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# Synthesis, Evaluation of Antimicrobial, Antioxidant, DNA Cleavage and Molecular Docking Studies of Pyridazine Derivatives

**H. Hanumanagouda[1], S. V. Bharathi[2] and K. M. Basavaraja[2]***

1. Department of Chemistry, KLE Society's P.C. Jabin Science College, Vidyanagara, Hubballi-580031, Karnataka, **INDIA**

2. Department of Chemistry, Vijayanagara Sri Krishnadevaraya University, Jnanasagara campus, Hospet road, Ballari- 583105, Karnataka, **INDIA**

Email:kmbasavaraja@gmail.com

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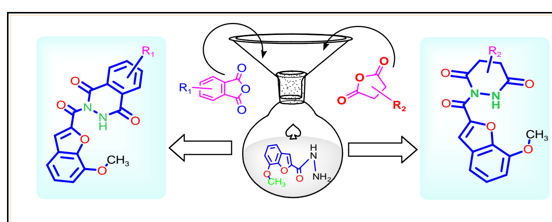
## ABSTRACT

*Literature survey reveals that the heterocyclic compounds involving Phthalazines and pyridazines possesses excellent biological properties. In the present work we report the synthesis of benzofuran moiety linked with Phthalazines and pyridazines. In continuation of our synthetic investigation, the present synthesis was achieved by the starting compound 7-methoxy-benzofuranyl carbohydrazide* ***(1)****. Carbohydrazide was condensed with phthalic anhydride and substituted phthalic anhydrides to obtain 2-(7-Methoxy-benzofuran-2-carbonyl)-substituted-2,3-dihydro-phthalazine-1,4-diones* ***(2a-h)****. Further on condensing with succinic anhydride and substituted succinic anhydrides we obtain 1-(7-Methoxy-benzofuran-2-carbonyl)-substituted-tetrahydro-pyridazine-3,6-diones* ***(3a-c)****. The structures of the newly obtained derivatives were confirmed by spectral and analytical data. Few compounds were screened for antibacterial, anti-fungal, anti-oxidant activity, DNA cleavage/protection properties and molecular docking studies. Some compounds exhibited encouraging of results.*

## Graphical Abstract:



Scheme of the syntheses of Pyridazine derivatives

**Keywords:** Benzofuran, Carbohydrazide, Phthalazines, Pyridazines, Antioxidant, Antimicrobial activity, Molecular docking and DNA cleavage.

## INTRODUCTION

Literature survey reveals that Phthalazine derivatives exhibit a broad spectrum of biological activities. Indeed, several Phthalazines derivatives have been reported to possess antitumor, antihypertensive,

antithrombotic, anticonvulsant, antidaiabetic, antimicrobial, anti-trypanosomal and anti-inflammatory activities. Phthalazin-1(2H)-ones are of considerable interest due to their antidaiabetic, antiallergic, Vasorelaxant, PDE4 inhibitors, VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer, antiasthmatic agents with dual activities of thromboxane A2 (TXA2) synthesize inhibition and bronchodialation, herbicidal, like activities. A number of established drug molecules like Hydralazine, Budralazine, Azelastine, Ponalrestat and Zopolrestat are prepared from the corresponding phthalazinones [1].

Sridhara, A. M *et.al* reported synthesis of a series of new 2-substituted [4-(1, 3, 4-oxadiazol-2-yl)methyl]phthalazin-1(2H)-one derivatives. They have synthesised the title compounds from methyl (4-oxo-3,4-dihydrophthalazin-1-yl)acetate, which was in turn prepared from phthalic anhydride and screened for their antimicrobial activities against various bacteria and fungi strains [2]. Rakholiya K. *et. al.* synthesised in a simple and convenient procedure for the preparation of 1-phthalazinone and 1,4-phthalazinedione derivatives using Schotten–Baumann reaction and Gabriel- Michael reaction respectively [3]. Agrawal M. *et. al* and Del Olmo E. *et. al.* reported the synthesis and vaso-relaxant activity of 4-benzyl phthalazinones and thiazolyl-phthalazinone derivatives as potent glucose uptake activators via high-throughput screening [4, 5].

Poly substituted Phthalazinones derivatives were synthesized and tested for their antifungal activity against a panel of pathogenic and clinically important yeasts and filamentous fungai [6]. Pyridazinone have been reported to exhibit wide range of pharmacological activities such as antidepressant, antihypertensive, antithrombotic, anticonvulsant, cardiotonic, antibacterial, diuretic, anti-HIV, aldose reductase inhibitors, hepatoprotective agents, anti-inflammatory and COX-2 inhibitors. Pyridazinone derivatives have also been reported to have remarkable anticancer activity [7]. Rathish I. G. *et.al.* synthesised pyridazinone substituted benzene sulfonylurea derivatives which exhibited glucose lowering effect [8]. Elagawany M. *et. al.* reported the design, synthesis, and molecular modeling of Phthalazinones and pyridazinone derivatives and as protein kinases inhibitors [9].

