DESIGN, SYNTHESIS, IN *VITRO* ANTIMICROBIAL, AND CYTOTOXIC STUDIES OF A NEW SERIES OF PYRROLO[2,3-*d*] PYRIMIDINEHYDRAZIDEHYDRAZIDE DERIVATIVES

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Abstract:
This study examines the design and synthesis of a newly manufactured pyrrolo[2,3-*d*]pyrimidinehydrazide (PPH) derivative. The PPH derivatives are designed to contain bromine atoms in positions 2, 3, 4, 5, and 6. The microwave technique is used as a novel and reliable method for the preparation of this type of PPH derivative. Spectral and elemental studies were used to provide a comprehensive description of the chemical structure of the PPH derivatives **2–7** that were constructed. By using the disc diffusion method, each drug was put through an in vitro antimicrobial test against six different microorganisms. In comparison to streptomycin, which had a concentration of 25.0 μg/ml, it was discovered that compounds 2, 4, and 5 exhibited the highest levels of activity against *E. coli* (11.5, 15.5, and 23.4 μg/ml, respectively). Furthermore, compound **7** demonstrated potential cytotoxic effects against *artemia salina*, with an IC_{50} value of 18.5 μg/ml.

Keywords: Pyrrolo[2,3-*d*]pyrimidinehydrazide, Elemental studies, Cytotoxic studies

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INTRODUCTION

As a result of the capability of nitrogen-containing heterocycles to generate hydrogen bonding, van der Waals forces, hydrophobic effects, π-stacking interactions, and dipole–dipole interactions with biological targets, the Food and Drug Administration (FDA) has approved more than eighty percent of the drugs that are currently on the market [1-5]. It is usual for pyrrolopyrimidine derivatives to exhibit a wide range of biological and pharmacological features, and these derivatives can be found in a variety of small molecule drug discovery programs [6]. These derivatives are nitrogen-fused heterocycles.

The pyrrolo[2,3-*d*]pyrimidinehydrazide derivatives hold a significant position among the pyrrolopyrimidine compounds. These compounds possess a wide range of biological properties, including anti-bacterial [7], anti-diabetic agents [8, 9], antiviral [10, 11], anti-inflammatory [12, 13], anti-hypertensive activity [14], anti-protozoal activity [15], and they have demonstrated potent anticancer activity, which makes them an effective tool for DNA interaction [16-18].

This work is a continuation of our research efforts that have been centered on the synthesis of novel nitrogen-containing heterocycles that possess anti-microbial activity [19–22]. In recent times, we have been paying close attention to the process of preparing new pyrrolo[2,3-*d*]pyrimidinehydrazide derivatives. These derivatives are predicted to exhibit biological activity, and they are prepared under conditions that are both environmentally friendly and time-saving via microwave assistance.

A new series of pyrrolo[2,3-*d*]pyrimidine hydrazide derivatives with the bromine atom on carbon 2, 3, 4, 5, and 6were prepared by us using our reliable method under microwave-assisted conditions. The purpose of this study was to evaluate the anti-microbial activity of these compounds as a first study for this particular type of pyrrolo[2,3-*d*]pyrimidinehydrazide as an anti-microbial agent. Additionally, the molecular docking study was displayed and discussed during the presentation.

