Neuroprotective effects of dimethyl fumarate against depression-like behaviors via astrocytes and microglia modulation in mice: possible involvement of the HCAR2/Nrf2 signaling pathway

Alana Gomes de Souza1 · Iardja Stéfane Lopes1 · Adriano José Maia Chaves Filho1 · Talita Matias Barbosa Cavalcante1 · João Victor Souza Oliveira1 · Michele Albuquerque Jales de Carvalho1 · Klistenes Alves de Lima1 · Paloma Marinheiro Jucá2 · Sabrina Silva Mendonça2 · Melina Mottin2 · Carolina Horta Andrade2 · Francisca Cléa Florenço de Sousa1 · Danielle S. Macedo1 · Marta Maria de França Fonteles1,3

Received: 18 February 2022 / Accepted: 27 April 2022 / Published online: 4 June 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
We postulated that dimethyl fumarate (DMF) exerts neuroprotective effects against depression-like behaviors through astrocytes and microglia modulation. To ascertain our hypothesis and define the mechanistic pathways involved in effect of DMF on neuroinflammation, we used the depression model induced by chronic unpredictable mild stress (CUMS), in which, the mice were exposed to stressful events for 28 days and from the 14th day they received DMF in the doses of 50 and 100 mg/kg or fluoxetine 10 mg/kg or saline. On the 29th day, the animals were subjected to behavioral tests. Microglia (Iba1) and astrocyte (GFAP) marker expressions were evaluated by immunofluorescence analyzes and the cytokines TNF-α and IL-1β by immunoenzymatic assay. In addition, computational target prediction, 3D protein structure prediction, and docking calculations were performed with monomethyl fumarate (DMF active metabolite) and the Keap1 and HCAR2 proteins, which suggested that these could be the probable targets related protective effects. CUMS induced anxiety- and depressive-like behaviors, cognitive deficit, decreased GFAP, and increased Iba1, TNF-α, and IL-1β expression in the hippocampus. These alterations were reversed by DMF. Thus, it is suggested that one of the mechanisms involved in the antidepressant effect of DMF is neuroinflammatory suppression, through the signaling pathway HCAR2/Nrf2. However, more studies must be performed to better understand the molecular mechanisms of this drug.

Keywords Depression · Neurogenic Inflammation · Dimethyl fumarate · Astrocytes · Microglia · Cytokines

Introduction
Major depressive disorder (MDD) is a common psychiatric condition and has been the leading cause of disability and premature death worldwide. Globally, more than 264 million people have MDD (World Health Organization 2020). As for the etiology of this disease, it is believed to be the result of molecular cellular abnormalities that interact with genetic and environmental factors (Krishnan and Nestler 2008). Stress is seen as the main environmental factor described in individuals’ predisposition to MDD (Andrews et al. 2011).

The neurobiological basis of MDD is not yet fully elucidated (Fu et al. 2012). To date, almost all drugs available for the treatment of MDD have been developed out of monoaminergic deficit. However, the limitations of treatment with these antidepressant drugs are becoming increasingly
evident (Rush et al. 2006; Insel and Sahakian 2012; Khan et al. 2012). Approximately 33% of patients do not respond adequately to conventional pharmacological therapy and even responsive patients have varied and unpleasant adverse effects that in some cases cause interruption of therapy. In addition, only 27% of patients reach remission within 12 weeks (Gaynes et al. 2009). Thus, understanding the role of other systems in the disease is important for the development of more promising drugs.

Clinical and preclinical data suggest that depression is associated with inflammation (Dantzer et al. 2011). Dowlati et al. (2010), in a meta-analysis, demonstrated that depressed patients have increased levels of inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-6.

Chronic unpredictable mild Stress (CUMS) is a validated model of depression, which induces neuroinflammation in stress-sensitive regions in the brain of mice. This model not only supports the inflammatory depression hypothesis but also provides further evidence of its strength as an experimental paradigm (Farooq et al., 2012).

Dimethyl fumarate (DMF) is a drug that has antioxidant and anti-inflammatory effects, being used for the treatment of psoriasis (Ockenfels et al. 1998) and was approved in 2013 for the treatment of multiple sclerosis (Xu et al. 2015; Al-Jaderi and Maghzachi 2016). After oral administration, DMF is rapidly metabolized to monomethyl fumarate (MMF), which crosses the blood-brain barrier and reaches detectable levels in the CNS (Kauppinen et al. 2013; Linker and Gold 2013).

DMF is an activator of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Loewe et al. 2002). Nrf2 is a nuclear transcription factor, which, under baseline conditions, forms a complex with the Keap1 protein (Kelch-like ECH associated protein-1), which leads to the ubiquitination and degradation of Nrf2 (McMahon et al. 2006). Keap1 comprises three domains: Kelch, broad complex, Tramtrack, and Bric-a-Brac (BTB) and intervening region (IVR) (Canning et al. 2015). When activated by fumarates, for example, Nrf2 translocates to the nuclear compartment and binds to antioxidant response elements (ARE), thus regulating the expression of antioxidant enzymes, as heme oxygenase 1 (HO-1), NADPH quinine oxidoreductase 1 (NQO1), and glutathione S-transferase (GST) (Zhang et al. 2013; Stefanson and Bakovic 2014).

Nrf2 has been shown to play an important role in diseases of the CNS. Nrf2 activation protects neurons against ischemic and hemorrhagic stroke, traumatic brain injury, and neurodegenerative disorders in animals (Iadecola and Anrather 2011; Magesh et al. 2012).

DMF has been reported to improve the survival of astrocytes and neurons subjected to oxidative stress in vitro (Linker et al. 2011; Albrecht et al. 2012). Moreover, DMF significantly alleviated CUMS-induced behavioral abnormalities in stressed rats, suppressing the expression of NF-κB, TNF-α, and IL-1β, reducing oxidative stress and positively regulating the hippocampal BDNF (Abd El-Fattah et al., 2018), serum serotonin, and GSH levels (Kortam et al. 2021). However, Nrf2-deficient mice also showed beneficial results after treatment with DMF, which was associated with anti-inflammatory results (Schulze-Topphoff et al. 2016). These observations suggest that immunomodulatory and anti-inflammatory responses initiated by DMF can occur between alternative pathways, regardless of Nrf2.

Thus, the present study aims to investigate the interaction between monomethyl fumarate and the Keap1/Nrf2 proteins, as well as to predict probable protein targets of this drug and its binding mode. As depression and other neuropsychiatric diseases have a pro-inflammatory phenotype (Haapakoski et al. 2016), we postulate that DMF has antidepressant effect, acting on neuroinflammation, and we aim to investigate molecular mechanisms involved in this effect.

**Methods**

**Animals**

Male adult Swiss mice weighing 25–30 g provided from the Animal House of the Federal University of Ceará were used. Briefly, the animals were maintained at a controlled temperature (22 ± 2 °C) with a 12-h dark/light cycle and food and water ad libitum. Mice were caged in groups of 8 in 41 × 34 × 16-cm cages. The animals were maintained and manipulated according to the NIH Guide for the Care and Use of Laboratory Animals and the experiments were performed after approval of the Ethics Committee on Animal Research of the Federal University of Ceará (protocol number 104/17).

**Drugs**

Dimethyl fumarate was obtained from Sigma (St. Louis, MO, USA) and fluoxetine from Eli Lilly. Dimethyl fumarate (50 and 100 mg/kg) was dissolved in carboxymethyl cellulose 0.5% (Abd El-Fattah et al. 2018) and fluoxetine (10 mg/kg) in distilled water. The drugs were made up freshly within 1–2 h of dosing and were administered orally in a volume of 0.1 ml/10 g body weight.

