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Imidazo[1,2-*a*]pyridines linked with thiazoles/thiophene motif through keto spacer as potential cytotoxic agents and NF-κB inhibitors

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ABSTRACT

A series of new imidazo[1,2-*a*]pyridine linked with thiazole/thiophene motif through a keto spacer were synthesized and tested for their cytotoxic potential against three human cancer cell lines including A549, HeLa and U87-MG using MTT assay. Compounds **A2**, **A3**, **A4**, **C1** and **C2** showed cytotoxicity against all the three cell lines. The selectivity index for compound **A4** for A549 and HeLa cells was comparable to that of doxorubicin. Among the synthesized compounds, **B5** showed the maximum inhibition of NF-κB activity as ascertained by NF-κB reporter assay (IC_{50} = 6.5 ± 0.6 μM). Treatment of NCI-H23 cells (EGFR overexpressed, KRAS G12V mutant) with erlotinib and gefitinib along with compounds **A4** and **B5** indicated synergistic and additive potential of combination therapy.

Cancer is a life threatening global health risk caused by abnormal functioning of multiple intracellular regulatory systems that occur in normal cells and characterized by aberrant cellular proliferation.[1,2] The disease is induced by a series of genetic and epigenetic changes, which can be triggered by both external (tobacco, alcohol, chemicals, infectious agents, and radiation)[3–5] and internal factors involving hormones, inherited mutations, cytokines and chemokines.[6–8] Among internal factors, NF-κB is an inducible transcription factor involved in the regulation of large number of normal cellular processes, such as immune and inflammatory responses, cellular growth and apoptosis.[9,10]

NF-κB proteins are a family of eukaryotic transcription factors. In unstimulated cells, NF-κB is present in the cytoplasm bound to its inhibitory subunit IκB. When these cells are stimulated by external stimuli or cellular signaling, active NF-κB is released from its cytoplasmic complex, translocates to nucleus and binds to DNA.[11,12] NF-κB binding to DNA regulates cellular signaling pertaining to cell survival, cell differentiation and cell growth. Uncoupling of NF-κB proteins from their regulators leads to their constitutive activation. Constitutively activated NF-κB is involved in various aspects of carcinogenesis such as proliferation of cancer cell, evading apoptosis and increased metastatic potential.[13,14] Hence, inhibition of NF-κB transcriptional activation is considered as an important target for developing anticancer agents.

A large number of structurally diverse molecules from natural products as well as from synthetic origin have been synthesized and evaluated for their NF-κB inhibitory activity (Fig. 1). For instance, curcumin, a major component of turmeric, and resveratrol, present in red grapes, have been reported to suppress the activation of NF-κB through inhibition of IKK activity.[15,16] Rugulactone and its derivatives exerted strong anti-proliferative effects in MDA-MB-231 cells through the inhibition of NF-κB.[17] 1,3,5-Triazine,[18] indoline,[19] benzoxathiole,[20] and coumarin[21] as core nucleus have been well documented as inhibitors of NF-κB. In addition, Choi et al., synthesized benzofuran and 2,3-dihydrobenzofuran-2-carboxamide derivatives and tested their NF-κB inhibitory activity in LPS-stimulated RAW 264.7 macrophage cells.[22] However, only benzofuran-2-carboxylic acid *N*-(4′-hydroxy)phenylamide was found to possess potent anti-proliferative as well as NF-κB inhibitory activity.

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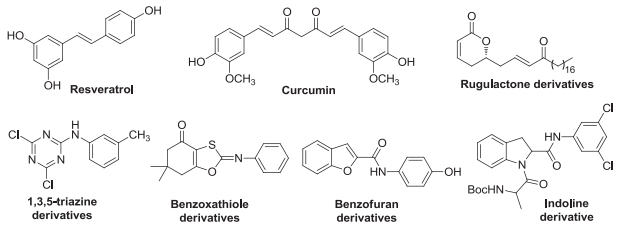


Fig. 1. Structures of some representative NF-κB inhibitors from natural and synthetic origin.

Imidazo[1,2-*a*]pyridine is one of the most important heterocyclic scaffold possessing diversified biological activities and has been considered as privileged structure because many marketed drugs such as zolpidem, alpedim, olprinone etc. contain it as a core template.[23] Moreover, imidazo[1,2-*a*]pyridine has emerged as an attractive pharmacophoric unit in the development of new anticancer agents.[24] Such imidazo[1,2-*a*]pyridine derivatives have shown promising anti-proliferative activity through inhibition of various cancer targets such as PI3Kα and/or mTOR,[25a,25b] tubulin,[25c] and cyclin-dependent kinase[25d] etc. Despite of the great advances in cancer chemotherapy, the past few years have witnessed increased awareness towards development of better anticancer drug candidate. In this context, pharmacological evaluation of imidazo[1,2-*a*]pyridine fused heterocycles represents a high-priority research area for the development of 'drug-like' or 'lead-like' molecules.

As a part of our ongoing research to discover and develop novel inhibitors of NF-κB mediated transcriptional activation as anticancer agents, we reported a series of 2-(2-alkylamino-4-substituted-thiazole-5-yl)-3-aryl-3*H*-quinazolin-4-one[26a,26b] and 2-thiophen-5-yl-3*H*-quinazolin-4-one derivatives[26c] as inhibitors of NF-κB and AP-1 mediated transcription. Owing to our long-standing interest in further exploration of these scaffolds, we became interested to evaluate the effect of hybridization of imidazo[1,2-*a*]pyridine ring with thiazole/thiophene nucleus into a single molecule, which should enable both the moieties to interact with the target protein without any restrictions. This is possible if we have a carbonyl/keto spacer, which enables the two ring scaffolds to be in a suitable conformation to interact with target protein as given in (Fig. 2). Moreover, oxygen having lone pair of electrons enable it for hydrogen bonding with the target protein. In order to investigate these conjugates, three series of imidazo[1,2-*a*]pyridine linked with thiazole (**A** and **B**) and thiophene (**C**) nucleus through a carbonyl spacer were synthesized with various substitutions. The synthesized compounds have been evaluated for their anti-proliferative activity, and some of them were further tested for NF-κB inhibition.

The preparation of the target compounds is shown in Scheme 1. Condensation of commercially available 2-aminopyridines **1a–c** with DMF-DMA and cyclization of resulting *N*,*N*-dimethyl-*N*'-(pyridin-2-yl)formimidamide **2a–c** with 1,3-dichloro-acetone gave 2-chloro-1-(imidazo[1,2-*a*]pyridin-3-yl)ethanone derivatives **3a–c**. Compounds **4a–d** were synthesized by slow addition of morpholine/*N*-methylpiperazine over a solution of the corresponding benzoyl isothiocyanate in ethyl acetate. Compounds **5a–g** and **6a–g** were obtained by reacting different aryl isothiocyanate with amidines/guanidine/alky 3-aminobut-2-enoate derivatives as previously described by our research group.[27] The key intermediates **4**, **5**, and **6** were further reacted with **3** to furnish target compounds (**A**, **B**, and **C**) with moderate to good yields. All the compounds were purified by column chromatography and characterized by various spectroscopic techniques (^{1}H NMR and ^{13}C NMR). For specific substitutions, refer Table 1.

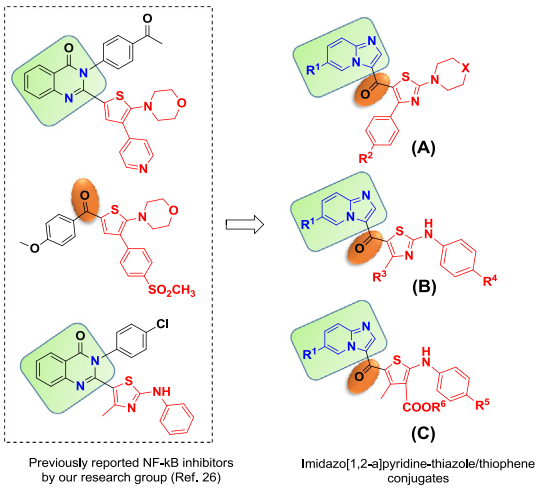
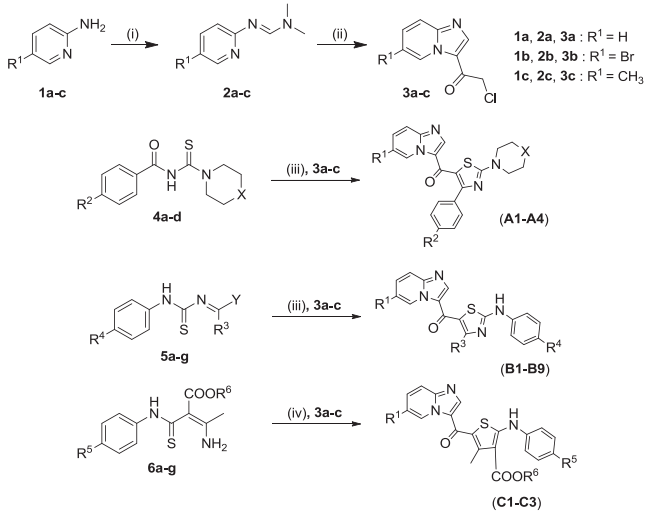


Fig. 2. Design of imidazo[1,2-*a*]pyridine-thiazole (**A** and **B**)/thiophene (**C**) conjugates.



Scheme 1. Synthesis of key intermediates and imidazo[1,2-*a*]pyridine-thiazole/thiophene conjugates: (i) DMF-DMA, MeOH, reflux; (ii) 1,3-dicholoacetone, CH_3CN, rt; (iii) TEA, DMF, rt; (iv) cat. KI, TEA, DMF, rt.

Out of 16 synthesized compounds, cytotoxicity of 14 compounds was evaluated against human lung cancer line (A549), human cervical carcinoma cell line (HeLa), human glioma cell line (U-87 MG) and mouse peritoneal macrophages using MTT assay.[28] Doxorubicin was used as reference agent. The IC_{50} values for anti-proliferative potency of the compounds are shown in Table 2.

The compounds **A2**, **A3**, **A4** and **B3** showed good anti-proliferative effect on A549 cell line with **B3** having the lowest IC_{50} (14.71 ± 1.81 μM). Compounds **A2**, **A3**, **A4**, **C1** and **C2** were more effective as anti-proliferative agents against HeLa cell line, **C1** being the most potent among them (IC_{50} = 13.51 ± 0.56 μM). In case of U-87MG cell line, the anti-proliferative effect of **A2**, **B4**, **C1** and **C2** was better than other compounds. Among these compounds, **C2** has the lowest IC_{50} (43.15 ± 0.35 μM) against U-87 MG cell line. The Selectivity Index (SI) was calculated for **A2**, **A3**, **A4**, **B3**, **B5**, **C1**, and **C2** as shown in Table 2. SI is the ratio of the cytotoxic potential of these compounds on rat peritoneal macrophages and the anti-proliferative potential of these compounds on different cell lines. SI of **A4** (2.7), **A2** (4.1) and **B3** (2.6) for A549 cell line is comparable with that observed for anticancer drug doxorubicin (2.56). SI of **C1**