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Synthesis of Pyrimidine, Pyran and Pyridine Derivatives and their Biological Evaluation and Molecular Docking Studies as Potential CDK-1 and GSK-3 Inhibitors

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ABSTRACT

Thirty five new compounds belonging to pyrimidine, pyran and pyridine classes were synthesized and tested against two protein kinases, CDK-1/cyclin B and GSK-3. Among these compounds, 2-amino-4-(2-butyl-5-chloro-4H-imidazol-4-yl)-6-(4-hydroxyphenyl)pyridine-3-carbonitrile 17d was identified as the most potent inhibitor of these kinases. To identify possible binding modes, docking of the most active compounds into the active sites of CDK-1/cyclin B and GSK-3 was carried out. The molecular modeling studies brought to the fore stereochemistry and nature of the central core ring as major determinants affecting CDK-1/cyclin B and GSK-3 activity.

Keywords : CDK-1, GSK-3, Molecular Docking

I. INTRODUCTION

In the last couple of decades the protein kinases have been found to be involved in a set of major human diseases, due to which they have become a major target for screening of leads in the pharmaceutical industry [1], and as a result a variety of pharmacophores for kinase inhibition have been described. Most kinase inhibitors currently being developed target the ATP-binding site, a ubiquitous domain in nature, and mimic mainly the H-bonding motif of the aminopyrimidine ring of ATP. Among the 518 human kinases [2], two classes have been particularly explored: first, the cyclin-dependent kinases (CDKs) which are involved in regulation of the cell division cycle, apoptosis, neuronal cell physiology, pain signaling, transcription, RNA splicing and insulin release, among other activities [3, 4] The second class glycogen synthesis kinase-3 (GSK-3), is a family of kinases involved in cell cycle control, insulin action, apoptosis, neuronal cell death and developmental regulation, among other processes [5, 6]. Both families of kinases have been implicated in various human diseases such as cancers, Alzheimer's disease, diabetes etc. and therefore both have been extensively used as targets in the identification of small molecular weight inhibitors that could be of potential therapeutic value. A recent mini-review also indicates that CDK/GSK-3 inhibitors may emerge as effective therapeutic agents for proliferative renal diseases, furthering the prospect that these inhibitors may emerge as effective therapeutic agents in the near future [7].

Over the past decades, several groups have identified and characterized a fair number of potent CDK inhibitors (olomoucine, roscovitine, purvalanols, indirubins, aloisines, hymenialdisine, etc.). Many of these molecules are derived from marine organisms. The most advanced compound, R-roscovitine (CYC-202) a purine analog, is a CDK2 selective inhibitor, and currently in Phase 2 clinical trials against non-small cell lung cancer, breast cancer and various B-cell malignancies; in Phase 1 against various kidney

inflammations (glomerulonephritis); in pre-clinical, animal tests against Alzheimer's disease and stroke. Interestingly, many of these molecules are in fact dual inhibitors of CDKs and GSK-3, due mainly to the high degree of homology (~86%) between the ATP-binding sites of the two kinases. In view of the potential therapeutic value of such compounds, the search for new CDK/GSK-3 inhibitors continues and remains an exciting challenge.

In this paper, we report the synthesis and biological evaluation of a series of pyrimidines, pyrans and pyridines as new families of CDK-1/GSK-3 inhibitors. Molecular docking studies have been carried out so as to address new pharmacomodulations likely to enhance the inhibitory activity of these compounds.

II. CHEMISTRY

The synthesis of pyrazolopyrimidinethione derivatives (**2a-e**, Scheme 1) and 6-aminotetrahydro-pyrimidine-5-carbonitrile derivatives (**3a-g**, Scheme 1) was accomplished by following the basic mechanism of multicomponent Biginelli reaction. Acid catalyzed one pot cyclocondensation of 3-aminopyrazolone and the appropriate aromatic aldehyde and thiourea afforded **2a-e**, while reaction between malononitrile, the appropriate aromatic aldehyde and urea afforded **3a-g**. Molecules **3a-e** on hydrolysis afforded 4-amino-2-hydroxy-6-(4-aryl)pyrimidine-5-carboxamides (**5a-e,** Scheme 1). Reaction of molecules **3a-c, 3f** and **3g** with 4-((5-chloro-2-butyl-1H-imidazol-4-yl)methylene)-2-phenyloxazol-5(4H)-one **6** in pyridine afforded pyrimidine-5-carbonitrile derivatives (**7a-c and 7f, 7g**, Scheme 1) respectively.
Reaction of ethyl 2-(4-carboxyphenylazo)acetoacetate **8** with substituted aldehydes afforded chalcones (**9a-h**, Scheme 2), while 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde on reaction with substituted acetophenones afforded chalcone derivatives (**14a-f**, Scheme 3). Chalcone derivatives **9a-e**, **14a-e** on treatment with malononitrile in pyridine furnished the corresponding pyran derivatives (**11a-e,** Scheme 2) and (**16a-e,** Scheme 3) respectively. While chalcones **9d-h** and **14a-d**, **14f** on reaction with malononitrile in the presence of excess of ammonium acetate, afforded the pyridine derivatives (**12a-e**, Scheme 2) and (**17a-d**, **17f**, Scheme 3) respectively.

