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SYNTHESIS AND BIOLOGICAL STUDY OF SUBSTITUTED 1,3,4-OXADIAZOLES AND 1,2,4-TRIAZOLES

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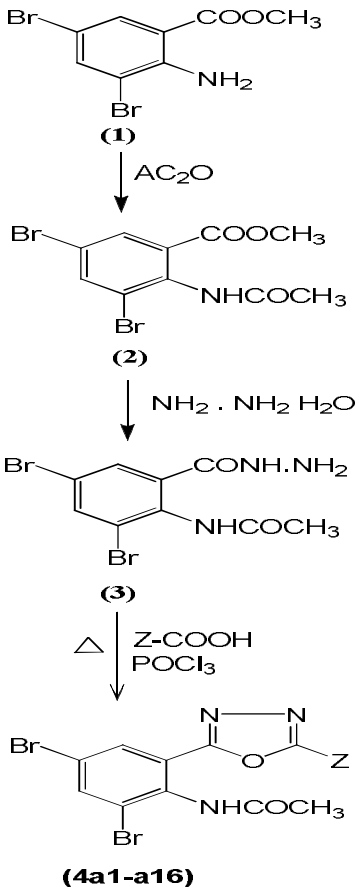
ABSTRACT : The 2-acetyl amino-3,5-dibromo methyl anthranilate when heated with excess of hydrazine hydrate, furnished the corresponding hydrazide in excellent yield. The hydrazide on heating with aryl/hetero aryl acids in presence of phosphorus oxychloride afforded 2-[3',5'-dibromo-2'-acetyl amino phenyl]-5-substituted-1,3,4-Oxadiazoles (**4a1-a16**). These oxadiazoles were utilized in the synthesis of 1,2,4-triazoles namely 4-amino-3-(3'5'-dibromo-2'-acetyl amino phenyl)-5-substited-1,2,4-triazoles (**5a1-a16**) and 4-(N-pyridyl carboxamido)-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted-1,2,4-triazoles (**6a1-a16**). The structures of the new compounds were established by elemental analysis, IR, ^{1}H NMR and mass spectral studies. The title compounds were screened for antimicrobial, anti-inflammatory and anlgesic activity by standard procedure and few of them showed significant activity.

KEY WORDS: 1,3,4-Oxadiazoles,1,2,4-Triazoles,Antibacterial,Antifungal and Anti-inflammatory.

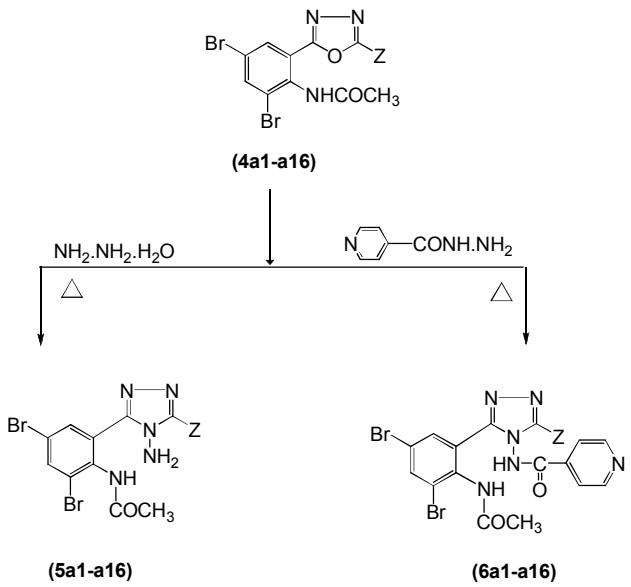
INTRODUCTION

Several five membered aromatic systems having three hetero atoms at symmetrical positions have been studied because of their interesting biological properties. Compounds having 1,3,4-Oxadiazole moiety are endowed with a variety of biological activities[1]. Further 1,2,4-triazoles have a wide range of therapeutical properties[2-6]. The wide spectrum of biological activities exhibited by various triazole derivatives have made them an important class of chemotherapeutic agents. The 1,2,4-triazole nucleus has recently been incorporated into a wide variety of therapeutically interesting drugs. In view of the above observations and in continuation of our studies in the field of oxygen and nitrogen heterocycles of medicinal interest, the present investigation deals with the synthesis of certain 1,3,4-oxadiazole and 1,2,4-triazole derivatives. The investigations appeared interesting because of compactness and planarity of oxadiazole and triazole ring systems, which may be additional factor for enhancing the biological profile.

Over the past several years the emergence of organisms resistant to nearly all the classes of antimicrobial agents has become a serious public health concern. The emergence of bacterial resistance to the variety of antimicrobial agents containing different biological active moieties is quite serious. 1,3,4-oxadiazole and 1,2,4-triazole derivatives are expected to have chemotherapeutic intervention against bacteria, virus and other pathogens. The synthesis of these agents has received considerable attention in recent years. Prompted by the above facts and as a part of our programme aimed at developing new biologically active compounds, a convenient synthesis of these unreported title compounds by incorporating potential pharmacophores with a view to get compounds of enhanced biological potency was devised. Apart from their chemical interest, these compounds could also be a subject of studies as pharmacological agents.



SCHEME- I



SCHEME-II

EXPERIMENTAL

All the compounds in the study were synthesized by following scheme-I and II. Melting points were taken in open glass capillary tubes by using Thiel's tube containing liquid paraffin and were uncorrected. IR spectra (KBr) were recorded on a Shimadzu 8400 FTIR Spectrophotometer. ^{1}H NMR spectra were recorded on Brucker Spectrophotometer (400 MHz) in DMSO-d_6/$CDCl_3$ using TMS as internal standard. (Chemical shifts are expressed in δ ppm). Mass spectra were recorded in Finnigan MAT 8230 Mass Spectrophotometer and micro analysis were recorded on Thermo Finnigan. All the synthesized compounds gave satisfactory elemental analysis.

