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Original Article

Synthesis, Characterization and Study of Some *N*-Substituted Aryl-2-({4-[(Substituted Aryl Carbamoyl) Methyl]-5-(Pyridin-4-yl)-4*H*-1, 2, 4-Triazol-3-yl} Sulfanyl) Acetamide

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Abstract

Pathogenic infections and inflammation are very common ailments humans suffer. Upsurge of resistant pathogens has impeded the antimicrobial drug development process in recent years and the search of new antimicrobial agents is clearly evident from the literature. In line with these developments the synthesis of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazol-3-yl} sulfanyl) acetamide derivatives as antimicrobial, antioxidant and anti-inflammatory had been undertaken. The synthesis of target derivatives was achieved in three steps viz. potassium-pyridine-dithiocarbazate (II) obtained from base catalyzed reaction of isoniazide with CS_2 which was further cyclized with hydrazine hydrate and CS_2 to afford 4-amino-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (III). This compound upon reaction with different aromatic N-substituted-α-chloroacetanilide afforded title compound *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazol-3-yl} sulfanyl) acetamides IV (KA1-KA15). All the newly synthesized derivatives were screened for i*n vitro* antibacterial activity carried out against four bacterial strains viz. *E.coli*, *K.pneumonia*, *S.aureus*, and *B. Subtilis* and antifungal activity against two fungal strains viz. *A.niger* and *S. cerevisiae* by minimum inhibitory concentration (MIC) method, *in vitro* antioxidant activity was carried out by hydrogen peroxide radical scavenging method, and *in vitro* anti-inflammatory activity by inhibition of protein denaturation method. From the results of screening, it is evident that most of the derivatives KA3, KA4, KA7, KA9, KA11, and KA14 were found to possess promising biological activities and the derivatives with presence of electron-withdrawing groups at *o*-, *m*- and *p*- position of the phenyl ring improve the activity considerably.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, MIC, 1, 2, 4-Triazoles.

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1. Introduction

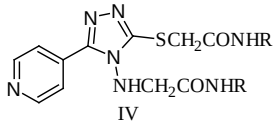
The upsurge of multi-drug resistant pathogens had made the treatment of microbial infections complicated [1]. In the past few decades, 1, 2, 4-triazoles and their fused heterocyclic compounds had received considerable importance due to their prevailing biological importance and many approved drugs contain 1, 2, 4-triazole ring system which include anti-inflammatory, antianxiety, cannabinoid, anticancer, antimicrobial, antiviral, and antiulcer agents [2-10]. In 1, 2, 4-triazole ring substitutions at 3, 4 and 5th position had been exploited in present work through the synthesis of series of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl} sulfanyl) acetamide derivatives. The synthesized derivatives were duly characterized by FT-IR, ^{1}H-NMR and GC-mass spectroscopy studies and evaluated for their antimicrobial, antioxidant and anti-inflammatory responses *in vitro.* The antimicrobial, antioxidant and anti-inflammatory activities were studied by minimum inhibitory concentration (MIC), hydrogen peroxide radical scavenging activity and inhibition of protein denaturation method respectively.

2. Materials and Methods

2.1. Materials

All reagents and solvents used in the present study were of analytical grade and procured from Loba chemie (India).The progress of the reaction was monitored by TLC using Merck silica gel precoated plate, with appropriate mobile phase, visualization by iodine vapour, UV chamber and products were purified by recrystallization technique. All the melting points were recorded on a Veego apparatus (Mumbai, India) and are uncorrected. All the synthesized derivatives were characterized by their FT-IR, ^{1}H-NMR, GC-Mass spectroscopy. FT-IR spectra were recorded in KBr on Bruker FT-IR instrument (Germany),^{1}H-NMR spectra were recorded on Bruker Avance ^{1}H-NMR spectrometer (Germany), at 400 MHz in DMSO-d6, by using varian instrument using TMS as internal standard and chemical shift values are given in ppm downfield to TMS (tetramethylsilane) and GC-Mass were recorded on GCMS-QP-5050 Schimadzu (Japan). The standard drugs Norfloxacin, Ketoconazole, Ascorbic acid and Ibuprofen were obtained as gift sample from Wockhardt Ltd., Aurangabad, India.

Table 1. Physicochemical characterization of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazol-3-yl} sulfanyl) acetamide.



