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Original article

Improvement of antibacterial activity of some sulfa drugs through linkage to certain phthalazin-1(2*H*)-one scaffolds

Hany S. Ibrahim [a, **], Wagdy M. Eldehna [a], Hatem A. Abdel-Aziz [b, c, *], Mahmoud M. Elaasser [d], Marwa M. Abdel-Aziz [d]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, Helwan 11829, Egypt
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[c] Department of Applied Organic Chemistry, National Research Center, Dokki, Cairo 12622, Egypt
[d] The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

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ABSTRACT

RAB1 **5** is a lead antibacterial agent in which trimethoprim is linked to phthalazine moiety. Similarly, our strategy in this research depends on the interconnection between some sulfa drugs and certain phthalazin-1(2*H*)-one scaffolds in an attempt to enhance their antibacterial activity. This approach was achieved through the combination of 4-substituted phthalazin-1(2*H*)-ones **9a**, **b** or **14a**, **b** with sulfanilamide **1a**, sulfathiazole **1b** or sulfadiazine **1c** through amide linkers **6a**, **b** to produce the target compounds **10a**–**d** and **15a**–**e**, respectively. The antibacterial activity of the newly synthesized compounds showed that all tested compounds have antibacterial activity higher than that of their reference sulfa drugs **1a**–**c**. Compound **10c** represented the highest antibacterial activity against Gram-positive bacteria *Streptococcus pneumonia* and *Staphylococcus aureus* with MIC = 0.39 μmol/mL. Moreover, compound **10d** displayed excellent antibacterial activity against Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* with MIC = 0.39 and 0.78 μmol/mL, respectively.

1. Introduction

Sulfa drugs are the oldest chemically synthesized antimicrobial agents, as they still are widely used today for treatment of various bacterial, protozoal and fungal infections [1]. Sulfonamides **1** (Fig. 1) work as competitive inhibitors for *dihydropteroate synthase* (DHPS), which is a key enzyme involved in folate synthesis [2]. Sulfa drugs are usually used in combination with *dihydrofolate reductase* (DHFR) inhibitors, to treat common bacterial infections such as urinary tract infections, nocardiosis, toxoplasmosis, blepharitis, conjunctivitis, septicemia, acute sinusitis, and chronic bronchitis, particularly for patients with penicillin allergy [3]. However, the most common side effects for this class of drugs are nausea, vomiting, diarrhea, anorexia and hypersensitivity reaction [4].

Resistance to sulfa drugs is considered an important factor that severely avoids the current clinical use of sulfa-based inhibitors of DHFR [5,6]. Sulfonamides resistance in *streptococcus* species have been reported since the Second World War, but the mechanism of resistance was discovered lately through mutational changes in the *folP* (*sulA*) gene of coding for DHPS of *Streptococcus pyogenes* [7] and *Streptococcus pneumonia* [8]. Furthermore, the appearance of methicillin-resistant *Staphylococcus aureus* (MRSA), which is considered a life-threatening nosocomial infection, is also resistant to sulfonamides [9]. In addition, characterization of a mutational altered dihydropteroate synthase contributes to sulfathiazole resistance in *Escherichia coli* [10,11]. The latter data shows the need of novel strategies to overcome such challenges.

On the other hand, phthalazin-1(2*H*)-ones is of valuable interest due to their diverse biological activity such as antidiabetic [12], antiallergic [13] and vasorelaxant agents [14]. Furthermore, phthalazin-1(2*H*)-ones were reported as PDE4 inhibitors [15], VEGF (vascular endothelial growth factor) receptor tyrosine kinases for cancer treatment [16], and as an antiasthmatic agent with dual activities of thromboxane A2 (TXA2) synthase inhibition and bronchodialation [17]. Furthermore, 2-substituted-phthalazin-1(2*H*)-

* Corresponding author. Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.
** Corresponding author. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, Helwan 11829, Egypt.
E-mail addresses: hany.s.ibrahim@gmail.com (H.S. Ibrahim), hatem_741@yahoo.com (H.A. Abdel-Aziz).

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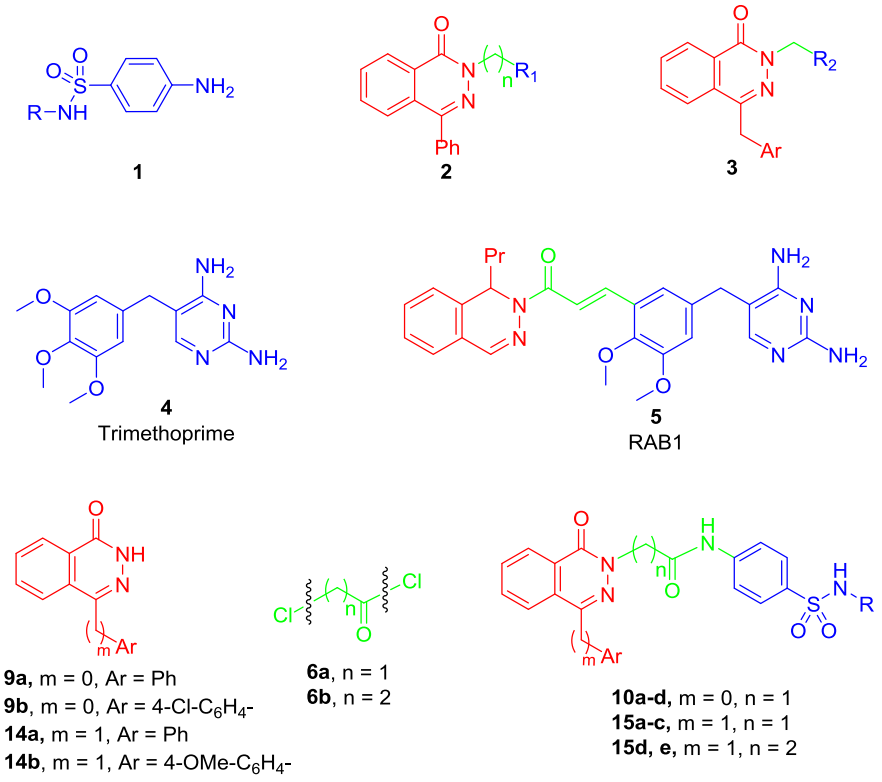
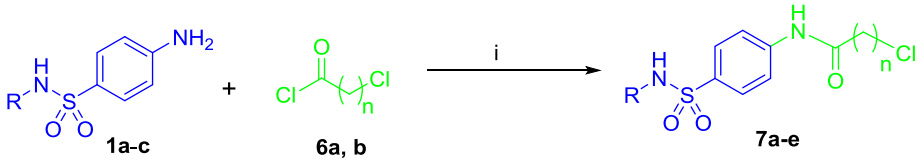


