Taylor & Francis
Taylor & Francis Group

Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lsyc20

Synthesis, antimicrobial studies, and molecular docking of some new dihydro-1,3,4-thiadiazole and pyrazole derivatives derived from dithiocarbazates

Amr Hassan Moustafa , Doaa H. Ahmed , Mohamed T. M. El-Wassimy & Mamdouh F. A. Mohamed

To cite this article: Amr Hassan Moustafa , Doaa H. Ahmed , Mohamed T. M. El-Wassimy & Mamdouh F. A. Mohamed (2020): Synthesis, antimicrobial studies, and molecular docking of some new dihydro-1,3,4-thiadiazole and pyrazole derivatives derived from dithiocarbazates, Synthetic Communications, DOI: 10.1080/00397911.2020.1843179

To link to this article: https://doi.org/10.1080/00397911.2020.1843179

SYNTHETIC COMMUNICATIONS®
https://doi.org/10.1080/00397911.2020.1843179

Taylor & Francis
Taylor & Francis Group

Synthesis, antimicrobial studies, and molecular docking of some new dihydro-1,3,4-thiadiazole and pyrazole derivatives derived from dithiocarbazates

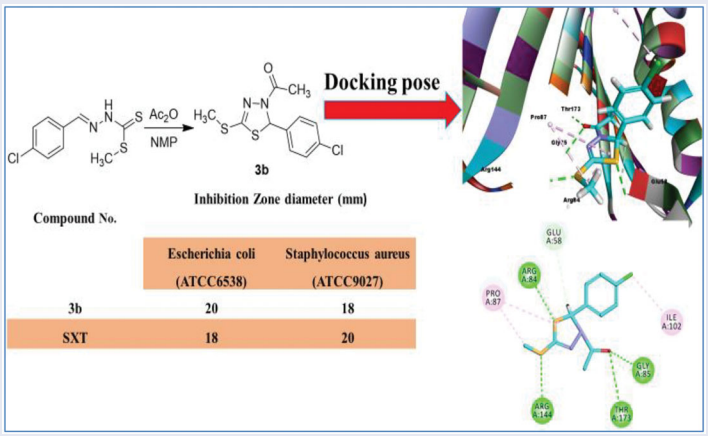
Amr Hassan Moustafa[a], Doaa H. Ahmed[a], Mohamed T. M. El-Wassimy[a], and Mamdouh F. A. Mohamed[b]

[a]Department of Chemistry, Faculty of Science, Sohag University, Sohag, Egypt; [b]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sohag University, Sohag, Egypt

ABSTRACT

A series of 3-acetyl-2-aryl-5-methylthio-2,3-dihydro-1,3,4-thiadiazoles **3a–g,** *N*- (4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide derivatives **5a–e** and *spiro*-compound **7** was prepared from starting material dithiocarbazates using *N*-methylpyrrolidone (NMP) /acetic anhydride mixture. Furthermore, a new series of 5-amino-3- (methylthio) -1-substituted-1*H*-pyrazole-4-carbonitrile derivatives **12a–d** was prepared using two synthetic routes: (i) *via* reaction of *bis* (methylthio) methylene malononitrile **8** with carbothiohydrazides **11a–d**, or (ii) *via* reaction of methyl 5-amino-4-cyano-3- (methylthio) -1*H*-pyrazole-1-carbodithioate **9a** with primary/secondary amines. The antimicrobial screening of newly synthesized compounds revealed that compounds **3b**, **7,** and **12d** are the most potent against the Gram-positive (*S. aureus*) and the Gram-negative (*E. coli*) bacteria compared to ciprofloxacin as reference drug. Mechanistically, the theoretical docking results of **3b, 7** and **12d** suggested that they may act as potent inhibitors of theDNA gyrase.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

Received 12 September 2020

KEYWORDS

Antimicrobial; carbothiohydrazides; 2,3-dihydo-1,3,4-thiadiazole; dithiocarbazates; pyrazole

CONTACT Amr H. Moustafa amr_hassanegypt@ymail.com Department of Chemistry, Faculty of Science, Sohag University, Sohag, Egypt

Supplemental data for this article is available online at https://doi.org/10.1080/00397911.2020.1843179

Introduction

Dithiocarbazates (DTCs) possessing both hydrazino and thione groups, are the active and the interesting arrangement structures for many scientists.[1] Their *S*-substituted are easily synthesized via one-pot multicomponent reaction of hydrazine hydrate, carbon disulfide and alkyl halides in aqueous basic medium.[2,3] They are valuable raw materials for the preparation of carbothiohydrazides,[4,5] carbothiohydrazones[6–8] and various aza-heterocyclic compounds such as pyrazoles,[2,9,10] 1,3,4-thiadiazoles,[11,12] 1,2,4-triazoles,[2]pyrazolo[1,5-*a*][1,3,5]triazines.[13,14]

Further, the 1,3,4-thiadiazole nucleus, which makes up the azole group, has a versatile application in medicinal, agricultural, and materials chemistry.[15–19] Their derivatives exhibit a wide variety of biological activities including antimicrobial,[20,21] anticonvulsant,[22] antioxidant,[23] antiparasitic,[24] antituberculosis,[25] antileishmanial,[26] anti-inflammatory,[27] antidepressant,[28] anticancer[29] and antiviral agents.[30] They also have gained great attention due to the large number of their reported diverse synthetic methods[16,31] for example: (i) via cyclization of thiosemicarbazides,[32,33] thiosemicarbazones,[34] aldehyde methylthio (thiocarbonyl) hydrazones[35] or acylhydrazines[36,37]; (ii) via transformation of 1,3,4-oxadiazoles.[38] In contrast to this, limited methods were succeeded for preparing them from DTCs via the reaction with chloroacetylchloride,[12] or hydrazonoyl bromide.[11]

On the other hand, pyrazole derivatives are found to be versatile compounds showing widespread biological and pharmaceutical activities[39,40] such as: antimicrobial,[41] anti-inflammatory,[42] analgesic,[43] antidiabetic,[44] antidepressant,[45] anticonvulsant,[45] antiviral,[46] and anticancer activities.[47] Many derivatives containing pyrazole cores have been applied in crop protection.[48]

Despite the large number of chemotherapeutics and antibiotics, the appearance of old and new antibiotic-resistant bacterial strains represents a substantial need for the synthesis and discovery of new safer and potent antimicrobial agents.[49]

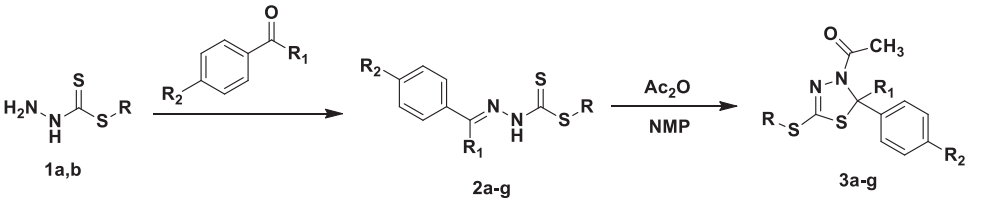
In the light of the foregoing data and as continuation of our program of identification of new biologically active leads,[50–57] the present work reports the new synthesis and the antimicrobial evaluation of 2,3-dihydro-1,3,4-thiadiazole and pyrazole derivatives starting from DTCs.

