Biological applications of imiquimod analogues: An update (Review)

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Abstract. The last decade witnessed intensive efforts in drug discovery and the emergence of thousands of molecules with potential for use as therapeutic targets. However, only a few hundred of these molecules reached the stage of clinical trials, with only a handful being translated from the bench to bedside. One such example is imiquimod, an immune response modifier belonging to the imidazoquinoline family. Imiquimod was first approved by the US Food and Drug Administration for the topical treatment of anal and genital warts and actinic keratosis, and later exhibited a potent antitumor activity. This promising activity inspired the development of several imidazoquinoxaline analogues and derivatives. The present review provides a comprehensive overview of the scientific literature on the chemical synthesis of imiquimod and several of its analogues, namely EAPB0203, EAPB0503 and EABP02303. The present review also discusses their preclinical properties and mechanisms of action in the context of cancer and parasitic infections, highlighting the worthiness of translating these activities into therapeutic drugs.

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1. Introduction

The immune system plays a dual role in cancer under the concept of the immune-editing theory (1-3). As such, the immune system can fight cancer, but can also shape cancer immunogenicity. In the first phase, the immune system may eliminate cancer, while in a later phase, such as in the equilibrium phase, a balance is established between antitumor and pro-tumor immunity until cancer cells escape the immune control and acquire metastatic potential (1-3). This underscores the importance of immunomodulatory therapy in boosting endogenous defense mechanisms to fight cancer and infections.

Immunomodulatory drugs include agents that modify the immune response by increasing (immunostimulators) or decreasing (immunosuppressives) the production of serum antibodies (4). Cancer-targeted small molecule-based immunomodulators are classified as inhibitors, agonists, or degraders (5). Another classification of cancer immunotherapeutics include checkpoint inhibitors, chimeric antigen receptor T-cells (CAR T-cells), monoclonal antibodies, cancer vaccines, cytokines, radio-immunotherapy and oncolytic virus therapy (6). Immunomodulators, one of four

immunotherapeutic classes, are themselves divided into four categories based on their mode of action as follows: Checkpoint inhibitors, cytokines, agonists and adjuvants. To date, 16 different immune-modulators [nine checkpoint inhibitors (7-10)], four cytokines (11-13) two adjuvants (5) and a small molecule with immunomodulatory properties (14) have been approved by the US Food and Drug Administration (FDA) for the treatment of several major cancer types. Imiquimod, one of the two approved adjuvants (5), targets Toll-like receptor (TLR)-7 and activates pathways involved in the innate immune system, which results in the induction of an adaptive immune response (15,16). In addition to its topical application for anal and genital warts, and actinic keratosis (17), imiquimod is used and approved for subsets of patients with basal cell carcinoma.

Imidazoquinoxalines, imiquimod analogues, have outperformed their parent compound and have exhibited potent antitumor properties with less adverse events in the preclinical research setting (18,19). In particular, the most promising analogues, from first and second generations, EAPB0203, EAPB0503 and EABP02303, have been evaluated in different types of cancer, such as melanoma (20-22), adult T-cell leukemia/lymphoma (ATL) (19,23), chronic myeloid leukemia (CML) (24) and acute myeloid leukemia (AML) (25). In addition, they have exhibited anti-parasitic activity, specifically against cutaneous leishmaniasis (26). At the molecular level, EAPB0203 and EAPB0503 act on tubulin, inhibit its polymerization, and consequently arrest the cells in the G_2/M phase (19,24,25), possibly leading to apoptosis (19,22). However, and unlike the two former compounds, EABP02303 does not bind to tubulin and its specific mode of action remains to be elucidated.

In the sections below, an overview of imiquimod is provided, including a detailed description of the chemical synthesis of imidazoquinoxalines and their therapeutic applications to date.

2. Imiquimod

Imiquimod (1-(2-methylpropyl)-1*H*-imidazo(4,5-*c*) quinolin-4-amine), also known as S-26308 or R-837, is the first non-osidic (i.e., contain no sugar) nucleoside analogue of the imidazoquinoline family. It is a tricyclic organic molecule with a nitrogen (N)-containing heterocyclic compound of four N atoms, a quinoline component, a 1H-imidazole ring, and a methyl-propyl group (27). Imiquimod belongs to the class of immune response modifiers (15,28), and is commercialized under the name of Aldara® or Zyclara®. Its topical use was approved by the US FDA in 1997, for the treatment of certain viral infections, such as perianal and genital human papilloma virus (HPV) disease (genital warts) by stimulating the host immune system (29,30). Imiquimod was also the first immune response modifier used for the treatment of infectious skin conditions due to its potent *in vivo* anti-viral and anti-tumor activities (16). In addition, it was effective as a topical therapy for certain types of skin cancer, including superficial basal cell carcinoma, Bowen's disease, superficial squamous cell carcinoma, certain superficial malignant melanomas and actinic keratosis (15,31-33). The systemic administration of imiquimod in mice has been shown to exert potent antitumor effects against various types of cancer, such as melanoma, lung sarcoma, mammary, colon, bladder carcinoma (19,34), basal cell carcinoma (16,35), actinic keratosis (36) and cutaneous B-cell lymphoma (19,33,37). Furthermore, its therapeutic spectrum has been extended to certain parasitic infections, such as cutaneous leishmaniasis (26,38) and toxoplasmosis (39). In a previous study on cutaneous leishmaniasis, imiquimod was clinically used, in combination with a systemic antimonial, and yielded a cure rate of 90% in patients with refractory cutaneous leishmaniasis, as compared to pentavalent antimonial treatment alone (40). In a clinical trial performed in Peru, Miranda-Verastegui *et al* (41) demonstrated that this combination was more effective than the placebo plus pentavalent antimony (41). Imiquimod has also been proven to be effective as a first line therapy for cutaneous leishmaniasis (42).

Chemical synthesis of imiquimod. Over the years, the chemical synthesis of imiquimod was performed using several methods. Conventional synthesis was based on the addition of imidazole group to the quinoline core (27) (Fig. 1).

However, this method presented several limitations, including the numerous and lengthy steps involved in the addition of N atoms. Thereafter, other strategies were adopted to save time and cost. One strategy began with 2-bromobenzaldehyde instead of quinolone (Fig. 2), yet it resulted in a low yield of 30%. Another approach used, in the first step, anthranilic acid, a commercially available compound. Several intermediates including benzoxazine, hydrazoic acid, tetrazoloquinoline and iminophosphorane were produced, and imiquimod was formed from iminophosphorane. While this route was synthetically interesting, it included the formation of a tetrazole derivative, a potentially explosive product (27).

Given the limitations of the aforementioned methods, new strategies are being developed to improve imiquimod synthesis, and to generate imiquimod derivatives with enhanced efficiency and reduced adverse effects (discussed below).

Biological activity and mechanisms of action of imiquimod. The exact mechanisms of action of imiquimod have not yet been fully explored. Nevertheless, it is well documented that imiquimod is a TLR-7 agonist (15,43), which ligates TLR-7 activating immune cells. TLR-7 is expressed on the endosomal surface of antigen presenting cells and is commonly involved in pathogen recognition (40). Other cell types activated by imiquimod include natural killer cells, macrophages, dendritic cells and B-lymphocytes, leading to the consequent activation of downstream protein kinases and transcription factors, including nuclear factor-κB (NF-κB), and the production of pro-inflammatory cytokines and chemokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-8 (44), IL-12, interferon (IFN)-α and IFN-γ (19,45-53). Cytokine induction and cellular infiltrates underlying cell-mediated immune responses result in the regression of warts caused by HPV infection (54). In the case of cutaneous leishmaniasis, imiquimod exhibits an anti-leishmanial activity via the activation of TLRs, the production of inflammatory cytokines and the induction of nitric oxide release (26). Recently, the mechanism of action of imiquimod against acute and chronic toxoplasmosis was elucidated. Indeed, imiquimod was shown to upregulate the expression of TLR-7, -11 and -12, and subsequently mediate the activation of the MyD88 pathway, resulting in the induction of

