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MONOGRAPH

AMITIFADINE HYDROCHLORIDE

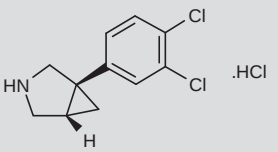
Prop INN; USAN

Triple Reuptake Inhibitor
Treatment of Depression

DOV-21947
EB-1010

(+)-(1*R*,5S)-1-(3,4-Dichlorophenyl)-3-azabicyclo[3.1.0]hexane hydrochloride

InChI: 1S/C11H11Cl2N.ClH/c12-9-2-1-7(3-10(9)13)11-4-8(11)5-14-6-11;/h1-3,8,14H,4-6H2;1H/t8-,11+;/m1./s1



$C_{11}H_{12}Cl_3N$

Mol wt: 264.579

CAS: 410074-74-7

CAS: 410074-73-6 (free base)

EN: 320202

SUMMARY

Amitifadine (EB-1010, DOV-21947) is a "triple reuptake inhibitor" that inhibits the reuptake of serotonin, norepinephrine and dopamine with relative in vitro potencies of 1:2:8. In addition to this in vitro receptor profile, in vivo microdialysis studies demonstrated that amitifadine increases extracellular concentrations of the three monoamines and reduces concentrations of monoamine metabolites. Amitifadine has also shown antidepressant potential in animal models, including the forced swim and tail suspension tests. As a result of these preclinical studies, amitifadine is currently in clinical development as an antidepressant and has demonstrated efficacy and tolerability in a randomized, placebo-controlled, proof-of-concept clinical trial in patients with major depressive disorder. The current monograph describes the chemical properties of amitifadine, as well as information gleaned from the preclinical and clinical studies to date. The premise behind the development of triple reuptake inhibitors is discussed.

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Key words: Triple reuptake inhibitor – Depression – Amitifadine hydrochloride – DOV-21947 –EB-1010

SYNTHESIS*

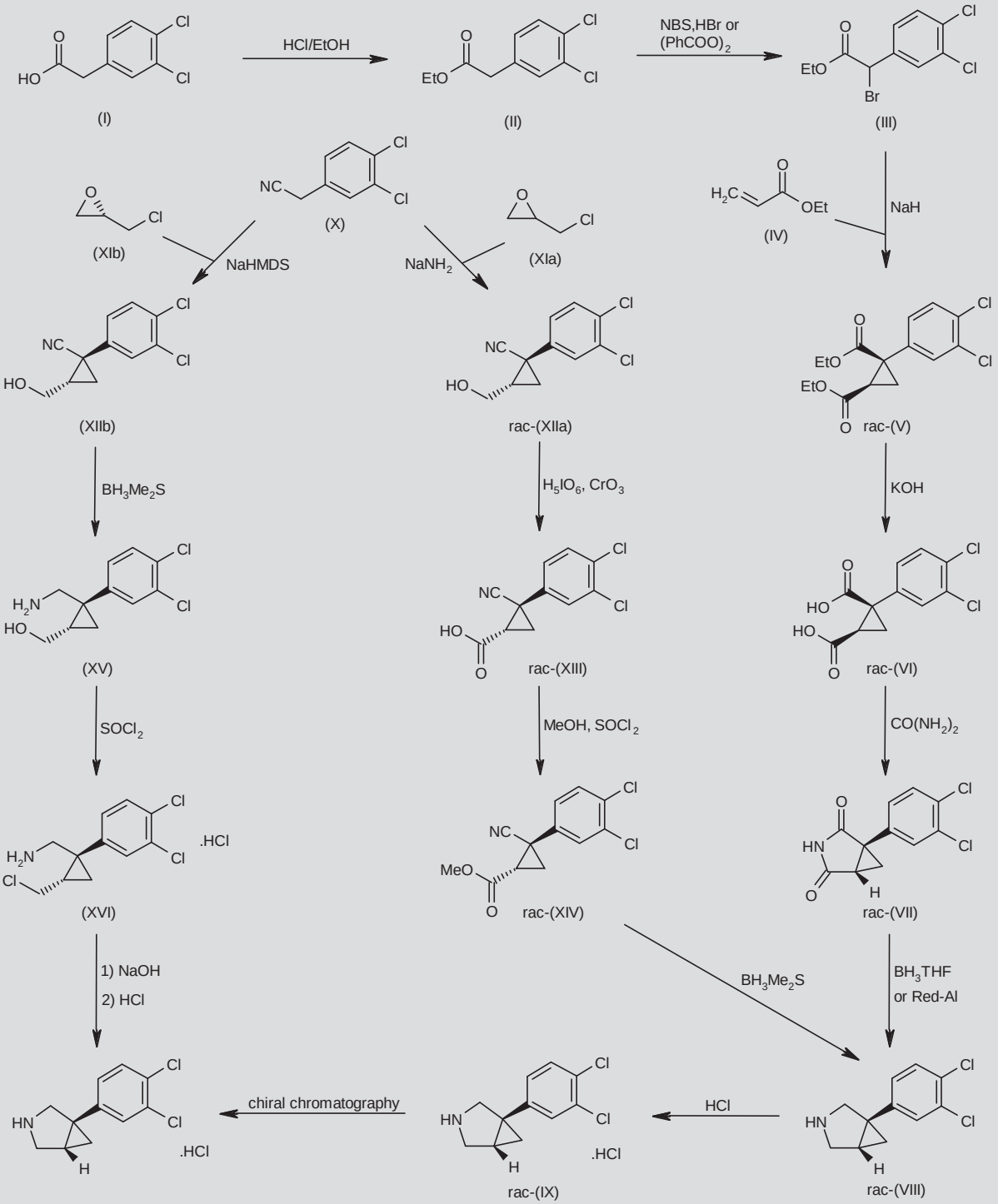
Amitifadine hydrochloride can be prepared by several related ways:

Esterification of 3,4-dichlorophenylacetic acid (I) with HCl/EtOH at reflux followed by radical bromination of the resulting ethyl ester (II) with NBS in the presence of HBr (1) or $(PhCOO)_2$ (2) in refluxing CCl_4 provides ethyl α-bromo-(3,4-dichlorophenyl)acetate (III). Tandem Michael addition and cyclization of ethyl bromoacetate (III) with ethyl acrylate (IV) by means of NaH and a catalytic amount of EtOH in ethyl ether gives diethyl *cis*-1-(3,4-dichlorophenyl)-1,2-cyclopropanedicarboxylate (V). After saponification with KOH in $EtOH/H_2O$, the resulting diacid (VI) is cyclized with urea in refluxing xylene to yield the bicyclic imide (VII). Subsequent reduction of imide (VII) with BH_3/THF or sodium bis(2-methoxyethoxy)aluminum hydride (Vitride, Red-Al) affords racemic 1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane (VIII) (1, 2), which is finally treated with HCl in Et_2O (1) and submitted to enantiomeric separation by chiral chromatography (3). Scheme 1.

Cyclocondensation of (3,4-dichlorophenyl)acetonitrile (X) with racemic epichlorohydrin (XIa) in the presence of $NaNH_2$ in THF gives 1-(3,4-dichlorophenyl)-2-(hydroxymethyl)cyclopropanecarbonitrile as a diastereomeric mixture enriched in the *cis*-isomers (XIIa). Oxidation of alcohol (XIIa) with H_5IO_6 and CrO_3 gives the carboxylic acid (XIII), which by esterification with MeOH in the presence of $SOCl_2$ affords the methyl ester (XIV). Finally, reductive cyclization of the cyano ester (XIV) using $BH_3{\cdot}Me_2S$ in refluxing THF affords 1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane (VIII) (4). Scheme 1.

In a stereospecific route, cyclization of nitrile (X) with (*S*)-epichlorohydrin (XIb) by means of NaHMDS in THF provides the *cis*-cyclopropanecarbonitrile (XIIb) as the major diastereoisomer, which is then reduced to aminoalcohol (XV) by means of $BH_3{\cdot}Me_2S$. Chlorination of alcohol (XV) with $SOCl_2$ in isopropyl acetate leads to the chlorinated amine (XVI), which is finally cyclized in the presence of NaOH, and then crystallized with HCl (5, 6). Scheme 1.

