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Original article

Synthesis and biological evaluation of some novel thiazole compounds as potential anti-inflammatory agents

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ABSTRACT

In the present investigation, furo[2,3-d]thiazol-5(2H)-one **5** was obtained from reaction of thiosemicarbazone derivative **2** with diethyl acetylene dicarboxylate. A series of newly synthesized 2-(hydrazinyl)thiazol-4(5H)-one **6, 7** & **8** and 2-(4-(substituted)-thiazol-2-yl)hydrazono derivatives **9a, b** & **10** were synthesized from treatment of thiosemicarbazone derivative **2** with appropriate α-halogenated compounds. Also, a one pot synthesis of thiazole derivatives **13** & **15** was achieved from three components reaction of hydrazone derivative **11** with phenyl isothiocyanate and α-halogenated compounds catalyzed by DMF/KOH. 4-(4-Morpholino phenyl) thiazol-2-amino **17** was obtained via the reaction of acetophenone derivative **1** with thiourea in presence of iodine. The reactivity of 2-aminothiazole **17** toward some electrophilic reagents was investigated. The structure of the newly compounds was confirmed on the basis of elemental analysis and spectral data. The antibacterial activity towards two Gram negative (*Proteus mirabilis* & *Serratia marcesens*) and two Gram positive (*Staphylococcus aureus* & *Bacillus cereus*) bacteria was investigated. The anti-inflammatory activity was also investigated and the inhibition of the carrageenin-induced oedema by these compounds was established.

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1. Introduction

Thiazolidinone has an important role as a widely exploited pharmacophore in medicinal chemistry [1] having varied biological activity such as antifungal [2], antibacterial [3,4], antimycobacterial [5], antipsychotic [6], anti-inflammatory [7]. Also, substituted thiazolidine derivatives represent important key intermediates for the synthesis of pharmacologically active drugs. It is well known that thiazole compounds have recently been grown up due to their biological activity [8]. They can be used as anticonvulsants [9], antibacterial [10,11] and antifungal agents [12]. Recently, some new thiazole compounds are used as anti-inflammatory [13]. In addition, morpholine is a simple heterocyclic compound with a great industrial importance. Many N-functionalized morpholines have found to possess diverse pharmacological activities. They are reported to exert a number of important physiological activities such as antidibetic [14], antihyperlipo-proteinemics [15], antiemetic [16], platelet aggregation inhibitors, bronchodilators, growth stimulates [17] and antidepressants [18]. These were also used in the treatment of inflammatory diseases, pain, migraine and asthma [19]. In view of the above facts and in continuation of our research program directed towards the development of a new, simple and efficient procedure for the synthesis of heterocyclic compounds [20–31]. It seems of considerable interest to synthesize newly the thiazole derivatives containing morpholine moiety. Additionally, our objective is also to study the antibacterial and anti-inflammatory activities of the synthesized compounds.

2. Results and discussion

2.1. Chemistry

The starting 1-(4-morpholinophenyl)ethanone **1** was obtained by nucleophilic substitution of 4-fluroacetophenone with appropriate morpholine in dimethyl sulphoxide (DMSO) in the presence of potassium carbonate as a base under reflux [32] (Scheme 1).

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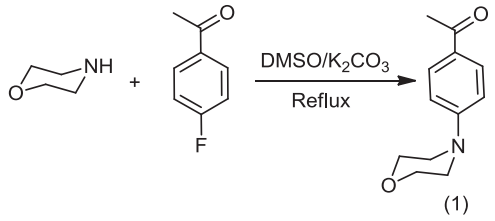
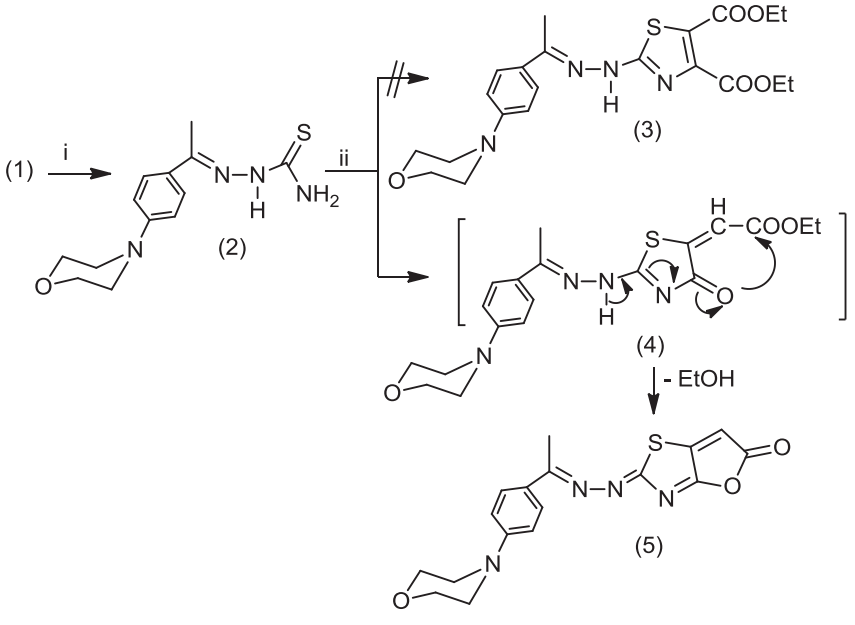


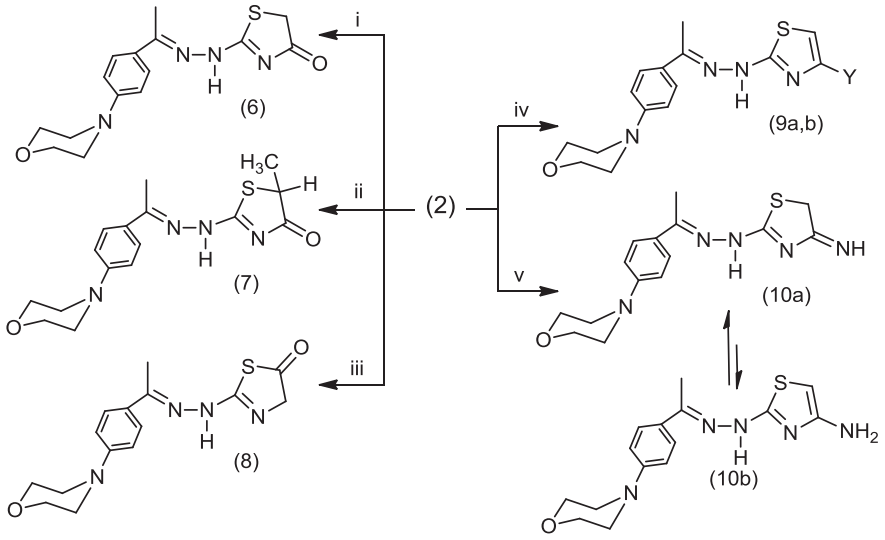
Scheme 1.

