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Neuroactive Steroids I: Positive Allosteric Modulators of the (γ-Aminobutyric Acid)$_A$ Receptor; Structure Activity Relationships of Heterocyclic Substitution at C-21.

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ABSTRACT: Neuroactive steroids (NAS) have been shown to impact Central Nervous System (CNS) function through positive allosteric modulation of the GABA$_A$ receptor (GABA$_A$-R). Herein, we report the effects on the activity and pharmacokinetic properties of a series of nor-19

pregnanolone analogs bearing a heterocyclic substituent at C-21. These efforts resulted in the identification of **SGE-516**, a balanced synaptic/extrasynaptic $GABA_A$ receptor modulator; and **SGE-872**, a selective extrasynaptic $GABA_A$ receptor modulator. Both molecules possess excellent drug like properties making them advanced leads for oral delivery of $GABA_A$ receptor modulators.

Introduction

Neuroactive steroids (NAS) are a compound class of both natural (**1-2**) and synthetic (**3-6**) origin, that have been shown to impact central nervous system (CNS) function through multiple mechanisms, including allosteric modulation of the (γ-aminobutyric acid)$_A$ receptor ($GABA_A$-R).[1–4] There have been extensive research activities on this class of compounds in the past where it was demonstrated that these act as positive allosteric modulators (PAMs) of $GABA_A$-R.[5,6] As the major inhibitory neurotransmitter in the nervous system, γ-aminobutyric acid (GABA), acting via an array of $GABA_A$-Rs, can influence a wide range of brain circuitry central to a variety of behavioral states. $GABA_A$-R dysregulation sits at the center of a range of diseases of the nervous system such as schizophrenia, mood and sleep-disorders, seizure disorders and epilepsy. Perhaps not surprisingly, given its critical role in the function of neuronal circuits, the $GABA_A$-R is the target for numerous neurotropic medications such as benzodiazepines and non-benzodiazepine analogs (e.g. Sonata), barbiturates, and certain anesthetics.

We have been interested in developing orally bioavailable $GABA_A$-R PAMs, belonging to the nor-19 structural class of synthetic NAS, and herein report the exploration of the SAR at the C-21 position in these molecules and our findings on modulation of synaptic *vs* extrasynaptic $GABA_A$-R selectivity.

There are a number of properties that make this class of NAS very attractive, primarily they have the potential to differentiate from classical $GABA_A$-R PAMs, such as benzodiazepines, by targeting different populations of $GABA_A$-R subtypes as will be discussed below. The $GABA_A$-R is a pentameric ion channel formed by assembling two alpha subunits (α1-α6), two beta subunits (β1-β3) and one additional subunit (γ1-γ3, δ, ε, π, or θ).[7] The subunit composition determines the biophysical and pharmacological characteristics of the channel and influences its location at synaptic or extra-synaptic sites. For example, γ subunits have been associated with clustering the receptor at synaptic sites and thus mediating fast inhibitory synaptic transmission. On the other hand, receptors that contain the δ subunit have been associated with extra-synaptic receptors that mediate tonic inhibitory conductances.[8] NAS have been suggested to putatively bind to 3 or 4 distinct sites on the $GABA_A$-R.[5,6] At low concentrations NAS bind to a site in the M3/M4 domains of the α subunit,[5] leading to allosteric modulation of the GABA induced current. This modulation could be in either a positive (PAM) or a negative (NAM) direction. At high concentrations, direct gating of $GABA_A$-R currents may be achieved in the absence of GABA via binding at the α/β interface near the GABA binding site.[5,6] On the other hand, benzodiazepines bind to an allosteric site distinct from the GABA binding site, at the interface of the α and γ subunits.[9-11] This limits their ability to potentiate synaptic GABA currents to receptor assemblies that must contain a γ subunit.[11] Conversely, NAS potentiate the $GABA_A$-R by binding to residues within the obligatory α subunit and so modulate receptors in a manner which is independent to their subunit composition. In this way, and unlike benzodiazepines, NAS are capable of targeting extra-synaptic $GABA_A$-Rs that include the δ subunit in addition to synaptic γ-containing receptors. As such, NAS may exhibit a therapeutic advantage over benzodiazepines, for example in a variety of seizure indications, including status epilepticus (SE).[12] A sub-

population of SE patients appears resistant to the action, or pharmacology, of first-line benzodiazepines that only target the synaptic $GABA_A$-Rs (as described above). While positively modulating or activating $GABA_A$-Rs results in a beneficial effect in some patients with seizures, in persistent SE, synaptic $GABA_A$-Rs are down-regulated, or weakened in their activity, and consequently, the effect of benzodiazepines is diminished or lost due to a reduction of the synaptic receptor population.[13] Interestingly, the extrasynaptic $GABA_A$-R population remains intact during these prolonged periods of seizure with no apparent down-regulation. Since select NAS can target both the extrasynaptic and synaptic $GABA_A$ receptors, we believe they can treat seizures that are otherwise benzodiazepine-resistant as they would still modulate a preserved population of $GABA_A$ receptors.

Furthermore, data also suggests that NAS can exert profound effects on expression levels of $GABA_A$-Rs.[14-16] For example, in the hippocampus, brief exposure to low concentrations of NAS can enhance tonic currents while having little effects on phasic synaptic currents even after a prolonged wash.[15,17] This suggests that NAS could influence the trafficking and surface expression of certain $GABA_A$-Rs. Consistent with this hypothesis, recent results suggest that NAS do indeed enhance trafficking of $GABA_A$-R subunits.[18] Such an effect would further differentiate NAS allosteric modulators of the $GABA_A$-R from benzodiazepines. The inability of benzodiazepines to promote $GABA_A$-R trafficking may contribute to the typical tolerance to their effects upon repeated dosing.[12]

Two examples of naturally occurring NAS compounds (Figure 1), allopregnanolone (3α-Hydroxy-5α–pregnane-20-one, **1**) and pregnanolone ((3α-Hydroxy-5β-pregnane-20-one, **2**) have emerged as agents for clinical development in a variety of indications.[19-21] However, due to their low oral bioavailability, these endogenous NASs have been clinically dosed as *i.v.* formulations

and are limited to parenteral administration. For **1**, the low oral bioavailability has been hypothesized to arise from high *in vivo* clearance due to rapid primary and secondary metabolism of the C3-hydroxyl *via* oxidation to the ketone and glucuronidation of the alcohol, respectively.[22,23,24] In the 1990's, the effects of substituents at the βC-3 were explored[25-29] with regard to both potency and DMPK[25,26] by a number of groups (e.g. alkanes, alkenes, alkynes, alkoxy and alkyl halides). In these studies introduction of an alkyl substituent at C-3 imposed steric and electronic constraints that reduced alcohol oxidation and conjugation. With this modification, CYP-mediated metabolism and reduced solubility become primary factors influencing oral pharmacokinetic properties. Additional modifications were also explored (Figure 1), including replacing the C-19 methyl with a hydrogen (nor-19 series),[30] e.g. **3** (Co26749)[31] and appending various heterocycles at C-21, e.g. **4** (Co134444) [32] and **5** (Co177843).[33] Several of these compounds reportedly have been tested in multiple *in vivo* models, which after oral administration, showed activity as anticonvulsants[25,32,34] and anxiolytics.[31,32] As in the case of **1**, minimal tolerance to the pharmalogical effect after chronic dosing has been shown with this family of analogs, differentiating NASs from the established benzodiazepine drug class.[12,35] One of the early synthetic NAS analogs ganaxolone (**6**)[36,23] (Figure 1), the βC-3 methyl derivative of **1**, has been reported to be safe[37] and active in adult patients with partial-onset seizures[38,39] and in children with epilepsy.[40] Two phase II clinical trials are ongoing with **6** in adults suffering from epilepsy with drug resistant partial onset seizures[41] and for the treatment of anxiety and attention deficits in children with Fragile X syndrome.[42] Due to its limited oral bioavailability, **6** oral doses range from 600 mg (t.i.d) to 900 mg (b.i.d).[41,42] Thus, the apparent blocking of C-3 metabolism of **1** by adding a methyl group to

this site hypothetically provides some measure of metabolic stability, but is clearly insufficient to provide robust oral bioavailability.

The work described herein addresses the limitations of the first generation endogenous and synthetic NASs described above through optimization of pharmacologic and pharmacokinetic properties. We report that modifying the C-21 position, among other things, enables exploration of receptor subtype selectivity while augmenting the requisite pharmacokinetic profile.

Results

The starting point for our SAR investigation was steroid **7**,[43] the nor-19 analog of **6**. Initial testing of **7** in the [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding assay[44] was performed in rat brain cortical membranes. The TBPS assay has been commonly used to identify compounds that bind to the $GABA_A$-R family, although, there is not necessarily a linear relationship between NAS potency and inhibition of TBPS binding since these activities result from binding to different sites of the receptor. Hence, additional functional assays are needed to fully characterize active compounds with respect to efficacy and selectivity. The pharmacological activity of **7** was evaluated in both recombinant $\alpha_1\beta_2\gamma_2$ $GABA_A$-R in LTK cells and $\alpha_4\beta_3\delta$ $GABA_A$-R in CHO cells using a Q-patch assay and a manual patch assay respectively to better understand the electrophysiologic activity and selectivity at $GABA_A$-Rs. The $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ subunit combinations were selected as they are representative of typical synaptic and extrasynaptic receptor populations.[45] Not unexpectedly, the TBPS potency of **7** is comparable to those of both **1** and **6** (Table 1). The functional activity in the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R is also similar with respect to potency and efficacy, with **1** being slightly more potent. However, the potency at $\alpha_4\beta_3\delta$ $GABA_A$-R dropped *ca.* 5 fold with a modest increase in efficacy. Finally, the physical and

pharmacokinetic properties of **7** are similar to those of **6**, showing low solubility and low microsomal stability *in vitro* (Table 3). Thus, our primary objective was to evaluate the SAR of heterocyclic ring analogs at the C-21 position with the goal of improving the physical and pharmacokinetic properties of the series, while maintaining the $GABA_A$-R functional activity.

