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Substituted 3-((*Z*)-2-(4-nitrophenyl)-2-(1*H*-tetrazol-5-yl) vinyl)-4*H*-chromen-4-ones as novel anti-MRSA agents: Synthesis, SAR, and in-vitro assessment

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ABSTRACT

In search for a new antibacterial agent with improved antimicrobial spectrum and potency, we designed and synthesized a series of novel 3-((*Z*)-2-(4-nitrophenyl)-2-(1*H*-tetrazol-5-yl) vinyl)-4*H*-chromen-4-ones **7a–h** by convergent synthesis approach. All the synthesized compounds were assayed for their in-vitro antibacterial activities against gram-negative and gram-positive bacteria. The preliminary structure-activity relationship, to elucidate the essential structure requirements for the antimicrobial activity that results into anti-MRSA (methicillin-resistant *S. aureus*) potential, has been described. Amongst the synthesized compounds **7d**, **7e, 7f and 7h** were found to possess activity against methicillin-resistant *S. aureus* in addition to the activity against other bacterial strains such as *E. faecalis, S. pneumoniae,* and *E. coli*.

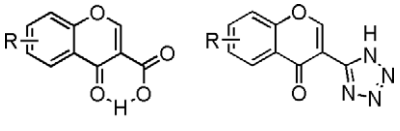
Owing to the emergence of multi-drug-resistant strains in recent decades, microbial diseases have become more complex to tackle as compared to the first half of the last century. To combat such microbial infections, various synthetic and semi-synthetic antimicrobial drugs have been discovered and used in the clinical practice. In spite of significant developments in the antimicrobial therapy, problems such as resistance development, spectrum of activity, potency and toxicity remain unresolved for most of the available antimicrobial drugs. Hence, the discovery and development of novel antimicrobial agents with optimized pharmacological profile together with increased activity towards resistant strains are highly desirable.

Chromones are a class of naturally occurring compounds that show interesting biological and pharmacological activities coupled with low toxicity.[1a,b]

Chromones having heterocyclic substituents at 2 and 3 positions have been reported to possess antiallergic activity[2] muscular relaxation effect and antimicrobial activity.[3] On the other hand, flavones and their derivatives are commonly used as precursors for many pharmaceutical products such as anticancer agents.[4] It is also well recognized that incorporation of tetrazole into the chromone skeleton can significantly enhance the antiallergic activity.[5]

Tetrazole is a class of heterocycles with wide range of applications that is receiving considerable attention.[6] This functionality has been frequently used as metabolically stable surrogates for carboxylic acid group as well as lipophilic spacers.[7] Attempts to replace the carboxylic acid group with a tetrazole ring have been made with chromones.[8a,b] Since 1*H*-tetrazole generally shows acidity comparable with Carboxylic acid (Fig. 1), 3-(1*H*-tetrazole-5-yl)-chromone derivatives **2** (R = H; $pK_a = 5.85$), which proved to be stronger acid than **1** (R = H; $pK_a = 8.85$), were found to be active, not only when administered intravenously but also orally. Inactivity of **1** was attributed to its weak acidity due to intramolecular hydrogen bond formation.[9]

In spite of possessing similar steric demand, fluorine and hydrogen analogues differ significantly in their chemical and biological properties. Fluorinated analogues are generally poorer substrates for metabolic enzymes, as compared to the hydrogen analogues. Moreover -F at 6-position in the quinolone structure has invariably



1 **2**

Figure 1. Chromones with Carboxylic acid and 1H-tetrazole.

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been associated with improved potency resulting in to the development and clinical usage of a range of fluoroquinolones for bacterial infections. It is widely accepted that one or just a few atoms in an organic molecule can dramatically alter its chemical and biological nature, including its potency, stability, lipophilicity and bioavailability. Thus fluorinated analogues are desired options for derivatization with the objective of arriving at a potent and pharmacologically acceptable new chemical entity. Various chemotherapeutic agents containing fluorine moiety are available for clinical use,[10] and some of them are depicted in Figure 2.

