Synthesis and Molecular Docking Studies of some Pyrano[2,3-c] Pyrazole as an Inhibitor of SARS-Coronavirus 3CL Protease

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Abstract: The widespread global COVID-19 pandemic due to the lack of specific treatment and the urgent situation requires the use of all resources to remedy this scourge. The current study aimed to use molecular docking tools to find potential drug candidates for treatment. The pyrano[2,3-c] pyrazole 5(a-e) was targeted against the Main protease (M\textsuperscript{pro}), which plays a vital role in the replication and transcription of the Corona viral genome. The 3CL Protease (PDB ID 6LU7) was modeled, and the compounds were docked using Autodock Vina software, and ADMET data have been studied. All synthesized compounds were well engaged into the active site of the main protease with strong hydrogen bond interaction and a good score of energy. The 5b have been classed as the best inhibitor with an energy score of -6.2 kcal/mol, similar to the one given by chloroquine (-6.2Kcal/mol). Moreover, the molecular interaction studies showed that protease structure had multiple active site residues for all studied compounds. Our finding confirms the potential of these derivatives as lead compounds against the selected target protein of coronavirus, which needs further analysis and dynamic simulation studies to propose then develop a new antiviral treatment.

Keywords: COVID-19; docking; protease.

1. Introduction

The coronavirus (Sars-Cov-2) causing COVID 19 diseases was detected for the first time in the Hubei province of china then spreading to several countries [1], which have created a real threat to the human population worldwide. In the past time, this new virus has been considered the main causal agent of respiratory disease in birds and mammals [2]. In humans, coronaviruses infect the lower respiratory tract and induce the common cold [1]. Besides that, the SARS-CoV is classified as the most virulent strain with 10-30% lethality, observed in immuno-compromised individuals and the healthy population [2–4]. This alarming situation worldwide needed the researchers' intervention to discover an effective drug therapy able to block the viral replication of this virus [1, 5–7].

The coronavirus can be defined as an enveloped and positive-stranded RNA. Its genome size is about 30 kb, larger than any other known RNA virus [8]. This novel coronavirus (SARS-
COV-2) encodes many important proteins such as 3CL Protease, which has been considered as the most attractive strategy in the treatment of many viruses such as hepatitis C [9] and the human immunodeficiency virus (HIV) [10]. With this in mind, we selected the main Mpro of coronavirus as a suitable target for the current investigation. This chymotrypsin-like cysteine protease has been recognized for its important role in mediating the replication of this virus, cleaving polyproteins into replication-related proteins, and to process the polypeptide into functional proteins. However, when an inhibitor interacts with the amino acids of the active site, it prevents the binding of substrates and affects the biological activity of the protease [6].

Until now, chloroquine has been recognized as a potent inhibitor of sars-cov2 by raising the pH of endosomal vesicles [11] inside the cells to enter this virus, thereby preventing fusion and circulation of the virus [11]. It also blocks the cytokine storm in the late phase of critically ill covid-19 patients. On the other side, the heterocyclic compounds, namely Pyrano[2,3-c]pyrazole have been used in the current study due to their wide biological deeds, including antiviral proprieties. The chemistry of these derivatives has received great interest since this pyrazole nucleus is the parent skeleton of several medicinal drugs. Pyrano[2,3-c]pyrazole derivatives showed their virucide activities against a wide spectrum of microorganisms. These compounds are used in the treatment of malaria. Furthermore, It has been proved in vitro to control the multiplication of herpes virus HSV1 [12].

Therefore, finding an inhibitor for COVID-19’s protease may be the first step to beating this contagious respiratory illness. In this regard, we describe herein an attempt to use some heterocycles as new inhibitors able to limit the propagation of this viral infection. Our efforts can be added to the literature surveys on the existing drugs that showed their potential to inhibit the replication of this virus, such as Favipiravir, Ribavirin, Remdesivir, Oseltamivir, hydroxychloroquine [13], and chloroquine [13].

![Figure 1. The hierarchical pyranopyrazoles docking protocol.](image-url)
behavior anti-COVID19. Hence, further investigation and validation of these inhibitors against coronavirus would be very helpful to bring these molecules to clinical settings.

