



HAL
open science

Improving the efficacy of hormone therapy in breast cancer: The role of cholesterol metabolism in SERM-mediated autophagy, cell differentiation and death

Julie Leignadier, Florence Dalenc, Marc Poirot, Sandrine Silvente-Poirot

► To cite this version:

Julie Leignadier, Florence Dalenc, Marc Poirot, Sandrine Silvente-Poirot. Improving the efficacy of hormone therapy in breast cancer: The role of cholesterol metabolism in SERM-mediated autophagy, cell differentiation and death. *Biochemical Pharmacology*, 2017, 144, pp.18-28. 10.1016/j.bcp.2017.06.120 . inserm-02380608

HAL Id: inserm-02380608

<https://inserm.hal.science/inserm-02380608>

Submitted on 26 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Research update

Classification:

(1) Antibiotics and chemotherapeutics or (3) metabolic disorders and endocrinology

Title:

Improving the efficacy of hormone therapy in breast cancer: the role of cholesterol metabolism in SERM-mediated autophagy, cell differentiation and death

Julie Leignadier^a, Florence Dalenc^{a,b}, Marc Poirot^{a§}, and Sandrine Silvente-Poirot^{a§}

^a*Cancer Research Center of Toulouse, UMR 1037INSERM-University of Toulouse, Toulouse, France;* ^b*Institut Claudius Regaud, Institut Universitaire du Cancer-Oncopole, Toulouse, France.*

[§]Address correspondence to these authors at the Department of Cholesterol Metabolism, Cancer Research Center of Toulouse, INSERM UMR 1037-University of Toulouse III, P.O. Box: 31017, Toulouse, France; Tel/Fax: +33-582741626; E-mail: marc.poirot@inserm.fr; sandrine.poirot@inserm.fr

Running title: Cholesterol metabolism and pharmacology of tamoxifen

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract: Breast cancer (BC) is one of the most common female cancers in the world, with estrogen receptor (ER)-positive BC the most frequent subtype. Tamoxifen (Tam) is an effective drug that competitively binds to the ER and is routinely used for the treatment of ER-positive BC. However, a number of ER-positive BC do not respond to Tam treatment and acquired resistance is often observed, constituting a major challenge for extending patient life expectancy. The mechanisms responsible for these treatment failures remain unclear, indicating the requirement for other targets and better predictors for patient response to Tam. One of Tam's off-targets of interest is the microsomal antiestrogen binding site (AEBS), a multiproteic complex made up of the cholesterol-5,6-epoxide hydrolase (ChEH) enzymes that are involved in the late stages of cholesterol biosynthesis. Tam and other selective ER modulators stimulate oxidative stress and inhibit the ChEH subunits at pharmacological doses, triggering the production and accumulation of cholesterol-5,6-epoxide metabolites responsible for BC cell differentiation and death. However, inhibition of the cholesterologenic activity of the AEBS subunits also induces the accumulation of sterol precursors, which triggers a survival autophagy to impair Tam's efficacy. Altogether, these studies have highlighted the involvement of cholesterol metabolism in the pharmacology of Tam that has provided new clues on how to improve its therapeutic efficacy in both BC and other cancers as well as offering a new rationale for developing more efficient drugs for BC treatment.

Keywords: Tamoxifen; AEBS; breast cancer; zymostenol; 5,6-epoxycholesterol

Chemical compounds:

1
2
3 Tamoxifen (PubChem CID: 2733526); toremifene (PubChem CID: 3005573); tesmilifene
4
5 (PubChem CID: 108092); PBPE (PubChem CID: 71311880); 4-hydroxytamoxifen (PubChem
6
7 CID: 449459); lazofoxifene (PubChem CID: 216416); raloxifene (PubChem CID: 5035);
8
9 bazedoxifene (PubChem CID: 154257); clomiphene (PubChem CID: 1548953); 17 β -estradiol
10
11 (PubChem CID: 5757); ICI 164,684 (PubChem CID: 104772). ICI 182,780 (PubChem CID:
12
13 44307470); RU 58,668 (PubChemCID: 119604); MER29, triparanol (PubChem CID: 6536);
14
15 boxidine (PubChem CID: 31742); dendrogenin A (PubChem CID: 9806490); cholesterol
16
17 (PubChem CID: 5997); desmosterol: (PubChem CID: 439577); zymostenol (PubChem CID:
18
19 101770); zymosterol (PubChem CID: 92746); 7-dehydrocholesterol (PubChem CID:
20
21 439423); 5,6 α -epoxycholesterol (PubChem CID: 227037); 5,6 β -epoxycholesterol (PubChem
22
23 CID: 108109); histamine (PubChem CID: 774).
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1. INTRODUCTION

Each year, breast cancer (BC) affects more than 1 million women worldwide and about 400,000 patients die from this disease [1]. Currently, only two biomarkers are used to decide upon BC therapeutic strategy irrespective of the stage of the disease: estrogen receptor α (ER) and the Human Epidermal growth factor Receptor 2 (HER2) (NCCN 2017, <https://www.nccn.org/>). Great strides have been made in BC treatment, through the use of targeted therapies such as endocrine therapies like Tamoxifen (Tam) (Fig. 1A) for BC expressing ER (70 to 80% of BC), or agents targeting overexpressed HER2 (15 to 18% of BC) [2]. However, not all BC respond to these therapies, and many develop resistance despite initial beneficial effects [3]. Therefore, there is an urgent requirement to further characterize the molecular actors involved in the pharmacology of Tam to improve the efficacy of hormone therapy and provide new molecular weapons to fight resistance.

Tam was initially developed to antagonize the tumor promoter effects of 17β -estradiol (E2) by competing with it for binding to ER [4, 5], and was shown to effectively inhibit ER⁽⁺⁾ BC development in animal models and patients [4]. Although first given as an adjuvant, Tam is now administered as first line treatment and has also been approved by the Food and Drug Administration (FDA) for chemopreventive use in subjects at risk [1]. Although Tam is well-tolerated compared to non-specific chemotherapy, it does produce some side effects, such as hot flushes, vaginal discharge, arthralgia, myalgia, weight gain and hypertriglyceridemia [1, 6, 7]. The binding of Tam to ER induces a specific conformational modification of the receptor, which affects the affinity of the ER-Tam complex for co-activators and co-repressors that drive the ER transcriptional response [8]. Thus, there is pool of cellular co-regulators that contribute towards driving the agonistic or antagonistic action of Tam, and this is why Tam and its analogues have been called selective estrogen receptor modulators (SERM) [8].

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

The estrogenic effects of Tam in bone tissue are beneficial to patients and it protects against osteoporosis in the long-term. However, the estrogenic actions of Tam on the uterus are believed to be responsible for the low but significant increase in endometrial cancer [9-11]. As a result of this, SERMs with no uterotrophic effects, such as raloxifene, lasofoxifene, and bazedoxifene were developed [12], but despite their good efficacy on ER⁽⁺⁾ BC, none of them were able to supplant Tam in the clinic [12]. Studies related to the action and metabolism of Tam in terms of its control of ER have been extensively reviewed and we encourage readers to look at them [1, 4, 13-21].

Hormonal therapies for BC, such as Tam, are given after first confirming the expression of one of two hormonal receptors, ER and/or the progesterone receptor (PR). The majority of ER⁽⁺⁾ BC are also PR⁽⁺⁾. The expression of ER and PR in BC tumors as justification for patient eligibility for hormone therapy has been successful, however not all patients respond to treatment and acquired resistance occurs systematically in metastatic settings and during adjuvant treatment, sometimes after 2 to 3 years or more. Aromatase inhibitors (AI) are inhibitors of 17 β -estradiol biosynthesis and are commonly used as for ER⁽⁺⁾ BC treatments. AI but not Tam or other SERMs, were shown to induce acquired resistance in which mutations were found on the ER [22]. In contrast, oxidative stress-neutralizing pathways and ligand-X-receptor (LXR)-driven cholesterol metabolic enzymes were found to be upregulated in animal models and patients with ER⁽⁺⁾ BC treated with Tam [15, 23-25]. Along the same lines, cholesterol-lowering medication during adjuvant endocrine therapy has also been shown to improve response to Tam [26].

Despite its efficacy in ER⁽⁺⁾ BC, Tam displays a complex pharmacology and several off-targets have been identified [27]. Studies of these off-targets can not only explain several side effects and thereby improve the whole clinical pharmacology of SERMs, but may also help in defining additional pathways involved in acquired and intrinsic resistance that could be

1 pharmacologically targeted. Treatment of patients with Tam gives a serum concentration at
2 equilibrium ranging from 1 μ M to 10 μ M [28], suggesting that targets of Tam with an affinity
3 within this concentration range should be considered in its anticancer pharmacology. Protein
4 kinase C (PKC) has been identified as a low affinity ($>$ μ M) target for Tam [27]. This enzyme
5 is a known target for tumor-promoting phorbol esters, calmodulin (which controls calcium-
6 dependent enzymes), and the acyl-coA-cholesterol acyl transferase (ACAT, or SOAT1 and
7 SOAT2). ACAT controls the biosynthesis of fatty acyl-cholesteryl esters, which have recently
8 been identified as tumor promoters, with the inhibition of cholesterol esterification appearing
9 to be a new promising target for cancer treatment [29-33]. Other molecular targets of Tam and
10 the SERM 4OHTam were shown to be inhibitors of phospholipase D1 and D2, respectively
11 [34-37]. Tam and 4OHTam are also inhibitors of glycosylceramide synthase [38, 39] and of
12 cyclin-dependent kinase 5 (CDK5) [40]. The relationship between these molecular targets and
13 the anticancer activity of Tam remains to be established in BC.

14 Tam and other SERMs have also been shown to interact with high affinity (nM) to an
15 intracellular and membranous binding site named the antiestrogen binding site (AEBS) [41],
16 whose molecular characterization has established a link between Tam and cholesterol
17 metabolism in its pharmacology [42]. The aim of the present article is to summarize the
18 available data on the structure and function of the AEBS and explain how this binding site can
19 account for the pharmacology of Tam, SERMs and AEBS ligands. We hope to shed light on a
20 new field of investigation, which is improving our understanding of the pharmacology of Tam
21 and will help in the development of the future generations of drugs for BC management.

2. The antiestrogen binding site (AEBS)

2.1. Pharmacological profile of the AEBS

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
The existence of the AEBS was first reported by Robert Sutherland and Jean-Charles Faye in the eighties [41, 43, 44]. Although initially detected in the cytosolic fractions of cells and tissues [41, 43-45], the AEBS is an intracellular microsomal Tam binding site [46, 47]. The name “antiestrogen binding site” was chosen because it was shown to bind to Tam and analogues but not 17 β -estradiol, which distinguished it from ER [41]. Synthetic estrogens such as diethylstilbestrol (DES) or pure steroidal antiestrogens (also named SERDs: for selective estrogen receptor destructors) [17, 18] such as ICI 164,384, ICI 182,780 (Faslodex) or RU 58,668, do not bind to the AEBS [27, 48]. Structure-affinity studies performed on the AEBS have shown that the presence of a phenolic group in the polyphenyl part of SERMs decreases their affinity for the AEBS [27, 49, 50] but improves their affinity for ER [51]. The presence of a cationic aminoalkyl side chain in drugs and drug candidates was also found to be required for high affinity binding to the AEBS [27]. Structure-affinity studies have also revealed structural elements of Tam that are required for AEBS binding (Fig. 1B), which are different from the structural determinants required for ER binding (Fig. 1C) [27, 49]. The selective affinity binding properties in the pharmacological profile of the AEBS are thus different from those of ER [47].

