Original Article

Synthesis, structural identification, and biological evaluation of naphthalene-based pyrimidine

Gurpreet Singh*, Vikramdeep Monga

Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India

Correspondence: Mr. Gurpreet Singh, Department of Pharmaceutical Chemistry, ISF College of Pharmacy, G.T. Road, Ghal Kalan, Moga - 142 001, Punjab, India. E-mail: gspreet47@gmail.com

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ABSTRACT

Increasing drug resistance in bacteria and cancer has alarmed the rings to develop newer, safer, and effective treatments against them. In a quest to identify new leads, we synthesized some naphthalene-based pyrimidines and evaluated their antibacterial and cytotoxic potential. The molecules were synthesized through the condensation of naphthalene-based chalcone with guanidine hydrochloride in methanol under reflux conditions. The antibacterial evaluation led to the identification of compound **5b** as the most potent molecule of the series. Compound **5c** was found to be the most potent against colo-205, while **5a** displayed the highest activity against A-549.

Keywords: Pyrimidines, naphthalene, antibacterial, cytotoxicity, chalcones

INTRODUCTION

Cancer is the most dangerous disease in which abnormal cells divide uncontrollably. This can result in tumors, damage to the immune system, and destroy the body tissues.[1] Despite various anticancer drugs available, the development of new anticancer agents for the treatment of cancer without side effects is a significant goal for scientists.[2-4] Moreover, the increase in multi-drug resistant pathogenic bacteria presents one of the most serious threats to human health globally, threatening to render application of numerous medical advances such as surgery and chemotherapy so life-threatening as to be impractical. Resistance has emerged to all clinical antibiotics, and the perceived low profitability of antibiotic development has resulted in an insufficient pipeline of new therapeutics.[5-9]The basic physiology of cancer is dissipated in Figure 1.

Traditional chemotherapeutic agents are cytotoxic to both cancer and normal cells. They usually cause oxidative stress that evokes apoptosis without selectivity leading to severe and sometimes life-threatening effects. Emerging drug resistance in cancers is alarming; the rings for developing new treatment and drug molecules.[10,11] Pyrimidine and its derivatives are prominent core structure present in a large variety of biologically active molecules, which includes anticancer, antiviral, antitumor, anti-inflammatory, antimicrobial, cyclin-dependent kinase inhibitors, tumor necrosis factors-α inhibition, PI-3 kinase inhibitor, Akt kinase inhibitors, and cytokine inhibitors.[12-16] Moreover, chalcones and their derivatives are recognized as potential biologically active compounds, including a wide array of biological properties such as antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic, and anti-inflammatory activities.[17-25]

Combining two pharmacologically important pharmacophores has been proven advantageous for developing a new drug molecule. In an attempt to obtain better and efficacious molecules against cancer, we designed and synthesized some new naphthalene-based pyrimidine derivatives and evaluated them for their possible antibacterial and anticancer activity.

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RESULTS AND DISCUSSION

Chemistry

Solvents and organic reagents were purchased from Sigma-Aldrich, Hi-media, and Loba-Chemie (India) and were used without further purification. Thin-layer chromatography was performed using commercially available pre-coated plates (Merck Kieselgel 60 F_{254} silica). Spots were visualized under ultraviolet light and iodine chamber. Mass spectra were recorded on gas chromatography–mass spectrometry (electrospray ionization [ESI]). Infrared (IR) spectra

(KBr pellets) were recorded on a Thermo Fourier transform IR spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) of the compounds were recorded on the JEOL or Bruker Advance II instrument at 300 MHz frequency, in $CDCl_3$ and tetramethylsilane (TMS) ($\delta = 0$) are used as an internal standard. The chemical shifts are reported in parts per million (δ) downfield from the signal of TMS added to the deuterated solvent. Spin multiplicities are given as s (singlet), b (broad), d (doublet), dd (double doublet), t (triplet), q (quartet), or m (multiplet). Microanalyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. Melting points were recorded with the Stuart SMP30 melting point apparatus and are uncorrected.

