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Research Article

Investigation of Experimental and *In Silico* Physicochemical Properties of Thiazole-Pyridinium Anti-Acetylcholinesterase Derivatives with Potential Anti-Alzheimer's Activity

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Abstract

Background: Physicochemical properties play important role in fundamental issues like absorption and distribution of pharmaceuticals to the target tissue. This is particularly important for drugs acting in central nervous system (CNS). In this study, physicochemical properties of previously synthesized thiazole-pyridinium derivatives with anti-acetylcholinesterase activity and possible anti-Alzheimer effect were studied.

Methods: Partition coefficient (n-octanol/water) and chromatographic R_f values for the studied compounds were determined using shake flask and high performance thin layer chromatography (HPTLC) methods, respectively. Different druglikeness properties of the compounds were also calculated using available software and web-servers.

Results: The experimentally determined logarithm of partition coefficients (log P) for the studied compounds were in the range of -1.00 to -0.38. The R_f values for the studied compounds under the applied chromatographic condition ranged between 0.38 to 0.58. Moreover, calculated physicochemical properties, and druglikeness scores of the studied thiazole-pyridinium derivatives and matching piperidine analogues were predicted. Furthermore, some ADMET features of studied compounds like toxicity and metabolism by CYP450 (2C9, 2D6, 3A4, 1A2 and 2C19) enzymes were predicted.

Conclusion: The ranges of experimental and calculated LogP values for the studied thiazole-pyridinums were close. However, the determined R_f values showed relatively better correlation to the predicted LogP values indicating the suitability of used chromatographic method for comparing the lipophilicity of the positively charged pyridinium derivatives. The studied compounds were predicted to pass GI membrane and reach the CNS where they can exert their effects. *In silico* studies indicate that the piperidine counterparts of the studied thiazole-pyridiniums may represent anti-Alzheimer agents with improved druglikeness properties.

Introduction

Alzheimer's disease (AD) is an age related neurodegenerative disorder of central nervous system (CNS) with increasing prevalence.[1-3] Gradual and progressive decrease of memory and disability result in incapacitation of various life aspects and altered behavioral distraction. All these physical and more importantly the mental and behavioral disabilities result in substantial cost of care for the public health providers and families. Diminished acetylcholine is critical in AD which happens in cholinergic synapses in certain areas of brain and causes deterioration of neuronal functions.[4,5] Furthermore, the accumulation of β-amyloid (Aβ) around neurons and the aggregation of protein tau inside the cells are other well-known hallmarks of chronic inflammatory events which occur in AD causing neurodegeneration and development of the disease.[6,7] Donepezil, galantamine, rivastigmine, memantine and tacrin are known anti-Alzheimer drugs[8,9] from which the latter drug, i.e., tacrin, was withdrawn

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from the market in 2013 due to liver toxicity.[10] Moreover, gastrointestinal problems and varied heart rate are common limitations of available therapeutics.[11-13] These drugs are aimed to inhibit the acetylcholinesterase at the initial stage of disease maintaining balanced acetylcholine level in CNS.[14] By gradual progression of the disease the routinely used drugs may not be effective. Therefore, limitation of the available effective therapeutic agents in the market has attracted life science researchers to investigate synthesis and development of novel drugs for AD. Thiazole containing compounds have been shown to exert cholinesterase inhibitory activity.[15,16] For example, Sun et al. have reported a series of thiazole acetamide derivatives showing anticholinesterase activity ranging from 3.14 to 32.45 nM for a possible role in the treatment of AD.[17] Apart from investigational compounds, many thiazole containing agents were approved for variety of medications including inflammation, peptic ulcer, cancer, and microbial and viral infections. Meloxicam, famotidine, dasatinib, cefexime, sulfathiazole, ritonavir are some well-known examples of such drugs. In line with the increased demand for new anti-Alzheimer drugs and by virtue of the observed anticholinesterase properties for the thiazole containing compounds, we have recently designed and synthesized a new series of thiazole-pyridinium compounds with promising *in vitro* activities.[18] Physicochemical properties are crucial when novel compounds are introduced particularly when the ultimate goal is the development of pharmaceutically active compounds. Chemical structure modification most often is used to change the physicochemical properties, which may also alter bioavailability of the drugs and hence play an important role in the metabolism, absorption and distribution of the drugs available in the market.[19,20] The relationship between pharmacokinetic profiles and physical features of the synthesized compounds designed for clinical uses is a complex function reflected in properties such as absorption, permeability, plasma protein binding and metabolism just to mention a few.[20] In contrast, the relationship between the physical properties and *in vitro* potency on a biological target is much more clear.[20] Studies have shown that the physico-chemical properties of drug candidates on passing through different phases of clinical trials change and converge toward that of the marketed drugs. For example, mean molecular weight, lipophilicity and rotatable bond counts of the candidate drugs decrease at the end of the development phase, while the H-bond donor count remain fairly constant. As outlined above, investigation of physicochemical properties of compounds exerting medically useful activities is one of the main emphases in medicinal chemistry. In the current study, some physicochemical properties were measured and/or predicted for the previously synthesized thiazole-pyridinium compounds in our group. Also, the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties were calculated for these compounds and the results were discussed in terms of druglikeness property.

