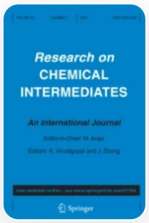
Design and synthesis of some new piritrexim analogs as potential anticancer agents

Published: 11 September 2017

Volume 44, pages 749–767, (2018) Cite this article



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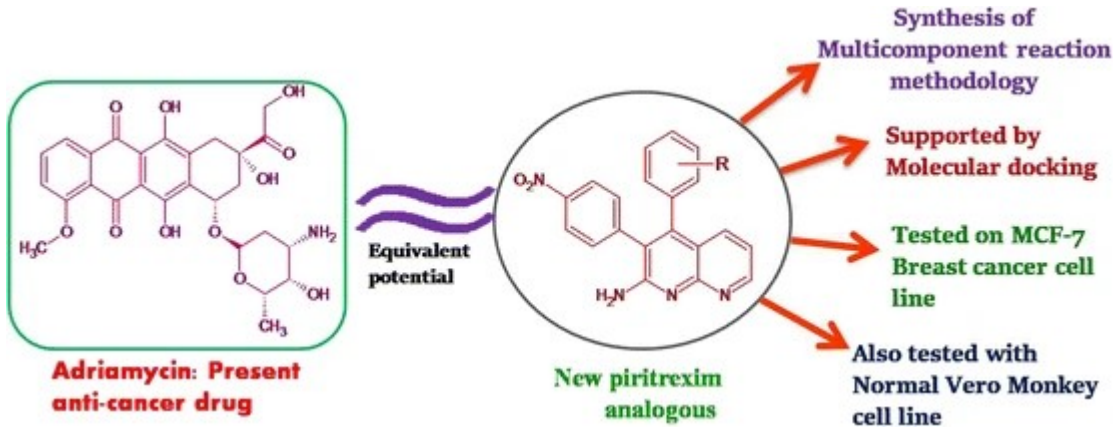
Abstract

The present study describes the synthesis and in vitro anticancer activity of novel piritrexim analogs against human breast cancer cell line MCF-7 and a normal Vero monkey cell line. The scheme involves the formation of 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine accomplished by the condensation reaction of 4-nitrophenyl acetonitrile, aromatic aldehyde,

and 2-aminopyridine using acetic acid as a catalyst. The selected synthesized compounds have been screened against a human breast cancer cell line MCF-7 and a normal Vero monkey cell line, out of which the compounds 4b, 4e, 4f exhibited higher potency (GI_{50} [<10 µg/mL)], and compounds 4a, 4c show moderate to good activity (GI_{50} 41.4 and 12.4 µg/mL) towards human breast cancer cell line MCF-7. Docking study on dihydrofolate reductase enzyme receptor (DHFR) and epidermal growth factor receptor reveals that compounds 4a, 4b, 4c, and 4e show moderate to good binding along with acceptable glide scores.

Graphical Abstract



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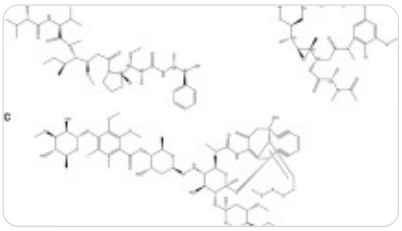

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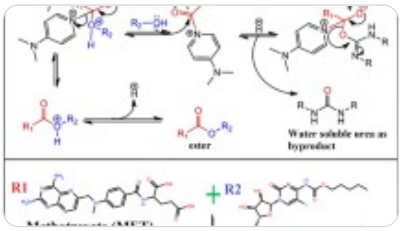

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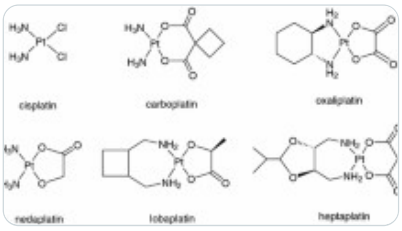

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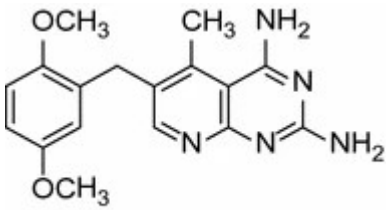
Introduction

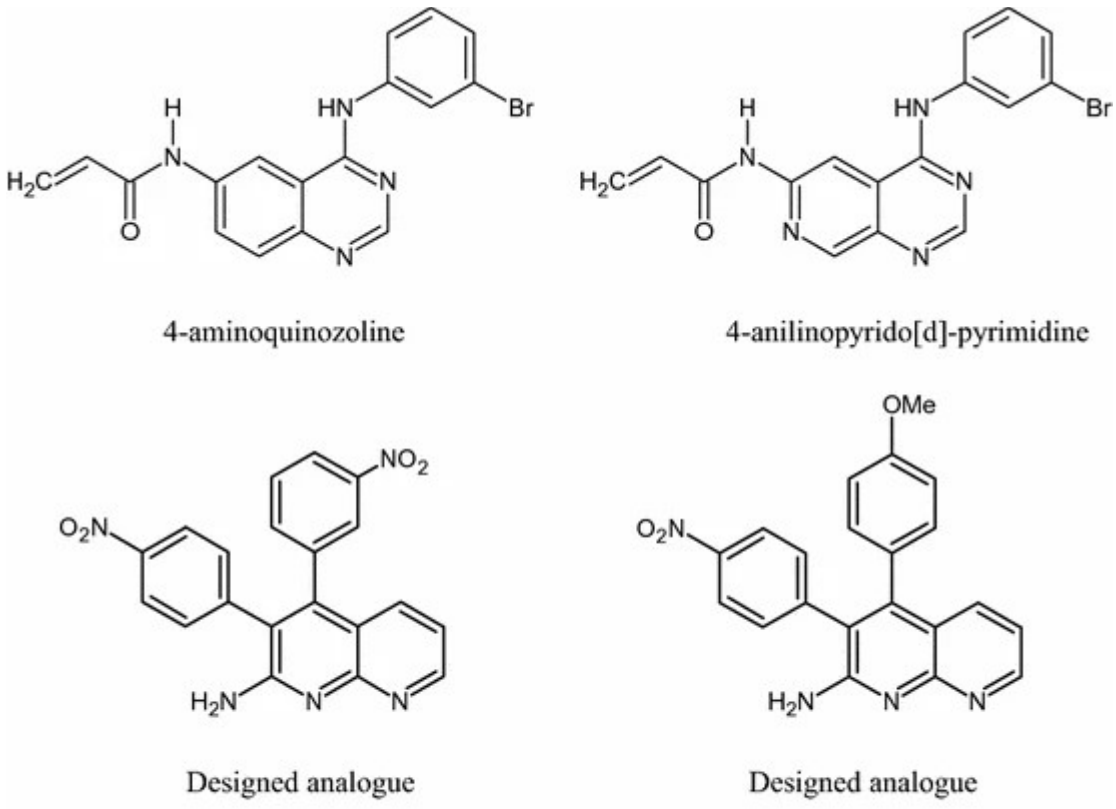
Piritrexim (2, 4-diamino-6-(2, 5-dimethoxybenzyl)-5-mehylpyrido [2, 3-d] pyrimidine, BW301U) is a lipophilic inhibitor of human dihyrofolate reductase (DHFR) [1], and mammalian cell growth [2] has been determined as an anticancer agent, first synthesized by Grivski et al. in 1980. Piritrexim is a small, lipid soluble molecule that crosses the cell membrane rapidly by a simple and rapid passive diffusion process [3]. A number of investigators have carried out large in vitro and in vivo studies by exploring the mechanism and scope of this molecule [4]. The activity of piritrexim in various tumors was explored in two-phase studies: phase I suggested that piritrexim was active against bladder cancer [5], and phase II showed that it was active against a broad panel of tumors, including malignant melanoma [6], head and neck cancer [7], soft tissue sarcoma [8], chemotherapy naive urothelial cancer [9], lung and colon cancer [10]. Because of its lipophilic nature, it was found to have high penetration in tissue.



