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Nicotinonitrile as an Essential Scaffold in Medicinal Chemistry: An Updated Review

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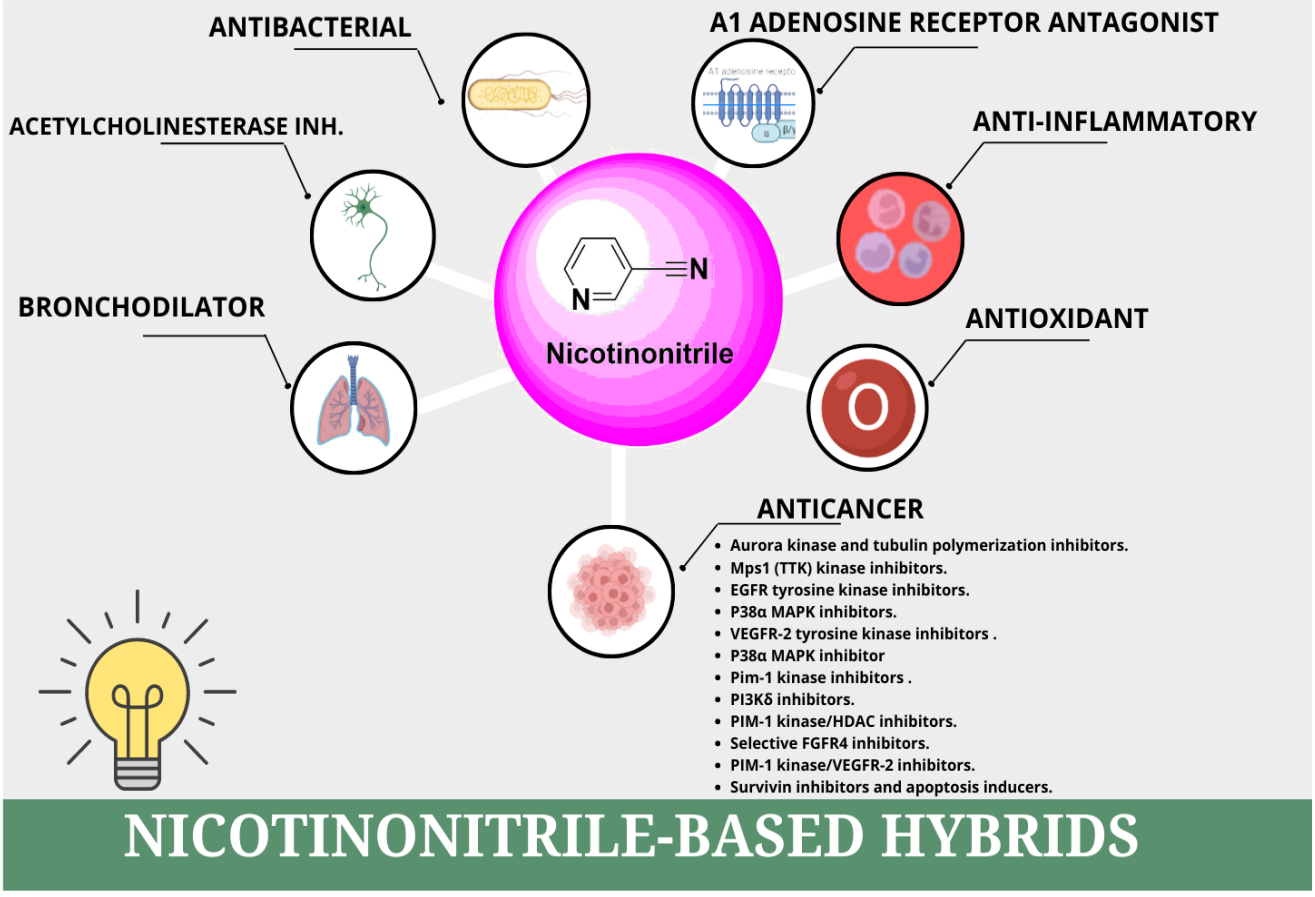
Abstract

Many studies have been carried out to find synthetic pathways to nicotinonitriles (3-cyanopyridines) and their analogs, which has resulted in a substantial amount of knowledge on their biological, therapeutic, and medical characteristics. Some of nicotinonitrile derivatives have emerged as marketed drugs such as bosutinib, milrinone, neratinib, and olprinone. In this review, we discuss the recently synthesized nicotinonitrile-based hybrids that possess important biological activity and will be useful for drug discovery and development.

Keywords
Nicotinonitrile, Hybrids, Anticancer



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1.Introduction

A diversified and highly functionalized nitrogen-containing heterocyclic compounds are core structural units in several natural products and synthetic drugs. These natural products and synthetic molecules possess tremendous applications in drug discovery and are valuable functional materials [1-3]. The pyridine ring system is one of the most common N-heteroaromatics found in a variety of physiologically active chemicals. It is also present in numerous natural compounds such as nicotinic acid, nicotinamide, and vitamin B6, all of which play important roles in metabolism. The 3-cyanopyridine **1** (nicotinonitrile) nucleus (**Fig. 1**) has attracted considerable attention recently due to its emergence as a derivative with diverse pharmacological activity, particularly in the medicinal field. Furthermore, 3-cyanopyridine can be fused with several functional groups forming fused cyanopyridines that was proved to have a broad spectrum of pharmacological activity [1]. Several 3-cyano-2-substituted pyridine hybrids were synthesized and shown to have various biological activities such as antioxidant activity [2]**,** anti-inflammatory activity [3], antiproliferative activity [4], antibacterial activity [5], acetylcholinesterase inhibitors [6], anti-tubulin activity [7], a bronchodilator [8] and A1 adenosine receptor antagonists [9]. Many drugs containing nicotinonitrile derivatives are available on the market, such as milrinone and olprinone (**Fig. 1**). This review article highlights the recently synthesized nicotinonitrile hybrids that possess important biological, therapeutic, and medicinal properties.

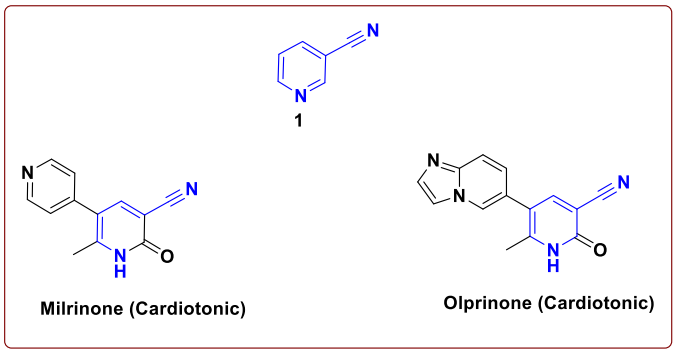


Figure 1. Structure of nicotinonitrile (**1**) and marketed nicotinonitrile-based drugs

2.Biological activity of Nicotinonitrile hybrids

Nicotinonitrile and their conjugates have been shown to play a critical function in a variety of biological processes in addition to their pharmacological and chemical significance. We summarize some of their potential activity:

2.1. Nicotinonitrile hybrids as antioxidant agents

In the body, free radicals of different types are continuously made for a specific metabolic need and then must get rid of them by an effective antioxidant network. When the production of free radicals exceeds the levels of antioxidant mechanisms this may lead to damage of cells, proteins, and other genetic materials, such as DNA. This can cause oxidative damage to tissues and organs, leading to many health problems. In the end, their effects cause severe long-term health issues, primarily degenerative diseases.