Preparation of 2-acetylamino-3,5-dibromo methyl anthranilate (2)

To 25 gm (0.164 mol) of 3,5-dibromo methyl anthranilate(1) in a 250 ml RB flask was added 35 ml of acetic anhydride and refluxed for 1 hr. The solution was poured on ice when the acetylated product separated out. mp: 96 °C, Yield: 80%.

Preparation of 2-acetylamine 3,5-dibromo anthranilic acid hydrazide (3)

Dissolve the ester 2-acetyl amino-3,5-dibromo methyl anthranilate (0.1 mol) in (30 ml) ethanol and hydrazine hydrate (0.2 mol) was added drop wise to the mixture with stirring. The resulting mixture was allowed to reflux for 12 to 16 hrs. excess ethanol was distilled out and contents were allowed to cool. The crystals formed filtered, washed thoroughly with water and dried. The completion of the reaction was monitored on TLC by using silica gel-G coated plates using chloroform and ethyl acetate (1 : 1) as the eluent and observed in UV light.

Preparation of 2-[2'-acetyl amino-3',5'-dibromo phenyl]-5-substituted 1,3,4-Oxadiazoles (4a1-a16).

The mixture of hydrazide **3** (0.01 mol), substituted aromatic heterocyclic acid (0.02 mol) and phosphorous oxychloride (10 ml) was refluxed on a steam bath for 6 to 8 hrs. the reaction mixture was cooled and poured on to crushed ice. The precipitated oxadiazole was filtered (in some cases the product was precipitated when pH was brought to 7 by adding liquor ammonia or sodium bicarbonate). The product was washed with cold water, dried and crystallized from ethanol. The completion of the reaction was monitored on TLC by using silica gel-G coated plates using chloroform and ethyl acetate (1:1) as the eluent and observed in UV light. The compounds thus prepared are listed in Table 1.

Table 1: Physical Characteristics of Oxadiazole and Triazole Derivatives

Compound	Z	MP °C	Yield
4a$_1$	pyrazinyl	162	62
4a$_2$	3-chloro pyrazinyl	137	64
4a$_3$	pyridyl	147	66
4a$_4$	2-chloropyridyl	122	69
4a$_5$	2-hydroxy-4-amino phenyl	128	66
4a$_6$	2-amino phenyl	115	70
4a$_7$	2-amino-3,5-dibromophenyl	133	59
4a$_8$	2-acetyl amino 3,5-dibromophenyl	145	65
4a$_9$	2-amino-4-nitro phenyl	128	58
4a$_{10}$	2-acetyl amino -4- nitro phenyl	114	60
4a$_{11}$	4-isobutylphenyl ethyl	142	62
4a$_{12}$	benzyl	130	68
4a$_{13}$	2-(2',6'-dichloro phenyl amino benzyl)	168	58
4a$_{14}$	phenoxy methyl	139	68
4a$_{15}$	4-chlorophenoxy methyl	129	64
4a$_{16}$	4-methyl phenoxy methyl	148	58
5a$_1$	pyrazinyl	240	72
5a$_2$	3-chloropyrazinyl	214	74
5a$_3$	pyridyl	2--	76
5a$_4$	2-chloropyridyl	242	78
5a$_5$	2-hydroxy-4-amino phenyl	224	68
5a$_6$	2-amino phenyl	248	66
5a$_7$	2-amino-3,5-dibromo phenyl	179	74
5a$_8$	2-acetyl amino 3,5-dibromo phenyl	174	75
5a$_9$	2-amino-4-nitro phenyl	176	68
5a$_{10}$	2-acetyl amino -4-nitro phenyl	210	70
5a$_{11}$	4-isobutyl phenyl ethyl	174	70
5a$_{12}$	benzyl	168	65
5a$_{13}$	2-(2'6'-dichlorophenylamino)benzyl	232	68
5a$_{14}$	phenoxymethyl	223	72
5a$_{15}$	4-chlorophenoxy methyl	268	74
5a$_{16}$	4-methyl phenoxy methyl	176	74

Table 1: Physical Characteristics of Oxadiazole and Triazole Derivatives

Compound	Z	MP °C	Yield
6a$_1$	pyrazinyl	226	64
6a$_2$	3-chloro pyrazinyl	244	62
6a$_3$	pyridyl	264	74
6a$_4$	2-chloro pyridyl	209	72
6a$_5$	2-hydroxy-4-amino phenyl	243	68
6a$_6$	2-amino phenyl	210	60
6a$_7$	2-amino-3,5-dibromo phenyl	233	68
6a$_8$	2-acetyl amino 3,5-dibromo phenyl	264	71
6a$_9$	2-amino-4-nitro phenyl	243	62
6a$_{10}$	2-acetyl amino-4-nitro phenyl	218	68
6a$_{11}$	4-isobutyl phenyl ethyl	204	70
6a$_{12}$	benzyl	198	68
6a$_{13}$	2-(2',6'-dichlorophenylamino) benzyl	218	65
6a$_{14}$	phenoxy methyl	222	62
6a$_{15}$	4-chlorophenoxy methyl	215	70
6a$_{16}$	4-methyl phenoxy methyl	201	74

4a2 IR (KBr) ν cm-^{1} 3260 (NH), 3055 (aromatic C-H stretching), 2905 C-H stretching CH_3, 1665 (C=O) 1608 (C=N), 1600 (C=C), 1460 (C-N), 1080 (C-O-C), 880 substituted phenyl ring, 640 (Br) 600 (Cl), **^{1}H NMR**(TMS) δ ppm 2.4 (3H,s,CH_3) 8.00-8.20 (2H, m, Ar-H) 8.60-8.80 (2H, m, heterocyclic protons), 10.8 (1H,s,NH of CONH), **Mass** m/z 473 (M^+) other prominent fragment ions are 432, 416, 235, 181, 143, 113, 80, 77, 70, 65 [found C, 35.48; H, 1.962; N, 8.76; $C_{14}H_8O_2N_3Br_2Cl$ requires C, 35.51; H, 1.69; N, 8.87%]

