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Synthesis and structural elucidation of a series of isoflavones-based on FPR antagonists

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ABSTRACT

Isoflavones are naturally occurring compounds well-known for their beneficial role in several diseases, such as cancer and inflammation. Recently some isoflavones derivatives were reported as potent and competitive antagonists of formyl peptide receptors (FPRs) with an important role in regulating the inflammatory process. As a result of their biological activities, there is a huge interest in developing synthetic procedures to obtain isoflavones. Surprisingly, and as far as our knowledge goes, the synthetic work and full characterisation of the isoflavones described as FPR antagonists weren't yet reported. The work herein described comprises the synthesis of two series of 2-trifluoromethyl isoflavones, including the ones described as FPR antagonists and their complete characterisation by 1D, 2D NMR techniques and high-resolution mass spectroscopy.

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Introduction

Isoflavones are naturally occurring compounds mainly found in soybeans, soy foods and vegetables. Chemically, they retain the typical $C_6C_3C_6$ skeleton of flavonoids, consisting of two aromatic rings being linked through an oxygenated heterocycle nucleus, being isomers of flavones. They are characterised by the presence of a double bond between carbons C2 and C3 and an aromatic ring in the C3 position.[1]

Dietary isoflavones are well-known by their ability to act as phytoestrogens.[1,2] Nevertheless, other health benefits have been attributed to this class of compounds namely, their beneficial role in cancer, such as hormone-dependent cancers, in osteoporosis, cardiovascular disease and inflammation, and several mechanisms have since been proposed to explain these activities.[3] Interestingly,a series of synthetic isoflavones were recently reported as potent and competitive antagonists of formyl peptide receptors (FPRs) having an important role in the regulation of inflammatory reactions implicated in disease pathogenesis.[4] Thus, the isoflavone backbone represents a promising scaffold for the development of novel FPR antagonists.

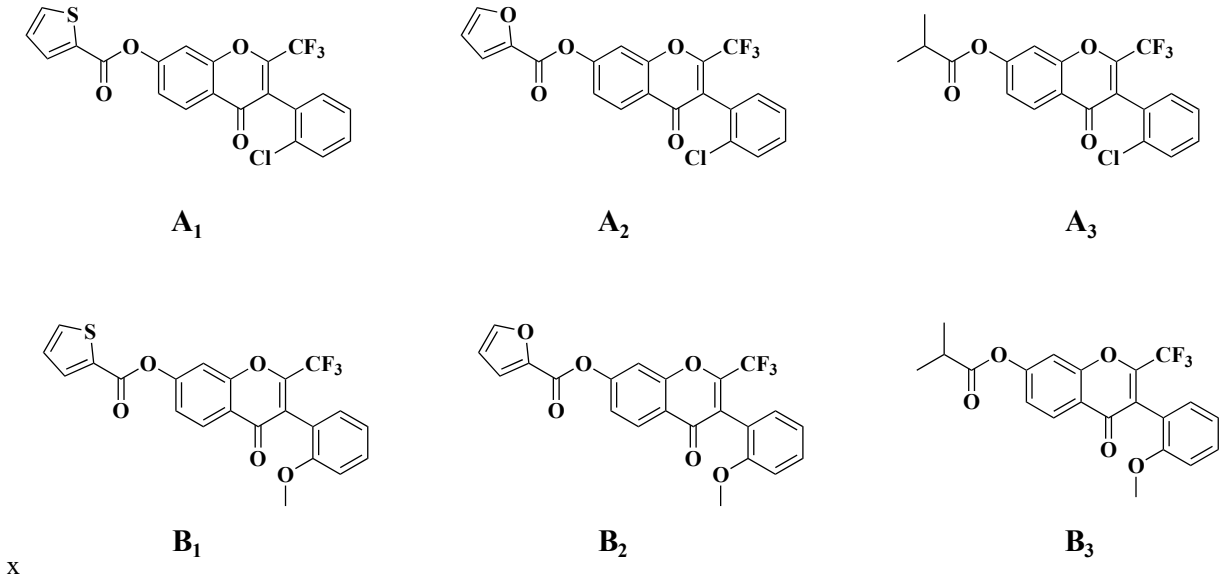
As a result of their biological activity, there is a huge interest in the development of synthetic procedures to obtain isoflavones and their derivatives for structure-activity relationship studies. Surprisingly, and as far as our knowledge goes, the synthetic work and full characterisation of the synthetic isoflavones described as FPR antagonists(compounds $\mathbf{A_1}$, $\mathbf{B_2}$ and $\mathbf{B_3}$, *figure 1*), weren't yet reported.

