Received: 5 February 2018 | Revised: 7 July 2018 | Accepted: 10 July 2018

DOI: 10.1002/med.21529

REVIEW ARTICLE

WILEY

Therapeutic potential of pteridine derivatives: A comprehensive review

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Funding information
Fundación Séneca, Grant/Award Number: Project 19249/PI/14; Secretaría de Estado de Investigación, Desarrollo e Innovación; Sistema Nacional de Garantía Juvenil, Grant/Award Number: PEJ-2014-A-95803; MINECO

Abstract

Pteridines are aromatic compounds formed by fused pyrazine and pyrimidine rings. Many living organisms synthesize pteridines, where they act as pigments, enzymatic cofactors, or immune system activation molecules. This variety of biological functions has motivated the synthesis of a huge number of pteridine derivatives with the aim of studying their therapeutic potential. This review gathers the state-of-the-art of pteridine derivatives, describing their biological activities and molecular targets. The antitumor activity of pteridine-based compounds is one of the most studied and advanced therapeutic potentials, for which several molecular targets have been identified. Nevertheless, pteridines are also considered as very promising therapeutics for the treatment of chronic inflammation-related diseases. On the other hand, many pteridine derivatives have been tested for antimicrobial activities but, although some of them resulted to be active in preliminary assays, a deeper research is needed in this area. Moreover, pteridines may be of use in the treatment of many other diseases, such as diabetes, osteoporosis, ischemia, or neurodegeneration, among others. Thus, the diversity of the biological activities shown by these compounds highlights the promising therapeutic use of pteridine derivatives. Indeed, methotrexate, pralatrexate, and triamterene are Food and Drug Administration approved pteridines, while many others are currently under study in clinical trials.

KEYWORDS
anti-inflammatory, antimicrobial, cancer, molecular target, pteridine, therapeutic potential

1 | INTRODUCTION

Pteridines are compounds based on a pyrimido[4,5-*b*]pyrazine ring system (Figure 1). These bicyclic compounds are produced by many living organisms, where they display different biological functions. Most naturally produced pteridines are referred to as pterins, as they present an amino and a carbonyl group at ring positions 2 and 4, respectively (Figure 1).

In regard to their synthesis, all natural pterins produced both in prokaryotic and eukaryotic organisms are formed from guanosine triphosphate (GTP) with the catalytic participation of the enzyme GTP cyclohydrolase. Thus, all living organisms have maintained and share the same metabolic synthesis pathway through evolution.[1]

Pterins were discovered as animal pigments in nature. This type of compounds was isolated for the first time from butterflies, such as those included in the genus *Colias*, as they are part of the pigments that give color to the butterflies' wings.[2] This is the reason why these compounds received their name, from the Greek word *Pteron*, which means wing.[1] Nevertheless, pteridines are not only found in butterflies' wings but also in the skin of other insects and vertebrate animals such as some colored fishes (salmon), reptiles (snakes), and amphibians. Thus, in nature, one of the common roles of pteridines is to be part of animal pigments, being present for instance in the colored eyes of *Drosophila melanogaster* in which, apart from other tryptophan-derived visual pigments named "ommochromes," there is a group of dimeric pteridines known as "drosopterins."[1]

However, not all pterins found in nature are pigments; in fact, other naturally synthesized pterins play essential metabolic roles as enzymatic cofactors, and are involved in the synthesis of nucleic acids, amino acids, neurotransmitters, nitrogen monoxides as well as purine and aromatic amino acids. This is the case of 5,6,7,8-tetrahydrobiopterin (BH_4), which together with neopterin are two of the most studied natural pterins (Figure 1).[3] BH_4 acts as cofactor for several aromatic amino acid hydroxylases as well as nitric oxide synthase (NOS), and its deficiency has been associated with several pathologies.[4] Other examples of pteridines with key metabolic roles are also the redox cofactor flavin adenine dinucleotide[5]; tetrahydrofolic acid (FH_4), key cofactor in the synthesis of nucleic acids and amino acids,[6] whose deficiency can cause megaloblastic anemia[7]; and molybdopterins (MPT or MoCo), the essential component of a group of redox enzymes.[8] Other noteworthy examples of natural pteridines are those included in the

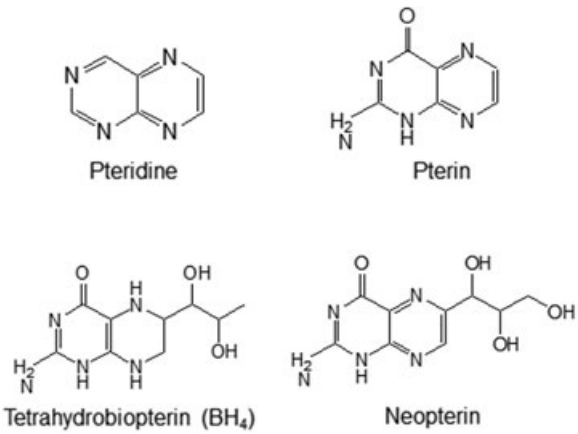


FIGURE 1 Chemical structure of pteridine, and the natural produced pteridines: pterin, neopterin, and tetrahydrobiopterin (BH_4)

TABLE 1 Schematic list of the pteridines' biological properties summarized in this study

Section no	Biological properties
3	Antitumor activity
4	Anti-inflammatory activity
5	Antimicrobial activity
5.1	Antibacterial activity
5.2	Antiviral
5.3	Antifungal
5.4	Antiparasite
6	Other activities
6.1	NOS inhibition
6.2	Hypertension
6.3	Neurodegeneration
6.4	Depression/anxiety
6.5	Ischemia/reperfusion injury
6.6	Osteoporosis
6.7	Diabetes complications
6.8	Ricin intoxication

Abbreviation: NOS, nitric oxide synthase.

series of B vitamins, such as folic acid or folate, also known as vitamin B9, and riboflavin (vitamin B2), which are considered important dietary supplements on the World Health Organization's List of Essential Medicines.[9,10]

In the case of neopterin, although its function is not totally clear, it is considered as a marker of immune activation, as it is mainly produced by monocyte-derived macrophages and dendritic cells upon activation by interferon γ (IFN-γ) produced by T_H1 cells.[11] Neopterin is used as a biomarker for cancer prognosis because it is increased in many cancer types and has been correlated with advanced tumor stages.[12]

Given the crucial role of pteridines in health and sickness conditions these compounds have long been in the spotlight of biomedical and medicinal chemistry. Hence, many pteridine derivatives have been synthesized and tested for their biological activities, demonstrating a great potential for drug development by targeting a wide array of human pathologies including, cancer, chronic inflammatory diseases, microbial infections, and many others.[13]

In the majority of cases, the synthesis of new pteridines has been directed to add novel substituents to the pteridine rings. Nonetheless, other approaches are based on the linkage of a pteridine derivative to metal complexes or to molecules known to inhibit a specific target, with the objective of increasing their potency by targeting two different enzymes. Nevertheless, since chemical synthesis of pteridine derivatives is not the focus of this review, information about synthetic methods explaining how pteridine-based compounds and drug candidates have been developed can be found in the comprehensive reviews by Suckling et al.[14,15]

The present review collects actualized data on the biological properties (Table 1) and targets of pteridine derivatives found in the current literature (summarized in Figure 3).

2 | METHODS

A bibliography search was performed on the Scopus and Science Direct databases using the terms "Pteridine" and "Biological Target" or "Molecular Target." Articles describing the biological activity or molecular targets of pteridine

compounds were selected. Articles that (i) were not related to pteridines, (ii) only described the synthesis but not the biological activity of pteridine derivatives, (iii) described nonbiologically active pteridines, and (iv) were reviews or not original research articles were excluded. After this first screening, 48 articles from Scopus, and 31 from ScienceDirect were selected, and a total of 63 unique papers were obtained after the exclusion of 16 repeated articles. Then, a second bibliographic screening was performed by a manual examination of the references and other cited articles, specifically searching for those compounds with a deeper and more advanced development. The same exclusion criteria as above were applied for this further bibliographic examination. Finally, a total of 120 original papers were selected. Information about clinical trials was obtained from ClinicalTrials.gov. The remaining references cited in this review were selected to establish the pteridines' therapeutic background.

3 | ANTITUMOR ACTIVITY

Cancer is the second leading cause of death worldwide, with a permanent increasing incidence, as the number of cases is expected to rise from 14 millions in 2012 to 22 millions in the next two decades.[16] Thus, there is a need for more efficient therapies that avoid tumor cell resistance and diminish the adverse effects of current treatments. In this sense, pteridine antitumor potential has been widely studied to search for novel anticancer drugs. We describe below those pteridine derivatives with antitumor potential, focusing on the different proteins and mechanisms targeted by these compounds.

Methotrexate (MTX) is probably the most used pteridine derivative since its first description in the 1950s.[17] Methotrexate is an antifolate that inhibits dihydrofolate reductase (DHFR),[18] although other targets have also been described lately.[19] DHFR catalyzes the NADPH-dependent dihydrofolate reduction to tetrahydrofolate, which is an essential reaction for the synthesis of purines and thymidine. Thus, MTX prevents DNA synthesis and, in turn, provokes cell death. MTX has been used as an anticancer agent during the past half-century, either alone or in combination with other chemotherapeutic agents to treat breast cancer, cutaneous T cell lymphoma, lymphocytic leukemia, lung cancer, and advanced-stages of non-Hodgkin lymphomas, among others (Figure 2).[20] To overcome some disadvantages of MTX treatment, such as its bioavailability or toxicity, several approaches for MTX delivery have been designed based on the use of dendrimers, liposomes, nanoparticles, or polymer conjugates (reviewed by Abolmaali et al).[21]

Pralatrexate (Figure 2) is another DHFR inhibitor with antitumor properties, specifically against lymphomas, with better results than MTX in both in vitro and in vivo studies.[22] It was approved in 2009 by the Food and Drug Administration (FDA) for the treatment of relapsed or refractory peripheral T-cell lymphoma.[23]

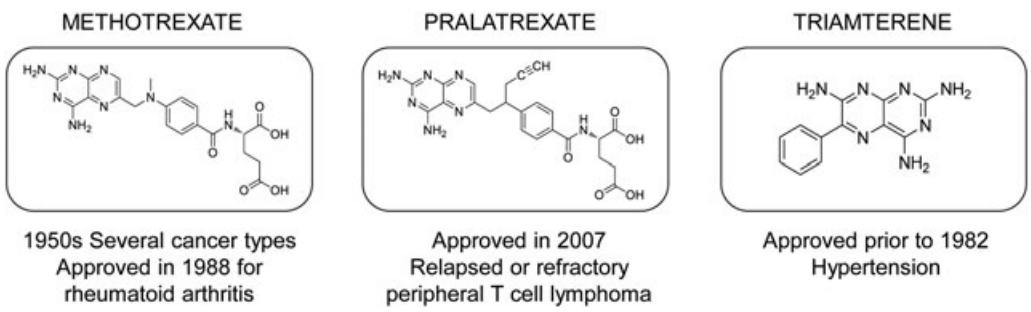


FIGURE 2 Chemical structure of FDA approved pteridine derivatives methotrexate, pralatrexate and triamterene, indicating date of approval and therapeutic use. FDA, Food and Drug Administration

Other potential targets for cancer treatment are carbonic anhydrases (CA), whose IX and XII isoforms are abundant in tumors and have been related to cancer progression.[24,25]

In a multitarget approach to inhibit both DHFR and CA, several arylsulfonamide- and arylsulfonate-diaminopteridine conjugates were synthesized and analyzed. Results from this study showed that benzene sulfonamide compounds (**1a-d**) were more active and selective against the CA IX isoform (K_i ranging from 2.1 to 4.7 nM; Table 2) than the reference compounds, acetazolamide and *N*-(4-sulfamoylphenylethyl)-4-sulfamoylbenzamide (K_i = 25 and 18 nM, respectively), which are drugs that are already proven to be active and are commonly used. Molecular docking also suggested that sulfonamide may bind more efficiently to the catalytic zinc in CA. In contrast to CA, the inhibitory potential of the same compounds (**1a-d**) against DHFR was weaker, finding in this case values of K_i in the micromolar range. Nevertheless, despite those remarkable results, when these compounds were tested in cell proliferation assays, they were just able to act as antitumor agents against nonsmall cell lung carcinoma (A549) and prostate carcinoma (PC3) cell lines when used at much higher doses, in the range of millimolar levels. Altogether, these results indicate that these compounds show promising antitumor activities, but they also point out the necessity of an improvement of their cell intake properties to be considered as really effective antiproliferative agents against tumor cells.[26]

Another dual-target approach was directed to inhibit both phosphatidylinositol 3 kinase α (PI3K-α) and mammalian target of rapamycin (mTOR). Both are part of the so-called PI3K-α/Akt/mTOR signaling pathway implicated in cell proliferation and survival, which is related to cancer.[27] Starting from a nonselective kinase inhibitor 2-aminopyridopyrimidone, a series of 4-methylpteridinones was synthesized and tested, resulting in activity against both enzymes. Structure-activity relationship (SAR) studies suggested that the C4 methyl group was critical for selectivity as it fills the unique PI3K/mTOR binding pocket and that heteroaryl groups at the C6 position improved the potency against PI3K. Compound **2**, the most active one (PI3K K_i = 2.8 nM and mTOR K_i = 6.8 nM), was orally dosed in a xenograft mouse model of U87 glioma cells, showing that the tumor volume of treated animals was indeed decreased down to 25% of the tumor volume observed in the control group of mice administered with the vehicle.[28]

Cancer cells have enhanced rates of glycolysis, producing excessive amounts of lactate, which is transported out of the cell by several monocarboxylate transporters (MCT1-4). MCT1 and MCT4 are highly expressed in most aggressive tumor types,[29] which make them suitable targets for cancer treatment. Some pteridine trione and dione derivatives (compounds **3** and **4a-c**, respectively) were active against MCT1, as they inhibited the proliferation of Raji Burkitt lymphoma cells expressing MCT1 but not MCT4, as well as MCF7 breast cancer cells engineered to overexpress mouse MCT1. C-lactate transport in these cells was also inhibited, which correlated with the antiproliferative effects. Compounds **3** and **4a-c** shared a 1-naphtylmethyl and an isobutyl substituent and have different hydroxyl groups present in alkyl and thioether-containing tethers that may be important for their activity, as was noted in the SAR studies. Other related sulfoxides, sulfones, amides, and triazoles, which differed from **3** and **4a-c** in the hydroxyl group, were also studied, but they were inactive.[30]

Another potential target for cancer treatment is FMS-like tyrosine kinase 3 (FLT3). FLT3 is involved in hematopoietic cell development through signal transduction implying the Ras/MAPK and PI3K/Akt pathways. FLT3 is frequently mutated in acute myeloid leukemia (AML), leading to constitutive ligand-independent activation of the target and conferring drug-resistance.[31] A SAR study of a series of pteridine-7(8*H*)one-based compounds lead to compound **5**, which included the following favorable structural features: (1) a 4-methylpiperazinyl group and a methyl group in the phenyl substituent, and (2) the absence of a Michael acceptor in the aminophenyl radical. Compound **5** inhibited FLT3 (IC_{50} = 1.56 nM, K_d = 0.25 nM) and some of its downstream signaling proteins. It was also cytotoxic to several cancer cell lines, including the AML cell line MV4-11, which contains an FLT3-activating mutation commonly found in AML patients (IC_{50} = 51 nM). This compound also caused cell cycle arrest in G_0/G_1 phase and cell death, as well as dose-dependent tumor growth inhibition in a MV4-11 xenograft mouse model.[32]

The same research group had previously synthesized and optimized a similar series of pteridine-7(8*H*)-dione derivatives and tested their inhibitory potential against wild-type and mutant epidermal growth factor receptor

(EGFR) tyrosine kinase. This protein is related to cell proliferation, adhesion, and differentiation, and its mutation also induces drug-resistance to EGFR tyrosine kinase inhibitors, such as gefitinib.[33] Compounds **6a** and **6b** were the most potent compounds in the series, inhibiting both wild $EGFR^{WT}$ (IC_{50} = 1.21 and 3.82 nM) and mutant enzyme $EGFR^{L858R/T790M}$ (IC_{50} = 0.68 and 1.07 nM).[34] Similar to compound **5**, compounds **6a** and **6b** have a 4-methylpiperazinyl group, but a methoxy group instead of a methyl one and have a Michael acceptor. They were optimized in a second round of SAR studies to get compound **7** (IC_{50} $EGFR^{L858R/T790M}$ = 0.3 nM and $EGFR^{WT}$ = 2 nM), with the same substituent as **6b** but a 6,7-dioxo-6,7-dihydropteridine core.[35] These compounds had antiproliferative effects in several cell lines carrying either $EGFR^{WT}$ (A431) or different mutant EGFR, including the gefitinib-resistant cell line H1975. Additionally, **6b** and **7** reduced the tumor volume when evaluated in a xenograft mouse model of H1975.

