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HETEROCYCLES 34. SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NEW POLYHETEROCYCLIC SCHIFF BASES AND MANNICH BASES

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ABSTRACT. New polyheterocyclic Schiff bases and Mannich bases containing 5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione moiety have been synthesized. The structures of the newly obtained compounds were confirmed by spectral analysis IR, ^{1}H NMR, ^{13}C NMR and MS. The obtained Schiff bases and Mannich bases were screened for their anti-inflammatory activity using the carrageenan-induced rat paw oedema test. Compounds 6c, 6f, 7b, 7c, 7d, 8a, 8c, 8d, 8f and 10 showed significant anti-inflammatory activity.

Keywords: *polyheterocyclic compounds, Schiff bases, Mannich bases, anti-inflammatory activity*

INTRODUCTION

Heterocyclic ring systems thiazole, 1,2,4-triazole and pyridine can be commonly found in the structure of many compounds of medicinal interest, presenting a diverse array of biological activities, including antimicrobial [1,2], anti-inflammatory [2c,3,4] enzyme inhibitory [5] and anticancer [6] properties. Moreover, the condensed ring system thiazolo-triazole was also found to be responsible for antimicrobial [7] and anti-inflammatory activity [7b,8].

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Schiff bases and Mannich bases are important classes of pharma cologically and chemically useful compounds due to their therapeutic potential and to the reactivity of their functional groups. In particular, Schiff bases and Mannich bases derived from 1,2,4-triazole were recently reported as potent anticancer [9], antimicrobial [10], anti-inflammatory and analgesic [11,12] agents. Consequently, polyheterocyclic Schiff bases and Mannich bases rejoining the above mentioned ring systems are becoming even more important in medicinal research.

As a part of our interest for the synthesis of new biologically active compounds containing azolic rings, herein we report the synthesis, charac terization and anti-inflammatory evaluation of some new polyheterocyclic Schiff bases and Mannich bases.

Mannich reaction has found broad application in synthetic organic chemistry, as a key step for new C-C and C-N bond forming. Besides the classical variant, which involves the use of an enolisable carbonyl compound as CH-acidic substrate, atypical Mannich reactions involving other compounds with mobile hydrogen have been already described. Encouraged by the recently reported regioselective aminoalkylation of 2H-1,2,4-triazole-3-thione derivatives [12], which afforded the corresponding N-Mannich bases in good yields, we decided to apply the N-aminoalkylation reaction in the series of azolic derivatives and Schiff bases containing the 5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione moiety, in order to obtain new biologically active polyheterocyclic Mannich bases.

RESULTS AND DISCUSSION

Synthesis of polyheterocyclic compounds

As illustrated in Scheme 1, the heterocyclic precursors **1-4** were obtained as previously described in the literature [13], starting from isonicotinic hydrazide.

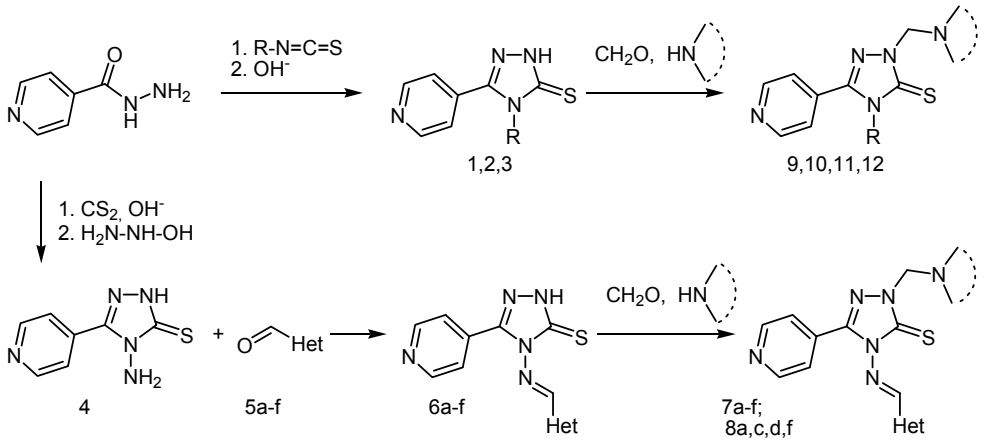
Condensation of 4-amino-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione 4 with various thiazole and thiazolo-triazole aldehydes **5a-f [14,15]**, in glacial acetic acid [10a], afforded the corresponding Schiff bases **6a-f** in good yield.