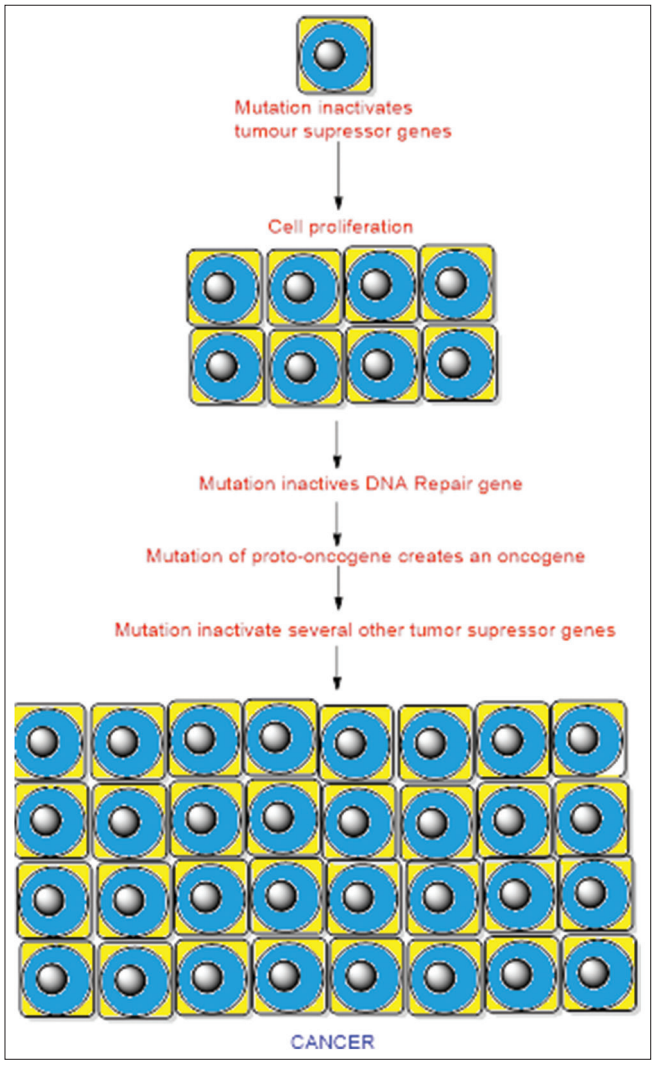


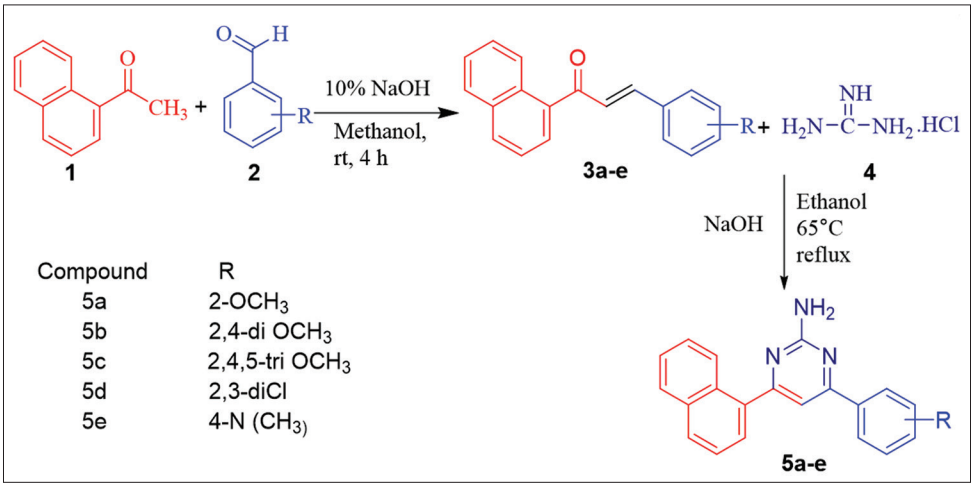
Figure 1: Basic physiology of cancer

A series of substituted naphthalene-based pyrimidine derivatives were prepared using solution-phase chemistry. The intermediate chalcones (3a–e) were synthesized through Claisen-Schmidt condensation of naphthyl ketone (1) with substituted aryl aldehydes (2). The intermediated obtained was treated with guanidine hydrochloride (4) under reflux conditions to obtain the required pyrimidine derivative [5a-e, Scheme 1]. The IR spectra displayed two characteristic peaks primary amine group at a value of 3507 cm^{-1}–3461 cm^{-1} (N-H asymmetrical stretching) and peak at 3270 cm^{-1}–3297 cm^{-1} characterized as symmetrical stretching of N-H group. The PNMR spectra showed a characteristic at 8.21–8.28 ppm which resembles to the aromatic proton of the naphthalene nucleus. The other protons were at the expected chemical shifts. Further, the mass and elemental analysis supported their structures. The physical parameters of pyrimidines have been summarized in Table 1.

