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Repurposing Antipsychotics of the Diphenylbutylpiperidine Class for Cancer Therapy

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Abstract

The recent development of high throughput compound screening has allowed drug repurposing to emerge as an effective avenue for discovering novel treatments for cancer. FDA-approved antipsychotic drugs fluspirilene, penfluridol, and pimozide are clinically used for the treatment of psychotic disorders, primarily schizophrenia. These compounds, belong to diphenylbutylpiperidine class of antipsychotic drugs, are the potent inhibitors of dopamine D_2 receptor and calcium channel. A correlation has been found that patients treated for schizophrenia have lower incidences of certain types of cancer, such as respiratory, prostate, and bladder cancers. These compounds have also been shown to inhibit cancer proliferation in a variety of cancer cells, including melanoma, lung carcinoma, breast cancer, pancreatic cancer, glioma, and prostate cancer, among others. Antipsychotic drugs induce apoptosis and suppress metastasis in *in vitro* and *in vivo* models through mechanisms involving p53, STAT3, STAT5, protein phosphatase 2A, cholesterol homeostasis, integrins, autophagy, USP1, wnt/β-catenin signaling, and DNA repair. Additionally, pre-clinical evidence suggests that penfluridol and pimozide act synergistically with existing chemotherapeutic agents, such as dasatinib, temozolomide, and cisplatin. Some studies have also reported that the cytotoxic activity of the antipsychotics is selective for dividing cells. Based on this growing body of evidence and the availability and previous FDA-approval of the drugs, the compounds appear to be promising anti-cancer agents.

1. Introduction

Drug approval by the Food and Drug Administration (FDA) requires around 10 months, and the total development cost for drugs is estimated to be between $868 million and $1.241 billion USD [1]. Advances in high throughput compound screening techniques have allowed

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drug repurposing to emerge as an alternative route to bring therapies to patients while saving time and money. For example, aspirin, a commonly prescribed anti-inflammatory agent, has been used for secondary prophylaxis of cerebrovascular ischemic stroke and cardiovascular disease [2]. Raloxifene, a chemotherapeutic agent for breast cancer, has been used for prophylaxis of osteoporotic fractures [3]. Thalidomide, a sleep-inducing agent, has been used for multiple myeloma chemotherapy [4]. Drugs with previous clinical approval have two primary advantages: they have a low toxicity to cells *in vivo* and they have known molecular targets for a specific disease [5].

The diphenylbutylpiperidine (DPBP) class of antipsychotics includes fluspirilene, penfluridol, and pimozide. The drugs are all used in the treatment of psychosis and have been reported to be more effective than other neuroleptics in reversing negative schizophrenic symptoms, such as emotional withdrawal and disruptions to affect [6]. For their on-label use, the drugs act as calcium channel antagonists by binding both nitrendipine binding sites, a calcium channel antagonist, and D_2 dopamine receptor binding sites with a high affinity [6]. The IC_{50} values for binding to the nitrendipine binding sites are 13 nM for pimozide, 21 nM for fluspirilene, and 30 nM for penfluridol, and the IC_{50} values for binding to the D_2 dopamine receptor binding sites are 4 nM for pimozide, 2.2 nM for fluspirilene, and 16 nM for penfluridol [6]. The compounds also block T-type and L-type calcium channels at low concentrations, leading to the inhibition of prolactin gene expression [7]. Though it is debated, prolactin may play a role in breast cancer carcinogenesis [8]. The antipsychotic ligand-based actions of the drugs on dopamine and serotonin receptors are likely to be different from the antiproliferative mechanisms of the compounds, however, as much higher drug concentrations are required to observe cytotoxic effects than are required to saturate the receptors [9, 10].

Interestingly, patients diagnosed with schizophrenia and treated with antipsychotic drugs have been shown to be less likely to develop cancer than the general population for certain cancer types [11]. One study reported that the incidence of respiratory, prostate, and bladder cancers in treated male schizophrenic patients is reduced compared to the general population while no difference was seen for treated female schizophrenic patients except for increases in the risk of breast and pancreatic cancer [12]. It has been shown that some antipsychotics may have carcinogenic properties in rodent models [11]. A separate study, however, found that neuroleptic medication use was associated with decreased risks for prostate cancer in males, colon cancer in females, and rectal cancer in both males and females [13]. It has also been shown that antipsychotics select for dividing cells while having a lower cytotoxic effect against normal cells [9]. This growing body of evidence suggests that DPBP's may be useful in the treatment of certain types of cancer. In the current review, we focus on antiproliferative effects, mechanisms of action, and toxicities of the three DPBP compounds in *in vitro* and *in vivo* models and suggest future directions for DPBP drug repurposing research.

2. Fluspirilene

Fluspirilene is primarily used to treat schizophrenia, and its antipsychotic activity is derived from a blockade of dopamine D_2 receptors [14]. The drug is administered via suspension in

water and subsequent intramuscular injection in humans, but oral administration *in vivo* for a subcutaneous mouse model was also effective [15, 16]. Of all three DPBP compounds, fluspirilene has the least published data with regard to repurposing the drug as an anti-cancer therapy.