In our synthetic plan, **7** served as an ideal key intermediate on which to expand the SAR at C-21 and served as the starting material for exploration. Acetyl intermediate **7** and its *cis* analog **8** were prepared in multi-gram quantities using methods previously described.[43,46] Straightforward bromination of the acetyl afforded a key intermediate ready for functionalization at C-21. Subsequent nucleophilic *N*-alkylation yielded analogs that allowed for a rapid SAR investigation (Scheme 1).

Table 1 highlights the results of our initial investigation of C-21 heterocyclic substitution on the *trans* A/B ring system series. In an effort to improve physical properties of the analogs while maintaining metabolic stability, we examined a series of small nitrogen containing heterocycles at the C-21 position. First, pyrrolidine substitution at C-21, yielding **9**, resulted in a loss of binding activity, suggesting that this level of basicity may not be tolerated at the binding site. We then turned our attention to aromatic and planar rings with muted basic properties and systematically increased the number of nitrogens, resulting in heterocycles with subtle differences in physicochemical profiles. Pyrazole **10** led to a recovery of binding potency; however, this did not result in functional efficacy at the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R. On the other hand, potency for this analog at the $\alpha_4\beta_3\delta$ $GABA_A$-R was retained relative to compound **7**. Although potent in the TBPS assay, loss of functional activity at both the $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ $GABA_A$-Rs was observed when the pyrazole ring was replaced with the more basic imidazole, compound **11**. Addition of a third nitrogen in the ring led to several triazole regioisomers, analogs **12-14**. The

most potent regioisomer, as measured solely by the TBPS assay, was 1,2,5-triazole **12**. At the $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ $GABA_A$-Rs, electrophysiological activities were similar for both 1,2,5- and 1,2,3- triazole analogs **12** and **13**, whereas analog **14** dramatically lost functional activity for both receptor subtypes. In summary, compound **12** was the only triazole analog with somewhat comparable activity to **7**. Interestingly, the trend continued in this C-21 mini series with the most basic triazole **14** as the least active. Thus far, increased polarity in conjunction with decreased basicity at C-21 has resulted in improvements in the functional activity at both $GABA_A$-Rs, but still inferior to the activity of **1**, **6** and **7**. We anticipated that the analogs bearing a tetrazole at C-21 could be optimal relative to previous analogs. While analog **15** was a reasonably potent compound, particularly at the $\alpha_4\beta_3\delta$ $GABA_A$-R, the overall anticipated trend did not hold for these analogs. Overall none of the heterocyclic analogs in the *trans* series described above provided significantly improved activity over starting compounds **1**, **6** and **7**.

We next turned our attention to explore similar C-21 SAR on the nor-19 pregnanolone series, i.e. βC-5 hydrogen or *cis*- series (Table 2). Our initial interest was driven by the expectation of improved physical properties of the *cis*- series over the trans- series due to a topology less favorable to stacking and a reduction in the overall lipophilicity of a des-methyl series of compounds. The starting point for the exploration of the *cis* nor-19 series was the C-21 unsubstituted lead molecule **8**.[43] The potency of **8** in the TBPS binding assay (55 nM) is comparable to that of **7**, as was the functional activity at $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ $GABA_A$-Rs. Similar to the nor-19 allopregnanolone series, or *trans*- series, the addition of a pyrrolidine at C-21 led to a considerable loss of activity - as measured in the TBPS assay. However, once aromatic heterocycles were introduced, all *cis* analogs were more potent in the TBPS assay than their corresponding *trans* counterparts (Figure 2, Table 1-2). The reason for this difference between

both series in the TBPS assay remains unclear, but represents a consistent finding. The poor functional activity at both $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ $GABA_A$-Rs exhibited by the imidazole **19** and isomeric triazole **22** appeared to be in agreement with the trend that more basic moieties at C21 are less tolerated. The introduction of the most polar and least basic heterocycles studied, yielded the tetrazole analogs **23** and **24**. Both tetrazoles showed comparable potency and efficacy at the $\alpha_4\beta_3\delta$ $GABA_A$-R functional assay, showing moderate potency and good efficacy, but still less potent than unsubstituted **8**. These data again suggests the trend that $\alpha_4\beta_3\delta$ $GABA_A$-R prefers polar and less basic heterocycles. This property was less clear with regard to activity at the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R. The optimal ring that appeared to combine the right balance of polarity, basicity, and regio-position of nitrogen, was the 1,2,5- triazole **20**. In the $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ $GABA_A$-Rs functional assays, **20** showed a level of potency, 100-250 nM, and efficacy, 570-660%, comparable to those of the first generation compounds, **1** and **6** (Figure 3). The corresponding *trans* analog **12** also showed the most promising activity among the series of analogs at the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R (Table 1). However, the *cis* analog was able to bring a favorable six-fold improvement at the $\alpha_4\beta_3\delta$ $GABA_A$-R.

Overall, the data shows that the *cis* series was more active in both TBPS binding (Figure 2) and functional activity, relative to the *trans* series. Triazole **20** was identified as the first analog with a level of *in vitro* activity comparable to both **1** and **6**. Additionally and most importantly, compound **20** possesses further significant improvements in DMPK over compounds **1** and **6**, which will be discussed in later sections of this manuscript.

Since the *cis* series was demonstrating more promising activity and DMPK, we chose to investigate the effect of adding a second ring to the pyrazole **18** (Table 2), as previous reports have suggested that larger groups may be tolerated at the C21 position.[33] To that end, addition of

a tetrahydrobenzopyrazole moiety to **8**, resulted in both the *N-1* linked analog **25** (linear) and the *N-2* linked analog **26** (angular). Both of these novel compounds showed excellent binding affinity. In the $\alpha_4\beta_3\delta$ $GABA_A$-R functional assay, the linear analog **25** showed excellent potency and efficacy, comparable to that of **1** and **6**. The improved activity of **25** over the pyrazole analog **18** potentially may be due to increased lipophilicity. Additionally, hints with regard to overall shape and topology of the molecule can be gleaned by the loss of activity with the *N-2* linked analog **26**. We next explored aromatization of the six membered ring leading to the more planar benzopyrazole analogs **27** and **28**. Although both benzopyrazole compounds showed excellent binding affinity in the TBPS assay, their activities in $\alpha_4\beta_3\delta$ $GABA_A$-R functional assay were inferior by 3-10 fold to that of the saturated system *N-1* linked tetrahydrobenzopyrazole **25**. The activity of all these bicyclic analogs was modest at the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R, illustrating the difficulty of modulating the synaptic receptor family.

Finally, the addition of a nitrogen atom into the phenyl ring of the benzopyrazole moiety was explored. It was speculated that this substitution by an additional nitrogen could potentially improve solubility by increasing slightly the pKa and could offer a hydrogen bond acceptor for a potential interaction with the $GABA_A$ receptor, in particular at the $\alpha_1\beta_2\gamma_2$ $GABA_A$-R. Thus, the four isomeric azabenzopyrazoles were installed at C-21, resulting in eight unique analogs, **29-36**. While all eight analogs showed poor activity in the $\alpha_1\beta_2\gamma_2$ $GABA_A$-Rs functional assay, we continued forward to evaluate the compounds for activity at the $\alpha_4\beta_3\delta$ $GABA_A$-R. Of the four *N-2* linked analogs **(30, 32, 34 36)** that were evaluated in the $\alpha_4\beta_3\delta$ $GABA_A$-R, the *7-N*-azabenzopyrazole, **34,** showed the highest potency, albeit modest (EC_{50} 1.4 μM). The other three *N-2* linked analogs, **30**, **32** and **36**, showed poor activity at the $\alpha_4\beta_3\delta$ $GABA_A$-R. The four *N-1* linked analogs were then tested in the $\alpha_4\beta_3\delta$ $GABA_A$-R assay; the *4-N*- and *6-N*-

azabenzopyrazoles **29** and **33** showed modest activity (EC_{50} 1.0 and 1.3 μM respectively). More significantly, the *5-N* and *7-N* azabenzopyrazoles, **31** and **35,** showed excellent potency and efficacy, comparable to that of first generation compounds **1** and **6**. The *7-N* analog **35** showed a moderate preference (>8 fold) for $\alpha_4\beta_3\delta$ *vs* $\alpha_1\beta_2\gamma_2$ $GABA_A$-R; the *5-N* analog **31** showed a strong preference for $\alpha_4\beta_3\delta$ $GABA_A$-R (>17 fold). To our knowledge, compound **31** represents one of the most selective NASs discovered to date (Figure 3). Overall, the addition of one nitrogen in the benzopyrazole ring did not lead to improved activity at the synaptic $GABA_A$-R, and in fact, for most of the compounds, that activity deteriorated. Despite these results, there were key analogs identified where the activity at the $\alpha_4\beta_3\delta$ $GABA_A$-R improved dramatically. It is therefore our hypothesis that the observations in this series of *N-1* linked compounds provide a reasonable starting point for the rational design of extrasynaptic preferring modulators.

Once we identified the optimally active compounds, their physical properties and metabolic stabilities were evaluated, **15**, **20**, **23**, **25, 31** and **35** (Table 3). The first generation neuroactive steroid **1** has a logD of 4.9 with modest 3.3 μM aqueous solubility at pH = 7.4. **6** is even more lipophilic, with a logD of 5.3, and perhaps, as a consequence exhibits a much lower solubility (0.7 μM). Early analog **7** also shows low solubility (1 μM) and a slightly lower logD than **6**, likely as a result of replacing the C-19 methyl by hydrogen. While substitution at C-21 of **7** with a tetrazole, yielding **15**, led to a decrease in logD (4.5), no apparent aqueous solubility improvement was observed. Potentially this could be attributed to the overall topology of the *trans* scaffold that leads perhaps to a more highly stacked crystal lattice than the *cis*. Therefore, the *cis* series would be expected to be more soluble, due to the reduced packing ability into the crystal lattice. Indeed, the *cis*- tetrazole analog **23** maintained the logD at 4.5 but the solubility improved 3 fold *vs* the *trans* analog **15**. The 1,2,5-triazole analog **20** showed an even more

improved solubility, 3 fold *vs* the tetrazole **23**, perhaps due to an increased pKa from the more basic heterocycle. Not surprisingly, the highly lipophilic analog **25** showed very poor solubility (0.3 μM). Finally, both azabenzopyrazole analogs **31** and **35** were more polar than **25**, *ca.* 0.5 logD units lower, with **35** showing good solubility, 12 μM, being 10 fold more soluble than **31**. This result is somehow surprising, since the likely higher pKa of **31** should favor its solubility over that of **35**. Microsomal stability of **1**, **6** and **7** predict high clearance in humans and rodent species for these compounds. The majority of the 2nd generation compounds described here appear to show improved stability in human microsomes, with the exception of **25** which bears a highly lipophilic and potentially metabolically unstable tetrahydrobenzopyrazole moiety. The bicyclic derivatives, **25**, **31** and **35**, seemed to be metabolically less stable in rodent species than the monocyclic analogs, **15**, **20** and **23**, which may be due to the presence of more basic nitrogen atoms and carbon atoms in the ring susceptible to CYP mediated oxidation.

