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ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL QUINAZOLINONES

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ABSTRACT

A series of 3-Amantadinyl-2-[(4-amino-3-aryl-5-ylthio)-1,2,4-triazolo]methyl)quinazolin-4-one 5(a-i) were prepared by the reaction of 3-Amantadinyl-2-[(4-acetamido-3-aryl-5-ylthio)-1,2,4-triazolo]methyl)quinazolin-4-one 4(a-i) with various 3-aryl-4-acetamido-5-mercapto triazoles. The compounds 5(a-i) biologically evaluated for their anti-inflammatory and antibacterial activities.Structures of the newly synthesized compounds were established on the basis of elemental and spectral (IR, ^{1}H-NMR and Mass) analysis. Furthermore the prepared pharmacophores have been investigated for their their antifungal, antibacterial, and anti-inflammatory activities. Compound 5h has shown most active anti-inflammatory and antifungal and antibacterial activities.

Keywords: Amantadinyltriazolyl quinazolinones, antifungal, antibacterial, anti-inflammatory activity.

INTRODUCTION

In general, the quinazolones are considered to be important versatile pharmacophore in the fields of pharmacy and biology. Quinazoline-4(3*H*)-ones are versatile nitrogen heterocyclic compounds, displaying a broad spectrum of biological and pharmalogical activities such as antiviral[1], antihistaminic[2], antihypertensive[3], anticancer[4], anti-fungal,[5] antihyperglycemic,[6] anti-HIV,[7] while on the other hand substituted triazole derivatives have occupied a unique position in heterocyclic chemistry due to their antimicrobial activities.[8,9] Various triazoles exhibited a wide range of therapeutical properties.[10] Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) was the first adamantane derivative introduced in medicine as effective therapy[11-13] against Asian A influenza virus. Among various substituents a growing interest in adamantyl derivatives is gaining prominence because of well known drugs like Rimantadine, Memantine, Adapalene, Adatanserin and others in clinical trials.[14,15] Furthermore, quinazoline-4(3*H*)-ones substituted at 2nd and 3rd position derivatives play a pivotal role in the hypotensive activity.[16-18] Microbial infections often produce pain and inflammation. The compounds possessing anti-inflammatory activity with antibacterial activities are not known. In the present study, we selected three pharmacophores i.e. quinazolones, 3-aryl-4-acetamido-5-mercapto triazoles and adamantyl precursors, to build up potent molecules possessing these three backbones, aiming to investigate their antibacterial, antifungal and anti-inflammatory activities.

EXPERIMENTAL

All reagents and solvents were generally used as received from the commercial supplier. Reactions were routinely performed in oven-dried glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) performed on silica gel G coated plate of

0.5 mm thickness. The TLC eluent was a mixture of different polar and nonpolar solvents in different proportions, and spots were visualized under iodine chamber. Elemental analysis (C, H, N) of all the compounds were determined through Perkin-Elmer 2400 elemental analyzer and results were found within ±0.4% of theoretical values. Infra red (IR) spectra were recorded in KBr on Perkin-Elmer-spectrum RX-I instrument and υ_{max} was recorded in cm^{-1}. ^{1}H-NMR spectra were recorded by Bruker DR-X-400 FT-NMR instrument using $CDCl_3$ and $DMSOd_6$ as solvent and tetramethysilane (TMS) an internal reference as δ (ppm).

Synthesis of 2-Methylbenzo(1,3) oxazin-4-ones 1.

These compounds were prepared according to the method of Bogert & Soil[19]. A mixture of anthranilic acid (1.0 mol) and acetic anhydride (0.02 mol), was refluxed for 2-3 h. with occasional stirring. The excess of acetic anhydride was distilled off. On cooling, a solid was separated out which was filtered and washed with petroleum ether (40-60^{0}C) and dried to furnish compound **1**: Yield 86% (petroleum ether),mp 81^{0}C. IR (KBr, υ_{max} cm^{-1}): 1701 (C=O), 1610 (C…C of aromatic ring), 1570 (C=N), 1302 (C-N).^{1}H NMR ($CDCl_3$, δ ppm): 6.65-7.73 (m, 4H, Ar-H), 1.31 (s, 3H, CH_3). MS: $[M]^+$ at *m/z* 628. Anal. Calcd for $C_9H_7NO_2$ C: 67.07; H, 4.38; N, 8.69%. Found: C, 66.82; H, 4.25; N, 8.51%.

