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Overview on Synthetic Strategies and Bioactivities of Pyrazolopyridines

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Abstract

There is undeniable evidence that many *N*-heterocycles exhibit a wide range of biological functions. Recently, derivatives with better bioactivities have been generated by condensing the different heterocycles. Pyrazolopyridines have been able to pique the interest of many researchers among many other condensed heterocycles due to the wide range of actions they exhibit. Furthermore, each type of condensed heterocycle has been specifically highlighted in this review in terms of design and biological activities. *In silico* methods and structure-activity relationship have also been discussed wherever reported.

***Keywords*:** Pyrazolopyridines; biological activities; SAR; Target, Kinase.

1. Introduction

It was discovered that heterocyclic motifs play a significant role in both natural and synthetic compounds. Most natural products contain heterocycles that are involved in biological activity.(1) Pyrazolopyridine is a class of nitrogen-containing heterocycles. Researchers have been paying more attention to pyrazolopyridines for many years due to their great biological potential and wide range of synthetic applications.(2)Pyrazolopyridines consist of pyrazole ring and pyridine ring fused together to form 5 different isomers which are Pyrazolo[1,5-*a*]pyridine, Pyrazolo[4,3-*c*]pyridine, Pyrazolo[3,4-*c*]pyridine, Pyrazolo[4,3-*b*]pyridine, and Pyrazolo[3,4-*b*]pyridine (Fig. 1)(3) Some of the marketed drugs containing pyrazolopyridine scaffold such as Cartazolate(4,5), Etazolate(6,7) and Tracazolate(8) (Fig. 2) which are used as anxiolytics, while Riociguat is used in treatment of pulmonary hypertension. (9,10) BMS-986236 is also being investigated as a potential treatment candidate after being identified as a kinase inhibitor.(11)

Pyrazolopyridines exhibited a variety of pharmacological impacts such as anti-cancer(12,13), antimicrobial(14,15), anti-biofilm(16), anti-TB(17), anti-Trypanosoma(18,19), antimalarial(20,21), anti-viral(22,23), analgesic(24), anti-hyperlipidemic(25,26), anti-Alzheimer(27,28), anti-leishmanial(29,30), antiplatelet(31), prevention the programmed

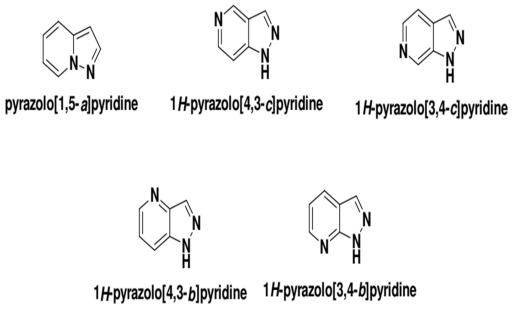

Figure 1: Different types of pyrazolopyridine isomers.

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death of cell (PD-1) signaling pathway(32), A_{2A} adenosine receptor antagonist(33), phosphoinositide-3-kinases (PI3 kinases) inhibitor(34), treatment of insomnia(35), chronic obstructive pulmonary disease COPD(36), parkinson's disease(37), pulmonary hypertension(38) and overactive bladder.(39) The most biologically active isomer of the pyrazolopyridine scaffold is Pyrazolo[4,3-*b*] pyridine. It exhibits several kinase inhibitory activities (Fig. 3) such as anaplastic lymphoma kinase (ALK)(40), B-Raf^{V600E} kinase(41), protein kinase B (Akt)(42), ataxia-telangiectasia and Rad3-related kinase (ATR)(43), TASK3 Kinase(44), mitogen-activated protein kinase (MAPK)(45), epidermal growth factor receptor (EGFR)(46), cyclin-dependent kinase1(47) and 2 (CDK1/CDK2)(48), cyclin-dependent kinase 5(CDK5)(49), cyclin-dependent kinase 8 (CDK8)(50), hepatocyte growth factor receptor (HGFR)(51), fibroblast growth factor receptor (FGFR)(52), Aurora-A kinase(53), cAMP-dependent protein kinase and cGMP-dependent protein kinase pathways(54), glycogen synthase kinase-3 (GSK-3)(55,56), activin-like kinase 5 (ALK5)(57), also, activation of adenosine 5'-monophosphate-activated protein kinase (AMPK).(58)

The current advancements of compounds involving the pyrazolopyridine ring, including the various synthetic pathways, the inhibition of different targets and structure activity relationship (SAR) of medications to produce potentially active molecules, were summarized in this review.

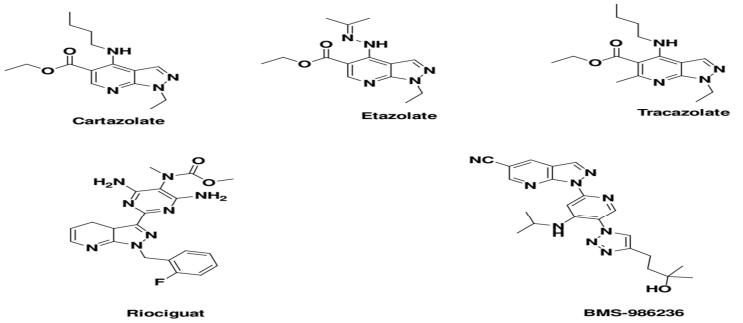


Figure 2: Biologically active pyrazolopyridine scaffold-baseddrugs

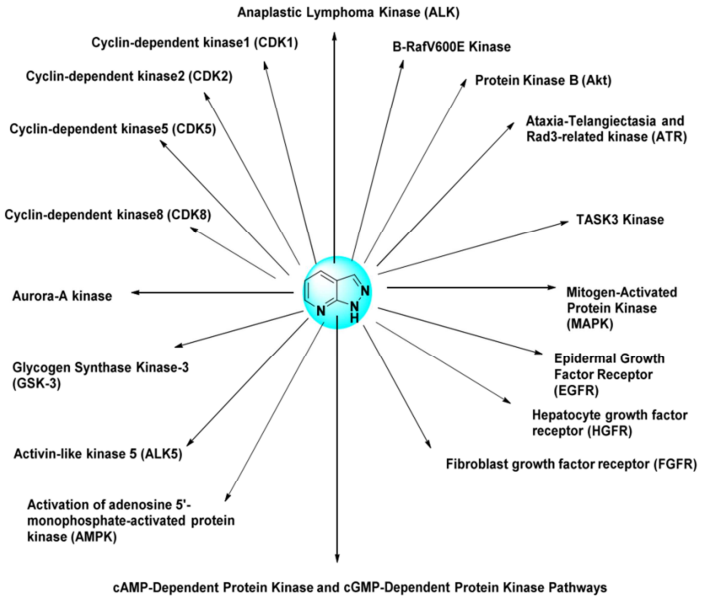
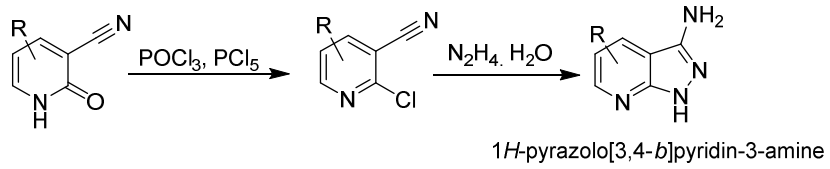


Figure 3: Different biological targets for pyrazolopyridine derivatives.

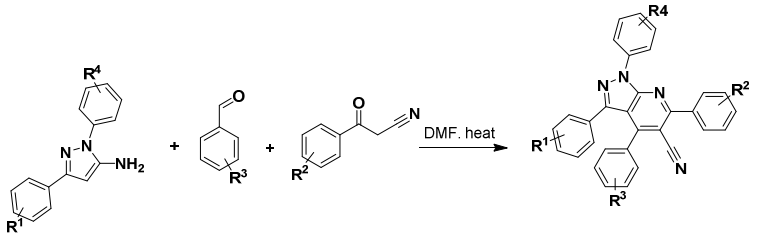
2. Synthetic strategies for pyrazolopyridines

Numerous synthetic approaches for the effective synthesis of pyrazolo[3,4-*b*] pyridines have been reported, including:

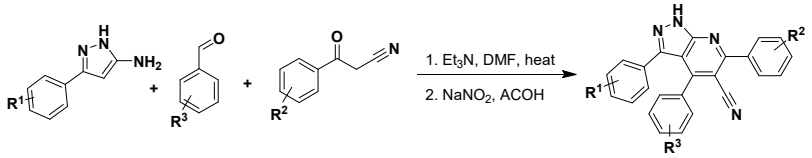
a) In 2003, *Witherington et al.* (59) synthesized pyrazolo[3,4-b] pyridines by halogenation of 3-cyano-2-pyridone followed by reaction with hydrazine hydrate.



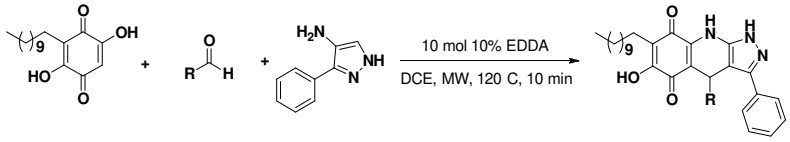
b) In 2022, *Barghasgh et al.*(60)established the preparation of penta-substituted pyrazolo[3,4-*b*] pyridines by reaction of 1,3-diphenyl-1*H*-pyrazol-5-amine with aldehydes and 2-cyano ketones.



c) In 2016, Hill(61) isolated a tetra-substituted pyrazole[3,4-*b*] pyridines from the reaction of 3-amino-5-arylpyrazoles with aldehydes and 2-cyano ketones.



d) Pedro Martín-Acosta (62)and his colleagues carried out three component reaction via Knoevenagel condensation of aldehyde and embelin, followed by subsequent 1,4-michael addition of aminopyrazole under microwave.



3. Biomedical applications of pyrazolopyridine scaffold

Scientists have been interested in the pyrazolopyridine scaffold for a long time due to its numerous medicinal benefits. For example, it serves as a scaffold for the synthesis of several compounds that have a variety of therapeutic effects on various disorders including anticancer, anti-inflammatory, and nervous system drugs.

3.1. Pyrazolopyridines as anti-tumor agents

3.1.1. Cell line studies

The antiproliferative activity of dihydro-1*H*-pyrazolo[1,3-*b*] pyridine embelin derivatives (**Fig. 4**) were assessed againstthree hematologic and five breast cancer cell lines. Leukemia cell lines generally exhibited the best results when compared to breast cancer cell lines, with IC_{50} values between 0.7 and 7.5 µM. Regarding leukemia cell lines, compound **1**, with a nitro group in para position, demonstrated the greatest cytotoxic effect against HL60 (IC_{50}= 0.70 ± 0.14 µM). Additionally, it displayed favorable inhibitory activity on HEL (IC_{50}= 1.05 ± 0.35 µM) and K-562 (IC_{50}= 1.25 ± 0.35 µM). Moreover, compound **2** with 4-CF_3 substituent exhibited the greatest IC_{50}value (IC_{50} = 1.00 ± 0.42 µM) on (HEL) while compound **3** (4-CN)displayed IC_{50} value of

0.92 ± 0.32 µM on K-562cell line, that was the best. variations affected the antiproliferative activity (**Fig. 5**). Phenyl substitution was crucial to the anti-tumor activity. Substitution at para position with electron Investigations were done on how structural withdrawing substituents was found to possess higher activity.(62)

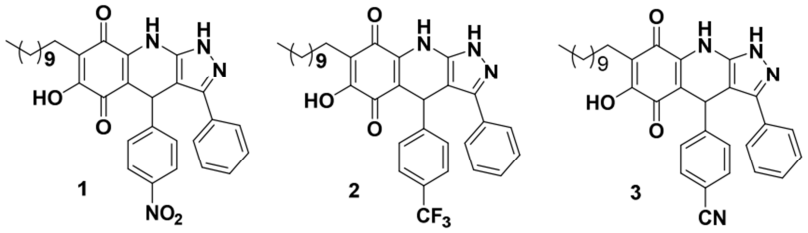


Figure 4: Dihydro-1*H*-pyrazolo[1,3-*b*]pyridinesas anticancer agents.

