IJDD

International Journal of Drug Discovery

ISSN: 0975–4423 & E-ISSN: 0975–914X, Vol. 3, Issue 2, 2011, pp-78-87

Available online at http://www.bioinfo.in/contents.php?id=24

INTRODUCING "SYNTHESIS ROUTE-BASED HIT IDENTIFICATION APPROACH" AS A TOOL IN MEDICINAL CHEMISTRY AND ITS APPLICATION IN INVESTIGATING THE ANTIPROLIFERATIVE AND ANTIMICROBIAL EFFECTS OF 2-AMINOPYRIMIDINE DERIVATIVES

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Received: June 18, 2011; Accepted: July 18, 2011

Abstract- A drug-like molecule could be considered as a congregation of small fragments which are chemically linked to each other and the biological effect of such molecule is due to the contribution of these small fragments working together. This molecular unit has been constructed gradually through a multi-step synthesis route. Assessment of the biological activity of all the intermediary compounds of a multi-step synthesis process helps to track down the emergence of biological activity alongside the synthesis process and the results provide valuable clues about the SAR of the compounds. Furthermore unanticipated findings may be encountered if such approach is exercised. A two-step synthesis route was employed to make a few 2-aminopyrimidines from their corresponding enamine precursors. Both aminopyrimidines and enamines were subjected to antiproliferative and antimicrobial tests. Hit compounds with acceptable activities were found in both aminopyrimidine and enamine series. With respect to antimicrobial activity, enamine series were found more promising. This experiment sets an example for the applicability of our new approach called "Synthesis route-based hit identification" as a tool in medicinal chemistry to increase the hit rate in drug discovery processes.

Keywords: synthesis route-based, hit identification, aminopyrimidine, medicinal chemistry, lead discovery

Introduction

Different methods of hit identification have been introduced to the field of lead discovery in medicinal chemistry during the past few decades such as combinatorial chemistry (High Throughput Screening, HTS),[1,2] Virtual Screening (VS)[3,4] and Fragment-Based Drug Discovery (FBDD)[5-7]. On the other hand, computational and computer-aided tools have been well exploited in medicinal chemistry. The ultimate goal of all these methods is shortening the time and decreasing the cost of drug discovery process.

Regardless of their biological effects, drug-like molecules could be considered as chemical entities which are synthesized through a synthetic route during which small pieces of chemicals undergo different reactions including condensation, functional group transformation, etc.

At the end of synthesis process, a drug-like molecule is obtained which could be considered as a congregation of small pieces (fragments) which are linked together. The drug-like candidate such obtained, owes its biological effect to the contribution of groups which are now working together as a unit.

In conventional medicinal chemistry studies, new structures are studied using various tools such as computational chemistry and QSAR to determine the pharmacophores in the compounds and the results of these studies will help to design and find newer structures [8, 9]. In these types of studies no attention is paid to the route of synthesis which has been used to make the compound. From chemical point of view the total structure of drug-candidate has been formed and constructed step by step during the multiple steps of synthesis route.

In the same way, from medicinal chemistry standpoint, the pharmacophore group(s) in a drug-candidate is emerged through the step by step transformation of the chemicals which have been used as the precursors of final drug candidate. Therefore if the assessment of biological activity is not exclusively dedicated to the final drug-like candidate and biological evaluation for each intermediate precursors are also implemented, the emergence of biological activity could be traced during the step by step chemical transformations through the

synthesis route process. The result of this type of approach could help the process of hit identification and better understanding of structure activity studies and pharmacophore determination.

The main objective of the present work was putting the above approach into examination, through the synthesis of a group of aminopyrimidine derivatives for their cytotoxic and antimicrobial activities. The idea is close to the rationale behind the fragment-based hit identification in which large molecules can be considered as the combination of two or more "Fragments" that contain all the features necessary for binding to the target protein. Fragments and large molecules have to obey the same set of molecular recognition rules when they bind to a target protein. During a binding event both fragments and large molecules have to overcome an entropic barrier associated with the loss of rigid body entropy on binding to the target. This barrier has only a small dependence on molecular size. Fragments can afford the entropic cost of binding only by using a high proportion of their atoms to form interactions. Therefore, fragments are considered efficient binders (high binding energies per unit of molecular mass) [5, 10].

Optimization of a week-binding fragment into a potent lead molecule can usually be achieved by adding extra atoms onto the fragment core. In 2001, Hann et al, [11] used a simple model of ligand-receptor interactions to investigate the impact of molecular complexity on binding to protein and on the probability of identifying hits or leads. Hann's model suggested that large and complex molecules have a great probability of forming suboptimal or repulsive interactions with the receptor. By contrast, fragments have a lower chance of forming mismatches and as a consequence, hit rates from Fragment Screening (FS) are often higher and the hits identified are generally more ligand efficient.

In the present study 2-aminopyrimidine nucleus was chosen as an attractive fragment to put the above approach into examination. Aminopyrimidine ring is part of the structure of many bioactive compounds (Fig. 1). A group of nonclassical dihydrofolate reductase inhibitors contain 2-aminopyrimidine in their structure [12].

Imatinib and Nilotinib are newly developed aminopyrimidine derivatives which conduct much of their activity by preventing activation of thyrosine kinase through autophosphorylation resulting in target-specific inhibition of cell proliferation [13]. 2-aminopyrimidine ring is also found in many cytotoxic natural products. Meridianins are marine alkaloids isolated from the south Atlantic tunicate Aplidium meridianum. Meridinanins A-G were successfully evaluated for their ability to inhibit various protein kinases and to display antitumor activity [14].

