The Action of Positive Allosteric Modulators of the $GABA_AR$ Can Be Reversed by Novel Spiro Barbiturates

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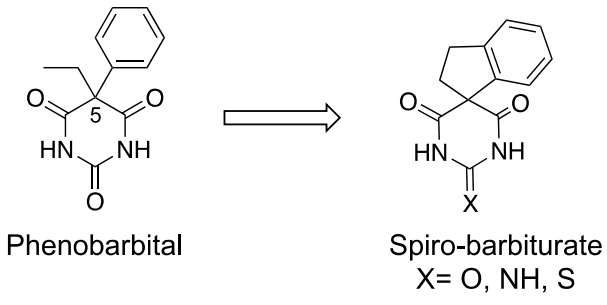
GRAPHICAL ABSTRACT



Conformational constraint of the 5-alkyl group in the sedative phenobarbital into the spiro-barbiturates leads to Null Allosteric Ligand (NAL) activity at the GABA-A receptor. Binding of spiro-barbiturates exerts global allosteric control over anesthetic TMD binding sites.

ABSTRACT

$GABA_AR$s are pentameric ligand-gated ion channels that play a major role in mediating inhibition in the CNS. They are the target of many widely used positive allosteric modulators (PAMs) of $GABA_AR$s such as general anesthetics, sedatives, antiepileptics, and anxiolytics. However, close structural analogs of these PAMs are negative allosteric modulators (NAMs) that cause excitation. Comparison of the SAR of inhibitory and excitatory barbiturates suggested that conformationally-constrained spiro-analogs of phenobarbital might have intermediate allosteric activity. More than 50 spiro-analogs were synthesized and characterized for their ability to enhance desensitization and reverse the action of anesthetics. A number of these reversed the action of anesthetics without having any action on GABA-induced desensitization. These constitute a new class of GABA-ergic drug that are null allosteric ligands. They offer the potential of reversing the sedative action of current PAMs and of modulating the behavior of diseases resulting from mutations in $GABA_AR$ subunits.

INTRODUCTION

Many general anesthetics, sedatives, anti-epileptics and anxiolytics are Positive Allosteric Modulators (PAMs) of γ-aminobutyric acid receptors ($GABA_ARs$), acting by shifting the GABA concentration-response curve to the left and prolonging synaptic currents by stabilizing the open state [1]. Synaptic $GABA_ARs$ consist of five homologous subunits arranged symmetrically around a central conductance pathway in the order β–α–γ–β–α anti-clockwise viewed from the extracellular side. On the time scale of structural and biochemical experiments, the PAM action of general anesthetics results in them binding with higher affinity to the desensitized state, which has higher affinity for agonists, than to the resting state. Photolabeling experiments revealed that anesthetics bind within a group of five homologous intersubunit binding sites in the transmembrane domain (TMD) and that GABA enhances photoincorporation into these sites [2, 3]. The location of these sites has subsequently been confirmed by structural studies [4].

However, the search for new agents many decades ago also revealed that certain closely related barbiturates and volatile agents were excitatory rather than sedative [5, 6]. Some of these excitatory agents were shown to target $GABA_ARs$ [7], shifting their GABA concentration-response curves to the right. The site of action of one such agent, *S*–mTFD-MPPB, a photoactivable derivative of the convulsant MPPB (**Fig. 1**) [8], photoincorporated within the same set of homologous intersubunit TMD sites as anesthetics [9]. However, unlike general anesthetics, which bind to two or more intersubunit sites in the desensitized state [10], *S*–mTFD-MPPB bound to a single site in the γ^+/β^- subunit interface in the resting state and photoincorporation was decreased by GABA [9]. *S*–mTFD-MPPB was a convulsant in mice and shifted the GABA concentration-response curve for activation to the right, whereas its enantiomer, *R*–mTFD-MPPB, was a sedative and shifted the GABA concentration-response curve for activation to the left [11]. Thus, agents binding in the TMD of $GABA_ARs$ can exert both positive and negative allosteric actions. We hypothesized that within this continuum, it should be possible to design agents near the null modulation point that might be mild PAMs, useful as sedatives

and anticonvulsants, or null allosteric ligands (NAL) [12] capable of attenuating the action of strong PAMs.

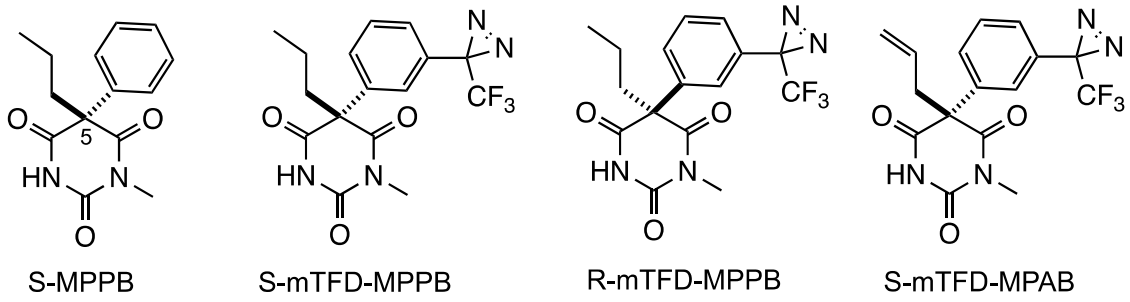


Figure 1. Structures of the discussed convulsant and anesthetic barbiturates.

Two observations suggested that the boundaries between positive and negative allosteric action are highly dependent on subtle changes caused by the C-5 aliphatic substituents. First, the contrast in actions of the enantiomers of mTFD-MPPB pointed to the orientation of the phenyl ring. Second, replacing the 5-propyl group of *S*–mTFD-MPPB by an allyl group, to give *S*–mTFD-MPAB, changes the action from convulsant to mild anesthetic[12]. Indeed, it is notable that there are no general anesthetic barbiturates with a 5-propyl group [5].

RESULTS

Design of conformationally constrained spiro-barbiturates

The starting point for our design of null allosteric ligands was the starkly different pharmacology of *S*–mTFD-MPAB (anesthetic) and *S*–mTFD-MPPB (convulsant). Photolabeling showed that the convulsant *S*–mTFD-MPPB was bound only in the γ+/β- interface in the resting state, whereas *S*–mTFD-MPAB was bound in both γ^+/β^- and α^+/β^- interfaces in the desensitized state [9]. Electrophysiology showed that the former caused a right-shift and the latter a left-shift of GABA dose-response curve [11]. Thus, changing the C-5 propyl to allyl has a profound effect on function. We concluded that the structure of an aliphatic substituent at the 5-position of the dihydropyrimidine ring and the orientation of the phenyl ring had large effects on the ligands' affinities, binding poses, and

interface binding selectivity for the TMD allosteric sites. Another reference point for our structural design was phenobarbital, a widely used antiepileptic drug [13] that is a mild sedative (a weak PAM). We hypothesized that changing the orientation of the phenyl ring and the ethyl group about the C5-C1" bond (see atom position numbering in **Fig. 2**) in phenobarbital might influence interaction of the new ligands with the receptor binding sites. This led us to explore conformationally constrained spiro-analogs with different sizes of the spiro-rings.

Molecular modeling using Chimera showed that in conformationally constrained spiro-analogs of phenobarbital, the phenyl ring changes both the orientation about the C5-C1' axis, as well as the tilt with regard to the dihydropyrimidine, depending on the size of the spiro-ring (**Fig. 2A and 2B**). The changes are relatively small in the five-membered ring (**BW-B-67**) as compared to six-membered (**BW-C-10**) and, in particular, the seven-membered ring compound (**BW-C-30**). To that effect, we synthesized a series of conformationally constrained analogs of phenobarbital and determined their pharmacological effects at $GABA_AR$ (**Fig. 2**). The conformational constraint of the 5-alkyl group by its connection to the phenyl ring causes gradual increase both in the angle the C5-C1' bond makes with the dihydropyrimidine ring and in the torsion angle the plane of the aromatic ring makes relative to the C2-C5 axis of the dihydropyrimidine ring. To that effect, we synthesized a series of conformationally constrained analogs of phenobarbital containing five-, six- and seven-membered rings (**Scheme 1**), **BW-B-67**, **BW-C-10** and **BW-C-30**, respectively, and determined their pharmacological effects on $GABA_ARs$.

Synthesis of spiro-barbiturates with varying ring sizes

Variously substituted phenylpropionic, phenylbutyric or phenylpentanoic acids **4** were converted into the corresponding acid chlorides and subjected to Friedel-Crafts cyclization into ketones **5** in the

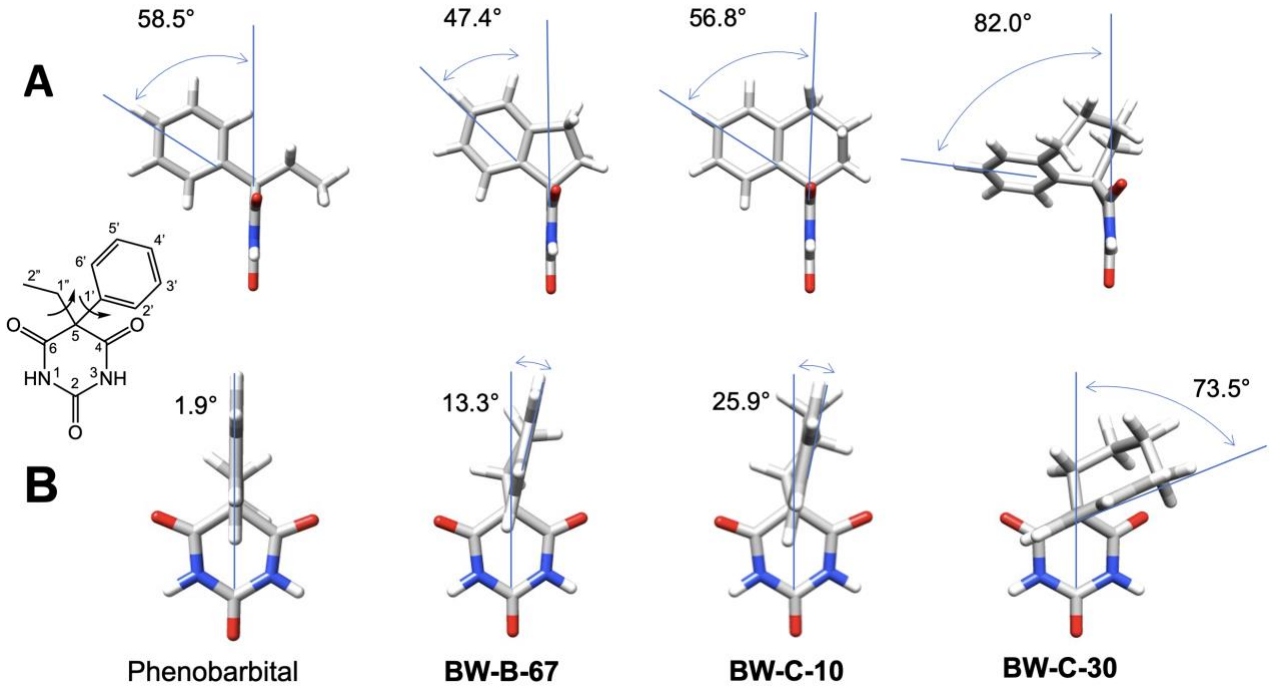
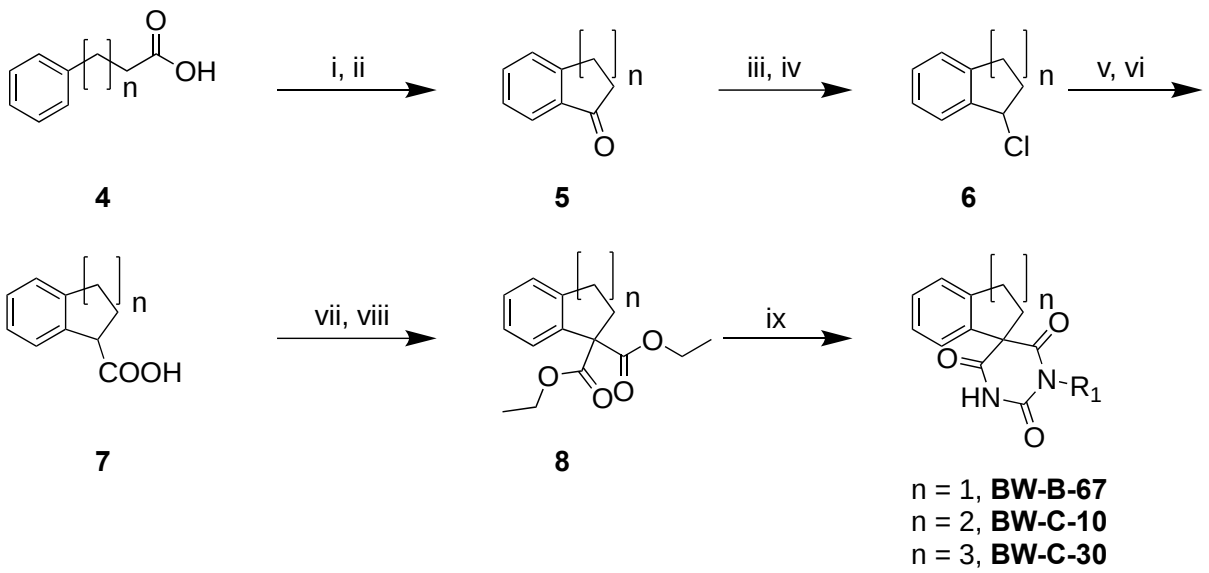


Figure 2. Comparison of structural features of five-, six-, and seven-membered spiro-analogs of phenobarbital. **Panel A:** Compounds oriented with the plane of the dihydropyrimidine ring perpendicular to the plane of the view. The angles shown indicate the tilt of the C5-C1' bond relative to the plane of the dihydropyrimidine ring. **Panel B:** Compounds oriented with the dihydropyrimidine ring in the plane of view. The torsion angles shown are between the plane of the phenyl ring and the C2-C5 axis of dihydropyrimidine ring. **Inset:** The atom numbering system used in this manuscript.

presence of aluminum chloride (**Scheme 1**). Ketones **5** were reduced with sodium borohydride and the corresponding alcohols were chlorinated with thionyl chloride into chlorides **6**. Chlorides **6** underwent nucleophilic displacement with sodium cyanide, and the cyanides were hydrolyzed into carboxylic acids **7** under acidic conditions. Acids **7** were converted into ethyl esters using thionyl chloride – ethanol, and the esters were subjected to acylation using LHDMS - ethyl chloroformate to provide diethyl malonates **8**. Finally, the assembly of a dihydropyrimidine ring was achieved by a base–catalyzed cyclization reaction of malonates **8** with urea to afford spiro-analogs **BW-B-67**, **BW-C-10** and **BW-C-30**.



n = 1-3; i: $SOCl_2$; ii: $AlCl_3$; iii: $NaBH_4$; iv: $SOCl_2$; v: NaCN; vi: aq. HCl; vii: $SOCl_2$, EtOH; viii: LHMDS, EtO(CO)Cl; ix: EtONa, $H_2N(CO)NH_2$

Scheme 1. Synthetic route to spiro-barbiturates **BW-B-67**, **BW-C-10**, **and BW-C-30**.

Assays of spiro-barbiturates activities

The principle of the assay used is that $GABA_A$Rs in the absence of ligands exist in a dynamic equilibrium between a resting and a desensitized state. The resting state predominates but agonists have approximately 100-fold higher affinity for the desensitized state than for the resting state [14]. The apparent affinity for the agonist muscimol is 50 nM [15], so that at 2 nM [^{3}H]muscimol binds only to desensitized receptors. Agents that increase desensitization increase 2 nM [^{3}H]muscimol binding. We define a null allosteric ligand (NAL) as one that does not modulate [^{3}H]muscimol binding when acting alone but that attenuates the enhancement of general anesthetic–induced [^{3}H]muscimol binding or equivalently anesthetic–induced desensitization.

Before considering the structure activity relationships amongst the many spiro derivatives synthesized, we illustrate the method with experiments shown in **Fig. 3**. *R*–mTFD-MPAB [11] is an efficacious PAM that enhances 2 nM [^{3}H]muscimol by 380% with an EC_{50} of 1.3 μM (**Fig. 3A**). We chose 10 μM *R*–mTFD-MPAB to represent maximum anesthetic–enhancement of [^{3}H]muscimol binding and scaled results between that value as one and zero (no anesthetic additions) (see the right axis of **Fig. 3A**). The

five–membered spiro-ring compound, **DK-B-21**, was a NAL because it both had no effect on [^{3}H]muscimol binding and inhibited *R*–mTFD-MPAB–enhanced [^{3}H]muscimol binding in a concentration dependent manner (**Fig. 3B**). In other cases, dual action was observed. **DK-B-4-2** acted both as a PAM on [^{3}H]muscimol binding and a NAM on anesthetic–induced desensitization, we term this action "mixed" (**Fig. 3C**). To compare the other spiro-analogs, we adopted a more efficient assay. Their activity was surveyed at 300 µM spiro-analog concentration under two conditions. First, at 2 nM [^{3}H]muscimol alone to see if they were PAMs and, second, at 10 µM *R*–mTFD-MPAB and 2 nM [^{3}H]muscimol to see if they could inhibit anesthetic–enhanced desensitization. Results are summarized in tables. Selected compounds were then studied in more detail.

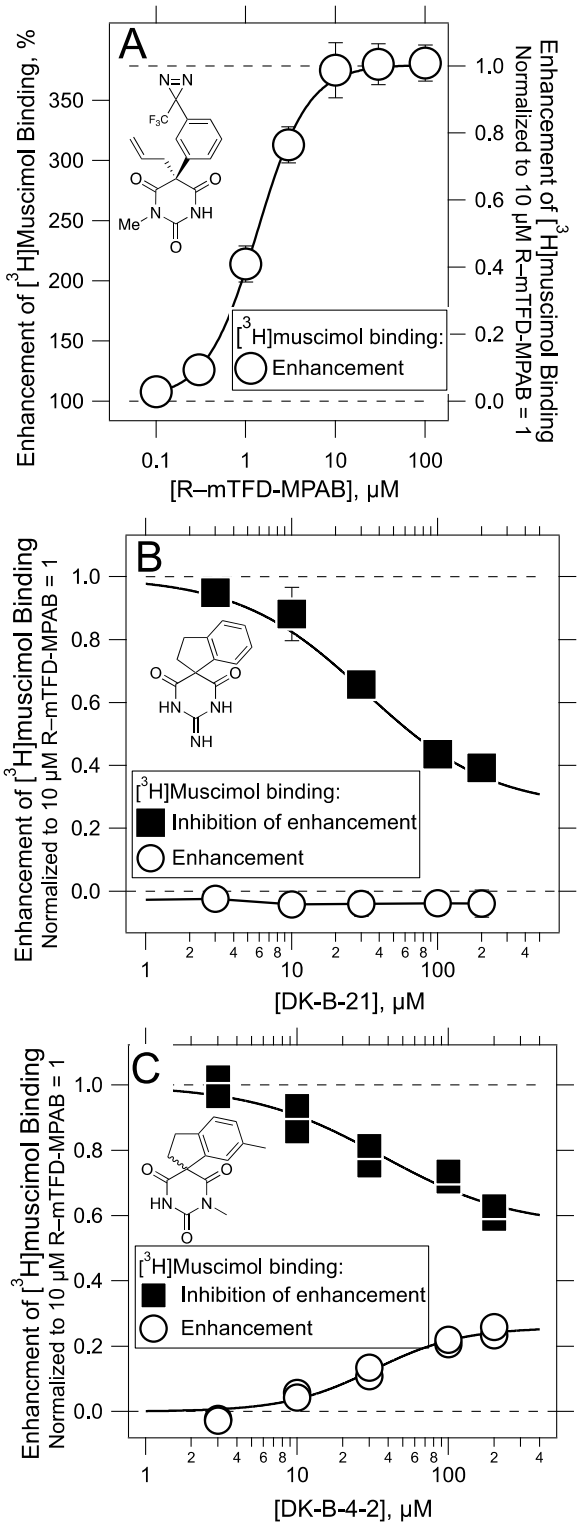


Figure 3.

Figure 3. The main ligand behaviors observed in this study in α1β3γ2L GABA$_A$R. (**A**) Representative titration of the enhancement of [^{3}H]muscimol binding by the PAM *R*-mTFD-MPAB (mean and standard deviation of 3 experiments). (**B**) The action of the NAL **DK-B-21** on [^{3}H]muscimol binding (mean and standard deviation of 3 experiments) and also on [^{3}H]muscimol binding in the presence of 10 µM *R*-mTFD-MPAB (mean and standard deviation of 4 experiments). (**C**) **DK-D-4-2** is both a PAM on [^{3}H]muscimol binding and a NAM on [^{3}H]muscimol binding enhanced by 10 µM *R*-mTFD-MPAB (individual points of 2 experiments).

