Chapter-II

Review of Literature
Flavonoids and breast cancer

The role of flavonoids in treatment of breast cancer has been reviewed by Beatrice Magne Nde et al., 2015. Commonly consumed fruits and vegetables are the rich sources of chemically diverse flavonoids. Among them dietary flavonols such as quercetin, myricetin and kaempferol; flavones such as tricin and tangeretin, flavopiridol (a semi-synthetic derivative of naturally occurring flavonoidal-alkaloid rohitukine); flavonols such as catechin, theaflavin, gallic esters of catechin and theaflavins; isoflavonoids such as genistein, daidzein, phenoxydol (synthetic analog of genistein), ME-143 (synthetic compound) and chalcones such as isoliquiritigenin and butein together with several synthetic chalcone derivatives have been investigated for their role in breast cancer treatment and prevention. Some of them have been clinically evaluated in form of dietary supplements and adjuvant therapies however, none of the naturally occurring flavonoids have been so far approved for the treatment in breast cancer.

In general, they have mostly contributed as chemical leads in the synthesis of cancer chemopreventive and/or chemotherapeutic agents [1].

In one of the reports, out of all the bioflavonoids studied excluding isoflavonoids and total flavonoids, the intake of flavonols and flavones was found to be associated with a decreased risk of breast cancer, especially among the post-menopausal women [2].

The anti-cancer effects exerted by flavonoids were found to be associated with the modulation of several pathways involved in the breast cancer pathogenesis such as aromatase inhibition and subsequent inhibition of the estrogen biosynthesis, inhibition of estrogen receptor-mediated signaling pathways and metabolizing enzymes, modulation of CYP1 and ATP-binding cassette (ABC) protein families, and induction of apoptosis and cell cycle arrest.

Further, their ability to work as phytoestrogens and anti-oxidants augments their overall anti-proliferative effects [3].
Flavopiridols and related piperidinyl analogs

Flavopiridol, \([\text{cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl) piperidinyl]}\)-1-benzopyran-4-one or Alvocidib was discovered in the late 1980’s through a research program on screening of natural products that inhibited phosphorylation of epidermal growth factor receptor tyrosine kinase (EGFR-TK). It is the first CDK inhibitor (CDK-1, -2, -4, -6, -7) to be tested in clinical trials. It is the semi-synthetic 2-chlorophenyl-derivative of flavoalkaloid rohitukine that is reported from the leaves and stems of \textit{Amoora rohituka} (syn. \textit{Aphanamixis polystachya}) and stem bark of \textit{Dysoxylum binectariferum} [4].

![Rohitukine](image1)

![Flavopiridol](image2)

According to the reports, in 2014 it was granted the status of orphan drug by USFDA for use in the treating patients with acute myeloid leukemia. Various mechanisms responsible for the anti-cancer activity shown by flavopiridol are reported such as the inhibition of CDKs, inhibition of EGFR-TK, protein kinase C, and protein kinase A, cell cycle arrest, induction of apoptosis, depletion of cyclin D1, down-regulation of VEGF, binding to cytosolic dehydrogenase and DNA inhibition of glycogen phosphorylase, interaction with MRP-1 and chemotherapy sensitization [4]. Tan and Swain 2002, reviewed the role of flavopiridol in breast cancer in various \textit{in vitro} and \textit{in vivo}, preclinical and clinical studies. It reportedly caused cell
cycle arrest, induced apoptosis, inhibited angiogenesis, and potentiated the effects of other chemotherapeutic agents [5]. However, compounds that belonged to the first-generation pan-CDK inhibitors such as flavopiridol, suffered certain limitations that included low-therapeutic index, lack of specificity and selectivity and high toxicities at the inhibitory concentrations. Therefore, they are followed by the improved and more specific second-generation CDK inhibitors namely palbociclib, abemaciclib and ribociclib [6, 7]. Nevertheless, according to the recent reports flavopiridol is undergoing phase III clinical trials [7]. Various analogs of flavopiridol that have been synthesized and evaluated for their anti-cancer activity are summarized below.

Kim et al., 2000 reported the synthesis of thio- and oxo-flavopiridol analogs possessing “O” or “S” atom as a linker between the chromone ring and the hydrophobic side chain and evaluated their CDK1 inhibitory activity. Two compounds, (3S,4R)-2-[(2-chlorophenyl)thio]-5,7-dihydroxy-8-(3-hydroxy-1-methyl-4-piperidinyl)-4H-benzopyran-4-one and (3S,4R)-2-(2-chlorophenoxy)-5,7-dihydroxy-8-(3-hydroxy-1-methyl-4-piperidinyl)-4H-1-benzopyran-4-one showed selective CDK1 inhibition with an IC$_{50}$ of 110 and 130 nM, respectively [8].

