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Research Articles

Efficient Synthesis of Densely Functionalized Pyrido[2,3-d]Pyrimidines via Three-component One-pot Domino Knoevenagel aza-Diels Alder Reaction and Induces Apoptosis in Human Cancer Cell Lines *via* Inhibiting Aurora A and B Kinases

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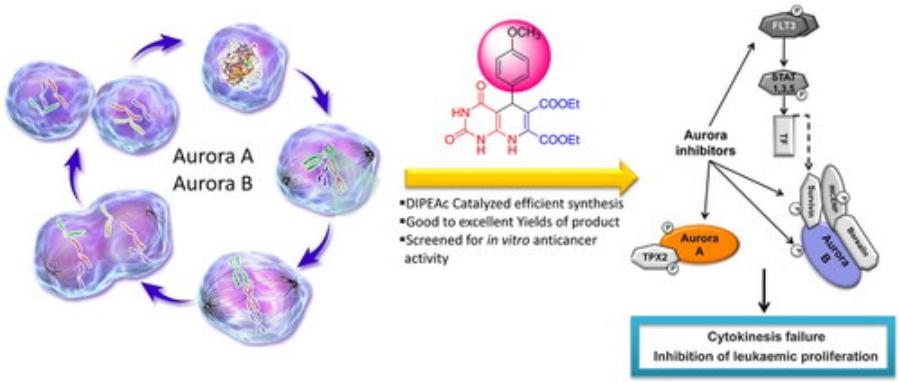
Abstract

The identification of novel aurora kinase inhibitors is one of the most attractive directions in the field of anticancer research and development. In our ongoing efforts to pursue the class of inhibitors, a series of pyrido[2,3-*d*]pyrimidines were synthesized in DIPEAc/EtOH media. The advantages of the present methodology include a one-pot multicomponent environmentally

reaction times, easy workup procedure and high yields. Synthesized compounds were screened against human lung carcinoma A549 cells, human hepatocellular liver carcinoma HepG2 cells and human cervical carcinoma epithelial HeLa cells. Compound **6 b** *i.e.* diethyl 5-(4-methoxyphenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6,7-dicarboxylate was found to be equipotent than the standard drug VX-680 against selected cancer cell lines. The selected compounds (**6b**, **6d** and **6h**) exhibit potent inhibition against Aurora A and B with IC_{50} values (9.4–25 mg/L). Molecular docking model showed that the compounds can bind well to the target by interacting with amino acid residues. It will provide some valuable information for the commercial Aurora Kinase inhibitors.

Graphical Abstract



Keywords: Pyrido[2,3-d]pyrimidines DIPEAc multicomponent aurora kinase inhibitor molecular docking

Introduction

The Aurora kinase family of serine/threonine kinases (Aurora A, B and C) is involved in multiple mitotic events, and aberrant expression of these kinases is associated with tumorigenesis.[1] Aurora A and Aurora B are validated anticancer targets, and the development of Aurora kinase inhibitors has progressed from preclinical to clinical studies. In humans, among the three members of the kinase family, Aurora-A, -B and -C, only Aurora-A and -B are expressed at detectable levels in all somatic cells undergoing mitotic cell division and have been characterized in greater detail for their involvement in cellular pathways relevant to the

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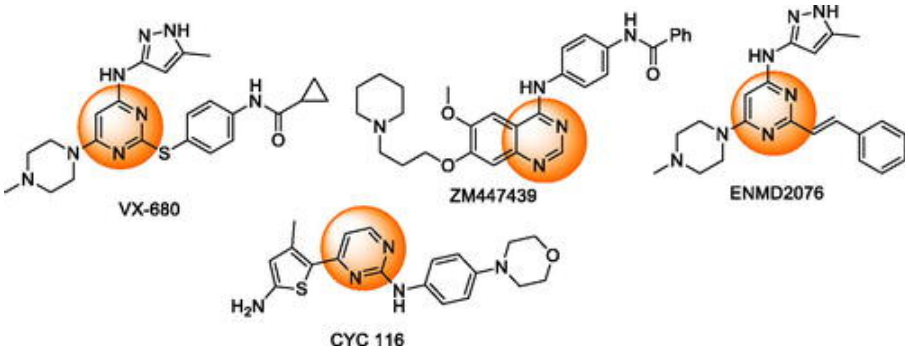
potential targets for anticancer therapy. Development of inhibitors against Aurora kinases as anticancer molecules gained attention because of the facts that aberrant expression of these kinases leads to chromosomal instability and derangement of multiple tumor suppressor and oncoprotein regulated pathways.[5]

A variety of A, B and C-Aurora kinase inhibitors (VX-680/MK-0457, ZM447439, Hesperadin, MLN8054, MLN8237 and AZD1152) have been developed and introduced into clinical trials (Figure 1).[6–9] Preclinical studies and early phase I and II clinical trials of multiple Aurora kinase inhibitors as targeted anticancer drugs have provided encouraging results. However, these drugs either lack sufficient efficacy or are associated with significant adverse effects such as febrile neutropenia, stomatitis, gastrointestinal toxicity, hypertension and fatigue. In a search for a aurora kinase target, we have identified pyrido[2,3-*d*]pyrimidines as a potential target for pharmacological intervention.