39 **2.2. Development of selective AEBS ligands**

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
Several research groups have developed Tam analogues and found some that bound preferentially to AEBS with negligible affinity for ER, including the cis isomer of centchroman [52] and a benzofuran analog of Tam [53]. The impacts of these compounds on other targets of Tam, such as ACAT, PKC and calmodulin, have not yet been studied. The synthetic high affinity AEBS ligands have been based upon the diphenyl methane (DPM) series of compounds, due to the seminal work of L.J. Brandes who first identified the DPM compound DPPE (tesmilifene, Fig. 1D) as a high affinity AEBS ligand [54]. Other DPM compounds have also been chemically synthesized and among them structure-affinity

1 studies have shown that PBPE (Fig. 1E) is one of the highest affinity ligands for the AEBS
2 [49]. DPPE and PBPE have been the most studied selective AEBS ligands [15, 27, 55-64],
3
4 and when DPPE was brought to the clinic under the trade name of toremifene it showed some
5
6 efficacy in cancer treatment [61, 65-69]. DPPE and PBPE were shown to be selective for the
7
8 AEBS with no impact on ER [49], ACAT [48], PKC [70, 71] or calmodulin [63, 72],
9
10 evidencing that DPM compounds are useful tools for defining the importance of the AEBS in
11
12 the anticancer pharmacology of SERMs.
13
14
15

16
17 In the quest to determine the physiological significance of the AEBS, several groups have
18
19 studied the lipid constituents of serum and found that 7-ketocholesterol (Fig. 1F) [73] and
20
21 unsaturated fatty acids [74, 75] are AEBS ligands. Structure-affinity studies were conducted
22
23 on a range of oxysterols, and only 6-ketocholestanol and/or 7-ketocholestanol were found to
24
25 be high affinity AEBS ligands, while side chain-oxysterols displayed a weak or unmeasurable
26
27 affinity for the AEBS [76, 77]. Two other ring B-oxysterols: 5,6 α -epoxycholesterol (5,6 α -EC)
28
29 (Fig. 1G) and 5,6 β -epoxycholesterol (Fig. 1H) (5,6 β -EC) have been shown to be high affinity
30
31 AEBS ligands [57]. 5,6 α -EC was also shown to be a ligand for LXR α and a modulator for
32
33 both the LXR α and β subtypes [78], however they have not been tested on other off-targets of
34
35 Tam or other known oxysterol targets such as AhR [79, 80], ROR [81-83], EBI2 [84],
36
37 Hedgehog component smoothened [85], oxysterol-binding protein-related proteins [86] or ER
38
39 [87, 88]. Histamine (Fig. 1I) was shown to be an endogenous AEBS ligand [89-91], and
40
41 recently a stereoselective conjugation product of the condensation of histamine and 5,6 α -
42
43 epoxycholesterol, named dendrogenin A (Fig. 1J), was identified and found to be an AEBS
44
45 ligand [92-97].
46
47
48
49
50
51
52
53

54 **2.3. Molecular characterization of the AEBS**

55 **2.3.1. The AEBS is made up of two enzymes involved in the late stages of** 56 57 **cholesterol biosynthesis** 58 59 60 61 62 63 64 65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Analysis of the structure of the known AEBS ligands suggested some links between AEBS and cholesterol metabolism. Tam and PBPE are both structurally related to cholesterol biosynthesis inhibitors that were developed in the sixties, MER-29 (Fig. 1K) and boxidine (Fig. 1L), which are inhibitors of the 3β -hydroxysteroid- Δ^{24} -reductase (DHCR24) [98] and the 3β -hydroxysteroid- Δ^7 -reductase (DHCR7), respectively. The fact that MER-29 and boxidine induce the accumulation of desmosterol (Fig. 1M) (the substrate of DHCR24) and 7-dehydrocholesterol (7DHC, Fig. 1N) (the substrate of DHCR7) [99], suggested that Tam and AEBS ligands could inhibit cholesterol biosynthesis at a post-lanosterol step (Fig. 2A). Indeed, Craste de Paulet's group in Montpellier (France) was the first to show that Tam inhibited cholesterol biosynthesis in BC cell lines [100]. Miettinen's group in Helsinki (Finland) also analyzed the sterol profile in the blood of BC patients treated with Tam and observed the appearance of zymostenol (zymo) (Fig.1O), suggesting an inhibition of cholesterol biosynthesis at the D8D7I (EBP, 3β -hydroxysteroid- Δ^8, Δ^7 -isomerase) step (Fig. 2B) [101]. Tests were conducted on breast cancer cell lines *in vitro* and the analysis of the sterol profile confirmed that Tam induced the accumulation of zymo, showing an inhibition of the EBP/D8D7I enzyme [42, 55, 59]. A subsequent evaluation of selective AEBS ligands from the DPM series showed that, in addition to zymo, DPPE and PBPE induced the accumulation of 7DHC suggesting that DHCR7 could be associated to the AEBS [42, 55, 59]. In line with this, 7-ketocholesterol, and 6- and 7-ketocholestanol were reported to inhibit EBP/D8D7I and induced zymo accumulation [42, 59]. However, no correlation was found between binding affinity to the AEBS and inhibition of cholesterologenic enzymes [42]. In addition, it was observed that AEBS ligands were not obligatory inhibitors of these cholesterologenic enzymes, and a shift between affinity for the AEBS and the doses required to inhibit these enzymes was found [42]. For example, weak affinity AEBS ligands such tBuPE did not inhibit cholesterol

1 biosynthesis in BC [42]. Phenolic SERMS that are ligands of the AEBS, such as
2 4OHTam, also induced the accumulation of desmosterol reflecting the inhibition of
3
4 DHCR24 with no impact on D8D7I, while raloxifene induced the accumulation of
5
6 zymosterol (Fig. 1P) reflecting a dual inhibition of D8D7I and DHCR24 [42, 55, 59, 60].
7
8
9 SERMs were reported to induce the accumulation of cholesterol precursors in cancer cell
10
11 lines of different tissue origins, such as melanoma cells, leukemia cells and neuroblastoma
12
13 cells [42, 55, 102-106].
14
15

16
17 Molecular reconstitution of the AEBS complex was done in Cos cells by transfection of
18
19 plasmids encoding human D8D7I and DHCR7. It was found that the expression of both
20
21 enzymes was required to reconstitute a high affinity Tam binding site displaying the
22
23 pharmacological profile of the AEBS [42]. Co-immunoprecipitation experiments showed
24
25 that EBP/D8D7I physically interacted with DHCR7 and bound Tam [42]. DHCR7 was
26
27 also recently reported to interact physically and functionally with DHCR24 [107]. The
28
29 AEBS is a multiproteic complex that involved EBP/D8D7I, DHCR7 and also DHCR24
30
31 and possibly other enzymes from the post-lanosterol cholesterol biosynthesis (Fig. 2A-C).
32
33
34
35
36

37 **2.3.2. The accumulation of cholesterol precursors by Tam and AEBS ligands in** 38 39 **BC cells triggered a survival autophagy.** 40 41

42
43 Among the AEBS ligands, those that are cholesterol biosynthesis inhibitors were shown to
44
45 induce a huge accumulation of free sterols in cells, which led to the appearance of
46
47 cytoplasmic multilamellar bodies (MLB), observed through transmission electron microscopy
48
49 (Fig. 2D). The accumulation of free sterols was visualized by fluorescence microscopy after
50
51 labeling with the free sterol chelating agent filipin (Fig. 2D) [55, 59]. Although the
52
53 significance of MLB formation has not yet been identified, it is possible that they are
54
55 aggregates of free sterols and sphingolipids such as sphingomyelin, since an increase in
56
57 sphingomyelin accumulation was measured in BC cells [60, 108]. MLB formation has been
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

observed in response to every AEBS ligand that inhibits cholesterol biosynthesis, independently of the nature of the accumulated cholesterol precursor [55, 59, 60].

Cholesterol intermediates have different physicochemical properties and may accumulate in different compartments of cells. Several cholesterol precursors restricted to FF-Mas [109, 110], desmosterol and zymosterol [111], were shown to display modulatory activities on the nuclear receptor LXR. Desmosterol was also shown to be an LXR α and LXR β ligand [111]. Other sterol intermediates, such as lanosterol, lathosterol and 7-dehydrocholesterol, did not display LXR modulatory activities [110, 111], and the impact of zymo on LXR has not been investigated to date. These observations suggest that the nature of the sterol intermediate that accumulate may have different consequences on cancer cells. For example, it was shown that 4OHTam, which induced desmosterol accumulation, triggered acquired resistance in MCF7 cells [112, 113] suggesting that LXR activation could be related to acquired resistance to Tam. 7DHC accumulation could reflect teratogenic risks or neurological disorders [105, 106]. The determination of a pharmacophore to predict the selectivity in the inhibition of cholesterologenic enzyme by AEBS ligands is thus important to study and will deserves further investigation.

Importantly, sterol intermediates are prone to oxygenation through radical chain oxidation and lipoperoxidation [114-116]. The oxygenation products of 7DHC have been studied in depth [114, 116], but other lipoperoxidation products of cholesterol biosynthesis intermediates remain to be chemically characterized and their biological properties defined. The positioning and the nature of the oxygenation group is thought to control the molecular targeting and biological properties of these compounds. Oxysterol binding proteins and receptors have been reported and, interestingly, display a high selectivity towards oxysterol subtypes [117].

1 The accumulation of sterols in cells treated with Tam has been linked to the induction of
2 autophagy, a self-digestion cellular mechanism [55, 58, 59, 118, 119]. It was initially
3
4 proposed that Tam induces a lethal autophagy [120, 121], however other studies have since
5
6 concluded that Tam and its analogues in fact induce a survival autophagy (Fig.2D), which
7
8 constitutes a mechanism of resistance to Tam [55, 58, 59, 112, 113, 122-125].
9
10

11 **2.3.3. The AEBS carries out the cholesterol-5,6-epoxide hydrolase (ChEH)** 12 13 **activity** 14 15 16 17

18 The natural high affinity AEBS ligands from the oxysterol series were also known as
19
20 extremely potent inhibitors of the microsomal cholesterol-5,6-epoxide hydrolase (ChEH)
21
22 [126], which catalyzes the hydration of 5,6 α -epoxycholesterols (5,6-EC) into cholestane-
23
24 3 β ,5 α ,6 β -triol (CT) (Fig.3A) [127]. It was found that 5,6-EC were high affinity and
25
26 competitive inhibitors of Tam at the AEBS [57]. AEBS ligands belonging to different
27
28 chemical families with affinities ranging from nM to μ M were tested alongside ChEH
29
30 substrates and inhibitors in our AEBS binding assays. Substrates of ChEH were potent and
31
32 competitive inhibitors of Tam binding on the AEBS, and a positive correlation between
33
34 AEBS affinity and inhibition of ChEH activity by the different tested compounds was
35
36 established. Consequently, compounds with no affinity for the AEBS did not inhibit ChEH.
37
38 This showed that ChEH and AEBS are pharmacologically identical [57]. However, although
39
40 the binding of a given compound to the AEBS can predict its inhibition of ChEH, and the
41
42 inhibition of ChEH can predict the AEBS affinity of a compound, the inhibition of ChEH
43
44 cannot predict post-lanosterol cholesterol biosynthesis inhibition. Knockdown of each AEBS
45
46 subunit (EBP/D8D7I an DHCR7) in the breast cancer cell line MCF-7 was also shown to lead
47
48 to a dual loss of AEBS and ChEH activities, and, conversely, overexpression of EBP/D8D7I
49
50 an DHCR7 in COS cells led to the reconstitution of ChEH activity with the expected
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 pharmacological profile [128]. Altogether, these results established that ChEH is molecularly
2 identical to the AEBS (Fig. 3A).
3
4
5
6

7 **2.4. The AEBS plays a role in the anticancer pharmacology of Tam**

8
9

10 The AEBS was proposed to play a role in the anticancer activity of Tam even before its
11 molecular identification was done. Several studies suggested that the AEBS might be of
12 interest since an increase or decrease in AEBS expression was associated with a loss of
13 sensitivity to Tam [45, 129]. Another study contradicted these findings [130], but this was
14 mainly based on the comparison of Tam with tBuPE, a selective but weak affinity AEBS
15 ligand [131], which was later established as not potent enough to induce similar effects to
16 Tam in terms of cholesterol metabolism perturbation [42]. Further studies evaluating selective
17 AEBS ligands showed that if they had no direct ER modulatory activity they were cytotoxic
18 and induced cancer cell differentiation [56, 59, 60, 63, 64, 71, 132-134].
19
20
21
22
23
24
25
26
27
28
29
30
31

