Synthesis and Anticancer activity of Flavone Derivatives against Estrogen Dependent Cancers by rational approach

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ABSTRACT
Aromatase and 17-βHSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Chalcones, Coumarins, Flavones, Isoflavones have been reported for such inhibition and are used for treatment of breast tumors. So in this topic, Flavone derivatives containing Imidathiadiazole, Thiadiazole, Triazole and benzimidazole hetrocycles synthesised by using simple laboratory reagents like 2-Hydroxy Acetophenone and 4-Hydroxy Benzaldehyde to convert chalcone leads to formation of Flavones by cyclization using Microwave and followed by attachment of different hetrocycles to form Flavone derivatives and characterized by IR, 1H NMR, 13C NMR spectroscopy and elemental analysis. These Flavone derivatives found to exhibit moderate to high inhibitory activity against Estrogen dependent cancers.

KEYWORD: Cancer, Flavones, Chalcones, Flavanones, Imidathiadiazole, Thiadiazole, Triazole, Estrogen, Aromatase, 17- β HSD

INTRODUCTION
Cancer [1] is a class of diseases characterized by uncontrolled division of cells and the ability of these cells to invade other tissues, either by direct growth into adjacent tissue (invasion) or by implantation into distant sites (metastasis). There are so many types of estrogen [2] dependent cancers in which here more emphasize on Breast cancer and uterine cancer. Breast cancer is one of the most common cancers in women. Due to various reasons, the estrogen receptors are over-expressed in these tumour cells and, hence, estrogenicity is enhanced by many folds leading to excessive proliferation. Estrogens are hormones which mainly act on female reproductive system. Estrogens have some beneficial effects as well as harmful effects. Like in females breast and uterine cell proliferation, milk production, cholesterol balance, increase bone strength. Estrogens can also be harmful in case like breast and uterine cell proliferation. Estrogen enhances breast or uterine cancer risk. Cancer is caused by DNA damage (mutations) in genes that regulate cell growth and division. Normal cells mutated by heredity, radiation chemicals or DNA error during cell cycle which cause the cancer. In addition in excess of estrogen normal cell divide to causes cancer. Estrogen can cause cell proliferation to normal cell as well as cancerous cells.

Mechanism of Estrogen, SERMs and SDR class of pharmacophores
Estrogens, Antiestrogens, SERMs, SDR, Aromatase class of drugs having different effect on Estrogen receptors. Estrogen molecules bind to the estrogen receptors then co-activators

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bind to the site and activate estrogen response element this leads to gene activation and gene expression takes place. This leads in next step transcription and translation to form specific proteins. These specific proteins act on cell system in different way. But in case of Antiestrogens and SERMs they bind to estrogen receptors but co-activators could not bind to the site. SDRs and Aromatase inhibitors act differently they stop estrogen bind to receptor by blocking biosynthetic pathways.

Figure 1

Figure 2: Mechanism of Estrogen and Antiestrogens/SERMs
Many of the currently available anticancer drugs are not able to differentiate between normal and cancerous cells or to overcome primary or secondary resistance mechanisms evolved in the cancer cells.[3]

Thus, there is a need for new anticancer agents which is having high potency, less toxicity in neoplastic cells, and unique target of action. Presently, cancer therapy interfering with a single biological molecule or pathway has been successfully utilized. However, there is general belief that agents modulating more than one target could have superior efficacy compared to single target drugs. Therefore, modulating multiple targets simultaneously can be achieved by the combination of multiple drugs with different mechanisms or by single chemical entity that could modulate several targets of a multifactorial disease. As a result, there is increasing interest in the discovery of agents that concomitantly address more than one biological target for cancer treatment.

Effects of phytoestrogens on human health have been reported for decades. These include not only beneficial action in cancer prevention but also endocrine disruption in males. Since then many molecular mechanisms underlying these effects have been identified. Targets of phytoestrogens comprise steroid receptors, steroid metabolising enzymes, elements of signal transduction and apoptosis pathways, and even the DNA processing machinery. Understanding the specific versus pleiotropic effects of selected phytoestrogens will be crucial for their biomedical application.

Phytoestrogens include chalcones, flavones and iso-flavones which are non-steroidal compounds possessing estrogenic activity. As earlier mentioned need for molecule which is having effect on several targets can be fulfilled by these flavones. This flavone class of drugs can effect on multiple receptors and may be acts like estrogen receptor activators, SERMs, aromatase inhibitors, 17-β HSD inhibitors and protein kinase B inhibitors.

