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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro antimicrobial activity of some newer quinazolinone–sulfonamide linked hybrid heterocyclic entities derived from glycine

Satish F. Vanparia · Tarosh S. Patel · Ritu B. Dixit · Bharat C. Dixit

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Abstract A novel series of 4-(amino or acetamido)-*N*-{[3-(substituted aryl)-4-oxo-3,4-dihydroquinazolin-2-yl]-methyl}benzenesulfonamide derivatives (**1–19**) were designed to assimilate 4-quinazolone and sulfonamide moieties in a single molecular framework. To derive entitled hybrid entities with structural diversity, an efficient multi-step synthetic approach initiated from glycine was developed, which involves milder conditions for emphasizing steps viz., reaction in aqueous-media, phosphazo-method of condensation, base mediated selective ester-cleavage, along with key-step, rapid and improved Grimmel's hetero-cyclization method. The structure of the synthesized compounds was confirmed by physico-chemical characteristics and spectroscopic investigations. All these compounds were screened for their in vitro antimicrobial activity. The minimum inhibitory concentrations of the synthesized compounds against various bacteria (*S. aureus*, *B. cereus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*) and fungus (*A. niger*, *C. albicans*) was measured by broth micro-dilution assay. Further, results on the preliminary biological activity indicated that most of the screened compounds have displayed varied degree of inhibitory actions.

Keywords 4-(Acetamidobenzenesulfonyl)amino acid · Methyl *N*-acylanthranilate · *N*-acylanthranilic acid · (4H)-3,1-benzoxazinone · 2,3-Disubstituted-4-quinazolin-(3H)-ones · Antibacterial and antifungal activity

Introduction

Currently, due to the microbial-resistance, a need for the development of newer antimicrobial agents with improved efficacy, reduced toxicity, and lower side effects (Cohen, 1992) has posed a great challenge to scientists worldwide. As a consequence, much more effort has gone into rational design of newer molecules for better results. However, it seems clear that selecting the appropriate molecules to synthesize is one of the most troublesome questions, though heterocyclic compounds remain the first choice ever to design a newer class of structural entities of medicinal importance.

Among the most frequently encountered N-heterocycles, derivatives of 4(3*H*)-quinazolinone core (Fig. 1, structure **Q**) has been extensively utilized as a drug-like template in medicinal chemistry (Thierry and Elizabeth, 2007). Quinazolin-4(3*H*)-one skeleton is associated as a building-block for several physiologically active molecules of natural and synthetic origin (Mhaske and Argade, 2006), including drug, methaqualone (Fig. 1, structure **Qm**) among others, (Tiwari *et al*., 2008). Hence, it has been considered to be "privileged substructure" like other organic scaffolds such as benzodiazepinone, isoxazole, sulfonamides, indole, etc. in their own right (Horton *et al*., 2003). The 4-quinazolin-(3*H*)-ones endowed with a vast

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S. F. Vanparia · T. S. Patel · B. C. Dixit (✉)
Chemistry Department, V. P. & R. P. T. P. Science College, Affiliated to Sardar Patel University, Vallabh Vidyanagar, Anand, Gujarat 388 120, India
e-mail: dixits20002003@yahoo.co.in

R. B. Dixit
Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), Affiliated to Sardar Patel University, New Vallabh Vidyanagar, Anand, Gujarat 388 121, India

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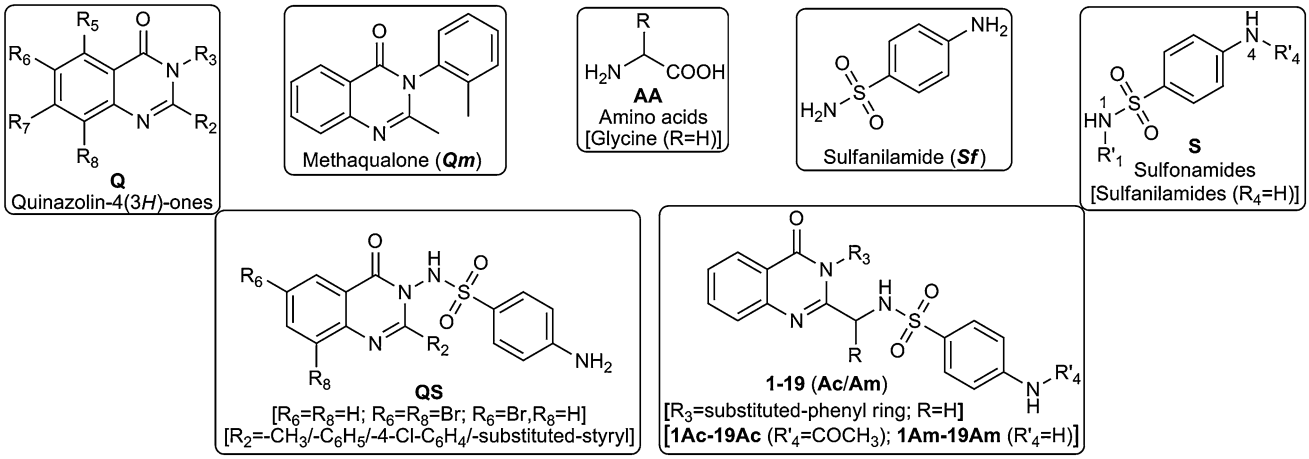


Fig. 1 General structure of some bioactive lead molecules (**Q**, **S**, **AA**), drug molecules of parent moieties (**Qm**, **Sf**), and structurally hybrid entities (**QS**, **1**–**19**)

array of pharmacological activities (Liu, 2007) such as, antiviral, antimalarial, antitubercular, anticancer, antiinflammatory, cardiovascular, diuretic, cardiotonic, antihistamine, and antidiabetic etc., activities. The (3*H*)-quinazolin-4-ones have been known to exhibit a range of CNS-depressant effects (Kashaw *et al*., 2009). In addition, 4-quinazolones can also act as antibacterial and antifungal agents (Wasfy, 2002; Grover and Kini, 2006; Pandey *et al*., 2009; Meyyanathan *et al*., 2010; Saravanan *et al*., 2012).

In addition, derivatives of sulfonamides (Fig. 1, structure **S**) have occupied a prominent position in medicinal chemistry, due to their wide range of applications as pharmaceutical agents (Anand, 1996; Reynolds, 1996) which exhibited significant pharmacological effects, such as antimicrobial (Chohan *et al*., 2010), antitumor, antimalarial, antiinflammatory, anticancer, and antiviral activities among others. Since the discovery of sulfanilamide (Fig. 1, structure **Sf**), the approaches to design newer sulfonamide ($–SO_2NH–$) derivatives have attracted continued interest of organic and medicinal chemists over the years (Silverman, 1992), and still utilize in active research (Smith and Jones, 2008; Wilden, 2010).

Besides the physiological importance, the proteinogenic α-amino acids are of enormous interest in synthetic chemistry as a versatile precursor because they display multi-functional character to facilitate various synthetic transformations to generate the heterocyclic skeletons (Calaza and Cativiela, 2010; Sardina and Rapoport, 1996). In particular, approaches to construct C-2 or N-3 or (C-2, N-3)-substituted 4(3*H*)-quinazolinone ring-skeleton by incorporation of C– or N– or both C,N-terminal of α-amino acid residues at 2nd or 3rd position, respectively is a subject of recent interest (Xu and Fu, 2011; Meyyanathan *et al*., 2010; Hirota *et al*., 2010; Zhichkin *et al*., 2007; Domínguez and Leónb, 2006; Liu, 2007).

In continuation of our pursuits toward the synthesis of newer sulfonamide linked heterocycles of biological interest (Vanparia *et al*., 2010, Dixit *et al*., 2010), we have recently reported (Jagani *et al*., 2011) various 2-(alkyl-/aryl-/styryl-)-3-(4-aminobenzenesulfonamido)-substituted-4(3*H*)-quinazolinone derivatives (Fig. 1, structure **QS**).