Spectral data (IR, ^{1}H-NMR and Mass) of all the newly synthesized compounds are in full agreement with the proposed structures.

III. BIOLOGICAL EVALUATION

Pyrimidines (**2a-e**, **5a-e, 7a-c and 7f, 7g**), pyrans (**11a-e, 16a-e**) and pyridines (**12a-e, 17a-d and 17f**) were assayed for kinase activity according to the methodology developed by the Cell Cycle Group of the Station Biologique CNRS, Roscoff, France. Kinases activities were assayed in Buffer A or C (unless otherwise stated), at 30^0 C, at final ATP concentration of 15 µM. Blank values were subtracted and activities calculated as p moles of phosphate were incorporated for a 10 min incubation. The activities are usually expressed in percentage of the maximal activity (in absence of the inhibitors). Controls were performed with appropriate dilutions of dimethylsulfoxide. GSK-3 was purified from porcine brain. It was assayed, following a 1/100 dilution in 1mg BSA/mL 10 mM DTT, with 5 µL, 40 µM GS-1 peptide as a substrate, in buffer A, in presence of 15 µM [γ-^{32}P] ATP (3000 Ci/mM; 1mCi/mL) in a final volume of 30 µL. After 30 min incubation at 30^0 C, 25 µL aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of Whatman P81phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid per liter of water. The wet filters were counted in presence of 1mL ACS (Amersham) scintillation fluid. CDK-1/ cyclin B was extracted in a homogenization buffer from M phase starfish (Marthasterias glacialis) oocytes and purified by affinity chromatography on P9^{CKShs1} sepharose beads, from which it was eluted by free P9^{CKShs1} as reported in the literature [8]. The kinase activity was assayed in buffer C, with 1 mg histone

H1/mL, in presence of 15 μM [γ-^{32}P]-ATP (3000 Ci/mmol; mCi/mL) in a final volume of 30 μL. After 10 min incubation at 30^0 C, 25 μL aliquots of supernatant were spotted onto P81phosphocellulose papers and treated as described above. The IC_{50} values were determined from dose-response curves; molecules with IC_{50} values are provided in Table 1.

The most active compound was **17d** (IC_{50}= 1μM) belonging to the pyridine class. Other pyridine derivatives **12d** and **17b** were found be more active towards CDK-1 than GSK-3. Compounds **2a** and **5e** belonging to the pyrimidine class demonstrated mild to moderate inhibitory action against both CDK-1 and GSK-3. Compounds **2d** and **7c, 7f, 7g** were slightly less active towards GSK-3, with 5- to 12-fold more activity towards CDK-1 than GSK-3. Pyran derivatives **11a-e** and **16e** also showed mild to moderate inhibitory action against CDK-1 and GSK-3.

IV. COMPUTATIONAL STUDIES

All the computational studies were carried out a Linux workstation with an AMD 3600+ processor and 3 GB physical memory running under the Red Hat Linux Enterprise WS4 operating system. The computations were carried out with the molecular modeling software – Sybyl v7.1 from Tripos Inc. St. Louis, MO [9], USA; InsightII v2005L from Accelrys Inc. San Diego, CA, USA [10]; MODELER 9v4 from University of California San Francisco, (UCSF), CA, USA, and GOLD Suite 4 from CCDC, UK [11].

