Dichlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′ yl]acetic acid* ***5a***

The crude product was purified by crystallization from *i*PrOH. Yield: 39%; m.p.: 128–130°C; $^1$H-NMR ($CDCl_3$) $\delta$ 2.17–2.37 (m, 1H, $CH_2$); 2.62–2.71 (m, 1H, $CH_2$); 3.55–4.17 (m, 4H, $CH_2$); 5.38–5.50 (m, 1H, CH); 6.93–7.10 (m, 1H, Ar); 7.24–7.74 (m, 6H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.12; 159.78; 155.60; 139.56; 139.74; 134.80; 131.18; 124.04; 122.65; 122.20; 117.88; 105.73; 104.99; 104.61; 77.90; 76.00; 60.37; 45.64; 41.90. Anal. calcd. for $C_{19}H_{15}Cl_2NO_5$: C 55.90; H 3.70; N 3.43; Found: C 56.15; H 3.55; N 3.47.

*[6-Bromo-2-(2,4-dichlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′[1,3]oxazolidin]-3′-yl]acetic acid* ***5b***

The crude product was purified by precipitation from AcOEt/hexane. Yield: 36%; m.p.: 190–192°C; $^1$H-NMR (DMSO) $\delta$ 2.28–2.41 (m, 1H, $CH_2$); 2.58 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 3.75–3.97 (m, 2H, $CH_2$); 4.01 (d, 2H, $J$ = 2.8 Hz, $CH_2$); 5.58 (d, 1H, $J$ = 11.5 Hz, CH); 6.96 (dd, 1H, $J$ = 1.1, 8.8 Hz, Ar); 7.47–7.77 (m, 5H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.35; 159.67; 155.90; 139.76; 139.90; 134.60; 130.81; 126.53; 122.99; 120.20; 114.45; 105.23; 104.89; 104.70; 77.54; 71.15; 60.31; 46.05; 40.19. Anal. calcd. for $C_{19}H_{14}BrCl_2NO_5$: C 46.85; H 2.90; N 2.88; Found: C 46.89; H 2.61; N 2.83.

*[6-Bromo-2-(2,4-difluorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***6b***

The crude product was purified by trituration with hexane. Yield: 63%; m.p.: 118–120°C; $^1$H-NMR ($CDCl_3$) $\delta$ 2.39–2.58 (m, 2H, $CH_2$); 3.84 (d, 1H, $J$ = 8.5 Hz, $CH_2$); 3.91 (d, 1H, $J$ = 8.5 Hz, $CH_2$); 4.16 (s, 2H, $CH_2$); 5.31–5.37 (m, 1H, CH); 6.81 (d, 1H, $J$ = 8.8 Hz, Ar); 6.86–7.01 (m, 2H, Ar); 7.37 (dd, 1H, $J$ = 2.4, 8.8 Hz, Ar); 7.51–7.59 (m, 1H, Ar); 7.62 (d, 1H, $J$ = 2.4 Hz, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.71; 159.41; 155.08; 134.45; 130.66; 126,49; 122.83; 120.17; 114.62; 112.87; 112.44; 105.35; 104.84; 104.32; 77.46; 71.09; 60.23; 46.03; 40.20. Anal. calcd. for $C_{19}H_{14}BrF_2NO_5$: C 50.24; H 3.11; N 3.08; Found: C 50.46; H 3.30; N 2.80.

*[6-Bromo-2-(4-bromo-2-fluorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetic acid* ***7b***

Yield: 30%; m.p.: 178–180°C; $^1$H-NMR ($CDCl_3$) $\delta$ 2.37–2.59 (m, 2H, $CH_2$); 3.81 (s, 2H, $CH_2$); 3.87 (d, 1H, $J$ = 4.1 Hz; $CH_2$); 4.13 (d, 1H, $J$ = 4.1 Hz, $CH_2$); 5.33 (d, 1H, $J$ = 10.6 Hz, CH); 6.82 (d, 1H, $J$ = 8.6 Hz, Ar); 7.26–7.53 (m, 4H, Ar); 7.61–7.62 (m, 1H, Ar) ppm; $^{13}$C-NMR ($CD_3COCD_3$): $\delta$ 171.50; 159.23; 155.00; 134.14; 130.53; 126,19; 122.63; 120.01; 114.57; 114.82; 112.45; 105.03; 104.66; 104.12; 77.23; 71.05; 60.10; 45.97; 40.11. Anal. calcd. for $C_{19}H_{14}Br_2FNO_5$: C 44.30; H 2.74; N 2.72; Found: C 44.30; H 2.69; N 2.51.

*2-(4-Methoxyphenyl)-2-methyl-2,3-dihydro-4H-chromen-4-one* ***11a***

To a solution of 2-hydroxy acetophenone **8a** (1.00 g, 7.35 mmol) in $CH_3CN$ (10 mL) was added 4-methoxy-acetophenone **9** (1.10 g, 7.35 mmol) and pyrrolidine (0.49 g; 7.35 mmol). The mixture was stirred at room temperature for 1 h, then refluxed for 48 h. The solvent was removed under reduced pressure and the residue diluted with AcOEt and washed with 1 N HCl, 1 N NaOH, and water. The organic layers were dried and evaporated. The crude product was purified by flash column chromatography eluting with AcOEt/hexane (3:7) to give **11a** (yield 19%); $^1$H-NMR ($CDCl_3$) $\delta$ 1.74 (s, 3H, Me); 3.08 (d, 1H, $J$ = 16.4 Hz, $CH_2$); 3.31 (d, 1H, $J$ = 16.4 Hz, $CH_2$); 3.74 (s, OMe); 6.80 (d, 2H, $J$ = 8.7 Hz, Ar); 6.88–6.95 (m, 1H, Ar); 7.03 (d, 1H, $J$ = 8.2 Hz, Ar); 7.32 (d, 2H, $J$ = 8.7 Hz, Ar); 7.41–7.49 (m, 1H, Ar); 7.76 (dd, 1H, $J$ = 1.6, 7.7 Hz, Ar) ppm.

*6-Bromo-2-(4-methoxyphenyl)-2-methyl-2,3-dihydro-4H-chromen-4-one* ***11b***

Compound **11b** was obtained from 5-bromo-2-hydroxy acetophenone **8b** (1.58 g, 7.35 mmol) and 4-methoxy-acetophenone **9** (1.10 g, 7.35 mmol) following the procedure described for **11a**. The crude product was purified by flash column chromatography eluted with AcOEt/hexane 3:7 to give **11b** (yield 22%); $^1$H-NMR ($CDCl_3$) $\delta$ 1.74 (s, 3H, Me); 3.04 (d, 1H, $J$ = 16.5 Hz, $CH_2$); 3.30 (d, 1H, $J$ = 16.5 Hz, $CH_2$); 3.75 (s, OMe); 6.81 (d, 2H, $J$ = 8.8 Hz, Ar); 6.92 (d, 1H, $J$ = 8.8 Hz; Ar); 7.30 (d, 2H, $J$ = 8.8 Hz, Ar); 7.51 (dd, 1H, $J$ = 2.6, 8.8 Hz, Ar); 7.85 (d, 1H, $J$ = 2.6 Hz, Ar) ppm.