Huda R. M. *et al*. synthesized a series of nicotinonitrile derivatives, including compound **2** (**Fig. 2**) demonstrating superior antioxidant activity than ascorbic acid measured by the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging method. The cyano group linked to the pyridine ring might have a unique capacity to scavenge reactive oxygen species, protecting cells from DNA damage [2].

In another study, Moustafa A. Gouda *et al*. colleagues synthesized compounds **3**, **4**, and **5** (**Fig. 2**) having a 3-cyano-pyridine moiety and showed that nicotinonitrile moiety has antioxidant activity and is more effective than ascorbic acid as measured by an *in vitro* ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay [10] .

Likewise, nicotinonitrile derivatives bearing a furan moiety were synthesized and evaluated for their antioxidant activity and protection of DNA damage. The data revealed that compounds **6**, **7** and **8** (**Fig. 2**) showed promising *in vitro* antioxidant activities using ABTS method. Compound **8** is the highest protective activity against DNA damage induced by the bleomycin iron complex [11].

The incorporation of substituted 3-cyanopyridine into phenothiazine derivatives is another strategy to get optimum antioxidant activity and showed that compounds **9** and **10** have good ABTS radical scavenging activity [12] (**Fig. 2**).

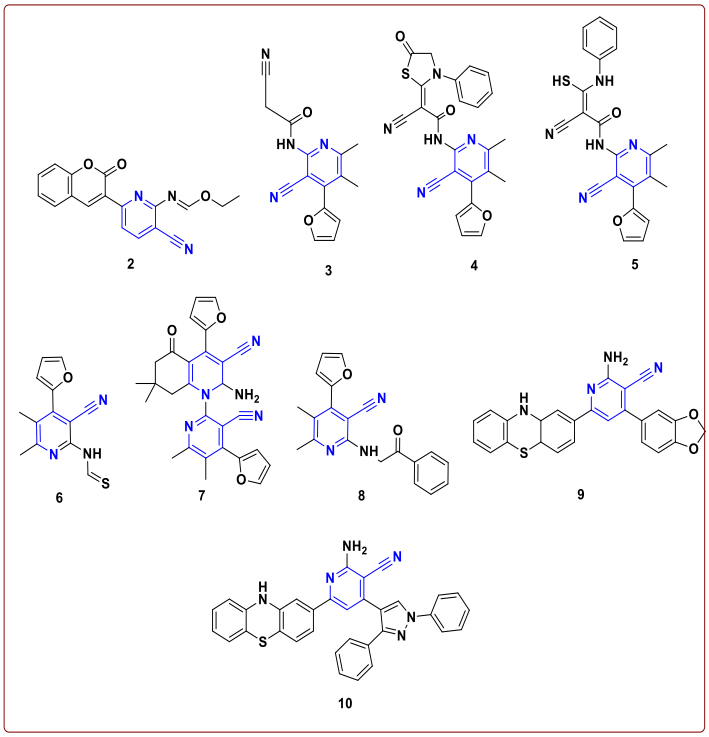


Figure 2. Structure of nicotinonitrile hybrids (**2-10**) showing antioxidant activities

2.2. Nicotinonitrile hybrids as anti-inflammatory agents

Nonsteroidal anti-inflammatory drugs act by inhibiting the activity of cyclooxygenase enzymes involved in the synthesis of prostaglandins, which play a role in inflammation. Medicinal chemists have developed many nicotinonitrile conjugates with potent anti-inflammatory activity.

The compounds **11a**, **11b**, and **12** were tested *in vivo* for their anti-inflammatory activity. The findings revealed that the *S*-alkyl derivative of 3-cyano-2-substituted pyridine **11a** and *N*-alkyl derivative **11b** were more effective than the reference indomethacin and relatively close to celecoxib in the degree of edema inhibition after 1, 2, and 3 hours. The anti-inflammatory effect of *S*-alkyl derivative **12** against edema was impressive (**Fig. 3**) [3].

Compared to nimesulide's activity (47.31% inhibition of paw edema), 3-cyano-2-pyridone derivative **13** exhibited comparable selective anti-inflammatory efficacy (46.90% inhibition of paw edema) against the cyclooxygenase-2 enzyme (**Fig. 3**) [13] .

Based on the structural features and binding sites of the COX-2 active site, Ved Prakash and colleagues designed a series of pyridone/pyridine-based building blocks and a series of polymethylene-containing heterocyclic compounds. Compound **14** inhibited up to 13.7 percent after 180 minutes, while the reference drug (nimesulide) inhibited up to 9.1 percent after the same time(**Fig. 3**) [14].

S.R. Shahinda *et al*. developed a series of 4,6-diaryl-3-cyanopyridin-2-ones and tested their COX-2 inhibition and anti-inflammatory properties. Compounds combining 4-methanesulfonylaminophenyl at C-6 of the pyridine ring and either 4-chorophenyl **15** or thienyl **16** at C-4 of the pyridine ring had better anti-inflammatory activity than celecoxib (edema inhibition percent 60 after 3 hours) (**Fig. 3**) [15].

