APPENDIX

LIST OF PUBLICATIONS

1. **Sivalingam Lakshmanan** & Narayanan Ramalakshmi, One-pot, four-component synthesis of benzylpyrazolyl naphthoquinone derivatives and molecular docking studies. *Synthetic Communications*, 2016, 46(24), 2045-2052.


**CONFERENCE**

1. **Oral presentation:** Design, green synthesis and molecular docking of benzopyrazole naphthoquinone derivatives via four component reaction. International Conference on Recent Advance In Material And Chemical Sciences, Gandhigram Rural Institute - Deemed University in Gandhigram

2. **Poster presentation:** A novel Turn on electrochemical & fluorescent chemosensor for the selective detection of Hg$^{2+}$ and their live cell imaging. International Conference on Recent Trends In Analytical Chemistry, University of Madras in Chennai.
Synthesis, molecular docking, DFT calculations and cytotoxicity activity of benzo[g]quinazoline derivatives in choline chloride-urea

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A B S T R A C T
Green and highly efficient one-pot three component approach for the synthesis of benzo[g]quinazoline derivatives (6a–g) using Choline chloride-urea (DES). Synthesized compounds 6b and 6g showed the most potent biological activity against A549 lung cancer cell line. Docking simulation was performed to position compounds 6b and 6g showed the greater affinity for anaplastic lymphoma kinase (ALK) receptor. Quantum chemical studies were carried out on these compounds to understand the structural features essential for activity using DFT/6-31G level of theory.

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1. Introduction

Cancer is one of the important contributors to GLOBOCAN 2012 estimation, about 14.1 million cancer cases and 8.2 million cancer deaths are estimated to have occurred worldwide in 2012 [1]. Lung cancer is a leading cause of cancer death accounting for approximately 26% of all female and 28% of all male cancer deaths in 2013 [2], with non-small cell lung cancer (NSCLC) accounting for 75–85% of lung cancer [3]. Most of the cancer is typically silent in early stages and incurable in late stages of lung cancer [4]. Non–small-cell lung cancer (NSCLC) and led to the development of tyrosine kinase inhibitors (TKIs), in what is now commonly referred to as precision oncology [5,6]. Rearrangements, either inversions or translocations, characterize the genomic changes involving anaplastic lymphoma kinase (ALK) that are observed in NSCLC [7,8]. A variety of ALK inhibitors has been developed and examined in clinical trials, such as alectinib, ceritinib, crizotinib. Among them, crizotinib (Xalkori) was the first small molecule inhibitor which was approved as a treatment of NSCLC including ALK fusion gene, EML4-ALK by FDA in 2011 [9,10]. Although crizotinib was very efficient for the treatment of ALK-positive NSCLC harbouring ALK rearrangements [11–14]. However, despite a high response rate of 60% in ALK-rearranged NSCLC, most patients develop resistance to crizotinib, typically within one to two years.

National cancer Institute has identified the quinone moiety as an important pharmacophoric element for cytotoxic activity. Naphthoquinones (NQs) have been the subject of much research owing to their pharmacological activities [15–17], anti-allergic [18], antibacterial [19], anti-fungal [20,21], anti-viral [22], apoptosis [23], anti-proliferative [24] and anticancer activity of 1,4-naphthoquinones [25–27]. The continuation of our synthetic efforts to develop bio-logically important of 1,4-naphthoquinones derivatives [28].

Recently, the synthesis of benzo[g]quinazoline derivatives has been reported [29]. In addition, derivatives of benzo[g]quinazoline have occupied a prominent position in medicinal chemistry, because of their well-known wide range of applications which exhibited significant pharmacological effects, such as antimicrobial, antitumor, antimalarial, anti-inflammatory, anticancer, and anti-viral activities among others [30]. A number of 1,4-naphthoquinone derivatives having nitrogen atom present in them received a great deal of attention for their anticancer activity. Therefore, the development of facile approaches to access these targets with
structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery [31,32]. As part of our effort using DES contribution as a solvent, as the catalyst and as a reactant for highly efficient one-pot three component approach for the synthesis of benzo[g]quinazoline derivatives green and cost-effective procedure with excellent yield, high purity to develop biologically important naphthoquinone derivatives [33].

2. Experimental

All of the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as received. Melting points were measured in open capillary tubes and are uncorrected. The 1H NMR, 13C NMR were recorded on a Bruker (Avance) 400 MHz NMR instrument using DMSO as an internal standard and DMSO as a solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (ppm) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60–80°C) and ethyl acetate as the eluent. Elemental analyses were performed on a Perkin-Elmer 2400 Series II Elemental CHNS analyzer. ESI mass was recorded using a Thermo Fleet-LC mass instrument.

2.1. Deep eutectic solvent preparation (DES)

Choline chloride-urea deep eutectic solvent was prepared according to the literature [34]. For DES preparation, urea (200 mmol) and choline chloride (100 mmol) were mixed, stirred and heated until a clear liquid appeared. The obtained deep eutectic solvent was used without any further purification (Scheme 1).

2.2. General procedure for the synthesis of benzo[g]quinazoline derivatives 6a–g

A mixture of Aromatic aldehyde (1 mmol), 2-hydroxy naphthoquinone (1 mmol) and deep eutectic solvent (1 mL) were added to the round bottom flask with stirring and the mixture was heated at 60°C for 15–30 min. After completion of the reaction, water (10 mL) was added while still hot. The DES being soluble in water comes in the water layer. The solid was separated by filtration and was washed with ethanol-water. The crude products were obtained in high purity after purification by recrystallization from ethanol.

2.2.1. 4-(4-Chlorophenyl)-3,4-dihydrobenzo[g]quinazoline-2,5,10(1H)-trione (6a)

Yellow solid, Yield 92%, M. P: 240–242°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 7.96 (s, 2H), 7.96 (dd, J = 14.9, 7.5 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.62 (t, J = 11.0 Hz, 2H), 5.89 (s, 1H), 5.16 (s, 1H), 13C NMR (75 MHz, DMSO, TMS, δ ppm) δ 183.61, 180.58, 160.72, 155.96, 148.31, 137.91, 135.60, 134.63, 130.01, 128.73, 128.14, 127.84, 126.12, 35.91. ESI-MS (M+1) calculated m/z 347.07 Found 348.97. Anal. Calcld for: C18H11ClN3O3: C, 58.07; H, 3.27; N, 12.03% found: C, 58.05; H, 3.25; N, 12.03%.

2.2.2. 4-(4-(Dimethylamino)phenyl)-3,4-dihydrobenzo[g]quinazoline-2,5,10(1H)-trione (6b)

Yellow solid, Yield 84%, M. P: 180–182°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 9.56 (s, 2H), 7.94 (d, J = 6.4 Hz, 2H), 7.83–7.69 (m, 2H), 7.14 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 6.18 (s, 1H), 2.94 (s, 6H), 13C NMR (75 MHz, DMSO, TMS, δ ppm) δ 189.83, 183.17, 181.89, 155.74, 154.19, 153.32, 126.15, 126.13, 125.92, 125.86, 127.07, 125.88, 124.56, 123.14, 111.05, 42.22, 35.69. ESI-MS (M+1) calculated m/z 347.13 Found 348.97. Anal. Calcld for: C20H17N3O3: C, 61.87; H, 3.10; N, 12.09%.

