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

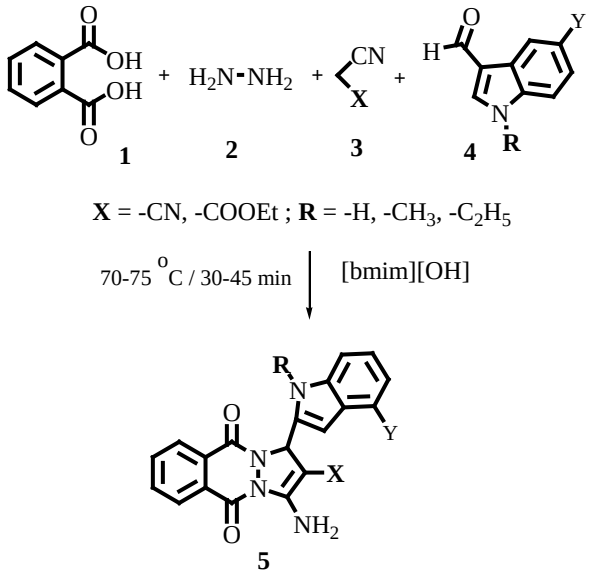
(s, 1H, -CH), 7.26-8.68 (m, 10H, Ar-H and NH_2), 11.87 (s, 1H, -NH,). ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 61.5, 69.1, 110.6, 111.5, 115.8, 115.9, 119.2, 122.9, 123.6, 127.6, 134.6, 135.6, 138.4, 144.6, 145.8, 161.6, 164.5. MS *m/z*: 400 $[M+H]^+$.

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.

