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Dual action spirobicycloimidazolidine-2,4-diones: Antidiabetic agents and inhibitors of aldose reductase-an enzyme involved in diabetic complications

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ABSTRACT

The desired 3-(arylsulfonyl)spiroimidazolidine-2,4-diones were synthesized by reacting spiroiminoimidazolidine-2,4-dione with arylsulfonyl chlorides. Spiroimidazolidine-2,4-dione was in turn synthesized from norcamphor. Structures of the synthesized molecules were established by modern spectroscopic techniques. The synthesized compounds were screened for in vivo antidiabetic activity and aldose reductase inhibition. Compounds **2a**, **2b** and **2g** exhibited excellent dual activity, compound **2a** being most prominent. These results reveal that the synthesized compounds may serve as the molecule of choice to treat diabetes and diabetic complications using a single medication.

Abnormal glucose metabolism causes an increase in the blood glucose level in diabetic patients. Prolonged hyperglycemia plays an important role in the development of diabetic complications such as atherosclerosis, neuropathy, end stage renal failure and blindness.[1] One of the important biochemical pathways that impair the function and structure of cells is the polyol pathway.[2] Polyol pathway consists of two steps, aldose reductase (ALR2, E.C.1.1.1.21) is the first enzyme involved in this pathway converting glucose into sorbitol using NADPH as a co-factor. Sorbitol dehydrogenase then converts sorbitol into fructose. Under normal glucose level in blood, glucose is converted into glucose-6-phosphate by hexokinases. As there is high affinity of hexokinases for glucose substrate, very small amount of glucose converts into sorbitol. In hyperglycaemic condition, aldose reductase converts glucose into sorbitol because of the saturation of hexokinases. Glucose influx is very low in this pathway under euglycemic conditions.[3,4] Once sorbitol is accumulated inside the cell, it cannot diffuse easily across the cell membrane causing increase in the osmotic pressure of the cell, which plays an important role in etiology of diabetic complications.[5]

Aldose reductase, the enzyme involved in the first step of polyol pathway, has been found in retina, nervous tissues, kidney, aorta and lens, that is tissues in which diabetic complications appear. Decrease in myo-inositol is observed in the peripheral tissues because of the accumulations of sorbitol in cells. Decreased myo-inositol causes the reduction in Na^+, K^+-ATPase activity, which plays an important role in nerve conduction.[6] Because of the activation of polyol pathway, as there is overutilization of NADPH, a lot of homeostatic processes are compromised. Depletion of NADPH results in reduction of nitric oxide causing circulatory abnormalities.[6] Blindness is due to the retinopathy or cataract formation. Diabetes is the major risk factor for the development of cataract and retinopathy. The risk of complications is lower if glucose level remains normal in blood, but strict glycemic control is extremely difficult.[7]

Inhibition of aldose reductase is, therefore, a potential target of drug action. The aim of the present work was to design and synthesize molecules to achieve the treatment of diabetics with a single medication that will not only control the glucose level but also reduce acute complications produced by abnormal glucose concentrations and increased aldose reductase activity. Although, thiazolidinediones and their analogues have long been used for the treatment of diabetes and to lower the aldose reductase activity, there are still a number of concerns regarding their long term safety.[8] Weight gain, hepatotoxicity[9] and edema[10] are some of the side effects of thiazolidinediones. Similarly, it has been reported that thiazolidinedione treated diabetic hypertensive patients are at a high risk for angina, congestive heart failure, cerebral vascular accident and myocardial infarction.[11] Imidazolidinediones, on the other hand, are cyclic urea derivatives and exhibit

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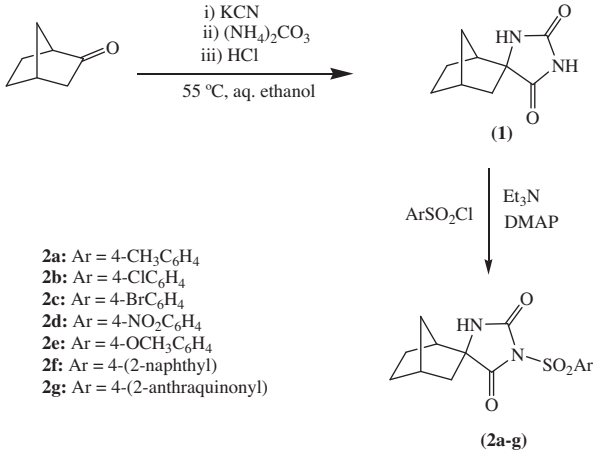
diverse biological activities, such as antitumor,[12] antiarrhythmic,[13] anticonvulsant[14] and herbicidal.[15] Therefore, we planned to combine the sulfonylureas and imidazolidinediones in a single molecule, arylsulfonylspiroimidazolidinediones, to further our previous work on antidiabetic agents.[16,17] The target compounds exhibited very good in vivo hypoglycemic activity and an excellent in vitro aldose reductae inhibition. As a result, the compounds have the potential to find use as hypoglycemic agents and in the treatment of diabetic complications, in a single medication.

