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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Morphological changes of some pathogenic microbial strains induced by novel thiadiazole derivatives

M. G. El-Gazzar · N. H. Zaher · S. Y. El-Tablawy

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Abstract In the quest for potent antimicrobial agents, some new 1,3,4-thiadiazole derivatives (**2–11**) were synthesized from the starting compound (**1**) 5-amino-1,3,4-thiadiazole-2(3H)-thione, with an aim to evaluate their antimicrobial effect on different pathogenic organisms isolated from microbial-infected cancer patients who had not yet received their radiation dose, compared to reference antibiotics. The titled compounds **5**, **6**, **10**, and **11** were found to possess comparable or more potent activity than the reference compounds amikacin and sulperazone. Compound **5** was the most distinctive derivative identified in the present study because of its remarkable antibacterial activity exhibited against the Gram +ve strains, it has been scanned under electron microscope to determine the morphological changes that has taken place in the bacterial cells of *Bacillus cereus* and non-irradiated and irradiated *Staphylococcus aureus*. MIC of compound **5** and/or gamma irradiation affected the morphology of the tested strains causing; membrane damage, deformity on the surface of some cells, disappearance of the septum, elongation of some cells, and shortening of others. It may be hoped that the present study will encourage efforts toward the development of novel antibacterial agents that could be better in terms of efficacy and safety.

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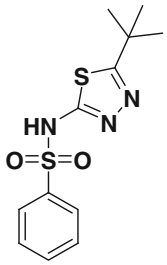
Introduction

Dealing with infectious diseases still considered a critical challenging issue. This is attributed to the emerging of new infectious diseases and increasing number of multidrug resistant (MDR) microbial pathogens. And this problem is more obvious for the Gram +ve bacteria (Tenover and McDonald, 2005; Pfeltz and Wilkinson, 2004). It is well-thought-out that this therapeutic problem is a crucial part in immunocompromised persons as cancer patients receiving gamma irradiation as therapy. They are more susceptible for different microbial infections (Sander and Bottger, 1999) and are in need to use effective antimicrobial agents. Besides Farrag and co-authors (Farrag *et al.*, 2002) had reported that the low doses of gamma irradiation had an effect on the resistance of some bacterial isolates to different antibiotics resulted in increasing difficulty in treating infections caused by these organisms.

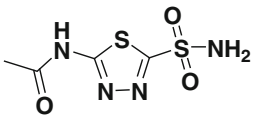
The need to design new compounds to deal with the evolving resistance to old and newfangled antimicrobials has become one of the most important areas of research today. A possible approach to overwhelmed MDR problem is to design novel agents with different mechanisms of action so that cross resistance with existing drugs cannot occur (Khan *et al.*, 2005).

Organic compounds integrating heterocyclic ring systems continue to draw considerable attention due to their wide range of biological activities. Among different five-membered heterocyclic systems thiadiazoles which act as H-binding domain with two electron donor system also as a constrained pharmacophores. The chemistry of 1,3,

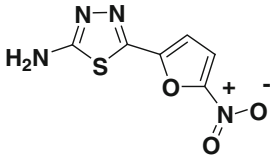
Fig. 1 Commercially available 1,3,4-thiadiazole drugs



Desaglybuzole



Acetazolamide



Furidiazine

4-thiadiazole derivatives has been of increasing interest as a versatile moiety, identified for its usefulness in medicine to be included in commercially available drugs as desaglybuzole, acetazolamide, and furidiazine (Guirgis *et al.*, 1976; Bashir *et al.*, 1990; Cohen *et al.*, 1975) (Fig. 1). 1,3,4-Thiadiazole is a widespread scaffold in a number of synthetic molecules exhibiting attractive biological consequences as antiviral, anticancer and cytotoxic, anticonvulsant, antihyperlipedemic, anti-inflammatory and analgesic, antidepressant, antioxidant, immuno-modulator, amebic, and antileishmanial (Hranjec *et al.*, 2011; Noolvi *et al.*, 2012; Zhang *et al.*, 2012; Guan *et al.*, 2012; Dogan *et al.*, 2002; Mougenot *et al.*, 2012; Özadal *et al.*, 2012; Chidananda *et al.*, 2012; Jubie *et al.*, 2012; Khan *et al.*, 2010; Aguilar *et al.*, 2012; Siddiqui *et al.*, 2012; Ardestani *et al.*, 2012). Many of thiadiazole compounds have found a lot of useful chemotherapeutic application as antimicrobial, antibacterial against many pathogenic bacteria and resistant mycobacterium, and also as antifungal agents (Sumangala *et al.*, 2012; Muralikrishna *et al.*, 2012; Plech *et al.*, 2012; Zoumpoulakis *et al.*, 2012; Almajan *et al.*, 2010; Barbuceanu *et al.*, 2012; Kolavi *et al.*, 2006).

Inspired by these findings, and in continuation of our ongoing efforts on the synthesis of heterocycles with potential antimicrobial activities (Ghorab *et al.*, 2011). The aim of this work to synthesize via simple convenient methods, a new series of 1,3,4-thiadiazoles having different radicals linked to amino group from 2 position, for evaluation of their antimicrobial profile. Also to study their effect on the morphology of some pathogenic microorganisms before and after irradiation to investigate the susceptibility of the tested bacterial strains to the synthesized compounds and studying the synergistic effect of gamma radiation. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the pharmacological activity of some pertinent chemotherapeutic agents such as sulfonamide, thiazole, pyrrole, aniline, acetophenone, and Schiff's base functionalities (Ghorab *et al.*, 2013; Sadek *et al.*, 2011; Mohamed *et al.*, 2009; Sivakumar *et al.*, 2008; Sabry *et al.*, 2013).