**Experimental design**

The chronic unpredictable mild stress (CUMS) procedure was carried out in mice as describe in earlier report (Willner 1997; Lu et al. 2006; Garza et al. 2012). This paradigm was designed to maximize unpredictability and mildness of the stress intensity. It consisted of a variety of stressors (showed in Table 1) applied randomly, once a day for 28 days. All
stresses were applied to animals outside of their housing area in a separate procedure room. After the animals were stressed, they were kept in the procedure room for 1–2 h to allow the stress odor to disappear. Mice were first divided into two groups, i.e., control and CUMS groups, which were subdivided into vehicle (carboxymethyl cellulose 0.5%), dimethyl fumarate 50 mg/kg (DMF50), dimethyl fumarate 100 mg/kg (DMF100), or fluoxetine 10 mg/kg (FLU) groups ((1) CONT + VEH, (2) CONT + DFM50, (3) CONT + DMF100, (4) CONT + FLU, (5) CUMS + VEH, (6) CUMS + DMF50, (7) CUMS + DMF100, (8) CUMS + FLU). Fourteen days after the beginning of the procedure, time required for the animal to develop depressive-like behaviors (He et al. 2016), treatments were started in all groups, 30 min before the application of the stress daily, in the CUMS groups. At the same time of day, control animals received the same treatments. The control groups were handled daily in the housing room.

**Behavioral tests**

**Open field test**

This test evaluates the influence of drugs on locomotor activity and exploratory behavior. An acrylic apparatus (transparent walls and black background, dimensions 30 × 30 × 15), divided into nine squares, was used (Archer 1973). The animal was placed in the center of the apparatus and the following parameters were observed over 5 minutes: the number of crossings (considering the four legs in the square) was measured as a direct locomotory activity marker, number of rearing (standing on hind legs), and number of grooming (self-cleaning movement). The apparatus was cleaned with 10% alcohol after each animal.

**Forced swimming test (FST)**

This test was conducted based on the method previously described (Porsolt et al. 1977). Mice were individually placed in an open cylindrical container (diameter, 22 cm; height, 40 cm) containing water at 20-cm height. The total time of immobility was recorded for 5 min after 1 min of habituation. Immobility was defined as the animal floating in the water without struggling and making only very minimal movements necessary to keep its head above the water. An increase in the duration of immobility is an indicative of depressive-like behavior.

**Sucrose preference test**

Before the test, the mice were trained to adapt to a sucrose solution (2%, v/v) by placing two bottles of a sucrose solution in each cage for 24 h. Then, one bottle containing water and another containing sucrose solution were placed on the cages for 24 h. After the adaptation, mice were housed in individual cages with free access to two bottles. One of them was containing 100 ml of sucrose solution (2% v/v) and another was containing 100 ml of water. The test started with the onset of the dark (active) phase of animals’ cycle. No food or water deprivation was applied before the test. After 24 h, volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated by the following formula: sucrose preference = (sucrose consumption/water consumption + sucrose consumption) × 100 (Willner et al. 1987).

**Novel object recognition test (NOR)**

Novel object recognition task is used to evaluate recognition memory. This test is based on the innate tendency of rodents to explore unfamiliar objects within their environment. This test assesses the mouse’s ability to discriminate between familiar and novel objects. Firstly, mice were individually habituated to an open field plexiglas box (30 × 30 × 15-cm size) for 5 min. After 15 min, mice were allowed to explore a set of two identical objects for 5 min (acquisition phase). These objects were suitably heavy and tall to guarantee that mice could neither move them nor climb over them. After a 5 min interval, mice were presented to a similar set of objects in the same environment with the replacement of one familiar object by a novel/unknown object (testing phase). The animals were allowed to freely explore the objects again for a 5-min period. A discrimination index was calculated as follows: (time exploring new object − time exploring familiar object)/ (time exploring new object + time exploring familiar object) (Ennaceur and Meliani 1988).

**Neurochemical assays**

**Immunoenzymatic assay for TNF-α and IL-1β concentrations**

For this test, hippocampus was homogenized in 8 volumes of phosphate-buffered saline (PBS) with protease (EMD

---

**Table 1** Chronic unpredictable mild stress protocol*

| Stressor | Description |
|----------|-------------|
| Restraint (2 h) | (55 ° for 8 h) |
| Tilted cage | (45 ° for 8 h) |
| Intermittent cycle between lights on and off | (18 h) |
| Constant light (24 h) | |
| Water deprivation (24 h) | |
| Wet cage (24 h) | |
| Electric shock | (1 mA, 2 s) |

*Garza et al. (2012)
Biosciences) and phosphatase (Sigma-Aldrich) inhibitors and centrifuged (12,000 rpm, 5 min). The concentration of each cytokine in 50-μl samples was determined by immunoenzymatic assay ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol and expressed in pg/g of tissue.

Immunofluorescence

Slices containing hippocampus were rinsed four times in PBS. They underwent an antigen recovery process and to were incubated overnight at 4 °C with the mouse monoclonal anti-GFAP (1:200, Santa Cruz Biotechnology) to mark astrocytes, and after, slices were rinsed four times with PBS and then incubated for 2 h at room temperature with AlexaFluor-594 conjugated goat anti-mouse IgG antibody (1:400; Invitrogen, Carlsbad, CA, USA). For microglia, slices containing hippocampus were incubated overnight at 4 °C with Iba1 (ionized calcium binding adapter protein 1) (E4O4W) XP® Rabbit mAb, Alexa Fluor® 555 Conjugate (1:300, Cell Signaling Technology). Subsequently, they were stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA, USA). Finally, slides were rinsed in PBS and cover slipped using Prolong Gold Antifade Mountant (ThermoFisher Scientific, Waltham, MA, USA). The methodology used was based on the manufacturers’ protocols. Slides were imaged using a Cytation 3 slide reader (Carl Zeiss, White Plains, NY, USA) through a magnification of 20 objective lens, at constant exposure, gain, and offset. The hippocampal subfields CA1, CA3, and DG were identified according to Paxinos and Franklin (2001) and four to five photomicrographs of each area for each group were analyzed. The experimenter who took the images was blinded to treatments. The fluorescence intensity analysis was semi-quantitative, using the ImageJ software package.

Statistical analysis

Statistical analysis was performed with GraphPad Prism for Windows (version 8.0, San Diego, USA). Data from behavioral and neurochemical tests are expressed as means ± SEM. For behavioral data, the analyses were performed by regular two-way analysis of variance (ANOVA), using as factors “CUMS model” (CUMS or control groups) and “treatment” (DMF50, DMF100, FLU) followed by Tukey’s test. For neurochemical data, the analyses were performed by regular one-way ANOVA followed by Tukey’s test. The significance level was set at P < 0.05.

Computational analysis

Target prediction

The prediction of biological targets of the MMF was performed through the SwissTargetPrediction (Gfeller et al. 2013) and Similarity Ensemble Approach (Keiser et al. 2007) servers. These servers estimate the most probable macromolecular targets of a small molecule, assumed as bioactive. The predictions are based on a combination of similarity with libraries of known actives.

3D protein structure prediction

The primary sequence of hydroxycarboxylic acid receptor 2 (HCAR2) was searched on the Uniprot server (Bateman et al. 2021) and submitted to the I-TASSER server (Yang and Zhang 2015) to build the template-based modeling of the HCAR2 tridimensional (3D) structure, using a threading method. The model generated by the server proceeded to the refinement stage on GalaxyRefine server (Heo et al. 2014). The statistical quality of the 3D protein was analyzed by the MolProbity server (Williams et al. 2018).

Molecular docking calculations

Molecular docking calculations were performed using Glide (Friesner et al. 2004) and CovDock (Zhu et al. 2014) programs, considering the ligand flexible and the protein rigid. The structure of the monomethyl fumarate was retrieved from the PubChem repository (PUBCHEM ID 5369209). The 3D structures of human Keap1 were obtained from the Protein Data Bank (Berman et al. 2000): PDB ID 6HWS (Talapatra et al. 2019) for the Kelch domain (Davies et al. 2016) and PDB ID 5GIT for the BTB domain (Wu et al. 2017). As the 3D structure of human HCAR2 was not available at PDB, it was built by template-based modeling, as previously described.

