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Synthesis, characterization and evaluation of antibacterial and antifungal activity of 2-mercaptobenzothiazole and 2-mercaptobenzoxazole derivatives

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Abstract: An efficient tandem route for unprecedented three component reaction involving 2-mercapto-benzoxazole or 2-mercaptobenzothiazole, salicylic acid and malononitrile was used for the development of new 2-mercapto-cromenopyridine derivatives. The synthesized new derivatives were characterized by various spectroscopic methods. In addition, these compounds were screened for *in vitro* antibacterial and antifungal activity against variety of bacterial and fungi strains respectively. Some of the synthesized derivatives were existed to be potent antibacterial derivatives against *S. aureus, E. coli, P. aeruginosa, B. subtilis, P. vulgaris* and antifungal derivatives against *C. coffeanum, A. niger, A. terreus and P. notatum*. All these findings suggested that MBT-III and MBT-VI may be further exploited as a new pharmacophore model for the development of anti-fungal agents.

Keywords: 2-Mercaptobenzothiazole, 2-mercaptobenzoxazole, salicylic acid, malononitrile, 10 mol% pyridine, antibacterial agent, antifungal agent

Introduction:

Multicomponent reaction (MCRs) is an interactive synthetic reaction used for the improvement of biological diversity of chemical derivatives [1]. MCRs are a simple and proficient methodology meant for the development of complex skeletons without tedious purification techniques and shielding group approaches. Therefore, it represents a potent methodology towards the synthesis of molecules of pharmaceutical values, valuable in therapeutic scaffolds and the drug-discovery method in medicinal industry [2],natural product synthesis and combinatorial chemistry [3].Thus, MCRs are well-known as extraordinarily beneficial reactions for the preparation of the wide range of heterocyclic compounds of biological importance [4]. A plethora of dissimilarities of MCRs occurs and in combination with post-condensation adjustments to a fairly various unique construction, that required wide

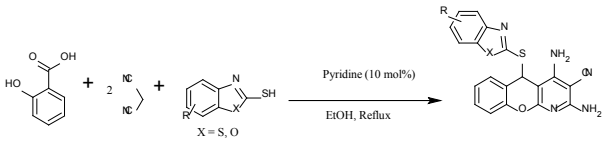
synthesis [4-5].

The thiazole type of heterocyclic chemistry found in numerous natural and synthetic compounds with a variety of biological activities like anticancer, antiviral, antibacterial, anti-fungal, anti-inflammatory, anticonvulsant and anti-parkinsonism that would be explained by the large number of drugs available in the market containing thiazole core[6]. The oxazole ring having heterocyclic compounds existed in several natural products with biological activities like antimicrobial [7], anticancer [8], anti-inflammatory [9], antihelministic [10], and anti-diabetic [11]. Furthermore, the thiazole rings also showed significant applications in other areas like liquid crystals [12], polymers [13], fluorescent dyes [14-15], photo nucleases [16], antioxidants [17], and insecticides [18]. In the natural products, thiazole rings [16], are existed as a sub-unit in a large number of global and oceanic chemistry, with diverse biological actions that signify very significant extent in drug chemistry.

Chromenopyridines frameworks have also been effectively operated for the generation of libraries with varied medical uses. These are fused heterocyclic compounds that show antibacterial [19-20], anti-asthmatic [21], anticancer [22], antiproliferative [23]. Some of the examples of Chromenopyridines like pranoprofen and amlexanox have been stated as potent NSAIDs and anti-allergic, respectively. Therefore, the improvement of a simple protocol for the preparation of chromenopyridines is of great attention to modern synthetic organic researchers.

The many synthetic routes used for the synthesis of substituted chromenopyridines mainly includes, the reaction of 2-amino-3-formylchromone with 2-amino-4-(phenylsulfanyl)- 4*H*-chromene-3-carbonitrile and malononitrile [24], 4-chlorocoumarin-3-carbaldehyde with malononitrile [25],the reaction of aryl aldehydes with 2,2-disubstituted chroman-4-onesand 2-cyanoacetamides[26], ethyl-3-aminocrotonate on reaction with 4-chloro-2*H*-3-chromene carbaldehydes [27],and 2,2-disubstituted chroman-4-one with combination of aryl aldehydes and 2-cyanoacetamide [28]. Some of the most attractive approaches for the synthesis of chromeno[2,3-*b*]pyridines includes the cyclocondensation of thiols, aldehydes and malononitrile. A few methods are such as using ZrP_2O_7 nanoparticles [29], SnO nanoparticles [30], Et_3N [31], and K_2CO_3[32], as catalysts which used in the synthesis of chromeno[2,3-*b*] pyridines.

Herein, we developed and characterized simple, new and facile procedure for the preparation of 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile of biological values using the MCRs, pyridine catalyzed with salicylic acid, malononitrile and 2-mercapto-benzothiazoles or 2-mercapto-benzoxazoles in ethanol(**Scheme 1**).



Scheme 1: Pyridine catalyzed synthesis of 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

Results and Discussions:

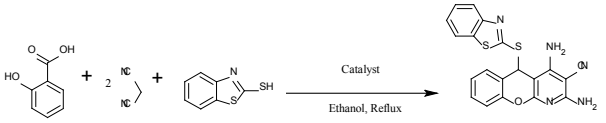
Initially, we focused efforts on optimization of reaction conditions. The model reaction between salicylic acid, malononitrile and 2-mercaptobenzothiazole was considered for exploring the various catalysts at concentration of 10 mol% under reflux in ethanol. We found higher product yield with pyridine as a catalyst (Table 1, entry 9). The efficiency of diverse

polar and non-polar solvents assessed by using a catalyst pyridine. Ethanol was found to be the best solvent for the anticipated conversion giving higher product yield (Table 2, entry 9). The other non-polar and polar solvents afforded moderate to low yield. The high reaction rate with ethanol recognized to the synchronization amongst ethanol and pyridine.

After screening the suitable catalyst giving the anticipated product with high yield, reactions were carried out using diverse stoichiometric conditions for the optimization of concentration of catalyst. The optimized concentration of catalyst is summarized in Table 3. The excellent product yield was obtained with 10 mol% concentration of pyridine (Table 3, entry 10). Furthermore, with increased concentration of catalyst (pyridine) above 10 mol%, we observed no significant change in the product yield (Table 3, entry 4-5).

After investigating the effect of diverse reaction factors on the same model reaction, we revolved our attention towards the preparation of 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrilederivativesusing salicylic acid, malononitrile and a variety of substituted 2-mercaptobenzothiazole or 2-mercaptobenzoxazole, and the results are presented in Table 4.

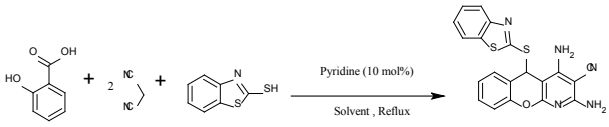
Table 1: Optimization of reaction condition using different catalyst for synthesis of MBT-I[a]



Entry	Catalyst	Time (min)	Yield (%)
1	DBU	120	30
2	$ZnCl_2$	120	41
3	Morpholine	120	45
4	Al_2O_3	120	51
5	NaOH	120	57
6	Na_2CO_3	120	45
7	KOH	120	48
8	CaO	120	57
9	pyridine	60	87

Reaction condition: salicylic acid (1 mmol), malononitrile (2 mmol), 2-mercaptobenzothiazole(1 mmol)and catalyst (5-20 mol%)
[a]Isolated yields

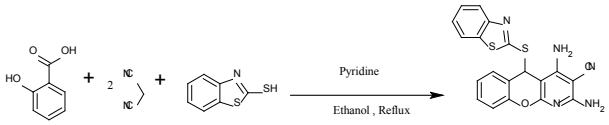
Table 2:Optimization of reaction condition for synthesis of MBT-I using different solvents[a]



Entries	Solvent	Time (min)	Yield[a] (%)
1	Me-OH	60	55
2	DCM	60	48
3	*Pr*-OH	60	61
4	*iBu*-OH	60	65
5	Water	60	26
6	Ethyl acetate	60	39
7	Acetone	60	45
8	THF	60	40
9	Et-OH	60	85
10	DMSO	60	61

Reaction condition: salicylic acid (1 mmol), malononitrile (2 mmol), 2-mercaptobenzothiazole(1 mmol) and pyridine (10 mol%) under reflux condition
[a]Isolated yields

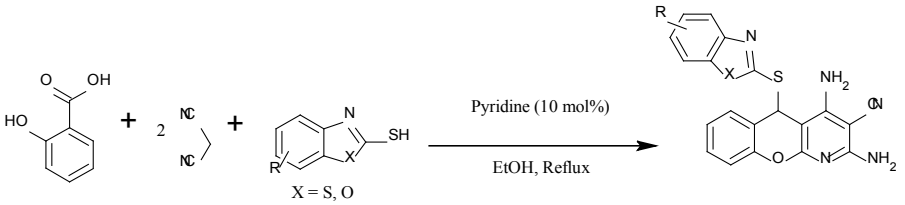
Table 3:Optimization of concentration of catalyst for synthesis of MBT-I[a]

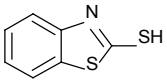
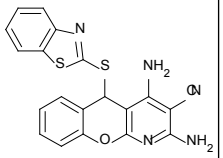
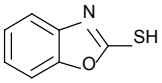
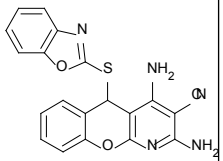
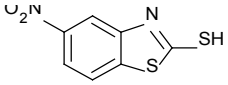
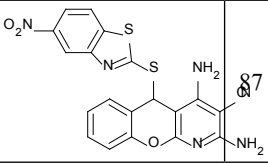
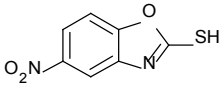
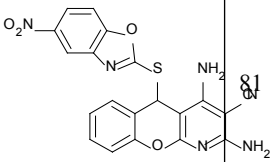
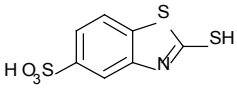
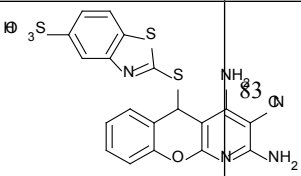
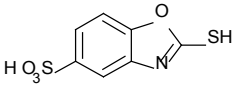
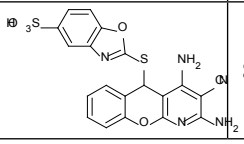


Entries	Concentration of catalyst	Time (min)	Yield[a] (%)
1	-	60	Trace
2	5	60	62
3	10	60	85
4	15	60	85
5	20	60	85

Reaction condition: salicylic acid (1 mmol), malanonitrile (2 mmol), 2-mercaptobenzothiazole (1 mmol) and Pyridineunder reflux condition in ethanol
[a]Isolated yields

Table 4: Synthesis of MBT-I to MBT-VI

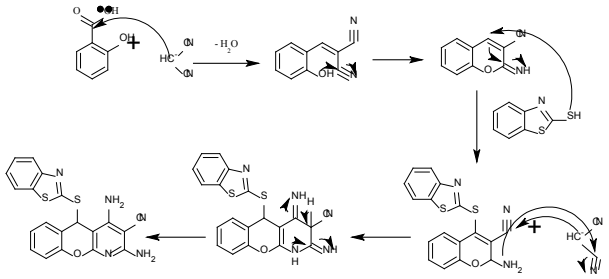


Entry	Substituted 2-mercaptobenzothiazole/2-mercapto-benzoxazole	Time (min)	Product	Yield[a]	M.P. (°C)
MBT-I		60		85	234-236
MBT-II		55		80	256-258
MBT-III		60		87	264-266
MBT-IV		50		81	216-218
MBT-V		60		83	248-250
MBT-VI		60		82	280-282

Reaction condition: salicylic acid (1 mmol), malononitrile (2 mmol), substituted 2-mercaptobenzothiazole/benzoxazole (1 mmol) and Pyridine (10 mol%) under reflux condition in ethanol
[a]Isolated Yield.

The plausible mechanism of pyridine catalyzed preparation of 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile from salicylic acid, malononitrile and 2-mercaptobenzothiazole with the probable sequence of events is shown in **Scheme 2**. Initially pyridine coordinates with the carbonyl group of salicylic acid and nitrile to accelerate the conjugate and through adding nucleophiles to equivalent substrates, which facilitates efficient nucleophilic attack of the pyridine on malononitrile leading to formation of the intermediate which loses a water molecule and undergoes subsequent steps to get the desired

product.



Scheme 2: The plausible mechanistic pathway for the synthesis of 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-b]pyridine-3-carbonitrile.

In vitro Antibacterial Evaluation

The results of *in vitro* antibacterial activity of the synthesized compounds are presented in Table 5. Some of the synthesized derivatives were found to be highly effective as antibacterial agents when compared to the standard drug streptomycin as observed by their low MIC values compared to standard drugs. Amongst the synthesized 2-mercaptobenzothiazole and 2-mercaptobenzoxazole derivatives, MBT-III and MBT-VI showed lower MIC against all the bacterial strains when compared to other derivatives and standard streptomycin. Therefore, derivatives MBT-III and MBT-VI were found to be the potent antibacterial agent against all the microbial strains than remaining derivatives and standard streptomycin. Derivative MBT-III and MBT-VI exhibited maximum antibacterial activity against *Staphylococcus aureus* 2079 with MIC of 4 and 3*μg/mL* respectively. The obtained results revealed that the synthesized benzothiazole derivatives possess antibacterial activity against the variety of microbial strains.

Table 5:Minimum inhibitory concentration values of the synthesized compounds against the growth of bacteria (in *μg*/*mL*)

Bacterial species	Strepto-mycin	MBT-I	MBT-II	MBT-III	MBT-IV	MBT-V	MBT-VI
Staphylococcus aureus 2079	5	8	10	4	12	10	3
Escherichia coli NCIM 2065	30	35	20	25	35	25	20
Pseudomonas aeruginosa MTCC 2297	10	15	15	5	15	15	8
S. aureus NCIM 5021	20	22	20	16	30	25	12
Bacillus subtilis NCIM 2423	10	8	15	7	20	15	5
E. coli NCIM 2931	20	15	20	14	25	15	15
Proteus vulgaris NCIM 2027	15	20	15	10	20	20	10
P. aeruginosa NCIM 5029	20	25	25	10	15	30	10

In vitro Antifungal Evaluation

The results of *in vitro* antifungal activity of the synthesized compounds are presented in Table 6. The antifungal potential of synthesized derivatives was measured in terms of diameter of zone of inhibition (DZI). We observed that some derivatives are active against some species of fungi and few are inactive against some species of fungi. Most of the synthesized derivatives showed potent antifungal activity similar to standard Griseofulvin (50 *μg/mL*) against *C. coffeanum, A. niger, A. terreus and P. notatum*. Amongst the synthesized derivatives, derivative MBT-I (DZI- 10,8*mm*), MBT-IV(DZI-8,8 *mm*) were found to be potent antifungal similar to that of standard Griseofulvin against *C. coffeanum, A. niger, A. terreus and P. notatum* respectively. The derivative MBT-III and MBT-VI were observed to be most potent antifungal derivative against a large number of fungi species when compared to other derivatives.

Therefore, the obtained results clearly revealed that the synthesized 2-Mercaptobenzothiazole and 2-Mercaptobenzoxazole derivatives possess potent antifungal activity against the variety of fungal strains.

Table 6: Anti-fungal activity (*μg/mL*) by zone of inhibition (*mm*)

Entry	Diameter of zone of inhibition (*mm*)			
	C. coffeanum	*A. niger*	*A. terreus*	*P. notatum*
MBT-I	20	10	8	23
MBT-II	22	26	30	19
MBT-III	10*	12*	32*	23*
MBT-IV	8	24	8	9
MBT-V	20	20	24	21
MBT-VI	8*	10*	8*	7*
Griseofulvin (50 *μg/mL*)	14	18	20	15

* = Significant activity

Concentration of test compound = 50 *μg/mL*

Conclusion

The new chromenopyridine derivatives were screened for antibacterial and antifungal activity. This scheme describes that new 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitriles were responsible for antibacterial and antifungal action, which shows low MIC value and maximum zone of inhibition respectively. Further work would be the assessment of the mechanism by which these derivatives shows the antibacterial and antifungal activities.

From the above data, it is determined that the new chromenopyridine derivatives may characterize a new antibacterial and anti-fungal agent which will be biologically active constituents that can create a systematic base for the expenditure of this in the recent drug. This information about the medicinal components treatment can also be prolonged to another area like a field of clinical pharmacology.

Experimental

Materials

All the chemicals were obtained from Sigma Aldrich and Thomas Baker. These chemicals were applied without extra purification procedures. The reactions were carried out in dried glassware. The chemicals of analytical grade were procured from commercial sources and used as such without further purification. Agilent FT-IR was used for functional group analysis. Bruker AC NMR spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) was used for NMR spectra using DMSO-d_6 as solvent and chemical shifts (δ) are mentioned in parts per million (ppm) values and TMS used as the internal reference with coupling constants expressing in hertz (Hz). Melting point apparatus with an open capillary was used for measurement of melting point and were unaffected. Shimadzu QP2010 GCMS was used for recording mass spectroscopy. Thin layer chromatography (TLC) was performed on silica gel Polygram SIL G/UV 254 plates. Media Mueller Hinton broth (MHB) for antibacterial activity was obtained from Hi-media Laboratories. Microbial type cell cultures (MTCC) for antibacterial activity were purchased from IMTECH, Chandigarh. Asthana hawker's medium was used for performing the antifungal activity.

Chemistry

The substituted 2-mercaptobenzothiazole (1 mmol) was selected as a starting material and salicylic acid (1 mmol) and malononitrile (2 mmol) in presence of pyridine were taken in 5 mL ethanol gave 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (MBT-I to MBT-VI). The complete general reaction procedure of the compound is stated in the experimental section. The physical characteristics of the prepared derivatives are represented in Table 4. The purity of the prepared derivatives was examined by TLC and the structures of all the derivatives were investigated by using FTIR, EIS-MS, ^{1}H and ^{13}C NMR spectroscopic techniques. The

obtained spectroscopic data are reliable with the anticipated molecular structures.

In vitro antibacterial evaluation

The *in vitro* antibacterial potential of the synthesized 2-mercaptobenzothiazole and 2-mercaptobenzoxazole derivatives was assessed by performing antibacterial assay against eight model organisms, *Pseudomonas aeruginosa* MTCC 2297, *Bacillus subtilis* NCIM 2423, *Escherichia coli* NCIM 2065, *Proteus vulgaris* NCIM 2027, *Staphylococcus aureus*2079, *E. coli* NCIM 2931, *S. aureus* NCIM 5021, *P. aeruginosa* NCIM 5029. The wells of a sterile 96-wells flat-bottomed tissue culture plate (HI-Media) were filled with 100 *µL* of sterile Mueller Hinton broth (MHB). To the first well of each column a 20 *µL* of the 2-mercaptobenzothiazole and 2-mercaptobenzoxazole derivative stock solution (dissolved in DMSO) was added and then serially diluted to establish a concentration ranging from 2 to 30 *µg/mL*. After dilution, a 5 *µL* of bacterial suspensions (0. 5 Macfarl and optical density adjusted) grown in MHB were added to each well. Negative and positive controls were also included in the assay. Further the plates were incubated for 18 *h* at 37±2˚C and the optical density measurements were carried at 540 *nm* using ELISA Microliter plate reader. Antibacterial activity was confirmed from the readings indicating reduction in optical density of the culture with increased concentration of 2-mercaptobenzothiazole or 2-mercaptobenzoxazole derivative. Further minimum inhibitory concentration (MIC) was determined by comparing the optical density of the tests and control wells. The percentage of growth was calculated with the help of following formula.

$$\%\ \text{Microbial inhibition} = \left(\frac{OD_0 - OD_c}{OD_0}\right) \times 100$$

Where, OD_0 is the optical density for negative control well and OD_c is the optical density for the test well with compound concentration c.

*In vitro a*ntifungal activity

All the synthesized derivatives (MBT-I to MBT-VI) have been tested for the antifungal activity against *Aspergillus niger, Aspergillus tevatus, Penicillium notatum and Collitricum coffeanum* using cup-plate agar diffusion technique. The potency of derivatives was assessed by measuring the diameter of zone of inhibition mm. Griseofulvin (50 *µg/mL in DMSO*) was selected as a standard drug for the antifungal activity. The Asthana hawker's medium was sterilized, cooled and inoculated with fungal suspension aseptically in individual portions and poured into sterile petri-dishes. The Petri-dish containing medium and suspension was allowed to solidify. Then the cavities of 8 mm diameter were prepared on solidified medium aseptically using sterile bore. All synthesized derivatives were added successively in the cavities with the help of micropipette and allowed to diffuse for the 1.0 h. DMSO was used as a solvent for all the compounds, and as a control. These plates were incubated at 28 ºC for 48 h. Finally, the diameter of zone of inhibition was observed and recorded.

Spectroscopic data:

Entry MBT-I: 2,4-diamino-5-(1,3-benzothiazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

M.P. 234-236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 7.63-7.59 (m ,2H, Chromenopyridine-H_6, Benzothiazole- H_4), 7.46-7.51 (m, 2H, Benzothiazole- H_7, Chromenopyridine-H_7), 7.37-7.40 (m, 2H, Chromenopyridine-H_8, Benzothiazole-H_5), 7.14-7.19 (m, 2H, Chromenopyridine- H_9, Benzothiazole-H_6), 6.04 (s, 4H, NH_2), 5.05-5.17 (m, 1H, Cromenopyridine-H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.8 (Benzothiazole- C'_2), 162.3 (Chromenopyridine-

C_{10a}), 158.6 (Chromenopyridine-C_2), 154.1 (Chromenopyridine-C_{9a}), 148.6 (Benzothiazole-C'_{3a}), 139.2 (Chromenopyridine- C_4), 136.8 (Benzothiazole- C'_{7a}), 131.4 (Chromenopyridine-C_8), 128.4 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 126.9 (Benzothiazole- C'_6), 126.2 (Chromenopyridine- C_6, C_7, Benzothiazole- C'_7), 124.3 (Benzothiazole- C'_5), 118.7 (CN), 115.2 (Chromenopyridine- C_9), 114.7 (Benzothiazole- C'_4), 54.1 (Chromenopyridine- C_5); IR (cm^{-1}): 3361, 2992, 2928, 2228, 1665, 1378, 1129, 972; MS (EI): m/z 403.2118 [M^+].

Entry MBT-II: 2,4-diamino-5-(1,3-benzoxazol-2-ylsulfanyl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

M.P. 256-258 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 8.20-8.23 (m, 4H, Chromenopyridine- H_6, H_7, Benzoxazole- H_4, H_7), 8.01-8.10 (m, 4H, Chromenopyridine- H_8, H_9, Benzoxazole- H_5, H_6), 7.87-7.90 (m, 2H, NH_2), 7.32-7.48 (m, 3H, NH_2, Chromenopyridine-H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.1 (Benzoxazole- C'_2), 163.0 (Chromenopyridine-C_{10a}), 158.9 (Chromenopyridine-C_2), 150.1 (Chromenopyridine-C_{9a}), 148.5 (Benzoxazole-C'_{3a}), 138.9 (Chromenopyridine- C_4), 134.1 (Benzoxazole- C'_{7a}), 130.5 (Chromenopyridine-C_8), 130.2 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 128.6 (Benzoxazole- C'_6), 126.7 (Chromenopyridine- C_6, C_7, Benzoxazole- C'_7), 122.1 (Benzoxazole- C'_5), 118.7 (CN), 116.9 (Chromenopyridine- C_9), 108.5 (Benzoxazole- C'_4), 55.1 (Chromenopyridine- C_5); IR (cm^{-1}): 3357, 3130, 2936, 2235, 1628, 1486, 1225, 801; MS (EI): m/z 388.7505 [M+H^+].

Entry MBT-III: 2,4-diamino-5-[(5-nitro-1,3-benzothiazol-2-yl)sulfanyl)]-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

M.P. 264-266 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm)10.16 (s, 1H, SO_3H), 7.14-7.20 (m, 6H, Chromenopyridine-H_6, H_7, H_8, Benzothiazole-H_4, H_7, H_6), 6.58-6.71 (m, 2H, Chromenopyridine- H_8, NH_2), 5.98 (s, 3H, NH_2), 5.35 (s, 1H, Chromenopyridine-H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.3 (Benzothiazole- C'_2), 162.8 (Chromenopyridine-C_{10a}), 157.9 (Chromenopyridine- C_2), 153.9 (Chromenopyridine- C_{9a}), 151.8 (Benzothiazole-C'_{3a}), 146.5 (Chromenopyridine- C_4), 136.9 (Benzothiazole- C'_{7a}), 131.6 (Chromenopyridine-C_8), 126.6 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 125.5 (Benzothiazole- C'_6), 121.5 (Chromenopyridine- C_6, C_7, Benzothiazole- C'_7), 119.5 (Benzothiazole- C'_5), 117.8 (CN), 116.5 (Chromenopyridine- C_9), 110.5 (Benzothiazole- C'_4), 46.9 (Chromenopyridine- C_5); IR (cm^{-1}): 3331, 3100, 2925, 2250, 1658, 1535, 1382, 1177, 1043; MS (EI): m/z 448.3358 [M^+].

Entry MBT-IV: 2,4-diamino-5-[(5-nitro-1,3-benzoxazol-2-yl)sulfanyl]-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

M.P. 216-218 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.30 (s, 1H, SO_3H), 8.20-8.23 (m, 3H, Chromenopyridine- H_6, H_7, Benzoxazole- H_4), 8.04-8.10(m,4H,Benzoxazole-H_7,H_6,Chromenopyridine-H_8, H_9), 7.87-7.90 (m, 2H, NH_2), 7.51-7.53 (m, 2H, NH_2), 5.23-5.28 (m, 1H, Chromenopyridine-H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8 (Benzoxazole- C'_2), 165.3 (Chromenopyridine-C_{10a}), 152.8 (Chromenopyridine-C_2), 149.2 (Chromenopyridine-C_{9a}), 144.4 (Benzoxazole-C'_{3a}), 139.9 (Chromenopyridine- C_4), 130.4 (Benzoxazole- C'_{7a}), 126.6 (Chromenopyridine-C_8), 124.5 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 123.1 (Benzoxazole- C'_6), 122.4 (Chromenopyridine- C_6, C_7, Benzoxazole- C'_7), 119.9 (Benzoxazole- C'_5), 115.8 (CN), 110.1 (Chromenopyridine- C_9), 109.8 (Benzoxazole- C'_4), 63.6 (Chromenopyridine- C_5); IR (cm^{-1}): 3353, 3104, 2921, 2224, 1661, 1531, 1464, 1386, 1173, 1032; MS (EI): m/z 432.1174 [M^+].

Entry MBT-V: 2,4-diamino-5-[(5-sulphonyl-1,3-benzothiazol-2-yl)sulfanyl]-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile

M.P. 248-250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 7.14-7.28 (m, 4H, Chromenopyridine-H_6, H_7, Benzothiazole- H_4, H_7), 6.71-6.74 (m, 4H, Chromenopyridine-H_8, H_9, Benzothiazole-H_6, NH_2), 5.99 (s, 3H, NH_2), 5.28 (s, 1H, Chromenopyridine-H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.0 (Benzothiazole- C'_2), 162.7 (Chromenopyridine-C_{10a}), 158.1 (Chromenopyridine-C_2), 155.6 (Chromenopyridine-C_{9a}), 150.1 (Benzothiazole-C'_{3a}), 142.5 (Chromenopyridine- C_4), 134.1

(Benzothiazole- C'_{7a}), 132.5 (Chromenopyridine- C_8), 131.9 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 130.5 (Benzothiazole- C'_6), 128.0 (Chromenopyridine- C_6, C_7, Benzothiazole- C'_7), 126.4 (Benzothiazole- C'_5), 118.1 (CN), 117.2 (Chromenopyridine- C_9), 111.6 (Benzothiazole- C'_4), 55.7 (Chromenopyridine- C_5); IR (cm^{-1}): 3338, 3051, 2917, 2366, 1647, 1527, 1468, 1386, 1162, 1035; MS (EI): m/z 484.0486 [$M+H^+$].

Entry MBT-VI: 2,4-diamino-5-[(5-sulphonyl-1,3-benzoxazol-2-yl)sulfanyl]-5*H*-chromeno[2,3-*b*] pyridine-3-carbonitrile

M.P.280-282 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 7.59-7.63 (m, 3H, Chromenopyridine- H_6, Benzoxazole- H_4, H_7), 7.46-7.51 (m, 4H, Chromenopyridine- H_7, H_8, H_9, Benzoxazole- H_6), 7.37-7.40 (m, 2H, NH_2), 6.37-6.49 (m, 2H, NH_2), 5.66 (s, 1H, Chromenopyridine- H_5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.0 (Benzoxazole- C'_2), 158.9 (Chromenopyridine- C_{10a}), 150.1 (Chromenopyridine-C_2), 148.5 (Chromenopyridine-C_{9a}), 132.7 (Benzoxazole- C'_{3a}), 130.2 (Chromenopyridine- C_4), 128.6 (Benzoxazole- C'_{7a}), 126.7 (Chromenopyridine- C_8), 126.6 (Chromenopyridine- C_{5a}, C_{4a}, C_3), 126.5 (Benzoxazole- C'_6), 125.5 (Chromenopyridine- C_6, C_7, Benzoxazole- C'_7), 123.3 (Benzoxazole- C'_5), 118.7 (CN), 116.9 (Chromenopyridine- C_9), 108.5 (Benzoxazole- C'_4), 55.1 (Chromenopyridine- C_5); IR (cm^{-1}): 3417, 3100, 2921, 2116, 1643, 1527, 1486, 1393, 1176, 1043; MS (EI): m/z 467.0495 [M^+].

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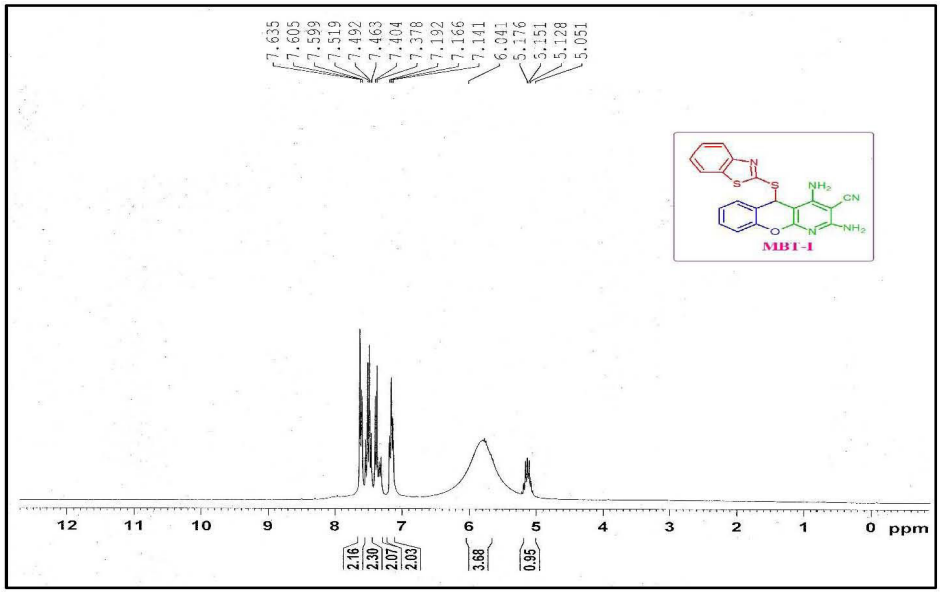
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7.599
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7.492
7.463
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7.378
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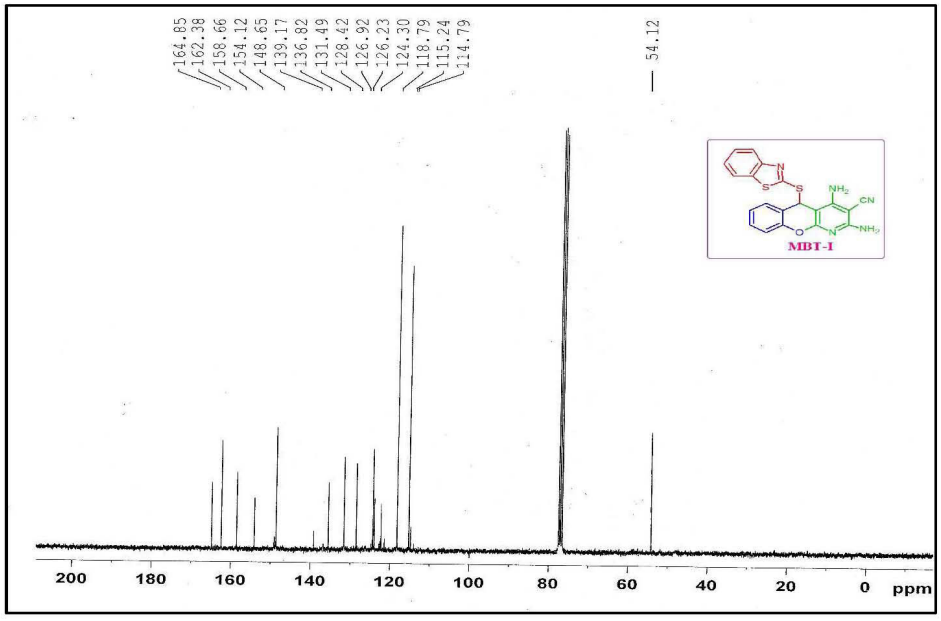
7.166
7.141
6.041
5.176
5.151
5.128
5.051
MBT-I
12
11
10
9
8
7
6
5
4
3
2
1
0 ppm
2.16
2.30
2.07
2.03
3.68
0.95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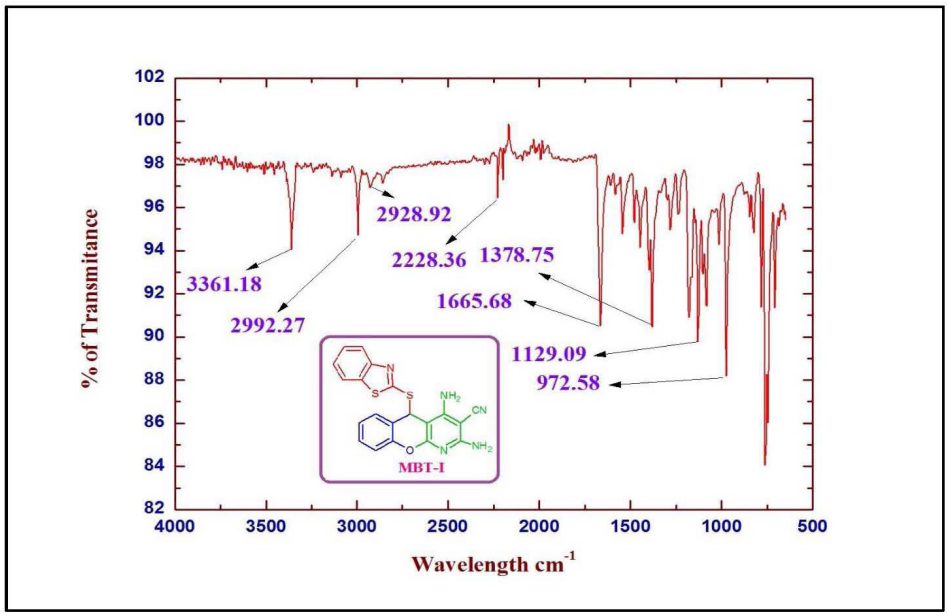
124.30
118.79
115.24
114.79
54.12
MBT-I
200
180
160
140
120
100
80
60
40
20
0 ppm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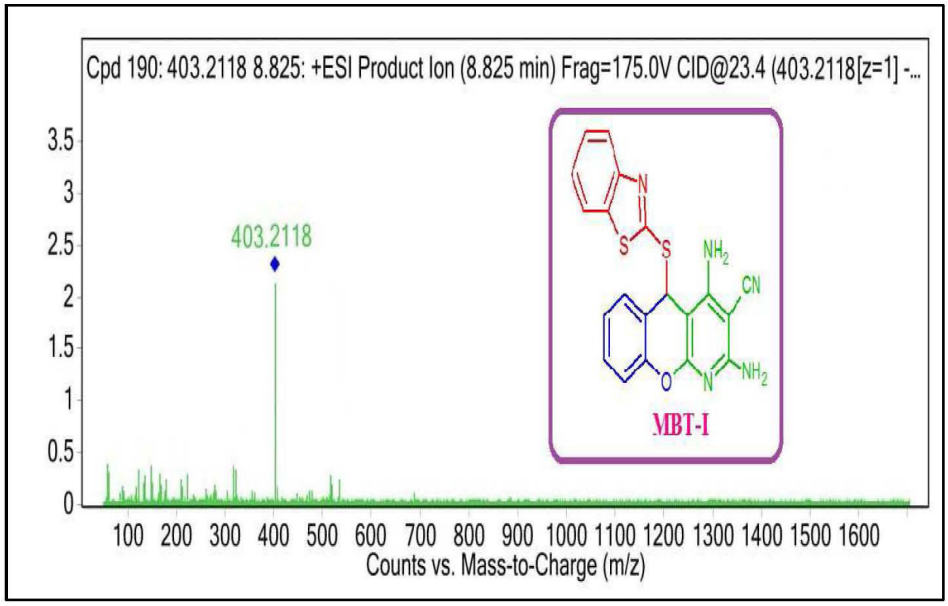
MBT-I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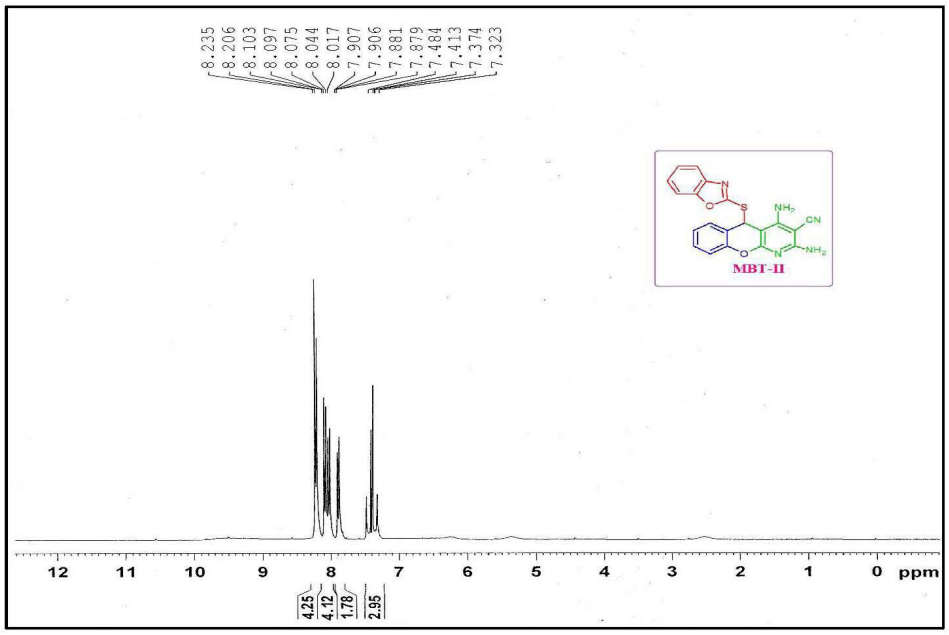
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8.044
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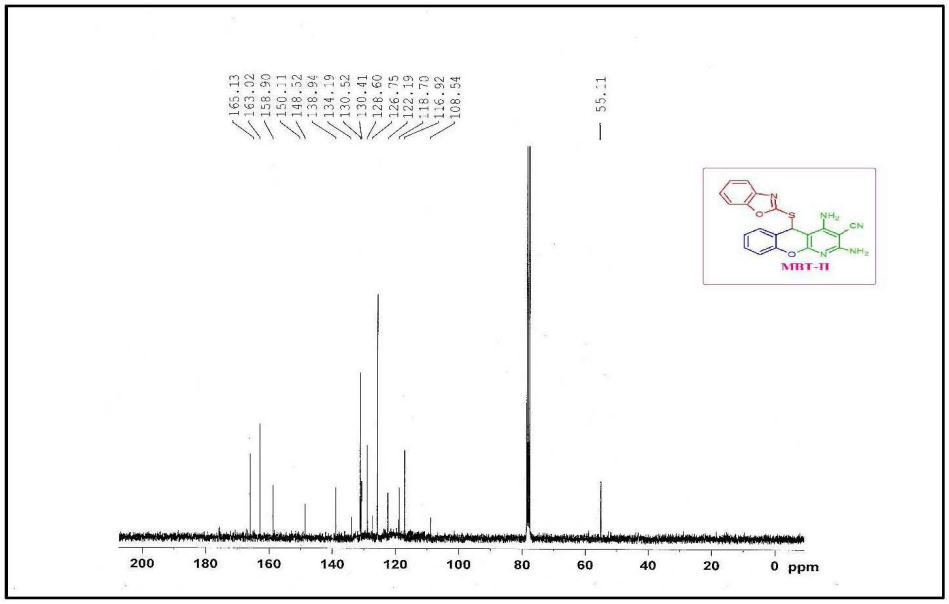
7.881
7.879
7.484
7.413
7.374
7.323
MBT-II
12
11
10
9
8
7
6
5
4
3
2
1
0 ppm
4.25
4.12
1.78
2.95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150.11
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138.94
134.19
130.52
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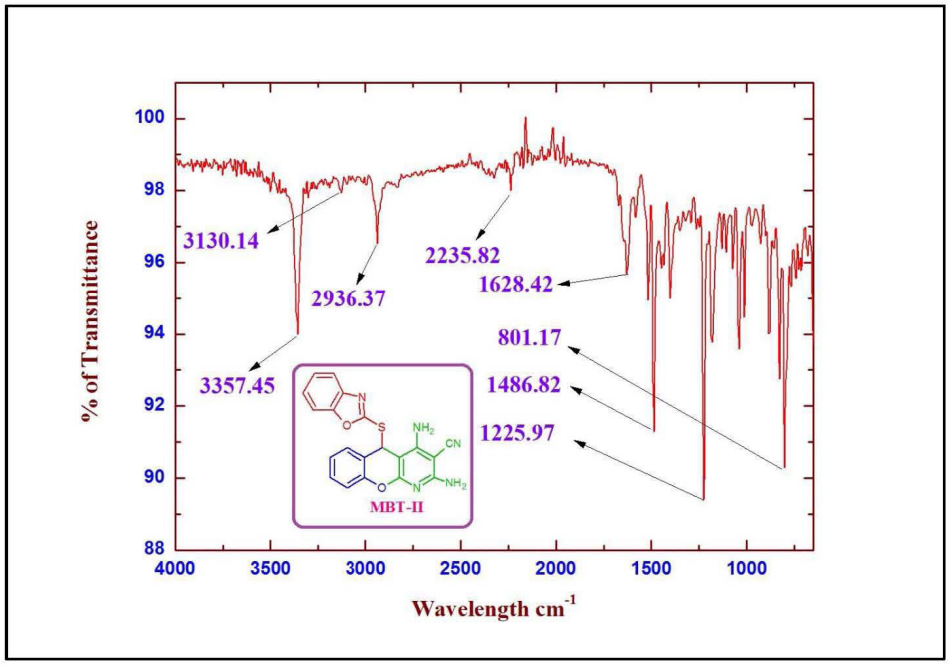
128.60
126.75
122.19
118.70
116.92
108.54
55.11
MBT-II
200
180
160
140
120
100
80
60
40
20
0 ppm


% of Transmittance
Wavelength cm-1
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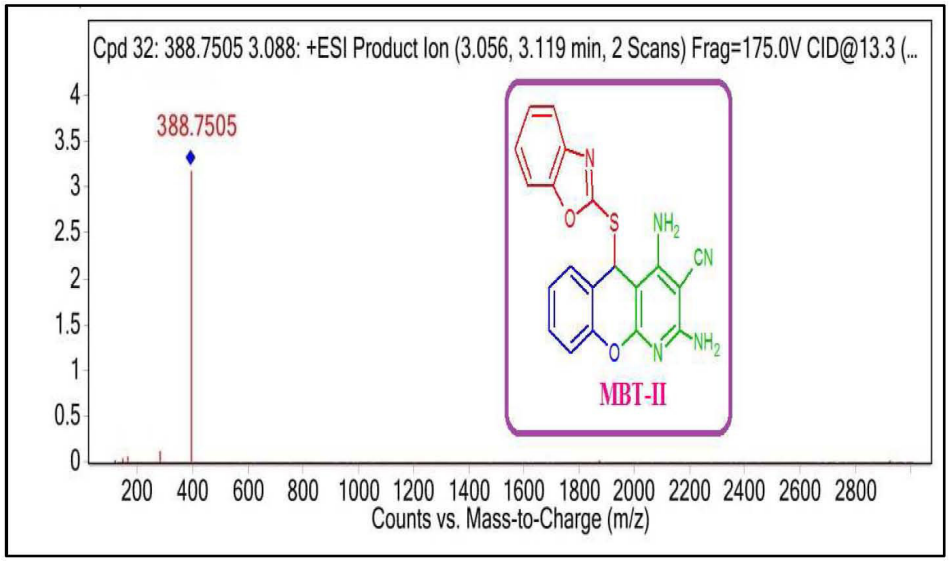
MBT-II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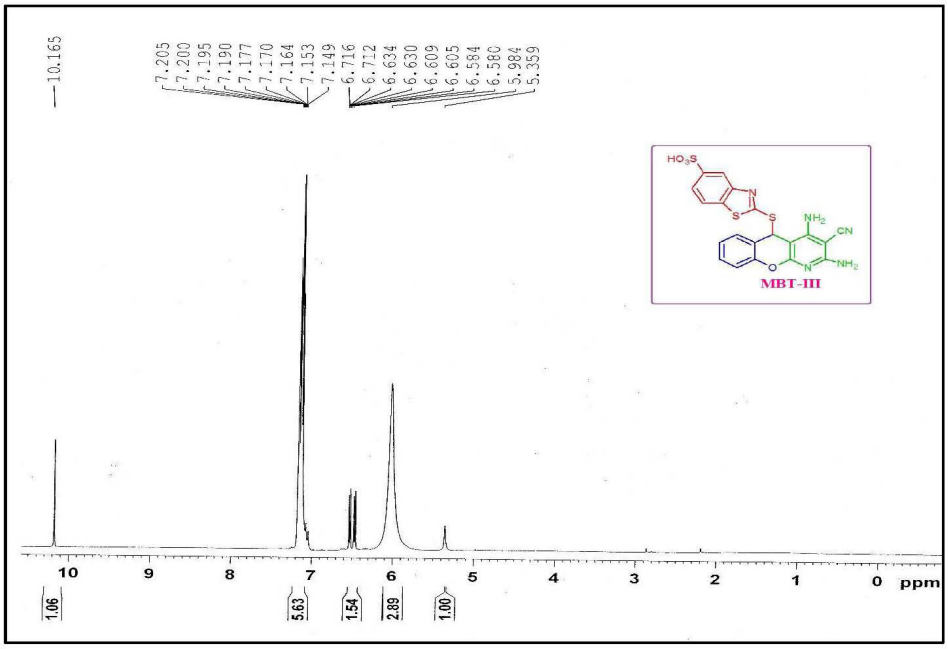
MBT-II
Counts vs. Mass-to-Charge (m/z)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7.177
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7.164
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6.630
6.609
6.605
6.584
6.580
5.984
5.359
MBT-III
10
9
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7
6
5
4
3
2
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0
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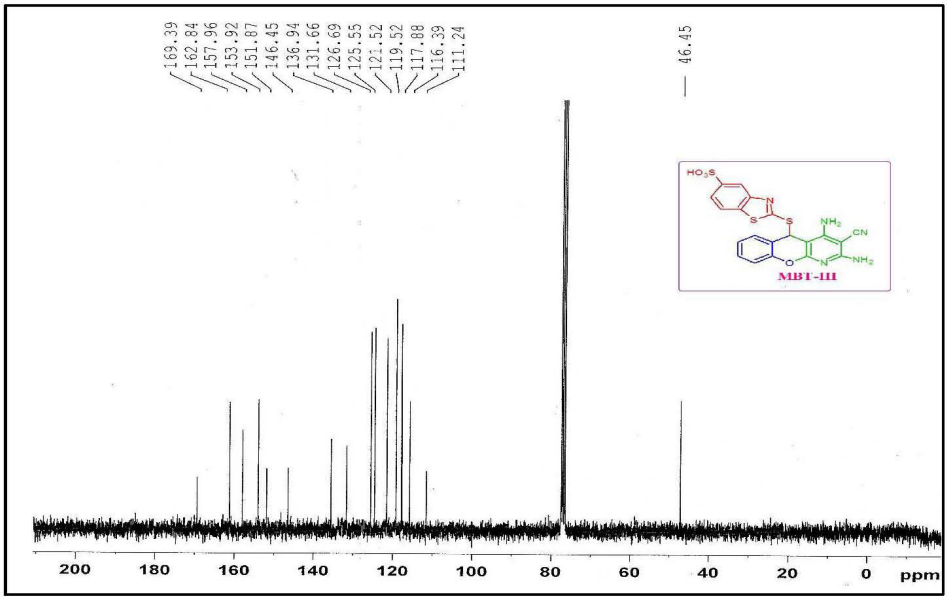
2.89
1.00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131.66
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125.55
122.52
119.52
117.88
116.39
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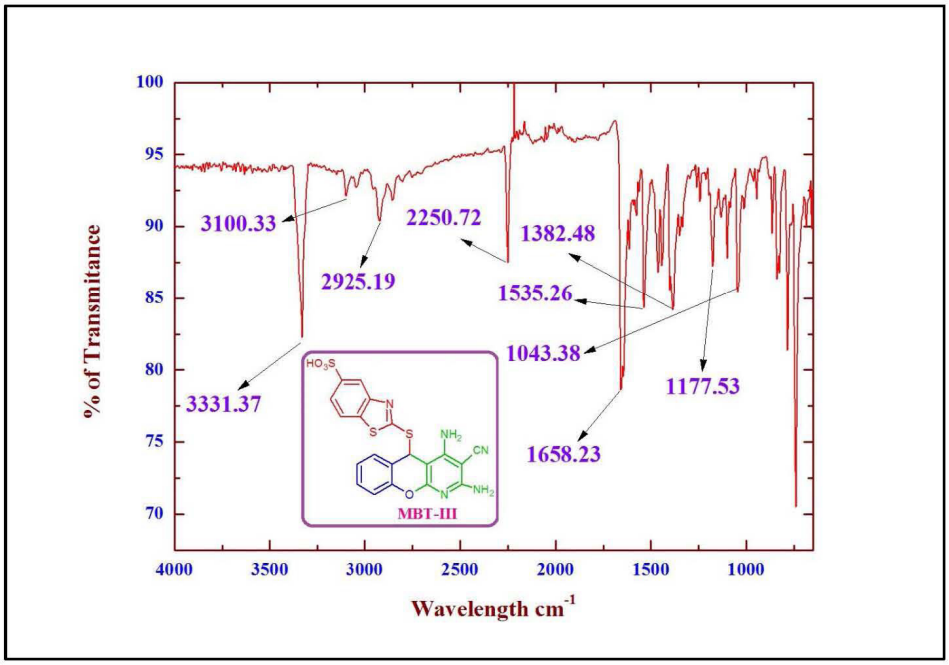
46.45
MBT-III
200
180
160
140
120
100
80
60
40
20
0
ppm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Wavelength cm-1
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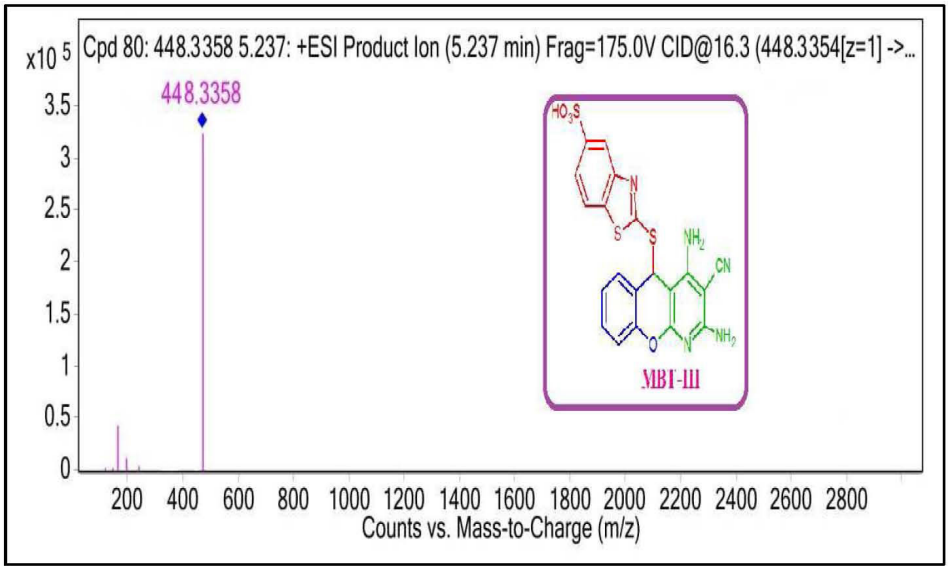
3331.37
MBT-III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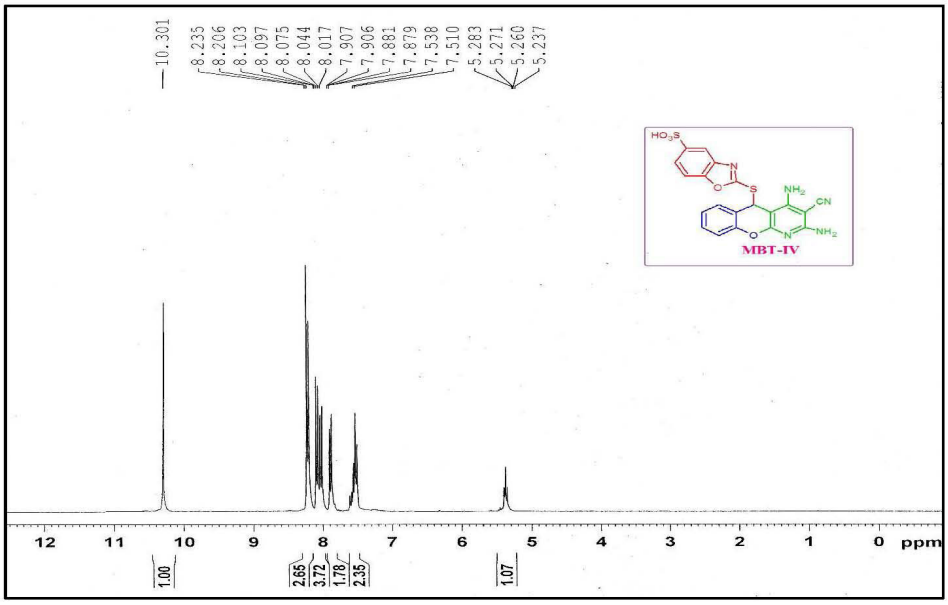
448.3358
Counts vs. Mass-to-Charge (m/z)
MBT-III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7.906
7.881
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7.538
7.510
5.283
5.271
5.260
5.237
MBT-IV
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
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2.65
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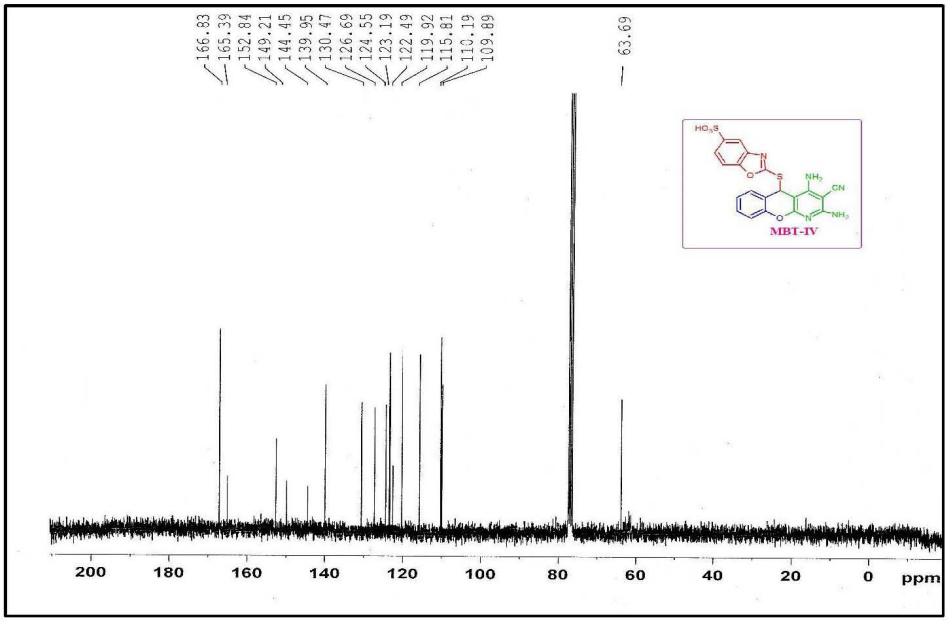
1.78
2.35
1.07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149.21
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139.95
130.47
126.69
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119.92
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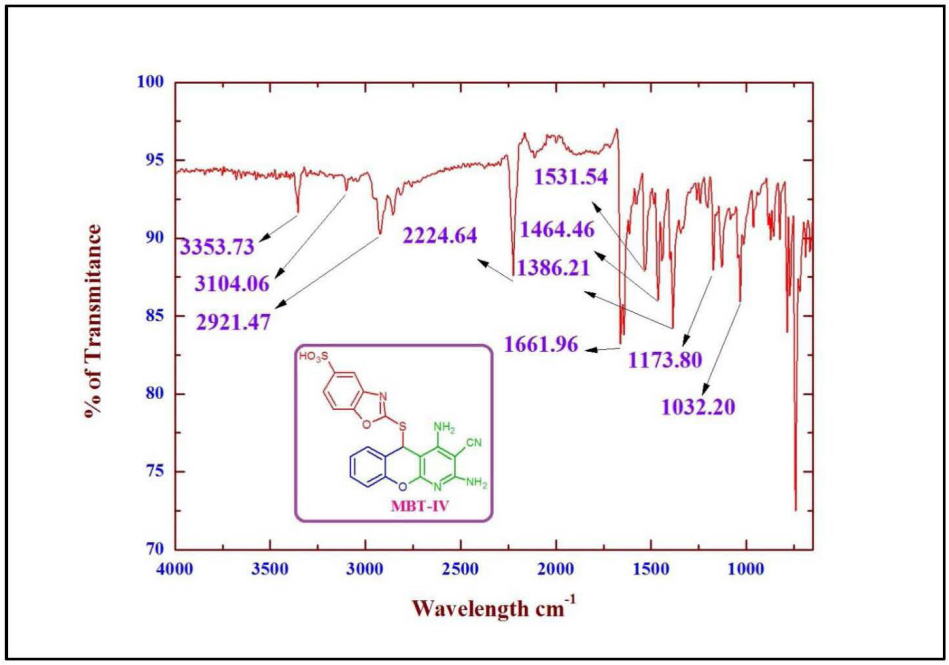
110.19
109.89
63.69
MBT-IV
200 180 160 140 120 100 80 60 40 20 0 ppm


100
95
90
85
80
75
70
% of Transmitance
4000
3500
3000
2500
2000
1500
1000
Wavelength cm-1
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2224.64
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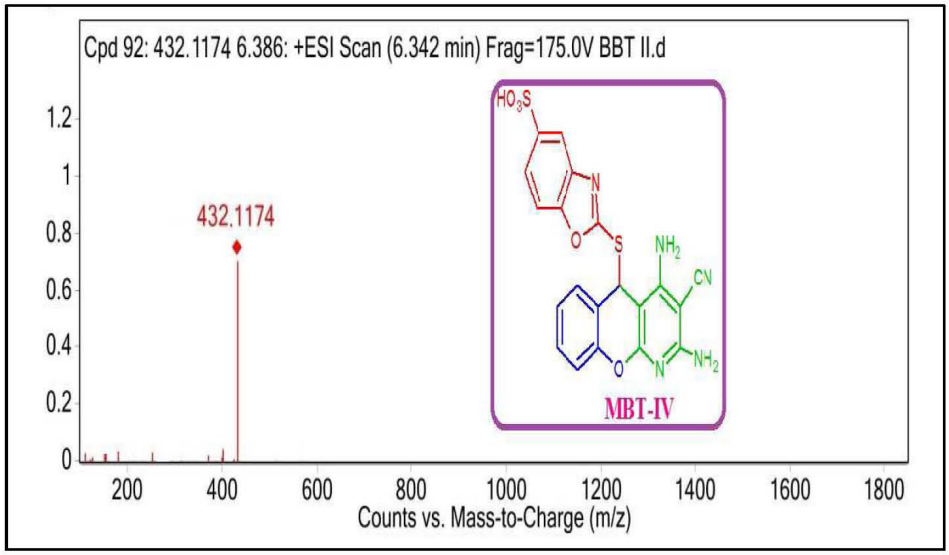
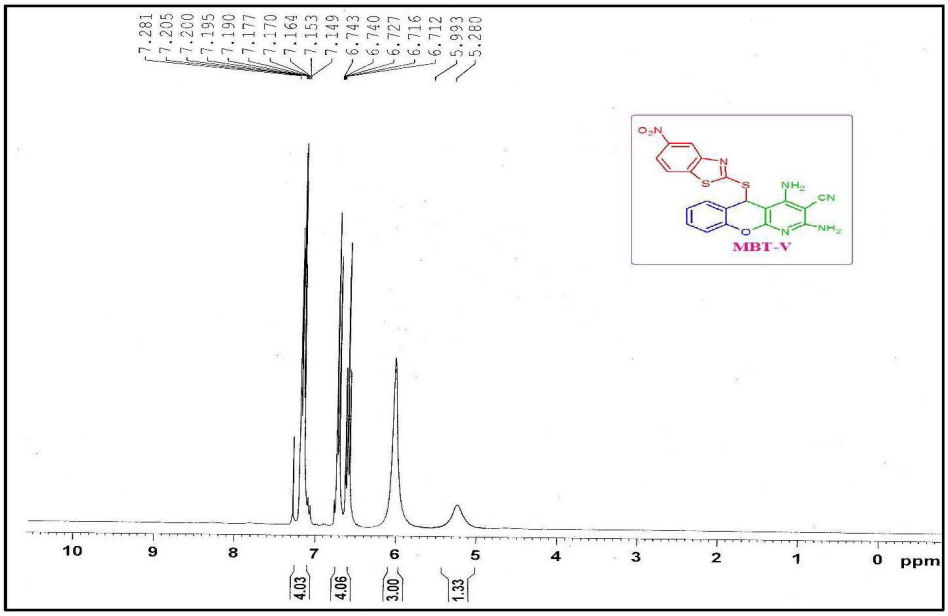
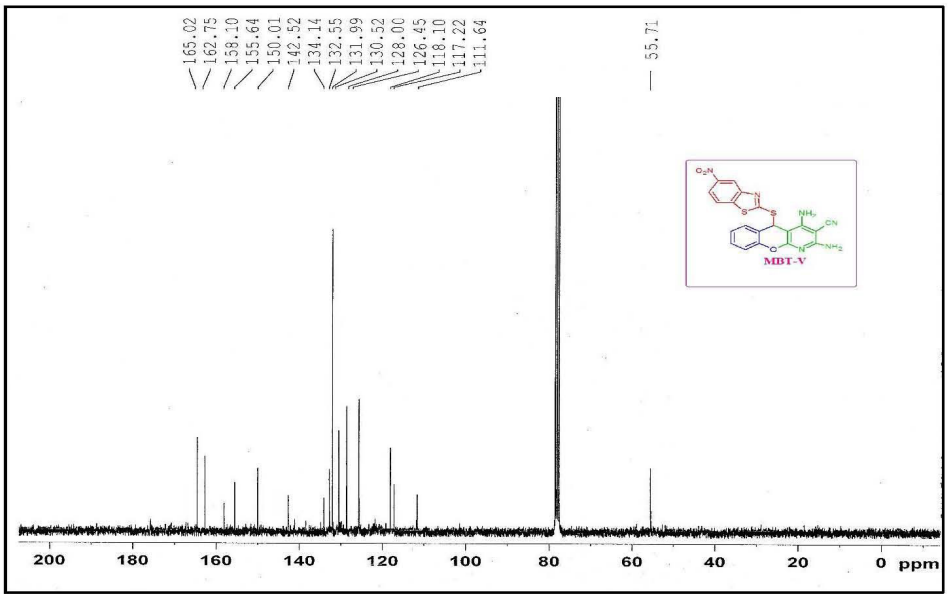
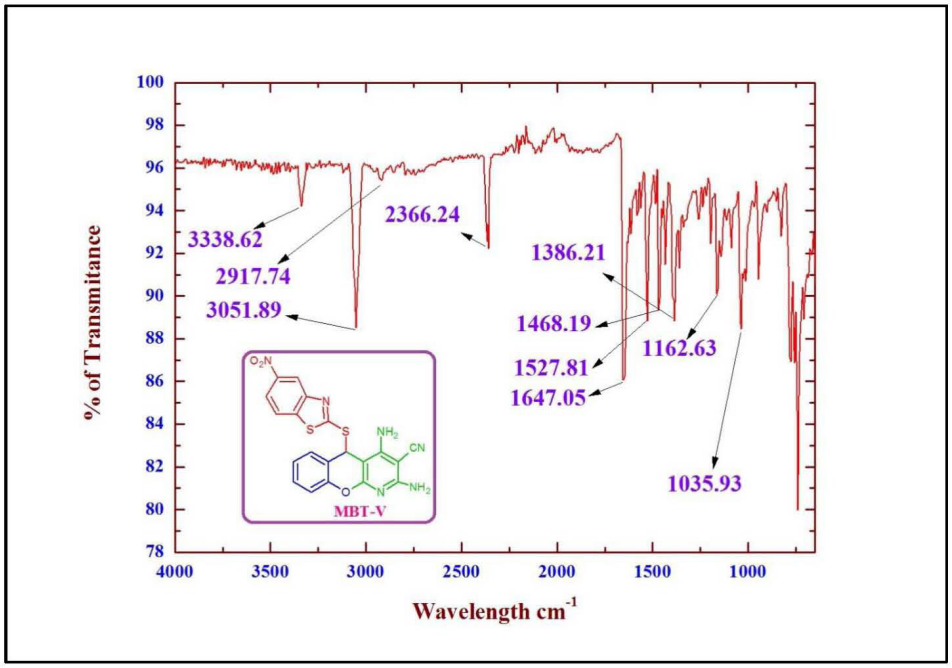
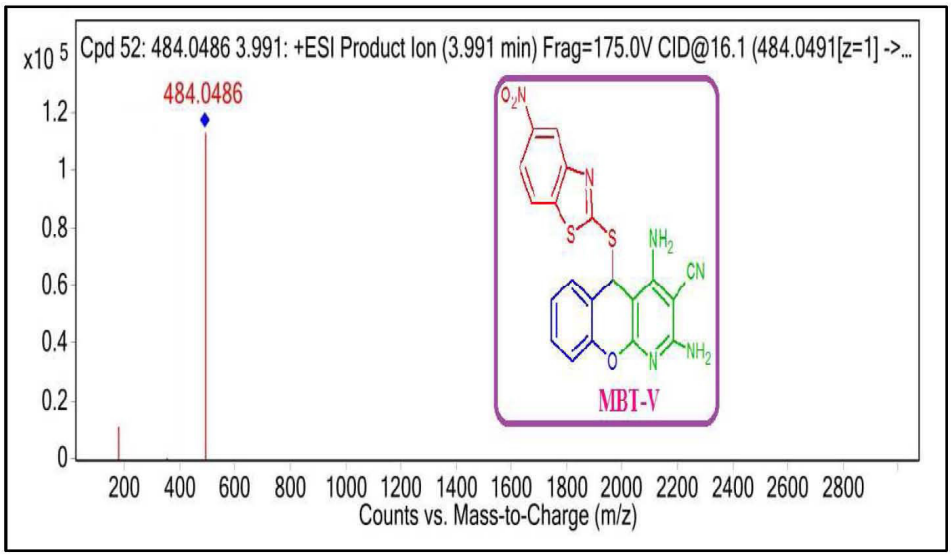
1661.96
1173.80
1032.20
MBT-IV

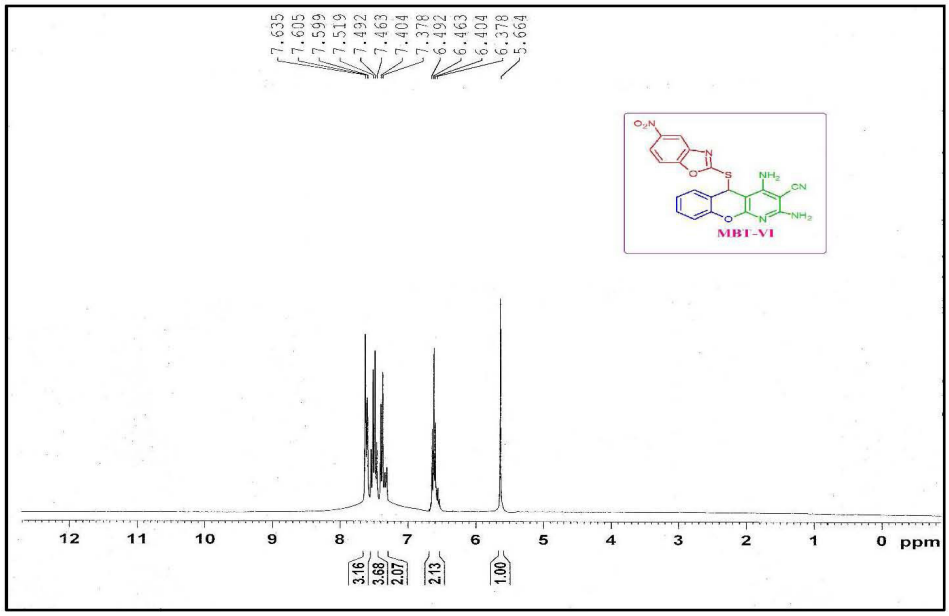

Cpd 92: 432.1174 6.386: +ESI Scan (6.342 min) Frag=175.0V BBT II.d
1.2
1
0.8
0.6
0.4
0.2
0
432.1174
200
400
600
800
1000
1200
1400
1600
1800
Counts vs. Mass-to-Charge (m/z)
MBT-IV

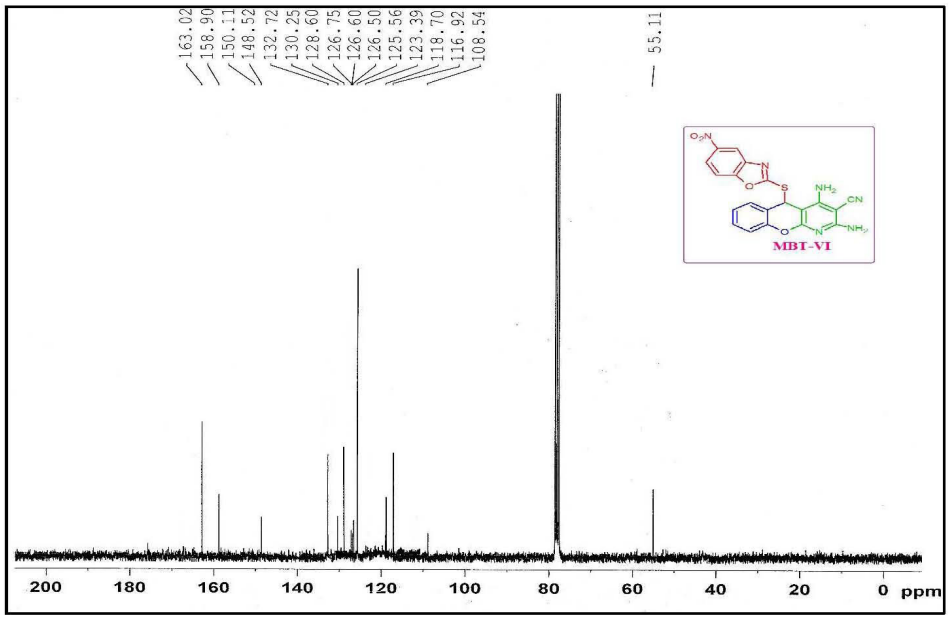

7.281
7.205
7.200
7.195
7.190
7.177
7.170
7.164
7.153
7.149
6.743
6.740
6.727
6.716
6.712
5.993
5.280
MBT-V
10
9
8
7
6
5
4
3
2
1
0 ppm
4.03
4.06
3.00
1.33

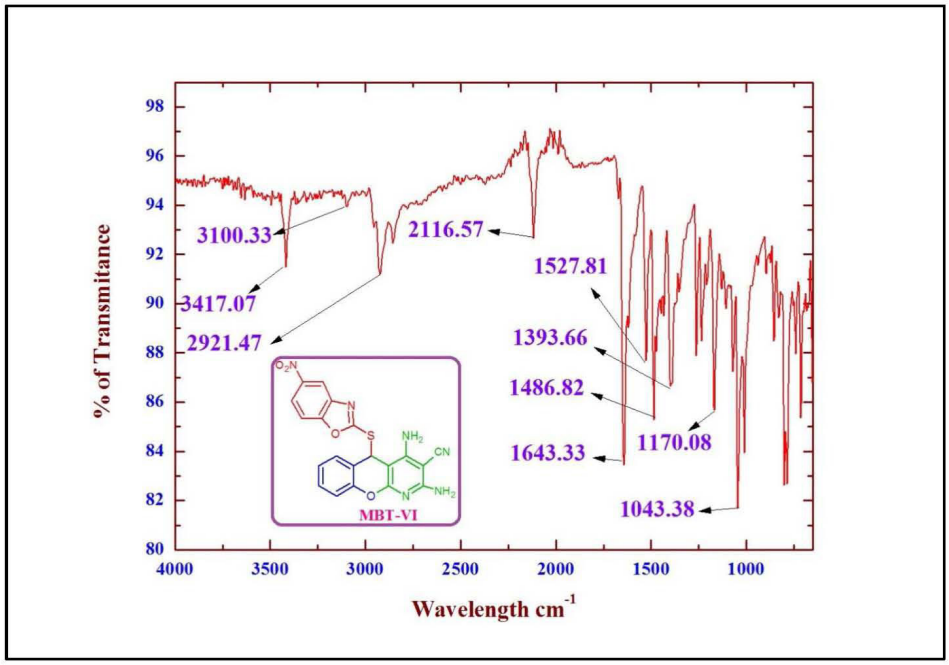

165.02
162.75
158.10
155.64
150.01
142.52
134.14
132.55
131.99
130.52
128.00
126.45
118.10
117.22
111.64
55.71
MBT-V
200
180
160
140
120
100
80
60
40
20
0 ppm

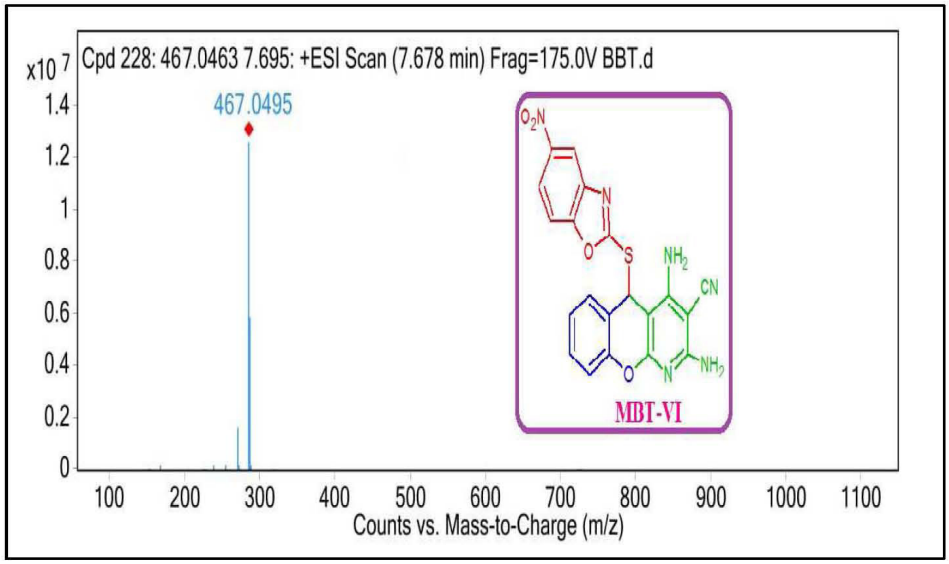

% of Transmitance
Wavelength cm-1
3338.62
2917.74
3051.89
2366.24
1386.21
1468.19
1527.81
1647.05
1162.63
1035.93
MBT-V


Cpd 52: 484.0486 3.991: +ESI Product Ion (3.991 min) Frag=175.0V CID@16.1 (484.0491[z=1] ->...
x10 5
484.0486
MBT-V
Counts vs. Mass-to-Charge (m/z)


7.635
7.605
7.599
7.519
7.492
7.463
7.404
7.378
6.492
6.463
6.404
6.378
5.664
MBT-VI
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
3.16
3.68
2.07
2.13
1.00


163.02
158.90
150.11
148.52
132.72
130.25
128.60
126.75
126.60
126.50
125.56
123.39
118.70
116.92
108.54
55.11
MBT-VI
200 180 160 140 120 100 80 60 40 20 0 ppm


% of Transmitance
Wavelength cm-1
3100.33
3417.07
2921.47
2116.57
1527.81
1393.66
1486.82
1643.33
1170.08
1043.38
MBT-VI


Cpd 228: 467.0463 7.695: +ESI Scan (7.678 min) Frag=175.0V BBT.d
x10 7
467.0495
Counts vs. Mass-to-Charge (m/z)
MBT-VI