Res Chem Intermed (2015) 41:8699–8711
DOI 10.1007/s11164-015-1922-8

Synthesis and biological evaluation of thiazolo and imidazo *N*-(4-nitrophenyl)-7-methyl-5-aryl-pyrimidine-6 carboxamide derivatives

Priyanka Dhiman · Neelam Malik · Prabhakar K. Verma · Anurag Khatkar

Received: 4 September 2014 / Accepted: 8 January 2015 / Published online: 1 February 2015

Abstract Two new series of thiazolo and imidazo *N*-(4-nitrophenyl)-7-methyl-5-aryl-pyrimidine-6 carboxamide derivatives were synthesized. All the synthesized compounds were evaluated for their antimicrobial activity against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441, Gram-negative bacterium: *Escherichia coli* MTCC 443 and antifungal activity against *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 and free radical scavenging activity. Compound **7e** was found the most active antimicrobial comparable to standards taken. Compounds **7a, 7c, 9a**, and **9d** also showed significant antibacterial and antifungal activity. Further, compounds **7f**, **9d**, and **9h** showed significant antioxidant activity with IC_{50} comparable with the standard compound. The synthesized compounds were confirmed for their structure by means of various spectrometric techniques like IR, ^{1}H NMR, mass, and elemental analysis.

Keywords Bigenelli-pyrimidine · Dihydropyrimidine · Antibacterial · Antifungal · Antioxidant

Introduction

Over the past few years, there has been an increased use of antimicrobial drugs and this has resulted in the development of resistance to these drugs. Different mechanisms in microbes contribute to the development of resistance to antibiotics. This leads to search for new heterocyclics as new antimicrobials with modified action [1]. Scientific evidence suggests that free metals and radicals become more harmful, which play an important role in the pathogenesis of many diseases [2].

P. Dhiman · N. Malik · P. K. Verma · A. Khatkar (✉)
Medicinal Chemistry Division, Faculty of Pharmaceutical Sciences, M.D. University, Rohtak 124001, India
e-mail: anuragpharmacy@gmail.com

Generally, compounds having antioxidant activity have been proven to exhibit anticancer [3], anti-hypertensive [4], anti-Alzheimer's, anti-inflammatory, and many other activities [5]. This fact attracted the special attention of researchers in the identification and development of novel antioxidants that can prevent radical-induced damages.

Pyrimidines with a vast literature and long history have been regarded as important constituents of nucleic acids and many biological processes. Pyrimidine moiety was also found to be embedded in a large number of alkaloids, drugs, antibiotics, agrochemicals, and antimicrobial agents as parent component [6]. 1, 2, 3, 4-tetrahydro pyrimidine derivatives synthesis has recently came out of a growing area of research to determine the most promising target molecules because of their important medicinal and pharmacological properties such as antiviral [7], anti-malarial [8], anticarcinogenic [9], hypoglycemic [10], and calcium channel modulators [11]. Hence, keeping in view of the facts mentioned above and continuing our research work on synthesis and biological evaluation of heterocyclic compounds [12–14] in our present work, we report the synthesis of two series of thiazolo and imidazo *N*-(4-nitrophenyl)-7-methyl-5-aryl-pyrimidine-6 carboxamide derivatives, which can be synthesized by taking *N*-(4-nitrophenyl)-3-oxobutamide (Biginelli reaction; a three-component cyclocondensation reaction is one of the methods described to synthesize dihydropyrimidine derivatives) with different aromatic aldehydes and thiourea, guanidine in the presence of concentrated HCl [15].

Materials and methods

Melting points were determined in open glass capillaries on a Sonar melting point apparatus and are uncorrected. The percentage yield was based upon the products obtained after recrystallization. Reaction progress was monitored by thin-layer chromatography (TLC) performed on silica gel G-coated plate in the solvent system toluene: ethyl acetate (7.5:2.5), and the spots were located by iodine. Infrared (IR) analysis was performed on a SHIMAZDU FT-IR instrument using KBr pellets and was recorded in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker DRX-300 FTNMR spectrometer using DMSO-d6 solvent and are expressed in parts per million (d, ppm) downfield from the internal standard TMS. Elemental analysis of all the synthesized compounds was carried out on a Thermo Scientific Flash 2000 CHN analyzer. Mass spectra were taken on a Bruker Compass Data Analysis 4.0 mass spectrometer.

Procedure for synthesis of *N*-(4-nitrophenyl)-3-oxobutamide (**3**) [16]

A mixture of ethylacetoacetate (0.01 mol) and 4-nitro aniline (0.01 mol) in toluene containing catalytic amount (0.05 ml, 40 %) of NaOH was refluxed on oil bath for 8 h. After completion of the reaction (TLC monitoring), the reaction mixture was kept overnight; separated solid was filtered, washed with petroleum ether and recrystallized from ethanol to obtain *N*-(4-nitrophenyl)-3-oxobutamide.

General procedure for synthesis of 5-(2-hydroxynaphthalen-1-yl)-7-methyl-*N*-(4-nitrophenyl)-3,5-dihydro-2*H*-thiazolo/imidazo[3,2-a]pyrimidine-6-carboxamide (**7f**, **9h**) [16]

A mixture of *N*-(4-nitrophenyl)-3-oxobutamide (0.01 mol), 2-hydroxynaphthaldehyde (0.01 mol), guanidine/thiourea (0.01 mol), and ethanol (8 ml), containing 0.4 ml of concentrated HCl was heated under reflux for 6–8 h. The solution was deep frozen overnight and precipitates were obtained. 1,2-dichloroethane (0.01 mol) was added to a boiling solution of intermediate obtained above (0.01 mol) in dimethyl formamide and then refluxed for 1 h. After completion of the reaction, the contents were poured on crushed ice. The precipitates obtained were filtered, dried, and recrystallized from ethanol.