2. Materials and Methods

2.1. Chemistry.

2.1.2. Catalyzed synthetic route to prepare the pyrano[2,3-c] pyrazoles.

The studied pyranopyrazoles derivatives 5 was synthesized by a four-component reaction between aromatic aldehyde 1, ethyl acetoacetate 2, hydrazine hydrate 3, and malononitrile 4 in the presence of the pyrophosphate Na$_2$CaP$_2$O$_7$ as a catalyst according to a previously described experimental procedure as shown in figure 2 (see also figure S1). The Reaction of pyranopyrazoles 5 was monitored by thin-layer chromatography (TLC), and all products were purified by recrystallization from ethanol. After that, the synthesized compounds were characterized by melting point and spectroscopic analysis (IR, $^1$H NMR, $^{13}$C NMR) (see Figure S2-S17 in supplementary material) [17], and we checked their purity by using the HPLC technique, and the analyses were carried out on a Shimadzu LC-20AT equipped with a C18, 5μm x 250 mm column, detection at λ = 254 nm.

![Figure 2. Synthetic route and structure of synthesized Pyrano[2,3-c] pyrazole 5(a-e).](https://nanobioletters.com/)

2.1.2. Preparation of the catalyst.

Na$_2$CaP$_2$O$_7$ was prepared as described in figure S18. The obtained catalyst was characterized using X-ray Diffraction (XRD) (Figure S18 and Table S1), Fourier Transform Infrared (FT-IR) spectroscopy (see Figure S19 and Table S2), Transmission Electron Microscopy(see Figure S21 and S22).

2.1.3. Spectroscopic characterization.

**6-Amino-3-methyl-4-phenyl-1,4-dihydropyran[2,3-c]pyrazole-5-carbonitrile (5a):**

White solid; m.p. 245-246 °C (lit. 244-245 °C); R$_f$ (20% AcOEt/hexane) 0.61; (HPLC): (t$_R$ = 3.63 min); $^1$H NMR (300 MHz, DMSO-d$_6$, ppm): δ 1.73 (s, 3H), 4.54 (s, 1H), 6.83 (s, 2H), 7.11-7.45 (m, 5H), 12.11 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-d$_6$, ppm): δ 9.7 (CH$_3$), 36.2 (pyran C$_4$), 57.2 (C$_5$-CN), 97.6 (C$_6$), 120.7 (CN), 126.7, 127.4, 127.5, 128.4, 128.5, 135.5 (aromatic carbons), 144.4 (C$_3$), 154.7 (C$_7$), 160.8 (C$_6$-NH$_2$); IR (KBr, cm$^{-1}$): 3473 (NH$_2$), 3170 (NH), 2191 (CN), 1649 (C=N), 1604 (Ar).

![https://nanobioletters.com/](https://nanobioletters.com/)
6-Amino-3-methyl-4-(4-methylphenyl)-1,4-dihydropyran-2,3-dipyrazole-5-carbonitrile 5b White solid; m.p. 205-207 °C (lit. 206-207 °C); Rf (20% AcOEt/hexane) 0.7; (HPLC): (tr = 3.53 min); 1H NMR (300 MHz, DMSO-d6, ppm): δ 1.75 (s, 3H, CH3), 2.22 (s, 3H, CH3), 4.51 (s, 1H, C4-H), 6.81 (s, 2H, NH2), 7.03 (m, 4H, HAr), 12.06 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6, ppm): δ 9.7 (CH3), 20.5 (CH3), 35.8 (pyran C4), 57.4 (C5-CN), 97.7 (C8), 120.7 (CN), 127.3-129.1, 136.7 (6 aromatic carbons), 141.4 (C3), 154.7 (C5), 160.7 (C6-NH2); IR (KBr, cm⁻¹): 3483 (NH2), 3113 (NH), 2193 (CN), 1641 (C≡N), 1602 (Ar).