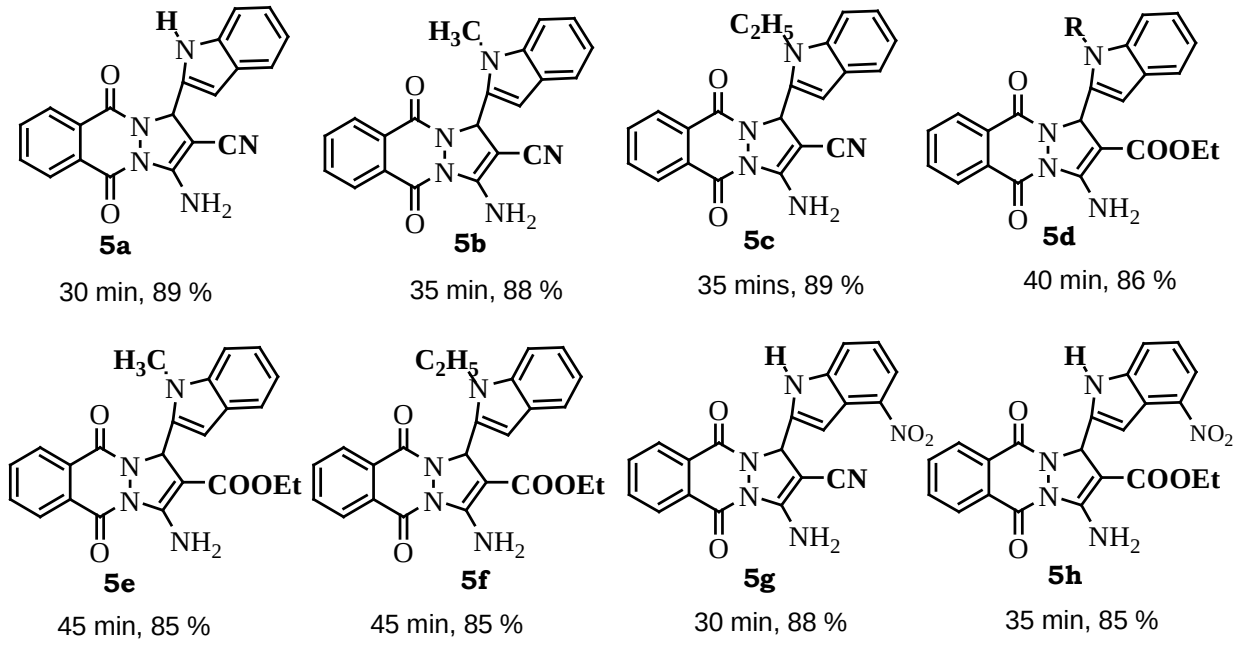
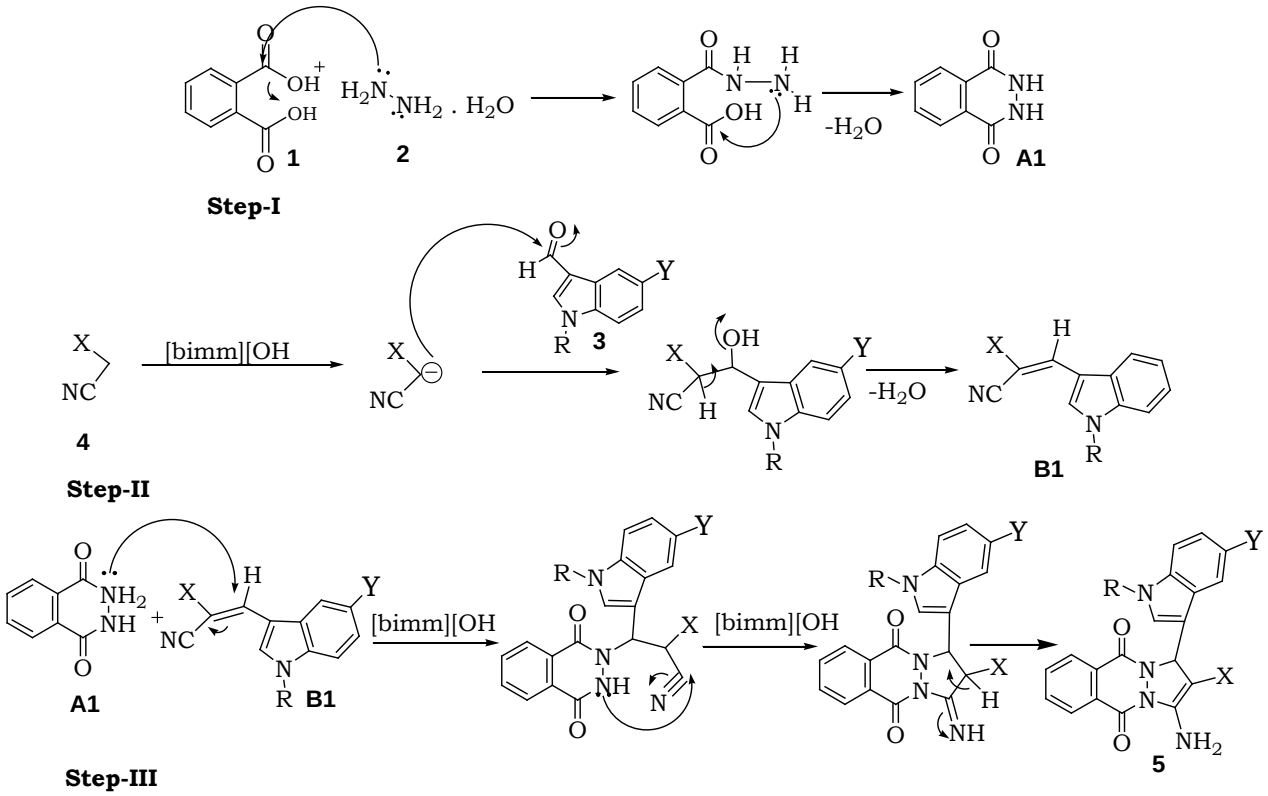


Figure 1. Structure of compounds **5a - 5h**.



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.

