Supporting Information

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New symmetrical acyclic and alicyclic *bis*urea derivatives of 4,4'-methylene*bis*(phenyl isocyanate): Synthesis, characterization, bioactivity and antioxidant activity evaluation and molecular docking studies

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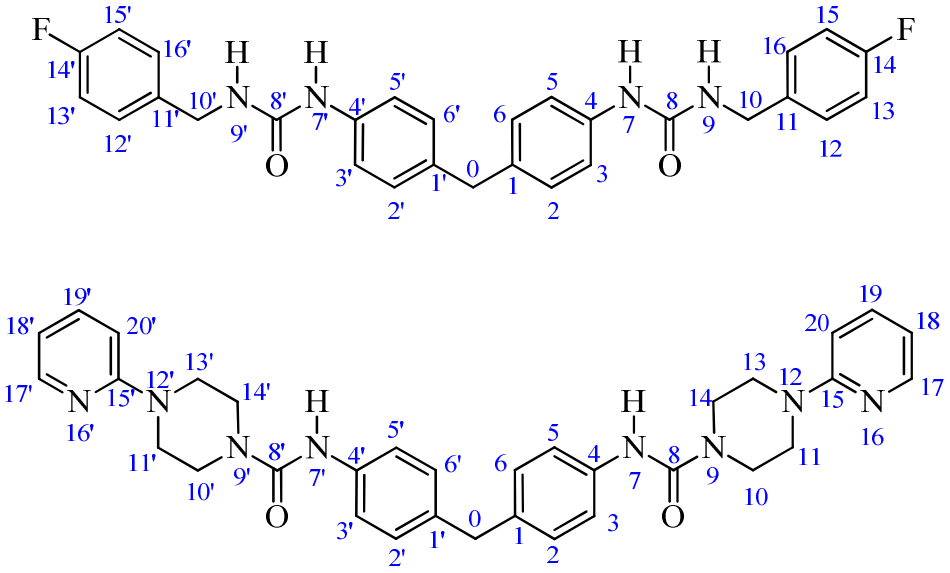
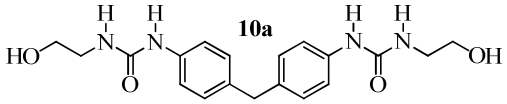
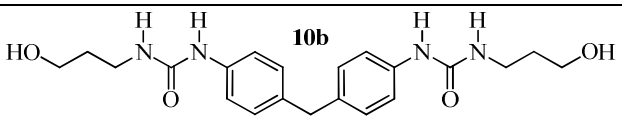
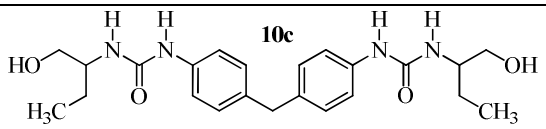
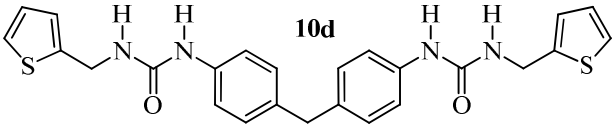
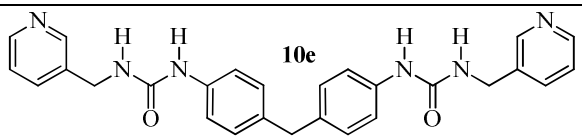
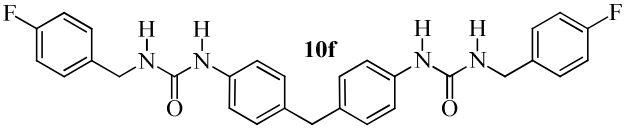
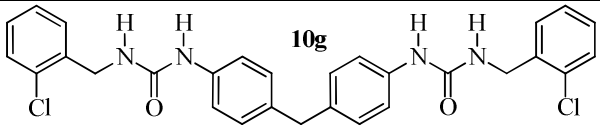
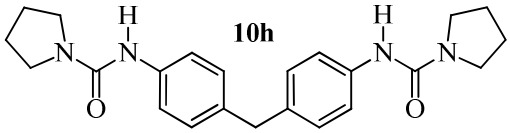
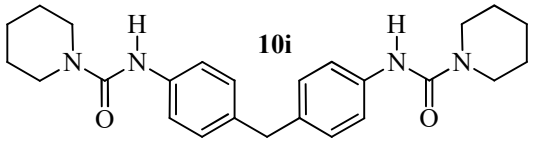
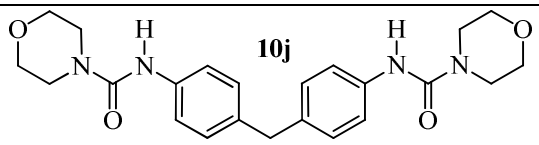
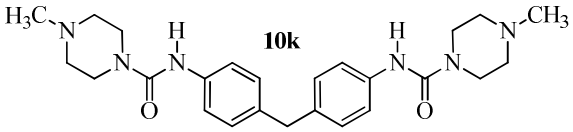
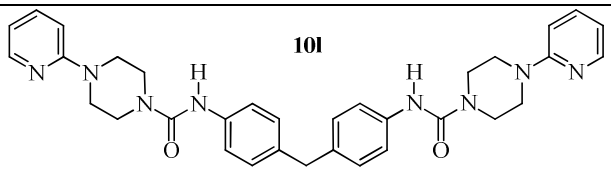
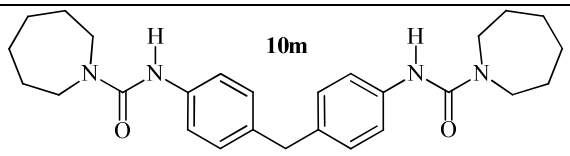
S.1. Numbering of Protons/Carbons for NMR Spectra



S.2. Products of scheme 1

Bisurea Derivative Structure and Name with formula	MW; *m/z* values and Elemental Analysis Data
 **10a** 1,1'-(methylenebis(4,1-phenylene))bis (3-(2-hydroxyethyl)urea) Chemical Formula: $C_{19}H_{24}N_4O_4$	Exact Mass: 372.18 Molecular Weight: 372.42 *m/z: 372.18 (100.0%), 373.18 (22.2%), 374.19 (2.1%), 374.18 (1.1%)* Elemental Analysis: C, 61.28; H, 6.50; N, 15.04; O, 17.18
 **10b** 1,1'-(methylenebis(4,1-phenylene))bis (3-(3-hydroxypropyl)urea) Chemical Formula: $C_{21}H_{28}N_4O_4$	Exact Mass: 400.21 Molecular Weight: 400.47 *m/z: 400.21 (100.0%), 401.21 (24.2%), 402.22 (3.4%)* Elemental Analysis: C, 62.98; H, 7.05; N, 13.99; O, 15.98
 **10c** 1,1'-(methylenebis(4,1-phenylene))bis (3-(1-hydroxybutan-2-yl)urea) Chemical Formula: $C_{23}H_{32}N_4O_4$	Exact Mass: 428.24 Molecular Weight: 428.52 *m/z: 428.24 (100.0%), 429.25 (25.4%), 430.25 (3.9%), 429.24 (1.5%)* Elemental Analysis: C, 64.46; H, 7.53; N, 13.07; O, 14.93
 **10d** 1,1'-(4,4'-methylenebis(4,1-phenylene))bis (3-(thiophen-2-ylmethyl)urea) Chemical Formula: $C_{25}H_{24}N_4O_2S_2$	Exact Mass: 476.13 Molecular Weight: 476.61 *m/z: 476.13 (100.0%), 477.14 (27.4%), 478.13 (9.5%),* 478.14 (4.5%), 477.13 (3.1%), 479.13 (2.7%) Elemental Analysis: C, 63.00; H, 5.08; N, 11.76; O, 6.71; S, 13.46
 **10e** 1,1'-(methylenebis(4,1-phenylene))bis (3-(pyridin-3-ylmethyl)urea) Chemical Formula: $C_{27}H_{26}N_6O_2$	Exact Mass: 466.21 Molecular Weight: 466.53 *m/z: 466.21 (100.0%), 467.22 (29.6%), 468.22 (4.6%), 467.21 (2.2%)* Elemental Analysis: C, 69.51; H, 5.62; N, 18.01; O, 6.86
 **10f** 1,1'-(methylenebis(4,1-phenylene))bis (3-(4-fluorobenzyl)urea) Chemical Formula: $C_{29}H_{26}F_2N_4O_2$	Exact Mass: 500.20 Molecular Weight: 500.54 *m/z: 500.20 (100.0%), 501.21 (31.7%), 502.21 (5.3%), 501.20 (1.5%)* Elemental Analysis: C, 69.59; H, 5.24; F, 7.59; N, 11.19; O, 6.39
 **10g** 1,1'-(4,4'-methylenebis(4,1-phenylene))bis (3-(2-chlorobenzyl)urea) Chemical Formula: $C_{29}H_{26}Cl_2N_4O_2$	Exact Mass: 532.14 Molecular Weight: 533.45 *m/z: 532.14 (100.0%), 534.14 (64.4%), 533.15 (31.7%), 535.14 (21.1%),536.14 (10.8%), 534.15 (5.3%), 537.14 (3.3%), 536.15 (3.1%), 533.14 (1.5%)* Elemental Analysis: C, 65.29; H, 4.91; Cl, 13.29; N, 10.50; O, 6.00

 **10h** *N*,*N*'-(methylenebis(4,1-phenylene))bis (pyrrolidine-1-carboxamide) Chemical Formula: $C_{23}H_{28}N_4O_2$	Exact Mass: 392.22 Molecular Weight: 392.49 *m/z: 392.22 (100.0%), 393.22 (26.4%), 394.23 (3.5%)* Elemental Analysis: C, 70.38; H, 7.19; N, 14.27; O, 8.15
 **10i** *N*,*N*'-(methylenebis(4,1-phenylene))bis (piperidine-1-carboxamide) Chemical Formula: $C_{25}H_{32}N_4O_2$	Exact Mass: 420.25 Molecular Weight: 420.55 *m/z: 420.25 (100.0%), 421.26 (27.5%), 422.26 (4.0%), 421.25 (1.5%)* Elemental Analysis: C, 71.40; H, 7.67; N, 13.32; O, 7.61
 **10j** *N*,*N*'-(methylenebis(4,1-phenylene))bis (morpholine-4-carboxamide) Chemical Formula: $C_{23}H_{28}N_4O_4$	Exact Mass: 424.21 Molecular Weight: 424.49 *m/z: 424.21 (100.0%), 425.21 (26.4%), 426.22 (3.9%)* Elemental Analysis: C, 65.08; H, 6.65; N, 13.20; O, 15.08
 **10k** *N*,*N*'-(methylenebis(4,1-phenylene))bis(4-methyl piperazine-1-carboxamide) Chemical Formula: $C_{25}H_{34}N_6O_2$	Exact Mass: 450.27 Molecular Weight: 450.58 *m/z: 450.27 (100.0%), 451.28 (27.5%), 452.28 (4.1%), 451.27 (2.2%)* Elemental Analysis: C, 66.64; H, 7.61; N, 18.65; O, 7.10
 **10l** *N*,*N*'-(methylenebis(4,1-phenylene))bis(4-(pyridin-2-yl) piperazine-1-carboxamide) Chemical Formula: $C_{33}H_{36}N_8O_2$	Exact Mass: 576.30 Molecular Weight: 576.69 *m/z: 576.30 (100.0%), 577.30 (36.2%), 578.30 (7.7%), 577.29 (3.0%)* Elemental Analysis: C, 68.73; H, 6.29; N, 19.43; O, 5.55
 **10m** *N*,*N*'-(methylenebis(4,1-phenylene))bis(azepane-1-carboxamide) Chemical Formula: $C_{27}H_{36}N_4O_2$	Exact Mass: 448.28 Molecular Weight: 448.60 *m/z: 448.28 (100.0%), 449.29 (29.7%), 450.29 (4.7%), 449.28 (1.5%)* Elemental Analysis: C, 72.29; H, 8.09; N, 12.49; O, 7.13

S.3. Biological Activity Studies

S3.1. Assay Parameters

Test Concentration	32 µg/mL or 20 µM ≤1% DMSO
QC	Duplicate (n=2) Control MIC: Pass
Plates	Non-Binding Surface, 384 well plate
Media Bacteria Fungi	Cation-adjusted Mueller Hinton broth Yeast Nitrogen Base
Read Out Bacteria *C. albicans* *C. neoformans*	OD_{600} OD_{530} Resazurin $OD_{600-570}$

S.3.2 Methods

S.3.2.1 Sample preparation

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 µg/mL or 20 µM (unless otherwise indicated in the data sheet), in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO. All the sample-preparation was done using liquid handling robots. Compounds that showed solubility issues during stock solution preparation are detailed in the data sheet.

S.3.3 Antimicrobial Assay

S.3.3.1 Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton Broth (**CAMHB**) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound containing plates, giving a cell density of $5x10^5$ CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

S.3.3.2 Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

S.3.4. Antifungal Assay

S.3.4.1 Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (**YPD**) agar at 30 °C. A yeast suspension of 1 x 10^6 to 5 x 10^6 CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of $2.5x10^3$ CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 24 h without shaking.

3.4.2 Analysis

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

S.3.5 Antibiotic standards preparation and Quality control

Colistin and Vancomycin were used as positive bacterial inhibitor standards for Gram-negative and Gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for *C. albicans and C. neoformans.* The antibiotics were provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384-well NBS plates.

The quality control (QC) of the assays was determined by the antimicrobial controls and the Z'-factor (using positive and negative controls). Each plate was deemed to fulfil the quality criteria (pass QC), if the Z'-factor was above 0.4, and the antimicrobial standards showed full range of activity, with full growth inhibition at their highest concentration, and no growth inhibition at their lowest concentration.

S .3.5.1. Assay materials

Material	**Code**	**Brand**	**Cat No.**
Compound preparation plate [Polypropylene]	PP	Corning	3364
Assay Plates [Non-binding surface]	NBS	384w Corning	3640
Growth media - bacteria	CAMHB	Bacto Laboratories	212322
Culture agar - fungi	YPD	Becton Dickinson	242720
Growth media - fungi	YNB	Becton Dickinson	233520
Resazurin		Sigma-Aldrich	R7017

S.3.5.2. Standards

Sample Name	**Sample ID**	**Full MW**	**Stock Conc. (mg/mL)**	**Solvent**	**Source**
Colistin - Sulfate	MCC_000094:02	1400.63	10.00	DMSO	Sigma; C4461
Vancomycin - HCL	MCC_000095:02	1485.71	10.00	DMSO	Sigma; 861987
Fluconazole	MCC_008383:01	306.27	2.56	DMSO	Sigma; F8929

S.3.5.3. Microbial Strains

ID	Batch	Organism	Strain	Description
GN_001	02	*Escherichia coli*	ATCC 25922	FDA control strain
GN_003	02	*Klebsiella pneumoniae*	ATCC 700603	MDR
GN_034	02	*Acinetobacter baumannii*	ATCC 19606	Type strain
GN_042	02	*Pseudomonas aeruginosa*	ATCC 27853	Quality control strain
GP_020	02	*Staphylococcus aureus*	ATCC 43300	MRSA
FG_001	01	*Candida albicans*	ATCC 90028	CLSI reference
FG_002	01	*Cryptococcus neoformans*	ATCC 208821	H99 - Type strain

S.3.5.4. Controls

Strain ID	***Species***	***Antibiotic***	***Pass/Fail***
GN_001:02	*E. coli*	Colistin	Pass
GN_003:02	*K. pneumonia* (MDR)	Colistin	Pass
GN_034:02	*A. baumannii*	Colistin	Pass
GN_042:02	*P. aeruginosa*	Colistin	Pass
GP_020:02	*S. aureus* (MRSA)	Vancomycin	Pass
FG_001:01	*C. albicans*	Fluconazole	Pass
FG_002:01	*C. neoformans* (H99)	Fluconazole	Pass

S.3.6 Method analysis

S.3.6.1 Antibacterial data collection

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

S.3.6.2 Antifungal data collection

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD_{530}), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD_{600-570}$), after the addition of resazurin (0.001% final concentration) and incubation at 35°C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. Table S1 depicts the antimicrobial activity with % inhibition values.

S.3.6.3 Inhibition

Percentage growth inhibition of an individual sample is calculated based on Negative controls (media only) and Positive Controls (bacterial/fungal media without inhibitors). Please note negative inhibition values indicate that the growth rate (or OD600) is higher compared to the Negative Control (Bacteria/fungi only, set to 0% inhibition). The growth rates for all bacteria and fungi has a variation of -/+ 10%, which is within the reported normal distribution of bacterial/fungal growth. Any significant variation (or outliers/hits) is identified by the modified Z-Score, and actives are selected by a combination of inhibition value and Z-Score.

S.3.6.4. Z-Score

Z-Score analysis is done to investigate outliers or hits among the samples. The Z-Score is calculated based on the sample population using a modified Z-Score method which accounts for

possible skewed sample population. The modified method uses median and MAD (median average deviation) instead of average and sd, and a scaling factor [Iglewicz, B. & Hoaglin, D. C. Volume 16: How to Detect and Handle Outliers. The ASQC Basic Reference in Quality Control: Statistical Techniques, 1993]: M(i) = 0.6745 * (x(i) - median(x))/MAD). M(i) values of > |2.5| (absolute) label outliers or hits.

S.3.6.5. Quality Control

All screening is performed as two replica (n=2), with both replicas on different assay plates, but from single plating and performed in a single screening experiment (microbial incubation). Each individual value is reported in the table (see ..1 and ..2). In addition, two values are used as quality controls for individual plates: Z'-Factor [1 - (3 * (sd(NegCtrl)+sd(PosCtrl))/(average(PosCtrl)-average(NegCtrl)))] and Standard Antibiotic controls at different concentrations (>MIC and < MIC). The plate passes the quality control if Z'-Factor >0.4 and Standards are active and inactive at highest and lowest concentrations, respectively. Data not supplied.

S.3.6.6 Selection of Actives

A - [Active] Samples with inhibition values equal to or above 80% and abs(Z-Score) above |2.5| for either replicate (n=2 on different plates) were classed as active.
P - [Partial Active] compounds with inhibition values between 50.9% - 79.9% or abs(Z-Sore) below |2.5|.
I - Inactive compounds with inhibition values below 50% and/or abs(Z-Sore) below |2.5|.

S. 3.6.7. Act XX

Act_XX: Indicates if a compound is active in any of the assays against a specific organism (Sa, Ec, Kp, Pa, Kp, Ca or Cn), or organism classes (GN: Gram-negative, GP: Gram-positive). Please note that the flag indicates single activities even if the average Inhibition values suggests otherwise, in which case a manual adjustment of the flag might be appropriate.

S.3.6.8. Act

Act: Indicates the number of organism-classes (GN,GP and FG) the compound has been found active against, 0 = no activity.

S.3.6.3.9. Sel

Sel: Indicates compounds that have been selected for further dose response studies, Hit-Confirmation. The selection includes all active as well as compounds with ambiguous results requiring confirmation of activity or inactivity.

Table S.1. Summary of Antimicrobial activity of the title compounds 12(a-m) against selected bacterial and fungal pathogens

CO-ADD Compound ID	Compound Code	CO-ADD Project ID	CO-ADD Run ID	Sel	Act	Sa	Ec	Kp	Pa	Ab	Ca	Cn	Conc
C0319639	**10a**	P0459	PSR00143	0	0	39.27	-3.35	19.41	21.49	0.04	12.27	-56.75	32 ug/mL
C0319649	**10b**	P0459	PSR00143	0	0	39.25	2.92	1.09	24.99	4.87	2.27	-57.49	32 ug/mL
C0319640	**10c**	P0459	PSR00143	0	0	27.37	1.53	11.19	20.89	0.62	-0.17	-38.07	32 ug/mL
C0319651	**10d**	P0459	PSR00143	0	0	38.55	-5.58	5.00	23.05	-3.62	6.29	-66.42	32 ug/mL
C0319646	**10e**	P0459	PSR00143	0	0	43.02	3.89	18.06	24.80	11.12	3.50	7.56	32 ug/mL
C0319647	**10f**	P0459	PSR00143	0	0	46.23	4.52	20.19	24.92	14.31	4.25	9.30	32 ug/mL
C0319648	**10g**	P0459	PSR00143	0	0	44.01	4.02	19.46	24.10	12.76	4.59	8.24	32 ug/mL
C0319641	**10h**	P0459	PSR00143	0	0	30.22	0.09	16.99	24.43	5.12	-2.82	-36.92	32 ug/mL
C0319642	**10i**	P0459	PSR00143	0	0	21.66	-3.07	6.07	19.72	-3.13	19.82	2.44	32 ug/mL
C0319643	**10j**	P0459	PSR00143	0	0	29.19	1.12	19.30	17.98	12.02	2.61	10.23	32 ug/mL
C0319644	**10k**	P0459	PSR00143	0	0	42.05	3.67	18.91	19.47	17.22	2.71	6.67	32 ug/mL
C0319650	**10l**	P0459	PSR00143	0	0	51.83	5.16	22.24	23.73	21.84	24.16	9.24	32 ug/mL
C0319645	**10m**	P0459	PSR00143	0	0	48.46	4.68	20.20	25.74	18.03	14.16	9.32	32 ug/mL

GN: Gram-negative species: Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumoniae* ATCC 700603, Ab: *Acinetobacter baumannii* ATCC 19606, Pa: *Pseudomonas aeruginosa* ATCC 27853, GP: Gram-positive species: Sa: *Staphylococcus aureus* ATCC 43300, FG: Fungal species: Ca: *Candida albicans* ATCC 90028 and Cn: *Cryptococcus neoformans* ATCC 208821; Sel: Selected, Act: Active.

S.4. Antioxidant Activity

Oxidation is a chemical reaction that can produce reactive oxygen species (ROS) such as H_2O_2, HOCl, HOBr, ROOH, HNO_2, ozone O_3, singlet oxygen O_2, free radicals like oxygen radical, $O_2^{\cdot}$, superoxide radical anion, $O_2^{\cdot-}$, hydroxyl radical, ·OH, perhydroxyl radical, ·OOH, alkoxy radical, $RO^{\cdot}$, peroxy radical, $ROO^{\cdot}$, and reactive nitrogen species (NOS) nitric oxide, $NO^{\cdot}$, Nitrogen dioxide, $NO_2^{\cdot}$ and peroxynitrite radical, $ONOO^{\cdot}$ leading to chain reactions that may damage cellular components such as proteins (enzyme inhibition, denaturation, degradation), lipids (lipid peroxidation) and DNA (mutations, cancer). An antioxidant is a molecule that prevents the oxidation of other molecules. Antioxidants terminate these chain reactions. Antioxidants are used for two different groups of substances: industrial chemicals added to products to prevent oxidation and natural chemicals found in food and body tissues which exert beneficial health benefits as well as redox signaling. It's a paradox in metabolism that oxygen is required for the existence of life on the Earth and it damages the living cells. Failure of the body to balance or counteract or detoxify the harmful effects of free radicals by means of antioxidants leads to the oxidative stress. Oxidative stress leads to many pathophysiological conditions which include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, neurodegeneration in motor neuron diseases, the pathologies caused by diabetes, rheumatoid arthritis, gene mutations and cancers, chronic fatigue syndrome, fragile X syndrome, heart and blood vessel disorders, atherosclerosis, heart failure, heart attack and inflammatory diseases. Several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase etc. protect DNA from oxidative stress. Dietary antioxidants, β-carotene, vitamin A, vitamin C, and vitamin E, glutathione (mother of antioxidants), flavonoids, catechins, polyphenols, selenium, etc, are found to exert a beneficial health effect if treated with.[1]

The beneficial effects of fruits, vegetables, beverages and newly synthesized drug molecules on human health have been attributed to their antioxidant activities. Therefore, antioxidant activity of food products is recognized as one of the important parameters in determining their functional values. Until now, antioxidant activity has been measured by various chemical and biological methods; 1. β-Carotene Bleaching (BCB) Test 2. Thiobarbituric Acid Reactive Species (TBARS) Assay 3. DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay 4. FRAP (The ferric Reducing Antioxidant Power) Assay and 5. Phycoerythyrin Assay. The present study employed DPPH Radical Scavenging Assay to assess the antioxidant activity of the title compounds.[2]

S.4.1. Antioxidant Activity by DPPH Scavenging Assay

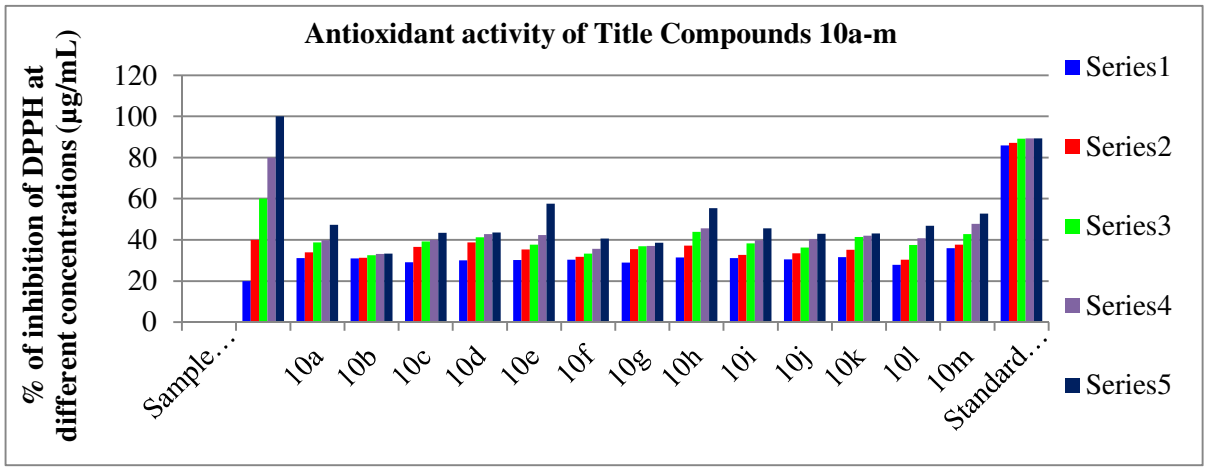


Figure S1. Relative Antioxidant Activity of the title compounds **12a-m**.

Antioxidant potential of the chemicals was estimated using modified DPPH free radical scavenging assay in 96 micro-well flat plates. Stock solutions of the chemicals were prepared as 1 mg/mL in DMSO. Each well was filled in with 200 µL chemical in DMSO starting from 100 µg/mL down to the lowest 20 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in methanol) was added to each well. After keeping the plate in the darkness for 30 minutes, the optical density of each well

was read using Tecan Infinite M 200 micro plate reader at wavelength 517 nm. Percentage of inhibition was calculated using the following formula:

$$\%Inhibition = \frac{OD\ (DPPH - Sample)}{OD\ (DPPH)} x100$$

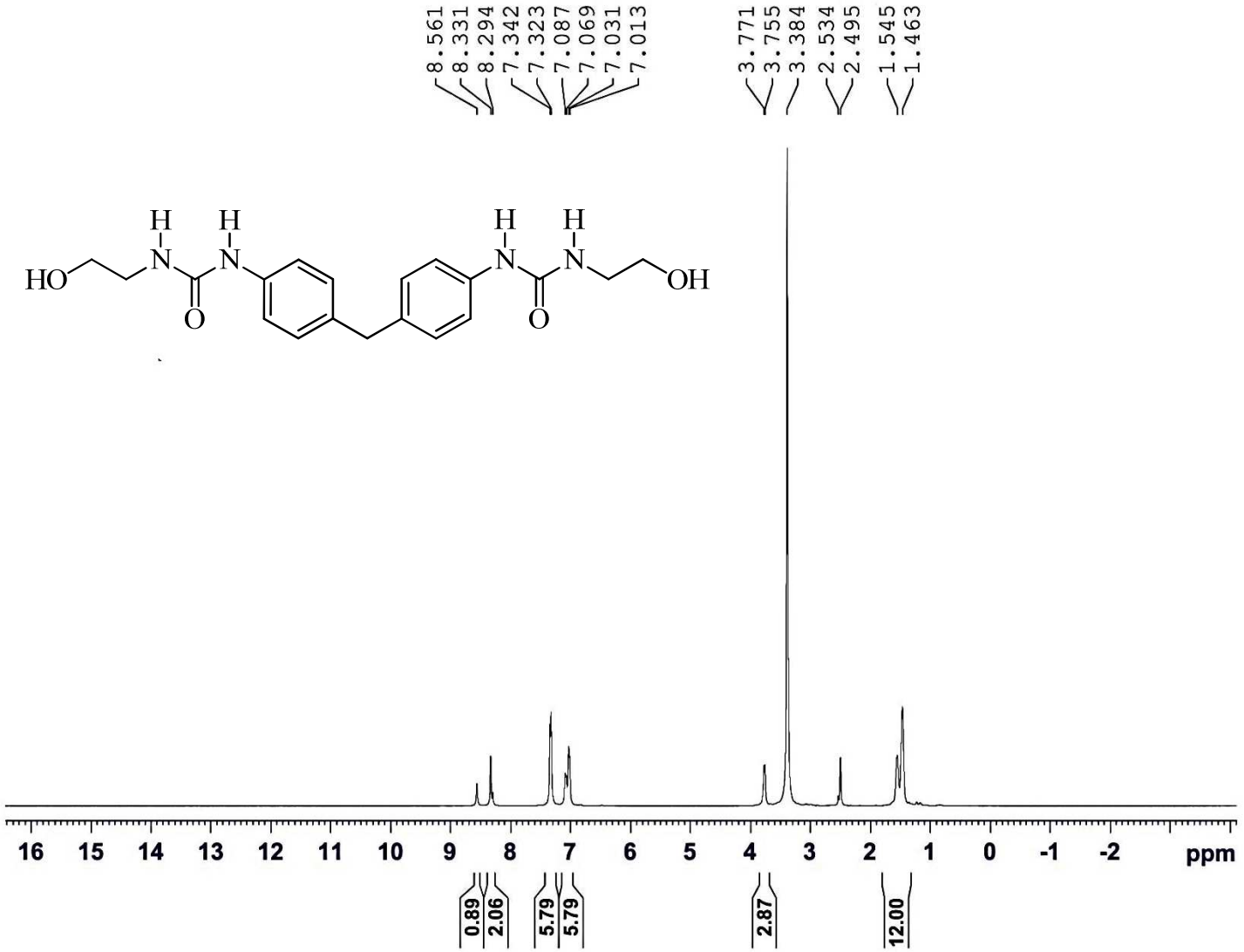
The radical scavenging effect was examined and compared with other natural antioxidant, ascorbic acid which was used as positive control. **Figure S1** depicts the relative antioxidant activity of the title compounds with respect to Vitamin C.

References

[1] Ronald L.; Prior, X. W.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290-4302.

[2] Antolovich, M.; Prenzler, D.P.; Patsalides, E.; Mc Donald, S.; Robards, K. Methods for testing antioxidant activity. *Analyst*, **2002**, *127*, 183–198.

SIF VIT VELLORE
PH-10a

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PROCNO                       1

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TD                       65536
SOLVENT                   DMSO
NS                          16
DS                           2
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FIDRES                0.125483 Hz
AQ                    3.9846387 sec
RG                        63.11
DW                       60.800 usec
DE                        6.50 usec
TE                       296.6 K
D1                  1.00000000 sec
TD0                          1

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P1                       14.25 usec
PLW1               14.00000000 W
SFO1               400.2604718 MHz

F2 - Processing parameters
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TI
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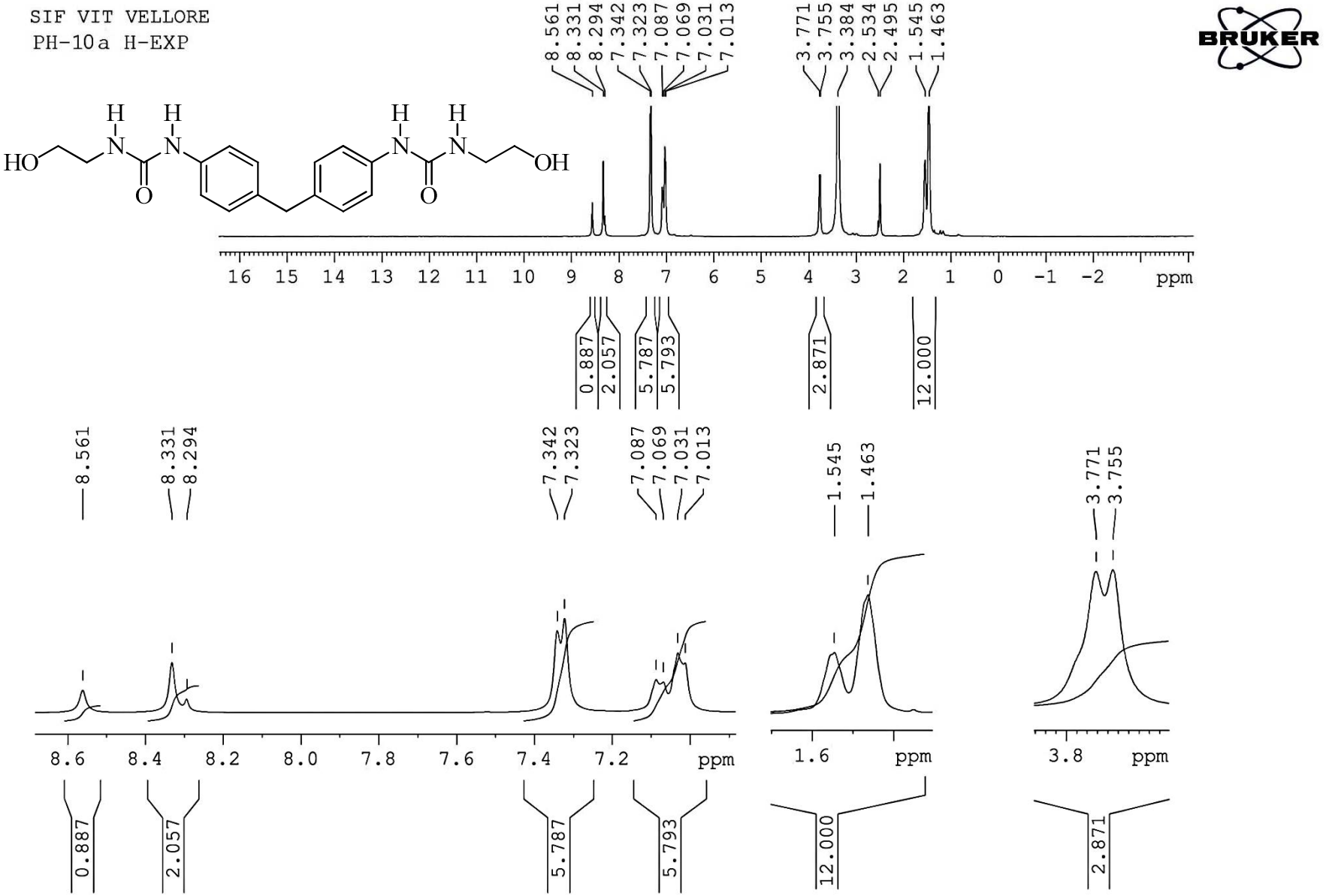
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PH-10a H-EXP
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8.294
7.342
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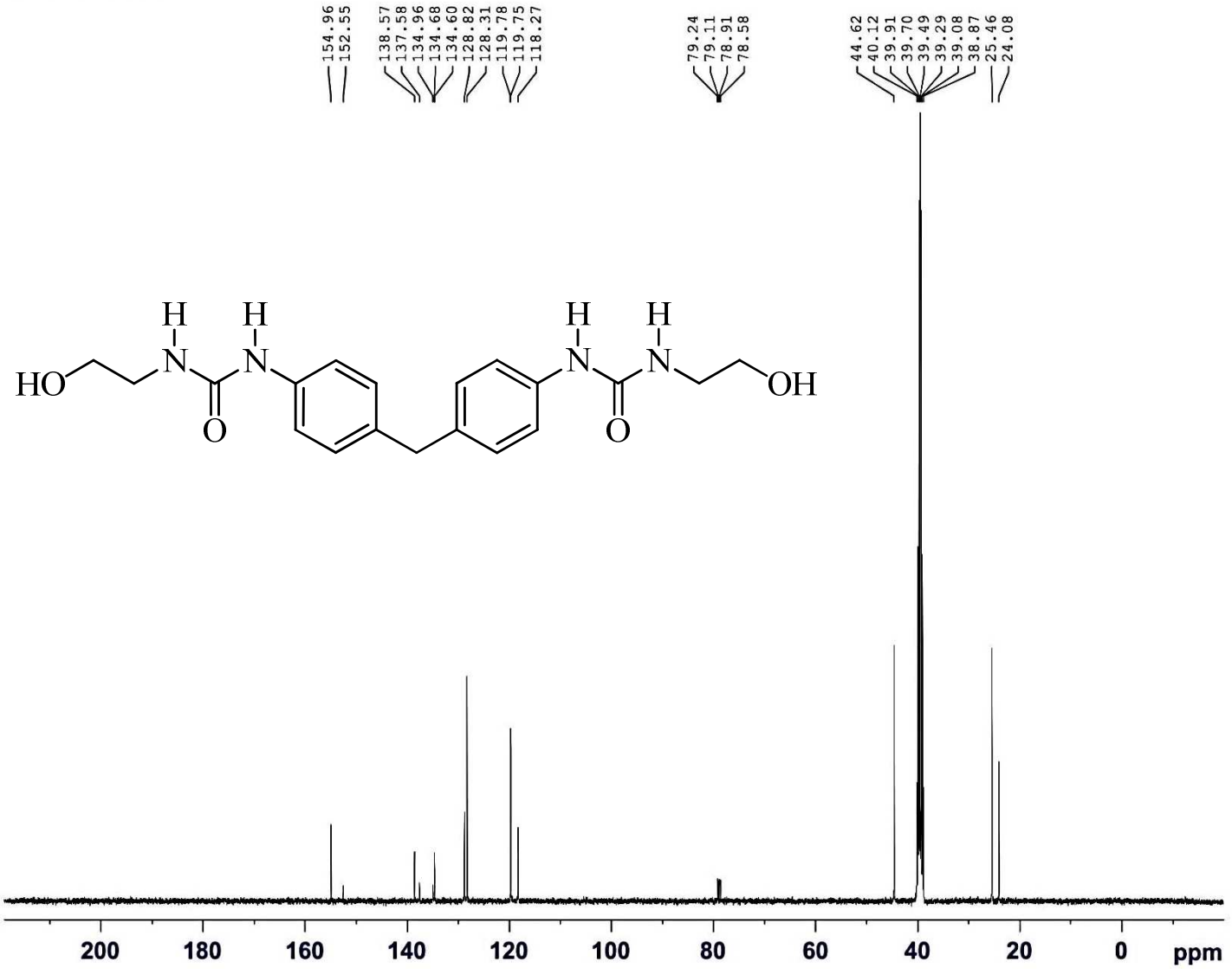
7.069
7.031
7.013
3.771
3.755
3.384
2.534
2.495
1.545
1.463
ppm
0.887
2.057
5.787
5.793
2.871
12.000

SIF VIT VELLORE
PH-10a 13C

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TD                      65536
SOLVENT                  DMSO
NS                         512
DS                           4
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FIDRES                0.366798 Hz
AQ                    1.3631988 sec
RG                        199.6
DW                       20.800 usec
DE                        6.50 usec
TE                       302.3 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
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P1                        9.80 usec
PLW1               58.00000000 W
SFO1              100.6550182 MHz

======== CHANNEL f2 ========
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NUC2                       1H
PCPD2                    90.00 usec
PLW2               14.00000000 W
PLW12               0.35097000 W
PLW13               0.28428999 W
SFO2              400.2596010 MHz

F2 - Processing parameters
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SF                100.6450057 MHz
WDW                         EM
SSB          0
LB                        1.00 Hz
GB           0
PC                        1.40
TI
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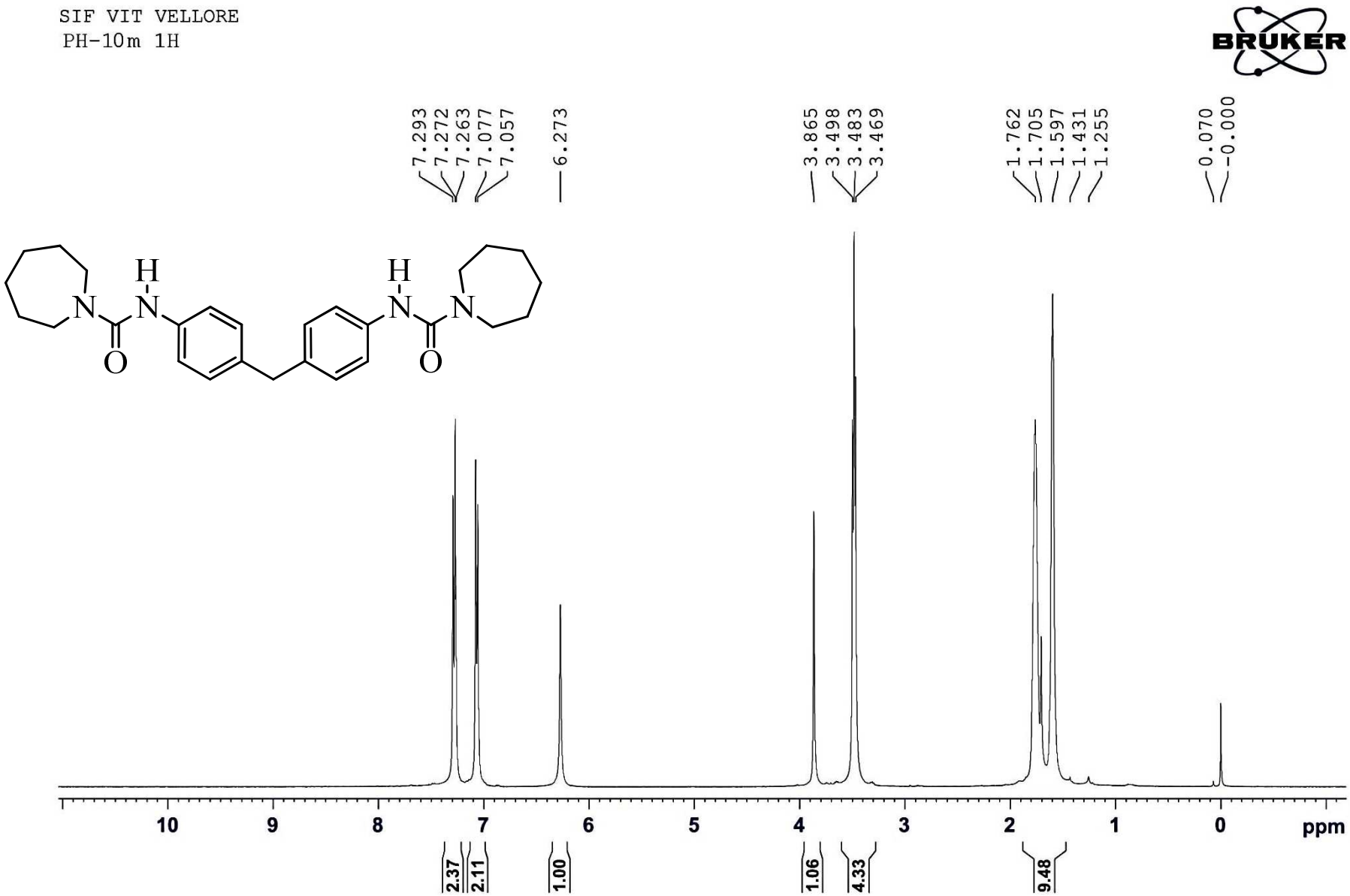
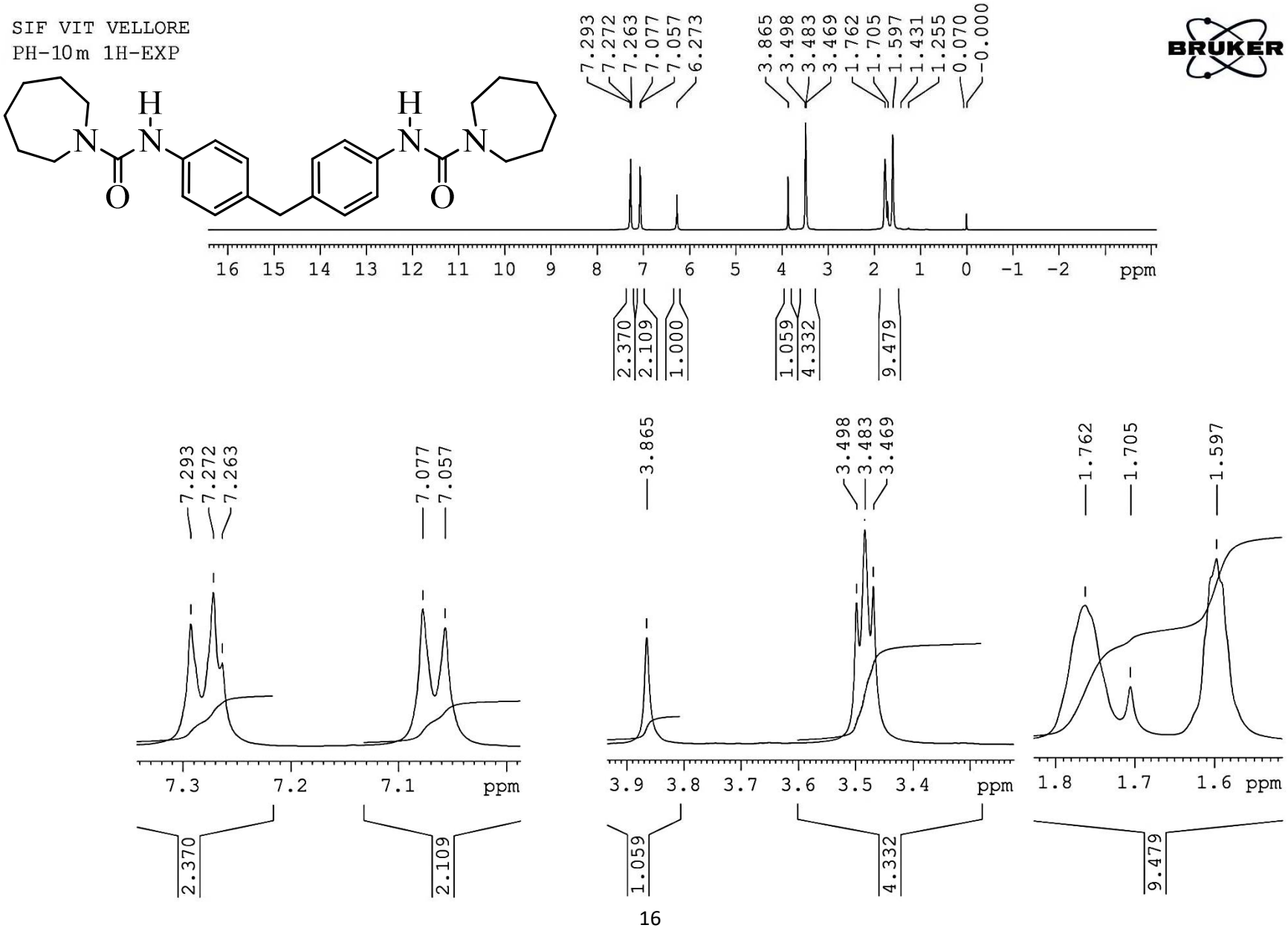
SIF VIT VELLORE
PH-10m 1H

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PH-10m 1H-EXP
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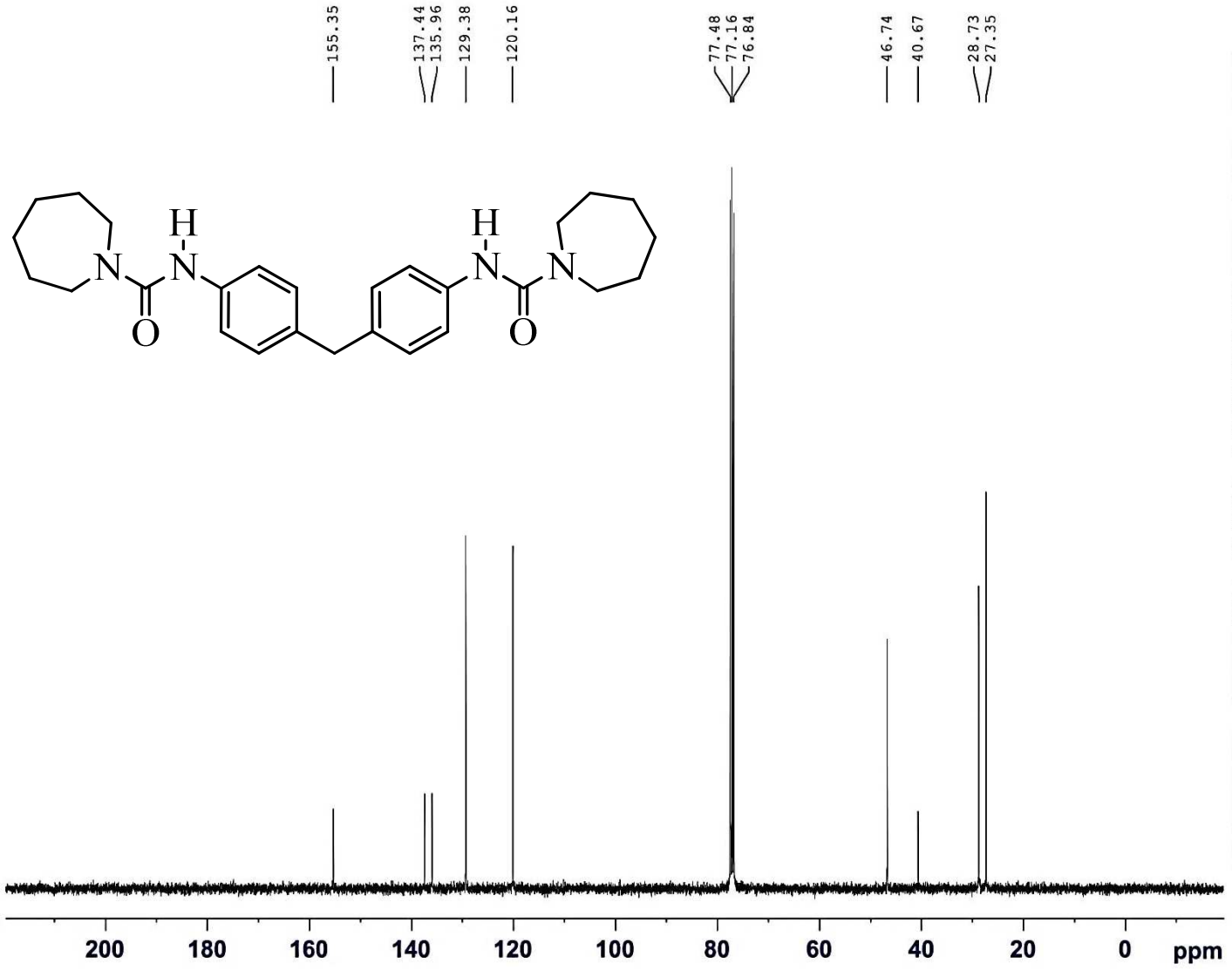
7.263
7.077
7.057
6.273
3.865
3.498
3.483
3.469
1.762
1.705
1.597
1.431
1.255
0.070
-0.000
ppm
2.370
2.109
1.000
1.059
4.332
9.479

SIF VIT VELLORE
PH-10m 13C

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PROCNO                 1

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NS                  512
DS                    4
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FIDRES         0.366798 Hz
AQ             1.3631988 sec
RG                199.6
DW               20.800 usec
DE                6.50 usec
TE                299.8 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 9.80 usec
PLW1        58.00000000 W
SFO1       100.6550182 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             90.00 usec
PLW2        14.00000000 W
PLW12        0.35097000 W
PLW13        0.28428999 W
SFO2       400.2596010 MHz

F2 - Processing parameters
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TI
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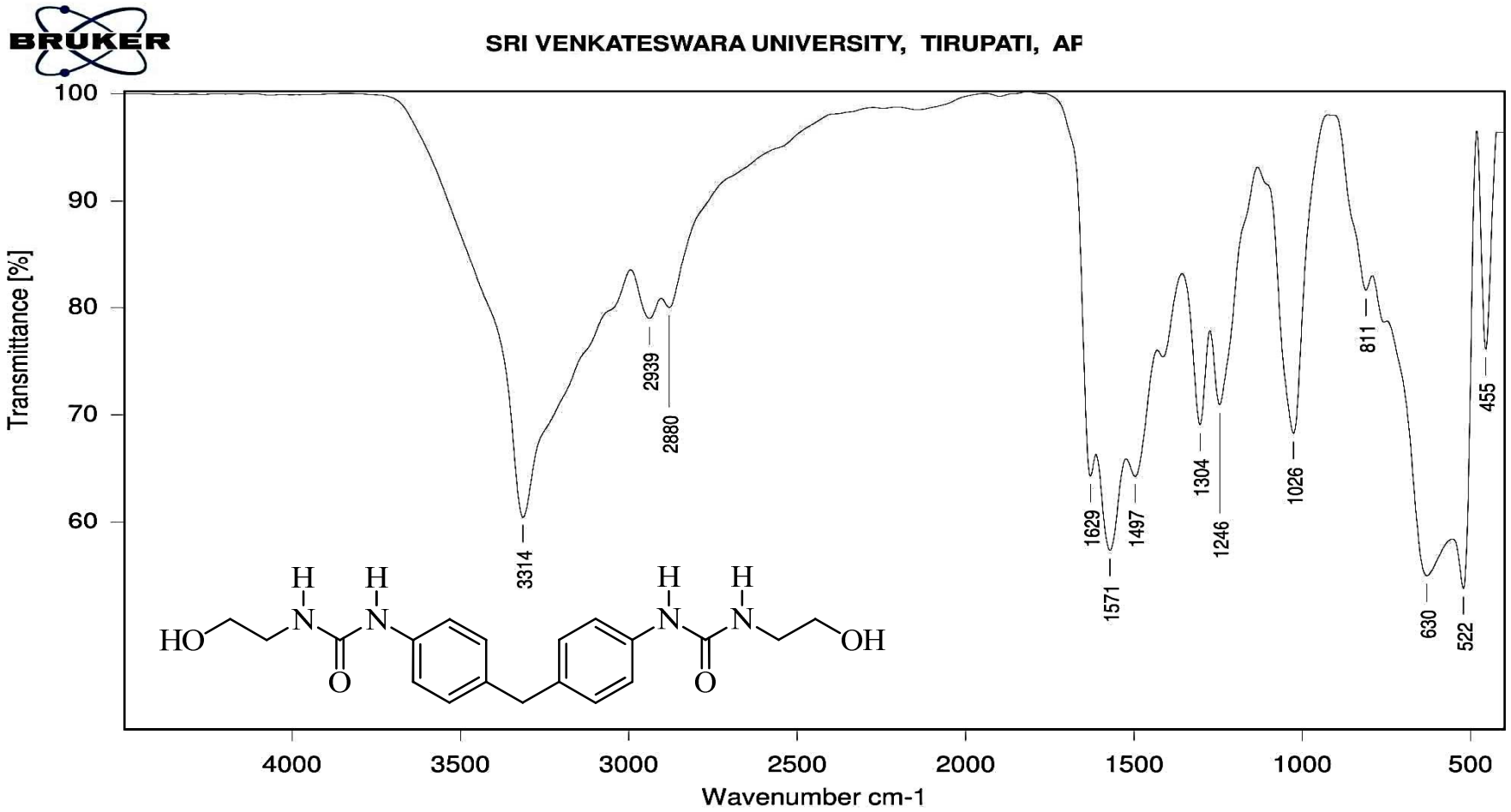

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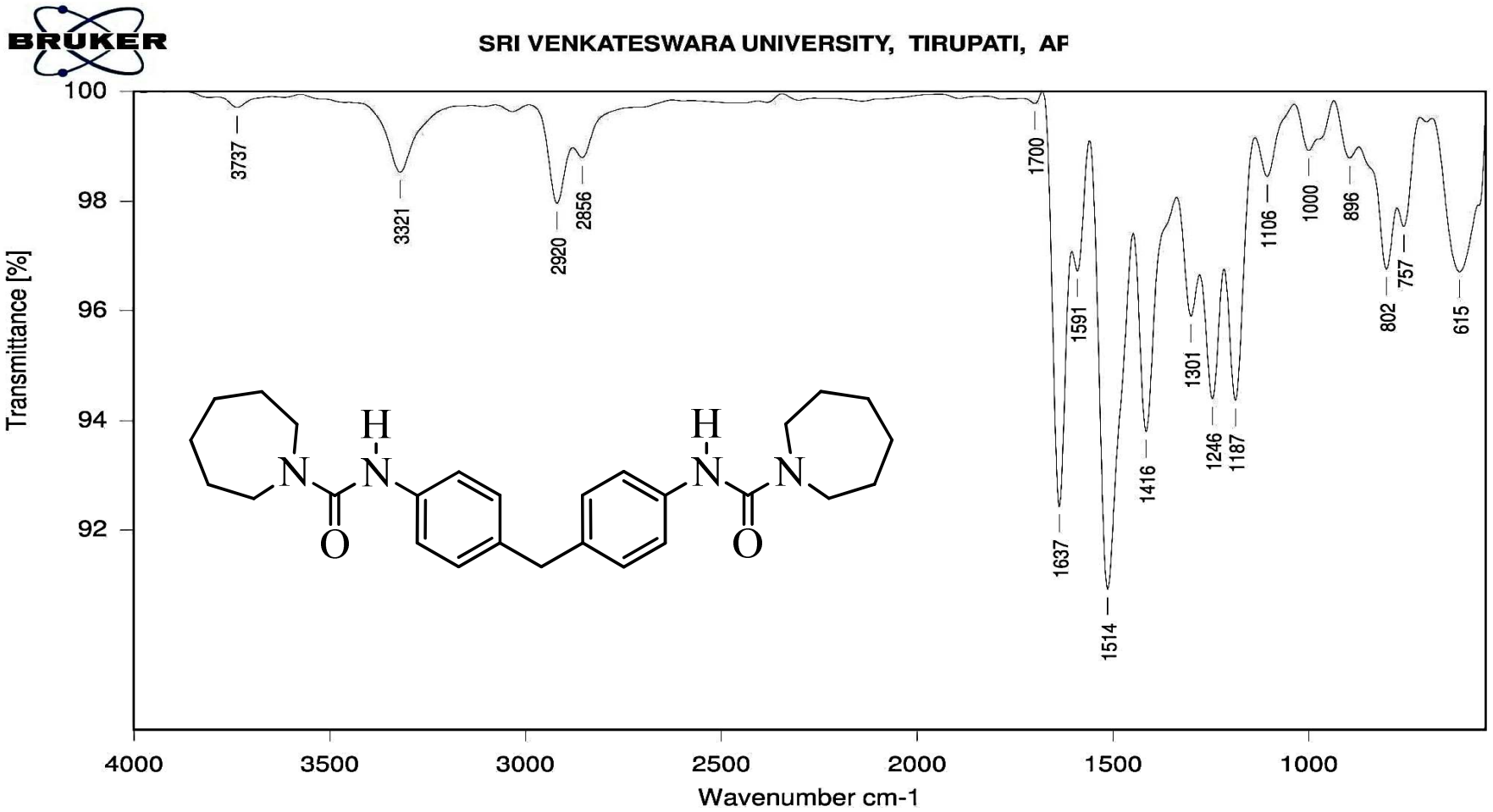
Resolution 4	Signal Gain, Sample
Number of Sample Scans 24	Scan time (sec) 29.77



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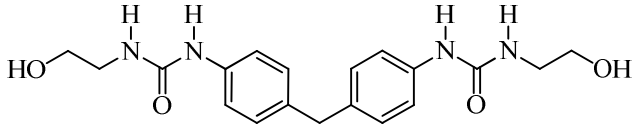
Date of Measurement 12/21/2014	Time of Measurement 12:43:37 PM
Resolution 4	Signal Gain, Sample
Number of Sample Scans 24	Scan time (sec) 29.81

LCMS-2010A DATA REPORT
SHIMADZU



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Sample	: 10a
Inj. Volume	: 5.000
Data Name	: G:\LCMSsolution\User\Data\PH-3.1A-APCI-POS1.qld
Method Name	: C:\LCMSsolution\User\Method\Copy of JAY-4-APCI.qlm

MS Spectrum

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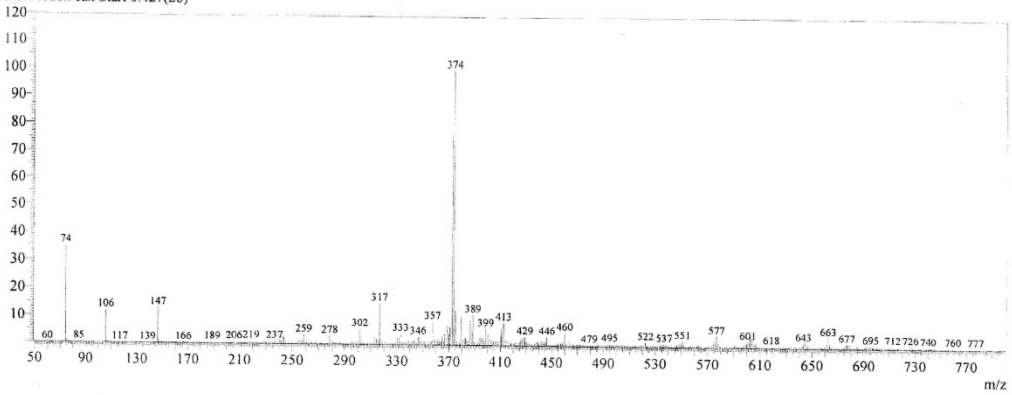
RawMode:Single 0.611(37)
BG Mode:Peak Start 0.427(26)



MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.611	0.427	0.843	513349669	52205433	9.83		100.00		373.85	4422585
				513349669	52205433			100.00			

FLASH EA 1112 SERIES CHN REPORT
THERMO FINNIGAN

Method filename:	C:\Program Files\Thermo Finnigan\Eager 300 for EA1112\DATA\Sys_data_ex
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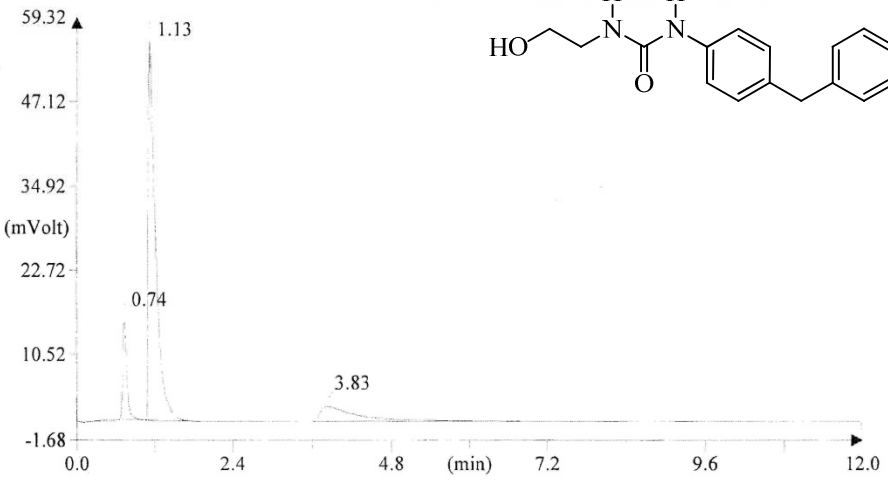
Chromatogram filename:	UNK-27012015-3.dat
Sample weight:	1.415



Element Name	Element %	Ret. Time
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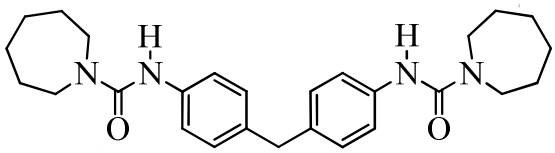
Carbon	61. 36	1. 13
Hydrogen	6. 54	3. 83

LCMS-2010A DATA REPORT
SHIMADZU



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Method Name	: C:\LCMSsolution\User\Method\esi.qlm

MS Spectrum

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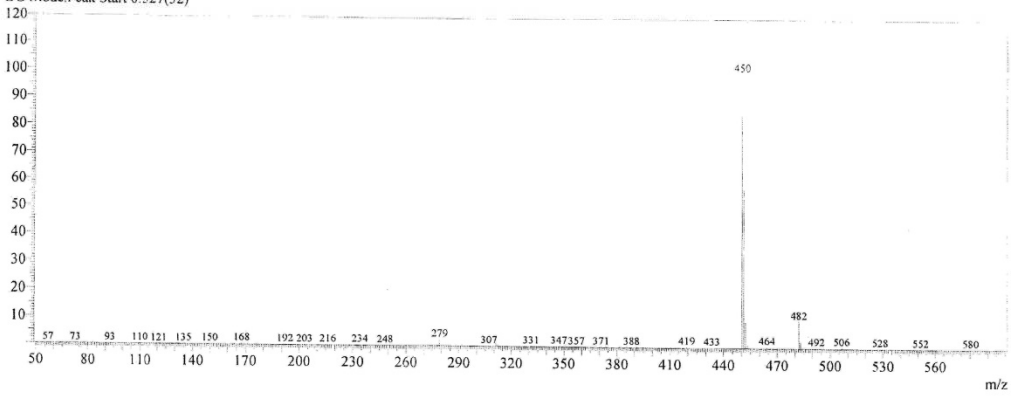
BG Mode:Peak Start 0.527(32)



MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
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				409293736	16473045			100.00			

FLASH EA 1112 SERIES CHN REPORT
THERMO FINNIGAN

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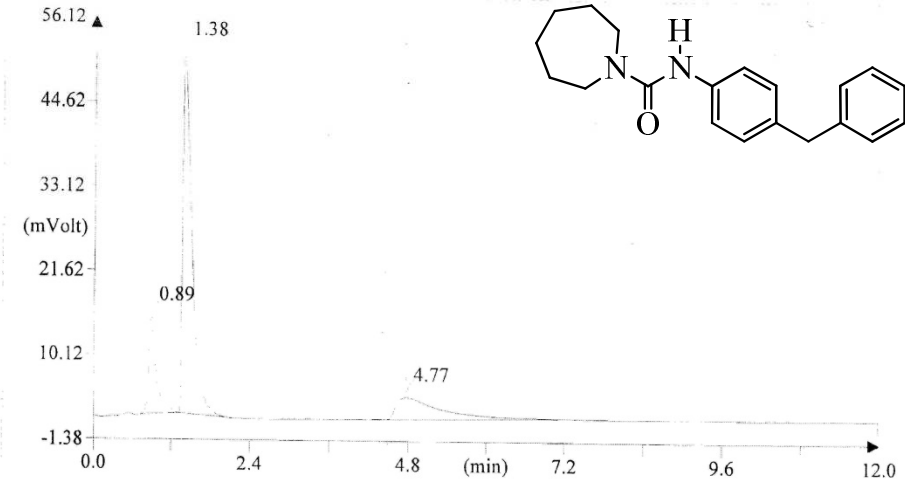

Element Name	Element %	Ret. Time
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Carbon	72. 15	1. 38
Hydrogen	8. 16	4. 77