Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(3):26-37
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, characterization, molecular docking and evaluation of antimicrobial and antiproliferative properties of 3-substituted chromen-2-one derivatives

Rajesh B. Patil* and Sanjay D. Sawant

Sinhgad Technical Education society's, Smt. Kashibai Navale College of Pharmacy, Pune-Saswad Road, Kondhwa (Bk), Pune, Maharashtra, India

---

ABSTRACT

Many coumarin derivatives have been reported to possess antimicrobial and antiproliferative activity. In view of this series of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one derivatives and 3-[3-(substituted amino)propanoyl]-2H-chromen-2-one derivatives were synthesised and characterized by FT-IR, ^{1}H-NMR and ESI-MS spectrometry. Molecular docking was carried out on epidermal growth factor receptor protein, a good target for many cancers using Autodock vina. In vitro antimicrobial studies were carried out on two gram-positive organisms viz. Bacillus Subtilis and Staphylococcus Aureus and two gram-negative organisms viz. Escherichia Coli and Pseudomonas Aeruginosa. In vitro cytotoxicity studies were carried out on HeLa cell lines. Compounds from PYR series possess moderate antimicrobial and antiproliferative activity. PYR10, was found most active amongst synthesised compounds.

Keywords: Coumarin, pyrimidine, antimicrobial, HeLa cell lines, Autodock Vina.

INTRODUCTION

Chromenones are important class of bioactive molecules consisting of chromen-2-ones, commonly called as coumarins and chromen-4-ones comprising of flavones, isoflavones, flavanoids and their derivatives. Chromenones possess derivatives possess diverse pharmacological activities including antitumor [1, 2], antivascular [3], antimicrobial [4,5], antioxidant [6], TNF-a inhibitor [7], antifungal [8], anticoagulant[9], antispasmolytic [10,11], estrogenic [12,13], antiviral[14], anthelminthic [15], anti-HIV [16], antitubercular [17, 18], anti-inflammatory [19, 20], herbicidal [21], analgesic [22] and anticonvulsant [23,24] activity. Protein tyrosine kinases (PTKs) are key mediators of the cellular signalling cascade which performs key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis [25]. They are classified as receptor tyrosine kinases (RTKs) and non receptor tyrosine kinases (NRTKs). RTK's like epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and nerve growth factor (NGF) have been identified as targets in various cancers. Genistein, a soya isoflavone, is well known protein tyrosine kinase (PTK) inhibitor which inhibits EGFR autophosphorylation at 2.6 µM concentration [26, 27]. Genistein also possess topoisomerase II and antioxidant activity [28, 29]. Coumarin derivatives like Daphnetin have been identified as EGFR-PTK inhibitors [30]. In view of this series of 3-substituted coumarin derivatives were synthesized. Heteroaryl ring and acyclic hetero atom containing chain at 3rd position of coumarin has been investigated in current work as shown in Figure 1. Computer docking technique helps in finding the important binding modes of ligand with its target protein. The analysis of important interactions like hydrogen bonds formed with important residues, hydrophobic interactions facilitate drug design process. In the present paper, we report the synthesis, characterization and docking studies of 3-substituted Coumarin derivatives. The antiproliferative activity of synthesized compounds was investigated on HeLa cell lines (human cervical adenocarsinoma cell lines) by Sulfo

Rhodamine B (SRB) assay. The antimicrobial activity was investigated on two gram-positive organisms *viz. Bacillus Subtilis* and *Staphylococcus Aureus* and two gram-negative organisms *viz. Escherichia Coli* and *Pseudomonas Aeruginosa*. The results of docking studies, antimicrobial activity and antiproliferative activities of synthesised compounds are reported in this paper.

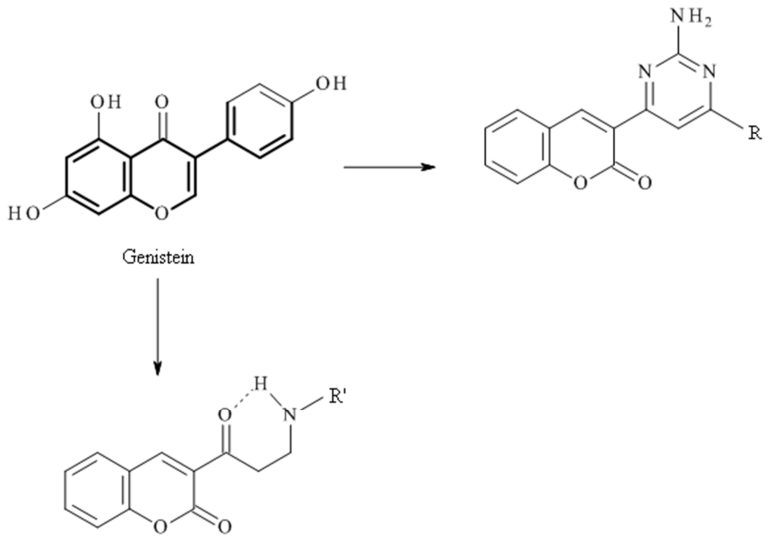
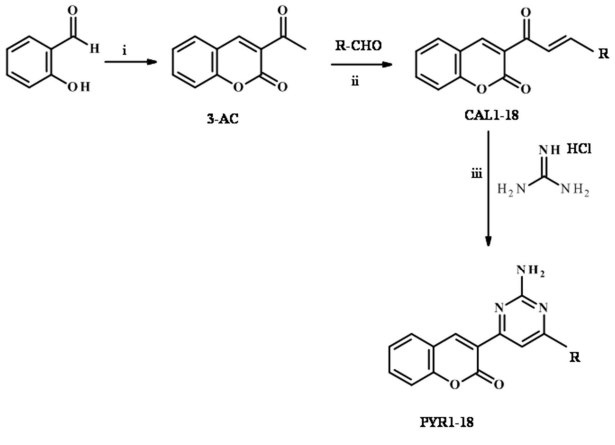


Figure 1: Rational behind synthesis of 3-substituted coumarins

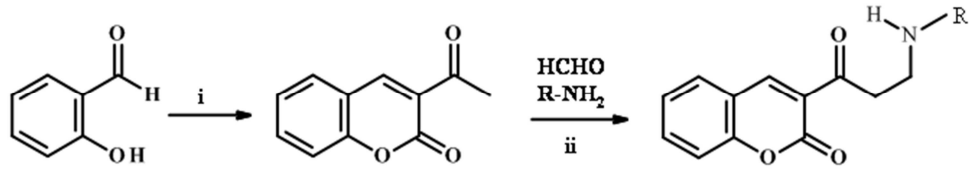
MATERIALS AND METHODS

Materials

The reagents used for synthesis were of laboratory grade and solvents were of analytical grade obtained from Thomas Baker and Loba Chemie respectively. The melting point of the compound was determined by open capillary method, expressed in °C. The reactions were monitored by preparative TLC's from Merck with the solvent system chloroform: methanol in the ratio of 9:1. Infra Red spectra were recorded on Shimadzu FT-IRAffinity-1 spectrophotometer by KBr pellet technique and are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker Avance 300 MHz FT-NMR spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts are expressed in δ ppm and splitting patterns are designated as s: singlet; d: doublet; q: quartet; m: multiplet. Mass spectra were recorded using Waters Quatropole Electrospray Mass Spectrophotometer. Scheme 1 & 2 depicts the strategy adopted to synthesize the compounds.



