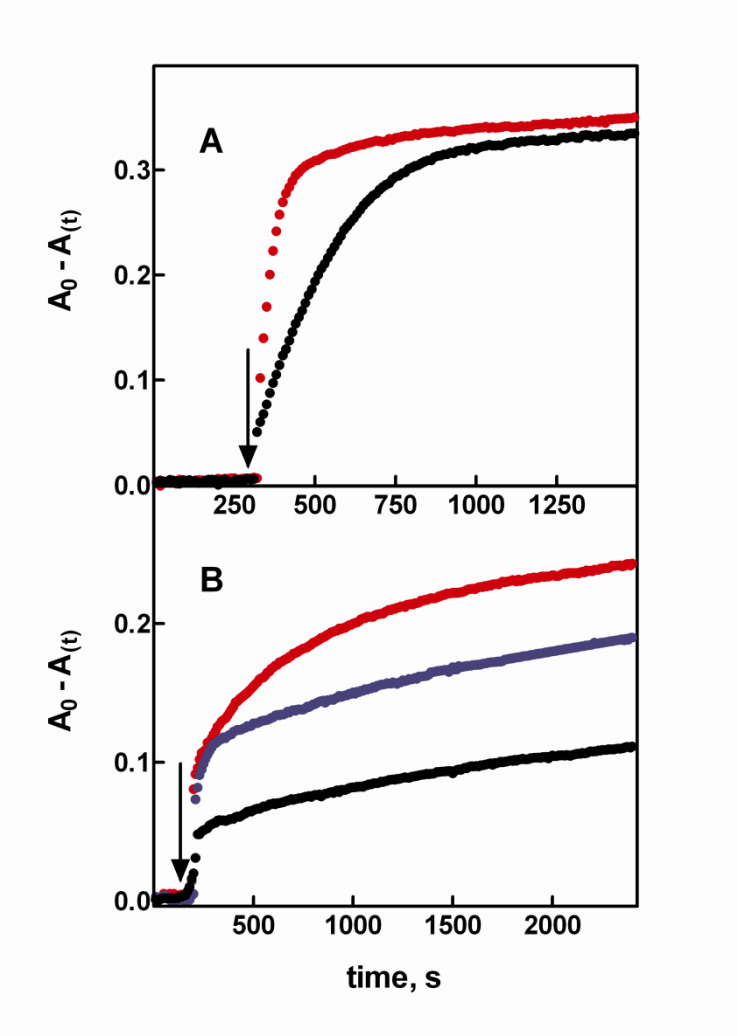
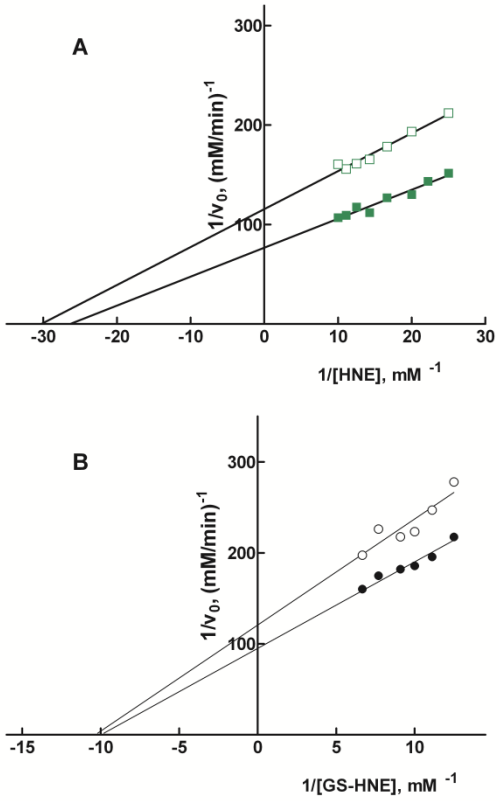
Figure S1 - Enzymatic titration of HNE and GS-HNE.



