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Synthesis and evaluation of diterpenic Mannich bases as antiviral agents against influenza A and SARS-CoV-2

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ABSTRACT

A chemical library was constructed based on the resin acids (abietic, dehydroabietic, and 12-formylabietic) and its diene adducts (maleopimaric and quinopimaric acid derivatives). The one-pot three-component CuCl2-catalyzed aminomethylation of the abietane diterpenoid propargyl derivatives was carried out by formaldehyde and secondary amines (diethylamine, pyrrolidine, morpholine, and homopiperazine). All compounds were tested for cytotoxicity and antiviral activity against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cells and SARS-CoV-2 pseudovirus in BHK-21-hACE2 cells. Among 21 tested compounds, six derivatives demonstrated a selectivity index (SI) higher than 10, and their IC50 values ranged from 0.19 to 5.0 μM. Moreover, two derivatives exhibited potent anti-SARS-CoV-2 infection activity. The antiviral activity and toxicity strongly depended on the nature of the diterpene core and heterocyclic substituent. Compounds 12 and 21 bearing pyrrolidine moieties demonstrated the highest virus-inhibiting activity with SIs of 128.6 and 146.8, respectively, and appeared to be most effective when added at the time points 0–10 and 1–10 h of the viral life cycle. Molecular docking and dynamics modeling were adopted to investigate the binding mode of compound 12 into the binding pocket of influenza A virus M2 protein. Compound 9 with a pyrrolidine group at C20 of 17-formylabietic acid was a promising anti-SARS-CoV-2 agent with an EC50 of 10.97 μM and a good SI value > 18.2. Collectively, our data suggested the potency of diterpenic Mannich bases as effective anti-influenza and anti-COVID-19 compounds.

1. Introduction

The search for new antiviral agents is one of the priority areas of research in modern medicinal chemistry due to the spread of a wide range of viral infections and the emergence of new dangerous viral diseases caused by pathogenic strains, such as coronaviruses, influenza A and B viruses, etc. For example, the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in China at the end of 2019 has generated a global response and is a significant health challenge (WHO, 2020; Li et al., 2020; Phelan et al., 2020).

An innovative approach to the development of new antiviral agents is the use of available plant metabolites as initial building blocks for the synthesis of libraries of derivatives and the study of the structure-antiviral activity relationship (Waziri, 2015; Hussain et al., 2017; Lima et al., 2021). Compounds of the terpene series are among the natural compounds that are promising for creating new antiviral agents (Pompei et al., 2009; González, 2014; Xiao et al., 2018; Hodon et al., 2019). Natural terpenoids and their synthetic analogs are a promising source of new drugs for treating various diseases. These compounds are characterized by a massive variety of molecular structures, low toxicity, and the ability to affect several specific targets inside cells, which determines a wide range of their biological activity, including anti-inflammatory, antitumor, and antiviral properties (Paduch and Kandele-Szerszen, 2014; Rodriguez-Rodriguez, 2015; Kazakova et al., 2017).
Terpene compounds, in particular diterpene acids, are promising for the development of antiviral drugs based on them. Therefore, diterpene derivatives of the abietane series can help treat or prevent an influenza viral infection where the virus is an enveloped virus that undergoes hemagglutinin-mediated fusion with a host cell and/or the resultant symptoms (Mauldin and Monroe, 2001). Dihydroquinopymaric acid amides and their 1β-succinyl and 1β-phthalyl derivatives containing amino acid residues show moderate activity against the reproduction of influenza virus A/FPV/Rostock/34 (H7N1) (Flekhter et al., 2009), while quinopimaric acid derivatives with esters fragments are characterized by more pronounced antiviral properties against influenza A/California/07/09 (H1N1) with selectivity indices of 10 and greater. The conjugate with cinnamic acid shows the highest SI = 56.6 (Vafina et al., 2015). Dihydroquinopimaric acid is found to be moderately active against influenza virus type A (H1N1) (EC₅₀ = 4.5; IC₅₀ > 100; SI > 22), whereas its oxidized product is moderately active against influenza virus type A (H1N1 and H3N2) (EC₅₀ = 3.2; IC₅₀ > 100; SI > 31 and EC₅₀ = 2.6; IC₅₀ > 100; SI > 38) and papilloma virus HPV-11 (Kazakova et al., 2010). An excellent antiviral activity of maleopimaric acid oxidized product and dihydroquinopimaric methyl-(2-methoxycarbonyl)ethylene amide is found toward HPV-11 with SIs 30 and 20, respectively. Methyl-(2-methoxycarbonyl)ethylene-, 1-hydroxy-5′-kapolaktamo- and 4-hydroxy-4,14α-epoxy-13(15)-ene-dihydroquinopimaric acid derivatives have also shown activity against replication of HCV nucleic acid low toxicity (Tret'yakova et al., 2015).