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyran-2,3-dipyrazole-5-carbonitrile 5c White solid; m.p. 215-218 °C; 1H NMR (300 MHz, DMSO-d6, ppm): δ 1.9 (s, 3H), 4.56 (s, 1H), 6.70 (2H), 6.94-7.18 (m, 5H), 11.00 (s, 1H); 13C NMR (75 MHz, DMSO-d6, ppm): δ 9.85, 28.64, 55.06, 104.95, 115.1, 120.79, 123.53, 124.25, 127.55, 128.93, 136.52, 148.39, 159.09, 160.08; IR (KBr, cm⁻¹): 3448, 3419, 3352, 2189, 1660, 1610.

6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyran-2,3-dipyrazole-5-carbonitrile 5d White solid; m.p. 210-212 °C; 1H NMR (300 MHz, DMSO-d6, ppm): δ 1.78 (s, 3H), 3.72 (s, 3H), 4.54 (s, 1H), 6.83 (2H), 6.86-7.08 (m, 4H), 12.09 (s, 1H); 13C NMR (75 MHz, DMSO-d6, ppm): δ 9.66, 35.51, 55.00, 57.9, 97.7 (CH3), 104.0, 115.1, 120.79, 123.52, 124.25, 127.55, 128.93, 136.52, 148.39, 159.09, 160.08; IR (KBr, cm⁻¹): 3483, 3255, 3118, 2193, 1641 (C≡N), 1602 (Ar).

6-Amino-3-methyl-4-(4-nitrophenyl)-1,4-dihydropyran-2,3-dipyrazole-5-carbonitrile 5e Wellow solid; m.p. 248-250 °C (lit. 248-249 °C); Rf (20% AcOEt/hexane) 0.64; (HPLC): (tr = 3.56 min); 1H NMR (300 MHz, DMSO-d6, ppm): δ 1.8 (s, 3H, CH3), 4.51 (s, 1H, C4-H), 7.05 (s, 2H, NH2), 7.46 (d, 2H, HAr), 8.21 (d, 2H, HAr), 12.20 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6, ppm): δ 9.6 (CH3), 35.8 (pyran C4), 55.9 (C5-CN), 96.5 (C8), 120.4 (CN), 123.8, 124.1, 128.8, 135.8 (5 aromatic carbons), 146.3 (C3), 152.0 (C7), 154.6 (C6-NO2), 161.1 (C6-NH2); IR (KBr, cm⁻¹): 3477, 3228 (NH2), 3118 (NH), 2196 (CN), 1651 (C≡N), 1595 (Ar).

2.2. Computational studies.

2.2.1. Determination of ADMET parameters.

The ADMET parameters of the synthesized compounds 5(a-e) were calculated using the freely accessible web server Swiss ADMET (http://swissadme.ch/index.php#undefined) [18]. These parameters provide drug likeness and pharmacokinetic data of the selected compounds. The values of the observed properties are presented in Tables 1 S3 and S4 (see ESI part).

<table>
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<th>Compounds</th>
<th>Log P</th>
<th>MM</th>
<th>Hydrogen bond donors</th>
<th>Hydrogen bond acceptors</th>
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<tr>
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<td>2.3</td>
<td>252.27 g/mol</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5b</td>
<td>2.7</td>
<td>266.3 g/mol</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5c</td>
<td>2.3</td>
<td>282.3 g/mol</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5d</td>
<td>2.0</td>
<td>268.27 g/mol</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>5e</td>
<td>2.1</td>
<td>297.27 g/mol</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

1 MM: Molar mass

2.2.2. The structure and active site of SARS-COV.

The structural data regarding the active binding site of the main protease (6LU7) can be used to design novel anti-COVID-19 inhibitors [19]. This Mpro enzyme exists in homodimer, and each monomer has three domains: domain I, II, and III [3]. The third domain and N-terminal finger are participating in the process of dimerization (Figure 3), which controls the catalytic activity of this enzyme. The Mpro of SARS-CoV-2 processes polyproteins using...
some catalytic residues viz histidine (His 41) and cysteine (Cys145) [3]. The active site of M\textsuperscript{pro} is located between domains I and II [1, 19].

![Figure 3](https://nanobioletters.com/)

**Figure 3.** The 3D representation of the main protease of COVID-19. The first protomer of the dimer is marked in light blue, and the second one is shown in orange. The three representative domains are labeled by Roman numbers. The catalytic residues “Cys 145 and His 41” are represented as yellow and blue spheres.

