Supplementary Material

Synthesis and anticancer studies of novel benzimidazole/benzoxazole-pyrazine derivatives

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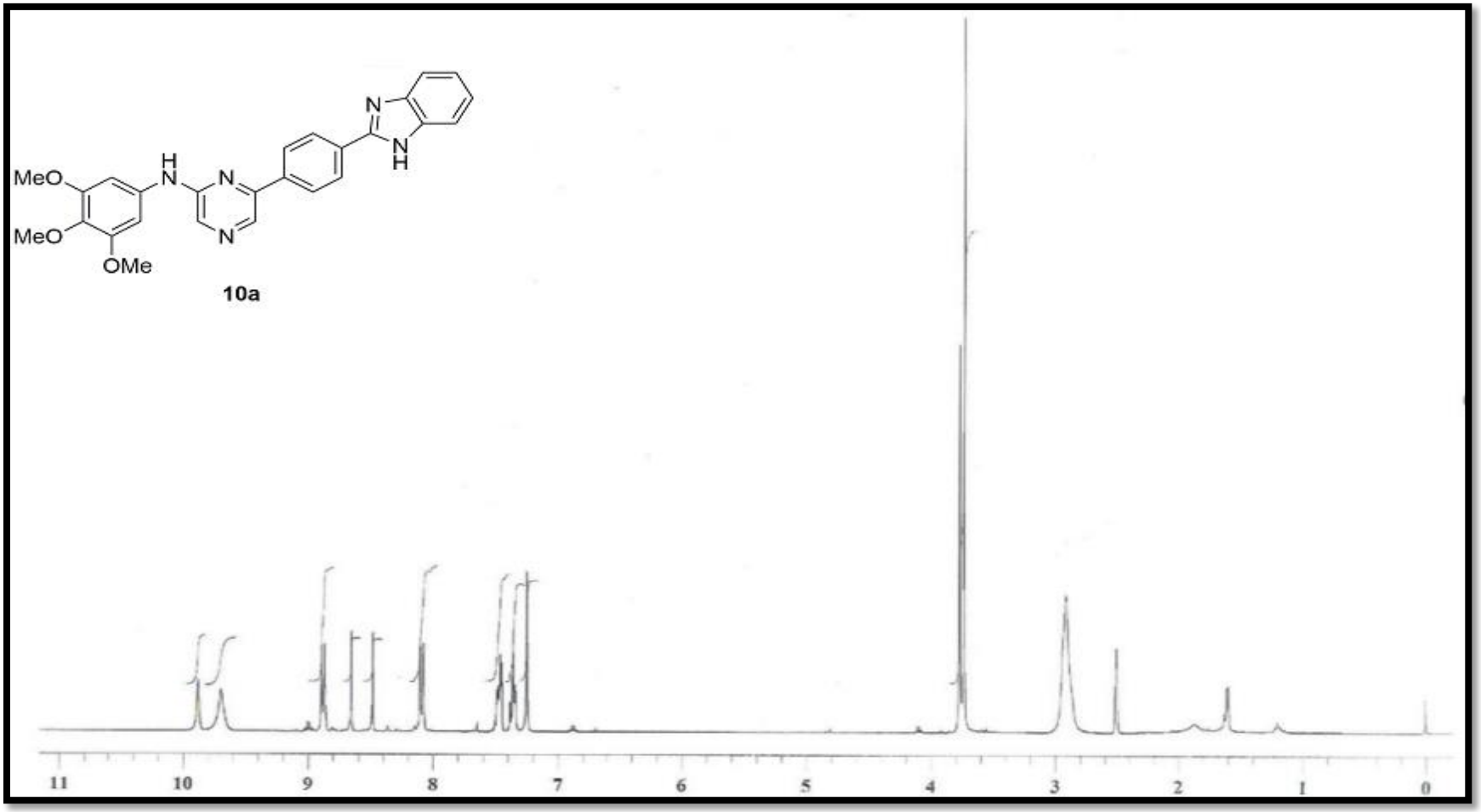

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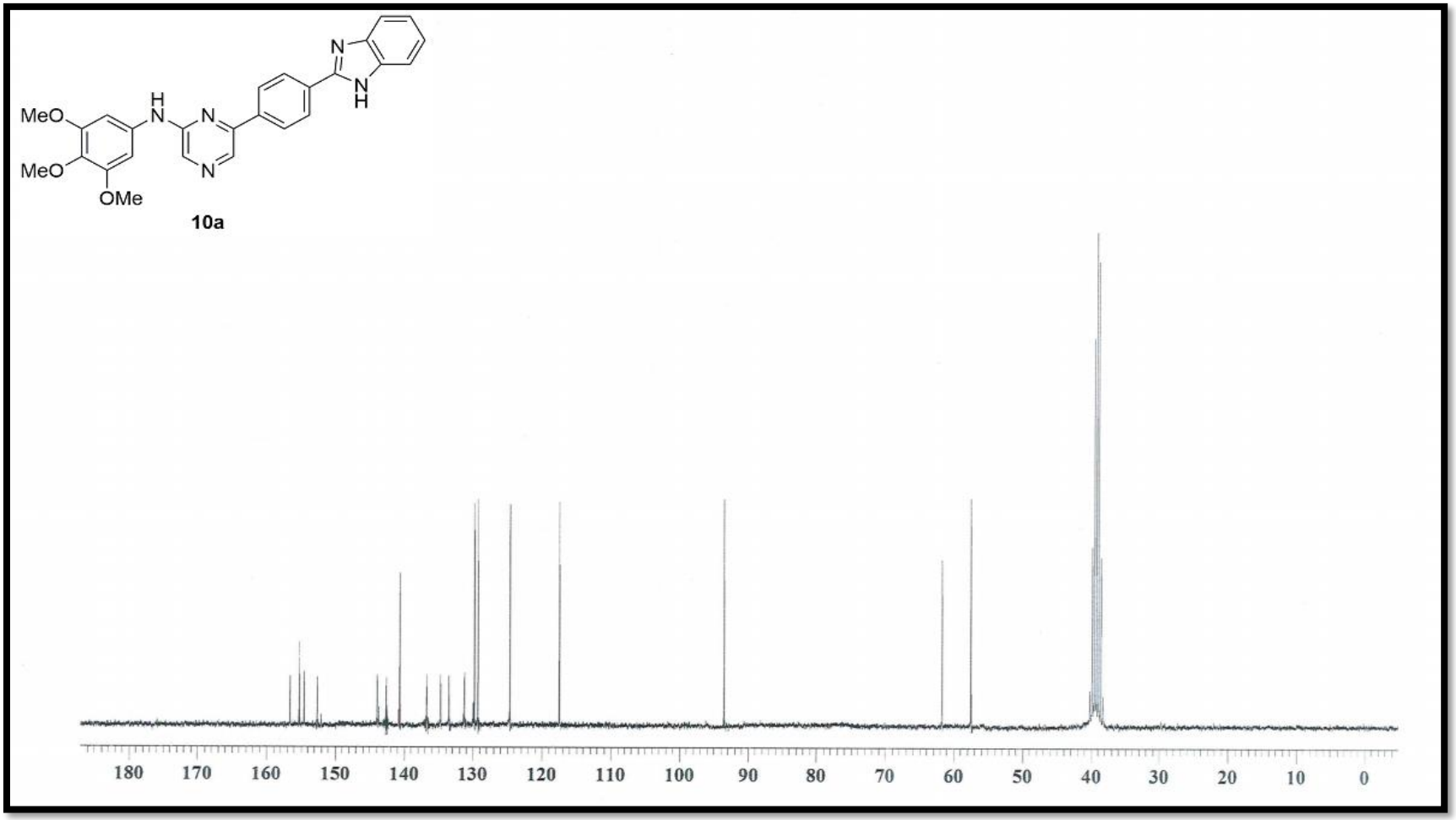

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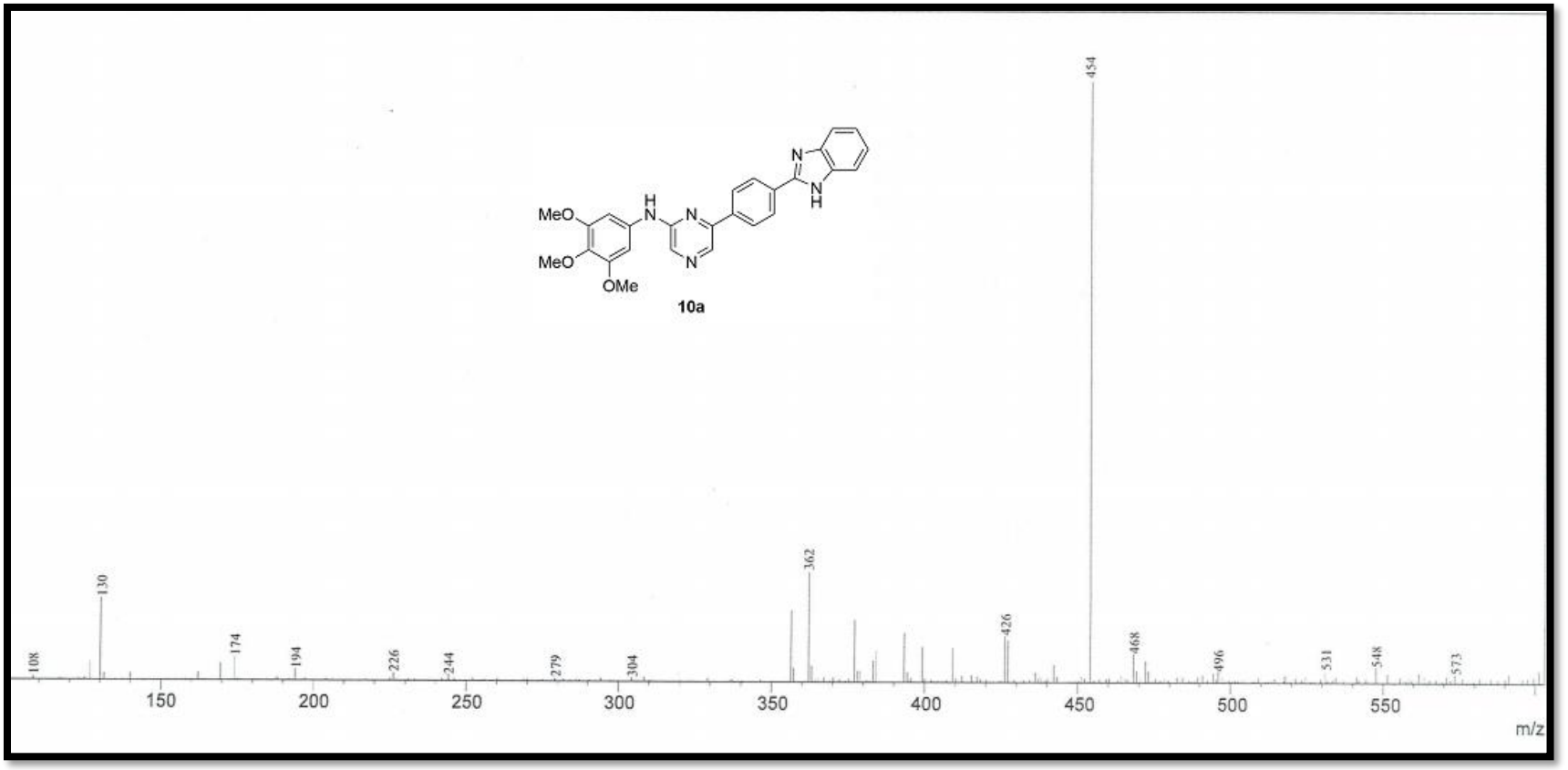
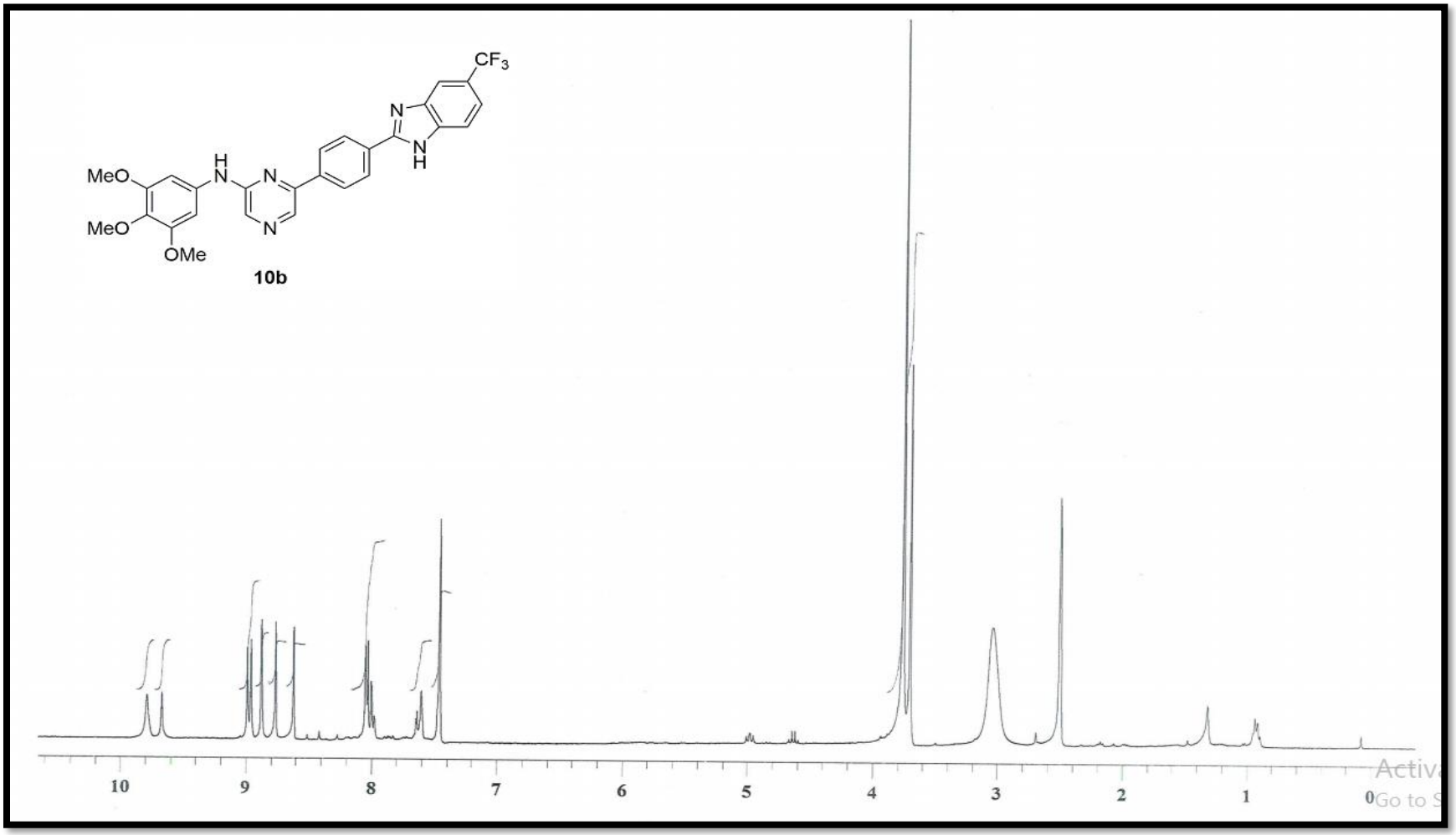


^{1}H-NMR Spectrum (DMSO-d_6, 400 MHz) of 6-(4-(5-(Trifluoromethyl)-1H-benzo[d]imidazol-2-yl)phenyl)-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amine(**10b**)



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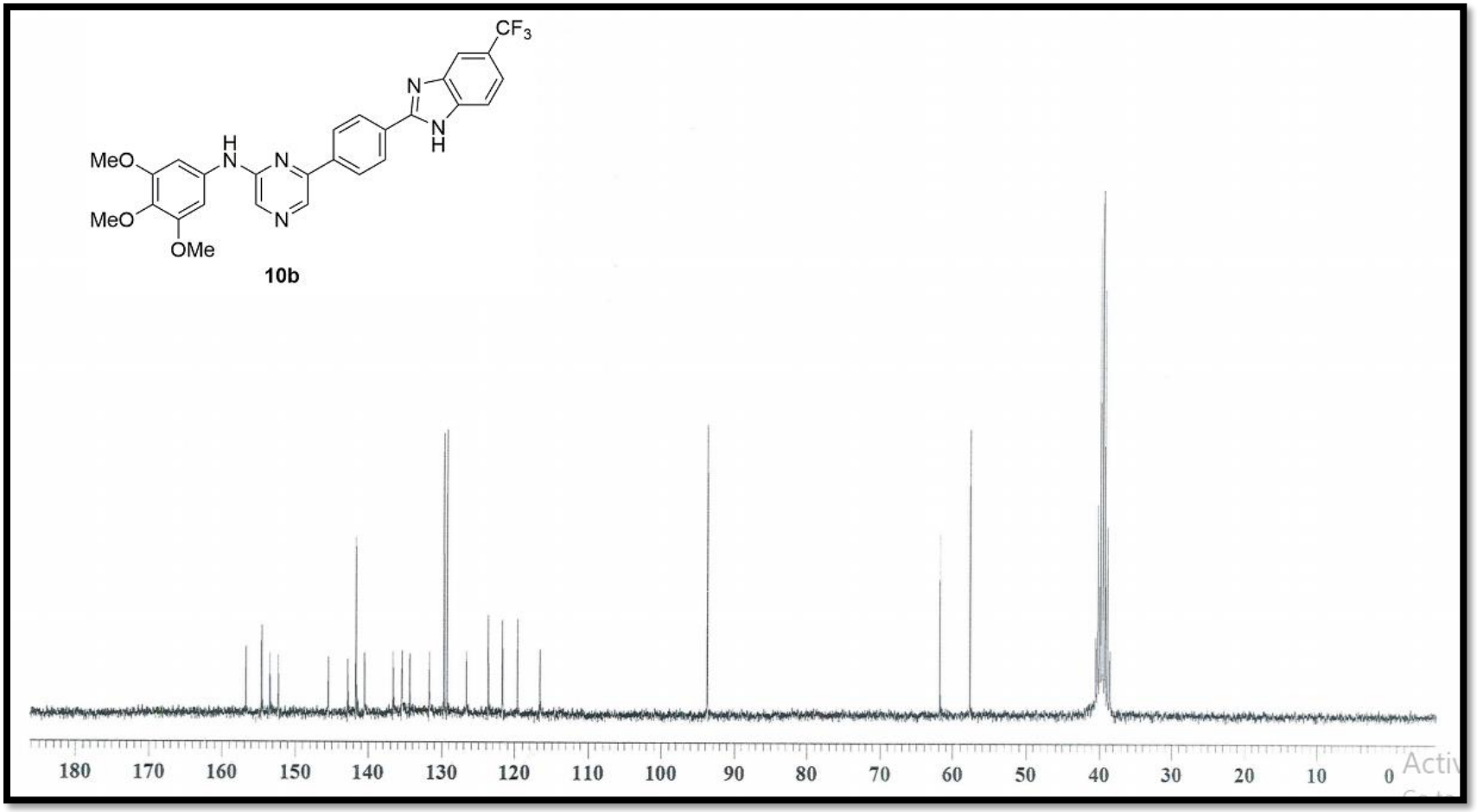

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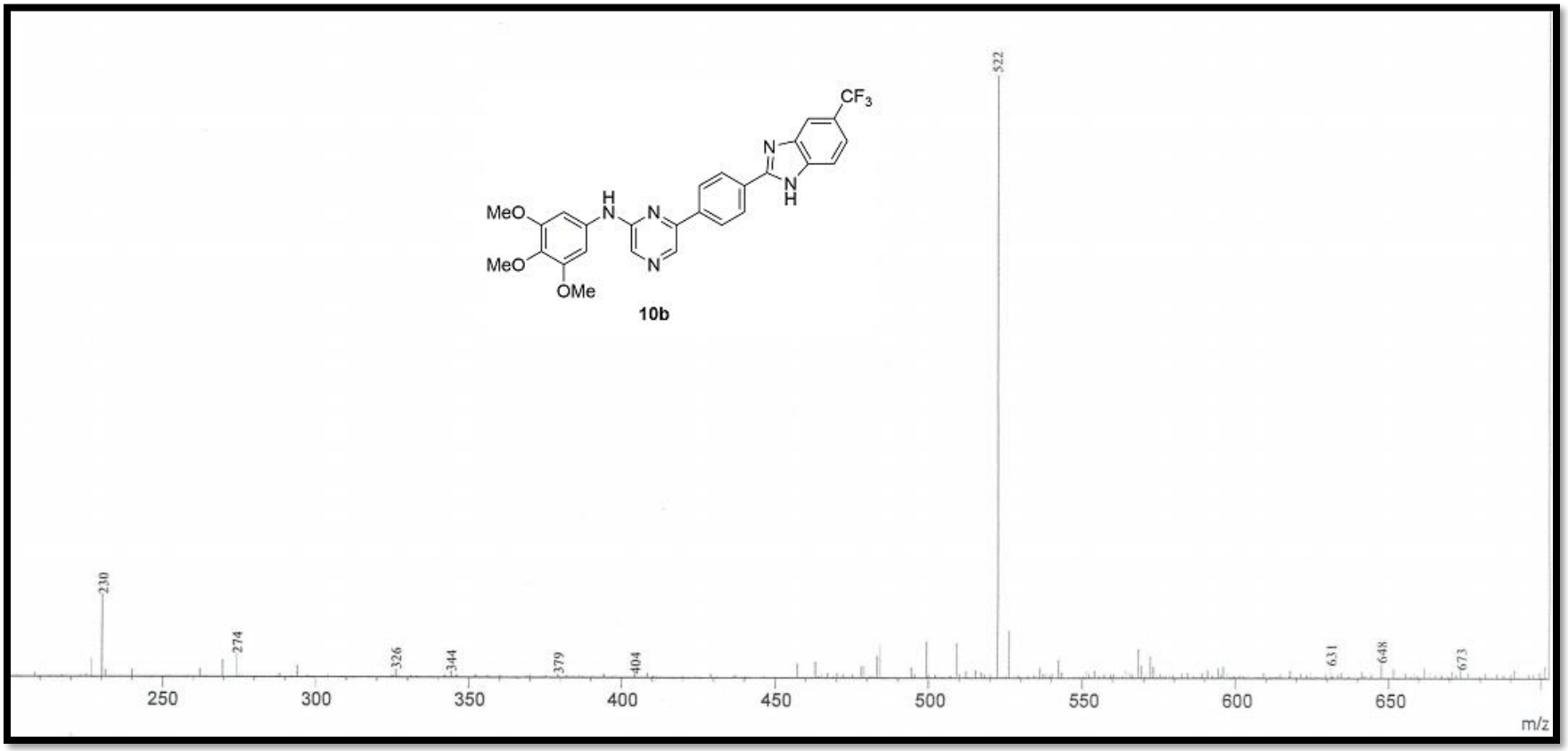
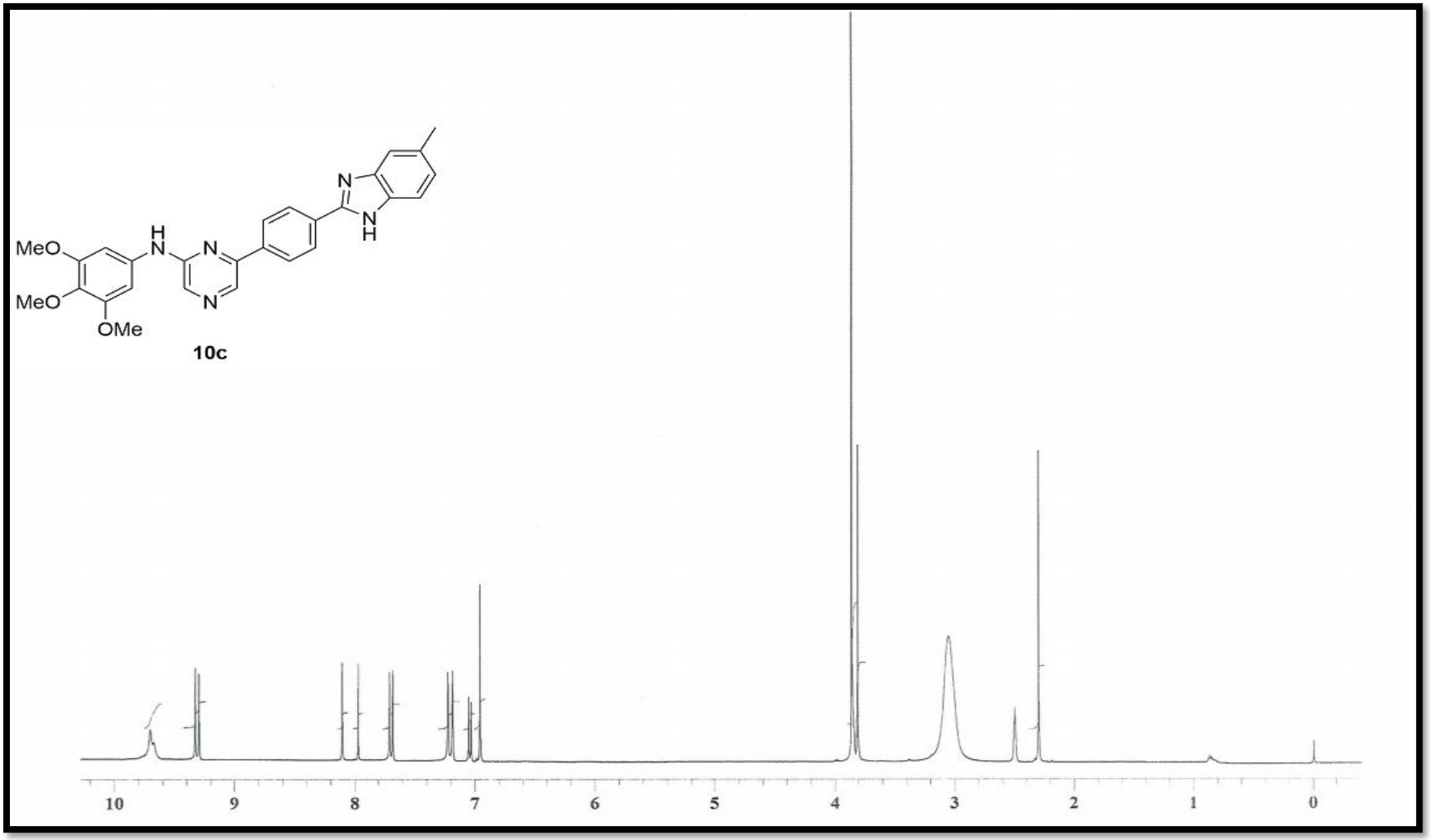


^{1}H-NMR Spectrum (DMSO-d_6, 400 MHz) of 6-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenyl)-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (**10c**)



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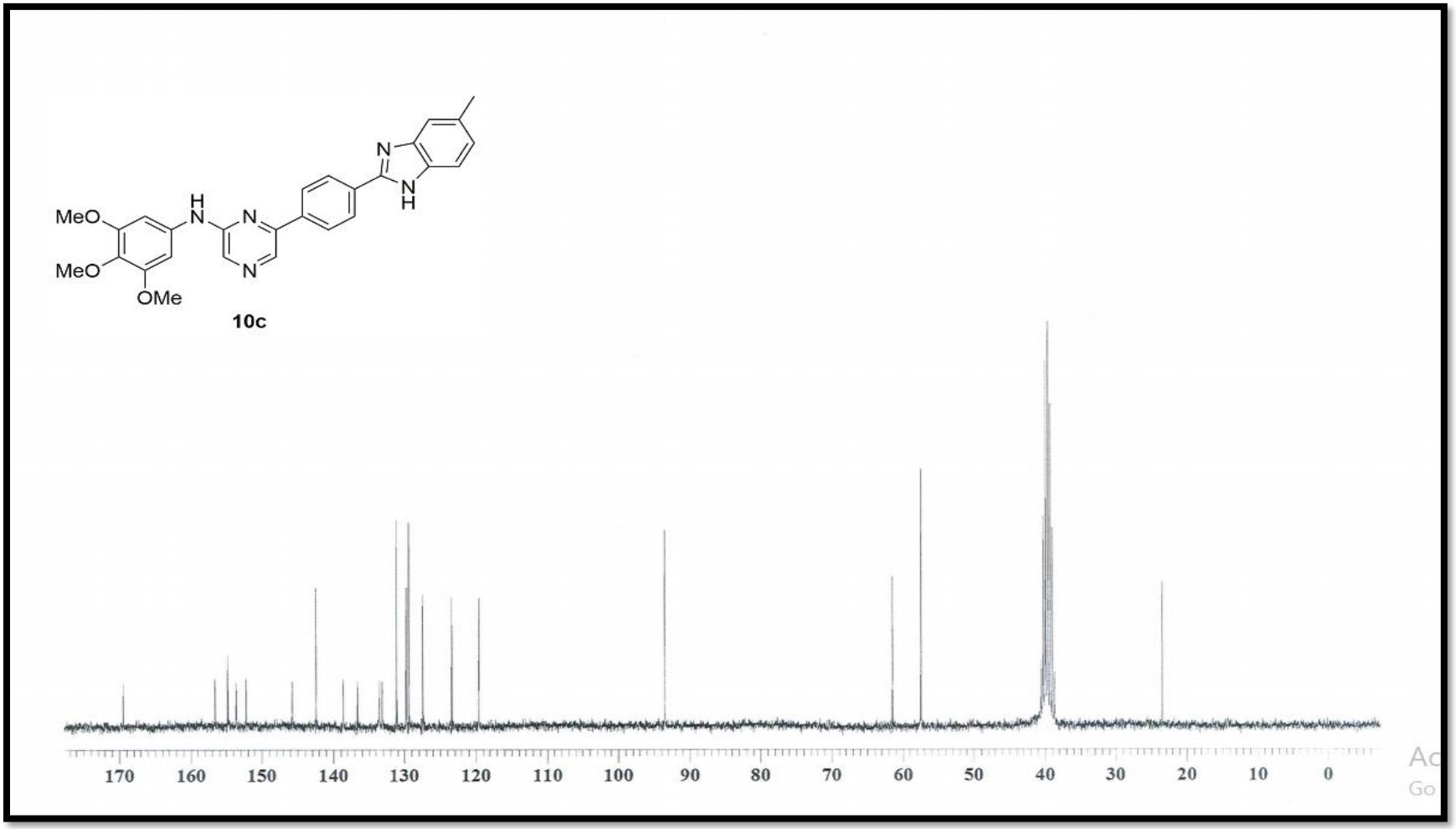

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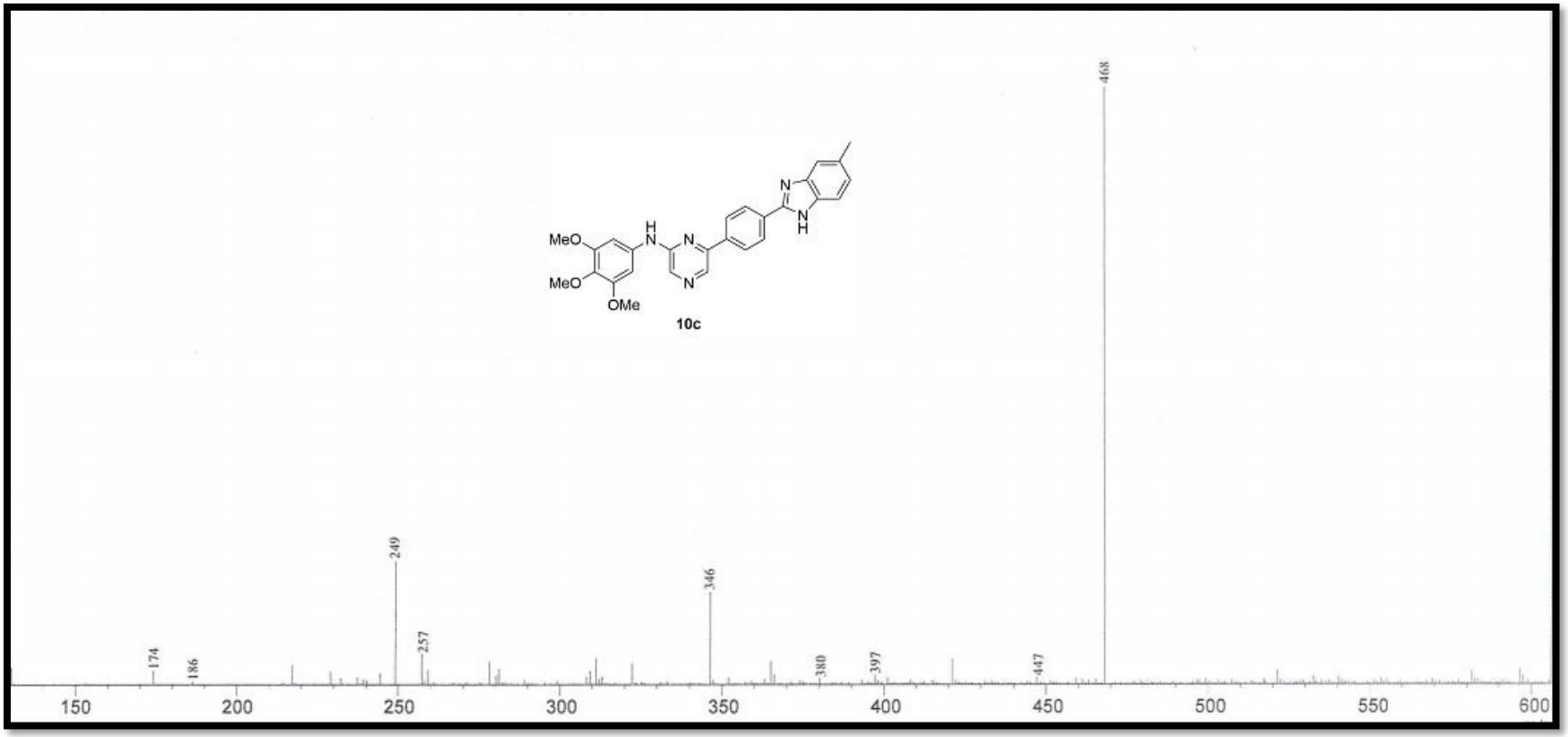

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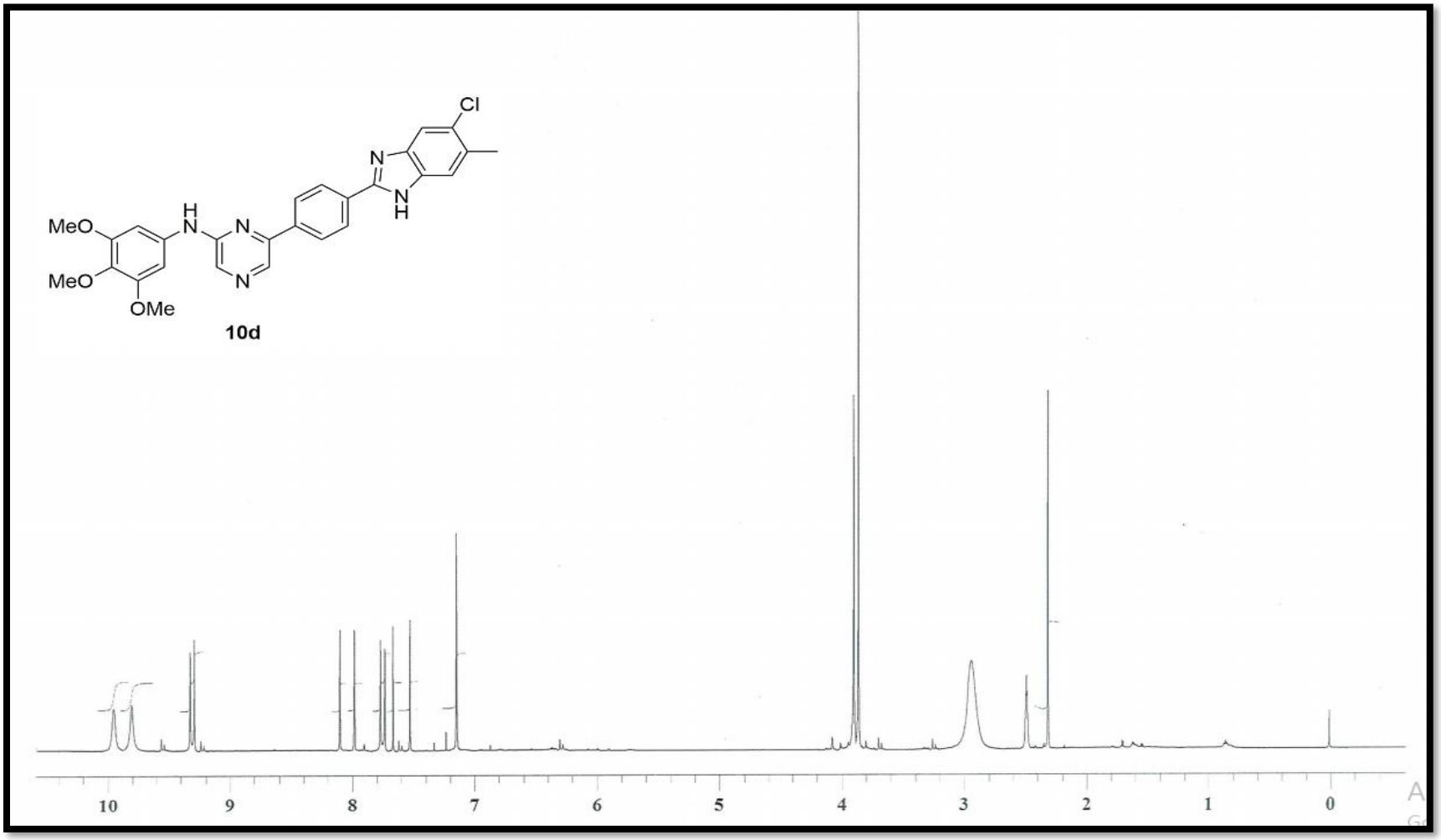

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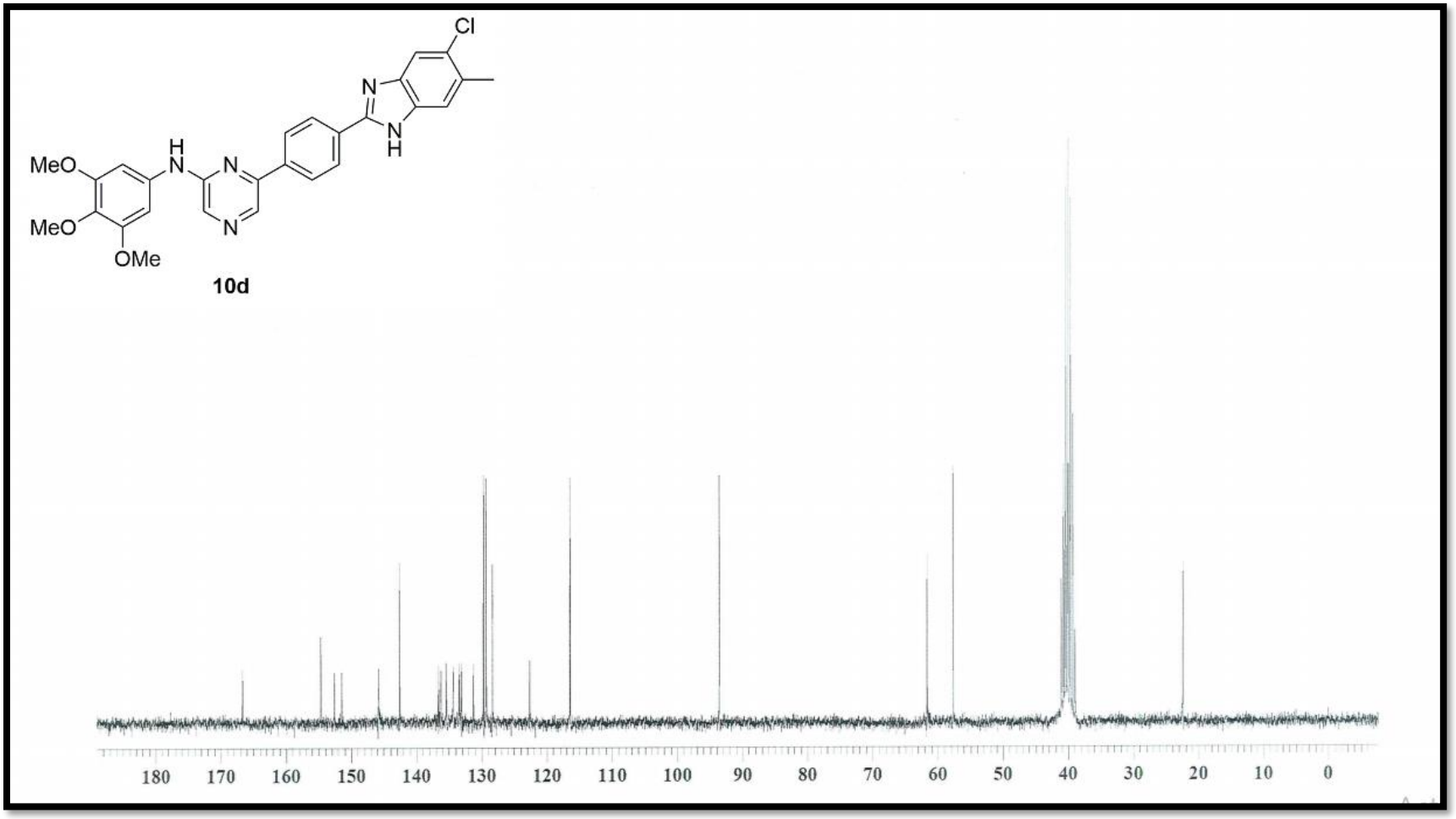

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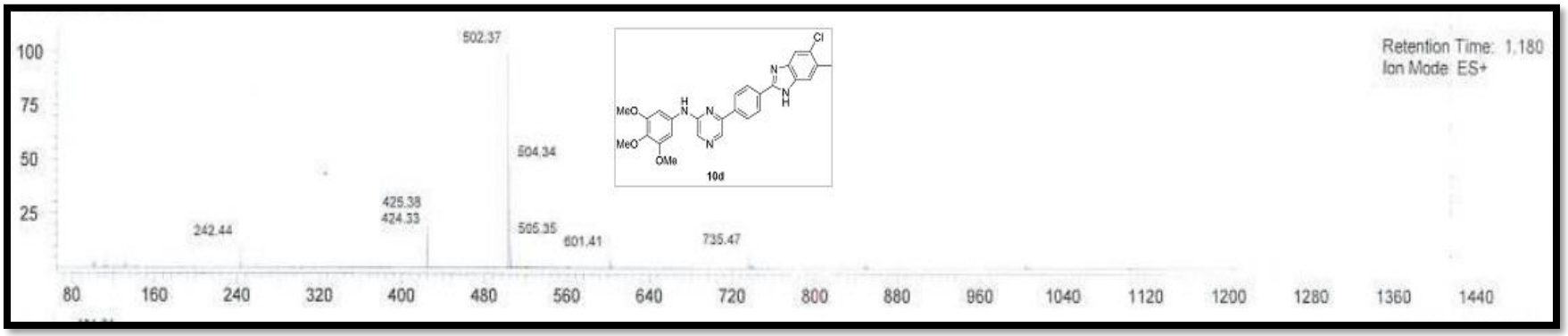

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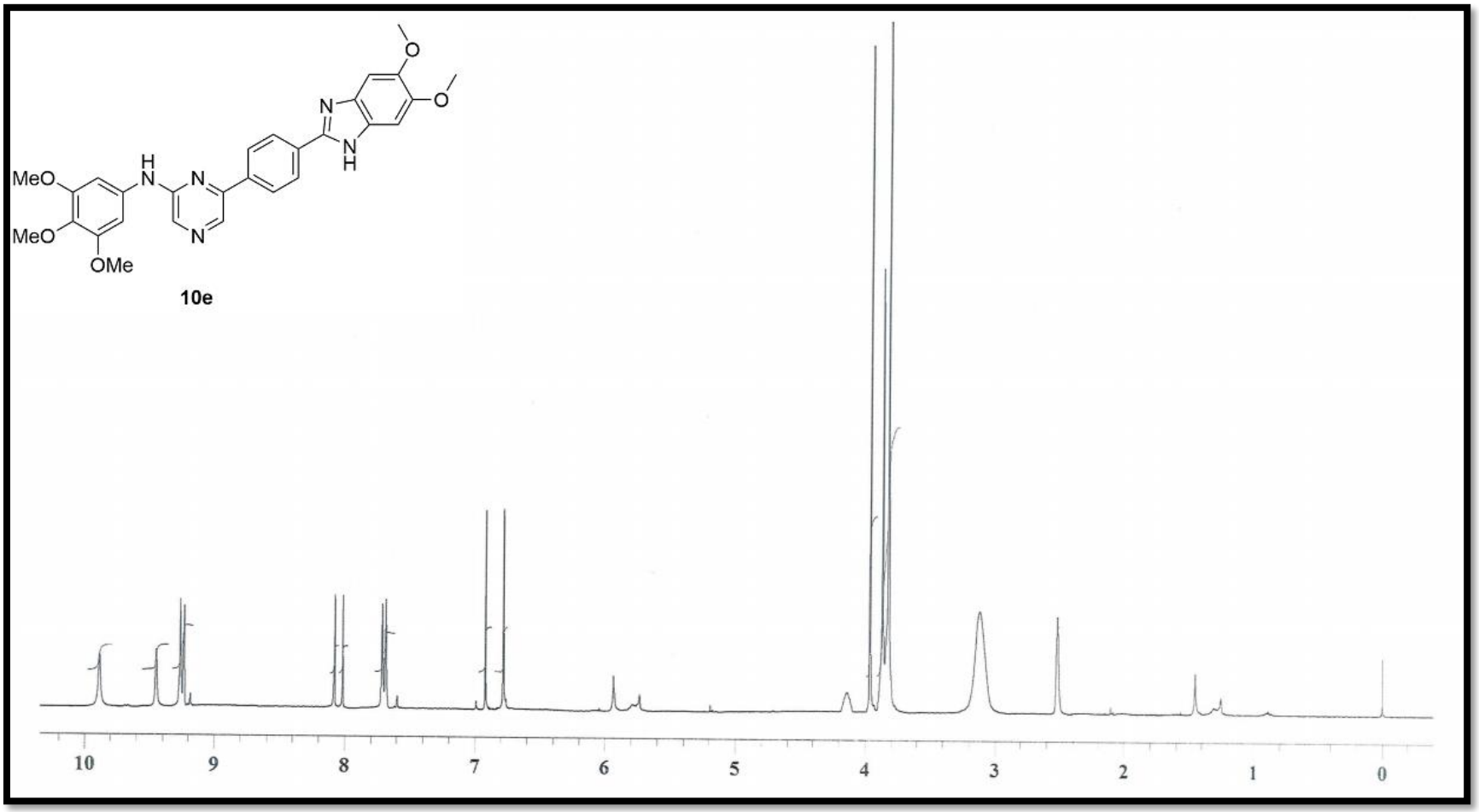

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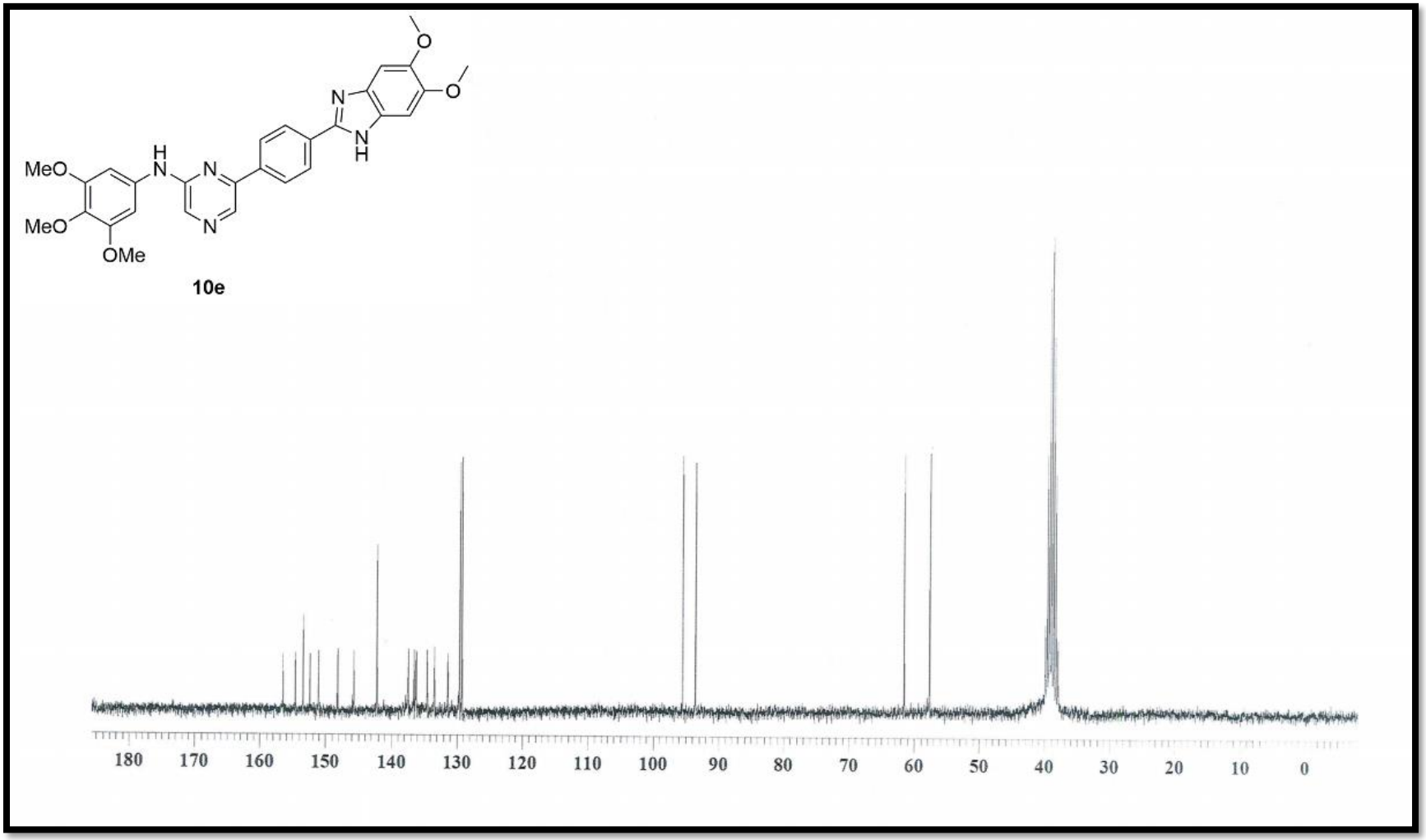

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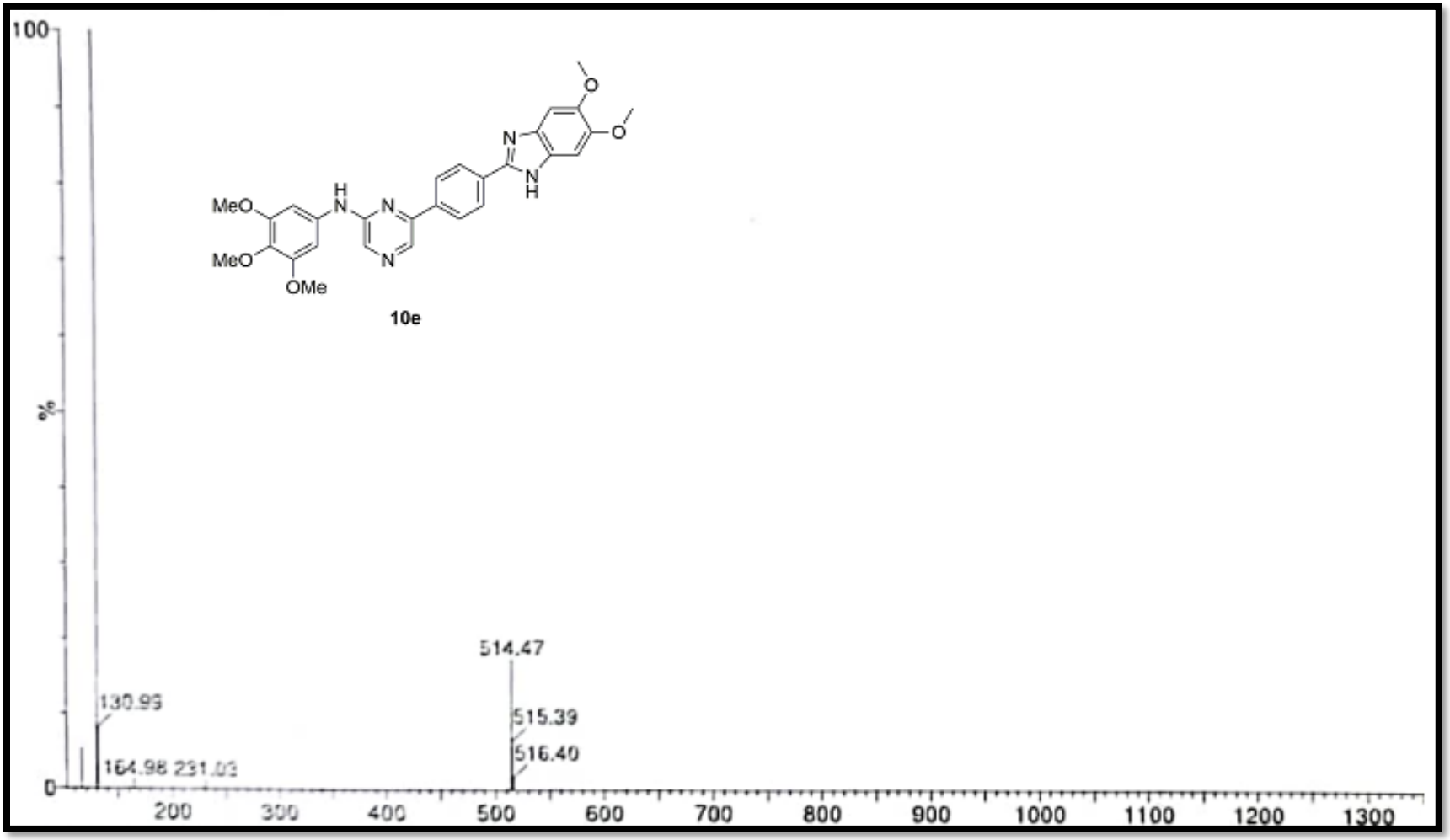

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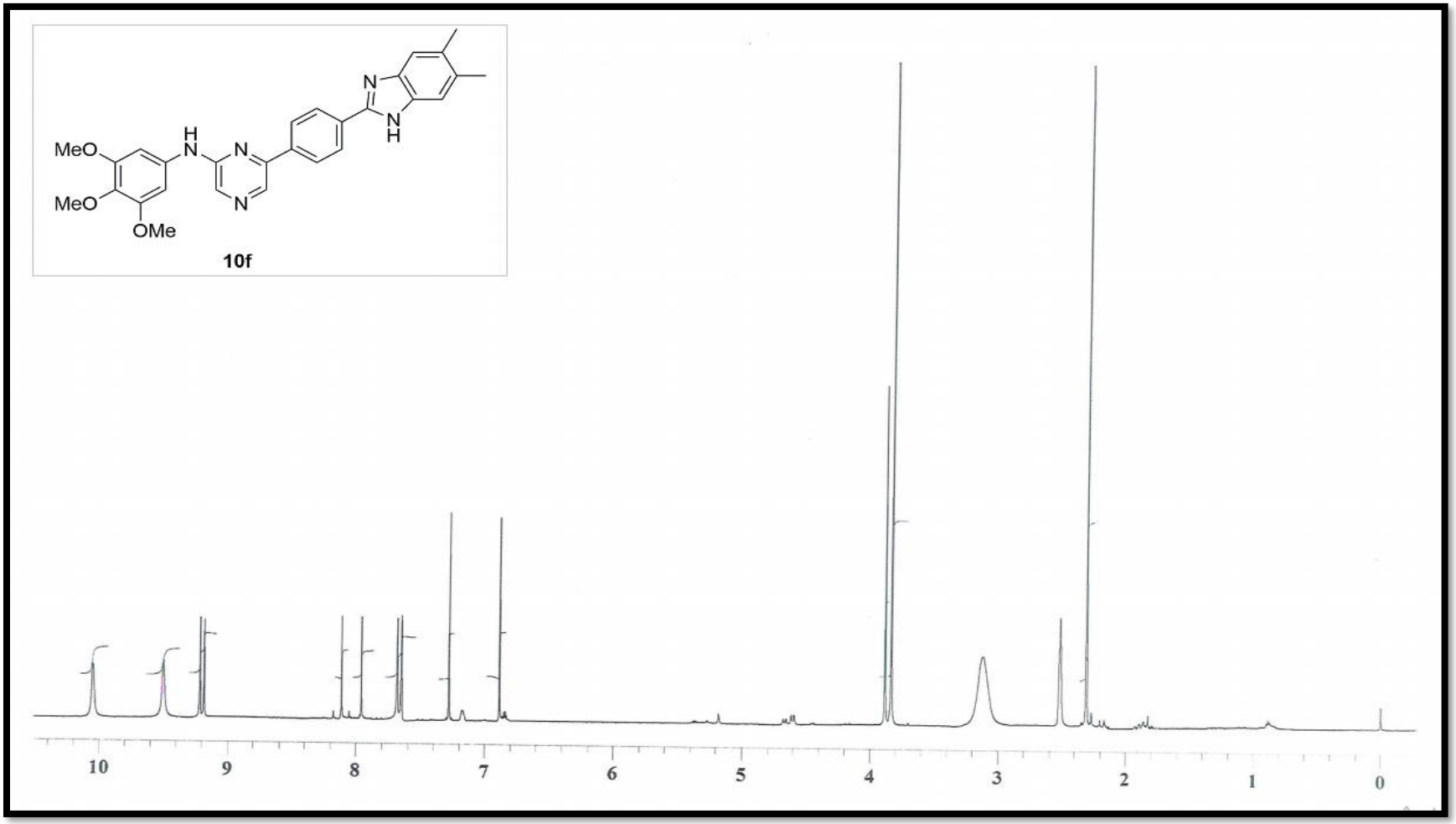

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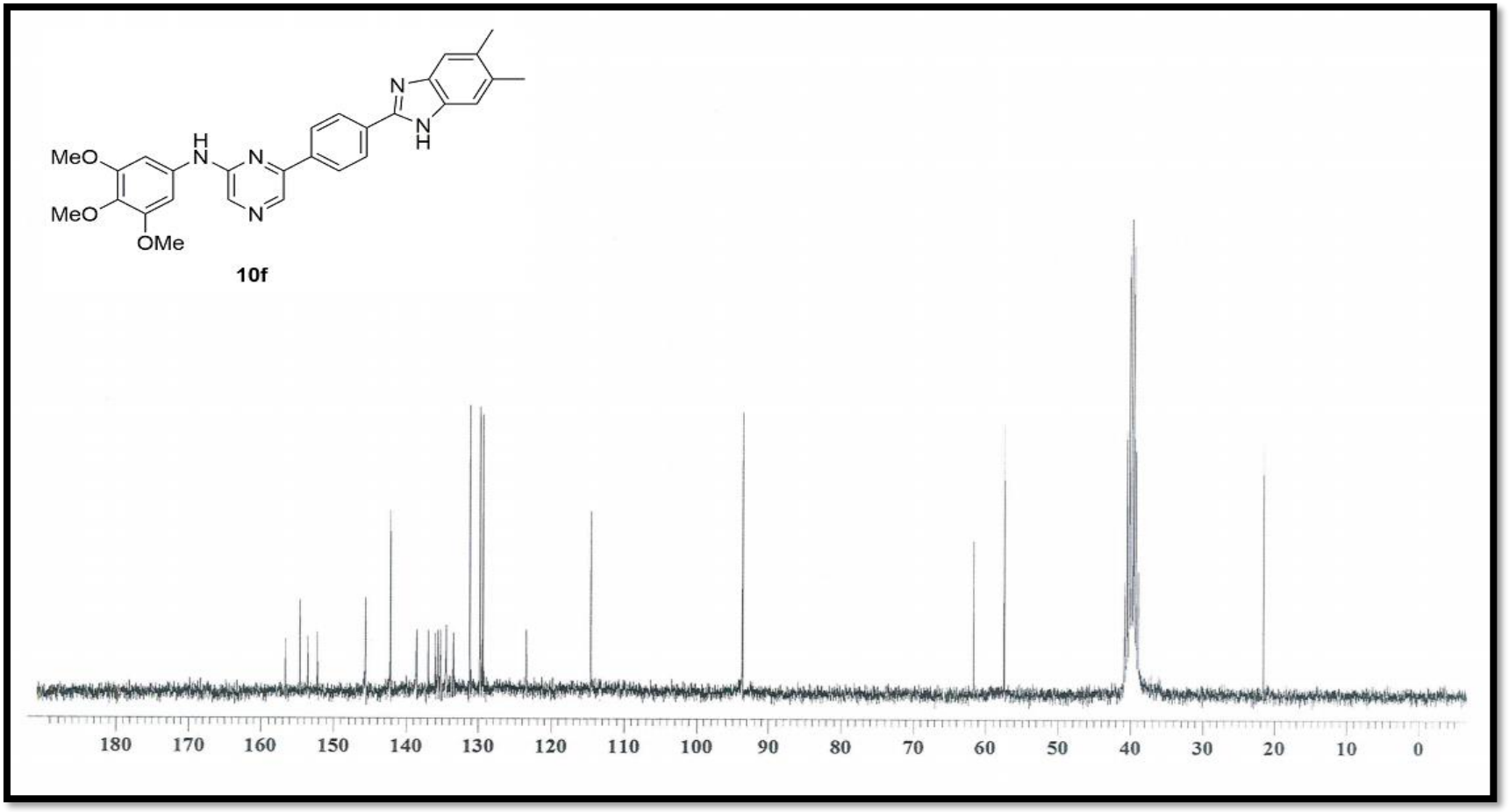

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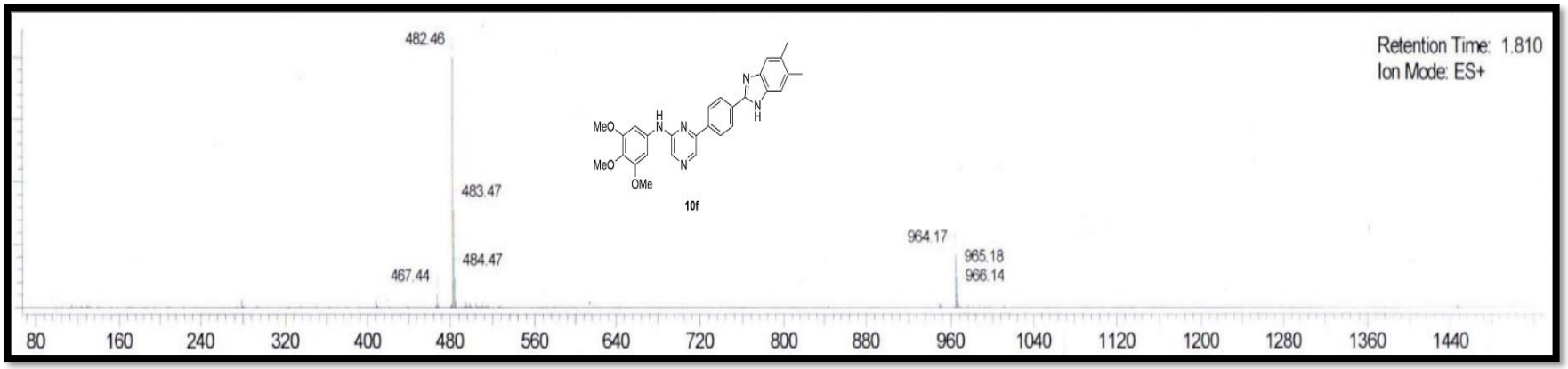

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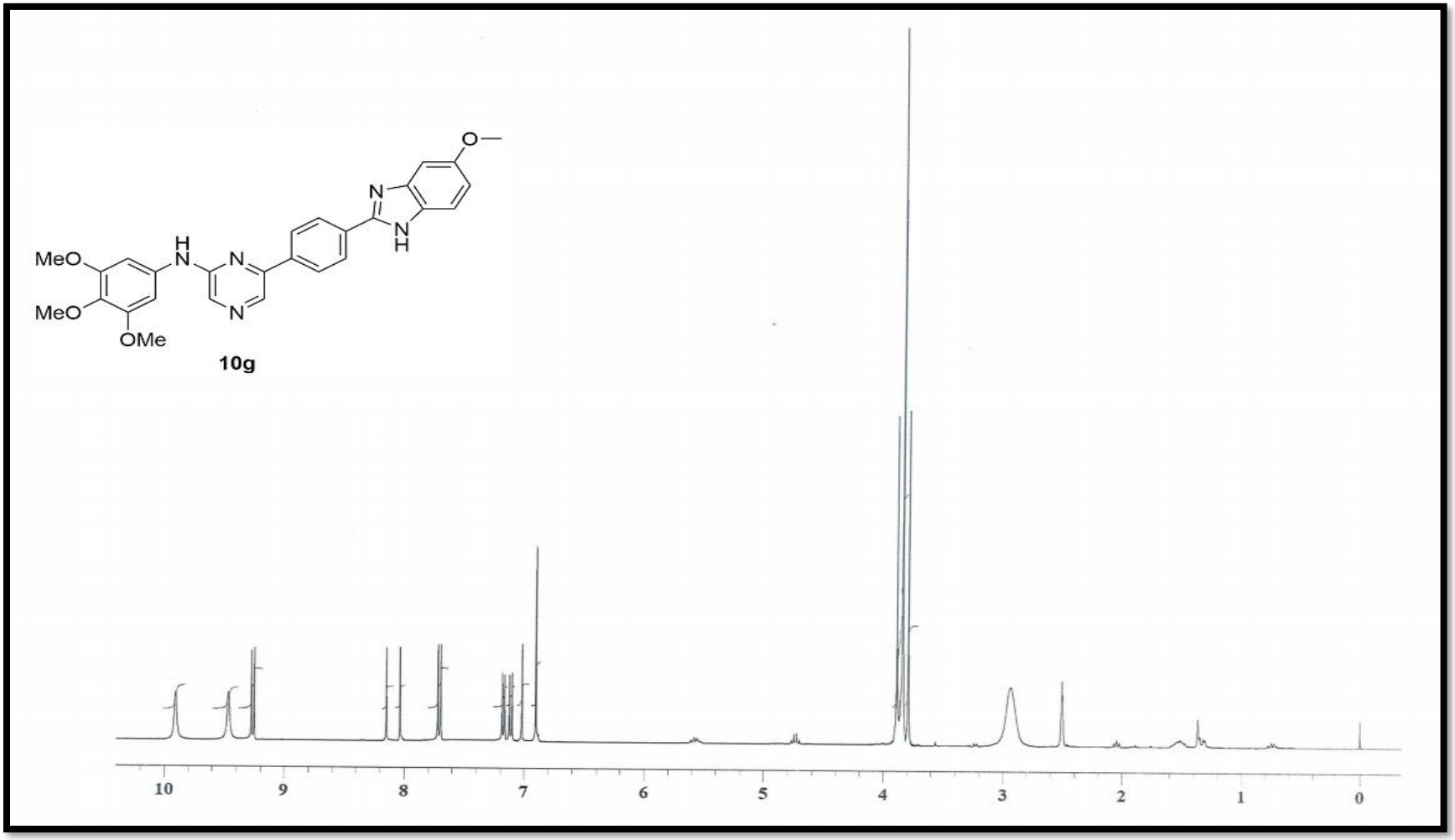
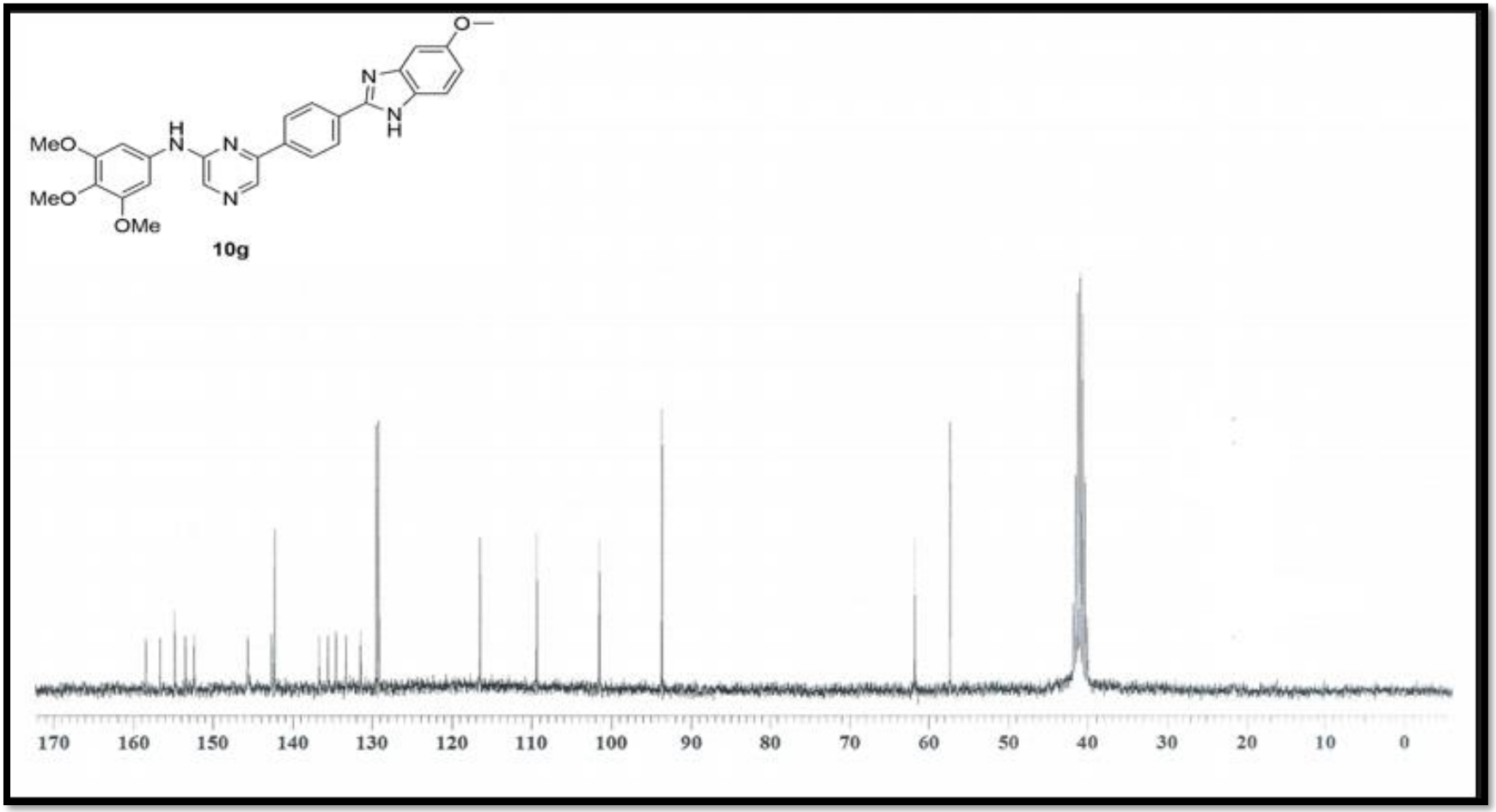


^{13}C-NMR Spectrum (DMSO-d_6, 100 MHz) of N-6-[4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl]-2-pyrazinyl-N-(3,4,5-trimethoxyphenyl)amine (**10g**)



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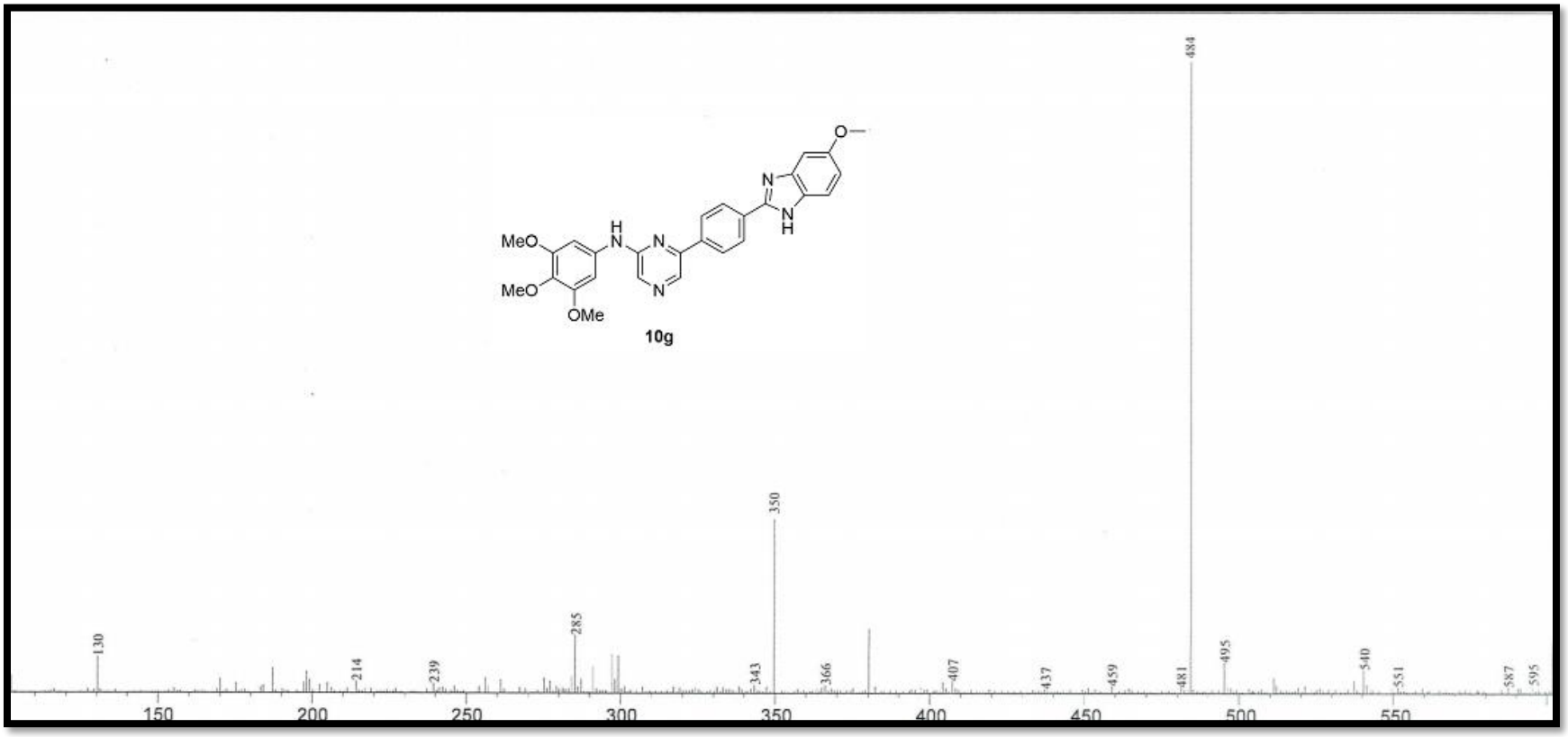
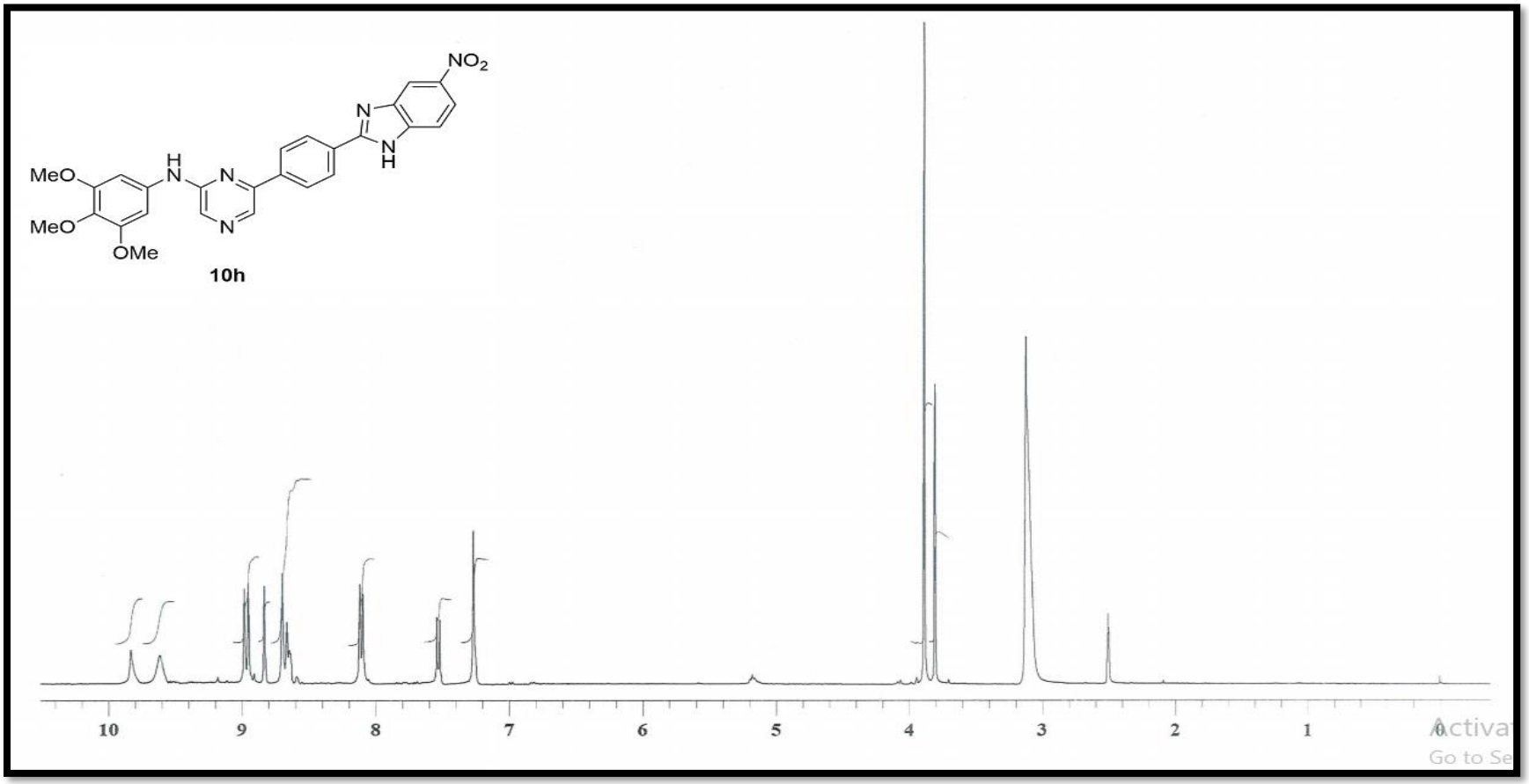


^{1}H-NMR Spectrum (DMSO-d_6, 400 MHz) of N-6-[4-(5-Nitro-1H-benzo[d]imidazol-2-yl)phenyl]-2-pyrazinyl-N-(3,4,5-trimethoxyphenyl)amine (**10h**)



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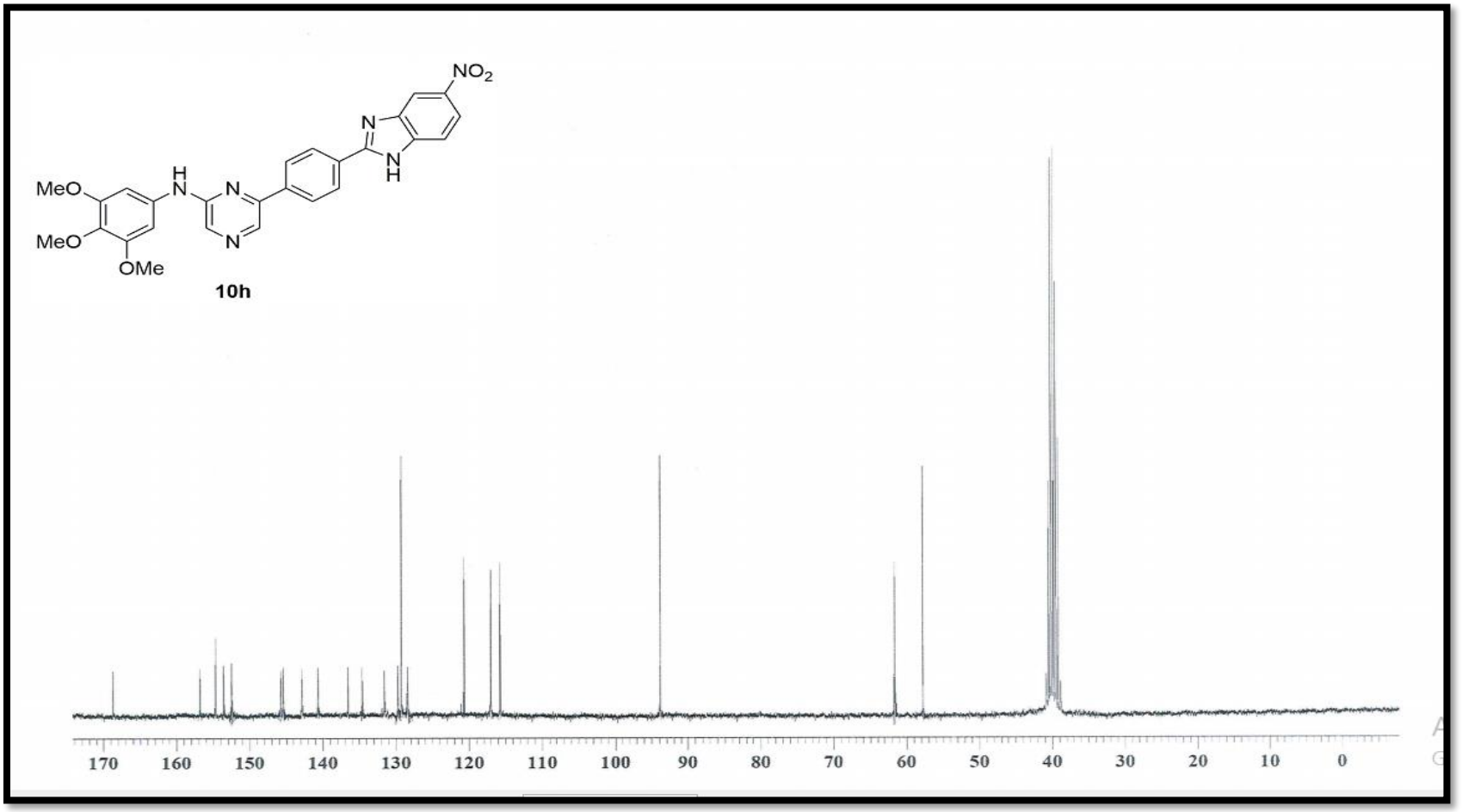

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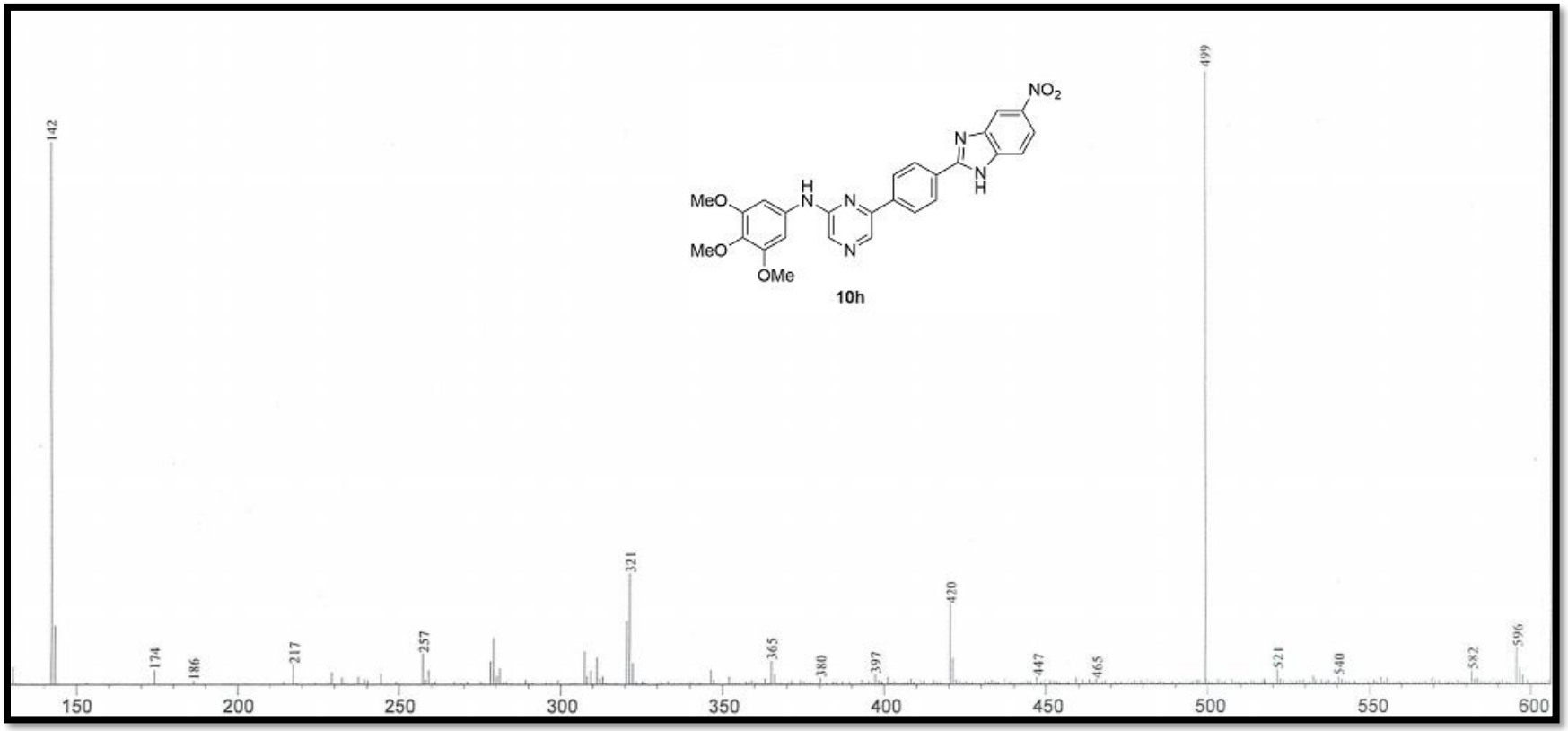

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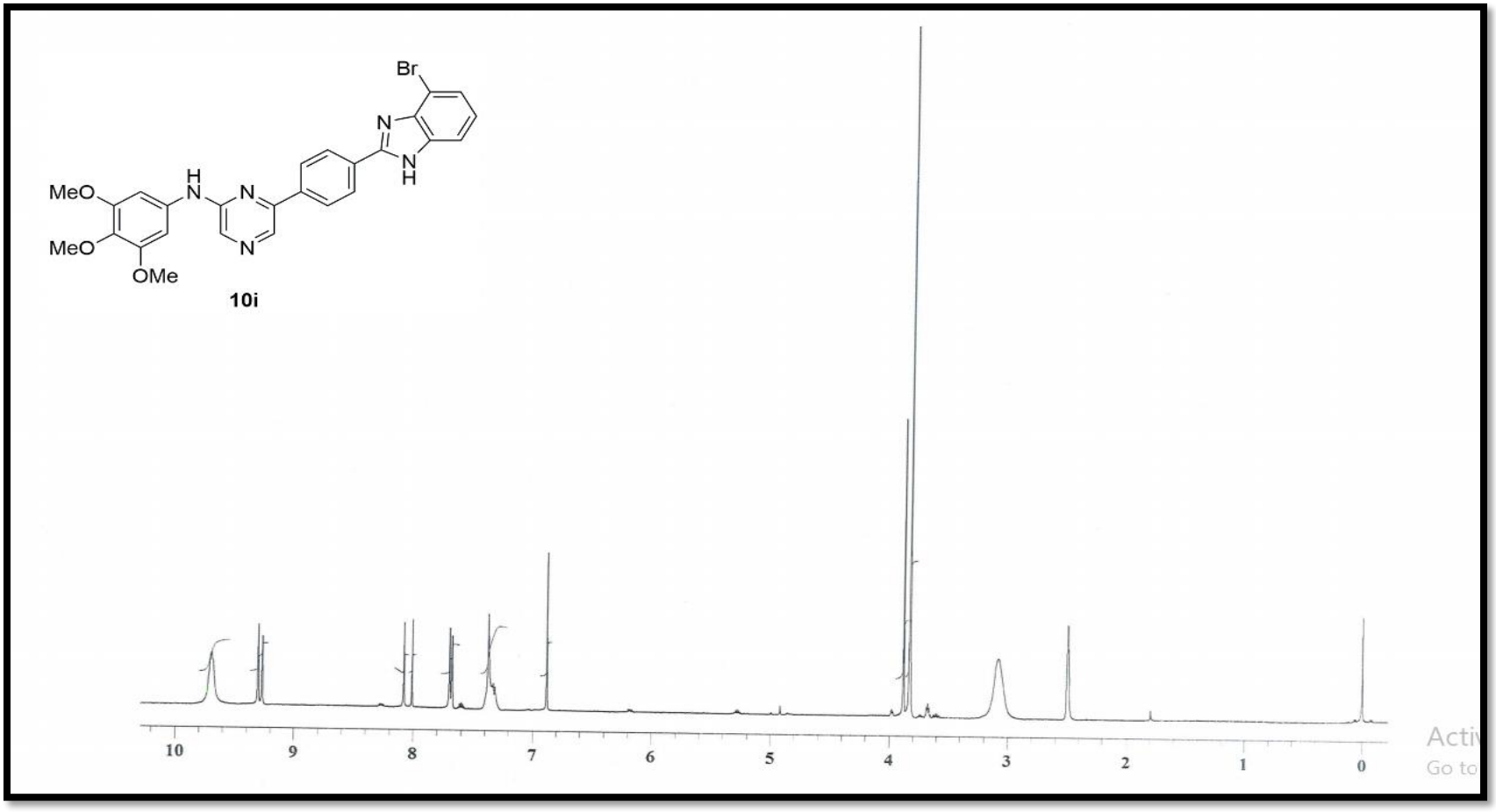

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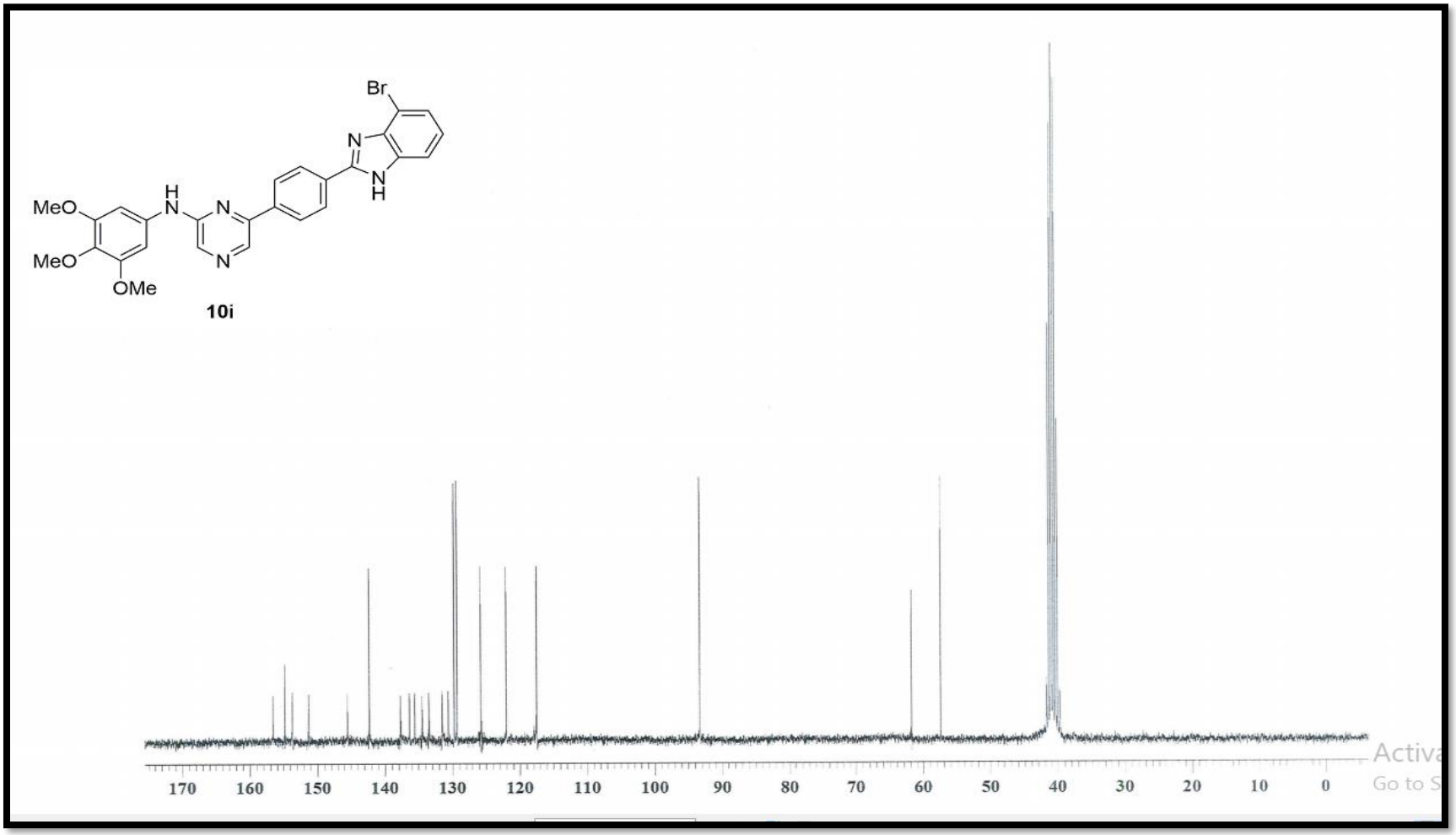

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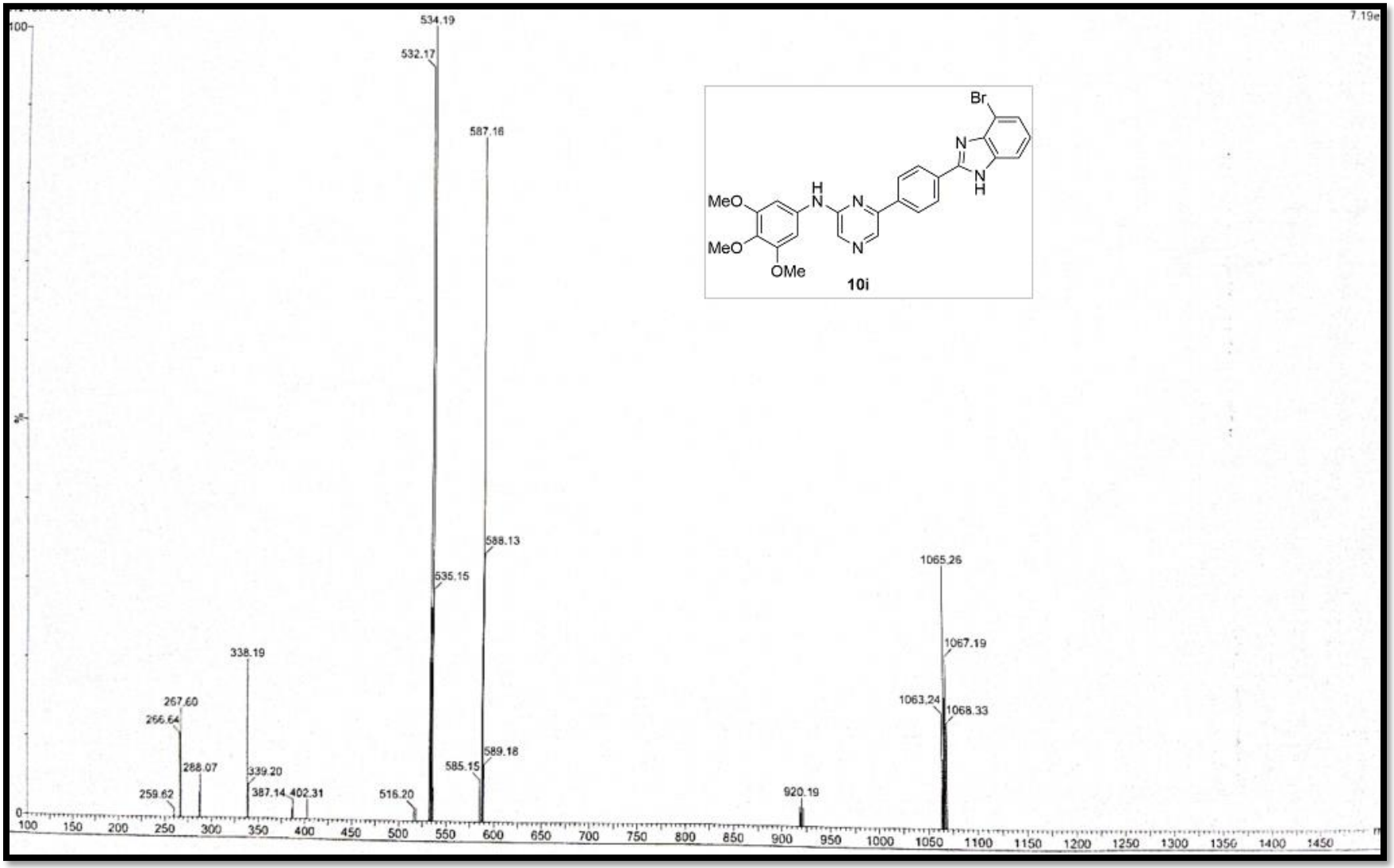

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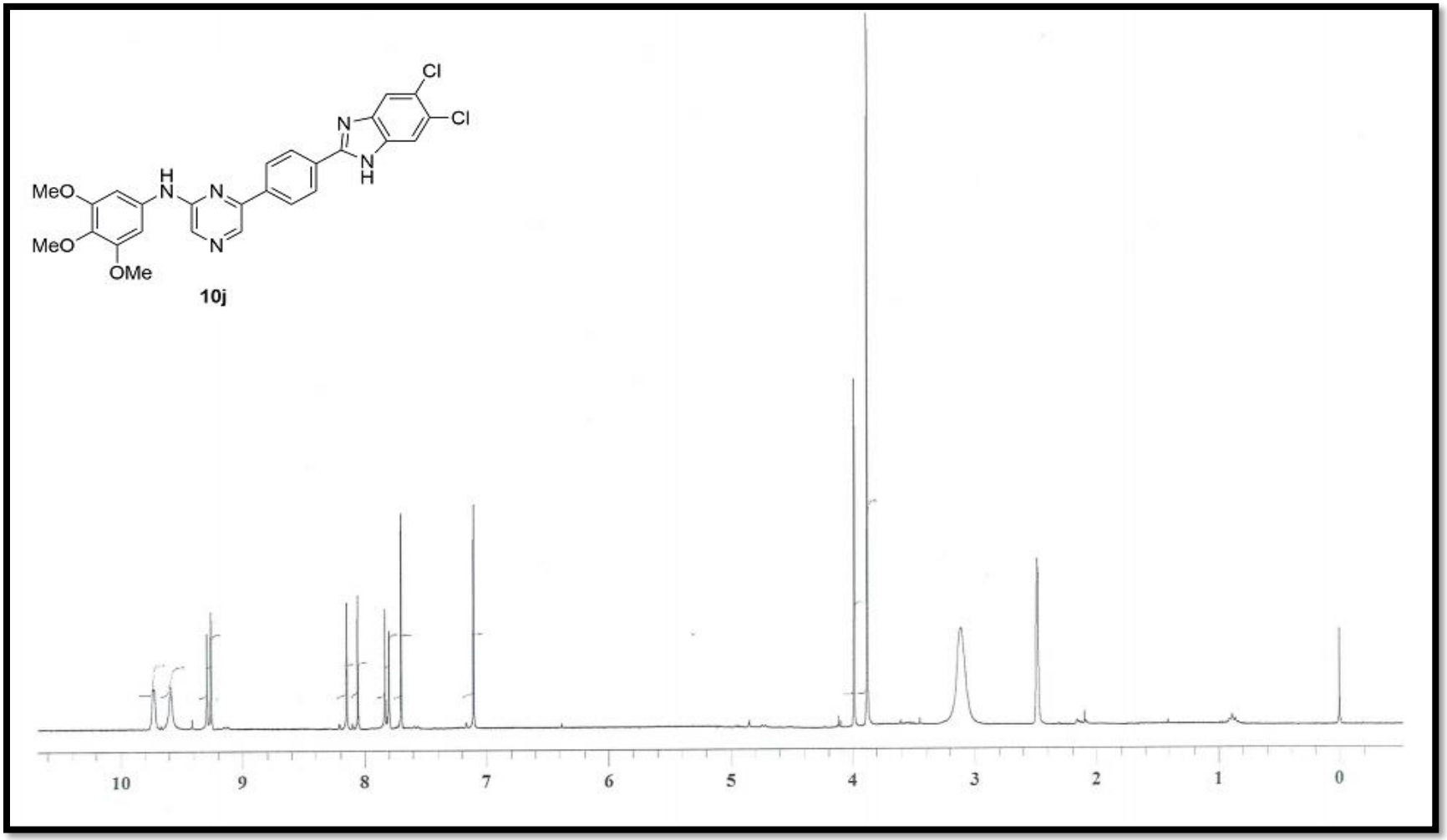

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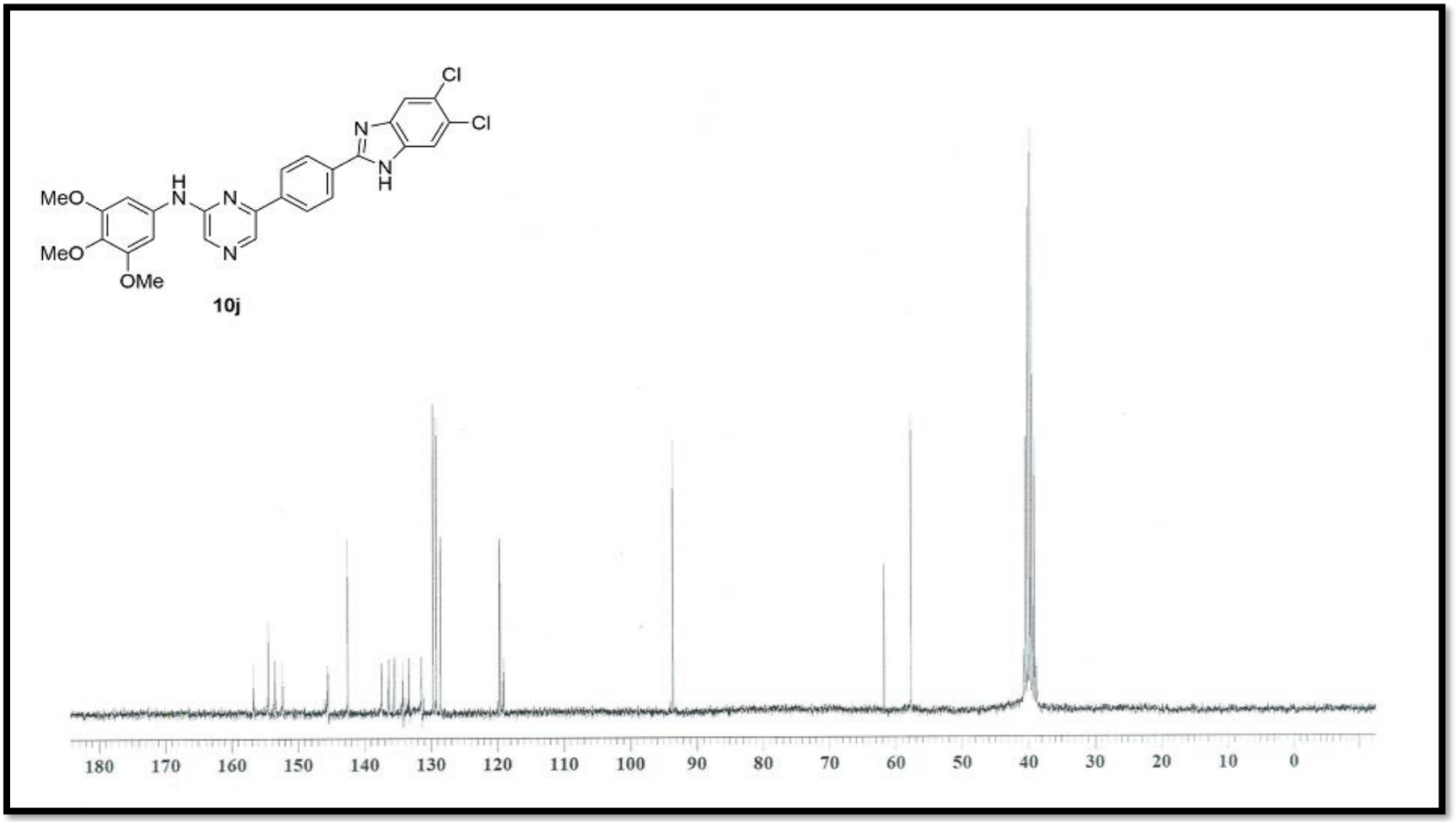

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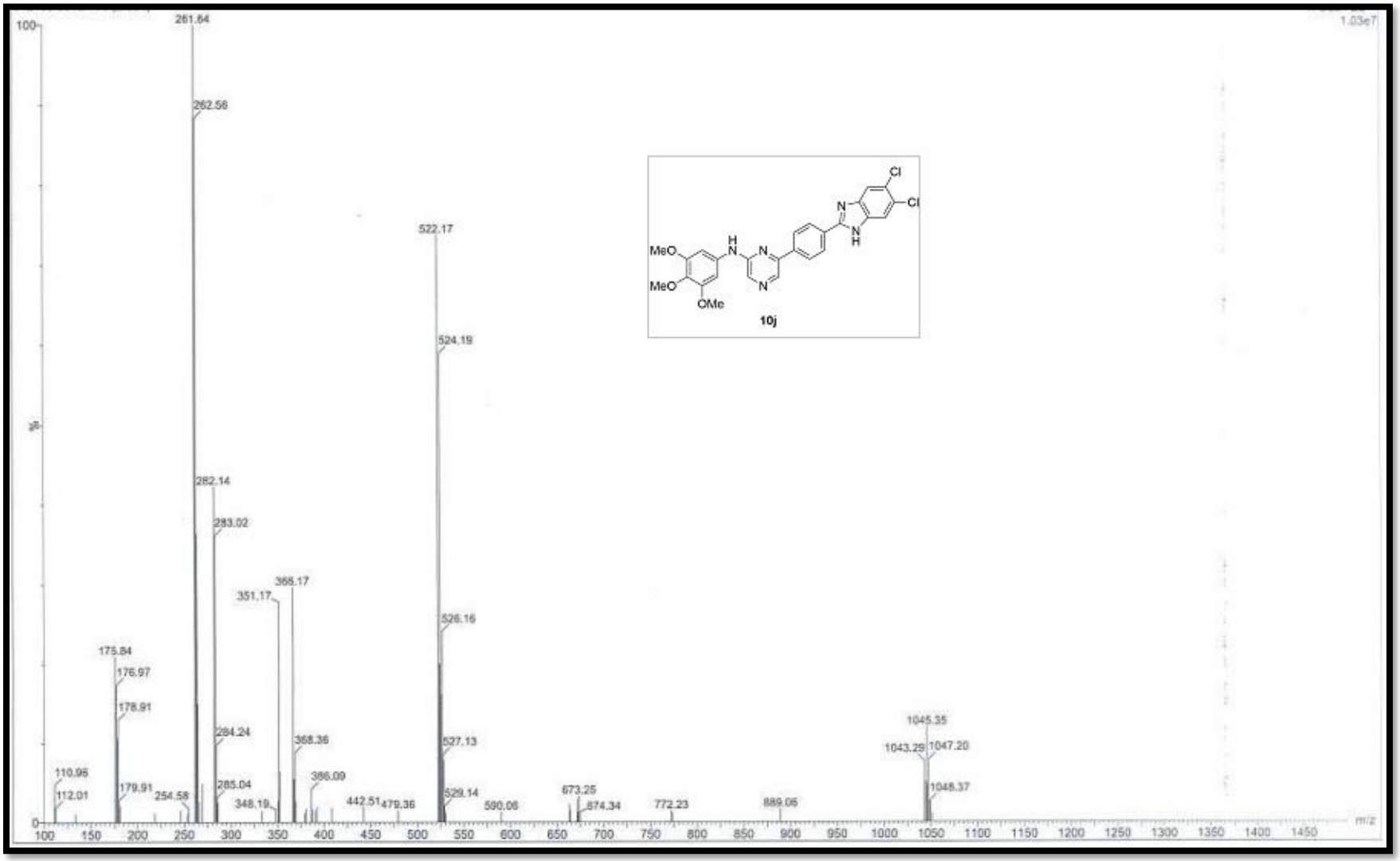
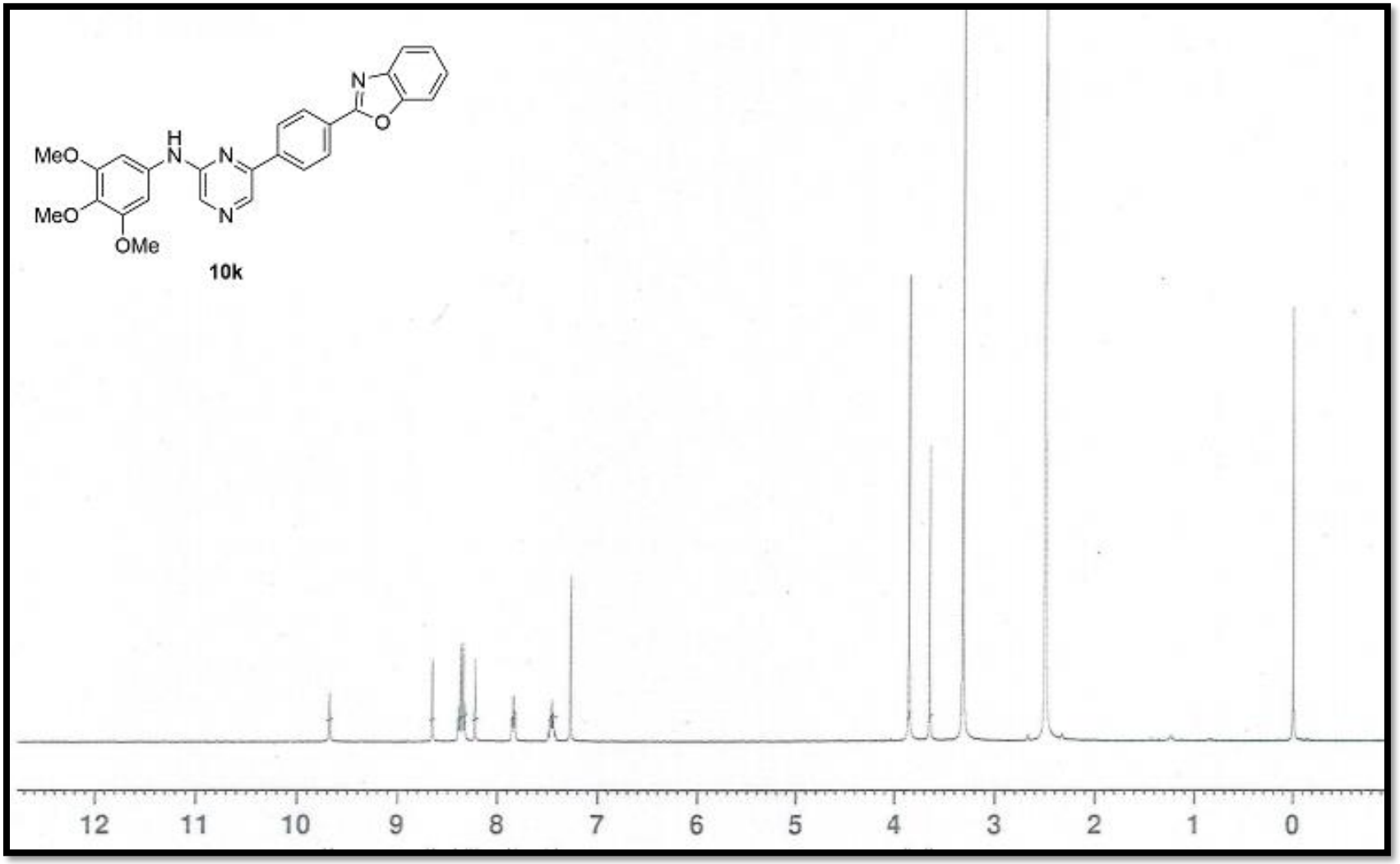


^{1}H-NMR Spectrum (DMSO-d_6, 400 MHz) of 6-(4-(benzo[d]oxazol-2-yl)phenyl)-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amine(**10k**)



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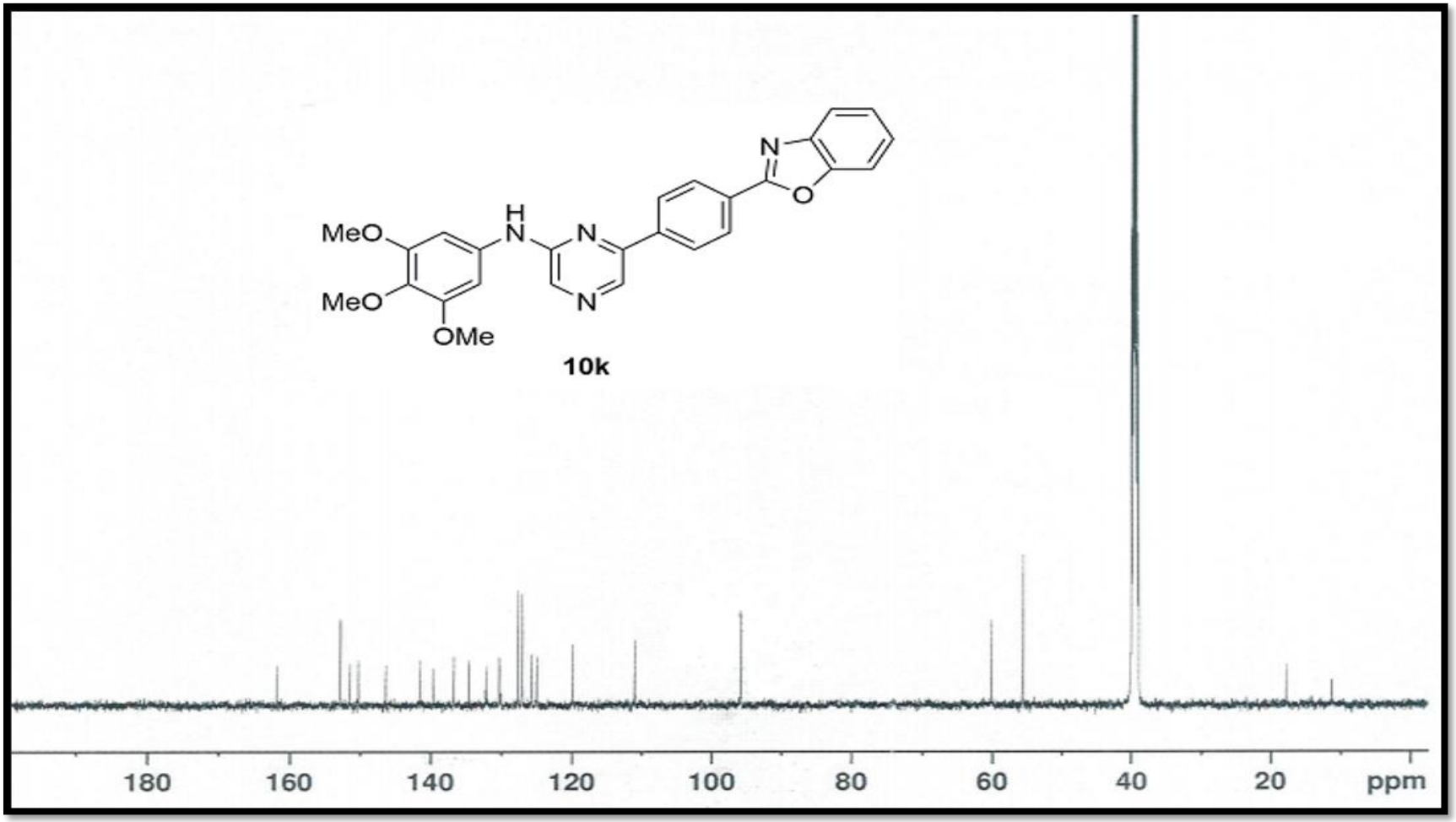

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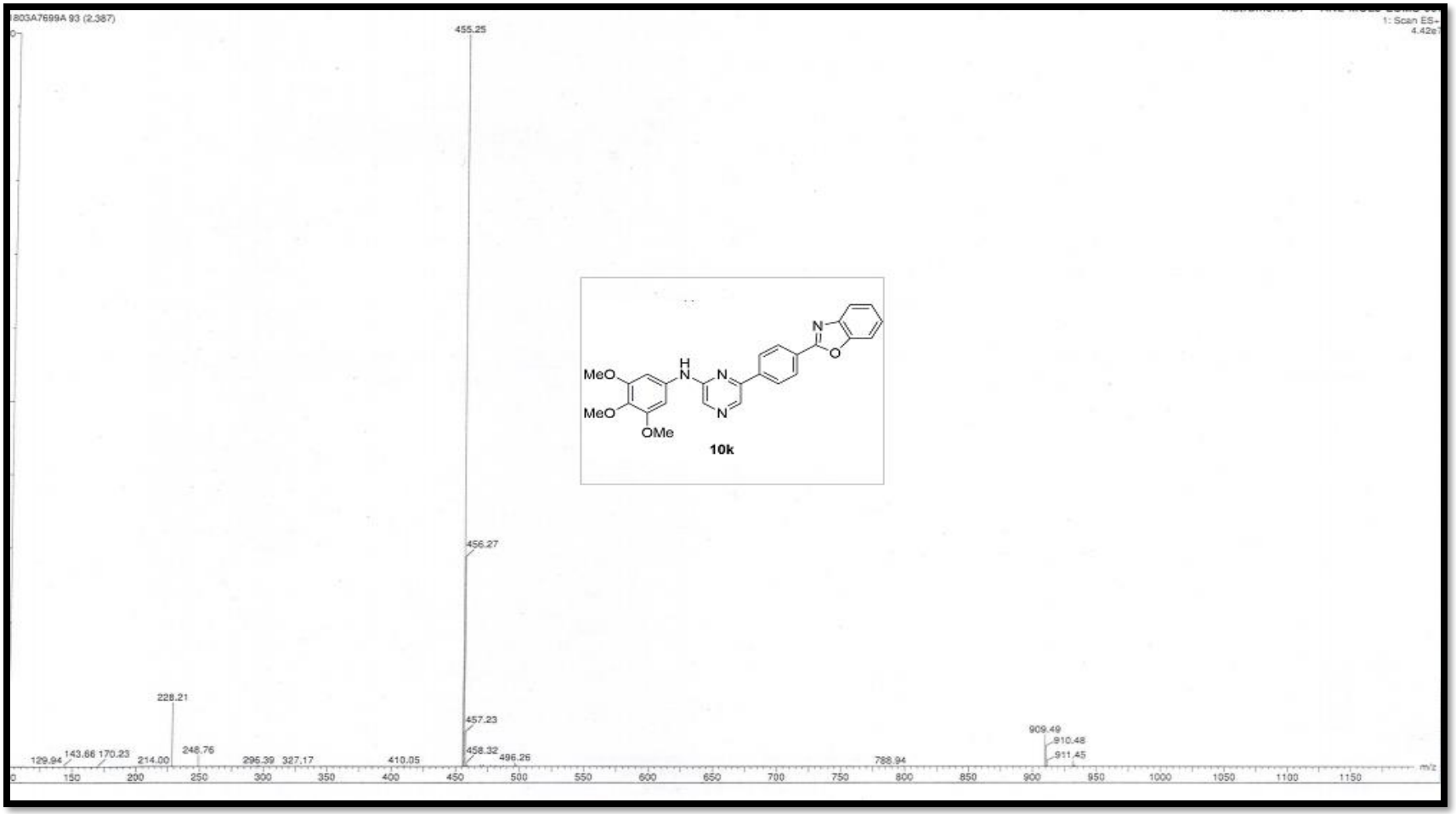
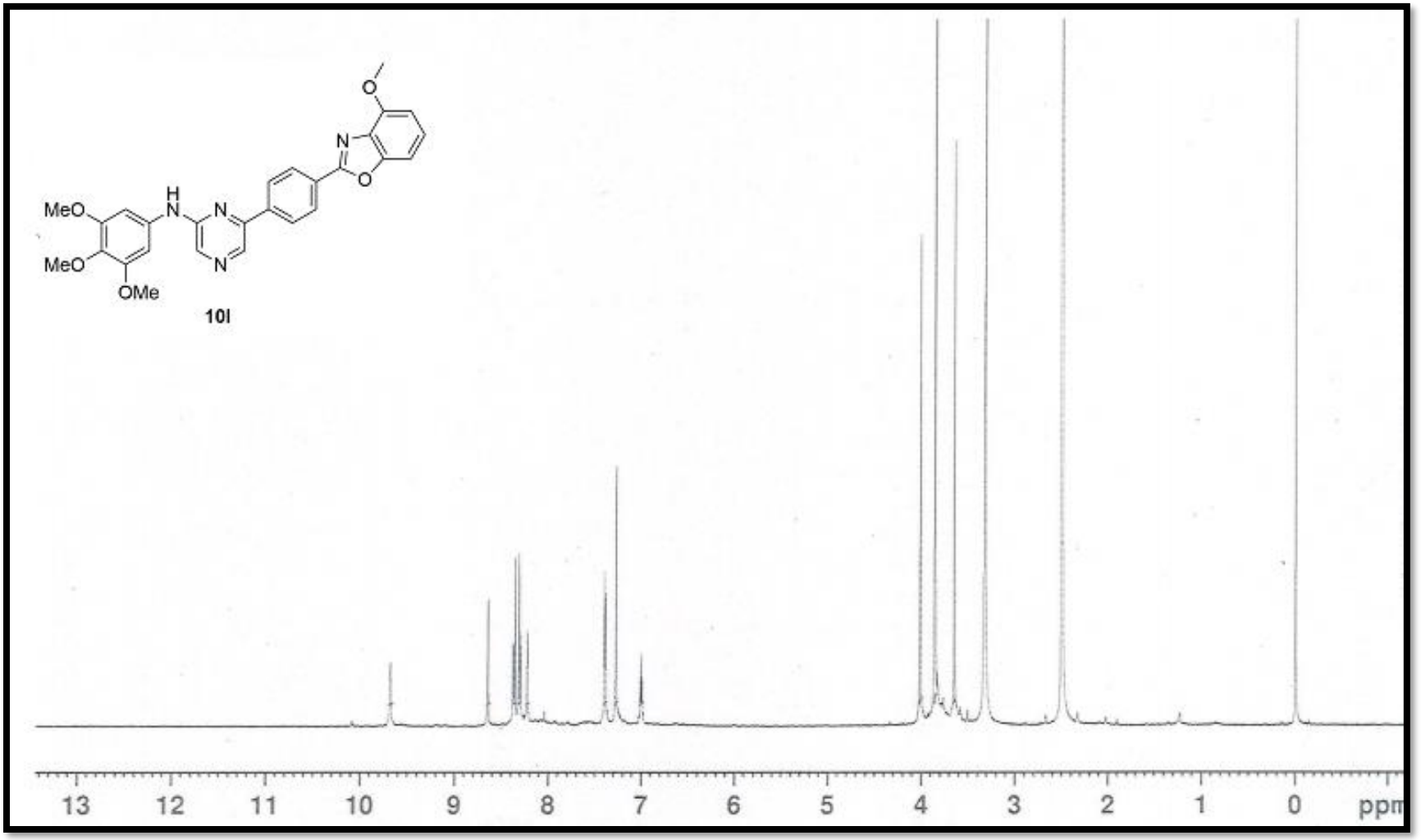


^{1}H-NMR Spectrum (DMSO-d_6, 400 MHz) of 6-(4-(4-methoxybenzo[d]oxazol-2-yl)phenyl)-N-(3,4,5-trimethoxyphenyl)pyrazin-2-amine (**10l**)



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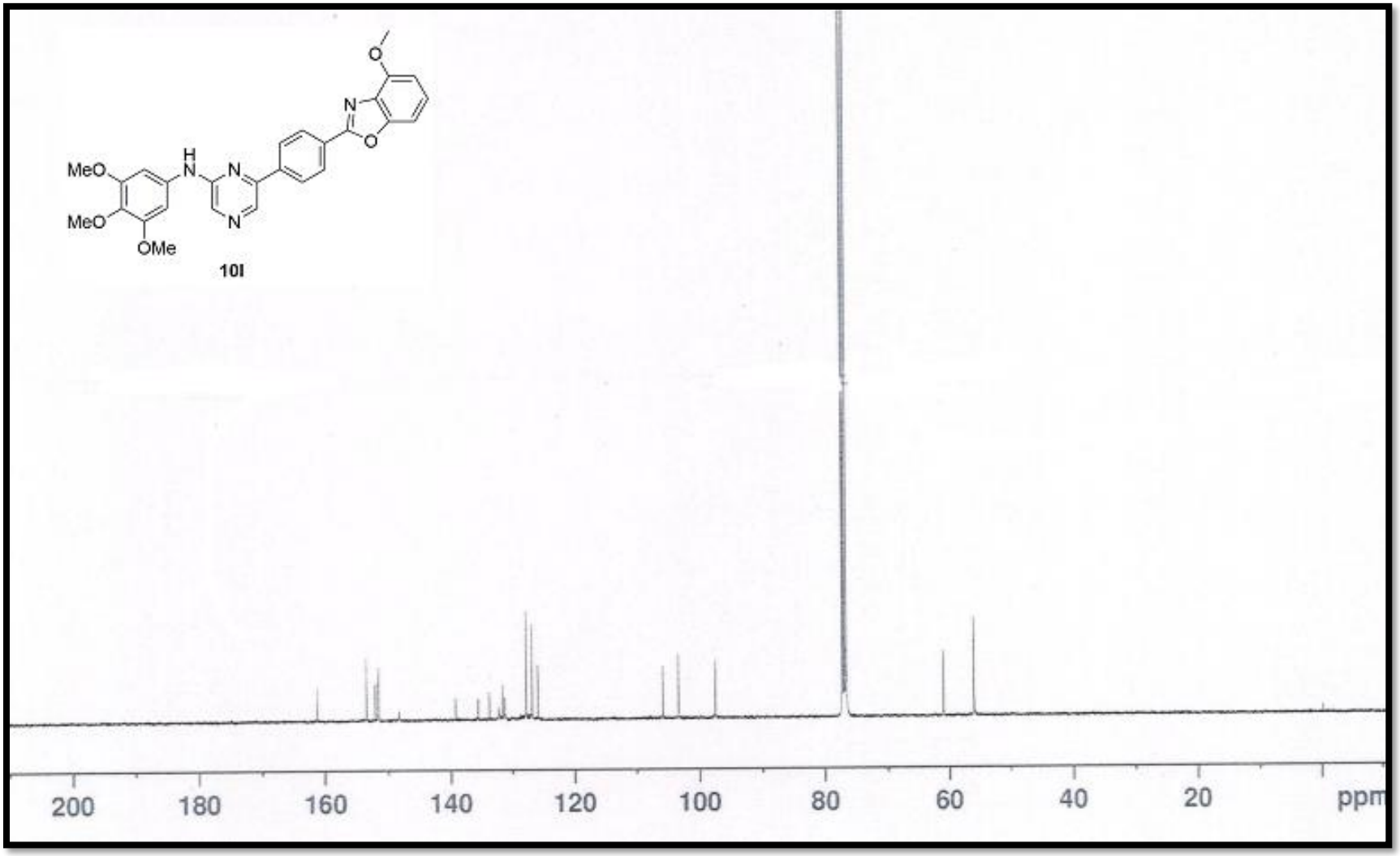

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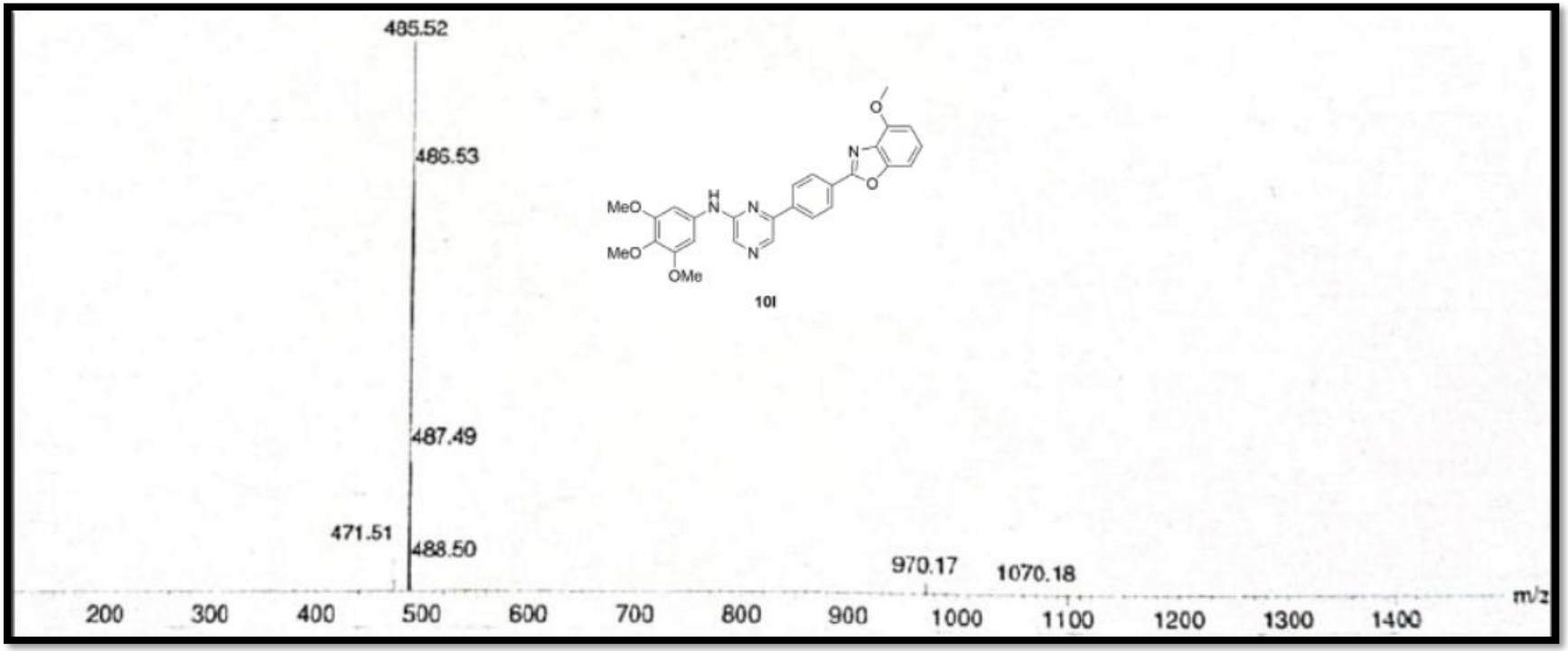

Cytotoxicity

The newly synthesized compounds **10a-j**were tested for *invitro*cytotoxicity against three human cancer cell lines MCF-7(breast), A-549(lung) and A-375(melanoma) for which *Doxorubicin* was used as standard drug, and compounds **10k**&**10l** were tested against four human cancer cell lines MCF-7(breast), MDA MB-231(breast), A-549(lung) and DU-145(prostate) by employing *Etoposide* as standard reference.Interestingly, the benzoxazole analogues **10k** & **10l**, displayed outstanding activity against all the three MCF-7, MDA-MB-231 and A-549 cell lines compared to *Etoposide*, the activity of these compounds against DU-145 cell line is low (**Table 2**). Among benzimidazole derivatives, compounds **10b, 10d, 10g, 10h** and **10j** showed superior activity against human breast cancer cell line MCF-7 with IC_{50} value of **0.19, 1.24, 1.9, 0.1** and **0.98 µM**,respectively, in comparison to *Doxorubicin* IC_{50} value of **2.02µM**.Activity of compound **10a** is on par with reference drug, with IC_{50} value of **2.9 µM**. Whereas the remaining compounds demonstrated good to moderate activity against MCF-7 cell line. Again, the samecompounds **10b, 10d, 10g, 10h** and **10j**displayed outstanding activity against human lung cancer cell line A-549 with IC50 value **0.76, 1.33, 0.89, 1.78** and **0.26µM** compared to *Doxorubicin* IC_{50} value of **2.18µM**.Compound **10c** (IC_{50} = 2.45µM**)** activity is comparable to *Doxorubicin*, and all other compounds exhibited good to moderate activity against A-549 cell line. In examination with human melanoma cell line A-375, except compounds 10a, 10c and 10e, all the compounds showed potent activity against A-375 cell line with IC_{50} value ranging between **0.1 – 3.56µM**compared to Doxorubicin IC_{50} value of **5.51µM.**

Table 1. IC_{50}values of target compounds **10a-j** in µM

Compound	MCF-7	A-549	A-375
10a	2.9±0.07	3.78±0.24	-
10b	0.19±0.02	0.76±0.03	0.12±0.02
10c	4.78±0.55	2.45±0.12	9.78±0.78
10d	1.24±0.10	1.33±0.05	0.56±0.02
10e	7.45±0.55	3.55±0.51	6.49±0.62
10f	5.12±0.57	10.3±0.03	2.65±0.07
10g	1.9±0.02	0.89±0.02	0.34±0.07
10h	0.1±0.03	1.78±0.07	0.22±0.03
10i	7.34±0.84	12.9±1.10	3.56±0.55
10j	0.98±0.01	0.26±0.05	0.11±0.02
Doxorubicin	2.02± 0.05	2.18±0.02	5.51±0.04

Table 2. IC_{50}values of target compounds **10k**& **10l** in µM

Compound	MCF-7	A-549	MDA-MB-231	DU-145
10k	0.23±0.04	0.34±0.05	0.92±0.02	1.45±0.04
10l	0.04±0.01	0.87±0.01	0.07±0.01	-
Etoposide	2.11±0.02	1.91±0.01	1.97±0.02	0.01±0.04