

## Supplementary data of:

# Image-based marker-free screening of GABA agonists, antagonists and modulators

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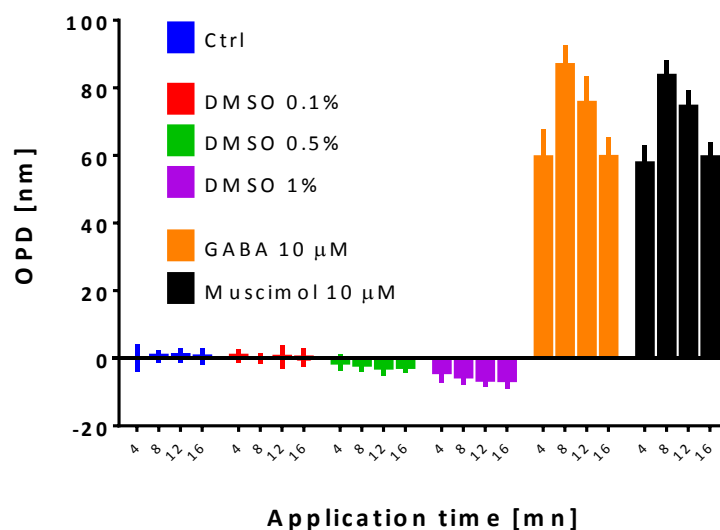
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| #  | Compound                                                                                                                      | Class                     | Original seed                 | Therapeutic group                              | Mechanism of action                                                                                                              |
|----|-------------------------------------------------------------------------------------------------------------------------------|---------------------------|-------------------------------|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 1  | Bemegride                                                                                                                     | Prestw original name seed |                               | CNS stimulant, Antidote, Respiratory stimulant | GABA antagonist                                                                                                                  |
| 2  | THIP Hydrochloride                                                                                                            | Prestw original name seed |                               | GABAergic Agonist                              | Schizophrenia                                                                                                                    |
| 3  | Thiocolchicoside                                                                                                              | Prestw original name seed |                               | Muscle relaxant, Spasmolytic                   | GABA agonist ?                                                                                                                   |
| 4  | Tracolate hydrochloride                                                                                                       | Prestw original name seed |                               | Anxiolytic, Anticonvulsant, Sedative           | GABA receptor modulator                                                                                                          |
| 5  | Ivermectin                                                                                                                    | Prestw original name seed |                               | Anthelmintic                                   | GABA ligand                                                                                                                      |
| 6  | Vigabatrin                                                                                                                    | Prestw original name seed |                               | Anticonvulsant                                 | GABA transaminase inhibitor                                                                                                      |
| 7  | Picrotoxin                                                                                                                    | Prestw original name seed |                               | Analeptic                                      | GABA channel blocker                                                                                                             |
| 8  | Clozapine                                                                                                                     | Prestw original name seed |                               | Antipsychotic                                  | 5-HT antagonist, Dopamine antagonist, GABA ligand                                                                                |
| 9  | Avermectin B1a                                                                                                                | Prestw original name seed |                               | Anthelmintic                                   | Ligand GABA receptors                                                                                                            |
| 10 | Topiramate                                                                                                                    | prestw original name seed |                               | anticonvulsant, antimigraine                   | enhances GABA-activated chloride channels, inhibits excitatory neurotransmission, through actions on kainate and AMPA receptors  |
| 11 | Chlorprothixene hydrochloride                                                                                                 | Prestw original name seed |                               | Neuroleptic, Antiemetic, Antihistaminic        | D2 dopamine receptor antagonist, GABAA receptors antagonist                                                                      |
| 12 | Viloxazine hydrochloride                                                                                                      | Prestw original name seed |                               | antidepressant                                 | up-regulation of GABA B receptors                                                                                                |
| 13 | Pentylenetetrazole                                                                                                            | Prestw original name seed |                               | CNS stimulant                                  | GABA antagonist                                                                                                                  |
| 14 | Acamprosate calcium                                                                                                           | Prestw original name seed |                               | Alcohol dependence treatment                   | blocking glutamatergic N-methyl-D-aspartate receptors, GABA type A receptors activation                                          |
| 15 | Baclofen (R,S)                                                                                                                | Prestw original name seed |                               | Antispasmodic                                  | GABA <sub>B</sub> agonist                                                                                                        |
| 16 | Zaleplon                                                                                                                      | Prestw original name seed |                               | sedative<br>hypnotic                           | binds to the benzodiazepine site on the $\alpha 1$ containing GABAA receptors which produces its therapeutic hypnotic properties |
