



Recent Studies on Aromatase and EGFR Involved in Breast Cancer and their Inhibitors

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ABSTRACT

One of the major issues affecting worldwide public health is cancer. According to the related occurrence and morbidity statistics, it is becoming more common in both economically developed countries and developing countries. Several diagnostics procedures and early treatment methods are essential in order to reduce the incidence rate of breast cancer. In this article, we introduce several reported strategies of treatment based on the tumor progression and breast cancer (BC) molecular subtypes in order to offer the most personalized treatment for BC patients.

Keywords: Aromatase, EGFR, Breast Cancer, Coumarin, Inhibition

1. Introduction

According to **Global Cancer Observatory** (GLOBOCAN), a web-based platform presenting global cancer statistics, breast cancer in 2020 is currently one of the most frequently diagnosed malignancies and the fifth cause of cancer-related deaths, with approximately 2.3 million new cases worldwide and is now also the leading cause of cancer death among females in economically developing countries compared to the developed ones (Sung et al., 2021). One in eight American women will have breast cancer at some point in their lives, according to the American Cancer Society (**Fig.1**).

In 2023, about 297,790 new cases of invasive breast cancer will be diagnosed in women. By 2050, there are projected to be 3.2 million new incidences of breast cancer year worldwide. These statistics illustrate the severity of the breast cancer incidence, which will have an impact on society in general and require immediate preventive measures (DeSantis, Ma, Bryan, & Jemal, 2014; Druesne-Pecollo et al., 2012; Tao et al., 2015).

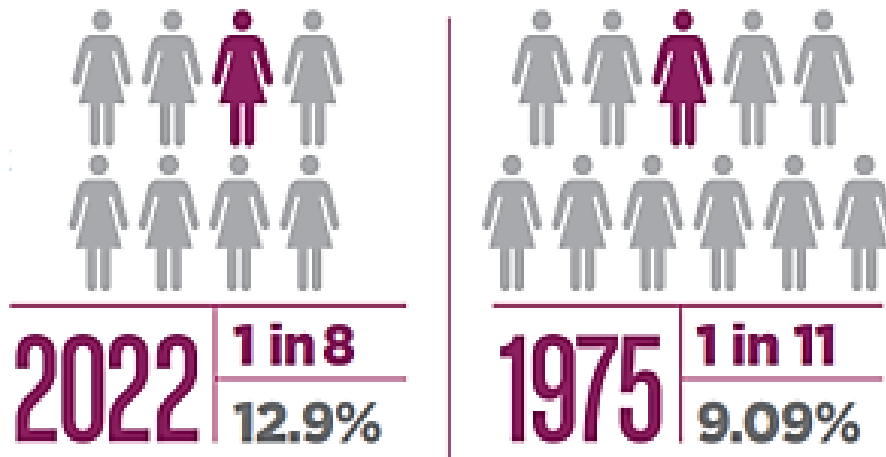


Fig.1: Breast cancer statistics

The Breast Health Global Initiative (BHGI) is currently in charge of creating appropriate recommendations and strategies to give the most effective breast cancer control achievable around the world (Duggan et al., 2020).

Risk factors for developing breast cancer include inherited and familial components. Breast cancer genes (BRCA1, BRCA2), and tumor protein (TP53) gene mutations are further risk factors for developing breast cancer (Yadav, Barmade, Tamboli, & Murumkar, 2015).

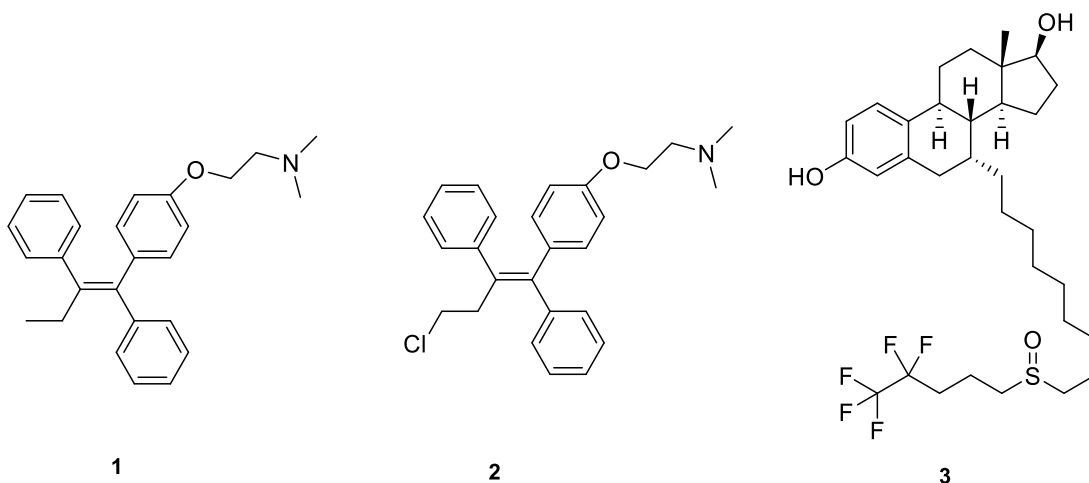
2. Material and methods

1.1. Current Personalized Treatments for Breast Cancer:

1.1.1. Hormone Therapy

In patients with the Luminal-molecular subtype of BC, endocrinal treatment may be administered either adjuvant or neoadjuvant therapy. The inhibition of estrogen receptors (ERs) through hormone therapy is frequently used to decrease estrogen levels or stop estrogen from stimulating breast cancer cells. Estrogen levels could be reduced by

- 1.1.1.1. Estrogen blockers:** such as selective estrogen receptor modulators (SERMs) (tamoxifen (1), toremifene (2)) and degraders (SERDs) (fulvestrant (3)).



1.1.1.2. Aromatase inhibitors (AIs):

1.1.1.2.1. Aromatase enzyme (regulation and expression) (Figure 2)

The cytochrome P (CYP) family member aromatase, also known as CYP19A1, which is characterized by the presence of a heme group, is engaged in the creation of estrogen from androgen via three consecutive hydroxylation reactions, to aromatic estrogens (estradiol-E2 and estrone-E1, respectively) and is in charge of catalyzing the final rate-limiting, critical, and important step for the biosynthesis of estrogen (Ji et al., 2014) (**Fig. 3**). These hormones are part of a class of steroids that support the growth and upkeep of the physical characters of women (Zubeldia-Brenner, Roselli, Recabarren, Gonzalez Deniselle, & Lara, 2016). Estrogen signaling pathways play a role in numerous processes, especially cell survival and proliferation. In addition to the reproductive system, estrogen plays an important role in the cardiovascular system, musculoskeletal system, and brain (A. Spinello et al., 2019).

Specifically, premenopausal women's ovaries, pregnant women's placentas, and postmenopausal women's adipose fibroblast cells all express the aromatase protein. Additionally, mesenchymal stromal (preadipocytes) and epithelial cells have been found to be the specific cell types that express aromatase in the breast (Miki et al., 2007). Compared to normal cells, some breast epithelial and stromal cancer cells show several-fold greater aromatase mRNA and protein levels (Lu et al., 1996).

