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PASS-assisted exploration of antidepressant activity of 1,3,4-trisubstituted-β-lactam derivatives

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ABSTRACT

Assisted by PASS predictions, the antidepressant activity of 1,3,4-trisubstituted monocyclic β-lactams in seasonal affective disorders is described.

The β-lactam class of antibiotics is well known for their neurotoxicity.[1] Since the first report of epileptogenic properties of penicillin in 1945 by the classic works of Johnson and Walker[2], adverse effects of penicillin and other β-lactam antibiotics on the central nervous system have become more widely recognized.[3] The neurotoxic effects of β-lactams have been credited to their structural resemblance to GABA as they mainly act as GABA antagonists.[4] It has been evidenced that different β-lactam antibiotics bind to $GABA_A$ ionophore site and lead to suppression of inhibitory postsynaptic responses at $GABA_A$-Receptor-inducing seizures.[5,6]

Interestingly, the beneficial effects of a family of β-lactam antibiotics were recently discovered by Jeffrey Rothstein and colleagues, who found that these antibiotics could protect against the dysfunctional effects of the neurotransmitter glutamate in mice by activating the expression of a glutamate transporter.[7,8] This neuroprotection has also been offered to ischemic insult in transgenic mouse models of neurodegenerative diseases. Based on the above neuroprotective mechanisms, it has been reported that the administration of a β-lactam antibiotic produces an antidepressant response in rodents.[9]

We have earlier reported some CNS active azetidinones in animal models of anxiety, catatonia and memory.[10] In continuation of these studies, we report here in antidepressant activity of some 1,3,4-trisubstituted monocyclic β-lactams, **3a**, **3b** and, **5a** and **5b** in dark-induced depression, an animal model of seasonal affective disorder (SAD). The compounds, **3a**, **3b** and, **5a** and **5b** were predicted for biological activity spectra software PASS (prediction of activity spectra of substances) as mentioned in our previous studies.[11,12] The CNS depression has been behaviorally assessed with forced swim test and biochemical assessment was made by measuring the brain serotonin levels. The compounds predicted to have the properties like GABA antagonist, serotonin release stimulant. (Table 1) showing their potential as antidepressant moieties were selected for this study.

1,3,4-Trisubstituted-β-lactam derivatives were prepared by the Lewis acid catalysed highly diastereoselective and π-facial-selective Diels–Alder cycloaddition reactions of *cis/trans*-3-butadienyl-2-azetidinones with dienophiles viz. maleic anhydride **2a**, ***N***-phenyl maleimide **2b**, *N-p*-tolylmaleimide **2c**, and benzoquinone **2d**. The reactions of **1a** with maleic anhydride **2a** and **1b** with *N*-phenyl maleimide **2b** gave better yields of adducts **3a** and **3b** with titanium(IV) chloride. However the best yields of the reactions of **4a** with **2c** and **4b** with **2d** were realized with the use of tin(IV) chloride at low temperature. The 1,3,4-trisubstituted azetidinone derivatives utilized for this biological screening were characterized with the help of analytical data, spectral evidences and X-ray crystallographic studies.[13,14] (see Scheme 1).

Evaluation of antidepressant effect in dark-induced depression. The animals (Mice) were kept in dark environment in isolated cages for whole day. Behavioral test (Forced swimming test) was performed immediately after first, third and fifth day of the dark treatment to assess adequate duration of dark treatment to induce significant depression. As the maximum depression was found after 5 days dark treatment, the protocol was used to induce depression. For the rest of studies, the experiments were

