European Journal of Medicinal Chemistry 45 (2010) 4983–4989

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antimicrobial activity of 2-substituted [4-(1,3,4-oxadiazol-2-yl methyl)] phthalazin-1(2*H*)-one derivatives

Ajjanna M. Sridhara [a,b], Kallam R. Venugopala Reddy [b,*], Jathi Keshavayya [c], Palusa Sanath Kumar Goud [a], Bankavadi C. Somashekar [a], Prosenjit Bose [a], Sanenahalli K. Peethambar [d], Satish Kumar Gaddam [a]

[a] *Advinus Therapeutics Pvt. Ltd., 21 & 22, Peenya II phase, Bangalore, 560 058 Karnataka, India*
[b] *Department of Studies in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaragatta, 577 451 Karnataka, India*
[c] *Department of Studies in Chemistry, Kuvempu University, Jnana Sahyadri, Shankaragatta, 577 451 Karnataka, India*
[d] *Department of Biochemistry, Kuvempu University, Jnana Sahyadri, Shankaragatta, 577 451 Karnataka, India*

ARTICLE INFO

Article history:
Received 25 April 2010
Received in revised form
21 July 2010
Accepted 1 August 2010
Available online 12 August 2010

Keywords:
Phthalazin-1(2*H*)-one
1,3,4-Oxadiazole
(3-Oxo-2-benzofuran-1(3*H*)-ylidene) acetic acid
(4-Oxo-3,4-dihydrophthalazin-1-yl)acetic acid
Biological assays
Antibacterial
Antifungal

ABSTRACT

A series of new 2-substituted [4-(1,3,4-oxadiazol-2-yl)methyl]phthalazin-1(2*H*)-one derivatives **7a**–**h** to **9a**–**h** were designed and synthesized from methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate (**4**), which in was turn prepared from phthalic anhydride. The structure of synthesized new compounds were characterized by spectral data and screened for their antimicrobial activities against various bacteria and fungi strains. Several of these compounds showed antimicrobial activity.

1. Introduction

Nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals. Phthalazin-1(2*H*)-ones and 1,3,4-oxadiazoles are important building blocks in the construction of new molecular systems for biologically active molecules [1–3]. The development of new and efficient methodologies for synthesis of potentially bioactive phthalazin-1(2*H*)-one derivatives is important.

Phthalazin-1(2*H*)-ones are of considerable interest due to their antidiabetic [4], antiallergic [5], Vasorelaxant [6], PDE4 inhibitors [7], VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer [8,9], antiasthmatic agents with dual activities of thromboxane A2 (TXA2) synthetase inhibition and bronchodialation [10], herbicidal [11], like activities. A number of established drug molecules like Hydralazine [12,13], Budralazine [14,15], Azelastine [16,17], Ponalrestat [18] and Zopolrestat [19] are prepared from the corresponding phthalazinones.

Disubstituted-1,3,4-oxadiazoles have been found to exhibit diverse biological activities such as antibacterial [20], antifungal [21], antitubercular [22], insecticidal [23], herbicidal [24], analgesic [25], anti-inflammatory [26], anticonvulsant [27] and anticancer [28].

The diverse biological activities of phthalazin-1(2*H*)-one, and 1,3,4-oxadiazole pharmacophores encouraged us to envisage the combination of both groups in a compact system. A new series of phthalazine-oxadiazoles substituted with heterocyclic moieties at C-2 position were developed by adopting efficient and well-versed methodologies (Fig. 1).

1,3,4-oxadiazoles were in turn substituted with different alkyl, aryl and heterocyclic groups. For preliminary research we selected 2-chloro-1,3-thiazol-5-ylmethyl, 6-chloropyridin-3-ylmethyl, and *N*-ethylmorpholine substituents as the heterocyclic groups. The incorporation of these substances is fundamentally due to their diverse biological, chemical characteristics and they are frequently used in medicine and in industry [29–31]. Encouraged by

* Corresponding author. Tel.: +91 8282 256228; fax: +91 8282 256255.
E-mail address: venurashmi30@rediffmail.com (K.R. Venugopala Reddy).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.08.005

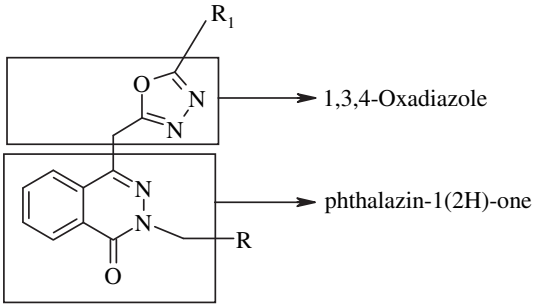


Fig. 1. 2-Substituted phthalazin-1(2*H*)-one oxadiazoles scaffold.

these observations it was envisaged to integrate the 1,3,4-oxadiazole moiety with a phthalazineframe work to study a potential additive effect of the combined molecule towards antimicrobial activity.

2. Chemistry

The key intermediate in the synthesis of new phthalazinone oxadiazoles **7a**–**h** to **9a**–**h** is methyl (4-oxo-3,4-dihydrophthalazin-1-yl) acetate (**4**), which was prepared from phthalic anhydride in good yield [32,33] (Scheme 1).

N-alkylation of compound **4** with different hetero alkyl halides in presence of base gave 3-substituted methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetates **5a**–**c.** The *N*-alkylation has been confirmed by Mass, IR, and NMR spectral studies. Addition of hydrazine hydrate to compounds **5a**–**c** in presence of ethanol as solvent afforded hydrazides **6a**–**c** in good yield. Cyclization of hydrazides **6a**–**c** with different acids in presence of phosphorous oxychloride gave 2-substituted [4-(1,3,4-oxadiazol-2-yl)methyl] phthalazin-1(2*H*)-one derivatives (Scheme 2).

3. Results and discussions

Investigation on antibacterial screening data (Table 1) showed some of the compounds were active against four human pathogenic bacteria. Compounds **7c, 7d, 7g, 7h, 8c, 8g, 8h,** and **9g** exhibited activity against *Escherichia coli*. Similarly compounds **7g, 7h, 8e** and **8g** showed activity against *Staphylococcus aureus.* The compounds **7h** and **9h** showed activity against *Bacillus subtilis.* Also the compounds **7g, 8d, 8e,** and **8g** showed activity against *Salmonella typhi.* From these results it could be generalized that, the chloro, fluoro, pyridine, and thiophene substituted oxadiazole derivatives shows higher activity compared to other analogues.

The antifungal results data (Table 2) revealed that, the synthesized compounds showed variable degree of inhibition against the tested fungi. Compounds **7g, 8g,** and **8h** possessed the highest antifungal activity against *Chrysosporium keratinophilum*. Compounds **7h, 8g, 8h,** and **9h** showed activity against *Candida albicans,* while the compounds **7g, 7h, 8e, 8d, 8g, 8h, 9g,** and **9h** showed activity of *Microsporum gypsum.* Also the compounds **7g, 8g, 8h, 9g,** and **9h** showed activity against *Aspergillums niger*. From these results it could be concluded that, the thiophene and pyridine substituted oxadiazole compounds showed higher activity and the others showed less activity against the fungi.

The antimicrobial data showed that, by changing the phthalazine substitution from 6-chloropyridin-3-ylmethyl to 2-chloro-1,3-thiazol-5-ylmethyl the activity increased but decreased by changing to 4-ethylmorpholine substitution. Compound **8g** which has thiazole and thiophene substitutions was the most active.

4. Conclusions

In this article we report the synthesis of (**7a**–**h** to **9a**–**h**), new phthalazine-oxadiazoles substituted at C_2 position with heterocyclic moieties, starting from commercially available phthalic anhydride. Investigation of their antimicrobial activity revealed that phthalazine with a 2-chloro-1,3-thiazol-5-ylmethyl substitutionand an oxadiazole with a thiophene substitution (**8g**) was the most active compound although it was significantly less than that of positive control. The fact that the compounds prepared in this study are chemically unrelated to the current medication suggests that the further work is clearly warranted.

