DESIGN, SYNTHESIS AND EVALUATION OF SOME NEW ISOXAZOLES, CYANO-PYRIDINES AND 1,5 BENZOTHIAZEPINES AS ANTIMICROBIAL AGENTS

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ABSTRACT: The title compounds (7a-f), (8a-f) and (9a-f) have been prepared from chalcones (6a-f) having s-triazine nucleus. These chalcones on cyclisation with hydroxyl amine hydrochloride in the presence of alkali and malononitrile in the presence of ammonium acetate give isoxazoles (7a-f) and cyanopyridines (8a-f) respectively. Chalcones (6a-f) on condensation with 2-amino thiophenol in the presence of glacial acetic acid give benzothiazepines (9a-f). Structures of newly synthesised compounds were established on the basis of their elemental analysis, IR and 1H NMR. All the synthesised compounds have been screened for their antimicrobial activity.

INTRODUCTION: In recent years, the synthesis of novel isoxazole derivatives remains a main focus of medicinal research. Isoxazoles have found wide application as pharmaceutical and agrochemical agents. The synthesis of isoxazole derivatives has attracted considerable attention from organic and medicinal chemists due to their considerable bioactivity. Various biological applications have been reported for isoxazoles such as antileukemia 1, anthelmintic 2, antimicrobial 3, antitubercular 4 etc… activities. Substituted pyridine derivatives like cyanopyridines have found to possess different biological activities such as antihistaminic 5, antiproliferative 6, antitumor 7, antihypertensive 8, cardiovascular 9 etc… activities. Benzothiazepine is a benzo - annelated example of thiazepine. Thiazepine is a seven membered ring compound containing N and S as hetero atoms. Literature survey reveals that 1,5-benzothiazepines possess wide spectrum of pharmacological activities such as hypertensive 10, spasmyltic 11, antiucler 12,13 etc… activities. In continuation of our work 14-17 and the scope for further studies on chalcones and its derivatives, we herein report some novel isoxazoles (7a-f), cyanopyridines (8a-f) and benzothiazepines (9a-f).

EXPERIMENTAL SECTION: All melting points were determined in open capillary and are uncorrected. The IR spectra were recorded on Perkin-Elmer 237 spectrophotometer. 1H NMR spectra were recorded on a Bruker Avance DPX 400 MHz spectrometer with CDCl3 as a solvent and tetramethylsilane (TMS) as internal standard.

Thin Layer Chromatography (TLC) analytical separation was conducted with Silica Gel 60 F-254 (Merck) plates of 0.25mm thickness eluted with visualized with UV (254nm) or iodine to check the purity of the synthesised compounds.

General procedure for the compounds (3), (4), (5) and (6): Compounds (3), (4), (5) and (6) were prepared by the reported method 18.
Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(2′-methoxyphenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7a) : Compound 6a (0.01 mol) was dissolved in alcohol (25ml) and hydroxylamine hydrochloride (0.01 mol) was added to it. Then solution of KOH (5ml of 40%) was added to the reaction mixture and refluxed for 8 hours. The progress of the reaction was monitored on TLC plate. After completion, the reaction mixture was cooled and poured into crushed ice and neutralised with dilute HCl. The product separated out was filtered, washed with water, dried and recrystallised from alcohol to give 7a.

Similarly, the remaining compounds (7b-f) were prepared by this method.

(7a): mp 165°C, IR (KBr) cm⁻¹: 3410 (N-H str.), 3077 (=CH str.), 804 (C-N str., s-triazine moiety), 831 (C-H bending), 1636 (C=N str, isoxazoles moiety), 1278 (C-O-C str.); ¹H NMR (CDCl₃) δ: 3.52 (t, 8H, oxazine ring), 3.63 (t, 8H, oxazine ring), 3.81 (3H, s, o- OCH₃), 6.81 (1H, s, -CH=), 7.0 – 8.0 ( 9H, m, Ar-H and -NH). (Found : C, 62.89; H, 5.62; N, 19.00. Calcd. for C₂₇H₂₉N₄O₃: C, 62.90; H, 5.67; N, 19.02 %).

Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(2′-nitrophenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7b): mp 165°C, IR (KBr) cm⁻¹: 3405 (N-H str.), 3066 (=CH str.), 809 (C-N str., s-triazine moiety), 822 (C-H bending), 1625 (C=N str, isoxazoles moiety), 1566 (C-NO₂ str.), 1095; ¹H NMR (CDCl₃) δ: 3.49 (t, 8H, oxazine ring), 3.60 (t, 8H, oxazine ring), 6.78 (1H, s, -CH=), 7.1 – 8.3 (9H, m, Ar-H and -NH). (Found : C, 70.09; H, 4.60; N, 16.80. Calcd. for C₂₆H₂₆N₈O₃: C, 64.32; H, 5.60; N, 20.19 %).

Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(3′′,4′′-methylphenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7c): mp 159°C, IR (KBr) cm⁻¹: 3400 (N-H str.), 3051 (=CH str.), 801 (C-N str., s-triazine moiety), 831 (C-H bending), 1645 (C=N str, isoxazoles moiety), 1250 (C-O-C str.); ¹H NMR (CDCl₃) δ: ¹H NMR (CDCl₃) δ: 3.52 (t, 8H, oxazine ring), 3.63 (t, 8H, oxazine ring), 3.81 (3H, s, m-OCH₃), 3.81 (3H, s, p-OCH₃), 6.81 (1H, s, -CH=), 7.0 – 8.0 ( 8sH, m, Ar-H and -NH). (Found : C, 61.60; H, 5.71; N, 17.94. Calcd. for C₂₈H₃₁N₇O₅: C, 61.64; H, 5.73; N, 17.97 %).

Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(3′-chlorophenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7d): mp 162°C, IR (KBr) cm⁻¹: 3405 (N-H str.), 3066 (=CH str.), 809 (C-N str., s-triazine moiety), 822 (C-H bending), 1625 (C=N str, isoxazolmoiety), 1096 (C-Cl str); ¹H NMR (CDCl₃) δ: ¹H NMR (CDCl₃) δ: 3.46 (t, 8H, oxazine ring), 3.69 (t, 8H, oxazine ring), 6.69 (1H, s, -CH=), 7.0 – 8.5 ( 9H, m, Ar-H and -NH). (Found : C, 60.04; H, 5.02; N, 18.83. Calcd. for C₂₆H₂₆ClN₇O₃: C, 60.60; H,5.04; N, 18.86 %).

Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(3′′,4′′-methylphenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7e): mp 132°C, IR (KBr) cm⁻¹: 3405 (N-H str.), 3066 (=CH str.), 809 (C-N str., s-triazine moiety), 822 (C-H bending), 1625 (C=N str, isoxazolmoiety), 1266 (C-O-C str.); ¹H NMR (CDCl₃) δ: ¹H NMR (CDCl₃) δ: 3.21 (3H, s, o-CH₃), 3.41 (3H, s, m-CH₃), 3.52 (t, 8H, oxazine ring), 3.63 (t, 8H, oxazine ring), 6.81 (1H, s, -CH=), 7.0 – 8.0 ( 8H, m, Ar-H and -NH). (Found : C, 65.45; H, 6.06; N, 19.07. Calcd. for C₂₉H₃₁N₇O₃: C, 65.48; H, 6.08; N, 19.09 %).

Preparation of 2,4-bis-(tetrahydro-1′,4′-oxazine)-6-[4′-{5"-(4′′-N,N-diethylphenyl)-isoxazol-3′-yl}] phenylamino]-s-triazine (7f): mp 215°C, IR (KBr) cm⁻¹: 3405 (N-H str.), 3066 (=CH str.), 809 (C-N str., s-triazine moiety), 822 (C-H bending), 1625 (C=N str, isoxazolmoiety); ¹H NMR (CDCl₃) δ: 3.32 (4H, q, N-CH₂), 3.32 (6H, t, -CH₃), 3.50 (t, 8H, oxazine ring), 3.61 (t, 8H, oxazine ring), 6.81 (1H, s, -CH=), 7.0 – 8.0 ( 9H, m, Ar-H and -NH). (Found : C, 64.35; H, 6.50; N, 20.11. Calcd. for C₃₀H₃₆N₈O₃: C, 64.37; H, 6.52; N, 20.13 %).

