

# Exploring standardized zembrin<sup>®</sup> extracts from the south african plant *sceletium tortuosum* in dual targeting phosphodiesterase-4 (pde-4) and serotonin reuptake inhibition as potential treatment in schizophrenia

## Abstract

Converging evidence suggests that Disrupted in Schizophrenia (DISC-1) is a recognized risk gene for schizophrenia. The interaction of DISC-1 with PDE-4 (Phosphodiesterase Subtype-4) plays a crucial role in neurodevelopment and neuro-inflammation in schizophrenia. Serotonin has drawn increased attention for its role in modulating cognition function. Hence dual targeting Selective Serotonin Reuptake site (SSRI) and PDE-4 may offer novel therapeutic paradigm towards augmenting antipsychotics in schizophrenia. Translational studies show that the family of mesembrane-related alkaloids isolated from the South African plant: *Sceletium tortuosum* have been shown to hit dual targets: SSRI and PDE-4 allosteric site. The promising findings warrant a proof-of-concept randomized controlled study Zembrin extract in schizophrenia.

**Keywords:** schizophrenia, disc-1 gene, serotonin, phosphodiesterase, zembrin extract

Volume 6 Issue 5 - 2017

Simon Chiu,<sup>1,4</sup> Hana Raheb,<sup>1</sup> Kristen Terpstra,<sup>1,5</sup> Josh Vaughan,<sup>1,6</sup> Autumn Carrie,<sup>1,7</sup> Michel Farina-Woodbury,<sup>2</sup> Farina Woodbury,<sup>1,8</sup> Zack Cernovsky,<sup>1,4</sup> Jirui Hou,<sup>1</sup> Vladimer Badmeav,<sup>9</sup> Mujeeb Shad,<sup>10</sup> Zach Suntras,<sup>8</sup> Nigel Gericke<sup>11</sup>

<sup>1</sup>Lawson Health Research Institute, Canada

<sup>2</sup>Department of Psychiatry, University of Puerto Rico, USA

<sup>3</sup>Department of Psychiatry, University British Columbia: University of Victoria medical campus, Canada

<sup>4</sup>Department of Psychiatry, University of Western Ontario, Canada

<sup>5</sup>Nursing Program, University of Toronto Faculty of Nursing, Canada

<sup>6</sup>Biomedical Sciences Honors Program, University Western Ontario, Canada

<sup>7</sup>Kinesiology Honors Program, University Western Ontario, Canada

<sup>8</sup>Northern Ontario Medical School, Thunderba, Canada

<sup>9</sup>Formerly Director of Medical and Scientific Affairs, USA

<sup>10</sup>Oregon Health & Science University, USA

<sup>11</sup>Department Psychiatry, Medical Director and founding Director of HG&H Pharmaceuticals (Pty) Ltd, South Africa

**Correspondence:** Simon Chiu, Lawson Health, Research Institute 268 Grosvenor Street; Lab FBI25, London Ontario N6A 4V2 Canada, Email [schiu3207@rogers.com](mailto:schiu3207@rogers.com)

**Received:** March 12, 2017 | **Published:** May 1, 2017

## Introduction

In schizophrenia treatment (SCZ) while the second generation of atypical antipsychotics are associated with fewer extra pyramidal motor side effects, the atypical antipsychotics prove to be no superior than the 1st generation of antipsychotics. The overall long term outcomes in area of social and vocational domains remain unfavourable.<sup>1</sup> Cognitive impairment precedes the emergence of positive and negative symptoms and appears to persist throughout the course of the disorder.<sup>2,3</sup> Expert consensus concludes that the negative outcomes are strongly related to the severity of impaired neuro-cognition (INC). The recent NIMH initiative, MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) outlines a strategic plan to address the issue through identifying promising molecular targets for future drug development.<sup>4,5</sup> Pharmacological interventions targeting various neurotransmitter networks: GABA, glutamate, dopamine (DA), serotonin (5-HT), norepinephrine have met with mixed findings.<sup>6</sup>

The issue of enduring negative symptoms carries equal weight in determining the overall quality of life in schizophrenia population. The primary deficit syndrome, defined as a disease entity characterized by the presence of primary enduring negative symptoms, appears to be relatively refractory to the newer atypical antipsychotics.<sup>7</sup> Primary negative symptoms are common in around 26% of the schizophrenia patient population and as high as 58% of the schizophrenia outpatients are estimated to experience the negative consequences of negative symptoms.<sup>8</sup> A recent systematic review of the pharmacological treatments in negative symptoms,<sup>9</sup> highlight the new approaches in targeting: 1) enhancing N-methyl-D-aspartic acid (NMDA) function through glycine binding site or glycine uptake inhibitor; 2) metabotropic glutamate receptor: mGluR2/3) allosteric modulator; 3) nicotinic cholinergic receptor. It has become a high priority to adopt a new approach to address the issues of neuro-cognition and negative symptoms. Recently, there has been increased interest in the emerging role of cAMP signaling in modulating neuro-cognition schizophrenia.<sup>10</sup>