Homology Modeling of CDK-1

The CDK-1 sequence (accession no. NP 001777) was obtained from NCBI. The homology model for CDK-1 was generated from CDK-2 crystal structures as templates. The sequence identity between CDK-1 and the CDK-2 templates is 66%. Consensus alignment was used rather than pair-wise sequence alignment as the models obtained from pair-wise alignment showed poor Profile-3D scores in the protein validation step. Blosum62 was used as the scoring matrix with the default value for the gap penalty. The CDK-1 sequence was aligned with sequences extracted from 22 CDK-2 crystal structures available in the Protein Data Bank (PDB) [12]. The PDB codes for the relevant CDK-2 crystal structures are 1B38, 1PXM, 1PXN, 1PXO, 1PXP, 1W8C, 2C5O, 2UZO, 2VTA, 2VTH, 2VTJ, 2VTL, 2VTM, 2VTN, 2VTO, 2VTP, 2VTQ, 2VTR, 2VTS, 2VTT, 2VU3 and 2VV9; these were the templates used for generation of the CDK-1 homology model. Thirty homology models were built using MODELER 9v4 [13-15]. The quality of all the models generated was judged by the energy based score DOPE (Discrete Optimized Protein Energy) [16] a part of the MODELER program, and the Profiles-3D [17] method (InsightII v2005L). DOPE is an atom based statistical potential in MODELER for evaluation of the model and structure prediction. The DOPE score of a protein is the conformational energy of the protein measuring the relative stability of one conformation with respect to the other conformations of the same protein. It helps in choosing the best model out of a set of predicted homology models generated by MODELER. Profiles-3D measures the compatibility of an amino acid sequence with a 3D protein structure. The Profiles-3D algorithm converts the 3D structure to a simplified 1D representation called the environmental string, which is then compared with the 1D amino acid primary sequence to check for the validity of the model and compatibility with the protein sequence. The Profiles-3D graph (Figure 2) portrays the properly folded and misfolded region(s) in the protein structure by performing the Eisenberg analysis of the model [18, 19]. The energy minimized explicit model of CDK-1 was then used for the docking studies (Figure 1).

Docking Simulations

The goal of molecular docking studies was to predict the binding poses of the CDK-1 inhibitors. There are several crystal structures and molecular modeling studies reported for CDK-2 inhibitors. However, matching studies for inhibitors of the CDK-1 protein

[20, 21] are far and few between. The inhibitors are believed to bind in the ATP-binding cleft. Using the homology model for CDK-1 and the crystal structures for GSK-3, the poses and the interactions between the molecules (Table 1) and the CDK-1 model was studied. These poses were carefully analyzed to identify possible modifications that may be made to the ligands to improve their activity.

For the modeling studies the molecules synthesized by us were grouped into the following classes:

A. – Pyrazolopyrimidinethione analogs,
B. – Substituted pyridinylmethyl diazenyl benzoic acids,
C. – Substituted pyranylmethyl diazenyl benzoic acids,
D. – 2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-6,6-phenylnicotinonitrile,
E. – 4-amino-6-(substituted tetraisoquinolinyl)-2-hydroxypyrimidine-5-carboxamide,
F. – (S,Z)-4-(4-((2-butyl-4-1H-imidazol-5-yl)methylene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)-2-hydroxy-6-phenyl-1,6-dihydropyrimidine-5-carbonitrile and
G. – N-(4-(6-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl-5-cyano-4H-pyran-2-yl)phenyl) benzenesulfonamide.

The molecules were built with the Builder module in Sybyl7.1. All the isomeric forms (E/Z & R/S) of the molecules were built and studied. The ligand geometries were optimized by energy minimization using the Powel gradient method with the Tripos force field and Gasteiger Hückel charges for all atoms, until a gradient of 0.01 kcal/mol/Å was reached. The dielectric constant was set to 1.0.

The parameters in GOLD modified/optimized for the docking studies were: (a) the dihedral angles of the ligand rotatable bonds; (b) the ligand ring geometries (flipping ring corners); (c) the dihedral angles of protein OH and NH_3 groups; and (d) mappings of the H-bond fitting points. At the start of a docking run, all these variables were randomized. The docking was carried for 40 GA runs, which was optimum to reproduce the pose of the ligand in the crystal structure (PDB code 2VV9). Most of the other GA parameters like the population size and the genetic operators were left at their default values. The docking protocol was validated by reproducing the binding pose of 2-{4-[4-({4-[2-methyl-1-(1-methylethyl)-1H-imidazol-5-yl]pyrimidin-2-yl}amino)phenyl]piperazin-1-yl}-2-oxoethanol found in the crystal structure (PDB Code 2VV9) which is shown in Figure 4. The validated protocol was then used for generating the docking poses for our CDK-1 inhibitors.