32 **2.5. Selective AEBS ligands control cancer cell death and differentiation**

33
34

35 High affinity AEBS ligands such as Tam and PBPE were shown to induce cytotoxicity in
36 ER⁽⁺⁾ and ER⁽⁻⁾ cancer cells of various tumor origins. From a number of BC cell lines, the
37 ER⁽⁺⁾ MCF-7 cell line was found to display the highest sensitivity to DPM compounds while
38 triple negative (ER⁽⁻⁾, PR⁽⁻⁾, HER2⁽⁻⁾) MDA-MB-231 cells were 10 times less sensitive to these
39 compounds and to Tam [56]. More specifically, analyses of the effects of Tam and AEBS
40 ligands showed that all of these compounds induced cell synchronization in the G0-G1 phase
41 of the cell cycle and characteristics of ductal cell re-differentiation, as observed with either the
42 histone deacetylase (HDAC) inhibitor SAHA [135] or *all trans*-retinoic acid [136, 137].
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 the presence of antioxidants such as vitamin E [56, 122]. Antioxidants such as vitamin E and
2 C are known to inhibit the cytotoxicity of cancer cells induced by Tam [58-60, 138-144],
3 suggesting possible adverse effects in patients who are being treated with Tam and taking
4 vitamin C or E as dietary complements.
5
6
7
8
9

10 **2.6. Cholesterol epoxidation is involved in BC cell differentiation and death induced** 11 **by Tam and AEBS ligands** 12 13

14
15 Analyses of the oxysterol profiles of BC cells treated with Tam, SERMs and AEBS
16 ligands have revealed that these compounds stimulate the appearance and accumulation of
17 5,6 α -EC and 5,6 β -EC, which are not normally present in cancer cells (Fig. 3C). Co-treatment
18 of cells with Tam or AEBS ligands and vitamin E inhibited both their pharmacological effects
19 and the accumulation of 5,6-EC, strongly suggesting that 5,6-EC could be second messengers
20 in Tam-induced BC cell death and differentiation (Fig. 3C). It was also shown that Tam and
21 AEBS stimulated lipoperoxidation [55, 56, 60, 122], which is responsible for cholesterol
22 epoxidation [96], and that their inhibition of ChEH blocked 5,6-EC metabolism to CT in
23 cancer cells [128]. Hydrogen peroxide and lipoperoxidation are involved in cholesterol
24 epoxidation and are therefore also responsible for the production of the second messengers
25 that are responsible for the induction of BC cell differentiation and death by Tam and AEBS
26 ligands. Indeed, several studies have identified a dysregulation in the expression of hydrogen
27 peroxide metabolic enzymes, both in a mouse BC model and in human BC tumors, which is
28 consistent with the involvement of 5,6-EC in the pharmacology of Tam [23, 25].
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 This mechanism of ROS-induced 5,6-EC production and its inhibition by vitamin E
51 provides potential routes by which cancer cells can protect themselves against the cytotoxicity
52 induced by Tam, other SERMS and AEBS-selective ligands (Fig. 3C-D) [58-60, 138-146].
53
54
55
56
57
58
59
60
61
62
63
64
65

2.7. Importance of the sulfotransferase SULT2B1b and the nuclear receptor LXR in cytotoxicity induced by Tam and AEBS ligands

Analyses of 5,6-EC biological properties and metabolism in BC cells have revealed that the diastereoisomers 5,6 α -EC and 5,6 β -EC both contribute to cell death induced by Tam or AEBS ligands but through different mechanisms (Fig. 3C). 5,6 β -EC induced a cytotoxicity linked with mitochondrial impairment (cytotoxicity route 1:CD1, Fig. 3C) and is 5,6 α -EC is responsible for the induction of BC cell differentiation and death, which was found to be mediated by the nuclear receptor LXR β and defined the cytotoxicity route 2 (CD2, Fig 3C) [56]. Accordingly, invalidation of LXR β expression induced a loss in BC cell differentiation and decreased the amplitude of cell death triggered by AEBS ligands. 5,6 α -EC is sulfated by the sulfotransferase SULT2B1b to give 5,6 α -epoxycholesterol-3 β -sulfate (5,6 α -ECS) (Fig. 3C and 4A) [56]. 5,6 α -ECS has been proposed to be a LXR antagonist [147] but it has now been accepted that in fact it is a modulator of LXR and activates the expression of certain LXR-responsive genes, leading to TG biosynthesis [56]. Under basal conditions (in the absence of oxidative stress and 5,6-EC), SULT2B1b gene invalidation reportedly blocked LXR α signaling [148, 149] and activated cell proliferation [150-154]. The effects of 5,6 α -ECS are thus peculiar among those reported for other sulfated sterols. Side chain-oxysterols were reported to stimulate cell proliferation [148, 150-152, 154] and to accumulate in prostate cancer, suggesting a link between this accumulation and carcinogenesis [155]. It should be noted that SULT2B1b displays different subcellular localizations and functions in prostate versus breast cancer cells, suggesting different biochemical properties under different intercellular conditions [156]. Knockdown of LXR β in MCF-7 cells was found to induce a loss of sensitivity to Tam, AEBS ligands and 5,6 α -ECS (Fig. 4B), establishing its importance in the pharmacology of these compounds. In the absence of the cytotoxic route 2 (CD2), the remaining sensitivity to Tam is due to the activation of the cytotoxic route 2 (CD2) and reach

1 the magnitude of that found in MDA-MB-231 in which only CD1 is possible (Fig. 4D).
2 Similarly, knockdown of SULT2B1b in MCF-7 cells induced a loss of sensitivity to Tam and
3
4 AEBS ligands due to the absence of CD2, however these cells remained sensitive to 5,6 α -
5
6 ECS, which can reactivate CD2 (Fig. 4C). This established that SULT2B1b contributes to the
7
8 sensitivity of cancer cells to Tam and AEBS ligands. Together, these observations suggest
9
10 that 5,6-EC metabolism, LXR β and SULT2B1b may represent new markers of sensitivity to
11
12 Tam and AEBS ligands. MDA-MB-231 are triple negative BC cells that are not sensitive to
13
14 the proliferative action of 17 β -estradiol. These cells are 10 times less sensitive than ER⁽⁺⁾
15
16 MCF-7 cells to Tam-induced cytotoxicity and are considered intrinsically resistant to Tam
17
18 (Tam^R, Fig. 4D), and only CD1 can be activated. They are also less sensitive to other AEBS
19
20 ligands compared to MCF-7 cells [56]. MDA-MB-231 cells express the AEBS and LXR β but
21
22 not SULT2B1b, thus they produce 5,6-EC but not 5,6 α -ECS following Tam treatment. Their
23
24 sensitivity to Tam is similar to that of MCF-7/LXR β - (Fig. 4B) which reflects the cytotoxicity
25
26 induced by 5,6 β -EC (CD2, Fig. 3C). These cells are as sensitive as MCF-7 cells to 5,6 α -ECS,
27
28 which can activate CD2 [56]. Interestingly, the ectopic expression of SULT2B1b in these
29
30 cells was shown to activate 5,6 α -ECS biosynthesis and sensitized cells to Tam and AEBS
31
32 ligands to the same level as MCF-7 cells (Fig. 4E) [56], confirming that 5,6-EC metabolism
33
34 actively contributes to the response to Tam and AEBS ligands in BC cells. In these cells, the
35
36 CD2 (Fig. 4E) involving 5,6 α -ECS and LXR is activated.
37
38
39
40
41
42
43
44
45
46
47
48

49 **3. Conclusion**

50
51
52 This update on Tam highlights the importance of cholesterol metabolism and the AEBS in the
53
54 anticancer pharmacology of Tam, other SERMs and AEBS ligands. The molecular
55
56 identification of the AEBS has opened up new research avenues and has identified a new
57
58 signaling pathway that is involved in the control of BC differentiation and death by Tam and
59
60
61
62
63
64
65

1 is centered on cholesterol metabolism. The identification of cholesterologenic enzymes as
2 targets of Tam and AEBS ligands is interesting since their inhibition, and the accumulation of
3 sterol precursors, have been associated with the induction of a protective autophagy, leading
4 to new hypotheses on the appearance of mechanisms of Tam resistance. Overexpression of
5 the AEBS was found to be predictive of acquired resistance to Tam in patients with Muc1⁽⁺⁾
6 ER⁽⁺⁾ BC [15, 24], however it is not known whether zymo levels in the blood of patients may
7 represent a predictive marker of response to Tam. Zymo has been shown to be prone to rapid
8 oxidation with a short half life (less than one week at minus 80°C) and it is not inhibited by
9 commonly used antioxidants [42, 157]. Thus, its sterol profile determined in biological
10 materials (cancer cells, blood) must be analyzed extemporaneously, otherwise the zymo signal
11 in analytical methods will be lost. This makes it difficult to analyze the blood sterol profile
12 and to quantify zymo in large cohorts of patients for which sera have been stored for long
13 periods of time. Identification of the end-product(s) of zymo oxidative metabolism may
14 represent an alternative that needs further exploration because these compounds will be stable
15 and measurable. As observed for zymo, 5,6-EC levels in the circulating blood of patients may
16 also represent a marker of Tam activity, and a feasibility study investigating this has very
17 recently been published by Dalenc et al. (OXYTAM, clinical Trial NCT 1553903) [158]. The
18 results from this study, although preliminary, indicate that Tam treatment modifies the
19 oxysterol profile in the blood of patients, with a tendency for increased 5,6-EC levels to be
20 associated with changes in levels of oxysterol markers, which are indicative of LXR
21 transcriptional activity. This study requires further investigation to validate it and to determine
22 whether the modification of oxysterol profiles in the blood of patients can be correlated with
23 clinical outcome, but it offers much potential, especially given that LXR was shown to be
24 central to the transcriptional signature associated with acquired resistance to Tam [24]. The
25 identification of new sterols (such as 5,6 α -ECS and dendrogenins) with specific properties

1 related to cancer also supports the future use of sterolomics approaches, which allow the
2 quantification of these species [96, 159]. Finally, since LXR, cholesterol metabolism and
3
4 oxysterols have been shown to play a role in cancer [160, 161] and in modulating both the
5
6 immune system and the tumor microenvironment [162-164], and since they are involved in
7
8 the anticancer pharmacology of Tam, further studies are also required to analyze the incidence
9
10 of Tam treatment on these parameters.
11
12

13
14
15 Another important point for future investigations arises from reports that vitamin E and
16
17 vitamin C inhibit Tam-induced BC cell death and/or differentiation and. Thus, considering
18
19 that BC patients are prone to automedication with “natural” products [165-167], this point
20
21 deserves further investigation in order to determine whether these may interfere with Tam
22
23 treatment in the clinic.
24
25

26
27
28 Altogether, this review has highlighted the unprecedented characterization of the
29
30 importance of cholesterol metabolism in the anticancer pharmacology of Tam in tumor
31
32 tissues, which has opened up new avenues for improving its clinical efficacy and testing
33
34 alternative approaches to fight against mechanisms of resistance to Tam.
35
36
37
38
39
40