Structure-activity relationship for spiro-barbiturates

Effect of the spiro-ring size

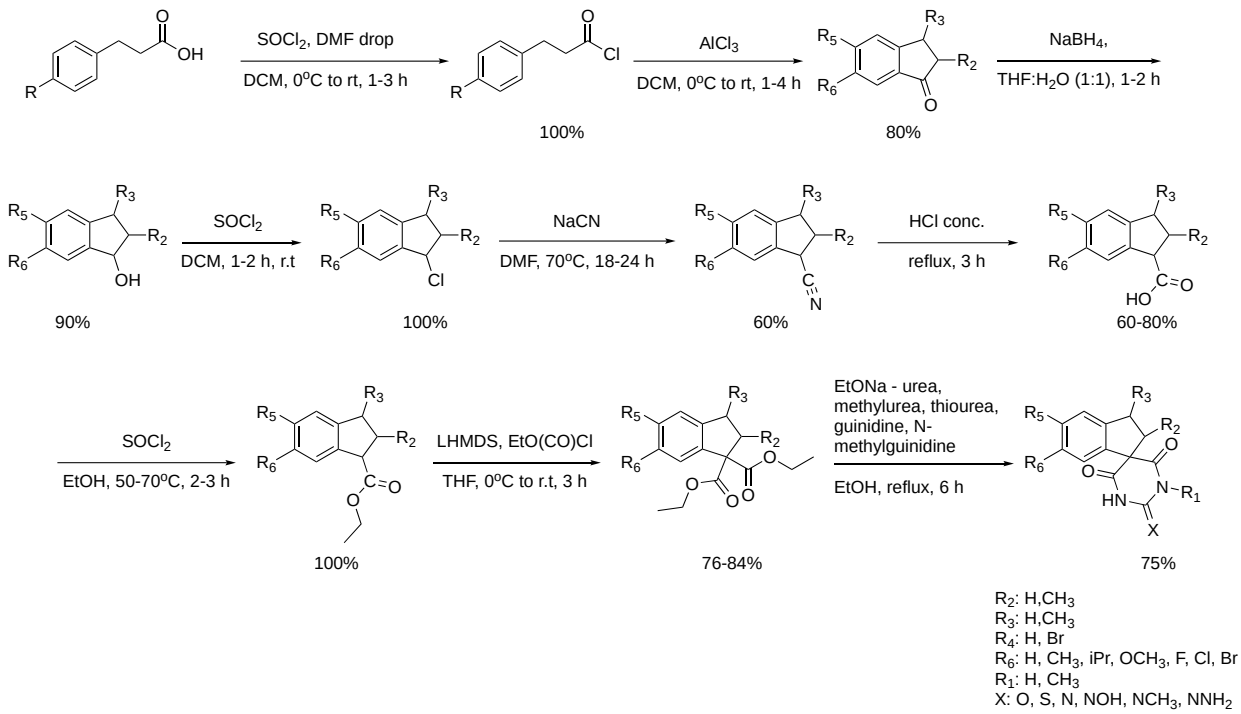
Overall, at 300 μM, as the ring size increased from five to seven (**BW-B-67**, **BW-C-10** and **BW-C-30**, respectively**)**, the ability to enhance [^{3}H]muscimol binding increased progressively (**Table 1**). On the other hand, only the five-membered ring compound was able to reverse the enhancement of [^{3}H]muscimol binding caused by 10 μM *R*–mTFD-MPAB. In contrast, phenobarbital, with its unrestrained C-5 ethyl, both enhanced [^{3}H]muscimol by 43%, consistent with its well know sedative action, but unexpectedly reduced anesthetic-enhanced desensitization by 12%.

Table 1. The effect of the spiro-ring size.[a]

Compound	Ring Size	R_1[b]	N[c]	Enhancement of [^{3}H]muscimol binding			Action on anesthetic–induced enhancement of [^{3}H]muscimol binding		
				Av	±	SD	Av	±	SD
BW-B-67	5	H	8	-0.02	±	0.03	0.53	±	0.06
BW-C-10	6	H	4	0.08	±	0.01	0.97	±	0.06
BW-C-30	7	H	4	0.22	±	0.02	0.98	±	0.04
Phenobarbital	N/A	H	4	0.43	±	0.05	0.88	±	0.02

[a]Ability of 300 μM of agent to attenuate the enhancement of 2 nM [^{3}H]muscimol binding by 10 μM *R*–mTFD-MPAB. [b]R^1 is defined in Scheme 2. [c]N is the number of replicates.

Because the five-membered ring compound was the best NAL, we focused our further efforts solely on determining the structure-activity relationships of the five-membered spiro-ring compounds. Toward this goal we have synthesized analogs of compound **BW-B-67** with substitutions in the dihydropyrimidine, phenyl, and the five-membered spiro-ring (**Scheme 2**) using the synthetic route shown in **Scheme 1**.



Scheme 2. Synthesis of substituted spiro-barbiturates used in determining SAR.

Effect of substitutions in the dihydropyrimidine ring

The activities of dihydropyrimidine analogs with substitutions at the C-2 carbon from oxo- (**BW-B-67)**, to imino- (**DK-B-21**), thio- (**DK-B-18**), hydroxyimino- (**DK-B-80**) or hydrazo- (**DK-B-82**) retained the NAL property (**Table 2**). When acting alone, they failed to enhance [^{3}H]muscimol binding, but they all reversed *R*–mTFD-MPAB–enhanced [^{3}H]muscimol binding from strongest to weakest in the order *N*-methylimino- = imino- > oxo- > hydroxyimino- = hydrazo- > thio-substituents. In this group of compounds, **DK-B-21** and **DK-D-7** had the best NAL properties. For reasons of synthetic expediency, we chose **DK-B-21** for more detailed studies.

Table 2. The effect of modification at C-2 of dihydropyrimidine ring (R_1, R_2, R_3, R_5 and R_6 = H).[a]

Compound	X	R_1[b]	N[c]	Modulation of [^{3}H]muscimol binding			Action on anesthetic–induced enhancement of [^{3}H]muscimol binding		
				Av	±	SD	Av	±	SD
DK-D-7	N-Me	H	4	-0.09	±	0.02	0.35	±	0.07
DK-B-21	NH	H	4	0.04	±	0.02	0.35	±	0.04
BW-B-67	O	H	8	-0.02	±	0.03	0.53	±	0.06
DK-B-80	N-OH	H	4	0.07	±	0.04	0.66	±	0.03
DK-B-82	N-NH_2	H	4	-0.02	±	0.02	0.66	±	0.01
DK-B-18	S	H	4	0.02	±	0.01	0.78	±	0.02

[a]Ability of 300 μM of agent to attenuate the enhancement of 2 nM [^{3}H]muscimol binding by 10 μM *R*–mTFD-MPAB. [b]R^1 is defined in Scheme 2. [c]N is the number of replicates.

Effect of substitution in the phenyl ring

We next examined the effect of substitutions in the meta-position of the aromatic ring. In the oxo-derivative series, all substitutions reversed enhancement, but compared to **BW-B-67** efficacy was lower with only the fluoro- and the methoxy-substituents exceeding 25% inhibition (**Table 3**). On the other hand, substitution in the phenyl ring had less than 10% effects on enhancement except for 6-Cl and the bulky 6-iPr substituents. Furthermore, there was no correlation with steric bulk or electrophilicity in the halogen series. Thus, in this group, although the methoxy-substituted derivative (**DK-B-5**) was the best NAL, it was still inferior to **BW-B-67**.

In general, substitution of sulfur for oxygen on the C-2 pyrimidine ring carbon increased the efficacy for enhancing [^{3}H]muscimol binding and decreased that for reversing *R*–mTFD-MPAB–enhanced [^{3}H]muscimol binding. In this series, the efficacy of [^{3}H]muscimol binding enhancement increased with substituent's bulk in the order Me < OMe < Br < iPr, with Me having no effect and iPr enhancing 54%. Only the bromo-derivative **DK-B-9** displayed 26% inhibition, but that was combined with 43% enhancement of [^{3}H]muscimol binding, making it a mixed agent. Thus, there were no efficacious NALs amongst this thio-series of compounds.

All of the imino-analogs examined in **Table 3** were NALs, having excellent inhibitory efficacies (> 40%) and enhancing [^{3}H]muscimol binding by less than 10%. Substitution of the methyl by the halogens F, Cl and Br reduced the inhibitory efficacy. On the other hand, substitution of a hydrogen by a methyl group into **DK-B-21**, to give **DK-B-51**, did not change the NAL efficacy.

Table 3. Effects of 6-substitution at the phenyl ring (R_1, R_2, R_3 and R_5 = H).[a]

Compound	X	R_6[b]	N[c]	Modulation of [^{3}H]muscimol binding			Action on anesthetic–induced enhancement of [^{3}H]muscimol binding		
				Av	±	SD	Av	±	SD
DK-B-64	O	F	4	0.07	±	0.01	0.73	±	0.06
DK-B-74	O	Cl	4	0.15	±	0.03	0.88	±	0.16
DK-B-2	O	Br	4	0.05	±	0.02	0.90	±	0.06
DK-B-1	O	Me	4	0.06	±	0.02	0.87	±	0.03
DK-B-5	O	OMe	4	0.00	±	0.03	0.69	±	0.04
DK-A-57	O	iPr	2	0.26	±	0.02	0.90	±	0.01
DK-B-9	S	Br	4	0.43	±	0.06	0.74	±	0.04
DK-B-8	S	Me	4	0.02	±	0.04	0.94	±	0.05
DK-B-19	S	OMe	4	0.20	±	0.02	1.04	±	0.05
DK-B-10	S	iPr	4	0.54	±	0.04	0.87	±	0.05
DK-B-51	NH	Me	4	-0.09	±	0.01	0.36	±	0.03
DK-C-56	NH	F	4	-0.02	±	0.04	0.58	±	0.06
DK-C-58	NH	Cl	4	0.02	±	0.04	0.53	±	0.02
DK-C-57	NH	Br	4	0.03	±	0.02	0.53	±	0.07

[a]Ability of 300 μM of agent to attenuate the enhancement of 2 nM [^{3}H]muscimol binding by 10 μM *R*–mTFD-MPAB. [b]R^6 is defined in Scheme 2. [c]N is the number of replicates.

Effect of *N*-1 substitution and chirality at C-5

Alkylation of nitrogen *N*-1 in unsymmetrically C-5-substituted barbiturates results in formation of a stereogenic center at C-5, and such enantiomers have been known to display different properties in their interactions with the GABA-A receptor [9, 11, 16]. We reasoned that the enantioselectivity of enhancing and inhibiting actions might differ, thus providing insight into the nature of their binding sites. *N*-Methylation of **BW-B-67** resulted in the two enantiomers of **BW-B-15**, which were separated by chiral chromatography and their absolute configurations determined by x-ray crystallography (see the **Supplemental Information**). Both enantiomers enhanced [^{3}H]muscimol binding moderately and

equally, whereas their inhibitory efficacies differed only modestly and both enantiomers were less efficacious inhibitors than the unmethylated **BW-B-67** (**Table 4**). In contrast, the seven-membered-spiro compound **BW-B-29** exhibited enantioselectivity in its PAM action, but the inhibitory activity of both the six- and seven-membered spiro-analogs **BW-C-11** and **BW-B-29** was too weak to characterize enantioselectivity.

N-Substitution of **BW-B-67** by ethyl, n-propyl or methylating both *N*-1 and *N*-3 nitrogen atoms resulted in agents with little or no inhibitory efficacy and, with one exception, unremarkable ability to enhance [^{3}H]muscimol binding. Only the enantiomers of the n-propyl derivative (**DK-C-66**) showed a large difference in [^{3}H]muscimol binding enhancement (5 vs 35%).

All phenyl ring-substituted derivatives of **BW-B-15** were PAMs, increasing [^{3}H]muscimol binding from 14% to a remarkable 80% for iso-propyl substituted **DK-A-58-1**. This is in contrast to the non-*N*-methylated series in **Table 3**, where only the iso-propyl compound **DK-A-57** was a PAM with 20% efficacy. Thus *N*-methylation enhances PAM action. The PAM efficacy was enantioselective in two of the four pairs; by 2.5- and 4.2-fold for the 6-methyl and 6-propyl substituted phenyl rings (**DK-B-4** and **DK-A-58**, respectively). Corresponding substitutions by 6-methoxy- or 6-bromo-group did not result in enantioselectivity. Thus, there was no clear correlation with the structure of the phenyl substituent. On the other hand, inhibition was more strongly influenced by C-5 chirality, especially for compounds with smaller substituents. The enantioselectivity in the inhibitory efficacies decreases in the order 6-H > 6-Me > 6-OMe = 6-iPr > 6-Br with the latter having no activity. The inhibitory efficacy of the best enantiomer of a pair declined with size in the same order H > Me > OMe = *i*Pr > Br, so these two measures are correlated. Significantly, the enantioselectivity for enhancing and inhibiting effects are unrelated, suggesting that they may be mediated by different sites of action.

One of the most interesting compounds in the *N*-methylated series was **DK-B-4-2**. It was the series most efficacious inhibitor but was also a good PAM. We therefore determined the concentration

dependence of these actions (**Fig. 3C**), finding that both enhancing and inhibiting actions occurred over the same concentration range, which adds further to the evidence suggesting that the two actions are mediated by separate sites.

Table 4. Enantioselectivity in *N*-1-substituted spiro-compounds (R_2, R_3 and R_5 = H).[a]

Compound*	Ring Size	R_1[b]	R_6[b]	N[c]	Modulation of [^{3}H]muscimol binding			Action on anesthetic–induced enhancement of [^{3}H]muscimol binding		
					Av	±	SD	Av	±	SD
BW-B-15-1	5	Me	H	4	0.08	±	0.07	0.78	±	0.11
BW-B-15-2	5	Me	H	4	0.16	±	0.06	0.67	±	0.04
BW-C-11-1	6	Me	H	4	0.13	±	0.04	0.90	±	0.02
BW-C-11-2	6	Me	H	4	-0.01	±	0.03	0.80	±	0.08
BW-B-29-1	7	Me	H	4	0.32	±	0.02	0.93	±	0.07
BW-B-29-2	7	Me	H	4	0.10	±	0.02	0.98	±	0.03
DK-C-65-1	5	Et	H	4	0.05	±	0.02	0.86	±	0.03
DK-C-65-2	5	Et	H	4	0.11	±	0.03	0.86	±	0.04
DK-C-66-1	5	nPr	H	4	0.31	±	0.04	0.95	±	0.02
DK-C-66-2	5	nPr	H	4	0.05	±	0.02	0.95	±	0.05
DK-C-69	5	Me2	H	4	0.07	±	0.02	0.98	±	0.02
DK-B-4-1	5	Me	Me	4	0.15	±	0.04	0.88	±	0.04
DK-B-4-2	5	Me	Me	6	0.37	±	0.15	0.54	±	0.08
DK-B-6-1	5	Me	OMe	4	0.36	±	0.01	1.06	±	0.10
DK-B-6-2	5	Me	OMe	6	0.37	±	0.05	0.73	±	0.05
DK-A-58-1	5	Me	iPr	4	0.80	±	0.04	0.73	±	0.02
DK-A-58-2	5	Me	iPr	4	0.14	±	0.04	0.97	±	0.05
DK-B-3-1	5	Me	Br	4	0.40	±	0.04	0.99	±	0.02
DK-B-3-2	5	Me	Br	4	0.35	±	0.08	1.09	±	0.07

[a]Ability of 300 μM of agent to attenuate the enhancement of 2 nM [^{3}H]muscimol binding by 10 μM *R*–mTFD-MPAB. [b]R^1 and R^6 are defined in Scheme 2. [c]N is the number of replicates. [b]Suffixes -1 and -2 denote the sequence of elution on the chiral chromatographic column, with compounds -1 eluting earlier than compounds -2. See the Experimental Section for enantiomer separation.

Effect of substitution at the five-membered ring

Substitution in the five-membered aliphatic ring was explored using **DK-B-21** as a starting point (**Table 5**). Substitution by a methyl group at either the 2- or 3-position removed the strong inhibitory action of **DK-B-21**. However, polar substitutions at the 3-position were more efficacious with amino and hydroxyl (**DK-D-27** and **DK-D-25-2**, respectively) producing inhibition of 19 and 45%, respectively. Both compounds are NALs because they also did not enhance [^{3}H]muscimol binding. In the case of the hydroxyl group, the chirality at the 3-position was important because the other enantiomer, DK-D-25-1, caused no inhibition within the error of measurement. However, compared to the parent **DK-B-21**, which has hydrogens in this position and causes 65% inhibition, these activities represent a decrease in inhibitory efficacy. Very modest enhancement (≤15%) of [^{3}H]muscimol binding was observed with methyl substitutions at either the 2- or 3-position, but only for the -2 enantiomer.

Table 5. Effect of substitutions at the five-membered ring (X = NH, R_1, R_5 and R_6 = H).[a]

Compound	R_3	R_2	X	N	Modulation of [^{3}H]muscimol binding			Action on anesthetic–induced enhancement of [^{3}H]muscimol binding		
					Av	±	SD	Av	±	SD
DK-D-1-1	Me	H	O	4	0.06	±	0.02	0.90	±	0.04
DK-D-1-2	Me	H	O	4	0.13	±	0.02	0.95	±	0.03
DK-D-25-1	OH	H	O	4	0.00	±	0.01	0.98	±	0.00
DK-D-25-2	OH	H	O	4	0.02	±	0.01	0.55	±	0.00
DK-D-27 (rac)	NH_2	H	O	4	0.10	±	0.02	0.81	±	0.02
DK-D-2-1	H	Me	O	4	0.06	±	0.02	0.96	±	0.05
DK-D-2-2	H	Me	O	4	0.15	±	0.03	0.99	±	0.04
DK-D-63-1	SCN	H	O	4	0.20	±	0.03	0.61	±	0.02
DK-D-63-2	SCN	H	O	4	0.30	±	0.02	0.67	±	0.02

[a]See footnote to Tables 1& 4. Assayed at 300 μM except for **DK-D-63-1** & **-2**, which were assayed at 1 mM.

Mechanism of action of reversal agents

Potency of spiro-barbiturate actions

Inhibitory potencies (IC_{50}s) were determined by titrating the reversal agent up to 300 μM against 2 nM [^{3}H]muscimol binding potentiated by a fixed concentration of 10 μM *R*-mTFD-MPAB. Enhancing EC_{50}s were determined by a similar titration against 2 nM [^{3}H]muscimol binding alone. Three agents each were surveyed in the three categories, NAL, PAM and mixed action (**Table 6)**, The NAL agents had IC_{50}s ranging from 28 to 113 μM, whereas the PAM agents' potencies varied from 22 to 38 μM. Two of the mixed agents had similar IC_{50}s and EC_{50}s, whereas DK-D-63-2 had too weak an enhancing efficacy to determine an EC_{50}.

Table 6. Potencies of some spiro-barbiturates for allosteric actions.

Action	Compound	[a]IC_{50}, μM	SD	[b]EC_{50}, μM	SD
NAL Agents					
	BW-B-67	18	± 4	—	
	DK-B-21	28	± 3	—	
	DK-D-7	99	± 27	—	
	DK-D-25-2	113	± 10	—	
PAM Agents					
	DK-A-57	—		22	± 3
	DK-A-58-1	—		32	± 6.5
	DK-A-58-2	—		38	± 11
Mixed Agents					
	DK-B-4-2	38	± 11	32	± 7
	DK-B-6-2	112	± 63	107	± 55
	DK-D-63-2	116	± 17	>300	

[a]IC_{50} ± SD for inhibition of 10 μM *R*–mTFD-MPAB–enhanced 2 nM [^{3}H]muscimol binding.
[b]EC_{50} ± SD for enhancing 2 nM [^{3}H]muscimol binding.

DK-B-21 does not modulate muscimol binding

To probe whether the observed effects could be related to changes in agonist binding, we studied the effect of the most efficacious NAL, **DK-B-21,** on the fractional occupancy of the agonist site (**Fig. 4**). At 1.6, 31.6 and 201.6 nM [^{3}H]muscimol, which give the calculated occupancies of 1.2%, 36% and

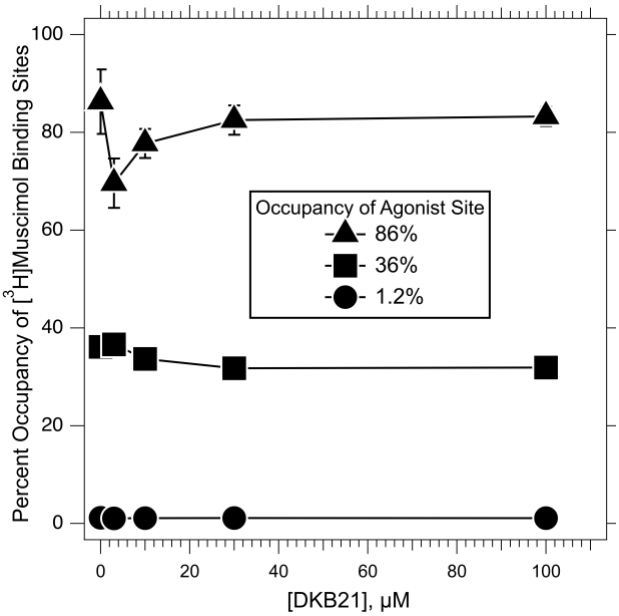


Figure 4. DK-B-21 does not change the fractional occupancy of the agonist site by [^{3}H]muscimol in α1β3γ2L GABA$_A$R.

86% using parameters in [1], there were no changes in the occupancy of the agonist site between 3 and 100 μM **DK-B-21**. From this data, we conclude that **DK-D-21** does not occupy the orthosteric agonist site nor does it allosterically modulate agonist binding.

DK-B-21 reverses the action of three anesthetics that act at different sites

To test whether **DK-B-21** acts in the TMD, we examined its ability to modulate three general anesthetics that bind selectively to different sites. Towards the outer end of the TMD, *R*–mTFD-MPAB binds in the α^+/β^- and γ^+/β^- subunit interfaces and etomidate in the two β^+/α^- subunit interfaces [11, 16]. Towards the intracellular end of the TMD, alfaxalone binds at a separate site in the two β+/α- subunit interfaces [3].

