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Drug Target Strategies in Breast Cancer Treatment: Recent Developments

Mayank and Vikas Jaitak*

Centre for Chemical and Pharmaceutical Sciences, Central University of Punjab, Bathinda (Pb), 151001, India

Abstract: Breast cancer (BC) is the leading cause of death among women all over the world. Estrogen receptor (ER) based therapy is one of the major approaches to target BC and is associated with various problems such as primary as well as secondary resistance. ER signaling is a complex pathway as many factors are involved; including several types of ERs and their associated co-regulators. Increasing understanding of ER signals results in new approaches targeting towards BCs. In this context, ER co-regulators have been explored and many modulators of ER co-regulators have been found out. EGFR and mTOR pathways also have significant impact on BC endocrine therapy because of the complex crosstalk mechanism which is responsible for primary and secondary resistance. Triple negative breast cancer (TNBC) is majorly associated with BRCA mutations. Currently there is no approved targeted therapy available in such form of cancer. Although PARP inhibitors seem to be suitable candidates for it. The present review is focused on the current scenario of ER, EGFR, as well as mTOR signaling target therapy. We have also discussed the current status of PARP inhibitors in BC chemotherapy.

Keywords: Breast cancer, chemotherapy, crosstalk, resistance, targeting.

INTRODUCTION

Breast cancer (BC) is the major cancer among women, having world's second highest morbidity rate of 10.9% [1]. Despite being various advancements in cancer chemotherapy, BC is still a major issue as more than three hundred thousand new cases and about forty thousand deaths have been detected in the United States alone during 2013 [2]. BC is a heterogeneous disease and three markers estrogen receptors (ER), progesterone receptor (PR) and erythroblastosis oncogene B2 (ErbB2 or EGFR2) are commonly used to classify BC [3, 4]. The major class is ER/PR positive which comprises of about 75% of all BCs whose growth is influenced by hormones and may be treated with hormonal therapy and thus categorized as a hormonal response positive (HR positive) tumor [5, 6]. Other forms of BC include tumor with overexpressed EGFR2 (20-25%) and triple negative BC (TNBC) (15%) which lacks all three markers [4, 7, 8, 9]. HR positive BC can be further sub-classified as luminal-A and luminal-B type. ER negative BC consists of basal type cells, some of which have overexpressed EGFR2 and other shows normal expression pattern [10]. Thus heterogeneity of BC seems to be much more complex as their response to therapy is diverse and individualization of therapy is required. Proper individualization of the therapy is impossible without a suitable classification system and for that more reliable markers are needed. Primary and secondary resistance towards BC chemotherapy as well as hormonal therapy are major problems in the desired clinical outcome of disease due to cross talk among various signaling cascades [11]. To understand BC and associated signalings, new targets have been explored [12]. In this review, we have discussed the estrogen signaling as a target for BC as well as current status of targeting approaches of EGFR and mTOR signaling as its crosstalk is important in modulating the signaling cascade of ER. The current status of PARP inhibitors and its emerging role in targeting TNBC is further mentioned in the review.

Estrogen Signaling in BC

First indication regarding involvement of steroid hormone signaling in BC comes from the observation that bilateral oophorectomy in patient with metastatic BC results in regression of tumor [13]. Now the involvement of estrogen signaling is well known and hormone therapy is commonly used to treat HR positive tumors. Estrogen signaling is mediated by classical as well as non-classical estrogen receptors (ER) [14]. Classical ER consists of ERα and ERβ which significantly control the cell proliferation and differentiation [15]. ERα is involved in cell proliferation as well as in cancer and ERβ has the opposite effect and thus may be considered as tumor suppressor [14, 16]. ERα which is overexpressed in 60-70% of BC is a ligand dependent transcription factor belonging to the nuclear receptor superfamily of protein with defined functional domains having the ability to activate or repress gene activity [17]. ER without ligand is in inactive state which is sequestered by multichaperone complex that involves heat shock protein 90 (Hsp 90) [18-20]. The complex system is generally present in the cytoplasm of the cell that may be associated partly with nuclear component structure [14]. Once bound to the ligand, which is estrogen in general, it allows the receptor to adopt such a conformation that the associated chaperone complex is dissociated, which releases Hsp90 along with related molecules and dimerization of receptor takes place which finally modifies the target gene expression [21]. Genes related to proteins such as cyclin D1, insulin like growth factor I receptor (IGF-IR), collagenase, PR, PS2, heat shock proteins, TGF-α, IGF-II and vascular endothelial growth factor (VEGF) represent the few among many genes regulated by ER [22]. The interaction of dimer with the promoter region of target genes is a critical step which can bind directly through specific estrogen response element (ERE) or indirectly through contact with other DNA bound transcription factors such as activation protein 1, specificity protein 1 or nuclear factor k-light-chain enhancer of activated B cell [12]. Once bound to the cognate response element in the promoter region of target genes, it allows proper recruitment of a series of co-regulators which control chromatin remodeling [14]. These co-regulators may be classified as a co-activator and co-repressor depending upon whether it activates or suppresses the target gene [23]. Although the classification of co-regulators is not the thumb rule as their behavior is cell specific and some co-repressors such as SMRT are must for the transcription in response to estrogen [14]. The response specificity of ligand depends upon the conformation that the receptor gains after binding to ligand because the recruitment profile of co-regulators depends on conformation gained by ligand bound receptor [14]. Another aspect of estrogen signaling is the existence of estrogen mediated rapid signaling which is known for

*Address correspondence to this author at the Centre for Chemical and Pharmaceutical Sciences, Central University of Punjab, Bathinda (Pb), 151001, India; Tel: +91-7814542436; Fax: +91-164-2240555;
E-mail: vikasjaitak@gmail.com

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many years [24]. Rapid signaling is mediated through membrane estrogen receptors (mER) and the pathway is commonly termed as a nongenomic pathway [25]. Rapid action of estrogen includes signaling through classical receptors that are present on the cell and through non-classical receptor that involves G protein coupled receptor 30 (GPR30) as well as ER-α36 [26]. Palmitoylation at cystine 447 is the key event in the localization of ERα to the plasma membrane where it activates MAPK and phosphatidylinositol 3 kinase (PI3K)/serine threonine protein kinase (Akt) pathways in BC cells [27]. Another mechanism involved in nongenomic signaling is methylation of ERα at arginine 260 in the DNA binding domain by protein arginine N-methyltransferase 1 (PRMT1) which facilitates Src/focal adhesion kinase mediated downstream transduction cascades [28]. These factors indicate the existence of a functional extranuclear signaling pathway for estrogen in BC cells, which appear to be a new inclusion in targeted therapy. Importance of understanding and exploring extranuclear targeting aspects of estrogen signaling can be realized by the fact that it has significant involvement in cell proliferation, migration, drug resistance and apoptosis inhibition [29, 30]. In a study, blocking of MAPK was demonstrated to show beneficial effects on BC, which further indicates the functional importance of rapid ERα mediated Src/MAPK pathway [12]. Integrin linked kinase is involved in extra-genomic signaling through PI3K pathways which regulates cell migration and thus inhibition of PI3K inhibits BC cell migration [31]. In another study ERα mediated regulation of deacetylation of tubulins in association with HDAC6 facilitate BC cell migration through the extragenomic signaling [32]. Tamoxifen induces tubulin deacetylation, suggesting extranuclear signaling through tubulin deacetylation confers endocrine resistance in BC [26]. Recent findings have revealed that PI3K/Akt activity associated with estrogen extranuclear signaling may be involved in blocking the apoptosis induced by TNF, hydrogen peroxide and serum withdrawal [33].