Histone deacetylases (HDACs) have also been postulated as potential targets in anticancer therapy. These proteins catalyze the deacetylation of lysine groups on histones and other proteins. The deacetylation of histones leads to chromatin condensation, impeding the transcription of the correspondent DNA section. Thus, cancer cells use HDACs to limit the transcription of proapoptotic cells.[36] As HDACs are expressed in many healthy cells, the drugs targeting HDACs present numerous side effects. A strategy to overcome this problem is to direct the compounds to cancer cells. Taking advantage of fact that several tumor cells display overexpressed folate receptor, Sodji et al[37] synthesized pteroate hydroxamate derivatives that were formed by a pteroate acid recognized by the folate receptor joined by a methylene linker to a zinc-binding group, which is essential for HDAC inhibition. The results showed that the pteroate-based hydroxamates were active against several HDAC isoforms, and the optimal length of the linker was five (**8a**) and six (**8b**) methylene groups (IC_{50} = 16.1 and 10.2 nM, respectively). As expected, compounds **8a** and **8b** were cytotoxic to cancer cells expressing folate receptor (ie, KB and HeLa cell lines), but not to cells lacking it (ie, A549 cells). Immunoblotting assays revealed that cytotoxicity was due to the simultaneous inhibition of both the HDAC1 and HDAC6 isoforms.

Some chemotherapeutic agents modify the O6-position of DNA guanine residues to produce a mutation that triggers cell death. Nonetheless, human cancer cells develop resistance to these drugs by the action of O6-alkylguanine-DNA alkyltransferase, a DNA repair protein.[38] Therefore, to block this resistance effect, alkyltransferase inhibitors should be used in combination with this kind of anticancer drugs. 2-Amino-O4-benzylpteridine derivatives are potent against the referred alkyltransferase, although the presence of DNA decreased its inhibitory potential (IC_{50} = 0.01 to 0.4 μM). Among the tested compounds, O4-benzylfolic acid (compound **9**) was the most potent one. Furthermore, these compounds enhanced the cytotoxic effect of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) against A549, KB, and HT-29 cells (ED_{90} of compound **9** = 5 μM with BCNU 40 μM), which are resistant to BCNU alone and are more effective in cells with higher expression levels of the α-folate receptor.[39]

Polo like kinase 1 (Plk1) controls multiple steps in mitosis and is highly expressed in proliferating tissues and thus in several cancers.[40] Some dihydropteridinones targeting Plk have been widely investigated. compound **10**, also known as Volasertib or BI-6727, was first described as an antitumor agent by Rudolph et al.[41] It principally inhibits Plk1 (IC_{50} = 0.87 nmol/L) but also Plk2 and Plk3 at lower levels (IC_{50} = 5 and 56 nmol/L, respectively). BI-6727 was active against several cancer cell lines and effective in various xenograft rodent models, presenting high levels of tissue penetration. In regard to cell cycle, BI-6727 provoked retention in G_2/M phase at 24 hours, followed by entrance into apoptosis and an increase of a sub-G_1 peak. Further studies focused on Volasertib biological activities, including clinical trials, have been completed (reviewed in Van den bossche et al[42]), either using it alone or in combination with other chemotherapeutic agents.[43] The first results established Volasertib as a promising treatment of AML, although a phase III study gave disappointing results, as the percentage of AML patients with objective response was not statistically significant between those treated with low-dose cytarabine (LDAC) plus placebo and those who received Volasertib plus LDAC. Indeed, the combined therapy showed a higher risk of fatal infections (Table 6).[44] BI-2536 (compound **11**) another dihydropteridinone, inhibited Plk1 (IC_{50} = 0.83 nM) as well, with a similar profile to Volasertib regarding the cell cycle. BI-2536 blocked the

proliferation of multiple cancer cell types and inhibited the growth of carcinomas in xenograft models.[45] Several phase I and II trials have been perfomed,[46–48] showing modest efficacy, which situates this compound below Volasertib (Table 6).

To identify highly selective Plk2 inhibitors, Plk1, 2, and 3 kinase-inhibitors complexes were aligned and compared. Based on the differences in the amino acid residues, researchers designed and synthesized two series of BI-2536 derivatives. In the first series, the BI-2536 amide group was replaced with a sulfamide group, while in the second series an indole group was added. The series' in vitro inhibitory potential against Plk isoforms 1, 2, and 3 was measured and a Plk2 selectivity index (SI) was calculated. In the case of the first series, the addition of a methyl group in the sulfamide (compound **12**) gave the best results (Plk2 IC_{50} = 3.4 nM), and bigger aliphatic groups worsened the inhibitory potential. In the second series, introducing short aliphatic groups into the amide gave good results, with compound **13** being the most potent (Plk2 IC_{50} = 4.88 nM), highlighting its strong selectivity against Plk2 compared to Plk3 (Plk3 SI = 910.16). In both series, the replacement of the 8-cyclopentane by a methyl-pyrazole moiety strongly reduced the inhibitory activity. The in vitro antiproliferative potential of the compounds against several cancer cell lines was also measured, revealing that compound **12** was generally more potent (IC_{50} = 0.099 to 1.41 μM) than **13** (IC_{50} = 0.418 to 1.12 μM).[49]

Kiryanov et al[50] have also investigated novel Plk1 inhibitors. In previous works, they had identified the interactions of chemical inhibitors with the Plk1 Lys82 via a bound water molecule. Based on this discovery, they designed and synthesized a series of 5,6-dihydroimidazol[1,5-*f*]pteridines that bound Lys82 and inhibited Plk1 activity under 50 nM. To further improve the ADME properties in rodents, they performed structural changes in the nitrile-containing compounds, obtaining compound **14**. This compound presented high microsomal stability, induced histone H3 phosphorylation, and had high absorption in xenograft mouse models.

Bromodomains are highly conserved proteins that recognize ε-acetylated lysine residues. The bromodomain-and-extra terminal domain (BET) family has four members, including bromodomain-containing protein 4 (BRD4), and is involved in several biological processes such as epigenetic regulation.[51] It has been shown that BI-2536 inhibits BRD4 as well as Plk1, which are implicated in AML. Since dual targeting molecules may be a potential strategy for anticancer agents, Chen et al[52] performed a SAR study with BI-2536 analogues. The compound that inhibited both enzymes at the highest level was **15a** (BRD4 K_i = 8.7 nM and Plk1 K_i = 5.8 nM), although when measured in a cell viability assay with MV4-11 cells, compound **15b** gave the lowest IC_{50} among the series (3 nM). Compound **15a** differs from BI-2536 in the replacement of the cyclopentyl group by a 3-bromobenzyl moiety, and comparison by molecular modeling showed that both compounds bind to BRD4 with a similar pattern, suggesting that this substituent could dictate the selectivity. Another conclusion of the SAR study is that the substitution of the pyrimidine NH with an oxygen atom converts the molecule into a BRD4-selective inhibitor. In the same line, Koblan et al evaluated the "BET-BRET" assay, which is a cell-permeable fluorophore-tagged BET bromodomain ligand in a bioluminescence resonance energy transfer (BRET) assay, to perform high-throughput screenings (HTS) of BET-inhibitors. They evaluated several compounds described in the Chen et al work[52] as well as new BI-2536 analogues. The most potent was compound **16**, which inhibited BRD4 (IC_{50} = 130 nM) but not Plk1. This enhanced selectivity is due to the methylation of the BI-2536 pyrimidine NH, which is similar to Chen et al SAR results. When compound **16** was tested in MV4-11 cells, it was cytotoxic (IC_{50} = 184 to 218 nM), although less than BI-2536, suggesting that the inhibitory activity of BI-2536 is mostly due to Plk1 inhibition.[53]

Ribosomal S6 kinases (RSKs) are involved in cell cycle progression and survival and play a role in cancer development.[54] Sapkota et al described BI-D1870 (compound **17**) as a specific inhibitor of the four RSK isoforms by competition with ATP at the N-terminal domain (up to 99% inhibition at 10 μM), confirming results on RSK-related pathways in HEK-293 and Rat2-2 cells.[55] After this first description, BI-D1870 has been widely used in cell-based studies.[56] Some results demonstrated the antitumor potential of BI-D1870, as it inhibited proliferation in myeloma,[57] medulloblastoma,[58] and oral squamous cell carcinoma, and induced G2/M arrest and apoptosis, ER stress, and ROS generation.[59] It also has a role as an inflammation regulatory agent, as BI-D1870 inhibited IL-6 and IL-10, but not tumor necrosis factor α (TNF-α), production from dendritic cells,[60] and has been related to Th17

differentiation in mice with induced encephalomyelitis.[61] Other suggested targets for BI-D1870 include Slk, Lok, and Mst1,[60] p21[62] and the mTORC1 signaling pathway.[56]

The normal function of transforming growth factor β (TGF-β) on epithelial cells is tumor suppressive; nonetheless, this molecule is double-faced, as it has been related to tumor progression and metastasis when acting on cancer cells. TGF-β causes the successive activation of TGF-β receptors I and II (TGFβRI and TGFβRII) kinase activity, Smad2 and 3 phosphorylation, and the formation of the Smad3/4 heterocomplex, which acts as a transcription factor for genes related to tumor progression and invasion.[63] SD-208 (compound **18**) was identified as a TGFβRI inhibitor (IC_{50} = 0.048 μmol/L).[64] This compound has been widely used to study TGF-β function in many systems, and the results confirmed its potential to treat and prevent metastasis. Several studies showed that cancer cells exposed to SD-208 had lower p-Smad3 and Smad3/4 complex levels, as well as less TGF-β-induced tumor progression gene expression.[64–66] Incubation of a coculture of irradiated glioma cells and peripheral blood lymphocytes or purified T cells in the presence of SD-208 restored the release of IFN-γ and TNF-α, while reducing secretion of IL-10 from immune cells.[64] Different xenograft mouse models treated with this compound showed a reduction in tumor growth and metastasis-preventing potential with major immune cell infiltration.[64–66] Nonetheless, other metastasis models obtained opposite results because the TGF-β effect in that case was actually tumor-protective,[67] thus highlighting the limitations of targeting this molecule.

Song et al[68] recently studied the potential of TG100-115 (compound **19**) as an inhibitor of migration and invasion in breast cancer cells. Wound-healing and transwell invasion assays with MDA-B-468 breast cancer cells and T-Rex-293 cells exposed to this compound showed an inhibition of migration and invasion, with no cytotoxicity. This effect may be due to the inhibition of the transient receptor potential channel subfamily M member 7 (TRPM7), a protein required for breast cancer proliferation and migration.[69] This was verified in in vitro kinase assays performed using the TRPM7 kinase domain and recombinant human CREB, where phosphorylation of CREB diminished in the presence of TG100-115. It also reversibly reduced TRPM7 channel activity, measured by a whole patch-clamp technique. Molecular docking analysis indicated that TG100-115 fits in the enzyme ATP binding pocket, and concentration-response curves confirmed a competitive binding mode. However, inhibition of PI3K p110δ could have also been caused by TG100-115 action.[68]

The heat shock protein Hsp90 has also been identified as a target for cancer therapy. Starting from a previously designed Hsp90 inhibitor, Li et al[70] performed a series of SAR studies focused on several positions in its core structure, 2-amino-7,8-dihydropteridin-6(5*H*)-one. Evaluation of Her2 (a Hsp90 client protein) degradation in SKBR-3 cells, affinity to Hsp90, and cytotoxicity in human tumor and normal cell lines lead to the identification of multiple compounds, among which compound **20** was active and displayed the highest selectivity for cancer cells. The SAR study identified the important role of the 6-carbonyl and the need for a hydrophobic group at the 5-position.

Janus-associated kinases (Jak) are a family of nonreceptor tyrosine kinases. Upon cell activation by cytokines or growth factors, autophosphorylated Jaks trigger signal pathways involving signal transducer and activator of transcription (STATs), which lead to cell proliferation and arrest of apoptosis.[71] Deregulation of Jak/STAT signaling is a common feature in myeloproliferative neoplasm patients. Thus, several approaches have been made to develop Jak inhibitors. Among them, 1-methyl-1*H*-imidazoles appear to be promising Jak-inhibitors; thus, Su et al[72] synthesized and tested some derivatives of this scaffold, including compound **21**, which has a pteridine ring as a substituent. It was able to inhibit Jak1 and Jak2 in vitro, as well as the proliferation of BaF3 TEL-Jak2 cells. However, compounds with different substituent groups gave even better results.

An extensive series of flavine derivatives were synthesized and analyzed as antitumor agents. Some of them showed cytotoxic activity against human tumor cell lines, including: NCI-H 460 (lung), HCT 116 (colon), A 431 (adenocarcinoma), CCRF-HSB-2 (T-cell acute lymphoblastoid leukemia), and KB (oral epidermoid carcinoma). Indeed, compounds **22a** and **22b** reached better results than cisplatin, the reference compound, in HCT 116 cells (IC_{50} = 1.8 and 0.72 μM, respectively). Molecular docking studies for binding to protein tyrosine kinase (PTK) showed low binding free energies (Δ*G*) and K_i values for several compounds, especially for 2-deoxo-2-phenylflavin-

5-oxides (**22**), which displayed ΔG values between −7.84 and −5.95 kcal/mol. The binding free-energy levels correlated with the IC_{50} values obtained against cancer cell lines.[73,74]

Malignant cells often induce an immunosuppressive state to avoid cell death programming. An experimental approach in cancer immunotherapy has been the generation of dendritic cell vaccines, in which blood monocytes from patients are differentiated to dendritic cells ex vivo.[75] These cells are stimulated with toll-like receptor (TLR) agonists to exert an immune response,[76] and finally reinoculated into the host. To identify new and easy-to-produce TLR2 agonists, an extensive library of compounds was analyzed in silico, with compound **23** (8-amino-1,3-dimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) being one of the retrieved compounds. To assess whether it activated the TLR2 intracellular signaling cascade, a nuclear factor-κB–dependent luciferase assay was performed in HEK293-TLR2 cells, showing that costimulation of cells with **23** together with the lipopeptide Pam_3CSK_4 increased TLR2 signaling. The absence of increased luciferase signal in the cells lacking TLR2 suggested the specificity of **23** for this membrane receptor. Moreover, TNF-α production increased when peripheral blood mononuclear cells (PBMCs) were preincubated with compound **23** before the addition of the lipopeptide. It also induced the production of IL-12 in in vitro differentiated dendritic cells, which is indicative of Th1 polarization. Finally, docking studies showed that the lipopeptide and compound **23** bind to the TLR1-TLR2 complex at different sites and that they are allosteric agonists.[77]

Many studies have proven the anticancer potential of pteridine derivatives, although the biological target has not been identified. In this sense, Chauhan et al synthesized a series of 6,7-dimethylpteridine derivatives having different heterocycles in position 2 linked through a thioether group or an alkylamino group at position 4. Some of them, including compound **24**, showed cytotoxicity against MCF7 (breast), NCI-H460 (lung), and SF-268 (central nervous system) cancer cell lines at 0.1 μM, although the maximum inhibitory effect upon the growth of the treated cells did not go lower than 57%.[78]

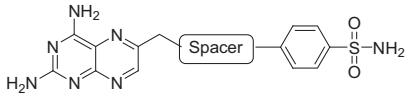
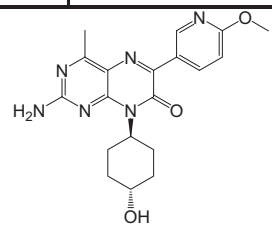
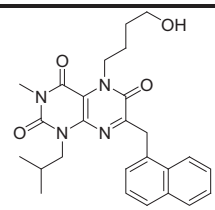
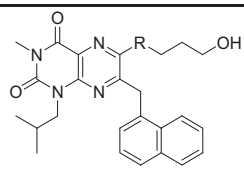
A novel series of 5,8-dihydropteridine-6,7-diones was recently synthesized and tested on several cancer cell lines, including MGC-803 and SGC-7901 (gastric cancer), A549 (lung), and PC-3 (prostate). Among the tested derivatives, those with piperazine substituent gave the best results. Concretely, **compound 25** presented the most potent antitumor activity, with IC_{50} values lower than 20 μM against all the tested cell lines. This compound also reduced MGC-803 cell colony formation (an indirect measurement of neoplastic transformation) and the migration of these cells in a wound-healing assay. Moreover, compound **25** showed a SI of 4.36 between MGC-803 cancer cells and the normal gastric epithelial cell line GES-1 (IC_{50} = 8.78 and 38.3 μM, respectively).[79]

