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Pixantrone Maleate

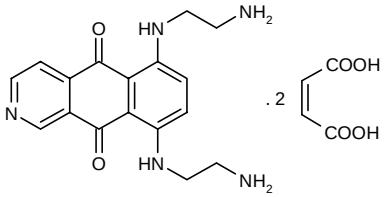
Rec INNM; USAN

Topoisomerase II Inhibitor
DNA Intercalating Agent
Oncolytic

BBR-2778

6,9-Bis(2-aminoethylamino)-5,10-dihydrobenz[*g*]isoquinoline-5,10-dione dimaleate

InChI=1/C17H19N5O2.2C4H4O4/c18-4-7-21-12-1-2-13(22-8-5-19)15-14(12)16(23)10-3-6-20-9-11(10)17(15)24;2*5-3(6)1-2-4(7)8/h1-3,6,9,21-22H,4-5,7-8,18-19H2;2*1-2H,(H,5,6)(H,7,8)/b;2*2-1-



$C_{25}H_{27}N_5O_{10}$
Mol wt: 557.5096

CAS: 144675-97-8

CAS: 144510-96-3 (free base)

CAS: 144675-98-9 (diHCl)

CAS: 175989-38-5 (monoHCl)

EN: 197776

Abstract

Pixantrone is a noncardiotoxic aza-anthracenedione antineoplastic agent related to mitoxantrone. They share the same cytotoxic mechanism of action, interacting with DNA as intercalating agents via inhibition of topoisomerase II. The efficacy of pixantrone as an antineoplastic drug has been demonstrated in phase II/III clinical studies. Pixantrone is also extremely interesting as a putative immunosuppressant; at equiactive doses it is less cardiotoxic than mitoxantrone and it shows similar *in vitro* and *in vivo* immunological effects.

Synthesis

Pixantrone can be prepared in five steps starting from pyridine-3,4-dicarboxylic acid (I) as follows. The first step (a) is dehydration of pyridine-3,4-dicarboxylic acid (I) to the corresponding anhydride (II). The reaction is performed in refluxing acetic anhydride and intermediate (II) is crystallized from *t*-BuOMe. In the second step (b), 1,4-difluorobenzene (III) is acylated, under Friedel Crafts conditions, with anhydride (II) by using $AlCl_3$ as Lewis acid. A mixture of 4-(2',5'-difluorobenzoyl)nicotinic acid (IVa) and 3-(2',5'-difluorobenzoyl)isonicotinic acid (IVb) is obtained. Oleum (20% SO_3) promotes a second intramolecular aromatic acylation at high temperature, producing in the third step (c) 6,9-difluorobenzo[*g*]isoquinoline-5,10-dione (V). The fourth step (d) is an aromatic nucleophilic substitution with ethylenediamine (VI) on the difluoro derivative (V). This reaction is carried out in THF at 50-55 °C. The last intermediate, 6,9-bis-[(2-aminoethyl)amino]-benzo[*g*]isoquinoline-5,10-dione hydrofluoride (VII), is generally recovered with quantitative yields by filtration from the reaction mixture. Finally, pixantrone is obtained in the last step (e) from intermediate (VII) and maleic acid (VIII) in water. Scheme 1.

Background

Pixantrone (BBR-2778) is an anthraquinone-based inhibitor of topoisomerase II, similar to the anthracycline doxorubicin and the anthracenedione mitoxantrone, but it lacks the 5,8-dihydroxy substitution pattern of mitoxantrone. Anthracyclines are the most active drugs in lymphoma therapy, but their use is limited by their cumulative and irreversible cardiotoxicity. Pixantrone was developed to improve the toxicity profile of the current anthracyclines and anthracenediones while maintaining their activity. Pixantrone showed no measurable cardiotoxicity compared with the parent compound mitoxantrone or other anthracyclines at equieffective doses in several animal models. Together with its superior cytotoxic activity in leukemia and lymphoma models, these features make the drug a promising candidate for clinical development in indolent and aggressive non-Hodgkin's lymphoma (NHL), as well as a potential potent immunosuppressant for human autoimmune diseases.