## DISC-1 gene and PDE: role in schizophrenia

Converging gene evidence suggests that the gene, Disrupted-in-Schizophrenia-1 (DISC1, MIM605210) is highly implicated as one of the important group of risk genes for schizophrenia.<sup>10,11</sup> The DISC locus was originally discovered in a large Scottish family pedigree with a balanced translocation (q42.1, q14.3); the chromosomal translocation co-segregates significantly not only in SCH, bipolar disorder (BPD) and severe recurrent depression.<sup>12</sup> Since then, various genetic association studies have replicated the original translocation family study. Genetic variants: single nucleotide polymorphisms (SNP) have been identified in a large sample from European population.<sup>13</sup> A meta-analysis of 9 different schizophrenia samples from different European populations identified 50 SNPs and found evidence for a common interval with the DISC1intron 4-6 defining the schizophrenia risk.

Significant advances have been made to delineate the role of DISC-1 in encoding a multifunctional scaffolding protein that specifically regulates 2nd messenger: cyclic adenosine monophosphate (cAMP) signaling via interactions with phosphodiesterase 4 (PDE4).<sup>10,14</sup> There is a growing body of evidence in support of the regulatory role of DISC-1 in neurodevelopment and neuro plasticity.<sup>15</sup> Dysregulation of synaptic plasticity due to SCZ at risk gene may be the underlying pathophysiology of SCZ. DISC-1 regulates neurogenesis, neuronal migration, dendritic arborization, and integration of cortical and hippocampal neurons. The family of phosphodiesterase hydrolyzes intracellular cAMP. More importantly, PDE4B has been shown to bind with DISC1.<sup>12</sup> Two DISC-1 strains of mutant mice have been demonstrated to exhibit reduced binding capacity and affinity to PDE-4B while exhibiting behavioral changes resembling SCZ and depression symptoms.<sup>16</sup>

There has been growing interest in the crucial role of cAMP-response element binding protein (CREB), coupled with the intracellular second messengers: cAMP and cGMP, in regulating multiple gene expressions.<sup>17-19</sup> Both cAMP and cGMP signal cascades are involved in neuronal survival, apoptosis, and synaptic strength and plasticity and neural network connectivity in CNS. In this respect the superfamily of 11 phosphodiesterases (PDE) widely distributed in the mammalian brain function regulate and fine-tune the homeostasis of cAMP-cGMP via sensing the intracellular levels of cyclic nucleotides.<sup>20</sup> There are 11 members of PDE- family: PDE-1, PDE-2, PDE-3, PDE -4, PDE-5, PDE-6, PDE-7, PDE-8, PDE -9, PDE-10 and PDE-11 with their characteristic substrate-specificities, tissue distribution and physiological functions. The PDE family has a total of 21 isoforms with multiple gene splice variants that encode more than 50 proteins expressed in mammalian cells. There is *in vitro* evidence that release of DISC-1-mediated transcription repression of PDE4D9 serves as a paradigm of feedback inhibition to inhibit dopaminergic signaling.<sup>21</sup> High levels of cAMP during stress exposure impaired prefrontal cortex (PFC) function and is consistent with the link of DISC1 with the negative impact of stress in neurodevelopment.<sup>22</sup>

PDE inhibitors are active in models of positive and negative symptoms of SCZ. PDE-4B knockout mice exhibited behavioral changes equivalent to negative symptoms and cognition deficits: decreased locomotor activity and attenuated prepulse inhibition accompanied by reduced dopamine and serotonin turnover.<sup>23</sup> Transgenic mice with forebrain restricted expression of mutant human DISC1 ( $\Delta$ hDISC1) showed abnormalities in oligodendrocyte (OLG) differentiation and OLG gene expression deficits.<sup>24</sup> premature OLG differentiation and increased proliferation of their progenitors

were identified. This finding suggests that PDE may be involved in remodelling of myelination defects in SCH.