| 17 | Etomidate                                                                                                                     | Prestw original name seed |                               | Hypnotic                                       | Gabaergic receptor agonist                                                                                                       |
| 18 | Gabazine bromide                                                                                                              | Prestw original name seed |                               |                                                | Antagonist GABAA                                                                                                                 |
| 19 | Gabapentin                                                                                                                    | Prestw original name seed |                               | Anticonvulsant                                 | GABA receptor ligand                                                                                                             |
| 20 | Valproic acid                                                                                                                 | Prestw original name seed |                               | anti-epileptic                                 | GABA transaminase inhibition                                                                                                     |
| 21 | Progabide                                                                                                                     | Others                    |                               |                                                | agonist GABAA and GABA <sub>B</sub>                                                                                              |
| 22 | Tiagabine Hydrochloride                                                                                                       | Others                    |                               |                                                | blocks GABA uptake                                                                                                               |
| 23 | Picrotoxin                                                                                                                    | Others                    |                               |                                                | antagonist GABAA                                                                                                                 |
| 24 | Picamilon                                                                                                                     | Others                    |                               |                                                | agonist GABAA                                                                                                                    |
| 25 | DIDS                                                                                                                          | Others                    |                               |                                                | anion exchange inhibitor                                                                                                         |
| 26 | Gabazine                                                                                                                      | Others                    |                               |                                                | antagonist GABAA                                                                                                                 |
| 27 | Saclofen                                                                                                                      | Others                    |                               |                                                | antagonist GABA <sub>B</sub>                                                                                                     |
| 28 | Piperidine-4-sulfonic acid                                                                                                    | Others                    |                               |                                                | agonist GABAA                                                                                                                    |
| 29 | (+)-Bicuculline                                                                                                               | Others                    |                               |                                                | antagonist GABAA                                                                                                                 |
| 30 | Isoгууvacine hydrochloride                                                                                                    | Others                    |                               |                                                | agonist GABAA                                                                                                                    |
| 31 | Muscimol                                                                                                                      | Others                    |                               |                                                | agonist GABAA                                                                                                                    |
| 32 | 3-(4-chlorophenyl)pentanedioic acid, 80<90%                                                                                   | Search (Maybridge)        | Baclofen (R,S)                |                                                |                                                                                                                                  |
| 33 | N1-(tert-butyl)-3,3-dimethylbutanamide                                                                                        | Search (Maybridge)        | Bemegride                     |                                                |                                                                                                                                  |
| 34 | disodium 5-nitro-2-[2-(4-nitro-2-sulfonatophenyl)vinyl]benzenesulfonate                                                       | Search (Maybridge)        | DIDS                          |                                                |                                                                                                                                  |
| 35 | 2-aminoheptanedioic acid                                                                                                      | Search (Maybridge)        | Gabapentin                    |                                                |                                                                                                                                  |
