Published in final edited form as:

Med Chem. 2012 May ; 8(3): 392–400.

Four-Component Synthesis of 1,2-Dihydropyridine Derivatives and their Evaluation as Anticancer Agents

Mohamed A. O. Abdelf-Fattah¹, Mahmoud A. M. El-Naggar¹, Rasha M. H. Rashied¹, Bernard D. Gary², Gary A. Piazza², and Ashraf H. Abadi^{1,*}

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt

²Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Avenue, Suite 3029, Mobile AL 36604-1405

Abstract

Two series of compounds with the general formula of 4,6-diaryl-2-oxo-1,2 dihydropyridine-3-carbonitriles and their isosteric imino derivatives were synthesized through a one pot reaction of acetophenone, aldehyde and ammonium acetate with ethyl cyanoacetate or malononitrile, respectively. The synthesized compounds were evaluated for tumor cell growth inhibitory using the human HT-29 colon and MDA-MB-231 breast tumor cell lines. Compound 4-(2-Ethoxyphenyl)-2-imino-6-(4-fluorophenyl)-1,2-dihydropyridine-3 carbonitrile (**6**) showed IC₅₀ value of 0.70 μ M versus HT-29. Meanwhile, compound 4-(2-Hydroxyphenyl)-2-imino-6-(4-fluorophenyl)-1,2-dihydropyridine-3-carbonitrile (**4**) showed IC₅₀ value of 4.6 μ M versus MDA-MB-231. Docking compound **10** to possible molecular targets, survivin and PIM1 kinase showed appreciable interactions with both, which suggest possible targets for the antitumor activity of this novel class of anticancer compounds.

Keywords

Multicomponent reactions; 1,2-dihydropyridine; cancer

INTRODUCTION

In times where a premium is put on speed, diversity, and efficiency in the drug discovery process, MCRs strategies offer significant advantages over conventional linear-type syntheses. The idea of MCRs is to create a multiplicity of compounds and then testing their biological activity on a range of molecular targets, so that large number of molecules are available to identify the most promising lead compounds for preclinical development. Not to mention also the new world-wide movement of adopting the philosophy of green chemistry that emphasizes the importance of saving initial reactants, costs and waste. Thus MCR is one of the ideal choices by chemists for drug discovery [1–4].

* Address correspondence to these authors at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt; Tel: 202-27090716; Fax: 202-27581041; ashraf.abadi@guc.edu.eg.

Compounds within the 3-cyano-4,6-diaryl-2(1*H*)imino or oxopyridines series represent a good example for those derivatives that can be developed utilizing MCR approaches. There is recently a great interest in this series of compounds due to their diverse pharmacological activities, such as antimicrobials against Gram positive and Gram negative microorganisms [5], antidepressant [6], cardiotoxic [7], and anticancer [8,9] activities.

The tumor cell regulatory proteins PIM1 kinase and survivin may serve as targets for the anticancer activity of this class of compounds.

PIM1 kinase is an oncogenic protein belonging to a family of proteins containing the homologues PIM-2 and PIM-3. PIM1 kinase is transcriptionally regulated by mitogens, cytokines, and various growth factors. PIM1 kinase is involved in the phosphorylation and activation of a large number of endogenous substrates that are associated with cell division and proliferation [10, 11].

Survivin is a member of a family of proteins known as Inhibitors of Apoptosis (IAP) and recent studies have shown that survivin is over expressed in many common human cancers and may be an important mediator of sensitivity to chemotherapy. Moreover, survivin has a very significant role in mitotic cell division where its expression pattern is cell cycle-dependent. In addition, survivin is one of the chromosomal passenger proteins; it complexes and localizes to Aurora B kinase in the mitotic machinery [12–14].

Different candidates belonging to 3-cyano-4,6-diaryl-2(1*H*) oxopyridines series have been recently shown promising anticancer activity through interfering with PIM1 kinase and surviving [11,12].

To explore the scope of this class of compounds, herein, we are reporting about thirty new derivatives with the general formula of 4,6-diaryl-2-imino (oxo)-1,2 dihydropyridine-3-carbonitriles and their evaluation for anticancer activity, structure activity relationships, and interaction with potential molecular targets.

RESULTS AND DISCUSSION

Chemistry

The general synthesis of the 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carbonitrile and their 2-imino counterparts is illustrated in Scheme 1, whereby the final products were obtained utilizing a multi-component reaction technique, a subtype of combinatorial reaction approach through refluxing the respective aldehyde with the corresponding ketone, ammonium acetate and ethyl cyanoacetate in ethanol for 16 to 18 hours, to afford the formation of the 2-pyridone derivative, or malononitrile to afford the iminopyridine derivative. All compounds were analyzed using ¹H-NMR, FT-IR spectroscopy, mass spectroscopy and elemental analysis. For mass spectroscopy, it was observed that in the majority of the compounds, the base peak was the molecular ion peak, indicating their stability.

Infrared spectra for the iminopyridine derivatives showed bands at the range of 3100 – 3400 cm⁻¹ corresponding to the NH stretching. A very characteristic band appeared at the range

of 2100–2250 cm⁻¹ corresponding to the cyano stretching. The 2-pyridone derivatives showed an extra band at the range of 1640–1710 cm⁻¹ for the carbonyl stretching.