## MATERIALS AND METHODS

**Materials:** All the chemicals were purchased from Sigma-Aldrich, Molchem Chemicals India Ltd and solvents were used without further purification. Melting points were determined in open capillary and are uncorrected. The purity of all the compounds was checked by TLC. IR spectra were taken on Perkin Elmer-237 by KBr disc method. The $^1$H NMR spectra were recorded on a Bruker Advance spectrometer (400MHz) using TMS as internal reference, $CDCl_3$ and DMSO-$d_6$ as solvents. Chemical shifts ($\delta$) are given in ppm. The mass spectra were recorded on a Joel GC mate GC-MS. Elemental analysis (C, H, N) was performed on Perkin Elmer-240 analyzer.

**Chemistry:** In the present investigation we are reporting the synthesis of [4-(1, 3, 4-oxadiazol-2-yl)methyl]phthalazin-1(2H)-one derivatives **(2a-h)** from 7-methoxy benzofuran-2 carbohydrazide (1) by condensation with phthalic anhydride and substituted phthalic anhydrides and by fusing compound **1** with succinic anhydride and substituted succinic anhydrides we synthesised 1-(7-methoxy-benzofuran-2-carbonyl)-substituted-tetrahydro-pyridazine-3,6-diones **(3a-c)**. The key intermediate 3-methoxy benzofuran-2 carbohydrazide **(1)** was prepared from 3 methoxybenzofuran-2-ethyl carboxylate as per the procedure reported earlier from this laboratory [10].

### Experimental procedure

**General procedure for the preperation of 2-(7-Methoxy-benzofuran-2-carbonyl)-substituted-2,3-dihydrophthalazine-1, 4-dione (2a-h):** A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **1** (0.01 mol) and phthalic anhydride/ substituted phthalic anhydrides (0.01 mol) was fused in an oil-bath at 150-160°C for 4 h. The reaction mixture was cooled, washed with 10% sodium carbonate solution and then with water. The solid thus obtained was filtered, dried and recrystallized

from suitable solvent. The physical constant, percentage yield, and analytical data of the products **2a-h** are summarized in the table 1.

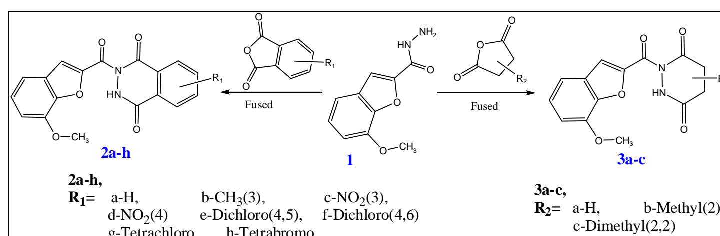



**Figure 1.** Synthesis phthalazinones and pyridazinediones.

**Table 1.** Analytical data of compounds 2a-h

| Comp. | Substituent 'R' | M.P. (°C) | Yield (%) | Solvent | Mol. formula | Found (calculated) % | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| **2a** | H | 189 | 86 | Aq. ethanol | $C_{18}H_{12}N_2O_5$ | 64.25 (64.29) | 3.58 (3.60) | 8.40 (8.33) |
| **2b** | 3-$CH_3$ | 210 | 88 | Aq. ethanol | $C_{19}H_{14}N_2O_5$ | 65.02 (65.14) | 4.00 (4.03) | 8.00 (8.03) |
| **2c** | 3-$NO_2$ | 200 | 89 | Ethanol | $C_{18}H_{11}N_3O_7$ | 56.65 (56.70) | 2.89 (2.91) | 11.01 (11.02) |
| **2d** | 4-$NO_2$ | 179 | 81 | Methanol | $C_{18}H_{11}N_3O_7$ | 56.68 (56.70) | 2.85 (2.91) | 11.00 (11.02) |
| **2e** | 4,5-Dichloro | 183 | 83 | Methanol | $C_{18}H_{10}C_{12}N_2O_5$ | 53.32 (53.36) | 2.45 (2.49) | 6.90 (6.91) |
| **2f** | 4,6-Dichloro | 195 | 79 | Ethanol | $C_{18}H_{10}C_{12}N_2O_5$ | 53.32 (53.36) | 2.35 (2.49) | 6.86 (6.91) |
| **2g** | Tetrachloro | 214 | 87 | Ethanol | $C_{18}H_8C_{14}N_2O_5$ | 45.55 (45.60) | 1.69 (1.70) | 5.89 (5.91) |
| **2h** | Tetrabromo | 218 | 85 | Aq. ethanol | $C_{18}H_8Br_4N_2O_5$ | 33.05 (33.16) | 1.21 (1.24) | 4.26 (4.30) |

**General procedure for the preperation of 1-(7-methoxy-benzofuran-2-carbonyl)-sub-stituted-tetrahydro-pyridazine-3, 6-diones (3a-c):** A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **(1)** (0.01 mol) and succinic anhydride/substituted succinic anhydride (0.01 mol) was fused in an oil-bath at 150-160°C for 5 h. The reaction mixture was cooled, washed with 10% sodium carbonate solution and then with water. The solid thus obtained was filtered, dried and recrystallized from suitable solvent. The physical constant, percentage yield, and analytical data of the products **3a-c** are summarized in the table 2.

