ONE-POT SYNTHESIS OF PHTHALAZINYL-2-CARBONITRILE INDOLE DERIVATIVES VIA [BMIM][OH] AS IONIC LIQUID AND THEIR ANTI CANCER EVALUATION AND MOLECULAR MODELING STUDIES

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Keywords: [bmim][OH]; environmentally benign synthesis; one-pot reaction; indol derivatives.

One pot four component, environmentally benign synthesis of 1*H*-indol-2-yl-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-b]phthalazine-2-carbonitrile derivatives was achieved in the presence of ionic liquid [bmim][OH]. The multi component reaction occurs with an initial formation of pthalazine followed by its reaction with the Knoevenagel cyclocondensation product of indole aldehyde and malononitrile/ethyl cyanoacetate in the presence of [bmim][OH] as ionic liquid at 70-75 ºC for 30-45 min. All the synthesized pthalazine indolyl analogues have been tested for their anti-cancer activity on breast and lung carcinoma cell lines. Among the tested derivatives, **5b**, **5c**, **5e**, and **5f** found to be active against the cancer cell lines. Further, molecular modeling studies were performed to understand binding pattern of the top active molecules with the target protein.

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INTRODUCTION

Multicomponent and eco-friendly reactions are major techniques for the efficient and rapid synthesis of a wide variety of heterocyclic molecules. These reactions are investigated widely in heterocylic synthesis, initially due to their ability to produce complex heterocyclic compounds with functionality groups form simple starting materials via multi component one-pot reactions.[1] In the past few decades, the preparation of new heterocyclic molecules has been the focal point of drug discovery research.[2] Among a wide variety of heterocyclic compounds, phthalazine scaffold has its significance due to its promising pharmacological and biological activities.

Phtalazine derivatives were reported to have anti-cancer,[3] cytotoxic,[4] antifungal,[5] anti-microbial[6] and anti-convulsant activities.[7] In addition, these molecules exhibited good promise as new fluorescence probes and luminescence materials.[8] Due to this reason, it was not surprising that many synthetic methods have been developed for the synthesis of wide verity of phthalazines. In one of these methods phthalhydrazide was used. This compound is usually used as an intermediate in the synthesis of many compounds with phthalazine molecule.[9] Although there were reports of the preparation of phthalazine derivatives,[10] their broad utility range have accentuated the need to make newer methods and newer derivatives of phthalazine moiety. Ionic liquid has attracted noteworthy attention by their significant attention due to their idiosyncratic properties like high thermal stability, easy recyclability, negligible vapour pressure, excellent chemical stability, wide liquid temperature range, and strong solvent power for a wide range of organic and inorganic molecules. By modification of cations and/or anions, the properties of ILs can be turned in many ways.[11]

1-(1*H*-Indol-2-yl)-1*H*-pyrazolo [1,2-*b*]phthalazine-5,10-diones were previously prepared in the presence of $InCl_3$ as catalyst in refluxing ethanol with dialkylphthalates.[12] However, the reported method suffers from the draw backs such as usage of costly catalyst and starting materials, apart from low yields. Further, the biological potential of the titled compounds have not been explored. In view of the potential scope to optimize the synthetic protocol, herein, we report synthesis of 1H-indol-2-yl-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile derivatives by one-pot reaction of phthalic acid, hydrazine hydrate, indolealdehydes and malononitrile/ethyl cyanoacetate in the presence of [bmim][OH], mediated at 70-75 ºC for 30-45 min with excellent yields. In addition, the anti cancer activity of the target compounds have also been evaluated.

EXPERIMENTAL

Melting points are determined on in open capillary tubes in sulphuric acid bath. FT-IR spectra are recorded on a VERTEX 70 Brucker by using KBr. A Bruker DRX-400 spectrometer 400 and 100 MHz was employed for recording ^{1}H NMR and ^{13}C NMR spectra respectively. DMSO-d_6 was used as solvent and TMS as an internal standard. Mass spectra were recorded on Agilent-LCMS instrument.

Molecular Docking

In silico molecular interactions of the selected test compounds with Bcl-2 protein were studied using MGL Tools 1.5.6 AutoDock Tools.[13] The initial ligand structures were created using Chem3D Ultra 16.0 software. Further,

the ligand energy was minimized using MOPAC (semi-empirical quantum mechanics), Job type with minimum 0.01 of RMS gradient and 100 iterations and saved in protein data bank (.pdb) format using Chem3D Ultra 16.0 software. The pre-downloaded PDB structure of BCL-2 protein co-crystallized with Venetoclax (PDB ID: 6O0K) was imported to the workspace. The Kollaman charges were included and the protein structure was prepared in Autodock. The size of the grid box in all the axes (X, Y, Z) was taken as 90 and analysed for further. PyMoL was used for the visualization of the output file generated from docking. The validation of the docking process was done with the comparison between the co-crystallized ligand (Venetoclax) and docked test compound. One pose per run was taken based on root mean square division clustering using a heavy atom threshold set at 1.0 Å and an energy penalty of 100. Each pose was examined manually, and the best poses were retained. LIGPLOT program was used to represent the hydrogen bonds and hydrophobic interactions of the ligand molecules with target protein.[14]

Cytotoxicity assay

The cytotoxicity of the synthesized compounds was tested against two different cancer cell lines A549 (Human lung carcinoma) and MCF 7 (Human breast carcinoma) using MTT assay.[15] Briefly, the cells were grown in 96-well microplates for a period of 24 h. After incubation, the cells were incubated with different concentrations of synthesized compounds along with doxorubicin (positive control) and incubated for 48 h. Subsequently, the cells were incubated again for 2 h with 250 µg/mL of MTT reagent. After incubation, the medium was replenished with 100 µL of DMSO and the absorbance was recorded at 570 nm on a microplate reader.

General procedure for preparation of 5

Phthalic acid (**1**) (10 mM) and hydrazine hydrate (**2**) (10 mM) was added in [bimm][OH] (50 mM) and heated at 70-75 ºC for 10-12 min to form phthalazine as intermediate. Then, to this reaction mixture indolealdehyde (**3a)** (10 mM) and malanonitrile/ethylcyano acetate (**4**) (10 mM) were charged and again heated for 20-35 min at the same temperature. The progress of reaction was monitored by TLC. After completion of the reaction, cooled the reaction mass to 30-35 ºC and charged cold water to the reaction mixture and stirred for 30 min. Solid part was separated by filtration to get crude. Finally, the product was recrystallised from ethanol solvent to obtain **5**.

3-Amino-1-(1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (5a)

M. P. >230 ºC. IR (KBr): 3116-3440 (broad, medium, -NH- group), 2218 (sharp, strong, -CN- group), 1669 (sharp, strong, -CO- of amide group), 1686 (sharp, strong, -CO-of amide group) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 5.67 (s, 1H, -CH), 7.26-8.68 (m, 11H, Ar-H & NH_2), 11.87 (s, 1H, -NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ = 61.0, 69.0, 110.1, 111.5, 115.9, 119.2, 122.9, 123.9, 127.3, 134.6, 135.8, 138.4, 144.6, 145.8, 161.0, 164.5 MS *m/z*: 355 $[M+H]^+$.

3-Amino-1-(1-methyl-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (5b)

M. P. >230 ºC. IR (KBr): 2215 (-CN-), 1669 (-CO-), 1685 (-CO-) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 2.22 (s, 3H, $-CH_3$), 5.32 (s, 1H, -CH), 7.20-8.69 (m, 11H, Ar-H & NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ = 23.5, 60.2, 68.1, 111.4, 111.6, 114.9, 118.2, 122.8, 123.4, 127.2, 133.3, 134.6, 138.4, 144.3, 145.8, 161.5, 164.6. MS *m/z*: 370 $[M+H]^+$.

3-Amino-1-(1-ethyl-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (5c)

M. P.: >230 ºC. IR (KBr): 2218 (-CN-), 1662 (-CO-), 1676 (-CO-) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 1.82 (t, 3H, CH_3) 2.23 (q, 2H, $-CH_2$), 5.27 (s, 1H, -CH), 7.23-8.95 (m, 11H, Ar-H & NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ = 19.4, 23.5, 60.6, 68.6, 111.5, 111.6, 114.3, 118.4, 122.5, 123.6, 127.2, 133.1, 134.2, 138.3, 144.2, 145.3, 161.6, 164.6. MS *m/z*: 384 $[M+H]^+$.

Ethyl-3-amino-1-(1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carboxylate (5d)

M. P. >230 ºC. IR (KBr): 3116-3440 (-NH-), 2204 (-CN-), 1668 (-CO-), 1672 cm^{-1} (-CO-) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 1.25 (t, 3H, $-CH_3$), 4.19 (q, 2H, $-CH_2$), 5.43 (s, 1H, -CH), 7.21-8.69 (m, 11H, Ar-H and NH_2), 11.79 (s, 1H, -NH,). ^{13}C NMR (DMSO-d_6, 400 MHz) δ = 15.2, 55.3, 60.6, 68.2, 110.4, 111.5, 115.2, 117.1, 122.1, 123.4, 127.3, 133.1, 134.8, 137.4, 142.6, 144.6, 150.2, 156.5. MS *m/z*: 403 $[M+H]^+$.

Ethyl-3-amino-1-(1-methyl-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carboxylate (5e)

M. P. >230 ºC. IR (KBr): 2214 (-CN-), 1665 (-CO-), 1683 (-CO-) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 1.13 (t, 3H, $-CH_3$), 2.25 (s, 3H, $-CH_3$), 4.01 (q, 2H, $-CH_2$), 5.43 (s, 1H, -CH), 7.20-8.60 (m, 11H, Ar-H and NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ = 15.2, 22.3, 56.4, 61.5, 67.1, 111.3, 113.5, 114.8, 118.1, 122.2, 122.8, 125.1, 132.3, 134.2, 138.3, 144.2, 143.6, 152.3, 153.6. MS *m/z*: 417 $[M+H]^+$.

Ethyl-3-amino-1-(1-ethyl-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carboxylate (5f)

M. P.: >230 ºC. IR (KBr): 2217 (-CN-), 1667 (-CO-), 1674 (-CO-) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 1.19 (t, 3H, $-CH_3$), 1.68 (t, 3H, CH_3) 2.38 (q, 2H, $-CH_2$), 4.16 (q, 2H, $-CH_2$), 5.25 (s, 1H, -CH), 7.20-8.92 (m, 11H, Ar-H and NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ = 15.3, 19.3, 23.5, 54.3, 60.2, 68.4, 111.3, 111.7, 114.0, 118.2, 122.3, 124.2, 126.8, 133.4, 134.8, 138.1, 144.2, 145.4, 151.6, 154.5. MS *m/z*: 431 $[M+H]^+$.

3-Amino-1-(5-nitro-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (5g)

M. P. >230 ºC. IR (KBr): 3116-3440 (broad, medium, -NH-group), 2218 (sharp, strong, -CN- group), 1669 (sharp, strong, -CO- of amide group), 1686 (sharp, strong, -CO-of amide group) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ = 5.67

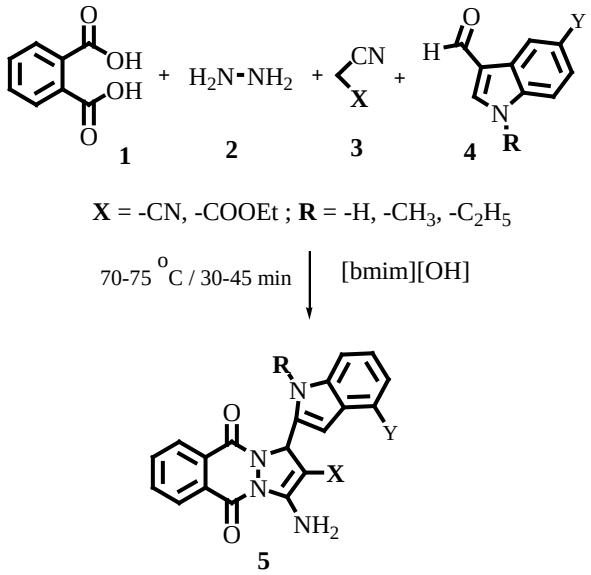
(s, 1H, -CH), 7.26-8.68 (m, 10H, Ar-H and NH_2), 11.87 (s, 1H, -NH,). ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 61.5, 69.1, 110.6, 111.5, 115.8, 115.9, 119.2, 122.9, 123.6, 127.6, 134.6, 135.6, 138.4, 144.6, 145.8, 161.6, 164.5. MS *m/z*: 400 $[M+H]^+$.

Ethyl-3-amino-1-(5-nitro-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carboxylate (5h)

M. P. >230 ºC. IR (KBr): 3362 (-NH-), 2296 (-CN-), 1661 (-CO-), 1665 cm^{-1} (-CO-) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 1.33 (t, 3H, $-CH_3$), 4.45 (q, 2H, $-CH_2$), 6.03 (s, 1H, -CH), 7.21-8.68 (m, 10H, Ar-H and NH_2), 11.78 (s, 1H, -NH,). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 14.1, 61.8, 69.3, 74.1, 110.5, 111.3, 115.8, 115.9, 119.0, 122.9, 123.7, 127.2, 134.6, 135.7, 140.5, 143.7, 151.5, 155.6. MS *m/z*: 402 $[M+H]^+$.

RESULTS AND DISCUSSION

The scheme (Scheme 1) of the optimization of the one-pot four-component synthesis, the reaction is initiated with phthalic acid **1** and hydrazine hydrate **2** to get phthalhydrazide intermediate via in-situ formation as in the presence of ionic liquids. To this reaction mixture, indole-3-carbaldehyde **3a** and malononitrile **4a** are charged for the synthesis of 3-amino-1-(5-nitro-1*H*-indol-2-yl)-5,10-dioxo-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **5a** in the presence of different ionic liquids ([DBUH][OAc], [bmim][OH] & [bmim][Br]) at different temperature as a simple model reaction. The results are summarized in Table 1. The best results are produced in the presence of bmim][OH] (5 eq) as ionic liquid at 70-75 ºC for 30-45 min to form title compound with good yields of 89 % by using **1** (1 eq), **2** (1 eq), **3a** (1 eq) and **4a** (1 eq)**]**. The structure of the compound **5a** has been confirmed by ^{1}H NMR, IR and Mass spectroscopy.



Scheme 1. Four-component synthesis of **5**.

In the next step, the model reaction was carried out in the presence of different amount of ionic liquid [bmim][OH] (3 eq, 5 eq and 8 eq) with respect to phthalic acid **1** (Table 2).

However, it was found that the one-pot reaction of **1** (1 eq), **2** (1 eq), **3a (1 eq)** and **4a** (1 eq) in the presence of [bmim][OH] as a medium (5 eq) for 30 min at 70-75 ºC gave the highest yield (89 %) (Table 1, entry 6).

Table 1. Effect of ionic liquid (5 eq) and temperature on reaction of **1**, **2, 3a** and **4a** to from **5a**.

Entry	Ionic liquid /5 eq	Temp. °C	Time, min	5a (%)
1	[bmim][Br]	40-45	600	80
2	[bmim][OH]	40-45	450	83
3	[DBUH][OAc]	40-45	600	81
4	[bmim][Br]	70-75	60	84
5	[bmim][OH]	70-75	30	89
6	[DBUH][OAc]	70-75	60	85
6	[bmim][Br]	80-85	60	83
8	[bmim][OH]	80-85	30	87
9	[DBUH][OAc]	80-85	60	82

Table 2. Effect of quantity of [bmim][OH] at 70-75 ºC on one-pot four component reaction of **1**, **2, 3a** and **4a** to from **5a**.

Entry	Quantity (eq)	Time, min	Yield, %
1	3	60	85
2	5	30	89
3	8	30	88

In the next step, the scope of the one-pot four component reaction process was explored, using the best optimized conditions by changing the aldehyde and the nitrile The structures of the products were assigned on the basis of their spectral properties -IR, NMR & Mass spectra (Figure 1).

The proposed mechanism for the synthesis of title compounds in the presence of [bmim][OH] is shown in scheme 2. This mechanism proceeds through three steps. In the first step, nucleophilic addition of hydrazine -NH_2 **(2)** to phthalic acid –CO **(1)** is followed by dehydration to form phthalazine **(A1).** In the second step, Knoevenagel condensation of indolealdehyde (**3)** and malononitrile/ethyl cyanoacetate (**4)** forms heterodyne **(B1).** In the third, Michael addition–cyclization reaction of phthalazine **(A1)** and heterodyne **(B1)** gives the desired 1 H-pyrazolo[1,2-b]phthalazine-5,10-dione (**5a–h**) is produced.

Cytotoxicity assay

A series of 8 conjugates of Phthalazinyl-2-carbonitrile indole derivatives were evaluated for their cytotoxicity against two different human cancer cell lines (A549 and MCF7) using MTT assay. The IC_{50} values of the synthesized compounds on two different cancer cell lines were tabulated and shown in the table 3. Most of the compounds showed significant reduction in the cancer cell viability in a dose dependent manner. Among synthesized, compound **5b** and **5f** exhibited good activity against tested cell lines. Compound **5f** showed significant activity against MCF-7 cells with an IC_{50} value of 10.7 µM while compound **5b** exhibited 10.9 µM against A549 cells.