| 36 | 6,8-dibromo-3-(4-fluorophenyl)-2-methyl-3,4-dihydroquinazolin-4-one                                                           | Search (Maybridge)        | Methaqualone                  |                                                |                                                                                                                                  |
| 37 | 6-bromo-3-(3,4-dichlorophenyl)-2-methyl-3,4-dihydroquinazolin-4-one                                                           | Search (Maybridge)        | Methaqualone                  |                                                |                                                                                                                                  |
| 38 | 3-(2-ethylphenyl)-2-mercaptopurine-4(3H)-one                                                                                  | Search (Maybridge)        | Methaqualone                  |                                                |                                                                                                                                  |
| 39 | 2-[8,11-dimethoxy-4,5,10,11-tetrahydro-2H-indol[7a,1-ajisoquinolin-7-yl]oxy]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol | Search (Maybridge)        | Thiocolchicoside              |                                                |                                                                                                                                  |
| 40 | N1-(1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[aj]heptalen-7-yl)acetamide                                            | Search (Maybridge)        | Thiocolchicoside              |                                                |                                                                                                                                  |
| 41 | 2,2-dimethylpentane-1,3,5-tricarboxylic acid                                                                                  | Search (Maybridge)        | Valproic acid                 |                                                |                                                                                                                                  |
| 42 | 5-methylbicyclo[3.3.1]nonane-1-carboxylic acid                                                                                | Search (Maybridge)        | Valproic acid                 |                                                |                                                                                                                                  |
| 43 | cyclopentane-1,2-dicarboxylic acid                                                                                            | Search (Maybridge)        | Valproic acid                 |                                                |                                                                                                                                  |
| 44 | 7-[3-(trifluoromethyl)phenyl]pyrazolo[1,5-a]pyrimidine-3-carbonitrile                                                         | Search (Maybridge)        | Zaleplon                      |                                                |                                                                                                                                  |
| 45 | Ethosuximide                                                                                                                  | Search (Prestwick)        | Bemegride                     |                                                |                                                                                                                                  |
| 46 | Zuclopenthixol hydrochloride                                                                                                  | Search (Prestwick)        | Chlorprothixene hydrochloride |                                                |                                                                                                                                  |
| 47 | Pimethixene maleate                                                                                                           | Search (Prestwick)        | Chlorprothixene hydrochloride |                                                |                                                                                                                                  |
| 48 | Tianexamic acid                                                                                                               | Search (Prestwick)        | Gabapentin                    |                                                |                                                                                                                                  |
| 49 | Aminocaproic acid                                                                                                             | Search (Prestwick)        | Gabapentin                    |                                                |                                                                                                                                  |
| 50 | Nystatine                                                                                                                     | Search (Prestwick)        | Ivermectin                    |                                                |                                                                                                                                  |
| 51 | Amphotericin B                                                                                                                | Search (Prestwick)        | Ivermectin                    |                                                |                                                                                                                                  |
| 52 | Colchicine                                                                                                                    | Search (Prestwick)        | Thiocolchicoside              |                                                |                                                                                                                                  |
| 53 | Oxprenolol hydrochloride                                                                                                      | Search (Prestwick)        | Viloxazine hydrochloride      |                                                |                                                                                                                                  |