Piritrexim

Piritrexim and heterocycles belonging to its class show significant properties such as lipid soluble inhibitors of dihydrofolate reductase, which are used in human medicine [11]. In 1976, a scientist named Carter described methotrexate as an effective treatment for metastatic breast cancer [12]. Lometrexol is a new antifolate that shows significant activity against a wide range of solid tumors in animal models [13]. These drugs have shown some disadvantages such as limited entry in cells because of their polarity and carrier mediated transport [14].

Piritrexim analogs such as 4-aminoquinozoline and 4-anilinopyrido[d]-pyrimidine have shown highly potent and selective inhibitors of epidermal growth factor receptor (EGFR) [15]. EGFR belongs to the family of receptor tyrosine kinases known as ErbB, and it activates the numerous signaling pathways that assist tumor growth [16]. EGFR has been strongly considered as responsible for the progression of human cancers, playing a significant role in regulation of cell growth, differentiation, and survival and has emerged as an imperative target in anticancer treatment [17].



4-aminoquinozoline and 4-anilinopyrido[d]-pyrimidine and designed analogues investigated in this work.

On the other hand the napthyridine derivatives, which are analogs of piritrexim are found to be of vital significance in medicinal and pharmaceutical fields due to their wide ranges of bioactivity such as anticancer agents, anti-infectives, and used as therapeutic drugs [18,19,20]. The structural significance of the napthyridines is exhibited by the presence of a hydrogen bond donor and acceptor site simultaneously, which improves the protein binding

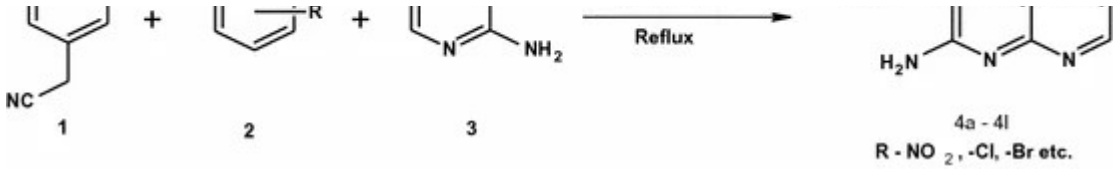
ability [21].

Moreover, napthyridines have been reported to possess anti-HCMV activity used in herpes treatment therapy [22]. Also, these vital molecules have been used as hepatitis C virus inhibitors and in DNA replication [23, 24]. Further, napthyridine derivatives have been also found to have strategic application in respiratory tract disorder treatment [25]. The selective hydrogenation of napthyridines has been successfully introduced as a first step in the synthesis of phenyl butyrates, which are vitronectin receptor antagonists used in the treatment of diseases such as osteoporosis and cancer [26].

The literature survey shows that there are reports available for the synthesis of napthyridine derivatives, which involve napthyridines prepared from starting material such as 2-amino nicotinic acid [27] and 2-amino nicotinealdehyde [28]. Ban et al. initiated napthyridines via Suzuki coupling reaction with aryl boronic acids to report a diverse library of these significant molecules [29]. Siddiqui et al. reported pyrido[2,3-d]pyrimidine-6-carbonitriles and 1,8]-naphthyridine-3-carbonitriles via reaction of aldehydes, 2-aminopyridine and malononitrile in the presence of thiamine hydrochloride as a catalyst [30].

Therefore, with a focus on the multicomponent approach, which proves to be an efficient and economic way to generate a wide library of analogues of piritrexim, an eminent need for the development of synthetic routes to obtain these vital moieties emerges. As part of our research program aiming at the design of new molecules through different pharmacophores, which leads to remarkable anticancer activity [31,32,33,34], herein we report a highly proficient and innovative method for the preparation of a new series of piritrexim analogues. This synthesized piritrexim analogues 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine (Scheme 1) were evaluated for their anticancer activity against human breast cancer cell line MCF-7 and normal Vero monkey cell line. In this work, we provide docking analysis of the compounds giving good activity against the cell lines on DHFR and EGFR enzymes.

Scheme 1



Synthesis of 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine

Results and discussion

Chemistry

Initially, we explored a one-pot reaction by taking equimolar amounts of 4-nitrophenyl acetonitrile (1 mmol), aromatic aldehydes (1 mmol), and 2-amino pyridine (1 mmol) in ethanol under reflux condition using different catalysts such as sulphuric acid, PTSA, sodium ethoxide, DABCO, and triethylamine along with acetic acid (Scheme 1). Acetic acid was found to be the proper catalyst for this transformation as it smoothly catalyzed the reaction in the aspects of reaction time and yield of the product. The reaction was completed within 2–4 h (monitored by TLC). The solid product was separated, filtered, and washed with pet ether to remove impurities. To our expectations the reaction progressed smoothly, yielding appropriate product in good to excellent yield. The afforded product was recrystallized in ethanol and further characterized using spectroscopic techniques IR, ^{1}HNMR, ^{13}CNMR and mass analyses. The synthetic route to 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine derivatives is shown in Scheme 1.

