

CASTELLANOS M. & CO
ABOGADOS
Calle 94A No. 7A-09
Bogotá D.C.

172
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-000562- -00006-0000

Fecha: 2009-04-17 15:14:41 Dep. 2020 NUECREACIONE
Fra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 444 RESPUESTAREQUE Folios: 33

Señora

JEFE DE LA DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

Ministerio de Desarrollo Económico

E. S. D.

Re.: Expediente No. **05.000.562**

Solicitud del examen de patentabilidad correspondiente a la Patente PCT
Entrada en Fase Nacional, para:

**"NUEVOS DERIVADOS DE GLICOSIDO DE TIOFENO, METODOS PARA LA
PREPARACION DE LOS MISMOS, MEDICAMENTOS QUE COMPRENDEN DICHOS
COMPUESTOS, Y SU USO"**

Solicitante: **SANOFI-AVENTIS DEUTSCHLAND GMBH**

RESPUESTA AL EXAMEN DE FONDO

Yo, **Luis Patiño Leyva**, identificado como aparece al pie de mi firma,
atentamente me permito presentar respuesta al oficio **No. 0669** notificado por
fijación en lista del 23 de enero de 2009, estando dentro del término legal en los
siguientes términos:

1. Claridad de la solicitud

- El oficio establece que en los ejemplos de la descripción no se encuentran sustentados todos los compuestos de las reivindicaciones, más específicamente, se menciona el sustituyente Cyc2.
- En cuanto a las reivindicaciones, el oficio establece que los posibles

radicales de la molécula de fórmula (I) o (II) son muy amplios y que los compuestos obtenidos tendrían diferentes características químicas y biológicas. Además, considera que la reivindicación 12 no se caracteriza correctamente y que la reivindicación 17 es muy general y amplia.

Con el objeto de superar las objeciones establecidas por el examinador, presento un nuevo pliego reivindicatorio en donde se han eliminado las reivindicaciones 1, 3, 4, 5, 12 a 17 de tal forma que la nueva reivindicación 1 (anterior 6) presenta específicamente los posibles radicales que sí tienen sustento descriptivo. Esto es ya no se incluye el radical Cyc2 en los compuestos reivindicados.

Así entonces, las objeciones a la claridad deben tenerse por superadas toda vez que las reivindicaciones objetadas (12 y 17) se han eliminado y adicionalmente, se han limitado los posibles sustituyentes para compuestos de fórmula (I) tal y como lo requirió el señor examinador.

2. Reivindicaciones relativas a un uso o a un segundo uso

El nuevo pliego reivindicatorio no recae en ésta categoría pues se han eliminado las reivindicaciones 13 a 16.

3. Nivel inventivo

Ese Despacho considera que la presente solicitud carece de nivel inventivo frente a los documentos **D1** (*Reactions of per-O-acetylglucosyl isothiocyanate with carbon bases. A new method for the stereocontrolled syntheses of nucleosides and glucosylaminothiophenes*) y **D2** (*Na⁺-glucose cotransporter inhibitors as antidiabetics. I. Synthesis and Pharmacological properties of 4'-dehydroxyphlorizin derivatives based on a new concept*).

El solicitante respetuosamente manifiesta su desacuerdo y considera que el documento D1 se refiere a métodos de preparación de glicósidos. Únicamente se hace alusión al campo farmacéutico en la introducción de D1 en donde se dice que los nucleosidos y aminotiofenos son herramientas quimioterapéuticas interesantes en el tratamiento de varias enfermedades infecciosas "chemoterapeutic tools in the treatment of various infectious diseases."... y... sobre la síntesis de análogos nucleosidos "synthesis of nucleoside analogues" (ver

página 2517, introducción). Es claro que D1 no da ninguna indicación dirigida a **medicamentos antidiabéticos o inhibidores de transportadores de glucosa (SGLT)** y, por lo tanto, una persona medianamente versada en la materia no partiría de D1 para resolver el problema planteado por la presente solicitud: proveer nuevos compuestos con los cuales fuese posible prevenir y tratar la diabetes de tipo 1 y de tipo 2.

En cuanto a los compuestos 30 y 31 de D1 (página 2523), el solicitante manifiesta que ambos compuestos difieren de los compuestos reivindicados en varias características, a saber: Los compuestos 30 y 31 son Aminoglicosidos, el anillo de tiofeno está sustituido en todas las posiciones posibles, el cual no es el caso de los compuestos presentados en el nuevo capítulo reivindicatorio. Los sustituyentes $R=CN$ y $R=CO_2Et$ que se enseñan para los compuestos 30/31 de D1 junto con NH_2 no son sustituyentes del tiofeno en la presente invención (R1). Resalto que dichos sustituyentes constituyen un elemento obligatorio en el método de preparación de D1, ver esquemas 2 y 3 (páginas 2522 y 2523). Así entonces, el documento D1 no enseña ninguna alternativa para los sustituyentes del tiofeno y en consecuencia D1 no da indicación alguna que lleve a modificar los compuestos 30/31 de tal manera que se llegue a los compuestos reivindicados. Las copias de los documentos D1 y D2 se adjuntan como referencia.

En la evaluación del nivel inventivo cabe resaltar que la pregunta a contestar es: "...sí teniendo en cuenta el estado de la técnica en su conjunto existe alguna indicación que lleve a la persona versada en la materia a modificar o adaptar el estado de la técnica más cercano para resolver el problema técnico, de tal manera que llegue a un resultado que estuviera incluido en el tenor de la (s) reivindicación (es)"

En consecuencia, D1 no afecta el nivel inventivo de la solicitud puesto que no indica a un técnico medio cómo modificar los sustituyentes que se enseñan en el estado de la técnica para llegar a los compuestos tal y como se reivindican en la presente solicitud y que tienen un efecto importante en la inhibición de SGLT.

En cuanto al documento D2, el examinador afirma que no se evidencia el efecto superior e inesperado al realizar los reemplazos en comparación con los divulgados anteriormente. El solicitante considera que D2 no afecta el nivel inventivo pues, como pasaré a explicar, los compuestos de acuerdo con la

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invención sí tienen un efecto superior e inesperado.

El documento D2 enseña derivados de phlorizina como inhibidores de SGLT lo cual está comprendido en el mismo campo técnico de la presente invención. Sin embargo, resalto que del SGLT se tienen dos subformas importantes en el tratamiento de la diabetes que son el SGLT1 y el SGLT2. El SGLT1 está ubicado principalmente en el intestino delgado. La inhibición de SGLT1 reduce el consumo de azúcar en el intestino delgado. El SGLT2 se localiza principalmente en el riñón (Zhou et al, J. Cellular Biochem. 90: 339-346 (2003)). La inhibición de SGLT2 lleva a la secreción de glucosa a través de la orina.

La phlorizina es un inhibidor de SGLT1/2 no selectivo en comparación con el compuesto del ejemplo 7 de la presente invención como se demuestra por los siguientes valores de IC50 in Vitro y la figura comparativa anexa:

	Phlorizina	Ejemplo 7
SGLT1	0,27 μ M	26,3 μ M
SGLT2	0,068 μ M	0,011 μ M

Se puede concluir, entonces, que los compuestos de la presente invención son inhibidores selectivos de SGLT2, mientras que D2 no hace alusión a la discriminación SGLT1/2.

En consecuencia, para una persona versada en la materia técnica que parta de las enseñanzas de D2, el problema a resolver sería proporcionar una alternativa para la inhibición del transportador sodio dependiente de la glucosa 2 para el tratamiento de la diabetes. Como lo indicó el señor examinador, los compuestos de D2 tienen diferentes características estructurales y, teniendo éste documento como el arte previo más cercano, el técnico medio en la materia no habría considerado los compuestos de D1 para utilizarlos en combinación con D2 por las razones anteriormente expuestas con relación al diferente campo técnico y las diferencias estructurales.

La posibilidad de reemplazar una o más de las características estructurales a partir de la combinación de los documentos D1 y D2, es una opinión "a posteriori", pues se está haciendo un juicio conociendo el resultado y este no es el concepto de nivel inventivo. El examinador debe partir del momento en que la

invencción no era conocida y establecer si las diferencias que resuelven el problema planteado se indicaban o sugerían en el campo técnico.

Teniendo en cuenta los argumentos expresados arriba, respetuosamente solicito, se conceda el título de patente a la presente invencción puesto que la misma cumple con los requisitos de los artículos 28, 30, 14, 21 y 18 de la Decisión 486.

4. ANEXOS

- Capítulo reivindicatorio
- Recibo de pago por modificaciones
- Formulario para modificaciones
- Copia de los documentos citados D1 y D2.
- Figura comparativa

Señora Jefe,



Luis Patiño Leyva
C.C. No. 2.730.616 de Bogotá
T.P.A. No.2698 del C. Sup. de la Judicatura
Calle 94A No. 7A - 09
Teléfonos: 6107420

FORMULARIO ÚNICO DE CORRECCIONES Y MODIFICACIONES

①

SOLICITUD DE:



Corrección de Error Material



Modificación a una solicitud

②

SOLICITANTE

Nombre: **SANOFI-AVENTIS DEUTSCHLAND GMBH**

Dirección: **Frankfurt 65926 Alemania**

Nacionalidad o Domicilio: **Frankfurt, Alemania**

Lugar de Constitución: **Alemania**

Teléfono:

Fax:

E-mail:

IDENTIFICACIÓN

C.C.



NIT



C.E.



Otro



Cual

Número

③

REPRESENTANTE
O APODERADO

Nombre: **LUIS PATIÑO LEYVA**

Dirección: **Calle 94A No. 7A-09**

Teléfono: **610 74 20** Fax: **610 07 06**

E-mail: **info@castellanosyco.com**

IDENTIFICACIÓN

C.C.



NIT



C.E.



Otro



Cual

Número

2.930.616

TPA

2698

④

Número de Expediente:

05.000.562

⑤

Descripción de la corrección o modificación solicitada:

Presento nuevo pliego reivindicatorio al cual solicito se otorgue el privilegio de patente.

⑥

Comprobante de pago No.

09-27,952

Fecha

14/04/2009

⑦

Anexos:



Comprobante de pago de la tasa.



Poderes si fuere el caso, junto con la sustitución de los mismos a mi favor.



Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas



Nuevo capítulo reivindicatorio

⑧

NOMBRE

LUIS PATIÑO LEIVA

FIRMA



CC 2.930.616 de Bogotá - TPA 2698 CSJ

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT: 800176089-2

RECIBO OFICIAL DE CAJA 1 09 - 27.952
FECHA 1 ABRIL 14 DE 2009

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Tra 11 PATENTEIYII Eve: 379 FASENACIONALII
Act 444 RESPUESTAREQUE Folios: 33

***** C O N C E P T O *****

DEPOSITANTE	TIPO PAGO	CANCO	CUENTA	No. PAGO	FECHA PGO	VAL PAGO
ALVARO CASTELLANOS	CONSIGNACION	BANCO DE BOGOTA	782784397	100793726	14/04/2009	111,000.00

***** C O N C E P T O *****

CANT. REQUISICION	CONCEPTO	TOTAL CONCEPTO
1 00005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
	12 MARCAS Y LEYAS COMERCIALES	111,000.00
	TOTAL	\$ 111,000.00

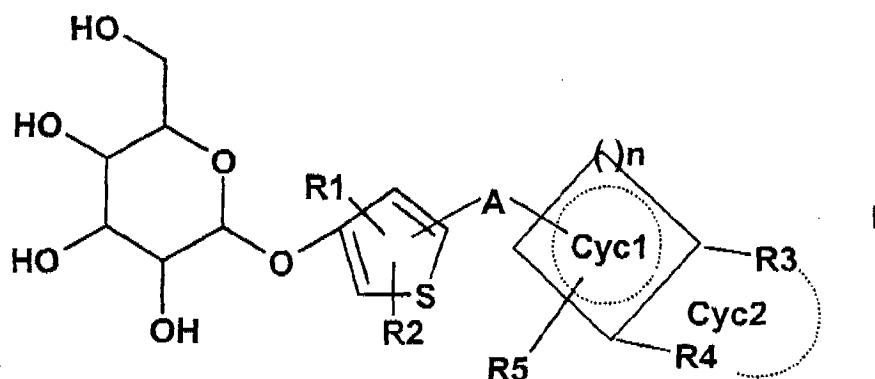
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RESPONSABLE:

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

REIVINDICACIONES MODIFICADAS

1. Un compuesto de fórmula I



- R1 es hidrógeno, CF₃, alquilo(C₁-C₄), fenilo,
- R2 es hidrógeno,
- A es -CH₂-, -C₂H₄-, -C₃H₆-, -CH(OH)-, -(C=O)-, -CH=CH-, -CH=CH-CH₂-, -CO-CH₂-CH₂- o bien -CO-NH-CH₂-;
- n es el número 2 ó 3;
- Cyc1 es fenilo o tienilo;
- R3, R4, R5 son hidrógeno, F, Cl, I, NO₂, OH, CN, alquilo(C₁-C₆), alcoxi(C₁-C₈), O-CH₂-fenilo, OCF₃, S-CH₃, COOH, o bien
- R3 y R4 son conjuntamente -CH=CH-O-, -O-(CH₂)_p-O-, con p = 1 ó 2, -O-CF₂-O-, -CH=CH-CH=CH-, y
- R5 es hidrógeno.

y sus sales farmacéuticamente aceptables.

2. Un compuesto de fórmula I según la reivindicación 1, en el cual A está unido al anillo de tienilo en la posición 2.

3. Un compuesto de fórmula I según las reivindicación 1, en el cual

A es -CH₂-, o bien -CH₂-CH₂-.

4. Un compuesto de fórmula I según las reivindicaciones 1 o 2, en el cual

Cyc1 es fenilo.

5. Un compuesto de fórmula I según las reivindicaciones 1 a 4, en el cual

Cyc1 es tienilo.

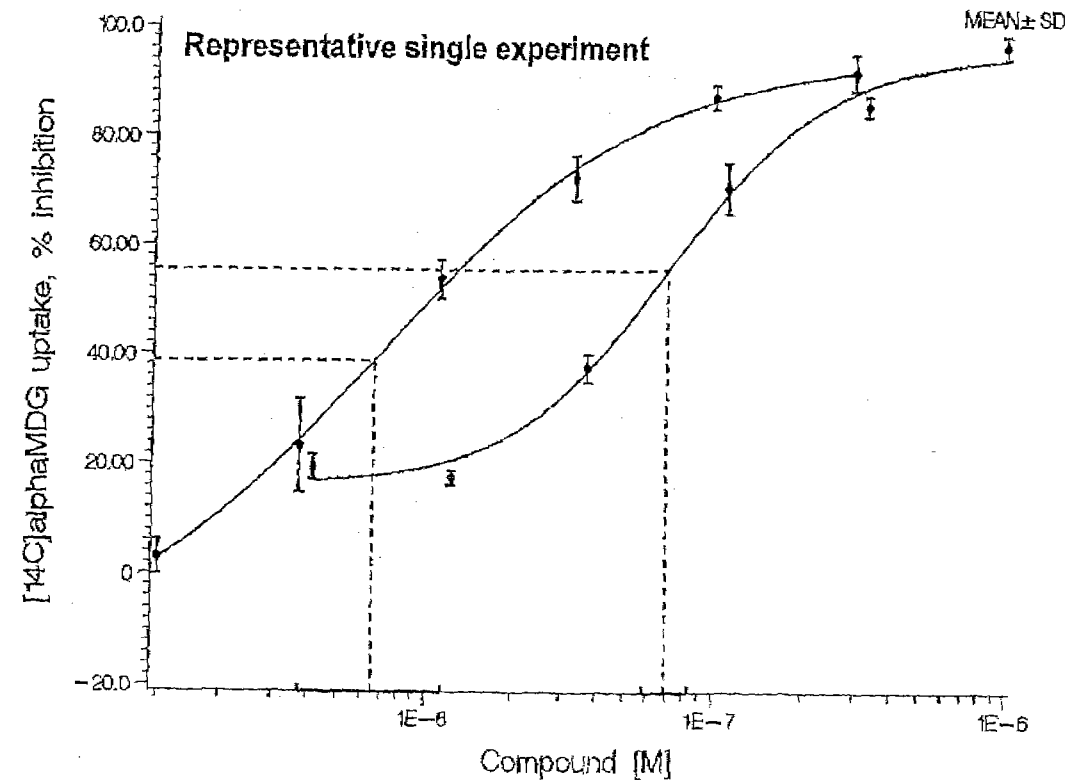
6. Un compuesto de fórmula I según las reivindicaciones 1 a 5, en el cual

Cyc1 está monosustituido.

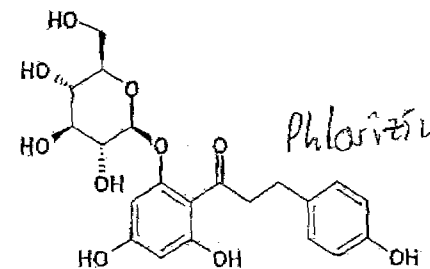
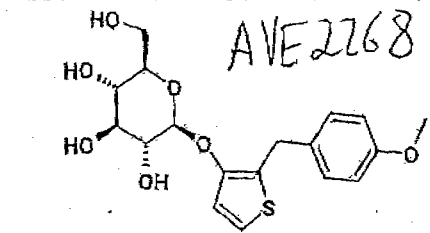
7. Un medicamento que contiene uno o más de los compuestos según una o más de las reivindicaciones 1 a 6.

Functional Effect of AVE2268 on the Cellular Level

Inhibition of recombinant human SGLT2 (SLC5A2) Comparison with phlorizin



IC₅₀(AVE2268): 11 nM (n=13)
IC₅₀(Phlorizin): 68 nM (n=3)
 n = number of single experiments performed
 in triplicate



sanofi aventis

Because health matters

Na⁺-Glucose Cotransporter Inhibitors as Antidiabetics. I. Synthesis and Pharmacological Properties of 4'-Dehydroxyphlorizin Derivatives Based on a New Concept

Kenji TSUJIHARA,* Mitsuya HONGU, Kunio SAITO, Masanori INAMASU, Kenji ARAKAWA,
Akira OKU, and Mamoru MATSUMOTO

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Received October 1995; accepted February 5, 1996

Based on our new concept that inhibitors of the Na⁺-glucose cotransporter (SGLT) would be useful as antidiabetics, 4'-dehydroxyphlorizin derivatives 1a–f were designed, synthesized, and examined for various pharmacological properties related to antidiabetic activity. In normal rats, 1a, e and phlorizin showed a strong SGLT-inhibitory effect and significantly increased urinary glucose on intraperitoneal administration at 10 mg/kg, though only 1a resulted in excretion of large quantities of urinary glucose on oral administration at 100 mg/kg. Compounds 1a, e, and phlorizin markedly inhibited glucose uptake in the small intestine during enteric perfusion in normal rats. Compound 1a had a significant reducing effect on blood glucose in the glucose tolerance test in mice when administered orally and also lowered blood glucose in streptozotocin-induced diabetic rats. The aglycons 2a, e of 1a, e, and 1a showed weak inhibitory effects on the facilitated glucose transporter-1 (GLUT-1) in human erythrocytes, while phloretin had a strong inhibitory effect on GLUT-1. Compound 1a caused no apparent renal damage in rats when administered orally at 1 g/kg for 4 successive weeks. Thus, 1a was considered to be a promising candidate as a lead compound for antidiabetics of a new type, and was selected for further pharmacological evaluation.

Key words antidiabetic; Na⁺-glucose cotransporter inhibitor; phlorizin; 4'-dehydroxyphlorizin

The incidence of diabetes mellitus, especially non-insulin-dependent diabetes mellitus (NIDDM), has been increasing markedly in the developed countries. One of the main reasons for this phenomenon is thought to be excess energy intake due to hyperphagia. Therefore, anti-obesity drugs such as appetite suppressants,¹⁾ absorption inhibitors,²⁾ and thermogenic stimulators³⁾ have been developed as antidiabetics. Blood glucose produces various glycosylated proteins in tissues non-enzymatically, and in the state of hyperglycemia, these glycosylated proteins give rise to the diabetes complications.^{4,5)} Therefore, a category of drug that would enable patients to escape from the state of chronic hyperglycemia is expected to be useful for the prophylaxis or treatment of diabetes. We have hypothesized that inhibitors of the Na⁺-glucose cotransporter (SGLT) would be suitable for this purpose. SGLT, which exists on the chorionic membrane of the intestine and the kidney, actively transports glucose by coupling with Na⁺.⁶⁾

Phlorizin was reported to be a selective inhibitor of SGLT,⁷⁾ but there has been no attempt to use phlorizin as an antidiabetic. We considered that compounds with an inhibitory effect on SGLT might be able not only to inhibit glucose uptake at the intestine, but also to stimulate

blood glucose excretion into urine directly at the kidney, and they might be useful in the treatment of diabetes, in preventing chronic hyperglycemia.

In this paper, we describe the design of SGLT inhibitors as candidate antidiabetic drugs. 4'-Dehydroxyphlorizin derivatives were synthesized and their pharmacological properties, such as SGLT-inhibitory effect, promotion of urinary glucose excretion, blood glucose reduction, inhibition of glucose uptake in the small intestine, etc., were examined in normal and diabetic rats and normal mice.

Drug Design Phlorizin was found in the last century and studied as a promoter of urinary glucose excretion. It is an inhibitor of SGLT, preventing renal tubular glucose reabsorption and promoting excretion of glucose, so that the blood glucose level is controlled.⁷⁾ Based on this action of phlorizin, it has been used as a blood glucose-lowering reagent to verify the glucose toxicity theory.⁸⁾ Namely, when the blood glucose level in diabetic animals is controlled to a normal level for a long period by subcutaneous daily administration of phlorizin without using insulin, the condition of the animals is normalized.

Phlorizin, however, is considered to have little effect when administered orally, because it is hydrolyzed into

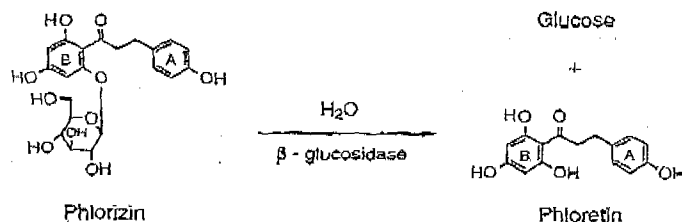


Chart 1

* To whom correspondence should be addressed.

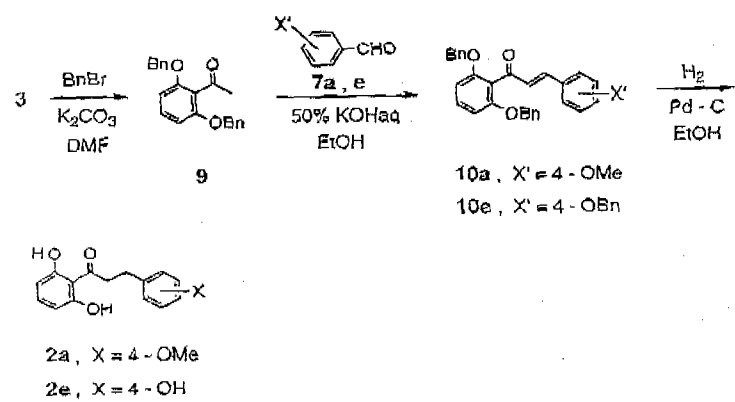


Chart 3

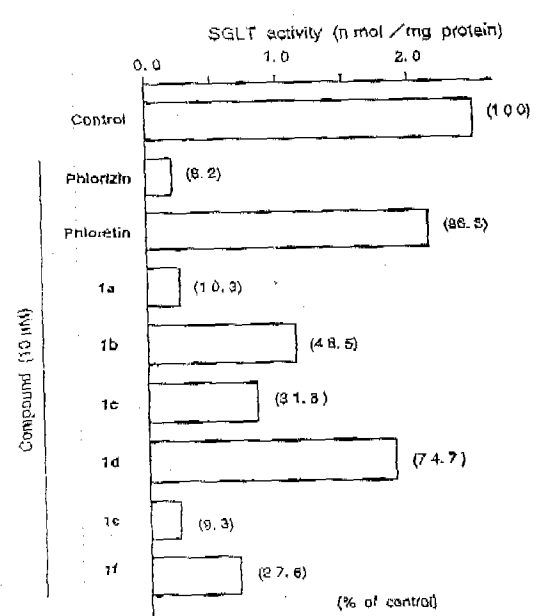


Fig. 1. Effect of 4'-Dehydroxyphlorizin Derivatives on Rat Kidney SGLT Activity

as shown in Chart 3. When the condensations of 3 with aldehydes were performed in aqueous EtOH containing KOH, none of the desired chalcone was obtained and 3 was decomposed. However, 9, the dibenzyl ether of 3, was condensed with 7a or 7e in an aqueous KOH solution and EtOH to give the chalcone 10a (94%) or 10e (91%), respectively. They were then hydrogenated to provide the dihydrochalcones 2a (85%) and 2e (82%), respectively.