Materials and Methods

Compounds used for physicochemical studies

The experimental and calculated physicochemical properties were obtained for compounds previously synthesized with the aim of developing novel anti-AD agents.[18] The biological activity evaluations revealed that these compounds (listed in Table 1) may be used as novel lead compounds applicable in the development of compounds effective in AD.

Melting point

Melting points (mp) for the studied compounds were reported previously,[18] but included here in a lookup table for possible use. Briefly, the uncorrected melting points of all studied derivatives were determined by Kofler hot stage apparatus.

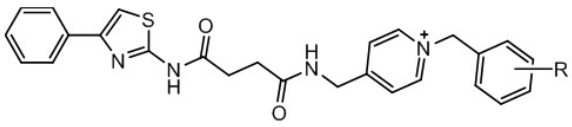
Partition coefficient determination

All chemicals were obtained from Merck Chemicals Co. (Darmstadt, Germany). Ultrapure water was obtained by Milli-Q water purification system (Millipore corporation, Bradford, MA, USA). UV spectrophotometric analysis was carried out by HALO DB-20 instrument (Switzerland). Determination of partition coefficient values for the studied compounds was carried out as follows. First, 200 mL of n-octanol and 200 mL of ultrapure water were mixed and agitated for 24 h at room temperature. Then, the mutually saturated solvents were separated. Next, stock solutions of the compounds were prepared by weighting 10 mg of each compound and dissolving in 10 mL of the presaturated solvents (i.e., water saturated n-octanol and n-octanol saturated water). Samples were prepared by using 200 µL of stock solution (stock solution of compound in either of saturated n-octanol or saturated water) and making up to 10 mL with the same solvent. Then, to the mixture was added 10 mL of the second solvent. The mixture was incubated at 25 °C while shaking at 180 rpm for 24 h. Two immiscible phases were separated and the concentrations of test compound were determined in both phases by UV spectroscopy at maximum absorption wavelength or lambda max (λ_{max}) of the compound using the calibration curve prepared in the same day. The λ_{max} values for the studied compounds in both solvents were ~261 nm. The calibration curves for each compound were constructed in both phases (saturated solvents) in the concentration range of 1.56-50 mg/L. The partition coefficient (PC) is the concentration of compound in n-octanol phase (C_o) over its concentration in aqueous phase (C_w), and was expressed in logarithmic scale as LogP values.

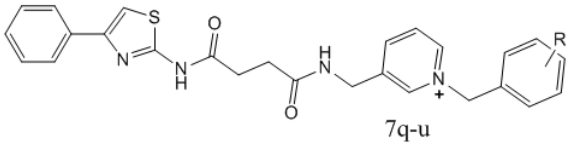
Retention factor (R_f) determination

High performance-thin layer chromatography (HPTLC) experiments were conducted by CAMAG system (Switzerland). For this purpose, glass backed silica plates 10×20 cm (Silica gel 60 F_{254}, Cat. 1.05642.0001) were

Table 1. Experimental partition coefficient (LogP) and R_f and melting point (mp values, as well as some physicochemical properties predicted for the studied compounds using ACD/Labs[a] and SwissADME[b].



7a-p



7q-u

Compound	R	Experimental LogP	[a]Predicted LogP	R_f	Mp °C	[b]MW g/mole	[b]HBD	[b]HBA	[b]tPSA	[b]RBC
7a	H	-0.63	-1.28±0.58	0.42	232-234	457.5	2	3	103.21	11
7b	2-NO_2	-0.38	-1.55±0.59	0.42	224-226	502.5	2	5	149.03	12
7c	4-NO_2	-0.56	-1.55±0.59	0.40	214-216	502.5	2	5	149.03	12
7d	2-F	-	-1.23±0.60	0.43	196-198	475.5	2	4	103.21	11
7e	3-F	-0.72	-1.23±0.60	0.42	226-228	475.5	2	4	103.21	11
7f	4-F	-0.41	-1.23±0.60	0.38	188-190	475.5	2	4	103.21	11
7g	2-Cl	-	-0.69±0.59	0.58	190-192	492	2	3	103.21	11
7h	3-Cl	-	-0.69±0.59	0.42	225-227	492	2	3	103.21	11
7i	4-Cl	-	-0.69±0.59	0.49	208-210	492	2	3	103.21	11
7j	2-Br	-0.68	-0.51±0.60	0.47	223-225	536.4	2	3	103.21	11
7k	3-Br	-0.66	-0.51±0.60	0.48	210-212	536.4	2	3	103.21	11
7l	4-Br	-	-0.51±0.60	0.41	221-223	536.4	2	3	103.21	11
7m	2-CH_3	-0.60	-0.82±0.58	0.52	224-226	471.6	2	3	103.21	11
7n	3-CH_3		-0.82±0.58	0.48	196-198	471.6	2	3	103.21	11
7o	4-CH_3	-0.86	-0.82±0.58	0.46	208-210	471.6	2	3	103.21	11
7p	3-OCH_3	-	-1.37±0.59	0.50	199-201	487.6	2	4	112.44	12
7q	H	-1.00	-1.28±0.58	0.48	158-160	457.5	2	3	103.21	11
7r	4-NO_2	-	-1.55±0.59	0.40	203-205	502.5	2	5	149.03	12
7s	4-F	-	-1.23±0.60	0.44	134-136	475.5	2	4	103.21	11
7t	4-Cl	-	-0.69±0.59	0.46	194-196	492	2	3	103.21	11
7u	4-Br	-0.70	-0.51±0.60	0.47	138-140	536.4	2	3	103.21	11