The docking solutions obtained by GOLD were then subjected to an energy minimization procedure that was limited to the ligand and active site residues defined by a 7Å radius from the ligand (centre). The amino acid residues in the active site were free to flex while the remaining part of the protein was held fixed. The minimization was carried out till an energy gradient of 0.001 kcal/mol/Å was achieved. The poses were evaluated based on consensus scoring using various scoring functions like ASP score [22], PLP1 [23], PLP2, LigScore 1, 2 [24], Jain [25], PMF [26], PMF04, and Ludi 1, 2, 3 [27]. The various scores are given in Table 2.

In a similar manner the same set of molecules were subsequently docked into the GSK-3 ATP binging site, with a prior validation of the docking protocol used on the crystal structure of 3-indolyl-4-arylmaleimide inhibitor in complex with the GSK-3 enzyme (PDB code 1R0E). Post docking geometry, optimization for the inhibitors followed by multiple scoring and consensus analysis was carried out in a similar way to that adopted for CDK-1 enzyme (Table 3).

V. RESULTS AND DISCUSSIONS

The best homology model (amongst 30 models) for CDK-1 (Figure 1) generated using MODELER was the one with the lowest energy value (DOPE) of –

35550.37 and with a score of 135.67 for Profiles-3D. Structure refinement of the homology model was done by energy minimization to a gradient of 0.001 kcal/mol/Å; the geometry optimized structure is shown in Figure 1. The Ramachandran plot for the model is depicted in Figure 3, with 99% of the , values falling in the allowed regions.

The general 3D organization of the CDK-1 protein is typical of the kinase family. The protein has a bilobar topology with one lobe mainly comprising helices and forming the C-terminal end while the N-terminal domain is composed of -sheets. The inhibitors of CDK-1 are primarily believed to bind to the ATP binding cleft present in the N-terminal domain. The residues that define the binding site for the inhibitors are lined by the -strands all around the pocket. The binding site comprises of (i) a hydrophilic region consisting of residues Glu12-Tyr15; (ii) a region containing residues Asp128, Lys130, Gln132 and Asn133 that are involved in electrostatic interactions with the inhibitor, with Asp146 capable of forming H-bonds, and (iii) the Phe80-Leu83 segment which lines the groove and is involved in hydrophobic interactions. The inhibitors were designed as purine base analogs with an expectation that they will bind in the ATP binding site.

Binding site analysis of the CDK-1 inhibitors

The right choice of parameters used for the docking of the molecules in the binding site of CDK-1 is shown by reproduction of the crystal structure pose (Figure 4) of the ligand (PDB code 2VV9) in the active site of CDK-2. The docking studies enabled a thorough binding site analysis to be carried out that helped to identify the different interactions between CDK-1 and the six classes of molecules. The molecules interact with the ATP binding site through H-bond and hydrophobic interactions. The H-bonding is seen to vary with the electropositive groups on the core of the molecule. However a common hydrophobic interaction pattern is seen across all the classes involving residues Ile10, Val18, Ala31, Val64, Phe80, Phe82 and Leu135, with some specific interactions being restricted to the individual classes. The studies revealed that the (R) isomers of Class [A] molecules have comparatively a higher degree of binding to the CDK-1 protein, which is understood to arise from H-bond interactions between the backbone amide carbonyls of Glu81 and Asp146 and the nitrogen on pyrazole ring and the thio group respectively a trend that has been exhibited by the Ludi Scores shown in Table 2. The molecules being more aromatic the Ludi scores provide a better rationale. In addition a weak stacking interaction of the pyrimidine ring with Phe80 is also seen as shown in Figure 5 [A]. The only active molecule belonging to Class [B] was docked, so determination of a trend in the activity with the scoring functions is not feasible. Examination the interaction pattern of the Class [B] with the protein the most active molecule exhibit H-bonding with the amide carbonyl of Leu83 and the hydroxyl group of Thr14 through the 6-amino, the 5-cyano and the carboxylic group on the inhibitors as depicted in Figure 5 [B]. Comparing the scores for the Class [C] molecules over the stereoisomerism the E/R isomers are looked into to have greater binding affinity than the E/S isomers. The Class [C] molecules show a single H-bond interaction with the -carboxylic function on Asp146, as compared to that seen with Class [B] molecules. Molecules in Class [C] exhibit similar hydrophobic interactions as Class [B], the dissimilarity is in the central rings which is the differentiating criteria of the two molecular classes, as seen in Figure 5 [C]. The Class [D] molecules bind to the CDK-1 through a H-bond formed by the chloro group with -amino group of Lys33 (Figure 5 [D]), while the 5-carboxamide function of Class [E] molecules, show H-bond interaction with the side chains of Lys133 and Asp146 (Figure 5 [E]). Of the active molecule docked the 1R, 4R isomers seem to be favored over other chiral analogs for this particular molecule in the Class [E]. In the Class [F] molecules, the imidazole nitrogen and the -carboxylate of Asp146 interact through an H-bond; the S isomers