The MMF ligand was docked into HCAR2 and Keap1 (Kelch domain) using the Maestro Glide software in extra-precision (XP) mode (Friesner et al. 2006). Covalent docking calculations were performed for the BTB domain of Keap1 protein, using Maestro CovDock program (Zhu et al. 2014). The protein structures were prepared using the Protein Preparation Wizard tool, adding the hydrogen atoms and minimizing the energy, using the OPLS-2005 force field. The MMF ligand was prepared through the LigPrep tool, correcting protonation, according to Epik, and performing energy minimization.

The protein grid coordinates for HCAR2 were centered at the residues Arg111, Arg251, and Thy284, provided by the I-TASSER server ligand binding site predictions for the homologous protein Nociceptin/orphanin FQ receptor (PDB...
ID 4EA3) (Yang and Zhang 2015) and which corroborate the binding site found by Offermanns and collaborators (2011). For the Keap1 Kelch domain, the protein grid coordinates were centered at Arg380, Ser363, Arg415, Gln530, Ser555, and Tyr572 residues (Unni et al. 2021) and for BTB domain were centered at Cys151 and Lys131 (Linker and Gold 2013). The PLIP server (Salentin et al. 2015) was used to identify non-covalent interactions between proteins and ligand. The Pymol program was used for the visual inspection of the docking poses and to render the 3D molecular images (Delano 2002).

Results

Behavioral results

Effect of dimethyl fumarate in the reversal of CUMS-induced changes in open field test parameters

There were no significant differences in the number of crossings between the groups studied, showing that the model and the treatment with DMF and FLU did not alter the locomotor activity of the animals (Fig. 1A).

The analysis of the number of rearings (Fig. 1B) in the open field test by two-way ANOVA revealed a significant interaction between the factors “CUMS model” and “treatment” ($F(3, 63) = 5.012, P = 0.0035$). In this regard, we observed a significant increase in the number of rearings in CUMS + VEH group compared to CONT + VEH ($P< 0.001$). Additionally, CUMS + FLU and CUMS + DMF100 groups presented a significant reduction in this parameter when compared to CUMS + VEH group ($P < 0.01$).

In the evaluation of the number of groomings (Fig. 1C), we observed a significant interaction between the factors ($F(3, 64) = 5.667, P = 0.0017$). CUMS + VEH group showed an increase in this parameter compared to the CONT + VEH ($P < 0.05$). DMF treatment at both doses reversed this increase ($P < 0.05$).

Effects of dimethyl fumarate in the reversal of CUMS-induced increase in the immobility time in the forced swimming test

Two-way ANOVA analysis demonstrated a significant interaction ($F(3, 66) = 11.30, P < 0.0001$) between factors with significant effect of “CUMS model” ($F(1, 66) = 4.104, P = 0.0468$) and “treatment” ($F(3, 66) = 10.93, P < 0.0001$). In Tukey’s test, a significant increase in immobility duration was observed in the group CUMS + VEH ($P < 0.001$) when compared to CONT + VEH. The treatment with DMF (50 and 100) and FLU was able to reverse the increase in immobility time ($P < 0.001$), as illustrated in Fig. 2.

Effects of dimethyl fumarate in the reversal of CUMS-induced decrease in the sucrose preference index in the sucrose preference test

In addition to the immobility time, the CUMS modified another parameter indicative of depressive-like behavior, the sucrose preference index, which was significantly reduced when compared to the control ($P < 0.01$),
indicating anecdotal behavior. The CUMS + DMF50 and CUMS + DMF100 groups led to an increase in this index in relation to the CUMS + VEH group \((P < 0.001\) and \(P < 0.05\), respectively), as shown in Fig. 3. The interaction between the factors was significant \(F(3, 64) = 7.628, P = 0.0002\).

**Effect of dimethyl fumarate in the reversal of CUMS-induced memory alteration in novel object recognition test**

In the evaluation of the recognition index, we observed a significant interaction between the factors \(F(3, 66) = 13.01, P < 0.0001\). CUMS + VEH group showed a decrease in this parameter compared to the CONT + VEH \((P < 0.001)\). The treatment with DMF at both doses \((P < 0.001)\) and FLU \((P < 0.01)\) was able to reverse this alteration caused by CUMS (Fig. 4).

**Neurochemical results**

**Effect of dimethyl fumarate in the reversal of CUMS-induced changes in astrocytic activation marker in hippocampus**

In CA1 area, GFAP expression was reduced in the CUMS group compared to the control \((P < 0.05)\) and treatment with DMF50 was able to reverse this reduction \((P < 0.05)\), as seen in Fig. 5A \((F(2, 10) = 8.16, P = 0.0079)\).

In CA3 \((F(2, 9) = 12.89, P = 0.0023)\) and DG \((F(2, 9) = 4.93, P < 0.0001)\) areas, the GFAP expression was reduced by CUMS compared to the control \((CA3: P < 0.05\) and DG: \(P < 0.001)\); however, the treatment with DMF50 did not interfere in this parameter, as seen in Fig. 5B and C, respectively.

**Effect of dimethyl fumarate in the reversal of CUMS-induced changes in microglial activation marker in hippocampus**

In the three studied areas CA1 (Fig. 6A), CA3 (Fig. 6B), and DG (Fig. 6C), the expression of Iba1 was increased in the CUMS group in relation to the CONT + VEH \((P < 0.001)\) and treatment with DMF50 was able to reverse this increase \((P < 0.001)\). CA1: \(F (2, 13) = 25.89, P < 0.0001\), CA3: \(F (2, 13) = 31.58, P < 0.0001,\) and DG: \(F (2, 11) = 76.90, P < 0.0001\).
Effect of dimethyl fumarate in the reversal of CUMS-induced increase in the concentration of the pro-inflammatory cytokines TNF-α and IL-1β in hippocampus

CUMS induced an increase in the expression of TNF-α in relation to the control ($P < 0.001$) and the treatments with DMF50 and FLU were able to reverse this increase ($P < 0.05$), as seen in Fig. 7A ($F (3, 27) = 21.56, P < 0.0001$).

Likewise, there was an increase in IL-1β in the CUMS group compared to the control ($P < 0.001$), which was reversed by treatments with DMF50 ($P < 0.001$) and FLU ($P < 0.01$), as seen in Fig. 7B ($F (3, 28) = 24.91, P < 0.0001$).

Computation results

Target prediction results for MMF

To estimate the most probable targets for MMF protective effects, we submitted the chemical structure of MMF to the SwissTargetPrediction and Similarity Ensemble Approach servers. These servers predict the most probable proteins, based on the similarity of MMF with chemical structures of the ChEMBL 27 (for SEA) and ChEMBL 23 (for SwissTargetPrediction) compounds databases, and rank proteins from the most to the less probable ones (Tables S1 and S2).

Based on both SwissTargetPrediction and SEA servers predictions, human HCAR2 was predicted as the most probable target of MMF. HCAR2 had the largest the quantitative probability rate and also presented known actives with similar chemical properties in SwissTargetPrediction compounds databases, and rank proteins from the most to the least probable ones (Tables S1 and S2).

3D-structure modeling and molecular docking of MMF against HCAR2 and Keap1 proteins

The 3D structure of HCAR2 and Keap1 was used to perform the docking calculations using Maestro programs. For HCAR2, there is no 3D structure available in PDB, and we firstly built the protein model using the I-TASSER server. This server used a compilation of the top ten templates (4MBS, 4XNW, 4MBS, 5ZH, 5ZKP, 6IBB, 5ZBH, 6DO1, 4XNV, 4YAY) and generated a model with C-score of −1.71. C-score values vary from −5 to 2, the higher C-score, the greater the model confidence. The model was analyzed on MolProbity server and showed a MolProbity score of 1.34 (a score normalized to be at the same scale as X-ray resolution). The analysis of the Ramachandran plot of the HCAR2 model (Fig. S1) showed that 99.65% of the residues lie in the most favorable regions, which was more than satisfactory, since ideally this value should be greater than 98%. The docking calculations against Keap1 protein, at both domains Kelch and BTB were performed using the 3D structures PDB ID 6HWS that present Kelch domain and PDB ID 5GIT that present the BTB domain.