Biological Results and Discussion

Using rat renal brush border membrane vesicles (BBMV), the inhibitory effects of the 4'-dehydroxyphlorizin derivatives 1a–f on SGLT were studied, and the results are shown in Fig. 1. Compounds 1a (4-OMe derivative) and 1e (4-OH derivative) showed the most potent inhibitory effects (around 90% inhibition), comparable to that of phlorizin. Compounds 1c (2,4-(OMe)₂ derivative) and 1f (3,4-(OH)₂ derivative) produced about 70% inhibition. Compound 1b (3,4-(OMe)₂ derivative)

Table 1. Effect of 4'-Dehydroxyphlorizin Derivatives on Urinary Glucose Excretion in Rats

Compound	Urinary glucose (mg/24 h)	
	10 mg/kg, i.p.	100 mg/kg, p.o.
Control	3 ± 1	3 ± 1
Phlorizin	320 ± 25	11 ± 6
1a	204 ± 8	380 ± 52
1b	7 ± 2	2 ± 1
1c	5 ± 0	3 ± 1
1d	3 ± 0	2 ± 1
1e	329 ± 7	60 ± 9
1f	43 ± 14	2 ± 1

Each value represents the mean ± S.E. (n = 3).

showed about 50% inhibition and 1d (3,4,5-(OMe)₃ derivative) was less potent. Therefore, it became clear that the substituent groups on the B-ring contributed to the inhibitory effect.

Next, the effect of these derivatives on urinary glucose excretion was investigated. Normal rats were given the compounds orally or intraperitoneally and glucose in urine collected for 24 h was measured. The results are shown in Table 1. When given intraperitoneally at 10 mg/kg, 1a, e and phlorizin induced marked urinary excretion of glucose, while 1f showed a weak effect, and 1b–d were inactive. The effects of these derivatives on urinary glucose excretion correlate with the SGLT-inhibitory activities, except in the case of 1e. This result strongly suggests that urinary glucose excretion is induced by the inhibition of SGLT in the kidney. On the other hand, when the compounds were given orally at 100 mg/kg, phlorizin had little effect on urinary glucose level. This is reasonable, because phlorizin is hydrolyzed to glucose and phloretin by β-glucosidase in the small intestine. However, 1a markedly increased urinary glucose and hence 1a was considered to be more stable to β-glucosidase than phlorizin. Compound 1e also moderately induced urinary glucose excretion, but the polysubstituted compounds 1b–d, f had no effect.

The inhibitory effects of those derivatives on glucose uptake in the small intestine were studied using an enteric perfusion technique in normal rats. The results are shown in Table 2. Compounds 1a, e had strong activities equivalent to that of phlorizin, and 1c, f were less active.

June 1996

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Table 2. Inhibitory Effects of 4'-Dehydroxyphlorizin Derivatives on Glucose Uptake in Rat Small Intestine

Compound	Inhibition (%)
Phlorizin	61.2 ± 7.9**
1a	50.5 ± 5.6**
1b	16.7 ± 6.8
1c	42.8 ± 3.6*
1d	11.4 ± 5.2
1e	64.5 ± 4.1*
1f	39.7 ± 1.7**

Each value represents the mean ± S.E. (n=4). * p<0.05, ** p<0.01 vs. control.

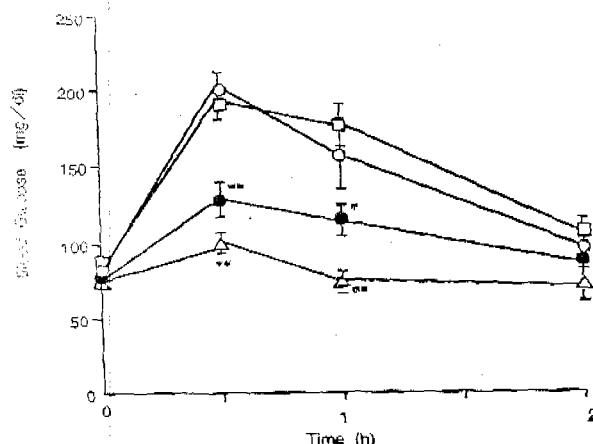


Fig. 2. Effect of 4'-Dehydroxyphlorizin Derivatives on Glucose Tolerance in Mice

Each sample was administered immediately before a subcutaneous load of glucose (1 g/kg). Points and bars represent the means and S.E. (n=6). O, control; ●, 1a (100 mg/kg, p.o.); □, 1d (100 mg/kg, p.o.); △, phlorizin (30 mg/kg, i.p.); ■, 1e (100 mg/kg, p.o.). Significantly different from control (* p<0.05, ** p<0.01).

These results showed a good correlation to SGLT-inhibitory activity in the kidney and also to urinary glucose excretion after intraperitoneal administration, except in the case of 1c.

Next, the effects of 1a, d on glucose tolerance in mice were examined and the results are shown in Fig. 2. Phlorizin, the positive control, strongly lowered the blood glucose level at 30 mg/kg on intraperitoneal administration. On the other hand, 1a which markedly induced urinary excretion of glucose, lowered the blood glucose level to a normal level on oral administration at 100 mg/kg. Compound 1d, which had no effect on urinary glucose, showed no effect. These results suggest that 1a lowered blood glucose level through enhancing urinary glucose excretion. Next, the effect of 1a on the blood glucose concentration in streptozotocin (STZ)-induced diabetic rats was examined. The results are shown in Fig. 3. The oral administration of 1a at 100 mg/kg lowered blood glucose to about 100 mg/dl 30 min after administration, and the effect lasted at least 2 h in STZ-induced diabetic rats.

The inhibitory effects of 2a, e and phloretin, aglycons of 1a, e and phlorizin, and 1a itself on GLUT-1 in human erythrocytes were also studied. As shown in Fig. 4, 2a, e and 1a itself had only a weak GLUT-1-inhibitory effect but phloretin inhibited GLUT-1 very strongly. Rosenberg

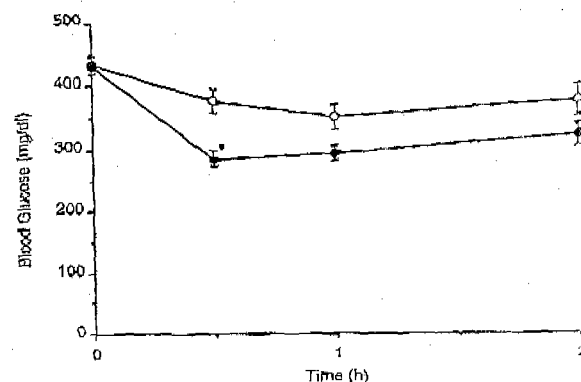


Fig. 3. Effect of Compound 1a on Blood Glucose Level in Streptozotocin-Induced Diabetic Rats

Points and bars represent the means and S.E. (n=5). O, control; ●, 1a (100 mg/kg, p.o.). Significantly different from control (* p<0.01).

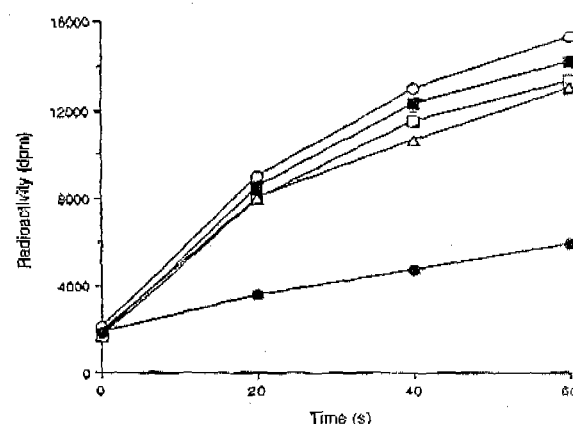


Fig. 4. Effect of Phloretin and Aglycons on Glucose Transport in Erythrocytes

O, control; ●, phloretin (10 μM); □, 2a (10 μM); △, 2e (10 μM); ■, 1a (10 μM).

and Wilbrandt^{9a)} reported that the four hydroxy groups of phloretin were necessary to inhibit the glucose transporter in human erythrocytes; the activity was reduced markedly when even one of four hydroxy groups was methylated. Therefore, it is reasonable that 2a and 2e, as 4'-dehydroxyphloretin derivatives, had only weak activities.

It must be considered that these compounds, as inhibitors of SGLT on the renal tubule, might induce kidney damage. Phlorizin has been reported to induce kidney injury,^{7a)} though no details were given. Recently, Gouvea¹⁶⁾ studied the effect of phlorizin on gentamicin-induced renal damage and reported that phlorizin induced marked excretion of urinary glucose, but showed no adverse influence on renal function or morphology in rats when subcutaneously administered at massive doses for 15 successive days. In our study, 1a produced no sign of renal damage when administered orally at 1 g/kg for 4 successive weeks in rats (data not shown). The inhibitory effect of phlorizin on SGLT has been reported to be reversibly antagonistic with respect to glucose.⁷⁾ Compounds that antagonistically and reversibly inhibit SGLT

may thus have no adverse effects on the kidney.

In conclusion, 1a inhibited glucose uptake in the small intestine and glucose reuptake in the kidney through inhibition of SGLT on these organs when administered orally, so that 1a was able to lower high blood glucose levels in normal mice and STZ-induced diabetic rats directly without mediation of its action by insulin. It did not induce either hypoglycemia or toxic signs, including renal damage in rats, and the GLUT-1-inhibitory effects of the aglycon 2a and 1a itself were very weak. Therefore, 1a seems to meet the five criteria for SGLT inhibitors to be usable as antidiabetics (see Drug Design section). Compound 1a is considered to be a suitable lead compound for new-type antidiabetics, and has been selected for further pharmacological evaluation.

Experimental

All melting points were determined on a Büchi 535 digital melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi IR-215 or an Analect FX-6200 FT-IR spectrophotometer. ¹H-NMR spectra were recorded on a Hitachi R-90H or a JEOL JNM-FX-200 spectrometer. Mass spectra were recorded on a Hitachi RMU-6 or a JEOL JMS-HX100 mass spectrometer. Microanalyses were performed on a Perkin-Elmer 2400 C, H, N analyzer.

2',6'-Dihydroxyacetophenone 2'-O-(2,3,4,6-O-Tetraacetyl-β-D-glucopyranoside) (5) A mixture of 2',6'-dihydroxyacetophenone 3 (50 g, 0.33 mol) and CdCO₃ (227 g, 1.32 mol) in toluene (4 l) was refluxed for 2 h with removal of the generated water in a Dean-Stark apparatus. Then acetobromoglucose 4 (270 g, 0.66 mol) was added and the whole was heated at reflux for 18 h. The hot mixture was filtered through a plug of Celite and the solid was washed with hot CHCl₃. The filtrate and the washing were combined and evaporated. The resultant residue was triturated in MeOH to provide a pale yellow solid, which was subjected to column chromatography to afford 5 (113 g, 71%) from the first eluate and the diglycoside 6 (21 g, 8%) from the second eluate. Compound 5: colorless needles, mp 200.5–201.5°C (lit.¹³) mp 201–203°C. IR (Nujol): 1760, 1740, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 1.96 (3H, s), 2.01 (6H, s), 2.02 (3H, s), 2.35 (3H, s), 4.05–4.30 (3H, m), 4.99 (1H, dd, *J* = 8.9, 9.3 Hz), 5.05 (1H, dd, *J* = 8.0, 9.7 Hz), 5.41 (1H, t, *J* = 9.6 Hz), 5.55 (1H, d, *J* = 8.1 Hz), 6.61 (1H, d, *J* = 8.3 Hz), 6.63 (1H, d, *J* = 8.3 Hz), 7.26 (1H, t, *J* = 8.3 Hz), 10.81 (1H, s). FAB-MS *m/z*: 483 (M+H)⁺. Compound 6: colorless needles, mp 182–189.5°C. IR (Nujol): 1750, 1740, 1710 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 1.96 (6H, s), 2.00 (6H, s), 2.01 (6H, s), 2.02 (6H, s), 2.21 (3H, s), 4.08 (2H, m), 4.22 (4H, m), 4.99 (4H, m), 5.39 (2H, t, *J* = 9.6 Hz), 5.53 (2H, d, *J* = 8.1 Hz), 6.90 (2H, d, *J* = 8.5 Hz), 7.41 (1H, t, *J* = 8.5 Hz). FAB-MS *m/z*: 835 (M+Na)⁺.

2',6'-Dihydroxy-4-methoxychalcone 2'-O-β-D-Glucopyranoside (8a) A solution of KOH (10 g) in H₂O (10 ml) was added to a suspension of 5 (9.65 g, 20 mmol) in EtOH (100 ml) and the mixture was stirred at room temperature for 10 min. Then *p*-anisaldehyde 7a (3.27 g, 24 mmol) was added and the whole was stirred at room temperature for 18 h. The reaction mixture was acidified with 10% HCl to pH 5 under ice-cooling. The resulting yellow needles were collected by filtration, washed with water and then Et₂O, and dried to give 8a (6.36 g, 74%), mp 136–140°C (lit.¹⁴) mp around 126°C. IR (Nujol): 3540, 3480, 3320, 3240, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 3.10–3.40 (4H, m), 3.47 (1H, m), 3.69 (1H, ddd, *J* = 1.7, 5.3, 11.5 Hz), 3.81 (3H, s), 4.58 (1H, t, *J* = 5.7 Hz), 5.03 (1H, d, *J* = 6.9 Hz), 5.04 (1H, d, *J* = 5.5 Hz), 5.06 (1H, d, *J* = 5.2 Hz), 5.09 (1H, d, *J* = 5.3 Hz), 6.59 (1H, d, *J* = 8.3 Hz), 6.72 (1H, d, *J* = 8.1 Hz), 6.98 (2H, d, *J* = 8.8 Hz), 7.29 (1H, t, *J* = 8.3 Hz), 7.40 (1H, d, *J* = 15.9 Hz), 7.47 (1H, d, *J* = 16.0 Hz), 7.71 (2H, d, *J* = 8.8 Hz), 11.02 (1H, s). FAB-MS *m/z*: 433 (M+H)⁺.

2',6'-Dihydroxy-4-methoxydihydrochalcone 2'-O-β-D-Glucopyranoside (1a) A solution of 8a (7.30 g, 16.90 mmol) in EtOH (150 ml) was hydrogenated over 10% Pd-C (2.00 g) under H₂ (1 atm) at room temperature for 1 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was crystallized from EtOH-H₂O to give 1a (6.57 g, 90%) as pale yellow needles, mp 105–107°C (lit.¹⁵) mp 106–108°C. IR (Nujol): 3440, 3400, 3240, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.84 (2H, t, *J* = 7.3 Hz), 3.19–3.49

(7H, m), 3.70 (1H, m), 3.71 (3H, s), 4.56 (1H, t, *J* = 5.4 Hz), 4.91 (1H, d, *J* = 7.3 Hz), 5.03 (1H, d, *J* = 4.9 Hz), 5.10 (1H, d, *J* = 4.4 Hz), 5.22 (1H, d, *J* = 4.9 Hz), 6.55 (1H, d, *J* = 8.3 Hz), 6.67 (1H, d, *J* = 8.3 Hz), 6.81 (2H, d, *J* = 8.8 Hz), 7.17 (2H, d, *J* = 8.8 Hz), 7.24 (1H, t, *J* = 8.3 Hz), 10.99 (1H, s). FAB-MS *m/z*: 435 (M+H)⁺. Anal. Calcd for C₂₂H₂₆O₉ · 1/4H₂O: C, 60.20; H, 6.08. Found: C, 60.36; H, 6.14.

Synthesis of 1a without Isolation of 8a 5 (3.31 g, 6.86 mmol) was condensed with 7a (1.12 g, 8.23 mmol) by the same procedure as described for the synthesis of 8a. The reaction mixture containing 8a was diluted with water and washed with toluene to remove excess 7a. The aqueous layer was hydrogenated over 10% Pd-C (0.15 g) at room temperature for 2 h. The catalyst was removed by filtration and the filtrate was neutralized with 10% HCl and extracted with AcOEt. The organic layer was washed with water and dried over MgSO₄. The solvent was removed and the residue was crystallized from EtOH-H₂O to give 1a (2.31 g, 78%), which was identical with the sample obtained as described above.

Compounds 1b–f were prepared in the same manner as described above.

1b: Yield 51% as pale yellow needles, mp 176–178.5°C (MeOH). IR (Nujol): 3560, 3490, 3460, 1620 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.84 (2H, t, *J* = 7.6 Hz), 3.10–3.55 (7H, m), 3.70 (3H, s), 3.71 (1H, m), 3.73 (3H, s), 4.56 (1H, t, *J* = 5.6 Hz), 4.92 (1H, d, *J* = 6.8 Hz), 5.03 (1H, d, *J* = 4.9 Hz), 5.09 (1H, d, *J* = 4.4 Hz), 5.22 (1H, d, *J* = 4.9 Hz), 6.55 (1H, d, *J* = 8.3 Hz), 6.68 (1H, d, *J* = 8.3 Hz), 6.74 (1H, dd, *J* = 2.0, 8.3 Hz), 6.78 (1H, d, *J* = 8.3 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 7.25 (1H, t, *J* = 8.3 Hz), 11.06 (1H, s). FAB-MS *m/z*: 465 (M+H)⁺. Anal. Calcd for C₂₃H₂₈O₁₀: C, 59.48; H, 6.08. Found: C, 59.54; H, 6.17.

1c: Yield 69% as pale yellow needles, mp 86–89°C (AcOEt-iso-Pr₂O). IR (Nujol): 3400, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.78 (2H, t, *J* = 7.3 Hz), 3.10–3.55 (7H, m), 3.70 (1H, m), 3.72 (3H, s), 3.76 (3H, s), 4.55 (1H, t, *J* = 5.6 Hz), 4.91 (1H, d, *J* = 7.3 Hz), 5.02 (1H, d, *J* = 4.9 Hz), 5.08 (1H, d, *J* = 3.9 Hz), 5.12 (1H, d, *J* = 4.9 Hz), 6.41 (1H, dd, *J* = 2.0, 7.8 Hz), 6.50 (1H, d, *J* = 2.0 Hz), 6.55 (1H, d, *J* = 8.3 Hz), 6.67 (1H, d, *J* = 8.3 Hz), 7.06 (1H, d, *J* = 7.8 Hz), 7.24 (1H, t, *J* = 8.3 Hz), 11.01 (1H, s). FAB-MS *m/z*: 465 (M+H)⁺. Anal. Calcd for C₂₃H₂₈O₁₀ · 1/2H₂O: C, 58.55; H, 6.17. Found: C, 58.56; H, 6.13.

1d: Yield 54% as a white powder, mp 136.5–139°C (Et₂O). IR (Nujol): 3420, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.81 (2H, t, *J* = 7.1 Hz), 3.10–3.55 (7H, m), 3.70 (1H, m), 3.73 (3H, s), 3.75 (3H, s), 3.77 (3H, s), 4.54 (1H, t, *J* = 5.6 Hz), 4.91 (1H, d, *J* = 6.8 Hz), 5.02 (1H, d, *J* = 4.9 Hz), 5.08 (1H, d, *J* = 3.9 Hz), 5.14 (1H, d, *J* = 4.9 Hz), 6.55 (1H, d, *J* = 7.8 Hz), 6.66 (1H, d, *J* = 2.4 Hz), 6.70 (1H, d, *J* = 2.9 Hz), 6.90 (1H, d, *J* = 8.3 Hz), 7.24 (1H, t, *J* = 8.3 Hz), 10.94 (1H, s). FAB-MS *m/z*: 495 (M+H)⁺. Anal. Calcd for C₂₆H₃₀O₁₁: C, 58.29; H, 6.12. Found: C, 58.22; H, 6.11.

1e: Yield 62% as pale yellow needles, mp 133–135°C (EtOH-H₂O) (lit.¹⁵) mp 134–136°C. IR (Nujol): 3520, 3400, 1620 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.78 (2H, t, *J* = 7.6 Hz), 3.20 (2H, t, *J* = 7.6 Hz), 3.10–3.55 (5H, m), 3.70 (1H, dd, *J* = 4.6, 11.0 Hz), 4.56 (1H, t, *J* = 5.6 Hz), 4.91 (1H, d, *J* = 6.8 Hz), 5.03 (1H, d, *J* = 4.9 Hz), 5.09 (1H, d, *J* = 3.9 Hz), 5.22 (1H, d, *J* = 4.9 Hz), 6.54 (1H, d, *J* = 8.3 Hz), 6.64 (2H, d, *J* = 8.8 Hz), 6.67 (1H, d, *J* = 8.3 Hz), 7.03 (2H, d, *J* = 8.3 Hz), 7.24 (1H, t, *J* = 8.3 Hz), 9.09 (1H, br), 11.00 (1H, br). FAB-MS *m/z*: 421 (M+H)⁺. Anal. Calcd for C₂₁H₂₄O₉ · 1/2H₂O: C, 58.74; H, 5.87. Found: C, 58.93; H, 5.87.

1f: Yield 52% as a pale yellow powder, mp 78–80°C (AcOEt-iso-Pr₂O). IR (Nujol): 3380, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.72 (2H, t, *J* = 7.6 Hz), 3.10–3.55 (7H, m), 3.70 (1H, d, *J* = 11.7 Hz), 4.55 (1H, br), 4.90 (1H, d, *J* = 7.3 Hz), 4.80–5.40 (3H, br), 6.45–6.62 (4H, m), 6.67 (1H, d, *J* = 8.3 Hz), 7.24 (1H, t, *J* = 8.3 Hz), 8.61 (2H, br), 11.02 (1H, br). FAB-MS *m/z*: 437 (M+H)⁺. Anal. Calcd for C₂₁H₂₄O₁₀ · 1/4H₂O: C, 57.21; H, 5.60. Found: C, 57.38; H, 5.90.

2',6'-Dibenzoyloxyacetophenone (9) A mixture of 3 (15.22 g, 100 mmol), benzyl bromide (68.42 g, 400 mmol) and K₂CO₃ (138 g, 1 mol) in *N,N*-dimethylformamide (DMF) (300 ml) was stirred at 80°C for 2.5 h. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in AcOEt, and the solution was washed with water and brine, dried, and evaporated *in vacuo*. The residue was crystallized from toluene-hexane to afford 9 (29.63 g, 89%) as colorless needles, mp 68.5–70°C. IR (Nujol): 1700 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.49 (3H, s), 5.08 (4H, s), 6.59 (2H, dd, *J* = 0.4, 8.3 Hz), 7.35 (11H, m). MS *m/z*: 332 (M⁺).

2',6'-Dibenzoyloxy-4-methoxychalcone (10a) A mixture of 9 (3.32 g, 10 mmol), 7a (1.63 g, 12 mmol), 50% aqueous KOH (5 ml) and EtOH (50 ml) was stirred at room temperature for 3 h. The mixture was evaporated and diluted with water. The precipitated solid was collected

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by filtration, washed with water, dried, and recrystallized from EtOH-Et₂O to give 10a (4.26 g, 94%) as yellow crystals, mp 120–121 °C. IR (Nujol): 1650 cm⁻¹. ¹H-NMR (CDCl₃) δ: 3.82 (3H, s), 5.08 (4H, s), 6.61 (2H, d, J = 8.4 Hz), 6.78–7.48 (17H, m). MS *m/z*: 450 (M⁺).

2,4,6-Tribenzoyloxychalcone (10e) 10e was prepared by the condensation of 9 (2.02 g, 6.08 mmol) with 7e (1.55 g, 7.30 mmol) in the same manner as described for the synthesis of 10a. Yellow crystals (2.91 g, 91%, recrystallized from AcOEt-Et₂O), mp 122–125 °C. IR (Nujol): 1670 cm⁻¹. ¹H-NMR (CDCl₃) δ: 5.07 (4H, s), 5.08 (2H, s), 6.61 (2H, d, J = 8.6 Hz), 6.68 (1H, d, J = 16.0 Hz), 6.95 (2H, d, J = 9.0 Hz), 7.12–7.47 (19H, m). FAB-MS *m/z*: 527 (M + H)⁺.

2,6-Dihydroxy-4-methoxydihydrochalcone (2a) A solution of 10a (3.30 g, 7.32 mmol) in EtOH (50 ml) and AcOEt (50 ml) was hydrogenated over 10% Pd-C (0.83 g) under H₂ (1 atm) at room temperature for 3 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was crystallized from toluene-hexane to give 2a (1.69 g, 85%) as pale yellow prisms, mp 128–131 °C. IR (Nujol): 3260, 1630 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.97 (2H, t, J = 7.6 Hz), 3.43 (2H, t, J = 7.6 Hz), 3.79 (3H, s), 6.38 (2H, d, J = 8.3 Hz), 6.83 (2H, ddd, J = 2.0, 2.7, 8.8 Hz), 7.16 (2H, dd, J = 2.4, 8.3 Hz), 7.22 (1H, t, J = 8.3 Hz), 9.51 (2H, s). MS *m/z*: 272 (M⁺). Anal. Calcd for C₁₆H₁₆O₄: C, 70.58; H, 5.92. Found: C, 70.51; H, 5.83.