Mannich bases **7a-f, 8a,c,d,f, 9-12** were obtained by the amino methylation of 4-substituted-5-(pyridine-4-yl)-2H-1,2,4-triazole-3-thiones **1-3** and **6a-f** with formaldehyde and secondary amines (pyrrolidine and piperidine) [10].

The structures of all newly synthesized polyheterocyclic compounds were established by their spectral analysis IR, ^{1}H NMR, ^{13}C NMR and MS.

The IR spectra of Schiff bases **6a-f** revealed the presence of the N-H absorption band in the range of 3446-3482 cm^{-1} and the C=S stretching

at 1263-1286 cm^{-1}, due to the existence of the thione tautomeric form. The absorption bands for the primary amino group -NH_2 were not observed, indicating the formation of the Schiff base. The -C=N- stretching vibration appeared in the range of 1603-1611 cm^{-1}. In the IR spectra of the Mannich bases **7a-f**, **8a,c,d,f**, **9-12**, the absence of the -NH- absorption band confirmed the formation of N-Mannich bases.



Compound	Het
5,6,7a-f, 8a,c,d,f	C_6H_5- (a); $p$$CH_3$-$C_6H_4$- (b); $m$$CH_3$-$C_6H_4$- (c); pCl-C_6H_4- (d); pBr-C_6H_4- (e); C_6H_5- (f)
Compound	—N
7a-f	—N
8a,c,d,f	—N

Compound	R	—N
1	$-CH_3$	–
2		–
3		–
9	$-CH_3$	—N
10		—N
11		—N
12		—N

Sheme 1. The synthesis of polyheterocyclic compounds

Previously reported spectral studies have already confirmed that 4-amino-5-(pyridine-4-yl)-2H-1,2,4-triazole-3-thione exists in the thione tautomeric form [13b]. In the ^{1}H NMR spectra of compounds **6a-f**, the NH proton resonated as a singlet at δ 14.53 ppm, indicating also in this case the existence of the thione tautomeric form. The formation of the Schiff bases **6a-f** was confirmed by the presence of the -N=CH- proton as a singlet at δ 9.93-9.97 ppm, whereas the signal due to the NH_2 protons was completely absent.

In the ^{1}H NMR spectra of products **7a-f**, **8a,c,d,f**, **9-12**, the signal at δ 14.53 ppm due to the NH proton was absent and the >N-CH_2-N< protons appeared as a singlet at δ 5.26-5.53 ppm, confirming the formation of the corresponding Mannich bases. The presence of the pyrrolidine/piperidine ring was confirmed by other characteristic signals in the aliphatic region.

In the ^{13}C NMR spectra of Schiff bases **6a-f**, the C=S carbon belonging to the thione tautomeric form was recorded at δ 168.52-169.26 ppm. For compounds **7a-f**, **8a,c,d,f**, **9-12,** formation of Mannich bases was confirmed by the presence of a characteristic signal around δ 65.94-71.18 ppm due to the >N-CH_2-N< carbon and by other aliphatic signals due to the pyrrolidine ring (2 signals at δ 23.84-23.95 ppm and 50.34-50.67 ppm), respectively the piperidine ring (3 signals at δ 23.77-23.98 ppm, 25.95-26.23 ppm and 51.89-52.13 ppm), which are absent in ^{13}C spectra of the precursors **1,2,3** and **6a-f**. The C=S carbon signal remained present in all ^{13}C spectra of Mannich bases, at δ 168.32-171.17 ppm.

The anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was found to be in the inflammatory oedema inhibition range of 10.74% - 58.87%, while standard drug Diclofenac showed 62.61% inhibition, after 4h (**Table 1**).

Among the tested compounds, Schiff base **6f** and Mannich bases **7b, 7d, 10, 8d** and **8f** displayed the most potent anti-inflammatory activity, the percentages of oedema inhibition being close to those of diclofenac. A moderate anti-inflammatory activity was observed for the Schiff base **6c** and the Mannich bases **7c, 8a** and **8c**.

