Synthesis and Biological Evaluation of Novel Pyrimidine Amine Derivatives Bearing Bicyclic Monoterpene Moieties

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Abstract: A series of novel pinanyl pyrimidine amine derivatives (1e-1n) and camphoryl pyrimidine amine derivatives (2b-2f) bearing bicyclic monoterpene moieties were designed and synthesized from natural and renewable nopinone and camphor. All chemical structures of target compounds were characterized by 1H NMR, 13C NMR and HRMS spectra analyses, and the antimicrobial activities were evaluated. The results indicated that most compounds showed considerable antibacterial and antifungal activities against Klebsiella pneumoniae, Streptococcus pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Methicillin-Resistant Staphylococcus aureus (MRSA), Bacillus cereus and Candida albicans. Among them, 1f showed potent antibacterial activity against all tested bacteria, 1i exhibited excellent inhibition against Streptococcus pneumoniae (1 µg/mL) and Escherichia coli (1 µg/mL), which was better than the control drug amikacin (2 µg/mL). As to antifungal activity against Candida albicans (C. albicans), compound 1f showed comparable activity (16 µg/mL) to the control drug ketoconazole. Furthermore, five active compounds with better antimicrobial activities also showed anti-inflammatory potencies against mouse mononuclear macrophages leukemia cells (RAW). Especially, 1f (IC50 = 1.37 µM) and 2f (IC50 = 1.87µM) are more potent than the control drug aspirin (IC50 = 1.91 µM).

Keywords: bicyclic monoterpene; pyrimidinamine derivatives; synthesis; antimicrobial activities; anti-inflammatory activity

1. Introduction

Heterocyclic compounds with nitrogen atoms were a vital class of organic compounds, most of them possessed various biological activities [1–4] and have been widely used in the medicine, pesticide, dye and food industries [5]. Pyrimidineamine derivatives characterized with two nitrogen atoms in the aromatic ring and a substituted amino outside showed valuable drug application, such as pyrimethamine (malaria prevention), imatinib (anti-tumor drug) and rosuvastatin (hyperlipidemic drug). Benefitting from the universal antibacterial and antiviral activities, tremendous novel pyridineamine compounds were developed in the pesticides, medicine and materials fields [6].

As for the pesticides, pyrimidine amine fungicides showed antimicrobial potencies by inhibiting the biosynthesis of methionine and the secretion of cell wall-degrading enzymes in fungi, thereby inhibited the invasion of bacteria into host cells. Pyrimidine amine fungicides have excellent prevention and control effects on various diseases caused by botrytis cinerea with the characteristics of high activity, low toxicity and drug resistance. The marketed pyrimidine amine bacteriostatic agents, such as pyrimoxamine, pyrimydamine, pyrimoxetine and flufenoxamine [7], etc. (Figure 1), have been used to control botrytis, powdery mildew, scab, and rust infestation. In recent years, developing progress on new pyrimidine amine bacteriostatic agents have emerged one after another [8–12], which has...
been expected to become a new type of market bactericide after methoxyacrylate and succinate dehydrogenase. However, it is difficult to industrialize most pyrimidine amine compounds due to severe toxicity and heavy pollution [13], which has resulted to a low market competitiveness.

In recent years, our research team has made some efforts to explore the application of pinane derivatives. Condensation of nopyrnone with thiosemicarbazide at exocyclic carbonyl group afforded nopyrnone thiosemicarbazone, then cyclized with α-halogenated ketone; new pinyl thiazole derivatives were obtained [29]. Biological evaluation results showed that these compounds showed certain insecticidal effect on Lagerstroemia aphid and displayed a concentration-dependent effect. In addition, insecticidal activities of nopyrnone-based pyrazole amides [30], antitumor activities of nopyrnone-based thiosemicarbazide derivative [31], amylase inhibition activities of nopyrnone-based thiazole hydrazones [32], and antitumor activities of camphor-based pyrimidine derivatives were also studied. These studies indicate that pinanyl heterocyclic derivatives have extensive biological activities, such as anticancer, anti-inflammatory, antioxidant, antibacterial, antidiabetic and antiviral, etc. Nevertheless, there are few studies reporting the synthesis of pyrimidine amine derivatives bearing bicyclic monoterpenic moiety. The aforementioned findings give us an impetus to design and synthesize novel pyrimidine amines bearing bicyclic monoterpenic moiety and explore their potential values as antimicrobial agents. Herein, we report the synthesis and biological evaluation of novel pinanylpyrimidine amines as antimicrobials. The design strategy of the target compounds is shown in Scheme 1:

Based on the above design strategy, a total of 15 novel pyrimidine amine derivatives with bicyclic monoterpenic units were synthesized by the synthetic route shown in
Scheme 2, and the structures of the compounds were characterized by $^1$H NMR, $^{13}$C NMR and HRMS. *K. pneumoniae*, *S. pneumoniae*, *P. aeruginosa*, *S. aureus*, *Escherichia coli* (*E. coli*), *methicillin*-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus* (*B. cereus*), and *Candida albicans* (*C. albicans*) were selected as test bacterial and fungi strains; the antibacterial activities and antifungal activity for the target compounds were evaluated. Antibacterial active compounds were selected for anti-inflammatory activity assay against mouse mononuclear macrophage leukemia cells (RAW), and the relationship between structures and activities were preliminarily discussed.

Scheme 1. Design strategy of the target compounds.

Scheme 2. Synthesis of pinanyl pyrimidine amines derivatives with bicyclic monoterpane units.

2. Results and Discussion

2.1. Chemistry

The synthetic route for the target compounds is illustrated in Schemes 2 and 3. Briefly, compounds 1a–1d were synthesized with a typical aldehyde-ketone condensation reaction [33]. The α-C of nopinone was converted to carbanion with the catalyst of alkali, then an addition reaction was performed between the carbanion and aldehyde carbonyl to generate α, β-unsaturated ketones. Based on the principle, nopinone as the starting material, condensed with *p*-hydroxybenzaldehyde, *p*-chlorobenzaldehyde, *p*-methoxybenzaldehyde and *p*-diethylaminobenzaldehyde, pinanyl ketene intermediates (1a–1d) were generated in the presence of sodium methoxide or potassium tert-butoxide.