IV

Comp	R	Mole. Formula	M.W.	M.P. (^{o}C)	Yield (%)
KA1	*4*-NO_2-Ph	$C_{23}H_{19}N_9O_6S$	549	120-121	75.64
KA2	*3*-NO_2-Ph	$C_{23}H_{19}N_9O_6S$	549	142-143	74.67
KA3	*4*-Cl-Ph	$C_{23}H_{19}Cl_2N_7O_2S$	528	205-206	68.24
KA4	*4*-OCH_3-Ph	$C_{25}H_{25}N_7O_4S$	519	209-210	77.06
KA5	*3*-CH_3-Ph	$C_{25}H_{25}N_7O_2S$	487	213-214	66.95
KA6	Ph	$C_{23}H_{21}N_7O_2S$	459	198-200	72.23
KA7	*4*-Br-Ph	$C_{23}H_{19}Br_2N_7O_2S$	617	204-205	75.30
KA8	*2*-NO_2-Ph	$C_{23}H_{19}N_9O_6S$	549	74-75	80.30
KA9	*3*-Cl-Ph	$C_{23}H_{19}Cl_2N_7O_2S$	528	170-171	60.40
KA10	*1*-Naphthalene	$C_{31}H_{25}N_7O_2S$	559	106-107	60.39
KA11	*4*-CH_3-Ph	$C_{25}H_{25}N_7O_2S$	487	217-218	65.93
KA12	2,4-$(NO_2)_2$-Ph	$C_{23}H_{17}N_{11}O_{10}S$	639	174-175	70.30
KA13	*2*-CO_2H-Ph	$C_{25}H_{21}N_7O_6S$	547	240-241	66.82
KA14	*2*-Cl-Ph	$C_{23}H_{19}Cl_2N_7O_2S$	528	225-226	60.90
KA15	*4*-CO_2H-Ph	$C_{25}H_{21}N_7O_6S$	547	236-237	70.30

2.2. Synthesis of Potassium-Pyridine-Dithiocarbazate (II)

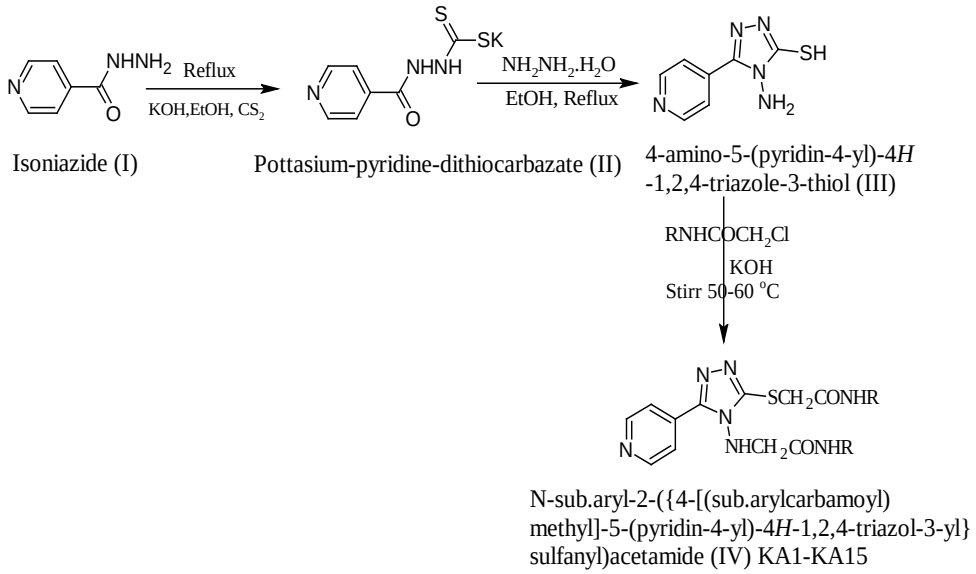
Isoniazide I (0.075mol, 10.28 g) was dissolved in a solution of potassium hydroxide (0.075mol, 4.2 g) in 100 ml of absolute ethanol and carbon disulphide (0.075 mol). The reaction mixture was agitated overnight and diluted with 200 ml of dry ether. The solid obtained was filtered and washed with dry ether, yield 80% [11].

2.2.1. Synthesis of 4-Amino-5-(pyridine-4-yl)-4H-1, 2, 4-Triazole-3-Thiol (III)

A mixture of potassium-pyridine-dithiocarbazate (II) (0.1mol, 25.1 g) and $NH_2NH_2.H_2O$ (0.1 mol, 5 ml) in 50 ml ethanol was refluxed for 2 h with occasional shaking and then solution was poured into the cold water. The solution acidified with 50 % HCl and precipitate obtained was filtered, dried and recrystalized from ethanol. m.p: 255-257 ^{0}C, TLC mobile phase- Ethyl Acetate: Methanol (70:30), yield 90% [11].

2.2.2. General Procedure for Synthesis of Aromatic N-Substituted- α -Chloroacetanilide

An aromatic primary amine (0.05mol) was dissolved in a mixture of glacial acetic acid (25 ml), saturated solution of sodium acetate (25 ml) and cooled to 5^{0}C. To this mixture, chloro acetyl



Scheme 1. Synthesis of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazol-3-yl} sulfanyl) acetamide (IV): Where R: *4*-NO_2-Ph, *3*-NO_2-Ph,*4*-Cl-Ph, *4*-OCH_3-Ph, *3*-CH_3-Ph, Ph, *4*-Br-Ph, *2*-NO_2-Ph, *3*-Cl-Ph, *1*-Naphthalene, *4*-CH_3-Ph, 2,4-$(NO_2)_2$-Ph, *2*-CO_2H-Ph, *2*-Cl-Ph, *4*-CO_2H-Ph.

chloride (0.075 mol, 6.2 ml) was added dropwise at 0-5^{0}C under constant stirring. Then it was left at room temperature for 6 h, the crude product that separated was filtered, washed with 50% acetic acid, cold water, dried and recrystalized from ethanol. TLC mobile phase- Ethyl Acetate: Petroleum ether: Methanol (2:2:1) [12].

2.2.3. Synthesis of N-substituted Aryl-2-({4-[(substituted Aryl Carbamoyl) Methyl]-5-(Pyridin-4-yl)-4H-1, 2, 4-Triazol-3-yl} Sulfanyl) Acetamide (IV)

The compound (III) (0.01 mol, 1.93 g) was dissolved in aqueous KOH (0.6%, 10 ml) under stirring till a clear yellow solution obtained, filtered to remove any suspended impurities and aromatic *N*- substituted-α-chloroacetanilide (0.02 mol) was added in small portions with stirring at 50-60^{0}C for 5 h. Reaction mixture was left overnight, the precipitate obtained filtered, washed with cold water, dried and recrystalized from ethanol. TLC mobile phase- Ethyl Acetate: Petroleum ether: Toluene (2:1:1) [12]. Scheme I and Table1.

2.2.3.1.N-(4-Nitrophenyl)-2-(4-(2-(4-Nitrophenyl amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA1)

FT-IR ν max (KBr, cm^{-1}): 3478 (N-H str), 2915 (C-H str, aliphatic),1626 (C=N str, aromatic), 1442 (C-N str, ring), 1572 (C=C str, aromatic),1626 (C=O str, amide), 3336 (N-H str, amide), 1502 (C-NO_2 str).^{1}H -NMR (DMSO, 400 MHz) δ: 2.10 (s,1H,NH), 10.56,

10.40 (s, 2H,sec. amide), 8.60-7.91 (d, J=1.4, 2.0 Hz, 2H, pyridine), 8.25-7.60 (m, 8H, aromatic),4.16-3.26 (d, 4H, methylene). MS: [M]+ at m/z 549.

2.2.3.2.N-(3-Nitrophenyl)-2-(4-(2-(3-Nitrophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-Yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA2)

FT-IR ν max (KBr,cm^{-1}): 3369(N-H str), 3065 (C-H str, aromatic), 2917 (C-H str, aliphatic), 1645 (C=N str, aromatic), 1433 (C-N str, ring),1513 (C=C str, aromatic),1645 (C=O str, amide), 3302 (N-H str, amide),1548 (C-NO_2, str). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.16 (s, 1H, NH), 10.76, 10.35 (s, 2H, sec. amide), 8.65-7.91 (d, J=6.2, 2.0 Hz, 2H, pyridine), 8.62-8.10-7.64 (m, 8H, aromatic), 4.14-3.16 (d, 4H, methylene). MS: [M]+ at m/z 549.

2.2.3.3. N-(4-Chlorophenyl)-2-(4-(2-(4-Chlorophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA3)

FT-IR ν max (KBr, cm^{-1}): 3342 (N-H str), 2916 (C-H str, aliphatic),1606 (C=N str, aromatic), 1450 (C-N str, ring),1552 (C=C str, aromatic),1671 (C=O str, amide),697(C-Cl str). ^{1}H-NMR (DMSO, 400 MHz) δ:2.19 (s,1H,NH),10.60,10.26 (s, 2H, sec. amide),8.23-8.01 (d, J=7.6, 4.4 Hz, 2H, pyridine),7.46-7.44 (m, 8H, aromatic),4.16-3.36 (d, 4H, methylene). MS: [M] + at m/z 528.

2.2.3.4.N-(4-Methoxyphenyl)-2-(4-(2-(4-Methoxyphenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA4)

FT-IR ν max (KBr, cm^{-1}): 3286 (N-H str), 3037 (C-H str, aromatic), 2914 (CH str, aliphatic),1650 (C=N str, aromatic),1406(C-N str, ring),1507(C=C str, aromatic),1650 (C=O str, amide), 3168 (N-H str, amide),1106 (C-O str, ether). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.50 (s,1H, NH), 10.71,10.47 (s, 2H, sec. amide), 8.72-8.05 (d, J=2.2, 2.8 Hz, 2H, pyridine),7.58-7.32 (m, 8H, aromatic),4.21-3.29 (d,4H, methylene),3.83-3.45 (t, 6H, methoxy). MS: [M]+ at m/z 519.

2.2.3.5.N-(3-Methylphenyl)-2-(4-(2-(3-Methylphenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1,2,4-Triazol-3-ylthio) Acetamides (KA5)

FT-IR ν max (KBr, cm^{-1}): 3293 (N-H str), 3012 (C-H str, aromatic),1658 (C=N str, aromatic),1406 (C-N str, ring),1547 (C=C str, aromatic),1587(C=O str, amide), 3170 (N-H str, amide). ^{1}H-NMR (DMSO, 400 MHz) δ:2.20 (s,1H,NH),10.82,10.36 (s,2H, sec. amide), 8.62-8.11 (d, J=1.08, 1.1 Hz, 2H, pyridine),7.41-7.37-7.35-6.85 (m, 8H, aromatic),4.16-3.39 (d, 4H, methylene), 2.50 (t, 6H, methyl). MS: [M]+ at m/z 487.

2.2.3.6.2-(4-(2-oxo-2-(Phenyl Amino) Ethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio)-N-Phenylacetamide (KA6)

FT-IR ν max (KBr, cm^{-1}): 3291 (N-H str), 3037 (C-H str, aromatic),1593 (C=N str, aromatic), 1405 (C-N str, ring),1540 (C=C str, aromatic),1653 (C=O str, amide), 3177 (N-H str, amide). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.50 (s, 1H, NH), 10.68, 10.47 (s, 2H, sec. amide), 8.70-8.00(d, J=1.4, 3.2 Hz, 2H, pyridine), 7.61-7.58-7.28 (m, 10H, aromatic), 4.16-3.37 (d, 4H, methylene). MS:[M]+ at m/z 459.

2.2.3.7.N-(4-Bromophenyl)-2-(4-(2-(4-Bromophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA7)

FT-IR ν max (KBr, cm^{-1}): 3282 (N-H str),3028 (C-H str, aromatic),1581 (C=N str, aromatic),1456 (C-N str, ring),1485 (C=C str, aromatic),1655 (C=O str, amide),3173 (N-H str, amide),677 (C-Br str). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.26 (s, 1H, NH), 10.59, 10.26 (s, 2H, sec. amide), 8.77-8.06 (d, J=0.7, 1.08 Hz, 2H, pyridine), 7.42-7.37 (m, 8H, aromatic), 4.19-3.34 (d, 4H, methylene). MS:[M]+ at m/z 617.

2.2.3.8 N-(2-nitrophenyl)-2-(4-(2-(2-Nitrophenyl Amino)-2-oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA8)

FT-IR ν max (KBr, cm^{-1}): 3472 (N-H str),1565 (C=N str, aromatic),1426 (C-N str, ring),1495 (C=C str, aromatic),1620 (C=O str, amide), 3339 (N-H str, amide),1342 (C-NO_2 str). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.14 (s, 1H, NH), 10.56, 10.39 (s, 2H, sec. amide), 8.71-8.10 (d, J=2.2, 2.08 Hz, 2H, pyridine), 8.11-7.93-7.88-7.61 (m, 8H, aromatic), 4.16-3.46 (d, 4H, methylene). MS:[M]+ at m/z 549.