Compounds **6c, 10, 8d** and **8f** proved to be more potent than diclofenac 1 hour after inducing inflammation (**Table 1**). Schiff base **6f** displayed anti-inflammatory activity comparable to diclofenac 2 and 3 hours after inducing inflammation, while the corresponding Mannich base **8f** displayed a better profile after 3 and 4 hours.

Table 1. Anti-inflammatory activity of Schiff bases and Mannich bases

Compound	Oedema volume in ml (average±SD) % inhibition			
	1h	2h	3h	4h
Control	0.69±0.16	1.48±0.21	1.78±0.28	2.14±0.37
Diclofenac	0.40±0.14* 42.02%	0.61±0.11* 58.78%	0.85±0.23* 52.24%	0.80±0.24* 62.61%
6a	0.55±0.12 20.28%	1.24±0.39 16.21%	1.25±0.28* 29.77%	1.87±0.47 12.61%
6b	0.71±0.23 -2.89%	1.12±0.39 24.32%	1.18±0.41* 33.70%	1.49±0.27* 30.37%
6c	**0.39±0.14*** **43.47%**	**0.98±0.3*** **33.78%**	1.40±0.36 21.34%	**1.47±0.38*** **31.3%**
6d	0.54±0.16 21.74%	1.37±0.41 7.43%	1.65±0.51 7.30%	1.82±0.27 14.95%
6e	0.51±0.14 26.08%	1.01±0.42* 31.75%	1.27±0.51 28.65%	1.32±0.45* 38.31%
6f	0.49±0.19 28.98%	**0.68±0.28*** **54.05%**	**0.86±0.27*** **51.68%**	**1.22±0.35*** **42.99%**
7a	0.69±0.31 0%	1.11±0.47 25%	1.33±0.44 25.28%	1.91±0.65 10.74%
7b	0.43±0.25 37.68%	**0.86±0.43*** **41.89%**	**1.02±0.38*** **42.69%**	**1.46±0.35*** **31.77%**
7c	0.57±0.15 17.39%	**1.21±0.13*** **18.24%**	**1.19±0.21*** **33.14%**	**1.31±0.3*** **38.78%**
7d	0.62±0.27 10.14%	**0.79±0.24*** **46.62%**	**1.07±0.35*** **39.88%**	**1.10±0.43*** **48.59%**
7e	0.49±0.06* 28.98%	1.26±0.43 14.86%	1.34±0.56 24.71%	1.12±0.46* 47.66%
7f	0.58±0.22 15.94%	1.03±0.34* 30.40%	1.33±0.43 25.28%	1.3±0.42* 39.25%
9	0.44±0.24 36.23%	1.47±0.30 0.67%	1.53±0.31 14.04%	1.48±0.35* 30.84%
10	**0.31±0.05*** **55.07%**	**0.90±0.32*** **39.18%**	**1.12±0.28*** **37.07%**	**1.03±0.18*** **51.86%**
11	0.51±0.24 26.08%	1.47±0.30 0.67%	1.35±0.31* 24.15%	1.28±0.40* 40.18%
8a	**0.36±0.13*** **47.82%**	**0.97±0.41*** **34.45%**	**1.26±0.37*** **29.21%**	**1.45±0.31*** **32.24%**
8c	**0.47±0.16*** **31.88%**	**1.18±0.25*** **20.27%**	**1.25±0.25*** **29.77%**	**1.39±0.29*** **35.04%**
8d	**0.38±0.15*** **44.92%**	**0.73±0.12*** **50.67%**	**1.00±0.25*** **43.82%**	**1.13±0.28*** **47.19%**
12	0.67±0.26 2.89%	1.64±0.65 -10.81%	1.82±0.70 -2.24%	1.85±0.59 13.55%
8f	**0.39±0.22*** **43.47%**	**0.73±0.26*** **50.67%**	**0.88±0.37*** **50.56%**	**0.88±0.32*** **58.87%**

* $p<0.05$ t-test

CONCLUSIONS

A serie of new polyheterocyclic compounds representing Schiff bases and Mannich bases containing the 5-(pyridine-4-yl)-2H-1,2,4-triazole-3-thione moiety have been synthesized, characterized and evaluated for their anti-inflammatory activity. Schiff bases **6c**, **6f** and Mannich bases **7b**, **7c**, **7d**, **8a**, **8c**, **8d**, **8f**, **10** significantly reduced the inflammatory response, their maximum percent inhibition ranging from 30 to 58.87%. Derivatization of Schiff bases **6a-f** at NH (2^{th} position of the 1,2,4-triazole ring) into the corresponding N-Mannich bases enhanced in most cases the anti-inflammatory activity.