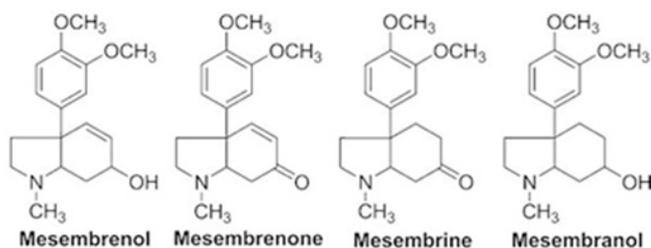
Rolipram, the PDE4 inhibitor, improved memory consolidation, working memory and information processing in rodent species subject to a variety of cognitive tasks: radial arm maze, passive avoidance, delayed arm water and visual -spatial tasks, working memory and information.<sup>25-28</sup> In the macaque monkey species, rolipram improved object retrieval performance suggesting positive interaction with executive function.<sup>26</sup> Furthermore, rolipram antagonized phencyclidine (PCP)-induced hyperactivity and cognitive deficits, reversed amphetamine -induced pre- pulse inhibition and facilitated extinction of cocaine-induced place preference.<sup>29,30</sup> The mechanism of the pro-cognitive action of PDE-4 inhibitor, rolipram, may be related to the co -expression of PDE-4 with DARPP -32 in D1 receptor-positive cortical pyramidal neurons in layer VI.<sup>31</sup> Rolipram was active in the sensori-motor gating test.<sup>31</sup> which may be explained by the enhanced DARPP-32 phosphorylation invoked by D1 receptor activation in the frontal cortex. Recent translational studies of PDE have also focused attention on PDE-10 and PDE-2A as putative targets for pro-cognitive strategy in SCZ and neurodegenerative disorders: Alzheimer dementia.<sup>33,34</sup> None of the PDE -10A or PDE-2 is in Phase II/III clinical trials in SCZ. Taken together, elevated cAMP contributes towards cognition deficits mediated through the prefrontal cortex and targeting PDE-4- phosphorylated cAMP Response-Element-Binding protein (CREB) (19) can be beneficial in SCZ.

Converging evidence suggests that cognitive impairment in schizophrenia is related to neuro-inflammation. The anti-inflammatory action of the prototypal PDE-4 inhibitor, roflumilast, currently indicated for the treatment of chronic obstructive pulmonary disease (COPD) is relevant to our understanding of the emerging role of PDE-4 in cognition.<sup>35</sup> Genetic knock- down of PDE-4D enzyme: PDE4d (-/-) mice displayed behavioral phenotype resembling anti-depressant treatment exhibited resistance to depression in the classical Porsolt force swim test and in the hyperactivity of the acoustic startle response.<sup>36</sup> Rolipram exhibits anti-depressant effect and reverses  $\beta$ -induced cognitive impairment and neuro-inflammatory and apoptotic responses in rats via PDE-4D or PDE-4B inhibition.<sup>37</sup> Cyclic AMP signaling stands at the cross-roads of immune cell (microglia) activation and inflammation. In the glia, pro-inflammatory cytokines are involved in regulating PDE-4-cAMP signaling in the glia; whereas PDE-cAMP-cascade can in turn modulate cytokine production: the family of interleukins and alpha-tumor necrosis factor.<sup>38</sup> The cross-talks between cytokine production and immune activation are part of the feedback loop coordinated through PDE-4-cAMP pathway.

Despite translational studies in support of the anti- psychotic efficacy of rolipram in schizophrenia and depressive disorders, serious adverse effects of nausea and vomiting have largely limited further therapeutic development. A single dose-finding study in 6 schizophrenic patients found an initial improvement of psychotic symptoms during the first week; however, psychotic symptoms recurred in parallel with serious nausea and vomiting.<sup>39</sup> Rolipram appeared to exhibit anti-depressant activity in major depressive disorder.<sup>40</sup>

**Zembrin extract and PDE- 4 allosteric modulator/5-HT Reuptake inhibitor:** In South Africa, extracts from South African succulent plant, *Sceletium tortuosum*, has been used for centuries as an orally available indigenous medicine, and for promoting well-being, enhancing mood and reducing stress.<sup>41-43</sup> The standardized extract S, *tortuosum*: Zembrin extract was developed by HG&H Pharmaceuticals Ltd. Bryanston, South Africa.

The cognitive enhancing effects of the key ingredients of Zembrin® extract are explained largely by the efficacy of alkaloids in competing for the PDE-4 binding. The chemical structures of mesembrenone alkaloids are fully characterized (Figure 1). In the PDE assay system using the human recombinant PDE-4B expressed in Sf9 cells), the extract Selenium tortuosum (Zembrin®) selectively inhibited phosphodiesterase 4 (PDE4) with IC<sub>50</sub> (inhibitory constant) value of 8.5µg/ml. Mesembrenone, a major alkaloid isolated from the extract (Figure 1) was the most active in inhibiting PDE4 with an IC<sub>50</sub> value of <1µM (44). Mesembrenone was 17 times more potent than mesembrine and 34 times more active than mesembranol. The IC<sub>50</sub> values for mesembrenone were 470nM, followed by mesembrine: 7800nM and mesembranol: 10,000 nM. Structure-Activity Relationship (SAR) studies show that cognition is likely to be selectively mediated via the long isoform of PDE-4D3.



**Figure 1** Chemical structures of alkaloids isolated from *Sceletium tortuosum*: mesembrine, mesembrenol, mesembrenone, mesembranol.