Scheme of synthesis 1: Synthesis of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one derivatives; (i) Ethylacetoacetate, piperidine, stirring; (ii) 0.2 ml piperidine, 90°C, stirring; (iii) Ethanol, Reflux, 5-6 hrs



R = -N(CH$_3$)$_2$, Ph, *p*-Cl Ph, 2,4-dinitro Ph, *p*-CH$_3$ Ph, *p*-NO$_2$ Ph, *o*-NO$_2$ Ph, *p*-OCH$_3$ Ph, *p*-OH Ph, 1-naphtyl, 2-pyrazinyl, 2-pyrimidinyl, 2-pyridinyl, *p*-COOH Ph, *o*-COOH Ph, 4-Br 2-COOH Ph, 2,6 dimethyl Ph, *o*-OCH$_3$

Scheme of synthesis 2: Synthesis of 3-[3-(substituted amino)propanoyl]-2H-chromen-2-one derivatives; (i) Ethylacetoacetate, Piperidine, stirring; (ii) Paraformaldehyde, substituted amines, conc. HCl, absolute ethanol, reflux 3-4 hrs

Synthesis

General procedure for the synthesis of 3-acetylcoumarin

To the mixture of salicyladehyde (18 mmol) and ethylacetoacetate (24 mmol) in 5 ml absolute ethanol, 0.2 ml piperidine was added with stirring. Stirring was continued till complete crystallization. The solid obtained was filtered and recrystallized from ethanol. Pale yellow solid, m.p 118 – 119 ^{0}C. Yield 70%.

General procedure for the synthesis of 3-[3-substituted prop-2-enoyl]-2H-chromen-2-one (CAL1-18)

The mixture of appropriate aromatic aldehydes (0.03 mol) and 3AC (0.01 mol) in 5 ml absolute ethanol was stirred and maintained at 45-50 ^{0}C. Piperidine (0.2 ml) was added drop wise with stirring until slight turbidity persists in the solution. The mixture was allowed to cool and solid obtained was filtered and washed with ethanol. The crude products were recrystallized from ethanol to afford CAL1-18 with different colors. Yield 50 – 80 %.

General procedure for the synthesis of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one derivatives (PYR1-18)

The mixture of CAL1-18 (1.8 mmol) and guanidine hydrochloride (1.8 mmol) in 15 ml absolute ethanol was refluxed for 5-6 hrs. The progress of reaction was monitored by TLCs using chloroform: methanol (9:1) mobile phase. The reaction mixture was neutralized with aqueous ammonia solution or dil. HCl. The solid obtained was filtered and recrystallized from ethanol.

General procedure for the synthesis of 3-[3-(substituted amino)propanoyl]-2H-chromen-2-one derivatives (MAN1-18)

Substituted anilines (5 mmol), paraformaldehyde (6 mmol) and 3AC (5 mmol) in 15 ml absolute ethanol was stirred for 3 – 4 hrs. The mixture was neutralized with aqueous ammonia. The solid obtained was filtered and recrystallized from ethanol.

Synthesis of 3-(2-amino-6-phenylpyrimidin-4-yl)-2H-chromen-2-one (PYR1)

Gray coloured solid, m.p. 150-152^{0}C. Yield 73%, Mol.Wt. 315.3. IR (KBr) ν (cm^{-1}): 1450 and 1500 (C-C in Ar), 3100 (C-H in Ar-H), 1320 (Ar-O-C), 1728 (C=O), 1610 (-C=N), 3500 (N-H). ^{1}H NMR (CDCl$_3$): (ppm) 1.58 (2H, s, NH$_2$), 7.39-7.93 (10H, m, Ar-H), 8.59 (1H, s, Pyran-H). MS: m/z = 340.4 (M$^+$ + Na + 2H)

Synthesis of 3-[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR2)

Pale yellow coloured solid, m.p. 169-171^{0}C. Yield 87%, Mol.Wt. 331.3. IR (KBr) ν (cm^{-1}): 1441 and 1516 (C-C in Ar), 3100 (C-H in Ar-H), 1280 (Ar-O-C), 1701 (C=O), 1188 (C-O in Ar-OH), 1602 (-C=N), 3500 (N-H). ^{1}H NMR (CDCl$_3$): (ppm) 3.46 (2H, s, NH$_2$), 6.85-8.14 (9H, m, Ar-H), 8.58 (1H, s, Pyran-H), 5.29 (1H, s, OH). MS: m/z = 356.4 (M$^+$ + Na + 2H)

Synthesis of 3-[2-amino-6-(4-hydroxy-3-methoxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR3)

Dark yellow coloured solid, m.p. 135-137^{0}C. Yield 65%, Mol. Wt. 361.35. IR (KBr) ν (cm^{-1}): 1424 and 1515 (C-C in Ar), 3100 (C-H in Ar-H), 1285 (Ar-O-C), 1718 (C=O), 1180 (C-O in Ar-OH), 1683 (-C=N), 3500 (N-H). ^{1}H NMR (CDCl$_3$): (ppm) 1.8 (2H, s, NH$_2$), 3.96 (3H, s, -OCH$_3$), 6.93-7.81 (8H, m, Ar-H), 8.57 (1H, s, Pyran-H), 2.8 (1H, s, OH). MS: m/z = 386.3 (M$^+$ + Na + 2H)

Synthesis of 3-[2-amino-6-[4-(dimethylamino)phenyl]pyrimidin-4-yl]-2H-chromen-2-one (PYR4)
Dark red coloured solid, m.p. 180-182^{0}C. Yield 80%, Mol. Wt. 358.39. IR (KBr) ν (cm^{-1}): 1420 and 1525 (C-C in Ar), 3100 (C-H in Ar-H), 1280 (Ar-O-C), 1710 (C=O), 1185 (C-O in Ar-OH), 1681 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.57 (2H, s, NH_2), 7.27-8.17 (8H, m, Ar-H), 8.57 (1H, s, Pyran-H)

Synthesis of 3-[2-amino-6-(4-chlorophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR5)
Pale yellow coloured solid, m.p. 110-112^{0}C. Yield 45%, Mol. Wt. 349.77. IR (KBr) ν (cm^{-1}): 1400 and 1500 (C-C in Ar), 3100 (C-H in Ar-H), 1250 (Ar-O-C), 1718 (C=O), 1610 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, NH_2), 7.34-7.97 (9H, m, Ar-H), 8.60 (1H, s, Pyran-H). MS: m/z = 374.3 (M^+ + Na + 2H)