Figure 1. Published Aurora Kinase inhibitors having pyrimidine pharmacophore.



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Pyrimidines is the important pharmacology core in many Aurora inhibitors, such as VX-680, ZM447439, CYC-116 and ENMD-2076.[6–9] To identify effective Aurora inhibitors, literature studies suggested that the pyrimidine core form hydrogen bonds with the hinge region of the kinase domain and show selectively inhibition to Aurora A and B.[10,11] Additionally pyrido[2,3-d]pyrimidines were reported to exhibit antitumor activity which may be attributed to inhibition of cyclin-dependent kinase,[12] check point kinase[13] or mammalian target of rapamycin.[14] Moreover, several derivatives having pyrido[2,3-d]pyrimidine core were found to induce apoptosis and/or reduce cell proliferation in different solid tumors and leukemia cell lines.[15,16] For example, a series of 2,4-bis substituted pyrido[2,3-d]pyrimidines I exhibited dose-

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pathways leading to cell cycle arrest and rapid apoptosis.[17] Moreover promising anticancer activity of structurally diverse pyrido[2,3-d]pyrimidine scaffolds was described in a number of recent publications and patents e.g. 2-(alkylsulfanyl)-N-alkylarylpyrido[2,3-d]pyrimidine derivatives showed good profile as caspase-3 activator and apoptosis inducers in breast, colon and bladder cancer cells lines.[17] Furthermore, the novel analog; 2-[(3-chloro-4-fluorophenyl)amino]-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one II potently inhibited p210Bcr-Abl tyrosine kinase and induced apoptosis of K562 leukemic cell line.[18]

Several approaches have been developed for the synthesis of pyridopyrimidines such as: the reaction of benzylidene derivatives of malononitrile with 6-amino-3, 4-dihydropyrimidine in refluxing ethanol,[19,20] the reaction of 6-amino-1-thio uracil with ethyl-3-phenyl-2-cyanoacrylate in absolute ethanol and in the presence of Et_3N by heating,[21,22] the three-component reaction of aldehydes, alkyl nitriles and aminopyrimidines in water and in the presence of KF-Al_2O_3 as catalyst,[23] the similar three-component reaction catalyzed by TE- BAC[24] or reaction of amino-uracil with *α*, *β*-unsaturated compounds in ionic liquid at 90 °C.[25] However, in spite of their potential benefits, many of these reported methods suffer from drawbacks such as use of environmentally harmful organic solvents, expensive catalysts, harsh reaction conditions difficult work-up, non-recyclability of solvents, commercial unavailability and low yields. Thus, the development of efficient method for the synthesis of biologically active compounds such as pyrido[2,3-*d*]pyrimidines, in one-step would be highly valuable and desirable.

Due to environmental and economically positive implications, one-pot multicomponent coupling reactions (MCRs)[26–32] have been proven to be a very elegant and rapid way to access complex structures in a single synthetic operation from simple building blocks. They also address fundamental principles of synthetic efficiency and reaction design, and show atom-economy and selectivity. MCRs are important tools for both combinatorial chemistry and diversity-oriented synthesis (DOS), and thus play a significant role in the development of methodology for drug discovery.[33–36] MCRs can be considered as an interesting topic for academic research, which also satisfies a practical interest of applied science.

Organic reactions using sustainable reaction media such as ionic liquids (ILs), avoiding the use of volatile organic solvents, have attracted a great deal of attention of synthetic organic chemist. ILs are not only easily available, environmentally safe, but also excellent catalysts and medium

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volatility, non-flammability, and are endowed with unique properties such as high thermal/chemical stability.[37,38] ILs act as 'neoteric solvents' for a broad range of chemical and industrial processes. Recently, ILs have been found to be useful as green media for numerous organic transformations.[39,40] The ability to dissolve many organic and inorganic substances makes ILs eco-friendly reaction media/catalysts.[41,42]

In continuation of our recent work aiming at the synthesis of a variety of heterocyclic systems with remarkable biological importance,[43–46] we report here on the utility of DIPEAc as ionic liquid promoter for the three-component one-pot domino Knoevenagel aza-Dielse Alder reaction resulted in 7-amino-5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-carbonitrile and diethyl 5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6,7-dicarboxylate derivatives. The synthesized compounds were evaluated against three cancer cell lines. Potent compounds also screened for Aurora-A and B kinase inhibitors as in order to explore new class of compounds that could be optimized for potent anticancer agents. In addition, docking study has been carried out to rationalize the biological activity.

Experimental

General

All the chemicals used were of laboratory grade. Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded with a BrukerAvance 400 spectrometer operating at 400 MHz using DMSO-d6 solvent and tetramethylsilane (TMS) as the internal standard and chemical shift in δ ppm. Mass spectra were recorded on a Sciex, Model; API 3000 LCMS/MS Instrument. The purity of each compound was checked by TLC using silica-gel, 60 F_{254} aluminum sheets as adsorbent and visualization was accomplished by iodine/ultraviolet light.

