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SYNTHESIS AND CHARACTERIZATION OF 3-ARYL-5H,13AH-QUINOLINO(3,2-F) (1,2,4)TRIAZOLO(4,3-B)(1,2-DIAZA-4-SULPHO)AZEPINES: IN VITRO ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepines [2a-i] have been prepared by the cyclisation of 5-aryl-4-amino-3-mercapto-1,2,4-triazole by reaction with 2-chloro-3-formylquinoline in catalytic presence of p-toluene sulphonic acid. All the synthesized compounds have been characterized by elemental and spectral (IR, ^{1}H- NMR and Mass) analysis. Furthermore, all compounds were evaluated for their antibacterial and antifungal activities against selected panel of pathogenic strains. Ampicillin trihydrate and fluconazole were used as standard drugs for antibacterial and antifungal activity, respectively. 3-(2-Chloro)phenyl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine [2h] was found, one of the most potent with lesser toxicity among the all prepared thiazepine derivatives.

Keywords: *Thiazepines; Antifungal; Antibacterial; Acute toxicity*

INTRODUCTION

Different heterocyles are highly essential to life due to their vital role in the metabolism of all living cells, e.g.- pyrimidines and purines are the bases of genetic material DNA, the essential amino acids like pyroline, histidine and tryptophan; vitamin and coenzyme precursors as thiamine, riboflavin, pyridoxine, folic acid and biotin; B_{12} and E families of vitamin. Heterocycles, whether natural or man-made, explored diversity in biological activity. Most common heterocyclic moieties consist of triazoles, thiazepines etc. Bulk of literature is available to illustrate the biological properties of 1, 2, 4-substituted triazoles [1-7]. Since few decades, triazole was found the unique position in medicinal chemistry as it constitutes important block in synthesis of different pharmacophore. Chemistry of quinolines [8-9] and its derivatives have gained much attention. Particularly substituted quinoline have been shown to possess antibacterial [10], antitumor [11], anticancerous [12], insecticidal [13], anti-tuberculosis [14] and anti-inflammatory [15] activities, while substituted thiazepines also possessed diversity in biological spectrum [16-17]. In continual search for biological useful newer derivatives, here we are reporting the cyclisation of 5-aryl-4-amino-3-mercapto-1,2,4-triazole by reaction with 2-chloro-3-formylquinoline in catalytic presence of p-toluene sulphonic acid to afford 3-aryl-5H,13aH-quinolino(3,2-f)(1,2,4) triazolo(4,3-b)(1,2-diaza-4-sulpho) azepines.

EXPERIMENTAL SECTION

Materials

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. The reference drugs Ampicillin trihydrate and fluconazole were procured from Ind-Swift, Pharmaceutical, Punjab, India and Dr. Reddy Lab., Hyderabad, India.

Equipment

The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyzer. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H- NMR spectra on Bruker DPX 200 using TMS as internal standard.

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Characterization of the synthesized compounds

Presence of absorption band at 670 cm^{-1} for C-S-C gp and 1624 (C=N) in IR spectra cleared the formation of 3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepines 2(a-i) which was also confirmed by the presence of signal at δ 6.24-6.32 for CH=N- group in ^{1}H-NMR spectra. Mass fragmentation of 2(a-i), cleared their formation.

General mass fragmentation of 3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepines

Scheme 2 explored the general mass fragmentation of parent compound i.e. substituted thiazepines. The molecular, base peak and other peak with their relative intensities were mentioned in Table 2. The mass spectral study of substituted thiazepines revealed that parent compounds cleaved by following two routes viz. route a and route b. Route a [18] furnished two daughter ions $[a]^+$ and $[b]^+$ at m/z 128 and 201 respectively, while route b also produced two daughter ions $[c]^+$ and $[d]^+$ at m/z 154 and 175 respectively. Liberation of CN from ion $[c]^+$ furnished ion $[g]^+$ i.e. quinoline ion, cleaved by employing two fragmentation subroutes i.e. I and II. Both fragmentation subroutes [19] I and II showed analogy with fragmentation modes of Michael et al. [20], which produced ions $[h]^+$ and $[i]^+$ at m/z 101 and 89 while ion $[i]^+$ further rearranged to give tropylium ion $[j]^+$. Loss of NCS [21] from ion $[b]^+$ afforded substituted triazole ion $[e]^+$ at m/z 143 which further released [22] CN to give ion $[f]^+$ at m/z 117. Ion $[d]^+$ released CN_2S to furnish ion $[k]^+$ at m/z 103 which on further loss of CN to generate ion $[l]^+$ at m/z 77.