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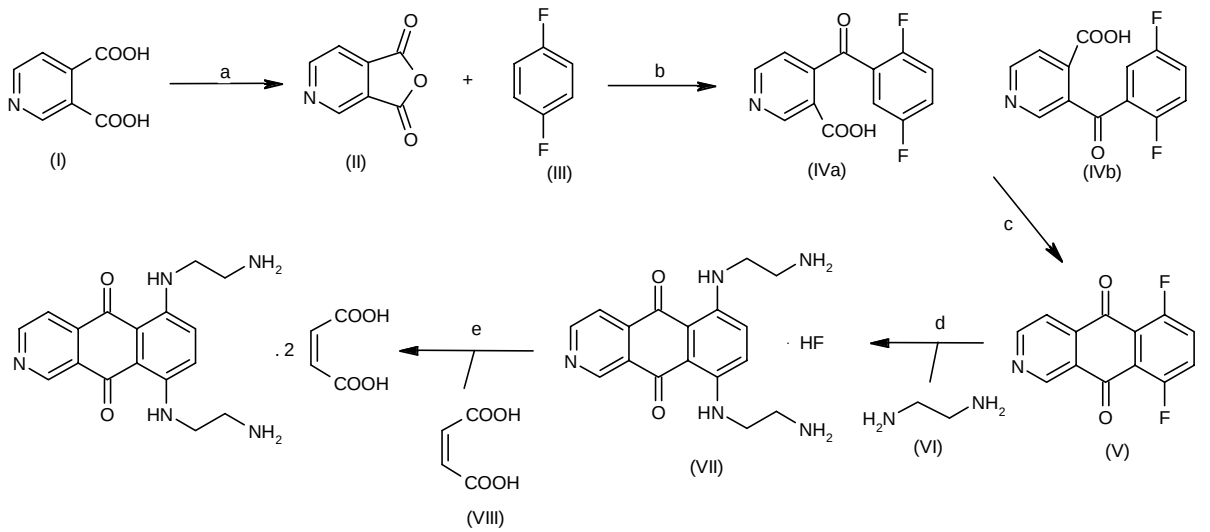
Scheme 1: Synthesis of Pixantrone Maleate



a: Ac_2O, reflux (with AcOH removal); *t*-BuOMe; 84-88% yield; **b**: $AlCl_3$, 1,4-difluorobenzene (III), reflux, 2 h; H_2O/AcOEt; 75-80% yield; **c**: oleum (20% SO_3), 140 °C; H_2O; AcOEt; about 70% yield; **d**: 1,2-ethylenediamine (VI, 8 eq.), THF, 55 °C, 5 h; r.t. 16 h; 90-95% yield; **e**: H_2O/AcOH; maleic acid (VIII), H_2O; H_2O/EtOH; 80-90% yield.

Preclinical Pharmacology

Like other topoisomerase II inhibitors, pixantrone stabilizes the normally transiently bound DNA-protein complex, referred to as the cleavable complex, resulting in the stimulation of topoisomerase II-mediated DNA cleavage (1, 2). *In vitro*, pixantrone has been shown to have a broad cytotoxic profile against human and murine tumor cells, including leukemia and non-small cell lung cancer (NSCLC) cells, with IC_{50} values ranging from 2.3 to 6.3 μg/ml (1-h exposure) in human cells. *In vivo*, pixantrone demonstrated antitumor activity comparable to that of mitoxantrone against several murine and human solid tumors and it was found to be curative in murine L1210 leukemia and murine YC-8 lymphoma, whereas mitoxantrone and doxorubicin showed only an increase in survival time. Toxicology studies in mice showed reversible myelosuppression.

We measured the *in vitro* action of pixantrone on mitogen- and antigen-induced human and animal mononuclear cells during experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis, in comparison to mitoxantrone. Pixantrone suppressed these responses in the nanomolar range, reduced the number of B-cells and antigen-specific anti-myelin basic protein (MBP) responses. At an equimolar range, pixantrone and mitoxantrone induced a similar frequency of apoptotic cells in rat lipopolysaccharide (LPS)-stimulated lymph node cells. Interferon gamma (IFN-γ) levels were reduced by pixantrone treatment in the supernatant of MBP-stimulated rat spleen cells, as was PHA-stimulated IFN-γ, IL-10 and tumor necrosis factor-α (TNF-α). Overall, these *in vitro* studies suggest a similar pattern of immunosuppression for pixantrone and mitoxantrone. Pixantrone was also effective in reducing the clinical symptoms and central nervous system (CNS) histopathology of both acute and chronic models of autoimmune demyelination, such as rat acute and chronic EAE, in the absence of cardiotoxicity (3, 4). These results provided further support for the use of the drug in multiple sclerosis and its potential in other human autoimmune diseases.