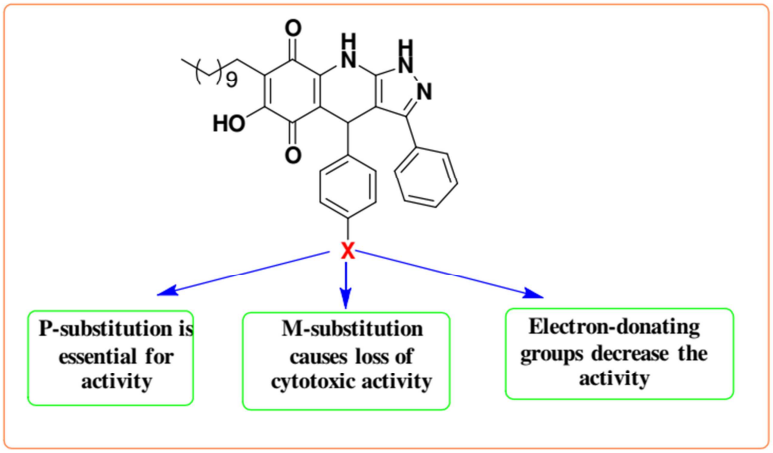


Figure 5: SAR study of 1*H*-pyrazolo[1,3-*b*]pyridinesas anti-cancer drugs.

Conversely, a library of tetra-phenyl substituted pyrazolo[3,4-*b*] pyridine derivative **4** was synthesized *via* one-pot three component reaction of aldehyde, cyano ketone, and 5-amino pyrazole (**Fig. 6**). Then, it was screened against NCI 60 cell lines and demonstrated superior growth suppression effects on breast cancer (HS 578T and MCF7), prostate cancer (PC-3), renal cancer (786-0), colon cancer (SW-620), non-small cell lung cancer (NCIeH460 and NCIeH522) and leukemia (RPMI-8226) cell lines with GI% equal 86, 85, 96, 97, 84, 99, 91 and 87%. respectively. Additionally, Compound **4** was found to be efficient against a wide range of cell lines. Hence, it is regarded as having a broad spectrum of antiproliferative action. Besides, it was evaluated for its inhibitory effect on CDK2 and Abl kinases and the results revealed a weak inhibitory activity against both kinases, hence, their mechanism of action are still unknown.(60)

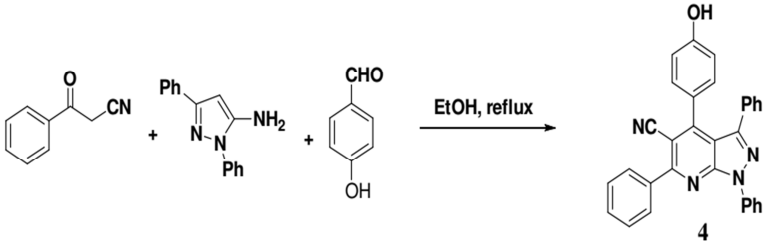


Figure 6: Synthesis of tetra phenyl-substituted pyrazolo[3,4-*b*]pyridine derivative**4**.

Some pyrazolo[4,3-*c*] pyridine derivatives have been synthesized and investigated on HepG2, MCF-7 and HCT-116 cell lines. Compound **5** (**Fig. 7**) was the most effective drug against both liver and breast cancer cell lines (IC_{50} = 3.695 and 1.937 µg/mL, respectively), compared to that of doxorubicin (IC_{50} = 4.749 and 2.527 µg /mL, respectively). While compound **6** (**Fig. 7**) demonstrated promising colon cancer (HCT116) inhibitory activity (IC_{50} = 2.914 µg /ml) relative to doxorubicin (IC_{50} = 3.641 µg/ml). SAR studies proved that the aryl group's substitution with electron donating or withdrawing groups, exhibited considerable antiproliferative activity against many different types of cancer cell lines. To determine the pattern of receptor binding, most of the series members were docked with the ERK2 enzyme's crystal structure (PDB ID: 5buj), and thedocking results have shown that these compounds are well bound with the active site. It showed that the compound 5 was the most active derivative, which may contribute, at least partially, to their antitumor activity.(63)

Figure 7: Pyrazolo[4,3-*c*] pyridine derivatives as anticancer agents

3.1.2. Tubulin polymerization inhibitors

Weige Zhang *et al.* adopted ring tethering strategy to develop novel series of 3,5-aryl-1*H*-pyrazolo[3,4-*b*] pyridine derivatives with enhanced conformational stability over precursor derivatives (**Fig. 8**). The compounds were evaluated for their antiproliferative activity against human gastric carcinoma (SGC-7901), lung carcinoma (A549) and cervical carcinoma (HeLa) cell lines using MTT assay. The majority of the reported compounds displayed promising inhibitory action. Compound **7** (**Fig. 8**) was found to be the most potent with IC_{50} = 0.013 ± 0.005, 0.082 ± 0.016, 0.045 ± 0.008 µM respectively. So, it was chosen to conduct a cell cycle analysis and a tubulin polymerization experiment to clarify the biological mechanism. Results revealed that compound **7** induced G2/M phase cell cycle arrest and could effectively inhibit tubulin *in vitro* and destroying the microtubule skeleton. M. Zhai *et al* synthesized the target compounds through the procedure that is depicted in **Fig. 9**. (64)

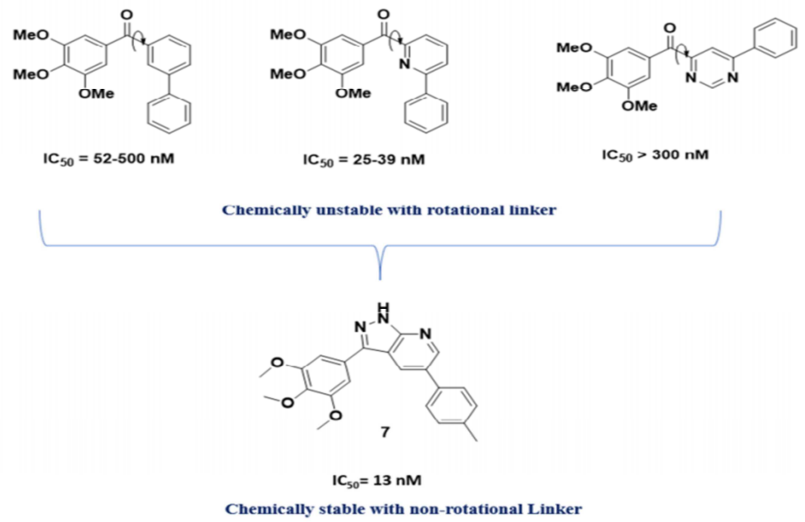


Figure 8: 3,5-Aryl-1*H*-pyrazolo[3,4-*b*] pyridine derivative **7** as tubulin polymerization inhibitor from ring tethering strategy.

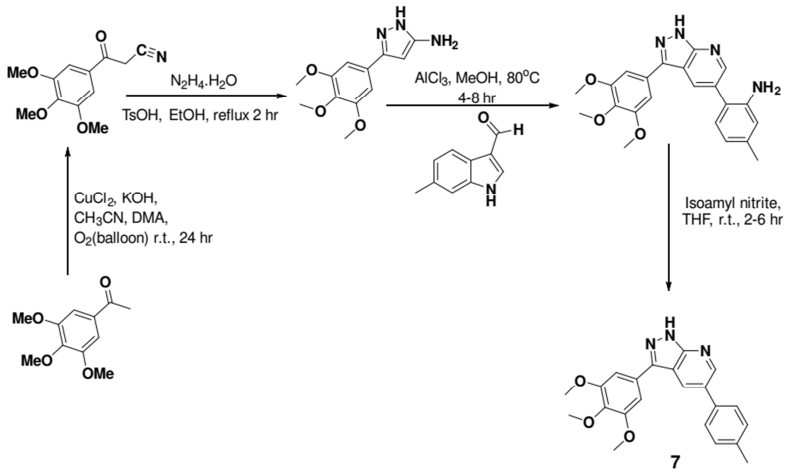


Figure 9: Synthesis of 3,5-aryl-1H-pyrazolo[3,4-*b*] pyridine derivative**7**.

Another molecular rigidification strategy has been adopted by Pei-Liang Zhao *et al* in the development of a series of 1,3-diarylpyrazolo[3,4-*b*] pyridineanalogues utilizing combretastatin A_4**8**and other reported inhibitors**7** and **9** of tubulin polymerization (**Fig. 10**). The whole series were screened *in vitro* to figure out their antiproliferative activities towards various cancer cell lines, which include mammary adenocarcinoma cells (MCF-7), breast cancer (MDA-MB-231), cervical cancer (HeLa), and esophageal squamous (Kyse150) cell lines. Compound **10 (Fig. 10)** that had3,4,5-trimethoxyphenyl substituent at the core's position-1 was compared with its analogue **11** that was substituted at the 3-position of the pyrazolo[3,4-*b*] pyridine ring, to investigate the impact of the 3,4,5-trimethoxyphenyl group's position on the activity (**Fig. 11**). Compound **10** exhibited IC_{50} values = 62.82 ± 2.52, 27.04 ± 6.42, 18.08 ± 1.48, 27.22 ± 2.31 μM against Kyse150, MDA-MB-231, HeLa and MCF-7, respectively. While compound **11** exhibited less antiproliferative activities than its analogue **10** (IC_{50} = 65.23 ± 1.75, > 100, 29.66 ± 0.57 and > 100, respectively), showing that the position of trimethoxyphenyl substituent was beneficial for activity. Compound **10**, one of the series' most potent antiproliferative agents was chosen to be tested for its ability to inhibit tubulin polymerization *in vitro*. Compound **10** was shown to have a significant anti-tubulin activity, with inhibition of 31%, suggestingthat this analogue was probably targeted against tubulin.(65)

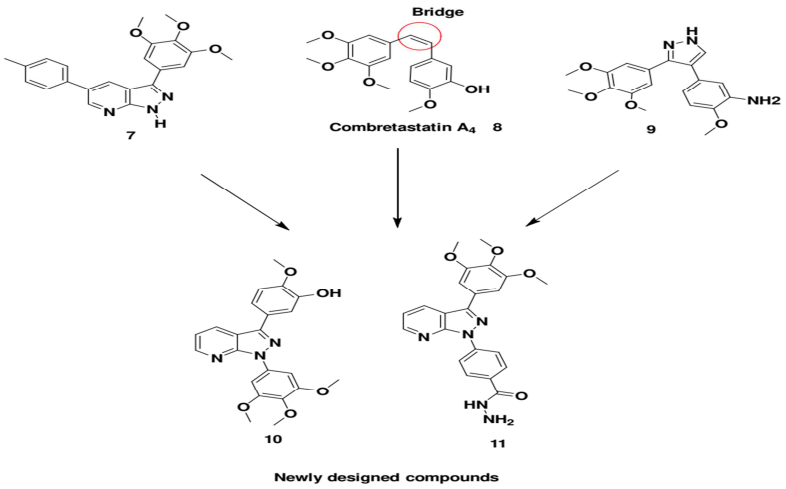


Figure 10: Development of a series of 1,3-diarylpyrazolo[3,4-*b*] pyridine by molecular rigidification strategy.