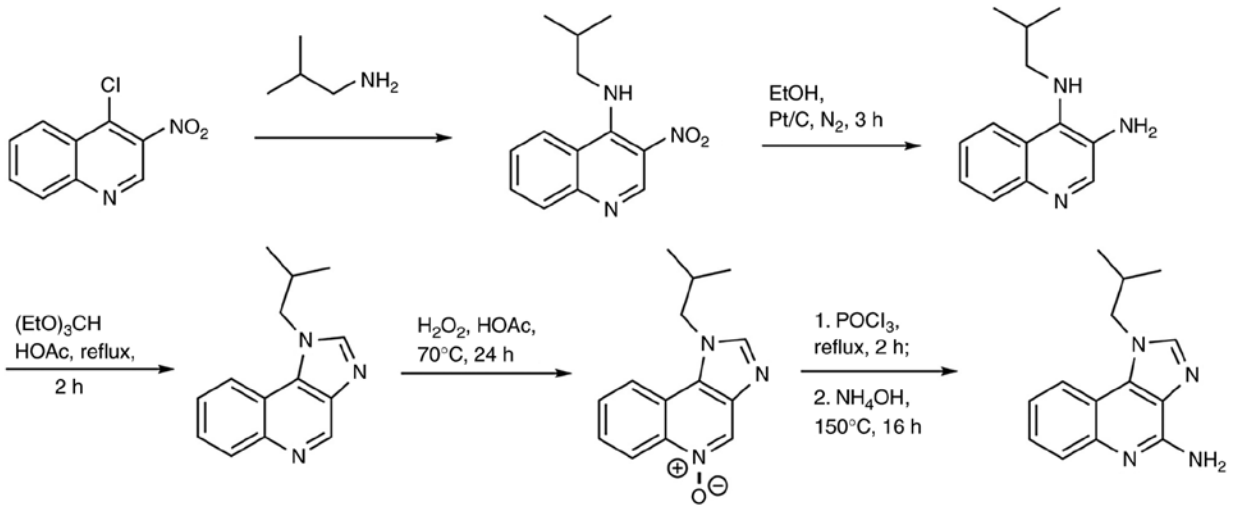


Figure 1. Conventional method for imiquimod synthesis (27). First, 4-chloro-3-nitroquinoline undergoes a nucleophilic aromatic substitution reaction, with isobutylamine to substitute isobutylamine for chlorine (Cl). This reaction is followed by the hydrogenation of the nitro group to form the imidazole ring in the resultant diaminoquinoline scaffold treated with triethyl orthoformate in glacial acetic acid to form the tricyclic core. Finally, imiquimod is obtained by the addition of the side amino group. The latter was added through a three-step sequence of reactions which involve formation of an N-oxide intermediate, chlorination and amination.

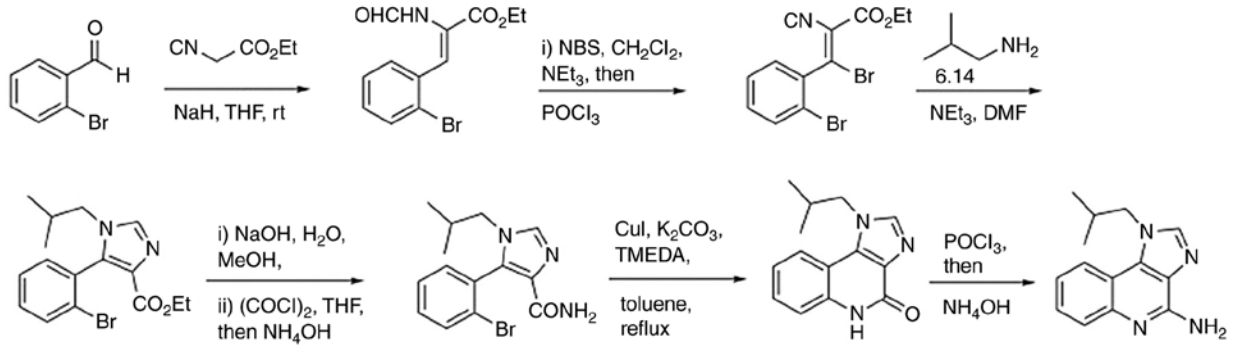


Figure 2. Synthesis of imiquimod. First, 2-bromobenzaldehyde is coupled with ethyl isocyanoacetate via a Knoevenagel coupling reaction and the resulting acrylate is brominated, and oxidized, followed by the addition of isobutylamine. This generates the imidazole ring. Then, several reactions are used to cyclize the quinoline moeity and provide the two missing N atoms: One N is added by a series of reactions replacing the ethyl-ether group by an amide forming group then the other N and the primary amide are added after a copper-mediated *N*-arylation reaction and Br substitution rendering the imidazoquinolinone scaffold (27,118).

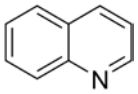
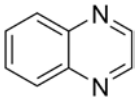
an immune response (39). There is also evidence to indicate that imiquimod, when applied to the skin, can lead to the activation of Langerhans cells, which then migrate to local lymph nodes to activate the adaptive immune system (40).

Independent of TLR-binding, imiquimod can also boost pro-inflammatory signaling by restricting the negative feedback on inflammation. In that sense, imiquimod inhibits adenylyl cyclase activity and consequently suppresses the adenosine receptor signaling pathways involved in negative regulation of inflammation (51,55). Imiquimod has also been shown to inhibit tumor progression in immunocompromised animals (34), and its topical application potently inhibits tumor induced-angiogenesis *in vivo*, in a dose and time-dependent manner (56). This effect was associated with the imiquimod-induced production of several cytokines, including IFN-γ, TNF-α and IL-18. This constitutes an effective response to the treatment of HPV-associated benign and premalignant tumor, but also of early malignant angiogenesis-dependent proliferative tumors (56).

Under the TLR-independent pathway, imiquimod induces direct apoptotic effects, through the intrinsic mitochondrial-and Bcl-2-dependent apoptotic pathway (18,50,57), leading to the translocation of cytochrome *c* into the cytosol and the activation of procaspase-3 and -9 (58). Notably, imiquimod selectively induces the apoptosis of transformed keratinocytes and melanoma cells, without any effect on their normal counterparts (19,49-51,59).

Clinical use of imiquimod and adverse effects. Imiquimod is approved and commercially available as a 2.5, 3.75 or 5% cream for the treatment of external genital warts and superficial basal cell carcinoma. However, it is also used for off-label conditions as follows: Melanocytic proliferations, such as lentigo maligna (60-62), atypical nevi (63), as well as lichen sclerosus, alopecia areata (64), Kaposi sarcoma (65,66) and Molluscum contagiosum (67). Research on imiquimod is still an active area. Indeed, >450 studies were published on the topic over the past year alone (as of May, 2023). A previous study evaluated

Table I. Quinoline and quinoxaline: Similarities and differences (82-89).

	Quinoline	Quinoxaline
Composition	Heterocyclic compound composed of a benzene and a pyridine ring	Heterocyclic compound composed of a benzene and a pyrazine ring
Structure		
Application	Anti-malarial Anti-viral Anti-bacterial Anti-fungal/anti-protozoan Anti-helminthic Local anesthetic Anti-asthmatic Anti-psychotic Anti-glaucoma Cardiotonic Anticancerous	Anti-malarial Anti-inflammatory Anti-HIV Anticancerous

HIV, human immunodeficiency virus.

the potentiating effect of imiquimod on dyphencyprone for the topical immunotherapy of alopecia areata and reported that 77% of patients exhibited an improvement in symptoms upon the addition of imiquimod (68). Imiquimod was also tested as a topical treatment in patients with HIV and high-grade squamous intraepithelial lesions, susceptible to develop into anal cancer (69). Compared to the active monitoring group, the rate of anal cancer onset was 57% lower in the treatment group; suggesting that the treatment of high-grade squamous intraepithelial lesions with medications such as imiquimod is protective against anal cancer (69). Another recent study described the efficacy of imiquimod loaded in phospholipid-free small unilamellar vesicles targeted to hepatocytes, in the treatment of chronic hepatitis B (70). In patients with lentigo maligna, imiquimod was shown to reduce the lentigo maligna surface after 1 month of treatment and could be prescribed to prevent the progression of these lesions to carcinogenesis (71). In a human melanoma cell line, imiquimod induced cell death by lysosomal membrane permeabilization and the release of lysosomal proteolytic enzymes, including cathepsins, which resulted in mitochondrial dysfunction (72). While imiquimod is an effective and safe therapy, certain adverse effects have been reported; these include local inflammatory reactions such as itching, burning, bleeding, erosions, ulcerations, excoriations, crusting, induration, edema and pain (73,74). Some patients have presented with systemic reactions, such as upper respiratory tract infections and flu-like symptoms, such as fever, sinusitis, headaches and tiredness (50,74,75). In the case of periocular skin lesions, imiquimod may cause conjunctivitis and ocular stinging (61). In addition, imiquimod can affect vitamin B12 levels (60). Imiquimod has also been shown to be associated with hypertrophic lupus erythematosus in an elderly patient (76). Additionally, a case report of actinic keratosis associated imiquimod with a severe case of bullous pemphigoid (77) and the occurrence of lupus-like reactions (78). In another study, when applied to a to a single lesion of *in situ* melanoma in a patient, imiquimod caused debilitating severe fatigue (79). Moreover, imiquimod caused localized skin ulceration in a patient with type-2 diabetes mellitus (80). Finally, women of reproductive age are advised to use contraception upon imiquimod treatment, due to the absence of definitive data on its teratogenic potential (74).