Since the strong electron donating effects of methoxy and diethylamine groups, the reactions of 1c and 1d were difficult and sodium methoxide were used instead of potassium tert-butoxide, and the reaction time were extended to 16~24 h with the yields of 84~86%. 1a–1c were purified by recrystallization from ethanol as solid products, 1d was oily product with diethylamino group, which was purified by column chromatography. Pinanyl pyrimidines (1e–1h) were obtained by cyclization 1a–1d with guanidine hydrochloride in the presence of sodium hydroxide. For the synthesis of 1g, the reaction time was long.
(30 h) and the yield was relatively low (35.5%) because of the methoxy deactivation effects. Pinanyl pyrimidine amines (1i–1n) were generated by further substitution of 1g or 1h with haloalkanes. The products were poorly dissolved in dichloromethane, ethanol and acetonitrile, and the reaction hardly occurred, thus the suitable solvent tetrahydrofuran was explored. For most instances, the substituted amination of haloalkanes and aminopyrimidines could react smoothly catalyzed by DIPEA. However, for preparing 1i by amination of 2-bromoethyl methyl ether and 1g, the reaction was difficult. A stronger base sodium hydride was used at 65 °C for 3 h, and the yield of the product was 79.8%. As for camphor, condensation of camphor and p-methoxybenzaldehyde occurred in tert-butanol solvent under the catalysis of potassium tert-butoxide, and the intermediate 2a was generated, followed by cyclization with guanidine hydrochloride, the camphorylpyrimidine intermediate 2b was afforded. According to the preparation method of pinanyl pyrimidineamines, camphoryl pyrimidineamine compounds (2c–2f) were obtained by substitution of 2b with alkyl halides in tetrahydrofuran solvent.

**Scheme 3.** Synthesis of camphoryl pyrimidine amine derivatives with bicyclic monoterpenic units.

### 2.2. Structural Characterization of Compounds

The characterization of target compounds mainly focused on the presence of pinanyl, camphoryl and pyrimidineamine functional groups in the structures.

For the pinanyl pyrimidine amines, in the 1H NMR spectrums, chemical shifts on the saturated carbons atoms of benzylidene nopinone intermediates were in the high fields (δ 0.81–2.99), including 6 H of methyl group and 6 H in the pinene ring. Hydrogen atom chemical shifts on the unsaturated carbons atoms appeared in the low fields with signals at δ 6.68–7.69 that corresponded to 4 H on the benzene ring and 1 H in the double bond of the pinene. For pinanyl pyrimidine amine products, the chemical shifts on the saturated carbons appeared in the range of δ 0.62–2.96, while those on benzene rings and pyridine rings appeared in the low fields with signals at δ 6.13–8.5. In the 13C NMR spectrums, the chemical shifts (δ) of the saturated carbons on the pinene ring appeared in high fields at δ 21.00–55.89, while those of unsaturated carbons on benzene, pyridine or pyrimidine rings appeared in low fields at δ 103.86–176.13. Because of the deshielding effects of C=O, chemical shifts of carbonyl carbon atoms were shifted to the lower fields at δ 201.54–203.85. Owing to the influences of fluorine atoms, F-C coupling phenomenon was observed in the 13C NMR spectra of compounds 1j and 1l. For compound 1j, fluorine atom coupled with three carbon atoms (δ 161.60, δ 137.58 and 115.22) on the fluorine-containing benzene ring, resulted two signals for every carbon atom.

For the camphorylpyrimidine amines, in the 1H NMR spectrums, chemical shifts on the saturated carbons atoms of benzylidene camphorone intermediates were in the high fields (δ 0.83–3.11) including 9 H of methyl group and 5 H in the camphor ring. Hydrogen atom chemical shifts on the unsaturated carbons atoms appeared in the low fields with signals at δ 6.93–7.48, that corresponded to 4 H on the benzene ring and 1 H in the double bond of the olefin. For camphoryl pyrimidine amine products, the chemical shifts on the saturated carbons appeared in the range of δ 0.54–3.08, while those on benzene rings and pyridine rings appeared in the low fields with signals at δ 6.26–7.80. In the 13C NMR spectrums, the chemical shifts (δ) of the saturated carbons on the camphor ring appeared in high fields at δ 10.04–71.65, while those of unsaturated carbons on the benzene, pyridine...
or pyrimidine rings appeared in low fields at δ 103.70–181.81. Affected by C=O, chemical shifts of carbonyl carbon atoms were shifted to the lower fields, and the F-C coupling phenomenons were also observed in the 13C NMR spectra of compounds 2d and 2f. The coupling splitting in the 13C NMR spectrum indicated the presence of fluorine atoms, which were consistent with the structures.

In the HRMS spectrums, the molecular ion peaks of the target compounds were consistent with the theoretical value with the deviation below 0.5%, which furtherly confirmed the structures of the target compounds.

2.3. Biological Evaluation

2.3.1. Antibacterial Activity Assay

Clinical isolates of MRSA are susceptible to amikacin at concentrations achieved by regional perfusion, amikacin was chosen to be the reference substance. The antibacterial activities of compounds 1e–1n and 2b–2f in vitro were evaluated by MIC assay with double dilution method [34]. The selected strains included K. pneumoniae, S. pneumoniae, Pseudomonas aeruginosa, P. aeruginosa, S. aureus, E. coli, MRSA, B. cereus and white Candida (C. albicans); the biological activity assay results are shown in Table 1:

Table 1. MIC (μg/mL) values of pinanyl pyrimidine amine and camphoryl pyrimidine amine derivatives.

| Compd. | R1      | R2         | Bacterial | Fungi |
|--------|---------|------------|-----------|-------|
|        |         |            | K. pneumoniae | S. pneumoniae | P. aeruginosa | S. aureus | E. coli | MRSA | B. cereus | C. albicans |
| 1e     | OH      | H          | 32        | 32   | 16   | 32   | 8     | 64   | 8     | >1024 |
| 1f     | Cl      | H          | 32        | 8    | 16   | 32   | 8     | 8    | 8     | >1024 |
| 1g     | OCH3    | H          | 64        | 32   | 64   | 1    | 8     | 8    | 32    | >1024 |
| 1h     | N(C2H5)2 | H          | 256       | >1024 | 128  | 16   | 128   | 8    | >1024 | >1024 |
| 1i     | OCH3    |            | 128       | 1    | 32   | >1024 | 1     | 64   | 64    | 32    |
| 1j     | OCH3    |            | 256       | 64   | 128  | 512  | 64    | 64   | >1024 | 32    |
| 1k     | OCH3    |            | 512       | >1024 | 128  | 512  | 128   | 64   | >1024 | 64    |
| 1l     | OCH3    |            | >1024     | >1024 | 128  | 16   | 512   | 8    | >1024 | 16    |
| 1m     | N(C2H5)2 |            | >1024     | 32   | 128  | 512  | 64    | 64   | >1024 | >1024 |
| 1n     | N(C2H5)2 |            | >1024     | >1024 | >1024 | >1024 | >1024 | >1024 | >1024 | >1024 |
| 2b     | OCH3    | H          | >1024     | 32   | 16   | >1024 | 8     | 64   | 32    | >1024 |
| 2c     | OCH3    |            | 64        | >1024 | 256  | 16   | 32    | 128  | >1024 | 64    |
| 2d     | OCH3    |            | >1024     | >1024 | 128  | 256  | 64    | 64   | >1024 | 64    |
| 2e     | OCH3    |            | >1024     | >1024 | 128  | 512  | 128   | 32   | >1024 | 128   |
| 2f     | OCH3    |            | 32        | 16   | 16   | 32   | 8     | 8    | 16    | 32    |
| amikacin | -      |            | 32        | 2    | 32   | 1    | 2     | 1    | 4     | -     |
| ketoconazole | -     |            | -         | -    | -    | -    | -     | -    | -     | 16    |