Results and discussion

Chemistry

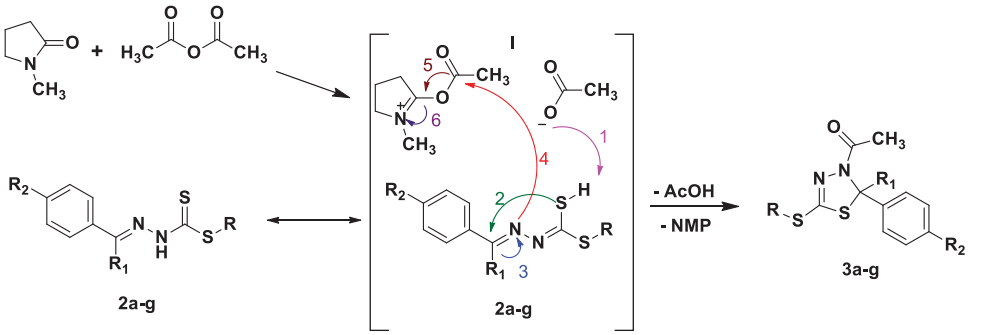
Herein, a series of 3-acetyl-2-aryl-5-methylthio-2,3-dihydro-1,3,4-thiadiazoles **3a–g** was synthesized by heating of methyl/benzyl 2-benzylidenehydrazinecarbodithioate derivatives **2a–g** with acetic anhydride in polar aprotic solvent of *N*-methylpyrrolidone (NMP) (Scheme 1). In this route, the starting materials thiocarbohydrazones **2a–g** were prepared according to the reported method carbothiohydrazones[7] and *via Schiff base* condensation between methyl/benzyl hydrazinecarbodithioate **1a,b** [3,5] with various aromatic aldehydes and/or ketones.

The formation of products **3a-g** was produced through acid anhydride-catalyzed intramolecular cyclization of intermediate **2′a-g** *via* nucleophilic lone pair addition of



1a, 2a, 3a; R = Me, R_1 = H, R_2 = H
1b, 2b, 3b; R = Me, R_1 = H, R_2 = Cl
1c, 2c, 3c; R = Me, R_1 = H, R_2 = OMe
1d, 2d, 3d; R = Me, R_1 = Me, R_2 = Cl
1e, 2e, 3e; R = Bn, R_1 = H, R_2 = H
1f, 2af, 3f; R = Bn, R_1 = H, R_2 = Cl
1g, 2g, 3g; R = Bn, R_1 = H, R_2 = OMe

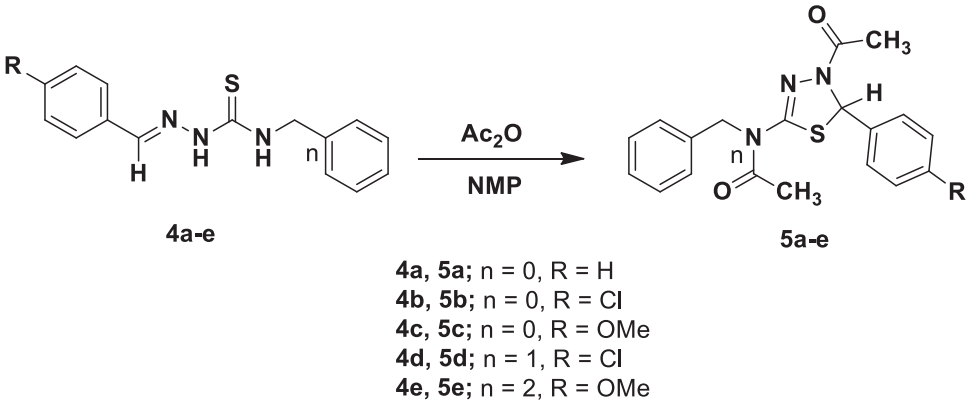
Scheme 1. The Synthetic route for the preparation of 2,3-dihydro-1,3,4-thiadiazoles **3a**–**g**.



Scheme 2. Plausible mechanism for the formation of **3a**–**g** using NMP and acetic anhydride.

sulfur at carbon *Schiff base* bond, followed by electrophilic acetylation at nitrogen *Schiff base* bond with elimination of acetic acid molecule (Scheme 2). The simple cyclic amide of NMP containing tertiary aliphatic amine is an excellent cleaning solvent and it can use to facilitate the acetylation reactions through the formation of intermediate **I** (Scheme 2).[58,59]

The chemical structures of the new 2,3-dihydro-1,3,4-thiadiazoles **3a-g** were confirmed based on their spectral (IR, ^{1}H, ^{13}C NMR) and elemental analyses (see Experimental part & Supporting Information). For example, the IR spectrum of **3 g** showed a characteristic absorption band at 1662 cm^{-1} for C=O; 2,90,62,92,82,949 cm^{-1} for the aliphatic C–H and 3,00,83,052 cm^{-1} for the aromatic C–H. Its ^{1}H NMR spectrum showed the presence of two singlet signals at δ 2.23 and 3.75 ppm characteristic of methyl and methoxy groups, respectively; two doublet signals at δ 6.87–6.90 and 7.42–7.44 ppm for four aromatic protons with coupling constant J = 8.6 and 7.0 Hz, respectively; also it exhibited doublet of doublets signals at δ 4.36–4.45 ppm with coupling constant J = 7.9, 13.6 Hz due to methylene group; and two multiplet signals at δ 7.13–7.15 and 7.30–7.37 ppmdue to six protons of aromatic and methine-SP^3 group. The ^{13}C NMR spectrum of **3g** showed ten signals at δ 114.4, 127.3, 128.0, 128.9, 129.5, 133.2, 137.1, 149.4, 159.7, 167.8 ppm, which are assigned to carbonyl group and aromatic carbons; two signals at δ 22.6, 55.6 ppm due to methyl and methoxy groups,



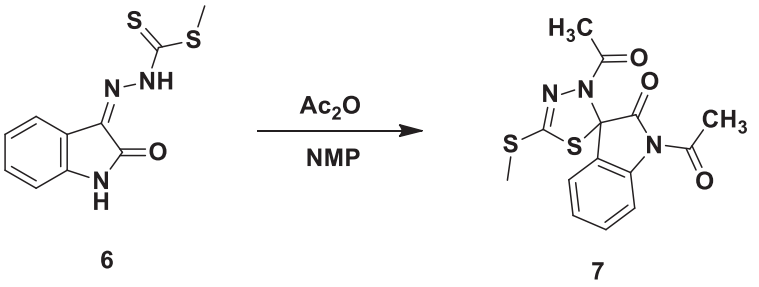
Scheme 3. Synthesis of dihydro-1,3,4-thiadiazoles **5a–e**.

respectively; while carbons of methylene and methine–SP^3 groups are characterized by signals at δ 37.1 (disappeared with *DEPT-135*) and 69.7 ppm, respectively.

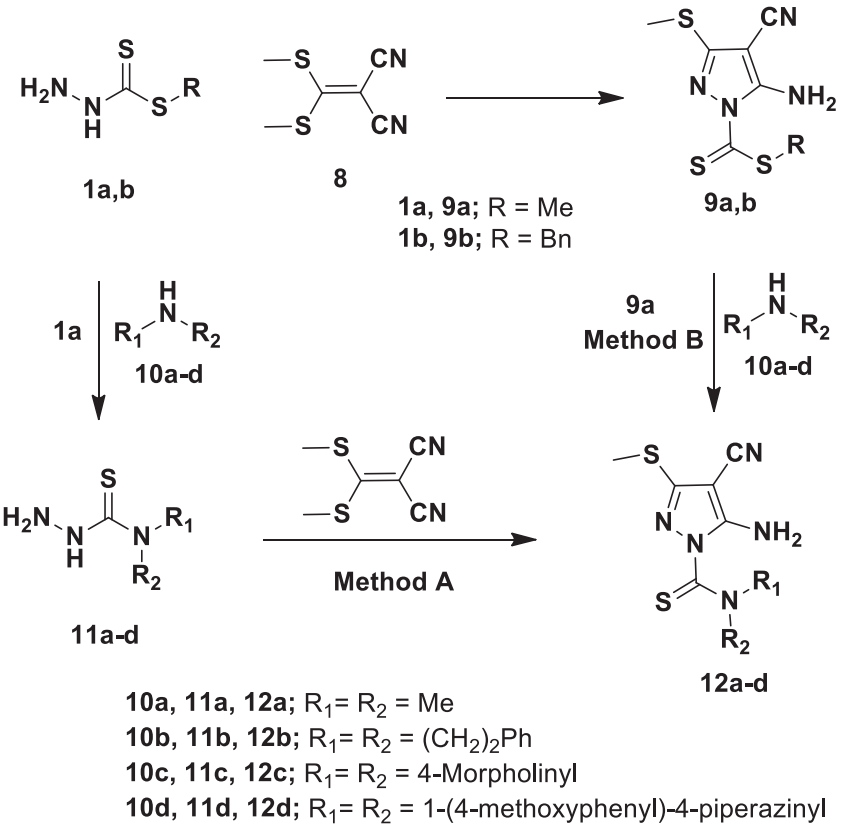
In the same manner, a new series of *N*- (4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide derivatives **5a–e** was produced by the heating of benzaldehyde, 4-chloro- or 4-methoxybenzaldehyde *N*-phenylthiosemicarbazones **4a–c**, 4-chlorobenzaldehyde *N*-benzylthiosemicarbazone **4d** and/or 4-methoxybenzaldehyde *N*- (2-phenylethyl) thiosemicarbazone **4e** in a mixture of acetic anhydride and NMP, respectively (Scheme 3). The structures of the newly synthesized compounds **5a–e** were confirmed by their spectral (IR, ^{1}H, ^{13}C NMR) and elemental analyses (see Experimental Part & Supporting Information).