Synthesis of 3-[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR6)
Dark yellow coloured solid, m.p. 180-182^{0}C. Yield 77%, Mol. Wt. 360.32. IR (KBr) ν (cm^{-1}): 1440 and 1510 (C-C in Ar), 3100 (C-H in Ar-H), 1280 (Ar-O-C), 1700 (C=O), 1600 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, NH_2), 7.34-7.97 (9H, m, Ar-H), 8.60 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(furan-2-yl)pyrimidin-4-yl]-2H-chromen-2-one (PYR7)
Off white solid, m.p. 152-154^{0}C. Yield 69%, Mol. Wt. 305.28. IR (KBr) ν (cm^{-1}): 1454 and 1557 (C-C in Ar), 3100 (C-H in Ar-H), 1291 (Ar-O-C), 1728 (C=O), 1682 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 4.19 (2H, s, NH_2), 6.51-7.81 (7H, m, Ar-H), 8.55 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(2-hydroxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR8)
Pale yellow coloured solid, m.p. 140-142^{0}C. Yield 50%, Mol. Wt. 331.32. IR (KBr) ν (cm^{-1}): 1456 and 1560 (C-C in Ar), 3100 (C-H in Ar-H), 1227 (Ar-O-C), 1714 (C=O), 1209 (C-O in Ar-OH), 1607 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 4.25 (2H, s, NH_2), 7.01-8.58 (8H, m, Ar-H), 8.67 (1H, s, Pyran-H), 2.89 (1H, s, OH). MS: m/z = 417.3 (M^+ + Isopropyl + Na +3H)

Synthesis of 3-[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR9)
Pale yellow coloured solid, m.p. 173-175^{0}C. Yield 65%, Mol. Wt. 345.35. IR (KBr) ν (cm^{-1}): 1400 and 1513 (C-C in Ar), 3100 (C-H in Ar-H), 1241 (Ar-O-C), 1718 (C=O), 1605 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.57 (2H, s, NH_2), 6.92-7.85 (8H, m, Ar-H), 8.59 (1H, s, Pyran-H), 3.87 (3H, s, OCH_3). MS: m/z = 370.4 (M^+ + Na + 2H)
Synthesis of 3-[2-amino-6-(3,4-dimethoxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR10)

Pale yellow coloured solid, m.p. 118-120^{0}C. Yield 70%, Mol. Wt. 375.37. IR (KBr) ν (cm^{-1}): 1400 and 1513 (C-C in Ar), 3100 (C-H in Ar-H), 1257 (Ar-O-C), 1730 (C=O), 1184 (C-O in Ar-OH), 1609 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.59 (2H, s, NH_2), 6.88-7.84 (7H, m, Ar-H), 8.59 (1H, s, Pyran-H), 3.94 & 3.96 (6H, s, OCH_3).

Synthesis of 3-[2-amino-6-(2-nitrophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR11)
Pale brown coloured solid, m.p. 183-185^{0}C. Yield 50%, Mol. Wt. 360.32. IR (KBr) ν (cm^{-1}): 1448 and 1507 (C-C in Ar), 3100 (C-H in Ar-H), 1257 (Ar-O-C), 1718 (C=O), 1345 and 1521 (N=O), 1607 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.57 (2H, s, NH_2), 6.37-8.50 (9H, m, Ar-H), 8.62 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(3-nitrophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR12)
Orange coloured solid, m.p. 147-149^{0}C. Yield 67%, Mol. Wt. 360.32. IR (KBr) ν (cm^{-1}): 1487 and 1507 (C-C in Ar), 3100 (C-H in Ar-H), 1276 (Ar-O-C), 1718 (C=O), 1347 and 1507 (N=O), 1662 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.57 (2H, s, NH_2), 6.37-8.50 (9H, m, Ar-H), 8.62 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(2-bromophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR13)
Pale yellow coloured solid, m.p. 167-169^{0}C. Yield 47%, Mol. Wt. 394.22. IR (KBr) ν (cm^{-1}): 1450 and 1560 (C-C in Ar), 3100 (C-H in Ar-H), 1285 (Ar-O-C), 1715 (C=O), 1660 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, NH_2), 7.34-7.97 (9H, m, Ar-H), 8.60 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(3-bromophenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR14)
Pale brown coloured solid, m.p. 133-135^{0}C. Yield 51%, Mol. Wt. 394.22. IR (KBr) ν (cm^{-1}): 1455 and 1559 (C-C in Ar), 3100 (C-H in Ar-H), 1284 (Ar-O-C), 1715 (C=O), 1662 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, NH_2), 7.34-7.97 (9H, m, Ar-H), 8.60 (1H, s, Pyran-H).

Synthesis of 3-[2-amino-6-(5-chloro-2-hydroxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR15)
Pale brown coloured solid, m.p. 109-111^{0}C. Yield 59%, Mol. Wt. 365.77. 1 IR (KBr) ν (cm^{-1}): 456 and 1559 (C-C in Ar), 3100 (C-H in Ar-H), 1272 (Ar-O-C), 1726 (C=O), 1181 (C-O in Ar-OH), 1607 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.55 (2H, s, NH_2), 6.70-7.66 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.72 (1H, s, OH).

Synthesis of 3-(2-aminopyrimidin-4-yl)-2H-chromen-2-one (PYR16)
Pale yellow coloured solid, m.p. 90-92^{0}C. Yield 61%, Mol. Wt. 239.22. IR (KBr) ν (cm^{-1}): 1456 and 1586 (C-C in Ar), 3100 (C-H in Ar-H), 1290 (Ar-O-C), 1729 (C=O), 1608 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 4.16 (2H, s, NH_2), 6.82-7.67 (6H, m, Ar-H), 8.50 (1H, s, Pyran-H). MS: m/z = 575.4 ($2M^+$ +4Na + 4H)

Synthesis of 3-[2-amino-6-(3-hydroxyphenyl)pyrimidin-4-yl]-2H-chromen-2-one (PYR17)
Pale brown coloured solid, m.p. 122-125^{0}C. Yield 63%, Mol. Wt. 331.32. IR (KBr) ν (cm^{-1}): 1488 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1288 (Ar-O-C), 1722 (C=O), 1184 (C-O in Ar-OH), 1639 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 4.22 (2H, s, NH_2), 6.89-7.92 (6H, m, Ar-H), 8.58 (1H, s, Pyran-H), 3.69 (1H, s, OH). MS: m/z = 356.3 (M^+ + Na +H)

Synthesis of 3-[2-amino-6-[(1E)-prop-1-en-1-yl]pyrimidin-4-yl]-2H-chromen-2-one (PYR18)
Pale brown coloured solid, m.p. 182-184^{0}C. Yield 45%, Mol. Wt. 279.29. IR (KBr) ν (cm^{-1}): 1488 and 1608 (C-C in Ar), 3100 (C-H in Ar-H), 1229 (Ar-O-C), 1729 (C=O), 1608 (-C=N), 3500 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.52 (2H, s, NH_2), 6.82-7.66 (5H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.8 (3H, d, allylic CH_3), 4.16 (2H, m, -CH=CH-).