2.2.3.9N-(3-Chlorophenyl)-2-(4-(2-(3-Chlorophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1,2,4-Triazol-3-ylthio) Acetamides (KA9)

FT-IR ν max (KBr, cm^{-1}): 3337 (N-H str), 3049 (C-H str, aromatic),1594 (C=N str, aromatic),1435 (C-N str, ring),1555 (C=C str, aromatic),1672 (C=O str, amide),3249 (N-H str, amide),681 (C-Cl str). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.17 (s, 1H, NH), 10.81, 10.47 (s, 2H, sec. amide), 8.79-8.21 (d, J=2.2, 1.4 Hz, 2H, pyridine), 7.90-7.59 (m, 8H, aromatic), 4.16-3.26 (d, 4H, methylene). MS:[M]+ at m/z 528.

2.2.3.10.N-(Naphthalene-1-yl)-2-(3-(2-(Naphthalene-2-ylamino)-2-Oxoethylthio)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-4-ylamino) Acetamides (KA10)

FT-IR ν max (KBr, cm^{-1}): 3252 (N-H str), 1598 (C=N str, aromatic), 1461 (C-N str, ring), 1546 (C=C str, aromatic), 1665 (C=O str, amide), 3059 (N-H str, amide). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.49 (s, 1H, NH), 10.34-10.20 (s, 2H, sec. amide), 8.69-8.04 (d, J=1.28, 8.16 Hz, 2H, pyridine), 8.24-7.46-7.88-7.78-7.88-7.50-7.44 (m, 14H, naphthalene), 4.33-3.37 (d, 4H, methylene). MS: [M]+ at m/z 559.

2.2.3.11.N-(4-Methylphenyl)-2-(4-(2-(4-Methylphenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-ylthio) Acetamides (KA11)

FT-IR ν max (KBr, cm^{-1}): 3476 (N-H str), 1582 (C=N str, aromatic), 1440 (C-N str, ring), 1466 (C=C str, aromatic),1626 (C=O str, amide),3350 (N-H str, amide). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.26 (s, 1H, NH), 10.62, 10.26 (s, 2H, sec. amide), 8.67-7.96 (d, J=1.16, 1.48 Hz, 2H, CH, pyridine), 7.46-7.09 (m, 8H, aromatic), 4.16-3.36 (d, 4H, methylene), 2.49 (6H, methyl). MS: [M] + at m/z 487.

2.2.3.12. N-(2, 4-dinitrophenyl)-2-(4-(2-(2, 4-Dinitrophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1,2,4-Triazol-3-ylthio) Acetamides (KA12)

FT-IR ν max (KBr, cm^{-1}): 3441(N-H str), 1579(C=N str, aromatic), 1421(C-N str, ring), 1492(C=C str, aromatic), 1622(C=O str, amide), 3325 (N-H str, amide), 1328 (C-NO_2 str).^{1}H-NMR (DMSO, 400 MHz) δ:2.01 (s,1H, NH), 10.74, 10.26 (s,2H, sec. amide),8.78-8.12 (d, J=2.6, 0.70 Hz, 2H, pyridine), 8.83-8.22-8.13 (m, 6H, aromatic), 4.10-3.34 (d, 4H, methylene). MS: [M]+ at m/z 639.

2.2.3.13. 2-(2-(4-(2-(2-Carboxyphenylamino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1,2,4-Tiazol-3-yithio) Acetamide) Benzoic Acid (KA13)

FT-IR ν max (KBr, cm^{-1}): 3342 (N-H str), 2916 (C-H str, aliphatic), 1606 (C=N str, aromatic), 1450 (C-N str, ring), 1552 (C=C str, aromatic), 1671 (C=O str, amide). ^{1}H-NMR (DMSO, 400 MHz) δ: 12.00 (s, 2H, carboxyl), 2.50 (s, 1H, sec. amine), 8.70-7.99 (d, J=1.0, 6.08 Hz, 2H, pyridine), 8.50-8.00-7.55-7.16 (m, 8H, aromatic), 4.31-3.48 (d, 4H, methylene). MS: [M] + at m/z 547.