As for the ¹H-NMR spectra, the diaryl substitution of the synthesized compounds led to the appearance of aromatic protons as multiplet peaks at chemical shifts (δ) 7.00–8.40 ppm. A singlet peak at around 6.50–7.00 ppm is characteristic for the aromatic proton at position 5 of the pyridone or iminopyridine ring. Compounds bearing methoxy substituent showed a characteristic singlet appeared at about 3.90 ppm, while those with the ethoxy substituent showed a characteristic triplet signal appeared at 1.25–1.30 ppm, and a quartet appeared at 4.03–4.13 ppm. Distances between peaks were described by coupling constants (J).

Biology

All synthesized compounds were tested for their *in vitro* tumor cell growth inhibitory activity using the human HT-29 colon cancer cell line and breast cancer cell line MDA-MB-231. Most of compounds were evaluated in 2 steps; first, the percentage inhibition at a screening dose of 50 μ M was performed in triplicate, then for compounds displaying a percentage of inhibition >50% were evaluated by testing a range of 10 concentrations with at least two replicates per concentration to calculate an IC₅₀ value. The results are summarized in Table 1.

In this study the effect of three major structure features on the anticancer activity of the synthesized candidates could be studied, namely the effect of the imino group on position 2 of the pyridine ring versus its 2-oxo isostere, the effect of the position of the fluorine atom on the phenyl ring at position 6 of the pyridine, and finally the nature of the substituent on the phenyl ring at position 4 of the pyridine ring.

Starting with the anticancer activity using the human HT-29 colon tumor cell line, 17 out of 30 synthesized candidates have showed appreciable tumor cell growth inhibitory activity. Generally, the presence of the imino group at position 2 of the pyridine ring gave higher potencies compared to the carbonyl isostere at the same position. This is clear when comparing the IC₅₀s of the imino derivatives to those of their 2-oxo analogues. The imino group may then be concluded as an important feature for the activity as it may interact with the target protein as both hydrogen bond donor and/ or acceptor.

In our synthesized candidates, the aryl group at position 4 of the pyridine ring carried either an oxygenated or halogenated substituent. As for the oxygenated substituents, they varied between 2-furanyl, 2-hydroxy phenyl, 2-methoxy phenyl, and 2-ethoxy phenyl, while the only halogenated substituent was 3,4-dichlorophenyl. The selection of the nature of the substituent at this position was based on previously published results by our group showing that candidates having negative electrostatic potential at this area exhibited anticancer activity [8, 9]. The 2-imino pyridine derivatives having the 2-ethoxy phenyl substituent at position 4 of the pyridine ring **6**, **16**, and **26** have showed the highest potency with IC₅₀s of 0.70, 1.5, 2.5 μ M respectively, followed by those having 2-methoxy phenyl or 2-furanyl substituent at the same position **8**, **2**, **28**, **14**, and **24** with IC₅₀s of 3.46, 6.3, 8.82, 9.3, 10.5 μ M respectively, and finally came the compounds having the 2-hydroxy phenyl substituent **4**, **12**, and **22** as the least potent with IC₅₀s of 12.7, >50, >50 μ M respectively. This may

suggest that although the presence of certain degree of negative potential at this area is an important determinant for activity, the bulkiness of the substituent at this position is also important. It may be concluded that the bulky ethoxy function may be involved in additional hydrophobic interaction with the target protein and highly affects the degree of non co-planarity between the phenyl at position 4 and the pyridine ring that also seems to be a crucial factor for activity. It also decreases the free rotation degree of the phenyl ring, which can increase affinity and selectivity towards the target protein.

As for the candidates with a halogenated nature at position 4 of the pyridine ring, the 2-imino derivatives with 3,4-dichloro phenyl at this position, namely **10**, **20**, and **30** displayed tumor cell growth inhibitory activity with IC₅₀s of 2.18, 3.96, and 5.74 μ M respectively. This confirms the necessity of having a certain degree of negative electrostatic potential at this area.

Regarding the position of the fluorine atom on the phenyl ring at position 6 of the pyridine ring, it was shown that the anticancer activity resides mainly in the candidates having the *para* or the *meta* fluoro substituent, while those with the *ortho* fluorine atom came as less active analogues. This is clear when comparing the potencies of compounds **6**, **15** against **25**, IC₅₀s 0.70, 10.2, 12.3 μ M respectively, compounds **18**, **8** against **26**, IC₅₀s 1.26, 3.46, 8.82 μ M respectively, and finally compounds **10**, **20** against **30**, IC₅₀s 2.18, 3.96, 5.74 μ M respectively. Actually, the majority of the synthesized compounds with the fluorine atom on the phenyl group at position 6 of the pyridine nucleus have generally shown to be more potent than other previously published analogues with bromine or chlorine atom in the same position, chart 2. This may show the importance of highly electronegative atom such as the fluorine one in this area also. The small size of the fluorine atom relative to the chlorine or the bromine ones may also affect the fitting of the synthesized candidate into its binding pocket on the target protein.

Moving to the MDA-MB-231 breast cancer cell line, the active candidates did not show certain trends when varying the substituents on position 4 of the pyridine ring nor when varying the positions of the fluorine atom on the phenyl ring at position 6. The highest potency was for compound **4**, having a 2-hydroxy phenyl at position 4 and *para* fluoro phenyl at position 6 of the pyridine ring. This compound came as one of the least active candidates on HT-29 colon cancer cell line and this may support the idea of having qualitative or quantitative differences of the different molecular targets in both cell lines.

Among the other possible targets for the synthesized candidates are PIM1 kinase and survivin. Consequently, docking experiments for one of the most active candidate **10** was performed to the active site of these proteins using MOE software [15].