*General procedure for synthesis of substituted N-(4-nitrophenyl)-6-methyl-4-phenyl-2-thioxo/imino-1,2,3,4-tetrahydropyrimidine-5-carboxamide derivatives (**6a–g**)(**8a–g**) [16]*

A mixture of *N*-(4-nitrophenyl)-3-oxobutamide (0.01 mol), different aromatic aldehyde (0.01 mol), guanidine/thiourea (0.01 mol), and ethanol (8 ml), containing 0.4 ml of concentrated HCl was heated under reflux for 6–8 h. The solution was deep frozen overnight and precipitates were obtained.

*General procedure for synthesis of 5-substituted N-(4-nitrophenyl)-7-methyl-3,5-dihydro-2H-imidazo/thiazolo [3,2-a]pyrimidine-6-carboxamide derivatives (**7a–g**)(**9a–g**) [16]*

1,2-dichloroethane (0.01 mol) was added to a boiling solution of substituted *N*-(4-nitrophenyl)-6-methyl-4-phenyl-2-thioxo/imino-1,2,3,4-tetrahydropyrimidine-5-carboxamide derivatives (0.01 mol) in dimethyl formamide and then refluxed for 1 h. After completion of the reaction, the contents were poured on crushed ice. The precipitates obtained were filtered, dried, and recrystallized from ethanol.

Spectral data

Intermediate *4-(4-chlorophenyl)-6-methyl-N-(4-nitrophenyl)-2-thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxamide* **8d** solid yellow; Anal. Calcd for $C_{18}H_{15}ClN_4O_3S$ C, 53.67; H, 3.75; N, 13.91 Found C, 53.58; H, 3.60; N, 13.92 IR (KBr pellets, cm^{-1}): 1,690 (C=O str., 2^0 amide), 3,343(N–H str., sec. amide), 1,581 (C=C str., skeletal vibration of phenyl nucleus), 860 (C–Cl), 1,211(C=S).

5-(2-hydroxyphenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide **7a** solid brown (yield: 82 %), solid yellow; m.p.: 264–266 °C *Rf* 0.42; Anal. Calcd for $C_{20}H_{19}N_5O_4$ C, 61.06; H, 4.87; N, 17.80 Found C, 60.68; H, 4.56; N, 16.83; IR (KBr pellets, cm^{-1}): 1,690 (C=O str., 2^0 amide), 3,046 (C=C–H asymmetrical str., aromatic), 3,156 (N–H str., sec. amide), 1,173 (C–N str., 3^0 aryl amine), 3,208 (OH str., phenyl), 1,583 (C=C str., skeletal vibration of phenyl nucleus), 1,397 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm

(DMSO-d6) 7.25–7.76(m, 4H, Ar–H), 7.95–8.34(m, 3H, Ar–H), 6.91 (s, 1H, CH, pyrimidine ring), 8.37(s, 1H, NH), 2.53–3.39(s, 2H, CH_2), 5.47(1H, OH) m/e: 394.15 (M+1).

5-(4-(dimethylamino)phenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide **7b** solid red; Anal. Calcd for $C_{22}H_{24}N_6O_3$ C, 62.84; H, 5.75; N, 19.99 Found C, 61.74; H, 5.95; N, 18.90; IR (KBr pellets, cm^{-1}): 1,687 (C=O str., 2^0 amide), 3,075 (C=C–H Asymmetrical str., aromatic), 3,170 (N–H str., sec, amide), 1,645 (C=N str., pyrimidine ring), 1,279 (C–N str., 3^0 aryl amine), 1,317 (C–N str., aryl 3^0 amine), 1,017 (C–O str., OCH_3), 1,573 (C=C str., skeletal vibration of phenyl nucleus), 1,514 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6)7.47–7.56 (m, 4H, Ar–H), 7.53–7.65(m, 3H, Ar–H), 6.91 (s, 1H, CH, pyrimidine ring), 8.05(s, 1H, NH), 2.67–3.91 (s, 2H, CH_2) m/e: 422.20 (M+2).

5-(4-hydroxyphenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7c**) solid yellow; Anal. Calcd for $C_{20}H_{19}N_5O_4$ C, 61.06; H, 4.87; N, 17.80 Found C, 60.98; H, 4.77; N, 17.84; IR (KBr pellets, cm^{-1}): 1,688 (C=O str., 2^0 amide), 3,077 (C=C–H Asymmetrical str., aromatic), 3,108 (N–H str., sec. amide), 1,073 (C–N str., 3^0 aryl amine), 1,290 (C–O str., OCH_3), 3,216 (OH str., phenyl), 1,584 (C=C str., skeletal vibration of phenyl nucleus), 1,502 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6) 7.14–7.74(m, 4H, Ar–H), 7.81–8.83(m, 3H, Ar–H), 6.91 (s, 1H, CH, pyrimidine ring), 8.76 (s, 1H, NH), 2.91–3.86 (s, 2H, CH_2), 5.03(1H, OH) m/e: 395.15 (M+2).

