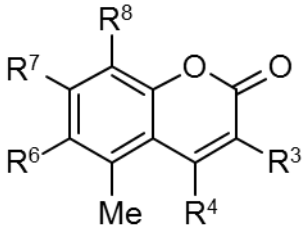
GRAPHICAL ABSTRACT



R^3 = H, Me, CH_2OH
R^4 = H, OMe, SMe, S(O)Me
R^6 = H, OH, OMe
R^7 = H, OH, OMe
R^8 = H, OH, OMe
Up to 5.5-fold enhancement of glucose-triggered release of insulin from β-cells

Stimulation of insulin secretion by 5-methylcoumarins and its sulfur analogues isolated from *Clutia lanceolata* Forssk.

Sarfaraz Ahmed [a], Mohammad Nur-e-Alam [a], Ifat Parveen [b], Simon J. Coles [c], Rahman M. Hafizur [d], Abdul Hameed [d], James B. Orton [c], Michael D. Threadgill [b,e], Muhammad Yousaf [a], Abdulaziz M. Alqahtani [a], Adnan J. Al-Rehaily [a]*

[a] *Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*

[b] *Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom*

[c] *UK National Crystallography Service, School of Chemistry, University of Southampton, University Road, Southampton, SO17 1BJ, United Kingdom*

[d] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi-75270, Pakistan*

[e] *Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom*

**Corresponding author.*

E-mail: ajalreha@ksu.edu.sa (A.J. Al-Rehaily)

Tel.: +966114677258. Fax: +966114677245.

Muhammad Yousaf: *Current address: Diteba Laboratories Inc. 1680 Tech Ave Unit #1, Mississauga, ON L4W 5S9 Canada*

Email: Muhammad.Yousaf@diteba.com

Abdul Hameed: Current address: Centre for Advanced Drug Research (CADR), COMSATS University Islamabad (CUI), Abbottabad, Pakistan

ABSTRACT

Clutia lanceolata Forssk. (*C. lanceolata*) is a medicinal plant native to sub-Saharan Africa and the Arabian Peninsula. Phytochemical investigation of the aerial parts of *C. lanceolata* yielded twenty-one coumarins including methylthio and methylsulfinyl-coumarins. Thirteen of these compounds are reported here for the first time, named as cluteolin A to M. The remaining eight compounds are known but have not been associated previously with *C. lanceolata*. The structures of the undescribed compounds were elucidated from their 2D NMR and MS spectra. Single crystal X-ray analyses confirmed the structures of eleven compounds. As, in Saudi Arabian tradition, *C. lanceolata* has been reported to have anti-diabetic and anti-fungal properties, the coumarins were examined for their biological activity. Seven compounds strongly enhanced the glucose-triggered release of insulin by murine pancreatic islets, with two compounds showing more than two-fold enhancement of insulin secretion, compared with the standard drug glimepiride.

Key words: *Clutia lanceolata* Forssk; Peraceae; Coumarins; Methylthiocoumarins; Single X-ray crystallography; Murine islets; Insulin.

Highlights:

- Thirteen undescribed 5-methylcoumarins isolated and characterised from *Clutia lanceolata*
- These include methylthio- and methylsulfinyl-coumarins
- Three compounds strongly stimulated glucose-triggered release of insulin from β-cells

1. Introduction

The genus *Clutia* belongs to the plant family *Peraceae* (formerly categorised as *Euphorbiaceae*), which are native to Eastern and sub-Saharan Africa and the Arabian Peninsula (Gilbert, 1992; Aynekulu et al., 2012; Parveen et al., 2016). In particular, in Saudi Arabia and nearby countries, several *Clutia* species have been used to treat diabetes and fungal infections (Aburashed et al., 2003; Mossa et al., 1990; Lulekal et al., 2014). Diterpenoids have been reported to be isolated from *C. richardiana*, (Mossa et al., 1988; Muhammad et al., 1994a,b; Muhammad et al., 1999; Mossa et al., 1996), including the novel labdane-like diterpenoid, Saudin (Mossa et al., 1985; Mossa, 1988). This compound showed a significant hypoglycaemic effect on non-alloxanised fasted mice (Mossa, 1988). Another species*, C. abyssinica*, has yielded diterpenoids (Waigh et al., 1990; Zerihun et al., 1987), flavonoids (Zerihun et al., 1987) and coumarins (Waigh et al., 1991). However, the components of *Clutia lanceolata* have been studied relatively rarely, with only four compounds (3,4-dihydroxy-2-methylbenzoic acid, 1,1'-binaphthyl-2,2'-diol, 1,3,8-trihydroxy-6-methylanthracene-9,10-dione and 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien- 3-one) being reported (Parveen et al., 2016).

The wide range of pharmacological and biological activities of coumarins of natural and synthetic origins has been reviewed (Menezes & Diederich, 2019; Zhu & Jiang, 2018; Hoult & Payá, 1996). Perhaps the most well-known and widely exploited of these therapeutic activities is the anticoagulant activity of warfarin and its analogues (Najmanová et al., 2015). Naturally occurring coumarins have also found applications outside medicine, including perfumery, dyes, laser technology and fluorescent indicators (Dittmer et al., 2005). The pharmacological activities of simple coumarins include anti-bacterial, anti-fungal, anti-retroviral, anti-inflammatory, anti-depressant and anti-cancer effects (Kumar et al., 2015). Coumarins have also been reported to decrease the glucose concentrations in blood (Marles & Farnsworth, 1995). Coumarins and terpenoids present in plant extracts from *Anacardium occidentale* caused a decrease in the blood glucose in diabetic rats (Ojewole, 2003). Further studies also suggested that a coumarin and its derivative, umbelliferone, reduced the blood glucose levels in diabetic rats (Marles & Farnsworth, 1995; Ojewole 2003; Ramesh & Pugalendi, 2006).

In the present work, a phytochemical analysis has been conducted on the coumarin components of *Clutia lanceolata* Forssk (Peraceae) as part of our continuing research into

the isolation and characterisation of previously undescribed specialised metabolites from medicinal plants found in Saudi Arabia. This plant is locally named as Laukh, A'lwah, or Saeer and is found in the regions Asir and Western Heights of Saudi Arabia (Rahman et al., 2004). The plant has been previously reported for hypoglycaemic activity (Mossa et al., 2000) and antifungal activity (Baka, 2010). We report herein the isolation and structure determination of twenty-one coumarins (Fig. 1), after extensive chromatography of extracts. Compounds **1-13** are previously undescribed and are named as cluteolin A to M. Eight known coumarins **14-21** are, for the first time, confirmed as natural compounds from *C. lanceolata*, although they have been reported as synthetic products or in isolates from other plants. Structural characterisation was achieved through a range of spectroscopic techniques and the structures of **1**,**8**,**9**,**11**,**12**,**14-17**, **19**,**20** (Fig. 2) have also been characterised by single-crystal X-ray analysis**.** Furthermore, **1-4**,**8-9**,**12**,**14-17, 19**,**20** were examined for their ability to stimulate the glucose-triggered release of insulin from pancreatic islets.

2. Results and Discussion

2.1. Structure determination

Compounds **1-21** were isolated and purified from the aerial parts of *C. lanceolata*. The structure of each compound was determined using a combination of HRESIMS, ^{1}H and ^{13}C NMR (including DEPT), 2D NMR techniques (COSY, HSQC, HMBC), IR spectroscopy and, where possible, (**1**,**8**,**9**,**11**,**12**,**14-17**,**19**,**20**), single-crystal X-ray analysis. Details of the various characterisation techniques for **1-13** are located in the electronic supplementary data (ESI), Figures S1 to S73. The spectroscopic data for **14-21** were consistent with those in the literature.

