GLYCEROL MEDIATED ONE-POT SYNTHESIS OF PYRAZOLE CONJUGATED TETRAHYDROQUINOLINE DERIVATIVES AND EVALUATION OF THEIR ANTICANCER ACTIVITY

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Pyrazole scaffold is an important building block in many of the medicinally active new chemical entities. In the current work, synthesis of pyrazole conjugated tetrahydroquinoline derivatives has been achieved by treating 3-methyl-1-phenyl-1H-pyrazol-5-amine (1), 5,5-dimethylcyclohexane-1,3-dione (2), and benzaldehydes (3) at 80-85 °C for 60-90 min using glycerol as green reaction medium. The anticancer activity of the synthesized pyrazole-conjugates was carried out on breast cancer (MCF-7) and liver cancer (A549) cell lines. Two among the tested compounds showed potential inhibition on A549 cell lines. Further, molecular modeling studies have performed and the binding interactions with the target protein have been observed. Additionally, pharmacokinetic properties such as bioavailability, log P, total polar surface area and blood brain barrier (BBB) have been predicted using SwissADME tools to get insight into the further structural optimization.

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INTRODUCTION

Pyrazol[3,4-b]quinoline derivatives were momentous for their pharmacological activities. In particular, they showed potential anticancer, anti-malarial, antiviral, and anti-inflammatory properties.1-3 These are also known for parasiticidic properties, antibacterial, antitumor, hypotensive, and vasodilation activities.1-3 In specific, pyrazoloannelated heterocyclic scaffold is being found in a diverse therapeutic drugs such as COX inhibitors, Phosphodiesterase 5 (PDE5) inhibitor (Sildenafil Citrate - Viagra), and mTOR signaling inhibitors (WYE-354) (Figure 1) etc.4 Due to their growing importance, various synthetic protocols have been reported in the recent past for the preparation of pyrazol[3,4-b]quinoline scaffolds using different homogeneous and heterogeneous materials such as FeNi2-ILs.5 PEGOSO3H,6 L-proline,7 and InCl38 as catalysts. However, the aforementioned methods have limitations and suffer from drawbacks such as prolonged reaction times, harsh reaction conditions, catalyst separation challenges, tedious workup, waste generation, toxic solvents, high reaction temperatures and low product yields. Therefore, there is a pressing need for newer methods that could surmount the above challenges.

Research for finding other alternate reaction media, which can substitute the hazardous, toxic, and inflammable organic solvents, which pose a serious threat to the environment, is gaining progress. Many environmentally compatible reaction media like green solvents,9 ionic liquids,10 supercritical fluids,11 and fluorous phases,12 are being used for several organic reactions. Each has its own advantages and is dependent on external factors like lipophilicity, pressure, and viscosity.

Glycerol was an environmental and biodegradable solvent produced as a by-product in the biodiesel industry.9 Given the high boiling point property of glycerol, reactions using this as a medium can be carried out at high temperature, thus allowing acceleration of the reaction or making possible reactions that do not proceed in low boiling point solvents.

Figure 1. Some therapeutically active compounds containing pyrazole scaffold.
In the bulk manufacturing of active pharmaceutical ingredients (API), the low toxicity of glycerol allows to be used as a solvent in the synthesis in which the toxicity and residue of solvent have to be carefully controlled. Due to the unique physico-chemical properties, there are a large number of reports on the applications of glycerol as efficient and convenient solvents in organic transformations.\textsuperscript{13}

In view of the importance of pyrazole scaffold, we report presently an efficient one-pot protocol for the synthesis of pyrazolo[3,4-b]quinolines using glycerol as green reaction medium. There have been no earlier reports for the preparation of pyrazolo[3,4-b]quinoline derivatives in glycerol as solvent.

### RESULTS AND DISCUSSION

Initially, using the one-pot three-component reaction of 3-methyl-1-phenyl-1H-pyrazol-5-amine (1) (1 mmol) with 5,5-dimethylcyclohexane-1,3-dione (2) (1 mmol), and benzaldehyde (3a) (1 mmol) was carried out at 80-85 °C as a model for synthesis of 3,7,7-trimethyl-1,4-diphenyl-6,7,8,9-tetrahydro-1H-pyrazolo[3,4-b]quinolin-5(4H)-one (4a). We examined the suitable solvents like glycerol, ethylene glycol, DMSO and DMF at different temperature in the presence of 1 equiv. TEA as catalyst at 80-85 °C. Results were summarised in Table 1. It is observed that the formation of 4a by one-pot three component reaction in glycerol at 80-85 °C for 60 min gave excellent yield 90 % compared to other conditions (Table 1 entry 1). The structure of 4a was confirmed by \textsuperscript{1}H, \textsuperscript{13}C-NMR, and mass spectroscopy.

Further, optimization studies were carried out by altering different catalysts such as piperidine and DBU for formation of 4a by using 1, 2 and 3a. It is noticed that usage of piperidine and DBU as catalyst for this reaction resulted in low yields (Table 1, entry 5 and 12). Lower and higher temperature also gave low yield in formation of 4a (Table 1, entry no 13 and 14).

In the continuous efforts to optimize the one-pot three component reaction, different catalyst amounts were used like 0.5 equiv., 1 equiv. and 2 equiv. of TEA and consistent optimized results were obtained with 1 equiv. of TEA. Results were summarised in Table 1 (Entry no 1, 15 & 16). However, finally formation of 4a in glycerol as solvent at 80-85 °C in the presence of 1 equiv. of TEA gave excellent yield for 60 min. Having optimised one-pot three component reaction conditions, we explored the scope and limitations with series of substituted anilines 3a-3h. It was found that the both electron-deficient and electron-rich anilines were applicable for this optimised conditions affording the corresponding benzothiazole derivatives yields 85-90 % (Figure 2). Encouraged by these results, the synthesis of 4a-4h were carried out in one-pot three component reaction by using 1, 2 and 3a-3h in glycerol at 80-85 °C in the presence of 1 equiv. TEA for 60-90 min (Scheme 1) with excellent yields of 85-90 %. Structures were confirmed by \textsuperscript{1}H and \textsuperscript{13}C NMR, and mass spectroscopy.

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Table 1. Optimization of reaction conditions for the synthesis of 4a.

| S. No. | Solvent   | Temp. °C | Catalyst       | Time h | Yield % |
|--------|-----------|----------|----------------|--------|---------|
| 1      | Glycerol  | 80-85    | TEA, 1 equiv.  | 1      | 90      |
| 2      | Ethylene glycol | 80-85    | TEA, 1 equiv.  | 2      | 80      |
| 3      | DMF       | 80-85    | TEA, 1 equiv.  | 2      | 60      |
| 4      | DMSO      | 80-85    | TEA, 1 equiv.  | 2      | 65      |
| 5      | Glycerol  | 80-85    | Piperidine, 1 equiv. | 1.5  | 85      |
| 6      | Ethylene glycol | 80-85    | Piperidine, 1 equiv. | 2.5  | 81      |
| 7      | DMF       | 80-85    | Piperidine, 1 equiv. | 3    | 65      |
| 8      | DMSO      | 80-85    | Piperidine, 1 equiv. | 3    | 68      |
| 9      | Glycerol  | 80-85    | DBU, 1 equiv.  | 1.5    | 87      |
| 10     | Ethylene glycol | 80-85    | DBU, 1 equiv.  | 2.5    | 83      |
| 11     | DMF       | 80-85    | DBU, 1 equiv.  | 2.5    | 68      |
| 12     | DMSO      | 80-85    | DBU, 1 equiv.  | 2.5    | 69      |
| 13     | Glycerol  | 60-65    | TEA, 1 equiv.  | 5      | 88      |
| 14     | Glycerol  | 90-95    | TEA, 1 equiv.  | 1      | 87      |
| 15     | Glycerol  | 80-85    | TEA, 0.5 equiv. | 1.5  | 86      |
| 16     | Glycerol  | 80-85    | TEA, 2 equiv.  | 1      | 85      |

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Glycerol/ TEA → 80-85 °C/ 60-90 min

**Scheme 1.** Synthesis of 4a-4h by one-pot synthesis.
Figure 2. Structures and yields of 4a-h synthesized by the one-pot reaction.

Figure 3. Molecular interactions of human Type I protein arginine methyltransferases (PRMTs) with different test compounds 4d, 4g and Doxorubicin. The interactions for the best docked pose for each ligand was showed in the image. The amino acids interacted with the docked ligands are illustrated using LigPlot.
Cytotoxicity assay

A series of 8 conjugates were synthesized and evaluated for their cytotoxicity against two different human cancer cell lines (A549 and MCF7) using MTT assay. IC50 values of the compounds against different cancer cell lines were tabulated and shown in the Table 2. Some of the compounds showed substantial reduction in the cell viability of cancer cells in a dose dependent manner. Compound 4d and 4g showed good activity against A549 cells with an IC50 value of 9.3 and 9.6 µM, respectively.

| Compound | IC50 value in µM (Mean ± S.D.) |
|----------|--------------------------------|
|          | MCF-7                        | A549                        |
| 4a       | >100                          | >100                        |
| 4b       | 14.3 ± 0.43                   | 16.7 ± 0.22                 |
| 4c       | >100                          | >100                        |
| 4d       | 12.4 ± 0.38                   | 9.3 ± 0.18                  |
| 4e       | 14.6 ± 0.42                   | 9.8 ± 0.38                  |
| 4f       | 24.6 ± 0.35                   | 19.6 ± 0.26                 |
| 4g       | 7.9 ± 0.12                    | 9.6 ± 0.16                  |
| 4h       | 21.4 ± 0.46                   | 17.5 ± 0.27                 |
| Doxorubicin | 0.68 ± 0.05                  | 8.63 ± 0.04 nM              |
| (Positive control) |                       |                             |

Molecular docking

The selected compounds (4d and 4g) from the preliminary screening were evaluated for in silico docking analysis. Molecular docking for the compounds 4d and 4g was performed against the human Type I protein arginine methyltransferases (PRMTs) active site. A total of ten different conformations were examined for each docked ligand. The binding energies for the best-docked pose of 4d and 4g compounds in the receptor active site were -8.2 and -8.0 kcal mol⁻¹, respectively. Whereas the positive control Doxorubicin showed -8.4 kcal/mol.

Table 3. The binding energies, RMSD values and amino acids in the receptor protein interacted with ligands were determined using autodock and LigPlot.

| Ligand               | Binding energy (kcal mol⁻¹) | RMSD  | H-bond/s | Protein–Ligand interactions |
|----------------------|-----------------------------|-------|----------|------------------------------|
| 4d                   | -8.2                        | 3.813 | Arg345   | Pro42, Phe54, Cys119, Lys145, Lys45 |
| 4g                   | -8.0                        | 4.853 | Arg345   | Phe54, Lys145, Arg181, Asp188, Asp187, Tyr258 |
| Doxorubicin (Positive control) | -8.4                        | 2.544 | Lys155, Asp182, Leu185, Ala186, Gly189 |
Cytotoxicity assay

Cytotoxicity of the synthesized compounds was tested against two different human cancer cell lines A549 (Human lung carcinoma) and MCF 7 (Human breast carcinoma) using MTT assay.17

Molecular Docking

In silico molecular binding of the synthesized compounds 4d and 4g with human Type 1 protein arginine methyltransferases (PRMTs) protein was evaluated using AutoDockTools.18 Based on cytotoxicity results, the selected ligand structures were generated using Chem3D Ultra 16.0 software. MOPAC (semi-empirical quantum mechanics) tool was used to minimize the energies of the ligand structures and the outcomes were saved in protein data bank (pdb) format using Chem3D Ultra 16.0 software. The PDB structure of PRMTs protein (PDB ID: 6NT2) was downloaded and imported to the workspace. The Kollaman charges were incorporated to the protein and were processed for further in AutoDock. Further, the grid box with a size of 90 in all the axes (X, Y, Z) was generated for the processed protein. The visualization of the output file generated from docking was analysed using PyMol. Doxorubicin was used as a positive control.

SwissADME

The physicochemical descriptors, pharmacokinetic properties and in silico drug likeness of the synthesized compounds were predicted using SWISSADME server. Lipophilicity and polarity of the compounds were predicted by BOILED-Egg (Brain Or Intestinal Estimated permeation) method.20,21 Doxorubicin was used as a positive control.

3,7,7-Trimethyl-1-(4-toly)-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4d)

Yield 90 %, m.p. 191-193 °C. 1H NMR (400 MHz, DMSO-d6) δ = 1.0 (s, 3H, CH3), 1.05 (s, 3H, CH3), 2.0 (s, 3H, CH2), 2.2-2.4 (d, 4H, CH2), 5.1 (s, 1H, CH), 6.5 (s, 1H, NH), 7.0-8.0 (m, 10H, Ar-H). 13C NMR (100 MHz, DMSO-d6) δ = 12.1, 27.4, 27.7, 27.8, 28.3, 28.9, 32.1, 36.1, 41.2, 48.7, 50.7, 104.7, 112.7, 121.5, 125.1, 126.4, 127.3, 127.6, 128.1, 129.2, 135.2, 145.9, 147.9, 195.0. MS: M+ = 384. 3,7,7-Trimethyl-1-(4-p-toly)-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4b)

Yield 88 %, m.p. >220 °C. 1H NMR (400 MHz, DMSO-d6) δ = 0.9 (s, 3H, CH3), 1.0 (s, 3H, CH3), 1.7 (s, 3H, CH3), 1.9-2.1 (d, 4H, CH2), 5.1 (s, 1H, NH), 7.0-8.0 (m, 9H, Ar-H). 13C NMR (100 MHz, DMSO-d6) δ = 11.8, 19.2, 26.8, 28.9, 30.0, 29.5, 40.6, 50.8, 104.8, 110.6, 120.9, 123.8, 125.3, 125.8, 126.6, 128.8, 129.5, 129.2, 134.5, 136.2, 138.4, 148.0, 148.2, 151.6, 193.8. MS: M+1 = 398.

