A review on BCRP inhibitors: An upcoming strategy for cancer treatment

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Abstract: Worldwide, Cancer is the most terrified disease and major cause of death. Every year approximately, 1.6 million new cases of cancer are being diagnosed worldwide and extensive research efforts are in going on for finding a cure to this life-threatening disease. Till date several thousand synthetic compounds and numerous natural products have been evaluated biologically as anticancer agents. The main disappointment of cancer treatment is multidrug resistance (MDR) which is linked with the over expression of certain protein transporters of ATP binding cassette (ABC). Among 48 ABC transporters, p-glycoprotein (p-gp), multidrug resistance protein 1(MDRP1), breast cancer resistance proteins (BCRP) are efflux transporters which are mainly associated with MDR. ABCG2/ BCRP is supposed to have importance in protection from regulating oral bioavailability, xenobiotics, and also known to have role in forming part of blood-testis barrier, blood-brain barrier and maternal-fetal barrier. BCRPs also expressed in stem cells, where it acts a role in protecting from xenobiotics. Due to broad substrate selectivity of BCRP, it might also impact the pharmacokinetics of many other unrelated drug including anti-HIV drugs, anticancer and endogenous compounds Breast cancer resistance protein (BCRP/ABCG2) is an ABC-transporter which is present in luminal layer of epithelial cells of intestine that limits absorption of many of drugs such as methotrexate, topotecan, mitoxantrone and doxorubicin (anticancers). In this review we want to present the role of BCRP in failure of chemotherapy and its inhibitors for the reversal of MDR. The main theme of the article will be BCRP inhibitors: An upcoming strategy for cancer treatment.

Keywords: Cancer, Multidrug resistant, BCRP, Chalcones, Quinazoline, Fumitremorgin.


1. INTRODUCTION
Cancer is one of the most dreaded diseases resulting in mortality worldwide. Every year, approximately 1.6 million new cases of cancer are being identified worldwide. Several considerable research efforts are on the way for finding a cure to this life-threatening disease.

Cancer exists in one or more than one forms in 200 different forms and claims over 8 million lives, taking a toll of around 22,000 every day. One in eight deaths occurring worldwide is due to cancer only. In India about 1,688,780 new cancer cases and 600,920 cancer deaths are estimated to occur in the United States. 20% rate of cancer incidence is higher in women than men while the cancer death rate is 40% higher in women.
It is believed that in near future the number of cancer patients is likely to increase in the developing and underdeveloped countries by up to 70%, a serious issue for one and all. It is estimated that 9 million people will be dying only from cancer in 2016 and 13.1 million in 2030. The most common cancers occurring in males are of lung, prostate, colorectum and stomach. In female cancer of breast, colorectum, cervical and skin are the most common cancers. In children, brain tumours and lymphoblastic leukaemia are the most general cancers.

Radiotherapy, chemotherapy, surgery and targeted therapy are different therapies, which are often used in combination for the treatment of cancer. The development of therapeutic tools for cancer treatment has been advanced in last few decades. The approaches for cancer treatment have gradually moved towards specific targets. These approaches for cancer cure include eradication of cancer cells without effecting normal cells. Inappropriately currently not even single available agent meets this target. Therefore concern research required for the discovery and development of novel selective anticancer agents that delay the progression of malignant tumours or prevent their recurrence.

1. Breast cancer resistance protein (BCRP/ABCG2)

MDR is allied with the over-expression of certain protein transporters of ATP-binding cassette (ABC). These ABC transport proteins are p-glycoprotein (p-gp), multidrug resistance protein (MDRP1) and breast cancer resistance protein. This polyspecific ABC transporter BCRP was first described by Doyle et al. in doxorubicin resistant MCF-7/AdrVp breast cancer cell lines, known as BCRP. ABC proteins are largest and most broadly expressed proteins among the ATP-binding cassette transporters. Among human beings, there are total 48 ABC genes which are categorised into seven super families A to G. For ABC transporters, hydrolysis and binding of ATP is required to provide energy for substrate movement across the cell transmembrane. BCRPs are present in many normal tissues and various solid tumours including adrenal gland, testis, stem cells, placenta, liver, blood-brain barrier and small intestine. BCRP is an 655 amino acid proteins having an ATP-binding domain and six-transmembrane domain with 72 kDa proteins. BCRP is an atypical half-transporter, consisting of only one hydrophobic membrane spanning domain (MSD), having 6 transmembrane domain along with single nucleotide membrane domain (NMD). Reduced drug uptake, the DNA activation repair pathways, induction of anti-apoptotic and the efflux of intracellular drugs are the few mechanisms that can confer MDR in cancerous cells.