EXPERIMENTAL SECTION

All chemicals (solvents and reagents) were purchased from Merck. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-D_6 solution on a Brucker Avance DPX spectrometer operating at 300 MHz and respectively 75 MHz. Chemical shifts are expressed in ppm values (δ scale) from TMS as internal standard. Mass Spectra were recorded on Agilent 1100 Ion Trap mass spectrometer operating at 70 eV, while IR spectra were recorded on a Brucker Equinox 55 FT-IR spectrometer. Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus.

General procedure for the synthesis of Schiff bases 6a-f

To a solution of 4-amino-5-(pyridine-4-yl)-2H-1,2,4-triazole-3-thione (**4**, 1 mmol) in 10 ml glacial acetic acid, the thiazolic aldehyde **5a-f** (1,5 mmol) was added. The mixture was refluxed for 2 h. The formed precipitate was isolated by filtration and washed with water and then with ethanol.

4-((2-phenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6a): Yield 86%; solid; m.p. 307.7-309.7^{0}C; IR (KBr, cm^{-1}): v = 3450 cm^{-1} (N-H secondary); 3085 cm^{-1} (C-H aromatic); 1603 cm^{-1} (C=N); 1271 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ = 7.54-7.56 (m, 3H); 7.93 (d, J = 6.0 Hz, 2H); 8.01-8.03 (m, 2H); 8.67 (s, 1H); 8.76 (d, J = 5.8 Hz, 2H); 9.96 (s, 1H); 14.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ = 122.41; 127.02; 128.00; 129.90; 131.48; 132.71; 133.14; 147.06; 149.83; 150.76; 159.77; 163.77; 169.18; ESI^{+}-MS: M^{+} found (M^{+} calculated for $C_{17}H_{12}N_6S_2$): 365.2 (364.4).

4-((2-*p*-tolylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6b): Yield 78%; solid; m.p. 313.3-315.5^{0}C; IR (KBr, cm^{-1}): v = 3450 cm^{-1} (N-H secondary); 3082 cm^{-1} (C-H aromatic); 1603 cm^{-1} (C=N); 1269 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ = 2.37 (s, 3H); 7.35 (d, J = 7.6 Hz, 2H); 7.89-7.92 (m, 4H); 8.63 (s, 1H); 8.76 (d, J = 4.4 Hz, 2H); 9.93 (s, 1H); 14.52 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ = 21.46; 122.41; 126.95; 127.60; 130.14; 130.42; 133.15; 141.18; 147.06; 149.70; 150.76; 159.88; 163.27; 169.27; ESI^{+}-MS: M^{+} found (M^{+} calculated for $C_{18}H_{14}N_6S_2$): 379.3 (378.5).

4-((2-*m*-tolylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6c): Yield 89%; solid; m.p. 310.3-314.7^{0}C; v = 3450 cm^{-1} (N-H secondary); 3055 cm^{-1} (C-H aromatic); 1611 cm^{-1} (C=N); 1263 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ= 2.39 (s, 3H); 7.34 (d, J = 7.5 Hz, 1H); 7.42 (t, J = 7.6 Hz, 1H); 7.78-7.82 (m, 2H); 7.92 (dd, J = 4.6 Hz, 1.5 Hz, 2H); 8.64 (s, 1H); 8.76 (d, J = 6.0 Hz, 2H); 9.96 (s, 1H); 14.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ= 21.32; 122.41; 124.22; 127.34; 127.75; 129.76; 132.13; 132.66; 133.14; 139.31; 147.05; 149.78; 150.74; 159.63; 163.29; 169.26; ESI$^+$-MS: M^+ found (M^+ calculated for $C_{18}H_{14}N_6S_2$): 379.3 (378.5).