Then, we performed the docking calculations against the predicted targets of MMF, in order to investigate the predicted binding affinities (measured by the docking score), the possible binding modes, and the interactions between MMF and the predicted targets. The more negative a docking score (equivalent to binding affinity energy), the more favorable is the ligand-protein binding interaction. We also performed docking of known HCAR2 activator and Keap1 inhibitors, using them as reference, for docking scores and protein-ligand interactions. As the studied compounds present different molecular weights, we calculated the relation between binding affinity (docking score) and number of heavy atoms (Table 2), through the ligand efficiency (LE) metric (Abad-Zapatero 2007). LE normalizes the affinity with respect to number of heavy atoms. Ligand efficiency is calculated by scaling the binding affinity ($\Delta G$) or docking score by the number of non-hydrogen atoms ($n$), according to Eq. 1:

$$LE = \frac{\Delta G}{n}$$

The widely accepted LE values for oral drugs/hits are $ \geq 0.3 \text{ Kcal-mol}^{-1}-\text{non-hydrogen atom}^{-1}$ (Abad-Zapatero 2007; Hopkins et al. 2014).

As we can see, MMF presented acceptable LE values (from 0.24 to 0.39 Kcal-mol⁻¹-non-hydrogen atom⁻¹) compared with nicotinic acid, sulfonyl-amino derivate, and britann, suggesting that it was efficient binding to HCAR2 and Kelch and BTB domain of Keap1, respectively. As MMF is a small compound, it could be considered a fragment since its molecular weight (MW) $\leq 300$ Da, it can easily bind to different binding sites. Recently, a new crystal structure of Mus musculus Keap1 (Kelch) (PDB ID 6LRZ) complexed with three DMF molecules, with good resolution, was released in the Protein Data Bank (Unni et al. 2021). Comparing and superposing this crystallographic structure with the calculated docking of human Kelch-MMF, we observe that MMF and DMF molecules acquire similar orientation and conformation (Fig. 8D). The root mean square deviation (RMSD) of ligands was 2.03 Å, indicating that docking of Kelch-MMF resulted in a pose very similar to the experimental Kelch-DMF.

MMF docked into HCAR2 presented a docking score of $-3.51$ Kcal-mol⁻¹. MMF made hydrophobic interactions with Leu104 residue and salt bridges (in blue) with Arg111, Lys166, and Arg251 (Fig. 8A). Docking calculation was also performed between HCAR2 protein and nicotinic acid, a potent HCAR2 activator, which had a docking score
of $-5.69\text{ Kcal}\cdot\text{mol}^{-1}$. Nicotinic acid made interactions with the Arg111 residue as MMF did (through salt bridge) and in addition it made a Hbond with the Ser179 (data not shown).

Docking calculations at BTB domain of Keap1 showed a docking score of $-3.05\text{ Kcal}\cdot\text{mol}^{-1}$ and the main interactions were a covalent bond with the Cys151 residue, hydrogen bonds with the Gly148 residue (in yellow), and
Methodology and to verify if docking was able to recover a known complex’s structure and interactions. Using the structure available in PDB 5GIT, we performed the docking calculation at Kelch domain of Keap1 in turn, docking calculations at Kelch domain of Keap1 showed that MMF binds to Kelch binding site with a docking score of $-2.12 \text{ Kcal}\cdot\text{mol}^{-1}$, similar to the MMF-BTB value. The ligand made a covalent bond with the Cys151 residue, as well as MMF, and a hydrogen bond with Tyr85. The RMSD was 0.426 Å. The RMSD quantifies and compares the docking pose of the ligand with its co-crystallized pose, values below 2.0 Å indicating similar structures and, thus, docking reliability. In turn, docking calculations at Kelch domain of Keap1 showed that MMF binds to Kelch binding site with a docking score of $-2.12 \text{ Kcal}\cdot\text{mol}^{-1}$ and the main interactions were hydrogen bonds with Gln530, Ser555 (in yellow), and structural waters (Fig. 8C). The redocking calculation was performed with the PDB 6HWS and the co-crystallized sulfonamido-derivative inhibitor. The sulfonamide-derivative presented a docking score of $-3.97 \text{ Kcal}\cdot\text{mol}^{-1}$ and, like MMF, made hydrogen bonds with Gln530, structural waters, hydrogen bonds with Arg415, Ser602, Asn414, and salt bridge with Arg415, as well as π-stacking interactions with the Tyr572 (data not shown). The RMSD calculated for sulfonamido-derivative was 1.85 Å, in relation to its coordinates at crystal structure, thus validating the docking method.

Discussion

Chronic stress is a precipitating factor for depression, and the changes in various body systems that occur in depression are similar to those seen in response to stress (Farhan et al. 2014). Thus, chronic unpredictable mild stress is a promising model for screening antidepressants.

CUMS group had an increase in the number of rearings, anxiety-like behavior, in the open field test, compared to the control group, and treatment with FLU and DMF100 was able to reverse this effect. This result is in line with the work of Ali et al. (2017), which also showed an increase in this parameter in the CUMS group, and a reduction after treatment with antidepressant drugs.

As already suggested in the literature (Shmelkov et al. 2010; Papaleo et al. 2011), grooming responses in mice may represent highly relevant traits for schizophrenia, anxiety, and depression, since increased grooming in rodents is usually consistent with stress responses and anxiety-like phenotypes. Our results corroborate this notion, since the CUMS group showed an increase in this parameter in relation to the control. Fajemiroye et al. (2015) showed that methyl fumarate was able to decrease the number of groomings in relation to depressive control, and in the present study, DMF had the same effect.

To assess depressive behaviors, the forced swimming and sucrose preference tests were performed. CUMS caused a significant increase in the immobility time and decrease in sucrose preference index, in relation to the control group, which corroborates with other findings (Ali et al. 2017; Abd El-Fattah et al. 2018; Liu et al. 2019). These results are indicative of depressive-like behaviors and the treatment with DMF was able to reverse them, which is also in line with the work of Abd El-Fattah et al. (2018). In open field test, there were no significant differences in the number of crossings between the groups studied, which corroborates the findings by Campolo et al. (2017) and Liu et al. (2019). Thus, it can be inferred that the alterations of immobility in the forced swimming test is not due to changes in locomotor activity.

As the anxiety/depression type phenotype is associated with cognitive deficit, the novel object recognition test was performed (Darcet et al. 2014). In this test, a reduction in the recognition index was observed in the CUMS group, which corroborates with Shehu et al. (2019) and Venkatesh et al. (2019), who also observed shorter exploration time on the new object compared to the old one by the stressed group, which is indicative of memory impairment. To the best of our knowledge, there are no studies that demonstrate the effect of DMF and FLU in mice subjected to CUMS in the novel object recognition test. In this study, the groups submitted to CUMS treated with FLU, DMF 50 and DMF 100 had reversion of reduction in the recognition index, that is, they were able to reverse the cognitive deficit caused by CUMS.

As in the behavioral tests, there was no difference between the results obtained by the different doses tested
(50 and 100 mg/kg) of DMF; we proceeded to the neurochemical tests only with the lowest dose. We measure the expression of glial fibrillar acid protein (GFAP), the main component of the intermediate filaments of the astrocytes cytoskeleton (Middeldorp and Hol 2011). There are divergences in the literature regarding the astrocytic response to depression. Miguel-Hidalgo et al. (2000) demonstrated a significant decrease in the density of astrocytes in the gray matter of the prefrontal cortex in younger depressed individuals. In contrast, older individuals showed an increase in the fraction of GFAP area in the prefrontal cortex, which may reflect a compensatory reaction to neuronal damage in MDD (Rajkowska et al. 2005).