General procedure for the synthesis of diisopropylethylammonium acetate (DIPEAc)

A mixture of glacial acetic acid (0.02 mol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.02 mol) was stirred at 0–10 °C for 30 min to obtain diisopropylethylammonium acetate as a viscous liquid.[47]

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thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4a-m)

A mixture of benzaldehyde (**1a**) (3 mmol), malanonitrile (**2**) (3 mmol), 6-aminouracil (**3**) (3 mmol) was added in DIPEAc (20 mol%) and ethanol (15 mL) and then the reaction mass was stirred at 80 °C. Progress of the reaction was monitored by TLC (ethyl acetate:n-hexane 1:9). After 2 h of stirring, reaction mixture was cooled to room temperature and poured on crushed ice. Thus obtained solid was filtered, dried and crystallized form ethanol.

Similarly the other compounds, (**4 b-m)** of the series were prepared. The melting points and the yields of the derivatives are recorded in Table 2.

General procedure for the synthesis of 5-(substituted phenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylester (6a-h)

A mixture of benzaldehyde (**1a**) (3 mmol), (3 mmol), 6-aminouracil (**3**) and (3 mmol) diethyl acetylenedicarboxylate (**5**) was added in DIPEAc (20 mol%) and ethanol (15 mL) and then the reaction mass was stirred at 80 °C. Progress of the reaction was monitored by TLC (ethyl acetate:n-hexane 1:9). After 2 h of stirring, reaction mixture was cooled to room temperature and poured on crushed ice. Thus obtained solid was filtered, dried and crystallized form ethanol.

Similarly the other compounds, (**6 b-h)** of the series were prepared. The melting points and the yields of the derivatives are recorded in Table 2.

In vitro anticancer screening

All the synthesized compounds **4a-m** and **6a-h** were evaluated for their *in vitro* anticancer activity against three human cancer cell lines A549, HePG2 and HeLa by MTT assay. The human cancer cell lines were grown in DMEM medium containing 10% fetal bovine serum and 0.7% antibiotics. Cells were seeded into 96 well microtiter plates in 100 µL of media at plating density of 5000 cells/well. Seeded cells were incubated at 37 °C, 5% CO_2, 95% air and 100% humidity for 24 h. At 24 h, old media was changed with fresh media followed by treatment with each compound at 10 µM, 1 µM and 0.1 µM. After 24 h treatment, cell viability was assessed by 3-(4,5-dimethylthiazol)-2,5- diphenyltetrazolium bromide (MTT), cell were incubated with 20 µL of MTT

dissolved in DMSO. MTT reduction was quantified by measurement of absorbance at 570 nm using a multimode reader, Synergy Mx of BioTek.[48]

Aurora kinases inhibitory activities

The synthetic compounds and reference compound were diluted to five concentrations (0.1, 1, 10, 100 and 1000 nM) in the PBS and then added 5 mL to the 50 mL reaction mixture (40 mMTris, pH = 7.4, 10 mM MgCl2, 1 mM DTT, 0.1 mg/mL BSA, 10 mM ATP, 0.2 mg/mL Kinase and 100 mMKemptide acetate salt), and then the kinase reactions were incubated for 30 min at 37 °C. Finally, we used Kinase-Glo luminescence kinase assay kit tested luminescent signal of the reaction mixture, gave IC_{50} values by GraphPad Prism software.

Spectral analysis of representative compounds

7-Amino-1,2,3,4-tetrahydro-1methyl-2,4-dioxo-5(4-nitrophenyl)pyrido[2,3-d]pyrimidine-6-carbonitrile (4 g)

IR (ATR υ_{max} cm^{-1}): Characteristic Absorption: 3398 (NH2 stretching), 3194 (N-H Stretching), 2885 (C-H stretching), 2202 (CN stretching), 1706 (C = O Stretching), 1522 (NO_2 stretching), 1454 (C = C,stretching). **LC-MS** (ESI m/z: 325.1 $(M + H)^+$. **^{1}H NMR**: (400 MHz, DMSO-d_6 δppm): 6.17 (s, 1H, -NH), 6.76–6.97 (m, 2H, Ar-H), 7.06 (s, 2H, -NH_2), 7.08–7.57 (m, 2H, Ar-H), 10.09 (s, 1H, -NH). Elemental Analysis for $C_{14}H_8N_6O_4$: Calculated C 51.86; H 2.49; N 25.92, Found C 51.75; H 2.38; N 25.89.

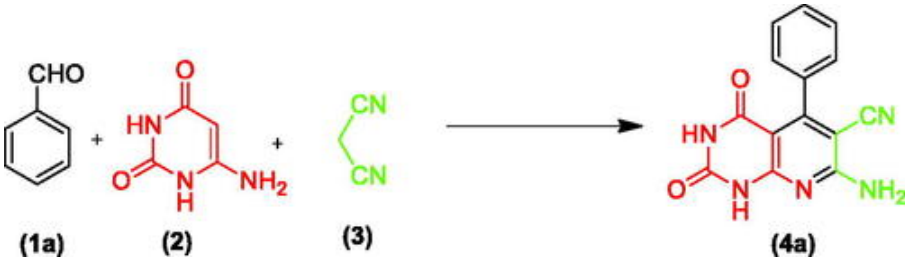
1,3-Dimethyl-2,4-dioxo-5-aryl/alkyl-1,2,3,4,5,8-hexadihydro[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylester (6a)