4-((2-*p*-chlorophenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6d): Yield 97%; solid; m.p. 307.3-308.4^{0}C; v = 3446 cm^{-1} (N-H secondary); 3058 cm^{-1} (C-H aromatic); 1604 cm^{-1} (C=N); 1276 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ= 7.62 (d, J = 8.1 Hz, 2H); 7.92 (d, J = 4.5 Hz, 2H); 8.05 (d, J = 7.9 Hz, 2H); 8.70 (s, 1H); 8.77 (d, J = 4.5 Hz, 2H); 9.97 (s, 1H); 14.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ = 120.20; 123.88; 126.97; 129.98; 133.29; 135.36; 137.52; 146.30; 149.16; 150.76; 162.83; 165.06; 168.52; ESI$^+$-MS: M^+ found (M^+ calculated for $C_{17}H_{11}ClN_6S_2$): 399.2 (398.9).

4-((2-*p*-bromophenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6e): Yield 87%; solid; m.p. 296.1-298.3^{0}C; v = 3482 cm^{-1} (N-H secondary); 3055 cm^{-1} (C-H aromatic); 1604 cm^{-1} (C=N); 1277 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ= 7.76 (d, J = 8.4 Hz, 2H); 7.92 (d, J = 5.7 Hz, 2H); 7.98 (d, J = 8.4 Hz, 2H); 8.71 (s, 1H); 8.77 (d, J = 5.5 Hz, 2H); 9.97 (s, 1H); 14.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ = 121.84; 124.35; 127.57; 128.44; 131.44; 132.39; 132.82; 146.60; 149.57; 150.27; 158.60; 162.92; 167.43; ESI$^+$-MS: M^+ found (M^+ calculated for $C_{17}H_{11}BrN_6S_2$): 443.3 (443.3).

4-((2-phenylthiazolo[3,2-*b*][1,2,4]-triazol-6-yl)methyleneamino)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3-thione (6f): Yield 77%; solid; m.p. 285.3-288.2^{0}C; v = 3461 cm^{-1} (N-H secondary); 3067 cm^{-1} (C-H aromatic); 1604 cm^{-1} (C=N); 1286 cm^{-1} (C=S); ^{1}H NMR (300 MHz, DMSO-D_6): δ= 7.51-7.56 (m, 3H); 8.12-8.16 (m, 2H); 8.29 (dd, J = 4.6 Hz, 1.5 Hz, 2H); 8.40 (s, 1H); 8.65 (d, J = 5.9 Hz, 2H); 9.97 (s, 1H); 14.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ= 121.42; 122.93; 123.95; 126.69; 127.04; 129.59; 130.32; 136.83; 137.42; 147.55; 148.45; 150.47; 151.64; 161.91; ESI$^+$-MS: M^+ found (M^+ calculated for $C_{17}H_{11}BrN_6S_2$): 404.3 (404.5).

General procedure for the synthesis of Mannich bases 7a-f; 8a,c,d,f; 9-12

1 mmol of the previously obtained 1,2,4-triazole derivatives (Schiff bases **6a-f** and triazole derivatives **1-3**) was suspended into a mixture of 2.5 ml DMF and 1 ml of absolute ethanol. To the obtained mixture were added 0.15 ml of formaldehyde solution 37% and 1 mmol of secondary amine (pyrrolidine, piperidine). The reaction mixture was stirred at room temperature for 48 h and then kept for 12h at 0ºC. The formed precipitate was filtered and washed with ethanol.

4-((2-phenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7a): Yield 50%; solid; m.p. 145.2-143.8^{0}C; v = 3093 cm^{-1} (C-H aromatic); 2964 cm^{-1} (C-H aliphatic); 1676 cm^{-1} (C=N); 1268 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.77 (m, 4H); 2.95 (m, 4H); 5.36 (s, 2H); 7.46-7.48 (m, 3H); 7.98-8.02 (m, 5H); 8.76 (d, J = 6 Hz, 2H); 10.46 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.02; 50.50; 66.10; 122.32; 124.61; 126.95; 129.21; 130.99; 132.80; 133.00; 145.95; 150.36; 150.43; 156.53; 164.33; 169.88.