41 LIST OF ABBREVIATIONS

42
43

44 AEBS, antiestrogen binding site; ChEH, cholesterol-5,6-epoxide hydrolase; Tam, tamoxifen,
45 ICI 46,474, nolvadex: (Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethylethanamine;
46 DPM, diphenylmethane; tesmilifene/DPPE, N,N-diethyl-2-[4-
47 (phenylmethyl)phenoxy]ethanamine; PBPE, 1-[2-[4-(phenylmethyl)phenoxy]ethyl]-
48 pyrrolidine; 4OHTam, 4-hydroxytamoxifen; lazofloxifen, (5R,6S)-6-phenyl-5-[4-(2-
49 pyrrolidin-1-ylethoxy)phenyl]-5,6,7,8-tetrahydronaphthalen-2-ol; raloxifene/keoxifen, [6-
50 hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4-(2-piperidin-1-
51 ylethoxy)phenyl]methanone; bazedoxifene, 1-[4-[2-(Azepan-1-yl)ethoxy]benzyl]-2-(4-
52 hydroxyphenyl)-3-methyl-1*H*-indol-5-ol; clomiphene, 2-[4-[(E)-2-chloro-1,2-diphenylethe
53 nyl]phenoxy]-N,N-diethylethanamine; triparanol/Mer-29, metasqualene,2-(4-chlorophenyl)-1-
54 [4-[2-(diethylamino)ethoxy]phenyl]-1-(4-methylphenyl)etanol; boxidine, (1-[2-[[4'-
55 (Trifluoromethyl)-4-biphenyl]oxy]ethyl]pyrrolidine); tBuPE, t-butylphenoxyethyl
56 diethylamine; dendrogenin A/DDA, 5 α -hydroxy-6 β -[2-(1*H*-imidazol-4-yl)-ethylamino]-
57 cholestan-3 β -ol; DHCR24, 3 β -hydroxysteroid- Δ^{24} -reductase; DHCR7, 3 β -hydroxysteroid- Δ^7 -
58
59
60
61
62
63
64
65

1 reductase; D8D7I/EBP, 3 β -hydroxysteroid- Δ^8,Δ^7 -isomerase; SC5D, 3 β -hydroxysteroid- Δ^5 -
2 desaturase; cholesterol, cholest-5-en-3 β -ol; desmosterol, cholest-5,24-dien-3 β -ol; zymostenol,
3 5 α -cholest-8-ene-3 β -ol; zymosterol, 5 α -cholesta-8,24-dien-3 β -ol; 7-dehydrocholesterol,
4 cholest-5,7-dien-3 β -ol; lanosterol, lanosta-8,24-dien-3 β -ol; 5,6 α -EC, 5,6 α -epoxycholesterol;
5 5,6 β -EC, 5,6 β -epoxycholesterol; 5,6 α -ECS, 5,6 α -epoxycholesterol-3 β -sulfate; HA,
6 histamine; ER, estrogen receptor; BC, breast cancer.
7
8
9
10

11 CONFLICT OF INTEREST

12
13
14
15 The authors declare no conflicts of interest.
16
17
18
19
20

21 ACKNOWLEDGEMENTS

22
23
24 All authors have contributed to the writing of this manuscript. This work was funded by the
25
26 Institut National de la Santé et de la Recherche Médicale, the université Toulouse III, the
27
28 Fondation de France (R11166BB), the Association pour la Recherche sur le Cancer (PJA
29
30 2013 12 00 342), the INCA (PRTK-K15-118), the association « pour Céline » and the
31
32 association « Flo ».
33
34
35
36
37
38
39
40
41

42 FIGURE LEGENDS

43
44
45 **Figure 1. Chemical structure of some synthetic and endogenous AEBS ligands and**
46 **pharmacophores from the tamoxifen series.** A) Tamoxifen. Molecular features that are
47
48 necessary for tamoxifen to recognize its two high affinity targets: B) the microsomal
49
50 antiestrogen binding site (AEBS) and C) ER. D-E) Selective AEBS ligands with no affinity to
51
52 ER. F-J) natural endogenous AEBS ligands. K) Mer29 is an inhibitor of DHCR24. L)
53
54 Boxidine is an inhibitor of DHCR7. M-P) Structure of cholesterol biosynthesis intermediates
55
56 that could accumulate in cancer cells treated with AEBS ligands.
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 2: The AEBS is made up of two cholesterologenic enzymes, and AEBS ligands induced the accumulation of cholesterol precursors, which triggered a survival autophagy in cancer cells. A) The AEBS consists of an association between D8D7I and DHCR7 linked to DHCR24. B) Binding of Tam to the AEBS led to the inhibition of D8D7I and to the accumulation of zymostenol (zymo). Binding of PBPE led to the accumulation of zymo and 7-dehydrocholesterol (7DHC). C) Binding of 4Ohtam induced the accumulation of desmosterol (desmo). D) Treatment of BC cells with Tam induced the accumulation of zymo in intracellular organelles. The accumulation of free sterols is revealed by filipin labeling under fluorescence microscopy. Ultrastructure analyses of cells through transmission electron microscopy (TEM) showed an accumulation of lysosomes (Lys) and the appearance of autophagosomes (AutoPh). These cellular events are associated with the induction of a survival autophagy in cancer cells. N: nucleus; C: cytoplasm.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 3: The AEBS carries out cholesterol-5,6-epoxide hydrolase (ChEH) activity and 5,6-ECs are second messengers of tam that are responsible for the induction of differentiation and death of breast cancer cells. A) ChEH catalyzes the hydration of cholesterol 5,6-epoxides (5,6 α EC and 5,6 β -EC) into cholestane-3 β ,5 α ,6 β -triol. B) Tam inhibits ChEH and induces the accumulation of the 5,6-ECs. C) AEBS ligands induced the biosynthesis and the accumulation of 5,6-EC. 5,6 β -EC contributes to route 1 cell death (CD1) through impairing mitochondrial activity. 5,6 α -EC is metabolized into 5,6 α -ECS by SULT2B1b and induces LXR β -dependent characteristics of differentiation and cell death (route 2 cell death, CD2). D) Vitamin E (Vit E) inhibits cholesterol epoxidation and blocks the induction of cancer cell differentiation and cancer cell death (CD1). N: nucleus; C: cytoplasm; Mito: mitochondria; TG: triacylglycerol; Lys: lysosome; Chol: cholesterol; ROS: reactive oxygen species; zymo: zymostenol.

Figure 4: The importance of SULT2B1b and LXR β on the sensitivity of BC cells to Tam.

1
2 Molecular mechanisms of BC cell death induced by Tam and AEBS ligands: A) MCF-7 cells
3
4 are sensitive to Tam (Tam^S) and 5,6-ECS (5,6-ECS^S); both the route 1 (CD1) and route 2
5
6 (CD2) are observable. B) MCF-7 cells invalidated for the expression of LXR β (MCF-
7
8 7/LXR β -) became less sensitive to Tam (Tam^R) and 5,6-ECS (5,6-ECS^R); only route 1 (CD1)
9
10 is observable. C) MCF-7 cells invalidated for the expression of SULT2B1b (MCF-
11
12 7/SULT2B1b-) are Tam^R and 5,6-ECS^S; only CD1 is observable with Tam but 5,6-ECS can
13
14 activate CD2. D) MDA-MB-231 cells are Tam^R and 5,6-ECS^S. MDA-MB-231 are less
15
16 sensitive than MCF-7 to tam cytotoxicity; only CD1 is observable. E) Transgenic MDA-MB-
17
18 231 cells expressing SULT2B1b (MDA-MB-231/SULT2B1b+) are Tam^S and 5,6-ECS^S; both
19
20 CD1 and CD2 occurred, these cells are as sensitive to Tam than MCF-7 cells. N: nucleus; C:
21
22 cytoplasm; Mito: mitochondria; TG: triacylglycerol; Lys: lysosome; Chol: cholesterol; ROS:
23
24 reactive oxygen species; zymo: zymostenol; CD1: cytotoxic route 1; CD2: cytotoxic route 2.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

REFERENCES

51
52
53
54
55
56
57
58 [1] Jordan VC. Chemoprevention of breast cancer with selective oestrogen-receptor modulators. Nat Rev
59
60 Cancer. 2007;7:46-53.
61
62
63
64
65

- 1 [2] Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26:v8-v30.
- 2 [3] Gu G, Dustin D, Fuqua SAW. Targeted therapy for breast cancer and molecular mechanisms of resistance to
3 treatment. *Curr Opin Pharmacol*. 2016;31:97-103.
- 4 [4] Jordan VC. Tamoxifen: a most unlikely pioneering medicine. *Nat Rev Drug Discov*. 2003;2:205-13.
- 5 [5] Poirot M. Four decades of discovery in breast cancer research and treatment - an interview with V. Craig
6 Jordan. *Int J Dev Biol*. 2011;55:703-12.
- 7 [6] Cuzick J, Sestak I, Bonanni B, Costantino JP, Cummings S, DeCensi A, et al. Selective oestrogen receptor
8 modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. *Lancet*.
9 2013;381:1827-34.
- 10 [7] Veronesi U, Maisonneuve P, Rotmensz N, Bonanni B, Boyle P, Viale G, et al. Tamoxifen for the Prevention
11 of Breast Cancer: Late Results of the Italian Randomized Tamoxifen Prevention Trial Among Women With
12 Hysterectomy. *J Natl Cancer Inst*. 2007;99:727-37.
- 13 [8] Smith CL, O'Malley BW. Coregulator Function: A Key to Understanding Tissue Specificity of Selective
14 Receptor Modulators. *Endocr Rev*. 2004;25:45-71.
- 15 [9] Droog M, Nevedomskaya E, Dackus GM, Fles R, Kim Y, Hollema H, et al. Estrogen receptor α yields
16 treatment-specific enhancers between morphologically similar endometrial tumors. *Proc Natl Acad Sci U S A*.
17 2017;114:E1316-E25.
- 18 [10] Jones ME, van Leeuwen FE, Hoogendoorn WE, Mourits MJE, Hollema H, van Boven HH, et al.
19 Endometrial cancer survival after breast cancer in relation to tamoxifen treatment: Pooled results from three
20 countries. *Breast Cancer Res*. 2012;14:R91.
- 21 [11] Swerdlow AJ, Jones ME. Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-
22 control study. *J Natl Cancer Inst*. 2005;97.
- 23 [12] Jordan VC, McDaniel R, Agboke F, Maximov PY. The evolution of nonsteroidal antiestrogens to become
24 selective estrogen receptor modulators. *Steroids*. 2014;90:3-12.
- 25 [13] Traboulsi T, El Ezzy M, Gleason JL, Mader S. Antiestrogens: structure-activity relationships and use in
26 breast cancer treatment. *J Mol Endocrinol*. 2017;58:R15-R31.
- 27 [14] Tryfonidis K, Zardavas D, Katzenellenbogen BS, Piccart M. Endocrine treatment in breast cancer: Cure,
28 resistance and beyond. *Cancer Treat Rev*. 2016;50:68-81.
- 29 [15] Poirot M, Silvente-Poirot S, Weichselbaum RR. Cholesterol metabolism and resistance to tamoxifen. *Curr*
30 *Opin Pharmacol*. 2012;12:683-9.
- 31 [16] de Medina P, Paillasse MR, Segala G, Khallouki F, Brillouet S, Dalenc F, et al. Importance of cholesterol
32 and oxysterols metabolism in the pharmacology of tamoxifen and other AEBS ligands. *Chem Phys Lipids*.
33 2011;164:432-7.
- 34 [17] Jordan VC. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 1.
35 Receptor interactions. *J Med Chem*. 2003;46:883-908.
- 36 [18] Jordan VC. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 2.
37 Clinical considerations and new agents. *J Med Chem*. 2003;46:1081-111.
- 38 [19] Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and molecular pharmacology of antiestrogen action
39 and resistance. *Pharmacol Rev*. 2001;53:25-71.
- 40 [20] Jordan VC. Biochemical pharmacology of antiestrogen action. *Pharmacol Rev*. 1984;36:245-76.
- 41 [21] Furr BJ, Jordan VC. The pharmacology and clinical uses of tamoxifen. *Pharmacol Ther*. 1984;25:127-205.
- 42 [22] Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations[mdash]a mechanism for
43 acquired endocrine resistance in breast cancer. *Nat Rev Clin Oncol*. 2015;12:573-83.
- 44 [23] Fiorillo M, Sotgia F, Sisci D, Cappello AR, Lisanti MP. Mitochondrial "power" drives tamoxifen resistance:
45 NQO1 and GCLC are new therapeutic targets in breast cancer. *Oncotarget*. 2017;8:20309-27.
- 46 [24] Pitroda SP, Khodarev NN, Beckett MA, Kufe DW, Weichselbaum RR. MUC1-induced alterations in a lipid
47 metabolic gene network predict response of human breast cancers to tamoxifen treatment. *Proc Natl Acad Sci U*
48 *S A*. 2009;106:5837-41.
- 49 [25] Schiff R, Reddy P, Ahotupa M, Coronado-Heinsohn E, Grim M, Hilsenbeck SG, et al. Oxidative stress and
50 AP-1 activity in tamoxifen-resistant breast tumors in vivo. *J Natl Cancer Inst*. 2000;92:1926-34.
- 51 [26] Borgquist S, Giobbie-Hurder A, Ahern TP, Garber JE, Colleoni M, Láng I, et al. Cholesterol, Cholesterol-
52 Lowering Medication Use, and Breast Cancer Outcome in the BIG 1-98 Study. *J Clin Oncol*. 2017;35:1179-88.
- 53 [27] de Medina P, Favre G, Poirot M. Multiple targeting by the antitumor drug tamoxifen: a structure-activity
54 study. *Curr Med Chem Anticancer Agents*. 2004;4:491-508.
- 55 [28] Trump DL, Smith DC, Ellis PG, Rogers MP, Schold SC, Winer EP, et al. High-dose oral tamoxifen, a
56 potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. *J Natl Cancer Inst*.
57 1992;84:1811-6.
- 58
59
60
61
62
63
64
65