2.2.3. 4-Dihydro-4-(4-nitrophenyl)benzo[g]quinazoline-2,5,10(1H)-trione (6c)

Yellow solid, Yield 85%, M. P: 246–248°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 9.79 (s, 2H), 7.96 (dd, J = 18.2, 7.0 Hz, 2H), 7.82 (dd, J = 14.9, 7.5 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.62 (t, J = 11.0 Hz, 2H), 5.89 (s, 1H), 5.16 (s, 1H), 13C NMR (75 MHz, DMSO, TMS, δ ppm) δ 183.61, 180.58, 160.72, 155.96, 148.31, 137.91, 135.60, 134.63, 130.01, 128.73, 128.14, 127.84, 127.42, 127.40, 126.12, 35.91. ESI-MS (M+1) calculated m/z 349.07 Found 350.62. Anal. Calcld for: C20H17N3O3: C, 61.89; H, 3.17; N, 12.03% found: C, 61.87; H, 3.10; N, 12.03%.

2.2.4. 4-Dihydro-4-(4-hydroxyphenyl)benzo[g]quinazoline-2,5,10(1H)-trione (6d)

Yellow solid, Yield 90%, M. P: 256–258°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 10.96 (s, 2H), 10.21 (s, 2H), 8.27–7.64 (m, 4H), 7.44 (dd, J = 28.3, 16.0 Hz, 2H), 7.26 (s, 1H), 5.74 (s, 1H), 13C NMR (75 MHz, DMSO, TMS, δ ppm) δ 183.46, 181.32, 155.71, 148.16, 137.42, 133.61, 130.91, 128.73, 128.14, 127.42, 127.40, 126.02, 35.89. ESI-MS (M+1) calculated m/z 347.13 Found 349.07. Anal. Calcld for: C18H11N3O4: C, 61.89; H, 3.17; N, 12.03% found: C, 61.87; H, 3.10; N, 12.03%.

2.2.5. 4-(4-Bromophenyl)-3,4-dihydrobenzo[g]quinazoline-2,5,10(1H)-trione (6e)

Yellow solid, Yield 85%, M. P: 238–240°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 10.00 (s, 2H), 7.95 (dd, J = 11.5, 3.9 Hz, 2H), 7.86–7.80 (m, 2H), 7.36 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 6.15 (s, 1H). ESI-MS (M+1) calculated m/z 382.20 Found 383.76. Anal. Calcld for: C18H13BrN2O2: C, 56.42; H, 2.89; N, 7.31%.

2.2.6. 4-(5-Bromo-2-hydroxy-3-methoxyphenyl)-3,4-dihydrobenzo[g]quinazoline-2,5,10(1H)-trione (6f)

Yellow solid, Yield 88%, M. P: 248–250°C 1H NMR (400 MHz, DMSO, TMS, δ ppm) δ 10.45 (s, 1H), 10.23 (s, 2H), 8.00–7.80 (m, 2H), 7.41–7.29 (m, 2H), 7.18 (s, 1H), 6.81 (s, 1H), 5.71 (s, 1H), 3.88 (s, 3H). ESI-MS (M+1) calculated m/z 428.22 Found 429.76. Anal. Calcld for: C19H13BrN2O2: C, 53.17; H, 3.05; N, 6.53%.

Scheme 1. Deep eutectic solvent preparation from Choline Chloride and urea.
2.2.7. 3, 4-Dihydro-4-(3,4-dimethoxyphenyl)benz[g]quinazoline-2,5,10(1H)-trione (6g)

Yellow solid, Yield 92%, M. P: 216–218 °C 1H NMR (400 MHz, DMSO, TMS, δ ppm) 6.984 (s, 2H), 7.95 (dd, J = 8.6, 2.8 Hz, 2H), 7.79 (dd, J = 14.0, 7.4 Hz, 2H), 6.83 (s, 1H), 6.74 (t, J = 10.3 Hz, 2H), 6.03 (s, 1H), 3.70 (s, 3H), 3.62 (s, 3H), ESI-MS (M+1) calculated m/z 364.11 Found 365.10. Anal. Calcd for: C20H16N2O5: C, 65.93; H, 4.43; N, 7.89.

2.3. Molecular docking

To investigate the potential binding mode of inhibitors, all the compounds were subjected to molecular docking using the AUTODOCK 1.5.6 docking program. Because of the critical roles of aberrant Signaling in cancer, anaplastic lymphoma kinase (ALK) receptor is an attractive oncology target for therapeutic intervention. To this end, the X-ray crystal structure of ALK in complex with crizotinib was downloaded from the protein data bank (PDBID: 2XP2) and was used for the docking study. Ligand 2D structures were drawn using ChemDraw Ultra 8.0. Chem3D Ultra 8.0 was used to convert the 2D structure into 3D and the energy minimized using the semi-empirical MM2 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .pdb file format for input to Auto Dock-Tools (ADT) version 1.5.6. All the ligand structures were then saved in PDBQT file format, for input into AUTODOCK version 4.2.

2.4. Biological evolution

2.4.1. Cytotoxicity activity

The human lung (A459) cell lines were purchased from the National Centre for Cell Science (Pune, India). Cell culture protocols method by following the procedure reported by Leland Booth et al. [35]. The medium was invigorated each two days. The cell growth medium was after that hatches underneath the humidified climate (CO₂) at RT. The specimen’s in assessments were sterilized in a castrate (autoclave) at 120 °C throughout 120 min and located in 96 well cell growth platters. The cytotoxicity towards human lung (A459) cancer cells was analyzed using drugs 5-FU as a control as-synthesized compounds 6a–g with the 3-(4,5-dimethilthiazol-2-yl) –2, 5-diphenyltetrazolium bromide (MTT) examine and Flou-rouracil as reference drug. Testing was carried out in 96-well cell culture plate (2 × 10⁴ cells/well). Subsequently, culture plates were incubated for one day with 50 μM of as-synthesized compounds 6a–g. After that, MTT solution was supplementary on culture well. Following one day incubation, the culture medium restraints unreached MTT was detached charily and calculated at optical microscope reader (Stat Fax-2100, AWARENESS, Palm City, USA) (570 nm).

2.4.2. Apoptosis analysis of A549 cells

To study the effect of MHT on the cytoskeletal organization of cells and to study the mechanisms of cell death induced by Confocal microscopy analysis of the cancer cells treated with targeted compounds 6b and 6g were performed. A549 cells were cultured on glass-base dishes for 24 h prior to treatment with the 10 μM concentration of compounds 6b and 6g. After treatment, the cells were washed three times with PBS to remove unbound with compounds 6b and 6g. The cells were stained with tubulin and actin markers and were viewed under a confocal microscope (Olympus IX81 in DU897 mode) using a 100 × oil objective and 488/561 nm excitation. Smash up to the cytoskeleton as a result of apoptosis, which was instigated by 6b and 6g was additionally examined by confocal microscopy in A549 cells.

3. Results and Discussion

3.1. Chemistry

The synthesis of benzog[quinazoline derivatives 6a–g was performed by Biginelli reaction. Initially, the 2-hydroxy naphthoquinone (1 mmol), Aromatic aldehyde (1 mmol) and deep eutectic solvent (DES) (2 mmol) was chosen as a catalyst as well as reaction medium and as the reactant for the Biginelli reaction to obtained good yield (Scheme 2). To our desire to a synthesis of benzog[quinazoline derivatives increase the capability is relatively inexpensive and recyclable and green methods. A probable mechanism for the formation of products 6a is the Knoevenagel reaction between 2-hydroxy naphthoquinone and aromatic aldehyde to form the Knoevenagel product I. The intermediate I undergoes nucleophilic addition of urea as a Choline chloride-urea and followed by cyclization and dehydration afforded benzog[quinazoline derivatives (Scheme 3). After addition of water, the DES comes in the water layer.