In light of these results, the opportunity to use this strategy to synthesize the new *spiro*-dihydro-1,3,4-thiadiazoles can be pursued. Thus 1,3′-diacetyl-5′- (methylthio) -3′*H*-*spiro*[indole-3,2′-[1,3,4]thiadiazol]-2 (1*H*) -one (**7**) was produced by heating of methyl 2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazinecarbodithioate (**6**) [8] in NMP/acetic anhydride (Scheme 4). The analytical data supports the structures (*see* Experimental Part & Supporting Information).

On the other hand, reaction of *bis* (methylthio) methylene malononitrile **8** with methyl hydrazinecarbodithioate **1a** and/or benzyl hydrazinecarbodithioate **1 b** was previously described[60] giving polyfunctionalized-pyrazoles **9a,b**, respectively (Scheme 5). Using this synthetic strategy is suitableto synthesize the new 5-amino-3- (methylthio) -1-substituted-1*H*-pyrazole-4-carbonitrile derivatives **12a–d** via reaction of *bis* (methylthio) methylene malononitrile **8** with carbothiohydrazides **11a–d**, which prepared through reported procedure *via* reaction of **1a** with primary/secondary amines **10a–d**,[4,5] respectively (Method A, Scheme 5). Formation of the products **9a,b** and **12a–d** has been confirmed by their spectral IR, NMR and elemental analyses. Compound **12c** was also confirmed using melting point comparison with reported synthesis by another method.[60] Also the structures of the synthesized compounds **12a–d** were chemically confirmed by their preparation through another route *via* the reaction of **9a** with primary/secondary amines **10a–d** namely, dimethylamine, phenylethylamine, morpholine, and 1- (4-methoxyphenyl) piperazine, respectively (Method B, Scheme 5).



Scheme 4. Synthesis of 1,3′-diacetyl-5′-(methylthio)-3′*H*-*spiro*[indole-3,2′-[1,3,4]thiadiazol]-2(1*H*)-one (**7**).



Scheme 5. The Synthetic route for the preparation of polyfunctionalized-pyrazole derivatives **9a,b** and **12a–d**.

IR spectrum of compound **12d** showed absorption peaks at 1616 cm^{-1} for C=N; 2215 cm^{-1} for C≡N; 2819, 2921, 2952 cm^{-1} for C–H aliphatic; 3032 cm^{-1} for C–H aromatic; and 3,30,03,393 cm^{-1} for NH_2 group. ^1H NMR spectrum of compound **12d** showed the presence of four singlet signals at δ 2.50, 3.23, 3.71 and 7.18 ppm characteristic of SCH_3, two CH_2, OCH_3 and NH_2 (exchangeable with D_2O) groups, respectively; broad singlet signal at δ 4.01 ppm for other two methylene groups of piperazine; also it exhibited two doublet signals at δ 6.84–6.87 and δ 6.92–6.94 ppm with coupling

constant $J = 8.9$ Hz for phenylene protons. Its ^{13}C NMR spectrum showed nine peaks at δ 73.9, 113.6, 115.1, 118.3, 144.9, 150.7, 154.1, 154.5, 176.0, which are assigned to SP^2 and SP carbon atoms; while the two methyl carbons (SCH3, OCH3) and four methylene carbons were characterized by peaks at δ 13.6, 49.8, 51.2 and 55.9 ppm.

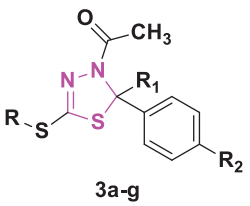
Antimicrobial evaluation

The *in vitro* antimicrobial activity of newly synthesized 2,3-dihydro-1,3,4-thiadiazoles **3a–g**, **5a–e**, **7** and polyfunctionalized-pyrazoles **9a,b**, **12a–d** was evaluated against *Staphylococcus aureus* (ATCC9027) *Escherichia coli* (ATCC6538) and *Candia albicans* (ATCC10231) using well diffusion method[15,61–63] and the results are presented in Table 1. Among the compounds tested, the *spiro*-compound **7**, was found to be the most active against both the Gram-positive (*S. aureus*) and the Gram-negative (*E. coli*) bacteria with inhibition zone 20 and 15 mm, respectively. However, compound **7** did not show significant antifungal activity against *C. albicans*.

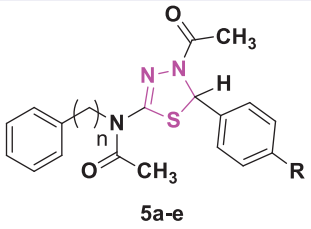
Regarding 3-acetyl-2-aryl-5-methylthio-2,3-dihydro-1,3,4-thiadiazoles derivatives **3a–g**, the antimicrobial screening showed that among this series compounds **3a**, **3b**, and **3c** are the most active. Compound **3b** showed the highest inhibition zone 18 and 20 mm against both the Gram-positive (*S. aureus*) and the Gram-negative (*E. coli*) bacteria, respectively. Compound **3c** exhibited strong activity against Gram-negative (*E. coli*) bacteria with 18 mm inhibition zone and moderate activity against the Gram-positive (*S. aureus*) bacteria with 12 mm inhibition zone. While compound **3a** exerted moderate activity against both *S. aureus* and *E. coli* with inhibition zone 12 mm. furthermore, compound **3e** and **3 g** showed weak activity against the Gram-negative (*E. coli*) bacteria only with inhibition zone 8 mm. With respect to the antifungal activity, all derivatives of this series did not exhibit notable antifungal activity.

Ongoing through the results of *N*- (4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide derivatives **5a–e**, the antimicrobial investigation revealed that compound **5b** has strong activity against Gram-negative (*E. coli*) bacteria with 18 mm inhibition zone and moderate activity against the Gram-positive (*S. aureus*) bacteria with 12 mm inhibition zone. In addition, **5e** has a moderate activity against both *S. aureus* and *E. coli* with inhibition zone 15 and 12 mm, respectively, and weak antifungal activity against *C. albicans* with 7 mm inhibition zone. Compounds **5a**, **5c** and **5d** did not exhibit notable antibacterial activity, however, **5c** showed weak antifungal activity against *C. albicans* with 6 mm inhibition zone. Finally, among the pyrazole derivatives **9a,b** and **12a–d**, compound **12d** exhibited strong activity against Gram-negative (*E. coli*) bacteria with 20 mm inhibition zone and moderate activity against the Gram-positive (*S. aureus*) bacteria with 12 mm inhibition zone without any notable antifungal activity. Also, compound **12c** showed a moderate activity against both *S. aureus* and *E. coli* with inhibition zone 12 mm and did not exhibit notable antifungal activity. Additionally, compound **12b** showed weak activity against Gram-negative (*E. coli*) bacteria with 8 mm inhibition zone and did not exert any activity against the Gram-positive (*S. aureus*) bacteria or against *C. albicans*. Compounds **9a**, **b**, and **12a** did not exhibit notable antimicrobial activity.

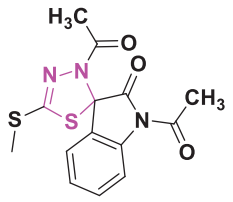
Table 1. The in *vitro* antimicrobial activity of 2,3-dihydro-1,3,4-thiadiazoles **3a–g**, **5a–e**, **7** and poly-functionalizedpyrazoles **9a,b** and **12a–d**.