- 1 [29] Paillasse MR, de Medina P, Amouroux G, Mhamdi L, Poirot M, Silvente-Poirot S. Signaling through
2 cholesterol esterification: a new pathway for the cholecystokinin 2 receptor involved in cell growth and invasion.
3 *J Lipid Res.* 2009;50:2203-11.
- 4 [30] de Medina P, Genovese S, Paillasse MR, Mazaheri M, Caze-Subra S, Bystricky K, et al. Auraptene is an
5 inhibitor of cholesterol esterification and a modulator of estrogen receptors. *Mol Pharmacol.* 2010;78:827-36.
- 6 [31] Yue S, Li J, Lee SY, Lee HJ, Shao T, Song B, et al. Cholesteryl ester accumulation induced by PTEN loss
7 and PI3K/AKT activation underlies human prostate cancer aggressiveness. *Cell Metab.* 2014;19:393-406.
- 8 [32] Li J, Gu D, Lee SS, Song B, Bandyopadhyay S, Chen S, et al. Abrogating cholesterol esterification
9 suppresses growth and metastasis of pancreatic cancer. *Oncogene.* 2016;35:6378-88.
- 10 [33] Geng F, Cheng X, Wu X, Yoo JY, Cheng C, Guo JY, et al. Inhibition of SOAT1 Suppresses Glioblastoma
11 Growth via Blocking SREBP-1-Mediated Lipogenesis. *Clin Cancer Res.* 2016;22:5337-48.
- 12 [34] Brown HA, Thomas PG, Lindsley CW. Targeting phospholipase D in cancer, infection and
13 neurodegenerative disorders. *Nat Rev Drug Discov.* 2017;16:351-67.
- 14 [35] Selvy PE, Lavieri RR, Lindsley CW, Brown HA. Phospholipase D: enzymology, functionality, and
15 chemical modulation. *Chem Rev.* 2011;111:6064-119.
- 16 [36] Scott SA, Selvy PE, Buck JR, Cho HP, Criswell TL, Thomas AL, et al. Design of isoform-selective
17 phospholipase D inhibitors that modulate cancer cell invasiveness. *Nat Chem Biol.* 2009;5:108-17.
- 18 [37] Eisen SF, Brown HA. Selective estrogen receptor (ER) modulators differentially regulate phospholipase D
19 catalytic activity in ER-negative breast cancer cells. *Mol Pharmacol.* 2002;62:911-20.
- 20 [38] Morad SA, Tan SF, Feith DJ, Kester M, Claxton DF, Loughran TP, Jr., et al. Modification of sphingolipid
21 metabolism by tamoxifen and N-desmethyltamoxifen in acute myelogenous leukemia--Impact on enzyme
22 activity and response to cytotoxics. *Biochim Biophys Acta.* 2015;1851:919-28.
- 23 [39] Morad SA, Cabot MC. Tamoxifen regulation of sphingolipid metabolism--Therapeutic implications.
24 *Biochim Biophys Acta.* 2015;1851:1134-45.
- 25 [40] Corbel C, Zhang B, Le Parc A, Baratte B, Colas P, Couturier C, et al. Tamoxifen inhibits CDK5 kinase
26 activity by interacting with p35/p25 and modulates the pattern of tau phosphorylation. *Chem Biol.* 2015;22:472-
27 82.
- 28 [41] Sutherland RL, Murphy LC, San Foo M, Green MD, Whybourne AM, Krozowski ZS. High-affinity anti-
29 oestrogen binding site distinct from the oestrogen receptor. *Nature.* 1980;288:273-5.
- 30 [42] Kedjouar B, de Medina P, Oulad-Abdelghani M, Payre B, Silvente-Poirot S, Favre G, et al. Molecular
31 characterization of the microsomal tamoxifen binding site. *J Biol Chem.* 2004;279:34048-61.
- 32 [43] Faye JC, Lasserre B, Bayard F. Antiestrogen specific, high affinity saturable binding sites in rat uterine
33 cytosol. *Biochem Biophys Res Commun.* 1980;93:1225-31.
- 34 [44] Sutherland RL, Foo MS. Differential binding of antiestrogens by rat uterine and chick oviduct cytosol.
35 *Biochem Biophys Res Commun.* 1979;91:183-91.
- 36 [45] Faye JC, Jozan S, Redeuilh G, Baulieu EE, Bayard F. Physicochemical and genetic evidence for specific
37 antiestrogen binding sites. *Proc Natl Acad Sci U S A.* 1983;80:3158-62.
- 38 [46] Watts CK, Sutherland RL. High affinity specific antiestrogen binding sites are concentrated in rough
39 microsomal membranes of rat liver. *Biochem Biophys Res Commun.* 1984;120:109-15.
- 40 [47] Watts CK, Murphy LC, Sutherland RL. Microsomal binding sites for nonsteroidal anti-estrogens in MCF 7
41 human mammary carcinoma cells. Demonstration of high affinity and narrow specificity for basic ether
42 derivatives of triphenylethylene. *J Biol Chem.* 1984;259:4223-9.
- 43 [48] de Medina P, Payre BL, Bernad J, Bosser I, Pipy B, Silvente-Poirot S, et al. Tamoxifen is a potent inhibitor
44 of cholesterol esterification and prevents the formation of foam cells. *J Pharmacol Exp Ther.* 2004;308:1165-73.
- 45 [49] Poirot M, De Medina P, Delarue F, Perie JJ, Klæbe A, Faye JC. Synthesis, binding and structure-affinity
46 studies of new ligands for the microsomal anti-estrogen binding site (AEBS). *Bioorg Med Chem.* 2000;8:2007-
47 16.
- 48 [50] Watts CK, Sutherland RL. Studies on the ligand specificity and potential identity of microsomal
49 antiestrogen-binding sites. *Mol Pharmacol.* 1987;31:541-51.
- 50 [51] Anstead GM, Carlson KE, Katzenellenbogen JA. The estradiol pharmacophore: Ligand structure-estrogen
51 receptor binding affinity relationships and a model for the receptor binding site. *Steroids.* 1997;62:268-303.
- 52 [52] Saeed A, Durani N, Durani S, Ray S, Kapil RS. Cis isomer of centchroman--a selective ligand for the
53 microsomal antiestrogen binding site. *Biochem Biophys Res Commun.* 1984;125:346-52.
- 54 [53] Teo CC, Kon OL, Sim KY, Ng SC. Synthesis of 2-(p-chlorobenzyl)-3-aryl-6-methoxybenzofurans as
55 selective ligands for antiestrogen-binding sites. Effects on cell proliferation and cholesterol synthesis. *J Med*
56 *Chem.* 1992;35:1330-9.
- 57 [54] Brandes LJ. A diphenylmethane derivative selective for the anti-estrogen binding site may help define its
58 biological role. *Biochem Biophys Res Commun.* 1984;124:244-9.

- 1 [55] Sola B, Poirot M, de Medina P, Bustany S, Marsaud V, Silvente-Poirot S, et al. Antiestrogen-binding site
2 ligands induce autophagy in myeloma cells that proceeds through alteration of cholesterol metabolism.
3 *Oncotarget*. 2013;4:911-22.
- 4 [56] Segala G, de Medina P, Iuliano L, Zerbinati C, Paillasse MR, Noguer E, et al. 5,6-Epoxy-cholesterols
5 contribute to the anticancer pharmacology of tamoxifen in breast cancer cells. *Biochem Pharmacol*.
6 2013;86:175-89.
- 7 [57] de Medina P, Paillasse MR, Segala G, Poirot M, Silvente-Poirot S. Identification and pharmacological
8 characterization of cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBS ligands. *Proc Natl
9 Acad Sci U S A*. 2010;107:13520-5.
- 10 [58] de Medina P, Silvente-Poirot S, Poirot M. Tamoxifen and AEBS ligands induced apoptosis and autophagy
11 in breast cancer cells through the stimulation of sterol accumulation. *Autophagy*. 2009;5:1066-7.
- 12 [59] de Medina P, Payre B, Boubekour N, Bertrand-Michel J, Terce F, Silvente-Poirot S, et al. Ligands of the
13 antiestrogen-binding site induce active cell death and autophagy in human breast cancer cells through the
14 modulation of cholesterol metabolism. *Cell Death Differ*. 2009;16:1372-84.
- 15 [60] Payre B, de Medina P, Boubekour N, Mhamdi L, Bertrand-Michel J, Terce F, et al. Microsomal
16 antiestrogen-binding site ligands induce growth control and differentiation of human breast cancer cells through
17 the modulation of cholesterol metabolism. *Mol Cancer Ther*. 2008;7:3707-18.
- 18 [61] Brandes LJ, Bracken SP, Ramsey EW. N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine in
19 combination with cyclophosphamide: an active, low-toxicity regimen for metastatic hormonally unresponsive
20 prostate cancer. *J Clin Oncol*. 1995;13:1398-403.
- 21 [62] Brandes LJ, Bogdanovic RP, Tong J, Davie JR, LaBella FS. Intracellular histamine and liver regeneration:
22 high affinity binding of histamine to chromatin, low affinity binding to matrix, and depletion of a nuclear storage
23 pool following partial hepatectomy. *Biochem Biophys Res Commun*. 1992;184:840-7.
- 24 [63] Poirot M, Garnier M, Bayard F, Riviere I, Traore M, Wilson M, et al. The anti-proliferative properties of 4-
25 benzylphenoxy ethanamine derivatives are mediated by the anti-estrogen binding site (ABS), whereas the anti-
26 estrogenic effects of trifluopromazine are not. *Biochem Pharmacol*. 1990;40:425-9.
- 27 [64] Fargin A, Bayard F, Faye JC, Traore M, Poirot M, Klæbe A, et al. Further evidence for a biological role of
28 anti-estrogen-binding sites in mediating the growth inhibitory action of diphenylmethane derivatives. *Chem Biol
29 Interact*. 1988;66:101-9.
- 30 [65] Raghavan D, Brandes LJ, Klapp K, Snyder T, Styles E, Tsao-Wei D, et al. Phase II trial of tesmilifene plus
31 mitoxantrone and prednisone for hormone refractory prostate cancer: high subjective and objective response in
32 patients with symptomatic metastases. *J Urol*. 2005;174:1808-13; discussion 13.
- 33 [66] Brandes LJ, Queen GM, LaBella FS. N,N-diethyl-2-[4-(phenylmethyl)phenoxy] ethanamine (DPPE) a
34 chemopotentiating and cytoprotective agent in clinical trials: interaction with histamine at cytochrome P450 3A4
35 and other isozymes that metabolize antineoplastic drugs. *Cancer Chemother Pharmacol*. 2000;45:298-304.
- 36 [67] Brandes LJ, Bracken SP. The intracellular histamine antagonist, N,N-diethyl-2-[4-(phenylmethyl)phenoxy]
37 ethamine.HCL, may potentiate doxorubicin in the treatment of metastatic breast cancer: Results of a pilot study.
38 *Breast Cancer Res Treat*. 1998;49:61-8.
- 39 [68] Brandes LJ, Simons KJ, Bracken SP, Warrington RC. Results of a clinical trial in humans with refractory
40 cancer of the intracellular histamine antagonist, N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine-HCl, in
41 combination with various single antineoplastic agents. *J Clin Oncol*. 1994;12:1281-90.
- 42 [69] Brandes LJ, LaBella FS, Warrington RC. Increased therapeutic index of antineoplastic drugs in combination
43 with intracellular histamine antagonists. *J Natl Cancer Inst*. 1991;83:1329-36.
- 44 [70] Issandou M, Faucher C, Bayard F, Darbon JM. Opposite effects of tamoxifen on in vitro protein kinase C
45 activity and endogenous protein phosphorylation in intact MCF-7 cells. *Cancer Res*. 1990;50:5845-50.
- 46 [71] Brandes LJ, Gerrard JM, Bogdanovic RP, Lint DW, Reid RE, LaBella FS. Correlation of the
47 antiproliferative action of diphenylmethane-derivative antiestrogen binding site ligands with antagonism of
48 histamine binding but not of protein kinase C-mediated phosphorylation. *Cancer Res*. 1988;48:3954-8.
- 49 [72] Brandes LJ, Bogdanovic RP, Cawker MD, Bose R. The antiproliferative properties of tamoxifen and
50 phenothiazines may be mediated by a unique histamine receptor (?H3) distinct from the calmodulin-binding site.
51 *Cancer Chemother Pharmacol*. 1986;18:21-3.
- 52 [73] Murphy PR, Breckenridge WC, Lazier CB. Binding of oxygenated cholesterol metabolites to antiestrogen
53 binding sites from chicken liver. *Biochem Biophys Res Commun*. 1985;127:786-92.
- 54 [74] Hwang PL. Interaction of unsaturated fatty acids with anti-oestrogen-binding sites. *Biochem J*.
55 1987;243:359-64.
- 56 [75] Hwang PL. Unsaturated fatty acids as endogenous inhibitors of tamoxifen binding to anti-oestrogen-binding
57 sites. *Biochem J*. 1986;237:749-55.
- 58 [76] Hwang PL. High-affinity binding sites for oxygenated sterols in rat liver microsomes: possible identity with
59 antiestrogen binding sites. *Biochim Biophys Acta*. 1990;1033:154-61.