a. No detection. b. Amikacin is positive control against bacterium; ketoconazole is positive control against fungus.
As shown in Table 1, most compounds showed moderate to excellent antibacterial activities against certain bacteria, while 1e, 1f, 1g, 1i and 2f exhibited favorable inhibition to most bacteria. Among them, 1i displayed excellent antibacterial activities against Streptococcus pneumoniae (S. pneumoniae) and Escherichia coli (E. coli) with MIC value of 1 µg/mL, which was superior to amikacin. 1e, 1f and 2f inhibited K. pneumoniae with MIC value of 32 µg/mL that was comparable to amikacin. 1f displayed better antibacterial effect against P. aeruginosa (16 µg/mL) than amikacin (32 µg/mL). 1f, 1g, 1h, 1l and 2f displayed weaker antibacterial effect against MRSA (8 µg/mL) than amikacin (1 µg/mL). As for antifungal activities, compound 1l exhibited the best activity against C. albicans with MIC value of 16 µg/mL, which was comparable to ketoconazole.

Preliminary structure–activity relationship analysis for compounds 1e~1n and 2b~2f showed that when R^2 was H, the target compounds had a certain inhibitory activity against most bacteria, but no inhibitory activity against fungi when R^2 was alkyl or aryl groups, compounds showed different antibacterial activities against bacteria and fungi. These results indicated the introduction of an electron-withdrawing group with F (such as 2f) would likely keep the antibacterial potencies while the introduction of an electron-donating group with diethylamine would decrease the antibacterial activities. From the data in Table 1, it can be seen that the antibacterial MIC values of 1i and 1f were lower than that of amikacin; the antifungal MIC of 1l was comparable to ketoconazole, which have potential applications in antimicrobial drug research.

So far, five compounds were determined with effective antibacterial activities. Especially, the promising compound 1i displayed better antibacterial activities against Strep- tococcus pneumoniae (S. pneumoniae) and Escherichia coli (E. coli) than amikacin. In addition, compound 1l exhibited comparable antifungal activity to ketoconazole. The properties of chemical structures, such as molecule weight, CLogP values, pKa values and tPSA values, affect lipophilicity, solubility, protein binding and the ability of a chemical to cross the cell membrane, thus governing the absorption, distribution, metabolism, excretion and toxicity. In order to illustrate the structure characters of active compounds, the properties comparison of compounds 1e, 1f, 1g, 1i, 2f and 1l were summarized in Table 2.

The data indicated that all the active compounds have smaller molecule weights than the control drugs, the predicted CLogP values were less than 7.26 and the predicted tPSA values were above 45.98 for all target compounds. As to antibacterial activities, compounds 1i (CLogP = 5.01, tPSA = 55.21) displayed excellent potencies with higher CLogP and lower tPSA than amikacin (CLogP = –4.12, tPSA = 331.94). For the available Pka values, amikacin is water-soluble with higher Pka and tPSA values but a lower CLogP value. As to the promising compound 1l, compared with ketoconazole (CLogP = 3.64, tPSA = 66.84), 1l (CLogP = 6.74, tPSA = 45.98) displayed similar antifungal potent to C. albicans with higher CLogP and lower tPSA value.

### Table 2. Properties of the target compounds and the control drugs.

| Compd. | MW | CLogP | pKa | tPSA |
|--------|----|-------|-----|------|
| 1e     | 281| 3.73  | 8.81|  70.97|
| 1f     | 300| 4.86  | -   |  50.74|
| 1g     | 295| 4.18  | -   |  59.97|
| 1l     | 353| 5.01  | -   |  55.21|
| 2f     | 435| 7.26  | -   |  45.98|
| amikacin | 585| –4.12 | 13.32| 331.94|
| 1l     | 421| 6.74  | -   |  45.98|
| ketoconazole | 531| 3.64  | -   |  66.84|

\[a\]: molecule weight (MW) was calculated from ChemBioDraw 20.0 and the digits were rounded. \[b\]: CLogP was predicted by ChemBioDraw 20.0. \[c\]: pKa was predicted by ChemBioDraw 20.0. \[d\]: tPSA (Topological polar surface area) was predicted by ChemBioDraw 20.0.

2.3.2. Anti-Inflammatory Activity Assay

To characterize whether compounds we synthesized can induce inflammatory cell apoptosis, the MTT assay was performed on the RAW cells. Based on the preliminary
screening of antibacterial activity, five compounds (1e, 1f, 1g, 1i and 2f) with outstanding antibacterial effects were selected for anti-inflammatory activity assay, and aspirin was used as the positive control drug. The anti-inflammatory activities of the five target compounds on mouse mononuclear macrophage leukemia cells (RAW) are shown in Table 3.

Table 3. Anti-inflammatory activity of compounds on RAW.

| Compd. | (Anti-Inflammatory Activity)/µmol L⁻¹ (IC₅₀ ± SD) |
|--------|-----------------------------------------------|
| 1e     | 2.32 ± 0.9                                    |
| 1f     | 1.37 ± 0.41                                   |
| 1g     | 3.0 ± 0.71                                    |
| 1i     | 4.86 ± 0.84                                   |
| 2f     | 1.87 ± 0.38                                   |
| Aspirin| 1.91 ± 0.35                                   |

From the results in Table 3, it can be seen that the five compounds 1e, 1f, 1g, 1i and 2f have obvious anti-inflammatory activities on RAW cells, especially, 1f (IC₅₀ = 1.37 µM) and 2f (IC₅₀ = 1.87 µM) displayed better inhibition than the control aspirin (IC₅₀ = 1.91 µM). Structure–activity relationship analysis: substituted amino R² has a great influence on the anti-inflammatory activity. While the electron withdrawing group (F) was introduced into the benzene para position, such as 2f, it is beneficial to maintain the anti-inflammatory activity. An electron-donating group such as ethoxymethyl was introduced to the substituted amino group; the anti-inflammatory activity was decreased.