*2-(4-Chlorophenyl)-2-methyl-2,3-dihydro-4H-chromen-4-one* ***12a***

Compound **12a** was obtained from 2-hydroxy acetophenone **8a** (5.00 g, 36.72 mmol) and 4-chloro-acetophenone **10** (5.68 g, 36.72 mmol) following the procedure described for **11a**. The crude product was purified by flash column chromatography eluting with AcOEt/hexane (5:95) to give **12a** (yield 40%); m.p.: 82–85°C; $^1$H-NMR ($CDCl_3$) $\delta$ 1.74 (s, 3H, Me); 3.08 (d, 1H, $J$ = 16.5 Hz, $CH_2$); 3.27 (d, 1H, $J$ = 16.5 Hz, $CH_2$); 6.91–6.99 (m, 1H, Ar); 7.05 (d, 1H, $J$ = 8.2 Hz, Ar); 7.26 (d, 2H, $J$ = 8.8 Hz, Ar); 7.35 (d, 2H, $J$ = 8.8 Hz, Ar); 7.44–7.52 (m, 1H, Ar); 7.76 (dd, 1H, $J$ = 1.6, 7.9 Hz, Ar) ppm.

*6-Bromo-2-(4-chlorophenyl)-2-methyl-2,3-dihydro-4H-chromen-4-one* ***12b***

Compound **12b** was obtained from 5-bromo-2-hydroxy acetophenone **8b** (7.89 g, 36.72 mmol) and 4-chloro-acetophenone **10** (5.68 g, 36.72 mmol) following the procedure described for **11a**. The crude product was used for the next step without further purification to give **12b** (yield 45%); m.p.: 90–92°C. $^1$H-NMR ($CDCl_3$) $\delta$ 1.74 (s, 3H, Me); 3.07 (d, 1H, $J$ = 16.6 Hz,

$CH_2$); 3.28 (d, 1H, $J = 16.6$ Hz, $CH_2$); 6.95 (d, 1H, $J = 8.8$ Hz, Ar); 7.24–7.34 (m, 4H, Ar); 7.54 (dd, 1H, $J = 2.6$, 8.8 Hz, Ar); 7.86 (d, 1H, $J = 2.6$ Hz, Ar) ppm.

### *(2E)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **26a***

To a solution of 4-methoxybenzaldehyde (1.51 g, 11.09 mmol) in absolute EtOH (20 mL) was added 2-hydroxyacetophenone (1.50 g, 11.09 mmol). The reaction mixture was stirred for 5 min and then NaOH (1.33 g, 33.29 mmol) was added. The resulting mixture was stirred for 5 h at rt and then 1 N HCl was added. The precipitate was filtered to give **26a** directly used in the next reaction without further purification. **26a** (2.75 g, 10.87 mmol, yield 99%) $^1$H-NMR ($CDCl_3$) $\delta$ 3.87 (s, 3H, OMe); 6.90–7.05 (m, 2H, Ar); 6.96 (d, 2H, $J = 8.8$ Hz, CH); 7.45–7.54 (m, 1H, Ar); 7.54 (d, 1H, $J = 15.3$ Hz, CH); 7.64 (d, 2H, $J = 8.8$ Hz, Ar); 7.87–7.94 (m, 1H, CH); 7.91 (d, 1H, $J = 15.3$ Hz, CH) ppm.

### *(2E)-1-(5-Bromo-2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **26b***

Compound **26b** was obtained from 5-bromo-2-hydroxyacetophenone (1.50 g, 6.97 mmol) and 4-methoxybenzaldehyde (0.95 g, 6.97 mmol) following the same procedure described for **26a**. The crude product was directly used in the next reaction without further purification. **26b** (2.3 g, 6.9 mmol, yield 99%) $^1$H-NMR ($CDCl_3$) $\delta$ 3.87 (s, 3H, OMe); 6.89–6.90 (m, 3H, Ar); 7.43 (d, 1H, $J = 15.3$ Hz, CH); 7.55 (dd, 1H, $J = 2.4$, 8.8 Hz, Ar); 7.60 (d, 2H, $J = 8.8$ Hz, Ar); 7.90 (d, 1H, $J = 15.3$ Hz, CH); 8.00 (d, 1H, $J = 2.4$ Hz, Ar) ppm.

### *(2E)-3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one **27a***

Compound **27a** was obtained from 2-hydroxyacetophenone (0.63 g, 4.65 mmol) and 4-chlorobenzaldehyde (0.65 g, 4.65 mmol) following the same procedure described for **26a** and used for the next reaction without further purification. **27a** (1.18 g, 4.56 mmol, yield 98%): $^1$H-NMR ($CDCl_3$) $\delta$ 6.91–7.05 (m, 2H, Ar); 7.40 (d, 2H, $J = 8.6$ Hz, Ar); 7.47–7.65 (m, 4H, Ar, CH); 7.82–7.92 (m, 2H, Ar, CH) ppm.

### *(2E)-1-(5-Bromo-2-hydroxyphenyl)-3-(4-chlorophenyl)prop-2-en-1-one **27b***

Compound **27b** was obtained from 5-bromo-2-hydroxyacetophenone (1.00 g, 4.65) and 4-chlorobenzaldehyde (0.65 g, 4.65 mmol) following the same procedure described for **26a** and used for the next reaction without further purification. **27b** (1.38 g, 4.09 mmol, yield 88%): $^1$H-NMR ($CDCl_3$) $\delta$ 6.95 (d, 1H, $J = 9.0$ Hz, Ar); 7.41–7.49 (m, 2H, Ar); 7.55–7.65 (m, 4H, Ar, CH); 7.89 (d, 1H, $J = 15.6$ Hz, CH); 7.99 (d, 1H, $J = 2.4$ Hz, Ar) ppm.

### *2-(4-Methoxyphenyl)-2,3-dihydro-4H-chromen-4-one **28a***

The solution of **26a** (2.75 g, 10.87 mmol) in glacial AcOH (20 mL) was refluxed for 72 h. After cooling to rt the reaction mixture was poured in $H_2O$ and extracted with EtOAc. The organic layer was dried and concentrated under vacuum to give **28a** without further purification (1.13 g, 4.46 mmol, yield 41%). $^1$H-NMR ($CDCl_3$) $\delta$ 2.86 (dd, 1H, $J = 3.1$, 16.8 Hz, $CH_2$); 3.12 (dd, 1H, $J = 13$, 16.8 Hz, $CH_2$); 3.84 (s, 3H, OMe); 5.44 (dd, 1H, $J = 3.1$, 13 Hz, CH); 6.94–7.09 (m, 4H, Ar); 7.38–7.55 (m, 4H, Ar); 7.93 (dd, 1H, $J = 1.7$, 8.1 Hz, Ar) ppm.