Variolin B is another marine alkaloid with aminopyrimidine ring and antiproliferative effect [15].

2-aminopyrimidine containing compounds have also been the subject of a few recently published patent applications regarding potent HSP-90 [16] and kinase inhibitors [17]. A two step synthesis process was used for the preparation of a few 2-aminopyrimidine derivatives: A group of methylketones are converted to their corresponding enamines in the first step and the final aminopyrimidines are obtained by cyclization of the enamines in the second step.

The aminopyrimidine derivatives and their corresponding enamine intermediates thus obtained, were all subjected to clonogenic cytotoxicity test against HepG2 cell line as well as antimicrobial tests against a group of selected bacteria and fungi.

Chemistry

The synthetic strategy to synthesize the 2-aminopyrimidines (**3a-h**) is depicted in **scheme.1**. Methylketones (**1a-h**) were allowed to react with dimethylforamide-dimethylacetate (DMF-DMA) to produce 3-(dimethylamino)-1-aryl-2-en-1-ones (**2a-h**) [18]. The key intermediates could be further condensed with guanidine hydrochloride to form the corresponding aminopyrimidine ring system (**3a-h**) [18] Structure confirmation of the synthesized intermediates and the final products was performed using IR, NMR, and Mass spectrometry.

Results and Discussion

The antiproliferative activities of enamines(**2a-h)** and their corresponding aminopyrimidines (**3a-h)** against five human cell lines were evaluated through a clonogenic assay procedure. Doxorubicin was used as a reference to evaluate the potency of the tested compounds. Seven different concentrations of each compound were used in the screening and the IC_{50} values were determined. The results are given in Table 1.

In another attempt, the enamines (**2a-h)** and aminopyrimidines (**3a-h)** were tested for their antimicrobial and antifungal activities against a selected group of pathogenic bacteria as well as Candida albicans. The results are presented in Table 2.

A few major points are apparent when antiproliferative activities of enamines are compared with those of the corresponding aminopyrimidines: First, compounds with antiproliferative activity are found in both enamines and aminopyrimidines series. Second, the order of antiproliferative activities is different for the two series. The order of activities for enamine series is **2b> 2c> 2d> 2g> 2h> 2e> 2a> 2f** and the order of activities for aminopyrimidines series is **3d> 3e> 3c> 3b> 3a> 3f> 3g> 3h**.

Third, the antiproliferative activity of some of the enamine derivatives is comparable with the activity of some aminopyrimidines.

Forth, for half of the compounds (**a, d, e, f**), the aminopyrimidines are more potent than the corresponding enamine reactant while for the other half (**b, c, g, h**), enamines are more potent than corresponding aminopyrimidines.

Different orders of antiproliferative activity for the two series are likely due to the different mechanisms of

action and/ or different interaction of these compounds with their biological targets.

Global physicochemical molecular parameters such as CLogP, refractivity, polarizability, molecular volume, and surface area for the compounds in both enamines and aminopyrimidines series were calculated and the results are presented in Table 3.

Despite the fact that the order of physicochemical molecular properties for enamine series is the same as that of aminopyrimidines, the difference in the order of antiproliferative activity for the two series reemphasizes the difference between these two groups of compounds in their modes of action.

In light of the above mentioned findings, active enamines and active aminopyrimidines could be both considered as promising fragments and they would be proper subjects to be used in fragment based drug discovery approaches. If the biological assays had been solely limited to target aminopyrimidine derivatives, active enamine fragments would have not been distinguished.

Evaluation of antibacterial and antifungal activities of both enamines and aminopyrimidines series, reveals another scenario: as it is apparent from Table 2, none of the 2-aminopyrimidines **3a-h** shows significant antibacterial/ antifungal activity but compounds **2c** and **2f** among the enamine series exhibit fair activities which could qualify them as antimicrobial fragment candidates. This is another evidence which supports the idea of synthesis route-based hit identification approach suggested in this article. In general fragment-based hit identification strategy has the advantage of making the process of hit identification more cost-effective and more efficient compared to the conventional high throughput screening (HTS) approach. Figure 2 shows the comparison between fragment-based hit identification and HTS approaches as it is depicted by Erlanson et al [19]. Synthesis route-based hit identification could be regarded as a complementary mean along with fragment-based approach. Figure 3 is a simplified illustration of the principle of this approach which is reproduced based on the figures suggested by Erlanson et al. In a multi-step synthesis process, the starting reactants are gradually transformed to intermediates and intermediates are converted to the final target compounds. Pharmacophore group is also formed gradually during these structural transformations. Therefore biological evaluation of each components of a multi-step synthesis process could help to map out the biologically active moieties (fragments) in the intermediary and/ or final structures. Higher hit rate could be expected in a synthesis route-based hit identification if more diverse bioassays are conducted for biological evaluation of intermediates and final compounds in a multi-step synthesis process. The weak absolute potency of fragments may be challenging in conventional bioassay tests but the recently approved biophysical screening methods such as NMR and crystallography could be priceless tools to overcome these problems.

Conclusion

The exponential growth of attractive novel molecular targets for potential drug therapy calls for more efficient and rapid hit identification methods in the process of drug discovery.