Scheme 1. Synthesis of Amitifadine Hydrochloride



BACKGROUND

Enhancement of monoamine neurotransmission has historically been the target of antidepressant drug development. The catecholamine hypothesis of depression was first described in the 1960s, and for more than two decades, clinicians treating depression relied on tricyclic antidepressants (TCAs), which inhibit the reuptake of norepinephrine and serotonin to varying degrees, and monoamine oxidase inhibitors (MAOIs), which inhibit the catabolism of serotonin, norepinephrine and dopamine. However, TCAs are "dirty drugs" with multiple side effects related to histaminergic, cholinergic and α-adrenoceptor antagonism; they also have a low therapeutic index as a result of quinidine-like cardiac conduction effects. Similarly, MAOIs have limitations by virtue of their potentially fatal interactions with dietary tyramine, stimulant medications and serotonergic medications. As a result of these issues, antidepressant drug development efforts in the past three decades have focused on improving safety and tolerability, which has led to molecules that specifically inhibit serotonin reuptake (i.e., selective serotonin reuptake inhibitors, SSRIs) or both serotonin and norepinephrine reuptake (i.e., serotonin–norepinephrine reuptake inhibitors, SNRIs). Other antidepressants have been developed which enhance norepinephrine and serotonin neurotransmission via other mechanisms; such medications include mirtazepine (presynaptic α_2-adrenoceptor antagonist), as well as trazodone and nefazodone (primarily presynaptic and postsynaptic 5-HT_2 antagonists). As a result, multiple highly tolerable antidepressant drugs are available, although these newer agents have failed to show advantages in efficacy compared to older agents (7, 8) or in onset of antidepressant response, which tends to lag 2-4 weeks behind drug initiation (9, 10). To date, only 65% of patients treated with antidepressants experience a therapeutic response (7, 8, 11, 12), even after multiple steps of antidepressant treatment, augmentation and switching, as noted in the Sequenced Treatment Alternatives to Relieve Depression (STAR-D) trial (13). An antidepressant with potential to provide superior efficacy or more rapid treatment response would be a welcome addition to the treatment arsenal.

Although most antidepressant drug development has focused on enhancement of serotonin and norepinephrine neurotransmission, multiple lines of research have suggested the relevance of dopamine. Data indicate the important role of mesolimbic dopamine in moderating motivation and reward-related behavior, which are typically disrupted in depression (14, 15), and researchers have proposed that anhedonia (a core symptom of depression) in particular may respond to interventions that enhance dopamine neurotransmission. Antidepressants have been shown to sensitize mesolimbic dopamine receptors in animal and human studies, leading to the hypothesis that enhancing synaptic dopamine availability may hasten antidepressant response (15). The dopamine and norepinephrine reuptake inhibitor bupropion was developed in the 1980s as an antidepressant (16), and it has since been repeatedly shown to boost the therapeutic response to noradrenergic and/or serotonergic antidepressants (and decrease sexual side effects) when used adjunctively (17-19). Additional data indicate that the stimulant class of medications, which induce release and block reuptake of dopamine and norepinephrine, augment and hasten the antidepressant response when combined with TCA (20-22), MAOIs (23, 24) and SSRIs/SNRIs (25-27). Finally, dopamine agonists themselves (bromocriptine, pergolide) have shown efficacy as augmenting agents with antidepressants in open-label studies (28, 29, cited in 15).

Thus, it appears that serotonin, norepinephrine and dopamine systems are all related to the pathophysiology of depression, and as such are relevant targets for pharmacological intervention. This premise has ushered the development of the "broad-spectrum" triple reuptake inhibitors, aiming to provide more reliable efficacy, particular benefit for anhedonia and more rapid therapeutic effect. Amitifadine is a result of this development trend. Of note, one triple reuptake inhibitor, GSK-372475, has been well studied in two placebo- and active (paroxetine, venlafaxine XR)-controlled clinical trials in patients with major depressive disorder (MDD) (30). However, GSK-372475 failed to separate from placebo and was neither as effective nor as well tolerated as the active controls. The explanation for this finding is elusive, although it is likely that the relative potencies at the three monoamine reuptake sites are relevant in determining the clinical effects of a triple reuptake inhibitor. The authors of the GSK-372475 study propose that excessive dopamine transporter occupancy may have contributed to its suboptimal tolerability, although from an efficacy standpoint it is unclear how such high occupancy would be detrimental. These issues appear to be simpler for the currently available dual reuptake inhibitors, which differ in their relative potencies at monoamine transporters but have all been shown to be effective for treating depression. Milnacipran blocks serotonin and norepinephrine reuptake equally, whereas greater selectivity at serotonin reuptake sites is characteristic of venlafaxine (30-fold) and duloxetine (10-fold) (31). Clinical ramifications of these in vitro differences in selectivity are poorly understood.

