DESIGN, SYNTHESIS & BIOLOGICAL EVALUATION OF NOVEL HYBRID DERIVATIVES OF THIADIAZOLE

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Abstract:

Novel hybrid derivative of quinoline-2-one substituted thiadiazole derivatives tested as antimicrobial activity on *Escherichia coli*, *Pseudomonas aeruginosa* and *gram-positive bacteria Staphylococcus aureus*, *Bacillus substilis* at 100 µg/ml concentration using cup-plate agar diffusion method. All derivatives of quinoline-2-one substituted thiadiazole were synthesized, purified and characterized using various physiochemical and spectral analysis. Among the all synthesized novel hybrid derivatives, three compounds 4b, 4c and 4f shows promising antibacterial activity as compared to standard drug Ciprofloxacin. The synthesis of new agents, active against resistant organism is of critical importance despite continued efforts to discover improved antimicrobial agents, there has been little success towards discover of antimicrobial agents. The compounds having fluoro substitution at 4th & 2,4-dichloro positions showed satisfactory antibacterial activity.

Keywords: Thiadiazole, Antibacterial

1. INTRODUCTION

Discovery of new drugs for systemic opportunistic microbial infections is a major challenge in infectious disease research. Microbial infections have increased in recent years, particularly those that are of nosocomial origin, leading to a broad use of agents with activity against pathogens [1]. Antimicrobial resistance of different pathogens also became widespread resistance of different pathogens also became widespread. Treatment of infectious diseases still remains an important and challenging problem because of a combination factor including

newly emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria [1-5]. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence new antibiotic resistant bacterial strains in the last decades constitutes a substantial need for new class of antibacterial agents [6]. During recent years, there have been intense investigations on thiadiazole and their substituted derivatives, many of which are known to possess interesting pharmacological properties such as anticancer [7], anti- tubercular [8], antibacterial [9], antifungal [10], antimicrobial and anti-inflammatory [11,12], analgesic and antimicrobial [13], anticonvulsant, analgesic [14], and antisecretory [15] activities. Moreover, much interest has also been focused on the cardiotonic [16], diuretic [17] and herbicidal [18] activities displayed by compounds incorporating this heterocyclic system. The varied biological activities of thiadiazole and their analogues have been known from the beginning of the 20th century. Thiadiazole become a pharmacologically important class of heterocyclic compounds. Chemical modification of these heterocycles has constantly resulted in compounds with broad spectrum of pharmacological activities [19]. In view of the high degree of bioactivity shown by the heterocyclic system, and initialization of our search for biological active heterocyclic compounds explore the additive effects towards their biological activities. Hence, we are reporting herein the synthesis of naval hybrid derivatives of thiadiazole derivatives and evaluation of their antibacterial [20].

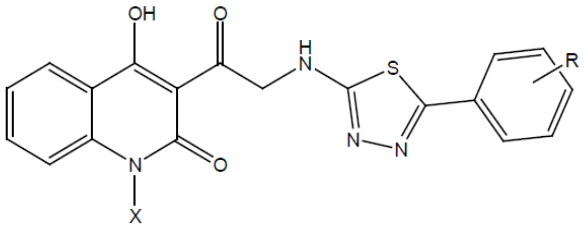
2. MATERIAL & METHODS

2.1 Procurement of chemicals

All chemical, reagents and solvents were procured from the authorize vendor of Sigma-Aldrich. Electro-thermal capillary devices were used to calculate melting point. Bruker was used to carried IR spectra. A Bruker 80 MHz (Bruker Bioscience, Billerica, MA, USA), in deutrated solvents such as CDCl3. Recorded H-NMR spectra, and the chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an interval standard.

2.2 Procedure

General procedure for synthesis of 3-(bromoacetyl)-4-hydroxy-1-methyl (2a)/phenyl (2b) quinolin-2(1H)-one: Substituted quinoline mixed with 50ml glacial acetic acid and gently heated at 80°C with continuous stirring for 30 min. The equimolar quantity of bromine (0.065 mol) in glacial acetic acid (10 ml) was slowly added in a drop wise manner for a period of 1 h and heating ofthe solution was continued until a slight change in the color of the solution was observed. The obtained mixture was cooled to room temperature. The products (2a and 2b) were obtained as yellow crystalline solid and recrystallized from ethanol [21].