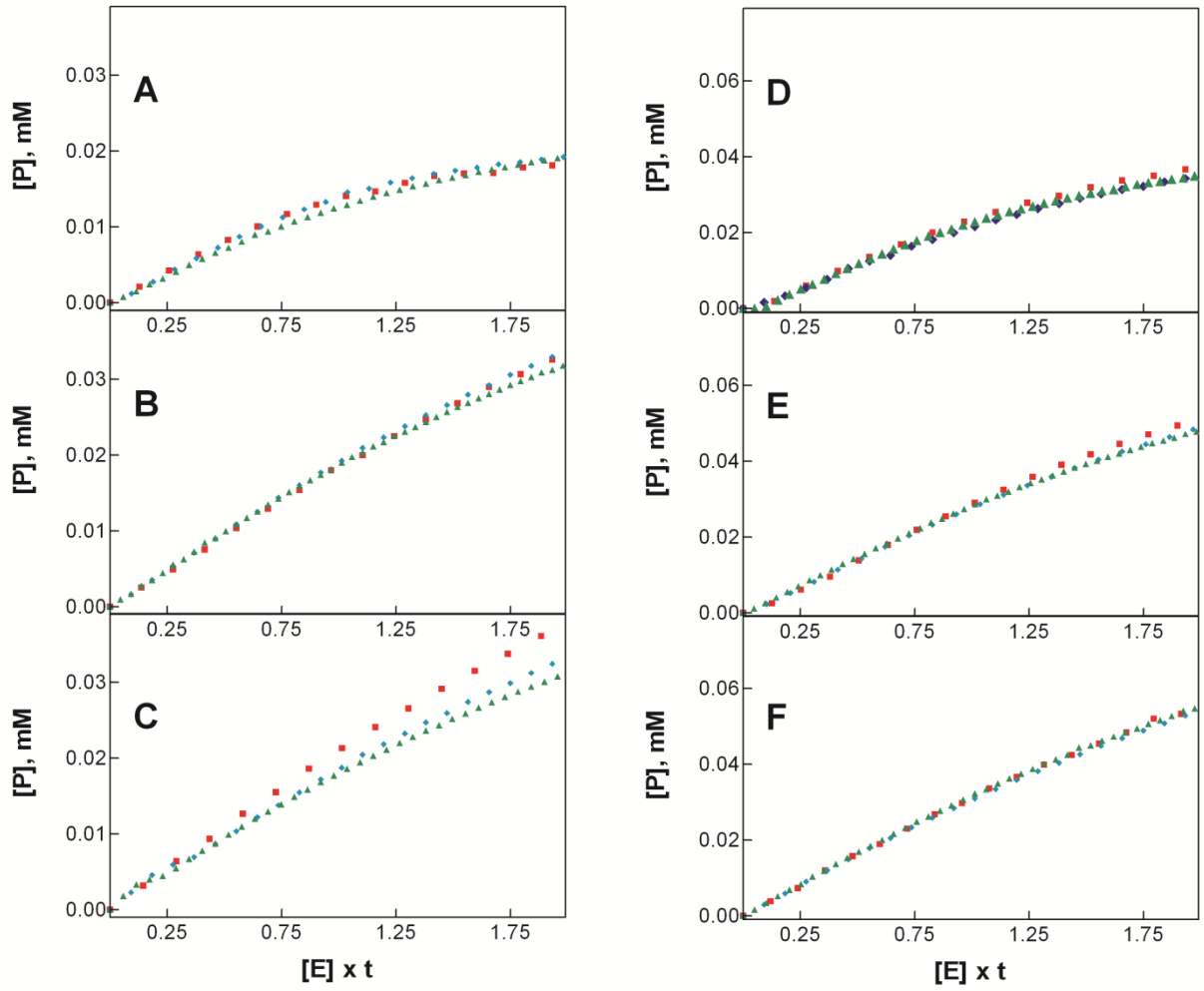
Solutions of HNE and GS-HNE were titrated through their reduction to the corresponding alchools catalyzed by AR, monitoring the decrease in absorbance at 340 nm associated to the NADPH oxidation (6.22 $mM^{-1}cm^{-1}$ extinction coefficient). The product concentration was determined by the difference of the absorbance measured at zero time (A_0) and the absorbance measured at each time corrected for the NADPH oxidation occurring in the absence of substrates (A_t). The latter reaction was considered as a first order kinetic reaction with a kinetic constant of 0.0048 min^{-1} (data not shown). *Panel A:* a 50 µM HNE solution, as measured by colorimetric assay (Gerard-Monnier et al. 1998. Chem. Res. Toxicol. 11, 1176), was incubated in the standard assay conditions at 37°C in the presence of either 8 mU (black curve) or 40 mU (red curve) of AR. The absorbance measured after 25 min accounted for a HNE concentration of 55 µM. *Panel B:* a 40 µM GS-HNE solution, as measured by colorimetric assay (Gerard-Monnier et al. 1998. Chem. Res. Toxicol. 11, 1176), was incubated in the standard assay conditions at 37 °C in the presence of 8 mU (black curve), 40 mU (blue curve) and 160 mU (red curve) of AR. The absorbance measured after 40 min in the presence the highest enzyme concentration (red curve) accounted for a GS-HNE concentration of 39 µM.
It is worth noting the higher amount of AR, with respect to the HNE titration, required to measure in a reasonable time the concentration of GS-HNE. This is consistent with the equilibrium to be displaced between GS-HNE hemiacetal and the free aldehyde.