Spiroimidazolidine-2,4-dione (**1**) consisting of norcamphoryl residue was obtained by employing Bucherer–Bergs reaction (Scheme 1).[18] Compound (**1**) was purified by repeated recrystallizations in ethanol-water solvent pair affording a yield of 81%. Spiroimidazolidine-2,4-dione was coupled with different arylsulfonyl chlorides in presence of triethylamine and DMAP as a catalyst to afford the desired 3-arylsulfonylspiroimidazolidine-2,4-diones (**2a**–**g**).[19]

The newly synthesized compounds were characterized by modern spectroscopic techniques. The spiroimidazolidine-2,4-dione (**1**) was identified in the IR spectrum by the presence of two peaks at 3270 and 3217 cm^{-1} assigned to the N–H of the imide and amide groups. The strong absorption bands at 1772 and 1704 cm^{-1} were assigned to two carbonyl groups. The synthesis of compound (**1**) was confirmed in the ^1H NMR spectrum where two broad singlets appeared at 10.56 and 8.44 ppm. These signals were assigned to the protons of imido and amido groups, respectively. ^{13}C NMR spectrum exhibited two low intensity signals at 157.1 and 179.8 ppm for two carbonyl group.

The coupling of spiroimidazolidine-2,4-dione (**1**) with arylsulfonyl chlorides was indicated in the IR spectra by the appearance of peaks for anti-symmetric and symmetric O=S=O absorptions in the range of 1392–1313 cm^{-1} and 1191–1141 cm^{-1}, respectively. The presence of only 1 peak for the N–H stretching in the range of 3300–3100 cm^{-1} also indicated the successful synthesis of 3-arylsulfonylspiroimidazolidine-2,4-diones (**2a**–**g**). The structures of 3-arylsulfonylspiroimidazolidine-2,4-diones (**2a**–**g**) were confirmed using ^1H NMR spectroscopy. The appearance of relatively broad N–H signal in the range of 7.94–9.12 ppm confirmed the coupling of arylsulfonyl group. The disappearance of the low field signal N-H signal confirmed that the arylsulfonylation has taken place at position 3 of spiroimidazolidine-2,4-dione. The norcamphoryl protons resonated in the region of 1.0–3.5 ppm. The aromatic protons were observed as two doublets for compounds **2a**–**e**, while as multiplets in case of compounds **2f** and **2g**. The methyl protons in compound **2a** resonated at $\delta = 2.46$, while the methoxy protons in **2e** appeared at $\delta = 3.87$ ppm. The synthesis of compounds **2a**–**g** was also confirmed in ^{13}C NMR spectra by the appearance of signals for C-2 and C-4 in the range of 150.2–151.6 and 172.7–173.8 ppm, respectively. The additional carbonyl carbons in compound **2g** resonated at $\delta = 181.6$ and 181.9 ppm. The synthesis of compounds **2a**–**g** was further confirmed in the mass spectra. A weak molecular ion peak was observed in all the cases. The characteristic loss of SO_2 and $ArSO_2$ was also observed for all the compounds. The isotopic M + 2 peaks were present in the mass spectra of compounds **2b** and **2c**, confirming the presence of halogen atoms in these molecules.

Plasma glucose measurements: The synthesized compounds were tested for their antidiabetic potential on male albino rats. Plasma glucose concentration dropped gradually and significantly on



Scheme 1. Synthesis of 3-arylsulfonylspiroimidazolidin-2,4-diones (**2a**–**g**).