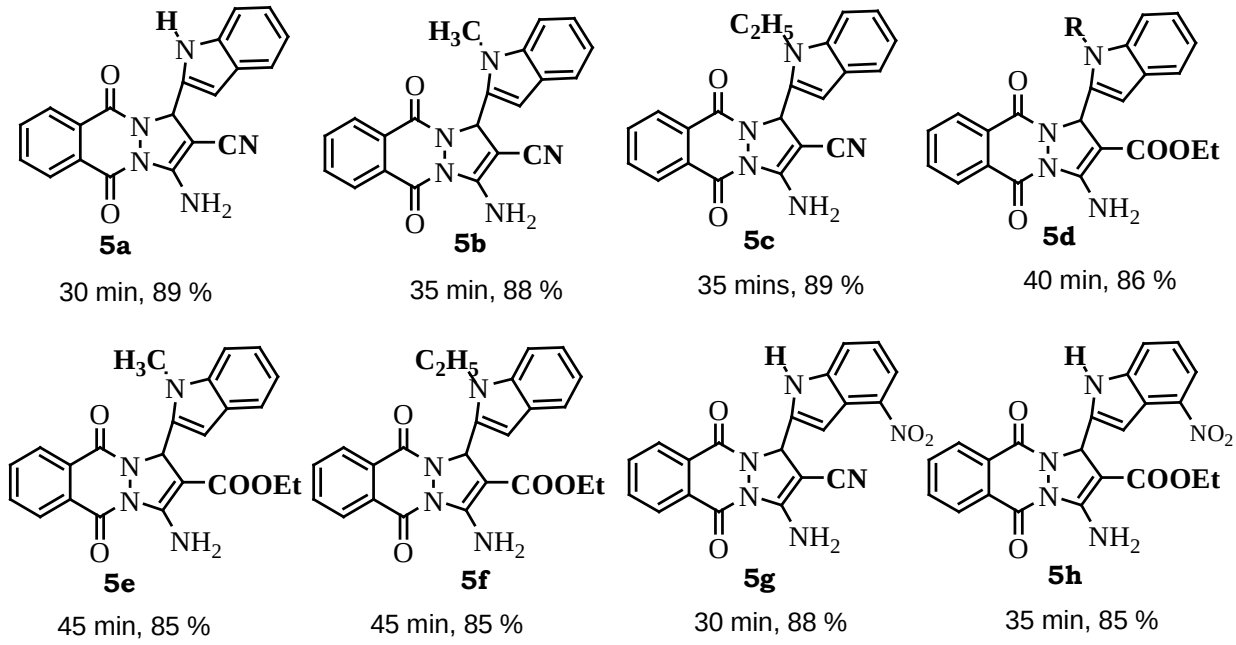
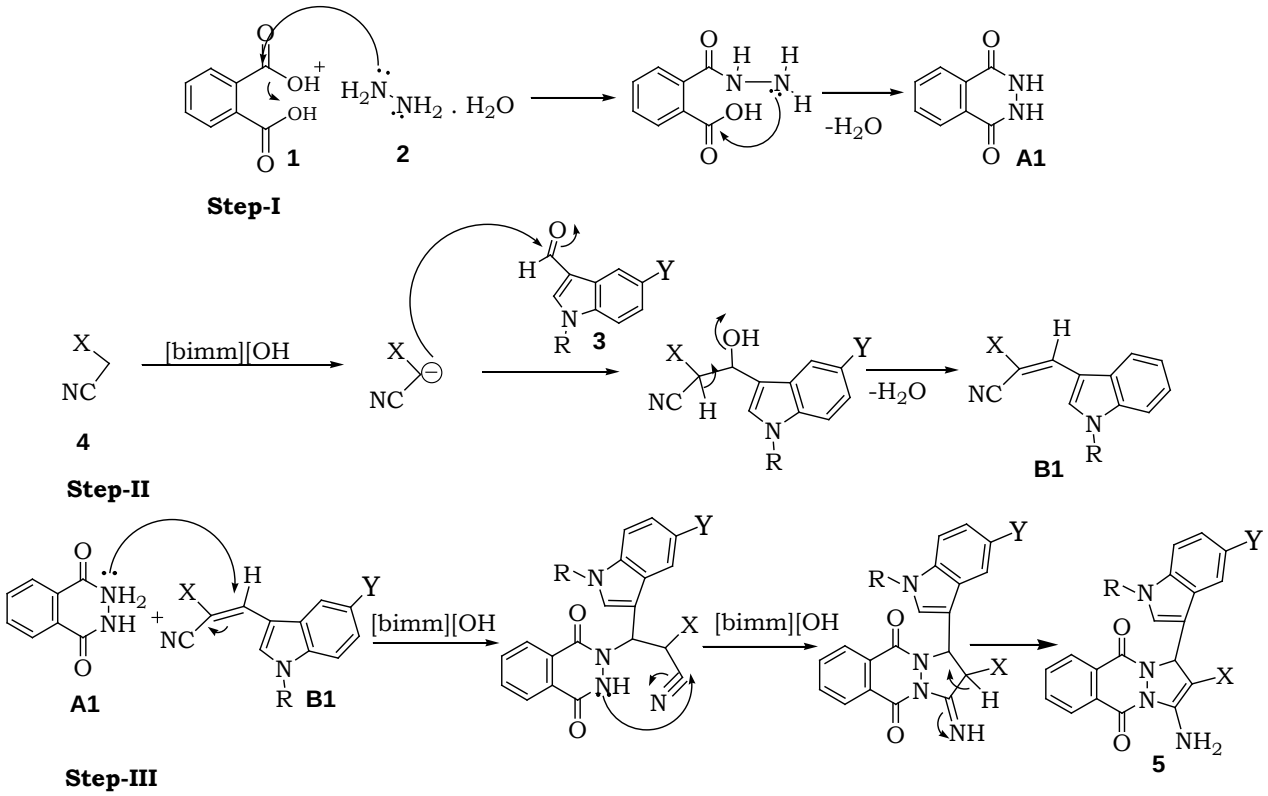


Figure 1. Structure of compounds **5a - 5h**.



Scheme 2. Possible mechanism of the formation of **5** from **1, 2, 3** and **4.**

Molecular Docking

Based on the cytotoxicity results, the most active compounds (**5b** and **5f**) were selected for *in silico* docking analysis. Molecular docking for the **5b** and **5f** compounds was performed against the active site of BCL-2 protein. A maximum of ten different conformations were examined for each docked ligand.

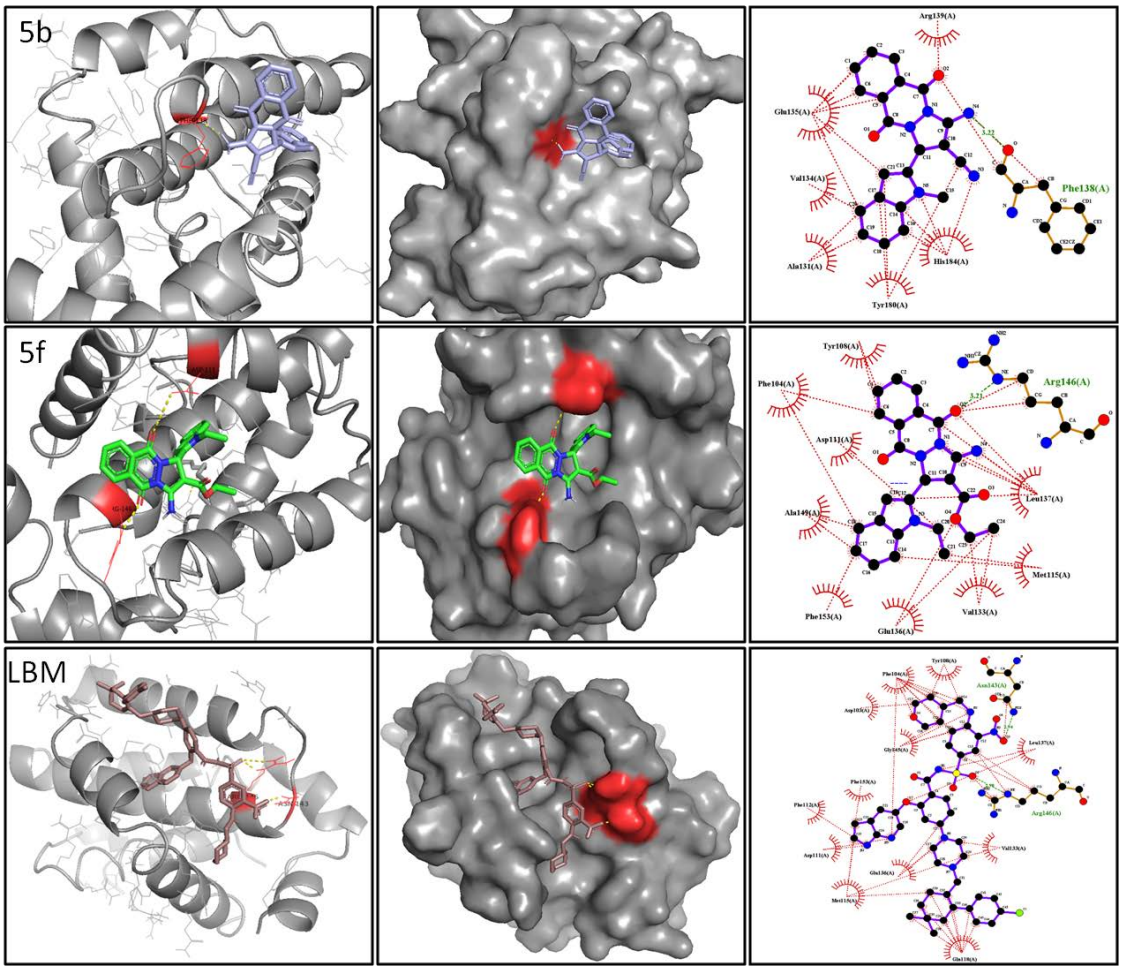


Figure 2. In silico docking of human BCL-2 protein with different compounds 5b, 5f and venetoclax (LBM). The binding interaction for the best docked pose for each ligand was showed in the image. The ligand binding site and the amino acids interacted with the ligands are illustrated using LigPlot.

Table 4. The binding energies and the RMSD values for the potential lead compounds were calculated using AutoDock. The amino acids interacted with ligands were determined using LigPlot.

Ligand	Binding energy kcal mol^{-1}	RMSD	H-bond/s	Protein–Ligand interactions
5b	-7.8	29.625	Phe138	Arg139, Glu135, Val134, Ala131, Tyr180, His184
5f	-8.2	6.211	Asp111, Arg146	Phe104, Tyr108, Asp111, Met115, Ala149, Phe153, Glu136,Val133, Leu137
Venetoclax (Positive control)	-9.8	13.286	Asn143, Arg146	Asp103, Phe104, Tyr108, Gly145, Phe153, Phe112, Asp111, Glu136, Met115, Val133, Leu137, Gln118

Table 3. Cytotoxic tests of the synthesized compounds

Test compound	IC_{50}(µM ± S.D)	
	MCF-7	A549
5a	>100	>100
5b	12.5 ± 0.18	10.9 ± 0.21
5c	12.5 ± 0.11	11.7 ± 0.13
5d	>100	>100
5e	15.5 ± 0.22	15.5 ± 0.18
5f	10.7 ± 0.12	12.7 ± 0.14
5g	>100	>100
5h	>100	>100
Standard	0.68 ± 0.08	0.86 ± 0.07

The docking results revealed that the overall binding energies for the best-docked pose of **5b** and **5f** compounds in the receptor active site were -7.8 and -8.2 kcal mol^{-1}, respectively, while the co-crystallized ligand venetoclax showed -9.2 kcal mol^{-1} binding energy. The binding of test compounds was mainly influenced by hydrophobic interactions as well as hydrogen bonds. The best docking poses were represented in the figure 2 and the resulting docking score and RMSD values were shown in Table 4. In addition, the amino acids that interacted with the target protein were shown in the Table 4. The ligand **5f** and venetoclax shared most common amino acid residues in hydrophobic interactions and hydrogen bonds.

CONCLUSION

In conclusion, we developed an efficient and environmental benign protocol for the synthesis of title compounds using an ionic liquid. This one-pot four component reaction proceeded in short time with high yields, straightforward work-up procedure and no need to use column purifications. In addition, the anti-cancer evaluation and molecular modelling studies gave an insight into their potential to act as anti-cancer agents and their binding pattern with the protein respectively. Further, optimization of hit compounds and detailed QSAR studies may result in lead like molecules.

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NOVEL OXIDATION DEGREE – Zn^{+3} IN THE MACROCYCLIC COMPOUND WITH *TRANS*-DI[BENZO]PORPHYRAZINE AND FLUORIDE LIGAND: QUANTUM-CHEMICAL CONSIDERATION

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Keywords: zinc(III), fluoride ligand, di[benzo]porphyrazine, DFT method.

Based on the results of a quantum chemical calculation using two variants of the DFT method, the possibility of the existence of a zinc heteroligand complex with *trans*-dibenzoporphyrazine and fluoride ion where oxidation degree of zinc is +3 that is unusual for the given chemical element, have been shown. The data on the key structural parameters and multiplicity of the ground state of this complex have been presented, too.

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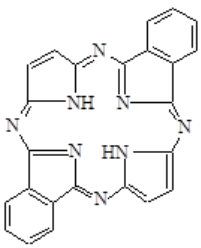
INTRODUCTION

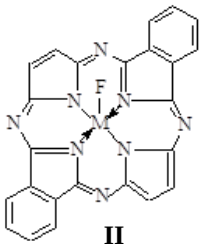
As it is well known for long time, the lightest of the d-elements of the Group II (XII) of the Mendeleev Periodic System of Chemical Elements, zinc, in all its chemical compounds, simple as well as coordination, has oxidation state of +2. The existence of compounds of Zn with higher oxidation states remains unproved in the experiment up to now.[1,2] A non-empirical quantum-chemical calculation revealed a very small probability of their existence, at least for ZnF_4, where this element has an oxidation state of +4.[3] Nevertheless, based on these data, we cannot exclude the possibility of the existence of zinc compounds with an intermediate oxidation state between +2 and +4, namely +3, which, in principle, may be realized in any macrocyclic coordination compounds containing ligands with fluorine donor atoms having a sufficiently high electronegativity.[4] On the other hand, such macrocyclic (NNNN)-donor atomic ligands as porphyrazine derivatives, in particular *trans*-di[benzo]porphyrazine (**I**), are capable of stabilizing the most diverse oxidation states of *d*-elements low as well as high. [5-9]



I

Figure 1. Strucutre of t*rans*-di[benzo]porphyrazine**.**

Therefore, it seems appropriate to use for the stabilization of the above oxidation state of zinc namely the combination of these two ligands which takes place in complexes of type **II** containing one F^- anion and double deprotonated form of *trans*-di[benzo]porphyrazine (where M is the atom of the *d*-element, in particular, Zn). At the present time, there is no information about such coordination compounds in the literature; however, it is possible to estimate the probability of their existence using modern quantum chemical calculation methods. This paper has been devoted to theoretical consideration of the given question.



II

Figure 2. Proposed structure of Zn(III) complex.

CALCULATION METHOD

Quantum-chemical calculations were performed by the two versions of DFT method. In the first of them (OPBE/TZVP), combining the common TZVP extended triple zeta split-valence basis set[10,11] and the OPBE non-hybrid functional,[12,13] which as shown earlier,[13–17] predicts in the case of 3*d* elements more adequately the relative energy stabilities of high-spin and low-spin states, and reliably characterizes key geometric parameters of corresponding molecular structures. In the second, B3PW91/TZVP, combining the common TZVP and B3PW91 functional,[18,19] which according to data,[20] has minimal value of so-called "normal error" in comparison with other variants of DFT method. This conclusion is in full harmony with the data of structural parameters of macrocyclic complexes of various 3*d*-elements with phthalocyanine obtained as a result of various DFT

quantum-chemical calculations and in experiment (see Supplemental material). Calculations were performed with the Gaussian09 program package.[21] The correspondence of the found stationary points to energy minima was proved in all cases by the calculation of second derivatives of energy with respect to atom coordinates. All equilibrium structures corresponding to minima of the potential energy surfaces had only real positive frequency values. Zn^{+3} has $3d^9$ electronic configuration, and, in this connection, spin multiplicities 2 and 4 were considered in calculation. Among the structures optimized at these multiplicities, the lowest-lying structure was selected. Parameters of molecular structures with the given multiplicities were calculated by the unrestricted methods (UOPBE and UB3PW91, respectively).

RESULTS AND DISCUSSION

According to the data obtained by us as a result of the quantum-chemical calculation carried out using the DFT OPBE/TZVP method as well as the DFT method B3PW91/TZVP, the Zn(III) complex of type **II** is capable to self-existence, at least in the gas phase. Molecular structure of this complex obtained by DFT B3PW91/TZV method, is shown in figure 1.

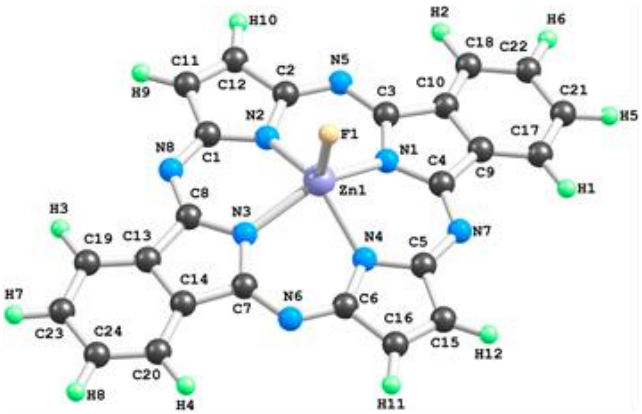


Figure 3. Molecular structure of Zn(III) complex of type **II** obtained by means of DFT B3PW91/TZVP quantum-chemical calculation.

Its molecular structure obtained by the DFT OPBE/TZVP method looks similar. The calculated chemical bond lengths between atoms and bond angles for this compound are presented in Table 1. It is apparent from these data that both the methods used by us give almost identical data for all structural parameters indicated above. As it can be seen from figure 3, the complex under examination has a tetragonal-pyramidal structure of the ZnN_4 chelate node with identical bond angles (NZnN), but rather such a significant (almost 30º) deviation from co-planarity. However, the grouping of nitrogen atoms (N1N2N3N4), which is part of the chelate node, is almost flat (deviation from coplanarity is only 0.2º). The Zn–N bond lengths in the chelate node are equal to each other only in pairs, but the difference between them is insignificant (Table 1). All four 6-membered metal-chelate rings are completely identically to each other in the lengths of bonds between the corresponding atoms as well as in the range of bond angles in them.

Table 1. Bond lengths and bond angles in the Zn(III) complex of type **II.**

Structural parameter	Calculated	
	DFT OPBE/TZVP	DFT B3PW91/TZVP
Zn–N bond lengths in chelate node, pm		
Zn1N1	210.1	210.0
Zn1N2	210.8	207.6
Zn1N3	210.1	210.0
Zn1N4	210.8	207.6
Bond angles in chelate node ZnN_4, *deg*		
(N1Zn1N2)	83.1	83.2
(N2Zn1N3)	83.1	83.2
(N3Zn1N4)	83.1	83.2
(N4Zn1N1)	83.1	83.2
Bond angles sum (BAS), *deg*	332.4	332.8
Non-bond angles between N atoms in N_4 grouping, *deg*		
(N1N2N3)	87.7	91.2
(N2N3N4)	91.9	88.8
(N3N4N1)	87.7	91.2
(N4N1N2)	91.9	88.8
Non-bond angles sum (NBAS), *deg*	359.2	360.0
Bond angles in 6-numbered ring (Zn1N1C4N7C5N4), *deg*		
(Zn1N1C4)	121.5	124.6
(N1C4N7)	127.2	127.7
(C4N7C5)	123.1	123.0
(N7C5N4)	127.3	127.8
(C5N4Zn1)	125.5	124.5
(N4Zn1N1)	83.1	83.2
Bond angles sum (BAS^6), *deg*	707.7	710.8
Bond angles in 5-numbered ring (C3N1C4C9C10), *deg*		
(C3N1C4)	106.8	109.2
(N1C4C9)	110.3	109.4
(C4C9C10)	106.3	106.0
(C9C10C3)	106.3	106.0
(C10C3N1)	110.3	109.4
Bond angles sum (BAS^5), *deg*	540.0	540.0
C–N bond lengths in 6-numbered chelate rings, *pm*		
N1C3	135.8	135.4
N1C4	135.8	135.4
N2C1	135.8	135.2
N2C2	135.8	135.2
N7C4	133.1	132.7
N7C5	133.1	132.6
C–C bond lengths in 5-numbered ring, *pm*		
C4C9	147.1	146.9
C9C10	135.2	140.0
C10C3	147.1	146.9
Zn–F bond length, *pm*		
Zn1F1	186.2	185.4
Bond angles between fluorine, copper and nitrogen atoms, *deg*		
F1Zn1N1	113.1	109.5
F1Zn1N2	108.0	110.8
F1Zn1N3	113.1	109.5
F1Zn1N4	107.9	110.9

A similar situation occurs for four 5-membered non-chelate rings with one nitrogen atom and four carbon atoms adjoining to 6-membered metal chelate rings. Besides, the 5-membered rings are coplanar, while the 6-membered ones are non-coplanar, the deviation from coplanarity in them depending used calculation method and is 12.3° (OPBE/TZVP) and 9.2° (B3PW91/TZVP) (Table 1). As can be seen when comparing the data obtained in the calculations using the above-listed DFT method variants (Table 1), each of the DFT method variants we used gives own individual (although slightly different) sets of bond angles in chelate nodes, metal chelate rings as well as non-chelate 5-membered cycles. However, the sums of these angles in each of these calculation methods are fully identical to each other.

