Synthesis of novel isoflavone/benzo-δ-sultam hybrids as potential anti-inflammatory drugs

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Abstract

A small series of novel isoflavone/benzo-δ-sultam hybrids was synthesised and evaluated as potential anti-inflammatory and neuroprotective drugs in LPS-activated BV2 microglia. The benzo-δ-sultam core was constructed in a two-step reaction by coupling 2-halobenzenesulfonamide derivatives with terminal alkynes, followed by a *6-endo-dig* cyclisation. The synthesised compounds, including precursors and hybrids, were tested for their ability to inhibit NO and TNF-α production in LPS-stimulated BV2 microglial cells, and the results are promising. The most potent hybrid reduces the NO production to 41%, and the TNF-α to 34% at 20 μM final concentration in the well.

Keywords: isoflavone, δ-sultam, anti-inflammatory, microglia, TNF-α

Neurodegenerative disorders are characterised by the progressive degeneration of the nervous system, which leads to dysfunctional neurons, and ultimately to neuronal death. The high volume of research that has been conducted in attempts to find a drug to delay or modify the course of neuronal degeneration has led only to drugs that alleviate the symptoms.[1-3] The same complexity leads to many potential targets to treat these diseases, targets for which the underlying mechanisms are largely unknown. Thus, in an effort to find a drug able to target multiple pathways of interest in treating these complex diseases, the synthesis of hybrid compounds has garnered a considerable interest.[4] Synthesising a hybrid that contains two or more biologically active pharmacophores might lead to a potent multi-target-directed ligand, able to tackle multiple targets of the underlying mechanism of the disease, with fewer side effects and increased permeability through the blood brain barrier and bioavailability. Two pharmacophores that possess interesting neuroprotective effects that might be able to treat the underlying disease are isoflavones and benzo-δ-sultams.

Several synthetic and naturally occurring isoflavones (see Figure **1**) have been shown to exhibit a wealth of properties, including anti-inflammatory and neuroprotective properties. There are multiple ways through which the isoflavones express their anti-inflammatory and neuroprotective activities, some of them involving

the inhibition of pro-inflammatory cytokines and mediators such as TNF-α and NO, inhibition of enzymes such as AChE (acetylcholinesterase) and BuChE (butyrylcholinesterase), inhibition of β-amyloid aggregation and antioxidant activity.[5, 6] A common mechanism is the inhibition of pro-inflammatory mediators which takes place through various signaling pathways such as NF-κB and MAPKs.[6, 7] One of the most potent isoflavones is genistein (Figure **1**). Genistein showed neuroprotection of the neurons affected by radical damage *via* an antioxidant effect.[8] Also, genistein prevented Aβ-induced neuroinflammation in BV2 and C9 cells by inhibiting the generation of pro-inflammatory cytokines and mediators *via* the TLR4 and NF-κB signaling pathway.[9] In LPS-stimulated BV2 microglia, genistein suppressed the production of pro-inflammatory cytokines by inhibiting the binding of LPS to toll-like receptor 4 (TLR4) and subsequent activation of NF-κB.[10]

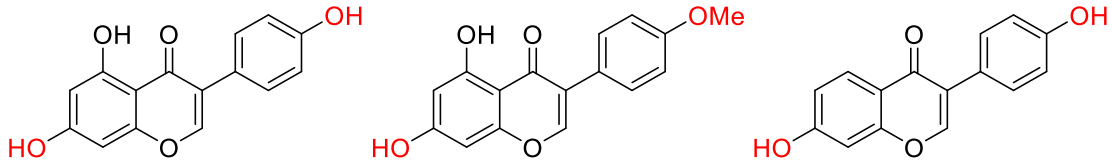


Figure 1. The chemical structures of some bioactive isoflavones.

Benzo-δ-sultams are well known nonsteroidal anti-inflammatory drugs (e.g. piroxicam, Figure **2**), that display a variety of biological activities such as anti-inflammatory, anticancer and antimicrobial, and help in reducing pain and inflammation in the body.[11-14] Piroxicam was reported to be neuroprotective in rotenone and L-dopa-treated rats by protecting the nigral neurons, and enhancing the motor function.[15] Meloxicam, another benzosultam NSAID, also shown in Figure **2**, was reported to inhibit the fipronil-induced apoptosis in human neuroblastoma SH-SY5Y cells by suppressing the pro-inflammatory cytokines and ROS generation,[16] and was found to be neuroprotective in intranigral LPS rat model by reducing microglial activation and dopaminergic neuron degeneration.[17]

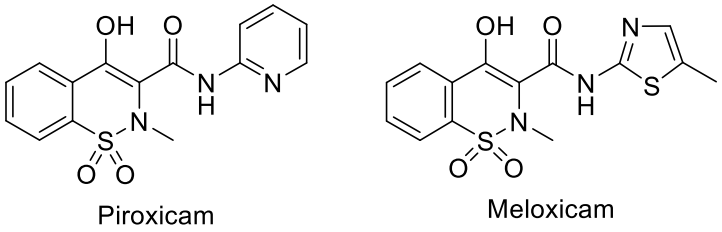


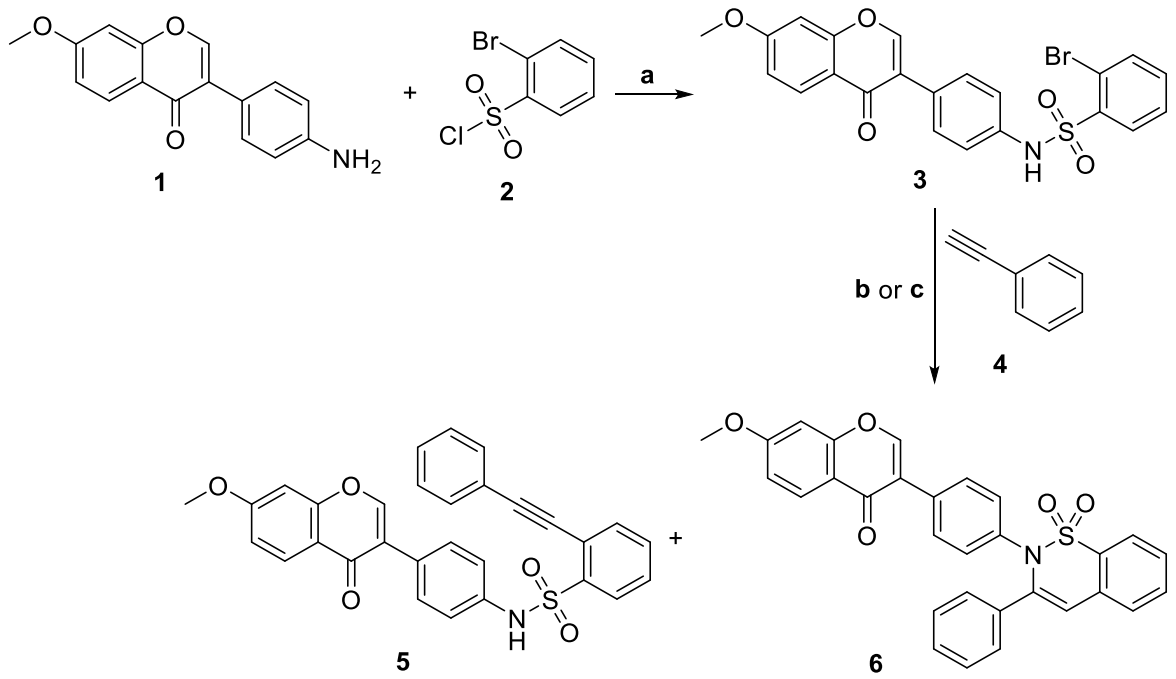
Figure 2. The chemical structures of some bioactive benzosultam derivatives.