![Thio- and oxo-flavopiridol analogs](image-url)
Murthi et al., 2000 studied the SAR of flavopiridol with respect to the ring D (3-hydroxy-1-methylpiperidinyl) in order to determine the key structural requirements for CDK inhibitory activity. It was found that, both the presence and the position of the nitrogen moiety on the D ring were essential for CDK inhibitory activity. The replacement with the tetrahydropyridyl moiety in the ring D showed promising CDK inhibitory activity. Further, it was also seen that the C ring could accommodate substitutions without a major loss in activity. It was also noted that, the quinolone and the isocoumarin rings were not effective flavone replacements [9].

![Tetrahydropyridyl derivative](image)

Gross et al., 2001 reported the stereoselective preparation of substituted piperidones and piperidines that resulted in the synthesis of flavopiridol ring D analogs [10].

Li et al., 2008 reported the synthesis of 2-aryl-8(piperidin-4-yl)-5,7-dimethoxy-4H-chromen-4-one derivative by the reaction of chalcone with arylaldehydes. The study showed that the reactions involving benzaldehydes substituted with electron donating group showed improved yield as compared to the other route of synthesis that involved β-diketone as a reaction intermediate [11].
Various reports on the synthesis and biological activity of some of the methoxylated and nitrogen-containing chalcones and aurones has been discussed below in brief.

**Chalcones**

Liu and Go 2006, reported that N-methylpiperidinyl bearing methoxylated chalcones inhibited the growth of human tumor cell lines (MCF, HCT 116, and Jurkat) at IC$_{50}$ values of <5 µM. The compound, 3-(2-chlorophenyl)-1-[2,4-dimethoxy-5-(N-methylpiperidin-4-yl)phenyl]-prop-2-en-1-one showed disruption of the cell cycle at G1 and G2/M phases and down regulation of cell cycle regulatory components such as CDK4, cyclin B, and E2F. The presence of the piperidinyl substitution in the chalcone was suggested to provide specificity to the mechanism of anti-proliferative activity, in addition to promoting a more desirable physicochemical profile [12].
Liu and Go 2007, synthesised a library of chalcones possessing different basic groups and evaluated them for their anti-proliferative activity against human breast cancer (MCF7) and colon cancer (HCT116) cell lines. It was believed that a single basic group on ring A was associated with improvement in activity. However, the results showed that an exception to this generalisation, a dibasic chalcone bearing 1-methylpiperidinyl on the ring A and 4-methylpiperazinyl on the ring B exhibited IC$_{50}$ value of < 10 µM and selectivity ratios of >2.5. However, it did not show any effect on inhibition of cell cycle progression as compared to chalcones with basic moiety on the ring A [13].

![Dibasic chalcone](image)

Liu et al., 2008 screened a library of chalcones with basic functionalities for inhibition of Pgp (ABCB1) by the calcein-AM accumulation assay on DCKII/MDR1 cells. Lipophilicity of chalcones as one of the factors in ascertaining Pgp inhibitory activity was also studied. Out of all the compounds screened, 3-(2,4-dimethoxyphenyl)-1-(4-(piperazin-1-yl) phenyl) prop-2-en-1-one inhibited both Pgp and BCRP and emerged out as a lead for the design of dual inhibitors [14].
Synthesis of novel tetrahydropyridinyl chalcones and aurones as useful therapeutic agents

Chapter II

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Bandgar et al., 2010a prepared a series of 2,4-dimethoxy and 3,4,5-trimethoxy chalcones by Claisen–Schmidt condensation and evaluated their anti-cancer, anti-inflammatory and anti-oxidant activities. Among these the 3,4,5-trimethoxychalcones showed growth inhibition of five human cancer cell lines namely ACHN, Panc1, Calu 1, H460 and HCT116 [15].

Further, Bandagar et al., 2010b prepared methoxylated chalcones containing nitrogen heterocyclics such as piperidine, morpholine, pyrrolidine and piperazine using Mannich reaction. The synthesised compounds were screened for anti-inflammatory related activities such as of cyclooxygenase-2 (COX-2), trypsin and β-glucuronidase inhibition. Chalcones with piperidine methyl substitution were found to be effective inhibitors of COX-2 as compared to COX-1 [16].

