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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(5): 60-66

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Synthesis, molecular docking and ADME prediction of some pyridine and pyrimidine derivatives as anti-colorectal cancer drugs

S.Gopalakrishnan*, E.Vadivel, P.Pon Sathieshkumar and M.S. Praba

Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

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ABSTRACT

Thymidylate synthase is the important enzyme used to generate thymidine monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair.. Thymidylate synthase inhibitors are chemical agents which inhibit the enzyme Thymidylate synthase and have potential as an anti-colorectal cancer chemotherapy. Pyridine and Pyrimidine derivatives are found to be the potent inhibitors of Thymidylate synthase enzyme were rapidly identified. The molecular modeling aspects of the pyridine and pyrimidine derivatives are also presented.

Keywards: Pyridine and Pyrimidine derivatives, Thymidylate synthase, molecular docking, ADME prediction.

INTRODUCTION

Cancer is inevitably one of the most studied but yet unsolved non- communicable human diseases [1]. It is an idiopathic disease and doctors and scientists are constantly trying to evolve new effective drugs for its treatment. There is no other disease which parallels cancer in diversity of its origin, nature and treatments.

Colorectal cancer is the fourth most common malignancy globally and the second leading cause of cancer deaths in Western countries, with approximately 300,000 new cases per annum diagnosed in the USA and Europe[2-4]. The three major types of therapy in Colorectal cancer are surgery, chemotherapy, and radiation therapy, each one of them applied differently, depending on whether the aim of treatment is curative or palliative. Approximately 50% of patients will ultimately die of locally advanced or metastatic disease. Only a minority of patients with metastases qualify for surgical resection. Consequently, the most widely used approach for this group is systemic or locoregional chemotherapy

combined with, where appropriate, palliative radiotherapy. Randomized trials have shown that chemotherapy improves both survival and quality of life in advanced Colorectal cancer. Treatment with 5-Flurouracil and calcium leucovorin has been the "standard" therapy for patients with Colorectal cancer for over a decade. Recently however, a number of new agents targeted against Thymidylate synthase have been synthesized and are in various stages of development. The purpose of this article is to review the currently available Thymidylate synthase inhibitors used in the treatment of advanced Colorectal cancer.

Pyridine and Pyrimidine [5-7] derivatives were associated with broad spectrum of biological activities including antituberculosis [8,9], anticonvulsant, anti-inflammatory [10], insecticidal [11,12], antifungal [13] and antitumor properties[14-16]. In the present study, two pyridine derivatives and two pyrimidine derivatives were synthesized and were docked with *Thymidylate synthase* enzyme to study the important binding orientations

EXPERIMENTAL SECTION

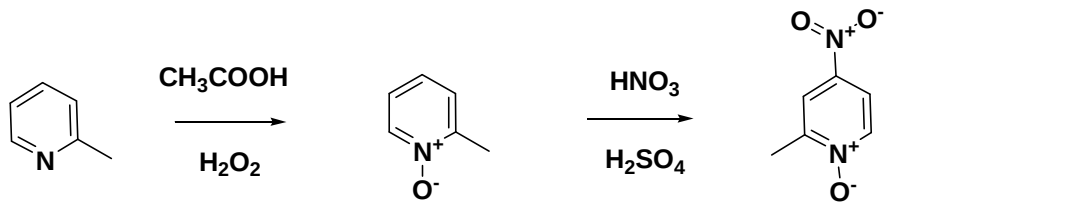
5-Bromo uracil, sodium methoxide, methanol and 2-picoline were purchased from Fischer Chemic Ltd, Phosphorus oxychloride, acetic acid, sulfuric acid, nitric acid and Hydrogen peroxide were purchased from Smilax Laboratories Ltd. N,N-Dimethyl aniline and silica gel GF254 were purchased from Merck Laboratories. All the solvents used were of commercial grade ^{1}H-NMR was recorded using Bruker NMR spectrometer, at 400 MHz in DMSO-d_6. The chemicals shifts were recorded in δ units (ppm) relative to Tetramethylsilane (TMS). The purity of the compounds was determined by HPLC using the Waters-alliance 2996 instrument. IR spectrum of the compounds was recorded using Perkin Elmer FTIR spectrometer from 4000 to 400 cm^{-1} using KBr pellet method.