Synthesis of 3-[3-(dimethylamino)propanoyl]-2H-chromen-2-one (MAN1)
Off white solid, m.p. 127-129^{0}C Mol. Wt. 245.27 IR (KBr) ν (cm^{-1}): 1454 and 1557 (C-C in Ar), 3100 (C-H in Ar-H), 1265 (Ar-O-C), 1733 (C=O), 1210 (-C=N), 3030 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.25 (1H, s, N-H), 7.31-7.88 (4H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-), 2.89 (6H, s, -CH_3). MS: m/z = 529.3 ($2M^+$ + K)

Synthesis of 3-[3-(phenylamino)propanoyl]-2H-chromen-2-one (MAN2)
Orange coloured solid, m.p. 145-147^{0}C Mol. Wt. 293.31 IR (KBr) ν (cm^{-1}): 1454 and 1557 (C-C in Ar), 3100 (C-H in Ar-H), 1240 (Ar-O-C), 1741 (C=O), 1210 (-C-N), 3030 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.25 (1H, s, N-H), 7.31-7.88 (8H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 294.4 (M^+ +H)

Synthesis of 3-[3-[(4-chlorophenyl)amino]propanoyl]-2H-chromen-2-one (MAN3)
Cream coloured solid, m.p. 117-119^{0}C Mol. Wt. 327.76 IR (KBr) ν (cm^{-1}): 1454 and 1559 (C-C in Ar), 3100 (C-H in Ar-H), 1227 (Ar-O-C), 1726 (C=O), 1203 (-C-N), 3030 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.43 (1H, t, N-H), 6.54-7.66 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.72 (4H, s, -CH_2-). MS: m/z = 686.2 ($2M^+$ + $3H_2O$ + 5H)

Synthesis of 3-[3-[(2,4-dinitrophenyl)amino]propanoyl]-2H-chromen-2-one (MAN4)
Pale yellow coloured solid, m.p. 142-145^{0}C Mol. Wt. 383.31 IR (KBr) ν (cm^{-1}): 1507 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1275 (Ar-O-C), 1731 (C=O), 1210 (-C-N), 3321 (N-H) 1329 and 1558 (N-O). ^{1}H NMR ($CDCl_3$): (ppm) 6.74 (1H, s, N-H), 6.91-8.23 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 763.1 ($2M^+$ + H)

Synthesis of 3-[3-[(4-methylphenyl)amino]propanoyl]-2H-chromen-2-one (MAN5)
Cream coloured solid, m.p. 163-165^{0}C Mol. Wt. 307.34 IR (KBr) ν (cm^{-1}): 1450 and 1550 (C-C in Ar), 3100 (C-H in Ar-H), 1275 (Ar-O-C), 1739 (C=O), 1210 (-C-N), 3320 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.41 (1H, t, N-H), 6.55-7.67 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.72 (4H, s, -CH_2-), 2.20 (3H, s, CH_3).

Synthesis of 3-[3-[(4-nitrophenyl)amino]propanoyl]-2H-chromen-2-one (MAN6)
Pale yellow coloured solid, m.p. 187-189^{0}C Mol. Wt. 338.31 IR (KBr) ν (cm^{-1}): 1454 and 1555 (C-C in Ar), 3100 (C-H in Ar-H), 1265 (Ar-O-C), 1728 (C=O), 1109 (-C-N), 3368 (N-H), 1320 and 1602 (N-O). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.66 (8H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 362.4 (M^+ + Na)

Synthesis of 3-[3-[(2-nitrophenyl)amino]propanoyl]-2H-chromen-2-one (MAN7)
Brownish coloured solid, m.p. 159-161^{0}C Mol. Wt. 338.31 IR (KBr) ν (cm^{-1}): 1454 and 1573 (C-C in Ar), 3100 (C-H in Ar-H), 1265 (Ar-O-C), 1739 (C=O), 1210 (-C-N), 3373 (N-H), 1344 and 1507 (N-O). ^{1}H NMR ($CDCl_3$): (ppm) 6.09 (1H, s, N-H), 6.66-8.42 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.72 (4H, s, -CH_2-).

Synthesis of 3-[3-[(4-methoxyphenyl)amino]propanoyl]-2H-chromen-2-one (MAN8)
Brownish coloured solid, m.p. 123-125^{0}C Mol. Wt. 323.34 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1264 (Ar-O-C), 1738 (C=O), 1210 (-C-N), 3386 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.40 (1H, s, N-H), 6.60-7.68 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-), 3.72 (3H, s, -OCH_3).

Synthesis of 3-[3-[(4-hydroxyphenyl)amino]propanoyl]-2H-chromen-2-one (MAN9)
Faint brownish coloured solid, m.p. 113-115^{0}C Mol. Wt. 309.31 IR (KBr) ν (cm^{-1}): 1454 and 1562 (C-C in Ar), 3100 (C-H in Ar-H), 1265 (Ar-O-C), 1738 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.34-7.68 (8H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-), 7.32 (1H, s, -OH). MS: m/z = 310.31 (M^+ + H)

Synthesis of 3-[3-[(naphthalen-1-yl)amino]propanoyl]-2H-chromen-2-one (MAN10)
Reddish brown coloured solid, m.p. 201-203^{0}C Mol. Wt. 343.37 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1230 (Ar-O-C), 1734 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 4.5 (1H, s, N-H), 7.30-7.67 (11H, m, Ar-H), 8.49 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 381.6 (M^+ + K)

Synthesis of 3-[3-[(pyrazin-2-yl)amino]propanoyl]-2H-chromen-2-one (MAN11)
White solid, m.p. 197-199^{0}C Mol. Wt. 295.29 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1230 (Ar-O-C), 1740 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 294.5 (M^+)

Synthesis of 3-[3-[(pyrimidin-2-yl)amino]propanoyl]-2H-chromen-2-one (MAN12)
Off white solid, m.p. 189-191^{0}C Mol. Wt. 295.29 IR (KBr) ν (cm^{-1}): 1450 and 1500 (C-C in Ar), 3100 (C-H in Ar-H), 1231 (Ar-O-C), 1740 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 294.5 (M^+)

Synthesis of 3-[3-[(pyridin-2-yl)amino]propanoyl]-2H-chromen-2-one (MAN13)
Off white solid, m.p. 181-183^{0}C Mol. Wt. 294.30 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1230 (Ar-O-C), 1740 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (8H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-). MS: m/z = 319.5 (M^+ + Na +2H)

Synthesis of 4-[[3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl]amino]benzoic acid (MAN14)
White solid, m.p. 133-135^{0}C Mol. Wt. 337.32 IR (KBr) ν (cm^{-1}): 1453 and 1557 (C-C in Ar), 3100 (C-H in Ar-H), 1231 (Ar-O-C), 1738 (C=O), 1210 (-C-N), 3350 (N-H), 3500 (O-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-).

Synthesis of 2-[[3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl]amino]benzoic acid (MAN15)
Pale yellow coloured solid, m.p. 147-149^{0}C Mol. Wt. 337.32 IR (KBr) ν (cm^{-1}): 1400 and 1525 (C-C in Ar), 3100 (C-H in Ar-H), 1225 (Ar-O-C), 1740 (C=O), 1200 (-C-N), 3300 (N-H), 3500 (O-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-).

Synthesis of 5-bromo-2-[[3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl]amino]benzoic acid (MAN16)
Brownish solid, m.p. 104-106^{0}C Mol. Wt. 416.22 IR (KBr) ν (cm^{-1}): 1454 and 1559 (C-C in Ar), 3100 (C-H in Ar-H), 1232 (Ar-O-C), 1741 (C=O), 1210 (-C-N), 3300 (N-H), 3500 (O-H). ^{1}H NMR ($CDCl_3$): (ppm) 1.60 (1H, s, N-H), 7.32-7.68 (7H, m, Ar-H), 8.51 (1H, s, Pyran-H), 2.73 (4H, s, -CH_2-).

Synthesis of 3-{3-[(2,6-dimethylphenyl)amino]propanoyl}-2H-chromen-2-one (MAN17)
White solid, m.p. 129-131^{0}C Mol. Wt. 321.36 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1231 (Ar-O-C), 1739 (C=O), 1210 (-C-N), 3300 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 6.76 (1H, s, N-H), 7.31-7.68 (7H, m, Ar-H), 8.50 (1H, s, Pyran-H), 2.72 (4H, s, -CH_2-), 2.13 (6H, s, -CH_3).