Fig. 1. Structure of compounds **1**–**5**, **6a**, **b**, **9a**, **b**, **14a**, **b**, **10a**–**d** and **15a**–**e**.

ones were reported as promising antimicrobial agents [18–22] such as 4-phenylphthalazin-1(2*H*)-ones **2** (R_1 = substituted-1,2,4-triazol-3-yl) [18,19] and 4-(heteroaryl-methyl)phthalazin-1(2*H*)-ones **3** (Ar = 1,3,4-oxadiazol-2-yl) [20] (Fig. 1).

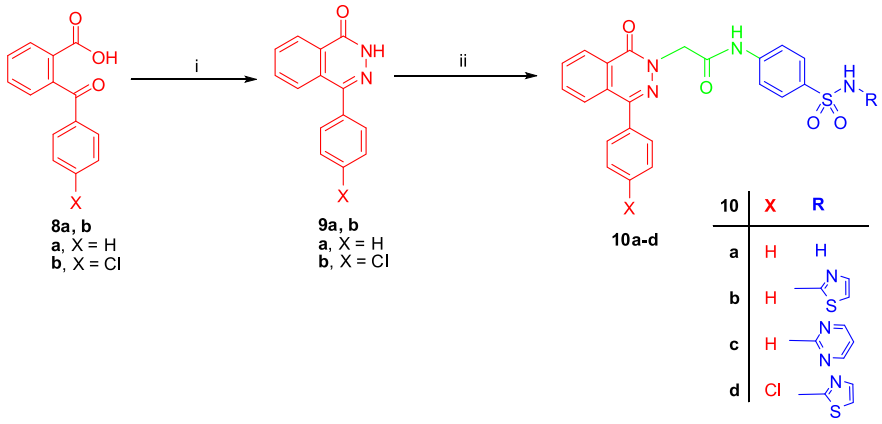
The combination of trimethoprim **4** and phthalazine scaffold lead to the discovery of a lead compound RAB1 **5** as a potent inhibitor of DHFR (Fig. 1), while the addition of phthalazine moiety provides a large hydrophobic anchor that embeds within the DHFR active site and accounts for its selective inhibitory activity against DHFR [23,24]. RAB1 **5** inhibits growth of both Gram-negative bacteria, *Francisella tularensis*, *Yersinia pestis*, *Brucella abortus*, and Gram-positive bacteria, *Bacillus anthracis*, and *S. aureus* indicating a broad-spectrum profile of activity [25]. Several research were made to make use of this idea in order to obtain a diverse inhibitory of broad-spectrum activity DHFR [26,27].

In this study, our goal is to use the same approach of RAB1 **5** through combining some sulfa drugs and certain 4-substituted-phthalazin-1(2*H*)-ones using amide linkers at position 2 of phthalazin-1(2*H*)-ones in an attempt to magnify the antibacterial activity of the selected sulfa drugs. Therefore, sulfanilamide **1a** (R = H), sulfathiazole **1b** (R = 1,3-thiazol-2-yl) and sulfadiazine **1c** (R = pyrimidin-2-yl) were selected to be combined with phthalazin-1(2*H*)-one **9a**, **b** ($m = 0$) and **14a**, **b** ($m = 1$) through linkers **6a**, **b** (n = 1, 2) to produce the target compounds **10a**–**d** and **15a**–**e**, respectively, as well as to investigate their *in vitro* antibacterial activity comparing it to their parent sulfonamides **1a**–**c** (Fig. 1).



7	n	R
a	1	H
b	1	
c	1	
d	2	H
e	2	

Scheme 1. Synthesis of compounds **7a**–**e**. Reagents and conditions: (i) dioxane/stirring r.t. 2 h.



Scheme 2. Synthetic pathway of compounds **10a–d**. Reagents and conditions: (i) $N_2H_4 \cdot H_2 \cdot SO_4$/NaOH/reflux 1 h; (ii) compounds **7a–e**/K_2CO_3/dry acetone/reflux 12 h or NaH/DMF/reflux 8 h.