The zinc-donor nitrogen atom and zinc-fluorine interatomic distances (Table 1) correspond in their size to single bonds Zn–N and Zn–F. The facts noted above, as well as the fact that the lengths of Zn–N and Zn–F bonds are different among themselves, allow us to assign the compound Zn(III) under study to the category of tetragonal pyramidal complexes (Figure 3). Besides, the bond angles formed by the fluorine, zinc atoms and the donor nitrogen atoms of the chelate site are slightly different from each other (although they are equal to each other in pairs). The given complex does not have a center of symmetry and therefore, for it a priori one can expect a sufficiently large value of the electric moment of the dipole. Indeed, the data for calculating this parameter (5.61 Debye units according to DFT OPBE/TZVP and 5.51 Debye units according to DFT B3PW91/TZVP) are in full accordance with such an expectation.

According to the data of our calculations, the ground state of the Zn(III) heteroligand complex containing *trans*-di[benzo]porphyrazine and F^- ion under examination according to both calculation methods used here is a spin doublet. It is quite expected for tetragonal-pyramidal complexes with $3d^9$ configuration, and a coordination number of a metal ion equal to 5. Besides, according to the data of both these methods, the nearest excited quartet state has much higher energy (by 157.4 kJ mol^{-1} in the case of DFT OPBE/TZVP and 192.8 kJ mol^{-1} in the case of DFT B3PW91/TZVP), which apparently, makes it impossible an availability of spin-crossover in this complex.

CONCLUSION

As can be seen from the data presented above, both variants of the DFT method used by us in this work, namely OPBE/TZVP and B3PW91/TZVP, quite definitely give evidence about the possibility of the existence of Zn(III) complex, namely [Zn**L**F] containing fluorine anion (F^-) and double deprotonated form ($\mathbf{L}^{2-}$) of *trans*-dibenzoporphyrazine ($H_2\mathbf{L}$). It should be noted in this connection that, according to our calculations of standard thermodynamic parameters $\Delta H^0_{f,\,298}$, $S^0_{f,\,298}$ and $\Delta G^0_{f,\,298}$ of the complex under study using described method,[22] all they are positive (298.7 kJ mol^{-1}, 959.0 kJ mol^{-1} K^{-1} and 515.4 kJ mol^{-1}, respectively) and hence, the given compound cannot be obtained from simple substances formed by chemical elements containing in its composition (zinc, fluorine, nitrogen, carbon and hydrogen). Nevertheless, both variants of the DFT method used by us, namely OPBE/TZVP and B3PW91/TZVP, predict the possibility of the existence of this complex, and the point is now to prepare it experimentally.

FUNDING INFORMATION

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest, financial or otherwise.

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IN VITRO ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF THREE EXTRACTS OF *AMARANTHUS HYBRIDUS* L. LEAF AND THEIR PHYTOCHEMICALS

Gloria Ihuoma Ndukwe,[a]* Poro David Clark[a] and Ibiba Reuben Jack[a]

Keywords: Maceration; *Amaranthus hybridus;* phytochemical constituents; antimicrobial; antioxidant; stigmasterol; quinazoline; capsaicin; methyl commate B.

The study sought to determine the phytochemical components, antioxidant and antimicrobial activities of n-hexane, ethyl acetate and methanolic extracts of *Amaranthus hybridus* L. leaf. Three extracts of *A. hybridus* were examined for antimicrobial activity using disc diffusion assay. The different extracts demonstrated varied concentration-dependent antimicrobial activities against the test organisms. All extracts studied in this work were active against *E. coli, S. aureus, B. cereus, T. mentagrophyte* and *A. niger*. The methanol extract showed potent inhibitory activity against *T. mentagrophyte* when compared to a standard antifungal agent, fluconazole. *In vitro* antioxidant activities were studied spectrophotometrically using vitamin C as standard. There were significant correlations between the methanolic extract and vitamin C for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, reducing ability, hydroxyl radical inhibitory and phosphomolybdate scavenging. The results of this study have shown that leaves of *A. hybridus* possess bioactive compounds which contributed to its antimicrobial and antioxidant properties.

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INTRODUCTION

Plants and plant-derived products have been a source of medicine for long. Even today, scientists and the general public recognize their value as a source of new and complementary medicines owing to their versatile applications.[1] Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agents.[2] Plants have been known to contain or possess abundant phytochemicals, antimicrobials and pharmacologically active principles, which include anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids.[3]

While orthodox medicine is generally accepted and preferred globally, the use of herbs and traditional medicines is often considered an equally acceptable alternative in many regions of the world.[4] Traditional medicine is commonly used in developing countries where the cost of orthodox medicine and access to medical care are not available to a part of the population.[4] The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led scientists to investigate the antimicrobial activity of medicinal plants.[5] Likewise, the use of synthetic antioxidants are suspected to cause or promote negative health effects, hence stronger restrictions are being placed on their application and a trend to substitute them with naturally occurring antioxidants is developing.[6] The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents.[7]

Keeping the above mentioned importance of medicinal plants in view, one of the medicinally important plants, *Amaranthus hybridus* L., also known as African spinach and 'terere' by most communities in Kenya, is cultivated in several regions of the world including South America, Africa, India, China, and the United States of America.[8] In Kenya, its leaves are eaten like spinach or green vegetables. In Nigeria, *Amaranthus hybridus* leaves combined with seasonings are used to prepare soup.[9] These leaves, when boiled and mixed with a groundnut sauce, are eaten as a salad in Mozambique or poured into a sauce and served over vegetables in West Africa.[10] The plant is also used in the treatment of intestinal bleeding, excessive menstruation and diarrhea.[11]

EXPERIMENTAL

Plant Material

The leaves of *Amaranthus hybridus* (Figure 1) were purchased from a market in Port Harcourt, Nigeria.

Figure 1. *Amaranthus hybridus* L. leaves.

The purchased leaves were identified and authenticated by Prof. (Mrs.) O. B. Green of the Department of Plant Science and Biotechnology, Rivers State University, Nigeria. The plant material was sorted out to obtain only fresh leaves which were washed with distilled water (without squeezing) to remove debris and dust particles. The washed leaves were air-dried for a few days under shade to prevent ultra-violet rays from altering the chemical constituents.[2,12] The dry leaves were later pulverized using a manual blender.

Extraction

The dry pulverized leaves of *A. hybridus* (610 g) was macerated using 2.1 L of n-hexane in an aspiratory bottle at room temperature for 48 h with frequent stirring.[13] Then the extract obtained was filtered into a conical flask using a funnel and a filter paper to obtain the n-hexane extract. The residue left was again subjected to second successive extraction with fresh n-hexane according to the procedure described above to obtain the second extract of n-hexane; this process was done 6 times to exhaustively extract the plant material. The same procedure was performed on the plant residue using 1.7 L of ethyl acetate and 1.2 L of methanol sequentially. The three extracts obtained were then separately concentrated using a rotary evaporator at 45 °C. The concentrated extracts were later weighed to obtain the yields and percentage yield for each extract was calculated.

Phytochemical Screening

Phytochemical examination was carried out on each of the extracts of *A. hybridus* leaf using standard methods. Each of the concentrated extract was subjected to qualitative tests via standard procedures to detect the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids and cardiac glycosides.[14,15]

Phytochemical Quantification

The methods of Wenkam and Wills were adopted for the preparation and extraction of *A. hybridus* leaf for GC-MS analysis.[16,17]

Antimicrobial Assay

Clinical isolates of two Gram-positive pathogenic bacteria (*Bacillus cereus* and *Staphylococcus aureus*), two Gram-negative pathogenic bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two pathogenic fungi (*Aspergillus niger* and *Trichophyton mentagrophyte*) were used for this study. The microbes were obtained from University of Port Harcourt teaching hospital, Nigeria.

Disc diffusion assay

Disc diffusion method of susceptibility testing, as described by Mahmodi *et al*. and Ndukwe *et al*. with some modifications was used to evaluate the antimicrobial properties of the extracts.[18,19] In this method, broth cultures of microorganisms were first prepared by inoculating a colony or colonial material of each microorganism separately, into 10 mL sterile nutrient broth for the bacteria and potato dextrose broth for the fungi. Inoculated nutrient broth tubes were incubated at 37 °C for 6 h to obtain a turbidity equivalent to 0.5 McFarland standards, while inoculated PDB broth tubes were incubated at 25 °C for 24 h. After incubation, the broth cultures of the bacteria were swab plated, separately, unto sterile nutrient agar plates. This was also done for the fungi using Sabouraud dextrose agar plates. The number of agar plates used corresponded to the number of dilutions of the investigated extracts, the number of microorganisms subjected to the testing, and the number of standard drugs (positive controls) used. Filter paper discs of 6 mm in diameter were impregnated separately with the *A. hybridus* leaf extracts and placed on the inoculated plates with the aid of sterile forceps. Antibiotic and antifungal impregnated discs were also placed on another set of inoculated plates to serve as positive controls. The plates were incubated at 37 °C for 24-48 h, after which the zones of inhibition around the discs were measured.

Antioxidant Assays

DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to the method reported by Sunil and Ignacimuthu with few modifications.[20] 1 mL of methanolic DPPH (0.15%) was mixed with 3 mL of each *A. hybridus* leaf extract at varying concentrations (0.25-2.5 mg L^{-1}) or vitamin C (reference antioxidant) and incubated in a dark room for 30 min. Thereafter, absorbance was measured at 515 nm. Scavenging activity of each extract and vitamin C was expressed as percentage and calculated using eqn. (1). Distilled water was used as blank.

$$\%\text{Scavenging activity} = \frac{A_c - A_s}{A_c} \times 100 \qquad (1)$$

where A_c is absorbance of the control and A_s is absorbance of the sample.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was investigated using the method described by Bera *et al.* with few modifications.[21] 1 mL of phosphate buffer (0.2 M, pH 7.2), 1 mL of test solution either *A. hybridus* leaf extract (0.25-2.5 mg L^{-1}) or vitamin C, 0.02 mL of ferric chloride (0.02 M) and 0.05 mL of phenanthroline (0.04 M) were introduced into a test tube. The reaction was triggered by adding 0.05 mL of 7 mM hydrogen peroxide. After 5 min of incubation at room temperature (25 °C), absorbance was measured at 560 nm using a UV spectrophotometer. Hydroxyl radical scavenging activity was expressed as percentage scavenging activity and calculated using eqn. (1). Methanol was used as blank.

Phosphomolybdate Assay

Free radical scavenging activity via the phosphomolybdate method was determined according to the method of Jayaprakash *et al.* as modified by Okoko and Diepreye.[22,23] 0.2 mL of either *A. hybridus* leaf extract (0.25-

2.5 mg L^{-1}) or vitamin C (reference antioxidant) was mixed with 1 mL of phosphomolybdate reagent (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid) and incubated in a water bath at 95 °C for 90 min. Absorbance was taken at 695 nm after allowing the content to cool. Free radical scavenging activity was expressed as percentage activity and calculated using eqn. (2). Distilled water was used as blank.

$$\% \text{ TAC} = \frac{A_c - A_s}{A_c} \times 100 \qquad (2)$$

where *TAC* is total antioxidant capacity.

Reducing Ability

The ability of the extract to reduce Fe^{3+} was investigated according to Oyaizu method as modified by Okoko and Diepreye.[24,23] *A. hybridus* leaf extract or vitamin C (0.5 mL was mixed with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide (1%) and incubated at 50 °C. After incubation for 20 min, 0.5 mL of trichloroacetic acid (10%) was added and centrifuged for 10 min at 3000 rpm. A portion of the upper layer (0.5 mL) was mixed with 0.5 mL distilled water and 0.1 mL ferric chloride (0.1%). After 10 min of incubation at room temperature, absorbance was measured at 700 nm. An increase in absorbance indicated greater reducing ability.

Statistical Analysis

Data obtained were expressed as means ± standard deviation (SD) of triplicates. All data were subjected to one-way analysis of variance (ANOVA) using SPSS (version 20) software. The values were considered to be significantly different when $p<0.01$. Means and standard deviations from the DPPH radical scavenging activity, hydroxyl radical inhibitory, reducing ability and phosphomolybdate assays are results of experiments performed in triplicate.

RESULTS AND DISCUSSION

Yields of extracts

Plants provide a large range of natural compounds belonging to different molecular families offering various medicinal properties.[25] Ethno-botanical information revealed that the plant selected in this study is traditionally used for various medicinal purposes.[11,26] Extraction of *A. hybridus* leaf was carried out starting with a non-polar solvent (n-hexane), followed by a semi-polar solvent (ethyl acetate) and finally a polar solvent (methanol). Percentage yields of *A. hybridus* leaf extracts are given in Table 1. Percentage of extractable compounds varied from 2.5 to 3.9 %. This observation agrees with the work of Ibrahim *et al.* who reported the percentage yield of extracts to be in the order, methanol > ethyl acetate > n-hexane.[27] However, the yields of the extracts, as well as the bioactivity of the extracts prepared by maceration method has been reported to vary in several studies.[28] It has also been suggested that the maceration method may be a better choice for the extraction of secondary metabolites.[28]

Table 1. Extraction yield of *A. hybridus* leaf.

Extract	Weight of extract, g	Percentage yield, %
n-Hexane	15.51	2.50
Ethyl acetate	19.92	3.20
Methanol	24.31	3.90

Qualitative and quantitative assessment of phytochemicals

The qualitative phytochemical screening of *A. hybridus* leaf extracts revealed the presence of secondary metabolites (Table 2). Alkaloids, saponins, steroids, terpenoids, and glycosides were present in *A. hybridus* leaf, but flavonoids and tannins were absent. This result corroborates the findings of Maiyo *et al.*, who noted that leaf extract of *A. hybridus* contained steroids, terpenoids and cardiac glycosides but lacked tannins.[28] Cardiac glycosides are an important class of natural drugs that are widely used in the modern treatment of congestive heart failure and for the treatment of arterial fibrillation and flutter.[29] Terpenoids have been demonstrated to be active against bacteria, fungi, viruses and protozoa.[30,31] Alkaloids have important pharmacological uses such as analgesics, antibacterial, antimalarial and anti-hypertensive.[32]

Table 2. Phytochemical groups present in *A. hybridus* leaf.

Phytochemical group	n-Hexane extract	Ethyl acetate extract	Methanol extract
Alkaloids	+	+	+
Saponins	-	+	+
Tannins	-	-	-
Flavonoids	-	-	-
Steroids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	-	+	+

Key: + Present; - Absent

Table 3. Phytochemical percentage composition of *A. hybridus* leaf.

Phytochemical group	Percentage composition (%)
Alkaloids	0.004
Glycosides	0.009
Saponins	0.002
Flavonoids	Nil
Tannins	Nil
Total	0.015

GC-MS is preferred for more precise information in both qualitative analysis and quantitative determination.[33]A quantitative estimate of the percentage of alkaloids, glycosides and saponins components are given in Table 3. Glycosides (0.009 %) had the highest phytochemical presence in *A. hybridus* leaf, followed by alkaloids (0.004 %), with saponins (0.002 %) as the lowest. The medicinal value of the plant may be related to their constituent phytochemicals. According to Varadarajan *et al.*,

the phytochemicals and other chemical constituents of medicinal plants account for their medicinal value.[34] GC-MS analysis of methanolic extract of *A. hybridus* leaf indicated the presence of 20 compounds. The names and structures of identified phytochemicals with their retention time, molecular formula, molecular weight and abundance (peak area in %) are presented in Table 4. Among the identified phytochemicals, squalene is a compound that possesses antitumor, antioxidant, anticancer, antimicrobial, chemo-preventive, pesticide and sun-screen properties; squalene has also been reported as an important precursor for the synthesis of phytosterols such as sitosterol, campesterol and stigmasterol. Capsaicin and dihydrocapsaicin are alkaloids and have been reported to have antioxidant activity, methyl commate B is a triterpene glycoside in nature. Triterpene glycosides are well known for their cytotoxic, antibacterial, antimicrobial, antiviral, insecticide, nematicide, anticoagulant, hemolytic, antiparasitic, wound healing and antitumor activities. Quinazoline is an alkaloid having anti-cancer, antifungal and antibacterial properties while stigmasterol exhibits anti-cancer activities.[35-40] Qualitative and quantitative studies have revealed that this plant is rich in phytochemical components and therefore could play important medicinal and physiological roles.

Antimicrobial activity of *A. hybridus* leaf extracts

The methanol extract of *A. hybridus* leaf demonstrated varied concentration-dependent antimicrobial activities against the test organisms (Table 5). The extract was active against *E. coli*, *S. aureus*, *B. cereus*, *A. niger* and *T. mentagrophyte*. However, there was no observable zone of inhibition against *P. aeruginosa.* Antimicrobial activities of the ethyl acetate extract of *A. hybridus* leaf are presented in table 6. It was observed that the ethyl acetate extract demonstrated weak activity against *S. aureus*, *B. cereus*, and *E. coli* when compared with the positive control (streptomycin and ofloxacin). Only 50-200 mg mL^{-1} of the extract had effect on *T. mentagrophyte* and *A. niger*, the other dilutions had no effect. There was no measurable zone of inhibition against *P. aeruginosa.* n-Hexane extract had little inhibitory effect on *S. aureus*, *E. coli* and *B. cereus.* There was no measurable inhibition zone against *P. aeruginosa.* However, the extract showed moderate antifungal activity in some of the concentrations compared to conventionally used fluconazole (Table 7). Several studies have indicated that Gram-negative bacteria are more resistant to antimicrobial agents than Gram-positive bacteria, due to the presence of a multilayered structure of Gram-

negative bacteria that is not present in Gram-positive bacteria.[41] Results of this study showed that the extracts of *A. hybridus* leaf were active against *E. coli*, *S. aureus*, *B. cereus*, *T. mentagrophyte and A. niger* but were ineffective against *P. aeruginosa* (Tables 5-7). *P. aeruginosa* is considered one of the most rapidly growing bacteria in its resistance to existing antibiotics.[19,42] The results of *A. hybridus* leaf indicate that methanol produced a more potent extract with increased antimicrobial activity, which inhibited greater number of bacterial strains and fungi. The observed antifungal potency of methanol extract can be attributed to two reasons. First, the nature of biologically active components (alkaloids, terpenoids and saponins) present.[43] Alkaloids, terpenoids and saponins, documented as plant metabolites, have been well known for their antimicrobial activities.[43] Secondly, the high polarity and strong extraction capacity of methanol may have given rise to a large number of active constituents responsible for the observed antifungal activity.

Table 4. Identified components of methanolic extract of *A. hybridus* leaf.

Compound	Molecular formula	Molecular weight	Retention time (min)	Peak Area
4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-(E)- 3-buten-2-one	$C_{13}H_{20}O$	192	9.7024	0.30
Quinazoline	$C_8H_6N_2$	130	10.441	0.61
N-(4-Methoxyphenyl)-2-hydroxyimino acetamide	$C_9H_{10}N_2O_3$	194	11.487	0.57
Spiro[4.5] decan-6-one	$C_{10}H_{16}O$	152	12.072	1.10
Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307	16.771	0.14
Nonivamide	$C_{17}H_{27}NO_3$	293	16.855	0.10
Capsaicin	$C_{18}H_{27}NO_3$	305	17.131	9.70
N-(4-Chlorophenyl)-4-nitrophenyl ester carbamic acid	$C_{13}H_9CIN_2O_4$	292	18.587	0.86
Squalene	$C_{30}H_{50}$	410	19.977	0.94
cis-1,2-Dicarboximine-4-n-butyl cyclohexane	$C_{12}H_{17}NO_2$	207	20.520	0.33
6-(4-Ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-isopropyl ester-1H-Indole-2-carboxylicacid	$C_{10}H_{11}NO_3$	193	20.901	0.33
2,7-Diphenylindole	$C_{20}H_{15}N$	269	21.931	0.24
N-[4-(Trifluoromethoxy)-phenyl]-1H-pyrazole-3-carboxamide	$C_{11}H_7F_3N_4O_4$	316	22.033	6.45
Olean-12-ene	$C_{30}H_{50}$	410	22.031	0.32
Lanosterol	$C_{30}H_{50}O$	426	22.977	1.97
N-Ethoxy-2-carbomenthyloxyaziridin	$C_{15}H_{27}NO_3$	116	26.644	0.41
(3β,22E)-Chola-5,22-dien-3-ol	$C_{24}H_{38}O$	342	27.441	0.17
6-Methyl-1,5-diazabicyclo[3.1.0]hexane	$C_5H_{10}N_2$	98	28.068	0.36
Stigmasterol	$C_{29}H_{48}O$	412	31.121	0.70
Methyl commate B	$C_{31}H_{50}O_3$	470	31.352	0.07

Table 5. Antimicrobial activity of methanol extract of *A. hybridus* leaf.

MCO	Zone of inhibition (mm)						
	200 mg mL^{-1}	100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}	12.5 mg mL^{-1}	6.25 mg mL^{-1}	PCT
S.a	10.1±0.23	9.7±0.12	8.9±0.21	8.1±0.21	7.7±0.12	7.7±0.12	28.9±1.05(ST)
B.c	10.1±1.10	9.0±0.20	8.6±0.12	8.3±0.20	8.1±1.01	8.1±0.17	19.8±0.12(ST)
E.c	9.2±0.31	8.9±0.21	8.6±0.23	8.2±0.40	7.8±0.29	8.1±0.25	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	9.9±0.21	8.8±0.06	8.5±0.15	8.0±0.06	7.9±0.15	7.4±0.06	13.9±0.12(FZ)
T.m	9.8±0.23	9.2±0.21	8.8±0.31	8.3±0.25	7.4±0.25	NA	12.0±0.10(FZ)

NA - no action, MCO – microorganisms, B. c – Bacillus cereus, S. a – Staphylococcus aureus, E. c –Escherichia coli, P. a – Pseudomonas aeruginosa, A. n Aspergillus niger, T. m – Trichophyton mentagrophyte, PCT – positive control: ST- Streptomycin, OF – Ofloxacin, FZ – Fluconazole.

Table 6. Antimicrobial activity of ethyl acetate extract of *A. hybridus* leaf.

MCO	Zone of inhibition (mm)						
	200 mg mL^{-1}	100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}	12.5 mg mL^{-1}	6.25 mg mL^{-1}	PCT
S.a	9.1±0.27	8.5±0.15	NA	NA	NA	NA	28.9±1.05(ST)
B.c	9.3±0.47	9.1±0.21	8.5±0.23	8.4±0.25	8.0±0.21	7.3±0.21	19.8±0.12(ST)
E.c	8.9±0.06	7.6±0.17	NA	NA	NA	NA	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	9.7±0.27	8.2±0.12	7.7±0.23	NA	NA	NA	13.9±0.12(FZ)
T.m	9.8±0.12	9.1±0.21	8.6±0.12	NA	NA	NA	12.0±0.10(FZ)

NA - no action, MCO – microorganisms, B. c – Bacillus cereus, S. a – Staphylococcus aureus, E. c –Escherichia coli, P. a – Pseudomonas aeruginosa, A. n Aspergillus niger, T. m – Trichophyton mentagrophyte, PCT – positive control: ST- Streptomycin, OF – Ofloxacin, FZ – Fluconazole.

Table 7. Antimicrobial activity of n-hexane extract of *A. hybridus* leaf.

MCO	Zone of inhibition (mm)						
	200 mg mL^{-1}	100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}	12.5 mg mL^{-1}	6.25 mg mL^{-1}	PCT
S.a	9.5±0.36	8.8±0.21	NA	NA	NA	NA	28.9±1.05(ST)
B.c	9.4±0.59	9.1±0.23	8.8±0.15	8.2±0.25	7.7±0.61	7.6±0.29	19.8±0.12(ST)
E.c	8.9±0.59	8.8±0.23	8.4±0.15	NA	NA	NA	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	9.0±0.21	8.5±0.12	7.7±0.15	NA	NA	NA	13.9±0.12(FZ)
T.m	9.9±0.10	8.9±0.10	NA	NA	NA	NA	12.0±0.10(FZ)

NA - no action, MCO – microorganisms, B. c – Bacillus cereus, S. a – Staphylococcus aureus, E. c –Escherichia coli, P. a – Pseudomonas aeruginosa, A. n Aspergillus niger, T. m – Trichophyton mentagrophyte, PCT – positive control: ST- Streptomycin, OF – Ofloxacin, FZ – Fluconazole.

Table 8. ANOVA Result for antioxidant activity of *A. hybridus* leaf extracts.

Extract	Method			
	HRIA (560 nm)	DPPH (515 nm)	Phosphomolybdate (695 nm)	Reducing ability (700 nm)
Vitamin C	0.41±0.15	0.54±0.14	0.45±0.04	0.33±0.07
Methanol extract	0.43±0.06	0.51±0.13	0.44±0.04	0.29±0.04
n-Hexane extract	0.34±0.06	0.37±0.10*	0.36±0.04*	0.27±0.08
Ethyl acetate extract	0.41±0.18	0.49±0.08	0.40±0.04	0.28±0.05
P-value	0.1667	<0.0001	<0.0001	0.1685
F-value	1.742	12.37	11.36	1.761
Summary	Ns	S	S	Ns

Summary of the six varying concentrations (0.25, 0.50, 1, 1.5, 2.0 and 2.5 mg L^{-1}) of different solvent extract of *A. hybridus* leaf. Values are the mean ± SD of samples in triplicate. Means present a significant difference ($p<0.01$). *Significance difference versus vitamin C, S-significant, NS – No significant difference.

Although results of the antibacterial assay were not very promising, an increase in the concentration of the extracts may improve the inhibition. In addition, the antimicrobial activities displayed against *E. coli, S. aureus, B. cereus T. mentagrophyte and A. niger* could be due to the synergistic effect of bioactive compounds such as squalene, quinazoline and methyl commate B, identified in the plant.

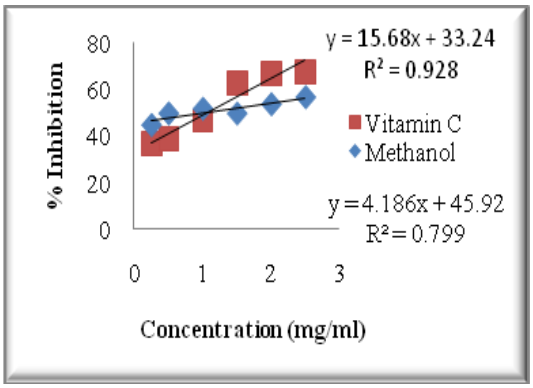

Figure 2. DPPH free radical scavenging activity of methanol extract of *A. hybridus* leaf.

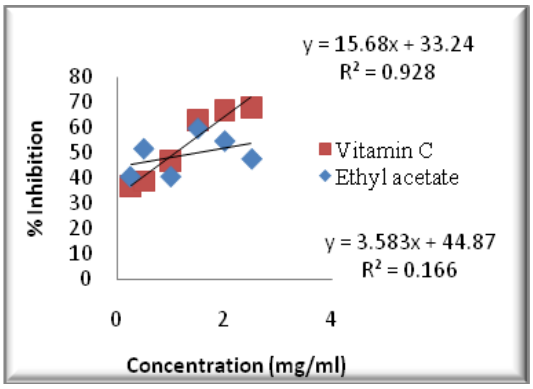

Figure 3. DPPH free radical scavenging activity of ethyl acetate extract of *A. hybridus* leaf.

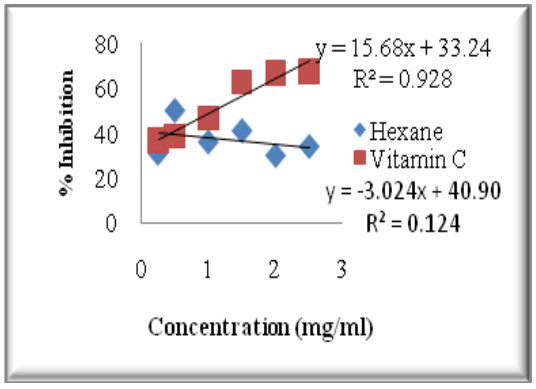

Figure 4. DPPH free radical scavenging activity of n-hexane extract of *A. hybridus* leaf.

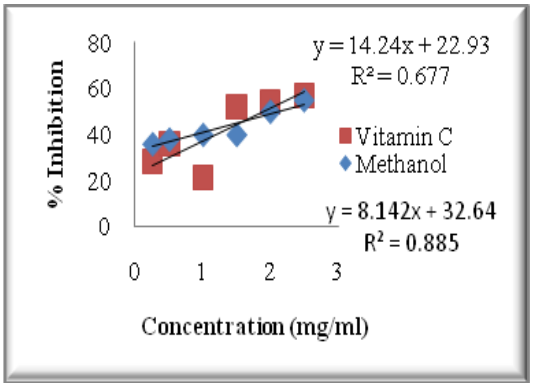

Figure 5. Hydroxyl radical inhibitory activity of methanol extract of *A. hybridus* leaf.

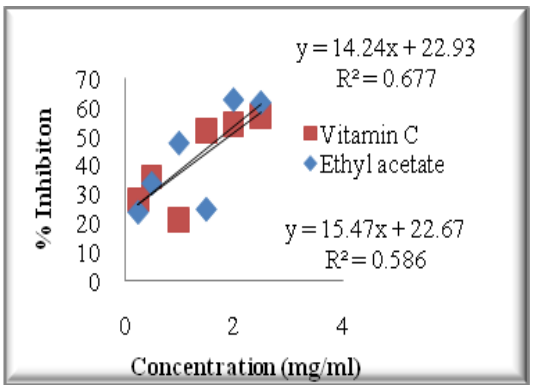

Figure 6. Hydroxyl radical inhibitory activity of ethyl acetate extract of *A. hybridus* leaf.

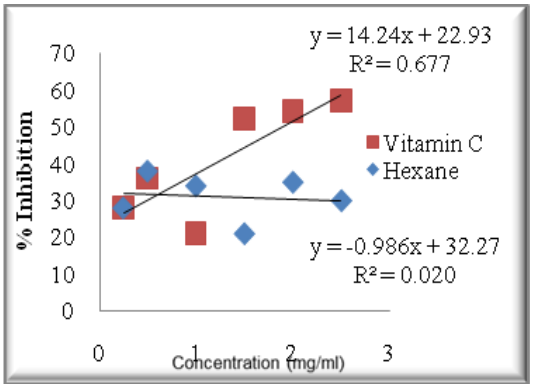

Figure 7. Hydroxyl radical inhibitory activity of n-hexane extract of *A. hybridus* leaf.

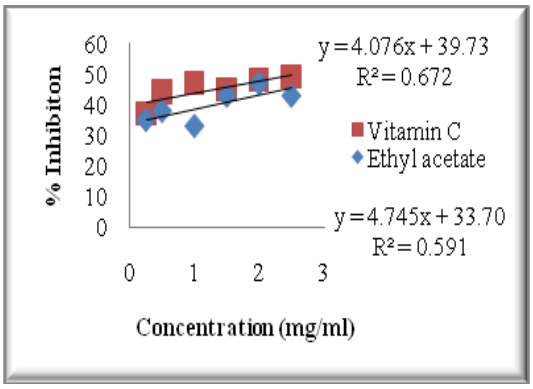

Figure 8. Phosphomolybdate assay of ethyl acetate extract of *A. hybridus* leaf.

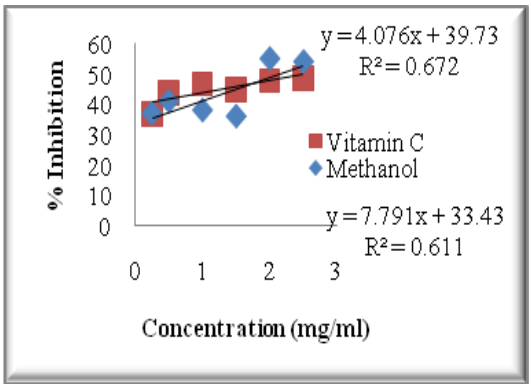

Figure 9. Phosphomolybdate assay of methanol extract of *A. hybridus* leaf.

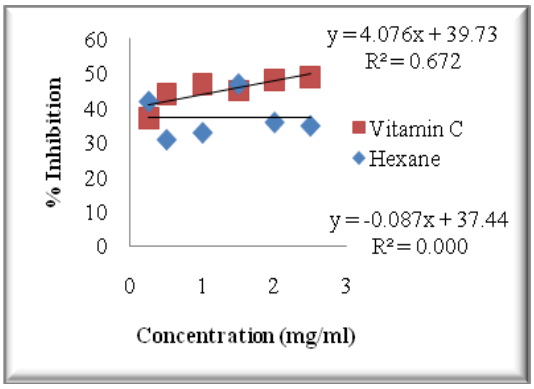

Figure 10. Phosphomolybdate assay of n-hexane extract of *A. hybridus* leaf.

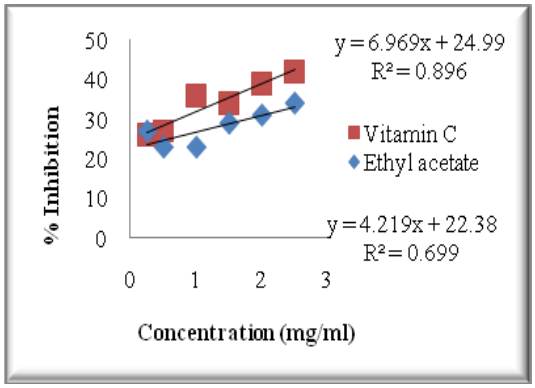

Figure 11. Reducing ability of ethyl acetate extract of *A. hybridus* leaf.

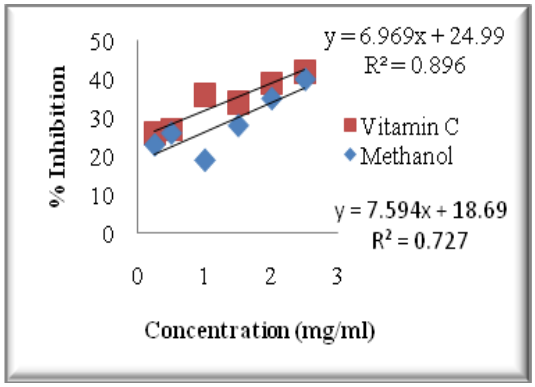

Figure 12. Reducing ability of methanol extract of *A. hybridus* leaf.

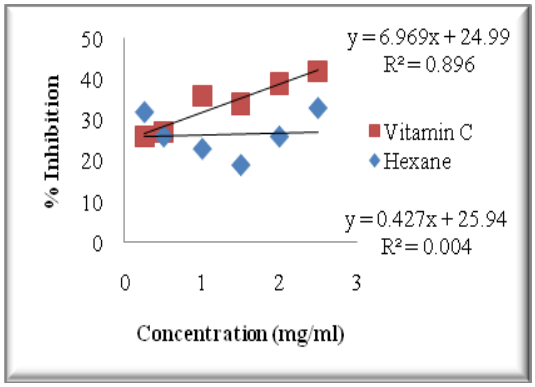

Figure 13. Reducing ability of n-hexane extract of *A. hybridus* leaf.

Antioxidant activity

Antioxidant activity of *A. hybridus* leaf was determined using DPPH radical scavenging, hydroxyl radical inhibitory, reducing power and phosphomolybdate assays. Previous researchers have reported that different methods for determining antioxidants activity give different results because of their different mechanistic principles.[44] DPPH assay is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity.[45] It is a stable free radical because of delocalization of its unpaired electron over the whole molecule. Donation of protons to the DPPH radicals results to a corresponding change from violet to pale yellow in solution. Comparable scavenging activities of the plant extracts were observed with those of the standard compound (Table 8). The result revealed significant differences with n-hexane extract while for ethyl acetate and methanolic extracts, there were no significant differences. It was also observed that the scavenging effect of the plant extracts on DPPH radical was in the order, methanol > ethyl acetate > n-hexane extracts. Scavenging effect of *A. hybridus* leaf extracts could be attributed to the presence of stigmasterol, an anticancer phytosterol.[35,40] It was observed that there exist a strong significant correlation (R^2 = 0.7996, Figure 2) in the activity of methanol extract of *A. hybridus* leaf when compared to vitamin C. In contrast, n-hexane extract showed a non-significant correlation with a negative slope (y = -3.0247x +40.907, R^2 = 0.1242, Figure 4) indicating that there was little or no scavenging activity in the n-hexane extract. The trend in the behaviour of methanolic extract was similar to that of vitamin C. Figure 3 shows that the radical scavenging activity is in positive correlation with the concentrations of ethyl acetate extract used. However, correlation in the case of n-hexane was found to be non-significant (Figure 4). This result is consistent with another report of a strong correlation of antioxidant activity in methanolic extract.[46]

Reducing power assay is often used to assess the ability of an antioxidant to donate an electron.[24] In this study, the ability of extracts was estimated in order to determine the potential of the extracts to reduce Fe^{3+} to Fe^{2+} by electron donation. This is associated with the presence of a redundant or complex molecule serving as an electron donor and/or free radical scavengers. In this study, the reducing power of the plant extracts were found in the order, methanol > ethyl acetate >n-hexane (Table 8), indicating that the methanolic extract has a better reducing power than other extracts. Methanolic extract of *A. hybridus* leaf may, therefore, contain high amount of reductants compared to n-hexane and ethyl acetate extracts. Therefore, methanolic extract can act as electron donor and could react with free radicals to convert them into more stable products and terminate the free radical chain reactions. This result confirms the findings of El-Hashasa *et al.*, who reported that the reducing power of a plant correlates with its phenolic content.[47] Figure 12 depicts that the reducing ability is in positive correlation with the concentrations of the extract (methanol) used. Significant positive correlations (Figure 11) were observed between the ethyl acetate extracts and vitamin C. While the n-hexane extract exhibited a non-significant correlation (Figure 13).

The hydroxyl radical is one of the most reactive oxygen species in living systems, which can react with all possible molecules in living organisms, especially proteins, DNA and

lipids.[48] Thus, removing OH radical is very important for the protection of biological systems. In this study, the methanol extract of *A. hybridus* leaf was more active than other extracts and was slightly better than vitamin C (Table 8). Correlation coefficient of methanol extract is R^2 = 0.885, showing that the inhibitory activity of the plant was slightly higher than vitamin C (R^2 = 0.6773, Figure 5). Ethyl acetate extract of *A. hybridus* leaf scavenged hydroxyl radical at concentrations of 2.0 mg mL^{-1} to 2.5 mg mL^{-1} which may be attributed to the presence of capsaicin. Capsaicinoids with phenolic structure can donate hydrogen to hydroxyl radical, thereby neutralizing it into water. The R-value of ethyl acetate appeared to be better than that of n-hexane (Figure 6 and 7). This could be as a result of the polarity index of n-hexane (0.1). There was a strong linear relationship between the activities of ethyl acetate and vitamin C (Figure 6). In contrast, n-hexane exhibited no correlation or relationship between the two variables (Figure 7). Alkaloids are major antioxidants in natural products, and their antioxidant activities have been proven in recent studies.[49,50] The activity of these plant extracts, especially methanol and ethyl acetate extracts are indications of the presence of phytochemicals that could be responsible for the observed antioxidant activity.

Phosphomolybdate test measures the ability of an extract to destroy free radicals by transferring an electron to the later. The antioxidants present in the extract reduced molybdate (VI) to molybdate (V) and this was measured spectrophotometrically at 695 nm.[22] Comparable scavenging activities of the plant extracts were observed with those of the standard compound (Table 8). The result revealed significant differences with n-hexane extract while for ethyl acetate and methanolic extracts, there were no significant differences. However, the antioxidant activity of vitamin C, an antioxidant used as the positive control, had a significantly better correlation than *A. hybridus* leaf (Figures 8-10). The observed differences in antioxidant properties may be due to the polarity of extraction solvents.

CONCLUSION

Methanolic extract of *A. hybridus* leaf showed significant antioxidant property. The leaf of *A. hybridus* is rich in various phytochemicals and had moderate to significant antifungal property but showed quite weak antibacterial activity. Bioactive compounds present in this leaf can be harnessed as natural antioxidants and pharmaceutical products.

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SYNTHESIS, CRYSTAL STRUCTURE AND MAGNETIC PROPERTIES OF BIS(3-AMINO-2-CHLOROPYRIDINE)DIBROMIDOCOPPER(II)

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Keywords: Copper(II); antiferromagnetism; crystal structure; 3-amino-2-chloropyridine.

The synthesis, X-ray crystal structure, and variable temperature magnetic properties of [(3-NH$_2$-2-Clpy)$_2$CuBr$_2$] **(**3-NH$_2$-2-Clpy = 3-chloro-2-aminopyridine) **(1)** are presented. The compound was characterized using combustion analysis, X-ray powder diffraction, single crystal X-ray diffraction, and temperature-dependent magnetic susceptibility measurements. Compound **1** crystallizes in the monoclinic space group $P2_1/n$. Inversion related molecules form a dimeric unit via short Cu…Br contacts. The dimers are linked into a step-like chain via short Br…Br contacts. Surprisingly, magnetic susceptibility measurements (1.8-325 K), which indicate weak antiferromagnetic interactions, are best fit by a uniform chain model suggesting that the exchange within and between dimers is nearly identical. Fitting the data with the S = ½ uniform chain model gave J = -3.38(2) K with C = 0.407(1).

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INTRODUCTION

The factors controlling the sign and strength of magnetic exchange in transition metal complexes has been a field of study for decades. Discoveries such as single-molecule magnets[1] and spin-crossover materials[2] as well as the recognition of the role played by magnetic interactions in the high temperature superconductor[3] have sparked a surge of interest. Copper(II) complexes have played a significant part in recent studies due to the single unpaired electron, making it a quantum system, and the plasticity of the copper(II) coordination sphere - allowing for a wide variety of structure types. In particular, pyridine and substituted pyridine complexes of Cu(II) halides have been investigated with an interest in understanding magnetostructural correlations in such compounds.[4] We have been particularly interested in the behaviour of such compounds with halogen substituents on the pyridine ring to study the influence of halogen bonding on the crystal packing and its resulting effect on the magnetic interactions in the sample.[5] Halogen bonds have a significant role in both intermolecular and interionic supramolecular structure of such compounds.[6] Superexchange pathways may be generated in these materials via the halide ions in either the bridging mode or via the two-halide pathway and these effects have been studied for many years.[7]

In particular, we have been examining the effects of substituents in the 2-position of the pyridine ring, especially halogen substituents.[8] The formation of supramolecular motifs such and dimers and chains through intermolecular interactions is strongly correlated with the conformation of the coordinated pyridine ligand. As part of this ongoing work, we report here the synthesis, structure and magnetic properties of bis(3-amino-2-chloropyridine)dibromido-copper(II).

EXPERIMENTAL

Copper (II) bromide was purchased from Sigma Aldrich and 3-amino-2-chloropyridine [3-NH$_2$-2-Clpy] from Matrix Scientific. Materials were used as received without further purification. X-Ray powder diffraction was carried out on a Bruker AXS-D8 X-ray Powder Diffractometer.

Synthesis

Bis(3-amino-2-chloropyridine)dibromidocopper(II) (**1**): 3-NH$_2$-2-Clpy (0.69 g, 5.2 mmol) and copper(II) bromide (0.56 g, 2.5 mmol) were dissolved in 20 mL of 50:50 methanol/water at room temperature. The solution was filtered to remove trace insoluble materials and left at room temperature for slow evaporation. After ~ 3 weeks, small black crystals of **1** were recovered by filtration, washed quickly with cold methanol and allowed to air dry to give 0.54 g (45 %). Elem. anal. for $C_{10}H_{10}Cl_2Br_2CuN_4$: found (calc.): C, 24.63(24.99); H, 2.41(2.10); N, 11.48(11.66).

X-Ray Structure Analysis

Data for **1** were collected at 87(2) K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) via φ by and ω scans. Cell parameters were retrieved using SMART[9] software and refined using SAINTPlus[10] on all observed reflections. Absorption corrections were applied using SADABS.[11] The structure was solved and refined using the SHELXS97 program[12] and refined via least-squares analysis via SHELXL-2018.[13] Non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms bonded to nitrogen atoms were located in the difference Fourier maps and their positions refined using fixed isotropic thermal parameters. Hydrogen atoms bonded to carbon atoms were placed in geometrically calculated positions and refined using a riding model with fixed isotropic thermal parameters. Data collection and refinement details are presented in Table 1.

Magnetic Susceptibility Data Collection

Magnetization data for **1** were collected using a Quantum Design MPMS-XL SQUID magnetometer. Finely ground crystals were packed into a #3 gelatin capsule and placed in a clear plastic straw for data collection. Data were collected as a function of field from 0 to 50 kOe at 1.8 K. As the field was reduced to 0 kOe several data points were recollected to check

for hysteresis effects; no hysteresis was observed. The M(H) response was linear beyond 10 kOe. Magnetization was also measured as a function of temperature from 1.8 to 325 K in a 1 kOe applied field. The data were corrected for the background signal (measured independently), the temperature independent paramagnetism of the Cu(II) ion and the diamagnetic contributions of the constituent atoms, estimated via Pascal's constants.[14] All data were fit using the Hamiltonian $H = -J\Sigma S_1 \cdot S_2$. Powder X-ray diffraction data for **1** were compared to the single crystal structure prior to magnetic data collection to ensure that the sample was the same phase as the single crystal structure. No impurities were detected.

RESULTS

Crystal Structure Analysis

Reaction of copper(II) bromide with 3-amino-2-chloropyridine in aqueous methanol gave compound **1** which crystallizes in the monoclinic space group $P2_1/n$. The molecular unit is shown in Figure 1. Selected bond lengths and angles are provided in Table 2. The Cu(II) ions is coordinated by two symmetry independent bromide ions and two symmetry independent 3-NH$_2$-2-Clpy ligands in a nearly square planar geometry (mean trans angle[15] = 173.8°). The mean deviation from the coordination plane is 0.107 Å, with the bromide ions and N atoms displaced to opposite sides as seen in Fig. 1. The 3-NH$_2$-2-Clpy ligands are in the *syn*-conformation with the chlorine substituents oriented to the same face of the coordination plane. The pyridine rings are nearly planar [mean deviations of constituent atoms = 0.0057 Å (N11 ring)=0.0066 Å] with the chlorine and amino substituents displaced slightly to opposite faces of the N11 ring and to the same face of the N21 ring. The pyridine rings are nearly perpendicular to the Cu-coordination plane and canted 11.8° to each other.

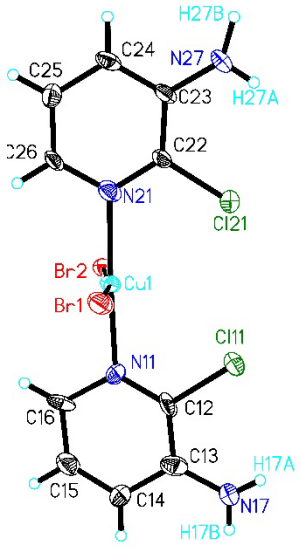


Figure 1. Thermal ellipsoid plot of the molecular unit of **1** showing 50% probability ellipsoids. Only the asymmetric unit, copper coordination sphere and those H-atoms whose positions were refined are labelled. Symmetry operation for Br1A and Br2A (1-x, y, ½-z).

Table 1. X-ray data of compound **1**.

Empirical formula	**$C_{10}H_{10}Br_2Cl_2CuN_4$**
Formula weight	480.48
Temperature	140(2) K
Wavelength	0.71073 Å
Space group	$P2_1/n$
a	8.9724(7) Å
b	15.6059(14) Å
c	10.5491(9) Å
α	90°
β	92.370(6)°
γ	90°
Volume	1475.8(2)Å^3
Z	4
Density (calculated)	2.162 Mg m^{-3}
Absorption coefficient	7.246 mm^{-1}
F(000)	924
Crystal size	0.51 x 0.30 x 0.30 mm^3
θ range for data collection	2.610 to 28.376°
Index ranges	$-11 \le h \le 11$, $-20 \le k \le 20$, $-13 \le l \le 13$
Reflections collected	20287
Independent reflections	3626 [*R*(int) = 0.1081]
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.277 and 0.8372
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3626 / 3 / 184
Goodness-of-fit on F^2	1.043
Final *R* indices [$I>2\sigma(I)$]	R^1 = 0.0618, wR_2 = 0.1431
R indices (all data)	R^1 = 0.1105, wR_2 = 0.1644
Largest diff. peak and hole	1.81 and -0.946 e Å^{-1} near Br2

Table 2. Selected bond lengths [Å] and angles [°] for **1**.

Bond	**Distance**	**Bond**	**Distance**
Cu1-Br1	2.4147(13)	Cu1-Br2	2.4365(13)
Cu1 N11	2.019(6)	Cu1 N21	2.020(6)
Bond	**Angle**	**Bond**	**Angle**
Br1-Cu1-Br2	170.94(5)	N11-Cu1-N21	176.7(3)
Br1-Cu1-N11	91.05(19)	Br1-Cu1-N21	91.0(2)
Br2-Cu1-N11	88.95(19)	Br2-Cu1-N21	89.42(19)

The bond lengths and angles are similar to those observed in similar $CuBr_2L_2$ complexes where L is a 2-halopyridine ligand [see the review in Ref. 8a].

As is commonly observed,[8a] the *syn*-conformation of the compound leads to the formation of centrosymmetric dimers via short Cu…Br contacts (see Figure 2 and Table 3). These interactions have the potential to provide a magnetic superexchange pathway. The dimers are further linked into a chain via the two-halide pathway, short Br… Br contacts, as also seen in Figure 2 (parameters in Table 3).

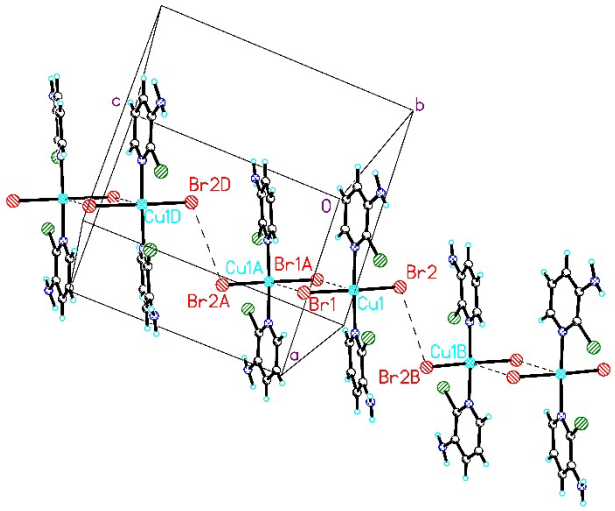
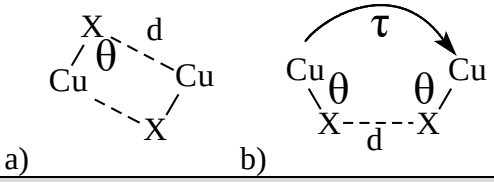


Figure 2. Chain formation via short Cu…Br and Br…Br contacts. Dashed lines represent Br…Br contacts and hydrogen bonds.

Table 3. Dihalide (a) and two-halide (b) superexchange pathway parameters from Figure 2.



a) b)

Bond	d (Å)	θ(°)	τ(°)
Cu1-Br2… Br2B-Cu1b	4.379	112.5/112.5	180
Cu1-Br1…Cu1A	3.844	99.1	

The lattice is further stabilized via weak halogen bonds between the bromide ions and chloro substituents of the organic moieties ($d_{Br1...Cl2B}$ = 3.821 Å, $\angle_{Cu1-Br1...Cl2B}$ = 159°) linking the dimers together into chains parallel to the *c*-axis.

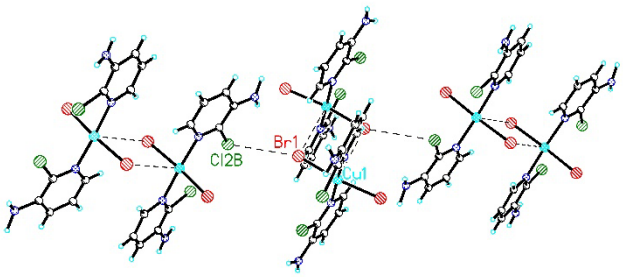


Figure 3. Halogen bonds in **1**.

Magnetic Study

Magnetization data as a function of applied field for **1** (Figure 4) show a linear response through most of the measured region and reach just over 4,000 emu/mol at 50 kOe, slightly below the expected saturation magnetization of ~ 5,800 emu mol^{-1} for a S=½ system with g slightly greater than 2, indicating the presence of weak antiferromagnetic interactions in the sample.

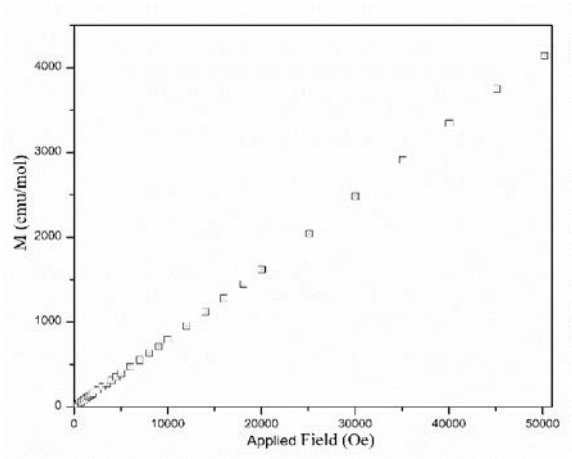


Figure 4. Magnetization as a function of applied field for **1**.

Magnetic susceptibility as a function of temperature is shown in Figure 5. $\chi(T)$ shows a monotonic increase with decreasing temperature until below 3 K where a modest turn, but no maximum, is seen. $\chi T(T)$ is more enlightening. The high temperature region suggests a Curie constant of slightly greater than 0.40. The downturn at low temperatures suggests weak antiferromagnetic interactions, in agreement with $M(H)$. The inset to Figure 5 shows the Curie-Weiss plot. The near zero intercept is again in agreement with weak antiferromagnetic interactions. The $1/\chi(T)$ data above 10 K were fit to the Curie-Weiss model yielding C=0.410(2) emu K mol^{-1} Oe^{-1} and a Weiss constant (θ) of -1.90(6) K (solid line in the inset to Figure 5).

A variety of models were used to fit the $\chi(T)$ and $\chi T(T)$ data including those for a dimer, a uniform chain and an alternating chain.[16] The best fit to the $\chi(T)$ data was given by the uniform chain model which yielded a Curie constant of 0.407(1) [0.409(2)] emu K mol^{-1} Oe^{-1} and an exchange constant, J, of -3.38(2) [-4.1(2)] K with a paramagnetic impurity of 0.7(1)% [1.9(2)%] (values in brackets were obtained from the fit of the χT(T) data to the uniform chain model).

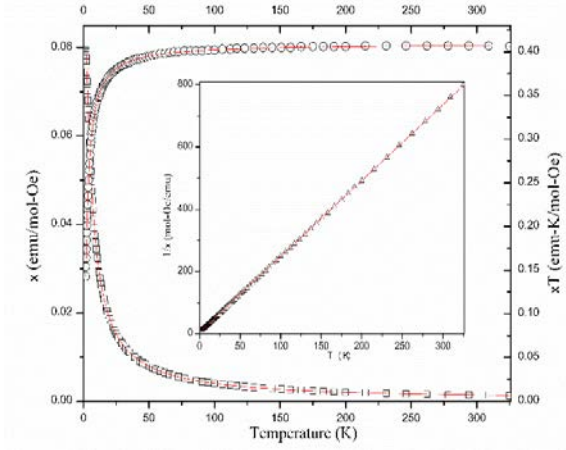


Figure 5. Susceptibility data for **1** plotted as $\chi(T)$ (□, left axis) and $\chi T(T)$ (○, right axis). The inset shows a plot of $1/\chi(T)$. The solid lines represent the best fits to the appropriate models (see text).

DISCUSSION

Compound **1** crystallizes in the *syn*-conformation with a slight deviation of the Cu(II) coordination sphere from planarity. Although several N-coordinated complexes of 3-NH_2-2-Clpy with tin and germanium have been reported,[17] only zinc complexes have been reported with first-row +2 transition metals.[18] As is frequently observed for 2-chloro-substituted pyridine ligands,[8c,19] the *syn*-conformation resulted in the formation of dimers via long Cu…X interactions, generating a dihalide superexchange pathway. A second interaction, via the two-halide pathway, was also possible (Figure 2) due to short intermolecular Br…Br contacts. However, attempts to fit the magnetic data to an antiferromagnetic dimer model, even with a Curie-Weiss correction for interdimer interactions via the two-halide pathway were unsuccessful; all attempts showed a maximum in $\chi(T)$, which was not observed in the data. This caused us to reexamine the structure and note that although the θ angles (112.5°) are not optimal (θ approaching 180° are best for exchange[15]), the τ angle of 180° is an ideal value and the Br…Br distance is well within range for measurable magnetic interactions. Thus we attempted a fit of the magnetic data to the alternating S=½ Heisenberg chain model[16] with fair results [Curie constant = 0.385(10) emu K mol^{-1} Oe^{-1}, J=-3.3 K and 1.6 % paramagnetic impurity] except that the fitted value of α, the ratio of the two exchange constants within the chain, was equal to 1.0(2). A value of α=1 would indicate that the chain is indeed a uniform chain and thus we attempted a fit to the uniform S=½ Heisenberg chain model. To our surprise, the fit was excellent (Figure 5, main figure). This suggests that although the structural pathways are different (dihalide vs. two-halide) within the chain, the magnitude of the exchange via each pathway is accidentally equivalent within the error, leading to an 'apparent' uniform magnetic chain.

CONCLUSIONS

We have successfully prepared and characterized the first coordination complex of 3-amino-2-chloropyridine with Cu(II). The compound adopts the *syn*-conformation and form alternating chains in the crystal via a combination of dihalide and two-halide bridges. Surprisingly, the variable temperature magnetic data are best fit by the uniform S = ½ chain model, suggesting accidental equivalence of the two magnetic superexchange pathways. Further work on the coordination chemistry of the ligand is in progress.

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CCDC 1996066 contains the supplementary crystallographic data for **1.** This data can be obtained free of charge via http://www.ccdc.cam.ac.uk/con-ts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk.

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MICROWAVE-INDUCED, EFFICIENT, CONVENIENT AND RAPID SYNTHESIS OF BENZYLOXYCHALCONES AS POTENT GROWTH INHIBITOR

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Keywords: Chalcones; Claisen-Schmidt condensation; microwave irradiation; antibacterial activity; antifungal activity.

A novel series of substituted chalcones containing benzyloxy moiety (**3a-3h)** was synthesized by microwave induced Claisen-Schmidt condensation of 2-acetyl-1-naphthol and its halo derivatives with different substituted aromatic aldehydes. All the synthesized chalcones were characterized by spectral analysis and screened for their antibacterial and antifungal effectiveness by using standard methods. It is found that the microwave irradiation technique is superior in terms of considerable increase in the reaction rate, yields and shortening the reaction time. The investigation of antimicrobial screening revealed that compounds (**3a-3d)** containing benzyloxy group at para position of aldehyde ring of chalcones possessing more potent antimicrobial activity.

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INTRODUCTION

Chalcone is generic term given to compounds bearing1,3-diphenyl-2-propen-1-one framework.[1] Chalcones and their derivatives are polyphenolic compounds of flavonoids family. They have been found in many plants as metabolic precursors of other flavonoids and isoflavonoids.[2] It is noteworthy to mention that the presence of chalcones have been reported in plants traditionally employed for therapeutical purposes.[3] Chemist have been attracted towards the nucleus of chalcones due to their relatively simple structures and wide variety of pharmacological activities.[4-7] Chalcone based compounds have been reported to exhibit anticancer,[8,9] anti-inflammatory activity,[10,11] anti-ulcerative,[12] analgesic,[13] anti-viral,[14] anti-fungal,[15] anti-malerial[16] and anti-bacterial activity[17] etc. which may be altered depending on the type of substituents on aromatic rings. Chalcones are synthesized by Claisen Schmidt condensation, which involve cross aldol condensation of suitable benzaldehyde derivatives and acetophenone derivatives by base catalysed or acid catalysed reactions followed by removal of water molecule. Synthetic and naturally occurring chalcones have been extensively studied and developed as one of the pharmaceutically important molecules. Therefore, in the present investigation it has been considered worthwhile to synthesize some new chalcone derivatives that may be of value in development of new, potent, selective and less toxic antimicrobial agent by conventional and microwave irradiation methods. Microwave induced enhancement of organic reaction is gaining popularity as a non- conventional technique for rapid synthesis.[18] The important features of this technique are easy access to high temperature, safe and environmentally benign techniques with shorter reaction time.

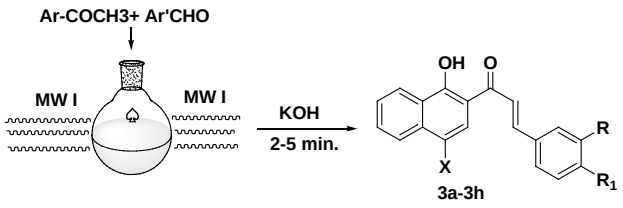
The synthesized compounds were purified by recrystalization and chromatography. The compounds were assigned on the basis of ^{1}H NMR, IR and GC-MS analysis. The compounds were tested for their anti-bacterial and anti-fungal activities by standard methods.

EXPERIMENTAL

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Gemini 300-MHz instrument in $CDCl_3$ as solvent and TMS as an internal standard. The mass spectra were recorded on EISHIMADZU-GC-MS spectrometer. Elemental analysis was carried out on a Carlo Erba 1108 analyzer. Synthon-3000, Anton Paar reaction system was used for microwave synthesis. The purity of products was checked by Thin Layer Chromatography (TLC) on silica gel. All solvents and chemicals were purchased from Alfa chemicals and used without further purification.

General procedure for synthesis of chalcones (3a-3h)

Equimolar quantities (0.001 mol) of 2-acetyl-1-naphthol or its halo derivative and respective aromatic aldehydes (0.001 mol) were mixed and dissolved in minimum amount (5 mL) of ethanol. To this, catalytic quantity of aqueous KOH solution was added slowly and mixed. The entire reaction mixture was microwave irradiated for about 2-5 min at 180 W. The reactions were monitored through TLC using solvent system benzene:ethyl acetate (8:2), when the reaction was complete the reaction mixture was cooled in an ice bath and product thus formed was filtered, washed with distilled water and recrystallized from ethanol.



Scheme 1. Synthesis of Chalcones under Microwave condition

A comparison (Table 1) of the results obtained from the two synthetic approaches indicate that the effect of microwave irradiation is not purely thermal, besides giving decreased reaction times and improved yield.

Table 1.Synthesis of chalcones under microwave irradiation.

No.	X	R	R_1	Time, min	M.P.[a] ^{0}C	Yield[b]
3a	H	OMe	OBn	3	150±2	90
3b	Br	OMe	OBn	4	155±2	92
3c	I	OMe	OBn	5	160±2	88
3d	Cl	OMe	OBn	5	152±2	90
3e	H	OBn	OMe	3.5	148±2	85
3f	Br	OBn	OMe	4	150±2	88
3g	I	OBn	OMe	5	158±2	92
3h	Cl	OBn	OMe	5	152±2	85

[a]M.P. refers to solvent- free and other technique.[27,28] [b]Yield of the isolated product in % term.

(Hydroxynaphthlen-2-en-1-one (3a)

Yellow solid. EI-MS *m/z* (rel. int. %): 410 (90) $[M+1]^+$; IR (KBr): 3420 (OH), 3059 (C-H), 1680 (C=O), 1570 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 14.0 (s, OH), 8.20 (d, C=CH, J= 15.6Hz), 7.30 (d, CO=CH, J= 15.6Hz), 7.25-6.60 (m, Ar-H), 5.16 (s, CH_2), 3.9(s, CH_3). ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 120, 118, 114, 111,71, 56. Anal. Calcd. for $C_{27}H_{22}O_4$: C, 79.02; H, 5.36. Found: C, 78.97; H, 5.32

2(*E*)-1-(4-Bromo-1-hydroxynaphthlen-2-yl)-3-(4-benzyloxy-3-methoxyphenyl)prop-2-en-1-one (3b)

Redish solid. EI-MS *m/z* (rel. int. %): 489 (60) $[M+2]^+$. IR (KBr): 3390 (OH), 3060 (C-H), 1676 (C=O), 1583 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 14.0 (s, OH), 8.20 (d, C=CH, J= 15.6Hz), 7.20 (d, CO=CH, J= 16.2Hz), 6.90-6.30 (m, Ar-H), 5.10 (s, CH_2), 3.9(s, CH_3); ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 118, 114, 111, 110, 71, 56, Anal. Calcd. for $C_{27}H_{21}O_4Br$: C, 66.25; H, 4.29; Br,16.15. Found: C, 66.22; H, 4.26; Br, 16.13.

2(*E*)-3-(4-Benzyloxy-3-methoxyphenyl)-1-(1-hydroxy-4-iodo-naphthalen-2-yl)prop-2-en-1-one (3c)

Brown solid. EI-MS *m/z* (rel. int. %): 536 (95) $[M+2]^+$. IR (KBr): 3410 (OH), 3050 (C-H0, 1678 (C=O),1583 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 14.0 (s, OH), 8.30 (d, C=CH, J= 15.6Hz), 7.25 (d, CO=CH, J= 16.2Hz), 6.90-6.50 (m, Ar-H), 5.15 (s, CH_2), 4.0(s, CH_3). ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 118, 114, 111, 92, 71, 56. Anal. Calcd. for $C_{27}H_{21}O_4I$: C, 60.44; H, 3.91; I, 23.69. Found: C, 66.42; H, 3.88; I, 23.65.

2(*E*)-3-(4-Benzyloxy)-3-methoxyphenyl)-1-(1-chloro-4-hydroxy-naphthalen-3yl)prop-2en-one (3d)

Orange solid. EI-MS *m/z* (rel. int. %): 445 (62) $[M+2]^+$. IR (KBr): 3380 (OH), 3020 (C-H), 1670 (C=O),1550 (C=C) $cm^{-1.}$ ^{1}H NMR(400MHz, $CDCl_3$) δ = 14.0 (s, OH), 7.81 (d, C=CH, J= 15.6Hz), 7.49 (d, CO=CH, J= 16.2Hz), 7.13-6.49 (m, Ar-H), 4.90 (s, CH_2), 3.73(s, CH_3) ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 118, 115, 114, 111, 71, 56. Anal. Calcd. for $C_{27}H_{21}O_4Cl$: C, 72.80; H, 4.71; Cl, 8.08. Found: C, 72.77; H, 4.68; Cl, 8.05.

2(*E*)-3-(3-(Benzyloxy)-4-methoxyphenyl)-1-(1-hydroxynaphtha-len-2yl) prop-2-en-1-one (3e)

Yellow solid. EI-MS *m/z* (rel. int. %): 410 (80) $[M+1]^+$. IR (KBr): 3410 (OH), 3050 (C-H), 1680 (C=O), 1570 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 14.0 (s, OH), 8.10 (d, C=CH, J= 15.6Hz), 7.20 (d, CO=CH, J= 16.2Hz), 7.10-6.75 (m, Ar-H), 4.90 (s, CH_2), 3.50(s, CH_3). ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 120, 118, 114, 111, 71, 56. Anal. Calcd. for $C_{27}H_{22}O_4$: C, 79.02; H, 5.36. Found : C, 78.97; H, 5.32.

2(*E*)-3-(3-(Benzyloxy)-4-methoxyphenyl)-1-(1-bromo-4-hyd-roxynaphthalen-3yl)prop-2-en-1-one (3f)

Faint yellow solid. EI-MS *m/z* (rel. int. %): 489 (70) $[M+2]^+$. IR (KBr): 3370 (OH), 3010 (C-H), 1680 (C=O,1560 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 13.70 (s, OH), 8.15 (d, C=CH, J= 15.6Hz), 7.35 (d, CO=CH, J= 16.2Hz), 7.60-6.89 (m, Ar-H), 5.20 (s, CH_2), 3.80(s, CH_3). ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 120, 118, 114, 111, 108, 71, 56; Anal. Calcd. for $C_{27}H_{21}O_4Br$: C, 66.25; H, 4.29; Br,16.15. Found: C, 66.22; H, 4.26; Br, 16.13

2(*E*)-3-(3-Benzyloxy-4-methoxyphenyl)-1-(1-hydroxy-4-iodo-naphthlen-2-yl)prop-2-en-1-one (3g)

Yellow solid. EI-MS *m/z* (rel. int. %): 536 (75) $[M+2]^+$. IR (KBr): 3420 (OH), 3030 (C-H), 1675 (C=O), 1560 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 13.96 (s, OH), 8.43 (d, C=CH, J= 15.6Hz), 7.98 (d, CO=CH, J= 16.2Hz), 7.80-7.25 (m, Ar-H), 5.19 (s, CH_2), 3.96(s, CH_3). ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 120, 118, 114, 111, 95, 71, 56; Anal. Calcd. for $C_{27}H_{21}O_4I$: C, 60.44; H, 3.91; I, 23.69. Found: C, 66.42; H, 3.88; I, 23.65.

2(*E*)-3-(3-Benzyloxy)-4-methoxyphenyl)-1-(1-Chloro-4-hyd-roxynaphthalen-3yl)prop-2en-one (3h)

Brown solid. EI-MS *m/z* (rel. int. %): 445 (60) $[M+2]^+$. IR (KBr): 3360 (OH), 3010 (C-H), 1670 (C=O), 1560 (C=C) cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ = 13.80 (s, OH), 8.20 (d,

C=CH, J= 15.6Hz), 7.90 (d, CO=CH, J= 16.2Hz), 7.73-7.49 (m, Ar-H), 4.98 (s, CH_2), 3.83 (s, CH_3) ^{13}C NMR ($CDCl_3$) δ = 192, 164, 149, 145, 144, 140, 138, 137, 136, 135, 134, 132, 131, 130, 129, 128, 127, 126, 125, 120, 118, 114, 112, 111, 71, 56; Anal. Calcd. for $C_{27}H_{21}O_4Cl$: C, 72.80; H, 4.71; Cl, 8.08. Found: C, 72.77; H, 4.68; Cl, 8.05.

Biological Activity

The newly synthesized compounds were screened for their antibacterial activity against Gram +ve bacterial strain of *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginasa* (ATCC-27853) and Gram-ve bacterial strain of *Klebsiella Pneumoniae* (ATCC-700603) *and Escherichia coli* (ATCC-25922) by disk diffusion method.[19,20] The sterile disks, previously soaked in a known concentration of the test compounds (50 mg ml^{-1}), were placed in nutrient agar medium. Solvent and growth controls were kept. Ofloxacin was used as positive control while the disk poured in DMSO was used as negative control. The plate was incubated for 24 h at 37 ^{0}C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram +ve and Gram -ve bacteria. Inhibition zone were measured and compare with controls.

Antifungal activity was performed by poison plate method.[21] The medium used was potato dextrose agar (Himedia). The medium was prepare and sterilized at 10 psi in autoclave for 15 min. The compounds to be tested were added to the sterile medium in aseptic condition so as to get final concentration of 1 %. A plate with DMSO was prepared as negative control, similarly a plate with 1 % Gresiofulvin was prepared as standard reference plate i.e. positive control. *Aspergilus niger, Penicillium chrysogenum, Fusarium monoliforme* and *Aspergilus flavus* were selected as test fungal cultures. They were allowed to grow on slant for 48 h so as to get profuse sporulation. 5 mL of 1:100 aqueous solution of Tween 80 was added to get the slant and spores were scraped with the help of nicrome wire loop to form suspension. The fungal suspension was spot inoculated on the plates prepared using compound with the help of nicrome wire loop. The plates were incubated at room temperature for 48 h, after the incubation plates were observed for the growth of inoculated fungi.

The minimum inhibitory concentration of compounds was obtained by Broth dilution method. In this the concentration of synthesized compounds were maintained at 8 mg mL^{-1} in the first tube containing 1 mL of broth. The tubes were vortexed to make the initial standard concentration. This were serially dilute in other tubes and finally 1 mL was discarded from the last tube to make the dilution of 1, 0.5, 0.25 mg mL^{-1}, respectively. To all these tubes, 0.1 mL of the long phase culture of target microorganism was added separately and incubated at 37 ^{0}C for 24-48 h for microbial growth.[22,23]

RESULTS AND DISCUSSION

Synthesis

In view of applications of chalcones and in continuation of our previous works reported on green synthetic protocol towards synthesis of bioactive compounds,[24-27] we synthesized new class of benzyloxy chalcone derivatives (**3a-3h**) under the condition of microwave assisted Claisen-Schmidt condensation of substituted aromatic aldehydes with 2-acetyl-1-naphthol/halosubstituted 2-acetyl-1-naphthol in presence of KOH in good yield.[28] We found that microwave technique has several advantages including clean and easy work-up procedure, short reaction time, high yield and eco-friendliness.

The structure of chalcone derivatives were characterized by recording their IR, ^{1}HNMR and GC-MS spectra. All the chalcones showed absorption band in region 1680-1640 cm^{-1} due to C=O stretching vibration. ^{1}H NMR spectra is best analyzing tool for the structural elucidation it showed two doublet in the region of δ = 8.10-8.40 ppm due to olefinic protons (-CH=CH-) and also showed a singlet in the range of 13.80-14.00 ppm due to hydroxyl group. ^{13}C NMR spectra of chalcones were recorded in $CDCl_3$ and are in good agreement with theoretical ^{13}C NMR spectra proposed for all compounds.

Antimicrobial activity

The investigation of antibacterial screening data revealed that all tested compounds (**3a-3h)** showed good to moderate bacterial inhibition against *S. aureus, P. aeruginasa, K .pneumoniae* and *E. coli* species. The good activity is attributed to the presence of pharmacologically active electron releasing methoxy and benzyloxy groups attached to aldehyde ring of chalcones. A comparative study also revealed that the compounds (**3a-3d)** containing benzyloxy group at *para* and methoxy group at *meta* position of aldehyde ring of chalcones showed more potent inhibitory activities against Gram +ve and Gram –ve bacteria than the compounds (**3e-3h)** having position of both groups interchanged. From this comparative study it is concluded that by changing the position of substituents of chalcones the microbial inhibition activities are altered. The bacterial zone of inhibition value are given in (Table 2)**.**

Antifungal screening data of all compounds **(3a-3h)** revealed good to moderate antifungal activity against all tested organisms. In term of structure activity relationship the compounds that contain benzyloxy group at *para* position of aldehyde ring of chalcones showed significant antifungal activities. Results were recorded as growth of fungi in percentage of zone of inhibition in (Table 2).

Table 2. Antimicrobial data of synthesized chalcones.

Compound	Antimicrobial activity[a]							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
3a	23	21	24	24	82	86	85	87
3b	25	22	23	26	80	85	83	85
3c	26	24	22	28	83	88	80	85
3d	24	23	26	27	90	80	80	78
3e	19	15	20	22	61	60	62	59
3f	19	14	16	22	60	70	60	62
3g	18	19	20	16	61	65	65	58
3h	17	17	16	18	59	60	61	60
Ofloxacin	26	25	28	30				
Griseofulvin					99	99	100	99

[a]**A:** *P.aeruginosa*, **B:** *S.aureus*, **C:** *K.pneumonie*, **D:** *E.coli*. Ofloxacin, **E:** *P.chrysogenum*, **F:** *F.moniliforme*, **G:** *A. flavus*, **H:** *A. niger*, Griseofulvin

Table 3. Minimum inhibitory concentration of synthesized chalcones.

A. Bacterial strains

Compound, mg mL^{-1}	*P. aeruginosa*			*S. aureus*			*K. pneumonie*			*E. coli*		
	1.0	0.5	0.25	1.0	0.5	0.25	1.0	0.5	0. 25	1.0	0.5	0.25
3a	-	-	-	-	-	-	-	-	-	-	-	-
3b	-	-	-	-	-	-	-	-	-	-	-	-
3c	-	-	-	-	-	-	-	-	-	-	-	-
3d	-	-	-	-	-	-	-	-	-	-	-	-
3e	-	+	+	-	+	+	-	+	+	-	+	+
3f	-	+	+	-	+	+	-	+	+	-	+	+
3g	-	-	+	-	-	+	-	-	+	-	-	+
3h	-	-	+	-	-	+	-	-	+	-	-	+

B. Fungal strains

Compound, mg mL^{-1}	*P. chrysogenum*			*F. moniliforme*			*A. flavus*			*A. niger*		
	1.0	0.5	0.25	1.0	0.5	0.25	1.0	0.5	0.25	1.0	0.5	0.25
3a	-	-	-	-	-	-	-	-	-	-	-	-
3b	-	-	-	-	-	-	-	-	-	-	-	-
3c	-	-	-	-	-	-	-	-	-	-	-	-
3d	-	-	-	-	-	-	-	-	-	-	-	-
3e	-	+	+	-	+	+	-	+	+	-	+	+
3f	-	+	+	-	+	+	-	+	+	-	+	+
3g	-	-	+	-	-	+	-	-	+	-	-	+
3h	-	-	+	-	-	+	-	-	+	-	-	+

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the synthesized compounds was evaluated at different concentration i.e. 1.0, 0.5, 0.25 mg mL^{-1}. The result of MIC are given in table 3, it is clear that the chalcones (**3a-3d**) shows more promising inhibition than chalcones (**3e-3h**) at minimum concentration (0.25 mg mL^{-1}) against all tested bacterial and fungal strains. The chalcones (**3g, 3h**) shows good inhibition against *P. aeruginosa*, *S. aureus* (Gram+ve) and *K. pneumonie*, *E. coli* (Gram –ve) strains, at minimum concentration 0.5 mg mL^{-1}. The chalcones **3e, 3f** showed moderate inhibition against all bacterial strains at minimum concentration 1.0 mg mL^{-1}. The chalcones **3g** and **3h** showed effective inhibitory potential against all fungal strains at minimum concentration 0.5 mg mL^{-1}. The *P. chrysogenum*, *F. moniliforme*, *A. flavus*, *A. niger* are prominently affected by chalcones **3e, 3f** at minimum concentration 1.0 mg mL^{-1}. The comparative analysis also revealed that the chalcones having electron releasing benzyloxy group at *para* position of ring **B** had more potent inhibition against all bacterial and fungal strains at minimum concentration 0.25 mg mL^{-1}.

CONCLUSION

In conclusion, salient feature of our approach is coupling microwave with keeping modernization and simplification over classical procedure for avoiding the generation of valuable toxic organic solvents, which are corrosive, and an efficient and cheap technology to synthesize chalcone derivatives. The evaluation of antimicrobial activities of chalcones carrying benzyloxy and methoxy group were reported. The activity results showed that compounds (**3a-3d)** possessing benzyloxy group at para position of aldehyde ring of chalcones are most active against all bacterial and fungal strains tested than the compounds (**3e-3h)** having position of benzyloxy group was changed from *para* to *meta*. This electronic effect played very important role in activity, as can be seen for the compounds having electron donor groups such as benzyloxy, methoxy. Thus in future this class of benzyloxy substituted chalcones may be used for the generation of better lead molecules to fight against bacterial and fungal strains.

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SYNTHESIS AND MOLECULAR DOCKING STUDIES OF NOVEL PYRIDINE-THIAZOLE-HYDRAZONE CONJUGATES AS ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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Keywords: Antimicrobial activity, antioxidant activity, thiazole, hydrazone, molecular docking study.

In the investigation, a series of new pyridyl and thiazolyl clubbed hydrazone derivatives have been synthesized. The newly synthesized compounds were evaluated for their in vitro antimicrobial and antioxidant activities. Some among the compounds have shown excellent antimicrobial activity against both bacterial and fungal pathogens. Two compounds among the series have exhibited excellent antioxidant activity. Furthermore, a molecular docking study has been performed against *DNA gyrase* to know the binding modes of these molecules and recorded good binding affinity. The ADME study has also been performed for predicting the pharmacokinetic profile, which expressed good oral drug-like behaviour.

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INTRODUCTION

Antibiotic resistance has to turn out to be one of the major issues of global health. The various medical treatments such as cancer chemotherapy, organ, transplantation, major surgery and diabetes management have become difficult without effective antimicrobial agents.[1] The available antimicrobial agents in the market have several drawbacks, including toxicity, low effectiveness, and environmental issues. Therefore, the exploration and progress of high-efficient antimicrobial agents with a different mode of action is of prime importance.[2]

The incorporation of two or more bioactive pharmacophores in a hybrid architecture is one of the important technique used in the new drug discovery.[3-7] Thiazole pharmacophore linked with different pharmacophores for the construction of new bioactive compounds has gained much consideration in current years.[8,9] The thiazole comprising molecules are reported to have encouraging α-glucosidase inhibiting,[10] antioxidant,[11] anti-inflammatory,[12] anti-Candida[13] and antifungal[14] activities. The pyridine nucleus is widespread in many naturally occurring bioactive products and is tremendously useful for the development of new bioactive synthetic compounds. Pyridine derivatives are known for biological activities such as antimicrobial,[15] anticancer,[16] anti-inflammatory, analgesic,[17]protein kinase inhibitor,[18]and antitubercularactivities.[19] Pyridine-thiazole clubbed molecules are designed with extensive applications in medicinal chemistry.[20,21] Compounds containing a *N*-acylhydrazone (NAH) pharmacophore have shown to possess anticancer, anti-HIV, antimicrobial,[22] antibacterial, antitubercular,[23] antioxidant,[24] anti-proliferative,[25] analgesic,[26] anti-inflammatory,[27] and anthelmintic activities.[28] Its significance and persistent presence in bioactive molecules have attracted many researchers towards the NAH moiety.[29-32] The representative bioactive hydrazones reported in the literature are shown in Figure 1.

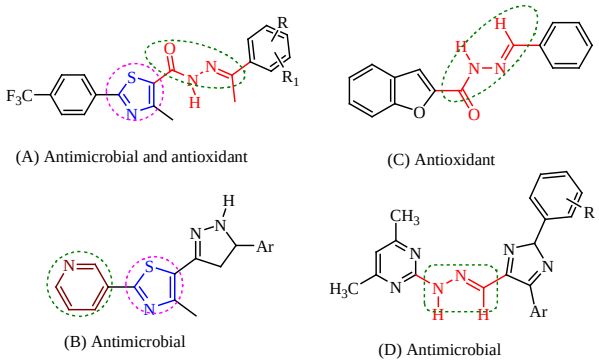


Figure 1. Some bioactive hydrazones.

In continuation, of our efforts to synthesize hydrazone derivatives having medicinal properties activities,[33-36] we have synthesized new hydrazone derivatives starting from ethionamide. The synthesized compounds were evaluated for their in vitro antimicrobial and antioxidant activities.

EXPERIMENTAL

The commercially available chemicals and reagents were used directly without further purification. The IR spectra (Neat) were recorded on Bruker FTIR spectrometer.The ^{1}H and ^{13}C NMR spectra were recorded on Bruker NMR (400 MHz) spectrometer. The chemical shifts were reported

in parts per million (ppm). TMS is used as a reference. The coupling constants (*J*) are reported in Hertz (Hz).

Procedure for the synthesis of ethyl 2-(2-ethylpyridin-4-yl)thiazole-4-carboxylate (3).

The mixture of 2-ethylpyridine-4-carbothioamide (**1**) (1.0 mmol) and ethyl bromopyruvate (**2**) (1.3 mmol) was refluxed in ethanol. The progress of the reaction was monitored by TLC. After completion of the reaction in 4 h, the ethanol was evaporated under a vacuum. The residue obtained was dissolved in ethyl acetate (50 mL) and neutralized by ammonia solution. The organic layer was washed with brine and dried over anhydrous sodium sulphate. The ethyl acetate was evaporated under vacuum and the obtained product was purified by column chromatography using ethyl acetate and petroleum ether to furnish ethyl 2-(2-ethylpyridin-4-yl)thiazole-4-carboxylate (**3**) as a thick oil. Yield 90 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.20 (t, *J* = 6.4 Hz, 3H, CH_3), 1.28 (t, *J* = 8.0 Hz, 3H, CH_3), 2.75 (q, *J* = 6.4 Hz, 2H, CH_2), 4.30 (q, *J* = 8.0 Hz, 2H, O-CH_2), 7.49 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 8.14 (s, 1H, Thiazolyl-H), 8.47 (s, 1H, Ar-H).

Procedure for the synthesis of 2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (4).

Compound **3** (1.0 mmol) and excess of hydrazine hydrate (3.0 mmol) were refluxed in ethanol. The progress of reaction was monitored by TLC. After completion of the reaction, the ethanol was evaporated under reduced pressure. The obtained residue was poured in ice-cold water. The solid obtained was filtered, washed with water and crystallized with ethanol to furnish 2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide(**4**). Yield: 75 %, mp: 97-99 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.28 (t, *J* = 7.2 Hz, 3H, CH_3), 2.84 (q, *J* = 7.2 Hz, 2H, CH_2), 4.63 (bs, 2H, NH_2), 7.80 (d, *J* = 4.1 Hz, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 8.41 (s, 1H, Thiazolyl-H), 8.62 (d, *J* = 4.1 Hz, 1H, Ar-H), 9.88 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 13.69, 30.64, 117.85, 118.45, 125.04, 139.47, 150.02, 150.10, 159.62, 164.19, 164.91.

General procedure for the synthesis of substituted (*E*)-*N*'-benzylidene-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide derivatives (6a-l)

The mixture of aromatic aldehydes (**5a-l**) (1.0 mmol) and **4** (1.0 mmol) was dissolved in diisopropyl-ethyl ammonium acetate (DIPEAc) (5 mL) and stirred at room temperature for 30 min. Then, the reaction mixture was poured on cold water. The solid obtained was filtered and washed with cold water. The products obtained were crystallized with ethanol to furnish the correspondingsubstituted (*E*)-*N*'-benzylidene-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (**6a-l**) with 82-95 % yields.

(*E*)-*N*'-(3-Bromobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide(6a)

Yield 84%, m.p. 138-140 °C. IR (Neat) : 3265, 3103, 2976, 2906, 1663, 1589, 1539, 1487, 1418, 1357, 1265, 1215, 1177, 1039, 929, 840, 791, 655 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.30 (t, *J* = 6.4 Hz, 3H, CH_3), 2.88 (q, *J* = 6.4 Hz, 2H, CH_2), 7.45 (t, *J* = 8.2 Hz, 1H, Ar-H), 7.66 (dd, *J* = 8.2 & 4.1 Hz, 1H, Ar-H), 7.75 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.88 (dd, *J* = 4.1 & 4.1 Hz, 1H, Ar-H), 7.94 (s, 2H, Ar-H), 8.62 (s, 1H, Thiazolyl-H), 8.67 (d, *J* = 4.1 Hz, 1H, Ar-H), 12.00 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 13.80, 30.69, 118.04, 118.64, 122.26, 126.48, 127.78, 129.18, 131.16, 132.83, 136.73, 139.36, 147.29, 149.60, 150.22, 156.88, 164.28, 165.37. HRMS (ESI)$^+$ calcd. for $C_{18}H_{15}BrN_4OS$ $[M+H]^+$ 415.0150. Found 415.0127.

(*E*)-2-(2-Ethylpyridin-4-yl)-*N*'-(4-fluorobenzylidene)thiazole-4-carbohydrazide (6b)

Yield: 87 %, m.p. 122-124 °C. IR (Neat): 3237, 3042, 2913, 2898, 2807, 1669, 1588, 1517, 1470, 1402, 1366, 1275, 1240, 1167, 1039, 959, 908, 821, 713, 661 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.30 (t, *J* = 7.5 Hz, 3H, CH_3), 2.85 (q, *J* = 7.5 Hz, 2H, CH_2), 7.32 (t, *J* = 8.3 Hz, 2H, Ar-H), 7.80-7.83 (m, 2H, Ar-H), 7.87 (dd, *J* = 3.9 & 3.9 Hz, 1H, Ar-H), 7.94 (s, 1H, Thiazolyl-H), 8.65 (s, 2H), 8.67 (d, *J* = 8.3 Hz, 1H), 11.88 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 13.82, 30.71, 115.94, 116.16, 118.04, 118.64, 127.54, 129.43, 129.52, 130.87, 139.39, 147.97, 149.74, 150.22, 156.77, 162.04, 164.31, 165.33. HRMS (ESI)$^+$ calcd. for $C_{18}H_{15}FN_4OS$ $[M+H]^+$ 355.0951. Found 355.1028.

(*E*)-*N*'-(4-Chlorobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6c).

Yield 83 %, m.p. 160-162 °C. IR (Neat): 3131, 3052, 2969, 2916, 1670, 1597, 1536, 1487, 1408, 1356, 1228, 1177, 1096, 1058, 1025, 977, 897, 828, 786, 705, 659 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.30 (t, *J* = 5.2 Hz, 3H, CH_3), 2.87 (q, *J* = 5.2 Hz, 2H, CH_2), 7.54 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.77 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.88 (dd, *J* = 4.2 & 4.2 Hz, 1H, Ar-H), 7.94 (s, 1H, Thiazolyl-H), 8.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 8.67 (d, *J* = 4.2 Hz, 1H, Ar-H), 11.93 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 13.81, 30.69, 118.03, 118.14, 118.63, 127.65, 128.89, 129.06, 133.21, 134.74, 139.37, 147.76, 149.67, 150.21, 156.79, 161.46, 164.37, 165.35.

(*E*)-*N*'-(4-Bromobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6d).

Yield 95 %, m.p. 168-170 °C. IR (Neat): 3223, 3055, 2969, 1669, 1596, 1536, 1483, 1408, 1353, 1229, 1113, 1060, 1010, 897, 827, 709, 661 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (t, *J* = 5.2 Hz, 3H, CH_3), 2.94 (q, *J* = 5.2 Hz, 2H, CH_2), 7.54 (d, *J* = 4.3 Hz, 2H, Ar-H), 7.63 (dd, *J* = 4.3 & 4.3 Hz, 1H, Ar-H), 7.67-7.69 (m, 3H, Ar-H &Thiazolyl-H), 8.37 (d, *J* = 8.2 Hz, 2H, Ar-H), 8.66 (d, *J* = 4.2 Hz, 1H, Ar-H), 10.41 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.00, 31.63, 117.96, 118.88, 125.19, 126.21, 129.32, 132.14, 132.61, 139.70, 147.86, 149.96, 150.45, 156.86, 165.19, 166.44. HRMS (ESI)$^+$ calcd. for $C_{18}H_{15}BrN_4OS$ $[M+H]^+$ 415.0150. Found 415.0231.

(*E*)-2-(2-Ethylpyridin-4-yl)-*N*'-(3-nitrobenzylidene)thiazole-4-carbohydrazide (6e).

Yield 92 %, m.p. 126-128 °C. IR (Neat): 3289, 3118, 3053, 2959, 2917, 2855, 1654, 1595, 1518, 1343, 1222, 1059, 944, 882, 802, 734, 671 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.39 (t, *J* = 6.3 Hz, 3H, CH_3), 2.94 (q, *J* = 6.3 Hz, 2H, CH_2), 7.59-7.64 (m, 2H, Ar-H), 7.69 (s, 1H, Ar-H), 8.82 (d, *J* = 8 Hz, 1H, Ar-H), 8.25 (dd, *J* = 4.3 & 4.3 Hz, 1H, Ar-H), 8.40 (s, 1H, Thiazolyl-H), 8.56 (s, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 8.67 (d, *J* = 8.2 Hz, 1H, Ar-H), 10.57 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.99, 31.63, 117.96, 118.87, 122.75, 125.07, 126.54, 129.95, 133.02, 135.70, 139.62, 146.42, 148.75, 149.72, 150.47, 157.07, 165.23, 166.61.

(*E*)-2-(2-Ethylpyridin-4-yl)-*N*'-(2-nitrobenzylidene)thiazole-4-carbohydrazide (6f)

Yield 90 %, m.p. 154-156 °C. IR (Neat): 3277, 3114, 3029, 2967, 2905, 2798, 1664, 1597, 1521, 1349, 1237, 1041, 928, 865, 755, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.37 (t, *J* = 6.2 Hz, 3H, CH_3), 2.93 (q, *J* = 6.2 Hz, 2H, CH_2), 7.53-7.58 (m, 1H, Ar-H), 7.63-7.68 (m, 2H, Ar-H), 7.70 (d, *J* = 3.8 Hz, 1H, Ar-H), 8.05 (t, *J* = 7.9 Hz, 1H, Ar-H), 8.35 (t, *J* = 7.9 Hz, 1H, Ar-H), 8.41 (d, *J* = 3.8 Hz, 1H, Ar-H), 8.65 (t, *J* = 7.9 Hz, 1H, Ar-H), 8.91 (s, 1H, Thiazolyl-H), 10.74 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.04, 31.58, 118.01, 118.88, 124.91, 126.61, 128.74, 129.50, 130.84, 133.82, 139.58, 144.02, 148.20, 149.64, 150.42, 157.04, 165.20, 166.57. HRMS $(ESI)^+$ calcd. for $C_{18}H_{15}N_5O_3S$ $[M+H]^+$ 382.0896. Found 382.0973.

(*E*)-*N*'-(3-Chlorobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6g)

Yield 88 %, mp: 140-142 °C. IR (Neat): 3193, 3043, 2973, 2897, 2831, 1667, 1597, 1528, 1468, 1418, 1349, 1270, 1224, 1180, 1106, 1061, 966, 902, 834, 790, 710, 692 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.30 (t, *J* = 6.1 Hz, 3H, CH_3), 2.87 (q, *J* = 6.1 Hz, 2H, CH_2), 7.51 (d, *J* = 4.3 Hz, 1H, Ar-H), 7.53 (d, *J* = 4.3 Hz, 1H,Ar-H), 7.70-7.72 (m, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.87 (dd, *J* = 4.3 & 4.3 Hz, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 8.63 (s, 1H, Thiazolyl-H), 8.67 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 12.00 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 13.81, 30.70, 118.04, 118.64, 126.06, 126.35, 127.78, 129.95, 130.90, 133.74, 136.51, 139.37, 147.20, 149.60, 150.22, 156.89, 164.31, 165.37.

(*E*)-*N*'-(2-Bromobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6h)

Yield 82 %, m.p. 162-164 °C. IR (Neat): 3225, 3121, 3052, 2966, 2919, 2855, 1667, 1595, 1534, 1483, 1407, 1353, 1269, 1227, 1179, 1111, 1061, 1017, 974, 896, 826, 786, 705, 657 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.39 (t, *J* = 6.3 Hz, 3H, CH_3), 2.94 (q, *J* = 6.3 Hz, 2H, CH_3), 7.55 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.63 (dd, *J* = 4.4 & 4.4 Hz, 1H, Ar-H), 7.66-7.69 (m, 3H, Ar-H, &Thiazolyl- H), 8.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 8.67 (d, *J* = 4.4 Hz, 1H, Ar-H), 10.41 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.00, 31.63, 117.97, 118.88, 125.20, 126.21, 129.32, 132.14, 132.61, 139.70, 147.86, 149.96, 150.45, 156.87, 165.19, 166.45. HRMS $(ESI)^+$ calcd. for $C_{18}H_{15}BrN_4OS$ $[M+H]^+$ 415.0150. Found 415.0230.

(*E*)-*N*'-(2-Chlorobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6i)

Yield 93 %, m.p. 128-130 °C. IR (Neat): 3140, 3017, 2951, 2918, 1665, 1589, 1533, 1485, 1427, 1343, 1214, 1159, 1115, 1037, 891, 815, 778, 653 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.39 (t, *J* = 5.5 Hz, 3H, CH_3), 2.95 (q, *J* = 5.5 Hz, 2H, CH_2), 7.30-7.37 (m, 2H, Ar-H), 7.38-7.42 (m, 1H, Ar-H), 7.66 (dd, *J* = 3.8 & 3.8 Hz, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 8.25 (dd, *J* = 4.0 & 4.0 Hz, 1H, Ar-H), 8.41 (s, 1H, Thiazolyl-H), 8.68 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 10.52 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.03, 31.62, 118.03, 118.95, 126.36, 127.30, 128.28, 129.91, 131.09, 131.71, 134.54, 139.73, 145.36, 149.93, 150.44, 156.90, 165.20, 166.50. HRMS $(ESI)^+$ calcd. for $C_{18}H_{15}ClN_4OS$ $[M+H]^+$ 371.8559. Found 371.0731.