Synthesis of 3-{3-[(2-methoxyphenyl)amino]propanoyl}-2H-chromen-2-one (MAN18)
Pale yellow solid, m.p. 151-153^{0}C Mol. Wt. 323.34 IR (KBr) ν (cm^{-1}): 1454 and 1558 (C-C in Ar), 3100 (C-H in Ar-H), 1231 (Ar-O-C), 1739 (C=O), 1210 (-C-N), 3030 (N-H). ^{1}H NMR ($CDCl_3$): (ppm) 3.46 (1H, s, N-H), 6.57-7.67 (8H, m, Ar-H), 8.49 (1H, s, Pyran-H), 2.72 (4H, s, -CH_2-), 2.07 (3H, s, -CH_3).

Evaluation of antimicrobial activity
The antimicrobial activity of all the synthesized compounds was examined against different Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) by measuring zone of inhibition. The antimicrobial activity was carried out by agar cup plate method at the concentration level 25 µg/ml. Ofloxacin was used as standard at concentration 25 µg/ml. Nutrient agar was used as culture media for antibacterial activity. Twenty four hrs old culture of bacterial pathogen was placed in nutrient agar and spread throughout the plate by spread plate technique. Wells were bored using sterile borer at equidistance. The plates were kept at room temperature for 30 minutes. The test compounds, standard and control were placed in respective wells and plates were incubated at 37^{0}C for 36 hrs. Zone of inhibition was measured by zone reader.

***In vitro* cytotoxicity activity by SRB Assay**

In vitro cytotoxicity activity of selected compounds was performed on HeLa cancer cell lines at Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. The cell viability was measured by SRB assay with triplicate measurements. Dimethylsulphoxide (DMSO) was used as a solvent. Briefly the SRB assay protocol included growing the cell lines in RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96 well micro titer plates in 90 µl at plating densities. After cell inoculation, the micro titer plates were incubated at 37°C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hr. Cell line was fixed with trichloroacetic acid (TCA) which represented a measurement of the cell viability at the time of compound addition (Tz). Compounds were dissolved in DMSO at 400-fold the desired final maximum concentration and stored frozen until use. An aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum concentration with complete medium containing compounds at a concentration of 10^{-4}. Additional three, 10-fold serial dilutions were made which include 10^{-5}, 10^{-6}, 10^{-7} concentrations. Aliquots of 10 µl of these solutions of compounds were added to the appropriate micro titer wells containing 90 µl of medium, resulting in the required final compounds concentrations. Plates were incubated at standard condition for 48 hr and cold TCA was added to terminate the assay. Cells were fixed by the addition of 50 µl of cold 30 % (w/v) TCA and incubated for 60 minutes at 4°C. The supernatant was discarded and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. Unbound dye and residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried and bound dye was subsequently solubilised with 10 mM unbuffered Tris base (pH 10.5). The absorbance was read on an Elisa plate reader at a wavelength of 540 nm. Percent growth was calculated for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells x 100. Using the total six absorbance measurements *viz.* time zero (Tz), control growth (C) with adrinamycin, and test growth in the presence of test compounds at the four concentration levels (Ti), growth inhibitory (50%) concentration (GI50), concentration of compound that produces total inhibition of the cells (TGI) and concentration of compound that kills 50% of the cells (LC50) were calculated.

Docking studies

In the present study, the X-ray crystal structure of the epidermal growth factor receptor (EGFR) protein in complex with 4-anilinoquinazoline inhibitor erlotinib was obtained from the RCSB Protein Data Bank (PDB ID: 1M17). Resolution of protein structure with 333 amino acid residues was 2.60 A^0. The protein was further processed by removing water and erlotinib. The resulted clean protein was further refined by energy minimization in UCSF Chimera [31] with Amber ff12SB force field. Combination of 10,000 steepest descent and conjugate gradient steps with 0.02 A^0 step size were used during energy minimization. The energy minimized protein structure was used for docking procedure. 2D structures of all the synthesized compounds (see scheme of synthesis) were drawn and converted to 3D structures using Marvin Sketch (a structure drawing program). Geometry optimization was carried out in ArgusLab 4.0.1 (from Thomson and Planaria Software LLC) on semi empirical quantum mechanical basis with parameterized model number 3 (PM3) hamiltonian, until restricted closed shell hartree-fock self consistent field formalism converses to 10^{-10} kcal/mol and steepest descent geometry search criteria until gradient converses to 10^{-6} kcal/mol. Gasteiger partial atomic charges of optimized molecules were computed in UCSF chimera and were updated in 3D structures. Docking simulation was carried out in Autodock Vina [32]. Polar and aromatic hydrogens and gasteiger charges were added in the protein using MGLtools1.5.4 [33] and the pdb file was subsequently converted to pdbqt format. Pre-optimized compounds were also pre-processed similarly and converted to pdbqt format. All the torsion angles in the small-molecules were set free so as to perform flexible docking. Grid box of size 48 x 66 x 50 with 1 A^0 spacing was defined along x, y and z axis. The defined grid box was large enough to cover active site of protein. The analysis of binding free energy and interactions of ligands with residues at active site was carried out by using Pymol and Discovery studio 3.5.

RESULTS AND DISCUSSION

Docking

The docking protocol adopted in this investigation was validated by docking of erlotinib to energy minimized EGFR protein. The residue Met769 is important in making hydrogen bond and Leu768, Gly768, Leu694 and Leu820 are important in hydrophobic interactions. The best conformer generated in docking showed same interactions as shown in Figure 1.

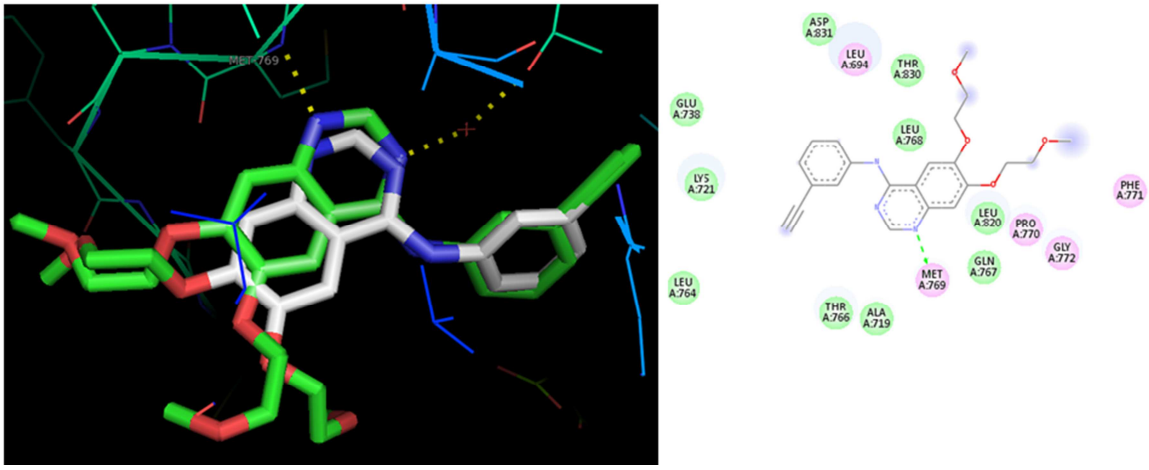


Figure 1: Docking of erlotinib (White stick represents docked conformer)

After docking designed molecules (PYR1-18 and MAN1-18) most of the compounds show interaction with Met769 along with other hydrophobic interactions. Coumarin ring occupies the position of quinazoline ring where as the nitrogen atom from the 3rd substitution makes the hydrogen bonds with important residues. The binding free energy in Kcal/mole and interactions are presented in Table 1. Compounds PYR1, PYR2, PYR3, PYR7, PYR10, PYR11, PYR13, PYR17, PYR18, MAN1, MAN4 show the hydrogen bond interaction with Met769. The aromatic ring attached to pyrimidine ring in PYR series accesses deeper in to the binding site of EGFR.