Pharmacokinetics and Metabolism

A dose-escalating phase I trial of pixantrone was conducted to determine the maximum tolerated dose (MTD), the dose-limiting toxicity (DLT) and the pharmacokinetic profile in patients with advanced solid tumors. Pixantrone was given as three consecutive weekly 30-min i.v. infusions over a 4-week cycle. The plasma dose-concentration curve fitted a biexponential profile, with a rapid distribution phase followed by a prolonged elimination phase (mean $t_{1/2z}$ = 12 h). Pixantrone displayed a large volume of distribution (9.7-29.7 l/kg) and a high plasma clearance rate (0.75-1.31 l/h/kg). Less than 10% of the dose was recovered in the urine as unchanged drug (5).

Safety

Interestingly, in contrast to mitoxantrone, at equiactive doses pixantrone produced no delayed cardiotoxic effect. In preliminary experiments after repeated i.v. injections in

mice, pixantrone did not induce any delayed myocardial toxic effects (personal communication), unlike mitoxantrone, which caused dose-dependent delayed histopathological myocardial lesions. To directly compare the cardiotoxicity of pixantrone and mitoxantrone, we therefore employed equiactive doses of both drugs in DA rats in an extensive heart pathological examination in a chronic animal model of multiple sclerosis, using objective and reliable semiquantitative scales for scoring cardiotoxicity. No cardiac damage was documented for pixantrone, while important pathological changes were detected in mitoxantrone-treated animal (3).

In the above-mentioned phase I study, the MTD was 150 mg/m^2/week and the DLT was neutropenia, typically occurring at day 14. Other toxicities were mild to moderate and were principally thrombocytopenia, lymphopenia, nausea and vomiting, alopecia and blue coloration of the skin and urine. No significant cardiac toxicity was observed (5).

In a recent phase I/II trial, 19 patients with NHL received protocol therapy consisting of pixantrone 80 mg/m^2 over 1 h on day 1, methylprednisolone 500 mg on days 1-5, cisplatin 25 mg/m^2 on days 1-4 and cytarabine 2000 mg/m^2 on day 5. Cycles were repeated every 21 days in the outpatient setting. DLT consisting of bone marrow suppression occurred at the first dose level (80 mg/m^2), which was defined as the recommended dose. Grade 3 and 4 toxicities were mainly hematological. Only 1 patient had grade 4 febrile neutropenia. Decreases in ejection fraction > 20% did not occur (6). The results are in agreement with the preclinical data indicating minimal or no cardiotoxicity.

Clinical Studies

The most recently reported phase I/II trial results (see above) indicated an overall response rate in relapsed/refractory, aggressive NHL of 58%, with 37% complete and 21% partial responses. Six of the 11 responders (55%) underwent stem cell transplantation. Median time to progression and overall median survival were 5.7 and 14.5 months, respectively. There was no significant interaction between pixantrone and the combined drugs (6).

Pixantrone 80-120 mg/m^2 was combined with fludarabine, dexamethasone and rituximab in patients with relapsed, indolent NHL in another phase I/II study. The overall response rate was 89% in 27 evaluable patients, and the estimated progression-free survival rate at 3 years was 50.4%. The primary toxicity was hematological (7).

In a previous phase II study in 33 patients with relapsed, aggressive NHL, confirmed responses included 5 complete and 4 partial remissions (8).

A total of 30 patients with relapsed, aggressive NHL enrolled in a phase II study were treated with cyclophosphamide 750 mg/m^2 on day 1, pixantrone 150 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1 and prednisone 100 mg on days 1-5. A complete response was noted in 14 patients, and a partial response in 8. Toxicity was acceptable (9).

Three clinical trials are under way: two phase III studies (EXTEND/PIX301) comparing pixantrone and other chemotherapeutic agents for third-line treatment of relapsed aggressive NHL (10, 11), and a phase II trial (RAPID/PIX203) comparing the standard CHOP-R regimen (cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab) to CPOP-R (the same but substituting pixantrone for doxorubicin) in patients with diffuse large B-cell lymphoma (12).

Acknowledgements

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Source

Cell Therapeutics, Inc. (IT, US).

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