PHARMACOPHORE DESIGNING, *IN-SILICO* METABOLISM AND TOXICITY STUDIES OF CHALCONE SEMICARBAZONES

Manmohan Singhal*[1], Arindam Paul[2], Hemendra Pratap Singh[3]

[*1]School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India
[2]GD Memorial College of Pharmacy, Jodhpur, Rajasthan, India
[3]B N College of Pharmacy, Udaipur, Rajasthan, India

Summary

In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model 'chalconesemicarbazone' which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc which may help in binding with receptors and plays an important role in pharmacological activities.. The pharmacophore of the synthesized compound was developed by using ligandscout 2.02 software by minimizing energy with MM3 force field. The possible metabolites and the toxicity of some selected synthesized chalconesemicarbazones were predicted by computational method using Pallas version-3.1 ADME-Tox prediction (metabolism prediction by Mexalert/RetroMex and toxicity prediction by Hazardexpert/ ToxAlert) software. Compounds 15, 26 and 28 have high probability of toxicity. The major pathway of metabolism was found to be p-hydroxylation and amide hydrolysis.

Keywords: Chalcones, Pharmacophore, Semicarbazones, Ligandscout, Pallas

For correspondence:
Email : manu_mpharm@yahoomail.com
Phone no: +91-9829153193

Introduction

Computational chemistry is a branch of chemistry that uses computers to assist in solving chemical problems. It uses the results of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of molecules and solids. While its results normally complement the information obtained by chemical experiments, it can in some cases predict unobserved chemical phenomena. It is widely used in the design of new drugs. Examples of such properties are structure (i.e. the expected positions of the constituent atoms), absolute and relative (interaction) energies, electronic charge distributions, dipoles and higher multipole moments, vibrational frequencies, reactivity or other spectroscopic quantities, and cross sections for collision with other particles[1,2].
Several major areas may be distinguished within computational chemistry:

- The prediction of the molecular structure of molecules by the use of the simulation of forces, or more accurate quantum chemical methods, to find stationary points on the energy surface as the position of the nuclei is varied.
- Storing and searching for data on chemical entities.
- Identifying correlations between chemical structures and properties (QSPR and QSAR).
- Computational approaches to help in the efficient synthesis of compounds.
- Computational approaches to design molecules that interact in specific ways with other molecules (e.g. drug design and catalysis).
- In pharmacophore identification.
- To predict *in silico* toxicity and metabolism prediction to refine a drug molecule towards its biological activity.
- In virtual screening of the new compounds.

To become an optimal drug, in addition to potency and selectivity, a compound must have appropriate ADME (absorption, distribution, metabolism, and excretion), safety, and developability characteristics. Relying solely on potency in the early stages of drug discovery can result in disproportionate attrition after clinical candidate selection contributing to the exorbitant costs of discovering and developing drugs. Only about one in ten of those diligently chosen, highly potent, and selective candidates that enter development reach the market often due to inadequate ADME properties [3].

Therefore, it is extremely important to consider the ADME characteristics of compounds earlier in the discovery process to wager bets on compounds that have a greater potential to survive the development and clinical trial stages of drug development. Increasing the odds of success to one in five (instead of ten) would reduce the total cost of bringing a new therapeutic to the market by 33% [4]. Experimental determination of ADME and pharmacokinetic (PK) characteristics is both expensive and time consuming, and is not practical for large numbers of compounds, especially when the pharmaceutical industry is under severe pressure to cut costs and improve efficiency. The price tag to support various ongoing discovery projects in a pharmaceutical company for synthesis and high throughput measurement of permeability, solubility, metabolic stability, and acute toxicity can run into millions of dollars at the rate of $5,000 - $10,000 per compound [5].