Preparation of 2, 4-bis-(tetrahydro-1′, 4′-oxazine)-6-[4′-{2″-amino-3″-cyano-4″-(2″-methoxyphenyl) – pyridin-6″-yl}] phenyl amino]-s-triazine (8a).

Compound 6a (0.01 mol) was dissolved in ethyl alcohol (25ml) malononitrile (0.01 mol) and ammonium acetate were added to it and Refluxed for 10 hours. The progress of the reaction was monitored on TLC plate. After completion, the reaction mixture was cooled and poured into crushed ice and neutralised with dilute HCl. The product separated out was filtered, washed with water, dried and recrystallised from alcohol to give 8a.
Similarly, the remaining compounds (8b-f) were prepared by this method.

(8a): mp 105°C, IR (KBr) cm⁻¹: 3410 (N-H str.), 3062 (=CH str.), 809 (C-N str, s-triazine moiety), 1618 (C=N str, cyanopyridine moiety), 1250 (C-O-C str.); ¹H NMR (CDCl₃) δ: 3.53 (t, 8H, oxazine ring ), 3.67 (t, 8H, oxazine ring ), 3.87 (3H, s, O-CHOCH₃ ), 6.8 (2H, s, -NH₂), 7.0 – 8.0 (10H, m, Ar-H and -NH); (Found : C, 63.67; H, 5.50; N, 22.25. Calcd. for C₃₀H₃₁N₉O₃: C, 63.70; H, 5.52; N, 22.29 %).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”-amino-3”-cyano-4”- (2”-nitrophenyl) – pyridin-6”-yl} phenyl amino]-s-triazine (8b): mp 168°C, IR (KBr) cm⁻¹: 3409 (N-H str.), 3065 (=CH str.), 811 (C-N str, s-triazine moiety), 1575 (C=N str., cyanopyridine moiety). 692 (C-NO₂ str.); ¹H NMR (CDCl₃): ¹H NMR (CDCl₃) δ: 3.55 (t, 8H, oxazine ring ), 3.69 (t, 8H, oxazine ring ), 6.91 (2H, s, -NH₂), 7.2 – 8.3 (10H, m, Ar-H and -NH); (Found: C, 59.96; H, 4.84; N, 24.08 Calcd. For C₂₉H₂₈N₁₁O₄: C, 59.99; H, 4.86; N, 24.12 %).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”-amino-3”-cyano-4”- (3”’,4”’-methyleneoxy phenyl) – pyridin-6”-yl} phenyl amino]-s-triazine (8c): mp 162°C, IR (KBr) cm⁻¹: 3407 (N-H str.), 3069 (=CH str.), 844 (C-N str, s-triazine moiety), 1590 (C=N str, cyanopyridine moiety), 1257 (C-O-C str.); ¹H NMR (CDCl₃): δ ppm: ¹H NMR (CDCl₃) δ: 3.53 (t, 8H, oxazine ring ), 3.67 (t, 8H, oxazine ring ), 3.71 (3H, s, O-CHOCH₃), 3.86 (3H, s, p-OCH₃), 6.8 (2H, s, -NH₂), 7.0 – 8.0 (9H, m, Ar-H and -NH); (Found: C, 62.48; H, 5.55; N, 21.13 Calcd. For C₃₁H₃₃N₁₀O₄: C, 62.51; H, 5.58; N, 21.16%).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”-amino-3”-cyano-4”- (3”-chlorophenyl) – pyridin-6”-yl} phenyl amino]-s-triazine (8d): mp 190°C, IR (KBr) cm⁻¹: 3432 (N-H str.), 3070 (=CH str.), 813 (C-N str, s-triazine moiety), 1580 (C=N str, cyanopyridine moiety), 798 (C-Cl str.); ¹H NMR (CDCl₃): δ ppm: ¹H NMR (CDCl₃) δ: 3.55 (t, 8H, oxazine ring ), 3.69 (t, 8H, oxazine ring ), 6.91 (2H, s, -NH₂), 7.2 – 8.3 (10H, m, Ar-H and -NH); (Found: C, 61.10; H, 4.92; N, 22.11 Calcd. For C₂₉H₂₈ClN₉O₂: C, 61.11; H, 4.95; N, 22.12 %).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”-amino-3”-cyano-4”- (3”’,4”’-methylphenyl) – pyridin-6”-yl} phenyl amino]-s-triazine (8e): mp 187°C, IR (KBr) cm⁻¹: 3488 (N-H str.), 3067 (=CH str.), 888 (C-N str, s-triazine moiety), 1578 (C=N str, cyanopyridine moiety); ¹H NMR (CDCl₃) ¹H NMR (CDCl₃) δ: 3.29 (3H, s, O-CHOCH₃), 3.47 (3H, s, m-CH₃), 3.55 (t, 8H, oxazine ring ), 3.69 (t, 8H, oxazine ring ), 6.91 (2H, s, -NH₂), 7.2 – 8.3 (9H, m, Ar-H and -NH); (Found: C, 66.04; H, 5.65; N, 22.34 Calcd. For C₃₁H₃₃N₁₀O₂: C, 66.06; H, 5.68; N, 22.36%).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”-amino-3”-cyano-4”- (4”-N,N-diethyl phenyl) – pyridin-6”-yl} phenyl amino]-s-triazine (8f): mp 155°C, IR (KBr) cm⁻¹: 3407 (N-H str.), 3070 (=CH str.), 813 (C-N str, s-triazine moiety), 1580 (C=N str, cyanopyridine moiety); ¹H NMR (CDCl₃): δ ppm: ¹H NMR (CDCl₃) δ: 3.32 (4H, q, N-CH₂), 3.32 (6H, t, -CH₃), 3.55 (t, 8H, oxazine ring), 3.69 (t, 8H, oxazine ring), 6.91 (2H, s, -NH₂), 7.2 – 8.3 (10H, m, Ar-H and -NH); (Found: C, 64.32; H, 5.59; N, 24.19 Calcd. For C₃₃H₃₈N₁₀O₂ : C, 64.34; H, 5.92; N, 24.21 %).

