Egypt. J. Chem. **Vol. 66,** No. **SI 13, 1001-1014** (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antitumor Potential and Possible Targets of Phenothiazine Derivatives: A Recent Review

Mona O. Sarhan[a*], Nosaiba A. Elsayed[b], Radwan El-Haggar[b], Wafaa A. Zaghary[b*]

[a] *Labelled Compounds Department, Hot Lab Centre, Egyptian Atomic Energy Authority.*

[b] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, 11795, Cairo, Egypt.*

Abstract

The unique chemical characteristics of the phenothiazine ring system have been a major driving force for the development of various phenothiazine-based compounds with a huge spectrum of industrial and biological significance. Literature is ever continuously growing discussing the different roles and synthetic routes of phenothiazines. A huge body of novel and repurposed phenothiazines have been reported to have antitumor action. Unfortunately, only few reports discuss the proper mechanism of such action. In this review, a comprehensive overview of the identified targets and/or mechanism of action of recently reported antitumor phenothiazine derivatives are reviewed.

Keywords: Phenothiazine, Antitumor, Molecular targets, Hybridization, Repurposing.

Introduction

Cancer is defined as the uncontrolled growth and spread of abnormal cells in any part of the body [1]. In 2020, the worldwide cancer burden is estimated to be 19.3 million new cancer diagnoses and ten million cancer deaths. These statistics are projected to grow to 28.4 million cases by 2040 [2].

Despite the fact that cancer has an infinite number of cell divisions, its pathogenesis is exceedingly complex and linked to various mechanisms [3]. Generally, the hallmarks of cancer consist of fourteen biological capabilities and enabling characteristics during its development. [4]. Upon spotting these hallmarks, the FDA identified 228 cancer therapies or cancer-related medications over the past 31 years [5]. However, chemotherapy usually fails due to acquired resistance, and most clinically useful anti-cancer agents are toxic and costly for patients, thus, a complete cancer cure remains a formidable obstacle for humans [6]. Therefore, the development of novel anti-cancer agents with low toxicity, and cost-efficiency has emerged as an increasingly challenging task for pharmaceutical researchers [7].

Phenothiazine, a thiazine class heterocyclic compound, is one of the most versatile scaffolds in term of biological activity [8]. Rooted in the earliest years of the chemistry of organic dyes, phenothiazines have been utilized in numerous medical fields, particularly psychopharmacology [9]. The anti-psychotic effects of phenothiazines are mostly attributed to their ability to inhibit dopamine receptors. Additionally, a wide variety of pharmacological actions have also been reported, including; antimalarial, antibacterial, antiviral, antioxidant, antiferroptotic agents, anti-inflammatory, anti-Alzheimer, antitubercular, and anti-prion effects [10-34]. Some antipsychotic phenothiazines have also been investigated as prospective cancer therapeutics [35]. For instance, chlorpromazine has been found to have promising activity against endometrial, lung, colorectal, and breast cancer [36-39].

In 2022, Brown summarized in his review the repurposed antipsychotic medications for cancer treatment [35]. On the other hand, different phenothiazine anticancer agents have been designed through molecular hybridization strategy [40,41]. This review attempted to shed light on the reported anticancer phenothiazines derivatives based on their cytotoxic mechanisms. The relevant structure activity relationships were also reviewed to serve as a guide for the researcher in cancer-fighting journey.

*Corresponding author e-mail:. monasarhan@windowslive.com ; (Mona O Sarhan).

Receive Date: 11 March 2023, Revise Date: 30 April 2023, Accept Date: 03 May 2023

DOI: 10.21608/ejchem.2023.199175.7715

1. Aromatase inhibitors

Aromatase (estrogen synthetase) is responsible for the aromatization of androgens in the biosynthesis of estrogens [42]. The pathogenesis and progression of female malignancies are linked to estrogen overproduction and the overexpression of the aromatase enzyme [43]. Thus, aromatase has been proven as an attractive target for estrogen-dependent malignancies [44]. Guided by structure-based lead optimization, a series of phenothiazine derivatives tagged with benzenesulfonamide moieties were designed based on the coumarin lead **1** (**Fig. 1**) [44]. In order to fit the hydrophobic pocket that is present in the enzyme, the new hybrids were designed to maintain the sulfonamide functional group and replacing the coumarin moiety with a phenothiazine one. Compounds **2a-b** (**Fig. 1**) showed potent anticancer potential compared to doxorubicin against T47D cell line with IC_{50} values of 8.1 and 8.8 µM *vs.* 9.8 µM, respectively. Also, **2a-b** showed aromatase inhibitory activity with IC_{50} values of 5.67 mM and 6.7 mM, respectively. Moreover, **2a-b** significantly downregulated the levels of the antiapoptotic proteins Bcl2 and Bcl-XL to an undetectable levels, while significantly upregulating the proapoptotic protein BAX by roughly 55000 times [44]. There is compelling evidence that the ratio of pro- and anti-apoptotic BAX and Bcl-2 proteins can predict the propensity of a cancer cell to undergo apoptosis [45]. It was shown that **2a-b** enhanced the BAX/Bcl2 ratio by almost 108 times proving the proapoptotic impact of both compounds.

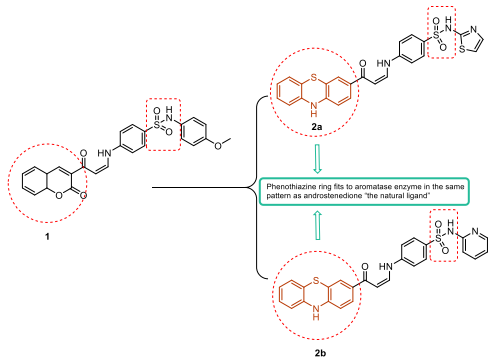


Fig.1. Phenothiazine-sulfonamide conjugates as aromatase inhibitors.

2. Epigenetic modulators

Epigenetics is the regulation of gene activity that doesn't involve alterations to the underlying sequence [46]. Epigenetic modulators are a key target for current cancer therapeutics owing to their crucial role in controlling biological system such as cellular proliferation, survival, and differentiation [46]. Histones are basic proteins associated with DNA condensation into chromatin and play a fundamental role in the regulation of gene expression [47]. Histone deacetylase enzymes (HDACs) regulate histone deacetylation at *N*-terminal lysine residues [48,49]. The HDAC family consists of eighteen different enzymes divided based on their structure into four different groups as discussed briefly in **Table 1** which have been a hot spot for anticancer research.

Table 1: Classification of HDAC family of enzymes.

Class	Enzyme
Class I	HDAC 1,2,3 and 8
Class II	Divided into two subclasses: IIa: HDAC 4, 5, 7 and 9. IIb: HDAC 6 and 10.
Class III (Sir2) (Also known as Sirutins, silence information regulators) [50]	SIRT 1-7
Class IV	HDAC11

2.1. HDAC6 inhibitors

HDAC6 is a promising anti-cancer therapeutic target due to its major contribution in multiple significant cellular processes such as cell motility, migration, the degradation of misfolded proteins, cell proliferation, and death [51]. Furthermore, the overexpression of HDAC6 plays a critical role in the pathogenesis of several cancers such as acute myeloid leukemia, ovarian, and breast cancers. Additionally, the inhibition of HDAC6 triggers apoptosis [52].

Based on the commonly accepted HDAC pharmacophore which includes a cap, a linker, and a zinc-binding group ZBG (**Fig. 2**) [47], Vögerl *et al.* synthesized a novel series of hydroxamate-based HDAC6 inhibitors using the phenothiazine ring as a cap group [53]. The lead compound **3a** demonstrated potent HDAC6 inhibition with IC_{50} of 22 nM (**Fig. 2**). Interestingly, the analysis of structure activity relationship demonstrated that; the addition of a nitrogen atom into the phenothiazine skeleton as observed in (**3b, Fig. 2**) results in significantly improved potency as indexed by IC_{50} value of 5 nM and increased selectivity for HDAC6 over other HDAC isoforms (more than 530- fold) [53].

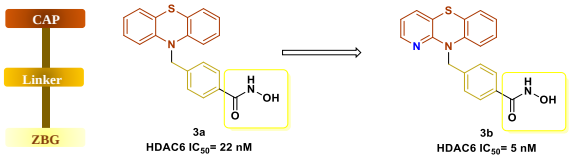


Fig. 2. Phenothiazine-hydroxamic acid derivatives as HDAC inhibitors.

2.2. SIRT1 inhibitors

The histone deacetylase SIRT1 is a NAD^+-dependent multiple function enzyme. It is responsible

for the regulation of apoptosis by deacetylating tumor suppressor proteins p53, which results in the inactivation of these proteins and an antiapoptotic effect [54]. The anticancer action of several SIRT1 inhibitors was mostly linked to p53- hyperacetylation [55]. Zhang and co-workers discovered the phenothiazine derivative inauhzin (**4**, **Fig. 3**), through computational screening of five hundred thousand commercially accessible compounds. *In vitro* studies showed that inauhzin induced hyperacetylation of p53 at a cellular level. Moreover, in a p53-dependent pattern, inauhzin was able to suppress the progression of lung and colon cancer cell line [56]. Similarly, Lee *et al*. investigated the repurposing of CPZ (**5, Fig. 3**) by studying the molecular mechanism underlying its cytotoxicity in colorectal cancer (CRC). P53 wild-type cells (HCT116 and LoVo) and p53 mutant cells (HCT15) were treated with CPZ, and both experienced significant growth suppression and apoptosis induction. It was found to increase the acetylation of Lys382 in p53 and this transcriptional activity was linked to inhibition of SIRT1 [38].