Accordingly, the work herein described comprises the synthesis of two series of 2-trifluoromethyl isoflavones (figure 1), including the ones described as FPR antagonists and their complete characterisation by 1D and 2D NMR techniques and by high-resolution mass spectroscopy.

Experimental

Materials

All reagents were purchased from Sigma-Aldrich Química, S.L. and Alfa Aesar, Thermo Fisher Scientific. All solvents were pro analysis grade from Merck, Carlo Erba Reagents and Scharlab. Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 acquired from Merck with layer thickness of 0.2 mm. The spots were visualized under UV detection at 254 and 366 nm. Column



x

Figure 1: 2-Trifluoromethyl-2′-chloro (**A_1-A_3**) and 2-trifluoromethyl-2′-methoxyisoflavone(**B_1-B_3**) based ester derivatives

chromatography was carried out with silica gel 60 0.040-0.063 mm acquired from Carlo-ErbaReactifs. Solvents were evaporated with a BuchiRotavapor.

NMR spectroscopy

1H and 13C NMR data were acquired on a Bruker Avance III 400 NMR spectrometer operating at 400.15 MHz and 100.62 MHz, respectively. For the 1H NMR experiments, the relaxation delay was 90° pulse, spectral width of 8012 Hz and 65 K data points. In the case of the 13C NMR experiments, the corresponding parameters were 30º pulse, 24038 Hz and 65 K, respectively, and 2.0 s relaxation delay. For the Distortionless Enhancement by Polarization Transfer (DEPT) sequence, the width of the 90º pulse for 13C was 7.7 µs, and the 90º pulse for 1H was 9.8 µs; the delay 2JC, H was set to 2.0 ms. For correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC), the data points were set to 2 K × 256 (t2 × t1) with a relaxation delay D1 of 1.5 s. The Heteronuclear Multiple Bond Connectivity (HMBC) was acquired with data points set to 4 K × 256 (t2 × t1) and relaxation delay D1 of 1 s. Furthermore, the long-range coupling time for HMBC was set to 71 ms.The data were processed using quadratic sine-bell weighting functions in both dimensions.

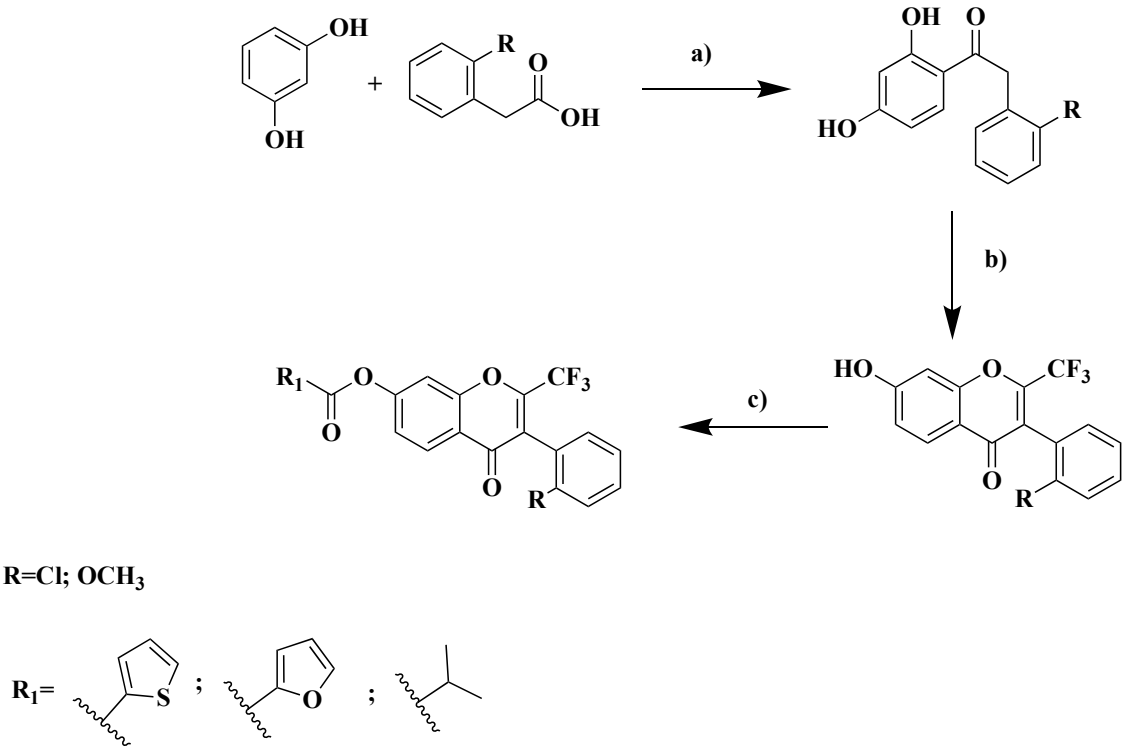
1H and 13C spectra of the samples were recorded at room temperature in 5 mm outer-diameter tubes. Samples were prepared in deuterated chloroform (CDCl3). Tetramethylsilane (TMS) was used as internal reference; chemical shifts (δ) were expressed in parts per million (ppm), and coupling constants (J) were given in Hertz (Hz). The 1H and 13C chemical shifts of CDCl3 were 7.26 and 77.2 ppm, respectively.