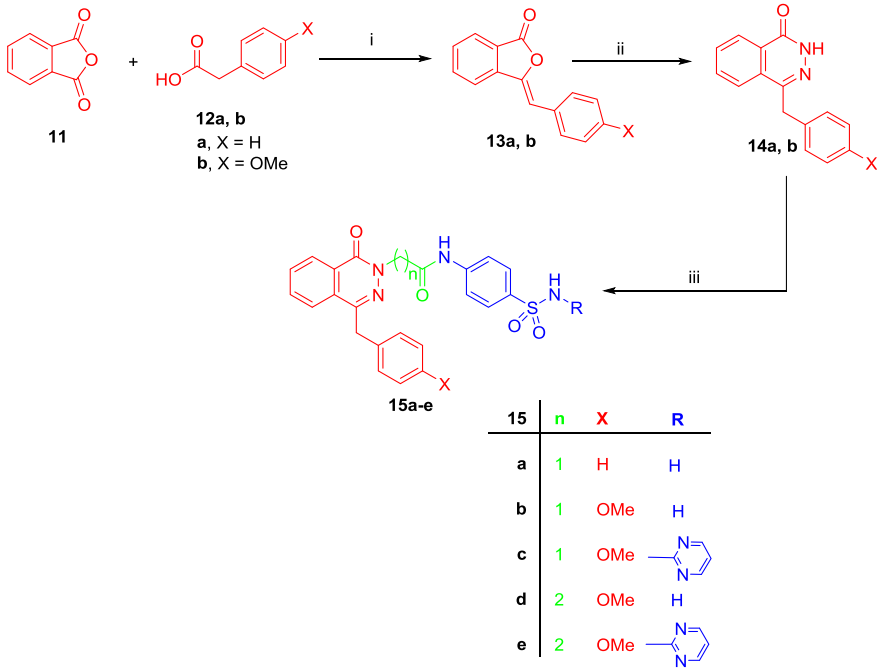
2. Results and discussion

2.1. Chemistry

The key intermediates **7a–e** were prepared by the reaction of sulfonamides **1a–c** with two acid chlorides **6a**, **b** in dioxane at ambient temperature [28–31] (Scheme 1). The IR spectrum of the unreported 3-chloro-*N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl) propanamide (**7e**) showed the absorption bands due to NH and C=O groups at 3353 and 1621 cm^{-1}, respectively, further two peaks of SO_2 group at 1330 and 1154 cm^{-1}. The ^1H NMR of **7e** revealed two triplet signals at δ 2.86 and δ 3.88 of two methylene groups. Moreover, it revealed the triplet signal ($J = 5$ Hz) of H-5 of pyrimidine at δ 7.04 and doublet signal ($J = 5$ Hz) integer to H-4 and H-6 of pyrimidine ring.

Phthalazin-1(2*H*)-ones **9a**, **b** were prepared by the reaction of 2-benzoylbenzoic acid (**8a**) or 2-(4-chlorobenzoyl)benzoic acid (**8b**) with hydrazine sulfate in the presence of sodium hydroxide [32] (Scheme 2). The target compounds **10a–d** were synthesized by the reaction of phthalazin-1(2*H*)-ones **9a**, **b** with intermediates **7a–e** in refluxed acetone in the presence of anhydrous potassium carbonate (Method A) or using DMF in the presence of sodium hydride (Method B) (Scheme 2).

On the other hand, phthalazin-1(2*H*)-ones **14a**, **b** where prepared through the fusion reaction of phthalic anhydride **11** and phenylacetic acid (**12a**) or 4-methoxyphenylacetic acid (**12b**) at 200 °C in the presence of anhydrous sodium acetate to yield phthalides **13a**, **b** [35] which consequently condensed with hydrazine sulfate (Scheme 3) [36]. The target compounds **15a–e** were synthesized by the reaction of phthalazin-1(2*H*)-ones **14a**, **b** with the appropriate intermediates **7a–e** under reaction condition (K_2CO_3/acetone or NaH/DMF), similar to that applied for compounds **10a–d** preparation.



Scheme 3. Synthetic pathway of compounds **15a–e**. Reagents and conditions: (i) CH_3COONa/fusion at 200 °C/5 h; (ii) $N_2H_4 \cdot H_2SO_4$/NaOH/reflux 3 h; (iii) compounds **7a–e**/K_2CO_3/dry acetone/reflux 12 h or NaH/DMF/reflux 8 h.

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

Compd.	Zone of inhibition[a]				
	Gram +ve			Gram −ve	
	Streptococcus pneumoniae	*Enterococcus faecalis*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*
10a	16.8 ± 0.37	15.9 ± 0.44	19.4 ± 0.25	16.8 ± 0.58	16.9 ± 0.36
15a	16.2 ± 0.44	15.3 ± 0.44	21.0 ± 0.25	17.6 ± 0.58	18.24 ± 0.44
15a	18.2 ± 0.44	16.2 ± 0.58	22.0 ± 0.30	19.6 ± 0.58	20.0 ± 0.72
15d	18.3 ± 0.17	16.7 ± 0.29	22.3 ± 0.44	19.9 ± 0.33	21.0 ± 0.58
Sulfanilamide	15.8 ± 0.44	14.2 ± 0.37	16.8 ± 0.19	15.8 ± 0.44	14.2 ± 0.37
10b	20.3 ± 0.39	17.0 ± 0.58	18.3 ± 0.45	16.3 ± 0.44	21.0 ± 0.37
10d	20.2 ± 0.17	17.2 ± 0.44	19.9 ± 0.45	22.2 ± 0.44	21.4 ± 0.37
Sulfathiazole	15.8 ± 0.58	14.9 ± 0.63	15.0 ± 1.22	13.8 ± 0.44	17.2 ± 0.25
10c	22.3 ± 0.25	20.3 ± 0.25	22.3 ± 0.58	16.1 ± 0.25	18.2 ± 0.58
15c	16.3 ± 0.25	15.2 ± 0.58	18.6 ± 0.17	16.9 ± 0.44	19.3 ± 0.25
15e	15.7 ± 0.33	17.2 ± 0.25	19.8 ± 0.34	16.2 ± 0.44	17.4 ± 0.67
Sulfadiazine	14.2 ± 0.25	13.7 ± 0.42	18.3 ± 0.44	14.2 ± 0.58	15.0 ± 0.58

[a] Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of clinically pathogenic microorganisms.