4a4 IR (KBr) ν cm-^{1} 3262 (NH), 3058 (aromatic C-H stretching), 2905 C-H stretching of CH_3, 1665 (C=O) 1610 (C=N), 1605 (C=C), 1462 (C-N), 1083 (C-O-C), 884 (Substituted phenyl ring), 642 (Br), 604 (Cl); **^{1}H NMR**(TMS) δ ppm 2.4 (3,H,s,CH_3) 8.00-8.20 (2H,m,Ar-H), 8.40-8.70 (3H,m,heterocyclic protons), 10.80 (1H, s, NH of CONH) **Mass** m/z (M^+) 472, 431, 415, 237, 180, 112, 77, 68 [Found C, 38.09; H, 1.86; N, 5.88' ; $C_{15}H_9O_2N_2Br_2Cl$ requires C, 38.13; H, 1.90; N, 5.93 %]

4a8 IR (KBr) ν cm-^{1} 3248 (NH), 3070 (aromatic C-H stretching), 2920 (C-H stretching of CH_3), 1665 (C=O of CONH), 1620 (C=N), 1610 (C=C), 1466 (C-N), 1085, (C-O-C), 885 substituted phenyl ring, 665 (Br), **^{1}H NMR**(TMS) δ ppm 2.60 (6H, s, 2 X CH_3), 8.00 – 8.20 (4H,m,Ar-H), 10.90 (2H,s,2x NH of NHCO), **Mass** m/z 652 (M^+), 570, 538, 510, 361, 354, 279, 235, 155, 91, 80, 77, 65 [Found C, 33.08; H, 1.75; N, 8.49; $C_{18}H_{12}O_3N_4Br_4$ requires C, 33.12; H, 1.84; N, 8.58%]

4a11 IR (KBr) ν cm-^{1} 3244 (NH), 3072 (Aromatic C-H stretching), 2840-2930 C-H stretching of CH_2 and CH_3 groups a symmetric and asymmetric, 1674 (C=O) 1621 (C-N), 1609 (C=C) 1486 (C-N), 1440-1390 C-H bending asymmetric and symmetric, 1089 C-O-C 840 (1,4-substituted benzene ring, 640 (Br), **^{1}H NMR**(TMS) δ ppm 0.90 (6H, d, 2x CH_3 of CH (CH_3)2), 1-30 (3H, d, CH_3 of CH-CH_3), 1-80 (1H, m, CH of CH (CH_3)2), 2.40 (2H, d, CH_2 of CH_2 CH (CH_3)2), 2.6 (3H,s,CH_3 of $NHCOCH_3$)3.70 (1H, q,CH of CH-CH_3) 7.60 – 7.90 (5H, m, Ar-H), 10.60 (1H ,s,NH of NHCO), **Mass** m/z 541 (M^+), 500, 484, 249, 235, 161, 134, 91, 80, 77, 70, 65 [Found C, 48.66; H, 4.19; N, 7.64; $C_{22}H_{23}O_2N_3Br_2$ requires C,48.79; H, 4.25; N,7.76%]

4a16 IR (KBr) ν cm-^{1} 3226 (NH), 3068 aromatic C-H stretching), 2860, 2958 (C-H stretching of OCH_2 & CH_3 group), 1670 (C=O of CONH), 1618 (C=N), 1602 (C=C), 1494 (C-N), 1H, 1446-1384 (C-H bending of OCH_2, CH_3 groups asymmetric and symmetric), 1092 (C-O-C), 840 (1,4 disubstituted benzene ring), 880 substituted benzene ring), 668 (Br), 592 (Cl), **^{1}H NMR**(TMS) δ ppm 1.10 (3H, s, CH_3), 2.40 3H, s, CH_3, of $COCH_3$), 4.20 (2H,s,OCH_2), 8.00 – 8.20 (6H, m, Ar-H), 10.90 (1H , s, NH of NHCO) **Mass** m/z 481 (M^+), 440, 424, 189, 91, 80, 77, 65 [Found C, 44.86; H, 3.07; N, 8.69; $C_{18}H_{15}O_3N_3Br_2$ requires C, 44.90, H.3.11, N, 8.73%]

Preparation of 4-amino-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted-1,2,4-triazoles (5a1-a16) and 4 (N-pyridyl carboxamido)-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted -1,2,4-triazoles (6a1-a16).

A mixture of 4a1 (0.01 mol) and hydrazine hydrate/INH (0.01 mol) in ethanol (25 ml) was refluxed for 8 to 10 hrs. Excess of ethanol was removed under reduced pressure and residual mass was poured into ice cold water. The separated solid was washed with dil acetic acid followed by water. The dried solid was crystallized from aq. ethanol. Following the same procedure the compounds of the series 5a2-a16 and (6a-a16) were prepared. The compounds thus obtained are listed in Table-1.

5a2 IR (KBr) ν cm-^{1} 3340 (NH_2) 3210, NH, 3063 (aromatic C-H stretching), 2870-2930 (C-H stretching of CH_3-), 1668 (C=O of CONH), 1616 (C=N), 1604 (C=C) 1464 (C-N), 880 substituted benzene ring 662, Br, 518 (Cl), **^{1}H NMR**(TMS) δ ppm 2.50 (2H,s,CH_3), 6.30 (2H,s,NH_2) 8.00 – 8.30 (2H,m,Ar-H), 8.60 – 8.80 (2H, m, heterocyclic) 10.20, (1H, s, N_4 of CONH), **Mass** m/z 487 (M^+) 446, 430, 235, 205, 160, 83, 80, 77, 65 [Found C, 34.36; H, 1.98; N, 20.09; $C_{14}H_{10}ON_7C_{l2}Br_2$ requires C.34-49, H, 2.05; N, 20.12%]

