doi.org/10.36721/PJPS.2024.37.3.REG.541-550.1

Development, synthesis and biological evaluation of imidazole [2,1-*b*] thiazole derivatives containing an indole ring for their potential anti-inflammatory properties

Yu-Cai Jiang[1]*, Hai-Feng Lin[2], Zhi-Wei Chen[3] and Li-Li Zheng[1]
[1]Department of Pharmacy, Affiliated Hospital of Putian University, Putian, China
[2]Department of Gastroenterology, Affiliated Hospital of Putian University, Putian, China
[3]Department of Pathology, Affiliated Hospital of Putian University, Putian, China

Abstract: In this study, in order to further search anti-inflammatory drugs with high efficiency and low toxicity, this study took the ring of indoles and imidazole [2,1-*b*] thiazole as the main skeleton. A total of nine new *N*-1-substituted derivatives of indole-2-carboxyamide-phenylimidazoles [2,1-*b*] thiazole (13-20) was synthesized through the processes of cyclization, amino reduction, ester hydrolysis, dehydration condensation, and acyl chloride substitution. These derivatives were then tested for their ability to reduce inflammation in RAW 264.7 macrophages. There was a significant majority of these compounds that effectively suppressed the production of NO, IL-6, and TNF-α in RAW 264.7 cells that were stimulated by LPS. One of these compounds, compound 19, was shown to be capable of efficiently lowering the levels of LPS-induced over expression of a number of inflammatory mediators. The inhibition rates for compound 19 were 54.66%, 68.82% and 43.74%, respectively. Additionally, an initial structure-activity relationship evaluation was carried out. The findings indicate that the incorporation of substituted benzyl moieties at the same position provided *N*-benzylation compounds with a positive anti-inflammatory effect. The electrophilicity of the substituent on the benzyl group had the potential to have an effect on the anti-inflammatory effect, which is something that calls for further investigation.

Keywords: Imidazole [2,1-*b*] thiazole, indole, anti-inflammatory activity.

INTRODUCTION

One of the most important physiological responses that is triggered to protect against illnesses and damage to tissues that are caused by pathogens is inflammation (Kim *et al.*, 2023). However, if these inflammatory responses persist for an extended period of time, they can increase the risk of developing a range of autoimmune disorders, respiratory conditions, osteoarticular ailments and rheumatoid joint diseases (Zhao *et al.*, 2018, Luan *et al.*, 2022). Macrophages play a crucial role in regulating host defense by producing pro-inflammatory mediators, including nitric oxide (NO), interleukin-6 (IL-6), in addition to tumor necrosis factor-α (TNF-α) (Das 2023, Wang *et al.*, 2018). While they contribute to antimicrobial responses, they can also have detrimental effects such as septic infection and acute lung injury (Wang *et al.*, 2023, Cohen 2002, Schulte *et al.*, 2013, Imam *et al.*, 2015). It has been confirmed that NO, IL-6 and TNF-α can regulate the occurrence of septic infection through various cytokine signaling pathways (Lazarov *et al.*, 2023, Naffaa *et al.*, 2013, Lv *et al.*, 2014). These observations strongly indicate that endogenous ignition contributes significantly to the septic infection's growth throughout its acute stage. Therefore, Targeting and inhibiting the pro-inflammatory factors may be an appropriate approach to developing new therapeutic strategies (Johnston and Webster *et al.*, 2009, Merrill *et al.*, 2011).

Recent years have seen a tremendous increase in the amount of attention paid to the chemistry and biology of fusion heterocyclic systems. This is mostly owing to the wide variety of physiological functions that these systems exhibit. (Cooper *et al.*, 2023, Bertamino *et al.*, 2015, Parrino *et al.*, 2015). Imidazole [2,1-*b*] thiazole compounds have attracted significant attention within the realm of heterocyclic compounds because of their wide array of pharmacological effects, which have been widely demonstrated to possess antibacterial activity (Guzeldemirci *et al.*, 2010, Cascioferro *et al.*, 2019), antituberculosis activity (Chitti *et al.*, 2022), antifungal activity (Sbenati *et al.*, 2021), antitumor activity (Kamal *et al.*, 2010), antiviral activity (Barradas *et al.*, 2011, Pehlivan *et al.*, 2018), anthelminthic activity (Shetty *et al.*, 2010), analgesic activity (Shahrasbi *et al.*, 2018), anti-inflammatory property (Bhongade *et al.*, 2016), hypotensive effects (Andreani *et al.*, 1992), cardiovascular impacts (Andreani *et al.*, 1985) as well as diuretic properties (Andreani *et al.*, 1987) and herbicide activities (Andreani *et al.*, 1996). The rise in the number of experimental investigations that have been carried out in this area may be attributed to the fact that, over the course of the last few years, there has been a rising curiosity among researchers in investigating the possible

**Corresponding author:* e-mail: taiwanhuigui2030@sina.com

medicinal applications of imidazole [2,1-*b*] thiazole derivatives. The anti-inflammatory properties of the compounds (1) and (2) have been proved to be outstanding in experimental animals (Shetty *et al.,* 2010).

The structural groups of indole derivatives are of utmost significance in the area of medicinal chemistry. This is due to the fact that these specific groups are often discovered in naturally occurring and bioactive compounds (Song *et al.,* 2019). In addition, a multitude of additional studies have showcased the compound's immense promise in the realm of disease management. Indole demonstrates remarkable anti-inflammatory activity and is extensively utilized as a key pharmacophore in the development of NSAIDs for treating clinically significant inflammatory diseases. For instance, in 1965, the U.S. approved indomethacin (3) for the treatment of pyrexia, analgesia, and inflammation-caused edema. Acetomethicine (4) has shown significant therapeutic effects in both rheumatoid arthritis and osteoarthritis. Pfizer manufactures the antirheumatic drug tenidap (5), which exhibits exceptional properties for treating rheumatic conditions. By suppressing the formation of prostaglandins and other substances that induce inflammatory symptoms, indole anti-inflammatory medications, which are extensively used in the medical profession, are able to successfully decrease fever, discomfort, stiffness and edema. However, despite their significant therapeutic benefits, these drugs have potential side effects including headaches, heartburns, diarrhea, skin redness or allergic reactions and intestinal gas.

We hypothesize that this chemical includes these two structures and speculate on its potential significance throughout an inflammatory agent treatment, based on the prevalence of imidazole [2,1-*b*] thiazoles and indole derivatives in nature and their influence on pharmaceutical chemistry. Given ongoing interest in discovering novel compounds with anti-inflammatory properties, we have developed and synthesized indole-ring derivatives of imidazole [2,1-*b*] thiazoles to investigate their in vitro anti-inflammatory activity by evaluating their intervention against the production of various pro-inflammatory factors (Scheme 1).

MATERIALS AND METHODS

Materials

In general, reagent grade chemical agents and organic solvents are typically not subjected to additional purification processes. (Changzheng Chemical Plant, Chengdu, Sichuan, China). TLC experiments were performed on 0.20 mm silica gel 60 F254 plates (Qingdao Marine Chemical Plant, Shandong, China). The melting point was determined using a WRS-1B melting device without correction. The nuclear magnetic resonance (NMR) spectra, which included ^{1}H and ^{13}C, were acquired by using a Bruker apparatus inside a DMSO-d_6 solution and running at either 400 or 100 MHz. The peak positions were determined by referencing them to tetramethylsilane parts per million (δ). *J* is measured in Hertz. Mass spectra were obtained using Agilent 6210 ESI/TOF or Agilent 6545 LC/Q-TOF mass spectrometers. All synthesized compounds were confirmed to have a purity level exceeding 95% through HPLC analysis.