Automated methods of compound synthesis and biological evaluation, high throughput screening (HTS), virtual screening and fragment-based strategies have been introduced to fulfill this need. The ever-growing cost of drug discovery processes from one hand and rising environmental concerns about the danger of production of chemicals by humans from the other hand, call for more efficient and environmentally acceptable strategies in medicinal chemistry.

"Synthesis route-based hit/pharmacophore identification" is a trouble-free and easy-to-implement method which could be considered as complementary tool in fragment-based drug discovery. In view of the fact that in this approach all the intermediate chemicals as well as the final compounds in a multi-step synthesis route are subjected to biological evaluation, higher hit rate will be expected at lower cost. When a group of compounds are considered for synthesis in medicinal chemistry, the intermediary reactants are often biologically ignored and no attention is paid to their biological activity. Synthesis route-based hit identification strategy may help to avoid this negligence to some extent and therefore it could be considered as a recycling tool along with all synthesis efforts in medicinal chemistry.

Experimental

General

All evaporations were carried out in vacuo with a rotary evaporator. Melting points (ºC) were determined by capillary method on an Electrothermal melting point apparatus. Infrared spectra were recorded as thin films on KBr plates with υ_{max} in inverse centimeters. Nuclear Magnetic Resonance spectra for proton (^{1}H NMR) were recorded on a Bruker DRX-Avance (500 MHz) spectrometer. Chemical shift values are expressed in ppm (parts per million) relative to Tetramethylsilane (TMS) as internal standard; s=singlet, d=doublet, dd=double doublet, t=triplet, q=quartet, m=multiplet, br s=broad singlet. Thin layer chromatography (TLC) was performed on Whatman Sil G/UV_{254} silica gel plates with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Mass analyses were performed with an Agilent 6400 Series equipped with an electrospray ionization source (capillary voltage at 4000V, nebulizing gas temperature at 300 ºC, nebulizing gas flow at 12 L/min). All the compounds were analyzed for C, H, N, and S on a Costech model 4010 and agreed with the proposed structures within ±0.4% of the theoretical values.

Representative procedure for the preparation of (2a-h) [18]

To a solution of acetophenone (84 mmol) in DMF (16ml), was added DMF-DMA (84 mmol) and the solution was heated under reflux for 24 h. Brine (25 ml) was added to the reaction mixture after cooling and the mixture was

then extracted with CH_2Cl_2 (3×25 ml). The combined organic fractions were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (12 ml), followed by addition of n-hexane (100 ml). The precipitate thus obtained was filtered and dried to give the pure product **2a** as a yellow solid (89%).

3-dimethylamino-1-(phenyl)prop-2-en-1-one (2a)
Yield 89%, mp 87-89 ºC; IR (KBr) υ_{max} 3401, 3011, 1637, 1577, 1548, 1541, 1426, 1355, 1238, 1211 1048, 897 767, 750, 663 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.87 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 5.77 (d, 1H, *J* = 12.27 Hz, COCH=CH), 7.37-7.40(m, 2H, ArH), 7.42-7.45(m, 1H, ArH), 7.67 (d, 1H, *J* = 12.27 Hz, COCH=CH), 7.83-7.85 (m, 2H, ArH); ESI-MS: Observed ($M+H^+$) = 176. Calcd for $C_{11}H_{13}NO$ = 175.23. Anal. Found: C, 75.37; H, 7.49; N, 7.97. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99 %.

3-dimethylamino-1-(4-fluorophenyl)prop-2-en-1-one (2b)
Yield 78%, mp 77-79 ºC; IR (KBr) υ_{max} 3067, 2924, 1643, 1595, 1547, 1440, 1359, 1240, 1155,1125, 1061, 903, 851, 786 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.87 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 5.77 (d, 1H, *J* = 12.23 Hz, COCH=CH), 7.17-7.22(m, 2H, ArH), 7.67 (d, 1H, *J* = 12.23 Hz, COCH=CH), 7.90-7.94 (m, 2H, ArH); ESI-MS: Observed ($M+H^+$) = 194. Calcd for $C_{11}H_{12}FNO$ = 193.22. Anal. Found: C, 68.45; H, 6.24; N, 7.25. Calcd for $C_{11}H_{12}FNO$: C, 68.38; H, 6.26; N, 7.25 %.

3-dimethylamino-1-(3-thienyl)prop-2-en-1-one (2c)
Yield 85%, mp 92-94 ºC; IR (KBr) υ_{max} 2997, 2804, 1662, 1520, 1478, 1417, 1262, 1035, 880, 759, 721 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.85 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 5.68 (d, 1H, *J* = 12.33 Hz, COCH=CH), 7.44-7.45(m, 1H, ArH), 7.46-7.48 (m, 1H, ArH), 7.59 (d, 1H, *J* = 12.33 Hz, COCH=CH), 8.12-8.13 (m, 1H, ArH); ESI-MS: Observed ($M+H^+$) = 182. Calcd for $C_9H_{11}NOS$ = 181.25. Anal. Found: C, 59.65; H, 6.10; N, 7.71; S, 17.74 . Calcd for $C_9H_{11}NOS$: C, 59.64; H, 6.12; N, 7.73; S, 17.69 %.