Another aspect in BC hormonal therapy is *de novo* as well as acquired resistance towards tamoxifen and related agents [11]. The main cause of such resistance includes crosstalk of ER signaling with multiple types of other signaling pathways [34]. Membrane ERα interacts and/or activates several kinases such as IGF-1R, Src, PI3K, MAPK, EGFR and EGFR2 [34-39]. Phosphorylation of co-activator by cytoplasmic kinases leads to modification of ERα activity [40-42]. Temoxifen and related SERM's which are agonists to membrane ERα (mERα) may interact with mER and activate multiple kinases which can phosphorylate ER and mediate ligand independent signaling [34, 39, 43]. Here we conclude that multiple

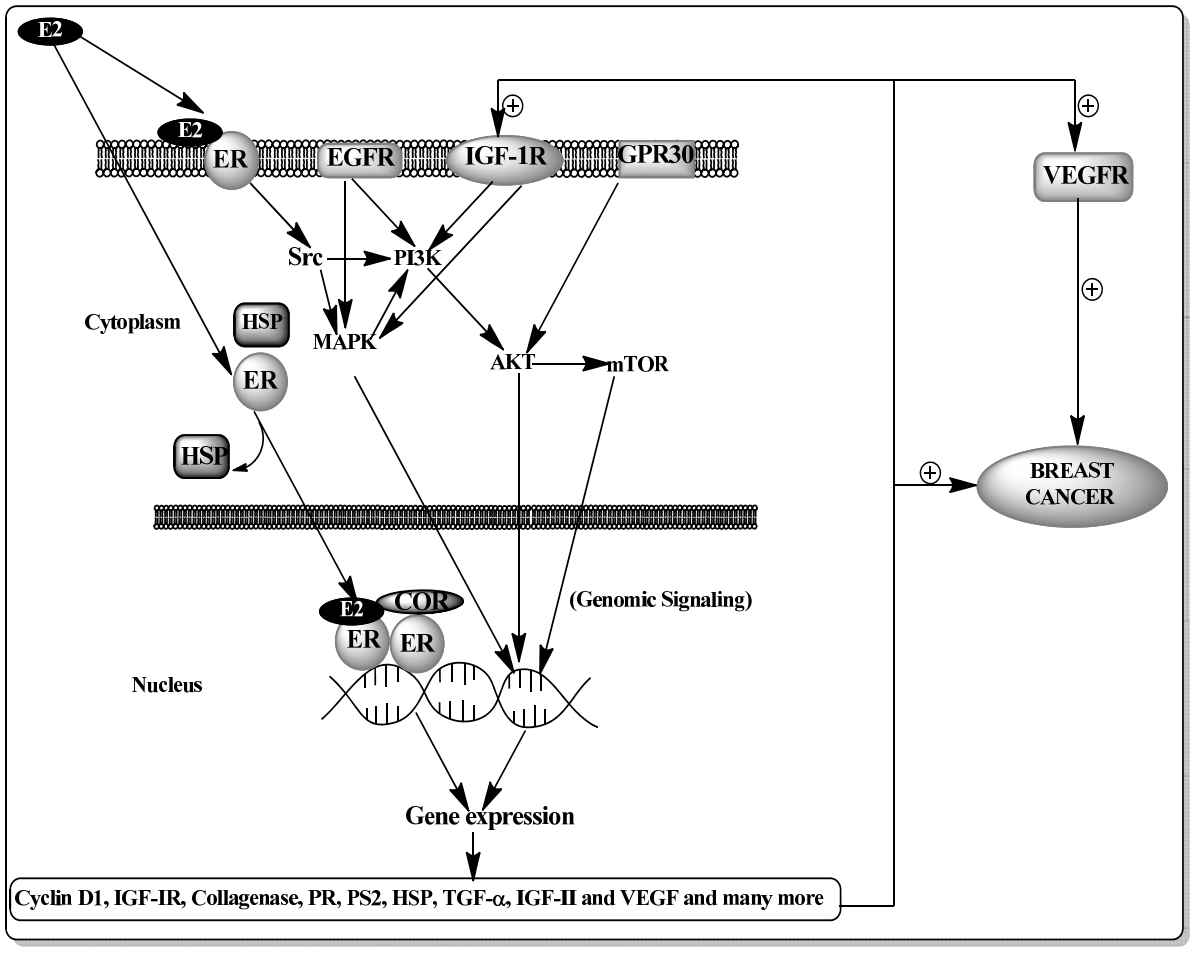

Fig. (1). Simplified model of deregulated estrogen signaling and associated risk of BC. Estrogen receptor signaling pathway is mediated through genomic and extranuclear signaling which is synchronized with various kinases. In genomic signaling, estrogen (E2) binds to ER, which allowes conformational changes in ER , dissociation of heat shock protein 90 (HSP) and related molecules from it, dimerization of ER receptors, interaction with DNA and recruitment of co-regulators (COR) to modify gene expression. The extranuclear signaling induces activation of PI3K/AKT/mTOR and Erk/MAPK pathway which can convey genomic signaling. Multiple induced transcriptions such as cyclin D1, IGF-IR, collagenase, PR, PS2, HSP TGF-α, IGF-II and VEGF may be responsible for cancer and cancer growth.