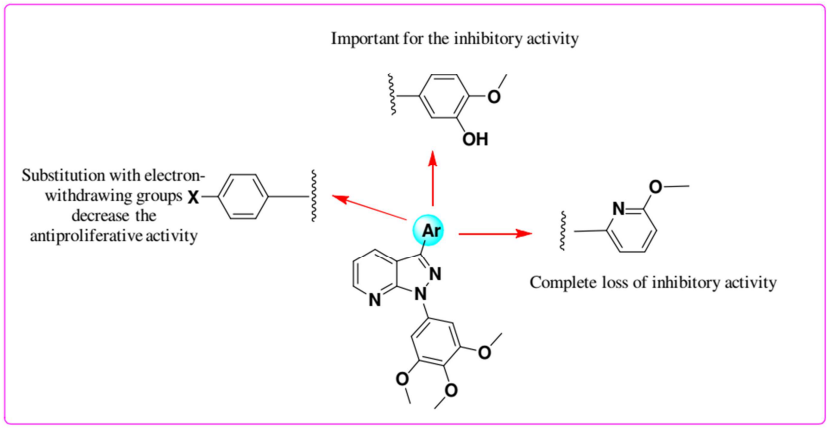


Figure 11: SAR study of 1,3-diarylpyrazolo[3,4-*b*] pyridine derivatives as tubulin polymerization inhibitors.

3.1.3. Anaplastic lymphoma kinase (ALK) inhibitors

A novel series of 3,6-diaryl-1*H*-pyrazolo[3,4-*b*] pyridineshave been designed and synthesized as inhibitors to ALK kinase. They demonstrated considerable potency against cellular proliferation and enzymatic assays. Compound **12** (**Fig. 12**) was the most effective ALK antagonist with IC_{50}= 1.58 nM (**Fig. 13**). In addition, compounds **13** exhibited good ALK inhibitory activity with IC_{50}= 47.40 nM (**Fig. 13**).(66)

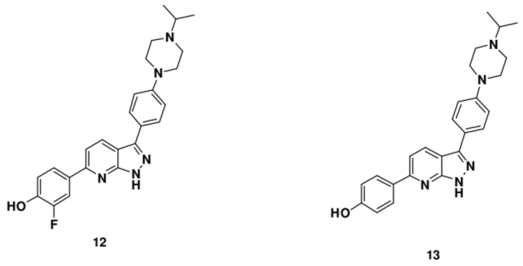


Figure 12: 3,6-Diaryl-1*H*-pyrazolo[3,4-*b*] pyridinesas inhibitors of ALK kinase.

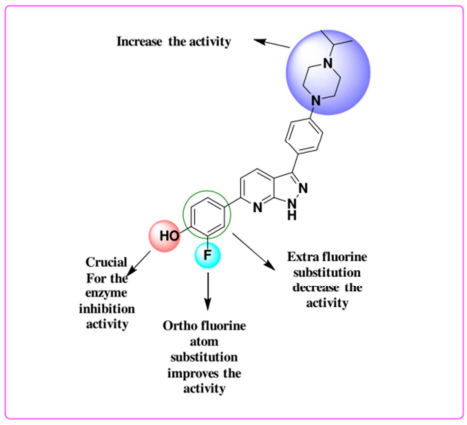


Figure 13: Figure 10: SAR of3,6-diaryl-1*H*-pyrazolo[3,4-*b*] pyridines as anticancer agents.

3.1.4. PIM-1 kinase inhibitors

Pyrazolo[3,4-*b*] pyridine derivatives have been synthesized as by Nafie and his co-workers by reaction of aminopyrazolo[3,4-*b*]pyridine-3,5-dicarbonitrile derivatives with hydrazinehydrate to give an intermediate, which is underwent diazotization reaction followed by reaction with either malononitrile or diethyl malonate as shown in **Fig. 14**. The whole series was subjected to cytotoxic screening against cell lines regarding breast cancer MDA-MB-231, MCF-7 and non-cancerous MCF-10A cells. Compounds **12, 13, 14, 15** and **16** (**Fig. 15**) exhibited cytotoxicity against MCF-7 (IC_{50} below 10 µM). These compounds were more selective to the estrogen dependent (MCF-7) rather than the non-estrogen dependent cell line (MDA-MB-231) or normal cells MCF-10A. The previous compounds were screened for their PIM-1 kinase inhibitory activity in comparison to staurosporin as a reference (IC_{50} = 17 nM) and the results revealed that the most efficient PIM-1 inhibitors were both compounds **15** and **16** with IC_{50} = 26 and 43 nM, respectively. The PIM-1 kinase was moderately inhibited by compounds **12** and **14**, whose IC_{50} values were 87 and 95 nM, respectively.(67)

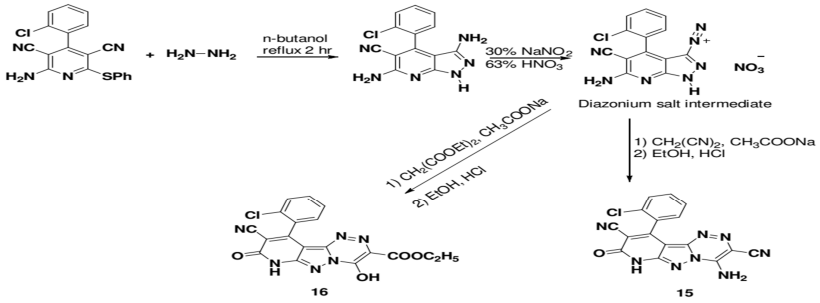


Figure 14: Synthesis of pyrazolo[3,4-*b*] pyridine by reaction of aminopyrazolo[3,4-*b*]pyridine-3,5-dicarbonitrile derivatives with hydrazine hydrate.

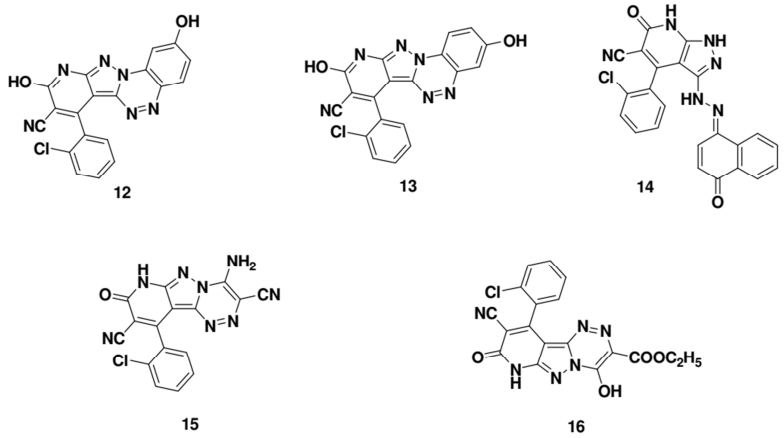


Figure 15: Pyrazolo[3,4-*b*] pyridine derivatives as PIM-1 kinase inhibitors.

3.1.5. Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) inhibitors

A series of mono and disubstituted 1H-pyrazolo[3,4-b] pyridine derivatives was synthesized as shown in **Fig. 16** by Park and his co-workers. The synthesized compounds were screened for their cytotoxicity against colorectal cancer cell lines. Most of the monosubstituted compounds showed moderate inhibitory activity. Compound 17 (**Fig. 17**) was the most effective compound from the monosubstituted series with DYRK-1B inhibitory effect (IC_{50} = 54 nM). Conversely, the inhibitory effect against DYRK1B was improved in the disubstituted series. The disubstituted derivative 18 demonstrated the strongest inhibitory effect with IC50 = 5 and 3 nM against DYRK1A and DYRK1B, respectively. The cytotoxic effect of compound 18 on colon cancer was measured on ten colon cancer cell lines. Compound 18 displayed significant effect against DLD-1, SW6205, RKO, SW480 and HCT116 cell lines. 3D spheroid model was used to assess the cell growth inhibition effect. After treatment with compound 18 for 10 days cancer cell size was reduced, the nuclei size got smaller, the number of viable cells decreased, and the number of dead cells increased.(68)

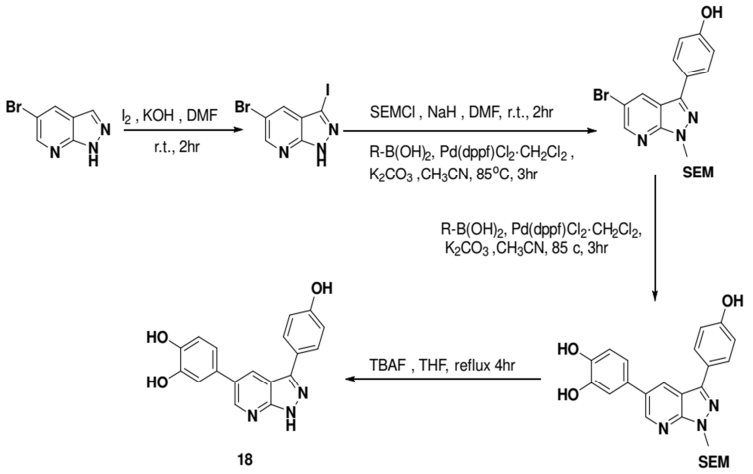


Figure 16: Synthesis of mono and disubstituted 1*H*-pyrazolo[3,4-*b*] pyridine derivatives.

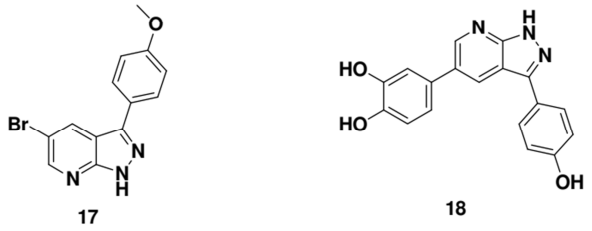


Figure 17: Figure 10: 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as DYRK1A/1B inhibitors.

3.1.6. Cyclin-Dependent Kinase (CDK) inhibitors

Cyclin-Dependent Kinases (CDKs) are believed to be among the most promising cancer therapeutic targets. Compound **19 (Fig. 18)** is an example of pyrazolo[3,4-*b*] pyridine derivative that demonstrated good CDK2inhibitory actions but poor cell line performance. The inability to enter the cells may be the cause of its poor activity. With some structural improvement, compound **20** was synthesized and demonstrated the highest potency against HCT-116 (Colon carcinoma), HepG2 (Liver carcinoma) and A-549 (lung carcinoma) cell lines with IC_{50} = 2.3, 2.6 and 2.9 µg/mL respectively. But compound **19** still has the upper hand on inhibition of the isolated enzyme. (69)

Compound **21** (**Fig. 19**) is another promising CDK2 inhibitor that showed a remarkablepotential activity against HepG2 and MCF7 cell lines with IC_{50} = 1.33 ± 0.06 and 2.65 ± 0.10 µM, respectively. Besides, compound **21** increased activation of caspase-3 in HepG2 by around 10 times when compared to the control and considered as an apoptotic inducer.(70)

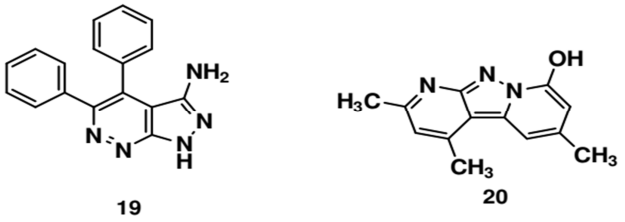


Figure 18: Pyrazolo[3,4-*b*] pyridine derivatives as CDK inhibitors.

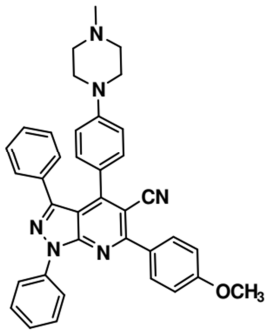


Figure 19: A pyrazolo[3,4-*b*] pyridine derivative as CDK inhibitor.