Synthesis of ethyl 6-(4-nitrophenyl) imidazole [2,1-b] thiazole-3-carboxylate (8)

In a solution of dioxane (100mL), the 2-aminothiazol-4-carboxylate (6) (4.2g, 24.2mmol) was thoroughly mixed with 2-bromoacetophenone) (5.9g, 24.2mmol). Over the course of 12 hours, the solution was refluxed at 110°C. After it had cooled to the temperature of the surrounding environment, the resultant precipitate that was produced was passed through filtering through silica gel. Following three 50mL ethanol washes, the hydrobromides were dried to produce the compound (8), which was a light yellow powder weighing 6.31g with a yield of 82%.

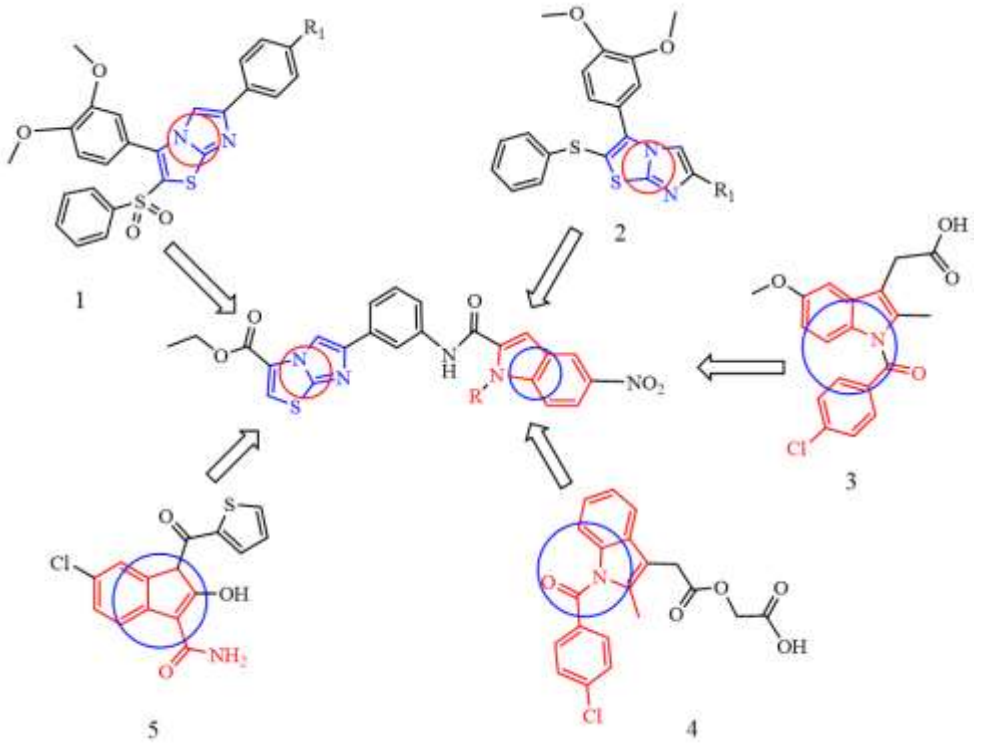
Synthesis of ethyl 6-(4-aminophenyl) imidazole [2,1-b] thiazole-3-carboxylate (9)

At room temperature, the tin chloride (II) dihydrate (3.5g, 15.6mmol) was then poured into concentrated hydrochloric acid (25mL) and the liquid was vigorously agitated. Subsequently, the mixed solution was supplemented with the compound (8) (1.0g, 3.2mmol). After that, There was a rise in temperature to 65°C and the entire mixture was swirled for ten minutes to generate a pink suspension. Afterward, the temperature was reduced and continued stirring for an additional hour before collecting the solid through suction filtration.

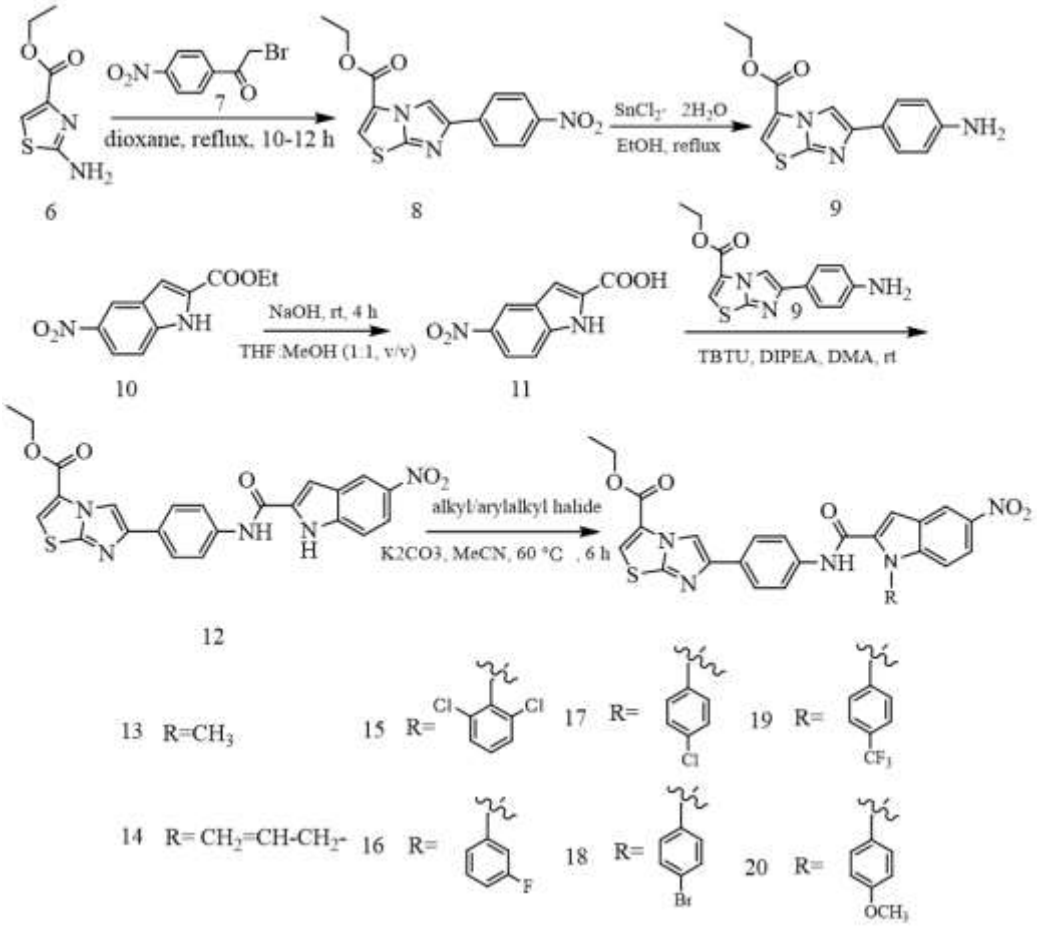
After dissolving the obtained solid in water, sodium hydroxide was added in order to get the pH level up to a level higher than 7. The resulting solution underwent extraction with water and dichloromethane, followed by drying of the organic phase using $MgSO_4$. After going through the filtering process, the solvent became highly concentrated, which led to the production of a solid that was light yellow in color and weighed 622mg. This solid was identified as the chemical called (9). The yield was 70.2%. mp157.1-158.2°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.41 (3H, t, *J* 7.2), 4.44 (2H, q, *J* 7.2), 5.17 (2H, s), 6.52-6.57 (1H, m), 7.08 (2H, d, *J* 6.6),7.18 (1H, s), 8.28 (2H, d, *J* 19.7); ^{13}C-NMR (DMSO-d_6, 101): 14.5, 62.3, 110.2, 111.0, 113.3, 113.7, 123.4, 124.7, 129.6, 134.7, 148.0, 148.4, 149.3, 158.0; HR-EI-MS, (*m/z*): 288.1137 $[M+ H]^+$ ($C_{14}H_{13}N_3O_2S$: 287.34).

