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Digest

# Breakthroughs in neuroactive steroid drug discovery

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ABSTRACT

Endogenous and synthetic neuroactive steroids (NASs) or neurosteroids are effective modulators of multiple signaling pathways including receptors for the γ-aminobutyric acid A ($GABA_A$) and glutamate, in particular *N*-methyl-D-aspartate (NMDA). These receptors are the major inhibitory and excitatory neurotransmitters in the central nervous system (CNS), and there is growing evidence suggesting that dysregulation of neurosteroid production plays a role in numerous neurological disorders. The significant unmet medical need for treatment of CNS disorders has increased the interest for these types of compounds. In this review, we highlight recent progress in the clinical development of NAS drug candidates, in addition to preclinical breakthroughs in the identification of novel NASs, mainly for $GABA_A$ and NMDA receptor modulation.



Neuroactive steroids (NASs) or neurosteroids are among the most potent and effective modulators of neuronal excitability. The term "neurosteroid" was first mentioned by Etienne Baulieu in 1980's and initially referred to endogenous steroids synthesized in the brain and central nervous system (CNS) from cholesterol.[1] Neurosteroids have been shown to impact CNS function primarily through allosteric modulation of the GABA (γ-aminobutyric acid)$_A$ receptor ($GABA_AR$) or the *N*-methyl-D-aspartic acid class of glutamate receptors (NMDAR). However, at high concentrations neurosteroids have been shown to act on other receptor systems like nicotinic acetylcholine, serotonin 5-$HT_3$, and sigma1 receptors. The term neurosteroid has been expanded to include synthetic and naturally-derived analogs that have CNS actions similar to endogenous neurosteroids. Increasing evidence[2,3] indicates that dysregulation of neurosteroid production plays a role in the pathophysiology of stress and stress-related psychiatric disorders, including mood and anxiety disorders. In addition to agonist or antagonist modes of action at different receptors, these receptors could be either positively (PAM) or negatively (NAM) modulated by NAS compounds at allosteric sites. For example, allopregnanolone (**1**) is a PAM at the $GABA_AR$ (Fig. 1).

Other endogenous NASs, such as 24(S) hydroxycholesterol (**2**) are PAMs at NMDA receptors.[4] Such mechanisms are providing novel approaches to treat CNS disorders, and the steroid field is showing a significant resurgence with multiple compounds advancing to clinical studies. Clinical trials currently underway are assessing the efficacy of various NASs for the treatment of diverse CNS disorders such as epilepsy,[5] super refractory status epilepticus (SRSE), Fragile X, traumatic brain injury and Alzheimer's disease.[3] In this BOMCL Digest, we will provide a brief perspective on recently disclosed $GABA_AR$ and NMDAR NASs that have either advanced to clinical studies or have been described preclinically within the last 3–5 years.

*Clinical NASs*. Ten NAS compounds have reached clinical development status since the 1970's (Table 1), however many have since been withdrawn. For example, an intravenous anesthetic combining two NASs, alphaxolone (**3**) and alphadolone in a cremophor vehicle was withdrawn from the market in 1984 due to issues with anaphylaxis. Later in 2001, it was determined that Cremophor EL was responsible for these anaphylactic reactions in humans.[7] Currently, alphaxolone alone is under additional human studies in a sulfobutyl ether-β-cyclodextrin formulation ("Phaxan") as an intravenous anesthetic.[8]

Minaxolone (CCI-12923, **4**), a $GABA_AR$ PAM, was developed as a water-soluble anesthetic NAS. It was withdrawn before registration due to toxicity observed in long-term studies in rats.[9] Early emphasis on water-soluble NASs led to the discovery of ORG-20599 (**5**), a potent $GABA_AR$ PAM. Unacceptable clinical profile, however led to withdrawal from further clinical development.[10] Marinus Pharmaceuticals has been developing ganaxolone (GX, **6**), a 3β methyl derivative of allopregnanolone for focal-onset

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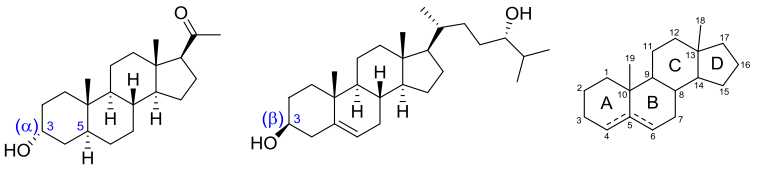


**Fig. 1.** Structures of Allopregnanolone (**1**) and 24 (S) hydroxycholesterol (**2**). Generic steroid nomenclature used in this manuscript. [a]Carbon numbering and ring designations of the steroid core.[6] Substituents above the plane of the paper are described as β and are shown as a solid line; those below the plane are described as α and are shown by a broken line. Carbons at positions 4, 5 and/or 6 may be saturated or unsaturated.

seizures in adults and in children with epilepsy. Focal-onset seizures are manifestations of abnormal epileptic firing of brain cells. It is estimated that ~65 million people worldwide are living with some form of epilepsy.[24] A disclosure in 2016 announced the discontinuation of phase 3 clinical studies for adult focal onset seizures and advancing **6** in status epilepticus and pediatric orphan indications.[11]

$GABA_AR$ antagonist sepranolone[12] (**7**, Table 1) is currently in clinical studies for the treatment of premenstrual dysphoric disorder (PMDD). This disease is a severe, debilitating form of premenstrual distress comprising emotional, physical symptoms and functional impairment. PMDD affects ~3–8% of women in fertile ages.[25] A phase 2b trial in PMDD patients was expected to begin in the second half of 2017 after an initial phase 1/2 study demonstrated a statistically significant difference between active treatment and placebo.