4-((2-*p*-tolylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7b): Yield 69%; solid; m.p. 151.2-153.9^{0}C; v = 3090 cm^{-1} (C-H aromatic); 2971 cm^{-1} (C-H aliphatic); 1676 cm^{-1} (C=N); 1266 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.78 (m, 4H); 2.43 (s, 3H); 2.97 (m, 4H); 5.38 (s, 2H); 7.29 (d, J = 6.7 Hz, 2H); 7.92 (d, J = 8.1 Hz, 2H); 8.01-8.02 (m, 3H); 8.76 (d, J = 6.1 Hz, 2H); 10.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.62; 24.03; 50.50; 66.10; 122.32; 124.40; 126.89; 129.89; 130.19; 133.01; 141.42; 145.96; 150.21; 150.44; 156.67; 164,40; 169.93.

4-((2-*m*-tolylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7c): Yield 69%; solid; m.p. 177.6-178.9^{0}C; v = 3031 cm^{-1} (C-H aromatic); 2967 cm^{-1} (C-H aliphatic); 1679 cm^{-1} (C=N); 1282 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.77 (m, 4H); 2.43 (s, 3H); 2.95 (m, 4H); 5.36 (s, 2H); 7.29-7.38 (m, 2H); 7.78 (d, J = 7.5 Hz, 1H); 7.84 (s, 1H); 7.98-8.01 (m, 3H); 8.76 (d, J = 6.1 Hz, 2H); 10.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.32; 23.87; 50.34; 65.94; 122.17; 124.03; 124.48; 127.31; 128.93; 131.66; 132.54; 132.85; 138.91; 145.79; 150.11; 150.26; 156.39; 164.24; 169.82.

4-((2-*p*-chlorophenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7d): Yield 57%; solid; m.p. 170.2-172.8^{0}C; v = 3031 cm^{-1} (C-H aromatic); 2972 cm^{-1} (C-H aliphatic); 1674 cm^{-1} (C=N); 1267 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.79 (m, 4H); 2.96 (m, 4H); 5.38 (s, 2H); 7.46 (d, J = 8.0 Hz, 2H); 7.94-8 (m, 4H); 8.05 (s, 1H); 8.76 (d, J= 4.3 Hz, 2H); 10.49 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.94; 50.43; 66.06; 122.24; 124.54; 128.05; 129.39; 131.20; 132.89; 136.94; 145.87; 150.35; 150.44; 156.27; 164.30; 168.39.

4-((2-*p*-bromophenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7e): Yield 58%; solid; m.p. 168.8-171.3^{0}C; v = 3035 cm^{-1} (C-H aromatic); 2972 cm^{-1} (C-H aliphatic); 1673 cm^{-1} (C=N); 1266 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.79 (m, 4H); 2.97 (m, 4H); 5.38 (s, 2H); 7.63 (d, J = 8.5 Hz, 2H); 7.90 (d, J = 8.5 Hz, 2H); 7.99 (d, J = 6.1 Hz, 2H); 8.06 (s, 1H); 8.77 (d, J = 6.1 Hz, 2H); 10.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.95; 50.43; 66.07; 122.24; 124.52; 125.31; 128.25; 131.63; 132.35; 132.89; 145.88; 150.36; 150.48; 156.08; 164.25; 168.38.

4-((2-phenylthiazolo[3,2-*b*][1,2,4]-triazol-6-yl)methyleneamino)-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (7f): Yield 50%; solid; m.p. 208.5-210.7^{0}C; v = 3043 cm^{-1} (C-H aromatic); 2964 cm^{-1} (C-H aliphatic); 1672 cm^{-1} (C=N); 1274 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.79 (m, 4H); 2.97 (m,

4H)); 5.38 (s, 2H); 7.48-7.51 (m, 3H); 7.66 (s, 1H); 8.19-8.22 (m, 2H); 8.38 (dd, J = 4.6 Hz, 1.6 Hz, 2H); 8.77 (dd, J = 4.6 Hz, 1.6 Hz, 2H); 11.16 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.20; 50.62; 66.11; 121.54; 122.66; 127.01; 128.05; 129.03; 130.42; 130.92; 132.84; 145.99; 146.68; 150.70; 157.53; 163.95; 168.36.

4-((2-*p*henylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((piperidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (8a): Yield 87%; solid; m.p. 188.3-188.9^{0}C; v = 3031 cm^{-1} (C-H aromatic); 2944 cm^{-1} (C-H aliphatic); 1669 cm^{-1} (C=N); 1270 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (m, 2H); 1.61 (m, 4H); 2.84 (m, 4H); 5.27 (s, 2H); 7.49 (m, 3H); 8.01-8.04 (m, 5H); 8.78 (d, J = 4.8 Hz, 2H); 10.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.77; 26.01; 51.89; 70.87; 122.25; 124.54; 126.88; 129.13; 130.91; 132.73; 132.96; 145.67; 150.29; 150.36; 156.51; 164.40; 169.64.

4-((2-*m*-tolylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((piperidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (8c): Yield 67%; solid; m.p. 151.8-155.5^{0}C; v = 3023 cm^{-1} (C-H aromatic); 2933 cm^{-1} (C-H aliphatic); 1676 cm^{-1} (C=N); 1270 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (m, 2H); 1.61 (m, 4H); 2.45 (s, 3H); 2.84 (t, J = 5.1 Hz, 4H); 5.27 (s, 2H); 7.28-7.40 (m, 2H); 7.80 (d, J = 7.5 Hz, 1H); 8.01-8.03 (m, 3H) overlapped with 7.81 (s, 1H); 8.79 (dd, J = 4.7 Hz, 1.5 Hz, 2H); 10.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.39; 23.78; 26.01; 51.90; 70.65; 122.25; 124.12; 124.57; 127.40; 129.02; 131.73; 132.63; 132.97; 138.89; 145.67; 150.20; 150.30; 156.54; 164.41; 169.91.

4-((2-*p*-chlorophenylthiazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-2-((piperidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (8d): Yield 58%; solid; m.p. 187.3-187.9 ^{0}C; v = 3103 cm^{-1} (C-H aromatic); 2930 cm^{-1} (C-H aliphatic); 1669 cm^{-1} (C=N); 1273 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (m, 2H); 1.60 (m, 4H); 2.84 (t, J = 5.0 Hz, 4H); 5.26 (s, 2H); 7.45 (d, J = 8.5 Hz, 2H); 7.94-8.00 (m, 4H); 8.05 (s, 1H); 8.78 (d, J = 6.0 Hz, 2H); 10.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.84; 26.07; 51.96; 70.96; 122.29; 124.60; 128.12; 129.45; 131.27; 132.99; 137.00; 154.73; 150.43; 150.51; 156.38; 164.45; 168.32.

4-((2-phenylthiazolo[3,2-*b*][1,2,4]-triazol-6-yl)methyleneamino)-5-(pyridin-4-yl)-2-((piperidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (8f): Yield 60%; solid; m.p. 212.4-215.7^{0}C; v = 3055 cm^{-1} (C-H aromatic); 2932 cm^{-1} (C-H aliphatic); 1670 cm^{-1} (C=N); 1261 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (m, 2H); 1.61 (m, 4H); 2.84 (t, J = 5.1 Hz, 4H); 5.26 (s, 2H); 7.49-7.51 (m, 3H); 7.66 (s, 1H); 8.19-8.22 (m, 2H); 8.38 (dd, J = 4.6 Hz, 1.6 Hz, 2H); 8.77 (dd, J = 4.6 Hz, 1.6 Hz, 2H); 11.16 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.98; 26.23; 52.13; 70.96; 121.50; 122.67; 127.03; 128.08; 129.04; 130.42; 130.93; 132.88; 145.94; 146.96; 150.72; 159.18; 164.31; 168.42.

4-methyl-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (9): Yield 59%; solid; m.p. 140.3-142.8^{0}C; v = 2964 cm^{-1} (C-H aliphatic); 1220 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.71-1.75 (m, 4H); 2.90 (t, J = 6.4 Hz, 4H); 3.71 (s, 3H); 5.27 (s, 2H); 7.54 (dd, J = 4.5, 1.5 Hz, 2H); 8.79 (dd, J = 4.5, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.86; 33.24; 50.47; 66.27; 122.17; 133.39; 147.95; 150.79; 169.88.