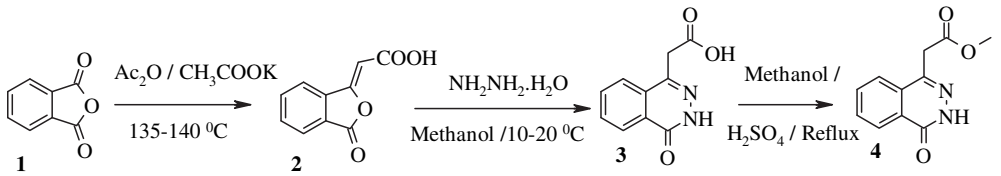
5. Experimental protocols

5.1. Chemistry

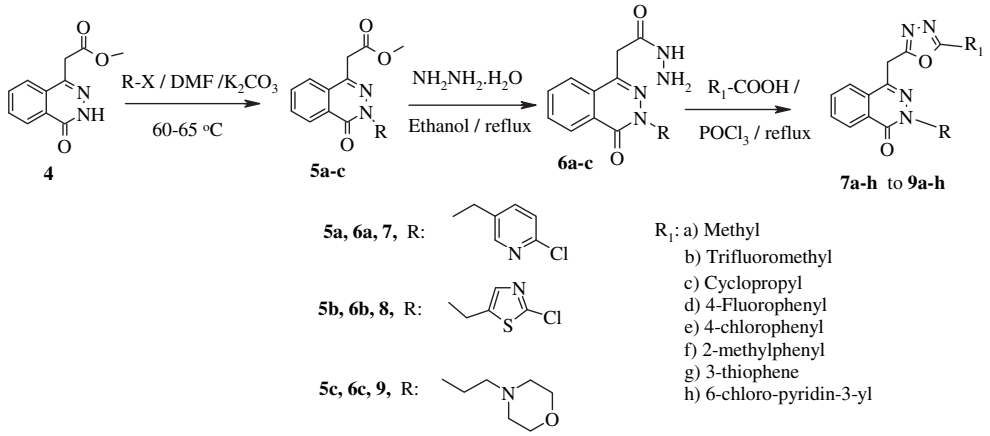
All chemicals used for the synthesis were of reagent grade and were procured from Sigma Aldrich Chemical Co, Bangalore; SDFCL, Mumbai; and the intermediates were prepared as per the known literature procedure. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz Varian-AS NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using PerkinElmer Spectrum 100 Series FT–IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Buchi melting point B-545 instrument and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using precoated silica 60 F_{254}, 0.25 mm aluminum plates (Merck). The crude compounds were purified by using CombiFlash® Companion® flash chromatography system, Teledyne Isco, Inc USA. Purity of compounds was assessed by HPLC method.

HPLC method for compounds 7a–h to 9a–h

HPLC instrument	Agilent 1200 series LC system
Mobile phase A	10 mm Ammonium acetate adjusted to pH: 3.0 with Acetic acid
Mobile phase B	Acetonitrile
Column	Inertsil ODS-3V 250 × 4.6 mm, 5 μ particle size
Detector	PDA at 254 nm
Sample concentration	10 mg/mL in methanol
Flow-rate	1.0 mL/min
Gradient	The gradient elution of the mobile phase was 50% B in 0–2 min, 50–90% in 2–10 min, 90% in 10–20 min, 90–50% in 20–25 min and 50% 25–30 min
Injection volume	20 μL



Scheme 1. Synthesis of methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate.



Scheme 2. Synthesis of 2-substituted phthalazin-1(2*H*)-one oxadiazoles **7a**–**h** to **9a**–**h**.

*5.2. Preparation of 3-substituted-methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate **5a**–**c***

A mixture of ester **4** (21.8 gm, 0.10 mol), dimethylformamide (250 mL), potassium carbonate (41.4 gm, 0.3 mol) and corresponding hetero alkyl halide (0.11 mol) was heated to 60–65 °C for 6 h. After completion of reaction, filtered the inorganics, the filtrate obtained was distilled completely under reduced pressure at 60–65 °C. The residue obtained was diluted with ice water (500 mL) and stirred for 30 min. The precipitated product was filtered, dried and recrystallized using isopropyl alcohol to yield **5a**–**c** as white solid.

*5.2.1. Methyl {3-[(6-chloropyridin-3-yl) methyl]–4-oxo-3,4-dihydrophthalazin-1-yl}acetate (**5a**)*

By using 2-chloro-5-(chloromethyl) pyridine, the title compound was obtained. Yield: 68.3%; M.p.: 178.2–183.5 °C; MS: $m/z = 344.1$ (M + 1); Purity: 98.2%; ^{1}H NMR(400 MHz, DMSO-d_6) δ: 3.64 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 7.48 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.79 (dd, $J = 8.0$, 2.4 Hz, 1H, Ar–H), 7.80–7.99 (m, 3H, Ar–H), 8.30–8.32 (m, 1H, Ar–H), 8.42 (d, $J = 2.4$ Hz, 1H, Ar–H).

*5.2.2. Methyl {3-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetate (**5b**)*

The compound was obtained by using 2-chloro-5-(chloromethyl)-1,3-thiazole. Yield: 60.3%; M.p.: 171.0–174.4 °C; MS: $m/z = 350.1$ (M + 1); Purity: 99.1%; ^{1}H NMR(400 MHz, DMSO-d_6) δ: 3.64 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 5.46 (s, 2H, CH_2), 7.77 (s, 1H, Ar–H), 7.89–7.99 (m, 3H, Ar–H), 8.31–8.33 (m, 1H, Ar–H).

*5.2.3. Methyl{3-[2-(morpholin-4-yl)ethyl]-4-oxo-3,4-dihydrophthalazin–1-yl}acetate (**5c**)*

By using 4-(2-chloroethyl)morpholine hydrochloride, the title compound was obtained. Yield: 70.9%; M.p.: 156.8–159.6 °C; MS: $m/z = 332.2$ (M + 1); Purity: 97.6%; ^{1}H NMR(400 MHz, DMSO-d_6) δ:

Table 1
Antibacterial activity of compounds **7a**–**h** to **9a**–**h**.