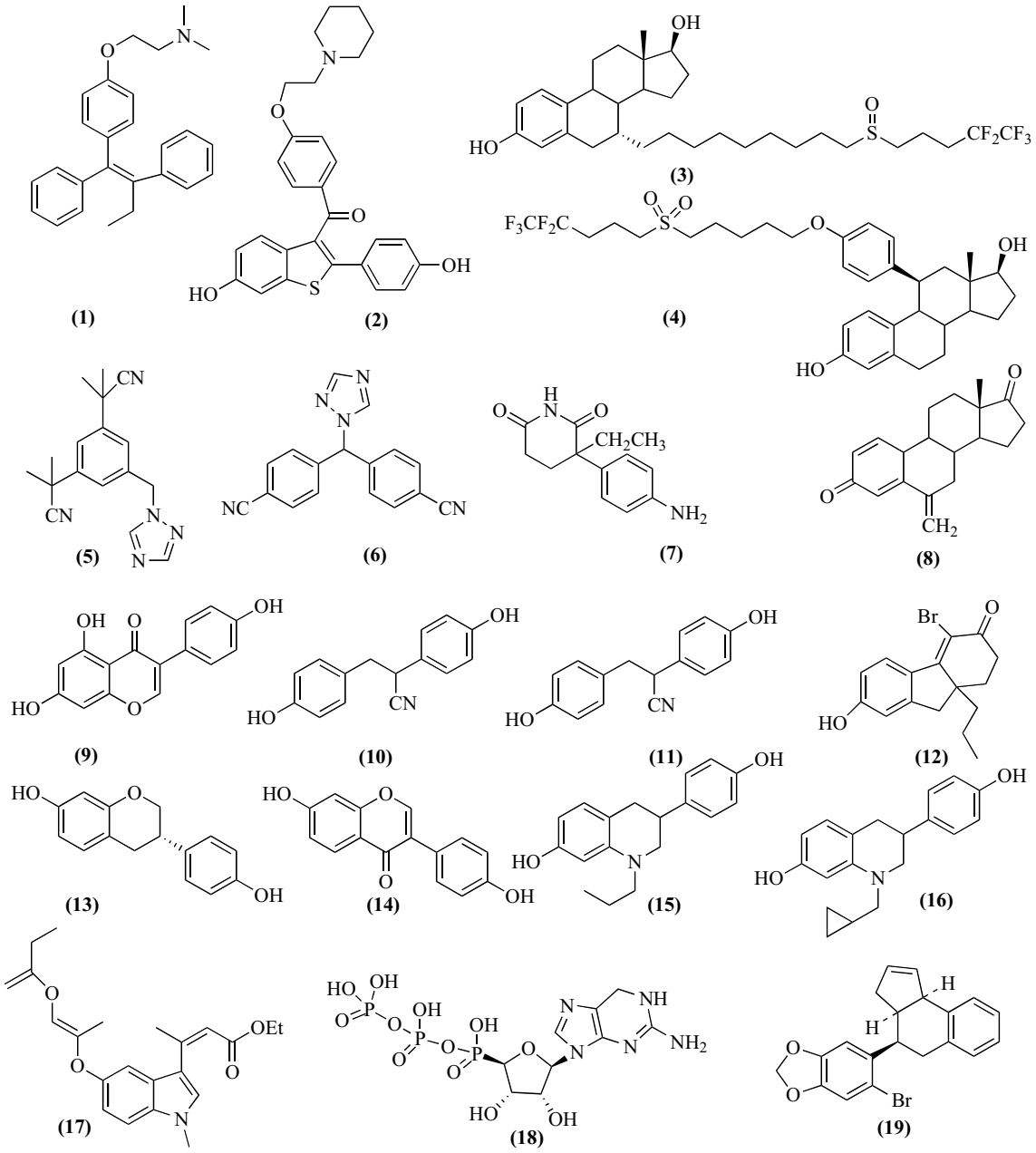

Fig. (2). Inhibitors/modulators of various types of ER mediated signaling.

classes of ER, related co-regulators, growth factors, and other kinases work together. Deregulation of these signalings results in multiple complications for chemotherapy as well as hormonal therapy Fig. (**1**).

Targeting Estrogen Signaling

Number of targeted approaches have been implemented and antiestrogens (AE) are among the oldest examples for BC [44, 45]. Tamoxifen **(1)** Fig. (**2**) is clinically most widely used selective estrogen receptor modulator (SERM) in HR positive BC and mechanism of which involves the recruitment of co-repressor in the final step in breast cell [14, 46]. Raloxifene **(2)** is the SERM which is clinically used for HR positive breast cancer [47]. Another important aspect of tamoxifen and raloxifene is their role in BC preventive therapy which is approved by the US Food and Drug Administration [48]. Another class of antiestrogen belongs to selective estrogen receptor down regulator (SERD) which includes Fulvestrant **(3)** and RU58668 **(4)** [49]. Fulvestrant is an FDA approved drug for treating postmenopausal women with HR positive advanced BC, which prevents ER dimerization, nuclear localization, blocks ER mediated transcriptional activity and induces receptor degradation [50-52]. Aromatase inhibitors (AIs) block the final step in the conversion of androgen to estrogen and thus reduce circulating estrogen level that seem to be the most suitable for postmenopausal women [14]. The agonistic activity of SERM on mER activates the EGFR signaling pathway and hence AIs seem to be superior to SERM. Anastrozole **(5)** and letrazole **(6)** are now commonly used for postmenopausal as adjuvant therapy for advance BC [53, 54]. Aminoglutetimide **(7)** and exemestine **(8)** are the other agents belonging to AIs category that are also in clinical practice [55, 56]. Increased understanding of estrogen signaling and problems associated with SERMs, SERD, and AIs result in evolvement of multiple new targeted approaches which include ERβ, mER, and co-regulators.

ERβ and GPER

ERβ opposes proliferation and tumor propagation associated with ERα and can be considered tumor suppressor. Suppression of angiogenesis and tumor growth in T47-D xenograft model by ERβ is a valid proof regarding its tumor suppressive properties [57]. BC is significantly associated with chromosome 12q loss which encodes ERβ indicating its antitumor action [58, 59]. siRNA mediated knockdown of ERβ results in increased gene transcription related to tumor proliferation [60]. ERβ mediated cell cycle arrest is also demonstrated, which results from the repression in c-myc, cyclin D1, cyclin E, cyclin A cdc25A related transcription as well as increase in expression of CDKp21 $^{waf1/cip1}$ and p27^{kip1} [16, 61-63]. Tamoxifen being antagonistic against ERβ may cause regression in the beneficial effect of SERM related therapy [64]. Thus ERβ seems to be an important target and a ligand which is selective antagonist towards ERα and agonist for ERβ which is theoretically ideal. Many ERβ specific ligands have been mentioned in literature which include genistein **(9)**, DPN **(10)**, ERB-041 **(11)** and tetrahydrofluorenone **(12)** [65].

Equol **(13)** which is a metabolite of daidzein **(14)** is reported to possess modest selectivity for ERβ. Its many derivatives have been synthesized such that compound **(15)**, **(16)** have been reported to showed antagonist activity against ERα and agonist activity against ERβ [65]. GPR-30 which is seven transmembrane G protein coupled estrogen receptor commonly known as a G protein estrogen receptor (GPER) is an important target for BC. Tamoxifen and other classical ER antagonists act as agonists towards GPER and play a critical role in activation of epidermal growth factor receptor (EGFR) as well as ERK. [66, 67]. MIBE **(17)** is a compound with antagonist activity against ERα and GPER [66]. G36 **(18)** and G15 **(19)** are other compounds which showed GPER antagonist activity [68]. Inclusion of ERβ and other non-classical ERs in targeted therapy for BC is comparatively a newer approach and theoretically seems to cause significant impact.

ER Co-Regulators

Co-regulators that include co-activators and co-repressor play an important role in the ER mediated gene expression. Steroid receptor co-activator 1 (SRC 1) was the first co-regulator to be identified [69]. Currently, multiple co-regulators are known and many of them have been found to be deregulated in BC. Over-expression of amplifier in breast cancer-1/SRC3, GRIP1, PELP1, MUC1, breast carcinoma amplified sequence3 (BCAS3) and Ciz1, have been found to be associated with BC [70, 71]. Moreover, overactivation of co-activators of ERα (AIB1 and BCAS3) have been found to be associated with tamoxifen resistance [26]. Deregulation in co-repressors which recruit histone deacetylase, prevent transcription and counterbalance the effect of coactivators have been observed in BC [72]. Thus targeting co-regulators seem to be a noble strategy for BC therapy, which is discussed in subsequent section.

SRC-1

Steroid receptor coactivator-1 (SRC-1) also known as NCOA1 was discovered in 1995 and it has broadened the knowledge about the physiology of ER action [73]. It belongs to p160 SRC family, other members of which are SRC2 and SRC3 [74]. SRC proteins have been found to be associated with BC as it was found that SRC-1 is over-expressed in 19% to 34% of BC cases which is positively correlated with EGFR2 overexpression and with poor clinical outcome of the disease [73]. In MCF-7 BC cells, SRC-1 is over expressed which is associated with increased proliferation in response to the estrogen indicating the importance of SRC-1 intensity in estrogen mediated tumor growth [75]. Moreover MCF-7 cells lacking SRC-1 are unable to show an increase in estrogen mediated SDF-1α expression, and reduce the ability of proliferation and invasion. It indicates the direct link of SRC-1 with SDF-1α/CXCL12 mediated autocrine/paracrine signaling which is responsible for the proliferation and invasion [76]. Furthermore, various studies indicate that SRC-1 coactivates PEA3 mediated twist expression and promotes epithelial-mesenchymal transition, migration, invasion and metastasis of mammary tumor cells [77]. Role of SRC-2, the second protein of SRC family is still uncertain. Many studies showed its positive correlation with estrogen induced tumor growth and spread where SDF-1α may be involved. Some other reports mentioned that downregulation of SRC-2 modulates estrogen-responsive genes and stimulates proliferation as found in MCF-7 BC cells, suggesting SRC-2 may have proliferative as well as antiproliferative function in BC cells [76, 78]. SRC-3 overexpression has been reported in a variety of cancers, including BC and promotes cancer initiation, expansion and metastasis [79]. Overexpressed SRC-3 status is responsible for the positive crosstalk with IGF-1 pathway and resistance towards classical hormonal therapy [80]. SRC-3 plays a critical role in activating P13K/AKT signaling in mammary tumorigenesis [81]. Role of SRC-2 in BC is still uncertain, but overactivation of SRC-1 and SRC-3 is widely associated with BC and can be a valid target for BC chemotherapy. Gossypol **(20)** Fig. (**3**) is a small molecule that acts as a selective inhibitor of SRC-1 and SRC-3 which has no effect on SRC-2 or other coregulators as observed in MCF-7 BC cells [80]. It reduces the concentration of SRC-3 in prostate, lung and liver cell lines and inhibits the cell viability in same cancer cell line where it promotes SRC-3 down-regulation. Additionally, its effect of sensitizing lung as well as BC cell lines towards chemotherapeutic agents and selective cytotoxicity towards cancer cells indicate the possibility of including SRC inhibitors in clinical consideration. Gossypol **(20)** represent a prototype of new class of chemotherapeutic agent against ER overexpressed BC and is under clinical trials for several types of cancer [80, 82].