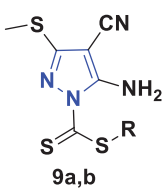
3a-g



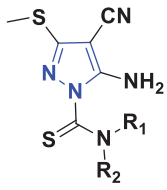
5a-e



7



9a,b



12a-d

Compound					Inhibition Zone diameter (mm)		
No.	R	R_1	R_2	*n*	S. A.	E. C.	C. A.
3a	Me	H	H	—	12	12	0
3b	Me	H	Cl	—	18	20	0
3c	Me	H	MeO	—	12	18	0
3d	Me	Me	Cl	—	0	0	0
3e	Bn	H	H	—	0	8	0
3f	Bn	H	Cl	—	0	0	0
3g	Bn	H	MeO	—	0	8	0
5a	H	—	—	0	0	0	0
5b	Cl	—	—	0	12	18	0
5c	MeO	—	—	0	0	0	6
5d	Cl	—	—	1	0	0	0
5e	MeO	—	—	2	15	12	7
7	—	—	—	—	20	15	0
9a	Me	—	—	—	0	0	0
9b	Bn	—	—	—	0	0	0
12a	—	Me	Me	—	0	0	0
12b	—	H	$-CH_2CH_2Ph$	—	0	8	0
12c	—	**R_1R_2N-**: 4-morphinyl		—	—	12	0
12d	—	**R_1R_2N-**: 1-(4-methoxyphenyl)-4-piperazinyl		—	—	20	0
Cipro					31	31	—
Fluc					—	—	25

C.A.: *Candida albicans*; Cipro: ciprofloxacin; E. C.: *Escherichia coli*; Fluc.: fluconazole; S. A.: *Staphylococcus aureus*.

Docking studies

The docking simulation experiments were performed using the bacterial DNA gyrase structure with the most active of newly synthesized compounds **3b**, **7,** and **12d**. The DNA gyrase structure was obtained from RCSB Protein data base (PDB ID: 4URO). DNA gyrase is a unique type II topoisomerase which is responsible for the super-coiling activity of DNA in bacteria.[64,65] We used the CDOCKER docking protocol, embedded in the Discovery Studio software 2.5 (San Diego, USA).

The docking results revealed that compound **3b**, nicely bound to the substrate binding pocket of 4URO (Figure 1) via incorporation of four hydrogen bonds with Arg84,

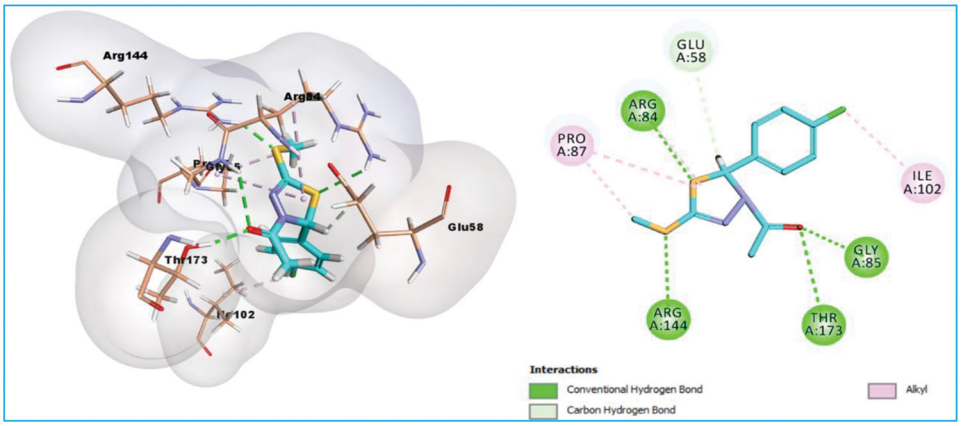


Figure 1. 3D and 2D docking and binding pattern of compound **3b** (cyan) into the active site of DNA gyrase (PDB entry: 4URO).

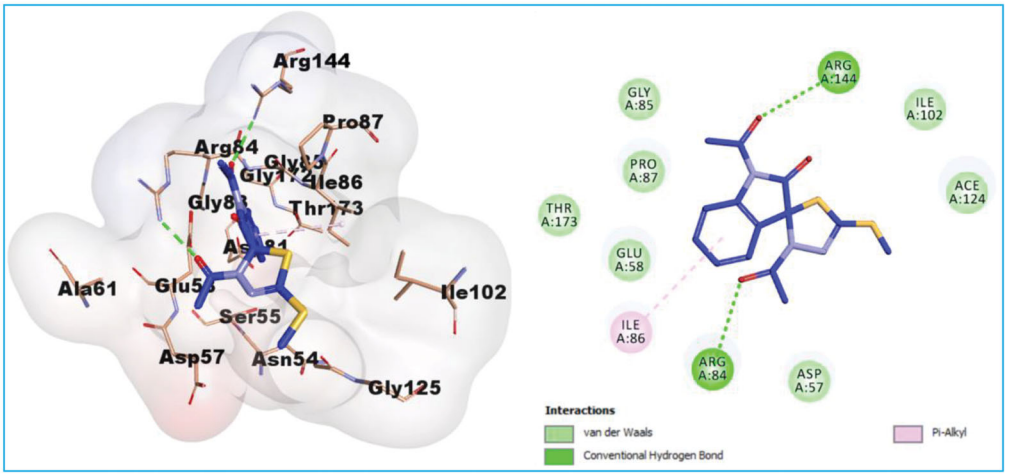


Figure 2. 3D and 2D docking and binding pattern of compound **7** (blue) into the active site of DNA gyrase (PDB entry: 4URO).

Gly85, Arg144 and Thr173. In addition to five hydrophobic interactions with Glu58, Arg84, Pro87 and Ile102 and this may explain the high antibacterial activity of compound **3b**.

Compound **7** engaged in two hydrogen bonds, one with Arg84 and Arg144. Additionally, compound **7** forms one hydrophobic interactions with Ile86 as shown in Figure 2.

Moreover, docking analysis of compound **12d** showed that it forms three hydrogen bonds with Gly85, Gln91, and Thr173 and seven hydrophobic interactions with the amino acid residues Gly83, Arg84, Ile86, Pro87, Ala98, and Ile102 (Figure 3). Interestingly, these theoretical docking results are in good agreement with experimental results which shows that **3b**, **7**, and **12d** may act as potent inhibitors of theDNA gyrase.

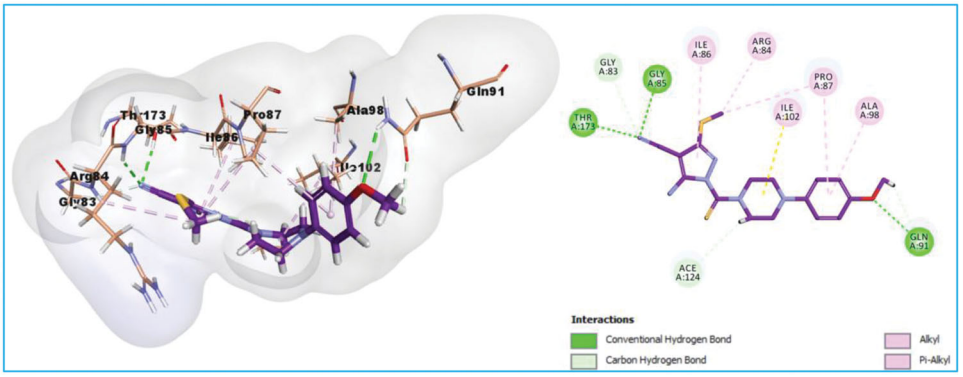


Figure 3. 3D and 2D docking and binding pattern of compound **12d** (violet) into the active site of DNA gyrase (PDB entry: 4URO).

Experimental

Chemistry

All commercially available reagents were purchased from Aldrich, Merck and Fluka and were used without further purification. For more details, *see* Supporting Information.