4-allyl-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (10): Yield 40%; solid; m.p. 110.5-111.9^{0}C; v = 3036 cm^{-1} (C-H vinyl); 2975 cm^{-1} (C-H aliphatic); 1267 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.73-1.78 (m, 4H); 2.91 (t, J = 5.5 Hz, 4H); 4.78-4.80 (m, 2H); 5.30 (s, 2H); 5.93-6.05 (m, 1H); 7.58 (dd, J = 4.5 Hz, 1.5 Hz, 2H); 8.77 (dd, J = 4.5 Hz, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.85; 47.85; 50.50; 66.24; 118.52; 122.20; 130.87; 133.33; 148.18; 150.73; 169.78.

4-phenyl-5-(pyridin-4-yl)-2-((pyrrolidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (11): Yield 61%; solid; m.p. 160.5-161.5^{0}C; v = 3031 cm^{-1} (C-H aromatic); 2957 cm^{-1} (C-H aliphatic); 1151 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.77-1.81 (m, 4H); 2.97 (t, J = 5.5 Hz, 4H); 5.35 (s, 2H); 7.18 (dd, J = 4.6 Hz, 1.5 Hz, 2H); 7.29-7.32 (m, 3H); 7.52-7.54 (m, 2H); 8.54 (dd, J = 4.6 Hz, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 23.92; 50.67; 66.54; 121.59; 129.21; 130.04; 130.29; 132.92; 134.72; 146.83; 150.34; 170.98.

4-phenyl-5-(pyridin-4-yl)-2-((piperidin-1-yl)methyl)-2H-1,2,4-triazole-3-thione (12): Yield 60%; solid; m.p. 204.5-206.30C; v = 3029 cm^{-1} (C-H aromatic); 2932 cm^{-1} (C-H aliphatic); 1273 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42-1.46 (m, 2H); 1.59-1.66 (m, 4H); 2.86 (t, J = 5.2 Hz, 4H); 5.27 (s, 1H); 7.21 (dd, J = 4.6 Hz, 1.5 Hz, 2H); 7.31-7.34 (m, 2H); 7.55-7.57 (m, 3H); 8.57 (dd, J = 4.6 Hz, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ =23.77; 25.95; 51.91; 71.18; 121.61; 128.24; 130.04; 130.29; 132.99; 134.75; 146.61; 150.32; 171.17.

The anti-inflammatory activity

All synthesized compounds were evaluated in order to determinate their anti-inflammatory activity, by performing the rat paw oedema test, according to the method of Winter et al (1962) modified by the introduction of a commercially available plethysmometer from Ugo Basile, Varese, Italy [16]. Male rats Winstar breed with an average weight around 175g were divided into 22 groups of 6 rats. All animals were housed in standard conditions with food and water *ad libitum*. The first group which represents the control one was injected intraperitoneally (i.p.) with 1 ml vehicle (Tween 80 and distilled water). The second group which represents the standard one was injected i.p. with diclofenac 20mg/kg as reference drug. In the 20 treated groups, all tested compounds were injected i.p. with doses of 20mg/kg. For all 22 groups the volume of the solution used for intraperitoneal administration was 1ml.

The inflammation was induced thirty minutes after intraperitoneal injection by administrating 0.1 ml carrageenan solution 1% intraplantar. Rats oedema were evaluated by measuring the rat hind left paw volume at hourly intervals from 1 to 4 hours. The rats paw volume was also measured before inducing inflammation.

The inhibition percent was calculated according to the following formula: % Inhibition of oedema = (1-Ēt/Ēm)x100, where Ēt represents the average value of the oedema in all treated groups 1 – 4 hours after the induced inflammation (in ml) and Ēm represents the average value of the oedema in control group 1 – 4 hours after after the induced inflammation (in ml).

Statistical analysis was performed by Student's 't' test, and *p*-value was choosen less than 0.05 for statistical significance.

The biological experiment was conducted according to the EC Directive 2010/63/EU, which regulates the use of laboratory animals.

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