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Synthesis, Characterization, and Anticancer Activities Evaluation of Compounds Derived from 3,4-Dihydropyrimidin-2(1H)-one

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Abstract: 3,4-dihydropyrimidin-2(1H)-one compounds (DHPMs) possess extensive biological activities and are mainly prepared via Biginelli reaction and N-alkylation. In the present study, selective alkylation of N¹ was investigated by using tetrabutylammonium hydroxide. In vitro cytotoxicity study on all synthesized compounds demonstrated that introduction of the aryl chain in the R³ as well as the low electron-donating group in the R¹ of DHPMs contributed to the anti-proliferative potency. A larger value of the partition coefficient (Log P) and suitable polar surface area (PSA) values were both found to be important in order to maintain the antitumor activity. The results from in vivo study indicated the great potential of compound **3d** to serve as a lead compound for novel anti-tumor drugs to treat glioma. Pharmacophore study regarding the structure-activity relations of DHPMs were also conducted. Our results here could provide a guide for the design of novel bioactive 3,4-dihydropyrimidin-2(1H)-one compounds.

Keywords: 3,4-dihydropyrimidin-2(1H)-ones; N¹-alkylation; structure-activity relationship; in vivo experiments; anticancer activities

1. Introduction

Noncommunicable diseases (NCDs) are a major threat to global health, causing a significant amount of death every year. Second only to cardiovascular disease, cancer is becoming a global burden which lead to an estimation of 8.7 million deaths in 2015 [1]. Moreover, cancer is expected to rank as the leading cause of death and the single most significant barrier for the increase of life expectancy in every country worldwide [2]. Contrary to common misperception, cancer is a major health challenge not only in high-income countries but also in low- and middle-income countries (LMICs), where the number of cancer occurrence is rapidly growing [3]. Unfortunately, almost all anticancer drugs are associated with serious side effects, making the search for novel chemical agents that are cytotoxic to cancer cells with less side effects an urgent need.

The interests of using DHPMs in medicinal chemistry is dramatically growing (Figure 1) due to their therapeutic and pharmacological properties [4,5]. It has been reported that DHPMs can possess various biological activities including antiviral [6,7], antitumor [8], anti-inflammatory [9], antidiabetic [10], antibacterial [11], antifungal [12], anti-epileptic [13], antimalarial [14], and

antileishmanial [15] and others upon suitable structural modification. The highly functionalized DHPM 10, termed MAL3-101, had been observed with effect of inducing breast cancer cell apoptosis [16]. More recently, DHPMs have emerged as the integral backbone of several calcium channel blockers [17,18], antioxidant molecules [19], and radical scavengers [20–22]. In addition, Barbosa et al. reported the synthesis and biological evaluation of a series of DHPMs functionalized with selenocyanides as potential multi-targeted therapeutics against Alzheimer's disease (AD) [23].

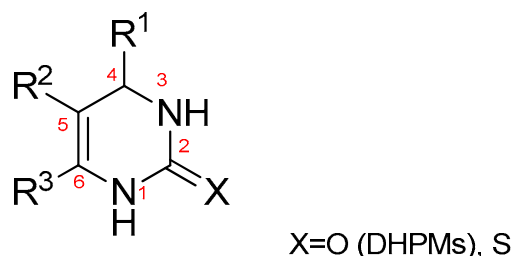


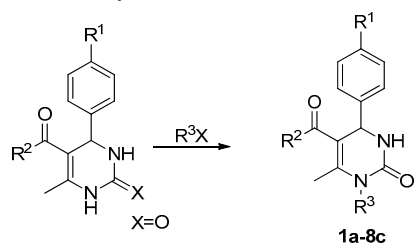
Figure 1. The structure of a 3,4-dihydropyrimidin-2(1H)-one compound (DHPM) and its thione derivatives.

In DHPMs, R^1 is usually an aryl group, such as phenyl or pyridyl, while R^2 is an ester or amide group and R^3 is an alkyl group, such as methyl or ethyl. General method for the synthesis of DHPMs starts with firstly obtaining the basic scaffold via Biginelli reaction followed by N alkylation. Mohammadi and Behbahani reviewed the synthesis of DHPMs and improved procedures for the preparation of DHPMs under solvent-free conditions or with the presence of solvent [24]. Dallinger and Kappe introduced a selective N^1 -alkylation method of DHPMs using Mitsunobu reaction [25]. However, the yield of Mitsunobu reaction was low, and the reagents were relatively expensive, making it not suitable for practical synthesis. Singh et al. provided another N-alkylation method catalyzed by inorganic strong base [26]. In the present report, not only did we find highly selective N^1 -alkylation of DHPMs in the presence of tetrabutylammonium hydroxide, but also we investigated the biological importance of the newly synthesized molecules both in vitro and in vivo.

2. Results and Discussion

2.1. Chemistry

The various non-alkylated DHPM moieties used in this report were synthesized through one pot Biginelli condensation reaction according to the reported method [27]. The effects of different choices of bases on the reaction, including sodium hydride (NaH), lithium hydroxide ($\text{LiOH}\cdot\text{H}_2\text{O}$), potassium carbonate (K_2CO_3) and some pKa similar organic base, were carried out to perform N^1 and N^3 dialkylation of DHPMs. While using a strong base, such as $\text{LiOH}\cdot\text{H}_2\text{O}$, NaH and potassium tert-butoxide (KTB), the reactions were proceeding very fast. However the dialkyl product was formed and detected even from the beginning of the reaction. In some reactions the yields of dialkylation were even higher than that of N^1 -alkylation. N-alkylation cannot be achieved when a weak base is used, such as K_2CO_3 , triethylamine (Et_3N), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and tetramethylguanidine (TMG). Interestingly, when tetrabutylammonium hydroxide was selected as the base, the yield was similar to that of cesium carbonate (Cs_2CO_3), while no dialkyl products was found (Table S1 in Supplementary Materials). A possible explanation for this phenomenon is that N^3 -alkylation of DHPMs had a large steric effect, so the steric-hindered base like tetrabutylammonium hydroxide would favor the mono-alkylation reaction. The yields of the DHPMs were reported in Table 1.

Table 1. Preparation of N¹-alkylated DHPMs with different halohydrocarbons.

DHPMs (1a–8e)	R ¹	R ²	R ³	N ¹ -Alkylation Yield (%) ^a	Log P (Partition Coefficient)
1a	Br	OEt		8.0	3.33
1b	Br	OEt		52.2	3.65
1c	Br	OEt		35.1	5.27
1d	Br	OEt		32.3	5.45
1e	Br	OEt		43.6	4.59
1f	Br	OEt		20.0	3.91
1g	Br	OEt		69.7	4.44
1h	Br	OEt		55.8	5.50
1i	Br	OEt		27.2	2.71
1j	Br	OEt		9.4	4.41
2a	OCH ₃	OEt		21.7	2.47
3a	Ph	OEt		33.5	4.32
3c	Ph	OEt		25.8	6.26
3d	Ph	OEt		13.3	6.43
3e	Ph	OEt		24.8	5.58
3g	Ph	OEt		19.9	5.43
3h	Ph	OEt		50.5	6.49
4a		OEt		20.3	1.70
5a	NO ₂	OEt		17.8	2.29
6a		OEt		21.7	1.07
7c	Br	NH ₂		14.6	3.92
7d	Br	NH ₂		58.2	4.09
7e	Br	NH ₂		73.8	3.24
7f	Br	NH ₂		21.7	2.55
8a	Br			25.2	4.36
8d	Br			16.7	6.47
8e	Br			28.7	5.62

^a The yields relate to the use of tetrabutylammonium hydroxide as a base.

2.2. Structure–Activity Relationship (SAR) Studies

2.2.1. Cytotoxicity Activities with SAR

Cytotoxic activity of the DHPMs are strongly dependent on their structure. Yadlapalli et al. screened 21 compounds in vitro anticancer screening against MCF-7 human breast cancer cells, and found the maximum GI₅₀ was 33.2 μ M. The results indicated that presence of thio-urea functional group in DHPMs enhanced the in vitro anticancer activity [28]. In vitro cytotoxicity of all synthesized compounds containing X = O were assayed on four cell lines, namely U87, U251 human malignant glioma cell lines, HeLa human cervical cancer cell line and A549 human lung cancer cell line. Cancer cell lines were exposed to drug solution at concentration of 10 μ M for 72 h, and results were summarized in Table 2. On the whole, no certain trend in inhibitory activity was observed in HeLa and A549 cell lines, indicating that these compounds were selective toward certain tumor types. Some of the tested compounds showed effective cytotoxicity in U87 and U251 cell lines.

Table 2. Survival rate of all compounds against U87, U251, HeLa, A549 cell lines at 72 h.