Therefore, in the present study, we introduced heterocyclic moieties into the diterpene skeleton by Mannich reaction (Fig. 1) and evaluated the in vitro antiviral activity and cytotoxicity of the target compounds against influenza virus A (H1N1) and SARS-CoV-2 pseudovirus.

2. Results and discussion

2.1. Chemistry

The target compounds bearing a heterocyclic moiety were synthesized, as shown in Scheme 1. The starting 1α,4α-dehydroquinopimaric acid 1 was prepared from quinopimaric acid in two steps as previously described (Shul’ts et al., 2009). For the synthesis of diterpene indole 5 with a morpholine fragment, primarily intermediate 2 was obtained as a result of the interaction of acid 1 with methylaminocrotonate by the Nenitzescu reaction (Tret’yakova et al., 2020), which was used in the further reaction. 1α, 4α-Dehydroquinopimaric acid propargylamide 3 was obtained by chloride method from acid 1 and propargylamine by heating in CH₂Cl₂ for 2 h. The maleopimaric acid propargylamide 4 was synthesized in a similar manner previously (Tret’yakova et al., 2016).

Key compounds 5–7 were prepared by aminomethylation of indole 2 and alkynes 3, 4 with the secondary amines and the formaldehyde at room temperature in a 1,4-dioxane medium in the presence of CuCl catalyst (Scheme 1).
The prepared compounds 5–25 were evaluated for their *in vitro* antiviral activity against the A/Puerto Rico/8/34 (H1N1) influenza virus (for MDCK cells). Oseltamivir carboxylate was used as a reference compound. The resulting data were expressed as virus-inhibiting activity (IC\textsubscript{50}), cytotoxicity (CC\textsubscript{50}), as well as selectivity index (SI), which is the ratio CC\textsubscript{50}/IC\textsubscript{50}, and presented in Table 1. Compounds with SIs greater than 10 were considered active (Smee et al., 2017).

Among 21 tested compounds, six of them were considered as prospective with SIs higher than 10 and IC\textsubscript{50} values from 0.19 to 5.0 μM. Analysis of the obtained data demonstrated that the antiviral activity against influenza virus A H1N1 depended on the nature of the heterocyclic moiety and the type of diterpene scaffold. Therefore, the Mannich bases obtained based on diterpene indole, as well as abietic and dehydroabietic acids 7–10, and 14–16 showed a moderate inhibitory activity (IC\textsubscript{50} 89–12.6 μM) and relatively high toxicity against cells (CC\textsubscript{50} 140–7.9 μM), which was expressed by the low values of the SI. Similar results were obtained for aminoalkylated analogs of 17-formylabietic, maleopimaric, and dihydroquinopimaric acids as well as methyl 1,4-dihydro-2,3-dihydroquinopimarate 23–25.

At the same time, diterpene Mannich bases with homopiperazine and pyrrolidine moieties 5, 6, 12, 18, 21, and 24 had SIs more than 10. Consequently, they can be recognized as prospective compounds. Among them, the 1α,4α-dehydroquinopimaric acid homopiperazine derivative 6, as well as the methyl 1β,4α-dihydroxydihydroquinopimarate containing morpholine and diethylamine moieties 13, 17, 19, 20, 22, 23, 25 (IC\textsubscript{50} 37.4–5.48 μM, CC\textsubscript{50} 92–19.8 μM, SI 5.45–0.49) except for compound 11, which showed low toxicity but weak antiviral activity (IC\textsubscript{50} 72.6 μM, CC\textsubscript{50} 718 μM, SI 9.89).