DK-B-21 was titrated from 0 to 200 μM against high affinity [^{3}H]muscimol binding in the presence of either 0, 3, 10 or 30 μM *R*–mTFD-MPAB (**Fig. 5A**). As the R–mTFD-MPAB concentration increased, the magnitude of the inhibition decreased. At 3 and 10 μM it fell 61% and 57%, respectively, whereas at 30 μM it only fell 17%. Fitting to the mass action equation yielded IC_{50}s that increased with R–mTFD-MPAB concentration from 12 ± 2 to 33 ± 4 to >200 μM at 3, 10 and 30 μM R–mTFD-MPAB, respectively. Although etomidate and alfaxalone were less efficacious enhancers, their actions were completely reversible at the lower concentrations, with IC_{50}s of 37 ± 9 and 39 ± 7 μM at 10 μM etomidate and 1.1 μM alfaxalone, respectively, whereas at the higher concentration inhibition was weak and the IC_{50}s >200 μM.

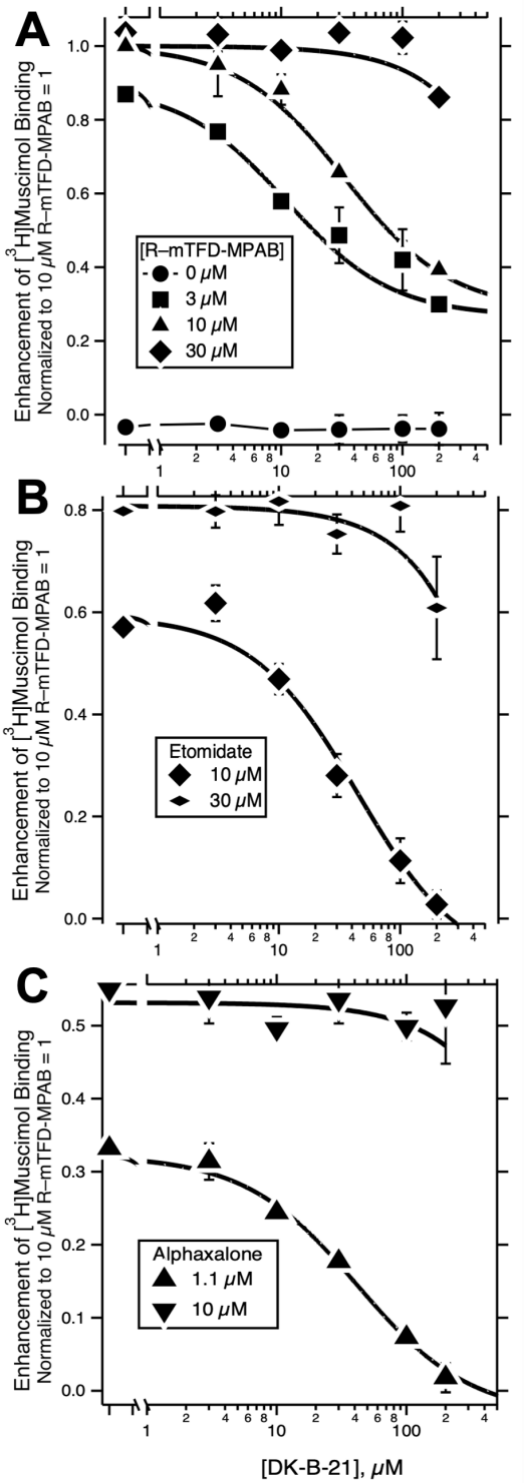


Figure 5. In α1β3γ2L GABA$_A$Rs **DK-B-21** inhibits [^{3}H]muscimol binding enhanced by (**A**) *R*-mTFD-MPAB, (**B**) etomidate and (**C**) alfaxalone.

Overall, the reversal was strongest at low anesthetic concentrations and could be overcome at high concentrations. Although this is formally consistent with either a negative allosteric interaction or competitive inhibition, it seems unlikely that **DK-B-21** would bind equally at three pairs of sites that exhibit strong selectivity for different structural classes of anesthetics.

DK-B-21 shifts anesthetic concentration-response curves to higher concentrations

To further characterize its mode of action, we determined the effect of **DK-B-21** on the potency of *R*-mTFD-MPAB–induced enhancement of [^{3}H]muscimol binding (**Fig. 6A**). *R*–mTFD-MPAB enhanced 2 nM [^{3}H]muscimol binding in a concentration–dependent manner reaching a plateau at 10 μM with an EC_{50} of 1.3 ± 0.1 μM. In the presence of 100 μM **DK-B-21**, the EC_{50} increased 6.4 ± 1.3-fold to 8.1 ± 1.5 μM. The Hill coefficient decreased from 1.6 ± 0.2 to 1.1 ± 0.2, whereas the maximum enhancement was unchanged at 375 ± 5 vs. 361 ± 12%. A similar pattern was also observed for etomidate, whose EC_{50} was increased 5.2 ± 0.7-fold from 1.1 ± 0.1 to 5.6 ± 0.5 μM by 100 μM

DK-B-21 (**Fig. 6B**) whereas its maximum effect was unchanged (288 ± 5 vs. 288 ± 9), and the Hill coefficient increased from 1.1 ± 0.1 to 1.5 ± 0.1.

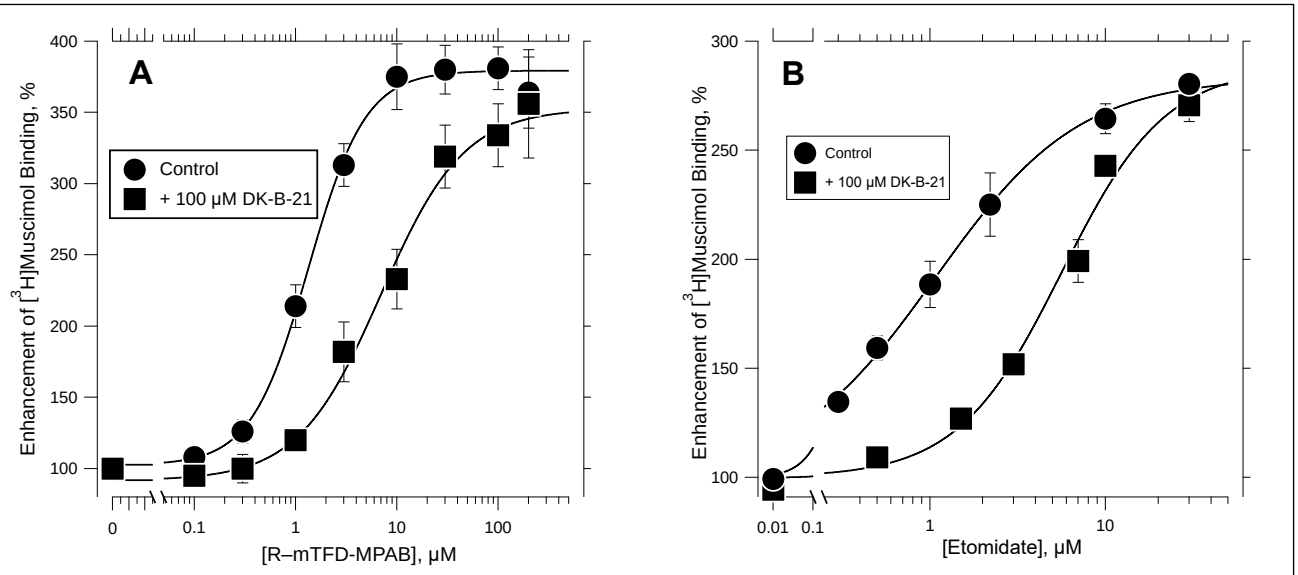


Figure 6. The NAL agent **DK-B-21** shifts anesthetic–induced desensitization curves to higher concentrations in α1β3γ2L $GABA_AR$s. (**A**) *R-m*TFD-MPAB titration of 2 nM [^{3}H]muscimol binding in α1β3γ2L $GABA_AR$s in the absence (filled circles) and presence of 100 µM **DK-B-21** (filled squares). (**B**) Etomidate titration of 2 nM [^{3}H]muscimol binding in α1β3γ2L $GABA_AR$s in the absence (filled circles) and presence of 100 µM **DK-B-21** (filled squares). The data points displayed are the mean and SD of 3 determinations.

Does the benzodiazepine site mediate inhibition of anesthetic–enhanced desensitization?

We examined fourteen compounds for their ability to modulate [^{3}H]flunitrazepam binding (**Table 7**). Overall, at 300 µM, all the compounds modestly reduced the normalized 1 nM [^{3}H]flunitrazepam binding from 12% to 57%. The 6- and 7- membered ring compounds, **BW-C-10** and **BW-C-30**, were the most efficacious. Amongst the rest, there was no correlation between inhibition and the nature of the 6-substituent. Enantioselectivity of inhibitory efficacy was not strong amongst the compounds examined. Substitution of the 6-position in **BW-B-15**, which itself had low enantioselectivity of 1.2-fold, with methoxy and isopropyl increased enantioselectivity to 1.4 and 1.6, whereas substitutions with bromo- and methyl residues erased enantioselectivity. Such small changes should not be overinterpreted.

Table 7. Effect of spiro-barbiturates on [^{3}H]flunitrazepam binding. (R^2, R^3 and R^5 = H).[a]

Compound	Ring size	X	R_1[b]	R_6[b]	N[c]	Inhibition of 1 nM [^{3}H]flunitrazepam binding (0 = full inh, 1 = no inh)		
						Av	±	SD
BW-C-30	7	O	H	H	4	0.43	±	0.02
BW-C-10	6	O	H	H	4	0.45	±	0.05
DK-B-6-1	5	O	Me	OMe	4	0.55	±	0.02
BW-B-15-1	5	O	Me	H	4	0.56	±	0.02
DK-B-21	5	NH	H	H	4	0.59	±	0.04
DK-A-58-2	5	O	Me	iPr	4	0.59	±	0.01
DK-B-3-2	5	O	Me	Br	4	0.61	±	0.02
DK-B-3-1	5	O	Me	Br	4	0.65	±	0.02
BW-B-15-2	5	O	Me	H	4	0.67	±	0.02
BW-B-67	5	O	H	H	4	0.68	±	0.06
DK-B-4-2	5	O	Me	Me	4	0.72	±	0.02
DK-B-4-1	5	O	Me	Me	4	0.76	±	0.01
DK-A-58-1	5	O	Me	iPr	4	0.84	±	0.03
DK-B-6-2	5	O	Me	OMe	4	0.88	±	0.02

[a]Ability of 300 μM of agent to attenuate the binding of 1 nM [^{3}H]flunitrazepam binding. [b]R^1 and R^6 are defined in Scheme 2. [c]N is the number of replicates.

The pharmacology of inhibition of flunitrazepam binding and inhibition of anesthetic–enhanced desensitization differed. For example, the six- and seven-membered ring compounds were the most efficacious in the former action and the least in the latter action. **DK-D-6-1** inhibited [^{3}H]flunitrazepam binding by 45% but had no effect on anesthetic–enhanced desensitization. Furthermore, the chirality of the two **DK-D-6** enantiomers on the two actions was reversed.

To resolve these complexities, we turned to α1β3 $GABA_A$Rs that lack the benzodiazepine site. **DK-B-21** was a NAL (**Fig. 7**). It did not modulate [^{3}H]muscimol binding and it reversed the enhancing action of 10 μM etomidate in a concentration dependent manner. Thus, the benzodiazepine site is not required for NAL action, but we cannot rule out contributions from the benzodiazepine site in the case of **DK-B-21** in α1β3γ2 receptors because **DK-B-21** inhibited [^{3}H]flunitrazepam binding by 41%.

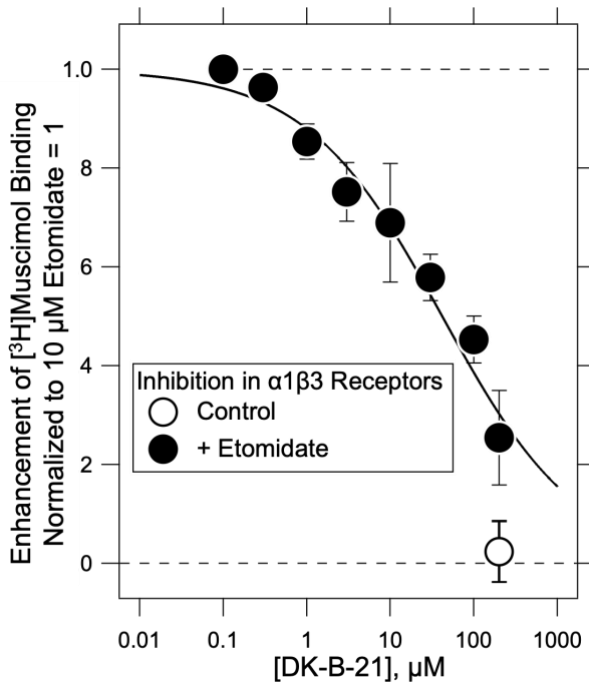


Figure 7. The pharmacology of **DK-B-21** in α1β3γ2 $GABA_AR$s is preserved in α1β3 $GABA_AR$s. The action of **DK-B-21** on [^{3}H]muscimol binding in the presence of 10 µM etomidate. The curve was fitted between 1 and zero, giving an IC_{50} of 36 ± 10 µM and a Hill coefficient of 0.48 ± 0.07 (N = 36).

Furthermore, in α1β3 receptors, we obtained a Hill coefficient of 0.5, whereas in α1β3γ2 the Hill coefficient was one (**Fig. 3B**).

DISCUSSION

Our expectation outlined in the Introduction that it might be possible to created null allosteric ligands (NALs) that have no action on the orthosteric site but that can allosterically reverse the action of allosteric ligands, specifically general anesthetics, that act in the transmembrane domain (TMD) of $GABA_AR$s was fulfilled. Several of the ligands we synthesized turned out to be NALs and one of the most efficacious, DK-B-21, was chosen for more detailed study. It was able to allosterically reverse the action of anesthetics on the orthosteric site without itself having any effect on agonist binding. This NAL action appeared to be global on the TMD because it was exerted on three general anesthetics each of which bind selectively to one of the three known pairs of anesthetic binding sites in that domain. *As far as we are aware, this is the first time such a pharmacology has been observed in $GABA_AR$s*. As such, it initiates a new departure that may have implications for reversal of sedation and for treating certain diseases that result from mutations in the TMD of $GABA_AR$ subunits.

The key structural parameter in this NAL action was the tilt of the C5-C1' bond relative to the plane of the dihydropyrimidine ring and the torsion angles between the plane of the phenyl ring and the C2-C5 axis of dihydropyrimidine ring. Both these parameters increased as the size of the ring increased (Fig. 2). The geometry of the five membered ring was optimal for NAL action with six- and seven-

membered rings (BW-C-10 and BWC-30, respectively) displaying only weak positive modulatory activity and no inhibitory activity (Table 1).

Following this discovery, we conducted an SAR campaign by modifying the initial 5-membered ring structure with substituents at the dihydropyrimidine, five-membered-spiro- and phenyl rings. In

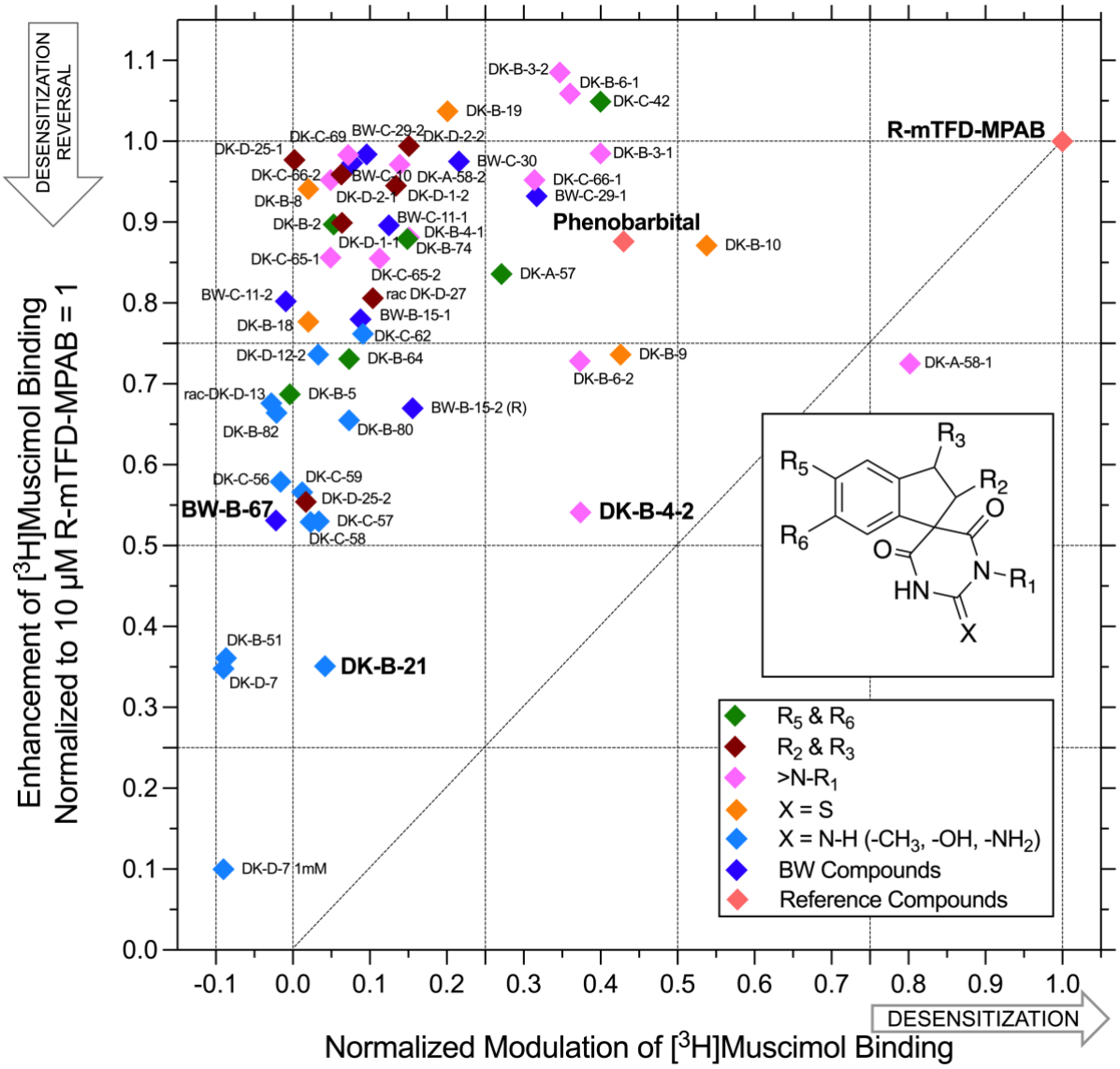


Figure 8. Two-dimensional representation of the properties of all synthesized and characterized spiro-barbiturates. Data points are color-coded to indicate different regions of structures where substituents were attached. For comparison, the data points for phenobarbital and R–mTFD-MPAB have been added.

general, NAL efficacy could be enhanced by polar substituents in the pyrimidine ring, whereas substitutions in the aromatic ring, especially larger ones were detrimental.

The actions of different analogs on agonist binding and on anesthetic–enhanced desensitization are listed in Tables 1-6. However, it is easier to comprehend the range of actions by considering the efficacies of these two actions for each ligand when it is plotted in two-dimensional space (**Fig. 8**). In this plot, (x,y: 1,1) represents a perfect general anesthetic, (0,0) a perfect NAL and (0,1) no activity. Differences of less than 0.1 should not be given too much weight. The position of substituents in the five-membered ring backbone are color-coded in the panels at the bottom right.

Consistent with our aims, all the agents synthesized lie above and to the left of the line of identity. A large group of compounds in the top left quartile are of no interest. These include most but not all of the agents with substituents in the aromatic and five-membered ring. The lack of activity for these compounds may stem from either weak interaction with their binding site, or the presence of two opposing effects. There are several compounds that display strong [^{3}H]muscimol binding enhancement and weak inhibitory activities such as **DK-B-10** and **DK-A-58-1**, typical of non-spiro barbiturates.

The data points located closest to the origin of the 2D-plot represent the best NAL agents, and they all have modifications in the pyrimidine ring. These includes **DK-B-21**, **DK-B-51** (6-methyl, imino-analog) and **DK-D-7** (*N*-methylated at the imino group). Above these, we found a sizable cluster of compounds with properties similar to that of **BW-B-67**, including **DK-C-56**, **DK-C-57** and **DK-C-58** (6-fluoro, 6-bromo- and 6-chloro-substituted analogs, respectively, with an imine at the 2-position), **DK-C-59** (5-bromo, imino analog), and **DK-D-25-2** (3-hydroxy, imino analog).

There are several compounds displaying combination of modest PAM and weak NAL activities such as **DK-C-66-1**, **BW-B-29-1**, and **DK-A-57**. They lie in a similar region of the graph to phenobarbital.

Phenobarbital, the most widely used antiepileptic drug [13], itself displayed rather significant potentiation of [^{3}H]muscimol binding combined with modest inhibitory activity, a result consistent with the well–known sedative side-effects of phenobarbital.

