Heterocyclic Letters
Vol. 10| No.3|447-457|May-July|2020
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS AND ASSESSMENT OF BIOLOGICAL ACTIVITY OF SOME NEW CHALCONES, AMINO PYRIMIDINES AND ISOXAZOLES DERIVATIVES INCORPORATING 1, 3, 5-TRIAZINE MOIETY

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Abstract
A series of new amino pyrimidines and isoxazoles derivativesof chalconeincorporating 1, 3, 5-triazine moiety as potential antimicrobial agents was designed, synthesized, and characterized by Elemental analysis, FTIR and NMR spectral techniques. All the synthesized compounds were screened in vitro against four bacterial strains (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa) and three fungal strains (Aspergillus nigerAspergillusclavatusand Candida albicans). The antimicrobial results indicated that some of the compounds showed remarkable activities comparable to the standard drugs.

Keywords
Chalcones, Amino pyrimidines, Isoxazoles, Spectral studies, Antimicrobial activity

Introduction

Chalcones are naturally occurring compound. These compounds go through many chemical reactions and it is helpful in the synthesis of heterocyclic compounds such as isoxazole, quinolinone, benzofuranone, indols and flavones, etc. Additionally, these compounds are vital intermediates in many addition reactions of nucleophiles owing to the inductive polarization of the carbonyl-functional group at the β-position. Many methods are reported for preparation of chalcones. They have a wide range of pharmacological activities depending on the substituted group of the two benzene rings of the chalcone[i]. Chalcone derivatives exhibit many medicinal activities like anti-inflammatory analgesic[ii,iii],anticancer[iv], antiviral[v], antifungal[vi,vii],antimicrobial[viii,ix], antioxidant[x], anti-histamine[xi] and anti-hyperglycemic activities[xii].

1,3,5-Triazine based chalcones and their derivatives have their own importance in heterocyclic chemistry due to their biological activities such asantimicrobial[xiii], antitubercular[xiv], antioxidant[xv], anticancer[xvi] etc. Chalcones are important precursors of many biologically active compounds such as pyrazoline, isoxazole, pyrimidine, indazole, benzodiazepine etc. Several pyrimidine derivatives have been developed as chemotherapeutic agents and have found wide clinical applications such as anticancer agents[xvi], antitumor[xvii], antiviral[xviii], anti-inflammatory[xix] etc. Five membered ring heterocycles like isoxazoles have been developing field within the realm of heterocyclic chemistry for the past several decades because of their wide range of biological activities such as antiprotozoal[xx], antiviral[xxi], antitubercular[xxii]. In view of this, it was thought of interest to synthesise some new isoxazoles, pyrimidines starting from chalcones.

Herein we wish to report on synthesis, spectral characterization, and evaluation of antimicrobial activity on some strains of microorganisms a series of novel amino pyrimidines and isoxazoles derivatives of chalcone, incorporating 1, 3, 5-triazine moiety. All new products were characterized by Elemental analysis, FTIR and NMR spectral data.

Experimental

Materials and measurements

All starting chemicals were commercially available and used asreceived, except for the solvents being purified by distillation.Purity of the compounds were checked by TLC using aluminum sheets Silica Gel 60 F-254 (Merck) plates of 0.25 mm thickness and detection of the components were made by exposure to UV light or keeping the plates in iodine chamber. Melting points of all synthesizedcompound were resolute in open capillary method and are uncorrected. Infrared spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using KBr pellets in the range 4,000-400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance (Bruker Scientific Corporation Ltd., Switzerland) DPX400 MHz spectrometer with $CDCl_3$ (^{1}H NMR) as a solvent and TMS as an internal standard at 400 and 100 MHz operating frequencies. IR, ^{1}H NMR and ^{13}C NMR data are consistent with the assigned structures. The chemical shifts are expressed in parts per million (ppm) downfield from the internal standard and signals are quoted as s (singlet), d (doublet) and m (multiplate). The coupling constants (J) are given in Hertz (Hz). All the newly synthesized compounds were analyzed for carbon, hydrogen and nitrogen by the PerkinElmer 2400 C H N series-II elemental analyser (PerkinElmer, USA).Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). Reference drugsare ampicillin, chloramphenicol, and ciprofloxacin as the standard antibacterial drugswhile Griseofulvin, Nystatinas standard antifungal drugs used of commercial grade.

General procedure for the compounds 1, 2, 3

The starting compounds **1**, **2** and **3** were prepared according to reported procedure[xxiii].