The computation was carried out in Schrodinger molecular modeling software. Molecular docking was performed using the GLIDE® integrated Maestro® 7.5 interface on the Linux operating system. The molecules were subjected to predict the Pharmacokinetic or ADME properties using the Qikprop® 2.5 module. The ChemOffice 2004 software was used to draw molecular structures and the conversion of the structure to 3D and PDB files. To study the interaction here we have used PyMol and VMD viewers were used.

RESULTS AND DISCUSSION

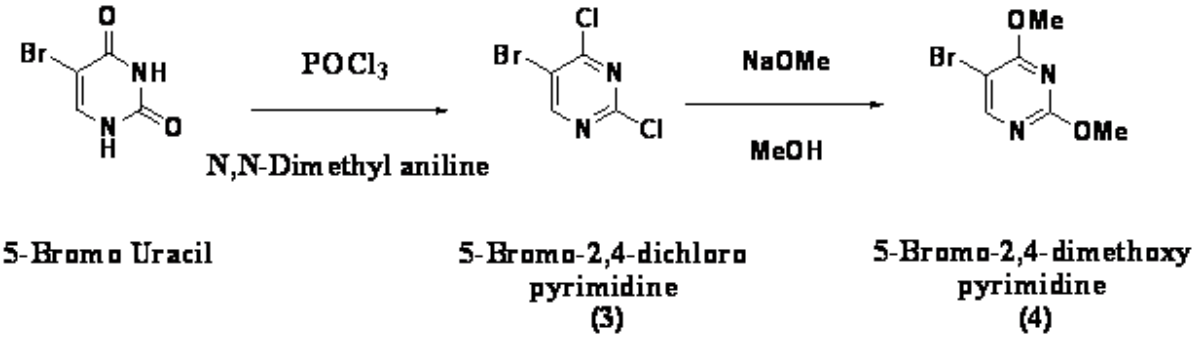
Synthesis of 2-Picoline-N-oxide (1)

The mixture of 300 g of 2-picoline and 2.6 l of acetic acid was warmed in a water bath at 65^0 C. To the mixture, 50% of hydrogen peroxide was added dropwise for 3 hr, then heated to 80° – 90°C and kept for 3 hr. The reaction mass was basified with 50% NaOH solution and was maintained the pH at 10. The reaction mixture was filtered and extracted with methylene dichloride.The organic layer was separated and evaporated under high vacuum. The colorless liquid was obtained.



2-Picoline **2-Picoline-N-oxide (1)** **4-Nitro-2-Picoline-N-Oxide (2)**

Scheme 1: Synthesis of pyridine derivatives



Scheme 2: Synthesis of Pyrimidine derivatives

Synthesis of 4-Nitro-2-picoline-N-oxide (2)

The mixture of sulphuric acid (492 ml) and 2-Picoline N-oxide (436 g) were taken in a multi-necked flask To this fuming nitric acid(667.6 ml) was added drop wise at 80^{o}C for 1 to 2 hr. Then the temperature was maintained at 90^{o}C for 4-5 hr. Then the reaction mass was quenched with crushed ice. Then the reaction mixture was basified with 50% NaOH solution to maintain the pH at 10. The reaction mixture was extracted with chloroform. The chloroform layer was separated and evaporated under high vaccum, when the yellow powder was obtained.

Synthesis of 5-Bromo-2,4-dichloro pyrimidine (3)

A mixture of 5-bromo-uracil (250 g) and N,N Dimethylaniline (300 ml) were taken in the 2 l multi-necked flask. Phosphorus oxychloride (600 ml) was added in drop wise for 1 hr at the 40°C. The temperature raised slowly and maintained at 120^{o}C for 6 hr. Then the mixture was extracted with methyl t-butyl ether. The organic layer was separated and evaporated under high vaccum. When a colorless liquid was obtained.

Synthesis of 5-Bromo-2,4-dimethoxy Pyrimidine (4)

Sodium methoxide solution was added to methanol in drop wise at room temperature in a round bottom flask. The temperature was reduced to 10°-15°C.The mixture of 30 g of 5-bromo-2,4-dichloro pyrimidine and 50 ml of methanol where taken in a R.B flask. The reaction mixture was stirred at 10°-15°C for 1 hr. The temperature was slowly raised to room temperature and was stirred for 18 hr. After 18 hr, completion of the reaction was checked by TLC. The reaction mass was filtered through celite bed. A white crude product was obtained by concentrating the filtrate

Characterization of synthesized compounds

The I.R, ^{1}H-NMR and ^{13}C-NMR of the synthesized Pyridine derivatives and Pyrimidine derivatives are presented in Table.1

Results of docking studies

The ten different orientations of the synthesized Pyridine derivatives and Pyrimidine derivatives to the receptor *Thymidylate Synthase* were carried out. The best orientation of the synthesized compounds are presented in Table.2. The hydrogen bonding and hydrophobic interactions of the best orientations are presented in Table 3 and Table 4 respectively and in Fig.1-6.