Figure S2 - Effect of pH on the reduction of HNE and GS-HNE catalyzed by AR.



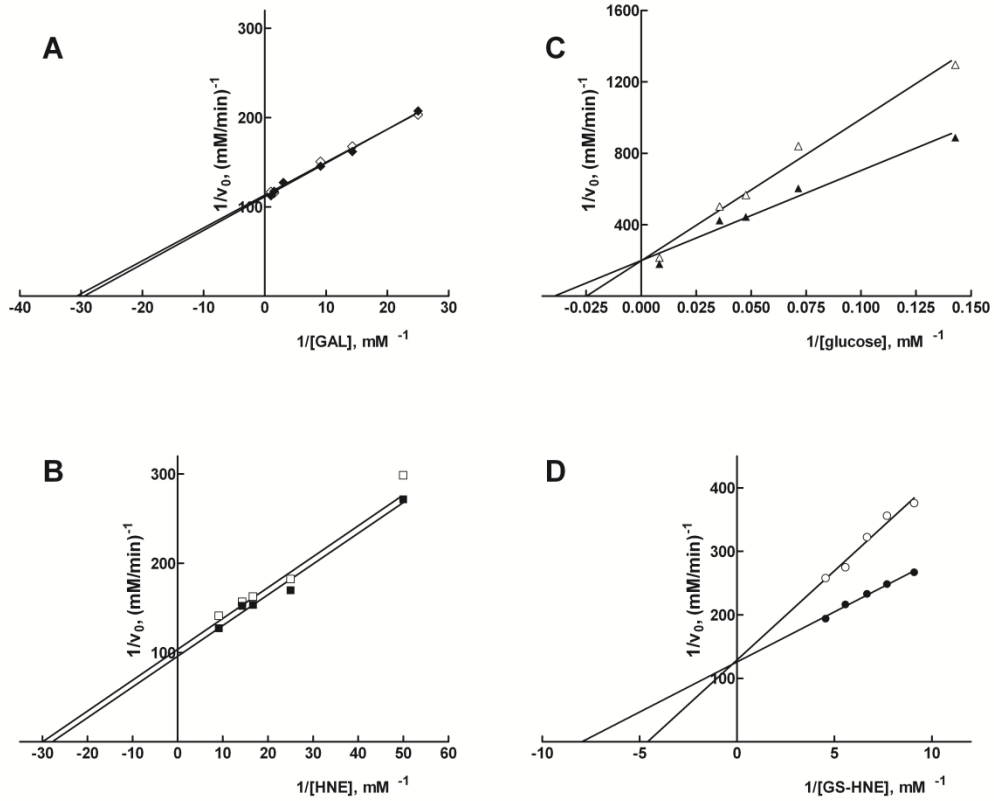
Double reciprocal plots of initial rate measurements of the AR catalyzed reduction of HNE (*Panel A*) and GS-HNE (*Panel B*). The assay was performed in standard conditions using 8 mU of purified AR at pH 6.8 (closed symbols) and pH 6.0 (open symbols).

Figure S3 - Selwyn test for AR acting on HNE and GAL as substrates.