dipped in 6% W/V NaBr solution in methanol and then completely dried at room temperature.[21] Then, 3 µL of 10 mg/mL solution of studied compounds in methanol were spotted on the plate by automatic TLC sampler (ATS4). Afterwards, the plate was air dried and automatically developed using the solvent system consisting of methanol-dichloromethane (1:9) by ADC2 instrument. The developed plate was visualized under UV light at 254 nm. The migration of the compounds under the used chromatographic condition was determined using TLC Scanner 4. The retention factors for the compounds were measured and reported by winCATS1.4.4 software.

Predicted physical properties

The following software and web servers were used to calculate different physicochemical and druglikeness properties. Briefly, ACD/Labs (ver 2017.2.1; Advanced Chemistry Development Inc., Toronto, Canada) software was used to calculate CLogP values of compounds. SwissADME (http://www.swissadme.ch/) web server was used to calculate molecular weight (MW), H-bond donor (HBD), H-bond acceptor (HBA), topological polar surface area (tPSA), and rotatable bond count (RBC). Blood brain barrier (BBB) permeability and human intestinal absorption (HIA) were predicted using admetSAR web server (http://lmmd.ecust.edu.cn:8000/predict/). Molecular properties prediction tool provided by Molsoft LLC (http://www.molsoft.com/mprop/) online web server was used to calculate druglikeness score of the studied compounds.

Results and Discussion

Partition coefficients of studied thiazole-pyridinium derivatives

Partition coefficient is regarded as the most important physical property of pharmaceutical substances and is evaluated as one of the criteria in almost all druglikeness indices and/or rules such as those proposed for example by Lipinski, Oprea, and Egan.[22-24] The huge interest in partition coefficient among the researchers in the field of drug design and discovery is not surprising and is attributed to the fact that the biological effectiveness of drugs is highly correlated to this property. Many other physical/biological properties such as water solubility, receptor binding and ADME features of drug substances mostly can be predicted by this very single property, i.e. partition coefficient. The results of partitioning between n-octanol and water phases were determined for the studied compounds and expressed as logarithm of partition coefficient values (LogP) presented in Table 1. The LogP values are in the range of -1.00 to -0.38, which is in the acceptable range defined by many druglikeness rules. For example, based on Lipinsk's rule the calculated LogP (CLog P) should not be

greater than 5 (or MLogP < 4.15), while Egan et al. defined the maximum acceptable LogP value to be less than 5.88.[25] Some other indices set the lower and upper limits for LogP values slightly different. For instance, based on Ghose, Muegge and Oprea druglikeness filters, the LogP ranges are -0.4 to 5.6, -2.0 to 5, and -2.0 to 4.5, respectively.[24,26,27] Based on the measured LogP values for our recently reported thiazole-pyridinium cholinesterase inhibitors,[18] their lipophilicity fall into the acceptable range, though a bit far from optimum value for the compounds sought to act at CNS. The measured LogP values were compared with those predicted by ACDLab software. The predicted LogP values for the studied compounds range between -1.55 to -0.51, which is in reasonable agreement with those determined experimentally. However, the predicted and experimental data do not show any significant correlation, which can be ascribed to errors associated with the shake flask method of LogP determination and more importantly the close lipophilicity (LogP values) of the studied compounds. These two factors have caused the lack of correlation between experimental and theoretically predicted LogP values for these derivatives. Nonetheless, the close agreement between the range of experimental and predicted values needs to be acknowledged.

Chromatographic retention factor (R_f) of studied thiazole-pyridinium derivatives

The other physical property determined for the studied compounds was retention factor (R_f) in normal phase thin layer chromatography using NaBr treated silica gel as the stationary phase and mixture of methanol:dichloromethane (1:9 v/v) as the mobile phase. The silica gel plates were treated with NaBr solution to provide counterion for the permanently positively charged thiazole-pyridinium derivatives and hence their movement on the silica gel become possible. The determined R_f values showed reasonably well correlation to the predicted LogP values (r=~0.7). Considering narrow range of R_f and LogP values for the studied compounds, such a relatively good correlation indicates that the applied chromatographic method may be used to compare the lipophilicity of the positively charged pyridinium derivatives. Furthermore, the studied compounds demonstrated sharp melting point in the range of 134-234 °C indicating their high purity.

