SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTI-MICROBIAL ACTIVITY OF NOVEL SUBSTITUTED QUINAZOLINE DERIVATIVES

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ABSTRACT

A series of novel substituted 8-bromo-2-(dimethylamino)-3-(3-(dimethylamino) propyl) quinazoline-4(3H)-one derivatives **2(a-h)** were prepared with excellent yields and evaluate their antimicrobial activity. These compounds **2(a-h)** are prepared the by the reactions between 8-bromo-3-(2-(dimethylamino) ethyl)-2-(methylthio) quinazoline-4(3H)-one and secondary amines. These all compounds characterization done by different spectroscopic methods PMR, IR, CMR, Mass Spectral and elemental data. All compounds were evaluated for their biological activity i.e., antibacterial and anti-fungal activity. The anti-bacterial activities were evaluated *in vitro* method. The antibacterial activity 8-bromo-2-(dimethylamino)-3-(3-(dimethylamino) propyl) quinazoline-4(3H)-one derivatives **2(a-h)** was assessed against three representative Gram-positive bacteria viz. *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus*, and three Gram-negative bacteria viz. *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum*, and antifungal activity against *Candida albicans*, *Aspergillus fumigates*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Among all these compounds most of the compounds were exhibit more potent anti-bacterial and anti-fungal activity against standard drugs.

Keywords: Synthesis, 8-bromo-2-(dimethylamino)-3-(3-(dimethylamino)propyl)quinazolin-4(3H)-one derivatives, Anti-microbial activity.

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INTRODUCTION

Past works report that so many heterocyclic compounds consist so many numbers of biological activity¹⁻⁸ due to those cycles containing heteroatoms. Anti-bacterial, anti-fungal, anti-inflammatory⁹⁻¹⁴. It is a multi-scale response to stress required for repair and regeneration after injury¹⁵⁻¹⁷. Many paths physiological path-ways like cytokines, interleukin, NF-kB, protein kinases (Adenosine Monophosphate-activated protein Kinase-AMPK), tyrosine kinases and various immunological responses regulate and mediate the process of inflammation¹⁸⁻²¹. In this process, prostaglandin synthesis is a vital step where cyclooxygenase 2 (COX-2) enzyme is one of the two key enzymes. In the second step, COX reduces PGG2 to PGH2^{22,24}. COX is majorly involved in the process of inflammation and is the targeted protein for most of the NSAIDs (non-steroidal anti-inflammatory drugs). It is also found to be a key protein in various physiological processes like gastric secretions²⁵ gastrointestinal motility and in other pathological conditions like inflammation, arthritis and colon cancer²⁶. Quinazolinone derivatives were previously reported as inhibitors of various enzymes involved in process of inflammation (COX, prostaglandin E2)²⁷, allergic reactions (Histamine H₃ receptor inhibition)²⁸, and also in the tumor-suppressing process through interacting with DNA, tubulin and thus acting as anticancer agents²⁹. Some series of Quinazolinone derivatives were also shown to have remarkable antimicrobial and antifungal properties³⁰. In addition, molecules with Quinazolinone scaffold act as regulators of calcium and sodium at cellular membranes by inhibiting sodium/calcium exchange process³¹.

Our interests in finding novel substituted quinazolinone leads with anti-microbial activity led to the design and synthesis of 8-Bromo-2-(dimethylamino)-3-(3-(dimethylamino) propyl) quinazoline-4(3H)-one derivatives. In the present investigation, we have to synthesized and evaluate tri-substituted quinazolinone derivatives as anti-microbial agents. Rationale behind the design and development of 8-bromo-2-(dimethylamino)-3-(3-(dimethylamino) propyl) quinazoline-4(3H)-one derivatives is to increase overall biological activity and in order to maintain the efficacy of the molecules, alkyl linked amino dialkyl groups were introduced at R₁ and aromatic /secondary amine systems were substituted at R₂. These substitutions are expected to increase the hydrophobic character. Further, we are also attentive to identify the possible efficient for the respective anti-bacterial and anti-fungal activity.