The structure of compounds **10a**–**d** and **15a**–**e** was confirmed under the basis of spectral analyses. For example, their IR spectra showed the absorption bands of NH in range 3476–3111 cm^{-1} in addition to the peaks of C=O functions around 1700 cm^{-1}, whereas SO_2 group bands appeared around 1330 and 1150 cm^{-1}. The ^1H NMR spectra of compounds **10a**–**d** and **15a**–**e** showed the signals of aliphatic $CO\underline{CH}_2$ protons within the range δ 4.48–5.07 while in case of **15d**, **e**, the signals of the aliphatic protons $CO\underline{CH}_2-\underline{CH}_2$ were observed as triplets near to δ 2.92 and δ 4.48, respectively. In addition, the $-\underline{CH}_2$ protons of benzylic moiety of **15a**–**e** appeared as a singlet signal in the range δ 4.14–4.87. The ^1H NMR spectra of compounds **10a**–**d** and **15a**–**e** revealed the D_2O exchangeable signal of amidic NH protons in the range δ 10.45–10.72. The ^{13}C NMR spectra of **10b** and **15e** showed signals resonating in the range δ 35.28–51.21 attributable for the aliphatic carbons of the linker while carbons of carbonyl groups appeared in the range δ 158.08–169.62. The ^{13}C NMR spectra of compounds **15e** showed the signals of benzylic methylene carbons in the range δ 36.87, while the signal $O\underline{CH}_3$ group of compound **15e** appeared at δ 54.87. The ^1H NMR of compounds **10a**, **15a**, **b**, **15d** showed the presence of D_2O exchangeable signal due to presence of $SO_2N\underline{H}_2$ group around δ 7.20, while the ^1H NMR of sulfathiazole containing compounds **10b** and **10d**, elaborated two doublets at δ 6.9–7.40 of thiazole ring. Moreover, the ^1H NMR of sulfadiazine derivatives **10c** and **15c**, **e** exhibited the signals of pyrimidine moiety as triplet and doublet signals near to δ 7.01 and 8.7, respectively.

2.2. Antibacterial activity

Antibacterial activity was performed at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Initially, all synthesized compounds and reference drugs were evaluated *in vitro* for their antibacterial activity, by inhibition zone technique, using three Gram-positive bacteria: *S. pneumonia* (RCMB 010010), *Enterococcus faecalis* (RCMB 010068) and *S. aureus* (RCMB 010028) with the addition of two Gram-negative bacteria: *E. coli* (RCMB 010052) and *Salmonella typhimurium* (RCMB 010072). Experiments were done using resistant Gram-negative *Pseudomonas aeruginosa* (RCMB 010043) but no activity obtained from both synthesized and reference drugs. The mean values of the inhibition zone diameter obtained for these compounds suggest that all synthesized compounds possess significant antibacterial activity against most test organisms used in these assays (Table 1), therefore minimum inhibitory concentration (MIC) of various synthesized compounds evaluated *in vitro* using twofold serial dilution technique, while the lowest concentration showed no growth as the MIC. Results of minimum inhibitory concentration reported in Table 2.

The first set of compounds, **10a**, **15 a**, **b** and **15d** exhibited antibacterial potency higher than that of their reference drug **1a**. Compound **15d** reaching highest potency with MIC = 6.25, 12.5, 0.39, 3.12 and 1.65 μmol/mL against *S. pneumonia*, *E. faecalis*, *S. aureus*, *E. coli* and *S. typhimurium*, respectively. In conclusion, 4-(4-

Table 2
Antimicrobial activity as MICs (μm/mL) of tested standards and synthesized compounds against tested microorganisms.

Compd.	Minimum inhibitory concentration				
	Gram +ve			Gram −ve	
	Streptococcus pneumonia	*Enterococcus faecalis*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*
10a	12.5	25	1.56	12.5	12.5
15a	25	50	0.78	6.25	3.12
15b	6.25	25	0.39	3.12	1.56
15d	6.25	12.5	0.39	3.12	1.56
Sulfanilamide	25	100	12.5	25	100
10b	1.56	25	12.5	25	0.78
10d	1.56	25	3.12	0.39	0.78
Sulfathiazole	25	50	50	100	12.5
10c	0.39	1.56	0.39	25	6.25
15c	25	50	6.25	25	3.12
15e	50	25	3.12	50	25
Sulfadiazine	100	100	6.25	100	50

methoxybenzyl)-phthalazin-1(2*H*)-one group increased the antibacterial activity of the first set compounds against tested bacteria when compared to the standard reference sulfanilamide **1a**.

The second set of compounds, **10b** and **10d**, elaborated 4-(4-chlorophenyl)-phthalazin-1(2*H*)-one group potentiate the antibacterial activity than 4-phenyl-phthalazin-1(2*H*)-one as reflected in the activity of both **10d** and **10b**, respectively. Compound **10d** represented the highest antibacterial activity against Gram-negative bacteria with MIC = 0.39 and 0.78 μmol/mL against *E. coli* and *S. typhimurium*, respectively.

The third set in which sulfadiazine **1c** is connected to 4-(4-methoxybenzyl)-phthalazin-1(2*H*)-one group-compounds **15c** and **15e**-exhibited an interesting results even with different linkers. Exceptionally, compounds **15c** and **15e** gave MIC = 6.25 μmol/mL and 3.12 μmol/mL, *S. aureus*, respectively, while **15c** showed MIC = 3.12 μmol/mL against *S. typhimurium*. However, the highest activity obtained from 4-phenyl-phthalazin-1(2*H*)-one group was compound **10c**, with MIC values 0.39 μmol/mL against *S. pneumonia* and *S. aureus*.