General procedure for the preparation of 2-(Ethylamino)-4-(tetrahydro-1',4'-oxazin)-6-[4'-{3''-(substitutedphenyl)-2''-propanon-1''-yl} phenylamino]-1,3,5-triazine

By applying classical Claisen-Schmidt condensation reaction,substituted acetophenone **(3)** (0.01 mol) and an appropriate various aldehyde (0.01 mol) dissolved in DMF in a 100 ml conical flask. 40% KOHsolution was added to make it alkaline. Then the reaction mixture was stirred for 24 hours on a magnetic stirrer at room temperature. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice, neutralized with dilute hydrochloric acid and the mixture was agitated a yellow solid was obtained. Finally, the product was isolated by filtration, crystallized from

ethanol to get product**(4a-4d)**.Synthetic pathway for preparation of title compounds is shown in reaction Scheme.

2-(Ethylamino)-4-(tetrahydro-1',4'-oxazin)-6-[4'-{3''-(2'''-methoxyphenyl)-2''-propanon-1''-yl} phenylamino]-1,3,5-triazine (4a):
Yellow solid, yield 81%, mp 108-110 °C. IR (KBr, ν_{max}, cm^{-1}): 3334 (NH), 3025 (=CH), 1654 (C=O), 1542 (C=C), 1433 (CH=CH), 1222 (C-O-C), 1045(C-F), 804 (C=N), 715 (C-H, 1,2-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.5 (4H, concealed t, CH_2), 3.4 (4H, concealed t, CH_2), 7.39 (1H, d, *J* = 9.8, CO-CH=), 8.17 (1H,CH=) 6.65 - 7.56 (8H, m, Ar-H), 8.2 (1H, d, *J*= 9.8, Ar-CH=), 8.3 (1H, s, NH), 1.56 (2H,q, CH_2), 0.96 (3H,t, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 46.5 (CH_2), 66.4 (CH_2), 156.7 (CF), 115.1 (CH), 129.5 (CH), 124.1 (CH), 128.0 (CH), 123.0 (C), 130.5 (CH), 127.4 (C), 116.8.0 (C), 148.5 (C), 23.0 (CH_2), 11.8 (CH_3), 163.6, 165.3 & 176.2 (C=N, 1,3,5-triazine), 1189.7 (CO), 121.5 (CH=), 145.0 (CH=); LCMS (m/z): 448.47 (M^+). Anal. calcd. for $C_{24}H_{25}N_6O_2F$:C 64.28; H 5.61; N 18.74 %. Found, %: C 64.25; H 5.59; N 18.70%.

2-(Ethylamino)-4-(tetrahydro-1',4'-oxazin)-6-[4'-{3''-(4'''-methoxyphenyl)-2''-propanon-1''-yl} phenylamino]-1,3,5-triazine (4b):
Yellow solid, yield 79%, mp 122-124 °C. IR (KBr, ν_{max}, cm^{-1}): 3336 (NH), 3029 (=CH), 1669 (C=O), 1538 (C=C), 1453 (CH=CH), 1356 (CH_3), 1219 (C-O-C), 1040 (C-F), 800 (C=N), 815 (C-H, 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.5 (4H, concealed t, CH_2), 3.4 (4H, concealed t, CH_2), 7.39 (1H, d, *J* = 9.8, CO-CH=), 8.17 (1H, CH=), 6.65-7.56 (8H, m, Ar-H), 8.2 (1H, d, *J*= 9.8, Ar-CH=), 8.3 (1H, s, NH), 1.56 (2H,q, CH_2), 0.96 (3H,t, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 46.5 (CH_2), 66.4 (CH_2), 156.7 (CF), 115.1 (CH), 129.5(CH), 124.1 (CH), 128.0 (CH), 123.0 (C), 130.5 (CH), 127.4 (C), 116.8.0 (C), 148.5 (C), 23.0 (CH2), 11.8 (CH_3), 163.6, 165.3 & 176.2 (C=N, 1,3,5-triazine), 189.7 (CO), 121.5(CH=), 145.0(CH=); LCMS (m/z): 448.47 (M^+). Anal. calcd. for $C_{24}H_{25}N_6O_2F$: C 64.28; H 5.61; N 18.74 %. Found, %: C 64.24; H 5.64; N 18.72 %.