To optimize the reaction condition and catalytic amount with proper solvent to enhance the yield and reduce the time of reaction, varieties of catalyst and solvent were examined (Table 1). As a result of altering the solvent with water, acetonitrile, THF, DMF, or ethanol, the reaction did not proceed further to product formation. In the case of DMF, TLC monitoring showed product formation; however, the obtained product was an oily, sticky mass. As per our investigation it was concluded that the use of ethanol as solvent was found to be beneficial because of its easy availability, it is comparatively inexpensive, and it has an efficient ability to produce the best product at reflux condition.

Table 1 Optimization of reaction condition

Based on these encouraging results, we focused attention on the optimization of catalytic amount for improve the yields (Table 2). From the result, it was evident that 30 mol% acetic acid was found in sufficient quantity to afford the desired product at maximum yield, after which there was no considerable increase in the yield of product.

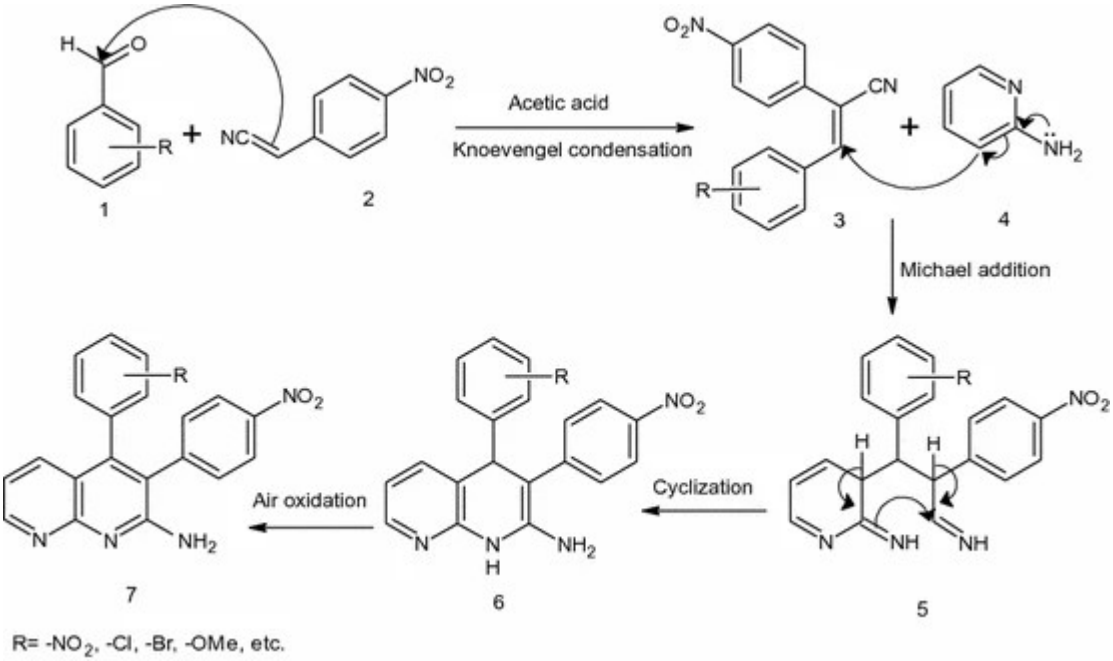
Table 2 Optimization of catalyst acetic acid

In order to expand the scope of this present method, the replacement of substituted aldehydes was examined. To our delight, all the reactions proceeded steadily to afford a new series of 1,8-naphthyridin-2-amine in admirable yields (Table 3). The structures of all the synthesized compounds were established on the basis of their spectroscopic data.

Table 3 Synthesis of 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine in the presence of acetic acid and its derivatives

On the basis of these results, a reasonable mechanism for the synthesis of 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine is outlined in Scheme 2. The formation of product expected to proceed initial Knoevenagel condensation of 4-nitrophenyl acetonitrile 2 with aromatic aldehydes 1 to form 2-(4-nitrophenyl)-3-phenylprop-2-enenitrile 3 due to electrophilic activation. The Michael additions of 2-amino pyridine 4 with Knoevenagel product 3 to give intermediatary Michael adduct 5, then subsequently, cyclization and further air oxidation to furnish preferred product 7. The essential role of acetic acid in the reaction is to catalyze the reaction and increase the rate of reaction, which gives excellent yield of product.

Scheme 2



Plausible mechanism for reaction

Molecular docking

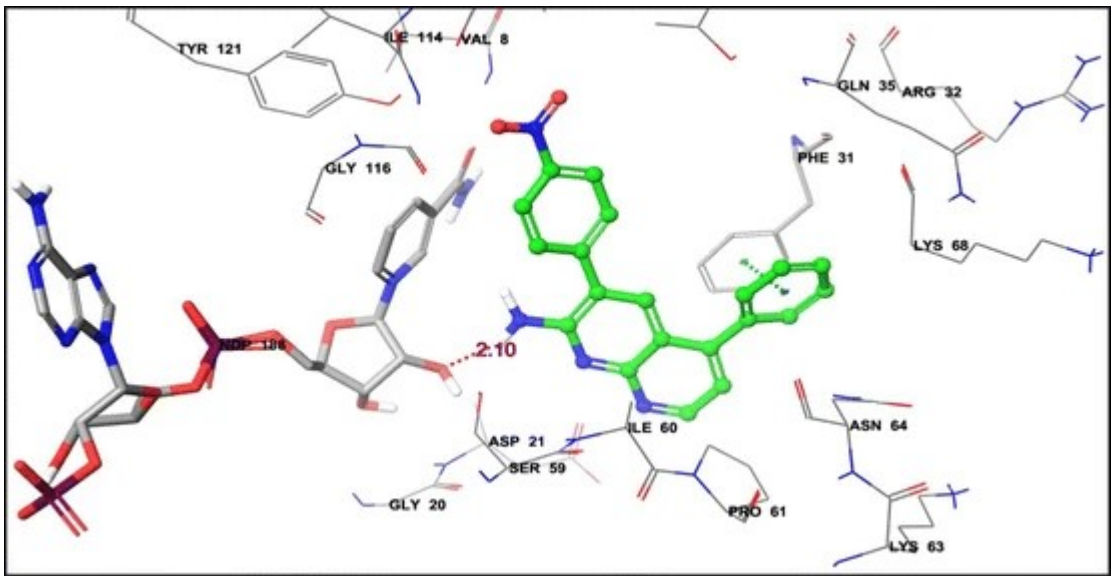
There are two critical differences between the bacterial and human DHFR active sites; Lys37 and Phe69 in the bacterial enzyme are replaced by Gln35 and Asn64, respectively, in the human enzyme [35]. The active site of DHFR comprises mostly of hydrophobic amino acids as Ile7, Val8, Trp24, Phe31, Phe34, Pro61, and Val115 [36].