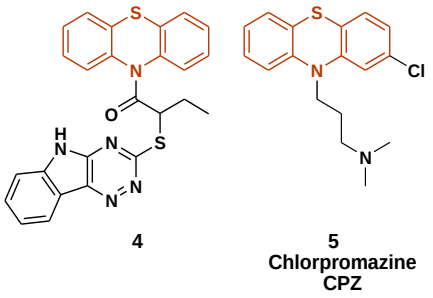


Fig. 3. Phenothiazine derivatives as SIRT1 inhibitors.

3. Tubulin polymerization inhibitor

Microtubules are composed of subunits made from a globular protein known as tubulin. They are responsible for the formation of mitotic spindles and chromosomal separation [57]. Inhibiting the polymerization of tubulin into microtubules results in apoptosis. Hence, tubulin is regarded as an attractive target for cancer chemotherapy [58].

In 2011, Prinz and co-workers synthesized 53 *N*-benzoylated phenothiazine and phenoxazine derivatives as a potential tubulin polymerization inhibitor. Compounds **6a-j** (**Fig. 4**) were examined for their capacity to inhibit cell proliferation, as well as their interaction with tubulin and their effect on the cell cycle. As presented in **Table 2**, the compounds efficiently suppressed tubulin polymerization (ITP) with activities greater than or equal to colchicine. Concentration-dependent flow cytometric studies demonstrated that the growth inhibition of the leukemic cell line (K562) was associated with G2/M arrest, which indicated mitotic blockade [59].

Another two sets of 1,2,3-triazole-phenothiazine hybrids (**7a-i**) and dithiocarbamate-phenothiazine hybrids (**8a-d**) were designed and synthesized by Liu *et al*. through molecular hybridization (**Fig. 4**). Conjugate **7h** showed cytotoxic activity superior to 5-fluorouracil against gastric cancer MGC-803 cells (IC_{50}=1.2 μM) and inhibited tubulin polymerization at a concentration of 2.87 μM. In addition, **7h** inhibited MGC-803 cell migration by regulation of the wnt/*β*-catenin pathway in a dose-dependent fashion. These findings promoted **7h** to be an excellent candidate for further clinical evaluation [58].

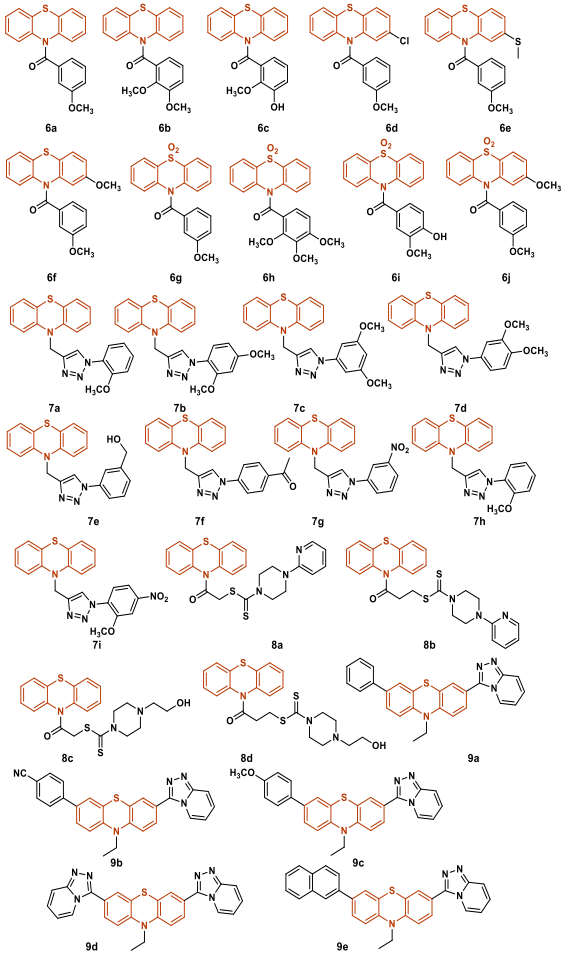


Fig. 4. Phenothiazine derivatives as tubulin polymerization inhibitors.

Table 2

Reported *in vitro* cytotoxic activity of compounds **6a-j** against K562 cell line and their inhibitory activity of tubulin polymerization (ITP).

	K562 IC_{50} (µM)	ITP IC_{50} (µM)
6a	0.33	2.23
6b	1.86	4.96
6c	0.21	1.40
6d	0.84	0.87
6e	3.62	1.43
6f	0.97	3.23
6g	0.28	2.53
6h	2.6	2.46
6i	0.8	1.05
6j	1.58	4.69

Novel phenothiazine bearing a [1,2,4]triazolo[4,3-a]pyridine substitution (**9a-e, Fig. 4**) were reported by Sachdeva and co-workers. The anticancer potential of the synthesized derivatives was investigated against five breast cancer cell lines (MDA-MB-231, MDA-MB-468, MCF7, T47D, and SKBR3). Compound **9a** exhibited the most promising activity with good apoptosis induction in human cancer cells. The docking study of **9a** revealed the presence of hydrophobic interaction between the phenyl ring, triazolopyridine, and phenothiazine moieties with adjacent residues inside the binding pocket of tubulin (**Fig. 5**) [60]. Additionally, one of the triazole ring's nitrogen (N_2) formed a hydrogen bond interaction with Thr276 which is similar to the interaction formed by taxane ring of paclitaxel [60,61]. These findings were further supported by G2/M arrest, indicating the disruption of the microtubule system [60].

In other context, Abuhaie *et al*. designed and synthesized a series of phenstatin (**10**) analogues (**11a-c, Fig. 6**), in which, the trimethoxy phenyl unit of phenstatin rings A and B were replaced by a phenothiazine ring and variable substituted aromatic ring systems, respectively (**Fig. 6**) [62]. Compounds **11a**, **11b,** and **11c** showed 95%, 99%, and 48% ITP with good cytotoxic activity against the full NCI-60 cell panel. Docking studies proposed the possible binding poses of the promising analogues at the colchicine binding site of tubulin. Compound **11a** adopted a conformation similar to phenstatin (**Fig. 6**). On the other hand, the NH_2 substitution in compound **11b** appeared to be beneficial for binding as shown by the formation of hydrogen bonding interactions of the carbonyl group and Val181 and Asn258. Finally, the introduction of the indole ring in compound **11c** limited the rotations around the carbonyl linker and enhancing hydrogen bond formation between Val238 and the carbonyl group (**Fig. 7**) [62].

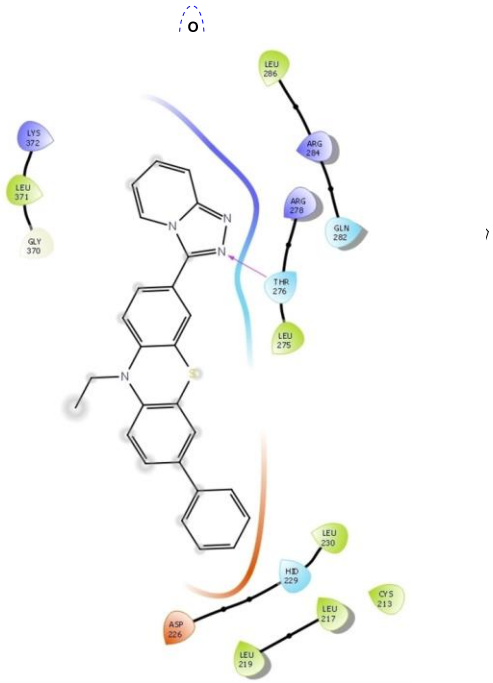


Fig. 5. Docked pose of **9a** in the tubulin binding site as reported by Sachdeva et. al [60].

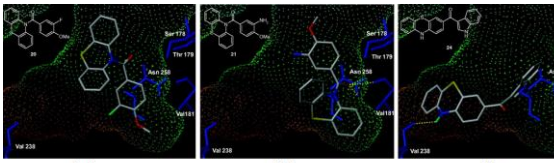


Fig. 7. Docked pose of **11a-c** in the colchicine binding site of tubulin as reported by Abuhaie *et al* [62].

4. Farnesyltransferase inhibitor

Ras protein is a low-molecular-weight GDP/GTP-binding guanine triphosphatase encoded by the Ras gene. It is essential for signal transduction of cell growth, proliferation, development and differentiation [63]. Due to its low hydrophobicity, Ras protein is unable to bind to the cell membrane to exert its effects. Therefore, prior farnesylation (transferring a prenyl unit (C_{15})) by farnesyl transferase (FTase) is essential for its function [64]. Ras mutations have been reported in 30% of cancers [64]. Hence, efficient farnesyl transferase inhibitors (FTIs) to block Ras protein and therefore prevent cancer is a promising research direction. [65]. This quest was pursued by Baciu-Atudosie and colleagues combined the phenothiazine and pyrazole rings to synthesize a series of phenothiazine derivatives (**12a-c, Fig. 8)** [66]. The synthesized compounds were assessed for their potential anti-cancer activity against the NCI-60 cell line panel. The compounds revealed promising FTase

inhibitory actvities ranging from 67.7% to 95.5% (**Table 3**). Compound **12a** emerged as a potent FTase inhibitor with IC_{50} of 3.73 µM in addition to promising *in vitro* cytostatic activity against HCT-116, LOX IMVI, and SK-MEL-5 cell lines. According to the elucidated SAR study, the addition of a carbonyl group immediately after the phenothiazine's nitrogen boosted its activity [66].