Moreover, a thorough literature review reveals that more efficacious antimicrobial agents can be designed by joining two or more bioactive moieties together in a single molecular framework (Xie and Seto, 2007). Hence, against the aforementioned background and as a part of general research program for the search of newer antimicrobial agents, the main objective of the present study is to unite two biodynamic agents of different class in a single molecular framework (Fig. 1, structure **1**–**19**), which might encompass encouraging pharmacological potential. That is to combine drugs of parent moieties, viz., sulfanilamide and Methaqualone into a single molecular structure, or to substitute active sulfonamide at position-2 of 4(3*H*)-quinazolinone moiety. Another objective of this study is to synthesize resultant quinazolinone–sulfonamide hybrid entities from glycine, and to derivatize further with different aryl substitutions at position-3. Besides this, it is of considerable interest to investigate the influence of structural variations on anticipated bio-activity (and not to study the mechanism of action of entitled molecules). This structural hybridization was on the basis of two other hypotheses which can lead to find a new class of potential antimicrobial agents: (i) for the 4(3*H*)-quinazolinones, the presence of cyclic ring substituent at position-3 and substituent groups like methyl or phenyl at position-2 is necessary requirement for medicinal properties (Panneerselvam *et al*., 2009), and the presence of pharmacophoric groups ($–NO_2$, –Br, –OH, –Cl, etc.) on the ring along with other substituents when placed at C-2 are reported to possess potential

antimicrobial activities (Rao *et al*., 1985; El-Sharief *et al*., 2001); while (ii) for the sulfonamides, the presence of N-heterocyclic ring as substituents at N-1 may lead to many times potent analogues than the parent sulfanilamide (Anand, 1996; Reynolds, 1996).

The privileged substructure concept suggests that while chemical diversity is almost infinite, biological activity in that space is clustered around substructure elements. Medicinal chemists have spent a great deal of effort on clustering privileged substructures and on the development of synthetic methods to generate organic equivalents (Horton *et al*., 2003). Hence, the exploration of aforesaid skeleton as "privileged" new chemical entities is of the paramount importance in current research to serve encouraging pharmacological potential. Therefore, we describe herein a milder synthetic approach for the rapid access to a series of hitherto unknown and newly designed 4-(amino or acetamido)-*N*-{[3-(substituted)-4-oxo-3,4-dihydroquinazolin-2-yl]methyl}benzenesulfonamide hybrid entities (**1**–**19**) starting from glycine, and the preliminary results on their in vitro evaluation for antimicrobial activities.

Results and discussion

Chemistry

Synthesis of compounds

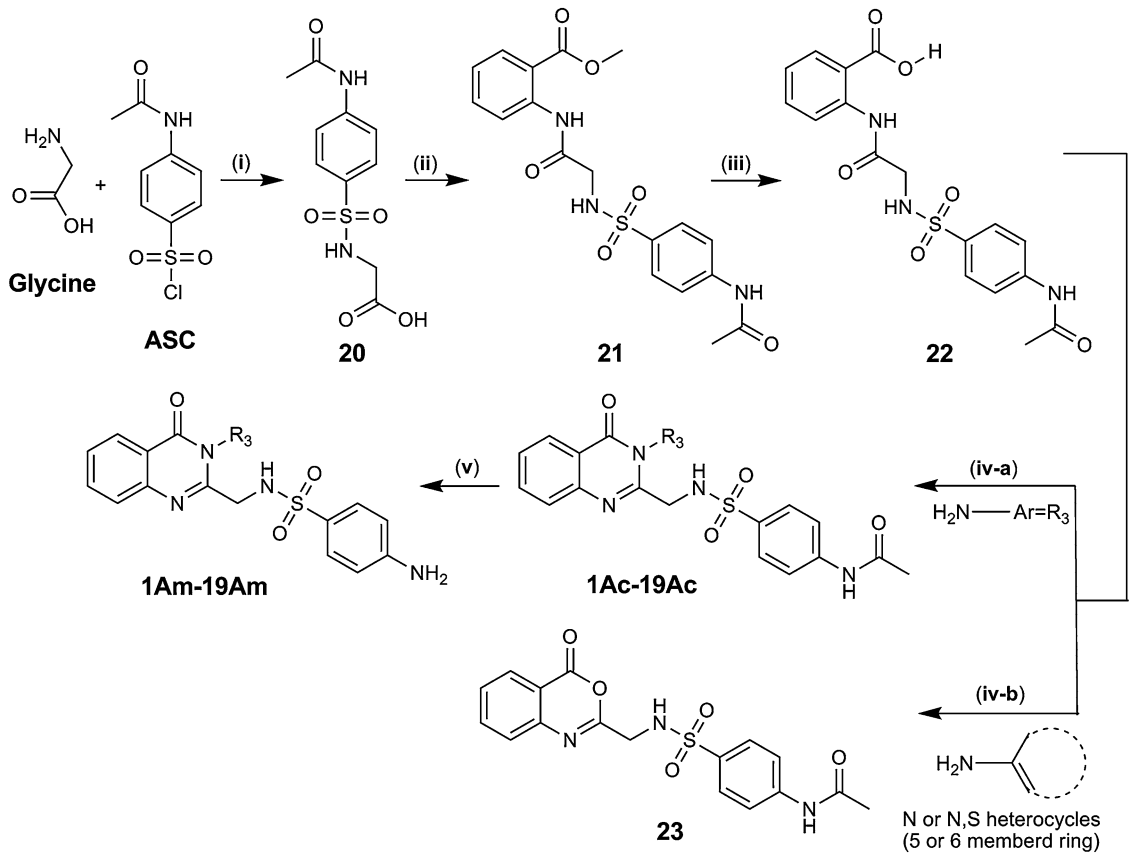
The efforts have been through to undertake synthesis of a novel series of some quinazolinone–sulfonamide hybrid entities as illustrated in synthetic pathways (Schemes 1, 2), which could assemble entirely the desired 2,3-disubstituted-4(3*H*)-quinazolinones (**1**–**19**) from glycine residue without any relevant protection–deprotection strategies and could tolerate substitution pattern at position-2 (C2) or position-3 (N3), respectively as well. Hence, to develop a more widely applicable approach for the synthesis of 4-quinazolinones (Witt and Bergman, 2003; Reddy *et al*., 2003; Connolly *et al*., 2005; Patil *et al*., 2011), we chose to evaluate two synthetic strategies based on the intermediate commonly opted in the reaction for the crucial ring-closure steps, particularly, via hetero-cyclization of N-acylanthranilic acid with primary amines, and via cyclo-condensation of 3,1-benzoxazin-4-one with nitrogen nucleophiles followed by subsequent dehydrative-cyclization.

For which, preferred starting material, 4-acetamidobenzenesulfonyl glycine (**20**) (Schröder *et al*., 2001) was prepared in improved yield (79 %) by a method involving the use of K_2CO_3 for reaction of *p*-acetamidobenzenesulfonyl chloride (**ASC**) with glycine in aqueous-media (Scheme 1, step-I). In the next step, reaction of **20** with methyl anthranilate afforded methyl *N*-acylanthranilate (**21**) in quantitative yield using the phosphazo method in the presence of PCl_3 as a condensing agent (Grimmel *et al*., 1946a; Itoh *et al*., 1998) in THF or Py (Scheme 1, step-II). The hydroxide-mediated ester cleavage of later compound (**21**) in the presence of milder base $Ba(OH)_2{\cdot}8H_2O$ in methanol without using any co-solvent (Anderson *et al*., 2004) afforded N-acylanthranilic acid (**22**) in higher yield (~94 %) (Scheme 1, step-III).