	Zone of Inhibition measured in mm							
	Escherichia coli ± S.D.*		*Staphylococcus aureus* ± S.D.*		*Bacillus subtilis* ± S.D.*		*Salmonella typhi* ± S.D.*	
Synthesized compounds	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
7a	3.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	0.0	0.0	0.0	2.5 ± 0.7	1.0 ± 0.0
7b	8.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	3.5 ± 0.7	2.0 ± 0.0	0.0	7.0 ± 0.0	4.0 ± 0.0
7c	11.5 ± 0.7	7.0 ± 0.0	4.5 ± 0.7	1.0 ± 0.0	6.5 ± 0.7	4.0 ± 0.0	6.0 ± 0.0	3.0 ± 0.0
7d	10.0 ± 0.0	8.0 ± 0.0	6.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	3.0 ± 0.0	7.5 ± 0.7	3.0 ± 0.0
7e	8.0 ± 0.0	5.0 ± 0.0	4.5 ± 0.7	1.0 ± 0.0	7.0 ± 0.0	3.5 ± 0.7	7.5 ± 0.7	4.0 ± 0.0
7f	7.0 ± 0.0	4.5 ± 0.7	6.5 ± 0.7	4.5 ± 0.7	2.5 ± 0.7	0.0	3.0 ± 0.0	1.0 ± 0.0
7g	15.5 ± 0.0	10.0 ± 0.0	11.5 ± 0.7	7.0 ± 0.0	6.0 ± 0.0	4.0 ± 0.0	15.5 ± 0.0	10.0 ± 0.0
7h	12.5 ± 0.7	7.5 ± 0.7	10.5 ± 0.7	7.5 ± 0.7	8.0 ± 0.0	5.5 ± 0.7	6.5 ± 0.7	2.5 ± 0.7
8a	5.5 ± 0.7	2.5 ± 0.7	1.0 ± 0.0	0.0	3.0 ± 0.0	1.00 ± 0.0	4.5 ± 0.7	2.5 ± 0.7
8b	6.5 ± 0.7	3.0 ± 0.0	7.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	3.0 ± 0.0	5.5 ± 0.7	3.0 ± 0.0
8c	11.5 ± 0.7	9.0 ± 0.0	4.5 ± 0.7	2.0 ± 0.0	5.5 ± 0.7	2.0 ± 0.0	7.5 ± 0.7	4.0 ± 0.0
8d	9.0 ± 0.0	5.0 ± 0.0	9.0 ± 0.0	5.0 ± 0.0	4.5 ± 0.0	2.0 ± 0.0	9.0 ± 0.0	5.0 ± 0.0
8e	9.0 ± 0.0	5.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	5.5 ± 0.7	3.0 ± 0.0	9.5 ± 0.7	5.0 ± 0.0
8f	6.0 ± 0.0	6.0 ± 0.0	2.0 ± 0.0	0.0	5.0 ± 0.0	2.0 ± 0.0	5.0 ± 0.0	3.0 ± 0.0
8g	16.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0	4.0 ± 0.0	12.0 ± 0.0	7.0 ± 0.0
8h	12.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	4.0 ± 0.0	6.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	3.0 ± 0.0
9a	2.5 ± 0.7	0.0	3.5 ± 0.7	0.0	1.0 ± 0.0	0.0	4.5 ± 0.7	1.5 ± 0.7
9b	3.5 ± 0.7	0.0	0.0	0.0	5.00 ± 0.0	2.0 0 ± 0.0	2.5 ± 0.7	0.0
9c	5.0 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	0.0	5.5 ± 0.7	3.5 ± 0.7	6.5 ± 0.7	2.5 ± 0.7
9d	2.0 ± 0.0	0.0	1.0 ± 0.0	0.0	6.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	3.5 ± 0.7
9e	7.0 ± 0.0	3.0 ± 0.0	8.0 ± 0.0	5.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	4.5 ± 0.7	3.0 ± 0.0
9f	5.5 ± 0.7	2.5 ± 0.7	3.5 ± 0.7	1.5 ± 0.7	4.5 ± 0.7	2.5 ± 0.7	5.0 ± 0.0	2.5 ± 0.7
9g	8.5 ± 0.7	3.5 ± 0.7	5.0 ± 0.0	3.5 ± 0.7	6.0 ± 0.0	3.0 ± 0.0	7.5 ± 0.7	4.5 ± 0.7
9h	5.5 ± 0.7	1.5 ± 0.7	7.0 ± 0.7	3.5 ± 0.7	9.0 ± 0.0	4.5 ± 0.7	6.5 ± 0.7	3.5 ± 0.7
Amoxicillin	18.5 ± 0.7	15.0 ± 1.4	16.5 ± 0.7	12.5 ± 0.7	14.5 ± 0.7	10.5 ± 0.7	20.5 ± 0.7	12.0 ± 0.0

*S.D = Standard deviation.

Table 2
Antifungal activity of compounds **7a**–**h** to **9a**–**h**.

	Zone of Inhibition measured in mm							
	Chrysosporium keratinophilum ± S.D.*		*Candida albicans* ± S.D.*		*Microsporum gypsum* ± S.D.*		*Aspergillus flavus* ± S.D.*	
Synthesized compounds	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
7a	0.0	0.0	3.5 ± 0.7	1.0 ± 0.0	2.00 ± 0.0	0.0	5.0 ± 0.0	1.0 ± 0.0
7b	4.5 ± 0.7	1.0 ± 0.0	4.5 ± 0.7	1.5 ± 0.7	5.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.7	0.0
7c	2.5 ± 0.7	0.5 ± 0.7	8.5 ± 0.7	5.5 ± 0.7	7.5 ± 0.7	4.5 ± 0.7	5.0 ± 0.0	2.5 ± 0.7
7d	1.5 ± 0.7	0.0	4.5 ± 0.7	2.5 ± 0.7	6.5 ± 0.7	4.0 ± 0.7	4.0 ± 0.0	2.0 ± 0.7
7e	3.0 ± 0.0	1.0 ± 0.0	5.5 ± 0.7	3.0 ± 0.0	4.0 ± 0.0	1.0 ± 0.0	5.0 ± 0.0	2.5 ± 0.7
7f	1.5 ± 0.7	0.0	3.00 ± 0.0	0.0	3.0 ± 0.0	1.0 ± 0.0	4.0 ± 0.0	1.0 ± 0.0
7g	10.5 ± 0.7	6.5 ± 0.7	7.5 ± 0.7	5.5 ± 0.7	11.0 ± 0.0	6.5 ± 0.7	7.5 ± 0.7	4.0 ± 0.0
7h	6.5 ± 0.7	4.5 ± 0.7	11.5 ± 0.7	8.5 ± 0.7	7.00 ± 0.0	4.0 ± 0.0	6.5 ± 0.7	3.5 ± 0.0
8a	2.5 ± 0.7	0.0	2.5 ± 0.7	1.0 ± 0.0	3.5 ± 0.7	0.0	3.0 ± 0.0	1.0 ± 0.0
8b	5.5 ± 0.7	2.0 ± 0.0	3.5 ± 0.7	0.5 ± 0.7	5.5 ± 0.7	3.0 ± 0.0	4.5 ± 0.7	1.5 ± 0.7
8c	3.5 ± 0.7	0.5 ± 0.7	5.5 ± 0.7	3.5 ± 0.7	7.0 ± 0.0	4.5 ± 0.7	6.0 ± 0.0	3.5 ± 0.7
8d	2.5 ± 0.7	0.0	5.5 ± 0.7	2.5 ± 0.7	8.5 ± 0.7	4.0 ± 0.7	5.0 ± 0.0	2.0 ± 0.7
8e	4.5 ± 0.7	2.0 ± 0.0	6.5 ± 0.7	3.0 ± 0.0	4.0 ± 0.0	1.0 ± 0.0	5.0 ± 0.0	2.5 ± 0.7
8f	3.5 ± 0.7	0.0	3.5 ± 0.7	0.0	3.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
8g	11.5 ± 0.7	5.5 ± 0.7	8.50 ± 0.7	4.5 ± 0.7	9.5 ± 0.7	6.5 ± 0.7	8.5 ± 0.7	3.5 ± 0.7
8h	8.5 ± 0.7	5.5 ± 0.7	10.5 ± 0.7	9.5 ± 0.7	7.5 ± 0.0	4.0 ± 0.0	8.0 ± 0.7	4.0 ± 0.0
9a	0.0	0.0	2.5 ± 0.7	1.0 ± 0.0	3.5 ± 0.7	0.0	3.5 ± 0.7	1.0 ± 0.0
9b	4.5 ± 0.7	2.0 ± 0.0	3.5 ± 0.7	0.5 ± 0.7	4.0 ± 0.0	2.0 ± 0.0	5.5 ± 0.7	2.5 ± 0.7
9c	2.5 ± 0.7	0.5 ± 0.7	4.5 ± 0.7	2.5 ± 0.7	6.0 ± 0.0	3.5 ± 0.7	5.0 ± 0.0	2.5 ± 0.7
9d	5.5 ± 0.7	2.5 ± 0.7	5.5 ± 0.7	2.5 ± 0.7	7.5 ± 0.7	4.0 ± 0.7	4.0 ± 0.0	2.0 ± 0.7
9e	1.5 ± 0.7	0.0	3.5 ± 0.7	1.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.0
9f	2.5 ± 0.7	0.0	1.5 ± 0.7	0.0	2.0 ± 0.0	1.0 ± 0.0	3.0 ± 0.0	1.5 ± 0.7
9g	6.5 ± 0.7	3.5 ± 0.7	7.50 ± 0.7	4.5 ± 0.7	10.5 ± 0.7	7.5 ± 0.7	9.5 ± 0.7	6.5 ± 0.7
9h	4.5 ± 0.7	1.5 ± 0.7	10.5 ± 0.7	6.5 ± 0.7	9.5 ± 0.0	4.0 ± 0.0	8.0 ± 0.7	4.5 ± 0.7
Metronidazole	14.0 ± 0.0	11.0 ± 1.0	19.5 ± 0.7	15.0 ± 0.0	17.0 ± 0.0	14.0 ± 1.0	17.5 ± 0.7	13.5 ± 0.7

*S.D = Standard deviation.

2.42 (m, 4H, mor–CH_2–N–CH_2–), 2.65–2.68 (t, J = 6.8 Hz, 2H, –CH_2), 3.50 (m, 4H, mor–CH_2–O–CH_2–), 3.64 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.23–4.25 (t, J = 6.8 Hz, 2H, –CH_2), 7.86–7.95 (m, 3H, Ar–H), 8.28–8.30 (m, 1H, Ar–H).

5.3. Preparation of 3-substituted (4-oxo-3,4-dihydrophthalazin-1-yl)acetohydrazide 6a–c

A mixture of esters **5(a–c)** (0.05 mol) and hydrazine hydrate (0.10 mol) in ethanol (200 mL) were heated under reflux for 10 h. After completion of reaction, cooled to room temperature, the precipitated product was filtered and washed with cold ethanol to give corresponding hydrazides **6a**–**c** as white solid.

5.3.1. 2-{3-[(6-chloropyridin-3-yl) methyl]-4-oxo-3, 4-dihydrophthalazin-1-yl} acetohydrazide (**6a**)

By using compound **5a**, the title compound was obtained. Yield: 86.9%; M.p.: 206.2–210.0 °C; MS: m/z = 344.1 (M + 1); Purity: 99.3%; ^{1}H NMR: (400 MHz, DMSO-d_6) δ: 3.79 (s, 2H, CH_2), 4.27 (brs, 2H, NH_2), 5.34 (s, 2H, CH_2), 7.48–7.50 (d, J = 8.4 Hz, 1H, Ar–H), 7.80–7.83 (dd, J = 8.4, 2.4 Hz, 1H, Ar–H), 7.86–7.98 (m, 3H, Ar–H), 8.28–8.30 (m, 1H, Ar–H), 8.43–8.44 (d, J = 2.4 Hz, 1H, Ar–H), 9.35 (brs, 1H, NH).

5.3.2. 2-{3-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetohydrazide (**6b**)

The title compound was obtained from **5b**. Yield: 83.2%; M.p.: 214.9–218.5 °C; MS: m/z = 350.1 (M + 1); Purity: 98.5%; ^{1}H NMR (400 MHz, DMSO-d_6): 3.80 (s, 2H, CH_2), 4.28 (brs, 2H, NH_2), 5.46 (s, 2H, CH_2), 7.78 (s, 1H, Ar–H), 7.86–7.98 (m, 3H, Ar–H), 8.30–8.32 (m, 1H, Ar–H), 9.38 (s, 1H, NH).

5.3.3. 2-{3-[2-(morpholin-4-yl)ethyl]-4-oxo-3,4-dihydrophthalazin-1-yl}acetohydrazide (**6c**)

By using **5c**, the title compound was obtained. Yield: 88.6%; M.p.: 187.0–191.7 °C; MS: m/z = 332.2 (M + 1); Purity: 99.1%; ^{1}H NMR(400 MHz, DMSO-d_6) δ: 2.44 (m, 4H, mor–CH_2–N–CH_2–), 2.66–2.69 (t, J = 6.8 Hz, 2H, CH_2), 3.51–3.53 (m, 4H, mor–CH_2–O–CH_2–), 3.78 (s, 2H, CH_2), 4.22–4.25 (t, J = 6.8 Hz, 2H, CH_2), 4.26–4.27 (brs, 2H, NH_2), 7.84–7.89 (m, 1H, Ar–H), 7.92–7.93 (m, 2H, Ar–H), 8.27–8.29 (m, 1H, Ar–H), 9.34 (brs, 1H, NH).

5.4. General procedure for the synthesis of 2-substituted [4-(1,3,4-oxadiazol-2-yl) methyl] phthalazin-1(2H)-one derivatives 7(**a**–**h**) to 9(**a**–**h**)

A solution of hydrazide **6a**–**c** (0.1 mmol), and corresponding acid (0.1 mmol) in phosphorous oxychloride (5 mL), was refluxed for 6–8 h. After cooling, the excess phosphorous oxychloride was evaporated under reduced pressure. The residue obtained was diluted with ice water (50 mL), neutralised with saturated sodium bicarbonate, and extracted with ethyl acetate (50 mL × 3). The combined organic layer was dried over anhydrous sodium sulphate. After filtration, the solvent was evaporated to get crude product, which was purified by using CombiFlash® Companion® flash chromatography system using ethyl acetate/hexane as mobile phase to get the corresponding phthalazin-1(2*H*)-one oxadiazole derivatives **7a**–**h** to **9a**–**h**.

5.4.1. 2-[(6-chloropyridin-3-yl)methyl]-4-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]phthalazin-1(2H)-one (**7a**)

White solid; Yield: 62.2%; Purity = 98.8%; M.p.: 175.9–179.4 °C; MS: m/z = 368.1 (M + 1); IR (KBr) cm^{-1}: 2924, 1718, 1656, 1587; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 4.49 (s, 2H, –C–CH_2–C–), 5.34 (s, 2H, –N–CH_2–C–), 7.27–7.29 (d, J = 8.4 Hz, 1H, Ar–H), 7.78–7.84 (dd, J = 8.4, 2.4 Hz, 1H, Ar–H), 7.86–7.88 (m, 3H, Ar–H), 8.44–8.46 (m, 1H, Ar–H), 8.49 (d, J = 2.4 Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.7, 163.7, 158.7, 150.1, 149.9, 141.9, 140.0, 134.3, 132.9, 132.7, 128.9, 127.6, 127.0, 126.0, 124.7, 50.9, 29.1, 10.9.

*5.4.2. 2-[6-(chloropyridin-3-yl)methyl]-4-{[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**7b**)*

White solid; Yield: 43.2%; Purity = 99.0%; M.p.: 117.5–119.8 °C; MS: m/z = 422.1 (M + 1); IR (KBr) cm^{-1}: 2830, 1719, 1652, 1599; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.64 (s, 2H, −C−CH_2−C−), 5.31 (s, 2H, −N−CH_2−C), 7.27–7.28 (d, J = 8.4 Hz, 1H, Ar−H), 7.72–7.74 (dd, J = 8.4, 2.4 Hz, 1H, Ar−H), 7.81–7.88 (m, 3H, Ar−H), 8.48–8.49 (d, J = 2.4 Hz, 2H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.8, 163.3, 158.4, 151.5, 150.2, 142.9, 141.2, 135.3, 132.9, 132.2, 128.5, 128.0, 126.8, 125.2, 124.9, 124.2, 50.8, 29.2.

*5.4.3. 2-[(6-chloropyridin-3-yl)methyl]-4-[(5-cyclopropyl-1,3,4-oxadiazol-2-yl)methyl] phthalazin-1(2H)-one (**7c**)*

White solid; Yield: 43.2%; Purity = 97.6%; M.p.: 158.9–161.8 °C; MS: m/z = 394.1 (M + 1); IR (KBr) cm^{-1}: 2923, 1718, 1656, 1587; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.98–1.09 (m, 4H, −CH_2−CH_2), 2.06–2.12 (m, 1H, Alk−CH), 4.46 (s, 2H, −C−CH_2−C−), 5.33 (s, 2H, −N−CH_2−C), 7.27–7.29 (d, J = 8.4 Hz, 1H, Ar−H), 7.76–7.78 (dd, J = 8.4, 2.4 Hz, 1H, Ar−H), 7.79–7.88 (m, 3H, Ar−H), 8.44–8.47 (m, 1H, Ar−H), 8.50 (d, J = 2.4 Hz, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.2, 161.8, 158.7, 149.8, 141.9, 139.9, 134.3, 132.9, 132.6, 129.5, 129.0, 128.9, 127.6, 126.0, 124.8, 50.8, 29.2, 17.0, 11.5.

*5.4.4. 2-[(6-chloropyridin-3-yl)methyl]-4-{[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)- one (**7d**)*

White solid; Yield: 70.4%; Purity = 99.1%; M.p.: 184.6–186.5 °C; MS: m/z = 448.1 (M + 1); IR (KBr) cm^{-1}: 2930, 1719, 1657, 1591; ^1H NMR (400 MHz, $CDCl_3$): δ 4.61 (s, 2H, −C−CH_2−C−), 5.35 (s, 2H, −N−CH_2−C−), 7.19–7.25 (m, 2H, Ar−H), 7.24–7.26 (d, J = 8.0 Hz, 1H, Ar−H), 7.74–7.76 (dd, J = 8.0, 2.4 Hz, 1H, Ar−H), 7.77–7.83 (m, 2H, Ar−H), 7.85–7.99 (m, 3H, Ar−H), 8.46–8.48 (m, 1H, Ar−H), 8.51–8.52 (d, J = 2.4 Hz, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 164.7, 163.7, 150.7, 150.1, 149.9, 141.9, 140.0, 134.2, 134.3, 132.9, 132.7, 128.9, 127.6, 126.7, 124.7, 123.2, 122.7, 49.8, 29.2.

*5.4.5. 4-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-[(6-chloropyridin-3-yl)methyl] phthalazin-1 (2H)-one (**7e**)*

White solid; Yield: 70.2%; Purity = 98.7%; M.p.: 168.3–170.1 °C; MS: m/z = 464.1 (M + 1); IR (KBr) cm^{-1}: 2928, 1718, 1658, 1592; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.61 (s, 2H, −C−CH_2−C−), 5.35 (s, 2H, −N−CH_2−C−), 7.19–7.21 (d, J = 8.0 Hz, 1H, Ar−H), 7.49–7.51 (d, J = 8.1 Hz, 2H, Ar−H), 7.74–7.77 (dd, J = 8.0, 2.4 Hz, 1H, Ar−H), 7.79–7.83 (d, J = 8.1 Hz, 2H, Ar−H), 7.85–7.93 (m, 3H, Ar−H), 8.46–8.48 (m, 1H, Ar−H), 8.51–8.52 (d, J = 2.4 Hz 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.8, 164.1, 163.6, 150.6, 150.0, 149.2, 141.1, 139.1, 133.2, 134.2, 132.8, 131.7, 128.9, 127.2, 126.6, 125.6, 124.8, 123.1, 122.6, 50.1, 29.1.

*5.4.6. 2-[(6-chloropyridin-3-yl)methyl]-4-{[5-(2-methylphenyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**7f**)*

White solid; Yield: 72.9%; Purity = 98.2%; M.p.: 159.2–162.3 °C; MS: m/z = 444.1 (M + 1); IR (KBr) cm^{-1}: 2924, 1792, 1653, 1589; ^1H NMR (400 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 4.62 (s, 2H, −C−CH_2−C−), 5.35 (s, 2H, −N−CH_2−C−), 7.17–7.19 (d, J = 8.0 Hz, 1H, Ar−H), 7.32–7.34 (m, 2H, Ar−H), 7.40–7.43 (m, 1H, Ar−H), 7.73–7.75 (dd, J = 8.0, 1.6 Hz, 1H, Ar−H), 7.79–7.95 (m, 4H, Ar−H), 8.46–8.48 (m, 1H, Ar−H), 8.51–8.52 (d, J = 1.6 Hz, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.8, 164.1, 163.6, 150.6, 150.0, 149.2, 141.1, 139.1, 133.2, 134.2, 132.8, 131.7, 128.9, 127.2, 126.8, 126.6, 125.8, 125.0, 124.8, 123.1, 122.9, 50.1, 29.1, 22.2.

*5.4.7. 2-[(6-chloropyridin-3-yl)methyl]-4-{[5-(thiophen-3-yl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**7g**)*

White solid; Yield: 72.9%; Purity = 99.2%; M.p.: 154.2–157.1 °C; MS: m/z = 436.1 (M + 1); IR (KBr) cm^{-1}: 2924, 1790, 1653, 1585; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.78 (s, 2H, −C−CH_2−C−), 5.30 (s, 2H, −N−CH_2−C−), 7.35–7.37 (m, 1H, Ar−H), 7.56–7.57 (m, 1H, Ar−H), 7.71–7.73 (m, 1H, Ar−H), 7.91–8.09 (m, 4H, Ar−H), 8.26 (m, 1H, Ar−H), 8.32–8.34 (m, 1H, Ar−H), 8.37 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.1, 161.8, 158.7, 149.9, 141.8, 139.9, 134.4, 132.9, 132.5, 129.5, 129.0, 128.8, 127.6, 127.1, 126.0, 125.8, 124.7, 124.6, 123.7, 50.8, 29.1.

*5.4.8. 2-[(6-chloropyridin-3-yl)methyl]-4-{[5-(6-chloropyridin-3-yl)-1,3,4-oxadiazol-2-yl] methyl}phthalazin-1(2H)-one (**7h**)*

White solid; Yield: 79.5%; Purity = 97.9%; M.p.: 188.6–190.2 °C; MS: m/z = 465.1(M + 1); IR (KBr) cm^{-1}: 2896, 1789, 1658, 1593; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.63 (s, 2H, −C−CH_2−C−), 5.34 (s, 2H, −N−CH_2−C−), 7.22–7.24 (d, J = 8.0 Hz, 1H, Ar−H), 7.51–7.53 (d, J = 8.4 Hz, 1H, Ar−H), 7.74–7.76 (dd, J = 8.0, 2.4 Hz, 1H, Ar−H), 7.80–7.92 (m, 3H, Ar−H), 8.20–8.23 (dd, J = 8.0, 2.4 Hz, 1H, Ar−H), 8.47–8.48 (d, J = 2.4 Hz, 2H, Ar−H), 8.49 (m, 1H, Ar−H) 8.99–9.00 (d, J = 2.4 Hz, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.9, 164.2, 163.5, 152.1, 150.6, 149.4, 141.2, 139.8, 133.1, 134.1, 132.2, 131.6, 128.0, 127.1, 126.3, 124.7, 123.1, 122.6, 50.1, 29.1.

*5.4.9. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl] phthalazin-1(2H)-one (**8a**)*

White solid; Yield: 62.6%; Purity = 98.5%; M.p.: 163.2–165.9 °C; MS: m/z = 374.0 (M + 1); IR (KBr) cm^{-1}: 3070, 1655, 1591, 1457; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 4.52 (s, 2H, −C−CH_2−C−), 5.43 (s, 2H, −N−CH_2−C−), 7.76 (s, 1H, Ar−H), 7.78–7.94 (m, 3H, Ar−H), 8.32–8.34 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.2, 163.5, 152.9, 140.2, 135.1, 134.2, 132.3, 131.7, 128.1, 127.1, 126.3, 124.6, 123.1, 122.6, 50.2, 29.3.

*5.4.10. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-{[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl] methyl}phthalazin-1(2H)-one (**8b**)*

White solid; Yield: 50.1%; Purity = 98.7%; M.p.: 140.3–143.5 °C; MS: m/z = 428.0 (M + 1); IR (KBr) cm^{-1}: 2830, 1657, 1591, 1417; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.54 (s, 2H, −C−CH_2−C−), 5.44 (s, 2H, −N−CH_2−C−), 7.76 (s, 1H, Ar−H), 7.71–7.96 (m, 3H, Ar−H), 8.32–8.34 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.8, 164.0, 158.4, 152.0, 140.9, 140.7, 135.5, 135.1, 134.0, 132.6, 128.6, 127.5, 127.1, 125.1, 46.8, 29.5.

*5.4.11. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-[(5-cyclopropyl-1,3,4-oxadiazol-2-yl)methyl] phthalazin-1(2H)-one (**8c**)*

White solid; Yield: 32.0%; Purity = 98.3%; M.p.: 129.1–132.7 °C; MS: m/z = 400.1 (M + 1); IR (KBr) cm^{-1}: 2833, 1660, 1561, 1420; ^1H NMR (400 MHz, DMSO-d_6) δ: 0.89–1.01 (m, 4H, −CH_2−CH_2−), 1.45 (m, 1H, Alk−CH), 4.57 (s, 2H, −C−CH_2−C−), 5.49 (s, 2H, −N−CH_2−C−), 7.77 (s, 1H, Ar−H), 7.89–8.99 (m, 3H, Ar−H), 8.31–8.33 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.2, 164.5, 158.2, 152.9, 140.2, 140.1, 135.6, 135.2, 134.1, 132.6, 128.6, 127.5, 127.1, 46.8, 29.5, 17.0, 11.5.

*5.4.12. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-{[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl] methyl} phthalazin-1(2H)-one (**8d**)*

White solid; Yield: 80.6%; Purity = 98.5%; M.p.: 156.5–158.9 °C; MS: m/z = 454.1 (M + 1); IR (KBr) cm^{-1}: 2865, 1660, 1607, 1498; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.84 (s, 2H, −C−CH_2−C−), 5.43 (s, 2H, −N−CH_2−C−), 7.43–7.50 (m, 2H, Ar−H), 7.68 (s, 1H, Ar−H), 7.93–7.97 (m, 1H, Ar−H), 7.99–8.04 (m, 3H, Ar−H), 8.11–8.13 (m, 1H, Ar−H), 8.34–8.36 (m, 1H, Ar−H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 168.7, 167.2, 166.1, 164.8, 163.7, 163.5, 162.6, 158.7, 152.8, 141.0, 140.7, 133.9, 132.5, 127.5, 124.4, 116.6, 116.5, 46.6, 29.6.

*5.4.13. 4-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-[(2-chloro-1,3-thiazol-5-yl) methyl] phthalazin-1(2H)-one (**8e**)*

White solid; Yield: 76.9%; Purity = 98.7%; M.p.: 163.2–165.2 °C; MS: m/z = 470.1; IR (KBr) cm^{-1}: 2860, 1667, 1620, 1480; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.84 (s, 2H, −C−CH_2−C−), 5.42 (s, 2H, −N−CH_2−C−), 7.24–7.26 (d, J = 8.0 Hz, 2H, Ar−H), 7.67 (s, 1H, Ar−H), 7.71–7.76 (m, 3H, Ar−H), 8.16–8.18 (d, J = 8.0 Hz, 2H, Ar−H), 8.32–8.34 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.6, 166.2, 166.1, 164.6, 163.7, 163.4, 162.6, 158.7, 152.5, 141.1, 140.0, 133.2, 128.2, 127.5, 124.4, 116.6, 116.1, 46.9, 29.5.

*5.4.14. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-{[5-(2-methylphenyl)-1,3,4-oxadiazol-2-yl] methyl} phthalazin-1(2H)-one (**8f**)*

White solid; Yield: 80.1%; Purity = 99.5%; M.p.: 134.6–136.9 °C; MS: m/z = 450.1 (M + 1); IR (KBr) cm^{-1}: 2924, 2887, 1653, 1589; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.62 (s, 3H, CH_3), 4.88 (s, 2H, −C−CH_2−C−), 5.45 (s, 2H, −N−CH_2−C−), 7.42–7.48 (d, J = 4.0, 2H, Ar−H), 7.56 (s, 1H, Ar−H), 7.83–7.90 (m, 3H, Ar−H), 8.22–8.25 (m, 2H, Ar−H), 8.47–8.49 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.2, 160.3, 158.4, 151.5, 142.3, 141.5, 138.0, 136.1, 134.4, 133.0, 132.2, 131.9, 129.4, 129.1, 128.9, 127.4, 126.9, 126.2, 123.0, 46.7, 29.2, 22.1.

*5.4.15. 2-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-{[5-(thiophen-3-yl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**8g**)*

White solid; Yield: 80.1%; Purity = 99.0%; M.p.: 139.2–140.6 °C; MS: m/z = 442.1 (M + 1); IR (KBr) cm^{-1}: 2924, 1653, 1585; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.82 (s, 2H, CH_2), 5.43 (s, 2H, −CH_2), 7.42–7.43 (m, 1H, Ar−H), 7.53–7.57 (m, 2H, CH_2), 7.69 (s, 1H, Ar−H), 7.75–7.85 (m, 2H, Ar−H), 7.99–8.22 (m, 2H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.6, 166.2, 166.1, 164.6, 163.7, 163.4, 162.6, 158.7, 152.5, 141.1, 137.6.0, 133.2, 128.2, 127.5, 124.4, 116.6, 116.1, 46.9, 29.5.

*5.4.16. 4-{[5-(6-chloropyridin-3-yl)-1,3,4-oxadiazol-2-yl]methyl}-2-[(2-chloro-1,3-thiazol-5-yl) methyl]phthalazin-1(2H)-one (**8h**)*

White solid; Yield: 64.3%; Purity = 98.8%; M.p.: 181.2–183.0 °C; MS: m/z = 471.0 (M + 1); IR (KBr) cm^{-1}: 2896, 1658, 1593, 1520; ^1H NMR (400 MHz, DMSO-d_6) δ: 4.66 (s, 2H, −C−CH_2−C−), 5.43 (s, 2H, −N−CH_2−C−), 7.68 (s, 1H, Ar−H), 7.77–7.79 (d, J = 8.0 Hz, 1H, Ar−H), 7.90–8.13 (m, 3H, Ar−H), 8.32–8.34 (m, 1H, Ar−H), 8.36–8.39 (dd, J = 8.0, 2.2 Hz, 1H, Ar−H), 8.98 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 163.1, 159.4, 158.7, 156.9, 152.8, 148.8, 141.0, 134.4, 133.9, 132.4, 132.1, 128.6, 127.6, 124.3, 123.2, 115.2, 114.9, 46.7, 29.6.

*5.4.17. 4-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]-2-[2-(morpholin-4-yl)ethyl]phthalazin-1(2H)-one (**9a**)*

White solid; Yield: 49.6%; Purity = 98.7%; M.p.: 158.2–160.3 °C; MS: m/z = 356.1 (M + 1); IR (KBr) cm^{-1}: 2941, 1787, 1722, 1652; ^1H NMR(400 MHz, $CDCl_3$) δ: 2.35 (s, 3H, CH_3), 2.53 (m, 4H, mor−CH_2−N−CH_2−), 2.77–2.80 (t, J = 6.8 Hz, 2H, −CH_2), 3.66 (m, 4H, mor−CH_2−O−CH_2−), 4.34–4.37 (t, J = 6.8 Hz, 2H, −CH_2), 4.65 (s, 2H, CH_2), 7.87–7.96 (m, 3H, Ar−H), 8.28–8.30 (d, J = 7.6 Hz, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.2, 163.7, 158.7, 150.1, 140.0, 134.3, 132.9, 132.7, 128.9, 124.7, 66.8, 57.7, 53.4, 41.6, 29.2, 10.6.

*5.4.18. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9b**)*

White solid; Yield: 31.8%; Purity = 98.3%; M.p.: 139.1–140.5 °C; MS: m/z = 410.1 (M + 1); IR (KBr) cm^{-1}: 2950, 2816, 1782, 1720, 1656; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.52 (m, 4H, −CH_2−N−CH_2−), 2.78–2.81 (t, J = 6.8 Hz, 2H, CH_2), 3.64–3.66 (m, 4H, −CH_2−O−CH_2−), 4.33–4.36 (t, J = 6.8 Hz, 2H, CH_2), 4.65 (s, 2H, CH_2), 7.42–7.88 (m, 3H, Ar−H), 8.48–8.49 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.1, 163.6, 158.7, 150.0, 140.0, 134.3, 132.8, 132.6, 128.9, 125.9, 124.6, 66.7, 57.6, 53.6, 41.9, 29.1.

*5.4.19. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(cyclopropyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9c**)*

Semi solid; Yield: 26.9%; Purity = 98.9%; MS: m/z = 382.2 (M + 1); IR (KBr) cm^{-1}: 2955, 2826, 1781, 1720, 1656; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.98–1.08 (m, 4H, −CH_2−CH_2−), 2.06–2.12 (m, 1H, Alk−CH), 2.55 (m, 4H, −CH_2−N−CH_2−), 2.80–2.85 (t, J = 6.8 Hz, 2H, CH_2), 3.62–3.66 (m, 4H, −CH_2−O−CH_2−), 4.31–4.35 (t, J = 6.8 Hz, 2H, CH_2), 4.67 (s, 2H, CH_2), 7.84–7.89 (m, 1H, Ar−H), 7.89–7.93 (m, 2H, Ar−H), 8.27–8.29 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.3, 163.8, 158.7, 149.8, 141.9, 134.3, 132.9, 132.6, 129.5, 124.8, 66.8, 57.5, 53.3, 41.6, 29.1, 17.1, 10.9.

*5.4.20. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9d**)*

White solid; Yield: 76.5%; Purity = 99.2%; M.p.: 150.2–154.3 °C; MS: m/z = 436.1 (M + 1); IR (KBr) cm^{-1}: 2956, 2812, 1787, 1653; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.51 (m, 4H, −CH_2−N−CH_2−), 2.78–2.79 (t, J = 6.8 Hz, 2H, CH_2), 3.80–3.81 (m, 4H, −CH_2−O−CH_2−), 4.51–4.55 (t, J = 6.8 Hz, 2H, CH_2), 4.61 (s, 2H, CH_2), 7.36–7.38 (m, 1H, Ar−H), 7.47–7.56 (m, 3H, Ar−H), 7.77–7.93 (m, 2H, Ar−H), 8.00–8.02 (m, 1H, Ar−H), 8.45–8.47 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1, 164.8, 163.7, 163.5, 162.6, 158.7, 152.8, 141.0, 140.7, 133.9, 132.5, 127.5, 127.0, 126.1, 66.5, 57.5, 53.4, 41.9, 29.4.

*5.4.21. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9e**)*

White solid; Yield: 70.1%; Purity = 97.8%; M.p.: 156.2–158.6 °C; MS: m/z = 448.1 (M + 1); IR (KBr) cm^{-1}: 2957, 2860, 1787, 1782, 1657; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.53 (m, 4H, −CH_2−N−CH_2−), 2.79–2.82 (t, J = 6.8 Hz, 2H, CH_2), 3.64–3.66 (m, 4H, −CH_2−O−CH_2−), 3.86 (s, 3H, CH_3), 4.34–4.38 (t, J=6.8 Hz, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.96–6.98 (d, J = 8.8 Hz, 2H, Ar−H), 7.77–7.82 (m, 2H, Ar−H), 7.92–7.93 (d, J = 8.8 Hz, 2H, Ar−H), 7.94–7.95 (m, 1H, Ar−H), 8.46–8.48 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.7, 164.5, 163.9, 163.5, 162.6, 158.6, 152.7, 143.8, 142.7, 141.0, 140.7, 133.9, 130.5, 127.5, 127.0, 126.1, 66.7, 57.5, 53.4, 41.9, 29.3, 22.3.

*5.4.22. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(thiophen-3-yl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9f**)*

White solid; Yield: 49.2%; Purity = 99.2%; M.p.: 153.5–156.8 °C MS: m/z = 424.1 (M + 1); IR (KBr) cm^{-1}: 2815, 1643, 1575, 1453; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.52 (m, 4H, mor−CH_2−N−CH_2−), 2.78–2.81 (t, J = 6.8 Hz, 2H, CH_2), 3.64–3.66 (m, 4H, mor−CH_2−O−CH_2−), 4.34–4.37 (t, J = 6.8 Hz, 2H, CH_2), 4.60 (s, 2H, CH_2), 7.44–7.45 (m, 1H, Ar−H), 7.61–7.62 (m, 1H, Ar−H), 7.77–7.99 (m, 4H, Ar−H), 8.46–8.49 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.1, 161.8, 158.7, 149.9, 141.8, 139.9, 134.4, 132.9, 132.5, 129.5, 129.0, 128.8, 127.6, 127.1, 66.2, 57.0, 53.2, 41.8, 29.1.

*5.4.23. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(5-bromopyridinyl-3-yl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9g**)*

Off white solid; Yield: 70.9%; Purity = 98.7%; M.p.: 145.6–149.1 °C; MS: m/z = 497.1 (M + 1); IR (KBr) cm^{-1}: 2955, 1653, 1576, 1452; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.53 (m, 4H, mor−CH_2−N−CH_2−), 2.77–2.80 (t, J = 6.4 Hz, 2H, CH_2), 3.66 (m, 4H, mor−CH_2−O−CH_2−), 4.34–4.37 (t, J = 6.4 Hz, 2H, CH_2), 4.65 (s, 2H, CH_2), 7.79–7.92 (m, 3H, Ar−H), 8.45–8.50 (m, 2H, Ar−H), 8.83 (m, 1H, Ar−H), 9.14 (m, 1H, Ar−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1,

164.8, 163.7, 163.5, 162.6, 158.7, 152.8, 150.7, 141.0, 140.7, 133.9, 132.5, 127.5, 127.0, 126.1, 66.5, 57.5, 53.4, 41.9, 29.4.

*5.4.24. 2-[2-(morpholin-4-yl)ethyl]-4-{[5-(6-chloropyridinyl-3-yl)-1,3,4-oxadiazol-2-yl]methyl} phthalazin-1(2H)-one (**9h**)*

White solid; Yield: 70.9%; Purity = 98.4%; M.p.: 138.6–140.3 °C; MS: $m/z = 453.1$ (M + 1); IR (KBr) cm^{-1}: 2955, 1653, 1576, 1452; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.52 (m, 4H, mor–CH_2–N–CH_2–), 2.76–2.80 (t, J = 6.8 Hz, 2H, CH_2), 3.64–3.66 (m, 4H, mor–CH_2–O–CH_2–), 4.33–4.37 (t, $J = 6.8$ Hz, 2H, CH_2), 4.65 (s, 2H, CH_2), 7.48–7.50 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.79–7.92 (m, 3H, Ar–H), 8.26–8.29 (dd, $J = 8.4$, 2.2 Hz, 1H, Ar–H), 8.47–8.50 (m, 1H, Ar–H), 8.98–8.99 (d, $J = 2.2$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1, 164.8, 163.7, 163.5, 162.6, 158.7, 152.8, 150.7, 141.0, 140.7, 133.9, 132.5, 127.5, 127.0, 126.1, 66.5, 57.5, 53.4, 41.9, 29.4.

5.5. Pharmacology

The antimicrobial activity of newly synthesized compounds **7a**–**h** to **9a**–**h** was determined by well plate method in nutrient agar (antibacterial activity) and Sabouraud dextrose agar (antifungal activity). The *in vitro* antibacterial activity was carried out against 24 h old cultures of bacterial strains and 72 h old cultures of fungal strains. In this work, *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, were used to investigate the antibacterial activities and *Chrysosporium keratinophilum, Candida albicans, Microsporum gypsum, A. niger* were used to investigate the antifungal activities.

The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 100 and 50 μg/mL. Approximately 1 cm^3 of a 24 h broth culture was placed in sterile Petri dishes. Molten nutrient agar kept at 45 °C was then poured into the Petri dishes and allowed to solidify. Six millimeter diameter holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. The inhibition zone that appeared after 24 h, around the holes in each plate were measured as zone of inhibition in mm. Experiments were duplicated and standard deviation was calculated. The antimicrobial results were compared with amoxicillin and summarized in Table 1.

The zone of inhibition of antifungal activity was determined using 72 h old broth culture. The results were compared with metronidazole and summarized in Table 2.

Acknowledgements

The author A.M. Sridhara is thankful to Advinus Therapeutics Pvt. Ltd. Bangalore, India, and Chairman, Department of Studies in Industrial Chemistry, Kuvempu University, Shimoga, India for their help and support to the research.

References

[1] B. Abd El-Fattah, M.I. Al-Ashmawi, S. El-Feky, E. Roder, Egypt. J. Pharm. Sci. 29 (1988) 259–268.
[2] B.E. Bayoumy, S.A. El-Feky, M. El-Mobayed, Egypt. J. Chem. 33 (1991) 267–275.
[3] M.A. Khalil, S.M. El-Khawss, M.G. Kassem, Sci. Pharma 48 (1980) 344–349.
[4] O.M. Boland, C.C. Blackwell, B.F. Clarke, D.J. Ewing, Diabetes 42 (1993) 336–340.
[5] Y. Hamamoto, K. Nagai, M. Muto, C. Asagami, Exp. Dermatol. 2 (1993) 231–235.
[6] E. Del Olmo, B. Barboza, M.I. Ybarra, J.L. Lopez-Perez, R. Carron, M.A. Sevilla, C. Boselli, A. San Feliciano, Bioorg. Med. Chem. Lett. 16 (2006) 2786–2790.
[7] M. Napoletano, G. Norcini, F. Pellacini, F. Marchini, G. Morazzoni, P. Ferlenga, L. Pradella, Bioorg. Med. Chem. Lett. 10 (2000) 2235–2238.
[8] G. Bold, K.H. Altmann, J. Frei, M. Lang, P.W. Manley, P. Traxler, B. Wietfeld, J. Brueggen, E. Buchdunger, R. Cozens, S. Ferrari, P. Furet, F. Hofmann, G. Martiny-Baron, J. Mestan, J. Roesel, M. Sills, D. Stover, F. Acemoglu, E. Boss, R. Emmenegger, L. Laesser, E. Masso, R. Roth, C. Schlachter, W. Vetterli, D. Wyss, J.M. Wood, J. Med. Chem. 43 (2000) 2310–2323.
[9] J.M. Arif, M. Kunhi, A.A. Bekhit, M.P. Subramanian, K. Al-Hussein, H.Y. Aboul-Enein, F.M. Al-Khodairy, Asian Pac. J. Cancer Prev. 7 (2006) 249–252.
[10] M. Yamaguchi, K. Kamei, T. Koga, M. Akima, A. Maruyama, T. Kuroki, N. Ohi, J. Med. Chem. 36 (1993) 4052–4060.
[11] Y.X. Li, Y.P. Luo, Z. Xi, C.W. Niu, Y.Z. He, G.F. Yang, J. Agric. Food Chem. 54 (2006) 9135–9139.
[12] F.H.H. Leenen, D.L. Smith, R.M. Faraks, R.A. Reeves, A. Marquez-Julio, Am. J. Med. 82 (1987) 969–978.
[13] J.M. Leiro, E. Alvarez, J.A. Arranz, E. Cano, F. Orallo, Int. Immunopharmacol 4 (2004) 163–177.
[14] S. Tanaka, M. Tanaka, A. Akashi, Stroke 20 (1989) 1724–1729.
[15] R. Moroi, K. Ono, T. Saito, T. Akimoto, M. Sano, Chem. Pharm. Bull. 25 (1977) 830–835.
[16] J.P. Kemp, E.O. Meltzer, H.A. Orgel, M.J. Welch, G.A. Bucholtz, E. Middleton, S.L. Spector, J.J. Newton, J.L. Perhach Jr., J. Allergy. Clin. Immunol. 79 (1987) 893–899.
[17] G. Scheffler, J. Engel, B. Kutscher, W.S. Sheldrick, P. Bell, Archiv. der. Pharmazie. 321 (1988) 205–208.
[18] P.F. Kador, J.H. Kinoshita, N.E. Sharpless, J. Med. Chem. 28 (1985) 841–849.
[19] B.L. Mylari, E.R. Larson, T.A. Beyer, W.J. Zembrowski, C.E. Aldinger, M.F. Dee, T.W. Siegel, D.H. Singleton, J. Med. Chem. 34 (1991) 108–122.
[20] A.A. El-Emam, O.A. Al-Deeb, M.A. Al-Omar, J. Lehmann, Bioorg. Med. Chem. 12 (2004) 5107–5113.
[21] M.M. Dutta, B.N. Goswami, J.C.S. Kataky, J. Heterocycl. Chem. 23 (1986) 793–795.
[22] H. Foks, D. Pancechowska-Ksepko, M. Janowiec, Z. Zwolska, E. Augustynowicz-Kopec, Acta. Pol. Pharm. 61 (2004) 473–476.
[23] T.P. Mohan, R. Vishalakshi, K.S. Bhat, K.S. Rao, G.N. Kendappa, Indian J. Chem. Sect. B. Org. Chem. Incl. Med. Chem. 43 (2004) 1798–1801.
[24] D.A. Kennedy, L.A. Summers, J. Heterocycl. Chem. 18 (1981) 409–410.
[25] M. Santagati, M. Modica, A. Santagati, F. Russo, A. Caruso, V. Cutuli, E. Di Pietro, M. Amico-Roxas, Pharmazie 49 (1994) 880–884.
[26] F.A. Omar, N.M. Mahfouz, M.A. Rahman, Eur. J. Med. Chem. 31 (1996) 819–825.
[27] A. Zarghi, S.A. Tabatabai, M. Faizi, A. Ahadian, P. Navabi, V. Zanganeh, A. Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1863–1865.
[28] D. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Bioorg. Med. Chem. Lett. 19 (2009) 4492–4494.
[29] P. Maienfisch, M. Angst, F. Brandl, W. Fischer, D. Hofer, H. Kayser, W. Kobel, A. Rindlisbacher, R. Senn, A. Steinemann, H. Widmer, Pest. Manag. Sci. 57 (2001) 906–913.
[30] G. Marciniak, D. Decolin, G. Leclerc, N. Decker, J. Schwartz, J. Med. Chem. 32 (1988) 2289–2296.
[31] M. Tomizawa, J.E. Casida, Annu. Rev. Entomol. 48 (2003) 339–364.
[32] T.G. Sinay Jr., R.J. Sysko, Euro. Pat. 331314, 1989.
[33] S. Foldeak, A. Puodziunas, A.N. Kost, P. Gribauskas, Khim. -Farm. Zh. 3 (1969) 5–8.