PRECLINICAL PHARMACOLOGY

DOV Pharmaceutical, Inc. (acquired in 2010 by Euthymics Bioscience) developed amitifadine and other related triple reuptake inhibitors from a class of azabicyclohexanes chemically related to bicifadine (32). Amitifadine blocks the transport of human recombinant norepinephrine, serotonin and dopamine transporters with potentially clinically relevant potency. More specifically, amitifadine has been shown to inhibit the reuptake of serotonin, norepinephrine and dopamine in HEK-293 cells expressing the corresponding human recombinant transporters, with IC_{50} values of 12, 23 and 96 nM, respectively. Additionally, amitifadine inhibited [^{125}I]-RTI-55 binding to membranes prepared from HEK-293 cells expressing recombinant human norepinephrine, serotonin and dopamine transporters, with K_i values of 262, 99 and 213 nM, respectively (33).

A subsequent in vivo study of the effects of amitifadine (10 mg/kg) on extracellular concentrations of monoamines and their metabolites in Wistar rat brain regions using microdialysis demonstrated that it markedly and persistently increased extracellular concentrations of serotonin, norepinephrine and dopamine in the prefrontal cortex and increased extracellular concentrations of dopamine in the dopamine-rich areas striatum and nucleus accumbens (34). Furthermore, microdialysis revealed decreases of extracellular concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and the dopamine metabolites 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic acid (HVA), consistent with a mode of action of uptake blockade activating inhibitory somatodendritic autoreceptors, which decrease the firing rate of the respective neu-

rons, thus decreasing cytoplasmic neurotransmitter levels available for monoamine oxidase metabolism. These in vivo studies confirm that amitifadine administration yields observable changes in brain monoamine levels at clinically relevant doses.

Amitifadine also demonstrated positive results in two widely used rodent models for antidepressant effects (35) by reducing immobility in the forced swim test and the tail suspension test (33). The minimum effective dose (MED) of oral amitifadine in the forced swim test was 5 mg/kg and a maximum reduction in immobility was observed at a dose of 20 mg/kg (comparable to that produced by the positive control, an intraperitoneal dose of imipramine 15 mg/kg). Orally administered amitifadine produced a dose-dependent reduction in immobility in the tail suspension test, with an MED of 5 mg/kg and a maximum reduction in immobility at a dose of 10-20 mg/kg (comparable to that produced by an intraperitoneal dose of imipramine 20 mg/kg).

PHARMACOKINETICS AND METABOLISM

Data have not been published related to the pharmacokinetics, pharmacodynamics or interactions between amitifadine and other drugs. However, amitifadine is the (+)-enantiomer of DOV-216303 (a racemic mixture) and according to in vitro radioligand studies is twice as potent at serotonin and norepinephrine inhibition and equipotent at dopamine reuptake inhibition compared to DOV-216303 (36). Amitifadine would be expected in some ways to have similar pharmacokinetics and pharmacodynamics to DOV-216303. Single- and multiple-dose pharmacokinetic studies of DOV-216303 revealed that it was rapidly absorbed, with a time to maximum plasma concentration (t_{max}) of 0.7-1.2 hours, an elimination half-life of 3.3-4.4 hours and dose-proportional maximum plasma concentrations (C_{max}) and area under the curve (AUC) during 10 days of administration (36, 37). Available publications do not address potential interactions of amitifadine or DOV-216303 with other drugs. As noted below, amitifadine has progressed in development from preclinical research to a recently completed proof-of-concept study in patients with MDD, and it can be reasonably inferred that amitifadine has a pharmacokinetic and pharmacodynamic profile (including drug interactions) that is favorable to its clinical use in humans.

SAFETY

Amitifadine was demonstrated to be safe and tolerable in the proof-of-concept study published by Tran et al. (N = 63). There was no difference between amitifadine and placebo groups related to the rate of discontinuation due to adverse events, and no serious adverse events or deaths were reported. In this study, two (6.1%) patients on amitifadine discontinued treatment related to adverse events; both of them discontinued due to rash. Two (7.1%) patients on placebo discontinued treatment due to adverse events; one patient terminated due to rash and another due to nausea and palpitations. A total of 43 adverse events were reported in 10 (30.3%) patients with amitifadine and 37 adverse events were reported in 11 (39.3%) patients with placebo. The most commonly reported adverse event was headache, occurring in 9.1% of patients with amitifadine and 10.7% with placebo. Other adverse events occurred at an overall incidence of 6.1% with amitifadine and at twice the rate of placebo, including diarrhea, nausea, rash and abdominal pain. Amitifadine treatment was associated with minor and likely clinically insignificant mean changes from baseline in vital signs and laboratory analytes, including a statistically significant difference (P = 0.017) in change from baseline in standing diastolic blood pressure (–3 mmHg with amitifadine compared to 2.8 mmHg with placebo). Mean heart rate increased by 1.55 bpm with amitifadine and decreased by 1.68 bpm with placebo. Mean body weight increased a nonsignificant 0.078 kg with amitifadine and 0.04 kg with placebo (38).

Side effects of sexual dysfunction are common with antidepressant treatment and are seen frequently in antidepressant clinical trials. The amitifadine proof-of-concept study measured sexual functioning at baseline and at weeks 2, 4 and 6 with The Derogatis Interview for Sexual Functioning - Self Report (DISF-SR) (39). DISF-SR scores were stratified by low mean baseline scores (< 25, indicating a significant rate of sexual dysfunction) versus high mean baseline scores (≥ 25, indicating a satisfying rate of sexual function). Results demonstrate no significant differences between amitifadine and placebo in both low baseline and high baseline groups (38).

In summary, based on clinical trial data it appears that amitifadine was well tolerated and associated with minor side effects, no significant laboratory or vital sign abnormalities and no worsening of sexual functioning.

CLINICAL STUDIES

The efficacy and tolerability of amitifadine were evaluated in a 6-week, multicenter, randomized, double-blind, parallel, placebo-controlled, proof-of-concept study in 63 adult patients with MDD, conducted at 11 sites in Romania, 2 sites in Serbia and 7 sites in the U.S. (38). Key inclusion criteria included age range of 18-65 inclusive, diagnosis of recurrent MDD according to criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Revised and confirmed by the MINI International Neuropsychiatric Interview, duration of current episode of at least 2 months, a history of previous significant clinical improvement with at least one antidepressant trial (i.e., not treatment-resistant), 17-item Hamilton Depression Rating Scale (HAMD-17) total score of at least 22 with severity score of at least 2 on item 1 at screening, body mass index (BMI) no greater than 35 kg/m^2 and body weight of at least 45 kg. Key exclusion criteria included exhibiting a risk of suicide, including scoring 4 on the HAMD-17 suicide item, a known history of being resistant to antidepressant treatment (i.e., failed two previous antidepressant treatments from different classes given for at least 4 weeks) or electroconvulsive therapy, a history of electroconvulsive therapy within 1 year before screening, and evidence of psychosis or other psychiatric disorders, including substance use disorder. Appropriate patients participated in a single-blind placebo run-in and were deemed eligible to randomize provided they were between 80% and 120% compliant and did not demonstrate an HAMD-17 score reduction of 15% or greater. Participants were randomized in a 1:1 ratio to placebo versus oral amitifadine dosed at 25 mg twice daily for 2 weeks, then 50 mg twice daily for 4 weeks. The results demonstrated that amitifadine achieved statistically significant superiority compared to placebo at endpoint on mean Montgomery Asberg Depression Rating Scale (MADRS) scores (18.2 vs. 22.0; P = 0.028) and some of the secondary variables, including the Clinical Global Impression Improvement Scale (CGI-I; P = 0.030) and the anhedonia factor

score derived from the MADRS, which included items of apparent sadness, reported sadness, concentration difficulties, lassitude and inability to feel ($P = 0.049$). The authors favorably compare the effect size (Cohen's d = 0.601) with those reported in meta-analyses of commercially available antidepressant medications, describing it as "about double the average effect size with standard antidepressant drugs". This comparison has been questioned due to differences in data analytic approaches (40); Tran et al. used Mixed Model Repeated Measures (MMRM) to analyze the amitifadine data, which can reduce standard deviation and inflate effect size, in contrast to the more traditionally used Last Observation Carried Forward (LOCF) method. Additionally, some of the differences between outcome measure scores at endpoint (week 6) are accounted for by higher scores in the placebo group at baseline (albeit not clinically significant). However, Tran et al. note that the study was discontinued early due to lack of funding, and it is likely that it was underpowered statistically as a result. If the planned number of subjects (100 in each group) were recruited, amitifadine may have outperformed placebo more consistently and robustly.

SOURCE

Euthymics Bioscience, Inc. (US).

DISCLOSURES

Dr. Marks is on the Speaker's Bureau of Sunovion and has received research support from Forest Laboratories and Titan Pharmaceuticals.

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