Neuroinflammation plays an important role in neurodegenerative disease pathogenesis, and is considered to be caused to some extent by activated microglia. Microglia, the resident macrophage cells of the CNS, are activated by various noxious agents and release pro-inflammatory cytokines and other mediators, causing inflammation. If at the beginning of Aβ plaque formation microglia contribute to Aβ plaque phagocytosis and clearance, continuous activation causes microglia to surround the Aβ plaques and contribute to Aβ plaque formation and subsequent neuronal damage.[18] It was noted that upon >95% microglia depletion for >6 months in the 5xFAD mouse model of Alzheimer Disease (AD), Aβ plaque formation was prevented. Thus, removing the activated and dysfunctional microglia may prevent Aβ plaque

formation. Therefore, anti-inflammatory drugs that prevent microglia activation and subsequent production of pro-inflammatory cytokines and mediators may have a beneficial effect in reducing the inflammation and neurodegeneration, and could be a potential treatment for neurodegenerative diseases as the healthy microglia cells would be able to engage in phagocytosis and remove the harmful agents.

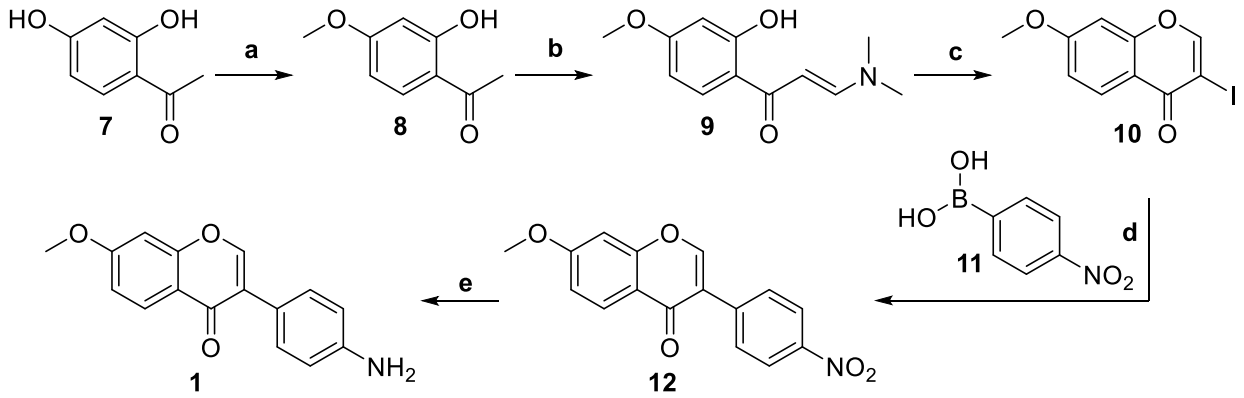
As discussed, isoflavones and benzo-δ-sultams have been shown by several studies to exhibit anti-inflammatory activity and neuroprotective effects by inhibiting neuroinflammation in BV2 microglia, but there was no clear relationship between the chemical structure of the compounds and neuroprotective activity.[19-21] Also, due to the complexity of the neurodegenerative diseases, the mechanism by which isoflavones and benzo-δ-sultams gain their neuroprotective effects is not yet fully understood. In this work, we describe the synthesis of a series of isoflavone/benzo-δ-sultam hybrids with potential anti-inflammatory and neuroprotective activity. The products were tested for their ability to inhibit NO and TNF-α production in LPS-stimulated BV2 microglial cells.

The synthesis of the hybrids was achieved through a cascade process involving cross-coupling and regioselective *6-endo-dig* cyclisation.[12, 22, 23] First, the benzo-δ-sultam was built on the (B) ring of the isoflavone (Scheme **1**). Reacting amine **1** with 2-bromobenzenesulfonyl chloride **2** in pyridine gave the previously unreported benzenesulfonamide **3**. One-pot Sonogashira coupling of **3** with phenylacetylene **4** and subsequent *6-endo-dig* cyclization through hydroamination,[22] led to the synthesis of isoflavone/benzo-δ-sultam hybrid **6** alongside its precursor **5**, the Sonogashira coupling product (method (b): **6**, 10%; **5**, 20%), both of which are new molecules. The slow addition of the alkyne **4** (to avoid potential homocoupling of the alkyne), and a better degassing of the solvent and reaction mixture (to avoid potential oxidation of the catalysts) led to compound **6** being obtained in greater yield (54%, method (**c**)). It can be noted that the coupling step is the step that determines the course of the reaction, so that improving the coupling reaction may lead to a better yield. When THF was used as the solvent instead of DMF, compound **6** was obtained only in trace amount.



Scheme 1. Synthesis of isoflavone/benzo-δ-sultam hybrid **6**. *Reagents and conditions*: (**a**) 1. pyridine, 0 °C; 2. 80 °C, 2 h, 78%; (**b**) 1. $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), Et_3N:DMF (1:2), N_2, RT, 15 min; 2. **4**, 70 °C, overnight (**6**, 10%; **5**, 20%); (**c**) 1. $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), Et_3N:DMF (1:2), N_2, RT, 30 min; 2. **4** (over 1 h, in DMF), 70 °C, overnight (**6**, 54%; **5**, traces).

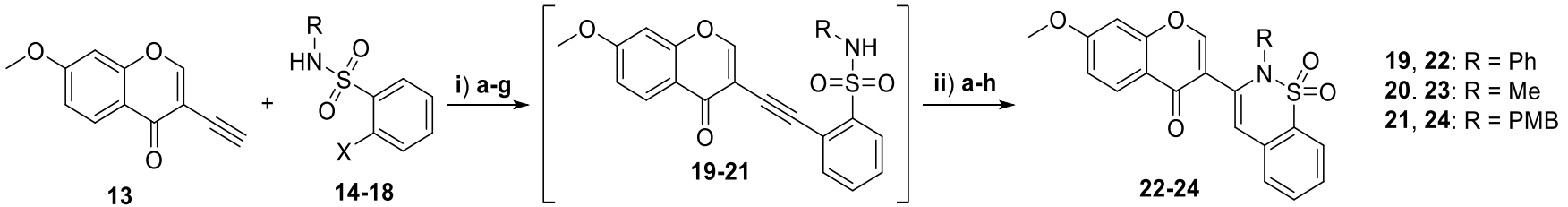
The known amine **1** was synthesised starting with the selective protection of 2,4-dihydroxyacetophenone **7** at the 4-hydroxy group using iodomethane to give **8**,[24] as shown in Scheme **1**. The protected compound **8** was treated with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) to furnish the enamino ketone **9**, which was subsequently cyclised using pyridine and iodine to give the desired 3-iodo-4*H*-chromen-4-one **10**, a known compound.[25, 26] Suzuki-Miyaura coupling of 3-iodochromone **10** with 4-nitrophenylboronic acid **11** in the presence of $[Pd(dppf)Cl_2]$ as catalyst and Na_2CO_3 as base, led to formation of the nitro-isoflavone **12**. The nitro-isoflavone **12** was reduced to amine **1** in the presence of iron powder and NH_4Cl in ethanol (Scheme **2**).[27]



Scheme 2. Synthesis of amine **1**. *Reagents and conditions*: (**a**) CH_3I (1 equiv.), K_2CO_3 (2 equiv.), acetone, RT, overnight; (**b**) $(CH_3)_2NCH(OCH_3)_2$ (1.5 equiv.), 95 °C, 3 h; (**c**) I_2 (2 equiv.), pyridine (1.1 equiv.), $CHCl_3$, RT, 12 h, 89% (over 3 steps); (**d**) $[Pd(dppf)Cl_2]$ (5 mol %), Na_2CO_3 (4 equiv.), toluene/ EtOH/H_2O (10:5:1), 70 °C, 19 h, 80%; (**e**) Fe powder, NH_4Cl (in H_2O), EtOH, 90 °C, 4 h, 95%.