We then chose to evaluate several of the most promising compounds in *in vivo* rat PK studies (Table 4). The first generation NAS **6** showed high clearance (CL >3 L/hr/kg) and low bioavailability (F <10%) confirming what has been reported previously.[47] The 1,2,5-triazole *cis* analog **20** exhibited a slightly improved profile, with moderate clearance (2.3 L/hr/kg) and good oral bioavailability (F > 25%) while the 1,2,3,5-tetrazole analogs, *trans*-**15** and *cis*-**23**, showed low clearance and excellent bioavailability. Unfortunately, both azabenzopyrazoles analogs, **31** and **35**, showed high clearance (CL > 3 L/hr/kg). Compound **31** did exhibit improved oral bioavailability in rodents (F = 40%). Lastly, Table 4 represents a sampling of a broader data set which establishes that this class of NAS displays excellent brain exposure; with the ratio of brain:plasma greater than 1.

Discussion and Conclusion

As mentioned above, first generation neuroactive steroids **1** and **6** are currently being developed to treat a variety of CNS disorders. Although potent, both compounds are limited with regard to the route of administration due to their DMPK and general physical properties. The high clearance and poor bioavalability of **6**, requires high daily oral dosing to achieve adequate exposures that produce a relevant pharmacological effect.[41,42] Both molecules possess a desirable $GABA_A$-R pharmacological profile where significant preclinical data supports their potential for improved efficacy in benzodiazepine resistant seizure disorders.[12,48] The blocking of the potential metabolism at the βC-3 position with a methyl group in **6** modestly improves clearance and bioavailability in rodents over those of **1** (Table 4). However, the added methyl group increases the lipophilicity, thus decreasing solubility (Table 3) which hampers the ability to effectively formulate **6**, particularly as a parenteral agent. Conversely, the higher solubility and rapid clearance of endogenous **1** makes it an ideally attractive candidate for development as an *i.v.* agent. For example in a cyclodextrin based *i.v.* formulation, such as SBECD, **1** showed >5 fold increased solubility versus **6** (unpublished results). Both first generation molecules lack the properties of an ideal drug candidate for oral administration. In this report, we have demonstrated that the addition of specific heterocycles at the C-21 position of βC-3-methyl, nor-19 allo- and pregnanolone analogs can favorably attenuate the physical and DMPK properties of these NASs, yielding 2nd generation NAS candidates more suitable for oral administration while maintaining the efficacy at the targeted $GABA_A$ receptors. Overall the A/B ring *trans*- series appeared to be less potent in $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta3\delta$ $GABA_A$-Rs (Table 1), with compound **15** being the lone exception. In addition, **15** showed low clearance and excellent bioavailability, but very low solubility. As discussed such solubility may be explained by the ability of the *trans* series to

tightly stack creating a more highly crystalline compound. However, the A/B ring *cis*- series can yield more active compounds in both $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta3\delta$ $GABA_A$-Rs (Table 2). The mono heterocyclic substitutions at C-21 led to polar and soluble analogs with low *in vitro* and *in vivo* clearances and excellent oral bioavailabilities (Table 3 and 4). A key example, the 1,2,5-triazole analog **20** (**SGE-516**)[46] showed potency and efficacy at both receptors comparable to the first generation NASs **1** and **6** (Figure 3) with improved solubility and pharmacokinetic properties (Table 3 and 4). The balance between potent pharmacology and optimized DMPK properties makes **20** an attractive molecule with a superior profile for oral administration. Furthermore, the hetero-bicyclic substitution at C-21 afforded potent compounds with a relative balanced activity profile in $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta3\delta$ $GABA_A$-Rs, e.g. **25** (Table 2). More interestingly, this *cis*- series produced compounds with preference for the extrasynaptic $\alpha_4\beta3\delta$ $GABA_A$-Rs (**31**, **34** and **35**), a property that is being fully explored in our laboratories. In particular, **31** (**SGE-872**)[49] is the most selective extrasynaptic $GABA_A$–R NAS discovered to date (Figure 3). Molecules of this type will aid in understanding the specific functional roles for extrasynaptic $GABA_A$ receptors. Our continuing research to explore novel 2nd generation NASs of the $GABA_A$ receptor will be the subject of future publications from these laboratories.

Experimental section

The synthesis of intermediates has been published elsewhere.[46,49] Experimental procedures and characterization data for compounds 7 and **8** can be found elsewhere.[43] LC/MS Method: Samples were analyzed by reversed phase LC-MS using Merck, RP-184 25 x 2 mm columns, eluting with mixtures of water (A, 4 L water, 1.5 mL TFA) and acetonitrile (B, 4 L, 0.75 mL TFA) with a gradient elution, 5 to 95% B over 1.5 min at 1.5 mL/min. The injection volume was

1 μL and column temperature 50 ^{0}C. Detection was based on electrospray ionization (ESI) in positive polarity using a Shimadzu2010 mass spectrometer , diode-array UV detector .

Preparative HPLC was carried out using a Waters RBridge 10 μM C18 column, 19*250 mm. Mobile phase: acetonitrile and water (30 L water, 24 g NH_4HCO_3, 30 mL $NH_3.H_2O$). Flow rate: 25 mL/min. NAS analogs typically eluted at 40-60% acetonitrile. All compounds, except for **9**, **10**, **11**, **17**, **18** and **19**, were obtained as mixtures of structure isomers and isolated using preparative HPLC.

^{1}H NMR spectra (δ) were recorded on a Bruker or Varian 400 MHz or Bruker 500 MHz instrument and TMS was used as an internal standard.

Accurate mass was measured using a time of flight mass spectrometer (TOF, Agilent) in ESI+ mode. Mobile phase: water - acetonitrile (10:90, 0.1% FA v/v); flow rate of 0.7 mL/min. A 1 μL volume of sample was injected. Nebulization gas: nitrogen; positive ion mode; drying gas: nitrogen; flow: 12 l/min; nebulizer pressure: 20 psig, 350 C^o; capillary voltage: 3.5 kV; fragmentor voltage 50V.

The purity of the final compounds was assessed on the basis of analytical LC-MS and the results were greater than 95%.

Compounds presented in Table 1 and Table 2 were synthesized using a general procedure exemplified by compounds **25** and **26**: To a suspension of 21-bromo-3α-hydroxy-3β-methyl-19-nor-5α-pregnan-20-one (500 mg, 1.26 mmol) and K_2CO_3 (348.3 mg, 2.5 mmol) in 10 mL anhydrous DMF, 4,5,6,7-tetrahydro-*2H*-indazole (307.9 mg, 2.5 mmol) was added under an atmosphere of nitrogen at room temperature (27 ^{o}C). The reaction mixture was stirred for 18 h at

this temperature. The reaction mixture was then poured into water, extracted with EtOAc (2 x 50 mL), the organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated, purified by preparatory HPLC to afford the target compound **25** (161.6 mg, yield: 29%) and **26** (112.7 mg, yield: 20%) as off-white powders.

3α-Hydroxy-3β-methyl-21-(pyrrolidin-1'-yl)-19-nor-5α-pregnan-20-one (9). Yield: 15 mg (42%) as an off-white solid. LC-MS: t_R = 0.77 min, *m/z* = 388.2 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.40 (AB, *J* = 17.9 Hz, 1H), 3.34 (AB, *J* = 17.9 Hz, 1H), 2.58-2.66 (m, 4H), 2.56 (t, J=9.0 Hz, 1H), 2.13-2.22 (m, 1H), 1.88-1.94 (m, 1H), 0.90-1.85 (m, 23H), 1.21 (s, 3H), 0.64-0.76 (m, 2H), 0.63 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.70 (CH_3), 22.94, 24.11, 24.26, 25.52, 25.74, 30.92 (6 x CH_2), 31.47 (CH_3), 33.43 (CH_2), 37.76 (CH), 38.83, 39.10 (2 x CH_2), 41.25 (CH), 45.18 (C), 46.44 (CH), 46.46 (CH_2), 47.72 (CH), 53.42 (CH_2), 56.02, 61.06 (2 x CH), 69.57 (C), 197.85 (C=O). HRMS *m/z* 388.3206, calcd for $C_{25}H_{42}NO_2$ 388.3210.

3α-Hydroxy-3β-methyl-21-(pyrazol-1'-yl)-19-nor-5α-pregnan-20-one (10). Yield: 12 mg (33%) as an off-white solid. LC-MS: t_R = 0.89 min, *m/z* = 385.5 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.42 (s, 1H), 6.34 (s, 1H), 4.97 (AB, *J* =18.1 Hz, 1H), 4.90 (AB, *J* =18.1 Hz, 1H), 2.59 (t, *J* = 8.8 Hz, 1H), 2.16-2.24 (m, 1H), 2.03-2.08 (m, 1H), 0.95-1.90 (m, 19H), 1.21 (s, 3H), 0.65-0.79 (m, 2H), 0.69 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.77 (CH_3), 23.11, 24.32, 25.56, 25.79, 30.95 (5 x CH_2), 31.49 (CH_3), 33.46 (CH_2), 37.80 (CH), 38.86, 39.08 (2 x CH_2), 41.30 (CH), 45.14 (C), 46.48 (CH), 46.51 (CH_2), 47.77, 56.00, 60.59 (3 x CH), 61.53 (CH_2), 69.58 (C), 106.27, 130.65, 139.83 (3 x CH), 204.03 (C=O). HRMS *m/z* 385.2836, calcd for $C_{24}H_{37}N_2O_2$ 385.2850.

