Journal of Sciences, Islamic Republic of Iran 33(3): 227 - 232 (2022) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Short Communication
Synthesis and Cytotoxic Assessment of Thieno pyridine Derivatives

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Received: 16 April 2022 / Revised: 1 October 2022 / Accepted: 30 October 2022

Abstract

Thieno[2,3-b]pyridine, a bicyclic heterocyclic, possesses various advantageous biological properties, including a cytotoxic effect. This study synthesized thieno pyridine scaffold derivatives with position **2** modifications. The reaction between 2-chloronicotinonitrile and methyl thioglycolate produced methyl amino thieno pyridine carboxylate **1**. As a result of hydrazinolysis, methyl amino thieno pyridine carbohydrazide **2** was synthesized as a key compound for preparing final derivatives. The reaction of compound **2** with phthalic anhydride yielded thieno pyridine-phthalimide. The 1,3,4-oxadiazole scaffolds were synthesized in two steps. During the initial reaction of compound **2** with aroyle chloride derivatives, the corresponding intercessor was cyclized to oxadiazole in the presence of phosphoryl chloride. Compound **2** was subsequently cyclized in boiling glacial acetic acid to produce a pyridine-pyrazole derivative. MTT assay was utilized to assess the cytotoxic activity against two cell lines. Except for compound **6**, the final compounds exhibited IC_{50} values <50 μM against both cell lines. Lipophilic compounds **4** and **5** exhibited significant cytotoxic effects compared to other compounds.

Keywords: Thieno pyridine; Anticancer activity; Oxadiazole, Phthalimide.

Introduction

Breast and cervical cancers, the most lethal cancers in women, are considered major global health issues. Despite the significant improvement and discovery of new drugs for treating these two cancers, many of them are associated with drug resistance and side effects. Consequently, it appears necessary to focus on synthesizing more effective drugs [1-3]. Thienopyridines are desirable scaffolds due to their antiviral [4, 5], anti-inflammatory [6, 7], antimicrobial [8], antiplatelet [9, 10], anti-hepatocellular carcinoma [11-13], anti-cancer [14-18], and antiangiogenic [17] properties.

This structure can function as a bioisoester for the benzofuran ring [16]. Various mechanisms, including inhibition of non-receptor tyrosine kinase c-Src [14], inhibition of eukaryotic elongation factor-2 kinase (eEF2) [15], phospholipase C (PLC-g2) [14], tubulin polymerization [19], and DRAK2 (DAPK-related apoptosis-inducing protein kinase) [20] have been proposed for anticancer activities of thieno[2,3-b]

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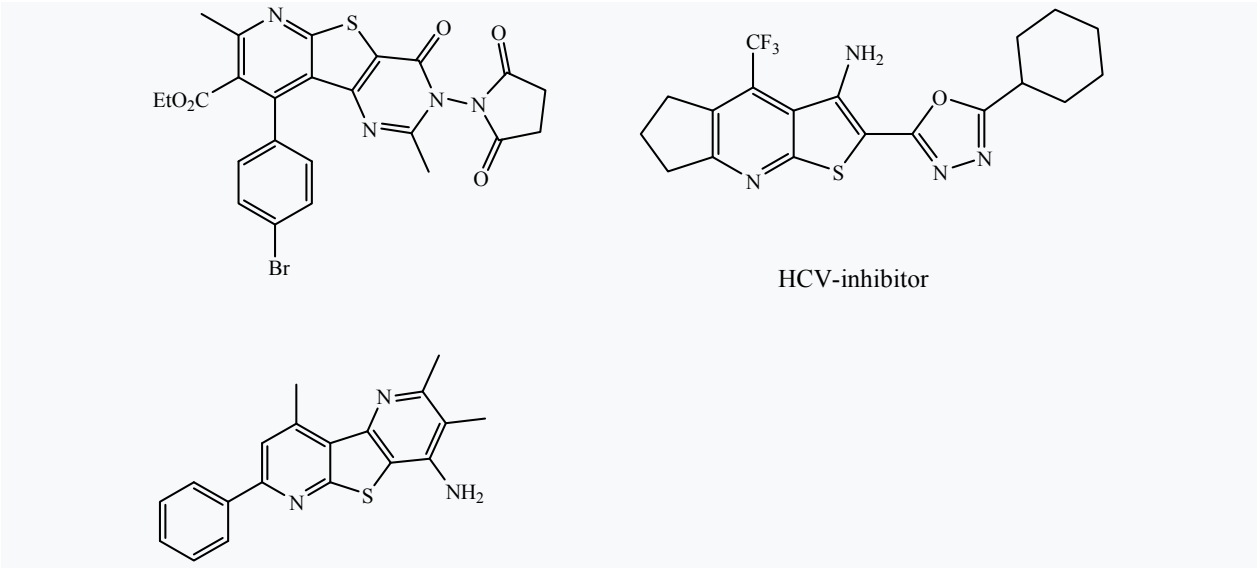


