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Design, Synthesis, Antibacterial and Antifungal Activity of Novel 2-[(*E*)- 2-aryl-1-ethenyl]-3-(2-sulfanyl-1*H*-benzo[*d*]imidazole-5-yl)-3,4- dihydro-4-quinolinones

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Abstract: The novel 2-[(*E*)- 2-aryl-1-ethenyl]-3-(2-sulfanyl-1*H*-benzo[*d*]imidazole-5-yl)-3,4- dihydro-4-quinolinones (**4a-j**) analogs were synthesized by Knoevenagel condensation of a solution of 2-methyl-3-(2-sulfanyl-1*H*-benzo[*d*]imidazole-5-yl)-3,4-dihydro-4-quinazolinone (**3**) with aromatic aldehyde in presence of catalytic amount of piperidine . Compounds (**4a-j**) showed significant biological activity against all the standard strains. All the synthesized compounds were characterized on the basis of their IR, ^{1}H NMR, MASS spectroscopic data and elemental analyses. All the compounds have been tested for antimicrobial and antifungal activity by the cup-plate method.

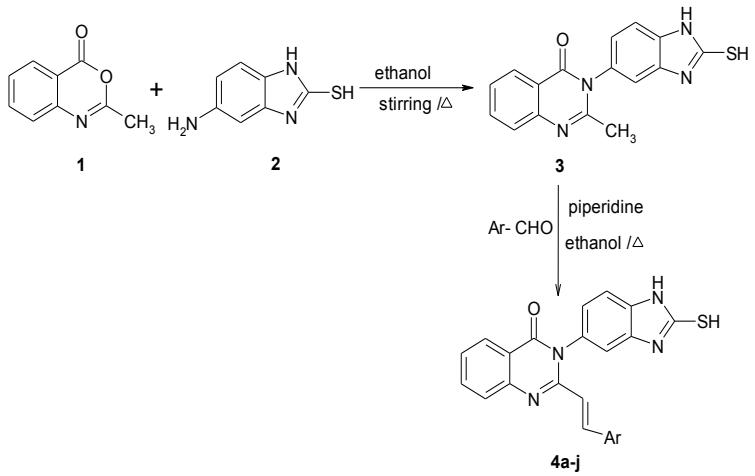
Keywords: Knoevenagel condensation, 2-Mercaptobenzimidazole, 3,4-dihydro-4-quinolinones, Antibacterial activity and Antifungal activity.

Introduction

In the present century, due to the advancement and changes in the culture and life style, new diseases are being existed among the human population which indicates that the search for better drugs is still necessary. Discovery of new drugs that is therapeutically useful and goes into clinics is a lifetime dream for medicinal chemist. Structural activity relationships of imidazole containing structure have dominated investigations in medicinal chemistry for active biological entities[1]. Many benzimidazole derivatives belong to a crucial structural motif that is seen in many pharmaceutically and biologically interesting molecules. They have been intensively used in medicinal chemistry as drugs such as antihistaminic[2], anti ulcerative[3], antihelmentic[4] and antipsychotic[5]. Some of their analogous shows an array of biological activities, including non-nucleosides HIV-1 reverse transcriptase inhibitor[6] and they are selective inhibitors of cyclooxy genase COX-2[7]. Several benzimidazoles have been

reported as antiviral[8], anticoagulant[9], anti-inflammatory[10], antibacterial[11], and anticancer agents[12]. Quinazoline-4-(3*H*)-ones have been reported to possess a wide range of biological activities such as anti-microbial [13-17], analgesic [18,19], anti-inflammatory[20], anti-convulsant[21,22], anti-cancer [23,24], anti-tubercular[25,26],anti-malarial[27], and antiviral[28,29] activities. Inspired with the biological profile of benzimidazole and quinazoline-4-(3*H*)-one, and their increasing importance in pharmaceutical and biological fields, it was thought worthwhile, to synthesize the title compounds with view to obtain certain new entities with two active pharmacophores in a single molecule frame work in order to prepare molecules having with potentially enhanced biological activities and to have then evaluated for their antimicrobial activity. On the other hand, to the best of our knowledge previously there is no report on the synthesis of novel 2-[(*E*)-2-aryl-1-ethenyl]-3-[2-sulfanyl-1*H*-benzo[*d*]imidazole-5-yl]-3,4-dihydro-4-quinolinones.

The synthesis of compounds **2**, **3** and **4** was accomplished by the synthetic sequence shown in Scheme 1. The reaction of 2-methyl benzoxazin -4-one (**1**) with 5-amino-1*H* – benzo[*d*]imidazole -2-thiol (**2**) in ethanol furnished 2-methyl-3-(2-sulfanyl-1*H*-benzo [*d*] imidazole-5-yl)-3,4-dihydro-4-quinazolinones(**3**). Compounds **3** on Knoevenagel condensation with substituted aromatic aldehydes in presences of piperidine in ethanol afforded novel 2-[(*E*)-2-aryl-1-ethenyl]-3-[2-sulfanyl-1*H*-benzo [*d*] imidazole-5-yl]-3,4-dihydro-4-quinolinones(**4**).The structures of products **3** and **4** have been elucidated on the basis of spectral (IR, ^{1}HNMR, and MS) and micro analytical data. Compound **3** displayed characteristic absorption bands in the IR spectra around 1700, 1619 and 699 cm^{-1}due to C=O, C=N, and C-S functional groups, respectively confirming the formation of sulfanyl benzo [*d*] imidazole-5-yl-dihydro-4-quinazolinone. ^{1}HNMR spectra of **3** exhibited a sharp singlet around δ 0.91, 3.54 due to CH_3, SH and 12.01 due to benzimidazole NH. Mass spectrum of the product **3**, agrees well with the structure, which showed a molecular ion peak at $[M]^+$ at *m/z* 308. The ^{1}H NMR spectrum of **4** displayed two distinct doublets around δ 6.6 and 6.8 due to styryl =CH protons, confirming the Knoevenagel condensation. The mass spectrum of **4** confirmed the structure by exhibiting the molecular ion peak at $[M]^+$ at *m/z* 396. Elemental analyses are satisfactory and confirmed elemental composition and purity of the newly synthesized compounds **4.**



Scheme 1. Synthesis of 2-((*E*)-2-aryl-1-ethenyl]-3-(2-sulfanyl-1*H*-benzo [*d*] imidazole-5-yl)-3,4-dihydro-4-quinolinones.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus and are Uncorrected. The IR spectra were recorded on Nicolet impact 410 FTIR spectrophotometer using KBr pellets.[1]HNMR spectra were recorded on bruker Ac 300 MHz instrument using $CDCl_3$ and with TMS as the internal standard. The mass spectra were obtained on a varian MATCH-7 instrument at 70eV Elemental analyses was carried out using Perkin-Elmer 240C CHN-analyzer.

Synthesis of 2-methyl -3-(2-sulfanyl-1H-benzo [d] imidazole-5-yl)-3,4-dihydro-4-quinazolinone (3)—General Procedure

A mixture of 2-methyl benzoxazin-4-one (**1**) (0.01 mol) and 5-amino-1*H*-benzo[*d*] imidazole-2-thiol (**2**) (0.01mol) were taken in ethanol (15 mL). The reaction mixture was refluxed while stirring for about 2 h. The residue was purified by recrystallization from ethyl acetate to produce **3**. yield 70%, m.p.:185-187^{o}C; Anal. Calcd. for $C_{16}H_{12}N_4OS$ (308.36): C, 62.32; H, 3.92; N, 18.17; S, 10.40. Found: C, 62.29; H, 3.94; N, 18.19; S, 10.38. IR (KBr, cm^{-})[1]: 1700 (C=O), 1619 (C=N), 699 (C-S); ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.91 (s, 3H, CH_3), 3.54 (s, 1H, SH), 6.31-7.54 (m, 7H, ArH), 12.01 (s, 1H, benzimidazole-NH D_2O exchangeable).EI-MS $[M]^+$ at *m*/*z* 308.

2-((E)-2-phenyl-1-ethenyl)-3-(2-sulfanyl-1H-benzo [d] imidazole-5-yl)-3,4- dihydro-4-quinazolinone (4a-j —General Procedure

To a solution of 2-methyl-3-(2-sulfanyl-1*H*-benzo[*d*]imidazole-5-yl)-3,4-dihydro-4-quinazolinone (**3**) (0.01mol) in ethanol (25 mL), aromatic aldehyde (0.01mol) was added with catalytic amount of piperidine (0.5 mL) and the contents were refluxed for 3 h. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distilled water, filtered, dried in vacuum and recrystallized from ethyl acetate (Scheme 1). Similarly remaining compounds (**4a-j**) was prepared by above method. The characterization data of these compounds is described in Tables 1 and 2.

Antibacterial Activity

Antibacterial activity of **4a-j** in DMSO was performed by the broth dilution method using nutrient agar against Gram-negative bacteria *Pseudomonas aeruginosa* (NCCS2200), *Escherichia Coli* (NCCS2065), and Gram-positive bacteria *bacillus cereus* (NCCS2106), and *Staphylococcus aureus* (NCCS2079) at 100μg/mL concentration. The minimum inhibitory concentration was done by the broth dilution method (*30*). Cefaclor was used as standard for comparison. The readymade nutrient broth media (Himedia, 24g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound was dissolved in DMSO and a concentration of 250 μg/mL of the test compounds was added in the first test tube, which was serially diluted. A fixed volume of 0.5 mL of overnight culture was added in all the test tube which were incubated at 37^{o}C for 24 h. After 24 h, these tubes were measured for turbidity. Results are given in Table 3.

Table 1. physical data of compounds **4a-j.**

Comp	Ar	M.F.	M. P °C	Yield , %	Elemental analysis, %					
					C		H		N	
					Found	Calcd	Found	Calcd	Found	Calcd
4a	C_6H_5	$C_{23}H_{16}N_4OS$	230	70	69.68	69.65	4.07	4.05	14.13	14.19
4b	2-OHC_6H_4	$C_{23}H_{16}N_4O_2S$	280	85	66.97	66.99	3.91	3.87	13.58	13.53
4 c	2- ClC_6H_4	$C_{23}H_{15}N_4OSCl$	270	65	64.11	64.08	3.51	3.54	13.00	13.03
4 d	4- ClC_6H_4	$C_{23}H_{15}N_4OSCl$	272	68	64.11	64.13	3.51	3.49	13.00	13.02
4 e	4-$OCH_3C_6H_4$	$C_{24}H_{18}N_4O_2S$	265	65	67.59	67.63	4.25	4.28	13.14	13.18
4 f	4- N $(CH_3)_2$ C_6H_4	$C_{25}H_{21}N_5OS$	210	70	68.32	68.37	4.82	4.83	15.93	15.09
4 g	4-$CH_3C_6H_4$	$C_{24}H_{18}N_4OS$	230	72	70.22	70.18	4.42	4.38	13.65	13.67
4h	2- BrC_6H_4	$C_{23}H_{15}N_4OSBr$	285	68	58.11	58.08	3.18	3.16	11.79	11.74
4i	4-$NO_2C_6H_4$	$C_{23}H_{15}N_5O_3S$	280	78	62.58	62.55	3.42	3.46	15.86	15.89
4 j	4- BrC_6H_4	$C_{23}H_{15}N_4OSBr$	284	78	58.11	58.09	3.18	3.20	11.79	11.76

Table 2. Spectral data of the compounds **4a-j.**

Compd.	IR, Cm^{-1}, (KBr)	^1H NMR ($CDCl_3$)	MASS (*m/z*) M^+
4a	1710 (C=O), 1605 (C=N), 618 (C-S)	3.52 (s, 1H, SH), 6.65(d, *J* =15.2 Hz, 1H, CH=CH), 6.85-7.86 (m, 12H, ArH and 1H, CH=CH), 12.10 (1H,benzimidazole-NH D_2O exchangeable)	396
4b	1705 (C=O), 1625 (C=N), 686 (C-S)	3.84 (s, 1H, SH), 6.31 (*d*, *J* = 15.2Hz, 1H, CH=CH), 6.93-7.84 (m, 11H, ArH and 1H, CH=CH), 8.43 (bs, 1H, OH, D_2O exchangeable), 11.12 (s, 1H, benzimidazole-NH D_2O exchangeable)	412
4 c	1720 (C=O), 1615 (C=N), 695 (C-S)	3.62 (s, 1H, SH), 6.58 (d, *J* =15.2 Hz, 1H, CH=CH), 7.35-7.89 (m, 11H, ArH and 1H, CH=CH),10.5 (s, 1H, benzimidazole-NH,D_2O exchangeable)	430
4 d	1715 (C=O), 1620 (C=N), 690 (C-S)	3.45 (s, 1H, SH), 6.81 (d, *J* =15.2 Hz, 1H, CH=CH), 7.26-8.17 (m, 11H, ArH and 1H, CH=CH), 9.83 (s, 1H, benzimidazole-NH D_2O exchangeable)	430
4 e	1710 (C=O), 1620 (C=N), 665 (C-S)	3.45 (s, 1H, SH), 3.62 (s, 3H, OCH_3), 7.06 (d, *J* =15.2 Hz, 1H, CH=CH), 7.18-8.18 (m, 11H, ArH and 1H, CH=CH),10.31 (s, 1H, benzimidazole-NH D_2O exchangeable)	426
4 f	1720 (C=O), 1610 (C=N), 690 (C-S)	3.12 (s, 6H, $2NCH_3$), 3.82 (s, 1H, SH), 6.72 (d, *J* =15.2 Hz, 1H, CH=CH), 7.22-8.14 (m, 11H, ArH and 1H, CH=CH), 10.25 (s, 1H, benzimidazole-NH,D_2O exchangeable)	439
4 g	1680 (C=O), 1615 (C=N), 682 (C-S)	2.8 (s, 3H, Ar-CH_3), 3.66 (s, 1H, SH), 6.80 (d, *J* =15.2 Hz,1H,CH=CH),7.22-7.85 (m, 11H, ArH and 1H ,CH=CH),9.85 (s, 1H, benzimidazole – NH, D_2O exchangeable)	410
4h	1695 (C=O), 1615 (C=N), 662 (C-S)	3.42 (s, 1H, SH), 6.80 (d, *J* =15.2 Hz, 1H, CH=CH), 7.15-7.93 (m, 11H, ArH and 1H ,CH=CH)	474
4i	1675 (C=O), 1610 (C=N), 665 (C-S)	4.01 (s,1H,SH), 6.70 (d, *J* =15.2 Hz, 1H, CH=CH), 7.22-7.80 (m, 11H, ArH and 1H, CH=CH)	441
4 j	1675 (C=O), 1618 (C=N), 685 (C-S)	3.85 (s, 1H, SH), 6.72 (*d*, *J* =15.2Hz, 1H, CH=CH),7.15-7.93 (m, 11H, ArH and 1H , CH=CH)	474