3-(2-(Substituted 1,3,4-thiadiazol-2-ylamino)acetyl)-4- hydroxy-1-methyl (4a-

4d)/phenyl(4e-4h)quinolin-2(1H)-ones

(Bromoacetyl)-4-hydroxy-1-methylquinolin-2(1H)-one (2a).

The compound 2a was prepared and purified as perthe above-mentioned procedure: yield 10.26 g (73%), mp 174-176 0C, IR (KBr, v, cm-1): 756.14 cm-1 and 1190.18 (CH -Br), 1615.47 cm-1 (-C=O amide), 3046.24cm-1 (aromatic -C-H stre). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): δ 3.78 (s, 3H, -N- CH_3), 4.98 (s, 2H, -CH_2-Br), 7.72 – 8.35 (m, 4H, Ar-H), 15.82 (s, 1H, -OH).

(Bromoacetyl)-4-hydroxy-1-phenylquinolin-2(1H)-one (2b)

The compound 2b was prepared and purified as perthe above mentioned procedure: yield 12.24 g (67.43%), mp 192-194 0, IR (KBr, v, cm-1): 786.11cm-1 and 1210.06 (CH2-Br), 1620.31 cm-1 (-C=O amide), 3058.11cm-1 (aromatic -C-H stre). 1H NMR (400MHz, CDCl3) δ (ppm): δ 5.23 (s, 2H, CH2-Br), 7.65 – 8.85 (m, 9H, Ar-H), 15.91 (s, 1H, -OH).

General procedure for synthesis of 3-(2-(substituted1,3,4-thiadiazolylamino)acetyl)-4-hydroxy-1- methyl (4a-4d)/phenyl(4e-4h)quinolin-2(1H)-ones.

A mixture of an appropriately substituted thiadiazole (one of 3a-3d, 0.004 mol) and 3-(2-bromoacetyl)-4-hydroxy-1-methylphenylquin olin-2(1H)-one (2a/2b, 0.004 mol) was dissolved in45 mL of glacial acetic acid with constant stirring. The obtained mixture was then heated at reflux temperature for about 6 to13 h. The progress of the reaction was monitored by the TLC. The obtained mixture was allowed to attain the room temperature. The solvent was removed undervacuum. The ethyl acetate was used to dissolve theobtained residue, washed twice with water, anddried over Na2SO4. Further, the final compounds were purified by removal of the solvent followed by column chromatography using 200-400 mesh silicagel eluting with methanol (12%) in dichloromethane [22].

3-(2-(5-Phenyl-1,3,4-thiadiazol-2-ylamino) acetyl)-4-hydroxy-1-methylquinolin-2(1H)-one (4a).

IR (KBr, v, cm^{-1}): 1620.9 cm-1 (-COCH2 stre), 1740.5 cm-1 (-C=O amide), 2968.4 cm-1 (Ar-CH stre), 3121.8 cm-1 (-NH), 3393.8 cm^{-1} (-OH); ^{1}HNMR (400MHz, DMSO-d6) δ (ppm): 2.21 (s, 3H,N-CH3), 6.28 (s, 1H, -NH), 6.54 (s, 2H, COCH2), 6.80–8.65 (m, 9H, Ar-H), 12.84 (s, 1H, -OH); LCMS: $C_{20}H_{16}N_4O_3S$ (M+) m/z 392.24; calcd. 392.09.

3-(2-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-ylamino)acetyl)-4-hydroxy-1 methylquinolin-2(1H)-one (4b).

IR (KBr, v, cm^{-1}): 1627.2 cm^{-1} (-COCH2 stre),1738.9 cm^{-1} (-C=O amide), 2943.7 cm^{-1} (Ar-CH stre), 3101.0 cm^{-1} (-NH), 3363.0 cm^{-1} (-OH); 1H NMR (400MHz, DMSO-d6) δ (ppm): 2.15 (s, 3H,
N- CH3), 6.21 (s, 1H, NH), 6.38 (s, 2H, COCH2), 6.90– 8.51 (m, 8H, Ar-H), 12.31 (s, 1H, -OH);LCMS: C H FN O S (M+) m/z 410.42; calcd 410

3-(2-(5-(2,4-Dichlorophenyl)-1,3,4-thiadiazol-2-ylamino)acetyl)-4-hydroxy-1-methylquinolin-2(1H)-one (4c).