Muller et al. (2001) detected reduction of astrocytes in Cornu Ammonis (CA) subfields CA1, CA2, of the hippocampus of patients with depression. These results are also demonstrated in preclinical studies, in which depression models, involving various types of stress, cause reduction of astrocytes in the hippocampus (Czéh et al. 2006; Leventopoulos et al. 2007). In the present study, GFAP expression was reduced by CUMS compared to control in the three areas of the hippocampus studied, CA1, CA3, and dentate gyrus. DMF treatment reversed this reduction only in CA1.

Regarding treatment with antidepressants, some studies show that they can reverse the reduction of GFAP caused by stress (Czéh et al. 2006; Banasr et al. 2010). However, a 4-week treatment with citalopram did not reverse the reduction in GFAP protein in the hippocampus of rats subjected to stress due to social defeat, although the animals’ behavior was normalized with the treatment (Araya-Callis et al. 2012).

Several studies have reported that melatonin, Nrf2 inducer, was able to negatively modulate inflammation in animal models of chronic neuroinflammation through the induction of reactive astrogliosis (Singhakumar et al. 2015; Ali et al. 2018; Permpoonputtana et al. 2018) and the change in polarization of the activated microglia from the pro-inflammatory or M1 phenotype to the anti-inflammatory or M2 state (Hu et al. 2019; Zhang et al. 2019).

Microglial activation is a key mediator of neuroinflammatory processes (Tronel et al. 2017), and neuroinflammation plays a crucial role in the pathogenesis of depression (Zhang et al. 2019). Hippocampal microglial activation promotes the release of inflammatory factors, which results in the interruption of neuroplasticity and cognitive impairment, thus contributing to the development of depression (Walker et al. 2013; Singhal and Baune 2017). In this study, Iba1 expression was increased by CUMS compared to control in the three areas of the hippocampus studied, CA1, CA3, and dentate gyrus, and treatment with DMF was able to reverse this increase in microglial activation.

It is well-known that treatment with ADs from distinct pharmacological classes can protect against brain damage associated with microglia reactivity and cytokine overproduction induced by chronic stress (Brooks et al. 2017; Lu et al. 2017). Animals treated with dimethyl fumarate showed...
fewer activated microglia cells in the peri-hematomal region compared to animals treated with vehicle, in a model of intracerebral hemorrhage (Iniaghe et al. 2015). To the best of our knowledge, there are no studies that have evaluated the microglial response to DMF in a depression model, so the present study is pioneering. Our results show that DMF reduced microglial activation, corroborating the results of Iniaghe et al. (2015).

Clinical studies have also shown that the abnormal profile of pro-inflammatory cytokines, especially IL-6, IL-1β, and TNF-α, may be associated with the initiation, relapse, and progression of depression (Young et al. 2014; Adzic et al. 2017). The increase in the expression of pro-inflammatory cytokines levels in the hippocampus of rats exposed to CUMS is well described in the literature (Abd El-Fattah et al., 2018; Zou et al. 2020). In the present study, the elevation of the hippocampal levels of TNF-α and IL-1β, caused by CUMS, was inhibited by DMF. Our results corroborate with Abd El-Fattah et al. (2018) and Quan et al. (2015) who demonstrated that the inhibition of these inflammatory mediators exerted therapeutic action in the depression-like behavior induced by CUMS in rats.

Nrf2 activation in the presence of xenobiotics can occur by two main mechanisms: interruption of the Kelch domain/Nfr2 interaction (Davies et al. 2016) and/or covalent binding to cysteine residues of the Keap1-BTB domain (Huerta et al. 2016). In this way, we performed the molecular docking of MMF into these two domains. The MMF-Keap1/Kelch docking showed some similar interactions to sulfonyl-amino derivate ligand and not so similar docking score. Comparing the LE value of MMF and sulfonyl-amino derivate (Table 2), MMF presented a better LE than sulfonyl-amino derivate, suggesting that MMF is more efficient than sulfonyl-amino derivate. In the other hand, MMF-Keap1/Kelch docking is less efficient than MMF-Keap1/BTB docking.

In turn, the MMF-Keap1/BTB covalent docking generated a docking score very similar to a potent Keap1/BTB inhibitor and similar interactions, including the covalent bonding to the Cys151 residue, occurred in both. The docking calculations suggested that MMF binds to BTB domain

| Target       | Compound         | Docking score (Kcal·mol$^{-1}$) | $n^5$ | LE* (Kcal·mol$^{-1}$·non-hydrogen atom$^{-1}$) |
|--------------|------------------|---------------------------------|------|-----------------------------------------------|
| HCAR2        | MMF              | $-3.51$                         | 9    | 0.39                                          |
|              | nicotinic acid   | $-5.69$                         | 9    | 0.63                                          |
| Kelch of Keap1 | MMF             | $-2.12$                         | 9    | 0.24                                          |
|              | sulfonamide derivate | $-3.97$                 | 76   | 0.09                                          |
| BTB of Keap1 | MMF              | $-3.05$                         | 9    | 0.33                                          |
|              | britain          | $-3.81$                         | 39   | 0.09                                          |

$^5$ Number of non-hydrogen atoms; $^*$ ligand efficiency

Fig. 8 3D intermolecular interactions of monomethyl fumarate (MMF) with A HCAR2 3D protein model; B BTB domain of Keap1; C Kelch domain of Keap1, obtained with molecular docking calculations and D the alignment of MMF Keap1/Kelch domain with the crystal DMF Keap1/Kelch domain, in grey. Hydrogen bonds are shown as yellow dashed lines, and salt bridges interactions as blue dashed lines. Atom colors: green (carbon atoms of MMF); deep salmon (carbon atoms of protein residues); red (oxygen); yellow (sulfur); dark blue (nitrogen), and white (hydrogen).
of Keap1 with similar protein-ligand interactions and similar docking score comparing with britain inhibitor. The LE values of MMF and britain (Table 2) showed that MMF is more efficient binding to BTB than britain. This indicates that Keap1 could be a promising target of MMF. The redocking calculations performed for both domains of Keap1, presented a RMSD below to 2.0 Å in both redockings, indicating the reliability of the docking calculations.

On the other hand, Schulze-Topploff et al. (2016) demonstrated that some immunological and clinical effects of DMF are independent of Nrf2. Thus, it was realized that there are other ways in which DMF produces its anti-inflammatory effect. Thus, to discover these alternative pathways and better understand the molecular mechanism involved in the antidepressant and anti-inflammatory effect of DMF, we made a target prediction study and found the HCAR2 protein as the most likely target. The hydroxycarboxylic acid receptor 2 (HCAR2) is a membrane receptor coupled to Gi protein known for suppressing pro-inflammatory activation and chemotactic recruitment of immune cells, such as dendritic cells and macrophages. HCAR2 is expressed in the mammalian brain, for example in rodent hypothalamic neurons (Fu et al. 2015; Rezq and Abdel-Rahman 2016). Chen et al. (2014) demonstrated that oral DMF treatment reduced neurological deficits and spinal cord demyelination in mice submitted to acute experimental autoimmune encephalomyelitis (EAE), an inflammatory model of multiple sclerosis. These effects were partially abolished in HCAR2-deficient mice, showing that the action of DMF is dependent on this protein.

The results of Tang et al. (2008) demonstrated that monomethyl fumarate (metabolite of dimethyl fumarate) binds to the same site in HCAR2 as nicotinic acid (niacin). This is consistent with the notion that the HCAR2 binding pocket requires low molecular weight carboxylic acid ligands (Offermanns 2006). Our docking results against HCAR2 showed that MMF presented protein-ligand interactions similar to nicotinic acid; thus, we suggest that the anti-inflammatory effects can be caused by dimethyl fumarate. Despite the better docking score of nicotinic acid, comparing the LE of MMF with nicotinic acid (Table 2), we can infer that MMF is as efficient as nicotinic acid in binding to HCAR2.