3. Conclusion

The interconnection between phthalazin-1(2*H*)-ones **9a**, **b** or **14a**, **b** and sulfonamides **1a**–**c** was a successful technique to produce potent broad-spectrum antibacterial agents. This takes place through reaction of key intermediates **7a**–**e** with **9a**, **b** and **14a**, **b** to produce targeted compounds **10a**–**e** and **15a**–**e**, respectively. In order to validate the enhancement of the antibacterial activity, inhibition zone for bacterial growth and MIC determination for the newly synthesized compounds was performed and showed an interesting antibacterial activity compared to their reference sulfa drugs.

4. Experimental

4.1. Chemistry

Melting points (°C, uncorrected) were determined using a Stuart melting point apparatus. The IR spectra (KBr) was recorded on a PerkinElmer FT/IR spectrometer. The NMR spectra recorded by Varian Gemini-300BB 300 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA). Chemical shifts were reported in parts per million (δ), and coupling constants (J) expressed in Hertz. TMS was used as an internal standard and chemical shifts were measured in δ ppm. ^{1}H and ^{13}C spectra were run at 300 and 75 MHz, respectively. Electron impact mass spectra was measured on a Varian MAT 311-A (70 e.v.).

4.1.1. Synthesis of compounds (*7a–e*)

To a stirred solution of the appropriate sulfa drugs **1a**–**c** (100 mmol) in dioxan (20 mL), 2-chloroacetyl chloride (**6a**) (0.113 g, 100 mmol) or 3-chloropropanoyl chloride (**6b**) (0.127 g, 100 mmol) was added drop wise at 0 °C during 30 min, and stirring continued for 2 h at room temperature. The obtained precipitate was filtered, dried and recrystallized from ethanol to yield **7a**–**e**. The physical properties and spectral data of **7a**–**d** were identical to those reported [28–31].

*4.1.1.1. 3-Chloro-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)propanamide (**7e**).* White crystals, 63% yield; mp 239–241 °C; IR (KBr) ν_{max}/cm^{-1} 3353 (NH), 1621 (C=O), 1584 (C=N), 1330, 1154 (SO_2), 646 (C–Cl); ^{1}H NMR (DMSO-d_6) δ 2.86 (t, 2H, J = 6.3 Hz, $-COCH_2-$), 3.88 (t, 2H, J = 6.3 Hz, $-CH_2Cl$), 7.04 (t, 1H, J = 5 Hz, H-5 of pyrimidine), 7.75 (d, 2H, J = 8.7 Hz, Ar–H), 7.91 (d, 2H, J = 8.7 Hz, Ar–H), 8.49 (d, 2H, J = 5 Hz, H-4 and H-6 of pyrimidine), 10.43 (s, 1H, CO–NH, D_2O exchangeable), 11.67 (s, 1H, SO_2–NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 38.66 ($-CH_2CO$), 38.94 ($-CH_2-Cl$), 30.13 ($-CH_3$), 115.71, 118.37, 128.86, 134.17, 142.73, 156.88, 158.26, 168.65 ($-C=O$).

*4.1.2. Synthesis of 4-phenyl-1(2H)-phthalazinone (**9a**) and 4-(4-chlorophenyl)-1(2H)-phthalazinone (**9b**)*

A solution of hydrazine sulfate (4.0 g, 30.4 mmol) and sodium hydroxide (2.4 g, 60.8 mmol) in water (20 mL) was heated on a steam bath for 20 min, then the latter was added to solution of **8a**, **b** (30.4 mmol) in water (20 mL). The reaction mixture was heated under reflux for 1 h, upon cooling the residue obtained was filtered, washed with water and crystallized from propanol to obtain the desired compounds **9a**, **b**, respectively [32].

*4.1.2.1. 4-Phenyl-1(2H)-phthalazinone (**9a**).* White crystals, 72% yield; mp 238–240 °C (Let. mp 212 °C [33]); ^{1}H NMR (DMSO-d_6) δ 7.52–7.58 (m, 5H, Ar–H), 7.64–7.70 (m, 1H, H-5 phthalazine), 7.84–7.90 (m, 2H, H-6 and H-7 of phthalazine), 8.32–8.35 (m, 1H, H-8 of phthalazine), 12.79 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ 126.03, 126.44, 127.88, 128.47, 128.87, 128.95, 129.25, 131.52, 133.50, 135.05, 146.34, 159.18 (C=O).

*4.1.2.2. 4-(4-Chlorophenyl)-1(2H)-phthalazinone (**9b**).* Pale yellow crystals, 77% yield; mp 270–271 °C (Let. mp > 250 °C [34]); ^{1}H NMR (DMSO-d_6) δ 7.66–7.70 (m, 1H, H-5 phthalazine), 7.62 (d, 2H, J = 7.4 Hz), 7.87 (d, 2H, J = 7.4 Hz), 7.89–7.93 (m, 2H, H-6 and H-7 phthalazine), 8.23–8.25 (m, 1H, H-8 phthalazine), 12.75 (s, 1H, NH, D_2O exchangeable).

*4.1.3. Synthesis of phthalide derivatives **13a, b***

Phthalic anhydride (**11**) (10.0 g, 67.5 mmol) was condensed with the proper phenylacetic acid derivative **12a**, **b** (81 mmol) in presence of freshly fused sodium acetate (0.26 g, 3.1 mmol) in a Dean–Stark apparatus for 4 h at 240 °C. After cooling, the solid reaction mixture was crystallized from ethanol to give phthalide derivatives **13a**, **b**, respectively [35].