IR (KBr, v, cm^{-1}): 1640.4 cm^{-1} (-COCH2 stre),1704.2 cm^{-1} (-C=O amide), 2988.7 cm^{-1} (Ar CH stre), 3124.0 cm^{-1} (-NH), 3394.5 cm^{-1} (-OH); 1H NMR (400MHz, DMSO-d6) δ (ppm): 2.48 (s, 3H, N-CH3), 5.98 (s, 1H, -NH), 6.18 (s, 2H, COCH2),7.21–8.64 (m, 7H, Ar-H), 12.85 (s, 1H, -OH)

3-(2-(5-Phenyl-1,3,4-thiadiazol-2-ylamino)acetyl) -4-hydroxy-1-phenylquinolin-2(1H)-one (4d).

IR (KBr, v, cm^{-1}): 1628.2 cm^{-1} (-COCH2 stre),1680.2 cm^{-1} (-C=O amide), 3012.7 cm^{-1} (Ar-CH stre), 3145.5 cm^{-1} (-NH), 3376.2 cm^{-1} (-OH); 1H NMR (400MHz, DMSO-d6) δ (ppm): 6.02 (s, 1H,
-NH), 6.32 (s, 2H, COCH2), 6.92–8.74 (m, 14H (M+) m/z 454.54; calcd. 454.11

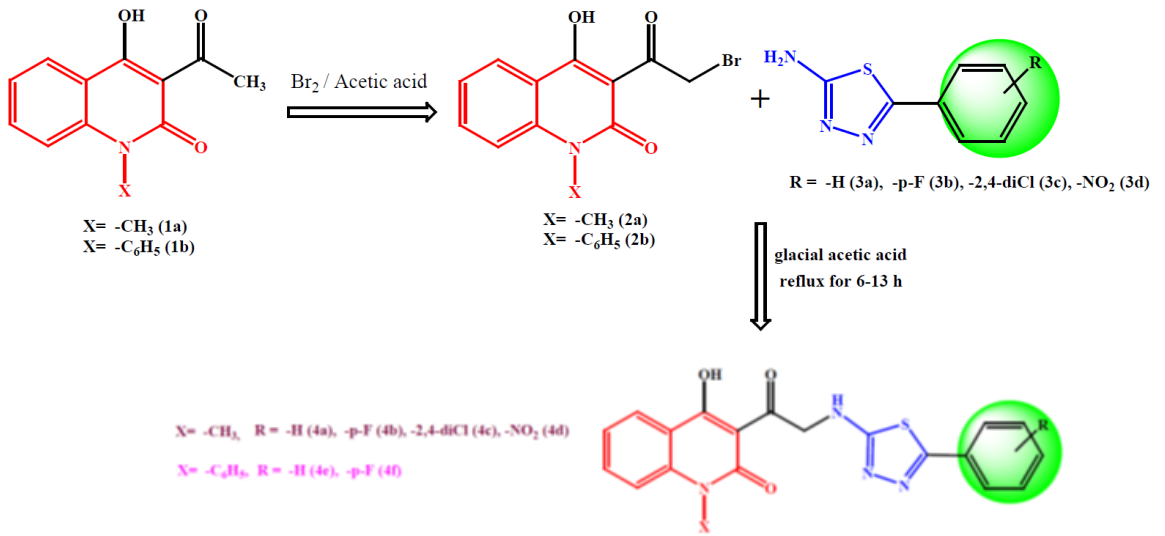
3-(2-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-ylamino)acetyl)-4-hydroxy-1 phenylquinolin-2 (1H)-one (4e).

IR (KBr, v, cm^{-1}): 1634.4 cm^{-1} (-COCH2 stre), 1694.4 cm^{-1} (-C=O amide), 3022.4 cm^{-1} (Ar-CH stre), 3112.5 cm^{-1} (-NH), 3354.8 cm^{-1} (-OH); ^{1}HNMR (400MHz, DMSO-d6) δ (ppm): 5.78 (s, 1H,-NH), 6.12 (s, 2H, COCH2), 6.88–8.59 (m, 13H, Ar-H), 13.24 (s, 1H, OH); (M+) m/z; 472.48 calcd. 472.10.