DHPMs (1a–8e)	Survival Rate of Four Different Cells (%)			
	U87 ^a	U251 ^a	HeLa ^a	A549 ^a
1a	87.34 ± 1.24	67.14 ± 4.61	69.81 ± 2.04	76.80 ± 1.76
1b	84.93 ± 0.72	78.25 ± 7.88	71.44 ± 0.67	59.48 ± 2.63
1c	97.83 ± 4.32	85.20 ± 1.16	71.96 ± 0.96	62.86 ± 0.97
1d	50.83 ± 0.25	51.07 ± 2.56	53.71 ± 1.08	73.26 ± 2.69
1e	89.41 ± 1.47	71.65 ± 4.64	51.05 ± 1.51	54.10 ± 1.44
1f	95.09 ± 12.76	74.75 ± 0.79	62.48 ± 1.41	61.86 ± 1.24
1g	84.04 ± 3.08	72.74 ± 9.08	51.05 ± 1.42	50.68 ± 1.22
1h	60.05 ± 1.55	56.40 ± 4.21	51.05 ± 0.63	59.28 ± 2.97
1i	94.46 ± 5.33	80.10 ± 7.98	62.48 ± 1.55	69.93 ± 0.34
1j	60.69 ± 1.89	63.27 ± 3.40	65.72 ± 0.39	66.60 ± 1.35
2a	85.54 ± 3.15	72.22 ± 9.37	67.90 ± 1.62	78.92 ± 1.62
3a	65.62 ± 3.77	48.23 ± 3.97	59.56 ± 3.87	58.92 ± 2.41
3c	100.69 ± 2.75	85.60 ± 4.04	73.81 ± 5.73	51.92 ± 3.35
3d	51.98 ± 1.64	49.49 ± 4.73	63.57 ± 2.74	64.94 ± 4.16
3e	70.94 ± 5.16	71.28 ± 3.76	57.38 ± 0.40	58.35 ± 4.25
3g	54.27 ± 0.88	51.07 ± 4.32	44.85 ± 1.08	43.70 ± 2.38
3h	60.37 ± 2.71	51.21 ± 0.58	46.02 ± 0.85	55.16 ± 2.43
4a	71.26 ± 2.08	77.28 ± 5.38	62.32 ± 2.26	81.36 ± 1.63
5a	75.53 ± 4.89	76.22 ± 5.48	47.96 ± 5.00	52.22 ± 0.19
6a	81.20 ± 4.44	82.03 ± 6.38	68.80 ± 5.39	68.04 ± 3.02
7c	81.02 ± 2.98	97.29 ± 3.47	74.26 ± 7.29	91.51 ± 1.21
7d	78.91 ± 4.81	84.79 ± 5.14	65.73 ± 3.44	74.22 ± 1.96
7e	76.22 ± 3.21	83.07 ± 2.22	66.91 ± 2.78	68.17 ± 7.73
7f	77.11 ± 6.22	81.09 ± 4.01	78.32 ± 2.47	71.78 ± 4.25
8a	81.22 ± 4.56	89.27 ± 3.64	66.91 ± 4.92	72.03 ± 5.56
8d	68.21 ± 2.16	71.53 ± 5.85	78.32 ± 2.28	71.29 ± 3.84
8e	81.86 ± 0.71	73.90 ± 2.22	79.80 ± 3.79	85.65 ± 2.39

^a U87, U251, HeLa, A549 cell lines were exposed at concentrations 10 μ M at 72 h.

Table 2 showed that compound **1a** resulted in cell viability of 87.34 ± 1.24 and 67.14 ± 4.61 in U87 and U251 cell lines. For SAR studies, we maintained the R¹ as Br, R² as ethyl acetate and explored R³ first with a series of halohydrocarbons. Compounds **1b**, **1f**, **1g**, and **1i**, with an alkyl side chain replacing the methyl 4-bromobutanoate of **1a**, were found to have similar activity as **1a**. Compound **1h**, with an 1-bromohexane in R³, displayed decent activity, indicating that the length of the alkyl side chain in the R³ would affect the potency. Compound **1d**, with a 4-bromobenzyl group in the R³, also demonstrated strong cytotoxicity, suggesting that the R³ may tolerate variations to some degree. We then explored the R² with the goal to compare the ester group and the amide group

on cell viability. Compared with **1e**, compound **7e** and **8e** differ in R², had no significant change in activity. Compounds **7c**, **7d**, **7f**, **8a**, and **8d** were also tested, and not satisfactory performances were observed, suggesting that the amide group may not be compatible in that position. For R¹ of DHPMs, we proposed that the capability of electron-donating group may affect the cell viability and the SAR of the R¹ group was explored. Compound **3a**, with a low electron-donating 4-biphenyl group instead of 4-phenylmorpholine group or 4-methoxyphenyl, was found to have better potency than **4a** and **2a**. Compound **1a**, with the 4-bromo phenyl group in the R¹, had significant activity in U251 cell lines, suggesting that a low electron-withdrawing group in the R¹ may contribute to augment the activity. Compound **5a** and **6a**, with a 4-nitrophenyl group and 4-pyridinylphenyl group in the R¹, were also not active in the cell study, suggesting that high electron-withdrawing substituent in the R¹ may not be tolerated. Studies on different electron-donating groups in the R¹ had shown that a maximum cytotoxic activity may be achieved for low electron-donating ability or low electron-withdrawing ability. Based on the above studies, the cell viability of compounds **3c**, **3d**, **3e**, **3g**, and **3h** were tested and compound **3d** and **3g**, with 4-biphenyl low electron-donating group in the R¹ and alkyl chain or 4-bromobenzyl in the R³, were found to have good activity.

2.2.2. Half Maximal Inhibitory Concentration (IC₅₀) Study of Compounds **1d**, **1h**, **3d** and **3g**

The values of 50% inhibitory concentration (concentration of drug yielding a 50% cell viability decrease, IC₅₀) measured for the distinct compounds investigated were comprised in Table 3, which confirmed that the active compounds can inhibit tumor cell growth. In tumor cells, inhibition of heat shock protein 90 (HSP90) results in the degradation of oncoproteins which is crucial to malignant progression [29]. Preclinical data suggest that synthetic HSP90 inhibitors such as BIIB021 may be active against tumors with acquired multidrug resistance [30]. All of the tested compounds had IC₅₀ in the micromolar range against U87 and U251 cell lines. These results evidenced that although compounds **1d** (9.72 ± 0.29 μM in U87 cell line, 13.91 ± 0.86 μM in U251 cell line), **1h** (9.3 ± 0.81 μM in U87 cell line, 14.01 ± 0.76 μM in U251 cell line), **3d** (12.02 ± 0.5 μM in U87 cell line, 6.36 ± 0.73 μM in U251 cell line), and **3g** (9.52 ± 0.81 μM in U87 cell line, 7.32 ± 0.86 μM in U251 cell line) did not display stronger cytotoxic activity on the U87 and U251 cell lines compared to positive control, they can still possess certain cytotoxic activity in micromolar range. In the present study, it was verified that the alkyl chain or aryl chain in the R³, and low electron-donating ability or low electron-withdrawing ability in the R¹ displayed obvious effect. As expected, all of the four compounds especially **3d** displaying high Log P (5.01) values and suitable PSA (58.64).

Table 3. Selected compounds studied for half maximal inhibitory concentration (IC₅₀) in U87 and U251 cell lines.

Compound	IC ₅₀ (μM)	
	U87	U251
1d	9.72 ± 0.29	13.91 ± 0.86
1h	9.30 ± 0.81	14.01 ± 0.76
3d	12.02 ± 0.5	6.36 ± 0.73
3g	9.52 ± 0.81	7.32 ± 0.86
BIIB021 ^a	2.07 ± 0.13	0.3 ± 0.043

^a BIIB021 as a positive control.

2.2.3. Effects on Xenograft Model of Compounds **3d** and **3g**

We further investigated the efficacy of compounds **3d** and **3g** in xenograft tumor model on the basis of their good membrane permeability and IC₅₀ value. In brief, GL261 mouse malignant glioma cells were inoculated subcutaneously in right flank regions. Mice were treated with either: control, positive control (30 mg/kg), or compound **3d** (100 mg/kg), or compound **3g** (100 mg/kg). The results of representative studies were summarized in Table 4, and examples were shown in Figure 2.

These data indicated that in xenograft tumor model, compound **3d** or **3g** were able to significantly inhibit tumor growth, with inhibition ratios (IR) of 54.9% and 34.3%, respectively. The compound **3d** produced a similar antitumor activity compared with BIIB021 (IR 59.7%). This study had shown that the aryl chain in the R³, and 4-biphenyl low electron-donating group in the R¹ had a moderate growth inhibitory effect on xenograft tumor model. The compound **3d** also had suitable Log P and PSA values. Although the compounds were less active when compared to the positive control, compound **3d** had the potential to serve as lead compound and be further optimized to improve activity.

Table 4. Inhibitory effects of compounds **3d** and **3g** on the xenograft tumor growth of GL261 in C57 mice.

