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DDD-028: A potent, neuroprotective, non-opioid compound for the treatment of diabetic neuropathy

Laura Micheli [a], Jagan Rajamoni [b], Lorenzo Di Cesare Mannelli [a], Parthasarathi Rajagopalan [c], Carla Ghelardini [a], Raghavan Rajagopalan [c,*]

[a] Department of Neuroscience, Psychology, Drug Research and Child Health, NEUROFARBA-Pharmacology and Toxicology Section, University of Florence, Florence, Viale Pieraccini 6, 50139 Florence, Italy
[b] Department of Chemistry and Biochemistry, University of Missouri, St. Louis, One University Blvd., St. Louis, MO 63121, USA
[c] Daya Drug Discoveries, Inc., 204 City Gate Lane, St. Louis, MO 63146, USA

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ABSTRACT

Diabetic neuropathy (DN) is a painful, chronic ailment that affects a large segment of diabetic population worldwide. Current medications such as pregabalin or duloxetine treat only the pain symptom associated with DN, but not the underlying nerve damage. DDD-028 (**1**) is a small molecule that displays potent pain-relieving activity in streptozotocin (STZ)-induced rodent model of DN. Combined with other studies indicating that DDD-028 suppresses astrogliosis and nerve damage induced by the anti-cancer drug, paclitaxel, the present study suggests that DDD-028 would be useful as a disease modifying therapeutic in the treatment of DN. The 3-dimensional structure of DDD-028 was confirmed by single crystal X-ray crystallography.

Diabetic neuropathy (DN) is a painful, chronic ailment that affects a large segment of diabetic population worldwide, and this number is expected to increase over the years due to substantial increase in diabetes caused by obesity, genetic and metabolic disorders, socioeconomic factors, and longevity. Among the four types of diabetic neuropathies, about 50% of the diabetic individuals is afflicted with peripheral neuropathy; about 30% with autonomic neuropathy; and the rest with focal and proximal neuropathies.[1–2] Further, neuropathic pain is also the primary cause of depression and other comorbid conditions among the diabetic population.[3] At present, there are no approved drugs for disease modifying treatment for diabetic neuropathy. Current medications for DN only relieve the pain symptom; they do not alleviate the underlying nerve damage.[4] Moreover, these medications are reported to be effective in only about 20–25% of the diabetic individuals suffering from DN.[5]

We have been extensively investigating the versatility of pentacyclic pyridoindole heterocyclic scaffolds **A-C** (Fig. 1) for their potential application in the treatment various CNS disorders such as schizophrenia, psychostimulant addiction, and neuropathic and inflammatory pain. Earlier, we had reported on the synthesis and receptor binding profiles of prototypical compounds **1**–**5**, and demonstrated that none of these compounds bind to any of the three opioid receptors or to any of the five dopamine receptors.[6] We further reported that DDD-028 (compound **1** of scaffold **A**) not only elicits a potent pain-relieving activity in various models of pain,[7,8] but, importantly, also displays a broad spectrum neuroprotective properties.[8–9] DDD-024 (compound **3** of scaffold **B**) has been reported to elicit anti-addictive property against psychedelic drugs, methamphetamine (Meth) and 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'), via dual serotonin 5-HT_{1A} agonism/5-HT_{2B} antagonism.[10–11] DDD-016 (compound **5** of scaffold **C**) has been proposed to elicit anti-psychotic activity via dual serotonin 5-HT_{2A}/D_2 antagonism.[12] In this paper, we wish to report that DDD-028 displays potent pain-relieving effect against streptozotocin (STZ)-induced DN.

Although the structure of DDD-028 was reported previously based on NMR data and the rote of its synthesis,[6] the three-dimensional molecular structure of DDD-028 has now been determined using single crystal X-Ray diffraction analysis. The molecular structure of DDD-028 with anisotropic displacemnt ellipsoid of atoms is shown in Fig. 2. The details of the structure refinement parameters (Table S1) and the geometrical parameters (Table S2) are given in the Supplementary Information. DDD-028 crystallizes from chloroform in orthorhombic spacegroup *Pbca* with eight molecules in the unit-cell. In the moelcular structure the heterocyclic six-membered ring associated with the carboline moiety exhibts a half-chair conformation, which can be understood from the

* Corresponding author.
E-mail address: raghavanr@daya-dd.com (R. Rajagopalan).

