HETEROCYCLES, Vol. 94, No. 2, 2017, pp. 314 - 325. © 2017 The Japan Institute of Heterocyclic Chemistry
Received, 7th November, 2016, Accepted, 12th January, 2017, Published online, 25th January, 2017
DOI: 10.3987/COM-16-13611

A MULTI-COMPONENT ONE-POT SYNTHESIS OF NOVEL (1,3,4-THIADIAZIN-2-YLAMINO)ISOINDOLINE-1,3-DIONES AS ANTIMICROBIAL AGENTS

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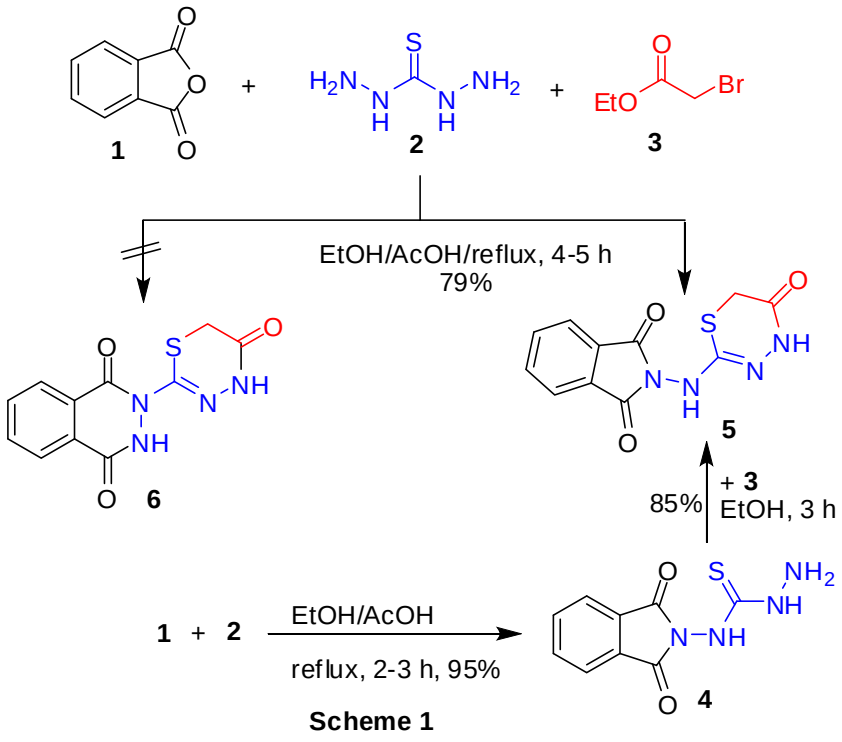
Abstract – A series of new (1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione derivatives **5**, **8**, **9**, **11a-d**, and **13a,b** were prepared *via* one-pot three component reaction of isobenzofuran-1,3-dione **1**, thiocarbohydrazide **2** and various *α*-halocarbonyl compounds. The chemical structure of the new compounds was elucidated by spectral analyses and X-ray single crystal technique. The newly synthesized compounds were also evaluated for their in vitro antimicrobial activity, and showed promising results.

Isoindoline-1,3-dione derivatives have received more attention because not only they are a core unit of wide range of natural products but also they have shown a broad spectrum of pharmacological properties such as antimicrobial,[1–7] hypolipidaemic,[8] antitubercular,[9] anticonvulsant,[10] anti-inflammatory,[11] antipsychotic,[12] anti-T. cruzi,[13] and analgesic activities.[14] Also, many 1,3,4-thiadiazines have biological importance such as matrix metalloproteinase inhibitors; phosphodiesterase IV inhibitors; cardiotonic and hypertensive activities.[15-17] These derivatives also used in agriculture as pesticides and insecticides.[18] On the basis of the above facts and in continuation of our research on the synthesis of novel heterocyclic systems,[19-22] we have synthesized new 2-(1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione derivatives *via* a one-pot synthetic approach for antimicrobial evaluation.

A multicomponent reaction of an equimolar mixture of isobenzofuran-1,3-dione **1**, thiocarbohydrazide **2**, and ethyl 2-bromoacetate **3** in anhydrous ethanol in the presence catalytic amount of acetic acid at reflux temperature gave white precipitate after 2 h. This white solid was isolated and identified by spectral

analyses and X-ray single crystal technique. The X-ray showed the structure of white solid is *N*-(1,3-dioxoisoindolin-2-yl)hydrazinecarbothioamide **4** (Figure 1). Continue the heating of the reaction mixture for additional 3 h afforded 2-(5-oxo-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione **5** as a single product in a good yield. While 2-(5-oxo-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-yl)-2,3-dihydrophthalazine-1,4-dione **6** was not formed[23,24] (Scheme 1).