Supplementary table 1: The “GABA library” composed of 53 compounds primarily from the PCL and the Maybridge libraries. The *Original seed* column indicates which compound was used to find related compounds in the PCL and MHF. The *Therapeutic group* and *Mechanism of action* columns are from the PCL annotations.

## DMSO tolerance



Supplementary figure 1: DMSO tolerance. The effect of 0.1, 0.5 and 1% DMSO is compared to the response of 10 µM of GABA and muscimol. Marginal effects start to appear at 0.5% DMSO, well above the 0.1% DMSO concentration used during the screen.

| Name                                                                    | Score ago    | SD   | HIT ago    |
|-------------------------------------------------------------------------|--------------|------|------------|
| Muscimol                                                                | 1.02         | 0.06 | YES        |
| Piperidine-4-sulfonic acid                                              | 0.96         | 0.00 | YES        |
| Isoguvacine hydrochloride                                               | 0.91         | 0.02 | YES        |
| Etomidate                                                               | 0.90         | 0.01 | YES        |
| Avermectin B1a                                                          | 0.86         | 0.15 | YES        |
| Ivermectin                                                              | 0.85         | 0.09 | YES        |
| Tracazolate hydrochloride                                               | 0.58         | 0.02 | YES        |
| THIP Hydrochloride                                                      | 0.35         | 0.06 | YES        |
|                                                                         | Score modul  | SD   | HIT modul  |
| Zaleplon                                                                | 0.86         | 0.12 | YES        |
| Colchicine                                                              | 0.84         | 0.08 | YES        |
| Oxprenolol hydrochloride                                                | 0.47         | 0.04 | YES        |
| Acamprosate calcium                                                     | 0.45         | 0.05 | YES        |
| disodium 5-nitro-2-[2-(4-nitro-2-sulfonatophenyl)vinyl]benzenesulfonate | 0.44         | 0.01 | YES        |
| Amphotericin B                                                          | 0.41         | 0.02 | YES        |
| N1-(tert-butyl)-3,3-dimethylbutanamide                                  | 0.38         | 0.02 | YES        |
| cyclopentane-1,2-dicarboxylic acid                                      | 0.38         | 0.00 | YES        |
| Vigabatrin                                                              | 0.35         | 0.01 | YES        |
|                                                                         | Score antago | SD   | HIT antago |
| Gabazine bromide                                                        | 1.05         | 0.05 | YES        |
| (+)-Bicuculline                                                         | 0.99         | 0.01 | YES        |
| Gabazine                                                                | 0.87         | 0.12 | YES        |
| Thiocolchicoside                                                        | 0.84         | 0.04 | YES        |
| Picrotoxin                                                              | 0.64         | 0.03 | YES        |

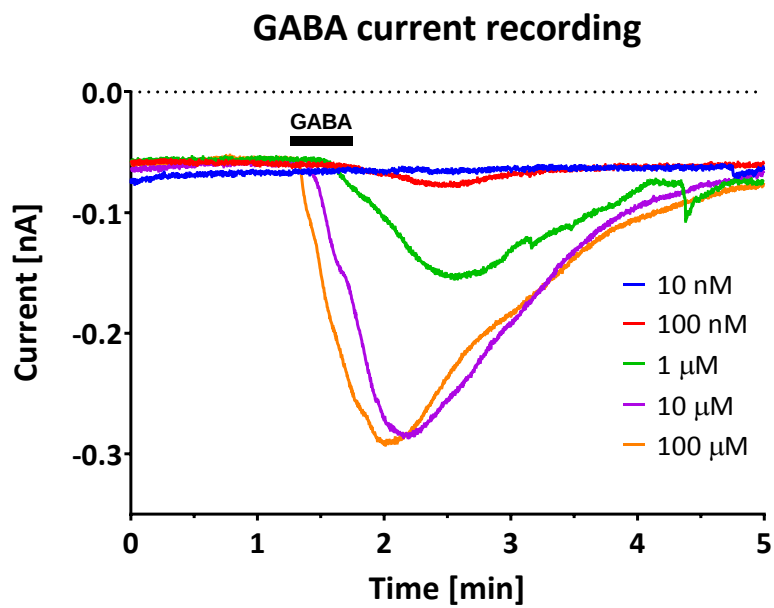
Supplementary table 2: Hits from the agonist/antagonist screen ranked according to their score. Agonist: 1 indicates the same effect as 10 µM GABA and 0 no effect. Antagonist: 1 indicates total inhibition (same as 0 µM GABA) and 0 indicates no inhibition (same response as 1.2 µM GABA, (EC<sub>70</sub> value)). Modulator, 1 indicates the same effect as 10 µM GABA and 0 no potentiation (same response as 0.4 µM GABA, (EC<sub>30</sub> value)). Modulators that also have a direct effect on GABA (all agonist hits with the exception of THIP) are not displayed.