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Cremer & Pople puckering parameter[13] values such as Q = 0.500, θ = 129.8 and φ = 206.8 respectively. The methyl group attached to the *N*-atom of the heterocyclic ring lies in the equitorial position which makes an angle of 81.92(14)° with the Cremer & Pople plane. Similarly, the puckering amplitude (q) of the seven membered hetercyclic ring with an endocyclic double bond is observed to q_2 = 0.6487 and q_3 = 0.3244 respectively. The calculated values of the puckering amplitudes q_2 and q_3 deviates significantly from the ideal chair or boat conformation, the puckering amplitude value shows that a conformational change between chair to boat has been taken place with the seven membered heterocyclic ring and is depicted in Fig. S1.[14,15] All bond length and angle values in the molecular structure are normal and is in agreemant with the ideal values (Table S2). In the crystal structure, the adjacent molecues are linked through a C—H…N hydrogen bond C16-H16…N2 [D…A = 3.418 (3)Å,H…A = 2.55 Å and C—H…N = 149°] which stablizes the molecular packing in the crystalline solid. Finalized crystal structure of DDD-028 has been deposited in Cambridge Crystallographic Data Centre (Reference Id is 'CCDC 2226620').

DDD-028 was previously reported to display potent pain-relieving activity in the chronic constriction injury (CCI)-induced neuropathic pain.[9,16] However, that study was based on small sample size (mice, n = 2) and without pregabalin, the 'gold standard' medication for diabetic neuropathy, as a positive control. Therefore, the CCI study was repeated using n = 10 mice per group to reaffirm DDD-028's potency and assess its pain-relieving effect compared to pregabalin. The pain-relieving effect against non-noxious stimuli (static von Frey Test) is shown in Fig.3. DDD-028 displays robust anti-allodynic activity at the low oral dose of 3 mg/kg. The pain-relieving effect of DDD-028 is at least comparable to that exerted by pregabalin based on the dosing level, and is also consistent with our earlier finding.[9] DDD-028 was then tested in the widely used model of STZ-induced diabetic neuropathy in rats.[17] Early STZ model using high dose of STZ has been criticized because in those studies the animals became very sick and, consequently, the data resulting thereof were not very reliable. Therefore, we used the multiple low-dose STZ model[18] in this study because the low-dose STZ results in negligible toxicity at later time points, and the animals live longer than those in the high-dose model. Importantly, this low-dose model closely resembles the pathology of DN in humans.[19] Detailed experimental procedure of STZ model is given in the Supplementary Information. Briefly, diabetic neuropathy was induced in rats by injecting STZ (50 mg/kg, i.p.) for five consecutive days. Pain threshold was monitored weekly until a clear difference between the vehicle and the STZ groups was observed, which occurred at about five weeks post STZ administration. Thereafter, DDD-028 was suspended in 1% CMC and acutely administered *per os* at three different doses: 1, 10, and 25 mg/kg. Both mechanical allodynia (electronic von Frey test) and mechanical hyperalgesia (Paw Pressure test) were assessed before and 15, 30, 45, 60, 90, and 120 min after DDD-028 treatment. The pain-relief response against non-noxious and noxious stimuli are shown in Figs. 4 and 5 respectively. The pain-relieving effect was apparent within 30 min even at a low dose of 1 mg/kg, but the effect persisted only for about 30 min. At the highest dose of 25 mg/kg, the pain-relieving effect lasted for at least 90 min.