The primary structure of EGFR is divided into the A, B, and C loops by the disulfide bonds Cys6–Cys20, Cys14–Cys31, and Cys33–Cys42. The A loop (residues 6–19) contains some helical structure, the B loop (residues 20–31) forms a two-stranded antiparallel sheet, and the C loop (residues 33–42) is a part of the second antiparallel sheet [37], while Asn32 located between two Cys residues, may function as a hinge between the two globules constituting EGFR [38].

On the basis of the docking study and glide score analysis, it was revealed that the molecular binding was moderate to good with the EGF receptor as compared to the DHFR enzyme

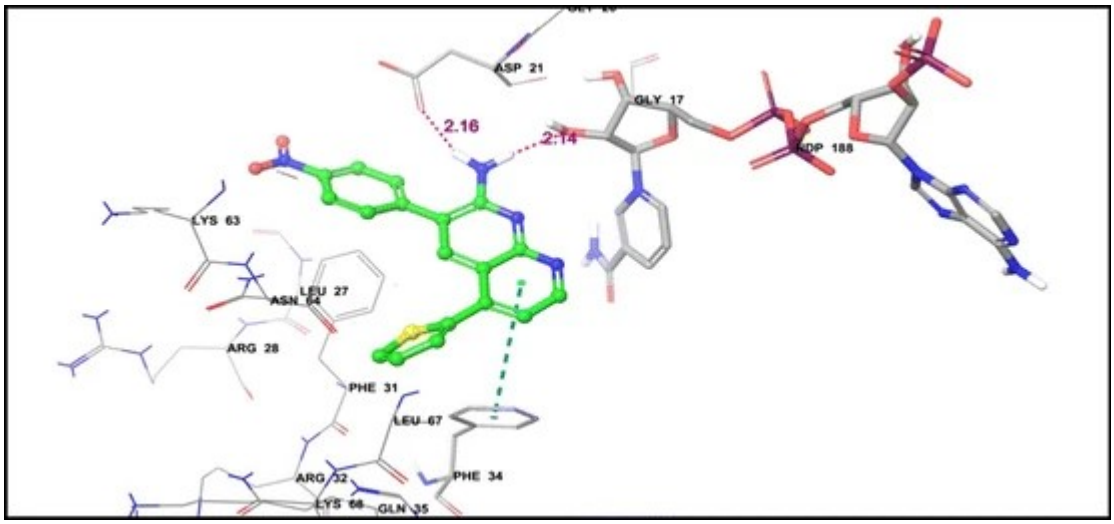
receptor. The molecular docking with DHFR is shown in Figs. 1, 2, and 3.