Results and discussion

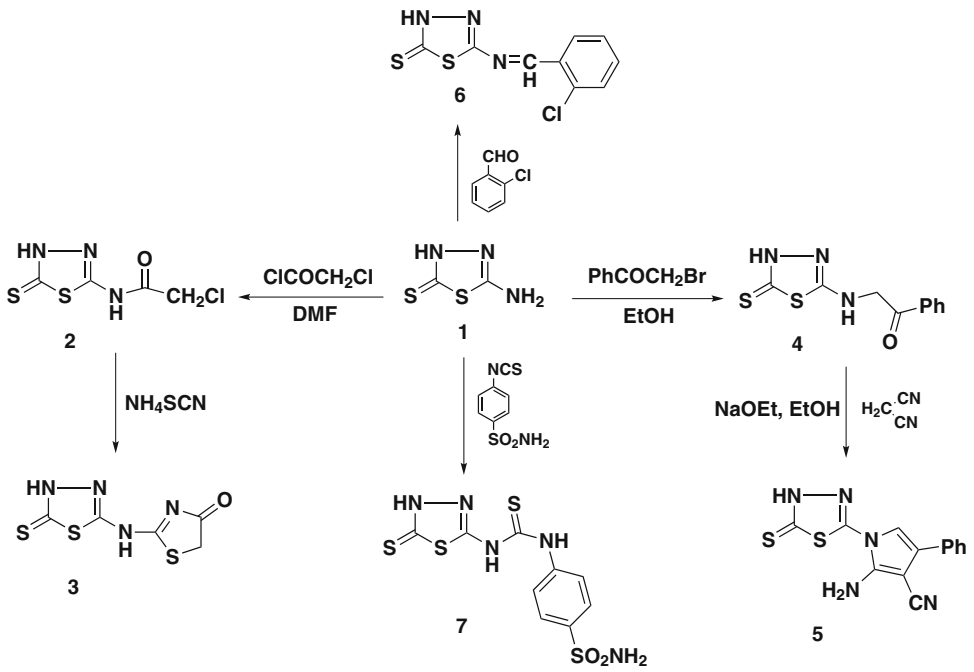
Chemistry

Considering the 5-amino-3H-[1,3,4]thiadiazole-2-thione (**1**) system as scaffold, we synthesized new derivatives. The synthesis of our target compounds (**2–7**), (**8–11**) is outlined in Schemes 1, 2 respectively. The starting compound (**1**) was prepared according to the method reported by (Cho and Kim, 1993), depending on the reaction between thiosemicarbazide and carbon disulfide in alkaline medium followed by acidification.

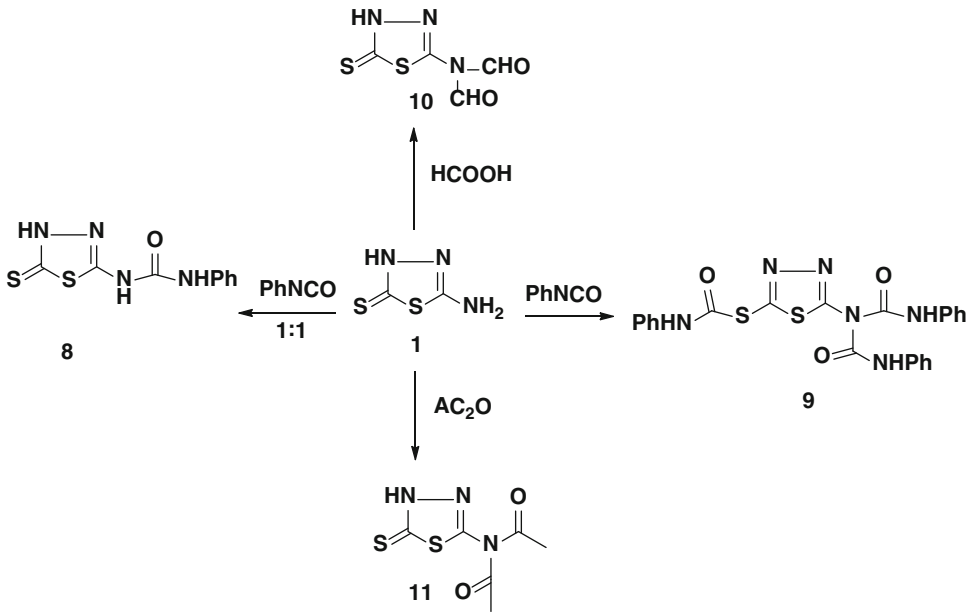
All structures of newly synthesized compounds were supported by physical, micro analytical, and spectral data. The target compounds 2-substituted-(5-thioxo-4, 5-dihydro[1, 3, 4]thiadiazol-2-yl) (**2–7**) were obtained through the reaction with different electrophilic reagents. Compound (**1**) was converted into (2-chloro-*N*-(5-thioxo-4,5-dihydro-[1, 3, 4]thiadiazol-2-yl)acetamide) (**2**), by nucleophilic acylation of amino group with chloroacetyl chloride in DMF stirred at room temperature. Where IR spectrum revealed characteristic strong intensity bands at 2,922, 1,682, and 825 cm^{-1} for the introduced (CH aliph, C=O and C–Cl stretching), respectively confirming the formation of the chloro acyl derivative. In addition, it showed absorption band at 3,187 cm^{-1} (N–H stretching) due to conversion of NH_2 into amide. ^{1}H-NMR spectrum displayed up-field signal at 4.34 ppm for the introduced CH_2 group and downfield shifted signal appearing at 7.90 ppm, due to the conversion of the free amino into amide. ^{13}C-NMR exhibited new up-field signal at 40.3 ppm for CH_2 and new signal at 167.19 ppm ascribed to C=O. Up-field shift experienced from 161(C-2 start compound **1**) to 154.1 ppm.

