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Early preclinical evaluation of dihydropyrimidin(thi)ones as potential anticonvulsant drug candidates

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ABSTRACT

Although significant advances are occurring in epilepsy research, about 30% of epileptic patients are still inadequately controlled by standard drug therapy. For this reason, it continues to be important to develop new chemical entities through which epilepsy could be effectively controlled. In this study, the anticonvulsant activity of forty-two dihydropyrimidin(thi)ones was explored and their efficacy was evaluated in rodents against the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole tests. The results of preliminary pharmacological screening after intraperitoneal injection in mice revealed that twenty-four compounds showed protection in half or more of the animals tested in the MES seizure model, being nine of them active at the lowest dose tested (30 mg/kg). Structurally, the most promising compounds (both urea and thiourea derivatives) presented smaller lateral chains and unsubstituted or *para*-substituted phenyl ring with a methyl group. Compounds **4**, **5** and **11** also protected against MES-induced seizures in 50–75% of rats after oral administration at 30 mg/kg. Moreover, the minimal motor and/or neurological impairment evaluated through the rotarod assay showed that around 52% of the compounds presented lower toxicity than the antiepileptic drugs lamotrigine, carbamazepine and phenytoin. In addition, the most active compounds did not show notable cytotoxicity in *in vitro* experiments conducted in several cell lines (relative cell proliferation higher than 50% at 30 μM), which can be relevant due to the fact that the toxicity is a common problem of the available antiepileptic drugs. Furthermore, additional computational studies indicated that all compounds respected the Lipinski's rule of five, which, together with the data of efficacy and toxicity, make them attractive compounds to be developed in the future as potential anticonvulsant agents.

1. Introduction

Epilepsy is a complex disorder of the brain function that affects around 60 million people worldwide (Shetty and Upadhya, 2016) and has a considerable impact in the patients' quality of life (Hosseini et al., 2016). A range of structurally diverse drugs is currently used for controlling both convulsive and non-convulsive epileptic seizures, which act through several molecular mechanisms and have different efficacy, pharmacokinetic and safety profiles (Santulli et al., 2016). Nevertheless, despite the availability of many AEDs already in clinical use, just 60–70% of the patients with epilepsy remains seizure-free when properly treated with current drugs (Bidwell et al., 2015). Therefore, the development of safer and more effective AEDs is critical and remains a challenge for medicinal chemists (Dalkara and Karakurt, 2012; Kowski et al., 2016).

In the process of discovery and development of new drug candidates, heterocyclic nucleus has received considerable attention in almost all drug classes (Taylor et al., 2016). In fact, there are several reports associating diverse heterocyclic systems, including pyrimidine ring systems, with anticonvulsant effects (Asif, 2015; Nusrat et al., 2014). In this context, the Biginelli reaction is a prominent multicomponent reaction that offers a straightforward approach to produce multifunctionalized dihydropyrimidines and related heterocyclic compounds (Kappe, 2003). Although this interesting chemical reaction has remained underexploited for decades, it gained prominence in the early 1990s with the advent of combinatorial chemistry, since this type of reaction was considered ideal to prepare large compound libraries in medicinal chemistry endeavours. Currently, the Biginelli scaffold has shown great pharmaceutical value and, for this reason, the search for novel dihydropyrimidines with important biological properties has been under intensive exploitation. Indeed, several members of this

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class of heterocycles have shown anticancer (Sośnicki et al., 2014), antimalarial (Chiang et al., 2009), antifungal and antibacterial (Ghodasara et al., 2013; Godhani et al., 2014), and antithyroid (Lacotte et al., 2013) properties, among many others. Regarding central nervous system (CNS) disorders, several Biginelli derivatives have been developed as inhibitors of the acetylcholinesterase enzyme (Arunkhamkaew et al., 2013). Moreover, there are some reports describing the antioxidant activity of these kinds of molecules (Da Silva et al., 2012; Gangwar and Kasana, 2012) and also a potential role in the treatment of Parkinson's disease (Kang et al., 2013). Concerning their potential anticonvulsant activity, up to date, to the best of our knowledge, only one *in vitro* study showed the ability of these compounds to modulate the GABAergic system (Lewis et al., 2010).