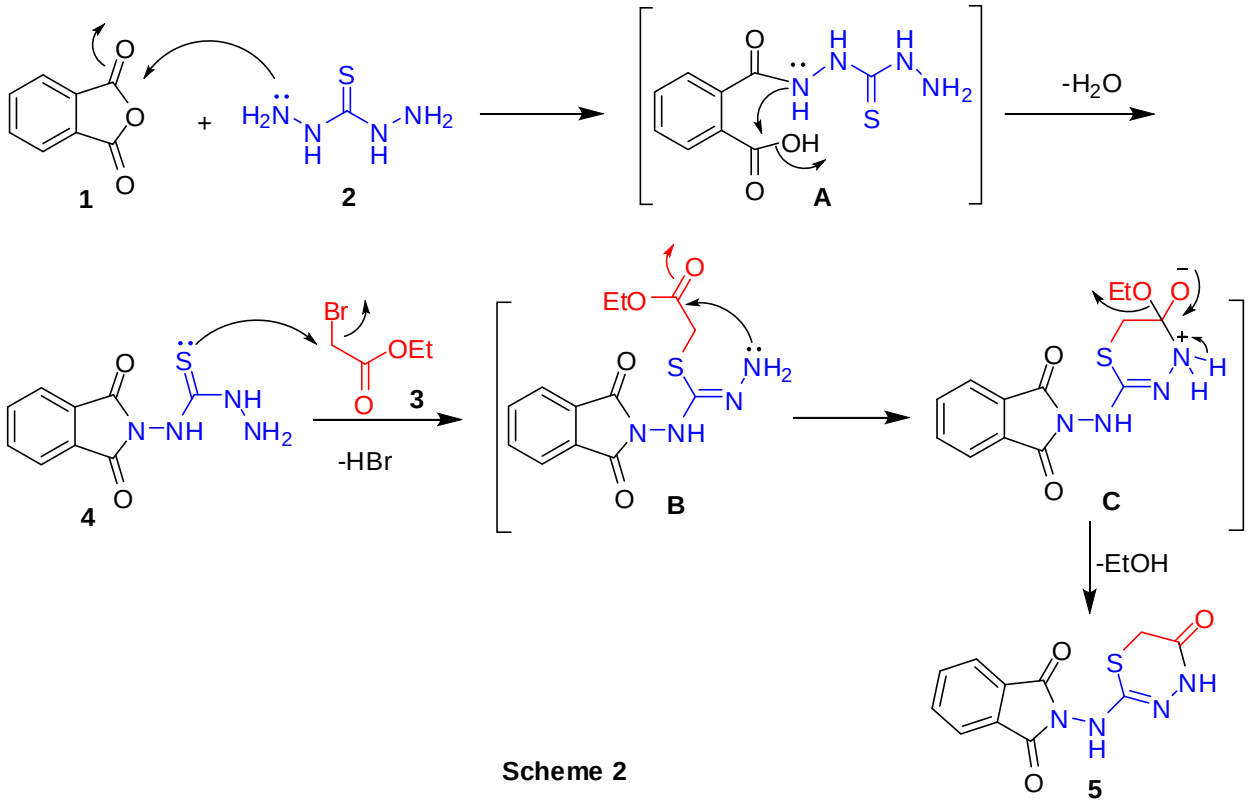


Scheme 1

The structure of compound **5** was elucidated using spectral data, where, IR spectra revealed, two peaks at ν 3331 3163, 1726, and 1647 cm^{-1} corresponding to two N-$H_{str.}$, C=$O_{str.}$ of phthalimide, and C=$O_{str.}$ of thiadiazine functions, respectively. ^{1}H NMR spectrum of **5** showed two –NH- protons at δ 2.60 and 11.64 ppm both of these singlet signals disappeared from the spectrum on addition D_2O to the solution, in addition the thiadiazine methylene protons signal was observed at 3.99 ppm as a sharp singlet integrating for two protons. ^{13}C NMR spectrum of **5** revealed signals at δ 32.6 and 173.3 ppm due to CH_2 and C=O, respectively of thiadiazine moeity, and disappeared of C=S from the spectrum at δ 182.5 ppm. On the other hand, the structure of compound **5** was further confirmed through alternative synthesis upon reaction of compound **4** with **3** in absolute ethanol under reflux condition as described in Scheme 1. Also, earlier publications have reported that 1-(1,3-dioxoisoindolin-2-yl)thiourea was synthesized from reaction of phthalic anhydride with thiosemicarbazide.[13]

The proposed mechanism for the formation of compound **5** is depicted in Scheme 2. It is believed that phthalic anhydride **1** first reacted with thiocarbohydrazide **2** to give the intermediate *N*-(1,3-dioxoisoindolin-2-yl)hydrazinecarbothioamide **4**, isolated and its structure proved with X-ray

analysis (Figure 1), which further undergoes S_N2 reaction followed by cyclization with ethyl 2-bromoacetate **3** to give the desired compound **5**.