Table 1: Docking score (Binding free energy in kcal/mol) and important interaction with residues

Sr. No.	Compound	Docking score (Binding free energy) kcal/mol	Interactions
1	PYR1	-10.1	**Met769 (H),** Asp831 (H), Lys721 (Pi)
2	PYR2	-9.5	**Met769 (H),** Asp831 (H), Lys721 (Pi)
3	PYR3	-9.6	**Met769 (H),** Lys721 (Pi), Phe699 (pi)
4	PYR4	-9.4	Lys721 (Pi), Leu694 (Pi)
5	PYR5	-9.4	Lys721 (Pi), Leu694 (Pi), Leu820 (Pi)
6	PYR6	-9.2	Lys721 (Pi), Leu820 (Pi), Arg817 (Pi)
7	PYR7	-9.3	**Met769 (H),** Asp831 (H)
8	PYR8	-10.1	Lys721 (Pi), Thr830 (Pi)
9	PYR9	-9.3	Lys721 (Pi), Leu694 (Pi), Leu820 (Pi)
10	PYR10	-9.0	**Met769 (H),** Lys721 (Pi)
11	PYR11	-10.9	**Met769 (H),** Asp831 (H), Lys721 (Pi)
12	PYR12	-9.9	Asp831 (H), Lys721 (Pi)
13	PYR13	-10.4	**Met769 (H),** Lys721 (Pi)
14	PYR14	-9.3	Lys721 (Pi)
15	PYR15	-10.5	Lys721 (Pi)
16	PYR16	-8.3	Lys721 (Pi)
17	PYR17	-10.5	**Met769 (H),** Asp831 (H), Lys721 (Pi), Leu764 (Pi)
18	PYR18	-8.6	**Met769 (H),** Lys721 (Pi)
19	MAN1	-7.3	**Met769 (H),** Lys721 (Pi)
20	MAN2	-8.3	Lys721 (Pi), Phe699 (Pi)
21	MAN3	-8.3	Lys721 (Pi), Phe699 (Pi)
22	MAN4	-8.5	**Met769 (H),** Phe699 (Pi)
23	MAN5	-8.5	Lys721 (Pi), Phe699 (Pi)
24	MAN6	-8.6	Asp831 (H), Lys721 (Pi)
25	MAN7	-9.0	Lys721 (Pi), Phe699 (Pi)
26	MAN8	-8.3	Asp831 (H), Lys721 (Pi)
27	MAN9	-8.4	Lys721 (Pi)
28	MAN10	-9.3	Lys721 (Pi), Phe699 (Pi)
29	MAN11	-7.7	Asp831 (H), Lys721 (Pi)
30	MAN12	-8.2	Lys721 (Pi)
31	MAN13	-8.2	Lys721 (Pi), Phe699 (Pi)
32	MAN14	-8.7	Asp831 (H), Lys721 (Pi)
33	MAN15	-8.9	Lys721 (H), Phe699 (Pi)
34	MAN16	-8.5	Lys721 (H), Phe699 (Pi)
35	MAN17	-8.9	Lys721 (Pi), Phe699 (Pi)
36	MAN18	-8.6	Lys721 (Pi), Phe699 (Pi)
37	Erlotinib	-7.5	**Met769 (H)**

Antimicrobial activity

The synthesised compounds were evaluated for antibacterial activity using agar cup plate method. Ofloxacin, a well known topoisomerase II DNA gyrase inhibitor, was used as a standard. The results are presented Table 2. The results show that compounds form PYR series are more active than MAN series. All the synthesised compounds exhibited poor activity against gram negative organisms as compared with standard drug ofloxacin.

Table 2: Antimicrobial activity of synthesized compounds

	Zone of Inhibition in mm (millimetre)			
Compounds	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***
MAN1	9.1±0.7	10.1±0.5	5.0±0.5	7.8±0.7
MAN2	9.6±0.5	10.6±0.2	5.6±0.2	6.6±1.1
MAN3	14.5±0.5	15.4±0.3	5.8±0.2	6.0±0.5
MAN4	14.8±0.7	15.5±0.5	6.6±0.2	7.6±0.5
MAN5	15.3±0.5	16.0±1.0	6.5±1.0	8.5±0.5
MAN6	9.6±1.5	14.0±3.1	7.1±0.7	6.1±0.5
MAN7	8.6±0.2	10.4±0.4	6.3±0.7	6.3±1.4
MAN8	17.5±1.3	16.8±0.7	9.0±0.8	8.8±0.7
MAN9	10.5±0.5	9.8±0.7	6.5±1.3	6.1±0.5
MAN10	8.8±0.2	10.8±0.2	5.6±0.2	5.0±0.5
MAN11	10.5±1.8	10.2±1.1	5.6±0.7	6.1±0.5
MAN12	14.0±1.5	14.5±0.5	7.3±0.2	8.5±0.5
MAN13	10.1±1.0	11.0±0.1	6.3±0.2	6.3±0.7
MAN14	11.0±2.2	10.7±0.2	6.1±0.5	6.0±0.8
MAN15	10.0±1.0	11.0±0.5	5.5±0.8	6.1±0.5
MAN16	10.3±1.5	10.6±.02	6.3±0.7	6.1±0.5
MAN17	14.1±1.2	15.0±0.5	6.6±1.2	7.6±0.2
MAN18	10.0±1.0	12.5±0.5	5.6±0.7	6.3±0.7
PYR1	13.6±0.7	15.5±1.0	12.6±0.7	12.3±0.2
PYR2	17.3±0.7	16.1±1.5	12.6±0.7	13.3±0.2
PYR3	18.1±1.3	19.1±0.5	14.3±0.2	14.1±0.2
PYR4	13.1±0.7	13.1±0.5	11.1±1.1	11.1±1.2
PYR5	15.1±0.7	14.3±0.2	13.1±0.5	13.1±0.5
PYR6	13.1±0.7	13.1±0.5	10.3±1.5	10.5±0.8
PYR7	14.6±0.2	14.8±0.5	13.3±0.7	13.3±0.2
PYR8	13.6±0.8	15.1±0.5	13.5±1.0	13.5±1.0
PYR9	12.6±0.2	13.1±0.2	11.8±0.5	12.3±0.2
PYR10	18.6±0.5	19.0±0.8	13.3±0.7	14.6±0.2
PYR11	13.3±0.7	11.1±1.1	12.0±0.8	10.6±0.2
PYR12	14.6±0.2	13.6±0.7	13.0±0.5	10.0±0.5
PYR13	12.6±0.2	13.3±0.7	13.3±0.7	9.8±0.5
PYR14	13.0±0.5	12.6±0.2	13.3±0.5	9.6±0.2
PYR15	15.3±0.7	14.5±0.8	14.0±1.3	12.6±0.2
PYR16	16.1±0.2	14.5±1.0	13.8±0.7	13.6±0.7
PYR17	13.1±0.5	13.3±0.7	10.6±1.1	11.6±0.2
PYR18	13.3±0.2	13.3±0.2	10.3±0.5	9.5±0.5
Ofloxacin	31.0 ± 0.711	28.8 ± 0.849	28.2 ± 0.205	27.9 ± 0.216