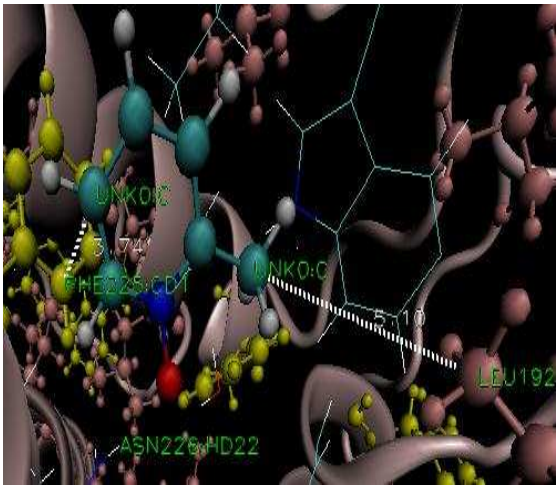


Fig 1 Hydrophobic interaction between (1) and Thymidylate synthase

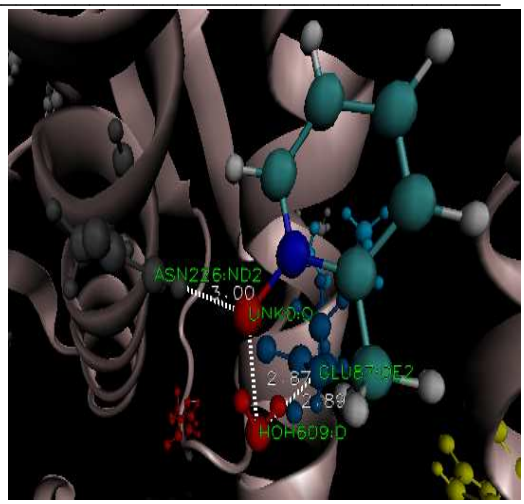


Fig.2. Hydrogen bonding interaction between (1) and Thymidylate synthase

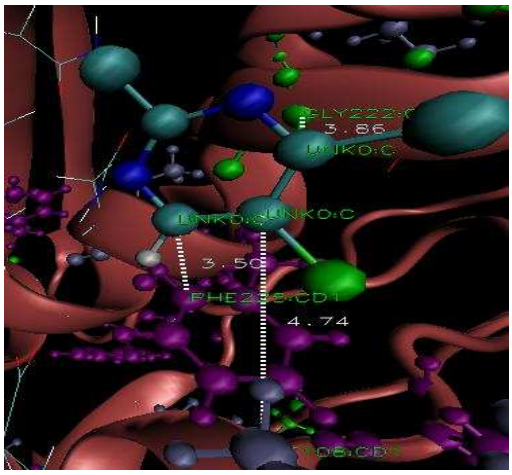


Fig. 3. Hydrophobic interaction between (2) and Thymidylate synthase

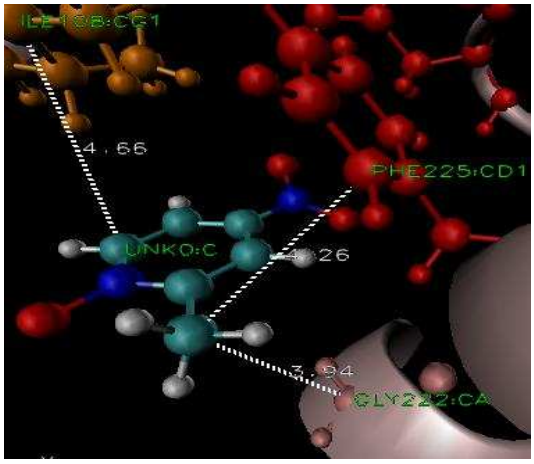


Fig 4. Hydrophobic interaction between(3) and Thymidylate synthase

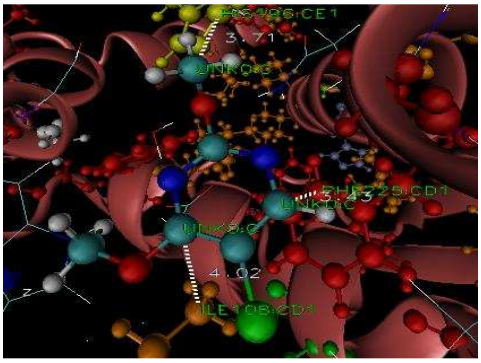


Fig. 5. Hydrophobic interaction between (4) and Thymidylate synthase

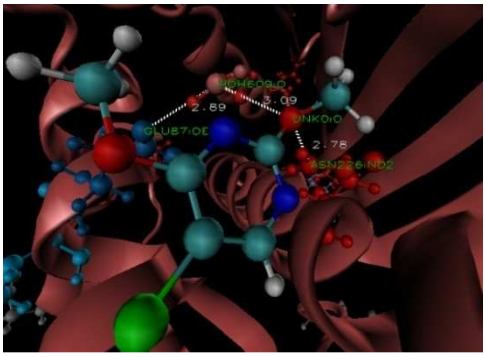


Fig .6. Hydrogen bonding interaction between (4) and Thymidylate synthase

ADME predictions of synthesized compounds

Physical descriptors and pharmaceutically relevant properties of pyridine pyrimidine derivatives were analyzed using Qikprop, significant descriptors were reported to predicting the drug-like properties of the molecules based on "Lipinski's rule of five violations". The results are presented in Table.5.

Table.1 Spectral data of the synthesized compounds

Compound	IR (v,cm^{-1})	^{1}H-NMR (DMSO-d_6)	^{13}C-NMR(ppm) (DMSO-d_6)
2-Picoline-N-oxide (1)	Aromatic C-H (3010) Aliphatic C-H (2895) N-O (1365)	2.33 (s,3H,CH_3) 7.21(m,4H, Aromatic protons)	17.94(**C**H_3) 118.0,120.56,139.95, 150.59 (5 Aromatic carbons)
4-Nitro-2-Picoline-N-Oxide (2)	Aromatic C-H (3040) Aliphatic C-H (2885) N-O (1385) -NO_2 (Asy 1550, Sym 1350)	2.40 (s,3H,CH_3) 8.05(m,3H, Aromatic proton)	22.16(**C**H_3) 119.0,120.22,137.60, 143.93 (5 Aromatic carbons)