The crystal structure of survivin (PDB ID code 1F3H) showed homodimeric chains and with no ligand. As such, we applied global docking and the automatic site finder in the software. The two techniques came almost to the same types of interaction; namely indirect H bonding of the NH and CN through the solvent contacts with Arg18 and Gln92, Fig. (1).

Docking compound **10** to the active site of PIM-1 (PDB ID code 2OBJ) reveals bidentate hydrogen bonding with the residue Lys67 and indirect H bonding through H₂O molecules with Glu89 and Asp186, Fig. (2).

These intensive interactions with survivin and PIM1 kinase indicates that one or both targets might be responsible for the anticancer activity of this series of compounds. Such cross reactivity to structurally similar to compounds as previously reported [11, 12].

EXPERIMENTAL

Chemistry

All reactions were performed with commercially available reagents and used without further purification. All reactions were monitored by thin-layer chromatography (TLC) carried out on pre-coated silica gel plates and detection of the components was made by short and long UV light. Melting points were determined in open capillaries using a Buchi Melting Point B-540 apparatus and are uncorrected. ¹H-NMR spectra were recorded on Varian Mercury spectrometer at 300 MHz using tetramethylsilane (TMS) as internal reference.

Chemical shift values are given in ppm at room temperature using DMSO-d₆ and Chloroform as solvents; chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS; multiplicities are abbreviated as: s: singlet; d: doublet; q: quartet; m: multiplet; dd: doublet of doublet; brs: broad singlet. Yields are not optimized. Elemental analyses were performed by the Microanalytical Unit, Faculty of Science, Cairo University; found values were within $\pm 0.4\%$ of the theoretical ones, unless otherwise indicated.

4.1.1 General Procedure for the Preparation of 4,6-diaryl-2-oxo-1,2

dihydropyridine-3-carbonitriles—The respective aromatic ketone (1 mmol), together with the respective aromatic aldehyde (1 mmol), ethyl cyanoacetate (1 mmol), and ammonium acetate (8 mmol) were dissolved in ethyl alcohol (30 ml) and put under reflux for 10 to 12 h. The precipitate obtained was filtered, washed with ethyl alcohol and dried. For the purification purpose, the precipitate was subjected to column chromatography on silica gel, eluting with wide range of eluting systems starting with methylene chloride to methylene chloride: methanol 9.85: 0.15.

6-(4-Fluorophenyl)-4-(2-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3- Carbonitrile (1): Yield: 85%; mp: 301 – 303 °C; IR (cm⁻¹): 3320, 2210, 1642; ¹H-NMR (CDCl₃): 3.91 (s, 3H, -OCH₃), 6.72 (s, 1H, C5-pyridone), 7.06 – 7.78 (m, 9H, aromatic & NH) ; MS (EI): m/z 320 (M⁺, 100%); Anal.(C₁₉H₁₃FN₂O₂) C, H, N.

6-(4-Fluorophenyl)-4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-Carbonitrile (3): Yield: 85%; mp: 319 – 321 °C; IR (cm⁻¹): 3494, 3339, 2100, 1705; ¹H-NMR (DMSO-d₆): 6.50 (s, 1H, C5-pyridone), 7.37–7.73 (m, 8H, aromatic); MS (EI): m/z 307 (M⁺, 100%); Anal. (C₁₈H₁₁FN₂O₂) C, H, N.

4-(2-Ethoxyphenyl)-6-(4-fluorophenyl)-2-oxo-1, 2-dihydropyridine-3-Carbonitrile(5): Yield: 70%; mp: 261–263 °C; IR (cm⁻¹): 3230, 2223, 1638; ¹H-NMR

(DMSO- d_6): 1.29 (t, 3H, $J = 6.9$ Hz, $-CH_3$), 4.12 (q, 2H, $J = 6.9$ Hz, $-OCH_2-$), 7.05 – 7.10 (m, 1H, aromatic), 7.17 – 7.24 (m, 1H, aromatic) 7.33–7.96 (m, 7H, aromatic & NH); MS (EI): m/z 334 (M^+ , 100%); Anal. ($C_{20}H_{15}FN_2O_2$) C, H, N.

6-(4-Fluorophenyl)-4-(furan-2-yl)-2-oxo-1,2-dihydro-pyridine-3-carbonitrile(7): Yield: 21%; mp: 307 – 310 °C; IR (cm^{-1}): 3150, 2221, 1657; 1H -NMR (DMSO- d_6): 6.85 (s, 1H, C5-pyridone), 7.05 (s, 1H, aromatic), 7.40–7.35 (m, 1H, aromatic), 7.42 (d, 1H, $J=2.1$ Hz, aromatic), 7.72–7.69 (m, 1H, aromatic), 7.98–7.90 (m, 2H, aromatic), 8.12–8.09 (m, 1H, aromatic), 12.63 (s, 1H, NH); MS (EI): m/z 280 (M^+ , 100%); Anal. ($C_{16}H_9FN_2O_2$) C, H, N.

4-(3,4-Dichlorophenyl)-6-(4-fluorophenyl)-2-oxo-1,2-di-hydropyridine-3-carbonitrile(9): Yield: 36%; mp: 328 – 330°C; IR (cm^{-1}): 3092, 2218, 1642; 1H -NMR (DMSO- d_6): 6.93 (s, 1H, C5-pyridone), 7.35–7.43 (m, 2H, aromatic), 7.74 (d, 1H, $J=9$ Hz, aromatic), 7.86 (d, 1H, $J=9$ Hz, aromatic), 7.97–8.07 (m, 3H, aromatic), 12.95 (s, 1H, NH); MS (EI): m/z 362 ($M^+ + 4$), m/z 360 ($M^+ + 2$), m/z 358 (M^+ , 100%); Anal. ($C_{18}H_9FN_2OCl_2$) C, H, N.