Procedure

General method of synthesis of 3-Aryl-4-amino-5-mercapto triazoles 1(a-i)

The starting triazoles were prepared according to the reported method [23-26].

General method of synthesis of 3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho) azepines 2(a-i)

The solution of compound **1(a-i)** (0.002 mol) in n-butanol was refluxed with 2-chloro-3-formylquinoline (0.002 mol) for 1-3 h. Excess of solvent was distilled off and the reaction mixture thus obtained was cooled, poured into ice cold water, washed with petroleum ether (40-60 °C) and recrystallised to furnish the product.

3-phenyl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo (4,3-b)(1,2-diaza-4-sulpho)azepine 2a: yield 64%; m.p. 230 °C; IR (KBr) cm^{-1} : 670 (C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 7.10-6.59 (m, 10H, ArH), 6.24 (s, 1H, CH=N). MS: m/z 329 $[M]^+$. Elemental analysis ($C_{18}H_{11}N_5S$); calcd: C 65.65,H 3.34, N 21.27; found C 65.60, H 3.30, N 21.25%.

3-(2-hydroxy)phenyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho) azepine 2b: yield 56%; m.p. 189 °C; IR (KBr) cm^{-1} : 675(C-S-C), 1250 (C-N), 1522 (N-N), 1571 (C—C of aromatic), 1625 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 9.65 (s, 1H, HO-Ar), 6.60-7.20 (m, 10H, ArH), 6.31 (s, 1H, CH=N).MS: m/z 345.38 $[M]^+$. Elemental analysis ($C_{18}H_{11}N_5SO$); calcd: C 62.60, H 3.21, N 20.28; found C 62.50, H 3.30, N 20.25%.

3-(3-hydroxy)phenyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2c: yield 51%; m.p. 219 °C; IR (KBr) cm^{-1} : 672 (C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 7.24-6.65 (m, 10H, ArH), 6.27 (s, 1H, CH=N). MS: m/z 345.38 $[M]^+$. Elemental analysis ($C_{18}H_{11}N_5SO$); calcd: C 62.60, H 3.21, N 20.28; found C 62.55, H 3.25, N 20.22%.

3-(4-hydroxy)phenyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2d: yield 49%; m.p. 232 °C; IR (KBr) cm^{-1} : 676(C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 7.14-6.55 (m, 10H, ArH), 6.29 (s, 1H, CH=N). MS: m/z 345.38 $[M]^+$. Elemental analysis ($C_{18}H_{11}N_5SO$); calcd: C 62.60,H 3.21, N 20.28; found C 62.58, H 3.20, N 20.30%.

3-(4-ethoxy)phenyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2e: yield 53%; m.p. 191 °C; IR (KBr) cm^{-1} : 671 (C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 7.18-6.60 (m, 9H, ArH), 6.25 (s, 1H, CH=N), 3.23 (q, 2H, $-H_2C-CH_3$), 1.10 (t, 3H, $-H_2C-CH_3$). MS: m/z 373.43 $[M]^+$. Elemental analysis ($C_{20}H_{15}N_5SO$); calcd: C 64.33,H 4.05, N 18.75; found C 62.48, H 4.10, N 18.70%.

3-(4-hydroxy)benzyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)aze- pine 2f: yield 57%; m.p. 172 °C; IR (KBr) cm^{-1} : 673 (C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 8.98 (s, 1H, HO-Ar), 7.06-6.50 (m, 9H, ArH), 6.30 (s, 1H, CH=N), 4.28 (s, 2H, $-CH_2$-triazole). MS: m/z 359.40 $[M]^+$. Elemental analysis ($C_{19}H_{13}N_5SO$); calcd: C 63.49, H 3.65, N 19.49; found C 63.58, H 3.60, N 19.50%.

3-(4-ethoxy)benzyl-5H,13aH-quinolino(3,2-f) (1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2g: yield 60%; m.p. 159 °C; IR (KBr) cm^{-1} : 675 (C-S-C),

Table 1. Antimicrobial evaluation of 3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho) azepines 2(a-i).