3. Quinolines and quinoxalines

Heterocyclic compounds are a cornerstone of current anticancer drug design due to their favorable pharmacokinetic and pharmacodynamic properties (higher lipophilicity, adjusted polarity and other physicochemical features including solubility, ionization state and hydrogen-bonding capacity). In 2015, ~30% of US FDA-approved anticancer drugs included one or more cyclic rings containing a nitrogen or an oxygen (81).

Quinolines and quinoxalines are N-containing heterocycles found in a number of natural compounds. In both quinoline and quinoxaline compounds, the main core of imiquimod is composed of a benzene ring. The difference is that, in quinoxalines, the pyridine ring of quinoline is replaced by a less alkaline component, the pyrazine ring (Table I) (82-84).

The quinoline compound has limited applications, while its derivatives span a wide range of activities, such as anti-malarial (quinine, quinidine, chloroquine, mefloquine, amodiaquinine, primaquine, etc.), anti-viral (saquinavir), anti-bacterial (fluoroquinolones such as ciprofloxacin, sparfoxacin, gatifloxacin, etc.), anti-fungal/anti-protozoan (clioquinol), anti-helminthic (oxamniquine), local anesthetic (dibucaine), anti-asthmatic (montelukast), anticancerous (camptothecin, irinotecan, topotecan, etc.), anti-psychotic (aripiprazole, brexpiprazole, etc.), anti-glaucoma (carteolol)

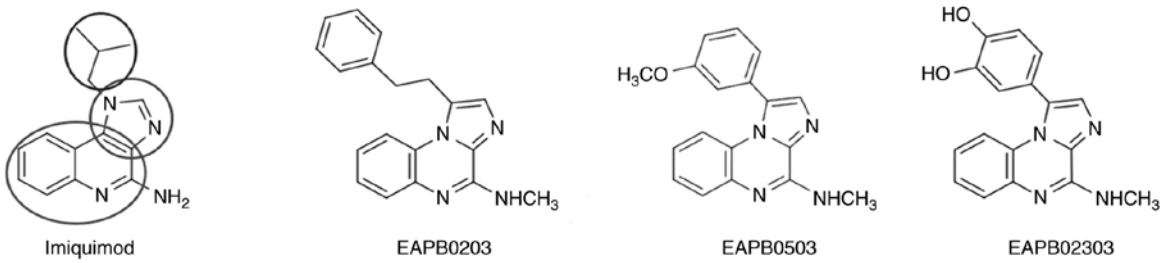


Figure 3. Structure of imiquimod, EAPB0203, EAPB0503 and EAPB02303. The heterocyclic scaffold platform of imiquimod is comprised of a quinoline component (a six-membered ring, lowermost ellipse) and a 1H-imidazole ring (a five-membered ring, middle ellipse). Analogue design is based on a modified heterocyclic scaffold, named imidazoquinoxaline, comprised of a quinoxaline (bicycle with two nitrogen atoms) fused on one of its face to an imidazole, leading to the presence of a bridgehead nitrogen which slightly modifies the general planarity of the molecule (90). The isopropyl group in imiquimod (uppermost ellipse) is substituted by a phenethyl in EAPB0203, a 3-methoxyphenyl in EAPB0503 or a 3,4-dihydroxyphenyl group in the new derivative EAPB02303. In the three imidazoquinoxaline analogues, the amino group of imiquimod is replaced by a methylamino group.

and cardiotonic (vesnarinone) activities. In the particular case of cancer, quinoline derivatives induce cell cycle arrest and apoptosis, the inhibition of angiogenesis, the disruption of cell migration and the modulation of nuclear receptor responsiveness in cancer cells (85). Similarly, quinoxaline compounds exibit a wide array of pharmacological activities, some of which include anti-malarial (86), anti-inflammatory (87), anti-HIV (88) and anticancer activities (89).

4. Imidazoquinoxalines

Imiquimod exerts a selective cytotoxic effect on cancer cells. However, the imiquimod-induced production of pro-inflammatory cytokines can have deleterious effects. Hence, a series of imiquimod analogues belonging to the quinoxaline family was synthesized, with the ultimate aim of enhancing the antitumor potential of imiquimod, while rendering it systemically available and curbing its toxicity profile (18).

A series of three imiquimod analogues were synthesized: The imidazo[1,2-*a*]quinoxalines, the imidazo[1,5-*a*]quinoxalines and the pyrazolo[1,5-*a*]quinoxalines. The present review focuses on three compounds of the imidazo[1,2-*a*]quinoxalines series, namely EAPB0503, EAPB0203 and EAPB02303. The first generation encompasses EAPB0203 [N-methyl-1-(2-phenylethyl) imidazo[1,2-*a*]quinoxalin-4-amine)] as an earlier derivative, followed by EAPB0503 [1-(3-methoxyphenyl)-N-methyl imidazo[1,2-*a*]quinoxalin-4-amine] (Fig. 3), and it paved the way for *in vitro* studies revealing considerable anticancer potential for this class of imidazoquinoxalines (19).

Recently, a newer imidazoquinoxaline derivative, EAPB02303 [1-(3,4-dihydroxyphenyl)imidazo[1,2-*a*]quinoxaline] (Fig. 3, right panel), as the lead compound of the second generation, was synthesized; this demonstrated a higher potency in a melanoma cell line model, with a 50% inhibitory concentration (IC_{50}) of 3 nM, as compared with IC_{50} of 383 nM for EAPB0503 (90).

Chemical synthesis of EAPB0203, EABP0503 and EAPB02303. EAPB0203 was first synthesized using the traditional method, which consists of the condensation of two imidazole derivatives coupled to *ortho*-fluoroaniline, followed by an intramolecular cyclisation and substitution with the appropriate amines (19). The synthesis of EAPB0503 is based on EAPB0203 synthesis, but was modified and optimized using the Suzuki reaction under basic conditions in a microwave-assisted reaction to yield greater purity with shorter synthesis time (Fig. 4). In summary, EAPB0503 was generated by replacing the phenethyl group in EAPB0203 with 3-methoxyphenyl (Fig. 3) (22). As regards EAPB02303, it was obtained by the substitution of 3-methoxyphenyl group at R1 position in EAPB0503 by 3,4-dihydroxyphenyl group on the 4-methylaminoimidazo(1,2-*a*) quinoxaline heterocyclic platform (Fig. 3) (90).

Pharmacokinetic and pharmacodynamic properties of EAPB0203, EAPB0503 and EAPB02303. The modifications introduced into the imidazoquinoxalines, EAPB0203, EAPB0503 and EAPB02303 (19), aimed to improve their polarity and binding properties.

Imidazo[1,2-*a*]quinoxalines, mostly EAPB0203 and EAPB0503, their metabolites, and their major oxygenated derivatives, were characterized and identified using nuclear magnetic resonance and liquid chromatography-mass spectrometry studies (91). Briefly, EAPB0203 and EAPB0503 are metabolized inside liver microsomes of numerous species, including mouse, human, dog and rat. Both EAPB0203 and EAPB0503 metabolites exhibit an increased polarity compared to their parent compounds, and have mostly shorter half-lives, apart from one metabolite (92). EAPB0203 and EAPB0503 biotransformation began with a demethylation step, yielding their two main metabolites EAPB0202 and EAPB0603, respectively. These metabolites are less potent than their parent molecule (92). Furthermore, ~98% of EAPB0203 and EAPB0503 bind to human serum albumin in the plasma, with very little compound remaining in the free form. Both compounds are eliminated from the plasma in two phases: an initial rapid decline phase, followed by a prolonged terminal phase. The terminal half-life is ~2 h. However, the systemic availability of EAPB0203 and EAPB0503, upon intraperitoneal administration is relatively modest, at 22.7 and 35% respectively (92); this motivates further research to enhance bioavailability and pave the way for more preclinical (and ultimately clinical) studies.