3α-Hydroxy--21-(imidazol-1'-yl)-3β-methyl-19-nor-5α-pregnan-20-one (11). Yield: 7 mg (35%) as an off-white solid. LC-MS: t_R = 0.76 min, *m/z* = 385.5 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 (s, 1H), 7.12 (s, 1H), 6.87 (s, 1H), 4.72 (AB, *J* = 17.8 Hz, 1H), 4.68 (AB, *J* = 17.8 Hz, 1H), 2.58 (t, *J* = 8.8 Hz, 1H), 2.16-2.24 (m, 1H), 1.85-1.98 (m, 1H), 0.95-1.80 (m, 19H), 1.21 (s, 3H), 0.68-0.77 (m, 2H), 0.67 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 12.52 (CH_3), 22.66, 23.87, 25.24, 25.58 (4 x CH_2), 30.35 (CH_3), 30.81, 33.37 (2 x CH_2), 37.41 (CH), 38.19, 38.37 (2 x CH_2), 41.32 (CH), 44.71 (C), 46.08 (CH_2), 46.56, 47.87, 55.77 (3xCH), 57.91 (CH_2), 60.50 (CH), 68.73 (C), 119.05, 123.28, 136.34 (3 x CH), 201.93 (C=O). HRMS *m/z* 385.2858, calcd for $C_{24}H_{37}N_2O_2$ 385.2850.

3α-Hydroxy-3β-methyl-21-(1',2',3'-triazol-2'-yl)-19-nor-5α-pregnan-20-one (12). Yield: 7 mg (19%) as an off-white solid. LC-MS: t_R = 0.889 min, *m/z* = 386.1 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (s, 2H), 5.26 (AB, *J* = 17.4 Hz, 1H), 5.23 (AB, *J* = 17.4 Hz, 1H), 2.59 (t, *J* = 9.0 Hz, 1H), 2.17-2.24 (m, 1H), 2.05-2.10 (m, 1H), 1.86-1.90 (m, 1H), 0.95-1.80 (m, 18H), 1.21 (s, 3H), 0.71 (s, 3H), 0.66-0.78 (m, 2H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.69 (CH_3), 23.06, 24.29, 25.54, 25.76, 30.93 (5 x CH_2), 31.48 (CH_3), 33.44 (CH_2), 37.77 (CH), 38.84, 38.99 (2 x CH_2), 41.27 (CH), 45.19 (C), 46.45 (CH), 46.48 (CH_2), 47.75, 55.99, 60.62 (3 x CH), 63.79 (CH_2), 69.57 (C), 134.97 (CH), 202.65 (C=O). HRMS *m/z* 386.2794, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',3'-triazol-1'-yl)-19-nor-5α-pregnan-20-one (13). Yield: 12 mg (33%) as an off-white solid. LC-MS: t_R = 0.85 min, *m/z* = 386.5 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.65 (s, 1H), 5.27 (AB, *J* = 18.0 Hz, 1H), 5.14 (AB, *J* = 18.0 Hz, 1H), 2.66 (t, *J* = 9.0 Hz, 1H), 2.18-2.27 (m, 1H), 2.04-2.11 (m, 1H), 1.85-1.92 (m, 1H), 0.95-1.80

(m, 18H), 1.21 (s, 3H), 0.66-0.81 (m, 2H), 0.68 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.80 (CH_3), 23.02, 24.27, 25.54, 25.76, 30.91 (5 x CH_2), 31.49 (CH_3), 33.41 (CH_2), 37.75 (CH), 38.83, 39.08 (2 x CH_2), 41.27 (CH), 45.38 (C), 46.42 (CH), 46.46 (CH_2), 47.72, 56.01 (2 x CH), 58.81 (CH_2), 61.14 (CH), 69.55 (C), 125.01, 133.98 (2 x CH), 201.78 (C=O). HRMS *m/z* 386.2785, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',4'-triazol-1'-yl)-19-nor-5α-pregnan-20-one (14). Yield: 15 mg (42%) as an off-white solid. LC-MS: t_R = 0.84 min, *m/z* = 386.1 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.96 (s, 1H), 5.01 (AB, *J* = 18.0 Hz, 1H), 4.93 (AB, *J* = 18.0 Hz, 1H), 2.63 (t, *J* = 8.9 Hz, 1H), 2.18-2.26 (m, 1H), 2.02-2.07 (m, 1H), 1.85-1.91 (m, 1H), 0.95-1.80 (m, 18H), 1.21 (s, 3H), 0.64-0.80 (m, 2H), 0.69 (s, 3H). ^{13}CNMR (CD_3OD, 100 MHz): δ 12.70 (CH_3), 22.59, 23.92, 25.21, 25.59 (4 x CH_2), 30.38 (CH_3), 30.79, 33.35 (2 x CH_2), 37.39 (CH), 38.15, 38.46 (2 x CH_2), 41.33 (CH), 44.94 (C), 46.03 (CH_2), 46.56, 47.78, 55.76 (3 x CH), 58.57 (CH_2), 60.51 (CH), 68.99 (C), 145.17, 150.26 (2 x CH), 203.93 (C=O). HRMS *m/z* 386.2816, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',3',4'-tetrazol-2'-yl)-19-nor-5α-pregnan-20-one (15). Yield: 7 mg (19%) as an off-white solid. LC-MS: t_R = 0.88 min, *m/z* = 387.1 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (s, 1H), 5.47 (AB, J=17.4 Hz, 1H), 5.45 (AB, *J* = 17.4 Hz, 1H), 2.66 (t, *J* = 8.7 Hz, 1H), 2.18-2.26 (m, 1H), 2.06-2.11 (m, 1H), 1.86-1.93 (m, 1H), 0.95-1.80 (m, 18H), 1.22 (s, 3H), 0.65-0.80 (m, 2H), 0.73 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.75 (CH_3), 23.05, 24.28, 25.55, 25.77, 30.92 (5 x CH_2), 31.51 (CH_3), 33.42 (CH_2), 37.77 (CH), 38.82, 39.06 (2 x CH_2), 41.27 (CH), 45.46 (C), 46.44 (CH), 46.48 (CH_2), 47.74, 56.03, 61.04 (3 x CH), 61.37

(CH_2), 69.58 (C), 153.20 (CH), 200.16 (C=O). HRMS *m/z* 387.2744, calcd for $C_{22}H_{35}N_4O_2$ 387.2755.

3α-Hydroxy-3β-methyl-21-(1',2',3',4'-tetrazol-1'-yl)-19-nor-5α-pregnan-20-one (16). Yield: 4 mg (11%) as an off-white solid. LC-MS: t_R = 0.86 min, *m/z* = 387.2 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.75 (s, 1H), 5.32 (AB, J=18.1 Hz, 1H), 5.19 (AB, J=18.1 Hz, 1H), 2.68 (t, J=8.9 Hz, 1H), 2.19-2.27 (m, 1H), 2.02-2.07 (m, 1H), 1.87-1.93 (m, 1H), 0.95-1.80 (m, 18H), 1.21 (s, 3H), 0.69-0.81 (m, 2H), 0.67 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.87 (CH_3), 23.05, 24.26, 25.54, 25.75, 30.90 (5 x CH_2), 31.52 (CH_3), 33.39 (CH_2), 37.75 (CH), 38.83, 39.14 (2 x CH_2), 41.27 (CH), 45.58 (C), 46.41 (CH), 46.45 (CH_2), 47.72, 56.06 (2 x CH), 56.69 (CH_2), 61.35 (CH), 69.58 (C), 143.63 (CH), 200.21 (C=O). HRMS *m/z* 387.2734, calcd for $C_{22}H_{35}N_4O_2$ 387.2755.

3α-Hydroxy-3β-methyl-21-(pyrrolidin-1'-yl)-19-nor-5β-pregnan-20-one (17). Yield: 12 mg (34%) as an off-white solid. LC-MS: t_R = 0.745 min, *m/z* = 388.6 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.39 (AB, *J* = 18.0 Hz, 1H), 3.32 (AB, *J* = 18.0 Hz, 1H), 2.56-2.65 (m, 4H), 2.56 (t, *J* = 8.8 Hz, 1H), 2.13-2.24 (m, 1H), 1.89-1.96 (m, 1H), 1.00-1.90 (m, 25H), 1.27 (s, 3H), 0.63 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.59 (CH_3), 23.14, 23.73, 24.37, 25.43, 25.71, 26.09 (7 x CH_2), 26.53 (CH_3), 31.36, 34.50 (2 x CH_2), 34.72, 37.64 (2 x CH), 39.11 (CH_2), 40.29 (CH), 41.16 (CH_2), 41.67 (CH), 44.91 (C), 54.02 (2 x CH_2), 55.93, 60.71 (2 x CH), 66.29 (CH_2), 72.04 (C), 207.79 (C=O). HRMS *m/z* 388.3220, calcd for $C_{25}H_{42}NO_2$ 388.3210.

3α-Hydroxy-3β-methyl-21-(pyrazol-1'-yl)-19-nor-5β-pregnan-20-one (18). Yield: 11 mg (31%) as an off-white solid. LC-MS: t_R = 0.88 min, *m/z* = 385.2 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 1.2 Hz, 1H), 7.43 (d, *J* = 1.2 Hz, 1H), 6.35 (s, 1H), 4.99 (AB, *J* = 17.8 Hz,

1H), 4.91 (AB, J = 17.8 Hz, 1H), 2.59 (t, J = 8.8 Hz, 1H), 2.15-2.25 (m, 1H), 2.03-2.10 (m, 1H), 1.05-1.90 (m, 21H), 1.27 (s, 3H), 0.69 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.75 (CH_3), 23.19, 24.36, 25.43, 25.67, 26.06 (5 x CH_2), 26.54 (CH_3), 31.34, 34.53 (2 x CH_2), 34.69, 37.61 (2 x CH), 39.09 (CH_2), 40.26 (CH), 41.14 (CH_2), 41.66 (CH), 45.19 (C), 55.88, 60.60 (2 x CH), 61.53 (CH_2), 72.03 (C), 106.30, 130.65, 139.85 (3 x CH), 204.04 (C=O). HRMS *m/z* 385.2848, calcd for $C_{24}H_{37}N_2O_2$ 385.2850.