2-(Ethylamino)-4-(tetrahydro-1',4'-oxazin)-6- [4'- {3''-(4'''-N, N'-dimethylaminophenyl)-2''-propanon-1''-yl} phenylamino]-1,3,5-triazine (4c):
Yellow solid, yield 72 %, mp 135-137 °C. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 3031 (=CH), 1690 (C=O), 1540 (C=C), 1456 (CH=CH), 1360 (CH_3), 1223 (C-O-C), 1522 (C-NO_2), 802 (C=N), 740 & 836 (C-H, 1,2 & 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.6 (3H, s, CH_3),1.9 (3H, s, CH_3), 3.3 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 6.5 (1H, d, *J* = 10.5, CO-CH=), 7.2 - 8.3 (8H, m, Ar-H), 8.4 (8H, d, *J*= 10.5, Ar-CH=), 8.6 (1H, s, NH);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 11.4 (CH_3), 46.2 (CH_2), 66.5 (CH_2), 111.2 (CH), 113.6 (CH), 115.2 (CH), 118.4 (CH), 120.7 (CH), 121.2 (=CH), 123.0 (CH), 128.3 (CH), 130.4 (C), 131.8 (CH), 133.3 (CH), 134.0 (C),137.8 (C), 139.1 (C), 143.2 (C), 145.5 (=CH), 147.8 (C), 165.2, 168.3 & 170.4 (C=N, 1,3,5-triazine), 187.6 (CO); LCMS (m/z): 475.48 (M^+). Anal. calcd. for $C_{24}H_{25}N_7O_4$: C 60.62; H 5.29; N 20.62 %. Found, %: C 60.61; H 5.28; N 20.59 %.

2-(Ethylamino)-4-(tetrahydro-1',4'-oxazin)-6-[4'-{3''-(4'''-methylphenyl)-2''-propanon-1''-yl} phenylamino]-1,3,5-triazine (4d):
Yellow solid, yield 78 %, mp 107-109°C. IR (KBr, ν_{max}, cm^{-1}): 3286 (NH), 3126 (=CH), 1680 (C=O), 1584 (C=C), 1489 (CH=CH), 1256 (C-O-C), 1520 (C-NO_2), 810 (C=N), 838 (C-H, 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 6.8 (1H, d, *J* = 7.5, CO-CH=), 7.0 - 8.2 (8H,

m, Ar-H), 8.4 (1H, d, *J*= 7.5, Ar-CH=), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm), 46.5 (CH_2), 66.4 (CH_2), 115.1 (CH), 129.5(CH), 124.1 (CH), 128.0 (CH), 123.0 (C), 130.5 (CH), 127.4 (C), 116.8.0 (C), 148.5 (C), 23.0 (CH2), 11.8 (CH_3), 1189.7 (CO), 121.5(CH=), 145.0(CH=) 165.7, 168.1 & 170.0 (C=N, 1,3,5-triazine), 186.3 (CO); LCMS (m/z): 475.48 (M^+). Anal. calcd. for $C_{24}H_{25}N_7O_4$: C 60.62; H 5.29; N 20.62 %. Found, %: C 60.60; H 5.30; N 20.60 %.