Finally, we found several ligands that lie close to the line of identity that display interesting dual activity having both moderate PAM and moderate NAM activities, such as **DK-B-4-2**, **DK-B-6-2** and **DK-B-9**. **DK-B-4-2** is of particular interest because, although it has a similar desensitizing efficacy as phenobarbital, it has a much greater effect on enhanced desensitization. Furthermore, its potency is an order of magnitude higher than that of phenobarbital (Table 6).

Overall, our SAR experiments indicate that the best NALs are imino derivatives, that phenyl ring and the spiro-ring substitutions have only modest effect, and are mostly detrimental to NAL activity, and that increased size of hydrophobic substituents at the phenyl ring in the oxo-series causes an emergence of the PAM activity.

Concluding Remarks. In conclusion, we report here the discovery of the first group of allosteric agents capable of reversing positive allosteric modulation of $GABA_AR$ by anesthetic drugs. It is now possible to contemplate the development of agents that are capable of reversing sedation and general anesthesia by pharmacological rather than physiological means, a goal that has been emphasized in recent reviews [17]. Our findings constitute the first step in the discovery of anesthesia reversal agents and possibly also new antiepileptic drugs without sedative side-effects. Importantly, we show that fine-tuning the structure of the compounds can provide a broad spectrum of compounds endowed with varying extents of sedative and inhibitory properties. Finally, this study did not determine the molecular mechanisms underlying these effects. They will require more detailed studies of a biophysical and structural nature. Such studies are currently being pursued.

EXPERIMENTAL SECTION

Materials and Methods

Starting materials and anhydrous grade solvents were from Aldrich and were not further dried or purified. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance spectrometer at 400 MHz and 100 MHz, respectively. NMR chemicals shifts were referenced indirectly to TMS for ^{1}H and ^{13}C NMR. HRMS experiments were performed with Q-TOF-2TM (Micromass) and Shimadzu Nexera UHPLC tandem with a TOF Bruker Impact II in the Institute of Tuberculosis Research at UIC College of Pharmacy. TLC was performed with Merck 60 F254 silica gel plates. All reactions were monitored by analytical thin-layer chromatography on silica gel 60 F254 glass plates and visualized by UV light at 254 nm. TLC plates were stained with aqueous solutions of phosphomolybdic acid and potassium permanganate. Compounds were purified by normal phase column chromatography on silica gel and/or preparative reverse phase HPLC, whereas enantiomers were separated by chiral HPLC using preparative chiral HPLC on Chiralpack IC, Chiralpack IA, or Regis IA columns.

Biochemical methods

Human full length α1β3γ2 GABA$_A$Rs, modified with an N-terminal Flag tag on the α1 subunit and a C-terminal 1D4 tag on the γ2 subunit, and human full length N-Flag–α1β3 GABA$_A$Rs were expressed in HEK 293–TetR cells as described in Dostalova et al [1, 2].

[^{3}H]Muscimol binding assays were performed as previously described [2]. Briefly, aliquots of HEK cell membranes (75 μg of membrane protein per sample) were incubated with 2 nM final concentration of [^{3}H]muscimol ((Perkin Elmer, Cat. # NET574250UC and specific activity 26.3 Ci/mmole) and appropriate ligands from stock solutions in DMSO and brought up to 2 mL with assay buffer (10 mM phosphate buffer, 200 mM KCl, and 1mM EDTA; pH 7.4). After incubation for 10 minutes, 500 μL aliquots were filtered on GF/B glass fiber filters (Whatman, Cat. #1821-025) pretreated for ≥ 1 h in 0.5% w/v poly(ethyleneimine). Filters were immediately washed twice under vacuum with 5 mL of

cold assay buffer and dried under a lamp for 60-80 minutes. They were added to Liquiscint (Atlanta, GA Cat. # LS-121) and counted in a Beckman LS-6500 liquid scintillation counter. Controls aliquots in the presence of 1 mM GABA were used to correct for nondisplaceable [^{3}H]muscimol binding. Corrected counts were normalized in either of two ways. First, they were normalized to the value in the absence of added anesthetic to give the percent enhancement of [^{3}H]muscimol. Second, they were double normalized between the control (Control cpm) and the value in the presence of 10 μM of either R–mTFD-MPAB or etomidate (Enhanced cpm)Double normalized value = (Sample cpm – Control cpm)/(Enhanced cpm – Control cpm).

Analysis. Survey experiments were determined in at least quadruplicate and compared to the expected result using the unpaired t-test. The expected result was zero for enhancement of [^{3}H]muscimol binding experiments or one for inhibition of anesthetic–induced enhanced [^{3}H]muscimol binding experiments. Concentration-response curves were fitted by nonlinear least squares to the Hill equation.

Materials: Dulbecco's Modified Eagle Medium containing nutrient mixture F-12 was from Gibco (Cat #, 11320-033). The other materials were from Fisher Scientific.

Computational methods

Statistical analysis and representation of the biochemical assays data were performed by Keith W. Miller, Xiaojuan Zhou and Dimosthenis Koinas on GraphPad Prism v9.2 and Igor v6.0.

Synthesis of five-membered spiro barbiturates

Synthesis of most five-membered spiro-barbiturates was accomplished as presented in Scheme 2.

Substituted indanones. Into a solution of the corresponding para-substituted-phenyl propionic acid (10 mmol) in anhydrous dichloromethane (20-40 mL) were added a drop of anhydrous DMF and thionyl chloride (12 mmol) dropwise at 0°C. The reaction was stirred at room temperature for 1-3 h. Then, the volatiles removed under reduced pressure and the flask was placed under high vacuum for

1-2 h. The corresponding acyl chloride dissolved in anhydrous dichloromethane (20 mL) and added to a suspension of aluminum chloride (11 mmol) in anhydrous dichloromethane (40 mL). The reaction was monitored by TLC every 15 min. The average reaction time was 1.5 h. The solution was transferred into a separation funnel charged with ice and 3N hydrochloride (200 mL) and shaken for 2 min. Ethyl acetate was added (200 mL) and layers were separated. The organic layer was washed with 3N hydrochloride (100 mL×1), saturated sodium bicarbonate solution (100 mL×3) and brine (100 mL×3). The organic layer was dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford the corresponding indanone as white crystalline solid (80%).

Reduction of indanones. To a solution of the corresponding indanone (8 mmol) in THF:H_2O (40 mL, 1:1) was added sodium borohydride (24 mmol) in portions while the reactions was stirred vigorously. The reaction was monitored by TLC every 15 min until the completion. The average reaction time was 1.5 h. The reaction was extracted with ethyl acetate (200 mL), washed with saturated sodium bicarbonate solution (100 mL×3) and brine (50 mL×2). The organic layer dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford the corresponding indanone as white crystalline solid or colorless oil (90%).

Substituted 2,3-dihydro-1H-indene-1-carbonitriles. To a solution of the corresponding indanol (7 mmol) in anhydrous dichloromethane (20 mL) was added dropwise thionyl chloride (8.4 mmol) at 0°C. The reaction was stirred at room temperature for 1-3 h. Then, the solvents were removed under reduced pressure and the residue was placed under high vacuum for 1-2 h. To a suspension of dry sodium cyanide (21 mmol) in anhydrous DMF (30 mL) was added a solution of the above chloride in anhydrous DMF (10 mL). The reaction was stirred at 70°C overnight. After cooling to room temperature, the reaction was quenched with saturated sodium bicarbonate. The aqueous layer was

extracted with EtOAc (50 mL×3), washed with saturated sodium bicarbonate solution (100 mL×3) and brine (50 mL×2). The organic layer was dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford the corresponding indene-carbonitrile as pale orange/yellow solid, oil or colorless oil (60%).

Substituted 2,3-dihydro-1H-indene-1-carboxylic acids. To a suspension of the corresponding indene-carbonitrile (5 mmol) in water (2 mL) was added concentrated HCl (6 mL, 12N). The mixture was stirred at 90°C for 3 h. After cooling to room temperature, the reaction was extracted with ethyl acetate (100 mL) and washed with hydrochloric acid 3N (2×100 mL). The organic phase was dried with anhydrous sodium sulfate and condensed under vacuum. The crude product was purified by flash chromatography on silica gel using hexane and ethyl acetate (Hex/EtOAc, 9:1 to 7:3) to afford the corresponding indene-carboxylic acid as a white solid (60-80%).

Substituted ethyl 2,3-dihydro-1H-indene-1-carboxylates. To a solution of the corresponding indene-carboxylic acid (4 mmol) in anhydrous ethanol (15 mL) was added dropwise thionyl chloride (4.8 mmol) at 0°C. Then, the reaction was stirred at 70°C for 3 h. After cooling to room temperature, the reaction was extracted with ethyl acetate (60 mL), washed with saturated sodium bicarbonate solution (60 mL×3) and brine (30 mL×2). The organic layer was dried with anhydrous sodium sulfate, concentrated under vacuum and the crude product was purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford the corresponding indene-carboxylate as a transparent oil or white waxy solid (100%).

Substituted diethyl 2,3-dihydro-1H-indene-1,1-dicarboxylates. To a solution of the corresponding indene-carboxylate (4 mmol) in anhydrous THF (10 mL) was added lithium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 4.1 mmol) dropwise at -78 °C. The reaction mixture was stirred at -78°C for 0.5 h. Ethyl chloroformate (4.8 mmol) was added at -78 °C. The reaction was stirred at -78°C for 0.5 h. Then, the reaction was warmed to room temperature and stirred for 3 h. The reaction was

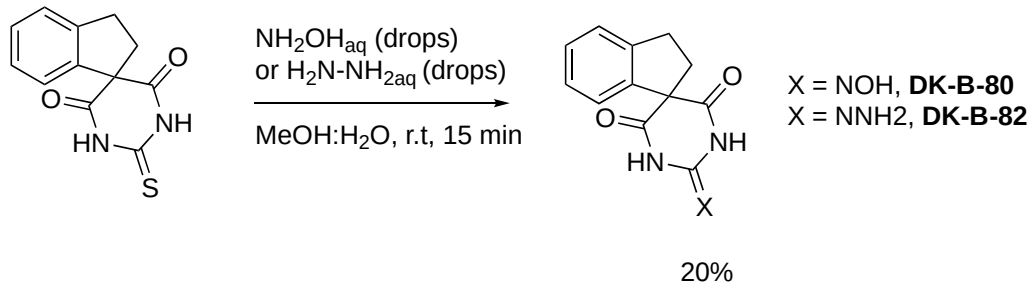
quenched with saturated ammonium chloride. The aqueous layer was extracted with ethyl acetate (30 mL×3), washed with saturated sodium bicarbonate solution (60 mL×3) and brine (30 mL×2). The organic layer dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (Hex/EtOAc, 95:5 to 90:10) to afford the corresponding indene-dicarboxylate as a transparent oil or white solid (76-84%).

Substituted 2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-triones. To a suspension of the corresponding malonate ester (0.5 mmol) and urea or methylurea or thiourea (5 mmol) in anhydrous ethanol (4 mL) was added freshly made sodium ethoxide solution (21% wt. in anhydrous ethanol, 5.5 mmol). The reaction mixture was refluxed for 5-6 h. After cooling to room temperature, the reaction was quenched with 1N hydrochloric acid (30 mL). The aqueous layer was extracted with ethyl acetate (15 mL×3), washed with 1N hydrochloric acid (20 mL×3). The organic layer dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (Hex/EtOAc, 9:1 to 3:7) to afford the corresponding barbituric acid as white solid (60%).

Substituted 2'-imino-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-diones. To a suspension of the corresponding diethyl malonate (0.5 mmol) and guanidine hydrochloride (5 mmol) in anhydrous ethanol (4 mL) was added freshly made sodium ethoxide solution (21% wt. in anhydrous ethanol, 15 mmol). The reaction mixture was refluxed for 4-5 h. After cooling to room temperature, 3N HCl in methanol (3 mL) were added to the reaction and the volatiles were removed under vacuum. The solids were resuspended in di water (20 mL), sonicated for 3 min following filtration and the wash, twice with deionized water (20 mL). The solids resuspended in a mixture of DCM:EtOAc:hexane (1:1:2), sonicated for 3 min following the removal of the supernatant, the process repeated twice to afford the hydrochloric salt of the imine analog as a white solid.

2'-(Methylimino)-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione hydrochloride (**DK-D-7**). To a suspension of the malonate **DK-B-16** (0.5 mmol) and *N*-methylguanidine hydrochloride (5 mmol) in anhydrous ethanol (4 mL) was added freshly made sodium ethoxide solution (21% wt. in anhydrous ethanol, 15 mmol). The reaction mixture was refluxed for 4 h. After cooling to room temperature, 3N HCl in methanol (3 mL) were added to the reaction and the volatiles were removed under vacuum. The solids were resuspended in di water (20 mL), sonicated for 3 min following filtration and they washed, twice, with di water (20 mL). The solids resuspended in a mixture of dichloromethane : ethyl acetate : hexane (2:1:4), sonicated for 3 min following the removal of the supernatant; the process was repeated twice to afford the hydrochloric salt of **DK-D-7** as a white solid (50%).

Certain spiro-barbiturates could not be synthesized using the general Scheme 2. These syntheses are delineated separately in the schemes and descriptions below.

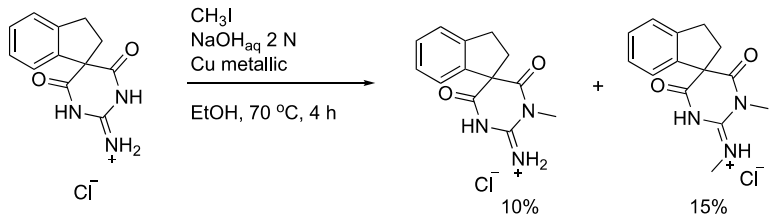


Scheme 3. Synthesis of **DK-B-80** and **DK-B-82**.

2'-(Hydroxyimino)-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione (DK-B-80). To a solution of **DK-B-18** (0.2 mmol) in methanol and water (1:1, 1 ml) was added several drops of hydroxylamine (50 wt. % in H_2O). The reaction stirred at room temperature for 10-15 min. To the reaction was quenched with 3N HCl in methanol (3 ml) and the volatiles were removed under vacuo. The solids were resuspended in di water (4 ml), sonicated for 3 min following filtration and the wash, twice, with di water (4 ml). The solids resuspended in a mixture of DCM:EtOAc:Hex (1:1:4),

sonicated for 3 min following the removal of the supernatant, the process repeated twice to afford the hydrochloric salts of **DK-B-80** as a white solid (20%).

2'-Hydrazineylidene-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione HCl (DK-B-82). Synthesis of **DK-B-82** was performed analogously to **DK-B-80**, except hydrazine (35 wt. % in H_2O was used).

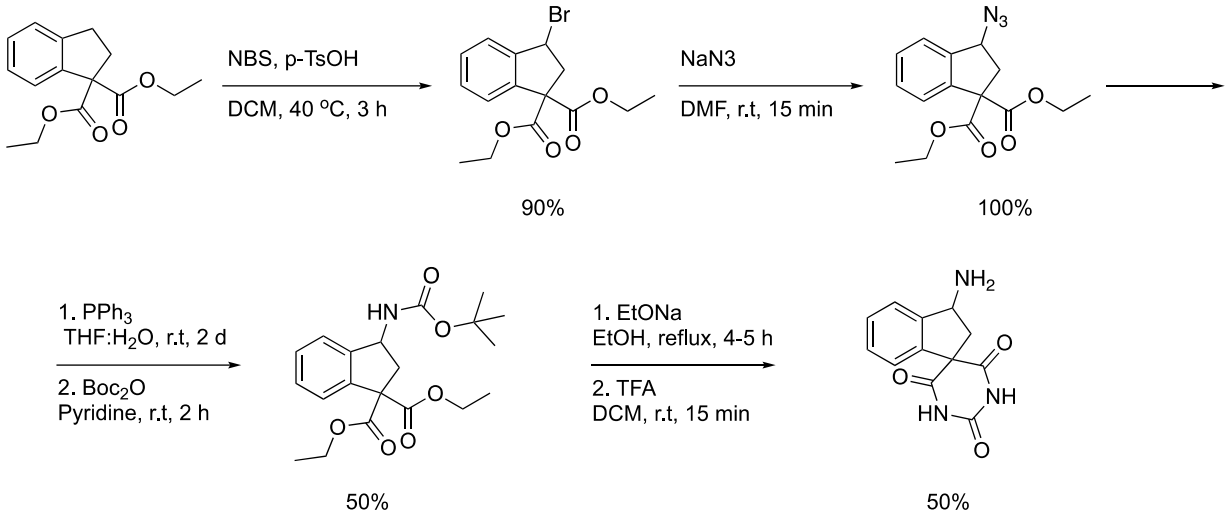


Scheme 4. Synthesis of **DK-D-12** and **DK-D-13**.

2'-Imino-1'-methyl-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione hydrochloride (**DK-D-12**). To a solution of **DK-B-21** (40 mg) in ethanol (4 mL) and 2N aqueous NaOH (1 mL) were added metallic copper (3.77 mg, 0.4 eq) and iodomethane (100 eq). The reaction mixture was heated at 70°C for 3h. After cooling to room temperature, the reaction filtered with PTFE O.25 μm and the solids washed with ethanol twice (2x2 mL). The filtrate condensed under vacuum, redissolved in 2N HCl in methanol (2 mL) and chromatographed on a prep-TLC plate using dichloromethane and methanol (9:1). The most polar of the two close products corresponds to the desired compound, **DK-D-12**. The band collected was extracted with methanol, filtered, reacidified with 2N HCl in methanol solution and condensed under vacuum to afford **DK-D-12** as white solid (10%).

1'-Methyl-2'-(methylimino)-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-D-13). To a solution of **DK-D**-7 (40 mg) in ethanol (4 mL) and 2 N aqueous NaOH (1 mL) were added metallic copper (3.77 mg, 0.4 eq) and iodomethane (100-300 eq). The reaction mixture was heated at 70°C for 3h. After cooling to room temperature, the reaction

filtered with PTFE O.25 μm and the solids washed with ethanol twice (2x2 mL). The filtrate condensed under vacuum, redissolved in 2N HCl in methanol (2 mL) and chromatographed on a preparative TLC plate using dichloromethane and methanol (9:1). The band was collected and extracted with methanol, filtered, reacidified with 2N HCl in methanol solution and condensed under vacuum to afford **DK-D-13** as white solid (40%).



Scheme 5. Synthesis of 3-amino-substituted spiro-barbiturate **rac-DK-D-27.**

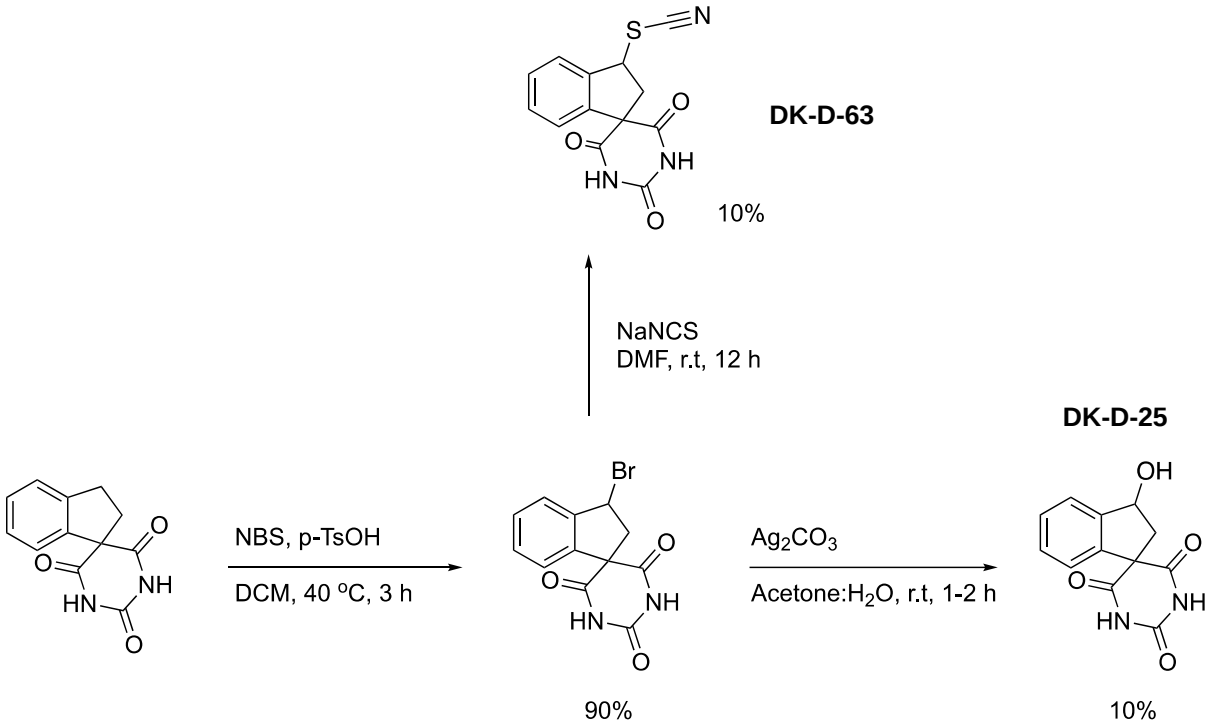
Diethyl 3-bromo-2,3-dihydro-1H-indene-1,1-dicarboxylate (**DK-D-11**). To a solution of **DK-B-16** (50 mg) in DMF (2 mL) were added N-bromosuccinimide (51 mg, 1.5 eq) and p-toluenesulfonic acid mono hydrate (36 mg, 1.5 eq). The reaction was heated at 40-44°C for 3 h (use reflux column). After cooling at room temperature, the reaction was extracted with EtOAc (20 mL) and washed with brine (3×30 mL). The organic layer dried over anhydrous sodium sulfate, filtered, concentrated in vacuum and, when needed, purified by flash chromatography on silica gel using hexane and ethyl acetate (95:5) to afford the **DK-D-11** as slightly orange oil (70-90%).