5-Bromo-2,4-dichloro Pyrimidine(3)	Aromatic C-H (3074) C-Cl (753)	9.07 (s,1H, Aromatic proton)	121.01,160.00,164.25, 168.60 (4 Aromatic carbons)
5-Bromo-2,4-dimethoxy Pyrimidine(4)	Aromatic C-H (3050) C-Br (605)	3.78 (s,3H, -OCH_3) 3.93 (s,3H, -OCH_3) 8.47(s,1H, Aromatic proton)	54.98 (-O**C**H_3) 54.84 (-O**C**H_3) 97.45,159.31,164.25, 171.60 (4 Aromatic carbons)

Table-2 Molecular docking results of the Pyridine and Pyrimidine derivatives compared with standard 5-Fluro uracil

Compound	G Score	Glide Hbond	Glide evdW	Glide Energy	Glide Ecoul	Glide emodel	Glide Lipo
2-Picoline-N-oxide (1)	-3.83244	-0.31432	-11.7895	-8.42836	3.361098	-13.20536	-1.05839
4-Nitro-2-Picoline-N-Oxide (2)	-4.88341	0	-17.2947	-18.3524	-1.05771	-25.3559	-1.09959
5-Bromo-2,4-dichloro Pyrimidine(3)	-4.87261	-0.55378	-16.2797	-21.3505	-5.07089	-27.68656	-0.75734
5-Bromo-2,4-dimethoxy Pyrimidine(4)	-5.21799	-0.59449	-19.3524	-22.4259	-3.07357	-29.5171	-1.26586
5-Fluro uracil	-6.12146	-0.96895	-15.5581	-23.0443	-7.48624	-31.2296	-0.38644

Glide score is calculated using the following equation:

GScore = 0.065*vdW + 0.130* Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

Where,

Glide HBond - Hydrogen-bonding term
Glide evdW - Van der Waal energy
Glide Ecoul - Coulomb energy
Glide emodel - Model energy
Glide Lipo - Lipophilic contact term

Table-3 Hydrogen bonding interactions of Pyridine and Pyrimidine derivatives compared with standard 5-Fluro uracil