Scheme 2

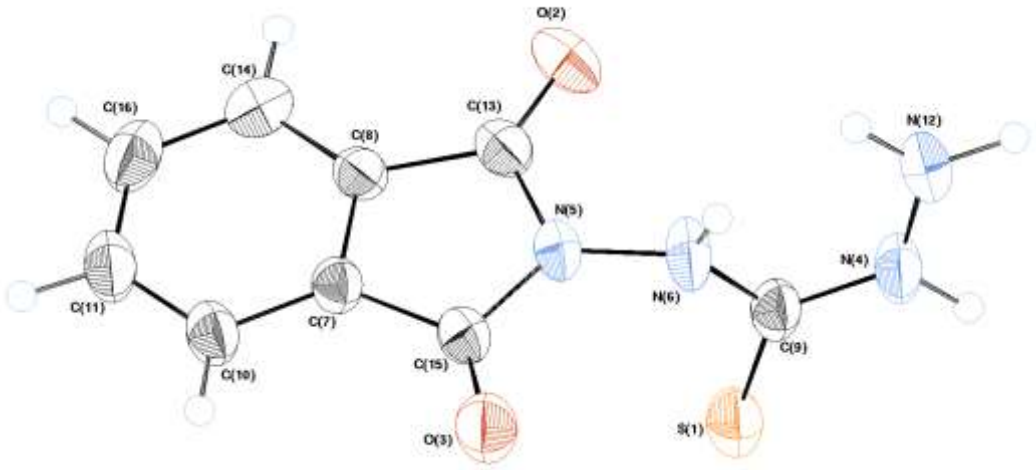
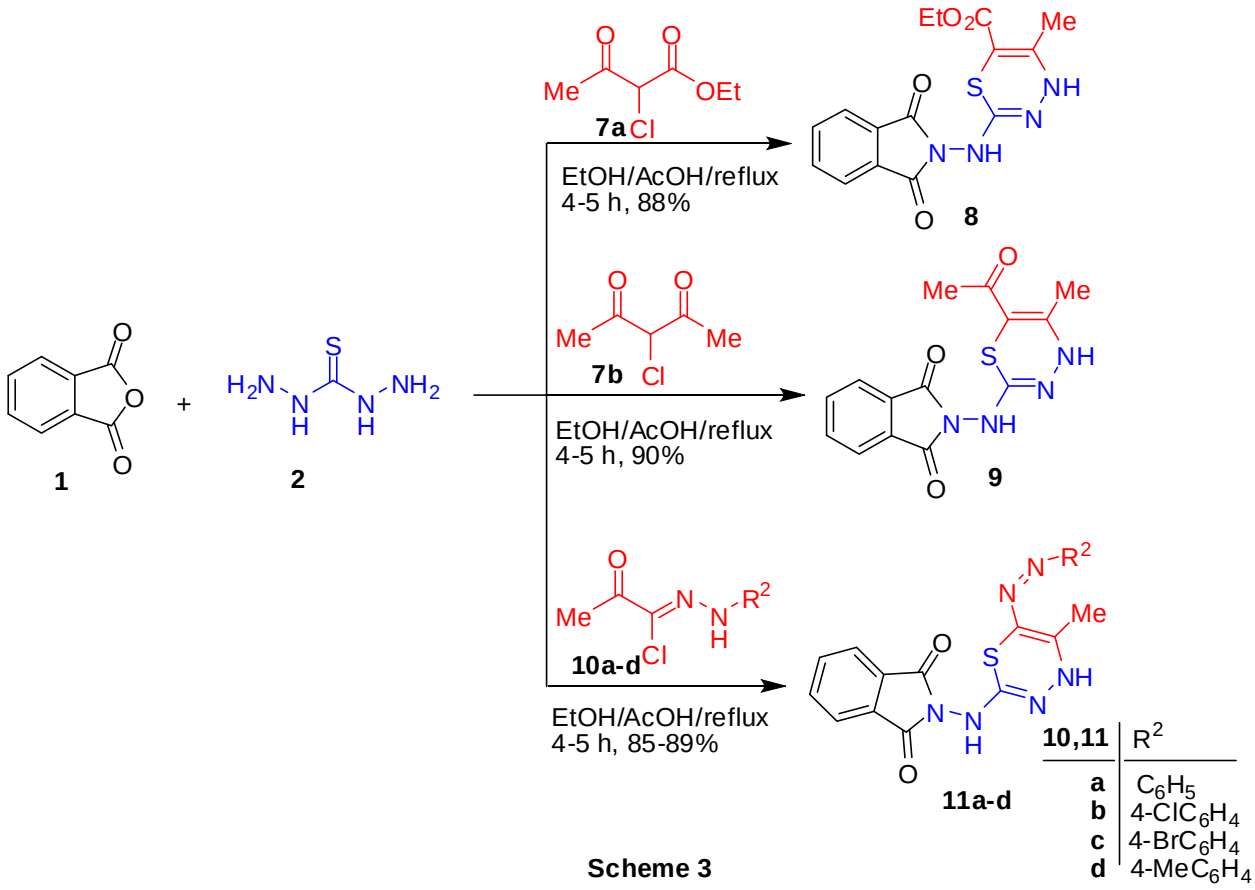


Figure 1. X-Ray structure of 4

The protocol was extended for synthesis anther 1,3,4-thiadiazine derivatives. The reaction of an equimolar amount of compounds **1**, **2**, and *α*-chloroketones namely, 3-chloropentane-2,4-dione **7a**, ethyl 2-chloro-3-oxobutanoate **7b**, and 2-oxo-*N*'-arylpropanehydrazonoyl chloride **10a-d** under typical reaction condition to afford 1,3,4-thiadiazines **8**, **9**, and **11a-d**, respectively, in good yields (Scheme 3).

