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Original Article

MOLECULAR MODELLING STUDIES, SYNTHESIS AND ANTIMICROBIAL SCREENING OF SOME NOVEL SULPHONAMIDE QUINAZOLIN-4(3H)-ONE FUSED DERIVATIVES.

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ABSTRACT

Objective: To synthesize some sulphonamide quinazolin-4(3H)-one fused derivatives as orally active antimicrobial agents, rationally designed by studying physico-chemical parameters and their binding mode with the active domain of DNA gyrase enzyme.

Methods: A series of sulphonamide incorporated Quinazolinone derivatives were subjected for molecular properties prediction, drug-likeness, lipophilicity, solubility parameters and drug score using Molinspiration and Osiris softwares. They were further docked into the active domain of DNA gyrase enzyme. The successful molecules were subjected for synthesis and antimicrobial screening by disd diffusion method.

Results: All the compounds passed the Lipinski "Rule of Five" (Ro5) and were chosen for the synthesis and antimicrobial screening as an oral bioavailable drugs/leads. The docking scores were also in good agreement with the antimicrobial screening results. It was observed that compounds showed moderate to good antibacterial activity, but their antifungal activity was somewhat moderate. Compound D10 and D7 showed pronounced activity against all bacterial and fungal strains.

Conclusion: We had noticed that compounds (D1, D10, D7) bearing methyl group in quinazoline ring either at 2 or 6 positions or at both the positions exhibited good antimicrobial properties and their predicted drug likeness model score were also admirable among the series. These results suggested that such compounds might be further derivatize to get more selective antimicrobial agents.

keywords: Sulphonamide, Quinazolinone, DNA Gyrase, Docking, Antimicrobial, Rule of five, Molecular Properties Prediction.

INTRODUCTION

The world is currently experiencing challenges of increased resistance development against available antimicrobials. The AIDS pandemic has also resulted in large numbers of immunocompromised patients susceptible to opportunistic bacterial and fungal infections. Toxicity of currently used antimicrobial drugs, such as amphotericin B which causes hepatotoxicity, is a limiting factor in their use. Additionally, the cost of effective antimicrobials plays a vital role in their availability, mainly in developing countries. Antimicrobial resistance is the ability of certain microorganisms to withstand attack by antimicrobials, and the uncontrolled rise in resistant pathogens threatens lives and wastes limited healthcare resources. Life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world. Owing to this increased microbial resistance, new classes of antibacterial agents with novel mechanisms are crucial need to combat with the multidrug-resistant infections. DNA gyrase is a major bacterial protein that is involved in replication and transcription and catalyzes the negative supercoiling of bacterial circular DNA. DNA gyrase is a known target for antibacterial agents since its blocking induces bacterial death [1]. The quinazolinone moiety is an important pharmacophore showing many types of pharmacological activities [2]. The quinazolinones are considered to be a "privileged structure" for drug development [3]. The chemistry of 4(3H)-quinazolinone has received an increasing interest because of its biological significance. Many derivatives of this system showed antibacterial [4-5] antifungal [6], anticancer [7], anti-inflammatory [8], anticonvulsant [9-10], analgesic [11], hypolipidemic [12], antiulcer [13], and antiproliferative [14] activities. Structure activity relationship studies of quinazolinone ring system revealed in various literatures [15]. Moreover, Sulfonamide derivatives have also been reported to possess significant antibacterial activities through competitive inhibition of dihydropteroate synthetase enzyme (DHPS) which is involved in folate synthesis [16]. So in present study we have incorporated both the sulphonamide and Quinazolin-4-one moiety together in one molecule to explore the antimicrobial activities. About 30% of oral drugs fail in development due to poor pharmacokinetics [17]. Among the pharmacokinetic properties, a low and highly variable bioavailability is indeed the main reason for stopping further development of the drug [18]. Thus, predictions of bioavailability and bioavailability-related properties, such as solubility, lipophilicity are important before actual synthesis, in order to reduce enormous wastage of expensive chemicals and precious time. An *in silico* model for predicting oral bioavailability is very important, both in the early stage of drug discovery to select the most promising compounds for further optimization and in the later stage to identify candidates for further clinical development [18]. In present investigation a series of 10 Quinazolinone sulphonamide analogues (D1-D10) were subjected to molecular properties prediction, drug-likeness by Molinspiration [19] & MolSoft (MolSoft 2007) softwares, lipophilicity and solubility parameter by using ALOGPS 2.1 program to filter the compounds for further synthesis and antimicrobial screening.

MATERIAL AND METHODS

Molecular Properties Calculations and Molecular Docking

Molecular properties, mainly hydrophobicity, molecular size, flexibility and the presence of various pharmacophoric features influence the Pharmacokinetic and pharmacodynamics behaviour of molecules in the living organism, including bioavailability. Thus in order to achieve good bioavailable drugs, we have subjected a series of quinazolinone derivatives **(D1-D10)** for the prediction of some basic pharmacokinetic properties under the Lipinski's "Rule of Five".