1.1 Role of BCRP in cancer management

Main failure of cancer treatment is the resistance to chemotherapeutic agents. This resistance is due to potential of ABC transporters to extrude the drugs from cancerous cells. BCRP actively efflux number of endogenous and exogenous substances across the cell membrane.

It is noted that by inhibiting BCRP, drugs are accumulated in the cells and able to show their anticancer effects. Therefore, one of the approach to overcome MDR in cancer therapy is to co-administration of anticancer drugs along with BCRP inhibitor. It is observed that there is no common recognized “pharmacophore” which can be used for the inhibition of three ABC transporters that are P-gp(ABCB1), BCRP(ABCG2) and MDR1(ABCC1). Figure 1 depicts various compounds with different pharmacological profile, which have also been explored broadly as BCRP substrates in various researches.
Now days, various advancements has been established for the treatment of cancer. Unfortunately, these are mostly associated with efflux of drugs by the tumour cells, that eradicate or minimize their anticancer property. The inhibitors of BCRP have been emerged as a new intention to overcome MDR. These inhibitors can be classified into various natural and synthetic derivatives. Therefore, it is prerequisite of development new molecules to maximize the anticancer activity without MDR.

**Figure 1** Molecules having BCRP inhibitory activity

### 1.2 BCRP INHIBITORS

Now days, various advancements has been established for the treatment of cancer. Unfortunately, these are mostly associated with efflux of drugs by the tumour cells, that eradicate or minimize their anticancer property. The inhibitors of BCRP have been emerged as a new intention to overcome MDR. These inhibitors can be classified into various natural and synthetic derivatives. Therefore, it is prerequisite of development new molecules to maximize the anticancer activity without MDR.

**Figure 1** Molecules having BCRP inhibitory activity

1. Elacridar (1)
2. Pantoprazole (2)
3. Biricoder (3)
4. Permethy epigallocatechin gallate (4)
5. GF120918 (5)
6. PD153036 (6)
7. Prazocin (7)
8. Gefitinib (8)
9. Gleevec (9)

1.2.1 Naturally BCRP inhibitors and their synthetic modifications

1.2.1.1 Fumitremorgin C (FTC)

FTC(10) is an indole alkaloid originated from a natural fungal origin and discovered as first generation inhibitor of the breast cancer resistance protein. But, clinical development of FTC was never undertaken due to its reported neurotoxicity. It also has tumour-inducing activity and cell cycle arrest at the G2/M-transitions. So there was a need to manage its selectivity, specificity and reduce the cytotoxicity. In second generation new forms of FTC were developed like ko143(11), which had less neurotoxicity as compared to natural FTC.11, 12

![Diagram of FTC](10)

![Diagram of FTC analogues](11)

Natural and semi synthetic fumitremorgin C derivatives are racemic mixtures, but it is known that only single enantiomer has desired effect. Here is need to improve selectivity and reduced neurotoxicity of FTC. For this purpose, Guofeng et al. designed new FTC analogues, in which tetracyclic core of native FTC was retained (12).

![Diagram of FTC analogue](12)

Diketopiperazine alkaloids is the class of all newly synthesized fumitremorgin analogues, containing 1,4-dioxo-2,3,4,7,12,12a-hexahydropyrazino [1',2':1,6]pyrido[3,4-b]indole skeleton. Natural FTC (10) is a pentacyclic structure, but in synthesized analogues, one ring (E-ring) is removed to develop new tetracyclic analogues, which are similar to ko132 and ko134. New analogues lack proline ring (E-ring) and methoxy group at C-18. Proline ring was replaced by acyclic substituents, which are supposed to have different confirmations. These different confirmations are suggested to be less neurotoxic than the native FTC. BCRP inhibitory potential was evaluated in MES-SA/Dx5 cell lines resistant to doxorubicin. In a study IC50 of doxorubicin alone and its combination with synthesized compound was compared. Compound 13 when given with doxorubicin was examined as potent BCRP inhibitor with IC50 = 0.66 µM.13

![Diagram of FTC analogue](13)
1.2.1.2 Bisbenzylisoquinoline alkaloids

These alkaloids belong to the largest family of natural occurring phytochemicals having bisbenzylisoquinoline moiety that are linked to each other by carbon-carbon bridge or ether bridge having broad potential clinical application. Neferine (14), isoliensinine (15), liensinine (16), dauricine (17) and tetradrine (18) are the five important bisbenzylisoquoline alkaloids with similar chemical structures in figure 2. 

![Bisbenzylisoquinoline derivatives](image)

**Figure 2** Bisbenzylisoquinoline derivatives

Tetradrine is obtained from *Stephenia tetrandra*. Dauricene is a bioactive compound isolated from the roots of *Menspermum dauricum*. Neferine, liensinine and isoliensinine are isolated from the embryos of *Neulumbo nucifera*. These alkaloids are reported as cardioprotective, reverse MDR and inhibition of pulmonary fibrosis. Yien *et al.* evaluated possible interaction between these five natural derivatives with BCRP, it was demonstrated that neferine, isoliensinine, tetrandrine accumulated in LLC-PK1 and LL-PK-1/BCRP cells in similar amount. Accumulation of tetrandine and neferine in cells were little high than isoliensinine because later was less lipophilic than tetrandine and neferine. In LLC-PK1 cells, liensinine and dauricine accumulation was more in comparison to LLC-PK1/BCRP cells. The study indicated that liensnine and dauricine are good substrates for BCRP. From the docking studies it was also revealed that the main problem associated with these alkaloids is limited cell permeability. Liensinine has direct hydrogen bonding with Arginine 482, which indicated it as a good substrate of BCRP.

1.2.1.3 Botryllamides

The BCRP inhibitory potential, ABCG2 high throughput screening method was applied to natural products extracted from marine sources repository by National Cancer Institute (NCI). Henrich *et al.* evaluated these extracts for their ability to accumulate BCRP substrates in the cells. These extracts were extracted...
from marine *Botryllus* *tyreus*, ascidian.\(^{20,21}\) Different botryllamides (A-H) were obtained from these extracts (Figures 3 and 4).

![Botryllamides A-D](image)

**Figure 3** Botryllamides A-D

It is noted that that capability to accumulate the BCRP substrates in cells, it was found that botryllamides A to G, I and J were the active BCRP inhibitors. Botryllamide C and H were inactive towards BCRP inhibition.\(^{22}\)

![Botryllamide E-J](image)

**Figure 4** BCRP inhibitors botryllamide E-J

1.2.1.4 Flavonoids

Flavonoids have also been reported for their BCRP inhibitory potential. So natural consumption of the flavonoids in the diet is known to be responsible for the inhibitory potential which may adverse the pharmacokinetics and influx of various drugs into the cancerous cells.\(^{23,24}\)
First flavonoids reported as BCRP Inhibitors

Two flavones, (Figures 5) retusin (19) and ayanin (20) have been reported as BCRP inhibitors and its activity slightly lesser than the potent Ko143 (11). Upto 6500 naturally occurring flavonoids are recognized till the date. Daily intake of about 200-1000 mg of the flavonoids in diet is good for health. Beverages (red wine, cola, coffee and tea), fruit juices, vegetables, nuts, sweet corns and potatoes are the major sources of flavonoids having antioxidant activity. Pick et al. identified new potent BCRP inhibitors belonging to different classes of flavonoids such as flavonones, flavones, flavonols, glycosides, isoflavones, and biflavonoids. It is reported that flavonols, isoflavones and flavonones.

Zhang et al. investigated that multiple natural flavonoids (Figures 6) like Apigenin (21), chrysin (22), kaempferol (23), biochanin A (24), genistein (25), hesperetin (26) and naringenin (27) for its inhibitory potential towards BCRP.

This predicted data suggested that flavonoids mediate interactions of anticancer drugs with BCRP and helps in reversal of MDR in cancer treatment. There are number of natural useful flavonoids such as quercetin, baicalein, rutin, morin, fisetin and ayanin, which have been reported to reverse MDR. Quercetin (28) chemically, it is 3,5,7,3′,4′-pentahydroxyflavone. Pharmacological effects of quercetin have been reported as anti-inflammatory, anti-allergic etc. Quercetin is also reported for its effectiveness in reducing risk of different cancers.
Conseil et al. SAR studies on flavonoids for modulating the MDR agents and the results advise that methylation of phenolic groups progress the P-gp inhibitory action and also act as important “pharmacophore” for reversal of MDR. Jianet al. synthesized quercetin derivatives and divided it into five subgroups. MDR reversal activity of synthesized compounds was evaluated by using four different drug resistant cell lines, (i) LCC6MDR, P-gp transfected human breast cancer cell lines resistant to paclitaxel; (ii) HEK293/R2, BCRP-transfected human embryonic kidney cell lines resistant to topotecan; (iii) MCF7-MX100, BCRP-transfected mitoxantrone selected breast cancer cell lines resistant to topotecan; (iv) 2008/MP1, MRP1-transfected ovarian cancer cell lines resistant to doxorubicin. On evaluation of quercetin and rutin against these four cell lines, it was seen that quercetin and rutin were less active chemo sensitizers and were not specific for the ABC transporters. Two types of tetra-alkylated derivatives of quercetin, which differ with respect to substitutions at 3 and 5 position were also synthesized. It was observed from the results that 5-O-non-methylation and 3-O-methylation contributes to the P-gp and BCRP modulating activities. Presence of a carbonyl linker between phenyl ring and methylated quercetin at 3-O position retained BCRP inhibitory activity. Other derivatives of quercetin with mono-, di- and trimethoxy groups substituted at terminal phenyl ring improved potency. Compounds 29 and 30 were found to be the most active among all the synthesized analogues. Compound 29 inhibited two cell lines LCC6MDR (resistant to paclitaxel) and HEK293/R2 (resistant to topotecan) with IC$_{50}$ = 24.2 µM and 34.7 µM and for compound 30 IC$_{50}$ was found to be 12.9 and 11.3 µM respectively.

