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PHARMACOKINETIC STUDY IN OXIDIZED O-AMINOPHENOL AND ITS BYPRODUCT COMPOUNDS: AN ORGANIC POLLUTANT

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ABSTRACT

Hazardous substances in environment generate toxicity in acute life of living beings. Pollutants are created by trash or run-off produced by the industries, Polluted water, air, and soil have an impact on not only human activities but crops and aquatic life also. Present study was done to demonstrate the pharmacokinetic analysis in o-aminophenol and byproduct compounds of oxidized o-aminophenol. *In silico* ADME/Tox and PASS prediction study was done on the o-aminophenol and byproduct compounds to evaluate their toxicity level. LD_{50} value of these compounds gives affirmation for the safety of these compounds. Some of byproduct compound 11-H dibenzo [b,e] [1,4]dioxepin-11-one, Phthalic acid and Phenol-2,4-bis (1,1-dimethylethyl) are non-carcinogenic in nature. Mostly byproducts are non-mutagenic, non-cytotoxic and non-hepatotoxic in nature. o-aminophenol likely to have carcinogenic nature than the byproduct compounds. These compounds depict drug likeliness property could be better used in pharma industries for the drug synthesis.

KEYWORDS: ADMET study, *In silico*, o-aminophenol, Organic pollutant, Pharmacokinetics.

1. INTRODUCTION

Life on this planet and human health are gifts from the natural environment. However, industrialization and continually improving technologies have exposed human life to numerous negative environmental effects.[1] Air, water, soil, and noise pollution are all examples of environmental contamination.[2] Most often, hazardous substances released

during industrial waste processes like heavy metals, organic dyes, phenols and nitro compounds etc., are to blame.[3,4,5,6] In the ceramics, paper industry, cosmetic, textile, food processing sectors, pharmaceutical, synthetic pigments, dyes, phenol and nitro compounds, are frequently employed.[7] Toxic impact has been reported on water habitat, terrestrial organisms, and wild life when concentrations of such toxic substances in range 1-10 mg/liter, which is considerably high in concentration to the environment.[8] Environmentalists and a number of chemists have shown interest in it. Because effluent containing harmful organic pollutants is directly receiving water stream, and produced water pollution, which is an important concern in society recently.[9] These harmful chemicals build up in the soil in significant amounts, and when used over an extended period of time, they can have deleterious consequences on crop productivity and, eventually, human health. For the eradication of organic pollutants, many conventional techniques are used, including adsorption,[10] Fenton's reagent,[11] ozonation,[12] electro-chemical oxidation,[13] and ion-exchange[14] among others. However, these techniques face challenges due to their high cost, sludge formation, lengthy process times, and difficult operational requirements. Many chemists are working in the field of oxidation of these harmful organic pollutants. Such as oxidation of o-aminophenol using hexacyanoferrate (III) claims its oxidative coupling into 2-aminophenoxazin-3-one.[15] The 2-aminophenoxazin-3-one is an anti-cancerous compound as reported. This study is enlightens the reaction specially concern with its by-products. To find out the possibility of these byproducts are in pharmacological activity as an application. Development of a new drug is costly and takes so much time and effort. Numerous computational aided prediction techniques seeking attention for the purpose of drug feasibility prediction at initial stage.[16] Nowadays *in silico* study for toxicity and drug likeliness prediction of chemical compound achieve a great attention during the earliest phase of drug discovery. It is recommended checking the bioavailability of compound before the clinical trial failure on the initial stage of drug discovery.[17] ADMET study predicts the in vivo, in vitro toxicity and pharmacokinetics of large database via computational modeling with improve in chemical characteristics of compounds.[18] The concept of biological potency of bioactive compounds was forecasted using Pass software. On the basis of structural formulae, the pharmacological effects and biochemical potential may be used efficiently to find new ligands for biological targets and vice versa using Pass chemo-informatics software.[19] This study emphasize the drug likeliness, pharmacokinetic forecasting and biological efficacy prediction in o-aminophenol and byproduct compounds observed in oxidative degradation of o-aminophenol an organic pollutant for further used in drug

synthesis in pharma industries. The goal of this publication is to check the feasibility of product with its side products using *in silico* study.