- [77] Hwang PL, Matin A. Interactions of sterols with antiestrogen-binding sites: structural requirements for high-affinity binding. *J Lipid Res.* 1989;30:239-45.
- [78] Berrodin TJ, Shen Q, Quinet EM, Yudit MR, Freedman LP, Nagpal S. Identification of 5alpha, 6alpha-epoxycholesterol as a novel modulator of liver X receptor activity. *Mol Pharmacol.* 2010;78:1046-58.
- [79] Son Y, Chun W, Ahn YT, Kim K, Lee CW, Kim JM, et al. 7-Ketocholesterol induces the reduction of KCNB1 in atherosclerotic blood vessels. *Biochem Biophys Res Commun.* 2015;457:324-7.
- [80] Savouret JF, Antenos M, Quesne M, Xu J, Milgrom E, Casper RF. 7-ketocholesterol is an endogenous modulator for the arylhydrocarbon receptor. *J Biol Chem.* 2001;276:3054-9.
- [81] Hu X, Wang Y, Hao LY, Liu X, Lesch CA, Sanchez BM, et al. Sterol metabolism controls T(H)17 differentiation by generating endogenous RORgamma agonists. *Nat Chem Biol.* 2015;11:141-7.
- [82] Soroosh P, Wu J, Xue X, Song J, Sutton SW, Sablad M, et al. Oxysterols are agonist ligands of RORgamma and drive Th17 cell differentiation. *Proc Natl Acad Sci U S A.* 2014;111:12163-8.
- [83] Wang Y, Kumar N, Solt LA, Richardson TI, Helvering LM, Crumbley C, et al. Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands. *J Biol Chem.* 2010;285:5013-25.
- [84] Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, et al. Oxysterols direct immune cell migration via EBI2. *Nature.* 2011;475:524-7.
- [85] Sever N, Mann RK, Xu L, Snell WJ, Hernandez-Lara CI, Porter NA, et al. Endogenous B-ring oxysterols inhibit the Hedgehog component Smoothed in a manner distinct from cyclopamine or side-chain oxysterols. *Proc Natl Acad Sci U S A.* 2016;113.
- [86] Suchanek M, Hynynen R, Wohlfahrt G, Lehto M, Johansson M, Saarinen H, et al. The mammalian oxysterol-binding protein-related proteins (ORPs) bind 25-hydroxycholesterol in an evolutionarily conserved pocket. *Biochem J.* 2007;405:473-80.
- [87] Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat Med.* 2007;13:1185-92.
- [88] Sato H, Nishida S, Tomoyori H, Sato M, Ikeda I, Imaizumi K. Oxysterol regulation of estrogen receptor alpha-mediated gene expression in a transcriptional activation assay system using HeLa cells. *Biosci Biotechnol Biochem.* 2004;68:1790-3.
- [89] Brandes LJ, LaBella FS, Glavin GB, Paraskevas F, Saxena SP, McNicol A, et al. Histamine as an intracellular messenger. *Biochem Pharmacol.* 1990;40:1677-81.
- [90] Brandes LJ, Bogdanovic RP, Cawker MD, LaBella FS. Histamine and growth: interaction of antiestrogen binding site ligands with a novel histamine site that may be associated with calcium channels. *Cancer Res.* 1987;47:4025-31.
- [91] Brandes LJ, Macdonald LM, Bogdanovic RP. Evidence that the antiestrogen binding site is a histamine or histamine-like receptor. *Biochem Biophys Res Commun.* 1985;126:905-10.
- [92] Silvente-Poirot S, de Medina P, Record M, Poirot M. From tamoxifen to dendrogenin A: The discovery of a mammalian tumor suppressor and cholesterol metabolite. *Biochimie.* 2016;130:109-14.
- [93] Poirot M, Silvente-Poirot S. When cholesterol meets histamine, it gives rise to dendrogenin A: a tumour suppressor metabolite. *Biochem Soc Trans.* 2016;44:631-7.
- [94] Dalenc F, Poirot M, Silvente Poirot S. Dendrogenin A: a Mammalian Metabolite of Cholesterol with Tumor Suppressor and Neurostimulating Properties. *Curr Med Chem.* 2015;22:3533-49.
- [95] Silvente-Poirot S, Poirot M. Cancer. Cholesterol and cancer, in the balance. *Science.* 2014;343:1445-6.
- [96] Poirot M, Silvente-Poirot S. Cholesterol-5,6-epoxides: chemistry, biochemistry, metabolic fate and cancer. *Biochimie.* 2013;95:622-31.
- [97] de Medina P, Paillasse MR, Segala G, Voisin M, Mhamdi L, Dalenc F, et al. Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties. *Nat Commun.* 2013;4:1840.
- [98] Avigan J, Steinberg D, Vroman HE, Thompson MJ, Mosettig E. Studies of cholesterol biosynthesis. I. The identification of desmosterol in serum and tissues of animals and man treated with MER-29. *J Biol Chem.* 1960;235:3123-6.
- [99] Gailani S, Holland JF, Glick A. Effects of boxidine on human serum sterols and neoplasms. *Clin Pharmacol Ther.* 1972;13:91-6.
- [100] Cypriani B, Tabacik C, Descomps B, Crastes de Paulet A. Role of estrogen receptors and antiestrogen binding sites in an early effect of antiestrogens, the inhibition of cholesterol biosynthesis. *J Steroid Biochem.* 1988;31:763-71.
- [101] Gylling H, Pyrhonen S, Mantyla E, Maenpaa H, Kangas L, Miettinen TA. Tamoxifen and toremifene lower serum cholesterol by inhibition of delta 8-cholesterol conversion to lathosterol in women with breast cancer. *J Clin Oncol.* 1995;13:2900-5.
- [102] Lindenthal B, Holleran AL, Aldaghlis TA, Ruan B, Schroepfer GJ, Jr., Wilson WK, et al. Progestins block cholesterol synthesis to produce meiosis-activating sterols. *FASEB J.* 2001;15:775-84.

- 1 [103] Moebius FF, Reiter RJ, Bermoser K, Glossmann H, Cho SY, Paik YK. Pharmacological analysis of sterol
2 delta8-delta7 isomerase proteins with [3H]ifenprodil. *Mol Pharmacol.* 1998;54:591-8.
- 3 [104] Holleran AL, Lindenthal B, Aldaghtas TA, Kelleher JK. Effect of tamoxifen on cholesterol synthesis in
4 HepG2 cells and cultured rat hepatocytes. *Metabolism.* 1998;47:1504-13.
- 5 [105] Korade Z, Kim HY, Tallman KA, Liu W, Koczok K, Balogh I, et al. The Effect of Small Molecules on
6 Sterol Homeostasis: Measuring 7-Dehydrocholesterol in Dhcr7-Deficient Neuro2a Cells and Human Fibroblasts.
7 *J Med Chem.* 2016;59:1102-15.
- 8 [106] Kim HY, Korade Z, Tallman KA, Liu W, Weaver CD, Mirnics K, et al. Inhibitors of 7-Dehydrocholesterol
9 Reductase: Screening of a Collection of Pharmacologically Active Compounds in Neuro2a Cells. *Chem Res
10 Toxicol.* 2016;29:892-900.
- 11 [107] Luu W, Hart-Smith G, Sharpe LJ, Brown AJ. The terminal enzymes of cholesterol synthesis, DHCR24 and
12 DHCR7, interact physically and functionally. *J Lipid Res.* 2015;56:888-97.
- 13 [108] Lusa S, Blom TS, Eskelinen EL, Kuismanen E, Mansson JE, Simons K, et al. Depletion of rafts in late
14 endocytic membranes is controlled by NPC1-dependent recycling of cholesterol to the plasma membrane. *J Cell
15 Sci.* 2001;114:1893-900.
- 16 [109] Gabitova L, Restifo D, Gorin A, Manocha K, Handorf E, Yang D-H, et al. Endogenous Sterol Metabolites
17 Regulate Growth of EGFR/KRAS-Dependent Tumors via LXR. *Cell Rep.* 2015;12:1927-38.
- 18 [110] Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated
19 by the nuclear receptor LXR alpha. *Nature.* 1996;383:728-31.
- 20 [111] Yang C, McDonald JG, Patel A, Zhang Y, Umetani M, Xu F, et al. Sterol Intermediates from Cholesterol
21 Biosynthetic Pathway as Liver X Receptor Ligands. *J Biol Chem.* 2006;281:27816-26.
- 22 [112] Schoenlein PV, Periyasamy-Thandavan S, Samaddar JS, Jackson WH, Barrett JT. Autophagy facilitates
23 the progression of ERalpha-positive breast cancer cells to antiestrogen resistance. *Autophagy.* 2009;5:400-3.
- 24 [113] Samaddar JS, Gaddy VT, Duplantier J, Thandavan SP, Shah M, Smith MJ, et al. A role for
25 macroautophagy in protection against 4-hydroxytamoxifen-induced cell death and the development of
26 antiestrogen resistance. *Mol Cancer Ther.* 2008;7:2977-87.
- 27 [114] Xu L, Porter NA. Free radical oxidation of cholesterol and its precursors: Implications in cholesterol
28 biosynthesis disorders. *Free Radic Res.* 2015;49:835-49.
- 29 [115] Porter NA. A perspective on free radical autoxidation: the physical organic chemistry of polyunsaturated
30 fatty acid and sterol peroxidation. *J Org Chem.* 2013;78:3511-24.
- 31 [116] Xu L, Korade Z, Porter NA. Oxysterols from free radical chain oxidation of 7-dehydrocholesterol: product
32 and mechanistic studies. *J Am Chem Soc.* 2010;132:2222-32.
- 33 [117] Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: From cholesterol metabolites to key
34 mediators. *Prog Lipid Res.* 2016;64:152-69.
- 35 [118] Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines
36 for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy.* 2016;12:1-222.
- 37 [119] Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, et al. Guidelines for
38 the use and interpretation of assays for monitoring autophagy. *Autophagy.* 2012;8:445-544.
- 39 [120] Scarlatti F, Bauvy C, Ventruti A, Sala G, Cluzeaud F, Vandewalle A, et al. Ceramide-mediated
40 Macroautophagy Involves Inhibition of Protein Kinase B and Up-regulation of Beclin 1. *J Biol Chem.*
41 2004;279:18384-91.
- 42 [121] Bursch W, Ellinger A, Kienzl H, Torok L, Pandey S, Sikorska M, et al. Active cell death induced by the
43 anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of
44 autophagy. *Carcinogenesis.* 1996;17:1595-607.
- 45 [122] Chakraborty A, Bodipati N, Demonacos MK, Peddinti R, Ghosh K, Roy P. Long term induction by
46 pterostilbene results in autophagy and cellular differentiation in MCF-7 cells via ROS dependent pathway. *Mol
47 Cell Endocrinol.* 2012;355:25-40.
- 48 [123] Qadir MA, Kwok B, Dragowska WH, To KH, Le D, Bally MB, et al. Macroautophagy inhibition
49 sensitizes tamoxifen-resistant breast cancer cells and enhances mitochondrial depolarization. *Breast Cancer Res
50 Treat.* 2008;112:389-403.
- 51 [124] Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast
52 cancer cells following DNA damage. *Cell Death Differ.* 2007;14:500-10.
- 53 [125] Clarke R. Cannibalism, cell survival, and endocrine resistance in breast cancer. *Breast Cancer Res.*
54 2011;13:311.
- 55 [126] Sevanian A, McLeod LL. Catalytic properties and inhibition of hepatic cholesterol-epoxide hydrolase. *J
56 Biol Chem.* 1986;261:54-9.
- 57 [127] Kodani SD, Hammock BD. The 2014 Bernard B. Brodie Award Lecture—Epoxide Hydrolases: Drug
58 Metabolism to Therapeutics for Chronic Pain. *Drug Metab Dispos.* 2015;43:788.
- 59 [128] Silvente-Poirot S, Poirot M. Cholesterol epoxide hydrolase and cancer. *Curr Opin Pharmacol.*
60 2012;12:696-703.