Synthesis of 3-Amantadinyl-2-methylquinazolin-4(3H)ones 2.

To a solution of compound **1** (0.02 mol), amantadine (0.02 mol) was added and the reaction mixture was heated on a free flame for 10-20 minutes in a conical flask. After the disappearance of water droplets in a conical flask it was kept at room temperature. On cooling a jelly like mass obtained which was dissolved in ethanol was refluxed and poured into water. The solid thus obtained was filtered, dried and finally recrystallized from appropriate solvent to obtain compounds 2: Yield 82% (DMF-water),mp 205 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1706 (C=O), 1615 (C…C of aromatic ring), 1571 (C=N), 1300 (C-N).^{1}H NMR ($CDCl_3$, δ ppm): 6.73-7.70 (m, 4H, Ar-H), 1.27 (s, 3H, CH_3); 1.30 (m, 15H, amantadinyl ring). MS: $[M]^+$ at *m/z* 294.39. Anal. Calcd for $C_{19}H_{22}N_2O$: C, 61.13; H, 5.67; N, 7.50; found: C, 61.02; H, 5.35; N, 7.33%.

Synthesis of 3-Amantadinyl-2-bromomethylquinazolin-4-(3H)ones 3.

Bromine (0.4mol) in acetic acid was added dropwise to the solution of compound **2a-ab** in acetic acid (50 ml). The reaction mixture was poured onto crushed ice then left overnight at room temperature. The precipitate thus obtained was recrystallized with suitable solvents to furnish compound **3**: Yield 76% (methanol),mp 198 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1619 (C…C of aromatic ring), 1579 (C=N), 1305 (C-N), 600(C-Br). ^{1}H NMR ($CDCl_3$, δ ppm): 6.67-7.72 (m, 4H, Ar-H), 1.36 (m, 15H, amantadinyl ring), 1.25 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 373.29. Anal. Calcd for $C_{19}H_{21}BrN_2O$: C, 61.47; H, 5.16; N, 7.55; found: C, 60.92; H, 5.45; N, 7.43%.

Synthesis of 3-Amantadinyl-2-[(4-acetamido-3-aryl-5-ylthio)-1,2,4-triazolo]methyl) quinazolin-4-one 4(a-i).

The solution of compound **3(a-i)** (0.002 mol) in isopropanol was refluxed with 3-aryl-4-acetamido-5-mercapto triazoles (0.002 mol) for 1-3 hours. Excess of solvent was distilled off and the reaction mixture thus obtained was cooled, poured into ice cold water, washed with petroleum ether (40-60°C) and recrystallised with appropriate solvents to furnish the products **4(a-i)** .

(**4a**): Yield 62% (methanol),mp 175 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1713 (C=O), 1620 (C…C of aromatic ring), 1580 (C=N), 1520 (N-N), 1304 (C-N), 1252 (C-N),670 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 8.02(bs, 1H, NH-CO-),6.60-7.75(m, 9H, Ar-H), 2.01(s,3H,CO-CH_3),1.42 (m, 15H, amantadinyl ring), 1.25 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 526.65. Anal. Calcd for $C_{29}H_{30}N_6O_2S$: C, 66.14; H, 5.74; N, 15.96; found: C, 66.18; H, 5.75; N, 15.95.

