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Original article

Synthesis and biological evaluation of flavones and benzoflavones as inhibitors of BCRP/ABCG2

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ABSTRACT

Multidrug resistance (MDR) often leads to a failure of cancer chemotherapy. Breast Cancer Resistance Protein (BCRP/ABCG2), a member of the superfamily of ATP binding cassette proteins has been found to confer MDR in cancer cells by transporting molecules with amphiphilic character out of the cells using energy from ATP hydrolysis. Inhibiting BCRP can be a solution to overcome MDR. We synthesized a series of flavones, 7,8-benzoflavones and 5,6-benzoflavones with varying substituents at positions 3, 3′ and 4′ of the (benzo)flavone structure. All synthesized compounds were tested for BCRP inhibition in Hoechst 33342 and pheophorbide A accumulation assays using MDCK cells expressing BCRP. All the compounds were further screened for their P-glycoprotein (P-gp) and Multidrug resistance-associated protein 1 (MRP1) inhibitory activity by calcein AM accumulation assay to check the selectivity towards BCRP. In addition most active compounds were investigated for their cytotoxicity. It was observed that in most cases 7,8-benzoflavones are more potent in comparison to the 5,6-benzoflavones. In general it was found that presence of a 3-OCH_3 substituent leads to increase in activity in comparison to presence of OH or no substitution at position 3. Also, it was found that presence of 3′,4′-OCH_3 on phenyl ring lead to increase in activity as compared to other substituents. Compound **24**, a 7,8-benzoflavone derivative was found to be most potent being 50 times selective for BCRP and showing very low cytotoxicity at higher concentrations.

1. Introduction

Cancer is the main cause of millions of deaths worldwide. Since decades chemotherapy has been a major form of the treatment for different types of cancers. Unfortunately, the majority of cancers are either resistant to chemotherapy or acquire multidrug resistance (MDR) during treatment. MDR is an acquired drug resistance by tumour cells that consists of simultaneous emergence of cellular resistance to toxic action of chemotherapeutic drugs originally used and to other chemicals having different chemical structure and mechanism of action. As a result of MDR, chemotherapeutic agents fail to target the tumour cells and cancer becomes untreatable by chemotherapy [1,2].

One of the mechanisms by which tumours develop multidrug resistance is over expression of efflux transport proteins, especially certain ATP-binding cassette (ABC) transporters in the plasma membrane of cancer cells. ABC transporters utilize energy obtained from hydrolysis of ATP to efflux chemically unrelated compounds [3–5]. Until now 48 ABC transporters have been identified in humans.

P-glycoprotein (P-gp) and the multidrug resistance associated protein 1 (MRP1) belonging to subfamily ABCC have been shown to confer resistance to a broad spectrum of chemotherapeutic agents. More recently BCRP (breast cancer resistance protein) which is also known as ABCG2 has been discovered and proved to cause resistance in tumour cells too.

Although BCRP was first cloned in doxorubicin resistant MCF-7 breast cancer cells [6], later it has been also found to confer resistance to mitoxantrone (hence named MXR) [7] and subsequently it was found in the placenta (hence named ABCP) [8]. BCRP is an ABC transporter protein consisting of 655 amino acids and having a molecular weight of 72 kDa. It is a half transporter containing a single NH_2-terminal nucleotide binding domain (NBD) followed by six transmembrane domains (TMD). It has been proposed that, to achieve its functionality, BCRP needs to be homo- or hetero-dimerized [6,7]. BCRP has been found to be present in many normal tissues other than in tumour cells, including apical membrane of placental syncytiotrophoblasts, endocrine cells of the pancreas, epithelial cells in the small intestine and colon, liver caniculi, blood–brain and blood–testis barrier, gallbladder epithelium and

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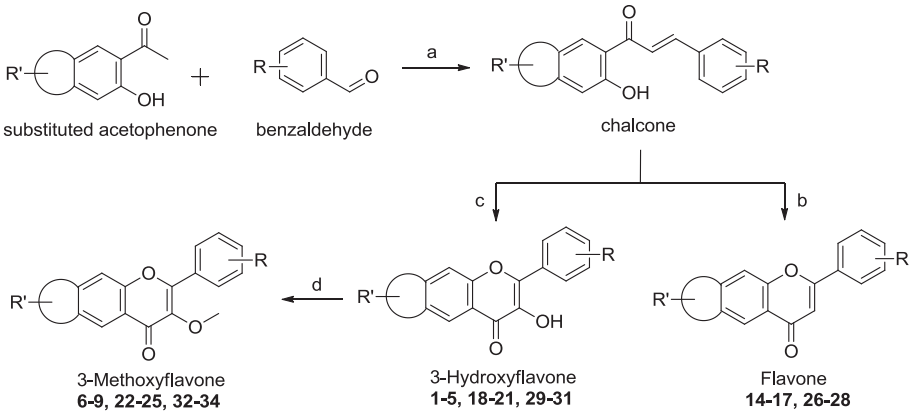


Scheme 1. General scheme for the synthesis of target compounds. Reagents and conditions: a) EtOH, NaOH or LiOH, rt, 5–72 h; b) DMSO, I_2, reflux, 7–8 h; c) EtOH, 25% H_2O_2, NaOH, rt, 24–48 h; d) acetone, CH_3I, K_2CO_3, reflux, 24 h.

stem cells [8–10]. In normal tissues BCRP protects the body from various endogenous and exogenous toxins.