General procedure for the preparation of2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(substitutedphenyl)-isoxazol-3''-yl} phenylamino]-1,3,5-triazine(5a-5d)
A mixture of synthesized chalcones **(4a-4d)** (0.01 mole) and hydroxylamine hydrochloride (0.01 mol) in alcohol was refluxed for 6 h in presence of 40% KOH. The reaction mixture was then cooled, poured into crushed ice and product separated out was filtered, washed with water until neutral pH, dried and recrystallized from alcohol to give **(5a-5d).**Synthetic pathway for preparation of title compounds is shown in reaction Scheme.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(2'''-methoxyphenyl)-isoxazol-3''-yl} phenylamino]-1,3,5-triazine (5a):
White solid, yield 82 %, mp 118-120 °C. IR (KBr, ν_{max}, cm^{-1}):3240 (NH), 3026 (=CH), 2825 (C-H), 1531 (C=N), 1526 (C=C), 1240 (C-O-C), 1022 (C-F), 804 (C=N, 1,3,5-triazine), 783 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.6 (4H, concealed t, CH_2), 3.8 (4H, concealed t, CH_2), 6.4 (1H, s, CH-C), 6.8 - 7.9 (8H, m, Ar-H), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.4 (CH_2, oxazine), 68.2 (CH_2), 46.0 (CH_3), 113.3 (CH), 115.4 (CH), 117.8 (CH), 119.4 (C), 120.3 (C), 126.3 (CH), 128.4 (CH), 130.3 (CH), 140.4 (C), 158.1 (C=N), 160.0 (C-Ar), 160.2, 163.7& 165.6 (C=N, 1,3,5-triazine); LCMS (m/z): 461.47 (M^+). Anal. calcd. for $C_{24}H_{24}N_7O_2F$: C, 62.47; H, 5.24; N, 21.25 % Found: C, 62.45; H, 5.25; N, 21.26 %.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(4'''-methoxyphenyl)-isoxazol-3''-yl} phenylamino]-1,3,5-triazine (5b):
White solid, yield 82 %, mp 118-120 °C. IR (KBr, ν_{max}, cm^{-1}):): 3244 (NH), 3024 (=CH), 2828 (C-H), 1530 (C=N), 1528 (C=C), 1239 (C-O-C), 1025 (C-F), 805 (C=N, 1,3,5-triazine), 825 (C-H, 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.7 (4H, concealed t, CH_2), 3.8 (4H, concealed t, CH_2), 6.3 (1H, s, CH-C), 6.7 - 7.9 (8H, m, Ar-H), 8.4 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.2 (CH_2, oxazine), 68.0 (CH_2), 46.2 (CH_3), 113.1 (CH), 115.3 (CH), 117.7 (CH), 119.3 (C), 120.1 (C), 126.2 (CH), 128.5 (CH), 130.4 (CH), 140.5 (C), 158.2 (C=N), 160.1 (C-Ar), 160.0, 163.5& 165.8 (C=N, 1,3,5-triazine); LCMS (m/z): 461.47 (M^+). Anal. calcd. for $C_{24}H_{24}N_7O_2F$: C, 62.47; H, 5.24; N, 21.25 % Found: C, 62.48; H, 5.27; N, 21.24 %.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6- [4'- {2''-amino-6''-(4'''-N, N'-dimethylaminophenyl)-isoxazol-3''-yl} phenylamino]-1,3,5-triazine (5c):
White solid, yield 68 %, mp 145-147 °C. IR (KBr, ν_{max}, cm^{-1}): 3244 (NH), 3024 (=CH), 2828 (C-H), 1530 (C=N), 1528 (C=C), 1239 (C-O-C), 1330 (C-NO_2), 805 (C=N, 1,3,5-triazine), 745 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.6 (4H, concealed t, CH_2), 3.7 (4H, concealed t, CH_2), 6.4 (1H, s, CH-C), 6.8 – 8.0 (8H, m, Ar-H), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.3 (CH_2, oxazine), 68.1 (CH_2), 46.2 (CH_3), 113.2 (CH), 115.3 (CH), 117.8 (CH), 119.4 (C), 120.2 (C), 126.3 (CH), 128.4 (CH), 130.6 (CH), 140.7 (C), 158.4 (C=N), 160.2 (C-Ar), 160.1, 163.6& 165.9 (C=N, 1,3,5-

triazine); LCMS (m/z): 488.48 (M^+). Anal. calcd. for $C_{24}H_{24}N_8O_4$: C, 59.01; H, 4.95; N, 22.94 % Found: C, 59.03; H, 4.97; N, 22.93%.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(4'''-methylphenyl)-isoxazol-3''-yl} phenylamino]-1,3,5-triazine (5d):
White solid, yield 73 %, mp 119-121 °C. IR (KBr, ν_{max}, cm^{-1}): 3244 (NH), 3024 (=CH), 2828 (C-H), 1530 (C=N), 1528 (C=C), 1239 (C-O-C), 1330 (C-NO_2), 805 (C=N, 1,3,5-triazine), 735 (C-H, 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.6 (4H, concealed t, CH_2), 3.7 (4H, concealed t, CH_2), 6.3 (1H, s, CH-C), 6.9 – 8.0 (8H, m, Ar-H), 8.4 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.4 (CH_2, oxazine), 68.3 (CH_2), 46.4 (CH_3), 113.1 (CH), 115.1 (CH), 117.5 (CH), 119.2 (C), 120.1 (C), 126.1 (CH), 128.4 (CH), 130.4 (CH), 140.5 (C), 158.2 (C=N), 160.1 (C-Ar), 160.2, 163.4& 165.2 (C=N, 1,3,5-triazine); LCMS (m/z): 488.48 (M^+). Anal. calcd. for $C_{24}H_{24}N_8O_4$: C, 59.01; H, 4.95; N, 22.94 % Found: C, 59.05; H, 4.93; N, 22.95%.

General procedure for the preparation of2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(substitutedphenyl)-pyrimidin-4''-yl} phenylamino]-1,3,5-triazine(6a-6d)
Entitled compound was prepared at reflux temperature by condensation reaction of prepared chalcone**(4a-4d)** (0.01mole), in 250ml round bottom flask with guanidine hydrochloride by using alcohol as solvent and 40% NaOH solution was added to make pH basic**.** The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice, neutralized with dilute hydrochloric acid and the mixture was agitated for 5 to 10 minutes. The product was separated by filtration and recrystallized from ethanol**(6a-6d)**.Synthetic pathway for preparation of title compounds is shown in reaction Scheme.