(**4b**): Yield 64% (ethanol),mp 143 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1621 (C…C of aromatic ring), 1580 (C=N), 1519 (N-N), 1302 (C-N), 1251 (C-N), 672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 10.22 (s,1H, OH-Ar), 8.07(bs, 1H, NH-CO-),6.62-7.85(m, 8H, Ar-H), 2.00(s,3H,CO-CH_3),1.35 (m, 15H, amantadinyl ring), 1.21 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 542.65. Anal. Calcd for $C_{29}H_{30}N_6O_3S$: C, 64.19; H, 5.57; N, 15.49; found: C, 66.18; H, 5.70; N, 15.55.

(**4c**): Yield 60% (acetic acid-water),mp 161 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1622 (C…C of aromatic ring), 1578 (C=N), 1521 (N-N), 1300 (C-N), 1250 (C-N), 668 (C-S-C). ^{1}H NMR ($CDCl_3$, δ

ppm): 10.19 (s,1H, OH-Ar), 8.00(bs, 1H, NH-CO-),6.66-7.87(m, 9H, Ar-H), 2.05(s,3H,CO-CH_3),1.40 (m, 15H, amantadinyl ring), 1.20 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 542.65. Anal. Calcd for $C_{29}H_{30}N_6O_3S$: C, 64.19; H, 5.57; N, 15.49; found: C, 66.18; H, 5.70; N, 15.55.

(**4d**): Yield 63% (ethanol),mp 156 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1621 (C…C of aromatic ring), 1580 (C=N), 1519 (N-N), 1302 (C-N), 1251 (C-N), 672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 10.23 (s,1H, OH-Ar), 8.07(bs, 1H, NH-CO-),6.62-7.85(m, 9H, Ar-H), 2.00(s,3H,CO-CH_3),1.49 (m, 15H, amantadinyl ring), 1.21 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 542.65. Anal. Calcd for $C_{29}H_{30}N_6O_3S$: C, 64.19; H, 5.57; N, 15.49; found: C, 66.18; H, 5.70; N, 15.55.

(**4e**): Yield 67% (DMF-water),mp 196 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1619 (C…C of aromatic ring), 1578 (C=N), 1518 (N-N), 1304 (C-N), 1250 (C-N), 669 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 8.00(bs, 1H, NH-CO-),6.68-7.81(m, 8H, Ar-H), 2.06(s,3H,CO-CH_3),1.36 (m, 15H, amantadinyl ring), 1.22 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 570.71. Anal. Calcd for $C_{31}H_{34}N_6O_3S$: C, 65.24; H, 6.00; N, 14.73; found: C, 65.18; H, 5.98; N, 15.00.

(**4f**): Yield 56% (DMF-water),mp 170 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1709 (C=O), 1620 (C…C of aromatic ring), 1580 (C=N), 1517 (N-N), 1302(C-N), 1253 (C-N), 671 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 10.10 (s,1H, OH-Ar), 8.00(bs, 1H, NH-CO-),6.68-7.81(m, 8H, Ar-H), 3.94(s, 2H,-CH_2- triazole),2.06(s,3H,CO-CH_3),1.40 (m, 15H, amantadinyl ring), 1.19 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 556.68. Anal. Calcd for $C_{30}H_{32}N_6O_3S$: C, 64.73; H, 5.79; N, 15.10; found: C, 65.00; H, 5.85; N, 15.02.

(**4g**): Yield 60% (methanol),mp 202 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1621 (C…C of aromatic ring), 1580 (C=N), 1519 (N-N),1302(C-N), 1251 (C-N), 672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 8.07(bs, 1H, NH-CO-),6.62-7.85(m, 8H, Ar-H), 4.10(s, 2H,-CH_2- CH_3), 3.88(s, 2H,-CH_2- triazole), 2.00(s,3H,CO-CH_3),1.45 (m, 15H, amantadinyl ring), 1.28 (s, 3H, CH_2- CH_3), 1.18 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 584.73. Anal. Calcd for $C_{32}H_{36}N_6O_3S$: C, 65.73; H, 6.21; N, 14.37; found: C, 65.78; H, 6.20; N, 14.45.