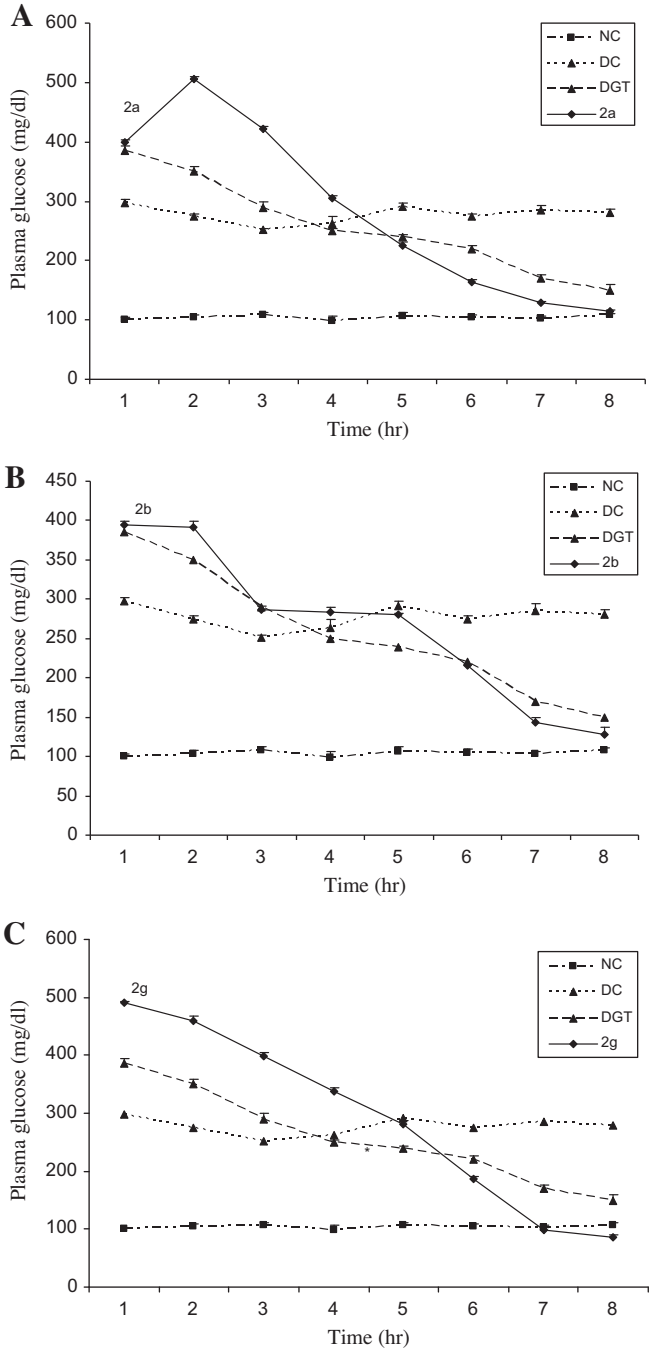


Figure 1. Significant lowering of plasma glucose in diabetic rats treated with compounds **2a**, **2b** and **2g**. $^{**}P < 0.001$.

Table 1
Plasma glucose concentration (mg/dl) in healthy rats, diabetic rats, diabetic rats treated with glibenclamide, and diabetic rats treated with compounds **2a–g**

Time (h)	NC	DC	DGT	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
0	100 ± 3.53	297 ± 5.38	385 ± 8.94	399 ± 4.11	395 ± 3.53	265 ± 3.62	388 ± 3.16	650 ± 5.38	330 ± 4.10	491 ± 2.91
1	104 ± 4.30	274 ± 4.73	350 ± 7.90	506 ± 3.45	392 ± 6.44	289 ± 1.93	399 ± 3.01	413 ± 2.94	210 ± 2.28	460 ± 7.07
2	108 ± 4.06	251 ± 2.91	289 ± 10.17	421 ± 4.30	287 ± 4.63	273 ± 3.15	429 ± 2.12	353 ± 1.42	389 ± 1.99	399 ± 5.78
3	98 ± 8.74	263 ± 10.67	250 ± 3.53	305 ± 3.53	283 ± 6.81	357 ± 3.27	482 ± 3.89	544 ± 5.32	393 ± 2.92	338 ± 5.14
4	106 ± 6.20	291 ± 6.40	239 ± 4.30	225 ± 6.51	280 ± 2.23	353 ± 4.42	421 ± 3.23	570 ± 7.69	471 ± 2.92	281 ± 6.51
5	105 ± 4.47	275 ± 3.53	220 ± 5.70	163 ± 4.63	215 ± 2.23	352 ± 1.99	349 ± 3.56	565 ± 2.42	401 ± 3.66	187 ± 4.63
6	103 ± 3.03	285 ± 8.66	170 ± 7.07	128 ± 2.55[a]	143 ± 6.81[a]	337 ± 4.82	502 ± 4.95	600 ± 6.39	414 ± 4.34	99 ± 2.91[a]
7	108 ± 3.52	280 ± 6.51	150 ± 9.35	114 ± 2.91[a]	128 ± 8.95[a]	245 ± 5.64	542 ± 6.66	620 ± 5.15	430 ± 2.80	86 ± 4.30[a]

NC: normal control; DC: diabetic control; DGT: diabetic glibenclamide treated; comparisons were made between and within treatment groups.
Values represent mean ± SEM.
[a] P <0.001.