Mesembrenone alkaloids distinguish from other putative PDE-4 modulators in exhibiting concomitant serotonergic property. The serotonergic property of mesembrine alkaloids synergizes with cAMP signal pathway in mediating the cognitive effects. Mesembrine was found to be the most potent compound in binding to the serotonin transporter site (5-HT site) with Hill coefficient of 1.4 nM. Mesembrenone and mesembranol were found to be much weaker in the 5-HT transport site *in vitro*.<sup>44</sup> The efficacy of Selective Serotonin Reuptake Inhibitors (SSRI) in the treatment of anxiety and depressive disorders is well documented.<sup>45</sup> More recently escitalopram, the putative SSRI, has been shown to improve cognition and facilitate post-stroke recovery.<sup>46</sup> Taken together, there is growing evidence to substantiate the role played by serotonin in decision making process within the cognitive domain of executive function.<sup>47</sup> High central serotonin levels are functionally associated with improved reversal learning, enhanced attentional set shifting and response inhibition while reducing delay discounting. Preliminary evidence suggests that drug development platform based upon serotonin receptor subtypes: 5-HT (1), 5HT (4), and 5-HT (6) may hold promise for the treatment of schizophrenia.<sup>48</sup>

Rolipram, despite its efficacy in SCH and depression models, fail to advance to final phases of clinical trial because of the serious side effects of nausea and vomiting. Investigators explore matching the preferred conformation at the regulatory site of PDE-4 with the potential candidates in designing a series of PDE-4 allosteric modulators.<sup>49</sup> It is perhaps fortuitous the HG & H Pharmaceuticals (Pty) Ltd discovered that the South Africa legendary plant, *Sceletium tortuosum* exhibited intrinsic activity as a PDE-4 allosteric modulator but devoid of serious emetic effects.<sup>41</sup> In this respect, the family of mesembrane-related alkaloids from Zembrin extract find the respected niche in comparison with the group of patented PDE-4 allosteric modulators with therapeutic potential for the treatment of schizophrenia, Alzheimer's dementia and depression: EHT0202 (etiolate hydrochloride).<sup>50</sup> L-454,560.<sup>51</sup> GEPR-7-b, derivative of rolipram.<sup>52</sup>

The functional MRI (fMRI) study conducted in Europe demonstrated for the first time that Zembrin passes through the BBB (Blood-Brain Barrier) to modify the functional responses towards environmental cues capable of generating fear.<sup>53</sup> In a double-blind, placebo-controlled, cross-over design, 16 healthy participants were scanned during performance in a perceptual-load and an emotion-matching task. The reactivity of the brain region, amygdala, to fearful faces under low perceptual load conditions was attenuated after a single 25mg oral dose of Zembrin. Follow-up connectivity analysis on the emotion matching task showed that the amygdala-hypothalamus coupling was adversely affected. This fMRI study clearly shows that *S. tortuosum* (later formulated as the standardize Zembrin extract) attenuates the emotional responses towards subcortical threat circuitry. The result is consistent with the action of Zembrin extract in dual targeting SSRI and PDE-4 in the acute anxiolytic effects. Earlier, in the rodent model of restraint-induced anxiety and behavioral stress, *S. Tortuosum* at the lower dosage of 5-10 mg/kg when given by the oral lavage, exerted a marginal anxiolytic effect.<sup>54</sup> Species differences in dose-response relationship of Zembrin may explain the discrepant findings.

Gericke.<sup>55</sup> reported for the first time on three case studies of Zembrin in patients diagnosed as major depression disorder (n=2) and dysthymia with co-morbid personality disorder (n=1) and major depressive disorder. In the 1st case, the male patient responded to daily oral dosage of 50mg Zembrin tablet with significant sustained reduction in relatively severe depressive symptoms: insomnia, anhedonia, and suicidal ideation and mood fluctuations. During the first week, transient anxiety symptoms were found. In the second case, the female patient reported that "her mood had lifted, feeling more focused and less anxious" within 10 days of starting on 50mg Zembrin tablet. Her hypersomnia was also resolved, in the third case, the patient who was non-responsive to St. John's Wort (*hypericum perforatum*) extract reported improvement in her mood. She noted that her hypersomnia amounting to 14 hr per day was changed to her normal pattern of 8hrs/day.

Recently, Chiu et al examined the cognitive effects of the standardized extract *Sceletium tortuosum* (abbreviated Zembrin®) in a RCT study of a cohort of cognitively healthy subjects (n=21, mean age: 54.6 yrs) administered Zembrin extract, at the daily oral dosage of 25mg, for three weeks.<sup>56</sup> The standardized extract *Sceletium tortuosum* (Zembrin®) was manufactured according to European Union Good Manufacturing practice (GMP).The extract was in the form of a fine dry powder with the dry plant material: extract ratio of 2:1, standardized to a total alkaloid content for the four main Selenium alkaloids (mesembrenone, mesembrenol, mesembrine and mesembranol) of 0.4%. The contents of the four alkaloids were quantified using High Pressure Liquid Chromatography (HPLC) analysis against validated analytical reference standards We demonstrated that Zembrin® treatment compared with the placebo group, selectively and significantly enhanced the cognitive measures of executive function (p< 0.032) and cognitive flexibility (p<0.022) in the computerized neurocognitive battery Vital Signs. Executive function encompasses higher-order cognitive processes: working memory, attention control, response inhibition and concept formulation, and is thought to be primarily driven by the prefrontal cortex. Zembrin® also improved processing speed, psychomotor speed and complex attention.

The subjective ratings of generalized well-being and positive mood states in our study.<sup>56</sup> agrees well with a recent safety and tolerability study on Zembrin® in healthy subjects who reported "uplifted spirits" and better coping with stress and depressing events.<sup>57</sup> Furthermore,

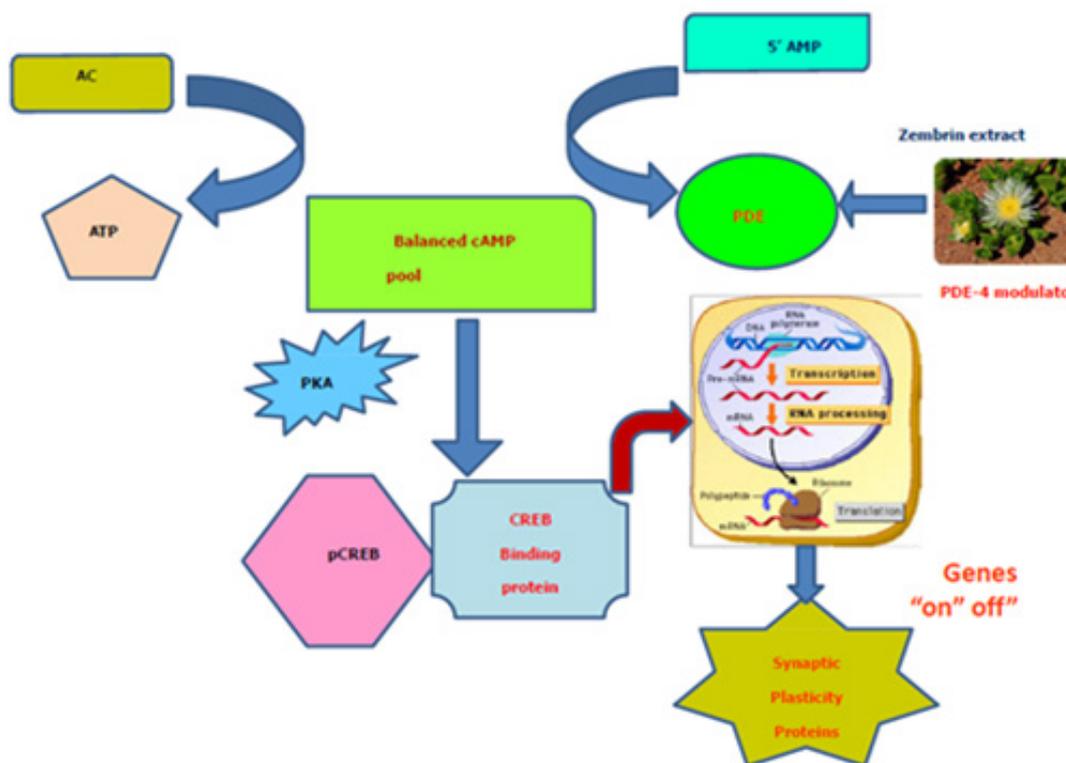
subjects taking Zembrin® reported improvement in the subjective quality of their sleep on the HAM -D subscale: the mean rating of sleep onset at baseline, 0.76 (SD = 1.14), changed to 0.43 (SD =0.93) at week 3 (paired t-test,  $t = 2.09$ ,  $df = 20$ ,  $p=0.049$ , 2-tailed). In both studies,<sup>53,56,57</sup> Zembrin® treated group found an overall improvement in the quality of sleep. In the three clinical studies in normal subjects,<sup>53</sup> Zembrin® was safe and well tolerated. There were no changes in blood pressure, pulse, temperature and weight in either the Zembrin® group or the placebo group. Intriguing enough, the study led by Neil et al.<sup>57</sup> found that the placebo group complained of increased frequency of adverse events than the Zembrin® group. The incidence of treatment emergent adverse events (TEAE) classed as “mild” and “moderate” was quite low

The pharmacological effects of Zembrin® in humans are consistent with the results of an EEG study in conscious freely moving rats.<sup>58</sup> The paradigm of electroencephalogram (EEG) was used to characterize the actions of Zembrin®. As a novel neurophysiological technique, EPG consists of frequency analysis of the field potentials in the presence of the active drug. The electrical signals were transmitted via the wireless mode and processed using a Fast Fourier Transformation (FFT). The frequency ranges are sensitive to the specific neurotransmitter system and spectral power was evaluated over the 8 frequency ranges: delta, theta, alpha, beta1a, beta1b, beta2, gamma power. Zembrin® at the oral dosage of 2.5mg/kg, 5mg/kg and 10 mg/kg, significantly reduced

alpha2 and beta1a which correlated with activation of dopaminergic and glutamatergic systems. Furthermore, Zembrin® attenuated the delta and theta frequency ranges which reflected changes in the cholinergic and norepinephrine systems as expected for drugs in neurodegenerative disorders. Zembrin induced the EEG changes typical of analgesic effects of standardized drugs being tested: theta wave reduction in common with the delta, alpha2 and beta1 attenuation. Zembrin® at 10mg/kg markedly attenuated theta waves which reflected the EEG effects of typical antidepressants.

## Conclusion and future directions

In summary, the findings from the translational studies support the potential pivotal roles of PDE-4/cAMP/CREB cascade and serotonin in schizophrenia and provide the pharmacological basis of exploring Zembrin extract via dual targeting both PDE- 4 and serotonin in improving the negative symptoms and cognition deficits in schizophrenia. The paradigm of exploring Zembrin extract in targeting PDe-4/cAMP/CREB signaling may present a novel direction in regulating gene expression in schizophrenia (Figure 2). The synergy of serotonin and PDE-4 have spinoffs on the likelihood of resetting the D-1 activation and NMDA hypo function, and warrant the design of randomized controlled trial of Zembrin extract in the cohort of treatment refractory schizophrenia exhibiting persistent negative symptoms and cognitive deficits.



**Figure 2** Model of PDE-4/cAMP/CREB Cascade in cognition.

Bi-directional regulation of cyclicAMP (cAMP) homeostasis is achieved through adenylate cyclase (AD) and phosphodiesterase (PDE) subtype 4. cAMP is activated via either PDE-4 inhibition or hormone/neurotransmitter-stimulated adenylate cyclase (AD). The cAMP-dependent protein kinase A (PKA), once activated through allosteric site, can phosphorylate cAMP response element binding protein (CREB) to form phosphorylated CREB: pCREB. pCREB associates with transcription co-activator; CREB Binding protein to initiate transcription and translation. The CREB-mediated gene expression contributes towards long- and short-term memory and synaptic plasticity. Zembrin extract modulates PDE-4 and participates in PDe- 4/cAMP/CREB cascade in cognition. The CREB-linked gene expression has been shown to be impaired in AD model. Abbreviations: ATP (Adenosine triphosphate); cAMP (Cyclic Adenosine monophosphate), Adenylate cyclase (AC), 5' AMP (5' adenosine monophosphate), CREB (cAMP Response Element Binding Protein); pCREB (phosphorylated CERB); PKA (Phosphokinase A)

## Funding

None

## Acknowledgments

None

## Conflicts of interest

The author declares that there are no conflicts of interest.