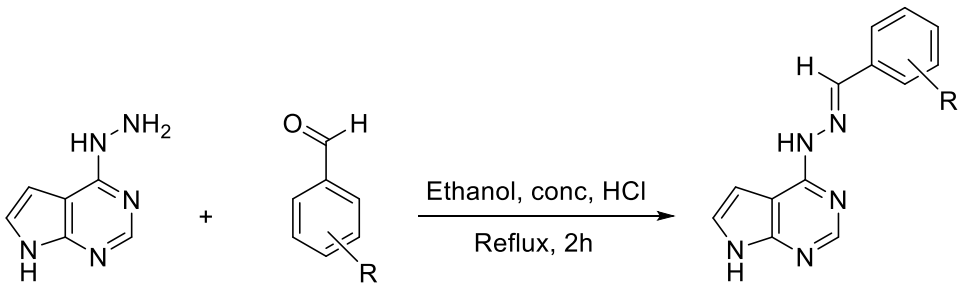
EXPERIMENTAL

Materials and methods

The melting points of all the compounds were recorded using a digital Gallen Kamp MFB-595 equipment, which may have been inaccurately calibrated. The following chemicals were utilized without additional purification: pyrrolo[2,3-*d*]pyrimidine, 2,3,4-tribromobenzaldehyde, hydrazine hydrate, 2,3-dibromobenzaldehyde, 2,4-dibromobenzaldehyde, 2,5-dibromobenzaldehyde, 2,6-dibromobenzaldehyde, 3,4-dibromobenzalde hyde, and 3,5-dibromobenzaldehyde. A Brucker spectrophotometer was used to record infrared spectra (cm^{-1}) with a KBr pellet. Bruker spectrometers operating in deuterated dimethyl sulfoxide (DMSO-d_6) were used to record the ^{1}H-NMR and ^{13}C-NMR spectra, at 400 MHz and 100 MHz, respectively. Chemical shift (δ ppm), multiplicity (*s*=singlet, *d*=doublet, *t*=triplet, *m*=multiplet), and coupling constant (*J* in Hz) were the following reported in ^{1}H-NMR spectra, which were allocated relative to deuterated solvent signals. At CIF Pune University's micro-analysis center, we documented elemental analyses. The recently produced chemicals were tested for purity using the TLC method [10].

Formulation of PPH compounds (1-7):

To prepare the compounds (**1-7**), one mmol of pyrrolo[2,3-*d*]pyrimidine-hydrazide and one mmol of substituted dibromobenzaldehydes were refluxed in 20 ml of ethanol for 2 hours. The cooled liquid was then dumped onto a bed of ice water. To get affords **1–7**, the precipitate was filtered off and recrystallized from ethanol.



Scheme 1: Preparation of compounds **1-7**

2.2.1. 4-{(2E)-2-[(2,3-dibromophenyl) methylide ne]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine(1).

Yield % 75.66, m.p. 189-190ºC, IR:υ_{max}/cm^{-1} 3110 (-NH- aromatic), 3178 (-NH- aliphatic), 2815 (-CH=), 1582/1479 (>C=C<), 1655 (>C=NN-), 1233 (C-F), 1070 (-N-N-), 741 (benzene ring), 688 (C-Br).^{1}H-NMR (DMSO-d_6) δ: 14.25 (s, 1H, -NH- aliphatic), 12.97 (s, 1H, NH, aromatic), 8.73 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.09-8.01 (m, 6H, aromatic-H)). Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 39.48; H, 2.17; Br, 40.09; N, 17.67.

2.2.2. 4-{(2E)-2-[(2,4-dibromophenyl)met hylidene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimi dine (2).

Yield % 73.79, m.p. 195-196ºC, IR:υ_{max}/cm^{-1} 3139 (-NH- aromatic), 3292 (-NH- aliphatic), 2884 (-CH=), 1590/1478 (>C=C<), 1661 (>C=NN-), 1291 (C-F), 1029 (-N-N-), 687 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 14.41 (s, 1H, -NH- aliphatic), 12.94 (s, 1H, NH, aromatic), 8.49 (s, 1H, -CH=), 7.20-7.56 (m, 6H, aromatic-H (7.20 (1H, *d*, (*J* = 3.88Hz), 7.34 (1H, *dd*, (*J* = 7.62, 1.62Hz), 7.45 (1H, *dd*, (*J* = 8.00, 1.75Hz), 7.48 (1H, *dd*, (*J* = 8.03, 1.62Hz), 7.56 (2H, *d*, (*J* = 3.88Hz)). Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 39.39; H, 2.26; Br, 40.11; N, 17.68.

