Rational design, synthesis and computational structure-activity relationship of novel 3-(4-chlorophenyl)-5-(3-hydroxy-4-ethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide

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Abstract
Densely functionalized 3-(4-chlorophenyl)-5-(3-hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide was synthesized in an expedient manner through specification and transamidation respectively, of ester-functionalized pyrazoles. This synthetic protocol allowed for three diversifying steps in which appendages on the pyrazole scaffold were adjusted to optimize inhibition of protein kinases. Computational design and study of novel 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide is reported. This computational prediction analysis will improve the understanding of candidate drugs and help in identifying its properties and effects on the human body. Simulation analysis of candidate drugs is necessary for providing clues about regulatory mechanisms, biochemical pathways and broader drug functions.
Keywords: Pyrazole carboxamide; CADD; Rule of 5; ADMET; Toxicity; Physiochemical properties

INTRODUCTION

Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms at adjacent positions and to the unsubstituted parent compound. Being so composed and having pharmacological effects on humans, they are classified as alkaloids. In medicine, pyrazoles are used for their analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamine oxidase inhibiting and antidiabetic. In organic chemistry carboxamides (or amino carbonyls) are functional groups with the general structure R-CO-NH_2, where R is an organic substituent (Wikipedia, 2008).

The dihydropyrazoles are important in the treatment and prophylaxis of anemia associated with kidney disease, as a combination therapy with chemotherapy, in preparation for autologous blood donation, and other cases of chronic anemia (Lange *et al*., 2004). These molecules can also be important for the treatment of ischemic heart disease, for treating peripheral vascular disease and for the enhancement of wound healing. Other uses of these compounds include: neuroprotection in cerebral ischemic conditions; and reducing or preventing hypoxia related disorders of cerebral, coronary or peripheral circulation (Srivastava *et al*., 2007).

Pyrazole carboxamide derivatives, pharmaceutical compositions containing them and their preparation are also used in the treatment of central nervous system disorders and affective conditions. More particularly, neuropeptide Y Y5 (NPY5) receptor is associat-

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ed with such disorders and conditions (Kordik *et al*., 2000). The pyrazole carboxamides are also used as medicaments, especially (oral) medicaments for the treatment of anemias. These carboxamides stimulate the Erythropoietin production (EPO) (erythropoiesis) using own blood donors (Kordik *et al*., 2000). The design processes of drugs can be streamlined by focusing on "drug-like" molecules. As a first step, it is necessary to identify biologically and pharmacologically relevant properties which are easily computable from the structure. Hence, it will be instructive to analyze the physicochemical, topological, and electronic properties of all known drugs and compare the properties of different classes of drugs (Stoltefuss *et al*., 2000).

In this paper the results of large-scale theoretical calculations were used for the study of the lipophilicity, solubility, absorption, polar surface area, solubility, bioavailability, partition coefficient, volume of distribution, gastro intestinal absorption, clearance and toxicity. Drug plasma-protein binding which is one of the many factors which influences bioavailability of a drug has been calculated and P-glycoprotein which plays a role in the protection of the organism against potentially toxic substances has also been calculated.

MATERIALS AND METHODS

Hartree-Fock calculations were performed using the Spartan' 06 program (Spartan, 2006). At the B3LYP (Becke, 1993), levels of theory with 6-31G** basis set (set of functions used to create the molecular orbitals and adds polarization functions to hydrogens to improve the total energy of the system) (Hehre *et al*., 1989). The compounds were built with standard bond lengths and angles using the PC SPARTAN Pro Ver 1.08 molecular modeling program. The molecular mechanical methods followed by the Hartree-Fock method at 6-31G** level were used to minimize energy of candidate compound. Molecular modeling and determination of molecular properties of drug structures were accomplished by Chem-Sketch, Molinspiration (Molinspiration, 2008) and MolSoft (Molsoft, 2007) softwares. Solubility, Log Kow, and dermal permeation coefficient were determined by the EpiSuite software (AllidSystems, Sylmar, CA). Drug likeness was determined by methods of Actelion and MolSoft (Actelion Ltd, 2007). Values of pKa were determined by using SPARC On-Line calculator for properties (Version August 2003, University of Georgia, Athens, GA, www.sparc.chem.uga.edu).

Molecular Modeling: In order to obtain the most stable conformation, a combination of molecular mechanics and quantum chemical calculations at the semi-empirical level were used. Structure of the molecule (Fig. 1) was built by HyperChem (Hyperchem Package, 2008) Release 8 for Windows (Hypercube Inc. Gainesville, Florida) using a molecular mechanics procedure under MM+ (Molecular mechanics) (Allinger, 1977). The geometry was optimized to an rms (root mean square) gradient of 0.001 in vacuo (Polak-Ribière method). Then a molecular dynamics program was run for 1 ps, with 0.001 ps steps and a relaxation time of 0.1 ps, at a simulation temperature of 300 K. This was followed by MM+ geometry optimization to an rms gradient of 0.2. The molecular dynamics run was repeated and a further MM+ protocol was carried out to a gradient of rms 0.004 on the selected drug. Angles and bond-lengths were measured on the models. Dipole moments were determined using the semi-empirical PM3 program (Stewart, 1989) by the singly-excited configuration interaction. The vibrational IR spectra were calculated using HYPER CHEM program at the B3LYP (Becke, 1993) levels of the theory with 6-31G** basis set. This method was employed to determine structural and electronic parameters, which were to be correlated with the psychoactivity. These parameters include bond distances, torsion angles, bond orders, ionization potential, energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) frontier orbital, etc. (Honorio *et al*., 2005).