6-(3-Fluorophenyl)-4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-Carbonitrile(11): Yield: 73%; mp: 317– 319 °C; IR (cm^{-1}): 3425, 2133, 1710; 1H -NMR (DMSO- d_6): 6.42 (s, 1H, C5-Pyridone), 7.39–7.47 (m, 4H, aromatic), 7.58 – 7.95 (m, 5H, aromatic & NH); MS (EI): m/z 307(M^+ , 100%); Anal. ($C_{18}H_{11}FN_2O_2$) C, H, N.

6-(3-Fluorophenyl)-4-(2-methoxyphenyl)-2-oxo-1, 2-dihydropyridine-3-Carbonitrile (13): Yield: 83%; mp: 301 – 303°C; IR (cm^{-1}): 3360, 2217, 1645; 1H -NMR ($CDCl_3$): 3.91 (s, 3H, $-OCH_3$), 6.50 (s, 1H, C5-pyridone), 7.06 – 7.13 (m, 3H, aromatic), 7.27– 7.78 (m, 6H, aromatic & NH); MS (EI): m/z 320 (M^+ , 100%); Anal. ($C_{19}H_{13}FN_2O_2$) C, H, N.

4-(2-Ethoxyphenyl)-6-(3-fluorophenyl)-2-oxo-1, 2-dihydropyridine-3-Carbonitrile(15): Yield: 87%; mp: 290 – 292°C; IR (cm^{-1}): 3281, 2222, 1645; 1H -NMR (DMSO- d_6): 1.42 (t, 3H, $-CH_3$, $J=6.9$ Hz, $-CH_3$), 4.16 (q, 2H, $-OCH_2-$, $J=6.9$ Hz, $-CH_2-CH_3$), 6.95 (s, 1H, C5-pyridone), 7.03–7.14 (m, 2H, aromatic), 7.23–7.37 (m, 2H, aromatic), 7.37 – 7.77 (m, 4H, aromatic); MS (EI): m/z 334 (M^+ , 50.19%), m/z 121 (100%); Anal. ($C_{20}H_{15}FN_2O_2$) C, H, N.

6-(3-Fluorophenyl)-4-(furan-2-yl)-2-oxo-1,2-dihydro-pyridine-3-carbonitrile(17): Yield: 30%; mp: 307–308 °C; IR (cm^{-1}): 3126, 2219, 1657; 1H -NMR (DMSO- d_6): 6.85 (s, 1H, C5-pyridone), 7.13 (s, 1H, aromatic), 7.41 (t, 1H, $J=9$ Hz, aromatic), 7.64–7.54 (m, 1H, aromatic), 7.80–7.71 (m, 3H, aromatic), 8.1 (s, 1H, aromatic), 12.65 (s, 1H, NH); MS (EI): m/z 280 (M^+ , 100%); Anal. ($C_{16}H_9FN_2O_2$) C, H, N.

4-(3,4-Dichlorophenyl)-6-(3-fluorophenyl)-2-oxo-1,2-di-hydropyridine-3-carbonitrile(19): Yield: 34%; mp: 331 – 334°C; IR (cm^{-1}): 3094, 2225, 1642; 1H -NMR (DMSO- d_6): 7.05 (s, 1H, C5-pyridone) 7.36– 7.45 (m, 1H, aromatic), 7.54– 7.63 (m, 1H, aromatic), 7.72 – 7.79 (m, 1H, aromatic), 7.82 (s, 1H, aromatic), 7.84 (d, 1H, $J=2.7$ Hz, aromatic), 7.87 (d, 1H, $J=2.7$ Hz, aromatic), 8.05 (s, 1H, aromatic), 12.92 (s, 1H, NH); MS (EI): m/z 362 ($M^+ + 4$), m/z 360 ($M^+ + 2$), m/z (M^+ , 100%); Anal. ($C_{18}H_9FN_2OCl_2$) C, H, N.

6-(2-Flourophényl)-4-(2-hydroxyphényl)-2-oxo-1,2-dihy-dropyridine-3-

Carbonitrile(21): Yield: 82%; mp: 312 – 314°C; IR (cm⁻¹): 3268, 2133, 1690; ¹H-NMR (DMSO-d₆): 6.80 (s, 1H, C5-Pyridone), 7.32–7.45 (m, 5H, aromatic), 7.60–7.77 (m, 4H, aromatic); MS (EI): m/z 307(M⁺, 100%); Anal. (C₁₈H₁₁FN₂O₂) C, H, N.

6-(2-Flourophényl)-4-(2-methoxyphényl)-2-oxo-1,2-dihy-dropyridine-3-

Carbonitrile(23): Yield: 91%; mp: 308 – 310°C; IR (cm⁻¹): 3414, 2223, 1646; ¹H-NMR (DMSO-d₆): 3.82 (s, 3H, -OCH₃), 6.86 (s, 1H, pyridone), 7.07–7.12 (m, 1H, aromatic), 7.19 – 7.22 (m, 1H, aromatic), 7.32 – 7.41 (m, 3H, aromatic), 7.48 – 7.72 (m, 4H, aromatic & NH); MS (EI): m/z 320 (M⁺, 100%); Anal. (C₁₉H₁₃FN₂O₂) C, H, N.