2,4,6-Trihydroxydihydrochalcone (2e) A solution of 10e (2.87 g, 5.46 mmol) in tetrahydrofuran (THF) (50 ml) was hydrogenated over 10% Pd-C (0.5 g) under H₂ (1 atm) at room temperature for 2.5 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was crystallized from iso-Pr₂O-toluene to give 2e (1.15 g, 82%) as pale yellow crystals, mp 169–172.5 °C (lit.¹⁷) mp 155.5–156 °C, from aqueous EtOH. IR (Nujol): 3280, 1630 cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ: 2.79 (2H, t, J = 7.6 Hz), 3.29 (2H, t, J = 7.8 Hz), 6.37 (2H, d, J = 8.3 Hz), 6.67 (2H, d, J = 8.8 Hz), 7.03 (2H, d, J = 8.3 Hz), 7.23 (1H, t, J = 8.3 Hz), 9.14 (1H, s), 11.67 (2H, s). MS *m/z*: 258 (M⁺). Anal. Calcd for C₁₄H₁₄O₄: C, 69.76; H, 5.46. Found: C, 69.79; H, 5.64.

Inhibition on Rat Kidney SGLT Activity Male Sprague-Dawley (SD) rats (10–13 weeks old) were used. Brush border membrane vesicles (BBMV) from the rat kidney were prepared according to the method of Nagasawa *et al.*¹⁸ The protein concentration was measured by use of a Coomassie protein assay kit (Pierce) and diluted with buffer A (10 mM HEPES/Tris (pH 7.4), 100 mM mannitol) to 4 mg/ml protein concentration.

Assay tubes containing 50 μl of BBMV suspension (0.2 mg, protein) and 100 μl of buffer A containing 1% (v/v) of DMSO or DMSO solution of the test compounds (final concentration, 10 μM) were preincubated at 37 °C for 2 min. Then 50 μl of buffer A containing 0.4 μM D-glucose (final concentration, 0.1 mM), 400 mM NaSCN (final concentration, 100 mM), and 20 μCi/ml [³H]glucose (final radioactivity, 1 μCi) was added. The assay tubes were incubated at 37 °C for 5 s. The incubation was terminated by addition of 1.5 ml of ice-cold stopping solution (10 mM HEPES/Tris (pH 7.4), 150 mM NaCl, 0.3 mM phlorizin) followed by rapid filtration through a membrane filter (nitrocellulose, 25 mm φ, pore size 0.45 μm, Advantec). The filter was washed with 4.5 ml of the stopping solution. Then the radioactivity of the pellet was measured with a liquid scintillation counter (Tricarb 2200CA, Packard).

Measurement of Urinary Glucose Excretion Male SD rats (6 weeks old) were used. Test compounds were administered twice, with an 8 h interval, at 10 mg/kg, i.p. or at 100 mg/kg, p.o. The volume of injection was kept at 5 ml/kg and an equal volume of the vehicle was given to the control group. Urine was collected for 24 h after first administration and urinary glucose was measured by use of a glucose analyzer (Apece).

Inhibition of Glucose Uptake in the Small Intestine Male SD rats (6–7 weeks old) were starved overnight. The glucose absorption in the small intestine was measured by the circulating method described by Kawaguchi.¹⁹ Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the abdomen was opened lengthwise. The small intestine at 2 cm from Treitz ligament was cannulated to introduce the circulating solution and the tube was connected with a peristaltic pump. The small intestine at 25 cm from that position toward the anus was cannulated to remove the circulating solution and the abdomen was closed. The circulating solution (5 mM glucose containing 0.9% NaCl and 1% DMSO, 20 ml) with or without a test compound (0.5 mM) was kept at 38 °C and circulated at 4 ml/min after flushing with 0.9% saline for 30 min. One drop of circulating solution was sampled at 0, 15, 30, 45, and 60 min, and the concentration of glucose was measured by glucose oxidase assay (new blood sugar test, Boehringer Mannheim). The inhibition (%) was calculated from the following equation.

$$\text{inhibition (\%)} = (A - B) / A \times 100$$

where *A* is the mean rate of glucose absorption from the circulating solution without test compound and *B* is that with test compound.

Blood Glucose-Reducing Effect in Glucose Tolerance Test Male ddY mice (8 weeks old) were starved overnight. Test compounds (100 mg/kg, p.o.) or phlorizin (30 mg/kg, i.p.) were administered immediately before subcutaneous loading of glucose (1 g/kg). The control group received glucose only. Blood was sampled from the tail vein at 0.5, 1, and 2 h later. Blood glucose levels were measured with the new blood sugar test (Boehringer Mannheim).

Effect of Compound 1a on Blood Glucose Level in STZ-Induced Diabetic Rats Male SD rats (6 weeks old) were starved overnight. Streptozotocin was administered at 50 mg/kg, i.v. After 1 week, compound 1a (100 mg/kg, p.o.) was administered and blood was sampled from the vein at 0.5, 1, and 2 h later. Blood glucose levels were measured with the new blood sugar test (Boehringer Mannheim).

Glucose Transport in Erythrocytes Fresh human blood from healthy donors was used. The erythrocytes were made substantially glucose-free by washing them four times with 10 volumes of phosphate-buffered saline (PBS; Dulbecco's formula without magnesium and calcium) and then resuspended in PBS to give a hematocrit value of 20%. The cell suspension (100 μl) was placed in a centrifuge tube and 100 μl of PBS containing 1 mM D-[³H]glucose (18.5 kBq), 2 mM D-glucose and test compound (20 μM) was added with mixing. After incubation of the tube for 0–60 s at 4 °C, 1 ml of ice-cold stopping solution (0.3 mM phloretin/PBS) was added. Erythrocytes were sedimented by centrifugation and washed once with the stopping solution. The pellet was lysed with 100 μl of H₂O and deproteinized with 0.6 ml of 5% trichloroacetic acid. An aliquot (0.4 ml) of supernatant was neutralized with 3 M NaOH and the radioactivity was measured by liquid scintillation counting.

Acknowledgment We are grateful to Emeritus Professor Jun Kawada of Tokushima University for his helpful comments and discussions.

References

- 1) Hadler A. J., *J. Clin. Pharmacol.*, **12**, 453–458 (1972).
- 2) Truscheit E., Frommer W., Junge B., Müller L., Schmidt D. D., Wingender W., *Angew. Chem. Int. Ed. Engl.*, **20**, 744–761 (1981); Horii S., Fukase H., Matsuo T., Kameda Y., Asano N., Matsui K., *J. Med. Chem.*, **29**, 1038–1046 (1986).
- 3) Arch J. R. S., Ainsworth A. T., Cawthorne M. A., Piercy V., Sennitt M. V., Thody V. E., Wilson C., Wilson S., *Nature (London)*, **309**, 163–165 (1984).
- 4) Baynes J. W., Monnier V. M., "The Maillard Reaction in Aging, Diabetes, and Nutrition," *Prog. Clin. Biol. Res.*, **304**, Alan R. Liss, Inc., New York, 1986.
- 5) Unger R. H., Grundy S., *Diabetologia*, **28**, 119–121 (1985); Review; Rossetti L., Giaccari A., DeFronzo R. A., *Diabetes Care*, **13**, 610–630 (1990); Kahn B. B., Shulman G. I., DeFronzo R. A., Cushman S. W., Rossetti L., *J. Clin. Invest.*, **87**, 561–570 (1991); Review: Dimitrakoudis D., Vranic M., Klip A., *J. Am. Soc. Nephrol.*, **3**, 1078–1091 (1992); Brichard S. M., Henquin J. C., Girard J., *Diabetologia*, **36**, 292–298 (1993).
- 6) Yano H., Seino Y., Imura H., *Diabetes Frontier*, **1**, 417–422 (1990).
- 7) a) Review: McKee F. W., Hawkins W. B., *Physiol. Rev.*, **25**, 255–280 (1945); b) Alvarado F., Crane R. K., *Biochim. Biophys. Acta*, **56**, 170–172 (1962); Alvarado F., Crane R. K., *ibid.*, **93**, 116–135 (1964); Togggenburger G., Kessler M., Semenza G., *ibid.*, **688**, 557–571 (1982).
- 8) Booth A. N., Jones F. T., DeEds F., *J. Biol. Chem.*, **233**, 280–282 (1958); Malathi P., Crane R. K., *Biochim. Biophys. Acta*, **173**, 245–256 (1969).
- 9) a) Rosenberg Th., Wilbrandt W., *Helv. Physiol. Acta*, **15**, 168–176 (1957); b) Kotyk A., Kolinshä J., Veres K., Szammer J., *Biochem. Zeitschrift*, **342**, 129–138 (1965).
- 10) Oldendorf W. H., Crane P. D., Lawner P. M., Braun L. D., *Stroke*, **14**, 388–393 (1983).
- 11) Diedrich D. P., *Biochim. Biophys. Acta*, **71**, 688–770 (1963).
- 12) Hase J., Kobayashi K., Kobayashi R., *Chem. Pharm. Bull.*, **21**, 1076–1079 (1973).
- 13) Diedrich D. P., *J. Med. Pharm. Chem.*, **5**, 1054–1062 (1962).
- 14) Dick W. E. Jr., *Carbohydr. Res.*, **70**, 313–318 (1979).

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- 15) Winget G. D., Izawa S., Good N. E., *Biochemistry*, **8**, 2067—2073 (1969).
- 16) Gouvea W. L., Alpert H. C., Kelley J., Pardo V., Vaamonde C. A., *Kidney International*, **35**, 1041—1048 (1989).
- 17) Skjevrak L., Solheim E., Scheftue R. R., *Xenobiotica*, **16**, 35—45 (1986).
- 18) Nagasawa M., Koide H., Ohsawa K., Hoshi T., *Nippon Jinzogaku Kaishi*, **26**, 11—20 (1984).
- 19) Kawaguchi N., *J. Yonago Med. Ass.*, **36**, 131—143 (1985).

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Tetrahedron: Asymmetry 9 (1998) 2517–2532

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Reactions of per-*O*-acetylglucosyl isothiocyanate with carbon bases. A new method for the stereocontrolled syntheses of nucleosides and glucosylaminothiophenes

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Received 29 May 1998; accepted 16 June 1998

Abstract

Reaction of 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate **5** with diethyl malonate in a basic medium gave the corresponding glucopyranosyl thioamide without significant deacetylation. This thioamide in solution presents *Z-anti* as the sole configuration. Reactions of **5** with carbanions which have an ethoxycarbonyl group are a way to prepare anomerically pure *N*-nucleoside derivatives of pyrrole and tetrahydropyridine. Reactions of **5** with carbanions stabilized by one cyano group are used to prepare glucosylamino thiophenes with only the β -configuration. Some other stereochemical aspects of the prepared compounds are discussed. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Sugar isothiocyanates are versatile starting materials in the syntheses of several types of glycoconjugates of biological interest.¹ Of the sugar isothiocyanates, the glycosyl ones are the most useful, which has prompted the development of both preparation procedures^{1–5} and synthetic applications.^{1,6–9} At the same time, the nucleosides and aminothiophenes are interesting chemotherapeutic tools in the treatment of various infectious diseases,¹⁰ which has originated an increasing demand for new compounds, and considerable effort is being directed towards the syntheses of nucleoside analogues. In the framework of a program aimed at the development of sugar isothiocyanate chemistry, in this paper we report on the reactions of 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate,¹¹ as an example of easily available glycosyl isothiocyanate, and different carbon bases. We also explore the use of the corresponding adducts in the preparation of pyrrole and pyridine nucleoside derivatives and of glucosylaminothiophenes. Many reactions of sugar isothiocyanates with *N*-, *O*- and *S*-nucleophiles have been studied;^{1,12} however, the data on reactions with carbon bases are very scarce and limited to enamines as nucleophiles.^{7,12}

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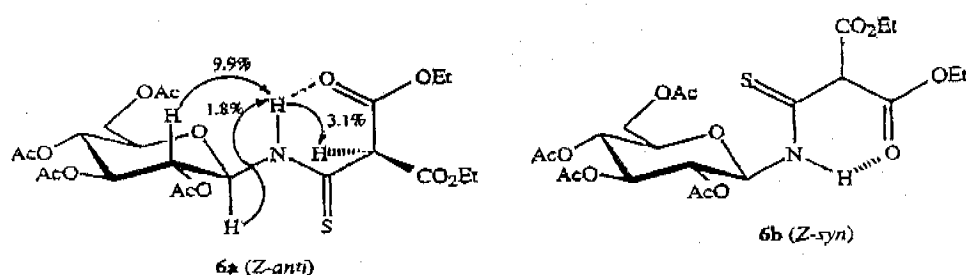
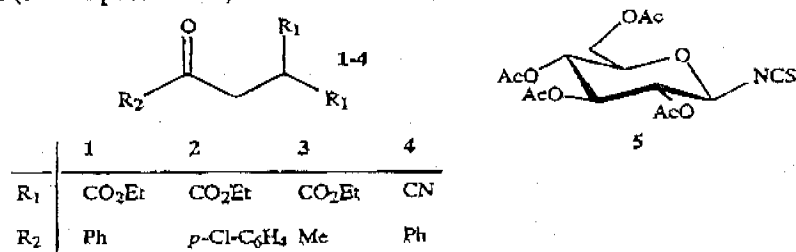


Fig. 1. Conformational stereoisomers by rotation around the C1'-N bond of 6

2. Results and discussion

Commercially available diethyl malonate, ethyl cyanoacetate, phenylthioacetonitrile, cyanoacetamide and malonodinitrile, and the newly synthesized phenacyl and acetonyl derivatives of diethyl malonate 1–3 and of malonodinitrile 4 were used to generate carbanions to study their reactions with 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate 5. Compounds 1–4 were prepared by alkylation of diethyl malonate or malonodinitrile with the corresponding α -haloketone in the presence of sodium hydride under a nitrogen atmosphere. The structures 1–4 were supported by IR, ^1H - and ^{13}C -NMR, and mass spectroscopic data (see Experimental).



2.1. Reactions of the glucosyl isothiocyanate 5 with carbanions having two ester groups

Reaction of the isothiocyanate 5 with diethyl malonate in a basic medium produced the *N*-glucosylthioamide 6 (Fig. 1), whose analytical and spectroscopic (Table 1 and Experimental) data were in agreement with the proposed structure. The hydrogen bond between the NH and an ethoxycarbonyl group, shown in Fig. 1, was evident from the chemical shift of NH (9.55 ppm, close to that for other strongly chelated amino groups⁶), and from the existence of two different resonances in the ^{13}C -NMR spectrum at 165 (CO chelated) and 164 ppm (CO free) for the ethoxycarbonyl groups.

The sugar amides and thioamides can exist in four configurational and conformational stereoisomers (Fig. 2), and because of the important biological role of many glycoconjugates with an amido function, such as glycopeptides, glycolipids, calicheamicins,¹³ and istamycins,¹⁴ it is very interesting to know the exact stereochemistry of any amidosugar. This can be a way to rationalize the role of these compounds in molecular recognition or enzyme inhibition phenomena. The aforementioned equilibrium has been evaluated for several types of amidosugar derivative¹⁵ and the *Z-anti* as sole conformer or mixtures of *Z-anti* and *Z-syn* conformers have been described as predominant stereoisomers. The recent observation¹⁶ that the value (9–11 Hz) for the $^3J_{\text{H,H}}$ between the NH and the vicinal CH sugar proton (H-1 or H-2) is compatible with both the antiperiplanar and synperiplanar arrangements of NH and CH has demonstrated

Table 1
Selected NMR data (δ , ppm; J , Hz) for compounds 6-14, 18-22, and 25,26 in CDCl_3

Comp.	δ_{NH}	$\delta_{\text{N-1}}$	$J_{\text{C-MH}}$	$J_{\text{C-2}}$	$\delta_{\text{C-1}}$	$\delta_{\text{C-5}}$
6	9.55d	5.75dd	8.1	9.5	81.7	193.7
7	11.21d	5.83t	8.2	8.2	81.9	196.5
8	11.15d	5.82t	8.2	8.2	81.9	195.4
9	10.80d	5.78dd	7.7	9.5	81.9	196.5
10 ^a	-	6.46d	-	9.6	81.3	193.8
	-	6.40bd	-	9.6	81.2	193.7
11	-	4.77d	-	8.6	92.0	166.3
12 ^b	-	4.77d	-	8.4	91.3	164.7
13	-	6.60d	-	9.6	83.1	196.7
14 ^c	-	4.88d	-	8.7	89.2	165.4
	-	4.71d	-	8.7	90.5	166.7
18	10.32d	5.59dd	7.8	9.4	82.8	-
19	7.10d	5.42dd	8.3	9.4	83.3	-
20	6.54d	4.65t	9.5	9.5	84.9	-
21	8.66d	4.66t	9.5	9.5	84.5	-
22	6.77d	4.77t	9.5	9.5	84.4	-
25	-	4.88d	-	9.5	89.2	-
26	-	4.71d	-	9.5	90.5	-

^aAs a pair of diastereomers. At room temperature. ^bIn $(\text{CD}_3)_2\text{SO}$.

a possible controversy in the published results, and new experiments have been carried out on both 2-amido-2-deoxy sugars¹⁶ and glycosylamides.¹⁷ In the case of the thioamido derivative 6, the presence of a sole stereoisomer was evident from the ^1H - and ^{13}C -NMR spectra which showed only one signal set. The above-discussed hydrogen bond is compatible with the *Z-anti* 6a and *Z-syn* 6b stereoisomers (Fig. 1) and rules out the *E-anti* and *E-syn* configurations. To distinguish between 6a and 6b the indicated NOE experiments were carried out. From these, the 1,3-*syn*-axial disposition between NH and C2'-H was evident, and consequently the *Z-anti* stereoisomer is the only possible one.

Treatment of the glucosyl isothiocyanate 5 with the diethyl phenacyl (acetonyl) malonates 1-3 and sodium hydride gave the glucosyl thioamide derivatives 7-9 respectively. The NMR spectra of compounds 7 and 8 had single sets of signals, the data being very close to those for 6, and supporting the same structure and stereochemistry. In these two cases, the chemical shift for the NH resonance was ≈ 11.2 ppm, corroborating the presence of a strong hydrogen bond. Compounds 7 and 8 were stable in solution in chloroform even after 10 days, and no formation of cyclic structures in the aglycon moiety were observed. This is probably due to the conjugation between the carbonyl and phenyl groups of the phenacyl radical. In the case of the reaction 5+3, the ^1H -NMR data of the final product demonstrated the structure 9; however the ^1H - and ^{13}C -NMR spectra showed the existence of an equilibrium between 9 and the two (5*R*,5*S*) hemiaminalic structures 10. The ratio of 9:10 at room temperature was 1.1:10 and did not change over time. The two diastereomers 10 were in a 1:1 ratio, which is consistent with the nonstereogenic character of C-2 and C-3 in the aglycon moiety of 9. With increasing temperature, the proportion of 9 in the equilibrium increased, the ratio of 9:10 reaching 1:4 at 50°C. Selected spectroscopic data of 10 are included in Table 1.

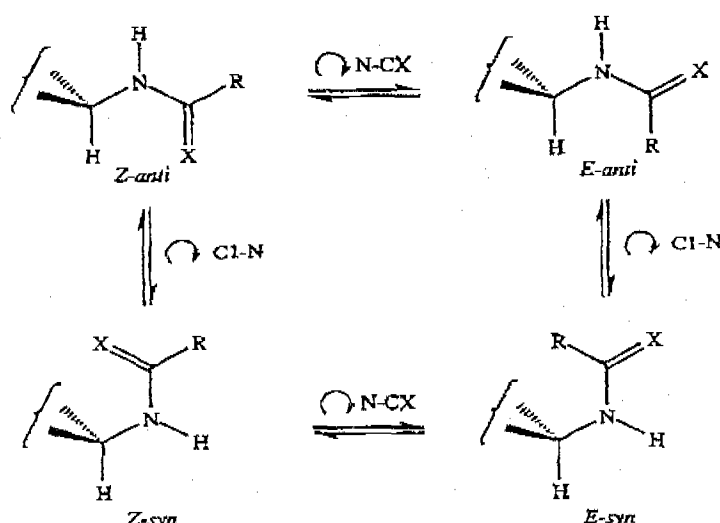
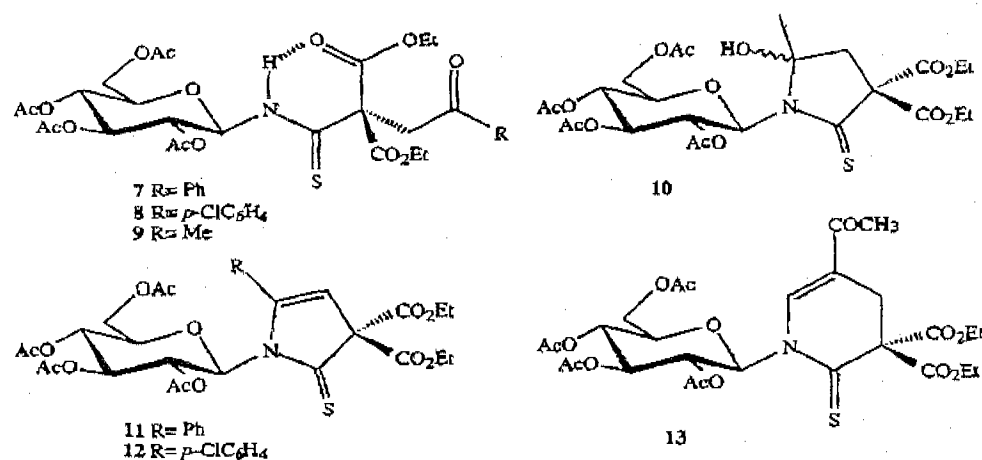


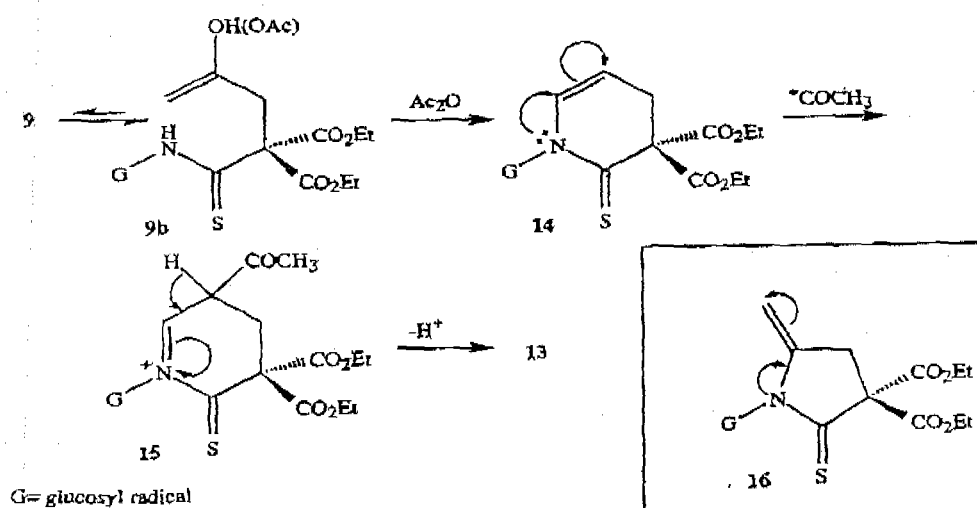
Fig. 2. Stereochemical equilibrium for the amide moiety of sugar amides ($X=O$) and thioamides ($X=S$)



Cyclodehydration of **7** and **8** with acetic anhydride and phosphoric acid gave the *N*-nucleoside derivatives of pyrroline-5-thione **11** and **12**. The resonance of the C=S group of **11** and **12** appeared at ≈ 165 ppm, a value very different to that for the starting materials, and close to that reported for related azolinethiones.¹⁸ The ¹³C resonances at ≈ 141 (C-2) and 116 (C-3) ppm and the ¹H resonance at 6.22 ppm supported the presence of the double bond in the aglycon moiety.