5. Immunomodulatory effect

A total of 22 imidazo(I,2-*a*)quinoxalines derivatives have been proven to be potent inhibitors of nucleotide phosphodiesterase

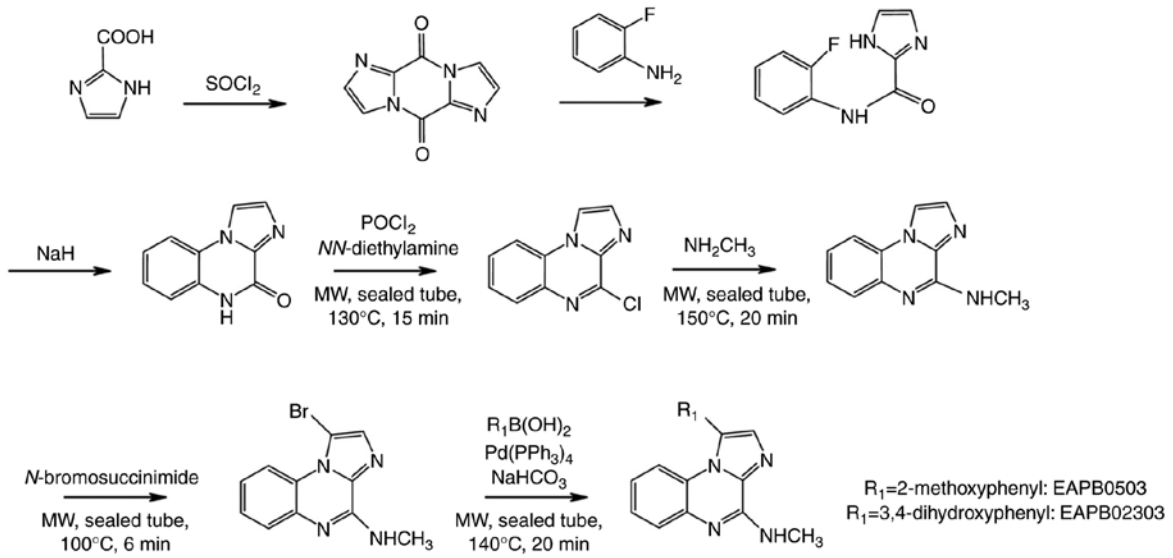


Figure 4. EAPB0503/EAPB02303 synthesis steps. Carbonyl-imidazole dimer that results from the bimolecular condensation of 2-imidazole carboxylic acid is coupled with ortho-fluoroaniline to give an intermediate, which is then cyclized to afford the new tricyclic core. Further treatments yield *N*-methylimidazo(1,2-*a*) quinoxaline-4-amine and Suzuki coupling performed on the 1-bromine derivative leads to either EAPB0503 or EAPB02303 products.

enzyme 4 extracted from human alveolar epithelial cells (93). It is considered that by inhibiting phosphodiesterase enzyme 4 activity, these compounds could lead to cAMP accumulation and transcription factor cAMP response element-binding protein activation inside the cell (31). In addition, since they are able to decrease TNF-α levels (19,93) they can consequently, modulate cytokine synthesis and/or production by immune cells, exerting therefore their anti-inflammatory activity (93). Moreover, it has been shown that imidazo[1,2-*a*]quinoxalines activate the p38 MAPK pathway and inhibit the PI3K pathway in a murine fibroblastic cell line (93).

6. Antitumor properties of EAPB0203, EAPB0503 and EAPB02303

Melanoma. Melanoma is the most aggressive form of skin cancer and is associated with a poor prognosis (94). Standard of care begins with surgical excision (when the tumor is resectable), systemic treatment (chemotherapy and/or immunotherapy), or radiation therapy for unresectable tumors or as adjuvant therapy (21). However, the mortality rates remain very high, urging the need for the identification of novel therapeutic targets. The activity of imiquimod and some of its derivatives was previously tested in melanoma cells *in vitro* and in animal models *in vivo* (50,95). EAPB0203 was 50-fold more potent than imiquimod and exhibited a remarkable cytotoxic activity against A375 melanoma cell line (19,96). EAPB0203 was also 110-fold more potent than fotemustine, a major treatment for metastatic melanoma (19,97). Of note, as previously demonstrated EAPB0503 exhibited a 7-9-fold greater antitumor and cytotoxic activity than EAPB0203 in the same cell line, and induced apoptosis by activating the NF-κB pathway (92). Indeed, both EAPB0203 and EAPB0503 induced the intrinsic apoptotic pathway characterized by the loss of mitochondrial membrane potential, the release of cytochrome *c*, the activation of caspases and the subsequent fragmentation of DNA. The potent cytotoxic activity of EAPB0503 was also observed in an *in vivo* mouse model of xenograft melanoma (19,96). In this regard, the antitumor activity of APB0503 was attributed to anti-proliferative and anti-mitotic effects, mediated by the inhibition of tubulin polymerization (18,22). This compound interacts with tubulin via a colchicine-binding site (18), with a 52% higher potency than colchicine, a well-known natural inhibitor of tubulin polymerization. Unlike imiquimod, the effect of EAPB0503 on tubulin polymerization in melanoma cells is not associated with its TLR-7 agonist activity, even at concentrations >300 μM (18). An EAPB0503 derivative, 1-(2-hydroxy-3-methoxyphenyl)-N-methylimidazo [1,2-*a*] quinoxalin-4-amine, exhibited a greater inhibition of tubulin polymerization in melanoma A375 cells (22).

Given encouraging data on melanoma *in vitro* and *in vivo* models, first-generation imidazoquinoxalines were evaluated in models of hematological malignancies (25) .

The next-generation derivative, EAPB02303, was unable to bind to tubulin at the colchicine site (90). Notably, EAPB02303 was still able to inhibit the growth of A375 melanoma cells *in vitro* at a nanomolar concentration range, independent of the inhibition of tubulin polymerization. The growth inhibition *in vitro* was, dose-dependently, associated with a reduction in size and weight of mouse-xenograft A375 melanoma cell tumors. In addition, growth inhibition was associated with a lower mitotic index, but not with necrosis (90). Transcriptomic analysis, in comparison with first-generation imidazoquinoxalines and a panel of 12 well-known anticancer drugs, suggested that EAPB02303 acts through a different mechanism. However, the exact mechanistic pathway remains to be elucidated (90).

Of note, the second-generation derivatives, EAPB02302 and EAPB02303, were even more cytotoxic on A375 cells, with IC_{50} values of 60 and 10 nM, respectively, versus an IC_{50} value of 1,570 and 200 nM for EAPB0203 and EAPB0503, respectively (96).

ATL. ATL is a rare and aggressive blood malignancy due to the transformation of T-cells by human lymphotropic virus type I.

ATL is associated with a poor prognosis, due to chemo-resistance and immunosuppression (98). Following more than four decades of research, the therapeutic management of ATL remains intricate and a cure for ATL is still out of reach in the majority of patients. The current strategies include the watch-and-wait policy, conventional chemotherapy, allogeneic hematopoietic cell transplantation (allo-HCT), and a combination of two antiviral agents, zidovudine and IFN-α (99-103). Although indolent ATL and a fraction of acute ATL exhibit a long-term survival subsequent to antiviral therapies and one third of patients with aggressive ATL undergo allo-HCT and <10% of those who received chemotherapy could have disease control for >5 years.

Several targeted therapies have been tested in ATL. Among these, EAPB0203 has been shown to induce growth inhibition, cell cycle arrest and apoptosis, selectively in ATL cell lines, while no effect has been reported in normal resting or PHA-activated peripheral blood mononuclear cells taken from two healthy donors (23). EAPB0203 induces apoptosis by decreasing the levels of the anti-apoptotic proteins, c-IAP-1 and Bcl-xL, thus triggering the intrinsic apoptotic pathway. In addition, EAPB0203 treatment stabilizes the tumor suppressor proteins p21 and p53 in a dose-dependent manner (19).

CML. CML is a clonal myeloproliferative disorder resulting from a reciprocal translocation between the *abl* proto-oncogene on chromosome 9 and the breakpoint cluster region (*bcr*) on chromosome 22, resulting in a shortened chromosome 22, known as the Philadelphia (Ph) chromosome. This translocation produces the recombined *bcr-abl* gene, encoding for BCR-ABL, a constitutively activated tyrosine kinase, responsible for CML leukemogenesis, maintenance and progression of the disease from chronic to blast phase, indicating a poor prognosis. The initial treatments for CML included busulfan and hydroxyurea. These were later replaced with IFN-α (104). In 2001, CML treatment witnessed a revolution with the use of imatinib mesylate (imatinib), the first tyrosine kinase inhibitor (TKI) (105-107). However, this treatment was associated with certain drawbacks, including intolerance to treatment, the lack of a therapeutic response or resistance, and this has motivated the development of second- and third-generation TKIs.

While TKIs significantly revolutionized CML treatment and prolonged survival of patients, CML cure is still out of reach, and bone marrow transplantation remains the only long-term curative option for CML patients. Both EAPB0203 and EAPB0503 were evaluated in an *in vitro* model of human blast crisis CML cell lines. The compounds induced cell cycle arrest at the G_2/M phase, accompanied by an increased level of histone 3 phosphorylation. Growth inhibition was more pronounced in three chronic-phase CML cell lines treated with EAPB0503 as compared to EAPB0203 (24), and was enhanced by the induction of the intrinsic apoptosis pathway. Molecular analysis revealed that EAPB0503-treated CML cells exhibited decreased BCR-ABL oncoprotein levels, which could explain growth inhibition. Notably, EAPB0503 appeared to circumvent imatinib resistance *in vitro*, presenting it as a potentially attractive therapeutic option in CML. The combination of imatinib (CML standard-of-care) and EAPB0503 synergized to inhibit CML cell line growth and proliferation *in vitro* (24). Collectively, these findings suggest that EAPB0503 is a potential therapeutic agent capable of inhibiting CML cell growth, synergizing with imatinib to alleviate the tumor burden, and overcoming resistance to imatinib and maybe other TKIs.