Similarly, another series of pteridine-7(8*O*)-one derivatives was synthesized[79] and tested on the MKN-45 and MGC-803 (gastric cancer), H1650 (lung cancer), and EC-109 (esophageal cancer) cell lines. Among the tested series, compound **26** displayed IC_{50} levels under 10 μM in every case. Further analysis suggested that it induced apoptosis, as demonstrated by a propidium iodide/annexin V assay and confirmed by increased Bax expression, together with decreasing levels of Bcl-2, and caspase-3 and 9 cleavage.[80]

Two novel 6-azapteridines, **27a** and **27b**, were synthesized and tested for their antiproliferative activities. Concretely, when tested in MCF7 and K562 cancer cells **27a** gave IC_{50} values of 8.3 and 12.9 μmol/L, while the IC_{50} values for **27b** were 7.2 and 14.6 μmol/L, respectively.[81] In contrast, two 2-(*N*,*N*-dimethyl-aminomethyleneamino)-3-pivaloylpteridin-4-ones, **28a** and **28b**, were tested in the Panc-1 cancer line. Compound doses from 2 mM (**28a**) and 400 μM (**28b**) decreased cell viability and augmented the cell death induced by UV-A irradiation. The results also showed that **28a** induced G2/M arrest.[82]

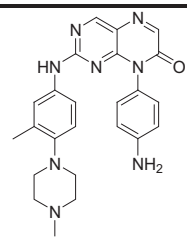
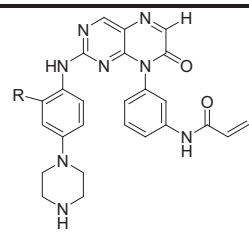
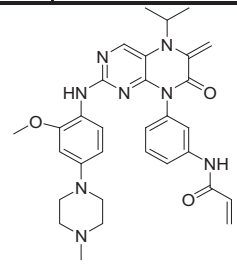
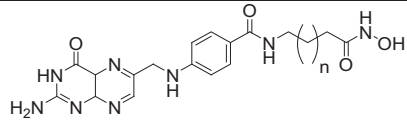
Lumazine is a pteridine-2,4(1*H*,3*H*)dione. Several approaches combining lumazines with metal complexes have been performed. One of them was the reaction of 6-acetyl-1,3,7-trimethyllumazine with $[ReCl(CO)_5]$ to give chloro-fac-tricarbonylrhenium(I) compounds **29a-c**. These rhenium complexes were tested against the NB69 neuroblastoma, U373 glioma, and the MCF-7 (hormone-dependent) and EVSA-T (hormone-independent) breast cancer tumor cell lines. In all cases, the lowest concentration tested (2 μM) led to an increased proliferation, whereas higher doses diminished cell growth when compared to the control. No further studies were performed to clarify the mechanism of this phenomenon.[83]

TABLE 2 Antitumor pteridine compounds

[a]	**Target**[b]	**Structure**	**Activity**[c]	**Method**[d]	**Ref**
1	CA IX		CA IX K_i = 2.1-4.7 nM DHFR IC_{50} = 1.8-20 μM	In vitro Docking	[26]
		Compound / **Spacer** **1a** / -NH- **1b** / -NH-CH_2-CH_2- **1c** / -*pABA*-NHCH_2-CH_2- **1d** / -NMe*p*ABA-NHCH_2-CH_2-			
2	PI3K mTOR		PI3K K_i = 2.8 nM mTOR K_i = 6.8 nM	In vitro In vivo	[28]
3	MCT1		Raji lymphoma cells: IC_{50} = 150 nM Lactate transport: IC_{50} = 548 nM	In vitro	[30]
4	MCT1		Raji lymphoma cells: IC_{50} = 37-70 nM MCF7 cells: IC_{50} = 439-570 nM		
		Compound / **R** **4a** / SCH_2 **4b** / CH_2CH_2 **4c** / *cis* CH=CH			

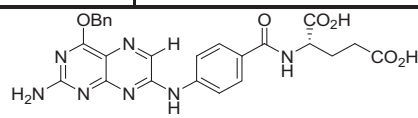
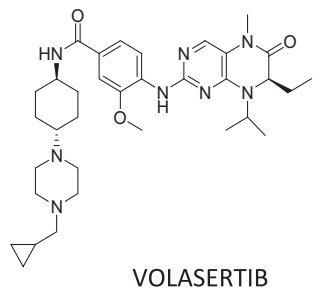
(Continues)

TABLE 2 (Continued)

5	FLT3		FLT3 IC_{50} = 1.56 nM MV4-11 cells. IC_{50} = 51 nM	In vitro In vivo Docking	[32]
6	EGFR	 **Compound** / **R** **6a** / H **6b** / OMe	$EGFR^{WT}$ IC_{50}: 6a = 1.21 nM 6b = 3.82 nM $EGFR^{L858R/T790M}$ IC_{50}: 6a = 0.68 nM 6b = 1.07 nM H1975 cell line. IC_{50}: 6a = 62.2 nM 6b = 59 nM	In vitro In vivo Docking	[34]
7	EGFR		$EGFR^{WT}$ IC_{50} = 2 nM $EGFR^{L858R/T790M}$ IC_{50} = 0.3 nM Cell lines: A431 IC_{50} = 7773 nM H1975 IC_{50} = 18 nM	In vitro In vivo Docking	[35]
8	HDAC	 **Compound** / **n**	HDAC1 IC_{50}: 8a = 16.1 nM 8b = 10.2 nM	In vitro	[37]

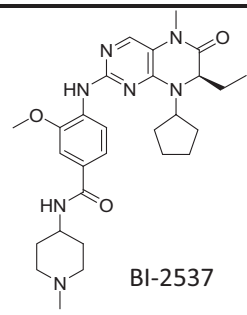
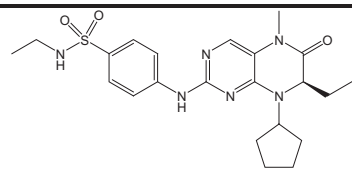
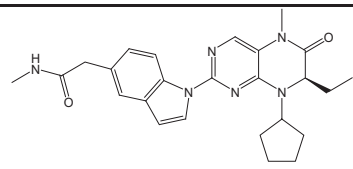
(Continues)

TABLE 2 (Continued)

		8a	5	Cell lines: KB IC_{50}: 8a = 30.3 μM 8b = 33 μM HeLa IC_{50}: 8a = 56.6 μM 8b = 35 μM		
		8b	6			
9	O^6-alkylguanine-DNA alkyltransferase			O6-Alkyltransferase IC_{50} = 0.01 μM HT29 cell line IC_{90} = 15 μM (+BDRU 40 μM)	In vitro	39
10	Plk1	 VOLASERTIB		Plk1 IC_{50} = 0.87 nmol/L Plk2 IC_{50} = 5 nmol/L Plk3 IC_{50} = 56 nmol/L Cell lines: HCT 116 IC_{50} = 23 nmol/L NCI-H460 IC_{50} = 21 nmol/L	In vitro In vivo	41

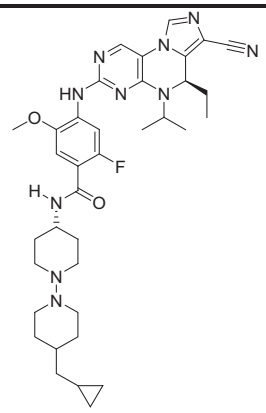
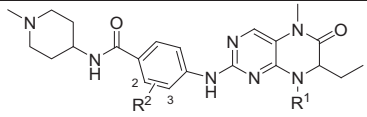
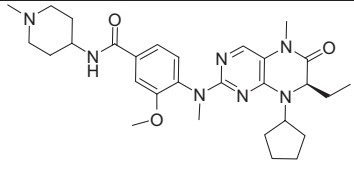
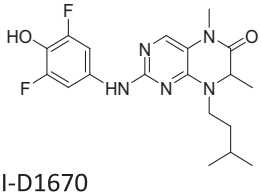
(Continues)

TABLE 2 (Continued)

11	Plk1	 BI-2537	Plk1 IC_{50} = 0.83 nM Plk2 IC_{50} = 3.5 nM Plk3 IC_{50} = 9 nM	In vitro In vivo	[45]
12	Plk2		Plk 2 IC_{50} = 3.4 nM Cell lines IC50: K562 = 0.222 μM MCF-7 = 0.151 μM HuH-7 = 0.099 μM A549 = 0.183 μM H1975 = 1.410 μM HeLa = 0.148 μM	In vitro Docking	[49]
13	Plk2		Plk 2 IC_{50} = 4.88 nM Cell lines IC50: K562 = 0.428 μM MCF-7 = 0.784 μM HuH-7 = 0.596 μM A549 = 1.120 μM H1975 = 0.882 μM HeLa = 0.418 μM		

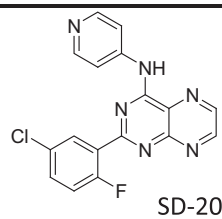
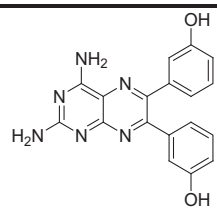
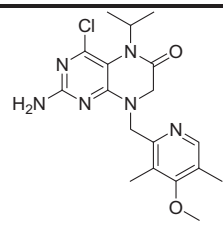
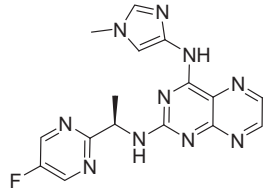
(Continues)

TABLE 2 (Continued)

14	Plk1		Plk1 IC_{50} = 3.9 nM HT29 cell line IC_{50} = 22 nM	In vitro In vivo Docking	[50]
15	BRD4	 **Compound** / **R^1** / **R^2** **15a** / 3-Br-$C_6H_4CH_2$ / 3-OMe **15b** / Cp / H	BRD4 K_i : 15a = 8.7 nM 15b = 60 nM Plk1 K_i : 15a = 5.8 nM 15b = ND MV4-11 cell line IC_{50}: 15a = 675 nM 15b = 3 nM	In vitro	[52]
16	BRD4		BRD4 IC_{50} = 130 nM MV4;11 cell line IC_{50} = 184-218 nM	In vitro Docking	[53]
17	Rsk	 BI-D1670	Rsk1 IC_{50} = 31 nM Rsk2 IC_{50} = 24 nM Rsk3 IC_{50} = 18 nM Rsk4 IC_{50} = 15 nM	In vitro	[55]

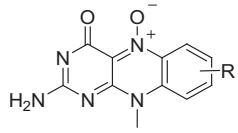
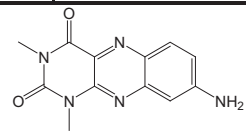
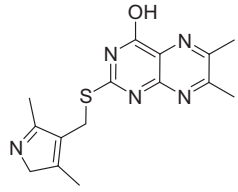
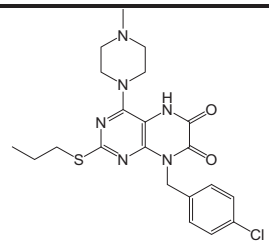
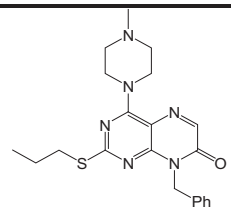
(Continues)

TABLE 2 (Continued)

18	TGF-βRI	 SD-208	TGF-βRI IC_{50} = 0.048 μmol/L	In vitro In vivo	64
19	TRPM7	 TG100-115	TRPM7 IC_{50} = 1.07 μM	In vitro Docking	68
20	Hsp90		Her2 degradation IC_{50} = 50 nM Cell lines: MCF-7 IC_{50} = 50 nM HT29 IC_{50} = 60 nM SKBR-3 IC_{50} = 20 nM BT474 IC_{50} = 30 nM hMEpicC IC_{50} = 320 nM	In vitro	70
21	Jak		Jak1 IC_{50} = 1.5 μM Jak2 IC_{50} = 0.8 μM BaF3 TEL-Jak2 cell line IC_{50} = 0.53 μM	In vitro Docking	72

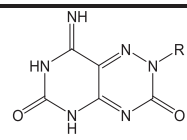
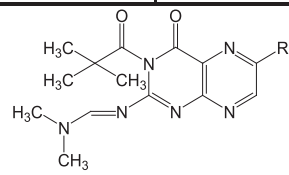
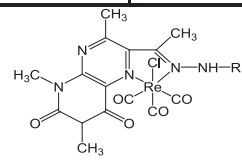
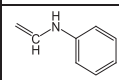
(Continues)

TABLE 2 (Continued)

22	PTK	 **Compound** / **R** **22a** / H **22b** / 7-OMe	IC_{50} HCT 116 cell line = 22a: 1.8 μM 22b: 0.72 μM	In vitro Docking	73,74
23	TLR2		IC_{50} = 5.26 nM	In vitro Docking	77
24	Tumor cell lines		Percentages of growth after cell lines treatment: MCF-7 = 57% NCI-H460 = 66% SF-268 = 62%	In vitro	78
25	Tumor cell lines		MGC-803 IC_{50} = 8.78 μM SGC-7901 IC_{50} = 17.89 μM A549 IC_{50} = 18.07 μM PC-3 IC_{50} = 11.76 μM GES-1 IC_{50} = 38.3 μM	In vitro	79
26	Tumor cell lines		MGC-803 IC_{50} = 7.01 μM MKN-45 IC_{50} = 4.32 μM H1650 IC_{50} = 9.92 μM EC-109 IC_{50} = 9.85 μM	In vitro	80

(Continues)

TABLE 2 (Continued)

27	Tumor cell lines		MCF7 IC_{50}: 27a = 8.3 μmol/L 27b = 7.2 μmol/L K562 IC_{50}: 27a = 12.9 μmol/L 27b = 14.6 μmol/L	In vitro	[81]
		Compound / **R** **27a** / phenyl **27b** / 4-bromophenyl			
28	Tumor cell line		ND	In vitro	[82]
		Compound / **R** **28a** / - **28b** / CHO			
29	Tumor cell lines		ND	In vitro	[83]
		Compound / **R** **29a** / H **29b** /  **29c**			

(Continues)

TABLE 2 (Continued)

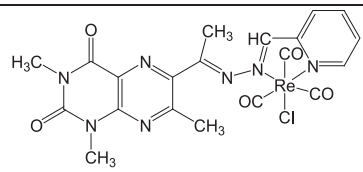
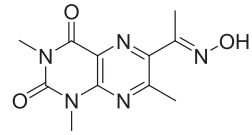
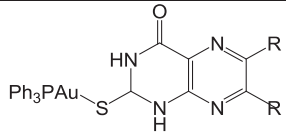
					
30	APA	$[Ag_2L_2X_2]_n$ L:  $X = ClO_4^-, NO_3^-, CF_3SO_3^-$	NB69 cell line IC_{50} = 5.18 – 16.37 μM U373-MG cell line IC_{50} = 5.25 – 7.90 μM	In vitro	[84]
31	Tumor cell lines	 **Compound** / **R** **31a** / H **31b** / CH_3 **31c** / 1, 8-Naphthylene	MCF7 IC_{50} = 0.5 μM A549 IC_{50} = 5 μM PC3 IC_{50} = 5 μM LOVO IC_{50} = 5 μM	In vitro	[86]

Table displays the structure and activity details of the pteridine derivatives that showed antitumor potential.
Abbreviations: IC_{50}, half-maximal inhibitory concentration; K_i, inhibition constant; ND, not determined, or the reference does not give exact data, or only shows data graphically.
[a]Compound number.
[b]Main molecular target tested.
[c]Main activity data extracted from the references.
[d]Method used to determine compound activity. In vitro biochemical assays or cell-based assays; in vivo assays in mice or rat models; molecular docking or crystallographic assays.