5a4 IR (KBr) ν cm-^{1} 3360, 3232 NH_2 and NH 3062 (Aromatic C-H stretching) 2902 C-H stretching of CH_3 group, 1660 (C=O), 1608 (C=N), 1602 (C=C), 1459 (C-N); 884 (substituted phenyl ring), 640, (Br), 600 (Cl), **^{1}H NMR**(TMS) δ ppm 2.40 (3H,s,CH_3); 6.20 (2H, s, NH_2), 8.00 – 8.20 (2H,m,Ar-H) 8.50-8.70 (3H,m,heterocyclic), 10.80 (1H, s, NH of CONH), **Mass** m/z 486, 445, 429, 235, 194 78, 77, 65 [Found C, 36.085, H, 2.17, N, 17.16; $C_{15}H_{11}ON_6Br_2Cl$ requires C, 37.03; H,2.26; N,17.25%]

5a8 IR (KBr) ν cm-^{1} 3354, 3226 (NH_2 & NH), 3064 (Aromatic C-H stretching) 2918 (C-H stretching of CH_3 group) 1664 (C=O), 1612 (C=N), 1604 (C=C), 1462 (C-N), 890 (substituted phenyl ring), 642 (Br), 602 (Cl) **^{1}H NMR**(TMS) δ ppm 2.60 (6H,s,2xCH_3) 6.20 (2H,s,NH_2), 8.00-8.20 (4H,m,Ar-H). 11.00 (2H, s, NH of NHCO), **Mass** m/z molecular ion peak at 666 is not observed, other prominent peaks are 585, 553, 470, 235, 155, 83, 80, 77, 65 [Found C, 32.38; H, 2.05; N, 12.46; $C_{18}H_{14}O_2N_6Br_4$ requires C, 32.43; H, 2.10; N, 12.61%].

5a11 IR (KBr) ν cm-^{1} 3354, 3236 (NH2 & NH) 3070 (aromatic C-H stretching) 2836 – 2940 (C-H stretching of CH_2 and CH_3 groups both asymmetric and symmetric), 1670 (C=O), 1619, (C=N), 1606 (C=C), 1489 (C-N), 1446, 1392 (C-H bending of CH_2 & CH_3 groups both asymmetric and symmetric), 842 (1,4 disubstituted phenyl ring) 886 (substituted phenyl ring), 642 (Br); **^{1}H NMR**(TMS) δ ppm 0.90 (6H, d, 2XCH_3 of CH-(CH_3)2), 1.30 (3H,d,CH_3 of (H-CH_3) 1.80, (1H, m, CH of –$CH(CH_3)2$), 2.40 (2H,d,CH_2 of CH_2CH (CH_3)2), 2.60 (3H,s,CH_3 of $NHCOCH_3$), 3.70 (1H, CH of CH-CH_3), 6.20 (2H,s,NH_2), 7.60-8.00 (6H,m,Ar-H),10.60 1H,s, NH of NHCO), **Mass** m/z 535, 494, 478, 243, 235, 161, 134, 91, 83, 80, 77, 65; [Found C, 49.24; H, 4.55; N, 12.88; $C_{22}H_{25}ON_5Br_2$ requires C,49.34; H, 4.67; N, 13.08%).

5a16 IR (KBr) ν cm-^{1} 3378, 3236 (NH_2, NH) 3066 (aromatic C-H stretching) 2861-2960 (C-H stretching of OCH_2 and CH_3 group), 1672 (C=O of CONH), 1620 (C=N), 1605 (C=C), 1490 (C-N), 1450-1390 (C-H bending of OCH_2 & CH_3 group) asymmetric and

symmetric), 840 (1,4 disubstituted phenyl ring), 884 (substituted phenyl ring) 665 (Br), 590 (Cl) **^{1}H NMR**(TMS) δ ppm 1.20 (3H,s,CH3), 2.40 (3H,s,CH_3 of $COCH_3$), 4.20 (2H,s,OCH_2) 6.50 (2H, s,NH_2), 8.00-8.30 (6H,m,Ar-H),11 (1H ,s,NH of NHCO) **Mass** m/z 495 (M^+), 454, 438, 374, 235, 121, 106, 91, 77, 65 [Found C, 43.57; H, 3.28; N, 14.09; $C_{18}H_{17}O_2N_5Br_2$ requires C, 43.63; H, 3.43; N, 14.14 %]

6a2 IR (KBr) ν cm-^{1} 3240 (NH), 3065 (aromatic C-H stretching), 2865, 2928 (C-H stretching of CH_3 asymmetric and symmetric) 1670 (C=O of CONH), 1614 (C=N) 1599 (C=C), 1478 (C-N) 1465-1380 (C-H bending of CH_3 asymmetric and symmetric), 880 and 740 (substituted phenyl rings) 644 (Br), 592 (Cl), **^{1}H NMR**(TMS) δ ppm 2.50 (2H,s,CH3), 7.60-7.80 (2H,m,Ar H), 8.50-8.80 (6H,m,pyridyl, pyrazinyl proton) 10.60 (1H,s,NH of $NHCOCH_3$), 10.80 (1H,s,NH of NHCO), **Mass**, m/z 592 (M^+), 551, 546, 311, 235, 176, 121, 113, 77, 65 [Found C, 40.48; H, 2.08; N, 18.87; $C_{20}H_{13}O_2N8Br_2Cl$ requires C,40.54; H,2.19;N,18.91 %]

6a4 IR (KBr) ν cm-^{1} 3235 (NH), 3058 (aromatic C-H stretching), 2862-2924 (C-H stretching of CH_3 group asymmetric & symmetric) 1664 (C=O of CONH), 1618 (C=N), 1602 (C=C), 1476 (C-N), 1454-1386 (C-H) bending of CH_3 asymmetric and symmetric)884 & 736 (substituted phenyl ring) 640 (Br) 594 (Cl-), **^{1}H NMR**(TMS) δ ppm 2.50 (2H,s,CH_3), 7.60-7.80 (2H,m,Ar-H), 8.50-8.90 (7H,m, pyridyl), 10.60 (1H ,s,NH of NH $COCH_3$) 10.80 (1H,s,NH of NHCO), **Mass** m/z 592, 551, 546, 301, 235, 189, 112, 107, 75, 77, 65 [found C, 42.46; H, 2.46, N, 16.49; $C_{21}H_{15}O_2N_7$ Br_2Cl requires C,42.56; H,2.53; N,16.55%]