IR (ATR υ_{max} cm^{-1}): Characteristic absorption: 3162 (N-H Stretching), 2885 (C-H, stretching), 1700 (C = O stretching), 1680 (C = O Stretching), 1463 (C = C, stretching). **LC-MS** (ESI *m/z*: 385.2 $(M + H)^+$. **^{1}H NMR**: (400 MHz DMSO-d_6 δppm):2.27–2.30 (m, 3H, -CH_3), 2.50–2.57 (m, 3H, -CH_3), 4.36–4.40 (m, 4H, -CH_2), 6.25 (s, 1H, -CH), 7.30–7.40 (m, 3H, Ar-H), 7.52–7.57 (m, 1H, Ar-H), 7.72–7.87 (m, 1H, Ar-H). Elemental Analysis for $C_{19}H_{19}N_3O_6$: Calculated C 59.22; H 4.97; N 10.90 Found C 59.30; H 4.89; N 10.82.

Chemistry

We began our optimization studies by using benzaldehyde (**1a**), 6-aminouracil (**2**), malanonitrile (**3**) as model substrates to identify the suitable reaction conditions for the proposed three-component reaction (Scheme 1). Very low yields were obtained even after heating and refluxing the reaction mixture for several hours in organic solvents like ethanol, methanol, DCM and DMF (Table 1). Since ionic liquids have many advantages including ready availability, non-toxicity, biodegradability and recyclability,[49–54] the synthesis of the model compounds were investigated in ChCl:urea and diisopropylethylammonium acetate (DIPEAc). The yield in DIPEAc was much improved in comparison with other solvents. The DIPEAc was prepared by stirring the equimolar mixture of acetic acid and N-ethyl-N-isopropylpropan-2-amine at 0-10 °C (Scheme 2).

Scheme 1. 7-Amino-2,4-dioxo-5-phenyl-1,2,3,4-tetrahydro pyrido [2,3-d] pyrimidine-6-carbonitrile (**4a**).



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Scheme 2. Synthesis of diisopropyl ethyl ammonium acetate (DIPEAc).

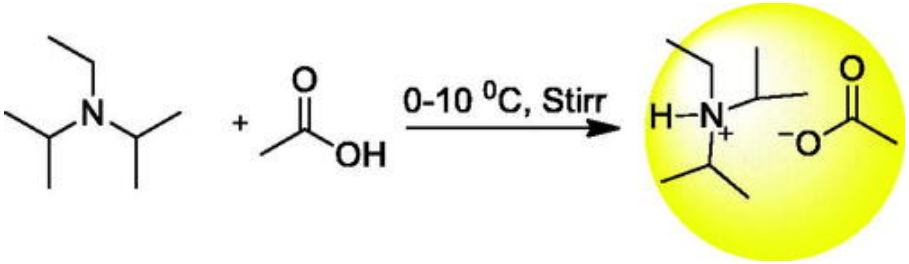

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Table 1. Screening of solvents and catalysts for the synthesis of 7-amino-1,2,3,4-tetrahydro-1-methyl-2,4-dioxo-5-phenylpyrido[2,3-d]pyrimidine-6-carbonitril (**4a**)

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Table 2. Physical data of 7-amino-1,2,3,4-tetrahydro-1methyl-2,4-dioxo-5-phenylpyrido[2,3-d]pyrimidine-6-carbonitrils (**4a-m**) and 5-(substituted phenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylesters (**6a-h**).[a]

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To investigate the catalytic activity of DIPEAc, the model reaction was carried out in several conventional solvents such as ethanol, methanol, acetonitrile, DMF each having DIPEAc (20 mol%). The cyclocondensations was best catalyzed by DIPEAc in ethanol and giving a good yield of **4a** 91% within 2 h (Table 1).

Ethanol is green solvent next to the water as it produced by the fermentation of sugars by yeasts or by petrochemical processes. As it is environmentally preferable solvent we select it as solvent of choice for further optimization. In the next step, the model reaction was performed in the presence of different quantities of DIPEAc in ethanol at 80 °C, with 20 mol % being the optimum, affording a quantitative yield of the desired product within 2 h (Table 1).

Having established by above results, the model reaction was performed using DIPEAc/Ethanol as a reaction medium at different temperatures. Model reaction in DIPEAc/Ethanol at 80 °C was found to proceed with excellent yield (91%) of the Pyrido[2,3-*d*]pyrimidines-6-carbonitril (**4a**) in 2 h (Table 1). It was also noted that under similar reaction conditions there was no condensation at room temperature. As temperature increased (40, 60, 80, 100 °C) the yield of the product was also increased (42, 82, 92, 92%).

After having optimized the reaction conditions, we turned to explore the scope and limitations of the three-component reaction by employing a variety of aryl/hetryl aldehydes (Scheme 3). Under the optimized conditions, irrespective of the substituent present on the aromatic ring of the aldehyde moiety, the corresponding products were obtained in high to excellent yields at 80 °C within 2 h. (Table 2).