When the same treatment with acetic anhydride and phosphoric acid was carried out on **9**, the *N*-glucosyl pyridine derivative **13** was obtained in 65% yield after purification. The ¹H- and ¹³C-NMR spectra of **13** showed a sole signal set, and no signals attributable to the other diastereomeric structure were observed. In contrast to **11** and **12**, the C=S group of **13** resonated at 196.7 ppm; the signals for the C=CH group appeared at 152.9 (C-5), 105.1 (C-6) and 6.49 (=CH) ppm. This latter resonance was a double doublet showing allylic couplings (2.2 and 1.7 Hz) with the protons on C-4. The resonance of the ketone C=O was observed at 199.6 and there was no signal for NH. A possible mechanism to explain the formation of the *N*-nucleoside analogue **13** is depicted in Scheme 1. The enolic form **9b**,

which can be acetylated, undergoes internal addition, followed by dehydration (if acetylated elimination of AcOH) to give **14**. This intermediate is an enamine analogue with strong nucleophilic character at the β -carbon (C-5). Consequently it can react at this position with an acetyl cation to give **13**, after loss of a proton. A possible structure **16** for the intermediate was considered, but the spectroscopic data and the existence of only one stereoisomer for **13** ruled out this formula. Related six-membered cyclizations of γ -oxothioureas have been described.¹⁹



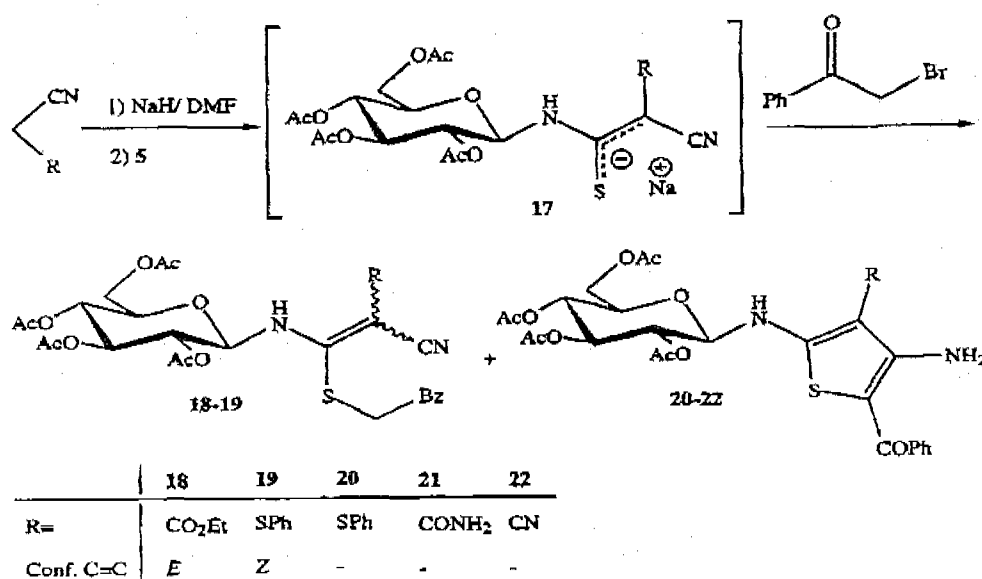
Scheme 1. Possible mechanism for the formation of **13**

2.2. Reactions of the isothiocyanate **5** with carbanions having one cyano group

When the carbanion from ethyl cyanoacetate reacted with the glucosyl isothiocyanate **5**, a non-isolatable salt (**17**) was formed (Scheme 2); which by reaction with phenacyl bromide gave the *S*-phenacyl derivative **18** in high yield, and no cyclization to a thiophene derivative was observed. The phenacyl group of **18** was evident from the IR band at 1684 cm^{-1} ($\text{C}=\text{O}$) and from the ^1H resonances at 4.96 and 4.63 ppm (AB system for CH_2 , J_{AB} 17.1 Hz) and ^{13}C resonances at 191.8 ($\text{C}=\text{O}$) and 42.7 ppm (CH_2).²⁰ The cyano group resonated at 117.3 ppm and its IR $\text{C}\equiv\text{N}$ bond appeared at 2212 cm^{-1} ; there were no signals for a $\text{C}=\text{S}$ group. The chemical shift for the resonance of the NH (10.32 ppm) demonstrated a strong hydrogen bond between this proton and the carbonyl of the ester group. The existence of this H-bond supported the *E* configuration assigned to the $\text{C}=\text{C}$ double bond.

In the case of the reaction of **5** with phenylthioacetone nitrile, and afterwards with phenacyl chloride, the *S*-phenacyl derivative **19** and the glucosylamino thiophene **20** were obtained after chromatography. The structural data of **19** closely resembled those of **18** (see Table 1 and Experimental), except the resonance for NH, which appeared at 7.10 ppm and corresponds to a weakly chelated amino proton. This fact is indicative of *Z* configuration of the $\text{C}=\text{C}$ double bond, with a hydrogen bond between the *cis* NH and $\text{C}\equiv\text{N}$ groups. Dilution studies confirmed the intramolecular chelated structure. Compound **20** (see structure discussion together with **21** and **22**) with a thiophene structure arises from the nucleophilic addition of the methylene of the phenacyl group onto the cyano group of the *E* stereoisomer of **19**.

When cyanoacetamide and malonodinitrile were used to generate the carbanions, the glucosylamino-thiophenes **21** and **22** were isolated. The IR spectra of **20**–**22** revealed the presence of the NH_2 stretching



Scheme 2. Reaction of compound 5 with cyanoderivatives

band at 3424–3322 cm^{-1} and, in the case of 22, a $\text{C}\equiv\text{N}$ absorption at 2212 cm^{-1} . The ^1H -NMR spectra showed broad signals for the NH_2 groups and a doublet for the NH at 6.54 ppm for 20 (nonchelated structure), 8.66 ppm for 21 (hydrogen bond between NH and CO groups) and 6.77 ppm (weak hydrogen bond between NH and $\text{C}\equiv\text{N}$). In contrast with what happened with 18 and 19, in the three cases 20–22, the H-1 of the sugar ring resonated at higher field than H-2, H-3 and H-4. No signals for a $\text{C}=\text{S}$ group were observed.

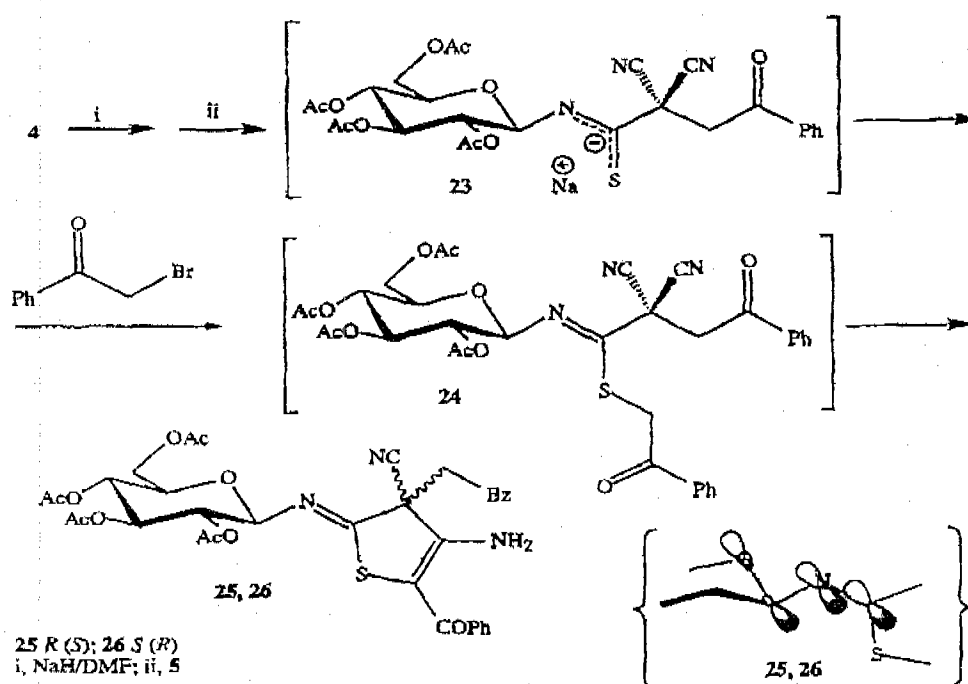
Some reactions between benzoyl isothiocyanate and methylene active reagents have been studied²¹ and a structure related to 17 has been proposed as an intermediate.

The reaction of 5 with a dicyano derivative has also been studied. Thus, when 5 was added on the carbanion proceeding from phenacylmalonodinitrile 4, and the resulting salt 23 was *S*-alkylated with phenacyl bromide, the diastomeric mixture (C-3 epimers) of glucosyliminothiophenes 25 and 26 was obtained. This mixture could be resolved chromatographically, and 25 (major) and 26 (minor) were isolated as pure products, although the configuration of C-3 could not be assigned. Compounds 25 and 26 are formed (Scheme 3) by nucleophilic addition of the methylene of the *S*-alkyl group of 24 on each one of the cyano groups. The ^1H -NMR spectra (Table 1 and Experimental) of 25 and 26 had no signals for NH, and H-1 of the sugar ring resonated as a doublet. The chemical shifts for the resonances of C-1 of the sugar ring and $\text{C}=\text{N}$ (C-2 of the thiophene ring) were close to those described for related glucosylimino derivatives.²² The resonances for the $\text{C}-\text{CH}_2$ group appeared at ≈ 42.5 ppm and the chemical shifts for the resonances of the NH_2 were ≈ 8.4 ppm, a value at a remarkably lower field than those for the same protons in 20–22; this suggests a possible seven-membered ring hydrogen bond with the carbonyl of phenacyl group.

As with other aldimines,²² the glucosylimines 25 and 26 probably prefer the conformation indicated in the Scheme 3, because this is stabilized by a $\pi-\sigma^*$ delocalization of the π electrons of the $\text{C}=\text{N}$ bond in the σ^* orbital of the $\text{C}-\text{O}$ bond in the pyranosyl ring.

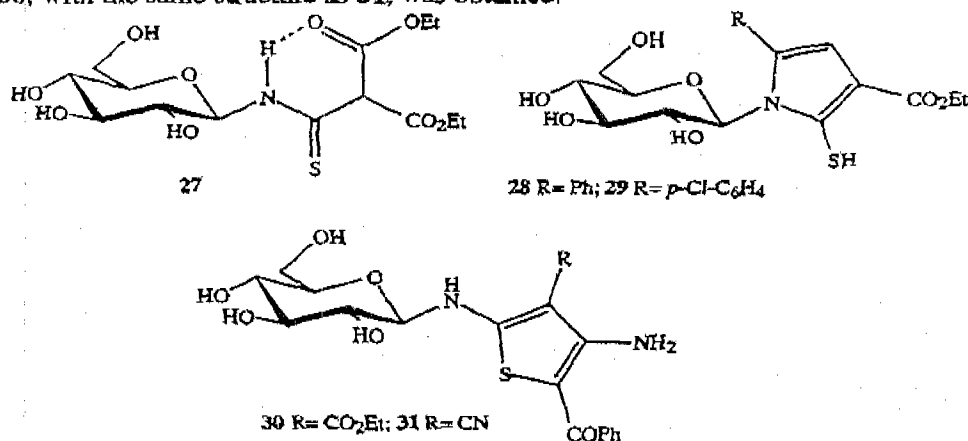
Conventional deacetylation of compounds 6, 11, 12, 18 and 22 gave the glucosyl thioamide 27, the *N*-nucleosides 28 and 29, and the glucosylaminothiophenes 30–31. The deacetylation of the phenylthio- and

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Scheme 3. Reaction of compound 5 with phenacyl malonodinitrile.

carbamoyl derivatives 19–21 was also tried but in these three cases decomposition took place, and it was not possible to isolate the corresponding deacetylated derivative. Compounds 27–31 were characterized by their NMR data (see Experimental). Compound 27 showed a strongly chelated structure (δ_{NH} 10.6 ppm). During the treatment with sodium methoxide of the *N*-nucleosides 11 and 12, saponification of an ethoxycarbonyl group and then decarboxylation took place, and in this way 28 and 29 had only one ethoxycarbonyl group. Compound 29 was isolated whereas 28 was only spectroscopically detected. In the case of compound 18, under deacylation conditions, the cyclization took place and the aminothiophene derivative 30, with the same structure as 31, was obtained.



In conclusion, a glycosyl isothiocyanate, the 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate 5, can react with methylene active compounds, in a basic medium, without significant deacetylation. These reactions open ways to prepare glycosylthioamides, nucleoside analogues of different heterocycles, and glycosylamino heterocycles. In all cases, the anomeric configuration is fixed by the C-1' configuration of the glucosyl isothiocyanate. In cases of the glucosylamino derivatives, the anomerization described for other free and *O*-protected glycosyl amines did not take place.^{23,24}

3. Experimental

3.1. General

Melting points are uncorrected. Optical rotations were measured for solutions in dichloromethane. FTIR spectra were recorded for KBr discs or thin films. ¹H-NMR (500 and 300 MHz) and ¹³C-NMR (125.7 and 75.4 MHz) spectra were obtained for solutions in CDCl₃, (CD₃)₂SO or MeOH-*d*₄. Assignments were confirmed by homonuclear 2D COSY and heteronuclear 2D correlated experiments. FAB-mass spectra were recorded with a Kratos MS-80RFA instrument with a resolution of 1000 (10% valley definition). Ions were produced by a beam of xenon atoms (6–7 kV) using a matrix consisting of thioglycerol or 3-nitrobenzyl alcohol and NaI as salt. HREIMS (70 eV), HRCIMS (150 eV) and HRDCIMS (150 eV) experiments were performed with a Micromass AutoSpecQ instrument with a resolution of 10 000 (5% valley definition). Isobutane was used as the reactive gas (500 mA, 8 kV). TLC was performed on silica gel HF₂₅₄, with detection by UV light or charring with H₂SO₄. Silica gel 60 (Merck, 230–400 mesh) was used for preparative chromatography.

3.2. General procedure for the preparation of 1–4

To a stirred solution of diethyl malonate (6.59 mmol) for 1–3 or malononitrile for 4 (6.59 mmol) in *v* mL of dry DMF over molecular sieves and under nitrogen, 9.88 mmol of NaH (as a suspension in mineral oil) are added. After 5 min, the corresponding α -haloketone (6.59 mmol) was added dropwise as a solution in 1.5 mL of dry DMF over molecular sieves and under nitrogen (for 1–3) or added directly as a solid (in the case of 4) onto the carbanion. The corresponding mixture was stirred at 30°C for 30 min, then diluted with ether (for 1–3) or dichloromethane (for 4), washed with water, dried (MgSO₄), filtered and evaporated to dryness.

3.2.1. Diethyl phenacylmalonate 1

v=1 mL. Column chromatography (ether:hexane=1:4) of the residue gave an oil (1.08 g, 59%); IR $\nu_{\max}$ 3063, 2984, 2934, 1732, 1690, 1451, 1368, 1267, 1155 and 1031 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 8.01–7.27 (m, 5H, Ph), 4.29–4.20 (m, 2H, CH₂CH₃), 4.06 (t, 1H, *J*_{HC,CH₂}=7.1, HC), 3.64 (d, 2H, CH₂COPh), 1.30 (t, 6H, ³*J*_{H,H}=7.1, 2CH₂CH₃) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 196.5 (COPh), 169.0 (2C, 2CO₂Et), 136.4–128.1 (6C, Ph), 61.7 (2C, 2CH₂CH₃), 47.1 (HC), 37.7 (CH₂COPh), 13.9 (2C, 2CH₂CH₃) ppm; HREIMS *m/z* obsd 278.1152, calcd for C₁₅H₁₈O₅ 278.1154.

3.2.2. Diethyl *p*-chlorophenacylmalonate 2

v=2 mL. Column chromatography (ether:hexane=1:4) of the residue gave an oil (1.65 g, 80%); IR $\nu_{\max}$ 3094, 2984, 2936, 1738, 1690, 1454, 1368, 1275, 1179 and 1032 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.96–7.42 (m, 4H, Ph), 4.27, 4.26 (each q, each 2H, ³*J*_{H,H}=7.1, 2CH₂CH₃), 4.07 (t, 1H, *J*_{HC,CH₂}=7.1,

HC), 3.61 (d, 2H, CH_2COPh), 1.31 (t, 6H, $2\text{CH}_2\text{CH}_3$) ppm; ^{13}C -NMR (75.4 MHz, CDCl_3): δ 195.3 (COPh), 168.8 (2C, $2\text{CO}_2\text{Et}$), 140.3–128.9 (6C, Ph), 61.7 (2C, $2\text{CH}_2\text{CH}_3$), 47.0 (HC), 36.7 (CH_2COPh), 13.9 (2C, $2\text{CH}_2\text{CH}_3$) ppm; HREIMS m/z obsd 312.0780, calcd for $\text{C}_{15}\text{H}_{17}\text{O}_5\text{Cl}$ 312.0765.

3.2.3. Diethyl acetonilmalonate 3

$v=1$ mL. Column chromatography (ether:hexane=1:4) of the residue gave an oil (0.60 g, 42%); IR ν_{max} 2986, 2940, 1738, 1458, 1366, 1271, 1159 and 1024 cm^{-1} ; ^1H -NMR (300 MHz, CDCl_3): δ 4.20 (q, 4H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$), 3.85 (t, 1H, $J_{\text{HC,CH}_2}=7.1$, HC), 3.06 (d, 2H, CH_2COCH_3), 2.21 (s, 3H, CH_2COCH_3), 1.27 (t, 6H, $2\text{CH}_2\text{CH}_3$) ppm; ^{13}C -NMR (75.4 MHz, CDCl_3): δ 204.8 (COCH_3), 168.7 (2C, $2\text{CO}_2\text{Et}$), 61.6 (2C, $2\text{CH}_2\text{CH}_3$), 46.8 (HC), 41.9 (CH_2COPh), 29.6 (COCH_3), 13.9 (2C, $2\text{CH}_2\text{CH}_3$) ppm; HREIMS m/z obsd 216.1004, calcd for $\text{C}_{10}\text{H}_{16}\text{O}_5$ 216.0998.

3.2.4. Phenacyl malonodinitrile 4

$v=2$ mL. Column chromatography (dichloromethane) of the residue gave a white solid (0.79 g, 65%) which gradually becomes brown at room temperature. This brown impurity could be removed by further column chromatography (ether:hexane=1:1); IR ν_{max} 3054, 2907, 1732, 2255, 1682, 1589, 1447, 1358 and 1217 cm^{-1} ; ^1H -NMR (300 MHz, CDCl_3): δ 8.03–7.56 (m, 5H, Ph), 4.48 (t, 1H, $J_{\text{HC,CH}_2}=6.8$, HC), 3.84 (d, 2H, CH_2) ppm; ^{13}C -NMR (75.4 MHz, CDCl_3): δ 192.2 (COPh), 134.7–128.0 (6C, Ph), 112.3 (2C, 2CN), 39.4 (CH_2COPh), 17.6 (HC) ppm; HREIMS m/z obsd 184.0635, calcd for $\text{C}_{11}\text{H}_8\text{ON}_2$ 184.0637.

3.3. Diethyl 2,3,4,6-tetra-O-aceryl- β -D-glucopyranosyl-aminothiocarbonyl malonate 6

To a stirred solution of **5**¹¹ (0.50 mg, 1.28 mmol) in dry DMF (4 mL) under argon at 40°C, diethyl malonate (0.19 mL, 1.28 mmol) and KOH (1.28 mmol) were added. The mixture was stirred for 1 h at 40°C, then diluted with dichloromethane, washed with water, dried (MgSO_4), filtered and evaporated to dryness. The resulting product was purified by recrystallization (ether:hexane). **6** was obtained as a crystalline solid (0.43 mg, 61%) which had mp 101–102°C (ether:hexane); $[\alpha] +52$ (c 1.0); IR ν_{max} 3308, 2982, 2942, 1750, 1537, 1420, 1370, 1225 and 1040 cm^{-1} ; ^1H -NMR (500 MHz, CDCl_3): δ 9.55 (d, 1H, $J_{\text{NH,H}}=8.1$, NH), 5.75 (dd, 1H, $J_{1,2}=9.5$, H-1), 5.35 (t, 1H, $J_{2,3}=J_{3,4}=9.5$, H-3), 5.21 (t, 1H, H-2), 5.12 (t, 1H, $J_{4,5}=9.5$, H-4), 4.97 (s, 1H, CH), 4.31–4.21 (m, 5H, H-6a and $2\text{CH}_2\text{CH}_3$), 4.11 (dd, 1H, $J_{5,6a}=2.1$, $J_{6a,6b}=12.5$, H-6b), 3.86 (ddd, 1H, $J_{5,6a}=4.5$, H-5), 2.09, 2.08, 2.04, 2.03 (each s, each 3H, 4Ac), 1.31, 1.28 (each t, each 3H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$) ppm; ^{13}C -NMR (125.7 MHz, CDCl_3): δ 193.7 (C=S), 170.5, 170.4, 169.7, 169.3 (4 COCH_3), 165.0, 164.0 ($2\text{CO}_2\text{Et}$), 81.7 (C-1), 73.7 (C-5), 72.4 (C-3), 69.8 (C-2), 68.0 (C-4), 66.2 (CH), 62.8, 62.7 ($2\text{CH}_2\text{CH}_3$), 61.4 (C-6), 20.5, 20.2 (2COCH_3), 20.4 (2C, 2COCH_3), 13.8 and 13.6 ($2\text{CH}_2\text{CH}_3$) ppm; FABMS m/z 572 (100, $[\text{M}+\text{Na}]^+$). Anal. calcd for $\text{C}_{22}\text{H}_{31}\text{O}_{13}\text{NS}$: C, 48.08; H, 5.69; N, 2.55; S, 5.83. Found: C, 47.94; H, 5.60; N, 2.66; S, 5.66.

3.4. General procedure for the preparation of 7–10

To a stirred solution of 0.26 mmol **1** for **7**, **2** for **8**, or **3** for **9** and **10** in v mL of dry DMF over molecular sieves and under nitrogen, 0.31 mmol of NaH (as a suspension in mineral oil) was added. After 5 min, 0.26 mmol of 2,3,4,6-tetra-O-aceryl- β -D-glucopyranosyl isothiocyanate **5** was added dropwise as a solution in 0.75 mL of dry DMF over molecular sieves and under nitrogen onto the carbanion. The mixture was stirred at 30°C for t min, then diluted with ether, washed with water, dried (MgSO_4), filtered and evaporated to dryness.

3.4.1. *N*-(2',3',4',6'-Tetra-*O*-acetyl- β -D-glucopyranosyl)-2,2-diethoxycarbonyl-4-phenyl-4-oxo-thiobutanamide 7

Column chromatography (ether:hexane=1:1 and 3:1) of the residue gave a white amorphous solid (69 mg, 40%) which had $[\alpha] +39$ (c 1.0); IR $\nu_{\max}$ 3214, 3030, 2980, 2942, 1751, 1688, 1537, 1452, 1370, 1225 and 1042 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 11.21 (d, 1H, $J_{\text{NH},1'}=8.2$, NH), 7.98–7.27 (m, 5H, Ph), 5.83 (t, 1H, $J_{1',2'}=8.2$, H-1'), 5.37–5.30 (m, 2H, H-2', 3'), 5.15 (m, 1H, H-4'), 4.70 (d, 1H, $J_{3a,3b}=18.6$, C-3a), 4.33–4.20 (m, 5H, H-6'a and $2\text{CH}_2\text{CH}_3$), 4.29 (d, 1H, C-3b), 4.09 (dd, 1H, $J_{5',6'b}=2.3$, $J_{6'a,6'b}=12.4$, H-6'b), 3.83 (ddd, 1H, $J_{4,5}=10.0$, $J_{5',6'a}=4.6$, H-5), 2.09, 2.06 (each s, each 3H, 2Ac), 2.03 (s, 6H, 2Ac), 1.22, 1.19 (each t, each 3H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$) ppm; $^{13}\text{C-NMR}$ (75.4 MHz, CDCl_3): δ 199.9 (C-4), 196.5 (C-1), 170.6, 170.0, 169.9, 169.4 (4COCH_3), 168.0, 166.2 ($2\text{CO}_2\text{Et}$), 135.9–128.1 (6C, Ph), 81.9 (C-1'), 73.9 (C-5'), 73.0, 69.9 (C-2', 3'), 68.3 (C-4'), 65.4 (C-2), 63.0, 62.9 ($2\text{CH}_2\text{CH}_3$), 61.6 (C-6'), 47.1 (C-3), 20.6, 20.4 (2COCH_3), 20.5 (2C, 2COCH_3), 13.6, 13.5 ($2\text{CH}_2\text{CH}_3$) ppm; FABMS m/z 690 (100, $[\text{M}+\text{Na}]^+$). Anal. calcd for $\text{C}_{30}\text{H}_{37}\text{O}_{14}\text{NS}$: C, 53.97; H, 5.59; N, 2.10; S, 4.80. Found: C, 53.97; H, 5.51; N, 2.21; S, 5.18.

3.4.2. *N*-(2',3',4',6'-Tetra-*O*-acetyl- β -D-glucopyranosyl)-4-(4-chlorophenyl)-2,2-diethoxycarbonyl-4-oxo-2-thiobutanamide 8

Column chromatography (ether:hexane=1:1 and 3:1) of the residue gave a white amorphous solid (108 mg, 59%) which had $[\alpha] +45$ (c 1.0); IR $\nu_{\max}$ 3206, 3041, 2984, 2942, 1755, 1684, 1539, 1368, 1225 and 1038 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 11.15 (d, 1H, $J_{\text{NH},1'}=8.2$, NH), 7.93–7.40 (m, 4H, Ar), 5.82 (t, 1H, $J_{1',2'}=8.2$, H-1'), 5.37–5.27 (m, 2H, H-2', 3'), 5.15 (t, 1H, $J_{3',4'}=J_{4',5'}=10.0$, H-4'), 4.66, 4.25 (each d, each 1H, $J_{3a,3b}=18.6$, H-3a, 3b), 4.24 (dd, 1H, $J_{5',6'a}=4.6$, $J_{6'a,6'b}=12.4$, H-6'a), 4.24 (q, 4H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$), 4.09 (dd, 1H, $J_{5',6'b}=2.3$, H-6'b), 3.83 (ddd, 1H, H-5'), 2.09, 2.07 (each s, each 3H, 2Ac), 2.04 (s, 6H, 2Ac), 1.23, 1.20 (each t, each 3H, $2\text{CH}_2\text{CH}_3$) ppm; $^{13}\text{C-NMR}$ (75.4 MHz, CDCl_3): δ 199.6 (C-4), 195.4 (C-1), 170.5, 169.4 (2COCH_3), 169.9 (2C, 2COCH_3), 167.8, 166.0 ($2\text{CO}_2\text{Et}$), 140.0–128.9 (6C, Ar), 81.9 (C-1'), 73.8 (C-5'), 72.9, 70.4 (C-2', 3'), 68.2 (C-4'), 65.3 (C-2), 63.0, 62.9 ($2\text{CH}_2\text{CH}_3$), 61.6 (C-6'), 46.9 (C-3), 20.6, 20.4 (2COCH_3), 20.5 (2C, 2COCH_3), 13.5 and 13.4 ($2\text{CH}_2\text{CH}_3$) ppm; FABMS m/z 724 (100, $[\text{M}+\text{Na}]^+$). Anal. calcd for $\text{C}_{30}\text{H}_{36}\text{O}_{14}\text{NSCl}$: C, 51.32; H, 5.17; N, 1.99; S, 4.57. Found: C, 51.21; H, 4.99; N, 2.05; S, 4.58.