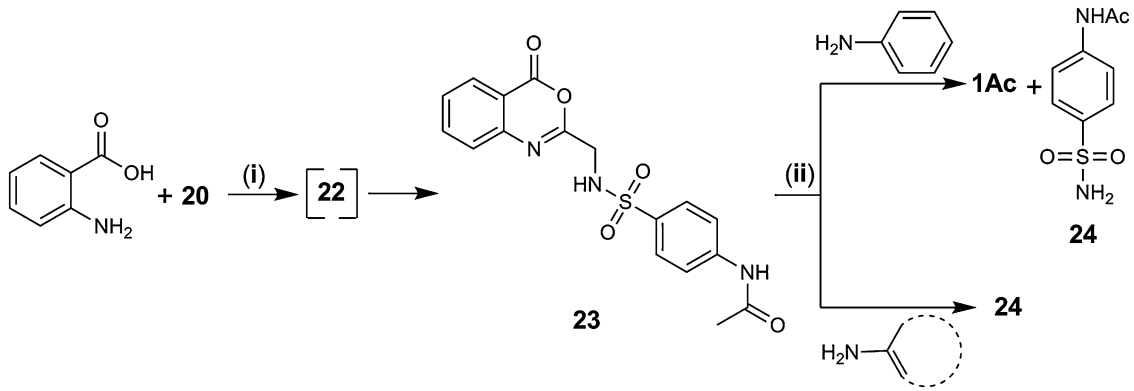
The first ring-closure approach applied in the synthesis of desired 4-quinazolinones commence with the cyclo-dehydration method for the reaction of N-acylanthranilic acids with primary amines, which was established by Grimmel *et al*. (1946b), modified by Xue *et al*. (2004) and chased recently by other investigators (Storelli *et al*., 2005; Giri *et al*., 2009), but this method still suffer with longer reaction time. Hence, this method was reinvestigated by considering the solvent and temperature effects on the efficiency of dehydrative-cyclization with the hope to identify an ideal combination that could improve reaction duration and contribute eventually the higher conversion to desired 2-[*N*-(4-acetamidobenzenesulfonamido)methyl]yl-3-(substituted)-4(3*H*)-quinazolinone derivatives (**1Ac**–**19Ac**) as shown in Scheme 1, step-IVa. Under the optimized conditions, PCl_3 (1 equiv.) in THF at 50–70 °C, reaction of **22** with aniline gave corresponding **1Ac** in excellent yield within short time. Effectively, a series of desired 4(3*H*)-quinazolinones (**1Ac**–**19Ac**) were synthesized in high yield (Table 1) in the presence of PCl_3using a simple, rapid, and milder experimental protocol via hetero-cyclization of N-acylanthranilic acid (**22**) with various aryl-amines such as, aniline and its *ortho*- or *meta*- or *para*- monosubstituted derivatives viz., methyl-, methoxy-, nitro-, chloro-, bromo-, fluoro-, and phenylhydrazine, respectively.

Further, under the optimized conditions, a range of heterocyclic amines, such as (2- or 3- or 4-)-aminopyridines, 2-aminothiazoles, 2-aminothiazoles, 2-aminobenzothiazoles, 2-aminotriazoles, 2-aminotetrazole, etc. were also examined (Scheme 1, step-IVb). However, in each case, reaction rendered with complete conversion of *N*-acylanthranilic acid (**22**) to led unexpected formation of corresponding benzoxazin-4-one (**23**) within stipulated time required for compounds **1Ac**–**19Ac**.

An alternative ring-closure approach effort for the synthesis of desired 4-quinazolinones involves cyclocondensation of 2-substituted-3,1-benzoxazin-4-one with nitrogen nucleophiles and subsequent dehydrative cyclization (Rabilloud and Sillion, 1980; Larksarp and Alper, 2000; Madkour, 2004; Jagani *et al*., 2011; 2012a, b), which merely requires harsh reaction conditions (Salehi *et al*., 2005; Kalusa *et al*., 2008; Liu *et al*., 2005). In initial attempts, aniline was reacted with **23** (Scheme 2, method A or B, Step-IIa). However, reaction proceeded sluggishly in our hands to



Scheme 1 Synthetic protocol via hetero–cyclization of N–acylanthranilic acid to 4(3*H*)–quinazolinone-sulfonamide hybrid entities **1**–**19** (**Ac**, **Am**). Reagents and conditions: **i** K_2CO_3, H_2O, Δ; **ii** methyl anthranilate, PCl_3, THF or Py, Δ; **iii** $Ba(OH)_2{\cdot}8H_2O$, methanol, rt or reflux; **iv-a** substituted aromatic-amines, PCl_3, THF, Δ; **iv-b** *N*– or *N,S*–amino–heterocycles (un–fused/benzo–fused 5– or 6–membered ring), PCl_3, or Py, Δ; **v** HCl, R–spirit–H_2O, Δ



Scheme 2 Alternative synthetic approach via cyclo–condensation of 3,1–benzoxazin–4–one to 4(3*H*)–quinazolinone. Reagents and conditions: Method A: **i** Triphenyl phosphite–$P(OPh)_3$, Py, Δ; **iia** aromatic-amines or **iib** heterocyclic amines; Δ. Method B: **i** *N*– or *N,S*–amino–heterocycles (un– fused/benzo–fused 5– or 6–membered ring), PCl_3, or Py, Δ; **iia** aromatic-amines or (**iib**) heterocyclic amines; DMF/Py/AcOH/Neat, Δ

afford corresponding 4-quinazolinone (**1Ac**), which was isolated in low yield (Method A, Step-IIa, 19 %; Method B, Step-IIa, 14 %) by column chromatography from the resulting complex reaction mixture along with unreacted **23** and unexpected major side-product, 4-acetamidobenzene-sulfonamide (**24**, ∼34 %). Despite this, condensation of **23** with other aromatic primary amines was experimented, but all efforts resulted with similar low isolated yields (∼15 %). Nevertheless, the formation of corresponding 4-quinazolinones was not detected in the case of aforesaid heterocyclic

Table 1 Physico-chemical characteristics of compounds (**1–23**)