Mass spectroscopy

Mass spectra (MS) were carried out on a Bruker Microtof (ESI) apparatus; the data were reported as m/z (% of the relative intensity of the most important fragments).

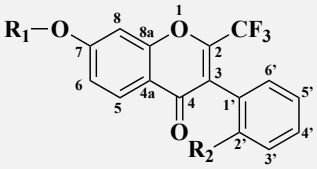
Synthesis of trifluoromethylisoflavones esters

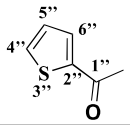
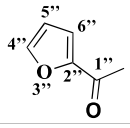
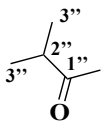
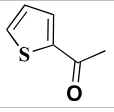
Compounds A1-A3 (Figure 1) were obtained by the cyclization of the 2-(2′-Chlorophenyl)-2′,4′-dihydroxyacetophenone, followed by its acylation with the corresponding acyl chloride, as depicted in scheme 1 (step b and c).5,6,7 Briefly, 2-(2′-Chlorophenyl)-2′,4′-dihydroxyacetophenone (1mmol) was cyclised, using trifluoroacetic anhydride (3mmol) and triethylamine (2mL). The reaction was refluxed for one hour, then poured into water, acidified (pH=3) and stirred at room temperature for another hour. The crude material was extracted with ethyl acetate (3x 10 mL). were dried over anhydrous sodium sulphate and the solvent evaporated. 7-hydroxy-3-(2-chlorophenyl)-2-(trifluoromethyl)-4H-chromen-4-one was recrystallised from ethyl acetate and obtained in a 65% yield. The acylation step was performed following the methodology described by Jayashree et al. 7: 7-hydroxy-3-(2-chlorophenyl)-2-(trifluoromethyl)-4H-chromen-4-one and (1mmol) and the corresponding acyl chloride (1.25 mmol), were dissolved in pyridine (7.5mL), and the solution refluxed for one hour (scheme 1, step c). The mixture was then cooled to room temperature and poured into water (30 mL), acidified to pH=5, and stirred at room temperature for two hours. Afterwards, the mixture was extracted with ethyl acetate (3x 10mL). The combined organic phases were dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure.

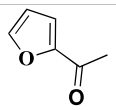
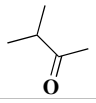


Scheme 1: Synthetic strategy followed for synthesis of isoflavone –based ester derivatives. Reagents and conditions: a) BF_3. Et_2O, 85° C, 90 minutes. b) Trifluoroacetic anhydride, triethylamine, reflux, 1 hour. c) Pyridine, reflux, 1 hour.