Biological evaluation

Antimicrobial evaluation

All the synthesized compounds were evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the disk diffusion method and ciprofloxacin was used as standard drug. The activity was measured as a zone of inhibition. All results were obtained in triplicate. None the compound was found equal of more active than the ciprofloxacin. The result revealed compounds 5b (having 2,4 diOCH_3) as the most active of the series displaying zone of inhibition of 12.5 mm and 16.5 mm against *S. aureus* and *E. coli*, respectively. An increase in the number of the methoxy group in compound 5c (2,4,5-tri OCH_3) led to the loss of activity against *S. aureus* and gave the highest activity against *E. coli*. The substitution



Scheme 1: Synthesis of naphthalene-based pyrimidine derivatives

Table 1: Physical data for synthesized compounds

S. No.	Chemical formula	Melting point	CHN
5a	$C_{21}H_{17}N_3O$	120-122°C	Calculated: C 77.04; H 5.23; N 12.84; Observed: C 76.98; H, 5.30, N 12.84.
5b	$C_{22}H_{19}N_3O_2$	124–126°C	Calculated: C 73.93; H 5.36; N 11.76; Observed: C 73.90; H 5.35.; N 11.76.
5c	$C_{23}H_{21}N_3O_3$	128–130°C	Calculated: C 71.30; H 5.46; N 10.85; Observed: C 71.25; H 5.40.; N 10.96.
5d	$C_{20}H_{13}Cl_2N_3$	124–126°C	Calculated: C 65.59; H 3.58; N 11.47, Observed: C 65.50; H 3.60; N 11.47
5e	$C_{22}H_{20}N_4$	130–132°C	Calculated: C 77.62; H 5.92; N, 16.46, Observed: C 77.65; H 6.00; N 16.39

with electron-withdrawing halogen in compound 5d and N-$(CH_3)_2$ in compound 5e led to weak inhibitory activity against both pathogens. The result obtained is summarized in Table 2.

Minimum inhibitory concentration (MIC)

MIC of the synthesized compounds was determined by serial dilution method at 125, 62.5, 31.25, 15.6, 7.8, and 3.9 μg/mL concentrations and ciprofloxacin was used as standard drug. The result revealed that none of the compounds was found potent as that of standard drug. Compound 5b was found to the most potent from the series, displaying MIC of 7.8 μg/mL and 15.6 μg/mL against *S. aureus* and *E. coli*. The results obtained are summarized in Table 3.

Cytotoxic evaluation

Cytotoxicity of the synthesized compounds was evaluated against two human cell lines, that is, Colo-259 and A-549, using 96 well plate methods. Compound 5b was found to have the highest cytotoxicity against both of the cell lines. None the compound was found to be equipotent as that of standard drug. Compound 5c was found to be the most potent against colo-205, while 5a displayed the highest activity against A-549. The other results are summarized in Table 4.

EXPERIMENTAL

General synthetic procedure of pyrimidine derivatives

1-acetylnaphthalene (1, 2 g, 0.011 mol) was dissolved in methanol (25 ml) in 100 ml round bottom flask. To the solution, substituted benzaldehyde (2, 0.011 mol) followed by 10% methanolic NaOH solution (5 ml) was added. The reaction mixture was kept in stirred conditions under ice-cold conditions. The progress of the reaction was monitored by thin-layer chromatography (TLC) (20% ethyl acetate in hexane). The reaction mixture was poured into ice; precipitated solid was filtered and recrystallized from methanol. A mixture of chalconoid (3a-e, 0.01 mol), guanidine hydrochloride (4, 0.02 mol), and NaOH (0.02 mol) was refluxed in ethanol (25 mL) for 6 h until the reactants disappeared. After completion of the reaction, as indicated by the TLC, the reaction mixture was quenched on ice mixture. Precipitated solid was filtered and purified by column chromatography or recrystallized from methanol.

4-(2-methoxyphenyl)-6-(naphthalen-1-yl)-pyrimidin-2-amine (5a)

Yellow crystals, 40% yield, I.R (ν_{max}, KBr, cm^{-1}): 3487 (N-H asymmetric str), 3270 (N-H symmetric str), 3170 (N-H bend overtone), 3002 (aromatic C-H str), 2918 (aliphatic C-H str), 1640(C=N str), 1585 (N-H bend), 1456 (aromatic C=C str), 1090 (C-N str), ^{1}HNMR (300MHz, CDC_{l3}, δ, TMS=0) : δ = 8.28 (1H, d, J = 8.1Hz), 7.99 (1H, d, J = 8.4Hz), 7.92 (1H, dd, J = 7.2Hz), 7.81 (1H, d, J = 8.1Hz), 7.56–7.33 (5H, m), 7.25 (1H, s), 6.88 (2H, m), 5.25 (2H, s), 3.95 (3H, s), ESI-MS for $C_{21}H_{17}N_3O$: Calculated [M^+]: 327.27, observed [M^+]: 327.