Sr. No.	Group (R_3)	Molecular formula	Formula weight	Elemental analysis (%), calcd. (found): C; H; N	Yield (%)[a]	M.p. (°C)[b–e]
1Ac	$-C_6H_5$	$C_{23}H_{20}N_4O_4S$	448.49	61.59 (61.61); 4.50 (4.49); 12.49 (12.56)	97	168–174
2Ac	$-C_6H_4$–(2-Me)	$C_{24}H_{22}N_4O_4S$	462.52	62.32 (62.42); 4.79 (4.78); 12.11 (12.15)	91	185–188
3Ac	$-C_6H_4$–(3-Me)	$C_{24}H_{22}N_4O_4S$	462.52	62.32 (62.49); 4.79 (4.81); 12.11 (12.18)	94	209–212
4Ac	$-C_6H_4$–(4-Me)	$C_{24}H_{22}N_4O_4S$	462.52	62.32 (62.47); 4.79 (4.80); 12.11 (12.17)	96	197–200
5Ac	$-C_6H_4$–(2-OMe)	$C_{24}H_{22}N_4O_5S$	478.52	60.24 (60.44); 4.63 (4.64); 11.71 (11.74)	90	212–216
6Ac	$-C_6H_4$–(3-OMe)	$C_{24}H_{22}N_4O_5S$	478.52	60.24 (60.41); 4.63 (4.62); 11.71 (11.73)	92	156–159
7Ac	$-C_6H_4$–(4-OMe)	$C_{24}H_{22}N_4O_5S$	478.52	60.24 (60.39); 4.63 (4.64); 11.71 (11.75)	95	158–164
8Ac	$-C_6H_4$–(2-NO_2)	$C_{23}H_{19}N_5O_6S$	493.49	55.98 (56.19); 3.88 (3.89); 14.19 (14.22)	84	227–230
9Ac	$-C_6H_4$–(3-NO_2)	$C_{23}H_{19}N_5O_6S$	493.49	55.98 (56.11); 3.88 (3.87); 14.19 (14.23)	85	165–169
10Ac	$-C_6H_4$–(4-NO_2)	$C_{23}H_{19}N_5O_6S$	493.49	55.98 (56.15); 3.88 (3.89); 14.19 (14.21)	88	176–180
11Ac	$-C_6H_4$–(2-Cl)	$C_{23}H_{19}ClN_4O_4S$	482.94	57.20 (57.40); 3.97 (3.98); 11.60 (11.63)	90	191–194
12Ac	$-C_6H_4$–(3-Cl)	$C_{23}H_{19}ClN_4O_4S$	482.94	57.20 (57.36); 3.97 (3.99); 11.60 (11.64)	92	201–204
13Ac	$-C_6H_4$–(4-Cl)	$C_{23}H_{19}ClN_4O_4S$	482.94	57.20 (57.39); 3.97 (3.98); 11.60 (11.65)	93	163–168
14Ac	$-C_6H_4$–(2-Br)	$C_{23}H_{19}BrN_4O_4S$	527.39	52.38 (52.48); 3.63 (3.64); 10.62 (10.65)	92	180–188
15Ac	$-C_6H_4$–(3-Br)	$C_{23}H_{19}BrN_4O_4S$	527.39	52.38 (52.51); 3.63 (3.62); 10.62 (10.61)	93	169–174
16Ac	$-C_6H_4$–(4-Br)	$C_{23}H_{19}BrN_4O_4S$	527.39	52.38 (52.46); 3.63 (3.66); 10.62 (10.60)	95	164–168
17Ac	$-C_6H_4$–(2-F)	$C_{23}H_{19}FN_4O_4S$	466.48	59.22 (59.41); 4.11 (4.10); 12.01 (12.05)	93	154–158
18Ac	$-C_6H_4$–(4-F)	$C_{23}H_{19}FN_4O_4S$	466.48	59.22 (59.39); 4.11 (4.09); 12.01 (12.03)	95	199–206
19Ac	–NH–C_6H_5	$C_{23}H_{21}N_5O_4S$	463.51	59.60 (59.46); 4.57 (4.58); 15.11 (15.13)	87	252–257
1Am	$-C_6H_5$	$C_{21}H_{18}N_4O_3S$	406.46	62.05 (62.29); 4.46 (4.47); 13.78 (13.82)	81	216–217
2Am	$-C_6H_4$–(2-Me)	$C_{22}H_{20}N_4O_3S$	420.48	62.84 (63.04); 4.79 (4.81); 13.32 (13.36)	84	184–190
3Am	$-C_6H_4$–(3-Me)	$C_{22}H_{20}N_4O_3S$	420.48	62.84 (63.09); 4.79 (4.80); 13.32 (13.37)	86	178–184
4Am	$-C_6H_4$–(4-Me)	$C_{22}H_{20}N_4O_3S$	420.48	62.84 (63.04); 4.79 (4.81); 13.32 (13.35)	79	171–174
5Am	$-C_6H_4$–(2-OMe)	$C_{22}H_{20}N_4O_4S$	436.48	60.54 (60.75); 4.62 (4.63); 12.84 (12.86)	76	172–174
6Am	$-C_6H_4$–(3-OMe)	$C_{22}H_{20}N_4O_4S$	436.48	60.54 (60.77); 4.62 (4.60); 12.84 (12.78)	72	174–176
7Am	$-C_6H_4$–(4-OMe)	$C_{22}H_{20}N_4O_4S$	436.48	60.54 (60.69); 4.62 (4.62); 12.84 (12.88)	75	176–178
8Am	$-C_6H_4$–(2-NO_2)	$C_{21}H_{17}N_5O_5S$	451.46	55.87 (55.99); 3.80 (3.81); 15.51 (15.55)	85	201–212
9Am	$-C_6H_4$–(3-NO_2)	$C_{21}H_{17}N_5O_5S$	451.46	55.87 (55.97); 3.80 (3.84); 15.51 (15.53)	78	208–212
10Am	$-C_6H_4$–(4-NO_2)	$C_{21}H_{17}N_5O_5S$	451.46	55.87 (55.98); 3.80 (3.83); 15.51 (15.56)	88	202–204
11Am	$-C_6H_4$–(2-Cl)	$C_{21}H_{17}ClN_4O_3S$	440.90	57.21 (57.41); 3.89 (3.93); 12.71 (12.74)	84	222–228
12Am	$-C_6H_4$–(3-Cl)	$C_{21}H_{17}ClN_4O_3S$	440.90	57.21 (57.39); 3.89 (3.90); 12.71 (12.75)	80	194–202
13Am	$-C_6H_4$–(4-Cl)	$C_{21}H_{17}ClN_4O_3S$	440.90	57.21 (57.36); 3.89 (3.92); 12.71 (12.73)	89	210–218
14Am	$-C_6H_4$–(2-Br)	$C_{21}H_{17}BrN_4O_3S$	485.35	51.97 (52.19); 3.53 (3.54); 11.54 (11.57)	83	218–226
15Am	$-C_6H_4$–(3-Br)	$C_{21}H_{17}BrN_4O_3S$	485.35	51.97 (52.25); 3.53 (3.57); 11.54 (11.55)	78	199–208
16Am	$-C_6H_4$–(4-Br)	$C_{21}H_{17}BrN_4O_3S$	485.35	51.97 (52.21); 3.53 (3.55); 11.54 (11.58)	81	178–181
17Am	$-C_6H_4$–(2-F)	$C_{21}H_{17}FN_4O_3S$	424.45	59.42 (59.65); 4.04 (4.05); 13.20 (13.25)	79	208–217
18Am	$-C_6H_4$–(4-F)	$C_{21}H_{17}FN_4O_3S$	424.45	59.42 (59.60); 4.04 (4.07); 13.20 (13.22)	85	176–184
19Am	–NH–C_6H_5	$C_{21}H_{19}N_5O_3S$	421.47	59.84 (59.67); 4.54 (4.55); 16.62 (16.65)	77	231–234
20	–	$C_{10}H_{12}N_2O_5S$	272.28	44.11 (44.35); 4.44 (4.45); 10.29 (10.27)	79	230–232
21	–	$C_{18}H_{19}N_3O_6S$	405.42	53.32 (53.52); 4.72 (4.74); 10.36 (10.39)	87	203–205
22	–	$C_{17}H_{17}N_3O_6S$	391.40	52.17 (52.45); 4.38 (4.39); 10.74 (10.77)	~94	253–254
23	–	$C_{17}H_{15}N_3O_5S$	373.38	54.68 (54.77); 4.05 (4.06); 11.25 (11.28)	>84	224–233

[a] Isolated yields of compounds synthesized via Scheme 1

[b–e] Lit. mp [°C]: **2Ac** [155], **8Ac** [175], **10Ac** [160] (Rao *et al*., 1985); **20** [232] (Schröder *et al*., 2001)

primary amines (Scheme 2, Method A or B, Step-IIb). Hence, the reaction conditions could not been optimized due to the formation of decomposition product (as shown in Scheme 2). Hence, there is need to efforts further to establish easy and efficient reaction procedure to improve reaction rate and yields as well.

Once the 4-quinazolinones (**1Ac–19Ac**) were synthesized, their hydrolysis to corresponding 4-amino-*N*-[(4-oxo-3-substituted aryl-3,4-dihydroquinazolin-2-yl)methyl] benzenesulfonamide derivatives (**1Am–19Am**) was not a difficult task and were produced in quantitative yield by the treatment of appropriate **1Ac–19Ac** with dilute HCl (40 % solution) in R-spirit and H_2O mixture (Jagani *et al*., 2011).

Analytical and spectroscopic investigations

The structural assignments to synthesized compounds were based on their physico-chemical characteristics and spectroscopic (FT-IR, ^{1}H-NMR, APT ^{13}C-NMR, and mass) investigations. All the compounds were synthesized in excellent yield and purity, under milder and rapid reaction conditions without any tedious workup procedure. The purity of products was checked by TLC, using $CHCl_3$: MeOH (95:5; for acids, 70:30) as an eluent to presumably provide R_f values ~0.5 of the respective products, and by elemental analysis. The results of elemental analyses on predicted molecular formula for **1–23** were in good agreement (±0.4 %) with their theoretical values.

The ESI–MS (positive mode) mass spectra of compounds (**1–23**) showed molecular ion peak $[M+H]^+$ corresponding to either exact mass or molecular weight of compound. The ESI–MS spectrum of **1Ac** exhibited a parent peak at *m/z* 449 $[M+H]^+$, which confirmed its proposed formula. While ESI–MS spectra of **11–16** (halogenated derivatives, except fluorine) exhibited molecular ion peak in characteristic two peak pattern, $[M+H]^+$and $[(M+H)+2]^+$ in almost 1:1 or 3:1 ratio due to the isotopic abundance nature of Br or Cl atoms, respectively, which supported the proposed formula of respective compounds. Compounds, **13Ac** (Cl-derivative) and **16Ac** (Br-derivative) exhibited ionization peaks ($[M+H]^+$ and $[(M+H)+2]^+$) at *m/z* (483, 485) and at *m/z* (527, 529), respectively. Further, ESI–MS spectra of compounds (**20** and **22**) appeared with $[M+Na]^+$ peak along with $[M+H]^+$ peak.