2.1. Antiproliferative/cell cycle effects

Fluspirilene has been shown to exhibit antiproliferative effects both *in vitro* and *in vivo* (Table 1). Mouse double minute 2 homolog (MDM2) targets p53 for ubiquitin-mediated degradation [14]. Fluspirilene was shown to inhibit the p53-MDM2 interaction by binding to the p53-binding pocket of the MDM2 protein, resulting in p53 activation and inhibition of human colon cancer cell growth [14]. In a separate study, a drug repurposing screen identified fluspirilene as the highest scoring small molecule drug for CDK2 inhibition out of 4,311 FDA-approved small molecule drugs [16]. Notably, in 2015, no CDK2 inhibitors had been FDA-approved for clinical use due to their high toxicity and off-target effects [16]. Shi et al. found that fluspirilene treatment decreased the expression of CDK2, p-Thr160 CDK2, cyclin E, Rb, and p-Ser795 Rb in hepatocellular carcinoma (HCC) cells, leading to G_1 cell cycle arrest [16]. The anti-proliferative effect was also seen *in vivo* in a subcutaneous Huh7 HCC cell model with oral fluspirilene treatment (15 mg/kg) compared to the standard of care drug 5-fluorouracil (10 mg/kg) [16]. A combinatorial therapy of both fluspirilene and 5-fluorouracil showed the highest response rate *in vivo* [16]. Fluspirilene treatment was correlated with decreased p-STAT levels and decreased cell viability, proliferation, and neurosphere formation in a dose-dependent manner in glioma stem cell (GSC) lines and glioma cell lines [15]. STAT3, which is required for proliferation of GSCs, was inhibited by fluspirilene in an orthotopic *in vivo* model [15, 17].

2.2. Toxicity

No toxicity was seen by intraperitoneal injection of fluspirilene (8 mg/kg) in male wistar rats [18]. Additionally, no significant body weight changes were observed following oral administration of fluspirilene (15 mg/kg) for 21 days in a mouse model [16].

3. Penfluridol

Penfluridol is clinically used to treat acute psychosis, chronic schizophrenia, and Tourette's syndrome [19]. The drug is administered orally, has a half-life of 70 hours, and is absorbed from the gastrointestinal tract and deposited in fatty tissue from which it is released [19, 20]. Importantly, the compound is also known to cross the blood-brain barrier [20–22].

3.1. Antiproliferative effects

B16/F10 melanoma, LL/2 lung carcinoma, 4T1 breast cancer, CT26 colon carcinoma [23], and pancreatic cancer cell *in vitro* proliferation was inhibited by penfluridol treatment [24]. One study found that penfluridol induced tumor suppressor protein phosphatase 2A in penfluridol-sensitive pancreatic cancer cells, leading to a downregulation of AKT, p70S6K, GSK3b, MYC ubiquitination and degradation, and cell death [24]. In a glioblastoma multiforme (GBM) model, penfluridol was shown to cause AKT phosphorylation at Ser473, reducing GLI1 expression in a dose-dependent manner for three cell lines (U-87MG, T98G,

U251) [21, 22]. Stem cell markers such as OCT4, Nanog, and Sox2 were also decreased in a dose-dependent manner in this model [22]. Antiproliferative effects of penfluridol were seen *in vivo* in mouse models of different cancer types. In a subcutaneous LL/2 lung tumor mouse model, penfluridol inhibited tumor growth (1514 ± 177 mm^3 in control vs. 608 ± 58 mm^3 in penfluridol treated mice) and increased survival time of the penfluridol-treated mice [23]. No anti-tumor effect, however, was seen *in vivo* in CT26 colon carcinoma models [23]. In a mouse orthotopic model of triple negative cancer, penfluridol suppressed tumor growth by 49% [20]. The same study also showed that penfluridol decreases tumor growth of metastatic brain tumors from intracardiac and intracranial injection of breast cancer cells by 90% and 72%, respectively [20]. In both an orthotopic and subcutaneous pancreatic cancer mouse model, penfluridol inhibited tumor growth [25].

3.2. Cell cycle and pro-apoptotic effects

Penfluridol has been shown to disrupt normal cell cycle progression in some cancer models. In a pancreatic cancer model, penfluridol treatment increased the G_2-M phase cell population in Panc1 and Panc0504 cell lines [24]. Correspondently, an increase in G_2-M arrest proteins cyclin B1 and p21 were observed along with a suppression of cyclin D and MYC [24]. The same study also showed that penfluridol treatment increased the pre-G_0 cell population in Pan0403, SU8686, and MiaPaCa2 cell lines [24]. Upregulation of pro-apoptotic proteins BIM, BAX, and PUMA and downregulation of anti-apoptotic protein Bcl-2 were also observed in pancreatic cancer cell lines following penfluridol treatment [24]. Penfluridol-induced pro-apoptotic cleavage of caspase 3 and PARP was observed in glioblastoma cells both *in vitro* and *in vivo* subcutaneous and orthotopic mouse models [22]. Interestingly, a similar efficacy of penfluridol was observed in both subcutaneous and orthotopic GBM models [22]. In penfluridol-treated triple-negative breast cancer cell models, cleavage of caspase 3 was followed by apoptosis [20]. Apoptosis following penfluridol treatment was also observed in three different pancreatic cancer cell lines [25].

