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Review Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2-[(SUBSTITUEDPHENYL)-5-(1-PHENYL-3-(PIPERAZINYL) PYRIDO[3,2-*f*]QUINAZOLIN-4(1*H*)-YL]-1,3,4-THIADIAZOLES

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ABSTRACT

Several novel 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl) pyrido[3,2-*f*] quinazolin-4(1*H*)-yl]-1,3,4 thiadiazole derivatives have been designed and synthesised by incorporating four known moieties such as 2-methylquinolin-5-ol, acetophenone, urea and 3-substitutedphenyl-4-amino-5-mercapto triazoles by using multi-step conventional reaction strategy. The synthesized derivatives were characterized by IR, ^{1}H-NMR, Mass and elemental analysis (C, H, N). Furthermore the synthesized 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl)pyrido[3,2-*f*] quinazolin-4(1*H*)-yl]-1,3,4-thiadiazoles 4a-g were screened for antibacterial and antifungal activities. The bacterial panel consisted of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, *Proteus vulgaris* while fungal panel included of *Aspergillus fumigatus* (plant isolate), *Candida glabrata*, *Candida albicans*, *Candida krusei*. Ampicillin trihydrate and fluconazole were used as reference drugs for antibacterial and antifungal activity, respectively. Bacterial and fungal inhibition determined by disk diffusion and serial plates dilution method, respectively. Pathogenic inhibitions were determined by measuring the diameter of the inhibition zone in mm. Compound 4c demonstrated significant antibacterial and antifungal spectrum against all the tested microbes.

Keywords- antibacterial, antifungal and substituted 1,3,4-thiadiazoles

INTRODUCTION

The heterocyclic derivatives have a great niche in the field of medicinal chemistry. Among the heterocyclic nuclei, 1,3,4-thiadiazole (Bekhit, *et al.,* 2008; Palaska, *et al*., 2002; Labanauskas *et al*., 2001; Onkol, *et al*., 2004; Schenone *et al*., 2001) and quinoline (Gokce, *et al*., 2001; Baldwin, *et al*., 1980) are found versatile biological moieties. Current global issue is the increasing number of multi-drug resistance and the solution is found continual drug development program. 1,3,4-thiadiazole, 1,2,4-triazole and quinoline (Gokce *et al*., 2001; Baldwin, *et al*., 1980) rings bearing derivatives have been extensively biologically studied for a number of pathological conditions including inflammation (Palaska, *et al*., 2002; Labanauskas, *et al*., 2001; El-Sayed, *et al*., 2004) pain (Onkol, *et al*., 2004; Gokce *et al*., 2001;), hypertension (Baldwin, *et al*., 1980), antibacterial (Varvaresou, *etal*., 1998, 2000), antimyco-bacterial (Foroumadi, *et al*., 2001; Mamolo, *et al*., 2001), antimicrobial (Abdelhamid *et al.,* 2016; Rezki, *et al.,* 2015; Kallur, *et al.,* 2012), antimycotic (Wujec, *et al*., 2004; Zamani, *et al*., 2004), antifungal (Chen, *et al*., 2000; Zou, *et al*., 2002), anxiolytic (Clerici *et al*., 2001), antiproliferative (Rezki, *et al.,* 2015) and antidepressant (Clerici *et al*., 2001) activities. In the present work, we have focused on quinolin-5-ol and 2-amino-5-phenyl-1,3,4-thiadiazole due to their significant pharmacological profiles with the hope that the targeted derivative will show higher biological efficacy.

MATERIALS AND METHODS

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. Reference drugs ampicillin trihydrate and fluconazole were procured from Ind-Swift Pharmaceutical, Panjab, India and Macleods Pharmaceutical, Mumbai, India respectively. 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 C H N rapid analyser. 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.