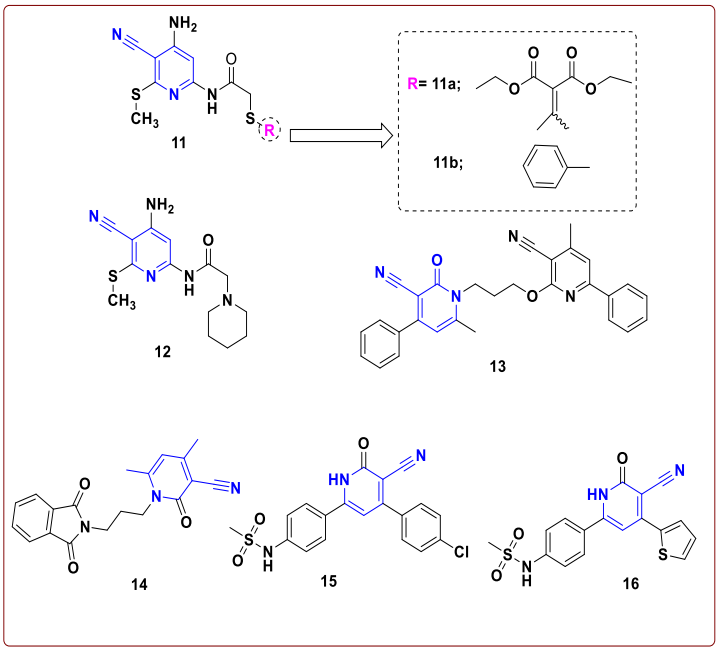


Figure 3. Structure of nicotinonitrile hybrids (**11-16**) exhibiting anti-inflammatory activities

2.3. Nicotinonitrile hybrids as antiproliferative agents

Cancer is still a rampant disease, and the malignant conditions associated with it motivate researchers to develop various targeted molecules. Various research efforts have been made to investigate the anticancer effects of different modified lead compounds. Nicotinonitrile hybrids have been shown to have anticancer activity through several mechanisms.

2.3.1. Nicotinonitrile hybrids as aurora kinase inhibitors and tubulin polymerization inhibitors

Aurora kinases are a group of highly conserved serine/threonine protein kinases [16] that have been linked to a variety of mitotic processes [17]. In humans, Aurora kinases have three homologs: Aurora A (A2k) and Aurora B kinase (A1k) that are found in all cells, whereas Aurora C kinase (A3k) is exclusively found in testes [18]. Aurora A and Aurora B kinase are promising cancer treatment targets. In clinical studies, certain Aurora kinase inhibitors have shown to be effective against a variety of tumor form [19].

The introduction of various substituted phenoxyethylamino or pyridyloxyethylamino groups into the 2- position of 3-cyano-4-methyl-6-(5-methyl-3-pyrazoloamino)-pyridine created a new class of dual inhibitor of Aurora kinase and tubulin polymerization. Compound **17** demonstrated remarkable protein kinase selectivity for Aurora A and B kinases by blocking phosphorylation of Ser10 of histone H3. Furthermore, it inhibited tubulin polymerization *in vitro*, had good cell membrane permeability, and had a favorable PK profile. It reduced the growth of HCT116 cells with an IC_{50} value of 0.001 µM (**Fig. 4**) [20].

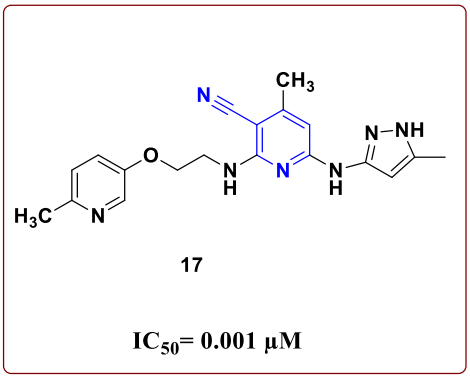


Figure 4. Structure of nicotinonitrile hybrid (**17**) as aurora kinase inhibitors and tubulin polymerization inhibitors

2.3.2. Nicotinonitrile hybrids as EGFR tyrosine kinase inhibitors

The epidermal growth factor receptor (EGFR/ErbB-1) and the associated human epidermal growth factor receptor-2 (ErbB-2/Her-2) are transmembrane growth factor receptor protein tyrosine kinases (PTKs) that regulate cell growth, differentiation, mitosis, and apoptosis [21]. Overexpression of these receptors has been observed in a number of malignancies, including breast, ovarian, colon, and non-small cell lung cancer (NSCLC) and has been associated with poor prognosis for patients [22].

Yongjun Mao and coworkers designed and synthesized 4-anilino-3-cyano-5-vinyl/ethynyl/phenyl pyridine derivatives and selectively tested them for EGFR and ErbB-2 kinases as well as growth inhibition of A-549 and HL60 cells. The results showed that compound **18** exhibited excellent EGFR inhibition with an IC_{50} value of 0.6 µM (**Fig. 5**) [23].

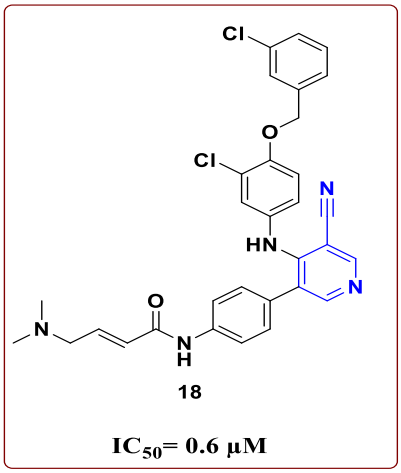


Figure 5. Structure of nicotinonitrile hybrid (**18**) as EGFR tyrosine kinase inhibitor

2.3.3. Nicotinonitrile hybrids as VEGFR-2 tyrosine kinase inhibitors

Vascular endothelial growth factor receptors (VEGFR) have been identified as an excellent target for curative agents in the development of anticancer drugs [24]. VEGFR-2 is a class V tyrosine kinase receptor (RTK) that is triggered by specific binding of the extracellular regulatory domain of VEGFR-2 in both endothelial and various tumor cells. When VEGFR-2 is stimulated, autophosphorylation occurs activating signaling pathways that lead to endothelial cell proliferation and tumor angiogenesis that promote tumor development [25].

The synthesized 3-cyanopyridine-sulfonamide hybrids were tested *in vitro* against 60 lines of human cancer cells and their enzyme inhibitory activity toward vascular endothelial growth factor receptor 2 was measured. Compound **19** showed potent anticancer activity with GI_{50} values of 1.06-8.92 µM against most of the cancer cell lines tested. Moreover, VEGFR-2 was effectively inhibited by compound **19** with a lower IC_{50} value of 3.6 µM compared to sorafenib (IC_{50}= 4.8 µM) (**Fig. 6**) [26].

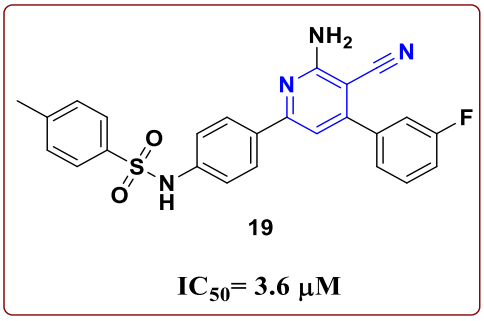


Figure 6. Structure of nicotinonitrile hybrid (**19**) as VEGFR-2 tyrosine kinase inhibitor

2.3.4. Nicotinonitrile hybrids as PIM-1 kinase inhibitors

PIM Kinases (provirus integration in Maloney) are significantly expressed in a variety of hematologic and solid malignancies. PIM Kinases, also called protooncogenes, modulate a network of signaling pathways important for cancer and growth, making them promise therapeutic targets [27]. PIM -1 regulates the cell cycle by phosphorylating Cdc25 phosphatases and cell cycle inhibitors [28, 29]. It also enhances Cdc25A-mediated cell transformation by phosphorylating and activating the phosphatase Cdc25A [30], which enhances the transformation of cells from G1 to S phase. PIM -1 is also associated with the development of drug resistance. Its expression has been found to be increased in cancer cell lines resistant to mitoxantrone and docetaxel [31, 32].