Synthesis of 5-nitro-1H-indole-2-carboxylic acid (11)

The compound (10) (23.6g, 0.1mol) was then immersed in a solution of tetrahydrofuran and methanol (V:V = 1:1) at the ambient temperature with a volume of 500mL.



Scheme 1: The concept of the design of the structural formula of the target compound



Scheme 2: The synthetic pathways of the structures that are the targets (13-20)

Subsequently, the combination then formed a dark reddish-brown solution with the addition of sodium hydroxide solution (40g, 1.0mol). After three hours at the temperature of the surroundings, water has been incorporated to the reaction mixture in order to quench it. Following the removal of tetrahydrofuran (THF) solvent by vacuuming, diluted hydrochloric acid was used to acidify the mixture until it reached a specific level of acidity (pH=1). The precipitate that was produced was collected using vacuum filtering and the residual dissolved products were extracted twice using a combination of tetrahydrofuran and ethyl acetate with a volume-to-volume ratio of 1:2, each time using 400mL. Ultimately, the organic layer obtained from the extraction procedure was mixed with the precipitate generated through filtration after it had been dissolved in THF. The dehydration of the composite organic layer is accomplished with the help of anhydrous magnesium sulfate, and the enrichment of the filtering process is carried out in a vacuum environment. Recrystallization of this concentrate utilizing tetrahydrofuran: ethyl acetate: hexane as solvents in a ratio of (V: V: V=1:1:1) yielded white solid product, the compound (11) weighing at 19.6 mg with a yield of 88.6%.

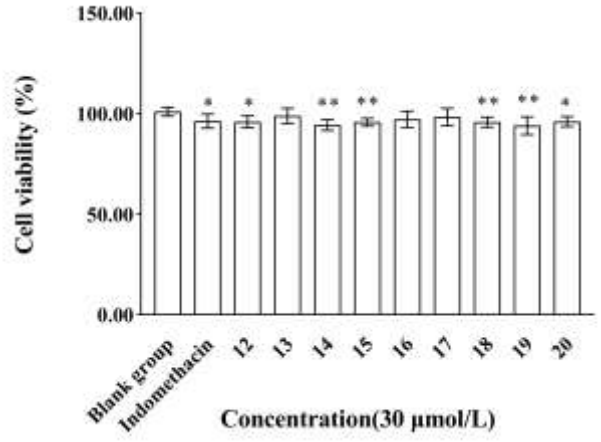


Fig. 1: The effects of title complexes on RAW264.7 macrophages that are responsible for cytotoxicity (**P*<0.05, ***P*<0.01, compare with blank group)

Synthesis of Ethyl 6-(4-(5-nitro-1H-indole-2-carboxamido) phenyl) imidazole [2,1-b] thiazole-3-carboxylate (12)

The compound (11) (0.5mg, 0.5mol) was dissolved in dimethyladipate (DMA) at 25°C. In the subsequent step, the suspension was gradually combined mixed with tributylthiourea (TBTU) and N, N-Diisopropylethylamine (DIPEA) for five minutes. Following that step, the mixture was left to stir overnight after adding compound (9) (143.5mg, 0.5mol). After concentration, it was diluted with the appropriate amount of aqueous sodium bicarbonate solution and ethyl acetate. Then, it was filtered, purified and resuspended in a 1 mol·L^{-1} aqueous sodium dihydrogen phosphate solution. This process of vacuum drying was repeated until a light yellow solid compound was obtained, which was then dried. The compound (12) weighing at 182.2mg with a yield of 76.51%. mp 143.1-145.1°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.39 (3H, t, *J* 7.2), 4.41-4.46 (2H, m), 7.64 (1H, d, *J* 9.1), 7.70 (1H, s), 7.88 (2H, d, *J* 8.7), 7.92 (2H, d, *J* 8.6), 8.12 (1H, dd, *J* 9.1 and 2.0), 8.26 (1H, s), 8.45 (1H, s), 8.77 (1H, d, *J* 1.9), 10.63 (1H, s), 12.63 (1H, s); ^{13}C-NMR (DMSO-d_6,101), δ_C: 14.52, 62.32, 106.87, 110.41, 113.45, 119.17, 119.76, 120.84×2, 123.45, 124.78, 125.80×2, 126.73, 129.86, 135.58, 138.37, 140.09, 141.79, 146.91, 148.69, 157.95, 159.22; HR-EI-MS, (*m/z*): 476.1028[M+ H]$^+$ ($C_{23}H_{17}N_5O_5S$: 475.110).

Standard method of operation for the synthesis of imidazole [2,1-b] thiazole derivatives (13-20)

In dry acetonitrile, compound (12) was dissolved. Equimolar alkyl or arylalkyl halides at room temperature were added after a suitable quantity of potassium carbonate (1mmol) was progressively added. The resultant solution was agitated for eight hours at 25°C. Under vacuum conditions, the solvent acetonitrile is allowed to evaporate at the conclusion of the procedure. The residue should be combined with 10mL of purified water and 20mL of ethyl acetate in a volume of three. To create a crude product, over anhydrous magnesium sulfate, the organic layer was allowed to dry, cleaned with a saturated sodium chloride solution (50mL), filtered and concentrated. With the use of silica gel column chromatography and a mixture of ethyl acetate and petroleum ether at a ratio of 1:5, compounds (13-20) were produced during the purification process.