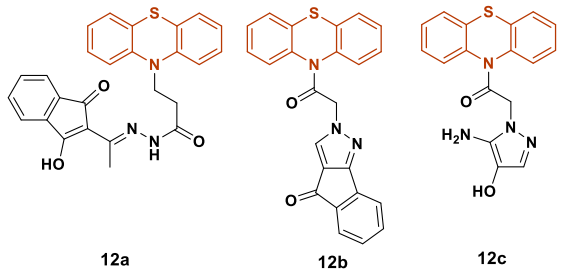


Fig. 8. Phenothiazine based FTIs.

Table 3
In vitro activity of compounds **12a-c** expressed as percent inhibition and determined IC_{50}.

	%Inhibition of FTase	IC50 (µM)
12a	95.5	3.73
12b	67.7	3.35
12c	94.9	17.21

5. Dual inhibitors of tubulin polymerization and farnesyltransferase

Phenothiazine core was observed in reported tubulin polymerization inhibitors such as **13a** (**Fig. 9**) as well as FTase inhibitors (**13b**, **Fig. 9**). Similarly, compounds sharing an indazoline ring displayed tubulin polymerization inhibition (**14a**, **Fig. 9**) as well as FTase inhibition (**14b**, **Fig. 9**).

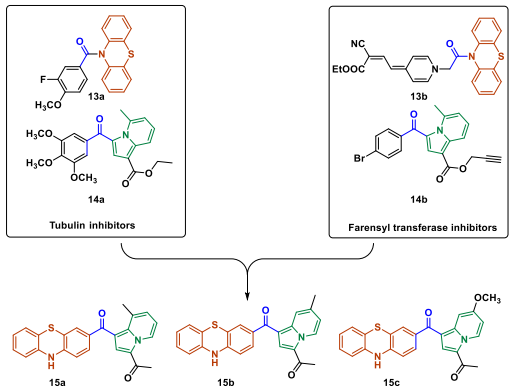


Fig. 9. Phenothiazine- indolizine hybrids as dual inhibitors of tubulin polymerization and FTase.

Accordingly, the hybridization of both scaffolds (indolizine and phenothiazine rings) was used as a rational strategy for the development of dual inhibitors. The cytotoxicity of the designed hybrids has been examined against the NCI-60 cancer cell lines and their inhibitory activities for tubulin polymerization and FTase were tested. Compounds **15a-c** (**Fig. 9**) showed the most promising dual inhibition for both targets as elaborated in **Table 4** [40].

Table 4
In vitro activity of compounds **15a-c** for tubulin polymerization and FTase expressed as percent inhibition and determined IC_{50}.

	%ITP	IC_{50}, µM (Tubulin)	%FTI	IC_{50}, µM (FTase)
15a	84	27.70	89	0.81
15b	77	3.32	96	0.25
15c	100	1.11	86	0.39

6. Protein phosphatase 2A activator

Protein phosphatase 2A (PP2A) is a protein serine/threonine phosphatase that removes phosphate group from these amino acid residues [67]. PP2A controls a variety of cellular processes, such as protein synthesis, cellular signaling, cell cycle, apoptosis, metabolism, and stress responses [68]. It is believed that PP2A is a tumor suppressor and is functionally inactive in cancer. This promoted PP2A activation as a therapeutic strategy for treating malignancies [69]

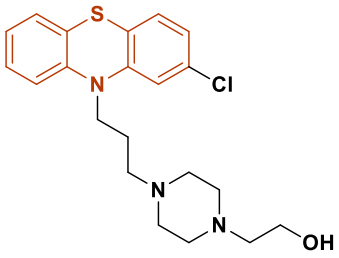
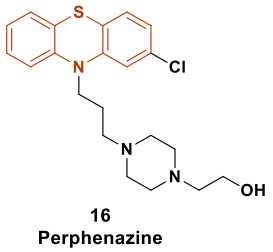


Fig. 10. Chemical structure of perphenazine.

Gutierrez *et al*. conducted a mechanistic study to explain the repeated identification of phenothiazines as anticancer agents. Using ligand-affinity chromatography coupled with mass spectroscopy to quantitatively evaluate drug-protein binding proteome-wide. The anticancer effects of perphenazine **16** (**Fig. 10**) were observed in T-cell acute lymphoblastic leukemia (T-ALL) cells *in vitro* and *in vivo* models. This compound triggered fast dephosphorylation of many PP2A sites that have been linked to the growth and survival of cancer [70]. Based on these findings, pharmacologic PP2A activation in T-ALL and other

malignancies that are mediated by hyperphosphorylated PP2A substrates may have positive implications for clinical practice.



16
Perphenazine

Fig. 10. Chemical structure of perphenazine.

7. Bcr-Abl kinase inhibitors

Abelson tyrosine kinase (c-Abl) is a non-receptor tyrosine kinase (TK) involved in cell development and proliferation and is often tightly regulated [71]. Nonetheless, 95% of chronic myeloid leukemia (CML) patients have a reciprocal translocation between the Abl gene from chromosome 9 and the breakpoint cluster (Bcr) gene from chromosome 22, resulting in an extra-long chromosome 9 and the Philadelphia chromosome containing the fused Bcr-Abl gene [72]. Philadelphia chromosome is the hallmark of CML and is responsible for producing Bcr-Abl kinase, a constitutively active TK that induces uncontrolled cellular proliferation [73]. The activity of the Bcr-Abl kinase involves the binding of ATP and the transfer of phosphate from ATP to tyrosine residues on a variety of substrates. This activity leads to an abnormally high rate of leukemic cell proliferation in CML and Ph+ ALL [74,75]. Bcr-Abl TK inhibitor (e.g., imatinib) blocks the binding of ATP to the Bcr-Abl TK; consequently, the subsequent cellular events are abrogated [73-75]. Since threonine 315 (T315) is required for hydrogen bonding between imatinib and Abl, a point mutation of T315 to isoleucine (T315I) disrupts this interaction [76]. To overcome this resistance, Subramanian *et al*. synthesized a novel series of phenothiazine-thiosemicarbazone, thiadiazole, and thiazolidinoyl hybrids as a Bcr-Abl TK inhibitors. Molecular docking simulation studies of the example compounds **17a-c** (**Fig. 11**) elucidated good fitting to T5131 Bcr-Abl kinase [77].

As demonstrated by docking studies, the phenothiazine moiety of **17a** fitted into the hydrophobic pocket containing Leu248, Ala269, Leu370, and Ile315 (**Fig. 12A**). Meanwhile, the NH group of the phenothiazine ring established a hydrogen bond with Met318. Furthermore, the phenothiazine moiety of **17b** was well placed inside the hydrophobic region containing Lys271, Ala269, Val256, and Leu248. Also, it formed four hydrogen bonds involving thiadiazole ring and the phenothiazine rings with different amino acids as presented in **Fig. 12B**. Finally, the phenothiazine ring of **17c** rested in the hydrophobic region lined with Ala269, Lys248, Leu370, Lys271, Ile315, and Ala380 (**Fig. 12C**). Trypan blue, MTT, and LDH assays were used to evaluate the anticancer efficacy of the novel hybrids against the leukemic K562 cell line [77].

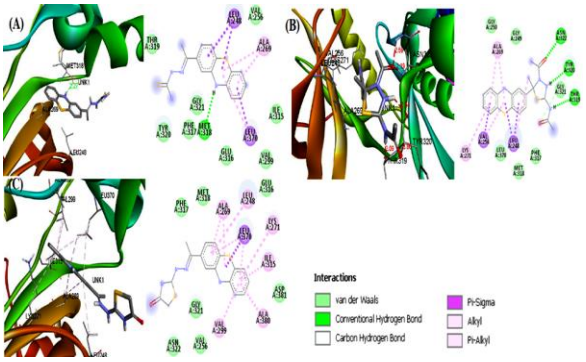
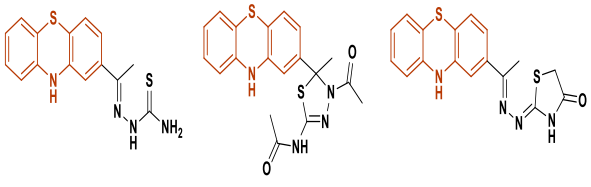


Fig. 12. Docked poses of different phenothiazine hybrids in the binding site of T5131 Bcr-Abl mutant kinase; (A) docking pose of **17a**; (B) docking pose of **17b**; (C) docking pose of **17c** (Adopted from reference [78]).

Additionally, several phenothiazine-based chalcones coupled with *N*-substituted rhodanines demonstrated potent antiproliferative action against the K526 cell line. Example compounds **18a-h** (**Fig. 13**) showed the highest antiproliferative activity and promising interaction with the active site of Bcr-Abl (T315I). The authors reported the predicted structural requirement after analyzing the results of the docking study as summarized in **Fig. 14** which concluded that a substitution at position 3 of the rhodamine ring (*N*-substitution) with amino acid moieties has an observed effect on affinity. Also, the NH of the phenothiazine ring system and the *N*-substituted groups in the rhodanine nucleus were involved in the formation of important hydrogen bond interactions [78].