Table 2: Zone of inhibition data of synthesized compounds

Compound	*S. aureus*	*E. coli*
5a	10±0.7	9.5±0.7
5b	12.5±0.7	16.5±0.7
5c	-	19±0.9
5d	9±1.1	8±1.1
5e	9±0.8	11±1.4
Ciprofloxacin	24±0.4	30±0.7
DMSO	-	-

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, DMSO: Dimethyl sulfoxide

Table 3: MIC of synthesized compounds

Compound (μg/mL)	*S. aureus*	*E. coli*
5a	31.25	125
5b	7.8	15.6
5c	62.5	31.25
5d	62.5	62.5
5e	62.5	125
Ciprofloxacin	0.25	0.12

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, MIC: Minimum inhibitory concentration

Table 4: Cytotoxic evaluation of synthesized compounds

Compound	Cell lines (IC_{50}, μM/mL)	
	Colo-205	A-549
5a	49.34	40.23
5b	76.38	79.24
5c	42.23	54.45
5d	50.54	49.56
5e	47.28	49.62
Docetaxel	22.29	19.92

4-(2,4-dimethoxyphenyl)-6-(naphthalen-1-yl)-pyrimidin-2-amine (5b)

White crystals, 40% yield, I.R (ν_{max}, KBr, cm^{-1}) : 3507 (N-H asymmetric str), 3288 (N-H symmetric str), 3147 (N-H bend overtone), 3004 (aromatic C-H str), 2907 (aliphatic C-H str), 1629 (C=N str), 1573 (N-H bend), 1450 (aromatic C=C str), 1113 (C-N str), ^{1}H NMR (300 MHz, $CDCl_3$, δ, TMS = 0): δ = 8.29 (1H, d, J = 8.1Hz), 7.99(1H, d, J = 8.4Hz), 7.92 (1H, d, J = 8.1Hz), 7.91 (1H, d, J = 8.1Hz), 7.68 (1H, d, J = 8.1Hz), 7.58–7.49 (4H, m),

6.64 (1H, dd, J = 2.4, 8.1Hz), 6.53 (1H, d, J = 2.4Hz), 5.19 (2H, s), 3.87 (3H, s), 3.84 (3H, s), ESI-MS for $C_{22}H_{19}N_3O_2$,: Calculated [M^+]: 357.14, observed [M^+]: 357.

4-(2,4,5-trimethoxyphenyl)-6-(naphthalen-1-yl)-pyrimidin-2-amine (5c)

Yellow crystals, 60% yields, I.R (ν_{max}, KBr, cm^{-1}): 3488 (N-H asymmetric str), 3290 (N-H symmetric str), 3180 (N-H bend overtone), 3010 (aromatic C-H str), 2958 (aliphatic C-H str), 1626 (C=N str), 1590 (N-H bend), 1470 (aromatic C=C str), 1090 (C-N str), ^{1}H NMR (300MHz, $CDCl_3$, δ, TMS = 0) : δ = 8.21 (1H, d, J = 8.1 Hz), 7.96 (1H, d, J = 8.4 Hz), 7.91 (1H, dd, J = 7.2Hz), 7.84 (1H, d, J = 8.1Hz), 7.68–7.58 (3H, m), 7.32 (1H, s), 6.78 (1H, s), 6.42 (1H, s), 5.19 (2H, s), 3.95 (9H, s), ESI-MS for $C_{23}H_{21}N_3O_3$,: Calculated [M^+]: 388.56, observed [M^+]: 388.

4-(2,3-dichlorophenyl)-6-(naphthalen-1-yl)-pyrimidin-2-amine (5d)

Yellow crystals, 62% yields, I.R (ν_{max}, KBr, cm^{-1}) : 3471 (N-H asymmetric str), 3297 (N-H symmetric str), 3162 (N-H bend overtone), 3044 (aromatic C-H str), 1641 (C=N str), 1581 (N-H bend), 1457 (C=C aromatic str), 1052 (C-N str), 775 (C-Cl str), ^{1}H NMR (300 MHz, $CDCl_3$, δ, TMS = 0): δ = 8.28 (1H, d, J = 8.0Hz), 7.95 (1H, d, J = 8.4Hz), 7.92 (1H, d, J = 8.4Hz), 7.69 (1H, d, J=7.2Hz), 7.58–7.52 (5H, m), 7.34 (1H, t, J = 8.0Hz), 7.21 (1H, s), 5.28 (2H, s), ESI-MS for $C_{20}H_{13}Cl_2N_3$,: Calculated [M^+]: 365.10, observed [M^+]: 365.