5-(4-formylphenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7d**) solid dark brown; Anal. Calcd for $C_{21}H_{19}N_5O_4$ C, 62.22; H, 4.72; N, 17.27 Found C, 61.98; H, 4.78; N, 17.29; IR (KBr pellets, cm^{-1}): 1,683 (C=O str., 2^0 amide), 3,072 (C=C–H asymmetrical str., aromatic), 3,212 (N–H str., sec. amide), 1,646 (C=N str., pyrimidine ring), 1,124 (C–N str., 3^0 aryl amine), 1,301 (C–O str., OCH_3), 1,725 (C=O str., aldehyde), 1,581 (C=C str., skeletal vibration of phenyl nucleus), 1,480 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6) 7.28–7.79(m, 4H, Ar–H), 7.95–8.35(m, 3H, Ar–H), 6.81 (s, 1H, CH, pyrimidine ring), 8.39(s, 1H, NH), 2.45–3.49(s, 2H, CH_2), 5.49(1H, OH) m/e: 406.15 (M+1).

7-methyl-5-(2-nitrophenyl)-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7e**) solid white; Anal. Calcd for $C_{20}H_{18}N_6O_5$ C, 56.87; H, 4.30; N, 19.90 Found C, 55.78; H, 4.36; N, 19.99; IR (KBr pellets, cm^{-1}): 1,684 (C=O str., 2^0 amide), 3,076 (C=C–H Asymmetrical str., aromatic), 3,171 (N–H str., sec. amide), 1,645 (C=N str., pyrimidine ring), 1,106 (C–N str., 3^0 aryl amine), 1,247 (C–O str., OCH_3), 1,572 (C=C str., skeletal vibration of phenyl nucleus), 1,440 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6) 7.45–7.77 (m, 4H, Ar–H), 7.93–8.35 (m, 3H, Ar–H), 6.97 (s, 1H, CH, pyrimidine ring), 8.38 (s, 1H, NH), 2.57–3.35(s, 2H, CH_2), 5.87(1H, OH) m/e: 423.14 (M+1).

5-(2-hydroxynaphthalen-1-yl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7f**) solid red; Anal. Calcd for $C_{24}H_{21}N_5O_4$ C, 65.00; H, 4.77; N, 15.79 Found C, 65.98; H, 4.79; N, 14.91; IR (KBr pellets, cm^{-1}): 1,685 (C=O str., 2^0 amide), 3,075 (C=C–H Asymmetrical str., aromatic), 3,168 (N–H str., sec. amide), 1,645 (C=N str., pyrimidine ring), 1,106 (C–N str., 3^0 aryl amine), 1,217 (C–O str., OCH_3), 1,571 (C=C str., skeletal vibration of phenyl nucleus), 1,440 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.55–7.73 (m,

4H, Ar–H), 7.94–8.34 (m, 3H, Ar–H), 6.96 (s, 1H, CH, pyrimidine ring), 8.38 (s, 1H, NH), 2.57–3.33(s, 2H, CH_2), 5.82(1H, OH) m/e: 444.16 (M+1).

5-(3,4-dimethoxyphenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7g**) solid brown; Anal. Calcd for $C_{22}H_{23}N_5O_5$ C, 60.40; H, 5.30; N, 16.01Found C, 60.87; H, 5.74; N, 16.56; IR (KBr pellets, cm^{-1}): 1,688 (C=O str., 2^0 amide), 3,025 (C=C–H Asymmetric str., aromatic), 3,168 (N–H str., sec. amide), 1,645 (C=N str., pyrimidine ring), 1,166 (C–N str., 3^0 aryl amine), 1,278 (C–O str., OCH_3), 1,575 (C=C str., skeletal vibration of phenyl nucleus), 1,440 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.55–7.78 (m, 4H, Ar–H), 7.83–8.34 (m, 3H, Ar–H), 6.98 (s, 1H, CH, pyrimidine ring), 8.35 (s, 1H, NH), 2.67–3.36 (s, 2H, CH_2), 5.86 (1H, OH) m/e: 438.17 (M+1).

5-(4-hydroxy-3-methoxyphenyl)-7-methyl-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide (**7h °**) solid yellow; Anal. Calcd for $C_{21}H_{21}N_5O_5$ C, 59.57; H, 5.00; N, 16.54 Found C, 57.92; H, 5.67; N, 16.76; IR (KBr pellets, cm^{-1}): 1,687 (C=O str., 2^0 amide), 3,077 (C=C–H Asymmetric str., aromatic), 3,108 (N–H str., sec. amide), 1,109 (C–N str., 3^0 aryl amine), 1,290 (C–O str., OCH_3), 1,584 (C=C str., skeletal vibration of phenyl nucleus), 3,216 (OH str., phenyl), 1,467 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.55–7.78 (m, 4H, Ar–H), 7.94–8.37 (m, 3H, Ar–H), 6.98 (s, 1H, CH, pyrimidine ring), 8.37 (s, 1H, NH), 2.67–3.37 (s, 2H, CH_2), 5.77 (1H, OH) m/e: 424.16 (M+1).