Thus, activation of HCAR2 upon MMF binding would lead to activation of the Gi-type G protein signaling cascade; this, in turn, would result in phospholipase C activation (Blad et al. 2011) and, thereby, in increased \( [\text{Ca}^{2+}]_i \), which, through phosphorylation of threonine 172 by calcium/calmodulin-dependent protein kinase 2 (CaM KK2), would lead to activation of AMPK (Hurley et al. 2005). This, in turn, would activate the SIRT1 protein, through the conversion of NAM to NAD+ by the enzyme nicotinamide b- o- syltransferase (NAMPT), the rate-limiting enzyme in the NAD biosynthesis pathway (Fulco et al. 2008).

Sirtuin-1 protein (SIRT1) is a member of sirtuins, a family of NAD+-dependent deacetylase proteins that regulate a large number of cellular processes, including aging, metabolism, redox homeostasis, cell survival, and inflammation (Singh et al. 2018). It is widely expressed in the CNS and involved in the maintenance of physiological brain functions and exhibits neuroprotective and anti-inflammatory effects in many neurodegenerative diseases. Several studies have shown that the deregulation of SIRT1 contributes to the pathogenesis of MDD (Alageel et al. 2018; Lu et al. 2018).

SIRT1 has shown to suppress NF-κB by direct deacetylation in NF-κB p65 lysine 310 (Yeung et al. 2004). This suppression of NF-κB leads to inhibition of the secretion of pro-inflammatory cytokines and to the switch microglia phenotype from pro- to anti-inflammatory.

The effects of the oligofucoidan on Nrf2 activation were inhibited, using a SIRT1 inhibitor, demonstrating that there
is a crosstalk between the SIRT1 and Nrf2 cascade (Yu et al. 2020). Shah et al. (2017) corroborated this notion, since melatonin exerted antioxidant and anti-inflammatory activities through the Nrf2 activation, dependent on SIRT1, in the brain of immature rats and in BV2 cells. When a SIRT1 inhibitor was used, the expressions of SIRT1 and Nrf2 were significantly decreased, suggesting that LPS-induced inhibition of Nrf2 is dependent on SIRT1 in vitro and in vivo.

The treatment with DMF was able to significantly increase the total number of GFAP-positive cells in the cortex and striatum of mice compared to the control group subjected to an ischemia-hypoxia model. These findings indicate that Nrf2 activation plays a substantial role in astrocytic activation and proliferation (Liu et al. 2019).

Finally, Zhang et al. (2017) showed redox-mediated transcriptional cross-talk between Nrf2 and NF-κB responses to LPS. Haghani et al. (2020) hypothesized involvement of Keap1, which can mediate IKKβ degradation and inhibit NF-κB nuclear localization (Kim et al. 2010). Other possible mechanisms are as follows: Nrf2 can inhibit NF-κB through reduction of reactive species (Cuadrado et al. 2014), and NF-κB can also inhibit Nrf2 activity through enhancing the recruitment of histone deacetylase to ARE region (Wakabayashi et al. 2010).

Thus, we hypothesized that the binding of monomethyl fumarate to HCAR2 leads to a signaling cascade, which culminates in astrocytic and microglial alterations and reduced production of pro-inflammatory cytokines (Fig. 9), results demonstrated in the present study.

**Conclusion**

The results of the present study revealed that dimethyl fumarate is a promising prodrug for the treatment of MDD and for comorbidities such as memory deficit. DMF modulated the neuroinflammatory pathway, increasing astrocytes expression, reducing microglia expression and the production of the pro-inflammatory cytokines IL-1β and TNF-α. The computational results suggested binding interactions of monomethyl fumarate (DMF active metabolite) with the Keap1 protein and consequent activation of Nrf2, as well as with the protein HCAR2, leading to a complex signaling cascade, which has a crosstalk with Nrf2 pathway.

Thus, it is suggested that one of the mechanisms involved in the antidepressant effect of DMF is neuroinflammatory suppression, triggered by the binding of its metabolite MMF to the HCAR2 protein. However, more studies must be carried out to better understand the molecular mechanisms involved in the antidepressant effect of this drug.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00210-022-02247-x.

**Author contributions** AGS, AJMCF, and MMFF conceived and designed research. AGS, ISL, TMBG, JYSO, MAJC, KAL, and PMJ performed the behavioral tests; AGS, ISL, and AJMCF performed neurochemical technics, AGS, SSM, MM, and CHA performed molecular modelling, DSM contributed new reagents or analytical tools. AGS, SSM, MM, and ISL analyzed data. AGS wrote the manuscript. All authors contributed, edited, read, and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

**Funding** The authors acknowledge the Brazilian National Research Council (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and Ceará State Foundation for Scientific and Technological Development Support (FUNCAP) for the financial support and scholarships that were essential to the conduction of the present study.

**Data availability** Materials were available by the Neuropharmacology Laboratory.

**Code availability** Not applicable.

**Declarations**

**Ethics approval** The experiments were performed after approval of the Ethics Committee on Animal Research of the Federal University of Ceará (protocol number 104/17), and in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA).

**Consent to participate** Not applicable.

**Consent for publication** All authors have agreed to publish this version of the manuscript.

**Conflict of interest** The authors declare no competing interests.

**References**

Abad-Zapatero C (2007) Ligand efficiency indices for effective drug discovery e ligand efficiency: a useful metric for lead selection. Expert Opin Drug Discovery 2:469–488. https://doi.org/10.1517/17460441.2.4.469

Abd El-Fattah AA, Fahim AT, Sadik NAH, Ali BM (2018) Resveratrol and dimethyl fumarate ameliorate depression-like behaviour in a rat model of chronic unpredictable mild stress. Brain Res 15:227–236. https://doi.org/10.1016/j.brainres.2018.09.027

Adzic M, Brkic Z, Mitic M et al (2017) Therapeutic strategies for treatment of inflammation-related depression. Curr Neuropharmacol 16. https://doi.org/10.2174/1570159115666170828163048

Alageel A, Tomasi J, Tersigni C et al (2018) Evidence supporting a mechanistic role of sirtuins in mood and metabolic disorders. Prog Neuro-Psychopharmacol Biol Psychiatry 86:95–101. https://doi.org/10.1016/j.pnpbp.2018.05.017

Albrecht P, Bouchachia I, Goebels N et al (2012) Effects of dimethyl fumarate on neuroprotection and immunomodulation. J Neuroinflammation 9. https://doi.org/10.1186/1742-2094-9-163

Ali SS, Abd El Wahab MG, Ayub NN, Suliaman M (2017) The antidepressant-like effect of Ocimum basilicum in a animal model of depression. Biotech Histochem 92:390–401. https://doi.org/10.1080/10520295.2017.1323276

Ali T, Rehman SU, Shah FA, Kim MO (2018) Acute dose of melatonin via Nrf2 dependently prevents acute ethanol-induced neurotoxicity in the developing rodent brain. J Neuroinflammation 15. https://doi.org/10.1186/s12974-018-1157-x
Al-Jaderi Z, Maghazachi AA (2016) Utilization of dimethyl fumarate and related molecules for treatment of multiple sclerosis, cancer, and other diseases. Front Immunol 7. https://doi.org/10.3389/fimmu.2016.00278

Andrews PW, Kornstein SG, Halberstadt LJ et al (2011) Blue again: perturbational effects of antidepressants suggest monoaminergic homeostasis in major depression. Front Psychol 159. https://doi.org/10.3389/fpsyg.2011.00159

Araya-Callís C, Hiemke C, Abumaria N, Flugge G (2012) Chronic psychosocial stress and citalopram modulate the expression of the glial proteins GFAP and NDRG2 in the hippocampus. Psychopharmacology 224:209–222. https://doi.org/10.1007/s00213-012-2741-x

Archer J (1973) Tests for emotionality in rats and mice: a review. Anim Behav 21:205–235. https://doi.org/10.1016/S0003-3472(73)80065-X

Banasr M, Chowdhury GM, Terwilliger R et al (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry 15:501–511. https://doi.org/10.1038/mp.2008.106