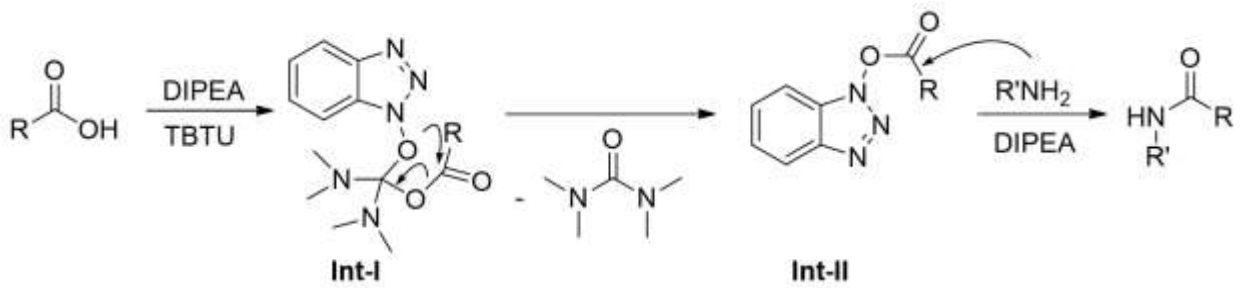
The study of bioactivity in vitro

Cell cultivation

Procell Co. Ltd. provided the RAW 264.7 macrophages and matching DMEMs (Wuhan, China). In the course of their incubation at 37 degrees Celsius and 5% carbon dioxide, the cells were given 10% fetal bovine serum (Hyclone, Logan, USA), 100mg/mL of streptomycin and 100U/mL of penicillin by the manufacturer. The passage of the cells occurred after they had reached a confluence of between 70% and 80%.

The cytotoxicity was assessed using the CCK8 assay

The RAW264.7 cells, which were in the logarithmic growth phase, were planted in 96-well culture plates at a density of 5×10^4 cells per ml. Each well received 100μL of the culture medium. The experiment was divided into three groups: a negative control group (complete medium without cells), a blank control group (complete medium with cells) and a drug treatment group (compound concentration of 30μmol·L^{-1}). After culturing the cells as described above for 24 hours at 37°C and 5% CO_2, each well was supplemented with 10μL of CCK-8 solution. The amount of absorption.



Scheme 3: The specific mechanism of preparing compound 12

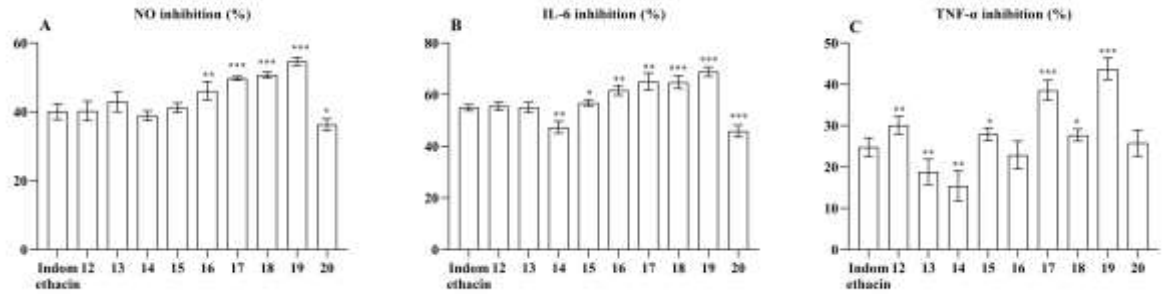


Fig. 2: The impact of title complexes on LPS-induced RAW264.7 macrophages' production of NO, IL-6, and TNF-α (**P*<0.05, ***P*<0.01, ****P*<0.001, compare with indometacin group)

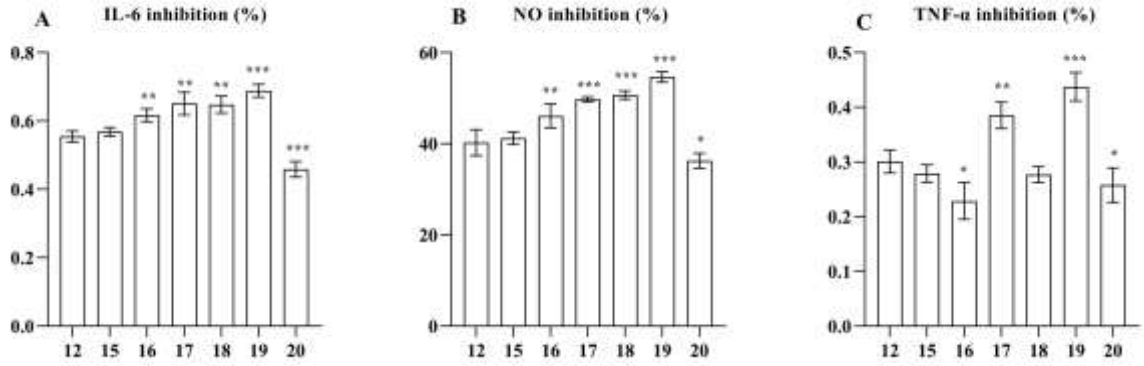


Fig. 3: The impact of N-benzyl derivatives (15-20) on LPS-induced RAW264.7 macrophages' production of NO, IL-6, and TNF-α (**P*<0.05, ***P*<0.01, ****P*<0.001, compare with compound 12)

A micro plate reader was used to measure a value at 450 nm in triplicate and the following formula was used to determine the cell viability of the samples. Cell viability = ($A_{\text{drug treatment group}}$ - $A_{\text{negative control group}}$) / ($A_{\text{blank control group}}$ - $A_{\text{negative control group}}$) ×100%.

The quantification of inflammatory mediators

The organization and intervention of cells

The experiment was carried out using three distinct groups: the blank group (LPS 0.5mg·L^{-1}), the control group (indomethacin 10μmol·L^{-1}) and the sample group (12-20 compound 10μmol·L^{-1}). Seeding was performed at a density of 4×10^5 cells per milliliter for RAW264.7 cells that were in the logarithmic growth phase in 24-well plates and incubated for 24 hours prior to drug administration. In the indomethacin and experimental groups, macrophages were 30 minutes, followed by stimulation with LPS (0.5mg·$L^{-1)}$ and then incubated for an additional 24 hours.

The impact of target compounds on the level of NO released from LPS-induced RAW264.7 cells

The Griess technique was used in order to determine the amount of nitric oxide (NO) that was present in the supernatant of RAW264.7 cells that had been stimulated by LPS. The NO content in each was determined by referencing the $NaNO_2$ standard curve. The experiment was conducted in both the blank group and the experimental group (the control group and the sample group). The inhibition rate, expressed as a percentage, was using the formula: ($NO_{\text{blank group}}$ - $NO_{\text{experimental group}}$) / $NO_{\text{blank group}}$ × 100%.

The impact of certain substances on the production of cytokines that promote inflammation in RAW264.7 cells that have been activated by lipopolysaccharide (LPS)

ELISA was utilized to evaluate the impact of LPS on the release of TNF-α and IL-6 in RAW264.7 from each group,

including the blank and experimental groups (The control group and the sample group), was meticulously collected and analyzed following kit instructions. Test samples were added to each well, along with HRP-labeled test antibodies in control and sample wells. After incubating at 37ºC for 60 minutes, reaction wells were sealed with a plate sealing membrane before being washed with a diluted concentrated solution. Substrates A and B (50μL) were then added to each well followed by incubation at 37ºC in the dark for 15 min; termination solution (50μL was subsequently added to stop the reaction before measuring A values at a wavelength of 450 nm for each well. Each experiment was repeated five times. Inhibition rate / % = $(A_{blank\ group} - A_{experimental\ group})/A_{blank\ group} \times 100\%$.

STATISTICAL ANALYSIS

We performed on the data obtained from the paper using GraphPad Prism 9.5.1. All analysis were carried out in triplicate. The results will be presented as mean ± SD, unless otherwise specified. Two-sided t-tests were used to assess differences between treatments. A statistically significant difference was considered when $p < 0.05$.