Compound (**2**) was cyclized into (2-(5-thioxo-4, 5-dihydro-[1, 3, 4]thiadiazol-2-ylamino)thiazol-4-one) (**3**) by treatment with ammonium thiocyanate in ethanol. The reaction proceeded via an intermolecular cyclization and liberation of hydrogen chloride molecule then rearrangement (Siavosh *et al.*, 2006). Where IR spectrum showed the disappearance of the peak at 825 cm^{-1} as a result of

Scheme 1 Synthetic pathways for compounds **2–7**



Scheme 2 Synthetic pathways for compounds **8–11**



cyclization and loss of HCl molecule. The 2 (NH) stretching bands (3,190 and 3,187 cm^{-1}) were shifted to lower frequency regions 3,167 and 3,034 cm^{-1}, respectively. ^{1}H-NMR spectrum displayed an up-field shift for CH_2, appearing at 2.98 ppm due to its inclusion in the 1,3 thiazole-2-yl ring formed. ^{13}C-NMR spectrum exhibited a new signal at 167.9 ppm ascribed to C-2 of thiazole ring formed, along with the up-field shifted values for C-5 thiazole at 36.4 ppm due to rearrangement.

Similarly, (1-phenyl-2-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylamino)-ethanone) **4** was prepared by the reaction of compound **1** with phenacyl bromide in refluxing ethanol with loss of one mole of hydrogen bromide. Where IR spectrum revealed the presence of characteristic absorption peaks at 3,011 and 2,932, 2,848 cm^{-1} for (CH aromatic and CH aliphatic stretching), respectively, and a strong intensity stretching band at 1,690 cm^{-1} ascribed for the (C=O) group introduced, confirming the ethanone derivative formation. ^{1}H-NMR spectrum displayed signals at 4.77 and 8.75 ppm which disappeared upon D_2O exchange, confirming the presence two (NH) groups, while the protons of the aromatic ring introduced showed sharp peaks at 7.42–7.99 ppm.

^{13}C-NMR spectrum showed new signals at 40.05 and 176.25 ppm corresponding to the introduced (CH_2), and (C=O), respectively. Whereas the aromatic carbon atoms appeared in the range of 129.3–135.6 ppm. C-2 thiadiazole exhibited up-field shifted frequency at 146.3 ppm. Compound (**4**) was cyclized into (2-amino-4-phenyl-1-(5-thioxo-4, 5-dihydro[1, 3, 4]thiadiazol-2-yl)-1H-pyrrole-3-carbonitrile) **5** by treatment with malononitrile in ethanol. Where IR spectrum revealed absorption bands at 3,245, 3,187 for the (NH_2 stretching) group formed and a characteristic medium intensity band at 2,222 cm^{-1} confirming the presence of carbonitrile group introduced. ^{1}H-NMR spectrum displayed signals at 3.52 ppm for the NH_2 group which disappeared upon D_2O exchange, and at 4.73 ppm ascribed for the (CH) group of the pyrrole ring. ^{13}C-NMR spectrum showed the absence of the signal ascribed for (C=O), instead new signals appeared at 108 and 111 ppm ascribed for C-3 pyrrole and C≡N, respectively. While C-2 and 4-pyrrole signals appeared with the aromatic ones.

The target compound (5-[(2-chloro-benzylidene)-amino]-3H-[1,3,4]-thiadiazole-2-thione) **6** is formed upon reaction of compound **1** with 2-chlorobenzaldehyde in refluxing ethanol through Schiff's base formation. Where IR spectrum revealed absorption bands at 3,022 and 2,956, 2,854 cm^{-1} for (CH aromatic and CH aliphatic stretching), respectively, and a characteristic strong intensity band at 834 cm^{-1} attributed to (C–Cl stretching). ^{1}H-NMR spectrum displayed signals at 7.32 ppm for the CH group of Schiff's base. The protons of the aromatic ring introduced showed sharp peaks at 7.34–7.78 ppm ascribed for chlorobenzene ring introduced. ^{13}C-NMR spectrum showed the presence of a new signal at 155.2 ppm assigned for (CH Schiff's base group), whereas the aromatic carbon atoms appeared in the range of 126.9–137.9 ppm.

The preparation of (4-[3-(5-thioxo-4,5-dihydro-[1,3,4] thiadiazol-2-yl)-thioureido]-benzenesulfonamide) **7** was attained by the reaction of compound **1** with 4-isothiocyanatobenzenesulfonamide in refluxing dimethylformamide. Where IR spectrum revealed absorption bands at 3,022, 1,387, 1,145 cm^{-1} assigned for CH aromatic and SO_2 groups-stretching, respectively. ^{1}H-NMR spectrum displayed signals at 5.50 ppm for the NH_2 and signals at 6.50, 6.70, 8.25 ppm for the 3NH groups which disappeared upon exchange with D_2O. The aromatic protons for the AB system of the benzene ring introduced appeared at 7.66 and 7.81 ppm. ^{13}C-NMR spectrum exhibited additional signal for the most de-shielded carbon atom at 200.01 ppm attributed to the thiocyanate C introduced. Whereas the aromatic carbon atoms appeared in the range of 122.2–139.4 ppm.

The target compound (1-phenyl-3-(5-thioxo-4,5-dihydro-[1,3,4]thiadiazol-2-yl)-urea) **8** was obtained by the unimolar reaction of compound **1** with phenylisocyanate in pyridine. Where IR spectrum revealed absorption band at 3,031 cm^{-1} due to the aromatic CH and strong intensity absorption band at 1,685 cm^{-1} assigned for C=O stretching. ^{1}H-NMR spectrum displayed signals at 7.28–7.48 ppm attributed to the aromatic protons of the phenyl ring introduced. ^{13}C-NMR spectrum showed additional signal for C=O at 169.69 ppm. While the aromatic carbon atoms appeared in the range of 118.16–139.69 ppm.

The polysubstituted target compound (2-di-(*N*-phenyl-formamide) amino-5-(*N*-phenyl-sulfinic amide) [1,3,4-thiadiazole]) **9** was obtained by the reaction of the starting compound **1** with excess phenylisocyanate in refluxing pyridine. Where IR spectrum revealed bands at 3,245 and 3,278 cm^{-1} ascribed for 3NH-stretching, 3,028 cm^{-1} due to CH aromatic-stretching and characteristic strong intensity absorption peaks at 1,654 and 1,676 cm^{-1} assigned for the three (C=O stretching) groups. The disappearance of the signal ascribed for (C=S), confirmed the polysubstitution. ^{1}H-NMR spectrum displayed signals for the three NH groups at 3.42 and 8.67 ppm which disappeared upon exchange with D_2O and the aromatic protons of the three phenyl rings introduced appeared as multiplets at range 6.97–7.48 ppm. ^{13}C-NMR spectrum revealed a great up-field shift for (C=S) at 165.43. An additional bands for (3C=O) introduced appeared at 149.6 and 152.48 ppm. The C-2 of thiadiazole encountered downfield shift at 170 ppm.

