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New Flavonoids from the Saudi Arabian Plant *Retama raetam* which Stimulate Secretion of Insulin and Inhibit α-Glucosidase

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Retama raetam is a bush which is a member of the family Fabaceae. It is used traditionally in North Africa and Saudi Arabia for the treatment of diabetes. Several flavonoids and alkaloids were already known from this plant. Chromatographic fractionation and purification led to the isolation of three new derivatives of prenylated flavones, retamasin C-E and four new derivatives of prenylated isoflavones, retamasin F-I, in addition to two isoflavones which had not previously been reported in this plant. Particularly interesting structures included isoflavones containing 3,5-dihydro-2*H*-2,5-methanobenzo[*e*][1,4]dioxepine and 3a,8b-dihydro-7-hydroxyfuro[3,2-*b*]benzo[2,1-*d*]furan units, both of which are new amongst natural product flavonoids. Five new examples (two flavones and three isoflavones) strongly enhanced the glucose-triggered release of insulin by murine pancreatic islets and one isoflavone was a potent inhibitor of α-glucosidase. This study may rationalise the traditional medicinal use of *R. raetam* and provides new leads for drug design in the treatment of diabetes.

Introduction

Retama raetam (Forsk.) Webb & Berthel is a plant common in North Africa, countries of the Eastern Mediterranean and in the Middle East,[1,2] including Saudi Arabia, and is a member of the family Fabaceae. It is particularly abundant in desert areas and is known locally as R'tm or broom bush. It flourishes in arid desert areas, where it stabilises the land and is a forage plant for camels, goats and sheep.[3] However, ingestion by livestock leads to toxicity.[2] In folk medicine in Saudi Arabia and Morocco, this plant has been used to treat diabetes and hypertension.[4] This traditional therapeutic activity has been borne out in experimental studies in rodents, where aqueous extracts of the plant lowered the concentrations of glucose and lipids in normal and streptozotocin-treated rats[4,5] and caused acute diuresis in normal rats.[6] Anti-oxidant, anti-microbial, anti-viral and insecticidal activities have also been reported.[4,7] One mechanism for the observed hypoglycaemic activity in rats has been suggested to be inhibition of renal re-uptake of glucose,[8] whereas a methanol extract showed antidiabetic activity in the same species which was attributed to increasing glucose-stimulated release of insulin by the pancreas and to diminution of absorption of glucose in the intestine.[9]

There is a need to identify the antidiabetic / hypoglycaemic compounds present in *R. raetam* for potential development into effective treatments for this major disease, a growing cause of death and disability across the world. Isolation of the active compounds is key, as the plant, crude extracts and folklore preparations have led to significant toxicity to animals[2] and humans.[10] Amongst other classes of compounds, quinolizidine and other alkaloids have been isolated (including cytisine, L-anagyrine, sparteine and lupinine), which may be responsible for this toxicity.[11] Pinitol, a cyclitol component of *R. raetam*, is a known hypoglycaemic agent.[12] However, the flavonoids have been most extensively studied as components of this plant. In addition to the simple compounds daidzein, naringenin, apigenin, kaempferol, quercetin and orientin (and their glycosides),[13] luteolin 4'-neohespiridoside has been identified as a more complex flavone-*O*-glycoside in this plant.[14,15] The flavone-*C*-glycoside saponarin was characterised by Kassem *et al.*,[15] whereas *C*-isoprenyl units can be recognised in the *R. raetam* pyranoflavones atalantoflavone[16] and 4',5-dihydroxy-(3'',4''-dihydro-3'',4''dihydroxy)-2'',2''-dimethylpyrano-(5'',6'':7,8)-flavone,[14] in the furanoflavone retamasin B[16] and in the open-chain flavones licoflavone C[16] and ephedroidin.[16] The isoprenyl unit is truncated in retamasin B.[16] The analogous *C*-glycosyl isoflavone genistein 8-*C*-glucoside[15] and the pyrano-isoflavones derrone[16] and hydroxyderrone[16] have also been found in *R. raetam*.

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Electronic Supplementary Information (ESI) available: [Table of NMR data for **4,9,10**; ^{1}H, ^{13}C, COSY, HSQC, HMBC NMR spectra for **1-10**; HRESIMS for **1-3,5-8**]. See DOI: 10.1039/x0xx00000x

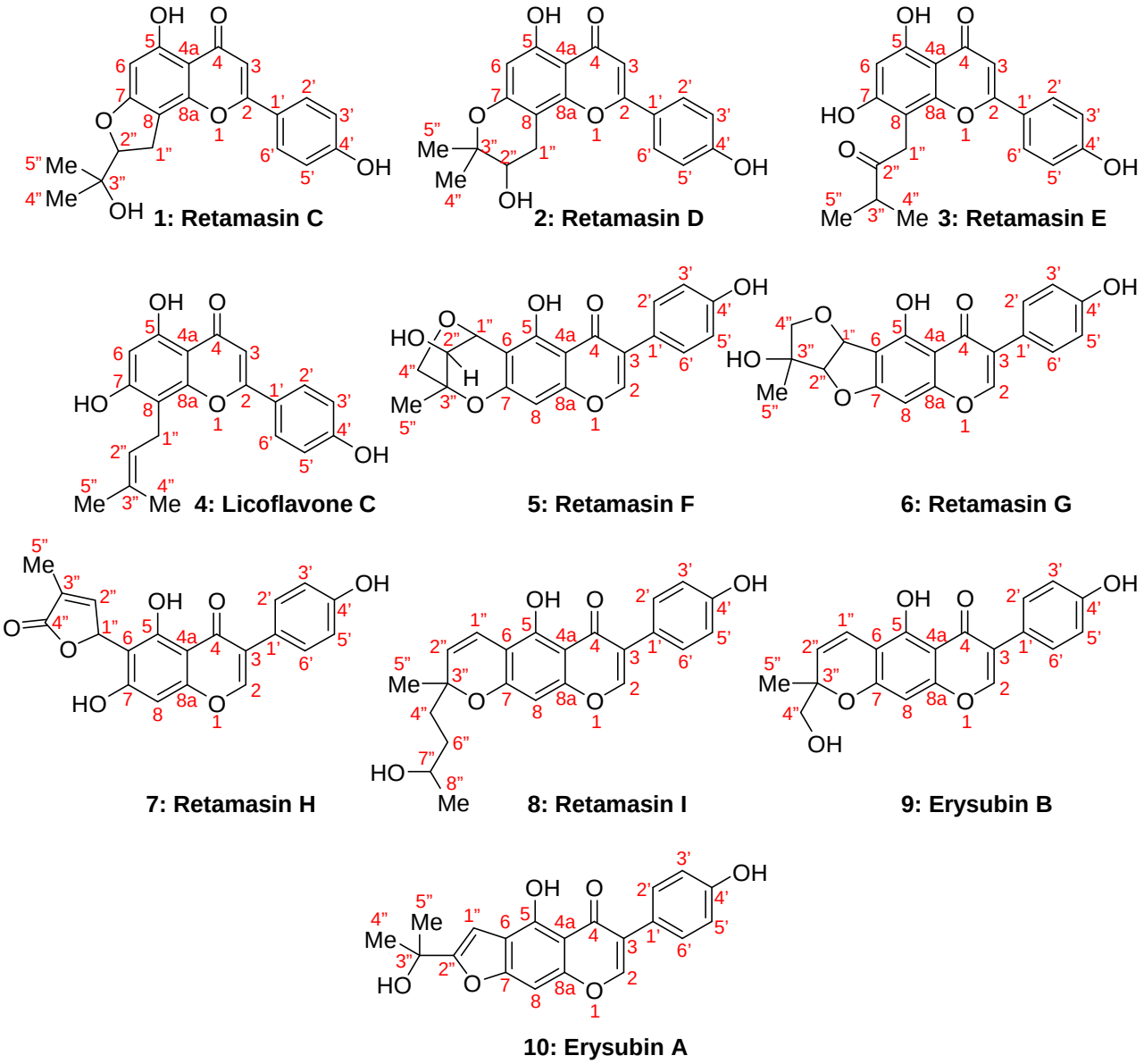

Figure 1. Structures of flavonoids newly isolated from *R. raetam*.