Lipophilicity

All the compounds were subjected to computational study in order to filter the drugs for biological screening. For good membrane

permeability logP value should be ≤5 [20]. All the title compounds **(D1-D10)** were found to have logP values in the range of 1.62–3.15.

Absorption, Polar surface area, and ''rule of five" properties

High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors), are important predictors of good oral bioavailability [21]. Molecular properties such as membrane permeability and bioavailability is always associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors counts in a molecule [22]. Lipinski [23] used these molecular properties in formulating his ''Rule of Five". The rule states that most molecules with good membrane permeability have logP ≤ 5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤ 5. This rule is widely used as a filter for drug-like properties. Table 1 contains calculated percentage of absorption (%ABS), molecular polar surface area (TPSA) and Lipinski parameters of the investigated compounds of the series (D1-D10). Magnitude of absorption is expressed by the percentage of absorption. Absorption percent was calculated [24] using the expression: %ABS =109 - 0.345 PSA. Polar surface area (PSA) was determined by the fragment-based method of Ertl and coworkers [25-26]. A poor permeation or absorption is more likely when there are more than 5 H bond donors, 10 H-bond acceptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability [27]. The series (D1-D10) under investigation had all compounds having hydrogen bond donor and acceptors in considerable range as shown in Table 1.

Number of rotatable bond is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels. It is revealed that for passing oral bioavailability criteria number of rotatable bond should be ≤10. The compounds in this series (D1-D10) possess lower range of 'number of rotatable bonds' i.e. (3-5) and therefore, exhibit low conformational flexibility.

Molecular polar surface area (TPSA) is a very useful parameter for the prediction of drug transport properties. TPSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. TPSA and volume is inversely proportional to % ABS. All the compounds under study have exhibited good %ABS except D6 having 26% Abs, But all the title compounds (D1-D10) followed the Lipinski ''Rule of Five''. The pharmacokinetic parameters were calculated online from Molinspiration Chemo informatics(http://www.molinspiration.com/cgi-bin/properties) and are given in Table 1.

Osiris Calculations

Structure based drug design is now very routine work as many drug fail to reach clinical phases because of ADME/TOX problem encountered. Therefore prediction of these problems before synthesis is rational approach to minimize cost production of expensive chemicals. The Osiris calculations are tabulated in Table 2. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physicochemical properties (cLogP, solubility, drug likeness and drug score) of compounds D1-D10 were calculated by the methodology developed by Osiris [28]. The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified. From the data evaluated from Osiris calculations it is obvious that, all compounds of the series are supposed to be non-mutagenic, non-irritating, non-tumorogenic with no reproductive effects when run through the mutagenicity assessment system in comparison with the standard drug except compound D6 having tumorogenic effect. The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water, is a well-established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values may cause poor absorption or permeation. It has been shown that for compounds to have a reasonable probability of good absorption, their logP value must not be greater than 5.0. On this basis, all the compounds D1-D10 possessed logP values in the acceptable range.

Aqueous solubility

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. In general a low solubility goes along with a poor absorption and therefore the general aim is to avoid poorly soluble compounds. Our estimated logS value is a unit stripped logarithm (base 10) of a compound's solubility measured in mol/litre. There are more than 80% of the drugs on the market have a (estimated) logS value greater than -4. In present series the values of logS are around -5. Further, Table-2 shows drug likeness of compounds D1-D10 which is in the acceptable zone to be drug like when compared with standard drug. We have calculated overall drug score (DS) for the compounds D1-D10 and compared with that of standard drug ciprofloxacin. The drug score combines drug likeness, cLogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the Compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with the equation (1):

$DS = \prod (1/2+1/2Si) \prod ti$

Where S; $(1/1+e^{ap+b})$

DS is the drug score, Si is the contributions calculated directly from miLogP; logS, molecular weight and drug likeness (pi) via the second equation, which describes a spline curve. Parameters a and b are (1,-5), (1, 5), (0.012, -6) and (1, 0) for cLogP, logS, molecular weight and drug likeness, respectively. The ti is the contributions taken from the four toxicity risk types and the values are 1.0, 0.8 and 0.6 for no risk, medium risk and high risk, respectively. The reported compounds D1-D10 showed moderate to good drug score as compared with standard drug used.

Molecular docking studies of the compounds using Pymol/Autodock vina Plugin:

The compounds in the study were subjected to dock in the active domain of DNA gyrase protein by using Pymol/Autodock vina Plugin software**.** Crystal structures of DNA gyrase protein in complex with Biocin (PDB ID: 1KZN) with resolution 3.5 Å was downloaded from RCSB Protein Data Bank to serve as the docking template [29]. The crystallographic water and ligand molecules were removed from the protein complex.

Pymol AutoDock vina plugin developed by Seeliger [30] was used on Linux ubuntu 12.0 installed on Pentium i3workstation. ChemDraw ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)] was used for construction of compounds which were converted to 3D structures using Chem3D ultra 8.0 software and the constructed 3D structures were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with AM1 mozyme geometry, 100 iterations and minimum RMS gradient of 0.10.