4-(4-(dimethyamino)phenyl)-6-(naphthalen-1-yl)-pyrimidin-2-amine (5e)

Yellow crystals, 60% yields, I.R (ν_{max}, KBr, cm^{-1}) : 3467 (N-H asymmetric str), 3286 (N-H symmetric str), 3154 (N-H bend overtone), 3010 (aromatic C-H str), 2910 (aliphatic C-H str), 1631 (C=N str), 1577 (N-H bend), 1444 (aromatic C=C str), 1192 (C-N str), ^{1}H NMR (300 MHz, $CDCl_3$, δ, TMS = 0): δ = 8.21 (1H, d, J = 8.2Hz), 8.00 (2H, d, J = 8.8Hz), 7.93–7.89 (2H, d, J = 7.6Hz), 7.66 (1H, d, J = 8Hz), 7.57–7.47 (4H, m), 6.76 (2H, d, J = 8.2 Hz), 5.13 (2H,s), 3.04 (6H,s), ESI-MS for $C_{22}H_{20}N_4$,: Calculated [M^+]: 340.26, observed [M^+]: 340.

Antibacterial assay of synthesized compounds

Synthesized compounds were assessed for their antibacterial activity against five pathogenic microbial strains, *S. aureus* (MTCC 96), *E. coli* (MTCC 82), and by disk diffusion method. The standard microbial strains were procured from the Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of synthetics was determined by observing the zone of inhibition in comparison to standard antibiotic (ciprofloxacin) disk. Test compounds were dissolved in dimethylsulfoxide (DMSO) to make a stock solution of 1 mg/ml. The fresh subculture of strains Luria Bertani Broth was spread over sterile assay medium (Nutrient Agar) at 40–45°C in Petri plates and allowed to stand for 30 min. Previously marked sterile paper disks (8 mm diameter) were placed on the surface of inoculated agar plates and 30 µL of each compound was pipetted onto the disks. The Petri plates were kept aside for 1 h and then incubated at 37°C for 24 h and the zone of inhibition was measured. Antimicrobial activity was determined in triplicates and DMSO was used as a negative control.

MIC

MIC of compounds was calculated using the serial dilution method. Different dilutions (125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/mL) of all selected compounds were prepared in DMSO. Five milliliters of nutrient broth was taken in previously marked test tubes and 100 µL of microbial suspension was added to these test tubes. One milliliter of different concentrations of compounds was added in test tubes and tubes were kept in an incubator at 37°C for 24 h and were viewed for assessing MIC of compounds against different test organisms. The concentration showing no growth was considered to be MIC of the respective compound against that strain.

Cytotoxic evaluation of synthesized compounds

The cytotoxicity of various drug solutions was determined by tetrazolium-based colorimetric assay (3-(4, 5-dimethylthiazol-2-yl) 2, 5 diphenyltetrazolium bromide [MTT] assay). Cells were plated in 96 well plates at 7×10^3 per 100 µL per well with density determined based on the growth characteristics of each cell line. After overnight incubation, triplicate wells were treated with varying concentrations of compounds ranging from 1 to 100 µm/mL and standard docetaxel incubated for 3 days. After 3 days, the medium was replaced with 2 µL of MTT solution (5 mg/mL) and cells were incubated for 3 h. Formazan crystals were dissolved in DMSO. The relative percentage of metabolically active cells compared with untreated controls was then determined based on the mitochondrial conversion of MTT to formazan crystals, which were dissolved in DMSO and spectrophotometric absorbance of the sample was determined by a microplate reader (BIORAD) at 570/630 nm.

CONCLUSION

Increasing bacterial resistance against antibiotics and life-threatening side effects of the anticancer drug has posed great challenges to the human race. There is an emergent need to identify new leads and subsequent development to drug molecules to obtain safer and effective treatments to reduce the burden of cancer and bacterias. In an attempt to identify new leads, we designed and synthesized naphthalene-based pyrimidines and evaluated their antibacterial and cytotoxic potential. Compound 5b was identified as the most potent antibacterial compound, was found to possess higher cytotoxicity. The other compounds were active against bacteria and displayed satisfactory results against tested cell lines.

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