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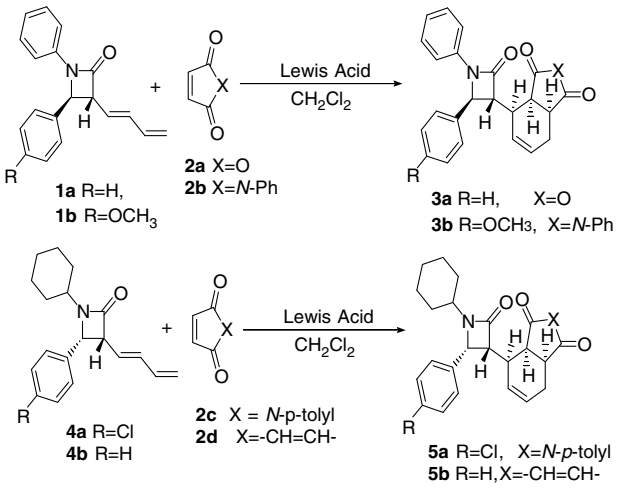
Table 1
PASS prediction score of the test compounds

S. No.	Compound	PASS prediction			
		$GABA_A$ antagonist		5-HT release stimulant	
		Pa	Pi	Pa	Pi
1	**3a**	0.680	0.029	0.564	0.065
2	**3b**	0.399	0.162	0.410	0.139
3	**5a**	0.367	0.182	np	np
4	**5b**	0.378	0.178	0.343	0.203

Np, not predicted by PASS.



Scheme 1.

performed by an observer unaware of the treatment administered. At the end of the behavioral test animals were sacrificed and the brains were removed for the serotonin content estimation.

Group 1: Control animals received vehicle only.

Group 2: Standard: Fluoxetine (20 mg/kg, IP) was administered 30 min prior to behavioral assessment for antidepressant activity.

Group 3–10 test. Test Compounds (1,3,4-trisubstituted-β-lactam derivatives, **3a, 3b, and 5a and 5b**) were administered on last day in different doses (15 and 30 mg/kg, ip) 30 min prior to behavioral assessment for antidepressant activity.

Drugs and treatment schedule. The following drugs were used:

The test compounds 1,3,4-trisubstituted-β-lactam derivatives **3a and 3b, and 5a** and **5b** and Fluoxetine were used as test compounds and standard, respectively. All the test compounds and reference drug Fluoxetine were suspended in 0.5% CMC solution. The drugs were administered intraperitoneally (ip) once daily for 4 days and 30 min, before the test on the 5th day. Appropriate amounts of the corresponding vehicles were given to the control animals. Control animals received a vehicle injection according to the same schedule.

Forced swimming test (FST) in mice. The experiment was carried out according to the method of Porsolt et al.[15] Briefly, mice (Albino Swiss) were individually placed in a glass cylinder (25 cm high and 10 cm in diameter) containing 6 cm of water maintained at 23–25 °C, and were left there for 6 min. Mice forced to swim for 6 min, and the total immobility duration was measured. After an initial 2-min period of vigorous activity, each animal assumed an immobile posture. A mouse was regarded as immobile when it remained floating passively on the water, making only small movements to keep its head above the water surface.

Serotonin estimation. Mice were sacrificed by decapitation 10 min after behavioral testing. The brains were removed and tissue homogenate was prepared in ice-cold 0.1 N perchloric acid with 0.2% ascorbic acid. Homogenate were centrifuged for 20 min at 15,000 rpm and supernatant was treated by the method of Snyder et al. modified for use with small amounts of material for estimation of serotonin level. [16]

Statistical analysis. All the results were expressed as means (±SEM). The data was evaluated by one-way analysis of variance (ANOVA) followed by Dunnet test. $p < 0.05$ was considered significant.

The increase in dark treatment significantly increased the depression as evidenced by increase in immobility period in forced swimming test, (Fig. 1). All the test compounds except **5a** showed significant ($p < 0.05$) antidepressant activity by decreasing the immobility period in the FST (Fig. 2). Further the biochemical

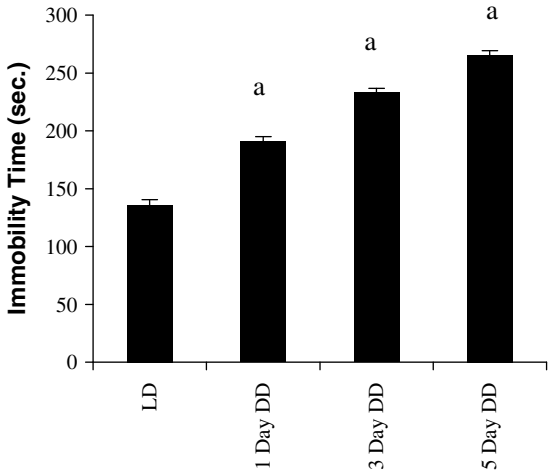


Figure 1. Effect of the duration of dark treatment on CNS depression. LD denotes 12:12 h light:dark cycle group and DD denotes 12:12 h dark:dark cycle group. All values are represented as means ± SEM; $n = 6$, here $a = p < 0.05$ when compared to LD group.