The target compound (*N*-formyl-*N*-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-formamide) **10** was attained by the neat reaction of the starting compound **1** with formic acid for 10 h. Where IR spectrum showed absorption peaks at 3,331 cm^{-1} assigned for NH-stretching. New absorption peaks appeared at 2,879 and 1,658 cm^{-1} due to stretching CH aliphatic and C=O, respectively. ^{1}H-NMR spectrum displayed only two signals for the NH group at 2.5 ppm which disappeared upon exchange with D_2O and signal attributed to CHO groups at 8.4 ppm. ^{13}C-NMR spectrum revealed three signals: an up-field shift for (C-2 thiadiazole) at 150.63 ppm, whereas the formyl carbon atoms appeared at 160.35 ppm and C-5 (C=S) appeared at 183.26 ppm.

Similarly, the preparation of disubstituted compound (*N*-acetyl-*N*-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide) **11** was achieved through the neat reaction of compound **1** in acetic anhydride. Where IR spectrum displayed signals at 2,897 cm^{-1} for aliphatic CH-stretching assigned for the introduced acetyl groups together with strong intensity absorption bands at 1,664 cm^{-1} attributed to the carbonyl groups of the acetyl moieties. ^{1}H-NMR spectrum displayed only two signals for the acetyl groups and NH at 2.02 and 12.29 ppm, respectively. ^{13}C-NMR spectrum revealed new up-field signal assigned for CH_3 groups at 22.35 ppm. C-2 thiadiazole encountered up-field shift at 152.21 ppm, whereas the carbon atoms of the acetyl groups appeared at 169.19 ppm.

Further, the EIMS of all the synthesized compounds are in conformity with the assigned structures along with the elemental analyses which were found within the limit of 0.4 % of theoretical values for all the synthesized compounds.

Antimicrobial screening

Structure activity relationship (SAR)

From the results obtained in (Table 1) we can observe that, the preliminary screening for the antimicrobial activity of the synthesized compounds affects Gram +ve bacteria and does not affect Gram −ve ones or the tested fungal strain. The most potent compound in this study was the thiadiazole derivative **5** bearing a pyrrole ring showing the highest antibacterial activity among the tested compounds and is more potent or nearly equal to that of the standard antibacterial agents. It was found to be nearly as potent as Amikacin and Sulperazone especially against *Staphylococcus aureus* and *Bacillus cereus* with inhibition zones nearly equal to that of the reference antibiotic amikacin. This comes in accordance that compounds bearing pyrrole ring posses potent antibacterial activity (Ghorab *et al.*, 2011; Mohamed *et al.*, 2009). In addition, it showed higher activity than compound **4** which proves the efficacy of cyclization and introduction of pyrrole ring on activity. Chloroacetylation of amino group in compound **2** resulted in a drastic drop in the activity which was improved by cyclization and formation of thiazolone ring in compound **3**. The Schiff's base derivative **6**, the thioureido derivative **7**, the mono- and polysubstituted ureido derivatives **8** and **9** showed moderate activity, while, disubstitution on amino group by formylation and acetylation in compounds **10** and **11** considerably increased the activity.

Effect of γ-irradiation on the susceptibility of Gram +ve bacterial strains to the synthesized compounds

The results in (Table 2) revealed that the most potent pyrrole derivative **5** revealed good activity against normal control and irradiated *S. aureus* with inhibition zones equal to that of Amikacin. While in case of normal control of *B. cereus*, it showed higher activity rather than the reference antibiotic Amikacin. For this, the minimum inhibitory concentration (MIC) and the effect of compound **5** on the morphology of *S. aureus* and *B. cereus* using scanning electron microscope (SEM) were carried out. Similarly, for the rest of the tested compounds, it was shown that irradiation did not affect the susceptibility of the bacterial strains and acted synergistically with the tested compounds. These results constitute a value for cancer patients receiving gamma radiotherapy. They are more susceptible for bacterial infection and in need for antimicrobial without affecting the bacteria susceptibility especially the Gram +ve ones. In this case compound **5** adds a merit of synergism with gamma radiation as antibacterial.

Scanning electron microscope study (SEM)

A control culture typical to the normal control *S. aureus* is shown in (Fig. 2a) as spherical cells with normal shape, size and complete septum (magnification 15,000), while cells treated with MIC of compound **5**, revealed membrane damage by the appearance of projecting cellular material and bleb formation. Furthermore, disappearance of septum

Table 1 Antimicrobial screening of thiadiazole derivatives (**2–10**)

Tested compounds	Inhibition zones (mm)[a]					
	P. aeruginosa	*K. pneumonia*	*S. aureus*	*S. epidermidis*	*B. cereus*	*C. albicans*
2	–	–	–	–	–	–
3	–	–	10	7	9	–
4	–	–	20	15	18	–
5	–	–	25	17	20	–
6	–	–	20	12	22	–
7	–	–	9	–	7	–
8	–	–	10	9	7	–
9	–	–	15	7	7	–
10	–	–	20	20	22	–
11	–	–	20	13	20	–
Amikacin[b]	15	11	20	25	20	NT
Sulperazon[c]	30	27	30	30	28	NT
Nystatine[d]	NT	NT	NT	NT	NT	7
DMF	–	–	–	–	–	–

NT not tested

[a] Slight activity: 12–14 mm (+); moderate activity: 15–18 mm (++); high activity: ≥19 mm (+++)

