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ECOLOGICAL OPTIMIZATION OF SYNTHESIS ROUTES FOR A NEW NON-COVALENT INHIBITOR OF SARS-COV-2 MAIN PROTEASE AS A PROMISING ACTIVE PHARMACEUTICAL INGREDIENT

Larysa Yevsieieva, Anna Geleverya, Nataliia Koval, Alexander Kyrychenko, Sergiy M. Kovalenko, Oleg Kalugin

When transitioning from laboratory synthesis to industrial production, comprehensive research involves more than just assessing quality and biological activity. A crucial aspect of developing a new active pharmaceutical ingredient (API) is the research and optimization of synthetic routes. This process must consider safety, environmental impact, and other parameters set by regulatory requirements.

In previous studies, we synthesized a new biologically active substance intended as a non-covalent inhibitor of the main protease of the SARS-CoV-2 virus. In this work, we analyzed and quantitatively assessed the environmental characteristics of the synthetic routes and optimized the synthesis method for further scaling of the technology. To identify the key factors affecting the environmental impact and efficiency of the process, we applied the fundamental principles of "green chemistry".

***The aim of the study** is to evaluate and optimize the environmental parameters involved in the synthesis of a new biologically active molecule: 1-(2-oxo-2-((pyridin-2-ylmethyl)(thieno[3,2-b]thiophene-2-ylmethyl)amino)ethyl)-5-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole-4-carboxylic acid (HIT). This compound has shown promise as a non-covalent inhibitor of SARS-CoV-2 proteases for potential treatment of COVID-19. The optimization process aims to enhance the synthesis efficiency while also improving the environmental aspects, considering the future scalability of production.*

***Materials and methods.** The study used computational methods, statistical and structural-logical methods, and the EcoScale and DataWarrior software tools.*

***Results.** While studying synthetic routes, alternative solvents were considered, the number of stages was reduced, and the intensity of the process mass was improved.*

***Conclusions**. A comprehensive approach to optimizing synthetic pathways has made it possible to improve the environmental parameters of the target molecule (HIT) synthesis scheme, increase the overall efficiency of the process, and develop safer and more efficient processes for scaling up and producing a new pharmaceutical substance*

***Keywords:** SARS-CoV-2 protease inhibitors, optimization of synthesis routes, pharmaceutical substance, environmental parameters*

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1. Introduction

1. 1. General principles for selecting synthesis routes for pharmaceutical substances

The development of environmentally friendly technologies aimed at reducing environmental impact is a requirement outlined in the latest documents from the European Commission and international environmental protection organizations [1]. This applies specifically to the production of active pharmaceutical ingredients (APIs). By developing synthesis methods for new hit molecules that incorporate green chemistry principles and comply with regulatory requirements, we lay the groundwork for research that can be applied in real-world production. This approach helps to minimize the environmental risks associated with the synthesis stage of pharmaceutical substances.

Initial research focused on discovering new biologically active molecules with specific properties typically involves synthesizing many closely related compounds in a short timeframe. At this initial stage, the efficiency of the synthetic pathways is not a primary concern. However, as the molecule progresses through its life cycle – from laboratory research to industrial production – the requirements for the synthesis process evolve. Once *in vitro* biological activity is confirmed, indicating the potential for further research on the molecule as an active pharmaceutical ingredient (API), the research direction shifts significantly. At this point, it becomes crucial to optimize the synthetic pathway of the molecule, considering safety, environmental impact, and various other factors as prescribed by regulatory requirements [2]. Additionally, the conditions for synthesizing an active molecule developed in the laboratory may need to be adjusted to successfully scale chemical processes.

Peter J. Harrington's influential book, «Pharmaceutical Process Chemistry for Synthesis: Rethinking the Routes to Scale-Up», examines and re-evaluates conventional methods of active pharmaceutical ingredient (API) synthesis. Processes designed in the laboratory are frequently inadequate for industrial-scale production and require careful analysis and modification. This adaptation must consider factors such as productivity, safety, economic viability, and environmental impact [3].

In 2006, a consortium of pharmaceutical manufacturers, including AstraZeneca, GSK, and Pfizer, introduced general principles for selecting and optimizing the route for active pharmaceutical ingredient (API) synthesis, known as the SELECT approach [4, 5]. This framework considers six key factors: safety, environmental impact, legal requirements, economics, control, and throughput. Each factor encompasses important aspects such as reagent availability, chemical yield, cycle time, minimization of intermediate stages and by-products, process stability, and overall cost, along with environmental considerations. Moreover, evaluating critical quality parameters of the API during the synthesis route development phase is essential for ensuring successful industrial-scale production of a pharmaceutical substance [6].

Pharmaceutical companies are actively implementing ISO 14000 Environmental Management Systems standards into their operations [7]. In the context of pharmaceutical substance synthesis, this includes:

– waste management: minimizing hazardous chemical waste;

– energy efficiency: optimizing production processes;

– solvent use: switching to less toxic or "green" solvents, which is one of the principles of green chemistry.

Considering the above, when selecting the optimal synthetic route for biologically active molecules during scaling, the following factors must be considered:

1. Chemical reaction yield: the amount of target product obtained compared to the theoretical maximum.

2. Cycle time: the time required to complete the synthesis process, including all stages of reaction, purification, and processing.

3. Cost and availability of starting materials and reagents.

4. Safety. Synthetic routes involving hazardous materials or reactions must be carefully evaluated and minimized.

5. Environmental impact. Environmental impact assessment in accordance with ISO 14000 requires companies to implement technologies that have a minimal impact on the environment. This includes the use of green chemistry approaches.