3-dimethylamino-1-(4-trifluoromethylphenyl)prop-2-en-1-one (2d)
Yield 90%, mp 101-103 ºC; IR (KBr) υ_{max} 1667, 1581, 1461, 1321, 1064, 1019, 854, 750, 711, 654 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.90 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 5.80 (d, 1H, *J* = 12.20 Hz, COCH=CH), 7.72-7.75 (m, 3H, COCH=CH, ArH), 8.02 (d, 2H, *J* = 8.09 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 244. Calcd for $C_{12}H_{12}F_3NO$ = 243.22. Anal. Found: C, 59.18; H, 4.98; N, 5.78. Calcd for $C_{12}H_{12}F_3NO$: C, 59.26; H, 4.97; N, 5.76 %.

3-dimethylamino-1-(4-phenylphenyl)prop-2-en-1-one (2e)
Yield 82%, mp 135-137 ºC; IR (KBr) υ_{max} 1637, 1562, 1531, 1413, 1049, 898, 763, 692 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.89 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 5.83 (d, 1H, *J* = 12.24 Hz, COCH=CH), 7.36 (t, 1H, *J* = 7.48 Hz, ArH), 7.45 (t, 2H, *J* = 7.53 Hz, ArH), 7.67-7.71 (m, 5H, COCH=CH, ArH), 7.94 (d, 2H, *J* = 8.23 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 252. Calcd for $C_{17}H_{17}NO$ = 251.32. Anal. Found: C, 81.25; H, 6.80; N, 5.56. Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57 %.

3-dimethylamino-1-(pyridin-4-yl)prop-2-en-1-one (2f)
Yield 81%, mp 113-115 ºC; IR (KBr) υ_{max} 1643, 1564, 1523, 1431, 1278, 1132, 784, 697, 661 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.90 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 5.79 (d, 1H, *J* = 12.16 Hz, COCH=CH), 7.70-7.76(m, 3H, COCH=CH, ArH), 8.63 (d, 2H, *J* = 5.82 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 177. Calcd for $C_{10}H_{12}N_2O$ = 176.22. Anal. Found: C, 68.09; H, 6.86; N, 15.95. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90 %.

3-dimethylamino-1-(pyridin-3-yl)prop-2-en-1-one (2g)
Yield 78%, mp 80-82 ºC; IR (KBr) υ_{max} 2931, 1644, 1576, 1540, 1414, 1252, 1069, 905, 783 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.90 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.82 (d, 1H, *J* = 12.20 Hz, COCH=CH), 7.40-7.43 (m, 1H, ArH), 7.72 (d, 1H, *J* = 12.20 Hz, COCH=CH), 8.15-8.18 (m, 1H, ArH), 8.61 (dd, 1H, J_1 = 4.80, J_2 = 1.61 Hz, ArH), 9.02 (d, 1H, *J* = 1.70 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 177. Calcd for $C_{10}H_{12}N_2O$ = 176.22. Anal. Found: C, 68.20; H, 6.85; N, 15.85. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90 %.

3-dimethylamino-1-(pyridin-2-yl)prop-2-en-1-one (2h)
Yield 75%, mp 128-130 ºC; IR (KBr) υ_{max} 1648, 1541, 1368, 1072, 912, 788, 767, 685 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 2.86 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 6.33 (d, 1H, *J* = 12.64 Hz, COCH=CH), 7.44-7.47 (m, 1H, ArH), 7.76 (d, 1H, *J* = 12.64 Hz, COCH=CH), 7.85-7.88 (m, 1H, ArH), 7.94 (d, 1H, *J* = 7.78 Hz, ArH), 8.58-8.59 (m, 1H, ArH); ESI-MS: Observed ($M+H^+$) = 177. Calcd for $C_{10}H_{12}N_2O$ = 176.22. Anal. Found: C, 68.20; H, 6.85; N, 15.90. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90 %.

Representative procedure for the preparation of (3a-h) [18]
To a solution of compound (**2a**) (2.68 mmol) in isopropanol (13.5 ml) were added sodium methoxide (10.7 mmol) and guanidine hydrochloride (4.02 mmol) and the solution was heated under reflux for 48 h. Distilled water (25 ml) was added to the reaction mixture after cooling and the mixture was then extracted with EtOAc (3×15 ml). The combined organic fractions were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (2 ml), followed by addition of n-

hexane (25 ml). The precipitate thus obtained was filtered and dried to give **3a** (75%).

4-phenylpyrimidine-2-amine (3a)
Yield 75%, mp 162-164 ºC; IR (KBr) υ_{max} 3320, 3155, 1657, 1562, 1551, 1475, 1344, 1218, 823, 776, 634 cm^{-1}; ^{1}HNMR (500 MHz, $CDCl_3$): δ 5.15 (br s, 2H, NH_2), 7.09 (d, 1H, *J* = 5.21 Hz, ArH), 7.51-7.52 (m, 3H, ArH), 8.03-8.05 (m, 2H, ArH), 8.40 (d, 1H, *J* = 5.21 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 172. Calcd for $C_{10}H_9N_3$ = 171.20. Anal. Found: C, 70.25; H, 5.29; N, 24.46. Calcd for $C_{10}H_9N_3$: C, 70.16; H, 5.30; N, 24.54 %.