Table 2
ALR2 inhibitory activity data

Compounds	IC_{50} ± SEM[a] (μM)
2a	1.8 ± 0.006
2b	5.59 ± 0.07
2c	15.4 ± 1.7
2d	13.1 ± 0.05
2e	4.4 ± 0.18
2f	3.8 ± 0.02
2g	4.5 ± 0.014
Sulindac	0.293 ± 0.08[b]

[a] n = 3.
[b] Reported IC_{50}: 0.374 μM by Zheng et al.[20]

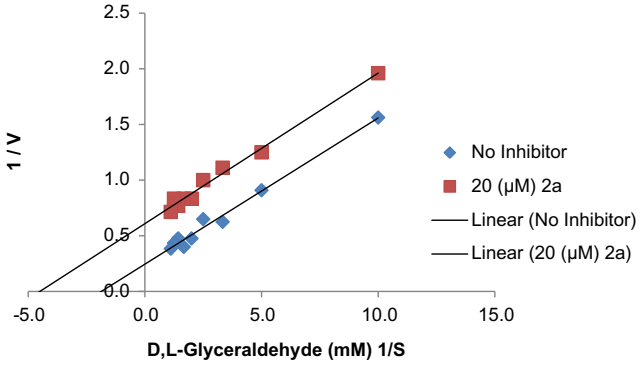


Figure 2. Inhibitory effect of compound **2a** on calf lens aldose reductase. Lineweaver–Burk plot in the presence and absence of Inhibitor (**2a**): (♦) no inhibitor; (■) 20 μM **2a** (uncompetitive type of inhibition).

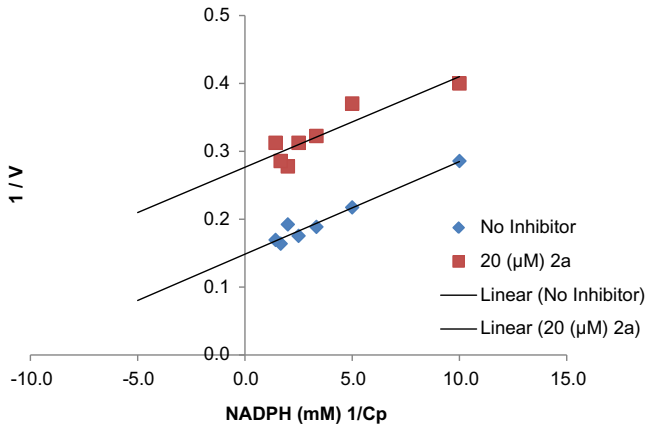


Figure 3. Inhibitory effect of compound **2a** on calf lens aldose reductase. Lineweaver–Burk plot by using NADPH, in the presence and absence of inhibitor (**2a**): (♦) no inhibitor; (■) 20 μM **2a** (uncompetitive type of inhibition).

Table 3
ALR1 inhibitory activity data

Compounds	IC_{50} ± SEM (μM)
2a	116.7 ± 2.97
2b	61.1 ± 0.85
2c	43.2% (0.6 mM)
2d	44.8 ± 1.99
2e	40.2 ± 0.07
2f	36.8% (0.6 mM)
2g	41.6% (0.6 mM)
Valproic acid	IC_{50}: 57.4 ± 0.89 (μM)[a]

[a] Reported IC_{50}: 56.1 (±2.7) by Stefek and co-workers.[22]