Groups	Dosage (mg/kg)	Number Initial/End	Body Weight (g)		Tumor Weight (g)	IR (%)
			Initial	End		
Control	0	8/8	20.2 ± 0.4	22.0 ± 1.2	0.83 ± 0.24	
3d	100	8/8	19.6 ± 0.6	21.5 ± 1.5	0.37 ± 0.19	54.9
3g	100	8/8	19.8 ± 0.5	21.8 ± 0.8	0.54 ± 0.25	34.3
BIIB021	30	8/8	20.1 ± 0.7	21.3 ± 1.0	0.33 ± 0.17	59.7

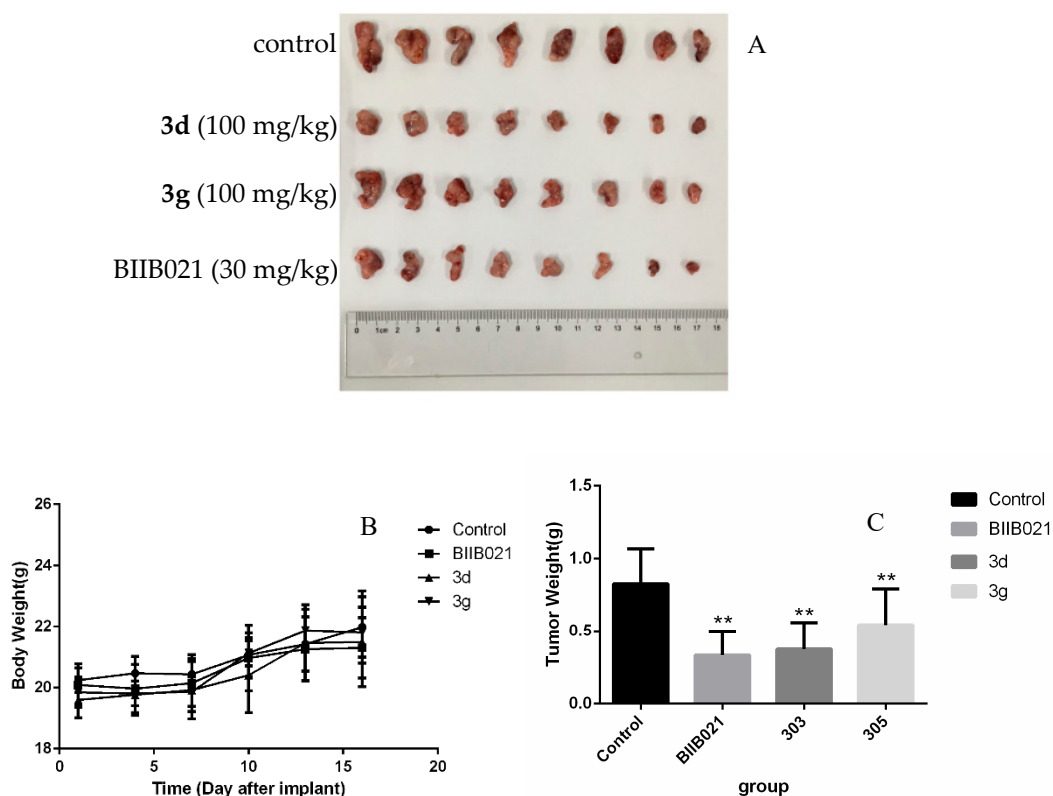


Figure 2. Effects of compounds **3d** and **3g** on the antitumor activity of GL261 xenograft tumors in C57 mice. (A): The tumors were stripped and photographed the experimental results after two weeks. (B): The body weight of the four groups of mice was changed for two weeks. (C): The tumor of the four groups of mice was weighed after two weeks. ** $p < 0.01$, compared with control group.

2.3. Pharmacophore Requirements

According to previous studies, thirteen substituted DHPMs with good anticancer activities were selected to generate pharmacophores and guide the design of novel DHPMs derivatives (Table S3 in Supplementary Materials). Galahad module of Sybyl-X 2.0 (Certara, Princeton, NJ, USA) was used to generate pharmacophore using population size of 20 and maximum generations as 10. Finally, 13 models were generated (Figure 3). The best pharmacophore model was chosen with low energy

and high value of steric and hydrogen bonding. Eight pharmacophore features, namely three acceptor atoms (AA-4, 5, 6), two donor atoms (DA-3, 8) and three hydrophobic center (HY-1, 2, 7) were identified. The two acceptor atoms were at the R² position and X position. Hydrophobic center is the DHPMs parent ring, R¹ position and R³ position.

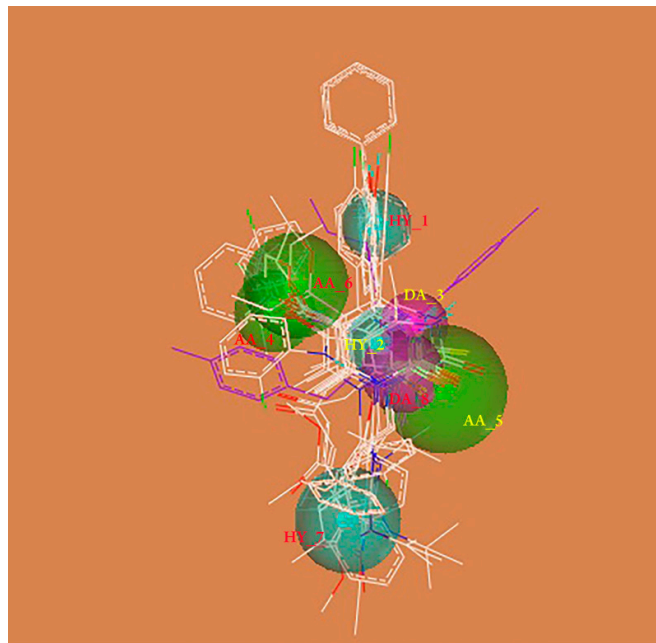


Figure 3. Pharmacophore Requirements.

3. Materials and Methods

3.1. General Information

All reagents were obtained from commercial suppliers and were used without any further purification unless otherwise stated. Column chromatography was performed using an SRL silica gel (200–300 mesh). Thin layer chromatography was performed using Merck silica gel GF₂₅₄ plates. Melting points were measured on an XT3A micro-melting point apparatus and are uncorrected (Beijing Keyi Company, Beijing, China). ¹H NMR and ¹³C NMR spectra were recorded with a Bruker AV-400 instrument or a Bruker AV-300 (Bruker, Ettlingen, Germany). Chemical shifts were reported as δ values (ppm) from internal reference tetramethylsilane (TMS). All coupling constants were reported in hertz (Hz), and proton multiplicities were labeled as br (broad), s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). HR-MS were performed on a Waters Vion IMS Q-tof (Waters, MA, USA).

3.2. Synthesis and the General Procedure for N1-alkylation

Tetrabutylammonium hydrogen sulfate (TBAS 0.1 eq) was added to a solution of ethyl 4-(4-bromophenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (200.0 mg, 0.59 mmol) and tetrabutylammonium hydroxide (0.26 mL, 1.0 mmol) in anhydrous DMF (4.0 mL). The mixture was stirred at 45 °C for 1.5 h under anhydrous condition. Then 2-chlorobenzyl chloride (0.13 mL, 1.06 mmol), potassium iodide (0.1 eq) were added to the reaction mixture slowly and stirred at 45 °C for 16 h. Saturated NaCl solution (25 mL) was added and then the reaction mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was washed with water (2 × 50 mL), followed by brine solution (1 × 50 mL). The organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure. Column chromatographic purification using a methanol in dichloromethane gradient (dichloromethane: methanol = 60:1–5:1) yielded compounds **1a–8e**.

3.2.1. N¹-Substituted Ethyl4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **1a–1j**

Ethyl 4-(4-bromophenyl)-1-(4-methoxy-4-oxobutyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1a). White solid, m.p.: 92.4–95.0 °C, yield: 8.0%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 7.14 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 5.53 (s, 1H, NH), 5.35 (d, J = 2.9 Hz, 1H, Ar-CH), 4.12 (q, J = 7.1 Hz, 2H, OCH₂), 3.98–3.88 (m, 1H, NCH₂), 3.71 (d, J = 5.3 Hz, 3H, OCH₃), 3.66 (dd, J = 9.5, 5.1 Hz, 1H, NCH₂), 2.57 (s, 3H, =CCH₃), 2.35–2.24 (m, 2H, COCH₂), 2.00–1.77 (m, 2H, CH₂), 1.20 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.35 (C=O), 165.98 (C=O), 153.11 (C=O), 150.50 (Ar-C), 143.74 (C-N), 131.86 (2 × Ar-C), 128.82 (2 × Ar-C), 120.95 (Br-Ar-C), 103.30 (C=C), 60.24 (CH₂), 52.28 (CH), 51.88 (CH₃), 41.43 (CH₂), 30.67 (CH₂), 24.98 (CH₂), 16.08 (CH₃), 14.56 (CH₃). HRMS (ESI): *m/z* calcd for C₁₉H₂₃BrN₂O₅ [M + H]⁺ 439.0869, found 439.0868.

Ethyl 4-(4-bromophenyl)-1-(3-ethoxy-3-oxopropyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1b). White solid, m.p.: 126.8–127.0 °C, yield: 52.2%, Rf value: 0.6 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.48–7.42 (m, 2H, 2 × Ar-H), 7.25 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 5.37 (s, 1H, Ar-CH), 4.19–4.05 (m, 4H, 2 × OCH₂), 3.83–3.73 (m, 1H, NCH₂), 3.29–3.20 (m, 1H, NCH₂), 2.70 (d, J = 16.4 Hz, 1H, CH₂), 2.49–2.38 (m, 1H, CH₂), 2.33 (s, 3H, =CCH₃), 1.25 (q, J = 7.3 Hz, 6H, 2 × CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 171.71 (C=O), 165.37 (C=O), 152.05 (C=O), 148.21 (Ar-C), 142.66 (C-N), 132.08 (2 × Ar-C), 129.58 (2 × Ar-C), 121.40 (Br-Ar-C), 100.21 (C=C), 60.57 (2 × CH₂), 59.97 (CH₂, CH), 33.09 (CH₂), 18.10 (CH₃), 14.54 (2 × CH₃). HRMS (ESI): *m/z* calcd for C₁₉H₂₃BrN₂O₅ [M + H]⁺ 439.0869, found 439.0870.