General procedure for the synthesis of 3-acetyl-2-aryl-5-methylthio-2,3-dihydro-1,3,4-thiadiazoles (3a-g);N- (4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide derivatives (5a–e) and 1,3′-diacetyl-5′- (methylthio) -3′H-spiro[indole-3,2′-[1,3,4]thiadiazol]-2 (1H) -one (7)

To a solution of thiocarbohydrazones **2a–g, 4a–e**, **6** (2 mmole) in 10 mL *N*-methylpyrrolidone (NMP), aceticanhydride (20 mmole, 1.9 mL) was added and there action mixture was heated and stirred insteam bath for 2 h. After complement of there action (monitored with TLC), there action mixture was then cooled to room temperature, poured into crushed ice and neutralized by ammonium hydroxide. The formed precipitate was collected by filtration, washed by distilled water, dried, and recrystallized from ethanol.

3-Acetyl-5- (benzylthio) -2- (4-methoxyphenyl) -2,3-dihydro-1,3,4-thiadiazole (3 g):

Yield 82%; beige solid; m.p.: 65–67 °C. IR (ATR) ν_{max} 3052, 3008 (CH aromatic), 2949, 2928, 2906 (CH aliphatic), 1662 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 4.36–4.45 (dd, $J = 7.9$, 13.6 Hz, 2H, CH_2), 6.87–6.90 (d, $J = 8.6$ Hz, 2H, $CH_{arom.}$), 7.13–7.15 (m, 3H, N–CH + $2CH_{arom.}$), 7.30–7.37 (m, 3H, $CH_{arom.}$), 7.42–7.44 (d, $J = 7.0$ Hz, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.6, 37.1 (exchangeable with DEPT-135), 55.6, 69.7, 114.4, 127.3, 128.0, 128.9, 129.5, 133.2, 137.1, 149.4, 159.7, 167.8. Elemental Analysis Calcd. (%) for $C_{18}H_{18}N_2O_2S_2$ (358.47): C, 60.31; H, 5.06; N, 7.81. Found: C, 60.21; H, 4.89; N, 7.76.

General procedure for the synthesis of 5-amino-3-(methylthio)-1-substituted-1H-pyrazole-4-carbonitrile derivatives (12a–d)

- **Method A**: A mixture of *bis* (methylthio) methylene malononitrile **8** (0.34g, 2mmol) and carbothiohydrazides **11a–d** (2mmol) was refluxed indioxane for 2h (monitored by TLC). ***A*** fter cooling the formed precipitate was collected by filtration, dried and recrystallized from dioxanetoafford **12a–d**.
- **Method B:** A mixture of methyl 5-amino-4-cyano-3-(methylthio)-1*H*-pyrazole-1-carbodithioate **9a** (0.49, 2 mmol) and primary/secondary amines **10a–d** (2 mmol) was refluxed indioxane for 4h (monitored by TLC). ***A*** fter cooling the formed precipitate was collected by filtration, dried and recrystallized from dioxane to afford **12a–d**.

5-Amino-1-{[4-(4-methoxyphenyl) piperazin-1-yl]carbonothioyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (12d):

Yield (method A: 92%, method B: 87%); gray solid; m.p.: 198–200 °C. IR (ATR)) ν_{max} 3393, 3300 (NH_2), 3032 (CH aromatic), 2952, 2921, 2819 (CH aliphatic), 2215 (C≡N), 1616 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.50 (s, 3H, SCH_3), 3.23 (s, 4H, $2CH_2$), 3.71 (s, 3H, OCH_3), 4.01 (br. s, 4H, $2CH_2$), 6.84–6.87 (d, $J = 8.9$ Hz, 2H, $CH_{arom.}$), 6.92–6.94 (d, $J = 8.9$ Hz, 2H, $CH_{arom.}$), 7.18 (s, 2H, NH_2, disappeared by D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.6, 49.8, 51.2, 55.9, 73.9, 113.6 (CN), 115.1, 118.3, 144.9, 150.7, 154.1, 154.5, 176.0. Elemental Analysis Calcd. (%) for $C_{17}H_{20}N_6OS_2$ (388.51): C, 52.56; H, 5.19; N, 21.63. Found: C, 52.84; H, 4.93; N, 21.44.

Biological activity

Antibacterial and antifungal activities

The *in vitro* antibacterial and antifungal activities of the newly synthesized 2,3-dihydro-1,3,4-thiadiazoles **3a–g**, **5a–e**, **7** and polyfunctionalized-pyrazoles **9a,b** and **12a–d** were screened using the well diffusion method.[15,61–63] For more details, *see* Supporting Information.

Docking methodology

Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, USA) was used for docking analysis.[66,67] For more details, see Supporting Information.

Conclusion

A series of 3-acetyl-2-aryl-5-methylthio-2,3-dihydro-1,3,4-thiadiazoles **3a–g**, *N*-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide derivatives **5a–e** and 1,3′-diacetyl-5′-(methylthio)-3′*H-spiro*[indole-3,2′-[1,3,4]thiadiazol]-2 (1*H*)-one (**7**) was produced by heating of thiosemicarbazone derivatives **2a–g, 4a–e, 6** in a mixture of NMP/acetic anhydride, respectively. Also, a series of 5-amino-3-(methylthio)-1-substituted-1*H*-pyrazole-4-carbonitrile derivatives **12a–d** was produced from *bis* (methylthio) methylene

malononitrile **8**, using two synthetic pathways. In addition, all synthesized compounds were screened for their antimicrobial activity against Staphylococcus aureus (ATCC9027) Escherichia coli (ATCC6538) and *Candia albicans* (ATCC10231) by means of paper disk diffusion method (9 mm) on Muller Hinton agar. Some of them were found to possess antibacterial activity and exhibited moderate-to-good activity. The results revealed that compounds **3b**, **7**, and **12d** are the most potent and elicited remarkable antibacterial activity against the Gram-positive (*S. aureus*) and the Gram-negative (*E. coli*) bacteria compared to ciprofloxacin as a reference drug. The molecular docking results of **3b**, **7**, and **12d** suggested that they may act as potent inhibitors of theDNA gyrase.

Full experimental detail, ^{1}H and ^{13}C NMR spectra. This material can be found via the "Supplementary Content" section of this article's webpage.

Acknowledgments

The authors thank Dr. EsamRashwan, Head of the confirmatory diagnostic unit VACSERA, Egypt, for carrying out the *in-vitro* antimicrobial screening. This research did not receive any specific grant from any funding agencies.

Disclosure statement

No potential conflict of interest was reported by the author (s).

ORCID

Amr Hassan Moustafa http://orcid.org/0000-0001-7242-6527

References

[1] Ali, N. H. S. O.; Hamid, M. H. S. A.; Putra, N. A.; Aqilah, M.; Asri; Adol, H. A.; Mirza, A. H.; Usman, A.; Siddiquee, T. A.; Hoq, M. R.; Karim, M. R. Efficient Eco-Friendly Syntheses of Dithiocarbazates and Thiosemicarbazones. *Green Chem. Lett. Rev.* **2020**, *13*, 129–140. DOI: 10.1080/17518253.2020.1737252.

[2] Hwang, J. Y.; Choi, H. S.; Lee, D. H.; Yoo, S. E.; Gong, Y. D. Solid-Phase Synthesis of 5-Amino-1- (Substituted Thiocarbamoyl) Pyrazole and 1,2,4-Triazole Derivatives via DithiocarbazateLinker. *J. Comb. Chem.* **2005**, *7*, 136–141. DOI: 10.1021/cc049931n.

[3] ShanmugaSundara Raj, S.; Yamin, B. M.; Yussof, Y. A.; Tarafder, M. T.; Fun, H. K.; Grouse, K. A. Trans-Cis S-Benzyl Dithiocarbazate. *Acta Crystallogr.C. Cryst. Struct. Commun.* **2000**, *56*, 1236–1237. DOI: 10.1107/s0108270100009276.

[4] Kulandaivelu, U.; Shireesha, B.; Mahesh, C.; Vidyasagar, J. V.; Rao, T. R.; Jayaveera, K.; Saiko, P.; Graser, G.; Szekeres, T.; Jayaprakash, V. Synthesis Antimicrobial and Anticancer Activity of N′-Arylmethylidene-Piperazine-1-Carbothiohydrazide. *Med. Chem. Res.* **2013**, *22*, 2802–2808. DOI: 10.1007/s00044-012-0279-4.