They were ranked according to their docking score as shown in Table-3. Compound D10 has shown maximum binding affinity having binding energy -9.5 while the least score was observed for compound D6, which had already got minimum score in Osiris calculations, but still it passed 'rule of five'. The redocked pose of the ligand biocin with the cocrystallized structure of the same has been shown in Fig-1a. The docked structure of all the compounds has been shown in Fig-1b.

Chemistry

The synthesis of sulphonamide incorporated quinazoline derivatives (D1-D10) as described in this study are outlined in scheme-1 and scheme-2. Their physical data is presented in Table 4. As shown in scheme-1(Fig-2a) the benzoxazinone derivatives were prepared from two alternative routes, one via amide formation (C1-C5) and another via direct cyclization with acetic anhydride (C6-C10). The Semicarbazide of sulphonamide was prepared by the reported procedure [31]. The benzoxazinones and Semicarbazide were fused

in glacial acetic acid to produce the titled compounds as shown in scheme-2 (Fig-2b). The compounds were recrystallized from ethanol. The purity of the compounds was checked by TLC. Spectral data ^{1}H-NMR, ^{13}CNMR, IR of all the synthesized compounds and Mass spectra of selected compounds were recorded and found in full agreement with the proposed structures. The elemental analysis results were within ± 0.4% of the theoretical values. *4.1.1. General Procedure for synthesis of 5-substituted-2-(4-substituted-benzamido benzoic acid 2(B1-B5) :*

5-substituted-anthranilic acid (20 mmol, 3.43 g) and 4-substituted-aryl/acyl chloride (22 mmol, 3.40 g) were stirred at room temperature in pyridine (80 ml) for 6 h. The solvent was removed under reduced pressure; the obtained residue was washed with acidulated water, filtered, washed with water, dried and recrystallized from ethanol.

2-[(cyclopropylcarbonyl)amino]benzoic acid:(B1)

m.p. 167-169 ˚C in 94% yield. IR (KBr, cm^{-1}) υ: 1762 (CO); 1H NMR (DMSO-d6): *δ* 12.07 (1H, s, NHCO), 11.5 1H, (s, OH), 8.1 (1H, d, Ar.), 7.8 (1H, d, Ar.), 7.1-7.5 (2H, m, Ar), 1.5 (s, 1H, CH), 1.1 (4H, m, CH_2). 13C NMR (DMSO-d6): δ 169.2, 165.0, 143.4, 140.5, 134.4, 131.9, 130.8, 130.0, 127.5, 126.8, 122.1, 118.8, 21.4. MS: (M^{+1}, 206).

2-[(thiophen-2-ylcarbonyl)amino]benzoic acid(B2):

m.p. 173-176 ˚C in 79% yield. IR (KBr, cm^{-1}) υ: 1763 (CO);^{1}H NMR (DMSO-d6): *δ* 11.95 (1H, s, NHCO), 11.55 (1H, s, OH), 8. 23 (1H,d, Ar.), 7.7 (1H, d, Ar.), 7.65 (1H,s, Ar), 7.63(1H, s, Ar), 7.3-7.56 (2H, m, Ar).

2-[(5-methyl-2-phenylcarbonyl)amino]benzoic acid(B3):

m.p. 173-176 ˚C in 79% yield. IR (KBr, cm^{-1}) υ: 1755 (CO);^{1}H NMR (DMSO-d6): *δ* 12.1 (1H, s, NHCO), 11.5 (1H,s, OH), 8.21 (1H, d, Ar.), 7.95 (2H, d, Ar.), 7.87 (1H, d, Ar.), 7.21- 7.58 (5H, m, Ar).

2-{[(4-methylphenyl)carbonyl]amino}benzoic acid(B4)

m.p. 173-176 ˚C in 79% yield. IR (KBr, cm^{-1}) υ: 1753 (CO);^{1}H NMR (DMSO-d6): *δ* 12.1 (1H, s, NHCO), 11.5 (1H, s, OH), 8.11 (1H, d, Ar.), 7.87(2H, d, Ar.), 7.83 (1H, d, Ar.), 7.85- 7.58(1H, m, Ar), 7.24(2H, d, Ar), 7.21(1H, m, Ar), 2.35 (s, 3H, CH3). MS: (M^{+1}, 256, 16.2%).

2-{[(4-nitrophenyl)carbonyl]amino}benzoic acid(B5)

m.p. 248-250 ˚C in 90% yield.1H NMR (DMSO-d6): *δ* 12.07 (1H, s, NHCO), 11.7 (1H, s, OH), 8.4(2H, d, Ar), 8.21 (2H, d, Ar.), 8.11 (1H, d, Ar.), 7.85(1H, d, J ¼ 2.0 Hz), 7.68-7.61 (2H, m), 7.34 (2H, d, J ¼ 8.0 Hz). 13C NMR (DMSO-d6): d 169, 164.0, 144.3, 140.5, 134.4, 131.9, 130.8, 130.0, 127.5, 126.8, 122.1, 118.8, 21.4. MS: (M^{+1}, 287, 16%).