| Compound                   | DHM                      |                |                            |                | Electrophysiology        |                |                           |                | Literature                                                                                 |                                                        |
|----------------------------|--------------------------|----------------|----------------------------|----------------|--------------------------|----------------|---------------------------|----------------|--------------------------------------------------------------------------------------------|--------------------------------------------------------|
|                            | EC <sub>50</sub><br>(μM) | 95% Conf. int. | Response ampl.<br>(% GABA) | 95% Conf. int. | EC <sub>50</sub><br>(μM) | 95% Conf. int. | Response ampl.<br>(% max) | 95% Conf. int. | EC <sub>50</sub><br>(μM)                                                                   | Response ampl.<br>(% GABA)                             |
| Muscimol                   | 0.24                     | 0.19 to 0.30   | 109                        | 102-105        | 0.18                     | 0.082 to 0.41  | 116                       | 90-142         | 3.8 <sup>1</sup> , 8.1 <sup>3</sup>                                                        | 97% <sup>3</sup>                                       |
| Ivermectin                 | 1.1                      | 0.87 to 1.5    | 58                         | 55-64          | 10                       | 6.1 to 18      | 50                        | 36-63          | 3 <sup>8</sup>                                                                             | N.A.                                                   |
| GABA                       | 1.2                      | 0.94 to 1.5    | 100                        | 92-108         | 1.2                      | 0.79 to 1.7    | 100                       | 84-116         | 20 <sup>1</sup> , 4.7 <sup>3</sup> , 8.3 <sup>4</sup> , 1.4 <sup>5</sup> , 12 <sup>6</sup> | N.A.                                                   |
| Avermectin B1a             | 1.3                      | 0.91 to 1.9    | 72                         | 62-79          | 13                       | 9.8 to 16      | 45                        | 40-50          | 1.9 <sup>9</sup>                                                                           | N.A.                                                   |
| Piperidine-4-sulfonic acid | 3.7                      | 2.8 to 5.0     | 94                         | 83-104         | 6.2                      | 3.7 to 10      | 89                        | 72-105         | 30 <sup>1</sup> , 4.6 <sup>3</sup> , 15 <sup>6</sup>                                       | 37% <sup>1</sup> , 38% <sup>3</sup> , 39% <sup>6</sup> |
| Etomidate                  | 5                        | 3.5 to 7.3     | 75                         | 66-87          | 10                       | 6.6 to 15      | 77                        | 62-93          | 3 <sup>10</sup>                                                                            | N.A.                                                   |
| Isoguvacine hydrochloride  | 7.8                      | 5.9 to 10      | 64                         | 58-72          | 9.6                      | 6.4 to 14      | 110                       | 89-132         | 48 <sup>6</sup> , 6 <sup>7</sup>                                                           | 95% <sup>6</sup>                                       |
| Trac唑ate hydrochloride     | 9.9                      | 7.9 to 12      | 43                         | 40-47          | 12                       | 7.0 to 21      | 49                        | 36-62          | 13.2 <sup>2</sup>                                                                          | N.A.                                                   |
| THIP Hydrochloride         | 11                       | 8.4 to 14      | 74                         | 66-81          | 9.3                      | 5.3 to 17      | 122                       | 89-156         | ~100 <sup>1</sup> , 3.8 <sup>3</sup> , 134 <sup>6</sup>                                    | 79% <sup>1</sup> , 76% <sup>3</sup> , 78% <sup>6</sup> |

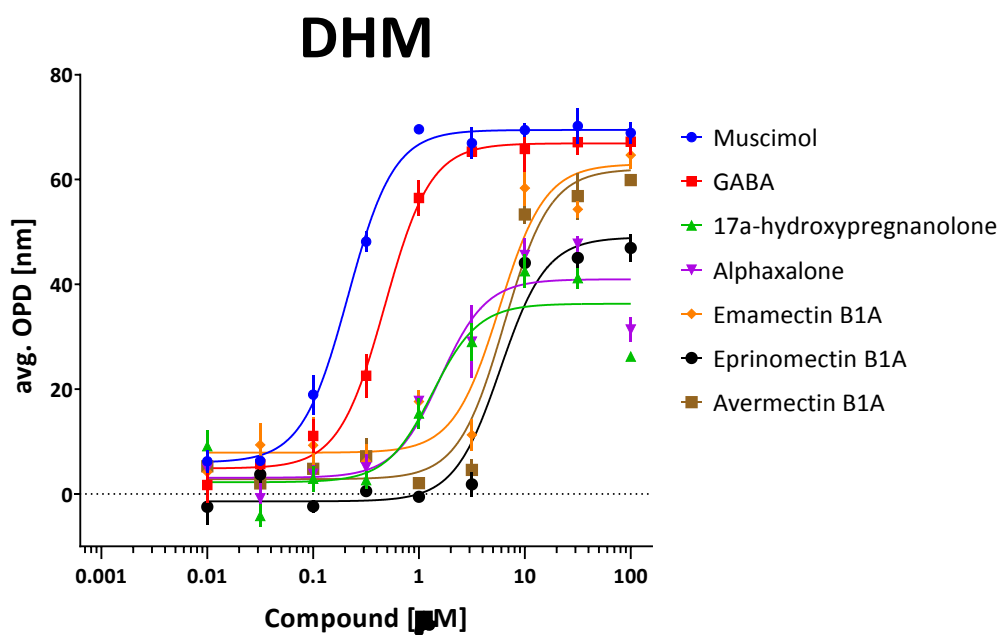
**Supplementary table 3: EC<sub>50</sub> ranking and amplitude of response measured with DHM and electrophysiology compared with the literature. N.A. not assessed.**