3.1.7. FMS-like tyrosine kinase-3 (FLT3) / CDK4 inhibitors

A novel family of FLT3 and CDK4 kinase-inhibiting 6-(pyrimidin-4-yl)-1*H*-pyrazolo[4,3-*b*] pyridine derivatives were synthesized by X. Li *et al*. The one with the best results on FLT3 and CDK4 in the nanomolar range was compound **22 (Fig. 20)** (IC_{50} = 7 and 11 nM, respectively). In addition, compound **22** was chosen to be tested against a panel of key kinases from the CMGC and TK families and demonstrated excellent selectivity over FLT3 and CDK4. The series was synthesized as illustrated in **Fig. 21**. (71)

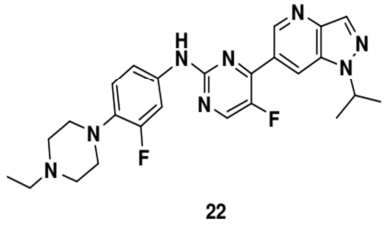


Figure 20: 6-(Pyrimidin-4-yl)-1*H*-pyrazolo[4,3-*b*] pyridine derivative as FLT3/CDK4 inhibitor.

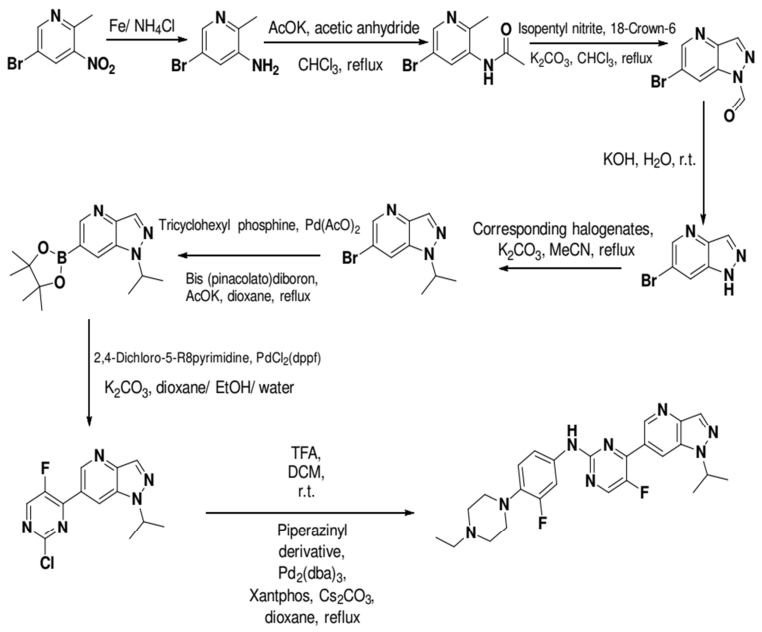


Figure 21: Synthesis of 6-(pyrimidin-4-yl)-1*H*-pyrazolo[4,3-*b*] pyridine derivatives.

3.1.8. Polo-like kinase 4 (PLK4) inhibitors

Polo-like kinases (PLKs) have been found as critical targets for treatment of multiple cancers. They play a major regulating role in different functions within the cell including cell mitosis, cellular stress response and maintenance of cell integrity. Researchers are mostly interested in PLK4 as it is overexpressed in lung, breast, pancreatic, colorectal cancer, and many others. PLK4 inhibitors having a 1*H*-pyrazolo[3,4-*b*] pyridinescaffold were assessed. Compound **23** (**Fig. 22**) is an example of potent PLK4 inhibitor (IC_{50} = 0.026 µM) that markedly reduced the breast cancer (MCF-7) cell line's growing (IC_{50} = 1.52 µM), although it had no repressive impact on normal cell. Moreover, it has been screened against 47 kinases, however only three kinases (PAK4, DDR2, and PLK4) were inhibited and showed > 90% inhibition against PLK4. Furthermore, the thorough biological analysis showed that compound **23** could inhibit PLK4 to cease cell division in the S/G2 phase, which would then have an impact on the production of proteins in the PLK4-regulated downstream signaling pathway. Compound **23** was synthesized as shown in **Fig. 23**. (72)

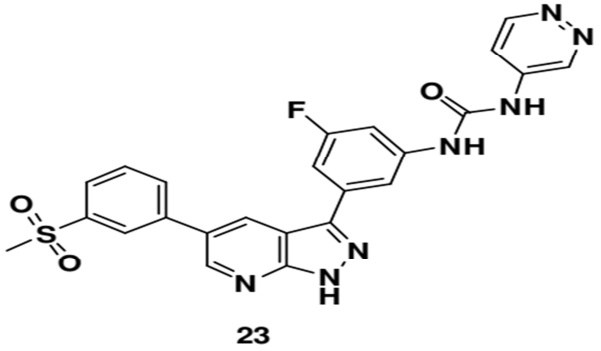


Figure 22: 1*H*-pyrazolo[4,3-*b*] pyridine as PLKs inhibitor.

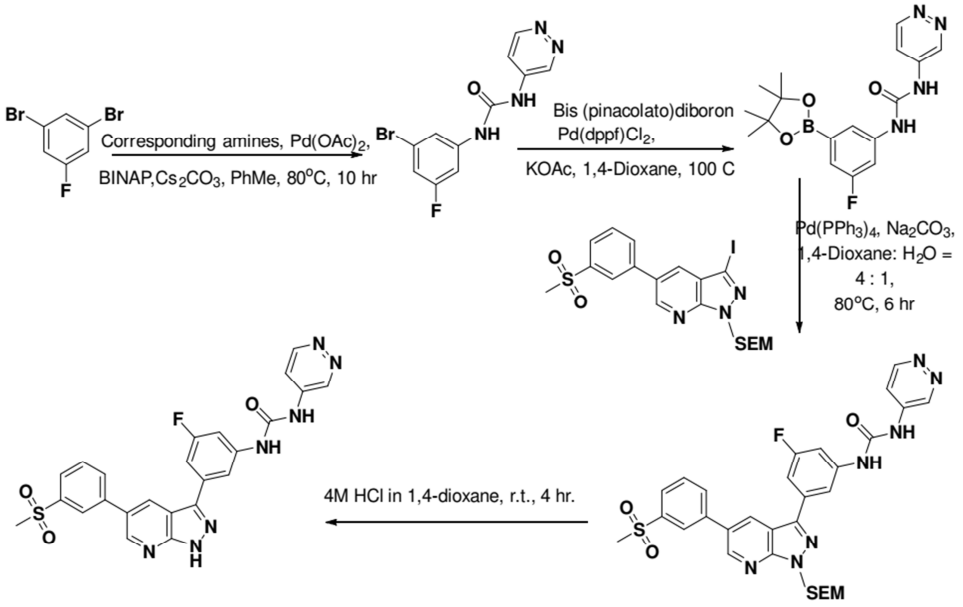


Figure 23: Synthesis of 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as polo-like kinase 4 (PLK4) inhibitors.

3.1.9. Sphingosine-1-phosphate receptor 2 (S1PR2) inhibitors

In HCT116 ^{DPD} cells, 5-fluorouracil (5-FU) resistance was reported to be effectively reversed by sphingosine-1-phosphate receptor 2 (S1PR2). SAR studies were performed to design novel ligands to act as (S1PR2) antagonists. Compound **24** (**Fig. 24)** was the most efficient S1PR2 inhibitor among the synthesized compounds. By preventing dihydropyrimidine dehydrogenase's expression in SW620/5-FU and HCT116 ^{DPD} xenografts, it reverses 5-FU resistance. In presence of compound **24,** the percentage of 5-FU inhibition was raised from 23.97% to 65.29% and 27.23% to 60.81%, respectively, without any toxicity on normal cells. Compound **24** was synthesized as illustrated in **Fig 25**.(73)

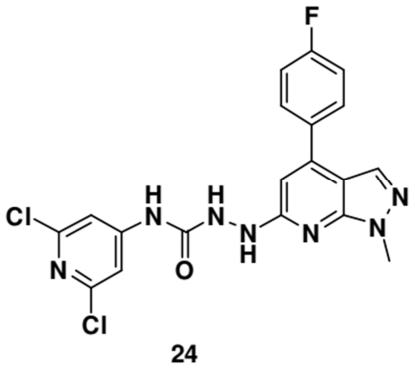


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Figure 24: Pyrazolo[4,3-*b*] pyridinederivative as S1PR2 inhibitor.

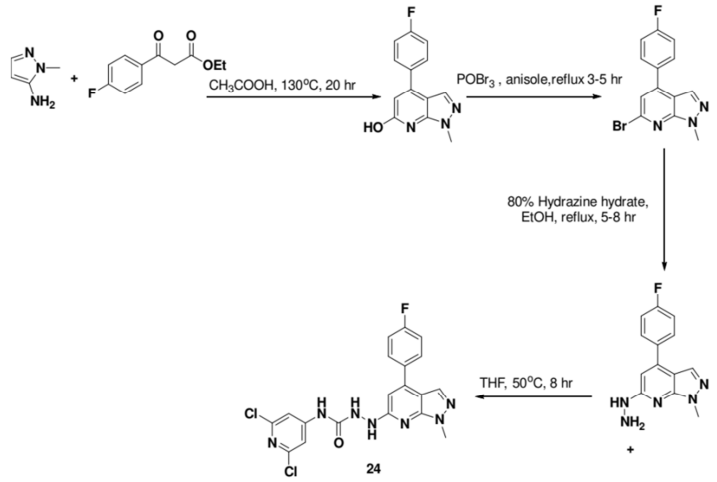

Figure 25: Synthesis of 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as sphingosine-1-phosphate receptor 2 (S1PR2) inhibitors.

3.1.10. Mitogen-activated protein kinase 4 (MKK4) inhibitors

Bent Pfaffenrot et al were able to synthesize new 1H-pyrazolo[3,4-*b*] pyridine derivatives with significant MKK4 selectivity.Compounds 25 and 26 were the first members of 1H-pyrazolo[2,3-*b*] pyridine class (**Fig. 26**) that demonstrated a great affinity in the low nanomolar concentrations to MKK4 and a superb selectivity profile. **Fig. 27** describes the synthesis of the most potent compounds 25 and 26 from the Weinreb-amide start Y. The authors used a combination of the classical Suzuki coupling conditions.(74) In **Fig. 28**, several of the significant SARs are highlighted.

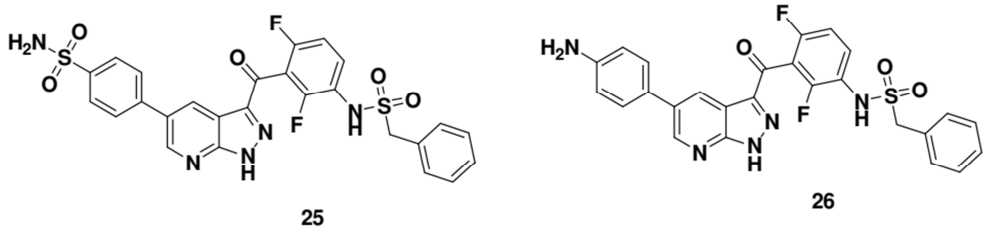

Figure 26: 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as MKK4 inhibitors.

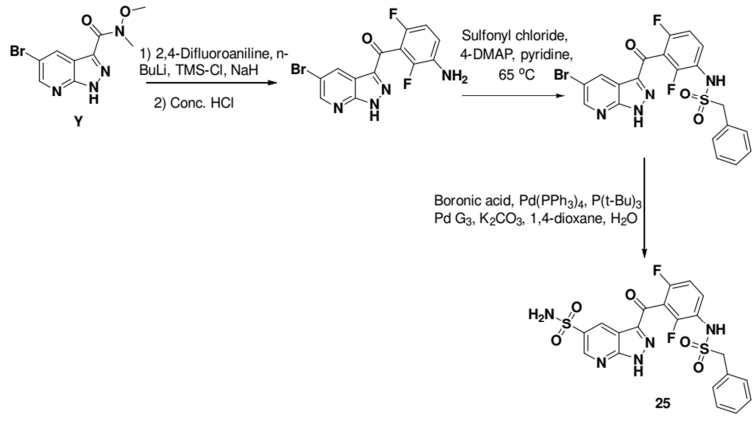

Figure 27: Synthesis of 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as (MKK4) inhibitors.