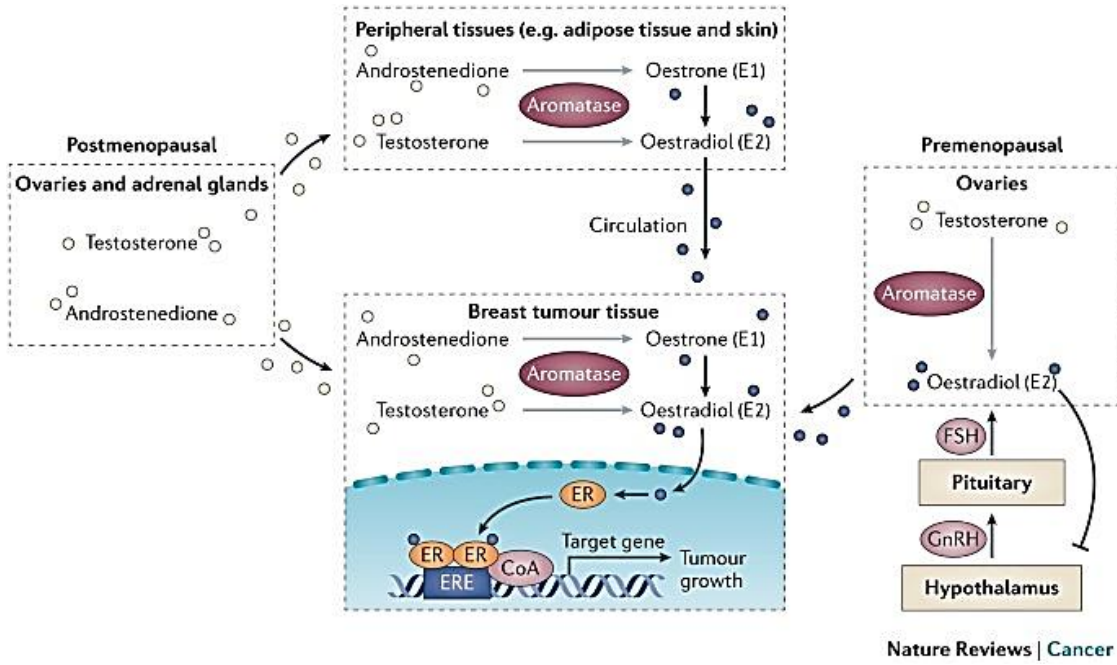


Fig. 2: Regulation and expression of aromatase enzyme.

In up to 50% of BC in postmenopausal women, estrogen synthesis by aromatase activity is principally responsible for the elevated estrogen levels in the tumor. Therefore, it is believed that inhibiting the aromatase enzyme is a promising treatment target for estrogen-dependent breast cancer (Simpson et al., 1994; Spinello, Ritacco, & Magistrato, 2019).

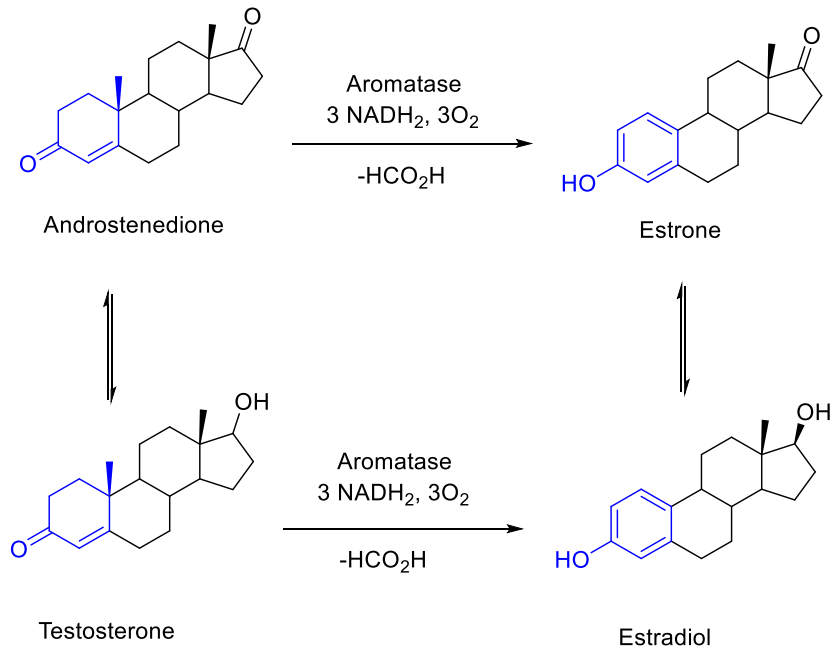
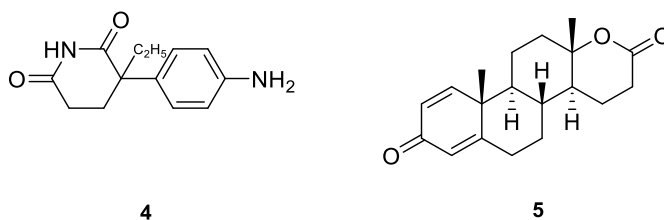


Fig. 3: Pathway of aromatase in estrogen biosynthesis

First Generation (aminoglutethimide (**4**) and testolactone (**5**)). They were approved by the FDA on 1980 for the treatment of breast cancer (Sayyad, Sabale, Umare, & Bajaj, 2022), then were discontinued later due to various side effects (Ghuge et al., 2020).



1.1.1.2.2. Classification of AIs:

Current AIs can be classified into steroidal and nonsteroidal classes:

- A) **Steroidal AIs or type I inhibitors**, having steroid-like structure similar to the substrate enzyme among them, formestane (**6**) The first selective second-generation steroidal aromatase inhibitor used to treat estrogen-receptor-positive breast cancer in postmenopausal women (Pérez Carrión et al., 1994) and exemestane (**7**), FDA approved drug. It is used as an adjunct treatment for (ER⁺) BC in postmenopausal women. Atamestane (**8**), minamestane (**9**), ORG 30958 (**10**) and plomestane (**11**) (Giudici et al., 1988; Henderson, Norbistrath, & Kerb, 1986; Johnston, 1987) covalently bind to the enzyme as a suicide inhibitor causing irreversible inactivation (**Fig.4**).

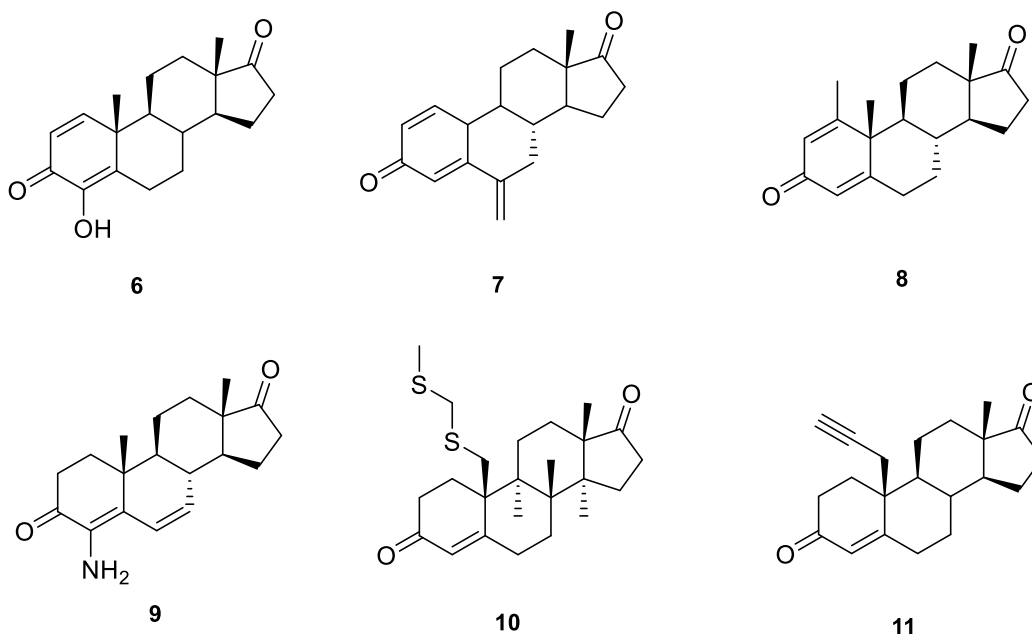


Fig. 4: Example of steroidal AIs

B) Non-steroidal AIs or Type II bind non-covalently to the heme motif of aromatase enzyme and prevent aromatase actions by competitive inhibition of androgens (Dellapasqua & Colleoni, 2010; Sanford & Plosker, 2008). Non-steroidal AIs include fadrozole (**12**), vorozole (**13**), rogletimide (**14**), letrozole (**15**) and anastrozole (**16**); among them, (FDA) has approved anastrozole (**8**) and letrozole (**7**) for the treatment of postmenopausal women with hormone-dependent breast cancer (Buzdar, 2002; Lamb & Adkins, 1998; Thürlimann et al., 2005) (**Fig. 5**).