4.1.2. 6-substituted-2-substituted-4H-benzo[d][1,3]oxazin-4-one ***3(C1-C10)***

The benzoic acid derivatives 2(B1-B5) and 1(A6-A10) were heated under reflux in acetic anhydride for 3 hr to furnish the intermediates from *3(C1-C10)*. The solid obtained was cooled, filtered, washed with diethyl ether and dried to be used for the next step of synthesis.

6-Chloro-2-methyl-4H-benzo[d][1,3]oxazin-4-one (C1)

m.p. 145-147 ˚C in 91% yield. IR (KBr, cm^{-1}) υ: 1767 (CO); 1H NMR (CDCl3): *δ* 8.15 (1H, s, Ar.), 7.64 (1H, d, Ar), 7.40 (1H,d, Ar), 2.53 (3H, s, CH3), ^{13}C NMR (DMSO-d6): *δ* 160.561, 158.198, 144.812, 136.54, 132.04, 126.678, 117.97, 20.914, MS: M+1 (241, 30.34%).

MS: M+1 (273, 18.34%).

6-bromo-2-methyl-4H-benzo[d][1,3]oxazin-4-one (C2)

m.p. 145-147 ˚C in 84% yield. IR (KBr, cm^{-1}) υ: 1760 (CO); 1H NMR (CDCl3): *δ* 8.17 (s, 1H, Ar.), 7.63 (d, 1H,Ar), 7.45 (d, 1H,Ar), 2.55 (s, 3H, CH3). ^{13}C NMR (DMSO-d6): *δ* 160.68, 158.065, 145.118, 139.344, 129.69, 128.356, 120.198, 118.307, 20.979, MS: M+1 (241, 30.34%).

6-iodo-2-methyl-4H-benzo[d][1,3]oxazin-4-one (C3): m.p. 167-169 ˚C in 89% yield. IR (KBr, cm^{-1}) υ: 1767 (CO); 1H NMR (CDCl3): *δ* 8.15 (s, 1H, Ar.), 7.7 (d, 1H,Ar), 7.51 (d, 1H,Ar), 2.56 (s, 3H, CH3). MS: M+1 (288, 29%).

2-Thiophene-2-yl-4H-benzo[d][1,3]oxazin-4-one (C4)

m.p. 173-176 ˚C in 81% yield. IR (KBr, cm^{-1}) υ: 1763 (CO);^{1}H NMR (DMSO-d6): *δ* 8. 23 (d, 1H, Ar.), 7.7 (d, 1H, Ar.), 7.65 (d, 1H,Ar), 7.63(d, 1H, Ar), 7.3-7.56 (m, 3H, Ar). M^{+1}, 231.

2-methyl-4H-benzo[d][1,3]oxazin-4-one (C5)

m.p. 178-180 ˚C in 79% yield. IR (KBr, cm^{-1}) υ: 1753 (CO); 1H NMR (CDCl3): *δ* 8.2 (d, 1H, Ar.), 7.7 (d, 1H,Ar), 7.51 (m, 2H,Ar), 2.9 (s, 3H, CH3). MS: M+1 (162, 31%).

2-(4-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one (C6)

m.p. 164-166 ˚C in 81% yield. IR (KBr, cm^{-1}) υ: 1767 (CO); 1H NMR (CDCl3): *δ* 8.75 (d, 2H, Ar.), 8.53 (d, 2H,Ar), 8.1 (d, 1H,Ar), 7.6 (d, 1H,Ar), 7.1 (d, 1H,Ar), 7.21-7.30(m, 2H,Ar). MS: M^{+1} (269).

2-methyl-4H-benzo[d][1,3]oxazin-4-one (C7)

m.p. 195-197 ˚C in 91% yield. IR (KBr, cm^{-1}) υ: 1750 (CO); 1H NMR (CDCl3): *δ* 8.1 (s,1H,Ar), 7.4 (d, 1H,Ar), 7.24 (d, 1H,Ar), 3.25 (s, 6H, CH3). MS: M^{+1} (176, 14%).

2-(4-tolyl)-4H-benzo[d][1,3]oxazin-4-one (C8)

m.p. 158-160 ˚C in 88% yield. IR (KBr, cm^{-1}) υ: 1755 (CO); 1H NMR (CDCl3): *δ* 8.12 (d, 1H,Ar), 7.5 (d, 3H,Ar), 7.42-7.34(m,2H,Ar), 7.24 (d, 2H,Ar), 3.25 (s, 3H, CH3). MS: M^{+1} (238, 16%).

2-cyclopropyl-4H-benzo[d][1,3]oxazin-4-one (C9)

m.p. 145-147 ˚C in 91% yield. IR (KBr, cm^{-1}) υ: 1753 (CO); 1H NMR (CDCl3): *δ* 8.15 (d, 1H,Ar), 7.5 (d, 1H,Ar), 7.45-7.35(m,2H,Ar), 1.3-1.05 (m, 5H, CH). MS: M^{+1} (188, 13%).

2,6-dimethyl-4H-benzo[d][1,3]oxazin-4-one (C10)

m.p. 198-201 ˚C in 90% yield. IR (KBr, cm^{-1}) υ: 1750 (CO); 1H NMR (CDCl3): *δ* 8.1 (s, 1H,Ar), 7.8 (d, 1H,Ar), 7.6(d,1H, Ar), 7.69-7.29(m,5H,Ar), 3.25 (s, 3H, CH3). MS: M^{+1} (238, 17%).

General procedure for synthesis of Semicarbazide derivative of sulphonamide:

0.01 M of sulphonamide(4) was dissolved in the 50 ml mixture of 10% glacial acetic acid in water. To this solution equimolar sodium cyanate dissolved in 50 ml mixture of 10% glacial acetic acid in water was added over a period of half an hr with continuous mechanical stirring. After one hr stirring the white crude product was filtered (5) and recrystallized from ethanol. The white urea derivative of sulphonamide was refluxed with hydrazine hydrate in ethanol for 6 hrs which yielded the corresponding Semicarbazide (6).

N-(4-sulfamoylphenyl)hydrazinecarboxamide

m.p. 218-220 ˚C in 90% yield. 1H NMR (CDCl3): *δ* 11.9(s,1H, NH), 11.5(s,1H, NH), 7.92 (d, 2H,Ar), 7.9(d,21H, Ar), 5.9(s,2H,NH_2), 4.9(s,2H, SO_2NH_2). MS: M^{+1} (231, 11%).

General procedure for synthesis of title compounds D1-D10:

Equimolar quantities of semicarbazide(6) and substituted benzoxazinones (C1-C10) were fused for 2 hour in glacial acetic acid. The solid product obtained was filtered washed with water,dried and recrystallized from ethanol to furnish the titled compounds.

4-{[(6-chloro-2-methyl-4-oxoquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D1)

m.p. -235˚C, 73% yield, IR (cm−1): 3337, 3274, 3219 (NH, NH2), 3085 (CH arom.), 1352, 1160 (SO2), ^{1}H NMR ($DMSOd_6$): *δ* 10.032(1H, s, NH, D_2O exchangeable), 9.345(1H, s, NH, D_2O exchangeable), 8.07 (1H, s, Ar), 7.91-7.88(1H, d, Ar), 7.76-7.63(5H, m, Ar), 7.256(2H, s, NH_2), 3.385 (3H, s, CH_3), ^{13}C NMR (DMSO-d6): *δ* 157.72(C=O), 145.38, 135.06, 130.85, 129.18, 126.79, 125.2, 21.4 (CH_3), MS: M^{+1} (408.8).

4-{[(6-bromo-2-methyl-4-oxoquinazolin-3(4H) yl)carbamoyl]amino}benzenesulfonamide(D2)

m.p. -246˚C, 84% yield,IR (cm−1): 3350, 3230 (NH, NH_2), 3080 (CH arom.), 1359, 1165 (SO2), 1H NMR (DMSOd_6): δ 10.034(1H, s, NH, D_2O exchangeable), 9.23(1H, s, NH, D_2O exchangeable), 8.89 (1H, s, Ar), 8.12-8.11(1H, d, Ar), 7.77-7.74(4H, d, Ar), 7.43-7.42(1H, d, J = 5 Hz, Ar), 7.31(2H, s, NH_2), 3.38 (3H, s, CH3), ^{13}C NMR (DMSO-d6): δ 158.12 (C=O), 145.69, 143.2,135.76, 131.05, 129.34, 126.79, 125.87, 91.45, 22.14(CH_3), MS: M^{+1} (362).

4-{[(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D3):

m.p. -264˚C, 78% yield,IR (cm−1): 3350, 86% yield, 3219 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6): δ 10.01(1H, s, NH,D_2O exchangeable), 9.323(1H, s, NH, D_2O exchangeable), 8.389 (1H, s, Ar), 8.16-8.143(1H, d, J = 8.5 Hz, Ar), 7.76-7.63 (4H, m, Ar), 7.48-7.46(1H, d, J = 10 Hz, Ar), 7.253(2H, s, NH_2), 3.369 (3H, s, CH3), ^{13}C NMR (DMSO-d6): δ 157.932 (C=O), 145.925, 143.295, 142.07, 134.43, 129.09, 126.79, 117.92,91.49, 21.5(CH_3), MS: M^{+1} (408.8).

4-{[(4-oxo-2-thiophen-2-ylquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide (D4):

IR (cm−1): 3355, 3220 (NH, NH_2), 3100 (CH arom.), 1350, 1165 (SO2), 1H NMR (DMSOd_6): δ 10.045(s,1H,NH,D_2O exchangeable), 9.1(s,1H,NH,D_2O exchangeable), 8.65-8.67 (d,2H,Ar), 8.66-7.2(m,11H, Ar+SO_2NH_2), ^{13}C NMR (DMSO-d6): δ 152.142, 142.435, 137.164, 130.85, 126.81, 117.68, MS: M^{+1} (443).

4-{[(2-methyl-4-oxoquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D5)

IR (cm−1): 3350, 3250 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6): δ 10.12(s,1H, NH), 9.21(s,1H, NH, D_2O exchangeable), 8.843-8.39 (d,2H,Ar), 8.64-7.2(m,8H, Ar+SO_2NH_2), ^{13}C NMR (DMSO-d6): δ 159.692, 145.435, 139.69, 132.57, 128.98, 118.68, MS: M^{+1} (374).

4-({[2-(4-nitrophenyl)-4-oxoquinazolin-3(4H)-yl]carbamoyl}amino)benzenesulfonamide(D6)

IR (cm−1): 3350, 3219 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6): δ 12.52(s, 1H, NH, D2O exchangeable), 9.91(s, 1H, NH, D_2O exchangeable), 9.43 (m, 2H, Ar), 8.64-8.62(d, 1H, Ar), 8.2-7.2(m, 11H, Ar+SO_2NH_2). MS: M^{+1} (480).

4-{[(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D7)

IR (cm−1): 3350, 3230 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6): δ 10.12(s, 1H, NH), 9.21(s, 1H, NH, D_2O exchangeable), 8.843-8.39 (d,2H,Ar), 8.64-7.2(m,8H, Ar+SO_2NH_2), 3.34 (s, 6H, CH3), ^{13}C NMR (DMSO-d6): δ 158.29, 146.69, 145.2,136.89, 131.05, 129.67, 128.71, 126.76, 92.465, 21.94, MS: M^{+1} (388).

4-({[2-(4-tolyl)-4-oxoquinazolin-3(4H)-yl]carbamoyl}amino)benzenesulfonamide(D8)

IR (cm−1): 3350, 3219 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6): δ 10.45(s, 1H, NH, D_2O exchangeable), 9.34(s, 1H, NH, D_2O exchangeable), 8.86-8.84 (d,2H,Ar), 8.64-7.2(m,12H, Ar+SO_2NH_2), 3.25(s,3H,CH_3) MS: M^{+1} (350).

4-{[(2,(cyclopropyl)-4-oxoquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D9)

IR (cm−1): 3365, 3200 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO2), 1H NMR (DMSOd_6)*:* δ 10.01(s, 1H, NH, D_2O exchangeable), 9.11(s, 1H, NH, D_2O exchangeable), 8.63-8.65 (d,2H,Ar), 8.56-7.2(m,8H, Ar+SO_2NH_2), 1.32-1.05 (m, 5H, CH), ^{13}C NMR (DMSO-d6): δ 159.21, 142.32, 140.2,133.76, 126.79, 125.87, 90.25, 21.214, MS: M^{+1} (388).

4-{[(6-methyl-4-oxo-2-phenylquinazolin-3(4H)-yl)carbamoyl]amino}benzenesulfonamide(D10)

IR (cm−1): 3350, 3219 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO_2), 1H NMR (DMSOd_6): δ 10.43(s, 1H, NH, D_2O exchangeable), 9.29(s, 1H, NH, D_2O exchangeable), 8.80-8.79 (d, 2H, Ar), 8.63-7.2(m, 12H, Ar+SO_2NH_2), 3.36 (s, 3H, CH3). MS: M^{+1} (350).

Pharmacology

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Bacillus Subtilis* (ATCC-6633) (recultured) bacterial strains by disc-diffusion method [32-33]. A standard inoculum (1-2 x10^7 c.f.u./ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 ˚C for 1 h. The sterile discs previously soaked with the test compound solution in DMSO of specific concentration 100 µg and 200 µg/disc were carefully placed on the agar culture plates. The plates were incubated at 37 ˚C and the diameter of the growth inhibition zones were measured after 24 h. The plates were inverted and incubated for 24 h at 37 ˚C. Ciprofloxacin was used as a standard drug. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 5. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 x 10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 ˚C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 h of incubation at 35 ˚C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 6.

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* in DMSO by agar diffusion method [34-35]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of 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 and each well was labeled. A control was also prepared in triplicate and maintained at 37 ˚C for 3-4 days. The fungal activity of each compound was compared with voriconazole as a standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 7. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6 x 10^4 – 6 x 10^4 c.f.u./ml. The cultures were incubated for 48 h at 35 ˚C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 ˚C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 8.

RESULTS AND DISCUSSION

Chemistry

The formation of titled compounds (D1-D10) has been confirmed mainly by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. In general IR

spectrum of compounds showed absorption at 3500-3200 cm^{-1} due to NH group and 1700-1682 cm^{-1} for two carbonyl group. The ^{1}H-NMR spectrum of compounds in general showed multiplet in the region of δ 6.62-8.52 due to aromatic proton. The most characteristics 2 peaks of proton (NH) of the urea portion appeared at δ 10-9 as a singlet. The other two broad singlet proton represented for N-CH=O and NHCO and the values of peak appeared at δ around 12. ^{13}C-NMR spectrum again proves the formation of title compounds by (NHC=O) appeared at δ close to 150 ppm. Mass spectrum of all the compounds reveals the parent ion peak with a considerable relative intensity value. The spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, MS) and C, H, N analysis of all the synthesized analogs are given in experimental part confirming their established structures.

Pharmacology

Antibacterial activity

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds D7, D1, D8 and D10 showed good bacterial inhibition. Most of them exhibited marked degree of activity largely against S.*aureus, E. Coli.* and *P. aeruginosa*. Compound D7 showed significant inhibition almost equivalent to standard and D10 showed moderate antibacterial activity. MBC of compound D10 was found to be same as MIC, against *S.aureus* and *E.coli* but in most of the compounds MBC was two, three or four folds higher than their corresponding MIC values. Compound D1showed good antibacterial activity against almost all bacterial strains.

Antifungal activity

Antifungal screening data indicates that most of the compounds showed moderate activity. Among the screened compounds, D10, D2 and D1 showed good inhibition against both of selected fungal strains i.e *Aspergillus niger* and *Candida albicans*. MFC of most of the compounds was found to be two to four folds higher than their corresponding MIC results.

CONCLUSION

The present work, through simple synthetic approaches, led to the development of novel hybrids of quinazoline containing sulphonamide pharmacophore that exhibited remarkable antimicrobial activities. The sulphonamide-quinazoline fused derivatives **(D1-D10)** were subjected for prediction of molecular properties and receptor binding affinity by different softwares in order to find suitable molecules for the synthesis and antimicrobial screening. Selected compounds were in full agreement with the ADME predicted properties as proved by '*Osiris*' calculations and "*Rule of Five*" predictions. Only compound D6 has medium risk of mutagenecity. The docking score of the compounds D10, D7 and D1 were in good agreement with the antimicrobial screening results. The designed molecules were subjected for the synthesis and antimicrobial screening. All the synthesized compounds were screened for antibacterial and antifungal activity by adopting standard protocol. On the basis of results obtained from antimicrobial screening it was found that compound D10, D1, D7and D8, were found active in the series. Examining closely on substitutions, it may be concluded that role of methyl group at sixth position of quinazoline ring has great influence on antimicrobial activity. Finally it is conceivable that further derivatization of such compounds will be of great interest with the hope to get more selective antimicrobial agents.

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REFERENCES

1. Reece RJ, Maxwell A. DNA gyrase: structure and function. Crit Rev Biochem Mol Biol. 1991; 26 (3-4):335-75.
2. Reddy PS; Reddy PP; Vasantha T: A review on 2-heteryl and heteroalkyl-4(3H)- quinazolinones. Heterocycles 2003; 60(1): 183- 226.
3. Horton DA, Bourne GT, Smythe ML: The combinatorial synthesis of bicyclic privileged structures or privileged substructures. Chem. Rev. 2003; 103(3): 893-930.
4. Shiba S, El-Khamry AA, Shaban ME, Atia KS. Synthesis and antimicrobial activity of some bis-quinazoline derivatives. Pharmazie 1997; 52:189.
5. Abdel-Hamid SG, Synthesis of some New Heterocyclic Systems Bearing 2-. Phenyl-6-iodo-4-(3H)-quinazolinon-3-yl Moiety as Antibacterial Agents. J. Indian Chem. Soc. 1997; 74: 613-618.
6. Bartroli J, Turm, E, Alguero M: Boncompte E, Vericant ML, Conte Ramis J, Merlos M, Gracia-Rafanell JF: New azole antifungals. 3. Synthesis and antifungal activity of 3-substituted-4(3H)-quinazolinones. J. Med. Chem. 1998; 41: 1869-82.
7. Barker A, Quinazoline derivatives and their use as anti-cancer agents. Eur. Pat. 635498, Chem. Abstr., 1995; 122: 214099.
8. Bekhit, A. A.; Khalil, M: Non-steroidal anti-inflammatory agents: synthesis of novel benzopyrazolyl, benzoxazolyl and quinazolinyl derivatives of 4(3 H)-quinazolinones, Pharmazie 1998; 53: 539.
9. Gursoy, A.; Karali, N. Synthesis and anticonvulsant activity of new acylthiosemicarbazides and thiazolidones, Farmaco; 1995; 50: 857.
10. Elhelby, A. A. and Abdel-Wahab M. H., Design and synthesis of some new derivatives of 3H-quinazolin-4-one with promising anticonvulsant activity. Acta Pharm.; 2003, 53: 127-38.
11. Fisnerova, L.; Grimova, J.; Roubal, Z.; Maturova E.; Brunova, B.(1986) Esters of 3-(2-hydroxyethyl)-4(3H)-quinazolinone with an analgesic effect, Cesk. Farm; 35: 447.
12. Kumar, A.; Sharma, S.; Bajaj, A. K.; Sharma, S.; Panwar, H.; Singh, N.; Srivastava, V. K. Some new 2,3,6-trisubstituted quinazolinones as potent anti-inflammatory, analgesic and COX-II inhibitors, Bioorg. & Med. Chem.; 2003, 11 : 5293-99.
13. Terashima, K.; Shimamura, H.; Kawase, A; Tanaka, Y.; Ishizuka, Y.; Sato, M. Studies on antiulcer agents. IV. Antiulcer effects of 2-benzylthio5,6,7,8-tetrahydro-4(3H)-quinazolinones and related compounds, Chem. Pharm. Bull.; 1995, 43: 2021.
14. Raffa, D.; Dailone, G.; Maggio, B.; Sehillaci, D.; Plescia, F. Synthesis and antiproliferative activity of novel 3-(indazol-3-yl)-quinazolin-4(3H)-one and 3-(indazol-3-yl)-benzotriazin-4(3H)-one derivatives, Arch Pharm.;199, 332: 317-320.
15. Hour, M. J.; Huang, L. J.; Kuo, S. C.; Xia, Y.; Bastow, K.; Nakanishi, Y.; Hamel, E.; Lee, K. H. 6-Alkylamino- and 2,3-dihydro-3'-methoxy-2-phenyl-4-quinazolinones and related compounds: their synthesis, cytotoxicity, and inhibition of tubulin polymerization, J. Med. Chem.; 2006, 43: 4479-87.
16. Skold, O., Sulfonamide resistance: mechanisms and trends. Drug Resist. Update; 2000, 3: 155–160.
17. Waterbeemd, H.V. de, Gifford E., ADMET in silico modelling: towards prediction paradise?, Nat. Rev. Drug Discov.; 2003; 2: 192-204.
18. Hou T., Wang J., Zhang W., Xu X., ADME evaluation in drug discovery. 6. Can oral bioavailability in humans be effectively predicted by simple molecular property-based rules, J. Chem. Inf. Model.; 2007, 47: 460-463.
19. <http://www.molinspiration.com>. 10/01/2013
20. Remko, M. Theoretical study of molecular structure, pK"a, lipophilicity, solubility, absorption, and polar surface area of some hypoglycemic agents, J. Mol. Struc: Theochem; 2009, 897: 73-82.
21. http://www.srcinc.com/what-we-do/databaseforms.aspx?id¼385/10/06/2013
22. Wang, R.X., Y. Fu, Lai, L.H., A new atom-additive method for calculating partition coefficients, J. Chem. Inf. Comput. Sci.; 1997, 37: 615-621.
23. Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev.;2001, 46: 3–26.
24. Wang, R.X., Y. Gao, L.H. Lai, Calculating Partition Coefficient by Atom- additive Method. Perspect. Drug Discov. Des.; 2000, 19: 47-66.
25. Zhao, Y.H., Abraham, M.H., Lee J., Hersey A., Luscombe C.H.N., Beck G., Sherborne B., Cooper I., Rate-limited steps of human

oral absorption and QSAR studies, Pharm. Res.; 2002, 19 1446-1457.

26. Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., and Kapple, K. D., Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem.;2002, 45: 2615–2623
27. Refsgaard, H. H., Jensen, B. F., Brockhoff, P. B., Padkjaer, S. B., Guldbrandt, M., and Christensen, M. S., In-silico prediction of membrane permeability from calculated molecular parameters. J. Med. Chem.; 2005, 48: 805–811.
28. http://www.organic-chemistry.org/prog/peo/
29. Lafitte, D., Lamour, V., Tsvetkov, P.O., Makarov, A.A., Klich, M., Deprez, P., Moras, D., Briand, C., Gilli, R.; DNA gyrase interaction with coumarin-based inhibitors: the role of the hydroxybenzoate isopentenyl moiety and the 5'-methyl group of the noviose, Biochemistry; 2002, 41: 7217-7223.
30. Seeliger D. and Bert L. de Groot, Ligand docking and binding site analysis with PyMOL and Autodock/Vina, J Comput Aided Mol Des.; 2010, 24(5): 417–422. doi: 10.1007/s10822-010-9352-6, PMCID: PMC2881210.
31. Pandeya S. N., Agarwal A. K., Anita Singh, Stables J. P., Design and synthesis of semicarbazones and their bioisosteric analogues as potent anticonvulsants: The role of hydrogen bonding Acta Pharm.; 2003, 53: 15–24
32. Collins, A.H., Microbiological Methods, second ed. Butterworth, London, 1976.
33. K. K. Jha, Abdul Samad, Kumar Yatendra, Shaharyar Mohd., Khosa R. L., Jain Jainendra, Kumar Vikash, Singh Priyanka, Design, Synthesis and Antimicrobial Evaluation of 1,3,4-oxadiazole derivatives, European Journal of Medicinal Chemistry; 2010, 45: 4963-4967.
34. Verma, R.S. (Ed.), Antifungal Agents: Past, Present and Future Prospects, National Academy of Chemistry & Biology, Lucknow, India, 1998.
35. Bakht M. Afroz, M. Shahar Yar, Sami Gaber Abdel-Hamid, Saleh I. Al Qasoumi, Abdul Samad, Molecular Properties Prediction, Synthesis and antimicrobial activity of Some Newer Oxadiazole derivatives, European Journal of Medicinal Chemistry; 2010, 45: 5258-5269.