HDACs

Histone deacetylases (HDACs) are the class of enzymes having 18 members, responsible for the removal of acetyl group from lysine amino acid on the histone which restores the positive charge on lysine and ultimately causes more strong wrapping of DNA by the histone [83-85]. Acetylation status of histone is controlled by the opposite activity of histone acetyl transferases (HATs) and HDACs [83]. Irregular HDAC activity is associated with various human cancers, including BC [86, 87]. The repressed state of ER in MCF-7 cells is associated with overexpressed HDAC1 and reactivation of ER takes place by exposure to HDAC inhibitor [88]. Triple negative BC can be sensitized towards tamoxifen by various HDAC inhibitors, which result from the release of HDAC1 from ERα promoter and thus restoring the ERα expression [88]. Impact of HDAC inhibitors on ERα status is somewhat complicated as short time inhibition of HDAC leads to acetylation as well as stabilization of receptor at the protein level, but long time exposure results in delocalization and proteasome mediated degradation of the receptor [14]. Furthermore, HDAC inhibitor mediated increased expression of ERβ has been observed in ER positive BC cells which itself having tumor suppressor activity [89]. Combination of HDAC inhibitor and hormonal therapy has been found to re-sensitize BC resistant to tamoxifen and is involved in down regulating ATK activity with induction of cell death [90]. Inhibition of HDAC results in decrease of EGFR mRNA in ER-negative MDA-MB-221 and *in vivo* along with re-sensitization of tamoxifen related therapy [91]. HDAC inhibitors are found to induce Wnt/β catenin mediated dedifferentiation of human BC cell [92].

HDAC inhibitors are important considerations in the management of multiple forms of BC including HR positive BC. Some HDAC inhibitors are already approved by FDA that includes varinostat **(21)** Fig. (**3**), belinostat **(22)**, panobinostat **(23)**, romidepsin **(24)**

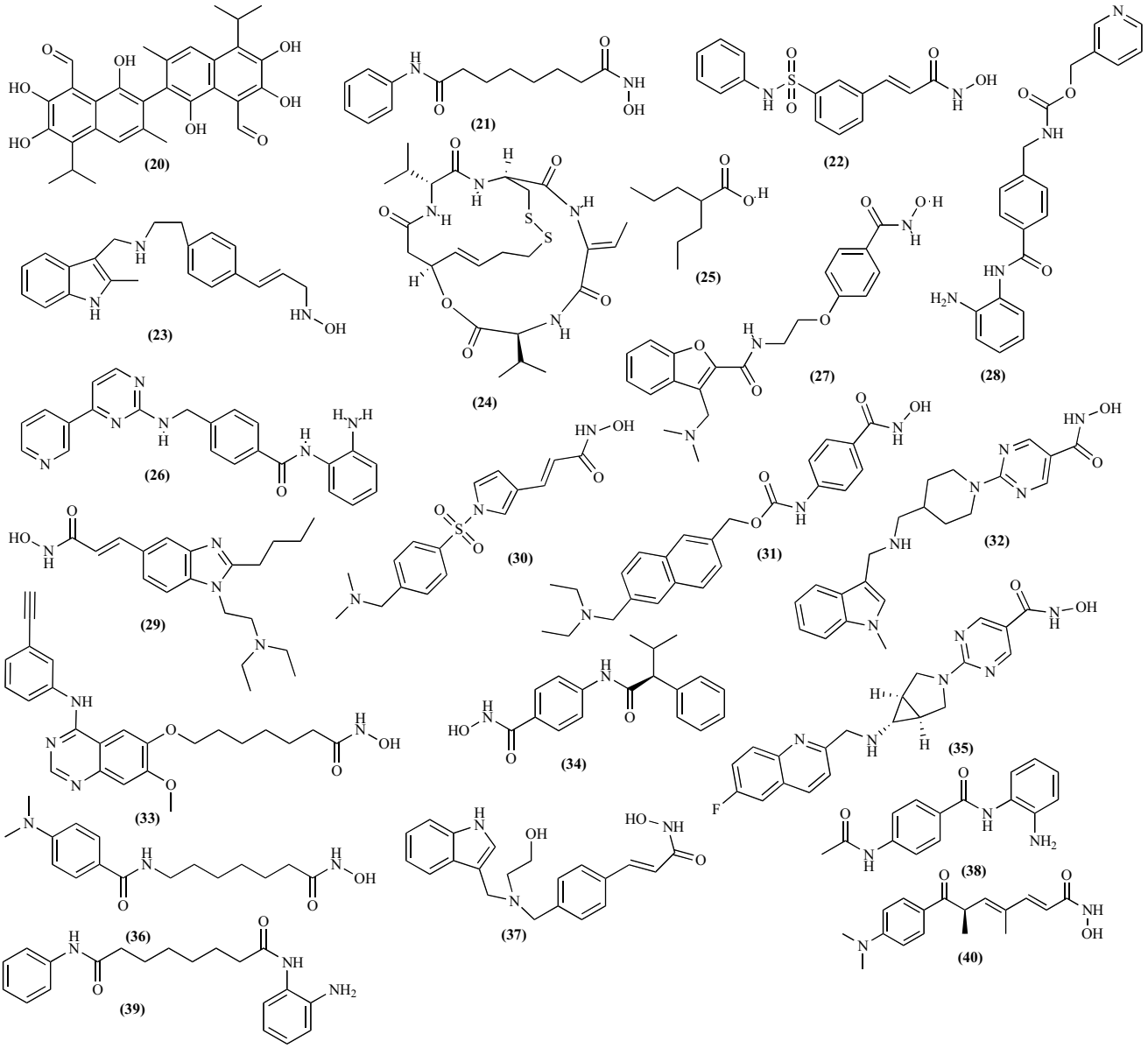

Fig. (3). Gossypol **(20)** a selective inhibitor of SRC-1, SRC-3 and HDAC inhibitors **(21-40)**.

[93, 94]. HDAC inhibitors such as valporic acid **(25),** mocetinostat **(26)**, abexinostat **(27)**, entinostat **(28)**, SB939 **(29)**, resminostat **(30),** givinostat **(31)**, quisinostat **(32),** CUDC-101 **(33)**, AR-42 **(34)**, CHR-3996 **(35)**, M344 **(36),** dacinostat **(37),** tacedinaline **(38),** BLM 210 **(39)** and trichostatin A **(40)** are under clinical trials for various forms of cancer (http://clinicaltrial.gov/).

