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Design, synthesis and biological evaluation of new 1,3-thiazole derivatives as potential anti-inflammatory agents

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ABSTRACT

Thiazoles are widely recognized as nuclei of great value for obtaining molecules with various biological activities, including analgesic, anti-inflammatory, anti-HIV, antidiabetic, antitumor and antimicrobial. A library of 26 thiazole derivatives as fragments were designed, synthesized and evaluated for their anti-inflammatory activities. Some screened compounds showed promising results and were found to be potent in the series by inhibiting LPS-induced TNFα and IL-8 release with IC_{50} values in µM range without cytotoxic activity.

1. Introduction

Small-ring heterocycles including nitrogen and sulfur have been under investigation for a long time on account of their synthetic diversity and therapeutic relevance. Among the wide range of heterocycles explored to privileged candidates in drug discovery, thiazoles have been identified to play a necessary role in medicinal chemistry.

1,3-Thiazole nuclei is already present in a variety of pharmacologically active substances such as antibiotics, antimycotics, antihistamines, vitamin B1 (thiamine) supplements and xanthine oxidase inhibitors for treatment of gout. Compounds with a thiazole nucleus that have potential for the treatment of tumors and as anti-inflammatory agents (Fig. 1) are also documented in literature [1-5]. Approximately 85% of all biologically active compounds contain a heterocyclic unit that can affect polarity, solubility, lipophilicity and ability to bind to target proteins by forming hydrogen bonds to active sites [6].

Taking into account a number of already available medicines [7,8] and novel compounds in the research phase that all contain a thiazole unit, it can be concluded that compounds with such an important unit still have a great potential in various applications.

In our current study series of thiazole derivatives were designed and synthesized as a starting fragments to be further developed in the drug-like molecules. Their *in vitro* anti-inflammatory activities have been screened in two assays. Compounds were screened in *in vitro* cell viability assays to evaluate potential cytotoxicity. Some screened compounds were found to be potent in the series by inhibiting LPS-induced TNFα and IL-8 release with IC_{50} values in µM range without cytotoxic activity. Prompted by obtained results a new series of thiazole derivatives are in plan to be designed in drug like molecules with anti-inflammatory activity.

2. Results and Discussion

2.1. Chemistry

In this work several classes of thiazole derivatives have been synthesized due to the evidenced great potential of biological activity of this heterocyclic core. New amides **1-10**, imines **11-15**, amines **16-20**, and methylated amides **21-23** and amines **24-26** have been successfully synthesized, spectroscopically characterized and their biological activity was investigated in potential anti-inflammatory activity by testing of compounds on lipopolysaccharide (LPS)-stimulated tumor necrosis factor alpha (TNFα) and interleukin 8 (IL-8) production in human peripheral blood mononuclear cells (PBMCs) (Schemes 1-4). Although some of these derivatives were mentioned in the literature earlier [9-19], they were obtained and isolated by different procedures and only some of them were surveyed for testing biological activity.

The amides **1-10** were synthesized from 2-amino thiazole and various carboxylic acids (Scheme 1) activated

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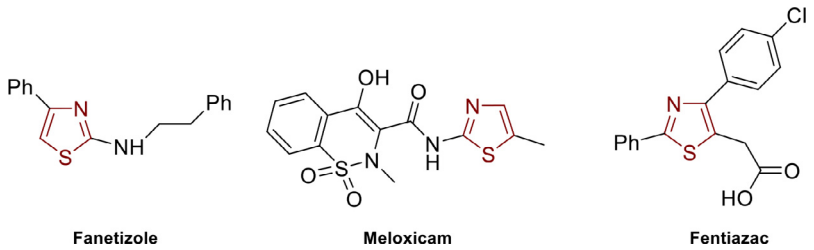
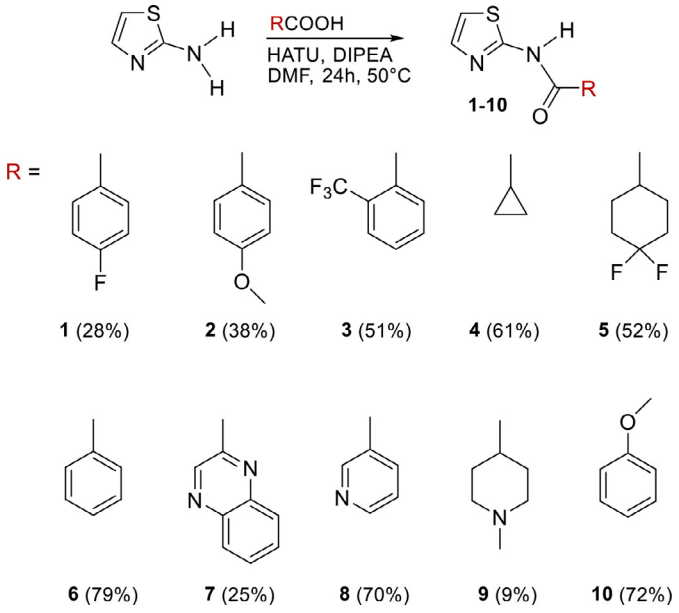


Fig. 1. Examples of thiazole bearing anti-inflammatory drugs. [3].



Scheme 1. Reaction pathway for obtaining amide derivatives **1-10**.

with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide-hexa-fluorophosphate (HATU), along with diisopropylethylamine (DIPEA) [19]. The activation of carboxylic acid takes place in 2 steps, of which in the first step the base, in this case DIPEA, first deprotonates the carboxylic acid, and the resulting carboxylate anion attacks the positively charged carbon atom in HATU. In the second step, the newly formed OAt anion reacts with the newly formed activated carboxylic acid to form an activated ester. After the formation of the activated ester, a further, third step is the formation of the desired amides **1-10** (Scheme 1) by reacting the amine deprotonated with DIPEA and the activated ester.

The relevant reactant in the synthesis of amides **1-5** was 2-aminothiazole, and the acid was added in excess, resulting in a lag of the HATU-activated acid complex that made it difficult to isolate the compounds by column chromatography. This was a direct cause of smaller amounts of isolated desired products. By reverse approach to the synthesis of further amides where the reactions for the preparation of compounds **6-10** were carried out with an excess of 2-aminothiazole, and the relevant reactant was a certain acid, the lag of the resulting complex was avoided and thus the isolation of these compounds was simpler. Therefore, desired product and higher yields were achieved as can be seen in Scheme 1. Amides **7** and **9** deviate from such a trend. In the isolation of product **7**, column chromatography was not required because it precipitated during the synthesis, but the yield was much lower than in other products. On the other hand, amide **9** bound to the silica gel column during column chromatography and the system used was not strong enough to elute from the column and the column was washed with the mixture of methanol and ammonium hydroxide for successfully isolation of very little amount of **9**. All synthesized amides **1-10** were characterized by NMR, UPLC and MS analyses (See SI) and the characteristic signals in the ^{1}H NMR spectra for products **7** and **8** are also shown on Fig. 2.

All imines **11-15** (Scheme 2) and amines **16-20** (Scheme 3) were synthesized by reductive amination reaction starting from 2-amino thiazole [9]. Three test reactions were performed, two Buchwald-Hartwig reactions with BrettPhos Pd G3 as a catalyst, and potassium *tert*-butoxide as a base, one of which was carried out with heating at 80°C in the conventional manner, on a magnetic stirrer overnight and was further heated at 100°C for 3 hours, and the second reaction was heated at 100°C by microwave irradiation for 30 minutes. The third test reaction, which proved to be the best for several reasons, was the reductive amination, carried out at 80°C on a stirrer with a heating element overnight.

Better conversion itself was one of the reasons why reductive amination was chosen for further reactions. Another very important reason is that reductive amination is a reaction in 2 steps where in the first step imines are formed which can be isolated as separate products (See Experimental). By such a reaction, it was achieved that 2 types of product were isolated, imines **11-15** from half of the reaction mixture, while the other part of the reaction mixture was reduced to the corresponding amines **16-19** with $NaBH_4$ as reducing agent (Scheme 3), only amine **20** has not been successfully synthesized using $NaBH_4$.

Although reductive amination has proven to be a good way of parallel synthesis of imines and corresponding amines, this approach makes it difficult to determine the exact yields because taking aliquots of the reaction mixture after imine formation, which is further used to reduce to amines, introduces a certain calculation error. All synthesized imines **11-15** and amines **16-19** were characterized by NMR, UPLC and MS analyses (See SI) and the characteristic signals in the ^{1}H NMR spectra for imine **15** and amine **18** are also shown on Figs. 3 and 4a.

To assess the influence of the NH group on biological activity, three amides **1-3** and their analogous three amines **17-19** were additionally methylated (Scheme 4). For this purpose, iodomethane with NaH was used as the base.

All methylated thiazole amides **21-23** and amines **24-26** were successfully isolated and characterized by NMR, UPLC and MS analyses (See SI) and additionally the characteristic signals in the ^{1}H NMR spectra methylated amine **25** are also shown on Fig. 4b along with the spectrum of the corresponding unmethylated amine **18** (Fig. 4a).