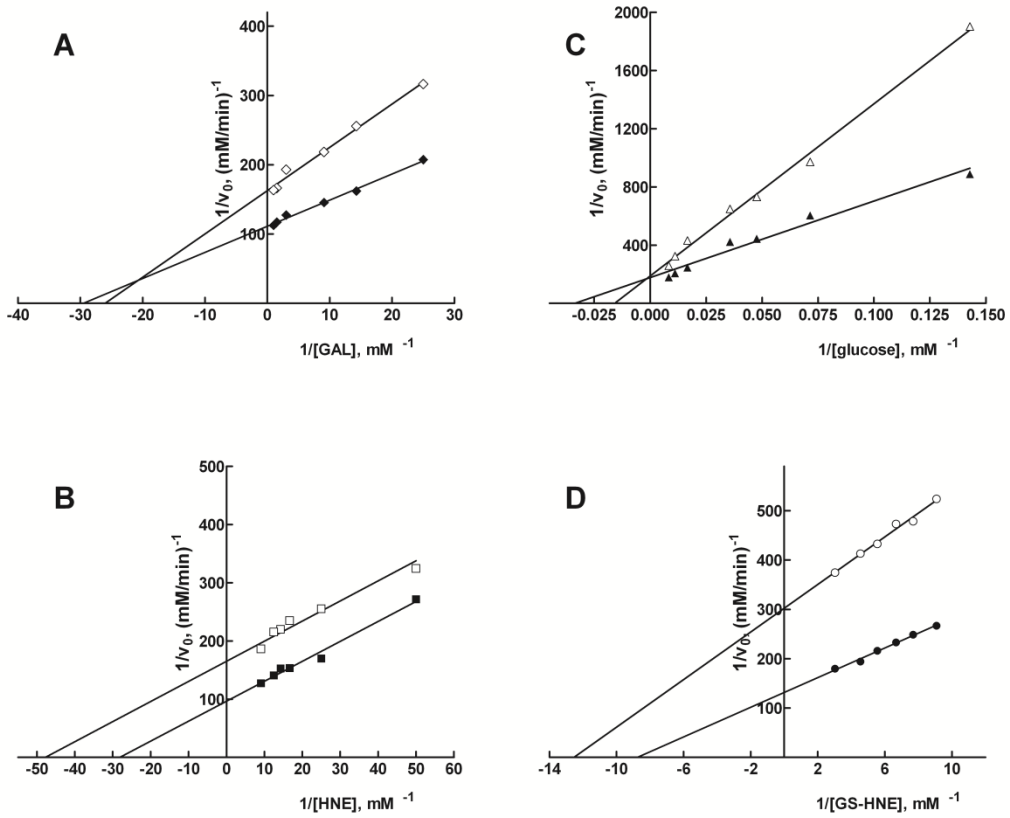
The AR catalyzed reaction was subjected to a Selwyn test using as substrates either HNE at a final concentration of 50, 110, 220 µM (Panels A, B and C, respectively) or GAL at a final concentration of 60, 110, 220 µM (Panels D, E and F, respectively).
In each panel the three curves refer to 3.5 mU (triangles), 5.6 mU (diamonds) and 8.8 mU (squares) of purified AR. The reaction was monitored following the decrease of absorbance at 340 nm linked to NADPH oxidation (extinction coefficient of 6.22 $mM^{-1}cm^{-1}$). The product concentration at different times was determined by the difference of the absorbance measured at zero time and the absorbance measured at each time corrected for the NADPH oxidation occurring in the absence of substrates. The latter reaction was considered as a first order kinetic reaction with a kinetic constant of 0.0048 min^{-1} (data not shown). The enzyme concentration was calculated on the basis of a molecular weight of 34 KDa.

Figure S4 - Inhibition models of compound 18 on the AR dependent reduction of different substrates.



Double reciprocal plots of initial rate measurements of the AR catalyzed reduction of different substrates either in the absence (closed symbols) or in the presence (open symbols) of 47 µM of compound **18**. The assay was performed in standard conditions using approximately 8 mU of purified AR.

Figure S5 - Inhibition models of compound 19 on the AR dependent reduction of different substrates.



Double reciprocal plots of initial rate measurements of the AR catalyzed reduction of different substrates either in the absence (closed symbols) or in the presence (open symbols) of 47 μM of compound **19**. The assay was performed in standard conditions using approximately 8 mU of purified AR.