4-(4-fluorophenyl)pyrimidine-2-amine (3b)
Yield 83%, mp 160-162 ºC; IR (KBr) υ_{max} 3327, 3158, 1652, 1567, 1551, 1427, 1221, 852, 814 cm^{-1}; ^{1}HNMR (500 MHz, $CDCl_3$): δ 5.16 (br s, 2H, NH_2), 7.05 (d, 1H, *J* = 5.25 Hz, ArH), 7.17-7.21 (m, 2H, ArH), 8.03-8.07 (m, 2H, ArH), 8.38 (d, 1H, *J* = 5.25 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 190. Calcd for $C_{10}H_8FN_3$ = 189.19. Anal. Found: C, 63.46; H, 4.27; N, 22.25. Calcd for $C_{10}H_8FN_3$: C, 63.49; H, 4.26; N, 22.21 %.

4-(3-thienyl)pyrimidine-2-amine (3c)
Yield 88%, mp 218-220 ºC; IR (KBr) υ_{max} 3354, 3204, 1663, 1577, 1479, 875, 817, 702 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 6.58 (br s, 2H, NH_2), 7.03 (d, 1H, *J* = 5.09 Hz, ArH), 7.64-7.66 (m, 1H, ArH), 7.70 (d, 1H, *J* = 5.03 Hz, ArH), 8.24-8.26 (m, 2H, ArH); ESI-MS: Observed ($M+H^+$) = 178. Calcd for $C_8H_7N_3S$ = 177.23. Anal. Found: C, 54.22; H, 3.97; N, 23.76; S, 18.05. Calcd for $C_8H_7N_3S$: C, 54.22; H, 3.98; N, 23.71; S, 18.09 %.

4-(4-trifluoromethylphenyl)pyrimidine-2-amine (3d)
Yield 94%, mp 168-170 ºC; IR (KBr) υ_{max} 3134, 1684, 1660, 1629, 1516, 1454, 1321, 1175, 1124, 1064, 798 cm^{-1}; ^{1}HNMR (500 MHz, $CDCl_3$): δ 5.36 (br s, 2H, NH_2), 7.02 (d, 1H, *J* = 5.20 Hz, ArH), 7.68 (d, 2H, *J* = 8.20 Hz, ArH), 8.08 (d, 2H, *J* = 8.20 Hz, ArH), 8.36 (d, 1H, *J* = 5.20 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 240. Calcd for $C_{11}H_8F_3N_3$ = 239.20. Anal. Found: C, 55.20; H, 3.36; N, 17.61. Calcd for $C_{11}H_8F_3N_3$: C, 55.23; H, 3.37; N, 17.57 %.

4-(4-phenylphenyl)pyrimidine-2-amine (3e)
Yield 69%, mp 222-224 ºC; IR (KBr) υ_{max} 3457, 3300, 3156, 1629, 1563, 1461, 1344, 814, 772, 733, 690 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 6.64 (br s, 2H, NH_2), 7.13 (d, 1H, *J* = 5.16 Hz, ArH), 7.35 (t, 1H, *J* = 7.35 Hz, ArH), 7.46 (t, 2H, *J* = 7.48 Hz, ArH), 7.71 (d, 2H, *J* = 7.22 Hz, ArH), 7.76 (d, 2H, *J* = 8.44 Hz, ArH), 8.13 (d, 2H, *J* = 8.44 Hz, ArH), 8.29 (d, 1H, *J* = 5.16 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 248. Calcd for $C_{16}H_{13}N_3$ = 247.29. Anal. Found: C, 77.78; H, 5.29; N, 16.93. Calcd for $C_{16}H_{13}N_3$: C, 77.71; H, 5.30; N, 16.99%.

4-(pyridin-4-yl)pyrimidine-2-amine (3f)
Yield 71%, mp 184-186 ºC; IR (KBr) υ_{max} 3400, 2947, 1650, 1572, 1553, 1471, 1074, 825, 641 cm^{-1}; ^{1}HNMR (500 MHz, $CDCl_3$): δ 5.26 (br s, 2H, NH_2), 7.13 (d, 1H, *J* = 5.10 Hz, ArH), 7.90 (d, 2H, *J* = 5.95 Hz, ArH), 8.48 (d, 1H, *J* = 5.10 Hz, ArH), 8.79 (d, 2H, *J* = 5.95 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 173. Calcd for $C_9H_8N_4$ = 172.19. Anal. Found: C, 62.91; H, 4.67; N, 32.42. Calcd for $C_9H_8N_4$: C, 62.78; H, 4.68; N, 32.54 %.

4-(pyridin-3-yl)pyrimidine-2-amine (3g)
Yield 68%, mp 182-184 ºC; IR (KBr) υ_{max} 3352, 3175, 1672, 1583, 1563, 1487, 1357, 1230, 1034, 822, 733, 668 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 6.72 (br s, 2H, NH_2), 7.16 (d, 1H, *J* = 5.12 Hz, ArH), 7.48 (m, 1H, ArH), 8.31 (d, 1H, *J* = 5.12 Hz, ArH), 8.35 (d, 1H, *J* = 7.99 Hz, ArH), 8.63 (m, 1H, ArH), 9.19 (s, 1H, ArH); ESI-MS: Observed ($M+H^+$) = 173. Calcd for $C_9H_8N_4$ = 172.19. Anal. Found: C, 62.71; H, 4.72; N, 32.57. Calcd for $C_9H_8N_4$: C, 62.78; H, 4.68; N, 32.54 %.