Much attention is being focused on the application of *in silico* screens to reliably predict ADME attributes solely from molecular structure. *In silico* prediction of ADME properties will not only reduce costs and development cycle times by wisely directing resources to essential experimental testing, but also bring forward their consideration earlier at the lead generation stage when compounds are being synthesized and tested

almost exclusively to meet pharmacological target potency levels. At the cost of experimental results indicated above, a mere 10-20% reduction in high throughput experimental measurement of permeability, solubility, metabolic stability, and acute toxicity through the use of *in silico* screens can lead to significant savings. Further, application of *in silico* screens offers an ideal 'fail-early-fail-cheaply' strategy for drug discovery because their application requires nothing more than inputting the basic structural information of a compound into a validated model.

Attrition during the drug development process is a serious economic problem for the pharmaceutical industry and it is often due to inappropriate ADME/Tox characteristics [6,7]. It has been estimated that 20-40% of the drug failures in investigational drug development phases are due to safety issues, not counting multiple incidents of adverse effects of existing drugs. The early drug discovery process needs to address in parallel not only potency but also pharmacokinetics and toxicological properties [8].

Materials and methods

A series of chalconesemicarbazones was synthesized, characterized and evaluated for pharmacological activities [9]. Structure and Physicochemical properties of the synthesized compounds are given in figure 1 and table 1.

The pharmacophore of the synthesized compound was developed by using ligandscout 2.02 software by minimizing energy with MM3 force field.

The possible metabolites and the toxicity of some selected synthesized chalconesemicarbazones were predicted by computational method using Pallas version-3.1 ADME-Tox prediction (metabolism prediction by Mexalert/RetroMex and toxicity prediction by Hazardexpert/ ToxAlert) software.

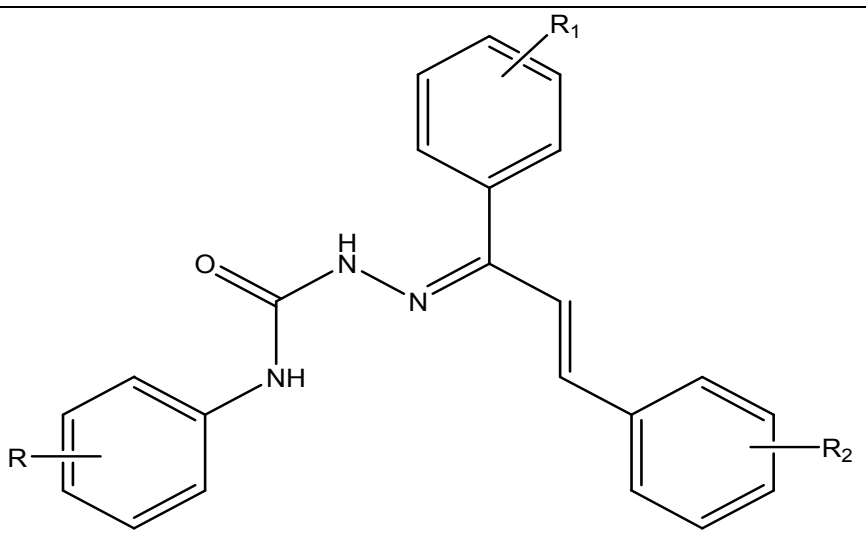

Figure 1: Structure of synthesized title compounds

Table 1: Physicochemical data of methyl semicarbazones

Comp no.	R	R_1	R_2	Yield (%)	Mol Wt.	Mol Formula	mp (ºC)	Rf value
4	2-CH_3	H	H	57	371	$C_{23}H_{21}N_3O_2$	150	0.78
5	2-CH_3	H	4”-OH	66	387	$C_{23}H_{21}N_3O_3$	145	0.71
7	2-CH_3	H	4”-$N(CH_3)_2$	58	414	$C_{25}H_{26}N_4O_2$	148	0.57
11	2-CH_3	5-OH	6”-OH	61	403	$C_{23}H_{21}N_3O_4$	135	0.63
15	4-CH_3	H	4”-OH	65	387	$C_{23}H_{21}N_3O_3$	188	0.63
24	2-CH_3	H	p-Cl	65	389.88	$C_{23}H_{20}ClN_3O$	115	0.49
25	o-CH_3	H	Cinnameldehyde	73	381.47	$C_{25}H_{23}N_3O$	126	0.51
26	o-CH_3	p-NH_2	p-Cl	61	404.89	$C_{23}H_{21}ClN_4O$	192	0.73
28	p-CH_3	p-NH_2	p-Cl	63	404.89	$C_{23}H_{21}ClN_4O$	173	0.72

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide **(4):**
1H-NMR (δ/ppm in $CDCl_3$): 2.12 (s, 3H, Ar-CH_3), 4.83 (s, 1H, 2-OH), 7.11-7.64 (m, J= 8.32 Hz, 12H, Ar-H) 7.7 (s, 1H, –CH=CH–), 7.9 (s, 1H, –CH=CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3480(–OH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 370; Elemental analysis calculated/found (%) C (74.37/74.26), H (5.70/5.48), N (11.31/11.12).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide **(5)**
1H-NMR (δ/ppm in $CDCl_3$): 2.18 (s, 3H, Ar-CH_3), 4.9 (s, 1H, 2-OH), 5.2 (s, 1H, 4-OH), 7.3-7.64 (m, J= 8.4 Hz, 11H, Ar-H) 7.8 (s, 1H, –CH=CH–), 8.0 (s, 1H, –CH=CH–), 8.44 (s, 1H, ArNH, D_2O exchangeable), 9.8 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3455 (NH), 3475(–OH), 3310–3245 (CONH), 1675 (–CH=CH–),1594 (C-N), 1615, 1556 (aromatic), 750, 695 (monosubstituted benzene); MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.24), H (5.46/5.35), N (10.85/10.47).

1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) semicarbazide **(11)**
1H-NMR (δ /ppm in $CDCl_3$): 2.24 (s, 3H, Ar-CH_3), 5.1 (s, 1H, 2-OH), 5.3 (s, 1H, 2, 4-OH), 7.2-7.78 (m, J= 8.35 Hz, 11H, Ar-H) ,7.8 (s, 1H, –CH=CH–), 8.2 (s, 1H, –CH=CH–), 8.78 (s, 1H, ArNH, D_2O exchangeable), 9.84 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3462 (NH), 3488(–OH), 3300–3240 (CONH), 1666 (–CH=CH–),1593 (C-N), 1618, 1554 (aromatic), 753, 694 (monosubstituted benzene); MS, m/z 386; Elemental analysis cal/fou (%) C (71.30/71.17), H (5.46/5.37), N (10.85/10.66).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide **(15)**
^{1}H-NMR (δ/ppm in $CDCl_3$): 2.17 (s, 3H, Ar-CH_3), 4.91 (s, 1H, 2-OH), 5.3 (s, 1H, 4-OH), 7.3-7.68 (m, J= 8.32 Hz, 11H, Ar-H) 7.79 (s, 1H, –CH=CH–), 8.1 (s, 1H, –CH=CH–), 8.42 (s, 1H, ArNH, D_2O exchangeable), 9.85 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3449 (NH), 3471(–OH), 3318–3245 (CONH), 1676 (–CH=CH–),1593 (C-N), 1618, 1559 (aromatic), 751, 696 (monosubstituted benzene); MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.25), H (5.46/5.33), N (10.85/10.58).

1-(1,5-diphenylpenta-2,4-dienylidene)-4-o-tolylsemicarbazide **(25)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 15H, Ar-H), 7.69 (s, 1H, –CH=CH–), 7.72 (s, 1H, –CH=CH–), 7.88-8.12 (dd, 2H, –CH=CH–), 8.34 (s, 1H, ArNH), 9.42 (s, 1H, CONH); IR (KBr/cm^{-1}): 3450 (NH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 380; Elemental analysis calculated/found (%) C (78.71/78.56), H (6.08/5.98), N (11.02/10.92).

1-[1-{4-aminophenyl-3-(4-chlorophenyl)}allylidene]-4-o-tolylsemicarbazide **(26)**
1H-NMR (δ/ppm in $CDCl_3$): 6.52 (s, 2H, NH_2), 7.10-7.65 (m, 13H, Ar-H), 7.72 (s, 1H, –CH=CH–), 7.94 (s, 1H, –CH=CH–), 8.32 (s, 1H, ArNH), 9.46 (s, 1H, CONH); IR (KBr/cm^{-1}): 3452 (NH), 3300–3246 (CONH), 1678 (–CH=CH–),1597 (C-N), 1626, 1567 (aromatic), 872 (Cl), 755, 697 (monosubstituted benzene); MS, m/z 403; Elemental analysis calculated/found (%) C (68.23/67.96), H (5.23/5.17), N (13.84/13.75).

Result and discussion

Pharmacophore Designing

In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model ‘chalconesemicarbazone’, which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc (figure 2), which may help in binding with receptors and plays an important role in pharmacological activities.

On these observations, we have designed a synthetic scheme to synthesize this pharmacophore, and also synthesize some lead compounds.

The pharmacophore of the synthesized compound was developed by using ligandscout 2.02 software by minimizing energy with MM3 force field.

The ligand scout application was initialized/started by double click on the icon. In the File menu (appeared at main window of ligand scout), the molecule to be analyzed was imported/opened whose structure was already saved in .mol file format, then with the help of molecule menu (appeared at main window of ligand scout) molecule was subjected for energy minimization. Form the *pharmacophore* menu, the desire p*harmacophore was* created and saved.

Table 2: Symbols showing pharmacophoric features in ligandscout

Depiction in LigandScout	*Pharmacophore Feature*
	Hydrogen Bond Donor
	Hydrogen Bond Acceptor
	Positive Ionizable Area
	Negative Ionizable Area
	Hydrophobic Interactions
	Aromatic Ring
	Metal Binding Feature
	Excluded Volume

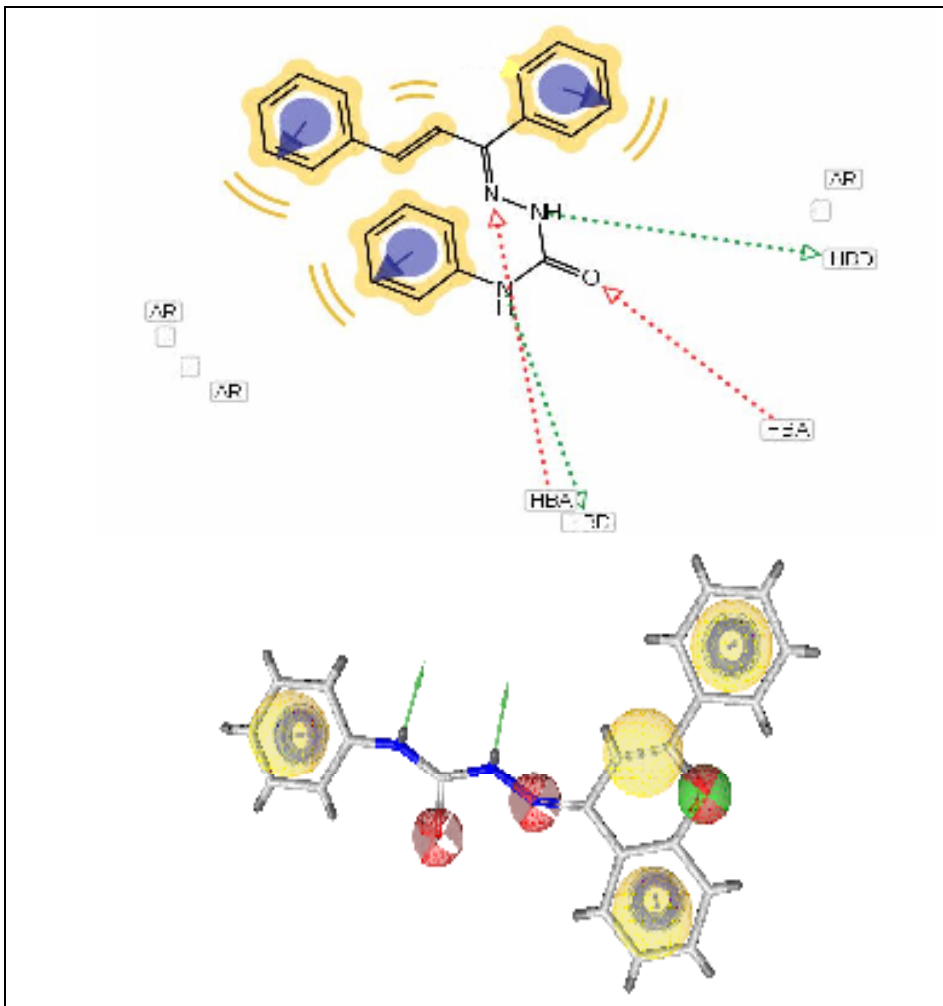

Figure 2: Pharmacophoric design of compound by ligandscout 2.02

In-Silico Toxicity Prediction/ Metabolism Prediction

The possible metabolites and the toxicity of some selected synthesized compounds were predicted by computational method using Pallas version 3.1 ADME-Tox prediction software and pentium IV processor.

The application was initialized/started by double click on the icon. In the New menu (appeared at main window of Pallas), the molecule to be analyzed was drawn by work sheet of Pallas, then with the help of select menu, molecule was subjected for prediction(option) of metabolism by Mexalert/RetroMex and toxicity by Hazardexpert/ ToxAlert respectively and noted.

Table 3: *In-silico* toxicity prediction of some selected compounds by Pallas 3.1 software

Compound	Toxicity	Overall toxicity	Oncogenicity	Mutagenicity	Teratogenicity	Irritability	Sensitivity	Immunotoxicity	Neurotoxicity
4	Not probable	17	0	0	17	0	0	0	0
5	Probable	53	0	29	17	53	0	0	29
7	Not probable	17	0	0	17	0	0	0	0
11	Not probable	15	0	0	15	0	0	0	0
15	Probable	53	0	29	17	53	0	0	29
24	Not probable	18	0	0	18	0	0	0	0
26	High probable	64	64	51	19	0	29	0	0
28	High probable	64	64	51	19	53	29	0	0

In-Silico toxicity prediction of the synthesized compounds is given in table 3 which shows that compounds 15, 26 and 28 have high probability of toxicity while compounds 4, 5, 7,11 and 24 have minimal probability of toxicity.

In-Silico metabolism prediction of the synthesized compounds is given in table 4 and figure 3. The major pathway of metabolism was found to be p-hydroxylation and amide hydrolysis however in some compounds glucuronide and sulfate conjugation may also occur.

Table 4: *In-silico* metabolism prediction of some selected compounds by Pallas 3.1 software

Com Code	Alert	count	Reactions				
			P-hydroxylation	Amide Hydrolysis	Phenol-sulphate Congugation	Formation of O-glucuronide	Formation of N-glucuronide
4	Prob.	5	3	3	-	-	-
5	Prob.	8	4	2	1	1	-
7	Prob.	4	2	2	-	-	-
11	Prob.	4	3	2	-	-	-
15	Prob.	7	3	2	1	1	-
24	Prob.	4	2	2	-	-	-
25	Prob.	5	3	2	-	-	-
26	Prob.	4	1	2	-	-	1
28	Prob.	3	-	2	-	-	1

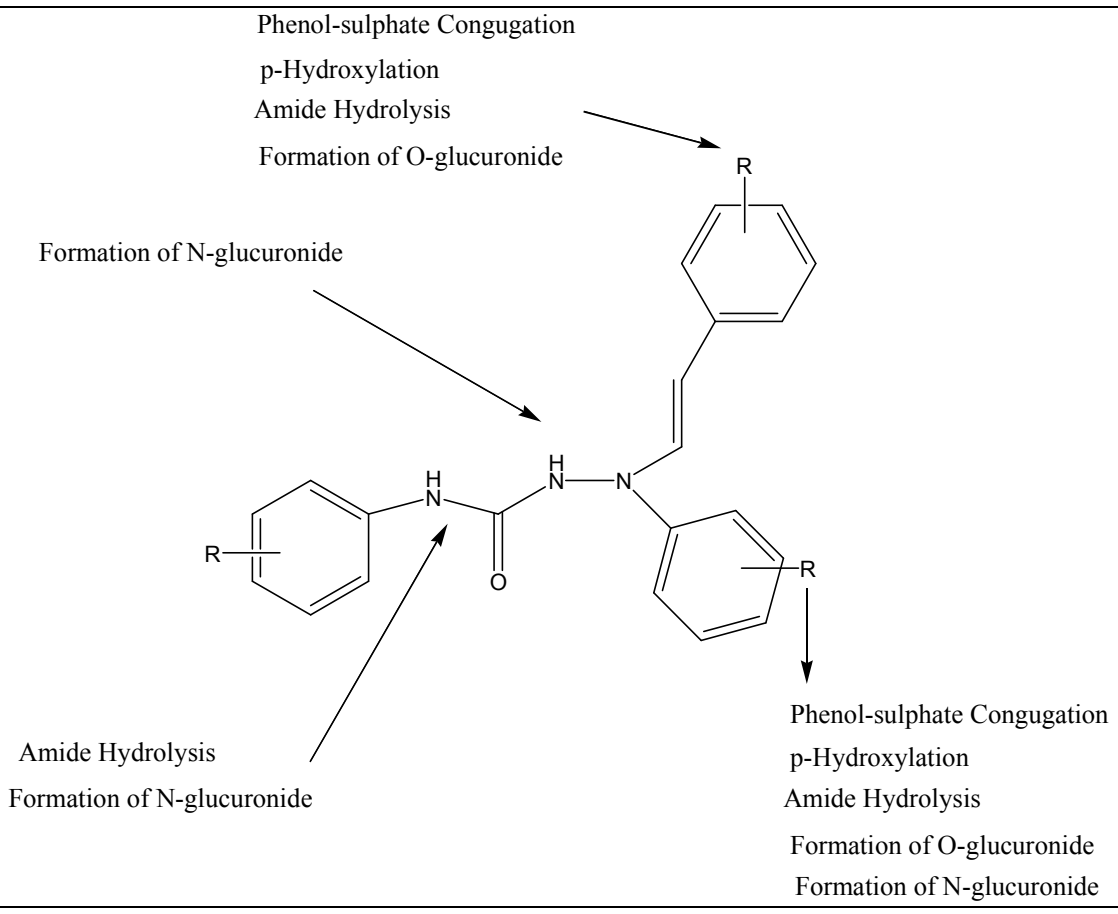

Figure 3: *In Silico m*etabolism of chalconesemicarbazone derivatives

Conclusion

In the present study by using ligandscout 2.02 software, we have designed a pharmacophore model 'chalconesemicarbazone' which is having hydrogen acceptor site, hydrogen donor site, lipophilic site etc which may help in binding with receptors and plays an important role in pharmacological activities. On these observations, we have designed a synthetic scheme to synthesize this pharmacophore, and also synthesize some lead compounds.

The possible metabolites and the toxicity of some selected synthesized chalconesemicarbazones were predicted by computational method using Pallas version-3.1 ADME-Tox prediction (metabolism prediction by Mexalert/RetroMex and toxicity prediction by Hazardexpert/ ToxAlert) software. Compounds 4, 7, 11 and 24 were found to be least toxic while compounds 26 and 28 were found to be highly toxic. Mostly compounds were metabolized mainly by P-hydroxylation and amide hydrolysis.

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