Synthesis and Molecular Docking of Isoquinoline derivatives as Potential Antitumor Agents

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Received on: 19-12-2013; Revised on: 17-01-2014; Accepted on: 08-02-2014

ABSTRACT

Aim: In an effort to establish new candidates with improved antitumor activity, series of Indolo[2,1-a]isoquinoline derivatives were synthesized and characterized. The synthesized compounds were docked in-silico onto the binding site of tyrosine kinase enzyme. Methods: The desired compounds 3a-l were synthesized regioselectively by the palladium-catalyzed preferential addition of N-heterocycles on to ortho-haloarylalkynes followed by intramolecular C-2 arylation without isolating enamine intermediate generated in in situ in the reaction, using an inexpensive and air stable 2-benzotriazolyl pyridine as novel bidentate ligand. Docking was performed by using the AutodockVina and integrated with Hex 6.0 to understand the binding preference of synthesized compound with target enzyme tyrosine kinase at default parameters. Result: The results obtained indicate that, out of our designed isoquinoline derivatives one has showed binding energy of -10.3 kcal/mol and it was showing best interaction with tyrosine kinase. To explore drug like behavior Quantum mechanical studies of that compound was also been carried out. Conclusion: The present investigation may find applications in developing an easy protocol for the formation of Indolo[2,1-a]isoquinoline derivatives and efforts to screen tyrosine kinase inhibitor using semi flexible manner by AutodockVina and Hex 6.0 at default parameters.

Keywords: Indolo[2,1-a]isoquinoline, tyrosine kinase inhibitor, Molecular docking

1. INTRODUCTION

Isoquinolines have a vast variety of substitution pattern that depends upon their biogenetic origin. Isoquinolines could be considered as simplest of isoquinoline alkaloids. Vast research has already been done on isoquinoline alkaloids. Derivatives of isoquinolines have effectively shown antimalarial, antibacterial, antifungal and anticancerous activities. Thiosemicarbazone possess a good spectrum of pharmacological properties including antitumor, antifungal, antibacterial, antiviral and antimalarial activities. The antitumor activity of such thiocompounds lies in their ability to inhibit ribonucleotide reductase, a necessary enzyme for DNA synthesis.

Currently 3-amino pyridine-2-carboxaldehyde thio carbazonetripane is being evaluated in human phase 11 trials as a cancer chemotherapeutic agent. The thiosemicarbazone analogs of 5-quinolone and 1-2 isoquinoline including the benzoylpyridinesemi carbazole 3 and 4 has shown moderate to excellent cytotoxicity against HuCCA-1, A549 and MOLT-3, HePG2 human cancer cell line. Receptor tyrosine kinases (RTKS) are a group of growth factors that controls a variety of cellular activities which is related to growth, metabolism and differentiation. RTK are high affinity cell surface receptor for many polypeptide growth factors cytokines and hormones. RTK mediated signals play pivotal and diverse role in regulation of various physiological functions ranging from cell proliferation, differentiation, cell adhesion, cell migration survival and apoptosis.In the human genome alone 58 RTK are encoded and categorized into 20 subfamilies. These include nerve growth factor receptor (NGFR), Tropomyosin-receptor-kinase (TrK) family receptors, epidermal growth factor receptors (EGFRs), Fibroblast growth factors (FGFR), glial derived neurotropic factor (GFR), and the insulin like growth factor (IGFR). RTK consist of four main domains: the extracellular ligand binding domain; the intracellular/ cytoplasmic highly conserved catalytic protein; tyrosine kinase domain which contains kinase inserts and sites for autophosphorylation.

Phosphorylation of tyrosine proteins catalyzes by tyrosine kinase, this phosphorylation leads to the different activity. Mutation in these tyrosine kinases lead to constitutive expression that may results to cancer. There is need to develop better methods that could be more effective and have significantly less side effects.

Insilico studies have been carried out with the aim of validating the effect of isoquinoline analogs on tyrosine kinase. In current study molecular docking has been done on different isoquinoline derivatives with the aim of identifying effective inhibitor of human tyrosine kinase.
2. Methodology

2.1. Target Selection

Target for computational analysis of binding energies was retrieved via protein data bank (PDB ID: 2OPL) having a resolution of 3.42 Å. The enzyme retrieved was having one polymer and two ligands.

2.2. Ligand preparation

Ligands were prepared by attaching various groups to native isoquinolines. The groups were added according to the free and reactive positions present on the main compound.

2.2.1. Chemicals

All the chemicals and reagents used to carry out research work were procured from Sigma Aldrich, Merck and S.D. Fine.