PRMTs

Nine protein arginine methyltransferases (PRMTs) are responsible for ω -*NG*-monomethylarginine (MMA), ω -*NG,NG*-asymmetric dimethylarginine (ADMA) and ω -*NG,N'G*-symmetric dimethylarginine (SDMA) [95]. PRMT1 is the main member of PRMT family and contribute for more than 90% of the methylarginine residues in mammalian cells and up-regulation of which is responsible for multiple forms of cancer including BC [95, 96]. PRMT2, CARM1 and PRMT7 are the other members, deregulation of which is responsible for BC induction and metastasis [95]. PRMT1 mediated transient methylation of ERα on Arg260 leads to exclusive cytoplasmic localization of receptor and trigger its interaction with p85 subunit of PI3K as well as Src which ultimately results in phosphorylation of AKT and activating the downstream cascade resulting in non-genomic effects of estrogens [14, 28]. Moreover, FAK adhesion protein interacts with R260 methylated ERα which indicates the possible role of the methylated status of ERα in BC migration and metastasis [14]. PRMT especially PRMT1 can be included in BC specific chemotherapy, which may provide better therapeutic outcomes. Many PRMT inhibitors are well documented in literature. Among them AMI1-AMI9 **(41-49)** Fig. (**4**) consists of nine compounds of AMI series identified by random screening having PRMT inhibitory activity [97]. AMI series of compounds have been taken as lead by several groups of researchers and a number of analogs have been synthesized, **(50-53)** which showed significant activity [98-100]. Another class of PRMT inhibitors belong to pyrazlo amide series. Compound **(54)** alongwith its analogs **(55-58)** has shown significant action [101, 102, 103]. Allantodapsone **(59),** stilbamidine **(60),** RM 65 **(61),** NS-1 **(62)**, TBBD **(63)** are other compounds reported in the literature with PRMT inhibitory activity [104]. Multiple PRMTs inhibitors are available which can be used to target PRMT in BC specific chemotherapy.

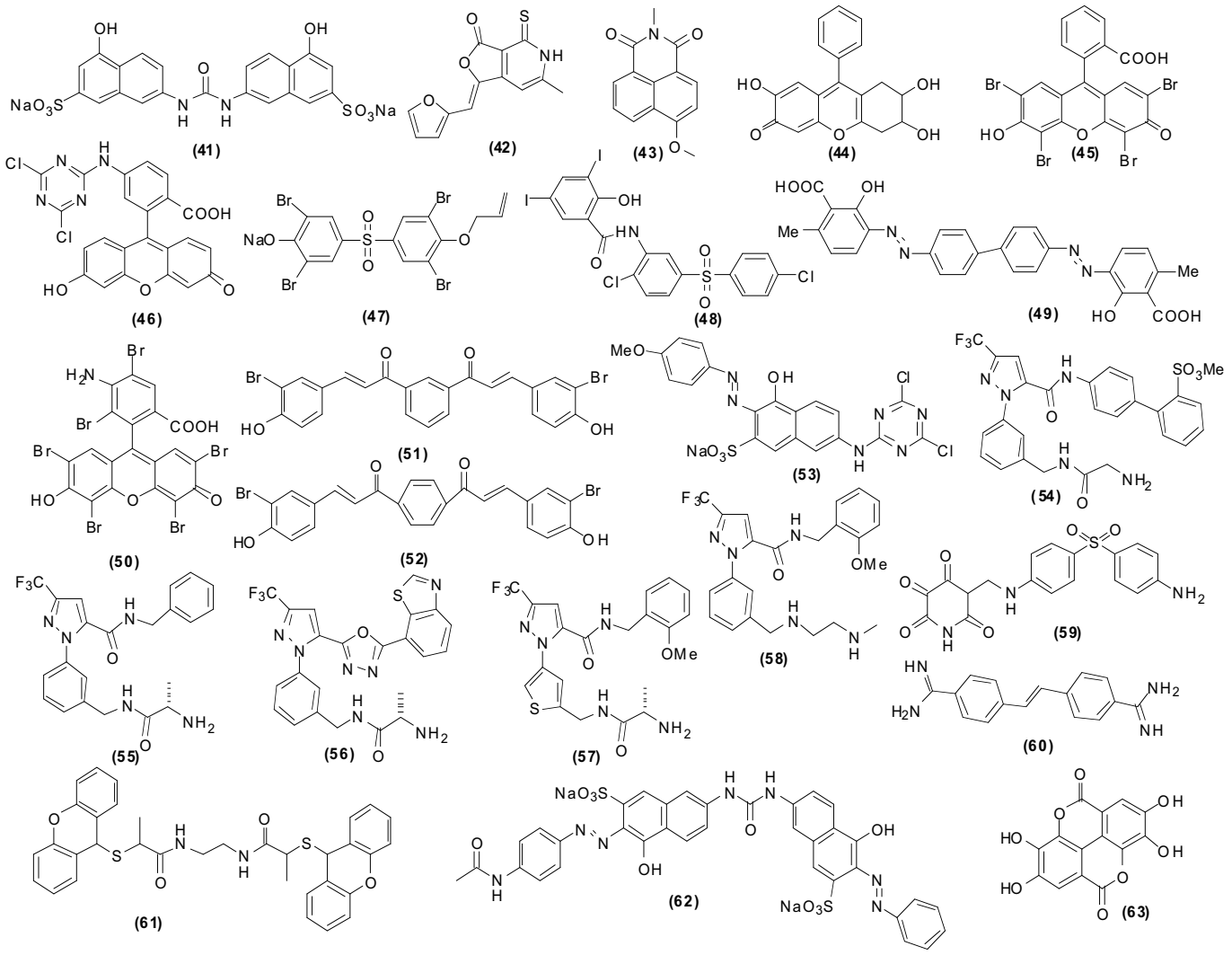

Fig. (4). Various PRMT inhibitors.

PAX-2

Paired Box-2 gene product (PAX-2) is a critical tamoxifen recruited transcription repressor of ErbB2 gene. Elevated AIB-1can lead to competition with PAX-2 binding of tamoxifen-ER complex which results in elevated ErbB2 expression. AIB-1 and ErbB-2 is related to the aggressive nature of BC. Thus PAX-2 may be the connecting link between BC and possible outcome of SERM related therapy [105]. PAX-2 seems to be an important target against BC, but our current understanding is very limited as no drug is known till date. Moreover, reports indicate that it could have tumor suppressor as well as oncogenic function [106]. Thus, proper generalization and validation of PAX-2 is the major requirement for its inclusion in chemotherapy.

TLE1

Transducin-like enhancer protein 1 (TLE1) is the modulator of transcription activity of ER, which exerts survival as well as anti-apoptotic function in various cell types and is involved in pathogenesis of cancer [107]. TLE1 has a regulatory effect on anoikis and it has been demonstrated that anoikis in untransformed mammary epithelial MCF10A cell was associated with significant down-regulation of TLE1 expression. Resistance in these cells towards anoikis has been found to be associated with forced expression of exogenous TLE1. In BC, significant up-regulation of TLE1 in a cell after detachment from the extracellular matrix has been seen [107]. TLE1 is responsible for anoikis resistance and anchorage-independent growth of BC cells, which is also correlated with genetic manipulation data of TLE1 *via* overexpression and down-regulation [107]. TLE1 inhibits Bit1 anoikis pathway by reducing the formation of proapoptotic Bit1-AES complex in part through sequestration of AES in the nucleus [107]. Significant overlaps of TLE1 binding sites in MCF-7 cells with ER target is associated with cell proliferation and can be down regulated by siRNAs [94, 108]. Thus, TLE1 is the novel target which can be implemented in the BC specific chemotherapy.