Khaled M. Abouzid research group synthesized a series of 2-amino-cyanopyridines and 2-oxo-cyanopyridines targeting PIM-1 kinase. Among the synthesized compounds, compound **21** showed potent antiproliferative activity with high PIM-1 kinase activity (IC_{50} value of 0.94 µM). Moreover, apoptosis studies were performed for compound **20** to evaluate the proapoptotic potential of synthetized compounds. The results showed that compound **20** increased the level of active caspase 3 by 5.6-fold compared to the control. It also increased the Bax/Bcl2 ratio by 22704-fold compared to the control (**Fig. 7)** [33].

Several 4,6-diaryl-3-cyano-2-pyridones were synthesized and evaluated for their cytotoxic activities against HepG2 and THLE-2 cell lines. Compound **21** was found to be more cytotoxic as it had the lowest IC_{50} value of 7.26 compared to 5- FU (IC50 = 6.98 µM). It had a 76.76% effect on cell proliferation. Moreover, it significantly stimulated apoptotic death of liver cancer cells by 49.78-fold (22.90% compared to 0.46% in control) by arresting the cell cycle Pre G1 in 35.16% of a cell population, compared to 1.57% in control. Moreover, it confirmed the intrinsic apoptosis of P53 overexpression with suppression of anti-apoptotic genes by PIM-1 inhibition (**Fig. 7)** [34].

Magda M.F. Ismail *et al.* developed 2-amino-6-(4-(benzyloxy)phenyl)-4-(4-(dimethylamino)phenyl)nicotinonitrile **22**, which is considered the most potent inhibitor of proliferation of PC-3 and HepG-2 cancer cell lines with IC_{50} values of 2.04 µM (selectivity index; SI = 78.63) and 3.73 µM (selectivity index; SI = 43.00), respectively among other compounds. In addition, it is the most potent PIM-1 kinase inhibitor with an IC_{50} of 0.47 µM compared to the IC_{50} of the reference compound 5-fluorouracile of 3.98 µM, and it induces G2/M cell cycle arrest (**Fig. 7)** [35].

In a recent study, two unique series of 6-(4-benzamido-/4-phthalimido)-3-cyanopyridine derivatives were designed and prepared as PIM-1 kinase inhibitors. These hits inhibited PIM -1 kinase 76.43 to 53.33 percent. Compounds **23** and **24** with rigid side chains (4-phthalimidophenyl) were the most potent sub-micromolar inhibitors of PIM-1 kinase activity (IC_{50}= 0.76 and 0.63 µM, respectively) (**Fig. 7)** [36]

2.3.5. Nicotinonitrile hybrids as dual acting PIM-1 kinase/HDAC inhibitors

Multitarget or smart hybrids with two or more pharmacophores targeting cancer [37, 38] are one of the most promising approaches in this field. Histone deacetylases Inhibitors (HDACIs) launched in recent years are considered as a potential therapeutic strategy against cancer.

Histone deacetylases (HDACs) are specialized enzymes that control chromatin remodeling. Currently, 18 HDAC genes are known to be involved in chromatin remodeling. HDACIs alter both histone and non-histone proteins, decrease cancer cell invasion, sensitize cancer cells to chemotherapy, promote apoptosis and immunogenicity [39]. In this scenario, suppression of HDAC activity (HDAC-1 and HDAC-2) altered chromatin occupancy of chromatin remodeling proteins during the DNA repair process [40].

Abuo-Rahma research group took advantage of this strategy and synthesized compounds that could be dual PIM-1/HDAC inhibitor hybrids by using 3-cyanopyridines as a cap moiety linked to an aliphatic linker bearing a hydroxamic acid moiety. Hybrids **25**, **26**, **27**, and **28** showed higher inhibitory activity against HeLa cell nuclear extract, HDAC 1 and 6 isozymes and exhibited strong PIM-1 inhibitory activity, especially compound **26** (IC_{50} 343.87 ± 16.6 nM), which was equipotent to the reference drug quercetin (IC_{50} 353.76 ± 17.1 nM). Hybrid **26** also showed obvious apoptosis-inducing potential in MCF-7 cell lines, causing apoptosis before G1 phase and cell cycle arrest in G2/M phase (**Fig. 8**) [41].

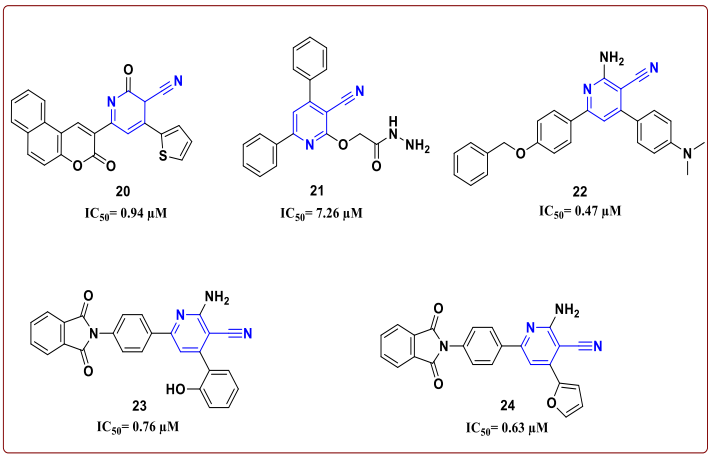


Figure 7. Structures of nicotinonitrile hybrids (**20-24**) as PIM-1 kinase inhibitors

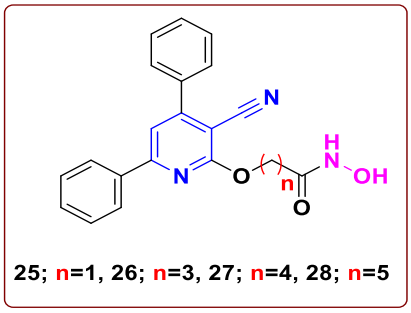


Figure 8. Structures of nicotinonitrile hybrids (**25-28**) as dual acting PIM-1 kinase/HDAC inhibitors

2.3.6. Nicotinonitrile hybrids as dual acting PIM-1 kinase/VEGFR-2 inhibitors

The combination of PIM kinase inhibitors with anti-VEGF therapy results in a synergistic antitumor response and a decrease in the frequency of hypoxia-mediated resistance, consistent with vascular responses to both drugs. This technique was used by Ola H. Rizk *et al.* to develop a series of 3-cyanopyridine derivatives. The results showed that compound **29** had greater anticancer potential than doxorubicin against all cell lines tested, especially HepG-2 with promising SI value. Mechanistic studies also showed that compound **29** induced caspase 3/7 and disrupted DNA more than doxorubicin. As shown by VEGFR-2 inhibition assay, compound **29** has about the same effect as quercetin (IC_{50} = 5.22 µM). The PIM-1 kinase inhibition assay showed that it was more effective than quercetin (IC_{50} = 9.23 µM). Real-time PCR assays showed that compound **29** had greater therapeutic potential than doxorubicin to alter VEGF, p53, and cyclin D levels (**Fig. 9**) [42].