Druglikeness of the studied compounds

Apart from the LogP, which is an important physical property in relation to the behavior of a compound as drug molecule, some other calculated physicochemical properties for the studied thiazole-pyridinium derivatives such as MW, HBD, HBA, tPSA and RBC are also predicted and shown in Table 1. The figures in the table indicate that the studied compounds have acceptable structural properties in terms of the above-named properties. The molecular weight for the derivatives varies between 457.5 to 536.4 Da, which is in the acceptable range according to most druglikeness rules. The number of functional groups capable of establishing hydrogen bonds as the acceptor or donor would be appropriate to be equal or less than 10 and 5 groups in drug molecules, respectively. As can be seen in Table 1, all compounds comply with these criteria. According to different rules, total polar surface area (tPSA) for drugs should range between 20 to 150 $Å^2$ and consistent with that, none of the molecules in the table violate this rule. However, the number of rotatable bonds in almost all of the compounds fall just outside the acceptable cutoff value (RBC≤10). It worth mentioning that according to some studies, the acceptable RBC can be up to 15 bonds.[27] Different physicochemical properties of a ligand molecule influence its biological effectiveness through both pharmacodynamic and pharmacokinetic features. For example, flexibility of a ligand may improve its interaction with the receptor by facilitating the adoption of appropriate conformation by the ligand for a better fit to the binding site of the receptor. Likewise, the lipophilicity of a ligand contributes towards the receptor binding through hydrophobic interactions. In the same way, other physical properties of the biologically active molecules are also important for the observed pharmacodynamic behavior. On the other hand, the pharmacokinetic features of the active molecules are highly governed by the same physicochemical properties influencing their pharmacodynamic aspects. There are numerous studies where the physicochemical properties of drug molecules are related to their ADMET features.[28,29] Such studies make it possible to have an idea about the faith of drug candidates along the developmental pipeline. Table 2 shows blood-brain barrier (BBB) permeability and human intestinal absorption (HIA) capability for the studied compounds predicted by admetSAR server.[30] According to the results, the compounds may be absorbed from GI tract and reach the CNS and subsequently demonstrate anti-cholinesterase, anti-Aβ and neuroprotective activities reported for them.[18] High GI absorption probability for the studied derivatives was also predicted by SwissADME web server.[31] However, according to this site, the compounds are devoid of BBB permeability. Also, our previous work showed that compound **7j,** one of the most potent derivatives**,** may pass the BBB based on the results of the determined effective permeability (P_e value for **7j** is 5.9 × 10^{-6} cm/s) in PAMPA-BBB (parallel artificial membrane permeability) assay.[18] Such controversy is not unusual due to the error prone nature of the ADMET predictions.[32] The more decisive answer regarding GI absorption and BBB penetration requires experimental studies. However, there are some experimental studies showing CNS penetration of structurally similar chemicals.[33-36] The druglikeness scores for the studied thiazole-pyridinium compounds were predicted by Molsoft druglikeness and molecular property prediction site (http://www.molsoft.com/mprop/) and compared with neutral matching piperidine derivatives. According to this site, the range of druglikeness score for drug and non-drug compounds is very broad. But, for the majority of drug molecules, the scores are positive values

Table 2. Blood-brain barrier (BBB) permeability and human intestinal absorption (HIA) capability predicted for the studied compounds using admetSAR web server.

Compound	BBB	Probability (BBB)	HIA	Probability (HIA)
7a	+	0.9615	+	0.6295
7b	+	0.8137	+	0.9261
7c	+	0.8730	+	0.9059
7d	+	0.9456	+	0.6554
7e	+	0.9697	+	0.6704
7f	+	0.9697	+	0.6704
7g	+	0.9285	+	0.6580
7h	+	0.9575	+	0.6726
7i	+	0.9575	+	0.6726
7j	+	0.9140	+	0.5705
7k	+	0.9496	+	0.5874
7l	+	0.9496	+	0.5874
7m	+	0.9090	+	0.7547
7n	+	0.9439	+	0.6737
7o	+	0.9410	+	0.7445
7p	+	0.8829	+	0.8083
7q	+	0.9432	+	0.7010
7r	+	0.8547	+	0.9306
7s	+	0.9529	+	0.7382
7t	+	0.9373	+	0.7397
7u	+	0.9260	+	0.6633

+: permeable , - : unpermeable

Table 3. Druglikeness scores predicted by Molsoft druglikeness and molecular property prediction site for the pyridinium derivatives and matching piperidines.