| CatalogID     | BatchID         | Name                                                              | Score | ScoreSD | HIT |
|---------------|-----------------|-------------------------------------------------------------------|-------|---------|-----|
| Riet_038      |                 | Muscimol                                                          | 0.965 | 0       | YES |
| STOCK1N-67743 | NP-IBS-20130627 | Avermectin B1A                                                    | 0.722 | 0.100   | YES |
| STOCK1N-56888 | NP-IBS-20130627 | 17α-hydroxypregnanolone                                           | 0.648 | 0.184   | YES |
| STOCK1N-51728 | NP-IBS-20130627 | Alphaxalone                                                       | 0.556 | 0.009   | YES |
| NP-008107     | 8/15/2013       | α-peltatin                                                        | 0.547 | 0.039   | YES |
| STOCK1N-65883 | NP-IBS-20130627 | Emamectin B1A                                                     | 0.539 | 0.267   | YES |
| NP-008106     | 8/15/2013       | Podophyllotoxin glucoside                                         | 0.528 | 0.089   | YES |
| STOCK1N-63866 | NP-IBS-20130627 | Eprinomectin B1A                                                  | 0.526 | 0.269   | YES |
| NP-008105     | 8/15/2013       | Teniposide related compound A                                     | 0.499 | 0.006   | YES |
| YB037         | 2014_05_21      | 2-iodo-N-(4-methoxyphenyl)-3-methylbenzamide                      | 0.460 | 0       | YES |
| NP-010309     | 8/15/2013       | Centaureidin                                                      | 0.402 | 0.153   | YES |
| STOCK1N-75293 | NP-IBS-20130627 | Vindesine                                                         | 0.322 | 0.002   | YES |
| STOCK1N-72689 | NP-IBS-20130627 | Colchicine                                                        | 0.315 | 0.035   | YES |
| NP-007926     | 8/15/2013       | Meleagrin                                                         | 0.311 | 0.003   | YES |
| YB124         | 2014_05_21      | 2-((4-cyano-2-(dimethylamino)phenyl)ethynyl)-N-(p-tolyl)benzamide | 0.283 | 0       | YES |

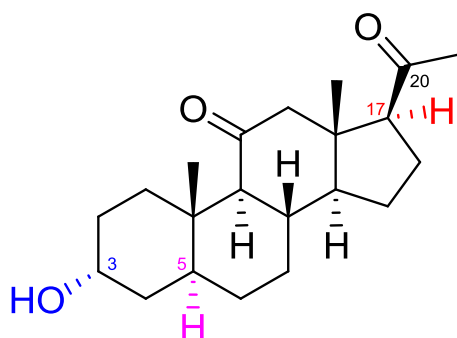
**Supplementary table 4: List of the 15 hits obtained in the blind primary screen of natural products and the Swiss chemical collections assayed at 10 μM, along with their associated score and standard deviation between the replicate (a value of zero indicate a single measure due to an error (pipetting/reading, etc.))**



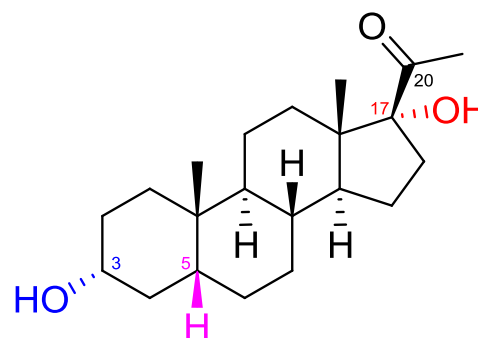
Supplementary figure 2: Typical current recordings at different GABA concentrations.