5-(4-hydroxyphenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9a**) solid green; Anal. Calcd for $C_{20}H_{18}N_4O_4S$ C, 58.53; H, 4.42; N, 13.65 Found C, 57.94; H, 4.49; N, 13.87; IR (KBr pellets, cm^{-1}): 1,690 (C=O str., 2^0 amide), 2,981 (C=C–H asymmetrical str., aromatic), 3o72 (N–H str., sec. amide), 1,082 (C–N str., 3^0 aryl amine), 3,209 (OH str., phenyl), 678 (C–S–C str., Thiazole ring), 1,577 (C=C str., skeletal vibration of phenyl nucleus), 1,465 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6) 7.05–7.45 (m, 4H, Ar–H), 7.70–7.86 (m, 3H, Ar–H), 6.89(s, 1H, CH, pyrimidine ring), 8.01 (s, 1H, NH), 2.66–3.92(s, 2H, CH_2), 5.50 (1H, OH) m/e: 411.11(M+1).

5-(2-formylphenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9b**) solid yellow; Anal. Calcd for $C_{21}H_{18}N_4O_4S$ C, 59.70; H, 4.29; N, 13.26 Found C, 59.76; H, 4.34; N, 13.67; IR (KBr pellets, cm^{-1}): 1,690 (C=O str., 2^0 amide), 3,076 (C=C–H asymmetrical str., aromatic), 3,120 (N–H str., sec. amide), 1,138 (C–N str., 3^0 aryl amine), 1,736 (C=O str., aldehyde), 1,232 (C–O str., OCH_3), 667 (C–S–C str., Thiazole ring), 1,537 (C=C str., skeletal vibration of phenyl nucleus), 1,414 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6)7.27–7.75 (m, 4H, Ar–H), 7.79–8.17(m, 3H, Ar–H), 6.92 (s, 1H, CH, pyrimidine ring), 8.78 (s, 1H, NH), 2.64–3.64 (s, 2H, CH_2), 9.89 (m, 1H, CHO) m/e: 423.11 (M+1).

5-(4-formylphenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9c**) solid brown; Anal. Calcd for $C_{21}H_{18}N_4O_4S$ C, 59.70; H, 4.29; N, 13.26 Found C, 59.87; H, 4.33; N, 13.35; IR (KBr pellets, cm^{-1}): 1,689 (C=O str., 2^0 amide), 3,068 (C=C–H asymmetrical str., aromatic), 3,280 (N–H str., sec. amide), 1,092 (C–N str., 3^0 aryl amine), 1,601 (C=N str., pyrimidine ring), 1,727 (C=O str., aldehyde), 1,260 (C–O str., OCH_3), 640 (C–S–C str., Thiazole ring), 1,506 (C=C str., skeletal vibration of phenyl nucleus), 1,384 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR, δppm (DMSO-d6) 7.42–7.87(m, 4H, Ar–H), 7.87–7.98 (m,

3H, Ar–H), 6.91 (s, 1H, CH, pyrimidine ring), 8.21 (s, 1H, NH), 2.49–3.84(s, 2H, CH_2), 10.48 (s, 1H, CHO) m/e: 423.11 (M+1).

5-(4-chlorophenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9d**) solid red; Anal. Calcd for $C_{20}H_{17}ClN_4O_3S$ C, 56.01; H, 4.00; N, 13.06 Found C, 55.57; H, 4.67; N, 13.12; IR (KBr pellets, cm^{-1}): 1,690 (C=O str., 2^0 amide), 3,024 (C=C–H Asymmetric str., aromatic), 3,165 (N–H str., sec. amide), 1,645 (C=N str., pyrimidine ring), 1,166 (C–N str., 3^0 aryl amine), 1,217 (C–O str., OCH_3), 1,570 (C=C str., skeletal vibration of phenyl nucleus), 760 (C–CI str., phenyl), 1,439 (C–NO_2 str., $C_6H_4NO_2$) 636 (C–S–C str., Thiazole ring), ^{1}H NMR δppm (DMSO-d6) 7.45–7.79 (m, 4H, Ar–H), 7.73–8.55 (m, 3H, Ar–H), 6.98 (s, 1H, CH, pyrimidine ring), 8.37 (s, 1H, NH), 2.67–3.55(s, 2H, CH_2), 5.97(1H, OH) m/e: 430.07 (M+2).

5-(4-(dimethylamino)phenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9e**) solid yellow; Anal. Calcd for $C_{22}H_{23}N_5O_3S$ C, 60.39; H, 5.30; N, 16.01 Found C, 60.40; H, 5.36; N, 16.23; IR (KBr pellets, cm^{-1}): 1,688 (C=O str., 2^0 amide), 3,024 (C=C–H asymmetric str., aromatic), 3,168 (N–H str., sec. amide), 1,645 (C=N str., pyrimidine ring), 1,167 (C–N str., 3^0 aryl amine), 1,279 (C–O str., OCH_3), 1,572 (C=C str., skeletal vibration of phenyl nucleus), 1,341 (C–N str., 3^0 aryl amine), 637 (C–S–C str., Thiazole ring), 1,440 (C–NO_2 str., $C_6H_4NO_2^1H$ NMR δppm (DMSO-d6) 7.45–7.77 (m, 4H, Ar–H), 7.93–8.35 (m, 3H, Ar–H), 6.97 (s, 1H, CH, pyrimidine ring), 8.38 (s, 1H, NH), 2.57–3.35 (s, 2H, CH_2), 5.89 (1H, OH) m/e: 438.16 (M+1).