AML. AML is a complex and heterogeneous hematological malignancy, characterized by the excessive proliferation of undifferentiated myeloid precursors, resulting in an impaired hematopoiesis and bone marrow failure. AML is associated with a highly variable, yet frequently poor prognosis, based on somatic genetic alterations (108). Due to the genetic complexity and heterogeneity of AML, treatment strategies remain variable among patients, apart from the AML subtype, acute promyelocytic leukemia and core binding-factor (CBF) AML, which have their own standard of treatment (109,110). In general, AML treatment remained unaltered for more than three decades, and relied on aggressive chemotherapy administered in two phases: An induction phase to eliminate AML blasts, and a consolidation phase to prevent relapse (111). Hematopoietic stem cell transplantation was used independently or sequentially, depending on the availability of stem cell donors and the overall clinical fitness of the patient (112).

Genome-wide studies, including gene and microRNA expression profiles, single nucleotide polymorphisms and gene copy number studies, have identified a growing number of recurrent genetic mutations of AML (113). Among the identified genes, mutations of nucleophosmin-1 *(NPM-1)* and fms-like tyrosine kinase 3 *(FLT3)* are prognostic markers of AML. NPM-1 is one of the most frequently mutated genes in AML (114). NPM-1 mutations alter the C-terminal DNA-binding domain of the protein, resulting in an aberrant nuclear export sequence and its retention in the cytoplasm, hence the NPM-1c nomenclature, referring to its cytosolic localization (115).

The pre-clinical efficacy of EAPB0503 and EAPB0203 has been evaluated in AML. EAPB0503 was shown to significantly and selectively inhibit the growth of NPM-1c-positive cells, at lower concentrations than EAPB0203 (25). Growth inhibition was associated with EAPB0503-induced early apoptosis in NPM-1c-, but not wild-type NPM-1-AML cells through caspase activation and p53 activation (25). More recently, EAPB0503 was shown to prolong the survival of mice with NPM-1c AML xenografts and a novel mechanism of action of this compound was found. Indeed, EAPB0503 induced NPM-1c SUMOylation, concomitant with the down-regulation of the de-SUMOylase, sentrin/SUMO specific peptidase 3, and paralleled by the upregulation of alternative reading frame (ARF) in NPM-1c-expressing cells (116). These results implicate EAPB0503 in the post-translational modifications of NPM-1c, demonstrating that therapies modulating NPM-1c post-translational modifications can be introduced to the management of NPM1c AML.

In conclusion, EAPB0503 displays a greater anti-leukemic activity than EAPB0203 in both CML and AML. This is hypothesized to be due to the ethyl linker that separates the imidazoquinoxaline heterocycle from the phenyl group in EAPB0203, but not in EAPB0503 (24).

7. Anti-parasitic activity of EAPB0503

The anti-parasitic activity of imidazoquinoxalines, was first reported for imiquimod (26). In the context of cutaneous

Table II. Mechanisms of action of imiquimod, EAPB0203, EAPB0503 and EAPB02303.

Compound	Disease/disease model	Mechanisms of action	(Refs.)
Imiquimod[a]	Genital warts	TLR-7: Innate and adaptive immune response activation	(15,43)
	Cutaneous	and cytokines secretion	(38)
	Leishmaniasis	Apoptosis: Bcl-2-dependent intrinsic apoptotic pathway	(26)
	Toxoplamosis	Nitric oxide production in response to pro-inflammatory cytokine	(39)
EAPB0203	ATL	Growth inhibition: Inhibition of tubulin polymerization	(19)
	Melanoma	Apoptosis: Anti-apoptotic proteins c-IAP-1 and Bcl-xL, downregulation triggering the intrinsic apoptotic pathway	(19)
EAPB0503	AML	Growth inhibition: Inhibition of tubulin polymerization	(25)
	CML	Apoptosis: Intrinsic apoptotic pathway	(24)
	Melanoma		(22)
EAPB02303	Melanoma	Growth inhibition: Unknown mechanism; independent from tubulin binding	(90)

[a]Currently used in clinical practice. TLR-7, Toll-like receptor 7; AML, acute myeloid leukemia; ATL, adult T-cell leukemia/lymphoma; CML, chronic myeloid leukemia.

leishmaniasis, imiquimod exerted its anti-amastigote activity via TLR-7 upregulation, leading to NF-κB activation and pro-inflammatory cytokine production. However, the effect of EAPB0503 on TLR-7 was less prominent (26). Noteworthy, findings suggest that EAPB0503 may act not only via TLR-7, but also through other TLRs (26). Moreover, the levels of macrophage inflammatory protein-1α and β, monocyte chemoattractant protein and some pro-inflammatory cytokines, such as IL-1β, IL-1, IL-6, IL-12 and TNF-α, which appear to be associated with resistance against leishmaniasis, are increased upon exposure to EAPB0503. This increase appears to be crucial for the clearance of cutaneous leishmaniasis and for the protective role of imiquimod derivatives against cutaneous leishmaniasis (26). Moreover, the production of the inducible nitric oxide synthase, normally induced in response to pro-inflammatory cytokines, increased in response to EAPB0503 further enhancing the leishmanicidal activity of macrophages infected with *Leishmania spp*. By contrast, anti-inflammatory cytokine levels, such as IL-10 and IL-4, are usually associated with disease progression (117), decreased by 4- and 15-fold, respectively, upon EAPB0503 exposure versus 4-fold upon exposure to imiquimod in *L. tropica*-infected macrophages (26). Moreover, EAPB0503 exhibited a more prominent anti-leishmanicidal activity than the clinically used glucantime, whether alone or combined with imiquimod (26)

8. Conclusions and future perspectives

As an immune response modifier, imiquimod has shed light on a new class of potential anti-cancer agents. Imidazoquinoxalines, imiquimod analogues, were synthesized in an attempt to mitigate the deleterious effects of the imiquimod-induced production of pro-inflammatory cytokines and to improve its antitumor properties. Earlier agents, EAPB0203 and EAPB0503, exhibited notable antitumor properties, mediated by the inhibition of tubulin polymerization, the inhibition of growth and the induction of apoptosis, in melanoma and/or leukemia models. In addition, they exhibited a potential to synergize with standard-of-care treatments. The next-generation derivative, EAPB02303, exhibited a more potent cytotoxic activity against melanoma cells, through a mechanism, which is independent of tubulin polymerization and which remains to be elucidated. A summary of the mechanisms of action of imiquimod and its analogues/derivatives is provided in Table II. Currently, imidazoquinoxalines are actively assessed in preclinical models of diseases, mainly cancer, in order to provide sufficient scientific evidence and promote their translation from bench to bedside.

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JO wrote the manuscript. Authors AS, HEH, CDM, PAB, MES and JS reviewed the different drafts, contributed to the writing and synthesized the available literature. JS designed the review and oversaw the writing process. All authors have

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Competing interests

The authors declare that they have no competing interests.