Flavonoids have a very wide range of biological and therapeutic activities. In this study, further clarification was sought of the compounds in *R. raetam* which are responsible for its antidiabetic activity. The chloroform extract of the aerial parts of the plant was fractionated chromatographically. Seven new flavonoids (**1-3,5-8**), one known flavone (**4**) and two known isoflavones (**9,10**) (Figure 1) were isolated and characterised spectroscopically, each derived from a flavone or isoflavone carrying a modified C_5 isoprene unit. All compounds were examined for stimulation of secretion of insulin from islets isolated from murine pancreas and seven were selected for assessment for inhibition of α-glucosidase.

Results & discussion

The dried aerial parts of *R. raetam* were extracted with chloroform and the solvent was evaporated. This residue was partitioned between hexane and acetonitrile. Chromatography of the acetonitrile fraction, followed by reverse-phase preparative HPLC on a C18 column, afforded ten pure compounds **1-10**. Their structures were elucidated using 1D and 2D NMR and HRESIMS data. The absolute configurations of the chiral compounds **1,2,5-9** cannot be determined from these data.

The HRESIMS of **1** showed a peak in positive mode at *m/z* 355.1156, corresponding to $[M + H]^+$ (calcd 355.1181) for the molecular formula $C_{20}H_{18}O_6$. Ions were also seen at *m/z* 377.0982 $[M + Na]^+$ (calcd 377.1001) and *m/z* 709.2250 $[2 M + H]^+$ (calcd 709.2285). The HRESIMS in negative mode

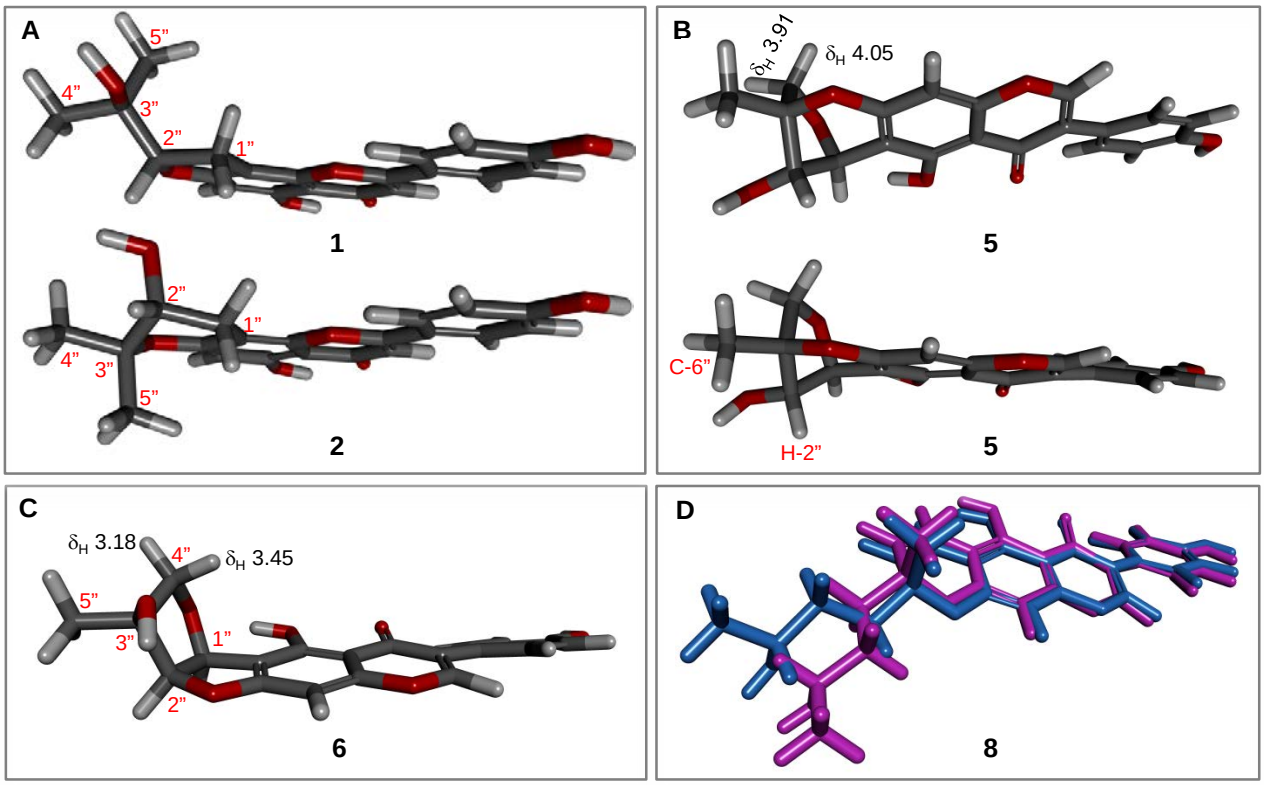

Figure 2. A: MM2-minimised structures of **1** and **2**, showing conformations of the dihydrofuran and dihydropyran rings, respectively. The *pseudo*equatorial location of H-2" in **2** is also shown. **B**: Two views of the MM2-minimised structure of the HO-*exo* diastereoisomer of **5**, showing tentative assignments of the ^{1}H NMR signals for H-5'' (upper) and the dihedral angle between (H-2")–(C-2") and (C-5")–(C-3") (lower), suggesting this diastereoisomer. **C**: Diastereoisomeric identity of **6** demonstrated by NOESY spectroscopy. **D**: Overlay of the MM2-minimised structures of the two diastereoisomers of **8**.

confirmed the formula, with peaks at *m/z* 353.1018 $[M - H]^-$ (calcd 353.1025), *m/z* 707.2125 $[2\ M - H]^-$ (calcd 707.2128), and *m/z* 753.2179 [2 M + formate]$^-$ (calcd 753.2183), in addition to a peak at 389 $[M + {}^{35}Cl]^-$. The NMR spectra showed the appropriate numbers of ^{1}H and ^{13}C signals. The core structure was shown, by a combination of NMR techniques, to be a flavone. Specifically, H-3 (δ_H 6.80) and H-6 (δ_H 6.28) both showed HMBC cross-peaks to C-4a (δ_C 104.68).

The ^{1}H NMR spectrum of **1** (Table 1) showed doublets at δ_H 7.94 (*J* 8.2 Hz) (δ_C 128.94) and δ_H 6.95 (*J* 8.2 Hz) (δ_C 116.49), each integrating for 2 H. These signals corresponded to H-2',6' (C-2',6') and H-3',5' (C-3',5'), respectively. The phenolic OH signal at δ_H 13.19 showed HMBC correlations with C-6 (δ_C 93.95), C-4a (δ_C 104.68) and C-5 (δ_C 161.78), with a weak cross-peak to the ketone (δ_C 182.30); these located the signal at δ_H 13.19 as being due to HO-5, which was deshielded by H-bonding to the ketone oxygen. The signal at δ_H 10.45 was thus due to the phenol at C-4', rationalising the δ_H chemical shifts for H-2',3',5',6'. These data, together with the δ_H and δ_C for ring A, confirmed that the flavone has a 4',5,7-oxygenation pattern. The methylene protons (δ_H 3.29, m) correlated with the ^{13}C signal (δ_C 26.49) in the HSQC spectrum and with C-7 (δ_C 166.74), C-8 (δ_C 104.48) and C-8a (δ_C 152.20) in the HMBC spectrum, which identified this methylene (CH_2-1") as attached at C-8. A COSY cross-peak from H-1" identified H-2" (δ_H 4.80), with HSQC linking this to C-2" (δ_C 92.05). H-2" gave HMBC cross-peaks to C-8 (δ_C 104.48) and C-7 (δ_C 166.74) of the flavone; both of these were three-bond correlations, which confirmed the fusion of the dihydrofuran ring. H-2" also correlated in HMBC through two bonds with C-1" (δ_C 26.49) and quaternary C-3" (δ_C 70.53) and through three bonds with the methyl carbons C-4" (δ_C 25.35) and C-5" (δ_C 26.25). These two methyl groups were diastereotopic; however, although the carbon chemical shifts were different, the ^{1}H chemical shifts were identical (δ_H 1.18). C-3" also carries an OH group (HO-3" δ_H 4.74), as shown by the downfield ^{13}C chemical shift. This OH signal shows HMBC correlations with C-2", C-3", C-4" and C-5". Together, these spectroscopic data confirmed the structure of **1** (retamasin C), a novel compound.