Kamal et al., 2010 synthesized and evaluated a new series of imidazo[2,1-b]pyridine/pyrimidine chalcone derivatives for their anti-cancer activity. They were substituted with nitrogen heterocycle on the ring B and exhibited activity with GI50 values ranging from 0.28 to 30.0 mM. Among all the chalcones, the compound bearing 3,4-dimethoxyphenyl substitution on ring B, induced G1 cell cycle arrest, down regulated G1 phase cell cycle
regulatory proteins such as cyclin D1, E1, and CDK2, showed apoptosis and down regulated procaspase-9 and could be considered as the potential lead for the development of anti-cancer agents [17].

Li et al., 2011 reported the synthesis of flavopiridol-based chalcones analogs and evaluated their CDK1 inhibition and cytotoxic activity against HCT116 cell lines. Chalcones were prepared by the aldol condensation of substituted acetophenone with various benzaldehydes. The acetophenone was prepared by Hoesch reaction and methylation. Chalcone was converted to final compound by Mannich reaction with morpholine. The results showed that compounds exhibited higher CDK1 inhibition as compared to the standard flavopiridol. Further, it was found that the piperidine ring of flavopiridol could be replaced with morpholine ring using Mannich base [18].

Juvale et al., 2012 synthesised and evaluated chalcones and benzochalcones as inhibitors of BCRP in MCF-7 MX and MDCK BCRP cell lines. Substitutions at positions 2´ and 4´ in the ring A were reported essential for the activity. The presence of 3,4-dimethoxy substitution on ring B yielded maximum inhibitory effect on BCRP. Among all the tested compounds, (E)-1-(2,4-Dihydroxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one and (E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-4,6-dimethoxy-phenyl)prop-2-en-1-one were most effective with IC₅₀ values in nanomolar range however, their activity was found to be threefold less than that of Ko143, a most potent BCRP inhibitor [19].
Solomon *et al.*, 2012 designed, synthesized, and evaluated series of heteroaryl chalcone possessing thiophene and furan substitutions on the ring B for their anti-proliferative effects on two breast cancer cell lines (MDA-MB231 and MDA-MB468) and a non-cancer breast cell line (184B5). The SAR suggested that the thiophene derivatives showed better anti-proliferative activity as compared to their bioisoteric furan derivatives on the MDA-MB231 cells, while, furanyl chalcones showed better anti-proliferative activity on the MDA-MB468 breast cancer cells. The compound, \((E)-1-(4\text{-chlorophenyl})-3-(5-(4\text{-methoxyphenyl})\text{furan-2-yl})\text{prop-2-en-1-one}\) showed differential anti-proliferative activity against cancer cells and was also found to exhibit a 3-7 fold higher activity on cancer cells as compared to non-cancer cells [20].
Further, Shenvi et al., 2013 reported the synthesis of 2,4,5-trimethoxy chalcones from substituted acetophenones and asaronaldehyde and their flavone, flavonol and flavanone analogs. The compounds screened for their anti-cancer activity against three human tumor cell lines (MCF-7, SW-982 and HeLa) using MTT assay, showed that chalcones bearing electron donor groups para to the carbonyl moiety of phenyl ring A exhibited better inhibitory activity. The \textit{in vitro} results were found to show 50 percent correlation with the \textit{in silico} SAR prediction [21].

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\begin{align*}
\text{R} & \quad \text{(2,4,5-trimethoxy chalcones derivatives)}
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Jayashree et al., 2016 reported the synthesis, anti-oxidant and anti-cancer activities of tetrahydropyridine chalcones. Tetrahydropyridine substituted 2′-hydroxy-4,6-dimethoxy chalcones bearing bioisosteric modifications on the ring B were prepared using Claisen-Schmidt condensation of the substituted acetophenone with the various aldehydes. They were found to show anti-cancer activity at IC\textsubscript{50} values less than 50 µM against human cancer cell lines such as A549, HepG\textsubscript{2}, HeLa, HCT-116, MCF-7 and MDA MB 231 and also inhibited cancer cell migrations in scratch wound assay. Two test compounds bearing naphthalene substitutions namely 1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one and 1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(naphthalen-2-yl) prop-2-en-1-one exhibited potent anti-cancer activity owing to their ability to induce apoptosis, inhibit cancer...
cell migrations, anti-angiogenic effects and also to some extent by reducing the oxidative stress [22].