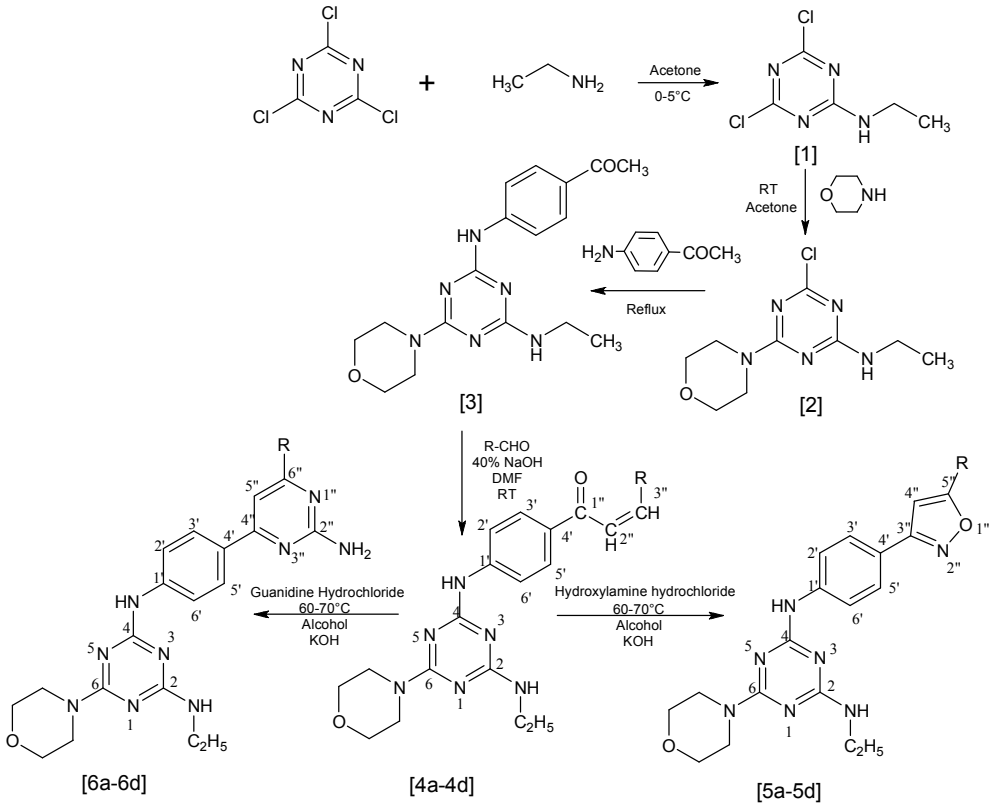
2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(2'''-methoxyphenyl)-pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (6a):
White solid, yield 78 %, mp 125-127 °C. IR (KBr, ν_{max}, cm^{-1}): 3442 (-NH_2), 3340 (-NH), 3010 (=CH), 2880 (C-H), 1570 (C=N), 1512 (C=C), 1230 (C-O-C), 1025 (C-F), 801 (C=N, 1,3,5-triazine), 771 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4 (4H, concealed t, CH_2), 3.7 (4H, concealed t, CH_2), 4.0 (-NH-), 3.0 (2H, q, CH_2), 1.2 (3H, t, CH_3), 6.52-7.23 (8H, Ar), 6.90 (-CH-), 4.0 (-NH_2);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 128.3 (-CH), 123.1 (C), 116.8 (CH), 143.1 (C), 23.3 (CH_2), 11.5 (CH_3), 46.3 (CH_2), 66.4 (C-O-C), 163.7 (C), 160.4 (C), 95.0 (CH), 163.6 (C), 158.4 (C-F), 116.0 (CH), 130.4 (CH), 124.9 (CH), 129.1 (CH), 123.5 (C)164.5, 165.4 & 167.1 (C=N, 1,3,5-triazine); LCMS (m/z): 487.51 (M^+). Anal. calcd. for $C_{25}H_{26}N_9OF$: C, 61.59; H, 5.37; N, 25.86 % Found: C, C, 61.57; H, 5.39; N, 25.84%.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(4'''-methoxyphenyl)-pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (6b):
White solid, yield 71 %, mp 131-133 °C. IR (KBr, ν_{max}, cm^{-1}): 3440 (-NH_2), 3342 (-NH), 3011 (=CH), 2879 (C-H), 1572 (C=N), 1513 (C=C), 1231 (C-O-C), 1024 (C-F), 800 (C=N, 1,3,5-triazine), 770 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.5 (4H, concealed t, CH_2), 3.6 (4H, concealed t, CH_2), 4.1 (-NH-), 3.1 (2H, q, CH_2), 1.1 (3H, t, CH_3), 6.50-7.24 (8H, Ar), 6.91 (-CH-), 3.9 (-NH_2);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 127.3 (-CH), 122.1 (C), 117.8 (CH), 144.1 (C), 23.1 (CH_2), 11.7 (CH_3), 46.0 (CH_2), 66.6 (C-O-C), 163.9 (C), 160.2 (C), 95.1 (CH), 163.4 (C), 158.3 (C-F), 116.0 (CH), 130.3 (CH), 124.8 (CH), 129.2 (CH), 123.6 (C), 164.4, 165.5 & 167.2 (C=N, 1,3,5-triazine);

LCMS (m/z): 487.51 (M^+). Anal. calcd. for $C_{25}H_{26}N_9OF$: C, 61.59; H, 5.37; N, 25.86 % Found: C, C, 61.56; H, 5.38; N, 25.85%.