[b] Inhibition of protein synthesis (Amikacin sensitive ≥17 mm). Reference antibiotics

[c] Inhibition of cell wall. Reference antibiotics

[d] Manufactured by Bristol–Myers Squibb, Giza, Egypt

Table 2 Effect of gamma irradiation on the susceptibility of tested Gram +ve strains to the biologically active compounds

Tested compounds	Inhibition zones (mm)[a]					
	S. aureus		*S. epidermidis*		*B. cereus*	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated
3	12	15	7	7	11	11
4	20	20	12	20	15	15
5	20	19	12	25	25	25
6	16	16	13	13	20	16
7	10	10	6	10	7	10
8	7	10	7	10	7	9
9	7	7	8	12	7	9
10	20	16	13	13	13	20
11	20	17	11	15	11	20
Amikacin	20	17	24	25	20	20
Sulperazon	40	26	30	35	28	32

[a] Slight activity: 12–14 mm (+); moderate activity: 15–18 mm (++); high activity: ≥19 mm (+++)

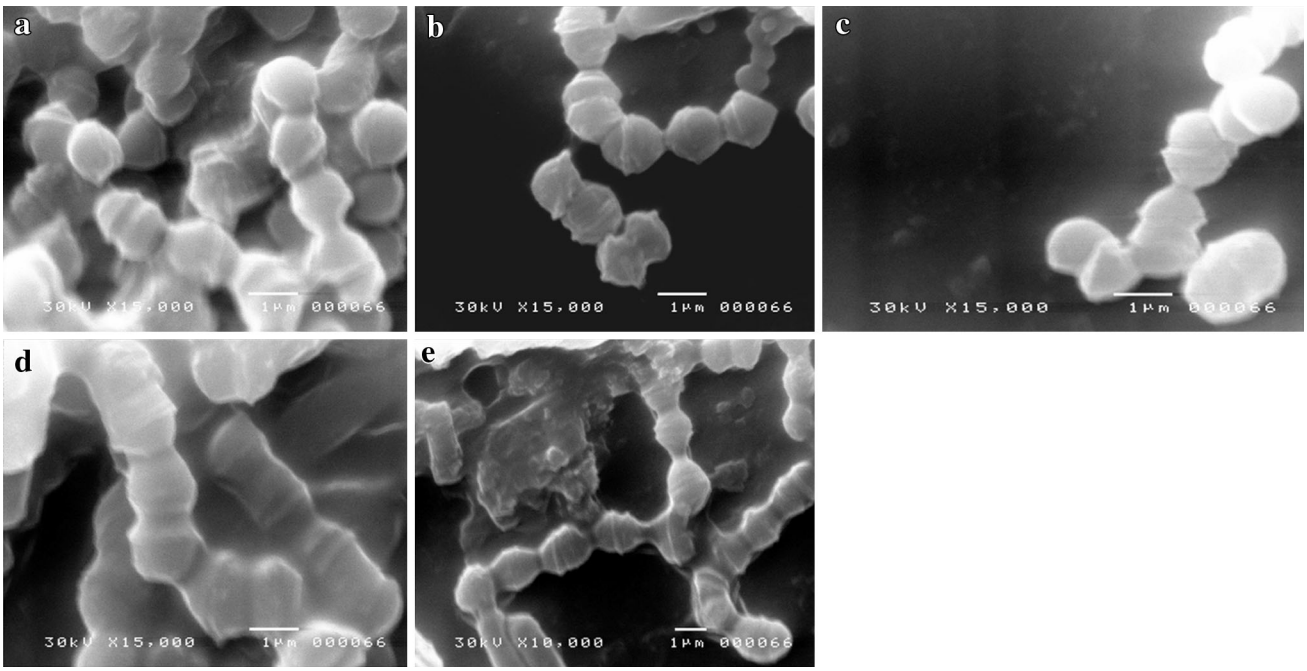


Fig. 2 **a**–**e** Scanning electron micrographs of untreated and treated *S. aureus*, with or without γ-irradiation. **a** Normal control *S. aureus*. **b**, **c** Cells treated with MIC of compound **5**. **d** *S. aureus* cells exposed to gamma radiation. **e** The synergistic effect of gamma irradiation and compound **5**

was noticed in some cells Fig. 2b, c (magnification 15,000).

Figure 2d showed *S. aureus* cells exposed to gamma radiation appeared longer and irregularly shaped than that of the normal control ones (magnification 15,000). Meanwhile, the synergistic effect of gamma irradiation and compound **5**, caused deformity of some cells' surface and elongation of others as shown in Fig. 2e (magnification 10,000).

As shown in (Fig. 3a; normal control *B. cereus*), cells are normal in shape and size (magnification 10,000), while (Fig. 3b; treated cells), displayed many morphological changes including elongation in some cells, shortening in others, disappearance of the septum, and aggregation of the cellular material at the poles of the cell (magnification 7,500).

Our results were in agreement with many investigators (Sanyal and Greenwood, 1993), when staphylococcal cells were treated with vancomycin. Also, Eltablawy and El-hifnawi (2010) found that there were morphological changes in *Pseudomonas aeruginosa* treated with garlic oil. It was reported (Rhayour *et al.*, 2003; Bennis *et al.*, 2004)

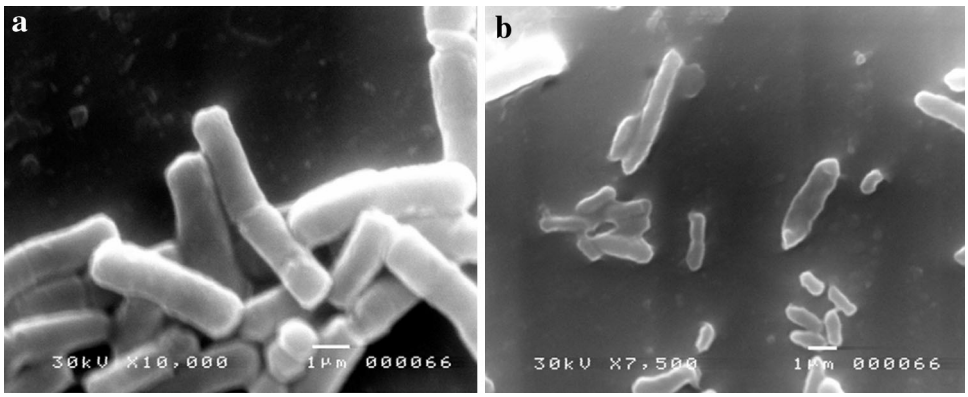


Fig. 3 **a**, **b** Scanning electron micrographs of untreated and treated *B. cereus*. **a** Normal control *B. cereus*. **b** Treated cells with MIC of compound **5**

that structures like blebs in the outside surface of treated cells, may represent collection of cytoplasmic constituents which were pushed through cracks produced in the cell wall.

Conclusions

The antimicrobial activity of the synthesized compounds may be due to the presence of versatile pharmacophore which might increase the lipophilic character of the molecules, by which it facilitates the crossing through the biological membrane of the microorganism and thereby inhibit their growth. Or acting additionally by different mechanisms of action for the hybrids. For this reason, we can see that compound **5** 2-amino-4-phenyl-1-(5-thioxo-4,5-dihydro[1,3,4]thiadiazol-2-yl)-1H-pyrrole-3-carbonitrile bearing the pyrrole moiety was found to exhibit the most potent antimicrobial activity against Gram +ve *S. aureus* and *B. cereus* when compared with Amikacin. At the same time it acts synergistically with gamma radiation.

We here in recommend further pharmacological studies to be conducted in future research to deal with the toxicity profiling and to perform the exact mechanistic assays to reveal the exact mechanism of action.

In conclusion, we reported here a simple and convenient route for the synthesis of some new 1,3,4-thiadiazole derivatives for antimicrobial, gamma synergistic and bacterial morphological evaluation.

Materials and methods

Chemistry

Measurement

The melting points were taken in an open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker 300 NMR spectrometer operating at 400.13 and 100.77 MHz, respectively. Microanalyses were obtained with an Elemental analyses system GmbH VarioEL V300 element analyzer. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF254, 200 mesh) aluminum plates (E Merk) and visualized in UV chamber. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). IR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis were consistent with the assigned structures.

Experimental protocol

*2-Chloro-N-(5-thioxo-4,5-dihydro-[1,3,4]thiadiazol-2-yl) acetamide (**2**)* A mixture of 5-amino-3H-[1,3,4]thiadiazole-2-thione **1** (0.5 g, 0.003 mol) and chloroacetyl chloride (0.3 g, 0.003 mol) in DMF was stirred at room temperature for 2 h, the reaction mixture was poured onto ice water and the solid obtained was recrystallized from ethanol to give **2**. Yield, 88 %, mp 280 °C. IR (KBr, cm^{-1}): 3,190, 3,187 (2NH), 2,922, 2,848 (CH aliph.), 1,682 (C=O), 1,230 (C=S), 825 (C–Cl). ^{1}H-NMR (DMSO-d_6, ppm): 4.34 [s, 2H, CH_2], 7.90, 9.10 [2 s, 2H, 2NH, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6, ppm): 40.3 (CH2), 154.1 (C-thiadiazole), 167.19 (C=O), 183.0 (C=S). MS: *m/z* 209 (M^+, 4.2 %), 76 (100 % base peak). Analysis calculated for $C_4H_4N_3OS_2Cl$ C, 22.91; H, 1.92; N, 20.04; found: C, 22.78; H, 1.62; N, 20.23.

*2-(5-Thioxo-4,5-dihydro-[1,3,4]thiadiazol-2-ylamino)thiazol-4-one (**3**)* A mixture of **2** (0.5 g, 0.002 mol) and ammonium thiocyanate (0.17 g, 0.002 mol) was refluxed in ethanol for 1 h. The reaction mixture was cooled, poured onto ice water and the solid obtained was recrystallised from dioxane to give **3**. Yield, 70 %, mp 280 °C. IR (KBr, cm^{-1}): 3,167, 3,034 (2NH), 1,682 (C=O), 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 2.98 [s, 2H, CH_2-thiazole], 4.03,

7.86 [2 s, 2H, 2NH, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6, ppm): 36.4 (C-thiazole), 154.9, 167.9, 175.01, 183.2. MS: *m/z* 232 (M^+, 8.76 %), 58 (100 % base peak). Analysis calculated for $C_5H_4N_4OS_3$: C, 25.85; H, 1.74; N, 24.12, found: C, 25.65; H, 1.56; N, 24.45.

*1-Phenyl-2-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-ylamino) ethanone (**4**)* A mixture of **1** (0.5 g, 0.003 mol) and phenacyl bromide (0.7 g, 0.003 mol) was refluxed in ethanol for 3 h. the reaction mixture was cooled, and the precipitated solid was recrystallised from dioxane to give **4**. Yield, 95 %, mp 204–205 °C. IR (KBr, cm^{-1}): 3,269, 3,124 (2NH), 3,011 (CH arom.), 2,932, 2,848 (CH aliph.), 1,690 (C=O), 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 4. 87 [s, 2H, CH_2], 4.77, 8.75 [2 s, 2H, 2NH, exchangeable with D_2O], 7.42-7.99 [m, 5H, Ar–H]. ^{13}C-NMR (DMSO-d_6, ppm): 40.05 (CH_2), 129.3, 129.7, 130.02, 135.6, 146.3 (C-thiadiazole), 176.25 (C=O), 183.1 (C=S). MS: *m/z* 251 (M^+, 1.76 %), 79 (100 % base peak). Analysis calculated for $C_{10}H_9N_3OS_2$: C, 47.79; H, 3.61; N, 16.72, found: C, 47.56; H, 3.81; N, 16.52.