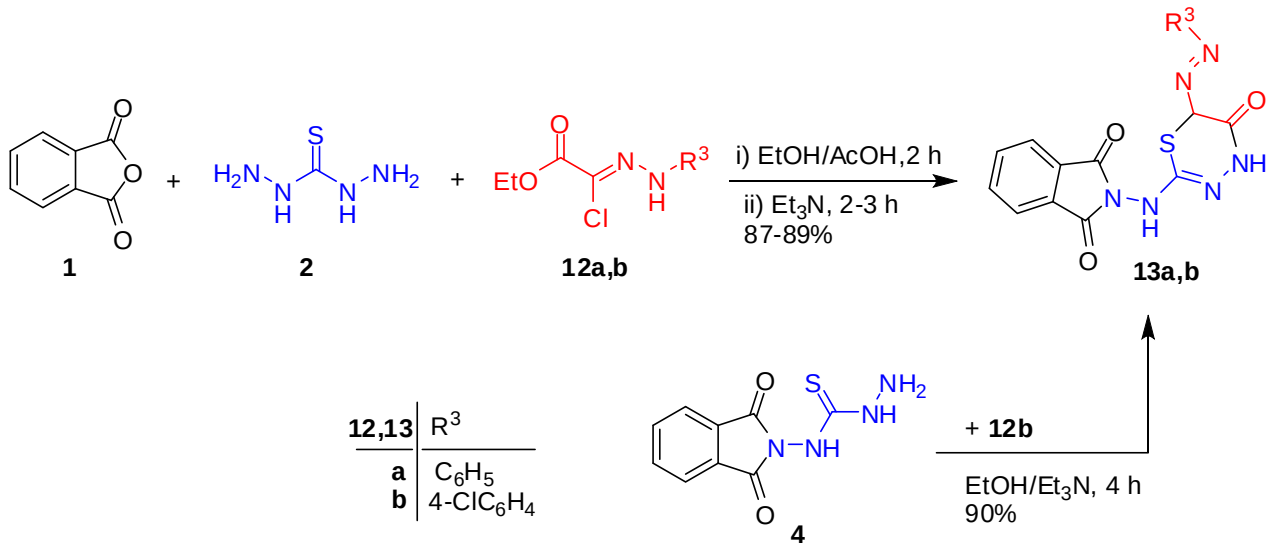


Scheme 3

The structure of the compounds **8**, **9**, and **11** was confirmed from their analytical and spectral data. For example, IR spectrum of compound **9** showed strong two absorption peaks at ν 3362 and 3198 cm^{-1} due to two NH functions, furthermore carbonyl of amidic and acetyl groups appears at 1742 and 1700 cm^{-1}, respectively. The ^{1}H NMR spectra of **9** revealed two singlets signals at δ 2.19 and 2.27 corresponding to methyl and acetyl protons, respectively, in addition showed two D_2O exchangeable signals at δ 2.19 and 8.56 ppm due to exocyclic and cyclic NH functions. Moreover, ^{13}C NMR spectrum of compound **9** showed two signals at δ 166.4 and 193.2 ppm due to C=O of phthalimide and C=O of acetyl group, respectively. Also, the mass spectra of compound **9** gave molecular ion peaks at *m/z* 316.

In contrast, one pot three-component reaction of an equimolar amount of compounds **1**, **2**, and ethyl 2-chloro-2-(2-arylhydrazono)acetate **12a,b** gave only compound **4** after boiling 2 h, the reaction stopped at this stage and does not further undergo cyclization with compound **12** because poor reactivity of carboethoxy group of hydrazonyl halides **12**. To overcome this problem we added few drops of triethylamine and boiling the mixture for another 2-3 h which furnished 2-(5-oxo-6-(aryldiazenyl)-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione **13a,b**, in good yields, respectively. On the other hand, compound **13b** was synthesized from reaction of compound **4** with **12b** using absolute ethanol as a solvent containing few drops of Et_3N (Scheme 4).

The structure of compound **13a,b** was characterized by IR and $^{1}H/^{13}C$ NMR spectroscopy, as well as by mass spectrometry techniques. The ^{1}H NMR of **13a** showed, besides an increasing in the intensity of the aromatic signals, a singlet at δ 3.43 ppm due to methine proton at C-6, and two D_2O exchangeable signals at δ 2.00 and 9.26 ppm due to exocyclic and cyclic NH functions. The mass spectra of compounds **13a** and **13b** also gave the anticipated molecular ion peaks.



Scheme 4

PHARMACOLOGICAL EVALUATION

ANTIBACTERIAL ACTIVITY

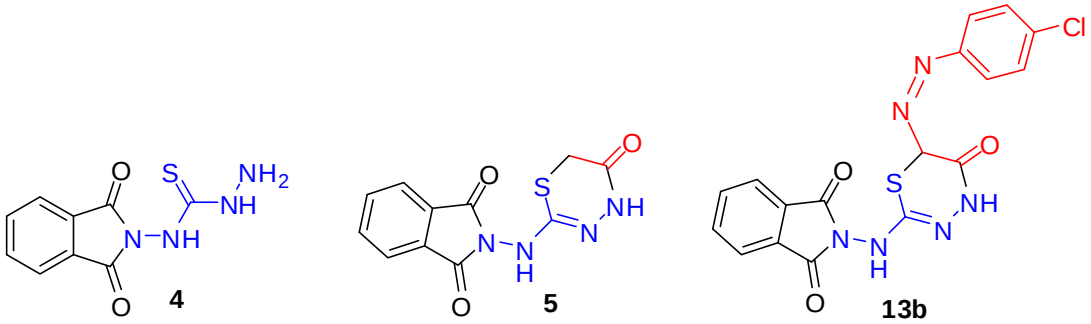
The antibacterial activity of the tested compounds was studied against *Staphylococcus aureus* (ATCC 29213) (SA), *Bacillus subtilis* (ATCC6633) (BS), and *B. megaterium* (ATCC 9885) (BM) as examples of Gram positive bacteria and *Klebseilla Peneumoniae* (ATCC13883) (KP), *Pseudomonas Aeuroginosa* (ATCC27953) (PA), *and Escherichia coli* (ATCC25922) (EC) as Gram negative bacteria. Vancomycine was used as control standard antibiotic agent. The results (Tables 1, 2) revealed that all the synthesized compounds showed excellent activity against all the tested microbes except compounds **8**, and **11a** showed weak activity and compound **11d** showed no activity. Compounds **4** and **13b** exhibited the highest activity against all tested microorganisms with inhibition zones range from 25 to 30 mm and minimum inhibitory concentrations (MIC) range between 65 to 125 µg /mL.

ANTIFUNGAL ACTIVITY

The compounds were tested for their antifungal activity against *Saccharomyces cervesia* (SC) and *Candida albicans* (NRRLY-477) (CA) using Ketoconazole as standard antifungal agent. The results displayed all compounds exhibit a good to an excellent antifungal activity. Compounds **5** and **13b** showed an excellent activity with inhibition zones range from 22 to 28 mm and MIC range between 62.5 to 125 µg /mL.

STRUCTURE–ACTIVITY RELATIONSHIP (SAR)

The structure–antimicrobial activity relationship of the synthesized compounds against the pathological strains of bacteria and fungi revealed that compound **4**, **5**, and **13b** have superior antimicrobial activities than other compounds. These can be correlated to the presence of, thiosemicarbazide group in compound **4**, and thiadiazin-5-one moiety in compounds **5**, **13b**. In addition the connection of 4-chlorophenyldiazene with thiadiazin-5-one moiety in compound **13b** increasing the potency.



In conclusion, we have applied a one-pot three components reaction for the synthesis of new 1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione derivatives using readily available materials. These derivatives may be of interest for pharmaceutical purposes yet to be explored.

Table 1. Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay

Cpds.	Gram positive bacteria			Gram negative bacteria			Yeast	
	(SA)	***(BS)***	***(BM)***	***(KP)***	***(PA)***	***(EC)***	***(SC)***	***(CA)***
4	25	25	27	28	27	29	21	22
5	20	22	27	23	22	22	26	22
8	16	16	16	17	18	18	18	16
9	20	18	21	22	22	24	20	16
11a	15	16	16	15	12	13	22	17
11c	20	22	25	24	23	24	23	19
11d	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	16	15
13a	23	24	24	26	25	28	25	22
13b	23	26	28	30	29	29	28	26
Vancomycin	28	30	24	25	24	22	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	23	22

The experiment was carried out in triplicate and the average zone of inhibition was calculated; N.A. (No activity).

Table 2. Minimum inhibitory concentration (μg/mL) against the pathological strains based on two fold serial dilution technique

Cpds.	Gram positive bacteria			Gram negative bacteria			Yeast	
	(SA)	***(BS)***	***(BM)***	***(SA)***	***(BS)***	***(BM)***	***(SA)***	***(BS)***
4	125	125	65	65	125	65	250	250
5	65	65	65	125	125	125	65	125
8	500	500	500	500	500	500	500	500
9	250	500	250	250	250	250	500	500
11a	-	500	500	-	-	-	500	500
11c	250	250	250	125	125	125	250	500
11d	-	-	-	-	-	-	500	-
13a	250	250	250	125	125	125	125	250
13b	125	65	65	65	65	65	65	125
Vancomycine	65	65	65	65	65	65	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	65	65

N.A. (No activity)

EXPERIMENTAL

All melting points were determined on digital Gallen-Kamp MFB-595 instrument using open capillary tubes and are uncorrected. IR spectra were recorded on Schimadzu FTIR 440 spectrometer using KBr pellets. Mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer provided with a data system. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model (500 MHz) Ultra Shield NMR spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ ppm units. The elemental analyses (% C, H, N) were done at the Microanalytical Center, Cairo University, Cairo, Egypt. Solvents were dried by standard techniques. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out and was run using thin layer chromatography (TLC) aluminum sheets silica gel 60 F_{254} (Merck).

General procedure for synthesis of compounds 4, 8, 9, and 11a-d. An equimolar mixture of phthalic anhydride **1** (5 mmol, 0.74 g), thiocarbohydrazide **2** (5 mmol, 0.53 g), and *α*-halocarbony compounds {ethyl bromoacetate **3** (5 mmol, 0.825 g), ethyl 2-chloro-3-oxobutanoate **7a** (5 mmol, 0.82 g), 3-chloropentane-2,4-dione **7b** (5 mmol, 0.67 g) and 2-oxo-*N*'-phenylpropanehydrazonoyl chloride **10a-d** (5 mmol)} was taken in anhydrous ethanol containing a catalytic amount of acetic acid (0.1 mL). The reaction mixture was heated at reflux for about 4–5 h and cooled to room temperature. The solid obtained was filtered, washed with EtOH, and recrystallized from EtOH to give 1,3,4-thiadiazine derivatives **5**, **8**, **9**, and **11a-d**, respectively.

Synthesis of *N*-(1,3-dioxoisoindolin-2-yl)hydrazinecarbothioamide (4). An equimolar mixture of phthalic anhydride **1** (5 mmol, 0.74 g), thiocarbohydrazide **2** (5 mmol, 0.53 g) was taken in absolute EtOH

containing a catalytic amount of acetic acid (0.1 mL). The reaction mixture was heated at reflux for about 2–3 h and cooled to room temperature. The solid obtained was filtered, washed with EtOH, and crystallized from EtOH to give **4**; a colorless powder; yield 95%; mp 220-221 ºC; IR (cm^{-1}): ν 3337, 3308 (NH_2), 3238, 3177 (2NH), 1788, 1738 (C=O); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 1.91 (s, 2H, NH, D_2O exchangeable), 7.92 (dd, 2H, *J* = 8.5, *J* = 3.4 Hz, Phthalimide-H), 7.95 (dd, 2H, *J* = 8.5, *J* = 3.4 Hz, Phthalimide-H), 9.63 (s, br, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δc (ppm): 123.6, 130, 135.13, 165.5, 182.5; E1-MS: (*m/z*, %): 236 (M^+, 6), 162 (100); Anal. Calcd for $C_9H_8N_4O_2S$ (236.037): C, 45.75; H, 3.41; N, 23.72. Found: C, 45.21; H, 3.19; N, 23.38%.

Method B: Synthesis of compound 5. A mixture of *N*-(1,3-dioxoisoindolin-2-yl)hydrazinecarbothioamide **4** (0.472 g, 2 mmol) and ethyl bromoacetate (0.33 g, 2 mmol) in absolute EtOH (20 mL) was refluxed for 3 h (TLC) and cooled to room temperature. The resulting precipitate was filtered, washed with EtOH, and crystallized from EtOH to give compound **5**.

2-(5-Oxo-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (5): a yellow powder, yield 79% (Method A), 85% (Method B); mp >300 ºC; IR (cm^{-1}): ν 3331, 3163 (2NH), 1726, 1647(C=O), 1600 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.60 (s, 1H, NH, D_2O exchangeable), 3.99 (s, 2H, CH_2), 7.97 (d, 2H, *J* = 6.5 Hz, Phthalimide-H), 8.10 (d, 2H, *J* = 6.5 Hz, Phthalimide-H), 11.64 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δc (ppm): 32.6, 125.7, 127.7, 133.2, 153.4, 166, 173.3; E1-MS: (*m/z*, %): 236 (M^+-CH_2CO (41), 8), 162 (100); Anal. Calcd for $C_{11}H_8N_4O_3S$ (276.032): C, 47.82; H, 2.92; N, 20.28. Found: C, 47.37; H, 2.64; N, 20.05%.