Data presented in Mean ±SD (N=3)

***In vitro* cytotoxicity activity by SRB assay**

Few compounds from MAN series (MAN3, MAN4, MAN5, MAN8, MAN12) showed moderate cytotoxic activity against HeLa cell lines in terms of growth inhibitory concentration (GI50). All the compounds from PYR series showed moderate activity in terms of GI50. The results are presented in Table 3 and Figure 2. GI50 values ranged from 51.5 – 98.4 µM for MAN series and 38.0 – 80.9 µM for PYR series. PYR10 with hydrophobic 3,4 dimethoxy substitution is most active among the synthesised compounds. In comparison to standard Adrinamycin all the compounds are inactive. For compounds from PYR series docking results are in agreement with the moderate cytotoxic activity.

Table 3: In vitro cytotoxicity of synthesized compounds against HeLa cells

Compound	LC50	TGI	GI50
MAN1	> 100	> 100	> 100
MAN2	> 100	> 100	> 100
MAN3	> 100	> 100	67.6
MAN4	> 100	> 100	75.1
MAN5	> 100	> 100	71.5
MAN6	> 100	> 100	> 100
MAN7	> 100	> 100	> 100
MAN8	> 100	98.6	51.5
MAN9	> 100	> 100	> 100
MAN10	> 100	> 100	> 100
MAN11	> 100	> 100	> 100
MAN12	> 100	> 100	98.4
MAN13	> 100	> 100	> 100
MAN14	NA	NA	NA
MAN15	NA	NA	NA
MAN16	NA	NA	NA
MAN17	> 100	> 100	> 100
PYR1	> 100	> 100	66.9
PYR2	> 100	> 100	61.6
PYR3	> 100	84.6	43.5
PYR4	NA	NA	NA
PYR5	> 100	> 100	58.2
PYR6	NA	NA	NA
PYR7	> 100	> 100	65.9
PYR8	> 100	> 100	58.3
PYR9	NA	NA	NA
PYR10	> 100	72.8	38.0
PYR11	NA	NA	NA
PYR12	> 100	77.6	40.9
PYR13	NA	NA	NA
PYR14	NA	NA	NA
PYR15	> 100	> 100	56.1
PYR16	> 100	> 100	80.9
PYR17	NA	NA	NA
PYR18	NA	NA	NA
Adrinamycin (Standard)	> 100	43.0	< 0.1

NA: Not tested; Data represented in µMolar concentrations. LC50: Concentration of compound that kills 50% of the cells; TGI: Concentration of compound that produces total inhibition of the cells; GI50: Growth inhibitory (50%) concentration.

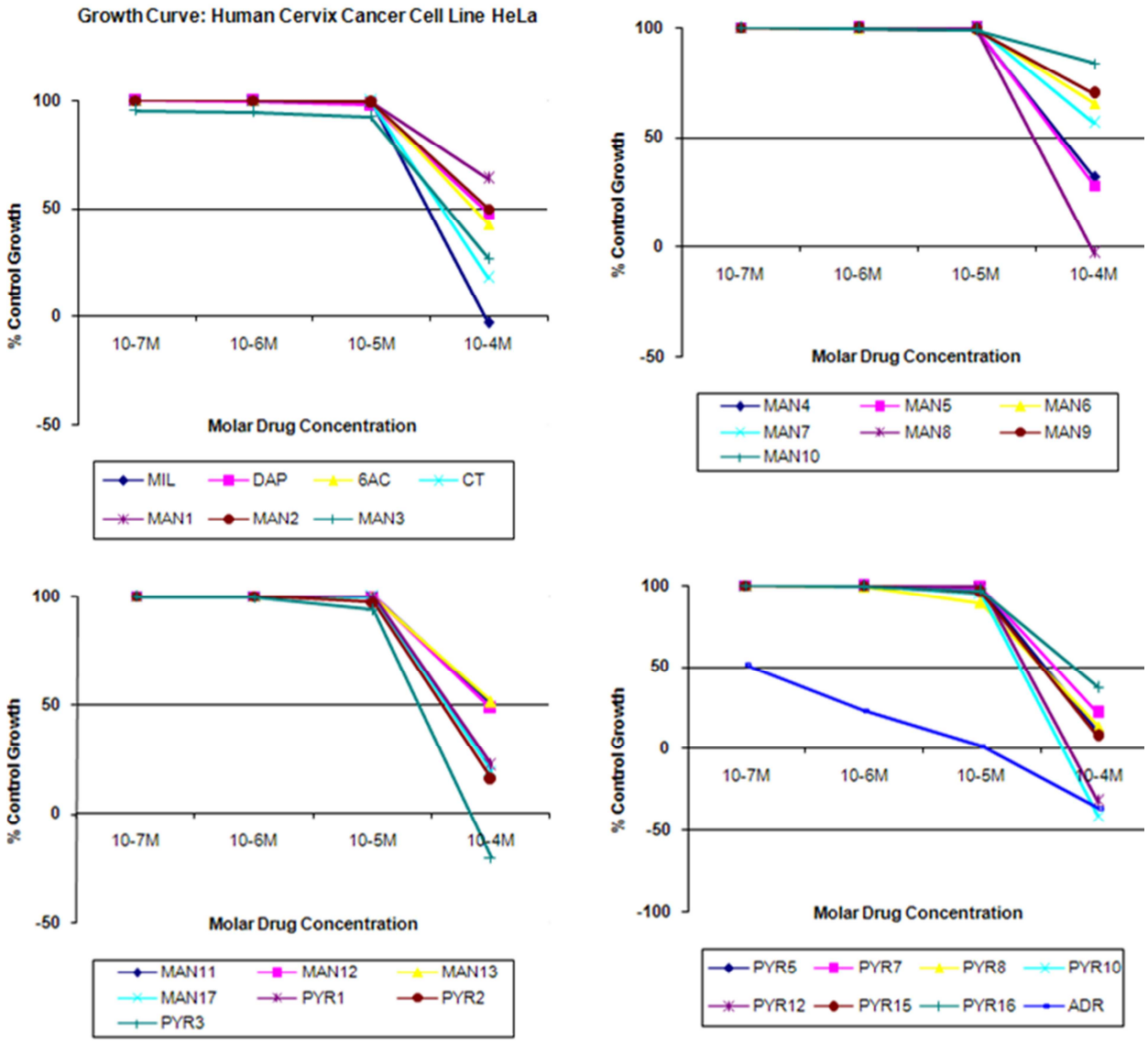


Figure 2: Plot of molar drug concentration against % control growth (ADR: Adrinamycin, MIL, DAP, 6AC, CT: Unreported compounds)

CONCLUSION

Series of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one derivatives and 3-[3-(substituted amino)propanoyl]-2H-chromen-2-one derivatives were synthesised and characterized by FT-IR, ^{1}H-NMR and ESI-MS spectrometry. Molecular docking in Autodock vina was carried out on epidermal growth factor receptor protein. *In vitro* antimicrobial study and *in vitro* cytotoxicity studies on HeLa cell lines were carried out and reported. Compounds from PYR series possess moderate antimicrobial and antiproliferative activity. PYR10 with 3,4-dimethoxy substituent was found most active amongst synthesised compounds.