2.2. Biology

Biological activity of 24 succesfully synthesized thiazole derivatives (**1-12, 14-19** and **21-26**) was evaluated *in vitro*. To assess potential anti-inflammatory activity, the effect of compounds on lipopolysaccharide (LPS)-stimulated tumor necrosis factor alpha (TNFα) and interleukin 8 (IL-8) production in human peripheral blood mononuclear cells (PBMCs) was tested. Out of 24 tested

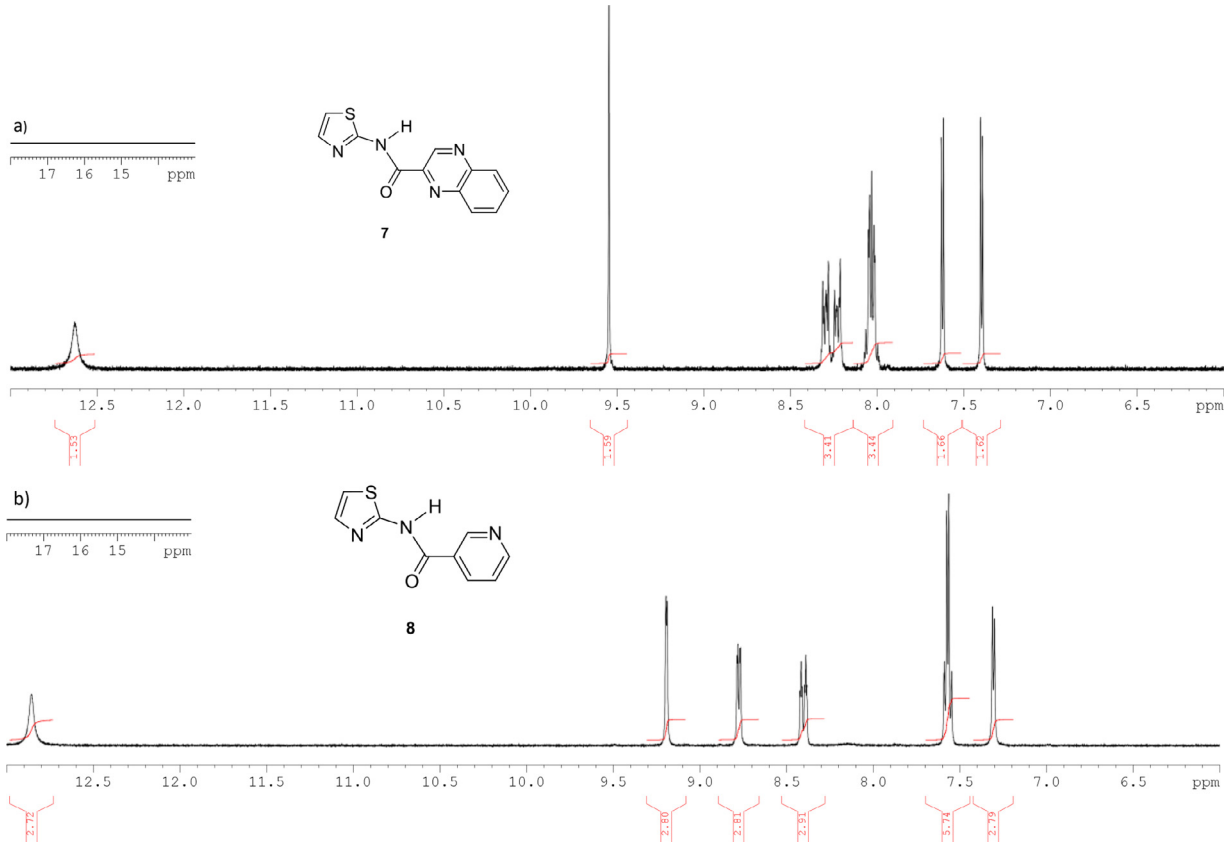
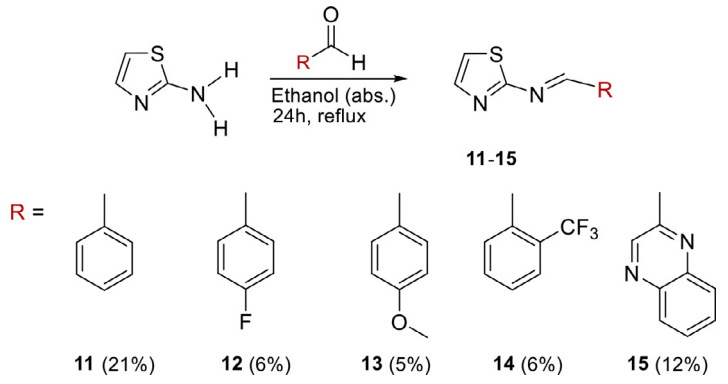
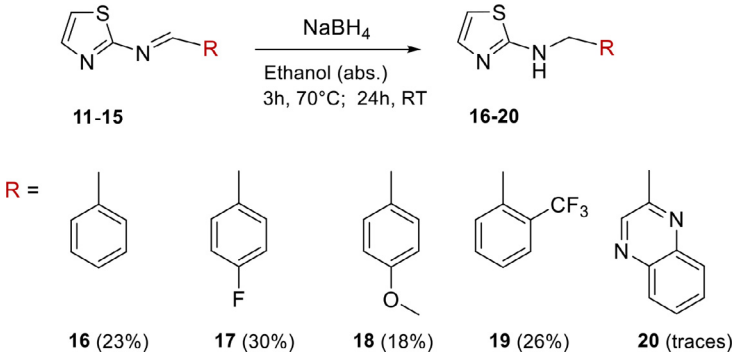


Fig. 2. ^{1}H NMR spectra (DMSO) of the thiazole derivatives **7** (a) and **9** (b).



Scheme 2. Synthesis of imines **11-15** by reductive amination reaction.



Scheme 3. Reduction reaction of imines to amines **16-19** and the planned amine **20**.

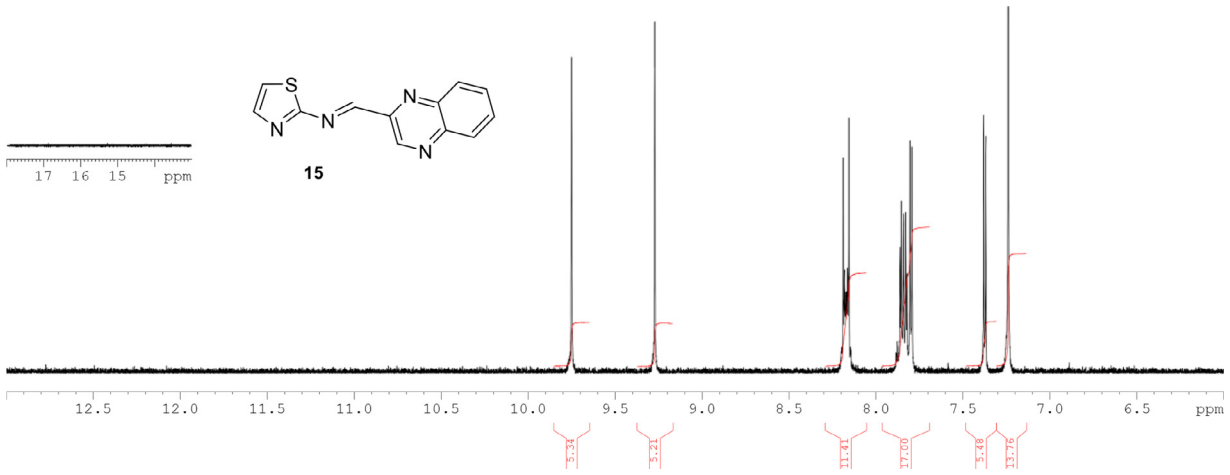
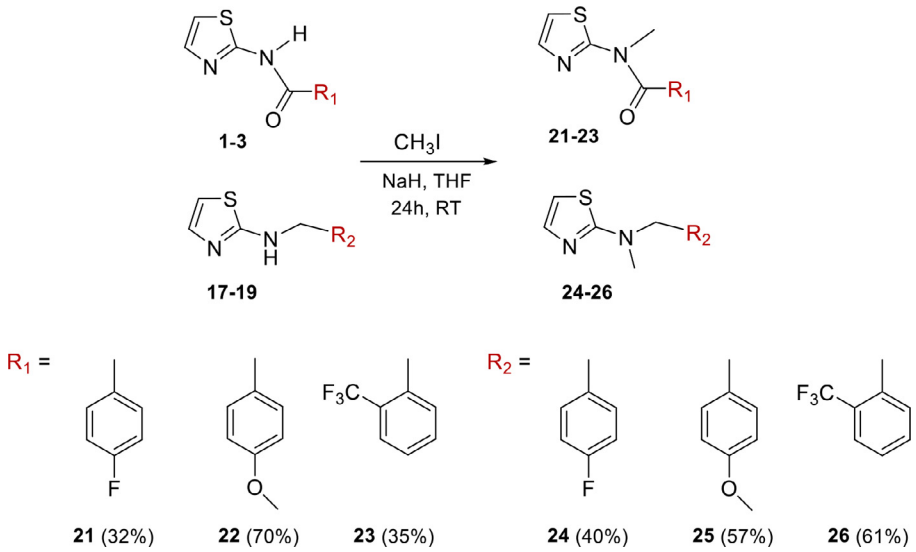


Fig. 3. ^{1}H NMR spectrum (DMSO) of thiazole imine derivative **15**.



Scheme 4. Products **21-26** obtained by methylation of the corresponding amides **1-3** and amines **17-19**.

compounds, 12 compounds inhibited LPS-induced TNFα release with IC_{50} values in μM range (Table 1 and Fig. 5). On the other hand, only compound **26,** at the highest concentration tested, inhibited IL-8 release (Scheme 5, Fig. 6).

In addition, the effect of compounds on viability of ovarian adenocarcinoma cell line SK-OV-3 was assessed. Briefly, cells were treated with compounds for 24 h and intracellular ATP levels were measured. Since ATP is produced only by live cells, the signals obtained were proportional to number of cells in wells. In this assay only compound **10** had some effect on viability of SK-OV-3 (Fig. 7).