FOXA1

Forkhead box protein A1 (FOXA1) also called hepatocyte nuclear factor 3-alpha (HNF-3α) is encoded by Foxa1 gene [109, 110]. FOXA1 is highly correlated with ER/PR and GATA3 positive BC along with hormonal signaling [111]. FOXA1 is the major factor behind estrogen-ER activity and endocrine response in BC cells as lack of FOXA1 in ERα positive BC is related to resistance to endocrine therapy [111-113]. In ER negative cancer, expression of FOXA1 is associated with the responsive nature of tumor to some endocrine therapy [114, 115]. Thus being a necessary factor for estrogen mediated signaling in BC and endocrine therapy, FOXA1 modulators may be investigated as a noble strategy against BC.

E6-AP

E6-associated protein (E6-AP) is a ubiquitin proteasome pathway enzyme which functions as a co-activator of steroid hormone receptor including ER [116, 117]. Expression of E6-AP decreases in human invasive breast as well as prostate cancer and down-regulation of which is associated with up-regulated ERα in BC and androgen receptor (AR) in prostate cancer. The E6-AP knockout animal model shows overexpressed ERα in mammary gland and AR in prostate [118]. In transgenic mice with overexpressed E6-AP, estrogen is unable to initiate mammary tumor, but tumor development occurs if overexpressed E6-AP is mutated [119]. All these findings suggest that E6-AP may have tumor suppressor activity.

HOXB7

Homeobox gene HOXB7 is overexpressed in multiple forms of cancer, including BC and promotes tumorigenesis by effecting proliferation, survival, invasion and angiogenesis [120]. In BC, overexpressed HOXB7 is associated with SERM resistance [121]. Long term exposure of MCF-7 cells to tamoxifen results in increased HOXB7 expression which ultimately up-regulate the EGFR activity by binding to EGFR promoter region. Furthermore, over-expressed HOXB7 significantly correlates with poor disease-free survival in ERα-positive breast cancer on adjuvant tamoxifen monotherapy [121]. Antagonist of HOXB7 can be an important tool to overcome SERM related resistance which may also result in increased disease free survival but no such lead is still available.

EGFR

Epidermal growth factor receptor (EGFR) family consists of four surface receptors, which include EGFR/ErbB1/HER1, ErbB2/HER2/EGFR2/Neu, ErbB3/HER3, and ErbB4/HER4 [122]. EGFR2 is the most important among them and is amplified in 20% of BC in general and about 10 % in ER positive BC [123]. EGFR2 amplified ER positive BC is harder to manage by endocrine therapy compared to EGFR2 negative ER positive breast cancer [124]. The hetero or homo-dimerization of EGFR allows autophosphorylation of receptor tyrosine to initiate downstream signaling which requires a proper ligand. EGFR2 is unique in itself as no ligand has been reported till date and can dimerize with the other three members to initiate downstream signaling [125]. Dimerization and autophosphorylation of receptors result in activation of multiple intracellular signaling such as phosphatidylinositol- 3 kinase (PI3K)-Akt, Ras-Raf- MEK-MAPK which maintains several cellular functions including cell division. HER signaling also plays an active role in resistance to therapy, especially endocrine therapy, which results from crosstalk between ER and HER signaling Fig. (**5**) [34]. EGFR2 amplified ER positive BC generally shows poor response to endocrine therapy which can be overcome by incorporating anti-EGFR2 therapy [5]. EGFR2 is highly explored in breast cancer and many agents have been approved for treatment. Trastuzumab was the first commercially available EGFR2 targeting monoclonal antibody for the treatment of BC [126]. Trastuzumab binds to the extracellular segment of EGFR2/Neu and inhibit proliferation of EGFR2 overexpressed BC [127]. Major drawback for trastuzumab

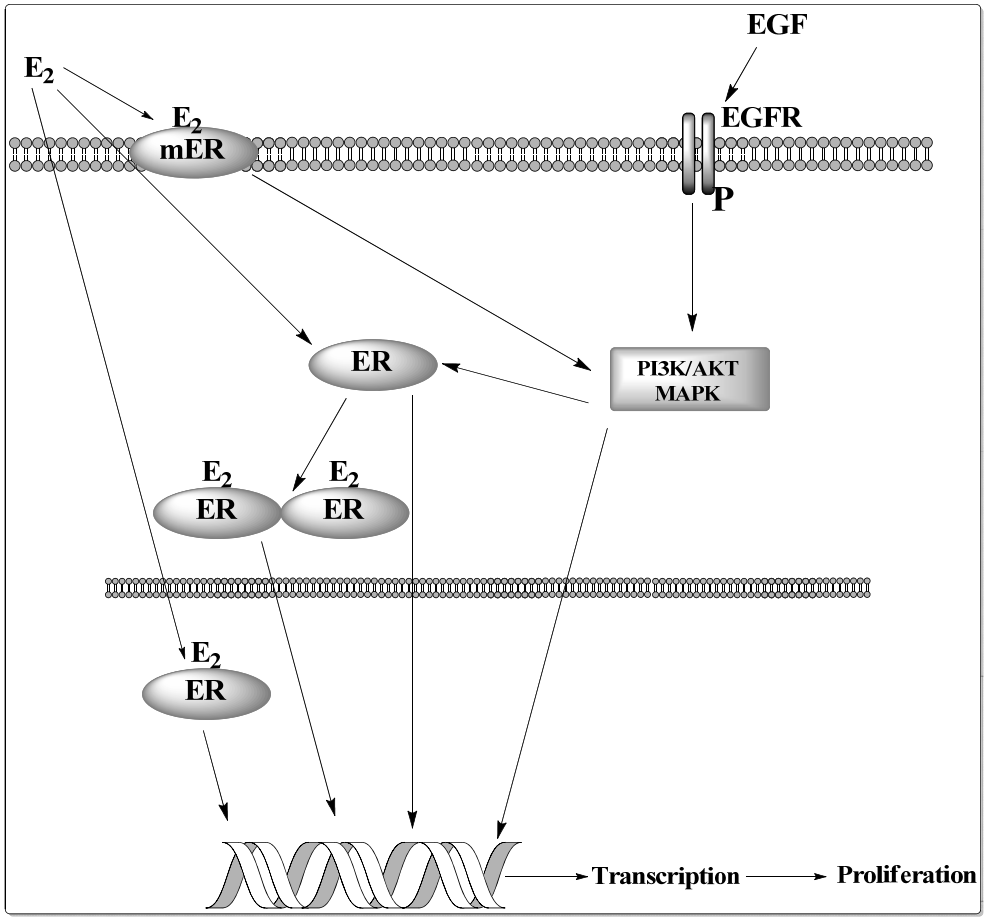

Fig. (5). Simplified model representing cross talk between ER and EGFR signaling. Activation of EGFR by epidermal growth factor (EGF) or by another ligand resulting in dimerization and autophosphorilation of EGFR, which activates multiple signaling pathways such as phosphatidylinositol- 3 kinase (PI3K)-Akt, Ras-Raf- MEK-MAPK and leads to ligand independent activation of estrogen receptor (ER). Membrane ER (mER) mediated signaling can also activate the downstream proteins of EGFR pathway. Thus the crosstalk is bidirectional and is the main cause of resistance towards endocrine cancer therapy.