Ethyl 4-(4-bromophenyl)-1-(2-chlorobenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1c). White solid, m.p.: 125.6–127.8 °C, yield: 35.1%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, J = 8.3 Hz, 2H, 2 × Ar-H), 7.36 (d, J = 7.7 Hz, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.16 (d, J = 8.2 Hz, 3H, 3 × Ar-H), 6.90 (d, J = 7.4 Hz, 1H, Ar-H), 6.48 (s, 1H, NH), 5.44 (s, 1H, Ar-CH), 5.06 (d, J = 7.8 Hz, 2H, NCH₂), 4.11 (q, J = 7.1 Hz, 2H, OCH₂), 2.38 (s, 3H, =CCH₃), 1.18 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 165.33 (C=O), 152.34 (C=O), 148.46 (2 × Ar-C), 141.90 (C-N), 134.59 (Ar-C-Cl), 132.88 (2 × Ar-C), 132.15 (2 × Ar-C), 130.04 (Ar-C), 129.59 (Ar-C), 129.34 (Ar-C), 128.00 (Ar-C), 121.56 (Br-Ar-C), 100.15 (C=C), 60.01 (CH₂), 59.16 (CH₂, CH), 18.21 (CH₃), 14.53 (CH₃). HRMS (ESI): *m/z* calcd for C₂₁H₂₀BrClN₂O₃ [M + H]⁺ 463.0424, found 463.0439.

Ethyl 1-(4-bromobenzyl)-4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1d). White solid, m.p.: 190.4–191.9 °C, yield: 32.3%, Rf value: 0.7 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, J = 8.3 Hz, 4H, 4 × Ar-H), 7.13 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 7.01 (d, J = 8.3 Hz, 2H, 2 × Ar-H), 5.72 (s, 1H, NH), 5.42 (d, J = 2.6 Hz, 1H, Ar-CH), 5.10 (d, J = 16.7 Hz, 1H, NCH₂), 4.87 (d, J = 16.3 Hz, 1H, NCH₂), 4.12 (q, J = 7.1 Hz, 2H, OCH₂), 2.44 (s, 3H, =CCH₃), 1.19 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 165.90 (C=O), 153.29 (C=O), 150.16 (Ar-C), 143.76 (C-N), 138.63 (Ar-C), 131.90 (4 × Ar-C), 128.98 (4 × Ar-C), 121.06 (Br-Ar-C), 120.43 (Br-Ar-C), 103.75 (C=C), 60.33 (OCH₂), 52.53 (CH-NH), 45.05 (N-CH₂), 16.54 (CH₃), 14.53 (CH₃). HRMS (ESI): *m/z* calcd for C₂₁H₂₀Br₂N₂O₃ [M + H]⁺ 508.9898, found 508.9901.

Ethyl 4-(4-bromophenyl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1e). White solid, m.p.: 136.8–139.7 °C, yield: 43.6%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.43–7.37 (m, 2H, 2 × Ar-H), 7.11 (d, J = 8.4 Hz, 2H, 2 × Ar-H), 7.05 (d, J = 8.6 Hz, 2H, 2 × Ar-H), 6.86–6.80 (m, 2H, 2 × Ar-H), 5.75 (s, 1H, NH), 5.39 (d, J = 2.9 Hz, 1H, Ar-CH), 5.16 (d, J = 15.9 Hz, 1H, NCH₂), 4.78 (d, J = 15.8 Hz, 1H, NCH₂), 4.09 (q, J = 7.1 Hz, 2H, OCH₂), 3.81 (s, 3H, OCH₃), 2.48 (s, 3H, =CCH₃), 1.18 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 165.95 (C=O), 158.70 (C=O), 153.42 (Ar-C), 150.43 (Ar-C), 143.86 (C-N), 131.83 (2 × Ar-C), 130.91 (2 × Ar-C), 128.99 (2 × Ar-C), 128.15 (Ar-C), 120.98 (Br-Ar-C), 114.41 (2 × Ar-C), 103.66 (C=C), 60.27 (CH₂), 55.68

(CH₃), 55.44 (CH), 52.49 (CH₂), 16.55 (CH₃), 14.53 (CH₃). HRMS (ESI): *m/z* calcd for C₂₂H₂₃BrN₂O₄ [M + H]⁺ 459.0919, found 459.0917.

Ethyl 4-(4-bromophenyl)-6-methyl-2-oxo-1-propyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1f). White solid, m.p.: 114.7–115.3 °C, yield: 20.0%, Rf value: 0.3 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.45 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.15 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 5.59 (s, 1H, NH), 5.36 (d, *J* = 2.8 Hz, 1H, Ar-CH), 4.12 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.88 (td, *J* = 9.9, 9.4, 5.0 Hz, 1H, NCH₂), 3.56 (ddd, *J* = 14.7, 9.9, 5.5 Hz, 1H, NCH₂), 2.54 (s, 2H, =CCH₃), 1.69–1.54 (m, 2H, CH₂), 1.27 (t, *J* = 7.1 Hz, 1H, OCH₂), 1.21 (t, *J* = 7.1 Hz, 2H, OCH₂), 0.92 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.36 (C=O), 154.39 (C=O), 151.87 (Ar-C), 145.26 (C-N), 133.17 (2 × Ar-C), 130.18 (2 × Ar-C), 122.22 (Br-Ar-C), 104.32 (C=C), 61.50 (OCH₂), 53.73 (CH-NH), 45.12 (N-CH₂), 24.38 (CH₂), 17.51 (CH₃), 15.90 (CH₃), 12.72 (CH₃). HRMS (ESI): *m/z* calcd for C₁₇H₂₁BrN₂O₃ [M + H]⁺ 381.0814, found 381.0807.

Ethyl 4-(4-bromophenyl)-1-butyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1g). White solid, m.p.: 117.5–119.3 °C, yield: 69.7%, Rf value: 0.3 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.13 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 5.39 (s, 1H, NH), 5.33 (s, 1H, Ar-CH), 4.10 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.90 (ddd, *J* = 15.1, 9.9, 5.9 Hz, 1H, NCH₂), 3.58 (ddd, *J* = 14.7, 9.6, 5.4 Hz, 1H, NCH₂), 2.52 (s, 3H, =CCH₃), 1.64 (s, 2H, CH₂), 1.30 (dd, *J* = 14.9, 7.4 Hz, 2H, CH₂), 1.18 (t, *J* = 7.1 Hz, 3H, CH₃), 0.92 (t, *J* = 7.3 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.02 (C=O), 153.12 (C=O), 150.58 (Ar-C), 143.92 (C-N), 131.81 (2 × Ar-C), 128.94 (Ar-C), 128.72 (Ar-C), 120.89 (Br-Ar-C), 103.17 (C=C), 60.18 (OCH₂), 52.30 (CH-NH), 31.90 (N-CH₂), 19.89 (CH₂), 16.16 (CH₃), 14.57 (CH₃), 14.10 (CH₃). HRMS (ESI): *m/z* calcd for C₁₈H₂₃BrN₂O₃ [M + H]⁺ 395.0970, found 395.0977.

Ethyl 4-(4-bromophenyl)-1-hexyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1h). White solid, m.p.: 161.3–161.6 °C, yield: 55.8%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, *J* = 8.1 Hz, 2H, 2 × Ar-H), 7.13 (d, *J* = 8.2 Hz, 2H, 2 × Ar-H), 5.50 (s, 1H, NH), 5.33 (s, 1H, Ar-CH), 4.10 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.90 (ddd, *J* = 14.8, 9.6, 5.9 Hz, 1H, NCH₂), 3.57 (ddd, *J* = 14.7, 9.6, 5.4 Hz, 1H, NCH₂), 2.52 (s, 3H, =CCH₃), 1.59 (s, 1H, CH₂), 1.49 (s, 1H, CH₂), 1.27 (s, 6H, 3 × CH₂), 1.19 (t, *J* = 7.1 Hz, 3H, CH₃), 0.88 (t, *J* = 6.6 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.04 (C=O), 153.20 (C=O), 150.68 (Ar-C), 143.84 (C-N), 131.79 (2 × Ar-C), 128.78 (2 × Ar-C), 120.91 (Br-Ar-C), 103.25 (C=C), 60.19 (OCH₂), 52.12 (CH-NH), 42.07 (N-CH₂), 31.51 (CH₂), 29.84 (CH₂), 26.35 (CH₂), 22.60 (CH₂), 16.17 (CH₃), 14.51 (CH₃). HRMS (ESI): *m/z* calcd for C₂₀H₂₇BrN₂O₃ [M + H]⁺ 423.1283, found 423.1270.

Ethyl 4-(4-bromophenyl)-1-(3-cyanopropyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1i). White solid, m.p.: 128.8–129.7 °C, yield: 27.2%, Rf value: 0.4 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (dd, *J* = 8.4, 1.9 Hz, 2H, 2 × Ar-H), 7.09 (dd, *J* = 8.3, 1.4 Hz, 2H, 2 × Ar-H), 5.33 (s, 1H, Ar-CH), 4.14–4.03 (m, 2H, OCH₂), 3.95–3.84 (m, 1H, NCH₂), 3.74 (dd, *J* = 9.0, 5.4 Hz, 1H, NCH₂), 2.52 (s, 3H, =CCH₃), 2.38–2.18 (m, 2H, CH₂), 1.96 (s, 1H, CH₂), 1.84 (s, 1H, CH₂), 1.16 (dd, *J* = 7.5, 6.8 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.04 (C=O), 153.20 (C=O), 150.69 (Ar-C), 143.84 (C-N), 131.78 (2 × Ar-C), 128.79 (2 × Ar-C), 120.91 (Br-Ar-C, C≡N), 103.24 (C=C), 60.19 (OCH₂), 52.11 (CH-NH), 42.07 (N-CH₂), 22.60 (CH₃), 16.17 (CH₂), 14.59 (CH₂), 14.43 (CH₃). HRMS (ESI): *m/z* calcd for C₁₈H₂₀BrN₃O₃ [M + Na]⁺ 428.0586, found 428.0577.

Ethyl 4-(4-bromophenyl)-6-methyl-1-(4-nitrobenzyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1j). White solid, m.p.: 183.3–184.6 °C, yield: 9.4%, Rf value: 0.6 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, *J* = 8.7 Hz, 2H, 2 × Ar-H), 7.47–7.44 (m, 2H, 2 × Ar-H), 7.29 (s, 2H, 2 × Ar-H), 7.17–7.10 (m, 2H, 2 × Ar-H), 5.69 (d, *J* = 3.0 Hz, 1H, NH), 5.45 (d, *J* = 3.1 Hz, 1H, Ar-CH), 5.10 (q, *J* = 17.1 Hz, 2H, CH₂), 4.11 (q, *J* = 7.1 Hz, 2H, OCH₂), 2.40 (s, 3H, =CCH₃), 1.18 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 165.88 (C=O), 153.21 (C=O), 149.95 (NO₂-Ar-C), 147.20 (Ar-C), 147.00 (C-N), 143.70 (Ar-C), 131.96 (2 × Ar-C), 129.02 (2 × Ar-C), 127.77 (2 × Ar-C), 124.25 (2 × Ar-C), 121.12 (Br-Ar-C), 103.87 (C=C), 60.39 (OCH₂), 52.59 (CH-NH), 45.51 (N-CH₂), 16.56 (CH₃), 14.52 (CH₃). HRMS (ESI): *m/z* calcd for C₂₁H₂₀BrN₃O₅ [M + H]⁺ 474.0665, found 474.0658.

3.2.2. N¹-Substituted Ethyl 4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **2a**

Ethyl 1-(4-methoxy-4-oxobutyl)-4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2a). White solid, m.p.: 87.8–90.4 °C, yield: 21.7%, R_f value: 0.4 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.15 (d, *J* = 8.6 Hz, 2H, 2 × Ar-H), 6.80 (d, *J* = 8.6 Hz, 2H, 2 × Ar-H), 5.77 (s, 1H, NH), 5.30 (d, *J* = 1.9 Hz, 1H, Ar-CH), 4.14–4.03 (m, 2H, OCH₂), 3.96–3.84 (m, 1H, NCH₂), 3.77 (d, *J* = 5.7 Hz, 3H, OCH₃), 3.67 (s, 3H, OCH₃), 3.63 (dd, *J* = 9.0, 4.6 Hz, 1H, NCH₂), 2.54 (s, 3H, =CCH₃), 2.29 (td, *J* = 7.0, 2.8 Hz, 2H, COCH₂), 1.98–1.87 (m, 1H, CH₂), 1.86–1.76 (m, 1H, CH₂), 1.20–1.13 (m, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.38 (C=O), 166.18 (C=O), 159.01 (Ar-C), 153.29 (C-N), 149.61 (Ar-C), 136.54 (Ar-C), 127.75 (Ar-C), 127.71 (Ar-C), 114.24 (Ar-C), 104.21 (C=C), 60.11 (OCH₂), 55.57 (OCH₃), 52.27 (OCH₃), 51.82 (CH-NH), 41.37 (N-CH₂), 30.72 (CH₂), 25.05 (CH₂), 16.06 (CH₃), 14.58 (CH₃). HRMS (ESI): *m/z* calcd for C₂₀H₂₆BrN₂O₆ [M + H]⁺ 391.1869, found 391.1869.

3.2.3. N¹-Substituted Ethyl 4-([1,1'-biphenyl]-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **3a**, **3c**, **3d**, **3e**, **3h**

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-(4-methoxy-4-oxobutyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3a). Yellow solid, m.p.: 141.5–142.7 °C, yield: 33.5%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.55 (dd, *J* = 10.1, 8.0 Hz, 4H, 4 × Ar-H), 7.44 (t, *J* = 7.5 Hz, 2H, 2 × Ar-H), 7.35 (dd, *J* = 11.1, 7.8 Hz, 3H, 3 × Ar-H), 5.87 (d, *J* = 2.8 Hz, 1H, NH), 5.44 (d, *J* = 2.9 Hz, 1H, Ar-CH), 4.21–4.09 (m, 2H, OCH₂), 4.04–3.90 (m, 1H, NCH₂), 3.76–3.68 (m, 1H, NCH₂), 3.68–3.65 (m, 3H, OCH₃), 2.63–2.54 (m, 3H, =CCH₃), 2.38–2.27 (m, 2H, OCH₂), 2.04–1.78 (m, 2H, CH₂), 1.23 (dd, *J* = 13.0, 5.9 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.29 (C=O), 166.08 (C=O), 153.25 (Ar-C), 150.06 (Ar-C), 143.48 (Ar-C), 140.20 (Ar-C), 139.68 (Ar-C), 129.37 (2 × Ar-C), 127.89 (2 × Ar-C), 127.21 (2 × Ar-C), 127.06 (2 × Ar-C), 103.72 (C=C), 60.16 (OCH₂), 52.46 (OCH₃), 51.74 (CH-NH), 41.41 (N-CH₂), 30.67 (CH₂), 24.99 (CH₂), 16.07 (CH₃), 14.56 (CH₃). HRMS (ESI): *m/z* calcd for C₂₅H₂₈N₂O₅ [M + Na]⁺ 459.1896, found 459.1903.

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-(2-chlorobenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3c). White solid, m.p.: 189.8–190.9 °C, yield: 25.8%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.57 (dd, *J* = 12.2, 4.8 Hz, 4H, 4 × Ar-H), 7.45 (t, *J* = 7.5 Hz, 2H, 2 × Ar-H), 7.41–7.33 (m, 4H, 4 × Ar-H), 7.15 (dtd, *J* = 13.6, 7.4, 6.0 Hz, 2H, 2 × Ar-H), 6.97 (d, *J* = 7.4 Hz, 1H, Ar-H), 5.77 (d, *J* = 3.0 Hz, 1H, NH), 5.55 (d, *J* = 3.0 Hz, 1H, Ar-CH), 5.11 (d, *J* = 2.5 Hz, 2H, NCH₂), 4.14 (q, *J* = 7.1 Hz, 2H, OCH₂), 2.40 (s, 3H, =CCH₃), 1.21 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.03 (C=O), 153.25 (C=O), 149.75 (Ar-C), 143.39 (Ar-C), 140.25 (C-N), 139.88 (Ar-C), 135.98 (Ar-C), 131.35 (Ar-C), 129.83 (2 × Ar-C), 129.42 (2 × Ar-C), 129.03 (2 × Ar-C), 127.93 (2 × Ar-C), 127.78 (2 × Ar-C), 127.30 (2 × Ar-C), 127.13 (Ar-C), 104.07 (C=C), 60.33 (OCH₂), 52.60 (CH-NH), 43.80 (N-CH₂), 16.12 (CH₃), 14.53 (CH₃). HRMS (ESI): *m/z* calcd for C₂₇H₂₅ClN₂O₃ [M + H]⁺ 461.1632, found 461.1631.

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-(4-bromobenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3d). White solid, m.p.: 175.3–177.8 °C, yield: 13.3%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.59 (dd, *J* = 5.2, 3.3 Hz, 2H, 2 × Ar-H), 7.56–7.52 (m, 2H, 2 × Ar-H), 7.47 (dd, *J* = 10.2, 4.7 Hz, 2H, 2 × Ar-H), 7.41 (dd, *J* = 7.7, 4.9 Hz, 2H, 2 × Ar-H), 7.38 (s, 1H, Ar-H), 7.33 (d, *J* = 8.2 Hz, 2H, 2 × Ar-H), 7.04 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 5.79 (d, *J* = 3.0 Hz, 1H, NH), 5.52 (d, *J* = 3.1 Hz, 1H, Ar-CH), 5.14 (d, *J* = 15.5 Hz, 1H, NCH₂), 4.88 (d, *J* = 16.1 Hz, 1H, NCH₂), 4.14 (q, *J* = 7.1 Hz, 2H, OCH₂), 2.46 (s, 3H, =CCH₃), 1.22 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.00 (C=O), 153.46 (C=O), 149.81 (Ar-C), 143.42 (C-N), 140.24 (Ar-C), 139.83 (Ar-C), 138.64 (Ar-C), 131.92 (2 × Ar-C), 131.82 (2 × Ar-C), 129.40 (2 × Ar-C), 128.94 (2 × Ar-C), 127.92 (2 × Ar-C), 127.24 (Ar-C), 127.12 (2 × Ar-C), 120.34 (Br-Ar-C), 104.13 (C=C), 60.27 (OCH₂), 52.59 (CH-NH), 44.97 (N-CH₂), 16.50 (CH₃), 14.52 (CH₃). HRMS (ESI): *m/z* calcd for C₂₇H₂₅BrN₂O₃ [M + Na]⁺ 527.0946, found 527.0945.