2.3.3. Molecular Docking Study

To explore the binding mode of compounds 1f with inflammation protein (PDB ID: 2AZ5), Auto Dock software was used to predict the putative binding modes. As shown in Figure 2, it can be seen that compound 1f bound to the active pocket of tumor necrosis factor protein, fitted well to the active pocket of the protein. The chlorophenyl ring fragment penetrated deep into the active cavity of the protein while the pyrimidine ring embedded in the upper active cavity of the TNF protein. Further analysis of the binding mode with amino acid residue in the protein pocket showed that nitrogen atoms in pyrimidine ring and chlorine atom in chlorobenzene unit formed hydrogen bonds with Ser60. In addition, the pyrimidine ring and the phenyl ring formed H–π interactions with Tyr59 and Gyl121, respectively. Compared with aspirin scored of −8.1, further flexible docking results indicated there is a strong biding ability between compound 1f and the TNF protein with the score of −7.9.

Figure 2. The binding mode of compound 1f with inflammation protein (PDB ID: 2AZ5).

3. Conclusions

In summary, from natural and renewable products nopinone and camphor, a series of novel pinanyl pyrimidine amine and camphoryl pyrimidine amine derivatives were de-
signed and synthesized; their antibacterial activity and anti-inflammatory activity in vitro were evaluated. Experimental results showed that most of the compounds showed broad-spectrum antibacterial activity against several bacterial species. Among them, compound 1l exhibited better inhibitory activity against *Streptococcus pneumoniae* (1 µg/mL) and *Escherichia coli* (1 µg/mL) than control amikacin (2 µg/mL), compound 1l showed excellent antifungal activity with the lowest inhibitory concentration of 16 µg/mL, which is comparable to ketoconazole. Five compounds with better antibacterial activity also showed obvious anti-inflammatory activities against RAW cells. Especially, compound 1f (IC$_{50}$ = 1.37 µM) and 2f (IC$_{50}$ = 1.87 µM) exhibited better anti-inflammatory activities than that of control aspirin (IC$_{50}$ = 1.91 µM). Molecular docking study exhibited that 1f could bind tightly to the inflammation protein that was consistent with the anti-inflammatory activity. All in all, it suggested that the target pyrimidine amines were expected to be a potential bacteriostat or anti-inflammatory agent, which is worthy of further study.

4. Experimental Section

4.1. Chemistry

All reagents and solvents were purchased from commercial sources and used without further purification. Flash chromatography was performed using silica gel with 200–300 mesh produced by Qingdao Ocean Chemical Factory. All reactions and processes of flash chromatography were monitored by the TLC method using silica gel plates with fluorescence F254 and iodine visualization. The melting points were determined with a YRT-3 drug melting point instrument produced by Tianjin Tianda Tianfa Technology Co., Ltd., and the thermometer was not calibrated. High-resolution mass spectrometry was analyzed by an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Brucker AV-400 spectrometer at 400 MHz and Brucker AV-500 spectrometer at 125 MHz using CDCl$_3$ and DMSO-$d_6$ as solvents, TMS as internal standard (Supplementary Materials). Coupling constants ($J$) are expressed in hertz (Hz). Chemical shifts (δ) are given in parts per million (ppm). The purity of the compounds was determined by Agilent 1260 liquid chromatograph fitted with an Inertex-C18 column. All target compounds have purity over 95%.

4.1.1. Synthesis of Intermediate 3-Arylbenzylidene Nopinones 1a–1d

In a solution of tert-butanol (30 mL) and norpinone (14.48 mmol), p-hydroxybenzaldehyde (14.48 mmol) and sodium methoxide (57.93 mmol) were slowly added under stirring. After feeding completed, the reaction mixture was refluxed for 6–7 h, the solvents were concentrated under reduced pressure, water (25 mL) was added to the residue, the reaction mixture was extracted with ethyl acetate (15 mL), and the organic layers were washed with saturated brine to neutrality. After solvent evaporation, the resulting crude product was recrystallized from methanol to give 1a. Intermediates 1b to 1d were prepared by the same method.

3-(4'-Hydroxybenzylidene)nopinone (1a): pale yellow crystal solid, yield: 76.7%, melting point: 206–207 °C. $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) δ: 10.00 (s, 1 H, Ar-OH), 7.52 (d, J = 8.6 Hz, 2 H, ArH), 7.49 (s, 1 H, ArCH = C), 6.86 (d, J = 8.6 Hz, 2 H, ArH), 2.94–2.81 (m, 2 H), 2.59 (dt, J = 10.7, 5.8 Hz, 1 H), 2.51 (t, J = 5.5 Hz, 1 H), 2.36–2.25 (m, 1 H), 1.37 (d, J = 10.2 Hz, 1 H), 1.32 (s, 3 H, CH$_3$), 0.81 (s, 3 H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) δ: 201.71, 158.60, 134.67, 132.78, 129.18, 126.25, 115.69, 55.14, 40.15, 30.53, 27.02, 25.83, 21.29. HRMS-ESI: 243.1385 calcd for C$_{16}$H$_{16}$O$_2$ [M+H]$^+$, found: 243.1386.

3-(4'-Chlorobenzylidene)nopinone (1b): The synthetic method of 1a was taken as reference, norpinone and p-chlorobenzaldehyde were selected as raw material, a light yellow crystal solid was obtained with the yield of 60.5%, melting point: 107–109 °C. $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) δ: 7.68 (d, J = 8.6 Hz, 2 H, ArH), 7.53 (s, 1 H, ArCH=C), 7.50 (d, J = 8.6 Hz, 2 H, ArH), 2.92 (t, J = 2.8 Hz, 2 H), 2.55–2.59 (m, 1 H), 2.28–2.33 (m, 1 H), 1.39 (d, J = 10.3 Hz, 1 H), 1.33 (s, 3 H, CH$_3$), 1.26 (d, J = 19.9 Hz, 1 H), 0.82 (s, 3 H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) δ: 201.54, 133.94, 133.67, 132.78, 126.25, 115.69, 55.14, 40.15, 30.53, 27.02, 25.83, 21.29. HRMS-ESI: 243.1385 calcd for C$_{16}$H$_{16}$O$_2$ [M+H]$^+$, found: 243.1386.
40.34, 38.76, 30.29, 26.83, 25.80, 21.33. HRMS-ESI: 261.1046 calcd for C_{16}H_{18}ClO [M+H]^+, found: 261.1043.