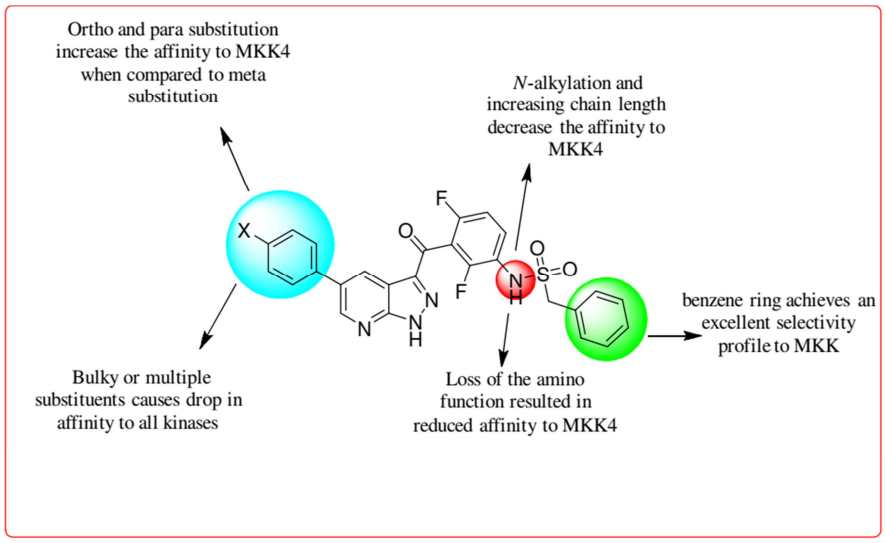

Figure 28: SAR of 1*H*-pyrazolo[3,4-*b*] pyridine derivatives as MKK4 inhibitors.

3.1.11. P38 mitogen-activated protein kinase (MAPK) inhibitors

A novel series of pyrazolo[3,4-*b*] pyridines was synthesized by Farahat *et al* and assessed for their antiproliferative activities against cervical HeLa, hepatocellular HepG2 carcinoma and NCI 60 cell lines. Compound **27** (**Fig. 29**) was found to be the most effective antiproliferative agent with a mean GI% of 60.3 that targets the NCI 60 cell lines. Additionally, it remarkably revealed excellent leukaemia growth suppression capabilities. (CCRFCEM, K562, and MOLT4), NSCLC (NCIH460), colon cancer (HCT116), melanoma (LOX IMVI), renal cancer (7860) with 85 to 90 % inhibition. Then, for a five-dose assay, drugs that demonstrated greater than 50% growth inhibition were chosen. The most effective analogues were compounds **28**, **29**, and **31**, with IC_{50} values of 5.5, 4.2, and 5.1 µM, respectively, which are comparable to doxorubicin's (IC_{50} = 1.7 µM). Moreover, compounds **27**, **28**, **29**, and **30** demonstrated effective inhibition of p38 kinase, with corresponding IC_{50} values ranges between 0.13 to 0.64 µM. Also, compounds **30**, **31** and **32** demonstrated good anticancer activity against most cell types (**Fig. 29**). Additionally, these compounds were synthesized with the general procedure using either aldehyde or isatin derivatives. (75)

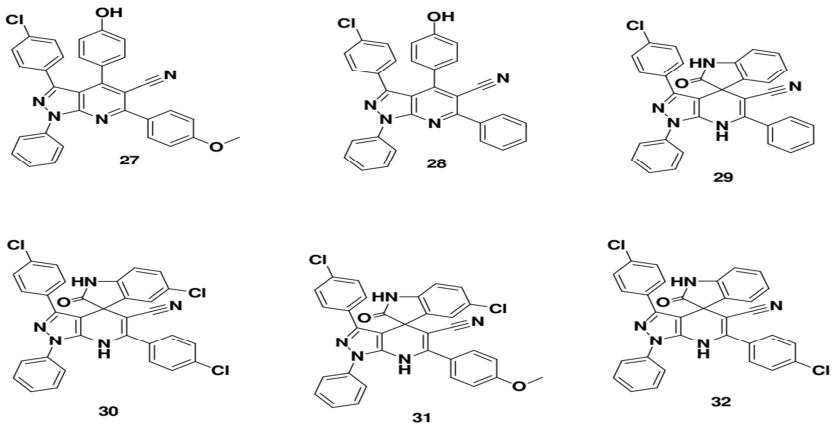

Figure 29: Pyrazolo[3,4-*b*] pyridine derivatives as p38α MAPK inhibitors.

3.1.12. The adenosine monophosphate-activated protein kinase (AMPK) activators

Novel pyrazolo[3,4-*b*] pyridinecompounds were synthesized and tested for lung cancer prevention potential. Compound **33** (**Fig. 30**) was tested for its ability *in vitro* to inhibit cell proliferation against small-cell and non-small-cell lung cancer cell lines. It demonstrated effective cytotoxicity (**Fig. 31**) with IC_{50} values less than 10 µM on five lung cancer cell lines. Moreover, it revealed more potency than reference A-769662 (IC_{50}= 45.29 ± 2.14 µM) at inhibiting the cell growth of A549 cells (IC_{50}= 3.06 ± 0.05 µM). To determine the compound's **33** influence on AMPK activation, Western blot analysis was employed to quantify the AMPK phosphorylation levels and ACC substrate, which was extensively utilized as an AMPK activation marker. Comparable to A-769662 reference's activity at 20 µM, compound **33** can decrease the phosphorylated ribosomal S6 kinase's (p70S6K) levels while increasing the AMPK phosphorylation levels.(76)**Fig. 32** describes the synthesis of the most potent compound **33** from the substituted 5-azido-1*H*-pyrazole-4-carbaldehyde start.

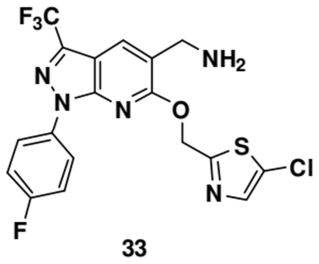

Figure 30: Pyrazolo [3,4-*b*] pyridine derivative as AMPK activator.

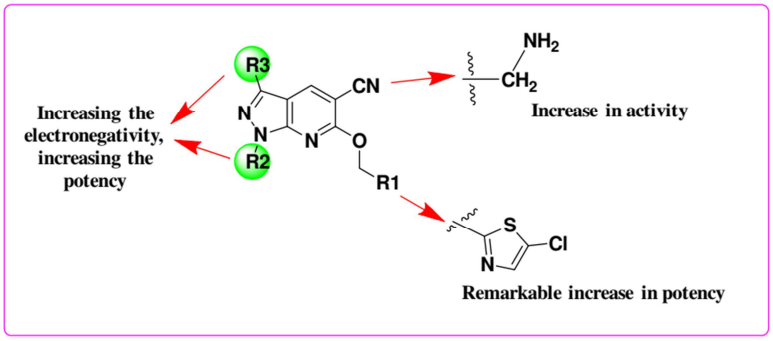

Figure 31: SAR of pyrazolo [3,4-*b*] pyridine derivatives as AMPK activators.

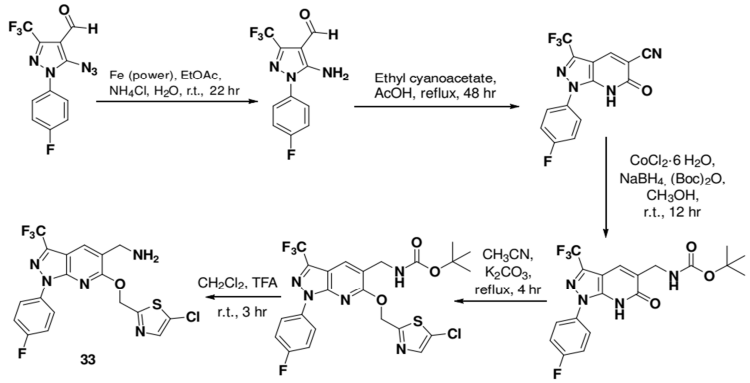

Figure 32: Synthesis of pyrazolo[3,4-*b*] pyridine derivatives as (AMPK) activators.

In addition, Bifeng Zheng *et al* synthesized novel pyrazolo[3,4-*b*] pyridine derivatives. The new derivatives were biologically estimated for their activation activity toward AMPK and the NRK-49F cell's antiproliferative activity. It was noteworthy that compound **34** (**Fig. 33**) demonstrated almost identical activation in comparison to A769662 (EC_{50} = 0.28 µM). Studies using the NRK-49F cell line revealed that strong enzyme activators might successfully limit cell growth, particularly for **34** (EC_{50} [AMPK-II] = 0.42 µM, effectiveness = 79%; IC_{50} [NRK-49F cell line] = 0.78 µM). Docking experiments for **34** with AMPK showed a promising binding profile, which offered knowledge for designing new AMPK activators.(77) In **Fig. 34**, several of the significant SARs are highlighted.

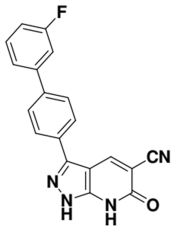

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Figure 33: Pyrazolo[3,4-*b*] pyridine derivatives as AMPK activator.

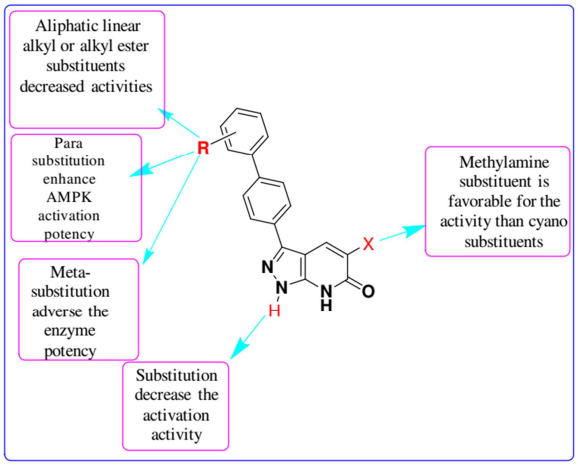
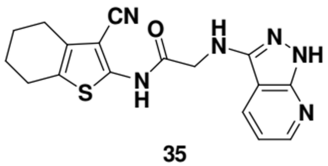


Figure 34: SAR of pyrazolo[3,4-*b*] pyridine derivatives as AMPK activators.

3.1.13. Dual human EGFR-related receptor 2 (HER2) / epidermal growth factor receptor (EGFR) Inhibitors

It was discovered that most antitumor quinazoline-based medications which specifically block overexpression of EGFR/HER2, lose their effectiveness over an extended period of time. In order to replace this quinazoline core while maintaining the benefit of having two nitrogen atoms required for interaction with important kinase active sites' amino acids, new scaffolds needed to be developed. Literature revealed that it is necessary for the EGFR/HER2 dual inhibitory activity to have a hydrophobic head, whereas hydrophobic tail at the bottom, is crucial for the kinase inhibition potency. Motivated by these findings, Ranza Elrayess *et al* synthesized a novel series of 1*H*-Pyrazolo[3,4-*b*] pyridin-3-ylamino derivatives in order to find powerful EGFR/HER2 dual inhibitors targeting non-small cell lung cancer cell line (H1299).

In comparison to gefitinib (IC_{50} = 40 µm), the synthesized compounds displayed IC_{50} values between 12 and 54 nM. In addition, all compounds demonstrated significant T790M EGFR's affinity, one of the primary mutations causing acquired drug resistance. Synthetic compound **35 (Fig. 35)** had the greatest *in vitro* cytotoxicity against H1299 (IC_{50} =12.5 nM). As well, EGFR and HER2 were respectively inhibited by 0.47 and 0.14 nM values which are equivalent to the IC_{50} of the authorized medication imatinib.(78)

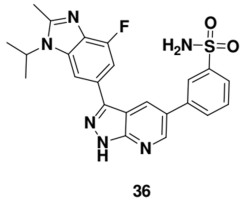


35

Figure 35: Pyrazolo[3,4-*b*] pyridine derivative as dual EGFR and HER2 inhibitor.