The positive-ion HRESIMS of **2** showed $[M + H]^+$ at *m/z* 355.1161 (calcd 355.1181), showing that it was an isomer of **1**. Ions were also observed at *m/z* 377.0982 $[M + Na]^+$ (calcd 377.1001) and *m/z* 337.1132 $[M + H - H_2O]+$ (calcd 337.1076); the latter indicated the presence of an aliphatic alcohol. In the negative-ion HRESIMS, ions were detected at *m/z* 353.1023 $[M - H]^-$ (calcd 353.1025), *m/z* 707.2126 $[2\ M - H]^-$ (calcd 707.2128) and *m/z* 743.1893 $[2\ M + {}^{35}Cl]^-$ (calcd 743.1900), confirming the formula. The NMR spectra (Table 1) showed the appropriate numbers of ^{1}H and ^{13}C signals. As for 1, H-3 (δ_H 6.82, s) and H-6 (δ_H 6.13, s) both correlated in the HMBC spectrum with C-4a (δ_C 104.98), confirming **2** as a flavone and not an isoflavone.

The IR spectrum of **2** indicated OH (3341 cm^{-1}) and a conjugated aryl ketone (1645 cm^{-1}). ^{13}C and HSQC NMR

Table 1. ^{1}H and ^{13}C NMR data for **1-3,5** in $(CD_3)_2SO$ [δ_H, multiplicity (*J* (Hz)); δ_C, type]

	1		**2**		**3**		**5**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		163.8, C_q		164.0, C_q		164.2, C_q	8.39, s	154.9, CH
3	6.80, s	103.2, CH	6.82, s	103.5, CH	6.78, s	103.5, CH		122.8, C_q
4		182.3, C_q		182.4, C_q		182.7, C_q		181.0, C_q
4a		104.7, C_q		105.0, C_q		104.00, C_q		105.5, C_q
5		161.8, C_q		159.3, C_q [a]		160.2, C_q		157.6, C_q [b]
6	6.28, s	93.995, CH	6.13, s	99.5, CH	6.28, s	9.99, CH		109.8, C_q
7		166.7, C_q		159.4, C_q [a]		161.8, C_q		160.2, C_q
8		104.5, C_q		99.8, C_q		101.4, C_q	6.49, s	94.1, CH
8a		152.2, C_q		154.8, C_q		155.5, C_q		157.7, C_q [b]
1′		121.5, C_q		121.7, C_q		122.0, C_q		121.4, C_q
2′,6′	7.94, d, *J* 8.1	128.9, CH	7.92, d, *J* 8.8	128.8, CH	7.85, d, *J* 8.8	129.0, CH	7.39, d, *J* 8.4	130.6, CH
3′,5′	6.95, d, *J* 8.1	116.5, CH	6.93, d, *J* 8.8	116.5, CH	6.91, d, *J* 8.8	116.4, CH	6.83, d, *J* 8.4	115.6, CH
4′		162.3, C_q		161.8, C_q		164.0, C_q		158.0, C_q [b]
1″	3.29, m	26.5, CH_2	2.73, dd, *J* 16.1, 6.8; 3.05 dd, *J* 16.1, 5.2	25.3, CH_2	3.95, s	35.2, CH_2	5.02, s	73.0, CH
2″	4.80, dd, *J* 8.6, 8.1	92.0, CH	3.75, brt, *J* 6	67.3, CH		211.5, C_q	4.17, d, *J* 3.8	73.9, CH
3″		70.5, C_q		79.4, C_q	2.84, m	~40, CH		88.4, C_q
4″	1.18, s	25.3, CH_3	1.32, s	21.5, CH_3	1.07, d, *J* 6.9	18.8, CH_3	3.91, d, *J* 10.3; 4.05, d, *J* 10.3	77.0, CH_2
5″	1.18, s	26.2, CH_3	1.24, s	25.6, CH_3	1.07, d, *J* 6.9	18.8, CH_3	1.50, s	16.4, CH_3
HO-5	13.19, s		12.70, s		12.96, s		13.37, s	
HO-7					10.7, br			
HO-3′			5.29, br					
HO-4′	10.45, br		10.4, br		10.4, br		9.64, s	
HO-2″							5.90, d, *J* 3.8	
HO-3″	4.74, br							

[a] Assignments could be reversed. [b] Assignments could be interchanged.

showed five methine signals, one methylene signal, two methyl signals and ten quaternary signals. The diastereotopic CH_2 protons resonated at δ_H 2.73 and δ_H 3.05, correlating in HSQC with the methylene C-1″ (δ_C 25.32). HMBC cross-peaks between these H-1″ signals and the downfield aromatic carbon signals at δ_C 159.3 (C-7) and δ_C 154.82 (C-8a) linked this methylene to C-8, supported by weaker two-bond HMBC cross-peaks from these H-1″ methylene protons to C-8. The COSY spectrum showed cross-peaks between these H-1″ signals and the signal at δ_H 3.75 (HSQC to δ_C 67.28). These chemical shifts indicated that this carbon (C-2″) also carried an oxygen, confirmed as OH by the COSY cross-peak to HO-2″ (δ_H 5.29). Three-bond HMBC correlations linked both H-1″ to a quaternary carbon signal (δ_C 79.44) for C-3″ linked to another oxygen. H-2″ was linked by HMBC to two methyl signals (C-4″ δ_C 21.53 and C-5″ δ_C 25.61). Weak four-bond HMBC cross-peaks from H-4″ (δ_H 1.32) and H-5″ (δ_H 1.24) to C-7 (δ_C 159.3) confirmed the six-membered dihydropyran ring fused at C-7 and C-8. NOESY cross-peaks showed that H-2″ is close in space to both H-4″ and H-5″; this is only possible if H-2″ is in a *pseudo*equatorial position. This conformation was supported by the ^{1}H NMR signals for both H-1″ being broad doublets, with only the ^{2}J value being large (>4 Hz); hence there were no *pseudo*axial / *pseudo*axial interactions with H-2″ and it adopted a *pseudo*equatorial conformation (Figure 2A). The geminal methyl groups in **2** resonated at different chemical shifts in both ^{1}H and ^{13}C spectra, which reflect their axial and equatorial positions in the *pseudo*chair of the dihydropyran conformation (Figure 2A). This contrasted with the almost planar conformation of the dihydrofuran in **1**, in which the CMe_2 unit was exocyclic and the signals for the diastereotopic methyl groups were accidentally coincident in both ^{1}H and ^{13}C spectra. These spectroscopic data confirmed the structure of **2** (retamasin D), a new compound. The CD spectrum (Supplementary Information) showed a positive peak in the region 260 nm – 400 nm; using the analysis of Wei et al.[17] suggests that **2** may have *R* configuration.

The positive-ion HRESIMS of **3** showed an $[M + H]^+$ peak at *m/z* 355.1159 (calcd 355.1181) for the molecular formula $C_{20}H_{18}O_6$. The negative-ion spectrum contained ions at *m/z* 353.1020 ($[M - H]^-$ calcd 353.1025), *m/z* 707.2126 ($[2 M - H]^-$ calcd 707.2129) and *m/z* 743.1894 ($[2 M + {}^{35}Cl]^-$ calcd

743.1895). The ^{13}C NMR spectrum (Table 1) contained seventeen discrete signals, of which ten were for quaternary carbons, five for methine carbons, one for a methylene carbon and one for two magnetically equivalent methyl carbons. The carbonyl resonance at δ_C 211.53 showed a dialkyl ketone. The IR spectrum showed absorbances for OH (3347 cm^{-1}), a conjugated aryl ketone carbonyl (1654 cm^{-1}) and a H-bonded dialkyl ketone carbonyl (1697 cm^{-1}).