1.2.2 Synthetic BCRP inhibitors

The naturally occurring anticancer agents have less selectivity and specificity. For the specificity of anticancer agents it is essential to focus over the different biological targets. As discussed above, several natural products were reported as potent inhibitors of BCRP but all had associated problems with their use. So various researchers had earned interest to design and synthesize new chemical entities as BCRP inhibitors. Various synthesized analogues which are found to inhibit BCRP are discussed below.
1.2.2.1 Quinazolines

Quinazoline (31) is the most widely active scaffold among all the bioactive compounds. Historically, the chemistry of quinazoline compounds is centuries old, but till now intense search for biologically active substances in this series began only in the last few decades.\textsuperscript{33}

Development of quinazolines started only with discovery of febrifugine, a quinazolinone alkaloids possessing antimalarial potential from the Chinese plant aseru (\textit{Dichora febribuga} Lour), which act as an impetus for initiation of the research on quinazolines.

![Quinazoline structure](image)

It is the fused form having six-membered simple aromatic rings, a benzene ring and a pyrimidine ring, called as benzopyrimidine. The first quinazoline was synthesized in the late 1860s from anthranilic acid and cyanogens to give the 2-cyanoquinazolinone (32).\textsuperscript{34} Quinazoline is considered as a driving force in the area of drug development as it possess wide range of biological actions such as anticancer, anti-inflammatory, antibacterial, anticonvulsant, antitubercular, diuretics and hypnотic.\textsuperscript{35}

According to the recent data, quinazoline nucleus has attracted the attention of medicinal chemists due to its potential as anticancer and many other substituted quinazoline derivatives have recently gained a lot of interest in chemotherapy. During the last few decades number of patents has been claimed for quinazolines derivatives as potential anticancer agents. In general, quinazoline scaffold is considered to be important in the field of pharmacy and biology.\textsuperscript{36} Several FDA approved anticancer quinazoline derivatives, e.g., Gefitinib, Erlotinib, Lapiitinib, Vandetanib are listed in Table 1. It is important to point out that cancer treatment strategies have evolved in the last few years, moving from the classical chemotherapy approach to the targeted therapy that aims at the inhibition of specific targets. From this point of view it is important to develop quinazoline derivatives, which are not only able to inhibit the specific targets but also able to reduce the main failure of chemotherapy that is MDR. Number of quinazoline based compound were reported to have remarkable inhibitory effect on BCRP.\textsuperscript{41,42,43}

### Table 1: Clinically used quinazoline derivatives as anticancer agents

<table>
<thead>
<tr>
<th>Quinazoline derivatives</th>
<th>Structure</th>
<th>Clinical uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td><img src="image" alt="Gefitinib Structure" /></td>
<td>Anticancer</td>
<td>37</td>
</tr>
</tbody>
</table>

\[\text{Annals of Tropical Medicine & Public Health} \quad \text{http://doi.org/10.36295/ASRO.2020.231550}\]
<table>
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<tr>
<th>Compound</th>
<th>Chemical Structure</th>
<th>Activity</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib</td>
<td><img src="image" alt="Erlotinib Structure" /></td>
<td>Anticancer</td>
<td>37</td>
</tr>
<tr>
<td>Vandenatib</td>
<td><img src="image" alt="Vandenatib Structure" /></td>
<td>Anticancer</td>
<td>38</td>
</tr>
<tr>
<td>Doxazocin</td>
<td><img src="image" alt="Doxazocin Structure" /></td>
<td>Anticancer</td>
<td>39</td>
</tr>
<tr>
<td>Alfuzosin</td>
<td><img src="image" alt="Alfuzosin Structure" /></td>
<td>Anticancer</td>
<td>40</td>
</tr>
</tbody>
</table>