Antimicrobial screening

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial and fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Bacterial inhibition diameter was measured in mm. Microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method (Cruickshank, *et al*., 1975; Collins, 1976) was used for determination of the preliminary antibacterial activity. Sterile 5mm Whatman no. 1 filter paper discs were used in the disc diffusion method. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140°C for 1h. The test compounds were prepared with different concentrations using DMF. One hundred millilitres of the microbial suspension was spread onto nutrient agar plates. 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 24h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted (Table I). On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against Aspergillus fumigatus (plant isolate), Candida glabrata, Candida albicans and Candida krusei in DMSO by the serial plate dilution method (Khan, 1997; Varma, 1998). Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1g), D-glucose (4g), and agar (2g) in distilled water (100mL) 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 3mL saline to get a suspension of the corresponding species. Agar media (20mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1h. Using an agar punch wells were made into each well labelled. 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 in mm (Table I).

Synthesis

Preparation of 8-methyl-1-phenyl-1*H*-[1,3]oxazino[5,6-*f*]quinolin-3(2*H*)one (1)

A mixture of 2-methylquinolin-5-ol (0.01mol), urea (0.01mol) and acetophenone (0.01mol) in ethanol was refluxed for 1h. The reaction mixture was cooled, poured into ice-water slowly with continuous stirring, filtered and recrystallized by methanol: Yield: 70%; m.p.: 215°C; R_f: 0.64 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 3410 (NH), 1745 (C=O), 1625 (C…C of aromatic ring), 1590 (C=N), 1290(C-O-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.60-7.42(m, 9H, Ar-H), 6.78 (bs, 1H, NH), 4.70 (s, 1H, CH-NH), 2.26 (s, 3H, Ph-CH_3). Anal. calcd. for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65; found C, 74.30; H,4.68; N,9.60. MS (m/z, %): 290.10.

Preparation of 3-chloro-8-methyl-1-phenyl-1*H*-[1,3]oxazino[5,6-*f*]quinoline (2)

Phosphorous oxychloride (0.01mol) added slowly to a solution of compound 1 (0.01mol) in toluene at R.T., stirred for 30min, after reaction mixture refluxed for 2h. Excess of solvent was distilled off. The reaction mixture cooled. The cooled mass poured into ice-water slowly with continuous stirring, neutralized with aqueous 3% KOH solution, filtered and recrystallized by ethanol: Yield: 69%; m.p.: 188°C; R_f: 0.70 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 3400 (NH), 1622 (C…C of aromatic ring), 1594 (C=N), 1288 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.52-7.55 (m, 9H, Ar-H), 4.75 (s, 1H, CH-N-C), 2.20 (s, 3H, Ph-CH_3). Anal. calcd. for

Table I. Antimicrobial screening of 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl) pyrido[3,2*f*] quinazolin-4(1*H*)-yl]-1,3,4-thiadiazoles (4a-g)

Compound	Substituent R	Antifungal activity (mm)			
		A. fumigatus	*A. flavus*	*C. albicans*	*C. glabrata*
1.					
2.					
3.		6	6	-	-
4a.	H	8	6	-	-
4b.	2-Cl	10	10	6	8
4c.	4-Cl	15	12	12	10
4d.	4-NO_2	8	6	-	-
4e.	2-OH	10	10	-	8
4f.	3-OH	8	6	-	6
4g.	4-OH	10	8	-	6
Ampicilline trihydrate		-	-	-	-
Fluconazole		20	20	15	15
DMF (control)		-	-	-	-

- means no activity.

Table II. Antimicrobial screening of 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl) pyrido[3,2*f*] quinazolin-4(1*H*)-yl]-1,3,4-thiadiazoles (4a-g)

Compound	Substituent R	Antibacterial activity (mm)			
		S. aureus	*E. coli*	*P. vulgaris*	*K. pneumoniae*
1.					
2.					
3.		-	-	6	-
4a.	H	-	-	6	-
4b.	2-Cl	12	8	10	12
4c.	4-Cl	15	12	15	18
4d.	4-NO_2	-	6	-	-
4e.	2-OH	8	-	10	10
4f.	3-OH	6	-	8	8
4g.	4-OH	8	-	8	6
Ampicilline trihydrate		16	16	20	20
Fluconazole		-	-		-
DMF (control)		-	-		-

- means no activity.

$C_{18}H_{13}N_2O_2Cl$: C, 70.02; H,4.24; N, 9.07; found C, 69.98; H,4.30; N, 9.16. MS (m/z, %): 308.07.