Compounds	Antibacterial inhibition (mm)				Antifungal inhibition (mm)			
	S. aureus	*E. coli*	*K. pneumoniae*	*P. vulgaris*	A. *fumigatus*	A. *niger*	C. *albicans*	C. *krusei*
@ Control	-	-	-	-	-	-	-	-
Ampicillin trihydrate	16	16	18	20	-	-	-	-
Fluconazole	-	-	-	-	29	25	15	-
2a.	12	-	-	10	15	18	15	14
2b.	10	14	06	08	12	15	12	10
2c.	15	-	15	16	25	14	16	19
2d.	10	12	20	15	10	15	08	12
2e.	12	-	-	-	-	16	-	8
2f.	12	09	16	12	-	24	21	18
2g.	15	10	12	08	15	20	22	15
2h.	18	20	25	23	14	25	16	12
2i.	16	16	14	18	12	12	10	14

- indicates no activity

Table 2. Mass spectral data for 3-phenyl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine

Selected ions	$[M]^+$	$[a]^+$	$[b]^+$	$[c]^+$	$[d]^+$	$[e]^+$	$[f]^+$	$[g]^+$	$[h]^+$	$[i]^+$	$[j]^+$	$[k]^+$	$[l]^+$
m/z	329	128	201	154	175	143	117	128	101	89	89	103	77
Relative intensity (%)	5	100	68	71	39	49	18	100	75	32	50	52	86

1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH); ^{1}H-NMR ($CDCl_3$)δ: 7.13-6.65 (m, 9H, ArH), 6.28 (s, 1H, CH=N), 4.41 (s, 2H, -CH_2-triazole), 3.45 (q, 2H, -H_2C-CH_3), 1.02 (t, 3H, -H_2C-CH_3). MS: m/z 387.46 $[M]^+$. Elemental analysis ($C_{21}H_{17}N_5SO$); calcd: C 65.10,H 4.42, N 18.08; found C 65.18, H 4.40, N 18.10%.

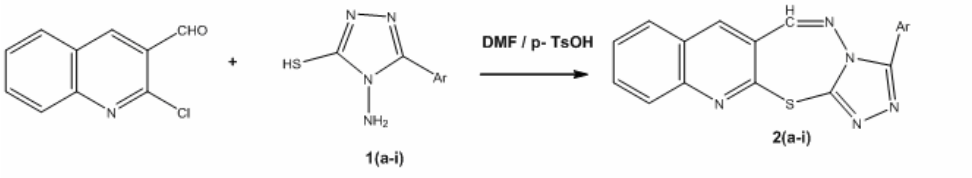
3-(2-chloro)phenyl-5H,13aH-quinolino(3,2-f)(1,2,4) triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2h: yield 50%; m.p.185 °C; IR (KBr) cm^{-1} : 673 (C-S-C), 1252 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 3100 (aromatic CH), 635 (C-Cl). ^{1}H-NMR ($CDCl_3$)δ: 7.15-6.58 (m, 10H, ArH), 6.32 (s, 1H, CH=N). MS: m/z 329 $[M]^+$. Elemental analysis ($C_{18}H_{10}N_5SCl$); calcd: C 59.42, H 2.77, N 19.25; found C 59.50, H 2.80, N 19.22%.

3-(4-chloro)phenyl-5H,13aH-quinolino(3,2-f)(1,2,4) triazolo(4,3-b)(1,2-diaza-4-sulpho)azepine 2i: yield 53%; m.p.203 °C; IR (KBr) cm^{-1} : 625 (C-S-C), 1250 (C-N), 1523 (N-N), 1575 (C—C of aromatic), 1625 (C=N), 3050 (aromatic CH), 630 (C-Cl). ^{1}H-NMR ($CDCl_3$)δ: 7.20-6.65 (m, 10H, ArH), 6.30 (s, 1H, CH=N). MS: m/z 329 $[M]^+$. Elemental analysis ($C_{18}H_{10}N_5SCl$); calcd: C 59.42, H 2.77, N 19.25; found C 59.50, H 2.80, N 19.22%.