3-(4-Methoxybenzylidene)-nopinone (1e): The synthetic method of 1a was taken as reference, nopinone and p-methoxybenzaldehyde were selected as raw material, a light yellow crystal solid was obtained with the yield of 85.5%, melting point: 71–72 °C. \(^{1}\)H NMR (400 MHz, CDCl\(_3\), ppm): \(\delta\) 7.69 (t, \(J = 2.0, 2.4\) Hz, 1 H, ArH), 7.59 (t, \(J = 2.0, 6.8\) Hz, 2 H, ArH, ArCH=C), 6.95–6.98 (m, 2 H, ArH), 3.87 (s, 3 H, OCH\(_3\)), 2.98 (t, \(J = 2.4, 3.2\) Hz, 2 H), 2.71–2.72 (m, 1 H), 2.61–2.69 (m, 1 H), 2.37–2.41 (m, 1 H), 1.53 (d, \(J = 10.4\) Hz, 1 H), 1.40 (s, 3 H, CH\(_3\)), 0.95 (s, 3 H, CH\(_3\)). \(^{13}\)C NMR (100 MHz, CDCl\(_3\), ppm): \(\delta\) 184.6%. Compounds 1f–1h were prepared in the same way as 1e.

4.1.2. Synthesis of Pinanealkylpyrimidineamines 1e–1n

To a 50 mL dry three-necked flask equipped with a thermometer and a condenser, tert-butanol (25 mL), 1a (4.1 mmol), guanidine hydrochloride (6.2 mmol) were added in sequence; the mixture of sodium hydroxide (7.8 mmol) and water (10 mL) was added below 50 °C. The reaction mixture was refluxed for 10 h then extracted three times with ethyl acetate (10 mL × 3), the combined organic layers were washed with saturated brine until neutral, the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 3/1) to give a yellow solid of pure 1e. Compounds 1f–1h were prepared in the same way as 1e.

7,7-Dimethyl-5,6,7,8-tetrahydro-4-(4'-hydroxyphenyl)-6,8-methylene-2-quinazolinamine (1e): light yellow yellow powder, yield: 82.5%, melting point: 271–273 °C. \(^{1}\)H NMR (400 MHz, DMSO-d\(_6\), ppm): \(\delta\) 7.50 (d, \(J = 8.7\) Hz, 2 H, ArH), 6.83 (d, \(J = 8.6\) Hz, 2 H, ArH), 4.00 (s, 2 H, NH\(_2\)), 2.61–2.81 (m, 2 H), 2.50–2.59 (m, 2 H), 2.24–2.26 (m, 1 H), 1.30 (s, 3 H, CH\(_3\)), 1.15 (d, \(J = 9.4\) Hz, 1 H), 0.62 (s, 3 H, CH\(_3\)). \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\), ppm): \(\delta\) 175.72, 162.40, 161.87, 161.69, 131.50, 130.44, 130.78, 130.16, 129.20, 128.58, 112.34, 50.06, 38.69, 29.81, 29.27, 26.09, 21.56. HRMS (m/z): 282.1606 calcd for C\(_{18}\)H\(_{20}\)N\(_2\)O [M+H]^+, found: 282.1605.

7,7-Dimethyl-5,6,7,8-tetrahydro-4-(4'-chlorophenyl)-6,8-methylene-2-quinazolinamine (1f): the synthetic method of 1e was taken as reference, 1b was selected as raw material, a white solid was obtained with the yield of 80.1%, melting point: 161–166 °C. \(^{1}\)H NMR (400 MHz, DMSO-d\(_6\), ppm): \(\delta\) 7.95 (d, \(J = 8.5\) Hz, 1 H, ArH), 7.69 (d, \(J = 8.5\) Hz, 1 H, ArH), 7.57 (d, \(J = 8.6\) Hz, 1 H, ArH), 7.51 (d, \(J = 8.4\) Hz, 1 H, ArH), 6.36 (s, 2 H, NH\(_2\)), 2.81 (dd, \(J = 16.4, 3.1\) Hz, 1 H), 2.70 (dd, \(J = 16.4, 2.6\) Hz, 1 H), 2.65 (t, \(J = 5.5\) Hz, 1 H), 2.62–2.59 (m, 1 H), 2.31–2.28 (m, 1 H), 1.36 (s, 3 H, CH\(_3\)), 1.24 (d, \(J = 8.4\) Hz, 1 H), 0.69 (s, 3 H, CH\(_3\)). \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\), ppm): \(\delta\) 176.13, 166.92, 161.87, 161.69, 132.84, 137.94, 133.86, 131.60, 129.58, 115.29, 112.63, 49.76, 40.27, 29.74, 29.63, 25.83, 21.33. HRMS (m/z): 300.1268 calcd for C\(_{19}\)H\(_{22}\)ClN\(_3\) [M+H]^+, found: 300.1271.

7,7-Dimethyl-5,6,7,8-tetrahydro-4-(4'-chlorophenyl)-6,8-methylene-2-quinazolinamine (1g): the synthetic method of 1e was taken as reference, 1c was selected as raw material, a white solid was obtained with the yield of 35.5%, melting point: 173–175 °C. \(^{1}\)H NMR (400 MHz, DMSO-d\(_6\), ppm): \(\delta\) 0.65 (s, 3 H, CH\(_3\)), 1.22–1.24 (m, 1 H), 1.34 (s, 3 H, CH\(_3\)), 2.27–2.30 (m, 1 H), 2.58–2.60 (m, 1 H), 2.61–2.66 (m, 1 H), 2.71–2.76 (m, 1 H), 2.83–2.87 (m, 1 H), 3.80 (s, 3 H, OCH\(_3\)), 6.21 (s, 2 H, NH\(_2\)), 7.00 (dd, \(J = 2.8, 8.8\) Hz, 2 H, ArH), 7.64–7.68 (m, 2 H, ArH), \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\), ppm): \(\delta\) 175.72, 162.40, 161.82, 160.05, 131.50, 130.44,
To a solution of THF (10 mL) and compound 1g (1.02 mmol), NaH (4.06 mmol) was added in portions below 0 °C with the protection of N₂. The solution was stirred for another 30 min, then 2-bromoethylmethyl ether (1.12 mmol) was added dropwise within 10 min and the reaction mixture was stirred at 65 °C for 3 h. After cooling to room temperature, the reaction mixture was poured into ice water and extracted with EtOAc (2 × 20 mL). The organic layers were washed with brine and dried over Na₂SO₄; after filtered, the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid of pure 1i. Compounds 1j–1n were prepared by the same method as 1i.