Preparation of 8-methyl-1-phenyl-3-piperazinyl-1*H*-[1,3]oxazino[5,6-*f*]quinoline(3)

A mixture of compound 2 (0.01mol), piperazine (0.015mol) and anhydrous sodium carbonate (1.25g) in absolute isopropanol was refluxed for 10h. The excess of amine and isopropanol was removed under reduced pressure and the residue was treated with 5% sodium bicarbonate solution to remove acidic impurities, filtered, washed with water properly and dried. It was crystallized from ethanol (95%) to give compound 3. Yield: 66%; mp 179°C; R_f: 0.66 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1620 (C…C of aromatic ring), 1586 (C=N), 1370 (N-N), 1312 (C-N), 1277 (C-O-C), 690 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.20-7.34 (m, 9H, Ar-H), 4.50 (s, 1H, CH-N-C), 3.08 (s, 4H, 2XCH_2), 2.53 (s, 3H, Ph-CH_3), 2.28

(s, 4H, 2XCH$_2$), 1.61 (s, 1H, NH). Anal. calcd. for $C_{22}H_{22}N_4O$: C, 73.72; H, 6.19; N, 15.63; found C, 73.70; H, 6.12; N,15.79. MS (m/z, %): 358.18.

Genertal preparation of 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl)pyrido[3,2-*f*] quinazolin-4(1*H*)-yl]-1,3,4-thiadiazoles (4a-g)

The ethanolic solution of compound 3 (0.01mol) and 2-amino-5-substitutedphenyl-1,3,4-thiadiazoles (0.01mol) heated to reflux for 2-3h. Excess of solvent was distilled off under reduced pressure and the residue cooled, poured into ice cold water, stirred, filtered, dried and recrystallised with appropriate solvents to yield compound 4a-g (Figure 1).

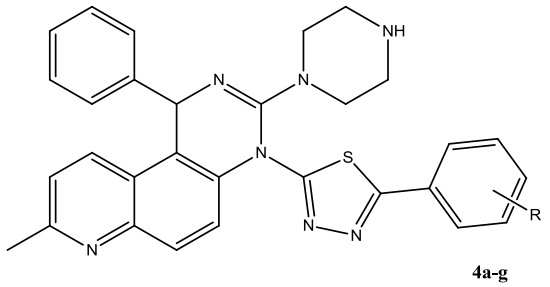


Figure 1. 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl)pyrido[3,2-*f*] quinazolin-4(1*H*)-yl]-1,3,4-thiadiazoles (4a-g)

Compound 4a: Yield: 67%; m.p.: 227°C; R_f: 0.72 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1625 (C…C of aromatic ring), 1590 (C=N), 1366 (N-N), 1302 (C-N), 1293 (C-O-C), 678 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.55-7.20 (m, 14H, Ar-H), 4.56 (s, 1H, CH-N-C), 3.10 (s, 4H, 2XCH$_2$), 2.55 (s, 3H, Ph-CH$_3$), 2.10 (s, 4H, 2XCH$_2$), 1.50 (s, 1H, NH). Anal. calcd. for $C_{30}H_{27}N_7S$: C, 69.61; H, 5.26; N, 18.94; found C, 69.68; H, 5.23; N,18.98. MS (m/z, %): 517.20.

Compound 4b: Yield: 56%; m.p.: 189°C; R_f: 0.66 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1627 (C…C of aromatic ring), 1594 (C=N), 1368 (N-N), 1300 (C-N), 1287 (C-O-C), 681 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.26-7.05 (m, 13H, Ar-H), 4.77 (s, 1H, CH-N-C), 3.00 (s, 4H, 2XCH$_2$), 2.50 (s, 3H, Ph-CH$_3$), 2.05 (s, 4H, 2XCH$_2$), 1.41 (s, 1H, NH). Anal. calcd. for $C_{30}H_{26}N_7SCl$: C, 65.26; H, 4.75; N, 17.76; found C, 65.38; H, 4.73; N,18.00. MS (m/z, %): 551.17.