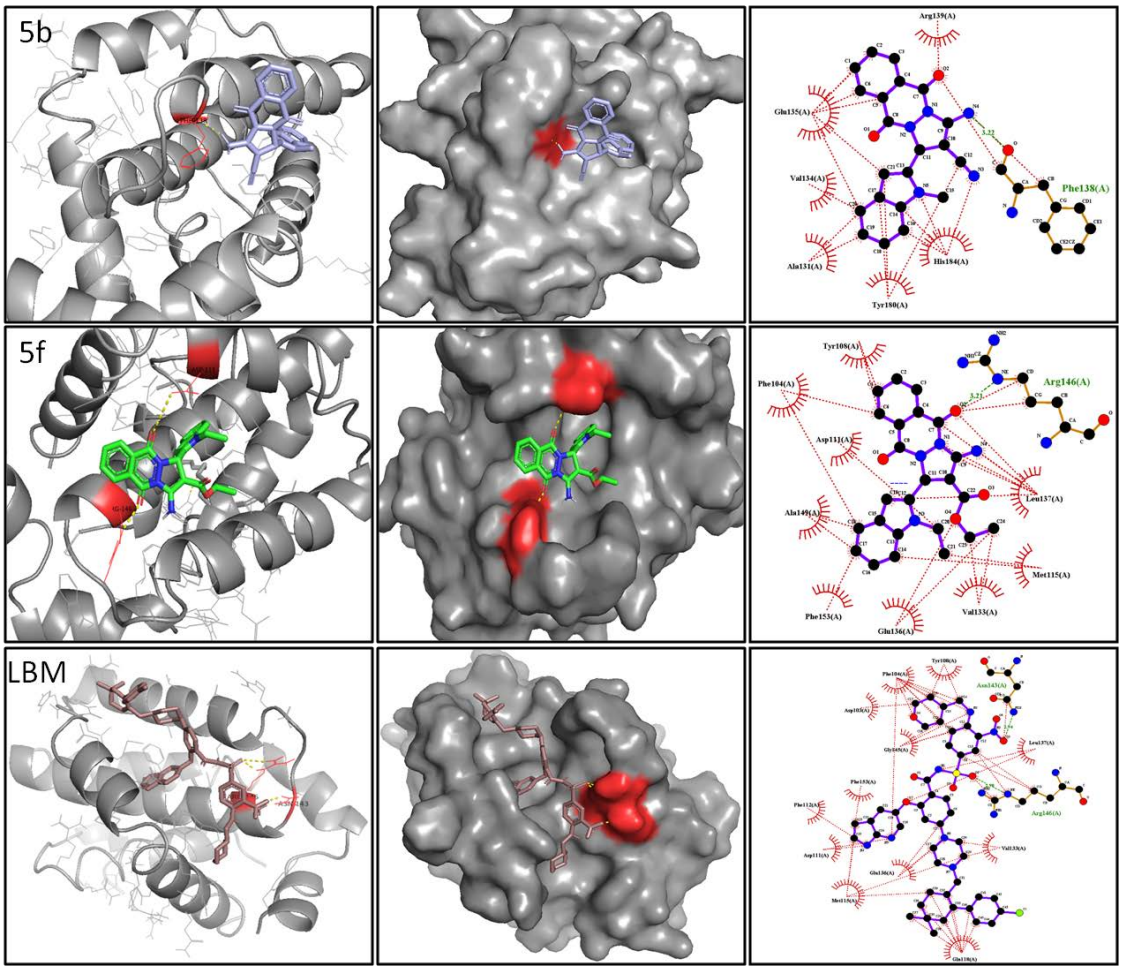


Figure 2. In silico docking of human BCL-2 protein with different compounds 5b, 5f and venetoclax (LBM). The binding interaction for the best docked pose for each ligand was showed in the image. The ligand binding site and the amino acids interacted with the ligands are illustrated using LigPlot.

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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REFERENCES

[1]Montagne, C., Shiers, J. J., Shipman, M., Rapid generation of molecular complexity using "sequenced" multi-component reactions: one-pot synthesis of 5,5′-disubstituted hydantoins from methyleneaziridines, *Tetrahedron Lett.*, **2006**, *47*, 9207. https://doi.org/10.1016/j.tetlet.2006.10.135

[2]Jain, R., Vederas, J. C. , Structural variations in keto-glutamines for improved inhibition against hepatitis A virus 3C proteinase, *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 3655. https://doi.org/10.1016/j.bmcl.2004.05.021

[3]Parivash, J., Manouchehr, M., Chemodivergent, multicomponent-tandem facile synthesis of novel 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione using acetic acid functionalized imidazolium salt {[cmdmim]I} as a recyclable catalyst, *New J. Chem.*, **2019,** *43*, 8266. https://doi.org/10.1039/C9NJ00993K

[4]Kim, J. S., Rhee, H. K., Park, H. J., Lee, S. K., Lee, C. O., Park Choo, H. Y., Synthesis of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones and evaluation of their cytotoxicity and topoisomerase II inhibition, *Bioorg. Med. Chem.*, **2008,** *16*, 4545–4550.