5-(4-methoxyphenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9f**) solid red; Anal. Calcd for $C_{21}H_{20}N_4O_4S$ C, 59.42; H, 4.75; N, 13.20 Found C, 60.55; H, 4.79; N, 13.26; IR (KBr pellets, cm^{-1}): 1,688 (C=O str., 2^0 amide), 3,026 (C=C–H asymmetric str., aromatic), 3,171 (N–H str., sec. amide), 1,640 (C=N str., pyrimidine ring), 1,161 (C–N str., 3^0 aryl amine), 1,218 (C–O str., OCH_3), 1,556 (C=C str., skeletal vibration of phenyl nucleus), 1,466 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.45–7.87 (m, 4H, Ar–H), 7.83–8.45 (m, 3H, Ar–H), 6.98 (s, 1H, CH, pyrimidine ring), 8.48 (s, 1H, NH), 2.57–3.37 (s, 2H, CH_2), 5.88(1H, OH) m/e: 426.12 (M+2).

5-(3,4-dimethoxyphenyl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9g**) solid white; Anal. Calcd for $C_{22}H_{22}N_4O_5S$ C, 58.14; H, 4.88; N, 12.33 Found C, 59.19; H, 4.85; N, 12.34; IR (KBr pellets, cm^{-1}): 1,687 (C=O str., 2^0 amide), 3,015 (C=C–H asymmetric str., aromatic), 3,155 (N–H str., sec. amide), 1,160 (C–N str., 3^0 aryl amine), 1,245 (C–O str., OCH_3), 1,597 (C=C str., skeletal vibration of phenyl nucleus), 686 (C–S–C str., Thiazole ring), 1,459 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.35–7.78 (m, 4H, Ar–H), 7.94–8.36 (m, 3H, Ar–H), 6.87 (s, 1H, CH, pyrimidine ring), 8.37 (s, 1H, NH), 2.47–3.38 (s, 2H, CH_2), 5.88 (1H, OH) m/e: 455.13 (M+1).

5-(2-hydroxynaphthalen-1-yl)-7-methyl-N-(4-nitrophenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxamide (**9h**) solid red; Anal. Calcd for $C_{24}H_{20}N_4O_4S$ C, 62.60; H, 4.38; N, 12.17 Found C, 62.76; H, 4.42; N, 12.76; IR (KBr pellets, cm^{-1}): 1,688 (C=O str., 2^0 amide), 3,047 (C=C–H asymmetric str., aromatic), 3,154 (N–H str., sec. amide), 1,169 (C–N str., 3^0 aryl amine), 1,208 (C–O str., OCH_3), 1,589 (C=C str., skeletal vibration of phenyl nucleus), 627 (C–S–C str., Thiazole

ring), 3,254 (OH str., phenyl), 1,484 (C–NO_2 str., $C_6H_4NO_2$) ^{1}H NMR δppm (DMSO-d6) 7.43–7.78 (m, 4H, Ar–H), 7.97–8.45 (m, 3H, Ar–H), 6.96 (s, 1H, CH, pyrimidine ring), 8.37 (s, 1H, NH), 2.53–3.37 (s, 2H, CH_2), 5.87 (1H, OH) m/e: 461.12 (M+1) (Tables 1, 2).

Biological screening of synthesized pyrimidine derivatives

Evaluation of antimicrobial activity

Determination of minimum inhibitory concentration (MIC) in-vitro antimicrobial activity of synthesized compounds was performed against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3,160, *Bacillus subtilis* MTCC 441, Gram-negative bacterium: *Escherichia coli* MTCC 443 and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 using tube dilution method using ciprofloxacin and fluconazole as standard drugs for antibacterial and antifungal activity, respectively [17]. Dilutions of test and standard compounds were prepared in double-strength nutrient broth—I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) [18]. The inoculated tubes were incubated at 37 ± 1 °C for 24 h (bacteria), at 25 ± 1 °C for 7 d (*A. niger*) and at 37 ± 1 °C for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest

Table 1 Arrangement of substituents used for targeted compounds

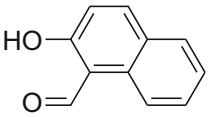
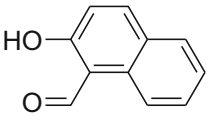
Compound	R_1	R_2	R_3	R_4	X
7a	OH	H	H	H	N
7b	H	H	$N(CH_3)_2$	H	N
7c	H	H	OH	H	N
7d	H	H	CHO	H	N
7e	NO_2	H	H	H	N
7f					N
7g	H	OCH_3	OCH_3	H	N
7h	H	OCH_3	OH	H	N
9a	H	H	OH	H	S
9b	CHO	H	H	H	S
9c	H	H	CHO	H	S
9d	H	H	Cl	H	S
9e	H	H	$N(CH_3)_2$	H	S
9f	H	H	OCH_3	H	S
9g	H	OCH_3	OCH_3	H	S
9h					S

Table 2 Physicochemical properties of synthesized pyrimidine derivatives

Compound	M. wt.	m. p.(C^0)	R_f value*	Yield (%)
7a	393.4	240–247	0.32	78.81
7b	420.4	275–279	0.47	66.25
7c	393.4	234–247	0.49	65.44
7d	405.4	216–219	0.40	67.17
7e	422.3	231–235	0.46	68.53
7f	443.4	251–256	0.45	47.40
7g	437.4	220–228	0.35	68.32
7h	423.4	274–276	0.37	79.27
9a	410.4	234–238	0.35	58.48
9b	422.4	135–137	0.39	78.63
9c	422.4	234–238	0.30	48.72
9d	428.5	175–177	0.43	55.11
9e	437.5	188–192	0.76	73.84
9f	424.4	234–236	0.38	83.25
9g	454.5	264–267	0.41	87.57
9h	460.5	255–260	0.43	76.68

*S.D = Standard Deviation (Average of three determinations)

concentration of test substance which inhibited the growth of microorganisms) given in Table 3.