[5] Lin, H. H.; Wu, W. Y.; Cao, S. L.; Liao, J.; Ma, L.; Gao, M.; Li, Z. F.; Xu, X. Synthesis and AntiproliferativeEvaluation of Piperazine-1-Carbothiohydrazide Derivatives of Indolin-2-One. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3304–3307. DOI: 10.1016/j.bmcl.2013.03.099.

[6] Abdelatef, S. A.; El-Saadi, M. T.; Amin, N. H.; Abdelazeem, A. H.; Omar, H. A.; Abdellatif, K. R. A. Design, Synthesis and Anticancer Evaluation of Novel

spirobenzo[h]chromene and Spirochromane Derivatives with Dual EGFR and B-RAF Inhibitory Activities. *Eur. J. Med. Chem.* **2018**, *150*, 567–578. DOI: 10.1016/j.ejmech.2018.03.001.

[7] Parul, N.; Subhangkar, N.; Arun, M. Antimicrobial Activity of Different ThiosemicarbazoneCompounds Against Microbial Pathogens. *Int. Res. J. Pharm.* **2012**, *3*, 350–363. https://irjponline.com/admin/php/uploads/1118_pdf.pdf.

[8] Pervez, H.; Iqbal, M. S.; Tahir, M. Y.; Choudhary, M. I.; Khan, K. M. Synthesis of Some N4-Substituted Isatin-3-Thiosemicarbazones. *Nat. Prod. Res.* **2007**, *21*, 1178–1186. DOI: 10.1080/14786410601129770.

[9] Hassan, S. M.; Emam, H. A.; Abdelall, M. M. Heteroaromatization with Ketene Dithioacetals: Part I. Synthesis of Some Novel 5-Amino-1- (1,3,4-Thiadiazol-2-yl) and 1-(1,3,4-Thiadiazin-2-yl) Pyrazole-4-Carbonitriles. *J. Chem. Res.* **2000**, *2000*, 544–545. DOI: 10.3184/030823400103166283.

[10] Trofimov, B. A.; Mal'kina, A. G.; Borisova, A. P.; Nosyreva, V. V.; Shemyakina, O. A.; Kazheva, O. N.; Shilov, G. V.; Dyachenko, O. A. Expedient Synthesis of Pyrazoles Substituted with Amino, Hydroxyl and Thioamide Groups. *Tetrahedron Lett.* **2008**, *49*, 3104–3107. DOI: 10.1016/j.tetlet.2008.03.046.

[11] Al-Omair, M. A.; Sayed, A. R.; Youssef, M. M. Synthesis of Novel Triazoles, Tetrazine, Thiadiazoles and Their Biological Activities. *Molecules.* **2015**, *20*, 2591–2610. DOI: 10.3390/molecules20022591.

[12] Wang, T.; Miao, W.; Wu, S.; Bing, G.; Zhang, X.; Qin, Z.; Yu, H.; Qin, X.; Fang, J. Synthesis, Crystal Structure, and Herbicidal Activities of 2-Cyanoacrylates Containing 1,3,4-Thiadiazole Moieties. *Chin. J. Chem.* **2011**, *29*, 959–967. DOI: 10.1002/cjoc.201190196.

[13] Gong, Y.-D.; Kim, J.-G. Solid-Phase Synthesis of Novel 7,8-Functionalized Pyrazolo[1,5-a][1,3,5]-2,4-Dithioxotriazine Derivatives on Dithiocarboxy Resin Bound Pyrazoles. *Bull. Korean Chem. Soc.* **2009**, *30*, 3085–3088. DOI: 10.5012/bkcs.2009.30.12.3085.

[14] Gong, Y. D.; Ryu, I. A. Solid-Phase Synthesis of Novel 7,8-Functionalized Pyrazolo[1,5-a][1,3,5]-2-Oxo-4-Thioxotriazine Derivatives via Cyclization Reactions of Dithiocarboxy Resin Bound Pyrazoles. *J. Comb. Chem.* **2009**, *11*, 626–630. DOI: 10.1021/cc900020j.

[15] El-Badry, Y. A.; Sallam, M. S.; El-Hashash, M. A. Efficient 1,3,4-Thiadiazole-4,5-Dihydropyridazin-3 (2H) -Ones as Antimicrobial Agents. *Chem. Pharm. Bull.* **2018**, *66*, 427–433. DOI: 10.1248/cpb.c17-00918.

[16] Hu, Y.; Li, C. Y.; Wang, X. M.; Yang, Y. H.; Zhu, H. L. 1,3,4-Thiadiazole: Synthesis, Reactions, and Applications in Medicinal, Agricultural, and Materials Chemistry. *Chem. Rev.* **2014**, *114*, 5572–5610. DOI: 10.1021/cr400131u.

[17] Jain, A. K.; Sharma, S.; Vaidya, A.; Ravichandran, V.; Agrawal, R. K. 1,3,4-Thiadiazole and Its Derivatives: A Review on Recent Progress in Biological Activities. *Chem. Biol. Drug Des.* **2013**, *81*, 557–576. DOI: 10.1111/cbdd.12125.

[18] Serban, G.; Stanasel, O.; Serban, E.; Bota, S. 2-Amino-1,3,4-Thiadiazole as a Potential Scaffold for Promising Antimicrobial Agents. *Drug Des. Dev. Ther.* **2018**, *12*, 1545–1566. DOI: 10.2147/dddt.s155958.

[19] Yusuf, M.; Jain, P. Synthesis and Biological Significances of 1,3,4-Thiadiazolines and Related Heterocyclic Compounds. *Arab. J. Chem.* **2014**, *7*, 525–552. DOI: 10.1016/j.arabjc.2011.02.006.

[20] Barbuceanu, S. F.; Saramet, G.; Almajan, G. L.; Draghici, C.; Barbuceanu, F.; Bancescu, G. New Heterocyclic Compounds from 1,2,4-Triazole and 1,3,4-Thiadiazole Class Bearing DiphenylsulfoneMoieties. Synthesis, Characterization and Antimicrobial Activity Evaluation. *Eur. J. Med. Chem.* **2012**, *49*, 417–423. DOI: 10.1016/j.ejmech.2012.01.031.

[21] Sahu, J. K.; Ganguly, S.; Kaushik, A. Synthesis of Some Novel Heterocyclic 1,2,4-Triazolo[3,4-b][1,3,4]ThiadiazoleDerivatives as Possible Antimicrobial Agents. *J. App. Pharm. Sci.* **2014**, *4*, 81. DOI: 10.7324/JAPS.2014.40214.

[22] Sharma, B.; Verma, A.; Prajapati, S.; Sharma, U. K. Synthetic Methods, Chemistry, and the Anticonvulsant Activity of Thiadiazoles. *Int. J. Med. Chem.* **2013**, *2013*, 348948. DOI: 10.1155/2013/348948.

[23] Jakovljević, K.; Matić, I. Z.; Stanojković, T.; Krivokuća, A.; Marković, V.; Joksović, M. D.; Mihailović, N.; Nićiforović, M.; Joksović, L. Synthesis, Antioxidant and AntiproliferativeActivities of 1,3,4-Thiadiazoles Derived from Phenolic Acids. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 3709–3715. DOI: 10.1016/j.bmcl.2017.07.003.

[24] Chauvière, G.; Bouteille, B.; Enanga, B.; de Albuquerque, C.; Croft, S. L.; Dumas, M.; Périé, J. Synthesis and Biological Activity of Nitro HeterocyclesAnalogous to Megazol, a Trypanocidal Lead. *J. Med. Chem.* **2003**, *46*, 427–440. DOI: 10.1021/jm021030a.

[25] Foroumadi, A.; Mirzaei, M.; Shafiee, A. Antituberculosis Agents II. Evaluation of *in Vitro* Antituberculosis Activity and Cytotoxicity of Some 2- (1-Methyl-5-Nitro-2-Imidazolyl) -1,3,4-Thiadiazole Derivatives. *Farmaco.* **2001**, *56*, 621–623. DOI: 10.1016/s0014-827x(01)01099-0.

[26] Alipour, E.; Emami, S.; Yahya-Meymandi, A.; Nakhjiri, M.; Johari, F.; Ardestani, S. K.; Poorrajab, F.; Hosseini, M.; Shafiee, A.; Foroumadi, A. Synthesis and AntileishmanialActivity of 5- (5-Nitroaryl) -2-Substituted-Thio-1,3,4-Thiadiazoles. *J. Enzyme Inhib. Med. Chem.* **2011**, *26*, 123–128. DOI: 10.3109/14756361003733654.