RESULTS

Synthesis

The synthetic route presented in experimental scheme 2 was successfully employed to synthesize the imidazole [2,1-*b*] thiazole derivatives (13-20) (table1). Firstly, the reaction between compound (6) and compound (7) yielded the intermediate nitrobenzene (8), which was then reduced to obtain the corresponding aniline. The successful synthesis of intermediate (8) represents a significant milestone in the overall synthetic process. To maximize the yield, we followed relevant literature and performed a reflux reaction at 110ºC for 12 hours after comparing different synthesis conditions. After achieving complete dissolution of 1,4-dioxane by stirring, we terminated the reaction, and we let it to return to ambient temperature. Finally, we extracted the resulting light yellow solid using ethanol. Subsequently, commercial compound (10) underwent hydrolysis under alkaline conditions to produce compound (11). This compound was coupled with the appropriate arylamine (9) to form the desired compound (12).

N-methylation at the *N*-1 position of compound (12)'s indole framework was easily achieved under mild alkaline conditions due to its relatively weak acidity. Finally, compounds ranging from (13) to (20) were obtained through this process. Because various alkyl or aryl alkyl halides have variable fat solubility, the solvent choice is critical during the synthesis of compounds (13-20). On top of that, the yield is very sensitive to the reaction time and temperature. Review of the literature indicates that acetonitrile is the best solvent to use in this experiment, with a reaction temperature range of 50-60 ºC and a duration range of 5-7 hours. By using thin-layer chromatography, one may track the reaction's progress. We're convinced that the compound (13-20) described here to be a first-of-its-kind find. The novel derivatives were characterized using techniques such as ^{1}HNMR, ^{13}CNMR and high resolution electrospray ionization mass spectrometry (HR-EI-MS).

The characterisation ^{1}H-NMR, ^{13}C-NMR, HR-MS data of target compounds (13-20) are presented in the Supporting Information.

Anti-inflammatory evaluation

Cell Viability study

The preliminary toxicity evaluation was conducted for all novel derivatives (13-20). As shown in fig. 1, the survival rates of RAW264.7 macrophages exceeded 94% at a concentration of 30μM. This observation implies that these compounds are non-toxic within the tested concentration range of 30μM and can be used for further investigation into their activity.

Initial evaluation of Effects on LPS-Induced NO, IL-6, and TNF-α Release

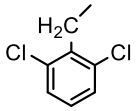
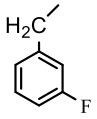
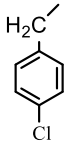
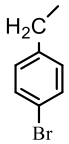
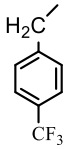
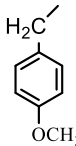
When evaluating the effectiveness of anti-inflammatory treatments, the use of inflammatory cytokines, such as NO, IL-6 and TNF-α, is often seen. These cytokines play a significant role in the onset of several inflammatory diseases. As shown in Fig. 2, the compound demonstrates significant inhibitory activity against inflammatory factors such as NO, IL-6 and TNF-α. In terms of NO and IL-6 inhibition experiments, all compounds except for 14 and 20 exhibit higher inhibitory activity than indomethacin. Additionally, most of these compounds show notable TNF-α inhibition surpassing that of indomethacin. The most remarkable compound is 19, which exhibits the highest inhibitory rates against NO (54.66%), IL-6 (68.82%) and TNF-α (43.74%).

DISCUSSION

Synthesis

Important reaction substrate, catalyst and pH conditions are required for the reduction of the nitro group to an amino group, a critical step in the synthesis of molecule (9). Potassium phosphate, sodium hydroxide, potassium carbonate, and cesium carbonate are among the most widespread bases in use. Powdered catalysts including cuprous iodide, iron, zinc, copper and tin (II) chloride dihydrate are often used. Finding the ideal reaction time and using the compound's lipid solubility to choose the right solvent are further critical steps. This step can be accomplished by adding tin (II) chloride dihydrate to an appropriate amount of concentrated hydrochloric acid at a controlled temperature of 65 ºC for 10 minutes while stirring, then lowering the temperature to room

Table 1: Physical properties of (13-20)

Compd.	R	C. F	$[M+H]^+$	Yield (%))	Melting point (°C)
13	$-CH_3$	$C_{24}H_{19}N_5O_5S$	490.0812	75.28	145.1-146.8
14	$-CH_2CHCH_2$	$C_{26}H_{21}N_5O_5S$	516.1349	76.51	210.1–212.1
15		$C_{30}H_{21}Cl_2N_5O_5S$	634.0720	80.49	145.5-148.7
16		$C_{30}H_{22}FN_5O_5S$	584.1411	76.1	232.1-233.5
17		$C_{30}H_{22}ClN_5O_5S$	600.1109	82.63	155.3-157.4
18		$C_{30}H_{22}BrN_5O_5S$	644.0610	83.21	232.1-235.5
19		$C_{31}H_{22}F_3N_5O_5S$	634.1365	80.1	232.5-234.5
20		$C_{31}H_{25}N_5O_6S$	596.1602	85.12	139.4-140.7

temperature and continuing to stir for 1 hour, according to relevant literature sources (Liu *et al.*, 2016, Trujillo *et al.*, 2020). At last, gather the solids and use sodium hydroxide to change the pH of the solution (making sure it's more than 7) so you can extract and separate them. Sodium hydroxide aqueous solution needs to be added drop wise under ice bath conditions for neutralization to avoid hydrolysis of ester groups in the product.

During the process of preparing compound (12**)**, an important discovery was made: The selection of condensation agents, solvents and bases, as well as the feeding sequence had a substantial influence on the amount of product that was produced. Using this method, it was discovered that the highest yielding compound 12 may be created by first adding the carboxylic acid compound, then adding TBTU, DMA and DIPEA into the reaction vessel. After that, stirring the mixture for five minutes before adding the amine. carboxylic acid generates carboxyl anion under the action of DIPEA (Early *et al.*, 2001, Lee *et al.*, 2008) and react with TBTU to form Int-I. Subsequently, molecular transfer reaction occurs to generate active ester Int-II. Finally, the amine (as nucleophilic) reacts with the active ester Int-II under the action of DIPEA to generate the target product. Among them, considering the poor solubility of raw materials and product, we chose DMA as the solvent (Scheme 3).

Structure-activity relationship (SAR) of substitutions at the N-1 Position of (12) in the initial structure

Based on these positive indicators, compound (12) was selected for further follow-up studies. In order to enhance the inhibitory effect, particularly on TNF-α secretion, we decided to optimize the *N*-1 substituent on the indole structure (Zablotskaya *et al.*, 2013, Sailaja *et al.*, 2019)

In page 8: As a consequence of this, the inclusion of imidazole [2,1-*b*] thiazole and indole as pharmacophore groups into a single molecule has resulted in the synthesis of a series of compounds that exhibit considerable anti-inflammatory action. The suggests that compounds featuring imidazole [2,1-*b*] thiazole and indole as the core backbone possess advantages in terms of their anti-inflammatory properties. Conformational analysis reveals that the *N*-1 substituent on the indole core influences the activity, while introducing benzyl derivatives with different electronic groups at various positions on the benzyl benzene ring may potentially affect activity (Song *et al.*, 2023, Liu *et al.*, 2016). Notably, compounds containing 4-trifluoromethyl substituents exhibit maximum activity. These findings can provide valuable

guidance for structural modifications and serve as a theoretical basis for designing new potent molecules essential for developing novel anti-inflammatory drugs.