### *6-Bromo-2-(4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one **28b***

Compound **28b** was obtained from **26b** (2.3 g, 6.9 mmol) following the procedure described for **28a**. The crude product was used for the next reaction without further purification. **28b** (1.15 g, 3.45 mmol, yield 50%): $^1$H-NMR ($CDCl_3$) $\delta$ 2.87 (dd, 1H, $J = 3.2$, 16.8 Hz, $CH_2$); 3.10 (dd, 1H, $J = 12.8$, 16.8 Hz, $CH_2$); 3.84 (s, 3H, OMe); 5.42 (dd, 1H, $J = 3.2$, 12.8 Hz, CH); 6.92–6.99 (m, 3H, Ar); 7.57 (dd, 1H, $J = 2.6$, 8.6 Hz, Ar); 7.66 (d, 2H, $J = 9.0$ Hz, Ar); 8.03 (d, 1H, $J = 2.6$ Hz, Ar) ppm.

### *2-(4-Chlorophenyl)-2,3-dihydro-4H-chromen-4-one **29a***

Compound **29a** was obtained from **27a** (1.18 g, 4.56 mmol) following the procedure described for **28a**. The crude product was directly used for the next reaction without further purification. **29a** (0.28 g, 1.09 mmol, yield 24%): $^1$HNMR ($CDCl_3$) $\delta$ 2.88 (dd, 1H, $J = 3.5$, 16.9 Hz, $CH_2$); 3.05 (dd, 1H, $J = 12.6$, 16.9 Hz, $CH_2$); 5.47 (dd, 1H, $J = 3.5$, 12.6 Hz, CH); 6.91–7.11 (m, 2H, Ar); 7.40–7.67 (m, 5H, Ar); 7.84–7.96 (m, 1H, Ar) ppm.

### *6-Bromo-2-(4-chlorophenyl)-2,3-dihydro-4H-chromen-4-one **29b***

Compound **29b** was obtained from **27b** (1.38 g, 4.09 mmol) purification following the procedure described for **26a**. The crude product was directly used for the next reaction without further purification. **29b** (1.05 g, 3.1 mmol, yield 76%): $^1$H-NMR ($CDCl_3$) $\delta$ 2.88 (dd, 1H, $J = 3.7$, 17.2 Hz, $CH_2$); 3.04 (dd, 1H, $J = 12.4$, 17.2 Hz, $CH_2$); 5.45 (dd, 1H, $J = 3.7$, 12.4 Hz, CH); 6.96 (d, 1H, $J = 8.8$ Hz, Ar); 7.38–7.44 (m, 4H, Ar); 7.59 (dd, 1H, $J = 2.5$, 8.8 Hz, Ar); 8.03 (d, 1H, $J = 2.5$ Hz, Ar) ppm.

### *2-(2,4-Dichlorophenyl)-2,3-dihydro-4H-chromen-4-one **30a***

To a solution of 2,4-dichlorobenzaldehyde (2.57 g, 14.68 mmol) in MeOH was added 2-hydroxy acetophenone (2.00 g, 14.68 mmol) and KOH (0.33 g, 5.87 mmol). The reaction mixture was refluxed for 7 h. The reaction mixture was refluxed for 7 h. The solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography

eluted with Hex/EtOAc (9:1) to give **30a** (4.00 g, 13.8 mmol, yield 94%): $^1$H-NMR ($CDCl_3$) $\delta$ 2.76–3.10 (m, 2H, $CH_2$); 5.82 (dd, 1H, $J$ = 13.0, 3.1 Hz, CH); 7.05–7.13 (m, 1H, Ar); 7.31–7.58 (m, 3H, Ar); 7.68–7.73 (m, 1H, Ar); 7.93–7.98 (m, 1H, Ar) ppm.

### *6-Bromo-2-(2,4-dichlorophenyl)-2,3-dihydro-4H-chromen-4-one* ***30b***

Compound **30b** was obtained from 5-bromo-2-hydroxyacetophenone (2.00 g, 9.30 mmol) and 2,4 dichlorobenzaldheyde (1.63 g; 9.30 mmol) following the procedure described for **30a**. The crude product was directly used for the next reaction without further purification. **30b** (2.2 g, 5.95 mmol, yield 64%): mp 133–135°C. $^1$H-NMR ($CDCl_3$) $\delta$ 2.82 (dd, 1H, $J$ = 13.2, 17.0 Hz, $CH_2$); 3.03 (dd, 1H, $J$ = 3.0, 17.0 Hz, $CH_2$); 5.80 (dd, 1H, $J$ = 3.0, 13.2 Hz, CH); 6.98 (d, 1H, $J$ = 8.8 Hz, Ar); 7.38 (dd, 1H, $J$ = 1.9, 8.4 Hz, Ar); 7.45 (d, 1H, $J$ = 1.9 Hz, Ar); 7.61 (dd, 1H, $J$ = 2.6, 8.8 Hz, Ar); 7.67 (d, 1H, $J$ = 8.4 Hz, Ar); 8.06 (d, 1H, $J$ = 2.6 Hz, Ar) ppm.

### *6-Bromo-2-(2,4-difluorophenyl)-2,3-dihydro-4H-chromen-4-one* ***31b***

Compound **31b** was obtained from 5-bromo-2hydroxyacetophenone (1.00 g, 4.65 mmol) and 2,4-difluorobenzaldehyde (0.66 g; 4.65 mmol) following the synthetic procedure described above for **30a**. The crude product was purified by crystallization from EtOH to give **31b** (0.38 g, 1.12 mmol, yield 24%): $^1$HNMR ($CDCl_3$) $\delta$ 2.85–3.12 (m, 2H, $CH_2$); 5.71 (dd, 1H, $J$ = 12.4, 3.7 Hz, CH); 6.83–7.02 (m, 3H, Ar); 7.53–7.65 (m, 2H, Ar); 8.05 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

### *6-Bromo-2-(4-bromo-2-fluorophenyl)-2,3-dihydro-4H-chromen-4-one* ***32b***

Compound **32b** was synthesized from 5-bromo-2-hydroxyacetophenone (1.00 g, 4.65) and 4-bromo-2-fluorobenzaldehyde (0.94 g, 4.65 mmol) following the synthetic procedure described for **30a**. The crude product was purified by flash column chromatography eluted with Hex/EtOAc (9:1) to give **32b** (0.65 g, 1.62 mmol, yield 35%): $^1$H-NMR ($CDCl_3$) $\delta$ 2.87–3.09 (m, 2H, $CH_2$); 5.70 (dd, 1H, $J$ = 4.8, 11.3 Hz, CH); 6.96 (d, 1H, $J$ = 8.8 Hz, Ar); 7.29–7.57 (m, 3H, Ar); 7.60 (dd, 1H, $J$ = 2.5, 8.8 Hz, Ar); 8.05 (d, 1H, $J$ = 2.5 Hz, Ar) ppm.

### *General procedure for preparation of compounds* ***13a,b, 14a,b, 33a,b-35a,b, 36b, 37b***

To a solution of the opportune chromanone (3.35 mmol) in $CH_2Cl_2$ (29 mL) was added trimethylsilylcyanide (TMSCN) (0.50 g, 5.02 mmol) and $ZnI_2$ (0.16 g, 0.50 mmol). The mixture was stirred at room temperature for 4 h, then $CH_2Cl_2$ was added and the solution was washed with water. The organic layer was dried and evaporated.