DOI: 10.1016/j.bmc.2008.02.049

[5]Ryu, C.K., Park, R. E., Ma, M. Y., Nho, J. H. Synthesis and antifungal activity of 6-arylamino-phthalazine-5,8-diones and 6,7-bis(arylthio)-phthalazine-5,8-dione, *Bioorg. Med. Chem. Lett.* **2007,** *17*, 2577–2580. DOI: 10.1016/j.bmcl.2007.02.003

[6]El-Sakka, S. S., Soliman, A. H., Imam, A. M., Synthesis, antimicrobial activity and Electron Impact of Mass Spectra of Phthalazine-1,4-dione Derivatives, *Afinidad*. **2009,** *66*, 167. https://core.ac.uk/download/pdf/39152404.pdf

[7]Grasso, S., DeSarro, G., Micale, N., Zappala, M., Puia, G., Baraldi, M., Demicheli, C., Synthesis and Anticonvulsant Activity of Novel and Potent 6,7- Methylenedioxyphthalazin-1(2H)-ones, *J. Med. Chem.*, **2000,** *43*, 2851. DOI: 10.1021/jm001002x

[8]Wu, H., Chen, X.-M., Wan, Y., Xin, H.-Q., Xu, H.-H., Ma, R., Yue, C.-H., Pang, L.-L., Synthesis and Luminescence of 7-amino-2H-indazolo[2,1-b]phthalazine-1,6,11(13H) triones catalyzed by silica sulfuric acid. *Lett. Org. Chem.* **2009,** *6*, 219–223. DOI: 10.2174/157017809787893127

[9]Mudumala,V. R., Chinthaparthi, R. R., Yeon, T. J., Sulphated alumina tungstic acid (SATA): a highly efficient and novel heterogeneous mesostructured catalyst for the synthesis of pyrazole carbonitrile derivatives and evaluation of green metrics, *Tetrahedron*. **2014,** *70*, 3762–3769. DOI: 10.1039/C9RA09013D

[10]Ramtohul, Y. K., James, M. N. G., Vederas J. C. Synthesis and Evaluation of Keto-Glutamine Analogues as Inhibitors of Hepatitis A Virus 3C Proteinase. *J. Org. Chem*. **2002,** *67*, 3169–3178. https://doi.org/10.1021/jo0157831

[11]Tavakoli, F., Mamaghani, M., Sheykhan, M., Mohammadipour, N., Rassa, M., Ultrasonic Activated, Highly Efficient and Regioselective Synthesis of a Novel Pyrrole- Linked benzo[f]chromene Scaffold in a Green Media, *Curr. Org. Synth.*, **2018,** *15*, 872–880. DOI: 10.2174/1570179415666180622122514

[12]Reddy, Y. D., Narayana, B. S., Reddy, CH. V. R., Dubey, P. K., Four Component Domino Reaction for the Synthesis of 1-(1H-indol-2-yl)-1H-pyrazolo [1,2-b]phthalazine-5, 10-Diones, *Synth. Commun.*, **2014**, *44*, 3037-3046. https://doi.org/10.1080/00397911.2014.928326

[13]Ghanbari-Ardestani, S., Khojasteh-Band, S., Zaboli, M., Hassani, Z., Mortezavi, M., Mahani, M., Torkzadeh-Mahani, M., The effect of different percentages of triethanolammonium butyrate ionic liquid on the structure and activity of urate oxidase: Molecular docking, molecular dynamics simulation, and experimental study, *J. Mol. Liq.*, **2019,** *292*, 111318. DOI: 10.1016/j.molliq.2019.111318

[14]Wallace, A. C., Laskowski, R. A., Thornton, J. M., LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, *Protein Eng. Des. Sel.*, **1995,** *8*, 127-134. DOI:10.1093/protein/8.2.127

[15]Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, **1983,** *65*, 55-63. DOI:10.1016/0022-1759(83)90303-4

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