3.3. Anti-migration/invasion effects

Integrins are the main class of cellular adhesion molecules and play an important role in cancer metastasis [26]. The protein family has been implicated in each step of cancer progression, including initiation, progression, local invasion, vasculature invasion, circulating tumor cell survival, extravasation into a secondary site, and colonization of the new tissue [26]. As such, integrins, which are activated by surface-exposed ligand binding, are an attractive therapeutic target for anti-cancer drugs [26]. Promising pre-clinical evidence for integrins inhibition has been less successful in clinical trials due to cellular redundancy and resistance pathways, meaning compounds that inhibit integrins along with other pro-cancer mechanisms may prove more useful for circumventing cellular resistance [26]. In three triple negative breast cancer cell lines (MDA-MB-231, HCC-1806, and 4T1), penfluridol treatment decreased integrin β4, integrin α6, integrin α5, and integrin β1 expression in a dose-dependent manner [20]. Downstream molecules in the integrin signaling pathway such as FAK, p-Paxillin (Y118), and Paxillin were also shown to be inhibited by penfluridol treatment in the triple negative breast cancer cell lines [20]. Studies regarding anti-angiogenic effects of penfluridol have complimented the anti-metastatic effects. Penfluridol reduced cell migration in a wound healing assay using Human Umbilical

Vein Endothelial Cells (HUVEC) as well as the breast cancer cell line MDA-MB-231. Similarly, reduced invasion of MDA-MB-231 cells was observed by penfluridol treatment. Low dosages of penfluridol also suppressed VEGF-induced angiogenesis *in vivo* in a Matrigel plug assay [Srivastava et al., Frontiers in Oncology, 2019; Under Review]. Mechanistically, another study showed that the aforementioned integrin β4/α6/α5/β1 repression was mediated by a penfluridol-induced reactive oxygen species (ROS) [27]. In the suppression pathway, the ROS was first shown to down-regulate c-Myc and c-Myc regulated microRNAs (miR-27a and miR-20a/miR-17). The ROS also induced Sp transcriptional repressors ZBTB10 and ZBTB4, causing down-regulation of Sp transcription factors Sp1, Sp3, and Sp4 [27]. The down-regulation of the Sp transcription factors decreased expression of Sp-dependent genes, including the integrin β4, integrin α6, integrin α5, and integrin β1 genes [27]. Taken together, penfluridol prevented cancer cell migration and invasion, and it inhibited brain metastasis in three *in vivo* triple negative breast cancer cell models partially through this mechanism of integrin transcriptional inhibition [27].

3.4. Cholesterol homeostasis

Dysregulation of cholesterol homeostasis has been shown to contribute to human cancer risk and progression. High intracellular cholesterol levels were shown to promote prostate cancer progression through upregulating *de novo* androgen synthesis and regulating the AKT signaling pathway [28]. More broadly, hypercholesterolemia increases the risk of aggressive prostate cancer through intratumoral steroidogenesis, increased inflammation and cellular proliferation, and changes in lipid rafts [29]. Statins, which is a class of commonly prescribed drugs that reduces lipid levels, have been shown to reverse these effects through decreasing cholesterol levels [29]. Penfluridol treatment caused accumulation of unesterified cholesterol and decreased cholesterol levels in a dose-dependent manner in melanoma (B16/F10), lung carcinoma (LL/2), and breast cancer (4T1) cell lines in both *in vitro* and *in vivo* models [23]. The exact mechanism for the dysregulation of cholesterol homeostasis via penfluridol treatment, however, is unknown [23].

3.5. Autophagy

Autophagy has a controversial role in cancer: it has been shown to suppress tumor formation and progression, and it has also been shown to enhance tumor cell survival during metabolic stress and promote metastasis [30]. Despite the unclear role, the FDA has approved anti-cancer drugs that induce autophagy, such as gemcitabine [25]. In a pancreatic cancer model, penfluridol treatment has shown to induce autolysosome formation and LC3B (a hallmark of autophagy) expression [25]. Mechanistically, it was found that penfluridol-induced endoplasmic reticulum stress in three pancreatic cancer cell lines (Panc-1, BxPC-3, and AsPC-1) was associated with the observed autophagy [31]. Endoplasmic reticulum stress markers such as binding protein (BIP), C/EBP homologous protein (CHOP), and inositol requiring 1α (IRE1α) were upregulated following penfluridol treatment, and high ER stress has been shown to activate the cell death program, leading to the observed apoptosis [31].

3.6. Immune system modulation

A growing body of evidence shows that the immune system plays a complex role in cancer development and progression. Innate immune system cells have been shown to induce DNA

damage through radical generation while also promoting angiogenesis and tissue remodeling [32]. Adaptive immune system cells can inhibit tumor growth via cytotoxic T-cell activity, but tumor growth can be promoted by regulatory T-cells that suppress the cytotoxic T-cell responses [32]. In line with these findings, penfluridol-mediated inhibition of GBM tumor growth in a subcutaneous mouse model was correlated with a reduction of regulatory T and myeloid-derived immune system suppressive cells [33]. In addition, an increase in tumor killing M1 macrophages, a reduction in chronic inflammation markers (CC14 and IFNγ) in the tumor microenvironment, and an increase in spleen size were seen in the penfluridol-treated mice [33].