3.2. Molecular docking

For the molecular docking study, protein structure was obtained from the Protein Data Bank: the ALK structure PDB ID was 2XP2. The co-crystallized ligand (crizotinib) in the ALK structure was removed. For the protein structure, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.6. Further ADT was used to remove crystal water, added Gasteiger charges to each atom, and adding polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen-donor angle was not less than 120°. The structures were then saved in PDBQT file format, for input into AUTODOCK version 1.5.4. A grid box with the dimension of 60 × 60 × 60 Å with 0.826 spacing and centred on 38.083, 46.914, 17.64 was created around the binding site of crizotinib on ALK protein using Auto Dock Tools. The centre of the box was set at crizotinib and grid energy calculations were carried out. For the AUTODOCK docking calculation, default parameters were used and 10 docked conformations were generated for each compound, the energy calculations were done using genetic algorithms. Docking of different ligands to protein was performed using AUTODOCK, same protocols used in as that of the validation study. All docking were taken into 2.5 million energy evaluations were performed for each of the test molecules. Docked ligand conformations were analyzed in terms of energy, hydrogen bonding, and hydrophobic interaction between ligand and receptor protein ALK. Detailed analyses of the ligand–receptor interactions were carried out, and final co-ordinates of the ligand and receptor were saved as pdb files. Docked structures were visualized using Discovery Studio Visualizer 2.5 (Accelrys Software Inc.). Synthesized compounds 6b, 6c, and 6g show very high binding energy with the ALK (2xp2) receptor. The small group substitution like CH₃ exhibit very high binding energy value –7.96 kcal/mol exhibited H-bonding with Cys1097, Tyr1096, Ala1274, Tyr1278 which results from six hydrogen bonds of crizotinib with Asp1349, Pro1331, Tyr1327 and Ala1148, Leu1256 (Fig. 1c).The moderate active compound 6f shows three H-bonding with Asp1349, Pro1331, Tyr1327 and...
The order of binding affinity of docked benzog[quinazoline Derivatives against the ALK receptor is $6_b > 6_e > 6_g > 6_a > 6_c > 6_f > 6_d$ with the range of binding energy being $-7.96$ to $-6.21$ kcal/mol. The free energy of binding (FEB) of all compounds was calculated (Table 1).

3.3. Density functional theory

Theoretical calculations were performed by the density functional theory (DFT) method at the B3LYP/6-31G level of theory in the Gaussian 03 package of programs [36]. According to the frontier molecular orbital theory, (HOMO) has the priority to provide electrons while LUMO can accept electrons first are the most important factors that affect the bioactivity [37]. Higher HOMO energy and lower LUMO energy in the drug molecule result in larger stabilizing interactions and hence, binding with the receptor.

It is incredible that compounds $6_b$ having the lowest LUMO energy value ($-3.38$) are more capable to accept electrons than those with higher energy LUMO and thus will show higher activity as

![Diagram of the synthesis of benzo[g]quinazoline derivatives (6a-g).]

Scheme 2. Synthesis of benzo[g]quinazoline derivatives (6a-g).

Scheme 3. The Knoevenagel and Biginelli mechanism for the benzo[g]quinazoline derivatives using DES.
compared to all other compounds. However, the energy gap (0.904) of this compound is also found lowest than the others; exhibit the highest cytotoxic activity. We also obtained a plot of the HOMO and LUMO of the molecules of each group to analyze the main atomic contributions for these orbitals. The importance of observing these plots was to determine which atoms were located at the possible sites of electronic transfer between the molecule under study and its biological target. The results illustrate that HOMO lobes are spread mainly over substituted aromatic rings; In contrast, the LUMO lobes are almost homogeneously spread over naphthoquinone moiety. The plots of the HOMO and LUMO of most active compounds 6b and 6g are articulate in (Fig. 2). The orbital energies of both HOMO and LUMO and their gaps were calculated for all the molecules and are reported in (Table 2). Molecular electrostatic potential (MEP) A molecule of the electrostatic potential map provides information about the electron acceptor and electron donor regions. The different values of the MEP at the surface are represented by different colours, red represents regions of most electro negative electrostatic potential, oxygen and nitrogen atoms have more negative charges. Most likely this region as recognized the docking results oxygen and nitrogen atoms have some interaction with the hydrogen bond receptor. The blue represents regions of most positive electrostatic potential were mainly distributed over the aromatic group which result from hydrophobic interaction (Fig. 3).

3.4. Biological evolution

3.4.1. Cytotoxicity activity

Among the compounds, 6b, 6e and 6g were found to have a high anticancer activity against A549 cells whereas other compounds had not so much effect at 50 μM concentration. The majority of compounds at 10 and 50 μM exhibited anti-proliferation effects on A549 cells. Consequently, the benzo[g]quinazoline derivatives suppressed the growth of A549 cells in dose. Moreover, compounds 6b, 6e and 6g exhibited more significant cytotoxicity and the inhibitory rate was almost up to 60–70% equivalent to 5-FU. Our finding demonstrated that all tested derivatives exhibited considerable cell growth inhibition at different concentrations (10, 25 and 50 μM) in which compounds 6b, 6e and 6g exerted optimal results and the others modest anti proliferation efficiency (Fig. 4), there by IC50 of the compounds were totally less than 50 μM (Table 1). Therefore, we selected 10 and 50 μM concentration for the following investigation according to the results of cell viability. Percent of cell viability was calculated and the inhibition of growth of human lung cancer cell line is defined by the nature of the substituent. The compounds having electron donating of methoxy

Table 1. IC50 and FEB values of compounds 6a–g.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (μM)</th>
<th>FEB (kcal/mol)</th>
<th>No. of H Bonds</th>
</tr>
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<tbody>
<tr>
<td>6a</td>
<td>23.03 ± 2.02</td>
<td>−7.24</td>
<td>2</td>
</tr>
<tr>
<td>6b</td>
<td>16.08 ± 0.06</td>
<td>−7.96</td>
<td>5</td>
</tr>
<tr>
<td>6c</td>
<td>24.29 ± 1.61</td>
<td>−6.95</td>
<td>2</td>
</tr>
<tr>
<td>6d</td>
<td>32.51 ± 2.19</td>
<td>−6.21</td>
<td>5</td>
</tr>
<tr>
<td>6e</td>
<td>19.63 ± 1.86</td>
<td>−7.38</td>
<td>3</td>
</tr>
<tr>
<td>6f</td>
<td>21.49 ± 2.37</td>
<td>−6.47</td>
<td>4</td>
</tr>
<tr>
<td>6g</td>
<td>19.26 ± 1.91</td>
<td>−7.62</td>
<td>6</td>
</tr>
<tr>
<td>5-FU</td>
<td>10.42 ± 0.01</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Free energy binding.

Fluorouracil used as a positive control.

Fig. 1. (a) and (b) Binding mode of the most active compound 6b, 6g with ALK receptor; (c) Binding mode of least active compound 6d with ALK receptor, (d) Binding mode of moderate active compound 6e with ALK receptor. The amino acids involved in hydrogen (blue dashed line), hydrophobic (white dashed line) interactions are highlighted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
and methyl group 6b and 6g exhibited significant cytotoxic activity with IC50 values having 16.08 and 19.26 μM against promising therapeutic agents for A549 lung adenocarcinoma cancer cell line.

3.4.2. Apoptosis analysis of A549 cells

The results observed that the inflexibility and the structure of actin and tubulin filaments were totally lost when the cells were treated with the as-synthesized compounds 6b and 6g (Fig. 5). Most of the cells showed a contracted morphology with disintegrated actin filaments. The disturbance of the actin and tubulin cytoskeleton is another real marker of the impelling of apoptosis, which prompts the demise of cells [44]. The cells were completely withered and exhibited horseshoe-shaped nuclei, which are unmistakable of apoptotic cell death.