Protocol S1

Synthesis of AR inhibitors

Chemistry. Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Routine nuclear magnetic resonance spectra were recorded in DMSO-d_6 solution on a Varian Gemini 200 spectrometer operating at 200 MHz. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Optical rotations were collected on a Perkin-Elmer 241 Polarimeter at 20±2 °C in water. Evaporations were performed in vacuo (rotary evaporator). Analytical TLCs were carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254) and on Merk RP-18 glass plates (RP-18 F-254s). Flash Master Personal from Biotage was used as chromatographic system, with Snap Cartridges KP-C18-HS or KP-Sil from Biotage as columns.

Synthesis of 4-substituted-4,7-dihydro-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylic acids (compounds 18 and 19). The commercially available 3-aminopyrazole (10.0 mmol) was dissolved in 20 mL of glacial acetic acid. Diethyl ethoxymethylenemalonate (2.42 mL, 12.0 mmol) was then added and the resulting mixture was refluxed under stirring until the disappearance of the starting material (TLC analysis). The so obtained ethyl 4,7-dihydro-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate, separated as a white solid, was collected by filtration, suspended in 5 mL of DMF and allowed to react, at 90 °C, with equimolar amounts of the suitable arylalkyl halide and anhydrous K_2CO_3 until the disappearance of the starting materials (TLC analysis). The solvent was then removed under reduced pressure and the resulting crude material was treated with crushed ice. The 4-substituted ester, separated as a pale yellow solid, was collected by filtration and hydrolyzed to the corresponding carboxylic acid through reaction with 5% NaOH, at 100° C, followed by acidification with concentrated HCl, under ice-cooling. The desired acid was collected, purified by crystallization from MeOH and characterized through physico-chemical and spectroscopic data.

Compound **18**: Yield: 74%. M.p. 242-245 °C. ^{1}H-NMR (δ, ppm): 12.70 (s, 1H, COOH, exc.), 9.01 (s, 1H, H_5), 8.02 (d, 1H, H_2), 7.42-7.35 (m, 5H, ArH), 6.54 (d, 1H, H_3), 5.49 (s, 2H, CH_2).

Compound **19**: Yield: 26%. M.p. 260°C dec. ^{1}H-NMR (δ, ppm): 9.13 (s, 1H, H_5), 8.40 (s, 1H, $H_{2'}$), 8.17 (d, 1H, $H_{4'}$), 8.00 (d, 1H, H_2), 7.87 (d, 1H, $H_{6'}$), 7.66 (t, 1H, $H_{5'}$), 6.56 (d, 1H, H_3), 5.63 (s, 2H, CH_2).

Synthesis of 2-(5-methyl-7-oxo-4-phenethyl-4,7-dihydropyrazolo[1,5-a]pyrimidin-6-yl)acetic acid (compound 20). The commercially available 3-aminopyrazole (10.0 mmol) was dissolved in 20 mL of glacial acetic acid. Diethyl acetylsuccinate (2.40 mL, 12.0 mmol) was then added and the resulting mixture was refluxed under stirring until the disappearance of the starting material (TLC analysis). The so obtained ethyl 2-(4,7-dihydro-5-methyl-7-oxopyrazolo[1,5-*a*]pyrimidin-6-yl)acetate, separated as a white solid, was collected by filtration, suspended in 5 mL of DMF and allowed to react, at 90 °C, with equimolar amounts of the suitable arylalkyl halide and anhydrous K_2CO_3 until the disappearance of the starting materials (TLC analysis). The solvent was then removed under reduced pressure and the resulting crude material was treated with crushed ice. The 4-substituted ester, separated as a pale yellow solid, was collected by filtration and hydrolyzed to the corresponding carboxylic acid through reaction with 5% NaOH, at 100° C, followed by acidification with concentrated HCl, under ice-cooling. The desired acid was collected, purified by crystallization from EtOH and characterized by physico-chemical and spectroscopic data.