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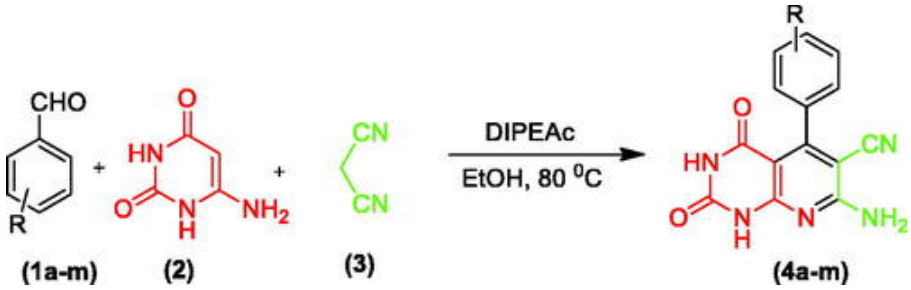
phenyl)pyrido[2,3-d]pyrimidine-6-carbonitril (**4a-m**).



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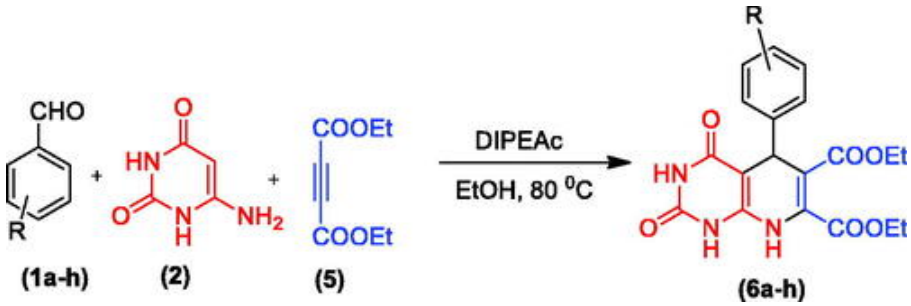
Next we sought to expand our strategy toward the synthesis of 1,3-dimethyl-2,4-dioxo-5-aryl/alkyl-1,2,3,4,5,8-hexadihydro[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylesters (**6a-h**) *via* one-pot three-component domino Knoevenagel Aza-Dielse Alder reaction of aldehydes (**1a-h**) 6-aminouracil (**2**) and diethyl acetylenedicarboxylate (**5**). To extend further, we used acetylenedicarboxylate instead of malanonitrile with wide variety of aldeydes under similar conditions and the corresponding 1,3-dimethyl-2,4-dioxo-5-aryl/alkyl-1,2,3,4,5,8-hexadihydro[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylesters (**6a-h**) were obtained in high yields (Scheme 4). DIPEAc has potential to make possible the synthesis of libraries under similar circumstances. The results are tabulated in Table 2.

Scheme 4. Synthesis of 1,3-dimethyl-2,4-dioxo-5-aryl/alkyl-1,2,3,4,5,8-hexadihydro[2,3-d]pyrimidine-6,7-dicarboxylic acid diethylester (**6a-h**).



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Plausible reaction mechanism

A tentative mechanistic rationale portraying the probable sequence of events is given in Scheme 5. On the basis of the experimental results and available literature,[55,56] postulated

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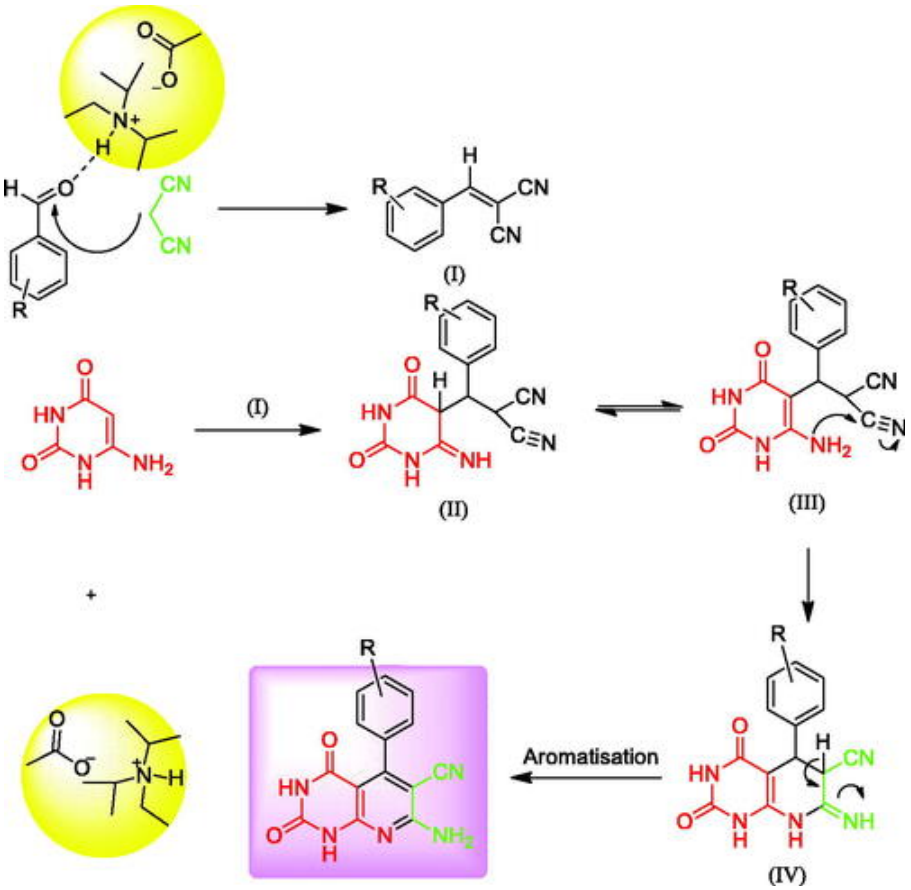
acetate (DIPEAc) is presented in Scheme 5. Rate of acceleration for the formation of pyrido[2,3-d]pyrimidine has been found to be enhanced due to the dual nature of IL, as a catalyst and reaction medium. IL might be helping to create a high initial concentration of the reactants *via* solvation.