6a8 IR (KBr) ν cm-^{1} 3238 (NH) 3059 (aromatic C-H stretching) 2830 2928 (C-H stretching of CH_3 groups asymmetric and symmetric) 1670 (C=O of NHCO), 1616 (C=N), 1603 (C=C), 1486 (C-N), 1458-1382 (C-H bending of CH_3 group) 890 (Substituted phenyl rings) 642 (Br), 595 (Cl), **^{1}H NMR**(TMS) δ ppm 2.50 (6H,s,2XCH_3) 7.60-7.90 (4H, m, Ar-H), 8.50-8.80 (4H, m, pyridyl proton) 10.60 (2H,s,NH of $NHCOCH_3$) 10.90 (1H,s,NH of CONH) **Mass** the molecular ion peak at m/z 771, is not observed the other prominence peaks are m/z. 479, 438, 292, 187, 106, 83, 77, 65 [found C, 37.28; H, 2.08; N, 12.66; $C_{24}H_{17}O_3N_7Br_4$ requires C, 37.35 ; H, 2.20 ; N. 12.71%]

6a11 IR (KBr) ν cm-^{1} :3243 (NH), 3068 (aromatic C-H stretching) 2940-2836 (C-H stretching of CH_2 & CH_3 groups asymmetric and symmetric) 1676 (C=O), 1622 (C=N), 1605 (C=C) 1490 (C-N), 1458-1398 (C-H bending of CH_2 & CH_3 groups asymmetric & symmetric) 840 (1,4 disubstituted phenyl ring) 890 (substituted phenyl ring) 644 (Br) **^{1}H NMR**(TMS) δ ppm: 0.90 (6H, d, 2xCH_3) of CH-(CH_3)2, 1.30 (3H, d, CH_3 of CH-CH_3) 1.80 (1H, m, CH of –CH (CH_3)2, 2.4 (2H, d, CH_2 of CH_2 – CH (CH_3)2) 3.70 (1H, q CH of CH – CH_3) 7.60-8.00 (6H, m, Ar-H), 8.40 – 8.70 (4H,m, pyridyl protons), 10.70 (1H,s,NH of $NHCOCH_3$) 10.90 (1H, s, NH of CONH. **Mass** parent ion peak at 640 is not observed other prominent peaks are at 599, 348, 292, 187, 161, 106, 82, 77, 65 [found C, 52.46; H, 4.28; N, 13.08; $C_{28}H_{28}O_2N_6Br_2$ requires C, 52.5; H, 4.37; N, 13.12%]

6a16 IR (KBr) ν cm-^{1} 3242 (NH), 3068 (aromatic C-H stretching), 2845 – 2938 (C-H stretching of OCH_2 and CH_3 groups both asymmetric and symmetric) 1672 (C=O), 1618 (C=N), 1604 (C=C), 1482 (C-N), 1446-1388 (C-H bending of OCH_2 & CH_3 groups, 838 (1,4-disubstituted phenyl ring), 888 (substituted phenyl ring), 652 (Br), 584 (Cl) **^{1}H NMR**(TMS) δ ppm 1.20 (3H,s,CH3) 2.50 (3H,s,CH_3 of $COCH_3$) 4.20 (2H,s,OCH_2), 7.80 – 8.10 (6H, m, Ar-H) 8.40 – 8.70 (4H, m, pyridyl protons) 10.60 (1H,s,NH of $NHCOCH_3$) 10.90 (1H,s,NH of CONH), **Mass** m/z molecular ion peak at m/z 600 is not observed, other prominent peaks are 479, 438, 235, 203, 188, 120, 91, 77, 65 [found C, 47.78; H, 3.26; N, 13.89; $C_{24}H_{20}O_3N_6Br_2$ requires C, 48.00 ; H,3.33, N, 14.00%).

Biological Screening

Antibacterial Screening

Standard nutrient agar medium

Meat extract (bacteriological)	1.0%
Peptone	1.0%
Sodium chloride	0.5%
Agar	2.0%
Water	100 ml

Meat extract was taken and made up the volume to 100 ml with water and to this were added weighed quantities of peptone, salt and agar. The contents were dissolved by heating and the mixture was filtered and pH was adjusted to 7.5. The medium was sterilized by autoclaving at 121° for 15 minutes, cooled to 45° and then poured in 20 ml quantities to petri dishes. A loopful of an overnight broth culture was spread evenly over the whole part with a sterile cotton-wool swab.

The culture plates were dried in the incubator with the lid until its surface was free from visible moisture without further delay, known concentration of the drug was applied as discs (prepared by uniformly punching out 6 mm discs from Whatmann filter paper (No. 41) and impregnating with drug (100 discs in 1 ml) with adequate spacing to the surface of the culture plates with sterile fine pointed forceps and pressed gently to ensure full contact with the medium. It was then transferred to the incubator for 24 hrs at 37°C. At the end of 24 hrs the diameter of zone of inhibition produced were measured (Table 2).

Antifungal Screening:

Composition of the sabouraud medium

Dextrose	2% w/v
Pancreative digest of casein	0.5% w/v
Peptic digest of animal tissue	0.5% w/v
Agar	2% w/v
Water	q.s
pH	5.2

Weighed quantities of dextrose, pancreative digest of casein, peptic digest of animal tissue were dissolved in water with gentle warming. The volume was made up to 250 ml with water. To this, the specified amount of agar was added and dissolved by heating on boiling water bath, and pH of the solution was adjusted to 5.2. The medium was distributed 100 ml each in two conical flasks and plugged with cotton. The broth culture of *Aspergillus niger/Candida albicans* was inoculated into each of the conical flasks.