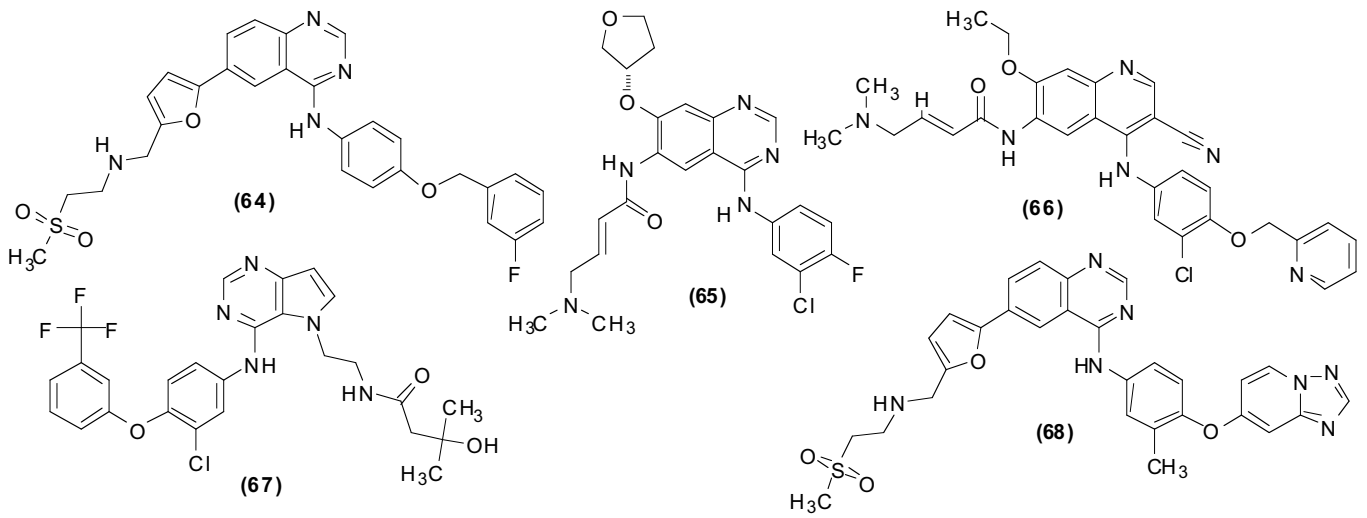

Fig. (6). Small molecule inhibitor/modulator of EGFR signaling in BC.

is the primary resistance as 68-88% of the patients show primary resistance and others may also get secondary resistance towards it [128]. Thus little fraction of patients with EGFR2 overexpressed BC can get a beneficial effect towards trastuzumab therapy that can be a good carrier for other drugs which may be conjugated with it. Trastuzumab as a carrier has been conjugated with emtansine which is a potent microtubule based cytotoxic agent allowing selective delivery of drugs to the EGFR2 overexpressed cells and is under various phases of clinical trials for BC [129, 130].

Pertuzumab is another humanized monoclonal antibody which binds to the extracellular domain of EGFR2, blocking dimerization and is approved by FDA in combination with docetaxel and trastuzumab for the treatment of metastatic BC [131, 132]. MM-302 is a liposome encapsulated doxorubicin attached to antibodies to selectively target EGFR2 overexpressed BC cells which are under clinical trials [133]. Ertumaxomab is a unique monoclonal antibody with dual target of CD3 antigen on T cell and EGFR2 on the tumor cell. It has the ability to form EGFR2-ertumaxomab-CD3 complex resulting in aggregation and activation of T cells, macrophages, dendritic cells and natural killer cells which ultimately lead to phagocytosis and death of tumor cells. It is under phase I and II of clinical trials for EGFR2/Neu positive advanced solid tumors [134]. MM-111 is an antibody fusion protein, which targets HER3/HER3 dimer and inhibit downstream signaling which is also under clinical trials [135]. All these antibodies and antibody conjugates attack the outer domain of the HER receptor. Small molecules have been reported which modulate EGFR signaling intracellular. Lapatinib **(64)** (Fig. **6**) is a small molecule that binds to ATP-binding pocket of EGFR2 protein kinase domain and inhibits self-phosphorylation which is critical for activity [7]. It is approved for the treatment of various forms of cancers, including EGFR2 over-expressed metastatic BC [136]. Afatinib **(65)**, Neratinib **(66)**, TAK-285 **(67)**, ARRY-380 **(68)** are the other small molecule inhibitors of EGFR, which are under clinical trials for HER overexpressed BC [137, 138, 139]. Other HER targeting agents include MM-121 which is a human monoclonal antibody against HER3 and neratinib that inhibit HER1 as well as HER4 receptor [140, 141].

PI3K/Akt/mTOR Signalling

mTOR signaling is critically involved in normal processes in many cell types including breast cells [139]. It involves PI3K mediated phosphorylation of certain components which ultimately phosphorylate and activate Akt. Once activated, Akt trigger several downstream signaling by activating mTOR results in increased cell survival, proliferation and cell growth [142]. mTOR maintain the resources needed by cells to increase nutrient uptake and angiogenesis when required [142]. It also involved in up-regulation of Akt activity resulting in increasing downstream effects. Proper regulation of mTOR signaling seems to be critical and is done by PTEN, which reverses the action of PI3K and inhibits activation of Akt [143].

This pathway has been found to be highly activated in many forms of cancer, including 50% of BC cases which are associated with mutations that activate this pathway [144, 145]. The major type of mutations involve amplification of PI3K, Akt and loss of PTEN function [146]. Thus PI3K/Akt/mTOR pathway is highly involved in BC and its modulation may provide benefits . Several mTOR inhibitors have been developed and among them Sirolimus **(69)**, everolimus **(70)** and temsirolimus **(71)** have been approved by FDA (Fig. **7**) [147]. Everolimus has been approved by FDA for the treatment of ER/PR positive, HER negative BC in combination with exemestane when 10 mg/day of letrozole or anastrozole failed to produce its effect [147]. Ridaforolimus **(72)**, NVP-BEZ235 **(73)**, SF1126 **(74)**, XL765 **(75)** and BGT226 **(76)** are other mTOR inhibitors, which are under clinical trials [147]. PI3K inhibitors can also be implemented for BC therapy. Some PI3K inhibitors such as SAR245408/SAR245409 **(77)** andGDC-0941 **(78)** are under clinical trials for BC [148]. Akt inhibitor can also be utilized to down-regulate mTOR signaling. Miltefosine **(79)** is an Akt inhibitor, which is under clinical practice [149]. MK-2206 **(80)**, AZD5363 **(81)**, Triciribine **(82)**, AR-67 **(83)**, GSK690693 **(84)** and VQD-002 **(85)** are the Akt inhibitors in clinical trials for various forms of cancers (http://clinicaltrial.gov/).

Poly (Adenosine Diphosphate [ADP])–Ribose) Polymerases (PARPs) Inhibitors

Poly (adenosine diphosphate [ADP])–ribose) polymerases (PARPs) are a family of enzymes that actively participate in DNA repair, gene transcription, chromatin architecture and apoptosis in normal cells [150-152]. PARP1 is the most abundant enzyme among them, which plays a critical role in single strand DNA base-excision repair. PARP1 inhibition results in single stranded break and subsequently double strand break, which ultimately leads to apoptosis [148].