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3e). White solid, m.p.: 163.5–165.8 °C, yield: 24.8%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, *J* = 7.2 Hz, 2H, 2 × Ar-H), 7.50 (d, *J* = 8.1 Hz, 2H, 2 × Ar-H), 7.44 (t, *J* = 7.5 Hz, 2H, 2 × Ar-H), 7.36 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.32 (t, *J* = 6.4 Hz, 2H, 2 × Ar-H), 7.08 (d, *J* = 8.5 Hz, 2H, 2 × Ar-H), 6.81 (d, *J* = 8.6 Hz, 2H, 2 × Ar-H), 5.71 (s, 1H, NH), 5.48 (s, 1H, Ar-CH), 5.17 (d, *J* = 15.8 Hz, 1H, NCH₂), 4.82 (d, *J* = 16.2 Hz, 1H, NCH₂), 4.11 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.75 (d, *J* = 3.9 Hz, 3H, CH₃), 2.46 (d, *J* = 14.7 Hz, 3H, OCH₃), 1.19 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.06 (C=O), 158.59 (C=O), 153.60 (Ar-C), 150.08 (Ar-C), 143.52 (C-N), 140.26 (Ar-C), 139.76 (Ar-C), 130.92 (2 × Ar-C), 129.40 (2 × Ar-C), 128.10 (2 × Ar-C), 127.91 (2 × Ar-C), 127.35 (2 × Ar-C), 126.96 (2 × Ar-C), 114.32 (2 × Ar-C), 104.05 (C=C), 60.21 (OCH₂), 55.40 (OCH₃), 52.59 (CH-NH), 44.75 (N-CH₂), 16.51 (CH₃), 14.52 (CH₃). HRMS (ESI): *m/z* calcd for C₂₈H₂₈N₂O₄ [M + Na]⁺ 457.2127, found 457.2127.

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-butyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3g). White solid, m.p.: 161.2–165.8 °C, yield: 19.9%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.58–7.50 (m, 4H, 4 × Ar-H), 7.43 (t, *J* = 7.6 Hz, 2H, 2 × Ar-H), 7.37–7.30 (m, 3H, 3 × Ar-H), 5.42 (s, 2H, NH, Ar-CH), 4.13 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.98–3.87 (m, 1H, NCH₂), 3.62 (ddd, *J* = 14.8, 14.3, 9.5 Hz, 1H, NCH₂), 2.55 (s, 3H, =CCH₃), 1.64–1.50 (m, 2H, CH₂), 1.31 (dt, *J* = 15.1, 7.5 Hz, 2H, CH₂), 1.21 (t, *J* = 7.1 Hz, 3H, CH₃), 0.92 (t, *J* = 7.3 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.12 (C=O), 153.25 (C=O), 150.18 (Ar-C), 143.64 (C-N), 140.24 (Ar-C), 139.67 (Ar-C), 129.38 (2 × Ar-C), 127.87 (2 × Ar-C), 127.18 (2 × Ar-C), 127.09 (2 × Ar-C), 127.08 (Ar-C), 103.52 (C=C), 60.12 (OCH₂), 52.49 (CH-NH), 41.87 (N-CH₂), 31.91 (CH₂), 19.88 (CH₂), 16.15 (CH₃), 14.56 (CH₃), 14.10 (CH₃). HRMS (ESI): *m/z* calcd for C₂₄H₂₈N₂O₃ [M + H]⁺ 393.2168, found 393.2178.

Ethyl 4-([1,1'-biphenyl]-4-yl)-1-hexyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3h). White solid, m.p.: 114.4–117.4 °C, yield: 50.5%, Rf value: 0.4 (CH₂Cl₂: CH₃OH=20:1). ¹H NMR (400 MHz, CDCl₃) δ 7.57–7.48 (m, 4H 4 × Ar-H), 7.42 (t, *J* = 7.5 Hz, 2H 2 × Ar-H), 7.33 (t, *J* = 8.0 Hz, 3H, 3 × Ar-H), 5.99 (d, *J* = 2.9 Hz, 1H, NH), 5.42 (d, *J* = 2.9 Hz, 1H, Ar-CH), 4.13 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.99–3.87 (m, 1H, NCH₂), 3.57 (ddd, *J* = 14.6, 9.5, 5.4 Hz, 1H, NCH₂), 2.54 (s, 3H, =CCH₃), 1.68–1.44 (m, 2H, CH₂), 1.25 (s, 6H, 3 × CH₂), 1.21 (t, *J* = 7.1 Hz, 3H, CH₃), 0.85 (t, *J* = 6.5 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.13 (C=O), 153.31 (C=O), 150.26 (Ar-C), 143.58 (C-N), 140.22 (Ar-C), 139.64 (Ar-C), 129.35 (2 × Ar-C), 127.86 (2 × Ar-C), 127.13 (3 × Ar-C), 127.04 (2 × Ar-C), 103.56 (C=C), 60.12 (OCH₂), 52.34 (CH-NH), 42.04 (N-CH₂), 31.48 (CH₂), 29.83 (CH₂), 26.3 (CH₂), 22.54 (CH₂), 16.15 (CH₃), 14.57 (CH₃), 14.33 (CH₃). HRMS (ESI): *m/z* calcd for C₂₆H₃₂N₂O₃ [M + H]⁺ 421.2491, found 421.2488.

3.2.4. N¹-Substituted Ethyl 6-methyl-4-(4-morpholinophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **4a**

1-(4-methoxy-4-oxobutyl)-6-methyl-4-(4-morpholinophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a). Orange solid, m.p.: 123.1–125.4 °C, yield: 20.3%, Rf value: 0.7 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (500 MHz, CDCl₃) δ 7.14 (d, *J* = 8.5 Hz, 2H, 2 × Ar-H), 6.83 (d, *J* = 8.0 Hz, 2H, 2 × Ar-H), 5.49 (s, 1H, NH), 5.29 (s, 1H, Ar-CH), 4.14–4.05 (m, 2H, OCH₂), 3.95–3.86 (m, 1H, NCH₂), 3.87–3.82 (m, 4H, 2 × OCH₂), 3.68 (s, 3H, OCH₃), 3.65 (dd, *J* = 8.9, 5.9 Hz, 1H, NCH₂), 3.16–3.09 (m, 4H, 2 × NCH₂), 2.55 (d, *J* = 16.9 Hz, 3H, =CCH₃), 2.38–2.25 (m, 2H, CH₂), 1.99–1.89 (m, 1H, CH₂), 1.84 (qd, *J* = 13.3, 6.9 Hz, 1H, CH₂), 1.22–1.14 (m, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.32 (C=O), 166.16 (C=O), 153.27 (Ar-C), 150.77 (C-N), 149.33 (Ar-C), 135.01 (Ar-C), 127.15 (2 × Ar-C), 115.33 (Ar-C), 104.18 (C=C), 66.52 (2 × OCH₂), 60.02 (OCH₃), 52.21 (N-CH₂), 51.79 (N-CH₂), 48.87 (N-CH₂), 41.32 (CH-NH), 30.69 (CH₂), 25.01 (CH₂), 16.00 (CH₃), 14.55 (CH₃). HRMS (ESI): *m/z* calcd for C₂₃H₃₁N₃O₆ [M + Na]⁺ 468.2111, found 468.2105.

3.2.5. N¹-Substituted Ethyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **5a**

Ethyl 1-(4-methoxy-4-oxobutyl)-6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5a). Yellow solid, m.p.: 108.4–111.2 °C, yield: 17.8%, Rf value: 0.7 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, CDCl₃) δ 8.16 (d, *J* = 8.7 Hz, 2H, 2 × Ar-H), 7.42 (d, *J* = 8.7 Hz, 2H, 2 × Ar-H), 5.66 (s, 1H, NH), 5.47 (d, *J* = 2.4 Hz, 1H, Ar-CH), 4.11 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.96–3.83 (m, 1H, NCH₂), 3.73–3.60 (m, 4H, NCH₂, OCH₃), 2.56 (s, 3H, =CCH₃), 2.32–2.23 (m, 2H, OCH₂), 1.98–1.74 (m, 2H, CH₂), 1.18 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.24 (C=O), 165.77 (C=O), 152.93 (Ar-C), 151.64 (C-N), 151.16 (Ar-C), 147.21 (Ar-C), 127.89 (2 × Ar-C), 124.28 (Ar-C), 102.758 (C=C), 60.30 (OCH₂), 52.37 (OCH₃), 51.75 (N-CH₂), 41.47 (CH-NH), 30.62 (CH₂), 16.08 (CH₃), 14.50 (CH₃) HRMS (ESI): *m/z* calcd for C₁₉H₂₃N₃O₇ [M + H]⁺ 406.1614, found 406.1611.

3.2.6. N¹-Substituted Ethyl 6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate **6a**

Ethyl 1-(4-methoxy-4-oxobutyl)-6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a). Yellow solid, m.p.: 97.8–100.3 °C, yield: 21.7%, Rf value: 0.6 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, CDCl₃) δ 8.54 (d, *J* = 5.8 Hz, 2H, 2 × Pyridine-H), 7.20 (d, *J* = 6.0 Hz, 2H, 2 × Pyridine-H), 6.00 (d, *J* = 3.4 Hz, 1H, NH), 5.39 (d, *J* = 3.5 Hz, 1H, Ar-CH), 4.15 (q, *J* = 7.1 Hz, 2H, OCH₂), 3.90 (ddd, *J* = 15.1, 9.7, 5.8 Hz, 1H, NCH₂), 3.67 (s, 3H, OCH₃), 3.66–3.60 (m, 1H, NCH₂), 2.56 (s, 3H, =CCH₃), 2.29 (td, *J* = 7.0, 3.9 Hz, 2H, CH₂), 1.79 (ddt, *J* = 13.3, 9.7, 6.6 Hz, 2H, CH₂), 1.21 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.14 (C=O), 165.73 (C=O), 153.04 (2 × Pyridine-C), 152.474 (C=O), 151.02 (2 × Pyridine-C), 150.27 (C-N), 121.45 (2 × Pyridine-C), 102.45 (C=C), 60.19 (CH₂), 51.75 (Ar-CH), 41.40 (CH₂), 30.57 (CH₂), 24.83 (CH₂ 2), 15.97 (CH₃), 14.41 (CH₃). HRMS (ESI): *m/z* calcd for C₁₈H₂₃N₃O₅ [M + H]⁺ 362.1716, found 362.1704.