4. Conclusion

Taken together, our investigation validated the in vitro therapeutic effect of benzo[g]quinazoline derivatives on human lungs cancer A549 cell line, indicating that benzo[g]quinazoline...
derivatives are a possible therapeutic drug for lungs cancer. Furthermore, benzo[g]quinazoline derivatives can inhibit A549 cancer cells growth through anti-proliferation and induce apoptosis. Results from molecular docking and biological activity of compounds 6b, 6e and 6g had bound well with the receptor of ALK active site also most potent anticancer efficiency. This is conceivable because nitrogen and oxygen atoms have higher negative charges (Electrostatic potential) in benzo[g]quinazoline derivatives. However, it will be valuable and necessary for the further consideration to explain the determined mechanism and study the effect in vivo.

Acknowledgment

Authors have no financial interest.

References

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One-pot, four-component synthesis of benzylpyrazolyl naphthoquinone derivatives and molecular docking studies

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One-pot, four-component synthesis of benzylpyrazolyl naphthoquinone derivatives and molecular docking studies

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ABSTRACT
A highly efficient, green, one-pot, four-component approach for the synthesis of benzylpyrazolyl naphthoquinone derivatives (5a–p) have been developed by the domino reaction of 2-hydroxy naphthoquinone, aromatic aldehyde, ethyl acetoacetate, and phenyl hydrazine derivatives in water and employed p-toluene sulfonic acid (p-TSA) as the right choice of catalyst at reflux. Docking simulation was performed to position compounds 5a, 5b, and 5g into the anaplastic lymphoma kinase (ALK) structure active site to determine the probable binding model.

GRAPHICAL ABSTRACT

ARTICLE HISTORY
Received 27 July 2016

KEYWORDS
2-Hydroxy naphthoquinone; molecular docking; one-pot synthesis

Introduction
Today, significant aspects of modern organic synthesis are necessary to develop methods with a low environmental impact. Multicomponent reactions (MCRs) have proven to be vital assets in organic and medicinal chemistry because of their simplicity, efficiency, and high selectivity. The development of organic reactions in water has become highly desirable in recent years to meet green considerations. MCRs have been developed based on the Knoevenagel reaction. Because Knoevenagel condensation can be conducted under conditions that are compatible with aqueous environments, it has often been involved in developing MCRs in water. Naphthoquinone represents an important class of biologically active molecules that are widespread in nature. It is also established that these compounds also exhibit a wide range of biological activities because of their abundance in medicinal scaffolds (namely, α-lapachone, β-lapachone, lapachol, and atovaquone). Many natural and synthetic naphthoquinones are known to be potent...
Pyrazolones are also important structural cores in many pharmaceutical industries in view of their medicinal activities. Heterocyclic nucleus containing pyrazolones (phenazone, propyphenazone, ampyrome, and metamizole) are useful antipyretic and analgesic drugs, while edaravone (MCI-186) has been used for treating brain and myocardial ischemia and is also used for treating diseases related to these enzymes, such as bone loss, cancer, and other proliferative diseases because of its antimicrobial, antibacterial, anti-inflammatory, and anticancer activities.

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) belonging to the insulin receptor superfamily, which includes insulin-like growth factor-1 receptor (IGF-1R) and leukocyte tyrosine kinase (LTK). The function of ALK in the normal human body is unclear. A possible role of ALK is in physiological development, and its expression in normal tissues is very restricted to the central nervous system. Lung cancers with ALK rearrangements are highly sensitive to ALK tyrosine kinase inhibition, further underscoring the notion that such cancers are addicted to ALK kinase activity. Therefore, the selective inhibition of ALK emerged as an attractive target for cancer therapies. For this reason, it would be of interest to evaluate the synthetic derivatives of this compound for their pharmacologic actions, which also in the future could potentially be used to treat disease.

Recently, the syntheses of naphthoquinone derivative have been reported. As part of our effort to develop biologically important benzylpyrazolyl naphthoquinone by a new synthetic method we here report a four-component approach because it makes the synthesis of benzylpyrazolyl naphthoquinone.

Table 1. Substrate scope for the synthesis of benzylpyrazolyl naphthoquinone (Sₐ–p).

<table>
<thead>
<tr>
<th>Entry</th>
<th>R₁</th>
<th>R₂</th>
<th>Products</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4-ClC₆H₄</td>
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Scheme 1. Synthesis of benzylpyrazolyl naphthoquinone.
modification of the pyrazolone nucleus in future development and production of new naphthoquinone derivatives (e.g., hydrazine 4, R2-substituted, Table 1, entry 16) easier. It was found that in the presence of p-toluene sulfonic acid (p-TSA), with water as a solvent, this is a green and cost-effective procedure with excellent yield and high purity (Scheme 1).

Results and discussion

The optimization of the reaction was studied by series of catalysts and solvents as well as under catalyst-free conditions with the hope to maximize the product yield in short reaction times. Initially, 2-hydroxy naphthoquinone 1, benzaldehyde 2, ethyl acetoacetate 3, and phenyl hydrazine 4 were refluxed in the presence of H2O, acetonitrile, chloroform, and ethanol as the solvent without any catalyst; however, the reaction even after 24 h failed to furnish any product. Inspired by this result, we have shifted our attention toward the optimization of the reaction conditions using various catalysts for the same model reaction using different solvents under reflux conditions. We then applied p-TSA (10 mol%) as catalyst in water and ultimately achieved satisfaction because the reaction proceed well, purveying the desired product in 84% yield within 20 min. p-TSA played a key and amazing catalytic role in this particular MCR compared with other organic acids applied, which can be attributed to its mild Brønsted acid. When chloroform, ethanol, and acetonitrile were used as the solvent, the product was formed in poor yields. It was evident that water showed superiority over other solvents and the yield of the desired product (5) was 84%. Therefore, water was chosen as the solvent for this reaction as the maximum yield. Hence, these optimized conditions were applied for all the experiments, taking equimolar amounts of substituted 2-hydroxy naphthoquinone (1.0 mol%), aromatic aldehyde (1.0 mol%), ethyl acetoacetate (1.0 mol%), and hydrazine (1.0 mol%) substituted under reflux conditions in the presence of 10 mol% p-TSA in water at 70 °C (Scheme 1), which afforded a library of benzylpyrazolyl naphthoquinone derivatives in good to excellent yields (70–84%). After optimizing the reaction conditions, a variety of aromatic aldehydes was employed under similar circumstances to evaluate the substrate scope of this reaction. The results are shown in Table 1. As expected from our original results, these reactions proceeded very cleanly at reflux in water and no undesirable side reactions were observed. Its agreement with green chemistry protocols make it a useful and attractive process for the synthesis of benzylpyrazolyl naphthoquinone derivatives.

The purity of the product was high enough for spectroscopic analysis without any further purification. It is a one-pot, multicomponent reaction, offering only water as the by-product after completion of the reaction, with tautomerisation. Pyrazolone could exist in three tautomeric forms (viz., the CH, OH, and NH forms; Fig. 1), but we observed that
tautomerisation occurred only in the NH form, which was established through $^1$H NMR and HRMS study. We conclude that the reaction is very product-selective, affording only benzylpyrazolylnaphthoquinone (5); bis-naphthoquinone (i), bis-pyrazolone (ii), and their annulated products (iii and iv) (Fig. 2) were not observed at all in this reaction.

The synthetic strategies adopted to obtain the target compounds are depicted in Scheme S2 (Supplementary Material). The four-component reaction seems to proceed following the mechanistic pathway. Initially, aryl hydrazine/hydrazine hydrate is reacted with ethylacetoacetate to generate the pyrazolone ring (I). Additionally, p-TSA catalyzed the Knoevenagel reaction between 2-hydroxy naphthoquinone and aromatic aldehyde to form the Knoevenagel product (II). Subsequently, during the Michael addition step, nucleophilic attack on II by the tautomeric form of pyrazolone (I) afforded the desired

Figure 2. Bis-naphthoquinone (i), bis-pyrazolone (ii), and their annulated products (iii and iv).