The structure, chemical name, yield, and mass spectroscopy data of synthesized compounds are depicted in Table **I.**



	R1	R2	NAME	YIELD* (%)	HRMS $[M+H]^+$ (calcd./found)
A_1		Cl	*3-(2-chlorophenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl thiophene-2-carboxylate*	60	451.0013/451.0008
A_2		Cl	*3-(2-chlorophenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl furan-2-carboxylate*	63	435.0242/435.0239
A_3		Cl	*3-(2-chlorophenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl isobutyrate*	59	411.0605/411.0603
B_1		OCH_3	*3-(2-methoxyphenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl thiophene-2-carboxylate*	60	447.0501/447.0497

B_2		OCH_3	*3-(2-methoxyphenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl furan-2-carboxylate*	64	431.0737/431.0733
B_3		OCH_3	*3-(2-methoxyphenyl)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl isobutyrate*	55	407.1101/407.1097

The structure, chemical name, yield, and mass spectroscopy data of synthesised compounds are depicted in table I.

Compounds B1-B3 (Figure 1) were obtained by the acylation of 7-hydroxy-3-(2-methoxyphenyl)-2-(trifluoromethyl)-4H-chromen-4-one with the corresponding acyl chloride, following the methodology described above and depicted in scheme 1 (step c). 7-hydroxy-3-(2-methoxyphenyl)-2-(trifluoromethyl)-4H-chromen-4-one was obtained following the procedure described in literature 8 (scheme 1, step a and b).

Results and Discussion

The complete structural characterisationof all synthesisedcompounds (**A_1- A_3 and B_1- B_3**) is depicted in tables **II and III**. The unambiguous assignment of all carbons and hydrogens was achieved by the combined expenditure of one- and two-dimensional nuclear resonance techniques.

The unequivocal assignment of the NMR data for compound **A_1** was performed as followed:

The hydrogens H-5 and H-8 of the benzopyran structure of compound **A_1** were readily assigned considering their splitting pattern and coupling constants (Table **II).** Thus, the peak at δ=8.31 ppm (d, *J*=8.8 Hz) was assigned to H-5 and the peak at δ=7.60 ppm (d, *J*=2.1 Hz) to H-8. The COSY experiment revealed an H-H interaction between these two peaks and the multiplet at δ=7.43-7.33 ppm (*Figure **2***), which integrated for three hydrogens. Furthermore, for this multiplet, an H-H interaction was observed with a peak at 7.52 ppm (ddd, *J*=0.4, 1.4, 7.9 Hz) (*Figure **2***). The construction of a splitting three diagram for the signal at δ=7.43-7.33 ppm enabled the identification of three hydrogens: one hydrogen at δ=7.41 ppm (H-4', ddd, *J*=1.7, 7.5, 7.9 Hz), other at δ=7.39 ppm (H-6, dd, *J*=2.1, 8.8 Hz) and another at δ=7.35 ppm (H-5', ddd, *J*=1.4, 7.4, 7.5 Hz).

The COSY experiment also showed an H-H interaction between two double doublets (at δ=8.05 ppm and 7.75 ppm) with the multiplet at δ= 7.24-7.21 ppm (which integrates for two hydrogens) (*Figure **2***). The splitting pattern along with the coupling constants enabled the attribution of the peak at δ=8.05 ppm to H-6'', and the peak at δ=7.75 ppm to H-4''. Moreover, by assembling a tree diagram of the multiplet at δ= 7.24-7.21 ppm, H-5'' and H6' were assigned to δ=7.23 ppm. Both hydrogens showed a double doublet multiplicity. However, H6'' showed characteristic coupling constants of a thiophene ring (*J*=3.8, 5.0 Hz) while H6' presented the typical *meta* and *ortho*constants of a phenyl ring (*J*=1.7, 7.4 Hz).

The carbons of compound **A_1** were assigned using HSQC experiment and depicted in table **II**. The CF_3 and the C-2 quaternary carbons were easily identified due to their typical coupling constants; the peak at δ= 118.9 ppm (d, *J*=277 Hz) was attributed to CF_3, and the signal at δ= 149.4 ppm (d, *J*=37 Hz) was assigned as C-2. The other quaternary carbons were then assigned based on the HMBC experiment and chemical shifts with the long-range interactions summarized in *Figure **3***. The attribution of the quaternary carbons from the benzopyran nucleus (C-4, C-4a, C-8a, C-7) was as follows: a)The peak at δ= 175.1 ppm was assigned to C-4 due to their chemical shift and a long-range interaction with H-5; b) the long-range interaction detected between H-5 and H-8 with the peaks at δ=155.4 and 155.6 ppm allowed their attribution to C-7 and C-8a, respectively; c) a long-range interaction was detected between H-6 and the peak at δ=155.4 ppm, corroborating its assignment to C-7. Additionally, a long-range interaction of H-8 with the peak at δ=120.9 ppm was observed, which was identified as C-4a (*Figure **4a**)*.