**Table 2,** Analytical data of compounds 3a-c

| Comp. | Substituent '$R_2$' | M.P. (°C) | Yield (%) | Solvent | Mol. formula | Found (calculated) % | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| **3a** | -H | 201 | 81 | Ethanol | $C_{14}H_{12}N_2O_5$ | 58.29 (58.33) | 4.15 (4.20) | 9.70 (9.72) |
| **3b** | 2- Methyl | 195 | 85 | Methanol | $C_{15}H_{14}N_2O_5$ | 59.55 (59.60) | 4.60 (4.67) | 9.25 (9.27) |
| **3c** | 2,2-Dimethyl | 210 | 79 | Aq. ethanol | $C_{16}H_{16}N_2O_5$ | 60.68 (60.75) | 5.00 (5.10) | 8.85 (8.86) |

## RESULTS AND DISCUSSION

In view of the above biological properties associated with phthalazinones and pyridazinone it was thought to synthesize benzofuranyl-phthalazinones and, pyridazinone derivatives and evaluation for

their various biological activities. All synthesized compound spectra like IR, $^{1}$H NMR and Mass are depicted in supplementary copy attached with this manuscript.

**2-(7-Methoxy-benzofuran-2-carbonyl)-substituted-2,3-dihydro-phthala zine-1,4-diones (2a-h):** 2-(7-Methoxy-benzofuran-2-carbonyl)-substituted-2,3-dihydrophthalazine-1,4-diones (**2a-h)** were prepared by heating the 7-methoxy-benzofuranyl carbohydrazide **1** with phthalic anhydride and various substituted phthalic anhydride in an oil bath.

The presence of only one absorption peak in the range of 3330-3441 cm$^{-1}$ due to NH group indicates the formation of products **2a-h** in their FT-IR spectrum (Table 03 and Figure 2 ). To provide the additional evidences for the proposed structures, the $^{1}$H NMR and mass spectrum of **2a** were recorded (Figure 3). The $^{1}$H NMR spectrum in DMSO-$d_6$ was exhibited a singlet at δ 3.99 ppm due to -$OCH_3$ protons, a multiplet in the range of δ 7.14-8.04 ppm were due to the aromatic protons. The NH was resonated as singlet at δ 11.61 ppm. The molecular ion peak was observed at *m/z* 336 confirmed the formation of **2a**.

**Table 3.** IR data of compounds (2a-h)

| Compound | Substituent 'R$_1$' | FT-IR data (cm$^{-1}$) | | |
|---|---|---|---|---|
| | | NH | C=O phthalazinones | C=O carbohydrazide |
| **2a** | H | 3336 | 1744 | 1693 |
| **2b** | 3-$CH_3$ | 3330 | 1736 | 1686 |
| **2c** | 3-$NO_2$ | 3378 | 1740 | 1682 |
| **2d** | 4-$NO_2$ | 3353 | 1732 | 1691 |
| **2e** | 4,5-Dichloro | 3441 | 1738 | 1694 |
| **2f** | 4,6-Dichloro | 3389 | 1731 | 1685 |
| **2g** | Tetrachloro | 3408 | 1742 | 1690 |
| **2h** | Tetrabromo | 3416 | 1730 | 1684 |

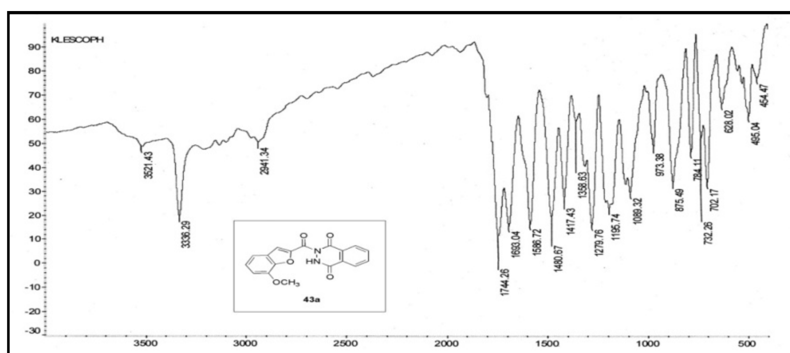



**Figure 2.** IR (KBr) spectrum of compound 2a

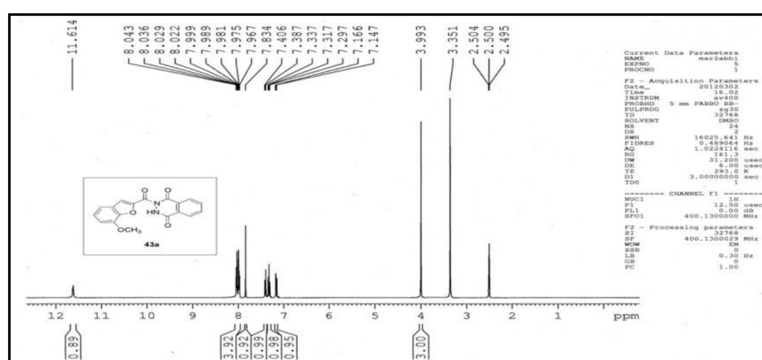



**Figure 3.** $^{1}$H NMR Spectrum of compound 2a.

**1-(7-Methoxy-benzofuran-2-carbonyl)-substituted-tetrahydro-pyridazine-3,6-diones (3a-c):** 1-(7-Methoxy-benzofuran-2-carbonyl)-substituted-tetrahydropyridazine-3,6-diones **(3a-c)** were prepared by heating benzofuranyl carbohydrazide **1** with succinic anhydride and substituted succinic anhydride in an oil bath.