4-(2-Ethoxyphényl)-6-(2-flourophényl)-2-oxo-1,2-dihy-dropyridine-3-

Carbonitrile(25): Yield: 91%; mp: 291–293°C; IR (cm⁻¹): 3392, 2222, 1643; ¹H-NMR (DMSO-d₆): 1.29 (t, 3H, J=6.95 Hz, -CH₃), 4.13 (q, 2H, J=6.95Hz, -OCH₂), 6.52 (s, 1H, C5-pyridone), 7.05–7.72 (m, 9H, aromatic & NH); MS (EI): m/z 334 (M⁺, 92.31%), m/z 63 (100%); Anal. (C₂₀H₁₅FN₂O₂) C, H, N.

6-(2-Flourophényl)-4-(furan-2-yl)-2-oxo-1,2-dihydro-pyridine-3-carbonitrile(27): Yield: 36%; mp: 304–307°C; IR (cm⁻¹): 3270, 2219, 1642; ¹H-NMR (DMSO-d₆): 6.84 (m, 1H, C5-pyridone), 6.92 (s, 1H, aromatic), 7.38 (m, 2H, aromatic), 7.72 – 7.57 (m, 3H, aromatic), 8.1 (t, 1H, J=1.8Hz, aromatic), 12.72 (s, 1H, NH); MS (EI): m/z 280 (M⁺, 100%); Anal. (C₁₆H₉FN₂O₂) C, H, N.

4-(3,4-Dichlorophényl)-6-(2-fluorophényl)-2-oxo-1,2-dihdropyridine-3-

carbonitrile(29): Yield: 31%; mp: 283–285°C; IR (cm⁻¹): 3125, 2221, 1642; ¹H-NMR (DMSO-d₆): 6.74 (s, 1H, C5-pyridone), 7.44–7.33 (m, 2H, aromatic), 7.78 – 7.57 (m, 3H, aromatic), 7.85 (dd, 1H, J=3Hz, aromatic), 8.02 (t, 1H, J=3Hz, aroamtic), 12.99 (s, 1H, NH),; MS (EI): m/z 360 (M⁺ + 4), m/z 360 (M⁺ + 2), m/z 358 (M⁺, 100%); Anal. (C₁₈H₉Cl₂FN₂O) C, H, N.

4.1.2 General Procedure for the Preparation of 4,6-diaryl-2-imino-1,2-

dihdropyridine-3-carbonitrile—The respective aromatic ketoe (1 mmol), together with the respective aromatic aldehyde (1 mmol), malononitrile (1 mmol), and ammonium acetate (8 mmol) were dissolved in ethyl alcohol (30 ml) and put under reflux for 16–18 hrs. The precipitate obtained was filtered, washed with ethyl alcohol and dried. For the purification purpose, the precipitate was subjected to column chromatography on silica gel, eluting with methylene chloride or methylene chloride: methanol 9.9: 0.1.

6-(4-Flourophényl)-2-imino-4-(2-methoxyphényl)-1, 2-dihdropyridine-3-

Carbonitrile(2): Yield: 56%; mp: 263– 265 °C; IR (cm⁻¹): 3360, 2221; ¹H-NMR (DMSO-d₆): 3.80 (s, 3H, -OCH₃), 6.88 (s, 1H, C5-pyridine). 7.06–7.11 (m, 1H, aromatic), 7.17–7.50 (m, 9H, aromatic & NH); MS (EI): m/z 319 (M⁺, 15%). m/z 121 (100%); Anal. (C₁₉H₁₄FN₃O) C, H, N.

6-(4-Flourophényl)-4-(2-hydroxyphényl)-2-imino-1,2-dihdropyridine-3-Carbonitrile

(4): Yield: 82%; mp: 293–295 °C; IR (cm⁻¹): 3497, 3367, 2217; ¹H-NMR (DMSO-d₆): 6.91

(s, 1H, C5-pyridine), 7.15–7.47 (m, 3H, aromatic), 7.64–7.70 (m, 1H, aromatic), 8.01 – 8.50 (m, 6H, aromatic & NHs); MS (EI): m/z 306(M⁺, 100%); Anal. (C₁₈H₁₂FN₃O) C, H, N.

4-(2-Ethoxyphenyl)-6-(4-fluorophenyl)-2-imino-1,2-dihydropyridine-3-Carbonitrile (6): Yield: 85%; mp: 285 – 287 °C; IR (cm⁻¹): 3373, 2215; ¹H-NMR (DMSO-d₆): 1.27 (t, 3H, J=6.9Hz, -CH₃), 4.10 (q, 2H, J=6.9 Hz, -OCH₂), 6.86 (s, 1H, C5-pyridine), 7.04–7.49 (m, 10H, aromatic & NHs); MS (EI): m/z 333(M⁺, 62.67%). m/z 305 (100%); Anal. (C₂₀H₁₆FN₃O) C, H, N.

6-(4-Fluorophenyl)-4-(furan-2-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile(8): Yield: 31%; mp: 158 – 159 °C; IR (cm⁻¹): 3329, 2208; ¹H-NMR (DMSO-d₆): 6.78 (s, 1H, C5-pyridine), 6.98 (s, 2H, aromatic), 7.32 – 7.58 (m, 4H, aromatic), 8.05 (s, 1H, aromatic), 8.15–8.22 (m, 2H, aromatic); MS (EI): m/z 279 (M⁺, 100%); Anal. (C₁₆H₁₀FN₃O) C, H, N.

4-(3,4-Dichlorophenyl)-6-(4-fluorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile(10): Yield: 35%; mp: 197- 198 °C; IR (cm⁻¹): 3353, 2220; ¹H-NMR (DMSO-d₆): 6.86 (s, 1H, C5-pyridine), 7.08 (s, 1H, aromatic), 8.05 (s, 1H, aromatic), 8.26–7.20 (m, 8H, aromatic); MS (EI): m/z 362 (M⁺+4), m/z 360 (M⁺+2), m/z 358 (M⁺, 100%); Anal. (C₁₈H₁₀FN₃Cl) C, H, N.

6-(3-Fluorophenyl)-4-(2-hydroxyphenyl)-2-imino-1,2-dihydropyridine-3-Carbonitrile(12): Yield: 64%; mp: 290–292 °C; IR (cm⁻¹): 3342, 2110; ¹H-NMR (DMSO-d₆): 6.27 (s, 1H, C5-pyridine), 7.27 (s, 2H, aromatic), 7.41 -7.98 (m, 8H, aromatic & NHs); MS (EI): m/z 306(M⁺, 100%); Anal. (C₁₈H₁₂FN₃O) C, H, N.

6-(3-Fluorophenyl)-2-imino-4-(2-methoxyphenyl)-1,2-dihydropyridine-3-Carbonitrile(14): Yield: 40%; mp: 291–293 °C; IR (cm⁻¹): 3349, 2221; ¹H-NMR (DMSO-d₆): 3.80 (s, 3H, -OCH₃), 6.92 (s, 1H, pyridine), 7.06–7.11 (m, 1H, aromatic), 7.18–7.36 (m, 4H, aromatic), 7.46–7.96 (m, 5H, aromatic & NHs); MS (EI): m/z 319 (M⁺, 100%); Anal. (C₁₉H₁₄FN₃O) C, H, N.

4-(2-Ethoxyphenyl)-6-(3-fluorophenyl)-2-imino-1,2-dihydropyridine-3-Carbonitrile (16): Yield: 45%; mp: 250 – 252 °C; IR (cm⁻¹): 3359, 2142; ¹H-NMR (DMSO-d₆): 1.40 (t, 3H, J=6.97Hz, -CH₃), 4.15 (q, 2H, J=6.97Hz, -OCH₂), 6.55 (s, 1H, C5-Pyridine), 7.03–7.81 (m, 10H, aromatic & NHs); MS (EI): m/z 333(M⁺, 82.5%). m/z 304 (100%); Anal. (C₂₀H₁₆FN₃O) C, H, N.

6-(3-Fluorophenyl)-4-(furan-2-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile(18): Yield: 28%; mp: 248 – 250 °C; IR (cm⁻¹): 3357, 2207; ¹H-NMR (DMSO-d₆): 6.78 (s, 1H, C5-pyridine), 7.01 (s, 2H, aromatic), 7.28–8.12 (m, 6H, aromatic); MS (EI): m/z 279 (M⁺, 100%); Anal. (C₁₆H₁₀FN₃O) C, H, N.

4-(3,4-Dichlorophenyl)-6-(3-fluorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile(20): Yield: 22%; mp: 215–216 °C; IR (cm⁻¹): 3355, 2216; ¹H-NMR (DMSO-d₆): 6.89 – 6.96 (m, 3H, aromatic), 7.42 – 7.31 (m, 1H, aromatic), 7.46–8.05 (m, 6H,

aromatic); MS (EI): m/z 361 ($M^{+}+4$), m/z 359 ($M^{+}+2$), m/z 357 (M^{+} , 100%); A-nal. ($C_{18}H_{10}FN_3Cl_2$) C, H, N.

6-(2-Fluorophenyl)-4-(2-hydroxyphenyl)-2-imino-1, 2-dihydropyridine-3-Carbonitrile (22): Yield: 77%; mp: 278–280 °C; IR (cm^{-1}): 3420, 3331, 2213; 1H -NMR (DMSO- d_6): 6.8 (s, 1H, C5-Pyridine), 7.34–7.44 (m, 4H, aromatic), 7.52–7.59 (m, 1H, aromatic), 7.64–7.96 (m, 5H, aromatic & NHs); MS (EI): m/z 306(M^{+} , 100%); Anal. ($C_{18}H_{12}FN_3O$) C, H, N.

6-(2-Fluorophenyl)-2-imino-4-(2-methoxyphenyl)-1, 2-dihydropyridine-3-Carbonitrile (24): Yield: 71%; mp: 289–291 °C; IR (cm^{-1}): 3301, 2100; 1H -NMR (DMSO- d_6): 3.80 (s, 3H, -OCH₃), 7.27–7.58 (m, 7H, aromatic), 7.76–7.79 (m, 1H, aromatic) 7.91–7.96 (m, 2H, aromatic & NHs); MS (EI): m/z 319(M^{+} , 25%), m/z 227 (100%); Anal. ($C_{19}H_{14}FN_3O$) C, H, N.

4-(2-Ethoxyphenyl)-6-(2-fluorophenyl)-2-imino-1, 2-dihydropyridine-3-Carbonitrile (26): Yield: 64%; mp: 248–250 °C; IR (cm^{-1}): 3330, 2113; 1H -NMR (DMSO- d_6): 1.29 (t, 3H, J = 6.9Hz, -CH₃), 4.11 (q, 2H, J = 6.9Hz, -OCH₂), 6.5 (s, 1H, C5-pyridine), 7.04–7.55 (m, 10H, aromatic & NHs); MS (EI): m/z 333(M^{+} , 100%); Anal. ($C_{20}H_{16}FN_3O$) C, H, N.

6-(2-Fluorophenyl)-4-(furan-2-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile (28): Yield: 27%; mp: 105–107 °C; IR (cm^{-1}): 3352, 2207; 1H -NMR (DMSO- d_6): 6.60 (s, 1H, C5-pyridine), 7.11 – 6.64 (m, 1H, aromatic), 7.18–8.08 (m, 7H, aromatic); MS (EI):m/z 279 (M^{+} , 100%); Anal. ($C_{16}H_{10}FN_3O$) C, H, N.

4-(3,4-Dichlorophenyl)-6-(2-fluorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile(30): Yield: 21%; mp: 107–109 °C; IR (cm^{-1}): 3351, 2213; 1H -NMR (DMSO- d_6): 6.98 (s, 1H, C5-pyridine), 7.20–7.83 (m, 7H, aromatic), 7.84–8.16 (m, 2H, aromatic); MS (EI):m/z 361 ($M^{+}+4$), m/z 359 ($M^{+}+2$), m/z 357(M^{+} , 100%); Anal. ($C_{18}H_{10}Cl_2FN_3$) C, H, N.

BIOLOGY

Cell Cultures

HT-29 tumor cells and MDA-MB-231 breast cancer cells were obtained from ATCC and were grown under standard cell culture conditions at 37 °C in a humidified atmosphere with 5% CO₂. Cells were grown in RPMI 1640 containing 5% fetal bovine serum. Cell count and viability was determined by Trypan blue staining followed by hemocytometry. Only cultures displaying >95% cell viability were used for experiments.

Growth Assays

Tissue culture treated microtiter 96-well plates were seeded at a density of 5000 cells/well. The plates were incubated for 18–24 h prior to any treatment. Cell viability was measured 72 h after treatment by the Cell Titer Glo Assay (Promega), which is a luminescent assay that is an indicator of live cells as a function of metabolic activity and ATP content. The assay was performed according to manufacturer's specifications.

MOLECULAR MODELING

Energy Minimization and Conformational Search Procedure

The compounds were drawn on ChemSketch 11 and saved as mol file, the latter were subjected to energy minimization using Force Field MMFF94x by Molecular Operating Environment (MOE) software [15].

Source of Target Proteins

The Crystal structure of Human PIM-1 kinase complexed with its inhibitor reference compound (PDB ID code: 2OBJ), survivin (PDB ID code: 1F3H), were downloaded from the Protein data bank and opened with MOE software. The complexed inhibitor, if any, was removed when using the synthesized compounds as ligands for docking.

Docking Procedure of PIM-1 Kinase

The co-crystallized compound was selected, and the binding site was identified according to residues. Ligand interactions were computed for the X-ray co-crystallized compound to reveal the different types of interaction as a validation for the coming docking procedure. The co-crystallized compound was then removed and replaced by the synthesized compound. Default settings of MOE-Dock were used, including “Rotate Bonds” option in order to allow flexible ligand-rigid receptor docking. The scoring function was London dG with a replacement of Alpha Triangle. Thirty conformers of the ligand were retained with highest and best score by default. The top score ligand-receptor docking was then demonstrated by 2D ligand-receptor interactions.

Docking Procedure of Survivin

Survivin is an identical dimeric protein. The binding site was defined by selecting the residues Leu6, Lys15, Phe86, Val89, Phe93, Leu96, Leu98, Phe101, Leu102 and Leu104 as reported in Literature [12]. The docking procedure was done by the default settings of MOE-Dock.

References

1. Ganem B. Strategies for innovation in multicomponent reaction design. *Acc Chem Res.* 2009; 42:463–472. [PubMed: 19175315]
2. Zhang W. Fluorous-enhanced multicomponent reactions for making drug-like library scaffolds. *Comb Chem High T Scr.* 2007; 10:219–229.
3. Sheldon AR. Atom efficiency and catalysis in organic synthesis. *Pure Appl Chem.* 2000; 72:1233–46.
4. Liu L, Wei L, Lu Y, Zhang J. One-pot tandem catalysis: a concise route to fused bicyclic scaffolds from acyclic β -ketoesters and alkynyl aldehydes. *Chemistry.* 2010; 16:11813–7. [PubMed: 20809555]
5. Abdel-Aziz A, El-Subbagh HI, Kunieda T. Lewis acid-promoted transformation of 2-alkoxypyridines into 2-aminopyridines and their antibacterial activity. Part 2: Remarkably facile C-N bond formation. *Bioorg Med Chem.* 2005; 13:4929–35. [PubMed: 15993091]
6. Abdel-Latif N. Synthesis and antidepressant activity of some new coumarin derivatives. *Sci Pharm.* 2005; 73:195–216.

7. Abadi AH, Al-Khamees HA. 3-Cyano-4,6-disubstituted-2(1H)-imino or oxopyridines: new antineoplastic agents with high selectivity towards leukemia cell lines. *Arch, Pharm (Weinheim)*. 1998; 331:319–24. [PubMed: 9844579]
8. Abadi AH, Abouel-Ella DA, Lehmann J, Tinsley HN, Gary BD, Piazza GA, Abdel-Fattah MA. Discovery of colon tumor cell growth inhibitory agents through a combinatorial approach. *Eur J Med Chem*. 2010; 45:90–97. [PubMed: 19836860]
9. Abadi AH, Ibrahim TM, Abouzid K, Lehmann J, Tinsley HN, Gary BD, Piazza GA. Design synthesis and biological evaluation of novel pyridine derivatives as anticancer agents and phosphodiesterase 3 inhibitors. *Bioorg Med Chem*. 2009; 17:5974–5982. [PubMed: 19628397]
10. Pogacic V, Bullock AN, Fedorov O, Filippakopoulos P, Gasser C, Biondi A, Meyer-Monard S, Knapp S, Schwaller J. Structural analysis identifies imidazo [1,2-b]pyridazines as PIM kinase inhibitors with in vitro antileukemic activity. *Cancer Res*. 2007; 67:6916–6924. [PubMed: 17638903]
11. Cheney IW, Yan S, Appleby T, Walker H, Vo T, Yao N, Hamatake R, Hong Z, Wu JZ. *Bioorg Med Chem Lett*. 2007; 17:1679–1683. [PubMed: 17251021]
12. Wendt MD, Sun C, Kunzer A, Sauer D, Sarris K, Hoff E, Yu L, Nettesheim DG, Chen J, Jin S, Comess KM, Fan Y, Anderson SN, Isaac B, Olejniczak ET, Hajduk PJ, Rosenberg SH, Elmore SW. Identification and structure-activity relationships of substituted pyridones as inhibitors of Pim-1 kinase. *Bioorg Med Chem Lett*. 2007; 17:3122–3129. [PubMed: 17391963]
13. Zhonghong L, Lianjie L, Changqing Z, Ying H, Yu J, Yan L. The influence of survivin shRNA on the cell cycle and the invasion of SW480 cells of colorectal carcinoma. *J Exp Clin Cancer Res*. 2008; 27:20. [PubMed: 18637206]
14. Rankin WV, Henry CJ, Turnquist SE, Turk JR, Beissenherz ME, Tyler JW, Rucker EB, Knapp DW, Rodriguez CO, Green JA. Identification of survivin, an inhibitor of apoptosis, in canine urinary bladder transitional cell carcinoma. *Vet Comp Oncol*. 2008; 6:141–150. [PubMed: 19178674]
15. MOE, Chemical Computing Group Inc. <http://www.chemcomp.com>

Fig. 1.
2D interaction of compound 10 with Survivin.

Fig. 2.
2D interaction of compound 10 with PIM1 Kinase.

Cpd	Fluoro Position	R	X	Cpd	Fluoro Position	R	X
1	4-F	2-methoxy phenyl	O	16	3-F	2-ethoxy phenyl	NH
2	4-F	2-methoxy phenyl	NH	17	3-F	2-furanyl	O
3	4-F	2-hydroxy phenyl	O	18	3-F	2-furanyl	NH
4	4-F	2-hydroxy phenyl	NH	19	3-F	3,4Dichlorophenyl	O
5	4-F	2-ethoxy phenyl	O	20	3-F	3,4Dichlorophenyl	NH
6	4-F	2-ethoxy phenyl	NH	21	2-F	2-hydroxy phenyl	O
7	4-F	2-furanyl	O	22	2-F	2-hydroxy phenyl	NH
8	4-F	2-furanyl	NH	23	2-F	2-methoxy phenyl	O
9	4-F	3,4Dichlorophenyl	O	24	2-F	2-methoxy phenyl	NH
10	4-F	3,4Dichlorophenyl	NH	25	2-F	2-ethoxy phenyl	O
11	3-F	2-hydroxy phenyl	O	26	2-F	2-ethoxy phenyl	NH
12	3-F	2-hydroxy phenyl	NH	27	2-F	2-furanyl	O
13	3-F	2-methoxy phenyl	O	28	2-F	2-furanyl	NH
14	3-F	2-methoxy phenyl	NH	29	2-F	3,4Dichlorophenyl	O
15	3-F	2-ethoxy phenyl	O	30	2-F	3,4Dichlorophenyl	NH

Scheme 1.

IC₅₀(Survivin): 0.8μM

IC₅₀(PIM1 Kinase): 13μM

Chart 1.

Some potential antitumor 3-cyano-4,6-diaryl-2(1H)imino or oxypyridines with their IC₅₀s against different possible molecular targets.

Chart 2.

Growth inhibitory potencies of some selected candidates relative to their brominated analogues versus HT-29 colorectal cancer cell lines [8,9].

Table 1

Tumor Cell Growth Inhibitory Activity of the Synthesized Compounds

Cpd #	HT-29Growth Inhibition IC ₅₀ ± SD(μM)	MDA-MB-231 Growth Inhibition IC ₅₀ ± SD (μM)	Cpd #	HT-29 Growth Inhibition IC ₅₀ ± SD (μM)	MDA-MB-231 Growth Inhibition IC ₅₀ ± SD (μM)
1	>50	>50	16	1.50±0.1	18.90±2.0
2	6.30±0.8	19.80 ± 2.0	17	>50	>50
3	42.50±1	9.30±0.7	18	1.26±0.2	>50
4	12.70±0.7	4.60 ±0.5	19	>50	>50
5	>50	16.60±1.2	20	3.96 ± 0.5	17.50±1.5
6	0.70±0.1	9.9±0.7	21	>50	>50
7	>50	>50	22	>50	>50
8	3.46±0.2	>50	23	>50	>50
9	>50	>50	24	10.50±0.4	13.30±1.5
10	2.18±0.1	4.63±0.5	25	12.30± 1.0	30.10±1.7
11	>50	>50	26	2.50±0.3	14.80±1.5
12	>50	>50	27	>50	14.90±1.0
13	12.70±1.2	32.50±1.5	28	8.82	17.90±1.0
14	9.30±0.8	20±0.5	29	>50	>50
15	10.20±1.2	22.80±0.8	30	5.74±0.4	6.98±0.8