15a 15b 15c

Fig. 11. Chemical structures of various phenothiazine derivatives designed as T5131 Bcr-Abl mutant kinase inhibitors.

8. Angiogenesis inhibitors

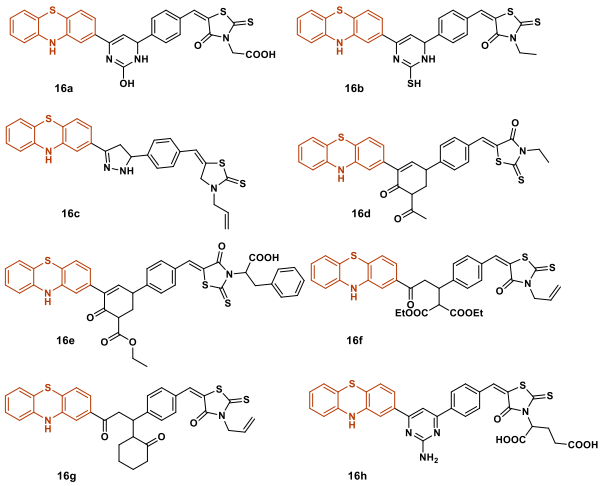


Fig. 13. Phenothiazine-chalcone-rhodanine conjugates with predicted Bcr-Abl (T5131) inhibitory activity.

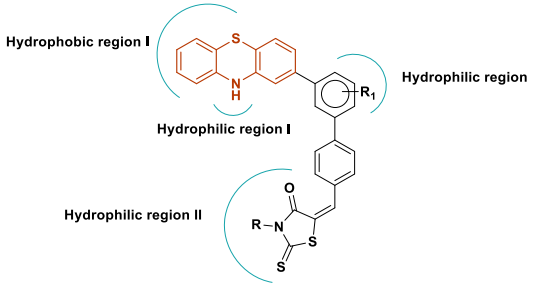


Fig. 14. Structural requirements of phenothiazine-chalcone-rhodanine hybrids for Bcr-Abl (T5131) inhibitory activity (Adopted from reference [78]).

Angiogenesis is the creation of new capillaries from existing vasculature [79]. This process is crucial in physiological (wound healing and the menstruation cycle) and pathological conditions (psoriasis, diabetic retinopathy, and cancer) [80]. In order to sustain high blood supply consistent with the high metabolic demands in cancer cells, VEGF, HIF1α, and related proangiogenic factors are highly expressed. These factors impact the endothelial cells, resulting in the formation of new blood vessels [81,82]. Park *et al* investigated the anti-angiogenic potential of the antipsychotic drug thioridazine (**19, Fig. 15**) against ovarian carcinoma in mouse xenografts [83]. Thioridazine showed anti-angiogenic activity mediated by suppressing PI3K/mTOR signaling pathway and decreasing VEGFR-2 phosphorylation. These findings provide solid evidence that thioridazine influences the activity of endothelial cells and consequent angiogenesis [83-85].

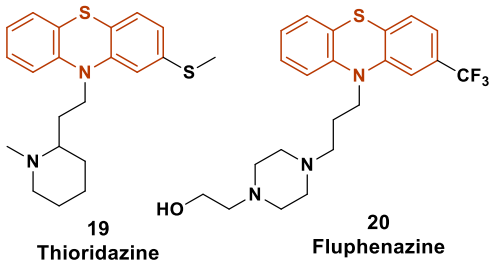


Fig. 15. Chemical structure of thioridazine and fluphenazine.

9. AKT/mTOR pathway inhibitor

The PI3K-AKT-mTOR pathway is a vital intracellular signaling pathway for cell cycle control [86]. PI3K phosphorylation stimulates AKT, which controls multiple downstream targets, including mTOR. Under physiological conditions, this pathway regulates glucose metabolism, cell proliferation, growth, size, and motility [87]. In many malignancies, oncogenic activation of the PI3K–Akt pathway supporting the anabolic demands of the abnormally growing cells [88]. The anti-cancer activity of CPZ (**5, Fig. 3**) has been established against human oral cancer cells (OSCC) and glioma cell line through repurposing strategy [89-90]. Shin *et al*. investigated the signal pathway responsible for CPZ-induced cytotoxicity in the U-87MG glioma cell line. CPZ caused a marked decrease of the phosphorylated AKT (Ser473) level and its downstream effectors GSK3 (Ser9) and mTOR (Thr2481) in a time-dependent pattern. The phosphorylation of p70S6K (Thr389), a mTOR downstream effector, was also reduced. The mentioned findings show that CPZ inhibits mTOR by inhibiting Akt [90]. In the same vein, OSCC cells treated with CPZ showed decreased levels of Akt and mTOR phosphorylation [89].

10. Lysosomal acid sphingomyelinase inhibitor

Sphingomyelin (SM) is an integral part of mammalian cell's plasma membrane . Sphingomyelinase (SMase) hydrolyzes the phosphodiester bond in SM, producing phosphocholine and ceramide (Cer) [91]. SMases are divided into three primary subtypes based on the ideal pH for their function activity: acid, neutral, and alkaline [92]. Acid SMase (ASM) and neutral SMase (NSM) are the most important contributors to Cer production caused by stress [93]. Inhibition of acid SMase results in hyperactivation of hypoxia stress-response pathways and that hypoxia-specific cell death is mediated by the stress-responsive transcription factor ATF4 [94]. Additionally, SM accumulation destabilizes the membrane integrity of lysosomes, thus, low SM levels is critical for protecting cancer cell lysosomes

against increased proteolytic activity [95]. Klutzny *et al*. revealed the molecular basis behind the hypoxia-selective cytotoxicity of fluphenazine (**20, Fig. 15**) using HCT116 tumor spheroid as a screening system. Fluphenazine exhibited an acid SMase inhibitory potential, resulting in cellular SM accumulation and induced cancer cell death caused by increased the pro-apoptotic signal of cellular stress-response pathways in the studied hypoxic tumor spheroids [94].

11. Cyclin dependent kinase inhibitors

The dysregulation of the cell cycle, which results in abnormal cell proliferation, is one of the key hallmarks of cancer [96]. Cyclin-dependent kinases (CDKs), a family of proteins involved in cell cycle regulation, are commonly overexpressed or mutated in cancer [97]. Therefore, drugs that target CDK have been developed and tested as potential cancer drugs [98]. Cyclin D, cyclin E, and their specific interacted CDKs are crucial regulators in the G1 phase [99]. The over expression of cyclin D and cyclin E is associated with oncogenesis and poor prognosis [100,101].

Fu *et al*. reported different phenothiazine derivatives comprising dithiocarbamate moiety with a piperazine ring as cell cycle blockers (**21a-g, Fig. 16**). The preliminary evaluation of the novel hybrids antiproliferative properties against EC-109, MGC-803, and PC-3 revealed a promising inhibitory activity. The highest inhibitory action was observed for compound **21a** (IC_{50} = 11.59 µM) when tested on PC-3 cells. Furthermore, **21a** arrested the cell cycle at G1 phase as indicated by cyclin D1 and CDK4 downregulation. The SAR of **21a-g** revealed that; the ethyl group on the piperazine ring is vital for CDK inhibition. Replacing the ethyl group with methyl, acetyl, pyridine, or a phenyl ring significantly decreases CDK inhibitory activity. Extending the carbon linker between the phenothiazine scaffold and the dithiocarbamate scaffold by one or two carbons reduced the antiproliferative efficacy [102].

Based on the accumulating evidence of the anticancer potential of trifluoperazine (**22, Fig. 16)** [103-106]**,** along with its ability to penetrate the blood-brain barrier (BBB), Feng *et al*. investigated the cytotoxic effects and the underlying molecular mechanisms of trifluoperazine against triple-negative breast cancer (TNBC) with brain metastasis using *in vitro* and *in vivo* models. The results indicate that TFP decreased the expression of cyclin D1, cyclin E, and their associated CDKs, including CDK4 and CDK2, in 4T1, MDA-MB-468, and MDA-MB-231 TNBC cell lines, causing G0/G1 cell cycle arrest. Additionally, trifluoperazine inhibited the growth of subcutaneous xenograft tumor and brain metastasis with no apparent adverse effects. Notably, **22** prolonged the survival of mice with brain metastasis [107]. Similarly, trifluoperazine's impact and molecular mechanism on CRC was investigated by Xia *et al*. Trifluoperazine suppressed the proliferation of CRC cells *in vitro* by downregulating CDK2, CDK4, cyclin D1, and cyclin E, resulting in a G0/G1 cell cycle arrest [108]. Congruent with this, Xu *et al*. addressed the repurposing CPZ (**5**) for CRC and pulmonary metastasis. CPZ successfully suppressed CRC by arresting the G2/M cell cycle which was attributed to reduced cdc2/cyclin B1 complex activity, decreased expression of cyclin B1, cdc2, cdc25c, and increased expression of phosphorylated cdc2 [109].