Bateman A, Martin MJ, Orchard S et al (2021) UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Res 49:D480–D489. https://doi.org/10.1093/nar/gkaa1100

Berman HM, Westbrook J, Feng Z et al (2000) The Protein Data Bank. Nucleic Acids Res 28:235–242. https://doi.org/10.1093/nar/28.1.235

Blad CC, Ahmed K, Ujerman AP, Offermanns S (2017) The neuroprotective effect of dimethyl fumarate in an MPTP-mouse model of Parkinson's disease: involvement of reactive oxygen species/nuclear factor erythroid 2-related factor 2 (KEAP1:NRF2) protein-protein interaction with high cell potency identified by fragment-based discovery. J Med Chem 59:3991–4006. https://doi.org/10.1021/acs.jmedchem.6b00228

Davies TG, Wixted WE, Coyle JE et al (2016) Monoacidic inhibitors of the Kelch-like ECH-associated protein 1: nuclear factor erythroid 2-related factor 2 (KEAP1:NRF2) protein-protein interaction with high cell potency identified by fragment-based discovery. J Med Chem 59:3991–4006. https://doi.org/10.1021/acs.jmedchem.6b00228

Dowlati Y, Herrmann N, Swardfager W et al (2010) A meta-analysis of cytokines in major depression. Biol Psychiatry 67:446–457. https://doi.org/10.1016/j.biopsych.2009.09.033

Ennaceur A, Meliani K (1988) A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav Brain Res 51:83–92. https://doi.org/10.1016/S0166-4328(05)80315-8

Fajemiroye JO, Polepally PR, Chaurasiai ND et al (2015) Oleandonic acid acrylate elicits antidepressant-like effect mediated by 5-HT 1A receptor. Sci Rep 5. https://doi.org/10.1038/srep11582

Farhan M, Ikram H, Kanwal S, Haleem DJ (2014) Unpredictable chronic mild stress induced behavioral deficits: a comparative study in male and female rats. Pak J Pharm Sci 27:879–884

Farouq RK, Isingrini E, Tanti A, Le Guisquet AM, Arlicot N, Minier F, Leman S, Chalon S, Belzung C, Camus V (2012) Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? Behav Brain Res 231(1):130–137. https://doi.org/10.1016/j.bbr.2012.03.020

Friesner RA, Banks JL, Murphy RB et al (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and Assessment of Docking Accuracy. J Med Chem 47:2739–2741

Fu Y, Yu S, Guo X et al (2012) Fluvoxamine induced glutamate release by activating both 5-HT 3 and sigma-1 receptors in prefrontal cortex of chronic restraint stress C57BL/6 mice. Biochim Biophys Acta, Mol Cell Res 1823:826–837. https://doi.org/10.1016/j.bbamcr.2012.01.008

Fu SP, Liu BR, Wang JF et al (2015) β-hydroxybutyric acid inhibits its growth hormone-releasing hormone synthesis and secretion through the GPR109A/extracellular signal-regulated 1/2 signaling pathway in the hypothalamus. J Neuroendocrinol 27:212–222. https://doi.org/10.1111/jnc.12256

Garza JC, Guo M, Zhang W, Lu X-Y (2012) Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3β/catenin signaling. Mol Psychiatry 17:790–808. https://doi.org/10.1038/mp.2011.161

Gaynes BN, Warden D, Trivedi MH et al (2009) What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. Psychiatr Serv 60:1439–1445. https://doi.org/10.1001/ps.2009.60.11.1439

Gfeller D, Michielin O, Zoete V (2013) Shaping the interaction landscape of bioactive molecules. Bioinformatics 29:3073–3079. https://doi.org/10.1093/bioinformatics/btt540

Haapakoski R, Ebmeier KP, Alenius H, Kivimäki M (2016) Innate and adaptive immunity in the development of depression: an update on current knowledge and technological advances. Prog Neuro-Psychopharmacol Biol Psychiatry 66:63–72. https://doi.org/10.1016/j.pnpbp.2015.11.012

Haghani A, Cacciottolo M, Doty KR et al (2020) Mouse brain transcriptome responses to inhaled nanoparticulate matter differed by sex and APOE in Nfr2-Nlk6b interactions. eLife 9:1–20. https://doi.org/10.7554/eLife.54822

He ZY, Wang WY, Hu WY, Yang L, Li Y, Zhang WY, Yang YS, Liu SC, Zhang FL, Mei R, Xing D, Xiao ZC, Zhang M (2016)
Gamma-H2AX upregulation caused by Wip1 deficiency increases depression-related cellular senescence in hippocampus. Sci Rep 6:34558. https://doi.org/10.1038/srep34558

Heo L, Shin WH, Lee MS, Seok C (2014) GalaxySite: ligand-binding-site prediction by using molecular docking. Nucleic Acids Res 42. https://doi.org/10.1093/nar/gku321

Hopkins A, Keserü G, Leeson P et al (2014) The role of ligand efficiency metrics in drug discovery. Nat Rev Drug Discov 13:105–121. https://doi.org/10.1038/nrd4163

Hu L, Zhang S, Wen H et al (2019) Melatonin decreases M1 polarization via attenuating mitochondrial oxidative damage depending on UCPC2 pathway in preinjured-treated microglia. PLoS One 14:e0212138. https://doi.org/10.1371/journal.pone.0212138

Huerta C, Jiang X, Trevino J et al (2016) Characterization of novel small-molecule NRFR2 activators: structural and biochemical validation of stereospecific KEAP1 binding. Biochim Biophys Acta Gen Subj 1860:2537–2552. https://doi.org/10.1016/j.bbagen.2016.07.026

Hurley RL, Anderson KA, Franzone J et al (2005) The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem 280:29060–29066. https://doi.org/10.1074/jbc.M503824200

Iadecola C, Anrather J (2011) Stroke research at a crossroad: asking the brain for directions. Nat Neurosci 14:1363–1368. https://doi.org/10.1038/nn.2953

Iniaghe LO, Krafft PR, Klebe DW et al (2015) Dimethyl fumarate confers neuroprotection by casein kinase 2 phosphorylation of Nrf2 in murine intracerebral hemorrhage. Neurobiol Dis 82:349–358. https://doi.org/10.1016/j.nbd.2015.07.001

Insel TR, Sahakian BJ (2012) A plan for mental illness. Nature 483:269. https://doi.org/10.1038/483269a

Kapuinnen A, Suuronen T, Ojala J et al (2013) Antagonistic crosstalk between NF-kB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal 25:1939–1948. https://doi.org/10.1016/j.cellsig.2013.06.007

Keiser MJ, Roth BL, Armbruster BN et al (2007) Relating protein pharmacology by ligand chemistry. Nat Biotechnol 25:197–206. https://doi.org/10.1038/nbt1284

Khan A, Faucett J, Lichtenberg P et al (2012) A systematic review of comparative efficacy of treatments and controls for depression. PLoS One 7. https://doi.org/10.1371/journal.pone.0041778

Kim JE, You DJ, Lee C et al (2010) Suppression of NF-κB signaling by KEAP1 regulation of IKKβ activity through autophagic degradation and inhibition of phosphorylation. Cell Signal 22:1645–1654. https://doi.org/10.1016/j.cellsig.2010.06.004

Kortam MA, Ali BM, Fathy N (2021) The deleterious effect of stress on UCP2 pathway in prorenin-treated microglia. PLoS One 7. https://doi.org/10.1371/journal.pone.0212138

Kortam MA, Ali BM, Fathy N (2021) The deleterious effect of stress on UCP2 pathway in prorenin-treated microglia. PLoS One 7. https://doi.org/10.1371/journal.pone.0212138

Offermanns S, Itoh K et al (1998) The antipsoriatic agent dimethylfumarate immunomodulates T-cell cytokine secretion and inhibits cytokines of the psoriatic cytokine network. Br J Dermatol 139:390–395. https://doi.org/10.1046/j.1365-2133.1998.02400.x