Figure 3. (a) Binding mode of the most active compound 5g with ALK receptor, (b) binding mode of intermediate active compound 5k with ALK receptor, (c) binding mode of least active compound 5m with ALK receptor, and (d) binding mode of moderately active compound 5i with ALK receptor. The amino acids involved in hydrogen (blue dashed line), hydrophobic (yellow dashed line), and halogen (red dashed line) interactions are highlighted.
product (5) via intermediate III. Alternatively, there is another possible reaction pathway for the reaction, via the formation of intermediate IV followed by reaction with 2-hydroxy naphthoquinone to afford benzylpyrazolyl naphthoquinone (5).

**Molecular docking**

Among all the compounds docked, 5a, 5b, 5c, and 5g show very high binding energy with the ALK receptor. The results exposed that compound 5g as the most active with a calculated binding energy of −9.56 kcal/mol compared with all the compounds docked with ALK receptors and interacts with five amino acids, namely, Ala1148, Val1130, Leu1256, Leu1122, and Glu1210 (Fig. 3a). The least binding energy was exhibited by compound 5m with a binding energy of −6.18 kcal/mol, which interacts with five amino acids, namely, His1244, Arg1248, Ile1246, Ala1274, and Phe1245 (Fig. 3b). The intermediary active compound 5k showed −7.54 kcal/mol of binding energy and interacts with three amino acids, namely, Ala1274, Ile1246, Phe1245, and His1244 (Fig. 3c). The moderately active compound 5i has a binding energy value of −7.80 kcal/mol, and it interacts with Ile1240, Arg1248, Ala1247, and Tyr1278 (Fig. 3d). The number of hydrogen bonds vary from zero to four with the receptor molecule that show other interactions like electrostatic and hydrophobic, which are responsible for forming strong bonds between ligands and receptor ALK (2XP2.pdb). The order of binding affinity of docked benzylpyrazolyl naphthoquinone derivatives against the ALK receptor is $5g > 5a > 5b > 5c > 5d > 5e > 5i > 5k > 5p > 5j > 5h > 5o > 5n > 5f > 5l$ and 5m with the range of binding energy being −9.56 to −6.18 kcal/mol (Table 2).

**Experimental**

All of the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as received. Melting points were measured in open capillary tubes and are uncorrected. The $^1$H NMR and $^{13}$C NMR were recorded on a Bruker (Avance) 300-MHz NMR instrument using tetramethylsilane (TMS) as an internal standard and

<table>
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<th>Compound</th>
<th>Binding energy (kcal/mol)</th>
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<tr>
<td>5b</td>
<td>−9.05</td>
<td>3</td>
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<td>5c</td>
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<tr>
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Table 2. Docking results of novel benzylpyrazol naphthoquinone derivatives with anaplastic lymphoma kinase (ALK) [2XP2.pdb] protein.
dimethylsulfoxide (DMSO) as a solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (δ scale) and the coupling constants are given in hertz (Hz). Silica-gel-G plates (Merck) were used for thin-layer chromatography (TLC) analysis with a mixture of petroleum ether (60–80 °C) and ethyl acetate as the eluent. Elemental analyses were performed on a Perkin-Elmer 2400 Series II Elemental CHNS analyzer. Electrospray ionization (ESI) mass was recorded using a Thermo Fleet-LC mass instrument.

**General procedure for the synthesis of compounds 5j**

To a mixture of 10 mol% p-TSA and 5 ml water, 2-hydroxy naphthoquinone 1 (1 mmol), P-hydroxybenzaldehyde 2 (1 mmol), ethyl acetoacetate 3 (1 mmol), and hydrazine 4 (1 mmol) were added and heated to reflux at 70 °C. The resulting clear solution, which gradually became turbid, was stirred for the stipulated time of 20 min. After completion of the reaction (indicated by TLC), the free-flowing solid was filtered and washed with ethanol (10 ml) to afford the desired products.

**2-((2,3-Dihydro-5-methyl-3-oxo-2-phenyl-1H-pyrazol-4-yl)(4-hydroxyphenyl)methyl)-3-hydroxynaphthalene-1,4-dione (5j)**

Dark green solid; mp 243–245 °C; 1H NMR (300 MHz, DMSO, δ ppm): δ 9.20 (s, 1H), 7.96 (dd, J = 19.1, 7.2 Hz, 2H), 7.93 (d, J = 7.0 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.46 (s, 1H), 7.30–7.16 (m, 2H), 6.97 (d, J = 8.3 Hz, 2H), 6.67–6.59 (m, 2H), 5.71 (s, 1H), 4.98 (s, 1H), 2.22 (s, 3H). 13C NMR (75 MHz, DMSO, TMS, δ ppm): δ 183.72, 181.90, 158.88, 152.19, 147.28, 141.91, 135.96, 134.60, 133.46, 131.96, 130.67, 129.39, 126.42, 125.90, 122.72, 120.58, 110.58, 107.18, 103.64, 28.23, 10.94. ESI-MS (M^+ + 1) calculated m/z 452.14. Found 452.16. Anal. calcd. for C_{27}H_{20}N_{2}O_{5}: C, 71.67; H, 4.46; N, 6.19%. Found: C, 71.69; H, 4.49; N, 6.21%.

**Conclusion**

In summary, we synthesized a series of heterocyclic benzylpyrazolyl naphthoquinone derivatives (5a–p) via one-pot, four-component reaction. This approach offers the advantages of clean and simple methodology, mild conditions, short reaction times, no column chromatography, low environmental impact, easy workup, and good yields. Molecular docking model was performed to position all the synthesized compounds into the ALK, and the results showed compounds 5a, 5b, and 5g could bind well with the ALK active site.

**References**


Synthesis, Quantum Chemical Studies and Cytotoxicity Activity of Diastereoselective trans-2,3-dihydronaphtho[2,3-b]furan Derivatives

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Post-Graduate and Research Department of Chemistry, Presidency College, Chennai-05, Tamil Nadu, India

ABSTRACT

A green, one-pot three component approach for the synthesis of diastereoselective trans-2,3-dihydronaphtho[2,3-b]furan derivatives (4a-g). Synthesized compounds were evaluated for their anticancer activity against A549 human lung adenocarcinoma cancer cell line. Among all the tested Compounds 4b and 4c showed the most potent biological activity against A549 lung cancer cell line. Docking simulation was performed to position compounds 4b and 4c showed greater affinity for anaplastic lymphoma kinase (ALK) receptor. Quantum chemical studies were carried out on these compounds to understand the structural features essential for activity using DFT/6-31G level of theory.

Keywords: One-pot, 2,3-dihydronaphtho[2,3-b]furan, Cytotoxicity, Molecular docking, DFT

INTRODUCTION

Cancer is one of the important contributors to deaths worldwide, corresponding to almost 1600 deaths per day in the United States. Lung cancer is a leading cause of cancer death accounting for approximately 26% of all female and 28% of all male cancer deaths in 2013 [1]. Among the anti-cancer strategies chemotherapy is available with drugs/chemicals which interfere with cell division, inhibit tumour angiogenesis or induce cancer cell death by various signalling pathways but have potential harm to normal cells [2-4]. However, it should be noted that many types of cancer develop resistance to chemotherapeutic drugs [5]. Therefore, the research of anti-cancer drugs with better activity and fewer side effects is becoming increasingly active.
Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) belonging to the insulin receptor superfamily, which includes insulin-like growth factor-1 receptor (IGF-1R) and leukocyte tyrosine kinase (LTK). A variety of ALK inhibitors have been developed and examined in clinical trials, such as alectinib, ceritinib, crizotinib. Among them, crizotinib (Xalkori) was the first small molecule inhibitor which was approved as a treatment of NSCLC including ALK fusion gene, EML4-ALK by FDA in 2011 [6,7]. Although crizotinib was very efficient for the treatment of ALK-positive NSCLC harboring ALK rearrangements [8,9], acquired drug resistance caused by point-mutations of ALK has been identified in patients treated with crizotinib [10,11]. The most abundant expression of ALK occurs in the neo-natal brain, suggesting a possible role for ALK in early brain development [12]. As a result, the selective inhibition of ALK emerged as an attractive target for cancer therapies [13,14].