*4.1.3.1. 3-Benzalphthalide (**13a**).* Orange crystals, 79% yield, mp 100–102 °C (Let. mp 100 °C, [35]); ^{1}H NMR δ 6.40 (s, 1H, –CH=), 7.16–8.06 (m, 9H, Ar–H).

*4.1.3.2. 4-Methoxybenzylidinephthalide (**13b**).* Orange crystals, 66% yield, mp 147–149 °C (Let. mp 147–148.5 °C [34]); ^{1}H NMR (DMSO-d_6) δ 3.81 (3H, s, OCH_3), 6.70 (s, 1H, –CH=), 7.16–8.06 (m, 8H, Ar–H).

*4.1.4. 4-(4-Benzyl/methoxybenzyl)phthalazin-1(2H)-one **14a**, **b***

A mixture of **13a**, **b** (0.05 mol) in ethanol (15 mL), hydrazine sulphate (7.8 g, 0.06 mol) in water (60 mL) was treated with NaOH solution (2 M, 50 mL), and the mixture was heated at 95 °C for 3 h and then cooled at room temperature. The reaction mixture was then diluted with water (300 mL) and the solid was collected by filtration, washed with water, dried and crystallized from ethanol to give **14a**, **b**, respectively [36].

*4.1.4.1. 4-Benzylphthalazin-1(2H)-one (**14a**).* Beige crystals, mp 200–202 °C (Let. mp = 201 °C [37]), 68% yield; ^{1}H NMR (DMSO-d_6) δ 4.29 (2H, s, CH_2), 7.15–7.37 (m, 5H, Ar–H), 7.77–7.91 (m, 2H, H-6 and H-7 of phthalazine), 7.94 (d, 1H, J = 7.8 Hz, H-5 of phthalazine), 8.23 (d, 1H, J = 7.5 Hz, H-8 of phthalazine), 12.56 (s, 1H, NH, D_2O exchangeable).

*4.1.4.2. 4-(4-Methoxybenzyl)phthalazin-1(2H)-one (**14b**).* Brown crystals, mp 193–195 °C (Let. mp 193.5–194.5 °C [38]), 58%

yield; ^{1}H NMR (DMSO-d_6) δ (ppm) 3.53 (3H, s, OCH_3), 4.20 (2H, s, CH_2), 6.82 (d, 2H, J = 8.7 Hz, Ar–H), 7.20 (d, 2H, J = 8.7 Hz, Ar–H), 7.75–7.89 (m, 2H, H-6 and H-7 of phthalazine), 7.92 (d, 1H, J = 7.8 Hz, H-5 of phthalazine), 8.23 (d, 1H, J = 7.5 Hz, H-8 phthalazine), 12.56 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 36.79 (benzylic $\underline{C}H_2$), 54.91 (O–$\underline{C}H_3$), 113.91, 118.93, 125.60, 125.94, 126.90, 127.90, 129.11, 129.45, 129.95, 131.28, 133.27, 145.42, 157.81, 159.37 ($\underline{C}$=O).

4.1.5. Synthesis of target compounds ***10a–d*** *and* ***15a–e***

Method A. To a solution of the appropriate amides **7a–e** (2 mmol) in acetone (20 mL), K_2CO_3 (0.69 g, 5 mmol) was added and reflux for 1 h. For this solution, the appropriate phthalazin-1(2*H*)-ones **9a**, **b** or **14a**, **b** (2 mmol) was added and the reflux was continued for 12 h, then left to cool at room temperature and poured into cursed ice. The resulted precipitate was filtered, dried and then recrystallized from DMF/H_2O to afford the targeted compounds.

Method B. To a stirred solution of the appropriate phthalazin-1(2*H*)-one **14a**, **b** (10 mmol) in dry DMF (20 mL), NaH (60%) (0.4 g, 10 mmol) was added and stirred for 30 min at room temperature. After that the appropriate intermediates **7a–e** (10 mmol) were added at room temperature then reflux for 8 h, then left to cool, poured into crushed ice the obtained precipitate, then filtered and washed with dioxan and recrystallization from DMF/H_2O gave the targeted compounds.

*4.1.5.1. 2-(1-Oxo-4-phenylphthalazin-2(1H)-yl)-N-(4-sulfamoylphenyl)acetamide (**10a**).* Method A, white crystals, 62% yield; mp 229–231 °C; IR (KBr) ν_{max}/cm^{-1} 3476–3273 (NH_2 + NH), 1673 (2C=O) 1643 (C=N), 1334, 1153 (SO_2); ^{1}H NMR (DMSO-d_6) δ 5.07 (s, 2H, $COCH_2$), 7.26 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.55–7.63 (m, 9H, Ar–H), 7.69–7.76 (m, 1H, H-5 phthalazine), 7.91–7.96 (m, 2H, H-6 and H-7 phthalazine), 8.37–8.40 (m, 1H, H-8 phthalazine), 10.68 and 10.81 (s, 1H, D_2O exchangeable, NHCO, *cis* and *trans* conformers).