As shown in figure chalcone, flavones and estradiol having almost same structural similarities and so such molecules reported to have above mentioned activity on estrogen dependent cancers.

Figure 3: Showing structural similarities between Chalcone, Flavone and Estradiol
Along with these drugs, some monoclonal antibodies are also in this class of estrogen dependent cancers. Like bevacizumab’s (Avastin), Pentuzumab (Perjeta), Everolimus (Afinitor).

In year 2011 Food and Drug Administration (FDA) took action on such class of drugs. FDA revoked bevacizumab’s (Avastin) indication for treatment of metastatic breast cancer citing the drug’s lack of effectiveness and safety. Data indicate that bevacizumab does not significantly improve patients’ survival time and that its risks outweigh its benefits. Bevacizumab remains approved for treating other types of cancer.

Two new drugs were approved in 2012: Pentuzumab (Perjeta) and Everolimus (Afinitor)

Like all previous molecules this proposed molecule also having Estrogen receptor domains and this rationale has led to work on the development of “multiple target” class of drugs that would modulate the action of estrogens and thereby interfere with, or even prevent, the proliferation of breast and uterine cancer cells. If it would have action like antiestrogen then act by binding to estrogen receptors so that the estrogen molecules themselves cannot bind to those receptors. In case of SERMs modulate the response of estrogen and 17-β HSD and Aromatase inhibitors by inhibiting the biosynthetic path way and enzyme inhibition. This also blocks estrogen from activating genes for specific growth-promoting proteins.
Acolbifene[5] formerly known as EM-652, and SCH 57068, is a third generation SERM acting as pure antiestrogen. Acolbifene is also the orally active antiestrogen which is the most potent of the known antiestrogens and exerts pure antiestrogenic activity in the mammary gland and endometrium.

**Chemistry**

* Antiestrogens
* SERM (selective estrogen receptor modulators)
  - Different effect on one type of cells & different on others.
* Aromatase inhibitors
* 17 β-HSD (hydroxysteroid dehydrogenase) inhibitors

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**Figure 5: Rationale showing comparison between Acolbifene and Proposed compound**

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**Figure 6: Biosynthetic pathway for Estradiol with inhibition of 17 β-HSDs and Aromatase enzymes**
17 β-hydroxysteroid dehydrogenase (17β-HSDs) catalyzes the NAD(P)H dependent reduction of the weak estrone (E1) to the biologically most active estradiol (E2). (Oxidation in opposite reaction; E2 to E1) This reaction, which represents the last step in E2 biosynthesis, takes place in target cells where the estrogens exert their effects via the estrogen receptors α and β. Besides their physiological effects, estrogens are involved in the development and the progression of estrogen dependent diseases (EDDs) like breast cancer and endometriosis.

Hydroxysteroid dehydrogenases (HSDs) belong to the NADPH/NAD+-dependent oxidoreductases, which interconvert ketones and the corresponding secondary alcohols. Most 17 β-HSDs belong to the short chain dehydrogenase/reductase (SDR) protein family. The only exception known so far is 17 β-HSD5 (AKR1C3) which belongs to the aldo-ketoreductases (AKR). In addition to a structured nomenclature for AKRs, the international SDR-initiative recently proposed a new gene-based nomenclature for enzymes of the SDR-family. The first number indicates one of the 48 families, the following letter stands for the SDR type (C, E, I, D, X, A) and the last number notifies the members of the neighboring genes present in that type. The two main types are “classical” (C) and “extended” (E) differing in subunit size and sequence patterns. For Ex. HSD17B1 (SDR28C1), HSD17B2(SDR9C2), HSD17B3(SDR12C2), HSD17B4(SDR8C1), ARK1C3(HSD type5), HSD17B6(SDR9C6) likewise 14 types of HSDs available which are responsible for different biological activities like, Breast cancer, prostate cancer, endometriosis, obesity, polycystic kidney disease, Alzheimer’s disease etc.

Aromatase inhibitors are two types: type-I are NADP(H) independent ex. Exemestane, FormestaneType-II are NADP(H) dependent ex. Anastrozole, vorozole, letrozole and aminoglutethimide class. Aromatase inhibitors such as letrozole and anastrozole, have been shown to be useful in the second-line therapy of estrogen-dependent breast cancer and have recently been approved as first line therapy in several countries.