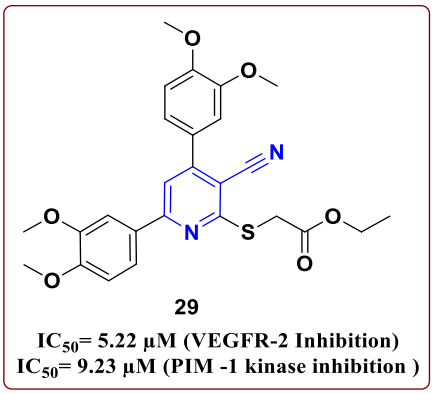


Figure 9. Structure of nicotinonitrile hybrid (**29**) as dual acting PIM-1 kinase/VEGFR-2 inhibitior

2.3.7. Nicotinonitrile hybrids as Mps1 (TTK) kinase inhibitors

Monopolar spindle 1 (Mps1), also known as TTK, is a dual-specificity kinase with an essential role for the spindle assembly checkpoint [43-47] that prevents cell cycle progression from metaphase to anaphase when chromosomes are not properly attached to the mitotic spindle. Increased mRNA and protein levels of Mps1 are observed in human cancer cells [48-50], thus selective inhibition of Mps1 should be a potential strategy for the development of cancer therapeutics.

In a recent study, an aminopyridine-based lead compound **30** that binds to a reverse peptide conformation in the Mps1 hinge region was used. Further development of the aminopyridine scaffold at the 2- and 6-positions led to the discovery of compound **31** that showed no significant inhibition for 287 kinases, whereas it enhanced cellular Mps1 and antiproliferative activity in A549 lung carcinoma cells (cellular Mps1 IC_{50} = 5.3 nM, A549 IC_{50} = 26 nM). A strong correlation between cellular Mps1 and antiproliferative IC_{50} values indicated that the antiproliferative activity found in A549 cells could be responsible for Mps1 inhibition (**Fig. 10**) [51].

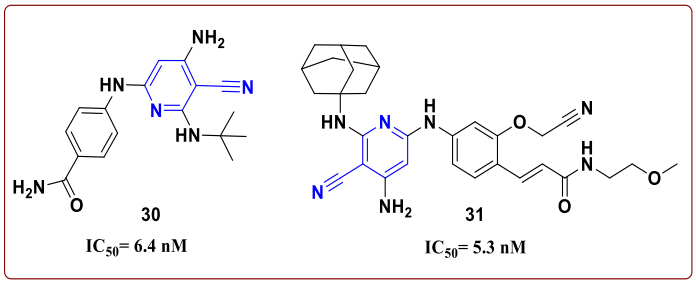


Figure 10. Structure of nicotinonitrile hybrids (**30-31**) as Mps1 (TTK) kinase inhibitors

2.3.8. Nicotinonitrile hybrids as P38α MAPK inhibitors

Breast cancer is the second leading cause of death in women and the most diagnosed disease [52]. Mitogen-activated protein kinases (MAPKs) are phosphorylating serine/threonine kinases that control signal transduction pathways and are involved in cell growth, differentiation, apoptosis, and transcription [53, 54]. There are four subfamilies of the MAP -kinase family: c-jun *N*-terminal or stress-activated protein kinases (JNK/SAPK), extracellular signal-regulated kinases (ERKs), ERK /big MAP kinase 1 (BMK1), and p38 protein kinases. The family of p38

protein kinases includes four isoforms (p38α, p38β, p38γ and p38δ) [55-57].
The p38α subtype is the best and first characterized isoform that plays an important role in cancer. High p38α levels have been associated with poor prognosis and highly invasive breast cancer. P38α is phosphorylated in approximately 20% of breast cancers and its selective activation occurs in intraductal grade breast tumors II or III, also its downregulation elicits antitumor responses [58, 59]. In addition, the development of p38α inhibitors has been declared as a potential therapeutic option for the treatment of ER negative breast cancer [60].
Nicotinonitrile-benzofuran hybrid **32** showed the best antiproliferative activities with an IC_{50} value of 1.18 µM against MCF -7, better than lapatinib as a reference standard (IC_{50}= 4.69 µM). When inhibitory potency against p38α MAPK was examined, compound **32** was found to exhibit significant activity as a p38α MAP kinase inhibitor (IC_{50} = 0.040 µM). Therefore, it can be concluded that the cytotoxic activity of compound **32** was due to the suppression of the target enzyme. Compound **32** induced preG1 apoptosis and cell growth arrest in G2/M phase, preventing the mitotic cycle. It also activated caspase 7, which implements apoptosis (**Fig. 11**) [61].