2. EXPERIMENTAL

2-aminophenol was purchased from CDH New Delhi, India. Thomas Baker (Chemicals) Pvt Limited Mumbai, India had provided analytical grade potassium ferricyanide. For this research analytical grade chemicals was used. GC-MS analysis was done by GC-MS spectrometer model number-QP2010 Plus. Sample was freshly prepared in double distilled water for the experimental study.

2.1 Experimental procedure

For the experimental study, prepare 2-aminophenol 2×10^{-4} M solution equipped with magnetic stirrer in 100 ml flask. Mix an appropriate amount of 2-aminophenol and oxidant in a 250 mL iodine flask. Further add required amount of hexacyanoferrate (III) ions with 2-aminophenol in iodine flask (250 ml) reaction mixture to commence the reaction. On oxidative degradation of 2-aminophenol, the byproducts formed was identified by GC-MS spectrometer. Those byproducts were further allowed to investigate the drug likeliness and toxicity present in it.

2.2 Software analysis

2.2.1 *In-silico* admet study

To evaluate ADMET study, the structure of compounds identified via GC-MS were drawn using Chem draw. To start the prediction, firstly the molecular formula of compound was transformed into SMILES format using pubchem. Pharmacokinetic characteristics of the compounds were forecasted using Swiss ADME software, according to the developed protocol.[20] The toxicity study was done using ProTox-II webserver. Toxicity in compounds was forecasted in the terms of different criteria i.e. oral toxicity, organ toxicity (hepatotoxicity) and toxological end points (mutagenicity, cytotoxicity, immunotoxicity and carcinotoxicity).

2.2.2 *In silico* pass prediction of biological potential

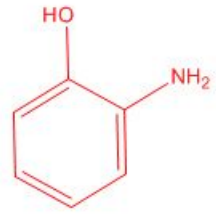
PASS software used to predict the biological activity of o-aminophenol and byproduct compounds. Pass is a chemoinformatic software to estimate the biological activities of compound on the basis of structural similarities to a large library of active components. Pac (chances to be active) or Pin (chances to be inactive) readings was used to evaluate the

bioactivity score. Pac> Pin, the predicted compound was likely active. Compounds were predicted to manifest distinct bioactivities (Pac> Pin).

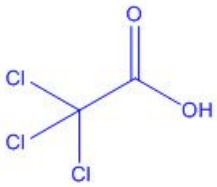
3. RESULTS AND DISCUSSION

3.1 GC-MS analysis

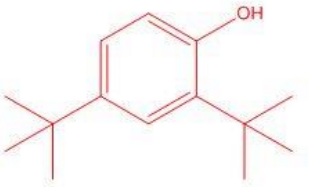
GC-MS analysis identified the compounds in oxidized o-aminophenol presented in **Fig 1**.



