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Synthesis and Biological Investigations of Different Derivatives of Chalconyl s-triazine

Lopa Mukeshbhai Patel

Assistant Professor, Department of Chemistry, V. S. Patel College of Arts and Science Bilimora
Email: *lopapatel.211[at]gmail.com*

Abstract: ***The biological actions of s-triazine derivatives, such as antibacterial, antifungal, antituburcular, and anti-protozoa properties, are well-known. Additionally, chalcones serve as an intermediary in the synthesis of several heterocycles, including pyrazoline and pyrimidine. Chalcones were created in this study using the Claisen-Schmidt reaction and then condensed with s-triazine. Additionally, at the appropriate temperature and conditions, morpholine and p-nitro aniline were added to the s-triazine nucleus. IR, NMR, and Mass spectrum investigations were used to characterise the synthesised molecules. A variety of bacterial and fungal strains were used to measure biological activity. SAR research had been performed and conclusions were derived.***

Keywords: Triazine, morpholine, chalcone, biological actions, antifungal and antibacterial properties

1. Introduction

Derivatives of s-triazine are a special heterocyclic scaffold that has been extensively studied for antibacterial, antifungal, antitubercular, and antiprotozoal properties. A variety of pharmacophores can be included for the creation of powerful bioactive compounds because the electron-deficient triazine core permits flexible nucleophilic substitution. Due to their broad range of biological action, s-triazine-based chalcones and their derivatives have generally been the subject of substantial research [1-14]. They are found to be effective as Synthesis and local-anesthetic activity [1], Pinocembrin chalcone: an antibacterial compound from Helichrysum trilineatum [2], Antibacterial chalcones- bioisosteric replacement of the 4′-hydroxy group[3], Antimalarial :*In vitro* antimalarial activity of chalcones and their derivatives [4], Antimalarial alkoxylated and hydroxylated chalones: structure− activity relationship analysis[5], Antiplasmodial chalcones inhibit sorbitol-induced hemolysis of Plasmodium falciparum-infected erythrocytes[6], and Synthesis, characterization and antimicrobial property of novel series of 3-(4-substituted phenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl) amino)-1, 3, 5-triazin-2-yl) amino) phenyl) prop− 2-en-1-one conjugates[7].

Due to their vast variety of chemical reactivity, broad spectrum of biological activity, and easy accessibility through synthesis, the study of derivatives of chalconyl s-triazine has been a developing subject within the field of heterocyclic chemistry for the past several decades. Further the research work was relevant to recent advances in the biological activity of s-triazine core compounds for pharmaceuticals [8], as well as anti-tuberculosis agents [9], anticancer [10] for human cell cycle phase distribution [11] and antifungal agents [12,13]. Chalcones' various characteristics have led us to synthesise them in order to investigate their biological functions and activities [14].

Sharma et al reported on Synthesis of medicinally important quinazolines and their derivatives[15], Liuet al mentioned on Targeting IGF2BP3 in cancer[16], Riyadhet al., reported on a Decade of Development of Ethylidenethiosemicarbazides as Building Blocks for Synthesis of Azoles and Azines [17], Gupta et al., studied on 1, 3, 4-Oxadiazole derivatives: targeting multiple bacterial pathways[18], Desai & Patel worked on Whispers beneath the skin and microneedles with Biomaterials Science [19], and Mehtaet al, studied on Therapeutic journey of Andrographis paniculata to synthetic and nanoformulations [20].

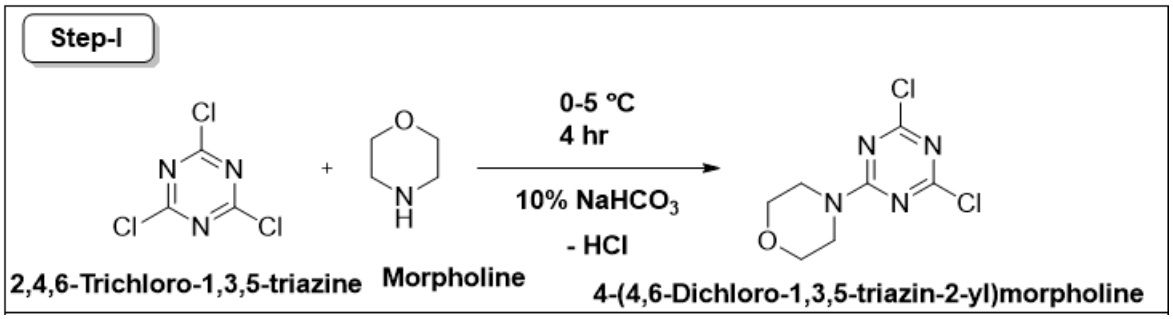
As precursors for pyrimidines, pyrazolines, and similar systems, chalcones are essential intermediates in heterocyclic synthesis. Because of the α,β-unsaturated carbonyl structure, chalcones have inherent antibacterial and anti-inflammatory effects in addition to their synthetic use. The most effective method for producing structurally varied chalcones is still Claisen-Schmidt condensation.

It has been demonstrated that adding electron-withdrawing groups like p-nitroaniline and heterocyclic amines like morpholine to the s-triazine nucleus increases biological efficacy by improving lipophilicity and enzyme interaction. Therefore, it is anticipated that chalcone and s-triazine pharmacophores will hybridise to produce compounds with synergistic antibacterial activity.

The current study synthesised chalcone-based s-triazine derivatives, characterised them using NMR analysis, and assessed their antibacterial and antifungal properties. To determine the impact of substituents on activity, connections between structure and activity were examined.

