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Synthesis and Antimicrobial Evaluation of New 5-Amino-2,3-dihydrophthalazine-1,4-dione Derivatives

Ewies F. Ewies[*1], Naglaa F. El-Sayed[1], Marwa El-Hussieny[1], Mohamed S. Abdel-Aziz[2]

[1]Organometallic and Organometalloid Chemistry Department, [2]Microbial Chemistry Department, National Research Centre, 33 El-Bohouth St. (former El Tahrir St.), P.O. 12622, Dokki, Giza, Egypt

Abstract

5-Amino-2,3-dihydrophthalazine-1,4-dione (Luminol **1**) reacted with some organophosphorus reagents such as phosphonium ylides, trialkyl phosphites, and tris(dialkylamino)phosphines to afford the corresponding olefinic products **5a-e**, alkoxy-, and dialkylamino derivatives of Luminol **6a-e**. 4-Thioxo-3,4-dihydrophthalazin-1(2*H*)-one derivative **7** was synthesized from the reaction of Luminol (**1)** with thiating Lawesson and Japanese reagents. Structures of the synthesized compounds were clarified based on their elemental and spectroscopic analyses. The synthesized compounds were evaluated for their antimicrobial activity against some bacterial and fungal strains.

***Keywords*:** Luminol, Wittig, Trialkyl phosphite, Antimicrobial activity

Introduction

Phthalazine and benzo[g]phthalazine derivatives[1] are nitrogen heterocyclic compounds that showed various biological activities, such as antimicrobial, [2] anti-inflammatory,[3] anticonvulsant,[4] leishmanicidal,[5] antibacterial,[6] and anticancer[7][8] and inhibitory effect to parasites.[9, 10] Also, they act as ligands for a variety of transition metals which have been used in chemosensor materials.[11-13]

Phthalazine is a vital moiety that forms the medication core of some drugs such as Hydralazine and Dihydralazine antihypertension which are used in treatment of hypertension and heart failure diseases.[14-16] There are many commercial drugs which have phthalazine moiety in their structure such as Zopolrestat which acts as aldose reductase inhibitor, and Azlastine; that treats intermittent and persistent Rhinitis[17] (Figure 1). Additionally, Luminol, **1** (5-amino-2,3-dihydrophthalazine-1,4-dione) is used in forensic medicine to detect blood in crime scenes, as a result of reacting with iron in heme and it used in biology to detect metals in cells.[18].

Although the significant progress in the production of antimicrobial drugs, microbes don't stop forming a resistance to the drugs which leads the scientists to have a continuous target to synthesize new and more effective antimicrobial drugs.[19-25] So, in this work, we try to synthesize some new compounds that may have antimicrobial activity starting from Luminol with consideration of our experience in the synthesis of new organophosphorus and heterocyclic compounds.[26-31]

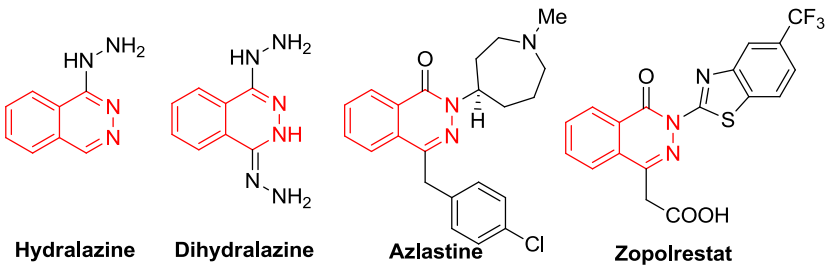

Fig. 1: Some commercial drugs of phthalazinone based structure

*Corresponding author e-mail: ewiesfawzy@yahoo.com.;ef.ewies@nrc.sci.eg (**Ewies F. Ewies**).
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Herein we studied the reactions of 5-amino-2,3-dihydrophthalazine-1,4-dione (Luminol **1**) with organophosphorus reagents, namely phosphonium ylides (**2a-e**), trialkyl phosphites (**3a-c**), tris(dialkylamino)phosphines (**3d,e**), thiating agents as Lawesson, and Japanese reagents (**4a,b**) to synthesize a series of phthalazine derivatives and evaluate their antimicrobial activities against some bacterial and fungal strains (Figure 2).