Compound 4c: Yield: 60%; m.p.: 196°C; R_f: 0.68 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1619 (C…C of aromatic ring), 1590 (C=N), 1364 (N-N), 1310 (C-N), 1290 (C-O-C), 675 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.20-7.12 (m, 13H, Ar-H), 4.80 (s, 1H, CH-N-C), 3.15 (s, 4H, 2XCH$_2$), 2.63 (s, 3H, Ph-CH$_3$), 2.12 (s, 4H, 2XCH$_2$), 1.32 (s, 1H, NH). Anal. calcd. for $C_{30}H_{26}N_7SCl$: C, 65.26; H, 4.75; N, 17.76; found C, 65.23; H, 4.80; N,17.72. MS (m/z, %): 551.17.

Compound 4d: Yield: 55%; m.p.: 243°C; R_f: 0.73 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1627 (C…C of aromatic ring), 1582 (C=N), 1359 (N-N), 1302 (C-N), 1298 (C-O-C), 680 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 8.25-7.17 (m, 13H, Ar-H), 4.72 (s, 1H, CH-N-C), 3.49 (s, 4H, 2XCH$_2$), 2.58 (s, 3H, Ph-CH$_3$), 2.14 (s, 4H, 2XCH$_2$), 1.44 (s, 1H, NH). Anal. calcd. for $C_{30}H_{26}N_8SO_2$: C, 64.04; H, 4.66; N, 19.92; found C, 64.15; H, 4.69; N,19.74. MS (m/z, %): 562.19.

Compound 4e: Yield: 60%; m.p.: 172°C; R_f: 0.70 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1630 (C…C of aromatic ring), 1585 (C=N), 1364 (N-N), 1310 (C-N), 1290 (C-O-C), 671 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 12.20 (ss, 1H, OH), 8.28-7.26 (m, 13H, Ar-H), 4.80 (s, 1H, CH-N-C), 3.14 (s, 4H, 2XCH$_2$), 2.62 (s, 3H, Ph-CH$_3$), 2.25 (s, 4H, 2XCH$_2$), 1.30 (s, 1H, NH). Anal. calcd. for $C_{30}H_{27}N_7SO$: C, 67.52; H, 5.10; N, 18.37; found C, 67.56; H, 5.20; N,18.27. MS (m/z, %): 533.20.

Compound 4f: Yield: 56%; m.p.: 155°C; R_f: 0.68 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1627 (C…C of aromatic ring), 1579 (C=N), 1372 (N-N), 1302 (C-N), 1285 (C-O-C), 678 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm): 12.30 (ss, 1H, OH), 8.22-7.20 (m, 13H, Ar-H), 4.82 (s, 1H, CH-N-C), 3.22 (s, 4H, 2XCH$_2$), 2.57 (s, 3H, Ph-CH$_3$), 2.12 (s, 4H, 2XCH$_2$), 1.23 (s, 1H, NH). Anal. calcd. for $C_{30}H_{27}N_7SO$: C, 67.52; H, 5.10; N, 18.37; found C, 67.54; H, 5.12; N,18.30. MS (m/z, %): 533.20.

Compound 4g: Yield: 62%; m.p.: 182°C; R_f: 0.72 (cycohexane: ethyl acetate). IR (KBr, cm^{-1}): 1630 (C…C of aromatic ring), 1585 (C=N), 1364 (N-N), 1306 (C-N), 1276 (C-O-C), 675 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ppm):

12.22 (ss, 1H, OH), 8.17-7.18 (m, 13H, Ar-H), 4.73 (s, 1H, CH-N-C), 3.17 (s, 4H, 2XCH_2), 2.68 (s, 3H, Ph-CH_3), 2.25 (s, 4H, 2XCH_2), 1.36 (s, 1H, NH). Anal. calcd. for $C_{30}H_{27}N_7SO$: C, 67.52; H, 5.10; N, 18.37; found C, 67.60; H, 5.24; N,18.29. MS (m/z, %): 533.20.