In both of these assays reference compounds were also tested. In LPS stimulated PBMC assay corticosteroid dexamethasone was used, due to its known anti-inflammatory effects. The activity on both TNFα and IL-8 release was reproducible, with IC_{50} values of 7.5 nM and 3.6 nM, respectively. Staurosporine, a pan-kinase inhibitor, was used as reference compound in cytotoxicity assay, and it concentration-dependently reduced number of live cells, with IC_{50} value 421 nM. Activities of both dexamethasone and staurosporine in corresponding assays are shown on Fig. 8.

Obtained results were summarized in structure-activity relationship overview (Fig. 9). In library design, 2-amino-1,3-thiazole was considered as starting point in synthesis to be further coupled by amide, imine and amine linkers with diversely substituted phenyl, pyridine, fused system such as quinoxaline, saturated rings (cPr, cyclohexyl) as well as saturated rings with basic centre (piperidone).

Analysing of activity on TNFα for the set of compounds with amide bond, compound **7** with fused quinoxaline system showed best activity, although with low inhibition plateau (47%). In comparison with compound **8** bearing pyridine, activity was lost 4 folds, not completely. Also, compounds with *para*-phenyl -EDGs such as methoxy (compound **2**) are beneficial for TNFα inhibition. In addition to explore importance of –CONH amide potential hydrogen bond for activity was explored by introducing of methyl group. Interestingly, by methylating compound **2** to obtain compound **22**, activity was completely lost showing importance of H-bond for activity. On the other hand, compound **3** that was inactive, by methylating amide, compound **23** showed activity.

For imine containing linker, compound **15** with quinoxaline unit was active, losing 2 folds activity in comparison to amide bond. Due to unsuccessful reduction of imine **15** to corresponding amine, library is lacking information about the activity of amine. Moreover, by reducing –C=O to obtain library of benzyl amine analogues, all compounds with variously substituted benzyl units were active leading to conclusion of importance of rotatable bonds for activity.

Compound **26** was the only one showing moderate activity on IL-8 inhibition in the range of activity for TNFα. Evaluation of com-

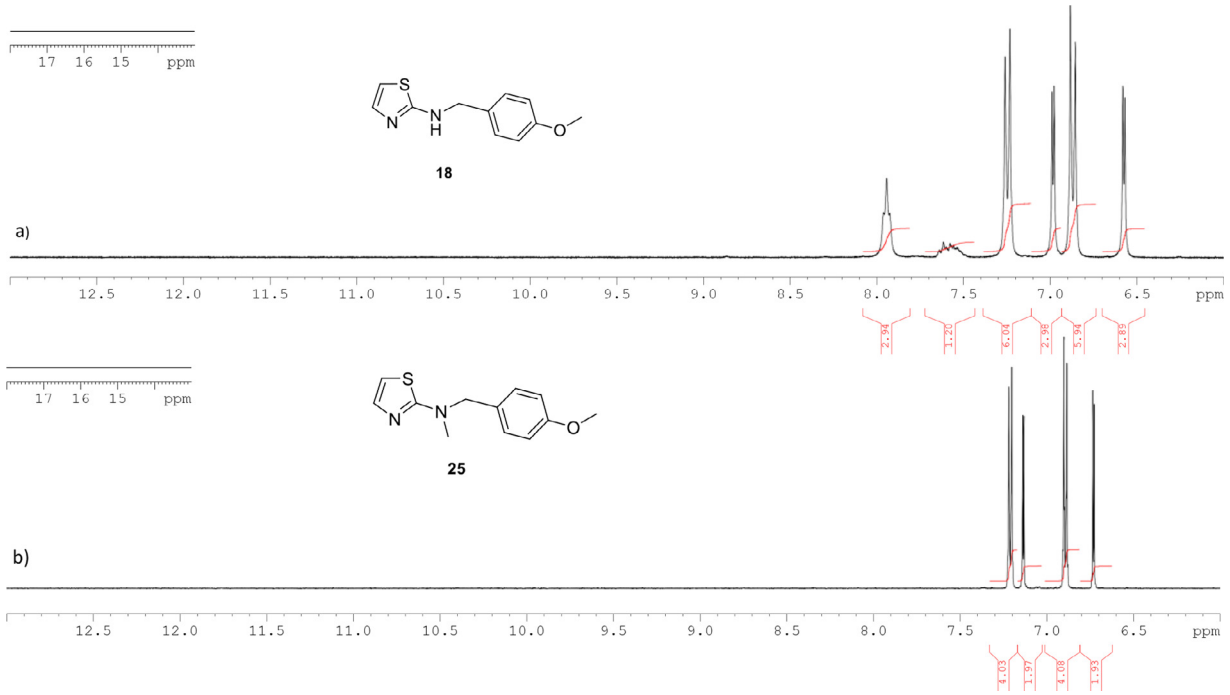


Fig. 4. Aromatic part of the ^{1}H NMR spectra (DMSO) of the unmethylated thiazole amine **15** (a) and the corresponding methylated amine **25** (b).

Table 1
Activities of 1,3-thiazole derivatives tested in two *in vitro* assays.

	IC_{50} value (μM)		
Compound	PBMC-LPS/ TNFα	PBMC-LPS/ IL-8	SK-OV-3 viability
1	>100	>100	>100
2	24	>100	>100
3	>100	>100	>100
4	>100	>100	>100
5	>100	>100	>100
6	>100	>100	>100
7	8[a]	>100	>100
8	>33	>100	>100
9	22[b]	>100	>100
10	>100	>100	>33
11	>100	>100	>100
12	>100	>100	>100
14	>100	>100	>100
15	17	>100	>100
16	>100	>100	>100
17	>33	>100	>100
18	>33	>100	>100
19	10	>100	>100
21	>100	>100	>100
22	>100	>100	>100
23	40	>100	>100
24	>33	>100	>100
25	25	>100	>100
26	56	47	>100

[a] – low inhibition plateau (47%)
[b] – compound was cytotoxic in PBMC assay at 100 μM

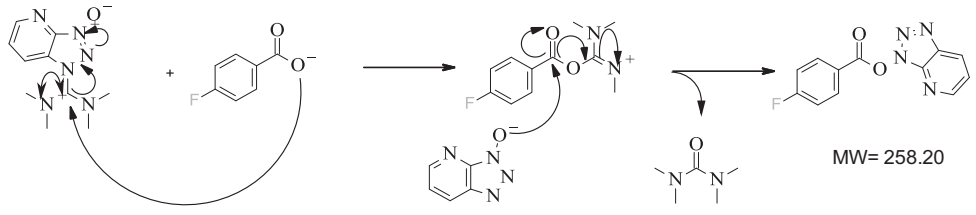
pounds by screening in *in vitro* cell viability assays on SK-OV-3 cells did not show cytotoxic effect. Encouraged by promising data obtained on small fragments a new series of thiazole derivatives are in plan to be developed in drug like molecules with anti-inflammatory activity aiming IC_{50} values in nM range with acceptable ADME-Tox properties.

3. Experimental Section

3.1. General

The amidation test reactions and the reductive amination reactions were carried out on a magnetic stirrer with a heating body, while part of the amidation reactions were carried out on a shaker with a heating body. Chromatographic separations were performed on a Biotage instrument using puriFlash silica gel-filled columns (4g, 15 μm and 12 g, 15 μm).

All products obtained were identified and characterized by ultra high efficiency liquid chromatography (UPLC) with mass and UV detectors and nuclear magnetic resonance (NMR). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Ultra Shield 300, Bruker Ultra Shield 400, Bruker Ultra Shield 500 and Bruker Ultra Shield 600 depending on the amount of compound and the desired spectra. The 2D-HH-COSY technique was also used to assign signals and determine interactions in the molecule. Ultra-high performance liquid chromatography monitoring the range of the reaction and determining the purity of the final



Scheme 5. Simplified demonstration of 4-fluorobenzoic acid activation with HATU.

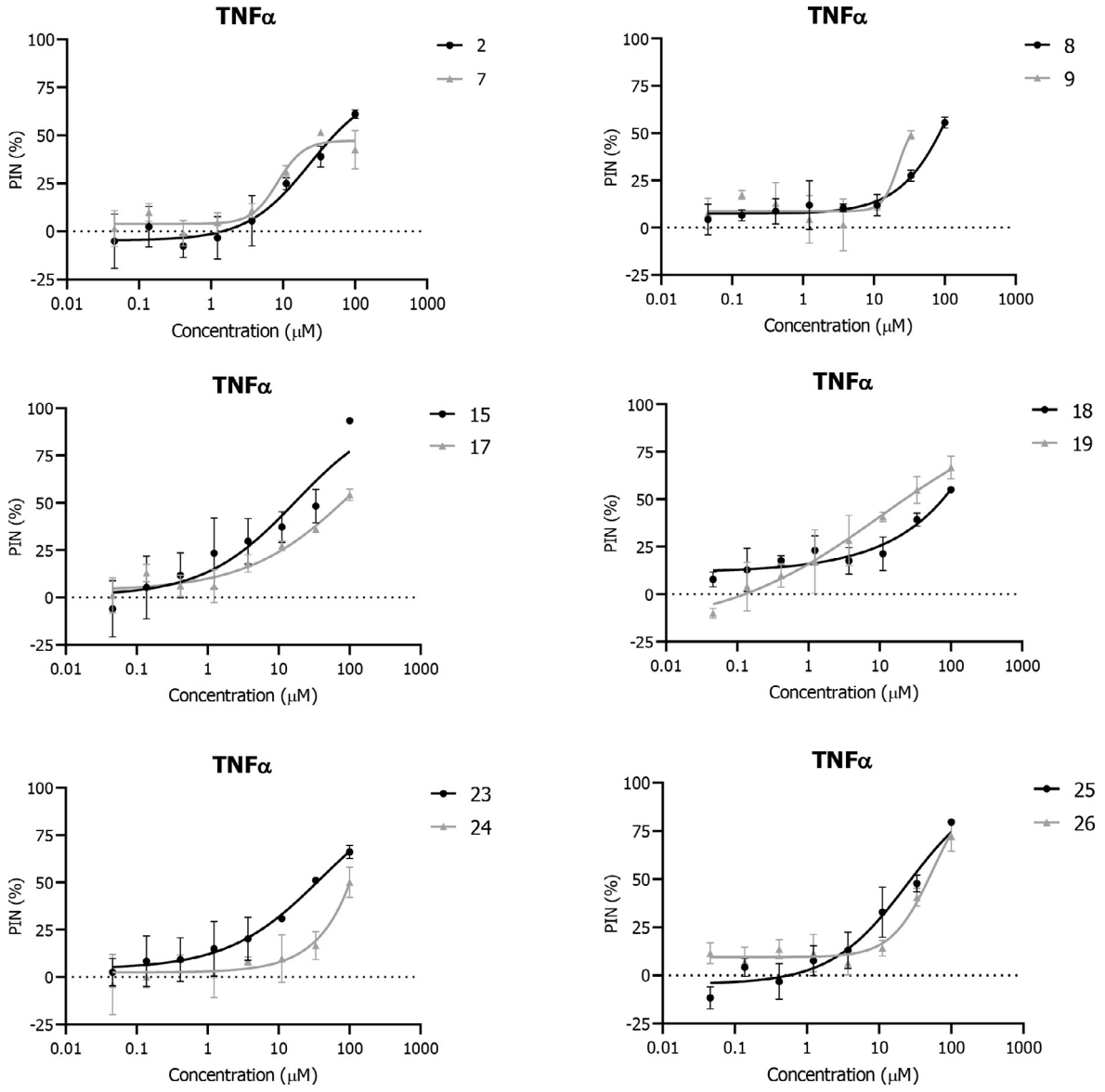


Fig. 5. Effect of compounds **2, 7, 8, 9, 15, 17**-**19** and **23-26** on LPS-stimulated TNFα production in hPBMCs.

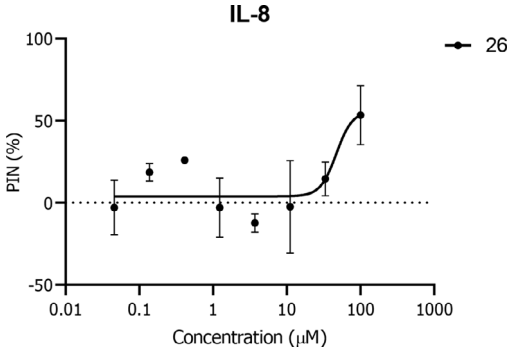


Fig. 6. Effect of compound **26** on IL-8 production in LPS stimulated PBMCs assay.

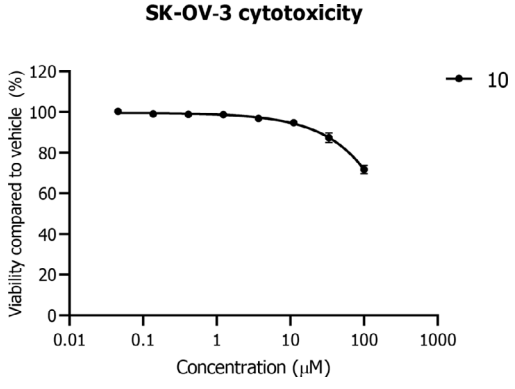


Fig. 7. Effect of compound **10** on SK-OV-3 cell viability.

product was performed on a Waters Acquity Ultra Performance LC with an SQD mass spectrometer (UPLC-MS/UV). Depending on the structure of the compound, two methods were used to monitor the range of the reaction: Method A - method duration = 2 min, acidic conditions (0.1% HCOOH in H_2O / 0.1% HCOOH in MeCN); Method B - method duration = 2 min, basic conditions (0.05% NH_3 in H_2O / 0.05% NH_3 in MeCN). The following abbreviations were used: s, singlet; d, doublet; t, triplet, dd, doublet of doublet; m, multiplet; DMF-dimethylformamide, DCM-dichloromethane,

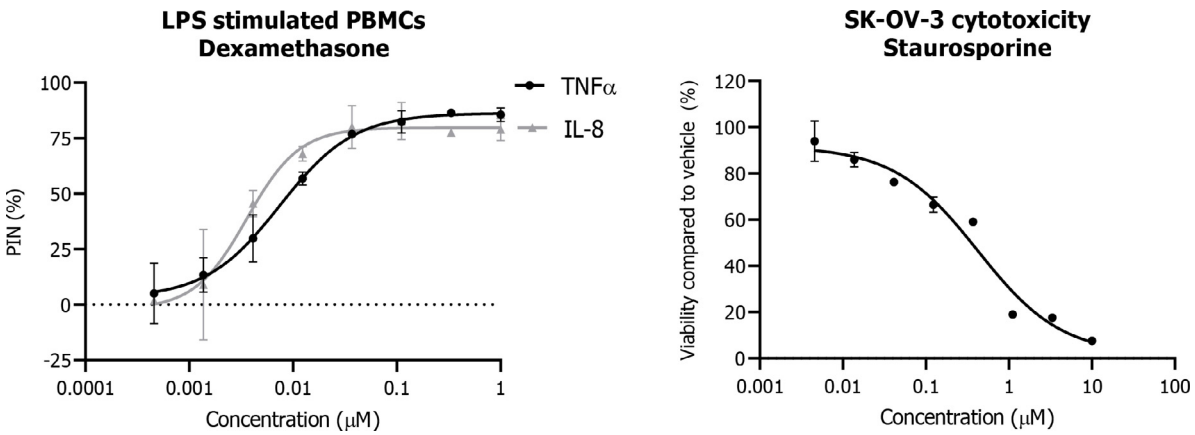


Fig. 8. Activity of reference compounds dexamethasone (A) and staurosporine (B) in LPS stimulated PBMCs and SK-OV-3 cytotoxicity assays, respectively.

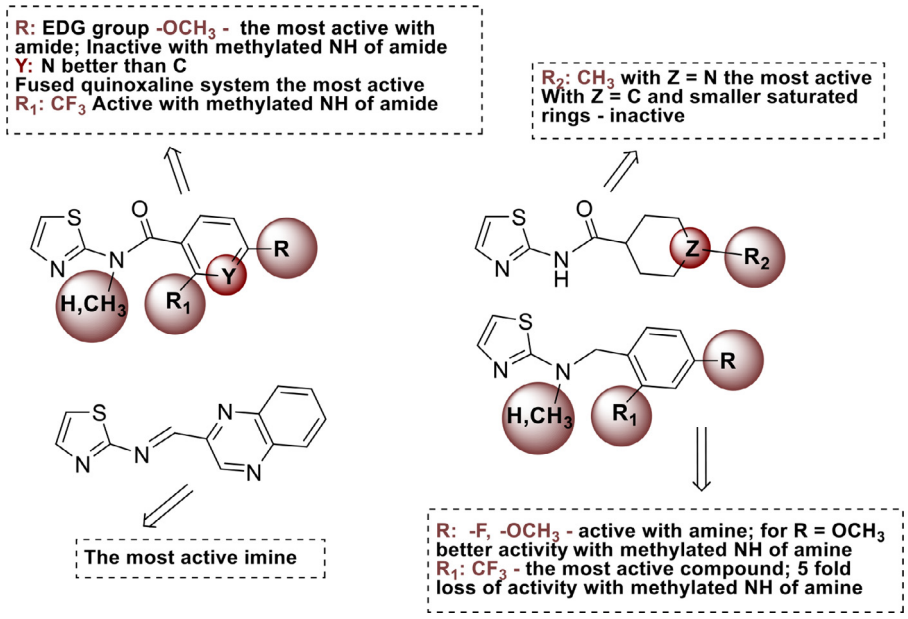


Fig. 9. SAR of thiazole fragment based library concerning their anti-inflammatory activity.

MeOH-methanol, MeCN-acetonitrile, DIPEA-diisopropylethylamine, HATU-1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexa-fluorophosphate.

3.2. Amidation reactions

Procedure for compounds 1-5: 1.2 equivalents of a certain carboxylic acid was dissolved in 1 mL of DMF in a round bottom flask and 1.2 equivalents of HATU and 1.2 equivalents of DIPEA were added to activate the acid present for subsequent reactions. After 15 minutes, 1 equivalent of 2-amino thiazole (50 mg, 0.499 mmol) was added to the reaction mixture, and the reaction mixture was stirred on a magnetic stirrer overnight at 50°C. The extent of the reaction was monitored by UPLC-MS/UV (Method A). The presence of HATU-activated acid intermediates was observed in all reaction mixtures (Fig. 10). The reaction was then stopped and extraction was performed. The reaction mixture was transferred to a separatory funnel and 10 mL of saturated aqueous ammonium chloride solution (NH_4Cl) and 10 mL of ethyl acetate were added. When the layers were separated, the aqueous layer was drained from the funnel, and the residual organic layer was further washed with 10 mL of saturated aqueous ammonium chloride solution and 2 × 10 mL of distilled water. After the extraction, the organic layer is additionally dried through a phase separator, through which the organic layer passes, and any water present remains in the separator. The reaction mixture thus treated was evaporated to dryness on a rotary evaporator.

The evaporated products were dissolved in a small amount of dichloromethane and applied to TLC plates developed in various solvent systems in order to find the best chromatographic separation system. Silica gel was then added to the solution, the mixture was evaporated to dryness and tested in a pre-column. Compounds were isolated by column chromatography on a Biotage device with puriFlash columns. Certain solvent systems, ie eluents, flow rates, columns used as well as elution time are shown in Table 2.

In previous procedures, the presence of HATU-activated acid complexes often meant difficult isolation of the resulting products. It was therefore decided to approach the following reactions getting products **6-10** in another way in which the relevant reactant was set up and 2-amino thiazole was added to the reaction in excess to ensure that all the resulting complex would be consumed in the reaction.

Procedure for compounds 6-10: 1 equivalent of a certain carboxylic acid was dissolved in 2 mL of DMF in an 8 mL vial followed by the addition of 1 equivalent of HATU and 1 equivalent of DIPEA to activate the acid present. The vial was then placed on a shaker.

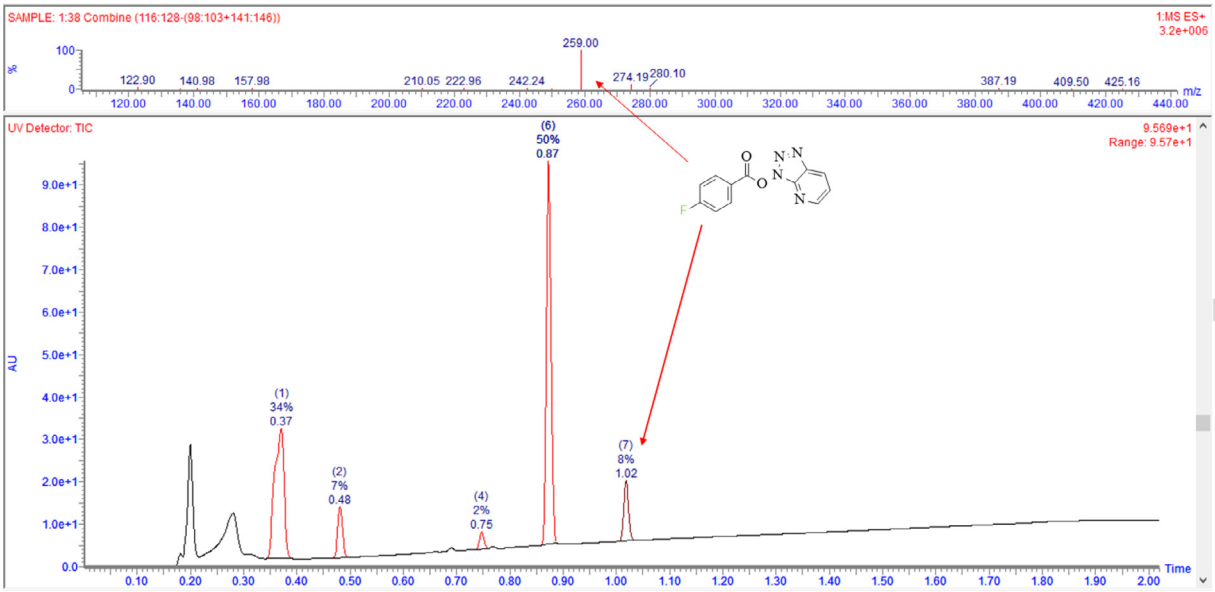


Fig. 10. Representation of the mass spectrum and TIC spectrum of the reaction range of the synthesis of 4-fluoro-*N*-thiazol-2-yl-benzamide (**1**).

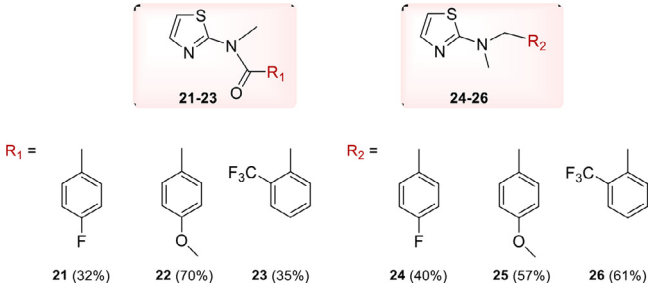
Table 2
Conditions for chromatographic separation of products **1-5**.

Product	Eluent and flow rate	Column	Elution time	Isolated quantity/mg
1	DCM:(DCM:MeOH=100:1) 0-100% 6 mL/min	puriFlash 4g, 15µm	30% DCM:MeOH= 100:1	30.6
2	DCM:(DCM:MeOH=100:1) 0-100% 6 mL/min	puriFlash 4g, 15µm	30% DCM:MeOH= 100:1	45.7
3	DCM:(DCM:MeOH:NH_4OH = 90:9:1.5) 0-100%	puriFlash 4g, 15µm	5% DCM:MeOH:NH_4OH = 90:9:1.5	68.99
4	DCM:(DCM:MeOH=100:1) 0-100% 6 mL/min	puriFlash 4g, 15µm	40% DCM:MeOH= 100:1	53.3
5	DCM:(DCM:MeCN=10:1) 0-100% 6 mL/min	puriFlash 4g, 15µm	35% DCM:MeCN= 10:1	127.3

After 15 minutes, 1.1 equivalent of 2-amino thiazole and 1.1 equivalent of DIPEA were added to the reaction mixture, and the reaction mixture was heated to 50°C and stirred on a shaker overnight. The extent of the reaction was monitored by UPLC-MS/UV (method A). By changing the ratio of reactants, the lag of the resulting HATU-activated acid complex was successfully avoided. The reaction mixture was then transferred to a separatory funnel after which 10 mL of distilled water and 10 mL of ethyl acetate were added. The aqueous layer was then drained from the funnel, the organic layer was transferred to a round bottom flask, and the aqueous layer was returned to the separatory funnel and extracted once more with 10 mL of ethyl acetate. The aqueous layer was again drained through a funnel, and the organic layer joined the previous one in a round-bottomed flask. The amount of product remaining in the aqueous layer was monitored by UPLC-MS/UV (method A) and it was observed that the product was present in traces in the aqueous layers and the aqueous layer was discarded, except for product **8** where the pH was adjusted to 6.5 with 1M aqueous HCl and product **9** in which the pH was adjusted to 9.1 with 1M aqueous NaOH to allow most of the resulting products to pass into the organic layer. The pH value at which the desired product has net charge zero, therefore higher solubility in the organic layer was determined using the ACD/*Percepta* software. The organic layer was then washed with 10 mL of aqueous lithium chloride solution, 10 mL of distilled water and 10 mL of aqueous sodium chloride solution, then dried over Na_2SO_4, filtered and evaporated to dryness on a rotary evaporator. The evaporated products were dissolved in a small amount of dichloromethane and applied to TLC plates developed in various solvent systems in order to find the best chromatographic separation system. Silica gel was then added to the solution, the mixture was evaporated to dryness and added to the pre-column. Chromatographic separation of products 1-10 was performed on a Biotage instrument on 4 g and 12 g puriFlash columns, depending on the amount of product obtained, in solvent systems at flow rates that proved to be best for individual products (Table 3). The only product that did not need to be isolated by column chromatography was product **7** because it precipitated during the synthesis in an amount sufficient for further testing.

After chromatographic separation, fractions were plotted on TLC plates to determine which fractions contained the desired product and whether any impurities were present. After selection, the fractions were transferred to a round bottom flask and evaporated to dryness on a rotary evaporator and dried in a vacuum oven overnight at 40°C.

The final products **1-10** were characterized by NMR and their purity was determined by UPLC-MS/UV with a blank.



4-fluoro-*N*-thiazol-2-yl-benzamide (1) [9]**:** white powder, m.p. 165 °C; R_f (DCM:MeOH=100:1) = 0.46, UPLC-MS/UV (method A) rt=0.87 min, *m/z*=223.00 ES+, purity (DAD)= 97.99%, ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm 12.66 (br s, 1H), 8.19-8.14 (m, 2H), 7.55 (d, J = 3.6 Hz, 1H), 7.41-7.34 (m, 2H), 7.28 (d, J = 3.6 Hz, 1H), ^{13}C NMR

Table 3
Conditions for chromatographic separation of products **6-10**.