EXPERIMENTAL

Material and Method

In this experiment, all reagents are used analytical reagent grade obtained from Sigma-Aldrich, Merck, SD fine and avra chemicals. With using standard procedures we purified Water, methanol, acetone, ether etc. 6- benzyl-3-(5-methyl-1-(naphthalene-1-yl)-1H-1,2,3-triazole-4-yl)-7H-[1,2,4] triazolo [3,4-b][1,3,4]thiadiazine compounds ¹H NMR and ¹³C NMR spectra were recorded on Bruker 300 MHz NMR instrument using tetra methyl silane (TMS).as internal standard compound and coupling constants (*J*) are reported in Hz units. IR spectra of the compounds were recorded using KBr pellets in the range 4000–600 cm⁻¹ on Perkin-Elmer Infrared model 337. The percentage composition of C, H, N of the compounds was determined by using micro analytical techniques on Perkin Elmer 240C (USA) elemental analyzer. All reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates from Merck, and compounds visualized either by exposure to UV light.

General Procedure

Preparation of methyl 3-bromo-2-(methylthiocarbonothioyl)benzoate (II)

To a vigorously stirring solution of mono bromo anthranilic acid ; 0.01 mole) in dimethyl sulfoxide (20 ml) sodium hydroxide (1.2 ml; 20 N) and carbon disulfide (0.015 moles) were added alternately, dropwise over a period of 30 min. After complete addition, the solution was stirred for another 30 m and was cooled to less than 15^oC-20^oC using a freezing mixture. To this solution dimethyl sulfate (0.01 mole) was added dropwise for 30 min. The solution was stirred for 3-4 hr at room temperature. The resulting mixture was poured onto crushed ice (100 ml) with stirring. The product was extracted with chloroform (4 x 20 ml), which on evaporation yielded a yellow resinous product. This crude product was used for the next step without purification³².

IR (cm⁻¹) $\nu_{\max}$:3353.2,3056.4,2960,1715.4,1670,1645.7,1070.8,783.2,586.4,¹H NMR (DMSO-d₆, 300 MHz) δ :2.0(s,3H,-SCH₃),3.88(s,3H,-OCH₃), 4.0(s,1H,NH),7.4-7.9(m, *J* = 8.4 Hz, 3H,Ar-H.),¹³C NMR (DMSO-d₆,75 MHz) δ :21.0, 51.5, 119.9, 122.6, 127.4, 129.2, 136.3, 143.9, 166.0, 199.9.Mass: *m/z* 319 (M⁺),321(M+2) *Anal.* Calcd. for C₁₀H₁₀N₄O₂S₂ Br: C, 62.12; H, 6.16; N, 18.14; S,7.23. Found: C, 65.78; H, 7.72; N, 14.37; S, 6.78. Yield: 78% , M.P: 150^oC.

Table-1

Compound No	X ₁	X ₂	n	R	R ¹	Molecular Formula	Molecular Weight	Yield (%)	M.P(^o C)
VIa	H	Br	2	CH ₃	CH ₃	C ₁₄ H ₁₉ BrN ₄ O	340.80	69	142-144
VIb	H	Br	2	CH ₃	C ₂ H ₅	C ₁₆ H ₂₃ BrN ₄ O	367.28	72	154-156
VIc	H	Br	3	CH ₃	CH ₃	C ₁₅ H ₂₁ BrN ₄ O	353.26	70	144-146
VI d	H	Br	3	CH ₃	C ₂ H ₅	C ₁₇ H ₂₅ BrN ₄ O	381.31	62	178-180
VIe	H	Br	2	C ₂ H ₅	CH ₃	C ₁₆ H ₂₃ BrN ₄ O	367.28	74	182-184