Analysis of the ^{1}H, ^{13}C, COSY, HSQC and HMBC NMR spectra of **3** indicated a structure similar to that of apigenin (4′,5,7-trihydroxyflavone). C-8 (δ_C 101.38) was quaternary (no HSQC correlations), showing that the substituent was attached to the apigenin core at this position. Three phenolic OH signals were seen: HO-5 (δ_H 12.96, s), HO-4′ (δ_H 10.4, br) and HO-7 (δ_H 10.7, br) H-bonded to the dialkyl ketone. The methylene protons (H-1″) resonated as a singlet (δ_H 3.95), thus the compound is achiral. This signal showed three-bond HMBC cross-peaks to C-7 (δ_C 161.79) and C-8a (δ_C 155.52) and a two-bond cross-peak to C-8 (δ_C 101.38), confirming that CH_2-1″ is attached at C-8 of the flavone. A weaker two-bond HMBC correlation connected the dialkyl ketone signal C-2″ (δ_C 211.53) to H-1″. The presence of an isopropyl group was shown by a doublet, integrating for 6 H, at δ_H 1.07 (H-4″ / H-5″) and a multiplet signal for H-3″ at δ_H 2.84. Three-bond HMBC cross-peaks were observed between the signals for H-4″ / H-5″ and the signal for the C-2″ ketone. Thus **3** (retamasin E, a novel compound) is identified as a 8-prenylated apigenin oxidised to a ketone at C-2″.

Compound **4** was identified as licoflavone C by comparison of the NMR spectra (Table S1, ESI) and melting point with the data reported for synthetic and natural product samples.[18,19] It should be noted that the NMR data should be compared with compound 10 in Al-Maharik and Botting[19] and not compound 6, as the spectroscopic data for these two compounds were erroneously transposed in that paper. IR data were also consistent with the structure.

The positive-ion HRESIMS of **5** showed that the molecular formula is $C_{20}H_{16}O_7$, with $[M + H]^+$ observed at *m/z* 369.0953 (calcd 369.0974), $[M + Na]^+$ *m/z* 391.0772 (calcd 391.0794), $[2 M + H]^+$ *m/z* 737.1833 (calcd 737.1870) and $[2 M + Na]^+$ *m/z* 759.1652 (calcd 759.1690). This was confirmed by negative-ion HRMS peaks at *m/z* 367.0818 $[M - H]^-$ (calcd 367.0818), *m/z* 403.0585 $[M + {}^{35}Cl]^-$ (calcd 403.0585), *m/z* 413.0874 $[M + HCO_2]^-$ (calcd 413.0873), *m/z* 771.1491 $[2 M + {}^{35}Cl]^-$ (calcted 771.1481) and *m/z* 781.1791 $[2 M + HCO_2]^-$ (calcd 781.1769).

Compound **5** was an isoflavone, as shown by the downfield chemical shift of the ^{1}H NMR singlet for H-2 (δ_H 8.39) (Table 1), which had HSQC connectivity with C-2 (δ_C 154.93). This proton showed a strong HMBC cross-peak with the signal for the carbonyl C-4 (δ_C 180.96) and a weaker two-bond correlation with quaternary C-3 (δ_C 122.85) but not with C-4a (δ_C 105.51). The latter was clearly identified by its HMBC correlation with H-8 (δ_H 6.49). H-8 made a two-bond correlation with C-8a (δ_C 157.63 / 157.66 / 158.02), which formed a three-bond connection with H-2. The 4-hydroxyphenyl unit at C-3 was demonstrated by HMBC correlations from H-2′ / H-6′ (δ_H 7.39) to C-3 (δ_C 122.85) and from H-2 to C-1′ (δ_C 121.41). HO-4′ (δ_H 9.64) was identified by a HMBC cross-peak with C-3′ / C-5′ (δ_C 115.56). In the IR spectrum, peaks were seen for the OH (3333 cm^{-1}) and conjugated aryl ketone (1643 cm^{-1}) groups. C-6 (δ_C 109.85) was quaternary and the chemical shift indicated substitution with a sp^3-hybridised carbon. It made a three-bond correlation with H-8 and a good two-bond correlation with H-1″ (δ_H 5.02). C-1″ was a methine, with the ^{13}C NMR signal at δ_C 73.03. H-1″ also showed a two-bond HMBC correlation with C-2″ (δ_C 73.90) and three-bond correlations with C-3″ (δ_C 88.38) and C-4″ (δ_C 77.01). Of these, C-3″ was quaternary, C-2″ was a methine and C-4″ was a methylene; the chemical shifts of each indicated an attached oxygen. Both of the diastereotopic H-4″ protons formed HMBC cross-peaks with C-1″ and C-2″ through three bonds, but only the signal at δ_H 3.91 formed a cross-peak with the methyl signal at δ_C 16.39 (C-5″). The lack of cross-peak from δ_H 4.05 was likely to be due to the dihedral angle along (C-3″)–(C-4″) between the (C-4″)–H bond to this proton and the (C-3″)–(C-5″) bond being *ca*. 90°. Thus the diastereotopic H-4″ ^{1}H NMR signals were tentatively assigned as shown in Figure 2B. A similar argument can be used to suggest that HO-2″ may be located in the *exo* position (Figure 2B), as the dihedral angle between the (H-2″)–(C-2″) bond and the (C-3″)–(C-5″) bond was also *ca*. 90° in this diastereoisomer, whereas it was *ca*. 30° in the other (HO-2″ *endo*) structure. Further evidence was provided by H-2″ being coupled only to HO-2″ and not to H-1″ and the lack of a corresponding COSY cross-peak; this was only possible when HO-2″ was located *exo* and the dihedral angle between (H-1″)–(C-1″) and (H-2″)–(C-2″) is ca. 90°. The HO-2″-*exo* diastereoisomer was confirmed by the observation of a strong NOESY cross-peak from the HO-2″ (δ_H 5.90) to the H-4″ signal (δ_H 4.05) and a weaker one to H-4″ (δ_H 3.91). This observation also assigned the signals for the diastereotopic H-4″, with H-4″ (δ_H 4.05) being on the face towards the OH. The methyl group (5″) was located as being attached at C-3″, as it was a singlet and through a two-bond HMBC cross-peak from H-5″ (δ_H 1.50) to C-3″ and a weak four-bond cross-peak to C-7 (δ_C 160.19). Taken together, these correlations demonstrated that there is a bicycle fused to the flavone core at C-6 and C-7 and that the structure of **5** (retamasin F, a novel compound) was as shown. From the positive value in the 260 nm – 400 nm region of the CD spectrum (Supplementary Information), we tentatively assign the configuration of **5** as 1″*S*, 2″*S*, 3″*R*.[17]

In positive-ion mode, HRESIMS of **6** showed a peak for $[M + H]^+$ was seen at *m/z* 369.0970 (calcd 369.0974), corresponding to the molecular formula $C_{20}H_{16}O_7$. Supporting this assignment were peaks for $[M + Na]^+$ at *m/z* 391.0772 (calcd 391.0794), $[2 M + H]^+$ at *m/z* 737.1832 (calcd 737.1870) and $[2 M + Na]^+$ at *m/z* 759.1651 (calcd 759.1690). Negative ions were observed for $[M + {}^{35}Cl]^-$ at *m/z* 403.0585 (calcd 403.0585), $[M + HCO_2]^-$ at *m/z* 413.0873 (calcd 413.0873) and $[2 M + {}^{35}Cl]^-$ *m/z* 771.1478 (calcd 771.1481). It is an isomer of **5**. The ^{13}C NMR spectrum (Table 2) showed eighteen discrete resonances, of which two were each for two carbons in magnetically equivalent environments. There were signals for ten quaternary carbons (including one carbonyl), eight methine carbons, one methylene carbon and one methyl carbon.