2.2.3. Optimization of the active site.

The X-ray crystallographic structure of the M\textsuperscript{pro} complexed with N3 was obtained from the Protein Data Bank (http://www.rcsb.org/, PDB (code 6LU7) [18]. To prepare the enzyme for docking studies, polar hydrogen atoms and charges were added to the systems [5].

2.2.4. Compound screening using Autodok Vina software.

We stimulated the docking interaction using the software Autodock 1.5.6 (MGL tools-1.5.6) [20, 21]. Polar hydrogen atoms and Kollman united charges were added to all target proteins, and the resulting file has been saved in pdbqt extension [22]. For the docking calculation, a grid box of 60×60×60 Å in x, y, z directions were created to cover the active site of the target [18, 23, 24]. The default grid points spacing was fixed to 0.375 Å and centered at x = 42.527 y = -46.679, z = 65.559. We used the Lamarckian Genetic Algorithm (LGA) [24] for flexible docking calculations in this work. The LGA parameters including size, energy screening, mutation rate, and crossover rate. After the calculation procedure, we selected the best conformations of the complex based on their binding energy scores [25].

2.2.5. Analysis and visualization.

Visual analysis of the complex interaction was displayed using Discovery Studio Visualizer, and the results were validated using Autodock-tools.

3. Results and Discussion

3.1. Molecular docking.

The docking results are summarized in Table 2 showed clearly that the 5b derivative was found to be the best molecule to interact at the target site with a binding affinity score similar to the chloroquine drug about -6.20 Kcal/mol. The interaction between the ligand 5b and the main protease (6LU7) was stabilized by forming four hydrogen bonds with various amino acid residues, including « Cyst145, Ser144, and GLY143 ».
Table 2. Representation of the 2D interaction between the tested compounds and the main protease of COVID-19.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Type of interaction</th>
<th>Binding Energy score</th>
</tr>
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<tbody>
<tr>
<td>5a</td>
<td>Thr 111, Lys5, and Gln 110</td>
<td>-5.4</td>
</tr>
<tr>
<td>5b</td>
<td>Cyst145 Ser144 and Gly143</td>
<td>-6.2</td>
</tr>
<tr>
<td>5c</td>
<td>His246 Thr111 and Lys5</td>
<td>-5.72</td>
</tr>
<tr>
<td>5d</td>
<td>Gln 110, His 246 and Thr111</td>
<td>-5.11</td>
</tr>
<tr>
<td>5e</td>
<td>Lys5 Gln 127 and ASP189</td>
<td>-5.14</td>
</tr>
<tr>
<td>Drug 1*</td>
<td>Thr 111</td>
<td>-6.20[19]</td>
</tr>
<tr>
<td>Drug 2**</td>
<td>Gln 110, Thr 292, Thr 111</td>
<td>-4.20[19]</td>
</tr>
</tbody>
</table>

*Chloroquine, and ** Favipiravir

Furthermore, the virtual screening of the 5a, 5c, 5d, and 5e compounds provided a good binding affinity with 6LU7 respectively -5.4, -5.72, -5.11, and 5.14 -Kcal/mol. Their energy value is better than the energy score given by favipiravir (-4.2kcal/mol) [13]; nevertheless, the 5b derivative has been considered a great inhibitor of the main protease because of its binding energy is similar to the chloroquine. Indeed, he interacted with the pocket of 6LU7 via hydrogen bonds (see figure 4), and the amino acid residues participating in the interaction are summarized in table 2.

According to our molecular docking analysis, we can classify the inhibition potential of the synthesized compounds, ranked by their binding affinity (ΔG) as follow: 5b > 5c > 5a>5d> 5e.

![Figure 4](https://nanobioletters.com/)

Figure 4. The ligand map showing the interaction between pyranopyrazoles 5(a-e) and residual amino acids of the main protease of COVID-19.

The M<sup>pro</sup> of coronavirus is a good target for treating COVID-19 because it is essential for the proteolytic maturation of this virus [3, 4]. The inhibition of M<sup>pro</sup> via blocking the active sites of the protein is one of the proposed hypotheses of the cellular mechanism of the pyrano[2,3-c] pyrazole and several drugs, including chloroquine [8] (see figure 5).