3.4.3. *N*-(2',3',4',6'-Tetra-*O*-acetyl- β -D-glucopyranosyl)-2,2-diethoxycarbonyl-4-oxothiopentanamide (9) and 2(R,S)-4,4-diethoxycarbonyl-2-hydroxy-2-methyl-1-(2',3',4',6'-tetra-*O*-acetyl- β -D-glucopyranosyl)-5-thioxopyrrolidine 10

Column chromatography (ether:hexane=3:1) of the residue gave a white amorphous solid 9+10 (58 mg, 37%).

Compound 9: $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 10.80 (d, 1H, $J_{\text{NH},1'}=7.7$, NH), 5.78 (dd, 1H, $J_{1',2'}=9.5$, H-1'), 5.39 (t, 1H, $J_{2',3'}=J_{3',4'}=9.5$, H-3'), 5.24 (t, 1H, H-2'), 5.12 (t, 1H, $J_{4',5'}=9.5$, H-4'), 4.30–4.18 (m, 4H, H-6'a, 6'b and CH_2CH_3), 3.86–3.83 (m, 1H, H-5'), 2.95–2.75 (m, 2H, H-3a, 3b), 2.09, 2.06, 2.02, 1.92 (each s, each 3H, 4Ac), 1.73 (s, 1H, CH_3COCH_2), 1.26 (t, 6H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$) ppm.

Compound 10: $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 6.38 (d, 1H, $J_{1',2'}=9.5$, H-1'), 5.35, 5.33 (each t, each 1H, $J_{2',3'}=J_{3',4'}=9.5$, H-2', 3'), 5.15 (t, 1H, $J_{4',5'}=9.5$, H-4'), 4.30–4.18 (m, 4H, H-6'a, 6'b and CH_2CH_3), 3.81 (dt, 1H, $J_{5',6'a}=J_{5',6'b}=3.3$, H-5'), 2.81–2.76 (m, 2H, H-3a, 3b), 2.07, 2.04, 2.01, 1.98 (each s, each 3H, 4Ac), 1.62 (s, 1H, CH_3COCH_2), 1.29 (t, 6H, $^3J_{\text{H,H}}=7.1$, $2\text{CH}_2\text{CH}_3$) ppm; $^{13}\text{C-NMR}$ (125.7 MHz, CDCl_3): δ 199.7, 195.8 (2CS), 171.9–166.0 (12C , $4\text{CO}_2\text{Et}$ and 8COCH_3), 84.2, 83.7 (2C-1'), 75.6, 74.9 (2C-5'), 73.0, 71.9 (2C-2'), 72.3, 70.4 (2C-3'), 67.9, 67.6 (2C-4'), 96.7, 95.2 (2C-2), 70.9, 70.2 (2C-4), 63.0–60.9 (2C-6' and $4\text{CH}_2\text{CH}_3$), 46.7, 44.9 (2C-3), 27.3, 25.0 (2CH_3), 20.7–20.2 (8C, 8COCH_3), 13.8,

13.7 (4C, 4CH₂CH₃) ppm; FABMS *m/z* 628 (100, [M+Na]⁺). HRFABMS *m/z* obsd 628.1665, calcd for C₂₅H₃₅O₁₄NSNa 628.1676. Anal. calcd for C₂₅H₃₅O₁₄NS: C, 49.58; H, 5.83; N, 2.31; S, 5.29. Found: C, 48.91; H, 5.47; N, 2.45; S, 5.25.

3.5. General procedure for the preparation of 11–13

The starting material (7 for 11, 8 for 12 or 9 for 13, 0.34 mmol) was dissolved in 3.4 mL of acetic anhydride and 0.17 mL of melted anhydrous H₃PO₄. The mixture was stirred at r.t. for 1 hour and then poured into ice–water, extracted with ether, washed with saturated aqueous NaHCO₃ and water, dried (MgSO₄) and concentrated. The residue was taken up in 96% ethanol and treated with basic resin Amberlist IR-45(OH), filtered and evaporated. The residue was purified by column chromatography (ether:hexane=3:1).

3.5.1. 4,4-Diethoxycarbonyl-2-phenyl-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-5-thioxo-2-pyrroline 11

White and amorphous solid (168 mg, 76%); *t*=12 h; [α] −7 (c 0.9); IR *v*_{max} 3067, 2980, 1750, 1653, 1447, 1371, 1225 and 1040 cm^{−1}; ¹H-NMR (500 MHz, CDCl₃): δ 7.52–7.39 (m, 5H, Ph), 6.22 (s, 1H, H-3), 5.32 (t, 1H, *J*_{2',3'}=*J*_{3',4'}=8.9, H-3'), 5.23 (dd, 1H, *J*_{1',2'}=8.6, H-2'), 5.20 (t, 1H, *J*_{4',5'}=8.9, H-4'), 4.77 (d, 1H, H-1'), 4.32–4.24 (m, 5H, H-6'a and 2CH₂CH₃), 4.20 (dd, 1H, *J*_{5',6'b}=2.2, *J*_{6'a,6'b}=12.3, H-6'b), 3.86 (ddd, 1H, *J*_{5',6'a}=5.1, H-5'), 2.09, 2.05, 2.01, 1.97 (each s, each 3H, 4Ac), 1.29, 1.28 (each t, each 3H, ³*J*_{H,H}=7.0, 2CH₂CH₃) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 170.7, 170.3, 169.3, 168.9 (4COCH₃), 166.3 (C-5), 165.3, 165.2 (2CO₂Et), 142.4 (C-2), 132.1–126.4 (6C, Ph), 115.0 (C-3), 92.0 (C-1'), 76.9 (C-4), 73.7 (C-5'), 73.5 (C-3'), 71.4 (C-2'), 68.4 (C-4'), 62.9, 62.8 (2CH₂CH₃), 62.1 (C-6'), 20.7 (COCH₃), 20.5 (3C, 3COCH₃), 13.9 and 13.8 (2CH₂CH₃) ppm; FABMS *m/z* 672 (100, [M+Na]⁺). Anal. calcd for C₃₀H₃₅O₁₃NS: C, 55.46; H, 5.43; N, 2.16; S, 4.94. Found: C, 55.43; H, 5.20; N, 2.17; S, 5.08.

3.5.2. 2-(4-Chlorophenyl)-4,4-diethoxycarbonyl-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-5-thioxo-2-pyrroline 12

White and amorphous solid (156 mg, 67%); *t*=10 h; [α] +54 (c 0.7); IR *v*_{max} 3075, 2980, 1753, 1655, 1437, 1371, 1225 and 1040 cm^{−1}; ¹H-NMR (300 MHz, CDCl₃): δ 7.42–7.40 (m, 4H, Ar), 6.22 (s, 1H, H-3), 5.32 (t, 1H, *J*_{2',3'}=*J*_{3',4'}=9.3, H-3'), 5.22 (dd, 1H, *J*_{1',2'}=8.4, H-2'), 5.19 (t, 1H, *J*_{4',5'}=9.3, H-4'), 4.77 (d, 1H, H-1'), 4.32–4.24 (m, 4H, H-6'a), 4.29 (q, 4H, ³*J*_{H,H}=7.1, 2CH₂CH₃), 4.20 (dd, 1H, *J*_{5',6'b}=2.4, *J*_{6'a,6'b}=12.4, H-6'b), 3.86 (ddd, 1H, *J*_{5',6'a}=4.6, H-5'), 2.09, 2.05, 2.01, 1.97 (each s, each 3H, 4Ac), 1.29, 1.28 (each t, each 3H, 2CH₂CH₃) ppm; ¹³C-NMR (125.7 MHz, DMSO-*d*₆): δ 170.0, 169.6, 169.3, 168.6 (4COCH₃), 164.8, 164.8 (2CO₂Et), 164.7 (C-5), 140.3 (C-2), 134.5–128.1 (6C, Ar), 116.4 (C-3), 91.3 (C-1'), 76.7 (C-4), 72.5 (C-5'), 72.3 (C-3'), 71.2 (C-2'), 68.0 (C-4'), 62.5, 62.4 (2CH₂CH₃), 61.8 (C-6'), 20.5, 20.4 (2COCH₃), 20.3 (2C, 2COCH₃), 13.7, 13.6 (2CH₂CH₃) ppm; FABMS *m/z* 706 (100, [M+Na]⁺). Anal. calcd for C₃₀H₃₄O₁₃NSCl: C, 52.67; H, 5.01; N, 2.05; S, 4.69. Found: C, 52.49; H, 4.94; N, 2.40; S, 4.59.

3.5.3. 5-Acetyl-3,3-diethoxycarbonyl-1,2,3,4-tetrahydro-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-2-thioxopyridine 13

Amorphous solid (139 mg, 65%); *t*=7 h; [α] −20 (c 0.4); IR *v*_{max} 2978, 1753, 1690, 1605, 1439, 1371, 1223, 1098 and 1040 cm^{−1}; ¹H-NMR (500 MHz, CDCl₃): δ 6.60 (d, 1H, *J*_{1',2'}=9.6, H-1'), 6.49 (dd, 1H, H-6), 5.66 (t, 1H, *J*_{2',3'}=9.6, H-2'), 5.42 (t, 1H, *J*_{3',4'}=9.6, H-3'), 5.25 (t, 1H, *J*_{4',5'}=9.6, H-

4'), 4.39 (dd, $J_{5',6'a}=3.7$, $J_{6'a,6'b}=12.7$, H-6'a), 4.33–4.21 (m, 4H, 2CH₂CH₃), 4.17 (dd, 1H, $J_{5',6'b}=2.0$, H-6'b), 3.93 (ddd, 1H, H-5'), 4.03 (dd, 1H, $J_{4a,4b}=19.1$, $J_{4a,6}=1.7$, H-4a), 3.75 (dd, 1H, $J_{4b,6}=2.2$, H-4b), 2.33 (s, 3H, =CCOCH₃), 2.13, 2.08, 2.04, 1.94 (each s, each 3H, 4Ac), 1.30, 1.29 (each t, each 3H, $^3J_{H,H}=7.1$, 2CH₂CH₃) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 199.6 (=CCOCH₃), 196.7 (C-2), 170.1, 169.6 (2COCH₃), 169.3 (2C, 2COCH₃), 166.4, 165.8 (2CO₂Et), 152.9 (C-5), 105.1 (C-6), 83.1 (C-1'), 74.8 (C-5'), 72.8 (C-3'), 69.9 (C-3), 67.2 (C-2'), 66.9 (C-4'), 62.8, 62.7 (2CH₂CH₃), 61.1 (C-6'), 36.6 (C-4), 32.1 (=CCOCH₃), 20.4, 20.0 (2COCH₃), 20.3 (2C, 2COCH₃), 13.7, 13.6 (2CH₂CH₃) ppm; FABMS *m/z* 652 (100, [M+Na]⁺). Anal. calcd for C₂₇H₃₅O₁₄NS: C, 51.50; H, 5.60; N, 2.22; S, 5.09. Found: C, 50.29; H, 5.71; N, 2.64; S, 5.47.

3.6. General procedure for the reaction of 5 with cyanocarbanions

To a stirred solution of 0.26 mmol of ethyl cyanoacetate for 18, phenylthioacetone for 19 and 20, cyanoacetamide for 21, malononitrile for 22, or 4 for 25 and 26 in 0.75 mL of dry DMF at 30°C and under nitrogen, 0.33 mmol of NaH (as a suspension in mineral oil) was added. After 5 min, 5 (0.26 mmol) was dropped, as a solution in 0.75 mL of dry DMF under nitrogen onto the carbanion. The mixture was stirred at 30°C for *t*₁ min, then 0.26 mmol of phenacyl bromide was added and stirred for *t*₂ min. The mixture was poured into saturated aqueous NH₄Cl, extracted with ether (except for the case of 21, which was extracted with dichloromethane), washed with water, dried (MgSO₄), filtered and evaporated to dryness.

3.6.1. (E)-Ethyl 2-cyano-3-phenacylthio-3-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylamino)acrylate 18

*t*₁=*t*₂=10 min. Column chromatography (ether:hexane=3:1) of the residue gave an amorphous solid (150 mg, 93%); [α]_D²⁰ +15 (c 1.0); IR *v*_{max} 3192, 3063, 2982, 2212, (CN), 1770, 1684 (CO of phenacyl), 1582, 1439, 1370, 1260, 1084 and 1032 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 10.32 (d, 1H, $J_{NH,1'}=7.8$, NH), 7.98–7.50 (m, 5H, Ph), 5.59 (dd, 1H, $J_{1',2'}=9.4$, H-1'), 5.35 (t, 1H, $J_{2',3'}=J_{3',4'}=9.4$, H-3'), 5.21 (t, 1H, H-2'), 5.09 (t, 1H, $J_{4',5'}=9.4$, H-4'), 4.96, 4.63 (each d, each 1H, $^2J_{H,H}=17.1$, CH₂COPh), 4.23 (dd, 1H, $J_{5',6'a}=4.8$, $J_{6'a,6'b}=12.4$, H-6'a), 4.23 (q, 2H, $^3J_{H,H}=7.1$, CH₂CH₃), 4.08 (dd, 1H, $J_{5',6'b}=2.2$, H-6'b), 3.85 (ddd, 1H, H-5'), 2.09, 2.06 (each s, each 3H, 2Ac), 2.04 (s, 6H, 2Ac), 1.30 (t, 3H, CH₂CH₃) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 191.8 (COPh), 170.5, 169.9, 169.8, 169.7, 169.3, 166.3 (4COCH₃, CO₂Et and C-3), 134.8–128.4 (6C, Ph), 117.3 (CN), 82.8 (C-1'), 81.5 (C-2), 73.3 (C-5'), 72.7 (C-3'), 69.9 (C-2'), 68.1 (C-4'), 61.5 (2C, C-6' and CH₂CH₃), 42.7 (CH₂COPh), 20.6, 20.4 (2COCH₃), 20.5 (2C, 2COCH₃), 14.1 (CH₂CH₃) ppm; FABMS *m/z* 643 (100, [M+Na]⁺). Anal. calcd for C₂₈H₃₂O₁₂N₂S: C, 54.19; H, 5.20; N, 4.51. Found: C, 54.63; H, 5.79; N, 4.47.

3.6.2. (Z)-3-Phenacylthio-2-phenylthio-3-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylamino)acrylonitrile 19 and 3-amino-2-benzoyl-4-phenylthio-5-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylamino)thiophene 20

*t*₁=*t*₂=30 min. Column chromatography (ether:hexane=2:1) of the residue gave 19 (56 mg, 33%) and 20 (29 mg, 17%).

19: [α]_D²⁰ -4 (c 0.3); IR *v*_{max} 3312, 3059, 2953, 2195 (CN), 1746, 1709 (CO of phenacyl), 1549, 1452, 1371, 1227 and 1036 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 8.04–7.08 (m, 11H, NH and 2Ph), 5.42 (dd, 1H, $J_{NH,1'}=8.3$, $J_{1',2'}=9.4$, H-1'), 5.32 (t, 1H, $J_{2',3'}=J_{3',4'}=9.4$, H-3'), 5.04, 5.00 (each t, each 1H, $J_{4',5'}=9.4$, H-2', 4'), 4.96, 4.48 (each d, each 1H, $^2J_{H,H}=16.9$, CH₂COPh), 4.22 (dd, 1H, $J_{5',6'a}=4.8$, $J_{6'a,6'b}=12.4$, H-6'a), 4.04 (dd, 1H, $J_{5',6'b}=2.2$, H-6'b), 3.79 (ddd, 1H, H-5'), 2.04, 2.02, 2.01, 1.78 (each s, each 3H, 4Ac) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 192.3 (COPh), 170.7, 170.6, 170.4, 169.7,

169.3 (C-3 and 4COCH₃), 134.8–125.7 (12C, 2Ph), 119.2 (CN), 83.3 (C-1'), 79.2 (C-2), 73.0 (C-5'), 72.4 (C-3'), 69.9 (C-2'), 68.2 (C-4'), 61.5 (C-6'), 42.0 (CH₂COPh), 20.5, 20.4, 20.0, 19.9 (4COCH₃) ppm; FABMS *m/z* 679 (100, [M+Na]⁺). Anal. calcd for C₃₁H₃₃O₁₀N₂S₂: C, 56.61; H, 5.06; N, 4.26; S, 9.75. Found: C, 56.30; H, 5.02; N, 4.20; S, 9.40.

20: [α] –11 (c 0.7); IR *v*_{max} 3322 (NH), 3057, 2918, 2851, 1748, 1460, 1371, 1227 and 1036 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 7.76–7.06 (m, 10H, 2Ph), 6.54 (d, 1H, *J*_{NH,1'} = 9.5, NH), 5.29 (t, 1H, *J*_{2',3'} = *J*_{3',4'} = 9.5, H-3'), 5.01 (t, 1H, *J*_{4',5'} = 9.5, H-4'), 4.99 (t, 1H, *J*_{1',2'} = 9.5, H-2'), 4.65 (t, 1H, H-1'), 4.21 (dd, 1H, *J*_{5',6'a} = 5.5, *J*_{6'a,6'b} = 12.4, H-6'a), 4.08 (dd, 1H, *J*_{5',6'b} = 2.3, H-6'b), 3.79 (ddd, 1H, H-5'), 2.02, 2.00, 1.88, 1.68 (each s, each 3H, 4Ac) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 186.1 (COPh), 170.8, 170.5, 169.8, 169.4 (4COCH₃), 162.7, 157.8 (2C, thiophene ring), 140.1–125.7 (12C, 2Ph), 97.8, 95.1 (2C, thiophene ring), 84.9 (C-1'), 73.1 (C-5'), 72.1 (C-3'), 70.4 (C-2'), 68.3 (C-4'), 61.6 (C-6'), 20.4 (2C, 2COCH₃), 20.3, 19.9 (2COCH₃) ppm; HREIMS *m/z* obsd 656.1510, calcd for C₃₁H₃₂O₁₀N₂S₂ 656.1498.

3.6.3. 3-Amino-2-benzoyl-4-carbamoyl-5-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylamino)thiophene 21

*t*₁ = 15 min, *t*₂ = 30 min. Column chromatography (dichloromethane:methanol = 40:1) of the residue gave 21 (71 mg, 46%) as an amorphous solid which had [α] –58 (c 0.7); IR *v*_{max} 3443 (NH), 3287, 3061, 2951, 1753, 1657, 1466, 1371, 1225 and 1036 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 8.66 (d, 1H, *J*_{NH,1'} = 9.5, NH), 7.69–7.45 (m, 5H, Ph), 5.75 (s, 2H, NH₂), 5.33 (t, 1H, *J*_{2',3'} = *J*_{3',4'} = 9.5, H-3'), 5.12 (t, 1H, *J*_{1',2'} = 9.5, H-2'), 5.05 (t, 1H, *J*_{4',5'} = 9.5, H-4'), 4.66 (t, 1H, H-1'), 4.19 (dd, 1H, *J*_{5',6'a} = 5.4, *J*_{6'a,6'b} = 12.4, H-6'a), 4.09 (dd, 1H, *J*_{5',6'b} = 2.2, H-6'b), 3.78 (ddd, 1H, H-5'), 2.07, 2.04, 2.03, 1.87 (each s, each 3H, 4Ac) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 187.1 (COPh), 170.4, 170.2, 169.9, 169.3 (4COCH₃), 164.9, 155.9 (2C, thiophene ring), 141.2–127.2 (6C, Ph), 103.2, 98.8 (2C, thiophene ring), 84.5 (C-1'), 73.2 (C-5'), 72.4 (C-3'), 70.6 (C-2'), 68.2 (C-4'), 61.6 (C-6'), 20.5, 20.3 (2COCH₃), 20.4 (2C, 2COCH₃) ppm; FABMS *m/z* 614 (100, [M+Na]⁺). Anal. calcd for C₂₆H₂₉O₁₁N₃S: C, 52.79; H, 4.94; N, 7.10; S, 5.42. Found: C, 52.64; H, 5.10; N, 6.96; S, 5.84.

3.6.4. 3-Amino-2-benzoyl-4-cyano-5-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylamino)thiophene 22

*t*₁ = 15 min, *t*₂ = 10 min. Column chromatography (ether:hexane = 6:1) of the residue gave 22 (136 mg, 91%) as an amorphous solid which had [α] –103 (c 0.5); IR *v*_{max} 3424 (NH), 3302, 3055, 2951, 2212 (CN), 1753, 1597, 1478, 1371, 1221 and 1040 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 7.70–7.43 (m, 5H, Ph), 7.10–6.85 (bs, 2H, NH₂), 6.77 (d, 1H, *J*_{NH,1'} = 9.5, NH), 5.34 (t, 1H, *J*_{2',3'} = *J*_{3',4'} = 9.5, H-3'), 5.07 (t, 1H, *J*_{4',5'} = 9.5, H-4'), 5.03 (t, 1H, *J*_{1',2'} = 9.5, H-2'), 4.77 (t, 1H, H-1'), 4.24 (dd, 1H, *J*_{5',6'a} = 5.2, *J*_{6'a,6'b} = 12.5, H-6'a), 4.12 (dd, 1H, *J*_{5',6'b} = 2.1, H-6'b), 3.84 (ddd, 1H, H-5'), 2.11, 2.04, 2.03, 1.91 (each s, each 3H, 4Ac) ppm; ¹³C-NMR (125.7 MHz, CDCl₃): δ 186.5 (COPh), 171.4, 170.4, 169.8, 169.3 (4COCH₃), 164.5, 157.8 (2C, thiophene ring), 140.1–127.1 (6C, Ph), 112.6 (CN), 97.0 (1C, thiophene ring), 84.4 (C-1'), 82.2 (1C, thiophene ring), 73.0 (C-5'), 72.1 (C-3'), 70.9 (C-2'), 67.9 (C-4'), 61.5 (C-6'), 20.6, 20.4 (2COCH₃), 20.5 (2C, 2COCH₃) ppm; FABMS *m/z* 596 (100, [M+Na]⁺). Anal. calcd for C₂₆H₂₇O₁₀N₃S: C, 54.44; H, 4.74; N, 7.33; S, 5.59. Found: C, 54.04; H, 4.95; N, 7.29.

3.6.5. 3R(S) and 3S(R)-4-Amino-5-benzoyl-3-cyano-2,3-dihydro-3-phenacyl-2-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylimino)thiophene 25 and 26

*t*₁ = *t*₂ = 20 min. Column chromatography (ether:hexane = 2:1 and 4:1) of the residue gave 25 (22 mg, 12%) and 26 (40 mg, 22%).

25: $[\alpha] -135$ (c 0.6); IR $\nu_{\max}$ 3395 (NH), 3283, 3063, 2955, 2208 (CN), 1751, 1684, 1613, 1481, 1371, 1227 and 1040 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.41 (bs, 2H, NH_2), 8.06–7.26 (m, 10H, 2Ph), 5.32 (t, 1H, $J_{2',3'}=J_{3',4'}=9.5$, H-3'), 5.15 (t, 1H, $J_{1',2'}=9.5$, H-2'), 5.08 (t, 1H, $J_{4',5'}=9.5$, H-4'), 4.88 (d, 1H, H-1'), 4.20 (dd, 1H, $J_{5',6'a}=5.7$, $J_{6'a,6'b}=12.3$, H-6'a), 4.18 (d, 1H, $^2J_{\text{H,H}}=18.6$, CHHCOPh), 4.10 (dd, 1H, $J_{5',6'b}=2.2$, H-6'b), 3.88 (d, 1H, CHHCOPh), 3.82 (ddd, 1H, H-5'), 2.04 (s, 6H, 2Ac), 2.03, 1.79 (each s, each 3H, 2Ac) ppm; $^{13}\text{C-NMR}$ (125.7 MHz, CDCl_3): δ 195.5 (CH_2COPh), 189.3 (COPh), 170.5, 170.1, 170.0, 169.5 (4COCH_3), 165.4, 162.6, 153.5 (3C, thiophene ring), 140.0–127.0 (12C, 2Ph), 115.5 (CN), 97.3 (1C, thiophene ring), 89.2 (C-1'), 74.1 (C-5'), 72.8 (C-3'), 71.2 (C-2'), 68.5 (C-4'), 62.1 (C-6'), 48.5 (CH_2Ph), 20.8, 20.6, 20.5, 20.4 (4COCH_3) ppm; HREIMS m/z obsd 691.1857, calcd for $\text{C}_{34}\text{H}_{33}\text{O}_{11}\text{N}_3\text{S}$ 691.1836.