*2.1.1. Compound **1***

Compound **1** was obtained as a colourless crystalline solid. Positive-ion HRESIMS showed a strong protonated molecular ion at *m/z* 221.0631 (calc. 221.0636), corresponding to the molecular formula $C_{12}H_{12}O_2S$. The ^{13}C NMR and DEPT spectra confirm the presence of twelve carbon signals, of which there are 3 × CH_3, 3 × CH and 6 × C_q (including one carbonyl). The ^{1}H and ^{13}C NMR assignments (Table 1) are based on the COSY, HSQC and HMBC spectra. The IR spectrum showed a strong peak at 1704 cm^{-1}, consistent with a lactone carbonyl

stretch. The spectra suggested a coumarin core for the structure (Waigh et al., 1991, Günther et al., 1975). The ^{1}H NMR signals at δ7.09 (d, *J* = 7.5 Hz) (H-6), δ 7.31 (t, *J* = 7.5 Hz) (H-7) and δ7.18 (d, *J* = 7.5 Hz) (H-8) were appropriately linked by COSY cross-peaks and comprised three adjacent protons on the carbocyclic ring. HSQC linked these signals to the ^{13}C signals at δ128.85 (C-6), δ129.88 (C-7) and δ115.48 (C-8), respectively. The chemical shift of the signal of C-8 identified this as being *ortho* to oxygen, thus the coumarin is substituted at C-3, C-4 and C-5. The ^{1}H signals for these substituents were consistent with $ArCH_3$ / $ArSCH_3$. One of the $ArCH_3$ signals (δ2.46) showed three-bond HMBC connectivity with the C-2 carbonyl (δ160.53) and is thus assigned the methyl group attached at C-3; HSQC then identified the corresponding methyl carbon at δ17.41. The other $ArCH_3$ (δ_H 2.89 / δ_C 24.98) was shown to be located at C-5 by a two-bond HMBC cross-peak from the CH_3 protons to C-5 (δ136.87) and three-bond cross-peaks to C-6 (δ128.85) and to C-4a (δ 120.18). The methylthio group (δ_H 2.36 / δ_C 20.21) was also located by HMBC, with its sole cross-peak to C-4 (δ151.66). The latter had been identified by its downfield chemical shift as the β-carbon of an enone. Furthermore, the structure of **1** was unambiguously confirmed by single crystal X-ray analysis. Thus **1** is firmly identified as 3,5-dimethyl-4-methylthiocoumarin and is named cluteolin A.

*2.1.2. Compound **2***

Compound **2** was a white powder. The molecular formula of $C_{13}H_{14}O_3S$ was established by observation of a protonated molecular ion at *m/z* 251.0737 for $[M + H]^+$ (calc. 251.0742). Thirteen discrete signals were seen in the ^{13}C NMR spectrum, comprising 4 × CH_3, 2 × CH and 7 × C_q. COSY, HSQC and HMBC spectra allowed assignment of all the NMR signals. The core heterocycle was a coumarin. The ^{1}H chemical shifts of the methyl protons (Table 1) indicated that one group was a methoxy (δ3.83) and that three were $ArCH_3$ or $ArSCH_3$ (δ2.35, δ2.43, δ2.86). An HMBC cross-peak from the methoxy protons to C-7 (δ160.47) confirmed the location of this group. The methylthio group (δ2.35) also only gave one HMBC correlation, which was to C-4 (δ151.65). One $ArCH_3$ (δ2.43) was located at C-3 through a three-bond HMBC correlation to the carbonyl C-2 (δ160.87). The other (δ2.86) was located at C-5 and gave HMBC cross-peaks to C-4a (δ113.91), C-5 (δ138.42) and C-6 (δ116.91). The position of this $ArCH_3$ group was confirmed additionally by observation of HMBC cross-peak

between H-6 (δ6.68) and Ar*C*H$_3$ (δ25.25). Compound **2** is thus shown to be 3,5-dimethyl-7-methoxy-4-methylthiocoumarin and is named as cluteolin B.

*2.1.3. Compound **3***

Compound **3** was also obtained as white powder. The positive-ion HRESIMS showed a sodium adduct ion at *m/z* 289.0500 for $[M + Na]^+$ (calc. 289.0511) and a protonated molecular ion *m/z* 267.0681 $[M + H]^+$ (calc. 267.0691), corresponding to the molecular formula $C_{13}H_{14}O_4S$. At higher mass, *m/z* 555.1112 corresponded to $[2 M + Na]^+$ (calc. 555.1123) and *m/z* 533.1294 corresponded to $[2 M + H]^+$ (calc. 533.1304). The ^{13}C NMR spectrum showed discrete signals for thirteen carbons, which were shown by DEPT to consist of 4 × CH_3, 2 × CH and 7 × C_q. Most of the ^{1}H and ^{13}C signals were assigned readily from HSQC and HMBC spectra and the core was established as a coumarin, consistent with a lactone carbonyl stretching band at 1719 cm^{-1} in the IR spectrum. The ^{1}H NMR spectrum (Table 1) showed only two ArH as singlets at δ5.88 (H-3) and at δ6.90 (H-6). The signal for H-3 was identified on the basis of its chemical shift and a two-bond HMBC cross-peak to the carbonyl C-2 (δ157.45). The other singlet was assigned as H-6 through three-bond HMBC correlations with the CH_3 at C-5 (δ24.77), with C-4a (δ111.62) and with C-8 (δ133.80). Interestingly, a weak four-bond correlation was observed to C-4 (δ159.79). The S-Me was shown to be attached to C-4 by an HMBC cross-peak from the 1-H signal at δ2.50 to C-4. Four-bond HMBC correlation was also seen to C-3 (δ103.70). One of the methoxy groups (δ_H 3.83, δ_C 56.16) is located at C-7, as shown by an HMBC cross-peak to C-7 (δ153.71). Similarly, the other methoxy group (δ_H 3.69, δ_C 60.62) was confirmed at C-8 (δ133.80) by an appropriate HMBC cross-peak. Therefore, **3** is 7,8-dimethoxy-5-methyl-4-methylthiocoumarin, which we name cluteolin C.

*2.1.4. Compound **4***

Compound **4** formed an amorphous white powder. The HRESIMS showed a protonated molecular ion at *m/z* 223.0421, being $[M + H]^+$ (calc. 223.0429) for the molecular formula $C_{11}H_{10}O_3S$. Correspondingly, eleven discrete signals were observed in the ^{13}C NMR spectrum. There were 2 × CH_3, 3 × CH and 6 × C_q. As for **1-3**, the core heterocycle was a coumarin. Signals for three ArH were evident in the ^{1}H NMR spectrum (Table 1). The singlet at δ6.09 was assigned readily to H-3 on the basis of its chemical shift. HSQC then identified

the C-3 signal at δ106.49. The other two ArH were doublets, δ6.97 (J = 8.3 Hz) and δ7.00 (J = 8.2 Hz), which are adjacent on the carbocyclic ring. A three-bond HMBC correlation from the δ6.97 signal to the Ar*C*H$_3$ (δ24.16) showed that these were also adjacent on the carbocyclic ring. The signal at δ6.97 also showed a weak four-bond HMBC cross-peak with C-4 (δ159.91), confirming that it corresponds to H-6. Thus, the signal at δ7.00 is for H-7 and locates the ArCH$_3$ at C-5. The S-Me protons (δ2.58) gave an HMBC cross-peak with C-4 (δ159.91). The broad NMR signal for the phenolic OH was seen downfield at δ9.97. Therefore, **4** is 8-hydroxy-5-methyl-4-methylthiocoumarin and is named cluteolin D.