Yield: 35%, m.p. 275-277 °C. ^{1}H-NMR: 7.92 (s, 1H, H_2), 7.28 (s, 5H, ArH), 6.45 (s, 1H, H_3), 4.32 (t, 2H, CH_2), 3.55 (s, 2H, CH_2), 3.02 (t, 2H, CH_2), 2.27 (s, 3H, CH_3)

Synthesis of D-glyceramide and L-glyceramide (compounds 21 and 22) . The suitable D- or L-glyceric acid calcium salt dihydrate (10.0 mmol), was converted to the corresponding acid by treatment with equimolar amount of HCl 0.1 N. Water was evaporated in vacuo and the resulting residue was diluted with methanol, filtered to remove salt, and allowed to react with trimethylsylil diazomethane, added dropwise until the persistence of a yellow colour, to obtain the key intermediate methyl D- or L-glycerate (TLC analysis). The solvent was then evaporated to dryness and the residue was diluted with 15.0 mL of 30% ammonium hydroxide and left under stirring at room temperature until the disappearance of the starting materials (TLC analysis). The solvent was then removed under reduced pressure and the resulting residue was diluted with methanol, filtered, and evaporated to dryness to obtain the target amide as a colorless syrup, which was purified by column chromatography (KP-C18-HS cartridge, CH_2Cl_2:MeOH = 9:1 as the eluant) and characterized by physico-chemical and spectroscopic data.

D-Glyceramide. Colourless syrup. Yield: 40%. $[\alpha]_D$: +14.8 (c=10%, H_2O). ^{1}H-NMR (δ, ppm): 7.15 (d, 2H, NH_2, exc.), 3.81-3.75 (m, 2H, CH_2), 3.55-3.39 (m, 1H, CH). *m/z*= [M^+] 105 (12), 55 (100).

L-Glyceramide. Colourless syrup. Yield: 54%. $[\alpha]_D$: -12.5 (c=10.4, H_2O). ^{1}H-NMR (δ, ppm): 7.17 (d, 2H, NH_2, exc.), 3.80-3.77 (m, 2H, CH_2), 3.59-3.39 (m, 1H, CH). *m/z*= [M^+] 105 (14), 55 (100).

Synthesis of L-erythronamide, D-arabinonamide and L-arabinonamide (compounds 23, 24 and 25). The suitable commercially available L-erythrose, D-arabinose or L-arabinose (10.0 mmol) was dissolved in 5 mL of water. Bromine (20.0 mmol) was added dropwise and the resulting orange solution was left overnight under stirring at room temperature. The excess of bromine was then quenched with 2% $Na_2S_2O_3$ and the solution was evaporated to dryness. The residue obtained was diluted with dry methanol, filtered, and concentrated under vacuum to a colorless syrup, which was allowed to react with 30% ammonium hydroxide (10.0 mL) until the disappearance of the starting material (TLC analysis). The reaction mixture was evaporated to dryness and the resulting residue was washed with dry methanol, filtered, and evaporated under reduced pressure. The so obtained amide was purified by column chromatography (KP-C18-HS cartridge, CH_2Cl_2:MeOH = 9:1 as the eluant) and characterized by physico-chemical data.

L-erythronamide. Yield 35%. M.p. 97-100 °C (Lit.[1] 91-92 °C). **D-arabinonamide**. Yield 35%. M.p.125-127 °C (Lit.[2]: 138-139 °C). **L-arabinonamide**. Yield 34%. M.p. 100-101 °C (Lit.[3] 132-133 °C).

Table S1. Compounds Tested as Differential Aldose Reductase Inhibitors.