26: $[\alpha] +89.2$ (c 0.7); IR $\nu_{\max}$ 3368 (NH), 3298, 3059, 2957, 2185 (CN), 1759, 1694, 1620, 1481, 1371, 1229 and 1036 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.36 (bs, 2H, NH_2), 8.04–7.26 (m, 10H, 2Ph), 5.31 (t, 1H, $J_{2',3'}=J_{3',4'}=9.5$, H-3'), 5.20 (t, 1H, $J_{1',2'}=9.5$, H-2'), 5.12 (t, 1H, $J_{4',5'}=9.5$, H-4'), 4.71 (d, 1H, H-1'), 4.25 (dd, 1H, $J_{5',6'a}=5.1$, $J_{6'a,6'b}=12.6$, H-6'a), 4.24 (d, 1H, $^2J_{\text{H,H}}=18.4$, CHHCOPh), 4.15 (dd, 1H, $J_{5',6'b}=2.2$, H-6'b), 3.82 (ddd, 1H, H-5'), 3.81 (d, 1H, CHHCOPh), 2.03, 2.01, 2.00, 1.99 (each s, each 3H, 4Ac) ppm; $^{13}\text{C-NMR}$ (125.7 MHz, CDCl_3): δ 194.4 (CH_2COPh), 189.0 (COPh), 170.8, 170.2, 169.5, 169.4 (4COCH_3), 166.7, 162.6, 153.6 (3C, thiophene ring), 139.8–126.9 (12C, 2Ph), 115.0 (CN), 95.5 (1C, thiophene ring), 90.5 (C-1'), 73.7 (C-5'), 73.0 (C-3'), 71.7 (C-2'), 68.2 (C-4'), 61.9 (C-6'), 48.1 (CH_2Ph), 20.9, 20.7 (2COCH_3), 20.6 (2C, 2COCH_3) ppm; HREIMS m/z obsd 691.1872, calcd for $\text{C}_{34}\text{H}_{33}\text{O}_{11}\text{N}_3\text{S}$ 691.1836.

3.7. General deacetylation method

The acetylated product (0.48 mmol) was dissolved in 6 mL of anhydrous methanol at r.t. and 1.0 mmol of NaOMe was added with stirring to the solution. The process was controlled by TLC (ether:hexane=6:1 and dichloromethane:methanol=4:1) until total deacetylation of the starting material. After t min, the reaction mixture was neutralized with acid resin Amberlite IR-120(H), filtered and the solvent evaporated under reduced pressure. The following products were prepared in this manner.

3.7.1. Diethyl β -D-glucopyranosylaminothiocarbonyl malonate 27

$t=15$ min. In this case, the ratio NaOMe/6, was three times that of the general method. $^{13}\text{C-NMR}$ (75.4 MHz, $\text{DMSO}-d_6$): δ 194.3 (CS), 164.9 (2C, $2\text{CO}_2\text{Et}$), 83.6 (C-1'), 79.0 (C-5'), 77.5 (C-3'), 72.4 (C-2'), 69.7 (C-4'), 64.8 (CH), 61.6 (2C, 2CH_2), 60.5 (C-6') ppm; FABMS m/z 404 (100, $[\text{M}+\text{Na}]^+$). HRCIMS m/z obsd 381.1060, calcd for $\text{C}_{14}\text{H}_{23}\text{O}_9\text{NS}+\text{H}$ 381.1094.

3.7.2. 4-Ethoxycarbonyl-1-(β -D-glucopyranosyl-2-phenyl-5-mercapto-2-pyrrole 28

$t=15$ min. This compound was not isolated pure. $^{13}\text{C-NMR}$ (125.7 MHz, $\text{MeOH}-d_4$): δ 166.6–162.4 (C-5, CO_2Et), 135.4–126.7 (6C, Ph), 122.2 (C-4), 109.2 (C-2), 89.0 (C-1'), 85.4 (C-3), 79.3 (C-5'), 78.9 (C-3'), 74.8 (C-2'), 71.6 (C-4'), 62.7 (C-6'), 61.1 (COCH_2CH_3), 14.8 (COCH_2CH_3) ppm; FABMS m/z 432 (100, $[\text{M}+\text{Na}]^+$). HRDCIMS m/z obsd 410.1292, calcd for $\text{C}_{19}\text{H}_{23}\text{O}_7\text{NS}+\text{H}$ 410.1273.

3.7.3. 2-Chlorophenyl-4-ethoxycarbonyl-1-(β -D-glucopyranosyl)-5-mercapto-pyrrole 29

$t=20$ min. $^{13}\text{C-NMR}$ (125.7 MHz, $\text{MeOH}-d_4$): δ 166.5, 164.0 (C-5, CO_2Et), 134.2–126.3 (6C, Ph), 122.5 (C-4), 109.2 (C-2), 88.9 (C-1'), 85.4 (C-3), 79.4, 78.9 (C-3', 5'), 74.7 (C-2'), 71.5 (C-4'), 62.6, 61.1 (C-6', COCH_2CH_3), 14.8 (COCH_2CH_3) ppm; FABMS m/z 443 (100, M^+). HRDCIMS m/z obsd 444.0891, calcd for $\text{C}_{19}\text{H}_{22}\text{O}_7\text{NSCl}+\text{H}$ 444.0884.

3.7.4. 3-Amino-2-benzoyl-4-ethoxycarbonyl-5-(β -D-glucopyranosylamino)thiophene 30

$t=10$ min. ^{13}C -NMR (75.4 MHz, $\text{MeOH}-d_4$): δ 187.5 (COPh), 171.1 (CO_2Et), 166.1, 159.8 (2C, thiophene ring), 142.7–128.1 (6C, Ph), 98.3, 97.0 (2C, thiophene ring), 87.4 (C-1'), 79.4 (C-5'), 78.5 (C-3'), 74.5 (C-2'), 71.1 (C-4'), 62.3 (C-6'), 61.8 (CH_2CH_3), 14.6 (CH_2CH_3) ppm; FABMS m/z 475 (100, $[\text{M}+\text{Na}]^+$). HRDCIMS m/z obsd 453.1340, calcd for $\text{C}_{20}\text{H}_{23}\text{O}_8\text{N}_2\text{S}+\text{H}$ 453.1331.

3.7.5. 3-Amino-2-benzoyl-4-cyano-5-(β -D-glucopyranosylamino)thiophene 31

$t=15$ min. ^{13}C -NMR (75.4 MHz, $\text{MeOH}-d_4$): δ 187.4 (COPh), 166.8, 159.6 (2C, thiophene ring), 142.2–128.1 (6C, Ph), 114.1 (CN), 96.9 (1C, thiophene ring), 87.6 (C-1'), 80.6 (1C, thiophene ring), 79.1, 78.7 (C-3', 5'), 73.8 (C-2'), 71.0 (C-4'), 62.3 (C-6') ppm; FABMS m/z 428 (100, $[\text{M}+\text{Na}]^+$). HRDCIMS m/z obsd 406.1088, calcd for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{N}_3\text{S}+\text{H}$ 406.1073.

Acknowledgements

We thank the Dirección General de Investigación Científica y Técnica of Spain for financial support (grant number PB 94/1440 C02-01) and the Fundación Cámara of the University of Seville for a grant to José L. Molina.

References

1. For a recent review see García Fernández, J. M.; Ortiz Mellet, C. *Sulfur Rep.* 1996, 19, 61–169.
2. Santoyo-González, F.; García Calvo-Flores, F.; Isac-García, I.; Hernández Mateo, F.; García Mendoza, P.; Robles Díaz, R. *Tetrahedron* 1994, 50, 2877–2894.
3. Lindhorst, T. K.; Kieburg, C. *Synthesis* 1995, 1228–1230.
4. Marino, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* 1997, 304, 257–260.
5. Pradera, M. A.; Molina, J. L.; Fuentes, J. *Tetrahedron* 1995, 51, 923–934.
6. Fuentes, J.; Molina, J. L.; Olano, D.; Pradera, M. A. *Tetrahedron: Asymmetry* 1996, 7, 203–218.
7. Fuentes, J.; Angulo, M.; Molina, J. L.; Pradera, M. A. *J. Carbohydr. Chem.* 1997, 16, 1457–1477.
8. García Fernández, J. M.; Ortiz Mellet, C.; Díaz Pérez, V. M.; Fuentes, J.; Kovacs, J.; Pinter, I. *Tetrahedron Lett.* 1997, 38, 4161–4164.
9. Guenther, W.; Kunz, H. *Angew. Chem. Int. Ed.* 1990, 29, 1050–1051.
10. For a review on nucleosides see Hurin, D. N.; Okabe, M. *Chem. Rev.* 1992, 92, 1745–1768. For pharmacological activities of the thiophene ring see for instance Xie, M.; Song, W.; *Yaoxue Xuebao* 1982, 17, 229–232. For wide and classical descriptions see *Nucleoside Analogues. Chemistry, Biology, and Medical Applications*, NATO Advanced Study Institute Series, Plenum Press: New York, 1979 and *Thiophene and its Derivatives*, S. Grenowitz, ed.; Wiley-Interscience: New York, 1986.
11. Babiano Caballero, R.; Fuentes Mota, J.; Galbis Pérez, J. A. *Carbohydr. Res.* 1986, 154, 280–288.
12. For a second review see Witczak, Z. *J. Adv. Carbohydr. Chem. Biochem.* 1986, 44, 91–145.
13. Ikeda, D.; Horiuchi, F.; Yoshida, M.; Miyasaka, T.; Kondo, S.; Umesawa, H. *Carbohydr. Res.* 1982, 109, 33–45.
14. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Siegel, M. M.; Morton, G. O.; Ellestad, G. A.; McGahren, W. J.; Border, D. B. *J. Am. Chem. Soc.* 1992, 114, 985–997.
15. Wawer, I.; Piekarska-Bartoszewicz, B.; Temmerius *Carbohydr. Res.* 1995, 267, 167–176 and references therein.
16. Fowler, P.; Berner, B.; Vasella, A. *Helv. Chim. Acta* 1996, 79, 269–287.
17. Avalos, M.; Babiano, R.; Carrero, M. J.; Cintas, P.; Higes, F. J.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron* 1998, 54, 615–628.
18. Katritzky, A. R. *Handbook of Heterocyclic Chem.*, Pergamon Press: Oxford, 1985; p. 105.
19. Shutalev, A. D.; Ignatova, L. A.; Unkovskii, B. V. *Khim. Geterotsikl. Soedin* 1982, 825–829; *Chem. Abstr.* 1982, 97, abstract number 163356u. See also *Specialist Periodical Reports of The Royal Society of Chemistry*, Vol. 16, Part I. N. R. Williams, Senior Reporter; The Royal Society of Chemistry: London, 1984; pp. 107–116.

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20. Fuentes, J.; Pradera, M. A.; Robina, I. *Tetrahedron* **1991**, *47*, 5797-5810.
21. Mohareb, R. M.; Aziz, S. I.; Abdel Sayed, N. I.; Shams, H. Z. *J. Chin. Chem. Soc.* **1992**, *39*, 181-187.
22. Kunz, H.; Sager, W.; Schanzenbach, D.; Decker, M. *Liebigs Ann. Chem.* **1991**, 649-654.
23. Cusack, I. D.; Robinson, D. H.; Rugg, P. W.; Shaw, G.; Lofthouse, R. *J. Chem. Soc., Perkin Trans. 1* **1974**, 73-81.
24. Fuentes Mota, J.; Pradera Adrián, M. A.; Ortiz Mellet, C.; García Fernández, J. M.; Babiano Caballero, R.; Galbis Pérez, J. A. *Carbohydr. Res.* **1988**, *173*, 1-16 and references therein.

Bioisosterism: A Rational Approach in Drug Design

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Received May 15, 1996 (Revised Manuscript Received July 25, 1996)

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I. Introduction

Years of cumulative research can result in the development of a clinically useful drug, providing either a cure for a particular disease or symptomatic relief from a physiological disorder. A lead compound with a desired pharmacological activity may have associated with it undesirable side effects, characteristics that limit its bioavailability, or structural features which adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach used by the medicinal chemist

for the rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive.¹

The prevalence of the use of bioisosteric replacements in drug design need not be emphasized. This topic has been reviewed in previous years.²⁻⁵ The objective of this review is to provide an overview of bioisosteres that incorporates sufficient detail to enable the reader to understand the concepts being delineated. While a few popular examples of the successful use of bioisosteres have been included, the

present review is focused primarily upon specific examples from current literature. The emphasis in this review was to outline bioisosteric replacements which have been used to advance drug development. No attempt was made to be exhaustive or to illustrate all of the specific analogues represented within a single study.

The ability of a group of bioisosteres to elicit similar biological activity has been attributed to common physicochemical properties. In this review an attempt has been made to quantitate, in specific instances, physicochemical effects such as electro-negativity, steric size, and lipophilicity and to correlate these values to the observed biological activity. Thus, an additional objective of this review was to demonstrate the opportunities that one has in employing bioisosteres to gain more specific insight into the quantitative structure-activity relationships (QSAR) associated with a specific class of drugs. While in some instances such associations were detailed by the authors of these literature examples, others were developed on the basis of evident correlations. To further explain and rationalize the biological activity observed with nonclassical bioisosteric groups, the observed biological activity has also been correlated with some substituent constants commonly employed in QSAR studies. These observations are consistent with the fact that bioisosteric replacements often provide the foundation for the development of QSAR in drug design.^{4,6} Recent advances in molecular biology, such as cloning of the various receptor subtypes, have enabled a clearer definition of the pharmacophoric sites. Bioisosteric replacements of functional groups based on this understanding of the pharmacophore and the physicochemical properties of the bioisosteres have enhanced the potential for the successful development of new clinical agents.

The bioisosteric rationale for the modification of lead compounds is traced back to the observation by Langmuir in 1919 regarding the *similarities of various physicochemical properties* of atoms, groups, radicals, and molecules.⁷ Langmuir compared the physical properties of various molecules such as N₂ and CO, N₂O and CO₂, and N₃⁻ and NCO⁻ and found them to be similar. On the basis of these similarities he identified 21 groups of *isosteres*. Some of these groups are listed in Table 1. He further deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Thus, isosteres were initially defined as those compounds or groups of atoms that have the same number and

Table 1. Groups of Isosteres as Identified by Langmuir

groups	isosteres
1	H ⁻ , He, Li ⁺
2	O ²⁻ , F ⁻ , Ne, Na ⁺ , Mg ²⁺ , Al ³⁺
3	S ²⁻ , Cl ⁻ , Ar, K ⁺ , Ca ²⁺
4	Cu ²⁻ , Zn ²⁺
↓	↓
8	N ₂ , CO, CN ⁻
9	CH ₄ , NH ₄ ⁺
10	CO ₂ , N ₂ O, N ₃ ⁻ , CNO ⁻
↓	↓
20	MnO ₄ ⁻ , CrO ₄ ²⁻
21	SeO ₄ ²⁻ , AsO ₄ ³⁻

arrangement of electrons. He further defined other relationships in a similar manner. Argon was viewed as an isostere of K⁺ ion and methane as an isostere of NH₄⁺ ion. He deduced, therefore, that K⁺ ions and NH₄⁺ ions must be similar because argon and methane are very similar in physical properties. The biological similarity of molecules such as CO₂ and N₂O was later coincidentally acknowledged as both compounds were capable of acting as reversible anesthetics to the slime mold *Physarum polycephalum*.⁸

A further extension to this concept of isosteres came about in 1925 with Grimm's Hydride Displacement Law.^{9,10} This law states: "Atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with one to four hydrogen atoms, in such a manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups one to four places respectively, to their right." Each vertical column as illustrated in Table 2, according to Grimm, would represent a group of isosteres.

Table 2. Grimm's Hydride Displacement Law

C	N	O	F	Ne	Na
	CH	NH	OH	FH	-
		CH ₂	NH ₂	OH ₂	FH ₂ ⁺
			CH ₃	NH ₃	OH ₃ ⁺
				CH ₄	NH ₄ ⁺

Erlenmeyer¹¹ further broadened Grimm's classification and redefined isosteres as atoms, ions, and molecules in which the peripheral layers of electrons can be considered identical (Table 3).

Table 3. Isosteres Based on the Number of Peripheral Electrons

no. of peripheral electrons				
4	5	6	7	8
N ⁺	P	S	Cl	ClH
P ⁺	As	Se	Br	BrH
S ⁺	Sb	Te	I	IH
As ⁺		PH	SH	SH ₂
Sb ⁺			PIH ₂	PIH ₃

The widespread application of the concept of isosterism to modify biological activity has given rise to the term *bioisosterism*. As initially defined by Friedman,² bioisosteres were to include all atoms and molecules which fit the broadest definition for isosteres and have a similar type of biological activity, which may even be antagonistic. More recently this definition has been broadened by Burger as "Compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties..."⁵ The critical component for bioisosterism is that bioisosteres affect the same pharmacological target as agonists or antagonists and, thereby, have biological properties which are related to each other.

Bioisosteres have been classified as either classical or nonclassical.¹² Grimm's Hydride Displacement Law and Erlenmeyer's definition of isosteres outline a series of replacements which have been referred to as classical bioisosteres. Classical bioisosteres have been traditionally divided into several distinct categories: (A) monovalent atoms or groups; (B)

such as benign prostatic hyperplasia and prostate cancer. Inhibition of steroid 5 α -reductase is of recent pharmaceutical interest in view of its role in the conversion of testosterone to dihydrotestosterone. Recent research has isolated and characterized two types of human 5 α -reductases, namely type I and type II. Therefore, a rational approach for treatment of these androgen-sensitive disease states can be envisioned by the inhibition of either one or both enzymes with specific inhibitors. 4-Substituted *N*-(1,1-dimethylethyl)-3-oxo-4-androstene-17 β -carboxamide analogues (**29**, Figure 20) with hydroxy, thiol, chlorine, or bromo substituents at the 4-position were evaluated and found to show intermediate inhibitory activity on human type II 5 α -reductase activity.⁴¹ From the data obtained (Table 16), it could be inferred that the enzyme has steric and electronic preferences at this position, resulting in significantly enhanced potency for the hydroxy and chloro substituted compounds.

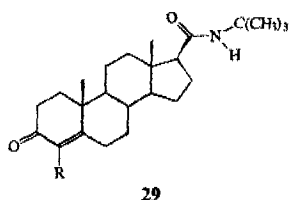


Figure 20.

Table 16. *In Vitro* Inhibition of Human Type II 5 α -Reductase of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17 β -carboxamides

compound	R	effective van der Waal's radii ³¹ (Å)	electro- negativity ²⁹	IC ₅₀ (nM)
29a	OH	1.53	3.51	172
29b	Cl	1.73	3.00	192
29c	SH	1.80	2.32	437
29d	Br	1.86	2.80	387

^a IC₅₀ values represent the concentration of compounds required to inhibit 5 α -reductase activity by 50%.

B. Divalent Isosteres

Divalent isosteres can be classified into two subgroups: (1) Those divalent bioisosteres which involve the interchange of atoms that are involved in a double bond, such as in the series: C=C, C=N, C=O, and C=S. And (2) those divalent isosteres where substitution of a different atom results in the alteration of two single bonds such as in the series; C-C-C, C-NH-C, C-O-C, and C-S-C. Both of these types of bioisosteric substitutions have been used extensively in the study of the structure-activity relationships of various pharmacologically active agents.

1. Divalent Replacements Involving Double Bonds

This subclass includes replacements such as C=S, C=O, C=NH, and C=C. As we have already discussed in the earlier sections, the presence of heterocyclic systems in most of the lead compounds under study as medicinal agents facilitates the tautomerization of these groups. As mentioned earlier, for the purpose of this review, the classification of

such analogues as divalent bioisosteres requires the absence of a mobile proton which can migrate within the ring system.

The replacement of C=S with C=O in Tolrestat (**30a**, Figure 21), an aldose reductase inhibitor currently under study in human subjects for the treatment of diabetic neuropathy, resulted in oxo-Tolrestat (**30b**), which retained activity both *in vitro* and *in vivo* (Table 17).⁴²

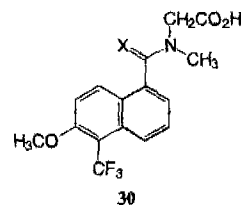


Figure 21.

Table 17. Aldose Reductase Inhibitory Activity of Tolrestat and Oxo-Tolrestat

compound	X	aldose reductase inhibition	
		<i>in vitro</i> ^a	<i>in vivo</i> ^b
30a	S	94	53
30b	O	86	56

^a Inhibition of enzyme activity in partially purified bovine lens preparation. ^b Inhibition of galactitol accumulation in the sciatic nerves of rats fed 20% galactose for 4 days.

Another illustration of the use of this class of divalent isosteres is with certain novel purine nucleoside analogues (**31**, Figure 22) that were tested *in vivo* for antiviral activity against Semliki Forest virus (SFV) infection in a mouse model.⁴³ Table 18 outlines the percentage of mice that survived for 21 days for the various bioisosteric analogues compared to the absence of any survivors in control infected mice. Replacement of the sulfur atom at C-8 with the oxygen or selenium atom, another well-known divalent isosteric replacement, resulted in weaker activity relative to the thio analogue.

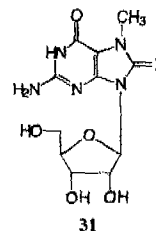


Figure 22.

Table 18. Activity of Guanosine Bioisosteric Analogues against Semliki Forest Virus

compound	X	% total survivors
31a	S	83 (10/12)
31b	O	67 (8/12)
31c	Se	58 (7/12)

2. Divalent Replacements Involving Two Single Bonds

The second major class of divalent bioisosteres represents those atoms or groups which are attached to different substituents. As these divalent bioisosteres are attached to two different substituents, the chemical and polar differences are less pronounced.

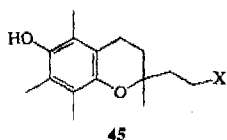


Figure 30.

Table 23. *In Vitro* and *ex Vivo* Inhibition of Lipid Autooxidation in Mouse Heart Homogenate

compound	X	<i>in vitro</i> ^a IC ₅₀ (μM)	<i>ex vivo</i> ^b ID ₅₀ (μM/kg)	IC ₅₀ /ID ₅₀
45a	-N ⁺ (CH ₃) ₃	19	11	1.7
45b	-P ⁺ (CH ₃) ₃	10	8	1.3
45c	-S ⁺ (CH ₃) ₂	7	6	1.3

^a Concentration that inhibits thiobarbituric acid reactive substances (TBARS) formation by 50%. ^b Dose that inhibits TBARS formation by 50% 1 h after sc administration.

E. Ring Equivalents

Ring equivalent bioisosteres represent the final subclass of classical bioisosteres that will be reviewed. Classical isosteric substitutions when applied within ring systems result in different heterocyclic analogues which can be effective bioisosteres. The use of the classical bioisosteres benzene, thiophene, and pyridine resulted in analogues with retention of biological activity within different series of pharmacological agents. One of the successful uses of this replacement resulted in the potent antihistamine mepyramine (47, Figure 31) which evolved by the replacement of the phenyl moiety in antegran (46) by a pyridyl group.

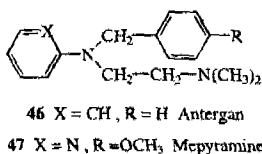
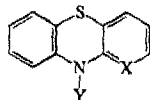


Figure 31.

Substitution of benzene by pyridine also improved activity in the tricyclic antihistamines [promethazine (48a) and isothipendyl (48b)] and neuroleptics [promazine (48c) and prothipendyl (48d)] (Figure 32) and resulted in a reduction of both sedative and extrapyramidal effects. In reviewing some more recent studies, this subclass of bioisosteres will be divided into (1) divalent ring equivalents and (2) trivalent ring equivalents.



- 48a Y = CH₂CH(CH₃)N(CH₃)₂ X = CH
 48b Y = CH₂CH(CH₃)N(CH₃)₂ X = N
 48c Y = CH₂CH₂CH₂N(CH₃)₂ X = CH
 48d Y = CH₂CH₂CH₂N(CH₃)₂ X = N

Figure 32.

1. Divalent Ring Equivalents

Some of these heterocyclic rings obtained by the replacement of a heteroatom within different ring systems will be discussed among the nonclassical category of bioisosteres on the basis of their ability to mimic the spatial conformation of certain other functional groups.

A copper-containing monooxygenase, dopamine β-hydroxylase, present in a variety of mammalian tissues, catalyses the benzylic hydroxylation of dopamine. Since dopamine β-hydroxylase plays an important role in the biosynthetic production of nor-adrenaline, it could be a target for the design of inhibitors as potential therapeutic agents for the modulation of adrenergic activity *in vivo*. Isosteric divalent ring replacements as obtained from Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity within a series of indane derivatives (49, Figure 33) evaluated as inhibitors of dopamine β-hydroxylase⁵³ (Table 24).

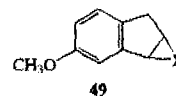


Figure 33.