*4.1.5.2. 2-(1-Oxo-4-phenylphthalazin-2(1H)-yl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (**10b**).* Method B, beige crystals, 42% yield; mp >300 °C; IR (KBr) ν_{max}/cm^{-1} 3275, 3194 (2NH), 1705 (2C=O), 1654 (C=N), 1356, 1133 (SO_2); ^{1}H NMR (DMSO-d_6) δ 4.86 (s, 2H, $COCH_2$), 6.89 (d, 1H, J = 4.8 Hz, H-6 of thiazole), 7.28 (d, 2H, J = 8.7 Hz, Ar–H), 7.39 (d, 1H, J = 4.8 Hz, H-5 of thiazole), 7.47 (d, 2H, J = 8.7 Hz, Ar–H), 7.58–7.95 (m, 9H, Ar–H), 10.47 (s, 1H, D_2O exchangeable, CONH), 10.72 (s, 1H, D_2O exchangeable, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 51.21 (–$\underline{C}H_2$CO), 105.90, 118.90, 126.03, 127.00, 128.57, 128.92, 129.18, 129.35, 137.43, 140.90, 164.82, 165.04 ($\underline{C}$=O), 166.79 ($\underline{C}$=O).

*4.1.5.3. 2-(1-Oxo-4-phenylphthalazin-2(1H)-yl)-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)acetamide (**10c**).* Method B, white crystals, 45% yield; mp 276–278 °C; IR (KBr) ν_{max}/cm^{-1} 3282, 3111 (2NH), 1695 (2C=O), 1625 (C=N), 1353, 1138 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.90 (s, 2H, N–CH_2), 4.98 (s, 2H, $COCH_2$), 6.87 (d, 2H, J = 9 Hz, Ar–H), 7.02 (t, 1H, J = 4.9 Hz, H-5 of pyrimidine), 7.47–7.51 (m, 9H, Ar–H), 7.71–7.76 (m, 1H, H-5 phthalazine), 7.95–7.98 (m, 2H, H-6 and H-7 phthalazine), 8.35–8.40 (m, 1H, H-8 phthalazine), 8.66 (d, 2H, J = 4.9 Hz, H-4 and H-6 of pyrimidine), 10.68 (s, 1H, D_2O exchangeable, NHCO),11.66 (s, 1H, D_2O exchangeable, SO_2NH).

*4.1.5.4. 2-(4-(4-Chlorophenyl)-1-oxophthalazin-2(1H)-yl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (**10d**).* Method B, white crystals, 53% yield; mp 274–276 °C; IR (KBr) ν_{max}/cm^{-1} 3276, 3195 (2NH), 1703 (2C=O), 1594 (C=N), 1359, 1137 (SO_2); ^{1}H NMR (DMSO-d_6) δ 4.85 (s, 2H, $C\underline{H}_2$CO), 6.90 (d, 1H, J = 4.8 Hz, H-6 of thiazole), 7.28 (d, 2H, J = 9 Hz, Ar–H), 7.39 (d, 1H, J = 4.8 Hz, H-5 of thiazole), 7.48 (d, 2H, J = 9 Hz, Ar–H), 7.62–7.95 (m, 8H, Ar–H), 10.47 (s, 1H, D_2O exchangeable, N$\underline{H}$CO) and 10.72 (s, 1H, D_2O exchangeable, SO_2NH).

*4.1.5.5. 2-(4-Benzyl-1-oxophthalazin-2(1H)-yl)-N-(4-sulfamoylphenyl)acetamide (**15a**).* Method A, white crystals, 58% yield; mp 212–214 °C; IR (KBr) ν_{max}/cm^{-1} 3470–3252 (NH_2 + NH), 1685 (2C=O) 1623 (C=N), 1330, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ 4.20 (s, 2H, CH_2 benzyl), 5.03 (s, 2H, $COCH_2$), 7.20 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.25–7.36 (m, 4H, Ar–H), 7.73–7.95 (m, 8H, Ar–H), 8.28 (d, 1H, J = 6.9 Hz, H-8 of phthalazine), 10.60 and 10.72 (s, 1H, D_2O exchangeable, CONH, *cis* and *trans* conformers).

*4.1.5.6. 2-(4-(4-Methoxybenzyl)-1-oxophthalazin-2(1H)-yl)-N-(4-sulfamoylphenyl)acetamide (**15b**).* Method A, pale yellow crystals, 59% yield; mp 219–221 °C; IR (KBr) ν_{max}/cm^{-1} 3482–3244 (NH_2 + NH), 1686 (2C=O), 1629 (C=N), 1336, 1153 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H, OCH_3), 4.25 (s, 2H, CH_2 benzyl), 5.03 (s, 2H, $COCH_2$), 6.82 (d, 2H, J = 8.6 Hz, Ar–H), 7.20 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.24 (d, 2H, J = 8.6 Hz, Ar–H), 7.73–7.96 (m, 7H, Ar–H), 8.27 (d, 1H, J = 7.2 Hz, H-8 phthalazine), 10.60 and 10.72 (s, 1H, D_2O exchangeable, NHCO, *cis* and *trans* conformers). MS *m*/*z* (%) 479 (M^++1, 30.8), 478 (M^+, 95), 308 (89.2), 266 (74.3), 252 (39.8), 172 (100), 121 (51.8).

*4.1.5.7. 2-(4-(4-Methoxybenzyl)-1-oxophthalazin-2(1H)-yl)-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)acetamide (**15c**).* Method B, yellow crystals, 37% yield; mp 240–242 °C; IR (KBr) ν_{max}/cm^{-1} 3322, 3275 (2NH), 1695 (2C=O) (C=N), 1335, 1135 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H, OCH_3), 4. 26 (s, 2H, CH_2 benzyl), 4.48 (s, 2H, $COCH_2$), 6.85 (d, 2H, J = 9 Hz, Ar–H), 7.03 (t, 1H, J = 4.8 Hz, H-5 of pyrimidine), 7.18 (d, 2H, J = 9 Hz, Ar–H), 7.63–7.73 (m, 3H, H-5, H-6 and H-7 of phthalazine), 7.82–7.95 (m, 2H, H-6 and H-7 of phthalazine), 7.96 (d, 1H, J = 7.2 Hz, H-5 of phthalazine), 8.41 (d, 1H, J = 6.9 Hz, H-8 phthalazine), 8.82 (d, J = 4.8 Hz, 2H, H-4 and H-6 of pyrimidine), 10.46 (s, 1H, D_2O exchangeable, CONH), 11.66 (s, 1H, D_2O exchangeable, SO_2NH).

*4.1.5.8. 3-(4-(4-Methoxybenzyl)-1-oxophthalazin-2(1H)-yl)-N-(4-sulfamoylphenyl)propanamide (**15d**).* Method A, beige crystals, 48% yield; mp 224–227 °C; IR (KBr) ν_{max}/cm^{-1} 3483, 3310 (NH_2 + NH), 1670 (2C=O) 1636 (C=N), 1335, 1101 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.93 (t, 1H, J = 6.9 Hz, N–CH_2), 3.69 (s, 3H, OCH_3), 4.25 (s, 2H, CH_2 benzyl), 4.48 (t, 2H, J = 6.9 Hz, $COCH_2$), 6.65 (d, 2H, J = 8.1 Hz, Ar–H), 6.83 (d, 2H, J = 8.1 Hz, Ar–H), 7.18 (d, 2H, J = 8.7 Hz, Ar–H), 7.23 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.71–7.93 (m, 5H, Ar–H), 8.27 (d, 1H, J = 8.7 Hz, H-8 of phthalazine), 10.60 and 10.72 (s, 1H, D_2O exchangeable, NHCO, *cis* and *trans* conformers); MS *m*/*z* (%) 493 (M^++1, 20.4), 492 (M^+, 67.8), 321 (100), 279 (70.3), 265 (100), 226 (59.8), 172 (100), 121 (100).

*4.1.5.9. 3-(4-(4-Methoxybenzyl)-1-oxophthalazin-2(1H)-yl)-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)propanamide (**15e**).* Method A, pale yellow crystals, 42% yield; mp 240–242 °C; IR (KBr) ν_{max}/cm^{-1} 3320, 3280 (2NH), 1695 (2C=O), 1637 (C=N), 1322, 1114 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.92 (t, 2H, J = 6.6 Hz, N–$C\underline{H}_2$), 3.56 (s, 3H, $OC\underline{H}_3$), 4.14 (s, 2H, $C\underline{H}_2$ benzyl), 4.46 (t, 2H, J = 6.3 Hz, $COC\underline{H}_2$), 6.61 (d, 2H, J = 9 Hz, Ar–H), 7.01 (t, 1H, J = 4.8 Hz, H-5 of pyrimidine), 7.15 (d, 2H, J = 9 Hz, Ar–H), 7.20 (s, 2H, $SO_2N\underline{H}_2$, D_2O-exchangeable), 7.73–7.80 (m, 3H, H-5, H-6 and H-7 of phthalazine), 7.80–7.89 (m, 2H, H-6 and H-7 of phthalazine), 7.85 (d, 1H, J = 7.2 Hz, H-5 of phthalazine), 8.24 (d, 1H, J = 6.9 Hz, H-8 of phthalazine) 8.46 (d, 2H, J = 4.8 Hz, H-4 and H-6 of pyrimidine), 10.46 (s, 1H, D_2O exchangeable, CONH) 11.66 (s, 1H, D_2O exchangeable, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 35.28 (–$\underline{C}H_2$CO), 36.87 (benzylic $\underline{C}H_2$), 46.44 (N–$\underline{C}H_2$–), 54.87 (O–$\underline{C}H_3$), 113.78,

115.69, 118.29, 125.70, 126.30, 127.52, 128.43, 128.81, 129.72, 131.55, 133.15, 134.00, 142.99, 145.12, 156.90, 157.76, 158.08 (C=O), 169.62 (C=O); MS *m/z* (%) 507 (M^++1, 38.6), 506 (M^+, 100), 321 (89.2), 293 (74.3), 279 (39.8), 267 (100), 251 (51.8), 239 (58.9), 121 (62.7).

4.2. Biological evaluation

4.2.1. Antibacterial activity

All strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Inhibition zones of bacterial growth calculated for the synthesized compounds and reference drugs using Hole-plate diffusion method. Six equidistant (1 cm diameter) holes were made using sterile cork borer in Muller–Hinton agar (MHA) agar sterile plates (16 × 16 cm), which was previously seeded with tested bacterial isolates. Holes were filled with 100 μL of the tested compound at concentration (100 μmol dissolved in 1 mL DMSO). Consequently, the plate incubated for 24 h at 37 °C. After incubation, antimicrobial activity of each set of compounds was evaluated by measuring the inhibition zone diameters against test organisms and compared with standard zone size ranges of their reference sulfa drug. The experiment was performed in triplicate and the average zone of inhibition was calculated.

4.2.2. Minimum inhibitory concentration

MIC was performed by a serial dilution technique described by Irobi et al. [39], starting with 100 μmol concentration of all compounds dissolved in 1 mL DMSO and then reduced by successive twofold dilutions of stock solution using a calibrated micropipette. The final solutions concentrations were 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.195 μmol/mL. In each case, triplicate tests were performed and the average was taken as final reading. All bacteria were incubated and activated at 30 °C for 24 h inoculation into nutrient. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 h for tested microorganisms (1×10^8 CFU/mL), each 5 mL received 0.1 mL of the above inoculum. MIC was expressed as the lowest concentration inhibiting test organism's growth [40].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.016. These data include MOL files and InChiKeys of the most important compounds described in this article.

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