Diethyl 3-azido-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-D-20). To a solution of **DK-D-11** (100 mg, 0.4 mmol) in DMF (3 mL) were added sodium azide (75 mg, 10 eq) and the reaction was stirred at room temperature for 15 min. The reaction was extracted with EtOAc (20 mL), washed with saturated sodium bicarbonate solution (30 mL×3) and brine (30 mL×2). The organic layer dried by

anhydrous sodium sulfate, concentrated in vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford the **DK-D-20** as colorless oil (90%).

Diethyl 3-amino-2,3-dihydro-1H-indene-1,1-dicarboxylate (**DK-D-22**). To a solution of **DK-D-20** (90 mg, 0.3 mmol) in THF (2 mL) were added triphenylphosphine (194 mg, 2.5 eq) and di water (0.5 mL). The reaction left to stir at room temperature for two days. After the consumption of the starting material the volatiles and the water were removed under vacuum. The crude was dissolved in anhydrous pyridine under inert atmosphere following the addition of Boc_2O (65 mg, 1 eq). The reaction was stirred for 2 h at room temperature. After the reaction run into completion, was quenched with saturated sodium bicarbonate, and extracted with EtOAc (30 mL), washed with saturated sodium bicarbonate solution (40 mL×3) and brine (40 mL×2). The organic layer dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (20:1) to afford **DK-D-22** as colorless oil (20%).

3-Amino-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**DK-D-27**). To a suspension of the **DK-D-22** (19 mg, 0.05 mmol) and urea (30 mg, 5 eq) in anhydrous ethanol (2 mL) was added freshly made sodium ethoxide solution (21% wt. in anhydrous ethanol, 10 eq.). The reaction mixture was refluxed for 5-6 h. After cooling to room temperature, the reaction was quenched with 1N hydrochloride (5 mL). The reaction was extracted with ethyl acetate (30 mL), washed with saturated sodium bicarbonate solution (40 mL×2) and brine (30 mL×2). The organic layer was dried by anhydrous sodium sulfate and concentrated under *vacuum*. The crude residue was dissolved in dichloromethane (1 mL) and TFA (0.2 mL) was added to the solution. The reaction left to stir for 15 min at room temperature and the solvents were removed under vacuum. The product precipitated from a solution of DCM:EtOAc:hexane (3:2:4) to afford the TFA salt of **DK-D-27** as a white solid (30%).



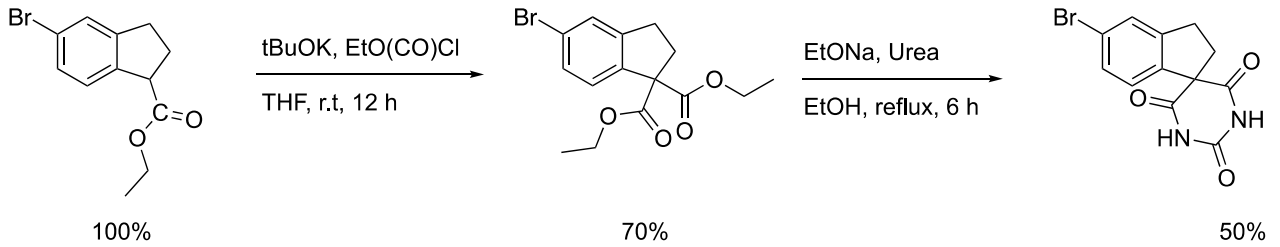
Scheme 6. Synthesis of 3-hydroxy- and 3-thiocyanato-substituted spiro-barbiturate **DK-D-25** and **DK-D-63**, respectively.

3-Bromo-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**DK-D-18**). To a suspension of **BW-B-67** (50 mg, 0.21 mmol) in dichloromethane (10 mL) were added N-bromosuccinimide (58 mg, 1.5 eq) and p-toluenesulfonic acid monohydrate (61 mg, 1.5 eq). The reaction was heated at 40-44°C for 3 h (use reflux column). After cooling at room temperature, the reaction was extracted with EtOAc (20 mL) and washed with 1N HCl aqueous solution (3×30 mL). The organic layer dried over anhydrous sodium sulfate, filtered, concentrated under vacuum and purified, when needed, by flash chromatography on silica gel using hexane and ethyl acetate (7:3) to afford the **DK-D-18** as slightly orange solid (70-90%).

Hydroxy-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**DK-D-25**). To a solution of **DK-D-18** (70 mg) in acetone (1 mL) were added water (0.05 mL) and silver carbonate (41 mg, 0.5 eq.). The reaction was stirred for 1-2 h at room temperature while monitored by TLC. The reaction was quenched with 2N HCl aqueous solution (3 mL) and extracted with EtOAc (10 mL). The organic layer was washed with 2N HCl aqueous solution (3x5 mL). The organic layer was dried by

anhydrous sodium sulfate, concentrated under vacuum, and purified by flash chromatography on silica gel using hexane - ethyl acetate (7:3 to 1:1) to afford the **DK-D-25** as white solid (10%). Separation of the enantiomers was achieved by chromatography on a Chiralpack IA semipreparative chiral column using hexane - ethanol (7:3), with elution times at 17.14 min for **DK-D-25-1** and 22.50 min for **DK-D-25-2**.

3-Thiocyanato-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**DK-D-63**). To a solution of **DK-D-18** (100 mg, 0.32 mmol) in DMF (2 mL) was added sodium thiocyanate (263 mg, 10 eq) and the reaction was stirred overnight at room temperature. The reaction was quenched with 2N HCl aqueous solution (3 mL) and extracted with EtOAc (10 mL). The organic layer was washed with 2N HCl aqueous solution (3x5 mL). The organic layer dried by anhydrous sodium sulfate, concentrated under vacuum and purified by flash chromatography on silica gel using hexane and ethyl acetate (7:3 to 1:1) to afford the **DK-D-63** as white solid (10%). Separation of the enantiomers was achieved by chromatography on Chiralpack IA semipreparative chiral column using hexane and ethanol (7:3), with elution times of 28.38 min for **DK-D-63-1** and 32.00 min for **DK-D-63-2**.



Scheme 7. Synthesis of 5-bromo-substituted spiro-barbiturate **DK-C-42.**

Diethyl 5-bromo-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-C-39). To a solution of DK-C-22 (3 mmol) in anhydrous THF was added tBuOK (2 eq). The reaction mixture was stirred at room temperature for 0.5 h. Ethyl chloroformate (3 eq) was added and the reaction was stirred for 1 h. The reaction was quenched with saturated solution of sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (30 mL×3), washed with saturated sodium bicarbonate solution (60 mL×3) and brine

(30 mL×2). The organic layer dried by anhydrous sodium sulfate, concentrated in vacuo and purified by flash chromatography on silica gel using hexane and ethyl acetate (Hex/EtOAc, 95:5 to 90:10) to afford the corresponding indene-dicarboxylate as a pale orange-brown oil in (30-50%).

Chiral separations

Purified racemic mixtures of the corresponding compounds dissolved in 2 ml of mobile phase and injected into an HPLC system equipped with the appropriate preparative of semipreparative chiral column. The column, composition of the mobile phase, flow rate and elution times of enantiomers are listed in Table 8. The enantiomers named based on their elution order as -1 and -2.

Table 8. Elution times of spiro-barbiturate enantiomers**.**

CHIRAL SEPARATIONS				
Compound	Column	Solvent System	RT* fast isomer	RT* slow isomer
BW-B-15-1/2	Chiralpack IC	Hex:EtOH (7:3) 10 mL/min	18.3	20.8
BW-C-11-1/2	Chiralpack IC	Hex:EtOH (7:3) 10 mL/min	21.69	37.9
BW-C-29-1/2	Chiralpack IC	Hex:EtOH (7:3) 10 mL/min	17.4	27.0
DK-A-58-1/2	Regis IA	Hex:iPrOH (95:5) 10 mL/min	23.5	24.63
DK-B-3-1/2	Chiralpack IC	Hex:iPrOH (8:2) 10 mL/min	15.0	21.2
DK-B-4-1/2	Chiralpack IC	Hex:iPrOH (8:2) 10 mL/min	21	23
DK-B-6-1/2	Chiralpack IC	Hex:iPrOH (7:3), 10 mL/min	20	55
DK-C-65-1/2	Chiralpack IC	Hex:iPrOH (8:2) 15 mL/min	17.2	18.7
DK-C-66-1/2	Chiralpack IC	Hex:iPrOH (8:2) 15 mL/min	15.2	18.65
DK-D-1-1/2	Chiralpack IC	Hex:iPrOH (8:2) 15 mL/min	14.56	16.30
DK-D-2-1/2	Regis IA	Hex:iPrOH (8:2) 15 mL/min	11.95	12.52
DK-D-25-1/2	Chiralpack IA	Hex:EtOH (7:3) 2.5 mL/min	28.38	32.00

*****Elution time in minutes

NMR and HR-MS Data

2,3-Dihydro-1H-inden-1-one (DK-B-11): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.7 Hz, 1H), 7.63 – 7.55 (m, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 3.18 – 3.09 (m, 2H), 2.73 – 2.64 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.19, 155.29, 137.24, 134.72, 127.42, 126.83, 123.86, 36.35, 25.94.

2,3-Dihydro-1H-inden-1-ol (DK-B-12): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (t, *J* = 7.1 Hz, 1H), 7.34 – 7.19 (m, 3H), 5.20 (t, *J* = 5.8 Hz, 1H), 3.04 (ddd, *J* = 15.9, 8.6, 4.7 Hz, 1H), 2.86 – 2.74 (m, 1H), 2.59 (s, 1H), 2.45 (dddd, *J* = 13.1, 8.3, 6.9, 4.8 Hz, 1H), 1.92 (dddd, *J* = 13.3, 8.6, 6.7, 5.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.06, 143.32, 128.25, 126.68, 124.87, 124.29, 76.28, 35.82, 29.81.

2,3-Dihydro-1H-indene-1-carbonitrile (DK-B-13): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.39 (m, 1H), 7.32 – 7.23 (m, 3H), 4.10 (t, *J* = 8.2 Hz, 1H), 3.09 (ddd, *J* = 15.9, 8.7, 4.3 Hz, 1H), 2.96 (dt, *J* =

16.0, 8.1 Hz, 1H), 2.57 (dtd, *J* = 12.4, 8.1, 4.3 Hz, 1H), 2.36 (dq, *J* = 12.8, 8.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.92, 137.59, 128.52, 127.23, 124.99, 124.27, 121.13, 34.48, 31.42, 31.18.

2,3-Dihydro-1H-indene-1-carboxylic acid (DK-B-14); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.32 (s, 1H), 7.47 (d, *J* = 7.0 Hz, 1H), 7.32 – 7.18 (m, 3H), 4.11 (dd, *J* = 8.3, 6.2 Hz, 1H), 3.21 – 3.08 (m, 1H), 3.03 – 2.88 (m, 1H), 2.54 – 2.43 (m, 1H), 2.38 (dtd, *J* = 13.1, 8.6, 6.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.79, 144.28, 140.14, 127.89, 126.66, 125.09, 124.83, 77.48, 77.16, 76.84, 50.14, 31.83, 28.75.

Ethyl 2,3-dihydro-1H-indene-1-carboxylate (DK-B-15); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.6, 6.3 Hz, 1H), 7.31 – 7.13 (m, 3H), 4.27 – 4.13 (m, 2H), 4.10 – 3.99 (m, 1H), 3.19 – 3.06 (m, 1H), 3.02 – 2.82 (m, 1H), 2.55 – 2.39 (m, 1H), 2.39 – 2.24 (m, 1H), 1.33 – 1.24 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.01, 144.23, 140.97, 127.58, 126.50, 124.84, 124.77, 60.83, 50.33, 31.87, 28.76, 14.39.

Diethyl 2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-B-16); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dt, *J* = 9.2, 4.9 Hz, 1H), 7.33 – 7.19 (m, 3H), 4.28 – 4.14 (m, 4H), 3.05 (t, *J* = 7.3 Hz, 2H), 2.75 – 2.64 (m, 2H), 1.27 (td, *J* = 7.1, 2.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 144.52, 139.35, 128.65, 126.77, 126.67, 124.66, 65.83, 61.71, 34.24, 30.97, 14.15.

2,3-Dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (BW-B-67/DK-B-17); ^{1}H NMR (400 MHz, CD_3OD) δ 7.42 – 7.29 (m, 2H), 7.28 – 7.19 (m, 2H), 3.27 (t, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.73, 146.27, 143.03, 130.19, 128.16, 126.57, 123.24, 33.73, 33.13. Chemical Formula: $C_{12}H_{10}N_2O_3$ Exact Mass: 230.0691, Found M- 229.0620.

1'-Methyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac-BW-B-15); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (brs, 1H), 7.46-7.26 (m, 2H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 3.51 -3.14 (m, 5H), 2.96-2.71 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.0, 150.3, 144.9, 140.8, 129.7, 127.4, 126.1, 122.1, 63.5, 33.5, 32.4, 28.6. HRMS: Calcd. for $C_{13}H_{11}N_2O_3$ $[M-H]^+$: 243.0770; Found: 243.0770.

2'-Thioxo-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione (DK-B-18); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (q, *J* = 7.5 Hz, 2H), 7.28 – 7.14 (m, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.89 – 2.77 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.79, 171.51, 146.06, 143.08, 130.29, 128.23, 126.68, 123.25, 64.78, 32.99. Chemical Formula: $C_{12}H_{10}N_2O_2S$ Exact Mass: 246.0463 Found M- 245.0390.

2'-Imino-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione HCl (DK-B-21); ^{1}H NMR (400 MHz, D_2O+NaOH) δ 7.39 (d, *J* = 7.1 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.26 – 7.18 (m, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 3.27 – 3.19 (m, 2H), 2.61 – 2.51 (m, 2H); **^{13}C-NMR**: ^{13}C NMR (101 MHz, CH_3OH+D_2O) δ 189.43, 146.32, 144.44, 128.75, 127.69, 125.72, 123.38, 62.41, 49.50, 37.44, 32.56.; TOF-MS $C_{12}H_{11}N_3O_2$: requires MH+, m/z 230.0885, found: MH-, m/z 228.0703. Chemical Formula: $C_{12}H_{11}N_3O_2$ Exact Mass: 229.0851 Found MH+ 229.0620.

1'-Ethyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac-DK-C-65); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.30 (q, *J* = 7.9 Hz, 2H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 3.94 (ddt, *J* = 20.6, 13.3, 6.7 Hz, 2H), 3.30 (t, *J* = 7.7 Hz, 2H), 2.84 (t, *J* = 7.4 Hz, 2H), 1.19 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.58, 170.15, 150.08, 144.78, 140.78, 129.52, 127.21, 125.97, 121.78, 77.36, 77.05, 76.73, 63.41, 37.15, 32.87, 32.29, 13.13. Chemical Formula: $C_{14}H_{14}N_2O_3$ Exact Mass: 258.1004 Found M- 257.0933.

1'-Propyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac-DK-C-66); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 12.6, 5.3 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 7.7 Hz, 1H), 3.83 (dddd, *J* = 28.2, 21.9, 11.0, 6.1 Hz, 2H), 3.29 (q, *J* = 6.7 Hz, 2H), 2.84 (q, *J* = 6.3 Hz, 2H), 1.60 (p, *J* = 7.7 Hz, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 170.31, 150.41, 144.81, 140.81, 129.50, 127.17, 125.95, 121.84, 77.38, 77.07, 76.75, 63.43, 43.37, 32.98, 32.30, 21.25, 11.15. Chemical Formula: $C_{15}H_{16}N_2O_3$ Exact Mass: 272.1161 Found M- 271.1095.

1',3'-Dimethyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (DK-C-69); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.25 (m, 2H), 7.16 (td, *J* = 7.1, 1.3 Hz, 1H), 6.96 (d, *J* = 7.7 Hz, 1H), 3.35 (s, 6H), 3.31 (t, *J* = 7.4 Hz, 2H), 2.86 (dd, *J* = 8.1, 6.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48, 144.72, 141.24, 129.42, 127.17, 125.96, 121.70, 77.36, 77.04, 76.72, 63.47, 33.52, 32.33, 29.15. Chemical Formula: $C_{14}H_{14}N_2O_3$ Exact Mass: 258.1004 Found M- 259.1095.

2'-(Methylimino)-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione hydrochloride (DK-D-7); ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (dt, *J* = 15.8, 8.2 Hz, 2H), 7.30 (s, 1H), 7.26 (t, *J* = 7.3 Hz, 1H), 3.27 (t, *J* = 7.5 Hz, 2H), 3.21 (s, 3H), 2.87 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.17, 169.39, 153.11, 144.91, 140.25, 129.29, 127.02, 125.44, 122.24, 64.19, 48.24, 48.03, 47.88, 47.82, 47.60, 47.39, 47.18, 46.97, 32.68, 31.38, 28.36. Chemical Formula: $C_{13}H_{13}N_3O_2$ Exact Mass: 243.1008 Found M- 242.0938.

2'-Imino-1'-methyl-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-D-12); ^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (d, *J* = 8.2 Hz, 2H), 7.32 – 7.23 (m, 3H), 3.46 (s, 4H), 3.27 (td, *J* = 7.4, 2.2 Hz, 3H), 2.88 (td, *J* = 7.3, 2.2 Hz, 3H), . ^{13}C NMR (101 MHz, CD_3OD) δ 169.32, 168.82, 155.02, 144.93, 139.98, 129.41, 127.06, 125.52, 122.27, 64.16, 48.24, 48.03, 47.81, 47.60, 47.39, 47.17, 46.96, 33.45, 31.28, 28.71. Chemical Formula: $C_{13}H_{13}N_3O_2$ Exact Mass: 243.1008 Found MH+ 244.1082.

1'-Methyl-2'-(methylimino)-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-D-13); ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 – 7.34 (m, 2H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 3.47 (d, *J* = 1.5 Hz, 3H), 3.28 (d, *J* = 9.2 Hz, 5H), 2.90 (ddd, *J* = 10.5, 5.8, 2.5 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 169.10, 144.90, 140.11, 129.33, 127.06, 125.41, 122.61, 122.38, 64.09, 48.25, 48.03, 47.88, 47.82, 47.67, 47.61, 47.46, 47.40, 47.18, 46.97, 33.89, 31.31, 29.46, 28.70. Chemical Formula: $C_{14}H_{15}N_3O_2$ Exact Mass: 257.1164 Found MH+ 258.1243.

2'-(Hydroxyimino)-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione (DK-B-33/DK-B-80); ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 – 7.25 (m, 2H), 7.21 (t, *J* = 7.1 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 3.23 (t, *J* = 7.5 Hz, 2H), 2.83 – 2.73 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.11, 144.67, 141.70, 128.89, 126.83, 125.28, 121.85, 63.40, 48.23, 48.02, 47.81, 47.60, 47.38, 47.17, 46.96, 31.60, 31.56. Chemical Formula: $C_{12}H_{11}N_3O_3$ Exact Mass: 245.0800. Found MH+ 246.0872.

2'-Hydrazineylidene-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione HCl (DK-B-82); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (d, *J* = 7.5 Hz, 1H), 7.33 – 7.25 (m, 1H), 7.21 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 3.23 (t, *J* = 7.4 Hz, 2H), 2.51 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.49, 145.58, 140.15, 128.38, 126.82, 124.76, 122.47, 69.87, 48.24, 48.03, 47.82, 47.73, 47.61, 47.51, 47.48, 47.39, 47.27, 47.24, 47.18, 46.97, 32.83, 31.41. Chemical Formula: $C_{12}H_{12}N_4O_2$. Exact Mass: 244.0960. Not stable in the MS.

6-Methyl-2,3-dihydro-1H-inden-1-one (DK-A-71); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.36 (dd, *J* = 18.1, 7.7 Hz, 2H), 3.12 – 3.00 (m, 2H), 2.70 – 2.60 (m, 2H), 2.38 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.25, 152.61, 137.28, 137.23, 135.92, 126.41, 123.67, 36.62, 25.48, 21.11.

6-Methyl-2,3-dihydro-1H-inden-1-ol (DK-A-82); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.15 (d, *J* = 7.4 Hz, 1H), 7.09 (d, *J* = 7.4 Hz, 1H), 5.19 (t, *J* = 5.7 Hz, 1H), 3.08 – 2.95 (m, 1H), 2.83 – 2.70 (m, 1H), 2.56 – 2.40 (m, 1H), 2.37 (s, 3H), 2.17 (s, 1H), 1.94 (td, *J* = 13.4, 7.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.23, 140.35, 136.41, 129.23, 124.83, 124.67, 76.42, 36.20, 29.79, 29.44, 21.32.

6-Methyl-2,3-dihydro-1H-indene-1-carbonitrile (DK-A-87); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 4.05 (t, *J* = 8.1 Hz, 1H), 3.08 – 2.99 (m, 1H), 2.91 (dt, *J* = 15.9, 8.1 Hz, 1H), 2.61 – 2.50 (m, 1H), 2.38 (s, 3H), 2.37 – 2.28 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.75, 137.65, 136.91, 129.48, 129.22, 128.93, 124.93, 124.66, 124.56, 124.24, 121.15, 34.25, 31.26, 31.09, 30.89, 29.61, 21.04.