Naphthoquinones (NQs) have been the subject of much research owing to their pharmacological activities [15,16], antiallergic [17], antibacterial [18,19], anti-neoplastic [20], antifungal [21], anti-hypoxic [22], antithrombotic [23,24] antiplatelet [25], antiviral [26,27], antiischemic [28], apoptosis [29,30], and Anticancer activity has also been reported for the 1,4-Naphthoquinones [31-34]. As continuation of our synthetic efforts to develop biologically important of 1,4-Naphthoquinones derivatives [35], molecular docking and DFT method is play an significant role in development of drug design [36,37].

MATERIALS AND METHODS

All of the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as received. Melting points were measured in open capillary tubes and are uncorrected. The 1H-NMR, 13C-NMR were recorded on a Bruker (Avance) 300 MHz NMR instrument using TMS as an internal standard and DMSO as a solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60-80°C) and ethyl acetate as the eluent. ESI mass was recorded using a Thermo Fleet-LC mass instrument.

General procedure for the synthesis of 2, 3-dihydronaphtho [2, 3-b]furan-4,9-dione

To a mixture of 10 mol% Et3N, 2-hydroxy-1,4-naphthoquinone (1mmol), Aromatic aldehyde (1mmol) and Phenacyl bromide (1 mmol), N-methyl Imidzolium (1mmol) in water (10 ml) was refluxed for 180 min at 90°C. After completion of the reaction (indicated by TLC), the free-flowing solid was filtered and washed with ethanol (10 ml) to afford the desired products as yellow solid in good yield.

2-(4-bromophenyl)-3-(4-chlorophenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (4a)

Yellow solid, M. P: 242-244°C. 1H NMR (300 MHz, DMSO): δ (ppm) 7.98 -7.50 (m, 4H), 7.42 (d, J=8.6 Hz, 2H), 7.32 (d, J=5.2 Hz, 4H), 5.66 (d, J=5.0 Hz, 1H), 4.57 (d, J=5.2 Hz, 1H); 13C NMR (75 MHz, DMSO, TMS): δ (ppm) 182.61, 179.76, 149.61, 138.27, 131.27, 128.87, 126.14, 124.17, 123.25, 78.73, 52.21, 49.39, 42.12. ESI-MS (M+1); Calcd. for C24H14BrClO3; 465.98. Found 466.52.

2-(4-bromophenyl)-2,3-dihydro-3-(3,4,5-trimethoxyphenyl)naphtho[2,3-b]furan-4,9-dione (4b)

Yellow solid, M. P: 232-234°C. 1H NMR (300 MHz, DMSO): δ (ppm) 7.89-7.44 (m, 4H), 7.11(s, 2H), 7.08-6.98 (m, 4H), 5.66 (d, J=5.1 Hz, 1H), 4.57 (d, J=5.2 Hz, 1H), δ (ppm) 183.71, 179.76, 149.61, 138.27, 131.27, 128.87, 126.14, 124.17, 123.25, 78.73, 52.21, 49.39, 42.12. ESI-MS (M+1); Calcd. for C27H21BrO6; 520.05. Found 521.65.

2-(4-bromophenyl)-3-(4-(dimethylamino)phenyl)-2,3 dihydronaphtho[2,3-b]furan-4,9-dione (4c)

Yellow solid, M. P.: 226-228°C. 1H NMR (300 MHz, DMSO): δ (ppm) 7.85-7.81 (m, 4H), 7.11(s, 2H), 7.08-6.98 (m, 4H), 5.66 (d, J=5.1 Hz, 1H), 4.57 (d, J=5.2 Hz, 1H), 3.66(s, 9H). 13C NMR (75 MHz, DMSO, TMS): δ (ppm) 183.98, 175.24, 154.73, 145.99, 133.92, 133.50, 132.6, 132.13, 131.28, 130.56, 128.56, 120.97, 131.11, 80.95, 55.28, 48.57. ESI-MS (M+1); Calcd. for C26H20BrNO3; 473.06 Found 473.98.

2-(4-bromophenyl)-2,3-dihydro-3-(4-hydroxyphenyl)naphtho[2,3-b]furan-4,9-dione (4d)

Yellow solid, M. P: 214-216°C. 1H NMR (300 MHz, DMSO): δ (ppm) 8.58 (s, 1H), 7.85-7.81 (m, 4H), 7.69-7.68 (d, J=3.6 Hz, 2H), 7.16-7.14 (d, J=8.4 Hz, 2H), 6.75-6.73 (d, J=8.4 Hz, 2H), 6.36-6.34 (d, J=5.4 Hz, 2H), 5.25 (d, J=5.2 Hz, 1H), 4.74 (d, J=5.2 Hz, 1H). 13C NMR (75 MHz, DMSO, TMS): δ (ppm) 183.54, 174.98, 156.63, 152.83, 140.71, 136.25, 135.47, 134.51, 134.16, 130.97, 129.65, 129.02, 122.65, 118.72, 83.91, 54.03. ESI-MS (M+1); Calcd. for...
C_{24}H_{15}BrO_{4}; 446.02 Found 447.38.

3-(5-bromo-2-hydroxyphenyl)-2-(4-bromophenyl)-2,3-dihyronaphtho[2,3-b]furan-4,9-dione (4e)

Yellow solid, M. P: 232-234°C. ¹H NMR (300 MHz, DMSO): δ (ppm) 8.91-8.90 (d, 2H), 7.66 (d, J=4.8 Hz, 2H), 7.14 (d, J=8.6 Hz, 2H), 6.70 (d, J=8.6 Hz, 2H), 6.35 (d, J=5.2 Hz, 2H), 5.24 (d, J=5.8 Hz, 1H), 4.69 (d, J=5.2 Hz, 1H), 2.88 (s, 6H). ¹³C NMR (75 MHz, DMSO, TMS): δ (ppm) 183.02, 177.34, 158.91, 148.47, 130.99, 130.96, 130.71, 130.68, 130.56, 130.21, 127.85, 118.68, 81.76, 52.26. ESI-MS (M+1); Calcd. for C_{24}H_{14}BrO_{4}; 446.02 Found 447.38.

2-(4-bromophenyl)-2,3-dihydro-3-(4-nitrophenyl)naphtho[2,3-b]furan-4,9-dione (4f)

Yellow solid, M. P: 246-248°C. ¹H NMR (300 MHz, DMSO): δ (ppm) 7.84 -7.80 (m, 4H), 7.67-7.66 (d, J=4.8 Hz, 2H), 7.30- 7.28 (d, J=8.7 Hz, 2H), 6.65-6.62 (d, J=8.8 Hz, 2H), 6.36-6.35 (d, J=5.0 Hz, 2H), 5.25 (d, J=5.2 Hz, 1H), 4.69 (d, J=5.2 Hz, 1H). ¹³C NMR (75 MHz, DMSO, TMS): δ (ppm) 183.63, 176.84, 147.21, 139.47, 136.52, 136.49, 135.78, 132.87, 131.43, 126.14, 124.17, 123.25, 121.45, 120.78, 118.23, 79.85, 52.21. ESI-MS (M+1); Calcd. for C_{24}H_{14}BrNO_{5}; 475.01 Found 476.84.