CONCLUSION

We have successfully synthesized a series of novel derivatives of imidazole [2,1-*b*] thiazole and conducted in vitro evaluations to assess their anti-inflammatory effects. The results demonstrated significant inhibition of pro-inflammatory factors such as NO, IL-6, and TNF-α in LPS-stimulated RAW264.7 cells by most of the compounds. Our initial structure-activity relationship study revealed that incorporating an indole ring into the structural framework containing imidazole [2,1-*b*] thiazole enhanced its anti-inflammatory effect. Furthermore, adding an electron-absorbing group at the *N*-1 position of the indole moiety further augmented this effect. Consequently, compound 19 exhibited the highest promise and potential among all tested compounds. Additionally, toxicological evaluation indicated negligible cytotoxicity for these new thiazole derivatives, suggesting a favorable safety and tolerability profile. The conclusion is that we have effectively created and tested a number of creative derivatives that give useful insights for future study on immune system regulation and treatment possibilities. In addition, these derivatives bring new potential and challenges to the area of medicine.

ACKNOWLEDGMENT

The research was funded by the following organizations: the Scientific Research Project of Putian University (2021031), the Fujian Provincial Natural Science Foundation in China (2021J011376), and the Educational Commission of Fujian Province of China (JAT210392).

SUPPORTING INFORMATION

Ethyl6-(4-(1-methyl-5-nitro-1H-indole-2-carboxamido) phenyl)imidazole [2,1-b]thiazole-3-carboxylate (13)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a light yellowish powder was formed. Yield 75.28%; mp 145.1-146.8°C; ^{1}H-NMR (DMSO-d_6, 400), (J=Hz) δ_H: 1.39 (3H, t, J 7.1), 4.09 (3H, s), 4.44 (2H, q, J 7.1), 7.57 (1H, s), 7.81 (1H, d, J 9.4), 7.84 (2H, d, J 8.9), 7.89-7.95 (2H, m), 8.17 (1H, dd, J 9.2 and 2.3), 8.26 (s, 1H), 8.45 (1H, s), 8.76 (1H, d, J 2.3), 10.63 (1H, s); ^{13}C-NMR (DMSO-d_6,101), δ_C: 14.54, 32.66, 62.31, 108.30, 110.49, 111.94, 119.13, 119.54, 120.89×2, 123.48, 124.82,,125.19, 125.80×2, 130.01, 136.12, 138.38, 141.59, 142.01, 146.91, 148.71, 157.98, 160.02; HR-EI-MS, (*m/z*): 490.0812 $[M+H]^+$ ($C_{24}H_{19}N_5O_5S$: 489.115).

Ethyl6-(4-(1-allyl-5-nitro-1H-indole-2-carboxamido) phenyl) imidazole [2,1-b] thiazole-3-carboxylate (14)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 76.51%; mp 210.1–212.1°C; ^{1}H-NMR (DMSO-d_6, 400), (J=Hz) δ_H: 1.39 (3H, t, J 7.2), 4.43 (2H, q, J 7.2), 4.86-4.95 (1H, m), 5.08-5.16 (1H, m), 5.32 (2H, d, J 2.6), 5.99-6.11 (1H, m), 7.62 (1H, m), 7.78 (1H, d, J 9.4), 7.84 (2H, d, J 8.7), 7.91 (2H, d, J 8.7), 8.16 (1H, dd, J 9.2 and 2.2), 8.25 (1H, s), 8.44 (1H, s), 8.78 (1H, d, J 2.2), 10.66 (1H, s); ^{13}C-NMR (DMSO-d_6,101), δ_C: 14.53, 47.18, 62.28, 108.78, 110.42, 112.12, 116.78, 119.27, 119.61, 120.84×2, 123.43, 124.79, 125.36, 125.76×2, 130.02, 134.69, 135.56, 138.35, 141.10, 142.16, 146.90, 148.65, 157.94, 159.88; HR-EI-MS, (*m/z*): 516.1349 $[M+H]^+$($C_{26}H_{21}N_5O_5S$: 515.13).

Ethyl6-(4-(1-(2,6-dichlorobenzyl)-5-nitro-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (15)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 80.49%; mp 145.5-148.7°C; ^{1}H-NMR (DMSO-d_6, 400), (J=Hz) δ_H: 1.39 (3H, t, J 7.1), 4.43 (2H, d, J 7.1), 6.19 (2H, s), 7.32-7.38 (2H, m), 7.38-7.44 (2H, m), 7.48 (2H, d, J 8.1), 7.58 (1H, s), 7.67 (2H, d, J 6.1), 8.1 (2H, dd, J 9.2 and 2.1), 8.27 (1H, s), 8.32 (1H, s), 8.41 (1H, s), 8.75 (1H, d, J 2.1), 10.72 (1H,s); ^{13}C-NMR (DMSO-d_6,101), δ_C: 14.54, 45.27, 46.00, 62.31, 108.46, 110.77, 111.62, 117.48, 119.40, 119.60, 119.85, 121.17, 123.43, 125.09, 125.81, 129.53, 129.79, 131.05, 131.87, 135.69, 135.54, 137.62, 139.44, 141.20, 142.10, 146.94, 148.76, 157.97, 160.46; HR-EI-MS, (*m/z*): 634.0720$[M+H]^+$($C_{30}H_{21}Cl_2N_5O_5S$: 633.06).

Ethyl6-(4-(1-(3-fluorobenzyl)-5-nitro-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (16)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 76.1%; mp 232.1-233.5°C; ^{1}H-NMR (DMSO-d_6, 400), (J=Hz) δ_H: 1.39 (3H, t, J 7.2), 4.42 (2H, d, J 7.2), 5.97 (2H, s), 6.95 (2H, d, J 7.6), 7.07 (1H, d, J 2.3), 7.34 (1H, d, J 6.6), 7.71 (1H, s), 7.77-7.82 (2H, m), 7.83 (1H, s), 7.90 (2H, d, J 8.7), 8.15 (1H, dd, J 9.2 and 2.3), 8.25 (1H, s), 8.43 (1H, s), 8.80 (1H, d, J 2.2), 10.75 (1H, s);^{13}C-NMR (DMSO-d_6, 101), δ_C: 14.53, 52.48, 62.27, 109.36, 110.45, 112.24, 113.73, 113.95, 114.53, 114.74, 119.72, 120.91, 122.99, 123.42, 124.82, 125.54, 125.77, 130.11, 131.08, 131.16, 135.49, 138.24, 141.29, 142.39, 146.87, 148.66, 157.94, 159.93, 161.46,163.89; HR-EI-MS, (*m/z*): 584.1411$[M+H]^+$ ($C_{30}H_{22}FN_5O_5S$: 583.13).