2.2.3. 4-{(2E)-2-[(2,5-dibromophenyl) methyl-dene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine (3).

Yield % 74.89, m.p. 189-192ºC, IR:υ_{max}/cm^{-1} 3096 (-NH- aromatic), 3195 (-NH- aliphatic), 2982 (-CH=), 1587/1477 (>C=C<), 1624 (>C=NN-), 1224 (C-F), 1001 (-N-N-), 731 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 11.82 (s, 1H, -NH- aliphatic), 11.69 (s, 1H, NH, aromatic), 7.51 (s, 1H, -CH=), 7.05-8.28 (m, 6H, aromatic-H (7.05 (1H, *d*, (*J* = 3.86Hz), 7.27 (2H, *dd*, (*J* = 8.40, 1.08Hz), 8.28 (2H, *s*), 7.48 (1H, *dd*, (*J* = 8.03, 1.62Hz), 7.56 (2H, *d*, (*J* = 3.88Hz)). Anal. Calcd. for Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 39.50; H, 2.27; Br, 40.33; N, 17.53.

2.2.4. 4-{(2E)-2-[(2, 6-dibromophenyl) methylidene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine (4).

Yield % 80.97, m.p. 196-198ºC, IR:υ_{max}/cm^{-1} 3127 (-NH- aromatic), 3289 (-NH- aliphatic), 2948 (-CH=), 1581/1475 (>C=C<), 1662 (>C=NN-), 1224 (C-F), 1023 (-N-N-), 682 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 14.42 (s, 1H, -NH- aliphatic), 12.94 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.11 (s, 1H, -CH=), 6.81-7.52 (m, 6H, aromatic-H (7.52 (1H, *d*, (*J* = 3.89Hz), 7.04 (1H, *dd*, (*J* = 1.64, 1.43Hz), 6.87 (2H, *dd*, (*J* = 1.68, 1.53Hz), 6.81 (1H, *d*, (*J* = 3.89Hz)). Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 39.40; H, 2.21; Br, 40.19; N, 17.70.

2.2.5. 4-{(2E)-2-[(3,4-dibromophenyl) methyl-dene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine (5).

Yield % 73.33, m.p. 187-188ºC, IR:υ_{max}/cm^{-1} 3046 (-NH- aromatic), 3290 (-NH- aliphatic), 2684 (-CH=), 1585/1476 (>C=C<), 1660 (>C=NN-), 1223 (C-F), 1023 (-N-N-), 682 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 14.52 (s, 1H, -NH- aliphatic), 12.48 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.09 (s, 1H, -CH=), 6.81-7.53 (m, 5H, aromatic-H (6.81 (1H, *d*, (*J* = 3.90Hz), 6.89 (1H, *d*, (*J* = 1.68Hz), 7.14 (1H, *d*, (*J* = 1.68Hz), 7.53 (1H, *d*, (*J* = 3.90Hz). Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 38.95; H, 2.26; Br, 40.22; N, 17.69.

2.2.6. 4-{(2E)-2-[(3, 5-dibromophenyl) methyl-dene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine (6).

Yield % 77.76, m.p. 183ºC, IR:υ_{max}/cm^{-1} 3109 (-NH- aromatic), 3194 (-NH- aliphatic), 2985 (-CH=), 1575/1506 (>C=C<), 1629 (>C=NN-), 1206 (C-F), 1046 (-N-N-), 695 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 11.87 (s, 1H, -NH- aliphatic), 11.74 (s, 1H, NH, aromatic), 8.28 (s, 2H, Ar H), 7.56 (s, 1H, -CH=), 6.93-7.70 (m, 3H, aromatic-H (6.93 (1H, *d*, (*J* = 3.86Hz), 7.34 (1H, *d*, (*J* = 0.54Hz). Anal. Calcd. for $C_{13}H_9Br_2N_5$(395.05): C, 39.52; H, 2.30; Br, 40.45; N, 17.73. Found: C, 38.99; H, 2.29; Br, 40.41; N, 17.72.

