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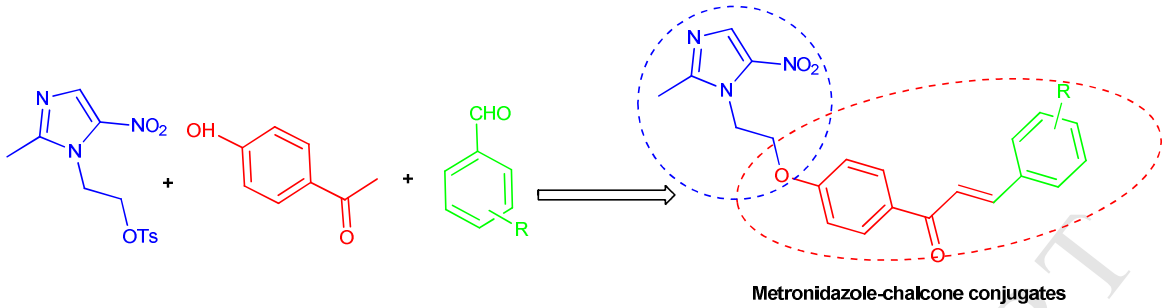
Graphical abstract



Metronidazole-chalcone conjugates

Novel metronidazole-chalcone conjugates with potential to counter drug resistance in *Trichomonas vaginalis*.

Amit Anthwal[a, b], U. Chinna Rajesh[b], M. S. M. Rawat[a,*], Bhavana Kushwaha[c], Jagdamba P Maikhuri[c], Vishnu L. Sharma[d], Gopal Gupta[c], Diwan S. Rawat[b,*]

[a]Department of Chemistry, H.N.B. Garhwal University (A Central University), Srinagar (Garhwal), Uttarakhand-246174, India.

[b]Department of Chemistry, University of Delhi, Delhi-110007, India.

[c]Division of Endocrinology and [d]Division of Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow–226031, India.

Corresponding authors. Tel:+91 9411104325; E-mail address: msmrawat@gmail.com (M.S.M. Rawat) and Tel: +91-11-27662683; *E-mail: dsrawat@chemistry.du.ac.in

Abstract: Trichomoniasis is the most prevalent, curable sexually transmitted disease (STD), which increases risk of viral STDs and HIV. However, drug resistance has been developed by some strains of *Trichomonas vaginalis* against Metronidazole (MTZ), the FDA approved drug against trichomoniasis. In the present study twenty two chalcone hybrids of metronidazole have been synthesized in a quest to get new molecules with higher potential against metronidazole-resistant *Trichomonas vaginalis*. All new hybrid molecules were found active against *Trichomonas vaginalis* with varying levels of activity against MTZ-susceptible and resistant strains. Eight compounds (**4a, 4c, 4d, 4e, 4f, 4h, 4q and 4s**) were found as active as the standard drug with an MIC of 1.56 µg/ml against MTZ-susceptible strain. However, compounds **4e, 4h** and **4m** were 4-times more active than MTZ against drug-resistant *Trichomonas vaginalis*, amongst which **4e** and **4h** were most promising against both susceptible and resistant strains.

Keywords: *Trichomonas vaginalis*, Metronidazole, Drug Resistance, HeLa cell line.

1. Introduction

Sexually Transmitted diseases have long been a big threat to mankind in developing as well as developed countries [1-3]. Nearly 340 million new cases of curable STI's are reported every year world-wide and in which trichomoniasis is a major contributor. Metronidazole (MTZ) is a drug of choice for trichomoniasis, which is also approved by the US-FDA [4]. However, the situation has been worsened by the resistance developed by microbes against MTZ. The *Trichomonas vaginalis* infection damages the vaginal epithelium, which increases the risk of women getting infected by HIV and chances of infected women transmitting HIV to her sexual partner(s) are also increased considerably [5,6]. The Concept of hybrid molecules is a rational approach to overcome the problem of drug resistance by introducing a new pharmacophore in a biologically active molecule to get new molecule of therapeutic importance [7,8]. In a quest to synthesize new hybrid molecules with better anti-trichomonas activity, especially against the resistant strain, we planned to modify the metronidazole framework at hydroxyl group. The rationale for selecting hydroxyl group was that due to its presence MTZ metabolizes into 1-acetic acid metabolite, which results in loss of activity [9]. Many research groups have attempted to modify MTZ at hydroxyl group in order to get a molecule with enhanced activity against *Trichomonas vaginalis* [10-14]. The chalcone is another widely studied pharmacophore with wide range of biological activities. The chalcones exhibit anticancer [15,16], anti-inflamatory [17,18], anti-oxidant [19,20] and antimicrobial [21] activities. As a part of our ongoing research towards development of biologically important molecules, we designed novel metronidazole-chalcone conjugates and anticipated that if such an active pharmacophore is attached to metronidazole the resulting hybrid molecules are likely to be more active especially against MTZ-resistant *Trichomonas*., we herein report synthesis and anti-trichomonas activity of 22 novel metronidazole-chalcone conjugates. The structure of MTZ was modified at hydroxyl group to attach chalcone moiety by covalent bond (figure1, scheme 1). The compounds (**4a-4v**) were synthesized by varying substituents on aromatic ring of chalcone moiety; these compounds were screened for anti-*trichomonas* activity against susceptible and resistant strains of *Trichomonas vaginalis*. The safety of the most promising molecules was evaluated against human cervical (HeLa) cell line.

2. Results and discussion

2.1. Chemistry

The novel metronidazole-chalcone conjugates were synthesized by multistep synthetic protocol. In first step hydroxyl group of MTZ was tosylated with toluenesulphonyl-chloride using pyridine as base and also as solvent to get 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl4-methylbenzenesulfonate **1**. Compound **1** on reaction with 4-hydroxyacetophenone in presence of base K_2CO_3 and DMF solvent yielded 1-(4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)phenyl)ethanone **3**. Furthermore, compound **3** was reacted with various substituted aldehydes in presence of base NaOH and methanol solvent to yield desired hybrid molecules **(4a-4v)** in good yield as shown in scheme 1.

2.2. Biology

All the chalcone-MTZ hybrids (**4a-4v**) exhibited anti-*trichomonas* activity against both the MTZ susceptible (MIC 1.56 – 25µg/ml) and resistant (MIC 3.125 – 100 µg/ml) strains whereas MTZ itself inhibited the growth of susceptible and resistant strain of *T. vaginalis* at MIC of 1.56 and 12.50 µg/ml respectively (Table 1).

The effect of various electron withdrawing (**4b-4q**) and electron donating (**4r-4u**) substituents in the aromatic ring of chalcone was studied. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-phenyl-propenone (**4a**), having unsubstituted aromatic ring was as active as MTZ against the MTZ susceptible strain of *T. vaginalis* but four times less active against resistant strain. The activity data against MTZ susceptible strain suggested that an introduction of electron withdrawing (**4b-4q**), donating (**4r-4u**) or an additional phenyl (**4v**) substituent in aromatic ring had marginal effect as seven compounds (**4c-4f**, **4h**, **4q**, **4s**) retained the activity while others were less active. As expected, all the compounds had higher MIC values (2 – 16 times) against resistant *T. vaginalis* like MTZ (8 times). The substituents in the aromatic ring played a significant role in exhibiting trichomonacidal activity against resistant strain. Compound **4a** having unsubstituted aromatic ring was found to be four times less active (MIC 50 µg/ml) than MTZ against resistant *T. vaginalis* (MIC 12.50 µg/ml) while the presence of a substituent enhanced the activity in eleven compounds, by two times (**4c**,**4d**, **4f**, **4i**, **4l**, **4n**, **4q**, **4t**) and four times (**4e**, **4h**, **4m**). Whereas four compounds were equipotent (**4g**, **4j**, **4s**, **4u**) to MTZ and the activity decreased in six compounds (**4b**, **4k**, **4o**, **4p**, **4r**, **4v**). The electron withdrawing substituents seems to be more desirable for trichomonacidal activity against resistant strain as compared to electron donating substituents as out of sixteen compounds thirteen have been found to be more potent than MTZ. Among fluoro substituted hybrids (**4b-4f**), 3,5-disubstitution (**4e**) enhanced the activity while in chloro (**4g-4k**) and

bromo (**4l-4n**) compounds 3-substitution (**4h**, **4m**) was most important. A trifluoromethyl (CF_3) substitution in aromatic ring (**4o**, **4p**) increased the activity as compared to compound having unsubstituted aromatic ring (**4a)** but remained less active with respect to MTZ. 4-Nitro substitution (**4q**) also increased the activity in comparison to **4a** and MTZ by eight and two folds respectively. Compounds with electron donating substituents (**4r**-**4u**) were more active than **4a** but only 3,5- dimethoxy compound (**4t**) was better than MTZ. A replacement of aromatic ring with naphthyl ring (**4v**) was also not fruitful.

The hybridization of MTZ and chalcone scaffolds resulted into enhancement of the activity of MTZ against resistant strain. Three compounds (**4e**, **4h**, **4m**) were four times more potent than MTZ. The presence of an electron withdrawing substituent in aromatic ring was detrimental for anti-*trichomonas* activity of these hybrid molecules. The activity of these chalcone-MTZ hybrids may be attributed to the nitro group of MTZ scaffold [22] and the alkylation of sulfhydryl groups[23] present over *T. vaginalis* via α,ß-unsaturated ketone group of chalcone moiety as reported[24] for protein SH-groups being specifically modified by α,ß-unsaturated compounds. Thus, it may be concluded that incorporation of chalcone moiety in MTZ scaffold may overcome the MTZ resistance. However, the present study has identified two very promising structures (**4e**, **4h**) that could overcome the MTZ-resistance of *Trichomonas vaginalis* by 4-times, without forfeiting activity against MTZ-susceptible strain (Table-1) and safety against HeLa (Table-2), *in vitro*. The lead optimization of these might lead to the identification of a candidate drug capable of overcoming the MTZ resistance of Trichomonas.

3. Experimental section

3.1. Chemistry

All the chemicals used in synthesis were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography (Merck TLC silica gel 60 F_{254}) was used to monitor the progress of reactions. The compounds were purified when needed by silica gel column (60-120 mesh). Melting points were determined on EZ-Melt automated melting point apparatus, Stanford Research systems and are uncorrected. IR (chloroform/film) spectra were recorded using Perkin-Elmer FT-IR spectrophotometer and values are expressed as υ_{max} cm^{-1}. Mass

spectral data were recorded in Thermo Finnigan LCQ Advantage max ion trap mass spectrometer /data system. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol ECX spectrospin at 400 MHz and 100 MHz respectively, in deuterated solvents with TMS as an internal standard. Chemical shift values are recorded on δ ppm and the coupling constants *J* are in Hz.

3.1.1. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl-4-methylbenzenesulfonate **(1)**

To a stirred solution of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol **(MTZ)** (5g, 0.029 mol) in anhydrous pyridine (75mL), *para*-toluenesulfonylchloride (11.13g, 0.0584 mol) was added and the reaction mixture was stirred at room temperature for 12 hours. The solid precipitated was filtered and crystallized from ethanol to give shining needle like crystals of compound **1** (scheme 1). Yield 85% (pale yellow solid); mp 151^{o}C; IR: 1596, 1526, 1460, 1430, 1366, 1174, 898; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3), 2.52 (s, 3H, -CH_3), 4.37 (t, 2H, -NCH_2), 4.54 (t, 2H, -OCH_2), 7.30 (d, 2H, *J*=8.3 Hz, ArH), 7.60 (d, 2H, *J*=8.3 Hz, ArH), 7.79 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.91, 21.01, 44.61, 68.38, 127.21, 129.24, 131.50, 133.04, 138.03, 145.22, 151.57.

3.1.2. 1-(4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)phenyl)ethanone **(3)**

To a stirred solution of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl-4-methylbenzenesulfonate (**1**) (5g, 0.0153 mol) in DMF (50 mL) was added 4-hydroxyacetophenone (**2**)(2.30g, 0.0169 mol) and reaction mixture was stirred overnight (12 hours) at room temperature (35-40^{o}C). After completion of reaction, the solid was precipitated by addition of water to reaction mixture. The precipitate was washed thoroughly with water to remove DMF and other impurities. The product was recrystallized from ethanol. Yield 74% (Pale brown solid); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.55 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.39 (t, 2H, *J*=4.76 Hz, NCH_2), 4.75 (t, 2H, *J*=4.76 Hz, OCH_2), 6.85 (d, 2H, *J*=9.52 Hz, ArH), 7.91 (d, 2H, *J*=9.52 Hz, ArH), 7.98 (s, 1H).

3.1.3. General procedure for synthesis of hybrid molecules **(4a-4v**):

To a stirred solution of 1-(4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)phenyl)ethanone (**3**) (200 mg, 0.6 mmol) in 10mL methanol corresponding substituted benzaldehyde / napthaldehyde (1.1equiv.) was added , followed by addition of NaOH (4 equiv.) and reaction mixture was stirred at room temperature for 3-4 hours. After completion of reaction monitored by T.L.C., the product precipitated was filtered, washed with water. The crude product obtained was recrystallized from methanol to get pure product (**4a-4v)** in good yield (scheme 1).

3.1.3.1. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-phenyl-propenone **(4a)**: Yield 68% (pale yellow solid); mp 185-186; IR ($CHCl_3$, cm^{-1}): 1658, 1602, 1468, 1362, 1256, 1220,1179, 833, 764; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.26 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.41-7.43 (m, 3H), 7.51 (d, 1H, *J*=16.11 Hz), 7.63-7.65 (m, 2H), 7.80 (d, 1H, *J*=16.11 Hz), 7.99 (d, 2H, *J*=5.86 Hz), 8.02 (s, 1H); ESI-MS *m/z* 378.14 $[M+1]^+$, Anal. Calcd for $C_{21}H_{19}N_3O_4$: C, 66.83; H, 5.07; N, 11.13. Found: C, 66.64; H, 5.19; N, 11.05.

3.1.3.2. 3-(3-Fluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4b)**: Yield 76% (pale brown solid); mp 147-148; IR ($CHCl_3$, cm^{-1}): 1659, 1600, 1529, 1467, 1360, 1240, 1177, 1029, 830, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.09-7.14 (m, 1H), 7.33 (d, 1H, *J*=9.52 Hz), 7.37-7.40 (m, 2H), 7.49 (d, 1H, *J*=15.38 Hz), 7.74 (d, 1H, *J*=15.38 Hz), 8.00 (s, 1H), 8.02 (d, 2H, *J*=8.79 Hz); ESI-MS *m/z* 396.18 $[M+H]^+$, Anal. Calcd for $C_{21}H_{18}FN_3O_4$: C, 63.79; H, 4.59; N, 10.63. Found: C, 63.53; H, 4.64; N, 10.78.

3.1.3.3. 3-(4-Fluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4c)**: Yield 81% (pale yellow solid); mp168-169; IR ($CHCl_3$, cm^{-1}): 2925, 2864, 1659, 1598, 1511, 1468, 1358, 1026, 825, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.90 (d, 2H, *J*=8.79 Hz), 7.09-7.13 (m, 2H),

7.43 (d, 1H, *J*=15.38 Hz), 7.61-7.65 (m, 2H), 7.76 (d, 1H, *J*=15.38 Hz), 7.99 (d, 2H, *J*=2.20 Hz), 8.02 (s, 1H); ESI-MS *m/z* 396.13 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}FN_3O_4$: C, 63.79; H, 4.59; N, 10.63. Found: C, 63.55; H, 4.67; N, 10.75.

3.1.3.4. 3-(3,4-Difluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4d)**: Yield 83% (pale brown solid); mp162-163; IR ($CHCl_3$, cm^{-1}) : 2927, 2852, 1658, 1601, 1515, 1468, 1260, 1180, 821, 754; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.90 (d,d, 2H, J_1=2.20 Hz, J_2=6.59 Hz), 6.97-7.00 (m, 1H), 7.33-7.39 (m, 2H), 7.42-7.45 (m, 1H), 7.69 (d,d, 1H, J_1=6.59 Hz, J_2=8.79 Hz), 7.98 (s, 2H), 8.01 (s, 1H); ESI-MS *m/z* 414.18 $[M+H]^+$, Anal. Calcd for $C_{21}H_{17}F_2N_3O_4$: C, 61.02; H, 4.15; N, 10.17. Found: C, 61.14; H, 4.02; N, 10.28.