6. Minimization of the number of synthesis steps.

7. Process productivity and reliability: the ability to carry out the reaction stably and reliably on a large scale.

8. Quality: guarantee of the purity and quality of the final product.

9. Intellectual property: guarantee that the chosen path does not infringe on existing patents.

10. Regulatory requirements: compliance with all relevant regulatory requirements for pharmaceutical or chemical production.

In 2025, the scientific review titled "Chemical Process Development in the Pharmaceutical Industry in Europe – Insights and Perspectives from Industry Scientists," prepared by representatives of the European pharmaceutical industry, highlighted the key factors that will impact the future scaling of chemical processes [8]. The development of active pharmaceutical ingredient (API) synthesis routes must focus on ensuring scalability, safety, cost-effectiveness, sustainability, and reliability of production. This approach is essential for transforming candidate molecules into effective drugs and creating viable business models.

1. 2. Selection of solvents for optimization of synthesis methods

The chemical synthesis of active pharmaceutical ingredients (APIs) involves a series of controlled chemical reactions that occur sequentially. This process utilizes organic solvents, reagents, and catalysts, ultimately producing the desired product along with several intermediate compounds, by-products, and waste materials.

Understanding the challenges and complexities of scaling up during the early stages of process optimization can significantly enhance laboratory research in addressing these issues. For instance, in a laboratory setting, there are typically no restrictions on the use of certain reagents and solvents that may be prohibited in large-scale production.

The ACS GCI Pharmaceutical Roundtable (ACS GCIPR) is a collaboration between representatives from the pharmaceutical industry and the Green Chemistry Institute. This consortium has proposed several guidelines and tools for promoting sustainable chemistry, including the CHEM21 Guide for Selecting Classic and Less Classic Solvents [9]. This guide ranks traditional solvents used in the pharmaceutical industry based on specific criteria related to hazards, health, and environmental impact. These criteria are in line with the standards set by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [10].

The twelve principles of green chemistry [11] underlie the requirements for a green solvent. A green solvent should meet several criteria [12]: it must be readily available on the market, competitively priced, recyclable, and of high quality – without the need for energy-intensive purification processes. Additionally, the production process should be environmentally friendly, and the solvent should pose minimal risks to human health and the environment. Furthermore, a "green" solvent must be biodegradable, derived from renewable raw materials, and exhibit good performance characteristics. It should also be thermally and (electro)chemically stable, convenient and safe to store and transport, and not flammable.

In recent years, numerous environmentally friendly alternative solvents have been proposed in the literature [13, 14]. However, finding a solvent that satisfies both environmental and production criteria remains challenging. Most traditional organic solvents used in chemical synthesis present fire hazards and have adverse effects on health and the environment.

When optimizing the synthesis routes for a pharmaceutical substance, it is essential to consider the critical quality parameters of the Active Pharmaceutical Ingredient (API). According to the "Quality by Design" approach [15], the quality of a medicinal product should be integrated into its design; it must be inherently built into or accounted for in the design process [16].

The Quality Target Product Profile (QTPP) should ensure that the required quality is maintained, beginning with the methods of Active Pharmaceutical Ingredient (API) synthesis. While the QTPP may not specify particular solvents used in the synthesis process, it does include Critical Quality Attributes (CQAs) that set requirements for the levels of residual organic solvents in APIs. This has a direct impact on the selection of solvents, particularly in the later stages of synthesis. It is important to avoid using solvents that are difficult to remove or highly toxic, specifically those classified as Class 1 and Class 2 according to ICH Q3C guidelines [17, 18]:

1. Class 1 solvents, solvents whose use should be avoided: benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethane, 1,1,1-trichloroethane. These solvents are known carcinogens, mutagens, or reprotoxicants.

2. Class 2 solvents. Solvents whose use should be restricted: acetonitrile, hexane, N,N-dimethylacetamide, N,N-dimethylformamide, 1,2-dimethoxyethane, 1,4-dioxane, dichloromethane, 2-dichloroethene, ethylene glycol, 2-ethoxyethanol, xylene, methanol, methyl butyl ketone, methylpyrrolidone, methylcyclohexane, 2-methoxyethanol, nitromethane, pyridine, sulfolane, tetralin, toluene, 1,1,2-trichloroethylene, formamide, chlorobenzene, chloroform, cyclohexane. These are solvents with a high risk of toxicity. Their use should be minimized.

3. Class 3 solvents (safe): anisole, acetone, 1-butanol, 2-butanol, butyl acetate, heptane, butane, dimethyl sulfoxide, ethanol, ethyl acetate, ethyl ether, ethyl formate, isobutyl acetate, isopropyl acetate, cumene, methyl acetate, 3-methyl-1-butanol, methyl isobutyl ketone, 2-methyl-1-propanol, methyl ethyl ketone, formic acid, pentane, 1-pentanol, 2-propanol, propyl acetate, acetic acid. These solvents with low toxicity risk are preferable for use in the technological process.

1. 3. Evaluating the efficiency of reactions as an environmental aspect of the synthesis process

The pharmaceutical industry is incorporating "green chemistry" principles into its production processes [19]. To optimize the synthesis of the final target molecule, it is essential to consider not only the environmental impact of the solvent but also the efficiency of the reaction. This includes minimizing the number of synthesis steps and addressing any potential impurities or by-products

Process Mass Intensity (PMI) is a key indicator in green chemistry that measures the efficiency of a chemical process (1). It is calculated as the ratio of the total mass of all materials used in the process to the mass of the final product [20, 21]. Typically, PMI can range from 100 kg/kg AFI to several thousand kg/kg, depending on the duration of the synthesis [8]. PMISolv (solvent mass intensity) (2) and PMIeag (reagent mass intensity) (3) are also calculated.

$$\text{PMI} = \frac{\sum \text{Total mass of all reagents and solvents}}{\text{Mass of final product}}, \quad (1)$$

$$\text{PMISolv.} = \frac{\sum \text{Mass of all solvents}}{\text{Mass of final product}}, \quad (2)$$

$$\text{PMIReag.} = \frac{\sum \text{Mass of all reagents}}{\text{Mass of final product}}. \quad (3)$$

Determining the parameters of the contribution of reagents, organic solvents, and process water to the process of obtaining the final target molecule allows for targeted comparison of processes and their optimization.