2.2.7. 4-{(2E)-2-[(2,3,4-tribromophenyl) methyl dene]hydrazinyl}-7H-pyrrolo[2,3-*d*]pyrimidine (7).

Yield % 79.04, m.p. 186ºC, IR:υ_{max}/cm^{-1}3021 (-NH- aromatic), 3118 (-NH- aliphatic), 2973 (-CH=), 1582/1480 (>C=C<), 1648 (>C=NN-), 1251 (C-F), 1026 (-N-N-), 731 (benzene ring).^{1}H-NMR (DMSO-d_6) δ: 11.93 (s, 1H, -NH- aliphatic), 11.66 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.12 (s, 1H, -CH=), 6.81-7.53 (m, 4H, aromatic-H (6.81 (1H, *d*, (*J* = 3.89Hz), 7.12 (2H, *d*, (*J* = 1.95Hz), 7.53 (1H, *d*, (*J* = 3.89Hz). Anal. Calcd. for $C_{13}H_8Br_3N_5$(473.95): C, 32.94; H,

1.70; Br, 50.58; N, 14.78. Found: C, 32.91; H, 1.69; Br, 50.55; N, 14.75.

Antimicrobial activity:

The study used gram-positive Staphylococcus aureus MCC 2010 and gram-negative Bacillus subtilis MCC 2010 microorganisms to test the antibacterial effectiveness of the chemicals that were produced. Bacillus subtilis (MCC 2010), Escherichia coli (MCC 2412), Staphylococcus aureus (MCC 2408), and Pseudomonas aeruginosa (MCC 2080) were the bacterial strains utilized in this investigation. To mimic the conditions usually used in an antibacterial test, the Muller Hilton agar medium was autoclaved at a pressure of 15 lbs/in2 for 15 minutes. To test whether the newly produced chemicals were effective against bacteria, the researchers used the disc diffusion method [19-22]. A decrease in the organism count to around L108 colony-forming units per milliliter (cfu/mL) was achieved by modifying the inoculum and suspending the culture in sterile distilled water to evaluate the antibiotic effect. Bacterial strains were cultured by swabbing 20 mL of Muller Hilton agar medium from Petri plates. The Petri dishes were then incubated for 15 minutes to allow the cultures to be absorbed. A 6-millimeter-diameter well was bored using a sterile borer. Afterward, 100 µ of a DMSO-reconstituted solution containing 4.0 mg/mL of each chemical was added to the polluted plates. An entire 24-hour period was spent incubating each plate at a temperature of 37 °C. An entire 24-hour period was spent incubating each plate at a temperature of 37 °C. To measure the antibacterial activity of each drug, the area of inhibition surrounding the test wells was measured. Dimethyl sulfoxide (DMSO) was used as the negative control, and streptomycin was used as the positive control [23-25].

Antifungal Activity:

Cup and plate experiments incorporating the compounds were conducted with two separate fungus species [25, 26]. The Candida albicans (MCC1439) and Saccharomyces cerevisiae (MCC1033) strains are the ones being studied. The discs, which were 1 mm thick and 5 mm in diameter, were filled with the test solution using a micropipette. The plates were then kept in an incubator set at 37 °C for a whole week. During this time, the test solution diffused throughout the body, preventing the injected fungus from growing. After incubating at 37°C for 36 hours, the inhibitory zone's diameter was measured. The inquiry included testing chemicals with substances that were thought to have antifungal characteristics using minimum inhibitory concentration (MIC) studies. After a total of 24 hours of incubation, the concentration of an antifungal drug at which all measurable microbial growth is successfully suppressed is called the minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) approach is used in diagnostic laboratories to validate microbial resistance to antimicrobial drugs and to evaluate the efficacy of newly developed antimicrobial agents.