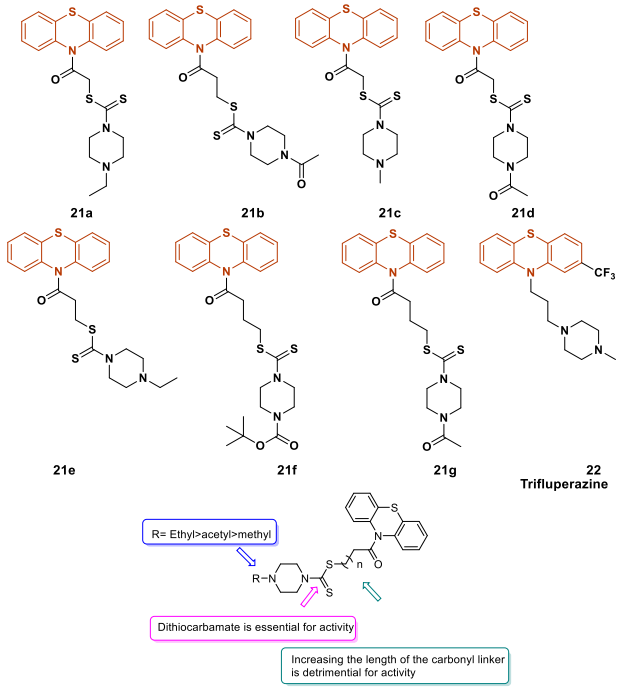


Fig. 16. Different phenothiazine derivatives as CDK inhibitors.

12. Estrogen Receptor *α* down modulator

Estrogen receptor (ER) is essential for the development of ER^+ breast cancer, which accounts for about 70% of all breast cancers [110]. Estrogen contributes to the progression of breast cancer by activating the PI3K/AKT signaling pathway [111]. ER^+ breast cancers are treated with endocrine therapy (ET) such as 4-hydroxy-tamoxifen (Tam) which inhibits ER signaling [112]. Unfortunately, significant number of women who have been treated with Tam suffered relapses with metastatic cancer that is resistant to Tam. Only fulvestrant, which is considered a second-line treatment ET, may be used on these patients [113]. Due to its low oral bioavailability and undesirable side effects, new medicines promoting ER degradation in Tam-resistant metastatic breast cancer are urgently required. Recently, Busonero *et al*. introduced the

selective modulation of ERα levels and degradation as a novel approach for Tam-resistant metastatic cancers. Thioridazine (**19, Fig. 15**) was identified through *in silico* screening of a library of sixty thousand compounds annotated in the iLINCS database as an ERα down modulators and as anti-proliferative agents. The impact of thioridazine on ERα levels and proliferation was validated in Tam-resistant MCF-7 cells and genome-edited Y537S ERα MCF-7 (Y537S-MCF-7) cells. The results revealed that 24h of thioridazine administration reduced intracellular ERα content by more than 90% and cause G1 cell cycle arrest in both cell lines [114].

13. NADPH Oxidase-1 inhibitors

NADPH oxidases (NOXs) comprise a family of enzymes that regulate the generation of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide. The NOX family is comprised of seven enzymes: five NOX isoforms (NOX1-5) and two dual oxidases (Duox1 and Duox2). NOX-derived ROS has many physiological functions starting with cellular division, differentiation, migration to angiogenesis and the synthesis of thyroid hormones [115]. In cancer, NOXs display key regulatory effects in tumorigenesis and angiogenesis through the promotion of oxidative stress and regulating various signaling pathways within the cell such as NRf2, p38 MAPK, JAK-STAT, PI3K, and RAS-RAF-MEK-ERK [116,117]. NOX isoforms showed high and specified expression in the panels of human cell lines. Some forms of CRC, gastric cancers, and adenocarcinomas show high expression of NOX1 [118-120], while high expression of NOX4 is observed in ovarian cancer, glioblastoma, melanoma, and renal carcinoma[121]. Hence, NOXs emerged as a potential target for cancer treatment and other ailments affected by NOXs dysfunction such as inflammation, cardiovascular diseases, and autoimmune diseases [122]. The effect of different NOX inhibitors as beneficial treatment for colon cancer, stomach cancer, and lung cancer was reported in references [123-124]. 2-Acetylphenothiazine (**23a, Fig. 17**) commonly known as ML171, is a probe chemical compound that was introduced by Scripps Research Institute during a HTS of 16000 compounds for new NOX1 inhibitors, while compounds **23b-d** were revealed during the context of the aforementioned study [125,126]. According to data deposited at PubChem (Assay ID: **AID2664** and **AID2808**) the reported compounds possessed IC_{50} ranging from 0.305-0.56 µM [125,126].

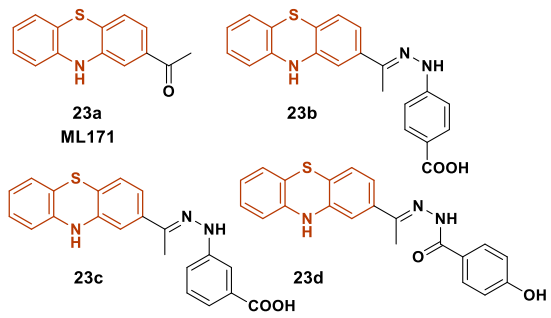


Fig. 17. PTZ derivatives reported as NOX1 inhibitors by Scripps institute.

Conclusion

Phenothiazine based derivatives showed great promise as anti-cancer therapeutics. However, specific molecular target(s) and mechanism of action of phenothiazine anticancer agents is rarely reported. In this review, we focused on reporting the multiple targets and mechanism of actions of recently developed phenothiazine antitumor agents in a trial to highlight the necessity of detailed mechanistic studies of such agents.

Conflict of interest

There are no conflicts to declare.

References

[1] R. A. Weinberg, "How cancer arises," *Sci. Am.*, vol. 275, no. 3, pp. 62–70, 1996.

[2] H. Sung *et al.*, "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, 2021, doi: 10.3322/caac.21660.

[3] X. Meng *et al.*, "A new hypothesis for the cancer mechanism," *Cancer Metastasis Rev.*, vol. 31, no. 1, pp. 247–268, 2012, doi: 10.1007/s10555-011-9342-8.

[4] D. Hanahan, "Hallmarks of Cancer: New Dimensions," *Cancer Discov.*, vol. 12, no. 1, pp. 31–46, 2022, doi: 10.1158/2159-8290.CD-21-1059.

[5] Q. Wu *et al.*, "Small-molecule inhibitors, immune checkpoint inhibitors, and more: FDA-approved novel therapeutic drugs for solid tumors from 1991 to 2021," *J. Hematol. Oncol.*, vol. 15, no. 1, p. 143, 2022, doi: 10.1186/s13045-022-01362-9.

[6] K. O. Alfarouk *et al.*, "Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp," *Cancer Cell Int.*, vol. 15, no. 1, p. 71, 2015, doi: 10.1186/s12935-015-0221-1.

[7] W. N. Hait, "Anticancer drug development: the grand challenges," *Nat. Rev. Drug Discov.*, vol. 9,