### *4-[(Trimethylsilyl)oxy]-2-(4-methoxyphenyl)-2-methyl-3,4-dihydro-2H-chromene-4-carbonitrile* ***13a***

Yield: 79%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.27 (s, 9H, Me); 1.71 (s, 3H, Me); 2.56 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.79 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 3.78 (s, 3H, OMe); 6.87 (d, 2H, $J$ = 8.7 Hz, Ar); 6.97–7.04 (m, 2H, Ar); 7.25–7.36 (m, 3H, Ar); 7.52 (dd, 1H, $J$ = 1.8, 8.0 Hz, Ar) ppm.

### *6-Bromo-4-[(trimethylsilyl)oxy]-2-(4-methoxyphenyl)-2-methyl-3,4-dihydro-2H-chromene-4-carbonitrile* ***13b***

Yield: 90%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.28 (m, 9H, Me); 1.70 (s, 3H, Me); 2.53 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 2.81 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 3.79 (s, 3H, OMe); 6.85–6.93 (m, 3H, Ar); 7.25–7.30 (m, 3H, Ar); 7.38–7.44 (m, 1H, Ar); 7.58–7.59 (m, 1H, Ar) ppm.

### *4-[(Trimethylsilyl)oxy]-2-(4-chlorophenyl)-2-methyl-3,4-dihydro-2H-chromene-4-carbonitrile* ***14a***

Yield: 37%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.28 (s, 9H, Me); 1.72 (s, 3H, Me); 2.61 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 2.77 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 6.99–7.07 (m, 2H, Ar); 7.27–7.39 (m, 5H, Ar); 7.52 (dd, 1H, $J$ = 1.6, 8.1 Hz, Ar) ppm.

### *6-Bromo-4-[(trimethylsilyl)oxy]-2-(4-chlorophenyl)-2-methyl-3,4-dihydro-2H-chromene-4-carbonitrile* ***14b***

Yield: 84%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.30 (s, 9H, Me); 1.70 (s, 3H, Me); 2.55 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.78 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 6.92 (d, 1H, $J$ = 8.8 Hz, Ar); 7.28–7.36 (m, 4H, Ar); 7.42 (dd, 1H, $J$ = 2.1, 8.8 Hz, Ar); 7.59 (d, 1H, $J$ = 2.1 Hz, Ar) ppm.

### *4-[(Trimethylsilyl)oxy]-2-(4-methoxyphenyl)-3,4-dihydro-2H-chromene-4-carbonitrile* ***33a***

The crude product was purified by flash column chromatography eluted with AcOEt/Hexane 1:9. Yield: 30%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.30 (s, 9H, Me); 2.42–2.68 (m, 2H, $CH_2$); 3.84 (s, 3H, OMe); 5.30–5.37 (m, 1H, CH); 6.87–7.07 (m, 2H, Ar); 6.97 (d, 2H, $J$ = 8.7 Hz, Ar); 7.23–7.32 (m, 1H, Ar); 7.40 (d, 2H, $J$ = 8.7 Hz, Ar); 7.57 (dd, 1H, $J$ = 1.6, 7.9 Hz, Ar) ppm.

### *6-Bromo-4-[(trimethylsilyl)oxy]-2-(4-methoxyphenyl)-3,4-dihydro-2H-chromene-4-carbonitrile* ***33b***

Yield: 77%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.32 (s, 9H, Me); 2.34–2.66 (m, 2H, $CH_2$); 3.81 (s, 3H, OMe); 5.38 (dd, 1H, $J$ = 3.4, 12.9 Hz, CH); 6.85–7.05 (m, 3H, Ar); 7.26–7.43 (m, 3H, Ar); 7.65–7.72 (m, 1H, Ar) ppm.

### *2-(4-Chlorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***34a***

Yield: 84%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.29 (s, 9H, Me); 2.36–2.67 (m, 2H, $CH_2$); 5.35 (m, 1H, CH); 6.89–6.93 (m, 1H, Ar); 7.01–7.09 (m, 1H, Ar); 7.30–7.42 (m, 5H, Ar); 7.65–7.72 (dd, 1H, $J$ = 1.5, 7.8 Hz, Ar) ppm.

*6-Bromo-2-(4-chlorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***34b***

The crude product was purified by flash column chromatography eluted with $CHCl_3$/hexane 1:1. Yield: 43%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.31 (s, 9H, Me); 2.32–2.44 (m, 1H, $CH_2$); 2.62 (dd, 1H, $J$ = 2.0, 13.4 Hz, $CH_2$); 5.36 (dd, 1H, $J$ = 2.0, 11.8 Hz, CH); 6.80 (d, 1H, $J$ = 8.8 Hz, Ar); 7.35–7.47 (m, 5H, Ar); 7.65 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*2-(2,4-Dichlorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***35a***

Yield: 69%; $^1$H-NMR($CDCl_3$) $\delta$ 0.21 (s, 9H, Me); 2.22 (dd, 1H, $J$ = 11.8, 13.6 Hz, $CH_2$); 2.70 (dd, 1H, $J$ = 1.9, 13.6 Hz, $CH_2$); 5.70 (dd, 1H, $J$ = 1.9, 11.8 Hz, CH); 6.98 (dd, 1H, $J$ = 1.1, 8.3 Hz, Ar); 7.07 (dt, 1H, $J$ = 1.1, 7.6 Hz, Ar); 7.32–7.46 (m, 3H, Ar); 7.59–7.65 (m, 2H, Ar) ppm.

*6-Bromo-2-(2,4-dichlorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***35b***

Yield: 60%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.31 (s, 9H, Me); 2.17 (dd, 1H, $J$ = 11.7, 13.4 Hz, $CH_2$); 2.82 (dd, 1H, $J$ = 1.7, 13.4 Hz, $CH_2$); 5.72 (dd, 1H, $J$ = 1.7, 11.7 Hz, CH); 6.84 (d, 1H, $J$ = 8.8 Hz, Ar); 7.33–7.38 (m, 1H, Ar); 7.40 (dd, 1H, $J$ = 2.4, 8.8 Hz, Ar); 7.46 (d, 1H, $J$ = 2.0 Hz, Ar); 7.57 (d, 1H, $J$ = 8.4 Hz, Ar); 7.66 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*6-Bromo-2-(2,4-difluorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***36b***

Yield: 48%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.31 (s, 9H, Me); 2.43 (dd, 1H, $J$ = 11.9, 13.3 Hz, $CH_2$); 2.68 (dd, 1H, $J$ = 2.0, 13.3 Hz, $CH_2$); 5.62 (dd, 1H, $J$ = 2.0, 11.9 Hz, CH); 6.80 (d, 1H, $J$ = 8.8 Hz, Ar); 6.84–7.01 (m, 2H, Ar); 7.39 (dd, 1H, $J$ = 2.4, 8.8 Hz, Ar); 7.45–7.57 (m, 1H, Ar); 7.65 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*6-Bromo-2-(4-bromo-2-fluorophenyl)-4-[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromene-4-carbonitrile* ***37b***

Yield: 89%; $^1$H-NMR ($CDCl_3$) $\delta$ 0.31 (s, 9H, Me); 2.40 (dd, 1H, $J$ = 11.7, 13.3 Hz, $CH_2$); 2.68 (dd, 1H, $J$ = 1.7, 13.3 Hz, $CH_2$); 5.61 (dd, 1H, $J$ = 1.7, 11.7 Hz, CH); 6.81 (d, 1H, $J$ = 8.8 Hz, Ar); 7.30–7.42 (m, 4H, Ar); 7.64 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*General procedure for preparation of compounds* ***15a,b, 16a,b, 38a,b–40a,b, 41b, 42b***

A solution of the opportune trimethylsilyl cyanohydrins (5.00 mmol) in THF (7 mL) was added dropwise at 0°C to a solution of 1 M $LiAlH_4$ in THF (10 mmol). The reaction mixture was stirred at room temperature for 1 h, then quenched with water and 1 N NaOH. The resulting lithium salts were filtered and the solution was evaporated.