2.2.2. Synthesis of Indolo[2,1-a]isoquinolines

As we previously communicated, we were intrigued by the possibility of developing a more convenient one-pot synthetic method of isoquinoline derivatives by simply mixing N-heterocycle 1, and substituted ortho-haloarylalkynes 2 under Pd catalyst without the prior preparation of the corresponding intermediate (Scheme 1).

Table 1 Optimization of the reaction conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cat. (mol %)</th>
<th>L(mol%)</th>
<th>Base</th>
<th>t (h)</th>
<th>Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pd(OAc) 2</td>
<td>-</td>
<td>KOt-Bu</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td>2.</td>
<td>Pd(OAc) 2</td>
<td>L1/2</td>
<td>KOt-Bu</td>
<td>12</td>
<td>35%</td>
</tr>
<tr>
<td>3.</td>
<td>Pd(OAc) 2</td>
<td>L2/2</td>
<td>KOt-Bu</td>
<td>12</td>
<td>45%</td>
</tr>
<tr>
<td>4.</td>
<td>Pd(OAc) 2</td>
<td>L2/2</td>
<td>KOt-Bu</td>
<td>12</td>
<td>52%</td>
</tr>
<tr>
<td>5.</td>
<td>Pd(OAc) 2</td>
<td>L3/2</td>
<td>KOt-Bu</td>
<td>08</td>
<td>66%</td>
</tr>
<tr>
<td>6.</td>
<td>Pd(OAc) 2</td>
<td>L3/2</td>
<td>K PO4</td>
<td>06</td>
<td>85%</td>
</tr>
<tr>
<td>7.</td>
<td>Pd(OAc) 2</td>
<td>L3/2</td>
<td>K PO4</td>
<td>06</td>
<td>76%</td>
</tr>
<tr>
<td>8.</td>
<td>Pd(OAc) 2</td>
<td>L2/2</td>
<td>K PO4</td>
<td>12</td>
<td>72%</td>
</tr>
<tr>
<td>9.</td>
<td>Pd(OAc) 2</td>
<td>L2/2</td>
<td>Cs CO3</td>
<td>06</td>
<td>54%</td>
</tr>
<tr>
<td>10.</td>
<td>Pd(OAc) 2</td>
<td>L3/2</td>
<td>K CO3</td>
<td>06</td>
<td>32%</td>
</tr>
<tr>
<td>11.</td>
<td>Pd(OAc) 2</td>
<td>L3/1</td>
<td>K PO4</td>
<td>12</td>
<td>49%</td>
</tr>
<tr>
<td>12.</td>
<td>Pd(OAc) 2</td>
<td>L3/2</td>
<td>K PO4</td>
<td>06</td>
<td>38%</td>
</tr>
<tr>
<td>13.</td>
<td>PdCl2/2</td>
<td>L3/2</td>
<td>K PO4</td>
<td>06</td>
<td>34%</td>
</tr>
<tr>
<td>14.</td>
<td>PdCl2/2</td>
<td>L3/2</td>
<td>K PO4</td>
<td>06</td>
<td>22%</td>
</tr>
</tbody>
</table>

*All reactions were performed with 1a (0.5 mmol) with 1-bromo-2-(4-ethylphenyl)ethynylbenzene 2a 1.1 equiv, in 2 mL of DMSO, at 110 °C under an nitrogen atmosphere. *Isolated yields. *DMF is used instead of DMSO in reaction.

The product of the reaction was fully characterized by 1H and 13C NMR and mass spectroscopic data. The presence of electron-releasing group (R’) on the arenes para to the triple bond increases the electron density on the distal end of the triple bond and favors the formation of 6-endodig cyclized products.

Table 2: Synthesis of indolo- and pyrrolo[2,1-a]isoquinolines

<table>
<thead>
<tr>
<th>N-Heterocycle:</th>
<th>Alkynes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a, R1 = Me, R2 = H</td>
<td>2a, R1 = p-MePh</td>
</tr>
<tr>
<td>1b, R1, R2 = H</td>
<td>2b, R1 = p-EtPh</td>
</tr>
<tr>
<td>1c, R1 = H, R2 = OMe</td>
<td>2c, R1 = p-OMePh</td>
</tr>
<tr>
<td>2d, R1 = p-BuPh</td>
<td></td>
</tr>
</tbody>
</table>

| 1. 3a, (1a+2a), 76% | 2. 3b, (1a+2b), 78% |

Scheme 1. Synthesis of Indolo[2,1-a]isoquinolines

Reaction conditions were optimized and tabularized in Table 1. The study revealed that the optimal condition for synthesis of diversely substituted indolo[2,1-a]isoquinoline was 2 mol% of Pd(OAc)2, 2 mol% L3, 1.4 equiv of K2PO4 in DMSO at 110 °C. The scope and limitations of this palladium-catalyzed tandem process were next examined by employing various substituted ortho-haloarylalkynes and substituted N-heterocycles (Table 2, entries 1-12). The nature of the N-heterocycles (electron rich, neutral and electron deficient) and the substituents attached to the triple bond has a major impact on the success of the developed chemistry.
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2.3. Binding site prediction

Binding site prediction was done using Q-site finder online tool. According to the preliminary study carried out using Hex 6.0 the ligand was found to bind near the predicted active sites.