Similarly, the same research group synthesized silver(I)/6-hydroxyminolumazine complexes (compound **30**). Again, the authors tested the effects of the silver-lumazine complexes against NB69 and U373-MG cells. All compounds were active in cell cultures, with IC_{50} values varying from 5.25 to 16.37 μM. The tested compounds promoted apoptosis in U373-MG cells, and induced death by necrosis in NB69 cells, demonstrating their in vitro antitumor activities.[84] Furthermore, these authors analyzed the effects of the complexes on the renin-angiotensin system. It is known that aminopeptidase A (APA) converts angiotensin II (AngII) to angiotensin III (AngIII), which in turn is converted to AngIV by aminopeptidase N (APN).[85] APA and APN activity assays showed an inhibitory effect

on APA activity, especially in U373-MG cells. In the case of APN, it was unaffected in NB69 cells and inhibited in U373-MG cells, suggesting a predominant action for AngII and a decrease in AngIII levels in the glioma cells.[84]

Mullice et al[86] designed an approach to improve the biological activity of pteridines in which they fused pteridine moieties to an "$Au(PR_3)$" core (R = PPh_3 or PCy_3) through a thiolate donor, obtaining compound **31**. Cytotoxicity assays were performed, observing that exposure of the MCF7 (breast), A549 (lung), PC3 (prostate), and LOVO (colon) adenocarcinoma cells lines to the gold-complexes resulted in growth inhibition in all cases (IC_{50} values ranging from 0.5 to 50 μM), with greater values for PPh_3 complexes.

4 | ANTI-INFLAMMATORY ACTIVITY

Inflammation is an immune process triggered by the presence of pathogens or damaged tissues. During the course of inflammation, the production of signaling molecules such as cytokines and the recruitment of immune cells help to eliminate pathogens and restore tissues. Nonetheless, most mechanisms directed to kill pathogens and/or to eliminate dead cells can also damage normal cells. In many diseases, such as rheumatoid arthritis, Crohn disease, asthma, or cirrhosis, the inflammatory process is dysregulated and continuously active leading to a chronic inflammation. To reduce inflammation, nonsteroideal anti-inflammatory drugs (NSAIDs) are commonly used, but due to their collateral effects they are not recommended to treat chronic diseases.

Recently, anti-TNF drugs have been developed to treat autoimmune inflammatory diseases. However, 40% of patients do not respond to these drugs, and side effects such as immunosuppression or neurological failure have been reported.[87] Therefore, research on new anti-inflammatory agents is of great interest,[88] and many pteridine derivatives have been investigated with this purpose. Those compounds with anti-inflammatory properties are listed in Table 3.

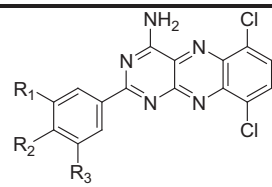
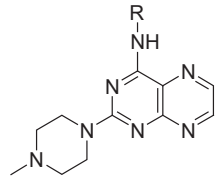
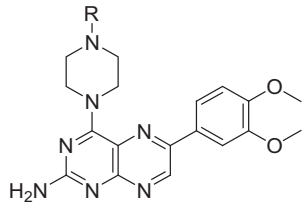
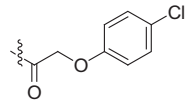
In addition to cancer treatment, methotrexate is also used in the treatment of the autoimmune diseases rheumatoid arthritis and psoriasis. In the first case, MTX is still the "anchor drug," as it is the most commonly used drug in rheumatoid arthritis, either alone or in combination with leflunomide or sulfasalazine.[89]

Our group synthesized a series of 4-amino-2-aryl-6,9-dichlorobenzo[*g*]pteridines with anti-inflammatory potential. Most pteridine derivatives from this series were able to in vitro inhibit the secretion of the proinflammatory cytokines TNF-α and IL-6 induced by lipopolysaccharide (LPS) treatment of a human macrophage-like cell model derived from HL-60 myeloid leukemia cells, without resulting in cytotoxicity. Compounds **32a**-**c** were the most active as they reached over 90% inhibition for both cytokines.[90]

Pontiki et al[91] synthesized a series of 2,4-aminopteridines that showed antioxidant activity measured by several approaches, including inhibition of soybean lipoxygenase, where 4-amino substituents played a crucial role in the inhibitory activity and 6,7-substituents generally showed lowered potency. Moreover, to gain insight into their anti-inflammatory activities, some 2-(4-methylpiperazin-1-yl)pteridin-4-amine compounds were tested in a rat model of ulcerative colitis and in the carrageenan paw edema model. In the first case, compound **33a** was the most potent compound, although further improvements should be made, since treated rats still exhibited several symptoms such as hyperemia and petechial bleeding. Regarding the paw edema, **33b** was able to reduce it by 41% after only 1 hour of treatment.

A series of 2-amino-4-*N*-piperazinyl-6-(3,4-dimethoxyphenyl)pteridine analogues were immunosuppressive when used in a mixed lymphocyte reaction (MLR), reaching in some cases nanomolar IC_{50} rates (up to 0.5 nM in the case of compound **34a**), as well as when used in in vitro assays with LPS-stimulated human peripheral blood mononuclear cells, in which they were also able to suppress TNF-α cell production, with compound **34b** reaching the lowest IC_{50} (10 nM).[92] The anti-inflammatory activities of compounds **34c** and **34d** were also confirmed in a mouse model of trinitrobenzenesulphonate (TNBS)-induced colitis used as a model for human Crohn disease, where histological scores, myeloperoxidase (MPO) activity in the colon and anti-TNBS IgG_1 antibody production were lower after treatment with **34c** and **34d**.[93,94] SAR studies performed with this series, its precursors, and related

TABLE 3 Anti-inflammatory potential of pteridine compounds

[a]	Target[b]	Structure	Activity[c]	Method[d]	Ref
32	IL-6 and TNF-α		TNF-α IC_{50} = 6-7.4 μM IL-6 IC_{50} = 22.9-25.7 μM	In vitro	[90]
		Compound / **R_1** / **R_2** / **R_3** **32a** / CH_3 / - / - **32b** / - / CH_3 / - **32c** / CF_3 / - / CF_3			
33	Lipoxygenase		Lipoxygenase IC_{50}: 33a = 37.5 μM 33b = 55 μM	In vitro In vivo Docking	[91]
		Compound / **R** **33a** / H **33b** / 			
34	TNF-α		MLR IC_{50} = 0.5-300 nM TNF-α IC_{50} = 10-80 nM	In vitro In vivo	[92–94]
		Compound / **R** **34a** / 			

(Continues)

TABLE 3 (Continued)

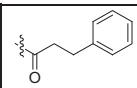
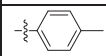
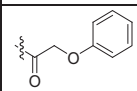
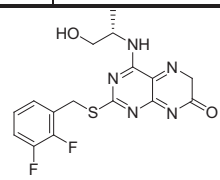
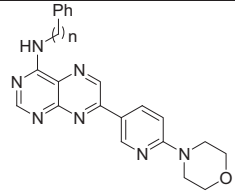
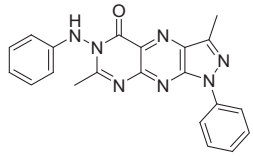
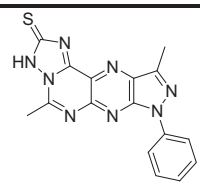
		34b 			
		34c 			
		34d 			
35	CXCR2		CXCR2 IC_{50} = 1 nM	In vitro In vivo	[96]
36	AK		AK IC_{50} = 72-120 nM	In vitro	[98]
		Compound / **n** **36a** / 2 **36b** / 3 **36c** / 4			
37	Edema		Reduction paw edema: 54.1%	In vivo	[104]
38	Edema		Reduction paw edema: 54.2%	In vivo	[104]

Table displays the structure and activity details of the pteridine derivatives that showed anti-inflammatory potential. Abbreviation: IC_{50}, half-maximal inhibitory concentration.

[a]Compound number.

[b]Main molecular target tested.
[c]Main activity data extracted from the references.
[d]Method used to determine compound activity. In vitro biochemical assays or cell-based assays; in vivo assays in mice or rat models; molecular docking or crystallographic assays.

compounds demonstrated that the 6-(3,4-dimethoxyphenyl) substituent is essential for the immunosuppressive and anti-inflammatory activities and that the derivatization of the piperazine moiety as amides or urea strongly lowered the MLR IC_{50} to sub-nM values.[92]

Inhibition of the chemokine-mediated recruitment of cells could also be used as a strategy for the treatment of inflammatory diseases.[95] A series of bicyclic compounds, including several pteridine-based compounds, were tested as antagonists of the CXCR2 receptor. Among the pteridine derivatives, compound **35** showed the best combination of properties, including good binding affinity IC_{50} values for CXCR2, rat oral bioavailability and clearance, pK_a, log *D* (distribution coefficient) and human plasma protein binding. Nevertheless, thiazolo[4,5-*d*] pyrimidine-2(3*H*)-one derivatives presented better characteristics than pteridines.[96]

Adenosine (ADO) is a short-life signaling molecule released from cells upon adverse conditions that induces a protective response; its concentration is regulated by the enzyme adenosine kinase (AK); thus, its inhibition could lead to an analgesic or anti-inflammatory effect due to increasing ADO levels.[97] The modification of a pyridopyrimidine previously identified as an AK inhibitor to obtain 4-amino-substituted pteridines (compounds **36a-c**) resulted in AK inhibition (IC_{50} values from 72 to 120 nM), although at lower inhibitory levels than the corresponding to the 5-substituted and 6-substituted pyridopyrimidines (IC_{50} values from 7.5 to 100 nM).[98]

Since the PI3K γ and δ isoforms are important mediators of inflammatory responses,[99] a dual inhibitor could have great interest in the treatment of asthma and chronic obstructive pulmonary disease (COPD), in which inflammation plays a crucial role.[100] TG100-115 (compound **19**) had been shown to inhibit both PI3K isoforms[101] (see Section 6.5 ischemia/reperfusion injury); thus, it was tested on mouse models of asthma and COPD. The results showed that aerosolized TG100-115 administered previously or after the induction of the pathology-reduced eosinophilia and hyperresponsiveness to bronchoconstrictors in the case of asthma and reduced neutrophil accumulation in COPD models.[102] The biological action of this compound was also studied in mice with concanavalin A-induced hepatitis, which is another model of inflammatory disease. In contrast with the COPD and asthma results, this study discarded TG100-115 as a therapeutic agent for hepatitis, as it increased transaminase levels, IL-2 production and hepatocyte apoptosis.[103]

Another series of pyrazolo[3,4-*g*]pteridines was synthesized and tested for their anti-inflammatory and antibacterial activities. Compounds **37** and **38** inhibited edema by 54% (90% potency compared with the reference compound indomethacin) in a carrageenan-induced rat paw edema model, although no further assays were performed to elucidate the mechanism of action responsible for this inhibition.[104]

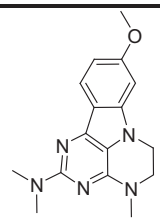
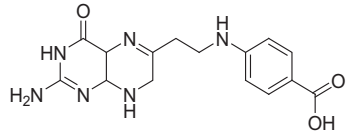
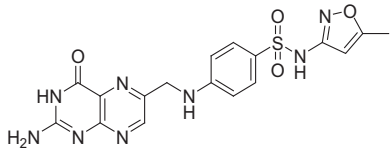
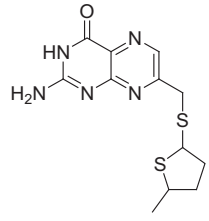
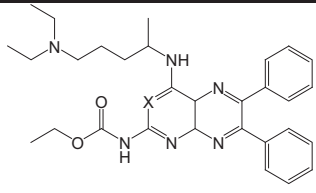
5 | ANTIMICROBIAL ACTIVITY

5.1 | Antibacterial activity

Although the number of newly developed antibiotic agents has slightly risen since 2011, there is still an urgent need for new antibiotics due to the inevitable development of bacterial resistance, which has led to the appearance of multidrug-resistant bacteria with no effective therapeutic treatments.[105] Several pteridine compounds have been investigated to define their possible antibacterial potential (Table 4).

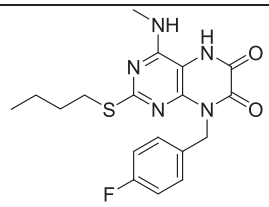
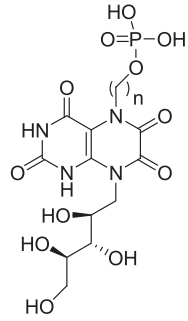
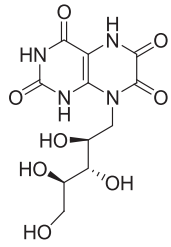
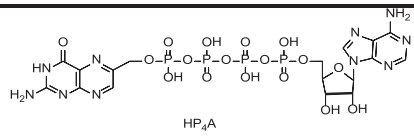
To identify drugs able to inhibit *E. coli* DNA topoisomerase I, a high throughput screening of a total of 49 268 compounds was conducted.[106] Assays were based on the principle that inhibition of this enzyme would impede DNA double strand rejoining, thus provoking the enhancement of DNA cleavage that reduces cell viability.[107] The

TABLE 4 Antimicrobial pteridine compounds

[a]	Target[b]	Structure	Activity[c]	Method[d]	Ref
39	DNA topoisomerase I		*E. coli* (YTOP) MIC = 40 μM *E. coli* (YTOPala) MIC = 60 μM *B. subtilis* MIC = 40 μM	In vitro	[106]
40	DHPS		*B. anthracis* DHPS IC_{50} = 25.9 μM	In vitro Docking	[109]
41	DHPS		*B. anthracis* DHPS (2mM PPi) IC_{50} = 3.4 μM *E. coli* K12 MIC = 10.9 μM	In vitro Docking	[110]
42	TGT		Ki = 0.45 μM	In vitro Docking	[112]
43	FtsZ (*M. tuberculosis*)	 **Compound** / **X** **43a** / C **43b** / N	MIC: 43a = 0.25 μg/mL 43b = 2 μg/mL FtsZ polymerization IC_{50}: 43a = 34.2 μM 43b = 38.1 μM	In vitro	[114]

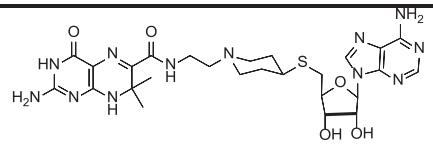
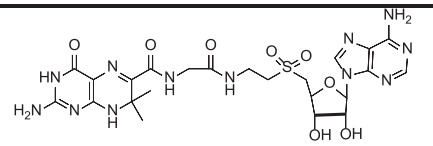
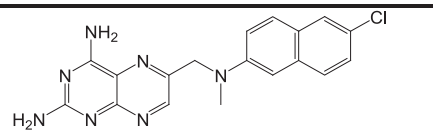
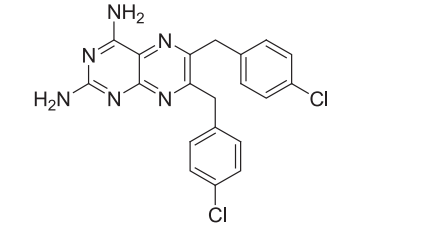
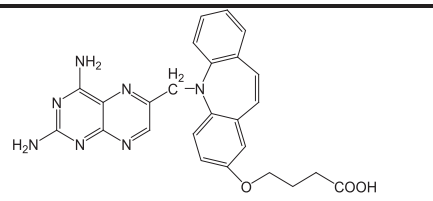
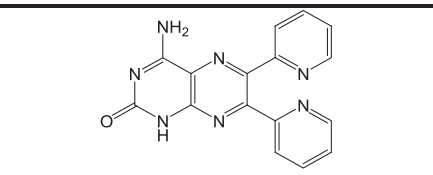
(Continues)

TABLE 4 (Continued)

44	MurI		*E. faecalis* IC_{50} = 0.9 μM *S. aureus* IC_{50} = 3.8 μM *S. aureus* MIC = 8 μg/mL	In vitro Docking	116
45	*M. tuberculosis* Lumazine synthase	 **Compound** / **n** **45a** / 4 **45b** / 5	45a K_i = 3.6 nM 45b K_i = 1.2 nM	In vitro Docking	119
46	*E. coli* riboflavin synthase		K_i = 6.2 nM	In vitro	119
47	HPPK	 HP_4A	HPPK K_d = 0.47 μM HPPK IC_{50} = 0.44 μM	In vitro Docking	121

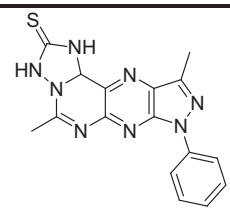
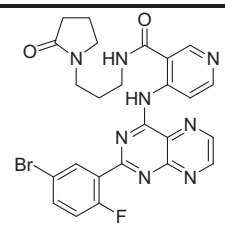
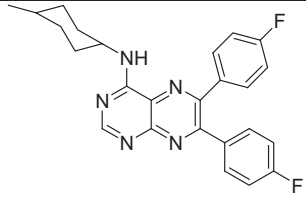
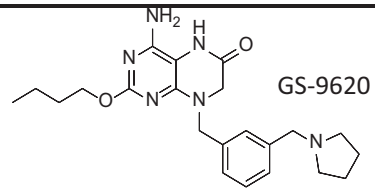
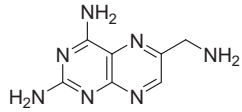
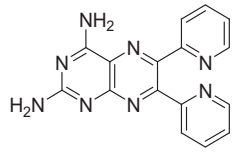
(Continues)

TABLE 4 (Continued)

48	HPPK		HPPK K_d = 2.55 μM HPPK IC_{50} = 3.16 μM	In vitro Docking	[122]
49	HPPK		HPPK K_d = 4.16 μM HPPK IC_{50} = 9.53 μM	In vitro Docking	[123]
50	*B. cereus*		IC_{50} = 2.2 μM	In vitro Docking	[124]
51	*E. coli* and *C. parvum* DHFR		*E. coli* DHFR: IC_{50} = 26 nM *C. parvum* DHFR: IC_{50} CpI = 1.4 μM	In vitro	[125]
52	DHFR		DHFR IC_{50}: *P. carinii* = 1.1 nM *T. gondii* = 9.9 nM *M. avium* = 2 nM Rat liver = 1500 nM	In vitro	[127]
53	*E. coli*		DIM = 29 mm	In vitro	[128]

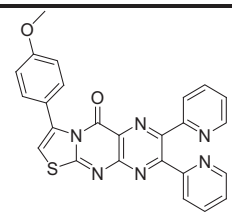
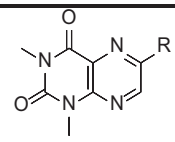
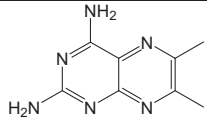
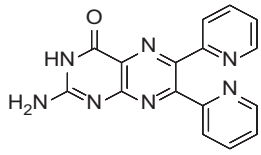
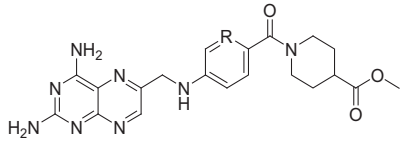
(Continues)

TABLE 4 (Continued)

38	*S. aureus* *B. cereus*		*S. aureus* MIC = 12.5 μg/mL *B. cereus* MIC = 6.5 μg/mL	In vitro	[104]
54	HCV VEGFR kinase		HuH7 replication IC_{50} = 64 nM Huh7 and MT4 cell line CC_{50} >32 μM	In vitro In vivo	[129]
55	NS5B RNA-dependent RNA polymerase		RNA polymerase IC_{50} = 1.3 μM Cell replicon assay IC_{50} = 18 μM Toxicity > 250 μM	In vitro	[130]
56	TLR7		IFN-α induction MIC = 3 nM TLR7 IC_{50} = 290 nM	In vitro In vivo Docking	[131]
57	TAR-Tat		IC_{50} = 50 μM	In vitro	[136]
58	HIV-1 (strain III_B)		HIV-1 IC_{50} > 3.23 μM CC_{50} = 10.52 μM	In vitro	[128]

(Continues)

TABLE 4 (Continued)

59	HIV-1 (strain III_B)		HIV-1 IC_{50} > 2.11 μM CC_{50} = 18.99 μM	In vitro	[128]
60	PPR virus		Reduction cytopathic effect: 60a = 14% 60b = 8%	In vitro	[137]
		Compound / **R** **60a** / $COCH_3$ **60b** / $COOC_2H_5$			
61	Orphan cytosine		Limit Of Detection: 13 nM	In vitro	[138]
62	*Candida spp.*		*C. albicans* DIM = 33 mm *C. tropicalis* DIM = 40 mm	In vitro	[128]
63	PTR1		LmPTR1 Ki: 63a = 100 nM 63b = 210 nM 63a: TcPTR1 K_i = 7 μM 63a: hDHFR K_i = 10 μM 63a: LmDHFR K_i = 4 μM	In vitro Docking	[142,144]
		Compound / **R** **63a** / C **63b** / N			

(Continues)

TABLE 4 (Continued)

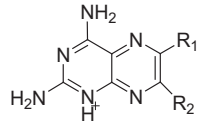
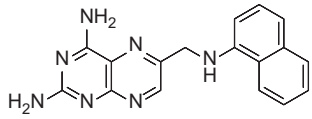
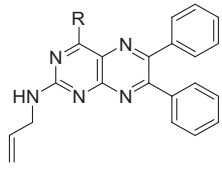
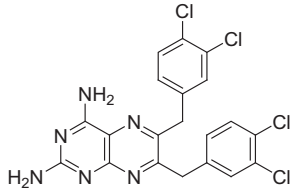
64	PTR1	 **Compound** / **R1** / **R2** **64a**: $CH(CH_3)_2$ / $CH(CH_3)_2$ **64b**: C_6H_5 / NH_2 **64c**: CH_3 / $(CH_3)_2$	LmPTR1 K_i: 64a = 0.24 μM 64b = 3.4 μM 64c = 12 μM TbPTR1 K_i: 64a = 3.3 μM 64b = 1.2 μM 64c >35 μM	In vitro Docking	[145]
65	PTR2		PTR2 K_i = 1.13 μM K_d = 0.83 μM	In vitro Docking	[146]
66	*T. brucei brucei*	 **Compound** / **R** **66a**: *n*-C_4H_9NH **66b**: $PhCH_2NH$ **66c**: Pyrrolidin-1-yl	MIC: 66a = 3.1 μM 66b = 3.1 μM 66c = 6.3 μM	In vitro	[147]
67	C. parvum DHFR		DHFR IC_{50} = 0.25 μM *C. parvum* IC_{50} = 0.6 μM	In vitro	[125]

Table displays the structure and activity details of the pteridine derivatives that showed antibacterial, antiviral, antifungal, or antiparasitary potential.

Abbreviations: CC_{50}, half-maximal cytotoxic concentration; DIM, diameter inhibition zone in millimeters; IC_{50}, half-maximal inhibitory concentration; K_i, inhibition constant; MIC, minimum inhibitory concentration .

[a]Compound number.

[b]Main molecular target tested.

[c]Main activity data extracted from the references.
[d]Method used to determine compound activity. In vitro biochemical assays or cell-based assays; in vivo assays in mice or rat models; molecular docking or crystallographic assays.

active compounds were first identified by the use of a luciferase reporter sensitive to the SOS response induced after DNA cleavage complex accumulation. Three compounds were identified with this technique, including compound **39** (10-methoxy-*N*,*N*,4-trimethyl-5,6-dihydro-4*H*-indolo[3,2,1-*de*]pteridin-2-amine), and then further analyzed; nonetheless, this compound did not actually inhibit *E. coli* growth, probably due to its inability to enter into the cell.[106]

Dihydropteroate synthase (DHPS) is involved in the bacterial folate biosynthesis pathway.[108] The characterization of the DHPS pterin pocket and its binding to several molecules suggested that compounds with a pteridine-scaffold might be potential inhibitors. A screening of previously reported pteridine-based compounds was performed, finding compound **40** as a potent antibacterial compound (IC_{50} values for *B. anthracis* DHPS = 25.9 nM).[109] The authors also created a pharmacophore model based on the pterin scaffold. According to it, the pyrimidine ring, which accesses residues deep in the pterin pocket, does not allow many modifications given that the C2 nitrogen and N8 should be unsubstituted. Additionally, the C4 carbonyl is essential for activity. In contrast, the pyrazine ring tolerates further modifications as it binds closer to the pterin pocket opening. There, an H or methyl should be at the N8 position, and a carboxyl group at C6 with no additional restrictions on this substituent. As can be observed, compound **40** fulfill these criteria. A series of pterin-sulfonamide derivatives were also able to inhibit DHPS, although when tested against *E. coli* K12, MIC inhibition was weaker for compound **41** (pterin-sulfamethoxazole; 10.9 μM) than the corresponding unmodified sulfonamides (0.8 μM for the most potent one).[110]

The enzyme tRNA-guanine transglycosylase (TGT) has been identified as a bacterial virulence factor. This enzyme catalyzes the addition of the nucleoside queuine to an anticodon loop, modulating the fidelity of tRNAs during translation.[111] Based on the structure of *Zymomonas* TGT, a hit-to-lead study was performed to identify TGT inhibitors. The "hot-spot" molecular docking study led to the synthesis of compound **42** (2-amino-7-((2-thienylthio) methyl)pteridin-4(3*H*)-one), the most potent among all the tested compounds, with a K_i value of 0.45 μM.[112] Its thiophene moiety exactly suits a region in the binding pocket that may be favorable for aromatic C-atoms.

Filamenting temperature-sensitive mutant Z (FtsZ) is a bacterial tubulin homolog implicated in bacterial septation.[113] In an attempt to improve the disadvantageous features of **43a**, previously identified as a *Mycobacterium tuberculosis* FtsZ inhibitor, compound **43b** (2-carbamic acid, 4-((4-(diethylamino)-1-methyl-butyl)-amino-6,7-diphenyl-ethyl ester, pteridine dihydrochloride) was designed. **43b** MIC was greater than the MIC showed by **43a** (2 and 0.25 μg/mL, respectively), with similar levels of FtsZ polymerization inhibition, although compound **43b** was less potent against FtsZ GTP hydrolysis and inhibited tubulin polymerization.[114]

Glutamate racemase (MurI) is an enzyme involved in the synthesis of bacterial peptidoglycan.[115] A SAR study of 8-benzyl pteridine-6,7-diones led to the identification of many compounds able to modestly inhibit MurI from several Gram-positive bacteria (ie, *S. aureus* and *E. faecalis*). The SAR approach was directed consecutively to the C2, N8, and C4 positions. The results suggested that lipophilic groups are preferred in positions 2 and 8, choosing *S*-*n*-butyl and 4-fluorobenzyl substituents at C2 and N8, respectively. Finally, to improve the physical properties, the SAR was directed to C4, obtaining compound **44**, which possesses a methyl group in this position. It was the compound with the best results among this series (*S. aureus* MIC = 8 μg/mL).[116]

Lumazine synthase catalyzes the later steps in riboflavin biosynthesis. Some pathogenic Gram-negative bacteria are not able to uptake riboflavin due to the lack of genes encoding riboflavin transporters[117]; thus, they must synthesize it. Therefore, inhibitors of lumazine and riboflavin synthase may be good antibacterial agents.[118] Several ribityllumazidiones containing alkyl phosphates were tested against *B. subtilis* and *M. tuberculosis* lumazine synthase and *E. coli* riboflavin synthase. The compounds were active against both enzymes, especially against the *M.*

tuberculosis lumazine synthase, over which compounds **45a** and **45b** (5-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl-)pentane 1-phosphates) showed a K_i of 3.6 and 1.2 nM, respectively, through a competitive inhibitory mechanism. Its precursor, 6,7-dioxo-8-ribityllumazine (compound **46**), was also active, principally upon *E. coli* riboflavin synthase (K_i = 6.2 nM).[119]

6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) is an enzyme involved in the folate biosynthetic pathway, catalyzing the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin.[120] This enzyme could be a target for antimicrobial therapy. The inhibitory potential of several bisubstrate analogues against HPPK was tested.[121] These compounds consisted of a pterin and an adenosine moiety linked through a chain of phosphoryl groups, varying the number of phosphoryl groups in the different compounds. Compound **47**, the one with the longer linker (chain with four phosphoryl groups), was the most potent compound (K_d with Mg^{2+} = 0.47 μM; IC_{50} = 0.44 μM), although the negative charges carried by the linkage may lead to poor bioavailability. In an attempt to improve the design, the linker was modified, either changing it to a piperidine linker and adding methyl groups at the pterin moiety (compound **48**)[122] or using a glycyl aminoethyl spacer and a sulfone group (compound **49**).[123] Although still active, none of the new compounds improved the K_d of compound **47** (compound **48** K_d = 2.55 μM and compound **49** K_d = 4.16 μM). In all cases, crystallographic studies were performed.

Several compounds of a different nature were screened against *B. cereus* DHFR. Although 2,4-diamino-5-deazapteridines were active (**50**; lowest IC_{50} = 2.2 μM), other structurally different compounds (quinazoline and 5-deazapteridines) reached better values, and thus, pteridines were not further analyzed in this case.[124] In another study, Nelson et al[125] tested the inhibitory potential of a diverse library of compounds upon *E. coli* DHFR, finding that compound **51** (6,7-di(4-chlorobenzyl)-2,4-diaminopteridine), showed a low IC_{50} of 26 nM. *Mycobacterium avium* DHFR inhibition was also examined, as it is an opportunistic infectious organism that often affects AIDS patients.[126] Among the diamino-methylpteridines synthesized by Rosowsky et al,[127] compound **52**, which contained a tricyclic substituent (2,4-diamino-6-[2′-*O*-(3-carboxypropyl)oxydibenz[*b*,*f*]-azepin-5-yl]methylpteridine), was the most potent one, inhibiting the different DHFRs assayed with an IC_{50} in *M. avium* DHFR of 2 nM and a high selectivity for bacterial over rat liver DHFR.

The antimicrobial properties of a series of dipyridylpteridine derivatives were evaluated by Abbas et al,[128] showing antiviral, antibacterial, and antifungal potential (see below). Among the tested compounds, compound **53** (4-amino-6,7-bis(2-pyridyl)-pteridin-2-one) showed the highest antibacterial potential, as it produced an inhibition zone of 29 mm against *E. coli* (ATCC 25922).

Some of the previously mentioned pyrazolo[3,4-*g*]pteridine derivatives also presented antibacterial potential. Again, one of the most potent was compound **38**, which inhibited *S. aureus* and *B. cereus* (MIC = 12.5 and 6.5 μg/mL, respectively). Activity against *E. coli* and *P. aeruginosa* was also measured, although in these cases those compounds gave poor results.[104]

5.2 | Antiviral

The antiviral properties of several pteridine derivatives have also been studied (Table 4). Based on the fact that SD-208 could inhibit hepatitis-C virus (HCV) replication (IC_{50} = 0.89 μM), Raboisson et al[129] synthesized a series of pteridine derivatives and tested their HCV-inhibitory activities against Huh7-Rep cells containing the subgenomic biscistronic replicon clone ET with a luciferase read out. Compound **54**, which contains a substituted nicotinamide and a bromo-fluorophenyl group as radicals, was found to be the most potent HCV-inhibitor (IC_{50} = 64 nM) with no cytotoxicity even at the highest dose. The results suggested that inhibition of *N* replication could be caused by an indirect mechanism that modulates the phosphorylation state of viral nonstructural protein 5A (NS5A) and may be linked to the inhibition of VEGFR-3 by compound **54**. Furthermore, the administration of **54** to mice showed good metabolism and pharmacokinetic (PK) properties.

SAR studies of pteridine derivatives against HCV NS5B RNA-dependent RNA polymerase showed that introducing a fluorine group at the *para*-position of an aryl substituent in the pteridine ring, which led to

compound **55**, greatly increased its potency, inhibiting the mentioned RNA-polymerase alone (IC_{50} = 1.3 μM). Moreover, when using a cell-based HCV subgenomic replicon assay, it was active (IC_{50} = 18 μM) without causing cytotoxicity.[130]

Roethle et al[131] first characterized the pteridinone GS-9620 (Vesatolimod, compound **56**). In their study, an extensive series of 64 pteridinone-based compounds was synthesized, and a SAR study was performed; the analysis of different substituent in the pteridinone core lead to the identification of GS-9620. This compound showed an induction of IFN-α in infected human PBMCs through an agonist effect for Toll-like receptor 7 (TLR7), while TNF-α stayed unaffected. Based on the hypothesis that IFN-α production upon TLR7 activation could inhibit hepatitis-B virus (HBV) replication, Lanford et al[132] tested GS-9620 on HBV chronically infected chimpanzees. Serum levels of HBV DNA decreased showing a mean reduction of 2.2 logs, together with a HBV viral DNA declination in the liver, and a reduction of hepatocytes positive for HBV core antigen and an increment of their apoptosis, as shown by immunohistochemical staining of liver sections. IFN-α levels increased in a dose-dependent manner, as well as TLR-7 levels did. GS-9620 has also been tested as an antiviral agent against HIV, suggesting that exposure of HIV-infected PBMCs to GS-9620 exerted an antiviral activity through the induction of IFN-α expression in plasmacytoid dendritic cells, which would block HIV replication before or during reverse transcription.[133] These promising results lead to the establishment of several clinical trials to study the potential of GS-9620 against HBV, HCV, and HIV (see Section 7.3 and Table 6).

TAR, a noncoding RNA sequence in HIV, has been identified as a target for antiviral drugs. It binds to Tat, a viral regulatory protein, to recruit the human positive transcription elongation factor (P-TEFb).[134,135] Compound **57**, 6-(aminomethyl)pteridine-2,4-diamine, showed an IC_{50} of 50 μM in a fluorimetric competition assay using a 31-mer TAR model and labelled Tat, although other nonpteridine-based compounds displayed better values.[136]

Dipyridylpteridine derivatives were active against HIV-1 (strain III_B) cultured in human T-lymphocyte (MT-4) cells. Compounds **58** and **59** achieved IC_{50} values >3.23 and >2.11 μM, respectively, while their CC_{50} were 10.52 and 18.99 μM for each compound.[128]

In the search for antiviral drugs, novel nonnucleoside xanthine, uracil and pteridine derivatives were synthesized. The first screening approach consisted of nucleic acid binding assays by agarose gel electrophoresis, followed by an in vitro antiviral assay. The 1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropteridine derivatives compounds **60a** and **60b** were able to bind both quelate and fragment DNA and RNA. Nevertheless, their antiviral activities were weak when tested against a Vero cell culture infected with *Peste des petits* ruminant virus (PPRV; 14% and 8% reduction of the cytopathic effect, respectively), with the uracil-based compounds being more active.[137]

A different case is fluorescent compound **61** (2,4-diamino-6,7-dimethylpteridine). It binds to the orphan cytosine (C) opposite to the abasic site (AP) in RNA duplexes. Thus, this RNA-binding ligand could be a tool for RNA functional studies and therapeutic approaches by targeting viral or bacterial RNAs.[138]

5.3 | Antifungal

The antifungal activities of pteridine-based compounds have been scarcely studied. Some compounds from the already mentioned dipyridylpteridine series have also shown antifungal properties; thus, compound **62** reached diameter of inhibition zone values over 33 mm against *C. albicans* and *C. tropicalis* (Table 4).[128]

The effect of compound **52** on *Pneumocystis carinii* DHFR, an AIDS opportunistic fungus,[126] was also studied, showing a very potent and highly selective effect in this case (IC_{50} = 1.1 nM).[127]

5.4 | Antiparasite

Leishmaniasis is caused by the protozoan parasite *Leishmania*. It is transmitted by infected phlebotomine sandflies. It is a climate-sensitive disease also related to malnutrition; thus, it mostly affects undeveloped countries.[139] Several approaches have been directed to inhibit *Leishmania* growth with pteridines (Table 4).

As already mentioned, DHFR is involved in the provision of reduced folate. In trypanosomatidic parasites, the N-terminal DHFR domain is linked to a thymidylate synthase domain, demonstrating its role as a bifunctional enzyme. The parasite DHFR domain share 25% to 40% homology with human DHFR.[140] Despite the fact that *Leishmania* is a folate auxotroph, DHFR inhibitors have shown a minor effect in the treatment of leishmaniasis due to the activity of pteridine reductase 1 (PTR1), which is overexpressed to supply reduced folates when DHFR is inhibited.[141] Thus, strategies to develop a new treatment should be directed at inhibiting both *Leishmania* DHFR and PTR1, while human DHFR activity remains unaffected. In vitro enzyme inhibition studies showed that piperidine-pteridine compounds **63a** and **63b**, which have a *p*-amino-benzoic acid (PABA) substituent, were able to inhibit *Leishmania major* PTR1 (K_i = 100 and 210 nM, respectively), while weakly affecting human DHFR. The combination of **63a** and **63b** with pyrimethamine (PYR) against other species such as *L. mexicana* and *L. major* resulted in a synergistic effect, aside from increasing the parasite sensitivity to oxidative stress. SAR studies revealed that a chain at N10 that can interact with LmPTR1 hydrophilic residues is necessary; they also indicated that substitutions on PABA allowing hydrophobic interactions in hDHFR should be avoided to increase selectivity.[142]

Trypanosoma cruzi causes Chagas disease, which affects 6 to 7 million people worldwide, mostly in Latin America. Currently, it can be treated with benznidazole or nifurtimox, although they are only active if administered soon after infection.[143] Inhibition of PTR1 by **compound 63a** was also tested against *Trypanosoma cruzi* (K_i = 7 μM), showing an increased ability to inhibit the growth of intracellular *T. cruzi* when combined with PYR, rising from 66% when used alone to 88% when combined. None of the compounds had cytotoxic effects on human Vero and MRC5 cells.[144]

A structure-based approach using known PTR1 inhibitors was performed to identify several scaffolds as potential drugs. One of the three determined scaffold was pteridine-based. Indeed, three commercially available diaminopteridines (compounds **64a-c**) were found to be active in vitro against both *T. brucei* and *L. major* PTR1.[145]

The activity of various 2,4-aminopterines on *T. cruzi* pteridine reductase 2 (TcPTR2) was also measured, with compound **65** being the most potent derivative (K_i = 1.13 μM). The researchers designed a docking approach to predict the activity of the compounds against TcPTR2. The results showed that the dock energy scores did not correlate accurately with the enzyme inhibitory values, as the K_d values calculated from the docked ligand conformations differed by an order of magnitude from the K_i values measured in vitro.[146]

Several 2,4-thibenzyl and 2,4-dialkylamino pteridine derivatives showed antiparasite activities against *T. brucei brucei* when tested in cell-based assays. The dialkylamino pteridines **66a** and **66b** reached MIC values of 3.1 μM, while **66c** had a MIC of 6.3 μM.[147]

Cryptosporidium parvum and *Toxoplasma gondii* are opportunistic parasites that can cause disease in AIDS patients.[126] A large number of compounds were tested against ***C. parvum*** DHFR, including several pteridines. Compound **67** (6,7-di(3,4-dichlorobenzyl)-2,4-diaminopteridine) was the only pteridine that showed selectivity between *C. parvum* and human DHFR. When tested in Madin-Darby canine kidney (MDCK) epithelial cells infected with intracellular forms of *C. parvum*, compound **67** showed an IC_{50} of 0.6 μM.[125] Similarly, a series of methylpteridines was tested against *T. gondii* DHFR, with compound **52** once again being the most potent (IC_{50} = 9.9 nM) and selective one for protozoan rather than for rat DHFRs.[127]

6 | OTHER ACTIVITIES

6.1 | NOS inhibition

Nitric oxide (NO) is a signaling molecule involved in many biological processes. NO is synthesized by three different NOS isoforms, including neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). nNOS is involved in neurotransmission and CNS blood pressure regulation and has been related to neurodegeneration. eNOS regulates vasodilatation and angiogenesis. Finally, iNOS is not constitutively expressed, but it is induced by several agents. During the inflammation process, macrophages generate large amounts of NO upon cytokine or LPS induction to kill pathogens, although as a collateral effect, healthy cells can also be affected.[148]

Since BH_4 is a NOS cofactor, several studies have been aimed to discover 2,4-aminopteridines that target the BH_4 binding pocket and inhibit the activity of these enzymes (Table 5). An approach involving a huge SAR study identified many porcine nNOS inhibitors. From a series of 2-amino-4-bialquil/diaralkyl-6-phenyl/anisyl-pteridines, the most potent compounds were **68a** and **68b** (2-amino-4-dibenzylamino-6-phenylpteridine and 2-amino-4-dibenzylamino-6-(4-methoxyphenyl)pteridine, respectively), which were able to completely inhibit enzyme activity with IC_{50} values ranging from 3 to 62 μM. This potential seems to be due to the increased interactions, both hydrophilic and hydrophobic, between the substituent at position 4 and the binding pocket, compared to those interactions of natural BH_4.[149] Among the compounds tested against the three NOS isoforms in another research, compound **69** was the most selective for nNOS, being 58 times most potent against this isoform than against iNOS. The most remarkable conclusions of the SAR study were that selectivity increased if substitutions at N4 and C6 were hydrophobic, and that compounds with a tetrahydropteridine scaffold, which is the case of compound **69**, were more active than those with an aromatic pteridine scaffold.[150] These and other 2-oxo and 2-aminopteridines, which are known as NOS inhibitors were used to design predictive models with 3D-QSAR techniques.[151]

Regarding iNOS, several 2,4-aminopteridines have also been shown to inhibit this isoform, even with better results than MTX.[152] Compounds **70a** (2-amino-4-((4-ethoxylcarbonylphenyl)amino)-6-phenylpteridine) and **70b** (2-amino-4-isopropylamine-6-(4-methoxyphenyl)-pteridine) were further analyzed in induced septic shock and immunological liver injury in rats. Treatment with these compounds elevated the mean blood pressure up to levels similar to the reference compound aminoguanidine, improved the symptoms of liver injury by decreasing the NO, GPT, and GOT levels and alleviated the development of ulcers, the mucosa membrane swelling and aggressiveness in rats.[153]

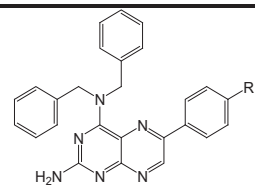
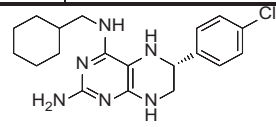
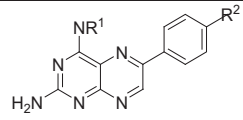
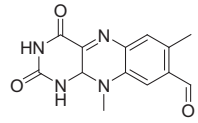
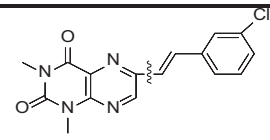
6.2 | Hypertension

Triamterene is a pteridinetriamine compound that acts as a potassium-sparing diuretic agent (Figure 2). It was approved by the FDA to treat hypertension. Triamterene blocks the sodium-potassium exchange pump (Na-K-ATPase), inhibiting reabsorption of sodium on the distal renal tubule. This compound is commonly used in combination with hydrochlorothiazide as a diuretic/antihypertensive drug since their effects are synergetic.[154]

6.3 | Neurodegeneration

Nerve growth factor (NGF) is a soluble signaling protein whose dysregulation has been associated with several pathologies, such as neurodegeneration and neuropathic pain. NGF interacts with two receptors, TrkA and p75NTR. Depending on the level of coexpression with TrkA, p75NTR can induce proliferation and differentiation signals or can induce apoptosis, which is the case when TrkA is absent.[155] The effect of compound **71** (Ro 08-2750: 7,10-dimethyl-2,4-dioxobenzo[*g*]pteridine-8-carbaldehyde) on NGF-receptor interactions was investigated on two neuronlike cell lines, PC-12 cells, which expresses both receptors, and SK-N-MC cells, which express only p75NTR.

TABLE 5 Other clinical activities of pteridine compounds

[a]	Target[b]	Structure	Activity[c]	Method[d]	Ref
68	nNOS	 **Compound** / **R**: **68a** / - ; **68b** / *p*-MeO	nNOS IC_{50}: 68a = 3 μM 68b = 5 μM	*In vitro*	[149]
69	NOS		nNOS IC_{50} = 3.68 μM iNOS IC_{50} = 214.2 μM eNOS IC_{50} = 31.71 μM	*In vitro* Docking	[150]
70	iNOS	 **Compound** / **R1** / **R2**: **70a** / HNC_6H_4COOEt / H ; **70b** / $HNCH(Me)_2$ / OMe	iNOS IC_{50}: 70a = 18.85 μM 70b = 24.08 μM	*In vitro* *In vivo*	[153]
71	NGF interactions		Interactions IC_{50} : NGF-$p75^{NTR}$ = 244 μM NGF-TrkA = 33 μM	*In vitro*	[156,157]
72	MAO-B		MAO-B IC_{50} = 0.314 μM MAO-B K_i = 0.0181 μM	*In vitro* Docking	[159]

(Continues)

TABLE 5 (Continued)

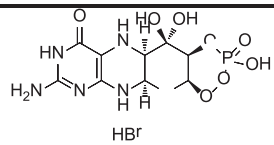
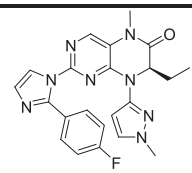
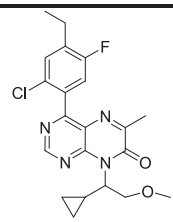
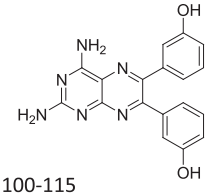
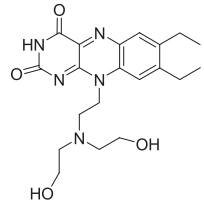
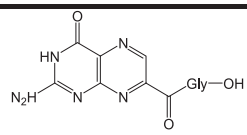
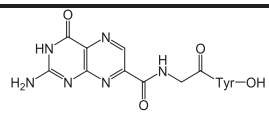
73	MoCo	 HBr	ND	In vitro	[163]
74	Plk-2		Plk-2 IC_{50} = 0.007 μM Plk-1 IC_{50} = 135 μM	In vitro In vivo	[165]
75	CRF-R1		rCRF-R1 IC_{50} = 7.5 nM	In vitro In vivo	[168]
19	PI3K	 TG100-115	PI3Kγ IC_{50} = 83 nM PI3Kδ IC_{50} = 235 nM	In vitro In vivo Docking	[101,102]
76	CatK		Type I collagen degradation: IC_{50} = 4.7 μM Eroded surface reduction: IC_{50} = 312 nM	In vitro *Docking*	[172]
77	ALR2		IC_{50} = 1.97 μM	In vitro Docking	[176]
78	RTA		IC_{50} = 6 μM	In vitro Docking	[178]

Table displays the structure and activity details of the pteridine derivatives that showed NOS inhibitory potential, or may serve as neurodegeneration, depression, ischemia, osteoporosis, diabetes or ricin intoxication treatment agents.

Abbreviations: IC_{50}, half maximal inhibitory concentration; K_i, inhibition constant; ND, not determined, or the reference does not give exact data, or only shows data graphically.
[a]Compound number.
[b]Main molecular target tested.
[c]Main activity data extracted from the references.
[d]Method used to determine compound activity. In vitro biochemical assays or cell-based assays; in vivo assays in mice or rat models; molecular docking or crystallographic assays.

The results showed that compound **71** bound to two NGF molecules and thus inhibited the p75NTR interaction at low concentrations, causing SK-n-MC cell death. This compound only affected the TrkA-NGF interactions at high concentrations. Thus, PC-12 cells treated with low doses of compound **71** could differentiate into neurons (Table 5).[156] In contrast, Kristen et al showed by using surface plasmon resonance spectroscopy that this compound blocks the interaction of NGF with TrkA more effectively than with p75NTR.[157]

Monoamine oxidase B has been associated with age-related neurodegeneration, such as Parkinson disease (PD). It is present in the brain among other tissues, where it metabolizes dopamine and forms ROS and other neurotoxic species.[158] Compound **72** (6-[(*E*)-2-(3-chlorostyryl)]-1,3-dimethyl-1*H*-pteridine-2,4-dione) showed inhibitory potential against baboon liver MAO-B at levels comparable to those of the reference compound (IC_{50} = 0.314 μM), although it also had some effect on NOS, which is also related to neurodegeneration (Table 5). Molecular docking studies revealed that compound **72** spans the MAO-B entrance and substrate cavity.[159]

Molybdenum cofactor (MoCo) forms the active site in many molybdenum enzymes that catalyze important redox reactions.[160] MoCo deficiency is a hereditary metabolic disorder characterized by neurodegeneration.[161] Cyclic pyranopterin monophosphate is an intermediate of MoCo biosynthesis. It had been previously observed that administration of pyranopterin monophosphate produced by *E. coli* could restore MoCo biosynthesis.[162] Clinch et al[163] synthesized hydrobromide pyranopterin monophosphate (compound **73**) and compared it to the bacterial compound. Both were converted in vitro to molybdopterin (MPT) using *E. coli* MPT synthase. The formed MPT was then further transformed into MoCo through the action of gephyrin and transferred to a MoCo-dependent human enzyme. Synthesized compound **73** produced similar enzyme activity compared with the *E. coli*-derived compound; thus, they had equal effectiveness (Table 5).

