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Short note

Ethyl 4-(2-fluorophenyl)-6-methyl-2-thioxo-1-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate

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Abstract: The Biginelli reaction is a highly versatile reaction, which leads to dihydropyrimidinones/thiones. This scaffold is reported as being a privileged structure due to its ability to interact with biological targets. Synthesis of ethyl 4-(2-fluorophenyl)-6-methyl-2-thioxo-1-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate was achieved through the Biginelli reaction using a functionalized thiourea. *In silico* studies demonstrated that the compound title showed good potential for interacting with ecto-5'-nucleotidase, which has been considered as a target in designs for anti-cancer drugs.

Keywords: Biginelli reaction; dihydropyrimidin-2-thiones; synthesis, virtual screening, drug design; LaSOM 282

1. Introduction

The Biginelli reaction involves the acid-catalyzed multicomponent synthesis of 3,4-dihydropyrimidin-2(1H)-ones(thiones) (DHPMs) [1]. Thus, this reaction is a powerful tool for the fast and easy generation of libraries with great structural diversity [2]. DHPMs are an example of privileged scaffolds, due to their interaction with numerous biological targets [3].

It was recently identified that N1 substituted Biginelli compounds were able to interact with the Eg5, a protein involved in chromosome separation during the cell cycle [4]. Another target for this heterocycle class is the enzyme ecto-5'-nucleotidase, a protein involved in hydrolysis of AMP to adenosine, a molecule which increases the cancer growth through immune system suppression [5]. The DHPM LaSOM 63, was identified as a prototype for ecto-5'-nucleotidase inhibitor development. LaSOM 63 was active against glioma cells and inhibited the phosphate liberation from cells treated with AMP [6]. In this context, in continuation with our studies focused on exploring the possibility of finding a DHPM ecto-5'-nucleotidase inhibitor, a new N1-aryl substituted DHPM has been designed and synthesized.

2. Results and discussion

A virtual library containing 528 compounds was constructed from dihydroprimidinone scaffolds. For this purpose, 22 thioureas and 24 aldehydes were included in the SMILIB program [7]. SMILIB is able to construct very large combinatorial compound libraries in SMILES format.

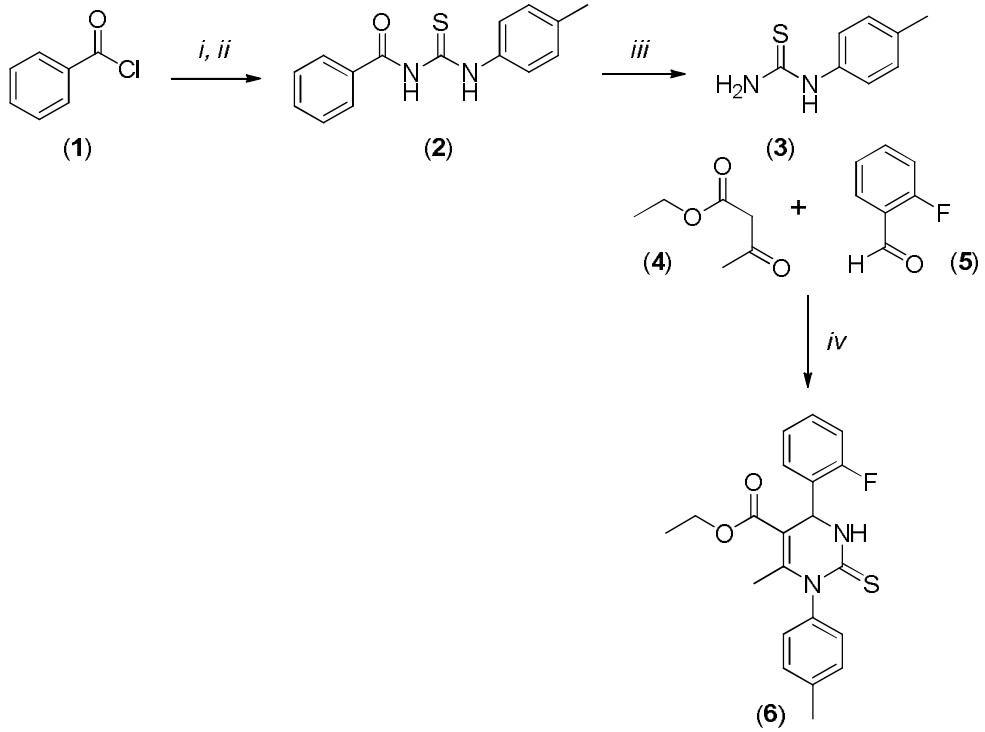
Following this, 87 compounds were selected for their physicochemical and pharmacokinetic properties. The filter was based on data obtained by the SwissADME server [8], using the Lipinski rule [9], and the ability to permeate the brain blood-brain barrier. These 35 selected compounds were submitted into two virtual screening routines: ligand-based, using the SHAFTS program [10]

(ShapeSim = 0.625) and structure-based, using the Autodock Vina program [11] (score = -6,41 kJ/mol). Ethyl-4-(2-fluorophenyl)-6-methyl-2-thioxo-1-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**6**) LaSOM 282 was selected by consensus rank-by-rank between compounds more similar to adenosine and the compound with greater affinity to the ecto-5'-nucleotidase receptor.

The title compound LaSOM 282 (**6**) was synthesized by the multicomponent Biginelli reaction promoted by trimethylsilane chloride (TMSCl), from condensation of ethyl acetoacetate (**4**), 2-fluorobenzaldehyde (**5**) and 1-(4-methylphenyl)thiourea (**3**), as shown in Scheme 1. The compound (**6**) was obtained with a high degree of purity and yielding (66%). The purification processes involved only recrystallization in ethanol. The compound (**3**) 1-(4-methylphenyl)thiourea was obtained in accordance with protocols previously published [4].