3.2.7. N¹-Substituted 4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide **7c, 7d, 7e, 7f**

4-(4-bromophenyl)-1-(2-chlorobenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7c). White solid, m.p.: 186.4–188.5 °C, yield: 14.6%, Rf value: 0.5 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, CDCl₃) δ 7.51 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.38 (dd, *J* = 5.4, 3.8 Hz, 1H, Ar-H), 7.24 (dd, *J* = 8.9, 6.7 Hz, 4H, 4 × Ar-H), 7.08–7.04 (m, 1H, Ar-H), 5.83 (s, 1H, NH), 5.36 (s, 1H, Ar-CH), 5.05 (s, 2H, NCH₂), 2.19 (s, 3H, =CCH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 169.09 (C=O), 153.52 (C=O), 143.36 (Ar-C), 138.64 (Ar-C), 136.65 (C-N), 131.83 (Ar-C-Cl), 131.39 (2 × Ar-C), 129.83 (2 × Ar-C), 129.28 (Ar-C), 129.00 (Ar-C), 127.82 (Ar-C), 127.30 (Ar-C), 121.10 (Br-Ar-C), 109.76 (C=C), 53.96 (CH), 43.34 (CH₂), 16.36 (CH₃). HRMS (ESI): *m/z* calcd for C₁₉H₁₇BrClN₃O₂ [M – H][−] 432.0114, found 432.0110.

1-(4-bromobenzyl)-4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7d). White solid, m.p.: 280.3–280.7 °C, yield: 58.2%, Rf value: 0.7 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO) δ 7.56–7.43 (m, 4H, 4 × Ar-H), 7.15 (s, 2H, 2 × Ar-H), 7.03 (d, *J* = 7.1 Hz, 2H, 2 × Ar-H), 5.17 (s, 1H, Ar-CH), 4.92 (d, *J* = 17.6 Hz, 1H, NCH₂), 4.67 (t, *J* = 14.5 Hz, 1H, NCH₂), 2.02 (s, 3H, =CCH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 169.06 (C=O), 153.73 (C=O), 143.42 (Ar-C), 139.33 (C-N), 138.70 (Ar-C), 131.80 (4 × Ar-C), 129.19 (2 × Ar-C), 129.07 (2 × Ar-C), 121.03 (Br-Ar-C), 120.29 (Br-Ar-C), 109.88 (C=C), 53.91 (CH), 44.61 (CH₂), 16.75 (CH₃). HRMS (ESI): *m/z* calcd for C₁₉H₁₇Br₂N₃O₂ [M + H]⁺ 477.9766, found 477.9769.

4-(4-bromophenyl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7e). White solid, m.p.: 256.4–257.9 °C, yield: 73.8%, Rf value: 0.6 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.80 (d, *J* = 3.0 Hz, 1H, NH₂), 7.48 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.34 (s, 1H, NH₂), 7.14 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 6.99 (d, *J* = 8.6 Hz, 3H, 2 × Ar-H, NH), 6.81 (d, *J* = 8.7 Hz, 2H, 2 × Ar-H), 5.16 (d, *J* = 2.4 Hz, 1H, Ar-CH), 4.92 (d, *J* = 16.5 Hz, 1H, NCH₂), 4.58 (d, *J* = 16.5 Hz,

1H, NCH₂), 3.69 (s, 3H, OCH₃), 2.04 (s, 3H, =CCH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 168.97 (C=O), 158.48 (Ar-C-O), 153.71 (C=O), 143.42 (Ar-C), 138.98 (C-N), 131.59 (2 × Ar-C), 131.52 (2 × Ar-C), 129.08 (2 × Ar-C), 128.01 (Ar-C), 120.81 (Br-Ar-C), 114.19 (2 × Ar-C), 109.58 (C=C), 55.44 (CH₃), 53.73 (CH), 44.29 (CH₂), 16.59 (CH₃). HRMS (ESI): *m/z* calcd for C₂₀H₂₀BrN₃O₃ [M + Na]⁺ 452.0586, found 452.0571.

4-(4-bromophenyl)-6-methyl-2-oxo-1-propyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7f). White solid, m.p.: 238.4–239.5 °C, yield: 21.7%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO) δ 7.60 (d, *J* = 3.0 Hz, 1H, Ar-H), 7.49 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.15 (t, *J* = 7.8 Hz, 2H, 2 × Ar-H), 5.09 (d, *J* = 2.6 Hz, 1H, Ar-CH), 3.73–3.61 (m, 1H, NCH₂), 2.47 (d, *J* = 1.6 Hz, 3H, =CCH₃), 2.13 (s, 3H, NCH₂, CH₂), 0.72 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 169.36 (C=O), 153.59 (C=O), 143.56 (Ar-C), 138.96 (C-N), 131.74 (2 × Ar-C), 129.06 (2 × Ar-C), 120.88 (Br-Ar-C), 109.67 (C=C), 53.71 (CH), 43.37 (CH₂), 23.24 (CH₂), 16.60 (CH₃), 11.46 (CH₃). HRMS (ESI): *m/z* calcd for C₁₅H₁₈BrN₃O₂ [M + Cl][−] 386.0271, found 386.0269.

3.2.8. N¹-Substituted 4-(4-bromophenyl)-6-methyl-2-oxo-N-phenyl-1,2,3,4-hydropyrimidine-5-carboxamides **8a**, **8d**, **8e**

Methyl 4-(4-(4-bromophenyl)-6-methyl-2-oxo-5-(phenylcarbamoyl)-3,4-dihydropyrimidin-1(2H)-yl)butanoate (8a). White solid, m.p.: 182.4–185.0 °C, yield: 25.2%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO) δ 9.89 (s, 1H, NH₂), 7.77 (d, *J* = 3.0 Hz, 1H, NH₂), 7.54 (dd, *J* = 15.5, 8.1 Hz, 4H, 4 × Ar-H), 7.27 (t, *J* = 7.9 Hz, 2H, 2 × Ar-H), 7.22 (d, *J* = 8.4 Hz, 2H, 2 × Ar-H), 7.03 (t, *J* = 7.4 Hz, 1H, Ar-H), 5.22 (s, 1H, Ar-CH), 3.86–3.76 (m, 1H, NCH₂), 3.59 (d, *J* = 4.8 Hz, 3H, CH₃), 3.43 (ddd, *J* = 14.4, 8.9, 5.6 Hz, 1H, NCH₂), 2.24 (d, *J* = 7.4 Hz, 2H, CH₂), 2.15 (s, 3H, =CCH₃), 1.80 (dd, *J* = 13.1, 5.2 Hz, 1H, COCH₂), 1.70–1.60 (m, 1H, COCH₂). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.33 (C=O), 166.18 (C=O), 153.36 (C=O), 143.19 (Ar-C), 139.38 (C-N), 138.66 (Ar-C-NH), 131.75 (2 × Ar-C), 129.02 (2 × Ar-C), 128.82 (2 × Ar-C), 123.80 (Ar-C), 120.91 (2 × Ar-C), 120.04 (Br-Ar-C), 110.23 (C=C), 53.86 (CH), 51.74 (CH₃), 40.95 (CH₂), 30.71 (CH₂), 25.16 (CH₂), 16.66 (CH₃). HRMS (ESI): *m/z* calcd for C₂₃H₂₄BrN₃O₄ [M + H]⁺ 486.1028, found 486.1016.

1-(4-bromobenzyl)-4-(4-bromophenyl)-6-methyl-2-oxo-N-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (8d). White solid, m.p.: 171.8–175.2 °C, yield: 16.7%, R_f value: 0.4 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.87 (s, 1H, NH₂), 7.94 (s, 1H, NH₂), 7.54–7.45 (m, 6H, 6 × Ar-H), 7.26–7.15 (m, 4H, 4 × Ar-H), 7.10 (d, *J* = 8.2 Hz, 2H, 2 × Ar-H), 6.98 (t, *J* = 7.3 Hz, 1H, Ar-H), 5.28 (s, 1H, Ar-CH), 4.93 (d, *J* = 17.0 Hz, 1H, NCH₂), 4.72 (d, *J* = 11.6 Hz, 1H, NCH₂), 2.00 (s, 3H, =CCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.58 (C=O), 152.83 (C=O), 144.02 (Ar-C), 139.51 (C-N), 139.06 (Ar-C-NH), 131.75 (Ar-C), 128.92 (4 × Ar-C), 123.55 (6 × Ar-C), 120.79 (Ar-C), 120.04 (4 × Ar-C), 105.40 (C=C), 55.00 (CH), 48.99 (CH₂), 17.44 (CH₃). HRMS (ESI): *m/z* calcd for C₂₅H₂₁Br₂N₃O₂ [M + H]⁺ 556.0058, found 556.0043.