*2-Amino-4-phenyl-1-(5-thioxo-4, 5-dihydro[1, 3, 4]thiadiazol-2-yl)-1H-pyrrole-3-carbonitrile (**5**)* A mixture of **4** (0.5 g, 0.001 mol) and malononitrile (0.13 g, 0.001 mol) was refluxed in ethanol containing sodium ethoxide (0. 04 g, 0.001 mol) for 3 h. The reaction mixture was cooled, poured onto ice water, acidified with dil. HCl and the solid obtained was recrystallised from dioxane to give **5**. Yield, 80 %, mp 110–112 °C. IR (KBr, cm^{-1}): 3,245, 3,187 (NH_2), 3,072 (CH arom.), 2,222 (CN), 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 3.52 [s, 2H, NH_2, exchangeable with D_2O], 4.73 [s, 1H, CH-pyrrole], 7.30 [s, 1H, NH, exchangeable with D_2O], 7.46–7.93 [m, 5H, Ar–H]. ^{13}C-NMR (DMSO-d_6, ppm): 108, 111 (C-pyrrole), 118 (CN), 120.2, 127, 128.4, 128.7, 133.6, 133.72, 169.77, 193.26, MS: *m/z* 299 (M^+, 0.19 %), 54 (100 % base peak). Analysis calculated for $C_{13}H_9N_5S_2$: C, 52.16; H, 3.03; N, 23.39, found: C, 52.45; H, 3.23; N, 23.67.

*5-[(2-Chloro-benzylidene)-amino]-3H-[1,3,4]thiadiazole-2-thione (**6**)* A mixture of **1** (0.5 g, 0.003 mol) and 2-chlorobenzaldehyde (0.47 g, 0.003 mol) was refluxed in ethanol containing 1 ml glacial acetic acid for 5 h. the reaction mixture was cooled, poured onto ice water and the solid obtained was recrystallised from dioxane to give **6**. Yield, 75 %, mp 200–202 °C. IR (KBr, cm^{-1}): 3,156 (NH), 3,022 (CH arom.), 2,956, 2,854 (CH aliph.), 1,230 (C=S), 834 (C–Cl). ^{1}H-NMR (DMSO-d_6, ppm): 7.32 [s, 1H, CH], 7.34–7.78 [m, 4H, Ar–H], 8.77 [s, 1H, NH, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6, ppm): 126. 9, 129.2, 131.3, 134.7, 135.4, 137.9, 165.7 (CH), 155.2, 181. 7. MS: *m/z* 255 (M^+, 23.4 %), 76 (100 % base peak). Analysis calculated for $C_9H_6ClN_3S_2$: C, 42.27; H, 2.36; N, 16.43, found: C, 42.47; H, 2.12; N, 16.71.

*4-[3-(5-Thioxo-4,5-dihydro-[1,3,4]thiadiazol-2-yl)-thioureido]-benzenesulfonamide (**7**)* A mixture of **1** (0.5 g, 0.003 mol) and 4-isothiocyanatobenzenesulfonamide (0. 72 g, 0.003 mol) was refluxed in DMF containing 3 drops of TEA for 5 h. the reaction mixture was cooled, poured onto ice water and the solid obtained was recrystallised from ethanol to give **7**. Yield, 73 %, mp 138–140 °C. IR (KBr, cm^{-1}): 3,367, 3,266, 3,176 (NH, NH_2), 3,022 (CH arom.), 1,230 (C=S), 1,387, 1,145 (SO_2). ^{1}H-NMR (DMSO-d_6, ppm): 5.50 [s, 2H, NH_2, exchangeable with D_2O], 6.50, 6.70, 8.25 [3 s, 3H, 3NH, exchangeable with D_2O], 7.66, 7.81 [2d, 4H, AB system, $J = 4.5$]. ^{13}C-NMR (DMSO-d_6, ppm): 122.2, 125.6, 133.6, 139.4, 152.1, 181.4 (C=S), 200.01. MS: *m/z* 347 (M^+, 5.1 %), 63 (100 % base peak). Analysis calculated for $C_9H_9N_5O_2S_4$: C, 31.11; H, 2. 61; N, 20.16, found: C, 31.34; H, 2.48; N, 20.37.

*1-Phenyl-3-(5-thioxo-4,5-dihydro-[1,3,4]thiadiazol-2-yl)-urea (**8**)* A mixture of **1** (0.5 g, 0.003 mol) and phenylisocyanate (0.36 g, 0.003 mol) was refluxed in pyridine for 8 h. The reaction mixture was cooled, poured onto ice water and the solid obtained was recrystallised from acetic acid to give **8**. Yield, 92 %, mp 241–243 °C. IR (KBr, cm^{-1}): 3,252, 3,198 (3NH), 3,031 (CH arom.), 1,685 (C=O), 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 7.28–7.48 [m, 5H, Ar–H], 8.70 [s, 1H, NH, exchangeable with D_2O], 8.96 [s, 2H, 2NH, exchangeable with D_2O], ^{13}C-NMR (DMSO-d_6, ppm): 118.16, 121.78, 128.75, 139.69 (C-aromatic), 152.52 (C-thiadiazole), 169.69 (C=O), 181 (C=S). MS: *m/z* 252 (M^+, 23.4 %), 253 (M^++1, 8.1 %), (65, 100 % base peak). Analysis calculated for $C_9H_8N_4OS_2$: C, 42.84; H, 3.20; N, 22.21, found: C, 42.56; H, 3.48; N, 22.61.

*2-Di-(N-phenylformamide)amine-5-(N-phenylsulfinic amide) [1,3,4-thiadiazole] (**9**)* A mixture of **1** (0.5 g, 0.003 mol) and phenylisocyanate (1 g, excess) was refluxed in pyridine for 8 h. The reaction mixture was cooled, poured onto ice water and the solid obtained was recrystallised from acetic acid to give **9**. Yield, 90 %, mp 229–230 °C. IR (KBr, cm^{-1}): 3,245, 3,278 (3NH), 3,028 (CH arom.), 1,685, 1,674 (C=O). ^{1}H-NMR (DMSO-d_6, ppm): 6.97–7.48 [m, 15H, Ar–H], 8.67 [s, 1H, NH, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6, ppm): 181.29, 121.72, 128.69, 139. 64 (C-aromatic), 149.6 (2 N–C=O), 152.48 (S–C=O), 165. 43 (C=S), 170.0 (C-thiadiazole). MS: *m/z* 490 (M^+, 4 0. 14 %), 491 (M^++1, 2.1 %), (65, 100 % base peak). Analysis calculated for $C_{23}H_{18}N_6O_3S_2$: C, 56.31; H, 3.70; N, 17.13, found: C, 56.55; H, 3.51; N, 17.49.