Fig. 1



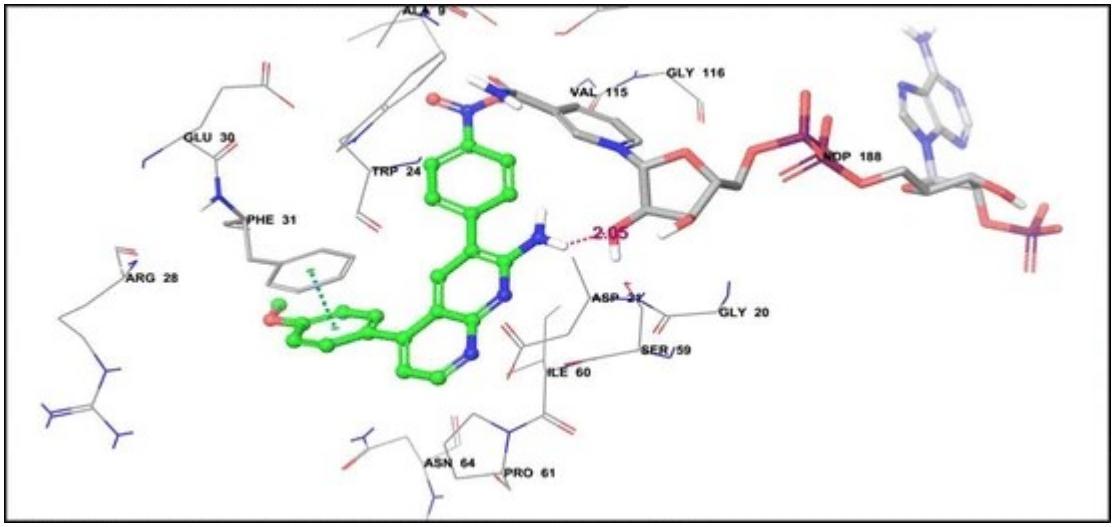
Compound 4a docked with DHFR

Fig. 2



Compound 4e docked with DHFR

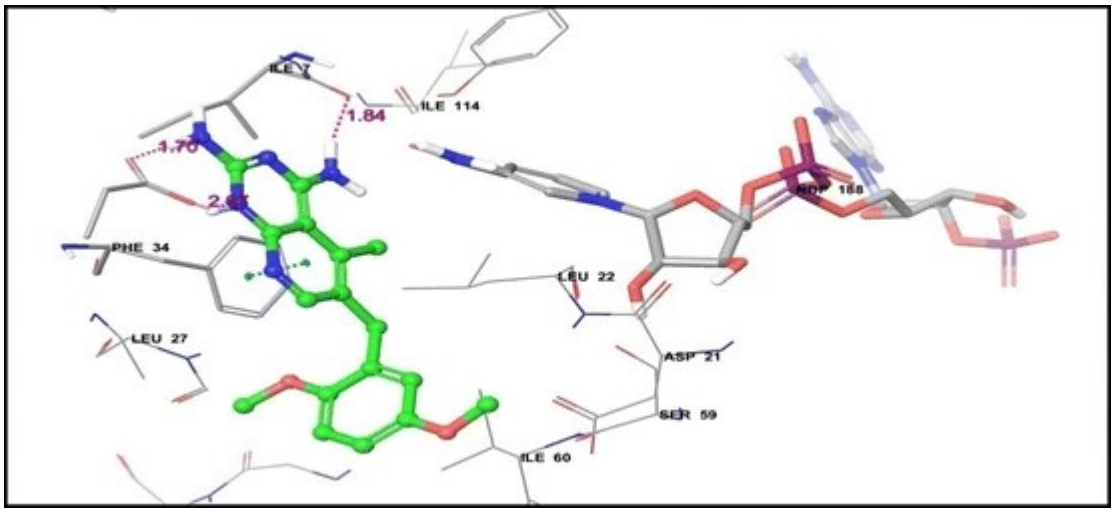
Fig. 3



Compound 4f docked with DHFR

Figure 4 shows the binding of standard drug piritrexim with a DHFR enzyme receptor, which demonstrates hydrogen bonding with Ile7 amino acid and Pi–Pi stacking interaction with Phe34. For a compound to be more potent than piritrexim, it should either be in close proximity to the co-factor NADPH or should possess some type of interaction with the same. The compounds 4a, 4e, and 4f (Figs. 1, 2, 3) show hydrogen bonding interaction with NADPH (amino acid residue Ndp188), which makes them more potent than piritrexim.

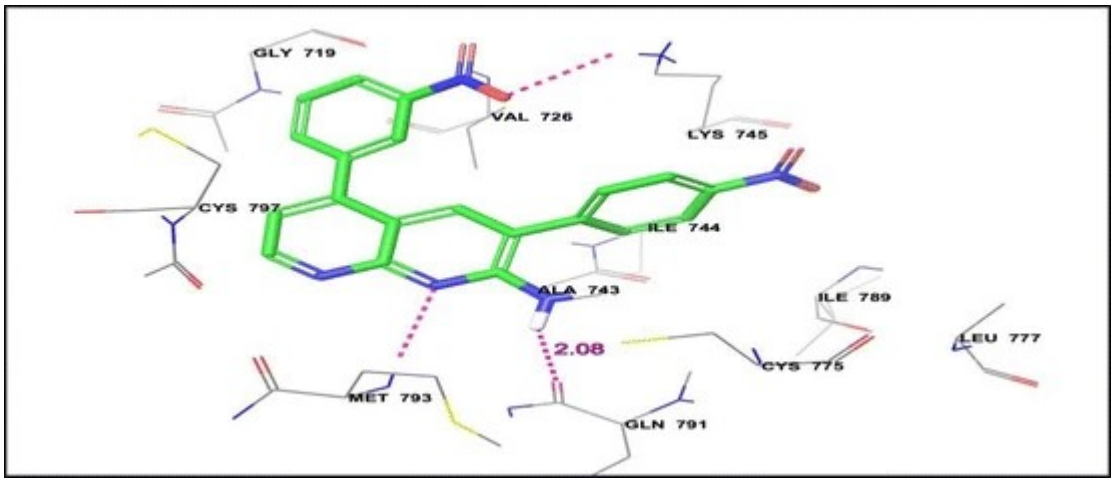
Fig. 4



Piritrexim docked on DHFR

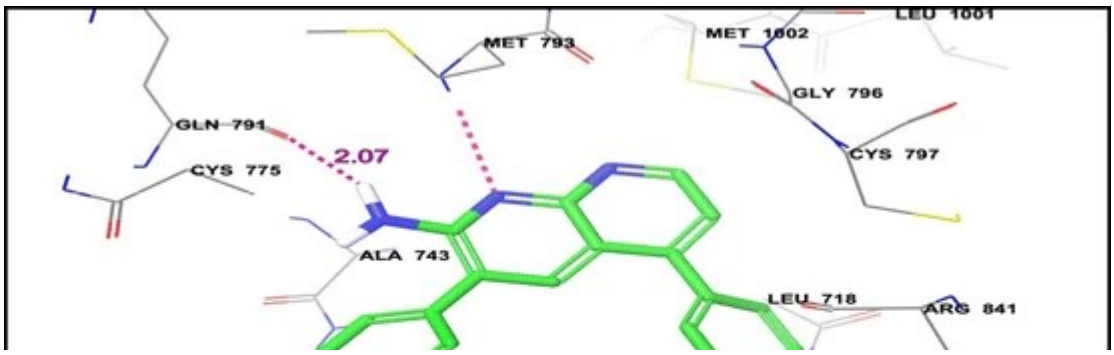
Increased glide scores were observed with EGF receptor docking study. The molecular docking with EGFR is shown in Figs. 5 and 7. The hydrogen bonding interaction is seen with amino acid residues Gln791, Lys745, and Met793 in compounds 4b and 4c (Figs. 5, 6) while no interaction with the mentioned amino acids was illustrated by compound 4f, which in turn showed decreased glide score (Fig. 7). The details of the molecular docking study are shown in Table 4.

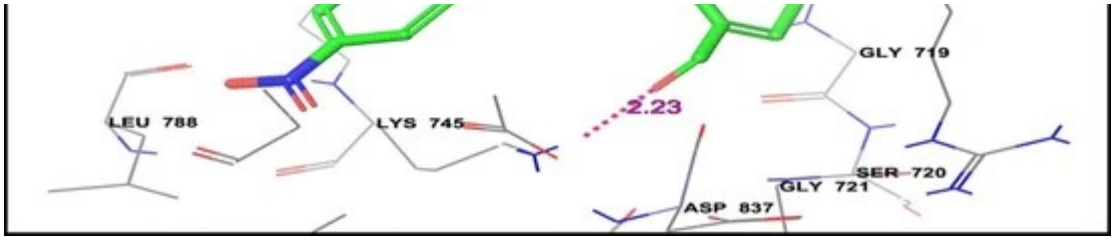
Fig. 5



Compound 4b docked on EGFR

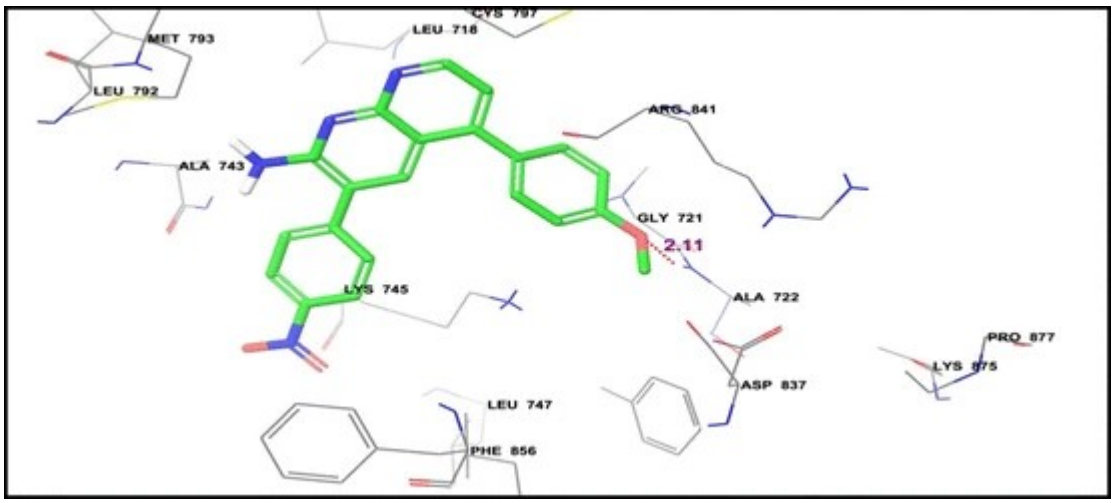
Fig. 6





Compound 4c docked on EGFR

Fig. 7



Compound 4f docked on EGFR

Table 4 Molecular docking of synthesized compound

We can see from the docking results (Table 4), as well as the anticancer activity results (Table 5), that the compounds that show good docking scores (compounds 4b, 4e, and 4f) when compared with the standard piritrexim (−8.890) also show excellent anticancer activity against a human breast cancer cell line. Hence, here we have tried to co-relate the similarity between the docked compounds and synthesized compounds.

Table 5 Anticancer activity of synthesized compounds on Human Breast cancer cell line MCF-7

The main difference between the interactions of EGFR and DHFR is that in DHFR the compounds show interaction with NADPH (Ndp 188 residue) whereas in EGFR the binding interaction is only with amino acid residues.

The binding modes were analyzed using the Glide XP scoring function, which is broken down into individual interaction energies (Table 4). These descriptors are viewable with the "XP Visualizer" program in Maestro. The score includes favorable energy terms such as the chemscore-lipophilic pair term (Lipophilic EvdW), hydrophobic enclosure (PhobEn), hydrophobic packing of hydrogen bonds (PhobEn HB), chemscore hydrogen-bond interactions (HBond), electrostatic interaction (Electro), and low molecular weight (Low MW). The cumulative penalty term (Penalty) includes intra-ligand contacts, polar atom burial, amide torsion, and desolvation along with the rapid site identification and ranking (Sitemap).

Moreover, the docking score is compared with the standard piritrexim. As seen from Table 4, the compounds that showed a dock score near to the score of piritrexim (−8.890) were considered active compounds. Also, with the chemscore hydrogen-bond interactions (HBond) as shown in Table 4, there is a smaller H-bond distance between the protein and the ligand, thus the interaction is good and results in excellent activity.

Biological evaluation

In vitro anticancer activity

The newly synthesized 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine derivatives were evaluated for their potential in vitro anticancer activity. The screening was done at the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India. The sulforhodamine B (SRB) assay [39, 40] was carried out to assess the anticancer effect of the compounds on human breast cancer cell line MCF-7 and a normal Vero monkey cell line

using adriamycine as positive control. The entire compounds were tested against human breast cancer cell line MCF-7. Interestingly, it was evident that 4b, 4e, and 4f exhibited potent activity with GI_{50} (<10 µg/mL) and entry 4a and 4c showed good to moderate anticancer activity with (GI_{50} 41.4 and 12.4 µg/mL). The other compounds were proved less potency compared to that of reference drug (Table 5).

In order to evaluate the anticancer activity further, the selected compounds were used on a normal Vero monkey cell line. The selected compounds were inactive towards the normal Vero monkey cell line. Overall, the comparison results of GI_{50} values for 4a, 4b, 4c, 4e, 4f indicates that the selected compound showed significantly higher influence towards human cancer cells than against the normal Vero monkey cells as evaluated with adriamycin (Table 6).

Table 6 Anticancer activity of synthesized compounds screened against normal Vero monkey cell line

Experimental section

Molecular docking

Molecular docking studies were performed in Maestro 9.1 using Glide v5.6 (Schrodinger, LLC, New York, NY, USA, 2010). All compounds were built using the Maestro build panel and optimized to lower energy conformers using Ligprep v2.4, which uses OPLS_2005 force field. The extra precision (XP) docking mode for compounds optimized by Ligprep was used on the generated grid of protein structure. The molecular docking of the synthesized compounds was performed on human dihydrofolate reductase (DHFR), as well as on epidermal growth factor receptor (EGFR). To perform the study, structures of DHFR and EGFR were taken from PDB entry 3GHW and 2RGP, respectively [41, 42].

General

All the chemicals and reagents used in synthesis were acquired commercially and used without further purification. The progress of reaction and purity of sample was checked using

silica gel 60-F 254 coated plates. Melting points were obtained by an open capillary tube apparatus and are uncorrected. The IR spectra were confirmed on Shimadzu IR-450 FT-IR spectrophotometer. The ^{1}HNMR and ^{13}CNMR spectra were performed on a Brucker Instrument at 300 MHz and 75 MHz with DMSO-d_6 as solvent and tetramethylsilane as an internal standard. The cumulative spectral data was found to be in good agreement with the proposed structures.

General procedure for the synthesis of 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine

To the mixture of 4-nitrophenyl acetonitrile (1 mmol), aromatic aldehydes (1 mmol), and 2-amino pyridine (1 mmol) in ethanol, 30 mol% of acetic acid as catalyst was added. The reaction mixture was stirred at reflux condition. The reaction was completed within 2–4 h (monitored by TLC). After completion of reaction the solid product was separated, filtered and washed with pet ether to remove impurities. The afforded product was recrystallized in ethanol to get desired product in good to excellent yield and further characterized using spectroscopic techniques IR, ^{1}HNMR, ^{13}CNMR and Mass analyses.

Conclusion

We have designed and synthesized a series of novel 3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine scaffolds, which show selective inhibitory activity on EGFR and DHFR. The most potent compounds 4b, 4e, and 4f exhibited excellent anticancer activity with GI_{50} (<10 µg/mL), and compounds 4a and 4c showed good to moderate anticancer activity with (GI_{50} 41.4 and 12.4 µg/mL) against human breast cancer cell line MCF-7 as compared to that of reference drug. Also, docking study on epidermal growth factor receptor (EGFR) reveals that compounds 4a, 4b, 4c, and 4e show moderate to good binding along with acceptable glide scores.

Spectroscopic data of the synthesized compounds

3-(4-nitrophenyl)-4-phenyl-1,8-naphthyridin-2-amine (4a)

Yellow solid; Yield: 89%, mp: 192–194 °C, IR ν_{max}: 1341, 1464, 2968, 3069 cm^{-1}; ^{1}H NMR

(300 MHz, DMSO-*d*6): δ 7.44–7.46 (t, 5H, $-H_{Ar}$),7.89–7.92 (m, 6H, $-NH_2$ and $-H_{Ar}$), 7.97 (s, 1H, $-H_{Ar}$), 8.23 (s, 1H, $-H_{Ar}$), 8.26 (s, 1H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 109.08, 117.96, 124.81, 127.49, 129.60, 130.02, 132.04, 133.64, 147.04; HRMS *m/z* calcd for $C_{20}H_{14}N_4O_2$,: 342.35076 Found: 342.37318, (Elemental Anal. calcd for $C_{20}H_{14}N_4O_2$ = C, 70.17; H, 4.12; N, 16.37; Found: C, 70.13; H, 4.08; N, 16.32%).

4-(3-nitrophenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4b)

Yellow solid; Yield: 88%; mp: 196–198 °C; IR ν_{max}: 1340, 1464, 2967, 3073 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*6): δ 7.68–7.80 (m, 2H, $-H_{Ar}$), 7.93–7.98 (t, 3H, $-H_{Ar}$), 8.12 (s, 1H, $-H_{Ar}$), 8.20–8.31 (m, 6H, $-NH_2$ and $-H_{Ar}$), 8.76 (s, 1H, $-H_{Ar}$);^{13}C NMR (300 MHz, DMSO-*d*6): δ 112.13, 116.63, 124.41, 124.46, 125.50, 127.39, 130.54, 134.87, 135.26, 139.63, 143.51, 148.11, 148.38; HRMS *m/z* calcd for $C_{20}H_{13}N_5O_4$: 387.34832 Found: 388.23638 (M + H^+) (Elemental Anal. calcd for $C_{20}H_{13}N_5O_4$ = C, 62.01; H, 3.38; N, 18.08; Found: C, 61.97; H, 3.34; N, 18.04%).

3-[2-amino-3-(4-nitrophenyl)-1,8-naphthyridin-4-yl]benzaldehyde(4c)

Yellow solid; Yield: 85%; mp: 205–207 °C; IR ν_{max}: 1365, 1434, 3073, 3108 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*6): δ 7.79–7.81 (d, 1H, $-H_{Ar}$), 8.05–8.08 (d, 4H, $-H_{Ar}$), 8.27–8.29 (d, 1H, $-H_{Ar}$), 8.34–8.40 (m, 6H, $-NH_2$ and $-H_{Ar}$), 8.47 (s, 1H, $-H_{Ar}$), 10.08 (s, 1H, CHO); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 110.61, 117.48, 124.86, 127.71, 130.20, 130.54, 132.94, 134.61, 135.22, 137.14, 140.14, 145.82, 148.09, 193.11; HRMS *m/z* calcd for $C_{21}H_{14}N_4O_3$: 370.36086 Found: 371.36985 (M + H^+) (Elemental Anal. calcd for $C_{21}H_{14}N_4O_3$ = C, 68.10; H, 3.81; N,15.13; Found: C, 68.05; H, 3.77; N, 15.09%).

4-(3-bromophenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4d)

Yellow solid; Yield: 86%; mp: 187–190 °C; IR ν_{max}:1387, 1445, 3123 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*6): δ 7.83–7.88 (t, 1H, $-H_{Ar}$), 8.03–8.06 (d, 3H, $-H_{Ar}$), 8.35–8.40 (m, 8H, $-NH_2$ and $-H_{Ar}$), 8.81(s, 1H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 110.59, 117.42, 124.86, 127.69, 128.35, 129.63, 131.47, 134.13, 135.76, 140.10, 145.28, 148.09; HRMS *m/z* calcd for $C_{20}H_{13}BrN_4O_2$: 421.24682, Found: 423.28520 (M + H^+) (Elemental Anal. calcd for $C_{20}H_{13}BrN_4O_2$ = C, 57.02; H, 3.11; N, 13.30; Found: C, 56.97; H, 3.07; N,13.26%).

3-(4-nitrophenyl)-4-(thiophen-2-yl)-1,8-naphthyridin-2-amine (4e)

Yellow solid; Yield 88%; mp: 204–206 °C; IR ν_{max}: 1373, 1405, 3046, 3091 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 7.22–7.25 (t, 2H, $-H_{Ar}$), 7.81–8.10 (m, 6H, $-NH_2$ and $-H_{Ar}$), 8.26–8.29 (d, 3H, $-H_{Ar}$), 8.43 (s, 1H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 124.46, 126.61, 128.45, 132.97, 137.48, 138.88, 140.29, 147.33; HRMS *m/z* calcd for $C_{18}H_{12}N_4O_2$:350.39436 Found: 349.28551 (M−H) (Elemental Anal. calcd for $C_{18}H_{12}N_4O_2$ = C,62.06; H, 3.47; N, 16.08; Found: C, 62.02; H, 3.42; N, 16.04%).

4-(4-methoxyphenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4f)

Yellow solid; Yield: 87%; mp: 199–201 °C; IR ν_{max}:1370, 1465, 2967, 3067, cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 2.46 (s, 3H,OCH_3), 7.32–7.35 (d, 3H, $-H_{Ar}$), 7.67 (s, 2H, $-H_{Ar}$), 7.84–7.89 (m, 4H, NH_2 and $-H_{Ar}$), 8.30–8.35 (m, 4H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 21.72, 108.18, 117.42, 124.36, 126.60, 129.86, 129.96, 130.23, 140.85, 142.69, 145.57; HRMS *m/z* calcd for $C_{21}H_{16}N_4O_3$: 372.37674 Found: 372.15905 (Elemental Anal. calcd for $C_{21}H_{16}N_4O_3$ = C, 67.73; H, 4.33; N, 15.05; Found: C,67.69; H, 4.28; N, 15.01%).

3,4-bis(4-nitrophenyl)-1,8-naphthyridin-2-amine (4g)

Yellow solid; Yield: 90%; mp: 200–202 °C; IR ν_{max}: 1391, 1465, 3063, 3181 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 7.44–7.46 (d, 3H, $-H_{Ar}$), 7.89–7.91 (d, 3H, $-H_{Ar}$), 7.92–7.93 (d, 3H, $-H_{Ar}$), 8.00 (s, 1H, $-H_{Ar}$), 8.24–8.27 (d, 3H, $-NH_2$ and $-H_{Ar}$);^{13}C NMR (300 MHz, DMSO-*d*6): δ 109.60, 117.20, 124.43, 127.18, 129.36, 131.37, 132.03, 136.88, 140.26, 144.98, 147.78; ESI *m/z* calcd for $C_{20}H_{13}N_5O_4$: 387.3494 Found MS m/z (ESI): 387.2 (Elemental Anal. calcd for $C_{20}H_{13}N_5O_4$ = C, 62.01; H, 3.38; N, 18.08; Found: C, 61.97; H, 3.34; N, 18.05%).

4-(3-chlorophenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4h)

Orange solid; Yield: 89%; mp: 215–216 °C; IR ν_{max}: 1368, 1465, 2967, 3072, cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 7.44–7.45 (d, 3H, $-H_{Ar}$), 7.85–8.01(m, 7H, $-NH_2$ and $-H_{Ar}$), 8.24–8.27 (d, 3H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): 110.61, 117.53, 124.82, 127.66, 128.39, 129.46, 131.46, 134.13, 135.66, 145.22; ESI *m/z* calcd for $C_{20}H_{13}ClN_4O_2$: 376.7963; Found MS m/z (ESI): 342.8; (Elemental Anal. calcd for $C_{20}H_{13}ClN_4O_2$ = C, 63.75; H, 3.48; N, 14.87; Found: C, 63.71; H, 3.43; N,14.83%).

4-(4-chlorophenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4i)

Yellow solid; Yield: 90%; mp: 220–222 °C, IR ν_{max}: 1391, 1464, 2967, 3071 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 7.60–7.63 (d, 2H, $-H_{Ar}$), 7.94–8.00 (t, 7H, $-H_{Ar}$), 8.22 (s, 1H, $-H_{Ar}$), 8.30–8.33 (d, 3H, $-NH_2$ and $-H_{Ar}$), ^{13}C NMR (300 MHz, DMSO-*d*6): δ 109.60, 117.28, 124.52, 127.30, 129.17, 129.42, 130.45, 131.48, 132.19, 136.75, 140.26, 145.15, 147.82; ESI *m/z* calcd for $C_{20}H_{13}ClN_4O_2$: 376.7963 Found MS m/z (ESI): 342.9; (Elemental Anal. calcd for $C_{20}H_{13}ClN_4O_2$ = C, 63.75; H, 3.48; N, 14.87; Found: C, 63.71; H, 3.44; N, 14.82%).

4-(4-bromophenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine (4j)

Yellow solid; Yield: 89%; mp: 195–197 °C; IR ν_{max}: 1338, 1484, 3061, 3098 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 8.02–8.05 (d, 2H, $-H_{Ar}$), 8.13–8.16 (d, 2H, $-H_{Ar}$), 8.33–8.36 (t, 6H, $-NH_2$ and $-H_{Ar}$), 8.38 (s, 3H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 113.06, 124.48, 124.83, 127.94, 130.99, 139.64; ESI *m/z* calcd for $C_{20}H_{13}BrN_4O_2$: 421.2473; Found MS m/z (ESI): 408.1; (Elemental Anal. calcd for $C_{20}H_{13}BrN_4O_2$ = C,57.02; H, 3.11; N, 13.30; Found: C, 56.97; H, 3.06; N, 13.26%).

3-(4-nitrophenyl)-4-(5-nitrothiophen-2-yl)-1,8-naphthyridin-2-amine (4k)

Yellow solid; Yield: 86%; mp: 200–204 °C; IR ν_{max}: 1328, 1494, 2848, 2922 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6): δ 7.82–7.83 (d, 2H, $-H_{Ar}$), 8.00–8.03 (d, 3H, $-H_{Ar}$), 8.13 (s 2H, $-H_{Ar}$), 8.31–8.33 (d, 3H, $-NH_2$ and $-H_{Ar}$), 8.57 (s, 1H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 110.20, 117.13, 124.92, 127.78, 130.31, 135.09, 138.05, 138.98, 143.09, 148.27, 153.49; ESI *m/z* calcd for $C_{18}H_{11}N_5O_4S$: 395.3930; Found: MS m/z (ESI): 339.6; (Elemental Anal. calcd for $C_{18}H_{11}N_5O_4S$ = C, 54.96; H, 2.82; N, 17.80; Found: C, 54.93; H, 2.78; N, (17.75%).

4-(4-methylphenyl)-3-(4-nitrophenyl)-1,8-naphthyridin-2-amine(4l)

Yellow solid; Yield: 85%; mp: 193–195 °C; IR ν_{max}: 1369, 1464, 2967, 3073, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$-*d*6): δ 3.91(s, 3H, CH_3), 7.02–7.05 (t, 2H, $-H_{Ar}$), 7.63 (s, 1H, $-H_{Ar}$), 7.82–7.85 (m, 4H, $-H_{Ar}$, 7.96–7.99 (t, 4H, $-NH_2$ and $-H_{Ar}$), 8.30–8.33 (m, 2H, $-H_{Ar}$); ^{13}C NMR (300 MHz, DMSO-*d*6): δ 21.70, 107.74, 117.62, 124.42, 127.04, 129.92, 130.05, 130.79, 140.81, 142.24, 146.44, 147.60; ESI *m/z* calcd for $C_{21}H_{16}N_4O_2$: 356.377; Found MS m/z (ESI): 360.3 (*M* + 4) (Elemental Anal. calcd for $C_{21}H_{16}N_4O_2$ = C, 70.77; H, 4.53; N, 15.72; Found: C, 70.72; H, 4.49; N, 15.68%).

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Acknowledgements

One of the authors (PPW) thanks the Department of Science and Technology, New Delhi, for INSPIRE Fellowship (No. DST/INSPIRE Fellowship/2013/197). We gratefully thank ACTREC, Mumbai, India, for providing anticancer screening of compounds.

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Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (DOCX 4474 kb)

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Cite this article

Warekar, P.P., Patil, K.T., Patil, P.T. *et al.* Design and synthesis of some new piritrexim analogs as potential anticancer agents. *Res Chem Intermed* 44, 749–767 (2018). https://

doi.org/10.1007/s11164-017-3132-z

Received	Accepted	Published
20 March 2017	22 August 2017	11 September 2017

Issue Date
February 2018

DOI
https://doi.org/10.1007/s11164-017-3132-z

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