Furthermore, the M<sup>pro</sup> structure has provided an immense opportunity to identify novel drug candidates for the treatment of coronavirus from natural products [2, 26] to synthetic compounds [1, 5, 27].

In our study, we have synthesized and characterized five pyrano[2,3-c] pyrazoles compounds 5(a-e), then we screened their antiviral capacity using the Autodock virtual tool [18]. The effective compounds were selected based on their best binding affinity score with COVID-19 major protease (6LU7) [8].
Molecular docking can be defined as a computational tool to identify non-Covalent binding between a protein (receptor) and a selected ligand (inhibitor) [24] by predicting their mode of interaction [13] and the amino acids involved in this interaction [22]. However, we can’t proceed to the virtual screening without passing by Lipinski estimation, and the selected compounds should fulfill Lipinski’s rule of five [24]. Thus, the major criterion for evaluating drug likeliness proprieties of these compounds is determining their molecular parameters, including absorption, distribution, metabolism, and excretion (ADMET) [21].

**In silico** study revealed that all the protease inhibitor drugs got docked with negative binding energy onto the target protein. These pyranopyrazoles compounds appeared to have the best potential to act against COVID-19, especially the 5b compound that gives an energy score of -6.2 Kcal/mol, which is similar to the energy score given by chloroquine (-6.2Kcal/mol) and better than the energy score given by hydroxychloroquine (-5.5 Kcal/mol) and favipiravir (-4.2kcal/mol). Moreover, the molecular interaction studies showed that protease structure had multiple active site residues for all studied compounds, including Gln 110, Thr 292, Thr 111, Asp 189and His 246.

In summary, innumerable and huge efforts have been taken by the scientific community to identify synthetic and natural drug candidates against the spread of SARS-CoV2. Hence, no coronavirus-specific inhibitor has accomplished a preclinical level. For that reason, the compounds reported in the current study deserve further investigation *in vitro* and *in vivo* to uncloak their antiviral proprieties.

4. **Conclusions**

This new virus has created a situation of stress in the medical and pharmaceutical sectors, for that reason, we have a strong motivation to find a potential treatment; as a consequence, our current investigation confirmed that pyran2,3-c pyrazole could act as Mpro inhibitors but need to be explored for more experimental assessment regarding the development of an active anti-SARS- Mpro drug.

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Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References


https://nanobioletters.com/


Supplementary files

General information

The crystalline structure of the Na$_2$CaP$_2$O$_7$ was identified by using X-ray diffraction analysis (XRD) (Bruker D8 Advance X-ray diffractometer with Cu-Kα radiation: $\lambda = 1.5406$ Å). The melting point of the pyranopyrazoles derivatives was determined using a Buchi 510 apparatus. The NMR spectra of $^1$H and $^{13}$C were recorded on a Bruker 300 MHz in DMSO-d$_6$. The chemical shifts (δ) are expressed in ppm. The IR spectra of the samples were acquired as KBr pellets on FTIR (IR Affinity - 1S, Fourier Transform Infrared Spectrophotometer, SHIMADZU). Analytical thin-layer chromatography was performed with Silica on TLC Alu foils purchased from Sigma Aldrich. Visualization of the developed chromatogram was performed by UV light (254 nm). All reactions were carried out under air. Solvents and starting materials (Aldrich) were used without further purification.

General procedure for preparation of pyranopyrazoles 5a-e

The catalyst Na$_2$CaP$_2$O$_7$ (20mol %) was added to a mixture of the aldehydes (1 mmol), ethyl acetoacetate (1mmol), malononitrile (1.2 mmol), and hydrazine hydrate (2 mmol), and 1 ml of water in a 5 ml flask fitted with a reflux condenser. The resulting mixture was heated to reflux (an oil bath) with stirring for 20 min. Acetone (2 ml) was added and the mixture was stirred for 2 min. The products and catalyst were isolated as described above and recrystallized from 96% ethanol (20 ml) to afford pyrano[2,3-c]pyrazoles 5a-e.