Thiosemicarbazone derivative **2** was obtained via condensation of ethanone derivative **1** with thiosemicarbazide in presence of a catalytic amount of conc. HCl in ethanol as the solvent affording in satisfactory yield (70%). IR spectrum of compound **2** revealed characteristic absorption bands at $\upsilon = 3418$, 3288 and 3167 cm^{-1} assignable for (NH_2 & NH), while its ^{1}HNMR spectrum (DMSO-d_6) indicated singlet signals at 2.23 ppm for CH_3, two triplet at 3.13, 3.73 ppm for morphonyl protons with two singlet at 8.17 and 10.07 ppm for NH and NH_2 respectively. Also, ^{13}CNMR (DMSO-d_6) of compound **2** revealed signals at δ 17.00 (CH_3), 39.98 (C3, C5 of morpholine), 77.62 (C2, C6 of morpholine), 147.55 (C=N) and 178.28 (C=S) ppm. Treatment of thiosemicarbazone derivative **2** with diethyl acetylene dicarboxylate afforded furo[2,3-d]thiazole derivative **5** and other expected structure **3** was ruled out on the basis of elemental analysis and spectral data. IR spectrum of compound **5** showed characteristic absorption band at 1703 cm^{-1} corresponding to carbonyl group. ^{1}HNMR spectrum ($CDCl_3$) of compound **5** revealed singlet signals at 6.80 ppm for CH furan with two triplets at 3.26 and 3.88 ppm for the morphonyl protons. Also, mass spectrum of **5** showed a molecular ion peak at m/z 356 (55%) with base peak at $m/z = 162$. The formation of compound **5** was assumed to proceed via nucleophilic attack of SH group on the activated triple bond of diethyl acetylene dicarboxylate followed by in situ heterocyclization with elimination of two ethanol molecule to afford compound **5** through non-isolable intermediate **4** (Scheme 2).

The behavior of thiosemicarbazone derivative **2** toward some α-halogenated compounds was investigated to synthesize versatile hitherto unreported thiazole derivative. Thus, the reaction of thiosemicarbazone derivative **2** with ethyl chloroacetate in glacial acetic acid containing a catalytic amount of fused sodium acetate afforded the corresponding 4-thiazolidinone derivative **6**. The molecular structure of compound **6** was established on the basis of its elemental analysis and spectral data. The infrared spectrum of **6** revealed characteristic absorption bands at 3140 and 1708 cm^{-1} for NH and carbonyl group, respectively. A molecular ion peak at $m/z = 318$ (35%) was observed in the mass spectrum of compound **6** with base peak at $m/z = 317$. The ^{1}HNMR spectrum of **7** showed signals at δ 2.37 (s, 2H, CH_3), 3.24, 3.71 (2t, 8H, morphonyl-H), 3.88 (s, 2H, SCH_2) and 9.42 ppm (s, 1H, NH). Similarly, reaction of thiosemicarbazone **3** with ethyl-α-chloropropionate resulted in the formation of 5-methyl-4-thiazolinone derivative **7** according to the spectral data of the isolated product (Scheme 3). The IR spectrum of compound **7** revealed intense absorption bands at 3179 (NH) and 1718 cm^{-1} (C=O). ^{1}HNMR spectrum (DMSO-d_6) of isolated product 7 showed signals at δ 1.70 (d, 3H, CH_3), 4.16 (q, 1H, CH) with singlet at 9.70 ppm for NH proton. The formation of compound **7** may be assumed to proceed through initial alkylation followed by intramolecular cyclization with elimination of ethanol. On the other hand reaction of thiosemicarbazone derivative **2** with chloro acetyl chloride afforded the corresponding 5-thiazolidinone derivative **8** (Scheme 3). ^{1}HNMR spectrum (DMSO-d_6) of **8** revealed singlet signal at 3.82 ppm for CH_2 thiazole. Also, the structure of compound **8** was confirmed on the basis of ^{13}CNMR which revealed signals at δ 14.35 for CH_3, 32.75 (C4 of thiazole), 47.62 (C3, C5 of morpholine moiety) and 173.91 ppm (thazole-C5). Cyclocondensation of thiosemicarbazone **2** with chloroacetone and phenacyl bromide in refluxing ethanol containing catalytic amount of fused sodium acetate resulted in the formation of 1,3-thiazoles **9a, b**. The ^{1}HNMR spectrum (DMSO-d_6) of the isolated products revealed in each case a singlet signal at 6.97 ppm for thiazole-H5. ^{13}CNMR spectrum (DMSO-d_6) of **9b** revealed signals at δ 14.35 (CH_3), 47.62 (C3, C5 of morpholine), 65.02 (C2, C6 of morpholine), 103.77 (thiazole-C5)



Scheme 2.

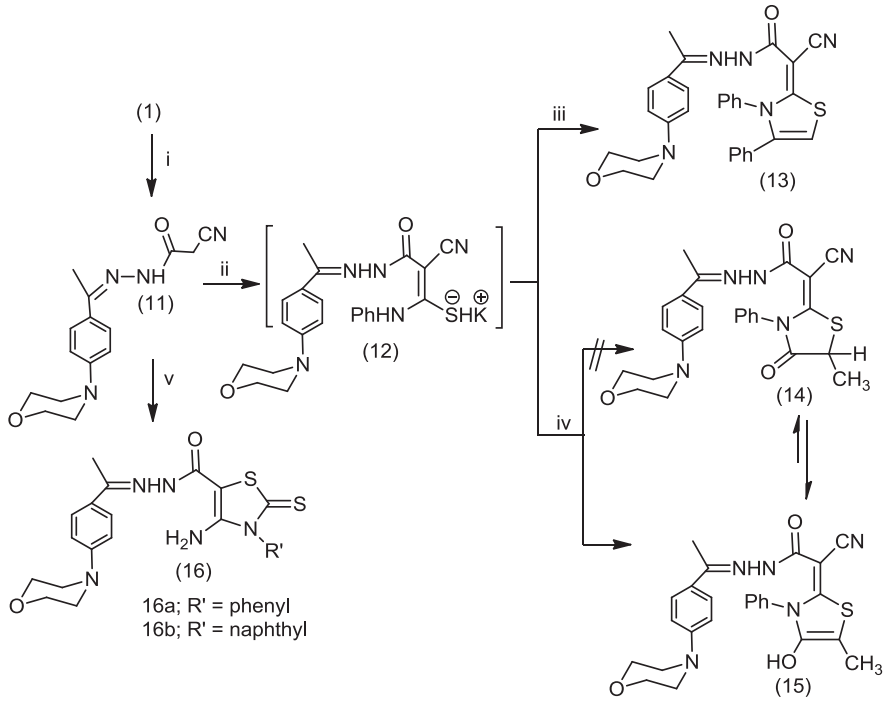


Scheme 3.

and 170.05 (thiazole-C2). In addition, interaction of thiosemicarbazone **2** with chloroacetonitrile afforded 4-aminothiazole derivative **10** (Scheme 3). The structure of **10** was confirmed on the basis of elemental analysis and spectral data. IR spectrum of compound **10** showed absorption bands at $\upsilon = 3316, 3104\ cm^{-1}$ for NH_2/NH groups. ^{1}HNMR spectrum (DMSO-d6) of **10** indicated that, the reaction product exist in the imino form **10a** rather than the amino form **10b**.