The quaternary carbons of the exocyclic phenyl ring were attributed based on the long-range correlation between the H-3', H-4' and H-6' and the quaternary carbon at δ= 134.3 ppm, which was assigned to C-2' (*Figure **4b**)*. Furthermore, the HMBC experiment showed an interaction between H-6' and a peak at δ= 123.7 ppm, which was thus assigned as C1'.

The signal at δ=159.4 ppm was assigned to carbon C-1'' by its chemical shift and long-range interaction with H-4'' and H-6''. Moreover, H-4'' and H-6'' also showed a long-range interaction with the peak at δ=131.7 ppm, which was attributed to C-2'' of the thiophene ring (*Figure **4c**)*. The structural assignment of compounds **A_2**and **A_3** were based onthe attributions performed for compound **A_1**.are depicted in table **II**.

Using the same approach, the unequivocal assignment of hydrogens and carbons of the C2'-methoxyl counterparts (compounds **B_1-B_3**), was accomplished and described in table **III**. Nevertheless, the hydrogens of the methoxyl group were readily assigned from ^{1}H NMR spectrum, by their chemical shift, integration and multiplicity at δ=3.77 ppm, δ=3.76 ppm and δ=3.75 ppm for compounds **B_1**, **B_2** and **B_3**, respectively. It is also important to stress that the chemical shift of exocyclic aromatic hydrogens and carbons was slightly affected by theaffected by the presence of methoxyl group at position C-2' instead of chlorine. All the hydrogens and carbons of compounds **B_1- B_3**, with exception off C-2', that appear with higher values of chemical shifts, suffered an upfield chemical shift, while C-2' appears at relative to their C2'-Cl counterparts (compounds **A_1- A_3**)

Table II: 1H and 13C NMR Data of Compounds A1-A3.

	compound A_1		compound A_2		compound A_3	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		149.4 (d, *J*=37 Hz)		149.4 (d, *J*=37 Hz)		149.4 (d, *J*=37 Hz)
3		128.5		128.5		128.6
4		175.1		175.1		175.2
4a		120.9		121.0		120.8
5	8.31 (d, *J*=8.8 Hz)	128.0	8.31 (d, *J*=8.8 Hz)	128.1	8.26 (d, *J*=8.7 Hz)	127.9
6	7.39 (dd, *J*=2.1, 8.8 Hz)	120.7	7.38 (1H, dd, *J*=2.0, 8.8 Hz)	120.7	7.23 (dd, *J*=2.0, 8.7 Hz)	120.7
7		155.4		155.7		155.9
8	7.60 (d, *J*=2.1 Hz)	111.4	7.59 (d, *J*=2.0 Hz)	111.4	7.43 (d, *J*=2.0 Hz)	111.3
8a		155.6		155.7		155.7
1'		123.7		123.8		123.7
2'		134.3		134.3		134.3
3'	7.52 (ddd, *J*=0.4, 1.4, 7.9 Hz),	129.5	7.51 (ddd, *J*=0.4, 1.4, 7.8 Hz),	129.5	7.51 (dd, *J*=1.4, 7.8 Hz)	129.5
4'	7.41 (ddd, *J*=1.7, 7.5, 7.9 Hz),	130.5	7.41 (ddd, *J*=1.8, 7.5, 7.8 Hz)	130.5	7.40 (ddd, *J*=1.8, 7.7, 7.8 Hz),	130.5
5'	7.35 (ddd, *J*=1.4, 7.4, 7.5 Hz)	126.7	7.35 (ddd, *J*=1.4, 7.5, 7.5 Hz),	126.7	7.35 (ddd, *J*=1.4, 7.5, 7.7 Hz)	126.7
6'	7.23 (dd, *J*=1.7, 7.4 Hz)	131.2	7.23 (dd, *J*=1.8, 7.5 Hz)	131.2	7.22 (dd, *J*=1.8, 7.5 Hz)	131.3
CF_3		118.9 (d, *J*=277 Hz)		119.0 (*J*=277Hz)		119.0 (d, *J*=277 Hz)
1''		159.4		155.1		174.6
2''		131.7		143.1	2.87 (1H, m, *J*=7.0 Hz)	34.3
3''					1.36 (6H, d, *J*=7.0 Hz)	18.8 (x2)
4''	7.75 (dd, *J*=1.3, 5.0 Hz	134.6	7.74 (dd, *J*=0.8, 1.7 Hz)	148.0		
5''	7.23 (dd, *J*=3.8, 5.0 Hz)	128.3	6.65 (dd, *J*=1.7, 3.5 Hz)	112.5		
6''	8.05 (dd, *J*=1.3, 3.8 Hz)	135.6	7.47 (dd, *J*=0.8, 3.5 Hz)	120.6		