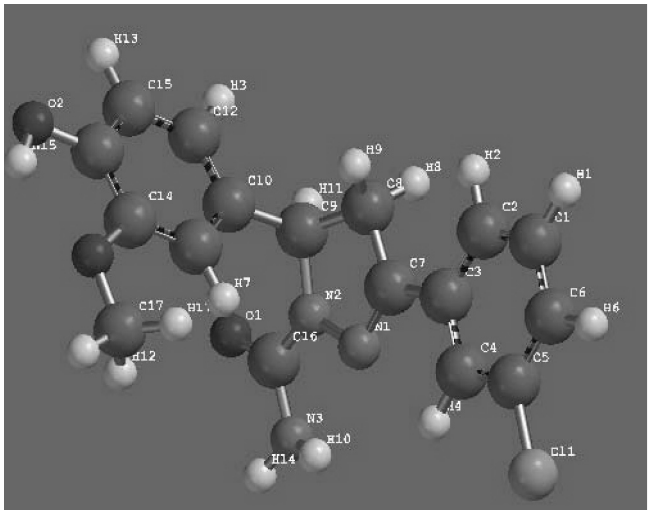


Figure 1. Structure of 3-(4-chlorophenyl)-5-(3-hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide.

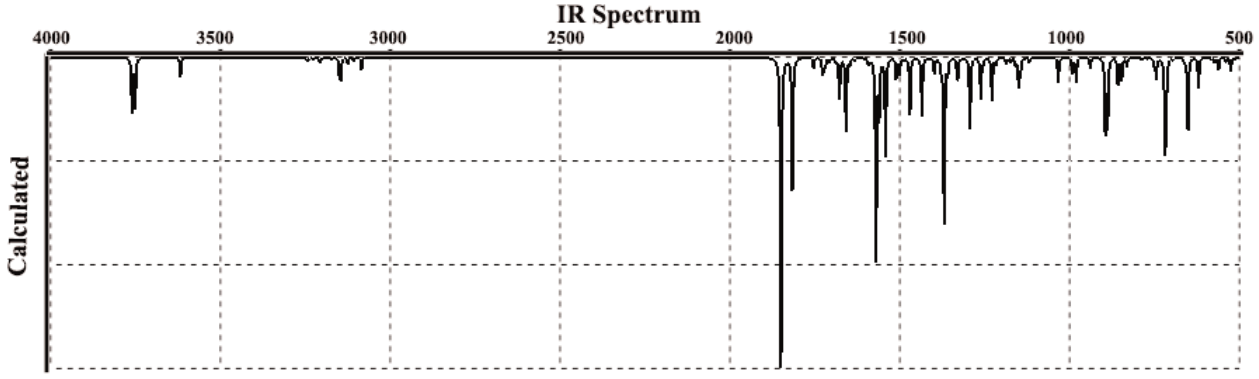


Figure 2. Theoretical Infra Red (IR) spectra of 3-(4-chlorophenyl)-5-(3-hydroxy-4-etoxyphenyl)-4, 5-dihydro-1H-pyrazole-1-carboxamide. (wavenumber (X-axis) vs percent transmittance (Y-axis).

RESULTS

Molecular mechanics and Infrared spectrum: The peak wavelengths and strengths of the calculated fourier transform infra red (FT-IR) absorption spectrum were mostly comparable with the observed spectrum (Fig. 2). The spectra exhibit a great number of bands, which is mainly due to the low symmetry of this molecule. The more intense bands were observed between 1700-1500 cm^{-1}, which is the usual region of stretching vibrations. According to the theoretical calculations, the candidate molecule has a planar structure of the point group symmetry (Cs). The observed FT-IR are shown in Fig. 3. Comparison of the frequencies graph at the MM+ and B3LYP levels (Fig. 2) with experimental graph (Fig. 3) reveals the over estimation of the calculated vibrational modes due to neglect of anharmonicity in the real system. Inclusion of electron

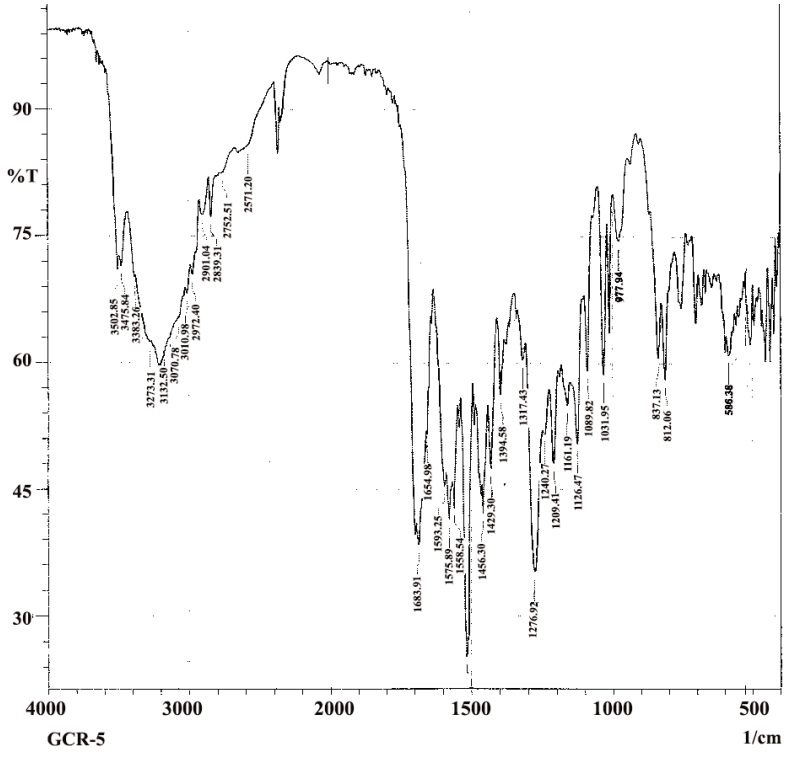


Figure 3. Experimental Infra Red (IR) spectra of 3-(4-chlorophenyl)-5-(3-hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide. (wavenumber (X-axis) vs percent transmittance (Y-axis).

correlation in density functional theory, to a certain extent, makes the frequency values smaller in comparison with the MM+ frequency data. Notwithstanding the level of calculations, it is customary to scale down the calculated harmonic frequencies in order to improve the agreement with the experiment. In this study, vibrational frequencies calculated at the B3LYP/6-31G** level were scaled by 0.96, Comparing the B3LYP and MM+ methods, above 3000 cm^{-1}, the predicted frequencies by B3LYP are larger than those by MM+; whereas under 3000 cm^{-1}, most of calculated frequencies by MM+ are larger than those by B3LYP.

Structural results: The calculated geometrical parameters (bonds lengths and valence angles) obtained in the different calculations are given in Table 1. The bond angles for the Density Functional Theory (DFT)-B3LYP method (Becke, 1993) are slightly better than MM+ when compared to experimental results of similar molecules (Kettmann and Svetlik, 2003). The computation underestimate the H-N-H angle. The best estimation for this angle is made with the B3LYP/ 6-31G** method. Based on the above comparison; although there are some differences between the theoretical values and the experimental values, the optimized structural parameters can well reproduce the experimental ones (Kettmann and Svetlik, 2003) and they are the basis for thereafter discussions.

The optimized bond lengths of C-C in the phenyl ring fall in the range from 1.362 to 1.482 A° for the MM+ method and 1.289 to 1.489 A° for the B3LYP

Table 1. Optimized bond lengths and angles of 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide.

Parameters	MM+	B3LYP (6-31G**)
Bond length		
C5-Cl1	1.721	1.701
C7-N1	1.301	1.289
C16-N3	1.362	1.357
N1-N2	1.379	1.422
C9-C10	1.522	1.588
C3-C7	1.482	1.489
C-O	1.369	1.402
N-H	1.008	1.012
N3-C16	1.362	1.57
O-H	0.977	0.998
C-H	1.087	1.070
Bond angles		
C1-C2-C3	120.00	120.87
C4-C5-C6	120.57	121.05
C15-C11-C14	121.60	12.98
C13-C10-C12	118.92	119.01
Cl1-C5-C6	119.68	119.54
C3-C7-N1	123.02	123.87
C3-C7-C8	121.77	121.19
N2-C16-O1	125.21	125.99
C8-C9-C10	111.23	112.07
C11-O2-H15	104.77	104.87
N2-C16-O1	125.21	126.45
N2-C16-N3	113.56	112.55
O1-C16-N3	121.13	121.90
N1-N2-C16	122.71	122.43
C17-03-C14	116.47	117.32

MM+: Molecular mechanics method, B3LYP (6-31G**): Becke 3-term correlation functional; Lee, Yang, and Parr exchange functional with 6-31G** basis set.

method which are in good agreement with those of experimentally reported values for the C-C bond length of the phenyl ring of similar molecules (Ketlmann and Stevlik, 2003). The results also show that for all calculations performed in the present work, there is a correlation between both N-C and C=O bond lengths. The C=O bond length is greater than that of the N-C. The N-N bond length is nearly equal to the C-C bond length and both are greater than the C-N bond length. The optimized C-Cl wavelengths by the two methods are 1.721 for the MM+ method and 1.701 for the B3LYP method, which are also in good agreement with the reported value.

Lipinski's rule of five: As per Lipinski's rule of five (Lipinski *et al*., 2001), an orally active drug has (i) not more than 5 hydrogen bond donors (OH and NH groups), (ii) not more than 10 hydrogen bond acceptors, (iii) a molecular weight under 500 and (iv) a partition coefficient log P under 5. A close study of the molecule of this study fulfills all requirements; hence it can be used as an oral drug.

H-Bond donors and acceptors: A poor permeation or absorption is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability (Refsgaard *et al*., 2005). Its abnormal increase may result in a considerably lowered absorption. The candidate drug has 3 H-bond donors and 4 H-bond acceptors.

Lipophilicity and partition coefficient (log P): An important consideration, although somewhat underrated for the predictive design of drugs, is their lipophilicity. On various occasions, this property has been interpreted as a measure of the permeation of drugs across cell membranes and their subsequent migration into the nucleus (Gnewuch and Sosnovsky, 2002).

Partition or distribution coefficients are critical elements in efforts designed to describe the uptake, distribution, biotransformation, and excretion of organic chemicals in biological systems (Jepson *et al*., 1993). High log P values imply high solubility and good penetration of lipid membranes, but by implication, low solubility in aqueous phases and hence the inability for the molecule to be transported through the body. Molecules with high log P also tend to be substrates of the metabolizing cytochrome P450 enzymes in the liver, in which case, first pass effects (phenomenon of drug metabolism) can remove much of the administered drug candidate before it can reach its target area (Vries *et al*., 2006). Log P value predicted by in this study for the target drug is 1.39.

Blood brain barrier: The blood-brain barrier (BBB) is of pivotal importance in maintaining homeostasis of the central nervous system (CNS), as it closely regulates the composition of the interstitial fluid in the brain (Nienke *et al*., 2006). The BBB at the level of brain microvessels creates the largest surface area known as the 'barrier interface' (12-20 m^2/1.3 kg of brain) and has the greatest influence on drug delivery to the brain. It is the most important site for regulating drug access to the brain, given its large surface area and the short diffusion distances from capillaries to neurons (10-15 μm) (Nienke *et al*., 2006).

The experimental determination of log*BB* (blood-brain barrier) is a time-consuming, expensive and difficult technique, requiring animal experiments and the synthesis of the test compounds, usually in radiolabeled form (Schlageter *et al*., 1999; Chikhale *et al*., 1994). It is of considerable value to predict log*BB* values of compounds from their physicochemical parameters or, ideally, from their molecular structures.

So, the value ascribed to this ability is calculated as demonstrated by Clark (Clark and Pickett, 2000).

LogBB = -0.0148 (PSA, Polar Surface Area) + 0.152 log P + 0.139

This value for the targeted drug is 1.616.

Drug dissolution (log S): The solubility of drugs in water is of central importance in the process of drug discovery and development, from molecular design to pharmaceutical formulation and biopharmacy because oral absorption is dependent on the compound dissolving in the aqueous of the gastrointestinal tract (dissolution) and then traversing the actual barrier of the gastrointestinal tract to reach the blood (Smith *et al*., 2006). Dissolution depends on the surface area of the dissolving solid and solubility of the drug at the surface of the dissolving solid. Yalkowsky (1999) has noted that log S correlates well with log P, but with an additional term involving the melting point (mp) for the crystalline solute, it is given as:

$$\text{Log S} = 0.8 - \text{log P} - 0.01(\text{mp}-25)$$

Virtually all drugs have aqueous solubilities of log S > -6[24].

Solubility of the candidate drug at different constituents of the body (at different pHs) is shown in Table 2.

Drug permeability and transport: Most drugs used to treat the CNS are lipid-soluble (lipophilic) and able to diffuse through the endothelial membranes (Bodor and Buchwald, 2003). The physicochemistry of different drugs that molecular weight (MW) and lipophilicity should be around MW ~280 amu, LogP octanol ~ 2.0 (Bodor *et al*., 2003; Asperen *et al*., 1999). Calculated values for this compound are as follows; MW = 357.18 amu and Log P octanol = 1.39), hence the designed drug can penetrate the BBB and will show activity at the target site of action.

The ATP binding cassette (ABC) family (P-glycoprotein (ABCB1)) and the multidrug resistance related poteins (MRPs, ABCC1, 2, and 5) can transport solutes out of the brain's endothelial cells, often with consumption of ATP (Chikhale *et al*., 1994).They are able to restrict the CNS entry of several potentially harmful, toxic or lipophilic agents circulating in the blood, derived from the diet or metabolism (Sveigaard and Dalgaard, 2000). As log P for the candidate drug is a low value of 1.39, the candidate drug is thus not lipophilic, hence the above discussed transporters can transport the candidate drug.

There are several potential routes for drug delivery to the brain. As, the candidate drug is not polar (hydrophilic), hence it can penetrate through tight junctions. Such drugs can unzip the junction locally as they can transmigrate, with minimal leakage, or can adhere in the region of the junction and then migrate through the cell (Chikhale *et al*., 1994).

Electrostatic potential maps: The electrostatic potential map shows the value of the electrostatic potential onto an electron density surface to get a description of the electrostatic characteristics of the target drug (Kermer *et al*., 2002). By convention, colors toward red depict negative potential, while colors toward blue depict positive potential and colors in between depict intermediate values of the potential. Thus, this drug has both, negative and positive well defined regions, which increase the interaction possibilities from an electrostatic point of view. Thus, especially when H-bonding (electrostatic in nature) is involved, the calculation of the electrostatic surfaces can be very useful in visualization of the sites of interaction in both hosts and guests to predict their affinities (Castro *et al*., 2006). Analysis of qualitative data obtained by electrostatic potential (EP) calculations also provides useful information for explaining the differential ability of molecular analogs to act with a common receptor. In the present work, electrostatic potential maps were constructed for the candidate drug to analyze the characteristics of the electrostatic potential. Thus, in this context, molecular electrostatic potential (MEP) maps were generated based on the density functional theory by the B3LYP/6-31G** method for the lowest minimum energy conformations. Map is shown in Figure 4.

Table 2. Solubility in buffer (log S) at different constituents of body body of 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide.

S.N.	Part of body	pH	Log S
1	Stomach	1.7	-4.29
2	Duodenum	4.6	-4.29
3	Jejunum and Ileum	6.5	-4.29
4	Blood	7.4	-4.29
5	Colon	8.0	-4.29

Comparison of these electrostatic potential maps reveals that both of them show two negative regions. One is closer to the oxygen atom of the C=O and the other one is closer to the oxygen atom of the O-H group. This region is nucleophilic and tends to form hydrogen bond interactions by accepting hydrogen from a donor counterpart. Electrostatic potential also

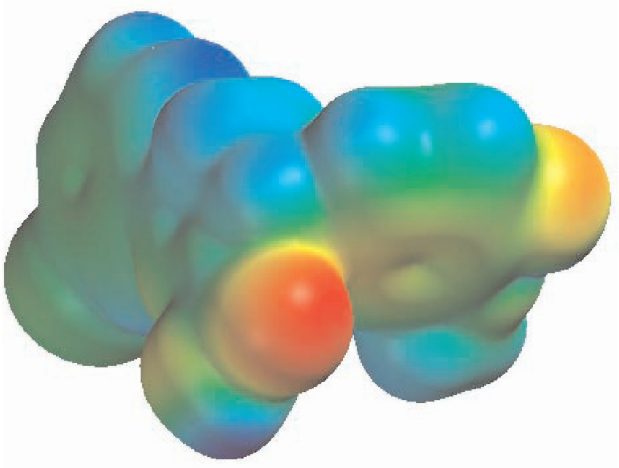


Figure 4. A 3D-view of electrostatic potential map of 3-(4-chlorophenyl)-5-(3-hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide.

shows four positive regions. Out of these, one is closer to the hydrogen atoms of the napthelene ring, another is closer to the hydrogen atom of the O-H group and the third one is nearer to hydrogen atoms of the amide group.

The candidate drug is a good drug, which can be encapsulated in hosts cells and the kind of drug whose electrostatic surface potential shows fair 'variety of region' to interact.

Aqueous solubility (log W): An insufficient aqueous solubility is likely to hamper bioavailability of the drugs. In recent years, high throughput screening (HTS), where collections of thousands of compounds are screened with the intention of finding relevant biological activity has proven valuable in finding new lead drugs (Banker *et al*., 2003). Estimated aqueous solubility of drug-like molecules with the quantitative structure-property relationship (QSPR) approach is found to be between -5.16 to 0.92. The molecule of this study has log w of - 4.29, so it is well within the range (Gao *et al*., 2002).

Polar surface area (PSA): Molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties. PSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. This parameter has been shown to correlate very well with human intestinal absorption, cell line for monolayer absorption (Caco-2) monolayer's permeability and blood-brain barrier penetration (Smith *et al*., 2000). Calculated surface characteristics of molecules have been correlated with several physicochemical properties of drug molecules including lipophilicity, the energy of hydration and the hydrogen bond formation capacity (Ooi *et al*., 1987). An increase in the value of PSA in the optimum model corresponded to an initial positive effect on bioavailability but then caused predicted bioavailability to drop substantially (Turner *et al*., 2003). PSA of the investigated molecule is 71.95 A^2.

P-Glycoprotein: P-Glycoprotein is a membrane-associated protein that has affinity for a variety of large, structurally unrelated, neutral or cationic amphipathic compounds. By pumping substrate drugs out of the cell, this protein decreases the intracellular drug accumulation, resulting in a diminished therapeutic efficacy (Asperen *et al*., 1999). The results of several studies also suggested that P-glycoprotein plays a role in the protection of the organism against potentially toxic substances, e.g., by limiting the absorption of orally ingested compounds, by mediating the elimination of substrates from the body, and by protecting crucial organs such as the brain and the testis against toxic substances in the circulation (Thiebaut *et al*., 1987; Kennedy, 1997).

For the molecule of this study, P-glycoprotin substrate has a weak H acceptor and Abraham's beta is less than 1.5. Probability of drug to be a P-gp (P-glycoprotein substrate is viewed as a constituent part of a compound's "pharmaceutical profiling" in drug design) substrate is 0.119; hence its reliability is low.

The candidate drug has an acid pKa > 5, MW < 360 and a logP < 2.5, so it's P-gP inhibitor probability is 0.242. Hence the candidate drug's reliability is high.

Plasma protein binding (PPB): Drug binding to plasma proteins is an essential step in both drug discovery and in clinical phases of drug development. Binding of drugs to plasma proteins is important in understanding the pharmacokinetics and pharmacodynamic relationship of a drug (Cheng *et al*., 2004; Fung *et al*., 2003). Therefore, PPB is normally recognized as an important factor in assessing drug disposition, efficacy and safety (Musteata *et al*., 2006). In PPB, propensity of a drug affects the amount of drug available to diffuse into target tissues, for example brain, the calculation of *in vivo* hepatic clearance and the interpretation of the drug's bioavailability (Kratochwil, 2002).

The strength of an interaction between plasma proteins and a drug is usually expressed as a %PPB value. This value for the target molecule is 83.96%. Similarly, the ability of a drug to bind to albumin, which is the most abundant carrier protein in human plasma is represented by an HSA (Human serum albumin is the most abundant protein in human blood plasma.) affinity constant (Kermer *et al*., 2002) ($LogK_A^{HSA}$). This value for the candidate drug is 3.97. As the drug under investigation is an acidic compound, in plasama, such drugs predominantly bind to human serum albumin.

Volume of distribution (V_d): Volume of distribution (V_d) is also an important parameter for characterizing drug disposition. V_d is a measure of relative partitioning of drugs between plasma (the central compartment) and the tissues. All tissues are considered as a single homogenous compartment. V_d is necessary for

simulating plasma concentration of a drug (C_p) and is a composite parameter, which depends on many chemical and biological factors. V_d is a function of the sum of binding interactions with various tissue components *vs* binding to plasma proteins. For of this parameter, a software developed by ap-algorithms was used. This value for the target molecule comes was calculated to be 1.66 l/kg.

Gastro-intestinal (GI) absorption: It is difficult to predict drug absorption after oral dosage due to complex drug-specific parameters and physiological processes, including: drug release from the dosage form and dissolution, aqueous solubility, GI motility and contents, pH, GI blood flow, membrane transfer or permeability and active transport systems, pre-systemic and first pass metabolism (Cummins *et al*., 2002; Martinez and Amidon, 2002). Drugs are categorized based on permeability, aqueous solubility and elimination mechanisms to improve the ability to anticipate transporter effects and food and drug-drug interactions (Wu and Benet, 2005; Amidon *et al*., 1995).

Watari *et al*. (1988) evaluated the pharmacokinetics of barbiturates in rabbits and found a linear relationship between the logarithms of k_a (drug absorption) and log P, as in equation:

$$\text{Log } K_a\text{: } 0.193 \log P + 0.0148$$

The value for the target drug molecule, calculated with the above equation, is 0.2346.

Clearence (Log CL_R): It is difficult to correlate clearance with physicochemical and molecular descriptors owing to the complexity of the biological system, the influence of transporters and the vast range of sites and mechanisms of drug biotransformation and elimination (Mager, 2006). Mayer *et al*. (1988) demonstrate the relation between renal clearance values and log D as in equation:

$$\text{Log } CL_R = -0.22 \text{ LogD } -0.84$$

Thus, there is a simple linear relationship between log D of barbiturates and the logarithm of intrinsic clearance, for the molecule of this study, this was: - 1.3504.

Toxicity: Traditionally, toxicity studies have been experimental in nature and in most cases have involved animal studies. Such studies can be time-consuming and expensive. As a result, computationally predicting the toxicity of a given molecule has been intensively studied, as a means to avoid animal testing (Roy *et al*., 2006).

The Ames test, which is used worldwide as an initial screen to determine genotoxic properties of new chemical entities (NCEs) for the pharma- and chemical industry is also used in this study. It is the short term bacteria reverse mutation test that is performed on various *S. typhimurium* and *Escherichia coli* strains. Ames genotoxicity is predicted from structure using the software developed by pharma-algorithms. Calculated probability of positive Ames test for the candidate drug is 0.121.

Predictions are displayed, in Table 3, in terms of color coded atomic/fragmental contributions ("color coded potentials"). This allows identifying and visualizing specific structural toxicophores: genotoxicity potential in the Ames test (green part is not involved in genotoxic activity, red part is associated with genotoxic properties).

Hansch and Clayton (1973) modeled the acute toxicity of barbiturates to the mouse using only the octanol-water partition coefficient (P), a measure of hydrophobicity:

$$\log 1/LD50 = 1.02\log P - 0.27(\log P)^2 + 1.86$$

$$n = 13 \; r^2 = 0.852 \; s = 0.113$$

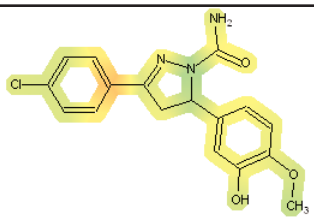
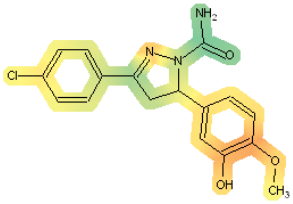
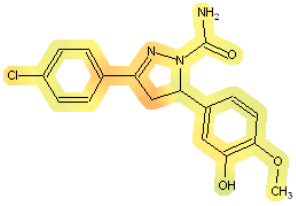
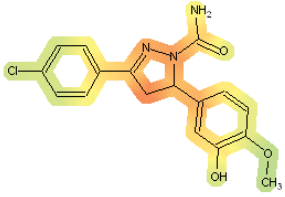
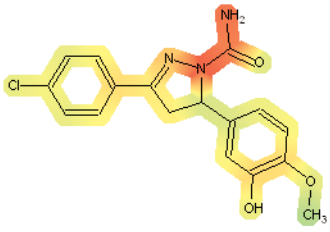
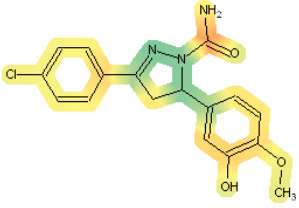
Where LD50 = dose to kill 50% of mice, n = number of compounds used in developing the QSAR (the training set), r = correlation coefficient, and s = standard error of the estimate.

Calculated value of LD50 and pLD50 (predicted LD) for mouse and rat are shown in Table 4 and 5, respectively. All the values for the drug taken through various routes are well in the range. The drug of this study can be used as intraperitoneal, oral and subcutaneous, since the value of LD50 is between 500-1000, so it is slightly toxic. This cannot be taken intravenously because the value of LD50 is 96 mg/kg, which shows that this drug is moderately toxic. According to Gosselin *et al*. (1984), if drug is slightly toxic, then the probable oral lethal dose for humans can be 5-15 gm/kg.

There are certain well known protein targets that can lead to toxicity, such as the human Ether-a-go-go related gene (hERG) (Hansch and Clayton, 1973). For such scenarios, one can apply a number of methods to decide whether a compound will be toxic by virtue of interacting with hERG.

Toxicity is also expressed through biological activity data (pIC50) defined as molar concentration of

Table 3. Probability of health effect due to toxicity on various parts of the body and color mapping highlighting structural features contributing to an adverse health effect of 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4,5-dihydro-1H-pyrazole-1- carboxamide.

S.N.	Part of Body	Probability	Color Mapping
1	Blood	0.32	
2	Cardiovascular system	0.73	
3	Gastrointestinal system	0.44	
4	Kidney	0.27	
5	Liver	0.13	
6	Lungs	0.23	

those chemicals necessary to displace 50% of radiolabeled tetrachlorodibenzo-p-dioxin (TCDD) from the aryl hydrocarbon (Ah) receptor.

Guha and Schurer (2008) used 775 pIC50 values, since they could not evaluate physiochemical and toxic descriptors for some of the molecules. They then selected a cutoff of 5.5, such that molecules with a pIC50 greater than this value were classified as toxic and the remainder as non-toxic. In this research, a model of the hERG force field, developed by quantum

Table 4. Acute toxicity LD5O for mouse.of 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4, 5-dihydro-1H-pyrazole-1- carboxamide.

	LD50 (mg/kg)	pLD50	Lower limit	Upper limit
Intraperitoneal	580	-0.23	-0.90	0.34
Oral	950	-0.44	-1.76	-0.08
Intravenous	96	0.56	-0.21	0.56
Subcutaneous	520	-0.18	-1.59	1.01

Table 5. Acute toxicity LD5O for rat of 3-(4-chlorophenyl)-5-(3hydroxy-4-etoxyphenyl)-4, 5-dihydro-1H-pyrazole-1-carboxamide.

	LD50 (mg/kg)	pLD50	Lower limit	Upper limit
Intraperitoneal	860	-0.39	-1.60	0.44
Oral	1300	-0.56	-2.15	0.21

pharmaceuticals, for predicting a molecular structure with respect to its inhibition constant for the hERG channels was used. This model is very useful for molecular acido-toxicity predictionThe valued for the target drug is 5.8, which is slightly greater than 5.5; hence this compound is slightly toxic.

Prediction of toxic properties of small drug like molecules is a big challenge both from theoretical and practical points of view. Quantitatively people use different measures of toxicity such as maximum recommended daily dose (MRDD) or lethal dose (LD50) (Kratochwil, 2002).

Organ specific health effects: In this investigation organ specific health effects were predicted using the software ToxBoxes V1.1 (Pharma Algogithms 2008). This software uses health effects' predictive algorithms based on long term toxicity studies with adverse effects reported on particular organs or organ systems. Data has been incorporated from chronic, subchronic, acute and carcinogenicity studies encompassing various species and routes of administration.

The structural features contributing to the adverse health effect are identified and highlighted using color mapping as shown in Table 3. Red sections are associated with the toxic action of the compound on a particular organ, while green sections of the molecule are not related to the health effect under investigation.

Bioavailability: The bioavailability of a drug is the rate at which the drug becomes available to the body and the extent to which the dose is ultimately absorbed after administration. The extent of bioavailability directly influences plasma concentrations, as well as the therapeutic and toxic effects resulting from oral drug administration. Drugs with poor bioavailability are inefficient because a major portion of a dose never reaches the plasma to exert a pharmacological effect. Low bioavailability is also associated with large inter-subject variability in plasma concentrations and effects. Incomplete oral bioavailability has various causes. These include poor dissolution or low aqueous solubility, degradation of the drug in gastric or intestinal fluids, poor intestinal membrane permeation, and presystemic intestinal or hepatic metabolism (Turner *et al*., 2003). Bioavailability values for drugs can be predicted by:

Bioavailability (%) = -45.20 + 5.08 (electron affinity) + 4.09 (aromatic ring count) -15.83 (HOMO) -3.34 (log *F*) -0.09 (molar volume) -0.72 (volumetric HLB (Hydrophile-Lipophile Balance)) - 4.75×10^{-7} (water solubility) + 1.18 (Hansen's hydrogen-bonding solubility parameter).

Predicted bioavailability of the drugs in the test set was used to evaluate the best overall predictive optimum performance model. The linear correlation between predicted and observed values is an indication

of the quality of the model predictions. Calculated bioavailability for the target drug molecule is above 70%. Probabilities of %F (Bioavailability) (Oral) > 30% is 0.849 and %F (Oral) > 70% is 0.756.

Druglikeness: It is a complex balance of various molecular properties and structural features which determine whether a particular molecule is similar to the known drugs. It generally means "molecules which contain functional groups and/or have physical properties consistent with most of the known drugs". These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecular size, flexibility and presence of various pharmacophoric features influence the behavior of molecules in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. The presence of structural fragments typically found in drugs molecules with scores between 2 and 7 are classified as drugs; otherwise they are classified as non-drugs (Walters and Murcko, 2002). Our candidate drug has a C=O functional group whose score is 3.4, hence it can be used as a drug. As this drug contains a single pharmacophoric group, it can attack the CNS. Therefore, because it has a single pharmacophoric group and contains an amine functional group, which is a part of pharmacophoric group, it can thus be classified as drug.

A more recent example of the functional approach to identify drug-like molecules is the work of Muegge *et al*. (2001). They assigned each molecule a score based on the presence of structural fragments typically found in drugs. Compounds containing specific single pharmacophoric groups can also be classified as drugs. One such group is the amine group. The candidate drug of this study contains an amine group; accordingly it can be classified as a drug. Some candidate drugs are also rejected because they contain nitro groups, which tend to activate aromatic rings and may increase a molecule's tendency to generate false positives under assay conditions (Tiwari and Pande, 2006). As the drug in this research does not have a nitro group, so there is less probability that it will be rejected.

For calculation of the drug-likeness score towards GPCR (G protein-coupled receptors); ligands, ion channel modulators, kinase inhibitors and nuclear receptor ligands based on Molinspiration technology was carried out. The score came out as 2.00.

DISCUSSION

Computational chemists have a wide array of tools and approaches available for the assessment of molecular diversity. Diversity analysis has been shown to be an important ingredient in designing drugs. So, computational sensitivity analysis and structural analysis have been used to study the drug-likeness of the candidate drug. Computer modeling has many plus points. One of the important advantages is the speed at which work can be carried out. The discussed structures can easily be manipulated and modified in a simulated environment. The theoretical study set out to determine stable conformation, pK_a, lipophilicity, solubility, absorption, blood brain barrier, hydrogen bond donors, hydrogen bond acceptors, drug dissolution, drug permeability, electrostatic potential map, p-glycoprotein, plasma protein binding, volume of distribution, gastrointestinal absorption, drug clearance, toxicity, bioavailability, drug-likeness and polar surface area of the candidate drug for which no experimental physicochemical data exits. Lipinski parameters for the target drug are within the general limit found for clinical uses. As good bioavailability can be achieved with an appropriate balance between solubility and portioning properties, the candidate drug has obtained a desirable level of bioavailability. Because of the enormous costs of drug failure due to toxicity found late in the development process, toxicity should be determined as early as possible, to guide synthesis. Hence, the use of computational prediction of toxicity, and several approaches to such prediction have also been demonstarted.

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