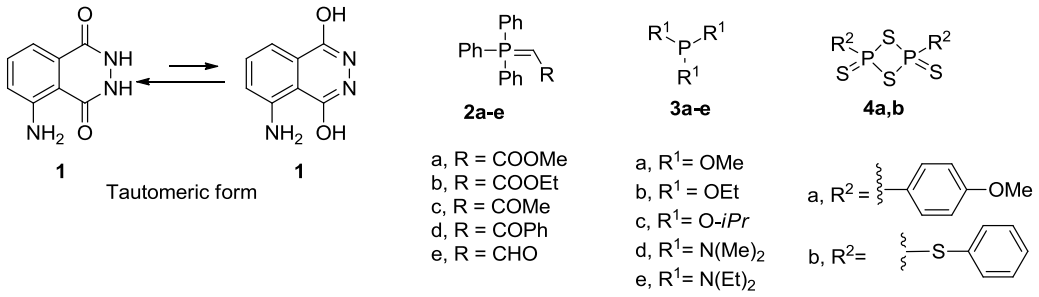
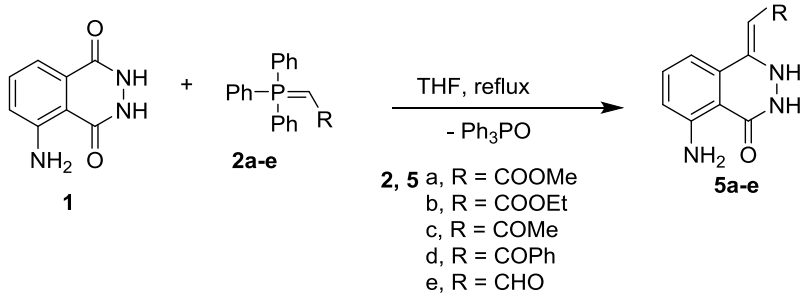


Fig. 2: starting material and reagents

Results and Discussion

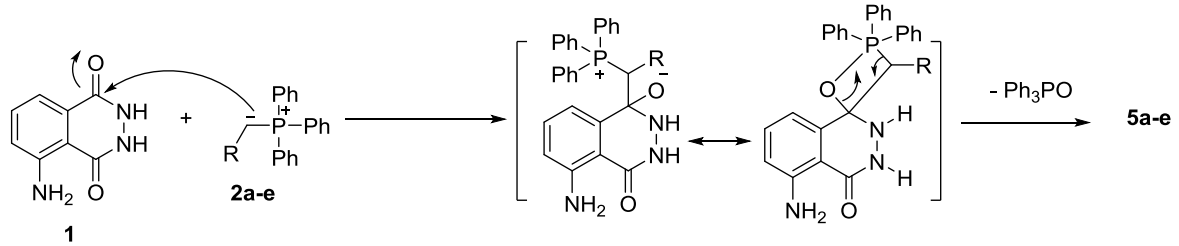
5-Amino-2,3-dihydrophthalazine-1,4-dione (Luminol **1**) reacted with various stabilized ylidenetriphenylphosphoranes (Wittig reagents, **2a-e**) in THF at reflux temperature for 10-12h (TLC monitoring) to afford the olefinic products **5a-e** in a good yield. Triphenylphosphine oxide (TPPO) was also isolated in each reaction and identified by comparing its m.p. and IR spectrum with those of a reference sample (Scheme 1).

Compatible elementary and spectroscopic results were gained for **5a-e**. The most characteristic features of compounds **5a-e** were the presence of =CH groups at around region of 5.90 ppm as singlet signals in ^{1}H NMR spectra and lacking the presence of absence of triphenyl phosphine group in both mass and NMR spectra. Also, ^{13}C NMR showed characteristic peaks of C=O group at characteristic peak of C=O group for each of the products at 168.3 (COOMe) **5a**, 168.2 (COOEt) **5b**, 194.2 (COMe) **5c**, 187.2 (COPh) **5d**, 174.2 ppm (CHO) **5e**, and amide at 161-164 ppm and carbonyl C=O at 161.2-164.5 ppm (*cf.* Experimental).



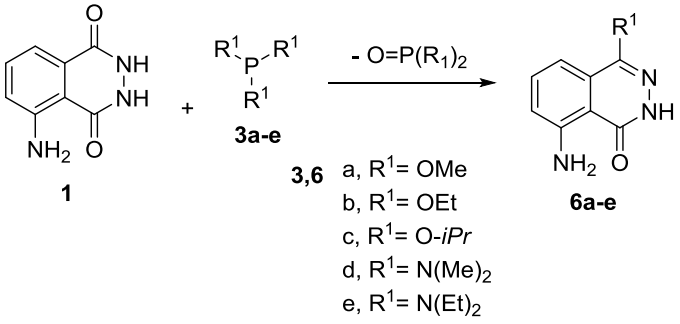
Scheme 1

The classical mechanism of Wittig reaction,[32] is applied to the formation of **5a-e**, through two-steps initiated by the nucleophilic attack of the ylides **2a-e** to less steric amide carbonyl-carbon to give the possible betaine oxaphosphetane intermediate. Decomposition of this oxaphosphetane subsequently occurs *via* a four-centered cyclic intermediate which ejects triphenyl phosphine oxide to afford the final products **5a-e** (Scheme 2).



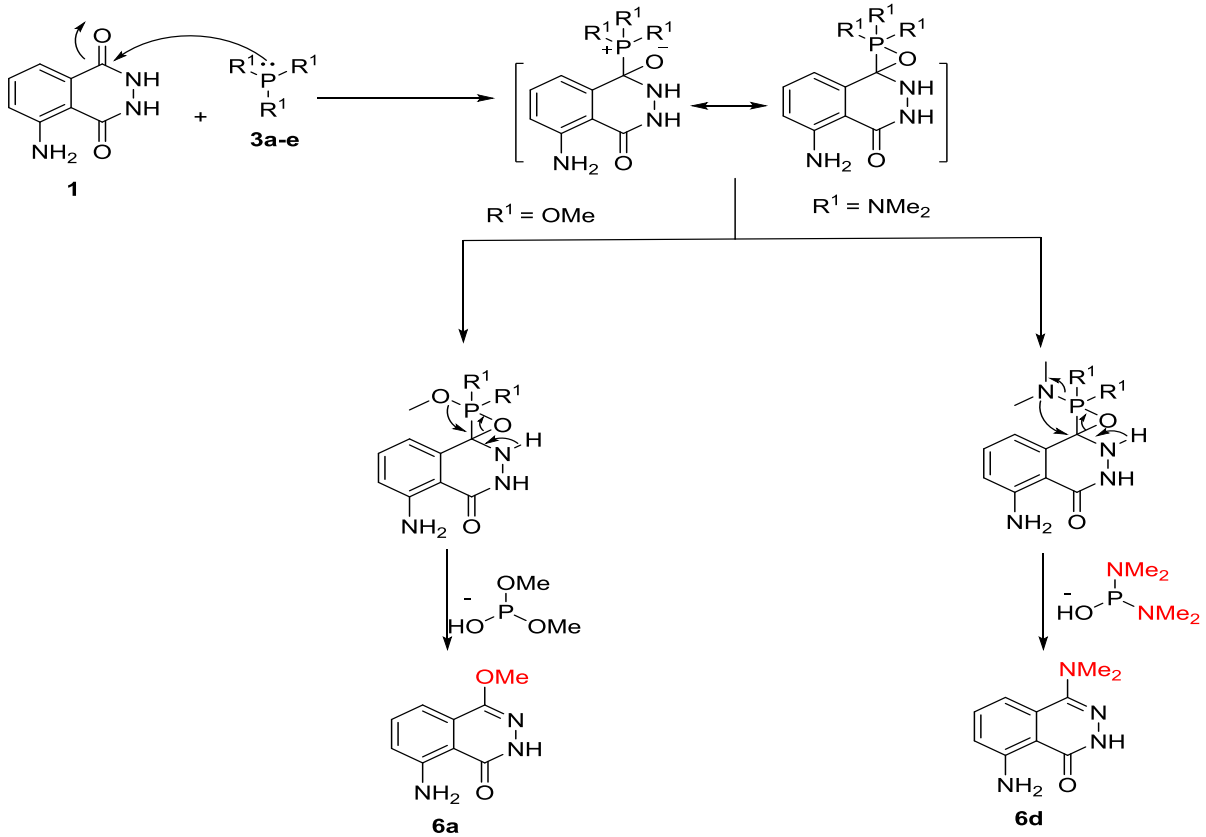
Scheme 2

On the other hand, the reaction of compound **1** with trialkyl phosphites **3a-c** or trisdialkylamino phosphines **3d,e** were proceeded in toluene or in THF at reflux temperature for 5-8h (TLC monitoring) to give the corresponding 8-amino-4-alkoxyphthalazin-1(2H)-one (**6a-c**) and 8-amino-4-(dialkylamino)-phthalazin-1(2H)-one (**6d,e**), respectively in excellent yield (Scheme 3). Structures of the products **6a-e** were elucidated on the basis and assignments of elemental and spectroscopic analysis (*cf.* Experimental).



Scheme 3

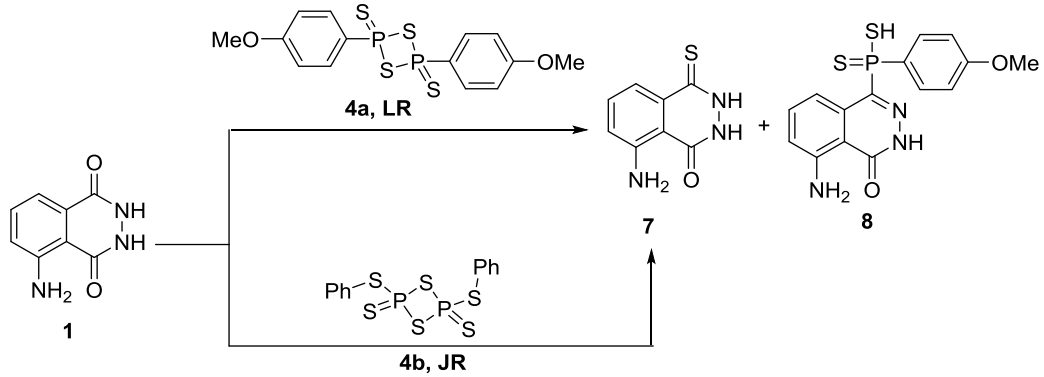
The acceptable mechanism is a nucleophilic attack of phosphorus lone pair to the carbonyl carbon to give the dipolar intermediate which cyclized to oxaphosphirane. This oxaphosphirane under influence of alkoxy or alkylamino group causes repulsion of dialkyl phosphonate or tetralkyl phosphinic diamide to give the final products **6a-e** (Scheme 4).



Scheme 4

The potentialities of Lawesson's reagent (LR, **4a**) and the Japanese reagent (JR, **4b**) as thiating agents have been tested among diverse classes of carbonyl compounds. [33] This promoted us to investigate the reaction of Lawesson's reagent (LR, **4a**) and Japanese reagent (JR, **4b**) with 5-amino-2,3-dihydrophthalazine-1,4-dione (**1**) in toluene at reflux temperature for 2h (TLC monitoring) which afford 8-amino-4-thioxo-3,4-dihydrophthalazin-1(2H)-one (**7**). The same yellow crystalline product in each reaction was obtained in good yield and no presence of the dithiophthalhydrazide[34], while phthalazin-1-yl-4-methoxyphenyl)phosphinodithioic acid derivative (**8**)

was isolated only with Lawesson's reagent in a 10% yield (Scheme 5). The structures of these new compounds were elucidated based on analytical and spectroscopic measurements (*cf.* Experimental).



Scheme 5

Antimicrobial Activity

Using agar cup plate method, most of our products were investigated for antimicrobial activity on four different test microbes namely: *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), *Candida albicans* (yeast) and *Aspergillus niger* (fungus). As shown in Table 1, the results indicated that, most of the tested compounds showed strong antifungal activity against *Aspergillus niger* by measuring the diameter of zone of inhibition (millimeter, mm).The descending order of the activity of compounds was **5e**>**6d**>**6c**>**6a**>**6b**>**1**>**5b**>**5a**>**5c**>**5d**. Also, they showed activity against *Candida albicans* (yeast) except Luminol **1** and **5e**, which have no activity. Compound **5b** showed a good activity against *Staphylococcus aureus* (Gram +ve) with inhibition zone of (28 mm), and showed a good activity against *Candida albicans* with inhibition zone of (25mm).Most of compounds showed no activity against *Escherichia coli* (Gram -ve).

Table 1. *In vitro* antimicrobial activity of the new synthesized compounds against a variety of pathogenic microorganisms

Sample Name	Clear zone (ϕmm)			
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***	***Aspergillus niger***
Luminol 1	**0**	**0**	**0**	19
5a	13	14	16	17
5b	**28**	16	**25**	18
5c	**0**	**0**	13	16
5d	12	16	14	15
5e	13	**0**	**0**	**30**
6a	12	**0**	12	**21**
6b	15	**0**	12	**20**
6c	12	**0**	12	**23**
6d	13	14	16	**28**
6e	**0**	12	15	**23**
8	**21**	**0**	16	**20**

Conclusion

The reactions of Luminol with different phosphorus and thiating reagents revealed formation of various products depending on the nature of the reagents. The preferred site of attack is the less steric carbonyl group of Luminol. The synthesized compounds were evaluated for their antimicrobial activities. The antimicrobial activity of most of the new compounds showed marked selectivity against fungi. Only Compound **5b** showed a good activity against *Staphylococcus aureus* and *Candida albicans.* The antimicrobial activity of the active compounds is a consequence of the biological and pharmacological activity due to presence of alkylated phthalazine moiety and not related to phtahlizine which has no antimicrobial activity.

Experimental

Chemistry

All chemicals were supplied by either Fluka or Aldrich chemical companies and were used without further purification. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementar and were found within ±0.4% of the theoretical values. **FT-IR** spectra were recorded with a Perkin-Elmer Frontier. Routine **NMR** spectra were recorded at room temperature on a Bruker Avance TM 300 spectrometer as solutions in dimethyl sulfoxide (DMSO). All chemical shifts are quoted in δ relative to the trace resonance of protonated dimethyl sulfoxide (δ2.50 ppm), DMSO (δ39.51 ppm) and external 85% aqueous H_3PO_4 (δ 0.0 ppm). The **mass** spectra were measured with a GC Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at λ_{254} nanometer for few seconds. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated by column chromatography. Solvents were dried/purified according to conventional procedures. 5-Amino-2,3-dihydrophthalazine-1,4-dione Luminol **1** was purchased from Sigma Aldrich.

Reaction of 5-Amino-2,3-dihydrophthalazine-1,4-dione (Luminol 1) with phosphonium ylides (2a-e) General procedures

Compound **1** (1 mmol) with phosphonium ylides **2a-e** (1 mmol) in around bottom flask and 30 mL THF were refluxed for 10h. After reaction completion (TLC monitoring), the solvent was evaporated under vacuum and the residue was chromatographed on silica gel column chromatography using ethyl acetate/petroleum ether (60-80°C) as eluent to give the desired products **5a-e**. Also, Triphenyl phosphine oxide was isolated and identified (TLC, m.p. and mix m.p.)

Methyl 2-(5-amino-4-oxo-3,4-dihydrophthalazin-1(2H)-ylidene)acetate (5a)

Eluent: petroleum ether (60-80°C) /EtOAc (70/30, v/v). Product **5a** was separated as deep green crystals, yield 60%. m.p 297-300°C. IR (KBr, cm^{-1}): 3422 (NH_2), 3386 (NH), 1735, 1654 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.78 (s, 1H, NH), 7.39 (t, *J* = 7.9 Hz, 1H, CH_{arom}), 7.29 (br. s. 2H, NH_2), 6.98 (d, *J* = 7.4 Hz, 1H, CH_{arom}), 6.83 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 5.73 (s, 1H, =CH), 3.73 (s, 3H, CH_3), 2.18 (s, 1H, NH).^{13}C NMR (75 MHz, DMSO) δ 168.3 (C=O), 161.2 (C=O), 153.2, 97.4 (C=CH), 150.5 (C-NH_2), 133.3, 128.2, 116.0, 111.0, 109.9 (C_{arom}), 63.3 (CH_3). MS (*m/z*): M^+ 233 (30%). Analysis for $C_{11}H_{11}N_3O_3$(233.22). Calced.: % C, 56.65; H, 4.75; N, 18.02. Found: %C, 56.42; H, 4.58; N, 18.25.

Ethyl 2-(5-amino-4-oxo-3,4-dihydrophthalazin-1(2H)-ylidene)acetate (5b)

Eluent: petroleum ether (60-80°C) /EtOAc (65/35, v/v). Product **5b** was separated as brown crystals, yield 55 %. mp 310-312°C. IR (KBr, cm^{-1}): 3420 (NH_2), 3380 (NH), 1695, 1640 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.80 (s, 1H, NH), 7.40 (t, *J* = 7.9 Hz, 1H, CH_{arom}), 7.30 (br. s. 2H, NH_2), 7.01 (d, *J* = 7.4 Hz, 1H, CH_{arom}), 6.80 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 5.94 (s, 1H, =CH), 4.43 (q, 2H, CH_2), 1.27 (t, 3H, CH_3), 2.18 (s, 1H, NH).^{13}C NMR (75 MHz, DMSO) δ 168.2 (C=O), 164.2 (C=O), 152.2, 90.4 (C=CH), 149.5 (C-NH_2), 135.3, 129.2, 115.0, 112.0, 105.9 (C_{arom}), 63.3 (CH_2), 14.3 (CH_3).MS (*m/z*): M^+ 247 (20%). Analysis for $C_{12}H_{13}N_3O_3$(247.25). Calced.: % C, 58.29; H, 5.30; N, 16.99. Found: %C, 58.42; H, 5.53; N, 16.68.

8-Amino-4-(2-oxopropylidene)-3,4-dihydrophthalazin-1(2H)-one (5c)

Eluent: petroleum ether (60-80°C) /EtOAc (70/30, v/v). Product **5c** was separated as brown crystals, yield 45%. mp 255-256°C. IR (KBr, cm^{-1}): 3422 (NH_2), 3386 (NH), 1665, 1654 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.39 (s, 1H, NH), 7.30 (t, *J* = 7.9 Hz, 1H, CH_{arom}), 7.10 (d, *J* = 6.6 Hz, 2H, NH_2), 6.81 (d, *J* = 7.4 Hz, 1H, CH_{arom}), 6.83 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 6.43 (s, 1H, =CH), 2.63 (s, 3H, CH_3), 2.17 (s, 1H, NH).^{13}C NMR (75 MHz, DMSO) δ 194.2 (C=O), 162.5 (C=O), 159.7, 98.2 (C=CH), 149.2 (C-NH_2), 135.3, 131.2, 115.0, 110.0, 105.9 (C_{arom}), 33.3 (CH_3).MS (*m/z*): M^+ 217 (30%). Analysis for $C_{11}H_{11}N_3O_2$(217.22). Calced.: % C, 60.82; H, 5.10; N, 19.34. Found: %C, 60.98; H, 5.36; N, 19.02.

8-Amino-4-(2-oxo-2-phenylethylidene)-3,4-dihydrophthalazin-1(2H)-one (5d)

Eluent: petroleum ether (60-80°C) /EtOAc (50/50, v/v). Product **5d** was separated as green crystals, yield 60 %. mp 243-245°C. IR (KBr, cm^{-1}): 3422 (NH_2), 3386 (NH), 1670, 1650 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.79-7.50 (m, 5H, CH_{arom}), 7.40 (t, *J* = 7.9 Hz, 1H, CH_{arom}), 7.31 (d, *J* = 7.4 Hz, 1H, CH_{arom}), 6.83 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 6.56 (s, 1H, =CH), 4.30 (br. s. 2H, NH_2), 3.53 (s, 1H, NH), 1.78 (s, 1H, NH).^{13}C NMR (75 MHz, DMSO) δ 187.2 (C=O), 164.5 (C=O), 151.7, 94.2 (C=CH), 149.4 (C-NH_2), 138.3, 135.2, 133.1, 131.8, 128.7, 128.4, 112.0, 105.9 (C_{arom}). MS (*m/z*): M^+279 (30%). Analysis for

$C_{16}H_{13}N_3O_2$ (279.29). Calced.: % C, 68.81; H, 4.69; N, 15.05. Found: %C, 68.55; H, 4.85; N, 15.20.

2-(5-Amino-4-oxo-3,4-dihydrophthalazin-1(2H)-ylidene)acetaldehyde(5e)

Eluent: petroleum ether (60-80ºC) /EtOAc (80/20, v/v). Product **5e** was separated as pale brown crystals, yield 65 %. 211-213ºC. IR (KBr, cm^{-1}): 3435 (NH_2), 3380 (NH), 1688, 1647 (C=O).^{1}H NMR (300 MHz, DMSO) δ 9.68 (s, 1H, CHO), 8.46 (s, 1H, NH), 7.42 (t, *J* = 7.9 Hz, 1H, CH_{arom}), 6.99 (d, *J* = 7.1 Hz, 1H, CH_{arom}), 6.85 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 6.05 (s, 1H, NH), 5.90 (s, 1H, =CH), 3.13 (br. s. 2H, NH_2). ^{13}C NMR (75 MHz, DMSO) δ 174.2 (aldehyde C=O), 161.6 (C=O), 153.3 (cyclic NH-*C*=), 150.9 (cyclic *C*-NH_2), 135.3, 130.5, 112.6, 112.2, 101.4 (C_{arom}), 99.6 (=CH). MS (*m/z*): M^+ 203 (30%). Analysis for $C_{10}H_9N_3O_2$ (203.20). Calced.: % C, 59.11; H, 4.46; N, 20.68. Found: % C, 58.89; H, 4.23; N, 20.82.

Reaction of 5-Amino-2,3-dihydrophthalazine-1,4-dione (1) with trialkylphosphites 3a-c and trisdilkylaminophosphines 3d,e

General procedures

In around bottom flask, Compound **1** (1 mmol) and reagents **3a-e** (1.5 mmol) were refluxed for 10h in toluene or THF for 5-8h (TLC monitoring). After reaction completion, the solvent is evaporated under vacuum and the residue was chromatographed on silica gel column chromatography using ethyl acetate/petroleum ether (60-80ºC) as eluent to give the desired products **6a-e**.

8-Amino-4-methoxyphthalazin-1(2*H*)-one (6a)

Eluent: petroleum ether (60-80ºC) /EtOAc (55/45, v/v). Product **6a** was separated as yellow crystals, yield 75 %. mp 195-197ºC. IR (KBr, cm^{-1}): 3429 (NH_2), 3390 (NH), 1661 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.62 – 7.55 (t, 1H, CH_{arom}), 7.28 (d, *J* = 7.1 Hz, 1H, CH_{arom}), 6.88 (d, *J* = 8.0 Hz, 1H, CH_{arom}), 3.42-3.33 (m, 5H, NH_2, CH_3), 2.87 (s, H, NH). ^{13}C NMR (75 MHz, DMSO) δ 161.3 (C=O), 155.0 (cyclic *C*-NH_2), 152.9 (cyclic *C*-O), 134.9, 127.6, 121.2, 116.2, 112.5 (C_{arom}), 51.9 (Me). MS (*m/z*): M^+ 191 (25%). Analysis for $C_9H_9N_3O_2$ (191.19). Calced.: % C, 56.54; H, 4.74; N, 21.98. Found: % C, 56.88; H, 4.51; N, 22.04.

8-Amino-4-ethoxyphthalazin-1(2H)-one (6b)

Eluent: petroleum ether (60-80ºC) /EtOAc (55/45, v/v). Product **6b** was separated as yellow crystals, yield 85 %. mp 188-190ºC. IR (KBr, cm^{-1}): 3430 (NH_2), 3385 (NH), 1660 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.62 – 7.55 (t, 1H, CH_{arom}), 7.28 (d, *J* = 7.1 Hz, 1H, CH_{arom}), 6.88 (d, *J* = 8.0 Hz, 1H, CH_{arom}), 4.42 (q, 2H, CH_2), 3.42-3.33 (br. s. 2H, NH_2), 2.87 (s, H, NH), 1.92 (t, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 161.9 (C=O), 154.0 (cyclic *C*-NH_2), 150.9 (cyclic *C*-O), 135.9, 126.6, 121.4, 116.5, 111.5 (C_{arom}), 65.9 (CH_2), 14.9 (Me). MS (*m/z*): M^+ 205 (25%). Analysis for $C_{10}H_{11}N_3O_2$ (205.21). Calced.: % C, 58.53; H, 5.40; N, 20.48. Found: % C, 58.76; H, 5.17; N, 20.14.

8-Amino-4-isopropoxyphthalazin-1(2H)-one (6c)

Eluent: petroleum ether (60-80ºC) /EtOAc (50/50, v/v). Product **6c** was separated as deep brown crystals, yield 50 %. mp 166-168ºC. IR (KBr, cm^{-1}): 3434 (NH_2), 3381 (NH), 1647 (C=O).^{1}H NMR (300 MHz, DMSO) δ 7.46 (t, *J* = 7.8 Hz, 1H, CH_{arom}), 6.95 (d, *J* = 7.5 Hz, 1H, , CH_{arom}), 6.89 (d, *J* = 8.0 Hz, 1H, CH_{arom}), 4.05 (br. s. 2H, NH_2), 3.95 (m, 1H, CH), 2.51 (d, 6H, CH_3), 1.87 (s, H, NH). ^{13}C NMR (75 MHz, DMSO) δ 161.80 (C=O), 151.93 (cyclic *C*-NH_2), 151.13 (cyclic *C*-O), 134.33, 127.04, 122.3, 116.89, 110.93, 109.92 (C_{arom}), 72.96 (CH), 23.56 (Me). MS (*m/z*): M^+ 219 (25%). Analysis for $C_{11}H_{13}N_3O_2$ (219.24). Calced.: % C, 60.26; H, 5.98; N, 19.17. Found: % C, 60.50; H, 5.75; N, 18.91.

8-Amino-4-(dimethyl amino)phthalazin-1(2H)-one (6d)

Eluent: petroleum ether (60-80ºC) /EtOAc (40/60, v/v). Product **6d** was separated as yellow crystals, yield 65 %. Mp 175-177ºC. IR (KBr, cm^{-1}): 3422 (NH_2), 3380 (NH), 1657 (C=O).^{1}H NMR (300 MHz, DMSO) δ 11.84 (s, 1H, NH), 7.41 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.33 (d, *J* = 1.0 Hz, 1H, CH_{arom}), 7.03 (d, 1H, CH_{arom}), 6.77 (s, 2H, NH_2), 2.61 (s, 6H, Me). ^{13}C NMR (75 MHz, DMSO) δ 159.84 (C=O), 151.13 (cyclic *C*-O), 147.16 (cyclic *C*-NH_2), 132.38, 130.73, 118.75, 113.00, 111.08 (C_{arom}), 43.29 (2 Me). MS (*m/z*): M^+ 204 (25%). Analysis for $C_{10}H_{12}N_4O$ (204.23). Calced.: % C, 58.81; H, 5.92; N, 27.43. Found: % C, 59.01; H, 6.12; N, 27.11.

8-Amino-4-(diethyl amino)phthalazin-1(2H)-one (6e)

Eluent: petroleum ether (60-80ºC) /EtOAc (50/50, v/v). Product **6e** was separated as yellow crystals, yield 65 %. mp 183-185ºC. IR (KBr, cm^{-1}): 3437 (NH_2), 3385 (NH), 1657 (C=O).^{1}H NMR (300 MHz, DMSO) δ 11.80 (s, 1H, NH), 7.40 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.30 (d, *J* = 1.0 Hz, 1H, CH_{arom}), 7.05 (d, 1H, CH_{arom}), 6.75 (br. s. 2H, NH_2), 3.61 (m, 4H, 2 CH_2), 1.21 (m, 6H, 2 CH_3). ^{13}C NMR (75 MHz, DMSO) δ 160.84 (C=O), 150.30 (cyclic *C*-NH_2), 145.16 (cyclic *C*-O), 132.38, 129.73, 116.75, 115.00, 106.08 (C_{arom}), 43.29 (2 CH_2), 13.5 (2 Me). MS (*m/z*): M^+ 232(15%). Analysis for $C_{12}H_{16}N_4O$ (232.38). Calced.: % C, 62.05; H, 6.94; N, 24.12. Found: % C, 62.30; H, 6.71; N, 24.45.

Reaction of 5-Amino-2,3-dihydrophthalazine-1,4-dione (1) with Lawesson's and Japanese reagents 4a,b

General procedures

Compound (**1**,1mmol) added to a solution of toluene containing thiating Lawesson or Japanese reagents **4a,b** (0.5 mmol) at reflux temperature for 2h (TLC monitoring). The reaction mixture filtered, the solvent evaporated under reduced pressure and the residue was chromatographed on silica gel column chromatography using ethyl acetate/petroleum ether (60-80ºC) as eluent to give the desired products **7** and **8** in case of Lawesson's reagent and compound **7** only in case of Japanese reagent.

8-Amino-4-thioxo-3,4-dihydrophthalazin-1(2H)-one (7)

Eluent: petroleum ether (60-80ºC) /EtOAc (35/65, v/v). Product **7** was separated as yellow crystals, yield 75 %. mp.215-217ºC. IR (KBr, cm^{-1}): 3422 (NH_2), 3385 (NH), 1662 (C=O), 1052 (C=S).^{1}H NMR (300 MHz, DMSO) δ 9.80 (s, 1H, NH), 7.41 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.31 (d, *J* = 1.0 Hz, 1H, CH_{arom}), 7.05 (d, 1H, CH_{arom}), 6.75 (s, 1H, NH), 4.61 (br. s. 2H, NH_2). ^{13}C NMR (75 MHz, DMSO) δ 187.8 (C=S), 164.8 (C=O), 148.3 (cyclic *C*-NH_2), 140.3, 137.7, 123.75, 115.0, 110.8 (C_{rom}). MS (*m/z*): M^+ 193 (15%). Analysis for $C_8H_7N_3OS$ (193.23). Calced.: % C, 49.73; H, 3.65; N, 21.75; S, 16.59. Found: % C, 49.96; H, 3.33; N, 21.51; S, 16.25.

(5-Amino-4-oxo-3,4-dihydrophthalazin-1-yl)(4-methoxyphenyl)phosphinodithioic acid (8)

Eluent: petroleum ether (60-80ºC) /EtOAc (35/65, v/v). Product **8a** was separated as yellow crystals, yield 10 %. mp 222-225ºC. IR (KBr, cm^{-1}): 3437 (NH_2), 3383 (NH), 1659 (C=O), 1004 (P=S). ^{1}H NMR (300 MHz, DMSO) δ 12.45 (s, 1H, NH), 7.55-7.38 (m, 3H, CH_{arom}), 6.94-6.82 (m, 4H, CH_{arom}), 4.60 (br. s. 2H, NH_2), 3.72 (s, 3H, CH_3), 1.19 (s, 1H, SH).^{13}C NMR (75 MHz, DMSO) δ 164.6 (cyclic C-O), 161.9 (C=O), 150.4 (cyclic *C*-NH_2), 137.7, 134.6, 133.7, 121.7, 118.0, 115.4, 114.5, 112.6 (C_{arom}), 95.0 (C-P), 56.03 (OMe).^{31}P NMR (121 MHz, DMSO) δ 22.47. MS (*m/z*): M^+ 363 (15%). Analysis for $C_{15}H_{14}N_3O_2PS_2$ (363.39). Calced.: % C, 49.58; H, 3.88; N, 11.56; S, 17.65. Found: % C, 49.21; H, 4.02; N, 11.69; S, 17.23.

Antimicrobial Activity

The samples were prepared by dissolving 10mg of products under investigation (**5a-e, 6a-e, 8**) in 2ml of methanol and 100µl of solution (containing 500µg of desired product) was used in this test. The antimicrobial activity of different samples was investigated by the agar cup plate method. Four different test microbes namely: *Staphylococcus aureus* (Gram +ve), *Escherichia coli* (Gram -ve), *Candida albicans* (yeast) and *Aspergillus niger* (fungus) were used. Nutrient agar plates were heavily seeded uniformly with 0.1ml of 105-106 cells/ml in case of bacteria and yeast. A Czapek-Dox agar plate seeded by 0.1ml the fungal inoculum was used to evaluate the antifungal activities. Then a hole (1cm diameter) was made in media by gel cutter (Cork borer) in sterile condition. Then one drop of melted agar was poured into hole and allowed to solidify to make a base layer. After that specific amount of tested sample (0.1 ml) was poured into the hole. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30oC for 48 hours in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded [35, 36].

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