Compound	Atom of the compounds involving interaction	Amino acid residue involving interaction	Atom of the amino acid residue involving interaction	Type of interaction	Distance (A^o)
2-Picoline-N-oxide (1)	(i) Oxygen	ASN276	ND2	D.I	3.00
	(ii) Oxygen	GLU87	OE2	(W.M:2.87)	2.89
4-Nitro-2-Picoline-N-Oxide (2)	NO INTERACTION				
5-Bromo-2,4-dichloro Pyrimidine(3)	(i) Nitrogen (1)	ASN 226	ND2	D.I	2.85
	(ii)Nitrogen (1)	GLU 87	OE2	W.M ($3.11A^o$)	2.89
5-Bromo-2,4-dimethoxy Pyrimidine(4)	(i) Oxygen (C2-O)	ASN 226	ND2	D.I	2.78
	(iii)Oxygen (C2-O)	GLU 87	OE2	W.M (3.09)	2.89
5-Fluro Uracil (Standard)	(i) Oxygen (C2-O)	GLN 214	NE2	D.I	3.26
	(ii)Nitrogen (3)	ASN 226	OD1	D.I	3.03
	(iii)Oxygen (C4-O)	ASN 226	ND2	D.I	2.90
	(iv)Oxygen (C4-O)	GLU 87	OE2	W.M (3.01)	2.89

D.I *– Direct interaction ;* ***W.M*** *– Water mediated interaction*

Table-4 Hydrophobic interactions of Pyridine and Pyrimidine derivatives compared with that of the standard 5-Fluro uracil

Compound	Hydrophobic interaction of compounds with Thymidylate Synthase (Distance in A^o)										
	PHE 225	**ILU 109**	**ILE 108**	**HIS 196**	**GLY 222**	**TRP 109**	**LEU 192**	**LEU 221**	**CYS 195**	**ILU 221**	**TYR 109**
2-Picoline-N-oxide (1)	3.74 (C5)	3.98 (C3)	–	–	3.86 (C6)		5.10 (Me)	–	–	–	4.32 (Me)
4-Nitro-2-Picoline-N-Oxide (2)	4.26 (Me)	–	4.66 (C6)	–	3.94 (Me)	–	–	–	–	–	–
5-Bromo-2,4-dichloro Pyrimidine(3)	3.50 (C6)	–	4.74 (C5)	4.61 (C2)	3.86 (C4)	–	–	5.04 (C4)	5.18 (C2)	–	–
5-Bromo-2,4-dimethoxy Pyrimidine(4)	3.43 (C6)	–	4.02 (C4)	3.71 (C2-OMe)	3.89 (C6)	3.70 (C4-OMe)	5.32 (C4-OMe)	4.96 (C6)	–	–	–
5-Fluro Uracil (Standard)	5.94 (C4)	–	–	4.24 (C4)	4.01 (C2)	–	4.96 (C5)	–	4.67 (C4)	–	–

CONCLUSION

Molecular docking is a key tool in structural molecular biology and computer assisted drug design. The goal of ligand–protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. The present study concludes that 5-Bromo-2,4-dimethoxy Pyrimidine(4) is found to be most active against Thymidylate Synthase. Coupling of these compounds with other known antineoplastic natural products for the finding of more promising anticolorectal cancer compounds are being investigated

Table.5 ADME properties of the synthesized compounds by Qikprob

Compound name	Mol-MW	QP Log Po/w	QP Log s	QP Log BB	QP PMDCK	Human oral absorption (%)	Rule of five
2-Picoline-N-oxide (1)	109.13	1.825	-1.307	0.245	2182	100	0
4-Nitro-2-Picoline-N-Oxide (2)	154.13	1.242	-1.603	0.615	215	82	0
5-Bromo-2,4-dichloro Pyrimidine(3)	227.87	2.123	-2.122	0.767	10000	100	0
5-Bromo-2,4-dimethoxy Pyrimidine(4)	219.03	1.881	-1.490	0.354	5941	100	0

Acknowledgement

The authors are grateful to Mr. A.Dhamotharan, Chief chemist, and Mr. M. Laddasamy, R&D Manager, Fischer Chemic Ltd., Thiruvallur for allowing to carry out the project cum training. The Authors are extend sincere thanks to Dr. K. Veluraja and Mr. J. Fermin angello selwin Centre for Bioinformatics, Department of Physics, Manonmaniam Sundaranar University, Tirunelveli for utilizing softwares for molecular docking studies.

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