treatment with compounds **2a**, **2b** and **2g**. Euglycemic concentrations were obtained 6 h after treatment with the respective compounds and decreased further at 7 h post dosage. Mean plasma glucose concentrations in compound **2a** treated rats at 6 and 7 h post treatment were 128 ± 1.55 and 114 ± 2.91, respectively, compared to 0 or 1 h post treatment (F = 1260.28; P <0.001; Fig. 1A). For compound **2b**, the mean plasma glucose concentrations were 143 ± 6.81 and 8.15 at 6 and 7 h, respectively (F = 335.90; P <0.001; Fig. 1B). Similarly, mean plasma glucose concentrations of compound **2g** treated rats were 99 ± 2.93 and 86 ± 4.30 at 6 and 7 h post treatment, respectively (F = 738.37; P <0.001; Fig. 1C). Glucose concentration did not decrease where rats were treated with compounds **2c–f**. Table 1 shows complete data sets in control and compound treated rats. The present results show that compounds **2a**, **2b** and **2g** possess potent glucose lowering activity, while compounds **2c–f** appear to be ineffective in lowering plasma glucose. Overall compound **2g** seems to be more promising than **2a** and **2b** in achieving euglycemic level. Importantly, all the three compounds showed a greater glucose lowering activity as compared to glibenclamide, a well known antidiabetic sulphonylurea drug.

Enzyme inhibition: Compounds **2a–g** were also evaluated for inhibition of the partially purified enzyme ALR2 extracted from the calf eye. Sulindac was used as a reference drug (Table 2).

In the next step, the enzyme kinetics were determined using compound **2a** which was found most active at a concentration of 20 μM. The type of inhibition was found to be uncompetitive when it was observed in relation with D,L-glyceraldehyde (Fig. 2) as a substrate and NADPH as a cofactor (Fig. 3). The K_m value of ALR2, was calculated as 0.537 mM, while the reported value is 0.585 mM.[21]

The K_m value of ALR2 using NADPH was found to be 91.05 μM, while reported value is 54 μM.[13] The series of arylsulfonylspiroimidazolidinediones was also tested on aldehyde reductase (ALR1),

highly homologous enzyme to ALR2, partially purified from kidneys of calf. The results are tabulated in Table 3 as IC_{50} values. Valproic acid was used as positive control to validate the ALR1 assay.

In this assay, the most potent compound against ALR2 was found to be **2a** with a methyl group attached at position 4 of the benzene ring. Compounds **2f** and **2g** also revealed excellent inhibitory activity, however, compound **2f** having a naphthyl group as an aryl moiety is more potent than **2g** where naphthyl group is replaced with anthraquinyl group. The activity decreases little bit, when halogen group is attached with the aryl moiety. The IC_{50} value of compound **2b** with a chlorine atom attaches to the *para*-position of aryl group was found to be 5.59 μM, while it decreased further to 15.4 μM when bromine atom is present as a substituent at the same position in compound **2c**. The activity remains almost the same when bromine was replaced with NO_2 group, hence compound **2d** showed IC_{50} value of 13.1 μM. When a methoxy group was used as a substituent again a good activity with IC_{50} value of 4.4 μM was observed. In the next step, we determined the enzyme kinetics using the most active compound (**2a**) in the series. A decrease in K_m and V_{max} values was observed in presence of inhibitor **2a**, suggesting an uncompetitive type inhibition mechanism. This means that in hyperglycaemic condition where the glucose level is high, the inhibitory efficiency of the drug would not decrease. The synthesized compounds were also evaluated for ALR1 inhibition. Compounds **2d** and **2e** showed a more potent activity than standard with an IC_{50} value of 44.8 μM and 42.2 μM, respectively, as compared to 57.4 μM of the standard. The activity decreases when a halogen atom is attached with the aryl group of arylsulfonylspiroimidazolidinediones. Methoxy and nitro group attachment caused the compound to become potent against ALR1.

It may be observed from the comparison of ALR1 and ALR2 activities that most of the synthesized compounds show selectivity against ALR2 as compared to ALR1. Compound **2g** is around 150-fold more potent inhibitor of ALR2 than ALR1. Similarly, compound **2a** is almost 100-fold and compound **2b** about 10-fold more potent towards ALR2 than ALR1. This selectivity towards ALR2 over ALR1 is desirable to minimize toxicity and side effects.

In conclusion, the synthesis of spirobicycloimidazolidine-2,4-diones was successfully accomplished. The synthesized compounds were evaluated for in vivo hypoglycemic activity on male albino rats and inhibition of ALR1 and ALR2 extracted from the kidney and eye of the calf, respectively. Compounds **2a**, **2b** and **2g** exhibited excellent hypoglycemic activity and were more potent than the standard drug glibenclamide, a well known 2nd generation antidiabetic drug. Compound **2a** was also the most potent inhibitor of ALR2. Rest of the compounds also revealed very good activity against ALR2. The activity against ALR1 was synthesized compounds also exhibit selectivity towards ALR2 over ALR1.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.039. These data include MOL files and InChiKeys of the most important compounds described in this article.

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