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"SYNTHESIS, CHARACTERIZATION, BIOLOGICAL, AND IN-SILICO TOXICITY STUDIES OF CHALCONES: A COMPREHENSIVE INVESTIGATION"

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ABSTRACT

"Five chalcones were successfully synthesized and purified via column chromatography. Their quantitative and qualitative characteristics were determined using NMR and IR spectroscopy. Biological evaluations, including anti-cancer, anti-inflammatory, anti- microbial, anti-cholesterol, and toxicity studies, demonstrated promising results. These findings suggest that the synthesized chalcones have potential as candidates for drug development, particularly in the field of anticancer medication."

1. INTRODUCTION

"Chalcones, known for their simple yet effective structure, have been widely explored in drug discovery [1-2]. These compounds, belonging to the flavonoid family of secondary metabolites, have shown diverse biological activities against various infectious diseases, including malaria [3-4]. Chalcone derivatives bearing substituents such as phenyl, allyl, alkoxy, and hydroxyl groups have demonstrated notable antimalarial activity, attributed to their increased lipophilicity, a crucial property for effective antimalarial action [5-6]. Additionally, the enone group within the chalcone structure, positioned between the phenyl rings, plays a significant role in antimalarial activity by facilitating better binding to the active site of the parasite [7-8].

Given the high demand for chalcone compounds, which cannot be met solely through natural sources, synthetic methods have been employed. Chalcones serve as valuable precursors for the synthesis of various heterocyclic compounds [9]. Cyclization of chalcone yields heterocyclic compounds containing nitrogen-containing rings like pyrazoline and pyrimidine, exhibiting potential antimalarial activity [10].

The biological evaluation of the synthesized chalcones holds promise for the development of novel drugs.

The findings of this research contribute to understanding the structural characteristics and their applications in medicinal contexts."

This study focuses on synthesizing potential chalcone compounds (1L–5L) via the Claisen-Schmidt condensation. The most promising compound was subjected to further investigation using molecular docking techniques to elucidate its activity.

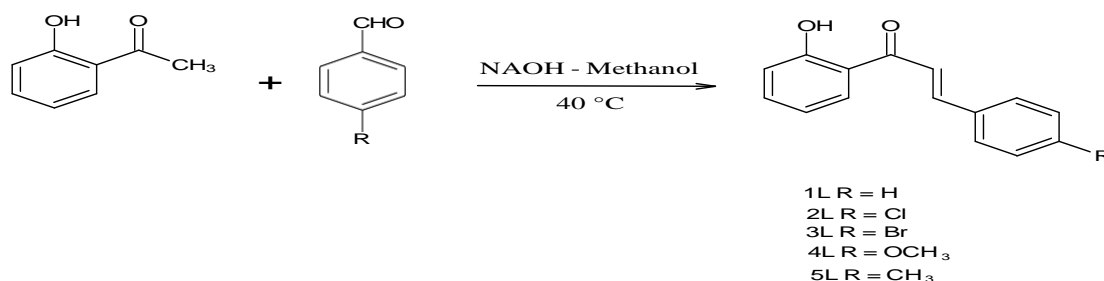
2. SYNTHESIS OF CHALCONES

2.1 Materials and methods

All chemicals utilized were of strictly analytical grade. Solvents underwent purification and drying procedures following established protocols [31]. Key chemicals, including 2-hydroxyacetophenone, benzaldehyde derivatives, and boron trifluoride etherate, were procured from Sigma Aldrich Laboratories."

2.2 General procedure for synthesis of chalcones (1L-5L):

The **chalcones (1L-5L)** were prepared by adding sodium hydroxide to the 2-hydroxyacetophenone and benzaldehyde derivatives in ethanol at 40°C. The mixture was stirred for 20 min and cooled to room temperature. The precipitate that formed was dissolved in water. The solution was made slightly acidic using dilute hydrochloric acid. The precipitate formed was filtered, washed with ice cold ethanol, and dried over calcium chloride to obtain crude chalcones. These were purified by column chromatography, eluting with a mixture of ethyl acetate and hexane (Scheme 1).



Scheme 1. Synthesis of chalcones (1L-5L)

3.0 Results and discussion

3.1 Physical measurements

The Electronic spectra were measured by using GBC UV-Vis double beam spectrophotometer in dichloro-methane solution in the 200-800 nm range. The FT-IR spectra were recorded on a Thermo Nicolet Avatar FT-IR spectrometer as Potassium Bromide powder in the frequency range 400–4000 cm^{-1} . The C, H and N contents were determined by Thermo-flash EA1112 series elemental analyzer. ^1H NMR and ^{19}F NMR spectra were recorded in Bruker AV 400 instrument.

3.1.1 , 2-Hydroxyphenyl)-3-phenyl-2-propen-1-one (1L): ^1H NMR (400 MHz, CDCl_3), [δ , ppm]: 12.79 (1H, s), 7.94-7.90 (3H, m), 7.68-7.64 (4H, m), 7.52-7.48 (1H, m), 7.45-7.42 (2H, m), 7.04-7.02 (1H, dd) and 6.96-6.92 (1H, m) (Figure 1). IR (KBr, cm^{-1}): 1635.5, 1565.8, 1199.4, 734.2 (Figure 2). Anal calcd for $\text{C}_{15}\text{H}_{12}\text{O}_2$, C 80.33%, H 5.35%. Found C 80.20 %, H 5.23%. UV-Vis: λ_{max} /nm (DCM) 317.48.

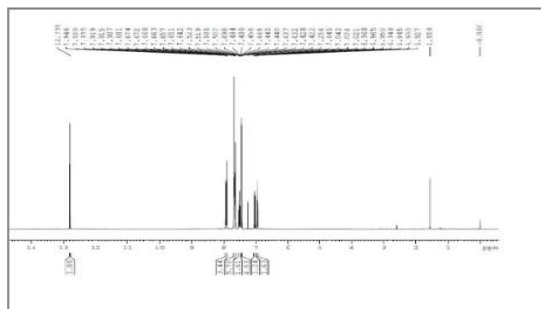


Figure 1. ^1H NMR spectra of ligand **1L**

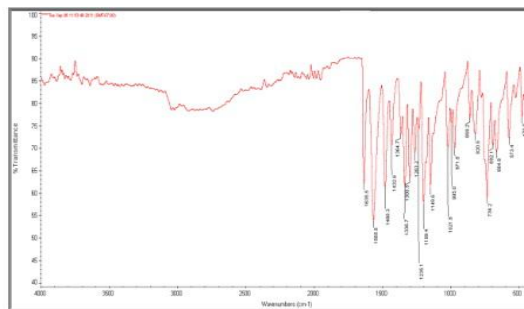
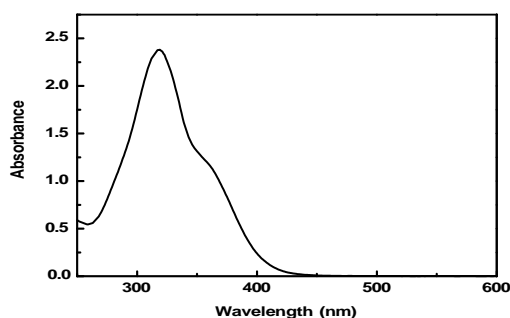


Figure 2. IR spectra of ligand **1L**



1. UV-Visible spectra of the ligand – 1L

3.1.2 , 2-Hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one (2L):¹HNMR (400 MHz, CDCl₃), [δ,ppm]: 12.72 (1H, s), 7.92-7.88 (1H, m), 7.84 (1H, s), 7.64 (1H, s), 7.61-7.58 (2H, m), 7.53-7.49 (1H,m), 7.43-7.40 (2H, m), 7.05-7.02 (1H, m) and 6.97-6.93 (1H, m) (figure 3). IR (KBr, cm⁻¹): 1636.9, 1561.8, 1201.6, 754.0 (figure 4). Anal calcd for C₁₅H₁₁ClO₂, C 69.63%, H 4.25%. Found C 69.43 %, H 4.18%. UV-Vis: λ_{max}/nm (DCM) 322.3.

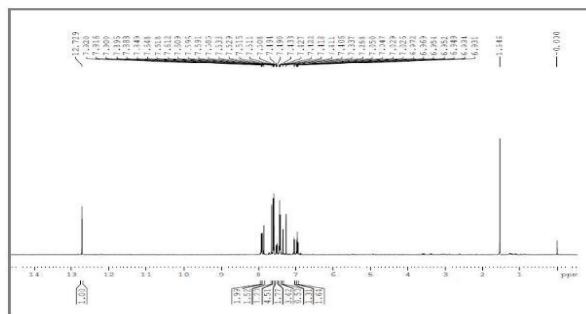


Figure 3. 1H NMR spectra of ligand **2L**.

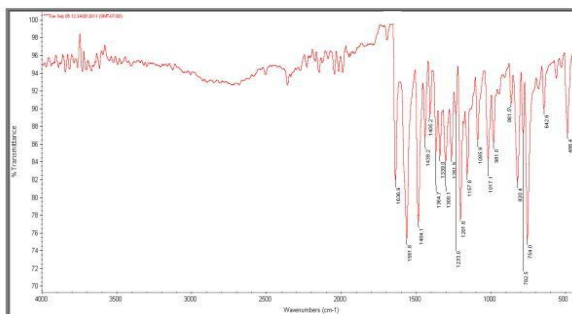
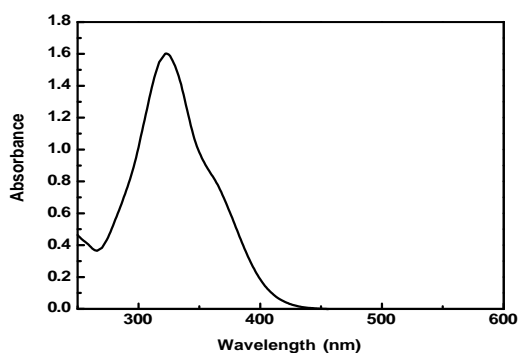


Figure 4. IR spectra of ligand **2L**.



UV-Visible spectra of the ligand - 2L

3.1.3 (2-Hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (3L)¹H NMR (400 MHz, CDCl₃), [δ, ppm]: 12.72 (1H, s), 7.91-7.89 (1H, dd), 7.87-7.83 (1H, d), 7.66-6.62 (1H, d), 7.59-7.49 (5H, m), 7.04-7.02 (1H, dd) and 6.97-6.93 (1H, m) (Figure 5). IR (KBr, cm⁻¹): 1639.3, 1561.6, 1201.8, 750.1 (figure 6). Anal calcd for C₁₅H₁₁BrO₂, C 59.42%, H 3.62%. Found C 59.23 %, H 3.40%. UV-Vis: λ_{max}/nm (DCM) 361.95.

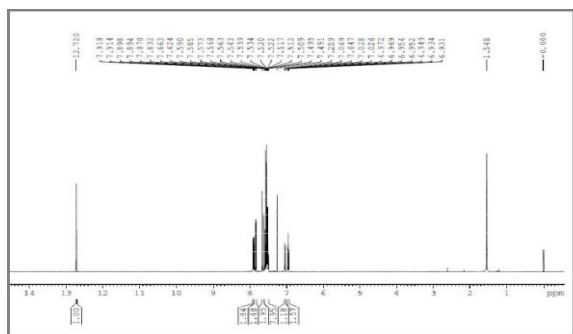


Figure 5. ¹H NMR spectra of ligand 3L.

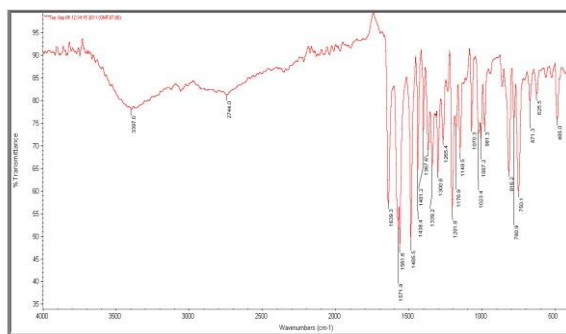
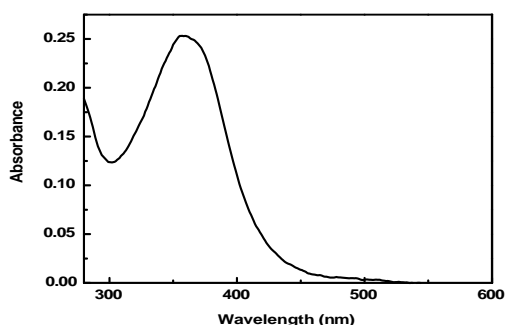


Figure 6. IR spectra of ligand 3L.



UV-Visible spectra of the ligand - 3L

3.1.4 , (2-Hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (4L):¹H NMR (400 MHz, CDCl₃), [δ,ppm]: 12.91 (1H, s), 7.93-7.88 (2H, m), 7.65-7.61 (2H, m), 7.56-7.52 (1H, d), 7.50-7.46 (1H, m), 7.03-7.01 (1H, m), 6.97-6.91 (3H, m) and 3.86 (3H, s) (Figure 7). IR (KBr, cm⁻¹): 1634.3, 1558.2, 1203.4, 757.3 (Figure 8). Anal calcd for C₁₅H₁₄O₃, C 75.57%, H 5.50%. Found C 75.32 %, H 5.22%. UV-Vis: λ_{max}/nm (DCM) 365.91.

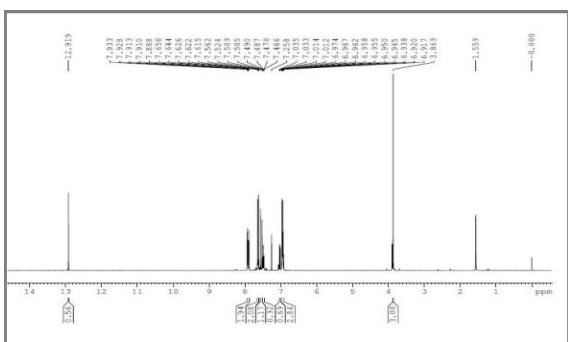


Figure 7. ¹H NMR spectra of ligand 4L.

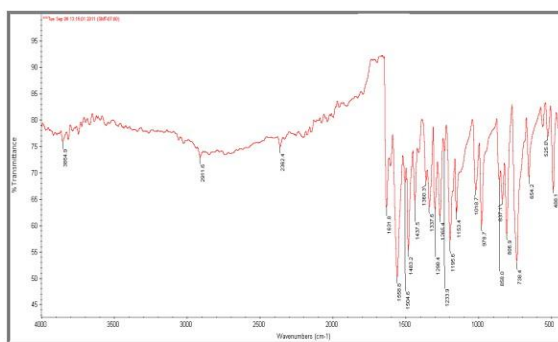
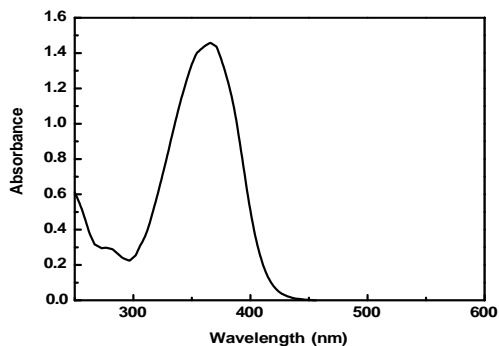


Figure 8. IR spectra of ligand 4L.



UV-Visible spectra of the ligand - 4L

3.1.5 , (2-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one (5L): ^1H NMR (400 MHz, CDCl_3), $[\delta, \text{ppm}]$: 12.85 (1H, s), 7.93-7.89 (2H, m), 7.64-7.60 (1H, d), 7.57-7.56 (2H, m), 7.51-7.47 (1H, m), 7.25-7.23 (2H, d), 7.03-7.01 (1H, s), 6.96-6.92 (1H, s) and 2.40 (3H, s) (Figure 9). IR (KBr, cm^{-1}): 1631.8, 1558.8, 1195.6, 738.4 (Figure 10). Anal calcd for $\text{C}_{15}\text{H}_{14}\text{O}_2$, C 80.64%, H 5.87%. Found C 80.62%, H 5.02%. UV-Vis: $\lambda_{\text{max}}/\text{nm}$ (DCM) 331.0.

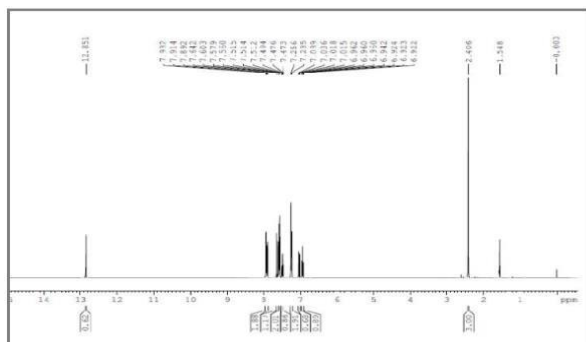


Figure 9. ^1H NMR spectra of ligand 5L

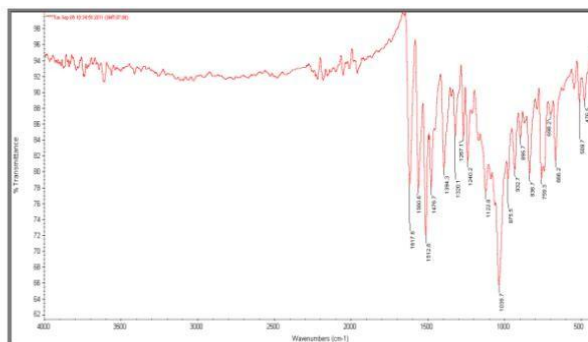
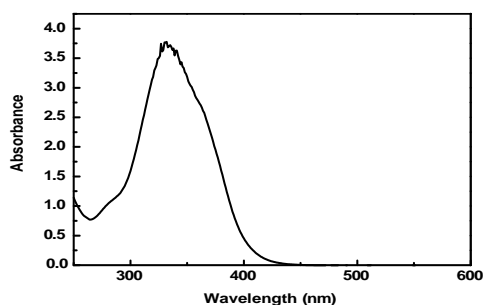


Figure 10. IR spectra of ligand 5L.



UV-Visible spectra of the ligand - 5L

4.0 Biological studies

In silico analysis of target protein based on lead molecule activity: The microbial, cancer, cholesterol esterase, fungal and inflammatory protein receptor structures were directly used for molecular docking. The 3D structure of the protein with the above origin were taken from RCSB PDB database; viz, 4B3Z with cancerous origin, 2LAO with cholesterol esterase origin, 2E5B with inflammatory origin, 2BRX with microbial origin, 4FPR with fungal origin and 2YT8 with neuronal origin were taken for this work.

Molecular docking studies: The docking simulation technique was considered as direct study on 3D structures of known functional characteristic proteins, which is a detailed study of intermolecular interaction with the ligands. The different functional characteristic of inflammatory, microbial and cancerous receptor-proteins was performed using PATCHDOCK server.

4.1 In silico analysis of target protein based on lead molecule activity: The selected protein structures are validated using Ramachandran Plot server at Uppsala University. Thus, stereo-chemical activity and quality were presented in table 1 [32]. The resultant overall modelled structures are potentially used for docking against the synthesized ligands [33].

Table 1. Ramachandran Plot Analysis

RCSB, PDB Data bases	Residues in Ramachandran Plot checked	In core regions (plus, signs)	Outlier
4B3Z	1709	1674	35
2LAO	217	212	5
2E5B	852	837	15
2BRX	387	374	13
4FPR	479	469	10
2YT8	1600	1533	67

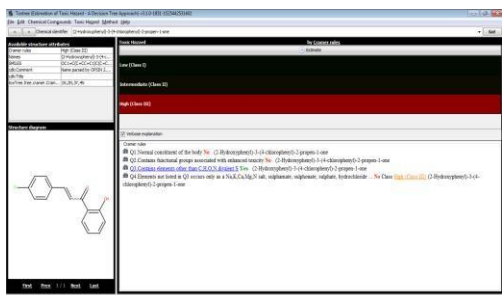
4.1.1 Molecular docking studies:

The different functional characteristic of inflammatory, microbial, and cancerous receptor-proteins was performed using PATCHDOCK server [34, 35]. The different functional characteristic of inflammatory, microbial, and cancerous receptor-proteins was performed using PATCHDOCK server [9]. The energy values are given in the above table 2.

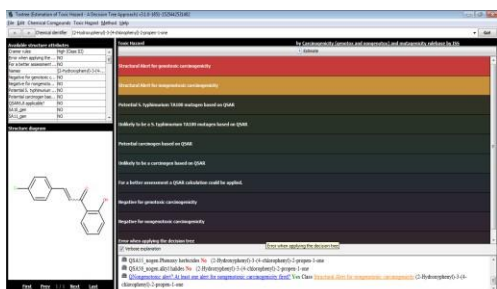
Table 2. Docking analysis

2LAO		Chalocones	Docking Score	2LAO	Complexes	Docking Score
			(Kcal/mol)			(Kcal/mol)
	Cholesterol	1L	3962	I. 2E5B	1L	5010
	Esterase	2L	4366	Inflammatory	2L	5136
		3L	4130	II.	3L	4824
		4L	4378		4L	5248
III.	4B3Z	5L	4334	2YT8	5L	5086
		1L	4546		1L	3782
	Cancerous	2L	4854	IV. NEURONAL	2L	3850
		3L	4564		3L	3742
	V.	4L	4836		4L	3970
		5L	4842	VI.	5L	3854
	2BRX	1L	4322	VII. 4FPR	1L	4696
VIII.	MICROBIAL	2L	4492	Fungal	2L	4866
		3L	4292	IX.	3L	4820
		4L	4650		4L	5194
	X.	5L	5238		5L	5000

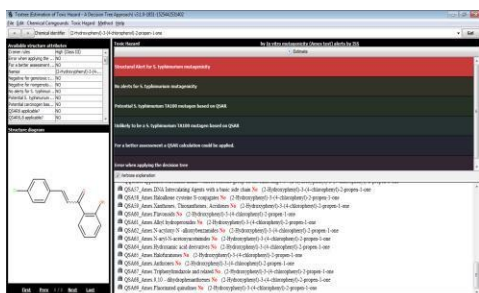
4.2.2 (2-Hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one



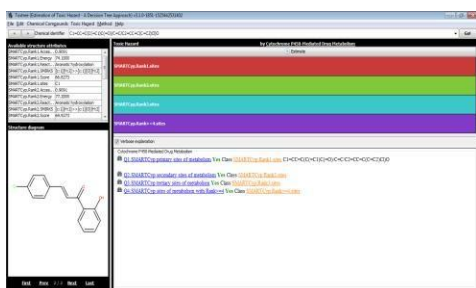
High class 3 toxicity



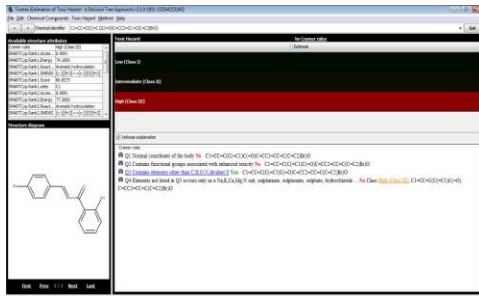
Structural alert for genotoxic carcinogenicity



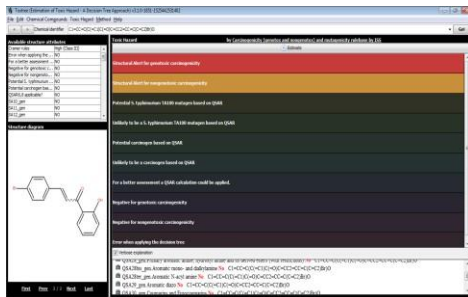
Structural alert for s. typhimurium mutagenicity



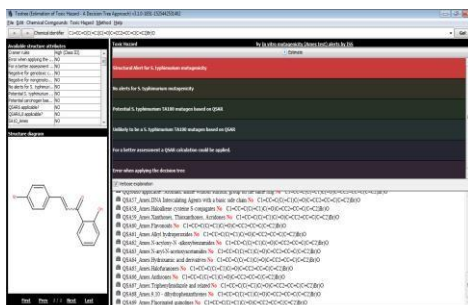
4.2.3 (2-Hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one



High class 3 toxicity



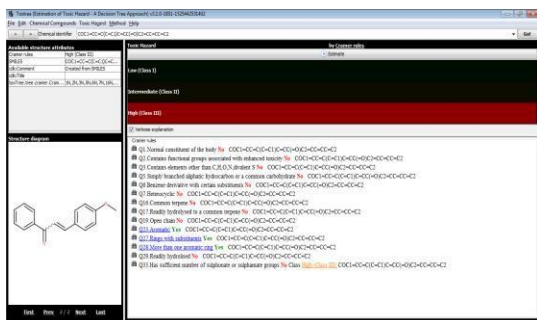
Structural alert for genotoxic and non-genotoxic mutagenicity



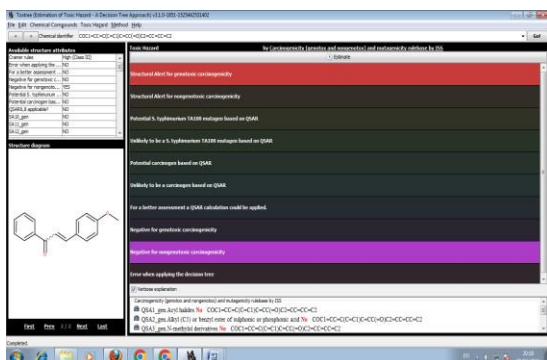
Structural alert for s. typhimurium mutagenicity



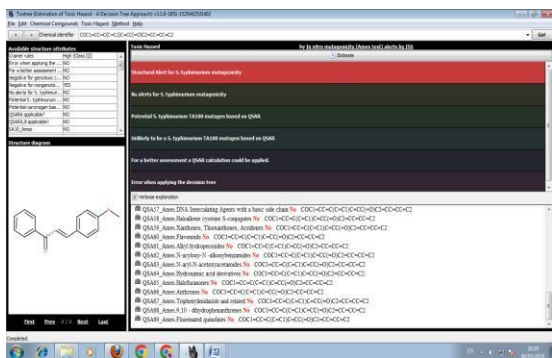
4.2.5 (2-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one



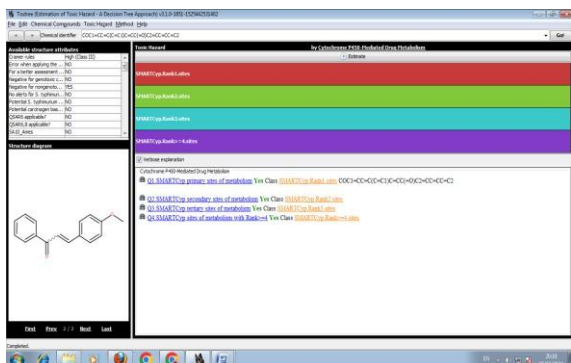
High class 3 toxicity



Structural alert for genotoxic mutagenicity



Structural alert for s. typhimurum mutagenicity



Toxicity Report:

The toxicity studies have been conducted using the Toxtree version 3 for analysis.

According to this software all the ligands are toxic to humans, but 1st, 2nd, 4th and 5th chalcones mentioned below are less toxic...

1. (2-Hydroxyphenyl)-3-phenyl-2-propen-1-one (1L):
2. (2-Hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one (2L):
3. (2-Hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (4L):
4. (2-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one (5L):

CONCLUSION

- The chalcones, synthesized are purified by column chromatography.
- All the synthesized chalcones were coloured.
- The elementary analysis was done by NMR, IR and UV.
- They were found to be soluble in CH₃OH, CH₃CN, C₆H₆, DMSO, DMF and CHCl₃.
- The biological activities are showing that, the chalcones are showing Anti-cancer, Anti- microbial and Anti-inflammatory, Anti-fungal, Anti-cholesterol, Anti neural properties, the results are mentioned in the above table.
- This present work is helpful in various new drug synthesis and reference for new complex formations in coordination chemistry.

References:

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