Chemistry of Flavones: In this search of new aromatase inhibitors, synthesized the new flavones derivatives, which would having significant anti-aromatase action. Some flavonoids are also known to be inhibitors of othersteroidogenic enzymes such as the 17b-HSD type 1(17b-hydroxysteroid dehydrogenase), which is involved in the regulation of the reversible interconversion of estrone to the potent estradiol.
MATERIALS & METHODS

Step-1: Synthesis of Flavone derivatives via clainsen-schmidt condensation

Substituted acetophenone react with benzaldehyde in presence of base to form condensed product chalcone which is in further step of reaction react with Methane sulfonic acid to give substituted flavone.

\[
\begin{align*}
 & \text{Substituted acetophenone} \quad 1a \\
 & \text{Substituted 4-Hydroxy Benzaldehyde} \quad 1b \\
 & \text{Chalcone Derivative} \quad 1c \\
 & \text{Flavone Derivatives} \quad 1d \\
\end{align*}
\]

Step-2: Reaction of Flavones with thiosemicarbazide to form thiadiazole ring system

Flavone derivatives from 1st step react with chloro acetic acid to give intermediates which on further react with thiosemicarbazide to give thiadiazole substituent on Flavone system.

\[
\begin{align*}
 & \text{Flavone Derivatives} \quad 1d \\
 & \text{Flavone Derivatives} \quad 1e \\
 & \text{Flavone Derivatives} \quad 1f \\
\end{align*}
\]
Step-3: formation of imidathidiazole ring system on Flavone derivatives$^{[11,12]}$

In step-3 as shown in Scheme-III two different reagent pairs [i] and [ii] react with step-2 product to give final Flavone derivatives.

Scheme-III

2-(4-(((benzylideneamino)-1,3,4-thiadiazol-2-yl)methoxy)phenyl)-23-dihyderochromen-4-one
Table 1: Derivative Identification codes for synthesized Flavone derivatives.

<table>
<thead>
<tr>
<th>No.</th>
<th>Derivative Id.</th>
<th>R</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>R⁵</th>
<th>R⁶</th>
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<td>1</td>
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<td>-</td>
<td>-</td>
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<td>H / OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>H / OCH₃</td>
<td>H / OCH₃</td>
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<td>OCH₃</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
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<td>H / OCH₃</td>
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<td>OH</td>
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<td>-</td>
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<td>OH</td>
<td>-</td>
<td>-</td>
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<td>H</td>
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<td>Br</td>
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<td>-</td>
<td>-</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
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<td>H / OCH₃</td>
<td>H / OCH₃</td>
<td>-</td>
<td>-</td>
<td>H</td>
<td>NO₂</td>
<td>H</td>
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</tbody>
</table>

Where, A = R, R¹ = H
A1 = R = H and R¹ = OCH₃
A2 = R = OCH₃ and R¹ = H
A3 = R = OCH₃ and R¹ = OCH₃

RESULTS AND DISCUSSION

Anticancer activity: Cancer is a disease characterized by uncontrolled growth of normal body cells. No specific cause was known that might be causing cancer but different factors like environmental factors, genetics, hereditary etc may be termed to define the cause of cancer in humans. In vitro cancer screening at NCI, USA, the screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10⁻⁵ M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well micro titer plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates are incubated at 37°C, with 5 % CO₂, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addiction (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50μg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate micro titer wells already containing 100 μL of medium, resulting in the required final drug concentrations.
Following drug addition, the plates are incubated for an additional 48 h at 37°C, with 5% CO2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air-dried. Bound stain is subsequently solubilized with 10 mM trizma (tris(hydroxymethyl) aminomethane) base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as: $\frac{[(Ti-Tz)/(C-Tz)] x 100}{[(Ti-Tz)/Tz] x 100}$ for concentrations for which Ti>=[Tz] and $\frac{[(Ti-Tz)/Tz] x 100}{[(Ti-Tz)/C-Tz] x 100}$ for concentrations for which Ti<Tz. Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

**CONCLUSION**

For specificity, new class of flavone derivatives prepare by using eco friendly reagents and prepare numbers of derivatives containing thiadiazole, imidothiadiazole and triazole ring system and check their anticancer potency on different cell lines.

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