Free radical scavenging activity

Free radical scavenging activity of synthesized compounds against stable free radical 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. Following the reduction, its deep violet color in methanol bleaches to yellow, and shows a significant absorption decrease at 517 nm. Fifty milliliters of various concentrations (25, 50, 75, and 100) μg/ml of the compounds dissolved in methanol was added to 5 ml of a 0.004 % methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Tests were carried out in triplicate, and ascorbic acid was used as a positive control. IC_{50} values were calculated (Table 4). Scavenging of DPPH free radical was calculated as: DPPH scavenging activity (%) = [(Ac–At)/Ac] × 100 where, Ac is the absorbance of the control reaction At is the absorbance of the test compound.

Results and discussion

Chemistry

The synthesis of two series of pyrimidine (**7a–h**) and (**9a–h**) derivatives was carried out as outlined in the Scheme 1. All the compounds were obtained in appreciable

Table 3 Minimum inhibitory concentration for the antimicrobial activity of newly synthesized pyrimidine derivatives

Compound	Minimum inhibitory concentration (μM)				
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
7a	11.53	11.24	10.34	11.27	10.53
7b	27.38	17.19	24.27	16.18	16.18
7c	11.17	12.17	11.36	11.32	12.15
7d	32.07	17.04	32.04	31.07	17.07
7e	10.46	09.27	10.46	09.27	10.46
7f	28.17	15.37	28.18	29.15	15.37
7g	23.47	22.75	24.47	22.44	22.75
7h	32.76	33.78	32.78	31.76	27.38
9a	13.32	12.67	13.34	12.32	12.67
9b	17.23	18.25	17.27	24.63	23.63
9c	21.32	21.33	12.26	20.37	23.27
9d	11.78	09.76	11.64	11.78	12.38
9e	31.19	26.38	26.38	26.38	24.38
9f	21.08	21.06	26.07	27.07	21.04
9g	22.67	22.35	24.67	22.34	22.69
9h	23.32	23.67	22.32	28.67	28.67
Std.	8.71[a]	8.71[a]	8.71[a]	10.09[b]	10.09[b]

[a] Ciprofloxacin

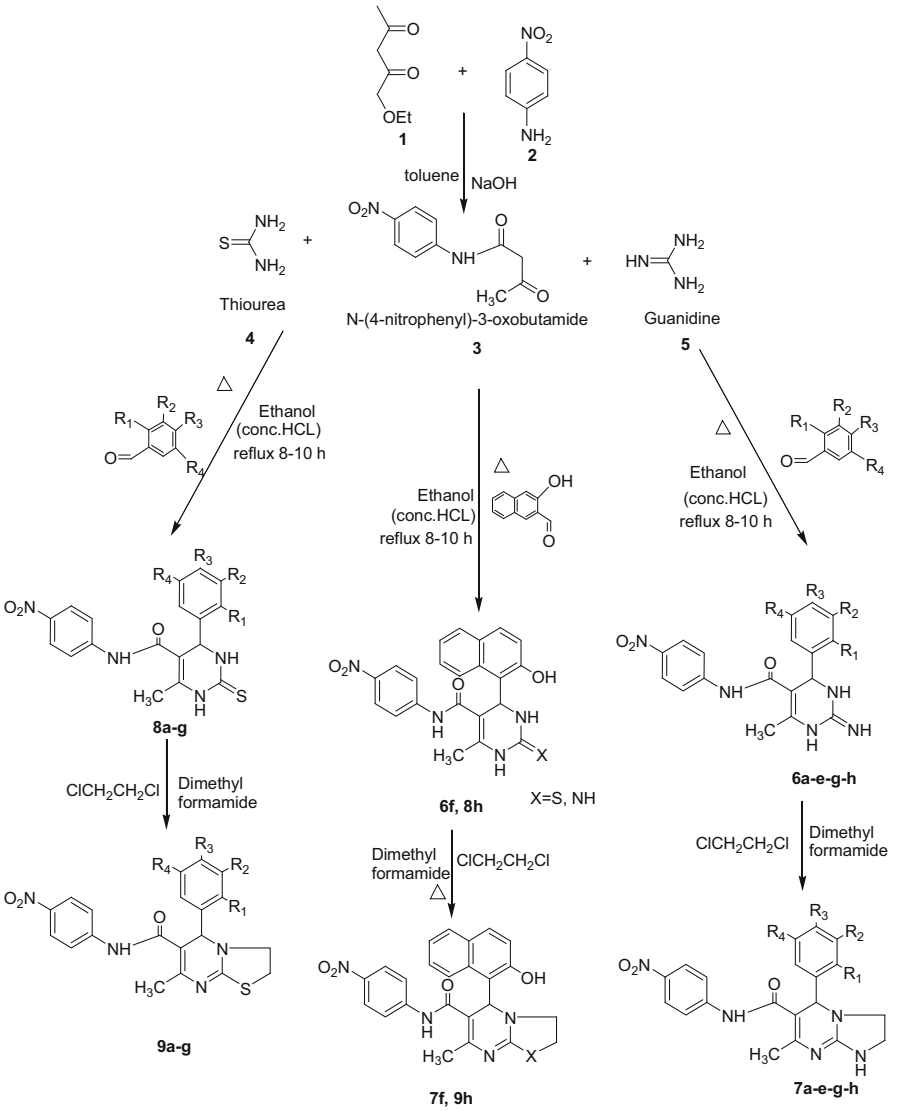
[b] Fluconazole

Table 4 Percentage inhibition and IC_{50} value for free radical scavenging activity of the synthesized pyrimidine derivatives

Compound	$IC_{50\ (mean \pm S.D.)}$* (μg/ml)	Compound	$IC_{50\ (mean \pm SD)}$* (μg/ml)
7a	52.43 ± 0.152	**9a**	75.4 ± 0.170
7b	47.2 ± 0.563	**9b**	51.7 ± 0.068
7c	55.2 ± 0.162	**9c**	64.2 ± 0.117
7d	47.5 ± 0.244	**9d**	41.6 ± 0.382
7e	52.9 ± 0.087	**9e**	58.8 ± 0.346
7f	42.8 ± 0.788	**9f**	52.3 ± 0.571
7g	62.2 ± 0.124	**9g**	60.2 ± 0.542
7h	57.8 ± 0.387	**9h**	42.3 ± 0.087
Ascorbic acid		41.9 ± 0.089	

*S.D = Standard Deviation (Average of three determinations)

yield and their physicochemical characteristics are presented in Table 2. The structures of the synthesized compounds were established on the basis of their consistent IR, NMR, and mass spectral characteristics in addition to elemental analysis (C, H, N), which were in full agreement with the assigned molecular structures and the data. IR spectrum showed a definite absorption at 1,690 cm^{-1} due



Scheme 1 Synthesis of methyl 5-substituted *N*-(4-nitrophenyl)-7-methyl-3, 5-dihydro-2*H*-thiazolo/imidazo[3,2-a]pyrimidine-6-carboxamide derivatives

to carboxamide group. The formation of compound **9d** was further supported by disappearance of the IR band at 1,211 cm^{-1} (**8d**) due to the C=S group and appearance of a new band at 636 cm^{-1} due to the C–S–C bond. The strong absorption bands appeared between 3,141 and 3,189 cm^{-1} in the IR spectra of compounds (**7a**–**h**) corresponding to secondary amine (N–H) group present in pyrimidine ring. The ^{1}H NMR data of compounds revealed signals between 6.84

and 6.97 δppm for CH of pyrimidine ring. The ^{1}H NMR data of compounds revealed signals between 8.07 and 8.19 δppm NH of pyrimidine ring.

Biological evaluation

Antimicrobial activity

All the synthesized compounds were screened for their antimicrobial activity and the results showed that *p*-chloro substituted pyrimidine derivative *7-methyl-5-(2-nitrophenyl)-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide* **7e** was found to be the most active antimicrobial comparable to standards taken. Compounds **7a, 7c, 9a** and **9d** also showed significant antibacterial and antifungal activity. This may be due to the presence of electron-withdrawing group present on the aromatic ring attached to pyrimidine moiety. The role of the electron-withdrawing group in increasing the antimicrobial activity is supported by the results of Mostafa et al. [19].

Free radical scavenging activity

Compounds concentration providing 50 % inhibition (IC_{50}) was calculated as per the method described by Bondet et al. [20]. Tests were carried out in triplicate, and ascorbic acid was used as a positive control. Standard curve was plotted for different concentrations of ascorbic acid. The results of antioxidant activity showed that few synthesized compounds exhibit considerable antioxidant activity. Chloro substitution **9d** at the *para* position of phenyl ring increases the antioxidant activity, which is also supported by Kumar et al. [21]. Substitution of bulky group like naphthol (**9h** and **7f**) also increases the activity, which is also favored by Bhalghat et al. [22].

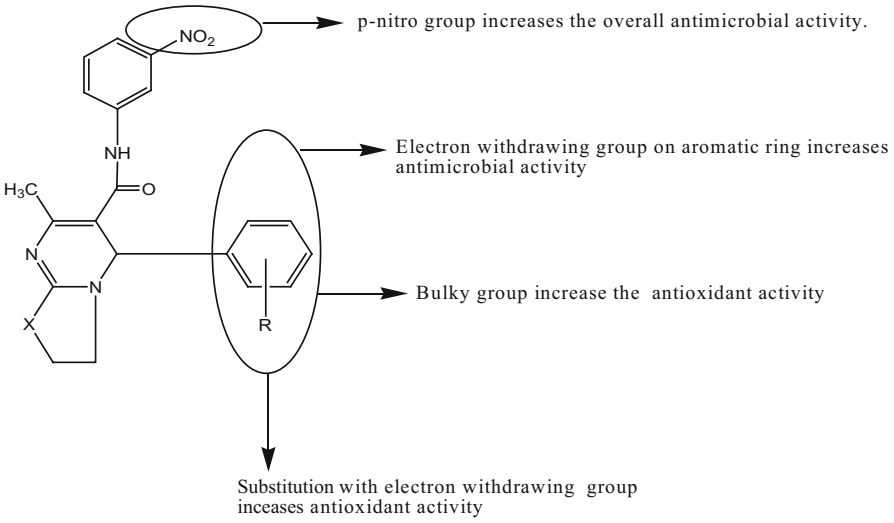


Fig. 1 Structure–activity relationship of the synthesized derivatives

Structure–activity relationship (SAR)

From the results of the antimicrobial and antioxidant activities of the synthesized substituted pyrimidine 6-carboxamide derivatives, the following structure–activity relationships can be derived (Fig. 1):

1. The results of antibacterial activity indicated that compound *7-methyl-5-(2-nitrophenyl)-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide* **7e** was found to be most potent among all the synthesized compounds. This may be due to the presence of the electron-withdrawing nitro group.
2. *p*-nitro group at *N*-substituted phenyl ring of all synthesized pyrimidine derivatives increases the overall antimicrobial activity.
3. Substitution of chloro group in compound **9d** at phenyl ring attached to pyrimidine ring increases the antioxidant activity.
4. Presence of bulky group in compound (**9h** and **7f**) also found to increase the antioxidant activity.

Conclusions

Two series of new thiazolo and imidazo pyrimidine derivatives were synthesized and their antimicrobial and antioxidant activities have been evaluated. Compound *7-methyl-5-(2-nitrophenyl)-N-(4-nitrophenyl)-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-carboxamide* **7e** was found to be the most active antimicrobial comparable to standards taken. Compounds **7a, 7c, 9a**, and **9d** also have shown significant antibacterial and antifungal activity. Compounds **7f**, **9d**, and **9h** have shown promising antioxidant activity with IC_{50} comparable with standard compound. Hence, this research represents a class of wide-spectrum antimicrobial agents and potential antioxidant agents, which can further be explored for future research.

References

1. M.K. Kathiravan, A.B. Salake, A.S. Chothe, P.B. Dudhe, R.P. Watode, M.S. Mukta, S. Gadhwe, Bioorg. Med. Chem. **20**, 5678–5698 (2012)
2. M.P. Patel, H.G. Kathrotiya, J. Chem. Sci. **125**, 993–1001 (2013)
3. B.A. Narayanan, N.K. Narayanan, G.D. Stoner, B.P. Bullock, Life Sci. **70**, 1821–1839 (2002)
4. B.B. Aggarwal, K.B. Harikumar, Int. J. Biochem. Cell Biol. **41**, 40–59 (2009)
5. A.C. Allison, R. Cacabelos, V.R. Lombardi, X.A. Alvarez, C. Vigo, Prog. Neuropsychopharmacol. Biol. Psychiatry **25**, 1341–1357 (2001)
6. K.S. Nimavat, A.D. Baldev, K.B. Vyas, K.B. Patel, J. Chem. Pharm. Res. **4**(6), 2972–2978 (2012)
7. J. Balzarini, C. Pannecouque, L. Naesens, G. Andrei, R. Snoeck, D.E. Clercq, Nucleosides, Nucleotides Nucleic Acids **23**, 1321–1327 (2004)
8. J. Morgan, R. Haritakul, P.A. Keller, Lett. Drug Des. Discov. **5**, 277–280 (2008)
9. A.E. Rashad, A.E. Mahmoud, M.M. Ali, Eur. J. Med. Chem. **46**, 1019–1026 (2011)

10. H.M. Faidallah, A.K. Khalid, M.A. Abdullah, J. Fluor. Chem. **132**, 870–877 (2011)
11. C.O. Kappe, Molecules **3**, 1–9 (1998)
12. S. Sigroha, B. Narasimhan, P. Kumar, A. Khatkar, K. Ramasamy, V. Mani, R.K. Mishra, A.A. Majeed, Med. Chem. Res. **21**, 3863–3875 (2011)
13. A. Khatkar, A. Nanda, P. Kumar, B. Narasimhan, Res. Chem. Intermed. **41**, 299–309 (2015)
14. A. Khatkar, A. Nanda, P. Kumar, B. Narasimhan, Arab. J. Chem. (2013) doi:10.1016/j.arabjc.2013.11.014 (in press)
15. C.O. Kappe, Acc. Chem. Res. **33**, 879–888 (2000)
16. J.D. Akbari, P.K. Kachhadia, S.D. Tala, A.H. Bapodra, M.F. Dhaduk, H.S. Joshi, K.B. Mehta, S.J. Pathak, Phosphorus, Sulfur Silicon Relat. Elem. **183**, 1911–1922 (2008)
17. J.G. Cappucino, N. Sherman, *Microbiology—A Laboratory Manual* (Addison Wesley, California, 1999), p. 263
18. Pharmacopoeia of India (Controller of Publications (Ministry of Health Department) Government of India, New Delhi, 2007), p. 37
19. A.H. Mostafa, M.A. Hussein, A.A. Radwan, A.N. Kfafy, Arch. Pharm. Res. **31**, 279–293 (2008)
20. V. Bondet, W. Brand-Williams, C. Berset, Food Sci. Technol. **30**, 609–615 (1997)
21. K.S. Kumar, A.V. Kanth, K.T. Reddy, G. Omprakash, J. Chem. Pharm. Res. **3**(5), 234–252 (2011)
22. C.M. Bhalgat, M.I. Ali, B. Ramesh, G. Ramu, Arab. J. Chem. **7**, 986–993 (2014)