3.1.14. TANK-binding kinase 1 (TBK1) inhibitors

All tissues highly express TBK1, which is known as NF-kB-activating kinase (NAK). and has become acknowledged as a potential therapeutic target due to its significant roles in cancer, autoimmune disorders, and metabolic diseases. By analyzing the binding mechanisms of compound TBK1 and URMC-099, Yin Sun and his group developed a series of 1*H*-pyrazolo[3,4-*b*] pyridine derivatives using the bioisostere technique with aid of computer-aided drug design (CADD). Optimized compound **36** (**Fig. 36**) exhibited an IC_{50} value of 0.2 nM and picomolar inhibition of TBK1as suggested by the *in vitro* enzyme activity experiments. To further assess the selectivity, the most potent inhibitor **36** at a dosage of 1.0 μM, underwent profiling of kinase selectivity against a panel of 31 kinases. The outcomes showed that compound **36** only generated less than 20% of kinase activity in TBK1. On the cell lines Panc0504, U87MG, A172, A2058 and A375, compound **36** had a powerful antiproliferative impact with an IC_{50} of micromole level.(79)



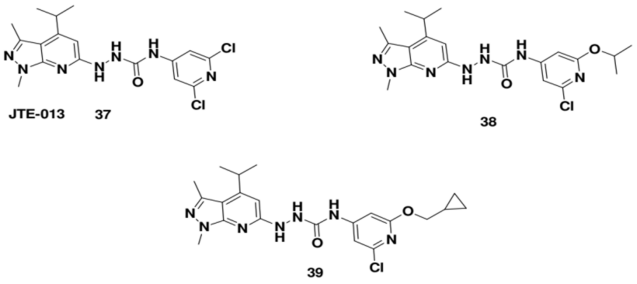
36

Figure 36: 1*H*-pyrazolo[3,4-*b*] pyridine derivative as TBK1 inhibitor.

3.2. Anti-inflammatory agents

3.2.1. Sphingosine 1-phosphate receptor 2 (S1PR2) inhibitors

Multiple sclerosis is an inflammatory disease accompanied by demyelination of neurons, resulting in damage to myelin and axons. Sphingosine 1-phosphate receptors (S1PRs) play acritical role in the regulation of the biological function throughout the cell. It is considered asignificant biological target in multiple sclerosis (MS). FTY720 was the first FDA approved drug to be used orally in the treatment of MS. Studies proved that it was inactive against S1PR2 but active against other four S1PR1 isoforms. So, there was an urgent need for discovering a drug with high selectivity to S1PR2. JTE-013 (pyrazolopyridine derivative) **37** (**Fig. 37**) was the first discovered S1PR2 antagonist with IC_{50} 58.4 ± 7.4 nM. JTE-013 was used as a lead and with further optimization to obtain additional S1PR2 ligands. Comparable to JTE-013 **37**, both pyrazolopyridine derivatives **38** and **39** exhibited S1PR2 binding affinities with IC_{50} values of 29.1 2.6 and 56.5 4.0 nM, respectively.(80)



JTE-013 37 38 39

Figure 37: Pyrazolo[3,4-*b*] pyridine derivatives as S1PR2 inhibitors.

3.2.2. Cyclooxygenase 2 (COX-2) inhibitors

A novel series of 2*H*-pyrazolo[4,3-*c*] pyridine derivatives was designed and synthesized to be exploited as anti-inflammatory agents. TNF-, IL-1, and IL-6, as well as the anti-inflammatory TNF-ά, IL-1β and IL-6 were measured in presence of indomethacin and compounds **40** and **41 (Fig. 38)**. The results revealed that TNF- ά, IL-1β, and IL6 were all significantly reduced by compounds **40** and **41**, and their effects were comparable to one another and superior to those of indomethacin. Additionally, at a 2 nM concentration, compounds **40** and **41** boosted IL-10 levels in comparison to the control group, and this increase was greater than that observed in the indomethacin-treated group.(81) It was also observed that the most potent inhibitors can be obtained from substituted thiophene rings (**Fig. 39**).

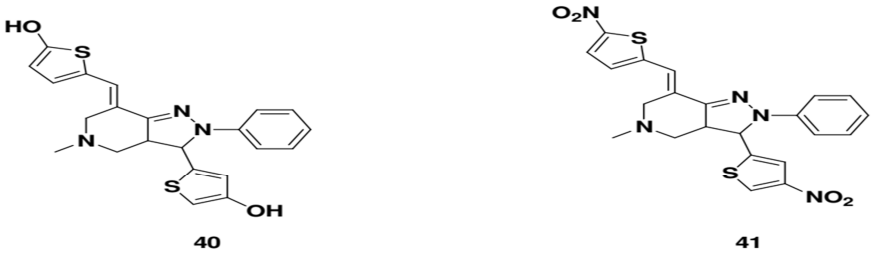


Figure 38: 2*H*-pyrazolo[4,3-*c*] pyridine derivatives as COX-2 inhibitors.

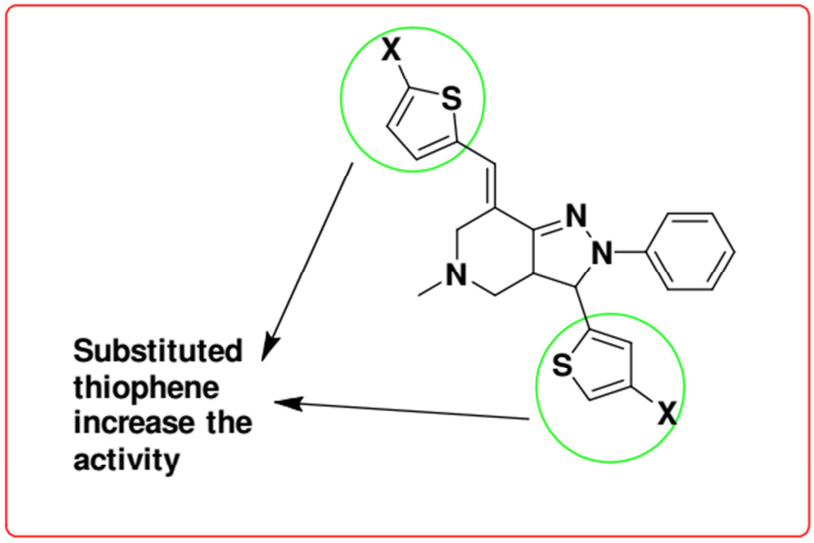
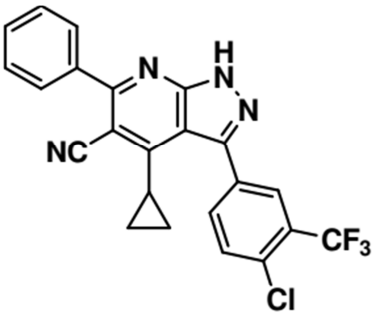


Figure 39: SAR of 2*H*-pyrazolo[4,3-*c*] pyridine derivatives as COX-2 inhibitors.

Nervous system agents

3.3.1. Metabotropic Glutamate Receptor 5 (mGluR5) Positive Allosteric Modulators (PAM)

The metabotropic glutamate receptor 5 (mGluR5) is a desirable target for schizophrenia treatment because it regulates glutamatergic signaling in combination with the *N*-methyl-D-aspartate receptor (NMDAR). Upon endogenous glutamate binding with mGluR5 receptor, neuronal signal transmission and *N*-methyl-D-aspartate receptor (NMDAR) activation are enhanced. Matthew D. Hill *et al* elucidated the synthesis of 1*H*-pyrazolo[3,4-*b*] pyridines, which lack intrinsic agonist activity, to serve as mGluR5 PAMs. 1*H*-pyrazolo[3,4-*b*] pyridine**42** (BMT-145027) (**Fig. 40**), was the first developed PAM as a result of SAR research. Compound **42** displayed good potency (EC_{50} = 47 nM), strong MsLM stability (85% surviving) and was able toenhance awareness in a mouse learning and memory preclinical paradigm.(82)



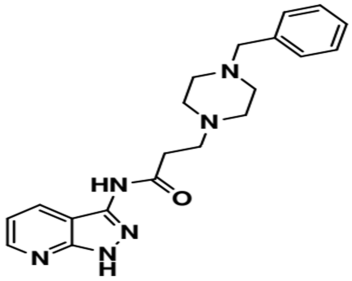
(BMT-145027)

42

Figure 40: 1*H*-pyrazolo[3,4-*b*] pyridine derivative as mGluR5 PAM.

3.3.2. Acetylcholine esterase (AChE) inhibitors

The brain's acetylcholine (ACh) levels are remarkably decreased in Alzheimer's disease brains. Subsequently, acetylcholine esterase (AChE) inhibition is the most successful strategy that is frequently utilized in disease therapy. A new series of multitargeted pyrazolopyridine derivatives were synthesized. Compound **43** (**Fig. 41**) was the most efficient AChE inhibitors in the series with IC_{50} = 0.034 ± 0.002 µM. Furthermore, it inhibited $A\beta_{1-42}$ self-aggregation and inhibiting reactive oxygen species (ROS) (cause severe damage in AD patients' brain areas) and is considered as bio-metals chelators. Additionally, it demonstrated BBB penetration *in vivo* with extensive safety (LD50 > 120 mg/kg).(83)



43

Figure 41: 1*H*-pyrazolo[3,4-*b*]pyridine derivative as AChE inhibitor.

4. Conclusions

A vital heterocyclic moiety for the discovery of potential inhibitors against a variety of targets is pyrazolopyridine. Each class of compounds possessed a broad range of therapeutic benefits encompassing anticancer, anti-inflammatory, nervous system modulators and others. For the discovery of target-based prospective drugs, multiple research projects are still required. Studies on the relationships between structure and activity may be useful in promising compounds' synthesis within a short time. In order to develop these promising compounds, we tried to study the structural characteristics of pyrazolopyridine along with its relation to numerous targets *via* the present review.

5. Competing interests

The authors declare that there are no personal or financial interests to declare.