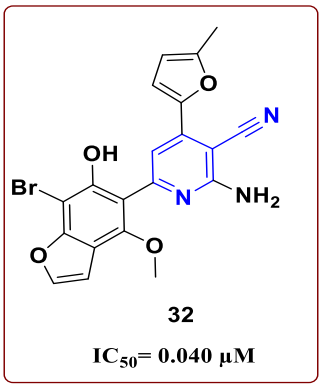


Figure 11. Structure of nicotinonitrile hybrid (**32**) as P38α MAPK inhibitor

2.3.9. Nicotinonitrile hybrids as potent phosphoinositide 3-kinase delta (PI3Kδ) inhibitors

Phosphoinositide 3-kinases (PI3Ks) are an important family of lipid kinases that play important regulatory roles in numerous cellular processes [62]. Based on structural differences and lipid substrate specificity, the PI3K family is generally divided into three distinct classes (including class I, II, and III), with class I PI3Ks further subdivided into IA and IB subgroups. There are three isoforms (PI3Kα, PI3Kβ, and PI3Kδ) in the class IA, which are heterodimers each containing a catalytic p110 subunit (p110α, p110β, and p110δ, respectively) and a regulatory p85 subunit, whereas only one isoform of PI3Kγ is in the class IB that consists of a catalytic p110γ and a regulatory p101 subunit [63]. Of these four PI3K isoforms, PI3Kδ has been shown to be predominantly expressed in B cells, where it catalyzes the phosphorylation of phosphatidylinositol-4,5-biphosphate to phosphatidylinositol-3,4,5-triphosphate and transports B cell receptor (BCR) signaling downstream, making PI3Kδ an essential signaling molecule for B cell proliferation, development, and survival [64]. Therefore, inhibition of PI3Kδ is considered therapeutically beneficial for hematologic malignancies such as chronic lymphocytic leukemia (CLL), follicular lymphoma (FL), and indolent non-Hodgkin's lymphoma (iNHL) [65].
A series of 6-aryl-substituted 4-pyrrolidinaminoquinazoline derivatives were developed and evaluated as potent PI3Kδ inhibitors. Among the synthesized derivatives, compound **33** showed equipotent PI3Kδ inhibition comparable to an IC_{50} value of 2.7 nM of reference drug Idelalisib. In addition, compound **33** exhibited beneficial PI3Kδ isoform selectivity against PI3Kα, PI3Kβ, and PI3Kγ and displayed distinct antiproliferation profiles against four human B cell lines of Ramos, Raji, RPMI-8226, and SU-DHL-6 [66]. In another research report, Minhang Xin and co-workers synthesized a series of 4-(piperid-3-yl)amino-substituted 6-pyridylquinazoline derivatives and found that compounds **34** and **35** exhibited significantly potent PI3Kδ inhibitory activities with IC_{50} values of 1.3 and 0.7 nM, respectively, which was equivalent or better than idelalisib (IC_{50} = 1.2 nM) (**Fig. 12**) [67].

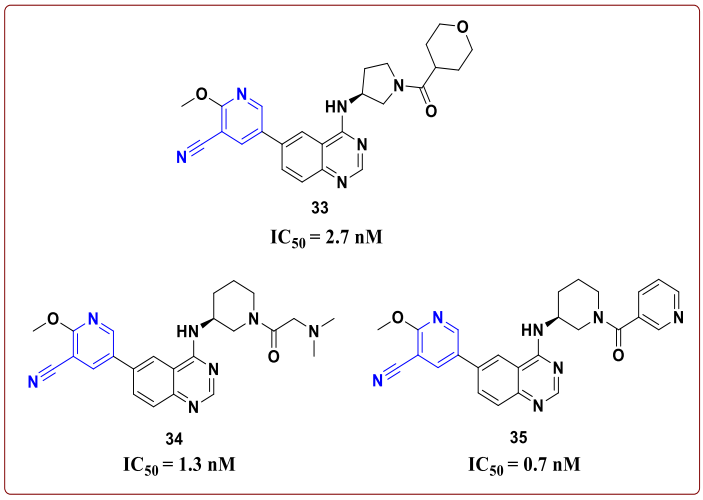


Figure 12. Structure of nicotinonitrile hybrids (**33-35**) as PI3Kδ inhibitors

2.3.10. Nicotinonitrile hybrids as potential selective FGFR4 inhibitors

Fibroblast growth factors (FGFs) signaling through FGF receptors (FGFRs) regulate basic developmental pathways and control events such as mesoderm patterning in the early embryo to the development of various organ systems [69]. FGF signaling extends to many physiological functions in the adult organism, including regulation of angiogenesis and wound healing. FGFRs are expressed on many different cell types and regulate important cell behaviors such as proliferation, differentiation, and survival, making FGF signaling vulnerable to subversion by cancer cells. There is compelling evidence for deregulated FGF signaling in the pathogenesis of many cancers arising from different tissue types. Impaired FGF signaling may promote tumor development by directly driving proliferation and survival of cancer cells and supporting tumor angiogenesis.
Compounds **36a-f** belonging to the 7-formyl naphthyridyl urea derivative **36**, were synthesized, and studied as FGFR4 kinase inhibitors. These compounds showed the strongest inhibitory effect on FGFR4 kinase and FGFR4 high-expressing tumor cells and possessed high selectivity toward FGFR4 over other kinases (**Fig. 13**).

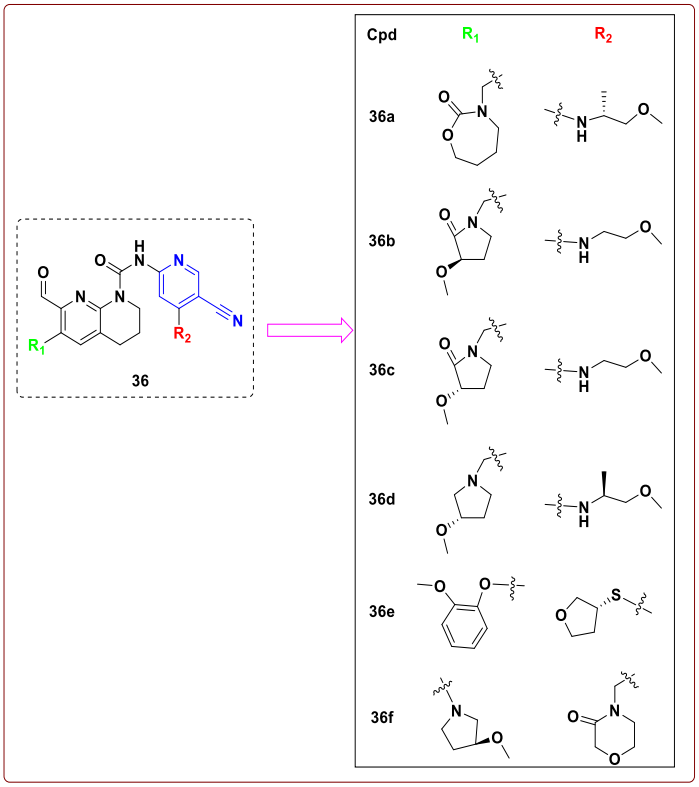
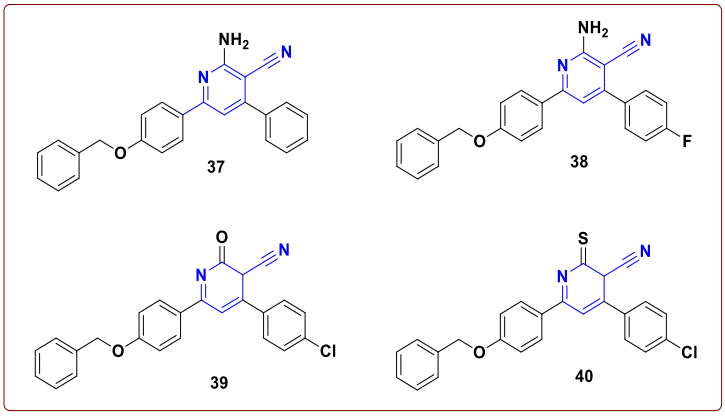