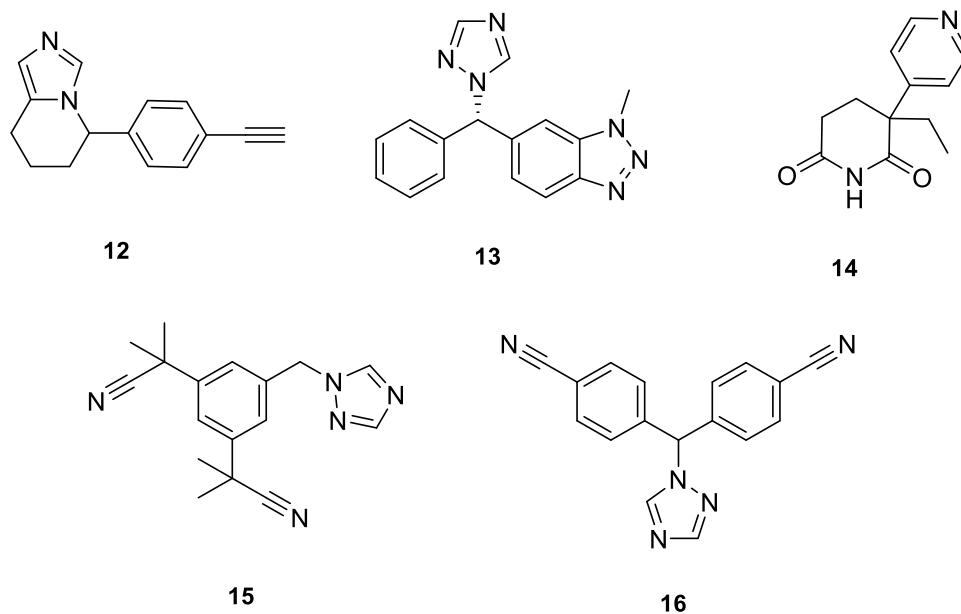


Fig. 5: Example of non-steroidal AIs

1.2. EGFRs in Breast Cancer

There are several members belonging to this family surface tyrosine kinase receptors (RTKs), class I of which includes the EGFR/ErbB family which are very often found overexpressed in several tumors, including breast cancers (Hsu & Hung, 2016; Wee & Wang, 2017) leading to dysregulation of their natural functions (regulation of cellular proliferation, differentiation, migration and survival) . This family comprises ErbB1/EGFR1, ErbB2/Her2, ErbB3/Her3 and ErbB4/Her4. (**Fig .6**)

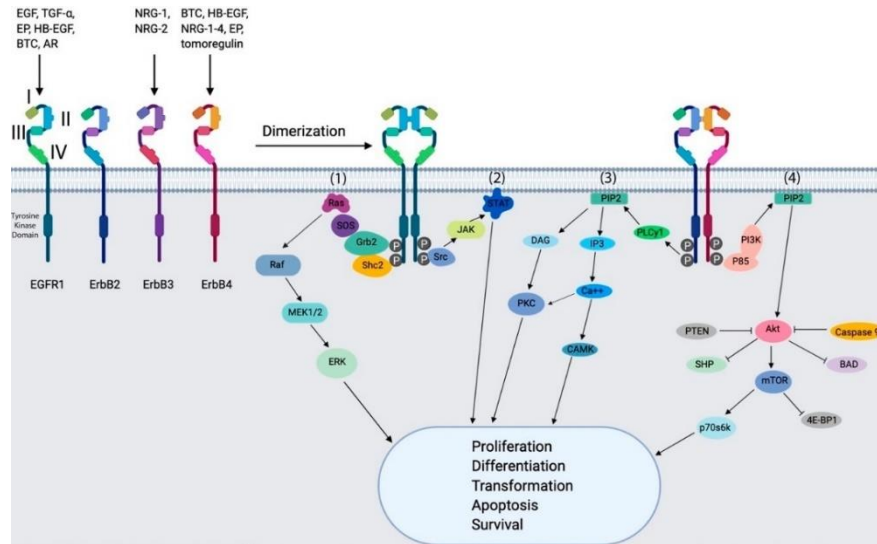


Fig. 6: Schematic representation of the four ErbB receptors.

1.2.1. Main therapeutic targets for EGFR: (Fig. 7)

1.2.1.1. Anti-EGFRs Monoclonal Antibodies (mAbs)

Cancer is targeted by mAbs that bind to the extracellular domain of EGFRs. Cytotoxic agents have been attached to mAbs leading to cell death and selective cancer therapies (Diamantis & Banerji, 2016).

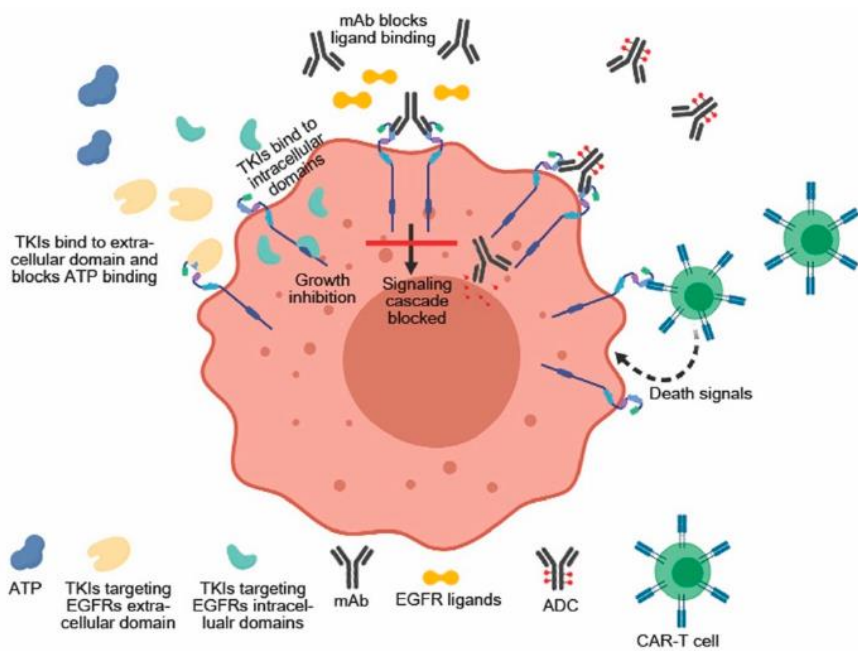


Fig. 7: EGFRs targeting therapy.

1.2.1.1.1. mAbs against EGFR1

EGFR1 receptor is commonly overexpressed, (in up to 25% of TNBC cases that is characterized by a reduced expression of estrogen (ER), progesterone (PR) and Her2 receptors (Choccalingam, Rao, & Rao, 2012; Gelmon et al., 2012; Nielsen et al., 2004).

Cetuximab (Fig. 8) and Panitumumab (Fig. 9) are FDA approved chimeric mAb that inhibits proliferation and signaling by binding with a high affinity to EGFR1. (Maennling et al., 2019) (Fig. 6).