being favored in interaction with CDK-1. The analysis reveals no specific H-bonding for Class [G] molecules within the ATP binding site of CDK-1.

The docking studies of pyrrolo[2,3-a]carbazoles [21] as reported in the literature indicate that the most potent molecules exhibit H-bond interactions with (i) the □-amino group of Lys33, (ii) the amide NH group of Thr14 with the carbonyl oxygen of the inhibitor and (iii) the □-amino group of Lys130 with the carbonyl of the carboxyl ester group of the inhibitor. The phenyl ring nestles in the hydrophobic pocket composed of Val18, Lys33 and Phe80. The substituted aminobenzimidazolopyrimidine [20] class of CDK-1 inhibitors form H-bonds through the sulphonamido group with □-amino group of Lys89 and the □-carboxylate group of Asp86, groups like –Br, or $–CF_3$ on the nucleus are found to be accommodated well by hydrophobic interactions within the region defined by Phe80, Val64, Leu135, and Ala145. Molecule **17d** belonging to Class [D] exhibits a binding pattern that is similar to that reported in the literature [20, 21].

Binding site analysis of GSK-3 inhibitors

The pose of the inhibitor (PDB code 1R0E) obtained by docking and the crystal structure are both shown in Figure 6, which reveals the quality and accuracy of the docking protocol used in this studies. The classes tested as GSK-3 inhibitors show affinity for the ATP binding site by virtue of their H-bonding and the hydrophobic interactions of varying degrees. The hydrophobic interactions mainly arise from contract with non-polar residues lining the pocket and lying adjacent to the ligand like Ile62, Phe67, Val70, Ala83, Tyr134, Val135, Tyr140 and Leu188 however specific interactions are reserved for the individual classes. The S-isomers belonging to Class [A] molecules exhibit a trend of biological activity with the docking scores especially with scoring functions like PLP1/2 and PMF/04. However the scoring function Ludi tends to make a controversial explanation with respect to the R-isomers. The nitrogen of the pyrazole ring of Class [A] molecules makes an H-bond with the □-carboxamide of Asn186, in addition a weak stacking of the pyrimidine ring with Phe86 is seen, as shown in Figure 7[A]. Not many molecules are active within Class [B] and hence the docking analysis is carried for the most active molecule. The central pyridine ring of Class [B] molecules weakly stacks with Phe67. No H-bonds are visible between the Class [B] molecules and the ATP binding site. The E/R isomers of Class [C] molecules are found to have higher scores compared to the E/S isomers. A consensus is exhibit by PLP1/2, PMF/04, ASP and Ludi 1/2/3 scoring functions. This could be due to the H-bond between the 4-hydroxy-4-phenyl group and the backbone carbonyl as well as with the amido group of Val135; other factors include a weak stacking of the pyran ring with Phe67 and a hydrophobic interaction of the 4-phenyl group with hydrophobic residues in its vicinity. Weak electrostatic interactions are also evident between the 4-carboxylate group on the phenyl group and the side chain guanidino group of Arg141. The central pyran ring of Class [C] molecules has a slight twist boat conformation which is a salient feature and can be associated with the 10-fold rise in the activity over molecules of Class [B] which have a central planar pyridine ring. The trend in scores for the molecules of Class [D] follows the trend in the biological activity for this class. H-bonding is seen between the 2-amino group and the □-carboxamide group of Asn186 and a second weak H-bond between 3-cyano group and □-carboxylate group of Asp200. Hydrophobic interactions are seen between the 2-butyl group and the cleft formed by Val70, Leu132, Ala83 and also between the 6-phenyl group and Tyr140. The R, R isomers of class [E] molecules are scored higher than the other isomeric forms. There is a weak stacking interaction between the 2-pyrimidine ring and Phe67. H-bonds between the 2-hydroxy group of Class [F] and the side chain carbonyl groups of Gln185 and Asn186 strengthen the interaction. A feeble trend in the score is seen with the ER-isomers with respect to the activity. The sulfonamide group in class [G] molecules shows H-bonds with the side chain

guanidino group of Arg141 which is complemented by a weak stacking interaction between Phe67 and the pyrimidine ring.