2-(4-bromophenyl)-2,3-dihydro-3-(3,4-dimethoxyphenyl)naphtho[2,3-b]furan-4,9-dione (4g)

Yellow solid, M. P: 228-230°C. ¹H NMR (300 MHz, DMSO): δ (ppm) 7.85-7.79 (m, 4H), 7.66 (d, J=4.8 Hz, 2H), 7.14 (d, J=8.6 Hz, 2H), 6.70 (d, J=8.6 Hz, 2H), 6.65 (s,1H), 5.24 (d, J=5.0 Hz, 1H), 4.69 (d, J=5.2 Hz, 1H), 3.69 (s, 6H). ¹³C NMR (75 MHz, DMSO, TMS): δ (ppm) 182.46, 179.85, 158.32, 144.29, 141.03, 136.84, 133.54, 131.78, 129.87, 128.65, 127.54, 125.77, 124.65, 118.78, 84.45, 59.73, 41.98. ESI-MS (M+1); Calcd. for C_{26}H_{19}BrO_{5}; 490.04 Found 491.65.

Cell culture

The human lung (A459) cell lines were purchased from the National Centre for Cell Science (Pune, India). Cell culture protocols method by following the procedure reported by Leland Booth et al. [38].

Cytotoxicity studies

The Cytotoxicity towards human lung (A459) cancer cells were analyzed using drugs 5-FU as a control as-synthesized compounds 4a-g with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) examine and Fluorouracil as reference drug. Testing was carried out in 96- well cell culture plate (1X10⁴ cells/well). Subsequently, culture plates were incubated for one day with 10 µM of as-synthesized compounds 4a-g. After that, MTT solution was supplementary on culture well. Following one day incubation, the culture medium restrain unreached MTT was detached charily and calculated at optical microscope (570 nm).

Molecular docking

To investigate the potential binding mode of inhibitors, all the compounds were subjected to molecular docking using the AUTODOCK 1.5.6 docking program. Because of the critical roles of aberrant Signalling in cancer, anaplastic lymphoma kinase (ALK) receptor is an attractive oncology target for therapeutic intervention. To this end, the X-ray crystal structure of ALK in complex with crizotinib was downloaded from the protein data bank (PDBID: 2XP2) and was used for the docking study. Ligand 2D structures were drawn using ChemDraw Ultra 8.0. Chem3D Ultra 8.0 was used to convert 2D structure into 3D and the energy minimized using semi-empirical MM2 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .pdb file format for input to Auto Dock-Tools (ADT) version 1.5.6. All the ligand structures were then saved in PDBQT file format, for input into AUTODOCK version 4.2.

For the molecular docking study, protein structure was obtained from the Protein Data Bank; the ALK structure PDB ID was 2XP2. The co-crystallized ligand (crizotinib) in the ALK structure was removed. For the protein structure, all hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.6. Further ADT was used to remove crystal water, added Gagteiger charges to each atom, and merged the non-polar hydrogen atoms to the protein structure. The structures were then saved in PDBQT file format, for input into AUTODOCK version 1.5.4. A grid box with dimension of 60 × 60 60 Å³ with 0.886 spacing and centred on 38.083, 46.914, 17.164 was created around the binding site of crizotinib on ALK protein using Auto Dock Tools. The centre of the box was set at crizotinib and grid energy calculations were carried out. For the AUTODOCK docking calculation, default parameters were used and 10 docked conformations were generated for each compound, the energy calculations were done using genetic algorithms. Docking of different ligands to protein was performed using AUTODOCK, same protocols used in as that of validation study. All docking were taken into...
2.5 million energy evaluations were performed for each of the test molecules. Docked ligand conformations were analyzed in terms of energy, hydrogen bonding, and hydrophobic interaction between ligand and receptor protein ALK. Detailed analyses of the ligand–receptor interactions were carried out, and final coordinates of the ligand and receptor were saved as pdb files. Docked structures were visualized using Discovery Studio Visualizer 2.5 (Accelrys Software Inc.). The free energy of binding (FEB) of all compounds were calculated.

RESULTS AND DISCUSSION

Chemistry

The trans-2,3-dihydronaphtho[2,3-b] furan derivatives were synthesized (Scheme 1, Table 1) as previously reported with some modification [39]. A plausible mechanistic explanation for this one - pot multicomponent reaction starts with a Knoevenagel condensation between 2-hydroxy-1,4-naphthoquinone (1 mm) and substituted aldehyde (1 mm) to form the intermediate. The next step is a Michael addition of Imidazolium ylide with enones affords the zwitterionic intermediate and SN₂ substitution reaction followed by cyclization to affords the stereoselective formation of trans-2,3-dihydronaphtho [2,3-b] furan-4,9-dione titled product. The structures of the prepared titled products were fully characterized by ¹H NMR spectra of 4a, the two protons at 2,3-position of dihydrofuran ring exhibit two doublets at 4.5 and 5.6ppm with the vicinal coupling constant J=4.8 Hz. The similar peak pattern and coupling constant less than 6.0 Hz were also seen in the other ¹H NMR spectra of prepared 2,3-dihydronaphtho[2,3-b]furan-4,9-dione derivatives. It has been established that in cis-2,3-dihydrofuran the vicinal coupling constant of the two methine protons J=7-10 Hz, while in trans-2,3-dihydrofuran vicinal coupling constant J=4-7Hz [40] which result trans-isomer is thermodynamically more stable than cis isomer is also agreement with lower heat of formation, as estimated using DFT/B3LYP:6-31G calculations [41] Table 2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compounds</th>
<th>R₁</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4a</td>
<td>4-CIC₆H₄</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>4b</td>
<td>3,4,5-(OMe)₃C₆H₂</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>4c</td>
<td>4-N(Me)₆C₂H₅</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>4d</td>
<td>4-HOC₆H₄</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>4e</td>
<td>5-Br₂-OHC₆H₃</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>4f</td>
<td>4-NO₂C₆H₄</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>4g</td>
<td>3,4-(OMe)₃C₂H₅</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 2: Physicochemical parameters of compound 2-(4-bromophenyl)-2,3-dihydro-3-(4-nitrophenyl) naphtha [2,3-b]furan-4,9-dione.
Biological Evaluation

Cytotoxicity activity

In the present work, we investigated the biological effects of a series of trans-2,3-dihydronaphtho[2,3-b]furan derivatives to determine their mechanism of action in human lung cancer cell line A549. The cytotoxicity of the as-synthesized compounds (4a-g) evaluated against A549 cells. The compounds 4b, 4c and 4e had obvious cytotoxicity, whereas other compounds had not so much effect at 10 µM concentration. The majority of compounds at 5 and 10 µM exhibited anti-proliferation effects on A549 cells. Consequently, the trans-2,3-dihydronaphtho[2,3-b]furan derivatives suppressed the growth of A549 cells in dose. Moreover, compounds 4b, 4c and 4e exhibited more significant cytotoxicity and the inhibitory rate was almost up to 70-80% equivalent to 5-FU. Our finding demonstrated that all tested derivatives exhibited considerable cell growth inhibition at concentrations 10 µM, in which compounds 4b, 4c and 4e exerted optimal results and the others modest anti-proliferation efficiency (Figure 1), there by IC\text{50} of the compounds were totally less than 10 µM (Table 3). Therefore, we selected 5 and 10 µM concentration for the following investigation according to the results of cell viability. Percent of cell viability was calculated and the inhibition of growth of human lung cancer cell line is defined by the nature of the substituent. The compounds having electron donating of three methoxy or methyl group 4b and 4c exhibited significant cytotoxic activity with IC\text{50} values having 4.01 and 4.69 µM against promising therapeutic agents for A549 lung adenocarcinoma cancer cell line.