The presence of tetrazole and other active groups like chromone in a single molecule has rarely been divulged. Owing to the versatile bioactivities exhibited by chromones, tetrazole and potential of fluorine, we directed our efforts to generate libraries of diverse chromones. In continuation of our work on bioactive chromones, we now wish to disclose our results towards the synthesis and in-vitro antibacterial assessment of substituted 3-((*Z*)-2-(4-nitrophenyl)-2-(1*H*-tetrazol-5-yl)-vinyl)-4*H*-chromen-4-ones by convergent strategies.

Condensation reactions of 3-formyl chromones with compounds containing active methylene group have been extensively studied.[11–14] Such kind of condensation reactions are achieved by using either acid or base catalyst. Similarly, the condensation reactions of 3-formyl chromones with hydrazine, mono substituted hydrazine, hydroxylamine and guanidine have also been studied.[15–17]

Based on such reports, we designed linear as well as convergent route to synthesize **7a–h**. In convergent fashion, *p*-nitrobenzylcyanide **4**[18] obtained by nitration of benzyl cyanide was converted into corresponding tetrazole derivative **5**[19] in 87% yield, by refluxing a mixture of **4**, zinc bromide and sodium azide in water for 24 h (Sharpless method)[20–22] Scheme 1. The compound **4** was then condensed swiftly with 3-formylchromones **6a–h**[23] in pyridine[24] under reflux to afford title compounds **7a–h**[25] selectively as a (***Z***) regioisomer in 40–88% yields Scheme 2. The (***Z***) regiochemistry of the double bond is confiremed by NOE experiment.[26]

However in linear fashion, the condensation of *p*-nitro-benzyl cyanide **4** was carried out with 3-formylchrom-ones **6a** in the presence of sodium acetate in acetic anhydride at 80 °C to give compound **8a**[27] in 81% yield. Conversion of the cyano group of **8a** into the tetrazole moiety **7a** was attempted under different conditions,[28a–e] but surprisingly the reactions failed in all the tested conditions Scheme 3.

All the tetrazolyl chromones **7a–h** were assayed for their in-vitro antibacterial activity by MIC determination against a panel of pathogenic bacterial strains such as *E. coli* (ATCC 25922), *S. aureus*

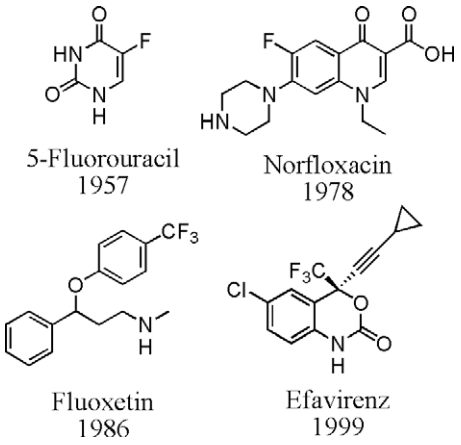
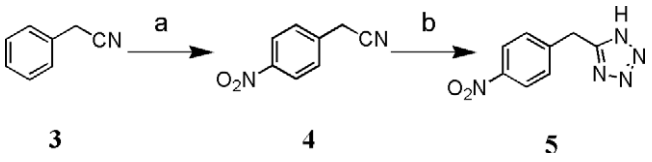
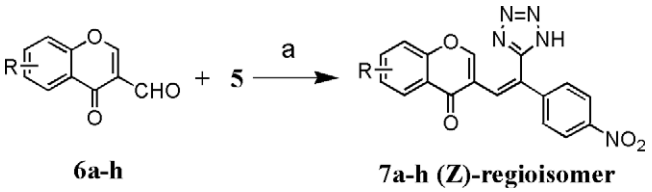


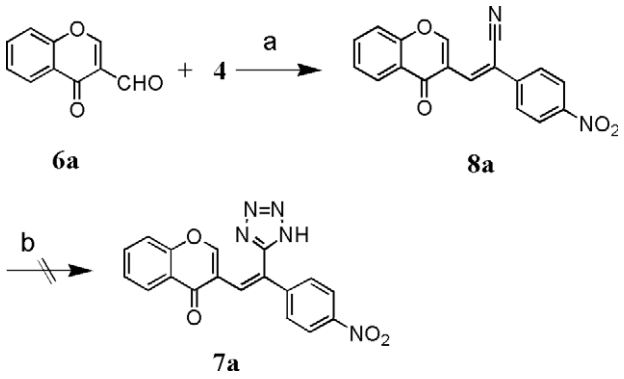
Figure 2. Fluorinated drugs.



Scheme 1. Synthesis of 5-(4-nitrobenzyl)-1H-tetrazole **5**. Reagents and conditions: (a) Conc. H_2SO_4/HNO_3, 0 °C, 58%; (b) NaN_3, $ZnBr_2$, water, reflux, 87%.



Scheme 2. Convergent approach. Reagent and condition: (a) Dry pyridine, reflux, 40–88%.

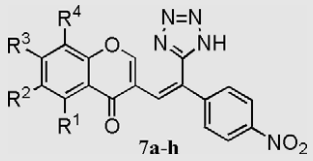


Scheme 3. Linear approach. Reagent and conditions: (a) Sodium acetate, acetic anhydride, 80 °C, 81%; (b) NaN_3, $ZnBr_2$, water, reflux.

(ATCC 13709 'Smith'), *S. aureus* 032, *E. faecalis* (ATCC 29212) and *S. pneumoniae* (ATCC 49619). *S. aureus* 032 is an methicillin-resistant *S. aureus* (MRSA) strain and was obtained as a clinical isolate,[29] and remaining strains were obtained from ATCC, USA. *S. aureus* (ATCC 13709 'Smith') is a wild type methicillin sensitive strain. Reference strains used as quality control for MIC testing included *S. aureus* ATCC 29213. Erythromycin, Gentamicin, Ampicillin and Ciprofloxacin were recovered from their commercial preparations. The purities and potencies of the agents recovered from commercial preparations were documented by ascertaining the purity of >98.5% by high-pressure liquid chromatographic analysis and by showing that the MICs of antibacterials were within acceptable limits against quality control strains.

MICs were determined as per CLSI (Clinical and Laboratory Standards Institute) recommendations on Mueller Hinton Agar containing serial twofold dilutions of drugs.[30] For each strain, approx. 10^4 CFU were applied per spot using a multipoint inoculator (Applied Quality Services, UK). Incubations were done at 35 °C, and growth was scored at 24 h. In MIC studies twofold differences were confirmed by a third repetition and more frequent results were reported. MICs employing *S. pneumoniae* were determined as per CLSI recommendations on Mueller Hinton Agar supplemented with yeast extract (0.25%, Difco, USA), glucose (0.5%, Sigma) and sheep blood (5%). The incubation was carried out in a CO_2 incubator (5% CO_2).[30] Dimethyl sulfoxide and potent antibacterial drugs Erythromycin, Ampicillin, Gentamicin and Ciprofloxacin were used as solvent control and standards, respectively. The results are furnished in Table 1.

Table 1
Antibacterial activities of substituted 3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-ones



7a-h

Entry	R^1	R^2	R^3	R^4	Antibacterial activity (MIC, μg ml^{-1}) Microorganism				
					S. aureus[a]	*S. aureus*[b]	*E. faecalis*	*S. pneumoniae*	*E. coli*
7a	H	H	H	H					
7b	H	Cl	H	H	>32	>32	>32	>32	>32
7c	H	Br	H	H	>32	>32	>32	>32	>32
7d	H	F	H	H	8.0	0.5	4.0	0.5	>32
7e	H	Cl	H	Cl	16	2.0	8.0	1.0	>32
7f	H	Cl	CH_3	H	16	4.0	16	16	>32
7g	H	CH_3	H	H	08	>32	>32	>32	>32
7h	H	CH_3	H	CH_3	8.0	2.0	16	16	>32
Erythromycin					0.5	>32	2.0	0.12	>32
Ampicillin					0.25	>32	0.5	0.06	>32
Gentamicin					0.25	0.5	>32	>32	>32
Ciprofloxacin					0.12	>32	0.5	2.0	>32

[a] *S. aureus* (ATCC 13709 'Smith').
[b] *S. aureus* 032 (Is an MRSA strain and was obtained as a clinical isolate).

The results indicate that among the tetrazolyl chromone analogues, **7a**, which is unsubstituted at **R^2** of the chromones ring, lacks significant activity. Introduction of **–Cl**, **–Br** and **–CH_3** at **R^2** of chromones ring, thereby producing analogues **7b**, **7c** and **7g**, respectively, did not alter the activity profile and continued to demonstrate the lack of activity similar to **7a** against the bacterial strains tested. However, **7g** demonstrated a modest indication of activity against methicillin sensitive strain of *S. aureus* (*S. aureus*†).

Amongst the halogens, **–F** at **R^2** shows distinct enhancement in the activity against all the bacterial strains with the exception of *E. coli*. Thus **–F** substituted compound, **7d**, exhibited significant drop in MICs (0.5 μg ml^{-1}) against *S. aureus*‡ and *S. pneumoniae* strains. As a result enhanced potency was observed with **7d** as compared to non-fluorinated analogues. This finding is in agreement with earlier report, wherein 6-fluorine substitution in Norfloxacin was shown to improve the gyrase inhibition up to 15- to 18-fold and cell penetration by another 3- to 4-fold, leading to 10- to 100-fold decrease in the MIC over its 6-hydrogen derivative.[31]

Introduction of **–Cl** at **R^4** of chromones ring, thereby producing **7e**, exhibited enhanced activity as compared to its mono-chloro analogue **7b**. Thus **7e** showed a further enhancement in the activity by at least onefold against *S. aureus*†, twofold against *E. faecalis*, fourfold against *S. aureus*‡ and fivefold against *S. pneumoniae* as compared to **7b**.

The considerable improvement in the activity was observed with **7f**, having **–CH_3** substitution at **R^3** of chromones ring. This chemical modification resulted in an increase in activity by at least onefold against *S. aureus*,† *E. faecalis* and *S. pneumoniae*, respectively, and threefold against *S. aureus*‡, as compared to **7b** an unsubstituted **R^3** analogue.

Similarly, augmentation in the activity was also noticed after introduction of **–CH_3** at **R^4** of chromones ring, thereby producing **7h**, a dimethyl analogue of **7g**. As compared to **7g**, single –CH_3 substitution resulted in an increase in activity by at least onefold against *E. faecalis* and *S. pneumoniae* and two to fourfold against *S. aureus* strains, respectively.

Amongst all the compounds synthesized **7d**, **7e**, **7f** and **7h** showed the potential to demonstrate anti-MRSA activity. Of particular interest was the compound **7d**, which showed remarkable activity against the multi-drug-resistant MRSA strain, comparable to the activity of Gentamicin. Erythromycin, Ampicillin and Ciprofloxacin were found to be inactive against this methicillin-resistant *S. aureus* strain indicating the presence of underlying quinolone as well as macrolide resistance.

As discussed above, with the exception of Gentamicin, other antibacterial agents studied here such as Erythromycin, Ampicillin and Ciprofloxacin did not show appreciable anti-MRSA activity. To date only Vancomycin, Linezolid, Tigecycline and Daptomycin possess indications for the treatment of MRSA infections. Ciprofloxacin is a broad-spectrum quinolone antibacterial used commonly for the treatment of infections caused by Gram-negative pathogens. Erythromycin, Ampicillin and Gentamicin are primarily used to treat Respiratory Tract Infections (RTI) infections caused by Gram-positive pathogens and certain atypical Gram-negative organisms.

Exploratory studies aimed at understanding the mechanism of action of **7f,** a representative compound described in this work, were performed employing genetically defined resistant mutants. Deleterious effect on the activity of **7f** against bacterial pathogens with decreased protein biosynthesis (brought about by addition of Chloramphenicol to the test medium) indicated a possible 'protein biosynthesis inhibition' mediated mechanism of action. However, the antibacterial activity of **7f** was found to be unaffected against a range of linezolid-resistant mutants of *S. aureus*. This result indicates that mechanism of action of **7f** is dissimilar to Oxazolidinones**.** Interestingly, activity of **7f** was compromised against macrolide-resistant strains suggesting a similarity of action between **7f** and the macrolides. Macrolides and Oxazolidinones such as erythromycin and linezolid, respectively, have been shown to demonstrate their activity by binding to Domain V of 23S rRNA and thereby inhibiting the protein biosynthesis process. Since Macrolides do not possess anti-MRSA activity,[32] it was very interesting to note that in spite of mechanism of action-based similarity with Macrolides, **7f** demonstrated a strong evidence of anti-MRSA activity. Amongst protein biosynthesis inhibitors only Linezolid and Tigecycline have been described to possess potent activity against MRSA.[33]

Moreover unlike other anti-MRSA agents such as Vancomycin and Daptomycin that act by complexing with cell wall, activity of **7f** was not affected in the presence of Gram-positive cell wall

lysate in the test medium suggesting a lack of 'cell wall-complexing' mediated antibacterial action.

In summary, convergent synthesis of substituted 3-((*Z*)-2-(4-nitrophenyl)-2-(1*H*-tetrazol-5-yl)vinyl)-4*H*-chrom-en-4-ones, **7a–h** were accomplished by combination of 3-formyl chromones **6** and 5-(4-nitrobenzyl)-1*H*-tetrazole **5**. However, most of these targeted chemical entities exhibited modest spectrum of antibacterial activities against pathogenic bacterial strains. Nevertheless, compounds **7d**, **7e**, **7f** and **7h** displayed significantly improved anti-MRSA activity providing a very noteworthy lead towards the optimization of the series described in this paper, with a potential to result into a broad spectrum anti-MRSA agent.

In conclusion, this study revealed that a range of diverse substituents on chromones ring, at position $\mathbf{R^2}$, $\mathbf{R^3}$ and $\mathbf{R^4}$ modulate the antibacterial activity. The study also resulted in diverse compounds, particularly active against MRSA strains, which could prove useful for further derivatization with the objective to design highly active compounds against difficult-to-treat multi-drug-resistant bacterial pathogens.