Figure 1. Structures of thieno pyridine derivatives with anticancer activity

pyridine-based structures. Multiple thienopyridine derivatives have been developed as potential cytotoxic agents [1, 21]. Zuo et al. synthesized a potent HCV inhibitor with a scaffold (thienopyridine-1, 3, 4-oxadiazole) [22]. Moreover, Leonczak et al. developed oxadiazole-thieno pyridine derivatives as inhibitors of DRAK2 [20].

Kadah et al. [23] synthesized multiple pyrido-thieno-pyrimidine imide scaffold-based effective anticancer agents. Hassan et al. developed several thieno-pyridine derivatives condensed with cytotoxic heterocyclics [24] (Figure 1). A number of thieno[2, 3-b]pyridine derivatives containing oxadiazole or imide were synthesized and tested against human cancer cell lines prompted by literature reviews on the cytotoxic properties of thienopyridine compounds.

Materials and Methods

Raw compounds and solvents were obtained from companies like Merck (Germany). 60 F_{254} plates (Merck, Germany) of silica gel were used for thin layer chromatography (TLC). Proton nuclear magnetic resonance (^{1}HNMR) were taken using a spectrometer (Bruker 400 MHz, Germany). IR (KBr discs) was acquired by a Perkin_Elmer infrared spectrophotometer (Germany). Melting points are uncorrected and were determined using electrothermal 9200 melting point instrument (England). Agilent Technologies 5975C mass spectrometer (USA) was used for mass spectra

Synthesis of methyl 3-aminothieno pyridine-2-carboxylate (1)

2-Chloronicotinonitrile (0.05 mol), methyl thioglycolate and potassium carbonate (0.1 mol) were stirred in ethanol at 80 °C for 24h.Then, the mixture was cooled, poured in to crushed ice and extracted with ethyl acetate which evaporated under vacuum to render a yellow powder [15] (Scheme 1).

Synthesis of methyl aminothieno pyridine-2-carbohydrazide (2)

Hydrazine hydrate 60% (0.04 mol) was added to a solution of **1**(0.02mol) in absolute ethanol (40mL) and reaction was continued for 16 h. Then, reaction container was cooled and the separated solid was filtered, washed with water to provide **2**[15] (Scheme 1).