2. Planning (methodology) of the study

The research methodology consisted of the following parts:

– consider methods for synthesizing a new biologically active HIT molecule [22], which has shown potential as an inhibitor of SARS-CoV-2 proteases for the treatment of COVID-19;

– analyze the environmental component of each stage of the HIT synthesis pathway using the EcoScale program;

– consider alternative solvents to improve environmental parameters;

– analyze the possibility of reducing the number of process stages and improving the "process mass intensity" parameter;

– propose an effective synthesis scheme for the target biologically active HIT molecule for scaling up and production as a new pharmaceutical substance.

3. Materials and methods

We utilized the EcoScale program, a publicly available tool specifically designed to evaluate the environmental aspects of synthesis methods. EcoScale provides a system for assessing various factors in the synthetic pathway of an active molecule, including waste generation, energy consumption, and the use of hazardous materials [20, 23]. This approach involves assigning penalty points to different parameters associated with these factors. The EcoScale rating ranges from 0 to 100, with higher scores indicating more environmentally friendly processes. This method en-

ables the comparison of multiple synthesis methods for the same product or stage in the synthetic pathway of a new molecule, based on safety, economic viability, and environmental attributes.

All chemical reagents and solvents were obtained from commercial suppliers and were used without further purification. The progress of the reactions was monitored using thin-layer chromatography (TLC) on Sorbfil UV-254 silica gel plates, with ethyl acetate and hexane mixtures as eluents. The compounds were purified using a Büchi Sepacore Flash Chromatography System, which includes an automated solvent delivery system. The eluents consisted of gradient mixtures of ethyl acetate and hexane, beginning with 100% hexane and gradually transitioning to 100% ethyl acetate. This method effectively separated the target compounds from impurities and unreacted materials. The purity of the final products was typically 95% or higher, as confirmed by analytical reverse-phase HPLC.

^{1}H NMR spectra were recorded on Agilent and JEOL spectrometers operating at 400 MHz. 13C NMR and 19F NMR spectra were obtained on a JEOL ECZL spectrometer at frequencies of 100 MHz and 376 MHz, respectively. The deuterated solvents used were DMSO-d6 and CDCl3. Chemical shifts are reported in δ (ppm) relative to the residual solvent peaks. Coupling constants (*J*) are expressed in Hz, and multiplicities are described using standard abbreviations: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), and *m* (multiplet).

High-resolution mass spectra were acquired using an Agilent 6230 TOF mass spectrometer, which is equipped with a diode array detector capable of detecting wavelengths at 210 and 254 nm. The mass spectrometer utilized electrospray ionization (ESI) in both positive and negative modes, covering a mass-to-charge ratio (m/z) range of 100 to 1200. Chromatographic separations were performed on an Agilent InfinityLab Poroshell 120 EC-C18 column, measuring 4.6 × 50 mm with a particle size of 2.7 µm. The mobile phases consisted of 0.1% trifluoroacetic acid (TFA) in water (denoted as A) and 0.1% TFA in acetonitrile (denoted as B). A linear gradient elution was carried out at a flow rate of 0.15 mL/min, with a total analysis cycle time of 25 minutes.

4. Research result

During the study, we analyzed the ecological component of the synthesis route for a new biologically active molecule, 1-(2-oxo-2-((pyridin-2-ylmethyl)(thieno[3,2-b] thiophene-2-ylmethyl)amino)ethyl)-5-(4(trifluoromethyl) phenyl)-1H-1,2,3-triazole- 4-carboxylic acid (HIT), which has shown potential as an inhibitor of SARS-CoV-2 proteases for the treatment of COVID-19 [22].

Laboratory method for synthesizing compound 1-(2-oxo-2-((pyridin-2-ylmethyl) (thieno[3,2-b]thiophen-2-ylmethyl)amino)ethyl)-4-(4-(trifluoromethyl) phenyl)-1H-1,2,3-triazole-5-carboxylic acid (HIT) involved a complex 7-step synthesis process (Fig. 1).