*2.1.4. Compound **5***

Compound **5** was again a white powder. In the positive-ion HRESIMS, a sodium adduct ion was observed at *m/z* 289.0498 ([M + Na]$^+$, calc. 289.0517) and a protonated molecular ion was seen at *m/z* 267.0679 ([M + H]$^+$, calc. 267.0697), showing the molecular formula to be $C_{13}H_{14}O_4S$. The negative-ion spectrum showed the ion [M - H]$^-$ at *m/z* 265.0536 (calc. 265.0541). The formula was supported by observation of thirteen discrete resonances in the ^{13}C NMR spectrum: 4 × CH$_3$, 1 × CH and 8 × C$_q$. The 1 D and 2D NMR data confirmed that the core was a coumarin. The IR spectrum contained absorption bands for hydroxy (3352 cm^{-1}) and lactone carbonyl (1703 cm^{-1}), the former pointing towards the presence of a phenolic OH. The methylthio group gave NMR signals at δ_H 2.36 and δ_C 19.78 (Table 2). There was an HMBC correlation from these protons to C-4 (δ151.35) only. There were two ArCH$_3$ groups, one of which (δ_H 2.29, δ_C 16.56) was assigned as attached to C-3 on the basis of HMBC correlation with C-4 and with the carbonyl carbon C-2 (δ159.41). The other ArCH$_3$ (δ_H 2.72, δ_C 24.34) was located at C-5 (δ130.83), using the two-bond HMBC cross-peak from the methyl signal to C-5 and the three-bond cross-peaks to C-4a (δ112.41) and C-6 (δ117.17). Weak four-bond HMBC cross-peaks were also seen to C-4 and to C-7 (δ146.58). An HMBC cross-peak from the sole ArH (δ_H 6.69) to the ^{13}C signal at δ130.83, identifying the latter as being due to C-8. C-8 is the point of attachment of the methoxy group, with an HMBC cross-peak from the OCH$_3$ signal at δ3.80. These data and inferences confirm that **5** is 3,5-dimethyl-7-hydroxy-8-methoxy-4-methylthiocoumarin, which is named as cluteolin E.

*2.1.6. Compound **6***

Compound **6** was isolated as white crystals. Sodium adduct and protonated molecular ions were evident in the HRESIMS in positive-ion mode at *m/z* 275.0340 ([M +

Na]$^+$, calc. 275.0354) and at *m/z* 253.0522 ([M + H]$^+$, calc. 253.0535) confirming the molecular formula as $C_{12}H_{12}O_4S$. Ions were also seen at *m/z* 527.0793 ([2 M + Na]$^+$, calc. 527.0811) and at *m/z* 505.0974 ([2 M + H]$^+$, calc. 505.0991). The IR spectrum showed a carbonyl absorption at 1729 cm^{-1}.

The core of the molecule was shown by combined use of spectroscopic techniques to be coumarin. The ^{1}H NMR signal (Table 2) for H-3 was identified by two-bond HMBC correlation with the carbonyl carbon C-2 (δ158.76) and C-4 (δ166.57). C-4 showed a strong HMBC cross-peak with the methyl signal at δ2.80. Thus the methylsulfinyl group is attached at C-4. The downfield chemical shift of the C-4 signal is consistent with being attached to an electron-withdrawing sulfoxide. Turning to the carbocyclic ring, an ArH signal was seen at δ 6.99 as a doublet (*J* = 2.9 Hz). This is *meta*-coupled to the other ArH at δ6.87 (m). The complex multiplicity of the latter signal is due to long-range coupling to the $ArCH_3$, which shows it to be due to H-6. Thus the doublet signal is due to H-8. The $ArCH_3$ is attached at C-5, with HMBC cross-peaks to C-5 (δ137.24), to C-4a (δ108.35) and to C-6 (δ115.98). The methoxy group gave resonances at δ_H 3.86 and δ_C 56.04 and was linked to C-7 (δ162.14), as shown by HMBC. Taken together, these data show that **6** is 7-methoxy-5-methyl-4-methylsulfinylcoumarin, named as cluteolin F.

*2.1.7. Compound **7***

Compound **7** was isolated as white solid. The positive-ion HRESIMS indicated that its molecular formula was $C_{13}H_{14}O_5S$, with a sodium adduct ion at *m/z* 305.0448 ([M + Na]$^+$, calc. 305.0460) and a protonated molecular ion at *m/z* 283.0629 ([M + H]$^+$, calc. 283.0640). Higher-mass ions were seen at *m/z* 587.1010 ([2 M + Na]$^+$, calc. 587.1022) and *m/z* 565.1190 ([2 M + H]$^+$, calc. 565.1202). Correspondingly, the ^{13}C NMR spectrum contained thirteen discrete resonances, comprising 4 × CH_3, 2 × CH and 7 ×C_q. Combined interpretation of the 2D NMR spectra again showed the core to be a coumarin. Two singlets were observed in the ^{1}H NMR spectrum in the ArH region. The singlet at δ7.22 (Table 2) showed HMBC cross-peaks only to the carbonyl C-2 (δ159.31), C-4 (δ165.62) and C-4a (δ108.57), confirming that it is due to H-3. The methylsulfinyl unit gave resonances at δ_H 2.77 / δ_C 43.59 and it was demonstrated to be attached to C-4 through an HMBC cross-peak. The downfield chemical shift of C-4 allowed its clear identification. The $ArCH_3$ (δ_H 2.47, δ_C 15.88) had HMBC cross-peaks to the previously identified C-4a, to C-5 (δ127.83) and to C-6 (δ145.15). The

downfield chemical shift of C-6 suggested that it carried an oxygen function, which was confirmed as a methoxy group (δ_H 3.80, δ_C 60.86) by HMBC. The remaining ArH was identified as H-8 by HMBC cross-peaks to C-4a, C-6, C-7 (δ156.59) and C-8a (δ152.19). Finally, the remaining methoxy group (δ_H 3.95, δ_C 56.37) was located at C-7 by HMBC. Thus, **7** is 6,7-dimethoxy-5-methyl-4-methylsulfinylcoumarin, named as cluteolin G.

*2.1.8. Compound **8***

Compound **8** was a colourless crystalline solid. In HRESIMS in positive-ion mode, a protonated molecular ion at *m/z* 205.0859 was evident, corresponding to $[M + H]^+$ (calc. 205.0847) for the molecular formula $C_{12}H_{12}O_3$. The ^{13}C NMR spectrum contained signals for twelve discrete carbons, of which there were 3 × CH_3, 3 × CH and 6 × C_q. The core structure was a coumarin. The IR spectrum features a carbonyl lactone at 1696 cm^{-1}. The ^1H NMR spectrum contained signals for three ArH, comprising a singlet at δ7.62 and a singlet (2 H) at δ6.66 (Table 2). The former was assigned as H-4 on the basis of its chemical shift, with C-4 resonating at δ136.65. One $ArCH_3$ (δ_H 2.20, δ_C 17.38) was identified as being attached at C-3 through HMBC correlations to C-4 and the carbonyl C-2 (δ162.82). The other $ArCH_3$ (δ_H 2.45, δ_C 18.48) was located at C-5 through HMBC cross-peaks with C-6 (δ113.86) and with C-4a (δ 112.28). H-6 also showed an HMBC link to C-8 (δ98.42). H-8 (δ6.66) correspondingly showed an HMBC interaction with C-6. The methoxy group (δ_H 3.83, δ_C 55.55) was attached at C-7 (δ161.45). Single crystal X-ray analysis confirmed the structure of **8** unambiguously. Taken together, these interpretations confirm that **8** is 3,5-dimethyl-7-methoxycoumarin, named cluteolin H.