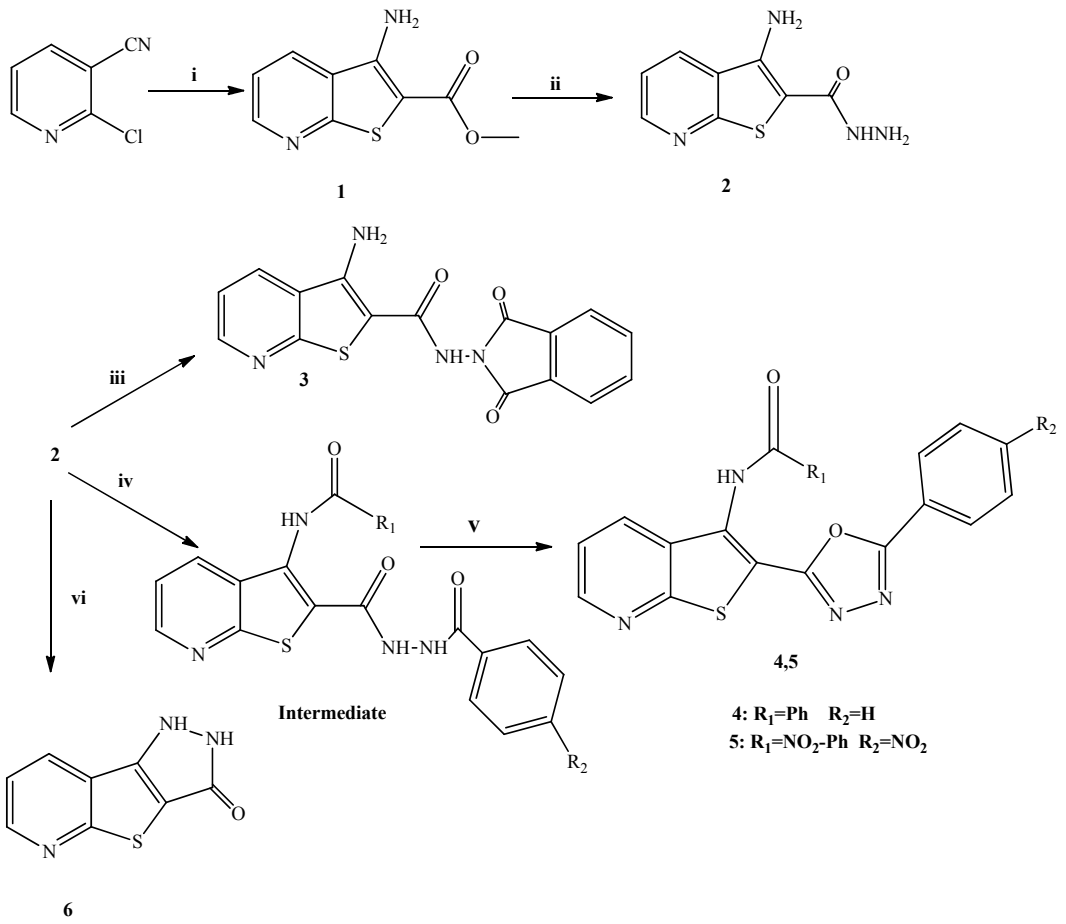
Preparation of 3-amino-N-(1, 3-dioxoisoindolin-2-yl) thieno pyridine-2-carboxamide (3)

Equimolar amount of compound **2** and phthalic anhydride (0.02mol) were refluxed in glacial acetic acid for 6 h. The obtained solid, was filtered and washed with water to obtain compound **3** (Scheme 1).

Synthesis of 2-(thieno pyridin-2-yl)-1, 3, 4-oxadiazole derivatives (4, 5)

Step IV**.** Equimolar amount of benzoyl chloride or 4-nitrobenzoyl chloride (0.02mol) was added to a solution of compound **2** (0.02mol) in THF solvent at 0 °C and stirred for 0.5-2 h in room condition, then the solvent was removed and the remainder was applied for next step.

Step V. The previous intermediate was dissolved in $POCl_3$ and refluxed for 1 then, ice water was added to



Scheme 1. (i) K_2CO_3, $SHCH_2COOMe$, EtOH, reflux; (ii) $N_2H_4.H_2O$, EtOH, reflux; (iii) Phthalic anhydride, Acetic acid glacial, reflux; (iv) THF, benzoyl chloride or 4-nitrobenzoyl chloride ;(V) $POCl_3$;(vi) Acetic acid glacial.

obtain precipitation which filtered off and water was used for washing , then recrystallized from ethyl acetate to render the final products [24]

Synthesis of pyridothienopyrazol derivative (6)

Compound **2** (0.02mol) was dissolved in glacial acetic acid and refluxed for 6 h. Resultant solid was filtered off, and crystallized to give **6** (Scheme 1)**.**

Cytotoxicity assay

Sample and* culture *media preparation

(RPMI-1640) supplemented with fetal bovine serum (FBS) 5% v/v and antibiotic (mixture of 100 units/mL penicillin /100 µg/mLstreptomycin) 1% was used for growing MCF-7 (breast cancer), and HeLa (cervical cancer) cell lines. Cells were cultured in a 96-well plate at a concentration of (5×10^4cells/mL) and incubated for 24 h. Different concentrations of the final products were added. Paclitaxel was used as the positive control. Negative Control cells contain cell suspension and DMSO (1%) without tested compounds. The blank wells were consisted of 200 µL of the culture medium. Incubation was continued for 48 hours at 37 °C. MTT dye (20 µL) was added to each well and incubated for 3 h. 150 µL of DMSO was used for dissolving formed formazan crystals and absorbance was measured using an ELISA plate reader at 570 nm [25,26].