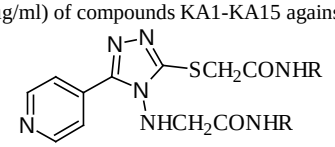
2.2.3.14.N-(2-Chlorophenyl)-2-(4-(2-(2-Chlorophenyl Amino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-yithio) Acetamides (KA14)

FT-IR ν max (KBr, cm^{-1}): 827(C-Cl str), 1392 (C=C str, aromatic), 1582 (C=O str, amide), 1490 (C-N str, ring). ^{1}H-NMR (DMSO, 400 MHz) δ: 2.14 (s, 1H, NH), 10.56, 10.21 (s, 2H, sec. amide), 8.67-8.25 (d, J=2.5, 1.4 Hz, 2H, pyridine), 7.61-7.93-7.49 (m, 8H, aromatic), 4.28-3.26 (d, 4H, methylene). MS: [M]+ at m/z 528.

2.2.3.15.4-(2-(4-(2-(4-Carboxyphenylamino)-2-Oxoethylamino)-5-(Pyridine-4-yl)-4H-1, 2, 4-Triazol-3-yithio) Acetamide) Benzoic Acid (KA15)

FT-IR ν max (KBr, cm^{-1}): 1550 (C=N str, aromatic),1397 (C-N str, ring),1487 (C=C str, aromatic),1601 (C=O str, amide),1671(C=O str, carboxyl).^{1}H-NMR (DMSO, 400 MHz) δ:2.14 (s, 1H, NH),10.79,10.33 (s, 2H, sec. amide), 8.71-7.93 (d, J=2.5, 2.12 Hz, 2H, pyridine),

Table 2. Minimum inhibitory concentration values (µg/ml) of compounds KA1-KA15 against microbes (Mean ± SD, N=6).



IV

Comp	R	*E. Coli.*	*K. pneumoniae*	*S.aureus*	*B.subtilis*	*A .niger*	*S.cerevisiae*
KA1	*4*-NO_2-Ph	37.83±0.68	58.66±0.40	51.25±0.27	41.66±0.40	58.83±0.25	46.80±0.27
KA2	*3*-NO_2-Ph	43.08±0.58	62.66±0.40	75.91±0.37	55.33±0.40	62.83±0.25	52.60±0.41
KA3	*4*-Cl-Ph	21.25±0.52	32.33±0.40	34.00±0.44	40.08±0.58	28.41±0.49	21.80±0.27
KA4	*4*-OCH_3-Ph	23.58±0.58	38.58±0.37	42.75±0.27	30.58±0.37	30.41±0.49	25.50±0.50
KA5	*3*-CH_3-Ph	31.25±0.52	49.58±0.37	54.75±0.27	42.66±0.40	46.66±0.60	40.80±0.57
KA6	ph	41.41±0.37	71.08±0.37	63.25±0.27	48.75±0.27	68.66±0.40	51.20±0.75
KA7	*4*-Br-Ph	18.33±0.40	42.75±0.27	30.66±0.40	18.91±0.37	22.83±0.25	34.70±0.44
KA8	*2*-NO_2-Ph	51.08±0.37	76.66±0.40	67.58±0.37	60.83±0.25	73.33±0.51	43.60±0.41
KA9	*3*-Cl-Ph	30.00±0.44	50.25±0.27	41.00±0.44	29.25±0.27	25.41±0.49	28.10±0.41
KA10	*1*-Naphthalene	54.41±0.37	90.25±0.27	78.83±0.25	59.75±0.52	79.66±0.40	62.70±0.44
KA11	*4*-CH_3-Ph	36.08±0.37	48.16±0.25	44.25±0.27	34.16±0.25	35.66±0.40	28.70±0.27
KA12	2, 4-$(NO_2)_2$-Ph	56.66±0.40	66.16±0.25	78.25±0.27	44.58±0.49	54.75±0.27	32.60±0.41
KA13	*2*-CO_2H-Ph	47.33±0.40	81.66±0.25	55.91±0.37	65.66±0.51	27.25±0.27	41.50±0.50
KA14	*2*-Cl-Ph	27.50±0.44	35.91±0.37	39.08±0.37	39.00±0.31	27.41±0.49	18.70±0.57
KA15	*4*-CO_2H-Ph	60.83±0.51	91.75±0.27	96.83±0.40	76.16±0.51	87.66±0.40	58.50±0.50
Norfloxacin	----	15.50±0.44	28.50±0.44	24.33±0.40	16.25±0.27	----	----
Ketoconazole	----	----	----	----	----	20.83±0.25	12.80±0.27

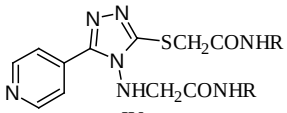
7.88-8.10 (m, 8H, aromatic), 4.16-3.36 (d, 4H, methylene). MS: [M]+ at m/z 547.