*2.1.9. Compound **9***

Compound **9** comprised colourless crystals. The positive-ion HRESIMS showed the protonated molecular ion for $[M+H]^+$ at *m/z* 221.0809 (calc. 221.0814), corresponding to the molecular formula $C_{12}H_{12}O_4$. Correspondingly, the ^{13}C NMR spectrum showed twelve discrete resonances: 3 × CH_3, 2 × CH and 7 × C_q. As expected, the core heterocycle was a coumarin, with the IR band for the lactone carbonyl at 1698 cm^{-1}. One $ArCH_3$ (δ_H 2.20, δ_C 17.44) (Table 3) was shown to be located at C-3 by HMBC cross-peaks to the carbonyl C-2 (δ161.83) and to C-4 (δ137.34). HSQC linked C-4 to H-4 (δ7.61). On the carbocyclic ring, C-5 was identified through HMBC correlation with H-4. C-5 showed a two-bond HMBC cross-peak with the remaining $ArCH_3$ (δ_H 2.41, δ_C 18.33). H-6 was characterised by two-bond HMBC

correlations with C-5 and C-7 (δ146.93) and three-bond HMBC correlations with C-4a (δ 112.58) and C-8 (δ131.76). Clearly, both C-7 and C-8 carry an oxygen function. These were differentiated by the phenolic OH resonating as a sharp singlet at δ6.03, which exhibited a two-bond HMBC cross-peak to C-7 (δ146.93) and three-bond HMBC cross-peaks to C-6 and C-8; therefore C-7 carries the hydroxy group. Finally, an HMBC correlation from the methoxy signal at δ_H 4.06 to C-8 confirmed its location at C-8. Single crystal X-ray analysis confirmed the structure of **9** unambiguously. Thus, **9** is 3,5-dimethyl-7-hydroxy-8-methoxycoumarion, named cluteolin I.

*2.1.10. Compound **10***

Compound **10** was a colourless powdery material. The molecular formula of $C_{13}H_{14}O_5$ was confirmed by a sodium adduct ion in the positive-ion HRESIMS at *m/z* 273.0726 ([M + Na]$^+$, calc. 273.0739) and a protonated molecular ion at *m/z* 251.0907 ([M + H]$^+$, calc. 251.0920). The ^{13}C NMR spectrum duly showed thirteen discrete signals: 4 $\times$ CH_3, 1 $\times$ CH and 8 $\times$ C_q. Together, these spectra showed a coumarin core. The IR spectrum supported this with a lactone carbonyl band at 1694 cm^{-1}. Also evident was the O–H stretch of an alcohol at 3355 cm^{-1}. Considering first the heterocyclic ring, the ArCH_3 signal at δ2.00 (Table 3) showed three-bond HMBC correlations to the carbonyl C-2 (δ163.38) and to C-4 (δ166.59); a two-bond HMBC cross-peak linked this methyl group to C-3 (δ109.87). Attached at the C-4 was a methoxy group (δ_H 3.80, δ_C 60.70), as demonstrated by HMBC. As for other compounds in this series an ArCH_3 was located at C-5, as demonstrated by a two-bond HMBC correlation from the methyl protons (δ2.49) to C-5 (δ119.48). The relatively upfield chemical shift of this carbon and the relatively downfield shift of C-4 in **10** are a consequence of the electronic properties of the methoxy group at C-4. Further HMBC correlations from the methyl at C-5 to C-4a (δ108.84) and C-6 (δ141.38) helped in characterising these resonances. The chemical shift of C-6 suggested attachment to an oxygen function but this was demonstrated to be hydroxy, rather than methoxy, by the absence of an HMBC cross-peak to the remaining methoxy group signal (δ_H 3.88). However, these methoxy protons were correlated by HMBC to the signal at δ_C 150.49, which was assigned as C-7. The sole ArH (δ6.93) showed two-bond HMBC correlations with C-7 and C-8a (δ147.54) and three-bond cross-peaks with C-6 and C-4a. The chemical shift of C-8 (δ97.64) was consistent with its location *ortho* to two

oxygen functions. Compound **10** is therefore demonstrated to be 4,7-dimethoxy-3,5-dimethyl-6-hydroxycoumarin, named as cluteolin J.

*2.1.11. Compound **11***

Compound **11** formed white crystals. In the HRESIMS in positive-ion mode, a protonated molecular ion at *m/z* 235.0962 ($[M+H]^+$ at *m/z* calc. 235.0970) corresponded to the molecular formula $C_{13}H_{14}O_4$. Ions corresponding to clusters of **11** with sodium were seen at *m/z* 491.1671 ($[2\ M+ Na]^+$ calc. 491.1682) and *m/z* 959.3453 ($[4\ M + Na]^+$, calc. 959.3466). Correspondingly, signals for thirteen carbons were seen in the ^{13}C NMR spectrum: 4 × CH_3, 2 × CH and 7 × C_q. The core of the molecule was a coumarin, with a carbonyl stretching band in the IR spectrum at 1711 cm^{-1}. An $ArCH_3$ group was evident in the NMR spectra at δ_H 2.09, δ_C16.69 (Table 3). HMBC cross-peaks from these protons were observed to carbonyl C-2 (δ 161.17), to C-3 (δ 120.66) and to C-4 (δ 137.57), locating this methyl at C-3. HSQC from C-4 and HMBC from this $ArCH_3$ identified H-4 (δ 7.93). From here, signals corresponding to the carbocyclic ring could be assigned. HMBC interactions from H-4 identified C-4a (δ 112.35), H-8a (δ 146.96) and C-5 (δ 130.80). C-5 carries a methyl group (δ_H 2.45, δ_C 18.03), as demonstrated by HMBC. Further HMBC from this methyl allowed assignment of C-6 (δ 110.50) and thus its attendant H-6 (δ 6.95). The latter chemical shifts indicate the presence of an *ortho* oxygen function. HMBC cross-peaks from H-6 confirmed C-4a and C-5 and identified C-7 (δ 153.58) and C-8 (δ 133.17), the latter two signals having been differentiated on the basis of comparison of their chemical shifts with those in the close analogue **3**. One methoxy group (δ_H 3.87, δ_C 56.21) was shown by HMBC to be attached to C-7, whereas the other (δ_H 3.75, δ_C 60.73) was attached at C-8. Single crystal X-ray analysis confirmed the structure of **11** unambiguously. Compound **11** is thus shown to be 7,8-dimethoxy-3,5-dimethylcoumarin, named cluteolin K.

*2.1.12. Compound **12***

Compound **12** was obtained as colourless crystals. The positive-ion HRESIMS showed a sodium adduct ion at *m/z* 243.0624 ($[M + Na]^+$, calc. 243.0633) and a peak for $[2\ M + Na]^+$ at *m/z* 463.1357 (calc. 463.1369), indicating the molecular formula $C_{12}H_{12}O_4$. Thus **12** is an isomer of **9**. However, the base peak in the mass spectrum was at *m/z* 203.0699 [$M + H - H_2O]^+$, calc. 203.0708), strongly suggesting the presence of $ArCH_2OH$. The core structure was

a coumarin. The ^{13}C NMR spectrum contained twelve discrete signals: 2 × CH_3, 1 × CH_2, 3 × CH and 6 × C_q. The IR spectrum showed a band at 3435 cm^{-1} for the hydroxy group. The lactone carbonyl stretching frequency was 1668 cm^{-1}, suggesting intramolecular H-bonding. The methylene protons resonate at δ 4.68 (Table 4). This signal has HMBC cross-peaks to carbon signals at δ 114.50 (C-3), at δ 164.39 and at δ 168.18. An HMBC cross-peak between the latter and the methoxy resonance at δ 4.06 distinguished these two carbon resonances and confirmed that the methoxy group was attached at C-4 (δ 168.18). The COSY spectrum indicated three adjacent protons on the carbocyclic ring. The $ArCH_3$ (δ_H 2.70, δ_C 22.61) was shown to be located at C-5 (δ 137.09) through a two-bond HMBC correlation. Three-bond correlations were observed to the C_q at δ 116.24 (C-4a) and to C-6 (δ 128.31). A weak four-bond HMBC cross-peak linked the $ArCH_3$ to C-4. HSQC from C-6 identified H-6 (δ 7.08). From here, strong three-bond HMBC cross-peaks were seen to C-4a and to C-8 (δ 115.40), whereas the two-bond HMBC cross-peaks to C-5 and to C-7 (δ 131.09) were weaker. The H-7 signal was identified as the triplet at δ 7.40 and H-8 resonated as a doublet at δ 7.21. Together, these data show that **12** is 3-hydroxymethyl-5-methylcoumarin. This structure is capable of forming an intramolecularly H-bonded six-membered ring. Single crystal X-ray analysis confirmed the structure of **12** unambiguously. This compound is named cluteolin L.

*2.1.13. Compound **13***

Compound **13** was obtained as a white solid. Positive-ion HRESIMS showed that the molecular formula was $C_{13}H_{14}O_5$, with a sodium adduct ion at *m/z* 273.0730 ($[M + Na]^+$, calc. 273.0734) and a protonated molecular ion at *m/z* 251.0911 (low abundance, $[M + H]^+$, calc. 251.0920). A cluster ion $[2 M + Na]^+$ was observed at *m/z* 523.1571 (calc. 523.1580) and the base peak corresponded to $[M + H - H_2O]^+$ (*m/z* 233.0805). The IR spectrum of **13** contained bands for OH (3410 cm^{-1}) and for lactone carbonyl (1707 cm^{-1}). This pattern was very similar to that for **12**, indicating that they were likely to be analogues. As in **1-12**, a methyl (δ_H 2.63, δ_C 22.78) was present at C-5, as demonstrated by HMBC cross-peaks to C-4a (δ 109.68), C-5 (δ 138.53) and C-6 (δ 116.48). HSQC then identified H-6 (δ 6.66) through a cross-peak to C-6. A two-bond HMBC cross-peak from H-6 revealed C-7 (δ 162.13), which also correlated with a methoxy group (δ_H 3.85). A three-bond HMBC cross-peak from H-6 identified C-8 (δ 98.94), with its attendant H-8 (δ 6.70). Taken together, these data demonstrate that **13** is 3-hydroxymethyl-4,7-dimethoxy-5-methylcoumarin, named cluteolin M.

*2.1.14. Compounds **14-21***

A further eight coumarins (**14-21**) were also separated and purified from the dichloromethane extract of *C. lanceolata*. The spectroscopic and other physical data for these compounds were consistent with those reported previously for samples which had been isolated from other plants or had been prepared synthetically. Furthermore, single crystal X-ray analysis confirmed the structures of **14-17, 19,20**. Compounds **14-18** were isolated from *C. abyssinica* (Waigh et al., 1991), while **15** has also been synthesised unequivocally (Bohlmann & Wienhold, 1979), leading to structural revision of earlier claimed natural products. Faiella et al. (2014) identified **19,20** (7-demethyl-8-methoxycoumarsabin and 8-methoxycoumarsabin, respectively) from *Sideritis pullulans*. The latter had also been identified as a constituent of *Leucas inflata* roots (Al Yousuf et al., 1999) and of *Juniperus sabina* (De Pascual et al., 1981); it has also been synthesised (Ahluwalia & Mukherjee, 1984). 8-Methoxypereflorin **21** has been isolated from *Perezia alamani* var. *oolepsis* (Joseph-Nathan et al., 1982) and from Mutisieae (Bohlmann & Zdero, 1977) and has been synthesised (Bohlmann &Wienhold, 1979).

2.2. Determination of the ability of the compounds to enhance release of insulin

Diabetes mellitus is considered as a metabolic disorder characterised by sustained increase in the concentration of glucose in blood. It is a chronic disease that occurs either by the deficiency in the glucose-triggered secretion of insulin by β-cells in the islets of the pancreas (Type 1 diabetes) or by lack of response of cells in the liver and of adipocytes to insulin (Type 2 diabetes). Type-2 diabetes is a 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. There can also be some loss of glucose-stimulated release of insulin by the β-cells of the islets of Langerhans. Clinically used drugs for the treatment of Type 2 diabetes either enhance the production of insulin by the β-cells (sulfonylureas, *e.g.* tolbutamide, glibenclamide, glimepiride) or render the peripheral cells more sensitive to insulin, thus stimulating uptake of glucose (*e.g.* metformin). Inhibitors of the tankyrases also increase insulin-stimulated uptake of glucose by adipocytes (Nathubhai et al. 2017). Type-1 diabetes also involves loss of glucose-stimulated release of insulin by the β-cells.

C. lanceolata is a traditional anti-diabetic medicinal plant; therefore, we have evaluated the anti-diabetic potential of compounds **1-4,8-9,12,14-17,19,20** using an assay that evaluates their effect on the glucose-triggered insulin-secretory activity of freshly isolated murine islets (Fig. 3). At 16.7 mM glucose, the insulin-secretory activity of control islets was 18.8 ± 3.0 ng $islet^{-1}$ h^{-1}. A similar value has been reported earlier (Hameed et al., 2018). Among the compounds tested at a single concentration (200 μM), **1,9,12,14,19,20** did not show any effect on the secretion of insulin. Some activity (broadly equivalent to that of the positive control glimepiride) was displayed by **2,8,15,17**. Strikingly, **3,4,16** showed potent enhancement of the glucose-triggered secretion of insulin by the isolated islets, showing 3.1-fold, 4.7-fold and 5.5-fold enhancement, respectively, with glimepiride only showing 2.1-fold enhancement. The strong stimulation of glucose-triggered release of insulin helps to rationalise the traditional use of the plant *C. lanceolata*. Thus this plant may be considered as a valuable herbal remedy for diabetes.

There is no clear *prima facie* structure-activity relationship distinguishing potent stimulators from weaker stimulators and their biochemical target is, as yet, unknown but these 5-methylcoumarins represent interesting new lead structures for further development of drugs to treat type-2 diabetes. Further studies will be needed to determine the mechanism(s) of their activity.

3. Conclusion

This phytochemical study into dichloromethane extract of the aerial parts of the plant *Clutia lanceolata* has yielded thirteen previously unreported 5-methylcoumarins, including sulphides and sulfoxides, namely cluteolin A to M (**1**-**13**), along with eight known coumarins (**14**-**21**). Our initial investigation demonstrates the anti-diabetic potential of the extracts and that the activity of stimulation of glucose-triggered secretion of insulin by murine pancreatic islets is evident for compounds **2**-**4**,**8**,**15**-**17**. In particular, **3**,**4**,**16** show very strong activity and warrant further study and development.

4. Experimental

4.1. Plant material

The plant material was procured from Shafat village, Baha, Kingdom of Saudi Arabia (KSA), in February 2015 in the wet season and was formally identified by Dr M. Yousuf

(taxonomist). A voucher specimen (16069) was logged in the Herbarium of the College of Pharmacy, King Saud University, Riyadh, KSA.

4.2. Extraction and isolation

Drying of the aerial portions of *Clutia lanceolata* Forssk, (Peraceae) was achieved in the shade. Once dried, the plant material was ground into a powder. Extractions with solvents were conducted at room temperature on the ground material (1.30 kg), using CH_2Cl_2 (3 × 5 L), followed by MeOH (3 × 5 L). The solvents were evaporated from the extracts at 40°C under reduced pressure using a Büchi rotary evaporator (model R-215). This yielded the CH_2Cl_2 extract (47.5 g) and the MeOH extract (41.8 g). The MeOH extract was suspended in distilled water and partitioned in sequence with EtOAc (3 × 1 L) and butan-1-ol (pre-saturated with water) (3 × 1 L), which provided an EtOAc fraction (8.9 g), a butan-1-ol fraction (21.4 g) and some material remaining in the water fraction (9.0 g). The CH_2Cl_2 extract (40.0 g) subjected to column chromatography over silica gel and eluted with gradient of hexane / EtOAc (1:0 → 1:3); the column was then flushed with MeOH. The fractions obtained were analysed by using TLC and the similar fractions were combined. This afforded forty-one fractions (Fr. 1 – Fr. 41). Further work on these fractions led to the isolation of thirteen previously unreported compounds, in addition to eight known compounds. Scheme 1 (Supplementary Information) depicts the detailed isolation scheme.

4.3. Isolation of islets and insulin secretion assay

Islets of Langerhans were isolated from the pancreata of male BALB/c mice from the animal house of the International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan. The animals were anaesthetised with sodium thiopental and, after surgical opening of the abdomen; the pancreas was distended by injection of 1.0 mg mL^{-1} collagenase solution (Sigma, St. Louis, MO, USA) into the bile duct. After careful removal, the distended pancreas was subjected to digestion with collagenase at 37°C for 15 min. The digested islets were further purified by centrifugation at 1000 rpm for 1 min, followed by filtration using a pre-wetted 70 µm cell strainer.