6. References

1. Devi Priya D, Nandhakumar M, Mohana Roopan S. Pyrazolo[1,5-a]pyridine: Recent synthetic view on crucial heterocycles. Vol. 50, Synthetic Communications. Bellwether Publishing, Ltd.; 2020. p. 3535–62.
2. Dyadyuchenko L v., Dmitrieva IG. [InlineMediaObject not available: see fulltext.] Microwave-assisted synthesis of pyrazolo[3,4-b]pyridine derivatives (microreview). Chem Heterocycl Compd (N Y). 2020 Nov 1;56(11):1414–.
3. Gouda MA, Berghot MA, el Ghani GEA, Khalil AGM. Recent Development in the Chemistry of Bicyclic 6+5 Systems, Part I: Chemistry of Pyrazolo[3,4-b]pyridines. Lett Org Chem. 2019 Apr 11;17(1):2–23.
4. Kripalani KJ, Dreyfuss J, Nemec J, Cohen AI, Meeker F. Biotransformation in the monkey of cartazolate (SQ 65,396), a substituted pyrazolopyridine having anxiolytic activity. Vol. 11, XENOBIOTICA. 1981.
5. Shi D, Padgett WL, Hutchinson KD, Moore SP, Daly JW. Pyrazolopyridines: Effect of structural alterations on activity at adenosine- and GABA(A)-receptors. Drug Dev Res. 1997 Sep;42(1):41–56.
6. Jindal A, Mahesh R, Bhatt S. Etazolate, a phosphodiesterase-4 enzyme inhibitor produces antidepressant-like effects by blocking the behavioral, biochemical, neurobiological deficits and histological abnormalities in hippocampus region caused by olfactory bulbectomy. Psychopharmacology (Berl). 2015;232(3):623–37.
7. Jindal A, Mahesh R, Bhatt S. Etazolate, a phosphodiesterase 4 inhibitor reverses chronic unpredictable mild stress-induced depression-like behavior and brain oxidative damage. Pharmacol Biochem Behav. 2013 Apr;105:63–70.
8. Ambati SR, Patel JL, Chandrakar K, Sarkar U, Penta S, Banerjee S, et al. One-pot, three-

component synthesis of novel coumarinyl-pyrazolo[3,4-b]pyridine-3-carboxylate derivatives using [AcMIm]FeCl4 as recyclable catalyst. J Mol Struct. 2022 Nov 15;1268.
9. Ghofrani HA, Galiè N, Grimminger F, Grünig E, Humbert M, Jing ZC, et al. Riociguat for the Treatment of Pulmonary Arterial Hypertension. New England Journal of Medicine. 2013 Jul 25;369(4):330–40.
10. Conole D, Scott LJ. Riociguat: First global approval. Drugs. 2013 Nov;73(17):1967–75.
11. Arunachalam PN, Kuppusamy P, Ganesan S, Krishnamoorthy S, Nimje RY, Jarugu LB, et al. Development of a Scalable Synthesis for the Potent Kinase Inhibitor BMS-986236; 1-(5-(4-(3-Hydroxy-3-methylbutyl)-1 H-1,2,3-triazol-1-yl)-4-(isopropylamino)pyridin-2-yl)-1 H-pyrazolo[3,4- b]pyridine-5-carbonitrile. Org Process Res Dev. 2019 May 17;23(5):912–8.
12. Eissa IH, El-Naggar AM, El-Hashash MA. Design, synthesis, molecular modeling and biological evaluation of novel 1H-pyrazolo[3,4-b]pyridine derivatives as potential anticancer agents. Bioorg Chem. 2016 Aug 1;67:43–56.
13. Stepanenko IN, Novak MS, Mühlgassner G, Roller A, Hejl M, Arion VB, et al. Organometallic 3-(1 H -benzimidazol-2-yl)-1 H -pyrazolo[3,4- b]pyridines as potential anticancer agents. Inorg Chem. 2011 Nov 21;50(22):11715–28.
14. El-Gohary NS, Gabr MT, Shaaban MI. Synthesis, molecular modeling and biological evaluation of new pyrazolo[3,4-b]pyridine analogs as potential antimicrobial, antiquorum-sensing and anticancer agents. Bioorg Chem. 2019 Aug 1;89.
15. Pradeep MA, Kumar NR, Swaroop DK, Reddy NS, Sirisha K, Kumar CG, et al. Design and Synthesis of Novel Pyrimidine/Hexahydroquinazoline-Fused Pyrazolo[3,4-b]Pyridine Derivatives, Their Biological Evaluation and Docking Studies#. ChemistrySelect. 2019 Jan 10;4(1):138–44.
16. Nagender P, Malla Reddy G, Naresh Kumar R, Poornachandra Y, Ganesh Kumar C, Narsaiah B. Synthesis, cytotoxicity, antimicrobial and anti-biofilm activities of novel pyrazolo[3,4-b]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives. Bioorg Med Chem Lett. 2014 Jul 1;24(13):2905–8.
17. Jamale DK, Vibhute SS, Undare SS, Valekar NJ, Patil KT, Warekar PP, et al. Unexpected formation of 4,5-dihydro-1H-pyrazolo[3,4-b]pyridine derivatives as a potent antitubercular agent and its evaluation by green chemistry metrics. Synth Commun. 2018 Nov 2;48(21):2750–60.
18. Ribeiro JLS, Soares JCAV, Portapilla GB, Providello M v., Lima CHS, Muri EMF, et al. Trypanocidal activity of new 1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine derivatives: Synthesis, in vitro and in vivo studies. Bioorg Med Chem. 2021 Jan 1;29.
19. Salvador RRS, Bello ML, Barreto IRL, Vera MAF, Muri EMF, de Albuquerque S, et al. New carbohydrazide derivatives of 1H-pyrazolo[3,4-b]pyridine and trypanocidal activity. An Acad Bras Cienc. 2016;88(4):2341–8.
20. Bernardino AMR, Romeiro GA, Mello H, De Souza MCB V, Ferreira VF. SYNTHESIS OF NEW 1//-PYRAZOLO[3,4-A|PYRIDINE DERIVATIVES.
21. Maria Menezes CS, Mauricio Sant CR, Rangel Rodrigues C, Barreiro EJ. Molecular modeling of novel 1H-pyrazolo[3,4-b]pyridine derivatives designed as isosters of the antimalarial me¯oquine [Internet]. Available from: http://www.farmacia.ufrj.br/lassbio.
22. Bernardino AMR, De Azevedo AR, Pinheiro LCDS, Borges JC, Carvalho VL, Miranda MD, et al. Synthesis and antiviral activity of new 4-(phenylamino)/4-[(methylpyridin- 2-yl)amino]-1-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carboxylic acids derivatives. Medicinal Chemistry Research. 2007 Dec;16(7–9):352–69.
23. Azevedo AR, Ferreira VF, De Mello H, Leäo-Ferreira LR, Jabor A V, Frugulhetti ICPP, et al. SYNTHESIS AND BIOLOGICAL EVALUATION OF Lff-PYRAZOLO [3,4-6] PYRIDINE-5 CARBOXYLIC ACIDS AGAINST VACCINIA VIRUS.
24. Rosaria Dias LS, Jos M, Alvim F, Carlos Freitas AC, Barreiro EJ, Luisa Miranda AP. PHARMACEUTICA ACTA HELVETIAE Synthesis and analgesic properties of 5-acyl-arylhydrazone 1-H pyrazolo [3,4-b] pyridine derivatives. Vol. 69, Pharmaceutica Acta Helvetiae. 1994.
25. Miyachi H, Yuzuriha T, Tabata R, Fukuda S, Nunomura K, Lin B, et al. Structural development of 1H-pyrazolo-[3,4-b]pyridine-4-carboxylic acid derivatives as human peroxisome proliferator-activated receptor alpha (PPARα)-selective agonists. Bioorg Med Chem Lett. 2019 Aug 15;29(16):2124–8.
26. Yoshida T, Oki H, Doi M, Fukuda S, Yuzuriha T, Tabata R, et al. Structural Basis for PPARα Activation by 1H-pyrazolo-[3,4-b]pyridine Derivatives. Sci Rep. 2020 Dec 1;10(1).

27. Sang Z, Wang K, Dong J, Tang L. Alzheimer's disease: Updated multi-targets therapeutics are in clinical and in progress. Vol. 238, European Journal of Medicinal Chemistry. Elsevier Masson s.r.l.; 2022.
28. Pan T, Xie S, Zhou Y, Hu J, Luo H, Li X, et al. Dual functional cholinesterase and PDE4D inhibitors for the treatment of Alzheimer's disease: Design, synthesis and evaluation of tacrine-pyrazolo[3,4-b]pyridine hybrids. Bioorg Med Chem Lett. 2019 Aug 15;29(16):2150–2.
29. de Mello H, Echevarria A, Bernardino AM, Canto-Cavalheiro M, Leon LL. Antileishmanial pyrazolopyridine derivatives: Synthesis and structure-activity relationship analysis. J Med Chem. 2004 Oct 21;47(22):5427–32.
30. Pawar S, Kumawat MK, Kundu M, Kumar K. Synthetic and medicinal perspective of antileishmanial agents: An overview. Vol. 1271, Journal of Molecular Structure. Elsevier B.V.; 2023.
31. Bawankule DU, Sathishkumar K, Sardar KK, Chanda D, Krishna AV, Prakash VR, et al. BAY 41-2272 [5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1H-pyrazolo[3,4-b]pyridine-3-yl]pyrimidin-4-ylamine]-induced dilation in ovine pulmonary artery: Role of sodium pump. Journal of Pharmacology and Experimental Therapeutics. 2005 Jul;314(1):207–13.
32. Dai X, Wang K, Chen H, Huang X, Feng Z. Design, synthesis, and biological evaluation of 1-methyl-1H-pyrazolo[4,3-b]pyridine derivatives as novel small-molecule inhibitors targeting the PD-1/PD-L1 interaction. Bioorg Chem. 2021 Sep 1;114.
33. Zhang J, Luo Z, Duan W, Yang K, Ling L, Yan W, et al. Dual-acting antitumor agents targeting the A2A adenosine receptor and histone deacetylases: Design and synthesis of 4-(furan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine derivatives. Eur J Med Chem. 2022 Jun 5;236.
34. Kendall JD, O'Connor PD, Marshall AJ, Frédérick R, Marshall ES, Lill CL, et al. Discovery of pyrazolo[1,5-a]pyridines as p110α-selective PI3 kinase inhibitors. Bioorg Med Chem. 2012 Jan 1;20(1):69–85.
35. Behnke D, Cotesta S, Hintermann S, Fendt M, Gee CE, Jacobson LH, et al. Discovery of 1H-pyrazolo[3,4-b]pyridines as potent dual orexin receptor antagonists (DORAs). Bioorg Med Chem Lett. 2015 Dec 1;25(23):5555–60.
36. Hamblin JN, Angell TDR, Ballantine SP, Cook CM, Cooper AWJ, Dawson J, et al. Pyrazolopyridines as a novel structural class of potent and selective PDE4 inhibitors. Bioorg Med Chem Lett. 2008 Jul 15;18(14):4237–41.
37. Maqbool M, Rajvansh R, Srividya K, Hoda N. Deciphering the robustness of pyrazolopyridine carboxylate core structure-based compounds for inhibiting α-synuclein in transgenic C. elegans model of Synucleinopathy. Bioorg Med Chem. 2020 Sep 1;28(17).
38. Li L, Zhang W, Lin F, Lu X, Chen W, Li X, et al. Synthesis and biological evaluation of pyrazolo[3,4-b]pyridine-3-yl pyrimidine derivatives as sGC stimulators for the treatment of pulmonary hypertension. Eur J Med Chem. 2019 Jul 1;173:107–16.
39. Umei K, Nishigaya Y, Kondo A, Tatani K, Tanaka N, Kohno Y, et al. Novel pyrazolo[1,5-a]pyridines as orally active EP1 receptor antagonists: Synthesis, structure-activity relationship studies, and biological evaluation. Bioorg Med Chem. 2017;25(9):2635–42.
40. Nam Y, Hwang D, Kim N, Seo HS, Selim KB, Sim T. Identification of 1H-pyrazolo[3,4-b]pyridine derivatives as potent ALK-L1196M inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):1426–38.
41. Wenglowsky S, Ren L, Ahrendt KA, Laird ER, Aliagas I, Alicke B, et al. Pyrazolopyridine inhibitors of B-RafV600E. Part 1: The development of selective, orally bioavailable, and efficacious inhibitors. ACS Med Chem Lett. 2011 May 12;2(5):342–7.
42. Zhu GD, Gong J, Gandhi VB, Woods K, Luo Y, Liu X, et al. Design and synthesis of pyridine-pyrazolopyridine-based inhibitors of protein kinase B/Akt. Bioorg Med Chem. 2007 Mar 15;15(6):2441–52.
43. Gorecki L, Muthna D, Merdita S, Andrs M, Kucera T, Havelek R, et al. 7-Azaindole, 2,7-diazaindole, and 1H-pyrazole as core structures for novel anticancer agents with potential chemosensitizing properties. Eur J Med Chem. 2022 Oct 5;240.
44. Ramírez D, Mejia-Gutierrez M, Insuasty B, Rinné S, Kiper AK, Platzk M, et al. 5-(Indol-2-yl)pyrazolo[3,4-b]pyridines as a new family of task-3 channel blockers: A pharmacophore-based regioselective synthesis. Molecules. 2021 Jul 1;26(13).
45. Bagley MC, Baashen M, Paddock VL, Kipling D, Davis T. Regiocontrolled synthesis of 3- and 5-aminopyrazoles, pyrazolo[3,4-d] pyrimidines, pyrazolo[3,4-b]pyridines and pyrazolo[3,4-b]quinolinones as MAPK inhibitors. Tetrahedron. 2013 Sep 30;69(39):8429–38.
46. Cheng H, Chang Y, Zhang L, Luo J, Tu Z, Lu X, et al. Identification and optimization of new dual inhibitors of B-Raf and epidermal growth factor receptor kinases for overcoming resistance

against vemurafenib. J Med Chem. 2014 Mar 27;57(6):2692–703.
47. Misra RN, Rawlins DB, Xiao HY, Shan W, Bursuker I, Kellar KA, et al. 1H-Pyrazolo[3,4-b]pyridine inhibitors of cyclin-dependent kinases. Bioorg Med Chem Lett. 2003 Mar 24;13(6):1133–6.
48. Duca JS, Madison VS. Insights from Ab initio quantum chemical calculations into the preferred tautomeric forms and binding affinities to CDK2 of substituted pyrazolopyridines. Biopolymers - Peptide Science Section. 2005;80(2–3):312–8.
49. Chioua M, Samadi A, Soriano E, Lozach O, Meijer L, Marco-Contelles J. Synthesis and biological evaluation of 3,6-diamino-1H-pyrazolo[3,4-b]pyridine derivatives as protein kinase inhibitors. Bioorg Med Chem Lett. 2009 Aug 15;19(16):4566–9.
50. Czodrowski P, Mallinger A, Wienke D, Esdar C, Pöschke O, Busch M, et al. Structure-Based Optimization of Potent, Selective, and Orally Bioavailable CDK8 Inhibitors Discovered by High-Throughput Screening. J Med Chem. 2016 Oct 27;59(20):9337–49.
51. Liu N, Wang Y, Huang G, Ji C, Fan W, Li H, et al. Design, synthesis and biological evaluation of 1H-pyrrolo[2,3-b]pyridine and 1H-pyrazolo[3,4-b]pyridine derivatives as c-Met inhibitors. Bioorg Chem. 2016 Apr 1;65:146–58.
52. Zhao B, Li Y, Xu P, Dai Y, Luo C, Sun Y, et al. Discovery of Substituted 1H-Pyrazolo[3,4-b]pyridine Derivatives as Potent and Selective FGFR Kinase Inhibitors. ACS Med Chem Lett. 2016 Jun 9;7(6):629–34.
53. Shi J, Xu G, Zhu W, Ye H, Yang S, Luo Y, et al. Design and synthesis of 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazoles and pyrazolo[3,4-b]pyridines for Aurora-A kinase inhibitors. Bioorg Med Chem Lett. 2010 Jul 15;20(14):4273–8.
54. Joshi CN, Martin DN, Fox JC, Mendelev NN, Brown TA, Tulis DA. The soluble guanylate cyclase stimulator BAY 41-2272 inhibits vascular smooth muscle growth through the cAMP-dependent protein kinase and cGMP-dependent protein kinase pathways. Journal of Pharmacology and Experimental Therapeutics. 2011 Nov;339(2):394–402.
55. Ye Q, Shen Y, Zhou Y, Lv D, Gao J, Li J, et al. Design, synthesis and evaluation of 7-azaindazolyl-indolyl-maleimides as glycogen synthase kinase-3β (GSK-3β) inhibitors. Eur J Med Chem. 2013;68:361–71.
56. Witherington J, Bordas V, Gaiba A, Naylor A, Rawlings AD, Slingsby BP, et al. 6-Heteroaryl-pyrazolo[3,4-b]pyridines: Potent and selective inhibitors of Glycogen Synthase Kinase-3 (GSK-3). Bioorg Med Chem Lett. 2003;13(18):3059–62.
57. Sabat M, Wang H, Scorah N, Lawson JD, Atienza J, Kamran R, et al. Design, synthesis and optimization of 7-substituted-pyrazolo[4,3-b]pyridine ALK5 (activin receptor-like kinase 5) inhibitors. Bioorg Med Chem Lett. 2017;27(9):1955–61.
58. Zheng B, Peng Y, Wu W, Ma J, Zhang Y, Guo Y, et al. Synthesis and structure–activity relationships of pyrazolo-[3,4-b]pyridine derivatives as adenosine 5'-monophosphate-activated protein kinase activators. Arch Pharm (Weinheim). 2019 Aug 1;352(8).
59. Witherington J, Bordas V, Haigh D, Hickey DMB, Ife RJ, Rawlings AD, et al. 5-Aryl-pyrazolo[3,4-b]pyridazines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). Bioorg Med Chem Lett. 2003 May;13(9):1581–4.
60. Barghash RF, Eldehna WM, Kovalová M, Vojáčková V, Kryštof V, Abdel-Aziz HA. One-pot three-component synthesis of novel pyrazolo[3,4-b]pyridines as potent antileukemic agents. Eur J Med Chem. 2022 Jan 5;227.
61. Hill MD. A Multicomponent Approach to Highly Substituted 1 H -Pyrazolo[3,4- b]pyridines. Synthesis (Germany). 2016 Jul 15;48(14):2201–4.
62. Martín-Acosta P, Amesty Á, Guerra-Rodríguez M, Guerra B, Fernández-Pérez L, Estévez-Braun A. Modular synthesis and antiproliferative activity of new dihydro-1H-pyrazolo[1,3-b]pyridine embelin derivatives. Pharmaceuticals. 2021 Oct 1;14(10).
63. Metwally NH, Deeb EA. Synthesis, anticancer assessment on human breast, liver and colon carcinoma cell lines and molecular modeling study using novel pyrazolo[4,3-c]pyridine derivatives. Bioorg Chem. 2018 Apr 1;77:203–14.
64. Zhai M, Liu S, Gao M, Wang L, Sun J, Du J, et al. 3,5-Diaryl-1H-pyrazolo[3,4-b]pyridines as potent tubulin polymerization inhibitors: Rational design, synthesis and biological evaluation. Eur J Med Chem. 2019 Apr 15;168:426–35.
65. Jian XE, Yang F, Jiang CS, You WW, Zhao PL. Synthesis and biological evaluation of novel pyrazolo[3,4-b]pyridines as cis-restricted combretastatin A-4 analogues. Bioorg Med Chem Lett. 2020 Apr 15;30(8).
66. Chen C, Pan P, Deng Z, Wang D, Wu Q, Xu L, et al. Discovery of 3,6-diaryl-1H-pyrazolo[3,4-b]pyridines as potent anaplastic lymphoma kinase

(ALK) inhibitors. Bioorg Med Chem Lett. 2019 Apr 1;29(7):912–6.
67. Nafie MS, Amer AM, Mohamed AK, Tantawy ES. Discovery of novel pyrazolo[3,4-b]pyridine scaffold-based derivatives as potential PIM-1 kinase inhibitors in breast cancer MCF-7 cells. Bioorg Med Chem. 2020 Dec 15;28(24).
68. Park A, Hwang J, Lee JY, Heo EJ, Na YJ, Kang S, et al. Synthesis of novel 1H-Pyrazolo[3,4-b]pyridine derivatives as DYRK 1A/1B inhibitors. Bioorg Med Chem Lett. 2021 Sep 1;47.
69. Mohamed MS, Awad YEED, El-Hallouty SM, El-Araby M. Design, Synthesis and Cancer Cell Line Activities of Pyrazolo[3,4-<i>b</i>]pyridine Derivatives. Open J Med Chem. 2012;02(03):78–88.
70. Hassan GS, Georgey HH, Mohammed EZ, George RF, Mahmoud WR, Omar FA. Mechanistic selectivity investigation and 2D-QSAR study of some new antiproliferative pyrazoles and pyrazolopyridines as potential CDK2 inhibitors. Eur J Med Chem. 2021 Jun 5;218.
71. Li X, Yang T, Hu M, Yang Y, Tang M, Deng D, et al. Synthesis and biological evaluation of 6-(pyrimidin-4-yl)-1H-pyrazolo[4,3-b]pyridine derivatives as novel dual FLT3/CDK4 inhibitors. Bioorg Chem. 2022 Apr 1;121.
72. Sun Y, Wang L, Sun Y, Wang J, Xue Y, Wu T, et al. Structure-based discovery of 1-(3-fluoro-5-(5-(3-(methylsulfonyl)phenyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)phenyl)-3-(pyrimidin-5-yl)urea as a potent and selective nanomolar type-II PLK4 inhibitor. Eur J Med Chem. 2022 Dec 5;243.
73. Luo D, Guo Z, Zhao X, Wu L, Liu X, Zhang Y, et al. Novel 5-fluorouracil sensitizers for colorectal cancer therapy: Design and synthesis of S1P receptor 2 (S1PR2) antagonists. Eur J Med Chem. 2022 Jan 5;227.
74. Pfaffenrot B, Klövekorn P, Juchum M, Selig R, Albrecht W, Zender L, et al. Design and synthesis of 1H-pyrazolo[3,4-b]pyridines targeting mitogen-activated protein kinase kinase 4 (MKK4) - A promising target for liver regeneration. Eur J Med Chem. 2021 Jun 5;218.
75. Farahat AA, Samir EM, Zaki MY, Serya RAT, Abdel-Aziz HA. Synthesis and in vitro antiproliferative activity of certain novel pyrazolo[3,4-b]pyridines with potential p38α MAPK-inhibitory activity. Arch Pharm (Weinheim). 2022 Feb 1;355(2).
76. Guo Y, Jiang X, Chang Q, Xiao Z, Chen Z, Jiang D, et al. Novel pyrazolo[3,4-b]pyridine derivatives: Synthesis, structure–activity relationship studies, and regulation of the AMPK/70S6K pathway. Arch Pharm (Weinheim). 2022 Jul 1;355(7).
77. Zheng B, Peng Y, Wu W, Ma J, Zhang Y, Guo Y, et al. Synthesis and structure–activity relationships of pyrazolo-[3,4-b]pyridine derivatives as adenosine 5'-monophosphate-activated protein kinase activators. Arch Pharm (Weinheim). 2019 Aug 1;352(8).
78. Elrayess R, Abdel Aziz YM, Elgawish MS, Elewa M, Yassen ASA, Elhady SS, et al. Discovery of potent dual egfr/her2 inhibitors based on thiophene scaffold targeting h1299 lung cancer cell line. Pharmaceuticals. 2021 Dec 1;14(1):1–21.
79. Sun Y, Tang H, Wang X, Feng F, Fan T, Zhao D, et al. Identification of 1H-pyrazolo[3,4-b]pyridine derivatives as novel and potent TBK1 inhibitors: design, synthesis, biological evaluation, and molecular docking study. J Enzyme Inhib Med Chem. 2022;37(1):1411–25.
80. Luo Z, Yue X, Yang H, Liu H, Klein RS, Tu Z. Design and synthesis of pyrazolopyridine derivatives as sphingosine 1-phosphate receptor 2 ligands. Bioorg Med Chem Lett. 2018 Feb 1;28(3):488–96.
81. Dennis Bilavendran J, Manikandan A, Thangarasu P, Sivakumar K. Synthesis and discovery of pyrazolopyridine analogs as inflammation medications through pro- and anti-inflammatory cytokine and COX-2 inhibition assessments. Bioorg Chem. 2020 Jan 1;94.
82. Hill MD, Fang H, Brown JM, Molski T, Easton A, Han X, et al. Development of 1H-Pyrazolo[3,4-b]pyridines as Metabotropic Glutamate Receptor 5 Positive Allosteric Modulators. ACS Med Chem Lett. 2016 Dec 8;7(12):1082–6.
83. Waly OM, Saad KM, El-Subbagh HI, Bayomi SM, Ghaly MA. Synthesis, biological evaluation, and molecular modeling simulations of new heterocyclic hybrids as multi-targeted anti-Alzheimer's agents. Eur J Med Chem. 2022 Mar 5;231.