VI f	H	Br	2	C ₂ H ₅	C ₂ H ₅	C ₁₈ H ₂₇ BrN ₄ O	395.34	72	134-136
VI g	H	Br	3	C ₂ H ₅	CH ₃	C ₁₇ H ₂₅ BrN ₄ O	381.31	68	176-178
VI h	H	Br	3	C ₂ H ₅	C ₂ H ₅	C ₁₉ H ₂₉ BrN ₄ O	409.36	66	156-158

Scheme-1

Compound VI

RESULTS AND DISCUSSION

General Procedure for the Synthesis of 8-bromo-3-(2-(dimethylamino)ethyl)-2-thioxo-2,3-Dihydroquinoline-4(1H)-one (III)

The crude product from the previous step (**II**; 0.01 mole) was taken and 15 ml of dry alcohol was added. This reaction mixture was refluxed for 8 hours after the addition of appropriate alkyl amine (0.012 moles). The course of the reaction was monitored every hour by TLC. Completion of the reaction was confirmed by filter paper soaked in sodium nitroprusside failing to give pink color when exposed at the top of the condenser. Refluxing was continued until an unpleasant smell disappeared. Contents of the flask were concentrated under reduced pressure and poured into crushed ice. The product formed was filtered and dried to get 8-bromo-3-(2-(dimethylamino)ethyl)-2-thioxo-2,3- Dihydroquinoline-4(1H)-one (**III**).

IR (cm⁻¹) $\nu_{\max}$: 3340.2,3040.1,2983.3,1790.1,1670,1645,1590.9,1425,1375,845,700,¹H NMR (DMSO-d₆, 300 MHz) δ : 2.27(s,6H,-(NCH₃)₂),2.66(t,2H,-NCH₂),3.55(t,2H,-NCH₂),4.0(s,1H,-NH),7.4-7.9(m, *J* = 8.3 Hz, 3H,Ar-H),¹³C NMR (DMSO-d₆,75 MHz) δ : 45.6,53.9,55.5,121.4,127.4,129.2,130.4,135.4,141.5, 161.5, 176.4,Mass: *m/z* 327 (M⁺),329(M+2),*Anal.* Calcd. for C₁₂H₁₄N₃OSBr: C, 71.12; H, 8.27; N, 12.75,S,4.24 Found: C, 64.23; H, 7.38; N, 13.57;S,5.79. Yield: 76% , M.P: 164°C.

General Method for the Synthesis of Potassium Salt of 8-bromo-3-(2-(dimethylamino)ethyl)-4-oxo-1,2,3,4-tetrahydro quinazoline-2-thiolate (IV)

8-bromo-3-(2-(dimethylamino)ethyl)-2-thioxo-2,3- Dihydroquinoline-4(1H)-one (**III** 0.01 mole) was taken in dry alcohol (10 ml) and potassium hydroxide (0.015 mole) was added to it. This mixture was refluxed gently for 4 h, until the product completely separated out. The progress of this reaction was monitored by TLC. The product is collected by decanting the liquid.

IR(cm⁻¹) $\nu_{\max}$: 3040.32,2923.16,2678.21,1790.35,1643.60,1541.18,1458.44,1219.29, 1017.72,770.88,¹H NMR DMSO-d₆, 300 MHz) δ : 2.27(s,6H,-(NCH₃)₂),2.66(t,2H,-NCH₂),3.55(t,2H,-NCH₂), 7.4-7.9(m, *J* = 8.4 Hz, 3H,Ar-H),¹³C NMR (DMSO-d₆,75 MHz) δ : 45.6,49.5,55.6,111.2,121.4,127.4,129.2,135.4, 161.5,163.5,Mass: *m/z* 346(M⁺),348(M+2),*Anal.* Calcd. for C₁₂H₁₃N₃OSBrK: C, 74.91; H, 9.71; N, 9.85; Found: C, 70.39; H,8.58;N,10.18. Yield: 73% , M.P: 156°C.

General Procedure for the Synthesis of 8-bromo-3-(2-(dimethylamino)ethyl)-2-(methylthio)quinazoline-4(3H)-one (Va-h)

The potassium salt (**IV**; 0.01 mole) was dissolved in 10 ml of dimethyl sulphoxide and cooled to 0-10°C. Dimethyl sulphate (0.01 mole) was added slowly in a drop-wise manner. Stirring was continued for 4 hours at 15-20°C. This was poured into crushed ice and extracted with chloroform. This was dried over anhydrous sodium sulphate. The chloroform extract was evaporated to get 8-bromo-3-(2-(dimethylamino)ethyl)-2-(methylthio)quinazolin-4(3H)-one. (**Va-h**).

Va: IR(cm⁻¹) $\nu_{\max}$:3040.32,2923.16,2990.25,2678.21,1790.35,1643.60,1541.18,1458.44,1219.29, 1017.72, 770.88,¹H NMR (DMSO-d₆, 300 MHz) δ :1.82(s,3H,S-CH₃),2.27(s,6H,-(NCH₃)₂),2.66(t,2H,-NCH₂),3.55(t,2H,-NCH₂), 7.4-7.9(m, *J* = 8.4 Hz, 3H,Ar-H),¹³C NMR (DMSO-d₆,75 MHz) δ : 37.5,45.6,47.2,49.5,55.6,111.2,121.4,127.4,129.2,135.4, 161.5,163.5,Mass: *m/z* 341(M⁺),343(M+2). *Anal.* Calcd. for C₁₃H₁₆N₃OSBr: C, 62.59; H,9.25; N,9.19;S,4.31; Found: C, 60.17; H, 6.91; N, 9.01;S,5.28. Yield: 68% , M.P: 172°C.

General Procedure for the Synthesis of 2-N, N-dialkyl amino 3-(N, N-dialkylamino) alkyl-6-bromo-2-methylthio-quinazoline-4(3H)-ones (VI)

8-bromo-3-(2-(dimethylamino)ethyl)-2-(methylthio)quinazoline-4(3H)-one (**V**; 0.01 mole) was dissolved in 10 ml of isopropyl alcohol and dialkylamine (0.015 mole) was added. This reaction mixture was refluxed till the unpleasant smell disappeared. The reaction mixture was poured into crushed ice. The product formed was filtered and recrystallized from isopropyl alcohol to get 2-N, N-dialkyl amino 3-(N, N-dialkylamino) alkyl-6-bromo-2-methylthio-quinazoline-4(3H)-ones (**VI a-h**).

VIa: 8-bromo-2-(dimethylamino)-3-(2-(dimethylamino)ethyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3040.32, 2998.25, 2925.10, 2678.21, 1790.35, 1643.60, 1017.72, 770.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.80(s,6H,N-(CH₃)₂), 2.27(s,6H,-(NCH₃)₂), 2.66(t,2H,-NCH₂), 3.55(t,2H,-NCH₂), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :40.2,43.3,47.1,48.4,49.1,54.3,127.4,129.2,130.4,131.4,132.5, 150.3, 159.3, 171.2,Mass: m/z 340.8 (M⁺), 342.8(M+2),*Anal.* Calcd. for C₁₄H₁₉BrN₄O: C,59.12; H,8.59; N,14.65; Found: C, 58.17; H, 8.95; N, 12.02.

VIb: 8-bromo-2-(diethylamino)-3-(2-(dimethylamino)ethyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3040.32, 2998.25, 2925.10, 2678.21, 1790.35, 1643.60, 1017.72, 770.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.74(s,6H,(CH₃)₂), 1.80(t,6H,(CH₃)₂), 1.87(q,4H,(CH₂)₂), 2.60(t,2H,-NCH₂), 3.55(t,2H,-NCH₂), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :38.2,41.3,46.3,46.2,46.0,52.3,127.4,129.2,130.4,131.4, 132.5, 150.3, 159.3, 171.2,Mass: m/z 367.2 (M⁺), 367.2(M+2),*Anal.* Calcd. for C₁₆H₂₃BrN₄O: C,60.12; H,8.12; N,12.55; Found: C, 58.20; H, 8.05; N, 12.22.