Prazocin (7), an α2 adrenergic blocker, is a quinazoline compound, which has been reported as a substrate for BCRP.44 Two other quinazoline based compounds such as gefitinib (8) and PD153035 (9) are tyrosine kinase inhibitors (TKIs) but also have BCRP inhibitory potential. Yanase et al. suggested that there may be interaction of gefitinib with BCRP expression.45

**Investigation on quinazolines as BCRP inhibitors:** Wiese et al. carried out the structure-activity relationship of quinazolines and in accordance with the results obtained from QSAR studies, various series of quinazoline based compounds were synthesized. From SAR analysis it was revealed that substitution of phenyl ring at position 2 is most significant among all the analysed positions 2, 4, 6 and 7 of quinazoline structure.46

BCRP inhibitory potential of quinazoline scaffold was further explored by same co-workers by synthesizing two new series of derivatives *i.e.* A and B (Figure 7). In series A, phenyl ring is substituted at position 2 of quinazoline. In series B, phenyl ring is substituted at the same position linked through an amine linker.

![Figure 7](image) Two different series of quinazolines designated as A and B
Both generated series were investigated for their capability to inhibit BCRP, by using two assays, named as Hoechst 33342 and Pheophorbide A assay. It was revealed that compounds bearing substituted phenyl at position 2 of the 4-anilinoquinazoline were the most potent. Substituents like nitro, cyano on the aniline ring at position 4 of the quinazoline increases the potency. An anilinoquinazoline compound bearing \textit{meta}-NO$_2$ group on phenyl ring at position 4 was potent with IC$_{50}$ = 0.013 µM in Hoechst 33342 assay. Other desired substituents for BCRP inhibition at various positions of quinazoline ring are explained in figure 8.

\textbf{Figure 8} Important structural features of quinazolines required for BCRP inhibition:

+ good activity, ++ very high activity, - decreased activity

Juvale \textit{et al.} extended their work on quinazoline based BCRP inhibitors by synthesizing 2-phenylquinazolines with different substituents at position 4 and investigated the resultant analogues for their BCRP inhibitory potential. Presence of a phenyl ring attached via a -NH- linker at 4 position resulted in most active inhibitor of BCRP. Compounds with a piperazine linker at same position of quinazoline ring were found to be less active. Among all these synthesized anilinoquinazolines and piperazinequinazolines analogues, nitro (33) and bromo (34) substitutions resulted in maximum activity with IC$_{50}$ = 0.13 µM and 5.12 µM, respectively.

Earlier studies by Wiese and coworkers on 4-anilino quinazoline derivatives as BCRP inhibitors also concluded that compounds with nitro, cyano, hydroxy, fluoro and acetamide substituents had high inhibitory potential.
Further this work was extended towards the synthesis and study of quinoline analogues inhibitory effect represented by structure 35.

It was concluded that 4-anilinoquinazoline analogues have better selectivity and inhibitory potency towards BCRP than 4-anilinoquinoline counterparts. Compound 36 with IC$_{50}$ = 80 µM was found to be most potent among synthesized derivatives.

![Structure 35](image)

1.2.2.2 Chalcones

Structurally, chalcones as represented in structure 37 are also known as α, β-unsaturated ketones. These are major components of the natural products. Synthetic analogues have diverse biological activities including anticancer, anti-inflammatory, antioxidant, analgesics, antihepatotoxic, antimalarial and antiallergic. Existence of double bond in conjugation with carbonyl functional group is believed to be responsible for the biological activity. Chalcones have tendency to exist in cis and trans-forms. Numerous synthetic routes are reported for the synthesis of chalcones. General synthetic methods involve Claisen-Schmidt condensation of an aldehyde and a ketone under homogenous conditions in presence of strong acid or base. Chalcones have been reported by different researchers as potent BCRP inhibitors.

A series of chalcone derivatives was synthesized by Juvale et al. and studied for their potential for BCRP inhibitory activity. Substituent at positions 2´ and 4´ on chalcone ring A were found to be essential for activity; however there was a great influence of substituent on ring B as well presence of 3,4-dimethoxy.
substituents on ring B was found to be optimal, while presence of chloro substituent at 2 and 4 position on ring A also showed a positive effect on BCRP inhibition.\(^{51}\)

In another study, Han et al. synthesized non-basic chalcone analogues and evaluated their capability to inhibit BCRP in MDA-MB-231/BCRP expressing cell lines. Compounds having 2,4-dimethoxy groups or 2,4-dihydroxy groups on ring A had greater potential to accumulate mitoxantrone in cancerous cells than an established BCRP inhibitor, fumitremorgin C. These non-basic chalcones had negligible effect on calcein accumulation in P-gp overexpressing MDCKII cells, indicating their potential as selective BCRP inhibitors.\(^{52}\)