1-(4-Methoxyphenyl)-3,7,7-trimethyl-4-phenyl-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4e)

Yield 90 %, m.p. >220 °C. 1H NMR (400 MHz, DMSO-d6) δ = 0.9 (s, 3H, CH3), 1.0 (s, 3H, CH3), 1.8-2.0 (d, 4H, CH2), 5.0 (s, 1H, CH), 7.0-8.0 (m, 9H, CH3), 11.9, 26.9, 27.7, 28.8, 31.8, 34.4, 47.6, 50.4, 54.8, 104.6, 110.0, 113.2, 120.5, 123.5, 126.2, 126.9, 129.8, 129.9, 130.3, 136.2, 138.3, 139.5, 145.3, 151.5, 156.9, 194.2. MS: M+1 = 414.

1-(4-Fluorophenyl)-3,7,7-trimethyl-4-phenyl-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4f)

Yield 90 %, m.p. >220 °C. 1H NMR (400 MHz, DMSO-d6) δ = 0.9 (s, 3H, CH3), 1.0 (s, 3H, CH3), 1.8-2.0 (d, 4H, CH2), 5.0 (s, 1H, CH), 7.0-8.0 (m, 9H, CH3), 11.9, 26.9, 27.7, 28.8, 31.8, 34.4, 47.6, 50.4, 54.8, 104.6, 110.0, 113.2, 120.5, 123.5, 126.2, 126.9, 129.8, 129.9, 130.3, 136.2, 138.3, 139.5, 145.3, 151.5, 156.9, 194.2. MS: M+1 = 414.

1-(4-Chlorophenyl)-3,7,7-trimethyl-4-phenyl-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4h)

Yield 90 %, m.p. 176-178 °C. 1H NMR (400 MHz, DMSO-d6) δ = 0.9 (s, 3H, CH3), 1.0 (s, 3H, CH3), 1.9 (s, 3H, CH3), 2.1-2.3 (d, 4H, CH2), 5.0 (s, 1H, CH), 6.5 (s, 1H, NH), 7.0-8.0 (m, 9H, Ar-H). 13C NMR (100 MHz, DMSO-d6) δ = 12.2, 27.3, 28.9, 30.0, 32.5, 42.5, 50.8, 104.3, 111.9, 121.1, 124.5, 127.5, 129.3, 129.5, 129.9, 131.2, 135.6, 137.5, 144.1, 147.5, 148.9, 195.2. MS: M+ = 417, M+2 = 419.

1-(4-Fluorophenyl)-3,7,7-trimethyl-4-phenyl-6,7,8,9-tetrahydro-1H-quinolin-5(4H)-one (4i)

Yield 90 %, m.p. >220 °C. 1H NMR (400 MHz, DMSO-d6) δ = 0.9 (s, 3H, CH3), 1.0 (s, 3H, CH3), 1.9 (s, 3H, CH3), 2.1-2.3 (d, 4H, CH2), 5.0 (s, 1H, CH), 6.5 (s, 1H, NH), 7.0-8.0 (m, 9H, Ar-H). 13C NMR (100 MHz, DMSO-d6) δ = 12.1, 27.3, 28.9, 30.0, 32.5, 42.5, 50.8, 104.5, 111.9, 121.0, 124.5, 127.5, 129.5, 129.9, 131.2, 135.6, 137.5, 144.1, 147.5, 148.9, 195.2. MS: M+1 = 417, M+2 = 419.
CONCLUSION

In summary, we have developed a simple and efficient green protocol for the synthesis of pyrazole-conjugated tetrahydroquinoline derivatives using 3-methyl-1-phenyl-1H-pyrazol-5-amine (1), 5,5-dimethylcyclohexane-1,3-dione (2) and benzaldehydes (3) as synths by exploiting the eco-friendly characteristic of glycerol as green reaction medium. These pyrazole conjugates have exhibited potential cytotoxic activity on breast cancer (MCF-7) and liver cancer (A549) cell lines. Further, molecular modelling studies gave an understanding about the target protein binding interactions with synthesized ligands. In addition, pharmacokinetic properties that were predicted using SwissADME tools gave details of the total polar surface area, BBB, ilogP and GI absorption. The information derived out of these would be helpful for the further structural optimization to get lead like molecules.

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