Biological Evaluation

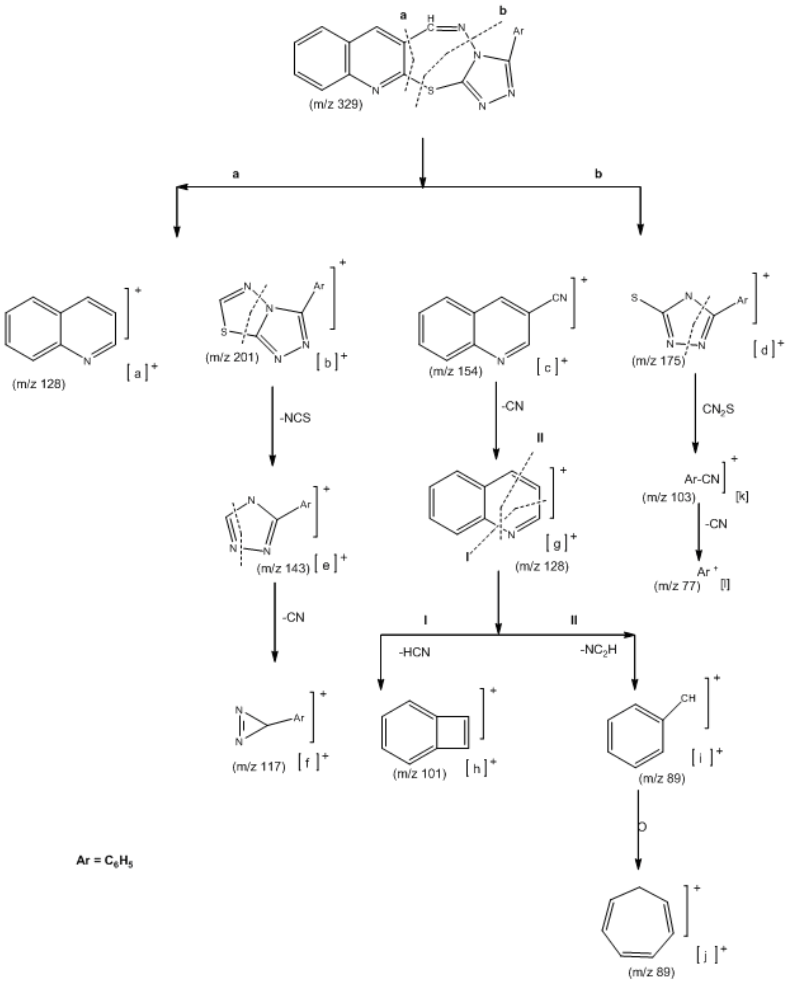
Antimicrobial screening

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial as well as fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. The microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [27] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ampicillin trihydrate used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The inhibition values of the tested compounds against the tested bacteria strains are recorded in Table 1. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata, Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method [28-29]. Fluconazole was employed as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Agar media (20 mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made into each



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

Scheme-1



Scheme-2

well labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The inhibition values of the tested compounds against the tested fungal strains are recorded in Table 1.

RESULT AND DISCUSSION

Synthesis

The synthetic work is outlined in scheme-1. Condensation of 3-aryl-4-amino-5-mercapto triazoles 1(a-i) with 2-chloro-3-formylquinoline in catalytic presence of p-toluene sulphonic acid afforded the target compounds i.e. 3-Aryl-5H,13aH-quinolino(3,2-f)(1,2,4)triazolo(4,3-b) (1,2-diaza-4-sulpho)azepines 2(a-i). The structure of these derived congeners were confirmed by spectral (I.R., ^{1}H-NMR and Mass) and elemental (C, H, N) analysis.

Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial and antifungal activity.

For antibacterial studies microorganisms employed were *S. aureus, E. coli, K. pneumoniae and P. vulgaris.* For antifungal, *A. fumigatus*, *C. glabrata, C. albacans* and *C. krusei* were used as microorganisms. Antibacterial and antifungal studies were assessed by disk diffusion and serial plate method respectively. The data are summarized in Table 1, and show that all compounds display certain activity against the tested microorganisms.

The tested compounds displayed mild to moderate inhibition. From SAR, it is cleared that incorporation of chloro substitution phenyl rings enhanced inhibitory properties of the prepared thiazepines against selected pathogens. Chloro substitution in the thiazepines [2h] and [2i] caused significant broad spectrum inhibitory properties in comparison of the remaining thiazepine derivatives. 2-Chloro phenyl substituted thiazepine [2h] displayed remarkable bacterial as well as fungal inhibitory activity and considering the potency of this derivative deserves further more investigation. We can see that the antibacterial and antifungal activity of the synthesized compounds may be due the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth.

Acute toxicity study

Lethal dose (LD_{50}) of most potent test compound was determined by the method of Carrol [30] in albino mice. After 24 h of drug administration, percent mortality in each group was observed from the data obtained LD_{50}. Data revealed that compound 2h does not show any toxicity upto dose of 9.75 mg/mL body weight in mice.

CONCLUSION

Hence it is cleared from the study of antimicrobial screening data and may be concluded that cyclization of substituted 1,2,4-triazoles into respective 9-substituted-3-aryl-5H,13aH-quinolino(3,2-f)(1,2,4) triazolo(4,3-b)(1,2-diaza-4-sulpho)azepines enhance antibacterial and antifungal activities. Presence of chloro group as substituent brought remarkable increase in biological activities and compound 2h was found the most potent compound.

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