4-(pyridin-2-yl)pyrimidine-2-amine (3h)
Yield 63%, mp 129-131 ºC; IR (KBr) υ_{max} 3497, 3326, 3168, 1633, 1579, 1476, 1356, 1255, 1010, 805, 768, 665 cm^{-1}; ^{1}HNMR (500 MHz, DMSO (d_6)): δ 6.69 (br s, 2H, NH_2), 7.42 (d, 1H, *J* = 4.40 Hz, ArH), 7.47 (br s, 1H, ArH), 7.92 (m, 1H, ArH), 8.26 (d, 1H, *J* = 7.57 Hz, ArH), 8.35 (d, 1H, *J* = 4.40 Hz, ArH), 8.65 (d, 1H, *J* = 3.38 Hz, ArH); ESI-MS: Observed ($M+H^+$) = 173. Calcd for $C_9H_8N_4$ = 172.19. Anal. Found: C, 62.80; H, 4.65; N, 32.55. Calcd for $C_9H_8N_4$: C, 62.78; H, 4.68; N, 32.54 %.

Clonogenic assay
Five cell lines consisting of HepG2 (human hepatocellular liver carcinoma), M1 (human fibroblast), MCF7 (human breast adenocarcinoma), SKHep1 (human liver adenocarcinoma), and Hela (human cervical carcinoma) which had been verified to be negative for Micoplasma contamination were used in this experiment. To apply the method of clonogenic assay [20], cells were plated in 6-well plates (200 cells/ well) for 24 h before treatment with the test compounds to allow attachment of the cells to wells. Seven different concentrations of each compound, doxorubicin (as reference), and DMSO 0.5% (applied solvent to dissolve the compound) were then added to the monolayer cells in triplicates. The plates were incubated for 10 days at 37 ºC in atmosphere of 5% CO_2. The media were removed after 10 days and the formed colonies were stained with a solution of 0.5% crystal violet in ethanol for 10 minutes and the number of colonies containing more than 50 cells was counted under microscope. The relation between the number of the colonies (as a percentage of the control containing DMSO 0.5%) and the concentrations of each compound were plotted to get survival curve of the cell lines and IC_{50} values were calculated.

Antimicrobial test
The antimicrobial assay was performed in tryptone beef extract agar, at pH 7.2, with an inoculum of 1-2 × 10^5 cells/mL. The antimicrobial activity of the test compounds was determined using the agar well diffusion method following a published procedure with slight modifications

[21,22]. Culture medium was thus inoculated with the given microorganism by spreading the bacterial inoculums in the media. Wells (7 mm diameter) were punched in the agar and filled with the test compounds with different concentrations. Control wells, containing neat DMSO (negative control) and standard antibiotic amikasin sulfate (50 mg/mL) for the tested bacteria, were also run parallel in the same plate. Bacteria were incubated at 37 ºC for 24 h. Antimicrobial activity was assessed by measuring the diameter of the zone of inhibition for the respective compound.

The minimum inhibitory concentration (MIC) was tested by broth macrodilution methods [23]. Briefly, serial 2-fold dilutions of the test compounds were prepared in DMSO, and 30 µL of each dilution was added to 3 mL of the above medium with the same inoculums of 1-2 × 10^5 cells/mL and under the same culture conditions. After the cultures were incubated at 37 ºC for 24 h, MIC was determined as the lowest concentration of the test compound that demonstrated no visible growth.

Antifungal test

The antifungal assay was performed in Sabouraud dextrose broth. The minimal inhibitory concentration (MIC) was determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) [24, 25].

Acknowledgement

The authors would like to express their thanks to the research deputy of Shahid Beheshti University of Medical Sciences for financial support of this research.

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Table 1- *Growth inhibition of cell lines by enamines (**2a-h**) and aminopyrimidines (**3a-h**)*

			EC50(µM)		
Compound	HepG2[a]	M1[b]	MCF7[c]	SKHep1[d]	Hela[f]
2a	129	252	260	143	298
3a	67	260	127	62	330
2b	12	18	37	12	24
3b	62	94	74	67	146
2c	18	22	25	12	31
3c	48	32	55	50	93
2d	61	55	42	53	62
3d	8	21	12	10	26
2e	84	82	76	90	97
3e	37	59	25	30	29
2f	302	ND	ND	312	298
3f	95	112	102	87	124
2g	73	88	53	68	66
3g	180	233	221	168	330
2h	74	61	83	75	88
3h	272	ND	220	256	ND
Doxorubicin	0.038	0.022	0.052	0.041	0.021

[a] human hepatocellular liver carcinoma cell line
[b] human fibroblast cell line
[c] human breast adenocarcinoma cell line
[d] human liver adenocarcinoma cell line
[f] human cervical carcinoma cell line

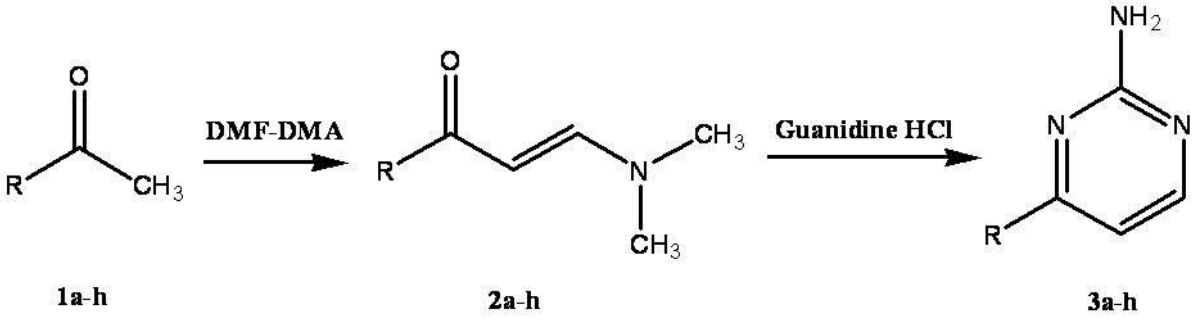
Table 2- *antimicrobial activities of enamines (**2c,2d,2f**) and aminopyrimidines (**3c,3h**)*