6-Methyl-2,3-dihydro-1H-indene-1-carboxylic acid (DK-A-90); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.85 (s, 1H), 7.30 (s, 1H), 7.19 (d, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 4.14 – 4.03 (m, 1H), 3.13 (dt, *J* = 12.3, 6.0 Hz, 1H), 3.07 – 2.77 (m, 1H), 2.59 – 2.45 (m, 1H), 2.44 – 2.31 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.99, 141.23, 140.29, 136.33, 128.76, 125.61, 124.52, 50.08, 31.43, 29.00, 21.33.

Ethyl 6-methyl-2,3-dihydro-1H-indene-1-carboxylate (DK-A-92); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 7.7 Hz, 1H), 4.27 – 4.16 (m, 2H), 4.05 – 3.98 (m, 1H), 3.13 – 3.01 (m, 1H), 2.88 (dt, *J* = 15.5, 7.6 Hz, 1H), 2.50 – 2.40 (m, 1H), 2.40 – 2.27 (m, 3H), 1.31 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.15, 141.19, 141.12, 136.11, 128.51, 128.43, 125.38, 124.44, 60.79, 50.26, 31.45, 29.09, 21.36, 14.39.

Diethyl 6-methyl-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-A-94); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.10 (q, *J* = 7.7 Hz, 2H), 4.26 – 4.15 (m, 4H), 2.98 (t, *J* = 7.2 Hz, 2H), 2.69 (t, *J* = 7.2 Hz, 2H), 2.35 (d, *J* = 9.4 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.94, 141.54, 139.40, 136.28, 129.65, 127.16, 124.34, 65.73, 61.67, 34.57, 30.57, 21.50, 14.18.

6-Methyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-B-1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (d, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.01 (s, 1H), 3.21 (t, *J* = 7.4 Hz, 2H), 2.80 (t, *J* = 7.4 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.82, 143.20, 138.19, 131.03, 126.28, 123.55, 64.31, 33.91, 32.72, 21.19; Chemical Formula: $C_{13}H_{12}N_2O_3$ Exact Mass: 244.0848 Found M- 243.0772.

1',6-Dimethyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (rac-DK-B-4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 7.7 Hz, 1H), 6.84 (s, 1H), 3.31 (d, *J* = 9.3 Hz, 3H), 3.23 (td, *J* = 15.4, 7.4 Hz, 2H), 2.89 – 2.76 (m, 2H), 2.29 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.36, 170.51, 150.66, 141.85, 141.00, 137.23, 130.59, 125.69, 122.48, 63.33, 33.82, 32.00, 28.55, 21.31; Chemical Formula: $C_{14}H_{14}N_2O_3$ Exact Mass: 258.1004, Found M- 257.0931.

6-Methyl-2'-thioxo-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione (DK-B-8); ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 6.97 (s, 1H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.81 (t, *J* = 7.5 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.82, 171.62, 143.30, 143.02, 138.28, 131.12, 126.39, 123.58, 64.69, 33.21, 32.74, 32.60, 23.69, 21.24, 14.41; Chemical Formula: $C_{13}H_{12}N_2O_2S$ Exact Mass: 260.0619, Found M- 259.0549.

2'-Imino-6-methyl-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-B-51); ^{1}H NMR (400 MHz, D_2O) δ 7.04 (d, *J* = 7.7 Hz, 1H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.58 (s, 1H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.35 (t, *J* = 7.6 Hz, 2H), 2.06 (s, 3H), 1.15 – 1.06 (m, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 187.02, 179.16, 158.67, 145.37, 141.83, 135.69, 127.96, 124.16, 122.38, 58.12, 48.24, 48.02, 47.81, 47.60, 47.39, 47.17, 46.96, 33.99, 31.89, 29.36, 22.87, 19.85. Chemical Formula: $C_{13}H_{13}N_3O_2$ Exact Mass: 243.1008.

6-Isopropyl-2,3-dihydro-1H-inden-1-one (DK-A-48a); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 1.1 Hz, 1H), 7.48 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.40 (dd, *J* = 7.9, 0.6 Hz, 1H), 3.13 – 3.07 (m, 2H), 2.72 – 2.67 (m, 2H), 1.27 (d, *J* = 6.9 Hz, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.50, 153.14, 148.60, 137.42, 133.92, 126.64, 121.07, 36.78, 34.02, 25.60, 24.12.

6-Isopropyl-2,3-dihydro-1H-inden-1-ol (DK-A-48b); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 7.16 (dt, *J* = 7.8, 4.6 Hz, 2H), 5.26 – 5.21 (m, 1H), 3.02 (ddd, *J* = 15.8, 8.5, 4.7 Hz, 1H), 2.93 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.83 – 2.74 (m, 1H), 2.50 (dddd, *J* = 13.1, 8.2, 6.9, 4.8 Hz, 1H), 1.95 (dddd, *J* = 13.7, 8.5, 6.6, 5.3 Hz, 1H), 1.74 (s, 1H), 1.26 (dd, *J* = 6.8, 3.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.90, 145.26, 140.88, 126.93, 124.85, 122.12, 76.69, 36.41, 34.20, 29.52, 24.41, 24.32.

6-Isopropyl-2,3-dihydro-1H-indene-1-carbonitrile (DK-A-47); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.20 (dd, *J* = 16.8, 7.8 Hz, 2H), 4.09 (t, *J* = 8.2 Hz, 1H), 3.12 – 3.01 (m, 1H), 3.00 – 2.87 (m, 2H), 2.65 – 2.53 (m, 1H), 2.37 (dq, *J* = 12.8, 8.4 Hz, 1H), 1.29 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.43, 140.30, 137.72, 126.89, 124.80, 122.15, 34.49, 34.02, 31.44, 31.05, 24.22, 24.09.

6-Isopropyl-2,3-dihydro-1H-indene-1-carboxylic acid (DK-A-50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.36 (s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.16 (dd, *J* = 7.8, 1.4 Hz, 1H), 4.11 (dd, *J* = 8.3, 6.0 Hz, 1H), 3.13 (ddd, *J* = 15.2, 8.5, 6.3 Hz, 1H), 3.01 – 2.87 (m, 2H), 2.51 (ddt, *J* = 12.9, 8.7, 6.0 Hz, 1H), 2.39 (dtd, *J* = 13.0, 8.6, 6.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.98, 147.67, 141.72, 140.24, 126.19, 124.63, 123.11, 50.16, 34.14, 31.46, 29.01, 24.46, 24.20.

Ethyl 6-isopropyl-2,3-dihydro-1H-indene-1-carboxylate (DK-A-52); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.16 (d, *J* = 7.7 Hz, 1H), 4.36 – 4.28 (m, 1H), 4.24 (ddd, *J* = 14.2, 8.2, 4.5 Hz, 1H), 4.12 – 4.05 (m, 1H), 3.18 – 3.08 (m, 1H), 3.02 – 2.89 (m, 2H), 2.59 – 2.47 (m, 1H), 2.39 (dtd, *J* = 14.1, 8.5, 5.6 Hz, 1H), 1.39 – 1.30 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.90, 147.30, 141.52, 140.99, 125.84, 124.52, 124.43, 122.77, 122.66, 122.49, 60.59, 50.21, 34.04, 31.37, 28.85, 28.67, 24.24, 14.32.

Diethyl 6-isopropyl-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-A-53); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.17 (t, *J* = 4.0 Hz, 2H), 4.26 – 4.19 (m, 4H), 3.01 (t, *J* = 7.3 Hz, 2H), 2.94 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.75 – 2.69 (m, 2H), 1.27 (dt, *J* = 7.1, 3.7 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.80, 147.38, 141.88, 139.32, 126.98, 124.73, 124.30, 65.73, 61.53, 34.43, 34.09, 30.52, 24.24, 14.11.

6-Isopropyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-A-57); ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (d, *J* = 7.8 Hz, 1H), 7.20 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.04 (s, 1H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.89 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.83 – 2.75 (m, 2H), 1.22 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.84, 152.30, 149.49, 143.68, 143.11, 128.48, 126.43, 120.90, 64.40, 35.24, 33.89, 32.71, 24.49; TOF-MS $C_{15}H_{16}N_2O_3$: requires MH-, m/z 271.1127, found: MH-, m/z 271.1058. Chemical Formula: $C_{15}H_{16}N_2O_3$ Exact Mass: 272.1161 Found M- 271.1086.

6-Isopropyl-1'-methyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (rac-DK-A-58) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.28 – 7.21 (m, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 6.86 (s, 1H), 3.33 (s, 3H), 3.31 – 3.22 (m, 2H), 2.90 – 2.75 (m, 3H), 1.19 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.35, 170.23, 150.46, 148.54, 142.24, 140.91, 127.87, 125.85, 120.00, 63.44, 34.12, 33.97, 32.03, 28.58, 24.23; Chemical Formula: $C_{16}H_{18}N_2O_3$ Exact Mass: 286.1317, found: M-, m/z ---.

6-Isopropyl-2'-thioxo-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione (DK-B-10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 1H), 7.04 (s, 1H), 3.21 (t, *J* = 7.4 Hz, 2H), 2.90 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.82 (t, *J* = 7.4 Hz, 2H), 1.23 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.89, 171.58, 149.57, 143.41, 143.20, 128.64, 126.52, 120.88, 64.82, 35.19, 32.97, 32.57, 32.23, 24.46; Chemical Formula: $C_{15}H_{16}N_2O_2S$ Exact Mass: 288.0932, Found M- 287.0860.

2'-Imino-6-isopropyl-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DKB34); ^{1}H NMR (400 MHz, D_2O_salt) δ 7.12 (d, *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 7.8 Hz, 1H), 6.66 (s, 1H), 2.99 (t, *J* = 7.7 Hz, 2H), 2.71 – 2.63 (m, 1H), 2.38 (t, *J* = 7.6 Hz, 2H), 1.00 – 0.92 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 188.84, 170.30, 149.49, 143.68, 143.11, 128.48, 126.43, 120.90, 63.45, 35.24, 33.89, 32.71, 24.49; Chemical Formula: $C_{15}H_{17}N_3O_2$ Exact Mass: 271.1321 Found MH+ 272.1406

6-Bromo-2,3-dihydro-1H-inden-1-one (DK-A-77); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 1.6 Hz, 1H), 7.68 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 3.14 – 3.03 (m, 2H), 2.76 – 2.66 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.38, 153.58, 138.94, 137.35, 128.25, 126.76, 121.57, 36.50, 25.51.

6-Bromo-2,3-dihydro-1H-inden-1-ol (DK-A-86); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.37 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 5.22 (d, *J* = 5.3 Hz, 1H), 2.99 (ddd, *J* = 16.0, 8.5, 4.5 Hz, 1H), 2.81 – 2.71 (m, 1H), 2.55 – 2.45 (m, 1H), 2.01 – 1.90 (m, 1H), 1.83 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.45, 142.28, 131.43, 127.61, 126.59, 120.45, 76.23, 36.37, 29.52.

6-Bromo-2,3-dihydro-1H-indene-1-carbonitrile (DK-A-89); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 4.07 (t, *J* = 8.2 Hz, 1H), 3.08 – 2.96 (m, 1H), 2.87 (dd, *J* = 16.1, 8.1 Hz, 1H), 2.61 – 2.50 (m, 1H), 2.40 – 2.29 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.94, 139.77, 131.57, 127.35, 126.46, 120.59, 120.39, 34.26, 31.30, 30.96.

6-Bromo-2,3-dihydro-1H-indene-1-carboxylic acid (DK-A-91); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.34 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 4.11 – 4.03 (m, 1H), 3.10 – 3.01 (m, 1H), 2.93 – 2.81 (m, 1H), 2.46 (ddt, *J* = 12.7, 8.7, 6.3 Hz, 1H), 2.42 – 2.32 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.55, 143.28, 142.34, 131.00, 128.33, 126.30, 120.30, 49.91, 31.39, 28.90.

Ethyl 6-bromo-2,3-dihydro-1H-indene-1-carboxylate (DK-A-93); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 4.25 – 4.15 (m, 2H), 4.03 – 3.97 (m, 1H), 3.07 – 2.97 (m, 1H), 2.89 – 2.78 (m, 1H), 2.45 (ddt, *J* = 13.2, 8.7, 6.6 Hz, 1H), 2.38 – 2.27 (m, 1H), 1.29 (dd, *J* = 12.9, 5.8 Hz, 3H^{13}C NMR (101 MHz, $CDCl_3$) δ 173.19, 143.15, 130.54, 128.00, 126.13, 120.04, 61.01, 50.07, 31.32, 28.86, 14.31.

Diethyl 6-bromo-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-A-95); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 1.7 Hz, 1H), 7.37 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.09 (d, *J* = 8.1 Hz, 1H), 4.30 – 4.16 (m, 4H), 2.96 (t, *J* = 7.3 Hz, 2H), 2.69 (dd, *J* = 13.8, 6.8 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR

(101 MHz, $CDCl_3$) δ 170.17, 143.50, 141.44, 131.73, 129.89, 126.08, 120.20, 65.65, 61.94, 34.35, 30.51, 14.11.

6-Bromo-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-B-2); ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, *J* = 7.1 Hz, 1H), 7.34 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 3.19 (t, *J* = 7.3 Hz, 2H), 2.79 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.20, 145.86, 144.96, 133.17, 128.12, 126.84, 121.23, 64.11, 35.56, 35.38, 32.60; Chemical Formula: $C_{12}H_9BrN_2O_3$ Exact Mass: 307.9797, Found M- 306.9729 and 308.9711.

6-Bromo-1'-methyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (rac-DK-B-3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.43 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.20 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 1.3 Hz, 1H), 3.34 (s, 3H), 3.24 (dd, *J* = 14.7, 7.3 Hz, 2H), 2.87 – 2.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.75, 169.53, 149.98, 144.13, 142.79, 132.81, 127.40, 125.56, 120.75, 63.05, 34.77, 32.04, 28.73; Chemical Formula: $C_{13}H_{11}BrN_2O_3$ Exact Mass: 321.9953, Found M- 320.9876 and 322.9855.

6-Bromo-2'-thioxo-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione (DK-B-9); ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.29 (d, *J* = 9.4 Hz, 2H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.82 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.54, 170.94, 145.65, 144.91, 133.29, 128.24, 126.79, 121.30, 64.49, 34.50, 32.49; Chemical Formula: $C_{12}H_9BrN_2O_2S$ Exact Mass: 323.9568, found: M-, m/z 322.9497 and 324.9475.

6-Bromo-2'-imino-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-C-57): ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 3.15 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 186.44, 170.83, 147.38, 144.43, 130.23, 126.38, 125.09, 119.28, 62.09, 48.38, 48.17, 48.10, 48.08, 47.95, 47.86, 47.83, 47.74, 47.62, 47.53, 47.31, 47.16, 47.10, 34.54, 31.82. Chemical Formula: $C_{12}H_{10}BrN_3O_2$ Exact Mass: 306.9956 Found M- 305.9886 and 307.9866.

6-Methoxy-2,3-dihydro-1H-inden-1-one (DK-A-76); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.9 Hz, 1H), 7.21 – 7.15 (m, 2H), 3.83 (s, 3H), 3.11 – 3.04 (m, 2H), 2.72 (dd, *J* = 6.6, 4.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.20, 159.52, 148.14, 138.38, 127.51, 124.19, 105.03, 55.74, 37.14, 25.25.

6-Methoxy-2,3-dihydro-1H-inden-1-ol (DK-A-85); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 1.7 Hz, 1H), 6.82 (dd, *J* = 8.2, 2.2 Hz, 1H), 5.21 (t, *J* = 6.1 Hz, 1H), 3.81 (s, 3H), 3.03 – 2.93 (m, 1H), 2.75 (dt, *J* = 15.3, 7.5 Hz, 1H), 2.51 (ddd, *J* = 12.8, 7.5, 4.6 Hz, 1H), 1.94 (dt, *J* = 20.8, 6.7 Hz, 1H), 1.79 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.19, 146.49, 135.23, 125.66, 115.19, 108.93, 76.81, 55.64, 36.74, 29.07.

6-Methoxy-2,3-dihydro-1H-indene-1-carbonitrile (DK-A-97); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 1.9 Hz, 1H), 6.83 (dd, *J* = 8.3, 2.2 Hz, 1H), 4.07 (t, *J* = 8.1 Hz, 1H), 3.81 (s, 3H), 3.01 (ddd, *J* = 15.3, 8.7, 4.1 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.61 – 2.52 (m, 1H), 2.37 (dq, *J* = 12.8, 8.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.38, 138.89, 134.75, 125.61, 121.11, 115.13, 109.20, 55.62, 34.78, 31.72, 30.64.

6-Methoxy-2,3-dihydro-1H-indene-1-carboxylic acid (DK-A-98); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.3 Hz, 1H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.79 (dd, *J* = 8.3, 2.4 Hz, 1H), 4.07 – 4.02 (m, 1H), 3.79 (s, 3H), 3.04 (ddd, *J* = 14.9, 8.4, 6.2 Hz, 1H), 2.86 (ddd, *J* = 15.2, 8.5, 6.4 Hz, 1H), 2.50 – 2.41

(m, 1H), 2.41 – 2.31 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.03, 158.94, 141.49, 136.24, 125.32, 114.29, 110.22, 55.64, 50.29, 30.99, 29.29.

Ethyl 6-methoxy-2,3-dihydro-1H-indene-1-carboxylate (DK-A-99); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 1.8 Hz, 1H), 6.78 (dd, *J* = 8.2, 2.2 Hz, 1H), 4.21 (tdd, *J* = 10.9, 7.1, 3.7 Hz, 2H), 4.04 – 3.98 (m, 1H), 3.79 (s, 3H), 3.08 – 2.98 (m, 1H), 2.89 – 2.80 (m, 1H), 2.46 (ddt, *J* = 13.1, 8.7, 6.6 Hz, 1H), 2.34 (dtd, *J* = 13.9, 8.5, 5.6 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.80, 158.80, 142.26, 136.12, 125.13, 113.72, 110.09, 60.78, 55.47, 50.46, 30.93, 29.19, 14.36.

Diethyl 6-methoxy-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-A-100); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 – 7.09 (m, 2H), 6.83 (dd, *J* = 8.3, 2.5 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 4H), 3.79 (s, 3H), 2.95 (t, *J* = 7.2 Hz, 2H), 2.74 – 2.67 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.62, 158.76, 140.49, 136.47, 125.02, 115.25, 111.66, 65.84, 61.62, 55.52, 34.75, 30.04, 14.10.

6-Methoxy-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-B-5): ^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (d, *J* = 8.4 Hz, 1H), 6.87 (dt, *J* = 11.4, 5.7 Hz, 1H), 6.69 (d, *J* = 2.3 Hz, 1H), 3.75 (d, *J* = 4.5 Hz, 3H), 3.14 (t, *J* = 7.4 Hz, 2H), 2.77 (dd, *J* = 9.2, 5.6 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.67, 160.70, 143.99, 138.09, 127.05, 116.06, 108.80, 55.99, 34.68, 32.23; Chemical Formula: $C_{13}H_{12}N_2O_4$ Exact Mass: 260.0797, Found M- 259.0730.

6-Methoxy-1'-methyl-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (rac-DK-B-6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 6.85 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.58 (d, *J* = 2.1 Hz, 1H), 3.75 (s, 3H), 3.32 (s, 3H), 3.27 – 3.18 (m, 2H), 2.84 (ddd, *J* = 12.7, 9.0, 5.1 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.14, 169.83, 159.32, 141.95, 136.80, 126.53, 115.10, 108.24, 63.51, 55.77, 34.43, 31.57, 28.62; Chemical Formula: $C_{14}H_{14}N_2O_4$ Exact Mass: 274.0954, Found M- 273.0884.

6-Methoxy-2'-thioxo-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-dione (DK-B-19); ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (d, *J* = 8.3 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.71 (s, 1H), 3.78 (s, 3H), 3.18 (t, *J* = 7.4 Hz, 2H), 2.82 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 184.08, 173.23, 160.74, 144.05, 137.82, 127.15, 116.19, 108.75, 55.98, 33.70, 32.10, 23.84; Chemical Formula: $C_{13}H_{12}N_2O_3S$ Exact Mass: 276.0569, Found M- 275.0506.

2'-Imino-6-methoxy-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione (DK-B-52); ^{1}H NMR (400 MHz, D_2O) δ 7.02 (d, *J* = 8.4 Hz, 1H), 6.64 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.27 (d, *J* = 2.5 Hz, 1H), 3.49 (s, 3H), 2.86 (t, *J* = 7.5 Hz, 2H), 2.29 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ 181.27, 164.08, 157.78, 138.87, 127.95, 116.80, 109.10, 53.07, 48.75, 23.25. Chemical Formula: $C_{13}H_{13}N_3O_3$ Exact Mass: 259.0957 Found MH+ 260.1042

6-Fluoro-2,3-dihydro-1H-inden-1-one (DK-B-46); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.3, 4.5 Hz, 1H), 7.37 (dd, *J* = 7.6, 2.4 Hz, 1H), 7.32 – 7.25 (m, 1H), 3.14 – 3.06 (m, 2H), 2.75 – 2.70 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.05, 163.64, 161.18, 150.58, 128.19, 128.11, 122.52, 122.29, 109.73, 109.52, 37.11, 25.38.