Preparation of 2,4-bis-(tetrahydro-1’,4’-oxazine)-6-[4’-{2”- (2”’-methyleneoxy phenyl) –2”’,3”’ -dihydro -1”’,5”-benzothiazepine-4”-yl} phenyl amino]-s-triazine (9a): Compound (6a) (0.01mol) and 2- amino thiophenol (0.01mol) in alcohol (30 mL) were refluxed for 12 hours in the presence of glacial acetic acid (5 mL).The progress of reaction was monitored on TLC plate. The reaction mixture was then cooled and poured into crushed ice. The product separated out was filtered, washed with water, dried and crystallised from alcohol to give (9a).

Similarly, the remaining compounds (9b-f) were prepared by this method.

(9a): mp 60°C, IR (KBr) cm⁻¹: 3025 (=CH str), 1556 (C=C str), 1573 (C=N str), 802 (C-N, s-triazine); ¹H NMR (CDCl₃) δ: 3.10 (dd, 1H, C₃-Ha benzothiazepine moiety), 3.35 (dd, 1H, C₃-Hb benzothiazepine moiety), 3.55 (t, 8H, oxazine ring), 3.69 (t, 8H, oxazine ring), 3.81 (3H, s, O-CHOCH₃), 5.00 (dd, 1H, -CH- benzothiazepine moiety), 6.90- 8.10 (m, 13H, 12 Ar-H and 1 NH); (Found: C, 64.98; H, 5.76; N, 16.04. Calcd. for C₃₃H₃₅N₇O₃S: C, 65.00; H, 5.79; N, 16.08 %).
Preparation of 2,4-bis-(tetrahydro-1',4'-oxazine)-6-[4'-{2''-(2''-nitrophenyl) -2'',3'' –dihydro - 1'',5''-benzothiazepine-4''-yl] phenyl amino]-s-triazine (9b): mp 66°C, IR (KBr) cm⁻¹: 3022 (=CH str), 1550 (C=C str), 1570 (C=N str), 810 (C-N, s-triazine) 695 (C-NO₂ str.); ¹H NMR (CDCl₃) δ: 3.12 (dd, 1H, C₃-H₃ benzothiazepine moiety), 3.32 (dd, 1H, C₃-H₃ benzothiazepine moiety), 3.51 (t, 8H, oxazine ring ), 3.71 (t, 8H, oxazine ring ), 5.12 (dd, 1H, -CH- benzothiazepine moiety), 7.0- 8.0 (m, 13H, 12 Ar-H and 1 NH) ; (Found : C, 67.17; H, 6.11; N, 16.12. Calcd. for C₃₄H₃₇Cl₂N₃O₂S: C, 67.19; H, 6.14; N, 16.13 %).