*N-Formyl-N-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl) formamide (**10**)* A solution of **1** (0.5 g, 0.003 mol) in formic acid was refluxed for 10 h. The reaction mixture was cooled where needle shaped crystals were filtered, dried and recrystallized from dioxane to give **10**. Yield, 87 %, mp 188–190 °C. IR (KBr, cm^{-1}): 3,331 (NH), 2,879 (CH aliph.), 1,658 (2C=O) and 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 2.50, [s, 1H, NH, exchangeable with D_2O], 8.42 [s, 2H, 2CHO], ^{13}C-NMR (DMSO-d_6, ppm): 150.63 (C-thiadiazole), 160.35 (2CHO), 183.26 (C=S). MS: *m/z* 189 (M^+, 5.43 %), 190 ($M^+ + 1$, 3.0 %), (161, 100 % base peak). Analysis calculated for $C_4H_3N_3O_2S_2$: C, 22.35; H, 1.88; N, 26.07, found: C, 22.61; H, 1.67; N, 26.46.

*N-Acetyl-N-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide (**11**)* A solution of **1** (0.5 g, 0.003 mol) in acetic anhydride was refluxed for 10 h. Where a solid formed on hot which was filtered, dried and recrystallized from dioxane to give **11**. Yield, 89 %, mp 175–177 °C. IR (KBr, cm^{-1}): 3,325 (NH-stretching), 2,897 (CH aliph.), 1,660 (2C=O) and 1,230 (C=S). ^{1}H-NMR (DMSO-d_6, ppm): 2.02 [s, 6H, 2CH3], 12.29 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6, ppm): 144.12 (C-thiadiazole), 168.65 (C=O), 183.38 (C=S). Analysis calculated for $C_6H_7N_3O_2S_2$: C, 33.17; H, 3.25; N, 19.34;, found: C, 33.21; H, 3.59; N, 19.62.

Antimicrobial screening

Five bacterial isolates representing Gram −ve and Gram +ve bacteria namely; *P. aeruginosa, Klebsiella pneumonia (K. pneumonia), S. aureus, Staphylococcus epidermidis (S. epidermidis), and B. cereus*, were recovered on Nutrient and MacConkey agar. One yeast fungus *Candida albicans (C. albicans)* was isolated on Sabouraud agar (oxoid). They were obtained from the Drug Microbiology Laboratory—Drug Radiation Research department—National center for radiation research and Technology (NCRRT).

The antimicrobial susceptibility testing was performed in vitro using the agar diffusion disk method (Barry, 1981). The tested compounds (10 mg) were dissolved in dimethylformamide (DMF, 1 ml) which showed no inhibition zone and used as negative control. Antimicrobial potentialities of the tested compounds were estimated by placing presterilized filter paper disks (6 mm in diameter) impregenated with the tested compound and placed on the solidified medium. Inhibition zones were measured after 24–48 h of incubation at 37 °C. Reference antibiotic disks (6 mm in diameter), Amikacin (AN 30 μg $disk^{-1}$), Sulprazon (Scf 105 μg $disk^{-1}$), and fungicide Nystatine (NS 30 μg $disk^{-1}$), were used as standards.

Effect of γ-irradiation on the susceptibility of Gram +ve bacterial strains to the synthesized compounds

This study was conducted to evaluate the effect of γ-irradiation on the susceptibility of the tested Gram +ve bacterial strains to the synthesized compounds. Irradiation process was carried out by using cesium 137 gamma cell, 40, Atomic energy of Canada Limited, Commercial product located at NCRRT and the dose rate was 0.775 rad/s. at the time of experiment. The Gram +ve bacterial strains were subjected to a dose level 24.4 Gray (Gy) which is biologically equivalent to in vitro fractionated multiple therapeutic dose of some cancer patients (Barton, 1995) in order to study the effect of irradiation to their susceptibility to the biologically active compounds.

The MIC determination for compound **5**

The MIC of the most promising compound **5** was determined by agar streak dilution method (Yousif *et al.*, 2011). A stock solution of the compound in DMF was prepared and graded quantities were incorporated in a specified quantity of molten sterile nutrient agar medium, then poured into a petri dish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 10^{5} CFU ml^{-1}, applied to the plates and incubated at 37 °C for 24 h. The MIC was considered to be the lowest concentration of the test compound exhibiting no visible growth of bacteria. MIC values were 125, 250, and 250 μl ml^{-1} for *S. aureus* (non-irradiated and irradiated) and *B. cereus* (non-irradiated), respectively, which are the most sensitive bacterial strains.

Scanning electron microscope (SEM) study

The effect of compound **5**, the most promising biologically active compound, on the morphology of the tested bacterial strains was studied using SEM (JSM-5400 JEOL) located at NCRRT. Tested bacterial strains were subcultured in nutrient broth (Difco). They were incubated for 24 h at 37 °C under static stirring. Compound **5** at its MIC was added directly in the medium after 3 h incubation. In case of *S. aureus*, the effects of irradiation at 24.4 Gy and synergistic effect of irradiation with MIC of compound 5 were also investigated. At the end of incubation, the cultures were prefixed with 2 % glutaraldehyde for 2 h at 4 °C. Post-fixation was carried out using a 2 % osmium tetroxide solution for 30 min at 30 °C. After each fixation the cells were washed twice with 0.1 M sodium cacodylate and the bacterial cells were prepared for electron microscopy according to the recommended method Philipp (1981).

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