Characterization of the compounds

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile 5a

Figure S1. The synthesis of pyranopyrazoles catalyzed by Na$_2$CaP$_2$O$_7$.

Figure S2. NMR $^1$H of pyranopyrazoles 5a.
6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5b.

Figure S3. NMR $^1$H of pyranopyrazoles 5b.

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5c.

Figure S4. NMR $^1$H of pyranopyrazoles 5c.
6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyrazole[2,3-c]pyrazole-5-carbonitrile 5d.

Figure S5. NMR $^1$H of pyranopyrazoles 5d.

6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyrazole[2,3-c]pyrazole-5-carbonitrile 5e.

Figure S6. NMR $^1$H of pyranopyrazoles 5e.
6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyran[2,3-c] pyrazole-5-carbonitrile 5a.

Figure S7. NMR $^{13}$C of pyranopyrazoles compound 5a.

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyran[2,3-c] pyrazole-5-carbonitrile 5b.

Figure S8. NMR $^{13}$C of pyranopyrazoles compound 5b.
6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5c.

![NMR spectrum of compound 5c](image)

**Figure S9.** NMR $^{13}$C of pyranopyrazoles compound 5c.

6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5d.

![NMR spectrum of compound 5d](image)

**Figure S10.** NMR $^{13}$C of pyranopyrazoles compound 5d.
6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5e.

Figure S11. NMR $^{13}$C of pyranopyrazoles compound 5e.

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5a

Figure S12. IR pattern of the synthesized compound of 5a.

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5b.
Figure S13. IR pattern of the synthesized compound of 5b.  

6-Amino-3-methyl-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile 5c.

Figure S14. IR pattern of the synthesized compound 5c.  

6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile 5d.

Figure S15. IR pattern of the synthesized compound 5d.
6-Amino-3-methyl-4-(4-methoxyphenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile 5e.

Figure S16. IR pattern of the synthesized compound 5e.

HPLC Analysis

(5a) $t_R = 3.63 \text{ min}$

(5b) $t_R = 3.53 \text{ min}$

(5c) $t_R = 3.56 \text{ min}$

(5d) $t_R = 3.704 \text{ min}$
Synthesis and characterization of the catalyst

Method for the preparation of Catalyst

The synthesis of the nano-structured diphosphate Na$_2$CaP$_2$O$_7$ has been carried by using the Na$_2$CO$_3$, CaCO$_3$, and NH$_4$H$_2$PO$_4$ in 1:1:2 proportions, respectively (purity of starting materials greater than 99%). These chemicals were mixed in an agate mortar and progressively heated in a porcelain crucible. The synthesis of Na$_2$CaPO$_7$ particles was confirmed by powder XRD, IR, SEM, and TEM studies. The steps of Na$_2$CaPO$_7$ synthesis are summarized in figure S17.

XDR of the catalyst

The powder obtained was analyzed by X-ray diffractometer, the diffractogram was recorded using a Bruker D8 Advance diffractogram. The spectrum of the Na$_2$CaP$_2$O$_7$ diphosphate is reproduced in Figure S18 the crystallographic data obtained are gathered in table S1. The diffractometer uses copper anticathode radiation Cu-K$_\alpha$ radiation: $\lambda = 1.5406$ A$^\circ$. The crystalline parameters were refined on a computer using the least-squares method via the AFPAR program. The acquisition is carried out at ambient temperature with a scanning mode 0/2 (and a Bragg angle 0 spanning from 10° to 50° (17).
**Figure S19.** The XDR pattern of the diphosphate Na$_2$CaP$_2$O$_7$.

**Table S1.** Allotment of the observed bands of Na$_2$CaP$_2$O$_7$ analyzed by X-ray diffractometer.

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</table>

**Infrared of the catalyst**

The infrared spectrum of the diphosphate Na$_2$CaP$_2$O$_7$ in powder form is shown in figure S19. The appearance of symmetrical vibration bands of P-O-P at 720 cm$^{-1}$ and anti-symmetrical vibration bands at 893 cm$^{-1}$ confirm the existence of P$_2$O$_7$ (Figure S19). Two vibration fields have proved the presence of the PO$_4$ group, the first at 996 cm$^{-1}$ and 1031 cm$^{-1}$ and the second going from 1130 to 1278 cm$^{-1}$ (17).
**Figure S20.** The infrared pattern of the catalyst was measured by using the FITR apparatus.