![Figure 1: Phase contrast images of as synthesized compounds (4a-4g) treated A549 cell.](image)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\text{50} (µM)</th>
<th>FEB' (kcal/mol)</th>
<th>No. of H Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>8.92 ± 2.02</td>
<td>-7.42</td>
<td>2</td>
</tr>
<tr>
<td>4b</td>
<td>4.01 ± 0.07</td>
<td>-8.95</td>
<td>5</td>
</tr>
<tr>
<td>4c</td>
<td>4.69 ± 0.09</td>
<td>-8.91</td>
<td>1</td>
</tr>
<tr>
<td>4d</td>
<td>15.36 ± 1.08</td>
<td>-7.63</td>
<td>1</td>
</tr>
<tr>
<td>4e</td>
<td>6.06 ± 0.03</td>
<td>-8.21</td>
<td>2</td>
</tr>
<tr>
<td>4f</td>
<td>18.59 ± 1.07</td>
<td>-7.38</td>
<td>3</td>
</tr>
<tr>
<td>4g</td>
<td>10.02 ± 1.09</td>
<td>-7.82</td>
<td>4</td>
</tr>
<tr>
<td>5-FUb</td>
<td>6.02 ± 0.04</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

\*a = Free energy binding. \*b = Fluorouracil used as a positive control.

Table 3: IC\text{50} and FEB values of compounds 4a–g.

Molecular docking

Synthesized compounds 4b, 4c and 4e show very high binding energy with the ALK receptor which exposed similarity with the cytotoxic activity of among all the tested derivatives. The most active compound 4b has a very high binding energy value -8.95 kcal/mol exhibited H- bonding with Met1199, Glu1132, Arg1120, Gly1121 and hydrophobic interaction with Leu1256, Ala1148, Leu1122 which results five hydrogen bonds with ALK receptor (Figure 2a) and also compounds 4c, has equal binding energy value -8.91 kcal/mol and interact with amino acids namely Ser1206, Leu122, Pro1260, Glu1210 (Figure 2b). The moderate active Compounds 4e has a very high binding energy value
-8.21 kcal/mol and interact with amino acids namely Gln1146, Leu1145, (Figure 2c). Interestingly, electron donating substituted compounds exhibit higher binding energy values compared with other groups decreased the binding energy, for example, compound 4f shows low binding energy value -7.38 kcal/mol in presence of electron withdrawing substituted (Figure 2d). The order of binding affinity of docked trans-2,3-dihydronaphtho[2,3-b] furan derivatives against the ALK receptor is 4b>4c>4e>4g>4d>4a> and 4f with the range of binding energy being -8.95 to -7.38 kcal/mol (Table 3). The numbers of hydrogen bonds vary from one to five with most key interacting residues, Leu1122 actively participate in the formation of hydrophobic bonding with the compounds 4b, 4c and 4e are responsible for forming strong bonds between ligands and receptor ALK (2XP2.pdb) to inhibit the function of enzyme and cytotoxic effect.

Figure 2: (a) and (b) Binding mode of the most active compound 4b, 4c with ALK receptor, (c) Binding mode of moderate active compound 4e with ALK receptor, (d) Binding mode of least active compound 4f with ALK receptor. The amino acids involved in hydrogen (blue dashed line), hydrophobic (white dashed line) interactions are highlighted.

Table 3: IC_{50} and FEB values of compounds 4a–g.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50}(µM)</th>
<th>FEB a (kcal/mol)</th>
<th>No. of H Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>8.92 ± 2.02</td>
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<td>2</td>
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<tr>
<td>4b</td>
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<td>-8.95</td>
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<tr>
<td>4c</td>
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<td>-8.91</td>
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<tr>
<td>4d</td>
<td>15.36 ± 1.08</td>
<td>-7.63</td>
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<tr>
<td>4e</td>
<td>6.06 ± 0.03</td>
<td>-8.21</td>
<td>2</td>
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<tr>
<td>4f</td>
<td>18.59 ± 1.07</td>
<td>-7.38</td>
<td>3</td>
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<tr>
<td>4g</td>
<td>10.02 ± 1.09</td>
<td>-7.82</td>
<td>4</td>
</tr>
<tr>
<td>5-FUb</td>
<td>6.02 ± 0.04</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

* = Free energy binding. b = Fluorouracil used as a positive control.

Density functional theory (DFT) study

Theoretical calculations were performed by the density functional theory (DFT) method at the B3LYP/ 6-31G level of theory in the Gaussian 03 package of programs [42]. According to the frontier molecular orbital theory, (HOMO) has the priority to provide electrons while LUMO can accept electrons first are the most important factors that affect the bioactivity [43,44]. Higher HOMO energy and lower LUMO energy in the drug molecule result in larger stabilizing
interactions and, hence, binding with the receptor. The orbital energies of both HOMO and LUMO and their gaps were calculated for all the molecules and are reported in Table 4. It is remarkable that compounds 4b, 4c, 4e and 4g having the lowest energy gap (ΔE) of 0.686, 0.615, 0.689, and 0.858 ev, respectively, exhibit the highest cytotoxic activity. We also obtained a plot of the HOMO and LUMO of the molecules of each group to analyze the main atomic contributions for these orbitals. The importance of observing these plots was to determine which atoms were located at the possible sites of electronic transfer between the molecule under study and its biological target. The results illustrate that HOMO lobes are spread mainly over substituted aromatic rings; In contrast, the LUMO lobes are almost homogeneously spread over naphthoquinone moiety (Figure 3). Molecular electrostatic potential (MEP) mapped surface of the molecules are calculated by DFT/ B3LYP/6-31G method at the 0.02 isovalue and 0.0004 density values. Molecular electrostatic potential and electrostatic potential are the useful quantities to display the charge distributions of molecules are used to visualize variably charged regions of a molecule. The electrostatic potential is used to find the reactive site of a molecule. Red represents regions of most electro negative electrostatic potential, blue represents regions of most positive electrostatic potential (Figure 4).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$E_{\text{total (kcal)}}$</th>
<th>$E_{\text{HF}}$</th>
<th>$\mu_{\text{(Debye)}}$</th>
<th>$E_{\text{HOMO}}$</th>
<th>$E_{\text{LUMO}}$</th>
<th>ΔE</th>
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<tr>
<td>4a</td>
<td>-4179.9504</td>
<td>5.5066</td>
<td>-6.316</td>
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<tr>
<td>4b</td>
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<td>5.1512</td>
<td>-4.129</td>
<td>-3.443</td>
<td>0.686</td>
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<tr>
<td>4c</td>
<td>-3853.9979</td>
<td>3.6909</td>
<td>-3.821</td>
<td>-3.206</td>
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<tr>
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<td>-5.873</td>
<td>-3.712</td>
<td>2.160</td>
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<tr>
<td>4e</td>
<td>-6366.1381</td>
<td>7.5424</td>
<td>-4.402</td>
<td>-3.713</td>
<td>0.689</td>
<td></td>
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<td>-6.805</td>
<td>-3.993</td>
<td>2.811</td>
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</tr>
<tr>
<td>4g</td>
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<td>3.8487</td>
<td>-4.581</td>
<td>-3.721</td>
<td>0.858</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Energies of both HOMO and LUMO and their gaps (in ev) calculated for all compounds.

Figure 3: Plots of the HOMO and LUMO density map of compounds 4b (left) and 4c (right).
CONCLUSION

In summary, theoretical quantum-chemical calculations correlate well with modern research related to trans-2,3-dihydronaphtho[2,3-b] furan derivatives (4a-g). This result also supports its higher activities and investigated their biological activities against human lung cancer cell line A549 in vitro. The results indicated that the as-synthesized compounds showed significant anticancer activities. Among all the compounds screened, 4b, 4c and 4e showed very high activity at 10µM concentration against A549 cell line. Molecular docking of the most potent inhibitor (4a-g) into binding site of ALK was performed, and the results showed compound 4b, 4c and 4e could bind well with the ALK active site.

CONFLICT OF INTEREST

The Authors declare no conflict interest.

REFERENCES