Figure 13. Structure of nicotinonitrile hybrids (**36a-f**) as FGFR4 inhibitors

2.3.11. Nicotinonitrile hybrids as survivin inhibitors and apoptosis inducers

The anti-apoptotic protein (survivin) belongs to the family of apoptosis inhibitors and is expressed almost exclusively in cancer cells during the G2/M phases of the cell cycle, whereas it is absent in most normal differentiated adult tissues. It promotes cancer cell survival by suppressing caspase-mediated apoptosis [70-74]. Overexpression of survivin is positively associated with cancer cell resistance to chemotherapy and poor patient prognosis. Therefore, survivin is considered one of the most important targets for the development of candidates with promising anticancer activity [75, 76]. Searching the literature for chemical scaffolds effective as survival inhibitors, it was found that 2-oxo-3-cyanopyridine derivative I exhibited significant anti-survivin activity [77]. Therefore, due to the remarkable anticancer activity against a variety of cell lines, great interest has been aroused in 3-cyanopyridine scaffolds [75-78].

Compounds **37**-**40** showed significant cytotoxic activities against three cancer cell lines: PC -3, HepG2 and MDA-MB -231 and were more effective than the reference drug 5- FU. Interestingly, they decreased Bcl-2 level by 1.9-3.8-fold and increased Bax level by 6.1-8.8-fold compared to control. They also increased the level of active caspase-3 by 7.1-8.5-fold compared with control. Further studies on the mechanism of action of most active compound **37**, revealed that it caused cell cycle arrest in G2/M phase and an increase in the percentage of pre-G1 apoptotic cells. In addition, Western blotting was performed with different concentrations of **37**. The results showed that **37** significantly suppressed the expression of survivin in PC -3 cells and caused a decrease in the ratio of caspase-7/cleaved caspase-7 and Bcl-2/Bax and an increase in cleaved PARP (**Fig. 14**) [79].



Figue 14. Structure of nicotinonitrile hybrids (**37-40**) as survivin inhibitors and apoptosis inducers

2.4. Nicotinonitrile hybrids as antibacterial agents

Cyanopyridine derivatives are well-known for their antimicrobial activity against Gram-negative and Gram-positive bacteria [80, 81]. Magda M. F. Ismail and colleagues synthesized a series of cyanopyridines and fused cyanopyridines and evaluated their antibacterial activity (minimum inhibitory concentration [MIC]) against *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro*, using amoxicillin and trimethoprim/sulfamethoxazole (TMP/SMX) as reference standards. It is worth noting that compounds **41**, **42**, and **43** demonstrated maximum antibacterial activity, suggesting that they might form a new class of antibacterial agents. According to the activity data, several test compounds exhibited the most promising broad-spectrum antibacterial properties [5].

Furthermore, the nicotinonitrile-coumarin hybrids were tested *in vitro* against three Gram-negative (*Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa*) and two Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) bacteria strains. Using ciprofloxacin as a reference drug, the values of inhibition zones and MIC were used to assess the strength of their antibacterial activity [82, 83]. When compared to ciprofloxacin, the nicotinonitrile-coumarin derivative **44** showed the most potent antibacterial properties, with MIC values of 1.9, 3.9, 3.9, 3.9 and 7.8 µg/mL against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Staphylococcus aureus* bacterial strains, respectively. Compound **45** also showed significant antibacterial activity against the investigated bacterial strains (MIC values = 3.9–15.6 µg/mL) (**Fig. 15**) [84].

When compared to ciprofloxacin, 2-((4-(Benzo[d][1,3]dioxol-5-yl)-3-cyano-6-(thiophen-2-yl)pyridin-2-yl)thio)acetamide **46** had the lowest MIC/MBC values of 4.8/9.6, 4.8/9.6, and 9.6/19.7 µM against *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus mutans*, respectively. Compound **46** also showed superior biofilm inhibition activity compared to ciprofloxacin, with IC_{50} values ranging from 5.2 to 7.3 µM against *S. aureus*, S. *mutans*, and *E. coli* bacterial strains (**Fig. 15**) [85].

Pyridine derivatives containing *p*-dimethylaminophenyl and *p*-bromophenyl moieties at positions 4 and 6 were synthesized and the findings revealed that compound **47** had the highest activity against Gram-negative bacteria (*Escherichia coli*) and moderate activity against Gram-positive bacteria (*Staphylococcus aureus*) (**Fig. 15**) [86].

The antibacterial activity of the synthesized 1,2,3-triazole-linked nicotinonitriles **48** was investigated, and compounds **48a** and **48b** had the highest activity against Gram-positive bacteria *S. aureus* with MIC= 17.6 and 16.8 g/mL, respectively. Compound **49** also had the best activity against Gram-negative bacteria *Escherichia coli* with MIC of 9.6 g/mL(**Fig. 15**) [87].

The antibacterial activity of a series of [1,2,4]triazolo[4,3-a]quinoxaline-nicotinonitrile hybrids was studied against a number of pathogenic organisms. Compound **50** having unsubstituted para-phenyl on the nicotinonitrile moiety showed broad antibacterial activity against most Gram-positive and Gram-negative strains with MIC= 3.9 µg/mL (**Fig. 15**) [88].

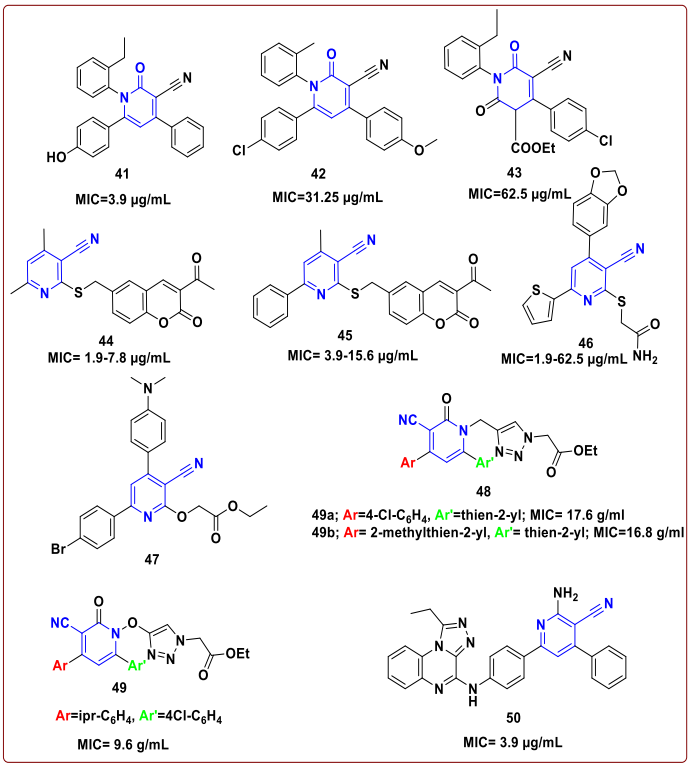


Figure 15. Structure of nicotinonitrile hybrids (**41-50**) showing antibacterial activities

2.5. Nicotinonitrile hybrids as acetylcholinesterase inhibitors

The gradual loss of cholinergic neurons in brain regions involved in cognitive activities provides strong evidence in Alzheimer's disease. The initial hypothesis that targets the serine hydrolase acetylcholinesterase (AChE) is one of the most promising treatments for Alzheimer's disease (AD) [89].