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6- [4'- {2''-amino-6''-(4'''-N, N'dimethylaminophenyl)-pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (6c):
White solid, yield 70 %, mp 115-117 °C. IR (KBr, ν_{max}, cm^{-1}): 3445 (-NH_2), 3341 (-NH), 3012 (=CH), 2878 (C-H), 1575 (C=N), 1514 (C=C), 1232 (C-O-C), 1330 (C-NO_2), 802 (C=N, 1,3,5-triazine), 774 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4(4H, concealed t, CH_2), 3.7 (4H, concealed t, CH_2), 4.0 (-NH-), 3.1 (2H, q, CH_2), 1.0 (3H, t, CH_3), 6.51-7.23 (8H, Ar), 6.92 (-CH-), 3.91 (-NH_2);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 127.4 (-CH), 122.2 (C), 117.7 (CH), 144.1 (C), 23.2 (CH_2), 11.8 (CH_3), 46.1 (CH_2), 66.5 (C-O-C), 163.8 (C), 160.1 (C), 95.2 (CH), 163.3 (C), 158.4 (C-F), 116.1 (CH), 130.2 (CH), 124.9 (CH), 129.1 (CH), 123.5 (C), 164.5, 165.4 & 167.1 (C=N, 1,3,5-triazine); LCMS (m/z): 514.52 (M^+). Anal. calcd. for $C_{25}H_{26}N_{10}O_3$: C, 58.36; H, 5.09; N, 27.22 % Found: C, 58.34; H, 5.10; N, 27.24

2-Ethyamino-4-(tetrahydro-1',4'-oxazin)-6-[4'-{2''-amino-6''-(4'''-methylphenyl)-pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (6d):
White solid, yield 76 %, mp 121-123 °C. IR (KBr, ν_{max}, cm^{-1}): 3443 (-NH_2), 3348 (-NH), 3015 (=CH), 2880 (C-H), 1580 (C=N), 1515 (C=C), 1235 (C-O-C), 1335 (C-NO_2), 805 (C=N, 1,3,5-triazine), 778 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.2 (4H, concealed t, CH_2), 3.5 (4H, concealed t, CH_2), 4.1 (-NH-), 3.1 (2H, q, CH_2), 1.1 (3H, t, CH_3), 6.55-7.23 (8H, Ar), 6.90 (-CH-), 3.89 (-NH_2);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 126.4 (-CH), 122.8 (C), 118.0 (CH), 144.0 (C), 23.0 (CH_2), 12.0 (CH_3), 46.3 (CH_2), 66.4 (C-O-C), 163.6 (C), 160.5 (C), 95.0 (CH), 163.6 (C), 158.1 (C-F), 116.6 (CH), 130.0 (CH), 125.0 (CH), 129.0 (CH), 123.8 (C), 164.7, 165.3 & 167.3 (C=N, 1,3,5-triazine); LCMS (m/z): 514.52 (M^+). Anal. calcd. for $C_{25}H_{26}N_{10}O_3$: C, 58.36; H, 5.09; N, 27.22 % Found: C, 58.33; H, 5.07; N, 27.25



R= C_6H_4-2-OCH_3, C_6H_4-4-OCH_3, C_6H_4-4-N $(CH_3)_2$, C_6H_4-4-CH_3

Scheme: Synthetic approach for the formation of design compounds

Results and discussion

The structures of all the synthesized compounds areconfirmed from their spectral and analytical data. As an example, in the IR spectrum of compound **4a**, a strong absorption band is observed at 1435 and 1660cm^{-1} which corresponds to the stretching vibration of the CH=CH and C=O functionality of α, β- unsaturated carbonyl group of chalcone moiety. The C-H bending vibrations for 1,2 disubstituted benzene rings were appeared at 705 cm^{-1}. The C=N stretching of 1,3,5-triazine nucleus and C=C functionality of aromatic ring were observed at 806and 1540 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **4a** showed a doublet at δ 7.29(*J* = 8.2 Hz) ppm for the -CO-CH= proton and at δ 7.46(*J* = 7.72 Hz)ppm for the Ar-CH= proton of α, β unsaturated carbonyl group protons. Finally, the ^{13}C NMR spectrum of the compound **4a** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structure. The most deshielded signal that appeared at δ 189.7 ppm was assigned to the carbonyl carbon of the chalcone moiety. The signal for CH=CH functionality of α, β- unsaturated carbonyl group was appeared at δ 121.0 and 145.0 ppm. The signals for aromatic carbons appeared between at δ 115.1-156.7 ppm in the ^{13}C spectrum. The IR spectrum of compound**5a** exhibited the disappearance of absorption at 1660 cm^{-1} corresponding to >C=O group of chalcone and exhibited a strong absorption band at 1535 cm^{-1} due to the C=N functionality of isoxazole unit. The C-H functionality of isoxazole unit was observed at 2820 cm^{-1}. The aromatic C=C stretching, C-H bending vibrations for 1,2-disubstituted benzene ring appeared at 1525 and 785 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core was observed at 802 cm^{-1}. The ^{1}H NMR spectrum of compound **5a** showed chiral C_4"-H proton of isoxazole ring which appeared as singlet at δ 6.4 for CH-C proton. The other remaining eight aromatic protons resonate as a multiplet signal at δ 6.5-7.4.

Finally, the ^{13}C NMR spectrum of compound **5a** showed a signal at δ 161.1 and 169.0 due to the -C=N and C-Ar carbon of isoxazole moeity which was also assigned to the isoxazole unit. The signals for aromatic carbons fall in the range of δ 114.8 to 157.2 in ^{13}C NMR spectrum. The IR spectrum of compound**6a** exhibited the disappearance of absorption at 1660 cm^{-1} corresponding to >C=O group of chalcone and exhibited a strong absorption band at 3442 cm^{-1} due to the NH_2 functionality of amino unit. The aromatic C=C stretching, C-H bending vibrations for 1,2-disubstituted benzene ring appeared at 1515 and 780 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core was observed at 804 cm^{-1}. The ^1H NMR spectrum of compound **6a**showed at 4.1 due to NH_2 group. Finally, the ^{13}C NMR spectrum of compound **6a** showed a signal at δ 163.3 due to C-NH_2.The signals for aromatic carbons falls in the range of δ 116-163.9 in ^{13}C NMR spectrum.Moreover, there are no absorptions in the region of 1600-1700 cm^{-1} in IR spectra of compound **5a** and **6a** which indicating the absence of a C=O group of chalcone moiety in these structures and further confirmed the cyclization of chalcone in toiosxazoles amino pyrimidines derivatives.
Furthermore, the mass spectrum of compounds **4a**, **5a** and **6a** showed M^+ peak at m/z 576.3, 666.4 and 588.3 (100%) respectively along with other fragment peaks, which further supported the structure of compounds **4a**, **5a** and **6a**. The obtained elemental analysis values are also in good agreement with theoretical data.

In vitro antimicrobial activity

The entire synthesized compounds (4a-4d), (5a-5d) and (6a-6d) were evaluated for their antibacterial and antifungal activities by using ampicillin, chloramphenicol as standard antibacterial drugs. Antifungal activity was screened against two fungal species by using griseofulvin and nystatin as the standard antifungal drug.

All the synthesised compounds were evaluated for their antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and two Gram-negative bacteria (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 441) by using ampicillin, chloramphenicol and ciprofloxacin as the standard antibacterial drugs. Antifungal activity was screened against three fungal species (*Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323) by using griseofulvin and nystatin as the standard antifungal drugs. The minimal inhibitory concentration (MIC) values of all the synthesised compounds were determined in terms of µg/ml by the Broth micro dilution method according to National Committee for Clinical Laboratory Standards[xxiv]. The results are summarised in **Table 1**.

The antibacterial screening of chalcones **[4a-4d]**, isoxazoles**[5a-5d]**and amino pyrimidines**[6a-6d]** pointed out that in Gram-positive bacteria, compound **6d** (MIC=62.5 µg/ml) showed outstanding activity and compounds **5b, 6a, 6c**(MIC=100 µg/ml) showed an outstanding inhibitory effect against *Staphylococcus aureus* as compared to ampicillin (MIC=250 µg/ml) and admirable to chloramphenicol and ciprofloxacin (MIC=50 µg/ml). Compounds **4b**and **6b** (MIC=125µg/ml) and**4c, 5a** (MIC=200 µg/ml) showed good potential activity to ampicillin (MIC=250 µg/ml) and**4a, 5c, 5d** possess equipotential activity to ampicillin (MIC=250 µg/ml) and modest to chloramphenicol and ciprofloxacin (MIC=50 µg/ml) against *Staphylococcus aureus* organism. In case of inhibiting *Streptococcus pyogenes*, compounds**4b, 4d, 5b** (MIC=62.5 µg/ml) exhibited excellent activity compared to ampicillin (MIC=100 µg/ml) while compound **4c**(MIC=100 µg/ml) demonstrate inhibitory effect as same as ampicillin (MIC=100 µg/ml) and less effective than chloramphenicol and ciprofloxacin (MIC=50 µg/ml) whereas compounds **6a**and **6d** (MIC=125 µg/ml) exerted