The presence of only one absorption peak in the range of 3361-3396 $cm^{-1}$ due to NH group indicates the formation of products **3a** in their IR spectrum. To provide the additional evidences for the proposed structures in table 4, the $^{1}H$ NMR and mass spectrum of **3a** were recorded (Figure 4). The $^{1}H$ NMR spectrum in $CDCl_3$ was exhibited two singlets at δ 2.91 and δ 3.99 ppm due to two $-CH_2$ and $-OCH_3$ protons, a multiplet in the range of δ 6.93-7.60 ppm were due to the aromatic protons. The -NH was resonated as singlet at δ 8.54 ppm. The molecular ion peak was observed at *m/z* 288 confirmed the formation of **3a** (Figure 5).

**Table 4.** IR data of compounds (3a-c)

| Compound | Substituent 'R$_2$' | FT-IR data ($cm^{-1}$) | | |
|---|---|---|---|---|
| | | NH | C=O pyridazinone | C=O carbohyrazide |
| **3a** | H | 3374 | 1732 | 1661 |
| **3b** | 3-$CH_3$ | 3396 | 1736 | 1657 |
| **3c** | 3-$NO_2$ | 3361 | 1739 | 1665 |

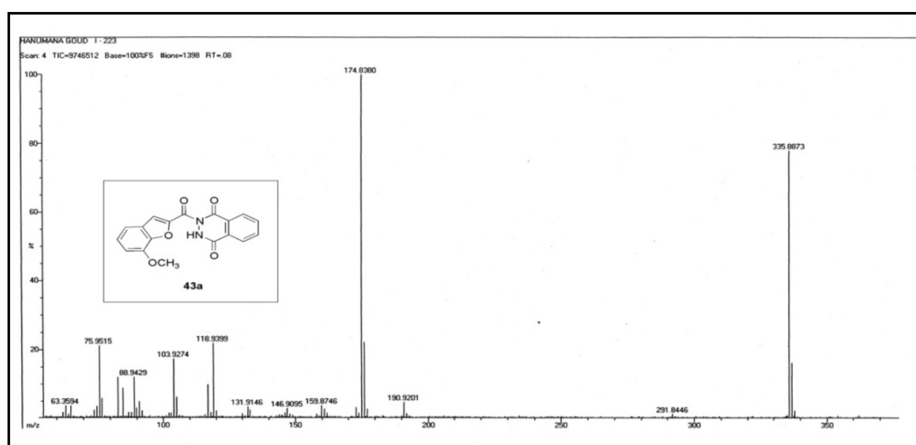



**Figure 4.** Mass spectrum of compound 2a.

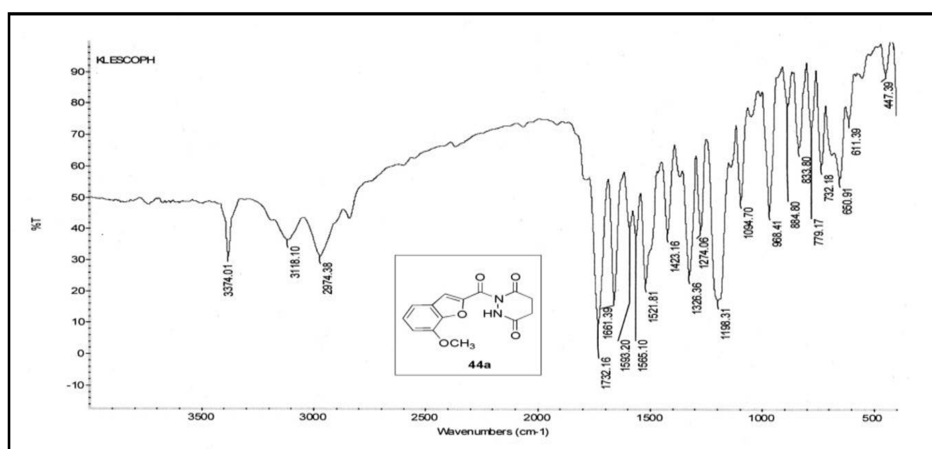



**Figure 5.** IR(KBr) spectrum of compound 3a.

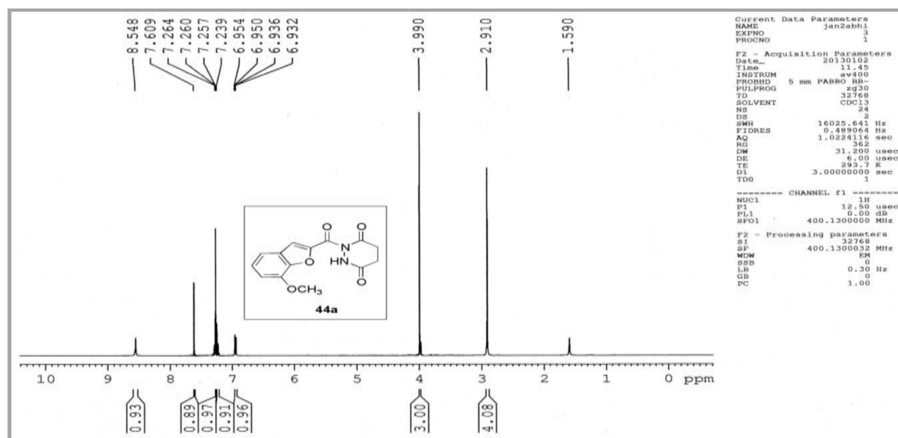



**Figure 6.** $^{1}$H NMR spectrum of compound 3a.

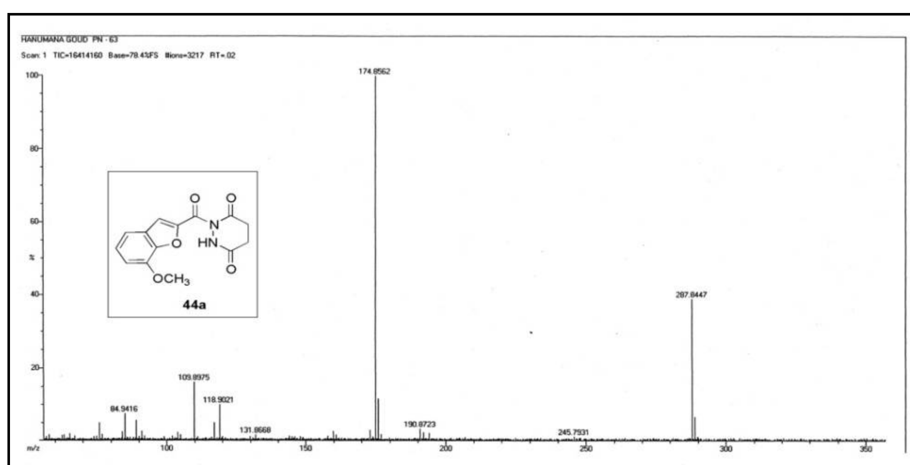



**Figure 7.** Mass spectrum of compound 3a.

## APPLICATION

**Antimicrobial screening:** Standard strains were procured from the microbial type culture collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The anti-bacterial activity of the synthesized compounds **2b, 2c** and **3a-c** were performed *in vitro* against (i) Gram-positive bacteria: *Streptococcus faecalis* (MTCC 3382), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 297) and (ii) Gram-negative bacteria: *Pseudomonas aureginosa* (MTCC 1034), *Klebsiella pneumoniae* (MTCC 3384) and *Escherichia coli* (MTCC 1089) by broth micro dilution method [11].

The MIC determination of the compounds was investigated in comparison with ciprofloxacin. Double dilutions of the test compounds and reference drugs were prepared in Muller-Hinton agar [12]. 