Naphthyl substituted tetrahydropyridyl chalcone derivatives

**Aurones**

Huang *et al.*, 2007 described the design and synthesis of a series of aurone analogs with piperazine moiety using Michael addition of the amine to the pyrone followed by the ring opening and intramolecular O-alkylation following nucleophilic substitution. The compounds were tested for their anti-tumor activity against HCCLM-7, Hep-2, MDA-MB-435S and SW-480 cancer cell lines using MTT assay. Two compounds, (Z)-2-((4-benzyl-piperazin-1-yl)methylene) benzofuran-3(2H)-one and (Z)-2-((4-(bis (4-fluorophenyl)-methyl) piperazin-1-yl)methylene) benzofuran-3(2H)-one showed prominent activity [23].

(Z)-2-((4-benzyl-piperazin-1-yl)methylene)benzofuran-3(2H)-one
Schoepfer et al., 2002 reported the design and synthesis of flavopiridol mimicking 2-benzylidene-benzofuran-3-ones. The (Z)-2-benzylidene-4,6-dihydroxy-7-(1-methyl piperidin-4-yl)-benzofuran-3-ones were synthesized from 4,6-dimethoxy-7-(1-methyl-piperidin-4-yl)-benzofuran-3-one and substituted benaldehydes following base-catalyzed aldol condensation. The compounds showed improved CDKs 1 and 2 inhibition and selectivity as compared to that of the standard flavopiridol [24].

Sim et al., 2008 described the synthesis and evaluation of a series of (Z)4,6-dimethoxyaurones for their ability to modulate breast cancer resistance protein (ABCG2)-mediated multidrug resistance in vitro. Aurones were synthesised by the reaction of 4,6-dimethoxybenzofuran-3(2H)-one with various benzaldehydes in a base-catalyzed aldol reaction. The SAR showed that substitution on the benzylidene ring B of the aurone was less important for ABCG2 inhibition while the same gave better inhibition of ABCB1 with a preference for the 3’ position on ring B. The 4,6-dimethoxyaurones were reported as promising ABCG2 inhibitors activity at submicromolar concentrations with limited anti-proliferative activity [25].
Guo et al. 2013, prepared a series of 2-aryl-yl(5-methacrylate)aurone analogs possessing furanyl and thienyl substitutions using multistep reaction involving esterification, nucleophilic substitution, aldol reaction and Heck coupling. Out of all the compounds, 2-(2-furanyl)methylene)-5-methacrylate-benzofuran-3(2H)-one exhibited inhibitory activities against K562 and HepG2 cell lines with an IC$_{50}$ values of 2.18 µM and 3.95 µM, respectively [26].

Nadri et al., 2013 reported a new series of 5,6-dimethoxybenzofuran-3-one derivatives bearing pyridinium moiety as potent dual inhibitors of acetylcholinesterase and butyrylcholinesterase. The compounds were prepared by the reaction between 5,6-dimethoxybenzofuran-3(2H)-one 3, pyridine-4-carbaldehyde and PTSA in the presence of dry toluene using Dean-Stark apparatus [27].
Narsinghani et al., 2013 synthesised substituted chalcones using Claisen–Schmidt condensation of substituted acetophenones with benzaldehydes and reported the subsequent aurones formation by their oxidative cyclization using mercury (II) acetate in dimethyl sulfoxide as catalyst. The anti-oxidant activity of the test compounds was found to increase in the presence of dimethylamino group on positions 4/4′ of ring B and hydroxyl group at 5/5′ positions on A-ring in both chalcones and aurones, respectively such as in chalcone 2′,5′-dihydroxy-4-dimethylamino and aurone 5-hydroxy-4′-dimethylamino [28].

5,6-dimethoxybenzofuran-3-one derivatives
Demirayak et al., 2015 synthesised 2-[3- or 4-(2-aryloxyethoxy)arylidene] benzofuran-3-one derivatives using multistep synthesis that involved the Claisen-Schmidt condensation of benzofuranone derivatives with benzaldehydes followed by the reaction with brominated acetophenones. The aurone derivative, 2-[4-[2-(4-methoxyphenyl)-2-oxoethoxybenzylidene] benzofuran-3-one exhibited the highest activity by showing a growth percentage -44.36% on UO-31 cell line [29].

![Chemical Structure](image-url)
References