Condensation of acetophenone derivative **1** with cyanoacetic acid hydrazide afforded hydrazone derivative **11** on the basis of spectral data which indicated the presence of characteristic absorption bands at $\upsilon = 3200$ and $2257\ cm^{-1}$ assignable for NH and C≡N groups in the infrared spectrum. ^{1}HNMR spectrum (DMSO-d_6) of compound **11** revealed singlet signals at δ 3.99 and 10.70 ppm for active methylene protons and NH proton. Also, the structure of compound **11** was confirmed on the basis of ^{13}CNMR (DMSO-d_6) which revealed signals at δ 13.14, 24.79 for CH_3 and CH_2, 66.21, 77.05 for morpholine moiety and 149.90, 196.64 for C≡N and C═O groups (Scheme 4). The non-isolable potassium sulphide salt **12** was achieved by the nucleophilic addition of active methylene group in compound **11** to phenyl isothiocyanate in dry dimethylformamide at room temperature in the presence of potassium hydroxide (Scheme 4). The potassium salt **12** was exploited to synthesize some new thiazolidine derivatives. Cyclocondensation of intermediate **12** with phenacyl bromide gave 4-phenylthiazole derivative **13** (Scheme 4). Infrared spectrum of compound **13** indicated the presence of NH, C≡N and C═O functional groups. ^{1}HNMR spectrum of compound **13** revealed singlet signals at δ 6.88 ppm for thiazole-H5. Also, the mass spectrum of compound **13** revealed a molecular ion peak at $m/z = 521$ (36%) which is characteristic for the molecular formula $C_{30}H_{27}N_5O_2S$. Treatment of intermediate **12** with ethyl-α-chloropropionate at room temperature gave 4-hydroxythiazole derivative **15**. The structure of compound **15** was preferred rather than the compound **14** according to the spectral data. IR spectrum showed abroad absorption band at 3420 cm^{-1} corresponding to hydroxyl group. ^{1}HNMR spectrum (DMSO-d_6) of compound **15** revealed singlet signals at δ 1.89, 2.29 (2s, 6H, $2CH_3$), 3.13, 3.72 (2t, 8H, morpholine moiety), 10.07 (s, 1H, NH) with singlet at δ 11.88 for OH group. The formation of **15** may be assumed to proceed through initial alkylation followed by intramolecular cyclization and elimination of ethanol molecule to afford the two isomeric structures **14** and **15**. In addition, ternary condensation of hydrazone derivative **11**, aryl isothiocyanate and sulfur metal in refluxing ethanol and in the presence of a catalytic amount of triethyl amine resulted in the formation of 4-aminothiazol-2-thione derivative **16a, b**. Infrared spectrum of isolated product **16a** revealed absorption bands at $\upsilon = 3327, 3310, 3200$ and $1672\ cm^{-1}$ corresponding to (NH_2/NH) and (C═O) groups, respectively. ^{1}H NMR spectrum (DMSO-d_6) of **16a** revealed singlet at δ 2.52 for CH_3, multiplet aromatic protons with NH_2 at δ 6.99–7.61 ppm with singlet signal at 9.00 ppm for imino group. Mass spectrum of compound **16b** showed a molecular ion peak at $m/z = 503$ (25%) with base peak at $m/z = 388$ (Scheme 4).

Furthermore, 4-(4-morpholinophenyl) thiazol-2-amino **17** was obtained via the reaction of acetophenone derivative **1** with thiourea in presence of iodine [33]. Compound **17** was characterized by its elemental analysis and spectral data. IR spectrum showed characteristic absorption bands at 3303, 3117 cm^{-1} assignable for amino group. ^{1}H NMR spectrum ($CDCl_3$) of **17** showed singlet signal at 6.58 assignable for thiazole-H_5 with singlet signal at 7.94 ppm assignable for NH_2. Also, ^{13}CNMR spectrum of **17** revealed signals at δ 102.00 (thiazole-C5), 150.30 (thiazole-C4), and 168.90 (thiazole-C2) (Scheme 5). The reactivity of 2-aminothiazole derivative **17** toward some electrophilic reagents was investigated. Thus, condensation of compound **17** with 4-methylbenzaldehyde in refluxing ethanol and in the presence of a catalytic amount of piperidine resulted in the formation of the imino derivative **18**. ^{1}HNMR spectrum (DMSO-d_6) of the isolated product **18** showed singlet signal at 2.17, 5.95 and 7.55 ppm assignable for CH_3, thiazole-H_5 and CH-benzylidine, respectively. Also, acetylation of 2-aminothiazole **17** with acetic anhydride on refluxing afforded N,N-(diacetyl)aminothiazole derivative **19** on the basis of spectral data. IR spectrum showed the absence of NH_2 group with the presence of a characteristic absorption band at $1691\ cm^{-1}$ assignable for carbonyl group. Also, ^{1}HNMR spectrum (DMSO-d_6) of **19** revealed two singlet signals 2.23, 2.40 ppm for two methyl with singlet at 6.92 ppm for thiazole-H5. Finally, condensation of 2-amino-4-phenyl thiazole with ethanone derivative **1** afforded the

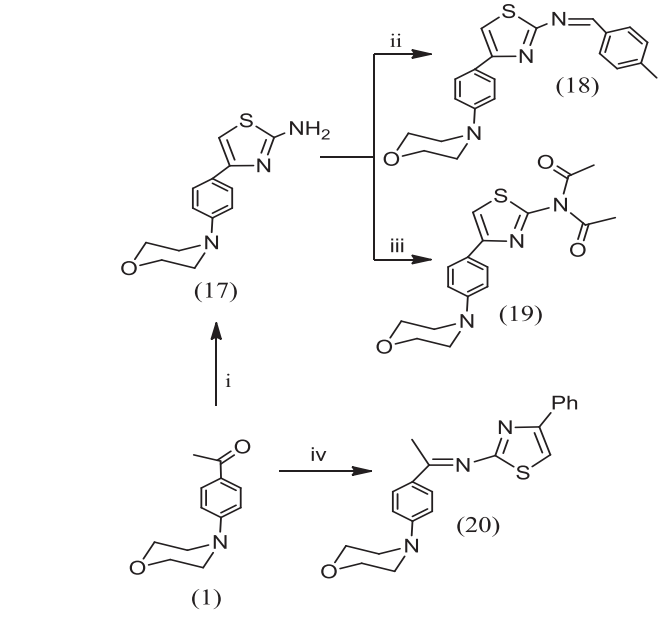


Scheme 4.

corresponding thiazole derivative **20**. ^{1}H NMR spectrum ($CDCl_3$) of the isolated product **20** showed singlet signal at 2.53 for CH_3 with signal at 5.22 ppm for thiazole-H5 (Scheme 5).

2.2. Molecular modeling calculations

In order to throw light on the molecular conformation of the synthesized compounds, energy minimization studies were carried out on the basis of the semi-empirical PM3 level provided by HyperChem 7.5 software. The calculated bond length and bond angles after geometrical optimization of compound **17** structure as a representative example of thiazole compounds are given in Table 1. The molecular structure of compound **17** structure along with the atom numbering scheme are given in Fig. 1.



Scheme 5.

2.3. Biological activity

2.3.1. Antibacterial activity

The synthesized compounds were tested for their inhibitory effects on the growth of two Gram negative (*Proteus mirabilis* & *Serratia marcesens*) and two Gram positive bacteria (*Staphylococcus aureus* & *Bacillus cereus*) bacteria in DMSO as solvent using *Ampicillin* as standard material because such organisms can achieve resistance to antibiotics through biochemical and morphological modification [34]. The antibacterial activity of the new compounds is listed in Table 2. The antibacterial activity was tested by using the disc diffusion method. The antimicrobial results showed that:

1. Only compound **11** were found to be the more active compounds against *S. aureus* (NCTC-7447) (Fig. 2).
2. None of the other tested compounds showed superior activity over the reference.
3. The activity index was calculated and the results are given in Table 3. The activity index of compound **11** reaches 75, suggesting that this compound be considered as the most promising potent broad spectrum antimicrobial compound (Table 4)
4. The tested heterocyclic compounds were more active against gram-positive than Gram-negative bacteria, it may be concluded that the antimicrobial activity of the compounds is related to cell wall structure of the bacteria. It is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in

Table 1
Bond distances (Å) and angles (°) for compound **17**.

Atoms	Bond distances (Å)	Atoms	Angle (°)	Atoms	Angle (o)
C(17)–H(33)	1.113	C(17)–O(18)–C(16)	110.6935	C(11)–C(7)–N(8)	115.6691
C(17)–H(32)	1.113	H(33)–C(17)–H(32)	110.7607	C(11)–C(7)–C(6)	122.1654
C(16)–H(31)	1.113	H(33)–C(17)–O(18)	108.3961	N(8)–C(7)–C(6)	122.1654
C(16)–H(30)	1.113	H(33)–C(17)–C(14)	108.3961	C(7)–C(6)–C(5)	120.0002
C(15)–H(29)	1.113	H(32)–C(17)–O(18)	108.9845	C(7)–C(6)–C(1)	120.0002
C(15)–H(28)	1.113	H(32)–C(17)–C(14)	108.9845	C(5)–C(6)–C(1)	119.9996
C(14)–H(27)	1.113	O(18)–C(17)–C(14)	111.3236	H(22)–C(5)–C(6)	120.0014
C(14)–H(26)	1.113	H(31)–C(16)–H(30)	110.7596	H(22)–C(5)–C(4)	120.0014
N(12)–H(25)	1.05	H(31)–C(16)–O(18)	108.3971	C(6)–C(5)–C(4)	119.9972
N(12)–H(24)	1.05	H(31)–C(16)–C(15)	108.3971	H(21)–C(4)–C(5)	119.9984
C(11)–H(23)	1.1	H(30)–C(16)–O(18)	108.9849	H(21)–C(4)–C(3)	119.9984
C(5)–H(22)	1.1	H(30)–C(16)–C(15)	108.9849	C(5)–C(4)–C(3)	120.0033
C(4)–H(21)	1.1	O(18)–C(16)–C(15)	111.322	N(13)–C(3)–C(4)	119.9999
C(2)–H(20)	1.1	H(29)–C(15)–H(28)	111.4295	N(13)–C(3)–C(2)	119.9999
C(1)–H(19)	1.1	H(29)–C(15)–C(16)	107.8376	C(4)–C(3)–C(2)	120.0002
N(13)–C(15)	1.4756	H(29)–C(15)–N(13)	107.8376	H(20)–C(2)–C(3)	120.0015
C(16)–C(15)	1.5364	H(28)–C(15)–C(16)	108.744	H(20)–C(2)–C(1)	120.0015
O(18)–C(16)	1.4334	H(28)–C(15)–N(13)	108.744	C(3)–C(2)–C(1)	119.9969
C(17)–O(18)	1.4333	C(16)–C(15)–N(13)	112.2766	H(19)–C(1)–C(6)	119.9986
C(14)–C(17)	1.5364	H(27)–C(14)–H(26)	111.4286	H(19)–C(1)–C(2)	119.9986
N(13)–C(14)	1.4756	H(27)–C(14)–C(17)	107.8384	C(6)–C(1)–C(2)	120.0029
C(1)–C(6)	1.3948	H(27)–C(14)–N(13)	107.8384		
C(5)–C(6)	1.3949	H(26)–C(14)–C(17)	108.7444		
C(4)–C(5)	1.3948	H(26)–C(14)–N(13)	108.7444		
C(3)–C(4)	1.3948	C(17)–C(14)–N(13)	112.2753		
C(2)–C(3)	1.3949	C(15)–N(13)–C(14)	108.2826		
C(1)–C(2)	1.3948	C(15)–N(13)–C(3)	125.8587		
C(7)–N(8)	1.3813	C(14)–N(13)–C(3)	125.8587		
C(9)–N(8)	1.3152	H(25)–N(12)–H(24)	120		
S(10)–C(9)	1.7161	H(25)–N(12)–C(9)	120		
C(11)–S(10)	1.7154	H(24)–N(12)–C(9)	120		
C(7)–C(11)	1.3788	H(23)–C(11)–S(10)	125.1462		
C(3)–N(13)	1.266	H(23)–C(11)–C(7)	125.1462		
C(9)–N(12)	1.266	S(10)–C(11)–C(7)	109.7076		
C(6)–C(7)	1.337	C(11)–S(10)–C(9)	89.2503		
		N(12)–C(9)–S(10)	121.8996		
		N(12)–C(9)–N(8)	121.8996		
		S(10)–C(9)–N(8)	116.2008		
		C(9)–N(8)–C(7)	109.1722		

the synthesis of peptidoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell

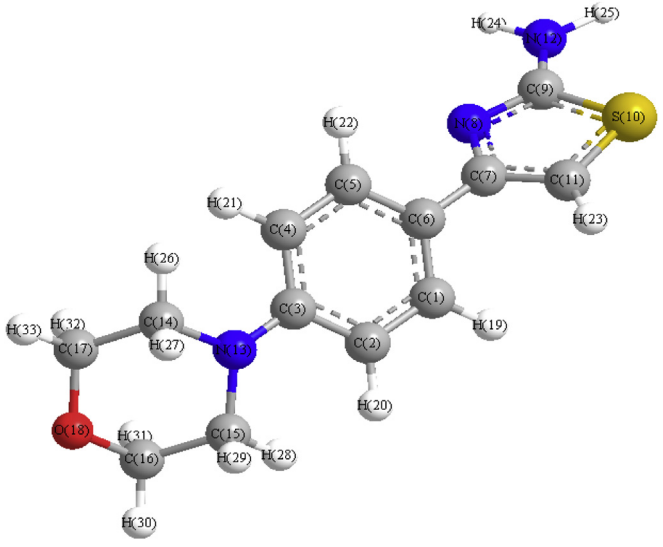


Fig. 1. Molecular modelling of compound **17**.

Table 2
Antibacterial activity of the synthesized compounds and inhibition zones (mm).

Compd. no.	G^+		G^-	
	Staphylococcus aureus (NCTC-7447)	*Bacillus cereus* (NCTC-14579)	*Proteus mirabilis* (NCTC-289)	*Serratia marcesens* (IMRU-70)
2	4	4	2	3
5	5	4	2	3
6	4	4	3	3
7	5	5	2	2
8	5	4	2	3
9a	5	3	2	2
9b	4	4	3	2
10	5	4	2	3
11	18	4	3	2
13	5	4	2	3
15	5	3	3	3
16a	4	3	2	2
16b	5	3	2	2
17	5	4	2	3
18	4	4	2	3
19	5	4	3	2
20	5	4	3	2
Standard (ampicillin)	24	23	21	21
DMSO (control)	0	0	0	0

G^+: Gram positive bacteria and G^-: Gram negative bacteria.

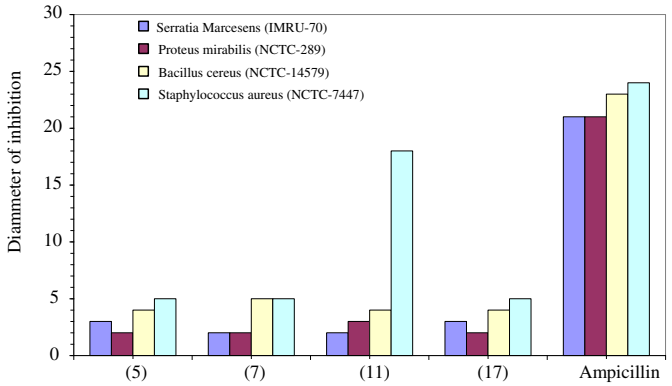


Fig. 2. Antibacterial activity of synthesized compounds.

wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and is infective against Gram-negative pathogens [35–37].

5. The MIC value of compound **11** is 40 μg/ml.
6. The mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine nitrogen atom (>C═N) with the active centers of cell constituents, resulting in interference with the normal cell process [34,36–38].
7. The variation in the effectiveness of different compounds against different organisms depends on either the impermeability of the cells of the microbes or on differences in ribosome of microbial cells [39].

2.3.2. Anti-inflammatory activity

The synthesized thiazole compounds were tested for their anti-inflammatory activity but only seven compounds (**2**, **5**, **6**, **8**, **9b**, **17** and **20)** were found biologically active with inhibition percent ranges from 35% to 87%. These seven compounds were screened for in vivo anti-inflammatory activity by inhibition of carrageenan induced rat paw edema method at the dose of 50 mg/kg orally.

Table 3
Activity index percent of the synthesized compounds and inhibition zones (mm).

Compound no.	G^+		G^-	
	Staphylococcus aureus (NCTC-7447)	*Bacillus cereus* (NCTC-14579)	*Proteus mirabilis* (NCTC-289)	*Serratia marcesens* (IMRU-70)
2	16.67	17.39	9.52	15.72
5	20.83	17.39	9.52	15.72
6	16.67	17.39	14.28	15.72
7	20.83	21.74	9.52	10.52
8	20.83	16.67	9.52	15.72
9a	20.83	13.04	9.52	15.72
9b	16.67	17.39	14.28	15.72
10	20.83	17.39	9.52	15.72
11	75	17.39	14.28	15.72
13	20.83	17.39	9.52	15.72
15	20.83	13.04	14.28	15.72
16a	16.67	13.04	9.52	15.72
16b	20.83	13.04	9.52	15.72
17	20.83	17.39	9.52	15.72
18	16.67	17.39	9.52	15.72
19	20.83	17.39	14.28	15.72
20	20.83	17.39	14.28	15.72
Standard (ampicillin)	24	23	21	19
DMSO (control)	0	0	0	0

G^+: Gram positive bacteria and G^-: Gram negative bacteria.

Results are presented in Table 3, Fig.3, as percent edema increase at the right hind paw and percent inhibition.

Carrageenin-induced edema is a nonspecific inflammation resulting from a complex of diverse mediators (Shen) [40]. Since edemas of this type are highly sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs), carrageenin has been accepted as a useful agent for studying new anti-inflammatory drugs (Winter et al.). This model reliably predicts anti-inflammatory efficacy of the NSAIDs, and during the second phase it detects compounds which are anti-inflammatory agents as a result of inhibition of prostaglandin amplification.

Significant anti-inflammatory activity was observed with inhibition in edema in the range of 35–87% after 4 h. The standard drug indomethacin has shown 91% inhibition after 4 h. Among all the screened compounds (**5** and **20**) were found to be potent in the series with 85 and 87% inhibition after 4 h, respectively, while the least potent one was compound (**6**) in the series with 35% inhibition after 4 h. The inflammatory compounds (**5** and **17**) have a high degree of inhibition reaches 85 and 87%, respectively. Thus these compounds can be considered as the most promising potent broad spectrum inflammatory compounds among the synthesized compounds.

3. Experimental

All melting points are uncorrected. IR spectra (KBr) were measured on Shimadzu 440 spectrometer, ^{1}H NMR spectra were obtained in DMSO on a Varian Gemini 600 MHz spectrometer using TMS as internal standard; chemical shifts are reported as (ppm). Mass spectra were obtained on GCMS/QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Department of Chemistry, Faculty of Science, King Abdul-Aziz University, Jeddah21589, KSA. Microbiology screening was carried out in Microbiology Department, Faculty of Pharmacy, Al-Mansoura University and Pharmacology Department, National Research Center, Cairo, Egypt BOX: 12622.

3.1. Chemistry

*3.1.1. 2-(1-(4-Morpholinophenyl)ethylidene) hydrazinecarbimidothioic acid **2***

Equimolar amounts of compound **1** (0.01 mol), thiosemicarbazide (0.01 mol) and a few drops of conc. HCl in ethanol (30 ml) was refluxed for 3 h. The solid product which produced on heating was collected and recrystallized from acetic acid as yellow crystals. Yield (70%); m.p. 215 °C; IR (KBr, cm^{-1}): 3418, 3288, 3167 (NH_2, NH), 2966, 2829 (aliph. CH) and 1205, 1050 (C═S). MS: 278 (M^+, 29%), 204 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.22 (s, 3H, CH_3), 3.13, 3.73 (2t, 8H, morphonyl-H), 6.88–7.82 (2d, 4H, Ar-H) and 10.07 (s, 1H, NH) ppm. ^{13}CNMR (600 MHz, DMSO-d_6): δ = 17.00 (CH_3), 39.98 (C3, C5 of morpholine), 77.62 (C2, C6 of morpholine), 114.46, 127.32, 129.78, 153.71 (phenyl-C), 147.55 (C═N) and 178.28 (C═S) ppm. Elemental analysis for $C_{13}H_{18}N_4OS$. Calcd: C, 56.09; H, 6.52; N, 20.13; Found: C, 56.00; H, 6.30; N, 19.90.

*3.1.2. 2-(2-(1-(4-Morpholinophenyl)ethylidene)hydrazinyl)furo [2,3-d]thiazo-l-5(2H)-one **5***

A mixture of compound **2** (0.01 mol) and acetylene dicarboxylate (0.01 mol) in ethanol (30 ml) was stirred under reflux for 24 h at 160 °C. The solid product which produced on heating was collected and recrystallized from acetic acid as yellow crystals. Yield (50%); m.p. 246 °C; IR (KBr, cm^{-1}): 2963 (aliph. CH), 1703 (C═O) and 1630 (C═N). MS: 356 (M^+, 55%), 162 (100%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.42 (s, 3H, CH_3), 3.26, 3.88 (2t, 8H, morphonyl-H), 6.80 (s, 1H, Furan-H) and 6.89, 7.85 (2d, 4H, Ar-H)

Table 4
Oedema inhibiting activity of synthesized compounds.

% Edema					% Inhibition			
Time (h)	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Control	54.8 ± 2.9	67.1 ± 4.4	79.5 ± 4.1	83 ± 4.7	–	–	–	–
Indomethacin	49.1 ± 4.2	36.8 ± 4.1*	19.5 ± 2.9*	7.2 ± 0.11	10.5	45.1	75.5	91.3
Compound **2**	74 ± 6.7	51.7 ± 6.4	35 ± 3.5*[a]	13.2 ± 2.6*	−35	23	55.7	83.6
Compound **5**	38 ± 3.6*[a]	30 ± 1.6*	18 ± 0.9*	11.8 ± 1.0*	23.3	50.6	75.7	85
Compound **6**	87.0 ± 1.57	72.5 ± 1.4	56 ± 4.0*[a]	53 ± 2.9*[a]	−58.6	−8	29	35.7
Compound **8**	38.7 ± 3.9	47.9 ± 2.7*	29.1 ± 3.0*	18.1 ± 1.8*	29.5	28.6	63.4	78.2
Compound **9b**	55 ± 4.4	43.6 ± 4.2*	30 ± 1.7*	17.4 ± 1.6*	−1.6	35	62.2	79.1
Compound **17**	64.1 ± 5	45.7 ± 4.3*	19.7 ± 2.2*	11 ± 1.3*	−17	31.7	75.3	86.8
Compound **20**	40 ± 2.5	51.8 ± 4.8	32 ± 1.9*	15 ± 1.5*	27.2	22.9	59.8	81.6

– Values are expressed as means ± SEM ($n = 6$).
* Significantly different from control group at $P < 0.05$.
[a] Significantly different from indomethacin group at $P < 0.05$.

ppm. Elemental analysis for $C_{17}H_{18}N_4O_3S$. Calcd: C, 56.97; H, 5.06; N, 15.63; Found: C, 56.60; H, 4.95; N, 15.46.

3.1.3. *Preparation of compounds (**6**, **7**, **8**, **9a, b** & **10**): general procedure*

A mixture of compound **1** (0.01 mol), appropriate α-halo compounds namely (ethylchloroacetate, ethyl α-chloropropionate, chloro-acetylchloride, chloroacetone, phenacyl bromide, chloroacetonitrile) (0.01 mol) and sodium acetate (0.01 mol) in acetic acid (30 mL) was refluxed for 4 h. The solid product which produced on heating was collected and recrystallized from the proper solvents.

3.1.4. *2-(2-(1-(4-Morpholinophenyl)ethylidene)hydrazinyl)thiazol-4(5H)-one **6***

Yield (75%); white solid (dioxane); m.p.200 °C; IR (KBr, cm^{-1}): 3140 (NH), 2949, 2860 (aliph. CH), 1708 (C=O). MS: 318 (M^+, 35%), 317 (100%). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.37$ (s, 2H, CH_3), 3.24, 3.71 (2t, 8H, morphonyl-H), 3.88 (s, 2H, SCH_2), 6.89, 7.82 (2d, 4H, Ar-H) and 9.42 (br, 1H, OH) ppm. ^{13}CNMR (600 MHz, $CDCl_3$): $\delta = 14.87$ (CH_3), 33.04 (thiazole-C5), 66.20 (C3, C5 of morpholine), 77.05 (C2, C6 of morpholine), 111.46, 114.02, 127.32, 128.21 (phenyl-C), 150.00 (C=N), 161.99 (thiazole-C2) and 172.48 (thiazole-C4) ppm. Elemental analysis for $C_{15}H_{18}N_4O_2S$. Calcd: C, 56.58; H, 5.70; N, 17.60; Found: C, 56.10; H, 5.40; N, 17.50.

3.1.5. *5-Methyl-2-(2-(1-(4-morpholinophenyl)ethylidene) hydrazinyl)thiazol-4(5H)-one **7***

Yield (65%); white solid (dioxane); m.p.205–207 °C; IR (KBr, cm^{-1}): 3179 (NH), 2962, 2831 (aliph. CH), 1718 (C=O). MS: 332 (M^+, 42%), 303 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): 1.70 (d, 3H, CH_3), 3.22, 3.88 (2t, 8H, morphonyl-H), 4.16 (q, 1H, CH), 6.88, 7.90 (2d, 4H, Ar-H), 9.80 (br, 1H, NH) ppm. Elemental analysis for $C_{16}H_{20}N_4O_2S$. Calcd: C, 57.81; H, 6.06; N, 16.85; Found: C, 57.50; H, 9.80; N, 16.40.

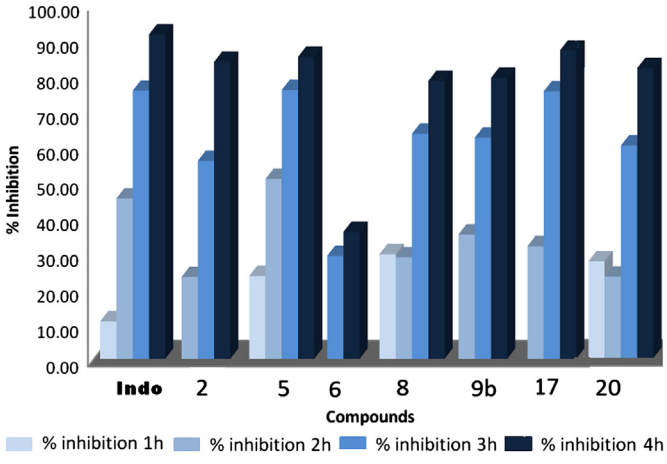


Fig. 3. Anti-inflammatory of synthesized compounds.

3.1.6. *2-(2-(1-(4-Morpholinophenyl)ethylidene)hydrazinyl)thiazol-5(4H)-one **8***

Yield (45%); white solid (ethanol); m.p. 267–68 °C; IR (KBr, cm^{-1}): 3100 (NH), 2983, 2825 (aliph. CH), 1715 (C=O). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 2.28$ (s, 3H, CH_3), 3.18, 3.73 (2t, 8H, morphonyl-H), 3.82 (s, 2H, SCH_2), 6.94, 7.70 (2d, 4H, Ar-H) and 11.86 (s, 1H, NH) ppm. ^{13}CNMR (600 MHz, DMSO-d_6): $\delta = 14.35$ (CH_3), 39.08 (SCH_2), 47.62 (C3, C5 of morpholine), 65.99 (C2, C6 of morpholine), 113.47, 114.04, 127.47, 127.98, 130.22 (phenyl-C), 152.09 (thiazole-C2), 159.92 (C=N) and 173.91(thiazole-C5) ppm. Elemental analysis for $C_{15}H_{18}N_4O_2S$. Calcd. C, 56.58; H, 5.70; N, 17.60; Found: C, 56.20; H, 5.50; N, 17.30.

3.1.7. *4-(4-(1-(2-(4-Methylthiazol-2-yl) hydrazono) ethyl) phenyl) morpholine **9a***

Yield (55%); white crystals (ethanol); m.p. 291 °C; IR (KBr, cm^{-1}): 3278, 3166 (NH), 2965 (aliph. CH.) and 1600 (C=N). ^{1}HNMR (600 MHz, DMSO-d_6): $\delta = 2.29$ (s, 6H, $2CH_3$), 3.20, 3.85 (2t, 8H, morphonyl-H), 6.97–7.79 (m, 5H, Ar-H + thiazole-H5) and 9.55 (s, 1H, NH) ppm. Elemental analysis for $C_{16}H_{20}N_4OS$. Calcd. C, 60.73; H, 6.37; N, 17.71; Found: C, 60.60; H, 6.20; N, 17.40.

3.1.8. *4-(4-(1-(2-(4-Phenylthiazol-2-yl) hydrazono) ethyl) phenyl) morpholine **9b***

Yield (40%); brown solid (ethanol); m.p.280–82 °C; IR (KBr, cm^{-1}):3109 (NH), 2958, 2854 (aliph. CH) and 1607 (C=N). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 2.25$ (s, 3H, CH_3), 3.14, 3.74 (2t, 8H, morphonyl-H), 6.94–7.87 (m, 10H, Ar-H + thiazole-H_5) and 11.07 (s, 1H, NH) ppm. ^{13}CNMR (600 MHz, DMSO-d_6): $\delta = 13.65$ (CH_3), 66.60 (C3, C5 of morpholine), 77.05 (C2, C6 of morpholine), 102.64 (thiazole-C5), 114.20, 125.81, 127.36, 127.48, 128.11, 130.31 (2 phenyl-C), 152.06 (thiazole-C2) and 169.40 (C=N)ppm. Elemental analysis for $C_{21}H_{24}N_4OS$. Calcd. C, 66.29; H, 6.36; N, 14.72; Found: C, 66.00; H, 6.10; N, 14.50.

3.1.9. *2-(2-(1-(4-Morpholinophenyl) ethylidene) hydrazinyl) thiazol-4(5H)-imine **10***

Yield (45%); brown solid (ethanol); m.p.136 °C; IR (KBr, cm^{-1}): 3316, 3104 (NH_2/NH), 2967, 2866 and (aliph. CH). MS: 317 (M^+, 39%), 301 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 2.25$ (s, 3H, CH_3), 3.22, 3.70 (2t, 8H, morphonyl-H), 3.87 (s, 2H, SCH_2), 6.40, 8.60 (2s, 2H, 2NH; cancelled with D_2O) and 6.86–7.90 (m, 4H, Ar-H) ppm. Elemental analysis for $C_{15}H_{19}N_5OS$. Calcd. C, 56.76; H, 6.03; N, 22.06; Found: C, 56.50; H, 5.80; N, 21.90.