As Biginelli compounds are multifunctionalized molecules and they are able to include several pharmacophores of the available AEDs (as illustrated in Fig. 1), in this work the anticonvulsant potential of this interesting class of molecules was investigated. Thus, a large set of Biginelli adducts was prepared, some of them for the first time, and their anticonvulsant properties were screened against rodent models of electrically and chemically-induced seizures, the maximal electroshock seizure (MES) test and the subcutaneous pentylenetetrazole (scPTZ) seizure test, respectively. Moreover, the neurotoxicity of these compounds was also explored *in vivo* on the rotarod performance test, and their cytotoxicity was also evaluated in *in vitro* conditions on neuronal, dermal, hepatic and intestinal cell lines. Finally, a computational study was also carried out to estimate several molecular and physicochemical properties of the target compounds in order to predict their druglikeness.

2. Material and methods

2.1. Chemistry

2.1.1. General remarks

The reagents urea, 5-methylfuraldehyde, 3-pyridinaldehyde, 5-chloro-2-furaldehyde, 2-thiophenaldehyde and anhydrous sodium sulphate were purchased from Acros Organics (New Jersey, USA); ethyl acetoacetate was acquired from Merck (Hohenbrunn, Germany) and bismuth (III) nitrate pentahydrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol 99.9% was purchased from Manuel Vieira & Cª (Torres Novas, Portugal); ethyl acetate and hydrochloric acid were acquired from Fisher Scientific (New Hampshire, USA); sodium hydroxide was purchased from Vencilab (Vila Nova de Gaia, Portugal); 5-chloro-2-thiophenaldehyde was purchased from Maybridge (Altrincham, United Kingdom) and deuterated DMSO (DMSO-d_6) was acquired from Armar Chemicals (Leipzig, Germany). The evaporation of solvents was carried out in a rotavapor (Büchi, Switzerland). Infrared (IR) spectra were collected on a Thermoscientific Nicolet iS10: smart iTR, equipped with a diamond ATR crystal. For ATR data acquisition, a sample of the solid compound was placed onto the crystal and the spectrum was recorded. An air spectrum was used as a reference in absorbance calculations. The sample spectra were collected at room temperature in the 4000–400 cm^{-1} range by averaging 16 scans at a spectral resolution of 2 cm^{-1}. Nuclear magnetic resonance (NMR) spectra (^1H NMR and ^{13}C NMR) were acquired on a Bruker Avance 400 MHz spectrometer and were processed with the software TOPSPIN 3.1 (Bruker, Fitchburg, WI, USA). DMSO-d_6 was used as solvent. Chemical shifts are reported in parts per million (ppm) relative to deuterated solvent as an internal standard. Coupling constants (J values) are reported in hertz (Hz) and splitting multiplicities are described as s = singlet; brs = broad singlet; d = doublet; dd = double doublet; t = triplet; q = quartet; dq = double quartet and m = multiplet. Electrospray ionization high resolution mass spectrometry (ESI-HRMS) spectra were performed by the microanalysis service on a QSTAR XL instrument (Salamanca, Spain).

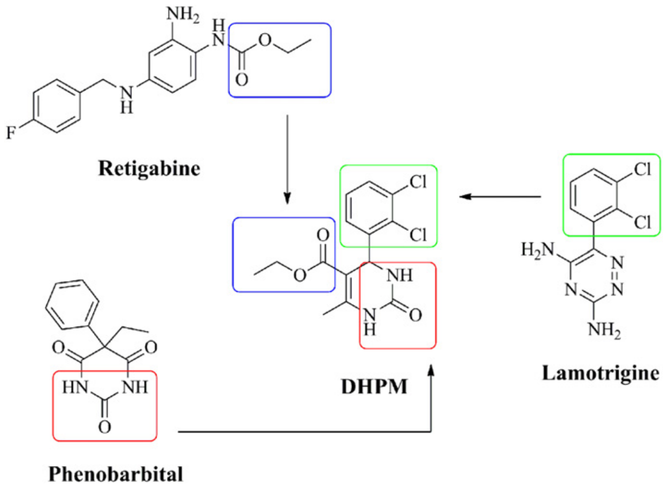

Fig. 1. Representative chemical structure of a 3,4-dihydropyrimidin-2(1*H*)-one (DHPM) and its structural similarities with the clinically available AEDs lamotrigine, phenobarbital and retigabine.