Table 24. Kinetic Inhibition Parameters for Dopamine β-Hydroxylase by Indan Derivatives

compound	X	K _i (mM) ^a
49a	-CH ₂ -	1.12
49b	-O-	0.65
49c	-NH-	0.4

^a Apparent binding constant K_i estimated by plotting 1/k_{obs} as a function of 1/[I].

The applicability of divalent ring-equivalent isosteres has also been demonstrated in other five- and seven-membered ring systems. The search for novel cardiotonic agents resulted in the successful development of two clinically useful agents, amrinone⁵⁴ (50) and milrinone⁵⁵ (51) (Figure 34). It is known that the positive vasodilatory actions of amrinone and milrinone are related to the inhibition of adenosine 3',5'-cyclic phosphate phosphodiesterase III (cAMP PDE III).

During SAR studies on amrinone (50), it was observed that a free amino group was not necessary for *in vitro* cAMP PDE III activity.⁵⁶ This prompted the design and synthesis of analogues with general structure 52.⁵⁷ Table 25 summarizes the *in vitro* cAMP PDE III activity of the divalent ring-equivalent

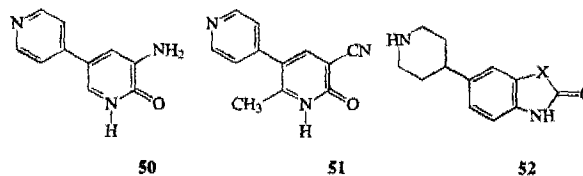


Figure 34.

Table 25. *In Vitro* cAMP PDE III Activity of Ring-Equivalent Bioisosteres of 5-(4-Pyridinyl)benzoxazol-2(3H)-one

compound	X	cAMP PDE III IC ₅₀ (μM) ^a
50	amrinone	28
51	milrinone	0.36
52a	-O-	9.8
52b	-CH ₂ -	5.3
52c	-NH-	1.3
52d	-S-	0.54

^a Concentration required to cause 50% inhibition of cAMP PDE III *in vitro*.

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2009

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
LUIS PATIÑO LEYVA
Apoderado

ASUNTO	Radicación	05-000562
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	6954
	Folios	08

Patente de Invención ☒ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☒ Cap. I ☐ Cap. II ☒

No. Sol. Internacional PCT/EP2003/006841
Fecha de Presentación Internal. 27 de Junio de 2003

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción: Folios 57 a 114 y 122 como se radicó inicialmente

Reivindicaciones: Folios 115 a 121 como se radicó inicialmente
Folios 179 a 180 modificados

Prioridad: Folios: 01
11 de Julio de 2002, DE, 10231370.9

Respuesta del solicitante: Folios 172 a 176 modificados

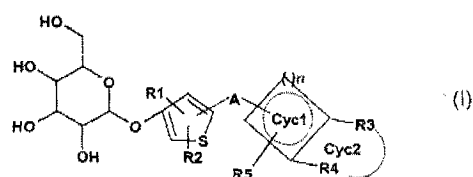
2. Análisis de la Prioridad

- La fecha de prioridad del 11 de Julio de 2002 y su correspondiente documento, corresponden con la materia solicitada de protección.

3. Objeto de la invención

- Título de la solicitud: **"NUEVOS DERIVADOS DE GLICÓSIDO DE TIOFENO, MÉTODOS PARA LA PREPARACIÓN DE LOS MISMOS, MEDICAMENTOS QUE COMPRENDEN DICHOS COMPUESTOS Y SU USO"**

El objeto de la presente solicitud de patente de invención consiste en obtener compuestos glicósidos de tiofeno para el tratamiento de la diabetes de fórmula I:



El problema planteado en esta solicitud es la necesidad de obtener compuestos glicósidos del tiofeno para el tratamiento de la diabetes

Para solucionar este problema técnico la solicitud en estudio, el solicitante proporciona compuestos según fórmula general I.

- **Clasificación Internacional de Patentes (IPC.):** C07H7/00; A61K31/7042; A61K31/7048

4. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

- Fecha de presentación de la solicitud prioritaria: 11 de Julio de 2002

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 11/07/2002 se encontraron los siguientes documentos del estado de la técnica relacionados, que pueden afectar la patentabilidad del objeto de la presente solicitud:

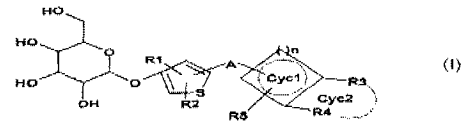
Nº	Documento	Título	Fecha de publicación
D2	Chem Phar. Bull 44 (6) 1174-1180 (1996), <i>Kenji Tsujihara et al</i>	"Na ⁺ -Glucose transporter inhibitors as antidiabetics. I. Synthesis and Pharmacological properties of 4'-dehydroxyphlorizin derivatives base don a new concept"	1996
D3	<i>Chem.Rev</i> , 1996, 96, 3147-3176	Bioisosterism: a Rational Approach in Drug Design	1996

5. Análisis de patentabilidad (artículo 14 D486)

Nivel inventivo (artículo18 D486)

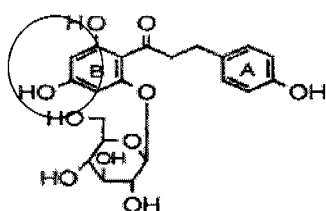
1) Respecto a la solicitud en estudio:

La reivindicación 1 se refiere a un compuesto de fórmula (I):



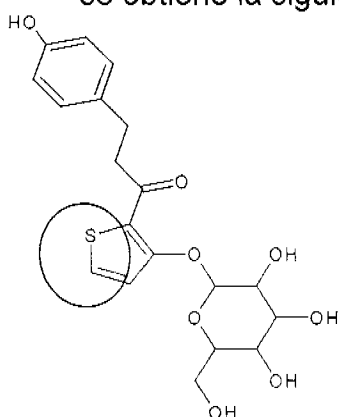
2) Respecto al estado de la Técnica:

D2 enseña previamente compuestos derivados de la florizina para el tratamiento de la diabetes con la siguiente estructura (Ver página 1174):



Phlorizin

Al realizar los reemplazos en la solicitud presentada cuando R^1 y R^2 son **hidroxi**, A es $-(C=O)CH_2CH_2$ (Ver ejemplos 1-6-folio 79) y Cyc1 es **fenilo** sustituido por R^4 y es $-OH$, se obtiene la siguiente estructura:



Solicitud presentada

D3 enseña previamente el empleo del bioisoterismo, el cual es usado para la modificación racional de compuestos líderes en agentes más seguros y clínicamente efectivos (Ver página 3147)

En la tabla 2, por ejemplo **D3** (Ver columna 2, página 3148) enseña los isósteros del oxígeno, los cuales pueden ser NH y CH_2

También divulga **D3** la clasificación de los isósteros divalentes en dos subgrupos (Ver columna 1, página 3155)

- 1) Aquellos que implican el intercambio de átomos que se encuentran involucrados en un doble enlace, como son las series: $C=C$, $C=N$, $C=O$ y $C=S$
- 2) Los isósteros divalentes donde la sustitución de un átomo diferente resulta en la alteración de los enlaces sencillos como son las series; $C-C-C$, $C-NH-C$, $C-O-C$ y $C-S-C$

Para las equivalencias de los anillos, **D3** (Ver columna 1, página 3158) enseña el uso de los bioisósteros clásicos tales como benceno, tiofeno y piridina resultando en la retención de la actividad dentro de series diferentes de los agentes farmacológicos.

3) Estado de la Técnica más cercano: **D2**

La diferencia de la solicitud presentada con **D2** es la presencia del anillo tiofeno en vez de un anillo y la presencia de grupos hidroxilo en el tiofeno.

En **D2**, por lo tanto ya se encontraba sugerida la estructura química de un glicósido y dos anillos aromáticos.

El solicitante varía los diferentes sustituyentes encontrados en el campo de la química orgánica en la molécula empleando el concepto de bioisosteria (**D3**):

En **D2** reemplaza el fenilo (**D2**) por un anillo análogo tal como es el tiofeno (**D3**) o de forma tal que se obtienen compuestos alternos a los previamente divulgados.

No se evidencia por lo tanto el efecto superior e inesperado al realizar este reemplazo comparativamente con los compuestos previamente divulgados, pues para el experto técnico en la materia al emplear los conceptos de bioisosteria, se derivarían de forma obvia y sin necesidad de utilizar habilidad inventiva los compuestos de la solicitud presentada

El Problema Técnico Objetivo fundamental el cual es “**obtener compuestos glicósidos del tiofeno para el tratamiento de la diabetes**”, sería fácilmente resuelto por el experto en la materia con las anterioridades encontradas, pues es evidente que con el documento **D2 y D3** se obtendrían los compuestos de la solicitud presentada como compuestos alternos frente a los ya conocidos, realizando las modificaciones al grupo farmacofórico ya conocido, por lo tanto carece de nivel inventivo.

De manera tal que los compuestos de la solicitud presentada y sus medicamentos carecen de nivel inventivo.

6. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D2	Chem Phar. Bull 44 (6) 1174-1180 (1996), Kenji Tsujihara et al	1-7	Nivel Inventivo
D3	Chem. Rev, 1996, 96, 3147-3176	1-7	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL

Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha

CONCEPTO TÉCNICO No. 01716	EXAMINADOR TÉCNICO Código No. 202070
----------------------------	---

CASTELLANOS M. & CO
ABOGADOS
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Bogotá D.C.

Señora

JEFE DE LA DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

Ministerio de Desarrollo Económico

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-000562- -00008-0000

Fecha: 2009-09-17 13:57:57 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALI
Act. 444 RESPUESTAREQUE Folios: 8

Re.: Expediente No. **05.000.562**

Solicitud del examen de patentabilidad correspondiente a la Patente PCT
Entrada en Fase Nacional, para:

**"NUEVOS DERIVADOS DE GLICOSIDO DE TIOFENO, METODOS PARA LA
PREPARACION DE LOS MISMOS, MEDICAMENTOS QUE COMPRENDEN DICHOS
COMPUESTOS, Y SU USO"**

Solicitante: **SANOFI-AVENTIS DEUTSCHLAND GMBH**

RESPUESTA AL EXAMEN DE FONDO

Yo, **Luis Patiño Leyva**, identificado como aparece al pie de mi firma, atentamente
me permito presentar respuesta al oficio **No. 6954** notificado por fijación en lista
del 19 de junio de 2009, en los siguientes términos:

Nivel inventivo

Ese Despacho considera que la presente solicitud carece de nivel inventivo frente
a los documentos **D2** (*Na⁺-glucose cotransporter inhibitors as antidiabetics. I. Synthesis and Pharmacological properties of 4'-dehydroxyphlorizin derivatives based on a new concept*) y **D3** (*Bioisosterism: a rational Approach in Drug Design*), porque la única diferencia de D2 con la solicitudes es la presencia del
anillo de tiofeno en vez de un arilo, reemplazo que resulta evidente a partir de las
enseñanzas de D3 y no evidencia un efecto técnico superior para los compuestos
reivindicados.

Presento un nuevo pliego reivindicatorio en donde los significados de A en la formula 1 se han limitado a -CH₂- y, se ha eliminado la reivindicación 3.

La limitación mencionada hace aún más notoria la diferencia de estructura química de los compuestos de la invención cuando se comparan con los derivados de Phlorizina. De tal manera que el enlazante A es totalmente diferente al elemento correspondiente en la Phlorizina y la estructura mostrada por el oficio No. 6954 cuando A es -(C=O) CH₂CH₂ no está contemplada en las nuevas reivindicaciones.

La limitación de A a CH₂ no es anticipada por las enseñanzas del documento D3 sobre bioisosterismo y es claramente una nueva definición de una clase de compuestos que no había sido divulgada ni sugerida en los documentos citados como estado del arte. Así entonces, no existe en el estado de la técnica en su conjunto ninguna indicación que lleve al técnico medio a modificar o adaptar el estado de la técnica más cercano (D2) de tal manera que se llegue a las definiciones dadas en las nuevas reivindicaciones.

En cuanto al efecto superior e inesperado de los compuestos reivindicados frente a los divulgados por D2 (Phlorizina), hago referencia a los argumentos presentados en la respuesta al anterior oficio de fondo en donde demostré que los compuestos de la invención son inhibidores selectivos de SGLT2, mientras que D2 no hace alusión a la discriminación SGLT1/ SGLT2. La inhibición selectiva del transportador de proteína SGLT2 tiene la ventaja de evitar los efectos laterales que están relacionados a la inhibición de SGLT1 en el intestino delgado. A este respecto, el documento WO01/27128, en el último párrafo de su página 4, hace énfasis en que la Phlorizina no es atractiva como un medicamento de administración oral. También afirma que la inhibición de SGLT1 está relacionada con un deterioro en la captación de glucosa en el intestino, diarrea y deshidratación.

Ahora, los siguientes resultados comparativos que también presenté ante su Despacho en la respuesta al oficio anterior,

	Phlorizina	Ejemplo 7
SGLT1	0,27µM	26,3 µM
SGLT2	0,068 µM	0,011 µM

evidencian que los compuestos de la invención sí tienen un efecto superior e inesperado sobre la Phlorizina (documento D2) que es un inhibidor de SGLT1/2 no selectivo en comparación con el compuesto del ejemplo 7 de la presente invención, el cual evita los efectos laterales relacionados con la inhibición de SGLT1 en el intestino delgado.

En este orden de ideas, la presente solicitud tal y como se modificó tiene altura inventiva por cuanto ninguno de los documentos citados enseña o sugiere la estructura reivindicada para los compuestos ni tampoco presentan indicación alguna que lleve al técnico medio a modificar la estructura de los compuestos de la anterioridad D2 con el objeto de lograr los compuestos reivindicados que constituyen una solución al problema técnico planteado sobre la necesidad de compuestos nuevos para prevenir y tratar la diabetes de tipo 1 y de tipo 2.

Teniendo en cuenta los argumentos expresados arriba, respetuosamente solicito, se conceda el título de patente a la presente invención puesto que la misma cumple con los requisitos del artículo 18 de la Decisión 486.

ANEXOS

- Capítulo reivindicatorio
- Recibo de pago por modificaciones
- Formulario para modificaciones

Señora Jefe,



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C.C. No. 2.930.616 de Bogotá
T.P.A. No.2698 del C. Sup. de la Judicatura
Calle 94A No. 7A - 09
Teléfonos: 7560770

FORMULARIO ÚNICO DE CORRECCIONES Y MODIFICACIONES

1		SOLICITUD DE:	
☐ Corrección de Error Material		☒ Modificación a una solicitud	
2 SOLICITANTE	Nombre: SANOFLAVENTIS DEUTSCHLAND GMBH	IDENTIFICACIÓN	
	Dirección: Frankfurt 65926 Alemania	C.C. ☐	NIT ☐
	Nacionalidad o Domicilio: Frankfurt, Alemania	C.E. ☐	Otro ☐
	Lugar de Constitución: Alemania	Cual _____	
	Teléfono: _____ Fax: _____	Número _____	
	E-mail: _____		
3 REPRESENTANTE O APODERADO	Nombre: LUIS PATIÑO LEYVA	IDENTIFICACIÓN	
	Dirección: Calle 94A No. 7A-09	C.C. ☒	NIT ☐
	Teléfono: 756 07 70 Fax: 756 01 58	C.E. ☐	Otro ☐
	E-mail: info@castellanosyco.com	Cual _____	
		Número 2.930.616	TPA 2698
4 Número de Expediente: 05.000.562			
5 Descripción de la corrección o modificación solicitada: Presento nuevo pliego reivindicatorio			
6 Comprobante de pago No. 09 -66,172 Fecha 04/09/2009			
7 Anexos: ☒ Comprobante de pago de la tasa. ☐ Poderes si fuere el caso, junto con la sustitución de los mismos a mi favor. ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas ☒ Nuevo capítulo reivindicatorio			
8			
NOMBRE LUIS PATIÑO LEIVA		FIRMA 	
		CC 2.930.616 de Bogotá - TPA 2698 CSJ	

20035

217

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 860176089-2

RECIBO OFICIAL DE CAJA : 09 - 66,172
FECHA : SEPTIEMBRE 4 DE 2009

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-000562- -00008-0000

Fecha: 2009-09-17 13:57:57 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALI
Act. 444 RESPUESTAREQUE Folios: 8

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
ALVARO CASTELLANOS	CONSIGNACION	BANCO DE BOGOTA	062754387	92622784	04/09/2009	10,415,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	111,000.00
		TOTAL :	\$ 111,000.00

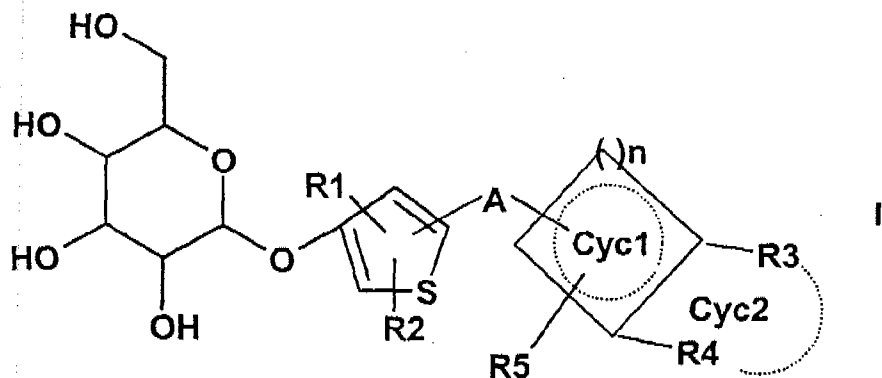
SON: CIENTO ONCE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

REIVINDICACIONES MODIFICADAS

1. Un compuesto de fórmula I



- R1 es hidrógeno, CF₃, alquilo(C₁-C₄), fenilo,
- R2 es hidrógeno,
- A es -CH₂-;
- n es el número 2 ó 3;
- Cyc1 es fenilo o tienilo;
- R3, R4, R5 son hidrógeno, F, Cl, I, NO₂, OH, CN, alquilo(C₁-C₆), alcoxi(C₁-C₈), O-CH₂-fenilo, OCF₃, S-CH₃, COOH, o bien
- R3 y R4 son conjuntamente -CH=CH-O-, -O-(CH₂)_p-O-, con p = 1 ó 2, -O-CF₂-O-, -CH=CH-CH=CH-, y
- R5 es hidrógeno.

y sus sales farmacéuticamente aceptables.

2. Un compuesto de fórmula I según la reivindicación 1, en el cual A está unido al anillo de tienilo en la posición 2.
3. Un compuesto de fórmula I según las reivindicaciones 1 o 2, en el cual
- Cyc1 es fenilo.

4. Un compuesto de fórmula I según las reivindicaciones 1 a 4, en el cual Cyc1 es tienilo.
5. Un compuesto de fórmula I según las reivindicaciones 1 a 5, en el cual Cyc1 está monosustituido.
6. Un medicamento que contiene uno o más de los compuestos según una o más de las reivindicaciones 1 a 5.

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PCT/US00/27187

However, the two transporters do vary in their substrate specificities for sugars such as galactose, which is a substrate for SGLT1 only.

Administration of phlorizin, a specific inhibitor of SGLT activity, provided proof of concept *in vivo* by promoting glucose excretion, lowering fasting and fed plasma glucose, and promoting glucose utilization without hypoglycemic side effects in several diabetic rodent models and in one canine diabetes model. No adverse effects on plasma ion balance, renal function or renal morphology have been observed as a consequence of phlorizin treatment for as long as two weeks. In addition, no hypoglycemic or other adverse effects have been observed when phlorizin is administered to normal animals, despite the presence of glycosuria.

Administration of an inhibitor of renal SGLTs for a 6-month period (Tanabe Seiyaku) was reported to improve fasting and fed plasma glucose, improve insulin secretion and utilization in obese NIDDM rat models, and offset the development of nephropathy and neuropathy in the absence of hypoglycemic or renal side effects.

Phlorizin itself is unattractive as an oral drug since it is a nonspecific SGLT1/SGLT2 inhibitor that is hydrolyzed in the gut to its aglycone phloretin, which is a potent inhibitor of facilitated glucose transport. Concurrent inhibition of facilitative glucose transporters (GLUTs) is undesirable since such inhibitors would be predicted to exacerbate peripheral insulin resistance as well as promote hypoglycemia in the CNS.

Inhibition of SGLT1 could also have serious adverse consequences as is illustrated by the hereditary syndrome glucose/galactose malabsorption (GGM), in which mutations in the SGLT1 cotransporter result in impaired glucose uptake in the intestine, and life-threatening diarrhea and dehydration. The biochemical differences between

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 1 270 584 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:

02.01.2003 Bulletin 2003/01

(21) Application number: 01912380.1

(22) Date of filing: 15.03.2001

(51) Int Cl.7: C07H 15/203, A61K 31/7034,

A61P 43/00, A61P 3/10,

A61P 3/04, C07C 69/78,

C07C 69/16, C07C 69/18,

C07C 323/18, C07C 39/15,

C07C 43/23

(86) International application number:

PCT/JP01/02041

(87) International publication number:

WO 01/068660 (20.09.2001 Gazette 2001/38)

(84) Designated Contracting States:

AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR

Designated Extension States:

AL LT LV MK RO SI

(30) Priority: 17.03.2000 JP 2000077304

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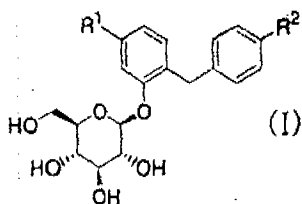
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(54) GLUCOPYRANOSYLOXY BENZYL BENZENE DERIVATIVES, MEDICINAL COMPOSITIONS CONTAINING THE SAME AND INTERMEDIATES FOR THE PREPARATION OF THE DERIVATIVES

(57) The present invention relates to glucopyranosyloxybenzylbenzene derivatives represented by the general formula:



wherein R¹ represents a hydrogen atom or a hydroxy (lower alkyl) group; and R² represents a lower alkyl group, a lower alkoxy group, a lower alkylthio group, a hydroxy(lower alkyl) group, a hydroxy(lower alkoxy) group, a hydroxy(lower alkylthio) group etc., and salts thereof, which have an excellent inhibitory activity in human SGLT2 and are useful as agents for the prevention or treatment of diabetes, obesity etc., and intermediates thereof.

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Test Example 1

Assay for inhibitory effect on human SGLT2 activity

5 1) Construction of the plasmid vector expressing human SGLT2

[0064] Preparation of the cDNA library for PCR amplification was performed by reverse transcription of a total RNA deprived from human kidney (Ori gene) with oligo dT as the primer, using SUPERSCRIP^T Preamplification System (Gibco-BRL: LIFE TECHNOLOGIES). DNA fragment coding for human SGLT2 was amplified by the Pfu DNA Polymerase (Stratagene)-used PCR reaction, in which the human kidney cDNA library described above was used as the template and the following oligo nucleotides 0702F and 0712R, presented as Sequence Numbers 1 and 2 respectively, were used as the primers. The amplified DNA fragment was ligated into pCR-Blunt (Invitrogen), a vector for cloning, according to standard method of the kit. The competent cell, *Escherichia coli* HB101 (Toyobo), was transformed according to usual method and then selection of the transformants was performed on the LB agar medium containing 50 μ g/mL of kanamycin. After plasmid DNA was extracted and purified from the one of the transformants, amplifying of the DNA fragment coding for human SGLT2 was performed by the Pfu DNA Polymerase (Stratagene)-used PCR reaction, in which the following oligo nucleotides 0714F and 0715R, presented as Sequence Numbers 3 and 4 respectively, were used as the primers. The amplified DNA fragment was digested with restriction enzymes, Xho I and Hind III, and then purified with Wizard Purification System (Promega). This purified DNA fragment was inserted at the corresponding restriction enzyme sites of pcDNA3.1 (-) Myc/His-A (Invitrogen), a vector for expressing of fusion protein. The competent cell, *Escherichia coli* HB101 (Toyobo), was transformed according to usual method and then selection of the transformant was performed on the LB agar medium containing 100 μ g/mL of ampicillin. After the plasmid DNA was extracted and purified from this transformant, the sequence of the DNA fragment inserted at the multi-cloning sites of the vector, pcDNA3.1 (-) Myc/His-A, was analyzed. This clone had a single base substitution (ATC which codes for the isoleucine-433 was substituted by GTC) compared with the human SGLT2 reported by Wells *et al* (Am. J. Physiol., Vol. 263, pp. 459-465 (1992)). Sequentially, a clone in which valine is substituted for isoleucine-433 was obtained. This plasmid vector expressing human SGLT2 in which the peptide presented as Sequence Number 5 is fused to the carboxyl terminal alanine residue was designated KL29.