(**4h**): Yield 63%(methanol), mp 175 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1713 (C=O), 1620 (C…C of aromatic ring), 1580 (C=N), 1520 (N-N),1304 (C-N), 1252 (C-N),670 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 8.02(bs, 1H, NH-CO-),6.60-7.75(m, 8H, Ar-H), 2.01(s,3H,CO-CH_3),1.42 (m, 15H, amantadinyl ring), 1.25 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 561.10. Anal. Calcd for $C_{29}H_{29}N_6O_2SCl$: C, 62.08; H, 5.21; N, 14.98; found: C, 62.10; H, 5.25; N, 15.96.

(**4i**): Yield 59% (methanol),mp 175 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1622 (C…C of aromatic ring), 1581 (C=N), 1519 (N-N),1301 (C-N), 1250 (C-N),672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 8.04(bs, 1H, NH-CO-),6.70-7.89(m, 8H, Ar-H), 1.98 (s,3H,CO-CH_3),1.46 (m, 15H, amantadinyl ring), 1.20 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 561.10. Anal. Calcd for $C_{29}H_{29}N_6O_2SCl$: C, 62.08; H, 5.21; N, 14.98; found: C, 62.10; H, 5.25; N, 15.96.

Synthesis of 3-Amantadinyl-2-[(4-amino-3-aryl-5-ylthio)-1,2,4-triazolo]methyl)quinazolin-4-one 5(a-i).

Compound **4(a-i)** (0.001 mol) was stirred in ethanolic–sodium hydroxide (100 ml) solution for 1-2 hours at room temperature. The reaction mixture dumped in crushed ice-water and appeared solid filtered, dried, triturated with petroleum ether to yield crude compounds **5(a-i)**.

(**5a**): Yield 60% (methanol),mp 185 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1714 (C=O), 1620 (C…C of aromatic ring), 1580 (C=N), 1520 (N-N),1304 (C-N), 1252 (C-N), 670 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 6.60-7.75(m, 9H, Ar-H), 4.56 (bs, 2H, NH_2), 1.43 (m, 15H, amantadinyl ring), 1.25 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 484.62. Anal. Calcd for $C_{27}H_{28}N_6OS$: C, 66.92; H, 5.82; N, 17.34; found: C, 66.88; H, 5.85; N, 17.35.

(**5b**): Yield 60% (methanol),mp 175 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1622 (C…C of aromatic ring), 1580 (C=N), 1521 (N-N),1302 (C-N), 1251(C-N),673 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 10.16 (s,1H, OH-Ar), 6.63-7.90(m, 8H, Ar-H), 4.48 (bs, 2H, NH_2), 1.47 (m, 15H, amantadinyl ring), 1.21 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 500.62. Anal. Calcd for $C_{27}H_{28}N_6O_2S$: C, 64.78; H, 5.64; N, 16.79; found: C, 64.88; H, 5.65; N, 16.75.

(**5c**): Yield 58% (DMF-water),mp 186 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1620 (C…C of aromatic ring), 1581 (C=N), 1520 (N-N),1304 (C-N), 1253 (C-N),674(C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 6.63-7.77(m, 8H, Ar-H), 4.56 (bs, 2H, NH_2), 1.44 (m, 15H, amantadinyl ring), 1.24 (s, 2H, CH_2). MS: $[M]^+$ at

m/z 500.62. Anal. Calcd for $C_{27}H_{28}N_6O_2S$: C, 64.78; H, 5.64; N, 16.79; found: C, 64.89; H, 5.64; N, 16.78.

(**5d**): Yield 62% (methanol),mp 201 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1621 (C…C of aromatic ring), 1579 (C=N), 1522 (N-N),1303(C-N), 1250 (C-N),672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 10.12 (s,1H, OH-Ar), 6.60-7.93(m, 8H, Ar-H), 4.43 (bs, 2H, NH_2), 1.49 (m, 15H, amantadinyl ring), 1.19 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 500.62. Anal. Calcd for $C_{27}H_{28}N_6O_2S$: C, 64.78; H, 5.64; N, 16.79; found: C, 64.88; H, 5.65; N, 16.75.

(**5e**): Yield 63% (ethanol),mp 160 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1712 (C=O), 1621 (C…C of aromatic ring), 1580 (C=N), 1519(N-N),1302 (C-N), 1251 (C-N), 672 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 6.62-7.85(m, 8H, Ar-H), 4.54 (bs, 2H, NH_2),3.94(s, 2H,-CH_2- triazole), 1.50 (m, 15H, amantadinyl ring), 1.34 (s, 3H, CH_2- CH_3), 1.21 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 528.67. Anal. Calcd for $C_{29}H_{32}N_6O_2S$: C, 65.88; H, 6.10; N, 15.90; found: C, 65.87; H, 6.11; N, 15.95.

(**5f**): Yield 57% (AcOH-water),mp 211 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1710 (C=O), 1623 (C…C of aromatic ring), 1581 (C=N), 1516 (N-N), 1300(C-N), 1253 (C-N), 673 (C-S-C). ^{1}H NMR ($CDCl_3$, δ ppm): 10.13 (s,1H, OH-Ar), 6.58-7.82(m, 8H, Ar-H), 4.64 (bs, 2H, NH_2),3.88 (s, 2H,-CH_2-triazole), 1.40 (m, 15H, amantadinyl ring), 1.19 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 514.64. Anal. Calcd for $C_{28}H_{30}N_6O_2S$: C, 65.35; H, 5.88; N, 16.33; found: C, 65.30; H, 5.85; N, 16.32.

(**5g**): Yield 60% (methanol),mp 234 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1709 (C=O), 1619 (C…C of aromatic ring), 1584 (C=N),1520(N-N),1304 (C-N),1250(C-N),669 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 6.62-7.85(m, 8H, Ar-H), 4.51 (bs, 2H, NH_2), 4.10(s, 2H,-CH_2- CH_3), 3.75(s, 2H,-CH_2- triazole), 1.45 (m, 15H, amantadinyl ring), 1.30 (s, 3H, CH_2- CH_3), 1.11 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 542.69. Anal. Calcd for $C_{30}H_{34}N_6O_2S$: C, 66.39; H, 6.31; N, 15.49; found: C, 66.38; H, 6.30; N, 15.45.

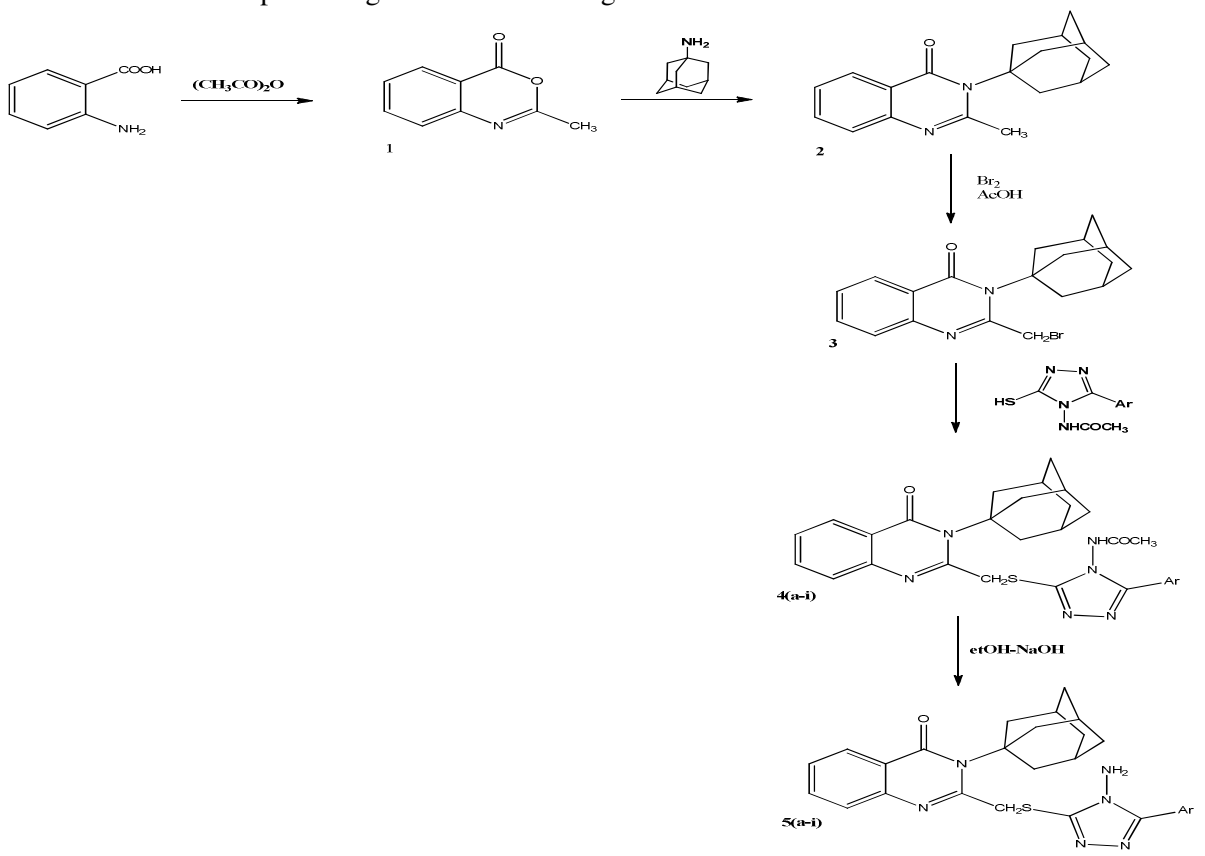
(**5h**): Yield 57% (methanol),mp 193 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1711 (C=O), 1622 (C…C of aromatic ring), 1584 (C=N), 1523 (N-N),1301(C-N), 1250 (C-N),673 (C-S-C). ^{1}H NMR ($CDCl_3$, δ ppm): 6.55-7.91(m,8H, Ar-H), 4.55 (bs, 2H, NH_2), 1.49 (m, 15H, amantadinyl ring), 1.25 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 519.06. Anal. Calcd for $C_{27}H_{27}N_6OSCl$: C, 62.48; H, 5.24; N, 16.19; found: C, 62.40; H, 5.25; N, 16.16.

(**5i**): Yield 53% (ethanol),mp 193 ^{0}C. IR (KBr, υ_{max} cm^{-1}): 1713 (C=O), 1620 (C…C of aromatic ring), 1582 (C=N), 1524 (N-N),1301 (C-N), 1250 (C-N),671 (C-S-C).^{1}H NMR ($CDCl_3$, δ ppm): 6.59-7.89(m,8H, Ar-H), 4.52 (bs, 2H, NH_2), 1.44 (m, 15H, amantadinyl ring), 1.23 (s, 2H, CH_2). MS: $[M]^+$ at *m/z* 519.06. Anal. Calcd for $C_{27}H_{27}N_6OSCl$: C, 62.48; H, 5.24; N, 16.19; found: C, 62.40; H, 5.25; N, 16.16.

Antimicrobial Evaluation

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. Microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. All microbial strains, i.e. fungal as well as bacterial; were clinical isolates, identified with conventional morphological and biochemical methods. Disk diffusion method[20,21] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 ^{0}C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The MIC (µg/ml) values of the tested compounds against the tested bacteria strains are recorded in Table **1**. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata, Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method.[22,23] Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A

loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 ^{0}C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The MIC (µg/ml) values of the tested compounds against the tested fungal strains are recorded in Table 1.



Ar = C_6H_5, 2-OH.C_6H_4, 3-OH.C_6H_4, 4-OH.C_6H_4, 4-C_2H_5O.C_6H_4, 4-OH.$C_6H_4CH_2$, 4-C_2H_5O.$C_6H_4CH_2$, 2-Cl.C_6H_4, 4-Cl.C_6H_4

Scheme-1

Anti-inflammatory activities

Compounds **5(a-i)** were tested for their anti-inflammatory and analgesic activities as well as for their ulcerogenicity and acute toxicity. The experiment was performed with albino rats of Charlese Foster strain of either sex, excluding pregnant females, of 60-90 days weighing 100-120 g. Food (chaw pallet) and water was given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Indomethacin and phenylbutazone were used as reference drugs for the comparison of anti-inflammatory, analgesic and ulcerogenic activities.

Anti-inflammatory activity against carrageenan induced rat's paw oedema: this study was done following the procedure of Winter.[24] The rats were divided into three groups (control, drug treated, and standard, drug of six animals each).A freshly prepared suspension of carrageenan (1% in 0.9% saline). 0.05 ml was injected under the planter aponeurosis of the right hind paw of each rat. Test compounds and standard drug were administered orally to the animals of drug treated groups and the standard drug group,

respectively, 1 h before the carrageenan injection. The paw volume of each rat was measured before 1 and after 3 h of carrageenan treatment with the help of a plethymometer. The percent anti-inflammatory activity was calculated according to the formula given below:

$$\text{Percentage of inhibition of oedema} = (1- V_t - V_c) \text{ X } 100$$

where, V_t and V_c are the mean increase in paw volume of rats of the treated and the control group, respectively. Considering, the potentiality of compounds 5h, this one was studied in detail at three graded doses 25, 50, 100 mg/kg p.o. Results obtained were statistically analyzed (Table 2).

Analgesic activity

Analgesic activity was performed following the method of Berkowitz[25]. This method is based on the property of the test compound to antagonize the phenylquinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonealy with 0.25 ml of a 0.02% solution of phenylquinone in ethanol (5%) 1 h after oral administration of the test compound. The number of writhes induced in each mouse was counted for 5 min (between 5 and 10 min) after injection of an irritant. The analgesic effect was expressed as percent protection in comparison to control (Table 2).

% protection= (1- mean no. of writhes in mice of test groups/ mean number of writhes in mice of control group) X 100

Ulcerogenic activity

Ulcerogenic labilities of newly synthesized compounds were checked with method of Verma.[26] Albino rats were fasted for 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment, and their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity (Table 2).

Acute toxicity activity

The test compounds were investigated for their acute toxicity (ALD_{50}) in albino mice, according to the method of Smith.[27] The test compounds were given orally at different dose levels in separate groups of animals. After 24 h of drug administration, percent mortality in each group was observed. ALD_{50} was calculated from the data obtained (Table 2).

COX-1 and COX-2 activity

The compounds prepared were tested for cyclooxygenase-1 and cyclooxygenase-2 inhibitory activities. The method of Copeland[28] was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of N,N,N`,N`-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H2 by COX-1 and COX-2 enzymes. COX-1 and COX-2 enzymes used in the assay were purified from microsomal fraction. The compounds were dissolved in DMSO and stock solution was diluted to required assay concentration. The assay mixture consists of Tris-HCl. Tris-HCl buffer (pH 8.0, 100 µM), hematin (15 µM), EDTA (3 µm), enzyme (COX-1 or COX-2, 100 µM) and test compound. The mixture was pre-incubated at 25 ^{0}C for 15 min and then the reaction was initiated by the addition of arachidonic acid (100 µM) and TMPD (120 µM) in total volume of 1.0 ml. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 s of the reaction following the increase in absorbance at 603 nm. IC50 values are calculated from four parameter least squares non-linear regression analysis of the log dose vs percentage inhibition plot. However, none of the compound studied here exhibited significant inhibitory activity when compared to standard inhibitors indomethacin (for COX-1) and celecoxib (for COX-2) (Table **2**).

RESULTS AND DISCUSSION

The entire synthesized compounds were evaluated for antifungal and antibacterial activity against the selected panel of pathogens. Antifungal activity was performed by serial plate dilution method and disk diffusion method was adopted for antibacterial activity. Among the compounds **5(a-i)**, compound **5h** was found the most potent candidates and showed better inhibition in comparison to reference drugs (Table 1).

Furthermore, these compounds also evaluated for anti-inflammatory at a dose of 50 mg/kg p.o. The results of anti-inflammatory activity of all the compounds are summarized in Table **2**. Out of all the tested compounds, only **5h** exhibited the most potent anti-inflammatory and analgesic activity in comparison to used reference drugs i.e. phenylbutazone and while it is less active then indomethacin respectively.

Table-1: Antibacterial and antifungal data for the synthesized compounds.

Antibacterial activity data in MIC (µg/ml)					Antifungal activity data in MIC (µg/ml)			
Comp. no.	*S. aureus*	*E.coli*	*K. pneumoniae*	*P. vulgaris*	*A. fumigatus*	*C. glabrata*	*C. albacans*	*C. krusei*
5a	-	-	-	-	-	-	-	-
5b	5	6	8	5	-	6	5	-
5c	6	8	10	6	-	8	-	4
5d	6	10	12	-	5	4	4	-
5e	8	12	16	-	12	-	1	-
5f	8	15	14	-	4	6	5	-
5g	12	15	16	10	10	-	10	8
5h	16	26	22	25	16	15	15	16
5i	15	24	20	18	-	10	8	10
Ampicillin trihydrate (std.)	16	20	20	20	-	-	-	-
Fluconazole (std.)	-	-	-	-	20	15	16	15
DMF (control)	-	-	-	-	-	-	-	-

- means no inhibition.

Table -2: Anti-inflammatory data of compounds 5(a-i).

Compd.	Ar	Anti inflammatory activity		Analgesic activity		UD_{50} (mg/kg/i.p.)	IC_{50} % of inhibition		ALD_{50} (mg/kg/i.p.)
		Dose (mg/kg/p.o.)	% inhibition of oedema	Dose (mg/kg/p.o.)	% Protection		COX-1	COX-2	
Phenyl-butazone		25 50 100	26.76*** 36.80*** 64.68**	25 50 100	14.26*** 32.50*** 54.58***	66.66	-	-	>800
Indo-methacin		5.0 7.5 10.0	52.20*** 63.10*** 93.20***	5.0 7.5 10.0	41.30*** 59.25*** 64.33***	54.60	-	-	>800
5a	C_6H_5	50	30.55***	50	21.00**	-	37.05***	76.64***	>800
5b	2-OH.C_6H_4	50	30.52***	50	20.96**		38.00***	72.24***	>800
5c	3-OH.C_6H_4	50	36.19***	50	35.00***	-	38.68***	64.28***	>800
5d	4-OH.C_6H_4	50	31.62***	50	21.27**	-	39.53***	66.26***	>800
5e	4-OC_2H_5.C_6H_4	50	32.67***	50	19.95**	-	39.27**	71.09***	>800
5f	4-OH.CH_2.C_6H_4	50	33.60***	50	22.67***	-	37.16**	65.82***	>800
5g	4-OC_2H_5.CH_2.C_6H_4	50	31.17***	50	18.76**		38.39**	63.56***	>800
5h	2-Cl.C_6H_4	25 50 100	28.75*** 41.23*** 66.76***	25 50 100	19.00*** 38.00*** 58.00***	133.33	69.13**	93.34***	>800
5i	4-Cl.C_6H_4	50	32.60***	50	19.98**	-	39.25**	71.10***	>800

*P< 0.05, **P<0.01, ***P<0.001, - denotes not tested
Propylene glycol standard for control group.

CONCLUSION

On the basis of study of biological activity data, it may be concluded that compounds **5**(a-i) displayed better antifungal and antibacterial activities. Presence of chloro group as substituent at 2nd position brought remarkable increase in biological activities. Compound **5h** explored the most potent antimicrobial spectrum as well as anti-inflammatory and analgesic activity and deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. ALD_{50} of all these tested compounds were >800 mg/kg p.o.

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