Insulin secretion studies were performed in static incubations. Islets were hand-picked into Eppendorf tubes and were pre-incubated in Krebs-Ringer bicarbonate (KRB) buffer solution containing bovine serum albumin (0.1%) and glucose (3.0 mM). Thereafter, batches of three size-matched islets were incubated for 60 min in KRB buffer with glucose

(16.7 mM), in the presence of individual test compounds. Chilling of the tubes on ice terminated the incubation. The supernatant solution was removed and stored at -40 °C until it was assayed for insulin. The secreted insulin was measured using an Ultra-Sensitive Mouse Insulin ELISA kit (Crystal Chem Inc., IL, USA) and was normalised for the number of islets. Test compounds were examined along with a reference (positive control) compound glimepiride (Sigma) and a vehicle control (negative control).

4.4. General experimental procedures

The NMR spectra were obtained using a Bruker Avance spectrometer operating at 700 MHz for ^{1}H NMR and 175 MHz for ^{13}C NMR. Conventional pulse sequences were used for COSY, HSQC and HMBC spectra. Chemical shift values stated in δ(ppm), using internal standard $SiMe_4$ or the residual solvent peak for calibration; coupling constants (*J*) are given in Hz. Ultra-high-accuracy mass analysis was performed on a Nano-Flow (Triversa Nanomate; Advion Biosciences Limited, Norfolk, UK) linear-trap quadrupole Fourier transformation ion cyclotron resonance mass spectrometry (FT-ICR-MS) Ultra device. The melting points were measured on a Büchi B-545 Melting Point apparatus. IR spectra recorded on a Bruker spectrometer. UV spectra were measured on UV.1601 PC (Shimadzu).

Column chromatography (CC) employed silica gel 60 (230-400 μm; Merck, Darmstadt) and LiChroprep* RP-18 (40–63 μm, Merck) Centrifugal Thin-Layer Chromatography (CTLC) was conducted on a Chromatotron (Ser. No.30 G, Harrison Research, USA). UV_{254} fluorescence indicator (Merck) was used for TLC plate analyses. Compounds were visualised under UV radiation in CAMAG UV Cabinet dual wavelength (254/366 nm) in addition to spraying with 4-methoxybenzaldehyde reagent followed by gentle warming with a heat gun. Semi-preparative HPLC was performed using Shimadzu system (Kyoto, Japan). This consisted of two columns: Shim-pack PREP-ODS (H) Kit (A) 250 mm × 4.6 mm i.d., 5 μm particles (B) 250 mm × 20 mm i.d; two LC-6AD solvent delivery pumps coupled with a Rheodyne manual injector; communications bus module CBM-20A; a multi-wavelength photodiode array detector (SPD-M20A) and an FRC-10A fraction collector. All connected to a computer system with Intel CoreDUO with Microsoft Windows XP and Shimadzu's LC solution software. Analytical HPLC was performed using the column under gradient conditions with the mobile phase ($MeCN/H_2O$) programmed linearly at 1.0 mL min^{-1}. The UV detection wavelengths were 210 and 254 nm. The chromatographic separation HPLC was

performed using column and preparative HPLC conditions as for analytical HPLC, except that the flow rate was 20 mL min^{-1}.

X-Ray crystal data were acquired upon a Rigaku 007HF diffractometer, equipped with: Varimax confocal mirrors; an AFC11 goniometer; a HyPix 6000 detector and an Oxford Cryosystems, low-temperature device, operating at T = 100(2) K. Crystals were mounted on a MITIGEN holder, with perfluoroether oil. The crystal structures were subsequently solved using the structure solution program Olex2 (Dolomanov et al., 2009), in conjunction with the ShelXT (Sheldrick, 2015a), using the Intrinsic Phasing solution method. The model was refined with version 2014/7 of ShelXL (Sheldrick, 2015b) using Least Squares minimisation.

*4.5. Summary of analytical data for compounds **1-13***

Cluteolin A (1): $C_{12}H_{14}O_2S$; mp 90.9°C; UV ($CHCl_3$) λ_{max} (log ε): 302.8 (4.10); IR ($CHCl_3$) ν_{max} 2922, 1704, 1600, 1455 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 1.

Cluteolin B (2): $C_{13}H_{14}O_3S$; mp 93.8°C; UV ($CHCl_3$) λ_{max} (log ε): 331.2 (3.84); IR ($CHCl_3$) ν_{max} 2920, 2851, 1710, 1610, 1538, 1450 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 1.

Cluteolin C (3): $C_{13}H_{14}O_4S$; mp 179.6°C; UV ($CHCl_3$) λ_{max} (log ε): 324.0 (4.27), 287.8 (4.26), 258.6 (4.23); IR ($CHCl_3$) ν_{max} 2919, 1719, 1599, 1539 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 1.

Cluteolin D (4): $C_{11}H_{10}O_3S$; mp 256.8°C; UV ($CHCl_3$) λ_{max} (log ε): 308.0 (4.13), 283.8 (4.16), 258.4 (4.20); IR ($CHCl_3$) ν_{max} 3368, 2918, 1703, 1542, 1418 cm^{1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 1.

Cluteolin E (5)*.* $C_{13}H_{14}O_4S$; mp 206.5°C; UV ($CHCl_3$) λ_{max} (log ε): 329.00 (4.01), 264.20 (3.65), 239.20 (3.75) ; IR ($CHCl_3$) ν_{max} 3352, 2924, 1703, 1593, 1448 cm^{1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 2.

Cluteolin F (6): $C_{12}H_{12}O_4S$; mp 150.1°C; UV ($CHCl_3$) λ_{max} (log ε): 333.40 (3.81), 261.00 (3.32), 239.40 (3.54) ; IR ($CHCl_3$) ν_{max} 2925, 2353, 1729, 1604, 1454 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 2.

Cluteolin G (7): $C_{13}H_{14}O_5S$; mp 215.4°C; UV ($CHCl_3$) λ_{max} (log ε): 343.2 (3.77), 283.8 (4.16), 239.2 (3.79); IR ($CHCl_3$) ν_{max} 2918, 2850, 1711, 1594, 1464 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 2.

Cluteolin H (8): $C_{12}H_{12}O_3$; mp 124.6°C; UV ($CHCl_3$) λ_{max} (log ε): 326.8 (3.83); IR ($CHCl_3$) ν_{max} 2918, 1696, 1608, 1450 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 2.

Cluteolin I (9): $C_{12}H_{12}O_4$; mp 152.2°C; UV ($CHCl_3$) λ_{max} (log ε): 324.4 (4.10); IR ($CHCl_3$) ν_{max} 3347, 1698, 1600, 1503, 1459 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 3.

Cluteolin J (10): $C_{13}H_{14}O_5$; mp 162.8°C; UV ($CHCl_3$) λ_{max} (log ε): 336.4 (4.01), 298.8 (3.98), 291.8 (3.99), 239.8 (3.98); IR ($CHCl_3$) ν_{max} 3355, 2928, 1694, 1611, 1451 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 3.

Cluteolin K (11)*:* $C_{13}H_{14}O_4$; mp 123.2°C; UV ($CHCl_3$) λ_{max} (log ε): 323.6 (3.70), 261.0 (3.36), 239.2 (3.75); IR ($CHCl_3$) ν_{max} 2953, 1711, 1593, 1505, 1450 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 3.

Cluteolin L (12): $C_{12}H_{12}O_4$; mp 166.4°C; UV ($CHCl_3$) λ_{max} (log ε): 286.8 (4.01); IR ($CHCl_3$) ν_{max} 3435, 2918, 2850, 1668, 1591, 1559, 1457 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 4.