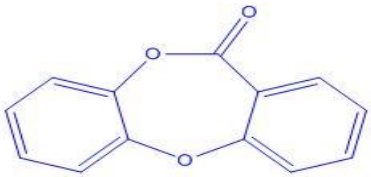
o-aminophenol



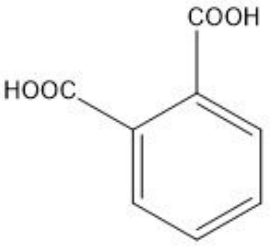
Trichloroacetic acid

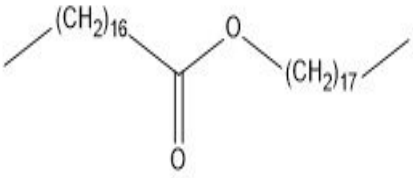


2,4-bis (1,1dimethylethyl)Phenol

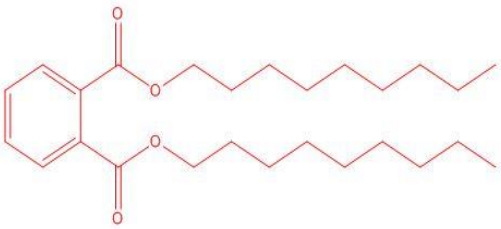


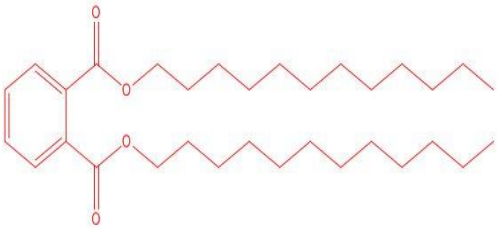
11-H dibenzo[b,e][1,4]dioxepin-11-one





Phthalic acid Octadecylester





dinonyl ester-1,2-Benzenedicarboxylic acid Didoceyl phthalate

Fig. 1: Chemical compounds identified in oxidized o-aminophenol

3.2 *In silico* admet study

The ADME attributes, pharmacokinetics and drug likeliness of compounds are assessed using Swiss ADME online software. To examine the drug properties, Lipinski's, Egan's and Veber's rule were followed.[21, 22, 23] As per as Lipinski's rule, the compound must have molecular weight (MW) is <500, TPSA (topological surface area) < 140, nOHD (no. of H-bond bonds) donors) ≤ 5, nOHA (no. of H bond acceptors) ≤ 5, and nRB (no. of rotatable ≤ 10 for the oral drugs. Present study depicts 5 compounds exhibits potent drug like properties subsequently follow the Lipinski's rule. A good brain penetration potential was observed with TPSA values less than 120 $Å^2$. Effective bioactive nature was confirmed by bioavailability score 0.55 observed in three compounds. P-glycoprotein (P-gp) substrate was found in o-aminophenol, octadecylester recommending poor fine intestinal absorption of compounds. The consensus Log Po/w (0.84-4.56) value predicts good lipophilicity character observed in Trichloroacetic acid, Phenol-2,4-bis (1,1-dimethylethyl), 1,2-Benzenedicarboxylic acid, 11-H dibenzo [b,e] [1,4]dioxepin-11-one and Phthalic acid. Trichloroacetic acid, Phenol-2,4-bis (1,1-dimethylethyl), Didoceyl phthalate, 1,2-Benzenedicarboxylic acid, dinonyl ester, 11-H dibenzo [b,e] [1,4]dioxepin-11-one and Phthalic acid demonstrated high gastrointestinal absorption power. Trichloroacetic acid, Phenol-2,4-bis (1,1-dimethylethyl) and 1,2-Benzenedicarboxylic acid intercross the blood-brain barrier (BBB). O-aminophenol and the byproduct compounds mainly interact with four isozymes of cytochrome group, i.e. CYP1A2 and CYP2C19, CYP2C9, CYPD6 attributing their potency, although possessing least toxicity. The GI absorption and drug like qualities in o-aminophenol and its side product were presented by boiled-egg prediction presented in **Fig 2**.

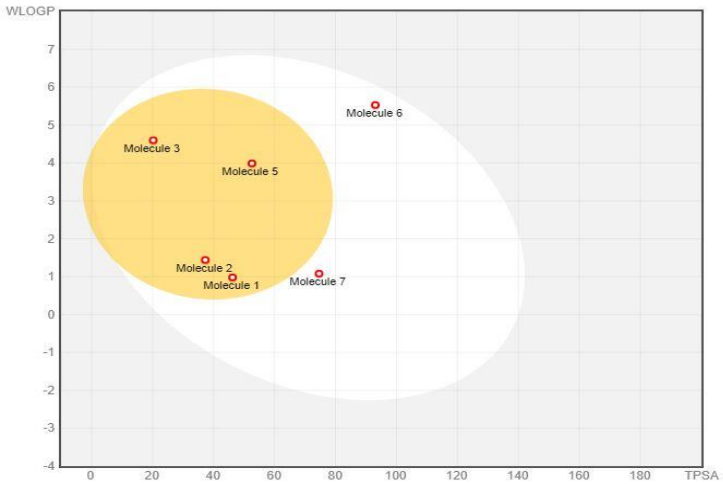


Fig. 2: Boiled-egg graph of oxidized o-aminophenol and byproduct compounds.