Acknowledgments

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19. 5-(4-nitrobenzyl)-1*H*-tetrazole **5**: A mixture of 4-nitrobenzyl cyanide (10 g, 0.062 mol), sodium azide (6 g, 0.092 mol), zinc bromide (7 g, 0.031 mol) and water (100 ml.) was refluxed in 250 ml. round-bottomed flask for 24 h with vigorous stirring. After completion of reaction, the reaction mixture was cooled to room temperature, the pH was adjusted to 1.0 with conc. HCl, and the reaction was stirred for 30 min. to break the complex $(p\text{-}NO_2PhCH_2CN)_2Zn$. The white solid was filtered, washed with 15 ml × 2 1 N HCl and dried at 90 °C to give 11 g of **2** as a white powder. Yield 87%; mp: 189–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 4.37 (s, 2H, CH_2), 7.50 (d, 2H, ArH, J = 8.0 Hz), 8.16 (d, 2H, ArH, J = 8.0 Hz); ESI(−ve): 204.5.
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25. General procedure for synthesis of **7(a–h)**: A mixture of 3-formylchromone **6** (150 mg, 0.86 mol), 5-(4-nitrobenzyl)-1H-tetrazole **5** (177 mg, 0.86 mol) and dry pyridine (2 ml) was refluxed in 15 ml round-bottomed flask for 24 h with stirring. The reaction mixture was cooled to room temperature and slowly poured into 20 ml of 2 N HCl. The pale-yellow product which then precipitated out was filtered and air dried to compound **7**.
3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl)vinyl)-4H-chromen-4-one ***7a****:* Yield: 77%; mp: 248–250 °C dec.; IR (KBr): 3110, 1630, 1570, 1518, 1480, 1353, 1245, 1204, 854, 762 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.99–7.05 (m, 2H, ArH), 7.52–7.57 (dt, 1H, ArH), 7.60–7.62 (dd, 1H, ArH, J = 1.6 and 7.6 Hz), 8.42–8.452 (dd, 2H, ArH, J = 2 and 9.2 Hz), 8.45 (s, 1H, CH), 8.50–8.53 (dd, 2H, ArH, J = 2 and 9.2 Hz), 9.68 (s, 1H, CH,), 10.62 (s, 1H, NH,); Anal. For $C_{18}H_{11}N_5O_4$: C 59.84, H 3.07, and N 19.38. Found: C 59.97, H 3.21, and N 19.51; ESI(−ve): 360.3.
6-Chloro-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7b****:* Yield: 88%; mp: 213–215 °C dec.; IR (KBr): 3085, 1632, 1517, 1467, 1421, 1351, 1155, 1076, 853, 756 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.03–7.05 (d, 1H, ArH, J = 9.2 Hz), 7.54–7.58 (m, 2H, ArH), 8.43–8.45 (d, 2H, ArH, J = 8.4 Hz), 8.46 (s, 1H, CH), 8.51–8.53 (d, 2H, ArH, J = 8.4 Hz), 9.75 (s, 1H, CH,), 10.7 (s, 1H, NH); Anal. For $C_{18}H_{10}ClN_5O_4$: C 54.63, H 2.55, and N 17.70. Found: C 54.69, H 2.57, and N 17.64; ESI(−ve): 394.2.
6-Bromo-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7c****:* Yield: 85%; mp: 230–232 °C dec.; IR (KBr): 3085, 1630, 1517, 1465, 1351, 1278, 1076, 853, 793 cm^{-1}; ^{1} H NMR(400 MHz, DMSO-d_6): δ 6.97–6.99 (d, 1H, ArH, J = 8.0 Hz), 7.65 (s, 1H, ArH), 7.68 (s, 1H, ArH), 8.43–8.45 (d, 2H, ArH, J = 8.0 Hz), 8.46 (s, 1H, CH), 8.51–8.53 (d, 2H, ArH, J = 8.0 Hz), 9.75 (s, 1H, CH,), 10.73 (s, 1H, NH); Anal. For $C_{18}H_{10}BrN_5O_4$: C 49.11, H 2.29, and N 15.91. Found: 49.34, H 2.40, and N 15.98; ESI(−ve): 440.2.
6-Fluoro-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7d****:* Yield: 80%; mp: 238–239 °C dec.; I.R.: 3110, 1620, 1518, 1479, 1421, 1351, 1215, 1194, 854, 759 cm^{-1}; ^{1}H NMR(400 MHz, DMSO-d_6): δ 7.02–7.05 (m, 1 H, ArH), 7.38–7.43 (m, 2H, ArH), 8.43–8.45 (dd, 2H, ArH, J = 2 and 7.6 Hz), 8.46 (s, 1H, CH), 8.51–8.53 (dd, 2H, ArH, J = 2 and 7.6 Hz), 9.74 (s, 1H, CH,), 10.45 (s, 1H, NH); Anal. For $C_{18}H_{10}FN_5O_4$: C 57.00, H 2.66, and N 18.46. Found: C 57.21, H 2.71, and N 18.38; ESI(−ve): 378.3.
6,8-Dichloro-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7e****:* Yield: 81%; mp: 233–235 °C dec.; IR (KBr): 3078, 1629, 1518, 1424, 1347, 1319, 1284, 1230, 857, 727 cm^{-1}; ^{1}H NMR(400 MHz, DMSO-d_6): δ 7.61–7.62 (d, 1H, ArH, , J = 2.4 Hz), 7.87–7.88 (d, 1H, ArH, J = 2.4 Hz), 8.43–8.45 (d, 2H, ArH, J = 8.4 Hz), 8.5 (s, 1H, CH), 8.52–8.54 (d, 2 H, ArH, J = 8.4 Hz), 9.85 (s, 1H, CH,), 10.77 (s, 1H, NH); Anal. For $C_{18}H_9Cl_2N_5O_4$: C 50.25, H 2.11, and N 16.28. Found: C 50.57, H 2.23, and N 16.38; ESI(−ve): 428.2.
6-Chloro-7-methyl-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7f****:* Yield: 78%; mp: 245–246 °C dec.; IR (KBr): 3070, 1634, 1517, 1470, 1342, 1321, 857, 722 cm^{-1}; ^{1}H NMR(400 MHz, DMSO-d_6): 2.35 (s, 3H, CH_3), 7.0 (s, 1H, ArH), 7.61 (s, 1H, ArH), 8.42–8.44 (d, 2H, ArH, J = 9.2 Hz), 8.45 (s, 1H, CH), 8.51–8.53 (d, 2H, ArH, J = 9.2 Hz), 9.95 (s, 1H, CH,), 10.7 (s, 1H, NH); Anal. For $C_{19}H_{12}ClN_5O_4$: C 55.69, H 2.95, and N 17.09. Found: C 55.91, H 3.01, and N 17.23; ESI(−ve): 408.2.
6-Methyl-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7g****:* Yield: 40%; mp: 246–247 °C dec.; ^{1}H NMR(400 MHz, DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.93–6.95 (d, 1H, ArH, J = 8 Hz), 7.37–7.4 (dd, 1H, ArH, J = 2 and 8.2 Hz), 7.42 (d, 1H, ArH, J = 2.2 Hz), 8.43–8.46 (d, 2H, ArH, J = 8.0 Hz), 8.5 (s, 1H, CH), 8.53–8.56 (d, 2H, ArH, J = 8.0 Hz), 9.67 (s, 1H, CH,), 10.4 (s, 1H, NH); Anal. For $C_{19}H_{13}N_5O_4$: C 60.80, H 3.49, and N 18.66. Found: C 60.97, H 3.57, and N 18.85; ESI(−ve): 374.25.
6,8-Dimethyl-3-((Z)-2-(4-nitrophenyl)-2-(1H-tetrazol-5-yl) vinyl)-4H-chromen-4-one ***7h****:* Yield: 69%; mp: 242–243 °C dec.; ^{1}H NMR(400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3),2.24 (s, 3H, CH_3), 7.38 (s, 1H, ArH), 7.40 (s, 1H, ArH), 8.43–8.46 (dd, 2H, ArH, J = 2.4 and 9.2 Hz),8.47 (d, 1H, CH, J = 1.6 Hz), 8.53–8.55 (dd, 2H, ArH, J = 2.4 and 9.2 Hz), 9.72-9.726 (d, 1H, CH, J = 1.6 Hz), 11.1 (s, 1H, NH); Anal. For $C_{20}H_{15}N_5O_4$: C 61.69, H 3.88, and N 17.99. Found: C 61.84, H 3.91, and N 18.04; ESI(−ve): 388.3.
26. NOE experiment is performed on **7b**. Irradiation of the methylene proton at C-2 of the chromone ring (δ = 9.75) showed no effect on other protons, whereas irradiation of the vinyl proton (δ = 8.46) showed an increase of aromatic protons (δ = 8.43–8.84). Thus, structure as well as regiochemistry (***Z***) of the double bond is confirmed.
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