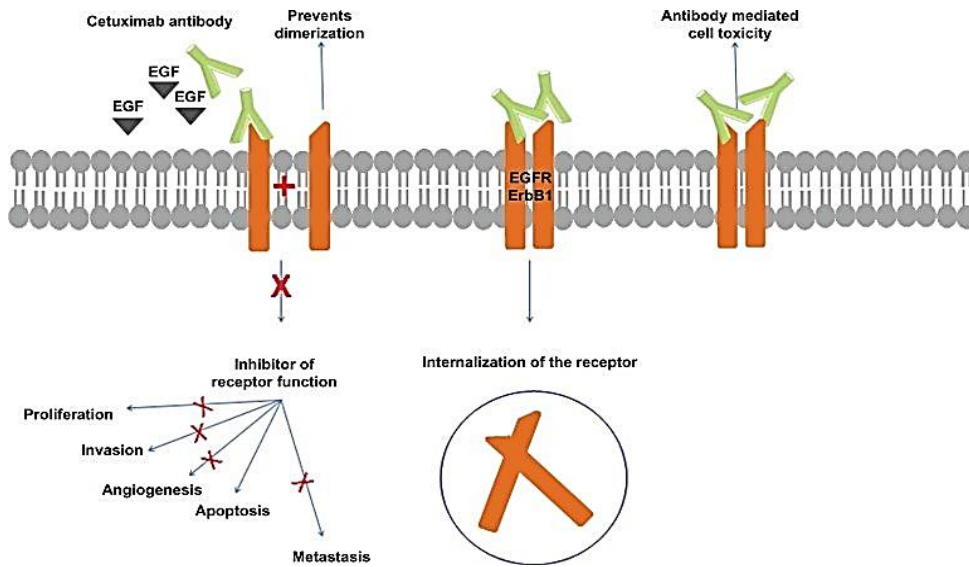


Fig. 8: Mechanism of action of Cetuximab

Panitumumab, as a completely human-derived monoclonal antibody, suppresses proliferation and signalling by binding to EGFR1. (Fig. 9)

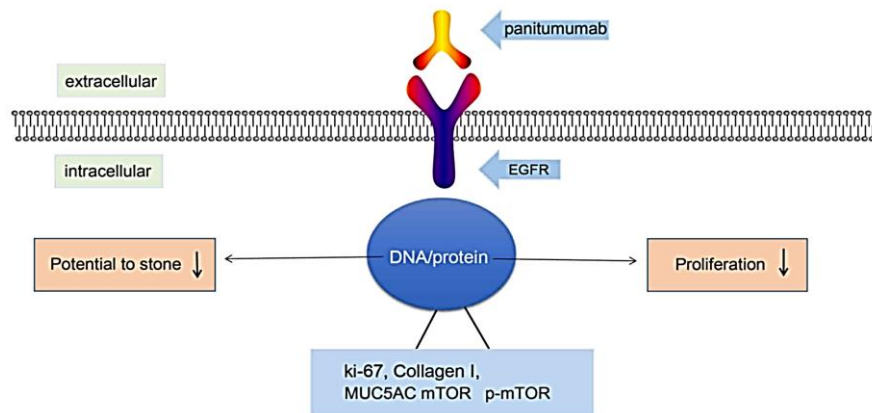
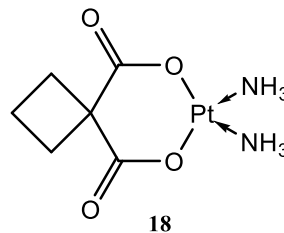
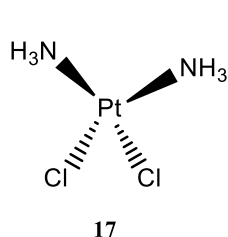
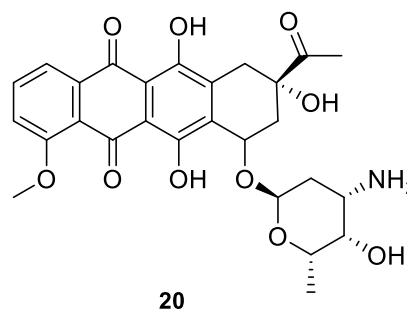
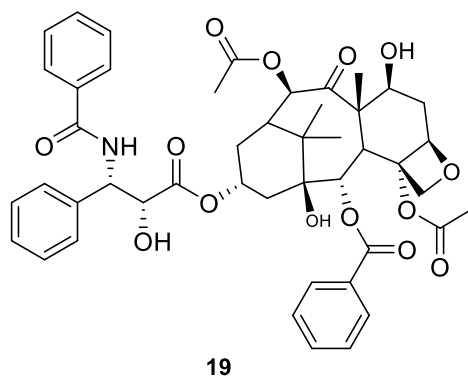


Fig. 9: Mechanism of action of panitumumab

Currently, the treatment of TNBC relies on a combination of surgery, chemotherapy and/or radiation therapy as there is no targeted therapy is approved for it due to the absence of targeted receptors (Liedtke et al., 2008; Silver et al., 2010). It was reported that the combination of cetuximab with cisplatin (**17**) or carboplatin (**18**) improved efficacy in TNBC treatment (Baselga et al., 2013).



Clinical trials of panitumumab and cetuximab in combination with a taxane (**19**)-anthracycline-containing (**20**) regimen for TNBC revealed better efficacy than either treatment alone (Nabholtz et al., 2014).



1.2.1.1.2. mAbs against Her2

Trastuzumab (**21**) and Pertuzumab (**22**) are recombinant humanized antibodies that have been approved for Her2-targeted therapy through various mechanisms of action (**Fig. 10**) (Llombart-Cussac et al., 2017; Tsang & Tse, 2020).

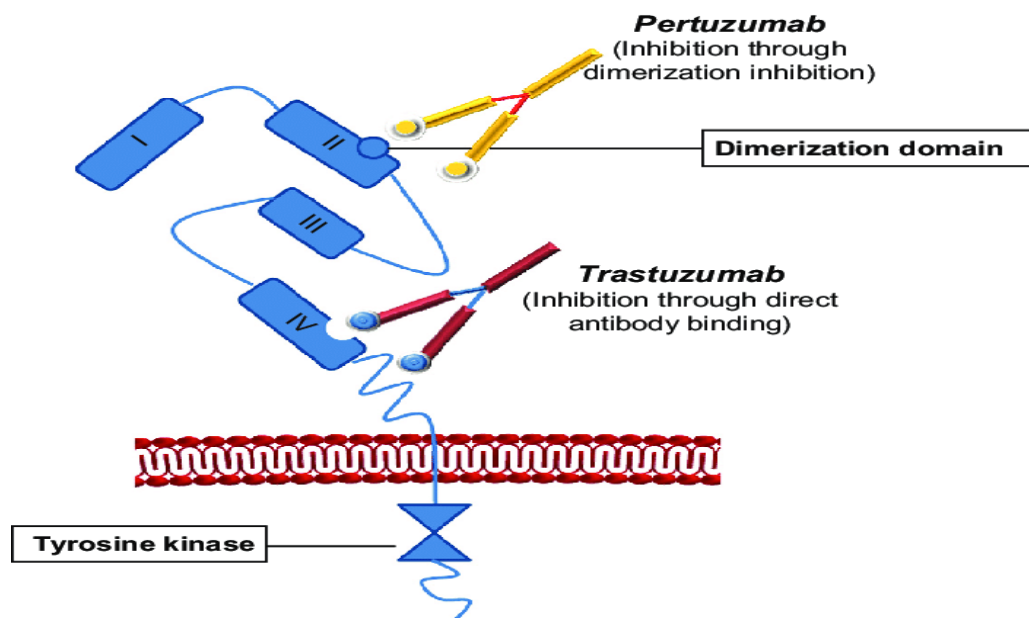
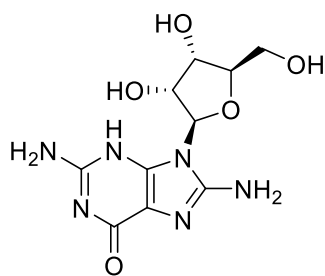
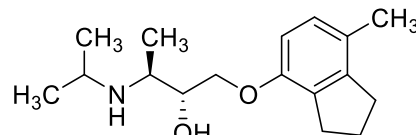


Fig. 10: Trastuzumab/pertuzumab mechanism of actions



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On the other hand, anti-HER2 antibodies may not be effective against all malignancies on their own. So, Trastuzumab was combined with several chemotherapeutic drugs, such as Trastuzumab-emtansine (T-DM1) (**23**), to overcome treatment resistance in human epidermal growth factor receptor 2 (Her2+) BC (Goutsouliak et al., 2020) (**Fig. 11**).