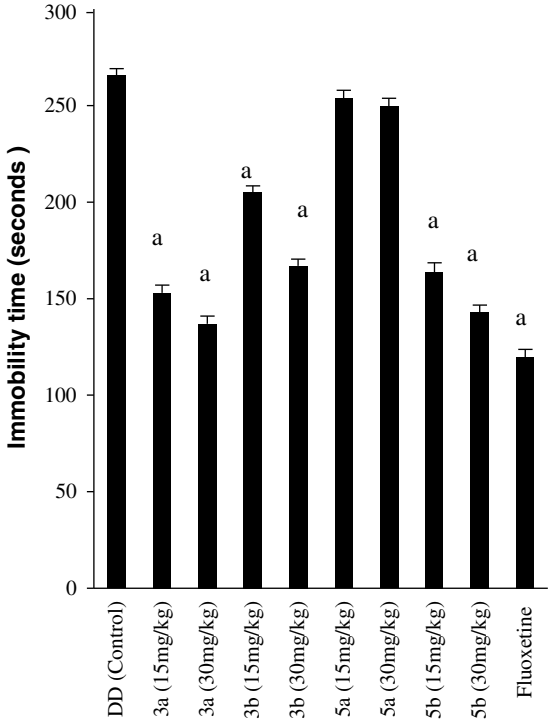


Figure 2. Ameliorative effect of test compounds on dark-induced depression. DD denotes 12:12 h dark:dark cycle group. All values are represented as means ± SEM; $n = 6$, $a = p < 0.05$ when compared to control DD group.

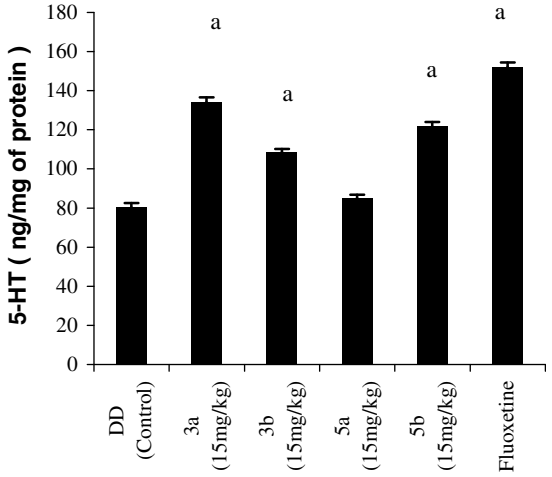


Figure 3. Effect on total brain serotonin content DD denotes 12:12 h dark:dark cycle group. All values are represented as means ± SEM; $n = 6$, $a = p < 0.05$ when compared to control DD group.

studies reveal corresponding increase in brain serotonin content (Fig. 3) which can be correlated well with antidepressant activity of test compounds in FST after dark treatment. Further these results were also in agreement with the PASS predictions corresponding to properties predicted by PASS.

From different Clinical and preclinical observations it is evident that the both inhibitory amino acid (IAA) neurotransmitter system and excitatory amino acid (EAA) neurotransmitter system play a critical role in pathophysiology and treatment of mood disorders.[17–19] It has been suggested that the loss or blockade of $GABA_B$ receptors may be a suitable target for the development of antidepressant agents as demonstrated by a study in which mice lacking $GABA_B(1)$ subunit (knockout mice) and treated with $GABA_B$ receptor antagonist CGP56433A displayed an antidepressant-like activity in the FST.[20,21,22]

In another study involving β-lactam predicted through GLT-1 by increasing the reuptake of glutamate.[9]

Therefore, it could be possible that antidepressant-like activity of test compounds may either be appropriated to GABAergic antagonism due to the structural similarity of the investigated compounds with GABA or through enhanced glutamate reuptake as reported in an earlier study by Rothstein et al.