Normal cells with intact homologous recombination double strand DNA repair pathway, the PARP blockage is bypassed. BC cells lacking BRCA1 and BRCA2, the inhibition of PARP leads to cell death [148]. BRCA1 mutation is majorly associated

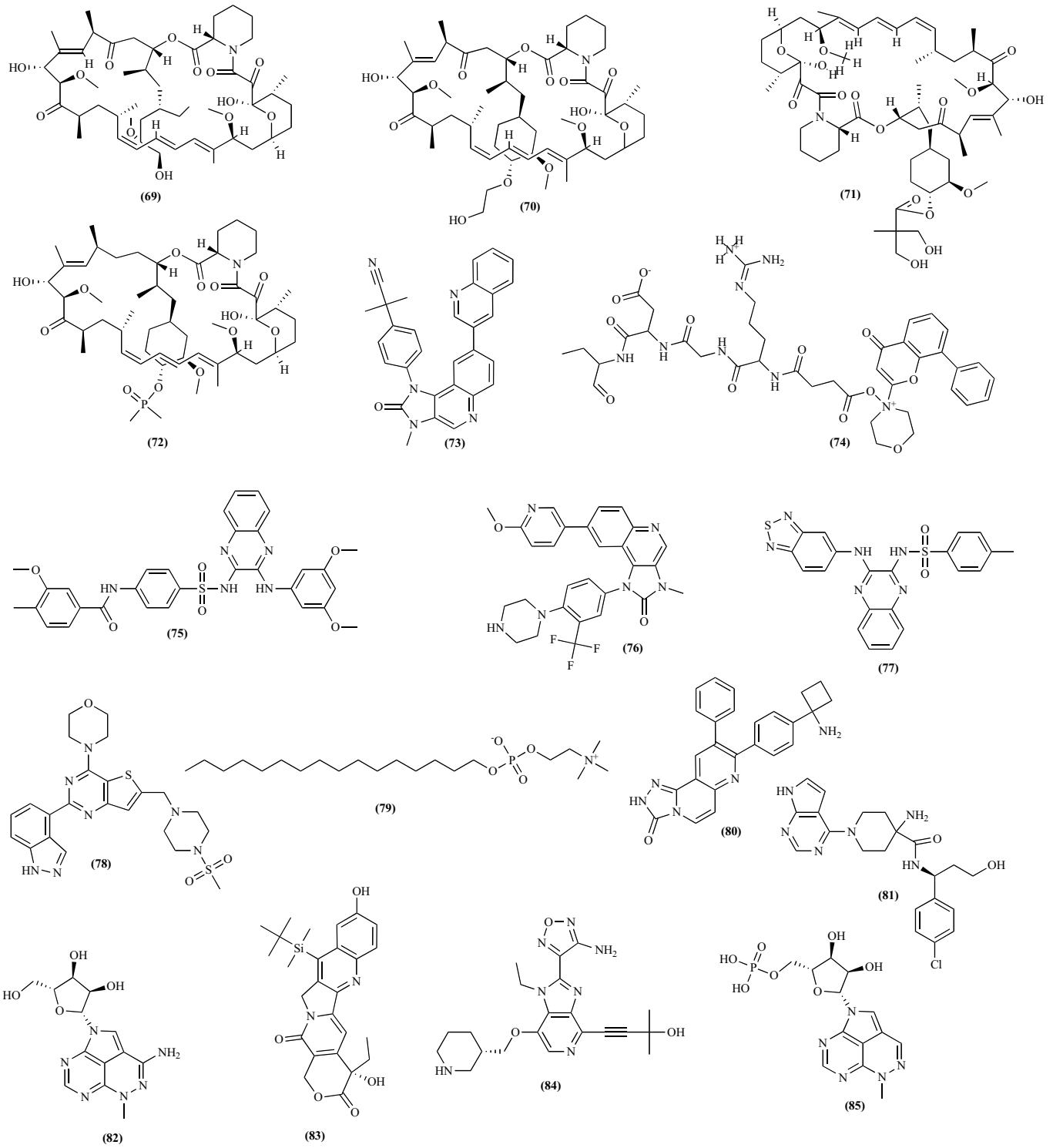

Fig. (7). Various inhibitors/modulators of PI3K/Akt/mTOR signaling.

with TNBC which is an aggressive form of cancer and still no proper targeting approach is available [148]. Moreover PARP is overexpressed in various forms of cancers, which seem to provide extra survival benefits to cancer cells from DNA damage and apoptosis [153]. It has also been found that cancers, especially BC with overexpressed PARP1 is associated with poor overall survival [153]. PARP inhibitors are the noble way to target BC cells by inducing DNA damage and apoptosis. Many PARP inhibitors are under clinical trials which include iniparib **(86)**, olaparib **(87)**, veliparib **(88)**, rucaparib **(89)**, MK-4827 **(90),** BMN-673 **(91)** and E7016 **(92)** (Fig.**8**) [151].

Thus other reported PARP inhibitors include nicotinamide **(93),** 3-AB **(94)**, quinazolinone **(95)**, CEP 8983 **(96)** and PJ-34 **(97)** [154, 155]. Although some PARP inhibitors have reached clinical trials, but phase 3 clinical trials are still lacking and data is still insufficient to compare with standard therapy [156]. Another

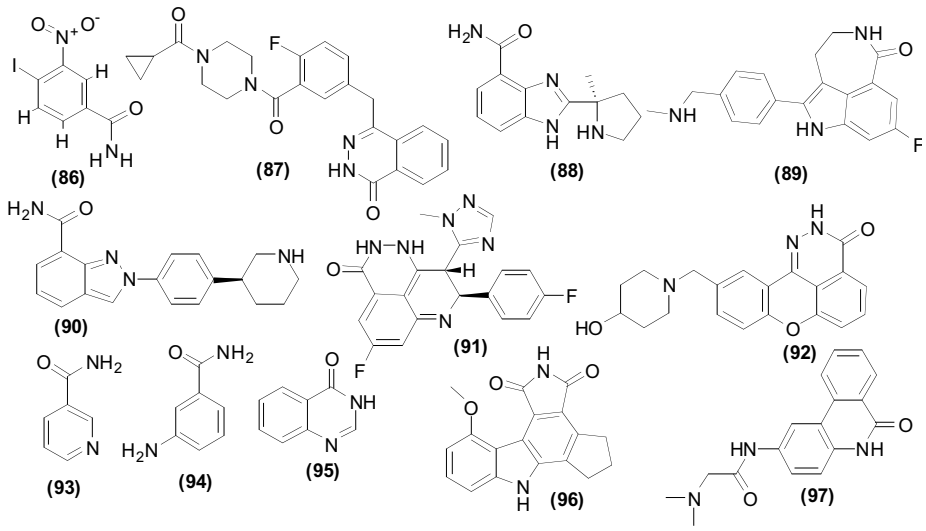

Fig. (8). PARP inhibitors against BC lacking BRCA1/BRCA2 mediated DNA repair.

limitation for implementing PARP inhibitors in clinical practice is the lack of well defined approach for selecting patients that are suitable for PARP inhibition approach [156]. Moreover the emergence of resistance towards PARP is still a key concern in the development of PARP inhibitors. Restoration of BRCA function, up regulation Non-Homologous End Joining, P-glycoprotein mediated efflux and loss of 53BP1 protein are among the causes of resistance towards PARP inhibitors [156, 157].

CONCLUSION

BC therapy is heading from nonspecific approach to targeted approach. Various new targets are identified and implemented for treatment. ER and hormonal therapy is an age old concept, but now various new downstream molecules are included. Many more targets such as co-regulators exist that need further investigation. Nonclassical ERs need to be explored in-depth so that can be utilized properly as anti-BC targeting approach. Interrelationship among different pathways is another issue to be explored as proper understanding of crosstalk among multiple signaling that will help to overcome primary resistance as well as increased efficacy of treatment. PARP inhibitors are an important advancement in cancer chemotherapy that can be combined with other DNA damaging agents to get synergic effect. Selectivity towards BRCA mutated BC cells as BRCA1 lacking cells may almost lose their ability to repair damaged DNA if PARP is inhibited, but normal cells have intact BRCA based repair machinery. Moreover, TNBC can be specifically targeted using PARP inhibitors. BC is a heterogeneous disease for which continuous evolution is going on regarding its targeting approach. In future with the availability of proper targeting approaches and marker based classification for proper selection of regimen can provide full proof treatment for BC.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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