	MIC(µg/ml)						
Compound	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. aureus*	*S. aureus* (MRSA)	*E. faecalis*	*C. albicans*
2c	100	400	400	200	400	400	>200
3c	400	400	200	400	>400	>400	>200
2d	200	200	200	>400	>400	>400	>200
2f	100	400	400	400	400	>400	50
3h	200	>400	>400	>400	>400	>400	>200
Amikacin	0.5	4	2	0.5	2	4	-
Fluconazole	-	-	-	-	-	-	8

Table 3- *Global molecular parameters for enamine and aminopyrimidine derivatives*

Compound	CLogP[a]	R[b]	P[c]	V[d]	SA[e]
2a	2.2974	55.26	20.85	547.5	391.29
3a	1.774	52.19	20.03	506.5	361.87
2b	2.516	55.67	20.76	565.5	396.54
3b	1.6262	52.6	19.94	524.5	367.68
2c	2.0739	54.2	20.38	503.5	378.44
3c	1.4698	51.13	19.55	462.5	347.71
2d	3.3127	61.77	22.42	646.5	433.05
3d	2.673	58.7	21.59	605.5	404.25
2e	4.1854	80.86	30.51	775.5	495.06
3e	3.662	77.79	29.69	734.5	468.87
2f	1.1964	53.73	20.14	540.5	388.46
3f	0.4098	50.67	19.32	499.5	353.65
2g	1.1964	53.73	20.14	540.5	388.88
3g	0.4098	50.67	19.32	499.5	354.59
2h	1.5964	52.77	20.14	540.5	392.08
3h	0.6198	49.71	19.32	499.5	360.99

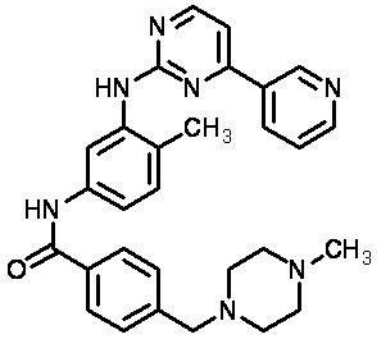
[a] ClogP values were calculated by using ChemDraw Ultra version 8.0.
[b]Refractivity ($Å^3$) values were calculated by using ChemDraw Ultra version 8.0.
[c] Polarizability ($Å^3$) values were calculated by using HyperChem version 8.0.3.
[d] Molecular volume ($Å^3$) values were calculated by using ChemDraw Ultra version 8.0.
[e] Surface area ($Å^2$) values were calculated by using HyperChem version 8.0.3.



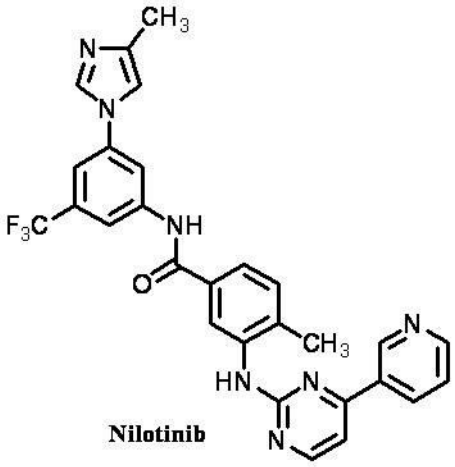
1a-h **2a-h** **3a-h**

1a, 2 a, 3 a: R = ph
1b, 2 b, 3 b: R = 4-F-ph
1c, 2 c, 3 c: R = 3-thienyl
1d, 2 d, 3 d: R = 4-(CF_3)-ph
1e, 2 e, 3 e: R = 4-(ph)-ph
1f, 2 f, 3 f: R = 4-pyridyl
1g, 2 g, 3 g: R = 3-pyridyl
1h, 2 h, 3 h: R = 2-pyridyl

Scheme 1- Reagent and conditions: (a) DMF, reflux, 24 h; (b) $NaOCH_3$, Isopropanol, reflux, 48 h

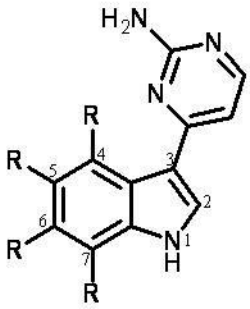


Imatinib



Nilotinib

Variolin B



	4	5	6	7
Meridianin A	OH	H	H	H
Meridianin B	OH	H	Br	H
Meridianin C	H	Br	H	H
Meridianin D	H	H	Br	H
Meridianin E	OH	H	H	Br
Meridianin F	H	Br	Br	H
MeridianinG	H	H	H	H