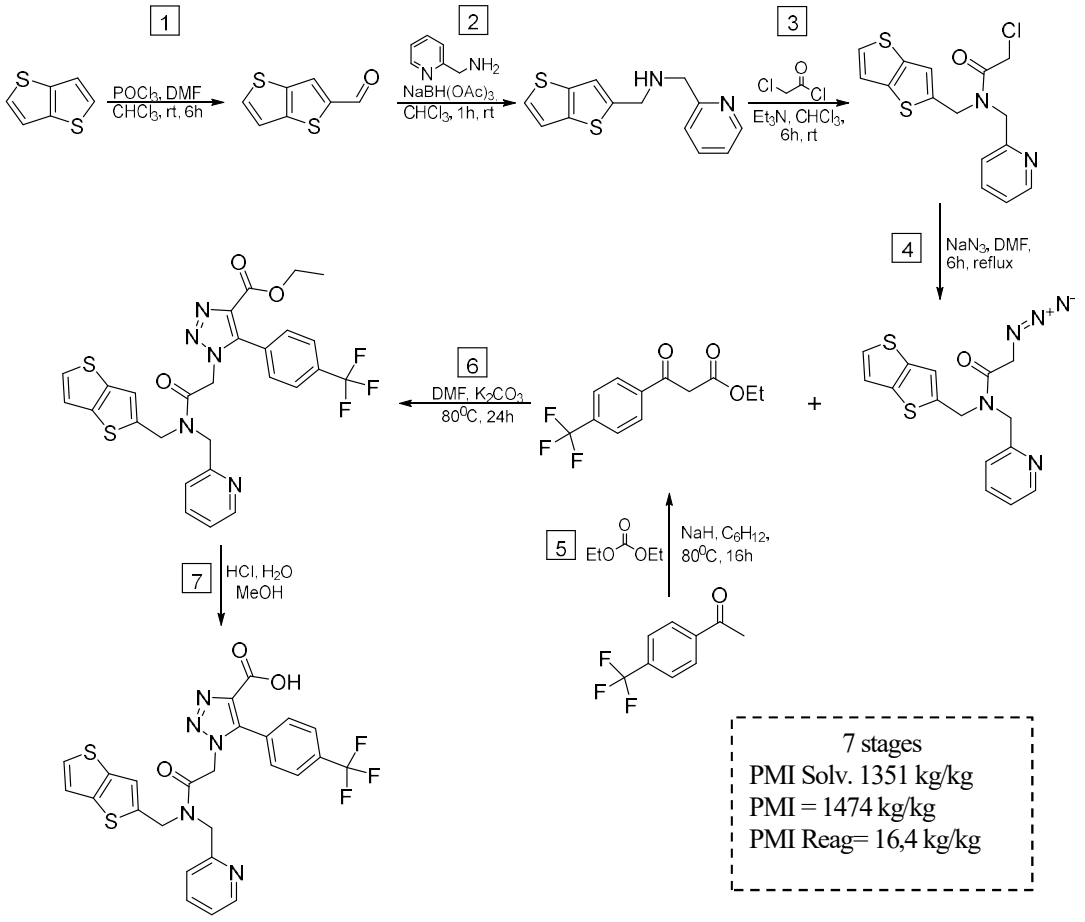
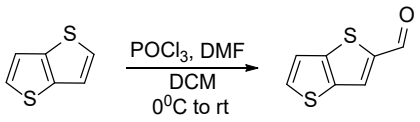


Fig. 1. Laboratory method for synthesizing the HIT compound

4. 2. Chemical synthesis

Synthesis of thieno[2,3-b]thiophene-2-carbaldehyde.

$POCl_3$ (1 equiv.) was added to a DMF (1 equiv.) solution in dichloromethane (3.0 mL) at 0°C, and the reaction mixture was allowed to reach rt [24]. The solution was then slowly added to a solution of thiophene derivative (1 equiv.) in dichloromethane (5.0 mL) at 0°C and allowed to reach rt. After stirring for 12 h, an excess of 3.0 M NaOH aqueous solution (5 equiv.) was added and stirred for 2 h at rt. The reaction mixture was extracted with dichloromethane (25 ml × 3), and the combined organic layers were dried over $MgSO_4$. Purification of the residue was accomplished by flash chromatography on silica gel using hexane/ethyl acetate (70:30) as the eluent, affording the product as a white solid. Yield 92%.

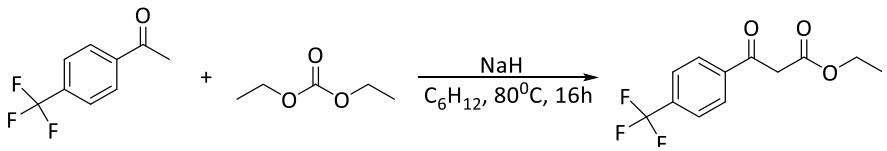


^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (*s*, 1H), 7.95 (*d*, *J* = 0.8 Hz, 1H), 7.70 (*d*, *J* = 5.3 Hz, 1H), 7.33 (*dd*, *J* = 5.3, 0.7 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 183.74, 145.85, 145.53, 139.28, 134.02, 129.25, 120.29.

MS (ESI+) m/z calculated for $C_7H_4OS_2$ $[M+H]^+$ 168.7, found 168.9.

Synthesis of ethyl 3-oxo-3-(4-(trifluoromethyl) phenyl)propanoate.



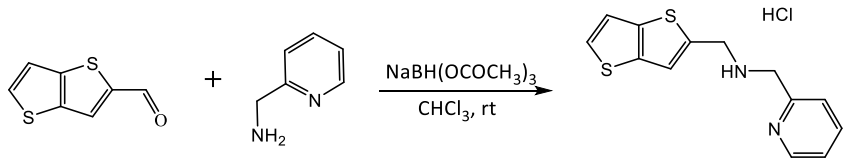
Following a literature procedure[1]: to a suspension of NaH (1.2 g, 30 mmol, 60% in mineral oil) in dry THF (10 mL) was added diethyl carbonate (4.85 mL, 40 mmol) in oven-dried glassware under Ar. A solution of the corresponding ketone (10 mmol) in dry C_6H_{12} (5 mL) was added slowly and the reaction mixture heated under reflux for 16 hours. The reaction mixture was quenched with glacial acetic acid (1 mL) and HCl (10%, 20 mL). The aqueous phase was extracted with ethyl acetate (3 × 10 mL) and the combined organic phase washed with saturated sodium hydrogen carbonate (10 mL), water (10 mL) and brine (10 mL). The combined organic phase was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to yield the crude product. This was purified by flash column chromatography on silica gel (gradient elution EtOAc in petroleum ether (0–25%)). Yield 1.701 g, 6.5 mmol, 65%, yellow oil. 3:2 enol:keto.

^{1}H NMR (400 MHz; $CDCl_3$): δ 12.57 (*s*, enol 1H), 8.06 (*m*, 2H), 7.88 (*m*, enol 2H), 7.76 (*m*, 2H), 7.68 (*m*, enol 2H), 5.72 (*s*, enol 1H), 4.31 (*q*, *J* = 7.1 Hz, enol 2H), 4.19 (*q*, *J* = 7.1 Hz, 2H) 4.01 (s, 2H), 1.39 (*t*, *J* = 7.1 Hz, enol 3H), 1.21 (*t*, *J* = 7.1 Hz, 3H).

^{19}F NMR (376 MHz; $CDCl_3$): δ –62.9 (enol 3F), –63.2 (3F).

^{13}C NMR (101 MHz; $CDCl_3$): δ 191.7, 173.0, 169.5, 167.1, 138.7, 136.9, 135.0 (*q*, *J* = 32.7 Hz), 132.8 (*q*, *J* = 32.7 Hz), 129.0, 126.5, 125.9 (*q*, *J* = 3.7 Hz), 125.6 (*q*, *J* = 3.7 Hz), 123.9 (*q*, *J* = 272.7 Hz), 123.6 (*q*, *J* = 272.7 Hz), 89.1, 61.8, 60.7, 46.2, 14.30, 14.10.

Synthesis of 1-(Pyridin-2-yl)-N-(thieno[3,2-b]thiophen-2-ylmethyl)-methanamine hydrochloride.

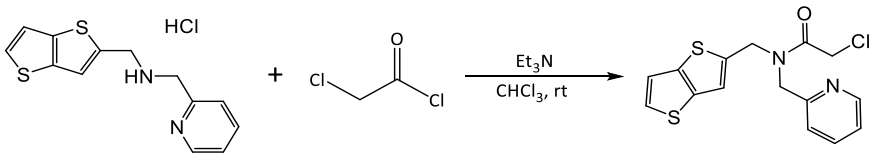


To chloroform solution (10 ml) were added successively aldehyde (1.0 equiv), amine (1.0 equiv), $NaBH(OAc)_3$ (1.2 equiv) at room temperature. The reaction mixture was stirred at room temperature for 1 h. Later, the solution was diluted with water (50 ml) and extracted with dichloromethane (3 × 20 ml). The combined organic layers were evaporated to give the crude product, which was purified by column chromatography on silica gel using hexane-ethyl acetate (10:1) as eluent to give pure products; amine was obtained in 84% yield, yellow solid.

^{1}H NMR (400 MHz, DMSO-D_6) δ 9.97 (*s*, 2H), 8.59 (*ddt*, *J* = 6.2, 2.8, 1.4 Hz, 1H), 7.83 (*tt*, *J* = 7.7, 1.8 Hz, 1H), 7.70 (*dd*, *J* = 5.3, 1.0 Hz, 1H), 7.65 (*dd*, *J* = 4.7, 2.6 Hz, 1H), 7.55–7.48 (*m*, 1H), 7.43 (*d*, *J* = 5.2 Hz, 1H), 7.38 (*dddt*, *J* = 6.0, 4.8, 2.3, 1.2 Hz, 1H), 4.49 (*d*, *J* = 1.6 Hz, 2H), 4.26 (*d*, *J* = 2.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-D_6) δ 152.53, 149.55, 140.82, 138.59, 137.83, 135.26, 129.79, 124.16, 124.09, 124.02, 120.58, 49.78, 45.69.

Synthesis of 2-chloro-N-(pyridin-2-ylmethyl)-N-(thieno[3,2-b]thiophen-2-ylmethyl)acetamide.



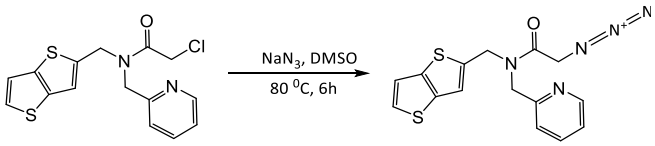
To a solution of 1-(pyridin-2-yl)-N-(thieno[3,2-b] thiophen-2-ylmethyl)-methanamine hydrochloride (1.0 equiv) in chloroform (20 ml) chloro acetyl chloride (1.2 equiv) was added dropwise at 0°C and stirred for 30 min, then stirred at rt for further 6 h. The reaction mixture was diluted with diethyl ether and washed with aq. HCl 1 M, aq. sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent evaporated. The crude product was purified by column chromatography (hexane/ethyl acetate 5/1) to give amide in 94% yield, yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74–7.62 (*m*, 1H), 7.25 (*s*, 4H), 6.98–6.89 (*m*, 1H), 6.88 (*dd*, *J* = 5.1, 3.5 Hz,

1H), 6.83–6.76 (*m*, 1H), 5.61 (*dd*, *J* = 15.0, 0.8 Hz, 1H), 4.34 (*d*, *J* = 14.9 Hz, 1H), 3.81 (*d*, *J* = 13.5 Hz, 2H), 3.72 (*d*, *J* = 13.6 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.48, 169.96, 166.51, 163.70, 161.21, 136.71, 136.63, 136.60, 130.22, 130.14, 128.23, 126.36, 124.49, 117.07, 116.84, 60.58, 48.72, 41.76, 40.93, 21.14, 14.27.