3.1.10. 2-Cyano-N′-(1-(4-morpholinophenyl) ethylidene) acetohydrazide ***11***

Equimolar amounts of compound **1** (0.01 mol), cyanoacetic acid hydrazide (0.01 mol) and a few drops of conc. HCl in ethanol (30 ml) was refluxed for 3 h. The solid product which produced on heating was collected and recrystallized from acetic acid as white solid. Yield (65%); m.p. 205 °C; IR (KBr, cm^{-1}): 3200 (NH), 2971, 2867 (aliph. CH), 2257 (C≡N) and 1671 (C=O). ^{1}HNMR (600 MHz, DMSO-d_6): $\delta = 2.24$ (s, 3H, CH_3), 3.20, 3.83 (2t, 8H, morphonyl-H), 3.96 (s, 2H, CH_2), 6.87, 7.86 (2d, 4H, Ar-H) and 10.70 (s, 1H, NH) ppm. ^{13}CNMR (600 MHz, DMSO-d_6): $\delta = 13.14$ (CH_3), 24.79 (CH_2), 66.21 (C3, C5 of morpholine), 77.05 (C2, C6 of morpholine), 114.01, 115.78, 127.78, 133.28 (phenyl-C), 149.90 (C≡N), 164.63 (C=N) and 196.64 (C=O). Elemental analysis for $C_{15}H_{18}N_4O_2$. Calcd. C, 62.92; H, 6.34; N, 19.57; Found: C, 62.60; H, 6.00; N, 19.20.

3.1.11. Preparation of compounds ***(13 & 15)****; general procedure*

To suspension of finally powdered potassium hydroxide (0.01 mol) in dry dimethylformamide (20 ml) the active methylene compound (**11**, 0.01 mol) and then the phenyl isothiocyanate (0.01 mol) were added in portions. The reaction mixture was stirred at room temperature for 1 h and then treated with α-halogenated compound (0.01 mol) and left at room temperature for 2 h; then it was poured into ice/water and acidified with 0.1 N HCl at pH 3–4. The resulting precipitate was filtered off, dried, and recrystallized from the proper solvent.

3.1.12. 2-Cyano-2-(3,4-diphenylthiazol-2(3H)-ylidene)-N′-(1-(4-morpholinoph-enyl) ethyl-idene) acetohydrazide ***13***

Yield (55%); yellow solid (ethanol); m.p. 180 °C; IR (KBr, cm^{-1}): 3100 (NH), 2210 (C≡N) and 1650 (C=O). MS: 521 (M^+, 36%), 237 (100%). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.53$ (s, 2H, CH_3), 3.20, 3.83 (2t, 8H, morphonyl-H), 6.86 (s, 1H, thiazole-H5), 6.88–7.90 (m, 15H, Ar-H + NH) ppm. Elemental analysis for $C_{30}H_{27}N_5O_2S$. Calcd. C, 69.08; H, 5.22; N, 13.43; Found: C, 68.80; H, 5.10; N, 13.10.

3.1.13. 2-Cyano-2-(4-hydroxy-5-methyl-3-phenylthiazol-2(3H)-ylidene)-N′-(1-(4-morph-olinophenyl) ethylidene) acetohydrazide ***15***

Yield (53%); brown solid (ethanol); m.p. 239–40 °C; IR (KBr, cm^{-1}): 3420 (OH), 3288, 3166 (NH), 1593 (C=N). MS: 475 (M^+, 73%), 461 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.89, 2.22 (2s, 6H, $2CH_3$), 3.13, 3.72 (2t, 8H, $4CH_2$), 6.87–8.17 (m, 9H, Ar-H), 10.07 (s, 1H, NH), 11.88 (s, 1H, OH) ppm. ^{13}C NMR (600 MHz, DMSO-d_6): δ = 13.80, 21.60 ($2CH_3$), 63.84 (C3, C5 of morpholine), 76.50 (C2, C6 of morpholine), 78.60 (C–C≡N), 80.10 (thiazole-C5), 111.80, 121.50, 122.80, 127.00, 129.40, 130.01, 142.00, 151.50 (2 phenyl-C), 114.50 (C–C≡N), 148.00 (C=N), 170.01 (C=O), 172.40 (thiazole-C2), 176.50 (thiazole-C4)ppm. Elemental analysis for $C_{25}H_{25}N_5O_3S$. Calcd. C, 63.14; H, 5.30; N, 14.73; Found: C, 62.90; H, 5.00; N, 14.50.

3.1.14. Preparation of compounds ***(16a & 16b)****; general procedure*

A mixture of compound **11** (0.01 mol), aryl isothiocyanate (0.01 mol), sulfur metal (0.01 mol) and catalytic amount of triethylamine were refluxed in ethanol (30 mL) for 6 h. The reaction mixture was poured into ice/water and acidified with 0.1 N HCl at pH 3–4 then the resulting precipitate was filtered off, dried, and recrystallized from the proper solvent.

3.1.15. 4-Amino-N′-(1-(4-morpholinophenyl)ethylidene)-3-phenyl-2-thioxo-2,3-dihydro-thiazole-5-carbohydrazide ***16a***

Yield (50%); brown solid (dioxane); m.p. 244–46 °C; IR (KBr, cm^{-1}): 3327, 3310, 3200 (NH/NH_2), 1672 (C=O). MS: 453 (M^+, 18%), 246 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 2.11$ (s, 3H, CH_3), 3.32, 3.86 (2t, 8H, $4CH_2$), 6.98–7.60 (m, 11H, Ar-H + NH_2), 9.00 (s, 1H, NH) ppm. Elemental analysis for $C_{22}H_{23}N_5O_2S_2$. Calcd. C, 58.26; H, 5.11; N, 15.44; Found: C, 58.00; H, 5.00; N, 15.20.

3.1.16. 4-Amino-N′-(1-(4-morpholinophenyl)ethylidene)-3-(naphthalen-2-yl)-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide ***16b***

Yield (55%); brown solid (acetic acid); m.p. 269–70 °C; IR (KBr, cm^{-1}): 3300, 3215, 3110 (NH_2/NH), 1650 (C=O). MS: 503 (M^+, 25%), 388 (100%). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 2.52$ (s, 3H, CH_3), 3.37, 3.86 (2t, 8H, $4CH_2$), 6.99–7.61 (m, 13H, Ar-H + NH_2), 9.00 (s, 1H, NH) ppm. Elemental analysis for $C_{26}H_{25}N_5O_2S_2$. Calcd. C, 62.00; H, 5.00; N, 13.91; Found: C, 61.80; H, 4.90; N, 13.70.

3.1.17. 4-(4-Morpholinophenyl)thiazol-2-amine ***17***

A mixture of acetophenone derivative **1** (0.1 mol), thiourea (0.2 mol) and Iodine (0.1 mol) was heated on a steam bath for 4 h. The hydroiodide separated, was filtered, washed with ether and dried. It was dissolved in hot water, filtered while hot and the clear solution neutralized with a strong solution of ammonia. The solid separated was filtered, washed with water and recrystallized from benzene as yellow crystals. Yield (65%); m.p. 227 °C; IR (KBr, cm^{-1}): 3303, 3117 (NH_2) 2970, 2838 (aliph. CH), 1606 (C=N). MS: 261 (M^+, 83%), 99 (100%). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.57$ (s, 2H, CH_3), 3.15, 3.82 (2t, 8H, morphonyl-H), 6.58 (s, 1H, thiazole-H_5), 6.87, 7.66 (2d, 4H, Ar-H), 7.94 (s, 2H, NH_2) ppm. ^{13}CNMR (600 MHz, $CDCl_3$): $\delta = 65.04$ (C3, C5 of morpholine), 75.05 (C2, C6 of morpholine), 102.00 (thiazole-C5), 112.80, 122.50, 128.30, 149.50 (phenyl-C), 150.30 (thiazole-C4), 168.90 (thiazole-C2) ppm. Elemental analysis for $C_{13}H_{15}N_3OS$. Calcd. C, 59.74; H, 5.79; N, 16.08; Found: C, 59.50; H, 5.40; N, 16.00.