RESULTS AND DISCUSSION

Chemistry

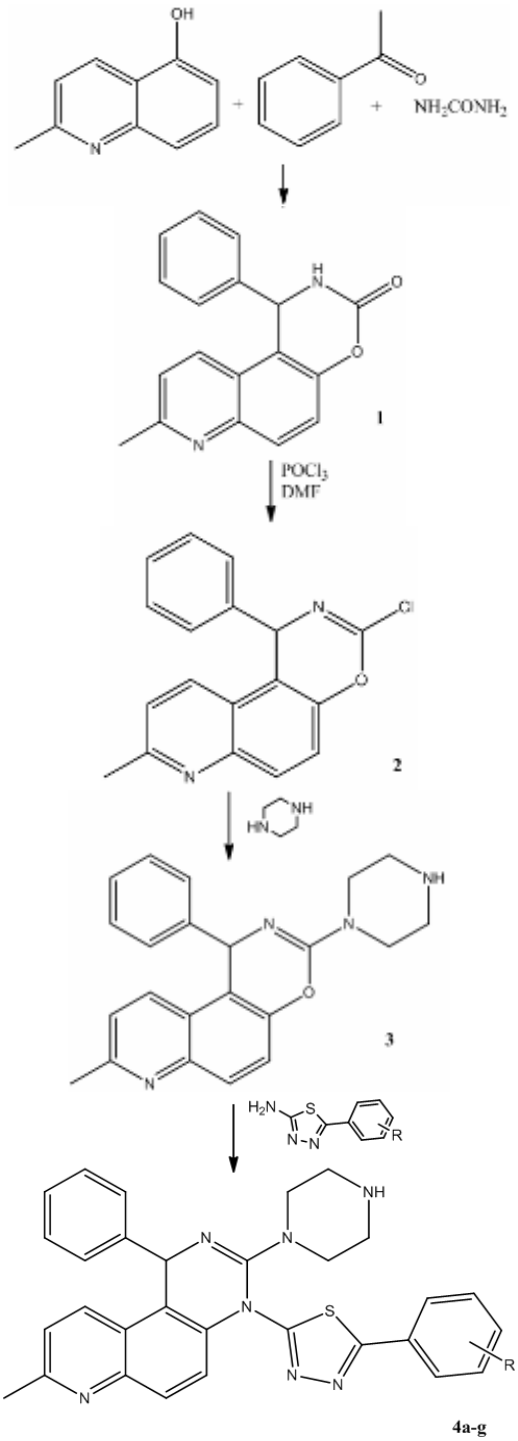
3-substitutedphenyl-4-amino-5-mercapto triazoles were prepared according to the reported method [20-23]. Ethanolic mixture of 2-methylquinolin-5-ol, urea and acetophenone refluxed to give 8-methyl-1-phenyl-1*H*-[1,3]oxazino[5,6-*f*]quinolin-3(2*H*)one (1) in good yield. Refluxing of compound 1 with phosphorous oxy chloride in DMF solvent furnished 3-chloro-8-methyl-1-phenyl-1*H*-[1,3]oxazino[5,6-*f*]quinoline (2) which on further reacted with piperazine to yield 8-methyl-1-phenyl-3-piperazinyl-1*H*-[1,3]oxazino[5,6-*f*]quinoline (3). Reaction of 3-substitutedphenyl-4-amino-5-mercapto triazoles with compound 3 resulted into targeted moieties 2-[(substitutedphenyl)-5-(1-phenyl-3-(piperazinyl)pyrido[3,2-*f*] quinazolin-4(1*H*)-yl]-1,3,4 thiadiazoles (4a-g) [**Scheme-1**].

Antimicrobial activity

Out of all the tested derivatives, compound 4c showed better antimicrobial activity. Screening data cleared that compound 1 and 2 were inactive against all the used pathogens. Compound 3 showed poor microbial inhibition. Among the derivative 4a-g; derivative 4a, 4d elicited poor inhibitions; 4e, 4f and 4g showed almost similar milder potency while derivative 4b, 4c exhibited moderate antimicrobial activity. Chloro substituted compounds 4b and 4c displayed significant broader microbial inhibition against pathogens.

CONCLUSION

Antimicrobial evaluation of all the synthesised compounds showed that conversion of compound **3** into substituted-1,3,4-thiadiazoles **4a-g** enhances antibacterial as well as antifungal activities. Compound **4c** possessing 4-chlorophenyl substitution; displayed the most potent antimicrobial potential against the used selected strains of pathogens.



R=H,2-Cl, 4-Cl, 4-NO_2, 2-OH, 3-OH,4-OH

Scheme-1

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