3.7. Combinatorial effects with existing chemotherapeutic agents/cancer therapies

Non-homologous end joining (NHEJ) is one mechanism by which tumor cells can become radioresistant by assisting in the repair of ionizing radiation-induced double-stranded breaks in the DNA [34]. HeLa cells were treated with 5 μM penfluridol followed by 8 Gy radiation, leading to increased levels of broken DNA and a disruption of DNA damage repair processes potentially mediated through inhibition of DNA-PKcs activation [34]. From these preclinical results, penfluridol may be a potential radiosensitizing agent [34]. Penfluridol has also shown synergistic effects with existing chemotherapeutic drugs. Dasatinib is used to treat pancreatic cancer, and penfluridol increased dasatinib activity in two pancreatic cancer cell lines (MiaPaCa2 and Panc0403) but was not synergistic with dasatinib in two dasatinib-resistant pancreatic cancer cell lines (Panc0327 and Panc1) [24]. In a glioblastoma model, combination therapy of penfluridol and temozolomide increased rates of apoptosis while decreasing GL1 and MGMT expression when compared to single-drug treatment [21].

3.8. Dosages/toxicity

A systematic review of human penfluridol dosages reported a mean dose of 39 mg/week (n=13 studies) and a range of 5 to 160 mg/week (n=18 studies) [19]. The authors of the review suggest that dosages of 40 to 80 mg/week are sufficient for clinical improvement for psychiatric patients based on their review [19]. Another study reports that dosages can range from 20–250 mg/week [20].

4. Pimozide

Pimozide is clinically used to treat schizophrenia [35]. It has also been used to treat lipodystrophy [36], Gilles de la Tourrette disease [37, 38], monosymptomatic hypochondriacal psychoses, body dysmorphic disorder, metastatic melanoma, trichotillomania, and trigeminal and postherpetic neuralgia [39]. Additionally, it has been used outside of the United States as an oral antipsychotic drug for several decades [38]. Pimozide derives its antipsychotic efficacy through dopamine D_2 receptor inhibition [36, 38]. Interestingly, some studies have shown that dopamine receptor activation is correlated with poor cancer prognoses [40]. Pimozide also has other targets that may be associated with its antipsychotic activity. It inhibits the release of corticotropin releasing factor, follicle stimulating releasing factor, and luteinizing hormone release factor in rats [41]. It is also a calmodulin antagonist [37] and a serotonin 5-HT_2 receptor antagonist [38, 42, 43]. Other studies have shown that the compound also inhibits both T-type and voltage-dependent

calcium channels [38, 44]. Once ingested, pimozide is metabolized by the liver [45], has a half-life of two days, and a common range of blood level concentrations of the drug is between 1 and 5 ng/mL [43].

4.1. Previous clinical studies

A case study was published in 1979 detailing the treatment of a post-menopausal woman diagnosed with metastatic pigmented malignant melanoma with a course of pimozide [36]. Oral pimozide was administered at 4 mg/day for the first week, 8 mg/day for the second week, and 12 mg/day for the subsequent weeks [36]. After eight weeks, lung lesions were found to have decreased, and the liver lesions decreased by 50% [36]. A larger phase II clinical trial was conducted in 1983 in which thirty patients who had previously treated metastatic melanoma were treated with a course of pimozide [46]. Six patients responded with poor drug tolerance, and another six patients responded with disease stabilization, a partial response, or a complete response in the soft tissue, lymph nodes, liver, and lungs for an over 17% response rate [46]. Some observed side effects are discussed in later section.

4.2. Antiproliferative effects

Pimozide has shown antiproliferative effects in several cancer cells lines both *in vitro* and *in vivo*. In breast cancer cells, pimozide inhibited cell growth in breast cancer cell lines (MCF-7, T47D, ZR75–1B, MDA-MB-231) in an estrogen, insulin, IGF-1, and EGF independent manner [37]. A separate study showed that pimozide inhibits cell growth in retinoblastoma and breast cancer epithelial cells in a dose-dependent manner [44]. In lung cancer cell lines NSCLCA549, H460, HCC4006, and H1437, pimozide inhibited cell proliferation [47]. In hepatocellular carcinoma cells, pimozide inhibited cell proliferation through downregulation of Bmi1, c-Myc, and Nanog expression levels [35, 45]. In osteosarcoma cells, pimozide suppressed Erk signaling [5]. STAT3 is a transcription factor with either pro-tumor growth or anti-cancer activity depending on a tumor's mutations and etiology [48]. Pimozide treatment of prostate cancer cells led to a reduction in tyrosine 705 phosphorylation of STAT3, leading to a suppression of STAT3 activation and a reversal of IL-6 induced STAT3 activation in prostate cancer cells and hepatocellular carcinoma cells (HCC) [35, 39]. One study showed that pimozide downregulates STAT3, leading to lower antioxidant enzyme catalase (CAT) levels and reactive oxygen species induction in osteosarcoma cells [5]. Pimozide was also shown to disrupt the wnt/β-catenin signaling pathway in HCCs, which led to a decrease in EpCAM and cyclin D1 expression [45]. This study proposes a mechanism in which pimozide inhibits PKA signaling, leading to G3K activation and a reduction of nuclear β-catenin levels and thus a reduction in wnt/β-catenin signaling pathway gene expression [45].