It has been demonstrated previously that DDD-028 does not bind to any of the opioid receptors.[6] To ascertain that DDD-028 is not acting via the opioid pathway, it was also subjected to acute pain model studies using the tail flick and hot plate tests in rats in comparison to morphine, the 'gold' standard for the opioid-based treatment of acute pain conditions. DDD-028 (1, 3, and 10 mg/kg) displayed no significant analgesic effect in both hot plate and tail flick tests at any of these doses, whereas morphine (3 mg/kg) showed robust analgesic effect (cf. Supplementary Information Figs. S3-S6 and Tables S6 and S7). Thus, the lack of binding of DDD-028 to any of the opioid receptors along with lack of analgesic

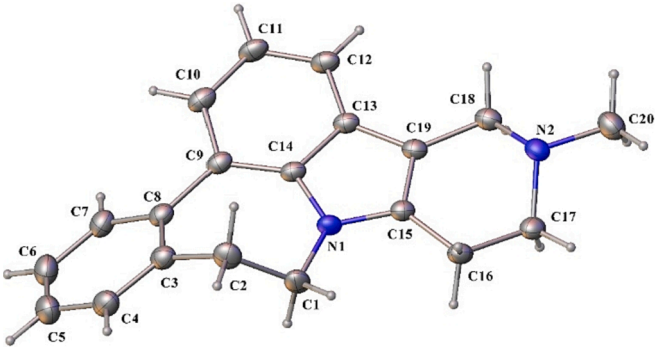


Fig. 2. X-ray crystal structure of DDD-028 (**1**).

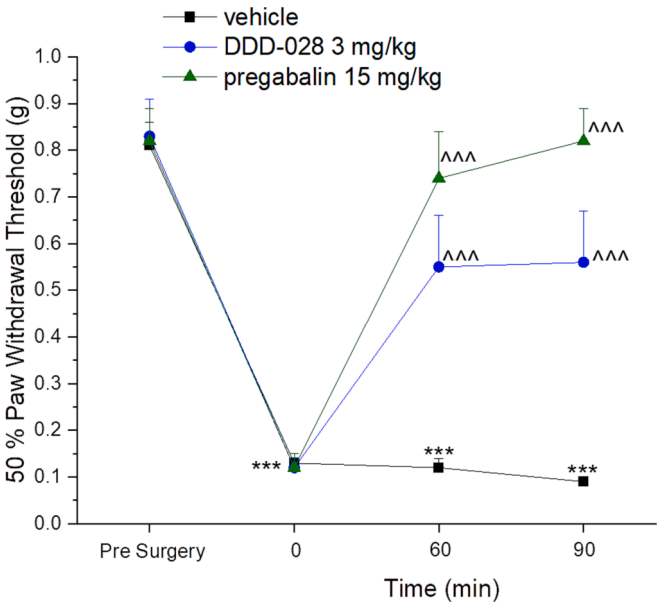
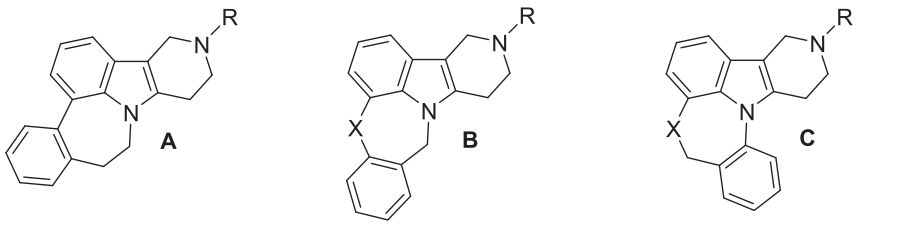


Fig. 3. Pain-relieving effect of *per os* acute administration of DDD-028 on CCI Induced Neuropathy – Mechanical Allodynia (static von Frey test). Results are reported as the Mean ± SEM of n = 10 mice per group. Statistical analysis: two-way repeated measures ANOVA followed by planned pairwise *post hoc* comparisons. ***p < 0.001 vs pre-surgery;^^^P < 0.001 vs vehicle.



1: R = Me, DDD-028
2: R = Me, X = CH_2, DDD-029
3: R = Me, X = O, DDD-024
4: R = Me, X = CH_2, DDD-025
5: R = Me, X = S, DDD-016

Fig. 1. Molecular structures of pentacyclic pyridoindole heterocyclic scaffolds **A-C**, and the prototypical compounds derived from these scaffolds (**1**–**5**).