- 1 [129] Pavlik EJ, Nelson K, Srinivasan S, Powell DE, Kenady DE, DePriest PD, et al. Resistance to tamoxifen
2 with persisting sensitivity to estrogen: possible mediation by excessive antiestrogen binding site activity. *Cancer*
3 *Res.* 1992;52:4106-12.
- 4 [130] Katzenellenbogen BS, Miller MA, Mullick A, Sheen YY. Antiestrogen action in breast cancer cells:
5 modulation of proliferation and protein synthesis, and interaction with estrogen receptors and additional
6 antiestrogen binding sites. *Breast Cancer Res Treat.* 1985;5:231-43.
- 7 [131] Sheen YY, Simpson DM, Katzenellenbogen BS. An evaluation of the role of antiestrogen-binding sites in
8 mediating the growth modulatory effects of antiestrogens: studies using t-butylphenoxyethyl diethylamine, a
9 compound lacking affinity for the estrogen receptor. *Endocrinology.* 1985;117:561-4.
- 10 [132] Deng T, Liu JC, Pritchard KI, Eisen A, Zacksenhaus E. Preferential killing of breast tumor initiating cells
11 by N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine/tesmilifene. *Clin Cancer Res.* 2009;15:119-30.
- 12 [133] Kedjouar B, Daunes S, Vilner BJ, Bowen WD, Klaebe A, Faye JC, et al. Structural similitudes between
13 cytotoxic antiestrogen-binding site (AEBS) ligands and cytotoxic sigma receptor ligands. Evidence for a
14 relationship between cytotoxicity and affinity for AEBS or sigma-2 receptor but not for sigma-1 receptor.
15 *Biochem Pharmacol.* 1999;58:1927-39.
- 16 [134] Delarue F, Kedjouar B, Mesange F, Bayard F, Faye JC, Poirot M. Modifications of benzylphenoxy
17 ethanamine antiestrogen molecules: influence affinity for antiestrogen binding site (AEBS) and cell cytotoxicity.
18 *Biochem Pharmacol.* 1999;57:657-61.
- 19 [135] Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM. The histone deacetylase
20 inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. *Cancer Res.*
21 2001;61:8492-7.
- 22 [136] Guilbaud NF, Gas N, Dupont MA, Valette A. Effects of differentiation-inducing agents on maturation of
23 human MCF-7 breast cancer cells. *J Cell Physiol.* 1990;145:162-72.
- 24 [137] Bacus SS, Kiguchi K, Chin D, King CR, Huberman E. Differentiation of cultured human breast cancer
25 cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. *Mol Carcinog.* 1990;3:350-
26 62.
- 27 [138] Mandlekar S, Yu R, Tan TH, Kong AN. Activation of caspase-3 and c-Jun NH2-terminal kinase-1
28 signaling pathways in tamoxifen-induced apoptosis of human breast cancer cells. *Cancer Res.* 2000;60:5995-
29 6000.
- 30 [139] Mandlekar S, Hebbar V, Christov K, Kong AN. Pharmacodynamics of tamoxifen and its 4-hydroxy and N-
31 desmethyl metabolites: activation of caspases and induction of apoptosis in rat mammary tumors and in human
32 breast cancer cell lines. *Cancer Res.* 2000;60:6601-6.
- 33 [140] Ferlini C, Scambia G, Marone M, Distefano M, Gaggini C, Ferrandina G, et al. Tamoxifen induces
34 oxidative stress and apoptosis in oestrogen receptor-negative human cancer cell lines. *Br J Cancer.* 1999;79:257-
35 63.
- 36 [141] Gundimeda U, Chen ZH, Gopalakrishna R. Tamoxifen modulates protein kinase C via oxidative stress in
37 estrogen receptor-negative breast cancer cells. *J Biol Chem.* 1996;271:13504-14.
- 38 [142] Peralta EA, Viegas ML, Louis S, Engle DL, Dunnington GL. Effect of vitamin E on tamoxifen-treated
39 breast cancer cells. *Surgery.* 2006;140:607-14; discussion 14-5.
- 40 [143] Khallouki F, de Medina P, Caze-Subra S, Bystricky K, Balaguer P, Poirot M, et al. Molecular and
41 Biochemical Analysis of the Estrogenic and Proliferative Properties of Vitamin E Compounds. *Front Oncol.*
42 2015;5:287.
- 43 [144] Subramani T, Yeap SK, Ho WY, Ho CL, Omar AR, Aziz SA, et al. Vitamin C suppresses cell death in
44 MCF-7 human breast cancer cells induced by tamoxifen. *J Cell Mol Med.* 2014;18:305-13.
- 45 [145] Harmalkar M, Upraity S, Kazi S, Shirsat NV. Tamoxifen-Induced Cell Death of Malignant Glioma Cells Is
46 Brought About by Oxidative-Stress-Mediated Alterations in the Expression of BCL2 Family Members and Is
47 Enhanced on miR-21 Inhibition. *J Mol Neurosci.* 2015;57:197-202.
- 48 [146] Denk F, Ramer LM, Erskine EL, Nassar MA, Bogdanov Y, Signore M, et al. Tamoxifen induces cellular
49 stress in the nervous system by inhibiting cholesterol synthesis. *Acta Neuropathol Commun.* 2015;3:74.
- 50 [147] Song C, Hiiipakka RA, Liao S. Auto-oxidized cholesterol sulfates are antagonistic ligands of liver X
51 receptors: implications for the development and treatment of atherosclerosis. *Steroids.* 2001;66:473-9.
- 52 [148] Cook IT, Duniec-Dmuchowski Z, Kocarek TA, Runge-Morris M, Falany CN. 24-hydroxycholesterol
53 sulfation by human cytosolic sulfotransferases: formation of monosulfates and disulfates, molecular modeling,
54 sulfatase sensitivity, and inhibition of liver x receptor activation. *Drug Metab Dispos.* 2009;37:2069-78.
- 55 [149] Chen W, Chen G, Head DL, Mangelsdorf DJ, Russell DW. Enzymatic reduction of oxysterols impairs
56 LXR signaling in cultured cells and the livers of mice. *Cell Metab.* 2007;5:73-9.
- 57 [150] Wang Z, Yang X, Chen L, Zhi X, Lu H, Ning Y, et al. Upregulation of hydroxysteroid sulfotransferase
58 2B1b promotes hepatic oval cell proliferation by modulating oxysterol-induced LXR activation in a mouse
59 model of liver injury. *Arch Toxicol.* 2017;91:271-87.
- 60
61
62
63
64
65

- 1 [151] Vickman RE, Crist SA, Kerian K, Eberlin L, Cooks RG, Burcham GN, et al. Cholesterol Sulfonation
2 Enzyme, SULT2B1b, Modulates AR and Cell Growth Properties in Prostate Cancer. *Mol Cancer Res.*
3 2016;14:776-86.
- 4 [152] Ren S, Ning Y. Sulfation of 25-hydroxycholesterol regulates lipid metabolism, inflammatory responses,
5 and cell proliferation. *Am J Physiol Endocrinol Metab.* 2014;306:E123-30.
- 6 [153] Zhang X, Bai Q, Xu L, Kakiyama G, Pandak WM, Jr., Zhang Z, et al. Cytosolic sulfotransferase 2B1b
7 promotes hepatocyte proliferation gene expression in vivo and in vitro. *Am J Physiol Gastrointest Liver Physiol.*
8 2012;303:G344-55.
- 9 [154] Zhang X, Bai Q, Kakiyama G, Xu L, Kim JK, Pandak WM, Jr., et al. Cholesterol metabolite, 5-cholesten-
10 3beta-25-diol-3-sulfate, promotes hepatic proliferation in mice. *J Steroid Biochem Mol Biol.* 2012;132:262-70.
- 11 [155] Eberlin LS, Dill AL, Costa AB, Ifa DR, Cheng L, Masterson T, et al. Cholesterol sulfate imaging in human
12 prostate cancer tissue by desorption electrospray ionization mass spectrometry. *Anal Chem.* 2010;82:3430-4.
- 13 [156] He D, Meloche CA, Dumas NA, Frost AR, Falany CN. Different subcellular localization of
14 sulphotransferase 2B1b in human placenta and prostate. *Biochem J.* 2004;379:533-40.
- 15 [157] Lamberson CR, Muchalski H, McDuffee KB, Tallman KA, Xu L, Porter NA. Propagation rate constants
16 for the peroxidation of sterols on the biosynthetic pathway to cholesterol. *Chem Phys Lipids.* 2017.
- 17 [158] Dalenc F, Iuliano L, Filleron T, Zerbinati C, Voisin M, Arellano C, et al. Circulating oxysterol metabolites
18 as potential new surrogate markers in patients with hormone receptor-positive breast cancer: results of the
19 OXYTAM study. *J Steroid Biochem Mol Biol.* 2017;169:210-8.
- 20 [159] Griffiths WJ, Abdel-Khalik J, Yutuc E, Morgan AH, Gilmore I, Hearn T, et al. Cholesterolomics: An
21 update. *Anal Biochem.* 2017;524:56-67.
- 22 [160] Kloudova A, Guengerich F, Soucek P. The Role of Oxysterols in Human Cancer. *Trends Endocrinol*
23 *Metab.* 2017;in press.
- 24 [161] Bovenga F, Sabba C, Moschetta A. Uncoupling nuclear receptor LXR and cholesterol metabolism in
25 cancer. *Cell Metab.* 2015;21:517-26.
- 26 [162] Fessler MB. The Intracellular Cholesterol Landscape: Dynamic Integrator of the Immune Response.
27 *Trends Immunol.* 2016;37:819-30.
- 28 [163] Traversari C, Sozzani S, Steffensen KR, Russo V. LXR-dependent and -independent effects of oxysterols
29 on immunity and tumor growth. *Eur J Immunol.* 2014;44:1896-903.
- 30 [164] Jakobsson T, Treuter E, Gustafsson JA, Steffensen KR. Liver X receptor biology and pharmacology: new
31 pathways, challenges and opportunities. *Trends Pharmacol Sci.* 2012;33:394-404.
- 32 [165] Helyer LK, Chin S, Chui BK, Fitzgerald B, Verma S, Rakovitch E, et al. The use of complementary and
33 alternative medicines among patients with locally advanced breast cancer--a descriptive study. *BMC Cancer.*
34 2006;6:39.
- 35 [166] Mills E, Ernst E, Singh R, Ross C, Wilson K. Health food store recommendations: implications for breast
36 cancer patients. *Breast Cancer Res.* 2003;5:R170-4.
- 37 [167] Cassidy A. Are herbal remedies and dietary supplements safe and effective for breast cancer patients?
38 *Breast Cancer Res.* 2003;5:300-2.
- 39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 1R1
[Click here to download high resolution image](#)