In FT-IR spectra of all the compounds (**1–23**), the presence of $–SO_2NH–$ group was evidenced by two strong bands (at 1,318–1,340 and 1,144–1,165 cm^{-1}) due to O=S=O stretching vibrations and absorption band (at ~3,250 cm^{-1}) assigned to $–SO_2N–H$ stretching as well. FT-IR spectra of **20–23** showed C=O stretching absorption bands at 1721, 1738, 1742, and 1750 cm^{-1} due to the presence of aliphatic –COOH, aromatic –COOH, –COOMe groups, and O–C=O of semi-anhydride type lactone ring, respectively. The disappearance of band associated with acid group of **22** confirmed the formation of 4-quinazolinones (**1Ac–19Ac**). Further, in FT-IR spectra of quinazolin-4-ones (**1–19**), strong intensity bands appeared at 1690–1670, 1610–1650, and 1495 cm^{-1} attributed to C=O, C=N, and C=C stretching vibrations respectively, provided a strong evidence for parent heterocyclic ring-skeleton.

In the ^{1}H-NMR spectra of **1–23**, protons of sulfonamide group ($–SO_2N\mathbf{H}–CH_2–$) was resonated at δ 7.9–8.42 ppm as a triplet (J = 4.8–6.0), except **1Am–19Am**, where it was observed at δ 7.36–7.60 ppm. The signal of methylene group protons ($–C\mathbf{H_2}–NH–$) was appeared in aliphatic region (at δ 3.3–4.2 ppm) as a doublet (J = 4.8–6.0). However, some derivatives (viz., **2**, **5**, **8**, **11**, **14**, **17**, **19**) have exhibited two-bond geminal coupling (H–C–H) of protons, and as a result two separate signals (dd; ~J = 16.0–18.0, 4.8–6.4) were appeared with considerable difference of frequency (δ ~0.2–0.3 ppm). The protons of (hetero)aromatic rings were resonated at expected downfield region (δ 6.3–8.4 ppm). The formation of compounds **1Am–19Am** were confirmed by the appearance of signal for protons of 4-NH_2 group (at δ 6.1–6.3 ppm), and by the disappearance of two signals (singlet at δ 2.3 ppm due to –N**H**CO, singlet at δ ~10.1 ppm due to $–COC\mathbf{H}_3$) for 4-NHAc group of **1Ac–19Ac** as well. It was noticed further for four protons of benzenesulfonyl ring that two separate doublets (J = 8.8/8.4) appeared with integral of two protons to each peak, when 4-NH_2 group (in **1Am–19Am**) was present. However, these four protons appeared as a broad multiplet (at δ ~7.6–7.8 ppm), when 4-NHAc group (in **1Ac–19Ac**) was present, which might have influenced the plane of symmetry.

The APT ^{13}C-NMR experiment was used to confirm interpretation of carbon resonances in characteristic patterns of positive peak and/or inverse peak, i.e., signals of primary–ternary (CH_3, CH) and secondary–quaternary (CH_2, C) carbons observed on the opposite side to base line. ^{13}C-NMR spectra of **1–19** were characterized by, two signals of quaternary carbons at low frequencies (δ 150–152 and 160–162 ppm) associated with cyclic –N–C=N carbon conjugated with C=O group respectively of cyclic amide or lactam ring of quinazolinone and, other signal for $\mathbf{C}H_2$ secondary carbon of glycine residue was observed at little higher frequency (δ 42 ppm) due to attachment with neighboring sulfonamide group. Further, formation of **1Am–19Am** was supported by the disappearance of signals observed at δ ~168 (C=O) and δ ~24 (CH_3) ppm due to the presence of acetamido group in **1Ac–19Ac**. All other carbons (CH, C) of aryl ring as well as quinazolin–4–one ring were resonated in the expected region (δ 120–150 ppm).

Biological activity

In vitro antimicrobial evaluations

All the synthesized compounds (**1–19**) were screened for their in vitro antibacterial and antifungal activities against

various bacterial and fungal strains to determine minimum inhibitory concentrations (MICs) by microdilution method (Murray *et al*., 1995). Methaqualone (**Qm**) (Wasfy, 2002) and sulfanilamide (**Sf**) (Vanparia *et al*., 2010) was used as reference drugs of parent moieties.

Antibacterial activity of **1–19** was performed against representative panel of Gram-positive bacteria (viz., *Staphylococcus aureus*, MTCC 2940; *Bacillus Cereus*, MTCC 1305) and Gram-negative bacteria (viz., *Escherichia coli*, MTCC 443; *Pseudomonas aeruginosa*, MTCC 1688; *Klebsiella pneumonia*, MTCC 139), where Ampicillin (**Ap**) was selected as a standard drug.

Antifungal activity of **1–19** was carried out against two fungus (viz., *Aspergilus Niger*, MTCC 282; *Candida albicans*, MTCC 227) using Ketoconazole (**Kz**) as standard drug.

The test bacterial and fungal cultures were maintained on Mueller–Hinton broth and Sabouraud–dextrose broth at 37 °C and 25 °C, respectively. Inoculums size of bacterial and fungal species were adjusted to 1.5×10^8 and 1.5×10^5 CFU mL^{-1} (colony forming unit per mL), respectively compared to 0.5 McFarland turbidity standard. DMSO was used as diluents to obtain desired concentration of compounds and drugs. The bacterial and fungal suspensions were added to MHB and SDB, respectively supplemented with varying concentrations of the test compounds, incubated at 37 °C (24 h) and 25 °C (48 h), respectively. The growth of test cultures was visualized using triphenyltetrazolium chloride (TTC, 0.5 % w/w) aqueous solution. MIC was defined as the lowest concentration of screened compounds that inhibited visible growth (red colored pellet on the bottom of the wells after the addition of TTC). The observed data on the in vitro antimicrobial evaluations of synthesized compounds (**1–19**) along with reference drugs are shown as MICs values (μg mL^{-1}) in Table 2.

The literature survey also revealed that most of the biologically active 4-(3*H*)-quinazolinones are either (C-2 or N-3)-monosubstituted or (C-2, N-3)-disubstituted derivatives. The presence of cyclic ring substituent at position-3 as well as substituent groups like methyl, phenyl, etc., at position-2 is necessary requirement for its medicinal properties (Panneerselvam *et al*., 2009). Furthermore, the presence of pharmacophoric groups (–NO_2, –Br, –OH, –Cl, etc.) on the ring along with other substituents at C-2 are reported to possess potential antimicrobial activities (Rao *et al*., 1985; El-Sharief *et al*., 2001). In addition, structural diversity for the sulfonamides can be imparted by the two equally important derivatization approaches, either, via 4-benzenenesulfonamido group (N^1-atom), i.e., variations of R_1-group ($R_1 \neq H$) has widely been carried out to obtain several times potent compounds (when R_1 = N-heterocycles) than the original Sulfanilamide, or via 4-anilino group (N^4-atom) i.e., variations of R_4-group ($R_4 \neq H$) has often executed to develop pro-drug for sulfonamide agents (Anand, 1996; Reynolds, 1996). This conception led us to synthesis a series of 2-{(4-substituted)benzenesulfonamide)}-3-{(un)substituted phenyl or unsubstituted aminophenyl}-4(3*H*)-quinazolinone hybrid entities (Fig. 1. **1–19**) by altering the various substituents (R_3) at position-3 (N3) as well as functionalities (R'_4) at position-4 of benzenesulfonamide ring bridged at position-2 (C2) of 4(3H)-quinazolinone ring, in order to investigate the pharmacophoric substituent effects, responsible for better activity.

The observation of results as summarized in Table 2 on the preliminary antimicrobial activity of synthesized compounds **1Ac–19Ac** and **1Am–19Am** revealed that all the screened compounds found to possess varied degree of antibacterial and antifungal activities as evidenced from their MIC, μg mL^{-1} values. Among the screened compounds, most of the compounds have shown more or equal antimicrobial activities compare to the reference drugs of parent moieties, Methaqualone (**Qm**) and Sulfanilamide (**Sf**), while a very fewer among the screened compounds found to be equipotent to the standard drugs, Ampicillin (**Am**) and Ketoconazole (**Kz**).

From in vitro antibacterial study, it was observed that compounds **13Am** (R_3 = –Ph–4-Cl; R'_4 = –4-NH_2), **15Am** (R_3 = –Ph–3-Br; R'_4 = –4-NH_2) and **12Am** (R_3 = –Ph–3-Cl; R'_4 = –4-NH_2), as well as compounds **10Am** (R_3 = –Ph–4-NO_2; R'_4 = –4-NH_2), **16Am** (R_3 = –Ph–4-Br; R'_4 = –4-NH_2), **18Am** (R_3 = –Ph–4-F; R'_4 = –4-NH_2), **7Ac** (R_3 = –Ph–4-OMe; R'_4 = –4-$NHCOCH_3$) have exhibited good to moderate activity against Gram-positive bacterial strains (*S. aureus* > *B. cereus*) respectively. While compounds **13Am** (R_3 = –Ph–4-Cl; R'_4 = –4-NH_2), **16Am** (R_3 = –Ph–4-Br; R'_4 = –4-NH_2), and **7Ac** (R_3 = –Ph–4-OMe; R'_4 = –4-$NHCOCH_3$), as well as compounds **7Am** (R_3 = –Ph–4-OMe; R'_4 = –4-NH_2), **15Am** (R_3 = –Ph–3-Br; R'_4 = –4-NH_2), **10Am** (R_3 = –Ph–4-NO_2; R'_4 = –4-NH_2), and **18Am** (R_3 = –Ph–4-F; R'_4 = –4-NH_2) displayed comparatively better to acquiescent activity against the corresponding Gram-negative bacterial strains (*E. coli* > *P. aeruginosa* > *K. pneumonia*). In general, compounds showed more selectivity against Gram-negative over Gram-positive species among all the bacterial strains.

From the results of antifungal activity data, it was found that compounds **7Ac** (R_3 = –Ph–4-OMe; R'_4 = –4-$NHCOCH_3$), **13Am** (R_3 = –Ph–4-Cl; R'_4 = –4-NH_2) and **15Am** (R_3 = –Ph–3-Br; R'_4 = –4-NH_2), as well as compounds **7Am** (R_3 = –Ph–4-OMe; R'_4 = –4-NH_2), **10Am** (R_3 = –Ph–4-NO_2; R'_4 = –4-NH_2), **12Am** (R_3 = –Ph–3-Cl; R'_4 = –4-NH_2), **16Am** (R_3 = –Ph–4-Br; R'_4 = –4-NH_2) and **18Am** (R_3 = –Ph–4-F; R'_4 = –4-NH_2) demonstrated high to fair activity against both the fungal species. In general, compounds were enormously active against the fungi species and particularly effective against *A. niger* than *C. albicans*.

Table 2 In vitro antimicrobial evaluation of compounds (**1–19**) and reference drugs

Minimum inhibitory concentration (MIC, μg mL^{-1}) values against various strains[a]									
Sr. no.	Compd. code[b]	Group at N-3 (R_3)	Gram-positive bacteria		Gram-negative bacteria			Fungi	
			S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumonea*	*A. niger*	*C. albicans*
1.	**1Am**	$-C_6H_5$	85	95	80	85	95	90	100
2.	**1Ac**	$-C_6H_5$	95	115	110	125	130	95	105
3.	**2Am**	$-C_6H_4$–(2-Me)	90	105	70	80	100	90	100
4.	**3Am**	$-C_6H_4$–(3-Me)	85	100	65	85	115	85	95
5.	**4Am**	$-C_6H_4$–(4-Me)	80	95	65	70	90	75	80
6.	**2Ac**	$-C_6H_4$–(2-Me)	100	115	90	120	125	80	90
7.	**3Ac**	$-C_6H_4$–(3-Me)	95	105	80	115	120	75	80
8.	**4Ac**	$-C_6H_4$–(4-Me)	95	105	85	105	115	70	85
9.	**5Am**	$-C_6H_4$–(2-OMe)	85	80	65	80	95	55	65
10.	**6Am**	$-C_6H_4$–(3-OMe)	80	75	60	85	90	50	50
11.	**7Am**	$-C_6H_4$–(4-OMe)	60	70	50	75	75	45	55
12.	**5Ac**	$-C_6H_4$–(2-OMe)	90	95	80	110	105	70	80
13.	**6Ac**	$-C_6H_4$–(3-OMe)	85	90	75	100	110	65	70
14.	**7Ac**	$-C_6H_4$–(4-OMe)	55	65	50	90	100	40	50
15.	**8Am**	$-C_6H_4$–(2-NO_2)	75	85	70	75	95	55	65
16.	**9Am**	$-C_6H_4$–(3-NO_2)	70	85	60	65	90	50	60
17.	**10Am**	$-C_6H_4$–(4-NO_2)	65	75	55	60	75	45	55
18.	**8Ac**	$-C_6H_4$–(2-NO_2)	85	90	75	100	115	65	80
19.	**9Ac**	$-C_6H_4$–(3-NO_2)	80	80	70	95	110	60	75
20.	**10Ac**	$-C_6H_4$–(4-NO_2)	80	85	70	90	105	55	70
21.	**11Am**	$-C_6H_4$–(2-Cl)	60	90	55	60	90	50	60
22.	**12Am**	$-C_6H_4$–(3-Cl)	55	65	50	55	80	50	55
23.	**13Am**	$-C_6H_4$–(4-Cl)	45	55	45	50	75	40	45
24.	**11Ac**	$-C_6H_4$–(2-Cl)	70	100	70	75	100	60	75
25.	**12Ac**	$-C_6H_4$–(3-Cl)	65	95	60	70	105	55	70
26.	**13Ac**	$-C_6H_4$–(4-Cl)	60	90	60	65	85	55	65
27.	**14Am**	$-C_6H_4$–(2-Br)	55	70	55	65	85	45	55
28.	**15Am**	$-C_6H_4$–(3-Br)	50	60	50	55	75	40	45
29.	**16Am**	$-C_6H_4$–(4-Br)	55	65	50	50	75	45	55
30.	**14Ac**	$-C_6H_4$–(2-Br)	70	95	65	75	95	60	70
31.	**15Ac**	$-C_6H_4$–(3-Br)	65	90	70	70	90	55	70
32.	**16Ac**	$-C_6H_4$–(4-Br)	60	95	65	65	90	50	60
33.	**17Am**	$-C_6H_4$–(2-F)	65	90	65	70	95	55	65
34.	**18Am**	$-C_6H_4$–(4-F)	60	55	55	65	85	45	60
35.	**17Ac**	$-C_6H_4$–(2-F)	85	105	80	95	95	70	85
36.	**18Ac**	$-C_6H_4$–(4-F)	80	95	75	85	95	55	80
37.	**19Am**	$-NH-C_6H_5$	100	105	90	95	115	105	115
38.	**19Ac**	$-NH-C_6H_5$	100	130	115	125	145	105	120
39.	**Qm**[c]	–	125	140	75	100	115	90	125
40.	**Sf**[c]	–	50	55	55	65	80	50	60
41.	**Ap**[d]	–	45	60	50	55	85	NT	NT
42.	**Kz**[d]	–	NT	NT	NT	NT	NT	40	50

[a] Mean values of triplicate results

[b] ***Am*** amino, ***Ac*** acetamido group substituent, [at position–4 (R'_4) on benzenesulfonamide ring (Fig. 1, **1–19**)]

[c] ***Sf*** sulfanilamide, ***Qm*** Methaqualone (reference drugs of parent moiety)

[d] ***Ap*** Ampicillin, ***Kz*** ketoconazole (standard drugs), *NT* not tested

Subsequently, reviewing these results on antimicrobial evaluations (Table 2), some initial conclusions on the co-relation of activity have been drawn with the sizable number of compounds, **1Ac–19Ac** and **1Am–19Am**.

At position-4 of benzenesulfonamide ring (bridged to quinazolin-4-one ring at position-2 via methylene linkage), the presence of amino group in the compounds **1Am–19Am** exhibited an enhancement in the antibacterial and antifungal activity than their acetylated derivatives **1Ac–19Ac**, respectively. Exceptionally, compound **7Ac** (R'_4 = –NHC-OCH_3; R_3 = 4-OMe–C_6H_4) showed better activity than corresponding compound **7Am**. Therefore, the presence of amino substituent was more favored than acetamido substituent for better antibacterial and antifungal activity.

At position-3 of the 4(3*H*)-quinazolinone ring, it has been observed that compound **19** having 3-aminophenyl was the least active in comparison with derivatives containing phenyl ring at N-3, which illustrated the need for an aromatic substituent at position-3. In addition to this, various substitutions (Br, Cl, F, NO_2, OMe, Me) placed on phenyl ring (at N-3) at *ortho*- or *meta*- or *para*- position showed significant effect on antibacterial and antifungal activity. The presence of halogenated phenyl substituents (bromo, chloro and fluoro) has more pronounced effect on activity than phenyl and non-halogenated phenyl substituents (nitro, methoxy and methyl). The order of activity for the compounds with different substituents on phenyl ring was Br $\geq$ Cl > F $\geq$ NO_2 > OMe > Me $\geq$ H. This observation clearly indicates the effect of electronic factors on activity. The electron-donating groups (alkyl or alkoxy) on the aromatic ring in general decreased antimicrobial activity of tested compounds compared to compounds having electron withdrawing groups (halogens and nitro). Furthermore, a clear preference for *para*- substitution over *meta*- $\approx$ *ortho*-substitution in phenyl ring at position N-3 was observed for the compounds **2–18** with same substitution. This led us to presume that the presence of the *meta*- or *ortho*-substituent might drive a change in local conformation, forcing the aryl ring out of co-planarity.

Among the explored compounds, the most active scaffolds **13Am** and **15Am** have 4-chloro-phenyl and 3-bromophenyl substitutions at position N-3, respectively and amino group at position 4 of benzenesulfonamide (R'_4). Further, quinazolinone (methaqualone) when taken in isolation were less active compared to standard drugs, but have shown enhanced antimicrobial activity when conjugated to sulfonamide (sulfanilamide) into quinazolinone–sulfonamide hybrid framework and in turn their derivatives have displayed higher potency revealing the latter's importance in arresting/inhibiting the bacterial as well as fungal growth.

Moreover, it has been found that, in comparison to the reference drugs of parent moieties, the screened compounds (**1–19**) exhibited more potent activity than Methaqualone and more or equally potent activity than sulfanilamide (sulfanilamide). The enhancement in the antimicrobial activity of **1–19** might be due to the additive effect of the presence of two bioactive moieties in a single molecular framework. This observation further supported the hypotheses that the presence of substituted aromatic ring at position-3 together with methyl group at second position of quinazolin-4(3*H*)-one is not always necessary for the medicinal activity and other groups when placed at this position can also lead to potent antimicrobial agents, as well as the presence of N-heterocyclic ring substitution at N-1 of sulfonamide moiety (which was bridged to 4(3*H*)-quinazolinone at position-2) of could enhance the antimicrobial activity. The scaffolds synthesized may be taken for future developmentto find the potential leads among such series.

Experimental

General considerations

Unless otherwise indicated, all common reagents and solvents (AR) were used as obtained from commercial suppliers without further purification. Alumina supported pre-coated silica gel 60 F_{254} Thin layer chromatography (TLC) plates were purchased from the E. Merck (India) Limited, Mumbai and were used to check purity of compounds and, to study the progress of the reaction whereby TLC plates were illuminated under Ultraviolet light (254 nm), evaluated in I_2 vapors and visualized by spraying with Draggendorff's reagent. Column chromatographic separations were carried out on silica gel (60–120 mesh). Infrared spectra (FT-IR) were obtained from KBr pellets in the range of 4,000–400 cm^{-1} with a Perkin Elmer spectrum GX spectrophotometer (FT-IR) instrument. ^{1}H-NMR spectra were acquired at 400.1 MHz, and ^{13}C-NMR at 100.6 MHz on a Bruker NMR spectrometer using DMSO-d_6 (residual peak at δ ~2.5 or ~39.5 ppm, 300 K) as a solvent as well as an internal reference standard. The electro-spray ionization mass spectra (positive mode) were recorded on a Shimadzu LC-MS 2010 eV mass spectrophotometer using methanol or acetonitrile. Micro analytical (C, N, H) data was obtainedusing a Perkin-Elmer 2400 CHN elemental analyzer. Melting points were taken by open-capillary method and are uncorrected.

Procedures for the synthesis of compounds (**1–22**)

*Synthesis of N-{[4-(acetylamino)phenyl]sulfonyl}glycine (**20**)*

The dry acetanilide (20 g, 0.148 mol) was added portionwise with stirring at 0 °C to chlorosulfonic acid (50.16 mL,

0.755 mol) over 30 min. After addition was complete, the reaction mixture was heated slowly to 70–80 °C for 1 h and then allowed to cool to room temperature. The purple reaction mixture was cautiously poured into stirred slurry of ice water (5 L). The resulting suspension of purple–white solid was stirred for several minutes to obtain an even suspension, the solid product was filtered out, washed successively with water, which was purified by extraction and recrystallization from $CHCl_3$ to yield purple–white crystals of the intermediate *p*-acetamidobenzenesulfonyl chloride (**ASC**, ~85 %).

The prepared **ASC** (11.683 g, 0.05 mol) was added portion-wise with stirring to a clear solution of amino acid (glycine, 3.753 g, 0.05 mol) in aqueous potassium carbonate (K_2CO_3, 6.91 g, 0.11 mol) solution (15 % or 2 mol L^{-1}). The mixture was then stirred vigorously and heated at 70–80 °C for 30–35 min. After completion of the reaction (TLC), the resulting clear solution was cooled to room temperature and then acidified in an ice-bath to pH ~2.5 with drop-wise addition of dil. HCl solution (2 mol L^{-1}). The precipitated product thus obtained was filtered off, washed with water, and recrystallized from R-spirit to afford *N*-(4-acetamidobenzenesulfonyl)glycine (**20**) in quantitative yield.

*Synthesis of methyl 2-[(N-{[4-(acetylamino)phenyl] sulfonyl}glycyl)amino]benzoate (**21**)*

To a mixture of *N*-(4-acetamidobenzenesulfonyl)glycine (13.614 g, 0.05 mol) and methyl anthranilate (8.314 g, 0.055 mol) in appropriate solvent (THF—165 mL or pyridine—125 mL), phosphorous trichloride (PCl_3, 6.86 g, 0.05 mol) in same solvent (10–15 mL) was added with continuous stirring over a period of 5 min. The resulting mixture/suspension was heated for an appropriate time till completion of reaction (TLC). Then, solvent was removed under reduced pressure (if, xylene was employed), and the content in the flask was diluted with water and treated with 10 % $NaHCO_3$ solution to give precipitate of methyl N-acylanthranilate (**21**), which was filtered off, washed with water, and recrystallized from methanol. (If xylene—220 mL then PCl_3, 17.166 g, 0.125 mol).

*Synthesis of 2-[(N-{[4-(acetylamino)phenyl] sulfonyl}glycyl)amino]benzoic acid (**22**)*

To a clear solution of **21** (20.271 g, 0.05 mol) in MeOH (500 mL), barium hydroxide octahydrate ($Ba(OH)_2{\cdot}8H_2O$, 23.659 g, 0.075 mol) was added. The reaction mixture was stirred at room temperature for 3 h or heated to reflux for 20–25 min. *Non-aqueous work-up* after completion of the reaction (TLC), solvent was recovered under reduced pressure, the residues were diluted with 400 mL EtOAc followed by the addition of HCl in EtOAc (1 M, 100 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo and the product obtained was recrystallized from methanol to afford N-acylanthranilic acid (**22**) in good yield. *Aqueous work-up* after completion of the reaction (TLC), the reaction mixture was diluted with distilled water and then acidified in ice-bath to pH ~2.5 with drop-wise addition of HCl solution (2 mol L^{-1}). The product so obtained was separated out, washed with distilled water, and then was recrystallized from methanol to afford **22** in the similar yield.