Table 3. Antibacterial activity of **4a-j**.

Compound	Minimum inhibitory concentration			
	Gram- Positive		Gram- Negative	
	S.aureus	*B.cereus*	*E.coli*	*P. aeruginosa*
4a	18	20	10	19
4b	17	21	14	17
4c	14	11	15	13
4d	11	22	11	18
4e	16	13	17	11
4f	19	16	18	16
4g	18	18	13	20
4h	15	19	16	15
4i	13	9	15	14
4j	15	20	12	17
Cefaclor	19	22	19	20

[a] Negative control (DMSO) – no activity. Values are indicated in µg/mL.

Anti fungal Activity

The antifungal activity of **4a-j** was performed by the agar cup bioassay method (*31*) using clotrimazole as the standard. The compounds were tested for their antifungal activity against two test organisms, *Aspergillus niger* (NCCS1196) and *Chrysosporium tropicum* (NCCS3474) using the agar cup bioassay method at 100 µg/mL concentration .The readymade potato dextrose agar media (Himedia, 39g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and Petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. The medium poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of the (week-old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone (100 µg/mL). Agar inoculation cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced .To each cup, 100 µg/mL of test solution was added. Controls were maintained with acetone and clotrimazole (100 µg/mL). The treated and the control were kept at room temperature for 72-96 h. Inhibition zones were measured and the diameter was calculated in millimeters. Three to four replicates were maintained for each treatment. The results are given in Table 4.

Table 4. Antifungal activity of **4a-j.**

Compound	Zone of inhibitory concentration	
	A. niger	*C. tropium*
4a	19	27
4b	50	45
4c	18	29
4d	23	26
4e	20	45
4f	15	30
4g	50	55
4h	35	19
4i	21	45
4j	45	40
Clotrimazole	29	30

[a]Negative control (acetone) – no activity.

Conclusion

From the antimicrobial screening it was observed that all the compounds exhibited activity against all organisms employed. The results of antibacterial screening (Table 3) reveal that compounds **4a-j** displayed a better activity and are more active than the standard drug cefaclor. In series **4**, compounds **4c**, **4e,** and **4i** possessing chloro, methoxy and nitro groups as substitutents on the benzene ring showed a better activity. However the degree of inhibition varied both with the test compound and with the bacteria used in the present investigation. In conclusion, almost all the compounds, **4a-j** exhibited the maximum activity by inhibiting the growth of all the four bacteria to a greater extent in comparison with the standard drug cefaclor. The antibacterial activity of some of these compounds is promising compared with standard cefaclor, and they can be exploited for the formation of bactericide after further study. The antifungal activity results (Table 4) indicated that compounds **4a-j** are significant toxic towards the two fungi and they are lethal at 100 µg/mL concentration. In series **4**, compounds **4b**, **4g,** and **4j** exhibited a high antifungal activity which may be due to the presence of hydroxyl, methyl and bromo substituents on the benzene ring. However the degree of spore germination inhibited varied with the test compounds as well as with the fungi. The antifungal activity of these compounds was compared with the standard drug clotrimazole, and they have a promising activity, when compared with standard drug clotrimazole, all the series of compounds, **4a-j** are highly toxic toward the fungi under

investigation and they are lethal even at 100 µg/mL concentration in comparison with standard clotrimazole at the same concentration.

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References

1. Gribble G W and Gilchrist T L**,** Progress in Heterocyclic Chemistry : Elsevier, United Kingdom., 2000, **121**.
2. Al-Muhaimeed H S, *J. Int. Med. Res.,* 1997, **25**, 175.
3. Richter J E, Am. J.Gastroenterol., 1997, 92, 30.
4. Hazelton J C, Iddon B, Suschitzky H and Woolley L H, Tetrahedron., 1995, 51, 10771.
5. Kyle D, Goehring R R and Shaoo B, WO. 2001039775.
6. Gardiner J M, Loyns C R Burke A, Khan A and Mahmood N, Bioorg. Med. Chem. Lett., 1995, 5, 1251..
7. Paramashivappa R, Phani Kumar P, Subba Rao P V and Srinivasa Rao A,. Bioorg. Med. Chem. Lett.,. 2003, 13, 657.
8. Li Y F, Wang G F, Luo Y, Huang W G, Tang W, Feng C L, Shi L P, Ren Y D, Zuo J P and Lu W, Eur. J. Med. Chem.,. 2007, 42, 1358.
9. Young W B, Sprengeler P, Shrader W D, Li Y, Rai R, Verner E, Jenkins T, Fatheree P, Kolesnikov A, Janc J W, Cregar L , Elrod K and Katz B, Bioorg. Med. Chem. Lett,. 2006, 16, 710.
10. Mertens A, Müller-Beckmann B, Kampe W, Holck J P and Vonder Saal W, J.
a. Med. Chem. 1987, 30,1279.
11. Vinodkumar R, Vaidya S D, Siva Kumar B V, Bhise U N, Bhirud S B and Mashelkar U C, Eur. J. Med. Chem., 2008, 43, 986.
12. Ramla M M, Omar M A Tokuda H and El.Diwani H I, Bioorg. Med. Chem., 2007, 15 ,6489.
13. Trivedi P B, Undavia N K, Dave A ,M Bhatt K N and Desai N C, Indian. J. Chem. Br., 1993, 32, 497..
14. Kant P, Indian. J. Heterocyc. Chem., 2006, 15, 221.
15. Srivastava M K, Mishra B and Nizamuddin, Indian. J. Chem. Br., 2001, 40,342.
16. Mishra P, Panneerselvam P and Jain S. J. Indian. Chem. Soc., 1995, 72, 559.
17. Jatav V, Jain S K, Kashaw S K and Mishra P, Indian. J. Pharm. Sci.,2006, 68, 360.
18. Alagarsamy V, Rajasolomon V, Vanikavitha G Paluchamy V, Ravi Chandran M, Arnold Sujin A, Biol. Pharm. Bull.,. 2002, 25, 1432.
19. Pannerselvam P, Pradeepchandran R V, Sridhar S K, Indian. J. Pharm. Sci., 2003, 65, 268.
20. Rani P, Archana Srivastava V K and Kumar A, Indian. J. Chem. Br.,.2002, 41, 2642.
21. Archana,Srivastava V K, Chandra R, Kumar A, Indian. J. Chem. Br., 2002, 41, 2371.
22. Alagarsamy V, Thangathirupathi A, Mandal S C Rajasekaran S Vijayakumar S and Revanthi R, Indian. J. Pharm. Sci.,2006, 68,108.
23. El-Hiti G A , Abdel-Megeed M F and Zied T, M. Indian. J. Chem. Br., 2002, 41,1519.
24. Murgan V, Thomas C C, Rama Sarma G V and Kumar E P , Indian. J. Pharm. Sci., 2003, 65, 386.
25. Pattan S R, Krishna Reddy V V, Manvi F V, Desai B G, Bhat A R, Indian.
26. J.Chem. Br., 2006, 45, 1778.

27. Nandy P, Vishalakshi M T and Bhat A R, Indian. J. Heterocycl. Chem., 2006, 15, 293.
28. Lakhan R, Singh O P and Singh R L , J. Indian. Chem. Soc., 1987, 64, 316.
29. Bishnoi A, Saxena R, Srivastava K, Joshi M N and Bajpai S K, Indian. J.
30. Heterocycl. Chem., 2006, 15, 307.
31. Pandey V K and Mukesh Tandon M, Indian. J. Heterocycl. Chem., 2006, 15, 399.
32. National Committee for Clinical Laboratory Standards (NCCLS) standard methods for Dilution antimicrobial susceptibility tests for bacteria, which grows aerobically;Nat.Comm.Clini Lab stands,Ltd., Villanova., 1982, 242.
33. Margery Linday,E.Practical Introduction to Microbiology;E & F.N. Spon Ltd., 1962, 177.