Product	Eluent and flow rate	Column	Elution time	Isolated quantity/ mg
6	DCM:(DCM:MeOH=100:1) 0-100% 6 mL/min	puriFlash 4g, 50μm	40% DCM:MeOH= 100:1	132.64
7	/	/	/	37.33
8	DCM:(DCM:MeOHl:NH_3 = 90:4:1) 0-100%, 8 mL/min	puriFlash12g, 15μm	40-45% DCM:MeOH:NH_3 = 90:4:1	116.77
9	MeOH, 8 mL/min	puriFlash 12g, 15μm	40% MeOH	13.50
10	DCM:(DCM:MeCN=10:0.5) 0-100% 6mL/min	puriFlash 4g, 15μm	5%DCM:MeCN=10:0.5	110.32

(400 MHz, DMSO-d_6) δ/ppm 165.8, 163.4, 131.1, 131.0, 129.0, 115.7, 115.5, 113.9;

4-methoxy-*N*-thiazol-2-yl-benzamide (2) [10]**:** white powder, m.p. 158 °C; R_f (DCM:MeOH=100:1) = 0.30, UPLC-MS/UV (method A), rt=0.86 min, *m/z*=235.04 ES+, purity (DAD)= 98.43%, ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.45 (br s, 1H); 8.12-8.06 (m, 2H); 7.53 (d, J = 3.6 Hz, 1H); 7.24 (d, J = 3.6 Hz, 1H); 7.09-7.03 (m, 2H); 3.84 (s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 166.0, 162.6, 137.0, 130.1, 124.0, 113.8, 113.7, 113.6, 55.9;

***N*-thiazol-2-yl-2-(trifluoromethyl)benzamide (3):** white powder, m.p. 171 °C; R_f (DCM:MeOH:NH_4OH = 90:9:1.5) = 0.80, UPLC/MS-UV (method A), rt=0.91 min, *m/z* =273.34 ES+, purity (DAD)= 99.82%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 12.78 (br s, 1H), 7.89-7.71 (m, 4H), 7.53 (d, J = 3.5 Hz, 1H), 7.32 (d, J = 3.4 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 165.5, 137.8, 133.7, 132.6, 130.8, 128.9, 126.4, 126.0, 125.0, 122.3, 114.2;

***N*-thiazol-2-ylcyclopropane carboxamide (4):** red-brown powder, m.p. 139 °C; R*f* (DCM:MeOH=100:1) = 0.19, UPLC/MS-UV (method A), rt=0.63 min, *m/z*=168.98 ES+, purity (DAD)= 96.33%, ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.34 (s, 1H), 7.44 (d, J = 3.7 Hz, 1H); 7.15 (d, J = 3.7 Hz, 1H); 1.97-1.90 (m, 1H); 0.91-0.84 (m, 4H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 171.8, 158.1, 137.6, 113.3, 13.5, 8.2;

4,4-difluoro-*N*-thiazol-2-yl-cyclohexane carboxamide (5): white powder, m.p. 162 °C; R_f (DCM:MeCN =10:1) = 0.49, UPLC/MS-UV (method A), rt=0.63min, *m/z* = 247.09 ES+, purity (DAD) 99.83%, ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm 12.16 (br s, 1H), 7.45 (d, J = 3.5 Hz, 1H), 7.19 (d, J = 3.5 Hz, 1H), 2.14-2.01 (m, 2H), 1.98-1.74 (m, 5H), 1.72-1.56 (m, 2H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 172.7, 157.9, 137.6, 113.4, 32.4, 32.0, 25.2, 24.9;

***N*-thiazol-2-yl-benzamide (6)** [11]**:** white powder, m.p. 152 °C; R_f (DCM:MeOH=100:1) = 0.45, UPLC/MS-UV (method A), rt=0.82 min, *m/z*=205.04 ES+, purity (DAD) = 99.29%, ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm 12.64 (br s, 1H), 8.09 (d, J = 7.3 Hz, 2H), 7.67-7.59 (m, 1H), 7.59-7.52 (m, 3H), 7.29 (d, J = 3.4 Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 164.0, 156.0, 132.5, 128.6, 128.5, 128.2, 128.1, 113.9;

***N*-thiazol-2-yl-quinoxaline 2-carboxamide (7)** [12]**:** yelow powder, m.p. 148 °C; R_f (DCM:MeOH = 100:1) = 0.56, UPLC/MS-UV (method A), rt=0,90 min, *m/z*=257.07 ES+, purity (DAD)=99.79%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 12.63 (s, 1H), 9.55 (s, 1H), 8.33-8.20 (m, 2H), 8.08-7.99 (m, 2H), 7.62 (d, J = 3.5 Hz, 1H), 7.40 (d, J = 3.5 Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 174.0, 154.0, 144.0, 143.7, 143.2, 140.0, 132.8, 131.7, 130.0, 129.6, 129.2, 114.8;

***N*-thiazol-2-yl pyridine 3-carboxamide (8)** [13]**:** white powder, m.p. 143 °C; R_f (DCM:MeOH:NH_3/MeOH = 90:4:1) = 0.26, UPLC/MS-UV (method A), rt=0.50 min, *m/z*=206.03 ES+, purity (DAD) = 100%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 12.86 (br s, 1H), 9.19 (d, J = 2.1 Hz, 1H), 8.78 (dd, J = 1.6, 4.9 Hz, 1H), 8.40 (m, J = 1.9, 8.0 Hz, 1H), 7.60-7.54 (m, 2H), 7.31 (d, J = 3.5 Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 162.0, 156.0, 152.8, 149.2, 136.1, 135.9, 135.8, 123.6, 114.1;

1-methyl-*N*-thiazol-2-yl-piperidine 4-carboxamide (9): brown-yellow powder, m.p. 155 °C; R_f (MeOH) = 0.29, UPLC/MS-UV (method A), rt=0.37 min, *m/z*=226.11 ES+, purity (DAD) = 95.80%, ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 11.46 (br s, 1H), 7.47 (d, J = 3.5 Hz, 1H), 7.04 (d, J = 3.5 Hz, 1H), 2.98 (d, J = 11.2 Hz, 2H), 2.51-2.40 (m, 1H), 2.33 (s, 3H), 2.05 (br s, 2H), 2.03-1.94 (m, 4H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 173.5, 158.0, 137.5, 113.2, 54.5, 46.1, 40.9, 28.1;

2-phenoxy-*N*-thiazol-2-yl-acetamide (10): white powder, m.p. 174 °C; R_f (DCM:MeCN=10:0.5) = 0.66, UPLC/MS-UV (method A), rt=0.87 min, *m/z*=235.06 ES+, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 12.34 (br s, 1H), 7.49 (br s, 1H), 7.38-7.17 (m, 3H), 6.95 (d, J = 7.5 Hz, 3H), 4.84 (s, 2H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 166.8, 157.8, 157.0, 137.8, 129.6, 121.3, 114.5, 113.9;

3.3. Reductive amination reactions

All imines and amines in this work were synthesized by reductive amination reaction. It is a two-step reaction where in the first step an imine is formed and in the second step the reduction of the imine to the desired amine is continued, with a reducing agent such as $NaBH_4$. The fact that imines and amines can be isolated by this reaction was the main reason why reductive amination was chosen before the Buchwald-Hartwig reaction which was also carried out. Although the Buchwald Hartwig reaction is very widespread as a way of amine synthesis, in this paper it proved to be a less interesting approach and the reaction profile looked worse.

3.3.1. Imine synthesis

Procedure for compounds 11-15: 1 equivalent (200 mg, 1.997 mmol) of 2-amino thiazole was dissolved in 7 mL of absolute ethanol followed by the addition of 1 equivalent of the desired aldehyde. The reaction mixture was heated to 80°C in a sealed Wheaton glass vial to prevent evaporation of the solvent. The reaction was performed for 3 hours after which was checked by UPLC-MS/UV (method B). It was observed that starting reagents were still present. The product **11** was then evaporated to dryness, while the other reactions were carried out overnight to improve the conversion followed by UPLC-MS/UV (method B). 3.5 mL of all reaction mixtures except product **1** which was weighed 150 mg, dissolved in 3-5 mL of absolute ethanol and separated in a round bottom flask to reduce the imines to the amine as described in section 4.3.2. The remaining reaction mixture was evaporated to dryness and triturated with diethyl ether to remove residual aldehyde after which the residual product precipitated. The product thus obtained was dissolved in dichloromethane, silica gel was added, the mixture was evaporated to dryness and added to the pre-column, and chromatographic separation was carried out as previously described in the amide synthesis. The solvent systems and chromatographic separation conditions are shown in Table 4. The amount of isolated products **12** and **13** after column chromatography was insufficient and their synthesis was repeated without the step of separating aliquots for the imine to amine reduction reaction.

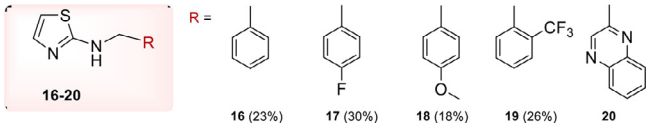
After chromatographic separation, the fractions in which the desired product is present were collected in a round-bottomed flask and evaporated to dryness. Due to the instability of the imine, aldehyde is often present in all fractions, even after chromatographic separation, which is why the evaporated products are

Table 4
Conditions for chromatographic separation of products **11-15**

Product	Eluent and flow rate	Column	Elution time	Isolated quantity/mg
11	DCM:(DCM:MeCN=10:0.5) 0-100% 6mL/min	puriFlash 4g, 15µm	20% DCM:MeCN=10:0.5	20.55
12	DCM:(DCM:MeCN=10:0.5) 0-100% 8mL/min	puriFlash 12g, 15µm	20-30% DCM:MeCN=10:0.5	23.67
13	DCM:(DCM:MeCN=10:0.5) 0-100% 8mL/min	puriFlash 12g, 15µm	40-50% DCM:MeCN=10:0.5	23.01
14	DCM:(DCM:MeCN=10:0.5) 0-100% 8mL/min	puriFlash 12g, 15µm	20-30% DCM:MeCN=10:0.5	17.60
15	DCM:(DCM:MeCN=10:1) 0-100% 7mL/min	puriFlash 12g, 15µm	40-60% DCM:MeCN=10:1	27.91

re-triturated with diethyl ether, except for products 1**2** and 1**3** which are triturated with pentane because they are soluble in diethyl ether. After trituration, the resulting mixture was centrifuged, the liquid portion was discarded, and the precipitate was dried in a vacuum oven at room temperature.

The final products **11-15** were characterized by NMR, but their purity could not be accurately determined by UPLC-MS/UV system due to the already mentioned instability and tendency to return to starting reagents while performing ultra high efficiency liquid chromatography system in water/acetonitrile system. The data below is given for the main isomer in the mixture.



1-phenyl-*N*-thiazol-2-yl-metanimine (11) [14-16]**:** yellow powder, m.p. 112 °C; R_f (DCM:MeCN = 10:0.5) = 0.76, UPLC/MS-UV (method B), rt=1.04 min, *m/z*=189.05 MS ES+, ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm 8.34 (d, J = 7.2 Hz, 1H), 7.49 (t, J = 7.2 Hz, 2H), 7.37 (m, 2H), 7.31 (t, J = 7.2 Hz, 2H), 7.02 (d, J = 3.7 Hz, 2H), 6.66 (d, J = 3.7 Hz, 2H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 167.5, 139.0, 129.6, 128.8, 128.3, 127.2, 127.1;

1-(4-fluorophenyl)-*N*-thiazol-2-yl-metanimine (12): white-yellow powder, m.p. 107 °C; R_f (DCM:MeCN = 10:0.5) = 0.82, UPLC/MS-UV (method B), rt=1.03 min, *m/z*=207.02 MS ES+, purity (NMR) 95%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 9.08 (s, 1H), 8.11 (t, J = 6.5 Hz, 2H), 7.72 (d, J = 3.8 Hz, 2H), 7.65 (d, J = 3.8 Hz, 2H), 7.39 (t, J = 8.4 Hz, 2H);

1-(4-methoxyphenyl)-*N*-thiazol-2-yl-metanimine (13): yellow powder, m.p. 97 °C; R_f (DCM:MeCN = 10:0.5) = 0.74, UPLC/MS-UV (method B), rt=1.01 min, *m/z*=219.06 MS ES+, ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm 9.11 (s, 1H), 7.11 (d, J = 7.8 Hz, 2H), 6.37 (d, J = 7.8 Hz, 2H), 6.22 (d, J = 3.5 Hz, 2H), 5.82 (d, J = 3.5 Hz, 2H), 2.98 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 167.0, 139.5, 139.0, 132.1, 128.5, 115.5, 114.6, 107.3, 56.0;

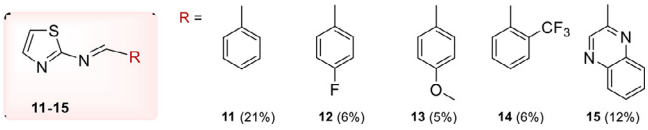
***N*-thiazol-2-yl-1-[2-(trifluoroethyl)phenyl]metanimine (14):** yellow powder, m.p. 115 °C; R_f (DCM:MeCN =10:0.5) = 0.83, UPLC/MS-UV (method B), rt=1.91 min and 2.31 min, *m/z*=257.01 MS ES+, purity (NMR) 95%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 9.38 (d, J = 2.3 Hz, 1H), 8.42 (d, J = 7.1 Hz, 1H), 7.95-7.77 (m, 5H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 171.0, 159.0, 157.8, 141.9, 133.3, 132.9, 132.0, 128.7, 126.5, 121.5, 106.5;

1-quinoxalin-2-yl-*N*-thiazol-2-yl-metanimine (15): red-brown powder, m.p. 95 °C; R_f (DCM:MeCN =10:1) = 0.72, UPLC/MS-UV (metoda B), rt=1.04 min, m/z=241.05 MS ES+, čistoća (NMR)=95+%, ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 9.63 (s, 1H), 9.21 (s, 1H), 8.27-8.16 (m, 2H), 7.91-7.84 (m, 2H), 7.36 (d, J = 3.5 Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 192.9, 159.0, 145.5, 144.3, 142.8, 135.1, 133.2, 133.1, 131.6, 130.2, 129.2, 128.9;

3.3.2. Amine synthesis

Procedure for compounds 16-20: To the reaction mixture from the previous imine synthesis was added 1 equivalent of $NaBH_4$ relative to the presumed amount of product present when the conversion of the previous reaction would be 100%. The reaction was then stirred on a magnetic stirrer overnight and the range was monitored by UPLC-MS/UV (method B). It was noticed that the range of reactions was quite low, which may be the result of the age of $NaBH_4$ and loss of its activity, so another 0.5 equivalents of $NaBH_4$ was added to the reaction mixture and the reaction mixture was heated to 70°C and stirred for another 3 hours. The range was re-checked by UPLC-MS/UV (method B) and it was observed that significantly more of the desired product was formed than before, although the starting reagents for the synthesis of the desired imines as well as the imine itself were still present. Despite the presence of starting reagents, the range of the reaction was satisfactory and the reactions were stopped. The reaction mixture was then transferred to a separatory funnel and 10 mL of ethyl acetate and 10 mL of brine were added. The aqueous layer was then drained and the organic layer was further washed with 10 mL of saturated aqueous NaCl solution and distilled water. The aqueous layers were all collected and checked by UPLC-MS/UV (Method B) to determine if the desired products were present. When it was concluded that there were no products in the aqueous layers or they were visible in traces, the aqueous layer was discarded. The organic layer was dried over $NaSO_4$, filtered and evaporated to dryness on a rotary evaporator. The product thus obtained was dissolved in dichloromethane, silica gel was added, the mixture was evaporated to dryness and the dry product on silica gel was transferred to a pre-column. Product **16**, unlike other products, was dissolved in a minimal amount of DCM and applied directly to the conditioned column. The obtained products were isolated by column chromatography on a Biotage instrument with 4g, and 12g puriFlash columns in different solvent systems seen in Table 5.

As in previous procedures after chromatographic separation, fractions were plotted on TLC plates to determine which fractions contained the desired product and whether any impurities were present. After selection, the fractions were transferred to a round bottom flask of known mass and evaporated to dryness and dried in a vacuum oven overnight at 40°C. The obtained final products were characterized by NMR and their purity was determined by UPLC-MS/UV using a blank.



***N*-benzylthiazole 2-amine (16):** white powder, m.p. 153 °C; R_f (DCM:MeCN=10:1) = 0.51, UPLC/MS-UV (method B) rt=0.98 min, *m/z*=191.06 ES+, purity (DAD)= 99.38%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 8.08-7.96 (m, 1H), 7.36-7.21 (m, 5H), 6.98 (d, J = 3.7 Hz, 1H), 6.59 (d, J = 3.7 Hz, 1H), 4.42 (d, J = 5.9 Hz, 2H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 169.0, 139.3, 138.6, 128.3, 127.4, 126.8, 106.2, 47.7;

***N*-[(4-fluorophenyl)methyl]thiazole 2-amine (17):** white powder, m.p. 169 °C; R_f (DCM:MeCN = 10:1) = 0.50, UPLC/MS-UV (method B) rt=1.02 min, *m/z*=209.05 ES+, purity (DAD) 99.12%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 8.03 (t, J = 5.5 Hz, 1H), 7.36 (dd, J = 5.5, 8.6 Hz, 2H), 7.13 (t, J = 8.9 Hz, 2H), 6.99 (d, J = 3.7 Hz, 1H), 6.60 (d, J = 3.7 Hz, 1H), 4.40 (d, J = 5.7 Hz, 2H), ^{13}C NMR

Table 5
Conditions for chromatographic separation of products **16-20**.

Product	Eluent and flow rate	Column	Elution time	Isolated quantity/mg
16	DCM:(DCM:MeCN=10:1) 0-100% 6 mL/min	puriFlash 4g, 15µm	15-25% DCM:MeCN=10:1	35.64
17	DCM:(DCM:MeCN=10:1) 0-100% 6 mL/min	puriFlash 12g, 15µm	20-30% DCM:MeCN=10:1	62.76
18	DCM:(DCM:MeCN=10:1) 0-100% 8 mL/min	puriFlash 12g, 15µm	50-60% DCM:MeCN=10:1	40.33
19	DCM:(DCM:MeCN=10:1) 0-100% 8 mL/min	puriFlash 12g, 15µm	20-30% DCM:MeCN=10:1	66.50
20	DCM:(DCM:MeOHl:NH_3=90:4:1) 0-100%, 8 mL/min	puriFlash12g, 15µm	60% DCM:MeOH:NH_3=90:4:1	27.91

(400 MHz, DMSO-d_6) δ/ppm 168.9, 162.4, 138.6, 135.5, 129.3, 114.9, 106.4, 46.9;

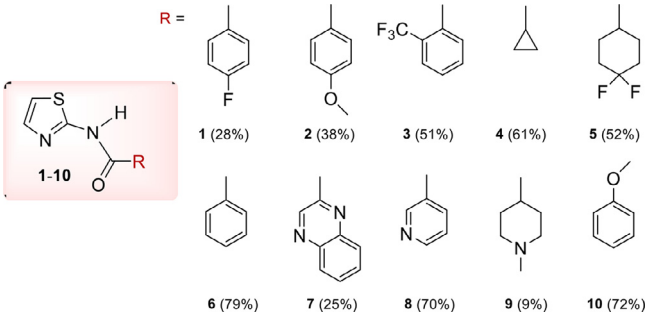
***N*-[(4-methoxyphenyl)methyl]thiazole 2-amine (18)** [17,18]**:** white powder, m.p. 149 °C; R_f (DCM:MeCN = 10:1) = 0.43, UPLC/MS-UV (method B) rt=0.90 min, *m/z*=221.43 ES+, purity (DAD) = 98.61%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 7.94 (t, J = 5.3 Hz, 1H), 7.31-7.19 (m, J = 8.7 Hz, 2H), 6.98 (d, J = 3.5 Hz, 1H), 6.91-6.83 (m, J = 8.5 Hz, 2H), 6.57 (d, J = 3.7 Hz, 1H), 4.33 (d, J = 5.7 Hz, 2H), 3.71 (s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 169.0, 158.0, 138.6, 131.1, 128.7, 113.6, 106.1, 55.0, 47.2;