Cell viability was calculated using (equation 1):

$$Cell\ Survival(\%) = \frac{\text{Mean absorbance of drug treated wells} - \text{Mean absorbance of blank}}{\text{Mean absorbance of negative control well} - \text{Mean absorbance of blank}} \times 100$$

The cytotoxic activities and IC_{50} values of compounds **3-6** against cell lines in comparison with paclitaxel are shown in Figures2, 3 and Table 1.

Results and Discussion

Methyl 3-aminothieno pyridine-2-carboxylate (1)

Yield: 76 %, Yellow powder, m.p. 195-196°C (lit:

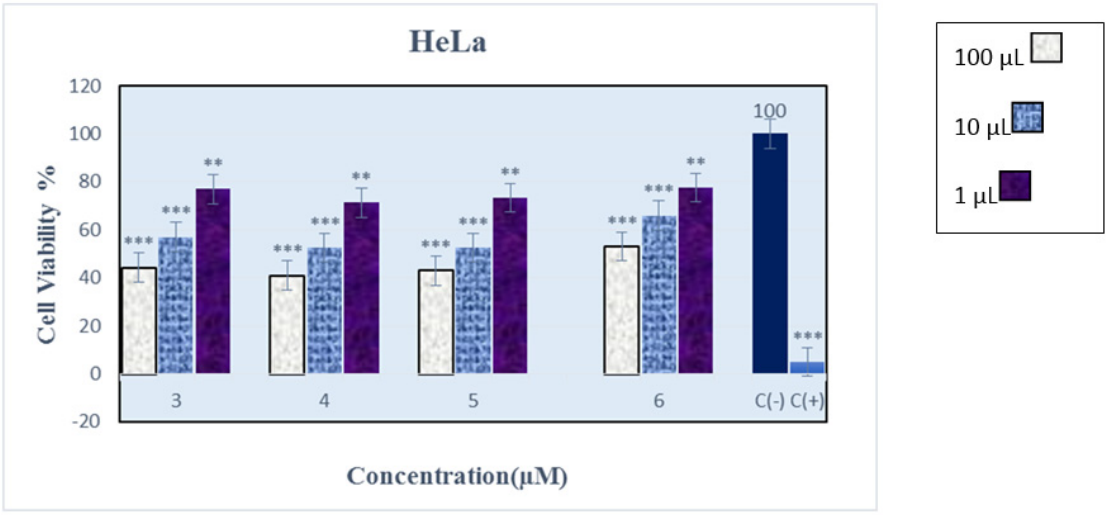


Figure 2. Cytotoxic effects of different concentrations (1, 10, and 100 μM) of compounds **3-6** against HeLa cells after 48h. ** P <0.01, *** P < 0.001 Show significant differences in comparison with negative control group, Data are presented as mean ± SD, n = 3.