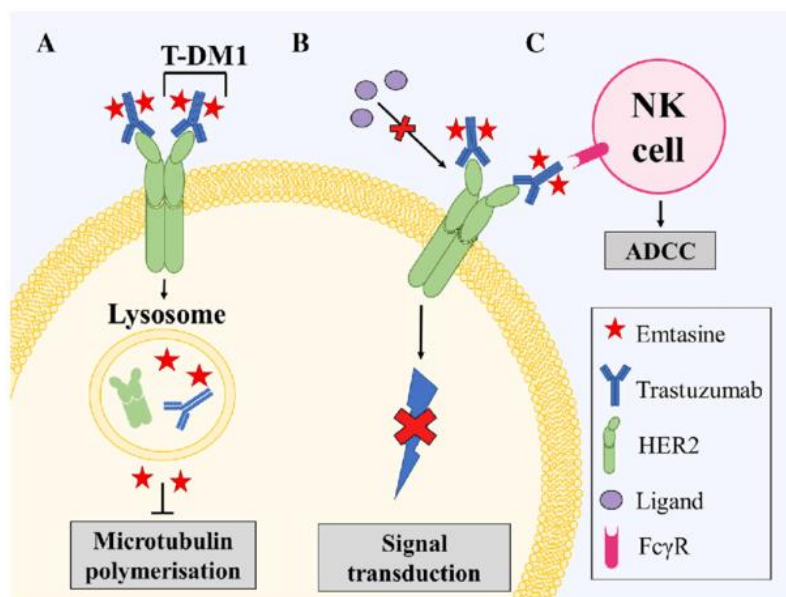
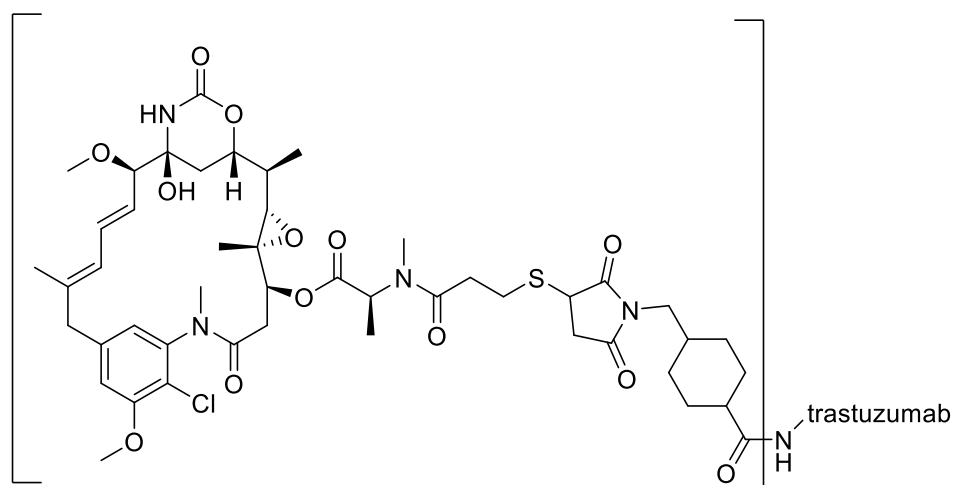


Fig. 11: (T-DM1) mechanism of action



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1.2.1.1.3. mAbs against Her3

Seribantumab and Lumretuzumab are humanized mAbs that block NRG1 from binding to Her3, resulting in the destruction of the target receptor (Mirschberger et al., 2013; Schoeberl et al., 2010) (Fig. 12).

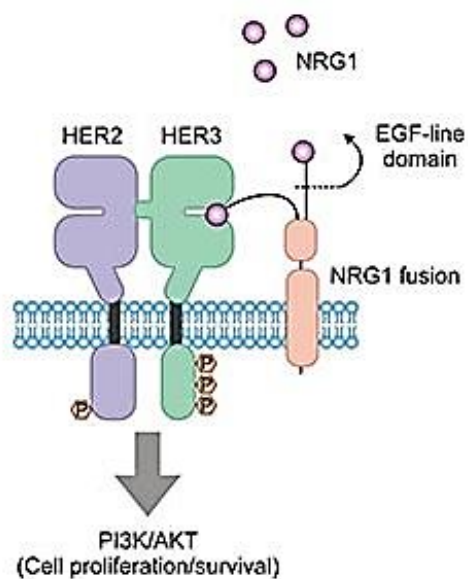


Fig. 12: HER 3 as therapeutic target in breast cancer

1.2.1.1.4. mAbs against Her4

Due to the under- and overexpression of Her4 under different scenarios in cancer, the biological importance of Her4 expression in cancer development is not completely understood (Hollmén, Määttä, Bald, Sliwkowski, & Elenius, 2009).

1.2.1.2. EGFRs Small Molecule Inhibitors (RTK Inhibitors)

Tyrosine kinase inhibitors can inhibit both the intracellular and extracellular domains of EGFRs while mAbs can only inhibit extracellular targets (Mishra, Patel, Alanazi, Yuan, & Garrett, 2018).

1.2.1.2.1. TKIs against EGFR1

Gefitinib (**24**) and Erlotinib (**25**) are reversible EGFR tyrosine kinase inhibitors that operate as ATP analogues by interfering with the ATP binding sites inside the EGFR receptors (Bareschino et al., 2007; Moyer et al., 1997). (**Fig. 13**)