3α-Hydroxy-21-(imidazol-1'-yl)-3β-methyl-19-nor-5β-pregnan-20-one (19). Yield: 10 mg (28.5%) as an off-white solid. LC-MS: t_R = 0.76 min, *m/z* = 385.1 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.12 (s, 1H), 6.88 (s, 1H), 4.74 (AB, J = 18.4 Hz, 1H), 4.73 (AB, J = 18.4 Hz, 1H), 2.59 (t, J = 8.8 Hz, 1H), 2.16-2.25 (m, 1H), 1.05-2.00 (m, 22H), 1.28 (s, 3H), 0.67 (s, 3H). ^{13}CNMR (100 MHz, CD_3OD): δ 12.50 (CH_3), 22.72, 23.89, 24.86 (2 x CH_2), 24.87 (CH), 25.47, 25.83, 31.18, 33.64 (4 x CH_2), 34.54 (CH_3), 37.61 (CH), 38.39, 40.40 (2 x CH_2), 40.44 (CH), 41.69 (CH), 44.76 (C), 55.63 (CH), 57.85 (CH_2), 60.53 (CH), 71.05 (C), 119.29, 123.19, 136.39 (2 x CH), 201.96 (C=O). HRMS *m/z* 385.2863 calcd for $C_{24}H_{37}N_2O_2$ 385.2850.

3α-Hydroxy-3β-methyl-21-(1',2',3'-triazol-2'-yl)-19-nor-5β-pregnan-20-one (20). Yield: 10 mg (15%) as an off-white solid. LC-MS: t_R = 0.87 min, *m/z* = 388.1 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.69 (s, 2H), 5.24 (AB, J = 17.3 Hz, 1H), 5.23 (AB, J = 17.3 Hz, 1H), 2.59 (t, J = 9.0 Hz, 1H), 2.16-2.24 (m, 1H), 2.05-2.11 (m, 1H), 1.05-1.90 (m, 21H), 1.27 (s, 3H), 0.71 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.69 (CH_3), 23.16, 24.34, 25.43, 25.65, 26.06 (5 x CH_2), 26.55 (CH_3), 31.34, 34.51 (2 x CH_2), 34.68, 37.61 (2 x CH), 39.02 (CH_2), 40.26 (CH), 41.13 (CH_2), 41.65, 45.26, 55.89, 60.63 (4 x CH), 63.81 (CH_2), 72.01 (C), 135.00 (CH), 202.67 (C=O). HRMS *m/z* 386.2784, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',3'-triazol-1'-yl)-19-nor-5β-pregnan-20-one (21). Yield: 15 mg (22%) as an off-white solid. LC-MS: t_R = 0.84 min, *m/z* = 386.1 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.65 (s, 1H), 5.27 (AB, *J* = 18.0 Hz, 1H), 5.14 (AB, *J* = 18.0 Hz, 1H), 2.66 (t, *J* = 9.0 Hz, 1H), 2.18-2.26 (m, 1H), 2.07-2.12 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.68 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.79 (CH_3), 23.11, 24.32, 25.41, 25.65, 26.03 (5 x CH_2), 26.55 (CH_3), 31.32, 34.50 (2 x CH_2), 34.65, 37.59 (2 x CH), 39.11 (CH_2), 40.24 (CH), 41.10 (CH_2), 41.65 (CH), 45.43 (C), 55.91 (CH), 58.82 (CH_2), 61.19 (CH), 71.99 (C), 125.02, 133.99 (2 x CH), 201.76 (C=O). HRMS *m/z* 386.2789, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',4'-triazol-1'-yl)-19-nor-5β-pregnan-20-one (22). Yield: 11 mg (31%) as an off-white solid. LC-MS: t_R = 0.829 min, *m/z* = 386.6 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.65 (s, 1H), 5.27 (AB, *J* = 17.9 Hz, 1H), 5.14 (AB, *J* = 17.9 Hz, 1H), 2.66 (t, *J* = 8.9 Hz, 1H), 2.17-2.27 (m, 1H), 2.06-2.12 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.68 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.79 (CH_3), 23.11, 24.32, 25.42, 25.66, 26.03 (5 x CH_2), 26.57 (CH_3), 31.32, 34.50 (2 x CH_2), 34.66, 37.60 (2 x CH), 39.14 (CH_2), 40.23 (CH), 41.11 (CH_2), 41.64 (CH), 45.39 (C), 55.92 (CH), 58.73 (CH_2), 61.11 (CH), 72.00 (C), 144.56, 151.79 (2 x CH), 202.03 (C=O). HRMS *m/z* 386.2810, calcd for $C_{23}H_{36}N_3O_2$ 386.2802.

3α-Hydroxy-3β-methyl-21-(1',2',3',4'-tetrazol-2'-yl)-19-nor-5β-pregnan-20-one (23). Yield: 10 mg (14%) as an off-white solid. LC-MS: t_R = 0.87 min, *m/z* = 369.1 (M -18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H), 5.46 (AB, *J* = 17.9 Hz, 1H), 5.45 (AB, *J* = 17.9 Hz, 1H), 2.65 (t, *J* = 9.0 Hz, 1H), 2.18-2.26 (m, 1H), 2.06-2.11 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.73 (CH_3), 23.11, 24.32, 25.41, 25.65, 26.03 (5 x CH_2), 26.56 (CH_3), 31.31, 34.49 (2 x CH_2), 34.65, 37.59 (2 x CH), 39.06 (CH_2), 40.23 (CH),

41.10 (CH_2), 41.64 (CH), 45.50 (C), 55.91, 61.07 (2 x CH), 61.37 (CH_2), 72.00 (C), 153.20 (CH), 200.15 (C=O). HRMS *m/z* 387.2738, calcd for $C_{22}H_{35}N_4O_2$ 387.2755.

3α-Hydroxy-3β-methyl-21-(1',2',3',4'-tetrazol-1'-yl)-19-nor-5β-pregnan-20-one (24). Yield: 8 mg (12%) as an off-white solid. LC-MS: t_R = 0.84 min, *m/z* = 369.1 (M - 18). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.74 (s, 1H), 5.31 (AB, *J* = 18.0 Hz, 1H), 5.19 (AB, *J* = 18.0 Hz, 1H), 2.68 (t, *J* = 8.8 Hz, 1H), 2.19-2.27 (m, 1H), 2.03-2.08 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.68 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.58 (CH_3), 22.98, 24.19, 25.18, 25.56 (4 x CH_2), 25.93 (CH_2+CH_3), 31.21, 33.94 (2 x CH_2), 34.43, 37.51 (2 x CH), 38.91 (CH_2), 40.14 (CH), 40.59 (CH_2), 41.55 (CH), 45.44 (C), 55.81 (CH), 56.72 (CH_2), 61.13 (CH), 71.68(C), 143.94 (CH), 200.69 (C=O). HRMS *m/z* 387.2745, calcd for $C_{22}H_{35}N_4O_2$ 387.2755.

3α-Hydroxy-3β-methyl-21-(4',5',6',7'-tetrahydroindazol-2'-yl)-19-nor-5β-pregnan-20-one (25). Yield: 161.6 mg (29%) as an off-white solid. LC-MS: t_R = 0.93 min, *m/z* = 439.2 (M + 1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.74 (s, 1H), 5.34-5.19 (m, 2H), 2.80-2.74 (m, 3H), 2.65-2.62 (m, 2H), 2.22-2.11 (m, 2H), 1.92-1.70 (m, 11H), 1.53-1.39 (m, 9H), 1.30-1.15 (m, 8H), 0.92-0.84 (m, 1H), 0.73 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz), δ 12.48 (CH_3), 19.28 (CH_2), 20.82, 21.61, 22.06, 22.76, 23.89, 24.86, 24.89 (CH_3), 25.46, 25.82, 31.18, 33.64 (CH_2), 34.54, 37.60, 39.39, 40.44 (CH_2), 40.45, 41.69, 44.79 (C), 55.62 (CH), 59.91 (CH_2), 60.34 (CH), 71.03 (C), 117.96 (C), 133.03 (CH), 147.24 (C), 202.67 (C=O). HRMS *m/z* 439.3322, calcd for $C_{28}H_{43}N_2O_2$ 439.3325.

3α-Hydroxy-3β-methyl-21-(4',5',6',7'-tetrahydroindazol-1'-yl)-19-nor-5β-pregnan-20-one (26). Yield: 113 mg (20%) as an off-white solid. LC-MS: t_R = 0.94 min, *m/z* = 439.2 (M + 1). ^{1}H NMR (MHz, CD_3OD): δ 7.57 (s, 1H), 5.14-4.99 (m, 2H), 2.80-2.75 (m, 1H), 2.60-2.48

(m, 4H), 2.22-2.09 (m, 2H), 1.91-1.72 (m, 11H), 1.58-1.39 (m, 9H), 1.32-1.26 (m, 5H), 1.22-1.12 (m, 3H), 0.72 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz), δ 12.60 (CH_3), 19.82 (CH_2), 20.47 (CH_2), 21.86 (CH_2), 22.24 (CH_2), 22.80 (CH_2), 23.92, 24.87, 24.88 (CH_3), 25.48, 25.83, 31.19, 33.66, (CH_2) 34.55, 37.62, 38.56 (CH_2), 40.45 (CH_2), 40.46 (CH_2), 41.69, 44.83 (C), 55.68 (CH), 57.92 (CH_2), 60.19 (CH), 71.04 (C), 117.16 (C), 134.78 (CH), 142.46 (C), 203.62 (C=O). HRMS *m/z* 439.3300, calcd for $C_{28}H_{43}N_2O_2$ 439.3325.