10mg of each test compounds were dissolved in 1 ml of dimethyl sulfoxide (DMSO) separately to prepare stock solution. Further, progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, 1 µg $mL^{-1}$. The Petri dishes were inoculated with 1-5 x $10^{4}$ colonies forming units (cfu $mL^{-1}$) and incubated at 37$^{o}$C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentrations of the tested compound that yield no visible growth on the plate were recorded in table 5 and 6. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments.

**Table 5.** Results of antibacterial activity

| Comp. | Diameter of the zone of inhibition in mm (Relative inhibition %) | | | | |
|---|---|---|---|---|---|
| | Gram negative | | | Gram positive | |
| | *E. coli* | *P. aeruginosa* | *K. pneumoniae* | *S. aureus* | *S. faecalis* |
| **2b** | 17 (94.4) | 23 (92) | 18 (90) | 17 (89.4) | 20 (100) |
| **2c** | 15 (83.3) | 18 (72) | 16 (80) | 15 (78.9) | 16 (80) |
| **3a** | 14 (77.7) | 20 (80) | 14 (70) | 14 (73.6) | 15 (75) |
| **3b** | 16 (88.8) | 22 (88) | 19 (95) | 18 (94.7) | 18 (90) |
| **3c** | 13 (72.2) | 19 (76) | 13 (65) | 15 (78.9) | 13 (65) |
| **Ciprofloxacin** | 18 (100) | 25 (100) | 20 (100) | 19 (100) | 20 (100) |

**Table 6.** Results of antifungal activity

| Comp. | Diameter of the zone of inhibition in mm (Relative inhibition %) | | | | |
|---|---|---|---|---|---|
| | *A. niger* | *A. fumigates* | *C. albicans* | *P. notatum* | *Rhizopus* |
| **2b** | 27 (90.0) | 23 (95.8) | 22 (91.6) | 24 (92.3) | 23 (88.4) |
| **2c** | 25 (83.3) | 15 (62.5) | 16 (66.6) | 20 (76.9) | 22 (84.6) |
| **3a** | 23 (76.6) | 18 (75.0) | 20 (83.3) | 19 (73.0) | 20 (76.9) |
| **3b** | 26 (86.6) | 16 (66.6) | 15 (62.5) | 17 (65.3) | 19 (73.0) |
| **3c** | 29 (96.6) | 22 (91.6) | 23 (95.8) | 22 (84.6) | 25 (96.1) |
| **Fluconazole** | 30 (100) | 24 (100) | 24 (100) | 26 (100) | 26 (100) |

The investigation of antibacterial screening data (Table **7** and **8**) revealed that all the tested compounds showed moderate to good microbial inhibition. The antibacterial and antifungal data revealed that all selected compounds exhibited moderate to good activity. The compounds **2b, 3b** and **3c** showed good activities.

**Antioxidant studies:** In vitro antioxidant activity of the synthesized compounds performed by ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method. ABTS solution I (2 mm of ABTS solution) and solution II (17 mm of potassium per sulfate) were prepared using distilled water. Solution II (0.3 mL) was added to 50 mL of solution I and the reaction mixture

**Table 7.** Results of antibacterial activities

| Compound | Gram negative | | | Gram positive | |
|---|---|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *K. pneumoniae* | *S. aureus* | *S. faecalis* |
| **2b** | 1 | 1 | 1 | 2 | 2 |
| **2c** | 16 | 16 | 31.25 | 31.25 | 16 |
| **3a** | 8 | 8 | 16 | 16 | 8 |
| **3b** | 2 | 2 | 2 | 4 | 4 |
| **3c** | 16 | 16 | 8 | 8 | 16 |
| **Ciprofloxacin** | 1 | 1 | 1 | 1 | 1 |

**Table 8.** Results of antifungal activities

| Compound | *A. niger* | *A. fumigates* | *C. albicans* | *P. notatum* | *Rhizopus* |
|---|---|---|---|---|---|
| **2b** | 8 | 4 | 8 | 8 | 4 |
| **2c** | 8 | 16 | 8 | 16 | 8 |
| **3a** | 16 | 8 | 16 | 8 | 8 |
| **3b** | 16 | 8 | 16 | 16 | 8 |
| **3c** | 8 | 4 | 8 | 4 | 4 |
| **Fluconazole** | 8 | 8 | 8 | 8 | 8 |

was left to stand at room temperature overnight in dark before use. Test solutions were prepared by dissolving drug samples and the standard (ascorbic acid) was accurately weighed (10 mg) separately

and dissolved in 1 mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions. Distilled DMSO (1 mL) was added to 0.2 mL of various concentrations of the drug samples or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. After 20 min, the absorbance was measured spectrophotometrically at 734 nm using ELISA reader. Blank was maintained without ABTS. $IC_{50}$ value obtained was the concentration of the sample required to inhibit 50% ABTS radical mono cation. The statistical analysis was performed by One way ANOVA followed by Tukey's post-hoc test was employed to analyze the results (Graph Pad Prism Software). The difference below the probability level of 0.05 was considered as statistically significant [13].

**Table 9.** Results of antioxidant activity

| Compound | $IC_{50}$ Value* Micro molar |
|---|---|
| **2b** | 54.36±1.42 |
| **2c** | 90.22±1.32 |
| **3a** | 86.96±1.36 |
| **3b** | 66.48±1.54 |
| **3c** | 62.25±1.04 |
| **Standard (Ascorbic acid)** | 12.10±0.51 |

The results are presented as Mean±SEM, n=5; $IC_{50}$ values of all the synthesized compounds are significantly different ($p<0.05$) from that of the standard (ascorbic acid).

The results (Table 9) indicated that all the compounds exhibited moderate to good antioxidant activity with ABTS method. Among the series, the compound **2b, 3b** and **3c** showed good activity. Ascorbic acid showed potent ability to inhibit free radicals with $IC_{50}$ values of 12.10±0.51 micromole concentration.

**Antioxidant studies:** In vitro antioxidant activity of the synthesized compounds performed by ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method. ABTS solution I (2 mm of ABTS solution) and solution II (17 mm of potassium per sulfate) were prepared using distilled water. Solution II (0.3 mL) was added to 50 mL of solution I and the reaction mixture was left to stand at room temperature overnight in dark before use. Test solutions were prepared by dissolving drug samples and the standard (ascorbic acid) was accurately weighed (10 mg) separately
and dissolved in 1 mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions. Distilled DMSO (1 mL) was added to 0.2 mL of various concentrations of the drug samples or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. After 20 min, the absorbance was measured spectrophotometrically at 734 nm using ELISA reader. Blank was maintained without ABTS. $IC_{50}$ value obtained was the concentration of the sample required to inhibit 50% ABTS radical mono cation. The statistical analysis was performed by One way ANOVA followed by Tukey's post-hoc test was employed to analyze the results (Graph Pad Prism Software). The difference below the probability level of 0.05 was considered as statistically significant [13].

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**DNA cleavage studies**

**Preparation of culture media:** DNA cleavage experiments were carried out according to the literature. Nutrient broth (peptone, 10 g $L^{-1}$; yeast extract, 5 g $L^{-1}$; NaCl, 10 g $L^{-1}$) was used for the culturing of *E. coli*. The 50 mL medium was prepared and autoclaved for 15 min at 121°C under 15-lb pressure. The autoclaved medium was inoculated with the seed culture. The *E. coli* was incubated for 24 h.

**Isolation of DNA:** The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was then dissolved in 0.5 mL of lysis buffer (100 mM Tris pH 8.0, 50 mM EDTA, and 10 % sodium dodecyl sulphate (SDS)). To this, 0.5 mL of saturated phenol was added and incubated at 55°C for 10 min. It was then centrifuged at 10,000 rpm for 10 min, and to the supernatant, an equal volume of chloroform: iso-amyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) were added. Then, this solution was centrifuged at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in a TAE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and stored in cold conditions.

**Agarose gel electrophoresis:** Cleavage products were analyzed by the agarose gel electrophoresis method. Test samples (1 mg $mL^{-1}$) were prepared in DMF. The samples (25 µg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37°C. Then 20 µL of DNA sample (mixed with bromophenol blue dye at a 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with a standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 MEDTA per 1 L) and finally loaded on agarose gel and a constant electricity of 50 V was passed for around 30 min. The gel was removed and stained with 10.0 µg $mL^{-1}$ ethidium bromides for 10–15 min and the bands observed under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage. Then, the results were compared with that of a standard DNA marker.

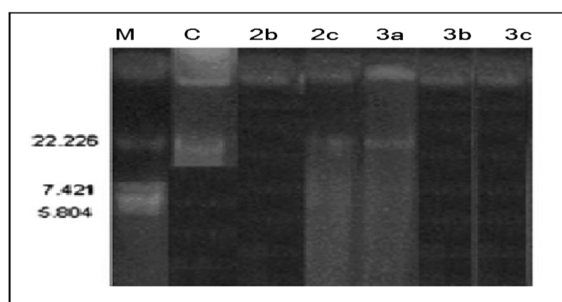


**Figure 8.** Gel electrophoresis of **2b, 2c, 3a, 3b** and **3c** on DNA of *E. coli* at 25µg Lane M: DNA marker; Lane C: Untreated DNA.

The compounds **2b, 3b** and **3c** act as a potent nuclease agents. As the compounds were observed to cleave the DNA, it can be concluded that, the compound inhibits the growth of the pathogenic organism by cleaving the genome. The gel containing *E. coli* DNA treated with compounds shows that after treatment, the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage was observed with **2b, 3b** and **3c**.

**DNA Protection:** Lane 1: blank, native DNA, Lanes **2b, c,** and **3a–c** test compounds and trolox. The compounds **2b**, **3b** and **3c** showed better activity than trolox regarding protection against 2, 2'-azobis (2-amidinopropane hydrochloride) (AAPH) induced DNA strand.

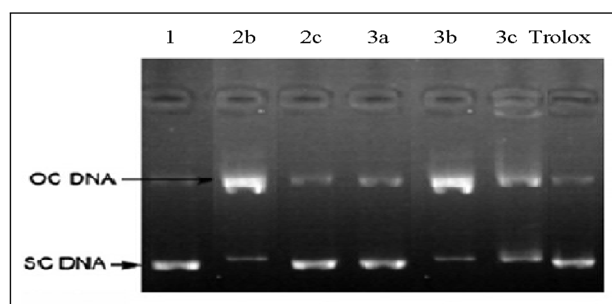


**Figure 9.** Protection against AAPH-induced pBR322 DNA strand.
(Breakage by compounds)

**Molecular Docking studies:** To examine the complete intermolecular interactions between the synthetic molecule and the object protein, a program auto-dock vina was used to determine the orientation of inhibitors bound in the active site of the enzyme antibacterial inhibitor. The protein structure file (PDB ID: 3mqo) was taken from PDB (www. rcsb. org/Pdb). The ligand molecules were designed and the structure was analyzed by using Chem.-Draw Ultra 6.0. 3D coordinates were prepared using OPENBABEL server. This protein and legend molecule Docking studies give a fair idea related to drug-receptor interactions [**14**] Docking study was gives docking affinity and Bond length, to sustain the interaction study of docking results with discovery studio visualize Program, estimate the bond angle and bond distance of amino acid residue mode of compounds **2a-f** and **3a-c,** docking scores and their interaction studies result are depicted in table 8.

**Table 8.** Results of molecular docking

| Compound name | Binding affinity (kcal mol$^{-1}$) | No. of H Bonds |
|---|---|---|
| **2a** | -8.4 | 3 |
| **2b** | -8.1 | 3 |
| **2c** | -7.9 | 5 |
| **2e** | -7.7 | 3 |
| **2f** | -8.4 | 4 |
| **3a** | -7.8 | 4 |
| **3b** | -8.5 | 5 |
| **3c** | -8.3 | 5 |

Docking and interaction study of compounds **2a** makes hydrogen bonding interactions at the protein hydrogen's of amino acids As depict in 3D and 2D images of compounds respectively in their figure 8. Compounds 3a and 3b figures also makes hydrogen bonding interaction at the protein hydrogen's of amino acids. As depicted in 3D and 2D images of the compounds respectively in their figures 9 and 10.

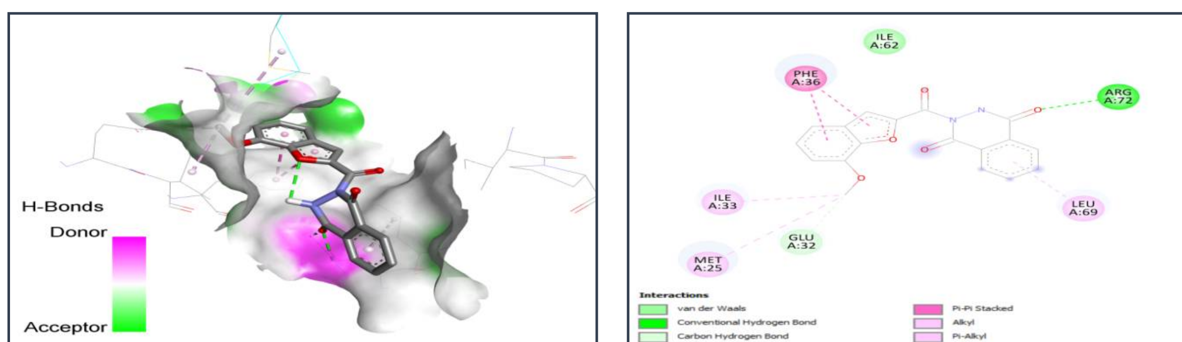


**Figure 10.** Molecular docking 3D and 2D image compound **2a.**

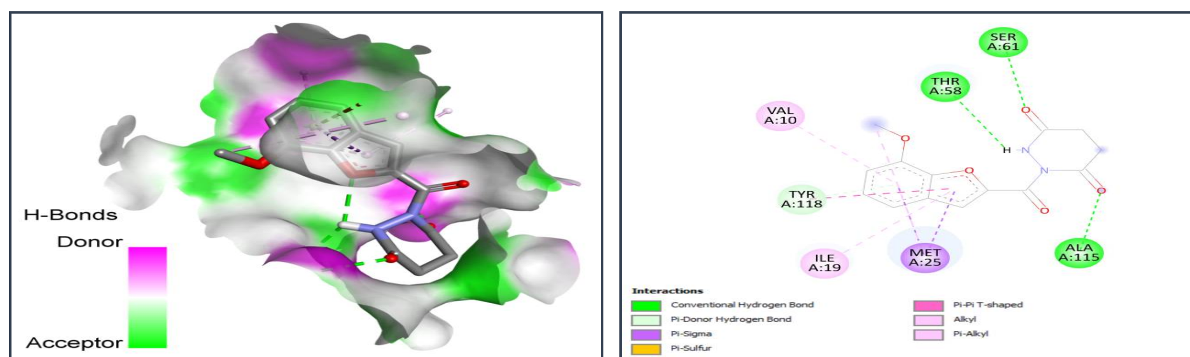


**Figure 11.** Molecular docking 3D and 2D images of compound 3a.

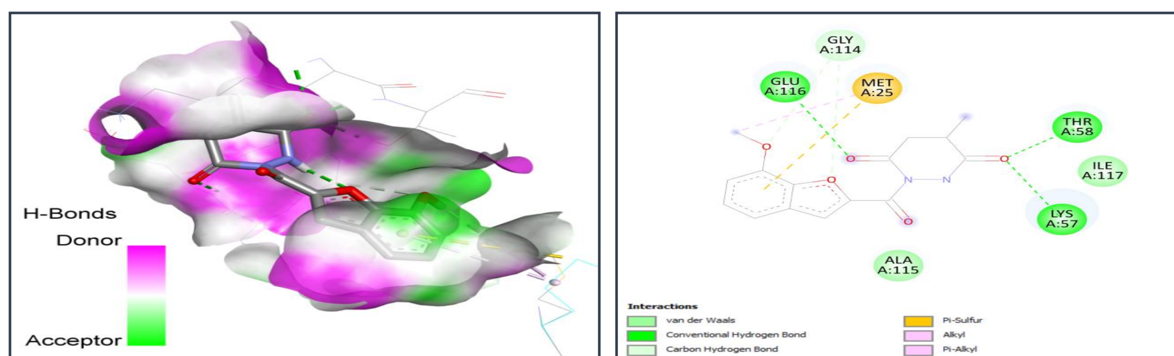


**Figure 12.** Molecular docking 3D and 2D images of compound 3b.

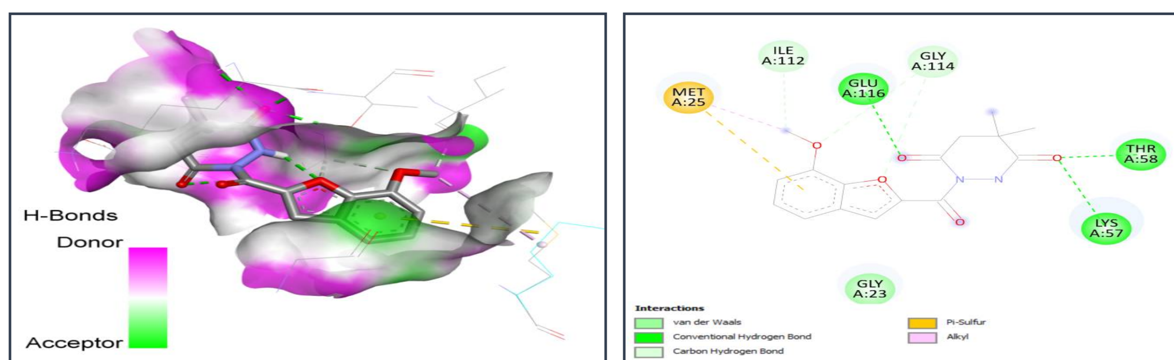


**Figure 13.** Molecular docking 3D and 2D images of compound 3c.

## CONCLUSION

In conclusion, we have synthesized substituted Phthalazines derivatives with simple experimental method in short reaction time. And these newly synthesized compounds are in concurrent with assigned structures which are purified by TLC and Confirmed by IR, $^{1}$H NMR, and Mass spectral data. Most of substituted compounds of benzofuranyl phthalazine derivatives exhibits Moderate to high activity against antioxidant, DNA cleavage, antibacterial organisms of *S.aureus, S.epidermies*, Pseudomonas, *E.colli* and antifungal organisms of Candida, Aspergillums. Some of compounds activity supported by molecular docking study of staphylococcal enterotoxin $C_2$ from *Staphylococcus aureus* proteins.

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**Conflict of Interest:** The authors declare that, there is no conflict of interest in financial and otherwise.

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