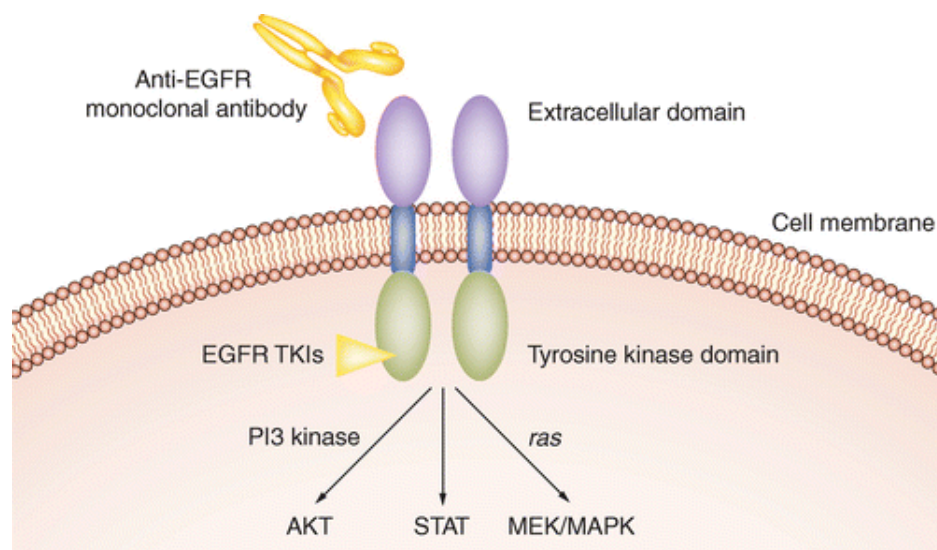
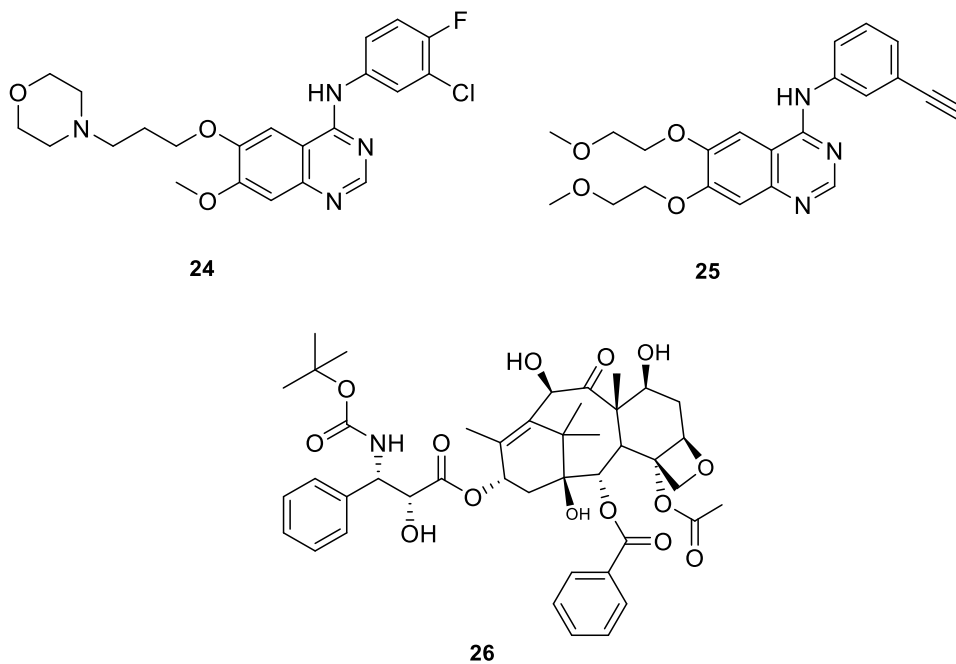


Fig.13: Mechanism of action of EGFR inhibitors

The FDA approved the synergistic effect for treating TNBC patients when combined docetaxel (**26**) or carboplatin (**18**) (Corkery, Crown, Clynes, & O'Donovan, 2009) with mABs cetuximab and panutumab as both cell lines were sensitive to the TKIs (El Guerrab et al., 2016).



1.2.1.2.2. TKIs against Her2

Lapatinib (**27**) and Neratinib (**28**) are EGFR and Her2 TKIs which operate as an ATP analogue at the ATP binding site, inhibiting the phosphorylation of EGFR and Her2 therefore inhibiting cell cycle progression and proliferation. (Blackwell et al., 2010; Figueroa-Magalhães, Jelovac, Connolly, & Wolff, 2014) (**Fig. 14**).

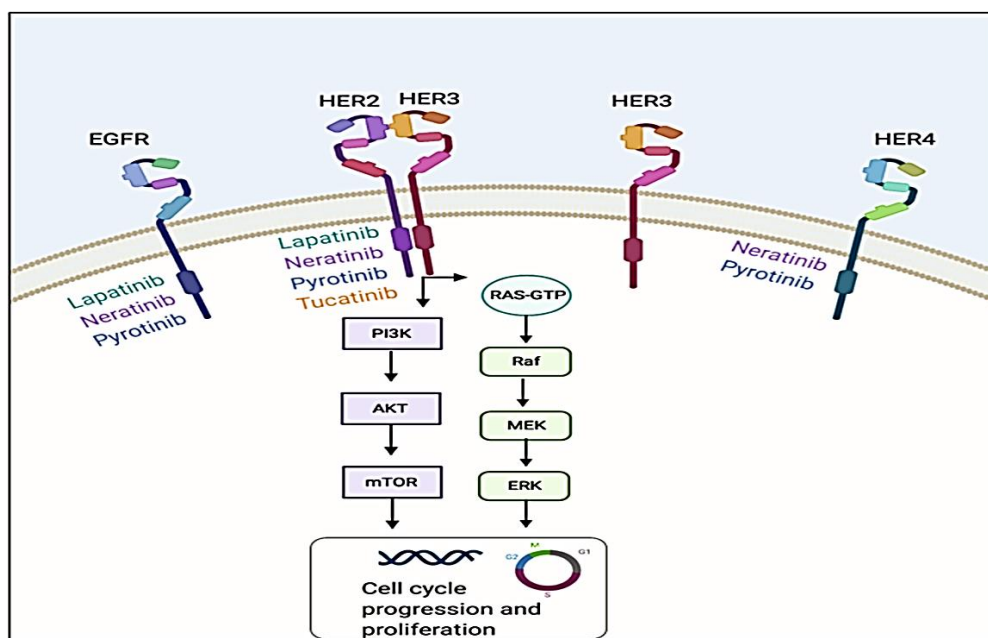
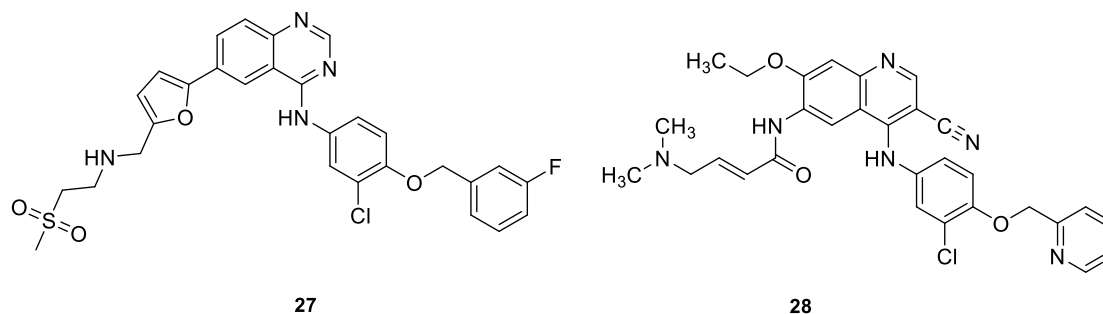


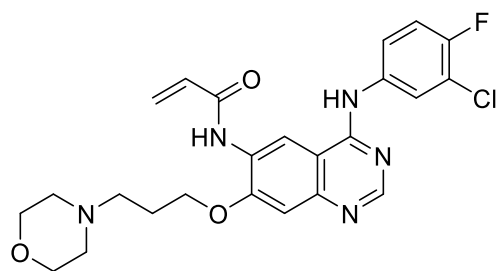
Fig. 14: Mechanism of action of HER2 inhibitors

1.2.1.2.3. TKIs against Her3

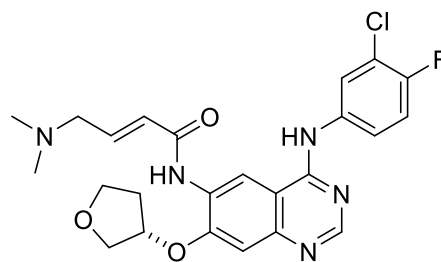
TKIs that target other EGFRs subsequently serve also as Her3 inhibitors (De Pauw et al., 2018)

1.2.1.2.4. TKIs against Her4

Such as Canertinib (**29**) and Afatinib (**30**) (De Pauw et al., 2016; Smaill et al., 2000) .