Ethyl 2-(1,3-dioxoisoindolin-2-ylamino)-5-methyl-4*H*-1,3,4-thiadiazine-6-carboxylate (8): an orange powder, yield 88%; mp 277–279 ºC; yellow; IR (cm^{-1}): ν 3361, (NH), 1736, 1686 (C=O), 1533 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 1.31 (t, 3H, CH_3), 2.15 (s, br, 1H, NH, D_2O exchangeable), 2.35 (s, 3H, CH_3), 4.25 (q, 2H, CH_2), 7.64 (d, 2H, *J* = 8.5 Hz, Phthalimide-H), 7.93 (d, 2H, *J* = 8.5 Hz, Phthalimide-H), 8.22 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 12.22, 14.87, 40.46, 59.77, 95.77, 123.86, 130.16, 135.48, 154.58, 161, 164.08, 166.51; E1-MS: (*m/z*, %): 260 (M^+-$CO_2C_3H_8$, 8), 255 (100), 162 (10); Anal. Calcd for $C_{15}H_{14}N_4O_4S$ (346.074): C, 52.02; H, 4.07; N, 16.18. Found: C, 51.89; H, 3.97; N, 16.02%.

2-(6-Acetyl-5-methyl-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (9): a yellow powder; yield 90%; mp >300 ºC; IR (cm^{-1}): ν 3362, 3198 (2NH), 1742, 1700 (2C=O), 1600, 1541 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.19 (s, br, 1H, NH, D_2O exchangeable), 2.23 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 7.93 (m, 4H, Phthalimide-H), 8.56 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 16.6, 19.60, 29.7, 123.8, 130.3, 135.4, 166.4, 193.2; E1-MS: (*m/z*, %): 316 (M^+, 5), 284 (100), 269 (40), 236 (13), 162 (17); Anal. Calcd for $C_{14}H_{12}N_4O_3S$ (316.063): C, 53.16; H, 3.82; N, 17.71. Found: C, 53.01; H, 3.69; N, 17.58%.

2-(5-Methyl-6-(phenyldiazenyl)-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (11a): an orange powder; yield 85%; mp 242–243 ºC; yellow; IR (cm^{-1}): ν 3390, 3292 (NH), 1732 (C=O), 1610, 1556 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.1 (s, 1H, NH, D_2O exchangeable), 2.35 (s, 3H, CH_3), 6.91-7.33 (m, 5H, Ar-H), 7.71-7.96 (m, 4H, Phthalimide-H), 11.45 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 12.9, 89, 123, 121.1, 123.8, 128.6 135.5, 146.9, 155.3, 165; E1-MS: (*m/z*, %): 378 (M^+, 5), 248 (100), 162 (20); Anal. Calcd for $C_{18}H_{14}N_6O_2S$ (378.090): C, 57.13; H, 3.73; N, 22.21, Found: C, 56.93; H, 3.64; N, 22.01%.

2-(6-((4-Chlorophenyl)diazenyl)-5-methyl-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (11b): a pale yellow; yield 87%; mp 260–262 ºC; IR (cm^{-1}): ν 3273, 3226 (2NH), 1735 (C=O), 1595, 1556 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.39 (s, 3H, CH_3), 7.30 (d, 2H, *J* = 7 Hz, Ar-H), 7.36 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.82 (d, 2H, *J* = 8, Phthalimide-H), 7.97 (d, 2H, *J* = 8.5, Phthalimide-H), 8.01 (s, br, 1H, NH, D_2O exchangeable), 10.40 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 14.8, 90.7, 123, 127.1, 130.4, 132.6 136.5, 146, 155.6, 165.5; E1-MS: (*m/z*, %): 412 (M^+, 9), 414 (M^+ + 2, 3), 162 (100); Anal. Calcd for $C_{18}H_{13}ClN_6O_2S$ (412.051): C, 52.37; H, 3.17; N, 20.36. Found: C, 52.05; H, 2.97; N, 20.21%.

2-(6-((4-Bromophenyl)diazenyl)-5-methyl-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (11c): a pale yellow; yield 89%; mp 259–260 ºC; IR (cm^{-1}): ν 3282, 3234 (NH), 1700 (C=O), 1595, 1558 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.25 (s, 3H, CH_3), 7.23 (d, 2H, *J* = 9 Hz, Ar-H), 7.29 (d, 2H, *J* = 9 Hz, Ar-H), 7.60 (d, 2H, *J* = 7.5 Hz, Phthalimide-H), 7.85 (d, 2H, *J* = 7.5 Hz, Phthalimide-H), 9.95 (s, br, 1H, NH, D_2O exchangeable), 10.39 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 13.9, 89.7, 122.8, 127.1, 131.5, 133.2 136.5, 146, 154.5, 164.5; E1-MS: (*m/z*, %):456 (M^+, 5), 458 (M^+ + 2, 4), 442(M^+- Me, 5), 290 (40), 171 (100), 162 (75); Anal. Calcd for $C_{18}H_{13}BrN_6O_2S$ (456.000): C, 47.28; H, 2.87; N, 18.38. Found: C, 47.05; H, 2.49; N, 18.09%.