*4-(Aminomethyl)-2-(4-methoxyphenyl)-2-methyl-3,4-dihydro-2H-chromen-4-ol* ***15a***

Yield: 94%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.68 (s, 3H, Me); 2.10 (d, 1H, $J$ = 13.3 Hz, $CH_2$); 2.24 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 2.49 (d, 1H, $J$ = 13.3 Hz, $CH_2$); 2.56 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 3.78 (s, 3H, OMe); 6.81–7.03 (m, 4H, Ar); 7.19–7.41 (m, 4H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(4-methoxyphenyl)-2-methyl-3,4-dihydro-2H-chromen-4-ol* ***15b***

Yield: 75%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.64 (s, 3H, Me); 2.02 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 2.19 (d, 1H, $J$ = 14.2 Hz, $CH_2$); 2.38 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 2.53 (d, 1H, $J$ = 14.2 Hz, $CH_2$); 3.76 (s, 3H, OMe); 6.80–6.90 (m, 3H, Ar); 7.21–7.32 (m, 3H, Ar); 7.48 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*4-(Aminomethyl)-2-(4-chlorophenyl)-2-methyl-3,4-dihydro-2H-chromen-4-ol* ***16a***

Yield: 97%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.67 (s, 3H, Me); 2.13 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 2.27 (d, 1H, $J$ = 14.1 Hz, $CH_2$); 2.41–2.57 (m, 2H, $CH_2$); 6.92–7.07 (m, 2H, Ar); 7.22–7.41 (m, 6H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(4-chlorophenyl)-2-methyl-3,4-dihydro-2H-chromen-4-ol* ***16b***

Yield: 90%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.66 (s, 3H, Me); 2.11 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 2.25 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.46 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 2.51 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 6.89 (d, 1H, $J$ = 8.7 Hz, Ar); 7.25–7.37 (m, 5H, Ar); 7.51 (d, 1H, $J$ = 2.2 Hz, Ar) ppm.

*4-(Aminomethyl)-2-(4-methoxyphenyl)-3,4-dihydro-2H-chromen-4-ol* ***38a***

Yield: 76%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.15–2.33 (m, 2H, $CH_2$); 3.02 (d, 1H $J$ = 13.2 Hz, $CH_2$); 3.11 (d, 1H, $J$ = 13.2 Hz, $CH_2$); 3.83 (s, 3H, OMe); 5.09 (dd, 1H, $J$ = 4.2, 10.4 Hz, CH); 6.73–7.08 (m, 4H, Ar); 7.15–7.23 (m, 1H, Ar); 7.38 (d, 2H, $J$ = 8.6 Hz, Ar); 7.47–7.50 (m, 1H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(4-methoxyphenyl)-3,4-dihydro-2H-chromen-4-ol* ***38b***

Yield: 43%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.90–2.55 (m, 2H, $CH_2$); 3.53–3.94 (m, 5H, $CH_2$, OMe); 4.96–5.13 (m, 1H, CH); 6.71–6.96 (m, 3H, Ar); 7.03–7.39 (m, 3H, Ar); 7.54–7.64 (m, 1H, Ar) ppm.

*4-(Aminomethyl)-2-(4-chlorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***39a***

Yield: 99%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.06–2.37 (m, 2H, $CH_2$); 2.89–3.25 (m, 2H, $CH_2$); 5.14 (dd, 1H, $J$ = 2.6, 12.3 Hz, CH); 6.85–7.02 (m, 2H, Ar); 7.16–7.25 (m, 1H, Ar); 7.33–7.41 (m, 4H, Ar); 7.49 (dd, 1H, $J$ = 1.7, 7.8 Hz, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(4-chlorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***39b***

Yield: 81%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.01–2.35 (m, 2H, $CH_2$); 2.89–3.13 (m, 2H, $CH_2$); 5.08–5.17 (m, 1H, CH); 6.75 (d, 1H, $J$ = 8.6 Hz, Ar); 7.16–7.50 (m, 5H, Ar); 7.60–7.62 (m, 1H, Ar) ppm.

*4-(Aminomethyl)-2-(2,4-dichlorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***40a***

Yield: 76%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.78–2.47 (m, 2H, $CH_2$); 2.96–3.35 (m, 2H, $CH_2$); 5.45–5.52 (m, 1H, CH); 6.88–7.04 (m, 2H, Ar); 7.18–7.66 (m, 5H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(2,4-dichlorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***40b***

Yield: 87%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.81–1.94 (m, 1H, $CH_2$); 2.41 (dd, 1H, $J$ = 1.8, 13.7 Hz, $CH_2$); 2.94 (d, 1H, $J$ = 13.4 Hz, $CH_2$); 3.23 (d, 1H, $J$ = 13.4 Hz, $CH_2$); 5.46 (dd, 1H, $J$ = 1.8, 12.8 Hz, CH); 6.79 (d, 1H, $J$ = 8.8 Hz, Ar); 7.29 (dd, 1H, $J$ = 2.4, 8.8 Hz, Ar); 7.36–7.41 (m, 2H, Ar); 7.58–7.62 (m, 2H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(2,4-difluorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***41b***

Yield 67%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.10–2.56 (m, 2H, $CH_2$); 2.93–3.20 (m, 2H, $CH_2$); 5.34–5.47 (m, 1H, CH); 6.77 (d, 1H, $J$ = 8.6 Hz, Ar); 6.85–7.02 (m, 2H, Ar); 7.17–7.30 (m, 1H, Ar); 7.39–7.64 (m, 2H, Ar) ppm.

*4-(Aminomethyl)-6-bromo-2-(4-bromo-2-fluorophenyl)-3,4-dihydro-2H-chromen-4-ol* ***42b***

Yield: 77%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.98–2.40 (m, 2H, $CH_2$); 2.92–3.21 (m, 2H, $CH_2$); 5.37–5.50 (m, 1H, CH); 6.77 (dd, 1H, $J$ = 2.6, 8.6 Hz, Ar); 6.90–7.62 (m, 5H, Ar) ppm.

*General procedure for preparation of compounds* ***17a,b, 18a,b, 43a,b–45a,b, 46b, 47b***

A solution of aminoalcohols (6.33 mmol) in THF (20 mL) was added dropwise to a solution of *N-N′*-carbonyl diimidazole (CDI) (1.02 g, 6.33 mmol) in THF (10 mL) at 0°C. The reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated and the residue diluted with AcOEt and washed with 1 N HCl and saturated aqueous $K_2CO_3$. The organic layers were dried and concentrated.