Papaleo F, Silverman JL, Aney J et al (2011) Working memory deficits, or PUMA-G) as a new therapeutic target. Trends Pharmacol Sci 32:36–43. https://doi.org/10.1016/j.tips.2010.06.008

Papaleo F, Silverman JL, Aney J et al (2011) Working memory deficits, or PUMA-G) as a new therapeutic target. Trends Pharmacol Sci 32:36–43. https://doi.org/10.1016/j.tips.2010.06.008

Permpoopuntana K, Tangweerasing P, Mukda S et al (2018) Long-term administration of melatonin attenuates neuroinflammation in the aged mouse brain. EXCLI J 17:634–646. https://doi.org/10.17179/excli2017-654

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Archives Internationales de Pharmacodynamie et de Therapie 229:327–336

Naunyn-Schmiedeberg’s Archives of Pharmacology (2022) 395:1029–1045

Springer
Quan W, Liu F, Zhang Y et al (2015) Antidepressant-like effects of magnesium lithospermate B in a rat model of chronic unpredictable stress. Pharm Biol 53:1168–1175. https://doi.org/10.3109/13880209.2014.967783

Rajkowska G, Miguel-Hidalgo JJ, Dubey P et al (2005) Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. Biol Psychiatry 58:297–306. https://doi.org/10.1016/j.biopsych.2005.04.013

Rezq S, Abdel-Rahman AA (2016) Central GPR109A activation mediates glutamate-dependent presessor response in conscious rats. J Pharmacol Exp Ther 356:456–465. https://doi.org/10.1124/jpet.115.229146

Rush AJ, Trivedi MH, Wisniewski SR et al (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. Am J Psychiatry 163:1905–1917. https://doi.org/10.1176/appi.ajp.2006.0511.229

Salentin S, Schreiber S, Haupt VJ et al (2015) PLIP: Fully automated protein-ligand interaction profiler. Nucleic Acids Res 43:W443–W447. https://doi.org/10.1093/nar/gkv315

Schulze-Topp Hoff U, Varrin-Doyer M, Pekarek K et al (2016) Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2. Proc Natl Acad Sci U S A 113:4777–4782. https://doi.org/10.1073/pnas.1603907113

Shah SA, Khan M, Jo MH et al (2017) Melatonin stimulates the SIRT1/Nrf2 signaling pathway counteracting lipopolysaccharide (LPS)-induced oxidative stress to rescue postnatal rat brain. CNS Neurosci Ther 23:33–44. https://doi.org/10.1111/cns.12588

Shehu A, Magaji MG, Yau J, Ahmed A (2019) Methanol stem bark extract of Adansonia digitata digerulates chronic unpredictable mild stress-induced depression-like behavior: involvement of the HPA axis, BDNF, and stress biomarkers pathways. J Basic Clin Physiol Pharmacol 31. https://doi.org/10.1515/jbcpp-2018-0153

Shmelkov SV, Hormigo A, Jing D et al (2010) Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. Nat Med 16:598–602. https://doi.org/10.1038/nm.2125

Singh CK, Chhabra G, Ndiaye MA et al (2018) The role of sirtuins in antidepressant and redox signaling. Antioxid Redox Signal 28:643–661. https://doi.org/10.1089/ars.2017.7290

Singhalumar R, Boontem P, Ekhawupranee K et al (2015) Melatonin attenuates methamphetamine-induced inhibition of neurogenesis in the adult mouse hippocampus: an in vivo study. Neurosci Lett 606:209–214. https://doi.org/10.1016/j.neulet.2015.09.011

Singhal G, Baune BT (2017) Microglia: An interface between the loss of neuroplasticity and depression. Front Cell Neurosci 11. https://doi.org/10.3389/fncel.2017.00270

Stefansson AL, Bakovic M (2014) Dietary regulation of Keap1/Nrf2/ARE pathway: focus on plant-derived compounds and trace minerals. Nutrients 6:3777–3801. https://doi.org/10.3390/nu6093777

Talapatra SK, Kozielski F, Wells G, Georgakopoulou ND (2019) Keap1 - inhibitor complex. Protein Data Bank. https://doi.org/10.2210/pdb6hws/pdb

Tang H, Lu JYL, Zheng X et al (2008) The psoriasis drug monomethyl-fumarate is a potent nicotinic acid receptor agonist. Biochem Biophys Res Commun 375:562–565. https://doi.org/10.1016/j.bbrc.2008.08.041

Trolotel C, Largue B, Ribeiro MJS et al (2017) Molecular targets for PET imaging of activated microglia: the current situation and future expectations. Int J Mol Sci 18. https://doi.org/10.3390/ijms18040802

Unni S, Deshmukh P, Krishnappa G, Kommu P, Padmanabhan B (2021) Structural insights into the multiple binding modes of dimethyl fumarate (DMF) and its analogs to the Kelch domain of Keap1. FEBS J 288(5):1599–1613. https://doi.org/10.1111/febs.15485

Venkatesh GM, Sankar V, Ramathan (2019) Molecular mechanism of tuberininfulidubular peptide of 39 on glucocorticoid receptor mediated glutamate/GABA imbalance and cerebral abnormalities against cognitive deficit model. J Pharm Pharmacol 71:996–1006. https://doi.org/10.1111/jphp.13085

Walker F, Nilsson M, Jones K (2013) Acute and chronic stress-induced disturbances of microglial plasticity, phenotype and function. Curr Drug Targets 14:1262–1276. https://doi.org/10.2174/138950111399902008

Williams CJ, Headl JJ, Moriarty NW et al (2018) MolProbity: more and better reference data for improved all-atom structure validation. Protein Sci 27:293–315. https://doi.org/10.1002/pro.3330

Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134:319–329. https://doi.org/10.1007/s002100050456

Willner P, Towell A, Sampson D et al (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93:358–364

World Health Organization (2020) Depression. https://www.who.int/news-room/fact-sheets/detail/depression

Wu G, Zhu L, Yuan X et al (2017) Britain ameliorates cerebral ischemia-reperfusion injury by inducing the Nrf2 protective pathway. Antioxid Redox Signal 27:754–768. https://doi.org/10.1089/ars.2016.6885

Xu Z, Zhang F, Sun FL et al (2015) Dimethyl fumarate for multiple sclerosis. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD011076

Yang J, Zhang Y (2015) I-TASSER server: new development for protein structure and function predictions. Nucleic Acids Res 43:W174–W181. https://doi.org/10.1093/nar/gkv342

Yeung F, Hoberg JE, Ramsey CS et al (2004) Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23:2369–2380. https://doi.org/10.1038/sj.emboj.7600244

Young JJ, Bruno D, Pomara N (2014) A review of the relationship between proinflammatory cytokines and major depressive disorder. J Affect Disord 169:15–20. https://doi.org/10.1016/j.jad.2014.07.032

Yi WC, Huang RY, Chou TC (2020) Oligo-fucoidan improves diabetes-induced renal fibrosis via activation of sirt-1, glp-1r, and nrf2/ho-1: an in vitro and in vivo study. Nutrients 12:1–15. https://doi.org/10.3390/nu12103065

Zhang C, Zhang YP, Li YY et al (2019) Minocycline ameliorates ischemia-reperfusion injury by inducing the Nrf2 protective pathway. Antioxid Redox Signal 29:1093–1107. https://doi.org/10.1089/ars.2019.858

Zhang M, An C, Gao Y et al (2013) Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. Prog Neurobiol 100:30–47. https://doi.org/10.1016/j.pneurobio.2012.09.003

Zhu K, Borrelli KW, Greenwood JR et al (2014) Docking covalent inhibitors: a parameter free approach to pose prediction and scoring. J Chem Inf Model 54:1932–1940. https://doi.org/10.1021/icc400118s

Zou T, Sugimoto K, Zhang J et al (2020) Geniposide alleviates oxidative stress of mice with depression-like behaviors by upregulating Six3os1. Front Cell Dev Biol 8. https://doi.org/10.3389/fcell.2020.553728

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.