A number of research papers has been published on molecular modeling studies of different classes of molecules like pyrazolo[3,4-b]pyrid[az]ine derivatives [28], and 3-anilino-4-phenylmaleimides [29] and pyrazolopyrimidine [30] with the GSK-3 enzyme. All these works report H-bond interactions between the ligand and the hinge region involving Val135 and Asp133 as the core interaction; this is also seen in our Class [C] molecules.

VI. CONCLUSIONS

Various pyrimidine, pyran and pyridine derivatives (**2a-e**, **5a-e**, **7a-c and 7f**, **7g**, **11a-e**, **12a-e**, **16a-e** and **17a-d**, **17f**) have been synthesized in reasonable yield. Screening for protein kinase (CDK-1 and GSK-3) inhibitory activities revealed that compound **17d** is the most potent against both protein kinases.

CDK-1 is an important target in the area of cancer and the fact that the crystal structure of CDK-1 is currently unavailable makes it necessary to build a homology model which will serve the goal of drug design. The 66% homology between CDK-1 and CDK-2 makes it a straightforward task to build a theoretical model of CDK-1 on the basis of the 22 crystal structures of CDK-2. Furthermore, since there is a precedence of the binding poses of some inhibitors with CDK-1, but none with CDK-2, a consensus scoring scheme is necessary to evaluate and rank the poses. The docking studies helped to throw light on the differences in the activities for the different class and the stereo isomers (R/S & E/Z) of the molecules synthesized.

Experimental protocols

General

Melting points were determined using a Kö-fler melting point apparatus and are uncorrected. Infra-Red spectra were recorded using a Perkin Elmer 1600 FT Spectrophotometer and only characteristic peaks are reported in cm^{-1}. ^1H NMR spectra were taken in DMSO-d_6, with TMS as internal standard using a Bruker AC-300F NMR Spectrometer (300 MHz). Chemical shifts (δ) are reported in parts per million (ppm) of the applied field. Mass spectra (CG/MS) were recorded on a Agilent Technologies 6890 / 5972A mass spectrometer. Elemental analysis was carried out using Heraus CHN rapid analyzer. Reactions were monitored over silica gel-G (Merck) TLC plates and spots were visualized by iodine vapor or by illumination with ultraviolet light (254 nm).

General procedure for the synthesis of 3-amino-4,5-dihydro-4-aryl)-1H-pyrazolo[3,4-d]pyrimidin-6(7H)-thione **(2a-e)**.

An equimolar mixture of 3-aminopyrazolone (0.01 mol), appropriate aldehyde (0.01 mol) and urea (0.01 mol) in methanol was heated under reflux for 8 hours in the presence of a catalytic amount of conc. HCl. The precipitated solid was filtered, dried and crystallized from the ethanol.

General procedure for the synthesis of 3-(2-butyl-4-chloro-1H-imidazol-5-yl)-1-(aryl)prop-2-en-1-one (**14a-f**).

A mixture of 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde (**13**) (0.01 mol) and the appropriate aldehyde (0.01 mol) in ethanol (20 ml) containing sodium hydroxide (3 ml of a 50% w/v aqueous solution) was stirred for 72 hours at room temperature. The reaction mixture was poured on to crushed ice and neutralized with diluted HCl. The solid mass that separated out was filtered, washed with water and crystallized from ethanol.

General procedure for the synthesis of 2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-6-aryl-4H-pyran-3-carbonitrile (**16a-e**).

A mixture of the appropriate 3-(2-butyl-4-chloro-1H-imidazol-5-yl)-1-phenylprop-2-en-1-one (**14a-e**)

(0.01 mol) and malononitrile (0.01 mol) in pyridine (20 ml) was heated under reflux for 10 hours. The reaction mixture was cooled, poured on to crushed ice and neutralized with diluted HCl. The solid mass that separated out was filtered, washed with water and crystallized from ethanol.

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