Scheme 5. Plausible mechanism for the synthesis of pyrido[2,3-d]pyrimidine in diisopropylethylammonium acetate (DIPEAc).



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In a plausible mechanism, it is assumed that the reaction may proceed initially through the Knoevenagel condensation between aryl aldehyde and malononitrile to form intermediate I. DIPEAc co-ordinate with the aldehyde to increase its electrophilic character. Next, Michael addition of 6-aminouracils to intermediate I afford II. Intermediate II converts to III after tautomerization. Then, Intermediate III converts to IV *via* cyclization. Finally, the desired product is obtained after aromatization from IV (Scheme 5).

Anticancer activity

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A series of 7-amino-5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-carbonitriles (**4a-m**) and diethyl 5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6,7-dicarboxylate derivatives (**6a-h**) were evaluated for their anticancer activity. The synthesized compounds were tested against human lung carcinoma A549 cells, human hepatocellular liver carcinoma HepG2 cells and human cervical carcinoma epithelial HeLa cells using MTT assay. The VX-680 has been used as standard drug. The results obtained for the anticancer screening study are revealed in Table 3 and schematically present in Figure 2.

Figure 2. Schematic presentation of potent anticancer agents and aurora kinase inhibitor.

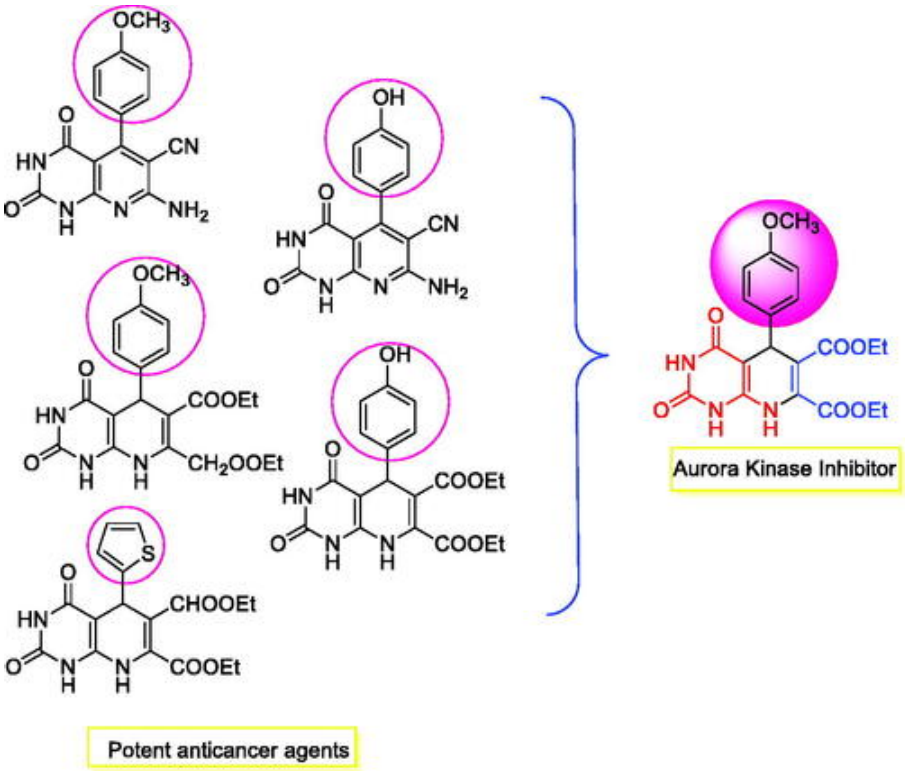

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Table 3. *In vitro* anticancer screening data.

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Table 3 explains that the **4b, 4f, 6b, 6d** and **6h** were found to be good anticancer compounds among the synthesized compounds. The compound **6b** i.e. diethyl 5-(4-methoxyphenyl)-2,4-

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standard drug VX-680 against selected cancer cell lines. The compound **6b** illustrated an IC_{50} values such as 10.1 μM, 14.5 μM and 8.0 μM against A549, HepG2 and HeLa, respectively. The compound **6h** is the second most potent synthesized compound with IC_{50} values such as 12.4 μM, 15.2 μM and 9.4 μM against A549, HepG2 and HeLa, respectively.