no. 4, pp. 253–254, 2010.
[8] A. Jaszczyszyn *et al.*, "Chemical structure of phenothiazines and their biological activity," *Pharmacol. Reports*, vol. 64, no. 1, pp. 16–23, 2012, doi: 10.1016/S1734-1140(12)70726-0.
[9] B. Varga *et al.*, "Possible biological and clinical applications of phenothiazines," *Anticancer Res.*, vol. 37, no. 11, pp. 5983–5993, 2017, doi: 10.21873/anticanres.12045.
[10] J. N. Domínguez *et al.*, "Synthesis and antimalarial effects of phenothiazine inhibitors of a Plasmodium falciparum cysteine protease," *J. Med. Chem.*, vol. 40, no. 17, pp. 2726–2732, 1997.
[11] L. Amaral *et al.*, "Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries," *Trop. Med. Int. Heal.*, vol. 6, no. 12, pp. 1016–1022, 2001.
[12] J. Liu *et al.*, "Antiferroptotic activity of phenothiazine analogues: A novel therapeutic strategy for oxidative stress related disease," *ACS Med. Chem. Lett.*, vol. 11, no. 11, pp. 2165–2173, 2020.
[13] D. Kumar *et al.*, "Synthesis of newer substituted thiadiazolyl and pyrazolyl phenothiazines as potent anti-inflammatory agents," *Orient. J. Chem.*, vol. 26, no. 2, p. 497, 2010.
[14] S. Maddila *et al.*, "Synthesis and anti-inflammatory activity of fused 1, 2, 4-triazolo-[3, 4-b][1, 3, 4] thiadiazole derivatives of phenothiazine," *Lett. Drug Des. Discov.*, vol. 10, no. 10, pp. 977–983, 2013.
[15] S. Sharma *et al.*, "Synthesis and anti-inflammatory activity of some heterocyclic derivatives of phenothiazine," *Die Pharm. Int. J. Pharm. Sci.*, vol. 60, no. 1, pp. 18–22, 2005.
[16] A. Kumar *et al.*, "Synthesis and anti-inflammatory activity of some potential cyclic phenothiazines.," *Boll. Chim. Farm.*, vol. 137, no. 5, pp. 152–156, 1998.
[17] K. L. Forrestall *et al.*, "Phenothiazines as dual inhibitors of SARS-CoV-2 main protease and COVID-19 inflammation," *Can. J. Chem.*, vol. 99, no. 10, pp. 801–811, 2021.
[18] X.-X. Wang *et al.*, "Synthesis and biological evaluation of selective histone deacetylase 6 inhibitors as multifunctional agents against Alzheimer's disease," *Eur. J. Med. Chem.*, vol. 225, p. 113821, 2021.
[19] S. Schwarthoff *et al.*, "Evaluation of γ-carboline-phenothiazine conjugates as simultaneous NMDA receptor blockers and cholinesterase inhibitors," *Bioorg. Med. Chem.*, vol. 46, p. 116355, 2021.
[20] R. I. VOIDING, "Phenothiazines to Treat Alzheimer's Disease," *Innov. Aging*, vol. 5, no. S1, p. 639, 2021.
[21] C. Yamali *et al.*, "Phenothiazine-based chalcones as potential dual-target inhibitors toward cholinesterases (AChE, BuChE) and monoamine oxidases (MAO-A, MAO-B)," *J. Heterocycl. Chem.*, vol. 58, no. 1, pp. 161–171, 2021.
[22] M. Kalkanidis *et al.*, "Novel phenothiazine antimalarials: synthesis, antimalarial activity, and inhibition of the formation of β-haematin," *Biochem. Pharmacol.*, vol. 63, no. 5, pp. 833–842, 2002.
[23] L. Gorecki *et al.*, "Phenothiazine-Tacrine Heterodimers: Pursuing Multitarget Directed Approach in Alzheimer's Disease," *ACS Chem. Neurosci.*, vol. 12, no. 9, pp. 1698–1715, 2021.
[24] S. Sharma and A. Singh, "Phenothiazines as anti-tubercular agents: mechanistic insights and clinical implications," *Expert Opin. Investig. Drugs*, vol. 20, no. 12, pp. 1665–1676, 2011.
[25] P. B. Madrid *et al.*, "Synthesis and antitubercular activity of phenothiazines with reduced binding to dopamine and serotonin receptors," vol. 17, pp. 3014–3017, 2007, doi: 10.1016/j.bmcl.2007.03.064.
[26] A. J. Warman *et al.*, "Antitubercular pharmacodynamics of phenothiazines," *J. Antimicrob. Chemother.*, vol. 68, no. 4, pp. 869–880, 2013.
[27] L. Zaccagnini *et al.*, "In silico/in vitro screening and hit evaluation identified new phenothiazine anti-prion derivatives," *Eur. J. Med. Chem.*, vol. 196, p. 112295, 2020.
[28] K. Venkatesan *et al.*, "Ultrasound-mediated synthesis of phenothiazine derivatives and their in vitro antibacterial and antioxidant studies," *Res. Chem. Intermed.*, vol. 41, no. 2, pp. 595–607, 2015, doi: 10.1007/s11164-013-1213-1.
[29] M. Otręba, *et al.*, "Antiviral activity of chlorpromazine, fluphenazine, perphenazine, prochlorperazine, and thioridazine towards RNA-viruses. A review," *Eur. J. Pharmacol.*, vol. 887, p. 173553, 2020.
[30] J. Lu *et al.*, "Screened antipsychotic drugs inhibit SARS-CoV-2 binding with ACE2 in vitro.," *Life Sci.*, vol. 266, p. 118889, Feb. 2021, doi: 10.1016/j.lfs.2020.118889.
[31] M. Plaze *et al.*, "Repurposing chlorpromazine to treat COVID-19: The reCoVery study.," *Encephale.*, vol. 46, no. 3, pp. 169–172, Jun. 2020, doi: 10.1016/j.encep.2020.05.006.
[32] R. Machado-Vieira *et al.*, "Convergent evidence for the antiviral effects of several FDA-approved phenothiazine antipsychotics against SARS-CoV-2 and other coronaviruses.," *Revista brasileira de psiquiatria (Sao Paulo, Brazil : 1999)*, vol. 43, no. 5. pp. 462–464, 2021, doi: 10.1590/1516-4446-

2020-0024.
[33] A. M. Chamoun-Emanuelli, *et al.*, "Phenothiazines inhibit hepatitis C virus entry, likely by increasing the fluidity of cholesterol-rich membranes," *Antimicrob. Agents Chemother.*, vol. 57, no. 6, pp. 2571–2581, 2013, doi: 10.1128/AAC.02593-12.
[34] R. G. Keynes *et al.*, "N10-carbonyl-substituted phenothiazines inhibiting lipid peroxidation and associated nitric oxide consumption powerfully protect brain tissue against oxidative stress," *Chem. Biol. Drug Des.*, vol. 94, no. 3, pp. 1680–1693, 2019.
[35] J. S. Brown, "Treatment of cancer with antipsychotic medications : Pushing the boundaries of schizophrenia and cancer ☆," *Neurosci. Biobehav. Rev.*, vol. 141, no. May 2021, p. 104809, 2022, doi: 10.1016/j.neubiorev.2022.104809.
[36] Y. Cui *et al.*, "Chlorpromazine Sensitizes Progestin-Resistant Endometrial Cancer Cells to MPA by Upregulating PRB," *Front. Oncol.*, vol. 11, p. 665832, 2021.
[37] R. Fujiwara *et al.*, "Chlorpromazine cooperatively induces apoptosis with tyrosine kinase inhibitors in EGFR-mutated lung cancer cell lines and restores the sensitivity to gefitinib in T790M-harboring resistant cells," *Biochem. Biophys. Res. Commun.*, vol. 626, pp. 156–166, 2022.
[38] W. Y. Lee *et al.*, "Repositioning antipsychotic chlorpromazine for treating colorectal cancer by inhibiting sirtuin 1," *Oncotarget*, vol. 6, no. 29, pp. 27580–27595, 2015, doi: 10.18632/oncotarget.4768.
[39] C.-E. Yang *et al.*, "The antipsychotic chlorpromazine suppresses YAP signaling, stemness properties, and drug resistance in breast cancer cells," *Chem. Biol. Interact.*, vol. 302, pp. 28–35, 2019.
[40] I. M. Moise *et al.*, "Indolizine-phenothiazine hybrids as the first dual inhibitors of tubulin polymerization and farnesyltransferase with synergistic antitumor activity," *Bioorg. Chem.*, vol. 103, no. 11, p. 104184, 2020, doi: 10.1016/j.bioorg.2020.104184.
[41] R. Reddyrajula *et al.*, "Molecular hybridization approach for phenothiazine incorporated 1,2,3-triazole hybrids as promising antimicrobial agents: Design, synthesis, molecular docking and in silico ADME studies," *Eur. J. Med. Chem.*, vol. 168, pp. 263–282, 2019, doi: 10.1016/j.ejmech.2019.02.010.
[42] P. R. Manna *et al.*, "Dysregulation of Aromatase in Breast, Endometrial, and Ovarian Cancers: An Overview of Therapeutic Strategies," in *Progress in Molecular Biology and Translational Science*, 1st ed., vol. 144, K. B. T.-P. in M. B. and T. S. Pruitt, Ed. Academic Press, 2016, pp. 487–537.
[43] D. Molehin *et al.*, "Regulation of aromatase in cancer," *Mol. Cell. Biochem.*, vol. 476, no. 6, pp. 2449–2464, 2021.
[44] M. M. Ghorab *et al.*, "Aromatase inhibitors and apoptotic inducers: Design, synthesis, anticancer activity and molecular modeling studies of novel phenothiazine derivatives carrying sulfonamide moiety as hybrid molecules," *Eur. J. Med. Chem.*, vol. 134, pp. 304–315, 2017, doi: 10.1016/j.ejmech.2017.04.028.
[45] S. Salakou *et al.*, "Increased Bax/Bcl-2 ratio up-regulates caspase-3 and increases apoptosis in the thymus of patients with myasthenia gravis," *In Vivo (Brooklyn).*, vol. 21, no. 1, pp. 123–132, 2007.
[46] J. Gräff, *et al.*, "Epigenetic Regulation of Gene Expression in Physiological and Pathological Brain Processes," *Physiol. Rev.*, vol. 91, no. 2, pp. 603–649, 2011, doi: 10.1152/physrev.00012.2010.
[47] N. Chessum *et al.*, *Recent advances in cancer therapeutics*, 1st ed., vol. 54. Elsevier B.V., 2015.
[48] M. Di Martile *et al.*, "The multifaceted role of lysine acetylation in cancer: prognostic biomarker and therapeutic target," *Oncotarget*, vol. 7, no. 34, p. 55789, 2016.
[49] M. E. Neganova *et al.*, "SIRT1 and p53, effect on cancer, senescence and beyond," in *Seminars in Cancer Biology*, 2022, vol. 83, pp. 452–471.
[50] T. Liu *et al.*, "The critical role of the class III histone deacetylase SIRT1 in cancer," *Cancer Res.*, vol. 69, no. 5, pp. 1702–1705, 2009.
[51] Y. Li *et al.*,"Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes," *FEBS J.*, vol. 280, no. 3, pp. 775–793, 2013.
[52] G. I. Aldana-Masangkay and K. M. Sakamoto, "The role of HDAC6 in cancer," *J. Biomed. Biotechnol.*, vol. 2011, 2010.
[53] K. Vögerl *et al.*, "Synthesis and Biological Investigation of Phenothiazine-Based Benzhydroxamic Acids as Selective Histone Deacetylase 6 Inhibitors," *J. Med. Chem.*, vol. 62, no. 3, pp. 1138–1166, 2019, doi: 10.1021/acs.jmedchem.8b01090.
[54] M. Schiedel *et al.*, "The Current State of NAD+ - Dependent Histone Deacetylases (Sirtuins) as Novel Therapeutic Targets," *Med. Res. Rev.*, vol. 38, no. 1, pp. 147–200, 2018, doi:

10.1002/med.21436.
[55] J. Yi and J. Luo, "SIRT1 and p53, effect on cancer, senescence and beyond," *Biochim. Biophys. Acta - Proteins Proteomics*, vol. 1804, no. 8, pp. 1684–1689, 2010, doi: https://doi.org/10.1016/j.bbapap.2010.05.002.
[56] Q. Zhang *et al.*, "A small molecule Inauhzin inhibits SIRT1 activity and suppresses tumour growth through activation of p53," *EMBO Mol. Med.*, vol. 4, no. 4, pp. 298–312, 2012, doi: 10.1002/emmm.201100211.
[57] L. Li *et al.*, "Recent advances in trimethoxyphenyl (TMP) based tubulin inhibitors targeting the colchicine binding site," *Eur. J. Med. Chem.*, vol. 151, pp. 482–494, 2018.
[58] N. Liu *et al.*, "Antitumor evaluation of novel phenothiazine derivatives that inhibit migration and tubulin polymerization against gastric cancer MGC-803 cells," *Invest. New Drugs*, vol. 37, no. 1, pp. 188–198, 2019, doi: 10.1007/s10637-018-0682-x.
[59] H. Prinz *et al.*, "N -Benzoylated Phenoxazines and Phenothiazines : Synthesis , Antiproliferative Activity , and Inhibition of Tubulin Polymerization," pp. 4247–4263, 2011, doi: 10.1021/jm200436t.
[60] T. Sachdeva *et al.*, "Design, Synthesis and Characterisation of Novel Phenothiazine-Based Triazolopyridine Derivatives: Evaluation of Anti-Breast Cancer Activity on Human Breast Carcinoma," *ChemistrySelect*, vol. 4, no. 43, pp. 12701–12707, 2019, doi: 10.1002/slct.201903203.
[61] S. Sengupta, *et al.*, "Interaction of a fluorescent paclitaxel analog with tubulin," *Biochemistry*, vol. 34, no. 37, pp. 11889–11894, 1995.
[62] C. M. Abuhaie *et al.*, "Synthesis and anticancer activity of analogues of phenstatin, with a phenothiazine A-ring, as a new class of microtubule-targeting agents," *Bioorganic Med. Chem. Lett.*, vol. 23, no. 1, pp. 147–152, 2013, doi: 10.1016/j.bmcl.2012.10.135.
[63] X. Dai *et al.*, "An overview on natural farnesyltransferase inhibitors for efficient cancer therapy," *J. Enzyme Inhib. Med. Chem.*, vol. 35, no. 1, pp. 1027–1044, 2020.
[64] S. G. Klochkov *et al.*, "Implications of farnesyltransferase and its inhibitors as a promising strategy for cancer therapy," in *Seminars in cancer biology*, 2019, vol. 56, pp. 128–134.
[65] R. M. O'Regan and F. R. Khuri, "Farnesyl transferase inhibitors: the next targeted therapies for breast cancer?," *Endocr. Relat. Cancer*, vol. 11, no. 2, pp. 191–205, Jun. 2004, doi: 10.1677/erc.0.0110191.
[66] L. Baciu-Atudosie *et al.*, "Synthesis and biological evaluation of new phenothiazine derivatives bearing a pyrazole unit as protein farnesyltransferase inhibitors," *Bioorganic Med. Chem. Lett.*, vol. 22, no. 22, pp. 6896–6902, 2012, doi: 10.1016/j.bmcl.2012.09.030.
[67] W. Chen *et al.*, "PP2A-mediated anticancer therapy," *Gastroenterol. Res. Pract.*, vol. 2013, 2013, doi: 10.1155/2013/675429.
[68] V. Janssens *et al.*, "PP2A: the expected tumor suppressor," *Curr. Opin. Genet. Dev.*, vol. 15, no. 1, pp. 34–41, 2005.
[69] M. A. Bassi *et al.*, "Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer," *Cancer Lett.*, vol. 335, no. 1, pp. 9–18, 2013.
[70] A. Gutierrez *et al.*, "Phenothiazines induce PP2A-mediated apoptosis in T cell acute lymphoblastic leukemia," *J. Clin. Invest.*, vol. 124, no. 2, pp. 644–655, 2014, doi: 10.1172/JCI65093.
[71] D. Mutluay and H. Oner, "The Abelson tyrosine kinase (c-Abl) localization in preimplantation mouse development.," *Rom. J. Morphol. Embryol. = Rev. Roum. Morphol. Embryol.*, vol. 58, no. 4, pp. 1385–1391, 2017.
[72] M. Y. Gordon, "Biological consequences of the BCR/ABL fusion gene in humans and mice.," *J. Clin. Pathol.*, vol. 52, no. 10, pp. 719–722, Oct. 1999, doi: 10.1136/jcp.52.10.719.
[73] Z.-J. Kang *et al.*, "The Philadelphia chromosome in leukemogenesis," *Chin. J. Cancer*, vol. 35, pp. 1–15, 2016.
[74] D. S. Krause and R. A. Van Etten, "Tyrosine Kinases as Targets for Cancer Therapy," *N. Engl. J. Med.*, vol. 353, no. 2, pp. 172–187, 2005, doi: 10.1056/NEJMra044389.
[75] G. D. Miller *et al.*, "Resistant mutations in CML and Ph+ ALL–role of ponatinib," *Biol. targets Ther.*, vol. 8, p. 243, 2014.
[76] T. O'Hare *et al.*, "Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia," *Blood*, vol. 110, no. 7, pp. 2242–2249, 2007, doi: 10.1182/blood-2007-03-066936.
[77] V. Subramanian *et al.*, "Novel thiadiazole derivatives as Bcr-Abl tyrosine kinase inhibitors," *J. Appl. Pharm. Sci.*, vol. 7, no. 3, pp. 68–76, 2017, doi: 10.7324/JAPS.2017.70311.
[78] S. A. Muhammad *et al.*, "Novel phenothiazine-based chalcone derivatives with various N-substituted rhodanines induce growth inhibition followed by apoptosis in leukemia cells," *Res. Chem. Intermed.*, vol. 43, no. 10, pp. 5637–5664, 2017, doi: 10.1007/s11164-017-2953-0.
[79] M. Rajabi and S. A. Mousa, "The role of angiogenesis in cancer treatment," *Biomedicines*,