4.3. STAT5 inhibition

STAT5 is a well-studied transcription factor, and it is known to be activated by tumorigenic kinases [49]. In chronic myelogenous leukemia (CML) cells, pimozide treatment decreased STAT5 tyrosine phosphorylation and subsequent expression of STAT5 target genes [10]. CML growth and CD34+ cell colony formation was inhibited by pimozide treatment independent of the presence of the T315I BCR/ABL mutation that generates kinase-inhibitor resistant cells [10]. Mouse models of acute myelogenous leukemia (AML) showed similar

results in which pimozide decreased STAT5 tyrosine phosphorylation, induced apoptosis, and reduced tumor burden [49]. An internal tandem duplication (ITD) of *FLT3* leads to STAT5 activation and resistance to PI3K/Akt pathway inhibition through protection of the mTOR/4EBP1/Mlc-1 pathway [50]. PI3K/Akt pathway inhibitor GDC-0941 treatment followed by pimozide treatment in acute myeloid leukemia (32D/ITD) cells was shown to decrease resistance to PI3K/Akt pathway inhibition [50]. In MCF7 breast cancer cells, pimozide treatment was shown to inhibit STAT5 phosphorylation, leading to decreased production of EGF family growth factors *Ereg, Epgn,* and *Nrg1* while also decreasing growth in Cuzd1-overexpressing mammary tumors in mouse models [51]. Pimozide derivatives were shown to have cytotoxic activity against BCR-ABL-positive and pSTAT5-overexpressing K562 cells through STAT5 inhibition [52]. Basally high levels of STAT3 and STAT5a in a prostate cancer cell line (22Rv1) were predictive of sensitivity to pimozide treatment while low levels of the two proteins in two cells lines (C4–2, PC3) may have helped mediate resistance to pimozide treatment [40]. T-cell leukemia cells (Ba/F3) with a ribosomal RPL10 R98S mutation were also sensitive to pimozide-mediated STAT5 inhibition through inhibition of STAT5 phosphorylation [53]. pSTAT5 inhibition also inhibited STAT5-mediated proliferation in T-cell prolymphocytic leukemia cells [54]. The same effect was observed in peripheral T-cell lymphoma cells, with pimozide leading to a 70% reduction in pSTAT5 expression and an induction of apoptosis through the TRAIL/DR4 extrinsic apoptotic pathway [55]. In a canine mastocytoma model, pimozide had only a small effect on pSTAT levels [56].

4.4. Cell cycle and pro-apoptotic effects

Pimozide treatment of MCF-7 breast cancer cells decreased S and G_2/M cells and led to an accumulation of G_0 and G_1 cells [37, 57]. Similar effects were seen in prostate cancer cell lines DU145 and LNCaP [39, 58], hepatocellular carcinoma cells [35], and osteosarcoma cells [5]. In the MCF-7 breast cancer cell lines, 2.5 μM pimozide treatment induced apoptosis [59]. Pimozide treatment of prostate cancer cells was found to induce apoptosis in a dose-dependent manner [40]. The role of caspase is pimozide-induced apoptosis is unclear, however. One study found that pimozide-induced apoptosis was caspase-3 and p53 independent [44]. A separate study reported that pimozide poorly inhibits caspase-1, caspase-3, and caspase-6 with IC_{50} values over 250 μM in each case [60]. A study in pancreatic cancer cells showed increased caspase-3 and caspase-7 levels following pimozide treatment [58]. Caspase-3 induction and PARP cleavage was also observed in pimozide-treated patient-derived glioblastoma cells but not in neural progenitor cells, indicating that pimozide may have selective anti-cancer activity [61]. Elevation of cleaved-PARP levels and induction of apoptosis was observed in osteosarcoma cells as well [5].

4.5. Anti-migration/invasion effects

Pimozide has been shown to inhibit prostate cancer cell (DU145, LNCaP) migration through downregulation of N-cadherin expression and upregulation of E-cadherin expression [39]. EMT was suppressed in HCC cells following pimozide treatment through N-cadherin and vimentin downregulation along with E-cadherin upregulation [35].

4.6. Cholesterol homeostasis

Pimozide can alter cellular membrane properties by disrupting cholesterol homeostasis through upregulation of cholesterol metabolism genes such as HMGCR, INSIG1, and LDLR [9]. The disruption of cholesterol homeostasis was reduced by the co-administration of cholesterol inhibitor mevastatin [9]. Pimozide and mevastatin had the greatest synergistic effects in the neuroblastoma cell lines, and the IC_{50}-value of pimozide was reduced by ~45% in the IMR32 cells [9]. Another experiment was done by analyzing the cytotoxicity of pimozide in lipoprotein-deficient medium, and the IC_{50}-value was reduced by ~80% for IMR32 [9]. Therefore, the diminished levels of extracellular cholesterol likely caused the cells to be more prone to pimozide alone compared to the mevastatin cotreatment experiment [9]. In a lipoprotein-depleted medium plus cotreatment with mevastatin, the IC_{50}-value of pimozide was reduced by more than 95% in the IMR32 cells [9].