Thiazole pyridinium derivatives	Druglikeness score	Matching Piperidine derivatives	Druglikeness score
7a	0.46	7a'	0.98
7b	-0.21	7b'	0.43
7c	0.01	7c'	0.57
7d	0.51	7d'	1.15
7e	0.61	7e'	1.16
7f	0.81	7f'	1.33
7g	0.72	7g'	1.32
7h	0.70	7h'	1.24
7i	0.99	7i'	1.48
7j	0.39	7j'	1.04
7k	0.45	7k'	1.02
7l	0.64	7l'	1.18
7m	0.52	7m'	1.13
7n	0.50	7n'	1.05
7o	0.43	7o'	1.01
7p	0.56	7p'	1.23
7q	0.55	7q'	0.91
7r	0.1	7r'	0.52
7s	0.90	7s'	1.27
7t	1.08	7t'	1.41
7u	0.73	7u'	1.12

up to 2, while the non-drugs often have negative values up to -3. As it is clear in Table 3, the pyridinium compounds with $-NO_2$ group in their structure show lower druglikeness score in comparison to the rest of the compounds, particularly when the nitro group is located in *ortho* position of benzyl moiety. Introduction of halogen atoms on the aromatic ring of benzyl moiety of the pyridinium compounds improves the druglikeness score and the best place for halogenation seems to be the *para* position. Moreover, among different halogens, Cl group in *para* position confers higher druglikeness score. Compared to pyridinium derivatives, proposed counterpart piperidines show higher druglikeness scores more likely due to the lack of permanent positive charge and it is assumed that they may represent anti-AD candidates with better ADME properties subject to having proper anticholinesterase activity akin to pyridinium derivatives.

One of the important futures evaluated for drug candidates during different phases of development process is the toxicity which is highly relevant to drug safety. Various *in vitro* and *in vivo* methods have been devised to determine the toxic effects of compounds. In addition, several *in silico* methods were also developed to use ample of available toxicity data for predicting toxic effects and screening for the possible early elimination of less promising drug candidates if required. Table 4 illustrates different toxicity parameters predicted for the studied compounds by admetSAR software directly from its web site. In general, according to the predictions, the studied thiazole-pyridinum derivatives may be considered compounds with low toxicity. However, a given derivative may show different toxicities based on different criteria. For example, compound **7a** is predicted to be a weak human ether-a-go-go-(hERG) encoded potassium ion channel inhibitor, non-toxic in AMES toxicity test, non-carcinogenic, a low toxic agent in honey bee toxicity test, whereas, it shows high toxicity in fish toxicity assay. It worth mentioning that nitro containing derivatives (**7b**, **7c** and **7r**) are predicted to be relatively more toxic. The results of neuroprotective assay on derivatives **7a, 7d, 7g, 7j** and **7m** revealed that some of these compounds can protect cultured PC12 cells against H_2O_2-induced cell toxicity indicating their *in vitro* non-toxic effects on the studied cells at the concentrations up to 100 μM.[18] These results in conjunction with the predicted toxic effects demonstrated in Table 4 point out to the fact that experimental assays are needed to complement the predictions. Development of novel drugs with effective influence on therapeutic targets with fewer side effects is the main goal of drug discovery. Parts of the observed side effects for drugs are related to their interaction with the metabolizing enzymes. Metabolism is the main elimination route for drugs leading to their biotransformation to metabolites often more susceptible for readily elimination from body. In most cases, the consequence of metabolism is detoxification and elimination of pharmaceuticals, but, it may also lead to the development of toxic metabolites contributing to the side effects and toxicity. The process of drug-metabolizing enzyme interaction may also result in drug-drug interaction by inducing and/or reducing the

Table 4. Various pharmaceutically important toxicity parameters predicted by admetSAR web server for the studied compounds.

Compounds	Human Ether-a-go-go-Related Gene	A M E S Toxicity	Carcinogens	Fish Toxicity	Honey Bee Toxicity	Acute Oral Toxicity	Carcinogenicity (Three-class)
7a	Weak I	Non	Non	H	L	III	Non-required
7b	Weak I	T	Non	H	L	III	Danger
7c	Weak I	T	Non	H	L	III	Danger
7d	Weak I	Non	Non	H	L	III	Non-required
7e	Weak I	Non	Non	H	L	III	Non-required
7f	Weak I	Non	Non	H	L	III	Non-required
7g	Weak I	Non	Non	H	L	III	Non-required
7h	Weak I	Non	Non	H	L	III	Non-required
7i	Weak I	Non	Non	H	L	III	Non-required
7j	Weak I	Non	Non	H	L	III	Non-required
7k	Weak I	Non	Non	H	L	III	Non-required
7l	Weak I	Non	Non	H	L	III	Non-required
7m	Weak I	Non	Non	H	L	III	Non-required
7n	Weak I	Non	Non	H	L	III	Non-required
7o	Weak I	Non	Non	H	L	III	Non-required
7p	Weak I	Non	Non	H	L	III	Non-required
7q	Weak I	Non	Non	H	L	III	Non-required
7r	Weak I	T	Non	H	L	III	Danger
7s	Weak I	Non	Non	H	L	III	Non-required
7t	Weak I	Non	Non	H	L	III	Non-required
7u	Weak I	Non	Non	H	L	III	Non-required

I: Inhibitor, H:high , L:low,T:Toxic , Non-require:Non carcinogenic, Acute Oral Toxicity: classI LD_{50}<50mg/Kg , classII 50<LD_{50}<500 , classIII 500<LD_{50}<5000, classIV LD_{50}>5000

metabolism of other co-administered drugs. Cytochrome P450 (CYP450) is the major drug metabolizing enzyme found as variety of isoforms. Various isoforms of CYP450 enzymes especially 2C9, 2D6, 3A4, 1A2, 2C9 and 2C19 are responsible for about 90% oxidative metabolic reactions.[37,38] Therefore, it is very important to evaluate the potential of a drug candidate to inhibit and/or induce CYP450 enzymes. As an important part of pharmaceutical drug discovery and development process the interactions of drug candidates with these enzymes are studied both experimentally and *in silico*. In the current investigation, the influence of studied thiazole-prydiniums on different drug metabolizing enzymes were evaluated using available online prediction tools, namely admetSAR and SwissADME web servers

Table 5. Effects of studied compounds on various CYP450 enzymes predicted by admetSAR web server.

Compounds	2C9 Substrate	2D6 Substrate	3A4 Substrate	1A2 Inhibitor	2C9 Inhibitor	2D6 Inhibitor	2C19 Inhibitor	3A4 Inhibitor
7a	N.S	N.S	N.S	I	I	N.I	I	I
7b	N.S	N.S	N.S	N.I	I	N.I	I	I
7c	N.S	N.S	N.S	N.I	I	N.I	I	I
7d	N.S	N.S	N.S	N.I	I	N.I	I	I
7e	N.S	N.S	N.S	I	I	N.I	I	I
7f	N.S	N.S	N.S	I	I	N.I	I	I
7g	N.S	N.S	N.S	N.I	I	N.I	I	I
7h	N.S	N.S	N.S	I	I	N.I	I	I
7i	N.S	N.S	N.S	I	I	N.I	I	I
7j	N.S	N.S	N.S	I	I	N.I	I	I
7k	N.S	N.S	N.S	I	I	N.I	I	I
7l	N.S	N.S	N.S	I	I	N.I	I	I
7m	N.S	N.S	N.S	N.I	I	N.I	I	I
7n	N.S	N.S	N.S	N.I	I	N.I	I	I
7o	N.S	N.S	N.S	I	I	N.I	I	I
7p	N.S	N.S	S	N.I	I	N.I	I	I
7q	N.S	N.S	N.S	N.I	I	N.I	I	I
7r	N.S	N.S	N.S	N.I	N.I	N.I	I	I
7s	N.S	N.S	N.S	N.I	I	N.I	I	I
7t	N.S	N.S	N.S	I	I	N.I	I	I
7u	N.S	N.S	N.S	I	I	N.I	I	I

N.S: Non Substrate , S: Substrate , I: Inhibitor , N.I: Non Inhibitor

Table 6. Effects of studied compounds on various CYP450 enzymes predicted by SwissADME web server.

Compounds	1A2 Inhibitor	2C19 Inhibitor	2C9 Inhibitor	2D6 Inhibitor	3A4 Inhibitor
7a	No	Yes	Yes	Yes	Yes
7b	No	Yes	No	No	Yes
7c	No	Yes	No	Yes	Yes
7d	No	Yes	Yes	Yes	Yes
7e	No	Yes	Yes	Yes	Yes
7f	No	Yes	Yes	Yes	Yes
7g	No	Yes	Yes	Yes	Yes
7h	No	Yes	Yes	Yes	Yes
7i	No	Yes	Yes	Yes	Yes
7j	No	Yes	Yes	Yes	Yes
7k	Yes	Yes	Yes	Yes	Yes
7l	Yes	Yes	Yes	Yes	Yes
7m	No	Yes	Yes	Yes	Yes
7n	No	Yes	Yes	Yes	Yes
7o	No	Yes	Yes	Yes	Yes
7p	No	Yes	Yes	Yes	Yes
7q	No	Yes	Yes	Yes	Yes
7r	No	Yes	No	No	Yes
7s	No	Yes	Yes	Yes	Yes
7t	No	Yes	Yes	Yes	Yes
7u	Yes	Yes	Yes	Yes	Yes

(Tables 5 and 6).

As shown in Tables 5 and 6, based on admetSAR, all derivatives were predicted as non inhibitor for 2D6 while being inhibitor of 2C9 (with the exception of **7r**), 2C19 and 3A4 isoenzymes. However, the effects on 1A2 isoenzyme are predicted to be structure dependent. The major difference in the results of predictions using SwissADME method is that the majority of the derivatives are predicted to be inhibitor of 2D6 isoenzyme. Biotransformation susceptibility of the studied compounds by major metabolizing enzymes predicted by different methods showed substantial contradictions. For example, admetSAR predicts that the studied compounds are not substrate for 2C9, 2D6 and 3A4 except for **7p** which is a substrate for 3A4. Further assessment of the metabolism susceptibility of the derivatives using RS-WebPredictor (ver 1.0) and BioTransformer web servers[39,40] indicates that the compounds may undergo metabolisms through different routes (please see Tables 7 and 8 for few representative biotransformation products). Overall, the results of predictions based on the above-mentioned two methods indicate that the studied compounds are not good substrates of CYP450 enzymes and may act as inhibitors of the metabolizing enzymes. Hence, one may deduce that they may show long duration of action *in vivo* due to not being substrate of metabolizing enzymes and may also lead to drug-drug interaction as the result of inhibiting various CYP450 isoenzymes. Such disagreements among the results obtained based on different algorithms necessitate experimental approaches for reliable conclusion on metabolic fate of the studies compounds. However, the results from *in silico* studies are very valuable and can help the researchers along the way.

Table 7. Number of metabolites predicted for the studied compounds using BioTransformer web server. A representative predicted metabolite was shown for each derivative as the example.

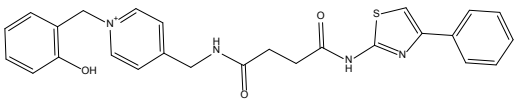
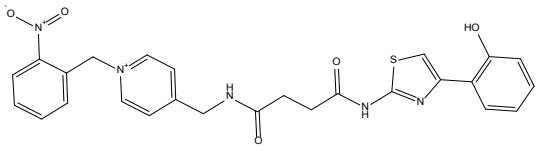
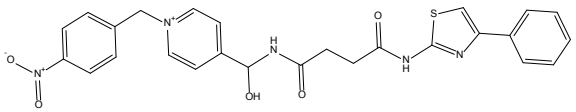
Compounds	Number of metabolites	Representative example
7a	11	
7b	11	
7c	10	

Table 7. Continued.

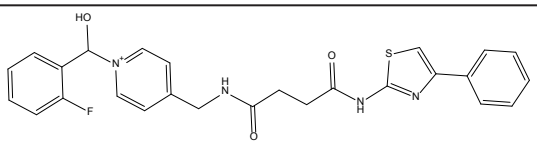
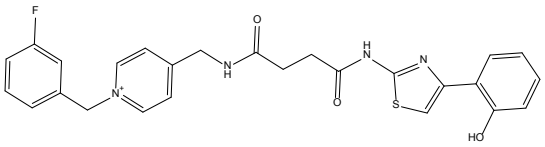
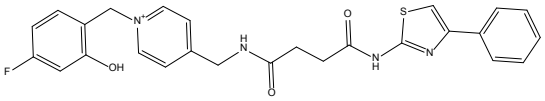
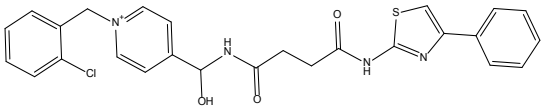
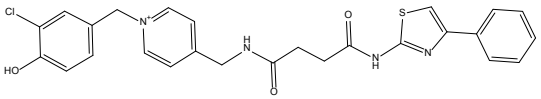
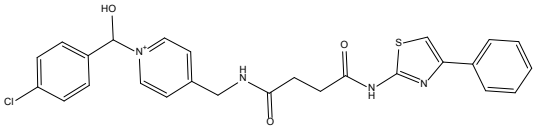
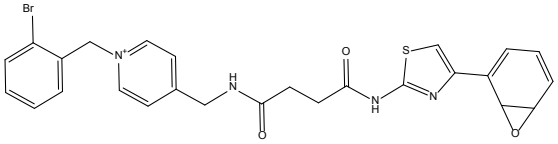
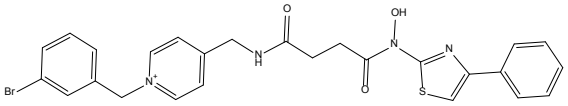
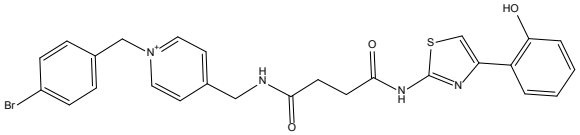
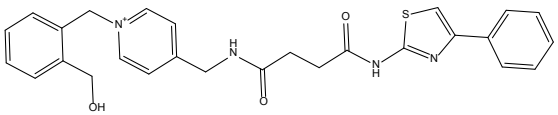
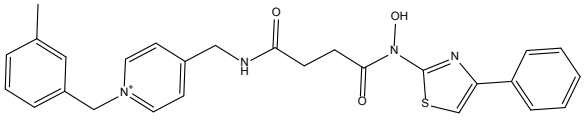
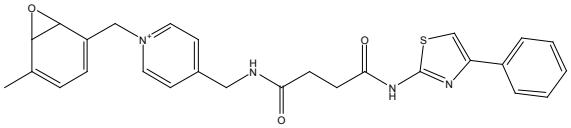
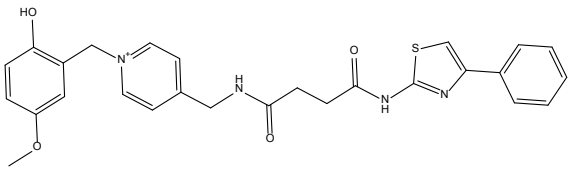
7d	13	
7e	13	
7f	11	
7g	13	
7h	13	
7i	11	
7j	13	
7k	13	
7l	11	
7m	14	
7n	13	
7o	12	
7p	13	

Table 7. Continued.

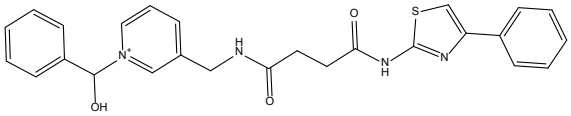
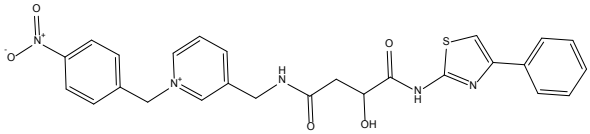
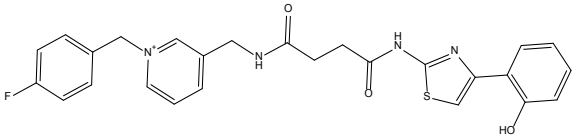
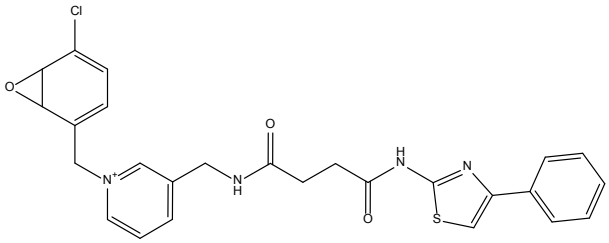
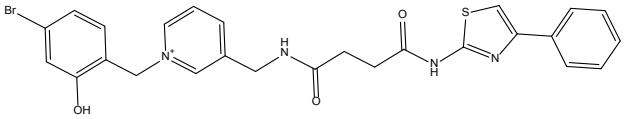
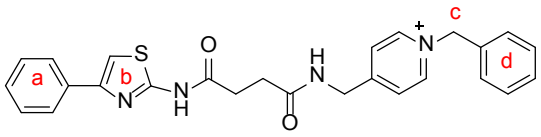
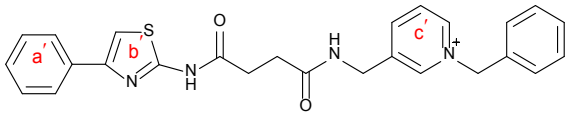
7q	11	
7r	10	
7s	11	
7t	11	
7u	11	

Table 8. The structural regions susceptible for metabolic attack identified by RS-WebPredictor web server. The regions predicted to be transformed by metabolizing enzymes were ordered based on the propensity of being metabolized.





	Metabolizing enzymes	
Compounds	**2D6 isoform**	**3A4 isoform**
7a	b-a-c	b-c-b
7b	b-a-c	b-b-c
7c	b-a-d	b-b-c
7d	b-a-c	b-c-b
7e	b-a-c	b-c-b
7f	b-a-c	b-c-b
7g	b-a-c	b-c-b
7h	b-a-c	b-c-b
7i	b-a-c	b-c-b
7j	b-c-a	b-c-b
7k	b-a-c	b-c-b
7l	b-a-c	b-c-b
7m	b-d-a	b-d-c
7n	b-d-a	b-d-c
7o	b-d-a	b-d-c
7p	d-b-a	b-d-c
7q	b'-a'-a'	b'-c'-c'
7r	b'-a'-d'	b'-c'-a'
7s	b'-a'-a'	b'-c'-c'
7t	b'-a'-a'	b'-c'-c'
7u	b'-a'-a'	b'-c'-c'

Conclusion

Some physicochemical properties were measured/predicted for few thiazole-pyridinium derivatives known to inhibit acetylcholinesterase designed as potential anti Alzheimera gents. The experimental results of LogP are in good accordance with the predicted ones. Moreover, the determined R_f values obtained from HPTLC analysis show reasonably well correlation to the predicted LogP values (r=~0.7). Considering narrow range of R_f and LogP values for the studied compounds, such a relatively good correlation indicates that the applied chromatographic method may be used to compare the lipophilicity of the positively charged pyridinium derivatives by the described method. According to the admetSAR web server predictions, the studied compounds were predicted to be absorbed from GI tract and reach the CNS and subsequently may demonstrate their anti-cholinesterase, anti-amyloid-β and neuroprotective activities. Besides, the comparison of the druglikeness scores of thiazole-pyridinium derivatives with those of the matching piperidines indicated higher druglikeness scores for the piperidine counterparts more likely due to lack of permanent positive charge in their structures. The toxicity studies show that the compounds are not overall toxic.

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Author Contributions

GG: Carried out all experiments. SD: Assisted in preparation of the compounds. AT: Assisted in conducting physicochemical experiments. MH: Assisted data analysis and *in silico* studies and helped in manuscript preparation. SD: Supervised the project, carried out the data analysis and interpretations of the results, and prepared the manuscript. All authors have read and agreed on the published version of the manuscript.

Conflict of Interest

The authors have no the conflict of interest.

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