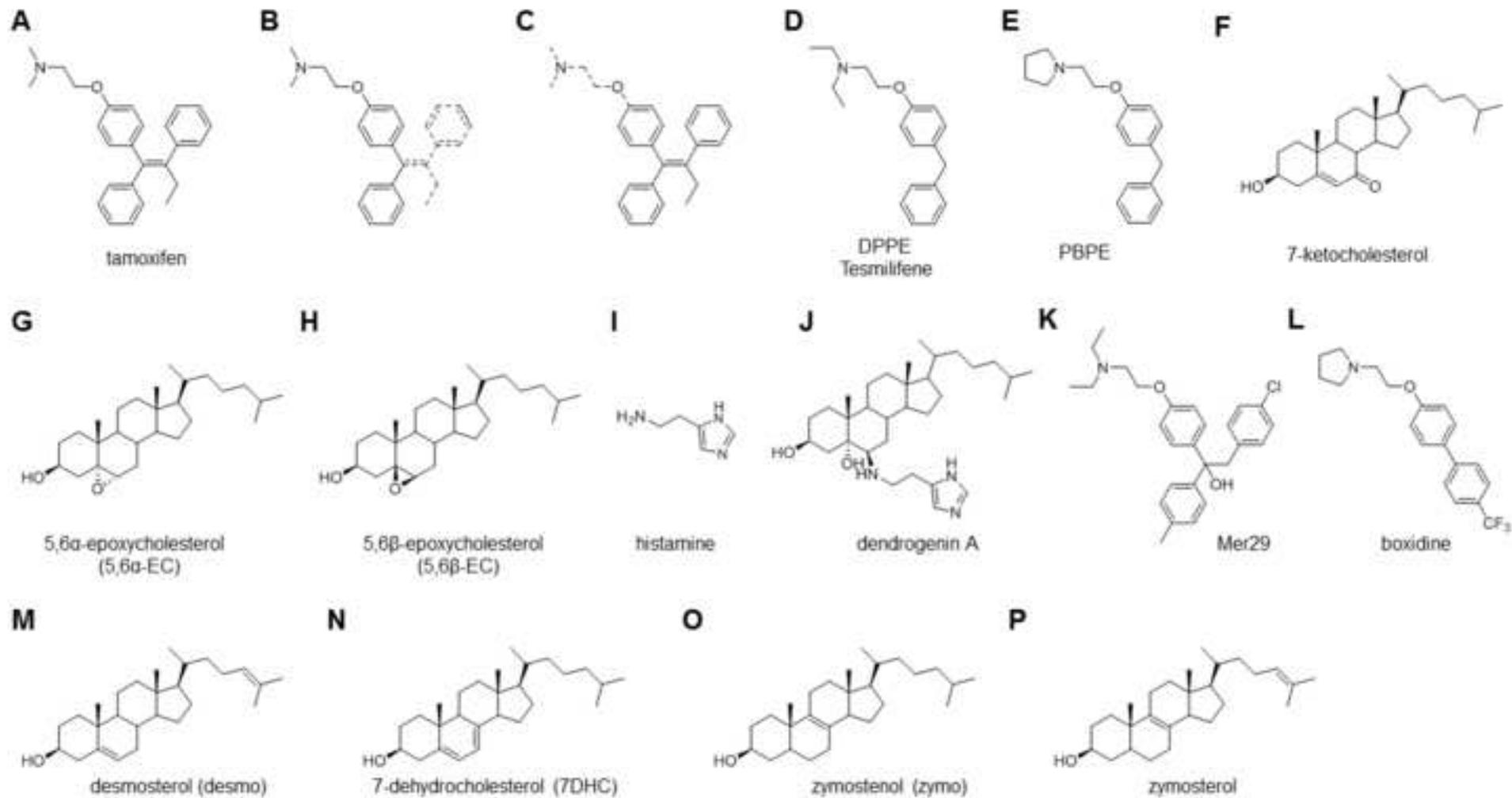


Figure 2R1
[Click here to download high resolution image](#)

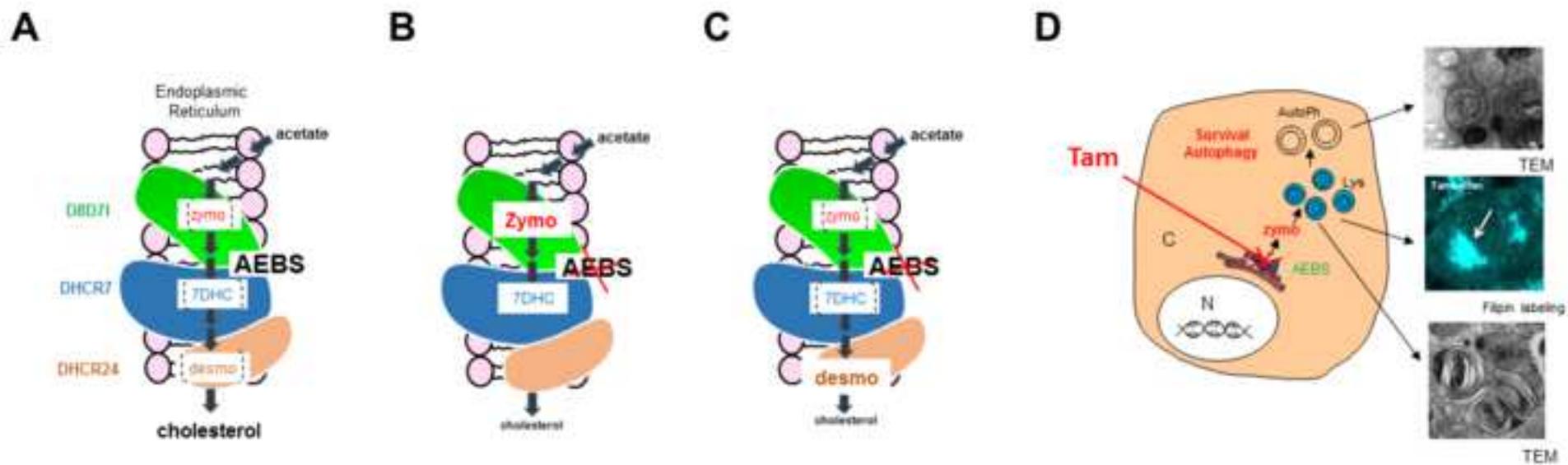


Figure 3R1
[Click here to download high resolution image](#)

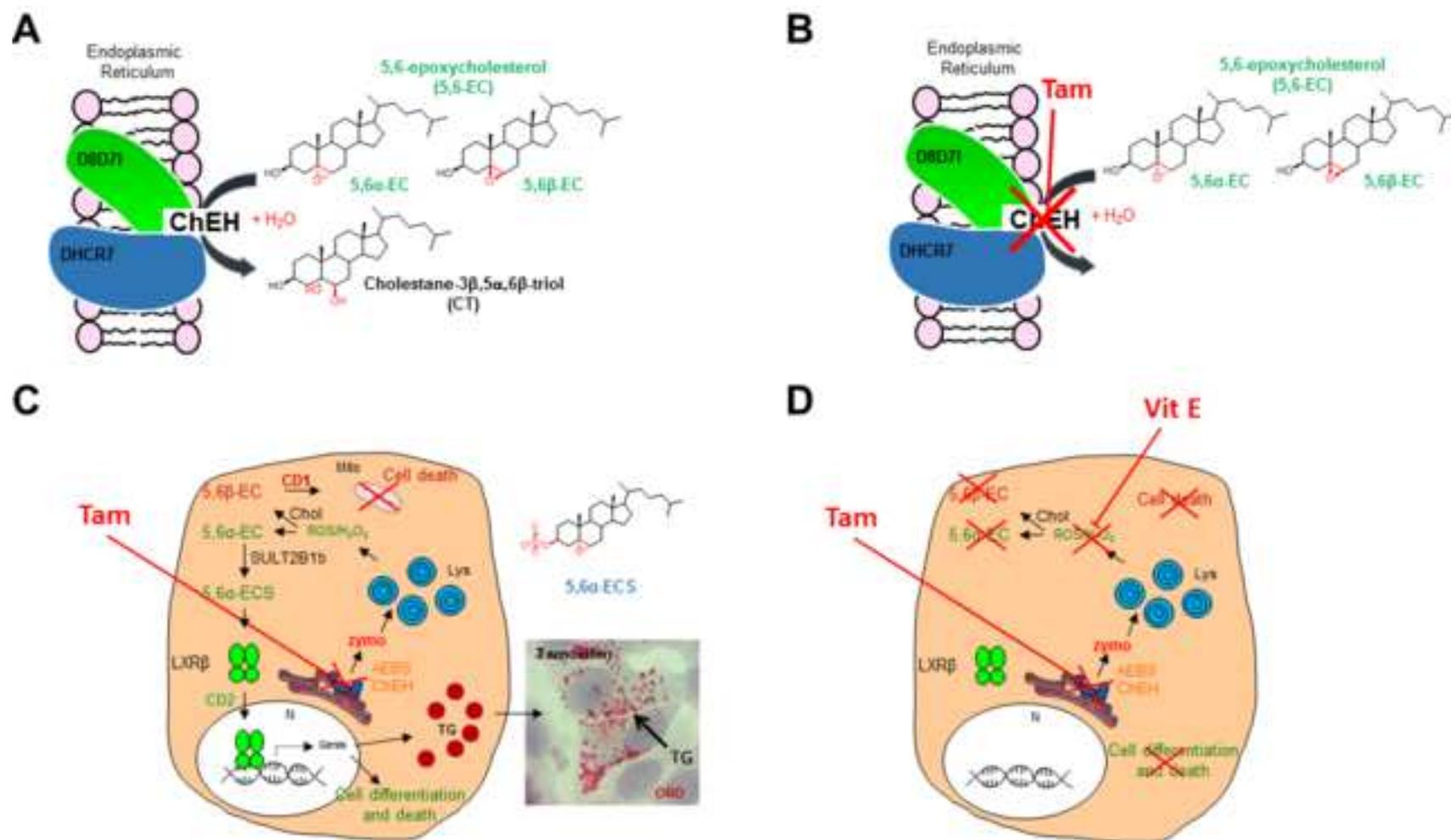


Figure 4R1

[Click here to download high resolution image](#)