It has been well established that the shortening of the immobility time in the FST depends on the enhancement of central monoamine neurotransmission,[23] primarily, on serotonin and noradrenaline neurotransmission.[24] The ability of the test compounds to raise brain serotonin content partly suggests that this may possibly be one of the mechanisms for their antidepressant activity.

Further investigations are in progress to elucidate the exact mechanism of the antidepressant activity of the test compounds.

It is concluded form the above discussion that the PASS predictions assisted in finding 1,3,4-trisubstituted azetidinones as the novel antidepressant molecules.

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14. *4-(2-Oxo-1,4-diphenyl-azetidin-3-yl)-3a,4,7,7a-tetrahydro-isobenzofuran-1,3-dione:* Mp 195–196 °C δ_H ($CDCl_3$, 500 MHz) 2.28 (ddddd, J = 3.0,3.0,3.0,8.2,15.8 Hz, 1H, H_{8b}), 2.81(ddd, J = 1.2, 7.0, 15.8 1H, H_{8a}),2.86 (ddddd, J = 3.0,3.0,3.0,5.8,11.0 Hz, 1H, H_5),3.49 (ddd,1.2, 8.2, 9.8 Hz,1H, H_9),3.97(dd, J = 2.2,11.0 Hz,1H, H_3), 4.05 (dd, J = 5.8, 9.8 Hz,1H, H_{12}), 4.75 (d, J = 2.2 Hz,1H, H_4), 5.89 (dd, J = 3.0, 9.2 Hz,1H, H_6), 6.10 (dddd, J = 3.0, 3.0, 7.0, 9.2 Hz, 1H, H_7), 7.24 (m, 10H, H, aromatic), δ_C ($CDCl_3$, 75 MHz) 24.7, 36.5, 40.4,42.9, 59.1, 61.2, 117.0, 124.2, 126.0, 128.9, 129.1, 129.4, 129.8, 130.7,137.0, 137.3, 165.8, 171.5, 173.8, m/z 373 (M^+). ν_{max} (KBr)/cm^{-1} 1727,1702, 1492,1385. Elemental Anal. Calcd for $C_{23}H_{19}N_1O_4$ (373.13): C, 73.98; H, 5.13, N, 3.75; found: C, 74.15; H, 5.33, N, 3.36.*4-[2-(4-Methoxyphenyl)-4-oxo-1-phenylazetidin-3-yl]-2-phenyl-3a,4,7,7a-tetrahydroisoindole-1,3-dione (**3b**):* Mp 215–216 °C. ^{1}H NMR (CDCl3, 200 MHz): δ_H = 2.29 (unresolved dddd, J = 3.0, 9.2,13.7 Hz, 1H, H8b), 2.87 (unresolved ddd, J = 6.5, 13.7 Hz, 1 H, H8a), 2.98 (unresolved dddd, J = 3.0, 5.5, 11.7 Hz, 1H, H5), 3.52(ddd, J = 3.1, 9.2, 9.9 Hz, 1H, H9), 3.79 (s, 3 H, $-OCH_3$), 3.99 (dd, J = 2.3, 11.7 Hz, 1H, H3), 4.09 (dd, J = 5.9, 9.9 Hz, 1H, H12), 4.81 (d, J = 2.3 Hz, 1H, H4), 5.85 (dd, J = 3.0, 9.8 Hz, 1H, H6), 6.07 (unresolved ddd, J = 6.7, 9.8 Hz, 1H, H7), 7.23 (m, 14H, arom.) ppm. ^{13}C NMR ($CDCl_3$, 60 MHz): δ_C = 26.0, 38.3, 39.7,41.3, 55.0, 60.2, 62.3, 116.2, 123.9, 126.7, 127.0, 128.3, 128.7, 128.9, 129.0, 129.3, 129.4, 129.5, 130.5, 137.8, 137.9, 166.9, 177.0, 178.6 ppm. IR (KBr): $\bar{\nu}$ = 1770, 1760, 1672, 1623, 1371, 1340,1176 cm^{-1}. m/z 478 [M]+. Elemental Anal. Calcd for $C_{30}H_{26}N_2O_4$ (478.19): C 75.30, H 5.48, N 5.85; found C 75.44, H 5.40, N 5.79.