*2-(4-Methoxyphenyl)-2-methyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***17a***

Yield: 25%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.65 (s, 3H, Me); 2.37 (d, 2H, $J$ = 14.5 Hz, $CH_2$); 2.69–2.94 (m, 2H, $CH_2$); 3.75 (s, 3H, OMe); 6.73–7.07 (m, 4H, Ar); 7.21–7.42 (m, 4H, Ar) ppm.

*6-Bromo-2-(4-methoxyphenyl)-2-methyl-2,3-dihydro-2′H-spiro[chromene-4,5′[1,3]oxazolidin]-2′-one* ***17b***

Yield: 75%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.73 (s, 3H, Me); 2.63–2.65 (m, 2H, $CH_2$); 3.06–3.20 (m, 2H, $CH_2$); 3.79 (s, 3H, OMe); 6.85 (d, 2H, $J$ = 8.9 Hz, Ar); 6.92 (d, 1H, $J$ = 8.8 Hz Ar); 7.23 (d, 2H, $J$ = 8.9 Hz, Ar); 7.40 (dd, 1H, $J$ = 2.3, 8.8 Hz, Ar); 7.51 (d, 1H, $J$ = 2.3 Hz, Ar) ppm.

*2-(4-Chlorophenyl)-2-methyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***18a***

Yield: 98%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.73 (s, 3H, Me); 2.55 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 2.72 (d, 1H, $J$ = 14.3 Hz, $CH_2$); 3.18 (d, 1H, $J$ = 9.2 Hz, $CH_2$); 3.36 (d, 1H, $J$ = 9.2 Hz, $CH_2$); 6.92–7.46 (m, 8H, Ar) ppm.

*6-Bromo-2-(4-chlorophenyl)-2-methyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***18b***

Yield: 88%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.72 (s, 3H, Me); 2.54 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 2.69 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 3.16 (d, 1H, $J$ = 9.2 Hz, $CH_2$); 3.31 (d, 1H, $J$ = 9.2 Hz, $CH_2$); 6.92 (d, 1H, $J$ = 8.8 Hz, Ar); 7.25–7.44 (m, 5H, Ar); 7.53 (d, 1H, $J$ = 2.2 Hz, Ar) ppm.

*2-(4-Methoxyphenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***43a***

The crude product was purified by flash column chromatography eluted by AcOEt/hexane 3:2. Yield: 30%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.41–2.69 (m, 2H, $CH_2$); 3.73–3.88 (m, 5H, $CH_2$, OMe); 5.00–5.07 (m, 1H, CH); 6.86–7.07 (m, 4H Ar); 7.23–7.30 (m, 1H, Ar); 7.39 (d, 2H, $J$ = 8.7 Hz, Ar); 7.44–7.48 (m, 1H, Ar) ppm.

*6-Bromo-2-(4-methoxyphenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***43b***

Yield: 92%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.04–2.65 (m, 2H, $CH_2$); 3.68–3.87 (m, 5H, $CH_2$, OMe); 5.00–5.13 (m, 1H, CH); 6.64–6.96 (m, 3H Ar); 7.03–7.39 (m, 3H, Ar) 7.54–7.64 (m, 1H, Ar) ppm.

*2-(4-Chlorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***44a***

Yield: 95%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.36–2.57 (m, 2H, $CH_2$); 3.73 (d, 1H, $J$ = 9.0 Hz, $CH_2$); 3.82 (d, 1H, $J$ = 9.0 Hz, $CH_2$); 5.04 (dd, 1H, $J$ = 3.6, 10.9 Hz, CH); 6.91 (dd, 1H, $J$ = 0.9, 8.2 Hz, Ar); 6.98–7.07 (m, 1H, Ar); 7.23–7.32 (m, 1H, Ar); 7.37–7.45 (m, 4H, Ar) ppm.

*6-Bromo-2-(4-chlorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***44b***

Yield: 73%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.41–2.62 (m, 2H, $CH_2$); 3.73–3.89 (m, 2H, $CH_2$); 5.01–5.11 (m, 1H, CH); 6.81 (d, 1H, $J$ = 8.8 Hz, Ar); 7.29–7.49 (m, 5H, Ar); 7.58 (d, 1H, $J$ = 2.3 Hz, Ar) ppm.

*2-(2,4-Dichlorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***45a***
Yield: 80%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.23–2.39 (m, 1H, $CH_2$); 2.58–2.69 (m, 1H, $CH_2$); 3.85–3.95 (m, 2H, $CH_2$); 5.40–5.54 (m, 1H, CH); 6.93–6.99 (m, 1H, Ar); 7.02–7.11 (m, 1H, Ar); 7.25–7.50 (m, 4H, Ar); 7.65–7.76 (m, 1H, Ar) ppm.

*6-Bromo-2-(2,4-dichlorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***45b***
The crude product was purified by crystallization from EtOH. Yield: 44%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.20–2.33 (m, 1H, $CH_2$); 2.61 (dd, 1H, $J$ = 1.4, 13.6 Hz, $CH_2$); 3.87 (s, 2H, $CH_2$); 5.41 (dd, 1H, $J$ = 1.4, 12.3 Hz, CH); 6.85 (d, 1H, $J$ = 8.9 Hz, Ar); 7.35–7.44 (m, 3H, Ar); 7.60 (d, 1H, $J$ = 2.4 Hz, Ar); 7.63 (d, 1H, $J$ = 8.4 Hz, Ar) ppm.

*6-Bromo-2-(2,4-difluorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***46b***
Yield: 66%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.45–2.54 (m, 2H, $CH_2$); 3.79 (d, 1H, $J$ = 8.9 Hz, $CH_2$); 3.86 (d, 1H, $J$ = 8.9 Hz, $CH_2$); 5.26–5.45 (m, 1H, CH); 6.74–7.14 (m, 3H, Ar); 7.21–7.67 (m, 3H, Ar) ppm.

*6-Bromo-2-(4-bromo-2-fluorophenyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one* ***47b***
The crude product was purified by crystallization from EtOH. Yield: 77%; $^1$H-NMR ($CDCl_3$) $\delta$ 2.31–2.56 (m, 2H, $CH_2$); 3.79 (d, 1H, $J$ = 9.1 Hz, $CH_2$); 3.86 (d, 1H, $J$ = 9.1 Hz, $CH_2$); 5.35 (dd, 1H, $J$ = 3.3, 10.7 Hz, CH); 6.82 (d, 1H, $J$ = 8.8 Hz, Ar); 7.28–7.53 (m, 4H, Ar); 7.59 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*General procedure for preparation of compounds* ***19a,b, 20a,b, 48a,b–50a,b, 51b, 52b***
A solution of (1.50 mmol) in dry THF (7 mL) was added dropwise, at −78°C, under $N_2$ atmosphere to a solution of *n*-BuLi (1.05 mL, 1.70 mmol, 1.6 M in hexane) and the reaction mixture was stirred for 1 h. Then, ethyl bromoacetate (0.25 g, 1.50 mmol) was added dropwise at −78°C and the resulting mixture was allowed to warm to room temperature and was stirred overnight. The mixture was quenched with $NH_4Cl$ and then the solvent was evaporated. The aqueous phase was extracted with AcOEt and the organic layer was dried and evaporated.