30

Sequence Number 1 ATGGAGGAGCACACAGAGGC

35

Sequence Number 2 GGCATAGAAGCCCCAGAGGA

40

Sequence Number 4 AACAAAGCTTGGCATAGAAGCCCCAGAGGA

45

Sequence Number 5 KLGPEQKLISEEDLNSAVDHHHHHH

2) Preparation of the cells expressing transiently human SGLT2

[0065] KL29, the plasmid coding human SGLT2, was transfected into COS-7 cells (RIKEN CELL BANK RCB0539) by electroporation. Electroporation was performed with GENE PULSER II (Bio-Rad Laboratories) under the condition: 0.290 kV, 975 μ F, 2×10^6 cells of COS-7 cell and 20 μ g of KL29 in 500 μ L of OPTI-MEM I medium (Gibco-BRL: LIFE TECHNOLOGIES) in the 0.4 cm type cuvette. After the gene transfer, the cells were harvested by centrifugation and resuspended with OPTI-MEM I medium (1mL/cuvette). To each well in 96-wells plate, 125 μ L of this cell suspension was added. After overnight culture at 37°C under 5 % CO₂, 125 μ L of DMEM medium (Gibco-BRL: LIFE TECHNOLOGIES) which is containing 10 % of fetal bovine serum (Sanko Jyunyaku), 100 units/mL sodium penicillin G (Gibco-BRL: LIFE TECHNOLOGIES), 100 μ g/mL streptomycin sulfate (Gibco-BRL: LIFE TECHNOLOGIES) was added to each well. After a culture until the following day, these cells were used for measurement of the inhibitory activity against the uptake of methyl- α -D-glucopyranoside.

3) Measurement of the inhibitory activity against the uptake of methyl- α -D-glucopyranoside

[0066] After removal of the medium of the COS-7 cells expressing transiently human SGLT2, to each well 200 μ L of the pretreatment buffer (a pH 7.4 buffer containing 140 mM choline chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris (hydroxymethyl)aminomethane) was added and the cells were incubated 37°C for 10 minutes. The pretreatment buffer was removed and 200 μ L of the same buffer was added again, and then the cells were incubated 37°C for 10 minutes. Seven μ L of methyl- α -D-(U-14C)glucopyranoside (Amersham Pharmacia Biotech) was added to 525 μ L of the buffer for uptake containing test sample (a pH 7.4 buffer containing 140 mM sodium chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 5 mM methyl- α -D-glucopyranoside, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris(hydroxymethyl)aminomethane), and this mixture was mixed, and then the buffer for measurement of uptake was prepared. For the control, the buffer for measurement of uptake without test compound was prepared. For estimate of the basal uptake in the absence of test compound and sodium, the buffer for measurement of the basal uptake, which contains 140 mM choline chloride in place of sodium chloride, was prepared similarly. After the pretreatment buffer was removed, 75 μ L of the buffer for measurement of uptake was added to each well, the cells were incubated at 37°C for 2 hours. After the buffer for measurement of uptake was removed, 200 μ L of the washing buffer (a pH 7.4 buffer containing 140 mM choline chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM methyl- α -D-glucopyranoside, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris(hydroxymethyl)aminomethane) was added to each well and immediately removed. After two additional washing, the cells were solubilized by addition of 75 μ L of 0.2 N sodium hydroxide to each well. After the cell lysates were transferred to the PicoPlate (Packard) and 150 μ L of MicroScint-40 (Packard) was added to each well, the radioactivity was measured with microplate scintillation counter TopCount (Packard). The difference in uptake was obtained as 100 % value by subtracting the radioactivity in the basal uptake from that in control and then the concentrations at which 50 % of uptake were inhibited (IC_{50} value) were calculated from the concentration-inhibition curve by least square method. The results are shown in the following Table 1.

[Table 1]

Test compound	IC_{50} value (nM)
Example 1	350
Example 2	450
Example 3	140
Example 4	500
Example 5	330
Example 6	370
Example 7	140
Example 8	8.1
Example 9	27
Example 10	210
Example 11	75
Example 12	110

Test Example 2

50 Assay for the facilitatory effect on urinary glucose excretion

[0067] As experimental animal, overnight fasted SD rats (SLC., male, 7 weeks of age, 180-240 g) were used. Ten mg of test compound was suspended or dissolved in 300 μ L of ethanol and then dissolved by adding of 1.2 mL of polyethylene glycol 400 and 1.5 mL of saline, and then 3.3 mg/mL solution was prepared. Three hundred μ L of this solution was dissolved with 2.7 mL of the solution for dilution (saline: polyethylene glycol 400:ethanol = 5:4:1) and then 0.33 mg/mL solution was prepared. After the body weights of rats were measured, the solution of test compound was intravenously injected to the tail vein at the dose of 3 mL/kg (1 mg/kg). For control, just only the solution (saline: polyethylene glycol 400:ethanol = 5:4:1) was intravenously injected to the tail vein at the dose of 3 mL/kg. Immediately

after intravenous injection to the tail vein, 200 g/L of glucose solution was orally administered to the rats at the dose of 10 mL/kg (2 g/kg). The intravenous injection to the tail vein was performed with 26 G injection needle and 1 mL syringe. The oral administration was performed with gastric tube for rat and 2.5 mL syringe. The head count in one group was 2 or 3. Collection of urine was performed in metabolic cage after the oral administration of glucose was finished. The sampling time for collection of urine was 24 hours after the oral administration of glucose. After the collection of urine was finished, the urine volume was recorded and the urinary glucose concentration was measured. The glucose concentration was measured with a kit for laboratory test: Glucose B-Test WAKO (Wako Pure Chemical Industries, Ltd.). The amount of urinary glucose excretion in 24 hours per 200 g of body weight was calculated from urine volume, urinary glucose concentration and body weight. The results are shown in the following Table 2.

[Table 2]

Test compound	Amount of urinary glucose excretion (mg)
Example 1	27.4
Example 7	109.1
Example 8	238.9
Example 10	69.5

Test Example 3

Acute toxicity test

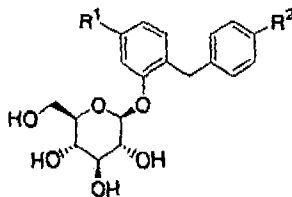
[0068] Five weeks old male ICR mice (CLEA JAPAN, INC. 29-34 g, 5 animals in each group) were fasted for 4 hours, and 666 mg/mL of a suspension which was prepared by adding saline: polyethylene glycol 400:ethanol (5:4:1) to 2-[4-(2-hydroxy-ethyl)benzyl]phenyl β -D-glucopyranoside (the compound described in Example 10) was subcutaneously administered at the dose of 3 mL/kg (2000 mg/kg). No death was observed until 24 hours after the administration.

Industrial Applicability

[0069] The glucopyranosyloxybenzylbenzene derivatives represented by the above general formula (I) of the present invention have an excellent inhibitory activity in human SGLT2. The present invention can provide agents for the prevention or treatment of diabetes, diabetic complications, obesity or the like. In addition, since compounds represented by the above general formula (II) are important as intermediates in the production of the compounds represented by the above general formula (I), the compounds represented by the above general formula (I) of the present invention can be readily prepared via such compounds.

Claims

1. A glucopyranosyloxybenzylbenzene derivative represented by the general formula:



wherein R¹ represents a hydrogen atom or a hydroxy(lower alkyl) group; and R² represents a lower alkyl group, a lower alkoxy group, a lower alkylthio group, a hydroxy(lower alkyl) group, a hydroxy(lower alkoxy) group, a hydroxy(lower alkylthio) group, a lower alkoxy-substituted (lower alkyl) group, a lower alkoxy-substituted (lower alkoxy) group or a lower alkoxy-substituted (lower alkylthio) group, or a pharmaceutically acceptable salt thereof.

D5 225



(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
12.06.2002 Bulletin 2002/24

(51) Int Cl.7: **C07H 17/02, C07D 231/20,**
A61K 31/7056, A61P 3/04,
A61P 3/10

(21) Application number: **00954963.5**

(22) Date of filing: **24.08.2000**

(86) International application number:
PCT/JP00/05678

(87) International publication number:
WO 01/16147 (08.03.2001 Gazette 2001/10)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **31.08.1999 JP 24680099**

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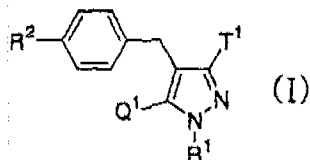
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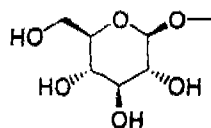
(54) **GLUCOPYRANOSYLOXYPYRAZOLE DERIVATIVES, MEDICINAL COMPOSITIONS**
CONTAINING THE SAME AND INTERMEDIATES IN THE PRODUCTION THEREOF

(57) The present invention relates to glucopyrano-
syloxy pyrazole derivatives represented by the general
formula:



while the other represents a lower alkyl group or a halo
(lower alkyl) group; and R² represents a hydrogen atom,
a lower alkyl group, a lower alkoxy group, a lower
alkylthio group, a halo(lower alkyl) group or a halogen
atom, or pharmaceutically acceptable salts thereof,
which have an inhibitory activity on human SGLT2 and
are useful as agents for the prevention or treatment of
diabetes, diabetic complications or obesity, and to phar-
maceutical compositions comprising the same and in-
termediates thereof.

wherein R¹ represents a hydrogen atom or a lower alkyl
group; one of Q¹ and T¹ represents a group represented
by the general formula:



2) Preparation of the cells expressing transiently human SGLT2

[0103] KL29, the plasmid expressing human SGLT2, was transfected into COS-7 cells (RIKEN CELL BANK RCB0539) by electroporation. Electroporation was performed with GENE PULSER II (Bio-Rad Laboratories) under the condition: 0.290 kV, 975 μ F, 2×10^6 cells of COS-7 cell and 20 μ g of KL29 in 500 μ L of OPTI-MEM I medium (Gibco-BRL: LIFE TECHNOLOGIES) in the 0.4 cm type cuvette. After the gene transfer, the cells were harvested by centrifugation and resuspended with OPTI-MEM I medium (1mL/cuvette). To each well in 96-wells plate, 125 μ L of this cell suspension was added. After overnight culture at 37 °C under 5 % CO₂, 125 μ L of DMEM medium which is containing 10 % of fetal bovine serum (Sanko Jyunyaku), 100 units/mL sodium penicillin G (Gibco-BRL: LIFE TECHNOLOGIES), 100 μ g/mL streptomycin sulfate (Gibco-BRL: LIFE TECHNOLOGIES) was added to each well. These cells were cultured until the next day and then they were used for the measurement of the inhibitory activity against the uptake of methyl- α -D-glucopyranoside.

3) Measurement of the inhibitory activity against the uptake of methyl- α -D-glucopyranoside

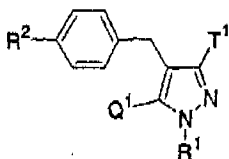
[0104] After a test compounds was dissolved in dimethyl sulfoxide and diluted with the uptake buffer (a pH 7.4 buffer containing 140 mM sodium chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 5 mM methyl- α -D-glucopyranoside, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris(hydroxymethyl)aminomethane), each diluent was used as test sample for measurement of the inhibitory activity. After removal of the medium of the COS-7 cells expressing transiently human SGLT2, to each well 200 μ L of the pretreatment buffer (a pH 7.4 buffer containing 140 mM choline chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris(hydroxymethyl)aminomethane) was added, and the cells were incubated at 37 °C for 10 minutes. After the pretreatment buffer was removed, 200 μ L of the same buffer was added again, and the cells were incubated at 37 °C for 10 minutes. The buffer for measurement was prepared by adding of 7 μ L of methyl- α -D-(U-14C)glucopyranoside (Amersham Pharmacia Biotech) to 525 μ L of the prepared test sample. For the control, the buffer for measurement without test compound was prepared. For estimate of the basal uptake in the absence of test compound and sodium, the buffer for measurement of the basal uptake, which contains 140 mM choline chloride in place of sodium chloride, was prepared similarly. After the pretreatment buffer was removed, 75 μ L of the each buffer for measurement was added to each well, the cells were incubated at 37 °C for 2 hours. After the buffer for measurement was removed, 200 μ L of the washing buffer (a pH 7.4 buffer containing 140 mM choline chloride, 2 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM methyl- α -D-glucopyranoside, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid and 5 mM tris(hydroxymethyl)aminomethane) was added to each well and immediately removed. After two additional washings, the cells were solubilized by addition of 75 μ L of 0.2 N sodium hydroxide to each well. After the cell lysates were transferred to the PicoPlate (Packard) and 150 μ L of MicroScint-40 (Packard) was added to each well, the radioactivity was measured with microplate scintillation counter TopCount (Packard). The difference in uptake was obtained as 100 % value by subtracting the radioactivity in the basal uptake from that in control and then the concentrations at which 50 % of uptake were inhibited (IC₅₀) were calculated from the concentration-inhibition curve by least square method. The results are shown in the following Table 1.

[Table 1]

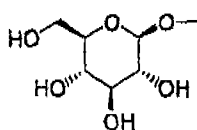
Test compound	IC ₅₀ value (nM)
Example 35	181
Example 36	441
Example 37	346
Example 38	702
Example 39	185
Example 43	84
Example 44	509
Example 45	441
Example 46	679
Example 48	415

Claims

1. A glucopyranosyloxypyrazole derivative represented by the general formula:

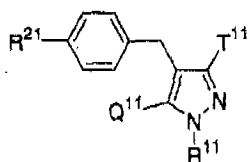


wherein R¹ represents a hydrogen atom or a lower alkyl group; one of Q¹ and T¹ represents a group represented by the formula:

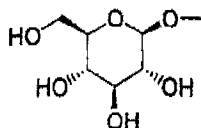


while the other represents a lower alkyl group or a halo(lower alkyl) group; R² represents a hydrogen atom, a lower alkyl group, a lower alkoxy group, a lower alkylthio group, a halo(lower alkyl) group or a halogen atom, or a pharmaceutically acceptable salt thereof.

2. A glucopyranosyloxypyrazole derivative as claimed in claim 1, represented by the general formula:



wherein R¹¹ represents a hydrogen atom or a straight-chained or branched alkyl group having 1 to 3 carbon atoms; one of Q¹¹ and T¹¹ represents a group represented by the formula:



while the other represents a straight-chained or branched alkyl group having 1 to 3 carbon atoms; and R²¹ represents a straight-chained or branched alkyl group having 1 to 4 carbon atoms, a straight-chained or branched alkoxy group having 1 to 3 carbon atoms or a straight-chained or branched alkylthio group having 1 to 3 carbon atoms, or a pharmaceutically acceptable salt thereof.

3. A glucopyranosyloxypyrazole derivative as claimed in claim 1, represented by the general formula:

Bioisosterism: A Rational Approach in Drug Design

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Received May 15, 1996 (Revised Manuscript Received July 25, 1996)

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George Patani graduated with a B.Pharm. in 1992 from the College of Pharmaceutical Sciences, Mangalore University at Manipal, India. In 1996, he received his M.S. in Pharmaceutical Science at Rutgers University under the direction of Professor Edmond J. LaVoie. He is presently pursuing graduate studies in pharmaceutics. His current research interests are focused on drug design and controlled drug delivery.



Edmond J. LaVoie received his B.S. in Chemistry from Fordham University in 1971 and his Ph.D. in Medicinal Chemistry from S.U.N.Y. at Buffalo under the direction of Dr. Wayne K. Anderson. After postdoctoral study with Dr. S. Morris Kupchan at the University of Virginia, he joined the American Health Foundation in Valhalla, NY. In 1988, he was appointed Professor of Medicinal Chemistry in the College of Pharmacy at Rutgers University. His current research interests are in the design and synthesis of cancer chemotherapeutics and in the elucidation of mechanism(s) of carcinogenesis.

I. Introduction

Years of cumulative research can result in the development of a clinically useful drug, providing either a cure for a particular disease or symptomatic relief from a physiological disorder. A lead compound with a desired pharmacological activity may have associated with it undesirable side effects, characteristics that limit its bioavailability, or structural features which adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach used by the medicinal chemist

for the rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive.¹

The prevalence of the use of bioisosteric replacements in drug design need not be emphasized. This topic has been reviewed in previous years.²⁻⁵ The objective of this review is to provide an overview of bioisosteres that incorporates sufficient detail to enable the reader to understand the concepts being delineated. While a few popular examples of the successful use of bioisosteres have been included, the

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
✓ **LUIS PATIÑO LEYVA**
Apoderado

ASUNTO	Radicación	05-000562
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	12862
	Folios	14

Patente de Invención ☒ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☒ Cap. I ☐ Cap. II ☒

No. Sol. Internacional PCT/EP2003/006841

Fecha de Presentación Internal. 27 de Junio de 2003

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción: Folios: 57 a 114 y 122 como se radicó inicialmente

Reivindicaciones: Folios 115 a 121 como se radicó inicialmente
Folios 179 a 180 modificados
Folios 218 a 219 modificados

Prioridad: Folios: 01
11 de Julio de 2002, DE, 10231370.9

Respuesta del solicitante: Folios 172 a 176; Folios 213 a 215

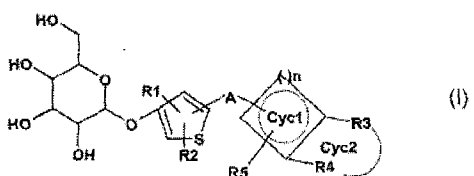
2. Análisis de la Prioridad

- La fecha de prioridad del 11 de Julio de 2002 y su correspondiente documento, corresponden con la materia solicitada de protección.

3. Objeto de la invención

- Título de la solicitud: **"NUEVOS DERIVADOS DE GLICÓSIDO DE TIOFENO, MÉTODOS PARA LA PREPARACIÓN DE LOS MISMOS, MEDICAMENTOS QUE COMPRENDEN DICHOS COMPUESTOS Y SU USO"**

El objeto de la presente solicitud de patente de invención consiste en obtener compuestos glicósidos de tiofeno para el tratamiento de la diabetes de fórmula I:



El problema planteado en esta solicitud es la necesidad de obtener compuestos glicósidos del tiofeno para el tratamiento de la diabetes

Para solucionar este problema técnico la solicitud en estudio, el solicitante proporciona compuestos según fórmula general I.

- **Clasificación Internacional de Patentes (IPC.):** C07H7/00; A61K31/7042; A61K31/7048

4. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

- Fecha de presentación de la solicitud prioritaria: 11 de Julio de 2002

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 11/07/2002 se encontraron los siguientes documentos del estado de la técnica relacionados, que pueden afectar la patentabilidad del objeto de la presente solicitud:

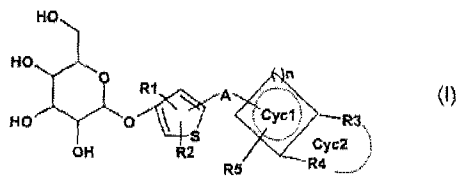
Nº	Documento	Título	Fecha de publicación
D4	EP 1270584 WO 01/068660	Glucopyranosyloxy benzylbenzene derivatives, medicinal compositions containing the same and intermediates for the preparation of the derivatives.	20/09/2001
D5	EP 1213296 WO/ 01/16147	Glucopyranosyloxy pirazole derivatives, medicinal compositions containing the same and intermediates in the production thereof	08/03/2001
D3	Chem.Rev, 1996, 96, 3147-3176	Bioisosterism: a Rational Approach in Drug Design	1996

5. Análisis de patentabilidad (artículo 14 D486)

Nivel inventivo (artículo18 D486)

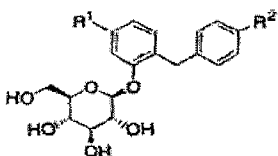
1) Respecto a la solicitud en estudio:

La reivindicación 1 se refiere a un compuesto de fórmula (I):

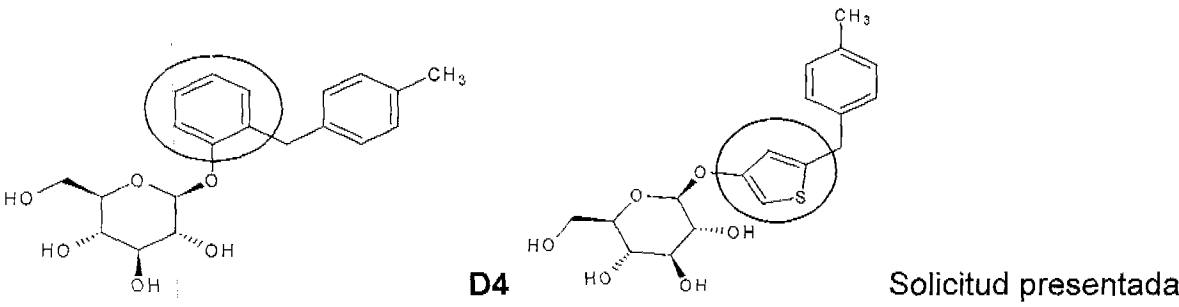


2) Respecto al estado de la Técnica:

D4 enseña previamente compuestos de fórmula I, útiles como agentes para la prevención o tratamiento de la diabetes, y obesidad (Ver resumen)



Al realizar las sustituciones planteadas en D4 se obtienen compuestos tales como y comparado con el de la solicitud presentada:



D5 enseña compuestos útiles para la prevención o tratamiento de la diabetes, sus complicaciones u obesidad (Ver resumen)

Al realizar las sustituciones planteadas en D5 se obtienen compuestos tales como y comparado con el de la solicitud presentada:

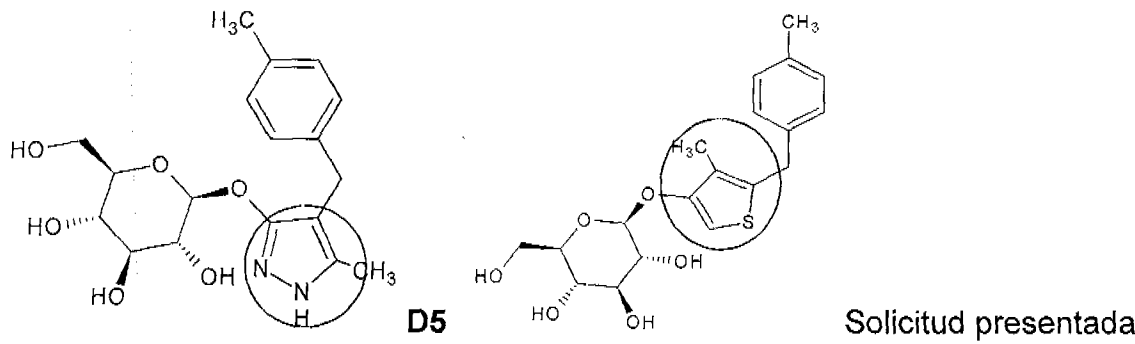


Tabla "Nivel Inventivo"		
Solicitud presentada	D4	D5
Glucopiranosil	Glucopiranosil	Glucopiranosil

El Problema Técnico Objetivo fundamental el cual es “**obtener compuestos glicósidos del tiofeno para el tratamiento de la diabetes**”, sería fácilmente resuelto por el experto en la materia con las anterioridades encontradas, pues es evidente que con los documentos **D4 y D3 o D5 y D3**; reemplazando un anillo heteroaromático análogo por tiofeno, en el grupo farmacofórico de **D4** o de **D5**, sin emplear habilidad inventiva.

No se evidencia por lo tanto el efecto superior e inesperado al realizar este reemplazo comparativamente con los compuestos previamente divulgados, pues para el experto técnico en la materia al emplear los conceptos de bioisosteria, se derivarían de forma obvia y sin necesidad de utilizar habilidad inventiva los compuestos de la solicitud presentada. Y puesto que al comparar los resultados de actividad biológica como inhibidores selectivos de SGLT2, se encuentra que:

D4 presenta resultados para el ensayo inhibitorio sobre la actividad SGLT2, valores de IC₅₀ de 8.1 y 27nM entre otros. (Ver páginas 16 y 17)

D5 presenta resultados para el ensayo inhibitorio sobre la actividad SGLT2, valores de IC₅₀ de 84nM entre otros.

El solicitante en el Folio 83 realiza los análisis sobre la medida de la absorción de la glucosa por vesículas de membrana de borde en cepillo y expresa los valores sobre IC₂₅, sin evidenciar su actividad inhibitoria selectiva sobre la actividad SGLT2 frente al estado de la técnica.

De manera tal que los compuestos de la solicitud presentada y sus medicamentos carecen de nivel inventivo.

6. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D4	EP 1270584 WO 01/068660	1-6	Nivel Inventivo
D5	EP 1213296 WO/ 01/16147	1-6	Nivel Inventivo
D3	Chem.Rev, 1996, 96, 3147-3176	1-6	Nivel Inventivo

7. Respuesta a los argumentos del solicitante:

El solicitante hace referencia en los folios 214 a 215, a que los compuestos de la solicitud presentada son selectivos sobre el transportador SGLT2, y presenta los resultados donde sobre un único compuesto (ejemplo 7), donde presenta una actividad de 0.011µM (11nM), sin evidenciarse en los demás compuestos en su conjunto dicha actividad. Además los valore no referencia el tipo de bioensayo utilizado en dicha actividad.

En el estudio de nivel inventivo arriba mencionado se reportan valores de 8.1nM para compuestos donde el anillo es un fenilo, y al comparar con la sustitución de tiofeno; éste no supera los valores reportados en **D4** por ejemplo.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar

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respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Directora de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha

20 NOV. 2009

CONCEPTO TÉCNICO No. 03448	EXAMINADOR TÉCNICO Código No. 202070
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