2-(5-Methyl-6-(*p*-tolyldiazenyl)-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (11d): a yellow powder, yield 88%; mp 239–240 ºC; IR (cm-1): ν 3273, 3240 (NH), 1789, 1735 (C=O), 1612, 1558 (C=N); ^{1}H NMR (DMSO-*d*$_6$) δ_H (ppm): 2.24 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.09 (d, 2H, *J* = 8 Hz, Ar-H), 7.23 (d, 2H, *J* = 8 Hz, Ar-H), 7.97 (d, 2H, *J* = 7.5 Hz, Phthalimide-H), 8.01 (d, 2H, *J* = 7.5 Hz, Phthalimide-H), 10 (s, br, 1H, NH, D_2O exchangeable), 10.67 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*$_6$) δ_c (ppm): 13.9, 21.6, 90.2, 122.8, 127.1, 131.5, 133.2 136.5, 146, 155.1, 165.3; E1-MS: (*m/z*, %): 392 (M^+, 3), 337 (5), 298 (25), 262 (100), 162 (25); Anal. Calcd for $C_{19}H_{16}N_6O_2S$ (392.106): C, 58.15; H, 4.11; N, 21.42. Found: C, 57.95; H, 4.01; N, 21.19%.

General procedure for Synthesis compounds 13a,b; Method A. An equimolar mixture of phthalic anhydride **1** (0.74 g, 5 mmol), thiocarbohydrazide **2** (0.53 g, 5 mmol), and ethyl 2-chloro-2-(2-arylhydrazono)acetate **12a,b** (5 mmol) was taken in anhydrous EtOH containing a catalytic

amount of acetic acid (0.1 mL). The reaction mixture was heated at reflux for about 2 h until white ppt. formed then added few drops of Et_3N (0.2 mL) and boiling the mixture for another 2-3 h, cooled to room temperature. The solid obtained was filtered, washed with EtOH, and recrystallized from aqueous EtOH to give **13a** and **13b**, respectively.

Method B: Synthesis of compound 13b. A mixture of *N*-(1,3-dioxoisoindolin-2-yl)hydrazine-carbothioamide **4** (0.472 g, 2 mmol) and ethyl 2-chloro-2-(2-(4-chlorophenyl)hydrazono)acetate **12b** (0.522 g, 2 mmol) in absolute EtOH (20 mL) was refluxed for 4 h (TLC) and cooled to room temperature. The resulting precipitate was filtered, washed with EtOH, and crystallized from EtOH to give **13b**.

2-(5-Oxo-6-(phenyldiazenyl)-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (13a): a yellow powder, yield 87%; mp 205–206 ºC; IR (cm^{-1}): ν 3338, 3238 (2NH), 1788, 1734, 1643 (3C=O), 1602, 1556 (C=N); ^{1}H NMR (DMSO-d_6) δ_H (ppm): 2.00 (s, br, 1H, NH, D_2O exchangeable), 3.43 (s, 1H, CH), 7.11-7.29 (m, 5H, Ar-H), 7.82 (d, 2H, *J* = 8.5 Hz, Phthalimide-H), 7.95 (d, 2H, *J* = 8.5 Hz, Phthalimide-H), 9.62 (s, br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ_c (ppm): 61.7, 124, 124.7, 124.9, 130, 135.6, 136.4, 156.3, 167, 183.4; E1-MS: (*m/z*, %):291.88 (M^+- C_6H_5N, 14), 256 (10), 162 (100); Anal. Calcd for $C_{17}H_{12}N_6O_3S$ (380.069): C, 53.68; H, 3.18; N, 22.09. Found: C, 53.32; H, 2.99; N, 21.87.

2-(6-((4-Chlorophenyl)diazenyl)-5-oxo-5,6-dihydro-4*H*-1,3,4-thiadiazin-2-ylamino)isoindoline-1,3-dione (13b): a yellow powder, yield (89%, Method A), (90%, Method B); mp 209–210 ºC; IR (cm^{-1}): ν 3338, 3240 (2NH), 1788, 1736, 1643 (3C=O), 1602, 1556 (C=N); ^{1}H NMR (DMSO-d_6) δ_H (ppm): 2.20 (s, br, 1H, NH, D_2O exchangeable), 7.36 (d, 2H, *J* = 8.5 Hz, *J* = 1.7 Hz, Ar-H), 7.38 (dd, 2H, *J* = 8.5 Hz, *J* = 2.5 Hz Ar-H), 7.92 (dd, 2H, *J* = 8.5 Hz, *J* = 3.4 Hz, phthalimide-H), 7.95 (dd, 2H, *J* = 8.5 Hz, *J* = 3.4 Hz, phthalimide-H), 10.66 (s, br, IH, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ_c (ppm): 62.31, 114.4, 116.1, 123.5, 126, 129.6, 130, 134.91, 141.7, 159.3, 165.3, 182.5; E1-MS: (*m/z*, %): 414 (M^+, 11), 260 (100); Anal. Calcd for $C_{17}H_{11}ClN_6O_3S$ (414.030): C, 49.22; H, 2.67; N, 20.26. Found: C, 48.87; H, 2.43; N, 20.03.

X-Ray crystallography of compound 4: A single crystal of compound **4** was obtained by slow evaporation at room temperature, from dimethylformamide (DMF). The crystal structure was solved and refined using MaXus (Bruker Nonius, Deflt and MacScience, Japan).[25] Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system is shown in Figure. 1. Crystal data for compound **4**: $C_9H_8N_4O_2S$, Mr, 236.253; system, Triclinic; Space group, P_1; unit cell dimensions, a,7.2533 (2)Å; b, 7.9510 (3)Å; c, 10.2813 (4)Å; α, 75.763 (2)°; β, 79.295 (2)°; γ, 64.837 (2)°; V, 517.95 (3)$Å^3$; Z, 2; D_x, 1.515 Mg m^{-3}; θ range for data collection, 2.910-30.034 °; μ (Mo- Kα), 0.30 mm^{-1}; T = 298 K; independent reflections, 3191; measured reflections, 2897; observed reflections, 1480; R(all), 0.097; R(gt), 0.047; wR(ref), 0.094; wR(all), 0.102; wR(gt), 0.094; S(ref), 0.961; S(all), 0.778; S(gt), 0.961; Δ/σmax, 0.024, Δρmax, 0.58e$Å^3$; Δρmin-0.69e$Å^3$.