2.2. Antiviral activity against influenza virus A

The prepared compounds 5–25 were evaluated for their *in vitro* antiviral activity against the A/Puerto Rico/8/34 (H1N1) influenza virus (for MDCK cells). Oseltamivir carboxylate was used as a reference compound. The resulting data were expressed as virus-inhibiting activity (IC\textsubscript{50}), cytotoxicity (CC\textsubscript{50}), as well as selectivity index (SI), which is the ratio CC\textsubscript{50}/IC\textsubscript{50}, and presented in Table 1. Compounds with SIs greater than 10 were considered active (Smee et al., 2017).

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At the same time, diterpene Mannich bases with homopiperazine and pyrrolidine moieties 5, 6, 12, 18, 21, and 24 had SIs more than 10. Consequently, they can be recognized as prospective compounds. Among them, the 1α,4α-dehydroquinopimaric acid homopiperazine derivative 6, as well as the methyl 1β,4α-dihydroxydihydroquinopimarate pyrrolidine derivative 24, showed high antiviral activity, exceeding the activity of the reference drug by 34 and 25 times, respectively, but exhibited high toxicity. For the maleopimaric acid Mannich bases with homopiperazine 5 and pyrrolidine fragments 18, antiviral activity was two times higher than the activity of oseltamivir, and the toxicity compared with the above compounds was decreased. Compounds 12 and 21 with the pyrrolidine fragment were the most effective against the influenza virus. The dihydroquinopimaric acid analog 21 with SI 146.8 was 13-fold more active than oseltamivir (IC\textsubscript{50} 0.47 μM) but still showed relatively high cytotoxicity (CC\textsubscript{50} 69 μM). Finally, 17-formylabietic acid derivative 12 exhibited potent viral inhibitory activity and low toxicity, indicating a high therapeutic index (SI 128.6) compared with the reference drug.
2.3. Time-of-addition experiments

We performed time-of-addition experiments to determine the sug-
gestive target for the virus-inhibiting activity of the most active com-
ounds in the virus life cycle (Fig. S5, Supporting Information). As
suggested from the data, compounds 12 and 21 showed the greatest
activity when added at time points 0–10 and 1–10, but not (−1)–10.
These results indicated that a possible target for compounds might be
the viral entry into the cell, uncoating, and the early stages of replica-
tion, but not the binding of the virus to the receptor.

Therefore, the possible target of the compounds might be M2-protein
or (less likely) HA-protein or NP. Moreover, it was impossible to exclude
cellular proteins involved in endocytosis.

2.4. Docking studies of compound 12

We investigated the binding mode of compound 12 into the binding
pocket of influenza A virus M2 protein (PDB code: 6BKL) by docking and
molecular dynamics simulation. The Schr"odinger program (version
2018) was employed, and the docked conformation of compound 12
was determined based on the minimum free energy analyses. According
to the computer-aided docking data, compound 12 occupied the M2 proton
channel formed by four adjacent subunits with an estimated binding
energy of $-5.47$ kcal/mol. Subsequently, we performed a 20.0-ns mo-
lecular dynamics simulation on the docking structure. The results
showed that compound 12 matched better to the binding site (Fig. S6,
Supporting Information).

HIS37 of subunit C formed a hydrogen bond with the hydroxyl of
compound 12, and SER31 of subunit D also formed a hydrogen bond
with another hydroxyl. In addition, multiple hydrophobic interactions
were observed between ligand and protein, namely residues VAL27,

Table 2
Antiviral activity of compounds 5–25 against SARS-CoV-2 pseudovirus in BHK-
21-hACE2 cells.