Supplementary figure 3: Dose-response curves of the 5 validated hits and GABA.

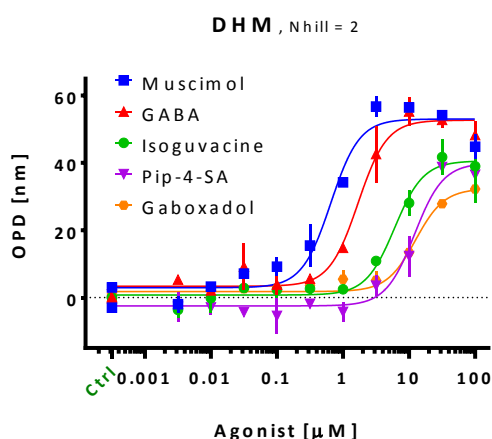


Alphaxalone



Hydroxypregnanolone

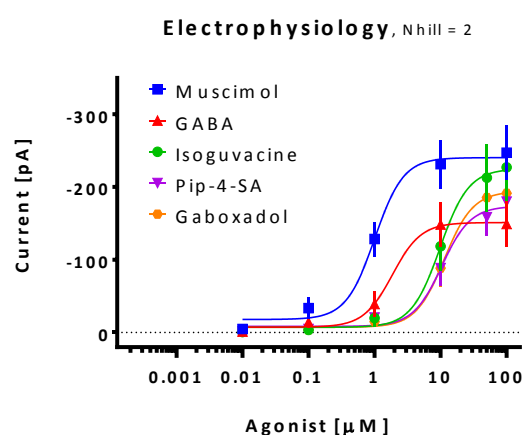
Supplementary figure 4: Structural requirements for neurosteroid modulation of GABA<sub>A</sub> receptors (adapted from <sup>11</sup>): 3α OH Hydrogen bond-donating group on A steroid ring. Hydrogen-bond accepting group (typically a keto moiety) on the D ring at either C20 of the pregnane steroid side chain or C17 of the androstane ring system. C5 Hydrogen orientation appears to be essential for increased potency, but less critical for activity.



|                  | Muscimol | GABA | Isoguvacine | Pip-4-SA | Gaboxadol |
|------------------|----------|------|-------------|----------|-----------|
| EC <sub>50</sub> | 0.65     | 1.7  | 6.0         | 12       | 13        |

95% Confidence Intervals

|                  | Muscimol     | GABA       | Isoguvacine | Pip-4-SA  | Gaboxadol |
|------------------|--------------|------------|-------------|-----------|-----------|
| EC <sub>50</sub> | 0.46 to 0.93 | 1.2 to 2.4 | 4.0 to 9.1  | 8.6 to 17 | 8.9 to 18 |



|                  | Muscimol | GABA | Isoguvacine | Pip-4-SA | Gaboxadol |
|------------------|----------|------|-------------|----------|-----------|
| EC <sub>50</sub> | 1.0      | 1.9  | 9.8         | 10       | 11        |

95% Confidence Intervals

|                  | Muscimol    | GABA        | Isoguvacine | Pip-4-SA  | Gaboxadol |
|------------------|-------------|-------------|-------------|-----------|-----------|
| EC <sub>50</sub> | 0.60 to 1.7 | 0.70 to 4.9 | 5.2 to 19   | 6.4 to 17 | 5.8 to 22 |

Supplementary figure 5: DHM vs electrophysiology EC<sub>50</sub> ranking. HEK-GABA expressing the α5β3γ2 receptor subtype.

## Supplementary references

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