Crystallographic data for the structures 4 have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 1499061. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk.

Antimicrobial activity: Chemical compounds were individually tested against a panel of Gram positive and Gram negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method[26] using 100 µL of suspension containing 1x108 CFU/mL of pathological tested bacteria and 1x106 CFU/mL of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 200 mg of the chemical compound in 1 mL of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. (Ciprofloxacin (50 mg/mL and Ketoconazole (50 mg/mL) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal inhibitory concentration (MIC) measurement: The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two fold serial dilution technique.[27] Two fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 500, 250, 125, and 65 µg/mL. Each 5 mL received 0.1 ml of the appropriate inoculum and incubated at 37 °C for 24 h and 48 h at 28 °C for fungi. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

REFERENCES

1. M. M. Raj, H. V. Patel, L. M. Raj, and N. K. Patel, *Int. J. Pharm. Bio. Sci.*, 2013, **3**, 814.
2. K. M. Amin, A. H. El-masry, N. A. Mohamed, G. E. A. Awad, and B. S. Habib, *Der Pharm. Chem.*, 2013, **5**, 97.
3. A. A.-H. Abdel-Rahman, O. M. Ali, and A. A.-S. Abdel-Megeed, *J. Heterocycl. Chem.*, 2013, **50**, 484.
4. R. Bamnela and S. P. Shrivastava, *Chem. Sci. Trans.*, 2012, **1**, 431.
5. S. Soni, M. Seth, and P. Sah, *Asian J. Biochem. Pharm. Res.*, 2012, **2**, 47.
6. N. S. El-Gohary and M. I. Shaaban, *Arch. Pharm. Chem. Life Sci.*, 2015, **348**, 666.
7. A. M. Khalil, M. A. Berghot, and M. A. Gouda, *Eur. J. Med. Chem.*, 2010, **45**, 1552.

8. A. A.-M. Abdel-Aziz, A. S. El-Azab, S. M. Attia, A. M. Al-Obaid, M. A. Al-Omar, and H. I. El-Subbagh, *Eur. J. Med. Chem.*, 2011, **46**, 4324.
9. D. Atukuri, P. Kattimani, and R. Kamble, *Org. Commun.*, 2011, **4**, 94.
10. V. Kathuria and D. P. Pathak, *Pharm. Innov.*, 2012, **1**, 55.
11. R. A. Pophale and M. N. Deodhar, *Pharm. Chem.*, 2010, **2**, 185.
12. R. Williams, J. T. Manka, A. L. Rodriguez, P. N. Vinson, C. M. Niswender, C. D. Weaver, C. K. Jones, P. J. Conn, C. W. Lindslev, and S. R. Stauffe, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1350.
13. P. A. T. de M. Gomes, A. R. Oliveira, M. V. de O. Cardoso, E. de F. Santiago, M. deOBarbosa, L. R. P. deSiqueira, D. R. M. Moreira, T. M. Bastos, F. A. Brayner, M. B. P. Soares, A. P. deOliveira Mendes, M. C. A. Brelaz de Castro, V. R. A. Pereira, and A. C. Lima Leite, *Eur. J. Med. Chem.*, 2016, **111**, 46.
14. A. M. Alanazi, A. S. El-Azab, I. A. Al-Suwaidan, K. E. H. ElTahir, Y.A. Asiri, N. I. Abdel-Aziz, and A. A.-M. Abdel-Aziz, *Eur. J. Med. Chem.*, 2015, **92**, 115.
15. J. Schroder, A. Henke, H. Wenzel, H. Brandstetter, H. G. Stammler, A. Stammler, W. D. Pfeiffer, and H. Tschesche, *J. Med. Chem.*, 2001, **44**, 3231.
16. H. Sugawara and M. Endoh, *Jpn. J. Pharmacol.*, 1999, **80**, 55.
17. H. M. Himmel, G. J. Amos, E. Wettwer, and U. Ravens, *J. Cardiovasc. Pharmacol.*, 1999, **33**, 301.
18. K. Rufenacht, *Helv. Chim. Acta*, 1973, **56**, 2186.
19. R. E. Khidre, I. A. M. Radini, and D. A. Ibrahim, *ARKIVOC*, 2016, **v**, 301
20. I. A. M. Radini, R. E. Khidre, and E. M. El-Telbani, *Lett. Drug Des. Discov.*, 2016, **13**, 921.
21. B. F. Abdel-Wahab, R. E. Khidre, and G. E. A. Awad, *J. Heterocycl. Chem.*, 2016, DOI: 10.1002/jhet.2610.
22. B. F. Abdel-Wahab, R. E. Khidre, and H. A. Mohamed, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2015, **190**, 1781.
23. V. S. R. Chunduru and R. R. Vedula, *Synth. Commun.*, 2013, **43**, 923.
24. V. S. R. Chunduru and R. R. Vedula, *Chem. Heterocycl. Compd.*, 2013, **49**, 586.
25. S. Mackay, C. J. Gilmore, C. Edwards, N. Stewart, K. Shankland "maXus Computer Program for the Solution and Refinement of Crystal Structures." Bruker Nonius, The Netherlands, MacScience, Japan & The University of Glasgow (1999).
26. C. Perez, M. Pauli, and P. Bazevque, *Acta Biol. Med. Exp.*, 1990, **15**, 113.
27. A. C. Scott, "Laboratory Control of Antimicrobial Therapy" In: J. G. Collee, J. P. Duguid, A. G. Fraser, and B. P. Marmion "Mackie and MacCartney Practical Medical Microbiology", 13th Ed., Churchill Livingstone, 1989, **2**, 161.