References

1. Dunn GP, Old LJ and Schreiber RD: The three Es of cancer immunoediting. Annu Rev Immunol 22: 329-360, 2004.
2. Abbott M and Ustoyev Y: Cancer and the immune system: The history and background of immunotherapy. Semin Oncol Nurs 35: 150923, 2019.
3. Kennedy LB and Salama AKS: A review of cancer immunotherapy toxicity. CA Cancer J Clin 70: 86-104, 2020.
4. Avorn J: Learning about the safety of drugs-a half-century of evolution. N Engl J Med 365: 2151-2153, 2011.
5. Wu Y, Yang Z, Cheng K, Bi H and Chen J: Small molecule-based immunomodulators for cancer therapy. Acta Pharm Sin B 12: 4287-4308, 2022.
6. Kumar AR, Devan AR, Nair B, Vinod BS and Nath LR: Harnessing the immune system against cancer: Current immunotherapy approaches and therapeutic targets. Mol Biol Rep 48: 8075-8095, 2021.
7. Liu SV, Reck M, Mansfield AS, Mok T, Scherpereel A, Reinmuth N, Garassino MC, Carpeno JD, Califano R, Nishio M, *et al*: Updated overall survival and PD-L1 subgroup analysis of patients with extensive-stage small-cell lung cancer treated with Atezolizumab, Carboplatin, and Etoposide (IMpower133). J Clin Oncol 39: 619-630, 2021.
8. Vaddepally RK, Kharel P, Pandey R, Garje R and Chandra AB: Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. Cancers (Basel) 12: 738, 2020.
9. Chen XY, Li YD, Xie Y, Cao LQ, Ashby CR Jr, Zhao H and Chen ZS: Nivolumab and relatlimab for the treatment of melanoma. Drugs Today (Barc) 59: 91-104, 2023.
10. Kang C: Retifanlimab: First approval. Drugs 83: 731-737, 2023.
11. Di Trolio R, Simeone E, Di Lorenzo G, Grimaldi AM, Romano A, Ayala F, Caracò C, Mozzillo N and Ascierto PA: Update on PEG-interferon α-2b as adjuvant therapy in melanoma. Anticancer Res 32: 3901-3909, 2012.
12. Qureshi YA, Karp CL and Dubovy SR: Intralesional interferon alpha-2b therapy for adnexal Kaposi sarcoma. Cornea 28: 941-943, 2009.
13. Rallis KS, Corrigan AE, Dadah H, George AM, Keshwara SM, Sideris M and Szabados B: Cytokine-based cancer immunotherapy: Challenges and opportunities for IL-10. Anticancer Res 41: 3247-3252, 2021.
14. Lamb YN: Pexidartinib: First Approval. Drugs 79: 1805-1812, 2019.
15. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K and Akira S: Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol 3: 196-200, 2002.
16. Kamath P, Darwin E, Arora H and Nouri K: A review on imiquimod therapy and discussion on optimal management of basal cell carcinomas. Clin Drug Investig 38: 883-899, 2018.
17. Tyring S: Imiquimod applied topically: A novel immune response modifier. Skin Therapy Lett 6: 1-4, 2001.
18. Courbet A, Bec N, Constant C, Larroque C, Pugniere M, Messaoudi SE, Zghaib Z, Khier S, Deleuze-Masquefa C and Gattacceca F: Imidazoquinoxaline anticancer derivatives and imiquimod interact with tubulin: Characterization of molecular microtubule inhibiting mechanisms in correlation with cytotoxicity. PLoS One 12: e0182022, 2017.
19. Moarbess G, Deleuze-Masquefa C, Bonnard V, Gayraud-Paniagua S, Vidal JR, Bressolle F, Pinguet F and Bonnet PA: In vitro and in vivo anti-tumoral activities of imidazo[1,2-a]quinoxaline, imidazo[1,5-a]quinoxaline, and pyrazolo[1,5-a]quinoxaline derivatives. Bioorg Med Chem 16: 6601-6610, 2008.
20. Deleuze-Masquefa C, Moarbess G, Khier S, David N, Gayraud-Paniagua S, Bressolle F, Pinguet F and Bonnet PA: New imidazo[1,2-a]quinoxaline derivatives: Synthesis and in vitro activity against human melanoma. Eur J Med Chem 44: 3406-3411, 2009.
21. Kwong A, Sanlorenzo M, Rappersberger K and Vujic I: Update on advanced melanoma treatments: Small molecule targeted therapy, immunotherapy, and future combination therapies. Wien Med Wochenschr 169: 314-322, 2019.
22. Zghaib Z, Guichou JF, Vappiani J, Bec N, Hadj-Kaddour K, Vincent LA, Paniagua-Gayraud S, Larroque C, Moarbess G, Cuq P, *et al*: New imidazoquinoxaline derivatives: Synthesis, biological evaluation on melanoma, effect on tubulin polymerization and structure-activity relationships. Bioorg Med Chem 24: 2433-2440, 2016.
23. Moarbess G, El-Hajj H, Kfoury Y, El-Sabban ME, Lepelletier Y, Hermine O, Deleuze-Masquéfa C, Bonnet PA and Bazarbachi A: EAPB0203, a member of the imidazoquinoxaline family, inhibits growth and induces caspase-dependent apoptosis in T-cell lymphomas and HTLV-I-associated adult T-cell leukemia/lymphoma. Blood 111: 3770-3777, 2008.
24. Saliba J, Deleuze-Masquéfa C, Iskandarani A, El Eit R, Hmadi R, Mahon FX, Bazarbachi A, Bonnet PA and Nasr R: EAPB0503, a novel imidazoquinoxaline derivative, inhibits growth and induces apoptosis in chronic myeloid leukemia cells. Anticancer Drugs 25: 624-632, 2014.
25. Nabbouh AI, Hleihel RS, Saliba JL, Karam MM, Hamie MH, Wu HCJM, Berthier CP, Tawil NM, Bonnet PAA, Deleuze-Masquefa C and El Hajj HA: Imidazoquinoxaline derivative EAPB0503: A promising drug targeting mutant nucleophosmin 1 in acute myeloid leukemia. Cancer 123: 1662-1673, 2017.
26. El Hajj R, Youness HB, Lachaud L, Bastien P, Masquefa C, Bonnet PA, El Hajj H and Khalifeh I: EAPB0503: An Imiquimod analog with potent in vitro activity against cutaneous leishmaniasis caused by Leishmania major and Leishmania tropica. PLoS Negl Trop Dis 12: e0006854, 2018.
27. Baumann M and Baxendale IR: An overview of the synthetic routes to the best selling drugs containing 6-membered heterocycles. Beilstein J Org Chem 9: 2265-2319, 2013.
28. Rudy SJ: Imiquimod (Aldara): Modifying the immune response. Dermatol Nurs 14: 268-270, 2002.
29. Miller RL, Gerster JF, Owens ML, Slade HB and Tomai MA: Imiquimod applied topically: A novel immune response modifier and new class of drug. Int J Immunopharmacol 21: 1-14, 1999.
30. Smith KJ, Hamza S and Skelton H: The imidazoquinolines and their place in the therapy of cutaneous disease. Expert Opin Pharmacother 4: 1105-1119, 2003.
31. Deleuze-Masquefa C, Gerebtzoff G, Subra G, Fabreguettes JR, Ovens A, Carraz M, Strub MP, Bompart J, George P and Bonnet PA: Design and synthesis of novel imidazo[1,2-a]quinoxalines as PDE4 inhibitors. Bioorg Med Chem 12: 1129-1139, 2004.
32. Del Rosso JQ: Topical imiquimod therapy for actinic keratosis: Is long-term clearance a realistic benefit? J Clin Aesthet Dermatol 1: 44-47, 2008.
33. Oumata N, Nguyen PH, Beringue V, Soubigou F, Pang Y, Desban N, Massacrier C, Morel Y, Paturel C, Contesse MA, *et al*: The toll-like receptor agonist imiquimod is active against prions. PLoS One 8: e72112, 2013.
34. Sidky YA, Borden EC, Weeks CE, Reiter MJ, Hatcher JF and Bryan GT: Inhibition of murine tumor growth by an interferon-inducing imidazoquinolinamine. Cancer Res 52: 3528-3533, 1992.
35. Sauder DN, Skinner RB, Fox TL and Owens ML: Topical imiquimod 5% cream as an effective treatment for external genital and perianal warts in different patient populations. Sex Transm Dis 30: 124-128, 2003.
36. Yokogawa M, Takaishi M, Nakajima K, Kamijima R, Digiovanni J and Sano S: Imiquimod attenuates the growth of UVB-induced SCC in mice through Th1/Th17 cells. Mol Carcinog 52: 760-769, 2013.
37. Spaner DE, Miller RL, Mena J, Grossman L, Sorrenti V and Shi Y: Regression of lymphomatous skin deposits in a chronic lymphocytic leukemia patient treated with the toll-like receptor-7/8 agonist, imiquimod. Leuk Lymphoma 46: 935-939, 2005.