As for **5**, this compound gave NMR resonances characteristic of an isoflavone substituted at C-6 (δ_C 109.10) with a sp^3-C substituent. C-2 was a methine and resonated downfield at δ_C 154.86. The H-2 signal was also downfield (δ_H 8.38) and was identified by HMBC cross-peaks to C-1′ (δ_C 121.38), C-4 (δ_C 181.30) and C-8a (δ_C 159.86). The 3-(4-hydroxyphenyl) unit was also located with a strong HMBC correlation from H-2′ / H-6′ (δ_H 7.36) to C-3 (δ_C 122.76). C-8 (δ_C 89.44) is a methine and H-8 (δ_H 6.59) formed two-bond HMBC correlations with C-7 (δ_C 167.38) and C-8a (δ_C 159.86) and three-bond correlations with C-4a (δ_C 106.22) and C-6 (δ_C 109.10). Two phenolic OH signals were present in the ^{1}H NMR spectrum, H-4′ at δ_H 9.58 and the intramolecularly H-bonded HO-5 at δ_H 13.47. One aliphatic OH signal was observed at δ_H 5.30, which was shown by HSQC to be a tertiary alcohol attached at C-3″ (δ_C 77.08). The IR spectrum contained peaks for the OH (3363 cm^{-1}) and conjugated aryl ketone (1655 cm^{-1}) groups, consistent with a hydroxylated isoflavone. Attached to C-6 was a methine carbon C-1″ (δ_C 78.68), with H-1″ forming an HMBC cross-peak with C-7 (δ_C 167.38). This carbon comprised one atom of a tetrahydrofuran, which was demonstrated as follows. H-1″ correlated with C-4″ (δ_C 72.79) in the HMBC spectrum and one of the methylene protons H-4″ (δ_H 3.45) correlates back with C-1″, joining the bridge across the oxygen. This H-4″ also formed a three-bond correlation with C-2″ (δ_C 91.91) and H-2″ (δ_H 4.76) showed a cross-peak with C-4″. The final carbon of the tetrahydrofuran, quaternary C-3″, was identified through two-bond HMBC correlations with both H-4″ methylene protons (δ_H 3.18 and δ_H 3.45) and with H-2″. The substituents at C-3″ were an alcohol (as noted above) and a methyl group. C-5″ (δ_C 25.12) of the methyl group correlated in HMBC with both H-4″ methylene protons, with HO-3″ and with H-2″, whereas the H-5″ protons formed cross-peaks with C-2′, C-4″ and, more weakly, with C-3″. As C-2″ was a methine and there were only two phenolic OH signals, then C-2″ must be attached to the oxygen at C-7 of the isoflavone core and the 6,7-substitution must be a tetrahydrofuranofuran. Thus **6** (retamasin G) had the new structure shown.

This structure has three chiral centres and thus four diastereoisomers were possible. NOESY cross-peaks between H-1″ and H-2″ confirmed that the tetrahydrofuran / dihydrofuran ring-fusion in **6** had *cis* configuration (Figure 2C). The coupling constant between these two protons $^3J_{1''\text{-}2''}$was 5.6 Hz, which is close to the corresponding $^3J_{cis}$ in the tetrahydrofuran / dihydrofuran fused system in panacene[20] (6.0 Hz) and in a related *cis*-3a,8b-dihydrofuro[3,2-*b*]-benzofuran-2(3*H*)-one (5.8 Hz).[21] Correlation was also seen in the NOESY spectrum between H-2″ and H-5″, which shows that this methyl is also *cis* to H-2″. The diastereotopic methylene proton signals were assigned by NOESY correlations HO-3″→H-4″ (δ_H 3.18) and H-5″→H-4″ (δ_H 3.45), as shown in Figure 2C.

The benzo-fused furanofuran unit in **6** is hitherto unknown in natural-product flavones and isoflavones, although it is present in the hexahydrofuranobenzofurans (+)-panacene (from the sea hare *Aplysia brasiliana*),[22] platypodantherone (from *Platypodanthera melissaefolia*),[23] and (+)-gynunone (from *Gynura elliptica*).[24] The motif is seen fused to coumarin in compounds from *Aegle marmelos*[25] and *Peucedanum praeruptorum*,[26] and to xanthones in psorofebrin and related compounds from *Psorospermum febrifugum*.[27] In all cases, the two furans are *cis*-fused, as in **6**. However, amongst compounds with Me and OH at the equivalent of C-3″, two examples have the same relative configuration as **6** (with Me *cis* to the ring-junction-H).[25,26] Two others have the opposite configuration (Me *trans* to ring-junction-H), although one lacks the OH.[24,27]

The positive-ion HRESIMS of **7** contained a peak for $[M + H]^+$ *m/z* 367.0800 (calcd 367.0818), corresponding to the molecular formula $C_{20}H_{14}O_7$. A peak for $[2\ M + Na]^+$ was seen at *m/z* 755.1344 (calcd 755.1377). The negative-ion spectrum contained peaks at *m/z* 365.0659 ($[M - H]^-$, calcd 365.0661), *m/z* 731.1399 ($[2\ M - H]^-$, calcd 731.1401) and *m/z* 767.1164 ($[2\ M + {}^{35}Cl]^-$, calcd 767.1168). The ^{13}C NMR spectrum (Table 2) contained eighteen discrete resonances, with two of the methine signals representing two equivalent carbons each. There were signals for eleven quaternary carbons, eight methine carbons and one methyl carbon. The ^{1}H spectrum contained signals for three phenolic OH groups, HO-4′ (δ_H 9.65), H-5 (δ_H 13.73) and HO-7 (δ_H 11.5).

Compound **7** was an isoflavone, with the singlet for H-2 at δ_H 8.37, the methine carbon C-2 at δ_C 154.70 and the quaternary C-3 at δ_C 122.97. The 3-(4-hydroxyphenyl) substituent was shown by signals at δ_H 7.40 (H-2′ / H-6′) and δ_H 6.84 (H-3′ / H-5′). The former correlated in the HMBC spectrum with C-4′ (δ_C 157.72 or 158.00) and with C-3. An HMBC cross-peak from H-3′ / H-5′ identified C-1′ (δ_C 121.44). IR peaks were observed for the OH (3322 cm^{-1}) and conjugated aryl ketone (1630 cm^{-1}) groups, consistent with a hydroxylated isoflavone. H-8 (δ_H 6.46) showed HMBC cross-peaks with C-4a (δ_C 104.11 or 104.40), 6-C (δ_C 104.11 or 104.40), C-7 (δ_C 167.59) and C-8a (δ_C 161.73) and weakly with C-2 through four bonds. The ^{1}H NMR signal at δ_H 6.36 was identified as being attached to sp^3-hybridised C-1″ (δ_C 74.16) and hence to C-6 by HMBC cross-peaks to C-5 (δ_C 157.72 or 158.00), C-6 and C-7. The unusually downfield chemical shift of this proton was consistent with its benzylic environment, with lactone oxygen and alkene groups adjacent. A strong two-bond HMBC cross-peak of H-1″ to C-2″ (δ_C 149.15) identified the alkene and the downfield chemical shift of the C-2″ signal indicated that the alkene was conjugated to a carbonyl. A weak three-bond cross-peak was seen between H-1″ and alkene carbon C-3″ (δ_C 128.48). H-2″ (δ_H 7.35) correlated in HMBC with the lactone carbonyl C-4″ (δ_C 175.03). Finally, the methyl-5″ was located as being attached to the lactone at C-3″ by HMBC correlations from H-5″ to C-3″, C-4″ and C-2″. Thus the structure of **7** (retamasin H) was confirmed as a novel isoflavone carrying a γ-lactone substituent at C-6.