A new series of chalcones for their inhibitory effect on transport of mitoxantrone was recently reported. The most potent compound had functionality N-methyl-1-indolyl or 6- hydroxyl-2,4- dimethoxy moiety on ring A and dimethoxy at positions 2’ and 4’ of ring B and showed inhibition with IC\(_{50}\) less than 0.5µM.\(^{53}\)

### 1.2.2.3 Chalcones and benzochalcones

Juvale et al. investigated the effect of substituent pattern on chalcone analogues for BCRP potential. SAR studies on compounds with diverse substituent at various positions on both phenyl rings of chalcone are described in table 2.

<table>
<thead>
<tr>
<th>S.No</th>
<th>STRUCTURE</th>
<th>SAR STUDIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image" alt="Chalcone" /></td>
<td>No substitution or presence of a single substituent on ring A resulted in decreased BCRP inhibition. Multiple substitutions on various positions 3’,4’,5’,6’ on phenyl ring showed increased activity towards BCRP. Chalcones with methoxy at positions 3 and 4 on ring A exhibited maximum inhibitory potency. Compounds bearing methoxy at position 3 had improved potency than methoxy at position 4. Substitution of hydroxyl group at position 2’ resulted in somewhat increased activity than the methoxy at same position. Chloro substituents at 2 and 4-positions of ring B also produced positive inhibitory effects but chloro at position 3,4 on ring A led to decreased in activity.</td>
</tr>
<tr>
<td>2.</td>
<td><img src="image" alt="3,4-Benzochalcones" /></td>
<td>Introduction of chloro at positions 2 and 4 of phenyl ring A resulted in negligible inhibitory effects of compounds. 3’,4’-Dimethoxy benzochalcone found to be the most potent compound. 3’-Methoxy substitution was found to be better than methoxy at 4’-position.</td>
</tr>
</tbody>
</table>
Three different series of compounds were evaluated for inhibition of BCRP using Hoechst 33342 accumulation assay in MCF-7 MX and MDCK cells expressing BCRP. Among all the synthesized series, compound 38 with IC$_{50} = 0.93$ and 0.85 µM and compound 39 with IC$_{50} = 0.75$ and 0.53 µM were found to be the most potent inhibitors of MCF-7 MX and MDCK BCRP cells. The potency was only 3 fold less than standard BCRP inhibitor ko143. It is observed from the results that 3',4'-benzochalcones and 5',6'-benzochalcones are less potent than the chalcone. Compounds with no substituents on ring A were inactive while 3,4-dimethoxy, 4-methoxy or 3-methoxy substitution is required for good inhibitory activity towards BCRP.

1.2.2.4 Chalcone hybrids
Earlier, it was reported that chalcones are potent and selective BCRP inhibitor, so chalcone moiety became lead for the researchers. Further studies were carried out by hybridizing chalcones with other moieties to improve its potency and selectivity towards BCRP.

1.2.2.5 Bifendate-chalcone derivatives
Bifendate-chalcones is the new class of potential inhibitors of both P-gp and BCRP. A number of studies have proven that TKIs act as substrate for P-gp and BCRP. These studies indicate that TKIs may be inactive in treatment of cancer patients whose tumours have over-expressing P-gp and BCRP. So it was essential to construct novel agents that could also inhibit P-gp well as BCRP. The six alkoxyl biphenyl moiety of bifendate was found to play an important role in its biological activities and chalcones are also known to as moieties having various significant biological activities, including P-gp inhibitory effects. Xiaoke et al. hybridized bifendate with chalcone to boost the inhibitory effect of bifendate as P-gp inhibitors. Newly generated bifendate-chalcone hybrid series not only exhibited potent P-gp inhibitory effects but also possess inhibitory effects equivalent to or even stronger than the verapamil (potent P-gp inhibitor). Substitution on benzene ring (ring-A) of chalcone played a vital role in the reversal of MDR mediated by P-gp transporter.
Different substituents studied on chalcone ring were methyl, methoxy, nitro and hydroxy. Compounds containing methoxy groups on ring-A showed more inhibitory activity than the hybrids bearing nitro and hydroxy group. Of the methoxy substituted compounds, ortho and meta-positions were found to be more important than para-position for activity. Replacement of methoxy group with methyl improved the P-gp inhibitory activity. Introduction of nitrile group into α,β-unsaturation of the bifendate-chalcone significantly reduced the P-gp inhibitory activity.