3α-Hydroxy-21-(indazol-2'-yl)-3β-methyl-19-nor-5β-pregnan-20-one (27). Yield: 12 mg (11%) as an off-white solid. LC-MS: t_R = 0.92 min, *m/z* = 435.6 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.67 (d, *J* = 8.7 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 5.22 (AB, *J* = 17.4 Hz, 1H), 5.17 (AB, *J* = 17.4 Hz, 1H), 2.64 (t, *J* = 8.8 Hz, 1H), 2.18-2.26 (m, 1H), 2.09-2.15 (m, 1H), 1.05-1.90 (m, 21H), 1.27 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, CD_3OD): δ 13.80 (CH_3), 23.25, 24.34, 25.43, 25.67, 26.04 (5 x CH_2), 26.52 (CH_3), 31.33, 34.53 (2 x CH_2), 34.68, 37.60 (2 x CH), 39.12 (CH_2), 40.26 (CH), 41.14 (CH_2), 41.66 (CH), 45.29 (C), 55.85, 60.92 (2 x CH), 62.81 (CH_2), 72.00 (C), 117.40, 120.33, 121.94 (3 x CH), 122.22 (C), 124.56, 126.35 (2 x CH), 148.78 (C), 202.75 (C=O). HRMS *m/z* 435.3002, calcd for $C_{28}H_{39}N_2O_2$ 435.3006.

3α-Hydroxy-21-(indazol-1'-yl)-3β-methyl-19-nor-5β-pregnan-20-one (28). Yield: 29 mg (25%) as an off-white solid. LC-MS: t_R = 0.95 min, *m/z* = 435.2 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.17 (t, *J* = 7.4 Hz, 1H), 5.15 (AB, *J* = 17.8 Hz, 1H), 5.14 (AB, *J* = 17.8 Hz, 1H), 2.64 (t, *J* =8.8 Hz, 1H), 2.09-2.24 (m, 2H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.77 (CH_3), 23.30, 24.37, 25.43, 25.71, 26.05 (5 x CH_2), 26.52 (CH_3), 31.33,

34.52 (2 x CH_2), 34.69, 37.61 (2 x CH), 39.19 (CH_2), 40.26 (CH), 41.13 (CH_2), 41.66 (CH), 45.24 (C), 55.89 (CH), 59.13 (CH_2), 60.28 (CH), 72.02 (C), 108.79, 120.81, 121.27 (3 x CH), 124.26 (C), 126.70, 134.06 (2 x CH), 140.32 (C), 204.45 (C=O). HRMS *m/z* 435.2992, calcd for $C_{28}H_{39}N_2O_2$ 435.3006.

3α-Hydroxy-3β-methyl-21-(pyrazolo[3',4'-b]pyridin-2'-yl)-19-nor-5β-pregnan-20-one (29). Yield: 30 mg (26%) as an off-white solid. LC-MS: t_R = 0.80 min, *m/z* = 436.6 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, *J* = 4.0 Hz, 1H), 8.06 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.98 (s, 1H), 7.05 (dd, *J* = 8.4, 4.0 Hz, 1H), 5.30 (AB, *J* = 17.3 Hz, 1H), 5.19 (AB, *J* = 17.3 Hz, 1H), 2.68 (t, *J* = 8.8 Hz, 1H), 2.13-2.27 (m, 2H), 1.05-1.90 (m, 21H), 1.27 (s, 3H), 0.71 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.79 (CH_3), 23.15, 24.33, 25.42, 25.67, 26.04 (5 x CH_2), 26.51 (CH_3), 31.32, 34.53 (2 x CH_2), 34.67, 37.60 (2 x CH), 39.08 (CH_2), 40.24 (CH), 41.12 (CH_2), 41.66 (CH), 45.48 (C), 55.91, 61.20 (2 x CH), 63.06 (CH_2), 72.01 (C), 114.42 (C), 117.95, 124.43, 129.82, 151.79 (4 x CH), 158.25 (C), 202.38 (C=O). HRMS *m/z* 436.2979, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[3',4'-b]pyridin-1'-yl)-19-nor-5β-pregnan-20-one (30). Yield: 27 mg (24.5%) as an off-white solid. LC-MS: t_R = 0.9 min, *m/z* = 458.2 (M + 23). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (dd, *J* = 4.5,1.6 Hz, 1H), 8.08 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.08 (s, 1H), 7.13 (dd, *J* = 8.0, 4.5 Hz, 1H), 5.32 (AB, *J* = 18.5 Hz, 1H), 5.31 (AB, *J* = 18.5 Hz, 1H), 2.70 (t, *J* = 9.0 Hz, 1H), 2.17-2.28 (m, 2H), 1.05-1.90 (m, 21H), 1.27 (s, 3H), 0.73 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.56 (CH_3), 23.09, 24.39, 25.44, 25.72, 26.07 (5 x CH_2), 26.52 (CH_3), 31.36, 34.54 (2 x CH_2), 34.71, 37.63 (2 x CH), 39.04 (CH_2), 40.29 (CH), 41.16 (CH_2), 41.67 (CH), 45.17 (C), 55.93 (CH), 56.89 (CH_2), 60.68 (CH), 72.03 (C), 115.54 (C), 116.99,

130.14, 132.97, 148.89 (3 x CH), 150.87 (C), 204.06 (C=O). HRMS *m/z* 436.2938, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[3',4'-c]pyridin-2'-yl)-19-nor-5β-pregnan-20-one (31). Yield: 10 mg (9.1%) as an off-white solid. LC-MS: t_R = 0.75 min, *m/z* = 458.2 (M + 23). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.26 (s, 1H), 8.17 (d, *J* = 6.0 Hz, 1H), 7.98 (s, 1H), 7.53 (d, *J* = 6.0, 1.2 Hz, 1H), 5.31 (AB, *J* = 17.5 Hz, 1H), 5.23 (AB, *J* = 17.5 Hz, 1H), 2.68 (t, *J* = 9.0 Hz, 1H), 2.20-2.28 (m, 1H), 2.11-2.16 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.83 (CH_3), 23.17, 24.32, 25.41, 25.66, 26.02 (5 x CH_2), 26.54 (CH_3), 31.30, 34.50 (2 x CH_2), 34.64, 37.59 (2 x CH), 39.15 (CH_2), 40.23 (CH), 41.10 (CH2), 41.63 (CH), 45.43 (C), 55.90, 61.22 (2 x CH), 63.16 (CH_2), 71.94 (C), 113.63 (CH), 124.15 (C), 124.39, 138.08, 144.41 (3xCH), 145.61 (C), 202.04 (C=O). HRMS *m/z* 436.2943, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[3',4'-c]pyridin-1'-yl)-19-nor-5β-pregnan-20-one (32). Yield: 12 mg (11%) as an off-white solid. LC-MS: t_R = 0.8 min, *m/z* = 436.2 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.34 (d, *J* = 5.5 Hz, 1H), 8.10 (s, 1H), 7.65 (dd, *J* = 5.5, 1.0 Hz, 1H), 5.26 (AB, *J* = 18.2 Hz, 1H), 5.25 (AB, *J* = 18.2 Hz, 1H), 2.69 (t, *J* = 8.9 Hz, 1H), 2.11-2.26 (m, 2H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.84 (CH_3), 23.22, 24.36, 25.43, 25.70, 26.04 (5 x CH_2), 26.56 (CH_3), 31.32, 34.50 (2 x CH_2), 34.66, 37.61 (2 x CH), 39.29 (CH_2), 40.23 (CH), 41.11 (CH_2), 41.65 (CH), 45.37 (C), 55.95 (CH), 59.36 (CH_2), 60.66 (CH), 72.01 (C), 114.81 (CH), 127.98 (C), 133.45, 133.65 (2 x CH), 137.06 (C), 139.09 (CH), 203.41 (C=O). HRMS *m/z* 439.2977, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[4',3'-c]pyridin-2'-yl)-19-nor-5β-pregnan-20-one (33). Yield: 5 mg (13%) as an off-white solid. LC-MS: t_R = 1.06 min, *m/z* = 436.3 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (s, 1H), 8.28 (d, *J* = 6.2 Hz, 1H), 8.16 (s, 1H), 7.53 (d, *J* = 6.2 Hz, 1H), 5.29 (AB, *J* = 17.6 Hz, 1H), 5.21 (AB, *J* = 17.6 Hz, 1H), 2.68 (t, *J* = 9.0 Hz, 1H), 2.10-2.28 (m, 2H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). HRMS *m/z* 436.2976, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[4',3'-c]pyridin-1'-yl)-19-nor-5β-pregnan-20-one (34). Yield: 9 mg (23%) as an off-white solid. LC-MS: t_R = 0.75 min, *m/z* = 458.2 (M + 23). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.14 (br, 1H), 8.44 (br, 1H), 8.19 (s, 1H), 7.14 (d, *J* = 5.6 Hz, 1H), 5.17 (AB, *J* = 18.0 Hz, 1H), 5.14 (AB, *J* =18.0 Hz, 1H), 2.67 (t, *J* = 9.0 Hz, 1H), 2.10-2.25 (m, 2H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, CD_3OD): δ 12.76 (CH_3), 22.61, 23.98 (2 x CH_2), 24.91 (CH+CH_2), 25.56, 25.86, 31.22, 33.68 (2 x CH_2), 34.56 (CH_3), 37.63 (CH), 38.68 (CH_2), 40.46 (CH_2+CH), 41.72 (CH), 45.00 (C), 55.69 (CH), 59.02 (CH_2), 60.50 (CH), 71.06 (C), 107.98 (CH), 120.87 (C), 133.57, 137.95, 140.08 (3 x CH), 144.74 (C), 203.20 (C=O). HRMS *m/z* 436.2953, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[4',3'-b]pyridin-2'-yl)-19-nor-5β-pregnan-20-one (35). Yield: 10 mg (9.2%) as an off-white solid. LC-MS: t_R = 0.81 min, *m/z* = 436.2 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (d, *J* = 4.1 Hz, 1H), 8.22 (s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.22 (dd, *J* = 8.9, 4.1 Hz, 1H), 5.27 (AB, *J* = 17.6 Hz, 1H), 5.20 (AB, *J* = 17.6 Hz, 1H), 2.68 (t, *J* = 8.9 Hz, 1H), 2.20-2.27 (m, 1H), 2.10-2.15 (m, 1H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.73 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.82 (CH_3), 23.23, 24.34, 25.43, 25.68, 26.04 (5 x CH_2), 26.54 (CH_3), 31.33, 34.52 (2 x CH_2), 34.66, 37.61 (2 x CH), 39.16 (CH_2), 40.25 (CH), 41.12