significant potential to ampicillin (MIC=100 µg/ml) and less potential to chloramphenicol and ciprofloxacin (MIC=50 µg/ml) against *Streptococcus pyogenes*.

In case of inhibiting Gram-negative bacteria, compound**6c** (MIC=62.5 µg/ml) demonstrated excellent activity compared to ampicillin (MIC=100 µg/ml) while compounds**4d, 5b, 6a** (MIC=100 µg/ml) showed equipotential to ampicillin (MIC=100 µg/ml) and less potential to chloramphenicol (MIC=50 µg/ml) and ciprofloxacin (MIC=25 µg/ml) against *Escherichia coli*. Compound **5c**(MIC = 62.5 µg/ml) showed excellent activity against ampicillin (MIC=100 µg/ml) and compound **4a, 4d, 5d**(MIC=100 µg/ml) exerted equipotent to ampicillin (MIC=100 µg/ml), mild to chloramphenicol (MIC=50 µg/ml) and modest ciprofloxacin (MIC=25 µg/ml) against *Pseudomonas aeruginosa*. The remaining compounds showed moderate to good activity to inhibit the growth of bacterial pathogens and were found less effective than the employed standard drugs. The antibacterial results revealed that most of the prepared compounds showed improved activity against the Gram-negative bacteria rather than Gram-positive bacteria.

From *in vitro* antifungal activity data, it is found that compounds **5b, 6b** (MIC=100 µg/ml) displayed highest antifungal activity against *Candida albicans* as compared to griseofulvin (MIC=500 µg/ml) and modest to nystatin (MIC=100 µg/ml) while compounds **4b, 4c, 4d**, **5a**, **5c, 5d, 6a and 6d** (MIC=500 µg/ml) showed the same potency as griseofulvin (MIC=500 µg/ml) against *Candida albicans*. Compound**4b** (MIC=100 µg/ml) depicted equipotent to griseofulvin (MIC=100 µg/ml) and nystatin (MIC=100 µg/ml) against *Aspergillus niger*. Compound**6c**(MIC=250 µg/ml) found comparable to griseofulvin (MIC=100 µg/ml) and nystatin (MIC=100 µg/ml) against *Aspergillus clavatus*.

Table 1
*In vitro*antimicrobialactivity of the synthesized compounds **[4a-4d], [5a-5d]** and **[6a-6d]**

***Antimicrobial* activity (MIC) µg/ml**							
Entry	**Antibacterial activity**				**Antifungal activity**		
	Gram Positive Bacteria		**Gram Negative Bacteria**		**Fungus**		
	S.aureus	***S.pyogenes***	***E.coli***	***P.aerug***	***C.albican***	***A. niger***	***A.clavatus***
4a	250	200	250	100	>1000	500	>1000
4b	125	62.5	125	50	500	100	>1000
4c	200	100	250	250	500	1000	1000
4d	500	62.5	100	100	500	1000	>1000
5a	200	250	125	200	500	1000	>1000
5b	100	62.5	100	125	100	500	500
5c	250	200	250	62.5	500	500	1000
5d	250	250	125	100	500	>1000	>1000
6a	100	125	100	125	500	1000	1000
6b	125	200	200	250	100	500	1000
6c	100	200	62.5	500	1000	250	500
6d	62.5	125	200	250	500	1000	1000
A	250	100	100	100	–	–	–
B	50	50	50	50	–	–	–

C	50	50	25	25	–	–	–
D	–	–	–	–	500	100	100
E	–	–	–	–	100	100	100

A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Greseofulvin, E: Nystatin

Conclusion

The aim of the present study is to develop an efficient protocol to obtain chalcones and it's derivatives with good to excellent yield. The FT-IR, ^{1}H NMR ^{13}C NMR and elemental analysis of all the newly synthesized compounds confirmed that purity of the entire synthesized compound is showing significant antimicrobial activity.

Acknowledgement

Author is thankful to the Atul Ltd. (Atul) Valsad, Gujarat for the FTIR analysis, RSIC Punjab University for the 1H NMR as well as elemental analysis.

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Received on April 10, 2020.