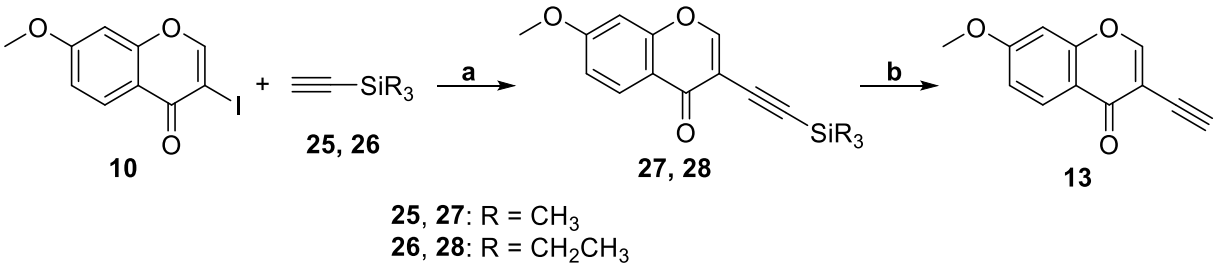
A second approach to isoflavone/benzo-δ-sultam hybrids was also used. For this, the alkyne **13** was cross-coupled with various 2-halobenzenesulfonamide derivatives **14-18**. Cyclisation of intermediates **19-21** in the presence of Cu(I) or Ag(I) allowed us to obtain the isoflavone/benzo-δ-sultam hybrids **22-24** (See Table **1**). The synthesis of intermediates **19-21**, and hybrids **22-24**, as can be seen in Table **1**, was influenced by several factors such as solvent, catalyst, halide, the degassing process and the method of addition of alkyne **13**. An optimization reaction between **13** and **14**/**16** to obtain **22** was initially attempted (Table **1**, methods **a**-**e**). As catalyst, $Pd(PPh_3)_2Cl_2$ worked best, and as solvent, DMF. Also, the slow addition of **13** (dissolved in DMF) facilitated the cross-coupling. Method (**c**) gave the best yield of the desired product **22** (44% yield). When method (**c**) was applied to the synthesis of compound **23**, only starting material **15** was recovered. Changing the solvent to THF, and the halide to the iodide **17** furnished **23** in 27% yield (method **f**). Changing the catalyst to 10% Pd/C, adding PPh_3 as ligand, and using 2-iodobenzenesulfonamide derivatives **16-18** (method **g**),[28] led to the synthesis of both intermediates **19-21**, and hybrids **22-24** in different ratios. The separation of the intermediates from the hybrids was possible for compounds **19** and **22**, but not for **20**/**23**, and **21**/**24**, these being obtained as mixtures. Treating the intermediate **19** and the mixtures **20**/**23**, **21**/**24** with $AgNO_3$ and Et_3N in EtOH,[23] afforded the cyclised compounds **22**, **23**, **24** in excellent yields (for the C–N bond forming reaction).[29]

Table 1. Synthesis of some hybrids of isoflavone with benzo-δ-sultam



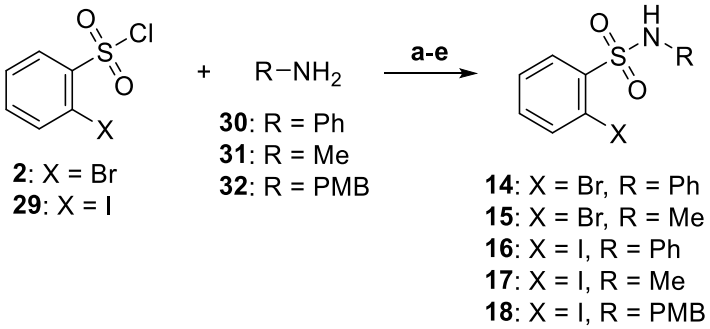
Step **i**, reagents and conditions	Sulfonamide	Compound (yield or recovery, %)	Step **ii**, reagents and conditions	Compound (yield, %)
(**a**) 1. $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), vacuum/N_2 (x3); 2. DMF, N_2, 10 min; 3. **14**, **13**, Et_3N, N_2, 70 °C, 7 h	**14**: X =Br, R = Ph	**22** (18%) **14** (72%)	-	-
(**b**) 1. $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), vacuum/N_2 (x3), THF, N_2, 5 min; 2. **14**, **13**, RT; 3. Et_3N, 0 °C; 4. N_2, 70 °C, 7 h	**14**: X =Br, R = Ph	**22** (5%) **14** (64%)	-	-
(**c**) 1. **14** or **15**, vacuum/N_2 (x3); 2. DMF/Et_3N (2:1), N_2, 10 min; 3. $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), 20 min; 4. **13** (in DMF, over 2 h), 70 °C; 5. 70 °C, 15 h	**14**: X =Br, R = Ph	**22** (44%) **14** (51%)	-	-
	15: X =Br, R = Me	**15** (86%)	-	-
(**d**) 1. **14**, vacuum/N_2 (x3); 2. DMF/Et_3N (2:1), N_2, 10 min; 3. $Pd(dppf)Cl_2$ (5 mol %), CuI (12 mol %), 20 min; 4. **13** (in DMF, over 1 h), 70 °C; 5. 70 °C, 15 h	**14**: X =Br, R = Ph	**19** (18%) **14** (67%) **13** (6%)	-	-
(**e**) 1. **16**, $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (12 mol %), vacuum/N_2 (x3); 2. DMF/Et_3N (2:1), N_2, 30 min; 3. **13** (in DMF, over 1 h), 70 °C; 4. 70 °C, 14 h	**16**: X =I, R = Ph	**19** (23%) **16** (50%)	-	-
(**f**) 1. **17**, $Pd(PPh_3)_2Cl_2$ (2 mol %), CuI (3 mol %), vacuum/N_2 (x3); 2. THF/Et_3N (5:1), N_2, 15 min; 3. **13** (in THF, over 15 min), RT, 1 h; 4. 60 °C, 40 h	**17**: X =I, R = Me	**23** (27%)	-	-
(**g**) 1. **16**-**18**, 10% Pd/C (3 mol %), PPh_3 (12 mol %), CuI (5 mol %), **13**, vacuum/N_2 (x3); 2. MeCN, 10 min, N_2, 0 °C; 3. Et_3N, N_2, 0 °C, 5 min; 4. 80 °C, overnight	**16**: X =I, R = Ph	**22** (32%) **19** (17%) **16** (32%)	(**h**) $AgNO_3$ (20 mol %), Et_3N, EtOH, N_2, 80 °C, 10 min	**22** (92%, from **19**)
	17: X =I, R = Me	**20**/**23** (1:1) **17** (36%)		**23** (41%, over 2 steps)
	18: X =I, R = PMB	**21**/**24** (10:1) **18** (11%)		**24** (31%, over 2 steps)

The known alkyne **13** was synthesised by a modified literature method which involved coupling of (trimethylsilyl)acetylene **25** or (triethylsilyl)acetylene **26** with 3-iodo-7-methoxy-4*H*-chromen-4-one **10** to give the protected derivatives **27** and **28** (Scheme **3**). Subsequent deprotection of **27** and **28** by using TBAF and D-camphor-10-sulfonic acid (CSA) in THF provided a high yield of the desired 3-ethynyl-7-methoxy-4*H*-chromen-4-one **13**.[30] When TBAF was used without CSA, and a mixture of methanol and THF (1:1) was used as solvent, the desired product was not obtained. It was reported in the literature that the deprotected derivative **13** undergoes hydrolysis and acetal formation in the presence of alcohols.[31] Also when only TBAF in THF was used, literature reported that the deprotected alkyne was obtained in 27% yield.[32] D-camphor-10-sulfonic acid (CSA) was synthesised as previously reported.[33]



Scheme 3. Synthesis of 3-ethynyl-7-methoxy-4H-chromen-4-one **13**. *Reagents and conditions*: (**a**) 1. $[Pd(PPh_3)_2Cl_2]$ (3 mol %), CuI (20 mol %), THF, TEA, N_2, 0 °C; 2. 3 h, RT, N_2, 82-88%; (**b**) 1. TBAF, CSA, THF, 0 °C; 2. RT, 3 h, 85-94%.