The structural elucidation of the compound (**6**) was made based on spectroscopic data, and the results are displayed in the experimental section and in the electronic supporting information. From the ^{1}H-NMR spectra (Figure S1), one broad singlet of the NH-3 proton of the DHPM core appeared at 7.3 ppm. Aromatic hydrogens of the two rings produced the next group of signals at 6.9 – 7.4 ppm. The benzylic hydrogen produced a signal at 5.7 ppm, while the singlet of allylic CH_3 resonated at 2.20 ppm. The signals that appeared as a triplet and quartet, respectively at 1.1 and 4.0 ppm, correspond to the H_3CCH_2 system. The methyl group linked to the aromatic ring at N1 produced a signal at 2.4 ppm.

In the APT ^{13}C-NMR spectrum (Figure S2), the most representative signals were the methyl carbons at 14 and 18 ppm, the methyne carbon at 49 ppm and the methylene carbon at 60 ppm. The ester carbonylic carbon produced a signal at 165 ppm, while the most downfield signals, which appeared at 178 ppm, correspond to the quaternary carbon of C=S bond carbonyl, which are situated between the two nitrogens. In addition, the *ipso*, *orto*, *meta* and *para* couplings between ^{19}F - ^{13}C were identified. The expansion of the aromatic region allowed for the calculation of the coupling ^{19}F-^{13}C constants and the assignment of the aromatic carbons of the ring linked to position 4 of the DHPM core.



Scheme 1 – Reactions and conditions: *i* NH_4SCN $(Me)_2CO$ 60ºC, 15 min; *ii* 4-$MePhNH_2$, $(Me)_2CO$, 60ºC 30 min; *iii* NaOH $_{aq}$ 2.5 M 90 ºC, 20 min; *iv* TMSCl, DMF, r.t, 72 h.

3. Materials and Methods

3.1. Chemical analysis

All the chemicals were purchased as reagent grade and used without further purification. Melting points were determined on a Fisatom 431 apparatus, and were uncorrected. Nuclear magnetic resonance spectra of carbon and hydrogen were recorded in a Bruker Ascend NMR with standard pulse sequences operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, using $CDCl_3$ as a solvent. FT-IR spectra was obtained in a Perkin Elmer spectrometer, opening in ATR mode.

3.2. Synthesis of 1-(4-methylphenyl)thiourea

For a solution of ammonium thiocyanate (1.1. equiv) in dry acetone at room temperature, benzoyl chloride (1 equiv) was added and the solution was heated at 70ºC for 15 minutes; Following this, *p*-toluidine (1 equiv) in acetone were dropsied and heating of the solution was maintained for 30 minutes. The reactional medium was poured into water at room temperature and the precipitate was filtered and submitted to alkaline hydrolysis in NaOH 2.50 M, 90 ºC for 20 minutes. Following this, the pH of the system was adjusted to 2 with HCl, and 8 with NH_4OH. The product was filtered and presented a high level of purity

3.3. Synthesis of ethyl 4-(2-fluorophenyl)-6-methyl-2-thioxo-1-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate – LaSOM 282

A mixture of ethyl acetoacetate (1equiv), 1-4(-methylphenyl)thiourea (1equiv) and a substituted benzaldehyde (1 equiv) were solubilized in DMF under ultrasound for 1 hour. TMSCl (6 equiv) was then added dropwise and the mixture was stirred at room temperature for 72 hours. After the mixture was poured over three volumes of water, it was submitted to ultrasound for one hour and the precipitate obtained was filtered and washed with water. The crude isolate was recrystallized from ethanol.

Etil 4-(2-fluorofenil)-6-metil-2-tioxo-1-(p-toluil)-1,2,3,4-tetrahidropirimidina-5-carboxilato (***6***)*:* melting point: 226-230 ºC; yield: 66%, recrystallized from ethanol. RMN ^{1}H ($CDCl_3$, 400 MHz, δ ppm): 1.14 (t, ^{3}J = 7.1 Hz, 3H); 2.18 (s, 3H); 2.40 (s, 3H); 4.08 (q, ^{3}J = 7.1 Hz, 2H); 5.72 (d, ^{2}J = 3.1 Hz, 1H); 7.00 (s, 1H); 7.06-7.20 (m, 3H); 7.22-7.34 (m, 4H); 7.53 (d, ^{2}J = 2,8 Hz, 1H). RMN ^{13}C ($CDCl_3$, 100 MHz, δ ppm): 13.97; 18.64; 21.32; 49.58; 60.61; 103.96; 116.16 (d, ^{2}J = 21.70 Hz); 124.46 (d, ^{4}J = 3.30 Hz); 128.40; 128.44; 128.57 (d, ^{2}J = 13.7 Hz); 130.17; 130.25; 138.08; 138.80; 147.65; 160.96 (d, ^{1}J = 248 Hz), 165.30; 178.45. FT-IR (cm^{-1}): 3192 (NH); 1704 (C=O); 1629 (C=C); 1164 (C-O).

Supplementary Materials: The following are available online: FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS spectra for product (**6**).

Author Contributions: synthesis NMR spectra obtaining, I.L.G, L.D., and A.F.S; design of compound, L.P.K, G.M.N and V.L.E.; writing—original draft preparation, I.LG, L.P.G, G.M.N., S.C.G., and V.L.E.; writing—review, S.C.G., and V.L.E.; supervision and project administration, V.L.E.

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Conflicts of Interest: The authors declare no conflict of interest.

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