Ethyl6-(4-(1-(4-chlorobenzyl)-5-nitro-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (17)

For the purpose of purifying the residue, a typical

approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 82.63%; mp 155.3-157.4°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.38 (3H, t, *J* 7.2), 4.42 (2H, q, *J* 7.2), 5.93 (2H, s), 7.14 (2H, d, *J* 8.5), 7.36 (2H, d, *J* 8.6), 7.68 (1H, s), 7.80 (3H, d, *J* 8.7), 7.90 (2H, d, *J* 8.7), 8.14 (1H, dd, *J* 9.2 and 2.3), 8.24 (1H, s), 8.43 (1H, s), 8.79 (1H, d, *J* 2.2), 10.70 (1H, s); ^{13}C-NMR (DMSO-d_6, 101), δ_C: 14.53, 47.49, 62.30, 109.34, 110.48, 112.27, 119.60, 119.74, 120.94×2, 123.45, 125.54×2, 125.78, 128.93×2, 129.04×2, 130.11, 130.62, 132.42, 135.43, 137.37, 138.22, 141.27, 142.36, 146.86, 148.69, 157.96, 159.91;HR-EI-MS, (*m/z*): 600.1109[M+ H]$^+$ ($C_{30}H_{22}ClN_5O_5S$: 599.10).

Ethyl6-(4-(1-(4-bromobenzyl)-5-nitro-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (18)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 83.21%; mp 232.1-235.5°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.39 (3H, t, *J* 7.1), 4.42 (2H, q, *J* 7.1), 5.92 (2H, s), 7.09 (2H, d, *J* 8.4), 7.49 (2H, d, *J* 8.4), 7.69 (1H, s), 7.80 (3H, dd, *J* 9.0 and 5.4), 7.90 (2H, d, *J* 8.7), 8.14 (1H, dd, *J* 9.2 and 2.3), 8.25 (1H, s), 8.43 (1H, s), 8.80 (1H, d, *J* 2.3),10.70 (1H, s); ^{13}C-NMR (DMSO-d_6, 101), δ_C: 14.54, 47.56, 62.28, 109.34, 110.46, 112.26, 119.59, 119.73, 120.91×2, 123.44, 124.81, 125.54, 125.77, 129.26×2, 130.11, 131.95×4, 135.42, 137.81, 138.23, 141.27, 142.36, 146.88, 148.66, 157.95,159.89; HR-EI-MS, (*m/z*): 644.0610 [M+H]$^+$($C_{30}H_{22}BrN_5O_5S$: 643.05).

Ethyl6-(4-(5-nitro-1-(4-(trifluoromethyl)benzyl)-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (19)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 80.1%; mp 232.5-234.5°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.39 (3H, t, *J* 7.1), 4.42 (2H, q, *J* 7.1), 6.05 (2H, s), 7.31 (2H, d, *J* 7.9), 7.68 (2H, d, *J* 8.1), 7.71-7.84 (4H, m), 7.89 (2H, d, *J* 8.4), 8.14 (1H, d, *J* 9.0), 8.23 (1H, s), 8.41 (1H, s), 8.81 (1H, s), 10.71 (1H, s); ^{13}C-NMR (DMSO-d_6, 101), δ_C (ppm): 14.50, 47.88, 62.27, 109.39, 110.41, 112.15, 119.67, 119.74, 120.93×2, 123.41, 124.78, 125.54, 125.73×2, 125.98, 127.58×4, 130.08, 135.34, 138.18, 141.33, 142.41, 143.21, 146.84, 148.66, 157.93, 159.81; HR-EI-MS, (*m/z*) : 634.1365[M+H]$^+$ ($C_{31}H_{22}F_3N_5O_5S$: 633.13).

Ethyl6-(4-(1-(4-methoxybenzyl)-5-nitro-1H-indole-2-carboxamido)phenyl)imidazole[2,1-b]thiazole-3-carboxylate (20)

For the purpose of purifying the residue, a typical approach that makes use of utilizing rapid silica gel column chromatography was used, a pale yellowish powder was formed. Yield 85.12%; mp 139.4-140.7°C; ^{1}H-NMR (DMSO-d_6, 400), (*J*=Hz) δ_H: 1.39 (3H, t, *J* 7.1 Hz), 3.67 (3H ,s), 4.43 (2H, q, *J* 7.2), 5.87 (2H, s), 6.84 (2H, d, *J* 8.7), 7.13 (2H, d, *J* 8.6), 7.62 (1H, s), 7.84 (3H, dd, *J* = 8.8 and 2.0), 7.91 (2H, d, *J* = 8.6), 8.14 (1H, dd, *J* = 9.2 and 2.3), 8.44 (1H, s), 8.24 (1H, s), 8.77 (1H, d, *J* 2.1), 10.70 (1H, s); ^{13}C-NMR (DMSO-d_6, 101), δ_C: 14.53, 47.40, 55.48, 62.28, 109.22, 110.45, 112.39, 114.44×2, 119.37, 119.62, 120.88, 121.49, 123.44, 124.80, 125.53, 125.80, 128.51, 128.62×2, 130.10, 130.20, 135.59, 138.32, 141.21, 142.21, 146.91, 148.67, 157.95, 159.03,160.09; HR-EI-MS, (*m/z*): 596.1602 [M+ H]$^+$ ($C_{31}H_{25}N_5O_6S$: 595.15).

REFERENCES

Andreani A, Rambaldi M, Bonazzi D, Bossa R and Galatulas I (1985). Cardiotonic activity of 2,5-dimethoxyphenylimidazo[2,1-b]thiazoles. *Arch. Pharm.* (Weinheim), **318**(11): 1003-1008.

Andreani A, Rambaldi M, Leoni A, Locatelli A, Bossa R, Fraccari A, Galatulas I and Salvatore G (1996). Potential antitumor agents. 24. Synthesis pharmacological behavior of imidazo[2,1-b]thiazole guanylhydrazones bearing at least one chlorine. *J. Med. Chem.*, **39**(14): 2852-2855.

Andreani A, Rambaldi M, Locatelli A, Cristoni A, Malrino S and Pifferi G (1992). Synthesis antihypertensive activity of imidazo[2,1-b]-thiazoles bearing a 2,6-dichlorophenyl group. *Acta. Pharm. Nord.*, **4**(2): 93-96.

Andreani A, Rambaldi M, Mascellani G and Rugarli PL (1987). Synthesis diuretic activity of imidazo[2,1-b]thiazole acetohydrazones. *Eur. J. Med. Chem.*, **22**(1): 19-22.

Barradas JSMI, Errea NB, D'Accorso, Sepulveda CS and Damonte EB (2011). Imidazo[2,1-b]thiazole carbohydrate derivatives: Synthesis antiviral activity against Junin virus, agent of Argentine hemorrhagic fever. *Eur. J. Med. Chem.*, **46**(1): 259-264.

Bertamino AS. Musella V. Di Sarno C, Ostacolo M, Manfra D, Vanacore P, Stiuso E, Novellino P, Campiglia IM and Gomez-Monterrey (2015). Dihydrithieno[2,3-b]naphto-4,9-dione analogues as anticancer agents: Synthesis in cell pharmacological studies. *Eur. J. Med. Chem.*, **102**(1768-3254 (Electronic)): 106-114.

Bhongade B, Talath AS, Gadad RA and Gadad AK (2016). Biological activities of imidazo[2,1-b][1,3,4]thiadiazole derivatives: A review. *J. Saudi Chem. Soc.*, **20**: S463-S475.

Cascioferro SB, Parrino GL, Petri MG, Cusimano D, Schillaci V, Di Sarno S, Musella E, Giovannetti G, Cirrincione and Diana P (2019). 2,6-Disubstituted imidazo[2,1-b][1,3,4]thiadiazole derivatives as potent staphylococcal biofilm inhibitors. *Eur. J. Med. Chem.*, **167**(1768-3254 (Electronic)): 200-210.

Chitti SK, Van Calster D, Cappoen A, Nikolla YM, Khetmalis P, Cos BK, Kumar S, Murugesan KV, Gowri Chra and Sekhar (2022). Design, synthesis biological evaluation of benzo-[d]-imidazo-[2,1-b]-thiazole imidazo-[2,1-b]-thiazole carboxamide triazole derivatives as antimycobacterial agents. *RSC Adv.*, **12**(35): 22385-22401.

Cohen J (2002). The immunopathogenesis of sepsis. *Nature*, **420**(6917): 885-891.

Cooper CR, Paul A, Alsaleh S, Washburn W, Rackers S, Kumar VN, Nesterov F, D'Souza SA, Vinogradov and Wang H (2023). Naphthodithiophene-fused porphyrins: Synthesis, characterization, impact of extended conjugation on aromaticity. *Chemistry*, **29**(57): e202302013.

Das UN (2023). Infection, inflammation, immunity in sepsis. *Biomolecules*, **13**(9): 1332.

Guillet CM, Fourcin S, Chevalier A, Pouplard H and Gascan (1995). ELISA detection of circulating levels of LIF, OSM, CNTF in septic shock. *Ann. NY Acad. Sci.*, **762**(0077-8923 (Print)): 407-409.

Guzeldemirci NU and Kucukbasmaci O (2010). Synthesis antimicrobial activity evaluation of new 1,2,4-triazoles 1,3,4-thiadiazoles bearing imidazo[2,1-b]thiazole moiety. *Eur. J. Med. Chem.*, **45**(1): 63-68.

Imam FN, Al-Harbi O, Al-Harbi MM, Ansari MA, Zoheir KM, Iqbal M, Anwer MK, Al Hoshani AR, Attia SM and SF Ahmad (2015). Diosmin down regulates the expression of T cell receptors, pro-inflammatory cytokines NF-kappaB activation against LPS-induced acute lung injury in mice. *Pharmacol. Res.*, **102**(1096-1186 (Electronic)): 1-11.

Johnston GR and Webster NR (2009). Cytokines the immunomodulatory function of the vagus nerve. *Br. J. Anaesth.*, **102**(4): 453-462.

Kamal AD, Dastagiri MJ, Ramaiah JS, Reddy EV, Bharathi C, Srinivas SN, Pushpavalli D, Pal M and Pal-Bhadra (2010). Synthesis of imidazothiazole-chalcone derivatives as anticancer apoptosis inducing agents. *Chem. Med. Chem.*, **5**(11): 1937-1947.

Kim YJ and Kang KS (2023). The phytochemical constituents of medicinal plants for the treatment of chronic inflammation. *Biomolecules*, **13**(8): 1162.

Lazarov TS, Juarez-Carreno N, Cox and Geissmann F (2023). Physiology diseases of tissue-resident macrophages. *Nature*, **618**(7966): 698-707.

Luan M, Wang H, Wang X, Zhang F, Zhao Liu Z and Meng Q (2022). Advances in anti-inflammatory activity, mechanism therapeutic application of ursolic acid. *Mini Rev. Med. Chem.*, **22**(3): 422-436.

Lv S, Han M, Yi R, Kwon S, Dai C and Wang R (2014). Anti-TNF-α therapy for patients with sepsis: A systematic meta-analysis. *Int. J. Clin. Pract.*, **68**(4): 520-528.

Merrill JC, You J, Constable C, Leeman SE and Amar S (2011). Whole-body deletion of LPS-induced TNF-alpha factor (LITAF) markedly improves experimental endotoxic shock inflammatory arthritis. *Proc. Natl. Acad. Sci. USA*, **108**(52): 21247-21252.

Naffaa MB, Makhoul F, Tobia A, Kaplan M, Aronson D, Saliba W and Azzam ZS (2013). Interleukin-6 at discharge predicts all-cause mortality in patients with sepsis. *Am. J. Emerg. Med.*, **31**(9): 1361-1364.

Parrino B, Carbone A, Ciancimino C, Spano V, Montalbano A, Barraja P, Cirrincione G, Diana P, Sissi C, Palumbo M, Pinato O, Pennati M, Beretta G, Folini M, Matyus P, Balogh B and Zaffaroni N (2015). Water-soluble isoindolo[2,1-a]quinoxalin-6-imines: *In vitro* antiproliferative activity molecular mechanism(s) of action. *Eur. J. Med. Chem.*, **94**(1768-3254 (Electronic)): 149-162.

Pehlivan E, Lieve N and Ulusoy Güzeldemirci N (2018). Synthesis antiviral activity evaluation of new 4-thiazolidinones bearing an imidazo[2,1-b]thiazole moiety. *Marmara Pharm. J.*, **22**(2): 237-248.

Sbenati RM, Semreen MH, Semreen AM, Shehata MK, Alsaghir FM and El-Gamal MI (2021). Evaluation of imidazo[2,1-b]thiazole-based anticancer agents in one decade (2011-2020): Current status future prospects. *Bioorg. Med. Chem.*, **29**: 115897.

Schulte W, Bernhagen J and Bucala R (2013). Cytokines in sepsis: Potent immunoregulators potential therapeutic targets - an updated view. *Mediators Inflamm.*, 165974.

Shahrasbi M, Azami Movahed M, Ghorban Dadras O, Daraei B and Zarghi A (2018). Design, synthesis biological evaluation of new imidazo[2,1-b]thiazole derivatives as selective cox-2 inhibitors. *Iran J. Pharm. Res.*, **17**(4): 1288-1296.

Shetty NSI, Khazi AM and Ahn CJ (2010). Synthesis, Anthelmintic anti-inflammatory activities of some novel imidazothiazole sulfides sulfones. *Bull. Korean Chem. Soc.*, **31**(8): 2337-2340.

Song Z, Zhou Y, Zhang W, Zhan L, Yu Y, Chen Y, Jia W, Liu Z, Qian J, Zhang Y, Li C and Liang G (2019). Base promoted synthesis of novel indole-dithiocarbamate compounds as potential anti-inflammatory therapeutic agents for treatment of acute lung injury. *Eur. J. Med. Chem.*, **171**(1768-3254 (Electronic)): 54-65.

Wang CY, Jang HJ, Han YK, Su XD, Lee SW, Rho MC, Wang HS, Yang SY and Kim YH (2018). Alkaloids from tetrastigma hemsleyanum their anti-inflammatory effects on LPS-induced RAW264.7 cells. *Molecules*, **23**(6): 1445.

Zhao BB, Guo HJ, Liu Y, Luo XY, Yang SX, Wang YT, Leng X, Mo CF and Zou Q (2018). K313, a novel benzoxazole derivative, exhibits anti-inflammatory properties via inhibiting GSK3beta activity in LPS-induced RAW264.7 macrophages. *J. Cell Biochem.*, **119**(7): 5382-5390.

Wang Z and Wang Z (2023). The role of macrophages polarization in sepsis-induced acute lung injury. *Front Immunol.*, **14**: 1209438.