*Ethyl[2-(4-methoxyphenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′[1,3]oxazolidin]-3′-yl]acetate* ***19a***
Yield: 38%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.20–1.32 (m, 3H, Me); 1.66 (s, 3H, Me); 2.04–2.32 (m, 2H, $CH_2$); 2.51–2.83 (m, 2H, $CH_2$); 3.14–3.40 (m, 2H, $CH_2$); 3.77 (s, OMe); 4.08–4.24 (m, 2H, $CH_2$); 6.75–7.05 (m, 4H, Ar); 7.19–7.43 (m, 4H, Ar) ppm.

*Ethyl[6-bromo-2-(4-methoxyphenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl] acetate* ***19b***
Yield: 88%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.21–1.35 (m, 3H, Me); 1.72 (s, 3H, Me); 2.67 (s, 2H, $CH_2$); 3.16 (d, 1H, $J$ = 9.0 Hz, $CH_2$); 3.29 (d, 1H, $J$ = 9.0 Hz, $CH_2$); 3.79 (s, 3H, OMe); 3.97 (s, 2H, $CH_2$); 4.18–4.29 (m, 2H, $CH_2$); 6.84 (d, 2H, $J$ = 8.6 Hz, Ar); 6.91 (d, 1H, $J$ = 8.8 Hz, Ar); 7.22–7.27 (m, 2H); 7.40 (dd, 1H, $J$ = 2.4, 8.6 Hz, Ar); 7.58 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*Ethyl[2-(4-chlorophenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***20a***
Yield: 63%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.20–1.37 (m, 3H, Me); 1.73 (s, 3H, Me); 2.61 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 2.73 (d, 1H, $J$ = 14.5 Hz, $CH_2$); 3.24 (d, 1H, $J$ = 8.9 Hz, $CH_2$); 3.49 (d, 1H, $J$ = 8.9 Hz, $CH_2$); 4.01 (s, 2H, $CH_2$); 4.22 (q, 2H, $J$ = 7.2 Hz, $CH_2$); 6.99–7.06 (m, 2H, Ar); 7.27–7.39 (m, 5H, Ar); 7.50–7.55 (m, 1H, Ar) ppm.

*Ethyl[6-bromo-2-(4-chlorophenyl)-2-methyl-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl] acetate* ***20b***
Yield: 76%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.21–1.34 (m, 3H, Me); 1.73 (s, 3H, Me); 2.40–2.73 (m, 2H, $CH_2$); 3.12–3.52 (m, 2H, $CH_2$); 4.01 (s, 2H, $CH_2$); 4.10–4.30 (m, 2H, $CH_2$); 6.90–7.05 (m, 1H, Ar); 7.25–7.45 (m, 5H, Ar); 7.54 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*Ethyl[2-(4-methoxyphenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***48a***
Yield: 63%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.22–1.33 (m, 3H, Me); 2.47–2.69 (m, 2H, $CH_2$); 3.78–3.94 (m, 5H, $CH_2$, OMe); 4.06–4.29 (m, 4H, $CH_2$); 4.99–5.07 (m, 1H, CH); 6.88–7.07 (m, 4H, Ar); 7.23–7.30 (m, 1H, Ar); 7.39 (d, 2H, $J$ = 8.4 Hz, Ar); 7.49–7.53 (m, 1H, Ar) ppm.

*Ethyl[6-bromo-2-(4-methoxyphenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***48b***
Yield: 70%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.25–1.58 (m, 3H, Me); 2.83–2.86 (m, 2H, $CH_2$); 3.79–3.89 (m, 5H, $CH_2$, OMe); 3.97–4.26 (m, 4H, $CH_2$); 5.46–5.50 (m, 1H, CH); 6.88–6.96 (m, 3H, Ar); 7.51–7.74 (m, 3H, Ar) 7.92–7.97 (m, 1H, Ar) ppm.

*Ethyl[2-(4-chlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***49a***
The crude product was purified by flash column chromatography eluted with AcOEt/hexane 2:3. Yield: 60%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.30 (t, 3H, $J$ = 7.1 Hz, Me); 2.45–2.62 (m, 2H, $CH_2$); 3.83 (d, 1H, $J$ = 8.5 Hz, $CH_2$); 3.91 (d, 1H, $J$ = 8.5 Hz, $CH_2$); 4.05 (d, 1H, $J$ = 18.1 Hz, $CH_2$); 4.15 (d, 1H, $J$ = 18.1 Hz, $CH_2$); 4.24 (q, 2H, $J$ = 7.1 Hz, $CH_2$); 5.00–5.15 (m, 1H, CH); 6.90–6.94 (m, 1H, Ar); 7.00–7.09 (m, 1H, Ar); 7.24–7.33 (m, 1H, Ar); 7.36–7.47 (m, 4H, Ar); 7.51 (dd, 1H, $J$ = 1.5, 7.8 Hz, Ar) ppm.

*Ethyl[6-bromo-2-(4-chlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***49b***
The crude product was purified by flash column chromatography eluted with AcOEt/hexane 2:8. Yield: 86%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.22–1.38 (m, 3H, Me); 2.24–2.62 (m, 2H, $CH_2$); 3.73–4.02 (m, 2H, $CH_2$); 4.05–4.30 (m, 4H, $CH_2$); 5.00–5.13 (m, 1H, CH); 6.80 (d, 1H, $J$ = 8.8 Hz, Ar); 7.24–7.40 (m, 5H, Ar); 7.60 (d, 1H, $J$ = 2.4 Hz, Ar) ppm.

*Ethyl[2-(2,4-dichlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***50a***
Yield: 65%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.25–1.34 (m, 3H, Me); 2.25–2.41 (m, 1H, $CH_2$); 2.62–2.70 (m, 1H, $CH_2$); 3.83–4.01 (m, 2H, $CH_2$); 4.10–4.13 (m, 2H, $CH_2$); 4.19–4.30 (m, 2H, $CH_2$); 5.40–5.51 (m, 1H, CH); 6.92–6.96 (m, 1H, Ar); 7.04–7.11 (m, 1H, Ar); 7.30–7.43 (m, 3H, Ar); 7.54 (d, 1H, $J$ = 7.7 Hz, Ar); 7.68 (d, 1H, $J$ = 8.4 Hz, Ar) ppm.

*Ethyl[6-bromo-2-(2,4-dichlorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***50b***
Yield: 79%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.21–1.34 (m, 3H, Me); 2.18–2.33 (m, 1H, $CH_2$); 2.57–2.68 (m, 1H, $CH_2$); 3.82–3.91 (m, 2H, $CH_2$); 4.06–4.20 (m, 2H, $CH_2$); 4.20–4.30 (m, 2H, $CH_2$); 5.38–5.41 (m, 1H, CH); 6.83 (d, 1H, $J$ = 8.8 Hz, Ar); 7.34–7.43 (m, 3H, Ar); 7.58–7.65 (m, 2H, Ar) ppm.

*Ethyl[6-bromo-2-(2,4-difluorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***51b***
The crude product was purified by flash column chromatography eluted with AcOEt/hexane 3:7. Yield: 52%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.31 (t, 3H, $J$ = 7.1 Hz, Me); 2.41–2.59 (m, 2H, $CH_2$); 3.84 (d, 1H, $J$ = 8.4 Hz, $CH_2$); 3.92 (d, 1H, $J$ = 8.4 Hz, $CH_2$); 4.14 (d, 2H, $J$ = 3.0 Hz, $CH_2$); 4.26 (q, 2H, $J$ = 7.1 Hz, $CH_2$); 5.31–5.38 (m, 1H, CH); 6.82 (d, 1H, $J$ = 8.8 Hz, Ar); 6.87–7.11 (m, 2H, Ar); 7.38 (dd, 1H, $J$ = 2.3, 8.8 Hz, Ar); 7.51–7.60 (m, 1H, Ar); 7.62 (d, 1H, $J$ = 2.3 Hz, Ar) ppm.

*Ethyl[6-bromo-2-(4-bromo-2-fluorophenyl)-2′-oxo-2,3-dihydro-3′H-spiro[chromene-4,5′-[1,3]oxazolidin]-3′-yl]acetate* ***52b***
Yield: 87%; $^1$H-NMR ($CDCl_3$) $\delta$ 1.32 (t, 3H, $J$ = 7.1 Hz, Me); 2.38–2.54 (m, 2H, $CH_2$); 3.81–3.95 (m, 2H, $CH_2$); 4.12 (s, 2H, $CH_2$); 4.26 (q, 2H, $J$ = 7.1 Hz, $CH_2$); 5.32–5.45 (m, 1H, CH); 6.83 (d, 1H, $J$ = 8.8 Hz, Ar); 7.07–7.16 (m, 1H, Ar); 7.33–7.54 (m, 3H, Ar); 7.62 (d, 1H, $J$ = 2.1 Hz, Ar) ppm.

## Biology

### Materials and Methods

Aldose reductase (ALR2) and aldehyde reductase (ALR1) were obtained from Sprague-Dawley albino rats, 120–140 g body weight, supplied by Harlan Nossan, Italy. To minimize cross-contamination between ALR2 and ALR1 in the enzyme preparation, rat lenses, in which ALR2 is the predominant enzyme, and kidney, where ALR1 shows the highest concentration, were used for the isolation of ALR2 and ALR1, respectively.

Pyridine coenzyme, D,L-glyceraldehyde, and sodium D-glucuronate were from Sigma-Aldrich. Sorbinil is a gift from Pfizer, Groton CT. Tolrestat was obtained from Lorestat Recordati, Italy. All other chemicals were of reagent grade.

## Enzyme preparation

### Aldose reductase (ALR2)

A purified rat lens extract was prepared in accordance with the method of Hayman and Kinoshita [20] with slight modifications. Lenses were quickly removed from rats following euthanasia and were homogenized (Glas-Potter) in 3 volumes of cold deionized water. The homogenate was centrifuged at 12 000 rpm at 0–4°C for 30 min. Saturated ammonium sulfate solution was added to the supernatant fraction to form a 40% solution, which was stirred for 30 min at 0–4°C and then centrifuged at 12 000 rpm for 15 min. Following this same procedure, the recovered supernatant was subsequently fractionated with saturated ammonium sulfate solution using first a 50% and then a 75% salt saturation. The precipitate recovered from the 75% saturated fraction, possessing ALR2 activity, was redissolved in 0.05 M NaCl and dialyzed overnight in 0.05 M NaCl. The dialyzed material was used for the enzymatic assay.

### Aldehyde reductase (ALR1)

Rat kidney ALR1 was prepared in accordance with method of Ward et al. [21]. Kidneys were quickly removed from normal killed rats and homogenized (Glas-Potter) in 3 volumes of 10 mM sodium phosphate buffer, pH 7.2, containing 0.25 M sucrose, 2.0 mM EDTA dipotassium salt, and 2.5 mM β-mercaptoethanol. The homogenate was centrifuged at 12 000 rpm at 0–4°C for 30 min, and the supernatant was subjected to a 40–75% ammonium sulfate fractionation, following the same procedure previously described for ALR2. The precipitate obtained from the 75% ammonium sulfate saturation, possessing ALR1 activity, was redissolved in 50 volumes of 10 mM sodium phosphate buffer, pH 7.2, containing 2.0 mM EDTA dipotassium salt and 2.0 mM β-mercaptoethanol, and was dialyzed overnight using the same buffer. The dialyzed material was used in the enzymatic assay.

### Enzymatic assays

The activity of the two test enzymes was determined spectrophotometrically by monitoring the change in absorbance at 340 nm, which is due to the oxidation of NADPH catalyzed

by ALR2 and ALR1. The change in pyridine coenzyme concentration/min was determined using a Beckman DU-64 kinetics software program (Solf Pack TM Module). ALR2 activity was assayed at 30°C in a reaction mixture containing 0.25 mL of 10 mM D,L-glyceraldehyde, 0.25 mL of 0.104 mM NADPH, 0.25 mL of 0.1 M sodium phosphate buffer (pH 6.2), 0.1 mL of enzyme extract, and 0.15 mL of deionized water in a total volume of 1 mL. All the above reagents, except D,L-glyceraldehyde, were incubated at 30°C for 10 min; the substrate was then added to start the reaction, which was monitored for 5 min. Enzyme activity was calibrated by diluting the enzymatic solution in order to obtain an average reaction rate of 0.011 (0.0010 absorbance units/min for the sample). ALR1 activity was determined at 37°C in a reaction mixture containing 0.25 mL of 20 mM sodium D-glucuronate, 0.25 mL of 0.12 mM NADPH, 0.25 mL of dialyzed enzymatic solution, and 0.25 mL of 0.1 M sodium phosphate buffer (pH 7.2) in a total volume of 1 mL. The enzyme activity was calibrated by diluting the dialyzed enzymatic solution in order to obtain an average reaction rate of 0.015 (0.0010 absorbance/min for the sample).

### Enzymatic inhibition

The inhibitory activity of the newly synthesized compounds against ALR2 and ALR1 was assayed by adding 0.1 mL of the inhibitor solution to the reaction mixture described above. All the inhibitors were solubilized in water and the solubility was facilitated by adjustment to a favorable pH. After complete solution, the pH was readjusted to 7. To correct for the non-enzymatic oxidation of NADPH and for absorption by the compounds tested, a reference blank containing all the above assay components except the substrate was prepared. The inhibitory effect of the new derivatives was routinely estimated at a concentration of $10^{-4}$ M. Those compounds found to be active were tested at additional concentrations between $10^{-5}$ and $10^{-8}$ M. The determination of the $IC_{50}$ values was performed by linear regression analysis of the log-dose response curve, which was generated using at least four concentrations of the inhibitor, causing an inhibition between 20% and 80%, with two replicates at each concentration. The 95% confidence limits (95% CL) were calculated from $t$ values for $n - 2$, where $n$ is the total number of determinations.

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