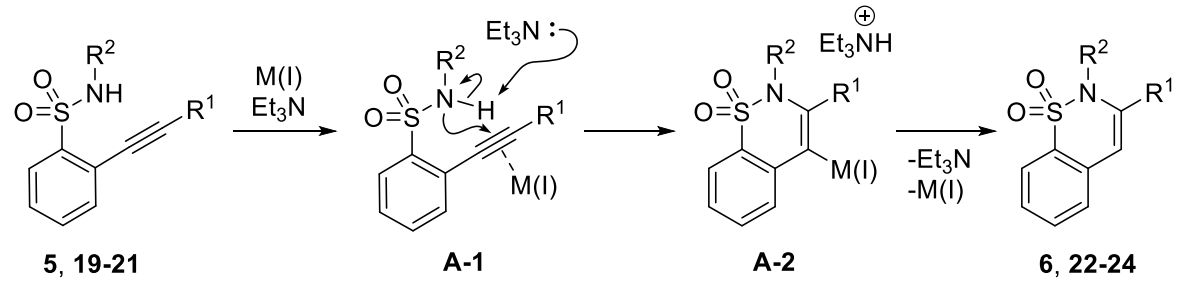
The required known sulfonamides **14-17** were readily obtained from 2-halobenzenesulfonyl chloride **2** or **29** and the corresponding amines **30** and **31** (Scheme **4**). With the hope of obtaining a benzo-δ-sultam with an NH, the previously unreported sulfonamide **18,** with PMB as a protecting group, was also synthesised.



Scheme 4. Synthesis of 2-bromo or 2-iodobenzenesulfonamide derivatives. *Reagents and conditions*: (**a**) **14**: X = Br, R = Ph; pyridine, 80 °C, 1 h, 96 %; (**b**) **15**: X = Br, R = Me; 1. $MeNH_2$ (40% in H_2O), THF, 0 °C, 10 min; 2. RT, 3 h, 90%; (**c**) **16**: X = I, R = Ph; 1. pyridine, DCM, 0 °C; 2. RT, overnight, 97%; (**d**) **17**: X = I, R = Me; 1. $MeNH_2$ (40% in H_2O), THF, 0 °C, 5 min; 2. RT, 3 h, 95%; (**e**) **18**: X = I, R = PMB; Et_3N, DCM, 0 °C; 2. RT, overnight, 97%.

A mechanism for the Cu- or Ag-mediated cyclization of the alkynes **5**, and **19-21** to give the benzo-δ-sultam **6**, and **22-24**, can be proposed based upon work by Barange et al., and starts with the coordination of the alkyne to M(I) and formation of a π-complex **A-1** (Scheme **5**).[28] The base, Et_3N, activates the *N* atom of the sulfonamide group, resulting in a regioselective *6-endo-dig* nucleophilic attack onto the π-complex **A-1**, and the formation of a M(I)-vinyl species **A-2**. Subsequent *in situ*

protonation furnishes the desired benzo-δ-sultams **6**, **22-24**. It has been reported that the M(I)-vinyl species may undergo allylation instead of protonation if some allyl halides are present in the reaction mixture.[28]



Scheme 5. Proposed mechanism for the formation of benzo-δ-sultam.[28]

The *6-endo-dig* ring closure was confirmed by 2D NOESY and HMBC, the *endo-dig* cyclisation being favoured possibly due to less geometric constraint.[28] The *6-endo-dig* ring closure was also confirmed by the single crystal X-ray analysis of **22** (Figure **3**).[34]

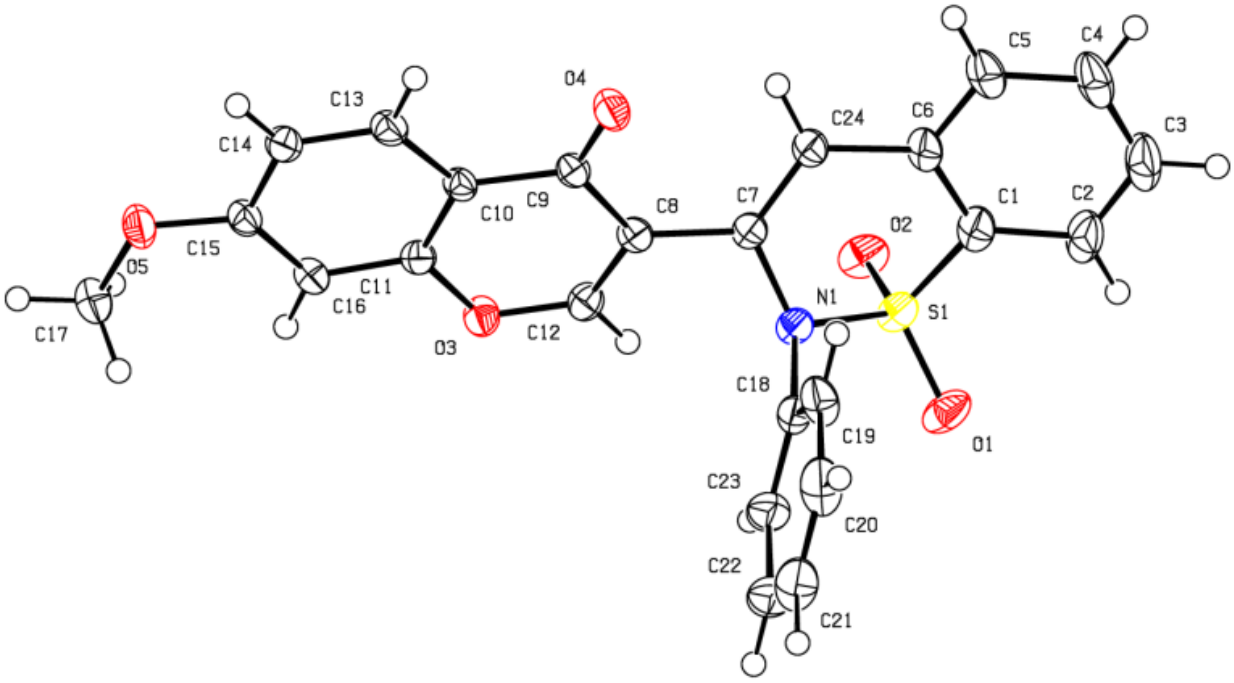
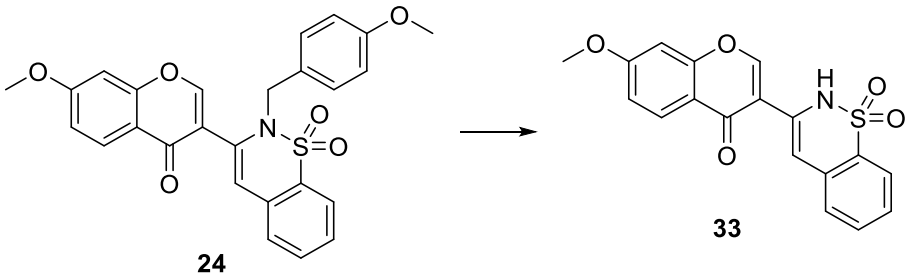


Figure 3. X-ray structure of isoflavone/benzo-δ-sultam hybrid **22**.