Cluteolin M (13)**:** $C_{13}H_{14}O_5$; mp 114.8°C; UV ($CHCl_3$) λ_{max} (log ε): 323.4 (3.97); IR ($CHCl_3$) ν_{max} 3410, 2851, 1707, 1598, 1456 cm^{-1}; for HRESIMS see text; for ^1H and ^{13}C NMR, see Table 4.

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Table 1. NMR data for **1-4** (700 MHz (^{1}H), 175MHz (^{13}C)).

Position	**1** ($CDCl_3$)		**2** ($CDCl_3$)		**3** ($(CD_3)_2SO$)		**4** ($(CD_3)_2SO$)	
	δ_H (*J* Hz)	**δ_C type**	**δ_H**	**δ_C type**	**δ_H**	**δ_C type**	**δ_H (*J* Hz)**	**δ_C type**
2		160.53 C_q		160.87 C_q		157.45 C_q		157.45 C_q

3		130.16 C_q		127.06 C_q	5.88 s	103.70 CH	6.09 s	106.49 CH
4		151.66 C_q		151.65 C_q		159.79 C_q		159.91 C_q
4a		120.18 C_q		113.91 C_q		111.62 C_q		117.90 C_q
5		136.87 C_q		138.42 C_q		132.23 C_q		125.49 C_q
6	7.09 d (7.5)	128.85 CH	6.68 s	116.91 CH	6.90 s	113.71 CH	6.97 d (8.3)	127.79 CH
7	7.31 t (7.5)	129.88 CH		160.47 C_q		153.71 C_q	7.00 d (8.2)	117.66 CH
8	7.18 d (7.5)	115.48 CH	6.68 s	99.03 CH		133.80 C_q		143.67 C_q
8a		152.22 C_q		154.13 C_q		147.49 C_q		142.11 C_q
R^3	2.46 s	17.41 CH_3	2.43 s	16.98 CH_3				
R^4	2.36 s	20.21 CH_3	2.35 s	20.13 CH_3	2.50 s	15.75 CH_3	2.58 s	15.81 CH_3
5-Me	2.89 s	24.98 CH_3	2.86 s	25.25 CH_3	2.70 s	24.77 CH_3	2.71 s	24.16 CH_3
R^6								
R^7			3.83 s	55.67 CH_3	3.83 s	56.16 CH_3		
R^8					3.69 s	60.62 CH_3	9.97 br	

Table 2. NMR data for **5-8** (700 MHz (^{1}H), 175MHz (^{13}C)).

Position	**5** ($(CD_3)_2SO$)		**6** ($(CD_3)_2SO$)		**7** ($CDCl_3$)		**8** ($CDCl_3$)	
	δ_H	**δ_C type**	**δ_H (*J* Hz)**	**δ_C type**	**δ_H**	**δ_C type**	**δ_H**	**δ_C type**

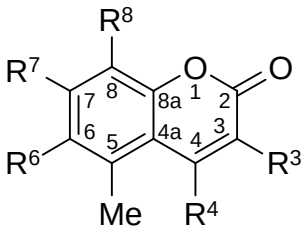
2		159.41 C_q		158.76 C_q		159.31 C_q		162.82 C_q
3		124.96 C_q	6.83 s	107.73 CH	7.22 s	110.06 CH		121.46 C_q
4		151.35 C_q		166.57 C_q		165.62 C_q	7.62 s	136.65 CH
4a		112.41 C_q		108.35 C_q		108.57 C_q		112.28 C_q
5		130.83 C_q		137.24 C_q		127.83 C_q		136.32 C_q
6	6.69 s	117.17 CH	6.87 m	115.98 CH		145.15 C_q	6.66 s	113.86 CH
7		146.58 C_q		162.14 C_q		156.59 C_q		161.45 C_q
8		130.83 C_q	6.99 d (2.9)	99.61 CH	6.86 s	99.74 CH	6.66 s	98.42 CH
8a		132.41 C_q		156.01 C_q		152.19 C_q		155.48 C_q
R^3	2.29 s	16.56 CH_3					2.20 s	17.38 CH_3
R^4	2.36 s	19.78 CH_3	2.80 s	43.21 CH_3	2.77 s	43.59 CH_3		
5-Me	2.72 s	24.34 CH_3	2.49 s	23.33 CH_3	2.47 s	15.88 CH_3	2.45 s	18.48 CH_3
R^6					3.80 s	60.86 CH_3		
R^7	10.2 br		3.86 s	56.04 CH_3	3.95 s	56.37 CH_3	3.83 s	55.55 CH_3
R^8	3.76 s	60.35 CH_3						

Table 3. NMR data for **9-11** (700 MHz (^{1}H), 175MHz (^{13}C)).

Position	**9** ($CDCl_3$)		**10** $(CD_3)_2SO$)		**11** ($CDCl_3$)	
	δ_H	δ_C **type**	δ_H	δ_C **type**	δ_H	δ_C **type**
2		161.83 C_q		163.38 C_q		161.17 C_q
3		121.11 C_q		109.87 C_q		120.66 C_q
4	7.61 s	137.34 CH		166.59 C_q	7.93 s	137.57 CH
4a		112.58 C_q		108.84 C_q		112.35 C_q
5		130.75 C_q		119.48 C_q		130.80 C_q
6	6.72 s	113.22 CH		141.38 C_q	6.95 s	110.50 CH
7		146.93 C_q		150.49 C_q		153.58 C_q
8		131.76 C_q	6.93 s	97.64 CH		133.17 C_q
8a		150.32 C_q		147.54 C_q		146.96 C_q
R^3	2.20 s	17.44 CH_3	2.00 s	10.35 CH_3	2.09 s	16.69 CH_3
R^4			3.80 s	60.70 CH_3		
5-Me	2.41 s	18.33 CH_3	2.49 s	12.40 CH_3	2.45 s	18.03 CH_3
R^6			8.79 br			
R^7	6.03 s		3.88 s	56.24 CH_3	3.87 s	56.21 CH_3
R^8	4.06 s	61.94 CH_3			3.75 s	60.73 CH_3

Table 4. NMR data for **12-13** (700 MHz (^{1}H), 175MHz (^{13}C)).

Position	**12** ($CDCl_3$)		**13** ($CDCl_3$)	
	δ_H (*J* Hz)	**δ_C type**	**δ_H (*J* Hz)**	**δ_C type**
2		164.39 C_q		164.83 C_q
3		114.50 C_q		111.60 C_q
4		168.18 C_q		168.71 C_q
4a		116.24 C_q		109.68 C_q
5		137.09 C_q		138.53 C_q
6	7.08 d (7.5)	128.31 CH	6.66 s	116.48 CH
7	7.40 t (7.9)	131.09 CH		162.13 C_q
8	7.21 d (8.3)	115.40 CH	6.70 s	98.94 CH
8a		154.26 C_q		156.25 C_q
R^3	4.68 s	56.25 CH_2	4.65 d (6.6)	56.28 CH_2
R^4	4.06 s	63.28 CH_3	4.04 s	63.22 CH_3
5-Me	2.70 s	22.61 CH_3	2.63 s	22.78 CH_3
R^6				
R^7			3.85 s	55.78 CH_3
R^8				



Cpd.	R^3	R^4	R^6	R^7	R^8
1	Me	SMe	H	H	H
2	Me	SMe	H	OMe	H
3	H	SMe	H	OMe	OMe
4	H	SMe	H	H	OH
5	Me	SMe	H	OH	OMe
6	H	S(O)Me	H	OMe	H
7	H	S(O)Me	OMe	OMe	H

Cpd.	R^3	R^4	R^6	R^7	R^8
8	Me	H	H	OMe	H
9	Me	H	H	OH	OMe
10	Me	OMe	OH	OMe	H
11	Me	H	H	OMe	OMe
12	CH_2OH	OMe	H	H	H
13	CH_2OH	OMe	H	OMe	H
14	Me	OMe	H	H	H
15	H	SMe	H	H	H
16	H	SMe	OMe	OH	H
17	H	SMe	OMe	OMe	H
18	Me	OMe	OMe	OMe	H
19	Me	OMe	H	OH	OMe
20	Me	OMe	H	OMe	OMe
21	H	OMe	H	H	OMe

Fig. 1. Structures of 5-methylcoumarins isolated from *Clutia lanceolata*. Compounds **1-13** are undescribed, whereas **14-21** are known but are isolated from this plant for the first time. Also shown is the chemical numbering scheme for the ring and its substituents.

