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Research Article

In silico prediction of selected pharmacokinetic and toxicity properties of some neuroactive steroids

K.K.Rajasekhar*,D.Ranganayakulu[1], V.Shankarananth[1], K.Rekha rani[1], K.Swetha harika[1] and K.Srividya[1].

** ,1-Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, Andhra pradesh, India.*

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ABSTRACT

Selected pharmacokinetic and toxic properties of some neuroactive steroids derived from pregnanolone and alphaxalone were predicted by in silico methods.Pregnanolone and alphaxalone were selected as lead molecules and a series of drug-like molecules derived from them were generated. Their pharmacokinetic and toxicity properties were calculated through the ADME and Tox boxes web version of pharma algorithms and ACD labs Chem. Sketch software version 12.0.All drug-like neuroactive steroids were predicted to be less toxic(LD_{50}900 to 1020 mg/kg and 940 to 1020 mg/kg) with an enhancement in lipophilicity (c log P 3.31 to 4.73 and 5.06 to 6.41),protein binding (64% to 91% and 56%to 92%) and oral bioavailability(64% to 88% and 52% to 90%).From this study, it can be concluded that all the drug-like neuroactive steroids possess marked lipophilicity, rate of absorption, volume of distribution, protein binding and oral bioavailability.

Keywords: Neuro active steroids, Pharmacokinetic properties, Toxicity, In silico methods.

INTRODUCTION

The postsynaptic $GABA_A$ receptor is a receptor complex containing a number of modulatory binding sites for drugs such as benzodiazepines, barbiturates, and neurosteroids. It regulates the influx of chloride ions in such a way that receptor activation causes hyperpolarisation of the cell membrane and,thus, decreased sensitivity of the neurone to excitatory input [1,2]. Neuroactive steroids are a novel class of positive allosteric modulators of the $GABA_A$ receptor that interact with a specific steroid recognition site on the receptor ion channel complex. Neurosteroids are synthesized in the brain, whereas other neuroactive steroids with pharmacological effects in the CNS are not necessarily synthesized in the CNS tissue. Both are important endogenous agents for influencing brain function by modulation of the activation of $GABA_A$ receptors. They include pregnanolone and its reduced metabolites such as 5α and 5β epimers. There steroids are rapidly biotransformed when administered exogenously due to metabolism of the 3-α-OH group at the 3rd position. Thus, they exhibit rapid onset and short duration of action. Several synthetic analogs such as alphaxalone have been developed and shows promising therapeutic effects. In general neuroactive steroids produce effects in animal models of CNS disorders similar to those of other positive allosteric modulators of the $GABA_A$ receptor without significant side-effects.[3]

Animal and human studies suggest that neurosteroids plays an important role in anaesthesia,epilepsy,anxiety,psychoses and memory impairment.[4,5,6,7,8]

Neuroactive steroids, appear to hold great promise for providing new anaesthetics. Pregnanolone is capable of providing general anaesthesia when used as the sole anaesthetic drug and produces minimal pain on injection. It appears to have some what less cardiovascular side effects than do barbiturates or propofol. However,recovery after induction with pregnanolone is significantly slower than after propofol[9].The clinical attraction of steroid anaesthetics includes the advantages of good overall safety,lack of toxicity and a relatively rapid metabolism by the liver that promotes a short duration of action. Their use in clinic has,however,been hampered by problems in formulation stunning from their solubility in water. A well known example was alphaxalone which enjoyed a widespread clinical use as a anaesthetic component of the formulation 'Althesin' in 1970's but anaphylactic reactions associated with the vehicle cremophor El led to alphaxalone's demise as an anaesthetic in human beings[10,11]. No neuroactive steroid has been approved subsequently for general anaesthesia. Nevertheless, neurosteroids have several properties that make them potentially useful parenteral anaesthetics, and newer agents have been examined in clinical trials [12,13].

Drug discovery and development are expensive undertakings. The research costs for a compound increase dramatically as it progress through clinical development, and therefore there are economic rea-

***Corresponding author.**

K.K.Rajasekhar,
Department of Pharmaceutical Chemistry,
Sri Padmavathi School of pharmacy,
Tiruchanoor,Tirupathi.A.P. India
Tel.: + 91-9440592854
Fax.
E-mail: kkrspsp@yahoo.co.in

sons for identifying and discontinuing the development of poor drug candidates at the earliest possible time. Even compounds that do eventually reach the market sometimes have less than ideal characteristics,complicating patient management. Poor pharmacokinetic properties are one of the main reasons for terminating the development of drug candidates[14,15,16].

The application of computational technology during drug discovery and development offers considerable potential for reducing the number of experimental studies required for compound selection and development and for improving the success rate. In this context, in silico approaches are being used today in drug discovery to assess the ADME properties of compounds at the early stages of discovery and development. This early assessment of ADME properties will help pharmaceutical scientists to select the best candidates for development as well as to reject those with a low probability of success[17]. In the present study, pregnanolone and alphaxalone were selected as lead molecules and 24 drug like molecules, with anticipated CNS activity were generated. Selected pharmacokinetic and toxicity properties such as clog P, polarisability, absorption,rate of absorption, metabolism,LD_{50}, volume of distribution,oral bioavailability, percentage protein binding, parachor and molar refractivity were predicted using softwares like ADME & TOX boxes web version of pharma Alogrithms and ACD labs chemsketch version12.0.

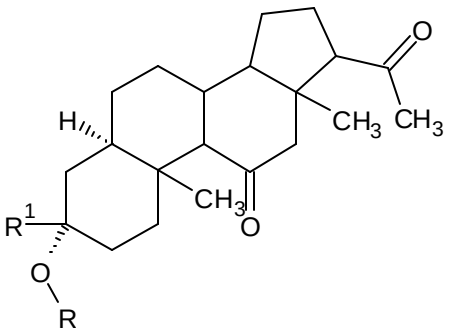
MATERIALS AND METHODS:

Selection of lead molecules:

Developing new neuroactive steroid agonist and antagonists, and understanding their mechanism of action are active areas of research in the quest for an ideal anaesthetic agent[18,19]. The mechanisms by which neuroactive steroids affect neuronal activity are not clear, but it should be noted that they may regulate neuronal excitability[20]. In an effort to develop an effective steroidal intravenous anaesthetic retaining the advantages of both pregnanolone and alphaxalone, we selected these two steroids as lead molecules to generate drug-like neuroactive steroids with anticipated CNS activity.

Generation of drug-like neuroactive steroids:

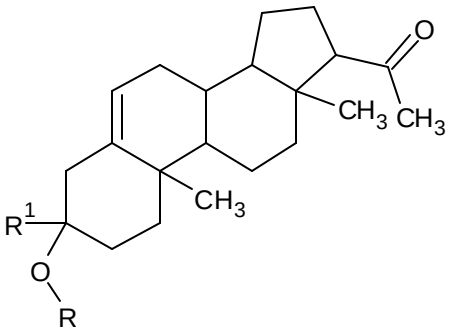
It is usual to start with molecules that appear to be drug-like at the outset rather than to make a hit drug-like later[21].Therefore, 24 drug-like neuroactive steroids were generated, 12 from pregnanolone and 12 from alphaxalone, by replacing 3-OH group with simple alkoxy and alkyl ester functionalities and by introducing simple alkyl groups at 3rd position. Structures of these drug-like molecules were drawn through chemsketch program[22]. Each 2D chemical structure was systematically built, that is, the basic nucleus was kept unaltered and the aqbove mentioned substituents were added accordingly. All these chemical



Alphaxalone R&R^1 = -H

Table No:1 Structural details and selected pharmacokinetic and toxicity properties of 12 drug like molecules derived from Alphaxalone

S.NO	Code	R	R^1	Molar Refractivity (cm^3)	Parachor (cm^3)	Polarisability X 10^{-24} (cm^3)	C log P	Absorption	Rate of absorption (min^{-1})	Volume of distribution V_d(L/Kg)	Meta-bolism	%protein Binding (%)	Oral Bioavailability (%)	LD_{50} in mice (mg/kg)
1.	A_1	$-CH_3$	-H	97.18	785.1	38.52	5.18	Passive	0.067	2.01	First pass	86	80	1000
2.	A_2	$-C_2H_5$	-H	101.81	825.2	40.36	5.71	Passive	0.074	2.21	First pass	74	72	960
3.	A_3	$-C_3H_7$	-H	106.44	865.2	42.19	6.24	Passive	0.081	2.32	First pass	92	90	945
4.	A_4	$-CH(CH_3)_2$	-H	106.42	863.2	42.19	6.06	Passive	0.078	2.41	First pass	78	75	965
5.	A_5	-H	$-CH_3$	97.01	780.9	38.45	5.06	Passive	0.069	2.12	First pass	68	65	1020
6.	A_6	-H	$-C_2H_5$	101.64	820.2	40.29	5.60	Passive	0.082	2.32	First pass	80	75	975
7.	A_7	-H	$-C_3H_7$	106.27	860.2	42.13	6.13	Passive	0.071	2.41	First pass	76	70	940
8.	A_8	-H	$-CH(CH_3)_2$	106.05	858.2	42.12	5.94	Passive	0.074	2.12	First pass	72	68	985
9.	A_9	$-COCH_3$	-H	101.93	830.3	40.41	5.38	Passive	0.068	2.12	First pass	65	65	970
10.	A_{10}	$-COC_2H_5$	-H	106.56	870.4	42.24	5.91	Passive	0.084	2.14	First pass	81	78	980
11.	A_{11}	$-COC_3H_7$	-H	111.17	908.4	44.07	6.26	Passive	0.091	2.31	First pass	88	84	965
12.	A_{12}	$-COCH(CH_3)_2$	-H	113.67	910.7	44.10	6.41	Passive	0.085	2.02	First pass	81	75	1000



Pregnanolone R&R^1 = -H

structures were saved and exported to ADME and TOX boxes web version of pharma Alogrithms [23].

Prediction of selected pharmacokinetic and toxicity properties:

Unfavourable ADME and toxicity properties have been identified as a major cause of failure for candidate molecules in drug development. Consequently, there is increasing interest in the early prediction of these properties, with the objective of increasing the success rate of compounds reaching development [24].The pharmacokinetic, toxicity and biopharmaceutical profiles of 24 drug-like neuroactive steroids were calculated through ACD labs chemsketch programme and ADME and TOX boxes of pharma Alogrithms.

RESULTS :

Tables 1 and 2 shows the structural details and the predicted pharmacokinetic and toxicity properties of 24 drug-like neuro active steroids derived from alphaxalone and pregnanlone respectively. As number of carbon atoms in alkoxy group increases from 1-3, lipophilicity also increases in all the 24 drug-like molecules.The polarisability also increases, as number of carbon atoms increase in alkoxy group.Structural modification do not shows much impact on type of absorption and metabolism. All molecules were predicted to be absorbed via passive diffusion and metabolised by first pass. Volume of distribution is slightly increased whereas protein binding,oral bioavailability and **LD**$_{50}$ (in mice) are varied. Increasing the steric bulkiness at 3rd position enhances the lipophilicity and rate of absorption. CompoundsA_1, A_5,A_{12} and P_5 were predicted to be less toxic in mice.

DISCUSSION:

The ultimate goal of the insilico prediction of pharmacokinetic properties is the accurate prediction of the invivo pharmacokinetics of a potential drug molecule in man,whilst it exists as only a virtual structure.This requires an integrated suite of models covering each of the processes involved and their incorporation into a full 'drug design' software package which combines ADME predictions with those for pharmacological properties,stability, chemical tractability,etc,to produce a molecule with the optimal combination of properties. This ideal, complete package does not yet exist. However, many of its component parts are already available and in some circumstances used for lead optimization screening, drug design and development[25].The lipophilicity of a drug is clearly related to its passive absorption and log P is complex and indirect[26].Volume of distribution is an important pharmacokinetic property that needs to be determined during drug development process.It is normally esti-

Table No:2 Structural details and selected pharmacokinetic and toxicity properties of 12 drug like molecules derived from Pregnanolone

S.N	Code	R	R^1	Molar Refractivity (cm^3)	Parachor (cm^3)	Polarisability X 10^{-24} (cm^3)	C log P	Absorption	Rate of absorption (min^{-1})	Volume of distribution V_d(L/Kg)	Meta bolism	%protein binding (%)	Oral Bioavail ability (%)	LD_{50} in mice (mg/kg)
1	P_1	$-CH_3$	-H	97.59	804.3	38.69	3.41	Passive	0.064	2.4	First pass	80	74	940
2	P_2	$-C_2H_5$	-H	102.23	844.4	40.52	3.94	Passive	0.071	2.31	First pass	73	72	920
3	P_3	$-C_3H_7$	-H	106.86	834.5	42.36	4.47	Passive	0.08	2.26	First pass	91	88	940
4	P_4	$-CH(CH_3)_2$	-H	106.83	882.4	42.35	4.28	Passive	0.076	2.41	First pass	74	72	960
5	P_5	-H	$-CH_3$	97.31	793.3	38.57	3.31	Passive	0.064	2.62	First pass	66	64	1020
6	P_6	-H	$-C_2H_5$	101.93	839.4	40.40	3.84	Passive	0.08	2.53	First pass	78	74	970
7	P_7	-H	$-C_3H_7$	106.56	879.5	42.24	4.37	Passive	0.071	2.42	First pass	74	69	935
8	P_8	-H	$-CH(CH_3)_2$	106.47	877.4	42.20	4.19	Passive	0.072	2.41	First pass	70	67	945
9	P_9	$-COCH_3$	-H	102.35	849.6	40.57	3.66	Passive	0.066	2.30	First pass	64	64	965
10	P_{10}	$-COC_2H_5$	-H	106.97	889.7	42.40	4.19	Passive	0.082	2.26	First pass	80	76	970
11	P_{11}	$-COC_3H_7$	-H	111.58	927.7	44.23	4.54	Passive	0.091	2.20	First pass	84	72	960
12	P_{12}	$-COCH(CH_3)_2$	-H	111.60	929.7	44.24	4.73	Passive	0.084	2.20	First pass	78	74	900

mated using animal scaling which is associated with certain levels of error[27].Hence, in drug development, the prediction of tissue distribution would help predict the in vivo pharmacokinetics of a compound prior to any experiments in animals or man. An understanding of tissue distribution, particularly when coupled with knowledge of the in vitro effects of the compound on biological targets of toxicological or pharmacological relevance, may also help in predicting the pharmacodynamic or toxicodynamic effects of a drug in specific tissues. Thus, in silico tools enabling the prediction of the pharmacokinetic profile would be invaluable for high-throughput screening and selection of compounds for in vivo testing.

Within the ADME processes, M,which stands for metabolism, certainly covers the largest, and still poorly understood aspect and consequently, the most difficult to evaluate and to predict. Infact , the metabolic fate of a compound depends on a large number of variables related to both the chemical itself and biological system[28].It is particularly difficult to develop reliable prediction software packages. The existing attempts are relatively 'Crude'under constant evolution and needing continuous refinement[29].No single approach can be used to predict the full range of ADME properties that are desired. A Challenge in this field is to identify the technique that is most suitable for modelling the property under investigation.Infact, a combination of two or more models for the same property, based on different principles, can give higher confidence in the results obtained for which they agree or identify areas of uncertainity where they differ.Insilico approaches will continue to evolve rapidly,just as invitro methods did in the past.Experience with latter showed that failure to adhere to best practice, such as elaboration of a prediction model and adequate validation,resulted in loss of confidence in their reliability and applicability, even when this was not warranted. It will be important to learn from this experience, and avoid repeating similar errors.

In summary, we believe that the data and methodologies exist to greatly improve ADME/TOX prediction of novel compounds early in the drug discovery process. The integration of modelling,high-throughput and systems biology approaches will allow true breakthroughs in in silico ADME/TOX assessment.

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