4.1.3. Synthesis of Pinanylpyrimidinamines 1i–1n

To a solution of THF (10 mL) and compound 1g (1.02 mmol), NaH (4.06 mmol) was added in portions below 0 °C with the protection of N₂, the solution was stirred for another 3 min, then 2-bromoethylmethyl ether (1.12 mmol) was added dropwise within 10 min and the reaction mixture was stirred at 65 °C for 3 h. After cooling to room temperature, the reaction mixture was poured into ice water and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine and dried over Na₂SO₄; after filtered, the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid of pure 1i. Compounds 1j–1n were prepared by the same method as 1i.

4.1.4. Synthesis of Oxygenated Pyrimidinamines 1o–1u

To a solution of THF (10 mL) and compound 1g (1.02 mmol), NaH (4.06 mmol) was added in portions below 0 °C with the protection of N₂, the solution was stirred for another 30 min, then 2-bromoethylmethyl ether (1.12 mmol) was added dropwise within 10 min and the reaction mixture was stirred at 65 °C for 3 h. After cooling to room temperature, the reaction mixture was poured into ice water and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine and dried over Na₂SO₄; after filtered, the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid of pure 1i. Compounds 1j–1n were prepared by the same method as 1i.

4.1.5. Synthesis of Pyridinylpyrimidinamines 1v–1z

To a solution of THF (10 mL) and compound 1g (1.02 mmol), NaH (4.06 mmol) was added in portions below 0 °C with the protection of N₂, the solution was stirred for another 30 min, then 2-bromoethylmethyl ether (1.12 mmol) was added dropwise within 10 min and the reaction mixture was stirred at 65 °C for 3 h. After cooling to room temperature, the reaction mixture was poured into ice water and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine and dried over Na₂SO₄; after filtered, the solvent was removed under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid of pure 1i. Compounds 1j–1n were prepared by the same method as 1i.

4.1.6. Synthesis of N-(3-Fluorobenzyl)-4-(4-methoxyphenyl)-7,7-dimethyl-5,6,7,8-tetrahydro-6,8-methylenequinazolin-2-amine 1i: the target compound was obtained as off-white solid with the yield of 89.8%, melting point: 125–127 °C, found: 386.2232 calcd for C$_{25}$H$_{27}$FN$_{3}$O$_{3}$ [M+H]$^+$, found: 386.2227.

N-(3-Fluorobenzyl)-4-(4-methoxyphenyl)-7,7-dimethyl-5,6,7,8-tetrahydro-6,8-methylenequinazolin-2-amine 1i: the target compound was obtained as off-white solid with the yield of 78.8%, melting point: 125–127 °C. $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) δ: 7.63 (d, J = 8.8 Hz, 2 H, ArH), 6.68 (d, J = 8.8 Hz, 2 H, ArH), 6.13 (s, 2 H, NH$_2$), 2.96–2.74 (m, 2 H), 2.70–2.54 (m, 2 H), 2.40 (d, J = 8.4 Hz, 2 H), 1.34 (s, 4 H, CH$_2$CH$_3$), 1.22 (d, J = 7.5 Hz, 2 H), 1.11 (t, J = 6.0 Hz, 7 H), 0.67 (s, 3 H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) δ: 175.26, 162.36, 161.64, 148.16, 130.50, 125.35, 111.39, 110.81, 50.07, 44.09, 38.54, 30.29, 29.92, 26.09, 21.49, 12.92. HRMS-ESI: 337.2392 calcd for C$_{21}$H$_{26}$N$_3$O$_3$ [M+H]$^+$, found: 337.2390.

N-(2,4-Difluorobenzyl)-4-(4-methoxyphenyl)-7,7-dimethyl-5,6,7,8-tetrahydro-6,8-diole Methylinquinazolin-2-amine 1i: the synthetic method of 1i was taken as reference, 1g and...
2,4-difluorobenzyl bromide were selected as raw materials, a gray solid was obtained with the yield of 40.0%, melting point: 125–126 °C. 1H NMR (400 MHz, DMSO-d₆, ppm) δ: 7.68 (dd, J = 2.0, 6.8 Hz, 2 H, ArH), 7.44 (q, J = 6.8 Hz, 1 H, ArH), 7.34 (t, J = 6.6 Hz, 1 H, ArH), 7.15–7.20 (m, 1 H, ArH), 6.99–7.03 (m, 3 H, ArH, NH), 4.52–4.53 (m, 2 H), 3.81 (s, 3 H, OCH₃), 2.80–2.87 (m, 2 H), 2.67 (t, J = 5.6 Hz, 1 H), 2.51–2.59 (m, 1 H), 2.28–2.32 (m, 1 H), 1.35 (s, 3 H, CH₃), 1.23 (d, J = 9.2, 1 H), 0.68 (s, 3 H, CH₃). 13C NMR (100 MHz, DMSO-d₆, ppm) δ: 175.86, 161.59 (d, J = 256.5 Hz), 162.73, 160.54 (d, J = 258.5 Hz), 161.70, 160.39, 160.19, 131.50, 131.16, 131.07 (d, J = 3.0 Hz), 131.00, 130.52, 124.26 (dd, J = 15.0, 3.0 Hz), 113.92, 112.40, 111.50 (dd, J = 20.9, 3.6 Hz), 103.86 (t, J = 25.6 Hz), 55.65, 50.25, 38.61, 37.97 (d, J = 3.9 Hz), 29.83, 26.04, 21.52. HRMS-ESI: 422.2044 calcd for C₂₅H₂₅F₂N₂O [M+H]+, found: 422.2046.

N-benzyl-4-(4-(diethylamino)phenyl)-7,7-dimethyl-5,6,7,8-tetrahydro-6,8-methyquinazolin-2-amine (1m): the synthetic method of 1m was taken as reference, 1f and benzyl chloride were selected as raw materials, a yellow white solid was obtained with the yield of 45.5%, melting point: 57–60 °C. 1H NMR (400 MHz, DMSO-d₆, ppm) δ: 7.66 (d, J = 8.6 Hz, 2 H, ArH), 7.28–7.36 (m, 4 H, ArH), 7.18–7.22 (m, 2 H, ArH), 6.68 (d, J = 8.6 Hz, 2 H, ArH, NH), 4.47–4.58 (m, 2 H), 3.33–3.40 (m, 4 H), 2.88 (qd, J = 48.9, 16.2, 3.2 Hz, 2 H), 2.64 (t, J = 5.5 Hz, 1 H), 2.55–2.61 (m, 1 H), 2.30–2.33 (m, 1 H), 1.35 (s, 3 H, CH₃), 1.22 (d, J = 9.2 Hz, 1 H), 1.11 (t, J = 6.9 Hz, 6 H, CH₂), 0.68 (s, 3 H, CH₃). 13C NMR (100 MHz, DMSO-d₆, ppm) δ: 174.79, 159.97, 147.72, 141.20, 130.05, 128.02, 127.20, 126.31, 124.97, 110.87, 110.34, 49.76, 44.06, 40.08, 38.04, 29.90, 29.41, 25.56, 21.01, 12.44. HRMS-ESI: 427.2862 calcd for C₂₈H₂₈F₂N₂O [M+H]+, found: 427.2877.

4-(4-(Diethylamino)phenyl)-7,7-dimethyl-N-(3-pyridylmethyl)-5,6,7,8-tetrahydro-6,8-methoxy quinazolin-2-amine (1n): the synthetic method of 1n was taken as reference, 1f and 3-chloromethylpyridine were selected as raw materials, a yellow solid was obtained with the yield of 50.2%, melting point: 139–141 °C. 1H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.50 (d, J = 4.8 Hz, 1 H, ArH), 7.72 (t, J = 8.0 Hz, 1 H, ArH), 7.62 (d, J = 8.4 Hz, 2 H, ArH), 7.33 (d, J = 7.9 Hz, 1 H, ArH), 7.19–7.24 (m, 2 H, ArH), 6.66 (d, J = 8.6 Hz, 2 H, ArH, NH), 4.56–4.68 (m, 2 H), 2.89 (q, J = 51.9, 15.5 Hz, 2 H), 2.64 (t, J = 5.6 Hz, 1 H), 2.56–2.61 (m, 1 H), 2.16 (s, 1 H), 1.35 (s, 3 H, CH₃), 1.23 (d, J = 9.0 Hz, 1 H), 1.10 (t, J = 6.9 Hz, 6 H), 0.68 (s, 3 H, CH₃). 13C NMR (100 MHz, DMSO-d₆, ppm) δ: 174.92, 160.17, 159.88, 148.60, 147.74, 136.42, 130.05, 124.93, 121.68, 120.70 111.16, 110.33, 49.75, 46.15, 45.38, 38.04, 29.90, 29.41, 25.56, 21.01, 12.43. HRMS-ESI: 428.2814 calcd for C₂₅H₂₅F₂N₂O [M+H]+, found: 428.2809.

4.1.4. Synthesis of Intermediate 3-(4-Methoxybenzylidene)-1, 7, 7-Trimethylbicyclo[2.2.1]Heptan-2-One (2a)

The synthetic method of 1a was taken as a reference, camphor and 4-methoxybenzaldehyde were selected as raw materials, light yellow crystals were obtained with the yield of 56.8%, melting point: 97–98 °C. 1H NMR (400 MHz, DMSO-d₆, ppm) δ: 7.45–7.48 (m, 2 H, ArH), 7.22 (s, 1 H, ArCH=C), 6.93–6.96 (m, 2 H, ArH), 3.86 (s, 3 H, OCH₃), 3.11 (d, J = 4.4 Hz, 1 H), 2.18–2.22 (m, 1 H), 1.76–1.82 (m, 1 H), 1.53–1.65 (m, 2 H), 1.02–1.05 (m, 6 H, CH₃), 0.83 (s, 3 H, CH₃). 13C NMR (100 MHz, DMSO-d₆, ppm) δ: 9.31, 18.40, 20.55, 25.92, 30.85, 46.79, 49.23, 55.35, 57.03, 114.18, 127.35, 128.30, 131.39, 140.07, 160.11, 208.27. HRMS-ESI: 271.1698 calcd for C₁₅H₂₃O₂ [M+H]+, found: 271.1700.

4.1.5. Synthesis of Camphor Pyrimidine Amines 2b–2f

4-(4-Methoxyphenyl)-8,9,9-trimethyl-5,6,7,8-tetrahydro-5,8-methyquinazolin-2-amine (2b): the synthetic method of 1e was taken as a reference, 2a was selected as raw material, a white solid was obtained with the yield of 30.8%, melting point: 183–184 °C. 1H NMR (400 MHz, DMSO-d₆, ppm) δ: 0.56 (s, 3 H, CH₃), 0.96 (s, 3 H, CH₃), 1.15 (s, 3 H, CH₃), 1.18–1.28 (m, 2 H), 1.84–1.90 (m, 1 H), 2.14–2.17 (m, 1 H), 3.05 (d, J = 3.6 Hz, 1 H), 3.81 (s, 3 H, OCH₃), 6.26 (s, 2 H, NH₂), 7.04 (d, J = 8.8 Hz, 2 H, ArH), 7.75 (d, J = 8.8 Hz, 2 H, ArH). 13C NMR (100 MHz, DMSO-d₆, ppm) δ: 10.71, 19.22, 20.15, 26.28, 32.08, 49.83, 54.01, 55.49,
Molecules 2022, 27, 8104

J. H, NH), 7.05–7.07 (m, 2 H, ArH), 7.80 (d, 3 H, ArH, NH), 7.15–7.20 (m, 1 H, ArH), 7.44–7.50 (m, 2 H, ArH), 7.74–7.77 (m, 2 H, ArH).

N-(2-Methoxyethyl)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-5,8-methoxyquinazolin-2-amine (2c): the synthetic method of 2i was as taken as a reference, 2a and benzyl chloride were selected as raw materials, a gray solid was obtained with the yield of 39.1%, melting point: 93–96 °C. 1H NMR (400 MHz, DMSO-d6, ppm): δ: 0.54 (s, 3 H, CH3), 0.96 (s, 3 H, CH3), 1.16–1.27 (m, 5 H, CH3, CH2), 1.87–1.90 (m, 1 H), 2.14–2.18 (m, 1 H), 3.07–3.08 (m, 1 H), 3.81 (s, 3 H, OCH3), 4.50–4.54 (m, 2 H), 7.02–7.05 (m, 2 H, ArH, NH), 7.18–7.19 (m, 1 H, ArH), 7.21–7.31 (m, 2 H, ArH), 7.38–7.39 (m, 3 H, ArH), 7.75–7.79 (m, 2 H, ArH). 13C NMR (100 MHz, DMSO-d6, ppm): δ: 10.64, 19.19, 19.20, 26.20, 32.06, 44.24, 49.85, 53.96, 55.45, 55.70, 113.44, 115.19 (d, J = 21.13 Hz), 123.04, 124.00, 128.86, 130.93, 137.72–137.75 (d, J = 3.0 Hz), 160.25, 160.44, 161.14–162.65 (d, J = 151.9 Hz), 181.20. HRMS-ESI: 418.2295 calcd for C26H20FN3O [M+H]+, found: 418.2296.