VIc: 8-bromo-2-(dimethylamino)-3-(3-(dimethylamino)propyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3032.12,2990.25, 2885.3, 2653.21,17850.35,1625.60,1010.72,765.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.80(s,6H,N-(CH₃)₂), 2.27(s,6H,-(NCH₃)₂), 2.66 (t,2H,-NCH₂), 2.87(m,2H,-CH₂), 3.55(t,2H,-NCH₂), 4.0(s,1H,-NH), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :40.2,43.3,47.1,48.4,49.1,54.3,56.2,127.4,129.2,130.4,131.4,132.5,150.3,159.3,171.2,Mass: m/z 353.28 (M⁺),355.8(M+2),*Anal.* Calcd. for C₁₅H₂₁BrN₄O: C,71.31; H,9.29; N,12.65; Found: C, 68.17; H, 9.95; N, 11.02.

VIId: 8-bromo-2-(diethylamino)-3-(3-(dimethylamino)propyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3040.32, 2998.25, 2925.10, 2678.21, 1790.35, 1643.60, 1017.72, 770.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.72(s,6H,N-(CH₃)₂), 2.25(t,6H,-(NCH₃)₂), 2.40(q,4H,N-CH₂)₂, 2.60(t,2H,-NCH₂), 2.82(m,2H,-CH₂), 3.52(t,2H,-NCH₂), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :38.2,41.3,46.1,46.4,48.1,52.3,121.4,124.2,128.4,130.4,131.5, 145.3, 156.3, 170.2,Mass: m/z 381.31 (M⁺),383.3(M+2),*Anal.* Calcd. for C₁₇H₂₅BrN₄O: C,74.12; H,8.01; N,12.35; Found: C, 69.17; H, 9.95; N,10.02.

VIe: 8-bromo-2-(dimethylamino)-3-(2-(diethylamino)ethyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3032.32, 2908.25, 2901.10, 2651.21,1775.35,1640.60,1010.72,765.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.70(t,6H,(CH₃)₂), 1.86(q,4H,N-CH₂)₂, 2.26(s,6H,-(NCH₃)₂), 2.62(t,2H,-NCH₂), 3.53(t,2H,-NCH₂), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :38.2,39.1,42.3,46.1,46.4,48.1,53.3,126.4,128.2,131.4,130.4,131.5,149.3,158.3,169.2,Mass: m/z 361.38 (M⁺),363.38(M+2),*Anal.* Calcd. for C₁₆H₂₃BrN₄O: C,76.12; H,9.36; N,11.01;Found: C, 74.17; H, 9.90; N, 10.01.

VIIf: 8-bromo-2-(diethylamino)-3-(2-(diethylamino)ethyl)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3040.32, 2938.25, 2928.10, 2688.21, 1771.35, 1621.60,1017.72,770.85, ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.65(t,6H,(CH₃)₂), 1.86(q,4H,N-CH₂)₂, 2.26(t,6H,-(CH₃)₂), 2.86(q,4H, N-CH₂)₂, 3.25(t,2H,-NCH₂), 3.55(t,2H,-NCH₂), 7.4-7.9(m, J = 8.2 Hz, 3H,Ar-H), ¹³CNMR(DMSO-d₆,75MHz) δ :32.2,36.3,41.1,43.4,46.1,52.3,121.4,123.2,126.4,127.4, 132.5,150.3, 159.3,171.2,Mass: m/z 395.3 (M⁺),397.8(M+2),*Anal.* Calcd. for C₁₈H₂₇BrN₄O: C,76.31; H,10.12; N,10.12; Found: C, 72.17; H, 10.95; N, 10.02.

VIg: 8-bromo-3-(3-(diethylamino)propyl)-2-(dimethylamino)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3021.32,2978.25,2915.10,2628.21,1760.35,1623.60,1005.72,770.85, ¹H NMR (DMSO-d₆, 300 MHz) δ :1.65(t,6H,(CH₃)₂), 1.86(q,4H,N-CH₂)₂, 2.26(t,6H,-(CH₃)₂), 2.86(q,4H, N-CH₂)₂,

3.20(m,2H,CH₂),3.55(t,2H,-NCH₂),7.4-7.9(m, $J = 8.2$ Hz,3H,Ar-H),¹³CNMR(DMSO-d₆,75MHz) δ :40.2,43.3,47.1,48.4,49.1,54.3,127.4,129.2,130.4,131.4,132.5,150.3,159.3,171.2,Mass: m/z 381.31 (M⁺),383.3(M+2),Anal. Calcd. for C₁₇H₂₅BrN₄O: C,59.12; H,8.59; N,14.65; Found: C, 58.17; H, 8.95; N, 12.02.

VIh: 8-bromo-3-(3-(diethylamino)propyl)-2-(diethylamino)quinazolin-4(3H)-one

IR (cm⁻¹) $\nu_{\max}$: 3040.32,2998.25,2925.10,2678.21,1790.35,1643.60,1017.72,770,¹H NMR (DMSO-d₆, 300 MHz) δ : 1.60(t,6H,(CH₃)₂),1.75(q,4H,N-CH₂)₂,2.20(t,6H,-(CH₃)₂),2.80(q,4H, N-CH₂)₂,3.20(m,2H,CH₂),3.55(t,2H,-NCH₂),3.67(t,2H,-NCH₂),7.4-7.9(m, $J = 8.2$ Hz,3H,Ar-H),¹³CNMR(DMSO-d₆,75MHz) δ :32.2,40.3,42.1,44.4,47.1,52.3,125.4,127.2,128.4,130.4, 131.5,150.3,159.3,171.2,Mass: m/z 409.3 (M⁺),411.3(M+2),Anal. Calcd. for C₁₉H₂₉BrN₄O: C,72.92; H,10.59; N,13.65; Found: C, 70.16; H, 10.95; N, 10.02.

Anti-Microbial Activity

The literature survey has revealed that 2-N, N-dialkyl amino 3-(N, N-dialkylamino) alkyl-6-bromo-2-methylthio-quinazoline-4(3H)-ones derivatives are well-known exhibits their wide range of biological activities. It is, therefore, considered worthwhile to evaluate the present Quinazoline derivatives of different types synthesized and their possible antimicrobial activities. The antimicrobial activity was determined by using disc diffusion method by measuring the zone of inhibition in mm. All the compounds, (a-h) were screened in-vitro at a concentration of 50 μ g/mL for antibacterial activity against three Gram-positive *Bacillus subtilis*(*B. subtilis*), *Bacillus sphaericus*(*B.sphaericus*) and *Staphylococcus aureus*(*S.aureus*), and three Gram-negative bacteria viz. *Pseudomonas aeruginosa*(*P.aeruginosa*), *Klebsiella aerogenesa*(*K. aerogenesa*) and *Chromobacterium violaceum*(*C.violaceum*). Standard antibacterial drug SM (Streptomycin), (50 μ g/disc) was also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken. Most of the compounds were found to display well to moderate antibacterial activity against different strains of bacteria. From the Table-3, it was observed that among all the compounds tested, sulphonyl derivatives **2a,2c,2e**,and **2f** showed good to excellent activity, while the remaining derivatives showed moderate activity against all the tested bacterial strains.The results of antibacterial activity and antifungal activity are expressed in Table-3 and Table-4. An attempt has also been made to correlate their structure and activity in view of the presence of different substituents.

Table 2: Antibacterial Activity of Compounds VI(a-h).

Compd.	Minimum Inhibitory Concentration (MIC) in μ g/mL					
	<i>B. subtilis</i>	<i>B. sphaericus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. aerogenes</i>	<i>C. violaceum</i>
VIa	32	30	30	28	28	30
VIb	20	24	24	22	24	28
VIc	38	34	32	30	28	36
VIId	18	20	18	24	26	26
VIe	28	30	32	28	32	32
VIIf	32	34	34	36	32	30
VIg	20	22	24	22	24	26
VIh	22	24	24	22	24	26
SM	30	28	30	32	30	28

SM = Streptomycin.

Antibacterial Activity

The *in vitro* antibacterial activity 2-N, N-dialkyl amino 3-(N, N-dialkylamino) alkyl-6-bromo-2-methylthio-quinazoline-4(3H)-ones **VI(a-h)** was assessed against three representative Gram-positive

bacteria viz. *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus*, and three Gram-negative bacteria viz. *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum* by the broth dilution method recommended by the National Committee for Clinical Laboratory Standards. Bacteria were grown overnight in Luria Bertani (LB) broth at 37°C, harvested by centrifugation and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 50 to 0.8 µg/mL. Ten micro liters of the broth containing about 10⁵ colony-forming units (cfu)/mL of test bacteria were added to each well of a 96-well micro titer plate. Culture plates were incubated for 24 h at 37°C, and the growth of bacteria was monitored by visually and spectrophotometrically. Penicillin and Streptomycin were also screened under identical conditions for comparison. The obtained data of compounds **VI(a-h)** are presented in Table-3. It has been observed that the compounds exhibit interesting biological activity, however, with a degree of variation. In the series of **VI(a-h)**, the compounds **VIa**, **VIc**, **VIe** and **VIh** are found to be the most active against Gram-positive bacteria and the Gram-negative bacteria. The remaining compounds showed moderate to good activity against all the Gram-positive bacteria and the Gram-negative bacteria.

Fig.-1: a Graphical form of Anti-Bacterial Screening

Antifungal Activity

The compounds **VI(a-h)** were also screened for their antifungal activity against *Candida albicans* (*C. albicans*) (ATCC 10231), *Aspergillus fumigatus* (*A. fumigatus*) (HIC 6094), *Trichophyton rubrum* (*T. rubrum*) (IFO 9185), and *Trichophyton mentagrophytes* (*T. mentagrophytes*) (IFO 40996) in dimethyl sulfoxide (DMSO) by disc diffusion method. Amphotericin B was used as a standard drug and the mean inhibition zone (MZI) data were measured and compared with controls, the MZI values of the compounds screened are given in **Table-4**. The antifungal screening data showed the appreciable activity of the test compounds. Among the screened compounds, compound **VIa**, **VIc** and **VIg** showed good antifungal activity. Remaining compounds showed moderate antifungal activity against test compounds.

Table-3: Antifungal Activity of Compounds VI(a-h)

Compnd	Mean zone inhibition (MZI) ^a in 10 µg/mL			
	<i>C. albicans</i>	<i>A. fumigatus</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>
VIa	28	28	26	26
VIb	18	20	18	20
VIc	28	32	30	28
VId	20	22	26	24

VIe	22	26	24	18
VI f	18	20	22	24
VIg	30	32	32	28
VIh	24	26	21	20
Amphotericin B	26	28	28	26

^aValues are mean (n = 3).

Fig.-2: a Graphical form of Anti-Fungal Screening

Inhibition Zone in mm (--indicates no inhibitory activity)Control inhibition zone (which indicates inhibition zone of solvent) was subtracted from the inhibition zone of compounds which gives actual inhibition zone of compounds.

CONCLUSION

In conclusion, a series of quinazolinone derivatives **VI(a-h)** were prepared. The antibacterial activity of these compounds was evaluated against various bacteria's; antifungal activity was evaluated against various fungi. The compounds showed a variable degree of antimicrobial activity. Among the screened compounds, **VIc**, **VI f** and **VIg** were found to be the most active against all the microorganisms employed both for antibacterial and antifungal activity. With this set of analogs, we are now in a position to investigate the multiple biological activities of these compounds.

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