***In vitro* cytotoxicity:**

A bioassay using brine shrimp was used to evaluate the cytotoxicity of the chemicals that were produced [27]. The shrimp eggs were placed in a specific area of the aquarium. The opposite half was filled with a fake seawater solution consisting of 38 g of NaCl and 1000 ml of water from the faucet. The entire process of hatching and developing into nauplii takes two days for shrimp. To perform a bioassay, the newly developed crustaceans were removed. Mixed with various strengths of dry components- 2.5, 5.5, 7.5, 10, and 12.5 mg/mL sample vials were filled. By dissolving the complexes in DMSO, their cytotoxicity was evaluated. Using a Pasteur pipette, ten live shrimp were added to each test tube. A control group was included to guarantee that the test technique and results obtained from the drug's cytotoxic activity were valid. After a day, the tubes were examined under a microscope to record any findings and count the number of nauplii that survived. Five separate replicates, each with three runs, made up the experiment. Many statistical tests, including LC50, LC90, chi-square, and 95% CI, were performed on the collected data. The numerical values were adjusted using Abbott's technique, as described in reference [28], whenever deaths occurred within the control group.

% deaths = [(test-control)/control] x 100.

RESULTS AND DISCUSSION

There are two methods for producing pyrrolo[2,3-*d*]pyrimidine (PP) hydrazones. The Schiff bases were produced when PPH was afforded a reasonable yield (80%) by refluxing PP over ethanol for around 12 hours. The Schiff bases of PPH were synthesized with an outstanding yield of 85% using the microwave technique (MW) in a reaction condition with ethanol for 2 hours at 60 °C. This process yielded compounds **1–7**. These findings demonstrated that the Schiff bases' reaction times were shortened by microwave

irradiation. In addition, compared to refluxing conditions, heating with a microwave is more efficient for creating pure chemicals in good to outstanding yields.

FT(IR) spectra:

To determine the degree of bonding between the molecule and the methoxy group of the benzaldehyde, the free PPH was compared to the FT(IR) spectra of the compounds that were formed. Using a limited spectrum of bands, the impact of PPH vibration on substituted bromobenzaldehydes was examined. No stretching vibrations have been detected in any of the produced compounds containing aldehyde (CHO) or amino (NH_2) groups, indicating that they are all fully developed. By contrast, the azomethine (HC=NN-) group generated a strong new band at 1573-1662 cm^{-1} [29]. The presence of an aromatic (NH) bandwidth in the 3054 - 3110 cm^{-1} region has led some to speculate that the prepared compounds must exist [30, 31]. All of the compounds have been classified based on the analysis of the aldehydic (-CH=) bands at 2815-2882 cm^{-1}. Two strong lines at 1493–1590 and 1441–1483 cm^{-1} are seen in the infrared spectra of**1–7** compounds; these lines are associated with the aromatic ring's >C=C group. The strongest bands are at 1318–1330, 728–738, and 654-687 cm^{-1}. Compounds **1-7** display FT(IR) spectra that are compatible with aromatic (C-N), di/ trisubstituted benzene ring, and monosubstituted benzene ring structures. The **1-7** molecule's aromatic C-Br group was identified by a band at 687-691 cm^{-1} in the FT(IR) spectra.

^{1}H NMR spectra:

All of the developed compounds' wide singlet signals in the ^{1}H NMR spectra, which range from 11.82 to 14.41 ppm, are due to the aromatic -NH- group in the pyrrolyl ring. All of the compounds that are made have an aldehydic -CH= group assigned to them in the 7.48-8.49 ppm range, and the aliphatic -NH- singlet peak is visible in the 11.69-12.94 ppm range. Since there is no broad singlet signal at 9.84 ppm (2H) corresponding to the -NH_2 of PPH, the 1H NMR spectra of all the produced derivatives show that the amino group was successfully substituted by the Schiff base [32]. A singlet for the pyrimidine proton between 6.98 and 8.28 ppm is visible in the ^{1}H-NMR spectra of compounds **1-7**. When looking at ^{1}H NMR spectra from different publications, you may find that they have the same bands [33].