Bruschettini SRL is developing tauroursodeoxycholic acid [**8**, TUDCA, the taurine conjugate of ursodeoxycholic acid (UDCA)] for the treatment of amyotrophic lateral sclerosis (ALS).[13] ALS is a progressive CNS disease where nerve cells in the brain and spinal cord that control voluntary movement gradually deteriorate, causing loss of muscle function and paralysis. Several studies have demonstrated that TUDCA imparts anti-apoptotic activity in a number of neurodegenerative diseases, including ALS, Alzheimer's disease, Parkinson's disease, and Huntington's disease.[26] It has been indicated that UDCA modifies the function of the bile salt export pump (BSEP, ABCB11) however underlying mechanisms remain unknown. On February 27th 2017, orphan designation was granted by the European Commission for TUDCA for the treatment of ALS.

The Shanghai Innovative Research Center of Traditional Chinese Medicine (SIRC-TCM) is developing S-111 (**9**, Yuxintine, or 20(*S*)-protopanaxadiol, Table 1), an active ginseng intestinal metabolite and an inhibitor of serotonin and norepinephrine uptake, for the treatment of depression, including major depressive disorder (MDD). MDD is a widely distributed medical condition that includes abnormalities of mood, appetite, sleep, cognition and psychomotor activity.[27] Preclinical antidepressant-like activity of orally administered S-111 was measured in various animal models of depression and demonstrated antidepressant-like activity with similar potency to fluoxetine.[28] The latest report in 2015, indicated the drug was in phase 2 development.[14,15]

Sage Therapeutics has recently disclosed phase 2 results for its first generation NAS, SAGE-547 (**10**, Brexanolone).[29] A parenteral, continuous infusion formulation of SAGE-547, was in phase 3 clinical trials for the treatment of SRSE, a life-threatening condition in which the brain is in a state of persistent seizure that fails to respond to standard treatments.[18,30,31] The study did not meet the primary endpoint,[32] comparing success in weaning of third-line agents and resolution of potentially life-threatening status epilepticus with brexanolone vs. placebo when added to standard-of-care. In addition, SAGE-547 has completed a phase 2 clinical trial in severe post-partum depression (PPD)[29,33] and an exploratory study in essential tremor (ET).[34] Simultaneous with the development program in SRSE, SAGE-547 was studied in a phase 3 program in moderate and severe post-partum depression.[29] There is considerable preclinical research supporting the potential for $GABA_AR$ modulation imparting benefits in a number of mood disorders. For post-partum depression in particular, there is evidence sustaining the potential utility of NASs, such as allopregnanolone, in depressive mood disorders through modulation of synaptic and extrasynaptic $GABA_ARs$.[35] This disease, with no approved drugs to date, is estimated to affect between 10 and 20% of women in the United States after childbirth.[36] In early November 2017, Sage Therapeutics announced positive top-line results for phase 3 studies in moderate and severe post-partum depression. Brexanolone achieved the primary endpoint in both trials with a mean reduction from baseline in the Hamilton Rating Scale for Depression (HAM-D) total score compared to placebo at 60 h.[37]

SAGE-547 is a potent $GABA_AR$ PAM, active at both synaptic and extrasynaptic $GABA_ARs$, and is ideally suited for parenteral administration, due to its high intrinsic clearance and low volume of distribution, yielding a fast on/fast off pharmacokinetic profile.[38] In searching for next generation NASs, Sage Therapeutics has developed a molecule with robust pharmacological PAM activity at $GABA_AR$ but with low intrinsic clearance, high oral bioavailability and potential for once daily dosing aiming to reach larger populations of patients. To this end, Sage Therapeutics reported the discovery of SAGE-217 (**11**, Table 1),[38,39] a clinical candidate which has now completed single and multiple ascending doses (SAD, MAD) phase 1 clinical trials and has progressed into phase 2 clinical trials for the treatment of $GABA_ARs$ mediated movement disorders such as essential tremor (ET) and Parkinson's disease as well as in mood disorders such as PPD and MDD.

In April 2017, Sage Therapeutics announced the advancement of SAGE-718 (**12**, Table 1) into phase 1 clinical studies.[23] SAGE-718 is a novel, oral, first-in-class oxysterol-based PAM of the NMDAR. Positive modulation of NMDARs has potential benefit in the treatment of a range of neurological disorders associated with a variety of cognitive, neurological and behavioral symptoms. SAGE-718 also has potential in the treatment of CNS disorders associated with a high prevalence of anti-NMDA antibodies or reduced levels of endogenous 24(*S*)-hydroxycholesterol (**2**). In preclinical studies, SAGE-718 improved social behavior in an animal model of NMDA hypofunction, and ameliorated both behavioral and electrophysiological deficits in a model of compromised cholesterol regulation.[23]

In general, development of NASs faces several challenges as seen from previous examples. Many of the issues are related to formulation[40] as those compounds tend to have physicochemical properties outside of the traditional small molecule drug-like properties (Table 1). Attempts were made to reduce lipophilicity of the compounds and increase aqueous solubility (**4**, **5**), however the compounds led to unacceptable margins of safety. It is important to highlight that the receptors are membrane bound. Chisari[41] postulated that NASs might require a membranous route of access to