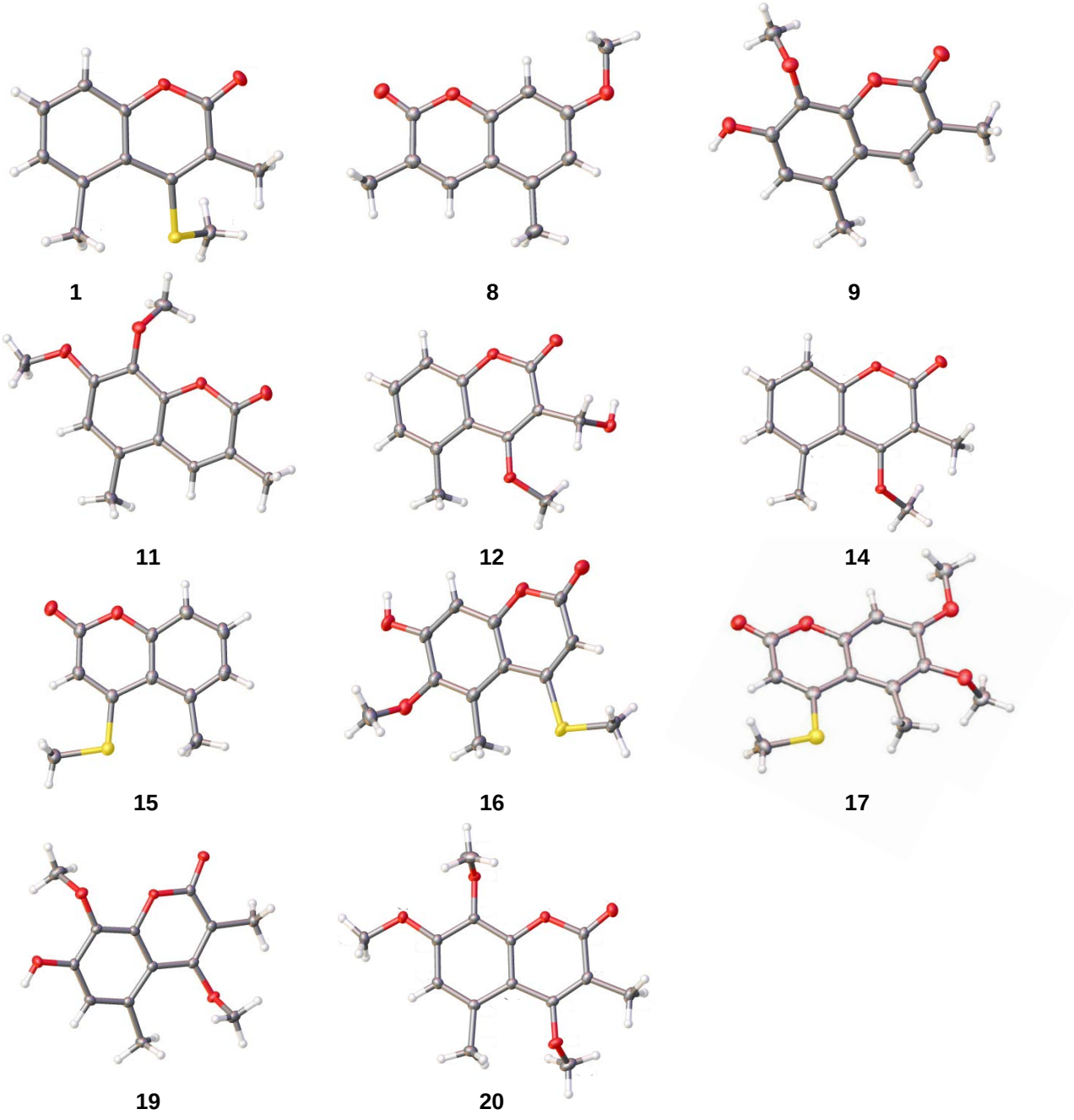


Fig. 2. X-Ray crystal structures determined for **1,8,9,11,12,14-17,19,20**. Atoms are shown as thermal ellipsoids drawn at the 50% probability level.

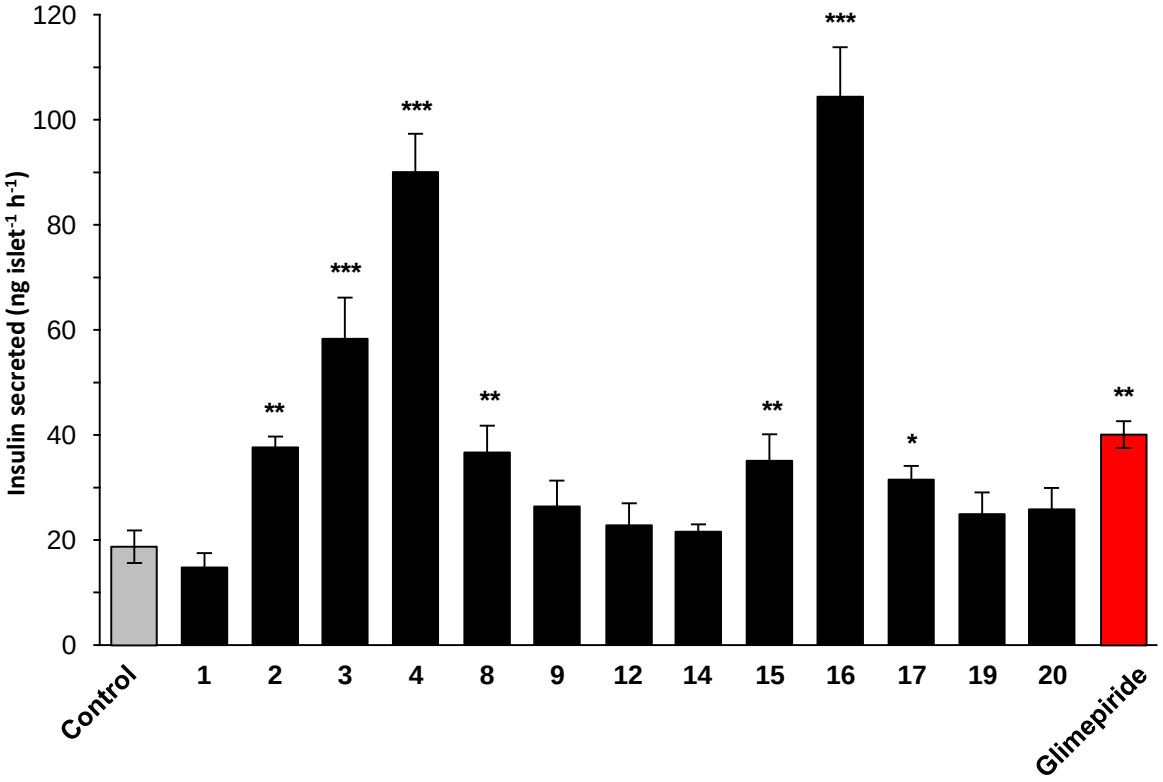


Fig. 3. Effects of test compounds (from *Clutia lanceoleta*) on the glucose-triggered secretion of insulin from murine islets. Islets were incubated for 1 h at 37°C in KRB buffer containing glucose (16.7 mM) in the absence (Control) or presence of test compounds and the secreted insulin was measured. Values are mean ± SD from three independent experiments. *P <0.05, **P <0.01, ***P <0.001 compared with the control value.