Table I: ^{1}H and ^{13}C NMR Data of Compounds **B_1-B**

	compound **B_1**		compound **B_2**		compound **B_3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		149.0 (d, *J*=36 Hz)		149.0 (d, *J*=36 Hz)		149.0 (d, *J*=36 Hz)
3		122.9		122.9		122.8
4		175.9		175.9		175.9
4a		121.1		121.1		120.9
5	8.29 (d, *J*=8.7 Hz)	128.0	8.29 (d, *J*=8.7 Hz).	128.1	8.24 (d, *J*=8.7 Hz)	127.9
6	7.35 (dd, *J*=2.1, 8.7 Hz)	120.3	7.34 (dd, *J*=2.1, 8.7 Hz)	120.3	7.19 (dd, *J*=2.0, 8.7 Hz)	120.3
7		157.3		157.3		157.2
8	7.55 (d, *J*=2.00 Hz)	110.8	7.55 (d, *J*=2.1 Hz)	110.8	7.39 (d, *J*=2.0 Hz)	110.8
8a		155.2		155.7		155.6
1'		118.1		118.1		118.2
2'		155.7		155.8		155.7
3'	6.98 (d, *J*=8.3Hz)	111.3	6.98 (d, *J*=8.3Hz)	111.3	6.97 (d, *J*=8.3 Hz)	111.2
4'	7.42 (ddd, *J*=1.7, 7.5, 8.3 Hz)	130.6	7.42 (ddd, *J*=1.0, 7.5, 8.3 Hz)	130.6	7.41 (ddd, *J*=1.7, 7.5, 8.3 Hz)	130.6
5'	7.03 (ddd, *J*=1.0, 7.5, 7.5 Hz)	120.4	7.03 (ddd, *J*=0.9, 7.5, 7.5 Hz)	120.4	7.02 (ddd, *J*=0.9, 7.5, 7.5 Hz)	120.4
6'	7.14 (dd, *J*=1.7, 7.5 Hz)	131.0	7.14 (dd, *J*=1.7, 7.5 Hz)	131.0	7.13 (dd, *J*=1.7, 7.5 Hz)	131.0
OCH_3	3.77 (3H, s)	55.6	3.76 (3H, s)	55.6	3.75 (3H, s)	55.6
CF_3		119.3 (d, *J*=276Hz)		119.2 (d, *J*=277 Hz)		119.3 (d, *J*=277 Hz)
1''		159.6		154.9		174.6
2''		131.8		143.1	2.86 (m, *J*=7.0 Hz)	34.3
3''					1.35 (6H, d, *J*=7.0 Hz)	18.8 (x2)
4''	7.74 (dd, *J*=1.3, 5.0 Hz)	134.5	7.73 (1H, dd, *J*=0.8, 1.7 Hz)	147.9		

5''	7.22 (dd, *J*=3.8, 5.0 Hz)	128.3	6.64 (1H, dd, *J*=1.7, 3.5 Hz)	112.5		
6''	8.04 (dd, *J*=1.3, 3.8 Hz)	135.5	7.46 (1H, dd, *J*=0.8, 3.5 Hz)	120.6		

Conclusions

This work describes for the first time an effective synthetic strategy to obtain trifluoromethylisoflavones derivatives, namely the ones that were described as FPR antagonists.[4]Theisoflavones(compounds $\mathbf{A_1}$-$\mathbf{A_3}$ and $\mathbf{B_1}$-$\mathbf{B_3}$) were synthesised in good **yields** and had been fully characterised by homo- and hetero-nuclear NMR and high-resolution mass spectrometry. The acquired data allowed the unambiguous identification of these compounds, providing a valuable database for the unequivocal identification of other analogue libraries.

Acknowledgments

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