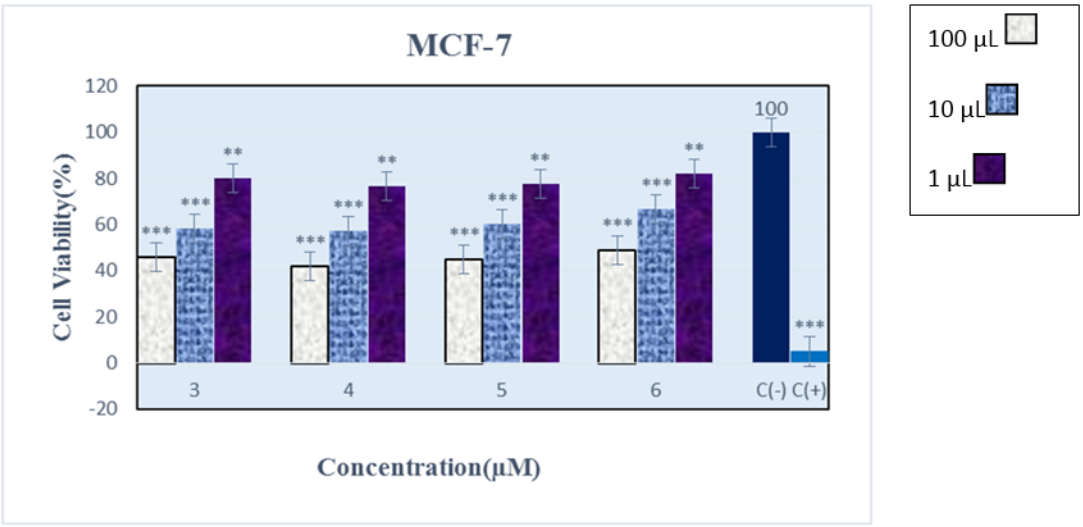


Figure 3. Cytotoxic effects of different concentrations (1, 10, and 100 μM) of compounds **3-6** against MCF-7 cells after 48h. ** P <0.01, *** P < 0.001 Show significant differences in comparison with negative control group, Data are presented as mean ± SD, n = 3.

Table 1. The IC_{50} (μM) of final compounds against cell lines

Final Compounds	3	4	5	6
HELA	47±2	38±4	40± 3	94±5
MCF-7	48±3	42±4	45±3	96 ± 2

194-196°C [15]), IR (ν_{max} cm^{-1}), 3424, 3319 (N-H), 1679 (C=O), 1626 (C=C), 1288 (C-O). ^{1}HNMR: (DMSO-d_6; 400 MHz): δ 8.78 (1H, dd, J=4Hz, J=1.6Hz, 6-CH), 8.65 (1H, dd, J=8Hz, J=1.6Hz, 4-CH), 7.56 (1H, dd, J=8Hz, J=4Hz, 5-CH), 7.44 (2H, s, NH_2), 3.9 (3H, s, CH_3).

3-Aminothieno [2, 3-b] pyridine-2-carbohydrazide (2)

Yield: 7 0 %, Yellow Solid, m.p. 214-216°C (lit: 215-217°C [15]), IR (ν_{max} cm^{-1}), 3424, 3319(N-H), 1690

(C=O), 1626 (C=C). ^{1}HNMR: (DMSO-d_6; 400 MHz): δ 9.08 (1H, s, NH), 8.62 (1H, d, *J*=8Hz, 6-CH), 8.41(1H, dd, *J*=8Hz, *J*=4Hz, 4-CH), 7.44 -7.42 (1H, m, 5-CH), 7.13 (2H, s, NH_2), 4.43 (2H, br, NH_2).

3-Amino-N-(1, 3-dioxoisoindolin-2-yl) thieno pyridine-2-carboxamide (3)

Yield: 50%, light yellow, m.p.99-103°C, IR) (cm^{-1}, ν_{max}), 3400, 3320, 3310 (N-H), 1733(C=O), 1640(C=N). ^{1}HNMR: (DMSO-d_6; 400 MHz): δ 10.6 (1H, s, NH), 8.83 - 8.82 (1H, m, 6-CH), 8.56 (1H, d, *J*=8Hz, 4-CH), 8.02 -7.99 (4H, m, H-Phthalic), 7.56 - 7.48 (3H, m, 5-CH, NH_2), MS (m/z): 338.1 (M^+); M.W. 338.3g/mole.

N-(2-(5-Phenyl-1, 3, 4-oxadiazol-2-yl) thieno pyridine-3-yl) benzamide (4)

Yield: 40%, yellow Powder, m.p.112-114 °C, IR (cm^{-1}, ν_{max}), 3100 (N-H), 1692 (C=O), 1282(C-O),^{1}HNMR: (400 MHz; $CDCl_3$): δ 9.11 (1H, s, NH), 8.83 (1H, dd, *J*=8Hz, *J*=4Hz, H-Pyridine), 8.64 (1H, dd, *J*=8Hz, *J*=4Hz, H-Pyridine), 7.85 - 7.83 (2H, m, H-Ar-oxadiazole), 7.72 (2H, d, *J* =8Hz, H-Ar-CONH), 7.61(1H, t, *J*=8Hz, H-Ar-oxadiazole), 7.53 -7.50 (4H, m, H-Ar-oxadiazole, H-Ar-CONH, H-Pyridine),7.39 (2H, t, *J*=8Hz, H-Ar-CONH), MS (m/z): 398(M^+); M.W. 398.4 g/mole.

4-Nitro-N-(2-(5-(4-nitrophenyl)-1, 3, 4-oxadiazol-2-yl) thieno pyridine-3-yl) Benz amide (5)

Yield: 40%, Orange Solid, m.p.139-141°C, IR (ν_{max}, cm^{-1}), 3279 (N-H), 1697 (C=O), 1334, 1533 (NO_2). ^{1}HNMR: (400 MHz; $CDCl_3$): δ 9.14 (1H, s, NH), 8.89 (1H, d, *J*=8Hz, H-pyridine), 8.66 (1H, d, *J*=8Hz, H-pyridine), 8.38-8.32 (4H, m, H-Ar), 8.02 (2H, d, *J*=8Hz, H-Ar),7.96 (2H, d, *J*=8Hz, H-Ar), 7.60 -7.56 (1H, m, H-pyridine), MS (m/z): 488(M^+); M.W.488g/mole.

Pyridothienopyrazol (6)

Yield: 45%, White Solid, m.p.132-134 °C, IR ($\nu_{max,}$ cm^{-1}), 3420, 3235 (N-H), 1657 (C=O), ^{1}HNMR: (400 MHz; $CDCl_3$): δ 8.81 (1H, dd, *J*=10Hz, *J*=4Hz, H-pyridine), 8.59 (1H, dd, *J*=8Hz, *J*=4Hz, H-pyridine), 8.16 (1H, s, NH), 7.54 - 7.50 (2H, m, H-pyridine, NH), MS (m/z): 191(M^+); M.W.191 g/mole.

Thienopyridine derivatives exhibited diverse biological properties and particularly cytotoxic effects [14-18]. Reaction 2-chloronicotinonitrile with methylthioglycolate underwent ring closure to give thieno pyridine core structure followed by treatment with hydrazine hydrate to yield key intermediate, used for the synthesis final products. Oxadiazole –based products were prepared through acyl hydrazide derivatives in a two step procedure. In this study, modification of structures focused on 2 position of thieno pyridine scaffold. The structure of compounds was established by ^{1}H-NMR, IR and Mass spectral data. The IR spectra of compound **3** revealed NH and CO bands at (3400, 3320, 3310 cm^{-1}) and 1733 cm^{-1}, respectively. Its ^{1}HNMR spectrum indicated signals between 8.02 and 7.99 ppm, corresponding to phthalic ring hydrogens. Compounds **4** and **5** displayed ether group of oxadiazole, NH, and carbonyl bands in their IR spectra. NO_2 bands were detected at 1334 and 1533 cm^{-1} for compound **5**. Observation bands at 3420 and 3235 for the NH group confirmed the structure of compound **6**, as previously reported for compounds with similar structures [27]. Compound **6** exhibited two regions at 7.54-7.50 and 8.16 ppm, corresponding to NH pyrazole and NH adjacent to the carbonyl group, respectively. Figures 2, 3, and Table 1 illustrate the cytotoxic activities and IC_{50} values of compounds **3-6** against cell lines. IC_{50} values against both cell lines ranged from 38 to 96 μM. Figures 2 and 3 depict significant differences in cell line viability relative to the negative control.

Cytotoxic results revealed that the connection of the oxadiazole ring to the thienopyridine scaffold yielded compounds **4** and **5** with IC_{50} values slightly less than compounds **3** and **6**. Adding a nitro group to the phenyl ring decreased the activity of oxadiazole-derived compounds against both cell lines. On the C_2 and C_3 regions of the thienopyridine core, a fused-ring strategy was used to produce compound **6**, for which a sharp loss of activity was observed.

It appears that lipophilicity could increase the potency of thienopyridine against tested cell lines, as demonstrated by oxadiazole-thienopyridine conjugates; this was confirmed by relatively hydrophilic thienopyridine-phthalimide and thienopyridine-pyrazole hybrids with high IC_{50} values. Further modifications should be investigated in the future to exploit the potential of thienopyridine derivatives fully.

Conclusion

The results of the cytotoxic tests indicated that lipophilicity of oxadiazole-derivatives might improve their cytotoxic activity.

References

1.Hung JM, Arabshahi HJ, Leung E, Reynisson J, Barker D. Synthesis and cytotoxicity of thieno [2, 3-b] pyridine and furo [2, 3-b] pyridine derivatives. Eur J Med Chem. 2014;86:420-37.

2. Liu Y, Wu L, Xue J, Lu Y, Yin L, Li M . Front. PD-1/PD-L1 inhibitors in cervical cancer. Frontiers in pharmacology

Pharmacol. 2019;10:65.
3. Madhusudana K, Shireesha B, Naidu VG, Ramakrishna S, Narsaiah B, Rao AR, et al. Anti-inflammatory potential of thienopyridines as possible alternative to NSAIDs. Eur J Pharmacol. 2012;678:48-54.
4. Nikkhoo AR, Miri R, Arianpour N, Firuzi O, Ebadi A, Salarian AA. Cytotoxic activity assessment and c-Src tyrosine kinase docking simulation of thieno [2, 3-b] pyridine-based derivatives. Med Chem Res. 2014;23(3):1225-33.
5. Schnute ME, Anderson DJ, Brideau RJ, Ciske FL, Collier SA. 2-Aryl-2-hydroxyethylamine substituted 4-oxo-4, 7-dihydrothieno [2, 3-b] pyridines as broad-spectrum inhibitors of human herpesvirus polymerases. Bioorg Med Chem Lett. 2007;17:3349-53.
6. Bernardino AM, da Silva Pinheiro LC, Rodrigues CR, Loureiro NI, Castro HC, Lanfredi-Rangel A, et al.Design, synthesis, SAR, and biological evaluation of new 4-(phenylamino) thieno [2, 3-b] pyridine derivatives. Bioorg & Med Chem. 2006;14(16):5765-70.
7. Nathan Tumey L, Boschelli DH, Lee J, Chaudhary D .2-Alkenylthieno [2, 3-b] pyridine-5-carbonitriles: Potent and selective inhibitors of PKCtheta. Bioorg Med Chem Lett. 2008;18:4420-3.
8. Rateb NM, Abdelaziz SH, Zohdi HF. Synthesis and antimicrobial evaluation of some new thienopyridine, pyrazolopyridine and pyridothienopyrimidine derivatives. J Sulfur Chem. 2011;32(4):345-54.
9. Freeman MK. Thienopyridine antiplatelet agents: focus on prasugrel. The Consultant Pharmacist. 2010;25(4):241-57.
10. Testa L, Biondi Zoccai GG, Valgimigli M, Latini RA, Pizzocri S, Lanotte S. et al Current concepts on antiplatelet therapy: focus on the novel thienopyridine and non-thienopyridine agents. Adva in hemato. 2010:1-7.
11. Wang NY, Zuo WQ, Xu Y, Gao C, Zeng XX, Zhang LD, et al. Discovery and structure–activity relationships study of novel thieno [2, 3-b] pyridine analogues as hepatitis C virus inhibitors. Bioorg & Med Chem Lett. 2014;24(6):1581-8.
12. Abreu RMV, Ferreira IC, Calhelha RC, Lima RT, Vasconcelos MH, Adega F, et al. Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno [3, 2-b] pyridine-2-carboxylate derivatives: In vitro evaluation, cell cycle analysis and QSAR studies. Eu J Med Chem, 2011;46(12):5800-06.
13. Zeng XX, Zheng RL, Zhou T, He HY, Liu JY,Zheng Y, et al. Novel thienopyridine derivatives as specific anti-hepatocellular carcinoma (HCC) agents: synthesis, preliminary structure–activity relationships, and in vitro biological evaluation. Bioorg Med Chem Lett. 2010;20(21):6282-5.
14. Pevet I, Brul C, Tizot A, Gohier A, Cruzalegui F, Boutin JA, et al.Synthesis and pharmacological evaluation of thieno [2, 3-b] pyridine derivatives as novel c-Src inhibitors. Bioorg Med Chem. 2011, 15, 19(8), 2517-28.
15. Lockman JW, Reeder MD, Suzuki K, Ostanin K, Hoff R, Bhoite L, et al. Inhibition of eEF2-K by thieno [2, 3-b] pyridine analogues. Bioorg Med Chem Lett. 2010;7:283-6.
16. Naguib BH, El-Nassan HB. Synthesis of new thieno [2, 3-b] pyridine derivatives as pim-1 inhibitors. J Enz Inhib Med Chem. 2016;31(6):1718-25.
17. Queiroz MJ, Calhelha RC, Vale-Silva LA, Pinto E, Lima RT, Vasconcelos MH. Efficient synthesis of 6-(hetero) arylthieno [3, 2-b] pyridines by Suzuki–Miyaura coupling. Evaluation of growth inhibition on human tumor cell lines, SARs and effects on the cell cycle. Eur J Med Chem. 2010;45(12):5628-34.
18. Queiroz MJR, Peixoto D, Calhelha RC, Soares P, dos Santos T, Lima RT et al. New di (hetero) arylethers and di (hetero) arylamines in the thieno [3, 2-b] pyridine series: Synthesis, growth inhibitory activity on human tumor cell lines and non-tumor cells, effects on cell cycle and on programmed cell death. Eur J Med Chem, 2013;69:855-862.
19. Romagnoli R, Baraldi PG, Kimatrai Salvador M, Preti D, Aghazadeh Tabrizi M, Bassetto M, et al. Synthesis and biological evaluation of 2-(alkoxycarbonyl)-3-anilinobenzo [b] thiophenes and thieno [2, 3-b] pyridines as new potent anticancer agents. J of Med Chem. 2013;56(6):2606-18.
20. Leonczak P, Gao LJ, Ramadori AT, Lescrinier E, Rozenski J, De Jonghe S, et al. Synthesis and Structure–Activity Relationship Studies of 2□(1, 3, 4□Oxadiazole□2 (3H) □thione) □3□amino□5□arylthieno [2, 3□b] pyridines as Inhibitors of DRAK2. Chem Med Chem. 2014;9(11):2587-601.
21. Mohareb RM, Abdallah AE, Abdelaziz MA. New approaches for the synthesis of pyrazole, thiophene, thieno [2, 3-b] pyridine, and thiazole derivatives together with their anti-tumor evaluations. Med Chem Res. 2014;23(2):564-79.
22. Zuo WQ, Wang NY, Zhu YX, Liu L, Xiao KJ, Zhang LD.; et al. A new series of HCV inhibitors based on a 2-(thieno [2, 3 b] pyridin-2-yl)-1, 3, 4-oxadiazole scaffold. RSC Adv. 2016;6(46):40277-86.
23. Kadah MS. Synthesis of some new thienopyridine and pyridothienopyrimidine derivatives with expected antitumor activity. Int J Med Sci. 2016;3(9):1-10.
24. Hassan AY, Sarg MT, Said MM, El-Sebaey SA. Utility of thieno [2, 3-b] pyridine derivatives in the synthesis of some condensed heterocyclic compounds with expected biological activity. Univers Org Chem. 2013;1(2):1-15.
25. Hassanzadeh F,Sadeghi-Aliabadi H, Nikooei S, Jafari E, Vaseghi G. Synthesis and cytotoxic evaluation of some derivatives of triazole-quinazolinone hybrids. Res Pharm Sci. 2019;14(2):130
26. Hassanzadeh F, Sadeghi-Aliabadi H, Jafari, E, Sharifzad A, DanaN. Synthesis and cytotoxic evaluation of some quinazolinone-5-(4-chlorophenyl) 1, 3, 4-oxadiazole conjugates. Res Pharm Sci 2019;14(5):408.
27. Attaby FA, Elneairy MA, Elsayed MS. Synthesis and antimicrobial evaluation of new pyridine, thienopyridine and pyridothienopyrazole derivatives. Arch Pharm Rese. 1999;22(2):194-201.