Compound **8** gave a $[M + H]^+$ peak in the HRESIMS at *m/z* 395.1473 (calcd 395.1495), corresponding to the molecular formula $C_{23}H_{22}O_6$. A further peak was seen for $[2\ M + H]^+$ at *m/z* 789.2872 (calcd 789.2911). The negative-ion HRMS confirmed the formula with a plethora of peaks for $[M - H]^-$ at *m/z* 393.1340 (calculated 393.1338), $[M + {}^{35}Cl]^-$ at *m/z* 429.1105 (calcd 429.1105), $[M + HCO_2]^-$ *m/z* at 439.1394 (calculated 439.1393), $[2\ M - H]^-$ at *m/z* 787.2752 (calcd 787.2755), [2 M +

Table 2. ^{1}H and ^{13}C NMR data for **6-8** in $(CD_3)_2SO$ [δ_H, multiplicity (*J* (Hz)); δ_C, type]

	6		**7**		**8**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	8.38, s	154.9, CH	8.37, s	154.7, CH	8.37, s	154.7, CH
3		122.8, C_q		123.0, C_q		122.9, C_q
4		181.3, C_q		181.0, C_q		180.9, C_q
4a		106.2, C_q		104.1, C_q [a]		105.8, C_q
5		158.9, C_q		157.7, C_q [b]		157.2, C_q
6		109.1, C_q		104.4, C_q [a]		105.0, C_q
7		167.4, C_q		167.6, C_q		159.7, C_q
8	6.59, s	89.4, CH	6.46, s	93.9, CH	6.50, s	94.8, CH
8a		159.9, C_q		161.7, C_q		158.0, C_q
1′		121.4, C_q	6.36, br	74.2, CH		121.5, C_q
2′,6′	7.36, d, *J* 8.5	130.7, CH	7.40, br	130.7, C_q	7.39, d, *J* 8.3	130.6, CH
3′,5′	6.81, d, *J* 8.5	115.6, CH	6.84, br	115.6, C_q	6.83, d, *J* 8.3	115.4, CH [c]
4′		158.0, C_q		158.0, C_q [b]		156.4, C_q
1″	5.71, d, *J* 5.6	78.7, CH	5.71, d, *J* 5.6	78.7, CH	6.66, brd, *J* 10.3	115.5, CH [c]
2″	4.76, d, *J* 5.6	91.9, CH	7.35, br	149.1, CH	5.74, 0.5 H, d, *J* 10.1; 5.75, 0.5 H, d, *J* 10.1	128.6, CH
3″		77.1, C_q		128.5, C_q		81.1, C_q
4″	3.18, d, *J* 8.5 3.45, d, *J* 8.5	72.8, CH_2		175.0, C_q	1.62, 0.5 H, m; 1.67, 0.5 H, m; 1.81, 0.5 H, m; 1.87, 0.5 H, m	36.9, CH_2
5″	1.29, s	25.1, CH_3	1.87, s	10.8, CH_3	1.41, s	27.4, CH_3
6″					1.4, m	33.7, CH_2
7″					3.56, septet, *J* 6.0	66.2, CH
8″					1.03, d, *J* 6.0	24.2, CH_3
HO-5	13.47, s		13.73, s		13.38,s	
HO-7			11.5, br			
HO-4′	9.58, br		9.65, br		9.62, s	
HO-3″	5.30, br					
HO-7″					4.407, 0.5.H, d, *J* 4.4; 4.414, 0.5 H, d, *J* 4.4	

[a] Assignments could be reversed. [b] Assignments could be reversed. [c] Assignments could be reversed.

$^{35}Cl]^-$ at *m/z* 823.2519 (calcd 823.2518) and $[2\ M + HCO_2]^-$ at *m/z* 833.2807 (calculated 833.2809). Twenty-one discrete ^{13}C NMR signals were recorded (Table 2), with two resonances corresponding to two equivalent carbons each. Ten signals were for quaternary carbons (all sp^2); there were nine methines (eight sp^2, one sp^3), two methylenes and two methyls. In the IR spectrum, peaks were seen for OH (3311 cm^{-1}) and aryl ketone (1630 cm^{-1}), consistent with a hydroxylated isoflavone.

The core of **8** was an isoflavone, with NMR signals for H-2 at δ_H 8.37 and for methine C-2 at δ_C 154.73. The singlet signal for H-8 (δ_H 6.50) correlated in the HMBC spectrum with C-4a (δ_C 105.76), C-6 (δ_C 105.02), C-7 (δ_C 159.72) and C-8a (δ_C 157.96), with a weaker four-bond cross-peak with the carbonyl C-4 (δ_C 180.91). ^{1}H NMR signals for two phenols were recorded: HO-4′ (δ_H 9.62) and H-5 (δ_H 13.38); thus the oxygen at the 7-position must be substituted. C-6 was shown to be quaternary, so a ring system connecting C-6 and O-7 was expected. H-1″ (δ_H 6.66) resonated as a broad doublet, coupled to H-2″ (δ_H 5.74, 0.5 H; δ_H 5.75, 0.5 H), as confirmed by a cross-peak in the COSY spectrum. Both protons formed cross-peaks in the HMBC spectrum with C-3″ (δ_C 81.10). Moving outwards in the substituent, C-3″ formed two-bond HMBC cross-peaks with both diastereotopic protons of the CH_2-4″ methylene (δ_H 1.65 m and δ_H 1.80 m; δ_C 36.95); another cross-peak linked C-4″ with H-2″. Strong COSY cross-peaks showed that this methylene was adjacent to another methylene, with both H-6″ protons resonating as a complex multiplet (δ_H 1.4) and the signal for C-6″ appearing at δ_C 33.66. Further COSY correlation showed that H-6′ is coupled to a methine proton (H-7″) at δ_H 3.56. This chemical shift, together with the chemical shift for C-7″ (δ_C 66.23), suggested an electronegative substituent here. Correspondingly, HO-7″ (δ_H 4.41) resonated as a doublet (*J* 2.9 Hz), with a COSY connection to H-7″ and with HMBC connections to C-6″, C-7″ and a methyl (C-8″; δ_C 24.17). The structure of this side-chain was tied together through further HMBC cross-peaks H-7″→C-4″ and H-8″→C-6″. The remaining methyl carbon was identified as C-5″ (δ_C 27.40) through HMBC correlations with H-2″; both H-4″ and H-5″ correlated with C-3″. Thus **8** (retamasin I) had the

Table 3. Percentage inhibition of the activity of α-glucosidase by flavonoids isolated from *R. raetam.*

Compound	% Inhibition of α-glucosidase activity at 50 μM
1	62.9 ± 1.9
2	43.6 ± 0.4
5	33.2 ± 1.6
6	61.0 ± 0.6
7	96.3 ± 0.2
9	46.7 ± 0.9
10	67.9 ± 0.7
Acarbose (positive control)	98.7 ± 0.9

structure shown. The detail of the ^{1}H and COSY NMR spectra showed that there were two overlapping signals for each of H-2", H-6" and HO-7", each integrating for 0.5 H. This may indicate that the novel compound **8** had been isolated as an equimolar mixture of diastereoisomers. Figure 2D shows an overlay of the MM2-minimised structures of these diastereoisomers and reveals that the environments of these protons were the most significantly different between these forms. The positive value in the 260 nm – 400 nm region of the CD spectrum (Supplementary Information) suggests that the configuration of **8** may be 3"*S*,[17] with a 50:50 mixture of epimers at C-7".

Compounds **9** and **10** were identified as erysubin B and erysubin A, respectively. Their ^{1}H and ^{13}C NMR spectra (Table S1, ESI) were almost identical to those reported for compounds isolated from the wood of *Erythrina suberosa* var. *glabrescens.*[28] The assignments were supported by COSY, HSQC and HMBC data and by comparison of mp with literature values.

C-Prenylation of flavones is well known in plants, although a review by Botta *et al*. suggested that *C*-prenylated isoflavones were less common, as isoflavone synthase and prenyltransferases were less widespread in nature.[29] The *C*-prenyl group is also found cyclised onto an *ortho*-phenol to form extensive ranges of furanoflavones and pyranoflavones.[30] One may speculate that the isomeric furanoflavone **1** and pyranoflavone **2** (also atalantoflavone[16]) arise through 5-*exo* and 6-*endo* cyclisations of a common oxidised derivative of **4**, such as 8-(3,3-dimethyloxiranylmethyl)-4',5,7-trihydroxyflavone. Retamasin A and retamasin B, previously reported in *R. raetam*,[16] are furanoflavones which may also derive from **4**.