6-Fluoro-2,3-dihydro-1H-inden-1-ol (DK-B-53); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 8.2, 5.1 Hz, 1H), 7.06 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.93 (td, *J* = 8.7, 2.5 Hz, 1H), 5.18 (t, *J* = 6.3 Hz, 1H), 3.02 – 2.92 (m, 1H), 2.80 – 2.70 (m, 1H), 2.50 (dddd, *J* = 12.7, 8.2, 7.0, 4.4 Hz, 1H), 2.22 (s, 1H), 1.94 (dddd, *J* = 13.0, 8.6, 7.0, 5.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.53, 161.11, 147.14, 147.07, 138.54, 125.99, 125.91, 115.49, 115.26, 111.26, 111.04, 76.27, 36.62, 29.16.

6-Fluoro-2,3-dihydro-1H-indene-1-carbonitrile (DK-B-57); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 8.3, 5.2 Hz, 1H), 7.04 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.91 (td, *J* = 8.6, 2.2 Hz, 1H), 4.05 (t, *J* = 8.2 Hz, 1H), 3.03 – 2.92 (m, 1H), 2.85 (dt, *J* = 15.8, 8.0 Hz, 1H), 2.60 – 2.48 (m, 1H), 2.30 (dq, *J* = 12.8, 8.3 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 163.12, 160.69, 139.40, 139.32, 138.30, 125.88, 125.80, 120.36, 115.42, 115.19, 111.18, 110.95, 34.26, 31.43, 30.36. ^{19}F NMR (376 MHz, $CDCl_3$) δ -115.51.

6-Fluoro-2,3-dihydro-1H-indene-1-carboxylic acid (DK-B-59); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.32 (s, 1H), 7.24 – 7.13 (m, 2H), 6.95 (td, *J* = 8.7, 2.4 Hz, 1H), 4.15 – 4.05 (m, 1H), 3.15 – 3.03 (m, 1H), 2.96 – 2.85 (m, 1H), 2.52 (ddt, *J* = 12.9, 8.7, 6.4 Hz, 1H), 2.48 – 2.37 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.37, 163.31, 160.89, 141.95, 141.87, 139.61, 139.59, 125.63, 125.55, 114.98, 114.75, 112.31, 112.08, 50.12, 30.96, 29.17.

Ethyl 6-fluoro-2,3-dihydro-1H-indene-1-carboxylate (DK-B-60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 8.2, 5.2 Hz, 1H), 7.09 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.89 (td, *J* = 8.7, 2.4 Hz, 1H), 4.25 – 4.16 (m, 2H), 4.01 (t, *J* = 7.5 Hz, 1H), 3.11 – 2.98 (m, 1H), 2.86 (dt, *J* = 15.5, 7.6 Hz, 1H), 2.48 (ddt, *J* = 13.3, 8.7, 6.7 Hz, 1H), 2.42 – 2.30 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.36, 163.31, 160.89, 142.91, 142.83, 139.55, 125.56, 125.47, 114.66, 114.44, 112.08, 111.85, 61.03, 52.21, 50.34, 31.05, 29.33, 29.24, 14.36. ^{19}F NMR (376 MHz, $CDCl_3$) δ -117.18.

Diethyl 6-fluoro-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-B-62); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.14 (dd, *J* = 8.0, 5.4 Hz, 1H), 6.95 (td, *J* = 8.7, 2.3 Hz, 1H), 4.21 (q, *J* = 6.7 Hz, 4H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 163.18, 160.76, 141.19, 141.10, 139.86, 125.46, 125.38, 115.92, 115.69, 113.86, 113.63, 65.73, 61.83, 52.90, 34.66, 30.15, 14.05. ^{19}F NMR (376 MHz, $CDCl_3$) δ -116.62.

6-Fluoro-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-B-64); ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (dd, *J* = 8.3, 5.2 Hz, 1H), 7.08 (td, *J* = 8.8, 2.4 Hz, 1H), 6.97 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.21 (t, *J* = 7.4 Hz, 2H), 2.83 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.27, 164.68, 162.26, 144.49, 144.41, 142.24, 127.65, 127.56, 117.14, 116.92, 110.87, 110.63, 64.31, 35.62, 32.28. ^{19}F NMR (376 MHz, CD_3OD) δ -117.94. Chemical Formula: $C_{12}H_9FN_2O_3$ Exact Mass: 248.0597, Found M- 247.0524.

6-Fluoro-2'-imino-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-C-56): ^{1}H NMR (400 MHz, CD_3OD) δ 7.20 (dd, *J* = 8.3, 5.2 Hz, 1H), 6.92 – 6.83 (m, 1H), 6.65 (dd, *J* = 9.1, 2.5 Hz, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 2.71 – 2.63 (m, 2H). ^{19}F NMR (376 MHz, CD_3OD) δ -119.38, -119.61, -119.67, -120.02. ^{13}C NMR (101 MHz, CD_3OD) δ 186.27, 170.96, 163.14, 160.74, 147.14, 147.06, 140.66, 125.51, 125.43, 113.97, 113.74, 108.93, 108.70, 62.41, 48.27, 48.20, 48.06, 47.84, 47.75, 47.72, 47.63, 47.54, 47.51, 47.42, 47.32, 47.30, 47.27, 47.20, 47.11, 47.08, 47.05, 46.99, 34.62, 31.49. Chemical Formula: $C_{12}H_{10}FN_3O_2$ Exact Mass: 247.0757, Found M- 246.0686.

6-Chloro-2,3-dihydro-1H-inden-1-one (DK-B-69); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 1.9 Hz, 1H), 7.53 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 3.15 – 3.07 (m, 2H), 2.75 – 2.67 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.58, 153.23, 138.73, 134.71, 133.89, 128.03, 123.72, 36.77, 25.57.

6-Chloro-2,3-dihydro-1H-inden-1-ol (DK-B-70a); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 1.2 Hz, 1H), 7.22 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 5.21 (q, *J* = 6.4 Hz, 1H), 3.01 (ddd,

J = 16.0, 8.6, 4.5 Hz, 1H), 2.84 – 2.73 (m, 1H), 2.51 (dddd, *J* = 12.9, 8.2, 6.9, 4.5 Hz, 1H), 2.02 – 1.90 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.05, 141.74, 132.56, 128.58, 126.15, 124.63, 76.27, 36.45, 29.45.

6-Chloro-2,3-dihydro-1H-indene-1-carbonitrile (DK-B-70b); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.42 (s, 1H), 7.27 – 7.24 (m, 1H), 7.29 – 7.16 (m, 2H), 7.20 (d, *J* = 8.1 Hz, 1H), 4.09 (t, *J* = 8.1 Hz, 1H), 4.09 (t, *J* = 8.1 Hz, 1H), 3.06 (ddd, *J* = 16.0, 8.7, 4.3 Hz, 1H), 3.06 (ddd, *J* = 16.0, 8.7, 4.3 Hz, 1H), 2.92 (dt, *J* = 16.1, 8.1 Hz, 1H), 2.92 (dt, *J* = 16.1, 8.1 Hz, 1H), 2.60 (dtd, *J* = 12.5, 8.1, 4.3 Hz, 1H), 2.60 (dtd, *J* = 12.5, 8.1, 4.3 Hz, 1H), 2.40 (dq, *J* = 12.9, 8.3 Hz, 1H), 2.40 (dq, *J* = 12.9, 8.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.49, 139.53, 133.21, 129.00, 126.18, 124.76, 120.51, 34.58, 31.61, 31.09.

6-Chloro-2,3-dihydro-1H-indene-1-carboxylic acid (DK-B-71); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.23 – 7.12 (m, 2H), 4.10 – 4.03 (m, 1H), 3.11 – 3.01 (m, 1H), 2.94 – 2.80 (m, 1H), 2.53 – 2.42 (m, 1H), 2.42 – 2.32 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.08, 142.74, 141.95, 132.44, 128.14, 125.85, 125.42, 49.90, 31.32, 29.00.

Ethyl 6-chloro-2,3-dihydro-1H-indene-1-carboxylate (DK-B-72); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.21 – 7.10 (m, 2H), 4.26 – 4.12 (m, 2H), 4.05 – 3.93 (m, 1H), 3.12 – 2.96 (m, 1H), 2.91 – 2.78 (m, 1H), 2.46 (ddt, *J* = 13.2, 8.7, 6.6 Hz, 1H), 2.34 (dtd, *J* = 14.1, 8.6, 5.7 Hz, 1H), 1.36 – 1.21 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.20, 142.79, 142.62, 132.13, 127.69, 125.68, 125.07, 61.00, 50.12, 31.25, 28.94, 14.31.

Diethyl 6-chloro-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-B-73); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J* = 1.8 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 3.99 – 3.88 (m, 4H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.42 (t, *J* = 7.3 Hz, 2H), 1.01 – 0.91 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 142.97, 141.10, 132.35, 128.89, 126.96, 125.64, 61.93, 53.03, 34.44, 30.44, 14.11.

6-Chloro-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-B-74); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (s, 2H), 7.21 (s, 1H), 3.22 (t, *J* = 7.4 Hz, 2H), 2.82 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.22, 151.96, 145.37, 144.64, 133.68, 130.24, 127.71, 123.89, 64.17, 35.43, 32.54. Chemical Formula: $C_{12}H_9ClN_2O_3$ Exact Mass: 264.0302, Found M- 263.0084.

6-Chloro-2'-imino-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-C-58): ^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (d, *J* = 8.1 Hz, 1H), 7.14 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 3.18 (t, *J* = 7.5 Hz, 2H), 2.69 – 2.61 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 186.20, 170.95, 147.22, 143.83, 131.51, 127.15, 125.74, 122.13, 62.21, 48.28, 48.06, 47.85, 47.76, 47.73, 47.64, 47.54, 47.51, 47.49, 47.42, 47.33, 47.30, 47.21, 47.09, 47.06, 47.00, 34.37, 31.76. Chemical Formula: $C_{12}H_{10}ClN_3O_2$ Exact Mass: 263.0462 Found M- 262.0399.

5-Bromo-2,3-dihydro-1H-inden-1-ol (DK-C-18a); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.32 (m, 2H), 7.27 (d, *J* = 7.6 Hz, 1H), 5.23 – 5.15 (m, 1H), 3.04 (ddd, *J* = 16.1, 8.5, 4.8 Hz, 1H), 2.87 – 2.74 (m, 1H), 2.49 (dddd, *J* = 13.2, 8.3, 6.9, 4.8 Hz, 1H), 1.95 (dddd, *J* = 13.8, 8.6, 6.7, 5.4 Hz, 1H), 1.76 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.67, 143.99, 129.86, 128.09, 125.73, 122.31, 77.34, 77.02, 76.70, 75.84, 36.07, 29.66.

5-Bromo-2,3-dihydro-1H-indene-1-carbonitrile (DK-C-18b); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.34 (m, 2H), 7.31 – 7.23 (m, 1H), 4.04 (t, *J* = 8.1 Hz, 1H), 3.07 (ddd, *J* = 15.6, 8.6, 4.3 Hz, 1H), 2.95 (dt, *J* = 16.2, 8.1 Hz, 1H), 2.64 – 2.50 (m, 1H), 2.38 (dq, *J* = 12.9, 8.4 Hz, 1H). ^{13}C NMR (101

MHz, $CDCl_3$) δ 145.29, 136.66, 130.42, 128.27, 125.75, 122.62, 120.50, 77.42, 77.10, 76.78, 34.12, 31.32.

5-Bromo-2,3-dihydro-1H-indene-1-carboxylic acid (DK-C-20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.29 (dt, *J* = 13.1, 6.5 Hz, 2H), 4.01 (dd, *J* = 8.1, 6.3 Hz, 1H), 3.18 – 3.01 (m, 1H), 3.00 – 2.83 (m, 1H), 2.57 – 2.27 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.42, 146.56, 139.00, 129.68, 127.93, 126.49, 121.82, 77.34, 77.02, 76.71, 49.40, 31.57, 28.75.

Ethyl 5-bromo-2,3-dihydro-1H-indene-1-carboxylate (DK-C-22); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 4.25 – 4.12 (m, 2H), 4.00 – 3.92 (m, 1H), 3.14 – 3.00 (m, 1H), 2.95 – 2.83 (m, 1H), 2.45 (ddt, *J* = 12.9, 8.7, 6.4 Hz, 1H), 2.39 – 2.27 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.32, 146.55, 139.89, 129.52, 127.84, 126.28, 121.44, 77.36, 77.04, 76.72, 60.91, 49.71, 31.62, 28.77, 14.26.

Diethyl 5-bromo-2,3-dihydro-1H-indene-1,1-dicarboxylate (DK-C-39); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.1 Hz, 1H), 7.36 (dd, *J* = 9.1, 1.2 Hz, 2H), 4.21 (qd, *J* = 6.9, 1.8 Hz, 4H), 3.01 (t, *J* = 7.3 Hz, 2H), 2.69 (t, *J* = 7.3 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 146.71, 138.32, 129.81, 128.15, 127.75, 122.76, 77.33, 77.01, 76.70, 65.23, 61.79, 34.11, 30.68, 14.02.

5-Bromo-2,3-dihydro-2'H-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (DK-C-42); ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (s, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 3.24 (t, *J* = 7.4 Hz, 2H), 2.80 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.91, 147.67, 140.80, 129.86, 128.24, 123.83, 122.60, 62.41, 33.35, 31.50. Chemical Formula: $C_{12}H_9BrN_2O_3$ Exact Mass: 307.9797 Found M- 306.9725 and 308.9704.

5-Bromo-2'-imino-2,2',3,3'-tetrahydro-4'*H*-spiro[indene-1,5'-pyrimidine]-4',6'(1'*H*)-dione hydrochloride (DK-C-59): Chemical Formula: $C_{12}H_{10}BrN_3O_2$ Exact Mass: 306.9956 Found M- 305.9883 and 307.9863

5-Ethyl-2-imino-5-phenyldihydropyrimidine-4,6(1*H*,5*H*)-dione [18] **hydrochloride (DK-C-62);** ^{1}H NMR (400 MHz, D_2O) δ 7.44 – 7.31 (m, 3H), 7.31 – 7.23 (m, 2H), 2.34 (q, *J* = 7.3 Hz, 2H), 0.85 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, D_2O_salt) δ 188.93, 141.79, 129.57, 128.09, 126.99, 59.35, 49.50, 29.37, 9.29. Chemical Formula: $C_{12}H_{13}N_3O_2$ Exact Mass: 231.1008 Found M- 230.0938. *DK-C-62 was synthesized followed the procedure for the Substituted 2'-imino-2,2',3,3'-tetrahydro-4'H-spiro[indene-1,5'-pyrimidine]-4',6'(1'H)-diones.*

2-Methyl-2,3-dihydro-1*H*-inden-1-ol (DK-C-91); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.31 (m, 2H), 7.26 – 7.15 (m, 5H), 4.70 (t, *J* = 7.2 Hz, 1H), 3.09 (dd, *J* = 15.7, 7.7 Hz, 1H), 2.94 (dd, *J* = 15.7, 7.4 Hz, 1H), 2.66 (dd, *J* = 15.7, 7.1 Hz, 1H), 2.45 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.23 (tt, *J* = 8.1, 6.8 Hz, 1H), 1.94 (d, *J* = 7.7 Hz, 1H), 1.24 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.02, 144.80, 143.36, 142.09, 128.43, 128.08, 126.68, 125.00, 124.77, 123.92, 82.85, 77.69, 77.39, 77.07, 76.75, 45.45, 39.30, 37.97, 37.89, 17.80, 13.66.

2-Methyl-2,3-dihydro-1*H*-indene-1-carbonitrile (DK-C-93); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (ddd, *J* = 6.7, 3.6, 1.4 Hz, 3H), 7.28 – 7.15 (m, 9H), 4.08 (d, *J* = 7.4 Hz, 1H), 3.60 (dd, *J* = 9.4, 1.5 Hz, 2H), 3.06 (ddd, *J* = 15.6, 12.9, 7.2 Hz, 3H), 2.81 – 2.62 (m, 4H), 2.54 (ddt, *J* = 15.5, 9.5, 1.1 Hz, 2H), 1.31 (d, *J* = 6.7 Hz, 6H), 1.26 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.62, 142.56, 137.82, 137.44, 129.14, 128.58, 128.50, 127.23, 125.30, 125.21, 124.96, 124.64, 124.48, 124.31,

124.03, 123.71, 120.73, 119.10, 77.97, 77.65, 77.33, 42.16, 42.12, 40.54, 39.55, 39.39, 37.98, 36.94, 18.50, 17.28, 15.38.

2-Methyl-2,3-dihydro-1*H*-indene-1-carboxylic acid (DK-C-95); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.40 (s, 1H), 7.54 – 7.41 (m, 1H), 7.32 (dq, *J* = 7.3, 4.0 Hz, 3H), 3.80 (d, *J* = 7.3 Hz, 1H), 3.34 (dd, *J* = 15.6, 7.9 Hz, 1H), 3.01 (dq, *J* = 14.8, 7.4 Hz, 1H), 2.70 (dd, *J* = 15.6, 7.5 Hz, 1H), 1.37 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.74, 143.54, 139.97, 127.85, 126.69, 126.58, 125.41, 125.05, 124.96, 124.91, 124.82, 124.73, 77.51, 77.20, 76.88, 57.79, 40.25, 38.53, 20.01.

Ethyl 2-methyl-2,3-dihydro-1H-indene-1-carboxylate (DK-C-97); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.33 (m, 1H), 7.32 – 7.18 (m, 3H), 4.37 – 4.18 (m, 2H), 3.70 (d, *J* = 7.7 Hz, 1H), 3.26 (dd, *J* = 15.6, 7.9 Hz, 1H), 3.04 – 2.84 (m, 1H), 2.62 (dd, *J* = 15.6, 7.8 Hz, 1H), 1.43 – 1.24 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.51, 143.46, 140.85, 127.50, 126.51, 124.73, 124.69, 124.60, 77.64, 77.32, 77.00, 60.69, 57.93, 40.20, 38.50, 19.86, 14.42.

Diethyl 2-methyl-2,3-dihydro-1*H*-indene-1,1-dicarboxylate (DK-C-99); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.7, 2.0 Hz, 1H), 7.30 – 7.18 (m, 3H), 4.32 – 4.12 (m, 4H), 3.34 – 3.19 (m, 2H), 2.74 – 2.64 (m, 1H), 1.27 (q, *J* = 7.0 Hz, 6H), 1.15 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.97, 169.65, 143.89, 139.25, 128.42, 126.77, 126.53, 124.68, 77.52, 77.21, 76.89, 69.09, 61.50, 61.40, 61.11, 52.07, 41.24, 41.15, 39.30, 39.26, 16.54, 16.51, 14.16, 14.02.

2-Methyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac DK-D-1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (s, 3H), 7.28 (d, *J* = 3.0 Hz, 1H), 7.19 (d, *J* = 7.4 Hz, 3H), 7.12 (d, *J* = 7.4 Hz, 2H), 3.37 (t, *J* = 2.0 Hz, 1H), 3.28 (q, *J* = 5.3 Hz, 2H), 3.14 (dd, *J* = 15.4, 7.4 Hz, 2H), 3.08 – 3.00 (m, 2H), 1.27 (dt, *J* = 7.0, 1.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.08, 170.37, 145.34, 141.92, 128.32, 126.96, 126.56, 124.35, 123.50, 66.05, 48.24, 48.03, 47.81, 47.78, 47.66, 47.64, 47.60, 47.42, 47.39, 47.35, 47.17, 46.96, 39.05, 13.40. Chemical Formula: $C_{13}H_{12}N_2O_3$ Exact Mass: 244.0848 Found M- 244.1082.

3-Methyl-2,3-dihydro-1*H*-inden-1-ol (DK-C-92); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.36 (m, 1H), 7.30 – 7.19 (m, 3H), 5.16 (q, *J* = 7.2 Hz, 1H), 3.04 (h, *J* = 7.1 Hz, 1H), 2.75 (dt, *J* = 12.6, 7.1 Hz, 1H), 1.82 (d, *J* = 7.3 Hz, 1H), 1.46 (ddd, *J* = 12.6, 8.8, 7.6 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.41, 145.05, 128.19, 126.91, 126.81, 123.82, 123.66, 123.37, 77.36, 77.04, 76.72, 75.21, 45.80, 36.31, 20.22.

3-Methyl-2,3-dihydro-1*H*-indene-1-carbonitrile (DK-C-94); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 14.8, 7.4 Hz, 2H), 7.39 – 7.24 (m, 5H), 4.17 (dd, *J* = 8.4, 5.4 Hz, 1H), 4.08 – 3.99 (m, 1H), 3.47 (q, *J* = 7.0 Hz, 1H), 3.22 (dt, *J* = 9.2, 6.8 Hz, 1H), 2.77 (dt, *J* = 12.6, 7.3 Hz, 1H), 2.60 (ddd, *J* = 13.0, 7.7, 5.6 Hz, 1H), 2.15 – 2.04 (m, 1H), 1.92 (dt, *J* = 12.5, 9.8 Hz, 1H), 1.40 (d, *J* = 6.8 Hz, 3H), 1.31 (dd, *J* = 7.0, 1.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.02, 147.27, 137.63, 137.05, 128.87, 128.59, 127.47, 127.34, 124.51, 124.11, 124.01, 123.72, 121.26, 77.76, 77.44, 77.12, 40.15, 39.37, 38.38, 38.25, 33.31, 33.15, 19.82, 19.15.