Antibacterial activity:

According to **Table 1**, all of the microorganisms that were tested were susceptible to the chemicals that were researched. The minimum inhibitory concentrations (MICs) for these compounds ranged from 0.5 to 64 µg/mL. The reference drug was *ciprofloxacin*, which is a broad-spectrum antibiotic that had a MIC of 10 µg/mL against the bacterial species. For Staphylococcus aureus, the inhibition zones ranged from 10 to 18 mm (MCC 2010), while for Pseudomonas aeruginosa, they ranged from 8 to 29 mm (MCC 2080).

Table 1: Antibacterial studies of **1-7**compounds

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	15	15	19	15
2	17	18	23	16
3	16	0	24	19
4	13	0	23	16
5	12	17	15	25
6	13	12	16	29
7	10	11	10	10
Ciprofloxacin	10	10	12	11

The most effective bacteria against S. aureus, when considering all bacteria collectively, are the **1**, **2**, and **7** strains. Compounds **1-6** showed superior efficacy compared to the industry benchmark ciprofloxacin. The compound **7** exhibited reduced antibacterial efficacy against P. aeruginosa. The results demonstrated that **6** surpassed the suggested method of delivery. The compounds **1-4** showed the highest efficacy against E. coli. Refer to sections **1**, **2**, and **4** for information on B. subtilis. The antibacterial activity is most likely caused by the more effortless penetration of the cell walls of lipophilic microorganisms. The molecule's capacity to traverse the lipid cell membrane of gram-negative bacteria is mainly attributed to the lipophilic alkyl chain. The results indicate that there is a negative correlation between the length of the carbon chain

and the antibacterial activity. This can happen when the size of the carbon chain is too large to traverse the bacterial cell membrane [34].

Antifungal activity:

In response to the reference drug fluconazole (MIC 50 µg/ml), the inhibition zones of Candida albicans (MCC1439) and Saccharomyces cerevisiae (MCC1033) were found to be 6-17 mm and 7-20 mm, separately. All of the substances tested showed substantially higher fungicidal activity than the reference medication, as proven by the data shown in **Table 2**. A minimum inhibitory concentration (MIC) of 54 µg/mL was found for all the substances that were tested against Candida albicans (MCC1439) and Saccharomyces cerevisiae (MCC1033).

Table 2: Antifungal studies of **1-7**compounds

Compound	*Candida albicans*	*Saccharomyces cerevisiae*
1	12	14
2	14	11
3	0	16
4	6	20
5	0	16
6	13	11
7	17	9
Fluconazole	9	12

***In vitro cytotoxicity*:**

Table 3 displays the findings of the evaluation of the cytotoxicity of the substances on Artemia salina. According to literature, the half-life (LD_{50}) values vary between 3.99 and 9.67 x 10-4 µM/mL, which represents the concentration at which 50% of the organisms were impacted.

Table 3: Brine shrimp bioassay of **1-7**compounds

Compound	LD_{50} (M)
1	$>6.45 \times 10^{-4}$
2	$>4.22 \times 10^{-4}$
3	$>5.25 \times 10^{-4}$
4	$>4.49\times 10^{-4}$
5	$>7.31\times 10^{-4}$
6	$>4.66\times 10^{-4}$
7	$>3.99\times 10^{-4}$

CONCLUSION

Various substituted bromo benzaldehydes were used to prepare several new derivatives of PPH. The results acquired from several analytical techniques, such as FT-IR, UV-vis, NMR spectral studies, and electrochemical data, support the creation of the proposed compounds. The compounds that were produced underwent a battery of analytical tests, including ^{1}H NMR, UV-vis, elemental analysis (C, H, N), and FT-IR spectroscopy. After collecting the spectra, they were examined. Based on the results, modified bromo benzaldehydes and PPH should be mixed in a ratio of 1:1. The antibacterial activity of all the compounds that were produced was substantial. Every one of the synthetic chemicals has extremely high cytotoxicity levels when applied to vulnerable cell types.

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