[27] Hussein, M. A.; Shaker, R. M.; Ameen, M. A.; Mohammed, M. F. Synthesis, Anti-Inflammatory, Analgesic, and Antibacterial Activities of Some Triazole, Triazolothiadiazole, and Triazolothiadiazine Derivatives. *Arch. Pharm. Res.* **2011**, *34*, 1239–1250. DOI: 10.1007/s12272-011-0802-z.

[28] Siddiqui, N.; Andalip; Bawa, S.; Ali, R.; Afzal, O.; Akhtar, M. J.; Azad, B.; Kumar, R. Antidepressant Potential of Nitrogen-Containing Heterocyclic Moieties: An Updated Review. *J. Pharm. Bioallied. Sci.* **2011**, *3*, 194–212. DOI: 10.4103/0975-7406.80765.

[29] Dawood, K. M.; Eldebss, T. M.; El-Zahabi, H. S.; Yousef, M. H.; Metz, P. Synthesis of Some New Pyrazole-Based 1,3-Thiazoles and 1,3,4-Thiadiazoles as Anticancer Agents. *Eur. J. Med. Chem.* **2013**, *70*, 740–749. DOI: 10.1016/j.ejmech.2013.10.042.

[30] Chen, Z.; Xu, W.; Liu, K.; Yang, S.; Fan, H.; Bhadury, P. S.; Hu, D. Y.; Zhang, Y. Synthesis and Antiviral Activity of 5- (4-Chlorophenyl) -1,3,4-Thiadiazole Sulfonamides. *Molecules.* **2010**, *15*, 9046–9056. DOI: 10.3390/molecules15129046.

[31] Li, Y.; Geng, J.; Liu, Y.; Yu, S.; Zhao, G. Thiadiazole-A Promising Structure in Medicinal Chemistry. *Chem. Med. Chem.* **2013**, *8*, 27–41. DOI: 10.1002/cmdc.201200355.

[32] Bhardwaj, V.; Noolvi, M. N.; Jalhan, S.; Patel, H. M. Synthesis, and Antimicrobial Evaluation of New Pyridine Imidazo[2,1b]-1,3,4-Thiadiazole Derivatives. *J. Saudi Chem. Soc.* **2016**, *20*, S406–S410. DOI: 10.1016/j.jscs.2012.12.007.

[33] Petrow, V.; Stephenson, O.; Thomas, A.; Wild, A. 3. Preparation and Hydrolysis of Some Derivatives of 1: 3: 4-Thiadiazole. *J. Chem. Soc.* **1958**, *1958*, 1508–1513. DOI: 10.1039/JR9580001508.

[34] Feng, H.; Ying, X.; Peng, Y.; Van der Eycken, E. V.; Liu, C.; Zhao, S.; Song, G. $FeCl_3$-Promoted Synthesis of 1,3,4-Thiadiazoles under Combined Microwave and Ultrasound Irradiation in Water. *Monatsh. Chem.* **2013**, *144*, 681–686. DOI: 10.1007/s00706-012-0846-x.

[35] Kubota, S.; Toyooka, K.; Ikeda, J.; Yamamoto, N.; Shibuya, M. Synthesis of 5- (Substituted Phenyl) 4-Acetyl-2-methylthio-Δ 2-1,3,4-Thiadiazolines and Their Oxidation with Potassium Permanganate. *J. Chem. Soc. Perkin Trans. I.* **1983**, *1983*, 967–971. DOI: 10.1039/P19830000967.

[36] Aryanasab, F.; Halimehjani, A. Z.; Saidi, M. R. Dithiocarbamate as an Efficient Intermediate for the Synthesis of 2-Amino-1,3,4-Thiadiazoles in Water. *Tetrahedron Lett.* **2010**, *51*, 790–792. DOI: 10.1016/j.tetlet.2009.11.100.

[37] Barot, K. P.; Manna, K. S.; Ghate, M. D. Design, Synthesis and Antimicrobial Activities of Some Novel 1,3,4-Thiadiazole, 1,2,4-Triazole-5-Thione and 1,3-Thiazolan-4-One Derivatives of Benzimidazole. *J. Saudi Chem. Soc.* **2017**, *21*, S35–S43. DOI: 10.1016/j.jscs.2013.09.010.

[38] Linganna, N.; LokanathaRai, K. Transformation of 1,3,4-Oxadiazoles to 1,3,4-Thiadiazoles Using Thiourea. *Synth.Commun.* **1998**, *28*, 4611–4617. DOI: 10.1080/00397919808004526.

[39] Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y. N.; Al-Aizari, F. A.; Ansar, M. Synthesis and Pharmacological Activities of PyrazoleDerivatives: A Review. *Molecules.* **2018**, *23*, 134. DOI: 10.3390/molecules23010134.

[40] Naim, M. J.; Alam, O.; Nawaz, F.; Alam, M. J.; Alam, P. Current Status of Pyrazole and Its Biological Activities. *J. Pharm.Bioallied Sci.* **2016**, *8*, 2–17. DOI: 10.4103/0975-7406.171694.

[41] Radini, I. A. M. Design, Synthesis, and Antimicrobial Evaluation of Novel Pyrazoles and Pyrazolyl 1,3,4-Thiadiazine Derivatives. *Molecules.* **2018**, *23*, 2092. DOI: 10.3390/molecules23092092.

[42] Yewale, S. B.; Ganorkar, S. B.; Baheti, K. G.; Shelke, R. U. Novel 3-Substituted-1-Aryl-5-Phenyl-6-Anilinopyrazolo[3,4-d]Pyrimidin-4-Ones: Docking, Synthesis and Pharmacological Evaluation as a Potential anti-Inflammatory Agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6616–6620. DOI: 10.1016/j.bmcl.2012.08.119.

[43] Gokulan, P. D.; Jayakar, B.; Alagarsamy, V.; Raja Solomon, V. Synthesis and Pharmacological Investigation of 5-Substituted-3-Methylsulfanyl-1H-Pyrazole-4-Carboxylic Acid Ethyl Esters as New Analgesic and anti-Inflammatory Agents. *Arzneimittelforschung.* **2012**, *62*, 457–462. DOI: 10.1055/s-0032-1321830.

[44] Faidallah, H. M.; Al-Mohammadi, M. M.; Alamry, K. A.; Khan, K. A. Synthesis and Biological Evaluation of Fluoropyrazolesulfonylurea and Thiourea Derivatives as Possible AntidiabeticAgents. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 157–163. DOI: 10.1080/14756366.2016.1180594.

[45] Abdel-Aziz, M.; Abuo-Rahma, G.; el, D.; Hassan, A. A. Synthesis of Novel Pyrazole Derivatives and Evaluation of Their Antidepressant and Anticonvulsant Activities. *Eur. J. Med. Chem.* **2009**, *44*, 3480–3487. DOI: 10.1016/j.ejmech.2009.01.032.

[46] El-Sabbagh, O. I.; Baraka, M. M.; Ibrahim, S. M.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Balzarini, J.; Rashad, A. A. Synthesis and Antiviral Activity of New Pyrazole and ThiazoleDerivatives. *Eur. J. Med. Chem.* **2009**, *44*, 3746–3753. DOI: 10.1016/j.ejmech.2009.03.038.

[47] Metwally, N. H.; Abdelrazek, F. M.; Eldaly, S. M. Synthesis and Anticancer Activity of Some New Heterocyclic Compounds Based on 1-Cyanoacetyl-3,5-Dimethylpyrazole. *Res. Chem. Intermed.* **2016**, *42*, 1071–1089. DOI: 10.1007/s11164-015-2074-6.

[48] Lv, X.-H.; Ren, Z.-L.; Li, D.-D.; Ruan, B.-F.; Li, Q.-S.; Chu, M.-J.; Ai, C.-Y.; Liu, D.-H.; Mo, K.; Cao, H.-Q. Discovery of Novel Double Pyrazole Schiff Base Derivatives as AntitobaccoMosaic Virus (TMV) Agents. *Chin. Chem. Lett.* **2017**, *28*, 377–382. DOI: 10.1016/j.cclet.2016.10.029.