3.1.3.5. 3-(3,5-Difluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4e)**: Yield 70% (pale yellow solid); 148-149; IR ($CHCl_3$, cm^{-1}): 3057, 2986, 1666, 1599, 1457, 1426,1215,1178,828,744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.43 (t, 2H, *J*=4.76 Hz), 4.77 (t, 2H, *J*=4.76 Hz), 6.85-6.88 (m, 1H), 6.91 (d, 2H, *J*=8.79 Hz), 7.14 (d, 2H, *J*=6.59 Hz), 7.48 (d, 1H, *J*=15.38 Hz), 7.67 (d, 1H, *J*=15.38 Hz), 7.98 (d, 2H, *J*=5.86 Hz), 8.01 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.61, 45.71, 66.78, 105.40, 110.90, 114.16, 123.79, 130.91, 131.36, 133.29, 138.22, 141.46, 151.60, 161.82, 164.43, 187.75; ESI-MS *m/z* 414.18 $[M+1]^+$, Anal. Calcd for $C_{21}H_{17}F_2N_3O_4$: C, 61.02; H, 4.15; N, 10.17. Found: C, 61.18; H, 4.07; N, 10.25.

3.1.3.6. 3-(2,5-Difluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4f)**: Yield 76% (pale yellow); mp 162-163; IR ($CHCl_3$, cm^{-1}): 3072, 2930, 1661, 1601, 1480, 1251, 1179, 830, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.43 (t, 2H, *J*=4.76 Hz), 4.77 (t, 2H, *J*=4.76 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.06-7.11 (m, 2H), 7.30-7.33 (m, 1H), 7.58 (d, 1H, *J*=16.11 Hz), 7.82 (d, 1H, *J*=16.11 Hz), 7.99 (d, 2H, *J*=3.66 Hz), 8.02

(s, 1H); ESI-MS *m/z* 414.18 $[M+1]^+$, Anal. Calcd for $C_{21}H_{17}F_2N_3O_4$: C, 61.02; H, 4.15; N, 10.17. Found: C, 61.15; H, 4.08; N, 10.30.

3.1.3.7. 3-(2-Chloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4g)**: Yield 69% (pale yellow solid); mp 176-177; IR ($CHCl_3$, cm^{-1}): 3013, 2933, 1658, 1594, 1517, 1428, 1252, 1167, 825,750; ^1H NMR (400 MHz, $CDCl_3$): δ 2.65(s,3H), 4.42 (t, 2H, *J*=4.76 Hz), 4.76 (t, 2H, *J*=4.76 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.31-7.34 (m, 2H), 7.43-7.47 (m, 2H), 7.73-7.75 (m, 1H), 7.99 (s, 1H), 8.01 (d, 2H, *J*=8.79 Hz), 8.15 (d, 1H, *J*=15.38 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.64, 45.73, 66.75, 114.07, 124.35, 127.00, 127.60, 130.20, 130.94, 131.05, 131.57, 133.18, 133.32, 135.32, 138.29, 140.06, 151.62, 161.37, 188.43; ESI-MS *m/z* 412.10 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}ClN_3O_4$: C, 61.24; H, 4.41; N, 10.20. Found: C, 61.37; H, 4.58; N, 10.05.

3.1.3.8. 3-(3-Chloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4h)**: Yield 77% (pale brown solid); mp 159-160; IR ($CHCl_3$, cm^{-1}): 3057, 2986, 1660, 1601, 1468, 1425, 1361, 1255, 1177, 831, 752; ^1H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.43 (t, 2H, *J*=5.13 Hz), 4.77 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.35-7.37 (m, 2H), 7.48-5.92 (m, 2H), 7.63 (s, 1H), 7.72 (d, 1H, *J*=15.38 Hz), 7.99 (s, 1H), 8.01 (d, 2H, *J*=9.52 Hz). ESI-MS *m/z* 412.10 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}ClN_3O_4$: C, 61.24; H, 4.41; N, 10.20. Found: C, 61.35; H, 4.51; N, 10.01.

3.1.3.9. 3-(4-Chloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4i)**. Yield 69% (pale yellow solid); mp 173-174; IR ($CHCl_3$, cm^{-1}): 3123, 2922, 1654, 1597, 1522, 1461, 1251, 1173, 1018, 810, 745; ^1H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.90 (d, 2H, *J*=8.79 Hz), 7.39 (d, 2H, *J*=8.79 Hz), 7.48 (d, 1H, *J*=15.38 Hz), 7.57 (d, 2H, *J*=8.05 Hz), 7.76 (d, 1H, *J*=15.38 Hz), 7.99 (d, 2H, *J*=4.39 Hz), 8.01 (s, 1H); ESI-MS *m/z* 412.10 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}ClN_3O_4$: C, 61.24; H, 4.41; N, 10.20. Found: C, 61.39; H, 4.52; N, 10.03.

3.1.3.10. 3-(2,4-Dichloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4j)**: Yield 73% (pale yellow solid); mp 192-193; IR ($CHCl_3$, cm^{-1}): 2924, 2864, 1658, 1602, 1470, 1373, 1255, 1179, 828, 754; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=4.76 Hz), 4.77 (t, 2H, *J*=4.76 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.30 (d,d, 1H, J_1=2.20 Hz, J_2=5.86 Hz), 7.42-7.47 (m, 2H), 7.67 (d, 1H, *J*=8.79 Hz), 7.99 (s, 2H), 8.01 (s, 1H), 8.07 (d, 1H, *J*=15.38 Hz); ESI-MS *m/z* 446.06 $[M+1]^+$, Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_4$: C, 56.52; H, 3.84; N, 9.42. Found: C, 56.38; H, 3.96; N, 9.29.

3.1.3.11. 3-(2,6-Dichloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4k)**: Yield 79% (pale brown); mp 212-213; IR ($CHCl_3$, cm^{-1}): 3003, 2927, 1666,1597, 1472, 1250, 1174, 828, 750; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.52 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.72 (t, 2H, *J*=5.13 Hz), 5.88-5.91 (m, 1H), 7.00 (d, 2H, *J*=8.79 Hz), 7.05 (d, 1H, *J*=8.79 Hz), 7.25-7.29 (m, 1H), 7.40 (d, 2H, *J*=8.05 Hz), 7.90 (d, 2H, *J*=8.79 Hz), 8.03 (s, 1H); ESI-MS *m/z* 446.06 $[M+1]^+$, Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_4$: C, 56.52; H, 3.84; N, 9.42. Found: C, 56.40; H, 3.92; N, 9.31.

3.1.3.12. 3-(2-Bromo-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4l)**: Yield 82% (pale yellow solid); mp 167-168; IR ($CHCl_3$, cm^{-1}): 3018, 1658, 1600, 1464, 1361, 1214, 1023, 973, 826, 747; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.25-7.26 (m, 1H), 7.34-7.41 (m, 2H), 7.64 (d, 1H, *J*=8.05 Hz), 7.72 (d, 1H, *J*=9.52 Hz), 7.99 (s, 1H), 8.01 (d, 2H, *J*=8.79 Hz), 8.10 (d, 1H, *J*=15.38 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.62, 45.70, 66.72, 114.05, 124.57, 125.71, 127.61, 127.76, 130.93, 131.18, 131.49, 133.28, 133.41, 134.90, 138.20, 142.50, 151.61, 161.36, 188.37;ESI-MS *m/z* 456.11 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}BrN_3O_4$: C, 55.28; H, 3.98; N, 9.21. Found: C, 55.39; H, 3.85; N, 9.32.

3.1.3.13. 3-(3-Bromo-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4m)**: Yield 65% (pale yellow solid); mp162-163; IR ($CHCl_3$, cm^{-1}): 3069, 2925,

2853, 1658, 1601, 1527, 1467, 1423, 1254, 1218, 1176, 829, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.30 (d, 1H, *J*=8.05 Hz), 7.49 (d, 1H, *J*=15.38 Hz), 7.53 (d, 2H, *J*=7.32 Hz), 7.70 (d, 1H, *J*=15.38 Hz), 7.78 (s, 1H), 7.99 (d, 2H, *J*=5.86 Hz), 8.02 (s, 1H); ESI-MS *m/z* 456.11 $[M+1]^{+}$, Anal. Calcd for $C_{21}H_{18}BrN_3O_4$: C, 55.28; H, 3.98; N, 9.21. Found: C, 55.37; H, 3.87; N, 9.33.

3.1.3.14. 3-(4-Bromo-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4n)**: Yield 79% (pale yellow solid); mp159-160; IR ($CHCl_3$, cm^{-1}): 3126, 2881, 1657, 1599, 1524, 1465, 1255, 1177, 812, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.90 (d, 2H, *J*=9.52 Hz), 7.48 (d, 1H, *J*=5.86 Hz), 7.51-7.56 (m, 4H), 7.72 (d, 1H, *J*=16.11 Hz), 7.99 (d, 2H, *J*=2.20 Hz), 8.01 (s, 1H); ESI-MS *m/z* 456.11 $[M+1]^{+}$, Anal. Calcd for $C_{21}H_{18}BrN_3O_4$: C, 55.28; H, 3.98; N, 9.21. Found: C, 55.39; H, 3.83; N, 9.37.

3.1.3.15. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-(2-trifluoromethyl-phenyl)-propenone **(4o)**: Yield 78% (pale brown solid); mp 208-209; IR ($CHCl_3$, cm^{-1}): 3043, 2968, 1662, 1602, 1468, 1321, 1255, 1171, 1119, 1065, 1019, 825, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=4.76 Hz), 4.76 (t, 2H, *J*=4.76 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.38 (d, 1H, *J*=15.38 Hz), 7.50 (t, 1H, *J*=8.05 Hz), 7.60 (t, 1H, *J*=8.05 Hz), 7.73 (d, 1H, *J*=7.32 Hz), 7.81 (d, 1H, *J*=8.05 Hz), 7.98 (s, 2H), 8.00 (s, 1H), 8.09 (d, 1H, *J*=15.38 Hz); ESI-MS *m/z* 446.12 $[M+1]^{+}$, Anal. Calcd for $C_{22}H_{18}F_3N_3O_4$: C, 59.33; H, 4.07; N, 9.43. Found: C, 59.45; H, 3.98; N, 9.32.

3.1.3.16. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-(4-trifluoromethyl-phenyl)-propenone **(4p)**: Yield 64% (pale yellow solid); mp 161-162; IR ($CHCl_3$, cm^{-1}): 3043, 2968, 1662, 1602, 1468, 1321, 1255, 1171, 1119, 1065, 1019, 825, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.43 (t, 2H, *J*=5.13 Hz), 4.77 (t, 2H, *J*=5.13 Hz), 6.92 (d, 2H,

J=8.79 Hz), 7.57 (d, 1H, *J*=16.11 Hz), 7.67 (d, 2H, *J*=7.69 Hz), 7.73 (d, 2H, *J*=8.79 Hz), 7.79 (d, 1H, *J*=16.11 Hz), 8.00 (d, 2H, *J*=8.42 Hz), 8.03 (s, 1H); ESI-MS *m/z* 446.12 $[M+1]^+$, Anal. Calcd for $C_{22}H_{18}F_3N_3O_4$: C, 59.33; H, 4.07; N, 9.43. Found: C, 59.47; H, 3.96; N, 9.35.

3.1.3.17. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-(4-nitro-phenyl)-propenone **(4q)**: Yield 74% (pale yellow solid); mp 231-232; IR ($CHCl_3$,cm^{-1}): 2920, 2852, 1663, 1599, 1517, 1473, 1344, 1256, 1179, 831, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.90 (d, 2H, *J*=8.79 Hz), 7.61 (d, 1H, *J*=16.11 Hz), 7.77-7.82 (m, 3H), 7.99 (s, 1H), 8.02 (d, 2H, *J*=8.79 Hz), 8.28 (d, 2H, *J*=8.79 Hz); ESI-MS *m/z* 423.12 $[M+1]^+$, Anal. Calcd for $C_{21}H_{18}N_4O_6$: C, 59.71; H, 4.30; N, 13.26. Found: C, 59.86; H, 4.20; N, 13.41.

3.1.3.18. 3-(4-Methoxy-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4r)**: Yield 65%(pale yellow solid); mp 169-170; IR ($CHCl_3$, cm^{-1}): 2926, 2851, 1655, 1597, 1511, 1467, 1256, 1172, 1030, 825, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 3.86 (s, 3H), 4.42 (t, 2H, *J*=4.76 Hz), 4.76 (t, 2H, *J*=4.76 Hz), 6.90 (d, 2H, *J*=8.79 Hz), 6.94 (d, 2H, *J*=8.79 Hz), 7.39 (d, 1H, *J*=15.38 Hz), 7.60 (d, 2H, *J*=8.05 Hz), 7.77 (d, 1H, *J*=15.38 Hz), 8.00 (d, 3H, *J*=8.79 Hz); ESI-MS *m/z* 408.15 $[M+1]^+$, Anal. Calcd for $C_{22}H_{21}N_3O_5$: C, 64.86; H, 5.20; N, 10.31. Found: C, 64.94.71; H, 5.08; N, 10.45.

3.1.3.19. 3-(3,4-Dimethoxy-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4s)**: Yield 73% (pale brown solid); mp136-137; IR ($CHCl_3$, cm^{-1}): 3014, 2927, 2844, 1654, 1598, 1513, 1466, 1259, 1027, 828, 754; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 3.93 (s, 3H), 3.95 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.91 (d, 3H, *J*=9.52 Hz), 7.15 (s, 1H), 7.22-7.24 (m, 1H), 7.36 (d, 1H, *J*=15.38 Hz), 7.75 (d, 1H, *J*=15.38 Hz), 7.99 (d, 2H, *J*=2.93 Hz), 8.02 (s, 1H); ESI-MS *m/z* 438.23 $[M+1]^+$, Anal. Calcd for $C_{23}H_{23}N_3O_6$: C, 63.15; H, 5.30; N, 9.61. Found: C, 63.28; H, 5.19; N, 9.48.

3.1.3.20. 3-(2,5-Dimethoxy-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4t)**: Yield 81% (yellow solid); mp107-108; IR ($CHCl_3$, cm^{-1}): 3007, 2950, 1654, 1596, 1461, 1422, 1358, 1168, 1032, 829, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 3.82 (s, 3H), 3.86 (s, 3H), 4.42 (t, 2H, *J*=4.76 Hz), 4.76 (t, 2H, *J*=4.76 Hz), 6.86-6.91 (m, 3H), 6.94 (d,d, 1H, J_1=2.93 Hz, J_2=5.86 Hz), 7.15 (d, 1H, *J*=2.93 Hz), 7.55 (d, 1H, *J*=16.11 Hz), 7.99 (s, 2H), 8.01 (s, 1H), 8.05 (d, 1H, *J*=16.11 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.51, 45.65, 55.69, 55.96, 66.62, 112.29, 113.64, 113.89, 116.93, 122.48, 124.30, 130.72, 131.93, 133.17, 138.22, 139.48, 151.60, 153.11, 153.31, 161.08, 188.96; ESI-MS *m/z* 438.23 $[M+1]^+$, Anal. Calcd for $C_{23}H_{23}N_3O_6$: C, 63.15; H, 5.30; N, 9.61. Found: C, 63.27; H, 5.17; N, 9.43.

3.1.3.21. 3-(3,4-Dimethyl-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone **(4u)**: Yield 77% (pale brown); mp164-165; IR ($CHCl_3$, cm^{-1}): 2929, 1656, 1601, 1469,1425, 1252, 1178, 820, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30 (s, 6H), 2.65 (s, 3H), 4.42 (t, 2H, *J*=5.13 Hz), 4.77 (t, 2H, *J*=5.13 Hz), 6.91 (d, 2H, *J*=8.79 Hz), 7.18 (d, 1H, *J*=7.32 Hz), 7.37-7.41 (m, 2H), 7.46 (d, 1H, *J*=16.11 Hz), 7.76 (d, 1H, *J*=16.11 Hz), 7.99 (d, 2H, *J*=3.66 Hz), 8.02 (s, 1H); ESI-MS *m/z* 406.12 $[M+H]^+$, Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.27; H, 5.81; N, 10.48.

3.1.3.22. 1-{4-[2-(2-Methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-3-naphthalen-1-yl-propenone **(4v)**: Yield 71 % (pale brown solid); mp 221-222; IR ($CHCl_3$, cm^{-1}): 3055, 3009, 1654, 1598, 1524, 1466, 1251, 1176, 1022, 903, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 4.43 (t, 2H, *J*=5.13 Hz), 4.76 (t, 2H, *J*=5.13 Hz), 6.92 (d, 2H, *J*=8.79 Hz), 7.50-7.62 (m, 4H), 7.88-7.94 (m, 3H), 7.99 (s, 1H), 8.06 (d, 2H, *J*=8.79 Hz), 8.25 (d, 1H, *J*=8.05 Hz), 8.65 (d, 1H, *J*=15.38 Hz); ESI-MS *m/z* 428.15 $[M+1]^+$, Anal. Calcd for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83. Found: C, 70.11; H, 4.81; N, 9.97.