*General synthesis of N-{4-[({[3-((un)substituted aryl)-4-oxo-3,4-dihydroquinazolin-2-yl]methyl}amino)sulfonyl] phenyl}acetamide derivatives (**1Ac–19Ac**)*

To a mixture of N-acylanthranilic acid (**22**) (0.783 g, 0.002 mol) and appropriate mono-(un)substituted aromatic-amines (0.0022 mol) {for instance, aniline and *o*-, *m*-, *p*-substituted derivatives of toluidine, anisidine, nitroaniline, chloroaniline, bromoaniline, fluoroaniline, and phenylhydrazine, respectively} in THF (25 mL), a solution of phosphorus trichloride (PCl_3, 0.275 g, 0.002 mol) in THF (5 mL) was added slowly with continuous stirring over a period of 5–10 min. The resulting mixture was allowed to warm at appropriate temperature (40–70 °C) for 25–35 min, after completion of the reaction as indicated by TLC, the mixture was allowed to cool at room temperature, poured into 100 mL ice-cold water and neutralized with 10 % $NaHCO_3$ solution. The solid product thus separated was filtered off, washed with distilled water, and recrystallized from R-spirit to give appropriate 4-quinazolone derivatives in excellent yields (84–97 %).

*General synthesis of 4-amino-N-[(4-oxo-3-substituted aryl-3,4-dihydroquinazolin-2-yl)methyl]benzenesulfonamides (**1Am–19Am**)*

An appropriate *N*-{4-[({[3-(substituted aryl)-4-oxo-3,4-dihydroquinazolin-2-yl]methyl}amino)sulfonyl]phenyl}acetamide derivatives, (**1Ac–19Ac**) (0.001 mol) was treated with dilute HCl solution (40 %) in R-spirit and H_2O (1:1) mixture (15–20 mL), and the reaction mixture was heated to reflux for 45–70 min till the clear solution obtained. After completion of the reaction, the mixture was cooled, poured into ice-cold water, and the pH was adjusted to 7.5–8 using saturated $NaHCO_3$ solution. The precipitated product was filtered off, washed with distilled water, and recrystallized from R-spirit to afford **1Am–19Am** in quantitative yield (72–89 %).

*Synthesis of N-[4-({[(4-oxo-4H-1,3-benzoxazin-2-yl)methyl]amino}sulfonyl)phenyl]acetamide (**23**)*

Method-I: To a mixture of **22** (0.783 g, 0.002 mol) and appropriate heterocyclic primary amine (*N*- or *N*,*S*-containing un-fused/benzo-fused 5- or 6-membered ring) (0.0022 mol) in appropriate solvent (THF—15 mL or pyridine—10 mL), a solution of phosphorus trichloride (PCl_3, 0.275 g, 0.002 mol) in same solvent (THF, 5-mL or pyridine, 3-mL) was added with stirring over a period of 5 min. The resulting mixture was warmed at 50 °C for 25–30 min. After completion of reaction (TLC), the solvent was recovered under reduced pressure, the residue was dissolved in ethyl acetate (100 mL), washed with water (10 × 2 mL) followed by 10 % $NaHCO_3$ solution (15 × 1 mL), water (10 × 2 mL), and brine. The organic layer was then dried over $MgSO_4$, concentrated in vacuo, and the solid product obtained was recrystallized from chloroform to afford **23** in good yield (0.656 g, 89 %).

Method-II: To a well-stirred solution of anthranilic acid (0.274 g, 0.002 mol) and **20** (0.545 g, 0.002 mol) in pyridine (10 mL), triphenyl phosphite ($P(OPh)_3$, 0.0022 or 0.0025 mol) was added and the resulting mixture was warmed at 55–70 °C for 4 h. After completion of reaction (TLC), the solvent was recovered under reduced pressure, the residue was dissolved in ethyl acetate (100 mL), washed with water (10 × 2 mL) followed by diluted aqueous base solution (15 × 1 mL), water (10 × 2 mL) and brine, then dried over $MgSO_4$, concentrated in vacuo, and the solid product obtained was recrystallized from chloroform to afford **23** in good yield (84 %).

Analytical data of the synthesized compounds (**1**–**22**)

All the analytical and spectral data of synthesized compounds are included in the supplementary material (Online Resource).

In vitro antimicrobial activity

The in vitro antimicrobial evaluations of newly synthesized compounds (**1**–**19**) was assessed against various species by microdilution method (Murray *et al*., 1995) using Methaqualone (**Qm**) (Wasfy, 2002) and Sulfanilamide (**Sf**) (Vanparia *et al*., 2010) as a reference drugs of parent moieties, along with the other standard drugs. The strains employed were procured from Institute of Microbial Technology (MTCC—*The Microbial Type Culture Collection and Gene Bank*), Chandigarh. The sulfonamide testing required a thymidine free media.

Antibacterial screening

The in vitro antibacterial screening of compounds **1**–**19** were determined against various Gram-positive bacteria [*S. aureus* (MTCC 2940); *B. Cereus* (MTCC 1305)] and against Gram-negative bacteria [*E. coli* (MTCC 443); *P. aeruginosa* (MTCC 1688); *K. pneumonia* (MTCC 139)] by broth-microdilution method. Ampicillin (**Ap**) was used as a standard drug along with the reference drugs of parent moieties. Mueller–Hinton broth medium was used to maintain the bacterial cultures and to dilute the compounds. Inoculum size of the bacterial strains was adjusted to 1.5×10^8 CFU mL^{-1} by comparing the turbidity with 0.5 McFarland turbidity standards.

Antifungal screening

The in vitro antifungal screening of compounds **1**–**19** were measured against two fungal species [*A. niger* (MTCC 282); *C. albicans* (MTCC 227)] by broth microdilution method. Ketoconazole (**Kz**) was used as standard antifungal drug along with the reference drugs of parent moieties. Sabouraud–dextrose broth medium was used to maintain the fungal strains and to dilute the compounds. Inoculum size of the fungi was adjusted to 1.5×10^5 CFU mL^{-1} by comparing the turbidity with 0.5 McFarland turbidity standards.

MICs determinations

The serial dilutions from stock solutions were prepared with DMSO and it was also used as a diluent/vehicle to obtain the desired concentrations of synthesized compounds and standard drugs. In pilot screening experiments, the prototype compounds (**1Ac**, **1Am**, **19Ac**, **19Am**) and drugs were tested against all micro-organisms and remainders were tested in secondary screening. To a series of tubes containing 5 mL medium suitable for the growth of the test organisms, 0.1 mL solution of different concentrations of test compounds and 0.1 mL of inoculum was added. These test tubes containing bacteria and fungi were incubated for 24 h (at 37 °C) and 48 h (at 25 °C), respectively and observed for the presence of turbidity. The growth of test cultures was visualized using triphenyltetrazolium chloride (TTC, 0.5 % w/w) aqueous solution. This method was repeated with standard as well as reference drugs for comparison. MIC was defined as the lowest concentration of the screened compounds that inhibited visible growth (red colored pellet on the bottom of the tubes after the addition of TTC). The highest dilution (lowest concentration) at which no visible growth (prevents appearance of turbidity) was observed, was considered as MIC (μg mL^{-1}) values, i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the

solvent had no influence on strain growth. The observed data as a mean value of triplicate results for the screened compounds and drugs are summarized in Table 2.

Conclusion

A facile, efficient and convenient multi-step synthetic approach involving milder conditions for the rapid access of a series of newly designed structurally diverse and hybrid heterocycles (**1–19**) was developed using amino acid as a precursor, which had assembled entirely the assimilation of quinazolinone and sulfonamide moieties in a single molecular framework, tolerated substitution pattern at C2 and N3 as well as eventually afforded higher yield. Further, the results of in vitro antimicrobial evaluations of the synthesized compounds **1–19** revealed that compounds **1Ac–19Ac** and **1Am–19Am** found to possess varied degree of antibacterial and antifungal activities. Among the compounds screened, compounds **13Am** and **15Am** emerged with more potent inhibitory action followed by compounds **7Ac**, **10Am**, **12Am**, **16Am**, and **18Am** demonstrated significant activity than the rest of the tested compounds with a moderate to mild activity against all species. The data also revealed that the presence of various groups on substituted phenyl ring at N-3 as well as 4-aminobenzenesulfonamide substituent bridged at C-2 of 4-(3*H*)-quinazolinone ring has more influence on the antimicrobial activity. The results obtained on the preliminary biological activity paving a way for the synthesis of additional hybrid entities for improving antimicrobial activity.

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