3-(2-(5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-ylamino)acetyl)-4-hydroxy-1-phenylquinolin-2(1H)-one (4f).

IR (KBr, v, cm^{-1}): 1645.4 cm^{-1} (-COCH2 stre),1692.4 cm^{-1} (-C=O amide), 3029.2 cm^{1} (Ar-CH stre), 3124.5 cm^{-1} (-NH), 3388.9 cm^{-1} (-OH); ^{1}H NMR (400MHz, DMSO-d6) δ (ppm): 5.84 (s, 1H,-NH), 6.02 (s, 2H, COCH2), 6.88–8.59 (m, 12H, Ar-H), 13.33 (s, 1H, OH).

Synthetic Scheme:



2.3 Antimicrobial activity [23]

All the synthesized compounds 4a-4h have been screened *in vitro* for their antibacterial activity against gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and gram-positive *bacteria Staphylococcus aureus, Bacillus substilis*, at 100 µg/ml concentration by cup-plate agar diffusion method using dimethyl sulfoxide as solvent. After 24h and 48h ofincubation at 37*C±1, the antibacterial was determined by measuring the zones of inhibition in mm. Standard ciprofloxacin were used under similar condition for comparison. Control test with solvent were performed for every assay but showed no inhibitionof microbial growth. The values of antibacterial shown in table no. 1

3. RESULT

The synthesis of title compounds 4a-4h was accomplished by condensing with different substituted thiadiazole. The newly synthesized compounds structures were confirmed by their spectral data. The spectral data of 4b exhibit IR band at 1627.2 cm^{-1} and 1738.9 cm^{-1} was due to amide carbonyl and acetyl groups. Another peak at2943.7 cm^{-1} mainly because of aromatic –C-H stretching. Further, a peak at 3101.0 cm^{-1} mainly because of –N-H stretching, and 3363.0 cm^{-1} of hydroxyl group indicates the completion of the reaction. Their structure was further confirmed by their ^{1}H NMR spectral data that exhibited three protons of N-methyl signal found at δ value 2.15 ppm, whereas aromatic protons signal showed the δvalue 6.90-8.51 ppm. The appearance of a signal atδ value 12.31 ppm indicates one proton of hydroxyl(-OH) group and δ value 6.21 ppm indicates one proton of amine (-NH) group. The mass spectra of the compound 4b (m+) found 410.42, which was one more proof for the confirmation of the title compound. The physicochemical data of the synthesized compounds are listed in Table 1. Someof these compounds have shown good antibacterial activity.

Table 1. Zone of inhibition in mm (100µg/ml)

Compounds	**Zone of inhibition**			
	Staphylococcus aureus	*Bacillus substilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
4a	08	10	13	15
4b	20	23	25	27
4c	21	25	32	30
4d	12	11	13	NA
4e	20	23	25	27
4f	09	07	10	11
Ciprofloxacin	23	26	34	31

NA=No activity, ND= Not determined.

4. DISCUSSION

The synthesized compounds of 3-(2-(substituted 1,3,4-thiadiazol-2-ylamino)acetyl)-4-hydroxy-1- methyl (4a-4d)/phenyl(4e-4f)quinolin-2(1H)-oneswere screened for their in vitro antibacterial activity against gram positive bacteria (*Staphylococcus aureus, Bacillus substilis*) and gram negative bacteria (*Escherichiacoli, Pseudomonas aeruginosa*) by measuring the zoneof inhibition at concentration 100 µg/mL (mm).Compounds 4b (20, 23, 25, 27mm), 4c (21, 25, 32, 30mm) and 4f (20, 23, 25, 27mm) showed promising antibacterial activity as compared with standard Ciprofloxacin (23, 26, 34, 31mm).

5. CONCLUSION

4-Hydroxy-1-methyl/phenyl – 3 - (substitute thiadiazole) quinoline – 2 (1H)-one compounds (4a-4h) were successfully synthesized and characterized on the basis of physicochemical and spectral studies. Further evaluated for their antimicrobial activity against *Staphylococcus aureus, Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa*. Among synthesized novel compounds 4b, 4c, 4f shows promising antibacterial activity as compared to Ciprofloxacin (100 µg/ml). Suitable molecular modification of these compounds may generate potent antimicrobial agents in future.

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CONFLICT OF INTEREST

No conflict of interest.

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