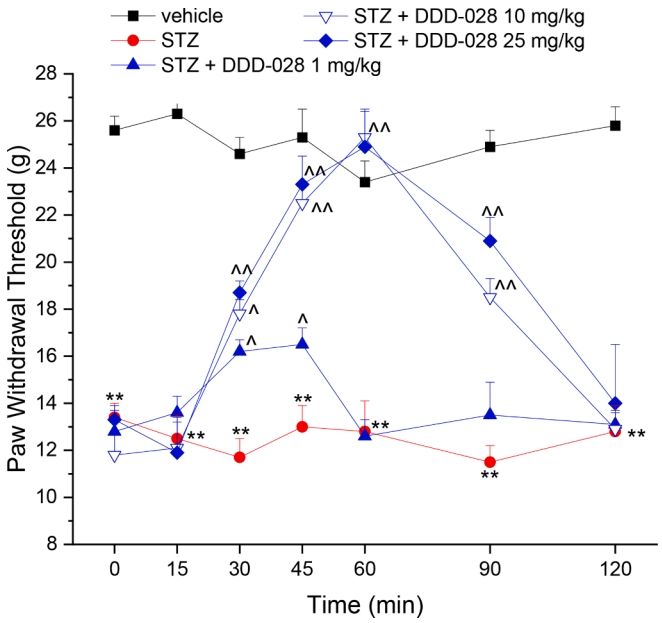


Fig. 4. Pain-relieving effect of *per os* acute administration of DDD-028 on STZ-induced diabetic neuropathy – Mechanical Allodynia (electronic von Frey test). Results are reported as the Mean ± SEM of n = 6 rats per group. Statistical analysis: one-way ANOVA followed by Bonferroni *post hoc* comparison. **P < 0.01 vs vehicle;^P < 0.05 and^^P < 0.01 vs STZ.

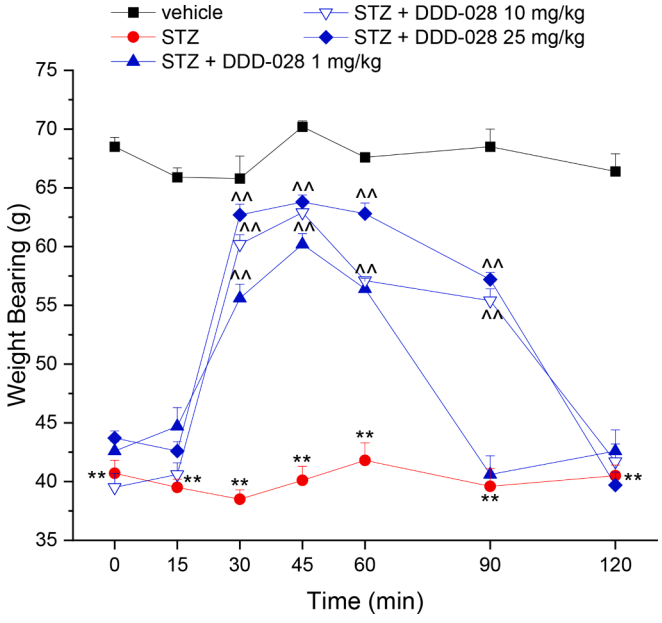


Fig. 5. Pain-relieving effect of DDD-028 (*per os*) on STZ-induced neuropathy – Mechanical Hyperalgesia (Paw Pressure test). Results are reported as the Mean ± SEM of n = 6 rats per group. Statistical analysis: one-way ANOVA followed by Bonferroni *post hoc* comparison. **P < 0.01 vs vehicle;^P < 0.05 and^^P < 0.01 vs. STZ.

effect in the acute nociception model clearly suggests that DDD-028′s pain-relieving activity is not mediated through opioid pathway.

The present study unequivocally demonstrated that DDD-028 elicits potent pain relief in the rodent model of DN. We previously reported[8,9] that DDD-028 also elicits neuroprotective properties by suppressing both astrogliosis and axonal damage. The results of the present study, taken together with those of the neuroprotection and acute pain model studies, indicates that DDD-028 would be useful as a non-opioid, disease modifying therapeutic for the treatment of DN. Our preliminary results[9] suggests that mechanism of action of DDD-028 is mediated through α7 nicotinic acetylcholine receptor (α 7nAChR). Further investigation is underway to determine if DDD-028 has a curative effect on the nerve damage caused by diabetes as well as to elucidate its precise mechanism of action.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2023.129472.

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