Polo-like kinase 2 (Plk-2) has been identified as a contributor to the phosphorylation of protein α-synuclein at Ser-129.[164] This protein is accumulated in the Lewy bodies commonly found in neurons in PD patients. Thus, blocking this phosphorylation may be a potential target for PD treatment. With this purpose, Bowers et al synthesized two extensive series of dihydriopteridinone derivatives and performed SAR studies on Plk-2 and Plk-1 inhibition, P-gp efflux and permeability. The SAR studies were directed to optimize the N8 substituent and the biaryl region at C2. Both aliphatic and aryl groups where tested at N8, as well as the cyclization of this substituent to obtain 7,8-tricyclic pteridinones. These reached high inhibitory values, although compound **74** ((7*R*)-7-ethyl-2-(2-(4-fluorophenyl)imidazol-1-yl)-5-methyl-8-(1-methylpyrazol-3-yl)-7*H*-pteridin-6-one) produced the best results and provoked 41% to 45% reduction of pS129-α-synuclein levels in Sprague-Dawley rat brains without affecting total α-synuclein levels (Table 5).[165,166]

6.4 | Depression/anxiety

Corticotropin releasing factor (CRF) is one of the main peptides that regulate the hypothalamic-pituitary-adrenal (HPA) axis. Upon binding to the CRF-R1 receptor, CRF triggers a pathway leading to the release of cortisol, which mediates metabolic and behavioral changes. The hypersecretion of CRF could lead to depression or anxiety[167]; thus, antagonists of CRF, which bind to CRF-R1, may be potential drugs to treat stress-related disorders. Two analogues series of pyrido[3,2-*b*]pyrazin-3(4*H*)-ones and pteridin-7(8*H*)-ones were compared in a SAR study. The first group of compounds was more potent than the pteridine-7(8*H*)-ones when measuring the IC_{50} using a CRF-R1

binding assay, although the pteridine derivatives had better plasma-free fractions. The most potent compound among this series of pteridine derivatives was 4-(2-chloro-5-fluoro-4-methoxyphenyl)-8-(1-cyclopropyl-2-methoxyethyl)-6-methylpteridin-7-one (compound **75**, IC_{50} = 7.5 nM; Table 5).[168]

6.5 | Ischemia/reperfusion injury

Ischemic tissue damage induces an overproduction of VEGF, which leads to vascular permeability and leakages and provoking myocardial infarcts, among other injuries. The Src and PI3K kinase pathways are involved in the loss of cellular junctions that occurs during ischemia, so these proteins are targets to treat ischemia/reperfusion injury.[169] In this context, the therapeutic potential of novel pteridine-based compounds was tested by Doukas et al.[101,170] A SAR study of PI3Kγ inhibitory potential revealed that the 2,4-diamino substituents, a 6′-regioselectivity and an *m*-hydroxyphenyl group in the pteridine scaffold, were required for PI3Kγ inhibition. Their assays showed that already mentioned TG100-115 (compound **19**) prevented vascular leakage. Further assays demonstrated that TG100-115 avoided VE-cadherin increasing levels as well as inhibited not only PI3Kγ but also the PI3Kδ isoform (IC_{50} = 83 and 235 nM, respectively) as well as some of their downstream proteins, with no effects on HUVEC cell proliferation (Table 5). Studies with rodents and porcine models showed that this compound inhibited VEGF-induced vascular permeability and reduced the infarct size down to 40% without affecting the leukocyte infiltration. TG100-115 got into a clinical trial to test its safety on patients who suffered myocardial infarction, although the last update was in 2008 and no further results have been published (Table 6).

6.6 | Osteoporosis

Osteoporosis is a metabolic bone disease characterized by high bone resorption by osteoclasts together with diminished bone formation by osteoblasts. Cathepsin K (CatK) is a cysteine protease with collagenase activity that plays a key role in osteoclast bone resorption, since collagen is the major component of bone matrix. CatK has multiple proteolytic activities, and the inhibition of the active site provokes diverse side effects.[171] The ectosteric site 1 in CatK, which is needed for the formation of protease oligomers during collagenase activity, is situated remotely from the active site, and its inhibition does not affect the function of CatK over other substrates; thus, targeting it would reduce the side effects. Law et al recently characterized the ectosteric site 1 and screened a huge library of compounds using three different docking algorithms (Surflex, Glide, and GOLD), identifying compound **76** (10-[2-[bis(2-hydroxyethyl)amino]ethyl]-7,8-diethylbenzo[*g*]pteridine-2,4-dione) as the most potent ectosteric inhibitor of CatK. This tricyclic compound inhibited type I collagen degradation through CatK (IC_{50} = 4.7 μM), but not the cleavage of Z-Phe-Arg-MCA and was innocuous for the proteases trypsin and matrix-metalloproteinase-1. Furthermore, in vitro osteoclast-bone resorption assays showed that compound **76** reduced the eroded trench surface (IC_{50} = 312 nM), with no significant changes in the number of osteoclasts and metabolic activity, suggesting an absence of toxicity (Table 5).[172]

6.7 | Diabetes complications

Aldose reductase (ALR2) is a member of the aldo-keto reductase (AKR) enzyme superfamily that reduces glucose to sorbitol.[173] ALR2 is related to diabetes complications,[174] so targeting it could be a useful strategy to prevent diabetes complications such as neuropathy or retinopathy. Nevertheless, other already assayed ALR2 inhibitors have shown various side effects, which could be due to the high similarity between ALR2 and other AKRs not related to diabetes; this is the case with aldehyde reductase (ALR1), which metabolizes toxic aldehydes. To overcome this problem, approaches to develop new ALR2 inhibitors should be directed to the ALR2 "specificity pocket," which differs from ALR1 at Leu300.[173,175] The ALR2 inhibitory potential of several amino acid-conjugated pterin-7-carboxamides was measured and compound **77**, which has a glycine side chain, showed the best IC_{50} value

(1.97 μM; Table 5). The results also indicated that L-amino acid residues were more potent than the corresponding D-residues and the inhibitory activity decreased with the increasing size of the residues. Moreover, docking studies revealed that the pterin carbonyl oxygen creates a hydrogen bound with Leu300, suggesting possible selectivity. Nonetheless, this point was not actually confirmed, as the synthesized compounds were not tested against other AKRs.[176]

6.8 | Ricin intoxication

Ricin toxin A (RTA) is a type 2 ribosome-inactivating protein. It is a toxic molecule found in castor beans that depurinates a specific adenosine in rRNA and a lectin B chain, inhibiting cellular uptake.[177] Pterin-7-carboxamides have been postulated as promising RTA inhibitors. An equal strategy as the one followed to find aldose reductase inhibitors (see previous Section 6.7, diabetes complications) was also used by the same research group against RTA. They synthesized several dipeptides and tripeptides conjugated to pterin-7-carboxamides and obtained good results in RTA inhibition assays, with IC_{50} values ranging from 6 to 115 μM, with the tyrosine-conjugated compound **78** being the most potent molecule (Table 5).[178]

7 | CLINICAL TRIALS

The availability of HTS techniques has facilitated the screening of huge and varied drug libraries against cell panels to identify drug candidates for clinical development.[179] In this sense, as shown in Table 6, several pteridine derivatives are under investigation in clinical trials as therapeutic agent candidates for different pathologies.

The corresponding Reference Numbers assigned by the US National Library of Medicine Clinical trials summarized herein are displayed below. Thus, further information about those trials can be found in ClinicalTrial.gov.[180]

7.1 | TG100-115

Since preclinical models showed that TG100-115 reduced the size of heart attack,[101,170] the clinical trial named "Safety of TG100-115 for Heart Attack Treated with Angioplasty to restore blood flow" (NCT00103350) was performed from 2005-2008 in a cohort of 100 enrolled participants. The multicenter, randomized, double-blind, placebo controlled, prospective study evaluated the safety and potential efficacy of single, increasing doses of TG100-115 (compound **19**) in subjects undergoing percutaneous coronary intervention for acute anterior ST elevation myocardial infarction. Nevertheless, as far as we know, no results have been reported so far.

7.2 | Inhibitors of Polo-like kinases

The two pteridine derivatives most widely assayed in clinical trials are the inhibitors of Polo like kinases: BI-2536 (compound **11**), an inhibitor of Plk1 to 3; and most recently Volasertib also known as BI-6727 (compound **10**), which mainly inhibits Plk1, but also Plk2 to 3 with a lower potency. As it has been described above, Plk1 has a main role in cell mitosis, although it also protects against apoptosis and is a regulator of tumor cell invasiveness. Furthermore, overexpression of Plk on tumor cells (observed in solid tumors and AML) has been correlated with bad prognosis, worst histologic degree, metastatic capacity, and low survival.[181–183] Since Polo like kinases are necessary for cell division, their inhibition have proven to exert antitumor activity in the comprehensive tumor cell line drug-screening study, the Cancer Genome Project (CGP), and also to slow down the growth of cancer in several preclinical murine models[45,182] and in a human AML xenograft model.[184] Furthermore, Volasertib has demonstrated a high level of distribution, indicating a good tissue penetration and bioavailability.[41]

7.2.1 | BI-2536

Regarding BI-2536, several phase I and II trials have been perfomed,[46–48] in patients with relapsed or refractory acute myeloid leukaemia (AML)[48] and solid tumors,[185] small-cell lung cancer,[186] nonsmall-cell lung cancer (NSCLC),[187,188] chemotherapy-naïve pancreatic cancer,[46] prostate cancer,[189] and non-Hodgkin lymphoma,[47] revealing modest or absent clinical effects, although it was related with a safety profile. These ineffective results were attributed to the PK pattern of BI 2536, especially its rather short half-life (~50 hours in individuals with advanced solid tumors)[190] and low intratumoral access.[191] Hence, clinical testing of BI-2536 was interrupted and antitumor activity of other Plk1 inhibitors are being tested in preclinical and clinical trials.[184]

7.2.2 | Volasertib (BI-2536)

Volasertib (aka BI-6727) is currently the most advanced of the investigational Plk inhibitors under clinical development. It was obtained by tailoring the dihydropteridinone structure of BI-2536.[41] Although the FDA (the US Food and Drug Administration) has not approved Volasertib as a treatment of any disease, it has shown promising results in clinical trials, which predict a forthcoming approval.

A total of 26 clinical trials with Volasertib have been reported (reviewed in Van den bossche et al[42]) either using it alone or in combination with other chemotherapeutic agents.[43] In the most advanced trials, Volasertib has been assayed against AML (NCT01721876),[44] nonsmall cell lung cancer (NSCLC; NCT00824408),[192] ovarian cancer (NCT01121406),[193] and urothelial cancer (NCT01023958).[194] With respect to the potential use of Volasertib against solid tumors several clinical trials have been completed (NCT01348347, NCT01145885, NCT00969761, among others), but still none have reached phase II. Others have been terminated (NCT02198482 by Boehringer Ingelheim due to manufacturing problems; and NCT02003573 unreported cause),[195] and three have been withdrawn (NCT02905994 due to lack of funding; NCT02527174, compound no longer available; and NCT02722135, unreported cause). Finally, two trials are active (NCT00804856, not recruiting[196]; and NCT01721876) and three completed (NCT01023958, NCT00824408, and NCT01662505, with reported results).

Earlier results established Volasertib as a promising treatment of AML.[44] According to previous results obtained in phase I, the efficacy and safety of Volasertib 350 mg plus LDAC vs LDAC alone has been further explored in a randomised phase IIa trial,[197] and in an ongoing confirmatory phase III trial in the same patients population (POLOAML-2; NCT01721876 with 666 participants from 122 worldwide locations and an estimated study completion date by 31 December 2019).[44,198]

In a recent report Ottmann et al,[196] have reported from trial NCT00804856, that Volasertib had antileukemic activity in patients with relapsed/refractory AML, a group of cancer patients with very scarce therapeutic options. Besides the antileukaemic activity, they analyzed the maximum tolerated dose (MTD), safety and PKs of intravenous Volasertib as monotherapy or in combination with subcutaneous LDAC in patients with relapsed/refractory AML considered unsuitable for intensive treatment.[44,198] Patients were treated with increasing doses of intravenous Volasertib on days 1 and 15 as monotherapy or in combination with a defined dose LDAC (2 × 20 mg/d subcutaneously on days 1 to 10) every 4 weeks. Complete remission with incomplete blood count recovery (CRi) was observed in six patients (18.8%) treated with the combined therapy and in five patients (8.9%) treated with monotherapy. The safety profile at the assayed doses was clinically manageable, and the PK and pharmacodynamic activities were similar to previous reported data.[194,199] The future challenge of this treatment is to uncover the mechanisms of the antileukemic activity and its potential to increment efficacy of other drugs targeting Plk.

Another phase II trial (NCT01023958) tested "Intravenous BI-6727 (Volasertib) in 2nd Line Treatment of Urothelial Cancer" from 50 participants from 19 November 2009 to 19 September 2011.[194] The primary objective of this trial was to evaluate the efficacy and safety of BI-6727 in patients with locally advanced, metastatic or recurrent urothelial cancer of the bladder, renal pelvis, or ureters after failure of first line or adjuvant/neoadjuvant

chemotherapy. Although Volasertib showed an acceptable safety profile, it only demonstrated a modest antitumor activity, as only 14% of patients showed a partial response, while 26% of patients had stable disease.

From 12 May 2010 to 13 August 2015, Volasertib was tested against ovarian cancer in an international randomized phase II trial in 110 participants (NCT01121406).[193] The aim of this trial was to assess the efficacy and the safety of BI-6727 vs investigator's best choice single cytotoxic agent in recurrent third and fourth lines platinum resistant/refractory ovarian cancer. A group of 100 patients were randomized at the study entry to receive either BI-6727 (50 patients) or nonplatinum single cytotoxic agent (50 patients). Disease control rates where 30.6% in the case of Volasertib, and 43.1% for the chemotherapy, although the number of patients with partial response was similar for both groups (seven and eight patients, respectively). Remarkably, although median progression-free survival (PFS) was lower in the case of Volasertib (13.1 weeks vs 20.6 weeks), six of the patients receiving Volasertib achieved PFS longer than 1 year, up to 192 weeks, while none of the patients treated with the other chemotherapy agent did overcome 54 weeks.

Volasertib has also been assayed against nonsmall-cell lung cancer (NCT00824408) as phase II clinical trial.[192] The objective was to evaluate whether BI-6727 monotherapy or in combination with pemetrexed might be effective in the treatment of advanced or metastatic NSCLC in patients who relapsed after or failed first-line platinum based therapy. The secondary objectives were to identify the acceptable dose of BI-6727 in combination with the antitumor agent Pemetrexed and to characterize the PK profiles of BI-6727 alone. The trial was completed by September 2016. Results showed that the combination of 300 mg of Volasertib with Pemetrexed did not improve the efficacy of Pemetrexed alone, while Volasertib monotherapy gave poor results. The adverse effects were similar in the case of combinatory treatment and monotherapy with Pemetrexed. Finally, PKs analyses discarded drug-drug interactions.

7.3 | Vesatolimod (GS-9620)

As mentioned above, the pteridinone GS-9620 (compound **56**) was first characterized by Roethle et al[131] in a SAR study. This compound induced the production of IFN-α in infected human PBMCs through an agonist effect for Toll-like receptor 7 (TLR7).[131] Based on the hypothesis that IFN-α production upon TLR7 activation could inhibit hepatitis-B virus (HBV) replication, a phase II clinical trial was performed (NCT02579382) from 19 October 2015 to 14 February 2018 entitled "A Study of the Safety, Tolerability, and Efficacy of Vesatolimod in Combination With Tenofovir Disoproxil Fumarate (TDF) in Adults With Chronic Hepatitis B (CHB) Infection Who Are Currently Not Being Treated." The primary objectives of this study were "to evaluate the safety, tolerability, and efficacy of Vesatolimod (formerly GS-9620) in adults with chronic hepatitis B (CHB) infection who were currently not being treated." First results showed that, as mentioned above, HBV DNA levels were not significantly reduced. Other results showed that, in most of the patients, the transient dose-dependent induction of ISG15 messenger RNA was not accompanied by an increase in the serum level of IFN-α. The authors attributed this fact to a likely elevation of IFN levels at a different time point from that of the samples collection, to limitations of the assay, or to the local production of this cytokine in the gut and liver, where IFN levels were not measured. On the other hand, serum IP-10 and ITAC levels were transiently elevated, as well as peripheral $CD24^{bright}CD38^{bright}CD19+$ Breg and $IgD^{+}CD10^{+}CD27\text{-}CD19^{+}$ T2 transitional B cells. Moreover, treatment with Vesatolimod was generally well-tolerated, with mild or moderate adverse events, with no dose-dependence.[200,201]

Vesatolimod has been also tested on patients with hepatitis C virus infection. During the phase I clinical trial (NCT01591668), the incidence of adverse events was evaluated in single and multiple doses of GS-9620. Results were similar to those obtained in HBV trials, as a transient dose-dependent induction of ISG15 was observed, with no significant decreases in HCV RNA. GS-9620 was well tolerated at all dose levels. Headache was the most frequently reported adverse event, and generally, adverse events were mild to moderate.[202]

As earlier commented, GS-9620 has also been tested as an antiviral agent against HIV, and two clinical trials have been performed, with no published results yet. The phase I trial entitled "Evaluate the Safety and

TABLE 6 Pteridine derivatives in clinical trials

Clinical trials	Agent	Pathology	Dosing[a]	N[b]	Design	Outcome[c]	Status[d]	Last update	Reference[e]
NCT00103350	TG100-115	Myocardial infarction	nd	100	Allocation: Randomized intervention model: Single Group Assignment Masking: Double Primary Purpose: Treatment	Safety and pharmacokinetics of TG100-115 Impact of TG100-115 on infarct size	Phase I/II, completed 2005-2008	May 2008	np
NCT00701766	BI-2536	Acute myeloid leukemia	50-400 mg	71	Intervention Model: Parallel Assignment Primary Purpose: Treatment	Maximum tolerated dose Best objective response (time frame: 3 wk, throughout the study period)	Phase II, completed 2006-2014	May 2014	[48]
NCT00526149	BI-2536	Advance solid tumor	200-250 mg	76	Allocation: Non-Randomized Masking: None (Open Label) Primary Purpose: Treatment	Confirmed objective response rate (complete and partial responses) as defined by RECIST	Phase II, completed 2007-2013	Oct 2013	[185]
NCT00412880	BI-2536	Small cell lung cancer	200-250 mg	23	Intervention Model: Single Group Assignment Primary Purpose: Treatment	Objective tumor response evaluated according to the RECIST criteria by tumor measurements using standard imaging (time frame: at least 6 wk)	Phase II, completed 2006-2014	May 2014	[187]
NCT00376623	BI-2536	Nonsmall cell lung cancer	200 mg 50-60 mg	96	Intervention Model: Parallel Assignment Primary Purpose: Treatment	Objective tumor response according to RECIST (time frame: every 6 wk)	Phase II, completed 2006-2014	May 2014	[188]
NCT00710710	BI-2536	Pancreatic cancer	200 mg 60 mg	89	Intervention Model: Parallel Assignment Primary Purpose: Treatment	Objective Response (RECIST) (time frame: 3 wk). PFS (time frame: every 6 wk)	Phase II, completed 2006-2014	May 2014	[46]
NCT00706498	BI-2536	Prostate cancer	200-250 mg	20	Intervention Model: Single Group Assignment Primary Purpose: Treatment	PSA response rate at 12 wk according to Prostate Specific Antigen Working Group (PSAWG) criteria. (Time frame: 12 wk)	Phase II, completed 2006-2014	May 2014	[189]

(Continues)

TABLE 6 (Continued)

Clinical trials	Agent	Pathology	Dosing[a]	N[b]	Design	Outcome[c]	Status[d]	Last update	Reference[e]
NCT00243087	BI-2536	Non-Hodking lymphoma	175 mg (MTD)	41	Masking: None (Open Label) Primary Purpose: Treatment	Maximum tolerated dose as measured by CTCAE v3.0 at days 1-22 of each course (time frame: up to 22 d of each course) Dose-limiting toxicity as measured by CTCAE v3.0 at days 1-22 of each course (time frame: up to 22 d of each course)	Phase I, completed 2005-2013	Nov 2013	[47]
NCT01721876	Volasertib Cytarabine	Acute myeloid leukemia	350 mg	666	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Double Primary Purpose: Treatment	Complete remission (CR) (time frame: 4 y) Complete remission with incomplete blood count recovery (CRi) (time frame: 4 y)	Phase III, active, not recruiting. 2012-/	May 2018	[44]
NCT00824408	Volasertib Pemetrexed	Nonsmall cell lung carcinoma	300 mg	143	Allocation: Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment	Progression free survival (PFS) time from the date of randomization to date of disease progression or death, whichever occurred first. (Time frame: from randomization until disease progression or death)	Phase II, completed 2009-2016	Sep 2016	[192]
NCT01121406	Volasertib	Ovarian cancer	300 mg	110	Allocation: Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment	Disease control rate at week 24 according to RECIT version 1.1 (time frame: week 24)	Phase II, completed 2010-2015	Aug 2015	[193]
NCT01023958	Volasertib	Urothelial cancer	250-350 mg	50	Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Objective tumour response according to RECIST criteria (time frame: from first drug administration until end of study, up to 2 y)	Phase II, completed 2009-2017	Nov 2017	[194]

(Continues)

TABLE 6 (Continued)

Clinical trials	Agent	Pathology	Dosing[a]	N[b]	Design	Outcome[c]	Status[d]	Last update	Reference[e]
NCT00804856	Volasetib Cytarabine	Acute myeloid leukemia	150-550 mg	180	Allocation: Randomized Intervention Model: Parallel Assignment Primary Purpose: Treatment	Phase I part: MTD of BI 6727 monotherapy and BI 6727 in combination with LDAraC (time frame: 4 wk) Phase IIa part: efficacy (complete remission, CR; complete remission with incomplete blood count recovery, CRi) (time frame: minimum 4 wk, maximum LPO)	Phase II, active 2008-/	Jun 2018	[196]
NCT02579382	Vesatolimod-Tenofovir Disoproxil Fumarate	Hepatitis B	1-4 mg	192	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Double (Participant, Investigator) Primary Purpose: Treatment	Mean change (Measured in log 10 IU/mL) in serum hepatitis B surface antigen (HBsAg) from baseline at week 24 (time frame: baseline; week 24)	Phase II, active 2015-/	Feb 2018	[201]
NCT01591668	Vesatolimod	HCV	0.3-4 mg	51	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment	Incidence of adverse events in single and multiple doses of GS-9620 (time frame: periodically day 1 to 6 months)	Phase I, completed 2012-2012	Aug 2013	[202]
NCT03060447	Vesatolimod ART	HIV	6 mg	30*	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Double (Participant, Investigator) Primary Purpose: Treatment	Overall safety profile as assessed by percentage of participants experiencing treatment-emergent serious adverse events (SAEs) and all treatment-emergent adverse events. (Time frame: up to 45 wk plus 30 d)	Phase I, active 2017-/	Jun 2018	np

(Continues)

TABLE 6 (Continued)

Clinical trials	Agent	Pathology	Dosing[a]	N[b]	Design	Outcome[c]	Status[d]	Last update	Reference[e]
NCT02858401	Vesatolimod ART	HIV	1-12 mg	72	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Double (Participant, Investigator) Primary Purpose: Treatment	Incidence of treatment-emergent serious adverse events (SAEs) and all treatment-emergent adverse events (time frame: up to 157 d) Maximum change from baseline in plasma log 10 HIV-1 RNA at any postdose timepoint (time frame: up to day 81 [Cohorts 1 to 3] or up to day 134 [Cohorts 4 to 9])	Phase I, active 2016-	May 2018	np

Table summarizes completed or currently active clinical trials involving the pteridine derivatives described. Data for different pathologies are referred to those clinical trial with the most advanced phase and last update.

[a]In the case of clinical trials involving two drugs, dose is referred to the pteridine-based compound. nd, not determined.

[b] Number of actual enrolled patients. (sr), estimated number, as Clinical Trial is active and still recruiting.

[c]Current primary outcome measures. RECIST, response evaluation criteria for solid tumors; PFS, progression-free survival; PSA, prostate specific antigen; MTD, maximum tolerated dose; CTCAE, common terminology criteria for adverse effects; ART, antiretroviral treatment.

[d] Study phase, recruitment status, first submitted date and actual primary completion date.

[e]Ref: Publications reporting the clinical trial results; np: no publications of the results have been found.

Efficacy of Vesatolimod in Antiretroviral Treated HIV-1 Infected Controllers" (NCT03060447) was scheduled from 23 February 2017 to 6 June 2018, with an estimated enrolment of 30 participants. The primary objective of this study was "to evaluate the safety and tolerability of a 10-dose regimen of Vesatolimod in HIV-1 infected controllers on antiretroviral treatment (ART) and during analytical treatment interruption (ATI) following Vesatolimod dosing."

The other phase I clinical trial against HIV-1 in infected patients was entitled "Safety and Biological Activity of Vesatolimod in HIV-1 Infected, Virologically Suppressed Adults" (NCT02858401) was active from 8 August 2016 to 4 May 2018. The primary objectives of this study were "to evaluate the safety and tolerability of Vesatolimod (formerly GS-9620) at escalating, multiple doses in HIV-1 infected virologically suppressed adults on antiretroviral therapy (ART), and to evaluate the virologic effect of Vesatolimod, as measured by changes in plasma HIV-1 RNA."

The great versatility and therapeutic potential of the pteridine derivatives highlights the need to continue searching for new compounds, as well as to deepen the knowledge of their targets and mechanisms of action, especially of those compounds that have demonstrated sufficient therapeutic potential in preclinical studies as well as in their corresponding clinical trials.

8 | RELEVANCE OF PTERIDINES FOR THE DEVELOPMENT OF NEW DRUGS

Pteridines are heterocycle compounds endowed with a wide range of therapeutic potentials, with a special emphasis on cancer treatment. In this sense, the establishment in the 1950s of methotrexate as an antitumor drug has motivated the search for new pteridine derivatives that can equal or exceed the actions of MTX, while suppressing its associated side effects. Translation of pteridine chemistry to medicinal chemistry seems to have been lower than with other heterocyclic scaffolds. However, reactions with probed suitability are broadly available for the synthesis of pteridines. Furthermore, applying novel radical substitution, side chain variation, nucleophilic substitution, and organometalic linking to the pteridine rings, a wide variety of reactions are available to search for diversity-oriented synthesis. With the aim of identifying new chemical agents to fight against the increased incidence of cancer, numerous pteridine derivatives have shown antitumor potential in either in vitro assays, generally using tumor cell lines, or xenograft mouse models. In most cases, the target molecule was identified, including a wide range of enzymes whose activity may be affected. This intensive research has led to the identification of pralatrexate for peripheral T cell lymphoma treatment, as well as other pteridine compounds, which are in currently clinical trials (Table 6).

Pteridine potential in chronic inflammation treatment has also been investigated. As a representative example, MTX is the anchor drug for rheumatoid arthritis treatment along with other therapeutic agents. In this context, similar strategies to those applied in the case of cancer research have been followed, although the development of pteridine compounds as anti-inflammatory agents has not advanced too much. However the increased prevalence of chronic inflammatory diseases associated to the higher life-expectancy of the current Western population will warrant a bigger effort in the search for new, more efficient and less harmful, anti-inflammatory drugs.

The antimicrobial activities of pteridine derivatives have been widely explored, identifying several compounds that may be useful antibiotics. Many articles have described pteridines directed to bacteria but also those against viruses or even parasites. Nevertheless, in vivo assays are scarce, and most articles just showed results derived from isolated enzyme-activity and cell-based assays, with the exception of GS-9620, which has been tested even in clinical trials for the treatment of hepatitis virus or HIV infection.

Finally, although the greatest effort and better results have been related to the potential antitumor functions of pteridine-based compounds, several other studies found in the literature indicate that pteridine-based drugs may

be of use to treat many other socially important diseases, including depression, osteoporosis, diabetes, hypertension, and ischemia, among others (Table 6).

9 | CONCLUSION

Over the past 60 years, evidence has accumulated to show that pteridine-derived compounds are important regulators of many physiological and pathological processes. The conservation of pterin structure throughout prokaryotic and eukaryotic cells leads to the possibility of new and not yet discovered roles for these molecules. As we have shown in this review, the range of target molecules of pteridine-based compounds and thus, the corresponding range of applications as therapeutic agents is currently expanding (Figure 3). In this regard, although not many potent drugs based on the pteridine structure have reached clinical trials and pharmaceutical use, it is also evident that, in recent years, several investigations have made important contributions in this field of research. Thus, many publications have pointed out that pteridines may be potentially used to treat several, highly prevalent and difficult to cure, diseases including not only cancer and inflammatory related diseases but also, neurodegenerative pathologies, cardiovascular diseases, depression, osteoporosis, and infectious diseases, among others. A deep understanding of both the diseases and the mechanisms being targeted in different pathologies is essential. Therefore, there are several aspects that need further investigation: the detailed dissection of the site of action of different compounds in vivo and in vitro models; SAR of newly synthesized compounds; thorough studies of the effects of different agents over the course of specific pathologies in animal models first, and eventually in the corresponding clinical trials.

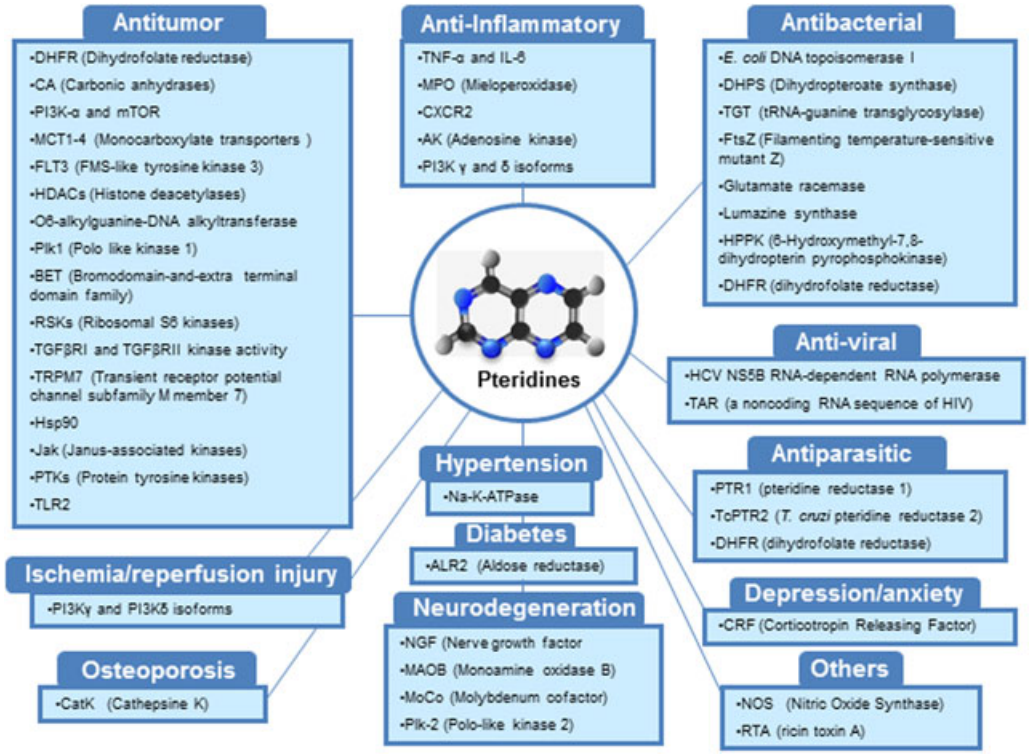


FIGURE 3 Schematic summary of pteridine targets [Color figure can be viewed at wileyonlinelibrary.com]

In conclusion, the accumulated evidence on the therapeutic potential of the pteridine-derivatives reviewed here, together with the development of new synthetic methods and bioinformatic tools, will guarantee significant advances in the pharmacological application of these compounds.

ACKNOWLEDGMENTS

Violeta Carmona-Martínez's salary has been supported by MINECO (Secretaría de Estado de Investigación, Desarrollo e Innovación, Sistema Nacional de Garantía Juvenil: PEJ-2014-A-95803). We gratefully acknowledge the financial support of the Fundación Séneca of the Comunidad Autónoma de la Región de Murcia (project 19249/PI/14).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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How to cite this article: Carmona-Martínez V, Ruiz-Alcaraz AJ, Vera M, Guirado A, Martínez-Esparza M, García-Peñarrubia P. Therapeutic potential of pteridine derivatives: A comprehensive review. *Med Res Rev*. 2018;1–56. https://doi.org/10.1002/med.21529