***N*-[[2-(trifluorophenyl)phenyl]methyl]thiazole 2-amine (19):** white powder, m.p. 160 °C; R_f (DCM:MeCN = 10:1) = 0.68, UPLC/MS-UV(method B) rt=1.20 min, *m/z*=259.04 ES+, purity (DAD) 99.50%, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 8.12 (t, J = 5.7 Hz, 1H), 7.73-7.57 (m, 3H), 7.50-7.43 (m, 1H), 6.99 (d, J = 3.5 Hz, 1H), 6.63 (d, J = 3.7 Hz, 1H), 4.63 (d, J = 5.9 Hz, 2H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm 168.0, 152.0, 138.6, 133.0, 132.6, 128.6, 127.4, 125.8, 125.7, 106.8, 43.9;

3.4. Synthesis of methylated amine derivatives 21-26

To determine the importance of the NH bond for the biological activity, some products were further methylated. Three amides and three similar matched pairs of amines were selected for methylation. The synthesis was performed under the same conditions and with the same reagents for all compounds. A certain amount of the corresponding amide or amine (10-20 mg) was dissolved in 0.5 mL of anhydrous THF in an 8 mL vial, and the reaction mixture was cooled to 0°C in an ice bath with stirring on a magnetic stirrer. After 15 minutes, 2 equivalents of NaH were added to the reaction mixture and the reaction mixture was cooled for a further 15 minutes. After 15 minutes, 2 equivalents of iodomethane were added to the reaction mixture, after which the reaction mixture was further cooled for 10 minutes and then removed from the ice and carried out at room temperature overnight. The extent of the reaction was monitored by UPLC-MS/UV (method A for methylated amides, method B for methylated amines). The reaction was then quenched and extracted in a vial. Then 0.5 mL of water and 2 mL of dichloromethane were added with stirring. The reaction mixture is then transferred to a phase separator, which passes the organic, in this case the lower layer, while the aqueous layer remains in the separator. The organic layer was then evaporated to dryness and weighed to determine the amount of product obtained. In all cases, it is seen that the desired product is not left in the aqueous layer and the aqueous layers are discarded. The resulting precipitates and oily products were then dissolved in a minimal amount of dichloromethane and applied directly to a conditioned puriFlash column followed by chromatographic separation. All products **21-26** were isolated by column chromatography on a Biotage instrument on a 4g, 15 μm puriFlash column with DCM: (DCM: ACN 10: 0.5) 1-100% system as eluent (Table 6).

After chromatographic separation, fractions were plotted on TLC plates to determine which fractions contained the desired product and whether impurities were present. After selection, the fractions were transferred to a round bottom flask and evaporated to dryness and dried in a vacuum oven overnight at 40°C. The final products **21-26** were characterized by NMR and their purity was determined by UPLC-MS/UV using blank sample.



***N*-methyl-*N*-thiazol-2-yl-2-(trifluoromethyl)benzamide (21)** [9]**:** white powder, m.p. 114 °C; R_f (DCM:MeCN = 10:0.5) = 0.67, UPLC/MS-UV (method A) rt=1.04 min, *m/z*=287.02 ES+, purity (DAD) = 99.87%, ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm 7.95 (d, J = 8.1 Hz, 1H), 7.89-7.84 (m, 1H), 7.82-7.77 (m, 2H), 7.65 (d, J = 3.5 Hz, 1H), 7.45 (d, J = 3.5 Hz, 1H), 3.38 (s, 3H);

4-fluoro-*N*-methyl-*N*-thiazol-2-yl-benzamide (22): white powder, m.p. 127 °C; R_f (DCM:MeCN = 10:0.5) = 0.48, UPLC/MS-UV (method A) rt=0.92 min, *m/z*=237.04 ES+, purity (DAD) = 100%, ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm 8.28 (t, J = 6.7 Hz, 2H), 7.58 (d, J = 4.6 Hz, 1H), 7.30 (t, J = 8.4 Hz, 2H), 7.07 (d, J = 4.6 Hz, 1H), 3.82 (s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6) δ/ppm (1 s missing because of the small quantity), 158.0, 131.2, 131.1, 129.0, 113.8, 113.7, 108.0, 55.8, 35.9;

4-methoxy-*N*-methyl-*N*-thiazol-2-yl-benzamide (23): white powder, m.p. 119 °C; R_f (DCM:MeCN = 10:0.5) = 0.37, UPLC/MS-UV (method A) rt=0.85 min, *m/z*=249.05 ES+, purity (DAD) = 100%, ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm 8.18 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 4.6 Hz, 1H), 7.02-7.00 (m, 3H), 3.83 (s, 3H), 3.80 (s, 3H);

***N*-[(4-fluorophenyl)methyl]-*N*-methyl-thiazole 2-amine (24):** colourless oil, R_f (DCM:MeCN = 10:0.5) = 0.56, UPLC/MS-UV (method B) rt=1.07 min, *m/z*=223.05 ES+, purity (DAD)=98.86%, ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm 7.32 (t, J = 6.6 Hz, 2H), 7.19-7.12 (m, 3H), 6.75 (d, J = 3.4 Hz, 1H), 4.67 (s, 2H), 3.00 (s, 3H);

***N*-[(4-methoxyphenyl)methyl]-*N*-methyl-thiazole 2-amine (25):** colourless oil, R_f (DCM:MeCN = 10:0.5)= 0.50, UPLC/MS-UV (method B) rt=1.04 min, *m/z*=235,08 min, purity (DAD) = 99.15%, ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm 7.23-7.19 (m, J = 8.5 Hz, 2H), 7.14 (d, J = 3.7 Hz, 1H), 6.91-6.87 (m, 2H), 6.73 (d, J = 3.7 Hz, 1H), 4.60 (s, 2H), 3.72 (s, 3H), 2.97 (s, 3H);

***N*-methyl-*N*-[[2-(trifluoromethyl)phenyl]methyl]thiazole 2-amine (26):** colourless oil, R_f (DCM:MeCN = 10:0.5)= 0.65, UPLC/MS-UV (method B) rt=1.21 min, *m/z*= 273.05 ES+, purity (DAD)= 99.53%, ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm 7.77 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 3.7 Hz, 1H), 6.78 (d, J = 3.7 Hz, 1H), 4.90 (s, 2H), 3.11 (s, 3H).

4. Conclusion

New 1,3-thiazole derivatives **1-19** and **21-26** were synthesized with one unsuccessful synthesis of product **20**. Ten amides were obtained by the reaction of 2-aminothiazole and HATU activated

Table 6
Conditions for chromatographic separation of products **21-26**.

Product	Eluent and flow rate	Column	Elution time	Isolated quantity/mg
21	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	20-30% DCM:MeCN=10:0.5	3.35
22	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	30-40% DCM:MeCN=10:0.5	11.11
23	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	5-10% DCM:MeCN=10:0.5	7.42
24	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	15% DCM:MeCN=10:0.5	8.56
25	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	30-40% DCM:MeCN=10:0.5	12.13
26	DCM:(DCM:MeCN=10:0.5) 0-100% 6 mL/min	puriFlash 4g, 15µm	10-15% DCM:MeCN=10:0.5	12.88

carboxylic acid and very good yields were observed in these reactions. After the synthesis of the first amides **1-5**, the reactions were optimized and the additional amides **6-10** were synthesized in parallel, in which an increase in yield was observed and a larger amount of compounds was isolated. The highest isolated yield of 79% was obtained in the case of *N*-thiazol-2-ylbenzamide (**6**). The purity of all synthesized amides is above 95% according to the UPLC analyses which was made them suitable for biological tests. In addition, five imines **11-15** were synthesized by the reductive amination reaction, part of which was used for reduction to the amines. Of the five planned amines **16-20**, four of them were successfully synthesized. Although for imines **11-15** the purity was estimated from ^{1}H NMR spectra, all synthesized amines **16-19** show purities above 95% according to DAD detector. The limiting factor for biological activity of imines is their tendency to hydrolyze and decompose in contact with water, which is why it is necessary to apply reaction optimization and translate such products into a more suitable form. Additionally, three analogous amides and amines were subjected to the methylation reaction to get methylated products **21-26**. The yields in these reactions range from 31% in the case of **22** to 70% for **23**.

Concerning the anti-inflammatory activity of synthesized 1,3-thiazoles, amide **7** with fused quinoxaline system showed the best activity. In comparison with its pyridine analogue **8**, activity was lost 4 times, but not completely. Compounds with *para*-phenyl - EDGs such as methoxy (compound **2**) are beneficial for TNFα inhibition. Methylating compound **2** to obtain analogue **22**, activity was completely lost showing the great importance of the hydrogen bond for the activity. On the other hand, compound **3** that was inactive, by methylating gave amide **23** with higher activity. For imine containing linker, compound **15** with quinoxaline unit was active, but weaker than its amide analogue. By reducing the carbonyl group to obtain library of benzyl amine analogues, all compounds with variously substituted benzyl units were active leading to conclusion of importance of rotatable bonds for activity. Methylated amine **26** was the only one showing moderate activity on IL-8 inhibition in the range of activity for TNFα. Evaluation of compounds by screening in *in vitro* cell viability assays did not show cytotoxic effect. Encouraged by promising data obtained on small fragments a new series of 1,3-thiazole derivatives are in plan to be developed in drug like molecules with anti-inflammatory activity aiming IC_{50} values in nM range with acceptable ADME-Tox properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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