*4-[2-(4-Chlorophenyl)-1-cyclohexyl-4-oxo azetidin-3-yl]-2-p-tolyl-3a,4,7,7a-tetrahydro isoindole-1,3-dione (**5a**):* Mp 241–242 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H = 1.40 (m, 10H, cyclohexyl), 1.52 (m, 1H, H8b), 2.31 (s, 3H, $-CH_3$), 2.39 (m, 1H, H8a), 2.57 (unresolved dd, J = 11.8 Hz, 1H, H5), 3.18 (unresolved ddd, J = 8.5,9.4 Hz, 1H, H9), 3.37 (m, 1H, cyclohexyl), 3.84 (dd, J = 5.5, 11.8 Hz, 1H, H3), 4.48 (dd, J = 5.8, 9.3 Hz, 1H, H12), 4.87 (d, J = 5.5 Hz, 1H, H4), 5.28 (m, 1H, H6), 5.67 (m, 1H, H7), 7.25 (m, 8H, arom.) ppm. ^{13}C NMR ($CDCl_3$, 60 MHz): δ_C = 19.8, 22.9, 25.0, 30.2, 32.56, 36.3, 40.6, 42.1, 52.2, 59.3, 62.3, 127.3, 127.4, 127.5, 128.5, 128.9, 129.7, 129.5, 130.2, 132.2, 137.3, 166.6, 176.8, 178.7 ppm. IR (KBr): $\bar{\nu}$ = 1750, 1720, 1648, 1320 cm^{-1}. m/z 502[M]+. Elemental Anal. Calcd for $C_{30}H_{31}N_2O_3$ (502.20): C 71.63,H 6.21, N 5.57; found C 71.78, H 6.36, N 5.23.*5-(1-Cyclohexyl-2-oxo-4-phenylazetidin-3-yl)-4a, 5,8,8a-tetrahydro-1,4-naphthoquinone (**5b**):* Mp 221–222 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H = 1.45 (m, 10H, H cyclohexyl), 2.09 (m, 1H, H8b),2.57 (m, 1H, H8a), 2.78 (unresolved ddd, J = 6.2, 10.4 Hz, 1 H,H5), 3.23 (m, 1 H, H9), 3.31 (m, 1H, cyclohexyl), 3.87 (dd, J = 5.5, 10.4 Hz, 1H, H3), 4.43 (dd, J = 6.2, 9.6 Hz, 1H, H14), 4.84(d, J = 5.5 Hz, 1H, H4), 5.32 (unresolved dd, J = 10.1 Hz, 1H,H6), 5.41 (dd, J = 9.9 Hz, 1H, H7) 6.50 (d, J = 10.5 Hz, 1H, H11),6.61 (d, J = 10.5 Hz, 1H, H12), 7.23 (m, 5H, arom. H) ppm. ^{13}C NMR ($CDCl_3$, 60 MHz): δ_C = 22.5, 25.1, 30.3, 32.1, 36.1, 40.5, 42.9, 52.1, 59.1, 61.2, 126.2, 126.9, 127.7, 128.2, 128.8, 137.0, 137.5, 141.0, 166.2, 199.4, 200.1 ppm. IR (KBr): $\bar{\nu}$ = 1756, 1723, 1634,1625, 1378, 1356, 1291, 1184 cm^{-1}. m/z 389 [M]+. Elemental Anal. Calcd for $C_{25}H_{27}NO_3$ (389.20): C 77.12, H 6.94, N 3.59; found: C 77.25, H 7.07, N 3.30.
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