| Compound | Inhibition rate (%) | Cell viability (%) | EC50, μM | CC50, μM |
|----------|---------------------|-------------------|----------|----------|
| 5        | 31.7                | 116.1             |          |          |
| 6        | ND                  | 0                 |          |          |
| 7        | 28.3                | 113.4             |          |          |
| 8        | 43.3                | 101.2             |          |          |
| 9        | 72.4                | 98.8              | 10.97    | >200     |
| 10       | 28.4                | 120.9             |          |          |
| 11       | ND                  | 41.7              |          |          |
| 12       | 19.7                | 89.3              |          |          |
| 13       | 48.1                | 103.1             |          |          |
| 14       | 47.7                | 86.0              |          |          |
| 15       | 59.4                | 91.5              | 11.13    | 86.34    |
| 16       | 37.8                | 108.2             |          |          |
| 17       | 38.9                | 148.2             |          |          |
| 18       | ND                  | 42.3              |          |          |
| 19       | 34.8                | 91.0              |          |          |
| 20       | 42.2                | 112.5             |          |          |
| 21       | 16.8                | 115.2             |          |          |
| 22       | ND                  | 44.1              |          |          |
| 23       | 39.9                | 81.5              |          |          |
| 24       | 50.7                | 114.4             |          |          |
| 25       | 34.0                | 116.7             |          |          |
| Amodiaquine | 92.4              | 107.3             | 3.17     | >100     |

* Measured at a concentration of 20.0 μM.
* EC50, effective concentration; the concentration resulting in 50% inhibition
  of virus replication.
* CC50, the 50% cell cytotoxicity concentration.
* ND, not detected.
* Measured at a concentration of 5.0 μM.

Fig. 3. Cytotoxicity and antiviral activity of compounds 9 and 15 against SARS-CoV-2 pseudovirus in BHK-21-hACE2 cells. Left: Antiviral activity of compounds 9
and 15 against SARS-CoV-2 by Firefly luciferase assay. Right: Cytotoxicity of compounds 9 and 15 in BHK-21-hACE2 cells was determined by CCK-8 Assay.
VAL28, ALA30, ILE32, ILE 33 of subunit A, VAL27, ALA30, ILE 33 of subunit B, VAL27, ALA30, ILE 33 of subunit C, and LEU26, VAL27, ALA30, ILE 33 of subunit D. Many van der Waals contacts were also observed (Fig. S7, Supporting Information).

2.5. Primary screening against SARS-CoV-2 pseudovirus

To explore the spectrum of antiviral activity, we further assessed the anti-SARS-CoV-2 pseudovirus activity of compounds 5–25 in BHK-21-hACE2 cells, and the primary screening results are summarized in Table 2. In addition, amiodarone, an effective inhibitor of viral entry of SARS-CoV-2 (St et al., 2021), was utilized as the positive control. Among the 21 tested compounds, four of them (6, 11, 18, and 22) showed a certain degree of cytotoxicity to BHK-21-hACE2 cells at a concentration of 20 µM with cell viability less than 50% and were not evaluated for inhibition of viral replication. Regarding the anti-antiviral effect, when BHK-21-hACE2 cells were exposed to SARS-CoV-2 pseudovirus and cultured in the presence of 20 µM compounds 9, 15, and 24, there was a significant reduction in the number of SARS-CoV-2 pseudovirus-infected cells.

Two compounds 9 and 15 showed significant antiviral activity with an inhibition rate over 60% at 20 µM and were selected for the dose-response assays (Fig. 3). Of particular note, compound 9 showed a higher anti-SARS-CoV-2 pseudovirus activity (EC50 10.97 µM) and lower cytotoxicity (CC50 > 200 µM) compared with compound 15.

3. Experimental

The synthetic protocols, spectral characteristics of compounds, and biological methods are described in Supporting Information.

4. Conclusions

In summary, we developed a practical approach to the novel abietane derivatives with high antiviral potency. The method was based on the three-component CuCl-catalyzed reaction of diolactone acetylethers with formaldehyde and secondary amines to form Mannich bases. The three-component CuCl-catalyzed reaction of diterpene acetylenes bearing a pyrrolidine fragment was investigated for the first time. The results showed that compound 9 could effectively inhibit pseudovirus replication with no apparent toxicity.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phytol.2022.07.010.

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