The medium was shaken thoroughly and distributed evenly in petridishes. It was kept aside for sometime for solidification and cups were made with sterile cork borer (internal diameter 8 mm). Thirteen cups were made in each petridish. 0.1 ml of the test drugs were added. The standards used were Griseofulvin 100 mg / 0.1 ml. All test drugs were dissolved in sterile dimethyl sulphoxide in concentrations being 100 µg/1 ml. To each place 0.1 ml of the standard drugs and control i.e., solvent DMSO were added. The plates were then incubated at room temperature (25°C) for 24 hours.

Anti-inflammatory activity:

The albino rats were divided into 11 groups containing 6 animals each. The animals were fasted for 12 hrs prior to the experiment and they were supplied with water.

On the day of the experiment, the animals were weighed and marked. A mark was made on the right hind paw just beyond the tibia-tarsal junction, so that every time the paw is dipped in the mercury column up to marked level to ensure constant paw volume. Then the paw volume of each rat was measured by mercury displacement method.

The animals of group-1 were treated with Acacia suspension as Control. The group-2 animals were treated with Ibuprofen 200 mg/kg as a standard drug, which was injected half an hour prior to the injection of formalin. The animals of the groups 3 to 11 were injected with 1,2,4-triazole derivatives, in a dose of 200 mg/kg body weight, half an hour prior to injection of formalin. Then 0.1 ml of formalin was injected subcutaneously into the right hind paw of all the animals in all groups. The paw volume of all animals in all groups was measured at 60, 120, 240 and 360 minutes intervals, after formalin administration.

The differences in the paw volumes (i.e. oedema volumes) of each animals of all the groups were calculated and compared with the changes in the oedema volumes of control and the drug treated animals. The results were expressed as percentage reduction in oedema volume, which can be calculated by using the formula:

$$\text{Percent reduction} = \frac{C_{vt} - t_{vt}}{C_{vt}} \times 100$$

Where,

C_{vt} = oedema volume of control animals at time 't'

t_{vt} =oedema volume of drug treated animals at time 't'

the results are compiled in the Table 3.

Analgesic Activity:

1) Albino mice of either sex were selected and divided into eleven groups, containing six animals in each group. These animals were fasted for twenty four hours, prior to the experiment.

2) Animals of Group – I considered as Control, were administed with 3% Acacia suspension.

3) Animals of Group-II were treated with standard drug, i.e. Ibuprofen (20 mg/kg), which is considered as standard group.

4) Animals of Group-III, IV, V, VI, VII, VIII, IX, X and XI were treated with synthesized agents (20 mg/kg) respectively.

5) The reaction time for each mouse was recorded at time interval of 0, 30, 60, 120, 240 and 360 minutes after the administration of test substances by using Eddy's hot plate.

The % analgesic activity (PAA) was calculated by the following formula

$PAA = (T_2/T_1) \times 100$

T_1 is the reaction time before treatment and T_2 is the reaction time after the treatment. The results are shown in Table 3.

RESULTS AND DISCUSSION

3,5-Dibromo methyl anthranilate procured as gift sample was used as starting material in the investigations carried out. The acetylation of this product produced corresponding N-acetyl derivative, which when refluxed with excess of hydrazine hydrate for a longer period resulted the required 3,5-dibromo-N-acetyl anthranilic acid hydrazide in excellent yield[7]. Condensation of hydrazide with various aryl/hetero aryl acids in presence of phosphorous oxychloride afforded 2-(3',5'-dibromo-2'-acetylamino phenyl)-5-substituted-1,3,4-Oxadiazoles (**4a1-a16**). There oxadiazole derivatives when heated with hydrazine hydrate and INH (Isonicotinic acid hydrazide) yielded 1,2,4-triazoles namely 4-amino-3-(3',5'-dibromo -2'-acetylamino phenyl)-5-substituted-1,2,4-triazoles (**5a1-a16**) and 4-(N-pyridyl carboxamide)-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted 1,2,4-triazoles (**6a1-a16**) respectively[8]. The structures of all the newly synthesized compounds were established on the basis of their elemental and spectral data.

a) Antibacterial and antifungal activity

The newly synthesized oxadiazole and triazole derivatives have been evaluated for antibacterial activity[9], against *Bacillces subtilis Staphylococcus aureus, Escherchia coli, Psuedomonos auriginosa* and antifungal activity against *Aspargillus niger* and *Candida albicans* using *Streptomycin* and *Greseofulvin* as standard for comparison of antibacterial and antifungal activity respectively at 50 and 100 µg/ml concentration, Dimethyl sulphoxide was used as solvent control.

2-(3'5'-dibromo-2'-acetyl amino phenyl)-5-substituted-1,3,4-oxadiazoles **4a2, 4a4, 4a7, 4a8, 4a10, 4a13, 4a15** have exhibited significant antibacterial activity while the rest of the compounds showed weak to moderate antibacterial activity. Antifungal screening

results indicate that the compounds were either weakly active or inactive. Triazole derivatives 4-amino-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted-1,2,4-triazoles and 4-(N-pyridyl carboxamido)-3-(3',5'-dibromo-2'-acetyl amino phenyl)-5-substituted-1,2,4-triazoles derived from 2-(3'5'-dibromo-2'-acetyl amino phenyl)-5-substituted oxadiazoles surprisingly exhibited antibacterial and antifungal activity much different than parent oxadiazoles. In contrast to oxadiazoles majority of triazole derivatives like 4-amino triazoles exhibited no activity, while 4-N-pyridyl carboxamindo triazole derivatives showed almost the same antibacterial activity as that of parent oxadiazoles.

However majority of triazole derivatives **5a5-5a10, 5a13-5a16** showed significant antifungal activity (24-27 mm), in comparison with the reference standard *Greseofulvin* (26-28 mm). Rest of derivatives like **5a1-5a4, 5a11** and **5a12** exhibited weak antifungal activity. The invitro antifungal activity of synthesized triazole derivatives (**6a1-a16**) showed weak antifungal activity at both the concentrations.

