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Original Research Article

Synthesis and *In Vitro* evaluation of 6-Fluoro benzothiazole substituted quinazolinones

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ABSTRACT

Purpose: The present work was hypothesized to synthesize Benzothiazole substituted diethyl amino Quinazolinone derivatives and evaluate their antimicrobial activity.
Materials and Methods: Quinazolinones were synthesized from condensation of 2-amino benzothiazole, anthranilic acid and acetic anhydride further followed by condensation with formaldehyde and diethyl amine. Antimicrobial activity was evaluated by agar disc diffusion using organisms *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Aspergillus flavus* and *Aspergillus niger. In vitro* anti-inflammatory and antioxidant activies were studied using albumin denaturation and hydrogen peroxide radical scavenging methods.
Results: The structures of the synthesized Quinazolinone derivatives were confirmed by spectral analysis, such as IR, ^{1}H-NMR and among the derivatives screened, the compounds 4d, 4f 4g, 4h have appreciable antimicrobial activity compared to standards. The compounds (4a-4h) have least anti-inflammatory activity while compound 4g have significant antioxidant activity as that of positive control.

1. Introduction

Formerly heterocyclic compounds have been investigated for their biological activities. 2-amino benzothiazole, a heterocyclic compound containing N and S atoms serve as an eccentric and a versatile scaffold for experimental drug design[1] and its hybrids have numerous biological activities like anti-inflammatory, antitumor,[2] anthelmintic,[3] anti-tubercular,[4] anticonvulsant,[5] antimicrobial[6] and anti-corrosion activity.[7] Quinazoline nucleus is the cardinal skeleton of umpteen compounds which can be synthesized from anthranilic acid. Quinazolines CBR417 and CBR490 acts as antifilarial by eliminating bacterial endo symbiont *Wolbachia* which is essential for worm viability and reproduction.[8] Quinazoline hybrids have an enormous number of biological activities like anti-hepatitis A,[9] histone deacetylase inhibition,[10] phosphoinositide-3-kinase inhibition, antiphospholipases, antiproteases, antidiabetic.[11,12] In the present work, a novel series of diethyl amino Quinazolinone derivatives were synthesized from benzothiazoles which were screened for antimicrobial activity.

2. Materials and Methods

Purification of the synthesized compounds was done by recrystallization. The melting points were determined by open capillary method and were uncorrected. The IR and NMR spectra were recorded on ABB BOMEM

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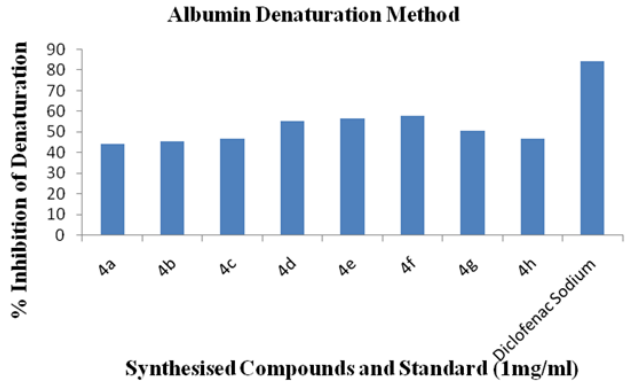

Fig. 1: Graph of in vitro anti inflammatory activity of synthesised compounds (4a-4h) and standard (Diclofenac sodium)

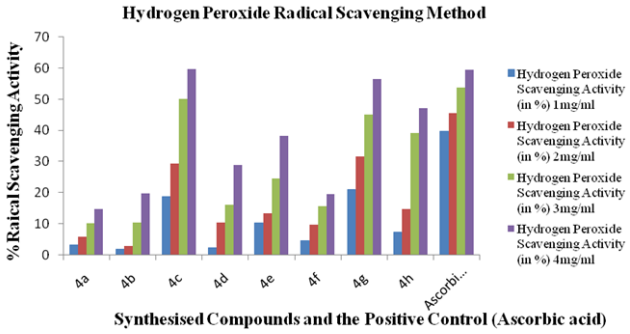

Fig. 2: Graph of *In vitro* antioxidant activity of synthesised compounds (4a-4h) and positive control (Ascorbic acid)

FTIR Spectrometer using a KBr disc and 400MHz NMR Spectrometer in DMSO using TMS as an internal standard respectively. Chemical shift (δ) was given in ppm. The absorbance of the solutions during in vitro studies were measured using UV-Visible Spectrophotometer SL-159, Elico India Ltd.

1. *Synthesis of 2-amino-6-fluoro-7-chloro-1, 3-benzothiazole*[13,14]*:* 8gm (0.08Mol) of potassium thiocyanate and 1.45g (0.01Mol) of 4-Fluoro-3-chloro aniline (Compound 1) was added to glacial acetic acid (20ml) cooled below room temperature. The above mixture was placed in an ice water bath on a magnetic stirrer. 1.6ml of bromine in 6ml of glacial acetic acid was added slowly in a dropwise manner into the mixture in such a way that the temperature never rise beyond room temperature. The stirring of solution for 2 hours below room temperature continued after complete addition of bromine. Then the mixture was further stirred a room temperature for 10 hours and allowed to stand overnight. The orange precipitate produced during overnight standing was heated with 10ml glacial acetic acid, heated to 85^0 C and filtered hot. The filtrates obtained were to 85^0C after addition of 6ml of water and filtered hot. Then again the orange residue was treated combined, cooled and neutralized with ammonia solution to a pH 6.0. The dark yellow precipitate collected was treated with activated charcoal, recrystallized with benzene and ethanol (1:1) mixture to obtain yellow crystals of 2-amino-6-fluoro-7-chloro-1, 3-benzothiazole (compound 2).
2. *Synthesis of 3-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one*[15,16]*:* 2-amino-7-chloro-6-fluoro benzothiazole (0.01 Mol) in glacial acetic acid was added to the mixture obtained from 4 hours reflux of anthranilic acid (0.01 Mol), acetic anhydride and refluxed for 4hrs. The reaction mixture was poured onto the crushed ice and kept overnight. The solid obtained was filtered, washed with cold distilled water, dried and re-crystallized from ethanol (95%).
3. *Synthesis of 1-((diethylamino)methyl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one*[17] *:* A mixture of Compound 2 (0.01mol), formaldehyde (40%, 1.5ml) and diethyl amine (0.01mol) were stirred for 4hrs in presence of methanol and left overnight at room temperature. The solid collected by filtration was washed with ethanol, dried and re-crystallized.
4. *Synthesis of 1-((diethyl amino) methyl)-3-(6-fluoro-7-(substituted phenyl amino) benzo [d] thiazol-2-yl)-2, 3-dihydro-2-methylquinazolin-4(1H)-one*[18] *:* Compound 3(0.01mol) dissolved in DMF, equimolar quantities of primary or secondary amine (0.01mol) was added and refluxed for 2hrs. The mixture was cooled and poured into crushed ice. The separated solid was filtered, dried and re-crystallized. TLC; mobile phase: n-butanol: ethyl acetate: benzene-1:2:1.

2.1. Antimicrobial screening[19]

The synthesized compounds were evaluated for antimicrobial activity by disc plate method. The antimicrobial activity was tested on Gram positive bacteria *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Gram negative bacteria Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 11775) and fungal strains *Aspergillus flavus* (NCIM 536) and *Aspergillus niger* (NCIM 548). Concentrations of synthesised compounds 50,100,150 μg/ml and 150, 200 μg/ml anti bacterial and antifungal activities were compared with standard drugs, Ciprofloxacin and Ketoconazole respectively using solvent control, DMSO. The results were described in Table.3 and 4.

2.2. *In vitro anti-inflammatory activity*[20]

The Quinazolinone derivatives (4a-4h) were screened for the antiinflammatory activity using inhibition of albumin denaturation technique. The 1mg/ml concentration of standard and test compounds were dissolved in a minimum amount of dimethyl sulphoxide (DMSO). The test solution (1 ml) containing sythesized compound was mixed with 1 ml of egg albumin solution (1% mM in phosphate buffer) and incubated at $37^0 \pm 1^0$C in incubator for 15 min. The denaturation of protein was induced by keeping the reaction mixture in a water bath for 10 min at $60^0 \pm 1^0$C. The turbidity produced after cooling was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Each experiment was done in triplicate and average was taken. The Diclofenac sodium was used as standard drug. Percentage of inhibition of denaturation was calculated from the formula below.

$$\% \text{ inhibition} = \left(\frac{\text{Absorbance of test}}{\text{Absobance of control}} - 1\right) \text{X } 100$$

2.3. *In vitro antioxidant activity*[21]

The Quinazolinone derivatives(4a-4h) were evaluated in vitro for antioxidant activity using hydrogen peroxide scavenging method. The concentrations of the synthesized compounds (4a-4h) 1-5μg/ml, the positive control(ascorbic acid) dissolved in DMSO were added to 2 ml hydrogen peroxide(40mM was added and the volume was made to 10 ml with phosphate buffer saline (pH-7.4). A control solution was prepared with DMSO in phosphate buffer saline without drug. The absorbance at 230nm was recorded using U.V spectrophotometer against blank (Phosphate buffer saline). The % inhibition of hydrogen peroxide scavenging activity was calculated using the following formula:

$$\% \text{ Radical Scavenging activity} = \left(\frac{\text{Absorbance of test}}{\text{Absorbance of control}} - 1\right) \text{X } 100$$

3. Results and Discussion

All the synthesized compounds were first purified by successive crystallization using appropriate solvents. The physicochemical properties of the synthesized compounds were tabulated in table no.1 and compounds were subjected to spectral analysis such as IR, ^{1}H-NMR.

3.1. *Spectral analysis*

1. 1-((diethylamino)methyl)-3-(7-(2-methoxyphenylamino)-6-fluorobenzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one: IR (KBr, cm^{-1}): 3039.42(Ar-NH), 1306.11(Ar-CH), 1630(C=N), 1196(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.2(s, Ar-H, 11H), 2.97(m, N-CH_2, 3H), 8.43(s, $CONH_2$, 1H), 3.1(d, NH, 1H), 5.28(s, CH_3, 1H).
2. 1-((diethylamino)methyl)-3-(7-(o-tolylamino)-6-fluorobenzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one: IR (KBr, cm^{-1}): 3041.56(Ar-NH), 1229.11(Ar-CH), 1604(C=N), 1643(ArC-O), 1190(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.4(m, Ar-H, 11H), 3.8(s, O-CH_3, 3H), 8.43(s, $CONH_2$, 1H), 3.31(d, CH-H_B, 1H), 5.28(s, CH-Cl, 1H), 3.1(d, Ar-NH_2, 2H), 2.93(t, N-CH_2-C, 2H).
3. 1-((diethylamino)methyl)-3-(6-fluoro-7-(phenethylamino)benzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one: IR (KBr, cm^{-1}): 3475 (Ar-NH), 1281(C-S), 1645(C=O), 1198(C-S). ^{1}H-NMR (DMSO, δ in ppm): 7-9(s, Ar-H, H), 2.97(m, N-CH_2, 3H), 8.43(s, $CONH_2$, 1H), 7.28(m, CO-CH, 1H), 7.48 (d, Ar-NH_2, 2H), 8-8.5(d, NH_2, 2H).
4. 1-((diethylamino)methyl)-3-(6-fluoro-7-morpholinobenzo[d]thiazol-2-yl)-2,3-dihydro-2-methylquinazolin-4(1H)-one: IR (KBr, cm^{-1}): 3012.56(Ar-CH), 1356.3(Ar-NH), 1595(C=N), 1160(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.5(m, Ar-H, 11H), 4.1(s, N-H, 1H), 8.43(d, $CONH_2$, 1H), 2.97(m, N-CH_2, 4H)