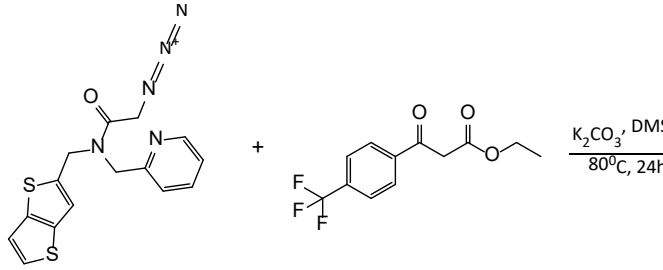
Synthesis of 2-azido-N-(pyridin-2-ylmethyl)-N-(thieno[3,2-b]thiophen-2-ylmethyl)acetamide.



2-Chloro-N-(pyridin-2-ylmethyl)-N-(thieno[3,2-b] thiophen-2-ylmethyl)acetamide (1.0 equiv.) and NaN_3 (2.0 equiv.) were dissolved in dimethylformamide (5 mL) in a round-bottomed flask. The resulting mixture was stirred at 80°C for 6 h, after which water (250 mL) and dichloromethane were added. The aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and then concentrated in vacuo to a residue, which was purified by flash column chromatography (hexane/ethyl acetate 4/1) to afford the title compound in yield 89%. White solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (*s*, 1H), 7.58 (*td*, *J* = 7.6, 1.7 Hz, 1H), 7.52 (*d*, *J* = 4.9 Hz, 1H), 7.32–7.25 (m, 2H), 7.17 (*ddd*, *J* = 7.3, 4.1, 1.4 Hz, 1H), 6.90 (*q*, *J* = 0.7 Hz, 1H), 4.74 (*d*, *J* = 1.0 Hz, 2H), 4.62 (*d*, *J* = 0.7 Hz, 2H), 3.73 (*s*, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.50, 154.60, 149.74, 143.07, 139.09, 138.89, 136.56, 131.91, 124.50, 122.92, 122.34, 122.24, 51.28, 50.03, 47.74.



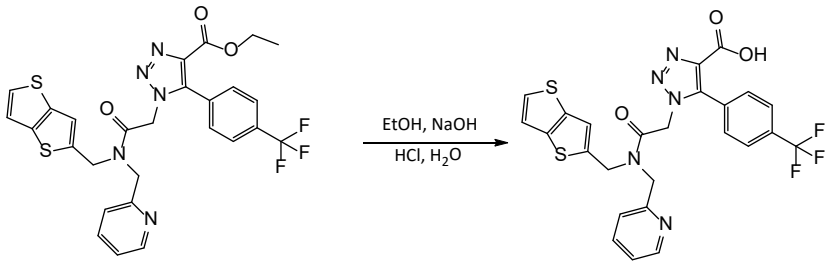
Synthesis of ethyl 1-(2-oxo-2-((pyridin-2-ylmethyl) (thieno[3,2-b]thiophen-2-ylmethyl)amino)ethyl)-5-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole-4-carboxylate.

To a solution of 2-azido-N-(pyridin-2-ylmethyl)-N-(thieno[3,2-b]thiophen-2-ylmethyl)acetamide (1.0 equiv) and ethyl 3-oxo-3-(4-(trifluoromethyl) phenyl)-propanoate (1.2 equiv) in DMSO (4 mL), K_2CO_3 (10.0 equiv) was added. The reaction mixture was stirred at 80°C for 24 hours. After cooling, water was added and the mixture was extracted with ethyl acetate, the organic layer was dried on $MgSO_4$ and after filtration was evaporated to dryness. The residue was purified by flash column chromatography (hexane/ethyl acetate 1/1) to obtain the product. The product yield 87%, yellow solid.

^{1}H NMR (400 MHz, DMSO-D_6) δ 8.58–8.50 (*m*, 1H), 7.82–7.69 (*m*, 3H), 7.67–7.61 (*m*, 2H), 7.56 (*d*, *J* = 5.0 Hz, 1H), 7.04 (*d*, *J* = 0.9 Hz, 1H), 5.08 (*s*, 2H), 4.68 (*d*, *J* = 0.8 Hz, 2H), 4.51 (*d*, *J* = 0.7 Hz, 2H), 4.39 (*q*, *J* = 6.4 Hz, 2H), 1.32 (*t*, *J* = 6.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-D_6) δ 168.51, 161.70, 157.02, 149.82, 142.68, 139.62, 139.11, 138.91, 138.09, 137.07, 133.45, 131.83 (*q*, *J* = 4.2 Hz), 131.29, 130.90 (*d*, *J* = 13.3 Hz), 127.08 (*q*, *J* = 4.8 Hz), 125.23, 125.01, 122.86–122.38 (*m*), 122.18, 61.14, 52.01, 50.63, 47.13, 14.09. MS (ESI+) m/z calculated for $C_{27}H_{22}F_3N_5O_3S_2$ $[M+H]^+$ 586.1, found 586.3.

Synthesis of 1-(2-Oxo-2-((pyridin-2-ylmethyl)(thieno[3,2-b]thiophen-2-ylmethyl)amino)ethyl)-5-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole-4-carboxylic acid.



Ethyl 1-(2-oxo-2-((pyridin-2-ylmethyl)(thieno[3,2-b]thiophen-2-ylmethyl)-amino)ethyl)-5-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole-4-carboxylate (1.0 equiv) was dissolved in ethanol (300 mL). Aqueous NaOH (2.0 M, 50.8 mL, 101.6 mmol, 2.0 equiv) was added, and the reaction mixture was stirred at 20°C. Reaction progress was monitored by TLC and was complete after 4 h. Methanol was removed under reduced pressure (bath temperature ≤ 40°C). The residue was diluted with water (30 mL) and acidified to pH 4 by slow addition of 2.0 M HCl (ice bath recommended to control exotherm). The resulting precipitate was collected by vacuum filtration, washed with cold water (2 × 20 mL), and dried in vacuo at 40°C to constant weight to give acids as a solid (79% yield).

^{1}H NMR (400 MHz, DMSO-D_6) δ 8.59–8.51 (*m*, 1H), 7.82–7.69 (*m*, 3H), 7.68–7.59 (m, 2H), 7.54 (*d*, *J* = 5.0 Hz, 1H), 7.36–7.28 (*m*, 3H), 7.04–6.99 (*m*, 1H), 5.08 (*s*, 2H), 4.68 (*d*, *J* = 0.8 Hz, 2H), 4.51 (*d*, *J* = 0.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-D_6) δ 168.49, 164.37, 157.05, 149.74, 142.68, 139.24–138.81 (*m*), 137.07, 136.28, 133.55, 131.86 (*q*, *J* = 4.2 Hz), 131.29, 130.92 (*d*, *J* = 13.3 Hz), 127.09 (*q*, *J* = 4.7 Hz), 125.23, 125.01, 122.86–122.38 (*m*), 122.18, 52.00, 50.65, 47.14. MS (ESI+) m/z calculated for $C_25H_{18}F_3N_5O_3S_2$ $[M+H]^+$ 558.0, found 558.2.

5. Discussion of research results

Analysis of the synthetic route using the EcoScale program indicated an average environmental impact score of 63.6 for the laboratory method of synthesizing the HIT compound, highlighting potential environmental issues in the processes.

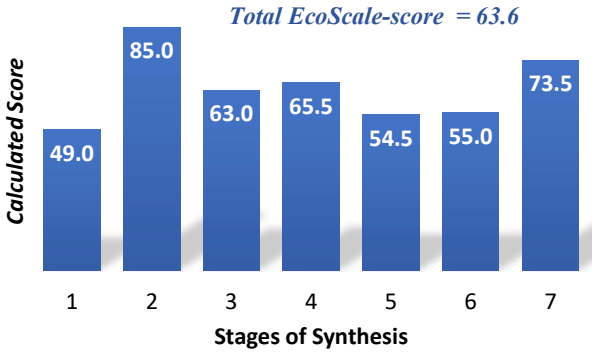


Fig. 2. Histogram comparing the environmental impact scores of different stages of synthesis (Fig. 1), calculated using the EcoScale program

Fig. 2 indicates that the most problematic stage is the first stage of synthesis (Fig. 1), which has a calculated score of 49 in the EcoScale program. The primary risks associated with this stage relate to the health and environmental hazards posed by the starting materials: thie-no(3,2-b)thiophene, phosphorus oxychloride, N,N-dimethylformamide, and dichloromethane. Due to these concerns, the EcoScale program has assigned penalty points to this stage. Additionally, this stage has a low yield (70%) of the intermediate product, thieno[3,2-b]thiophene-2-carbaldehyde. However, this product is produced on an industrial scale and is relatively inexpensive to purchase [25]. Consequently, it may be possible to exclude stage 1 from the process.

Stage 5 of the synthesis process also demonstrates low calculated environmental impact scores. In this stage, ethyl 3-oxo-3-(4-trifluoromethylphenyl) propanoate (CAS 106263-53-0) is produced as the starting material for stage 6. This compound is commercially available and is manufactured on an industrial scale. Additionally, it can be sourced from multiple manufacturers [25], allowing for the possibility of excluding stage 5 from the process.

Additionally, stages 6 and 7 can be combined because modifying the synthesis conditions enables the hydrolysis of ethyl ester into the target acid without needing to separate it from the reaction medium.

The use of problematic solvents, such as chloroform, DMF, cyclohexane, and methanol, as well as hazardous chemicals like sodium hydride and sodium azide, negatively impacts the environmental profile of the synthesis process. In our optimization of the synthesis (as shown in Fig. 1), we eliminated steps 1 and 5 by substituting the production of the starting material with a commercially available product. Additionally, we combined stages 6 and 7 into one step, which allowed us to avoid using solvents like cyclohexane, DMF, and methanol. For stages 4 and 6, we replaced DMF with DMSO, since both are commonly used polar aprotic solvents; however, DMSO has superior environmental characteristics [26].

The use of chloroform in large-scale synthesis is undesirable because it is classified as a chlorofluorocarbon, which falls under the regulations of the Montreal Protocol on Substances that Deplete the Ozone Layer [27]. As a result, the use of first-level ozone-depleting substances, such as chlorofluorocarbons (CFCs), and second-level ozone-depleting substances, like hydrochlorofluorocarbons (HCFCs), has been almost entirely phased out. While fluorinated alkenyl esters, such as hydrofluoroolefins (HFOs) and hydrofluoroethers (HFEs), are more environmentally friendly and easily degradable, their widespread adoption is still a long way off [28, 29]. Alternatives to chloroform, such as 2-methyltetrahydrofuran (2-MTF) [30] and tert-butyl methyl ether [31], may be suitable for certain applications, but they are not exact substitutes for chloroform. This topic requires further research.

We optimized the initial laboratory synthesis scheme (Fig. 1) and developed an improved version (Fig. 3). The optimized synthesis scheme consists of four stages and demonstrates better performance indicators, including total mass intensity of the PMI process, PMI for reagents, PMI for solvents, and a higher calculated total EcoScale score.

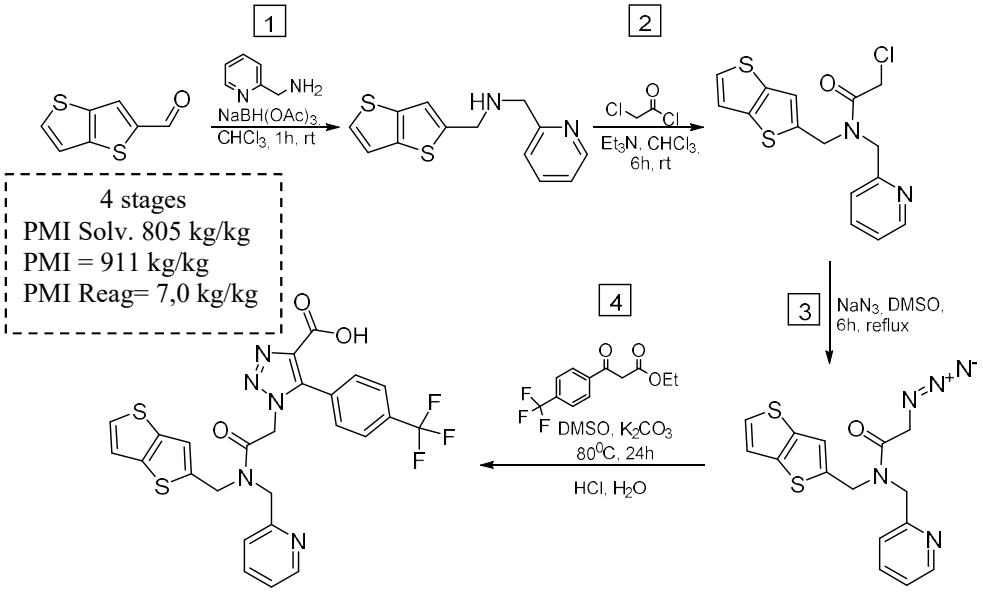


Fig. 3. Optimized method for synthesizing HIT compounds

Analysis of the synthetic route in accordance with Fig. 3, using the EcoScale program, revealed an average EcoScale score of 72.6, as summarized in Fig. 4. This indicates that the optimization of the synthetic route enhanced both the environmental performance and efficiency of the synthesis process.

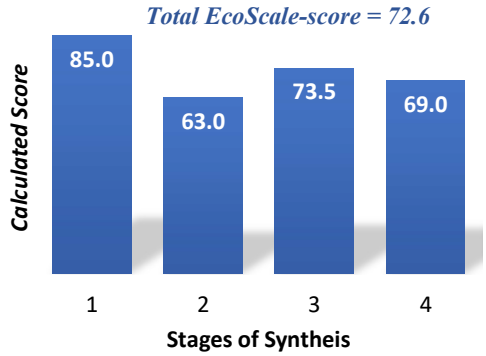


Fig. 4. Histogram comparing the environmental impact scores of different stages of synthesis (Fig. 3), calculated using the EcoScale program

Practical relevance. The practical relevance of our study is twofold. First, we provide an optimization of synthetic pathways by using alternative solvents and reducing the number of synthesis stages. This aims to improve the environmental aspects of producing the target molecule (HIT). Second, our optimization procedure can be scaled up to create a general protocol for developing safer and more efficient processes, ultimately leading to the production of various new pharmaceutical compounds.

Limitations of the study. EcoScale software is intended for laboratory-scale synthesis of substances. It utilizes a calculation method based on specific algorithms and databases. This assessment method provides a quick overview of the environmental aspects of the synthesis process at the early stages of research, facilitating faster decision-making. However, it is important to note that this approach cannot fully capture all the complexities of the actual industrial process.

Prospects for further research. We see the prospect for further research in conducting additional optimization of synthesis when scaling the process, considering safety, environmental impact, and critical quality parameters laid down in regulatory requirements.

6. Conclusions

The development of a new non-covalent inhibitor of the SARS-CoV-2 main protease (M^{pro}) is highly important for several reasons, primarily due to its potential to offer an improved therapeutic option over existing treatments [32–35]. By analyzing and quantitatively assessing the environmental characteristics of the synthetic routes and optimized the synthesis method of compound HIT for further scaling of the technology we found the following:

1. The efficiency and environmental friendliness of the synthesis processes of a new molecule, 1-(2-oxo-2-((pyridin-2-ylmethyl)(thieno[3,2-b]thiophene-2-ylmethyl)amino)ethyl)-5-(4(trifluoromethyl) phenyl)-1H-1,2,3-triazole-4-carboxylic acid (HIT), which has the potential to become an active pharmaceutical ingredient in drugs as an inhibitor of the main protease of the SARS-CoV-2 virus.

2. The change in the number of synthesis stages and the replacement of solvents with more environmentally friendly and safer ones is justified.

3. Using the EcoScale program, the coefficients (EcoScale scores) of the environmental component of each stage and the overall synthesis scheme were calculated, which made it possible to assess their environmental friendliness.

4. Calculated process mass intensity (PMI) values, including PMI for reagents and solvents

5. The synthetic route for the synthesis of a new promising pharmaceutical substance was optimized, which made it possible to improve both environmental performance and process efficiency for further scaling up of the technology.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in creating the submitted work.

Authors’ contributions

Anna Geleverya, Nataliia Koval: Investigation, Formal analysis, Data curation. **Larysa Yevsieieva**: Conceptualization, Validation, Writing – review & editing, Writing – original draft, Methodology. **Alexander Kyrychenko**: Conceptualization, Validation, Writing – review & editing, Writing – original draft, Methodology. **Sergiy M. Kovalenko**: Conceptualization, Supervision, Writing – review & editing. **Oleg Kalugin**: Conceptualization, Supervision, Project administration, Funding acquisition.

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