3.2. Biology

3.2.1. Anti-Trichomonal Assays

Clinical isolates of metronidazole-susceptible *T. vaginalis* collected at Post Graduate Institute of Medical Research and Education, Chandigarh, India, were obtained from the laboratory of Divya Singh [25], and a metronidazole-resistant strain of *T. vaginalis* (CDC085 [ATCC 50143]) was procured from the American Type Culture Collection (ATCC, USA). Both strains were cultured under partial anaerobic condition in TYM medium (0.1% K_2HPO4, 0.06% KH_2PO_4, 0.5% NaCl, 0.5% glucose, 2.0% yeast extract, 0.2% L-lysine, 0.15% tryptone [pH 6.8]) supplemented with 10% (V/V) heat-inactivated fetal bovine serum, 2% vitamin mixture, 100 U of penicillin/ml, and a 100-µg/ml streptomycin solution in 15-ml screw-cap sterile tubes, followed by incubation at 37°C in a CO_2 incubator.

The *T. vaginalis* parasites to be used in drug susceptibility assays were grown in TYM medium for 1 day following regular subculturing and were in the log phase of growth. *In vitro* drug susceptibility assays were carried out according to the standard procedure [26]. Stock solutions (10.0 mg/ml) of test compounds were prepared in dimethyl sulfoxide (DMSO) and diluted with TYM medium to obtain a concentration of 100 µg/ml, and then further serially diluted with the same medium to 0.78 µg/ml in a 48-well plate. DMSO (0.05%) in TYM was used as vehicle in control wells. Parasites (5 X 10^3 trophozoites/well) were added to these wells and incubated anaerobically at 37°C. Trophozoite growth and viability in drug-containing wells were monitored by trypan blue staining and cell number score on a daily basis, in comparison to the control. Assay results were clearly defined after 24 h in terms of the MIC (the lowest concentration of compound at which all trophozoites became nonviable). Metronidazole (Sigma-Aldrich) was used as reference standard.

3.2.2. Cytotoxicity of compounds toward human cervical (HeLa) cells.

HeLa cells were procured from National Centre for Cell Sciences, Pune, India, and grown in Dulbecco modified Eagle medium (DMEM; Sigma-Aldrich) supplemented with fetal bovine

serum (10%), and antibiotics (a penicillin-streptomycin mixture [100 U/ml]). The cultures were maintained in a CO_2 incubator at 37°C in a 5% CO_2–95% air atmosphere. An MTT (3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyltetrazolium bromide)-based colorimetric assay for evaluation of the cytotoxicity of drug formulations against the human cervical cell line (HeLa) was used [26]. Cells seeded at a density of 5 X 10^4 per well in 96-well plates were incubated in culture medium (DMEM with 10% fetal calf serum) for 24 h at 37°C in a 5% CO2–95% air atmosphere. After 24 h, the culture medium was replaced with fresh medium containing dilutions of test compounds (starting with 1.0 mg/ml) in experimental wells and 0.05% DMSO in culture medium in control wells. After incubation for another 24 h, 5 µL of MTT solution (5 mg/ml in PBS [pH 7.4]) was added to each well. The formazan crystals formed inside the viable cells were solubilized in DMSO, and the optical density at 540 nm (OD_{540}) was recorded in a microplate reader (Microquant; BioTek, USA).

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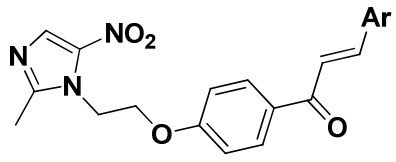
List of figure captions:

Figure 1: Metronidazole-chalcone conjugates

Tables

Table 1: The anti-*Trichomonas* activity of chalcone-MTZ hybrids (**4a-4v**)



Entry	Ar	Compound	MIC against *T. vaginalis*	
			Mtz-Susceptible (µg/ml)	Mtz-Resistant (µg/ml)
1	C_6H_5	**4a**	1.56	50
2	3-FC_6H_4	**4b**	25	100
3	4-FC_6H_4	**4c**	1.56	6.25
4	3,4-FC_6H_3	**4d**	1.56	6.25
5	3,5-FC_6H_3	**4e**	1.56	3.125

6	2,5-FC_6H_3	**4f**	1.56	6.25
7	2-ClC_6H_4	**4g**	3.125	12.5
8	3-ClC_6H_4	**4h**	1.56	3.125
9	4-ClC_6H_4	**4i**	6.25	6.25
10	2,4-ClC_6H_3	**4j**	6.25	12.5
11	2,6-ClC_6H_3	**4k**	6.25	25
12	2-BrC_6H_4	**4l**	3.125	6.25
13	3-BrC_6H_4	**4m**	3.125	3.125
14	4-BrC_6H_4	**4n**	6.25	6.25
15	2- $CF_3C_6H_4$	**4o**	6.25	25
16	4-$CF_3C_6H_4$	**4p**	3.125	25
17	4-$NO_2C_6H_4$	**4q**	1.56	6.25
18	4-$OMeC_6H_4$	**4r**	3.125	25
19	3,4-$OMeC_6H_3$	**4s**	1.56	12.5
20	2,5-$OMeC_6H_3$	**4t**	12.5	12.5
21	3,4-MeC_6H_3	**4u**	6.25	12.5
22	1-naphthyl	**4v**	6.25	25
MTZ	-	-	1.56	12.5

Table 2: The cyto-toxicity of the most promising molecules, **4e** & **4h** against human cervical (HeLa) cell line in vitro

Compound	**IC_{50} against *HeLa* (µg/ml)**
4e	>1000
4h	>1000
MTZ	>1000

Figures and Schemes

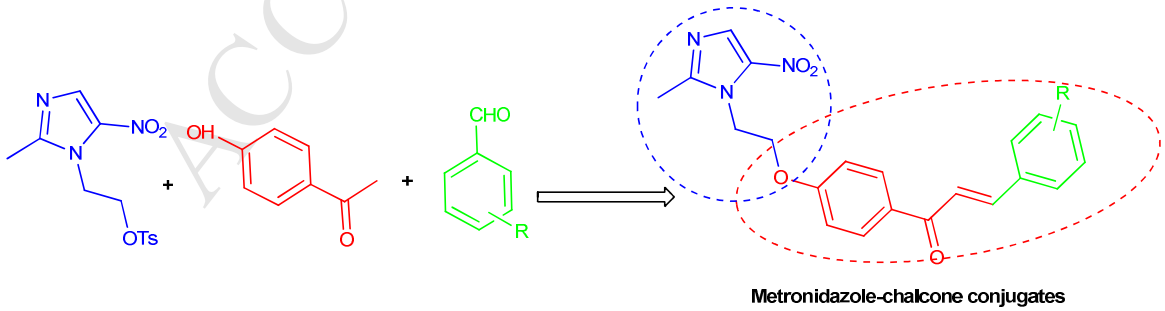
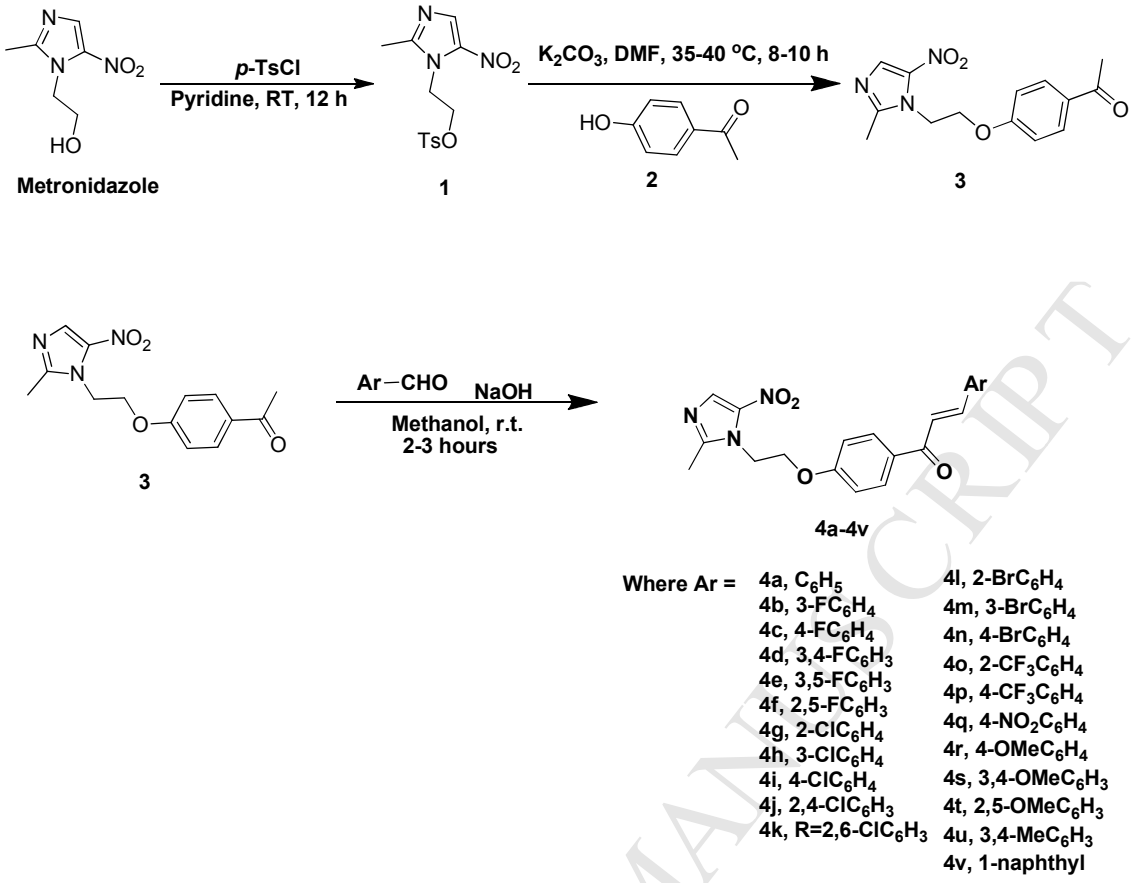


Figure 1

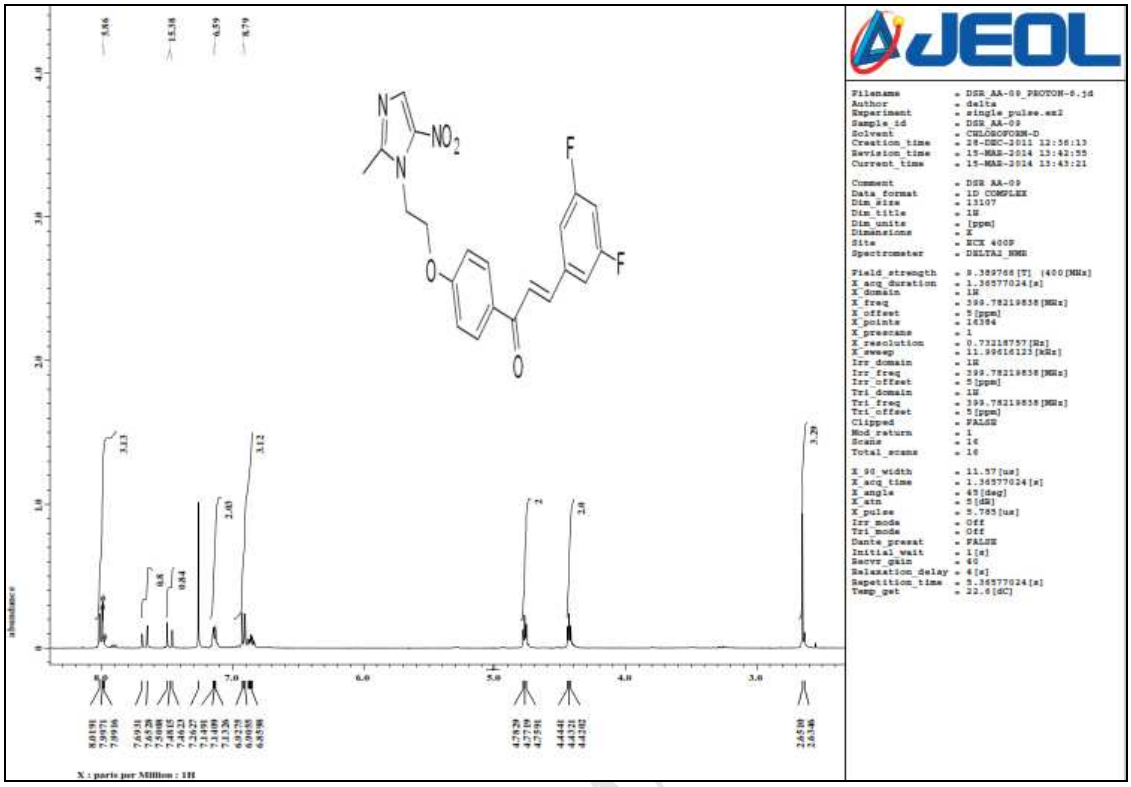


Scheme 1

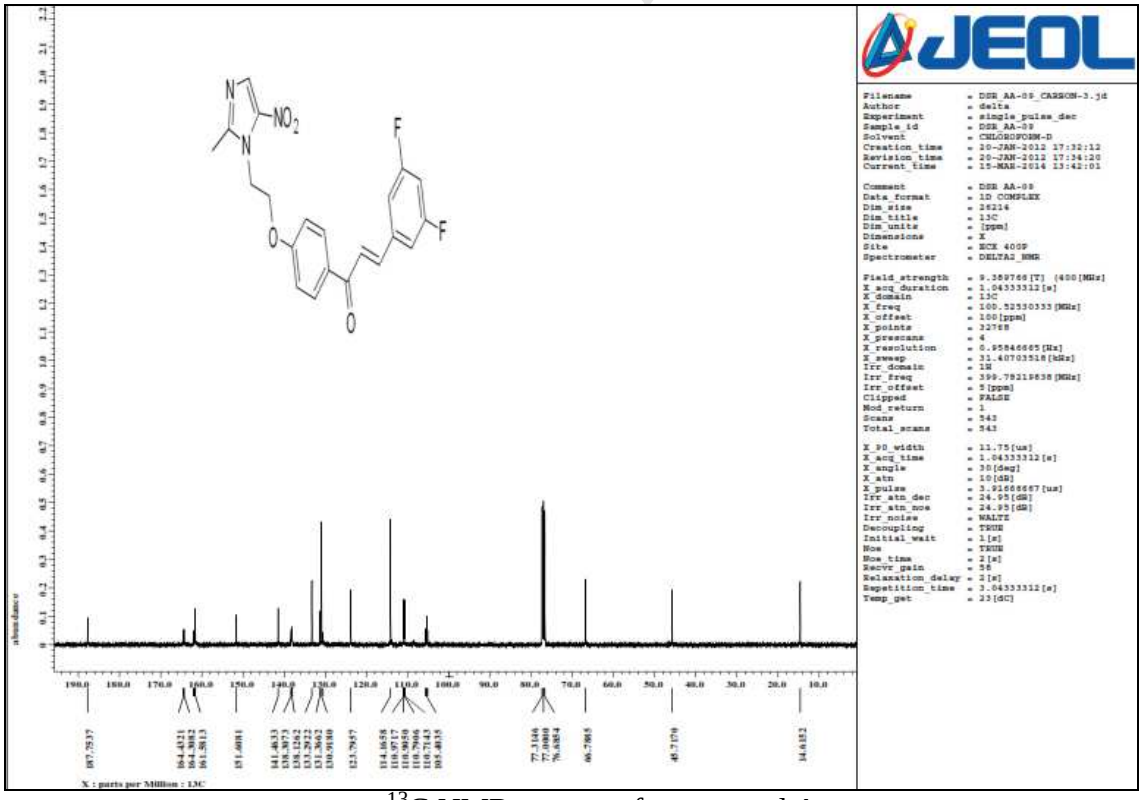
Research Highlights

- All hybrid molecules were found active against susceptible and resistant strain of *T. vaginalis.*
- Eight compounds were found as potent as standard drug against metronidazole susceptible strain of *T. vaginalis*.
- Five, seven and three compounds were found equipotent, 2-fold and 4-fold active than standard drug respectively.
- *In vitro* cytotoxicity of two most active compounds is much above than MIC values.

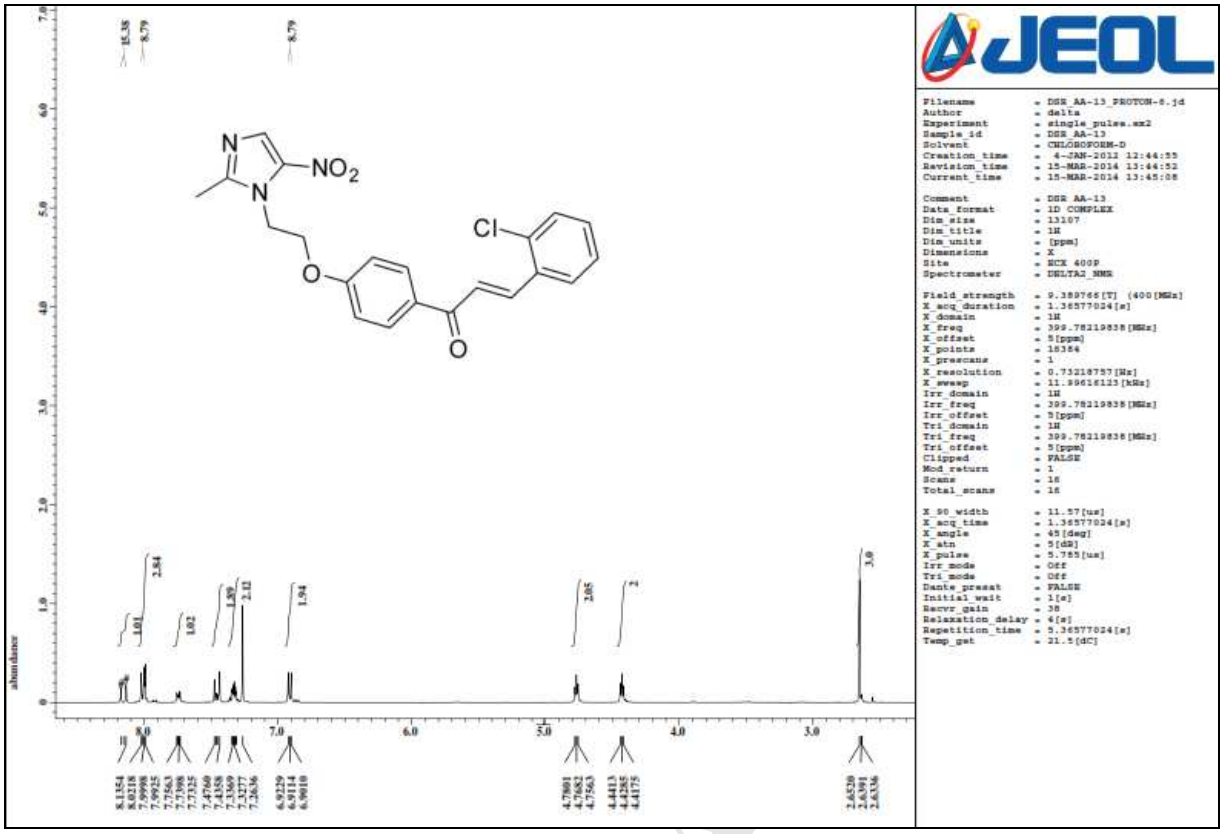
Supporting Information:



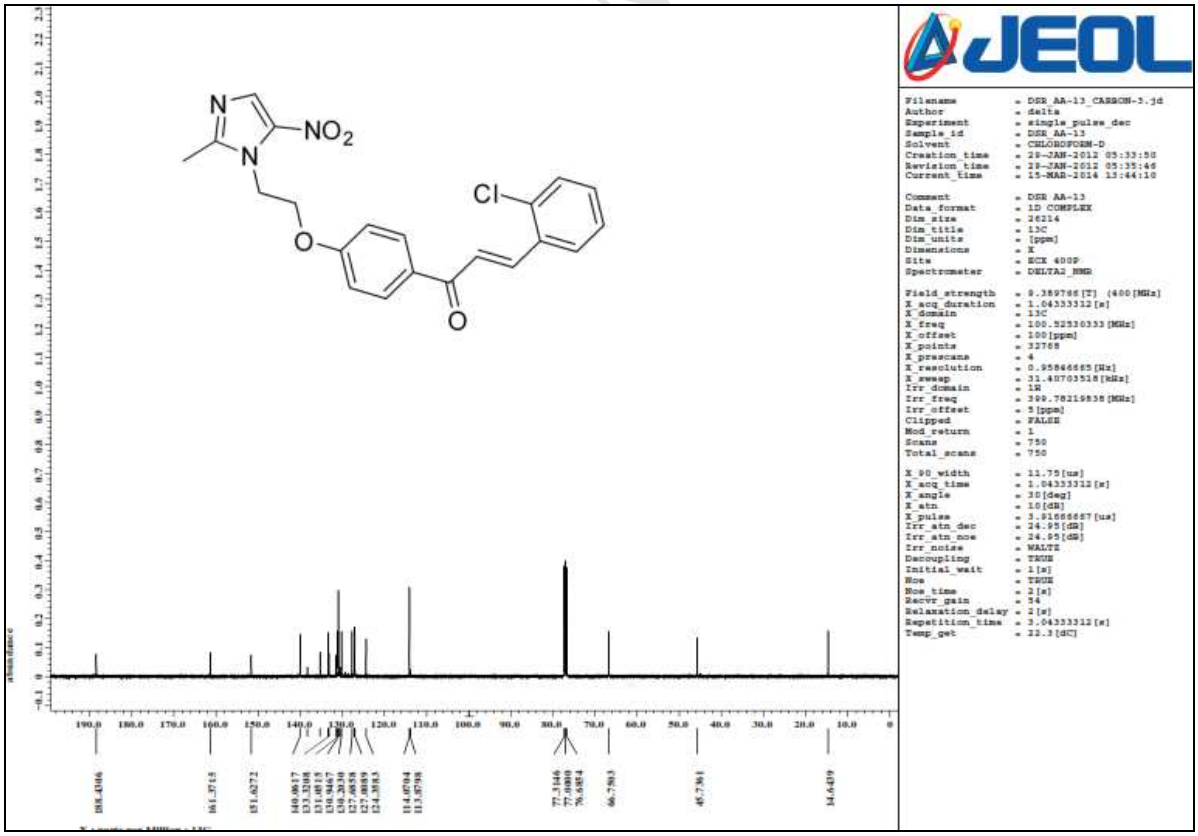
^{1}H NMR spectra of compound **4e**



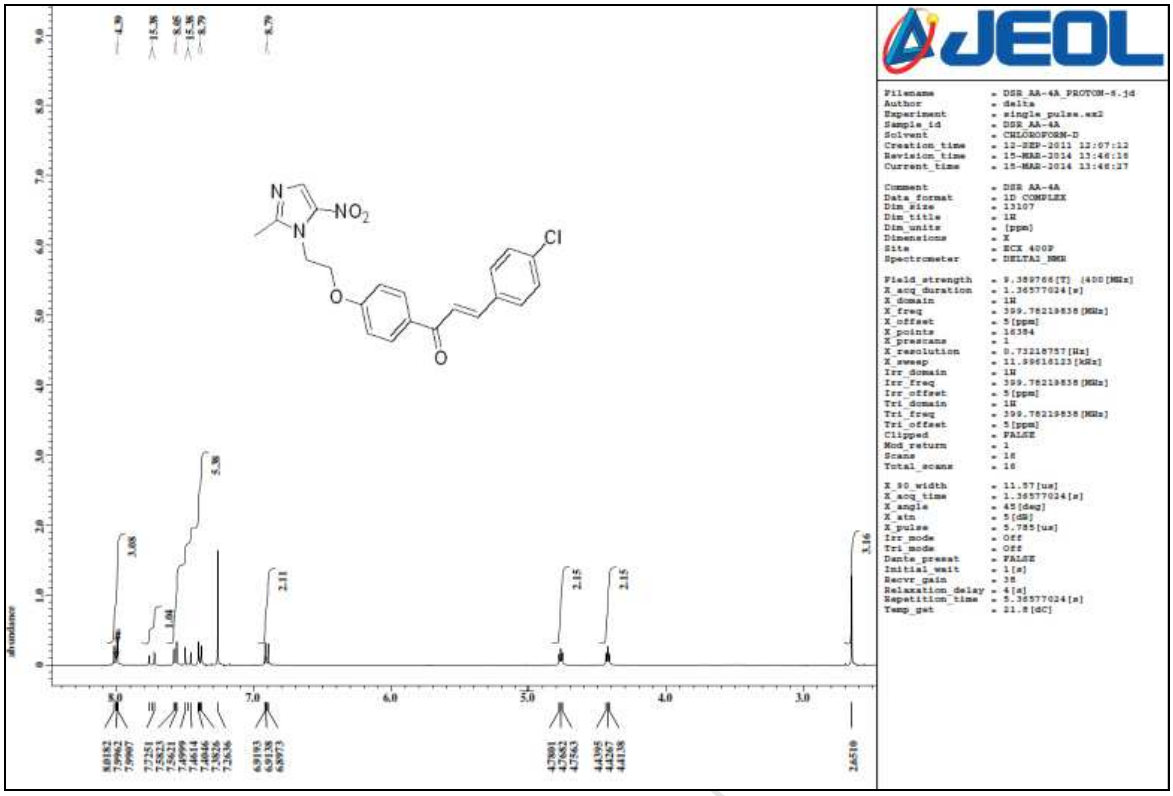
^{13}C NMR spectra of compound **4e**



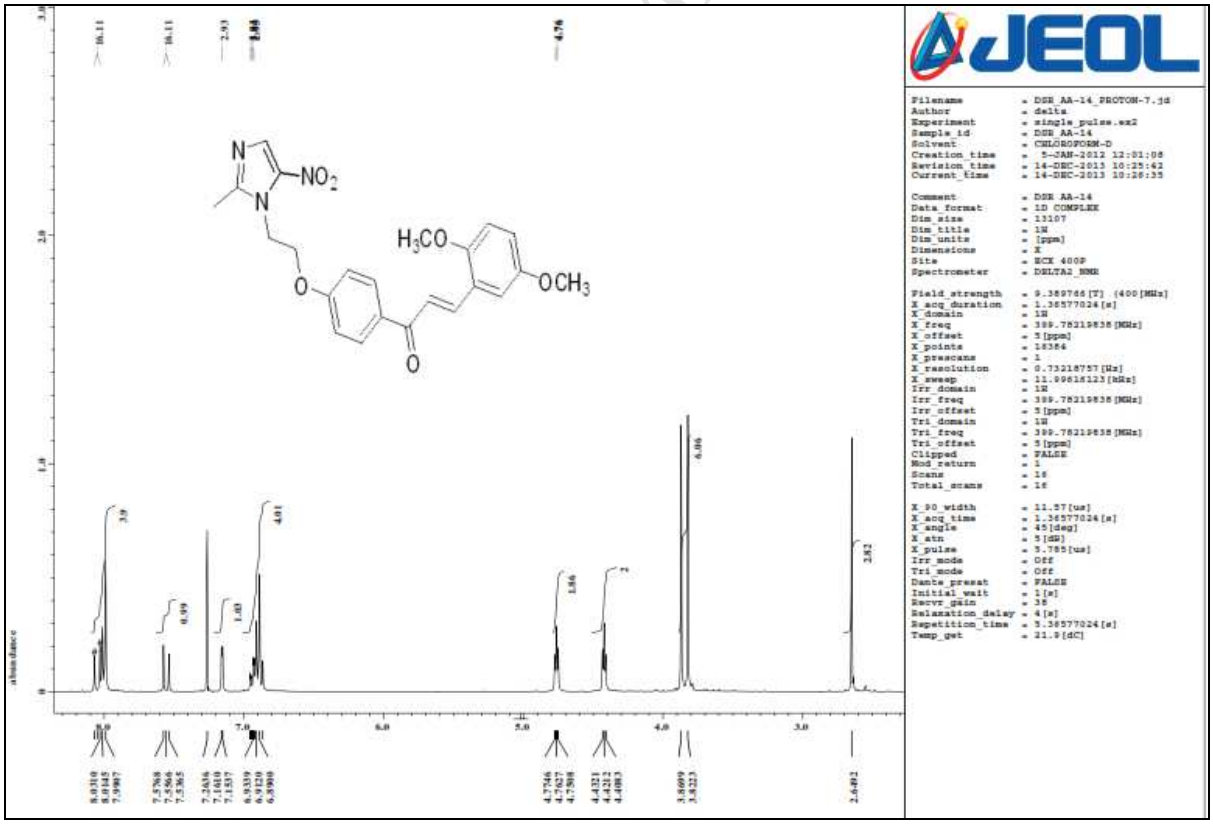
^{1}H NMR spectra of compound **4g**



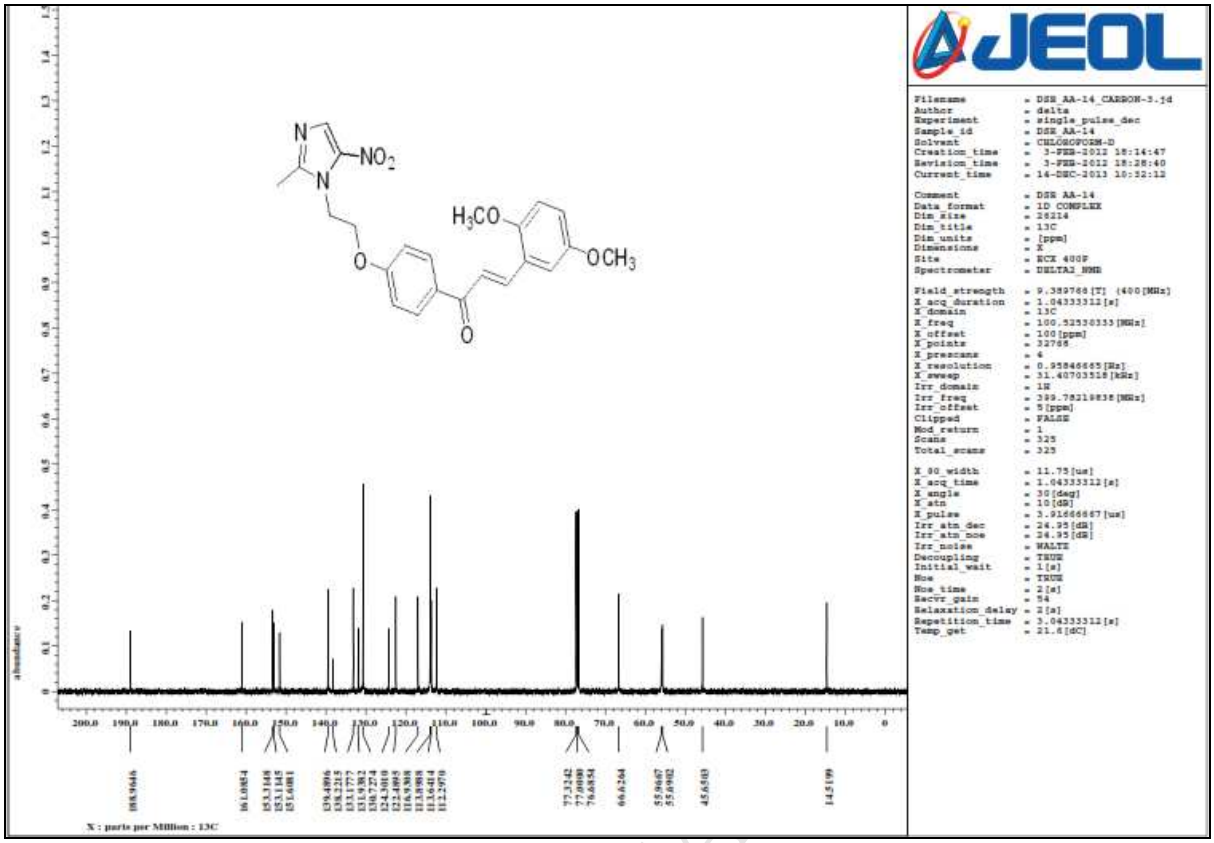
^{13}C NMR spectra of compound **4g**



^{1}H NMR spectra of compound **4i**



^{1}H NMR spectra of compound **4t**



^{13}C NMR spectra of compound **4t**