(CH_2), 41.65 (CH), 45.37 (C), 55.90, 61.05 (2 x CH), 63.43 (CH_2), 71.99 (C), 121.47, 125.68, 125.81 (3 x CH), 139.05, 141.93 (2 x C), 148.38 (CH), 202.22 (C=O). HRMS *m/z* 436.2980, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

3α-Hydroxy-3β-methyl-21-(pyrazolo[4',3'-b]pyridin-1'-yl)-19-nor-5β-pregnan-20-one (36). Yield: 17 mg (15.5%) as an off-white solid. LC-MS: t_R = 0.85 min, *m/z* = 436.6 (M + 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.60 (d, *J* = 4.3 Hz, 1H), 8.28 (s, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.30 (dd, *J* = 8.5, 4.3 Hz, 1H), 5.18 (AB, *J* = 18.0 Hz, 1H), 5.14 (AB, *J* = 18.0 Hz, 1H), 2.67 (t, *J* = 9.0 Hz, 1H), 2.10-2.24 (m, 2H), 1.05-1.90 (m, 21H), 1.28 (s, 3H), 0.72 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 13.83 (CH_3), 23.17, 24.35, 25.42, 25.71, 26.03 (5 x CH_2), 26.54 (CH_3), 31.32, 34.50 (2 x CH_2), 34.66, 37.59 (2 x CH), 39.24 (CH_2), 40.25 (CH), 41.11 (CH_2), 41.65 (CH), 45.34 (C), 55.92 (CH), 59.40 (CH_2), 60.63 (CH), 71.79 (C), 117.04, 120.91 (2 x CH), 133.30 (C), 134.89 (CH), 142.08 (C), 145.66 (CH), 203.82 (C=O). HRMS *m/z* 436.2961, calcd for $C_{27}H_{38}N_3O_2$ 436.2959.

Figure 1. Structures for endogenous and synthetic NASs: allopregnanolone (**1**), pregnanolone (**2**), Co26749/WAY-141839 (**3**), Co134444 (**4**), Co177843 (**5**), ganaxolone (**6**).

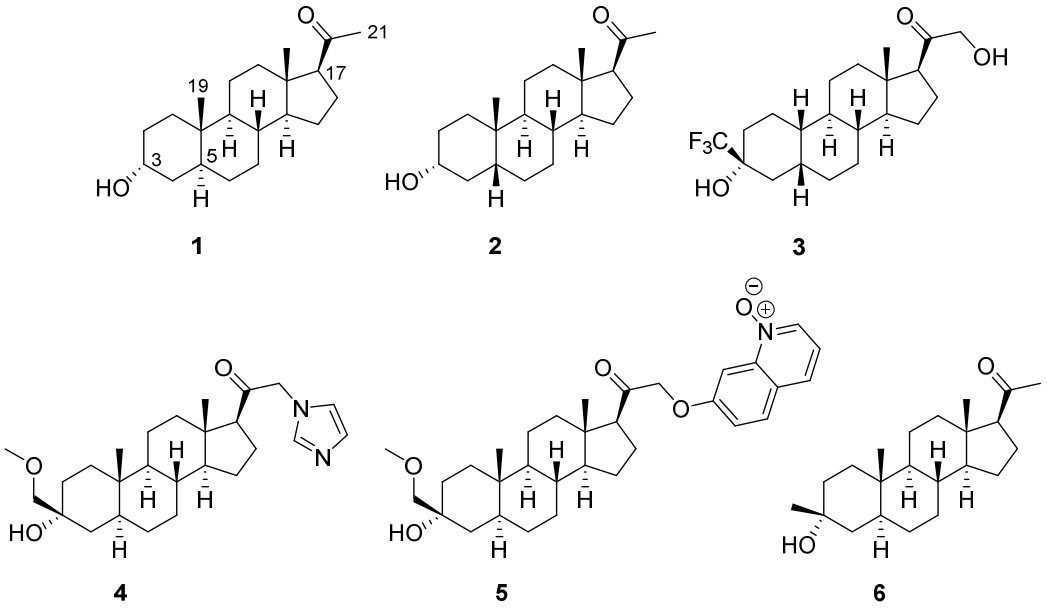


Figure 2. Correlation of [^{35}S]TBPS activity of *trans*- *vs cis* nor-19 series with 5 membered ring substutuents at C-21. [^{35}S]TBPS data is reported in Table 1 and 2.

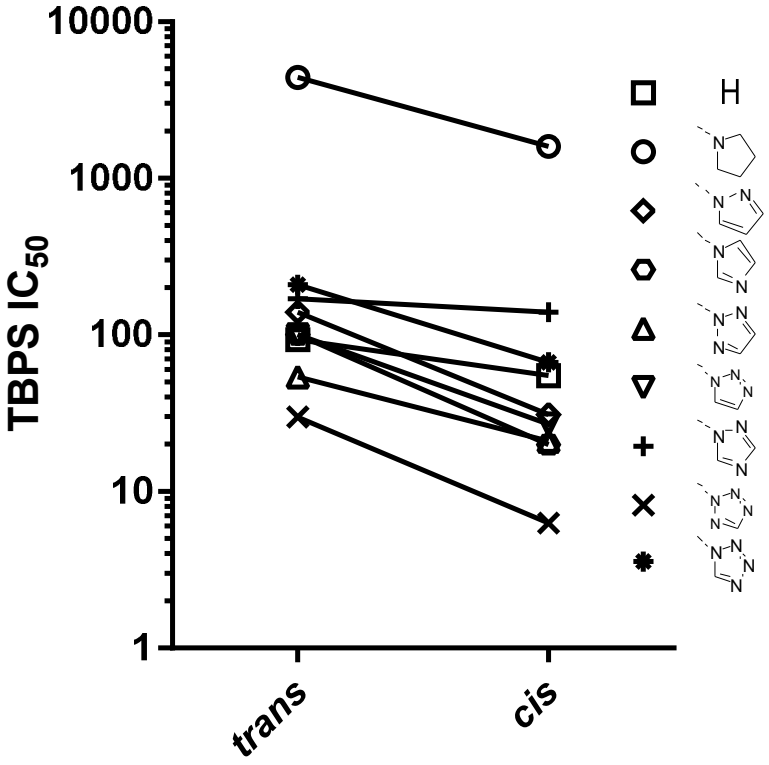
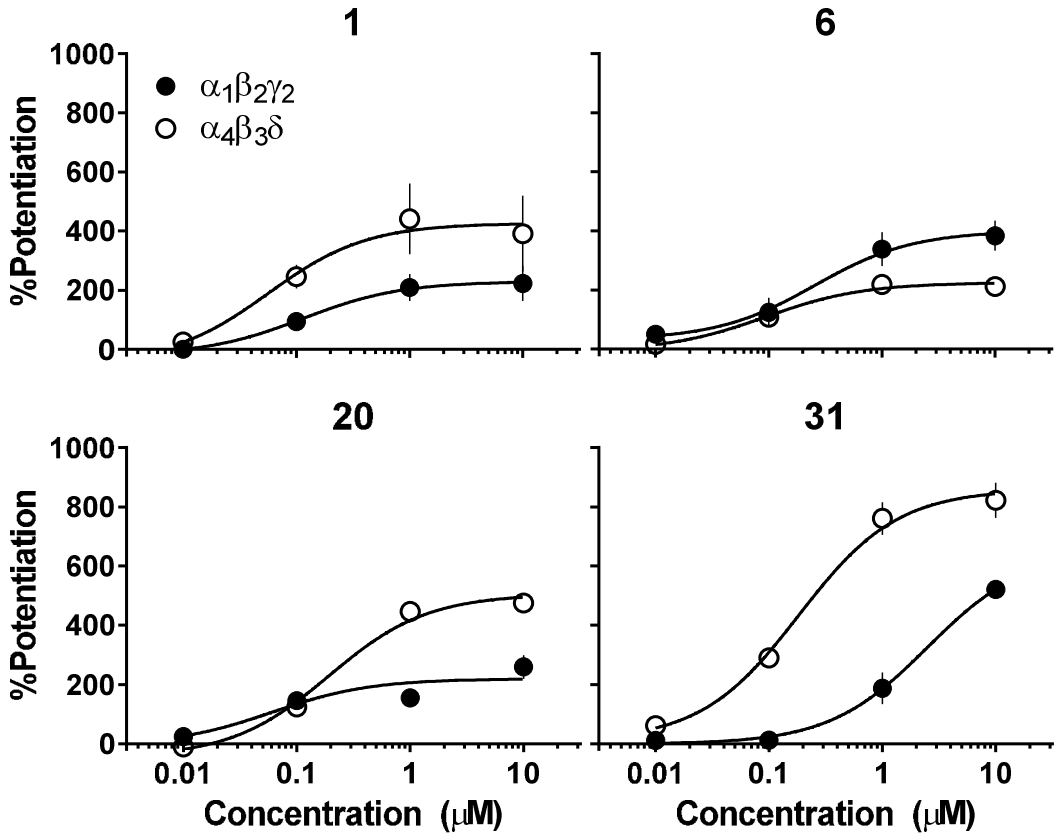

10000
1000
100
10
1
TBPS IC50
trans
cis
H

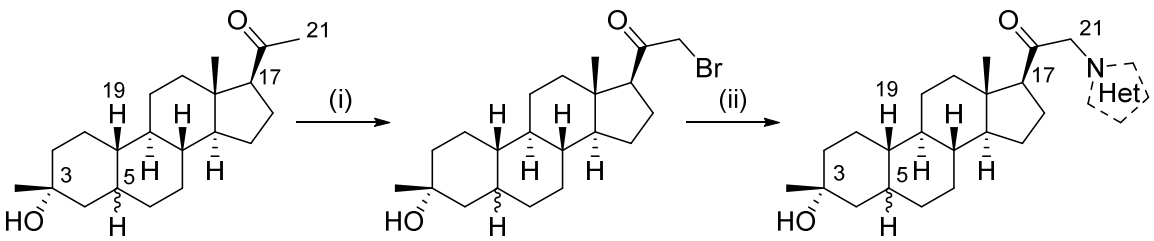
Figure 3. $\alpha_1\beta_2\gamma_2$[a] and $\alpha_4\beta3\delta$[b] $GABA_A$–R dose response curves using manual patch (n=3) for **1, 6, 20** and **31**.



[a]$\alpha_1\beta_2\gamma_2$ EC_{50} (nM, 95% CI) / E_{max} (%, 95% CI) / R^2: **1**, 115 (33-398) / 229 (185-273) / 0.9; **6**, 256 (91-720) / 400 (340-460) / 0.93; **20** 61 (10-370) / 219 (171-267) / 0.8; **31,** 2501 (1425-4390) / 652 (551-753) / 0.98.

[b]$\alpha_4\beta_3\delta$ EC_{50} (nM, 95% CI) / E_{max} (%, 95% CI):**1**, 75.5 (24-235) / 430 (339-521) / 0.8; **6**, 103 (68-155) / 225 (207-243) / 0.97; **20**, 240 (153-377) / 579 (526-631) / 0.97; **31,** 178 (129-244) / 858 (804-912) / 0.98.

Scheme 1. Synthesis of heterocyclic nor-19 NAS analogs.

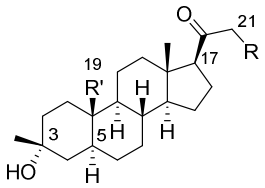


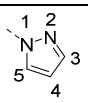
trans-5α: **7**
cis-5β: **8**

trans-5α: **9-16**
cis-5β: **17-35**

(i) Br_2, CH_3OH ; (ii) Heterocycle, K_2CO_3, DMF or THF

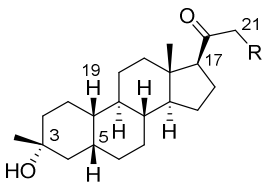
Table 1. [^{35}S]TBPS and $GABA_A$-R pharmacology of trans A/B ring analogs. $\alpha_1\beta_2\gamma_2$ EC_{50} (nM) / E_{max} (%) values were obtained using automated patch clamp. $\alpha_4\beta_3\delta$ EC_{50} (nM) / E_{max} (%) values were obtained using manual patch clamp.



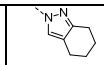
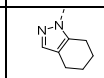
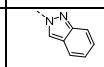
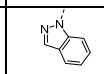
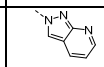
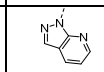
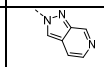
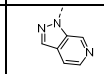
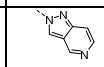
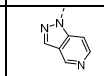
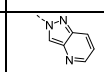
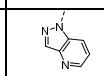
Cmpd.	R'	R	[^{35}S]TBPS IC_{50} (nM) (95%CI)	$\alpha_1\beta_2\gamma_2$		$\alpha_4\beta3\delta$	
				EC_{50} (nM) (95%CI)	E_{max} (%) (95% CI)	EC_{50} (nM) (95%CI)	Emax (%) (95% CI)
1	CH_3	H	22 (20-26)	237 (107-527)	510 (428-592)	75.5 (24-235)	430 (339-521)
6	CH_3	H	42 (31-57)	266 (130-543)	309 (263-534)	103 (68-155)	225 (207-243)
7	H	H	74 (47-116)	355 (99-1272)	289 (213-367)	553 (376-814)	399 (363-435)
9	H		2886 (1805-4615)	-	-	-	-
10	H		149 (125-177)	>3000	>226	1146 (417-3151)	210 (152-268)
11	H		108 (84-138)	>3000	>422	>3000	>405
12	H		54 (39-74)	592 (171-205)	323 (229-418)	1419 (986–2043)	370 (332 –408)
13	H		102 (89.5-116)	2738 (1025-7313)	514 (355-672)	1325 (698-2517)	300 (247-354)
14	H		174 (148-205)	>3000	>238	781 (467-1306)	561 (488-633)
15	H		31 (26-37)	945 (290-308)	433 (299-568)	292 (142-599)	430 (366-494)
16	H		214 (173-264)	>3000	>451	2136 (848-5379)	276 (200-352)

R^2 for [^{35}S]TBPS was >0.98 for all compounds; R^2 for $\alpha_1\beta_2\gamma_2$ was 0.8 for **7**, **15** and 0.85 for **12**, all others >0.91-0.99; R^2 for $\alpha_4\beta_3\delta$ was 0.8 for **1** and 0.9 for **10**, all others >0.93-0.99. [^{35}S]TBPS IC_{50} values were fitted using two replicates per concentration. $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ EC_{50} and E_{max}(%) values were obtained from three replicates.

Table 2. [^{35}S]TBPS and $GABA_A$-R pharmacology of *cis* A/B ring analogs. $\alpha_1\beta_2\gamma_2$ EC_{50} (nM) / E_{max} (%) values were obtained using automated patch clamp. $\alpha_4\beta_3\delta$ EC_{50} (nM) / E_{max} (%) values were obtained using manual patch clamp.



Cmpd.	R	[^{35}S]TBPS IC_{50} (nM) (95%CI)	$\alpha_1\beta_2\gamma_2$		$\alpha_4\beta3\delta$	
			EC_{50} (nM) (95%CI)	E_{max} (%) (95% CI)	EC_{50} (nM) (95%CI)	Emax (%) (95% CI)
8	H	55 (46-67)	371 (227-607)	444 (398-491)	119 (79-178)	553 (509-597)
17		1289 (820-2027)	-	-	-	-
18		32 (25-40)	945 (290-3085)	433 (299-568)	1458 (944-2251)	592 (519-665)
19		21 (18.5-24)	>3000	>999	3130 (1786-5484)	738 (603-873)
20		11 (9-13)	125 (36-426)	663 (503-823)	240 (153-377)	579 (526-631)
21		27 (21-35)	1836 (702-4800)	585 (421-749)	1834 (1513-2223)	981 (926-1036)
22		140 (108-182)	>3000	>331	>3000	>398
23		6 (4-10)	1151 (473-2802)	698 (529-867)	345 (207-575)	404 (360-447)
24		69 (60-79)	2198 (1479-3267)	792 (698-886)	394 (190-815)	408 (344-472)

25		24 (21-29)	724 (413-1270)	467 (402-532)	178 (53-599)	455 (345-565)
26		50 (31-81)	1491 (455-4887)	396 (263-529)	>3000	>882
27		11 (10-12.5)	1902 (1437-2517)	938(860-1015)	2523 (1615-3943)	611 (527-696)
28		15 (12-18.5)	1534 (790-2977)	770 (624-915)	508 (294-878)	557 (488-626)
29		42 (35-51)	>3000	>928	1021 (844-1233)	596 (566-626)
30		16 (12-20)	1749 (732-4177)	696 (521-872)	1866 (836-4163)	978 (745-1207)
31		16 (14-19)	>3000	>744	178 (129-244)	858 (804-912)
32		17 (12.5-23)	>3000	>408	>3000	>809
33		24 (22-26.5)	>3000	>1757	1353 (569-3216)	532 (403-660)
34		33 (29-39)	>3000	>257	1405 (885-2230)	765 (665-864)
35		6 (5-6)	>3000	>1037	343 (168-702)	436 (371-502)
36		25 (18-35)	2515 (1058-5978)	772 (566-978)	>3000	1461

R^2 for [^{35}S]TBPS was >0.98 for all compounds; R^2 for $\alpha_1\beta_2\gamma_2$ was 0.8 for **18**, **20**, **26**, all others >0.91-0.99; R^2 for $\alpha_4\beta_3\delta$ was 0.8 for **25**, all others >0.91-0.99. [^{35}S]TBPS IC_{50} values were fitted using two replicates per concentration. $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_3\delta$ EC_{50} and E_{max}(%) values were obtained from three replicates.

Table 3. LogD, aqueous solubility (pH = 7.4) and *in vitro* metabolic stability (Cl_{hep} L/hr/kg) properties for selected analogs.

Cmpd.	$LogD_{pH7.4}$	Aq. Sol. (μM)	Hu (Cl_{hep})	Rat (Cl_{hep})	Mouse (Cl_{hep})
1	4.9	3.3	0.50	3.34	4.05
6	5.3	0.7	0.39	3.34	4.05
7	5.1	1.0	0.5	3.17	4.05
15	4.5	0.9	0.10	0.86	2.03
20	4.6	7.3	0.19	2.70	4.05
23	4.5	2.5	0.33	2.36	4.05
25	5.7	0.3	0.65	3.34	4.05
31	4.7	1.2	0.27	3.34	2.03
35	4.7	12.4	0.27	3.34	3.60

LogD and Aq Sol are single point estimates. Microsomal stability is estimated by calculating rate of elimination from 5 time points sampled over 60 minutes.

Table 4. *In vivo* parameters for selected compounds in rats, following *i.v.* (5 mpk) and oral (20 mpk) administration. B:P ratios are obtained by single point at 30 min post *i.v.*

Cmpd.	Clearance (L/hr/kg)	Oral Bioavailability (%)	Brain:Plasma Ratio
1	4.8	2.3	1.8
6	3.1	10	4
15	0.5	67	2.0
20	2.3	27	1.8
23	1.1	41	2.3
31	3.5	40	2.1
35	4.4	1.5	1.3

Clearance, oral bioavailability, and Brain:Plasma ratios calculated from mean plasma concentrations from 2 rats.

ASSOCIATED CONTENT

Supporting Information.

Protocols for biological assays, details of animal care and pharmacokinetic experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest. B.L.H and S.J.G. are consultants for SAGE Therapeutics.

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ABBREVIATIONS

NAS, neuroactive steroid; $GABA_A$-R, $(\gamma$-aminobutyric acid$)_A$ receptor; [^{35}S]TBPS, t-butylbicyclophosphorothionate; PAM, positive allosteric modulator.

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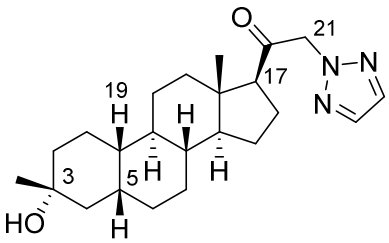
Table of Contents Graphic



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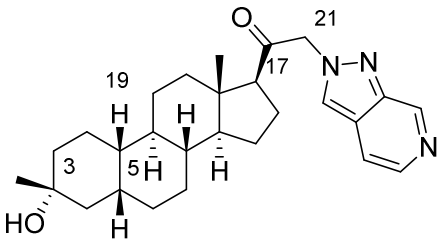

31