## References

1. Vingerhoets WA, Bloemen OJ, Bakker G, et al. Pharmacological interventions for the MATRICS cognitive domains in schizophrenia: what's the evidence? *Front Psychiatry*. 2013;157:1–22.
2. Kroken RA, Løberg EM, Drønen T, et al. A critical review of pro-cognitive drug targets in psychosis: convergence on myelination and inflammation. *Front Psychiatry*. 2014;5:11.
3. Holmén A, Juuhl–Langseth M, Thormodsen R, et al. Neuropsychological profile in early-onset schizophrenia–spectrum disorders: Measured with the MATRICS battery. *Schizophr Bull* 2009;36(4):852–859.
4. Marder SR. Lessons from MATRICS. *Schizophr Bull*. 2011;7: 49–52.
5. Harvey PD, Green MF, Nuechterlein KH. Latest developments in the MATRICS process. *Psychiatry (Edmont)*. 2010;7(6):49–52.
6. Keefe RS, Buchanan RW, Marder SR, et al. Clinical trials of potential cognitive-enhancing drugs in schizophrenia: what have we learned so far? *Schizophr Bull*. 2013;39(2):417–435.
7. Bobes J, Arango C, Garcia–Garcia M, et al. CLAMORS study collaborative group prevalence of negative symptoms in outpatients with schizophrenia spectrum disorders treated with antipsychotics in routine clinical practice: findings from the CLAMORS study. *J Clin Psychiatry*. 2010;71(3):280–286.
8. Hovington CL, Bodnar M, Joobar R, et al. Identifying persistent negative symptoms in first episode psychosis. *BMC Psychiatry*. 2012;12:224–228.
9. Chue P, Lalonde JK. Addressing the unmet needs of patients with persistent negative symptoms of schizophrenia: emerging pharmacological treatment options. *Neuropsychiatr Dis Treat*. 2014;10:777–789.
10. Wang ZZ, Zhang Y, Zhang HT, et al. Phosphodiesterase: An interface connecting cognitive deficits to neuropsychiatric and neurodegenerative diseases. *Curr Pharm Des*. 2015;21(3):303–3216.
11. Porteous DJ, Millar JK, Brandon NJ, et al. Sawa DISC at 10: connecting psychiatric genetics and neurosciences. *Trends Mol Med*. 2011;17(12):699–705.
12. Millar JK, MacKie S, Clapcote SJ, et al. Disrupted in schizophrenia 1 and phosphodiesterase 4B towards an understanding of psychiatric illness. *J Physiol*. 2007;584(Pt 2):401–405.
13. Millar JK, Pickard BS, Mackie S, et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that require cAMP signaling. *Science*. 2005;31(5751):1187–1191.
14. Schumacher J, Laje G, Abou Jamra R, et al. The DISC locus and schizophrenia: evidence from an association study in a central European sample and from a meta-analysis across different European Populations. *Hum Mol Genet*. 2009;18(14):2719–2727.
15. Pickard BS, Thomason PA, Christoforou A, et al. The PDE4B gene confers sex-specific protection against schizophrenia. *Psychiatr Genet*. 2007;17(3):129–133.
16. Balu DT, Coyle JT. Neuroplasticity signaling pathways linked to the pathophysiology of schizophrenia. *Neurosci Biobehav Rev*. 2010;35(3):848–870.
17. Clapcote SJ, Lipina TV, Millar JK, et al. Behavioral phenotypes of DISC1 missense mutations in mice. *Neuron*. 2007;54(3):387–402.
18. Xu Y, Zhang HT, O'Donnell JM. Phosphodiesterases in the central nervous system: implications in mood and cognitive disorders. *Handb Exp Pharmacol*. 2011;204:447–485.
19. Benito E, Barco A. CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. *Trends Neurosci*. 2010;33(5):230–240.
20. Blokland A, Menniti FS, Prickaerts J. PDE inhibition and cognition enhancement. *Expert Opin Ther Pat*. 2012;22(4):349–354.
21. Siuciak JA. The role of Phosphodiesterases in Schizophrenia. Therapeutic implications. *CNS Drugs*. 2008;22(12):983–993.
22. Soda T, Frank C, Ishizuka K, et al. DISC-ATF4 transcriptional repression complex: dual regulation of the cAMP-PDE-4 cascade by DISC1. *Mol Psychiatry*. 2013;18(8):898–908.
23. Arnsten AF. Prefrontal cortical network connections: key site of vulnerability in stress and schizophrenia. *Int J Dev Neurosci*. 2011;29(3):215–223.
24. Nave KA, Ehrenreich H. Myelination and oligodendrocyte functions in psychiatric diseases. *JAMA Psychiatry*. 2014;71(5):582–584.
25. Rutten K, Van Donkelaar EL, Ferrington L, et al. Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats. *Neuropsychopharmacology*. 2009;34(8):1914–1925.
26. Rutten K, Basile JL, Prickaerts J, et al. Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl)*. 2008;196(4):643–648.
27. Rodefer JS, Saland SK, Eckrich SJ. Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. *Neuropharmacology*. 2012;62(3):1182–1190.
28. Wiescholleck V, Manahan–Vaughan D. PDE4 inhibition enhances hippocampal synaptic plasticity *in vivo* and rescues MK801-induced impairment of long-term potentiation and object recognition memory in an animal model of psychosis. *Transl Psychiatry*. 2012;2:e89.
29. Kaner SJ, Tokarczyk J, Siegel SJ, et al. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. *Neuroscience*. 2007;144(1):239–246.
30. Zhong P, Wang W, Yu F, et al. Phosphodiesterase 4 inhibition impairs cocaine-induced inhibitory synaptic plasticity and conditioned place preference. *Neuropsychopharmacology*. 2002;37(11):2377–2387.
31. Kuroiwa M, Snyder GL, Shuto T, et al. Phosphodiesterase 4 inhibition enhances the dopamine D1 receptor/PKA/DARPP-32 signaling cascade in frontal cortex. *Psychopharmacology (Berl)*. 2012;219(4):1065–1079.
32. Wang C, Yang XM, Zhuo YY, et al. The phosphodiesterase-4 inhibitor rolipram reverses Aβ-induced cognitive impairment and neuroinflammatory and apoptotic responses in rats. *Int J Neuropsychopharmacol*. 2012;15(6):749–766.
33. Redrobe JP, Jørgensen M, Christoffersen CT, et al. *In vitro* and *in vivo* characterisation of Lu AF64280, a novel, brain penetrant phosphodiesterase (PDE) 2A inhibitor: potential relevance to cognitive deficits in schizophrenia. *Psychopharmacology (Berl)*. 2014;231(16):3151–3167.
34. Banerjee A, Narayana L, Raje FA, et al. Discovery of benzo[d]imidazo[5,1-b]thiazole as a new class of phosphodiesterase 10A inhibitors. *Bioorg Med Chem Lett*. 2013;23(24):6747–6754.
35. Rabe KF. Update on roflumilast, a phosphodiesterase 4 inhibitor for the treatment of chronic obstructive pulmonary disease. *Br J Pharmacol*. 2011;163(1):53–67.

36. Schaefer TL, Braun AA, Amos-Kroohs RM, et al. A new model of Pde4d deficiency: genetic knock-down of PDE4D enzyme in rats produces an antidepressant phenotype without spatial cognitive effects. *Genes Brain Behav.* 2012;11(5):614–622.
37. Wang C, Yang XM, Zhuo YY, et al. The phosphodiesterase-4 inhibitor rolipram reverses Aβ-induced cognitive impairment and neuroinflammatory and apoptotic responses in rats. *Int J Neuropsychopharmacol.* 2012;15(6):749–766.
38. Ghosh M, Garcia-Castillo D, Aguirre V, et al. Proinflammatory cytokine regulation of cyclic AMP–phosphodiesterase 4 signaling in microglia *in vitro* and following CNS injury. *Glia.* 2012;60(12):1839–1859.
39. Pietzcker A, Muller-Oerlinghausen B, Kehr W. Antipsychotic activity of rolipram in schizophrenia patients: a pilot study [abstract]. *Naunyn Schmiedebergs Arch Pharmacol.* 1979;308:R44.
40. Scott AI, Perini AF, Shering PA, et al. In-patient major depression: is rolipram as effective as amitriptyline? *Euro J Clin Pharmacol.* 1991;40(2):127–129.
41. Gericke N, Viljoen AM. Scelletium—A review update. *J Ethnopharmacology.* 2008;199(3):653–663.
42. Gericke N. Plants, Products and people: Southern African perspectives. In: Iwu M & Wootton JC (Eds.), *Ethnomedicine and Drug Discovery* Elsevier. Amsterdam, Netherlands 2002.
43. Gericke N, Van Wyk BE. Pharmaceutical compositions containing mesembrine and related compounds. *US Patent.* 1999;6288104.
44. Harvey AL, Young LC, Viljoen AM, et al. Pharmacological actions of the South African medicinal and functional food plant *Scelletium tortuosum* and its principal alkaloids. *J Ethnopharmacol.* 2011;137(3):1124–1129.
45. Cipriani A, Purgato M, Furukawa TA, et al. Citalopram versus other anti-depressive agents for depression. *Cochrane Database Syst Rev.* 2012;7:CD006534.
46. Jorge RE, Acion L, Moser D, et al. Escitalopram and enhancement of cognitive recovery following stroke. *Arch Gen Psychiatry.* 2010;67(2):187–196.
47. Homberg JR. Serotonin and decision making processes. *Neurosci Biobehav Rev.* 2012;36(1):218–236.
48. Meltzer HY, Roth BL. Lorcaserin and pimavanserin: emerging selectivity of serotonin receptor subtype-targeted drugs. *J Clin Invest.* 2013;123(12):4986–4991.
49. Burgin AB, Magnusson OT, Singh J, et al. Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. *Nat Biotechnol.* 2010;28(1):63–70.
50. Vellas B, Sol O, Snyder PJ, et al. EHT0202/002 study group. EHT0202 in Alzheimer's disease: a 3-month, randomized, placebo-controlled, double-blind study. *Curr Alzheimer Res.* 2011;8(2):203–212.
51. Lacombe P, Charet N, Claveau D, et al. Alkyl-bridged substituted 8-arylquinolines as highly potent PDE IV inhibitors. *Bioorg Med Chem Lett.* 2009;19(17):5266–5269.
52. Bruno O, Fedele E, Prickaerts J, et al. GEBR-7b, a novel PDE4D selective inhibitor that improves memory in rodents at non-emetic doses. *Br J Pharmacol.* 2011;164(8):2054–2063.
53. Terburg D, Syal S, Rosenberger LA, et al. Acute effects of *Scelletium tortuosum* (Zembrin), a dual 5-HT reuptake and PDE4 inhibitor, in the human amygdala and its connection to the hypothalamus. *Neuropsychopharmacology.* 2013;38(13):2708–2716.
54. Smith C. The effects of *Scelletium tortuosum* in an *in vivo* model of psychological stress. *J Ethnopharmacol.* 2011;133(1):31–36.
55. Gericke N. Clinical application of selected South African medicinal plants. *Australian Journal of Medical Herbalism.* 2001;13:3–17.
56. Chiu Simon, Gericke Nigel, Farina-Woodbury M, et al. Proof-of-Concept Randomized Controlled Study of Cognition Effects of the Proprietary Extract *Scelletium tortuosum* (Zembrin). Targeting Phosphodiesterase-4 in Cognitively Healthy Subjects: Implications for Alzheimer's Dementia. *Evid Based Complement Alternat Med.* 2014;682014.
57. Nell H, Siebert M, Chellan P, et al. A randomized, double-blind, parallel-group, placebo-controlled trial of Extract *Scelletium tortuosum* (Zembrin) in healthy adults. *J Altern Complement Med.* 2013;19(11):898–904.
58. Dimpfel W, Schombert L, Gericke N. Electropharmacogram of *Scelletium tortuosum* extract based on spectral local field power in conscious freely moving rats. *J Ethnopharmacology.* 2016;177:140–147.