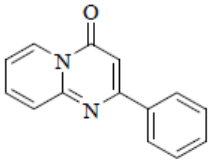
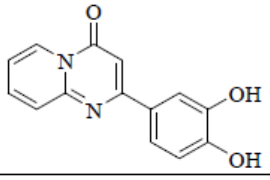
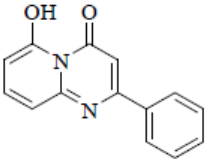
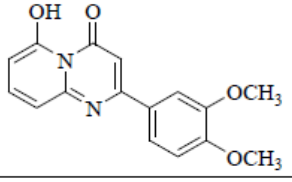
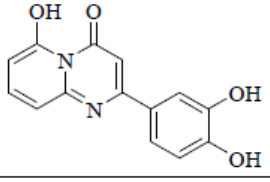
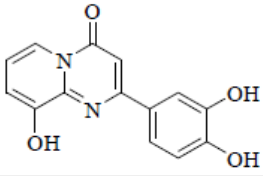
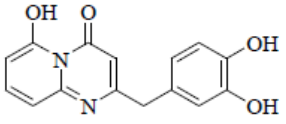
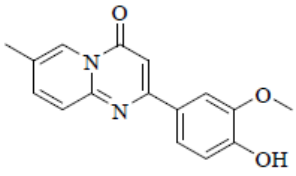
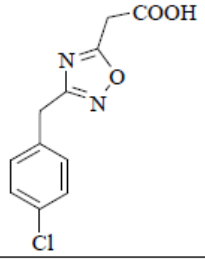
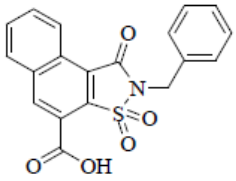
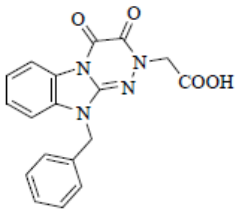
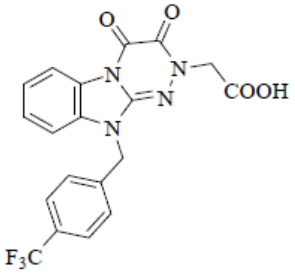
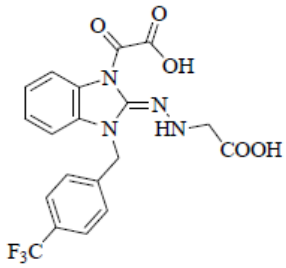
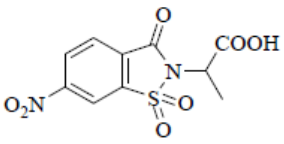
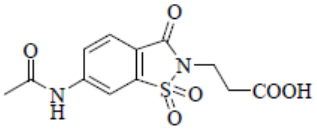
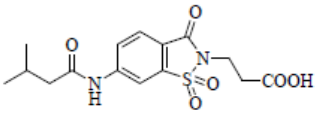
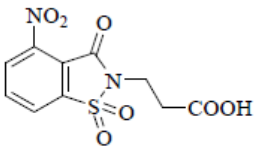
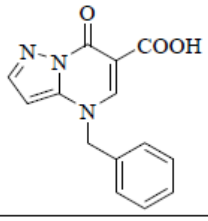
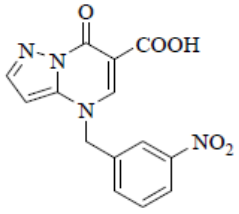
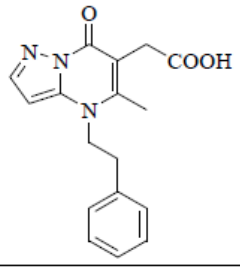
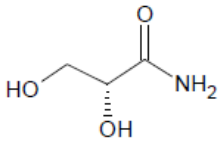
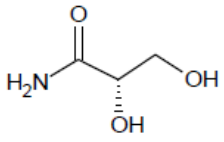
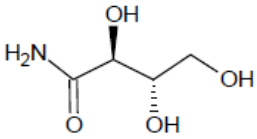
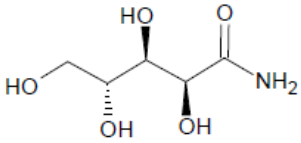
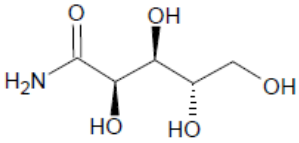
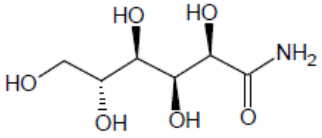
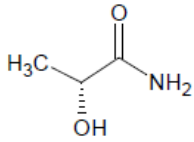
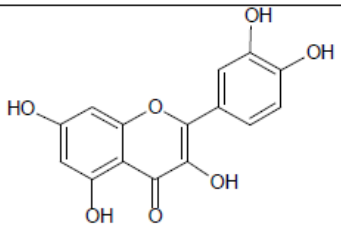
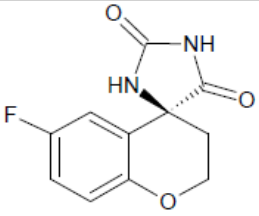
1		**2**		**3**	
4		**5**		**6**	
7		**8**		**9**	
10		**11**		**12**	
13		**14**		**15**	
16		**17**		**18**	
19		**20**			

Table S2. Compounds Tested as Differential Aldose Reductase Inhibitors.

21		**22**		**23**	
24		**25**		**26**	
27		**28**		**29**	
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