4.7. Ubiquitin-mediated degradation pathway effects

USP1 is a deubiquitinase that has been implicated in cancer progression. It is known to deubiquitinate inhibitor of DNA-binding 1 (ID1), which is a transcription factor that causes cell proliferation in leukemia, and thereby activates ID1 [62]. In a leukemia cell model, pimozide inhibited USP1, promoted ID1 degradation, and inhibited growth of the cells [62]. In GBM cells, pimozide treatment also inhibited USP1, leading to decreased levels of ID1, Sox2, Olig2, and CHEK1 [61]. The same GBM model also showed increased p-gH2AX foci, likely due to inhibition of DNA repair processes [61]. A USP1/WDR48 complex was shown to promote stem cell maintenance and DNA damage repair [63]. Additionally, a USP/AUF1 complex is involved in the Fanconi anemia pathway and helps mediate the DNA damage response [60, 64]. The same study reported that pimozide inhibited the USP/AUF1 complex weakly and noncompetitively [60, 64].

4.8. Dopamine receptor inhibition

The antipsychotic efficacy of pimozide is tied to its dopamine D_2 receptor and calcium channel antagonism. The dopamine D_2 receptor, however, has also been shown to be overexpressed in human pancreatic ductal adenocarcinoma cell lines (MiaPaCa-2 and BxPC-3), and inhibition with pimozide selectively suppressed cellular proliferation of the cancerous cell lines when compared to normal fibroblasts [58]. In the same study, it was reported that pimozide treatment increased pERK levels, which activated PKA and was indicative of ER stress in the pancreatic cancer cells [58]. Epidemiological studies have also shown that schizophrenic patients receiving dopamine D_2 receptor antagonists have lower incidences of rectum, colon, and prostate cancers when compared to the general population [58].

4.9. Combinatorial effects with existing chemotherapeutic agents/cancer therapies

Pimozide radiosensitized breast cancer MCF-7 cells through enrichment of sigma 2 sites, which are shown to be highly concentrated in several tumor types and to bind neuroleptic drugs [44, 59]. Pimozide may also serve as a radiosensitizer in glioma as GBM mouse models treated with irradiation and pimozide survived twice as long as groups with single treatments or no treatment [61]. Bleomycin is a chemotherapeutic agent used to treat

leukemia and ovarian carcinoma, and bleomycin A2 activity acted synergistically with pimozide [65]. A 5 mM pimozide treatment was shown to increase bleomycin-induced lethality by 3.2-fold in human ovarian carcinoma cells [66]. Pimozide acted synergistically with leukemia kinase inhibitors imatinib and nilotinib in inhibiting pSTAT5 and inducing apoptosis in leukemic cells [66]. Sunitinib is used to treat kidney, gastrointestinal, and pancreatic neuroendocrine tumors, and synergistic effects were observed for pimozide and sunitnib [49]. Cisplatin is used to treat a variety of human cancers, including testicular cancer, ovarian cancer, breast cancer, bladder cancer, heck and neck cancer, and lung cancer, among others. Pimozide showed synergistic activity with cisplatin in a cisplatin-resistant non-small cell lung cancer (NSCLC) cell line (H596) but not in a cisplatin-sensitive (NSCLC) cell line (H460) [67]. Lastly, oxaliplatin is used in colorectal cancer, and pimozide was shown to reverse oxaliplatin-mediated neurotoxicity in rat models [68].

4.10. Dosages/toxicity

Several values have been reported for pimozide dosages. For acutely ill schizophrenic patients, low dosages of 2–6 mg/day have a lower rate of extrapyramidal adverse effects than 10 mg/day [38]. The extrapyramidal effects include Parkinsonian symptoms such as tremor, bradykinesia, and shuffling gait [38, 46]. The clinical dosing is not recommended to exceed 10 mg/day [55], but a 24 mg oral dose of pimozide reaches a peak plasma concentration of only 0.04 μM, which is a much lower concentration than used in many of the anti-cancer studies [45]. Another study reports, however, that a dosage of 25 mg/kg used for *in vivo* studies is low when compared to the dose used to treat CNS diseases [5]. The LD_{50} of pimozide is unknown in humans, but it is 228 mg/kg in mice, 5120 mg/kg in rats, 188 mg/kg in guinea pigs, and 40 mg/kg in dogs [5]. Mice treated with low dosages of pimozide (10 mg/kg/day) survived without adverse effects [61], and changes in body weight and short or long term satiety were not observed in rats given pimozide dosages of 0.1 mg/kg/day [69]. Pimozide also has no effect on hematopoietic progenitors derived from healthy human donors [49]. One study reported, however, that pimozide use in mouse models may be limited by the toxicity of the drug as its maximum tolerated dose may be insufficient for anti-cancer activity in leukemic mouse models [62]. In humans, the side effects can be lethargy, xerostomia, xerophtalmia, extrapyramidal manifestations, akinesia, depression, anorexia, and tremor [36, 38, 61]. Rapid administration of pimozide has also led to ECG changes cardiac arrhythmia risk, hypertension, sudden cardiac death, prolongation of the QT interval, and possible ventricular arrhythmias [38, 57, 70].