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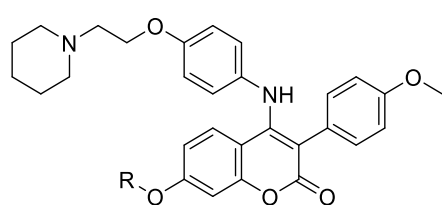


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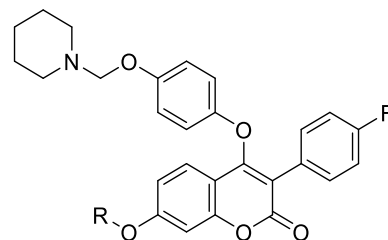
Results

Coumarins in breast cancer

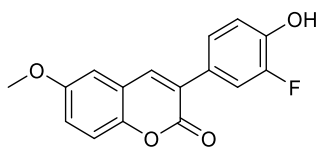
It has been shown that 3-phenylcoumarins (**31-35**) (G. Luo et al., 2017; Guoshun Luo et al., 2017; S. Niinivehmas & Pentikäinen, 2021) are able to suppress BC by blocking specific hormonal enzymes like hormonal estrogen receptor- α (ER- α) as it can mimic steroid compound binding in ER and thus offer a solution to affect ER activity (Kumar, Sunita, Jha, & Pattanayak, 2018; S. P. Niinivehmas, Manivannan, Rauhamäki, Huuskonen, & Pentikäinen, 2016).



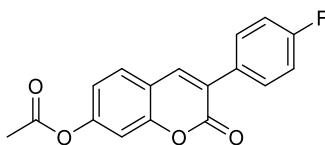
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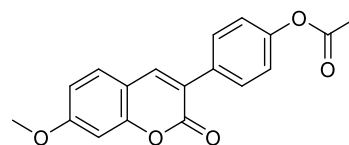
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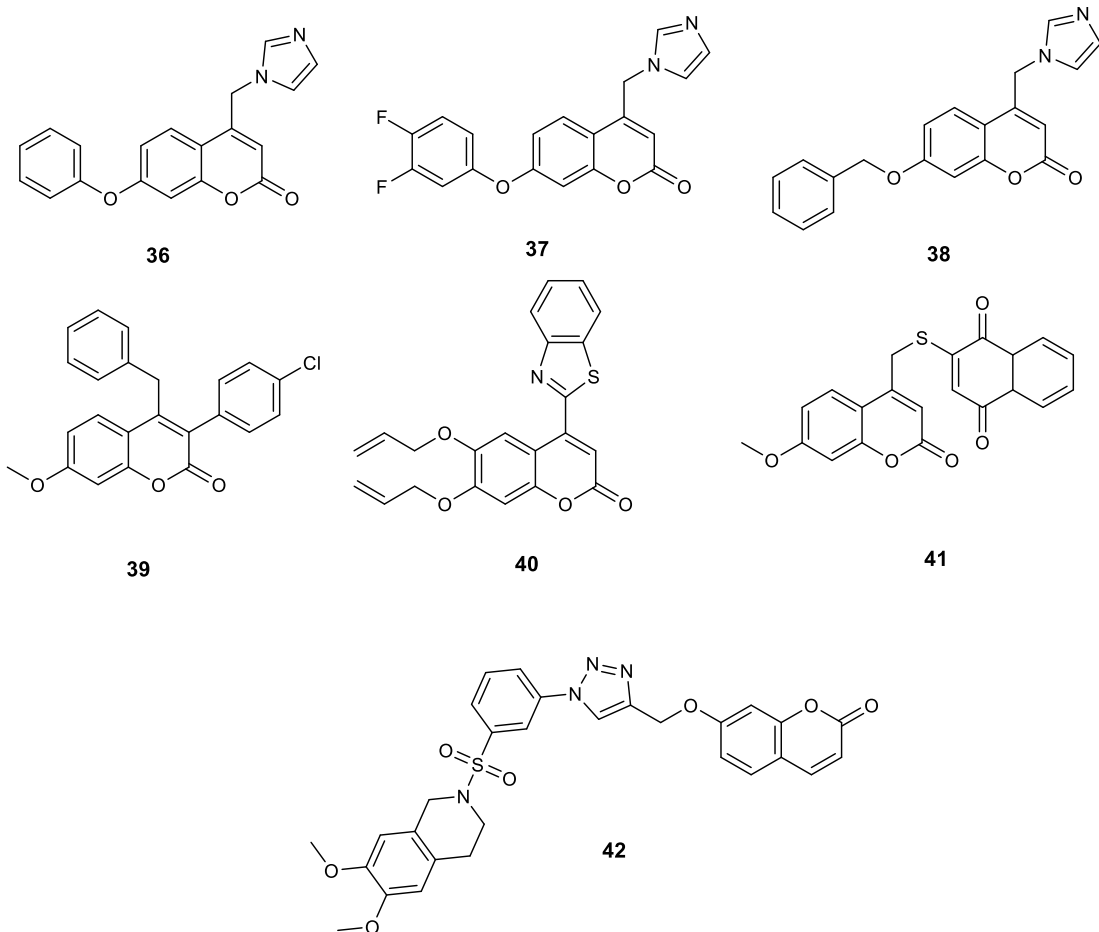
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In literature, coumarin scaffold was described as the best core as aromatase inhibitors bearing imidazole (**36-88**) which displayed the highest aromatase inhibitory Potency (F. Leonetti et al., 2004; Stefanachi et al., 2011).

Also, presence of phenyl moiety at C³ or C⁴ (**39-42**) (Bana et al., 2015; Chen, 2004. ; Kini, Choudhary, & Mubeen, 2012; Francesco Leonetti et al., 2004; Liu et al., 2014; Zhu et al., 2012) showed some potency as an aromatase inhibitor. The open chain coumarin sulphonamides showed significant aromatase inhibitory activity as compound **52** can be used as a potential lead anti-aromatase agent for future development (Pingaew et al., 2015).



It has been demonstrated that **Steroid sulfatase** STS produces 5-androstenediol, an androgenic substrate with estrogenic characteristics, which controls the amounts of active estrogens and androgens, playing a significant role in estrogen dependent breast cancer (**Fig. 15**). Estrone sulphatase activity in malignant breast tissue is now known to be considerably higher than in normal breast tissue. Sulfatase inhibitors are therefore considered promising therapeutic target for breast cancer (Billich, Nussbaumer, & Lehr, 2000; Geisler, Sasano, Chen, & Purohit, 2011).