Fig. 1- Chemical Structures of some bioactive aminopyrimidine derivatives

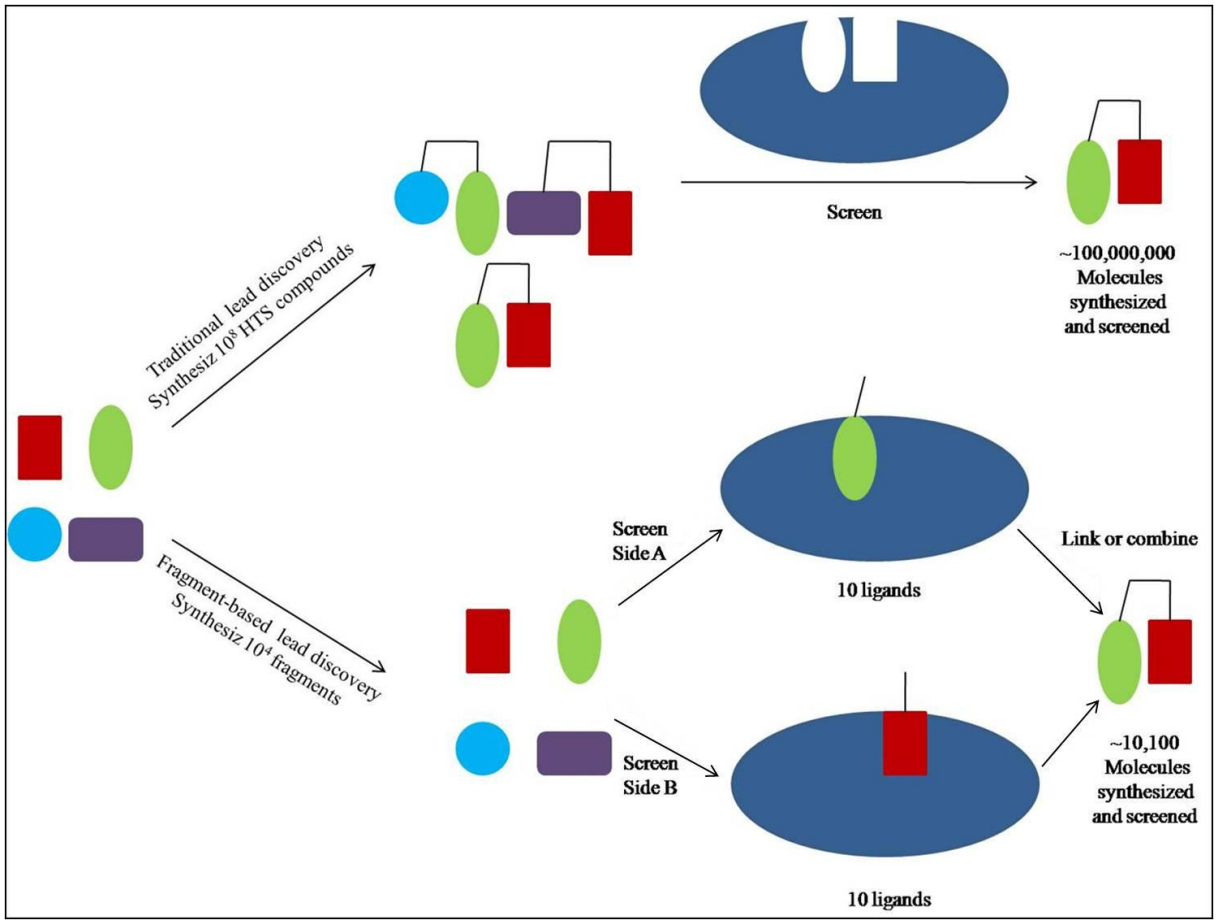


Fig. 2- Traditional lead discovery (top) and Fragment-Based Lead Discovery (bottom) [19]

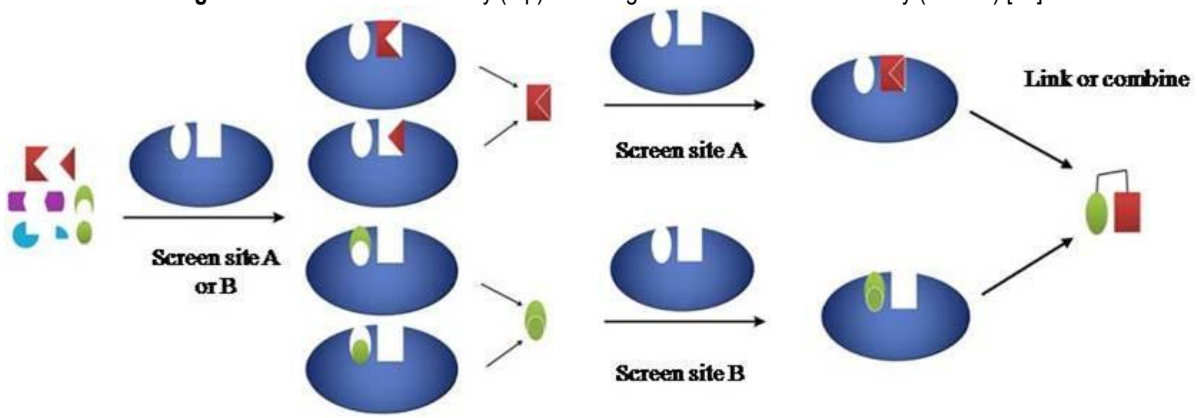


Fig. 3- Schematic illustration of synthesis route-based hit identification approach