5. Conclusion

This paper discusses the existing literature regarding the anti-cancer mechanisms and characteristics of the three drugs in the diphenylbutylpiperidine class: fluspirilene, penfluridol, and pimozide. All three drugs are FDA-approved for use as antipsychotic agents, but studies have shown that the drugs may also possess potent and selective anti-cancer activity at higher dosages. The drugs have also been seen to act synergistically with a number of existing chemotherapeutic agents. Given the high cost associated with new drug research, development, and FDA approval, these drugs are an attractive area for further research. The compounds were shown to inhibit cellular proliferation, induce cell cycle

arrest, and induce apoptosis both *in vitro* and *in vivo*. In penfluridol and pimozide, which have been more extensively studied, several mechanisms of action have been reported. Dysregulation of cholesterol homeostasis was reported with both compounds as an observed decrease in cholesterol levels was associated with inhibition of tumor growth. Penfluridol was also shown to act as anti-tumor agent by inducing autophagy and acting as an immune system modulator that increased tumor killing M1 macrophages. Pimozide was shown to derive part of its anti-cancer activity through STAT5 inhibition and USP1 deactivation.

Since the dosage for anti-cancer activity is much higher than the dosage required for antipsychotic activity, effective yet safe dosages may be difficult to obtain in the clinic, especially with regards to pimozide. Pimozide has been shown to have severe adverse effects when administered rapidly and at a high concentration. To find safer alternatives, the drugs could be used to potentially find more selective and less toxic derivatives. The new compounds, however, would also require FDA approval in addition to preclinical testing.

A very promising future direction for these compounds is in combinatorial therapies. Penfluridol and pimozide were shown to exhibit synergistic activity with several chemotherapeutic agents, potentially allowing their dose and thus toxicity to be reduced when placed in cocktail therapies with other drugs. This optimistic speculation, however, needs to be proven with experimental evidence, especially *in vivo*. Additionally, drug sensitivity markers are beginning to be uncovered for many cancer therapies, and they should also be uncovered for these diphenylbutylpiperidine compounds to help select for DNA and tumor fingerprints that may be responsive. Thus, more work should be done in these regards to help determine whether bringing these promising compounds into the clinic could provide a benefit to cancer patients.

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Table 1.

Fluspirilene

Cancer type	Cell line	Model used	Molecular targets	Efficacy	IC_{50}	Reference
Hepatocellular carcinoma	HepG2	*In vitro*	CDK2, p-Thr160 CDK2, cyclin E, Rb, and p-Ser795 Rb	G1 arrest	4.017 μM	[16]
	Huh7	*In vitro,* subcutaneous mouse model	CDK2, p-Thr160 CDK2, cyclin E, Rb, and p-Ser795 Rb	Tumor growth inhibition	3.468 μM	[16]
Colon cancer	HCC116-$p53^{+/+}$	*In vitro*	p53-MDM2 complex	Cell cycle arrest		[14]
Glioma	U251, SNB19, T98, U87, TGS01, KGS01	*In vitro*	STAT3, p-Ser727 STAT3	Anti-proliferation		[15]
	TGS04	*In vitro*, orthotopic mouse model	STAT3, p-Ser727 STAT3	Tumor growth inhibition		[15]

Table 2.

Penfluridol

Cancer type	Cell line	Model used	Molecular targets	Efficacy	IC_{50} (μM)	Reference
Melanoma	B16/F10	*In vitro*, subcutaneous mouse model	Cholesterol, unesterified cholesterol	Anti-proliferation, Tumor growth inhibition	2.51	[23]
Lung carcinoma	LL/2	*In vitro*, subcutaneous mouse model	Cholesterol, unesterified cholesterol	Anti-proliferation, Tumor growth inhibition	2.45	[23]
Colon carcinoma	CT26	*In vitro*, subcutaneous mouse model	Cholesterol, unesterified cholesterol	Anti-proliferation	2.74	[23]
Breast cancer	4T1	*In vitro*; subcutaneous, orthotopic, metastatic, and intracranial mouse models	Cholesterol, unesterified cholesterol Integrins α5, α6, β4, β1, FAK, p-Tyr118 Paxillin, Paxillin	Anti-proliferation, Apoptosis, Anti-invasion, Tumor growth inhibition	3.19–3.7	[20, 23] [27]
	HCC-1806	*In vitro*	Integrins α5, α6, β4, β1, FAK, p-Tyr118 Paxillin, Paxillin	Anti-proliferation, Anti-invasion	4–5	[20]
	MDA-MB-231	*In vitro*, orthotopic mouse model	Integrins α5, α6, β4, β1, ROS, c-Myc, miR-27a, miR-20a/miR-17, ZBTB10, ZBTB4, Sp1, Sp3, Sp4	Anti-proliferation, Anti-invasion	4–5	[20] [27]
	SKBR3	*In vitro*	Integrins α5, α6, β4, β1, ROS, c-Myc, miR-27a, miR-20a/miR-17, ZBTB10, ZBTB4, Sp1, Sp3, Sp4	Anti-proliferation, Anti-invasion		[27]
Pancreatic cancer	Panc1, Panc0504, Panc0403, SU8686 MiaPaCa2 Panc0203 Panc0327 BxPc3 HPDE AsPc1	*In vitro*	Protein phosphatase 2A, AKT, SRC, p-70S6K, GSK3b, MYC, BIM, BAX, PUMA, Bcl-2, BIP, CHOP, IRE1α, ER stress	Cell cycle arrest: G_2-M and G_0, Apoptosis, Anti-proliferation	2.9–12.0 21.0 35.8 9.3 36.9 8.9 10.5 4.8–16.2 54.4 3.5-resistant	[24] [25] [25] [31]
	Panc1005				resistant	
Glioblastoma multiforme	U87-MG T98G U251 GBM: 43, 10, 44, 28, 14 SJ-GBM2 CHLA-200	*In vitro*, subcutaneous and orthotopic mouse models	p-Ser473 AKT, GLI1, OCT4, Nanog, Sox2, caspase 3, PARP	Apoptosis, Tumor growth inhibition	6 5.5 9 4–10	[21, 22]

Table 3.

Pimozide

Cancer type	Cell line	Model used	Molecular targets	Efficacy	IC_{50} (μM)	Reference
Breast cancer	MCF-7	*In vitro,* orthotopic mouse model	Sigma 2 sites, p-STAT5, Ereg, Epgn, Nrg1, Cuzd1	Radiosensitization, Anti-proliferation, Apoptosis, Cell cycle arrest	6	[37, 51, 57, 59]
	T47D, ZR75–1B, MDA-MB-231	*In vitro*		Anti-proliferation		[37]
Retinoblastoma	Y79 WERI-Rb-1	*In vitro*	Ca^{2+} T-channels	Anti-proliferation, Apoptosis, Cell cycle arrest	0.9 1.2	[44]
Rat glioma	C6	*In vitro*	Ca^{2+} T-channels	Anti-proliferation, Apoptosis, Cell cycle arrest	8.0	[44]
Glioblastoma	Patient-derived cell lines	*In vitro,* orthotopic mouse model	Caspase 3, PARP, USP1, ID1, Sox2, Olig2, CHEK1, p-gH2AX	Anti-proliferation, Apoptosis		[61]
Leukemia	K562	*In vitro,* subcutaneous mouse model	USP1, ID1	Anti-proliferation, Tumor growth inhibition		[62]
Chronic myelogenous leukemia	K562, KU812	*In vitro*	STAT5, p-STAT5, MAPK, p-MAPK	Apoptosis, Cell cycle arrest		[10]
Acute myelogenous leukemia	MV411 Ba/f3 FLT3 ITD 32D/ITD	*In vitro,* orthotopic mouse model	p-Tyr STAT5, STAT5, FL3-ITD, PI3K, Akt, mTOR, 4EBP1, Mcl-1	Anti-proliferation, Apoptosis, Tumor growth inhibition	3–5	[49] [50]
T-cell leukemia/ lymphoma	Ba/F3 Kit225 HuT102	*In vitro*	p-STAT5, STAT5, TRAIL/DR4	Anti-proliferation, Apoptosis	 15 11	[53] [54] [55]
Lung cancer	NSCLC A549, H460, HCC4006, H1437, H520	*In vitro*	USP1	Anti-proliferation		[47] [67]
	H322, SKLU-1, SW1573, H522, H1299, H1703,				5.0–13.7	
Hepatocellular carcinoma	MHCC-97L Hep 3B Hep G2 Huh7	*In vitro,* subcutaneous mouse model (MHCC-97L only)	Bmi1, c-Myc, Nanog, STAT3, IL-6, N-cadherin, vimentin, E-cadherin, wnt/β-catenin, EpCAM, cyclin D1, PKA, GSK3	Anti-proliferation, Cell cycle arrest, Anti-invasion, Tumor growth inhibition	6.15 1.81 1.14 8.44	[35, 45]
Osteosarcoma	U2OS	*In vitro*	ERK, PARP, STAT3, catalase, ROS	Anti-proliferation, Apoptosis, Cell cycle arrest	13.78	[5]
Prostate cancer	DU145 LNCaP PC3M BPH-1 22RV1	*In vitro*	p-Tyr705 STAT3, STAT3, STAT5A, IL-6, N-cadherin, E-cadherin	Anti-proliferation, Apoptosis, Cell cycle arrest, Anti-invasion	6.541 7.21 9.19 7.78 11.12	[39] [40]

Cancer type	Cell line	Model used	Molecular targets	Efficacy	IC_{50} (μM)	Reference
Pancreatic cancer	K562, Panc-1 MiaPaCa2 BxPC-3	*In vitro,* orthotopic mouse model	Caspase 3/7, DRD2, p-STAT5, p-ERK, PKA, ER stress	Anti-proliferation, Apoptosis, Tumor growth inhibition	5	[58] [52]