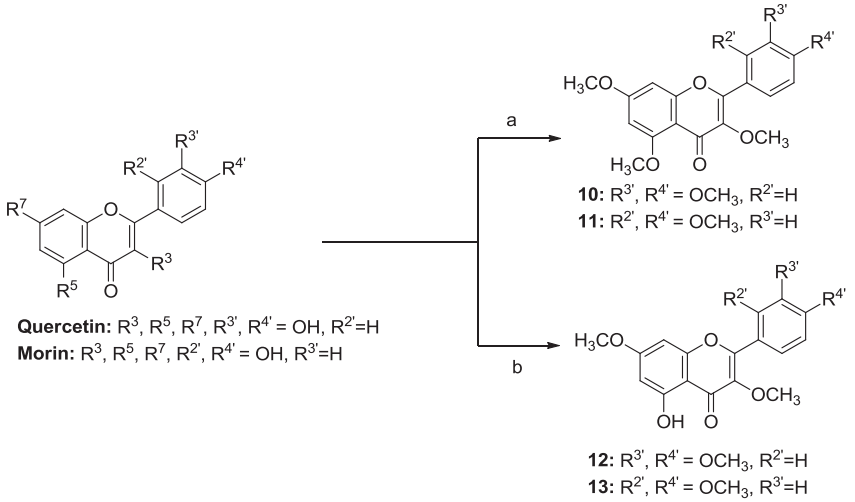
Several substrates of BCRP have been identified, which include anticancer drugs such as mitoxantrone [11], podophyllotoxins etoposid and teniposid, flavopiridol [12], topotecan, irinotecan [13] and its active metabolite SN-38. To overcome MDR acquired by over expression of BCRP several efforts have been made by investigating inhibitors of BCRP. These structurally unrelated compounds include naturally occurring flavonoids [14–17], broad spectrum inhibitors like tariquidar [18] and elacridar, chromons [19] and methoxystilbenes [20]. Several research groups have investigated chalcones (precursor of flavonoids) for their inhibitory effect [21–24]. Fumitremorgin C was found to be a very potent inhibitor of BCRP [13], but its use was limited due to neurotoxicity. Its non-toxic analogue Ko143 is the most potent and selective inhibitor of BCRP known today.

Recently a flavonoid, the 7,8-benzoflavone was identified as very potent inhibitor of BCRP [15,25]. To further investigate benzoflavones for their potential to inhibit BCRP, we synthesized several 7,8- and 5,6-benzoflavones bearing varying substitutions at positions 3, 3′ and 4′. Also several substituted synthetic flavones were investigated to allow comparison of their inhibitory potential with that of the benzoflavones. These compounds were checked for their inhibitory effect on BCRP in Hoechst 33342 and pheophorbide A accumulation assays. The compounds were also investigated for their effect on P-gp and MRP1. Compound **24**, a 7,8-benzoflavone bearing three methoxy substituents at positions 3, 3′,4′ was found to be most potent having an inhibitory concentration only about 2 fold less than Ko143 which is the most potent specific BCRP inhibitor [26], while being almost 3 fold more potent than the unsubstituted 7,8-benzoflavone.

2. Result and discussion

2.1. Chemistry

In the current study we synthesized and investigated several flavones (**1**–**13**), 7,8-benzoflavones (**14**–**25**) and 5,6-benzoflavones (**26**–**34**). Flavones (**1**–**9**) and both type of benzoflavones were synthesized from their precursor chalcones and benzochalcones. The precursors were synthesized by Claisen–Schmidt condensation using substituted acetophenones and benzaldehydes in presence of NaOH or LiOH as base in ethanol as reported earlier [23]. The synthesis of the studied compounds is depicted in Scheme 1 and Scheme 2. Synthesis of benzoflavones occurred via one of two routes. The first route involved cyclization of a precursor benzochalcone to the corresponding benzoflavone in presence of iodine in DMSO to give benzoflavones with no substituent at



Scheme 2. General scheme for the synthesis of compounds **10**–**13**. Reagents and conditions: a) acetone, CH_3I, K_2CO_3, reflux, 24 h; b) acetone/water (2:1), dimethyl sulphate, KOH, reflux, 3 h.