2.3. Biological Evaluation

2.3.1. Antimicrobial Activity

All the synthesized derivatives were screened for *in vitro* antimicrobial activity against two gram positive strains *S .aureus* (*S.aureus*, NCIM 2079), *B. subtilis* (*B. subtilis*, NCIM 2711) and two gram negative strains *E.coli* (*E.coli*, NCIM 2685), *K. pneumonia* (*K. pneumoniae*, NCIM 2957*)* and two fungal strains *A. nigar* (*A. nigar*, NCIM 596), *S. cerevisiae* (*S. cerevisiae*, NCIM 3102), using the broth micro dilution method. Minimum inhibitory concentration (MIC) was determined and compared with standard drugs Norfloxacin for antibacterial and Ketoconazole for antifungal activity and statistical analysis was performed using ANOVA to find the significance of the test (Table 2) [13].

Table 3. Anti-inflammatory activity by % inhibition of protein denaturation and antioxidant activity by hydrogen peroxide radical scavenging for compounds KA1-KA15(Mean ± SD, N=6).



IV

Comp	R	Anti-inflammatory effect (%)		Scavenging effect (%)	
		100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
KA1	*4*-NO_2-Ph	47.14±0.12	45.10±0.27	32.23±0.48	16.41±0.07
KA2	*3*-NO_2-Ph	51.07±0.06	47.41±0.12	37.14±0.03	26.22±0.23
KA3	*4*-Cl-Ph	59.34±0.05	56.22±0.47	45.31±0.03	38.81±0.27
KA4	*4*-OCH_3-Ph	55.14±0.04	53.55±0.05	41.73±.03	34.14±0.05
KA5	*3*-CH_3-Ph	54.59±0.14	51.75±0.22	40.49±.05	31.66±0.08
KA6	Ph	48.30±0.05	45.54±0.07	34.58±0.07	21.75±0.16
KA7	*4*-Br-Ph	61.45±0.05	59.83±0.14	47.38±0.34	40.42±0.06
KA8	*2*-NO_2-Ph	49.60±0.05	44.30±0.12	35.62±0.02	23.52±0.06
KA9	*3*-Cl-Ph	62.65±0.19	61.54±0.44	50.37±0.17	45.42±0.06
KA10	*1*-Naphthalene	39.55±0.02	37.30±0.15	30.55±0.13	15.26±0.12
KA11	*4*-CH_3-Ph	61.51±0.21	58.48±0.13	48.35±0.18	42.94±0.06
KA12	2,4-$(NO_2)_2$-Ph	47.96±0.23	43.50±0.12	33.45±0.15	19.89±0.13
KA13	*2*-CO_2H-Ph	57.47±0.10	53.91±0.03	43.38±0.08	36.40±0.01
KA14	*2*-Cl-Ph	63.77±0.24	58.31±0.07	50.71±0.14	48.50±0.09
KA15	*4*-CO_2H-Ph	52.65±0.05	47.14±0.09	39.59±0.09	27.60±0.10
Ibuprofen	-------	66.67±0.15	49.57±0.08	------	------
Ascorbic acid	-------	------	------	57.01±0.03	52.10±0.10
Control		------	------	------	------

2.3.2. Antioxidant Activity by Hydrogen Peroxide Radical Scavenging Activity

1ml of (20-200 µg/ml) test derivative or standard (Ascorbic acid) was added to 0.6ml of hydrogen peroxide solution in phosphate buffer (pH-7.4). After incubating for 10 min at 37^0C the absorbance was measured at 230nm, corresponding blanks were taken. The absorbance of hydrogen peroxide in phosphate buffer as control was measured at 230nm. The scavenging effect (%) measured using following equation. Hydrogen peroxide produces hydroxyl radicals in cells, scavenging of these radicals by the test drug is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230nm with increasing concentration of the test drug and statistical analysis was performed using ANOVA to find the significance of the test (Table 3) [14].

Scavenging Effect (%) =

$$\frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

2.3.3. In Vitro Anti-Inflammatory Activity by Inhibition of Protein Denaturation

The standard drug and synthesized derivative were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5 %. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° $\pm$ 1^0C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0\pm$ 1^0C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer). Percentage inhibition of denaturation was calculated from control where no drug was added and the Ibuprofen was used as standard drug.

The percentage inhibition of denaturation was calculated by using following formula and statistical analysis was performed using ANOVA to find the significance of the test (Table 3) [15].

$$\% \text{ of Inhibition} = 100 \text{ X } [1- \text{Vt} / \text{Vc}]$$

Where, Vt = Mean absorbance of test sample, Vc = Mean absorbance of control

3. Results and Discussion

The synthesis of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazol-3-yl} sulfanyl) acetamide derivative is depicted in Scheme 1. The FT-IR spectras, reveals that functional groups present in the molecule appeared at their characteristic frequency N–H, str. between 2914-2917 cm^{-1}, C-H str, aliphatic between 3252-3478 cm^{-1}, C=N str, aromatic between 1520-1658 cm^{-1}, C=C str, aromatic between 1392-1572 cm^{-1}, C-N, str. ring between 1397-1490 cm^{-1}, C=O, str, amide between 1582-1671

cm^{-1}, C-NO_2 str, between 1328-1548 cm^{-1}, C-Cl str, between 681-827 cm^{-1}, C-Br str, at 677 cm^{-1}, C=O str, carbocyl between1671-1693 cm^{-1}. The chemical shift (δ) for secondary amine hydrogen was observed in the range of 2.01-2.50 ppm, δ value for secondary NH amide two hydrogen was observed in the range of 10.20 - 10.82 ppm, δ value for C-H, pyridine two hydrogen was observed in the range of 8.77-7.91 ppm, δ value for aromatic hydrogen was observed in the range of 7.09-8.83 ppm, δ value for methylene hydrogen was observed in the range of 3.16-4.28 ppm. The m/e value was observed, e.g., in case of KA1-KA15 at 459-639 $(M)^+$.

3.1. Antimicrobial, Antioxidant and Anti-Inflammatory Activity

From *in vitro* antibacterial activity, In case of *E. coli, K. pneumonia*, *S.aureus* and *B. subtilis* derivatives KA3, KA4, KA7, KA9 and KA14 (*p*-Cl-Ph, *m*-Cl-Ph, *o*-Cl-Ph and *p*-OCH_3-Ph) were found to have significant activity which is 1-2 folds less than the standard drug Norfloxacin. In case of *in vitro* antifungal activity against *A. nigar* and *S. cerevisiae*, derivatives KA3, KA4, and KA14 (*p*-Cl-Ph, *m*-Cl-Ph and *p*-OCH_3-Ph) were found to have significant activity which is 1-2 folds less than the standard drug Ketoconazole. *In vitro* anti-inflammatory activity, derivatives KA3, KA4, KA5, KA7, KA9, KA11, KA13 and KA14 (*p*-Cl-Ph, *p*-OCH_3-Ph , *m*-CH_3-Ph, *p*-Br-Ph, *m*-Cl-Ph, *p*-CH_3-Ph, *o*-CO_2H-Ph and *o*-Cl-Ph) at 100 µg/ml were found to have significant activity which is at least 80 % of the standard drug Ibuprofen. In case of *in vitro* antioxidant activity, derivatives KA3, KA7, KA9, KA11 and KA14 (*p*-Cl-Ph, *p-Br*-Ph, *m*-Cl-Ph, *p*-CH_3-Ph and *o*-Cl-Ph) at 100 µg/ml were found significant active which is at least 80 % of the standard drug ascorbic acid. Thus from the obtained antibacterial, antifungal, antioxidant and anti-inflammatory activity data it has been observed that electron withdrawing groups at specific position on phenyl ring i.e., (*p*-Cl-Ph, *m*-Cl-Ph, *o*-Cl-Ph, *p*-OCH_3-Ph, *m*-CH_3-Ph, *p*-Br-Ph, *p*-CH_3-Ph, and *o*-CO_2H-Ph) are contributed positively for these activities.

4. Conclusion

A series of *N*-substituted aryl-2-({4-[(substituted aryl carbamoyl) methyl]-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl} sulfanyl) acetamide had been synthesized in quantitative yields with the use of conventional method and evaluated for their *in-vitro* antimicrobial, antioxidant and anti-inflammatory activity result are shown in Table 2 and 3. On the basis of observed results, we could conclude that additions of different functional groups have varying effects on these activities. Considerable increase in activity was observed when the electron withdrawing groups were incorporated at *o*-, *m*- and *p*- position of the phenyl ring.

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