2.4. Molecular docking

Docking simulations were performed in rigid as well as semi flexible manner by AutodockVina and Hex 6.0 at default parameters. The molecular distances for confirmation of hydrogen bonds was done using the complex file of Hex 6.0 saved in .pdb file format. Visualization was accomplished using Swiss PDB viewer tool.

2.5 Quantum mechanical characterization

GAMESS was used for DFT based calculations of the lead molecule. The graphs for Hartee-fock energy values and dipole moment were calculated using MoCalc.

3. Analytical data

6-(4-Methylphenyl)-12-methylindolo[2,1-a]isoquinoline (3a)
6-(4-Ethylphenyl)-12-methylindolo[2,1-a]isoquinoline (3b)
6-(4-Methoxyphenyl)-12-methylindolo[2,1-a]isoquinoline (3c)
6-(4-Tert-butylphenyl)-12-methylindolo[2,1-a]isoquinoline (3d)
6-p-Tolyindolo [2,1-a]isoquinoline (3e)
6-(4-Ethylphenyl)indolo[2,1-a]isoquinoline (3f)
6-(4-Methoxyphenyl)indolo[2,1-a]isoquinoline (3g)
6-Phenyindolo [2,1-a]isoquinoline (3h)
6-Methoxy-6-(4-ethylphenyl)indolo[2,1-a]isoquinoline (3i)
6-Methoxy-6-(p-tolyl)indolo[2,1-a]isoquinoline (3j)
6-Methoxy-6-(4-methylphenyl)indolo[2,1-a]isoquinoline (3k)
6-Methoxy-6-(p-methoxyphenyl)indolo[2,1-a]isoquinoline (3l)

All the reactions were carried out using 0.5 mmol of the N-heterocycle 1 and 1.1 equiv of 2-haloalkyne 2 in the presence of Pd(OAc)₂ (2.0 mol%), L₃ (2.0 mol%), base K₃PO₄ (1.4 equiv), in 2.0 ml DMSO at 110 °C for 6 h.

*All the reactions were carried out using 0.5 mmol of the N-heterocycle 1 and 1.1 equiv of 2-haloalkyne 2 in the presence of Pd(OAc)₂ (2.0 mol%), L₃ (2.0 mol%), base K₃PO₄ (1.4 equiv), in 2.0 ml DMSO at 110 °C for 6 h.
4. RESULTS

3.1. Prediction of binding site
Binding sites were visualized through the Jmol tool of Q-site finder. Jmol is a java based application that is embedded in the webpage of Q-site finder. The file used as input to Q-site finder was the file generated by Hex 6.0 after docking at default parameters. The compound was treated as ligand and colored locations were visualized as binding sites in Fig 1.

Fig. 1. Binding site prediction using Q-site finder

3.2. Docking studies
Molecular docking was undertaken to study the binding affinity of tyrosine kinase with various drugs and analogs of isoquinoline as shown in Fig. 1-6.

Fig. 2. Dasatinib: Insilico docking of dasatinib with tyrosine kinase

Fig. 3. Gefitinib: Insilico docking of gefitinib with tyrosine kinase

Fig. 4. Imatinib: Insilico docking of imatinib with tyrosine kinase

Fig. 5. Lapatinib: Insilico docking of lapatinib with tyrosine kinase
Various result of Autodock Vina were verified by considering the docking score. The docking results were ranked according to least binding energy of investigated drugs and synthesized compounds. Different drugs were selected according to their mode of action (tyrosine kinase inhibitors). Between the screened drugs and our designed molecules the later one has showed binding energy of -10.3kcal/mol and hence it is showing best interaction with tyrosine kinase. Fig. 7 depicts the hydrogen bonds formed by the isoquinoline analogue with tyrosine kinase.