Preparation of 2,4-bis-(tetrahydro-1',4'-oxazine)-6-[4'-{2''-(3''-4'' -N,N-diethylphenyl) -2'',3'' –dihydro - 1'',5''-benzothiazepine-4''-yl] phenyl amino]-s-triazine (9f): mp 108°C, IR (KBr) cm⁻¹: 3022 (=CH str), 1550 (C=C str), 1570 (C=N str), 810 (C-N, s-triazine); ¹H NMR (CDCl₃) δ: 3.32 (4H, q, N-CH₂), 3.32 (6H, t, -CH₃), 3.21 (dd, 1H, C₃-H₃ benzothiazepine moiety), 3.40 (dd, 1H, C₃-H₃ benzothiazepine moiety), 3.57 (t, 8H, oxazine ring), 3.67 (t, 8H, oxazine ring ), 5.18 (dd, 1H, -CH- benzothiazepine moiety), 7.1- 8.5 (m, 13H, 12 Ar-H and 1 NH) ; (Found : C, 66.41; H, 6.47; N, 17.19. Calcd. for C₃₆H₄₂N₆O₂S: C, 66.44; H, 6.50; N, 17.22% ).

RESULTS AND DISCUSSION: Minimum inhibitory concentration (MIC) of all the synthesised compounds have been screened by Broth dilution method against four different strains, viz. two Gram positive bacteria (S. aureus MTCC 96 and S. pyogenes MTCC 442) and two Gram negative bacteria (E. coli MTCC 443 and P. aeruginosa MTCC 1688) and compared with standard drug: Ampicillin. Antifungal activity against C. albicans MTCC 227, A. niger MTCC 282 and A. clavatus MTCC 1323 organisms was determined by same method and compared with standard drug: Griseofulvin.

Antibacterial Activity: From the screening results (Table 1), In Gram positive bacterial strains compounds 7e, 7f, 8f and 9f showed good activity (25 – 150 µg/ml) against S. aureus ; whereas compounds 7e, 9c and 9f showed good to very good activity (62.5 – 100 µg/ml) against S. pyogenes compared with Ampicillin.

In Gram negative bacterial strains, compounds 7e, 7f, 8c and 9b exhibited good activity (25 – 125 µg/ml) against E. coli; compound 9b exhibited good activity (50 – 100 µg/ml) against P. aeruginosa.

All others compound show moderately active or less active against all bacterial strains.
Antifungal Activity: From the screening results (Table 1), compounds 7b, 7f, 8b, 8d and 9c showed very good activity against C. albicans, while compounds 7c, 7d, 8c, 8e and 9b showed good activity against C. albicans compared with Griseofulvin.

**TABLE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY DATA OF COMPOUNDS 7(A−F), 8(A−F) AND 9(A−F)**

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<th>Minimal bactericidal concentration µg/ml</th>
<th>Minimal fungicidal concentration µg/ml</th>
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<tr>
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CONCLUSION: Compound having ethyl group have exhibited more antimicrobial activity. These results suggest that the chalcone derivatives have excellent scope for further development as commercial antimicrobial agents.

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REFERENCES: