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Comparative Studies on Conventional and Microwave Assisted Synthesis of Novel Quinoxalinone Derivatives as Antimicrobial Agents

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ABSTRACT

A facile green protocol has been developed for the synthesis of a series of 3-methyl-quinoxalin-2 (1*H*) -one substituted 1,2,4-triazole derivatives under microwave irradiation. Besides, these compounds were also synthesized by conventional heating procedures for comparison. In a relative study, microwave-assisted synthesis lead to higher yields of target compound within very short reaction time than conventional counterparts. The structures of these derivatives were ascertained through FTIR, Mass, NMR and elemental analysis. These target analogues were also found to exhibit considerable inhibitory activity against disease causing microorganisms.

1. Introduction

Resistance to antimicrobial agents poses a "major comprehensive risk" to public health, says a new report by the World Health Organization (WHO) which specifically reveals that this severe threat is no longer a prediction for the hereafter. It is taking place right now in every part of the globe and holds the potential to strike anyone, of whatsoever age, in any state. The report disclosed that key antibiotics, no longer work in more than half of people being treated in some countries**[1]**. Therefore, this situation emphasized more new antibiotics need to be developed with novel mechanisms of action as well as structural modifications to improve both their binding affinity and their spectrum of activity.

In this regard N-bridged heterocyclic compounds, such as quinoxalinone, pyrazolone, pyrazine, triazole etc., has received considerable attention in recent years owing to the extensive array of pharmacological activities associated with them**[2,3]**. Triazole and its derivatives are known to exhibit various pharmacological properties such as antimicrobial**[4,5,6]**, antitubercular**[7,8]**, anticancer**[9,10,11]**, anticonvulsant**[10,11]**, anti-inflammatory**[12]**, analgesic**[13]** and antiviral**[14,15].** They have also been incorporated in a wide variety of therapeutically interesting drugs including H1/H2 histamine receptor blockers**[16]**, CNS stimulants, anti-anxiety agents**[17]** and sedatives**[18]**. The most important use, however, is as antimycotics such as fluconazole, itraconazole and voriconazole**[19]**.

Naturally occurring compounds, is well known for its broad range in the field of medicine as well as for its applications in the pharmaceuticals as antifungal**[20]**, antibacterial**[21]**, antitubercular**[22]**, neuropharmacological**[23]**, anticancer**[24]** and anti-inflammatory agents**[25]**etc.

In recent medicinal chemistry perspective, a combination of two different active fragments in one molecule via covalent bridge is gaining attention. This strategy has been regarded as Hybrid approach, where various drug moieties of the single skeleton have been designed to bind independently to different biological targets to produce beneficial effects and fewer chances to face resistance.

Thus, in connection with the studies of both the nuclei *i.e.*, quinoxalinone and 1,2,4-triazole, it is conceivable to develop a series of hybrid quinoxalinone substituted triazole derivatives by conventional as well as microwave assisted approach**[26]** with the aim of investigating their antimicrobial properties and determining the efficient synthetic protocol.

2. Material and Methods

Reagents, chemicals and solvents used in the study are of analytical grade and used without any prior purification.

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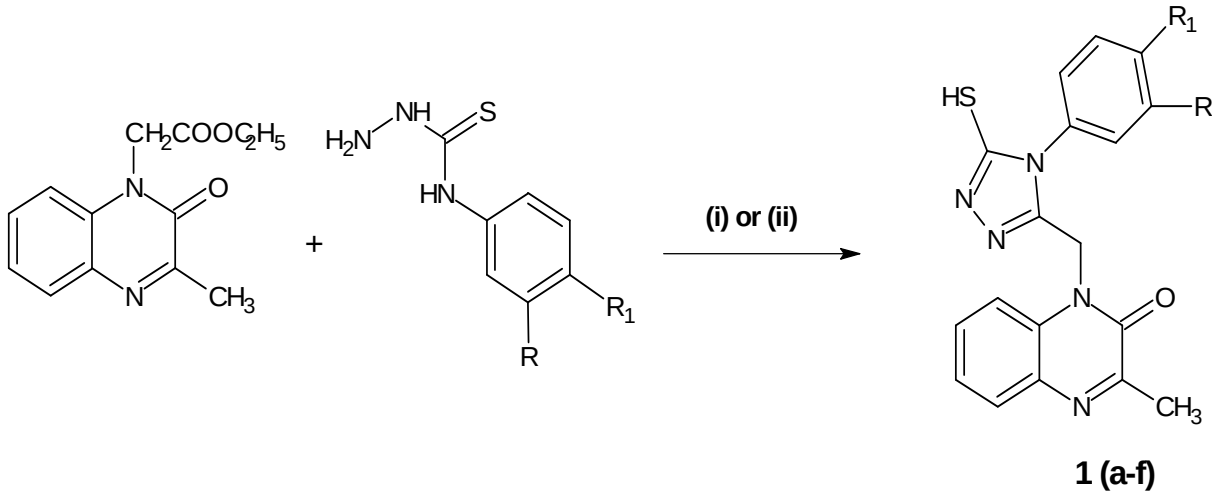
All the microorganisms were collected from Microbial Type Culture Collection and Gene Bank (MTCC; Institute of Microbial Technology, Chandigarh, India). The organisms were *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 734) and *Candida albicans* (MTCC 227).

Determination of percentage yield and melting point range of synthesized compounds was performed with open capillary tube on a melting point apparatus – 1020 (Arya Labware). Infra red spectrums were recorded as KBr pellets on FTIR – Spectrometer: 8000 (Shimadzu) while ^{1}H NMR spectrums on Bruker model DRX-400 & 300 MHz NMR Spectrometer in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as the internal reference. Elemental Analysis was carried on Perkin Elmer (PE – 2400) elemental analyzer. The progress of reaction in both the techniques was monitored by TLC on silica gel plate using hexane**:**ethylacetate**:**methanol**:**dichloromethane (in different proportions) as the solvent system. Microwave assisted synthesis was carried on Catalyst – 2000 scientific microwave (Catalyst).

2.2 Reaction schemes

The synthetic procedures were initially carried out by the conventional methodology and after establishment of its procedure; the microwave assisted technique was adapted in order to improve the reaction yield, reaction time, and minimal usage of solvent. In present study, the compounds were synthesized by both conventional and microwave irradiation technique, therefore, spectral data of the same compound obtained by both the techniques was more or less similar. Hence the data of the compounds synthesized by microwave irradiation technique has been incorporated.

As depicted in Scheme 1, acetate derivative of 3-methyl-quinoxalin-2(1*H*)-one i.e. ethyl (3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetate was reacted with 6 different substituted phenyl hyrazinecarothioamide derivatives in different environment of conventional and microwave synthesis to furnish substituted triazole derivatives of 3-methyl-quinoxalin-2(1*H*)-one **1(a-f).** The preliminary antimicrobial studies of **1(a-f)** was performed in order to deduce the potential of the designed skeleton as an antimicrobial agent. Further to ascertain the role of 5-mercapto, in the antimicrobial activity of the title analogues **(1 (a-f))**, the 5-mercapto group of each analogue was substituted with a polar group and non-polar group *i.e*., benzyl and acetate group (Scheme 2). This had figured the role of polarity on the activity shown by the compounds. Different reaction environment were provided for conventional and microwave technique in order to achieve the synthesis of the target compound.



Reagents: (i) 4N NaOH, 4N HCl and ethanol, reflux 3-4 hr (ii) PPA, 500W 4-6 min.

Scheme 1: Synthesis of 1-{[4-(3,4-disubstituted phenyl)-5-mercapto-4*H*-1,2,4-triazol-3-yl]methyl}-3-methylquinoxalin-2(1*H*)-one

2.3 General method for the synthesis of 1-{[5-mercapto-4-(aryl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methylquinoxalin-2(1*H*)-one, 1 (a – f)

Conventional Method

Ethyl (3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetate (0.01 mol) and *N*-(aryl)hydrazinecarbothioamide (0.01 mol) was dissolved in 20 ml ethanol, freshly prepared solution of sodium hydroxide (4N, 4 ml) was added which resulted in clear solution. It was refluxed for 3 – 4 h and treated with decolourising charcoal and filtered. The filtrate was cooled and pH was adjusted to 4-6 with dil. HCl. The product was precipitated which was filtered, dried and purified by column chromatography.

Microwave Method

Ethyl (3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetate (0.01 mol), *N*-(aryl)hydrazinecarbothioamide (0.01 mol) and 1 g PPA were mixed in 50 ml Erlenmeyer Pyrex Flask. The Erlenmeyer Pyrex Flask was kept in microwave at 500 W for 4-6 minutes. After completion, confirmed by TLC, the reaction mixture was poured onto crushed ice, precipitated by sodium carbonate solution. The precipitate obtained was filtered, dried and purified by column chromatography.

2.4 General method for the synthesis of 1-{[5-(benzylthio)-4-(aryl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2 (a –f)

Conventional method

A mixture of **1(a – f)** (0.01 mol) and benzyl chloride (0.02 mol) in ethanolic potassium hydroxide (0.08 g KOH in 20 ml aq. ethanol) was refluxed for 4 hr. Excess solvent was distilled off and on cooling the reaction mixture a crude precipitate was obtained, which was purified by column chromatography.

Microwave method

To the mixture of **1 (a – f)** (0.01 mol) and benzyl chloride (0.02 mol), 2 ml of DMF was added in 50 ml Erlenmeyer Pyrex Flask. The Erlenmeyer Pyrex Flask was kept in microwave at 500 W for 4-5 minutes. After completion of reaction, confirmed by TLC, the reaction mixture was cooled at room temperature to obtain crude product, purified by column chromatography.

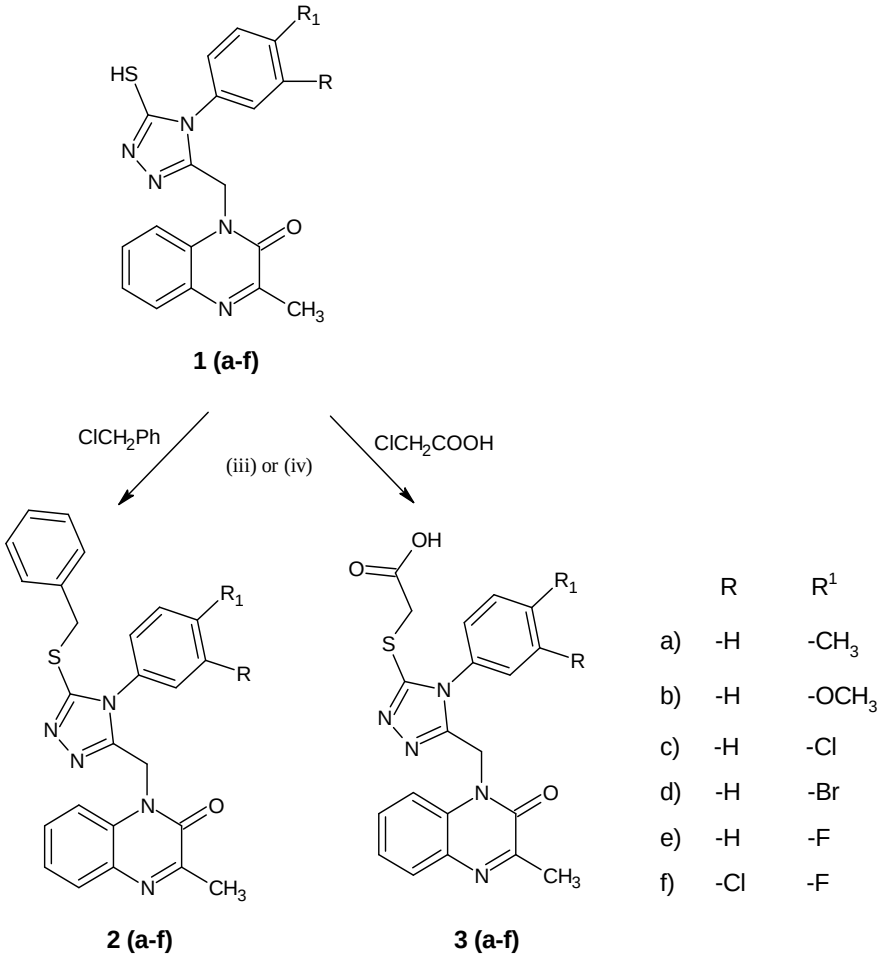
2.5 General method for the synthesis of ({4-(aryl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3 (a –f)

Conventional method

A mixture of **1 (a – f)** (0.01 mol) and chloroacetic acid (0.02 mol) in ethanolic potassium hydroxide (0.08 g KOH in 20 ml aq. ethanol) was refluxed for 3 – 3.5 hr. Excess solvent was distilled off and on cooling the reaction mixture a crude precipitate was obtained, which was recrystallized from ethanol and purified by column chromatography.

Microwave method

To the mixture of **1 (a – f)** (0.01 mol) and chloroacetic acid (0.02 mol), 2 ml of DMF was added in 50 ml Erlenmeyer Pyrex Flask. The Erlenmeyer Pyrex Flask was kept in microwave at 500 W for 4-5 minutes. After completion of reaction, confirmed by TLC, the reaction mixture was cooled at room temperature to obtain a crude product, purified by column chromatography.



Reagents: Benzyl chloride and chloroacetic acid (iii) ethanolic KOH, reflux 4 hr (iv) DMF, 500W, 4-5 min.

Scheme 2: Synthesis of final motifs i.e. 1-{[5-(benzylthio)-4-(aryl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one **2(a-f)** and ({4-aryl-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid **3 (a-f).**

2.6 Structural determination and characterization of synthesized compounds

The structure of all the synthesized compounds i.e. from the first synthesized product (1) of Scheme 1 to the final products i.e. 2 (a-f) and 3 (a-f) was confirmed by their spectroscopic as well as elemental analysis data. In the mass spectral data the molecular ion peak of most of the compounds was found in agreement with its molecular mass, for example, the mass spectral data of **1a** ($C_{19}H_{17}N_5OS$) showed molecular ion peak at m/z 362 (7%), base peak at m/z 188 (100%). Other peaks appeared at m/z 271, 129, and 94 with relative intensities 40%, 2%, and 30% respectively due to some fragmentation pattern.

The result of elemental showed a minimum difference of not more than 0.5% between calculated and observed values for carbon, hydrogen and nitrogen for compound.

2.7Antimicrobial Activities

Minimum inhibitory concentration (MIC) test was carried on all the synthesized motifs i.e. 1 (a-f), 2 (a-f) and 3 (a-f). The clinical isolates (or the test organisms) used include three Gram positive bacteria i.e. *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 430) & *Staphylococcus aureus* (MTCC 3160), three Gram negative bacteria i.e. *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) & *Salmonella typhi* (MTCC 734) and one fungal strain of *Candida albicans* (MTCC 227). Ciprofloxacin and fluconazole were used as reference standards for antibacterial and antifungal activities respectively.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

Stock solution of the standard drug (ciprofloxacin/fluconazole – 250 µg/ml each in DMSO)/derivatives synthesized (1000 µg/ml) were prepared. Sterilized nutrient broth was added to seven test tubes. 2 ml of the stock solution of the standard drug/test drug was added to the first test tube. Further two fold dilutions was made of the standard/test drug. Lowest dilution prepared was 7.8 µg/ml. 0.1 ml of the revived microorganism was added to each test tube, plugged with cotton and packed with aluminium foil. These test tubes were incubated for 18 h at 37 ^{0}C (25 ^{0}C for *C.albicans*). Turbidity was observed in the test tube where the growth of microorganism took place**[27]**.

3. Result and Discussion

Solvents used were purified and dried by standard methods. The time required for completion of reaction by both the methods and the respective percentage yield along with the melting point of the product is summarized in Table 1.

Table No.1: Physical data of the synthesized compounds

Compd.	R	R[1]	% Yield & Reaction Time		M.P. (^{0}C)
			MW Method (min)	Conventional Method (Hr)	
1a	H	CH_3	78.93 (4)	75.48 (2.5)	260 – 262
1b	H	OCH_3	75.26 (6)	58.16 (2.5)	271 – 273
1c	H	Cl	77.89 (4)	38.72 (3)	218 – 220
1d	H	Br	74.34 (4)	69.95 (3)	235 – 237
1e	H	F	85.63 (4)	74.89 (3)	268 – 270
1f	Cl	F	80.54 (4)	81.21 (3)	289 – 291
2a	H	CH_3	83.62 (7)	60.27 (4)	315 – 317
2b	H	OCH_3	87.82 (9)	54.22 (4)	328 – 330
2c	H	Cl	84.56 (7)	52.47 (4)	>340
2d	H	Br	76.46 (7)	42.66 (4)	>340
2e	H	F	79.21 (7)	60.0 (4)	>340
2f	Cl	F	80.23 (7)	33.69 (4)	>340
3a	H	CH_3	82.39 (7)	26.19 (3.5)	332 – 334
3b	H	OCH_3	74.84 (8)	48.15 (3.5)	338 – 340
3c	H	Cl	71.39 (5)	44.79 (3)	>340
3d	H	Br	78.47 (5)	38.98 (3)	>340
3e	H	F	81.66 (5)	21.80 (3)	>340
3f	Cl	F	74.73 (5)	43.04 (3)	>340

3.1 Spectral data of Synthesized compounds

3.1.1 Spectral data of 1-{[5-mercapto-4-(aryl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methylquinoxalin-2(1*H*)-one, 1 (a – f)

(i). 1-{[5-mercapto-4-(4-methyl phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methylquinoxalin-2(1*H*)-one, 1a

Yield: 2.73 g (conventional), 2.85 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 3303.8 (N – H), 2850.6 (C – H), 1590.2 (C=C), 1560.3 (C=N), 1637.5 (C=O), 1281.6 (C=S).

^{1}H NMR (300 MHz, DMSO d_6): δ 2.613 (s, 3H, CH_3), 7.532 – 8.262 (m, 8H, Ar – H), 2.097 (s, 3H, CH_3), 3.142 (s, 2H, N – CH_2), 5.417 (s, 1H, NH; D_2O exchangeable). MS: in m/z [rel.%]: 362 [M^+, 7%] 271 [40%], 188 [100%], 129 [2%], 94 [30%]. Anal. Calc for $C_{19}H_{17}N_5OS$: C, 62.79%; H, 4.71%; N, 19.27%. Observed: C, 62.40%; H, 4.90%; N, 19.22%.

(ii). 1-{[5-mercapto-4-(4-methoxy phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methyl- quinoxalin-2(1*H*)-one, 1b

Yield: 2.20 g (conventional), 2.85 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 3303.8 (N – H), 2848.7 (C – H), 1608.5 (C=C), 1556.5 (C=N), 1635.5 (C=O), 1244.0 (C=S). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.461 (s, 3H, CH_3), 7.892 – 8.206 (m, 8H, Ar – H), 3.917 (s, 2H, N – CH_2), 5.686 (s – br, 1H, NH; D_2O exchangeable), 4.557 (s, 3H, OCH_3). MS: in m/z [rel.%]: 378 [M^+, 3%], 314 [18%], 225 [22%], 136 [100%]. Anal. Calc for $C_{19}H_{17}N_5O_2S$: C, 60.14%; H, 4.52%; N, 18.46%. Observed: C, 60.25%; H, 4.63%; N, 18.42%.

(iii). 1-{[5-mercapto-4-(4-chloro phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methyl- quinoxalin-2(1*H*)-one, 1c

Yield: 1.65 g (conventional), 2.99 g (microwave); green crystals. IR (cm^{-1}, KBr): ν_{max} 3151.5 (N – H), 2852.5 (C – H), 1589.2 (C=C), 1550.3 (C=N), 1664.4 (C=O), 1245.0 (C=S), 1139.9 (C – Cl). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.585 (s, 3H, CH_3), 7.142 – 7.991 (m, 8H, Ar – H), 3.449 (s, 2H, N – CH_2), 6.614 (s, 1H, NH; D_2O exchangeable). MS: in m/z [rel.%]: 383 [M^+, 12%], 385 [3%], 262 [11%], 144 [100%], 72 [26%]. Anal. Calc for $C_{18}H_{14}N_5OSCl$: C, 56.32%; H, 3.68%; N, 18.24%. Observed: C, 56.18%; H, 3.61%; N, 18.28%.

(iv). 1-{[5-mercapto-4-(4-bromo phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methyl- quinoxalin-2(1*H*)-one, 1d

Yield: 2.99 g (conventional), 3.18 g (microwave); purple crystals. IR (cm^{-1}, KBr): ν_{max} 3219.0 (N – H), 2852.5 (C – H), 1610.5 (C=C), 1548.7 (C=N), 1674.1 (C=O), 1244.0 (C=S), 671.2 (C – Br). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.481 (s, 3H, CH_3), 7.522 – 8.297 (m, 8H, Ar – H), 3.344 (s, 2H, N – CH_2), 6.198 (s, 1H, NH; D_2O exchangeable). MS: in m/z [rel.%]: 427 [M^+, 6%], 429 [7%], 190 [100%], 104 [23%], 84 [2%]. Anal. Calc for $C_{18}H_{14}N_5OSBr$: C, 50.48%; H, 3.29%; N, 16.35%. Observed: C, 50.56%; H, 3.38%; N, 16.41%.

(v). 1-{[5-mercapto-4-(4-fluoro phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methyl- quinoxalin-2(1*H*)-one, 1e

Yield: 2.74 g (conventional), 3.14 g (microwave); green crystals. . IR (cm^{-1}, KBr): ν_{max} 3307.7 (N – H), 2852.5 (C – H), 1591.2 (C=C), 1554.5 (C=N), 1641.3 (C=O), 1247.9 (C=S), 1107.4 (C – F). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.592 (s, 3H, CH_3), 6.941 – 7.463 (m, 8H, Ar – H), 3.424 (s, 2H, N – CH_2), 5.996 (s, 1H, NH; D_2O exchangeable). MS: in m/z [rel.%]: 366 [M^+, 27%], 348 [42%], 273 [28%], 161 [100%], 131 [21%]. Anal. Calc for $C_{18}H_{14}N_5OSF$: C, 58.84%; H, 3.84%; N, 19.06%. Observed: C, 58.95%; H, 3.85%; N, 19.17%.

(vi). 1-{[5-mercapto-4-(3-chloro, 4-fluoro phenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-3-methyl- quinoxalin-2(1*H*)-one, 1f

Yield: 3.25 g (conventional), 3.24 g (microwave); cream coloured crystals. IR (cm^{-1}, KBr): ν_{max} 3222.8 (N – H), 2923.9 (C – H), 1608.5 (C=C), 1548.7 (C=N), 1634.6 (C=O), 1245.9 (C=S), 1169.3 (C – Cl), 1101.3 (C – F). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.599 (s, 3H, CH_3), 7.170 – 7.449 (m, 8H, Ar – H), 3.381 (s, 2H, N – CH_2), 5.872 (s, 1H, NH; D_2O exchangeable). MS: in m/z [rel.%]: 401 [M^+, 17%], 403 [6%], 179 [100%], 144 [60%], 99 [5%]. Anal. Calc for $C_{18}H_{13}N_5OSClF$: C, 53.80%; H, 3.26%; N, 17.43%. Observed: C, 53.84%; H, 3.48%; N, 17.45%.

3.1.2 Spectral data of 1-{[5-(benzylthio)-4-(aryl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2 (a – f)

(i). 1-{[5-(benzylthio)-4-(4-methyl phenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2a

Yield: 2.73 g (conventional), 3.79 g (microwave); cream coloured crystals. IR (cm^{-1}, KBr): ν_{max} 2848.7 (C – H), 1606.6 (C=C), 1564.2 (C=N), 1664.5 (C=O). ^{1}H NMR (300 MHz,

DMSO d_6): δ 2.211 (s, 3H, CH_3), 7.612 – 8.432 (m, 13H, Ar – H), 3.591 (s, 2H, N – CH_2), 2.415 (s, 2H, CH_2 – Ar). MS: in m/z [rel.%]: 453 [M^+, 3%], 358 [6%], 316 [26%], 274 [100%], 121 [30%], 52 [9%]. Anal. Calc for $C_{26}H_{23}N_5OS$: C, 68.85%; H, 5.11%; N, 15.44%. Observed: C, 68.92%; H, 5.02%; N, 15.65%.

(ii). 1-{[5-(benzylthio)-4-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2b

Yield: 2.54 g (conventional), 4.12 g (microwave); cream coloured crystals. IR (cm^{-1}, KBr): ν_{max} 2896.9 (C – H), 1604.5 (C=C), 1558.0 (C=N), 1656.4 (C=O). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.462 (s, 3H, CH_3), 7.270 – 7.489 (m, 13H, Ar – H), 3.254 (s, 2H, N – CH_2), 2.051 (s, 2H, CH_2 – Ar), 4.498 (s, 3H, OCH_3). MS: in m/z [rel.%]: 469 [M^+, 9%], 297 [42%], 123 [100%], 90 [6%], 66 [13%]. Anal. Calc for $C_{26}H_{23}N_5O_2S$: C, 66.50%; H, 4.94%; N, 14.91%. Observed: C, 66.72%; H, 5.08%; N, 14.65%

(iii). 1-{[5-(benzylthio)-4-(4-chloro phenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2c

Yield: 2.48 g (conventional), 4.0 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2852.2 (C – H), 1596.4 (C=C), 1560.3 (C=N), 1660.8 (C=O), 1188.5 (C – Cl). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.447 (s, 3H, CH_3), 7.128 – 8.251 (m, 13H, Ar – H), 3.842 (s, 2H, N – CH_2), 2.751 (s, 2H, CH_2 – Ar). MS: in m/z [rel.%]: 473 [M^+, 16%], 475 [4%], 214 [100%], 129 [36%], 75 [12%]. Anal. Calc for $C_{25}H_{20}N_5OSCl$: C, 63.50%; H, 4.25%; N, 14.78%. Observed: C, 63.55%; H, 4.39%; N, 14.85%.

(iv). 1-{[5-(benzylthio)-4-(4-bromo phenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2d

Yield: 2.21 g (conventional), 3.96 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2822.7 (C – H), 1599.6 (C=C), 1548.6 (C=N), 1650.5 (C=O), 612.1 (C – Br). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.208 (s, 3H, CH_3), 8.184 – 8.881 (m, 13H, Ar – H), 3.642 (s, 2H, N – CH_2), 2.019 (s, 2H, CH_2 – Ar). MS: in m/z [rel.%]: 518 [M^+, 11%], 520 [12%], 254

[100%], 136 [26%], 96 [4%]. Anal. Calc for $C_{25}H_{20}N_5OSBr$: C, 57.92%; H, 3.89%; N, 13.51%. Observed: C, 57.96%; H, 3.90%; N, 13.56%.

(v). 1-{[5-(benzylthio)-4-(4-fluoro phenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2e

Yield: 2.74 g (conventional), 3.61 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2915.4 (C – H), 1610.1 (C=C), 1568.2 (C=N), 1646.6 (C=O), 1109.1 (C – F). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.326 (s, 3H, CH_3), 8.210 – 8.872 (m, 13H, Ar – H), 3.892 (s, 2H, N – CH_2), 2.970 (s, 2H, CH_2 – Ar). MS: in m/z [rel.%]: 457 [M^+, 7%], 365 [14%], 188 [22%], 173 [100%], 159 [39%]. Anal. Calc for $C_{25}H_{20}N_5OSF$: C, 65.63%; H, 4.41%; N, 15.31%. Observed: C, 65.64%; H, 4.30%; N, 15.62%.

(vi). 1-{[5-(benzylthio)-4-(3-chloro, 4-fluoro phenyl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one, 2f

Yield: 1.65 g (conventional), 3.94 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2925.6 (C – H), 1606.5 (C=C), 1550.7 (C=N), 1659.5 (C=O), 1107.7 (C – F), 1164.3 (C – Cl). ^{1}H NMR (300 MHz, DMSO d_6): δ 2.894 (s, 3H, CH_3), 7.583 – 8.622 (m, 13H, Ar – H), 3.421 (s, 2H, N – CH_2), 2.346 (s, 2H, CH_2 – Ar). MS: in m/z [rel.%]: 491 [M^+, 14%], 493 [5%], 212 [100%], 195 [4%], 171 [19%]. Anal. Calc for $C_{25}H_{19}N_5OSClF$: C, 61.03%; H, 3.89%; N, 14.24%. Observed: C, 61.14%; H, 3.96%; N, 14.25%.

3.1.3 Spectral data of ({4-(aryl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3 (a –f)

(i). ({4-(4-methyl phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3a

Recrystallization: ethanol/DMF; Yield: 1.10 g (conventional), 3.46 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2896.9 (C – H), 1628.5 (C=C), 1568.0 (C=N), 1666.4 (C=O), 1730.0 (C=O, acid). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.594 (s, 3H, CH_3), 8.198 – 8.841 (m, 13H, Ar – H), 3.875 (s, 2H, N – CH_2), 4.972 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]: 420 [M^+, 6%], 314 [9%], 274 [3%], 196 [100%], 90 [24%]. Anal. Calc for $C_{21}H_{19}N_5O_3S$: C, 59.84%; H, 4.54%; N, 16.62%. Observed: C, 59.86%; H, 4.91%; N, 16.65%

(ii). ({4-(4-methoxy phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3b

Recrystallization: ethanol/DMF; Yield: 2.10 g (conventional), 3.27 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2856.6 (C – H), 1601.7 (C=C), 1558.4 (C=N), 1655.5 (C=O), 1710.5 (C=O, acid). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.280 (s, 3H, CH_3), 8.485 – 8.998 (m, 13H, Ar – H), 3.377 (s, 2H, N – CH_2), 4.352 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]: 436 [M^+, 2%], 356 [11%], 218 [100%], 161 [34%], 150 [29%]. Anal. Calc for $C_{21}H_{19}N_5O_4S$: C, 57.66%; H, 4.38%; N, 16.01%. Observed: C, 57.50%; H, 4.45%; N, 15.82%

(iii). ({4-(4-chloro phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3c

Recrystallization: ethanol/DMF; Yield: 1.97 g (conventional), 3.15 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2846.7 (C – H), 1604.7 (C=C), 1566.1 (C=N), 1666.4 (C=O), 1751.2 (C=O, acid), 1191.9 (C – Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.592 (s, 3H, CH_3), 7.955 – 8.549 (m, 13H, Ar – H), 3.328 (s, 2H, N – CH_2), 4.792 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]: 441 [M^+, 5%] 443 [2%], 258 [100%], 199 [32%], 181 [24%]. Anal. Calc for $C_{20}H_{16}N_5O_3SCl$: C, 54.36%; H, 3.65%; N, 15.85%. Observed: C, 54.39%; H, 3.58%; N, 15.80%.

(iv). ({4-(4-bromo phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3d

Recrystallization: ethanol/DMF; Yield: 1.89 g (conventional), 3.80 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2850.6 (C – H), 1589.2 (C=C), 1558.0 (C=N), 1635.5 (C=O), 1716.5 (C=O, acid), 635.5 (C – Br). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.167 (s, 3H, CH_3), 7.367 – 8.153 (m, 13H, Ar – H), 3.218 (s, 2H, N – CH_2), 4.438 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]:485 [M^+, 5%], 487 [7%], 222 [100%], 157 [48%], 76 [20%]. Anal. Calc for $C_{20}H_{16}N_5O_3SBr$: C, 49.39%; H, 3.32%; N, 14.40%. Observed: C, 49.70%; H, 3.45%; N, 14.84%.

(v). ({4-(4-fluoro phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3e

Recrystallization: ethanol/DMF; Yield: 0.92 g (conventional), 3.47 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2920.9 (C – H), 1589.2 (C=C), 1560.4 (C=N), 1640.4 (C=O), 1720.5 (C=O, acid), 1145.6 (C – F). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.477 (s, 3H, CH_3), 7.270 – 7.489 (m, 13H, Ar – H), 3.380 (s, 2H, N – CH_2), 4.486 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]: 426 [M^+, 8%], 241 [100%], 239 [18%], 158 [6%], 94 [23%]. Anal. Calc for $C_{20}H_{16}N_5O_3SF$: C, 56.46%; H, 3.79%; N, 16.46%. Observed: C, 56.48%; H, 3.71%; N, 16.65%

(vi). ({4-(3-chloro, 4-fluoro phenyl)-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-yl}thio)acetic acid, 3f

Recrystallization: ethanol/DMF; Yield: 1.97 g (conventional), 3.43 g (microwave); white crystals. IR (cm^{-1}, KBr): ν_{max} 2850.6 (C – H), 1593.1 (C=C), 1558.4 (C=N), 1635.5 (C=O), 1716.5 (C=O, acid), 1145.6 (C – F), 1188.1 (C – Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.445 (s, 3H, CH_3), 6.970 – 7.519 (m, 13H, Ar – H), 3.680 (s, 2H, N – CH_2), 4.461 (s, 2H, CH_2 – COO^-). MS: in m/z [rel.%]: 459 [M^+, 2%], 461 [9%], 300 [2%], 161 [100%], 96 [12%]. Anal. Calc for $C_{20}H_{15}N_5O_3SFCl$: C, 52.23%; H, 3.29%; N, 15.23%. Observed: C, 52.35%; H, 3.24%; N, 15.46%.

3.2 Minimum inhibitory concentration data

The MIC of the compounds varied between 250 µg/ml – 7.8 µg/ml (Table 2). The results depicted that final motifs i.e. 1-{[5-(benzylthio)-4-(aryl)-4*H*-1,2,4-triazol-3-yl]-methyl}-3-methyl quinoxalin-2(1*H*)-one **2(a-f)** and ({4-aryl-5-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)methyl]-4*H*-1,2,4-triazol-3-

yl}thio)acetic acid **3(a-f)** with the halogen substitution illustrated prominent antibacterial activities on both Gram positive and Gram negative bacteria. Compounds 2(f) and 3(f) i.e. the chloro and fluoro substituted derivative showed the maximum potency against Gram negative bacteria i.e. the concentration of the compound required was minimal to inhibit the growth of bacteria as compared to the other derivatives.

Table No.2: Results of minimum inhibitory concentration (MIC) in µg/ml

Compound No.	B. s	B. c	S. a	P. a	E. c	S. t
1a	125	125	62.5	31.25	31.25	31.25
1b	125	62.5	125	62.5	31.25	31.25
1c	62.5	62.5	31.25	31.25	15.6	15.6
1d	15.6	31.25	15.6	15.6	7.8	7.8
1e	31.25	31.25	15.6	7.8	15.6	7.8
1f	31.25	62.5	62.5	15.6	7.8	15.6
2a	250	125	125	62.5	125	62.5
2b	250	250	125	125	62.5	31.25
2c	62.5	62.5	31.25	31.25	62.5	62.5
2d	31.25	31.25	62.5	31.25	15.6	31.25
2e	62.5	62.5	62.5	15.6	15.6	15.6
2f	62.5	31.25	62.5	31.25	31.25	62.5
3a	250	250	250	125	125	125
3b	250	250	250	125	125	250
3c	125	62.5	125	62.5	31.25	31.25
3d	62.5	62.5	125	31.25	15.6	15.6
3e	62.5	31.25	125	62.5	31.25	15.6
3f	62.5	62.5	125	62.5	31.25	31.25
Ciprofloxacin	15.6	15.6	15.6	7.8	7.8	7.8

Furthermore, the antimicrobial sensitivity testing of the six triazole derivatives i.e. **1 (a – f)** were assayed using agar diffusion technique against the test organisms.

The antimicrobial screening results of **1 (a – f)** derivatives depicted them to be potentially sound motifs. Therefore further substitution on 5-mercapto position was done in order to establish its role in the antimicrobial activity. Antimicrobial screening in a similar way as done for **1 (a – f)** was carried out for **2 (a – f)** and **3 (a – f)**. The result of antimicrobial testing measured as zone of inhibition (in mm) and reported in Table 3.

Table No.3: Results of Antimicrobial Screening with zones of inhibition in mm

	Zone of inhibition (in mm)						
Compound No.	**B. s**	**B. c**	**S. a**	**P. a**	**E. c**	**S. t**	**C. a**
1a	11	10	7	16	16	19	15
1b	8	9	6	18	16	13	14
1c	20	21	19	25	26	33	30
1d	25	22	26	33	37	38	36
1e	26	28	24	32	35	37	38
1f	22	20	20	29	29	34	31
2a	4	6	4	6	4	6	0
2b	2	9	5	8	8	10	0
2c	15	13	15	19	18	21	13
2d	22	20	16	22	25	29	30
2e	19	18	21	20	23	22	23
2f	16	19	13	18	20	21	24
3a	4	5	8	11	12	11	14
3b	0	0	6	9	9	7	5
3c	14	15	19	19	17	23	21
3d	23	24	23	27	30	33	28
3e	19	22	21	23	25	27	22
3f	16	20	16	22	25	25	25
Ciprofloxacin	20	22	19	28	26	33	NA
Fluconazole	NA	NA	NA	NA	NA	NA	34

Gram positive bacteria: B. s = *Bacillus subtilis* (MTCC 441), B. c = *Bacillus cereus* (MTCC 430), S. a = *Staphylococcus aureus* (MTCC 3160), Gram negative bacteria: E. c = *Escherichia coli* (MTCC 443), P. a = *Pseudomonas aeruginosa* (MTCC 424), S. t = *Salmonella typhi* (MTCC 734), Fungus: C. A = *Candida albicans* (MTCC 227), NA = not applicable

Fluconazole a 1,2,4-triazole containing clinically used drug was taken as reference for antifungal activity[28,29] and only **1d** and **1f** were found to possess more or less similar antifungal activity. Ciprofloxacin was used as reference standard[30,31] for antibacterial activity. The results showed that halogen containing derivatives i.e. **1c, 1d, 1e, 1f, 2c, 2d, 2e, 2f, 3c, 3d, 3e, 3f** have better antibacterial potential than those without halogen. Whereas, 5-mercapto substitution either by a polar or non – polar groups significantly resulted in decrease in activity. **1d** and **1f** showed better antibacterial activity than the standard ciprofloxacin.

4. Conclusion

The synthetic procedure was carried on by both conventional and microwave assisted technique and it was inferred that microwave assisted approach is highly efficient for the preparation of various 3-methyl-quinoxalin-2(1*H*)-one substituted 1,2,4-triazole derivatives i.e. compounds 2 (a-f) and 3 (a-f). The structure activity relationship (SAR) can be explained as substitution on 5-mercapto group lowers the activity. While, substitution with a benzyl group on 5-mercapto position of the triazole nucleus lowers the activity to the greatest extent as compared to the acetate group. The methyl and methoxy group on the phenyl ring of the triazole ring lowers the activity. The highest antimicrobial activity was

observed in the bromo substituted derivative (comparable to standard) while fluoro group also showed considerable activity. Whereas, the presence of chloro does not impart significant bioactivity. It even lowers the activity of the fluoro derivative as observed in case of 3-chloro,4-fluoro derivative. **1d** and **1f** emerged as the most active antimicrobial agent. Therefore this work can be very useful for further studies in terms of biological and pharmacological properties.

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