The PMB-protected hybrid **24** was deprotected using trifluoroacetic acid[35] to furnish δ-sultam **33** in fair yield (53%, Scheme **6**).



Scheme 6. Deprotection of PMB. *Reagents and conditions*: CF_3COOH, N_2, RT, 6 h, 53%.

A rapid screening of the synthesised compounds was performed on LPS-activated BV2 mouse microglial cells to determine compound potential as anti-inflammatory drugs.[36] Comparisons were made to the known[37-39] anti-inflammatory isoflavone natural products daidzein and biochanin A, the structures of which are shown in Figure **1**. Cell viability and NO inhibitory activity were evaluated at 20 µM compound concentration for the synthesised compounds, and compared to those of daidzein and biochanin A, as shown in Figures **4** and **5** (daidzein is compound **34**, and biochanin A is compound **35**). We also determined TNF-α inhibitory activity for the most active compounds, based upon comparisons to the controls daidzein **34** and biochanin A **35**, as described below. Daidzein[37] and biochanin A[38, 39] were chosen as they have good cell viability and are known to inhibit the production of NO and TNF-α in LPS-activated BV2 cells. The screening results revealed that most of the synthesised compounds may have anti-inflammatory activity, and therefore be neuroprotective by inhibiting the pro-inflammatory mediators that damage neurons. This way, it is expected that the synthesised compounds may be able to inhibit the microglia activation or shift the potential neurodegenerative role of activated microglia back to its neuroprotective role.

Cell viability was evaluated using the XTT assay after incubating the cells for 24 h in the presence or absence of synthesised compounds.[40] The assay was performed to show that the tested compounds (20 µM compound concentration) are not cytotoxic to the BV2 cells, and to demonstrate that the reduction of NO and TNF-α production is not due to cytotoxicity of the compounds. In most cases the compounds were non-toxic as there was no significant difference between control cells and the cells treated with the synthesised compounds (Figure **4**). Intermediates **5** and **19**, and hybrids **6** and **33** slightly affected the viability of the cells, while precursor **13** (49% cell viability) affected significantly the viability of the cells. With the control compound biochanin A having a cell viability of ~76% ± 6% (Figure **4**), we selected ≥80% as the threshold for an acceptable cell viability (as seen in Figure **4**, daidzein showed a cell viability much higher than 80%). Other studies[37-39] have also shown that daidzein and biochanin A show low cell toxicity to BV2 cells at these concentrations.

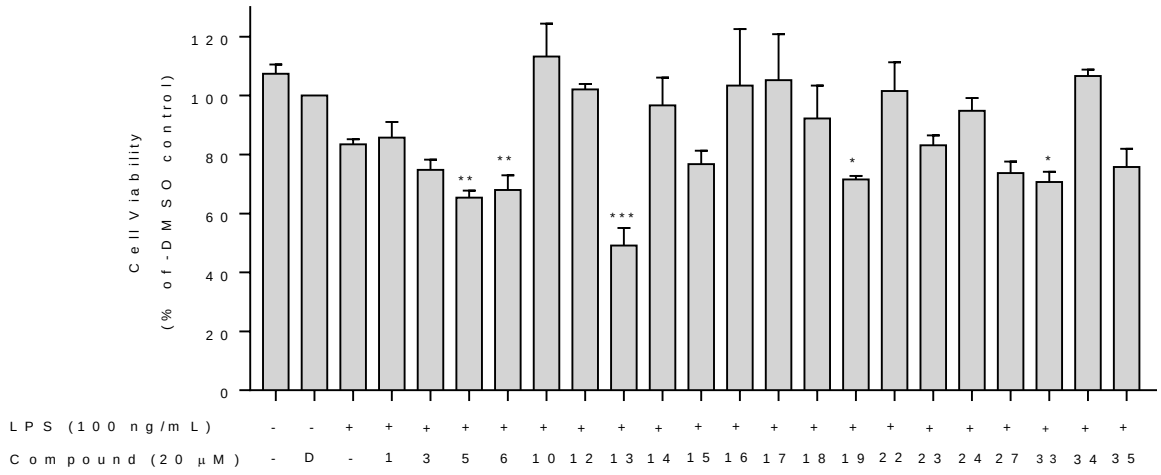


Figure 4. Cell viability of BV2 microglia cells treated with the synthesised compounds. LPS-activated BV2 microglia cells were treated or not with the synthesised compounds (20 μM) for 24 h, then with XTT solution for 2 h. Values are expressed as mean ± SEM for three experiments. Statistical analysis was performed using one-way ANOVA with post-hoc Tukey's multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with to negative control cells that contained the amount of DMSO used to add the compound solutions in the cell medium (- D, 100%).

Nitric oxide (•N=O) production was measured indirectly through NO_2^- production, a stable NO breakdown product, by using the Griess Assay.[41] NO is a multifunctional mediator found in most cells of the body.[42] NO is generated from L-arginine by NO synthase, and is a powerful neurotransmitter, an immune defence molecule, and endothelium-derived relaxing factor. In inflammation, NO is produced in higher quantities and acts as a cytotoxic effector molecule to remove noxious agents. However, overproduction damages the healthy tissues and NO becomes an inflammatory mediator leading sometimes to cell death. It can be seen in Figure **5** that NO is produced in small amounts in normal cells (20% NO production), but when the cells are LPS-activated, NO production increases significantly (100% NO production). There is a great interest in compounds that inhibit NO production, since they may have beneficial effects in various diseases including neurodegenerative diseases.

Synthesised compounds (20 μM) were tested for their ability to inhibit NO production in LPS-activated BV2 microglial cells and were again compared to the isoflavone natural products daidzein and biochanin A (Figure **5**), chosen because of their known[37-39] ability to inhibit the production of NO in LPS-activated BV2 cells. Activating the cells with LPS led to a significant increase in NO production compared to control cells. Treating the LPS-activated cells with the synthesised compounds resulted in a significant inhibition of NO production, with **10** (21% NO production) and **27** (21%) being the most active, followed by hybrids **33** (24%) and **24** (41%), and isoflavones **3** (26%) and **1** (42%). The benzo-δ-sultam/isoflavone hybrids **6**, **22**-**24**, and **33** were active, significantly decreasing the NO production.

In general, the sulfonamides **3**, **5**, and **19** showed reasonable NO inhibition but showed some level of cell toxicity. The *N*-aryl substituted sultams **22** and **24** were active as NO inhibitors and showed good cell viability. Replacement of the *N*-aryl group with an NH (**33**) improved NO inhibition, but decreased cell viability, whereas replacement with *N*-methyl (**23**) significantly decreased NO inhibition activity. *N*-methyl sulfonamides (**15**, **17**, **23)** in general showed poor NO inhibition. The *p*-nitro-substituted

isoflavone **12** was not toxic to cells, but did not inhibit NO, whereas the corresponding *p*-amino compound **1** showed good inhibitory activity and is perhaps the compound that is most similar to the natural isoflavones. The simple alkynyl compound **21** was very active, but also cytotoxic, whereas the corresponding trimethylsilyl alkyne **27** was just as active but less toxic. The simple iodo compound **10** was one of the least cytotoxic and most potent NO inhibitors tested, revealing that simple substituted chromones still offer interest.

