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Study Of Chemoselective Reaction Of Active Methylene Compounds With Imine Derivatives Of 3-Aminoacetophenone And Antifungal Potential Of The Synthesized Compounds

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Abstract: –

Active methylene compounds, including cyanoacetic acid (I), ethyl cyanoacetate (II), malonitrile (III), dimethyl malonate (IV), diethyl malonate (V), ethyl acetoacetate (VI), acetylacetone (VII), malonamide (VIII), and nitromethane (IX), exhibited chemoselective reactions with the carbon–nitrogen double bond of 1-(3-(3-phenylallylideneamino)phenyl)ethanone. This resulted in the formation of mono addition–elimination products (Ia–IXa) while preserving the carbon–oxygen double bond, considered to be more reactive, even in the presence of two moles of active methylene compounds. The products (Ia–IXa) were characterized through elemental analysis and spectral studies. Evaluation against two phytopathogenic fungi, *Helminthosporium oryzae* and *Colletotrichum capsici*, revealed that 2-cyano-5-phenylpenta-2,4-dienoic acid (Ia), ethyl 2-cyano-5-phenylpenta-2,4-dienoate (IIa), and 2-(3-phenylallylidene)pentane-2,4-dione (VIIa) exhibited ED₅₀ values of 22, 27, and 32 µg/ml, respectively—significantly lower than the ED₅₀ value of Indofil M-45 (46 µg/ml) against *H. oryzae*.

Key words: 1-(3-(3-Phenylallylideneamino) phenyl) ethanone, active methylene compounds, acetyl aniline, diene derivatives.

Introduction

In recent years, the field of multiple bonds in chemistry has witnessed significant advancements, driven by the utilization of these compounds as key substrates in synthesizing both industrial and biologically relevant compounds. Compounds featuring carbon–carbon bonds, carbon–oxygen, and carbon–nitrogen have been recognized for their diverse biological activities, including antimicrobial, antifungal, antitumor, and nematicidal properties. Active methylene compounds, in particular, undergo condensation reactions with carbonyl compounds. When reacting with imines, these compounds yield either addition products or addition–elimination products. This reactivity profile is pivotal in the observed chemoselective reactions, enabling the creation of mono addition–elimination products while keeping the carbon–oxygen double bond intact, given the specific reaction conditions. This study focuses on investigating the reaction of active ethylene compounds with 1-(3-(3-phenylallylideneamino) phenyl) ethanone—a compound containing carbon–oxygen double bonds, carbon–nitrogen, and carbon–carbon. The paper also presents unexpected findings related

to the antifungal activity of the synthesized compounds.

Experimental

The products' homogeneity was assessed through TLC. Satisfactory elemental analysis results were obtained for the compounds. The uncorrected melting points were calculated using an "electric melting point" instrument. The IR spectra were acquired on a "Perkin-Elmer FT-IR spectrometer" and a "KBr disc". The ¹H NMR spectra in CDCl₃ were procured by using "Bruker Spectrospin 300 MHz spectrometer using TMS as the internal standard". Mass spectra was collected using a "Perkin-Elmer Clarus 500 mass spectrometer".

Synthesis 1-(3-(3-phenylallylidene amino)phenyl)ethanone:

3-Acetylaniline (0.1 mole) was mixed in 150ml of ethanol. Then cinnamaldehyde (0.1 mol) added in solution, and the mixture warmed for 5 minutes, resulting in a transparent solution, and was kept standing all night (at room temperature). The resulted crude material was filtered & subjected to recrystallization from ethanol. This process yielded crystals of 1-(3-(3-phenylallylideneamino)phenyl)ethanone with a MP of 95°C. The overall yield of the product was 93%.

General method for active methylene compounds condensation with 1-(3-(3-phenylallylideneamino)phenyl) ethanone:

A solution was prepared by combining 0.01 mol of an active methylene compound with 0.01 mol of 1-(3-(3-phenylallylideneamino) phenyl)ethanone in 25 ml of dry benzene within a 100 ml conical flask. Followed by pyridine (few drops), the mix underwent reflux and vigorous shaking for 1-2 hours. Later solution was cooled and closed flask stored for 24hrs at room temperature. To get the desired diene product, the resultant crude solid was filtered and recrystallized from benzene (Ia-IXa). The solvent evaporated from the filter, leaving a gel-like material. TLC examination of this compound revealed 3-acetylaniline and unreacted starting components. "Reaction of active methylene compounds (I-IX) with 1-(3-(3-phenylallylideneamino) phenyl) ethanone in 2:1 molar ratio was carried by following the above procedure".

Antifungal activity:

The test compounds (Ia-IXa) and a standard fungicide, Indofil M-45, were each dissolved in 0.5ml of alcohol(absolute) to create stock solutions. The total volume was 10 ml of sterilised distilled water. These stock solutions, prepared on an active ingredient basis at a concentration of 2000 µg/ml, were subjected to serial dilution by the addition of distilled water as needed.

The spores and cultures of the test fungi *H. oryzae* and *C. capsici* were isolated paddy leaves and chilli fruit, respectively. The specimens were cleaned in flowing tap water for 5-10 minutes before being divided into tiny segments using a sterile blade. These little contaminated pieces were disinfected with 0.1% mercuric chloride for one minute, then rinsed three times with sterilised distilled water. The surface-sterilized infected fragments were then transferred to PDA slants under aseptic conditions and placed in incubation at 25±1°C. A spore suspension (1×10⁶ spores/ml) was obtained by collecting cultures of the test fungus that were actively developing at 10 days. Equal volumes (0.02 ml each) of the test solution and spore suspension were applied to cavity slides. The slides were then placed in Petri plates with moist filter paper and incubated at 24±1°C for 24 hours. The spore germination was monitored, and the percentage of spore germination inhibition was obtained, allowing ED₅₀ values to be derived.

Results and Discussion

Condensation of active methylene compounds *viz.* cyanoacetic acid (I), ethyl cyanoacetate (II), malonitrile (III), dimethyl malonate (IV), diethyl malonate (V), ethyl acetoacetate (VI), acetylacetone (VII), malonamide (VIII) and nitromethane (IXa) with 1-(3-(3-phenylallylideneamino)phenyl)ethanone,

synthesized by condensing 3-acetylaniline with cinnamaldehyde, in equimolar ratio in the presence of pyridine results in crude solids formation (Ia-IXa) and then were sanitized by recrystallization from benzene. The products were characterized (Fig. 1) as 2-cyano-5-phenylpenta-2,4-dienoic acid (Ia), ethyl 2-cyano-5-phenylpenta-2,4-dienoate (IIa), 2-(3-phenyl allylidene)malononitrile (IIIa), dimethyl 2-(3-phenylallylidene) malonate (IVa), dimethyl 2-(3-phenylallylidene)malonate (Va), ethyl 2-acetyl-5-phenylpenta -2,4-dienoate (VIa), 2-(3-phenylallyli dene)pentane-2,4-dione (VIIa), 2-(3-phenylallyli dene)malonamide (VIIIa) and (4-nitrobuta-1,3-dienyl)benzene (IXa) based on spectral and elemental data. The IR spectra of the products showed bands in the region 1000-950 cm^{-1} and in the range of 1590-1575 cm^{-1} depicting the presence of $-\text{CH}=\text{CH}-$ (trans) and $-\text{CH}=\text{C}<$ linkage respectively. Usual bands for other functions were also satisfactorily indicated. For example, a sharp band between 2225-2215 cm^{-1} in compounds Ia-IIIa was due to $-\text{CN}$ group. The IR spectra of all the compounds except IIIa contained bonds due to $>\text{C}=\text{O}$ group. The ^1H NMR spectra of the compounds in CDCl_3 contained an eight proton multiplet between 6.8-8.0 δ accounting for five aromatic protons and three olefinic protons³. The protons from the functional groups resonated within the anticipated range. The mass spectra of the compounds indicated that the molecular ion peak was also a significant contributor to the base peak. Details of the products, including their physical attributes and spectral data, are documented in Table 1. Condensation of active methylene compounds (I-IX) with 1-(3-(3-phenylallylideneamino) phenyl)ethanone in 2:1 molar ratio also yielded the same products Ia-IXa respectively. The generation of products Ia-IXa is elucidated through the carbanion's attack, resulting from the removal of a proton from the active methylene group on the carbon-nitrogen double bond of 1-(3-(3-phenylallylidene amino)phenyl)ethanone. This process forms initially unstable addition products, which, in turn, undergo the loss of 3-acetylaniline, ultimately producing stable addition-elimination products (Scheme 1).

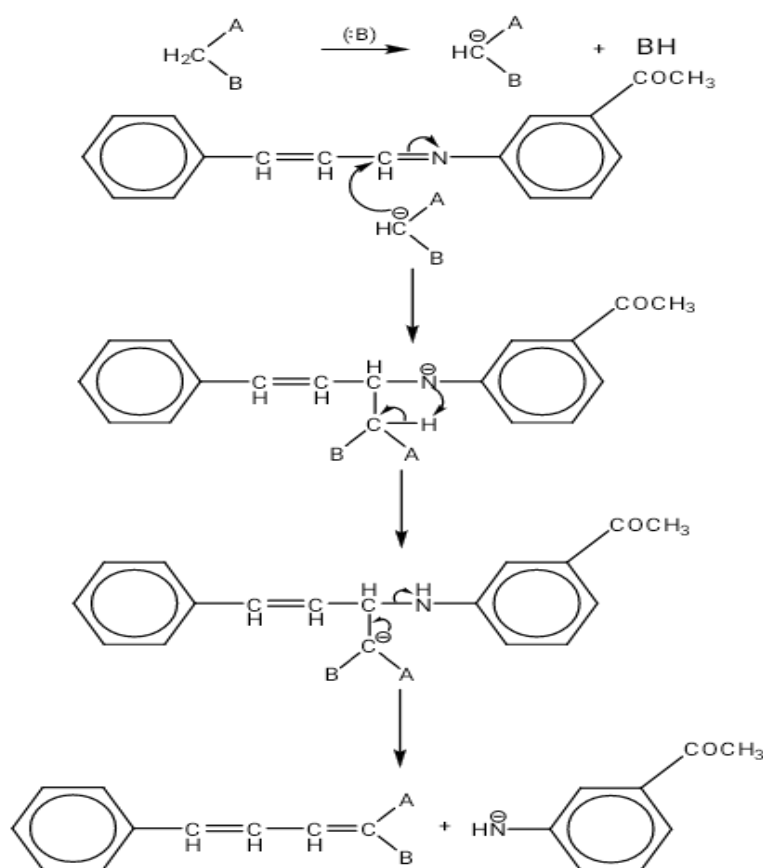
The synthetic compounds (Ia-IXa) were evaluated in vitro for antifungal activity versus two phytopathogenic fungi such as *H. oryzae* and *C. capsici* by spore germination inhibition technique¹. The results were expressed in terms of "ED₅₀ values".

Among the test compounds (Ia-IXa), all the compounds showed ED₅₀ values less than 1000 $\mu\text{g}/\text{ml}$ except compound IVa against *H. oryzae*. The ED₅₀ values of 22, 27 and 32 $\mu\text{g}/\text{ml}$ possessed by compound 2-cyano-5-phenylpenta -2,4-dienoic acid (Ia), ethyl 2-cyano-5-phenylpenta-2,4-dienoate (IIa) and 2-(3-phenylallyli dene)pentane-2,4-dione (VIIa) against the fungus were much lower than that of standard "fungicide Indofil M-45 (46 $\mu\text{g}/\text{ml}$)". Compounds 2-(3-phenylallylidene)malononitrile (IIIa), dimethyl 2-(3-phenylallyli dene)malonate (Va) and 2-(3-phenylallylidene)malonamide (VIIIa) possessed good potential with ED₅₀ values of 90, 75 and 80 $\mu\text{g}/\text{ml}$. Test compound VIa was also found to be quite effective with ED₅₀ value of 155 $\mu\text{g}/\text{ml}$. Poor antifungal activity was shown by (4-nitrobuta-1,3-dienyl)benzene (IXa) with ED₅₀ value of 625 $\mu\text{g}/\text{ml}$. Five test compounds were found to be active against test fungus *C. capsici*. Among these test compounds the compound 2-(3-phenylallylidene)malononitrile (IIIa) and (4-nitrobuta-1,3-dienyl)benzene (IXa) were found to possess promising activity against *C. capsici* with ED₅₀ value of 76 and 70 $\mu\text{g}/\text{ml}$ respectively. Moderate potential was shown by compound dimethyl 2-(3-phenyl allylidene)malonate (IVa) and 2-(3-phenylallylidene)pentane-2,4-dione (VIIa) with ED₅₀ values of 183 and 200 $\mu\text{g}/\text{ml}$. The compound IIa was possessed poor antifungal potential with ED₅₀ value of 675 $\mu\text{g}/\text{ml}$ (Table 2).

Conclusions

Active methylene compounds (I-IX) underwent chemoselective reaction with the carbon-nitrogen

double bond of 1-(3-(3-phenylallylideneamino)phenyl) ethanone. Under the provided reaction circumstances, this reaction preserved the carbon-oxygen double bond, which is thought to be more reactive, as well as the carbon-carbon double bond. This reaction produced mono-addition-elimination products (Ia-IXa). Interestingly, even when active methylene compounds were used in the condensation, the mono addition-elimination products (Ia-IXa) outperformed bis assault at both carbon-nitrogen and carbon-oxygen double bonds. Synthesized compounds were tested against *H. oryzae* and *C. capsici* for antifungal activity. The best compounds of the present study were 2-cyano-5-phenylpenta-2,4-dienoic acid (Ia), ethyl 2-cyano-5-phenylpenta-2,4-dienoate (IIa) and 2-(3-phenylallylidene)pentane-2,4-dione (VIIa) having ED₅₀ values of 22, 27 and 32 µg/ml lower than the ED₅₀ value of Indofil M-45 against *H. oryzae* and the most effective compounds against *C. capsici* were 2-(3-phenylallylidene)malononitrile (IIIa) and (4-nitrobuta-1,3-dienyl) benzene (IXa) with ED₅₀ value of 76 and 70 µg/ml respectively.

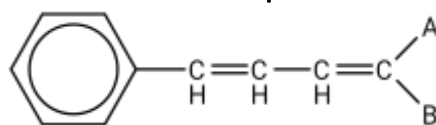


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Table 1. Physical characteristics and spectral data of diene derivatives



Compd	A	B	m.p. °C	Yield %	¹ H NMR spectrum of functional group (δ)	M ⁺ m/z
Ia	CN	COOH	207	52	-	199
IIa	CN	COOC ₂ H ₅	79	65	1.3 (t, 3H, COOCH ₂ CH ₃) 4.5 (q, 2H, COOCH ₂ CH ₃)	227
IIIa	CN	CN	121	80	-	180
IVa	COOCH ₃	COOCH ₃	116	55	3.8 (s, 6H, 2 x COOCH ₃)	246
Va	COOC ₂ H ₅	COOC ₂ H ₅	107	62	1.3 (t, 6H, 2 x COOCH ₂ CH ₃) 4.2 (q, 4H, 2 x COOCH ₂ CH ₃)	274
VIa	COCH ₃	COOC ₂ H ₅	80	58	2.3 (s, 3H, COCH ₃) 1.3 (t, 3H, COOCH ₂ CH ₃) 4.2 (q, 2H, COOCH ₂ CH ₃)	244
VIIa	COCH ₃	COCH ₃	103	51	2.3 (s, 6H, 2 x COCH ₃)	214
VIIIa	CONH ₂	CONH ₂	149	63	7.2 (s, 4H, 2 x CONH ₂)	216
IXa	NO ₂	H	127	67	7.14(d, 1H, 1 x H)	175

Table 2. Antifungal potential of 1-(3-(3-phenylallylideneamino)phenyl) ethanone derivatives

Compd	ED ₅₀ values (µg/ml) against	
	<i>H. oryzae</i>	<i>C. capsici</i>
Ia	22	*
IIa	27	675
IIIa	90	76
IVa	*	183
Va	75	*

Vla	155	*
VIIa	32	200
VIIIa	80	*
IXa	625	70
Indofil M-45**	46	30

*more than 1000 µg/ml

** Standard fungicide against *H. oryzae* and *C. capsici*.