In the isoflavone series, **5**, **6**, **7**, **9** and **10** can all be identified as derivatives of a 6-prenylated isoflavone which have been oxidised and cyclised in different modes. The 6-prenyl unit can also be identified as C-1", C-2", C-3", C-4" and C-5" in **8** but a three-carbon extension (C-6", C-7" and C-8") is also present. The biosynthetic origin of these three carbons is unclear but they may be the stump of another prenyl unit which has been partly trimmed down. The benzo-fused bicyclic system in **5** is new amongst natural products but has been identified synthetically as the product of treatment of 2-(1-hydroxy-2-methyl-2-(oxiran-2-yl)ethyl)phenols (oxiranes derived from an arylisoprene) with acid at low temperature.[31] More vigorous treatment with acid triggers an elimination reaction to give 2-hydroxymethylchromenes. It may be that **5** is biosynthesised from a 6-prenylisoflavone and subsequently undergoes acid-catalysed elimination to give **9**.

Type-2 diabetes is a long-term metabolic disease in which the ability of peripheral cells to take up glucose from the blood in response to insulin is decreased ("insulin resistance"), leading to an increase in the concentration of glucose in the blood and thence to a range of pathologies. There is also some loss of glucose-stimulated release of insulin by the β-cells of the Islets of Langerhans in the pancreas. Clinically used drugs for treatment of this disorder mostly address either the production of insulin by the β-cells (sulfonylureas, *e.g.* tolbutamide, glibenclamide) or increase the sensitivity of the peripheral cells to insulin (stimulating uptake of glucose, *e.g.* metformin). Type-1 diabetes also involves loss of glucose-stimulated release of insulin by the β-cells. Thus the flavonoids **1-10** isolated from *R. raetam* were evaluated for their ability to increase the secretion of insulin by isolated murine pancreatic islets.

Figure 3 shows the amount of insulin secreted per islet in the presence of the compounds (200 μM), with tolbutamide (200 μM, positive control) and in the absence of test compound (negative control). The insulin secretory activity of control islets was 8.5 ± 0.38 ng islet^{-1} h^{-1} upon stimulation by glucose (16.7 mM). This secretion was enhanced significantly (p<0.01) in the presence of the standard sulfonylurea drug tolbutamide (19.49 ± 1.69 ng islet^{-1} h^{-1}). Isoflavone **7** had little effect and flavones **2** and **4** inhibited the release of insulin under these conditions. However, the remaining flavonoids stimulated the glucose-triggered release of insulin significantly, with **8** and **9** raising the production of insulin *ca*. 3-fold and **1,3,5,6,10** raising secretion by *ca*. 5-fold. There is no clear *prima facie* structure-activity relationship distinguishing potent stimulators from weaker stimulators or inhibitors and the biochemical target is unknown but these flavones / isoflavones represent interesting new lead structures for further development of drugs to treat Type-2 diabetes and may explain the traditional use of this plant.[4]

Inhibitors of α-glucosidase, a key enzyme in the digestion of polysaccharides, are also used in the management of diabetes. These inhibitors have diverse structures. Acarbose, a mimic of a tetrasaccharide, is particularly effective in populations with a diet high in carbohydrate.[32] 2-(3-Ethoxy-4-hydroxyphenyl)quinazolin-4-one was the most potent inhibitor of α-glucosidase amongst a series of quinazolinones[33] and the natural product sarcoviolin β is potently inhibitory.[34] Table 3 lists the percentage inhibition of α-glucosidase activity by flavonoids **1,2,5-7,9,10** at 50 μM; acarbose was used as a positive control. All test compounds showed some activity. The most potent flavonoid **7** showed IC_{50} = 36.3 ± 1.7 μM for inhibition of this enzyme.

Conclusions

This paper reports major extension of the number of flavonoids isolated and characterised from *R. raetam*. Three new 8-

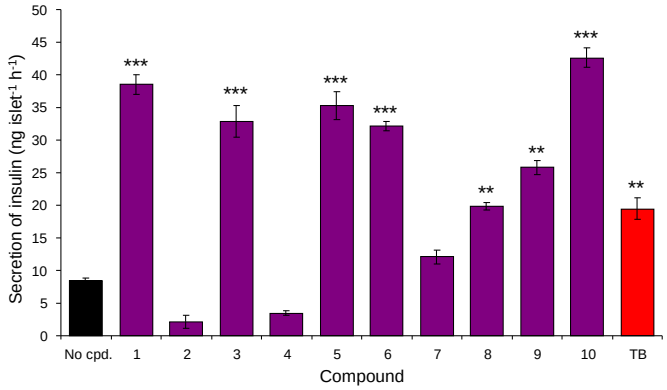

Figure 3. Glucose-stimulated secretion of insulin from murine pancreatic islets by compounds **1-10**. Freshly isolated islets were incubated for 1 h at 37°C in KRB buffer containing glucose (16.7 mM) in the absence (negative control) or presence of different test compounds and the amount of insulin secreted was measured. Values are mean ± SEM from three independent experiments. **P < 0.01, ***P < 0.001, compared with the value for the negative control. TB = tolbutamide.

prenylated flavone derivatives **1-3** and four new 6-prenylated isoflavone derivatives **5-8** are reported, along with identification of two known isoflavones **9,10** newly observed in this plant. The fused bicyclic heterocycles in **5** and **6** are demonstrated in naturally occurring flavonoids for the first time. Although only modest activity was seen in many of the new flavonoids in inhibition of the catalytic activity of α-glucosidase, **7** showed similar potency to acarbose and may be worthy of further investigation. More importantly, five of the new flavonoids (**1,3,5,6,10**) showed strong stimulation of the glucose-triggered release of insulin from pancreatic islets, with effects much greater than that of the widely used antidiabetic drug tolbutamide. Although they are isomers, **1** and **2** showed very different activities in the insulin secretion assay (Figure 3); this reflects the very different conformations of the saturated heterocycles and the presentation of the functional groups to the receptor(s). These biological observations rationalise the traditional use of *R. raetam* in folk medicine and provides new structural leads for drugs development for treatment of this rapidly increasing major worldwide disease, adding to the range of therapeutic activities known for prenylated flavonoids.[35]

Experimental

General experimental procedures

IR spectra were recorded on a Perkin-Elmer FTIR 600 series spectrometer. UV spectra were obtained from an HP 8453 diode array spectrophotometer. The 1D and 2D NMR (COSY, HSQC, HMBC) spectra using standard pulse programs were recorded at room temperature on a Bruker Avance DRX 700 spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C), on a Bruker Avance 500 FT spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), and on an Agilent 400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard $SiMe_4$ and coupling constants (*J*) are given in in Hz. HRESIMS were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using normal-phase silica gel (Merck; 230-400 μm) and reversed-phase silica gel (LiChroprep RP-18, Merck; 40-63 μm). Preparative HPLC was performed on a Shimadzu system (Kyoto, Japan), consisting of two LC-6AD Semi-Preparative Solvent Delivery pumps connected with Rheodyne manual injector, communications bus module CBM-20A, a multi-wavelength photo-diode array detector (SPD-M20A), FRC-10A fraction collector, and Shimadzu LC solution software. The column was a Shim-pack PREP-ODS(H) Kit 250 mm × 20 mm, with 5 μm particles. The gradient elution used a mobile phase consisting of acetonitrile and water (2:3) programmed linearly to 100% MeCN over 25 min at the flow rate 20 mL min^{-1}. The UV detection wavelength was 254 nm. Analytical HPLC was performed using the same system but with a Shim-pack PREP-ODS(H) Kit (A) 250 mm × 4.6 mm with 5 μm particles and flow rate 1.0 mL min^{-1}. TLC was carried out on silica gel sheets (Alugram Sil G/UV254, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F254S, Merck, Germany), with visualisation by 10% aq. H_2SO_4, followed by heating.

Plant material

The aerial parts of *Retama raetam* were collected in April 2010 near Al-looz Mountain, Tabook, Saudi Arabia and were identified by Dr. M. Yusuf, taxonomist, College of Pharmacy, King Saud University (KSU), Riyadh, Saudi Arabia. A voucher specimen (No.15567) has been deposited at the herbarium of the College of Pharmacy, KSU.