**Table S2.** Attribution of the observed bands of Na$_2$CaP$_2$O$_7$ analyzed by FTIR spectroscopy.

<table>
<thead>
<tr>
<th>Observed band (cm$^{-1}$)</th>
<th>Awarding</th>
</tr>
</thead>
<tbody>
<tr>
<td>407</td>
<td>δ(P$_2$O$_7$)</td>
</tr>
<tr>
<td>419</td>
<td>Distortion vibrations</td>
</tr>
<tr>
<td>483</td>
<td></td>
</tr>
<tr>
<td>511</td>
<td>υ$_{sym}$(P-O-P)</td>
</tr>
<tr>
<td>548</td>
<td>υ$_{antisym}$(P-O-P)</td>
</tr>
<tr>
<td>577</td>
<td></td>
</tr>
<tr>
<td>626</td>
<td></td>
</tr>
<tr>
<td>720</td>
<td>υ$_{sym}$(PO$_4$)</td>
</tr>
<tr>
<td>893</td>
<td>Valence vibrations</td>
</tr>
<tr>
<td>996</td>
<td></td>
</tr>
<tr>
<td>1031</td>
<td></td>
</tr>
<tr>
<td>1130</td>
<td>υ$_{antisym}$(PO$_4$)</td>
</tr>
<tr>
<td>1175</td>
<td></td>
</tr>
<tr>
<td>1278</td>
<td></td>
</tr>
</tbody>
</table>

**TEM and SEM**

The morphological studies of the surface of Na$_2$CaP$_2$O$_7$ are performed by using the scanning electron microscope HIROX SH-4000M. To visualize the microstructure of the Na$_2$CaP$_2$O$_7$, Transmission Electronic Microscopy (TEM) was used on an FEI microscope operated at 120 kV (17).
Figure S21. SEM images of Na$_2$CaP$_2$O$_7$.

Figure S22. TEM images of Na$_2$CaP$_2$O$_7$.

**ADMET/TOX screening**

Table S3. *In silico* physicochemical parameters for good oral bioavailability of synthesized compounds 5(a-e).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Log P</th>
<th>MM (g/mol)</th>
<th>Hydrogen bond donor</th>
<th>Hydrogen bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>2.3</td>
<td>252.27</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5b</td>
<td>2.7</td>
<td>266.3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5c</td>
<td>2.3</td>
<td>282.3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5d</td>
<td>2</td>
<td>268.27</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>5e</td>
<td>2.1</td>
<td>297.27</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

All the ADMET parameters were found to be favourable for all selected compounds 5(a-e).

Table S4. Drug likeness predictions of tested compounds 5(a-e).

<table>
<thead>
<tr>
<th></th>
<th>Absorption</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIA (%)</td>
<td>Cells Caco-2 (nm sec$^{-1}$)</td>
</tr>
<tr>
<td>5a</td>
<td>86.259135</td>
<td>8.35134</td>
</tr>
<tr>
<td>5b</td>
<td>86.801884</td>
<td>0.414056</td>
</tr>
<tr>
<td>5c</td>
<td>86.421462</td>
<td>7.08002</td>
</tr>
<tr>
<td>5d</td>
<td>76.953830</td>
<td>21.1136</td>
</tr>
<tr>
<td>5e</td>
<td>68.466285</td>
<td>17.972</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>81.478448</td>
<td>0.630713</td>
</tr>
</tbody>
</table>
HIA: Human intestinal absorption (HIA, %); BBB: *in vivo* blood-brain barrier penetration (C.brain/C.blood); Caco-2 cell: *in vitro* Caco-2 cell permeability (nm/sec); MDCK: *in vitro* MDCK cell permeability (Mandin Darby Canine Kidney)

Intestinal absorption has been predicted to be more than 80% for compounds 5a (86.25%) 5b (86.8%) and 5c (86.42%), which is greater than reference drugs Ampicillin (81.478%).