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Synthesis, anti-cancer and anti-inflammatory activity of novel 2-substituted isoflavenes

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ABSTRACT

Fifteen novel 2-substituted isoflavenes were synthesised via nucleophilic addition to isoflavylium salts. Twelve of the newly synthesised isoflavenes, along with the unsubstituted parent isoflavene, were tested in cell viability assays against the SHEP neuroblastoma and MDA-MB-231 breast adenocarcinoma cell lines. While the 2-substituted isoflavenes displayed a range of anti-proliferative activities, in most cases they were less active that the unsubstituted isoflavene (IC_{50} = 9.9 μM vs SHEP; IC_{50} = 33 μM vs MDA-MB-231). However, compound **7f**, derived from the reaction between isoflavylium salt **5** and *para*-methoxyacetophenone, showed improved anti-proliferative activity against breast cancer cells (IC_{50} = 7.6 μM). Furthermore, compound **7f**, as well as analogues **7a**, **7c**, **11d** and **14**, inhibited the production of interleukin-6 in LPS-activated RAW 264.7 cells.

1. Introduction

Isoflavonoid compounds are the subject of considerable research interest due to their range of biological activities in humans. These include agonistic and antagonistic interactions with estrogen receptors,[1–6] as well as anti-inflammatory[7–13] and anti-cancer activity.[14–20] The isoflav-3-ene **1** (Fig. 1) has demonstrated cytotoxic, anti-proliferative and anti-angiogenic activity against a variety of cancer types, notably ovarian and prostate cancers.[15,16] Isoflavene **1** is also highly selective in its mode of action, exhibiting minimal toxicity against normal cells. More recently, a series of oxazinyl isoflavenes and isoflavans were shown to inhibit the growth of several cancer cell lines in vitro.[17] Furthermore, isoflavonoid compounds have been shown to induce apoptosis in ovarian cancer stem cells via inhibition of the mammalian target of rapamycin.[18–20] Cancer stem cells are implicated in the development of recurrent and chemoresistant tumours and are resistant to conventional chemotherapies.[21] The development of isoflavonoid anti-cancer agents therefore represents a promising new strategy for cancer treatment.

The anti-inflammatory properties of isoflavonoid compounds have been reported in a variety of in vitro and in vivo models of conditions such as asthma,[7] arthritis,[8] UV-induced skin damage[9] and cardiovascular disease.[10] Isoflavonoid compounds have been reported to inhibit the production of a variety of inflammatory signalling molecules, such as interleukin (IL)-6, tumour necrosis factor (TNF)-α and nitric oxide.[8,9,11] In addition to these modes of action, isoflavonoid compounds can prevent tissue damage via their antioxidant capability.[3,12,13] The polyphenolic character of many isoflavenes facilitates both free radical scavenging[12] and the chelation of transition metals.[13]

Many biologically active isoflavonoid compounds bear one or more aryl substituents on the pyran ring. The 4-aryl isoflavan **2** is a potent cytotoxic and anti-proliferative agent currently under investigation as a treatment for pancreatic and bile duct cancers.[22] The 2-aryl isoflavene **3** is a selective estrogen receptor modulator developed for the treatment of hormone-dependent breast cancer.[23,24] The 2,3-diarylbenzopyran motif is shared by a number of other biologically active species.[25–27] The aim of the present study was to explore the chemistry, anti-cancer activity and anti-inflammatory activity of a variety of 2-substituted isoflavenes, with a particular focus on those with aryl moieties on the C2 substituent.

Several synthetic routes to 2-substituted isoflavonoid compounds have previously been reported. Some of the strategies

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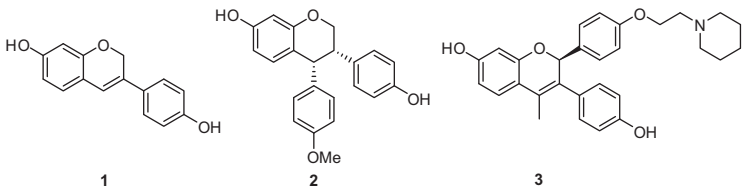


Figure 1. Structures of some biologically active isoflavonoid compounds.

employed include the cyclisation of deoxybenzoins,[28,29] condensation of enamines with salicylaldehydes[30] and Grignard addition to coumarins.[31] The treatment of isoflavylium salts with nucleophiles has been established as a versatile route to 2-substituted isoflavenes. Previously, isoflavylium salts have been reported to react with a variety of nucleophiles including amines,[32] thiols,[33] alcohols and trimethylsilanes.[34] We have expanded upon this chemistry to generate a variety of novel products. Herein, we report the synthesis, characterisation and in vitro biological activities of a series of newly synthesised 2-substituted isoflavenes.

2. Results and discussion

2.1. Synthesis of 2-substituted isoflavenes

An isoflavylium hexafluorophosphate salt was prepared using the hydride abstraction strategy employed by Faragalla et al.[34] Isoflavene **1** was acetylated to give compound **4**, which was treated with tritylium hexafluorophosphate in CH_2Cl_2 at room temperature to generate salt **5** in a yield of 91% (Scheme 1). The formation of the salt was confirmed by ^{1}H NMR spectroscopy. In the isoflavene, the two protons at C2 gave rise to a signal at δ 5.15 which appeared as a fine doublet (J = 1.4 Hz) due to allylic coupling with H4. The H4 proton appeared as a broad singlet at δ 6.76. In the isoflavylium salt, H2 and H4 appeared as doublets (J = 2.0 Hz) at δ 9.93 and 9.85.

Salt **5** was treated with a series of α-methyl ketones (Scheme 2). These reactions proceeded in the absence of any added base, indicating that the isoflavylium salt itself may have initiated the formation of the reactive enolate species. The yield of **6b** was significantly lower than those of the other analogues. This is thought to have been the result of a side reaction at the second α-carbon on the methyl butyl ketone. The structure of analogue **6a** was established via ^{1}H NMR spectroscopy. The H2 proton appeared at δ 5.93, which is downfield of the corresponding signal (δ 5.15) in the spectrum of the parent isoflavene **4**. The H2 proton appeared as a doublet of doublets, due to coupling with the diastereotopic protons of the neighbouring methylene group. The methylene protons appeared as doublets of doublets at δ 3.16 and 2.36. Similar chemical shifts and splitting patterns were observed for analogues **6b–i** and the deacetylated products **7a–i**, which were obtained by treatment with methanolic potassium hydroxide.

The carbonyl group in isoflavenes **6a–i** and **7a–i** provides a starting point for further chemical elaboration. One facile example is the formation of a hydrazone. Isoflavene **6a** was treated with 2,4-dinitrophenylhydrazine at reflux in ethanol to give the hydrazone **8** in 67% yield (Scheme 3). The phenolic analogue **9** was obtained in 91% yield by deacetylation of compound **8** with potassium hydroxide.

When the isoflavylium salt **5** was treated with 2-acetylpyrrole, the reaction occurred not at the α-methyl group, but at position 4 on the pyrrole ring, to give product **10a** (Scheme 4). In the ^{1}H NMR spectrum of **10a**, H2 appeared as a broad singlet at δ 6.19. The protons on the pyrrole ring appeared as two multiplets (each integrating for one proton) at δ 6.93 and 6.87. The methyl group appeared as a singlet at δ 2.25. In a similar manner, the isoflavylium salt **5** reacted with 3-substitued indoles and 2,6-dimethylaniline to generate 2-arylisoflavenes **10b–d**. Compounds **10a–d** were treated with aqueous potassium hydroxide to give the phenolic isoflavenes **11a–d**.

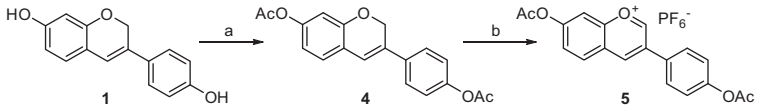
As 4-substituted isoflavonoid compounds are known to possess anti-cancer properties,[22] the synthesis of a 4-substituted isoflavylium salt was investigated. Attempts to form the isoflavylium salt of 4-substituted isoflavene **12** under the hydride abstraction conditions described in Scheme 1 were complicated by the failure of the salt to precipitate from the reaction mixture. A complex mixture of products was observed, believed to have been the result of reactions between the isoflavylium salt and the isoflavene **12**. Instead, isoflavene **12** was treated with thallium trifluoroacetate in trifluoroacetic acid, followed by concentrated HCl to give the isoflavylium chloride salt **13** in 50 % yield (Scheme 5). The TBDMS groups were cleaved during the synthesis of **13**, as indicated by the absence of signals at <2 ppm in the ^{1}H NMR spectrum. Salt **13** was treated with acetone to generate the 2,4-disubstituted isoflavene **14**.

2.2. Biological activity

2.2.1. Anti-cancer activity

The anti-proliferative properties of isoflavenes **7a–i**, **9**, **11d**, and **14** were assessed in vitro using MDA-MB-231 breast adenocarcinoma cells and SHEP neuroblastoma cells. As shown in Figure 2, all compounds inhibited cell proliferation in a dose-dependent manner.

The newly synthesised 2-substituted isoflavenes exhibited IC_{50} values ranging from 33 μM to 377 μM against SHEP cells (Table 1), and were therefore less active than the parent isoflavene **1** (IC_{50} = 9.9 μM). The most active of the 2-substituted compounds were **7f** and **7g**, derived from *para*-methoxy- and *para*-bromoacetophenone, respectively. The substituent on the phenyl ring in compounds **7c–h** has a significant impact on anti-proliferative activity. For example, substitution of chlorine (**7h**) for bromine



Scheme 1. Reagents and conditions: (a) Ac_2O, K_2CO_3, acetone, reflux; (b) Ph_3CPF_6, CH_2Cl_2, rt.