Extraction and isolation

The air-dried aerial parts (1.0 Kg) of *Retama raetam* were percolated at room temperature with $CHCl_3$ and the solvent was evaporated to yield a dark brown residue (30.0 g). This extract was partitioned between hexane and MeCN to afford hexane fraction (10.0 g) and MeCN fraction (14.0 g). The MeCN fraction (13.0 g) was subjected to silica gel chromatography (5.5 × 50 cm) with a linear gradient elution of methanol and $CHCl_3$ to give fractions A–T. Fraction D (150 mg) was separated by reversed-phase silica gel column chromatography (MeOH / H_2O, 4:1 → MeOH) to give **1** (8.0 mg), **2** (5.0 mg) and **3** (2.1 mg). Fraction E (414 mg) was separated by reversed-phase chromatography on a C_{18} column (MeCN / H_2O, 13:7 → MeCN) to yield **4** (2.7 mg), **5** (4.1 mg) and **6** (1.2 mg), Fraction F (495 mg) was separated by reversed-phase silica gel column chromatography (MeOH / H_2O, 3:1 → MeOH) to give **7** (4.1 mg), **8** (2.1 mg) and **9** (4.0 mg). Fraction H (241 mg) was further purified by reversed-phase chromatography on a C_{18} column (MeOH / H_2O, 7:3 → MeOH) to yield **10** (0.8 mg).

*Retamasin C (**1**):* yellow powder; mp 195-196°C; $[\alpha]^{25}_D$ −18.6 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 311 (3.68), 263 (3.62) nm; IR (KBr) ν_{max} 3315, 1650, 1601, 1589, 1511 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS: *m/z* 355.1156 [M + H]$^+$ (calcd for $C_{20}H_{19}O_6$, 355.1181).

*Retamasin D (**2**):* yellow powder; mp 204-206°C; $[\alpha]^{25}_D$ −10.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 324 (3.60), 263

(3.54) nm; IR (KBr) ν_{max} 3341, 1645, 1544, 1473 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS: *m/z* 355.1161 $[M + H]^+$ (calcd for $C_{20}H_{19}O_6$, 355.1181).

Retamasin E (***3***)*:* yellow powder; mp 231-232°C; UV (MeOH) λ_{max} (log ε) 333 (3.62), 263 (3.52) nm; IR (KBr) ν_{max} 3347, 1697, 1654, 1416 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS: *m/z* 355.1159 $[M + H]^+$ (calcd for $C_{20}H_{19}O_6$, 355.1181).

Licoflavone C (***4***)*:* powder; mp 172-174°C (lit.[19] mp 173-174°C); UV (MeOH) λ_{max} (log ε) 326 (ε 3.62), 305 (3.56) nm; IR (KBr) ν_{max} 3267, 1651, 1423 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Information.

Retamasin F (***5***)*:* yellow powder; mp 224-225°C; $[\alpha]^{25}_D$ −22.6 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 315 (3.64), 263 (3.63); IR (KBr) ν_{max}: 3333, 1643, 1608, 1574, 1521 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS: *m/z* 369.0953 $[M + H]^+$ (calcd for $C_{20}H_{17}O_7$, 369.0974).

Retamasin G (***6***)*:* yellow powder; mp 233-234°C; $[\alpha]^{25}_D$ −26.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 333 (3.61), 263 (3.55) nm; IR (KBr) ν_{max} 3363, 1655, 1530, 1425 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS: *m/z* 369.0970 $[M + H]^+$ (calcd for $C_{20}H_{17}O_7$, 369.0974).

Retamasin H (***7***)*:* yellow powder; mp 247-248°C; $[\alpha]^{25}_D$ −41.8 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 321 (3.62), 263 (3.52); IR (KBr) ν_{max} 3322, 1630, 1525, 1412 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS: *m/z* 367.0800 $[M + H]^+$ (calcd for $C_{20}H_{15}O_7$, 367.0818).

Retamasin I (***8***)*:* yellow powder; mp 218-219°C; $[\alpha]^{25}_D$ −2.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 325 (3.62), 263 (3.52) nm; IR (KBr) ν_{max} 3311, 1621, 1534, 1433 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS: *m/z* 395.1473 $[M + H]^+$ (calcd for $C_{23}H_{23}O_6$, 395.1495).

Erysubin B (***9***)*:* powder; mp 248-249°C (lit.[32] mp 247-249°C); $[\alpha]^{25}_D$ −17.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 284 (3.56), 225 (3.62); 205 (3.65) nm; IR (KBr) ν_{max} 3342, 1621, 1427 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Information.

Erysubin A (***10***)*:* powder; mp 231-232°C (lit.[32] mp 231-233°); UV (MeOH) λ_{max} (log ε) 353 (3.62), 267 (3.60); 212 (3.57) nm; IR (KBr) ν_{max} 3355, 1598, 1419 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Information.

Sources of materials for biological assays

Tolbutamide (TB), collagenase V and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). Mouse Ultrasensitive Insulin ELISA kit was purchased from Crystal Chem Inc. (IL, USA).

Isolation of pancreatic islets

Primary pancreatic islets were isolated from male BALB/c mice (28-36 g) from the International Center for Chemical and Biological Sciences (ICCBS) Animal Research Facility, University of Karachi, Pakistan. The handling of animals and harvesting of tissues were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and with prior approval from the Animal Use Committee of ICCBS (Protocol number: 2015-0020). Pancreatic islets were isolated by digestion with collagenase V and islet-picking methods as described earlier.[36] Briefly, mice were anaesthetised with sodium thiopental (30 mg Kg^{-1}) and the distended whole pancreas was digested at 37°C in collagenase solution (1.0 mg mL^{-1}) for 15 min. The digested islets were further purified by centrifugation at 1000 rpm for 1.0 min, followed by filtration using a pre-wetted 70 μm cell strainer. Finally, the islets were manually picked with a siliconised Pasteur pipette under a NIKON SMZ-745 stereomicroscope. The isolation and purification medium used was Hank's Balanced Salt Solution (HBSS) without calcium, magnesium and phenol red. Diameters were measured using a camera mounted on a stereomicroscope.

Insulin secretion assay

Freshly isolated islets (n = 3) of similar diameter (120–160 μm) were pre-incubated for 45 min at 37°C in Krebs–Ringer bicarbonate (KRB) buffer solution containing BSA (0.1%) and glucose (3.0 mM) in Eppendorf tubes. Each was performed in triplicate. The islets were washed with KRB buffer before being transferred into KRB buffer (300 μL) containing glucose (16.7 mM), supplemented with either test compound (pre-dissolved in DMSO, 200 μM) or tolbutamide (pre-dissolved in DMSO, 200 μM; a standard insulin secretagogue used as a positive control) and were incubated at 37°C for 1.0 h. At the end of incubation, an aliquot (200 μL) was placed on ice-cold water and kept at -40°C until the insulin was assayed. Concentrations of insulin in the collected aliquots were measured using the mouse ultrasensitive insulin ELISA kit according to the high-range protocol. The reading is the concentration of insulin in ng mL^{-1} of medium. After calculation of the absolute amount of insulin in the medium, this amount was divided by three (the number of islets in the tube).

Assay of inhibition of α-glucosidase

The assays of inhibition of α-glucosidase were conducted according to the method reported elsewhere[33] with slight modification. The reaction mixture contained sodium phosphate buffer (50 mM, pH 6.8) in a total volume of 300 μL. α-Glucosidase (40 mU) from *Saccharomyces cerevisiae* (Sigma Chemical Co., St. Louis, MO, USA) was incubated with target compounds for 15 min at 37°C. Compounds were dissolved in DMSO; the final concentration of this solvent in the assay mixture was 3%. After addition of 4-nitrophenyl-α-D-glucopyranoside (0.7 mM), the enzyme reaction was continuously monitored at 400 nm in 96-well microplates using SpectraMax Plus 384 microplate reader (Molecular Devices, CA, USA). Acarbose was used as a positive control. The activity of the compounds was measured as percent inhibition of α-glucosidase activity relative to controls with no test compounds. The assays were performed in quadruplicate and the mean percentage inhibitions were calculated, along with their respective SEM (standard error of the mean) values.

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