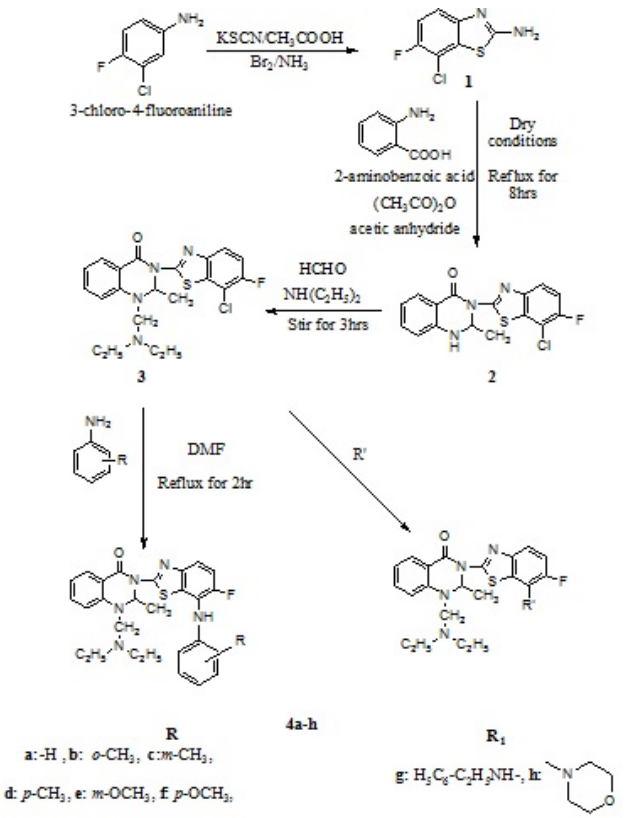

Table 1: Physical characterization of the synthesized compounds

Compound	Molecular Formula	Molecular Weight	R_f	M.P (^{O}C)	% Yield	Elemental analysis (Calculated)		
						C	H	N
4a	$C_{28}H_{30}O_2SN_5F$	519	0.65	107	65%	59.82	4.05	13.42
4b	$C_{28}H_{30}O_2SN_5F$	519	0.64	108	67%	59.82	4.05	13.42
4c	$C_{28}H_{30}O_2SN_5F$	519	0.67	110	70%	59.82	4.05	13.42
s4d	$C_{28}H_{30}OSN_5F$	503	0.71	160	68%	61.72	4.18	13.84
4e	$C_{28}H_{30}OSN_5F$	503	0.71	159	59%	61.72	4.18	13.84
4f	$C_{28}H_{30}OSN_5F$	503	0.73	162	69%	61.72	4.18	13.84
4g	$C_{28}H_{32}OSN_5F$	505	0.72	103	71%	62.36	4.46	13.47
4h	$C_{32}H_{38}O_4SN_5F$	607	0.69	96	69%	56.85	4.36	14.41

Table 2: Antibacterial activity of the synthesized compounds

Compound	Diameter of zone of Inhibition in millimeters											
	Bacillus subtilis			Staphylococcus aureus			Klebsiella pneumonia			Escherichia coli		
	Concentration (μg/ml)											
	50	100	150	50	100	150	50	100	150	50	100	150
4a	-	8	10	11	9	8	9	9	9	9	8	7
4b	10	11	12	8	11	12	10	7	9	9	7	-
4c	9	10	12	14	11	10	14	10	-	-	10	12
4d	11	12	12	11	8	10	14	11	12	11	10	11
4e	10	11	9	6	8	-	11	8	6	13	10	9
4f	9	9	14	11	8	9	12	10	11	12	12	10
4g	9	12	12	12	12	10	11	13	11	12	12	12
4h	-	13	14	9	8	11	14	12	14	13	11	13
Ciprofloxacin	15	13	14	13	11	13	15	13	13	15	15	14

Table 3: Antifungal activity of the synthesized compounds

Compound	Diameter of zone of inhibition (in mm)			
	Aspergillus flavus		Aspergillus niger	
	Concentration in μg/ml			
	150	**200**	**150**	**200**
4a	6	9	7	10
4b	8	12	9	11
4c	11	13	11	12
4d	7	8	6	10
4e	6	9	7	11
4f	11	13	10	13
4g	7	11	8	12
4h	7	13	9	13
Ketoconazole	11	13	15	17
Control	6	9	7	10

Table 4: *In Vitro* anti inflammatory activity of the synthesized compounds

S. No	Compound	Absorbance value (Mean)	Inhibition of denaturation (in %)
1	4a	0.120	44.57
2	4b	0.121	45.78
3	4c	0.122	46.98
4	4d	0.129	55.42
5	4e	0.130	56.62
6	4f	0.131	57.83
7	4g	0.125	50.60
8	4h	0.122	46.98
9	Control	0.083	-
10	Diclofenac Sodium	0.153	84.33

Table 5: In Vitro antioxidant activity of the synthesized compounds

S.No	Compound	Hydrogen Peroxide Scavenging Activity (in %)				
		1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
1	4a	3.1	5.9	10.3	14.9	19.8
2	4b	1.83	2.99	10.58	19.9	28.4
3	4c	18.8	29.5	50.1	59.7	65.4
4	4d	2.3	10.5	16.3	28.9	36.5
5	4e	10.23	13.36	24.6	38.3	49.3
6	4f	4.6	9.7	15.7	19.7	26.9
7	4g	20.9	31.7	45.2	56.5	66.9
8	4h	7.4	14.8	39.3	47.2	57.8
9.	Ascorbic acid	39.6	45.7	53.9	59.5	68.3

4. Conclusion

The novel benzothiazole substituted Quinazolinone derivatives (4a-h) synthesized were characterized and structures were confirmed by spectral characterization like FTIR, ^{1}H NMR. The synthesized compounds were evaluated for anti microbial activity, in vitro anti-inflammatory and antioxidant activity. The compounds substituted with p-anisidine, o-toludine and morpholine at 7^{th} position on benzothiazole showed significant antibacterial activity at lower concentration (50 μg/ml) while p-anisidine, p-toludine substituted quinazolinones have significant antifungal activity. In vitro studies reveals that the synthesised compounds have less significant anti-inflammatory activity while the compound subatituted with phenyl ethyl amine showed significant antioxidant activity as that of the positive control. Further evaluation and structural modification of the compounds with significant activity produces a lead with antimicrobial and antioxidant activity.

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6. Source of Funding

None.

7. Conflict of Interest

None.

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