From the anticancer screening data as illustrated in Table 3 we can conclude that the diethyl 5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6,7-dicarboxylate derivatives are good anticancer agents than those of 7-amino-5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-carbonitrile derivatives. The replacement of phenyl ring by other heteryl ring was beneficial for anticancer activity. In the compound **6a** the phenyl ring has been replaced by thiophene ring which gave compound **6 h**, this was found to be more potent than **6a**.

The compound **6 b** with the para-methoxy group on the phenyl ring was found to be most potent compound among the synthesized compounds. In the 7-amino-5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-carbonitrile series the compound **4 b** bearing the para-methoxy group on the phenyl ring was found to be most potent compound. This observation explains that the para-methoxy group on the phenyl ring nepotism the good anticancer activity.

Aurora kinases inhibitory activities

The potent compounds such as **6b**, **6d** and **6h** were evaluated for their ability to inhibit aurora kinase. The VX-680 has been used as standard drug. The inhibition activity against Aurora kinases was performed by Kinase-Glo luminescent kinase assay *in vitro*.[58] As observed in Table 4 that the compound **6b** is the most potent Aurora kinase inhibitor. The results of Aurora kinases inhibitory activities suggest that the synthesized compounds exert their anticancer activity by inhibiting aurora kinase enzyme.

Table 4. The kinase inhibitions of selected compounds *in vitro*.

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Molecular docking study was performed in Maestro 9.1 using Glide v. 6.8 (Schrodinger LLC). All compounds were built using Maestro build panel and optimized to lower energy conformers using Ligprep v3.5 (Schrodinger, Inc., New York, NY, USA). The coordinates for EGFR enzyme were taken from RCSB Protein Data Bank and prepared for docking using 'protein preparation wizard' in Maestro v10.3. The bond orders and formal charges were added for hetero-groups and hydrogen's were added to all atoms in the structure. Side chains that are not close to the binding cavity and do not participate in salt bridges were neutralized and termini were capped by adding ACE and NMA residue. After preparation, the structure was refined to optimize the hydrogen bond network using OPLS_2005 force field. The minimization was terminated when the energy converged or the RMSD reached a maximum cut-off of 0.30 Å. The extra precision (XP) docking mode for all compounds was performed on generated grid of protein structure.[59] The final evaluation of ligand–protein binding was done with Glide score.[60]

Aurora kinases are a class of serine/threonine protein kinase family which helps in the process of mitosis for healthy cell proliferation. The Aurora A kinase is associated with centrosome maturation and separation and there by regulates spindle assembly and stability. The Aurora B kinase is a chromosome passenger protein and regulates chromosome segregation and cytokinesis. As Aurora kinases are considered to be potential targets for novel small molecule inhibitors we here studied the docking of the designed aurora kinase inhibitors on Aurora A and B kinase enzymes, respectively.

The docking studies were performed on Aurora kinase A (PDB ID: 1MQ4) and Aurora kinase B (PDB ID: 4B8M), respectively. It was observed that the inhibitor molecules were attached into the pocket of the receptor enzyme through different hydrogen and hydrophobic interactions. The binding affinity and pose of compounds **6b**, **6d** and **6h** were found to be correlating with that of the standard molecule pose VX-680 in Aurora A as well Aurora B kinases. The main amino acid residues of Aurora A kinase which were involved in the binding interaction were polar Lys 258, Lys 162, Lys 143 and hydrophobic amino acid Trp 277 whereas in Aurora B kinase the key amino acid residues which were involved for hydrogen bonding interactions were Lys 180, Glu 177 and Arg 175 which indicated that compound **6h** was highly potent Aurora A and B kinase inhibitor among the others. It was also observed that the compound **6h** showed the salt bridge interaction and metal co-ordinate bonding with the amino acid residue Lys 162 and Mg^{2+} ions possessed highest binding affinity with good docking score in Aurora A kinase. Figure 3

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image of highly potent compound **6h** on Aurora B kinase.

Figure 3. **2D** image of compound **6h** on Aurora A kinase.