Acknowledgement
Authors are thankful to Prof. M. N. Navale, Founder President, Sinhgad Technical Education Society for constant encouragement. The authors thank Diya Labs, Mumbai for providing ^{1}H NMR and ESI-Mass spectra. Authors are also thankful to Advance Center for Treatment, Research & Education in Cancer (ACTREC), Mumbai for *in vitro* cytotoxicity studies.

REFERENCES

[1] A. Shafiee, R. Motamedi, O. Firuzi, S. Meili, A. Reza Mehdipour, R. Miri, *Med. Chem. Res*., **2011**, 20, 466-474.
[2] R. K. Reddy, S. P. Rao,J. G. Dev, Y. Poornachandra, C. Ganesh Kumar, S. P. Rao, B. Narsaiah, *Bioorg. Med. Chem. Lett*., **2014**, 24(7), 1661-1663.

[3] H. Gourdeau, L. Leblond, B. Hamelin, C. Desputeau, K. Dong, I. Kianicka, D. Custeau, C. Boudreau, L. Geerts, S. Cai, J. Drewe, D. Labrecque, S. Kasibhatla, B. Tseng, *Mol. Cancer. Ther*., **2004**, 3, 1375-1384.
[4] M. Mladenovic, N. Vukovic, S. Sukdolak, S. Solujic, *Molecules*., **2010**, 15, 4294-4208.
[5] T. Serbetci, S. Birteksoz, S. Prado, S. Michel, F. Tillequin, *Nat. Prod. Commun.*, **2012**, 7(7), 891-894.
[6] A. A. Fadda, M. A. Berghot, F. A. Amer, D. S. Badawy, N. M. Bayoumy, *Arch. Pharm. (Weinheim).*, **2012**, 345(5), 378-385.
[7] J. F. Cheng, A. Ishikawa, Y. Ono, T. Arrhenius, A. Nadzan, *Bioorg. Med. Chem. Lett.*, **2003**, 3(13), 3647-3650.
[8] T. A. Ali, S. A. Abdel-Aziz, H. M. El-Shaaer, F. I. Hanafy, A. Z. El-Fauomy, *Turk. J. Chem.*, **2008**, 32, 365-374.
[9] I. Zghab, B. Trimeche, M. Mansour, M. Hassine, D. Touboul, H. Jannet, *Arabian Journal of Chemistry*., Forthcoming 2013.
[10] DrugBank version 4.1 [Internet]. DrugBank: Open Data Drug and Drug Target Database; http://www.drugbank.ca/drugs/DB01148.html
[11] D. Nardi, A. Leonardi, R. Pennini, A. Tajana, P. Cazzulani, R. Testa, *Arzneimittelforschung*., **1993**, 43(1), 28-34.
[12] W. Sun, L. D. Cama, E. T. Birzin, S. Warrier, L. Locco, *Bioorg. Med. Chem. Lett.*, **2006**, 16(6), 1468-1472.
[13] M. K. Hussain, M. I. Ansari, N. Yadav, P. K. Gupta, A. K. Gupta, R. Saxena, I. Fatima, M. Manohar, P. Kushwaha, V. Khedgikar, J. Gautam, Ruchir Kant, P. R. Maulik, R. Trivedi, A. Dwivedi, K. Ravi Kumar, A. K. Saxena, K. Hajela, *RSC Adv.*, **2014**, 4, 8828-8845.
[14] J. Mori, M. Iwashima, M. Takeuchi, H. Saito, *Chem. Pharm. Bull.*, **2006**, 54(3), 391-396.
[15] B. Naga Sudha, C. Sridhara, C. Girija Sastry, Y. S. R. Reddy, O. Sreevidya, S. Lavanya, V. Asha Jyothia, V. Nagesha, S. Sen, R. Chakraborty, *Indian. J. Chemistry.*, **2013**, 52B, 422-427.
[16] D. Bhavsar, J. Trivedi, S. Parekh, M. Savant, S. Thakrar, A. Bavishi, A. Radadiya, H. Vala, J. Lunagariya, M. Parmar, L. Paresh, R. Loddo, A. Shaha, *Bioorg. Med. Chem. Lett.*, **2011**, 21(11), 3443-3446.
[17] D. C. Mungra, M. P. Patel, D. P. Rajani, R. G. Patel, *Eur. J. Med. Chem.* **2011**, 46(9), 4192-4100.
[18] P. Vijaya Kumar, V. Rajeswar Rao, *Indian. J. Chemistry.*, **2008**, 47B, 106-111.
[19] J. J. Chen, T. Y. Wang, T. L. Hwang, *J. Nat. Prod.* **2008**, 71(2), 212-217.
[20] A. L. Asrondkar, V. N. Patil, N. U. Mishra, A. S. Bobade, A. S. Chowdhary, *Der Pharma Chemica.*, **2013**, 5(4), 288-292.
[21] L. Bin, X. Long-Guan, X. Xiao-Hua, L. Yong-Hong, *Chin. J. Org. Chem.*, **2011**, 31(12), 2067-2073.
[22] K. K. Sivakumar, A. Rajasekaran, *International Journal of Research in Pharmacy and Chemistry*, **2014**, 4(3), 517-527.
[23] M. I. Hegab, M. M. Abdulla, *Arch Pharm (Weinheim).*, **2006**., 339(1), 41-47.
[24] P. M. Ronad, V. S. Maddi, B. C. Koti, Y. V. Kurhe, A. Swamy, Swamy Ahmt, M. S. Jaji, *Indian Journal of Novel Drug delivery*., **2010**., 2(4), 158-161.
[25] S. R. Hubbard, J. H. Till, *Annu. Rev. Biochem.*, **2000**, 69, 373-398.
[26] K. Polkowski and A. P. Mazurek, *Acta Poloniac Pharmacutica – Drug Research*, **2000**, 57 (2):135-155.
[27] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukauri, *The Journal of Biological Chemistry*., **1987**, 262 (12): 5592-5595.
[28] F. Schmidt, C. B. Knobbe, B. Frank, H. Wolburg, M. Weller, *Oncology Reports.*, 2008, 19: 1061-1066.
[29] C. E. Park, H. Yun, E. B. Lee, B. I. Min, H. Bae, W. Choe, I. Kang, S. S. Kim, J. Ha, *J. Med. Food*., **2010**, 13(4):815-20.
[30] E. Yang, Y. Zhao, K. Zhang, P. Mack, *Biochemical and Biophysical Research Communications*, **1999**, 260:682–685.
[31] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S, Couch, *J. Comput. Chem.*, **2004**, 25, 1605-1612.
[32] O. Trott, A. J. Olson, *J. Comput. Chem.*, **2010**, 31(2), 455–461.
[33] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, *J. Computational Chemistry*, **2009**, 16, 2785-2791.