N-(3-Fluorobenzyl)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-5,8-methoxyquinazoline-2-Amine (2e): the synthetic method of 2i was as taken as a reference, 2a and benzyl chloride were selected as raw materials, a gray solid was obtained with the yield of 44.2%, melting point: 114–115 °C. 1H NMR (400 MHz, DMSO-d6, ppm): δ: 0.55 (s, 3 H, CH3), 0.96 (s, 3 H, CH3), 1.24–1.25 (m, 5 H, CH3, CH2), 1.85–1.90 (m, 1 H), 2.14–2.18 (m, 1 H), 3.07–3.08 (m, 1 H), 3.81 (s, 3 H, OCH3), 4.50–4.54 (m, 2 H), 7.02–7.05 (m, 2 H, ArH, NH), 7.18–7.19 (m, 1 H, ArH), 7.21–7.31 (m, 2 H, ArH), 7.38–7.39 (m, 3 H, ArH), 7.75–7.79 (m, 2 H, ArH). 13C NMR (100 MHz, DMSO-d6, ppm): δ: 10.66, 19.16, 20.20, 49.86, 53.96, 55.70, 114.33, 122.95, 126.84, 127.96, 128.51, 129.86, 141.60, 160.63 (d, J = 39.24 Hz). HRMS-ESI: 400.2389 calcd for C26H20N3O [M+H]+, found: 400.2395.

N-(2,4-Difluorobenzyl)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-5,8-Methylquinazolin-2-amine (2f): the synthetic method of 2i was as taken as a reference, 2a and 2,4-difluorobenzyl bromide were selected as raw materials, a gray solid was obtained with the yield of 43.3%, melting point: 59–61 °C. 1H NMR (400 MHz, DMSO-d6, ppm): δ: 0.54 (s, 3 H, CH3), 0.96 (s, 3 H, CH3), 1.16–1.26 (m, 5 H, CH3, CH2), 1.87–1.88 (m, 1 H), 2.16–2.17 (m, 1 H), 3.07–3.08 (m, 1 H), 3.81 (s, 3 H, OCH3), 4.52–4.55 (m, 2 H), 7.02–7.05 (m, 3 H, ArH, NH), 7.15–7.20 (m, 1 H, ArH), 7.44–7.50 (m, 2 H, ArH), 7.74–7.77 (m, 2 H, ArH). 13C NMR (100 MHz, DMSO-d6, ppm): δ: 10.02, 19.05, 19.99, 25.91, 31.88, 39.07 (d, J = 4.0 Hz), 49.97, 54.06, 55.35, 55.74, 103.47 (t, J = 25.0 Hz), 110.88 (dd, J = 4.0, 17.1 Hz), 113.83, 122.85 (d, J = 4.0 Hz), 124.29, 129.83, 131.07 (dd, J = 6.0, 7.0 Hz), 159.90, 160.55, 160.66, 161.09 (d, J = 259.8 Hz), 162.04 (d, J = 247.5 Hz), 181.73. HRMS-ESI: 436.2200 calcd for C26H25F2N3O [M+H]+, found: 436.2203.

4.2. Antibacterial Activities Assay

K. pneumoniae, S. pneumoniae, P. aeruginosa, S. aureus, Escherichia coli (E. coli), methicillin-resistant Staphylococcus aureus (MRSA), Bacillus cereus (B. cereus), and Candida albicans (C. albicans) were selected as test bacterial and fungi strains, which were provided by the Laboratory of the Third People’s Hospital of Yancheng City. Ketoconazole and amikacin were selected as the control drugs for inhibiting fungi and bacteria; the antibacterial activities of the synthesized pinanyl pyrimidine amine derivatives and camphorphyll pyrimidine amine derivatives were evaluated by the micro-dilution method. A total of 100 µL of.
purified water was added into 96-well plate from the 2nd to the 12th well firstly; then, the test compounds 1e∼1n and 2b∼2f solutions, the positive control drug ketoconazole and amikacin solutions at the concentration of 100 µg/mL with methanol were added to the first well in the volume of 100 µL. The test compounds and positive control drugs were diluted twice on 96-well plates, and a series of concentration gradients (1024–1.0 µg/mL) were prepared from the first to the 12th well. Pure DMSO as a reference, 100 µL of pre-prepared bacterial suspension, was added to achieve the required final concentration in a volume of 200 µL, and the solutions were mixed well. Finally, the 96-well plate was kept under 5% CO₂ at 37 °C, the bacteria were cultured for 24 h, and the fungi were cultured for 48 h. The lowest concentration without turbidity was taken as the minimum inhibitory concentration of the sample against the test bacteria. Each sample was repeated three times for each test bacteria, the experimental data were recorded, and the results were averaged.

4.3. Anti-Inflammatory Activities Assay

The MTT method was used to detect the cell survival rate. Cells were evenly seeded into 96-well tissue culture plate at a density of 5 × 10⁴ cells per well and incubated for 12 h. Attachment of cells was controlled under the microscope. 10 mmol/L test compounds solution was diluted into 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.125 µM, 1.56 µM, 0.8 µM with serum-free medium. The cell supernatant was discarded, and the drug-containing solution was added into the 96-well plate as the required final concentration in a volume of 200 µL, the same volume of medium was added to both the positive control group and the negative control group, and the incubation was continued for 12 h. The cell supernatant was discarded, 5 µg/mL LPS serum-free medium was added, after an incubation period of 24 h, the MTT reagent was added (20 µL of a 0.5 mg/mL solution) to each well. Plates were further incubated for 1 h and then the assay was terminated by removing supernatant. Viability of cells was determined spectrophotometrically by measuring absorbance at 492 nm and background correction at 710 nm using a BMG POLARstar microplate reader; the data obtained were used to calculate the cell viability using SPSS software.

4.4. Molecular Docking

The complex crystal of tumor necrosis factor (TNF) with trifluorotolyl indole (PDB ID: 2AZ5) was chosen as the template to elucidate the binding mode of 1f and TNF. Protein structure was downloaded from the PDB database (http://www.rcsb.org (accessed on 12th August 2022)) and saved in PDB file format. Pymol and Auto Dock software were used for molecular docking. The TNF kinase was defined as a receptor after the preparation of adding hydrogen atoms and deleting waters, adding charge and force fields, and the protein structure was optimized. The active cavity was defined as a grid box module: a grid box centered at (19.112, 7.014, 9.290) with a size of 18 × 18 × 18, the spaces of grid points were 1 Å. The images of binding mode were prepared using Auto Dock Vina software.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/molecules27228104/s1, The ¹H-NMR, ¹³C-NMR and HRMS spectra of the target compounds.

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