3.1.18. N-(4-methylbenzylidene)-4-(4-morpholinophenyl)thiazol-2-amine ***18***

Equimolar amounts of compound **17** (0.01 mol), 4-methylbenzaldehyde (0.01 mol) and a few drops of piperidine in ethanol (30 ml) were refluxed for 3 h. The solid product which produced on heating was collected and recrystallized from the acetic acid as white solid. Yield (65%); m.p. 251 °C; IR (KBr, cm^{-1}): 2900, 2851 (aliph. CH), 1620(C=N). MS: 363 (M^+, 17%), 86 (100%). ^{1}HNMR (600 MHz, DMSO-d_6): $\delta = 2.17$ (s, 3H, CH_3), 3. 14, 3.83 (2t, 8H, $4CH_2$), 5.95 (s, 1H, thiazole-H5), 6.68–7.27 (m, 8H, Ar-H), 7.55 (s, 1H, benzyldine-CH) ppm. Elemental analysis for $C_{21}H_{21}N_3OS$. Calcd. C, 69.39; H, 5.82; N, 11.56; Found: C, 69.10; H, 5.60; N, 11.30.

3.1.19. N-acetyl-N-(4-(4-morpholinophenyl) thiazol-2-yl) acetamide ***19***

A mixture of compound **17** (0.01 mol) and acetic acid anhydride (30 ml) was refluxed for 24 h. The solid product which produced after cooling was collected and recrystallized from ethanol as white solid. Yield (50%); m.p. 272 °C; IR (KBr, cm^{-1}): 2973, 2860 (aliph. CH), 1961(C=O) and 1608 (C=N). ^{1}HNMR (600 MHz, DMSO-d_6): $\delta = 2.23, 2.40$ (2s, 6H, $2CH_3$), 3. 19, 3.87 (2t, 8H, $4CH_2$), 6.92-7.78 (m, 5H, Ar-H + thiazole-H_5) ppm. Elemental analysis for $C_{17}H_{19}N_3O_3S$. Calcd. C, 59.11; H, 5.54; N, 12.17; Found: C, 59.00; H, 5.30; N, 11.90.

3.1.20. N-(1-(4-morpholinophenyl)ethylidene)-4-phenylthiazol-2-amine ***20***

Equimolar amounts of compound **1** (0.01 mol), 4-phenylthiazol-2-amine (0.01 mol) and a few drops of piperidine in ethanol (30 ml) was refluxed for 5 h. The solid product which produced on heating was collected and recrystallized from the acetic acid as white solid. Yield (65%); m.p.304–6 °C; IR (KBr, cm^{-1}): 2967, 2838 (aliph. CH), 1645 (C=N). MS: 363 (M^+, 100%), 303 (100%). ^{1}HNMR (600 MHz, $CDCl_3$): $\delta = 2.53$ (s, 2H, CH_3), 3.29, 3.86 (2t, 8H, morphonyl-H), 6.71 (s, 1H, thazole-H_5), 6.85- 7.88 (m, 9H, Ar-H) ppm. Elemental analysis

for $C_{21}H_{21}N_3OS$. Calcd. C, 69.39; H, 5.82; N, 11.56; Found: C, 69.10; H, 5.50; N, 11.50.

3.2. Molecular modeling

An attempt to gain a better insight on the molecular structure of these synthesized thiosemicarbazone compounds, geometric optimization and conformation analysis has performed using PM3 force field as implemented in HyperChem 7.5 [41].

3.3. Biological activity

3.3.1. Antibacterial activity

Antimicrobial activity of the tested samples was determined using a modified Kirby–Bauer disc diffusion method [42]. Briefly, 100 μl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 108 cells/ml [43]. A 100 μl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method of the National Committee for Clinical Laboratory Standards (NCCLS) [44]. Among the available media available, NCCLS recommends Mueller–Hinton agar due to: it results in good batch-to-batch reproducibility. Plates inoculated with Gram (+) bacteria as *S. aureus* and *B. cereus*; Gram (−) bacteria as *P. mirabilis* and *S. marcesens*, they were incubated at 35–37 °C for 24–48 h and then the diameters of inhibition zones were measured in millimeters [42]. Standard discs of ampicillin (antibacterial agent served as positive controls for antimicrobial activity but filter discs impregnated with 10 μl of solvent were used as a negative control. The agar used is Mueller–Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher and Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 μl of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar, it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or "Clear Zone". For the disc diffusion, the zone diameters were measured with slipping calipers of the (NCCLS) [44], results are presented in Table 3. Agar-based methods such as E-test and disk diffusion can be good alternatives because they are simpler and faster than the broth-based methods [45,46]. The diameter of the zone of inhibition was measured after 24 h of incubation. The antibacterial activity of a common standard antibiotic ampicillin was also recorded maintaining the same protocol as above and at the same concentration and solvent. The antibacterial activity results of the compounds were compared with the standard and % activity index for the heterocyclic compounds was calculated by using the formula as given below:

$$\%\ \text{Activity index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

3.3.2. Determination of minimum inhibitory concentration (MIC) value

The antibacterial screening concentrations of the compounds to be used were estimated from the minimum inhibitory concentration (MIC) value. The MIC was determined using the disc diffusion technique.

3.3.3. Anti-inflammatory (in vivo)

All the synthesized compounds were screened for the in vivo anti-inflammatory activity by carrageenan induced rat paw edema method.

- Method: Inhibition of carrageenan induced inflammation in rat paw.
- Animals used: Albino Wister rats.
- Number of animals used: 6.
- Dose of test compounds: 50 mg/kg.
- Dose of standard drug: 10 mg/kg (indomethacin).
- Route of administration: oral (1% w/v Tween 80 suspension).
- Carrageenan suspension: sub planter (0.1 ml of 1% w/v suspension in 0.9% saline solution).

The method developed by Winter et al. [47] was employed. Albino Wistar rats of either sex (130–150 g) were divided into various groups (6 rats per group). Animals were deprived of food for 12 h prior to experiment and only water was given *ad libitum*. First group was used as a control group (treated with 1 ml of 20% v/v DMSO solution), the second group (treated with 1 ml of 20% v/v DMSO solution of indomethacin (10 mg/kg) orally) and the rest of groups (treated with DMSO solution of test compounds at a dose of 50 mg/kg orally). One hour after the administration of the compounds, carrageenan suspension (0.1 ml of 1% w/v suspension in 0.9% saline solution) was injected into the plantar region of left hind paw of animals. Immediately, the paw volume was measured using plethysmometer (UGO Basile 21025 Comerio, Italy, initial paw volume, V_c). Thereafter, the paw volume was measured after 1–4 h after carrageenan administration. The difference between initial and subsequent readings gave the change in edema volume for the corresponding time. Edema volume of control (V_c) and volume of treated (V_t) were used to calculate percentage (%) inhibition and (%) edema volume by using following formula.

$$\%\ \text{Inhibition} = [1 - (V_t/V_c)] \times 100$$

$$\%\ \text{Edema volume} = 100 \times (\text{Edema volume after drug treatment}/\text{Initial volume}).$$

3.3.3.1. Statistical analysis. Values were expressed as means ± S.E. Comparisons between means were carried out using one-way ANOVA followed by least significant difference (LSD) and Turkey multiple comparisons test.

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