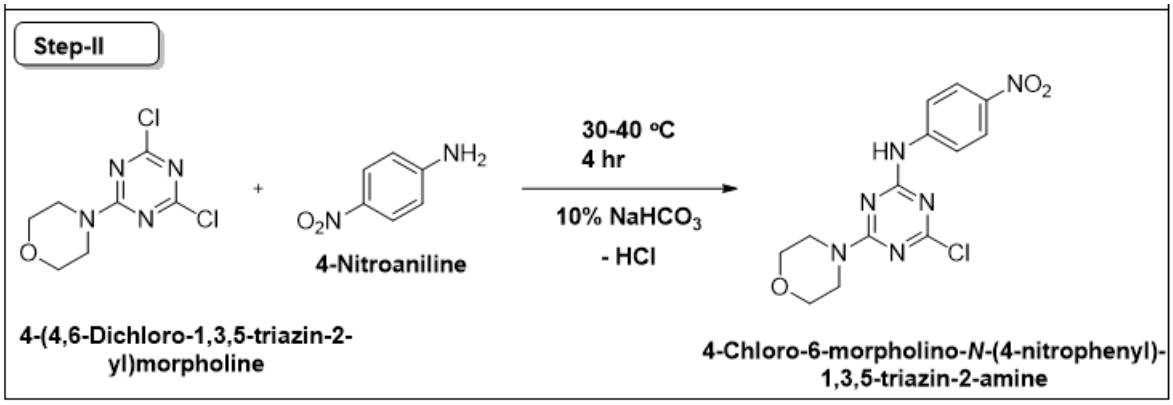
2. Materials and Method

Step-1:



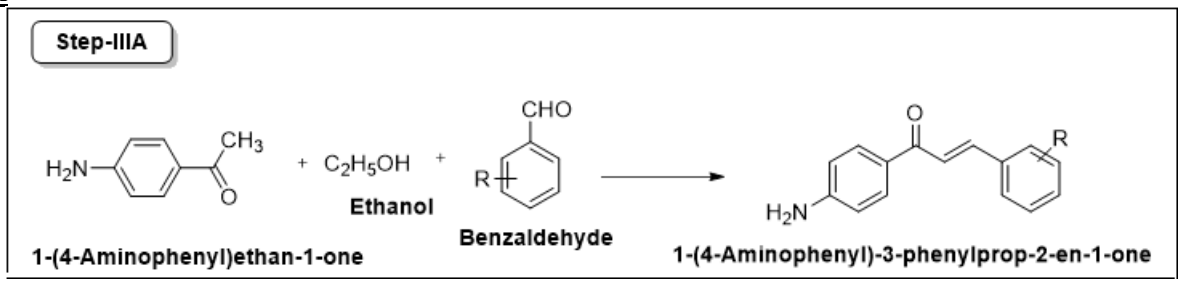
Scheme 1

Step-2



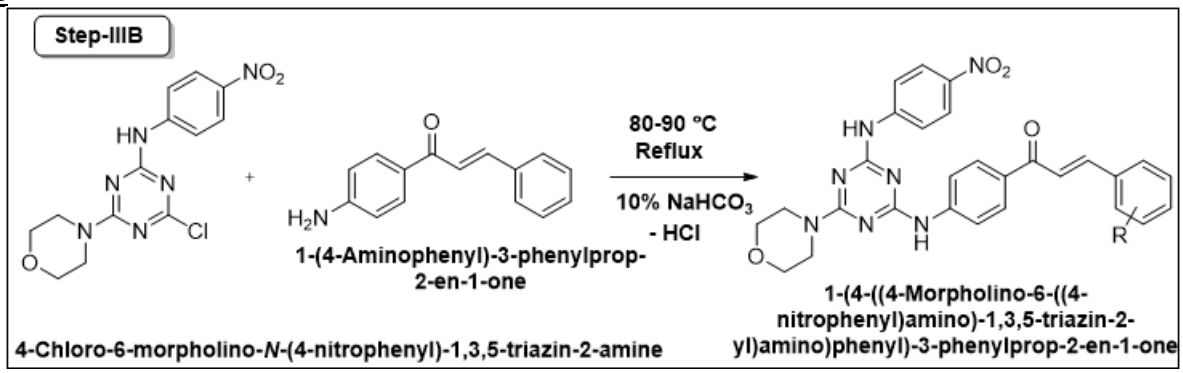
Scheme 2

Step-3A



Scheme 3

Step-3B



Scheme 4

3. Results and Discussion

Biological screening of the synthesized heterocycles:
In order to evaluate their potential as therapeutic agents, the synthesised compounds were put through a series of biological tests. Among other things, the assays included assessments for antibacterial, antifungal, antitubercular, anticancer, and anti-inflammatory properties. These substances' biological properties were compared to well-known, conventional drugs in the pertinent therapeutic areas.

Analysis of Structure-Activity Relationship (SAR): The novel compounds' SAR was carefully examined in light of the biological activity findings. Carefully examined was the

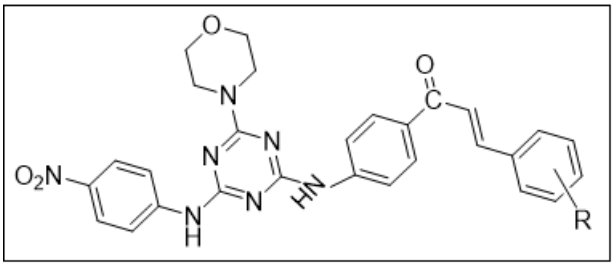
relationship between the chemical structure and the subsequent biological effects. To increase the biological activity of potential drugs, suggestions for molecular structural alterations were made.

Antibacterial activity data

The antimicrobial activity of reported compounds in terms of MIC values is summarized in Table 1. The MIC values of resulted compounds are observed in the varied range (62.5 to 500 µg/mL) to antibacterial

Biological Activity:

1) 3-(Phenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one



Minimum Inhibitory Concentration

		Antibacterial activity				Antifungal activity	
		Gram Positive		**Gram Negative**			
Comp. No.	**R**	***S.aureus*** MTCC 96 µg/mL	***S.pyogenus*** MTCC 443 µg/mL	***P.aeruginosa*** MTCC 741 µg/mL	***E.coli MTCC 442 µg/ mL***	***C.albicans*** **MTCC 227 µg/mL**	***A.niger*** **MTCC 282 µg/mL**
a	*4*-hydroxy	500	500	250	250	500	250
b	*2*-hydroxy	200	200	125	100	250	500
c	*2*-hydroxy & *3*-bromo	100	100	100	125	250	500
d	Styrene substituted	200	200	250	250	250	>1000
e	4-fluorophenyl	250	250	100	125	1000	500
f	4-aminophenyl	250	250	200	200	1000	1000
g	3,4-dihydroxyphenyl	250	250	62.5	100	500	1000
h	4-trifluoromethylphenyl	250	250	200	250	1000	1000
Standard Drug	**Ciprofloxacin**	50	50	25	25	-	-
	Nystatin	-	-	-	-	100	100

Structural activity relationship (SAR)

Through electronic, steric, and lipophilic effects, substitution on the 3-phenyl ring greatly affects the biological profile of the chalcone–triazine hybrid. While excessive polarity may decrease membrane permeability, electron-donating groups like 4-hydroxy, 2-hydroxy, 4-amino, and 3,4-dihydroxy boost electron density and hydrogen-bonding capacity, resulting in improved contact with enzyme targets and generally higher antibacterial effectiveness. While the 2-hydroxy-3-bromo derivative gains from the synergistic impact of hydrogen bonding and halogen bonding, increasing lipophilicity and potency, the 2-hydroxy substituent can generate intramolecular hydrogen bonding with the enone carbonyl, adding conformational rigidity.

Anticancer activity and cell penetration are favoured by electron-withdrawing groups like 4-fluoro and 4-trifluoromethyl, which enhance metabolic stability and hydrophobic interactions. It is anticipated that styryl substitution may increase cytotoxic potential by extending π-conjugation and encouraging π–π stacking with DNA or protein residues. Overall, 4-hydroxy, 3,4-dihydroxy, and 4-fluoro analogues may be best for antimicrobial activity due to a balance between electron donation for target binding and electron withdrawal for permeability, while CF_3, styryl, and bromo-substituted derivatives are better for anticancer applications.

4. Characterisation

Spectral analysis ^{1}H NMR & ^{13}C NMR

1) **3-(4-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5- triazin-2-yl)amino)phenyl)prop-2-en-1-one**
^{1}H NMR (400 MHz, DMSO-*d6*) δppm: 9.00(s, 1H), 8.80(S, 1H), 8.71(m, 2H), 8.00-7.90(m, 9H), 7.72-7.66(m, 5H), 3.76-3.38(m, 8H).
^{13}C NMR (100 MHz, DMSO-*d6*) δppm: 190.98, 163.07, 161.85, 157.07, 145.35, 141.65, 141.56, 140.05, 131.63, 131.33, 128.73, 128.71, 127.37, 121.83, 120.64, 120.63, 118.31, 117.76, 117.54, 65.59, 47.06.

2) **3-(2-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5- triazin-2-yl)amino)phenyl)prop-2-en-1-one**
^{1}H NMR (400 MHz, DMSO-*d6*) δppm: 8.65(s, 1H), 8.33(S, 1H), 8.24(m, 1H), 7.65-7.62(m, 2H), 7.43-7.27(m, 12H), 3.39-3.34(m, 8H).
^{13}C NMR (100 MHz, DMSO-*d6*) δppm: 191.08, 162.07, 161.85, 159.30, 145.35, 144.36, 141.65, 131.63, 129.98, 128.73, 127.63, 127.37, 122.95, 118.31, 117.54, 116.37, 65.59, 47.06.

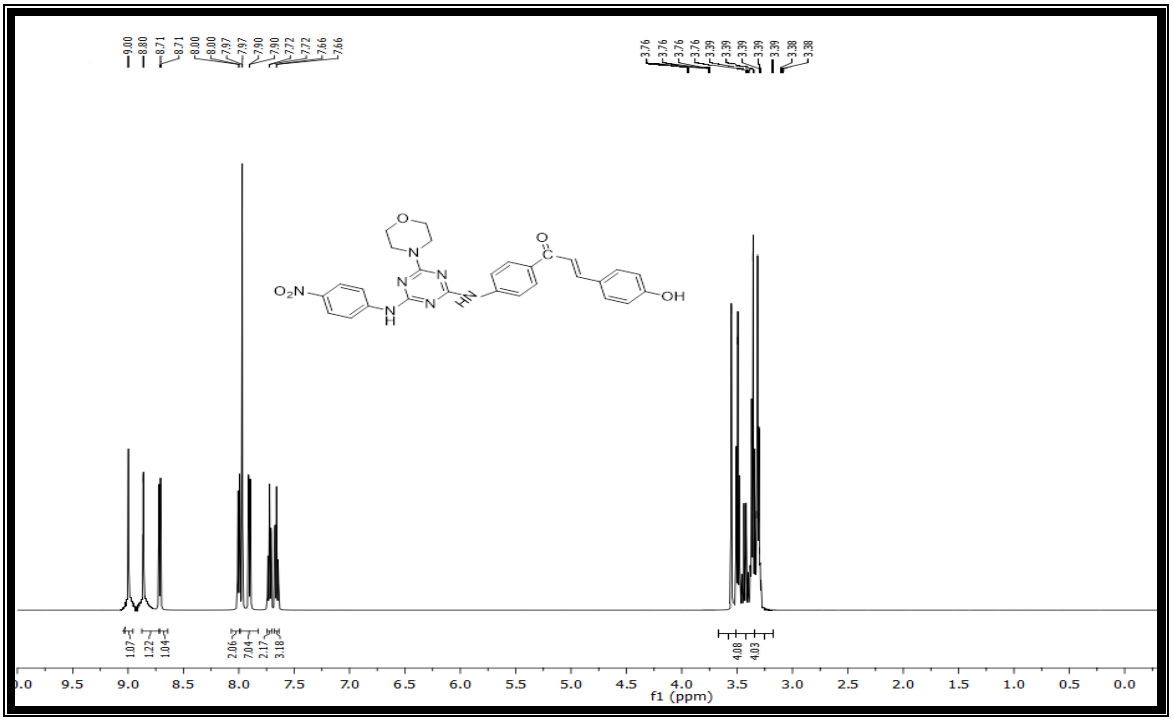
3) **3-(5-Bromo-2-hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)- 1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one**
^{1}H NMR (400 MHz, DMSO-*d6*) δppm: 8.14(s, 1H), 8.03(S, 1H), 7.50(m, 2H), 7.33-

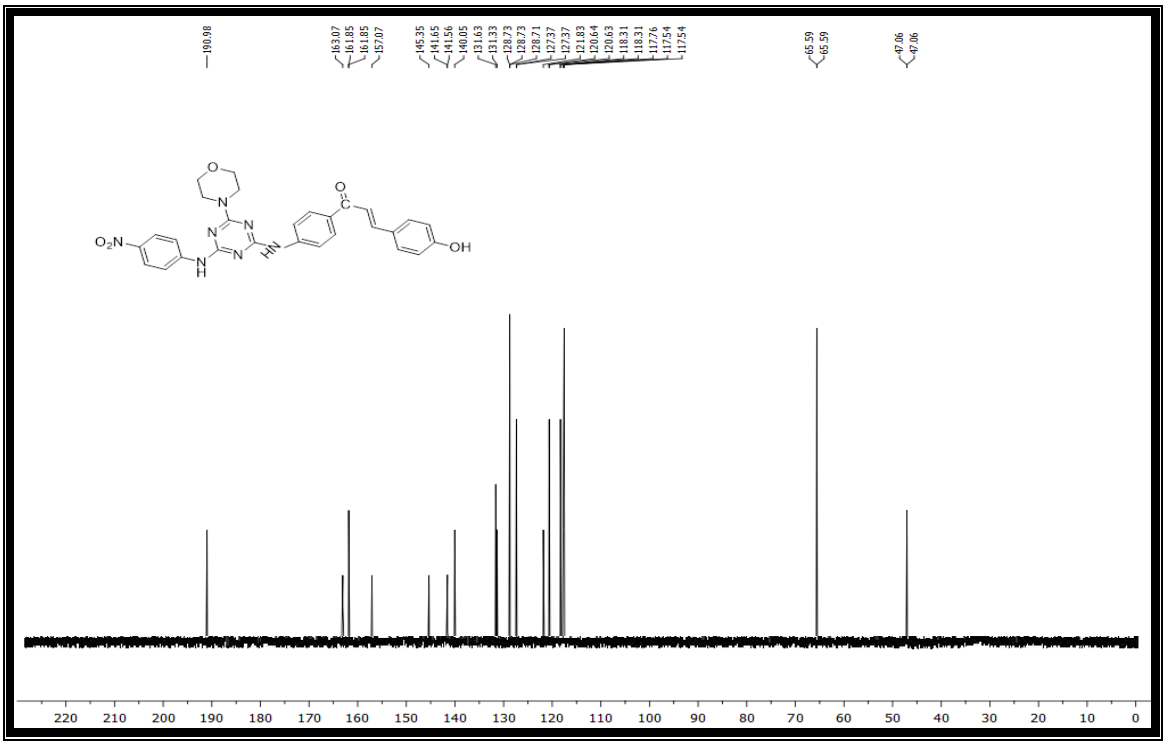
7.04(m, 18H), 3.39-3.34(m, 8H).
^{13}C NMR (100 MHz, DMSO-*d6*) δppm: 191.80, 163.07, 161.85, 131.63, 129.12, 128.76, 128.73, 127.63, 127.56, 127.37, 118.31, 117.54, 65.59, 47.06.

4) **1-(4-((4-Morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)- 5-phenylpenta-2,4-dien-1-one**
^{1}H NMR (400 MHz, DMSO-*d6*) δppm: 9.07(s, 1H), 8.88(s, 1H), 8.71(s, 1H), 7.50-7.21(m, 12H), 3.39-3.22 (m, 8H).
^{13}C NMR (100 MHz, DMSO-*d6*) δppm: 190.98, 163.07, 161.85, 156.19, 145.65, 145.56, 139.39, 128.73, 127.37, 120.21, 120.11, 119.18, 118.31, 117.54, 112.70, 65.59, 47.06.

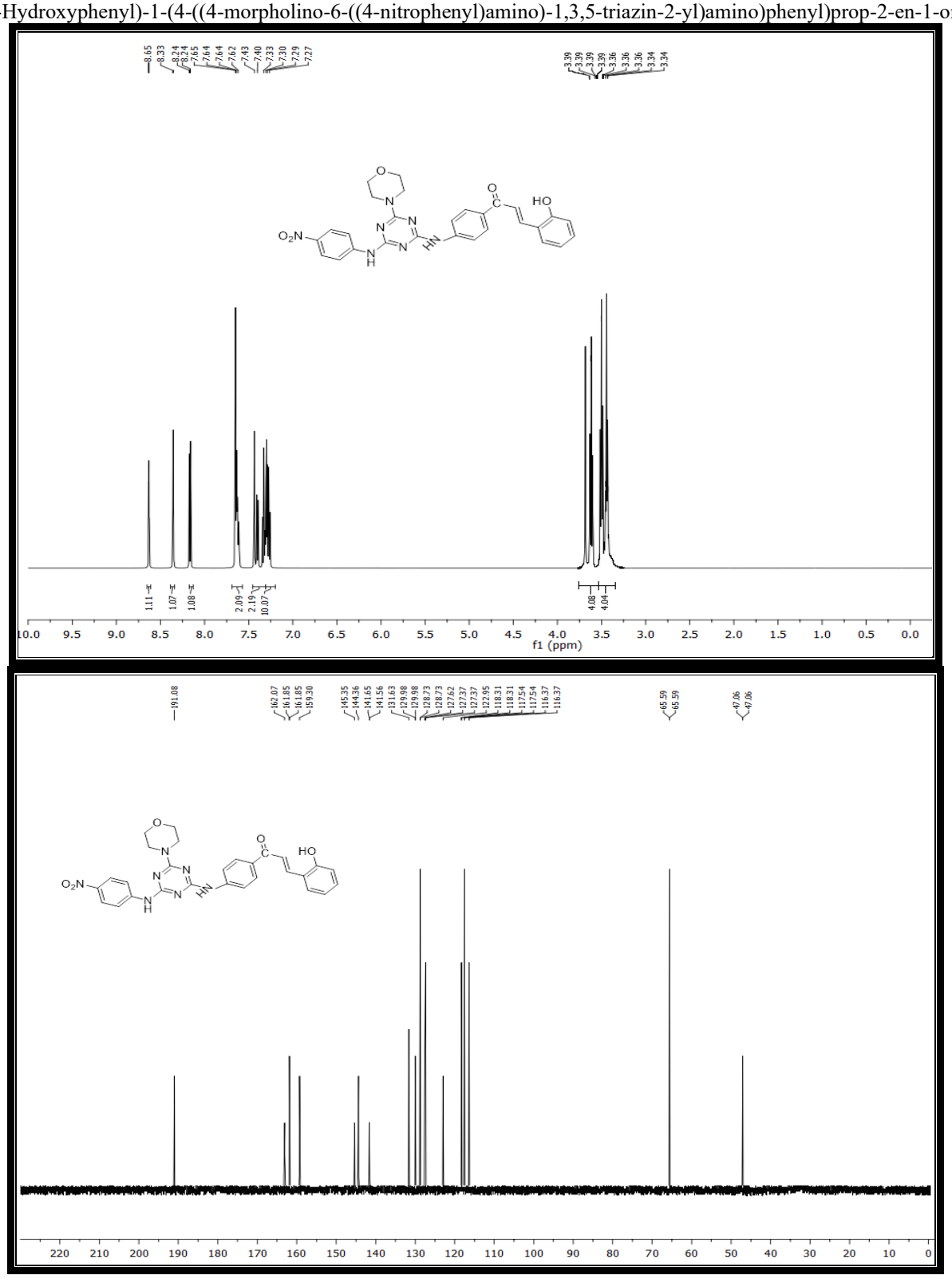
^{1}H NMR & ^{13}C NMR

1) 3-(4-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one

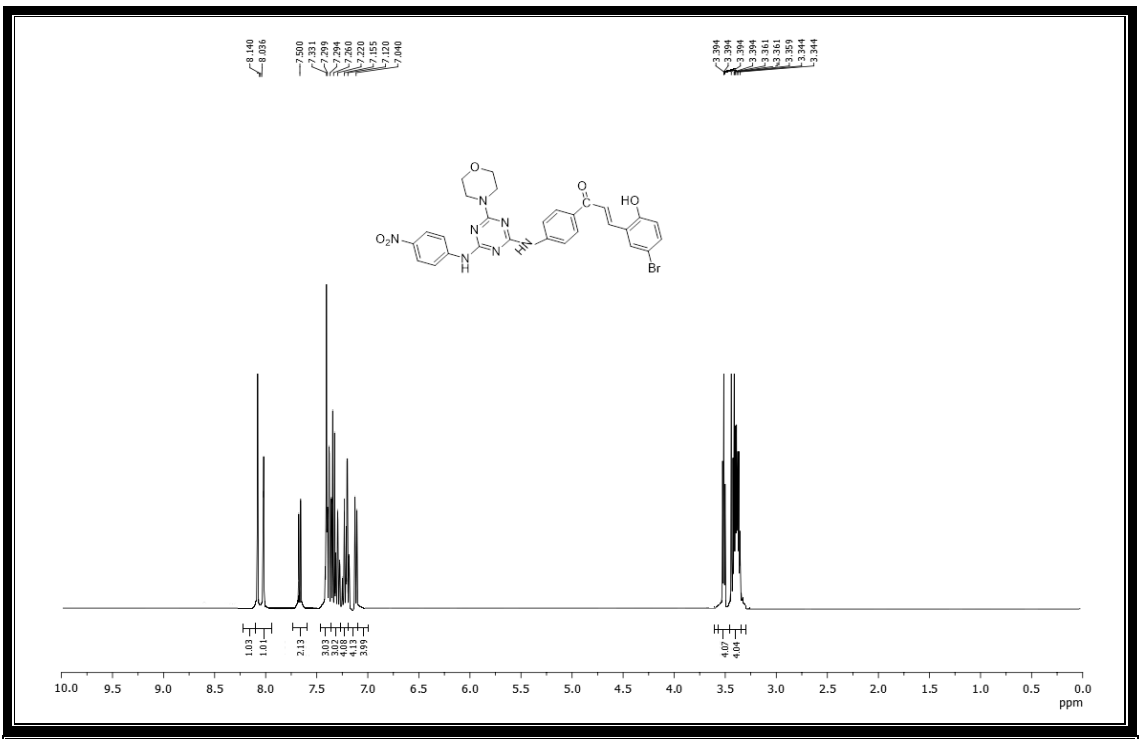


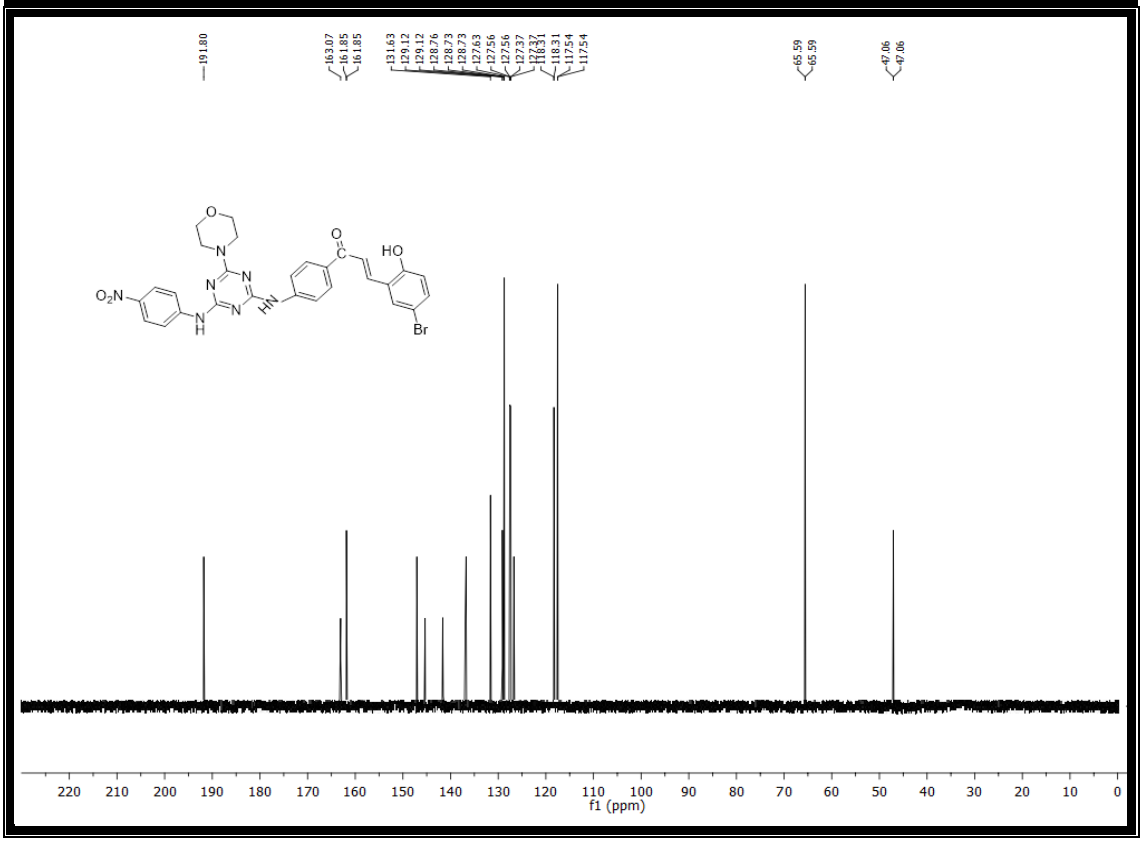


2) 3-(2-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one

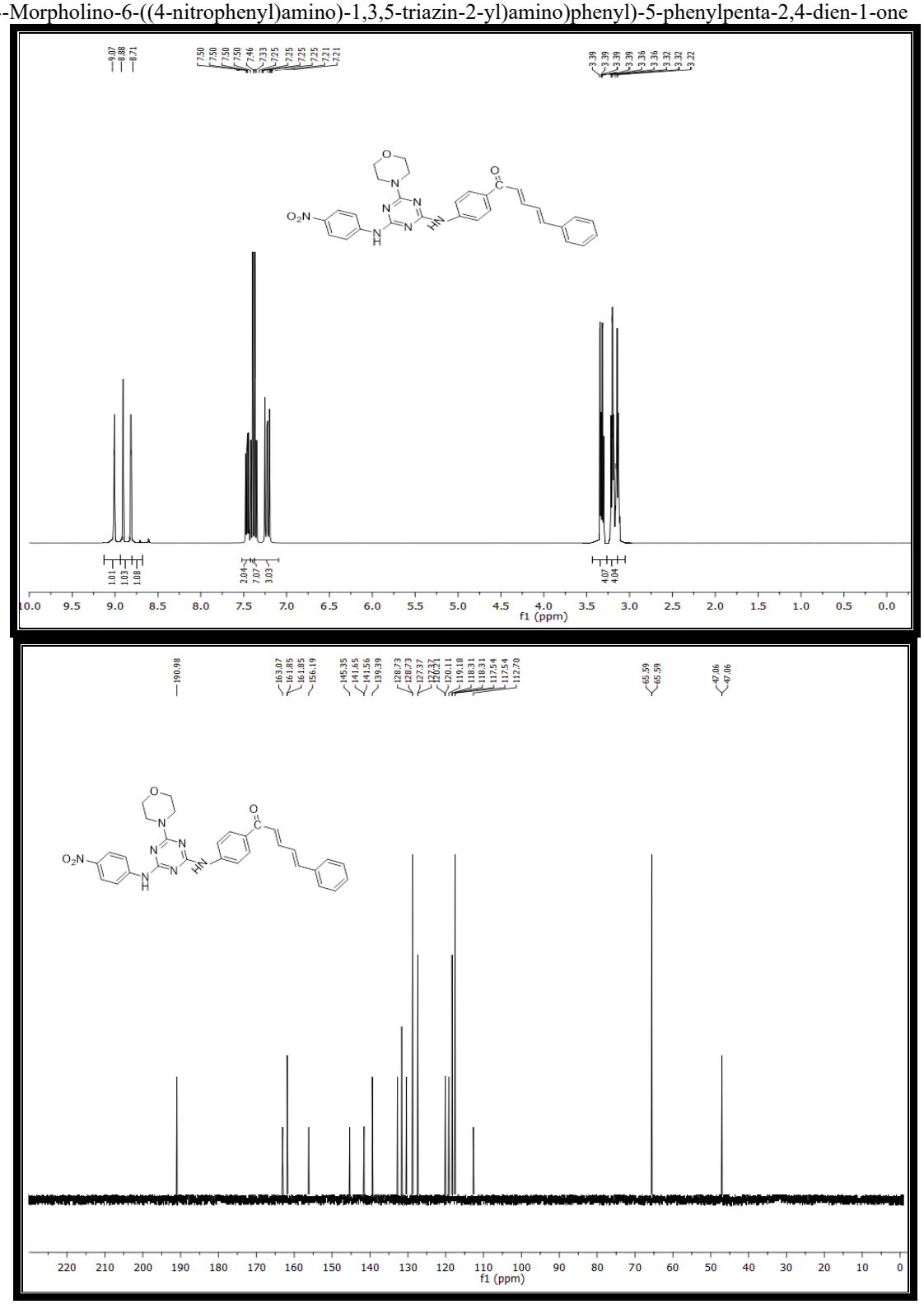


3) 3-(5-Bromo-2-hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one



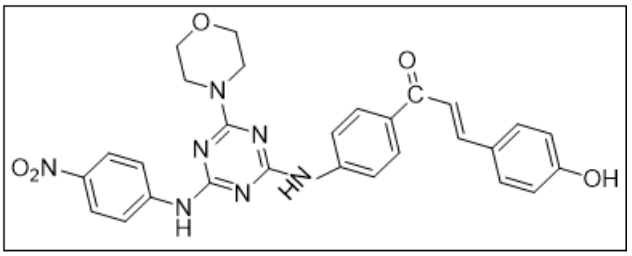


4) 1-(4-((4-Morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)-5-phenylpenta-2,4-dien-1-one



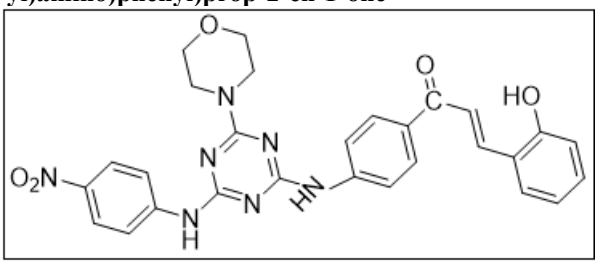
IR

1) **3-(4-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one**



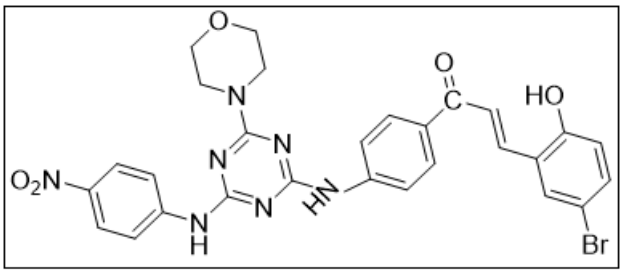
- Broad O–H (phenolic): 3450 cm^{-1}, broad, medium–strong — phenolic OH stretch
- Aromatic / =C–H stretches (sp^2 C–H): 3099 cm^{-1}, weak — aromatic and alkene C–H.
- N–H (secondary amine): 3430 cm^{-1}
- Aliphatic C–H (morpholine): 2920 cm^{-1}, medium — CH_2 stretches of morpholine.
- Strong conjugated carbonyl (α, β-unsaturated C=O): 1677cm^{-1}, strong — chalcone-type C=O; conjugation with the double bond and aromatic rings.
- C=C (conjugated alkene / aromatic C=C): 1595 cm^{-1} (conjugated C=C often near 1600–1620),
- Nitro (NO_2):
  - asymmetric NO_2 stretch: 1555 cm^{-1}, strong (strong band).
  - symmetric NO_2 stretch: 1379 cm^{-1}, strong.
- C–O (phenol / aryl–O): 1249 cm^{-1}, strong — aryl–OH (C–O stretching).
- C–N / aromatic amine stretches (triazinyl–NH, aryl–NH, morpholine C–N): 1299 cm^{-1}, medium (overlaps with other bands).
- C–O–C (ether, morpholine ring): 1075 cm^{-1}, strong — morpholine ether/ether-like vibrations and C–O stretch.

2) 3-(2-Hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one



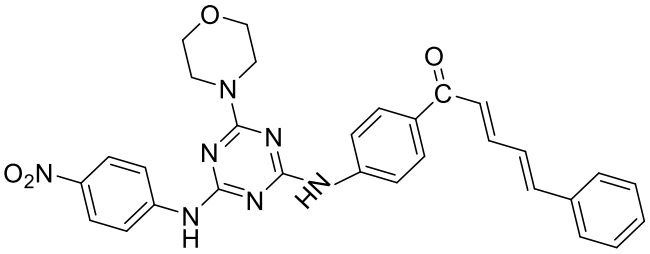
- **O–H (phenolic)**: **–3450 cm^{-1}, broad**
- **N–H (aryl/anilino)**: **3439 cm^{-1}, medium** — may overlap with O–H region; intensity/shape depends on H-bonding/ exchange.
- **Aromatic / =C–H (sp^2 C–H)**: **3129 cm^{-1}, weak** — aromatic and vinylic C–H stretches.
- **Aliphatic C–H (morpholine CH_2)**: **2859cm^{-1}, medium**.
- **Conjugated carbonyl (α, β-unsaturated C=O)**: **~1635cm^{-1}, strong**.
- **C=C (conjugated alkene / aromatic C=C)** and **aromatic stretches**: **~1550 cm^{-1}, medium**.
- **Nitro (NO_2)**:
  - **asymmetric stretch**: **1525 cm^{-1}, strong**.
  - **symmetric stretch**: **1355 cm^{-1}, strong**.
- **C–O (phenolic C–O stretch)**: **1266 cm^{-1}**,.
  - Intramolecular H-bonding can alter the exact position and intensity of the C–O band (sometimes shifts slightly).
- **C–N / aromatic amine (aryl-NH, triazine-NH, morpholine C–N)**: **1296 cm^{-1}, medium** (overlaps with C–O and NO_2 bands).
- **C–O–C / morpholine (ether-like) stretch**: **1055 cm^{-1}, strong**.
- **aromatic C–H bending (para/ortho substitution patterns)**: **799 cm^{-1}** region- diagnostic patterns of substituted phenyl rings

3) 3-(5-Bromo-2-hydroxyphenyl)-1-(4-((4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)prop-2-en-1-one



- **O–H (phenolic, ortho, intramolecularly H-bonded): ~3233 cm^{-1}, broad, medium → strong**
- **N–H (anilino / triazinyl–NH): ~3445 cm^{-1}, medium**
- **Aromatic / vinylic =C–H (sp^2 C–H): 3090 cm^{-1}, weak**.
- **Aliphatic C–H (morpholine CH_2): 2927 cm^{-1}, medium**.
- **Conjugated carbonyl (α,β-unsaturated C=O, chalcone): ~1660 cm^{-1}, strong**
- **Aromatic C=C / conjugated C=C stretches: ~1545 cm^{-1}, medium** (overlaps with nitro asymmetric band region).
- **Nitro (NO_2):**
- **Asymmetric NO_2 stretch: ~1522 cm^{-1}, strong**.
- **Symmetric NO_2 stretch: ~1339cm^{-1}, strong**.
- **C–O (phenolic C–O): ~1267 cm^{-1}, strong–medium** — position/intensity can shift slightly because of H-bonding.
- **C–N / aromatic amine / triazine stretches: ~1296cm^{-1}, medium.**
- **C–O–C (morpholine / ether-like): ~1055 cm^{-1}, strong.**
- **Aromatic C–H out-of-plane bending (substitution pattern diagnostics): ~655 cm^{-1}, medium → strong**
- **C–Br stretch (aryl bromide): ~597 cm^{-1}, weak → medium.**

4) 1-(4-((4-Morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)-5-phenylpenta-2,4-dien-1-one



- **Conjugated carbonyl (α,β,γ,δ-unsaturated ketone / dienone C=O): ~1635cm^{-1}, strong**
- **Conjugated C=C (vinylic / extended conjugation / aromatic C=C): ~1599 cm^{-1},**
- **Nitro (NO_2):**
- **asymmetric stretch: ~1555 cm^{-1}, strong**.
- **symmetric stretch: ~1356 cm^{-1}, strong**.
- **N–H (aryl/anilino linking nitrophenyl → triazine): ~3444 cm^{-1}, medium** — may be sharper or somewhat broadened depending on H-bonding / sample conditions; can overlap with other weak bands if present.
- **Aromatic & vinylic sp^2 C–H stretches: ~3088 cm^{-1}, weak**- from phenyl rings and the dienyl C–H.

- **Aliphatic C–H (morpholine CH_2): ~2938 cm^{-1}, medium**.
- **C–N / aromatic amine / triazine ring stretches: 1350 cm^{-1}, medium** (overlaps with NO_2 symmetric band and C–O bands).
- The triazine C=N/C–N type vibrations often fall in the **1559 cm^{-1}** region as weak–medium contributions and can further complicate that region.
- **C–O–C (morpholine ether-like)** and **C–O (if any O present in ring)**: **~1055 cm^{-1}, strong** — morpholine ring C–O and C–N–C skeletal stretches.
- **Aromatic C–H out-of-plane bending (substitution patterns): ~779 cm^{-1},**

5. Conclusion

Compounds with morpholine substitution on the s-triazine nucleus shown increased antibacterial and antifungal activity in the derivatives of this series, whereas compounds without morpholine showed relatively lower efficacy. While electron-donating substituents on the chalcone ring produced moderate activity, the addition of a p-nitro group on the aniline moiety was advantageous for antifungal activity. Additionally, the combined presence of morpholine on the triazine core and electron-withdrawing groups on the chalcone phenyl ring greatly enhanced the overall antibacterial potency. These findings suggest that increased biological activity requires proper substitution on both the chalcone and s-triazine fragments.

Data accessibility: Every piece of information has been included in the manuscript and the addendum.

Declaration of generative AI and AI-assisted technologies in the writing process: The authors employed GPT 4 in order to enhance the manuscript's language and readability during the preparation process. Following their use of this tool/service, the writers took full responsibility for the publication's content and reviewed and amended it as necessary.

Author contributions statement: Writing – original draft, methodology, data curation, conceptualization.

Conflict of Interest: No any conflicts of interest.

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