Among this new series of bifendate-chalcones, compound 40 (IC\textsubscript{50}>100 µM) was found to be the most active compound. Notably, from recent reports it is revealed that several chalcone derivatives could inhibit not only P-gp but also other ABC transporters such as BCRP and MRP1. Based on these research findings, previously synthesized bifendate-chalcones were also evaluated for their reversal properties of breast cancer resistance protein mediated multidrug resistance using HEK293/BCRP cells by the same research group. It was noted that the compound, which showed potent inhibition of P-gp, equally inhibits BCRP. Three compounds 41-43 also notably enhanced mitoxantrone accumulation in BCRP expressing cells by means of inhibiting BCRP mediated drug effluxing effects, with IC\textsubscript{50} = 37.47, >100 and 20.36 µM respectively.

1.2.2.6 Quinazoline-Chalcone conjugates
During the past few years, number of studies has been carried out to overcome the ABC drug transporters mediated problem of MDR in cancer treatment. These ABC transporters are the ATP-binding cassette, which result in the efflux of different drugs from cell membrane.
Due to the over-expression of these ABC transporters, influx of anticancer drug is reduced in the cancerous cells, which lead to resistance of cancerous cells to that particular anticancer agent. Chalcone derivatives have been reported as potent inhibitors for BCRP. Quinoxaline-chalcone derivatives, reported by Winter et al. in 2014, form a new class of BCRP inhibitors. Chalcones and quinazolines both are most promising inhibitors of ABC transporters. So, there is a possibility to enhance their individual potency by combination of both moieties. Combination of both may result in new more potent and selective scaffolds for BCRP inhibition. Therefore Stefanie et al. combined chalcone and quinazoline moieties in a single molecule to produce new heterodimeric derivatives and investigated them as BCRP inhibitors. To produce compounds with 2-substitued or 2-unsubstitued quinazoline ring, two methods were developed. SAR studies on various quinazoline-chalcone conjugates have been compiled in Table 3.

**1.2.2.7 Flavones and benzoflavones**

Flavones, 7,8-benzoflavones and 5,6-benzoflavones were synthesized and studied by Wiese et al. as BCRP inhibitors. Compounds with methoxy groups at position 3 have showed potency than those with hydroxy group. *Meta* and *para* substituted methoxy...
phenyl at 2-position led to increase in the activity of compound. Addition of hydroxy group at position 5 further enhanced the inhibitory potency. Compound 44 was found to be the most potent among the flavones with \(IC_{50} = 0.540\) and \(0.570\) \(\mu\)M in Hoechst 33342 assay and Pheophorbide A assay, respectively. Both 5,6-benzoflavones and 7,8-benzoflavones were found to be active inhibitors of BCRP. Presence of hydroxyl group at position 3 in both cases led to decrease in activity, however introduction of methoxy group at this positions resulted in sharp increase in BCRP inhibitory activity.

Addition of halogens as also influenced the activity. 5,6- Benzoflavone based compound 45 was most potent with \(IC_{50} = 0.426\) and \(0.468\) \(\mu\)M in Hoechst 33342 and Pheophorbide A assay, respectively. In 7,8-benzoflavone based series of compound 46 showed maximum activity with \(IC_{50} = 0.590\) and \(0.458\) \(\mu\)M in Hoechst 33342 and Pheophorbide A assay respectively.
1.3 Miscellaneous BCRP inhibitors

1.3.1 Tariquidar derivatives

Structurally tariquidar is related to anthranilic acid and is a derivative of anthranilamide. It is in clinical trial phase II, for the inhibition of BCRP Xu et al. generated a series of tariquidar derivatives and evaluated them for their reversal activity on two different MDR transporters i.e. P-gp and BCRP.\(^\text{64,65}\)

It was postulated from the study that tariquidar binds to the same binding site where the P-gp substrate binds. The structure of tariquidar comprises three pharmacophoric components.\(^\text{66}\)

i) Anthranilamide group, which facilitates hydrogen bonding with the transporter
ii) Substituted phenyl moiety providing an important hydrophobic site for interaction,
iii) Quinolinyl substituent believed to have binding with additional site of transporter.

![Different pharmacophore of Tariquidar](image)

Tariquidar is believed to be highly toxic to normal cells, so there is a necessity to generate derivatives of tariquidar which will be less or least toxic to the normal cells. New derivatives of tariquidar were synthesized by incorporating different substituent in the building blocks particularly in BB3. Introduction of amide or sulphonamide in BB3 generated compounds with low toxicity than the lead compound tariquidar.\(^\text{67}\)

The reported results revealed that compounds with sulphonamide substitution were least toxic and exhibited better aqueous solubility than the tariquidar itself. Compounds of amide series produced dual inhibitors of P-gp and BCRP mediated drug efflux. Compound 47 was found to be the most active among the new derivatives of tariquidar with IC\(_{50}\)>100.

![Compound 47](image)

1.3.2 Naphthalenyl derivatives

Recently, PET studies using the corresponding \(^{11}\)C-radiolabelled ligands, demonstrated that tariquidar and elacridar displayed high P-gp and moderate BCRP inhibition.\(^\text{68,69}\)

SoColabufa et al. developed naphthalenyl...
derivatives substituted with oxazole, thiazole and furan heterocyclic nuclei to obtain various bioisosters as shown in figure 10. All derivatives were investigated for the activity towards the MDR transporters (BCRP/P-gp/MRP1). Compounds for developing biphenyl derivatives to obtained potent inhibitors of P-gp that also had moderate inhibition activity for BCRP and MRP1. Aryloxazole and arylthiazole derivative series have been developed for improving the P-gp selectivity. The P-gp activity of these derivatives was decreased and showed increased BCRP and MRP1 inhibition activity. Different substituent on the aryl chain has been substituted for modulating the activity towards the MDR transporters (BCRP/P-gp/MRP1).

**Figure 10** Naphthalenyl derivatives

### 1.3.3 Alkoxyl biphenyl derivatives

Bifendate derivatives having dibenzo-[c,e]azepine structural component with potential more than verapamil for reversing P-gp mediated MDR were described by Gu Xiaoke and co-workers. The potential of synthesized compounds for reversal of resistance mediated by BCRP was evaluated. The compound represented by general structure 48 has inhibitory potency same or little less than the standard Ko143. Intrinsic cytotoxicity was increased in combination with Ko143. These compounds increase the drug influx into the cancerous cells by inhibiting BCRP, instead of decreasing level of BCRP-expression in cancer cells and help to reduce MDR.

### 1.3.4 Acridone derivatives

Acridone (49) is a naturally occurring agent, which is chemically aza-analogue of xanthones. Acridone has inhibitory activity towards MRP and P-gp MDR transporters.
An acridone derivative, GF120918 (5), act as inhibitor of BCRP as well as P-gp. A natural flavonoid, tectochrysin having structural similarity with acridone has also been reported for its inhibitory effect on MDR-transporters.\textsuperscript{73} Compound 50 depicted high BCRP inhibitory potency with IC\textsubscript{50} = 0.35 µM.

1.3.5 Substituted chromones
Valdameriand coworker continued research from acridone derivatives to chromone derivatives as BCRP inhibitors. Acridone moiety was replaced by chromones to produce compounds with more potency and selectivity for BCRP and also to reduce the cytotoxicity.\textsuperscript{74} Compound 51 was depicted maximum activity among the synthesized derivatives of chromones with IC\textsubscript{50} = 0.11 µM.

1.3.6 Methoxystilbenes
Glaucio \textit{et al}. identified good interacting flavonoid binding sites with tectochrysin and 6-prenylchrysin, which produced inhibition with significant cyotoxicity. Due to good affinity, inhibition of BCRP was high but there was need to synthesize compounds less toxic than 6-prenylchrysin. Resveratrol (52), 3,5,4\textsuperscript{'}-trihydroxy \textit{trans}-stilbene, is a natural plant polyphenol, reported with good affinity towards BCRP.\textsuperscript{75,76} To generate more potent and selective inhibitors of BCRP hydroxyl groups of resveratrol were replaced with methoxy groups, which resulted in highly potent compound 53 with IC\textsubscript{50} = 0.16 µM.
2.1 Conclusions: A series of anticancer drugs are reported till now. But to a greater extent new anticancer drugs are required to synthesize because all are mostly associated with one and more side effects. The main problem associated with anticancer drugs is resistance. So there is need to generate new molecules which are able to deal with this multi drug resistances problem that is linked with the over expression of certain protein transporters of ATP binding cassette like BCRP. This BCRP is promotes the efflux of anticancer drugs from target side. So the new inhibitors of all of ATP binding cassette are requisite to synthesize. In this review we discuss about all the molecules that are inhibitors of BCRP. Natural and synthetic compounds are equally able to deal with it, but both are required to modify to reduce toxicity. Chalcone and Quinazoline based moieties are more active to inhibit these proteins and their combination is able to boost their action. For the future work it will become to a greater extent relevant to develop not only anticancer drugs but also to enlarge new non toxic compounds to inhibit the efflux function of ABC transporters.

3.1 CONFLICT OF INTEREST
The author declares no conflict of interest.

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5.1 REFERENCES