Series of nicotinonitrile-coumarin hybrid molecules [6] bearing arene moieties and linked *via* thioethers were synthesized and evaluated for their capability as potential acetylcholinesterase inhibitors using the Ellman method [90]. Compound **51** showed more potent inhibitory activity than the reference donepezil with IC_{50} of 13 nM. Compound **52**, linked to the 6-(4-chlorophenyl) group was the second in inhibitory strength toward the AChE enzyme and showed IC_{50} of 25 nM compared with donepezil (IC_{50} = 14 nm) [6]. Compound **53**, linked to 6-(4-nitrophenyl) group, exhibited more activity than donepezil with an inhibition percentage of 94.1. The second in AChE inhibitory strength was compound **54**, bearing 6-(4-chlorophenyl) moiety with an inhibition percentage of 72.3 (**Fig. 16**) [91].

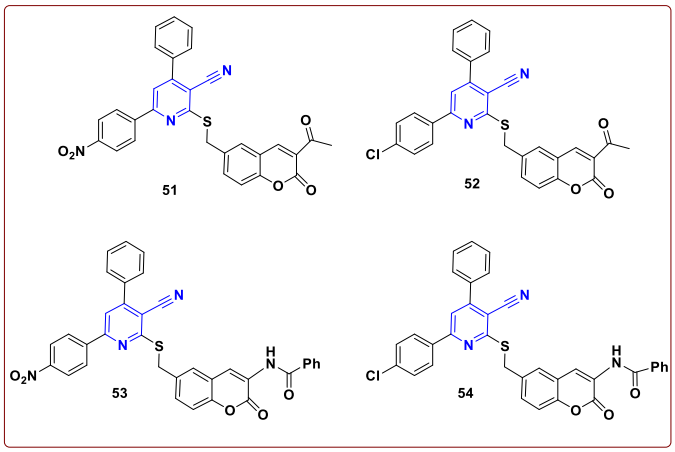


Figure 16. Structure of nicotinonitrile hybrids (**51-54**) as acetylcholinesterase inhibitors

2.6. Nicotinonitrile hybrids as a bronchodilators

The nicotinonitrile ring system appears to be an attractive bioactive scaffold for developing bronchodilator agents. The bronchodilation properties of synthesized nicotinonitrile-containing compounds were evaluated using isolated guinea pig tracheal rings pre-contracted with the histamine standard technique [92, 93]. Compound **55** (IC_{50}= 3.28 µM) is approximately threefold more potent than theophylline (IC_{50}= 11.57 µM). Additionally, compound **56** exhibits promising activity (IC_{50} = 5.34 µM, approximately twofold that of the standard reference, theophylline) (**Fig. 17**) [8].

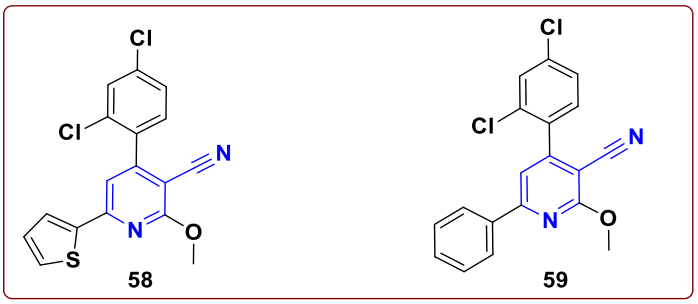


Figure 17. Structure of nicotinonitrile hybrids (55-56) as bronchodilators

2.7. Nicotinonitrile hybrids as A1 adenosine receptor antagonists

Endogenous nucleoside Adenosine plays a role in many human physiological processes by binding to particular receptors in the body. It is well known that G protein-coupled receptor (GPCR) superfamily includes adenosine receptors (ARs) [94]. The genetic structure and tissue distribution of four distinct AR subtypes have been found: A1, A2A, A2B, and A3 [94].

A1AR is extensively expressed in the central nervous system and peripheral tissue, where it exerts protective effects against brain and kidney ischemia. It was reported that, A1AR antagonists effectively treat Alzheimer's disease (AD), heart disease, and renal disease [95-97].

As determined by radioligand binding experiments, the binding affinities (pKi) of compound **57** were 100 times greater for the A1AR (pKi = 6.68 mol/L) than for the A2AAR. Additionally, compound **58** was discovered to have a 1000-fold higher affinity

for A1AR (pKi = 7.13 mol/L) than for A2AAR (pKi < 4 mol/L) (**Fig. 18**) [9].

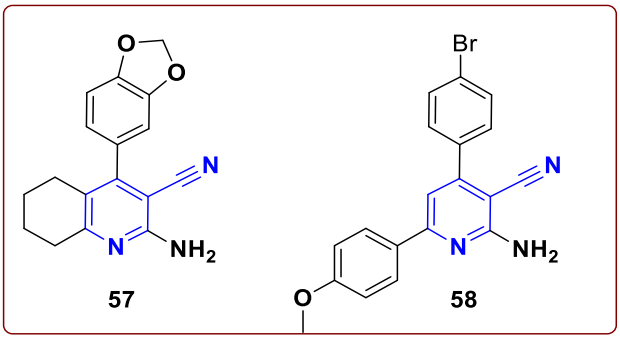


Figure 18. Structure of nicotinonitrile hybrids (**57-58**) as A1AR antagonists

3. Conclusion

Nicotinonitriles (3-Cyano-2-substituted pyridines) is considered a spirited scaffold in medicinal chemistry for synthesizing different hybrids with variable biological activities. Additionally, it may inspire medicinal chemists in discovering and effective therapies by modifying the structure of existing drugs. Specifically, we focused on nicotinonoitrile (3-Cyanopyridine) candidates that are appealing and have potential activity for various disorders. A study of the structure-activity connections of this moiety and the mechanisms of action and *in vivo* investigations of these compounds would be required in future research. Consequently, it is possible that additional encouraging findings may be obtained, leading to the adoption of a currently effective lead compound against various types of illnesses.

4. References

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