vol. 5, no. 2, p. 34, 2017.
[80] Y. Yang *et al.*, "HIFs, angiogenesis, and cancer," *J. Cell. Biochem.*, vol. 114, no. 5, pp. 967–974, 2013, doi: 10.1002/jcb.24438.
[81] T. Tonini *et al.*, "Molecular basis of angiogenesis and cancer," *Oncogene*, vol. 22, no. 42, pp. 6549–6556, 2003.
[82] R. Lugano *et al.*, "Tumor angiogenesis: causes, consequences, challenges and opportunities," *Cell. Mol. Life Sci.*, vol. 77, pp. 1745–1770, 2020.
[83] M. S. Park *et al.*, "Thioridazine inhibits angiogenesis and tumor growth by targeting the VEGFR-2/PI3K/mTOR pathway in ovarian cancer xenografts," *Oncotarget*, vol. 5, no. 13, p. 4929, 2014.
[84] H.-J. Byun *et al.*, "Anti-angiogenic effects of thioridazine involving the FAK-mTOR pathway," *Microvasc. Res.*, vol. 84, no. 3, pp. 227–234, 2012.
[85] N. E.-S. Ibrahim *et al.*, "The comparative effect of nisin and thioridazine as potential anticancer agents on hepatocellular carcinoma," *Reports Biochem. Mol. Biol.*, vol. 9, no. 4, p. 452, 2021.
[86] D. Miricescu *et al.*, "PI3K/AKT/mTOR signaling pathway in breast cancer: From molecular landscape to clinical aspects," *Int. J. Mol. Sci.*, vol. 22, no. 1, p. 173, 2020.
[87] G. Hoxhaj and B. D. Manning, "The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism," *Nat. Rev. Cancer*, vol. 20, no. 2, pp. 74–88, 2020.
[88] D. A. Fruman, *et al.*, "The PI3K pathway in human disease," *Cell*, vol. 170, no. 4, pp. 605–635, 2017.
[89] A. J. Jhou *et al.*, "Chlorpromazine, an antipsychotic agent, induces G2/M phase arrest and apoptosis via regulation of the PI3K/AKT/mTOR-mediated autophagy pathways in human oral cancer," *Biochem. Pharmacol.*, vol. 184, no. October 2020, p. 114403, 2021, doi: 10.1016/j.bcp.2020.114403.
[90] S. Y. Shin *et al.*, "The antipsychotic agent chlorpromazine induces autophagic cell death by inhibiting the Akt/mtor pathway in human U-87MG glioma cells," *Carcinogenesis*, vol. 34, no. 9, pp. 2080–2089, 2013, doi: 10.1093/carcin/bgt169.
[91] H. Xiang *et al.*, "Physiological functions and therapeutic applications of neutral sphingomyelinase and acid sphingomyelinase," *Biomed. Pharmacother.*, vol. 139, p. 111610, 2021, doi: 10.1016/j.biopha.2021.111610.
[92] D. Milhas *et al.*,"Sphingomyelin metabolism at the plasma membrane: implications for bioactive sphingolipids.," *FEBS Lett.*, vol. 584, no. 9, pp. 1887–1894, May 2010, doi: 10.1016/j.febslet.2009.10.058.
[93] N. H. T. Petersen *et al.*, "Transformation-Associated Changes in Sphingolipid Metabolism Sensitize Cells to Lysosomal Cell Death Induced by Inhibitors of Acid Sphingomyelinase," *Cancer Cell*, vol. 24, no. 3, pp. 379–393, 2013, doi: 10.1016/j.ccr.2013.08.003.
[94] S. Klutzny *et al.*, "Functional inhibition of acid sphingomyelinase by Fluphenazine triggers hypoxia-specific tumor cell death," *Cell Death Dis.*, vol. 8, no. 3, pp. 1–15, 2017, doi: 10.1038/cddis.2017.130.
[95] N. Fehrenbacher *et al.*, "Sensitization to the lysosomal cell death pathway by oncogene-induced down-regulation of lysosome-associated membrane proteins 1 and 2," *Cancer Res.*, vol. 68, no. 16, pp. 6623–6633, 2008.
[96] M. Zhang *et al.*, "CDK inhibitors in cancer therapy, an overview of recent development.," *Am. J. Cancer Res.*, vol. 11, no. 5, pp. 1913–1935, 2021.
[97] P. Łukasik *et al.*, "Cyclin-Dependent Kinases (CDK) and Their Role in Diseases Development-Review.," *Int. J. Mol. Sci.*, vol. 22, no. 6, Mar. 2021, doi: 10.3390/ijms22062935.
[98] S. Vijayaraghavan *et al.*, "Inhibiting CDK in cancer therapy: current evidence and future directions," *Target. Oncol.*, vol. 13, no. 1, pp. 21–38, 2018.
[99] C. Bertoli *et al.*, "Control of cell cycle transcription during G1 and S phases.," *Nat. Rev. Mol. Cell Biol.*, vol. 14, no. 8, pp. 518–528, Aug. 2013, doi: 10.1038/nrm3629.
[100] J. K. Kim and J. A. Diehl, "Nuclear cyclin D1: an oncogenic driver in human cancer.," *J. Cell. Physiol.*, vol. 220, no. 2, pp. 292–296, Aug. 2009, doi: 10.1002/jcp.21791.
[101] H. C. Hwang and B. E. Clurman, "Cyclin E in normal and neoplastic cell cycles," *Oncogene*, vol. 24, no. 17, pp. 2776–2786, 2005, doi: 10.1038/sj.onc.1208613.
[102] D.-J. Fu *et al.*, "Molecular diversity of phenothiazines: design and synthesis of phenothiazine–dithiocarbamate hybrids as potential cell cycle blockers," *Mol. Divers.*, vol. 21, no. 4, pp. 933–942, 2017.
[103] C. Wu *et al.*, "Pharmacological exploitation of the phenothiazine antipsychotics to develop novel antitumor agents – A drug repurposing strategy," *Nat. Publ. Gr.*, no. June, pp. 1–11, 2016, doi:

10.1038/srep27540.
[104] A. Pulkoski-Gross *et al.*, "Repurposing the antipsychotic trifluoperazine as an antimetastasis agent," *Mol. Pharmacol.*, vol. 87, no. 3, pp. 501–512, 2015, doi: 10.1124/mol.114.096941.
[105] J. Jiang *et al.*, "Trifluoperazine Activates FOXO1-Related Signals to Inhibit Tumor Growth in Hepatocellular Carcinoma," *DNA Cell Biol.*, vol. 36, no. 10, pp. 813–821, 2017, doi: 10.1089/dna.2017.3790.
[106] Q. Y. Chen *et al.*, "Molecular mechanism of trifluoperazine induces apoptosis in human A549 lung adenocarcinoma cell lines.," *Mol. Med. Rep.*, vol. 2, no. 5, pp. 811–817, 2009, doi: 10.3892/mmr_00000177.
[107] Z. Feng *et al.*, "The antipsychotic agent trifluoperazine hydrochloride suppresses triple-negative breast cancer tumor growth and brain metastasis by inducing G0/G1 arrest and apoptosis," *Cell Death Dis.*, vol. 9, no. 10, pp. 1–15, 2018, doi: 10.1038/s41419-018-1046-3.
[108] Y. Xia *et al.*, "Antipsychotic drug trifluoperazine suppresses colorectal cancer by inducing G0/G1 arrest and apoptosis," *Front. Pharmacol.*, vol. 10, p. 1029, 2019.
[109] F. Xu *et al.*, "Repurposed antipsychotic chlorpromazine inhibits colorectal cancer and pulmonary metastasis by inducing G2/M cell cycle arrest, apoptosis, and autophagy," *Cancer Chemother. Pharmacol.*, vol. 89, no. 3, pp. 331–346, 2022, doi: 10.1007/s00280-021-04386-z.
[110] I. Dimauro *et al.*, "Estrogen-Receptor-Positive Breast Cancer in Postmenopausal Women: The Role of Body Composition and Physical Exercise.," *Int. J. Environ. Res. Public Health*, vol. 18, no. 18, Sep. 2021, doi: 10.3390/ijerph18189834.
[111] S. E. Nunnery and I. A. Mayer, "Targeting the PI3K/AKT/mTOR Pathway in Hormone-Positive Breast Cancer.," *Drugs*, vol. 80, no. 16, pp. 1685–1697, Nov. 2020, doi: 10.1007/s40265-020-01394-w.
[112] S. Briest and V. Stearns, "Tamoxifen metabolism and its effect on endocrine treatment of breast cancer," *Clin. Adv. Hematol. Oncol.*, vol. 7, pp. 185–192, Apr. 2009.
[113] G. Wang, "Fulvestrant as a reference antiestrogen and estrogen receptor (ER) degrader in preclinical studies: treatment dosage, efficacy, and implications on development of new ER-targeting agents.," *Transl. Cancer Res.*, vol. 9, no. 8, pp. 4464–4468, Aug. 2020, doi: 10.21037/tcr-20-2166.
[114] C. Busonero, S. Leone *et al.*, "In silico screening for ERα down modulators identifies thioridazine as an anti-proliferative agent in primary, 4OH-tamoxifen-resistant and Y537S ERα-expressing breast cancer cells," *Cell. Oncol.*, vol. 41, no. 6, pp. 677–686, 2018, doi: 10.1007/s13402-018-0400-x.
[115] J. D. Lambeth, "Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy," *Free Radic. Biol. Med.*, vol. 43, no. 3, pp. 332–347, 2007.
[116] I. P. Harrison and S. Selemidis, "Understanding the biology of reactive oxygen species and their link to cancer: NADPH oxidases as novel pharmacological targets," *Clin. Exp. Pharmacol. Physiol.*, vol. 41, no. 8, pp. 533–542, 2014.
[117] X. Gào and B. Schöttker, "Reduction–oxidation pathways involved in cancer development: a systematic review of literature reviews," *Oncotarget*, vol. 8, no. 31, p. 51888, 2017.
[118] K. Tominaga *et al.*, "Evidence for cancer-associated expression of NADPH oxidase 1 (Nox1)-based oxidase system in the human stomach," *Free Radic. Biol. Med.*, vol. 43, no. 12, pp. 1627–1638, 2007.
[119] N. Oshima *et al.*, "Induction of cancer stem cell properties in colon cancer cells by defined factors," *PLoS One*, vol. 9, no. 7, p. e101735, 2014.
[120] M. Fukuyama *et al.*, "Overexpression of a novel superoxide-producing enzyme, NADPH oxidase 1, in adenoma and well differentiated adenocarcinoma of the human colon," *Cancer Lett.*, vol. 221, no. 1, pp. 97–104, 2005.
[121] G. Teixeira *et al.*, "Therapeutic potential of NADPH oxidase 1/4 inhibitors," *Br. J. Pharmacol.*, vol. 174, no. 12, pp. 1647–1669, 2017.
[122] M. M. Konaté *et al.*, "Inhibiting the activity of NADPH oxidase in cancer," *Antioxid. Redox Signal.*, vol. 33, no. 6, pp. 435–454, 2020.
[123] M. Chocry and L. Leloup, "The NADPH oxidase family and its inhibitors," *Antioxid. Redox Signal.*, vol. 33, no. 5, pp. 332–353, 2020.
[124] J. Zhang *et al.*, "Small molecules regulating reactive oxygen species homeostasis for cancer therapy," *Med. Res. Rev.*, vol. 41, no. 1, pp. 342–394, 2021.
[125] D. Gianni *et al.*, "Optimization and characterization of an inhibitor for NADPH oxidase 1 (NOX-1)," *Probe Reports from NIH Mol. Libr. Progr. [Internet]*, 2011.
[126] D. Gianni *et al.*, "A novel and specific NADPH oxidase-1 (Nox1) small-molecule inhibitor blocks the formation of functional invadopodia in human colon cancer cells," *ACS Chem. Biol.*, vol. 5, no. 10, pp. 981–993, 2010.