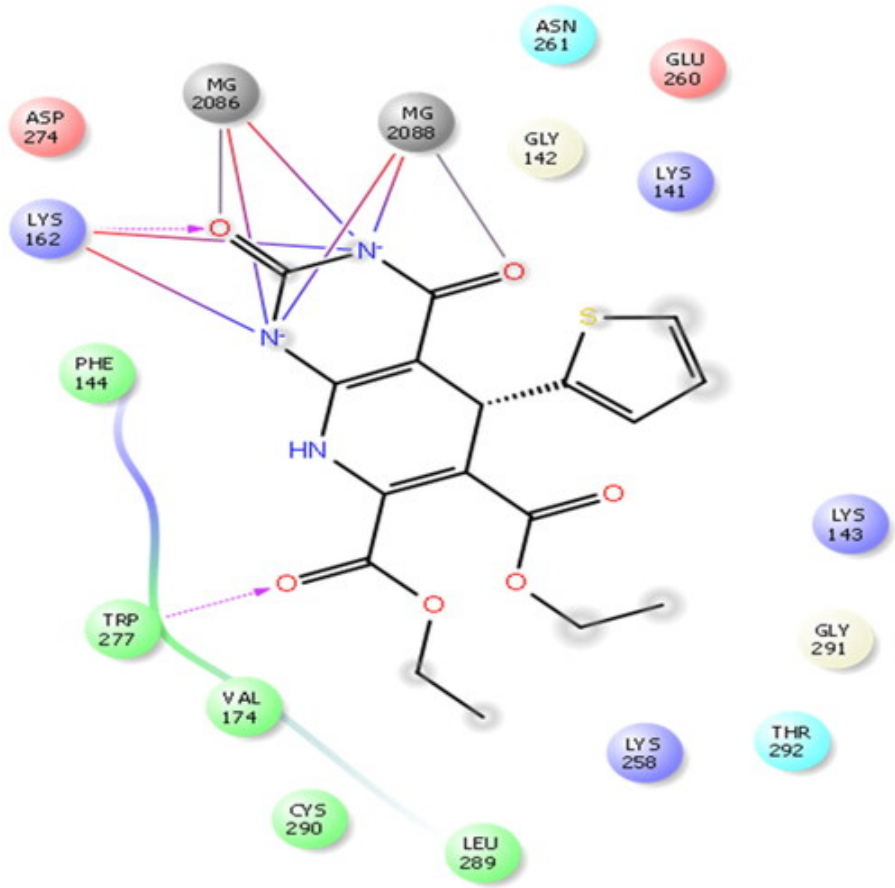

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Figure 4. **2D** image of compound **6h** on Aurora B kinase.

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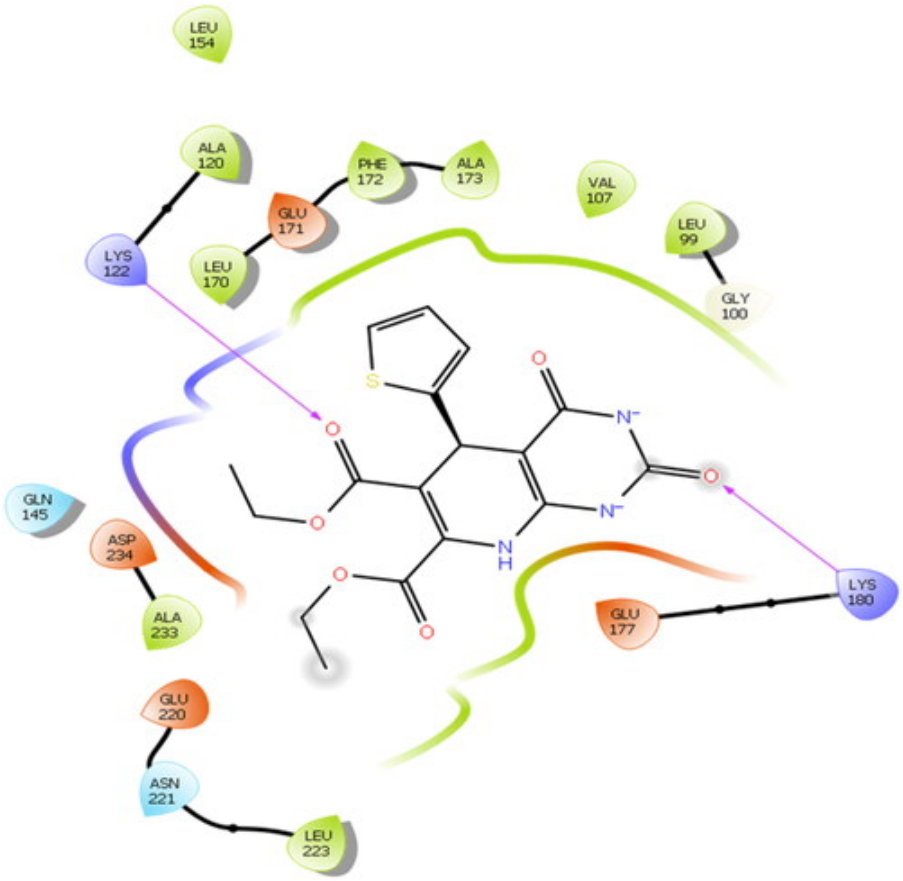

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Conclusion

A series of 7-amino-5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-carbonitriles (**4a-m**) and diethyl 5-(substitute phenyl/heteryl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6,7-dicarboxylate derivatives (**6a-h**) were synthesized by the efficient one-pot multicomponent reaction. This strategy involves the use of DIPEAc as a promoter and EtOH as an eco-friendly reaction medium. The merits of this protocol are mild reaction conditions and good yields of desired products in short reaction times. All the synthesized compounds evaluated for their anticancer activity against three cancer cell lines, namely lung carcinoma A549 cells, HepG2 cells and human cervical carcinoma epithelial HeLa cells. The results revealed that compounds **4b**, **6b**, **6d** and **6h** displayed good growth inhibitory activity against A549 and HepG2 cells. The potent compounds **6b**, **6d** and **6h** were also evaluated for their ability to inhibit aurora kinases A and B. Molecular modeling study was carried out in order to rationalize the *in vitro* anticancer results.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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