(*E*)-2-(2-Ethylpyridin-4-yl)-*N*'-(4-nitrobenzylidene)thiazole-4-carbohydrazide (6j)

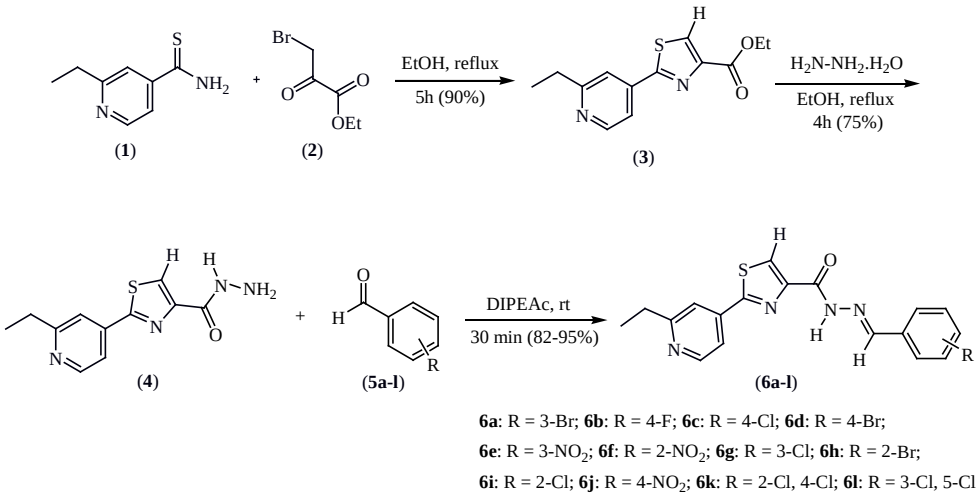
Yield 80 %, m.p. 188-190 °C. IR (Neat): 3255, 3081, 2978, 2905, 2851, 1667, 1562, 1523, 1367, 1217, 1047, 956, 867, 814, 754, 661 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.40 (t, *J* = 6.3 Hz, 3H, CH_3), 2.95 (q, *J* = 6.3 Hz, 2H, CH_2), 7.64 (dd, *J* = 4.2 & 4.2 Hz, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 7.99 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.29 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.42 (s, 1H, Thiazolyl-H), 8.57 (s, 1H, Ar-H), 8.68 (d, *J* = 4.2 Hz, 1H, Ar-H), 10.56 (s, 1H, Amido-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.01, 31.65, 117.97, 118.89, 124.21, 126.68, 128.47, 139.62, 139.80, 146.20, 148.94, 149.65, 150.51, 157.05, 165.27, 166.70.

(*E*)-*N*'-(2,4-Dichlorobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6k)

Yield 87 %, m.p. 144-146 °C. IR (Neat): 3247, 2993, 2944, 2831, 1662, 1598, 1522, 1489, 1418, 1349, 1265, 1214, 1183, 1058, 971, 841, 776, 722, 656 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.38 (t, *J* = 7.1 Hz, 3H, CH_3), 2.94 (q, *J* = 7.1 Hz, 2H, CH_2), 7.30 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.41 (t, *J* = 4.5 Hz, 1H, Ar-H), 7.65 (d, *J* = 4.5 Hz, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 8.19 (dd, *J* = 4.5 & 4.5 Hz, 1H, Ar-H), 8.41 (s, 1H, Thiazolyl-H), 8.66 (d, *J* = 4.5 Hz, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 10.57 (s, 1H, Amido- NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.03, 31.59, 118.01, 118.95, 126.54, 127.86, 129.03, 129.71, 129.76, 134.93, 137.06, 139.69, 144.24, 149.76, 150.42, 156.94, 165.20, 166.53.

(*E*)-*N*'-(3,5-Dichlorobenzylidene)-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide (6l).

Yield 85 %, m.p. 174-176 °C; IR (Neat): 3244, 3082, 3021, 2954, 2871, 1668, 1561, 1547, 1491, 1417, 1359, 1268, 1233, 1188, 1056, 1003, 917, 849, 812, 780 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.38 (t, *J* = 6.1 Hz, 3H, CH_3), 2.94 (q, *J* = 6.1 Hz, 2H, CH_2), 7.48 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.62 (d, *J* = 4.3 Hz, 2H, Ar-H), 7.68 (s, 1H, Ar-H), 7.91



Scheme 1. Synthesis of (*E*)-*N*'-benzylidene-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide derivatives.

(s, 1H, Thiazolyl-H), 8.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 8.67 (d, *J* = 4.3 Hz, 1H, Ar-H), 10.45 (s, 1H, Amido-NH) ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.00, 31.63, 117.96, 118.88, 126.39, 126.90, 129.30, 130.91, 133.41, 133.76, 134.77, 139.65, 146.44, 149.80, 150.45, 156.92, 165.20, 166.52.

RESULTS AND DISCUSSIONS

The reaction sequence followed for the synthesis of target compounds has been depicted in **Scheme 1**. In the first step, 2-ethylpyridine-4-carbothioamide (**1**) (1.0 mmol) and ethyl bromopyruvate (**2**) (1.3 mmol) were refluxed in ethanol to obtain ethyl 2-(2-ethylpyridin-4-yl)thiazole-4-carboxylate (**3**) with 90 % yield. Then, ethyl 2-(2-ethylpyridin-4-yl)thiazole-4-carboxylate (**3**) (1.0 mmol) and excess of hydrazine hydrate (3.0 mmol) was refluxed in ethanol to furnish the important intermediate 2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide(**4**) with 75 % yield. The condensation of various substituted aromatic aldehydes (**5a-l**) with 2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide(**4**) furnished the corresponding substituted (*E*)-*N*'-benzylidene-2-(2-ethylpyridin-4-yl)thiazole-4-carbohydrazide(**6a-l**) with 82-95 % yields. Diisopropyl-ethyl ammonium acetate (DIPEAc) is used as solvent and catalyst in the condensation reaction.[37]

The structures of the newly synthesized compounds were assigned by their IR, ^{1}H NMR, ^{13}C NMR and HRMS spectral data. In the IR spectrum, the peaks in the range of 1660 to 1670 cm^{-1} was observed, which confirms the presence of hydrazone carbonyl. The C=N stretching band appears in the range of 1590 to 1600 cm^{-1}. The band at 3150-3210 cm^{-1} is assigned for the N-H stretching. The ^{1}H NMR spectrum of these compounds have displayed a triplet-quartet pattern for ethyl substituent at δ 1.30-1.40 and 2.85-2.95. The amide N-H signal has appeared in the range of δ 10.41 to 12.00. The characteristic signal at δ 166 ppm in the ^{13}C NMR spectrum, confirms the presence of the carbonyl group (*N*-acylhydrazone). Finally, the HRMS data strengthen the structure assigned to newly synthesized compounds.

Antioxidant Activity

Free radicals are considered to be an important performers in physiological mechanisms.[38,39] The radical reacts comprehensively with nearly every kind of biomolecules seen in the living cell and may deviate the cells from their normal physiological roles. Antioxidants are the substances capable of scavenging free radicals. The antioxidant properties of the hydrazones (**6a-l**) were calculated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method.[40,41]

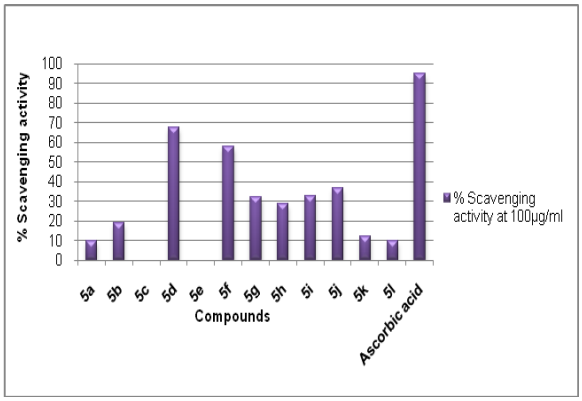


Figure 2. DPPH scavenging activity (%) of hydrazone derivatives (**6a-l**).

Ascorbic acid was used as a reference for antioxidant assay. According to the results obtained from the DPPH test, the best performing hydrazones were **6d** (67 %) and **6f** (57 %). The moderate antioxidant activity (10-36 %) was shown by hydrazones **6g**, **6h**, **6i** and **6j** (Figure 2). Free radical scavenging activity was measured in terms of percent inhibition.

Antimicrobial Activity

The agar well diffusion method was used for screening of the antimicrobial activity of the hydrazone derivatives (**6a-l**).[42]

Table 1. Antimicrobial activity of hydrazone derivatives (**6a-l**).

S.No.	Pathogens	Compounds											Standard
		6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l	
1	*S. typhi*	23	-	-	05	-	-	-	-	12	-	-	-	33
2	*E. aerogenes*	17	12	-	-	05	-	-	09	05	-	-	06	33
3	*B. subtilis*	26	12	-	-	-	-	05	-	06	-	07	-	32
4	*B. cereus*	18	06	-	-	-	09	-	-	07	-	-	-	33
5	*P. aeruginosa*	18	06	-	-	-	07	-	-	10	-	-	08	32
6	*S. abony*	16	07	-	-	-	05	-	-	06	-	-	-	32
7	*E. coli*	-	09	-	-		-	-	-	-	-	08	-	29
8	*S. aureus*	16	08	-	05	-	-	06	-	-	-	05	-	29
9	*S. boydii*	18	08	-	-	-	-	-	-	06	-	-	-	34
10	*C. albicans*	06	05	08	-	07	-	-	-	15	-	-	-	30
11	*S. cerevisiae*	15	10	10	-	-	-	-	05	05	-	-	-	30
12	*A. niger*	15	05	-	-	-	-	-	-	-	-	-	05	30

- = no activity

Table 2. MIC value of most potent hydrazone derivatives **6a**, **6b** and **6i** (µg mL^{-1}).

S.No.	Pathogens	Compounds			Ceftazidime	Fluconazole
		6a	6b	6i		
1	*B. subtilis*	40 ± 0.35	85 ± 0.35	150 ± 1.50	24 ± 0.65	NA
2	*B. cereus*	65 ± 1.20	95 ± 1.60	135 ± 0.90	35 ± 1.35	NA
3	*E. aerogenes*	60 ± 0.40	110 ± 1.30	280 ± 2.30	30 ± 0.55	NA
4	*S. cerevisiae*	120 ± 0.85	170 ± 1.10	310 ± 0.80	NA	20 ± 0.80
5	*C. albicans*	90 ± 0.45	215 ± 1.40	190 ± 1.12	NA	30 ± 1.50

NA = Not available

Both the Gram-positive and Gram-negative bacterial pathogens were used for evaluating antibacterial activity. *Staphylococcus aureus* ATCC 6538, *Bacillus megaterium* ATCC 2326, *Bacillus subtilis* ATCC 6633 were Gram-positive pathogens used in this study. *Escherichia coli* ATCC8739, *Salmonella typhi* ATCC9207, *Shigella boydii* ATCC 12034, *Enterobacter aerogenes* ATCC13048, *Pseudomonas aeruginosa*ATCC9027 and *Salmonella abony* NCTC6017 were the Gram-negative pathogens used. Antifungal activity of synthesized compounds was determined against *Aspergillusniger*ATCC 16404, *Saccharomyces cereviseae*ATCC 9763, and *Candida albicans* ATCC10231 fungal pathogens. Ceftazidime and fluconazole were used as antibacterial and antifungal standard reference compounds, respectively. The hydrazone derivatives**6a**, **6b**, and **6i**were displayed remarkable antibacterial and antifungal activity against almost all pathogens. The zones were measured and recorded by using the scale in millimeter (Table 1).

The MIC values were calculated for the most active hydrazones. The hydrazones **6a**, **6b** and **6i** were found effective against many pathogens; hence these hydrazones were selected for MIC determination studies. The MIC was determined against *B. subtilis*, *E. aerogenes* and *C. albicans.*It was determined by using the method and rules of the Clinical and Laboratory Standard Institute (CLSI).[43]All experiments were performed in triplicates and results are expressed as mean ± SD in µg mL^{-1} (Table 2).

Molecular Docking Study

A molecular docking was performed to predict the molecular mechanism of action of hydrazone derivatives (**6a-l**).[44,45] The crystal structure of the *DNA gyrase* (PDB ID1QX1) was used for docking analysis. All the hydrazone derivatives showed excellent binding potential with *DNA gyrase*, which indicates the antimicrobial potential of hydrazone derivatives. Hydrazone **6a** has shown hydrogen bonding interaction with ARG342, and VdW interactions with ARG630, GLU634, GLY178, ARG342, LEU345 (Figure 3). Hydrazone **6b** has displayed hydrogen bonding interactions with ARG342, hydrophobic interaction with MET27, VAL31 and VdW interactions with GLU634, ALA637, MET27, VAL31, GLY341, ARG342, PRO343 (Figure 4). While hydrazone **6i** has shown hydrogen bonding interactions with ARG630, hydrophobic interaction with ARG342 and VdW interactions with GLU627, ARG630, GLU634, ARG342, PRO343, LYS344 (Figure 5).

ADME Prediction

The hydrazone derivatives (**6a-l**) were scrutinized for ADME prediction using Swiss ADME portal.46All the hydrazone derivatives were displayed excellent ADME parameters with low Lipinski violation, which is needed for the oral absorption of drug molecules, and the outcomes are summarized in Table 3.

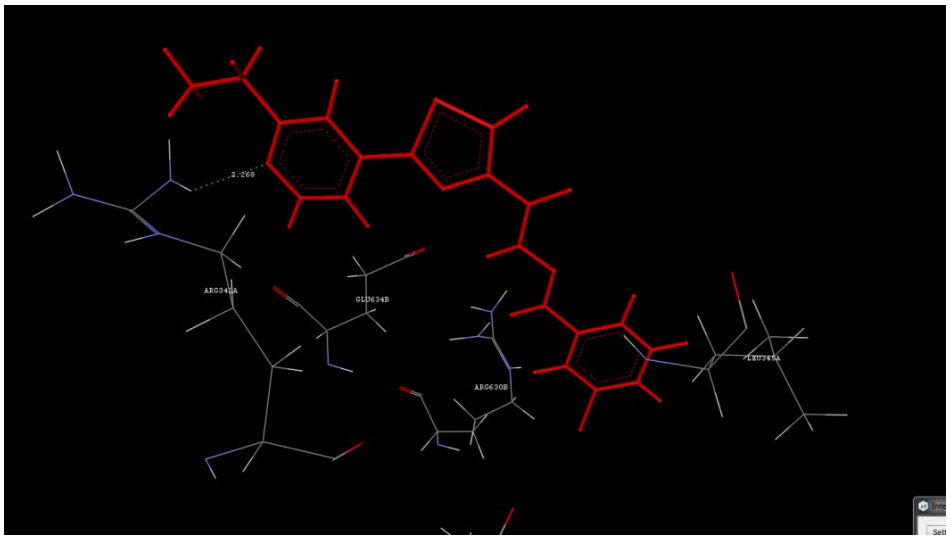


Figure 3. Binding mode of hydrazone **6a** into the active site of DNA gyrase.

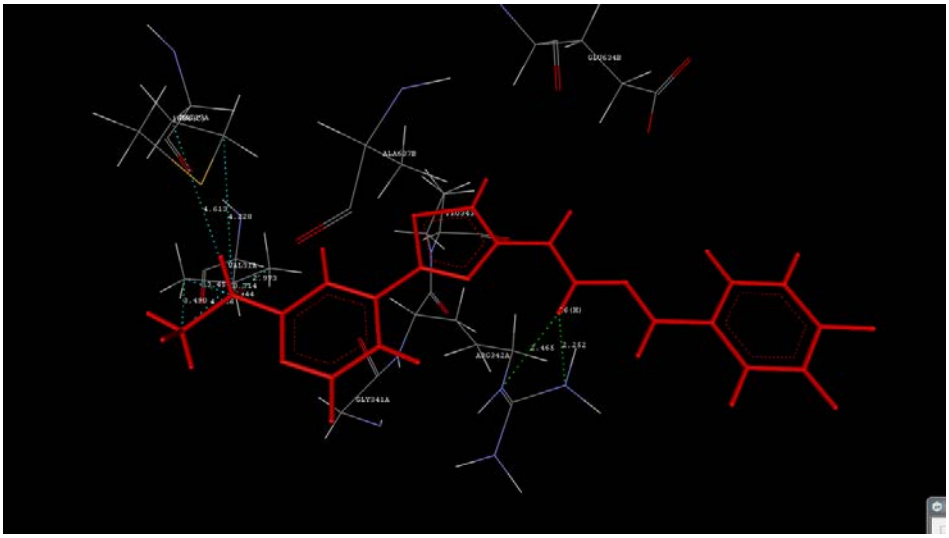


Figure 4. Binding mode of hydrazone **6b** into the active site of DNA gyrase.

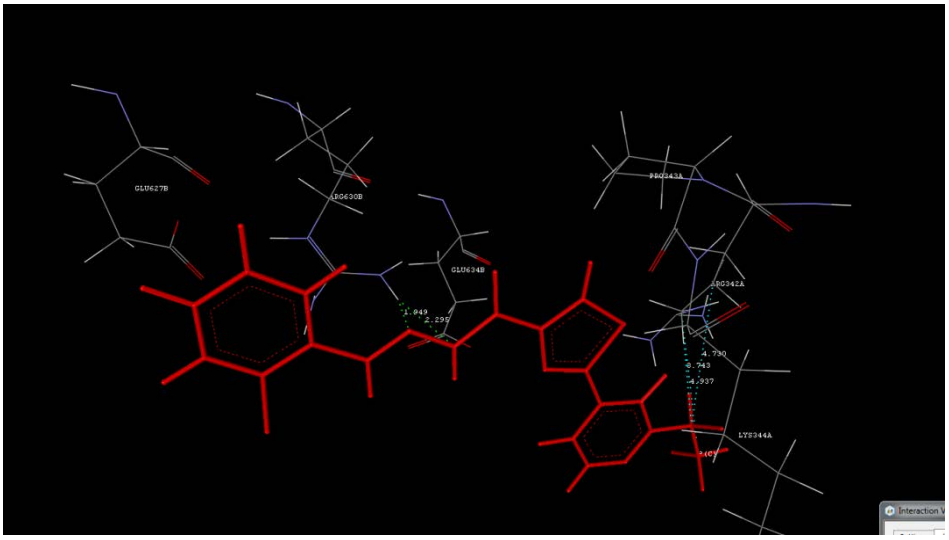


Figure 5. Binding mode of hydrazone **6i** into the active site of DNA gyrase.

Table 3. Pharmacokinetic parameters of hydrazone derivatives (**6a-l**).

Entry	Mol. Wt.	Rotatable bonds	H-bond acceptors	H-bond donors	LOG P	Bioavailability Score
6a	414.32	6	3	1	3.93	0.55
6b	353.41	6	4	1	3.65	0.55
6c	369.87	6	3	1	3.81	0.55
6d	414.32	6	3	1	3.9	0.55
6e	380.42	7	5	1	3.23	0.55
6f	380.42	7	5	1	3.18	0.55
6g	369.87	6	3	1	3.78	0.55
6h	414.32	6	3	1	3.93	0.55
6i	369.87	6	3	1	3.83	0.55
6j	380.42	7	5	1	3.24	0.55
6k	404.31	6	3	1	4.04	0.55
6l	404.31	6	3	1	3.94	0.55

CONCLUSION

In conclusion, a series of new pyridyl and thiazolyl clubbed hydrazone derivatives (**6a-l**) were synthesized starting from ethionamide. All the newly synthesized hydrazone derivatives were screened for their *in vitro* antimicrobial activity. The hydrazone derivatives **6a**, **6b** and **6i** have displayed excellent antimicrobial activities. Antioxidant potential of synthesized hydrazone derivatives has also been evaluated by DPPH scavenging method. The hydrazone derivatives **6d** and **6f** have shown good antioxidant activity. A molecular docking study was performed to investigate the binding modes of these molecules by using *DNA gyrase*. These compounds were showed excellent binding potential, which indicates the antimicrobial potential of synthesized hydrazone derivatives.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

The supporting information of this article containing ^{1}H NMR, ^{13}C NMR and HRMS spectrums of new compounds are available for the authorized users.

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