4-(4-bromophenyl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-N-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (8e). White solid, m.p.: 191.7–193.5 °C, yield: 28.7%, R_f value: 0.5 (CH₂Cl₂: CH₃OH=10:1). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.90 (s, 1H, NH₂), 7.92 (d, *J* = 3.0 Hz, 1H, NH₂), 7.53 (d, *J* = 8.4 Hz, 4H, 4 × Ar-H), 7.29–7.20 (m, 4H, 4 × Ar-H), 7.09 (d, *J* = 8.6 Hz, 2H, 4 × Ar-H), 7.03 (d, *J* = 7.4 Hz, 1H, Ar-H), 6.88 (d, *J* = 8.7 Hz, 2H, 2 × Ar-H), 5.31 (s, 1H, Ar-CH), 4.97 (d, *J* = 16.0 Hz, 1H, NCH₂), 4.69 (d, *J* = 16.2 Hz, 1H, NCH₂), 3.74 (s, 3H, OCH₃), 2.06 (s, 3H, =CCH₃). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.19 (C=O), 158.68 (Ar-C-O), 153.74 (C=O), 143.43 (Ar-C), 139.48 (C-N), 139.05 (Ar-C-NH), 131.86 (2 × Ar-C), 131.61 (2 × Ar-C), 129.15 (2 × Ar-C), 129.10 (2 × Ar-C), 128.21 (Ar-C), 123.95 (Ar-C), 121.09 (2 × Ar-C), 120.28 (Br-Ar-C), 114.39 (2 × Ar-C), 109.96 (C=C), 55.60 (CH₃), 54.35 (CH), 44.57 (CH₂), 16.94 (CH₃). HRMS (ESI): *m/z* calcd for C₂₆H₂₄BrN₃O₃ [M + H]⁺ 506.1079, found 506.1069.

3.3. N¹ and N³-Substituted Ethyl 4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates

Ethyl 4-(4-bromophenyl)-1,3-bis(2-chlorobenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate. White solid, m.p.: 145.1-146.6 °C, yield: NA. ¹H NMR (400 MHz, CDCl₃) δ 7.48-7.41 (m, 2H, 2 × Ar-H), 7.41-7.33 (m, 3H, 2 × Ar-H), 7.31-7.24 (m, 3H, 2 × Ar-H), 7.24-7.12 (m, 4H, 4 × Ar-H), 5.38 (s, 1H, Ar-CH), 5.29 (d, J = 15.7 Hz, 1H, NCH₂), 5.12 (s, 2H, NCH₂), 4.11 (q, J = 7.1 Hz, 2H, OCH₂), 4.05 (d, J = 15.7 Hz, 1H, NCH₂), 2.39 (s, 3H, =CCH₃), 1.19 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆) δ 165.47 (C=O), 153.08 (C=O), 149.52 (Ar-C), 140.96 (C-N), 135.74 (Ar-C), 134.47 (Ar-C), 133.00 (Ar-C), 132.20 (Ar-C), 131.56 (2 × Ar-C), 130.12 (2 × Ar-C), 129.95 (Ar-C), 129.87 (Ar-C), 129.78 (Ar-C), 129.41 (Ar-C), 129.22 (Ar-C), 127.95 (Ar-C), 127.83 (Ar-C), 127.43 (Ar-C), 121.70 (Br-Ar-C), 104.15 (C=C), 60.54 (CH), 57.84 (CH₂), 47.89 (CH₂), 45.28 (CH₂), 16.29 (CH₃), 14.48 (CH₃). HRMS (ESI): m/z calcd for C₂₈H₂₅BrCl₂N₂O₃ [M+H]⁺ 587.0504, found 587.0520.

3.4. The Information on Reaction Condition Optimization

In order to investigate the effects of reaction conditions on N¹ selectivity, we designed orthogonal tests table of different aspects to optimize the reaction, including temperature, time, amount of solvent, dosing interval, the presence of a phase transfer catalyst, different mole equivalent of base and halohydrocarbons. Each condition set include two variables of low dose and high dose (Table S2 in Supplementary Materials). Orthogonal test is widely used in everyday studies and research, because it could handle a complex issue with much lower cost and less time. Orthogonal tests L₈(2⁷) were applied to analyze the influence of the seven factors above on the selectivity and yield of N¹-alkylation of DHPMs.

Results of orthogonal tests for N¹-alkylation of DHPMs in tetrabutylammonium hydroxide system were shown in Table S2 and statistics analysis was carried out with the extremely analysis method. The change of all conditions had no effect on N¹-alkylation selectivity. According to the importance of influence, they were sorted into temperature (R 36.9), dosing interval (R 22.3), reaction time (R 19.5), phase transfer catalyst (R 16.5) and mole ratio of halohydrocarbons (R 11.9). Two other factors, the mole equivalent of base and the amount of solvent, had little effect on yield (R < 10). Therefore, the yield could be increased by raising the reaction temperature and prolonging the reaction time. Meanwhile the selectivity was maintained.

In accordance with the requirements of saving raw materials and lowering costs, 1.7 equivalents of tetrabutylammonium hydroxide, and other conditions selected better factors for orthogonal tests were selected. The N¹-alkylation of DHPMs was synthesized from compound 1, compound 2. The optimal conditions were the mole ratio 1:1.8:1.7 (compound 1, compound 2 and tetrabutylammonium hydroxide), DMF 20 mL/g, TBA was added as a catalyst. After the reaction of compound 1 and TBA in DMF for 1.5 h, compound 2 and potassium iodide (KI) were added to the mixture and stirred for 16 h at 45 °C.

3.5. In Vitro Studies

3.5.1. Cell Culture

U87, U251, Hela and A549 cell lines were obtained from Stem Cell Bank, Chinese Academy of Sciences. They were maintained in 75 cm² culture flasks at 37 °C in a humidified air incubator with 5% CO₂. The high-glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gemini, New York, NY, USA), and penicillin-streptomycin (Solarbio; Beijing, China) was used to culture cells. For all cell lines, the medium was renewed every 2 days until cells reach approximately 90–95% confluence. Then, they were detached by trypsin (Beyotime, China) and before the experiments, cells were counted using a hemocytometer and suitably diluted in the adequate complete culture medium.

3.5.2. Preparation of Compounds Solutions

BIIB021 was purchased from Aladdin Industrial Co. (Shanghai, China). The remaining compounds were synthesized in the authors laboratory. All compounds were dissolved individually in DMSO in a concentration of 10 mM and stored at $-20\text{ }^{\circ}\text{C}$. From this stock solution, the various working solutions of the compounds in different concentrations were prepared by adequate dilutions in the complete culture medium before each experiment.

3.5.3. MTT Assay

The *in vitro* antiproliferative effects were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St Louis, MO, USA) assay [31,32]. After reaching confluence, cells were trypsinized and counted using a hemocytometer. Then, cell suspension/well with density of 4×10^4 cells/mL was seeded in 96-well culture plates and left to adhere for 24 h. After adherence, the medium was replaced by the several solutions of the compounds in study (10 μM for preliminary studies and 1.25, 2.5, 5, 10, 20, and 40 μM for concentration-response studies) in the appropriate culture medium for approximately 72 h. Untreated cells were used as the negative control. Each experiment was performed in triplicate and independently repeated. Then, the medium was removed, 20 μL of the MTT solution (5 mg/mL), prepared in the appropriate serum-free medium, was added to each well, followed by incubation for approximately 3 h at $37\text{ }^{\circ}\text{C}$. Then, the MTT containing medium was removed and the formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm using a microplate reader Bio-rad Xmark spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). After background subtraction, cell proliferation values were expressed as percentage relatively to the absorbance determined in negative control cells.

3.6. *In Vivo* Studies on Xenograft Model

C57 mice (6–8 weeks old, male) were used to establish the xenograft tumors following our published Protocol [33,34]. In brief, GL261 cells (5×10^6) were inoculated subcutaneously in right flank regions. The mice were divided into four groups randomly as control, BIIB021 (30 mg/kg), 303 (100 mg/kg), and 305 (100 mg/kg) with eight mice per group. The mice were intra-gastric administration once a day for compound **3d** and **3g** starting from the second day, and body weights was measured every 3 days. At the end of treatment, animals were euthanized and the tumors were stripped and weighed after two weeks. All data were expressed as mean \pm SD ($n = 5$). * $p < 0.05$, compared with control group. The use of animals was approved by the Animal Experimentation Ethics Committee of Yantai University (protocol number 20180601) in accordance with the guidelines for ethical conduct in the care and use of animals.

3.7. Log P Properties

The logarithm of the partition coefficient (Log P) properties of compounds were calculated by ACD/labs 6.00.

3.8. Pharmacophore Requirements

The GALAHAD module of Sybyl-X 2.0 (Certara, Princeton, NJ, USA) was used to generate pharmacophore. Thirteen DHPMs derivatives were selected with good activity against anticancer. All the structures are attached in Table S3 [20,35,36]. The final pharmacophore models were achieved with follow operations, including a population size value of 20, a maximum generation value of 100 and the value of molecular required hitting was 8.

4. Conclusions

A wide range of organic bases had been selected to study the N^1 and N^3 dialkylation of DHPMs. Selective alkylation of N^1 was achieved with the use of tetrabutylammonium hydroxide. All the

synthesized derivatives were screened for their anti-proliferative activity in U87, U251, HeLa and A549 cell lines using the MTT assay. The study demonstrated that these compounds were more selective toward glioma tumor types. Introduction of the aryl or alkyl chain in the R³, and low electron-donating group in the R¹ of DHPMs exhibited potent anti-proliferative activity. The in vivo efficacy study showed that compound **3d** may have the potential to serve as lead compound for novel anti-tumor drugs to treat glioma. The study may provide a foundation for the future development of DHPMs as a new anti-tumor drug.

Supplementary Materials: The following are available online. Copies of the ¹H-NMR and ¹³C-NMR spectra of the compounds and Tables S1, S2 and S3 are available online.

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Sample Availability: Samples of the compounds are available from the authors.



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