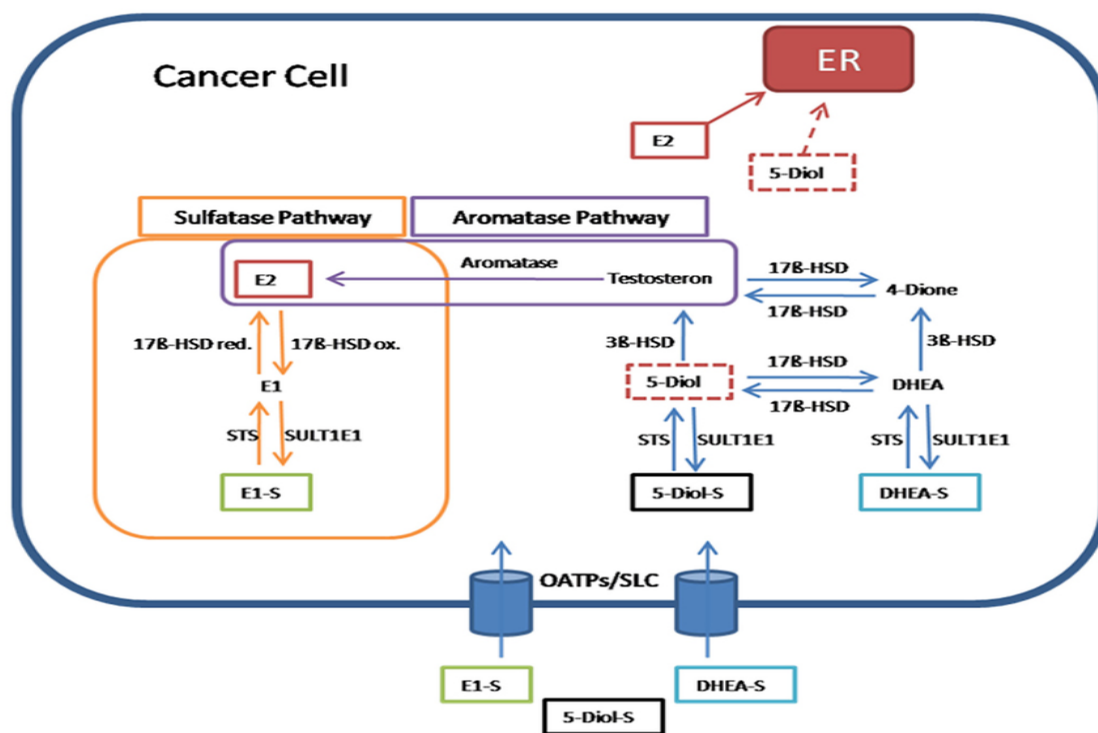
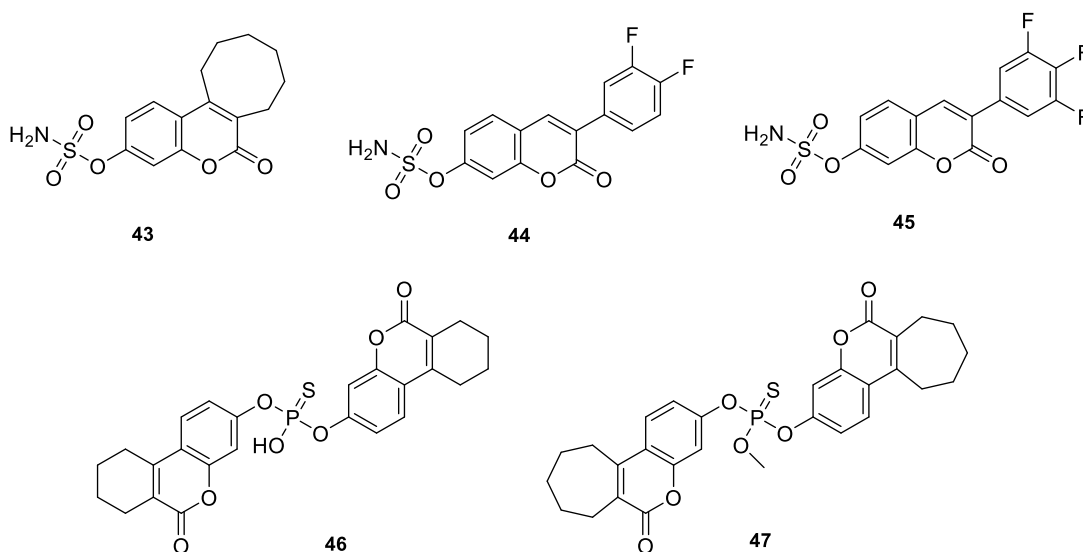
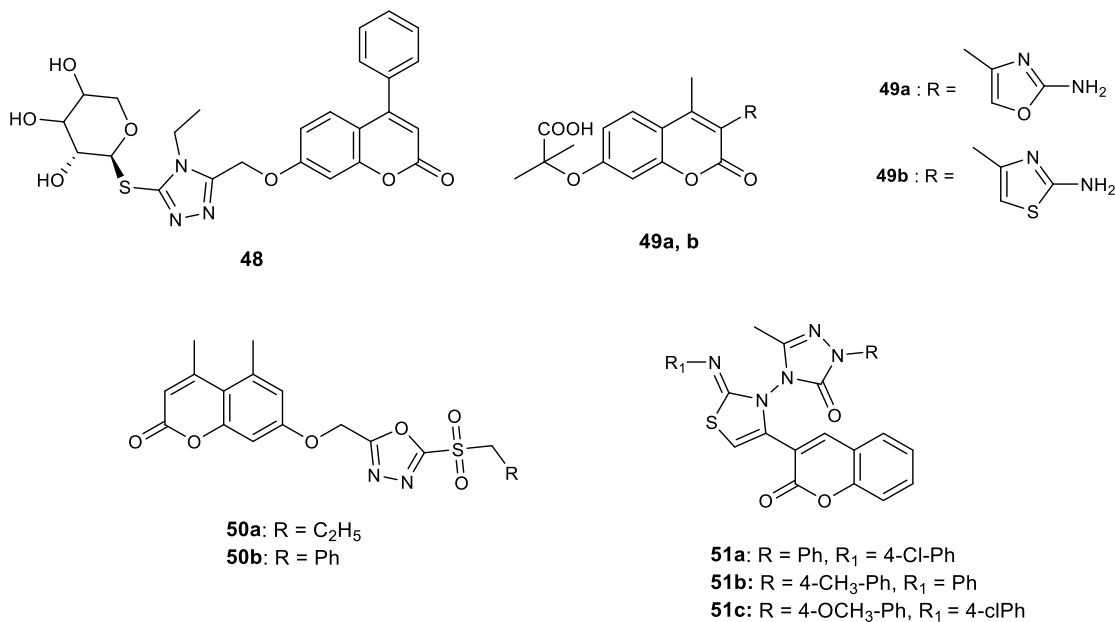


Fig. 15: Steroid sulfatase pathway

Moreover, the coumarin-based irosustat (nonsteroidal STS inhibitor) (**43**) is the first-generation steroid sulfatase inhibitor in breast cancer, revealing the potential of coumarin derivatives as novel anti-breast cancer agents. Modification of 3-position with a phenyl substituent by Sebastian *et al.* to develop potent STS inhibitors (compounds **44** and **45**) (Demkowicz *et al.*, 2016). The thiophosphate bis-coumarin derivatives were evaluated by Demkowicz *et al.* for STS inhibition. Compounds **46** and **47** are the Potent STS inhibitors of this class (Demkowicz *et al.*, 2015; Sardari *et al.*, 2017).



Coumarin based derivatives possess promising anti-breast activity as kinase inhibitors. In literature, 1,2,3-Triazole-coumarin-glycosyl hybrid **48** (El-Sayed et al., 2022), substituted coumarins **49 a, b** (Sairam, Gurupadayya, Vishwanathan, Chandan, & Nagesha, 2016) and coumarin tethered 1,3,4-oxadiazole derivatives **50a, b** (Dhawan et al., 2018) illustrated excellent EGFR kinase inhibitory activity against MDA-231 cell lines. Moreover, a series of coumarin-3-yl-thiazol-3-yl-1,2,4-triazolin-3-ones analogs **51a, b and c** exhibited excellent inhibitory action towards MDA-MBA-231 cells (Shaikh et al., 2018).



Conclusion

In this report, we critically reviewed the structure and functions of aromatase and EGFR enzymes along with their inhibitors and their major role in controlling the developing of breast cancer. In addition, we mentioned the importance of coumarin derivatives as an ubiquitous in natural and synthetic bioactive compounds, and coumarin derivatives readily interact with a variety of enzymes and receptors in breast cancer cells.

Disclosure

The author reports no conflicts of interest in this work.

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