2.1.2. Synthesis and structural characterization

*General procedure for compounds **1**–**37***: The compounds were synthesized, accordingly as previously described for thiourea (Matias et al., 2017) and urea (Matias et al., 2016) derivatives.

*General procedure for compounds **38**–**41***: To a mixture of an aldehyde (1 mmol), ethyl acetoacetate (1 mmol) and urea (1.3 mmol) was added bismuth (III) nitrate pentahydrate [$Bi(NO_3)_3 \cdot 5H_2O$, 10 mol%] and the reaction was heated with stirring at 70 °C in a preheated oil bath for 8–15 min. The reaction was considered completed when the mixture solidified and, after being cooled to room temperature, was poured onto cold water and stirred during 20–30 min. The solid separated was filtered under suction, washed with ice-cold water, dried and then recrystallized from ethanol 99.9% to afford the pure product. All the products were characterized by IR, ^1H and ^{13}C NMR. HRMS were also obtained for the new compounds.

Ethyl 6-methyl-4-(5-methylfuran-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (compound **38**). Yellow solid, yield: 69%, IR (υ_{max}/cm^{-1}): 3225, 3100, 2982, 2923, 1703, 1652, 1218, 1090; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.14 (t, 3H, J = 7.06 Hz, OCH_2CH_3), 2.21 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.98–4.07 (m, OCH_2), 5.13 (d, 1H, J = 3.24 Hz, CH), 5.92–5.96 (m, 2H, ArH), 7.71 (brs, 1H, NH), 9.18 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.39, 14.16, 17.74, 47.71, 59.18, 96.81, 106.00, 106.33, 149.21, 150.70, 152.38, 154.21, 165.06.

Ethyl 4-(5-chlorofuran-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (compound **39**). Brown solid, yield: 67%, IR (υ_{max}/cm^{-1}): 3351, 3233, 3115, 2976, 1694, 1644, 1230, 1098; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.14 (t, 3H, J = 7.08 Hz, OCH_2CH_3), 2.24 (s, 3H, CH_3), 3.97–4.10 (m, 2H, OCH_2), 5.16 (d, 1H, J = 3.33 Hz, CH), 6.19 (d, 1H, J = 3.30 Hz, ArH), 6.36 (d, 1H, J = 3.33 Hz, ArH), 7.82 (brs, 1H, NH), 9.30 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.13, 17.76, 47.78, 59.31, 95.93, 107.51, 107.98, 133.54, 149.88, 152.13, 155.98, 164.85. HRMS (ESI-TOF): m/z [M^+ + H] calcd for $C_{12}H_{14}N_2O_4Cl$: 284.6956; found 285.0637.

Ethyl 6-methyl-2-oxo-4-(thiophen-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (compound **40**). White solid, yield: 83%, IR (υ_{max}/cm^{-1}): 3235, 3104, 2981, 1711, 1650, 1208, 1088; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.14 (t, 3H, J = 7.09 Hz, OCH_2CH_3), 2.22 (s, 3H, CH_3), 4.06 (q, 2H, J = 7.10 Hz, OCH_2), 5.41 (d, 1H, J = 3.46 Hz, CH), 6.89 (d, 1H, J = 3.27 Hz, ArH), 6.92–6.95 (m, 1H, ArH), 7.35 (dd, 1H, J_1 = 5.03 Hz, J_2 = 1.17 Hz, ArH), 7.90 (brs, 1H, NH), 9.31 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ:14.16, 17.68, 49.35, 59.36, 99.77, 123.50, 124.63, 126.66, 148.66, 148.79, 152.24, 165.02.

Ethyl 4-(5-chlorothiophen-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (compound **41**). White solid, yield: