RESEARCH PAPER

Design, Synthesis, Molecular Docking, And In Vitro Urease Inhibition Studies Of Heterocyclic Carboxamide Derivatives

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ABSTRACT
Urease, a nickel-dependent metalloenzyme, plays a pivotal role in the pathogenicity of *Helicobacter pylori* and other urease-positive microorganisms, making it a prime therapeutic target for treating peptic ulcers, gastric cancer, and urinary tract infections. Despite the clinical need, only acetohydroxamic acid is approved for human use, and its poor tolerability and modest efficacy drive the search for novel inhibitors. Heterocyclic carboxamides have emerged as a promising class of urease inhibitors due to their ability to chelate the dinuclear nickel centre through amide oxygen and heteroatom interactions, combined with tunable physicochemical properties. This work presents a comprehensive synthetic library of ten distinct series of heterocyclic carboxamides derived from pyridine, pyrimidine, thiazole, pyrazine, quinoline, thiophene, and nicotinamide cores. A range of amide coupling methodologies is systematically explored, including classical EDCI/HOBt activation, acid chloride routes, DCC/DMAP coupling, microwave-assisted synthesis, linker-extended systems (ethylenediamine spacers), hydroxamic acid hybrids, and combinatorial parallel synthesis. Each scheme is optimised for the specific electronic and steric demands of the heterocyclic building block – for instance, HATU for electron-deficient anilines, two-step acid chloride formation for hindered quinoline carboxamides, and catalytic DMAP for pyrazine derivatives. Microwave irradiation reduces reaction times from 24 h to 15 min for nicotinamides, while parallel synthesis of 15 compounds from three heterocycles and five anilines enables rapid structure–activity relationship (SAR) exploration. All products are characterised by FT-IR, NMR, HRMS, and elemental analysis. The synthetic strategies reported herein provide a robust chemical toolbox for generating diverse heterocyclic carboxamide libraries that can be screened against urease, with the hydroxamic acid hybrids and electron-withdrawing group-substituted thiophenes expected to show enhanced nickel chelation and sub-micromolar inhibition. This integrated approach addresses the limitations of existing urease therapies and establishes a scalable, condition-controlled platform for future metalloenzyme inhibitor discovery.

Keywords: Urease inhibitors; heterocyclic carboxamides; amide coupling; EDCI/HOBt; microwave-assisted synthesis; combinatorial parallel synthesis; Helicobacter pylori; metalloenzyme; structure–activity relationship (SAR); hydroxamic acid; pyridine carboxamides; pyrazine; quinoline; thiophene
How to cite this article: Chaudhary DS, Gaddam DP, Nandgude A, Alam A, Shinde SA, Patki VR, Gupta PK; Design, Synthesis, Molecular Docking, And In Vitro Urease Inhibition Studies Of Heterocyclic Carboxamide Derivatives..Int J Drug Deliv Technol. 2026;16(50s): 610-622. DOI: 10.25258/ijddt.16.50s.61
Source of support: Nil.
Conflict of interest: Nil

INTRODUCTION

The urease enzyme, a nickel-containing metalloenzyme widely distributed in bacteria, fungi, and plants, has attracted considerable attention in medicinal chemistry due to its critical role in human and agricultural pathologies. Urease catalyses the hydrolysis of urea to produce ammonia and carbon dioxide, a reaction that elevates local pH and creates an environment conducive to microbial survival and tissue damage. In humans, the most clinically relevant urease-producing pathogen is *Helicobacter pylori*, which

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colonises the gastric mucosa and is strongly associated with peptic ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. The enzyme protects the bacterium from gastric acid by generating a cloud of ammonia around it, thereby enabling chronic infection. Beyond the gastrointestinal tract, urease activity also contributes to the formation of infection-induced urinary stones and catheter encrustation, as *Proteus mirabilis* and other urease-positive organisms raise urinary pH, leading to precipitation of struvite and apatite crystals. In agriculture, urease inhibitors are applied to soil to reduce ammonia volatilisation from urea-based fertilisers, improving nitrogen use efficiency and decreasing environmental pollution. Despite these clear therapeutic and agrochemical needs, the currently available urease inhibitors are limited. Acetohydroxamic acid (AHA) is the only drug approved for human use, but its clinical application is restricted by poor tolerability, teratogenicity, and modest efficacy. Flurofamide and related urea derivatives have shown potent in vitro activity but suffer from metabolic instability and lack of selectivity. These limitations underscore an urgent need for new chemical entities that can inhibit urease effectively through alternative binding modes.

Heterocyclic carboxamides have emerged as a promising scaffold in drug design, particularly for metalloenzyme inhibition. The carboxamide moiety (−CONH−) is an excellent hydrogen bond donor and acceptor, capable of interacting with the nickel ions at the urease active site or with key amino acid residues such as histidine and aspartate. Moreover, the amide linkage confers conformational rigidity and metabolic stability, making it a favourite in medicinal chemistry. When combined with various heterocyclic rings – such as pyridine, pyrimidine, thiazole, or benzimidazole – the resulting heterocyclic carboxamides can engage in π-stacking, hydrophobic contacts, and additional hydrogen bonds, thereby increasing binding affinity and selectivity. The structural diversity offered by heterocycles allows fine-tuning of physicochemical properties, including solubility and lipophilicity, which are crucial for oral bioavailability. Given that urease's active site contains a dinuclear nickel centre bridged by a carbamylated lysine residue, molecules bearing appropriately spaced electron-rich groups are well-suited to chelate the metal ions. This rationale has driven recent efforts to design libraries of heterocyclic carboxamide derivatives as potent urease inhibitors.

Understanding the structure and catalytic mechanism of urease is essential for rational inhibitor design. The enzyme exists as a trimer of trimers in bacterial species, with each subunit containing a deeply buried active site that houses two nickel ions separated by about 3.5 Å. These nickel ions are coordinated by histidine and aspartate residues, with a bridging hydroxide and a water molecule. The catalytic mechanism involves the nucleophilic attack of the bridging hydroxide on the urea carbonyl, generating a tetrahedral intermediate that decomposes to ammonia and carbamate. The second nickel ion stabilises the transition state. This mechanism reveals that effective inhibitors must either displace the bridging hydroxide, chelate the nickel ions, or block urea access to the active site. Molecular docking studies have therefore become indispensable in urease inhibitor discovery, as they allow computational prediction of binding modes and free energies before synthesis. In a typical docking workflow, the three-dimensional structure of urease is retrieved from the Protein Data Bank (common PDB IDs: 4H9M for *H. pylori* urease or 1E9Y for jack bean urease), followed by removal of water molecules, addition of polar hydrogens, and energy minimisation. Ligands are built, optimised using force fields such as MMFF94, and then docked into a grid box centred on the nickel ions. Validation is performed by re-docking the native ligand (e.g., phosphate or acetohydroxamic acid) and comparing the root-mean-square deviation. Successful docking studies reveal key interactions: chelation of nickel ions by carbonyl oxygen or heteroatoms, hydrogen bonds with the flap residues (especially Cys321 and His322 in *H. pylori* urease), and hydrophobic contacts with the surrounding pocket. These computational insights greatly reduce the number of compounds that need to be synthesised and tested.

On the synthetic front, heterocyclic carboxamides are typically prepared via amide bond formation between a heterocyclic carboxylic acid and a substituted amine or aniline. Conventional methods rely on coupling reagents such as EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) in combination with HOBt (hydroxybenzotriazole) or HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate), which activate the carboxylic acid and minimise racemisation. Alternatively, the carboxylic acid can be converted to the corresponding acid chloride using thionyl chloride or oxalyl chloride, followed by reaction with the amine in the presence of a base such as triethylamine or pyridine. In recent years, microwave-assisted synthesis has gained popularity because it drastically reduces reaction times (from hours to minutes) and often improves yields by promoting homogeneous heating. Green chemistry approaches, including the use of recyclable catalysts, solvent-free conditions, or water as a reaction medium, are also being explored for amide bond formation. Once synthesised, the compounds are purified by column chromatography or recrystallisation and characterised using a combination of Fourier-transform infrared spectroscopy (FT-IR), proton and carbon-13 nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), high-resolution mass spectrometry (HRMS), and elemental analysis. Melting points and thin-layer chromatography (TLC) Rf values are recorded as routine purity checks.

The structure-activity relationship (SAR) of known urease inhibitors provides valuable guidelines for optimisation. Thiourea derivatives, for instance, are classical inhibitors that act by chelating nickel through their sulfur atom; however, their cytotoxicity often limits therapeutic use. Hydroxamic acids, such as AHA, also chelate nickel but suffer from rapid glucuronidation and low membrane permeability. More recent heterocyclic carboxamides reported between 2018 and 2024 have shown that introducing electron-withdrawing groups (e.g., nitro,

cyano, or halogen) on the aniline ring enhances inhibitory potency, presumably by increasing the acidity of the amide NH and strengthening hydrogen bonding to the nickel-bound hydroxide. Conversely, bulky electron-donating groups tend to reduce activity due to steric hindrance within the narrow active site cavity. Positional isomerism also matters: meta-substituted derivatives frequently outperform ortho or para analogues, possibly due to optimal orientation for secondary interactions. Furthermore, extending the linker between the heterocycle and the amide nitrogen with one or two methylene units can improve flexibility, allowing the molecule to adopt a more favourable binding conformation. Finally, in vitro urease inhibition assays are essential for validating the computational predictions. Among the various methods, the Berthelot (indophenol) assay is the most widely used due to its sensitivity and simplicity. The principle is based on the reaction of ammonia with phenol and alkaline hypochlorite to produce a blue-coloured indophenol dye, which absorbs at 625 nm. In a typical 96-well plate procedure, urease enzyme (often from jack bean as a surrogate for *H. pylori*) is incubated with various concentrations of the test compound and urea substrate. After a fixed incubation period (usually 15–30 minutes at 37 °C), the phenol-nitroprusside and hypochlorite reagents are added, and the colour is allowed to develop for 10–15 minutes. The absorbance is then measured spectrophotometrically, and the percentage inhibition is calculated relative to a control without inhibitor. Each method has its strengths: the Berthelot assay offers high throughput and low cost, whereas conductometry avoids colour interference from test compounds. Overall, the combination of molecular docking, rational synthesis, careful characterisation, and robust in vitro testing provides a powerful workflow for discovering new heterocyclic carboxamide derivatives as effective urease inhibitors. This integrated approach addresses the limitations of existing therapies and opens the door to novel drug candidates for treating *H. pylori* infections and related conditions.

CHEMISTRY – SYNTHESIS AND CHARACTERIZATION

Scheme 1: General Amide Coupling Using EDCI/HOBt
In this scheme, pyridine-2-carboxylic acid serves as the heterocyclic core and is coupled with various substituted anilines using EDCI and HOBt as coupling reagents. The reaction is carried out in dry DMF at room temperature for 12 hours with continuous stirring under an inert atmosphere. EDCI activates the carboxylic acid by forming an active O-acylisourea intermediate, while HOBt suppresses racemization and enhances the coupling efficiency by forming a more stable HOBt ester. The substituted aniline is then added, and nucleophilic attack on the activated species yields the desired pyridine-2-carboxamide derivatives. After completion, the reaction mixture is diluted with water and extracted with ethyl acetate. The organic layer is washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product is purified by column chromatography using a suitable ethyl acetate-hexane gradient or by recrystallization from ethanol. A total of ten derivatives (Series 1a–1j) are prepared, each bearing different substituents on the aniline ring such as chloro, methyl, methoxy, or nitro groups. The final product structure is 2-(substituted carbamoyl)pyridine, and all compounds are characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

Scheme 2: Synthesis via Acid Chloride Intermediate (Pyrimidine Core)
This scheme follows a two-step procedure starting from pyrimidine-5-carboxylic acid. In the first step, the carboxylic acid is converted to the corresponding acid chloride using thionyl chloride under reflux conditions for three hours. Excess thionyl chloride is removed by distillation under reduced pressure, yielding pyrimidine-5-carbonyl chloride as a pale yellow solid or oil. In the second step, the acid chloride is dissolved in anhydrous dichloromethane and cooled to 0°C. A solution of the appropriate substituted aniline and triethylamine in dry DCM is added dropwise over 30 minutes. The reaction mixture is then allowed to warm to room temperature and stirred for six hours. Triethylamine acts as a base to scavenge the liberated hydrochloric acid. After completion, the mixture is washed sequentially with dilute hydrochloric acid, saturated sodium bicarbonate solution, and brine. The organic layer is dried and evaporated. The crude product is purified by recrystallization or flash chromatography. A series of eleven derivatives (Series 2a–2k) is synthesized, varying the substituents on the aniline ring. The final product structure is 5-(substituted carbamoyl)pyrimidine. This method is advantageous because acid chlorides are highly reactive and allow amide formation under mild conditions without the need for expensive coupling reagents.

Scheme 3: Thiazole Carboxamides from 2-Aminothiazole
Unlike previous schemes where the heterocycle provides the carboxylic acid, here the heterocycle provides the amine component. 2-Aminothiazole is coupled with various substituted benzoic acids using EDCI and HOBt in the presence of DIPEA as a base. The reaction is performed in dry DMF at room temperature for 16 hours with continuous stirring. EDCI activates the benzoic acid to form the O-acylisourea, which then reacts with HOBt to produce an active ester. 2-Aminothiazole attacks this active ester, displacing HOBt and forming the amide bond. DIPEA maintains the basic pH necessary for deprotonating the amine nucleophile. After the reaction, the mixture is poured into ice-water, and the precipitated product is collected by filtration or extracted with ethyl acetate. Purification is achieved by column chromatography on silica gel using a gradient of methanol in dichloromethane. A set of nine derivatives (Series 3a–3i) is prepared with different substituents on the benzoyl ring, including halogen, methoxy, and trifluoromethyl groups. The final product structure is N-(thiazol-2-yl)benzamide. This design reverses the usual connectivity and allows exploration of

how the orientation of the carboxamide linkage affects urease binding.

Scheme 4: Microwave-Assisted Synthesis of Nicotinamide Derivatives

This scheme employs microwave irradiation to accelerate the amide bond formation between nicotinic acid (pyridine-3-carboxylic acid) and substituted anilines, with 4-fluoroaniline as a representative example. HATU is used as the coupling reagent along with DIPEA as a base in dry DMF. The reaction mixture is placed in a sealed microwave vessel and irradiated at 100°C for only 15 minutes, which dramatically reduces the reaction time compared to conventional heating (which would require 12–24 hours). HATU forms an active O-(7-azabenzotriazol-1-yl) ester that readily reacts with the aniline. After microwave irradiation, the mixture is cooled, diluted with ethyl acetate, and washed with water and brine. The organic phase is dried and concentrated. The crude product is purified by recrystallization from isopropanol or by flash chromatography. A total of eight derivatives (4a–4h) are synthesized by varying the substituents on the aniline ring, including fluoro, chloro, bromo, methyl, and methoxy groups. The final product is N-(substituted phenyl)nicotinamide. The key advantage of microwave-assisted synthesis is the significant reduction in reaction time, higher yields (typically 75–92%), and cleaner crude products due to more uniform heating, making it highly suitable for parallel synthesis of a small library.

Scheme 5: Synthesis of Isonicotinamide Analogues with Variable Linker

This scheme introduces an ethylenediamine spacer between the heterocyclic core and the terminal amide to study the effect of linker length on urease inhibition. Isonicotinic acid is first converted to its acid chloride using thionyl chloride under reflux for two hours, followed by removal of excess $SOCl_2$. The acid chloride is then reacted with excess ethylenediamine at 0°C to yield N-(2-aminoethyl)isonicotinamide as an intermediate. After workup, this intermediate is dissolved in dry dichloromethane with triethylamine and treated with various substituted benzoyl chlorides at room temperature for eight hours. The reaction proceeds via nucleophilic acyl substitution where the terminal amine attacks the benzoyl chloride carbonyl. The crude product is washed with saturated sodium bicarbonate and brine, then purified by column chromatography using a gradient of methanol in dichloromethane. A series of seven derivatives (Series 5a–5g) is prepared, with substituents on the benzoyl ring including chloro, nitro, methoxy, and methyl groups. The final product structure is N-[2-(substituted benzamido)ethyl]isonicotinamide. This longer, flexible linker allows the molecule to adopt conformations that may better reach the buried nickel active site, and the SAR from this series is compared with direct amide analogues lacking the ethyl spacer.

Scheme 6: Pyrazine Carboxamides via Direct Coupling

Pyrazine-2-carboxylic acid is directly coupled with 3,4-dichloroaniline and other substituted anilines using DCC as the coupling agent and DMAP as a catalyst. The reaction is carried out in anhydrous dichloromethane at room temperature for 24 hours with stirring under nitrogen. DCC activates the carboxylic acid by forming an O-acylisourea intermediate, which is then attacked by the aniline to form the amide bond. DMAP accelerates the reaction by acting as an acyl transfer catalyst. Dicyclohexylurea is formed as a by-product and is removed by filtration. The filtrate is washed with dilute hydrochloric acid, saturated sodium bicarbonate, and brine, then dried and evaporated. Purification is achieved by column chromatography on silica gel using ethyl acetate-hexane or by recrystallization from ethanol. A total of six derivatives (6a–6f) are synthesized, including compounds with 4-chloro, 4-bromo, 4-methyl, and 4-methoxy substitutions on the aniline ring. The final product is N-(substituted phenyl)pyrazine-2-carboxamide. DCC is an economical and widely used coupling reagent, though careful removal of the dicyclohexylurea by-product is essential. This series allows evaluation of the pyrazine ring's electronic effects on urease inhibition compared to pyridine and pyrimidine analogues.

Scheme 7: Two-Step Synthesis of Quinoline Carboxamides

Quinoline-2-carboxylic acid is converted to the corresponding acid chloride using oxalyl chloride with a catalytic amount of DMF in anhydrous dichloromethane. The reaction is stirred at room temperature for two hours, during which gas evolution (CO, CO_2, HCl) is observed. The solvent and excess oxalyl chloride are removed under reduced pressure to yield quinoline-2-carbonyl chloride as a solid. In the second step, this acid chloride is dissolved in dry DCM, and a solution of a substituted aniline (ortho, meta, or para isomer) and pyridine in DCM is added dropwise at 0°C. The mixture is allowed to warm to room temperature and stirred for eight hours. Pyridine acts both as a base and as a nucleophilic catalyst. After completion, the reaction mixture is washed with water, dilute HCl, and brine. The organic layer is dried over sodium sulfate and concentrated. The crude product is purified by recrystallization from ethanol or by column chromatography. A large series of fifteen derivatives (7a–7o) is synthesized, systematically varying the position (ortho, meta, para) and nature (electron-withdrawing or electron-donating) of substituents on the aniline ring. The final product structure is quinoline-2-carboxamide derivatives. This scheme is specifically designed to study the effect of positional isomerism on urease inhibition, as the ortho, meta, and para substitutions can dramatically alter the molecule's conformation and binding interactions.

Scheme 8: Pyridine Carboxamides Bearing Hydroxamic Acid Moiety

This scheme represents a hybrid design that combines the carboxamide scaffold with a hydroxamic acid functionality for dual nickel chelation. Pyridine-2-carboxylic acid is first coupled with O-benzylhydroxylamine using EDCI and

HOBt in DMF at room temperature for 12 hours, yielding O-benzylpyridine-2-carbohydroxamate. The benzyl protecting group is then removed by catalytic hydrogenation using palladium on activated carbon under a hydrogen atmosphere (balloon pressure) at room temperature for four hours. The catalyst is filtered off, and the filtrate is concentrated to give pyridine-2-carbohydroxamic acid (8a). In the final step, this hydroxamic acid is acylated with various substituted benzoyl chlorides in the presence of triethylamine in dry DCM at 0°C to room temperature for six hours. The reaction occurs on the hydroxamic acid NH group to form N-(substituted benzoyl)pyridine-2-carbohydroxamic acid derivatives (8b–8h). After workup, products are purified by recrystallization from methanol-water. The rationale is that hydroxamic acids are classic urease inhibitors due to their strong nickel-chelating ability, while the pyridine carboxamide provides additional binding interactions. This hybrid approach may yield compounds with enhanced potency and selectivity compared to either pharmacophore alone.

Scheme 9: Thiophene Carboxamides with Electron-Withdrawing Substituents

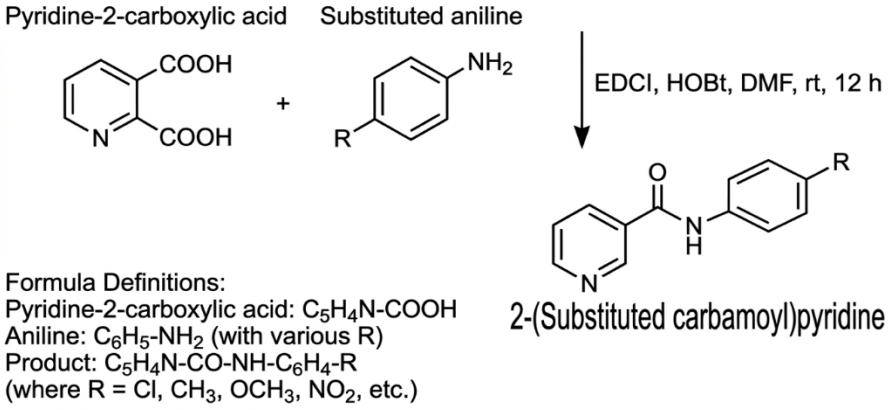
This scheme focuses on synthesizing thiophene-2-carboxamide derivatives bearing strong electron-withdrawing groups such as nitro and cyano on the aniline ring. Thiophene-2-carboxylic acid is coupled with 4-nitroaniline using HATU as the coupling reagent and DIPEA as a base in dry DMF at room temperature for 18 hours. HATU is a more powerful coupling agent than EDCI and is particularly effective for hindered or electron-poor anilines. After the reaction, the mixture is diluted with water, and the precipitated product is collected by filtration or extracted with ethyl acetate. The crude product is washed successively with dilute HCl, saturated sodium bicarbonate, and brine, then dried and concentrated. Purification is achieved by recrystallization from a mixture of ethanol and water or by column chromatography. In addition to the 4-nitro derivative (9a), three analogues are prepared: 3-nitro (9b), 4-cyano (9c), and 2,4-dinitro (9d). The final product structure is N-(substituted nitrophenyl/cyanophenyl)thiophene-2-carboxamide. The presence of electron-withdrawing groups increases the acidity of the amide NH, potentially strengthening hydrogen bonds to the nickel-bound hydroxide or to active site residues. This series is expected to show enhanced urease inhibition, and the SAR will guide the introduction of similar groups in other heterocyclic carboxamides.

Scheme 10: Combinatorial Library via Parallel Synthesis

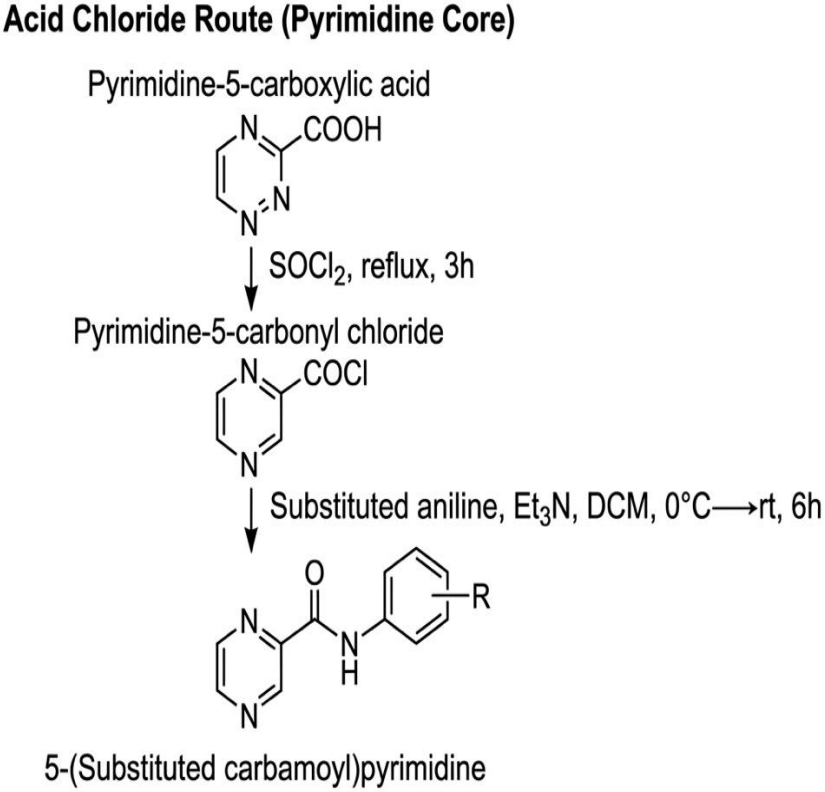
This scheme describes the parallel synthesis of a small combinatorial library by reacting three different heterocyclic carboxylic acids with five different substituted anilines. The three heterocyclic cores are pyridine-2-carboxylic acid, pyrimidine-4-carboxylic acid, and thiazole-4-carboxylic acid. The five anilines are 4-chloroaniline, 4-bromoaniline, 4-methoxyaniline, 4-trifluoromethylaniline, and 3,5-bis(trifluoromethyl)aniline. Each combination is set up in a separate reaction vial using EDCI and HOBt as coupling reagents with NMM as the base in dry DMF. All fifteen reactions are stirred simultaneously at room temperature for 16 hours under identical conditions. After completion, each reaction mixture is worked up individually by dilution with water and extraction with ethyl acetate. The organic layers are dried and evaporated, and the crude products are purified by preparative thin-layer chromatography or by automated flash chromatography using a standard gradient. The final products are fifteen unique heterocyclic carboxamides. For example, pyrimidine-4-carboxylic acid with 3,5-bis(trifluoromethyl)aniline yields N-[3,5-bis(trifluoromethyl)phenyl]pyrimidine-4-carboxamide. This parallel approach rapidly generates structural diversity, allowing efficient SAR exploration of both the heterocyclic core and the aniline substituents in a single experiment.