[49] Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial Resistance: A Global Multifaceted Phenomenon. *Pathog. Glob. Health.* **2015**, *109*, 309–318. DOI: 10.1179/2047773215y.0000000030.

[50] Abdelbaset, M. S.; Abuo-Rahma, G. E. A.; Abdelrahman, M. H.; Ramadan, M.; Youssif, B. G. M.; Bukhari, S. N. A.; Mohamed, M. F. A.; Abdel-Aziz, M. Novel Pyrrol-2 (3H) -Ones and Pyridazin-3 (2H) -Ones Carrying Quinoline Scaffold as anti-Proliferative Tubulin Polymerization Inhibitors. *Bioorg. Chem.* **2018**, *80*, 151–163. DOI: 10.1016/j.bioorg.2018.06.003.

[51] Abou-Zied, H. A.; Youssif, B. G. M.; Mohamed, M. F. A.; Hayallah, A. M.; Abdel-Aziz, M. EGFR Inhibitors and Apoptotic Inducers: Design, Synthesis, Anticancer Activity and Docking Studies of Novel Xanthine Derivatives Carrying Chalcone Moiety as Hybrid Molecules. *Bioorg. Chem.* **2019**, *89*, 102997. DOI: 10.1016/j.bioorg.2019.102997.

[52] Al-Sanea, M. M.; Gotina, L.; Mohamed, M. F.; Grace Thomas Parambi, D.; Gomaa, H. A. M.; Mathew, B.; Youssif, B. G. M.; Alharbi, K. S.; Elsayed, Z. M.; Abdelgawad, M. A.; Eldehna, W. M. Design, Synthesis and Biological Evaluation of New HDAC1 and HDAC2 Inhibitors Endowed with Ligustrazine as a Novel Cap Moiety. *Drug Des. Dev. Ther.* **2020**, *14*, 497–508. DOI: 10.2147/dddt.s237957.

[53] Ibrahim, T. S.; Sheha, T. A.; Abo-Dya, N. E.; AlAwadh, M. A.; Alhakamy, N. A.; Abdel-Samii, Z. K.; Panda, S. S.; Abuo-Rahma, G. E. A.; Mohamed, M. F. A. Design, Synthesis and Anticancer Activity of Novel ValproicAcid Conjugates with Improved Histone Deacetylase (HDAC) Inhibitory Activity. *Bioorg. Chem.* **2020**, *99*, 103797. DOI: 10.1016/j.bioorg.2020.103797.

[54] Mohamed, M. F. A.; Shaykoon, M. S. A.; Abdelrahman, M. H.; Elsadek, B. E. M.; Aboraia, A. S.; Abuo-Rahma, G. Design, Synthesis, Docking Studies and Biological Evaluation of Novel ChalconeDerivatives as Potential Histone Deacetylase inhibitors. *Bioorg. Chem.* **2017**, *72*, 32–41. DOI: 10.1016/j.bioorg.2017.03.005.

[55] Mohamed, M. F. A.; Youssif, B. G. M.; Shaykoon, M. S. A.; Abdelrahman, M. H.; Elsadek, B. E. M.; Aboraia, A. S.; Abuo-Rahma, G. E. A. Utilization of Tetrahydrobenzo[4,5]Thieno[2,3-d]Pyrimidinone as a Cap Moiety in Design of Novel Histone DeacetylaseInhibitors. *Bioorg. Chem.* **2019**, *91*, 103127. DOI: 10.1016/j.bioorg.2019.103127.

[56] Youssif, B. G. M.; Abdelrahman, M. H.; Abdelazeem, A. H.; Abdelgawad, M. A.; Ibrahim, H. M.; Salem, O. I. A.; Mohamed, M. F. A.; Treambleau, L.; Bukhari, S. N. Design, synthesis, mechanistic and histopathological studies of small-molecules of novel indole-2-carboxamides and pyrazino[1,2-a]indol-1 (2H) -ones as potential anticancer agents effecting the reactive oxygen species production. *Eur. J. Med. Chem.* **2018**, *146*, 260–273. DOI: 10.1016/j.ejmech.2018.01.042.

[57] Youssif, B. G. M.; Mohamed, M. F. A.; Al-Sanea, M. M.; Moustafa, A. H.; Abdelhamid, A. A.; Gomaa, H. A. M. Novel Aryl Carboximidamide and 3-Aryl-1,2,4-Oxadiazole Analogues of Naproxen as Dual Selective COX-2/15-LOX Inhibitors: Design, Synthesis and Docking Studies. *Bioorg. Chem.* **2019**, *85*, 577–584. DOI: 10.1016/j.bioorg.2019.02.043.

[58] Fritz, J. S.; Schenk, G. H. Acid-Catalyzed Acetylation of Organic Hydroxyl Groups. *Anal. Chem.* **1959**, *31*, 1808–1812. DOI: 10.1021/ac60155a034.

[59] Gowda, M. S.; Pande, S. S.; Ramakrishna, R. A.; Prabhu, K. R. Acylation of Grignard Reagents Mediated by N-Methylpyrrolidone: A Remarkable Selectivity for the Synthesis of Ketones. *Org. Biomol. Chem.* **2011**, *9*, 5365–5368. DOI: 10.1039/c1ob05780d.

[60] Hassan, S. M.; Emam, H. A.; Abdelall, M. M. Heteroaromatization with Ketene Dithioacetals: Part II. Synthesis of Some Novel 5-Aminopyrazole-3-Carbonitrile, 3-Carboxamide and Pyrazolo[3,4-d] Pyrimidin-4-One Derivatives as Antimicrobial Agents. *Phosphorus Sulfur Silicon Relat. Elem.* **2001**, *175*, 109–127. DOI: 10.1080/10426500108040260.

[61] Jagadale, S.; Chavan, A.; Shinde, A.; Sisode, V.; Bobade, V. D.; Mhaske, P. C. Synthesis and Antimicrobial Evaluation of New Thiazolyl-1,2,3-Triazolyl-Alcohol Derivatives. *Med. Chem. Res.* **2020**, *29*, 989–999. DOI: 10.1007/s00044-020-02540-5.

[62] Mali, A. B.; Joshi, M.; Kulkarni, V. Phytochemical Screening and Antimicrobial Activity of Stevia RebaudianaLeaves. *Int. J. Curr. Microbiol. App. Sci.* **2015**, *4*, 678–685. DOI: 10.1016/S2222-1808(14)60572-7.

[63] NCCLS. 2000. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A7. NCCLS, Wayne, PA

[64] Bax, B. D.; Chan, P. F.; Eggleston, D. S.; Fosberry, A.; Gentry, D. R.; Gorrec, F.; Giordano, I.; Hann, M. M.; Hennessy, A.; Hibbs, M.; et al. Type IIA Topoisomerase Inhibition by a New Class of Antibacterial Agents. *Nature.* **2010**, *466*, 935–940. DOI: 10.1038/nature09197.

[65] Berger, J. M.; Gamblin, S. J.; Harrison, S. C.; Wang, J. C. Structure and Mechanism of DNA Topoisomerase II. *Nature.* **1996**, *379*, 225–232. DOI: 10.1038/379225a0.

[66] Abd El-Kader, A. M.; Mahmoud, B. K.; Hajjar, D.; Mohamed, M. F. A.; Hayallah, A. M.; Abdelmohsen, U. R. A. M.; Mahmoud, B. K.; Hajjar, D.; Mohamed, M. F.; Hayallah, A. M.; Abdelmohsen, U. R. AntiproliferativeActivity of New PentacyclicTriterpene and a Saponin from Gladiolus Segetum Ker-GawlCorms Supported by Molecular Docking Study. *RSC Adv.* **2020**, *10*, 22730–22741. DOI: 10.1039/D0RA02775H.

[67] Ahmed, E. A.; Mohamed, M. F.; Omran, A.; Salah, H. Synthesis, EGFR-TK Inhibition and Anticancer Activity of New Quinoxaline Derivatives. *Synth. Commun.* **2020**, *50*, 2929–2940. DOI: 10.1080/00397911.2020.1787448.