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SYNTHESIS OF *N*-(4-(((6-NITROXINAZOLIN-4-YL)AMINO)METHYL)PHENYL)CYCLOHEXANECARBOXAMIDE

Abstract*: Quinazoline derivatives are heterocyclic compounds of significant importance in organic synthesis and drug design, among which 4-aminquinazoline derivatives are particularly noteworthy. In this study, 4-amino-6-nitroquinazoline (**7**), a key precursor for targeted syntheses, was efficiently prepared, and the results were confirmed using physicochemical methods.*

Keywords*: heterocyclic compounds, 4-aminquinazoline, drug development, nucleophilic substitution, condensation.*

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N-(4-(((6-NITROXINAZOLIN-4-IL)AMINO)METIL)FENIL)SIKLOGEKSANKARBOKSAMID SINTEZI

Annotatsiya*: Xinazolin hosilalari organik sintez va dori vositalari dizaynida muhim ahamiyatga ega bo‘lgan geterosiklik birikmalar hisoblanadi, ulardan ayniqsa 4-aminoxinazolin hosilalari alohida e’tiborga loyiqdir. Ushbu tadqiqotda maqsadli sintezlar uchun muhim prekursor bo‘lgan 4-amino-6-nitroxinazolin hosilasi (**7**) yuqori hosildorlik bilan sintez qilindi va natijalar fizik-kimyoviy usullar yordamida tasdiqlandi.*

Kalit so‘zlar*: geterosiklik birikmalar, 4-aminoxinazolin, dori vositalari dizayni, nukleofil almashinish, kondensatsiya.*

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СИНТЕЗ *N*-(4-(((6-НИТРОКСИНАЗОЛИН-4-ИЛ)АМИНО)МЕТИЛ)ФЕНИЛ)ЦИКЛОГЕКСАНКАРБОКСАМИДА

***Аннотация**: Производные хиназолина представляют собой гетероциклические соединения, имеющие значительное значение в органическом синтезе и разработке лекарственных средств, среди которых особенно выделяются 4-аминохиназолиновые соединения. В данном исследовании была эффективно синтезирована 4-амино-6-нитрохиназолиновая производная (7), являющаяся ключевым прекурсором для целевых синтезов, а результаты были подтверждены физико-химическими методами.*

***Ключевые слова**: гетероциклические соединения, 4-аминохиназолин, разработка лекарственных средств, нуклеофильное замещение, конденсация.*

Introduction

The quinazoline ring is a fused heterocyclic system formed by the junction of a pyrimidine and a benzene ring. Due to their unique properties, quinazoline derivatives have long attracted the interest of chemists. Moreover, these compounds hold a prominent place in medicinal chemistry and drug design. In recent years, the study of signaling pathways and drug targets in the human body has highlighted the importance of target-based drug design for disease treatment and the discovery of new therapeutic agents [1, 2]. Consequently, several quinazoline derivatives with inhibitory activity against various targets have been approved by the U.S. Food and

Drug Administration (FDA), including a number of 4-amino-6-substituted quinazolines (Figure 1) [3]:

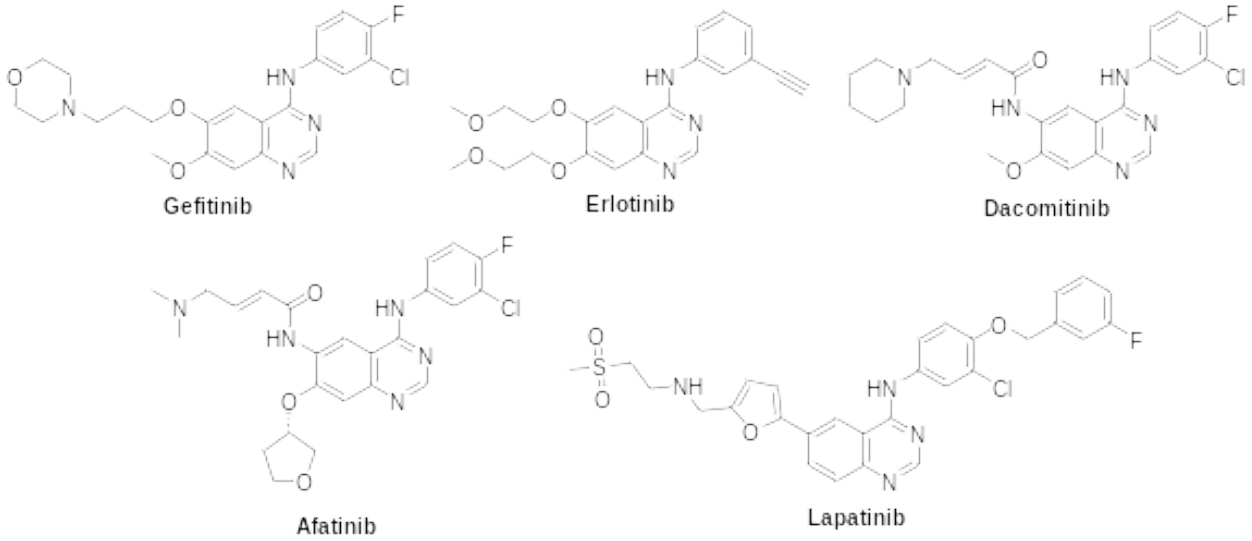


Figure 1. Quinazoline derivatives approved by FDA

Gefitinib, erlotinib, and dacomitinib were approved in 2003, 2004, and 2018, respectively, as EGFR inhibitors for the treatment of non-small-cell lung cancer (NSCLC). Afatinib (approved 2012) and lapatinib (approved 2013) act as dual EGFR/HER2 inhibitors and are used in breast cancer and NSCLC therapy [4, 5]. Analysis of FDA-approved drugs further indicates that quinazoline-containing compounds occupy a prominent position among fused heterocyclic scaffolds in medicinal chemistry [6, 7].

Literature review

In recent years, extensive research has been devoted to the synthesis, structural modification, and biological evaluation of quinazoline derivatives, with particular emphasis on establishing their structure–activity relationships (SAR) [8-10]. Notably, Shao et al. designed and synthesized a series of novel 4,6-diaminoquinazoline derivatives, among which a lead compound exhibited potent EGFR inhibitory activity along with significant anticancer efficacy [11]. Similarly, Zuo and co-workers reported the development of several 4,6-disubstituted quinazoline derivatives, where the target compounds demonstrated strong EGFR inhibition and pronounced in vivo

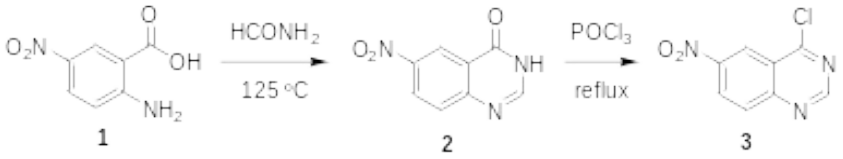
antitumor activity [12]. In addition, Hou et al. synthesized a variety of quinazoline-based compounds and systematically investigated their biological activities [13]. Furthermore, Tu and co-workers prepared a series of 4,6-aminoquinazoline derivatives, identifying several compounds with potent EGFR inhibitory activity and high antiproliferative effects against human cancer cell lines [14, 15]. Moreover, ongoing studies continue to explore 4,6-diaminoquinazoline derivatives, with their inhibitory and antiproliferative activities being actively investigated [16, 17]. Collectively, these findings underscore the continued importance of developing new and efficient key precursors for the synthesis of 4,6-disubstituted quinazoline derivatives, which remains a significant objective in medicinal chemistry research.

Research methodology

The quinazoline scaffold contains multiple reactive centers, which makes it a highly versatile framework for chemical modification. Among these, 4,6-disubstituted quinazoline derivatives are of particular interest due to their recognized biological relevance, and the development of new and efficient precursors in this area remains an important objective. In the present study, we focused on the synthesis of a 4-amino-6-nitroquinazoline derivative, leading to the preparation of compounds that can serve as key synthons for further structural diversification. This work provides a practical foundation for the development of novel 4,6-disubstituted quinazoline derivatives. Within this study, 4-chloro-6-nitroquinazoline was successfully synthesized and subsequently transformed into the key precursor *N*-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide, obtained in good yield via a straightforward and efficient synthetic route.

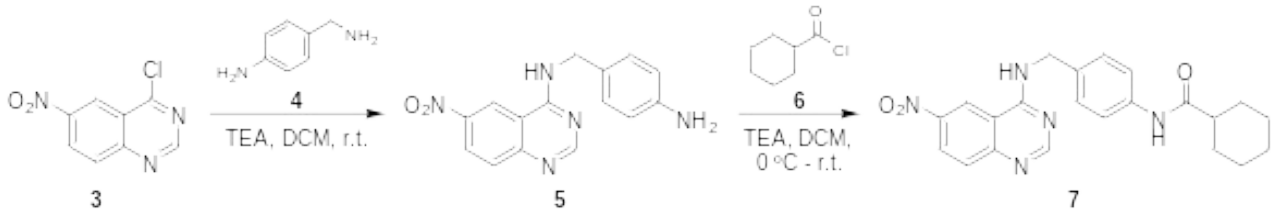
The synthesis commenced from commercially available 6-nitroanthranilic acid (**1**). In the first step, 6-nitroanthranilic acid was cyclized under solvent-free conditions with formamide via the Niementowski reaction at 125 °C, affording 6-nitroquinazolin-4(3*H*)-one (**2**). In the subsequent step, compound 2 was chlorinated

under solvent-free conditions using $POCl_3$ as the chlorinating agent to give 4-chloro-6-nitroquinazoline (**3**) (Scheme 1):



Scheme 1. Synthesis of 4-chloro-6-nitroquinazoline (**3**)

Intermediate (**3**) underwent selective nucleophilic substitution with 4-aminobenzylamine (**4**) under basic conditions (triethylamine, TEA) in dichloromethane (DCM) at room temperature to afford intermediate (**5**). In the final step, *N*-(4-aminobenzyl)-6-nitroquinazolin-4-amine (**5**) was acylated by the gradual addition of cyclohexanecarbonyl chloride (**6**) in an ice bath under basic conditions (TEA) in DCM, yielding the key precursor *N*-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide (**7**) (Scheme 2):



Scheme 2. Synthesis of key precursor *N*-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide (**7**)

Analysis results

The starting materials were obtained from commercial suppliers and used without further purification. All solvents, including ethanol, petroleum ether, toluene, ethyl acetate, and 1,2-dichloroethane, were anhydrous to avoid moisture interference. Reaction progress was monitored by thin-layer chromatography (TLC) on $HSGF_{254}$ silica plates (2.5 × 5 cm), with samples applied using 0.3 × 100 mm glass microcapillaries. Chromatograms were visualized under UV light at 254 and 365 nm or by iodine staining. Tetramethylsilane (TMS) was used as an internal standard in NMR analysis. Structural characterization was carried out using ^{1}H and ^{13}C NMR

spectra recorded on a VARIAN 400 MHz spectrometer at room temperature. HRMS was performed on a QTRAP® 6500+ LC-MS system to confirm molecular composition and structure.

*Preparation of 6-nitroquinazolin-4(3H)-one (**2**)*

The starting material **1** (0.182 g, 1.0 mmol) was dissolved in formamide (5 mL), and the resulting mixture was heated under reflux conditions with continuous stirring for 5 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was allowed to cool to room temperature, leading to the formation of a solid precipitate. The resulting crystals were collected by vacuum filtration, thoroughly washed with distilled water, and dried under reduced pressure to afford compound **2**. Yield 85%, light brown solid, Mp 133–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 2.7 Hz, 1H), 8.53 (dd, J = 9.0, 2.7 Hz, 1H), 8.32 (s, 1H), 7.85 (d, J = 9.0 Hz, 1H).

*Preparation of 4-chloro-6-nitroquinazoline (**3**)*

The intermediate **2** (0.191 g, 1.0 mmol) was carefully added to phosphorus oxychloride ($POCl_3$, 5 mL), and the reaction mixture was heated under reflux conditions with continuous stirring for 6 h. The progress of the chlorination reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool slightly and then slowly poured onto crushed ice–water with vigorous stirring. The resulting solid precipitate was formed immediately, collected by vacuum filtration, washed thoroughly with cold water to remove residual reagents, and dried under reduced pressure to afford compound **3**. Yield 73%, light yellow solid, Mp 140–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 2.7 Hz, 1H), 8.56 (dd, J = 8.9, 2.7 Hz, 1H), 8.36 (s, 1H), 7.88 (d, J = 9.0 Hz, 1H).

*Preparation of N-(4-aminobenzyl)-6-nitroquinazolin-4-amine (**5**)*

The intermediate **3** (0.209 g, 1.0 mmol) and 4-(aminomethyl)aniline (**4**) (0.146 g, 1.2 mmol) were dissolved in DCM (5 mL). After the addition of triethylamine (0.202 g, 2.0 mmol), the reaction mixture was stirred at room temperature overnight.

The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with distilled water and extracted with DCM (3×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography using DCM/MeOH as the eluent to afford intermediate **5**. Yield 91%, light yellow solid, Mp 145–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (d, J = 2.5 Hz, 1H), 9.38 (t, J = 5.8 Hz, 1H), 8.59 (s, 1H), 8.46 (dd, J = 9.2, 2.5 Hz, 1H), 7.81 (d, J = 9.2 Hz, 1H), 7.06 (d, J = 8.3 Hz, 2H), 6.52 (d, J = 8.3 Hz, 2H), 5.01 (s, 2H), 4.62 (d, J = 5.6 Hz, 2H).

*Preparation of N-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide (**7**)*

The intermediate **5** (0.295 g, 1.0 mmol) was dissolved in DCM (5 mL) and the solution was cooled to 0 °C using an ice bath. Triethylamine (0.303 g, 3.0 mmol) was then added under stirring, followed by the slow dropwise addition of cyclohexanecarbonyl chloride (**6**) (0.294 g, 2.0 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into water and extracted with DCM (3×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting crude product was purified by silica gel column chromatography using DCM/MeOH as the eluent to afford compound **7**. Yield 68%, off-white solid, Mp 145–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 9.46 (t, J = 5.8 Hz, 1H), 9.41 (d, J = 2.5 Hz, 1H), 8.59 (s, 1H), 8.48 (dd, J = 9.2, 2.4 Hz, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 8.3 Hz, 2H), 4.74 (d, J = 5.7 Hz, 2H), 2.30 (t, J = 11.5 Hz, 1H), 1.83 – 1.59 (m, 5H), 1.46 – 1.13 (m, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.17, 160.15, 158.26, 152.86, 144.05, 138.45, 132.92, 129.14, 127.86, 126.33, 120.65, 119.04, 114.08, 44.79, 43.56, 29.12, 25.38, 25.22. HRMS (ESI) calcd for $C_{22}H_{23}N_5O_3$ [M+H]+ 405.1801 found 405.1809.

Conclusion

In summary, the key intermediate *N*-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide (**7**) was successfully synthesized using a straightforward and efficient method. The stepwise synthesis, including Niementowski cyclization, selective nucleophilic substitution, and acylation under controlled conditions, afforded the target compound in good yields and high purity. Structural elucidation by ^{1}H and ^{13}C NMR, as well as HRMS, confirmed the identity of all intermediates and final product. The synthesized *N*-(4-(((6-nitroquinazolin-4-yl)amino)methyl)phenyl)cyclohexanecarboxamide (**7**) serves as a key intermediate and a versatile scaffold for the preparation of structurally diverse quinazoline derivatives. This intermediate provides a valuable platform for the design of novel inhibitors and bioactive compounds, supporting further medicinal chemistry efforts in the development of therapeutically relevant quinazoline-based agents.

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