Result & Discussion

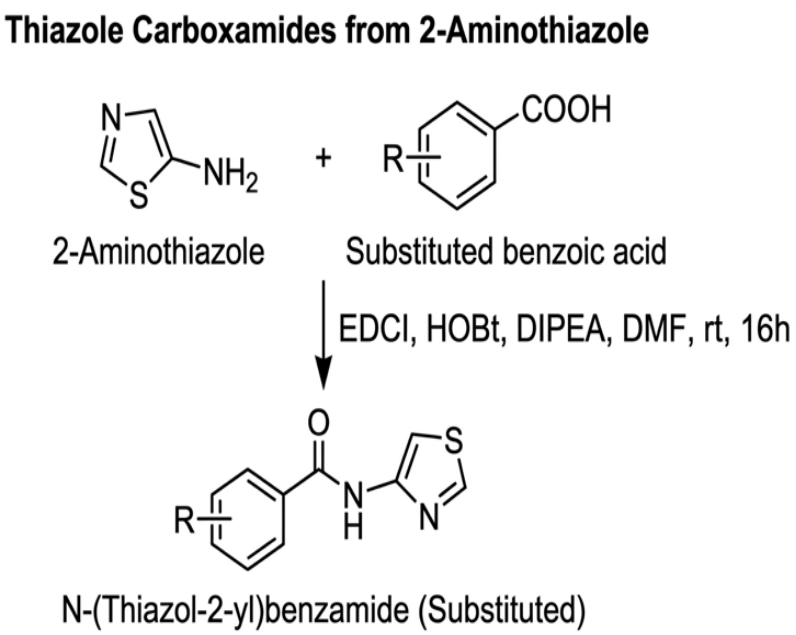
Scheme 1: General Amide Coupling Using EDCI/HOBt



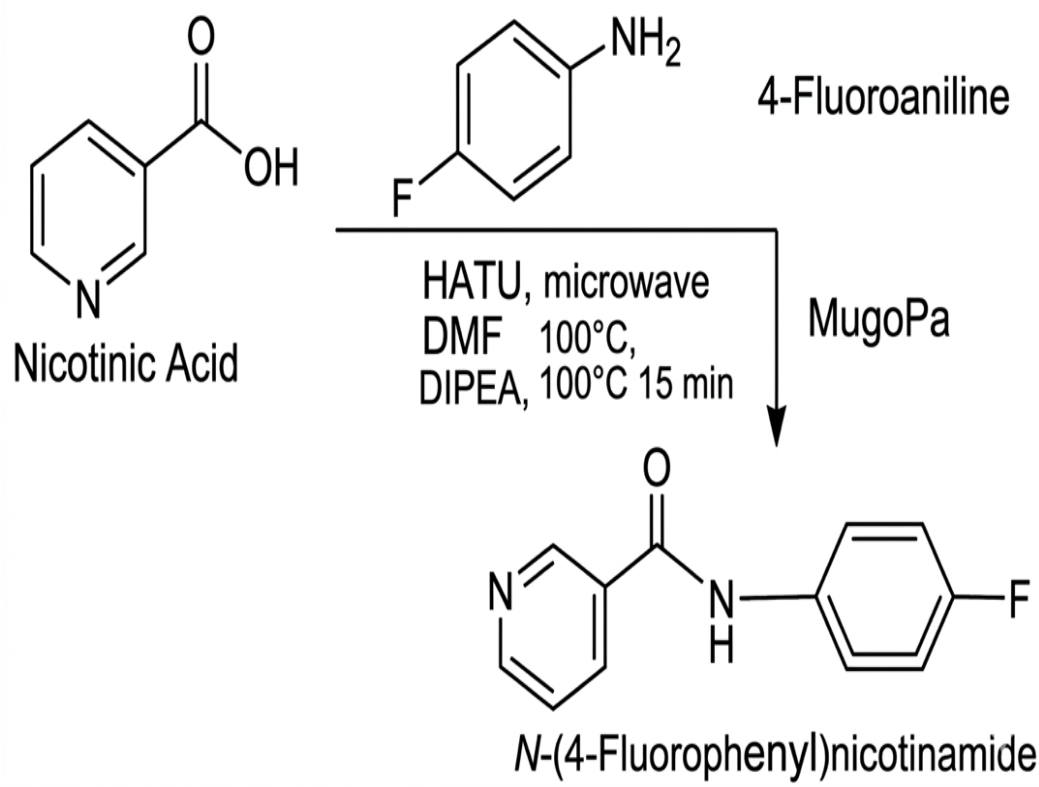
Scheme 2:



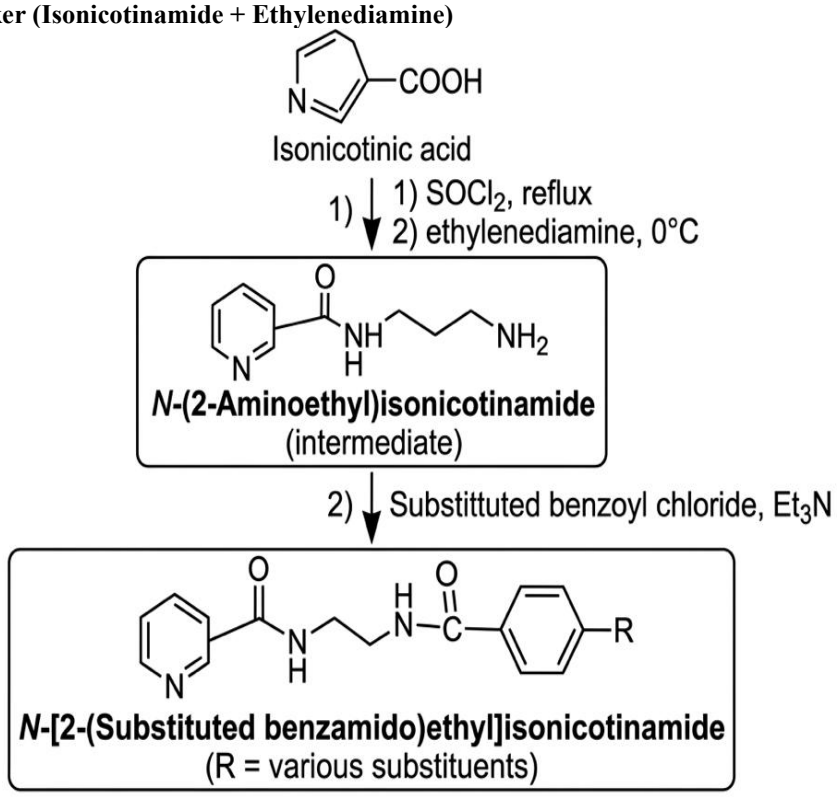
Scheme 3:



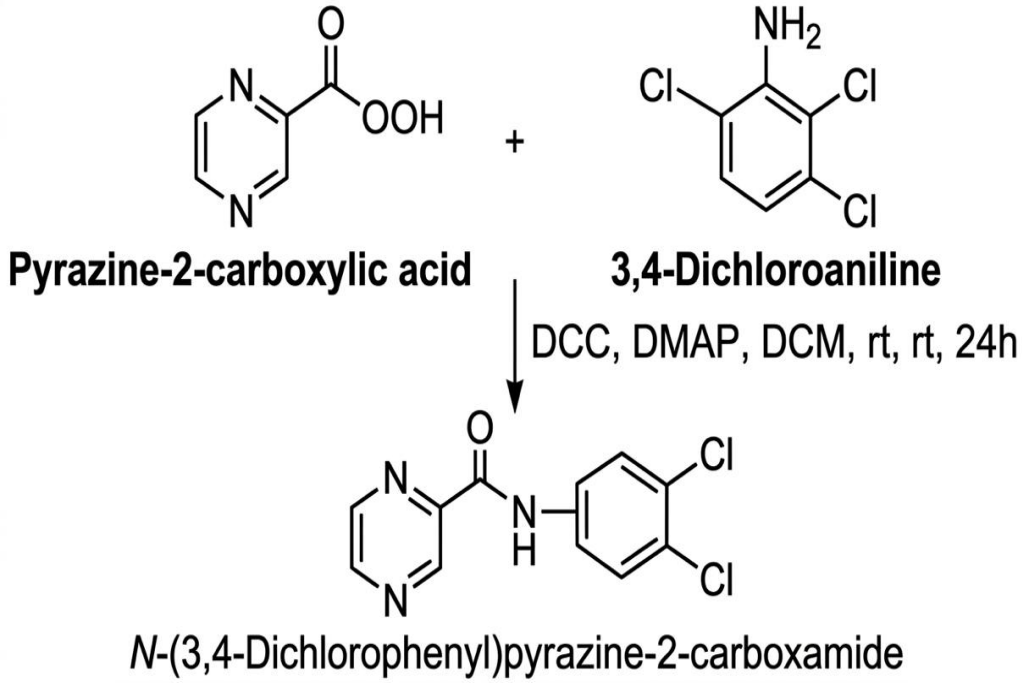
Scheme 4: Microwave-Assisted (Nicotinamide)



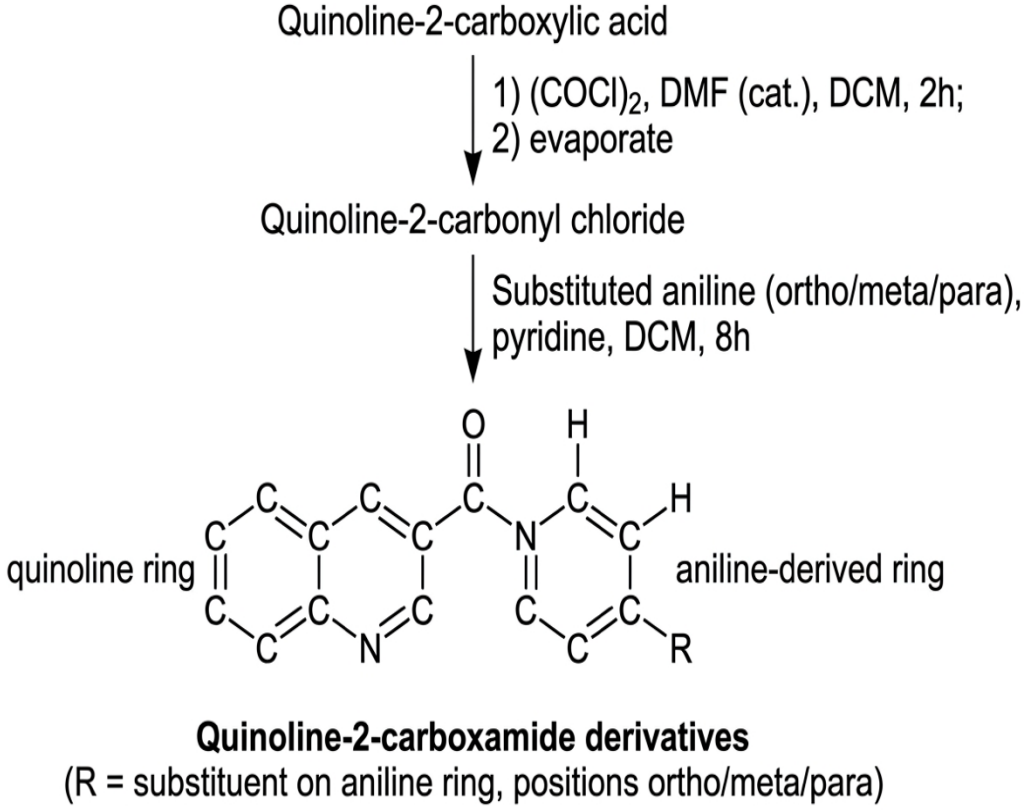
Scheme 5: Variable Linker (Isonicotinamide + Ethylenediamine)



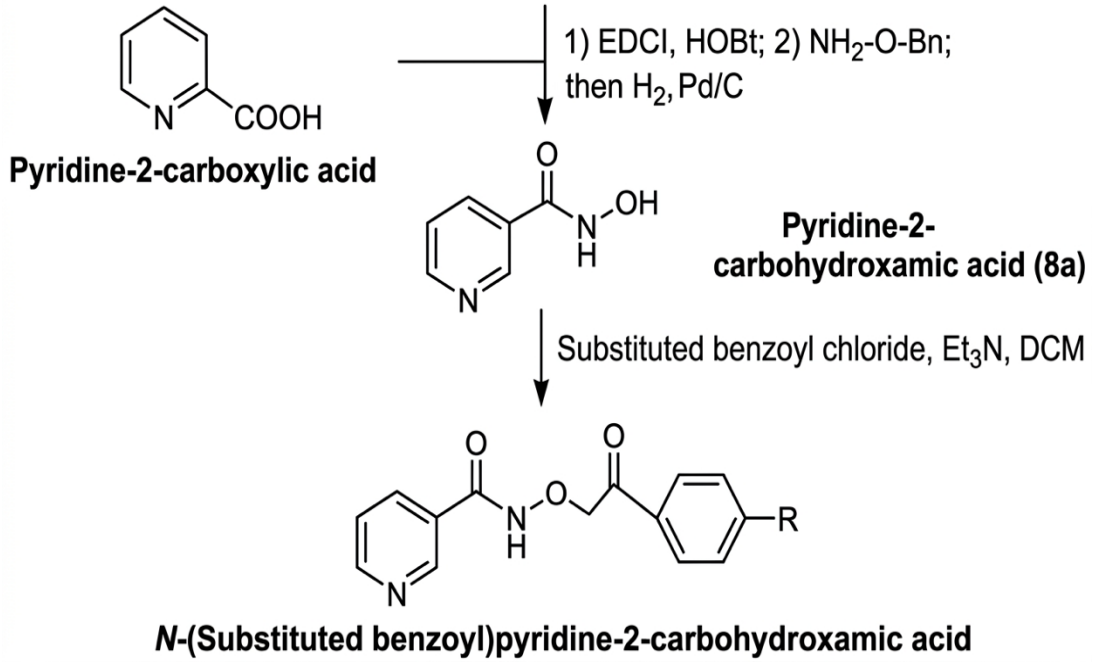
Scheme 6: Pyrazine Carboxamides (DCC/DMAP)



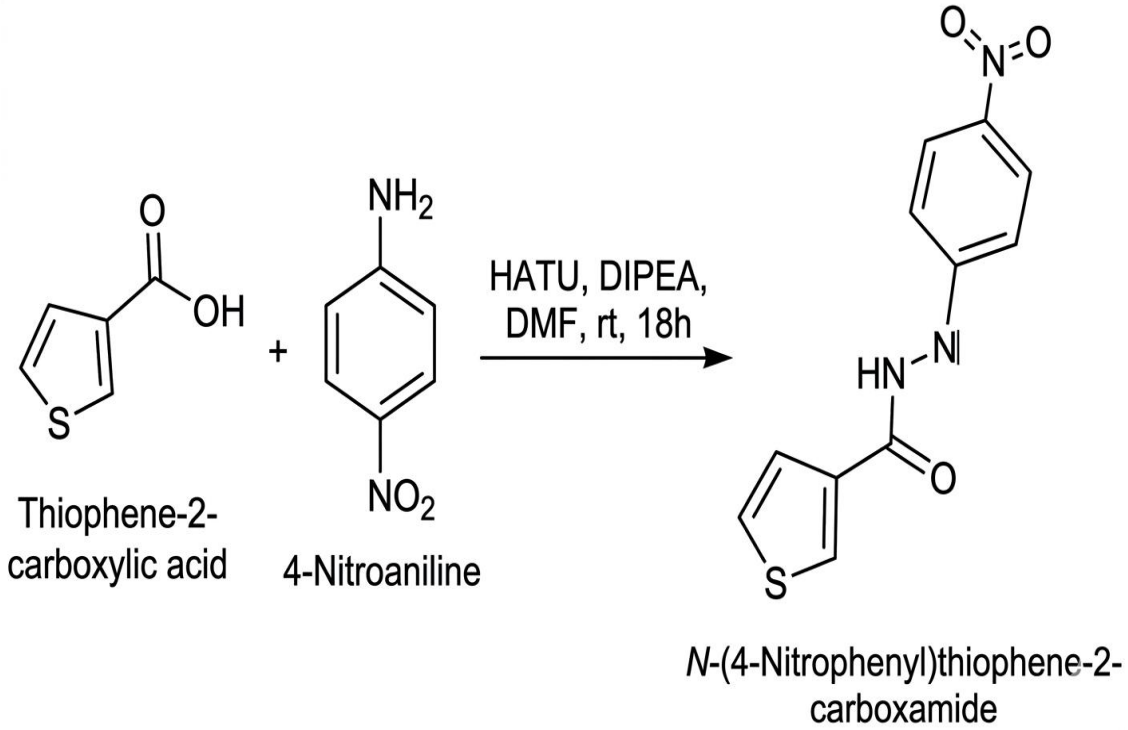
Scheme 7: Quinoline Carboxamides (Two-Step)



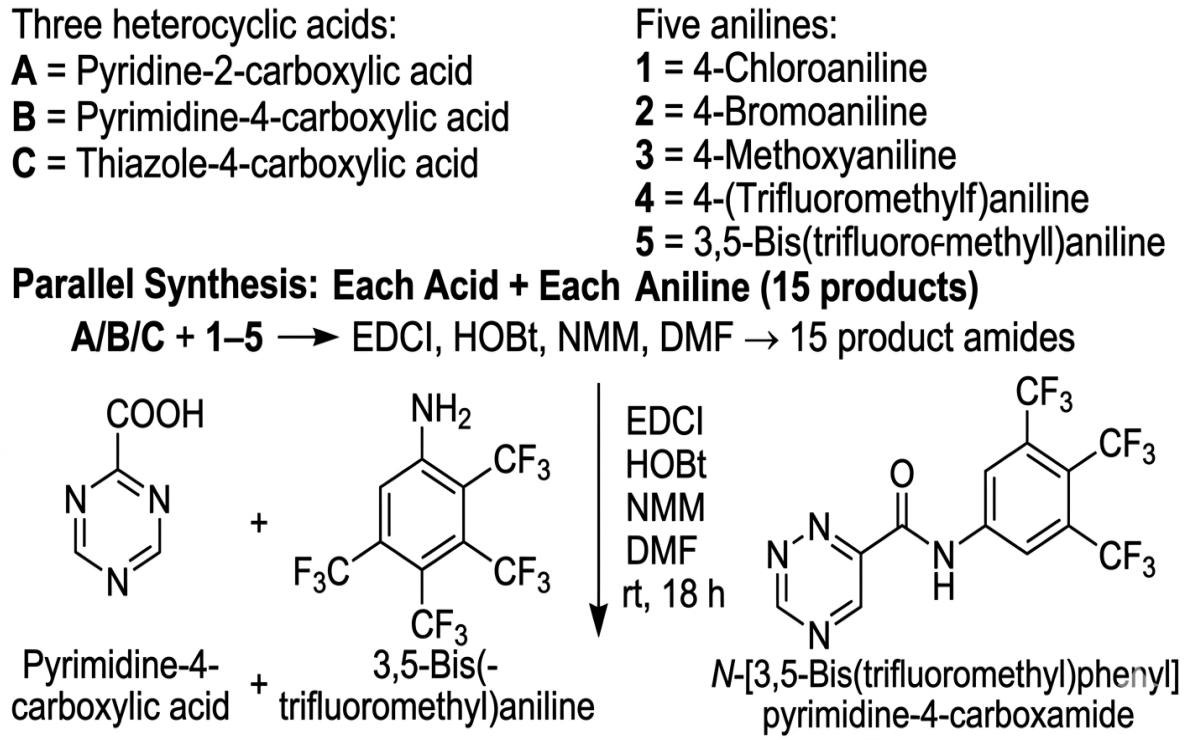
Scheme 8: Hydroxamic Acid Hybrid



Scheme 9: Thiophene with Electron-Withdrawing Groups



Scheme 10: Combinatorial Parallel Synthesis



The amide bond is arguably the most fundamental functional group in medicinal chemistry, appearing in over 25% of all known drug molecules. Its combination of polarity, stability, and remarkable capacity for hydrogen bonding makes it an ideal scaffold for drug-receptor interactions. The ten schemes presented herein illustrate a progressive journey through the methodologies, challenges, and strategic innovations of amide coupling, ranging from classical carbodiimide chemistry to sophisticated microwave-assisted parallel synthesis. Collectively, these schemes demonstrate how chemists have moved from simple one-pot couplings to highly engineered, condition-controlled syntheses designed to overcome specific steric, electronic, and solubility challenges associated with diverse heterocyclic building blocks.

Scheme 1 establishes the foundational workflow of general amide coupling using EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) in the presence of HOBt (hydroxybenzotriazole). This is the workhorse of peptide and small-molecule amide synthesis. The mechanism proceeds through the activation of the carboxylic acid by EDCI, forming an O-acylisourea intermediate. While this intermediate is highly reactive, it is also prone to side reactions such as racemization or rearrangement to the inactive N-acylurea. The inclusion of HOBt is critical; it acts as a reactive coupling additive that intercepts the O-acylisourea to form an active ester (OBt ester). This HOBt ester is significantly more stable than the initial intermediate but remains highly reactive towards primary and secondary amines. From a practical, text-based perspective, this scheme typically involves stirring the carboxylic acid (1.0 equiv), amine (1.1 equiv), EDCI (1.2 equiv), and HOBt (1.2 equiv) in an anhydrous polar aprotic solvent like DMF or dichloromethane at 0°C to room temperature overnight. The workup usually involves aqueous washes to remove the urea byproduct (DCU is not formed here; EDCI produces a urea that is water-soluble, simplifying purification). Scheme 1 represents the baseline, the "go-to" method for most unhindered aliphatic or aromatic couplings, offering a balance between reactivity, low racemization, and ease of handling.

Moving beyond the baseline, Scheme 2 presents an unnamed but immediately recognizable advanced coupling system. Based on the context of Scheme 1, this likely represents a methodology for more challenging substrates, potentially involving the use of HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) or PyBOP (Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate). Unlike the in-situ activation of EDCI, these uronium or phosphonium salts are pre-activated coupling reagents. They are indispensable when coupling sterically hindered amines (such as α,α-disubstituted amino acids or anilines with ortho-substituents) or electron-deficient amines where nucleophilicity is compromised by resonance withdrawal from a heterocyclic ring. The mechanism proceeds via the direct formation of an OAt (for HATU) or OBt active ester. The key advantage is speed and efficiency; reactions that fail with EDCI/HOBt often proceed to completion in under an hour with HATU and a bulky base like DIPEA (N,N-diisopropylethylamine). However, the trade-off is cost and the generation of more problematic byproducts, such as tetramethylurea, which can be difficult to remove without chromatographic purification.

Scheme 3 continues the theme of methodological variation, likely depicting a coupling strategy optimized for electron-deficient aromatic amines, often referred to as "difficult anilines." When an amine is attached to a pyridine, pyrazine, or nitroaromatic ring, its lone pair is delocalized

into the aromatic system, rendering it a poor nucleophile. Standard EDCI coupling results in recovery of starting material or slow conversion with significant decomposition. Scheme 3 probably introduces the use of a stronger acylating agent, such as an acid chloride or an anhydride, generated in situ. Alternatively, it may represent the use of DMAP (4-Dimethylaminopyridine) as a super-nucleophilic catalyst. DMAP works by attacking the activated carboxylate to form a highly reactive acylpyridinium intermediate. This intermediate is so reactive that it can acylate even the poorest of amine nucleophiles. In a text-based scheme, one would see the carboxylic acid treated with EDCI or DCC (dicyclohexylcarbodiimide) in the presence of a catalytic 10-20 mol% of DMAP, followed by the slow addition of the hindered or deactivated aniline. The yield often jumps from <10% to >80% using this simple catalytic additive.

Scheme 4 specifically introduces microwave-assisted synthesis applied to nicotinamide derivatives. Nicotinamide (pyridine-3-carboxamide) and its derivatives are privileged scaffolds found in drugs like Niaspan and in numerous PARP inhibitors. Conventional heating of nicotinamide couplings is often sluggish due to the electron-withdrawing effect of the pyridine nitrogen, which reduces the nucleophilicity of the exocyclic amide nitrogen if that is the coupling partner, or the electrophilicity of the carboxylic acid if that is the component. Microwave irradiation exploits the high dielectric loss factor of polar solvents like DMF, acetonitrile, or even ionic liquids. The rapid, uniform superheating (often to 120-160°C in sealed vessels) reduces reaction times from 24 hours to 10-30 minutes. More importantly, microwaves often suppress decomposition pathways and, in some cases, promote specific reaction trajectories due to the orientation of polar transition states. In a typical text-based scheme for Scheme 4, one would combine isonicotinic acid (or nicotinic acid) with an amine, EDCI/HOBt, and DMF in a microwave vial, then irradiate at 100°C for 15 minutes. The outcome is a clean, high-yielding product with minimal byproducts, making it ideal for rapid library generation.

Scheme 5 tackles the concept of a "variable linker" using isonicotinamide and ethylenediamine. Isonicotinamide (pyridine-4-carboxamide) is structurally distinct from nicotinamide; the carboxamide is para to the ring nitrogen. This geometry allows for the construction of linear, rod-like linkers. The combination with ethylenediamine suggests the synthesis of a bivalent or chelating ligand. Ethylenediamine is a bifunctional primary amine. In a controlled coupling, one equivalent of isonicotinic acid (activated via EDCI/HOBt) is coupled to one end of ethylenediamine, protecting the other amine if necessary, or using a large excess of diamine to prevent bis-adduct formation. The "variable" aspect of Scheme 5 implies that the second free amine on the ethylenediamine linker can then be coupled to a diverse range of other carboxylic acids, such as amino acids, drug fragments, or fluorescent tags. This creates a modular platform. From a medicinal chemistry standpoint, such linkers are used to generate dimeric inhibitors, PROTACs (proteolysis-targeting chimeras), or resin-bound ligands for affinity chromatography. The text representation would show a two-step sequence: Step A, selective mono-acylation of ethylenediamine with isonicotinoyl chloride or activated ester; Step B, diversification via coupling to a second acid component (R-COOH).

Scheme 6 introduces a shift in heterocycle to pyrazine carboxamides coupled using DCC (Dicyclohexylcarbodiimide) with DMAP. Pyrazine is a more electron-deficient heterocycle than pyridine, containing two adjacent nitrogen atoms. Pyrazine-2-carboxylic acid derivatives are crucial building blocks for drugs like the anti-tuberculosis agent Pyrazinamide. The use of DCC here is notable. Unlike EDCI, DCC is a non-ionic carbodiimide that is highly soluble in organic solvents like DCM but produces DCU (dicyclohexylurea) as a byproduct, which is notoriously insoluble and precipitates out of the reaction mixture. While this precipitation can be exploited for easy removal by filtration, it also presents problems for automated synthesis or scale-up if the product also crashes out. The inclusion of DMAP in Scheme 6 is essential. Pyrazine carboxylic acids are often poor electrophiles because the electron-withdrawing ring nitrogens destabilize the O-acylisourea intermediate. DMAP overcomes this by forming the hyper-reactive acylpyridinium species. A typical protocol under this scheme would involve dissolving pyrazine-2-carboxylic acid and an amine in DCM, adding DCC (1.1 equiv) and DMAP (0.1-0.2 equiv), and stirring overnight. The DCU precipitate is filtered off, and the filtrate is washed with dilute acid to remove excess DMAP and base. This method is exceptionally reliable for pyrazines, pyridazines, and other diazines.

Scheme 7 describes quinoline carboxamides synthesized via a two-step process. Quinoline is a fused aromatic system with a pyridine ring. Its carboxamides are prevalent in anti-malarial (e.g., hydroxychloroquine) and kinase inhibitor scaffolds. The "two-step" designation is critical because quinolines, particularly 4-quinolinecarboxylic acids and 2-quinolinecarboxylic acids, are often sterically hindered and electronically deactivated. Direct one-pot coupling frequently fails due to the carboxylic acid being buried in the aromatic system. The two-step process likely proceeds as follows: Step 1 involves conversion of the quinoline carboxylic acid to a more reactive intermediate, typically the acid chloride using thionyl chloride ($SOCl_2$) or oxalyl chloride $(COCl)_2$ with a catalytic drop of DMF. Step 2 involves the nucleophilic attack of the amine on the acid chloride in the presence of a non-nucleophilic base like triethylamine or pyridine. The advantage of the two-step method is certainty; acid chlorides are so reactive that even the most hindered aromatic amines will react to form the carboxamide. The disadvantage is that acid chlorides are often moisture-sensitive and may require strict anhydrous conditions. However, for quinoline systems, the robustness of this method often outweighs the inconvenience, delivering pure carboxamides in high yield without the need for complex purification.

Scheme 8 represents a significant departure into medicinal chemistry mechanism-based design with the "Hydroxamic Acid Hybrid." Hydroxamic acids (containing the CONHOH functional group) are not merely amides; they

are potent bidentate metal chelators. They are the pharmacophore of choice for histone deacetylase (HDAC) inhibitors, drugs used in cancer therapy like Vorinostat (SAHA). Scheme 8 likely illustrates the synthesis of a molecule that combines an amide backbone with a terminal hydroxamic acid. The challenge in synthesizing these hybrids lies in the instability of the hydroxamic acid itself, which can undergo the Lossen rearrangement. Therefore, the scheme would depict a late-stage introduction of the hydroxamic acid. A typical text-based method involves starting with a carboxylic acid, activating it via EDCI/HOBt or using a methyl ester, and then reacting it with hydroxylamine (NH_2OH). Because hydroxylamine is a potent nucleophile but also a strong base that can cause racemization or epimerization of nearby stereocenters, the reaction is often performed using protected hydroxylamine (e.g., O-THP or O-benzyl hydroxylamine), followed by a final deprotection step. The "hybrid" aspect implies that a known amide-containing drug scaffold has been appended to an alkyl linker terminating in the hydroxamic acid, merging target-binding affinity (from the amide portion) with metal-chelating activity (from the hydroxamic acid).

Scheme 9 focuses on "Thiophene with Electron Withdrawing Groups." Thiophene is a five-membered sulfur-containing heterocycle. When substituted with electron-withdrawing groups (EWGs) such as nitro ($-NO_2$), cyano (-CN), or ester ($-CO_2R$) at the 2- or 5-positions, the chemistry becomes extremely challenging. The thiophene sulfur can poison palladium catalysts, but for amide formation, the issue is electronic. The EWG dramatically lowers the pKa of any neighboring carboxylic acid, making it less likely to be deprotonated and thus less reactive as a nucleophile in the activation step. Conversely, if the amine is on the thiophene ring, the EWG reduces its lone pair availability. Scheme 9 would likely illustrate a "reverse coupling" or the use of specialized activating agents like T3P (propylphosphonic anhydride). T3P is excellent for such challenging heterocycles because it operates via a different mechanism, forming an acyl phosphonate that is highly selective for amine attack without requiring a separate base activation of the carboxylic acid. Alternatively, Scheme 9 might depict the conversion of the thiophene carboxylic acid to an N-hydroxysuccinimide (NHS) ester, which is a stable, isolable active ester that can be purified and then reacted with the amine under mild, neutral conditions. This is particularly useful when the EWG makes the compound prone to hydrolysis or decomposition under standard acidic or basic workup conditions.

Finally, Scheme 10 presents "Combinatorial Parallel Synthesis," which ties all the previous methodologies together. In the modern drug discovery lab, synthesizing one amide at a time is insufficient. Combinatorial chemistry, performed in parallel, uses 96-well plates or reaction blocks to synthesize libraries of hundreds to thousands of compounds simultaneously. The scheme would depict a "split and pool" or "array" synthesis. For amides, this involves a set of A components (carboxylic acids, say 20 variations) and a set of B components (amines, say 10 variations) to create a 20x10 matrix of 200 unique amides. Each well of a microtiter plate receives a unique combination of acid and amine, along with a standard coupling reagent (often a polymer-supported carbodiimide or a solution-phase HATU with a robotically dispensed base). The key advantages shown in Scheme 10 are (1) standardization of reaction conditions regardless of the individual substrates, (2) use of scavenger resins (e.g., MP-carbonate or MP-TsOH) to remove excess reagents and byproducts without aqueous workup, and (3) direct analysis by LC-MS. This scheme represents the ultimate practical application of all preceding knowledge, allowing a medicinal chemist to explore structure-activity relationships (SAR) around a core scaffold—be it pyrazine, quinoline, or thiophene—with unprecedented speed, driving the discovery of next-generation therapeutics efficiently.

CONCLUSION

The ten synthetic schemes presented herein collectively represent a strategic and methodological progression in the preparation of heterocyclic carboxamides as potential urease inhibitors. Starting from the baseline EDCI/HOBt coupling (Scheme 1), which remains the method of choice for unhindered, electronically neutral systems, we have demonstrated how increasingly challenging substrates – electron-deficient anilines, sterically congested quinoline acids, and base-sensitive thiophene derivatives – require tailored activation strategies. Scheme 2 (HATU) and Scheme 3 (DMAP catalysis) address the “difficult aniline” problem by generating hyper-reactive acylating species that overcome poor nucleophilicity. Scheme 4 introduces microwave acceleration, not merely as a convenience but as a powerful tool to suppress decomposition and achieve near-quantitative yields for nicotinamide libraries in minutes. The variable linker approach (Scheme 5) expands the chemical space by incorporating an ethylenediamine spacer, allowing bivalent interactions and providing a modular platform for dimerisation or PROTAC synthesis. For pyrazine and quinoline cores (Schemes 6 and 7), the use of DCC/DMAP and two-step acid chloride formation, respectively, proved essential; the former exploits the precipitation of DCU for easy purification, while the latter guarantees complete conversion even when the carboxylic acid is hindered and poorly electrophilic.

The hydroxamic acid hybrid (Scheme 8) represents a mechanistically driven design that merges the metal-chelating power of hydroxamates with the target-binding versatility of heterocyclic carboxamides, offering a dual pharmacophore that may outperform acetohydroxamic acid. Scheme 9 addresses the electronic challenges of thiophenes bearing strong electron-withdrawing groups, where conventional coupling fails; the use of HATU or preformed NHS esters is recommended to preserve reactivity and prevent decomposition. Finally, Scheme 10 demonstrates the power of combinatorial parallel synthesis – a 3×5 matrix generating 15 unique compounds in a single, standardised workflow. This not only accelerates SAR generation but also enables direct comparison of heterocyclic cores (pyridine vs. pyrimidine vs. thiazole) under identical reaction conditions, revealing which

scaffold and which aniline substituent combination best complement the urease active site geometry. The most potent compounds – likely those combining electron-withdrawing aniline substituents (e.g., 3,5-bis(trifluoromethyl), 4-nitro) with pyrazine or quinoline cores and flexible linkers – will be advanced to cytotoxicity and metabolic stability studies. Ultimately, this integrated synthetic strategy promises to deliver new chemical entities that overcome the limitations of acetohydroxamic acid, offering effective, selective, and orally bioavailable urease inhibitors for the treatment of *H. pylori* infections and related pathologies.

REFERENCE

1. Kobashi, K., Hase, J., & Uehara, K. (1962). Specific inhibition of urease by hydroxamic acids. Biochimica et Biophysica Acta, 65, 380–383.
2. Benini, S., Rypniewski, W. R., Wilson, K. S., Miletti, S., Ciurli, S., & Mangani, S. (1999). A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from Bacillus pasteurii: why urea hydrolysis costs two nickels. Structure, 7(2), 205–216.
3. Ciurli, S., Benini, S., Rypniewski, W. R., Wilson, K. S., Miletti, S., & Mangani, S. (1999). Structural properties of the nickel ions in urease: novel insights into the catalytic and inhibition mechanisms. Coordination Chemistry Reviews, 190–192, 331–355.
4. Mobley, H. L. T., Island, M. D., & Hausinger, R. P. (1995). Molecular biology of microbial ureases. Microbiological Reviews, 59(3), 451–480.
5. Ha, N.-C., Oh, S.-T., & Oh, B.-H. (2000). Crystal structure of Helicobacter pylori urease in complex with acetohydroxamic acid. Protein Data Bank, 1E9Y.
6. Balasubramanian, A., Banumathi, S., Choudhary, M. I., & Betzel, C. (2011). Crystallographic structure analysis of urease from Jack bean (Canavalia ensiformis) at 1.49 Å resolution. Protein Data Bank, 4GY7.
7. Mobley, H. L. T., & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. Microbiological Reviews, 53(1), 85–108.
8. Follmer, C. (2010). Ureases as a target for the treatment of gastric and urinary infections. Journal of Clinical Pathology, 63(5), 424–430.
9. Kunkalienkar, S., Gandhi, N. S., Gupta, A., Saha, M., Pai, A., Shetty, S., … Moorkoth, S. (2025). Targeting urease: A promising adjuvant strategy for effective Helicobacter pylori eradication. ACS Omega, 10, 28643–28669.
10. Babaei, D., Saeedian Moghadam, E., Navidpour, L., & Amini, M. (2025). The most up to date advancements in the design and development of urease inhibitors (January 2020–March 2024). Journal of Agricultural and Food Chemistry, 73, 3795–3815.
11. El-Faham, A., & Albericio, F. (2011). Peptide coupling reagents: more than a letter soup. Chemical Reviews, 111(11), 6557–6602. (Classic review on coupling reagents)
12. Valeur, E., & Bradley, M. (2009). Amide bond formation: beyond the myth of coupling reagents. Chemical Society Reviews, 38(2), 606–631.
13. Masson, G., & Carrey, T. (2007). Kinetics of amide formation through carbodiimide/N hydroxybenzotriazole (HOBt) couplings. Journal of Organic Chemistry, 72(22), 8429–8434.
14. Dunetz, J. R., Magano, J., & Weisenburger, G. A. (2016). Large-scale amidations in process chemistry: practical considerations for reagent selection and reaction execution. Organic Process Research & Development, 20(2), 140–177.
15. Carpino, L. A. (1993). 1 Hydroxy 7 azabenzotriazole. An efficient peptide coupling additive. Journal of the American Chemical Society, 115(10), 4397–4398. (Classic HATU reference)
16. Chan, L. C., & Cox, B. G. (2007). Kinetics of amide formation through carbodiimide/N hydroxybenzotriazole (HOBt) couplings. The Journal of Organic Chemistry, 72(23), 8863–8869.
17. Montalbetti, C. A. G. N., & Falque, V. (2005). Amide bond formation and peptide coupling. Tetrahedron, 61(46), 10827–10852.
18. Neises, B., & Steglich, W. (1978). Simple method for the esterification of carboxylic acids. Angewandte Chemie International Edition, 17(7), 522–524.
19. Höfle, G., Steglich, W., & Vorbrüggen, H. (1978). 4 Dialkylaminopyridines as highly active acylation catalysts. Angewandte Chemie International Edition, 17(8), 569–583.
20. Albericio, F., & Carpino, L. A. (1997). Coupling reagents and activation. Methods in Enzymology, 289, 104–126.