Fig. 7. Hydrogen bonds less than 4Å distance with tyrosine kinase

3.3. Quantum mechanical analysis for drug like property
Quantum mechanical studies have been conducted to explore drug like behavior of synthesized compound. GAMESS (Gordon group) has been utilized to peep into quantum mechanical characteristics. Comparative analysis of drugs and compounds has revealed that relationship exists between them in fields of total energy (Hartree) and dipole moments. Dipole moment can be illustrated as the magnitude of charges upon distance square. These dipole moments play vital role in constituting different interactions. The comparative analysis by GAMESS has given a value of dipole moment as 2.1 as shown in Fig. 8 for our synthesized molecule that is least in comparison to the dipole moments of chosen drugs. Total energy (Hartree) was found to be in between highest and lowest value. The total energy (Hartree) value of synthesized compound was found to be around -1000 as in Fig. 9.

Fig. 8. Comparative study of dipole moment

Fig. 9. Comparative study of total energy (Hartree)

4. DISCUSSION
Isoquinolines are well established for their anti-cancerous property. Substantial discrepancies still persist with their properties related to efficiency, ADME properties, toxicity and problem of huge side effects. To overcome these discrepancies novel derivatives with enhanced properties are required. The current study addresses these problems to enhance drug like properties through an insilico approach. Tyrosine kinase (1opl) was simulated for binding with various derivatives drawn based on isoquinoline pharmacophore. Comparatively five anti cancerous drugs namely, Imatinib, Gefitinib, Lapatinib, Dasatinib and Ruxolitinib were also simulated for binding to check
the efficiency of binding in forms of binding affinity. Out of various derivatives 6-(4-tert-butyphenyl)-12-methylindolo[2,1-α]isoquinoline was found to have lowest binding affinity -10.3 kcal/mol with tyrosine kinase (1opl). The grid for Autodock vina was decided using the results of Q-site finder. Binding site in autodock vina was center_x = 77.485; center_y = 11.553; center_z = 40.827; size_x = 34; size_y = 42; size_z = 48. The binding pocket of analogue comprised of GLU301, PHE302, LEU292, GLU24, TYR272, PHE401 amino acids. Three H bonds were also detected using SPDBV. First bond was formed between O of GLU489 (45.930, 58.926, 66.762 and 58.61 SPDBV coordinates) and H of analogue (47.254, 59.785, 69.125 and 99.99 SPDBV coordinates) at a distance of 2.84 Å. Second H bond was formed between OE1 of GLU489 (50.544, 61.851, 65.969 and 91.97 SPDBV coordinates) and H of analogue (49.316, 60.995, 68.103, 99.99 SPDBV coordinates) at a distance of 2.61 Å. Third H bond was formed between OE2 of GLU489 (52.291, 60.503, 65.967 and 91.67 SPDBV coordinates) at a distance of 3.62 Å. The binding pocket of drugs and analogue were found to have PHE302, LEU292 and PHE401 in common. Total energy (Hartree) was calculated by density functional theory calculations stated that analogues having total energy between that of ruxolitinib and lapatinib will have anti-cancerous drug like properties. The dipole moments stated that analogue is soluble in organic solvents only with low polar nature. Dipole moment of analogue enhances the drug likedness by illustrating its nature. The low polar nature also elucidates that analogue may have low penetration power thus decreasing the bioavailability. On the basis of insilico simulations of binding, prediction of H bonds, pharmacokinetic and pharmacodynamic properties calculated through DFT proposes the isoquinoline analogue as a better compound than available drugs for the inhibition of tyrosine kinase.

5. CONCLUSION

In conclusion, we described herein a facile and direct approach for the regioselective synthesis of indolo[2,1-α]isoquinolines in good yield by the hydroamination of alkynes followed by intramolecular C-2 arylation. An inexpensive compound BtPy (L3), is used as a ligand, along with Pd(II), increasing the overall utility of this reaction. Out of various derivatives 6-(4-tert-butyphenyl)-12-methylindolo[2,1-α]isoquinoline was found to have lowest binding affinity -10.3 kcal/mol with tyrosine kinase (1opl). The grid for Autodock vina was decided using the results of Q-site finder. Comparatively five anticancerous drugs namely, Imatinib, Gefitinib, Lapatinib, Dasatinib and Ruxolitinib were also simulated for binding to check the efficiency of binding in forms of binding affinity.

Conflicts of interest

All authors have none to declare.

ACKNOWLEDGEMENT

We gratefully acknowledge G. B. Pant Engineering College, Pauri Garhwal for financial support and providing instrumentation facilities. P.T. and S.M. are thankful to AICTE (All India Council for Technical Education) and TEQIP (Technical Education Quality Improvement Program) respectively for fellowship.

REFERENCES

17. Laurie AT, Jackson RM, Q-Site Finder: an energy- based

Source of support: Nil, Conflict of interest: None Declared