3-Methyl-2,3-dihydro-1*H*-indene-1-carboxylic acid (DK-C-96); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.24 (s, 1H), 7.47 (dd, *J* = 10.8, 7.5 Hz, 2H), 7.34 – 7.20 (m, 6H), 4.16 – 4.03 (m, 2H), 3.51 (h, *J* = 7.3 Hz, 1H), 3.23 (h, *J* = 7.3 Hz, 1H), 2.73 (ddd, *J* = 13.2, 7.7, 3.7 Hz, 1H), 2.62 (dt, *J* = 12.7, 7.8 Hz, 1H), 1.98 (ddt, *J* = 41.3, 13.0, 8.6 Hz, 3H), 1.41 (d, *J* = 6.9 Hz, 3H), 1.33 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.00, 180.81, 178.34, 148.94, 148.43, 139.79, 139.73, 128.11, 127.81, 126.79, 126.75, 125.20, 124.68, 123.71, 123.50, 77.48, 77.16, 76.84, 48.98, 48.95, 38.41, 38.36, 37.82, 37.72, 20.17, 19.81.

Ethyl 3-methyl-2,3-dihydro-1H-indene-1-carboxylate (DK-C-98); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.43 (m, 2H), 7.38 – 7.23 (m, 4H), 4.41 – 4.15 (m, 3H), 4.20 – 4.03 (m, 2H), 3.56 (h, *J* = 7.2 Hz, 1H), 3.33 – 3.19 (m, 1H), 2.80 (ddd, *J* = 12.9, 7.7, 3.9 Hz, 1H), 2.64 (dt, *J* = 12.6, 7.7 Hz, 1H), 2.10 (dt, *J* = 12.7, 9.3 Hz, 1H), 1.95 (ddd, *J* = 12.9, 8.6, 7.4 Hz, 1H), 1.52 – 1.40 (m, 4H), 1.45 – 1.29 (m, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.82, 173.68, 148.88, 148.37, 140.72, 140.54, 127.80, 127.55, 126.68, 126.65, 126.62, 124.97, 124.44, 123.66, 123.43, 77.69, 77.37, 77.05, 60.76, 60.68, 49.11, 49.04, 49.01, 38.46, 38.32, 37.87, 37.83, 20.33, 19.79, 14.42, 14.31.

Diethyl 3-methyl-2,3-dihydro-1*H*-indene-1,1-dicarboxylate (DK-C-100); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.37 – 7.20 (m, 3H), 4.34 – 4.12 (m, 4H), 3.41 (h, *J* = 7.3 Hz, 1H), 3.07 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.21 (dd, *J* = 13.1, 8.5 Hz, 1H), 1.40 – 1.28 (m, 6H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.87, 170.45, 148.78, 138.97, 128.75, 128.69, 126.77, 126.72, 126.56, 123.36, 77.57, 77.25, 76.93, 64.75, 61.65, 61.56, 52.73, 52.67, 42.93, 37.38, 19.71, 14.10, 13.99.

3-Methyl-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac-DK-D-2); ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.27 (m, 2H), 7.20 (dd, *J* = 14.9, 7.3 Hz, 2H), 3.67 (h, *J* = 7.2 Hz, 1H), 2.88 (dd, *J* = 13.1, 8.2 Hz, 1H), 2.44 (dd, *J* = 13.1, 8.4 Hz, 1H), 1.42 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 172.39, 172.11, 150.82, 149.20, 141.29, 128.96, 127.00, 124.23, 121.78, 62.33, 48.26, 48.05, 47.84, 47.63, 47.41, 47.20, 46.99, 41.02, 38.61, 19.08. Chemical Formula: $C_{13}H_{12}N_2O_3$ Exact Mass: 244.0848 Found M- 243.0774.

3-Bromo-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (DK-D-18) crude; ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 (d, *J* = 7.6 Hz, 1H), 7.47 (td, *J* = 7.5, 1.1 Hz, 1H), 7.41 – 7.35 (m, 1H), 7.26 (d, *J* = 7.8 Hz, 2H), 5.84 (t, *J* = 7.6 Hz, 1H), 3.39 – 3.34 (m, 2H), 3.25 (dd, *J* = 14.0, 7.5 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.51, 170.42, 144.15, 139.88, 129.73, 129.29, 126.83, 121.60, 62.67, 48.81, 48.28, 48.07, 47.86, 47.71, 47.65, 47.43, 47.22, 47.01, 43.47.

3-Hydroxy-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (rac-DK-D-25); ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.39 – 7.31 (m, 2H), 7.24 (d, *J* = 7.7 Hz, 2H), 5.57 – 5.48 (m, 2H), 3.05 (dd, *J* = 13.4, 7.4 Hz, 2H), 2.67 (dd, *J* = 13.4, 6.2 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.84, 171.65, 150.70, 145.89, 140.62, 129.28, 128.76, 125.32, 121.69, 74.11, 61.41, 48.25, 48.04, 47.83, 47.70, 47.61, 47.40, 47.19, 46.97, 41.79 Chemical Formula: $C_{12}H_{10}N_2O_4$ Exact Mass: 246.0641 Found M- 245.0577.

Diethyl 3-bromo-2,3-dihydro-1*H*-indene-1,1-dicarboxylate (DK-D-11); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 6.9, 2.1 Hz, 1H), 7.47 – 7.40 (m, 1H), 7.42 – 7.32 (m, 2H), 5.56 (dd, *J* = 7.2, 4.7 Hz, 1H), 4.35 – 4.14 (m, 4H), 3.48 (dd, *J* = 14.7, 7.2 Hz, 1H), 3.07 (dd, *J* = 14.7, 4.7 Hz, 1H), 1.27 (dt, *J* = 14.3, 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.63, 169.47, 143.80, 138.31, 129.61, 129.55, 129.13, 126.65, 126.53, 125.70, 77.43, 77.11, 76.80, 64.64, 62.25, 62.09, 61.59, 48.71, 45.04, 45.00, 30.87, 14.02.

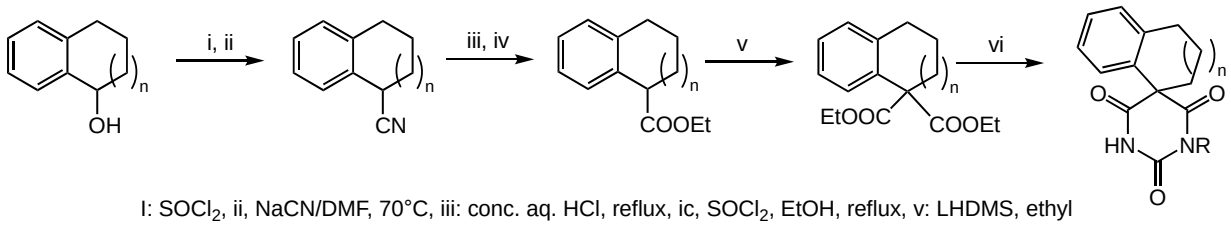
Diethyl 3-azido-2,3-dihydro-1*H*-indene-1,1-dicarboxylate (DK-D-20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (tt, *J* = 4.5, 2.4 Hz, 1H), 7.46 – 7.34 (m, 3H), 5.02 (dd, *J* = 7.4, 5.7 Hz, 1H), 4.30 – 4.13 (m, 4H), 3.21 (dd, *J* = 13.9, 7.4 Hz, 1H), 2.61 (dd, *J* = 13.9, 5.7 Hz, 1H), 1.26 (dt, *J* = 15.5, 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.79, 169.56, 141.20, 138.85, 129.33, 129.29, 129.27, 129.23, 129.19, 127.05, 127.02, 124.49, 77.47, 77.15, 76.83, 63.95, 63.40, 62.15, 62.09, 62.03, 40.64, 14.09, 14.06, 14.00, 13.97, 13.91, 13.88.

Diethyl 3-((*tert*-butoxycarbonyl)amino)-2,3-dihydro-1*H*-indene-1,1-dicarboxylate (DK-D-22); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.56 (m, 1H), 7.40 (d, *J* = 5.2 Hz, 1H), 7.38 – 7.29 (m, 2H), 5.34 (td, *J* = 8.6, 4.6 Hz, 1H), 5.17 (d, *J* = 9.3 Hz, 1H), 4.28 – 4.11 (m, 4H), 3.03 (dd, *J* = 14.0, 8.1 Hz, 1H), 2.49 (dd, *J* = 14.1, 4.7 Hz, 1H), 1.46 (s, 10H), 1.26 (dt, *J* = 10.7, 7.1 Hz, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 170.05, 155.52, 144.33, 138.47, 129.36, 128.49, 126.58, 124.83, 79.51, 77.37, 77.05, 76.73, 62.06, 61.83, 53.69, 41.57, 28.42, 14.00.

3-Amino-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione trifluoroacetate (rac-DK-D-27); ^{1}H NMR (400 MHz, D_2O salt) δ 7.63 (d, *J* = 7.4 Hz, 1H), 7.52 (dt, *J* = 15.3, 7.2 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 1H), 5.23 – 5.13 (m, 1H), 3.31 (dd, *J* = 15.1, 8.1 Hz, 1H), 2.75 (dd, *J* = 14.7, 3.4 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 172.05, 170.38, 150.26, 142.55, 138.82, 130.82, 130.10, 129.82, 125.91, 122.48, 62.05, 54.31, 36.82, 29.34. Chemical Formula: $C_{12}H_{11}N_3O_3$ Exact Mass: 245.0800 Found M- 244.0732.

3-Thiocyanato-2,3-dihydro-2'*H*-spiro[indene-1,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (DK-D-63): ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, *J* = 7.6 Hz, 1H), 7.49 (td, *J* = 7.6, 1.2 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 5.26 (t, *J* = 7.5 Hz, 1H), 3.43 – 3.32 (m, 2H), 3.04 (dd, *J* = 14.1, 6.7 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.04, 170.39, 150.58, 141.49, 141.32, 129.95, 129.73, 125.51, 122.07, 111.23, 62.05, 51.06, 48.24, 48.02, 47.93, 47.90, 47.81, 47.72, 47.69, 47.60, 47.50, 47.48, 47.38, 47.29, 47.26, 47.23, 47.17, 46.96, 39.90. Chemical Formula: $C_{13}H_9N_3O_3S$ Exact Mass: 287.0365 Found M- 286.0297.

Synthesis of six- and seven-membered spiro-barbiturates



I: $SOCl_2$, ii, NaCN/DMF, 70°C, iii: conc. aq. HCl, reflux, ic, $SOCl_2$, EtOH, reflux, v: LHDMS, ethyl cyanoformate, THF, NaOEt/EtOH, urea or methyurea

Scheme 8: Synthetic route to spiro-barbiturates BW-C-10, and BW-C-30.

1,2,3,4-Tetrahydronaphthalene-1-carbonitrile. This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.35 (m, 1H), 7.25-7.18 (m, 2H), 7.17-7.09 (m, 1H), 3.99 (t, *J* = 6.3 Hz, 1H), 2.96-2.73 (m, 2H), 2.24-1.98 (m, 3H), 1.93-1.78 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.5, 129.9, 129.8, 128.9, 128.1, 126.6, 121.9, 30.9, 28.5, 27.4, 20.9.

1,2,3,4-Tetrahydronaphthalene-1-carboxylic acid. This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.56 (brs, 1H), 7.26-7.07 (m, 4H), 3.86 (t, *J* = 5.7 Hz, 1H), 2.95-2.68 (m, 2H), 2.30-2.14 (m, 1H), 2.12-1.92 (m, 2H), 1.87-1.72 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 137.4, 132.6, 129.8, 129.6, 127.2, 126.0, 44.6, 29.2, 26.6, 20.5.

Ethyl 1,2,3,4-tetrahydronaphthalene-1-carboxylate. This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.01 (m, 4H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.82 (t, *J* = 5.7 Hz, 1H), 2.93-2.65 (m, 2H), 2.21-2.08 (m, 1H), 2.07-1.92 (m, 2H), 1.85-1.70 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 137.4, 133.6, 129.6, 129.4, 126.9, 125.9, 60.8, 45.0, 29.3, 26.8, 20.8, 14.4.

Diethyl 3,4-dihydronaphthalene-1,1(2*H*)-dicarboxylate. This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.38 (m, 1H), 7.24-7.07 (m, 3H), 4.27-4.17 (m, 4H), 2.82 (t, *J* = 6.5 Hz, 2H), 2.47-2.38 (m, 2H), 1.91-1.79 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 137.2, 132.3, 130.6, 129.5, 127.7, 125.7, 61.8, 59.1, 31.2, 29.5, 20.0, 14.2.

3,4-Dihydro-1'*H*,2*H*-spiro[naphthalene-1,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-10). This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.44 (brs, 2H), 7.23 -7.07 (m, 3H), 7.00 (d, *J* = 7.1 Hz, 1H), 2.76 (t, *J* = 6.1 Hz, 2H), 2.28-2.19 (m, 2H), 1.96-1.84 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 173.3, 150.2, 138.2, 132.8, 129.4, 128.4, 127.4, 126.2, 54.1, 33.9, 28.4, 18.5. HRMS: calculated for $C_{13}H_{11}N_2O_3$ $[M-H]^+$: 243.0770; Found: 243.0763.

6,7,8,9-Tetrahydro-1'*H*-spiro[benzo[7]annulene-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-30). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.42 (brs, 2H), 7.24-7.05 (m, 3H), 6.99-6.87 (m, 1H), 3.08-2.87 (mz, 2H), 2.40-2.25 (m, 2H), 1.88-1.63 (m, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 173.4, 149.9, 142.2, 136.5, 131.1, 129.7, 127.6, 126.4, 60.3, 33.1, 31.0, 25.5, 20.6. HRMS: Calculated for $C_{14}H_{13}N_2O_3$ $[M-H]^+$: 257.0926; Found: 257.0926.

1'-Methyl-3,4-dihydro-1'*H*,2*H*-spiro[naphthalene-1,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-C-11). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (brs, 1H), 7.26-7.08 (m, 3H), 6.82 (d, *J* = 7.8 Hz, 1H), 3.34 (s, 3H), 2.90 (t, *J* = 6.2 Hz, 2H), 2.42-2.32 (m, 2H), 2.14-2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.7, 150.2, 138.5, 131.7, 130.4, 128.5, 127.8, 126.8, 55.6, 35.2, 28.8, 28.6, 18.9. HRMS: Calculated for $C_{14}H_{15}N_2O_3$ $[M-H]^+$: 257.0926; Found: 257.0929.

1'-Methyl-6,7,8,9-tetrahydro-1'*H*-spiro[benzo[7]annulene-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-30). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.25-7.08 (m, 3H), 6.81 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H), 3.20-3.00 (m, 2H), 2.50-2.37 (m, 2H), 2.02-1.82 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 171.8, 145.0, 142.6, 135.6, 132.1, 129.3, 128.7, 126.8, 61.7, 34.7, 31.9, 28.4, 26.1, 21.2. HRMS: Calculated for $C_{15}H_{17}N_2O_3$ $[M+H]^+$: 273.1239; Found: 273.1185

1,2,3,4-Tetrahydronaphthalene-1-carbonitrile.[1] This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.35 (m, 1H), 7.25-7.18 (m, 2H), 7.17-7.09 (m, 1H), 3.99 (t, *J* = 6.3 Hz, 1H), 2.96-2.73 (m, 2H), 2.24-1.98 (m, 3H), 1.93-1.78 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.5, 129.9, 129.8, 128.9, 128.1, 126.6, 121.9, 30.9, 28.5, 27.4, 20.9.

1,2,3,4-Tetrahydronaphthalene-1-carboxylic acid.[2] This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.56 (brs, 1H), 7.26-7.07 (m, 4H), 3.86 (t, *J* = 5.7 Hz, 1H), 2.95-2.68 (m, 2H), 2.30-2.14 (m, 1H), 2.12-1.92 (m, 2H), 1.87-1.72 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 137.4, 132.6, 129.8, 129.6, 127.2, 126.0, 44.6, 29.2, 26.6, 20.5.

Ethyl 1,2,3,4-tetrahydronaphthalene-1-carboxylate. This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.01 (m, 4H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.82 (t, *J* = 5.7 Hz, 1H), 2.93-2.65 (m, 2H), 2.21-2.08 (m, 1H), 2.07-1.92 (m, 2H), 1.85-1.70 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 137.4, 133.6, 129.6, 129.4, 126.9, 125.9, 60.8, 45.0, 29.3, 26.8, 20.8, 14.4.

Diethyl 3,4-dihydronaphthalene-1,1(2*H*)-dicarboxylate.[3] This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.38 (m, 1H), 7.24-7.07 (m, 3H), 4.27-4.17 (m, 4H), 2.82 (t, *J* = 6.5 Hz, 2H), 2.47-2.38 (m, 2H), 1.91-1.79 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 137.2, 132.3, 130.6, 129.5, 127.7, 125.7, 61.8, 59.1, 31.2, 29.5, 20.0, 14.2.

3,4-Dihydro-1'*H*,2*H*-spiro[naphthalene-1,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-10). This compound was obtained analogously to that described for indane derivative. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (brs, 2H), 7.23 -7.07 (m, 3H), 7.00 (d, *J* = 7.1 Hz, 1H), 2.76 (t, *J* = 6.1 Hz, 2H), 2.28-2.19 (m, 2H), 1.96-1.84 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 173.3, 150.2, 138.2, 132.8, 129.4, 128.4, 127.4, 126.2, 54.1, 33.9, 28.4, 18.5. HRMS: calculated for $C_{13}H_{11}N_2O_3$ $[M-H]^+$: 243.0770; Found: 243.0763.

6,7,8,9-Tetrahydro-1'*H*-spiro[benzo[7]annulene-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-30). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.42 (brs, 2H), 7.24-7.05 (m, 3H), 6.99-6.87 (m, 1H), 3.08-2.87 (mz, 2H), 2.40-2.25 (m, 2H), 1.88-1.63 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 149.9, 142.2, 136.5, 131.1, 129.7, 127.6, 126.4, 60.3, 33.1, 31.0, 25.5, 20.6. HRMS: Calculated for $C_{14}H_{13}N_2O_3$ $[M-H]^+$: 257.0926; Found: 257.0926.

1'-Methyl-3,4-dihydro-1'*H*,2*H*-spiro[naphthalene-1,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-C-11). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (brs, 1H), 7.26-7.08 (m, 3H), 6.82 (d, *J* = 7.8 Hz, 1H), 3.34 (s, 3H), 2.90 (t, *J* = 6.2 Hz, 2H), 2.42-2.32 (m, 2H), 2.14-2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.7, 150.2, 138.5, 131.7, 130.4, 128.5, 127.8, 126.8, 55.6, 35.2, 28.8, 28.6, 18.9. HRMS: Calculated for $C_{14}H_{15}N_2O_3$ $[M-H]^+$: 257.0926; Found: 257.0929.

1'-Methyl-6,7,8,9-tetrahydro-1'*H*-spiro[benzo[7]annulene-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (BW-B-30). This compound was obtained analogously to that described for indanone derivative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.25-7.08 (m, 3H), 6.81 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H), 3.20-3.00 (m, 2H), 2.50-2.37 (m, 2H), 2.02-1.82 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 171.8, 145.0, 142.6, 135.6, 132.1, 129.3, 128.7, 126.8, 61.7, 34.7, 31.9, 28.4, 26.1, 21.2. HRMS: Calculated for $C_{15}H_{17}N_2O_3$ $[M+H]^+$: 273.1239; Found: 273.1185.

SUPPORTING INFORMATION

The supporting information is available free of charge. Figure S1, X-ray structure of BW-B-15-2 and Figure S2: Structures of all synthesized and characterized spiro-barbiturates in this work; ^{1}H, ^{13}C and HR MS spectra for all new synthesized compounds.

AUTHOR CONTRIBUTION

Project design: K.S.B and K.W.M; Search for funding: K.W.M Chemical syntheses and compound characterization, D.K. and B.W.; bioassays, X.Z. , manuscript writing and revision, K.W.M, K.W.M, D.K. All authors have given approval to the final version of the manuscript.

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NOTES

The authors declare no competing interests.

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ABBREVIATIONS

CNS, central nervous system; DMSO, methyl sulfoxide; EC_{50}, concentration required for 50% of full effect; GABA, γ-aminobutyric acid; GABAAR, GABAA-type receptor; ECD: Extracellular domain; TMD: Transmembrane domain; IC_{50}, concentration required for 50% of full inhibitory effect; nH, Hill coefficient; SD, standard deviation; TFD, trifluoromethyldiazirine; rac: racemic; BDZ: benzodiazepine; NAL, Null Allosteric Ligand; PAM, Positive Allosteric Ligand; NAM, Negative Allosteric Ligand; MPPB, 1-methyl-5-phenyl-5-propylpyrimidine-2,4,6(1H,3H,5H)-trione; MPAB: 5-allyl-1-methyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione; R-mTFD-MPAB, (R)-5-allyl-1-methyl-5-(3-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2,4,6(1H,3H,5H)-trione; S-

mTFD-MPAB, (S)-5-allyl-1-methyl-5-(3-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2,4,6(1H,3H,5H)-trione; R-mTFD-MPPB, (R)-1-methyl-5-propyl-5-(3-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2,4,6(1H,3H,5H)-trione; S-mTFD-MPPB, (S)-1-methyl-5-propyl-5-(3-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2,4,6(1H,3H,5H)-trione; EtOAc, ethyl acetate; DCM: dichloromethane; THF: tetrahydrofuran; DMF: dimethylformamide; EtOH: ethanol; Hex: hexane; iPrOH: isopropanol; $SOCl_2$: thionyl chloride; $AlCl_3$: aluminum chloride; LHMDS: Lithium bis(trimethylsilyl)amide; EtONa: sodium ethoxide; r.t.: room temperature; o/n: overnight; PPh_3: triphenylphosphine; NaCN: sodium cyanide

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