Oxadiazoles which showed significant antibacterial activity failed to show potent antifungal activity. Perhaps the substitutant groups at positions 2 & 5 of the oxadiazole ring system do not contribute in attaining and in increasing the antifungal property of the compounds. However the oxadiazoles when converted to 4-amino triazole derivatives (**5a1-a16**) and 4-(N)-pyridyl carboxamido triazoles (**6a1-a16**) behaved totally different in exhibiting their antibacterial and antifungal activity. Conversion of oxadiazole to 4-amino triazoles resulted in the loss of antibacterial property. The compounds showed, weak or no activity. However oxadiazoles when converted to 4-N-pyridyl carboxamido triazoles showed surprisingly different behavior in their antibacterial activity. The compounds showed activity almost comparable to these of parent oxadiazoles. Perhaps in addition to the substituents at 3 & 5 positions the CONH group at 4th position of triazole may also contribute in the enhancement of antibacterial activity as the presence of CONH group generally increases the antibacterial activity of the compounds.

Majority of 4-amino triazole derivatives showed potent antifungal activity while 4-N-pyridyl carboxamido derivatives failed to show significance antifungal activity, while 4-N-pyridyl carboxamido triazole derivatives failed to show significant antifungal activity. Perhaps the substituents at 3,4 and 5 positions may act as potential. Pharmacophores which may lead to get compounds of enhanced biological potency. The molecule designed consists of different substitutents on aromatic/heterocyclic ring. The atoms/groups in the substituents at 5th position of oxadiazole ring system believed to act as carrier for toxic agents into cells of pathogenic organisms, leading to high intracellular concentration of toxicant which ultimately causes cell death.

The electron withdrawing groups like Cl, Br, NO_2 etc in oxadiazole derivatives play vital role in exhibiting antibacterial activity of the compounds. It is quite interesting to note that oxadiazole derivatives having these electron withdrawing group present in the ring system as substitutients at position 5 of oxadiazole moiety exhibited potent activity. This clearly suggests that the bromine atoms at positions 3',5' of acetanilide ring at position 2 of oxadiazole do not have any role in influencing the antibacterial property. It is because 2,5-dibromo acetanilide moiety is present at position 2 of oxadiazole ring in all the compounds of the series. However 4-amino-3,5-disubstituted triazoles (**5a1-a16**) failed to show significant antibacterial activity. Perhaps the replacement of oxygen of oxadiazole by Nitrogen to obtain triazole ring system may cause lowering of antibacterial property. It is generally observed that oxygen atom increases the oxygen uptake of organisms. Perhaps the replacement of it may be the reason for reduced antibacterial character.

Anti-inflammatory[10,11] and analgesic activity[12].

The compounds from the series (**4a1-a16**) (**5a1-a16**) and (**6a1-a16**) were screened for their anti inflammatory activity using rat hind .paw method of Winter et al., modified by Dhawan and Srimal. The compounds were also screened for analgesic activity using Eddy's hot plate technique. Ibuprofen was used as standard drug for screening of both activities. Among oxadiazole series **4a10, 4a11, 4a13, 4a16** showed much significant anti-inflammatory activity in comparison with the other members of the series. However the detailed study revealed that they posses moderate activity when compared with the standard drug. The 4-amino triazole derivatives like **5a5, 5a7, 5a8, 5a10, 5a11, 5a13, 5a16** showed significant activity. The activity of the compounds **5a11, 5a13** and **5a16** almost comparable with the standard drug. This justifies that the conversion of oxadiazole to 4-amino triazole derivatives has resulted the compounds with much enhanced potency. Perhaps the structural changes in the triazole moiety as a whole and the influence of the substituents on the ring at position 5 of the triazole may be responsible for enhanced activity of the compounds. Interestingly the 4-N-pyridyl carboxamido triazole derivatives like **6a8, 6a9, 6a10, 6a11**, & **6a13** exhibited slightly better anti-inflammatory activity then their parent oxadiazoles. Thus in general triazole derivatives derived from oxadiazoles exhibited significant activity. Rest of the compounds of all the series exhibited much weaker activity of not worth mentioning. In the literature it is shown that triazole scaffold containing compounds exhibited anti-inflammatory activity mediated through inhibition of cyclooxygenase I and II (COX-1 and II) enzyme depending upon the position and kind of substituents on the 1,2,4-triazole ring system. Investigations are pending to demonstrate their inhibitory potencies towards COX-I and COX-II inhibition.

The analgesic studies of oxadiazole derivatives (**4a1-a16**), triazole derivatives (**5a1-a16**) and (**6a1-a16**) revealed that **4a10, 4a11, 4a13, 5a10, 5a11, 5a13, 5a16, 6a10, 6a11, 6a13** and **6a16** exhibited moderate analgesic

activity in comparison with the standard drug used for the study. However the analgesic activity of these compounds is much significant when compared with the rest of the compounds of each series. The detailed study on anti-inflammatory and analgesic activity revealed that the compounds which showed significant anti-inflammatory activity are also associated with analgesic activity with varied degree.

Table 2. Antibacterial and antifungal activity of Oxadiazole and triazole derivatives (4a1-a16, 5a1-a16 and 6a1-a16)

Sr. No.	Compound	Antibacterial activity con. In μg/mL (inhibition zone in mm)								Antifungal activity con.in μg/mL zone of inhibition in mm			
		Bacillus subtilis		Staphylococcus aureus		Escherichia coli		Pseudomonas auriginosa		Aspargillus niger		Candida albicans	
		50	100	50	100	50	100	50	100	50	100	50	100
1	**4a$_1$**	09	12	06	10	07	09	05	09	14	17	12	16
2	**4a$_2$**	19	22	18	22	18	22	15	18	15	18	12	16
3	**4a$_3$**	13	16	12	15	11	15	11	14	10	14	10	13
4	**4a$_4$**	18	21	18	21	19	22	17	22	12	15	11	15
5	**4a$_5$**	10	14	11	14	11	14	10	13	10	14	09	13
6	**4a$_6$**	11	14	09	12	10	14	11	14	06	09	08	11
7	**4a$_7$**	18	22	20	23	19	22	18	22	11	14	09	12
8	**4a$_8$**	20	23	21	23	19	23	19	22	08	12	06	09
9	**4a$_9$**	13	17	12	16	12	15	11	15	09	13	08	12
10	**4a$_{10}$**	19	22	18	21	18	23	18	21	10	13	08	13
11	**4a$_{11}$**	14	16	12	15	12	16	12	16	08	12	05	09
12	**4a$_{12}$**	09	12	08	12	08	12	10	13	07	11	07	10
13	**4a$_{13}$**	19	23	20	23	18	21	18	21	12	15	09	12
14	**4a$_{14}$**	10	13	09	13	10	13	09	13	09	12	06	10
15	**4a$_{15}$**	20	23	19	22	18	23	15	18	13	16	12	15
16	**4a$_{16}$**	13	16	10	13	10	13	08	12	08	12	06	10
17	**5a$_1$**	08	11	09	12	09	12	07	11	12	15	11	14
18	**5a$_2$**	10	14	10	14	08	11	06	10	13	16	11	14
19	**5a$_3$**	07	10	06	09	07	11	06	09	10	13	09	12
20	**5a$_4$**	09	13	10	13	10	14	08	11	11	14	09	13
21	**5a$_5$**	08	12	09	13	09	12	07	10	23	27	20	24
22	**5a$_6$**	09	12	07	11	06	09	06	09	22	25	21	24
23	**5a$_7$**	11	14	10	13	10	13	11	15	23	26	22	25
24	**5a$_8$**	10	13	12	15	09	12	10	13	24	27	22	26
25	**5a$_9$**	07	10	08	12	06	09	07	10	22	26	20	23
26	**5a$_{10}$**	09	12	10	13	10	13	08	11	23	26	21	24
27	**5a$_{11}$**	08	11	08	12	07	11	06	09	12	16	11	14
28	**5a$_{12}$**	07	11	08	12	08	11	07	10	14	17	10	14
29	**5a$_{13}$**	10	14	11	15	07	10	09	13	23	26	21	24
30	**5a$_{14}$**	08	12	10	13	06	10	06	09	20	24	20	23
31	**5a$_{15}$**	09	13	10	13	10	13	08	11	20	23	21	24
32	**5a$_{16}$**	07	10	08	13	08	12	09	12	21	24	20	24
33	**6a$_1$**	08	11	07	10	08	11	07	10	10	13	09	12
34	**6a$_2$**	18	21	18	21	17	21	16	19	08	12	11	14
35	**6a$_3$**	11	14	11	14	11	14	10	13	09	12	08	12
36	**6a$_4$**	19	22	18	21	18	21	18	20	10	13	09	12
37	**6a$_5$**	10	13	09	13	09	12	08	11	11	14	10	13
38	**6a$_6$**	10	14	09	12	08	11	10	13	19	13	09	13
39	**6a$_7$**	18	22	19	22	20	23	16	20	12	14	11	14
40	**6a$_8$**	20	23	20	23	18	21	17	21	10	13	11	15
41	**6a$_9$**	11	14	11	15	10	13	10	13	12	15	10	13
42	**6a$_{10}$**	18	21	18	21	17	20	16	20	13	16	10	13
43	**6a$_{11}$**	14	17	12	15	11	14	08	12	10	14	08	12
44	**6a$_{12}$**	09	13	08	11	07	11	07	10	11	14	09	12
45	**6a$_{13}$**	18	21	19	22	18	21	17	20	12	15	10	13
46	**6a$_{14}$**	10	13	09	12	09	12	08	12	10	13	19	13
47	**6a$_{15}$**	19	22	19	23	18	21	16	19	12	16	12	15
48	**6a$_{16}$**	12	15	10	13	09	13	09	12	13	16	12	15
49	Streptomycin	20	23	21	24	19	23	17	20	-	-	-	-
50	Greseofulvin	-	-	-	-	-	-	-	-	25	28	23	27

Table 3. Anti inflammatory and Analgesic activity of Oxadiazole and Triazoles

Sr. No.	Compound	Anti-inflammatory activity			Analgesic activity		
		Dose mg /kg	Paw edema volume	% Reduction	Dose mg/kg	Maximum average reaction time (sec)	Analgesic activity
1	Control	-	0.59 ± 0.03	-	-	3.83 ± 0.31	-
2	Ibuprofen	200	0.15± 0.02	74.58	20	9.83 ± 0.33	256.66
3	**4a10**	200	0.22± 0.05	42.80	20	5.33 ± 0.33	169.71
4	**4a11**	200	0.21± 0.09	44.60	20	6.83 ± 0.31	178.40
5	**4a13**	200	0.17± 0.62	45.40	20	6.82 ± 0.54	159.64
6	**4a16**	200	0.21± 0.03	49.60	20	5.17 ± 0.33	134.62
7	**5a5**	200	0.12± 0.02	65.50	20	4.83 ± 0.31	126.12
8	**5a7**	200	0.14± 0.01	62.40	20	5.18 ± 0.36	132.00
9	**5a8**	200	0.13± 0.02	61.50	20	5.33 ± 0.33	139.00
10	**5a10**	200	0.11± 0.02	66.00	20	7.33 ± 61	191.40
11	**5a11**	200	0.08± 0.01	73.20	20	8.00 ± 0.32	202.50
12	**5a13**	200	0.11± 0.02	69.80	20	5.33 ± 0.33	187.24
13	**5a16**	200	0.12± 0.12	71.90	20	6.50 ± 0.34	169.71
14	**6a8**	200	0.17± 0.04	46.20	20	5.33 ± 0.33	139.00
15	**6a9**	200	0.16± 0.01	45.00	20	5.17 ± 0.53	128.46
16	**6a10**	200	0.15± 0.02	48.00	20	6.50 ± 0.34	170.00
17	**6a11**	200	0.16± 0.04	46.60	20	7.32 ± 0.60	184.00
18	**6a13**	200	0.15± 0.01	48.90	20	6.50 ± 0.32	172.00
19	**6a16**	200	0.15± 0.02	47.16	20	5.33 ± 0.34	185.00

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