38. Raman VS, Duthie MS, Fox CB, Matlashewski G and Reed SG: Adjuvants for Leishmania vaccines: From models to clinical application. Front Immunol 3: 144, 2012.
39. Hamie M, Najm R, Deleuze-Masquefa C, Bonnet PA, Dubremetz JF, El Sabban M and El Hajj H: Imiquimod targets toxoplasmosis through modulating host toll-like receptor-MyD88 signaling. Front Immunol 12: 629917, 2021.
40. Arevalo I, Ward B, Miller R, Meng TC, Najar E, Alvarez E, Matlashewski G and Llanos-Cuentas A: Successful treatment of drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator. Clin Infect Dis 33: 1847-1851, 2001.
41. Miranda-Verastegui C, Tulliano G, Gyorkos TW, Calderon W, Rahme E, Ward B, Cruz M, Llanos-Cuentas A and Matlashewski G: First-line therapy for human cutaneous leishmaniasis in Peru using the TLR7 agonist imiquimod in combination with pentavalent antimony. PLoS Negl Trop Dis 3: e491, 2009.
42. Arevalo I, Tulliano G, Quispe A, Spaeth G, Matlashewski G, Llanos-Cuentas A and Pollack H: Role of imiquimod and parenteral meglumine antimoniate in the initial treatment of cutaneous leishmaniasis. Clin Infect Dis 44: 1549-1554, 2007.
43. Walter A, Schäfer M, Cecconi V, Matter C, Urosevic-Maiwald M, Belloni B, Schönewolf N, Dummer R, Bloch W, Werner S, *et al*: Aldara activates TLR7-independent immune defence. Nat Commun 4: 1560, 2013.
44. Kono T, Kondo S, Pastore S, Shivji GM, Tomai MA, McKenzie RC and Sauder DN: Effects of a novel topical immunomodulator, imiquimod, on keratinocyte cytokine gene expression. Lymphokine Cytokine Res 13: 71-76, 1994.
45. Weber A, Zimmermann C, Mausberg AK, Kieseier BC, Hartung HP and Hofstetter HH: Induction of pro-inflammatory cytokine production in thymocytes by the immune response modifiers Imiquimod and Gardiquimod. Int Immunopharmacol 17: 427-431, 2013.
46. Wolf IH, Kodama K, Cerroni L and Kerl H: Nature of inflammatory infiltrate in superficial cutaneous malignancies during topical imiquimod treatment. Am J Dermatopathol 29: 237-241, 2007.
47. Wong JG, Toole JWP, Demers AA, Musto G and Wiseman MC: Topical 5% imiquimod in the treatment of lentigo maligna. J Cutan Med Surg 16: 245-249, 2012.
48. Schon M and Schon MP: The antitumoral mode of action of imiquimod and other imidazoquinolines. Curr Med Chem 14: 681-687, 2007.
49. Schon MP and Schon M: The small-molecule immune response modifier imiquimod-its mode of action and clinical use in the treatment of skin cancer. Expert Opin Ther Targets 10: 69-76, 2006.
50. Bilu D and Sauder DN: Imiquimod: Modes of action. Br J Dermatol 149 (Suppl 66): 5-8, 2003.
51. Levine, E.N.a.V., Role of Topical Therapy: Imiquimod. 2017.
52. Wagstaff AJ and Perry CM: Topical imiquimod: A review of its use in the management of anogenital warts, actinic keratoses, basal cell carcinoma and other skin lesions. Drugs 67: 2187-2210, 2007.
53. Megyeri K, Au WC, Rosztoczy I, Raj NB, Miller RL, Tomai MA and Pitha PM: Stimulation of interferon and cytokine gene expression by imiquimod and stimulation by Sendai virus utilize similar signal transduction pathways. Mol Cell Biol 15: 2207-2218, 1995.
54. Sauder DN: Immunomodulatory and pharmacologic properties of imiquimod. J Am Acad Dermatol 43: S6-S11, 2000.
55. Schon MP, Schon M and Klotz KN: The small antitumoral immune response modifier imiquimod interacts with adenosine receptor signaling in a TLR7- and TLR8-independent fashion. J Invest Dermatol 126: 1338-1347, 2006.
56. Majewski S, Marczak M, Mlynarczyk B, Benninghoff B and Jablonska S: Imiquimod is a strong inhibitor of tumor cell-induced angiogenesis. Int J Dermatol 44: 14-19, 2005.
57. Denning DP and Hirose T: Anti-tubulins DEPendably induce apoptosis. Nat Cell Biol 16: 741-743, 2014.
58. Schön MP and Schön M: Immune modulation and apoptosis induction: Two sides of the antitumoral activity of imiquimod. Apoptosis 9: 291-298, 2004.
59. Bong AB, Bonnekoh B, Franke I, Schön MP, Ulrich J and Gollnick H: Imiquimod, a topical immune response modifier, in the treatment of cutaneous metastases of malignant melanoma. Dermatology 205: 135-138, 2002.
60. Heikkinen AK and Susitaival P: Severe systemic reaction to topical imiquimod. Acta Derm Venereol 91: 594-595, 2011.
61. Cannon PS, O'Donnell B, Huilgol SC and Selva D: The ophthalmic side-effects of imiquimod therapy in the management of periocular skin lesions. Br J Ophthalmol 95: 1682-1685, 2011.
62. Benson E: Imiquimod: Potential risk of an immunostimulant. Australas J Dermatol 45: 123-124, 2004.
63. Somani N, Martinka M, Crawford RI, Dutz JP and Rivers JK: Treatment of atypical nevi with imiquimod 5% cream. Arch Dermatol 143: 379-385, 2007.
64. Hanna E, Abadi R and Abbas O: Imiquimod in dermatology: An overview. Int J Dermatol 55: 831-844, 2016.
65. Rosen T: Limited extent AIDS-related cutaneous Kaposi's sarcoma responsive to imiquimod 5% cream. Int J Dermatol 45: 854-856, 2006.
66. Ezzell TI, Fromowitz JS and Ramos-Caro FA: Recurrent pyogenic granuloma treated with topical imiquimod. J Am Acad Dermatol 54 (5 Suppl): S244-S245, 2006.
67. Barba AR, Kapoor S and Berman B: An open label safety study of topical imiquimod 5% cream in the treatment of Molluscum contagiosum in children. Dermatol Online J 7: 20, 2001.
68. Díaz-Guimaraens B, Saceda-Corralo D, Hermosa-Gelbard A, Moreno-Arrones ÓM, Dominguez-Santas M, Suarez-Valle A and Vañó-Galván S: Imiquimod-enhanced immunotherapy with diphencyprone for patients with alopecia areata. Dermatol Ther 35: e15516, 2022.
69. Palefsky JM, Lee JY, Jay N, Goldstone SE, Darragh TM, Dunlevy HA, Rosa-Cunha I, Arons A, Pugliese JC, Vena D, *et al*: Treatment of anal high-grade squamous intraepithelial lesions to prevent anal cancer. N Engl J Med 386: 2273-2282, 2022.
70. Al Fayez N, Rouhollahi E, Ong CY, Wu J, Nguyen A, Böttger R, Cullis PR, Witzigmann D and Li SD: Hepatocyte-targeted delivery of imiquimod reduces hepatitis B virus surface antigen. J Control Release 350: 630-641, 2022.
71. Daude M, Dinulescu M, Nguyen JM, Maillard H, Duff FL, Machet L, Beylot-Barry M, Legoupil D, Wierzbicka-Hainaut E, Bedane C, *et al*: Efficacy of imiquimod in the management of lentigo maligna. J Eur Acad Dermatol Venereol 27: 10.1111/jdv.19141, 2023.
72. Chang SH, Lin PY, Wu TK, Hsu CS, Huang SW, Li ZY, Liu KT, Kao JK, Chen YJ, Wong TW, *et al*: Imiquimod-induced ROS production causes lysosomal membrane permeabilization and activates caspase-8-mediated apoptosis in skin cancer cells. J Dermatol Sci 107: 142-150, 2022.
73. Urquhart JL and Weston WL: Treatment of multiple trichoepitheliomas with topical imiquimod and tretinoin. Pediatr Dermatol 22: 67-70, 2005.
74. Cantisani C, Lazic T, Richetta AG, Clerico R, Mattozzi C and Calvieri S: Imiquimod 5% cream use in dermatology, side effects and recent patents. Recent Pat Inflamm Allergy Drug Discov 6: 65-69, 2012.
75. Pasadyn SR and Cain R: Topical imiquimod induces severe weakness and myalgias after three applications: A case report. J Clin Aesthet Dermatol 12: 58-59, 2019.
76. Safadi MG, Hassan S, Patel V, Viglione M and Zahner SL: Imiquimod-induced hypertrophic lupus erythematosus-like reaction. Dermatol Online J 28: 10.5070/D328458526, 2022.
77. Li HO, Aw M and Glassman SJ: Imiquimod-induced bullous pemphigoid: A case report. SAGE Open Med Case Rep 11: 2050313x231164222, 2023.
78. Arias NM, Bonino CB, Feal PP, Rico MLP, Peñaranda JMS and Osorio IV: Lupus-like reaction following imiquimod treatment for actinic keratoses. Dermatol Ther 35: e15700, 2022.
79. Raman J, Bisbee E, Missall TA and Saikaly SK: A case of topical imiquimod induced fatigue. J Dermatolog Treat 33: 3202-3204, 2022.
80. McKinzie AH and Christman MA: Imiquimod-associated localized skin ulceration in a patient with uncontrolled diabetes. Obstet Gynecol 140: 316-319, 2022.
81. Martins P, Jesus J, Santos S, Raposo LR, Roma-Rodrigues C, Baptista PV and Fernandes AR: Heterocyclic anticancer compounds: Recent advances and the paradigm shift towards the use of nanomedicine's tool box. Molecules 20: 16852-16891, 2015.
82. Kumar S, Bawa S and Gupta H: Biological activities of quinoline derivatives. Mini Rev Med Chem 9: 1648-1654, 2009.
83. Chu XM, Wang C, Liu W, Liang LL, Gong KK, Zhao CY and Sun KL: Quinoline and quinolone dimers and their biological activities: An overview. Eur J Med Chem 161: 101-117, 2019.
84. Balderas-Renteria I, Gonzalez-Barranco P, Garcia A, Banik BK and Rivera G: Anticancer drug design using scaffolds of β-lactams, sulfonamides, quinoline, quinoxaline and natural products. Drugs advances in clinical trials. Curr Med Chem 19: 4377-4398, 2012.

85. Afzal O, Kumar S, Haider MR, Ali MR, Kumar R, Jaggi M and Bawa S: A review on anticancer potential of bioactive heterocycle quinoline. Eur J Med Chem 97: 871-910, 2015.
86. Bonilla-Ramirez L, Rios A, Quiliano M, Ramirez-Calderon G, Beltrán-Hortelano I, Franetich JF, Corcuera L, Bordessoulles M, Vettorazzi A, de Cerain AL, *et al*: Novel antimalarial chloroquine- and primaquine-quinoxaline 1,4-di-N-oxide hybrids: Design, synthesis, Plasmodium life cycle stage profile, and preliminary toxicity studies. Eur J Med Chem 158: 68-81, 2018.
87. Burguete A, Pontiki E, Hadjipavlou-Litina D, Ancizu S, Villar R, Solano B, Moreno E, Torres E, Pérez S, Aldana I and Monge A: Synthesis and biological evaluation of new quinoxaline derivatives as antioxidant and anti-inflammatory agents. Chem Biol Drug Des 77: 255-267, 2011.
88. Fabian L, Porro MT, Gómez N, Salvatori M, Turk G, Estrin D and Moglioni A: Design, synthesis and biological evaluation of quinoxaline compounds as anti-HIV agents targeting reverse transcriptase enzyme. Eur J Med Chem 188: 111987, 2020.
89. El Newahie AMS, Nissan YM, Ismail NSM, El Ella DA, Khojah SM and Abouzid KAM: Design and synthesis of new quinoxaline derivatives as anticancer agents and apoptotic inducers. Molecules 24: 1175, 2019.
90. Patinote C, Deleuze-Masquéfa C, Kaddour KH, Vincent LA, Larive R, Zghaib Z, Guichou JF, Assaf MD, Cuq P and Bonnet PA: Imidazo[1,2-a]quinoxalines for melanoma treatment with original mechanism of action. Eur J Med Chem 212: 113031, 2021.
91. Lafaille F, Banaigs B, Inguimbert N, Enjalbal C, Doulain PE, Bonnet PA, Masquefa C and Bressolle FMM: Characterization of a new anticancer agent, EAPB0203, and its main metabolites: nuclear magnetic resonance and liquid chromatography-mass spectrometry studies. Anal Chem 84: 9865-9872, 2012.
92. Khier S, Deleuze-Masquéfa C, Moarbess G, Gattacceca F, Margout D, Solassol I, Cooper JF, Pinguet F, Bonnet PA and Bressolle FMM: Pharmacology of EAPB0203, a novel imidazo[1,2-a]quinoxaline derivative with anti-tumoral activity on melanoma. Eur J Pharm Sci 39: 23-29, 2010.
93. Morjaria S, Deleuze-Masquefa C, Lafont V, Gayraud S, Bompart J, Bonnet PA and Dornand J: Impairment of TNF-alpha production and action by imidazo[1,2- alpha] quinoxalines, a derivative family which displays potential anti-inflammatory properties. Int J Immunopathol Pharmacol 19: 525-538, 2006.
94. Lideikaitė A, Mozūraitienė J and Letautienė S: Analysis of prognostic factors for melanoma patients. Acta Med Litu 24: 25-34, 2017.
95. Drobits B, Holcmann M, Amberg N, Swiecki M, Grundtner R, Hammer M, Colonna M and Sibilia M: Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. J Clin Invest 122: 575-585, 2012.
96. Chouchou A, Patinote C, Cuq P, Bonnet PA and Deleuze-Masquéfa C: Imidazo[1,2-a]quinoxalines derivatives grafted with amino acids: Synthesis and evaluation on A375 melanoma cells. Molecules 23: 2987, 2018.
97. Quereux G and Dreno B: Fotemustine for the treatment of melanoma. Expert Opin Pharmacother 12: 2891-2904, 2011.
98. Hermine O, Ramos JC and Tobinai K: A review of new findings in adult T-cell leukemia-lymphoma: A focus on current and emerging treatment strategies. Adv Ther 35: 135-152, 2018.
99. Tsukasaki K, Hermine O, Bazarbachi A, Ratner L, Ramos JC, Harrington W Jr, O'Mahony D, Janik JE, Bittencourt AL, Taylor GP, *et al*: Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: A proposal from an international consensus meeting. J Clin Oncol 27: 453-459, 2009.
100. Bazarbachi A, Suarez F, Fields P and Hermine O: How I treat adult T-cell leukemia/lymphoma. Blood 118: 1736-1745, 2011.
101. Cook LB, Fuji S, Hermine O, Bazarbachi A, Ramos JC, Ratner L, Horwitz S, Fields P, Tanase A, Bumbea H, *et al*: Revised adult T-cell leukemia-lymphoma international consensus meeting report. J Clin Oncol 37: 677-687, 2019.
102. Tsukasaki K, Marçais A, Nasr R, Kato K, Fukuda T, Hermine O and Bazarbachi A: Diagnostic approaches and established treatments for adult T cell leukemia lymphoma. Front Microbiol 11: 1207, 2020.
103. El Hajj H, Tsukasaki K, Cheminant M, Bazarbachi A, Watanabe T and Hermine O: Novel treatments of adult T cell leukemia lymphoma. Front Microbiol 11: 1062, 2020.
104. Hehlmann R, Heimpel H, Hasford J, Kolb HJ, Pralle H, Hossfeld DK, Queisser W, Löffler H, Hochhaus A and Heinze B: Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. The german CML study group. Blood 84: 4064-4077, 1994.
105. Bisen A and Claxton DF: Tyrosine kinase targeted treatment of chronic myelogenous leukemia and other myeloproliferative neoplasms. Adv Exp Med Biol 779: 179-196, 2013.
106. Krause DS and Van Etten RA: Bedside to bench: Interfering with leukemic stem cells. Nat Med 14: 494-495, 2008.
107. Okimoto RA and Van Etten RA: Navigating the road toward optimal initial therapy for chronic myeloid leukemia. Curr Opin Hematol 18: 89-97, 2011.
108. Lagunas-Rangel FA, Chávez-Valencia V, Gómez-Guijosa MA and Cortes-Penagos C: Acute myeloid leukemia-genetic alterations and their clinical prognosis. Int J Hematol Oncol Stem Cell Res 11: 328-339, 2017.
109. Yilmaz M, Kantarjian H and Ravandi F: Acute promyelocytic leukemia current treatment algorithms. Blood Cancer J 11: 123, 2021.
110. Borthakur G and Kantarjian H: Core binding factor acute myelogenous leukemia-2021 treatment algorithm. Blood Cancer J 11: 114, 2021.
111. Molica M, Breccia M, Foa R, Jabbour E and Kadia TM: Maintenance therapy in AML: The past, the present and the future. Am J Hematol 94: 1254-1265, 2019.
112. Kassim AA and Savani BN: Hematopoietic stem cell transplantation for acute myeloid leukemia: A review. Hematol Oncol Stem Cell Ther 10: 245-251, 2017.
113. Lin WY, Fordham SE, Hungate E, Sunter NJ, Elstob C, Xu Y, Park C, Quante A, Strauch K, Gieger C, *et al*: Genome-wide association study identifies susceptibility loci for acute myeloid leukemia. MedRxiv 2021: 2021.07.22.21259893, 2021.
114. Falini B, Brunetti L, Sportoletti P and Martelli MP: NPM1-mutated acute myeloid leukemia: from bench to bedside. Blood 136: 1707-1721, 2020.
115. Wang AJ, Han Y, Jia N, Chen P and Minden MD: NPM1c impedes CTCF functions through cytoplasmic mislocalization in acute myeloid leukemia. Leukemia 34: 1278-1290, 2020.
116. Skayneh H, Jishi B, Hleihel R, Hamie M, El Hajj R, Deleuze-Masquefa C, Bonnet PA, El Sabban M and El Hajj H: EAPB0503, an imidazoquinoxaline derivative modulates SENP3/ARF mediated SUMOylation, and induces NPM1c degradation in NPM1 mutant AML. Int J Mol Sci 23: 3421, 2022.
117. Sacks D and Noben-Trauth N: The immunology of susceptibility and resistance to Leishmania major in mice. Nat Rev Immunol 2: 845-858, 2002.
118. Ley SV and Thomas AW: Modern synthetic methods for copper-mediated C(aryl)(bond)O, C(aryl)[bond]N, and C(aryl)[bond]S bond formation. Angew Chem Int Ed Engl 42: 5400-5449, 2003.