The biochanin A and daidzein control compounds in Figure **5** inhibit NO production at levels of 41% ± 4% and 35% ± 2%, respectively. In order to select isoflavone/chromone compounds for further study, we used the ≥80% cell-viability figure from above together with the NO control data for biochanin A and daidzein, which led us to use ≤45% NO production as the NO selection criteria.

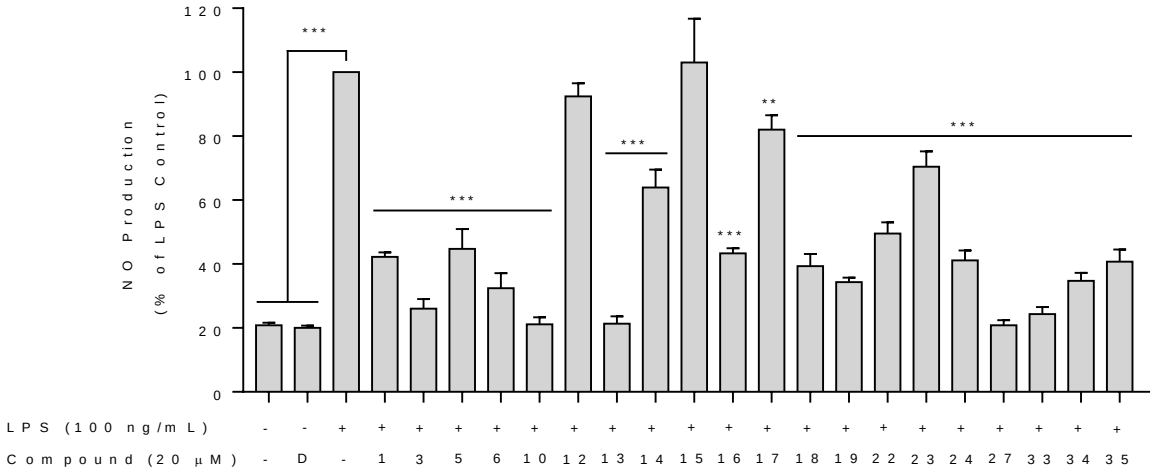


Figure 5. NO production in LPS-activated BV2 microglia cells. LPS-activated BV2 microglia cells were treated or not with the synthesised compounds (20 µM) for 24 h. The supernatants were collected, and NO was assessed using Griess assay. Values are expressed as mean ± SEM for three experiments. Statistical analysis was performed using one-way ANOVA with post-hoc Tukey's multiple comparisons test. **p < 0.01, ***p < 0.001 in comparison with LPS control.

An important pro-inflammatory cytokine in activated microglia and subsequently in neuroinflammation is tumour necrosis factor α (TNF-α). TNF-α, in low concentrations, may protect the brain and act as a defence mechanism.[43] However, in higher concentrations TNF-α may promote neuronal degeneration.[44]

TNF-α production was assessed using the enzyme-linked immunosorbent assay (ELISA) on supernatants collected from LPS-activated BV2 microglia cells treated or not with the corresponding compounds.[45] On the basis of the rapid screening results discussed above, just three isoflavone/chromone compounds showing good cell viability (≥ 80%) with high inhibition of NO production (≤ 45% production of NO) were assessed. The results are presented in Figure **6**. The negative control presented a small TNF-α production of 12%, while the activation of the cells with LPS (100 ng/mL) led to a significant increase in TNF-α production and acted as the positive control. Treating the LPS-activated cells with simple isoflavone **1**, TNF-α production was reduced to 55%, **1** showing only moderate inhibitory activity of TNF-α. The 3-iodochromenone **10** and the only benzosultam screened, compound **24**, showed good inhibitory activity, significantly reducing the TNF-α production

to 26% and 34% respectively. At the same concentration, biochanin A was assessed as bringing about a similarly significant reduction (to ~33%) in the production of LPS-induced inflammatory TNF-α in BV2 cells,[39] and also showed significant activity at lower concentrations.[38] Daidzein[37] is less active.

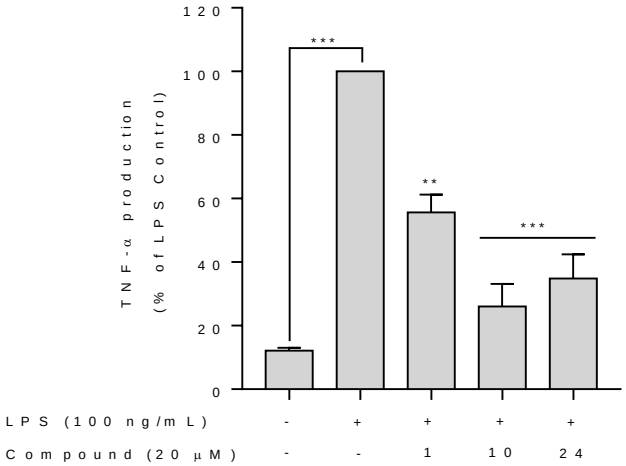


Figure 6. TNF-α production in LPS-activated BV2 microglia cells. LPS-activated BV2 microglia cells were treated or not with compounds **1**, **10** or **24** (20 μM) for 24 h. The supernatants were collected and TNF-α was assessed using ELISA. Data are expressed as mean ± SEM for three experiments. Statistical analysis was performed using one-way ANOVA with post-hoc Tukey's multiple comparisons test. **p < 0.01, ***p < 0.001 in comparison with LPS control.

In order to provide us with some further information into the potential of these compounds, we used the predicted pharmacokinetic properties presented in Table **2**. Whilst actual pharmacokinetic experiments were beyond the scope of this study, these *in silico* predictions offer some useful insights. Thus, most of the synthesised compounds are predicted to permeate the blood-brain barrier (BBB), an important factor for CNS drugs.[46] Also, most of the compounds present good predicted human intestinal absorption, and low to good aqueous solubility. These factors indicate that the compound might be administered orally, in relatively small concentrations, and are predicted to be in solution at the place of absorption, with good predicted absorption.[47]

We find it of interest that compounds **1**, **3**, **10, 24**, **27** and **33**, all of which showed significant anti-inflammatory activity, also exhibit promising predicted BBB permeability (medium level) and good solubility and absorption, making them good candidates for further development of neuroprotective drugs. Compounds **10** and **24**, our most promising compounds, show acceptable predicted pharmacokinetic data, and also show non-experimental molecular descriptors (MW ≤ 1000, HBA ≤ 16, HBD ≤ 6, RB ≤ 20, PSA ≤ 250) that are within recently assessed sensible ranges for potential drug candidates and leads.[48, 49]

Table 2. *In silico* prediction of some pharmacokinetic properties for synthesised compounds

Compound No	MW	HBD	HBA	RB	AlogP98	PSA 2D	BBB Level	SL	AL
1	267.28	1	4	2	2.10	61.70	2	3	0
3	486.34	1	5	5	4.09	82.57	2	2	0
5	507.56	1	5	7	5.45	82.57	4	1	1

6	507.56	0	5	4	4.98	73.12	1	1	0
10	302.07	0	3	1	1.65	35.16	2	3	0
12	297.26	0	5	3	2.74	77.98	3	2	0
13	200.19	0	3	1	2.46	35.16	1	3	0
14	312.18	1	2	3	3.07	47.41	1	2	0
15	250.11	1	2	2	1.49	47.41	2	3	0
16	359.18	1	2	3	2.90	47.41	2	3	0
17	297.11	1	2	2	1.32	47.41	2	3	0
18	403.24	1	3	5	2.89	56.34	2	3	0
19	431.46	1	5	6	3.93	82.57	2	2	0
22	431.46	0	5	3	3.46	73.12	2	2	0
23	369.39	0	5	2	1.89	73.12	3	3	0
24	475.51	0	6	5	3.45	82.05	2	2	0
27	272.37	0	3	3	3.30	35.16	1	2	0
33	355.37	1	5	2	1.68	82.57	3	3	0

MW: molecular weight; HBD: H-bond donors; HBA: H-bond acceptors; RB: rotatable bonds; AlogP98: lipophilicity (logarithm of the partition coefficient between octanol and water, atom-type value); PSA-2D: two-dimensional polar surface area; BBB level: blood-brain barrier permeation level (0 = very high; 1 = high; 2 = medium; 3 = low; 4 = undefined); SL: solubility level, predicted level of aqueous solubility in water at 25 °C (1 = very low; 2 = low; 3 = good; 4 = optimal; 5 = very soluble); AL: absorption level, predicted level of human intestinal absorption (0 = good; 1 = moderate; 2 = low; 3 = very low).

To conclude, the synthesis of five novel benzo-δ-sultam/isoflavone hybrids **6, 22-24** and **33** was achieved using a two-step reaction by coupling 2-halobenzenesulfonamide derivatives with terminal alkynes, followed by a 6-endo-dig cyclisation. The anti-inflammatory activity of the benzo-δ-sultam/isoflavone hybrids and some of their precursors was assessed using LPS-stimulated BV2 cells. Cell viability, and NO inhibition assays results showed that several compounds are active and not cytotoxic at the concentrations investigated. Three of the most active, non-cytotoxic compounds performed better than the daidzein and biochanin A controls, and so were evaluated for their inhibitory activity towards TNF-α production, revealing precursor **10** and benzo-δ-sultam/isoflavone hybrid **24** as the most active, with activity levels comparable to those reported for biochanin A: these compounds will be taken forward for further pharmacological evaluation.

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Electronic supplementary material

The experimental data, ^{1}H and ^{13}C-NMR spectra for precursors and new compounds are available in the supplementary material.

References and notes

1. S. O. Bachurin, E. V. Bovina and A. A. Ustyugov, *Med. Res. Rev.*, 2017, **37**, 1186-1225.
2. A. Martinez, M. D. Palomo Ruiz, D. I. Perez and C. Gil, *Expert Opin. Investig. Drugs*, 2017, **26**, 403-414.

3. M. L. Bolognesi, *Future Med. Chem.*, 2017, **9**, 707-709.
4. P. Bawa, P. Pradeep, P. Kumar, Y. E. Choonara, G. Modi and V. Pillay, *Drug Discov. Today*, 2016, **21**, 1886-1914.
5. J. Yu, X. Bi, B. Yu and D. Chen, *Nutrients*, 2016, **8**, 361.
6. C. Spagnuolo, S. Moccia and G. L. Russo, *Eur. J. Med. Chem.*, 2018, **153**, 105-115.
7. L. Kole, B. Giri, S. K. Manna, B. Pal and S. Ghosh, *Eur. J. Pharmacol.*, 2011, **653**, 8-15.
8. K. P. Ho, L. Li, L. Zhao and Z. M. Qian, *Mol. Cell. Biochem.*, 2003, **247**, 219-222.
9. K. P. Devi, B. Shanmuganathan, A. Manayi, S. F. Nabavi and S. M. Nabavi, *Mol. Neurobiol.*, 2017, **54**, 7028-7041.
10. J.-W. Jeong, H. H. Lee, M. H. Han, G.-Y. Kim, W.-J. Kim and Y. H. Choi, *Chem. Biol. Interact.*, 2014, **212**, 30-39.
11. S. Xu, C. A. Rouzer and L. J. Marnett, *IUBMB Life*, 2014, **66**, 803-811.
12. K. C. Majumdar and S. Mondal, *Chem. Rev.*, 2011, **111**, 7749-7773.
13. S. Debnath and S. Mondal, *E. J. Org. Chem.*, 2018, **2018**, 933-956.
14. R. N. Brogden, R. C. Heel, T. M. Speight and G. S. Avery, *Drugs*, 1984, **28**, 292-323.
15. A. M. Teema, S. A. Zaitone and Y. M. Moustafa, *Neuropharmacology*, 2016, **107**, 432-450.
16. J. H. Park, Y. S. Park, J.-B. Lee, K.-H. Park, M.-k. Paik, M. Jeong and H. C. Koh, *J. Appl. Toxicol.*, 2016, **36**, 10-23.
17. Y. Sui, D. Stanić, D. Tomas, B. Jarrott and M. K. Horne, *Neurosci. Lett.*, 2009, **460**, 121-125.
18. E. Spangenberg, P. L. Severson, L. A. Hohsfield, J. Crapser, J. Zhang, E. A. Burton, Y. Zhang, W. Spevak, J. Lin, N. Y. Phan, G. Habets, A. Rymar, G. Tsang, J. Walters, M. Nespi, P. Singh, S. Broome, P. Ibrahim, C. Zhang, G. Bollag, B. L. West and K. N. Green, *Nat. Commun.*, 2019, **10**, 3758.
19. N. T. Hiep, J. Kwon, D. W. Kim, B. Y. Hwang, H. J. Lee, W. Mar and D. Lee, *Phytochemistry*, 2015, **111**, 141-148.
20. X. Wang, S. Chen, G. Ma, M. Ye and G. Lu, *Neuroreport*, 2005, **16**, 267-270.
21. R. Velagapudi, M. Aderogba and O. A. Olajide, *Biochim. Biophys. Acta, Gen. Subj.*, 2014, **1840**, 3311-3319.
22. S. Debnath and S. Mondal, *J. Org. Chem.*, 2015, **80**, 3940-3948.
23. D. Rambabu, P. V. N. S. Murthy, K. R. S. Prasad, A. Kandale, G. S. Deora, M. V. Basaveswara Rao and M. Pal, *Tetrahedron Lett.*, 2012, **53**, 6577-6583.
24. J. Tummatorn, S. Ruchirawat and P. Ploypradith, *Chem. Eur. J.*, 2010, **16**, 1445-1448.
25. N. Gavande, N. Karim, G. A. Johnston, J. R. Hanrahan and M. Chebib, *ChemMedChem*, 2011, **6**, 1340-1346.
26. R. Gammill, *Synthesis*, 1979, **1979**, 901-903.
27. W. Wang, Y. He, P. Xu, Q. You, H. Xiao and H. Xiang, *Bioorg. Med. Chem.*, 2015, **23**, 4428-4433.
28. D. K. Barange, T. C. Nishad, N. K. Swamy, V. Bandameedi, D. Kumar, B. R. Sreekanth, K. Vyas and M. Pal, *J. Org. Chem.*, 2007, **72**, 8547-8550.
29. Step **i**. Into a Schlenk flask were added **18** (85 µmol, 34.2 mg), 10% Pd/C (2.55 µmol, 2.7 mg, 3 mol %), PPh_3 (10.2 µmol, 2.7 mg, 12 mol %), CuI (4.25 µmol, 0.81 mg, 5 mol %) and **13** (97.75 µmol, 19.6 mg), and the mixture was placed under vacuum and backfilled with N_2 three times. The cap was changed with a septum stopper under a positive pressure of N_2 and the flask was placed on an ice bath. Dry MeCN (1.5 mL, degassed) was added, and the resulting suspension was degassed with N_2 for 10 min at 0 °C. Et_3N (255 µmol, 36 µL) was added dropwise at 0 °C, and stirred for 5 min. The reaction mixture was allowed to warm to RT, the septum stopper was replaced with a lid under a positive pressure of N_2, and after the reaction was stirred overnight at 80 °C. The resulting mixture was filtered through Celite™, washed with DCM (25 mL), and the filtrate concentrated under vacuum to dryness. The crude was purified by flash chromatography (CH_2Cl_2/acetone, 100:1) to give a mixture of the intermediate **21** with the final product **24** (approximately 10:1, 14 mg) as a pale yellow solid; R_f = 0.35 (CH_2Cl_2/acetone, 98:2). The starting **18** (4 mg, 11%) was also separated. Step **ii**. The obtained mixture (14 mg) was dissolved in dry ethanol (1 mL) under N_2. Et_3N (88 µmol, 12 µL) and $AgNO_3$ (6 µmol, 1 mg) were added, and the mixture was stirred at 80 °C for 10 min. The resulting mixture was filtered through Celite™, washed with CH_2Cl_2 (20 mL), and the filtrate concentrated under vacuum to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/acetone, 100:1) to give **24** (12.5 mg, 31% yield over two steps) as a white solid; mp = 212-213 °C; R_f = 0.35 (CH_2Cl_2/acetone, 98:2); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.25 – 8.18 (m, 2 H), 7.75 (app d, J = 7.6 Hz, 1 H), 7.53 (s, 1 H), 7.46 (td, J = 1.3, 7.5 Hz, 1 H), 7.40 (td, J = 1.2, 7.5 Hz, 1 H), 7.30 (app d, J = 7.0 Hz, 1 H), 7.05 (dd, J = 2.3, 8.9 Hz, 1 h), 6.91 – 6.85 (m,

3 H), 6.49 (d, J = 8.7 Hz, 2 H), 4.55 (s, 2 H), 3.94 (s, 3 H), 3.62 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 174.6 (qC), 164.4 (qC), 159.1 (qC), 157.4 (qC), 156.7 (CH), 133.6 (qC), 133.3 (qC), 132.5 (qC), 131.9 (CH), 130.2 (2 CH), 128.5 (CH), 128.3 (CH), 127.6 (CH), 125.7 (qC), 122.3 (CH), 120.3 (CH), 118.2 (qC), 117.9 (qC), 115.3 (CH), 113.1 (2 CH), 100.2 (CH), 55.9 (CH_3), 55.1 (CH_3), 52.8 (CH_2); HRMS (Dual AJSESI): calc m/z for $C_{26}H_{21}NO_6S$: 475.1090 [M], 476.1162 $[M+H]^+$; found: 475.1098 [M], 476.1171 $[M+H]^+$; FT-IR (cm^{-1}): υ = 3076, 2981, 2835, 1641, 1596, 1513, 1436, 1337, 1245, 1172, 1074, 853, 763, 665; Hybrids **22** and **23** were synthesised in a similar manner.

30. M. O. Akram, S. Bera and N. T. Patil, *Chem. Commun.*, 2016, **52**, 12306-12309.
31. P. Bhattacharya and A. Basak, *Tetrahedron Lett.*, 2013, **54**, 5137-5139.
32. T. Patonay, I. Pazurik and A. Ábrahám, *Aust. J. Chem.*, 2013, **66**, 646-654.
33. P. D. Bartlett and L. Knox, *Organic Synth.*, 1965, 12-12.
34. CCDC deposit number 2020033. Crystallographic data has been deposited at the Cambridge Crystallographic Data Centre. Copies of this data can be obtained free of charge on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, UK at www.ccdc.cam.ac.uk/data_request/cif.
35. Z. Bluke, E. Paass, M. Sladek, U. Abel and V. Kauss, *J. Enzyme Inhib. Med. Chem.*, 2016, **31**, 664-673.
36. The BV2 mouse brain microglial cells, cell line ICLCATL03001, was purchased from Interlab Cell Line Collection - Banca Biologica e Cell Factory, Italy. The cells were cultured in Gibco™ Roswell Park Memorial Institute (RPMI) 1640 Medium (Life Technologies). The RPMI medium was supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Sigma), 1 mM sodium pyruvate (Sigma), and streptomycin (100 units/mL) – penicillin G (100 mg/mL) (Sigma) to obtain the complete RPMI medium. The cells were stimulated with LPS from *Salmonella typhimurium*, S-form TLRpure™ Sterile Solution (Innaxon Biosciences). The cells were maintained at 37 °C in 5% CO_2 humidified atmosphere.
37. Y. Geng, S. Zhu, P. Cheng, Z.-M. Lu, H.-Y. Xu, J.-S. Shi and Z.-H. Xu, *Phytomedicine*, 2017, **26**, 55-61.
38. W.-Y. Wu, Y.-Y. Wu, H. Huang, C. He, W. Z. Li, H.-L. Wang, H.-Q. Chen and Y.-Y. Yin, *Int. J. Mol. Med.*, 2015, **35**, 391-398.
39. Y. Zhang and W.-a. Chen, *Neurochem. Res.*, 2015, **40**, 165-171.
40. BV2 cells were seeded out in a 96-well plate at a concentration of 2×10^5 cells/mL and cultured at 37 °C in 5% CO_2 humidified atmosphere until 80% confluency. Prior to experimentation, the culture medium was changed to serum free RPMI. Subsequently the BV2 cells were treated with the synthesised compounds (20 μM) 30 min prior to stimulation with LPS (100 ng/mL) and incubated for 24 h. XTT assay was conducted by adding XTT solution (concentration) to cells in each well containing 100 μL culture medium, and then incubated for 2 h at 37 °C in 5% CO_2. Thereafter, the absorbance was read using the microplate reader at 450 nm.
41. Griess Assay was conducted using the Griess Assay System (Promega). 50 μL of culture supernatants were pipetted into each well of a 96-well plate. Then, the culture medium was incubated with 50 μL sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) in the dark for 10 min. Subsequently, 50 μL of NED solution (0.1% N-1-napthylethylenediamine dihydrochloride in water) was added, and the plate was incubated in the dark for 10 min. After, the absorbance was read at 540 nm.
42. H. Kolb and V. Kolb-Bachofen, *Immunol. Today*, 1992, **13**, 157-160.
43. M. T. Heneka and M. K. O'Banion, *J. Neuroimmunol.*, 2007, **184**, 69-91.
44. V. H. Perry, J. A. R. Nicoll and C. Holmes, *Nat. Rev. Neurol.*, 2010, **6**, 193.
45. BV2 cells were seeded out in a 48-well plate at a concentration of 2×10^5 cells/mL and cultured at 37 °C in 5% CO_2 humidified atmosphere until 80% confluency. Prior to experimentation, the culture medium was changed to serum free RPMI. Subsequently the BV2 cells were treated with the synthesised compounds (20 μM) 30 min prior to stimulation with LPS (100 ng/mL) and incubated for 24 h. After, supernatants were collected, centrifuged at 2500 rpm for 5 min at 4 °C, and analysed for levels of TNF-α using Mouse TNF-α ELISA MAX™ Deluxe Set (BioLegend, UK).
46. The values for *in silico* prediction of some pharmacokinetic properties were obtained using the ADMET Descriptors in BIOVIA Discovery Studio 2016 (BIOVIA, San Diego, USA).
47. K. T. Savjani, A. K. Gajjar and J. K. Savjani, *ISRN pharmaceutics*, 2012, **2012**.
48. M. D. Shultz, *J. Med. Chem.*, 2019, **62**, 1701-1714.
49. Bradley C. Doak, B. Over, F. Giordanetto and J. Kihlberg, *Chem. Biol.*, 2014, **21**, 1115-1142.