The yellow part in boiled- egg diagram can infuse via BBB (blood-brain barrier), and the drug-likeness of compounds was shown in bioavailability radar graphs presented in **Fig 3.**

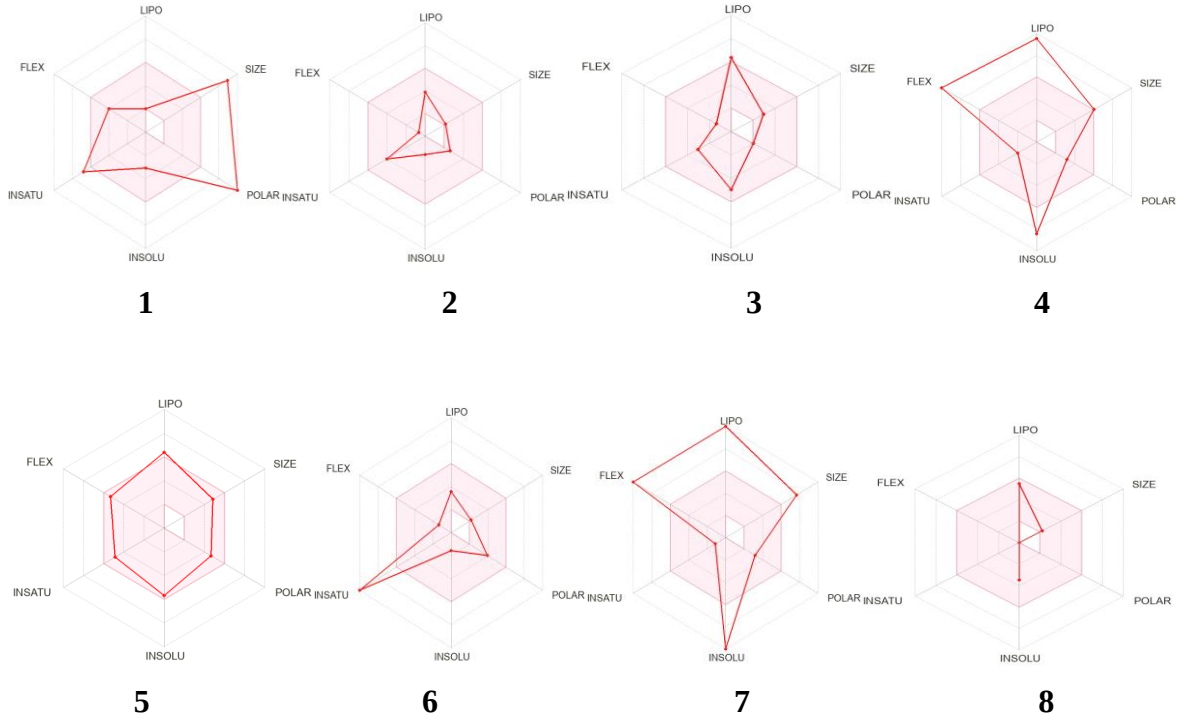


Fig. 3: Bioavailability radar graph (pink area depicts the drug likeliness property) 1: o-aminophenol, 2: Trichloroacetic acid, 3: Phenol-2,4-bis (1,1-dimethylethyl), 4: Didoceyl phthalate, 5: dinonyl ester-1,2 Benzenedicarboxylic acid, 6: 11H dibenzo [b,e] [1,4] dioxepin-11-one, 7: Phthalic acid, 8: Octadecylester.

A web server ProTox-II was used to predict the ADME study and toxicity parameter of organic compounds presented in **table 1, 2.** Trichloroacetic acid, Didoceyl phthalate, 1, 2 Benzenedicarboxylic acid, dinonyl ester, Octadecyl ester was predicted to be carcinogenic in nature. O-aminophenol is hepatotoxic and 11H dibenzo [b,e] [1,4]dioxepin-11-one is immunotoxic in nature. These compounds were forecasted to exhibits non-cytotoxic and non-mutagenic. To assure the safety of compounds, LD_{50} value was also calculated.

Table 1: *In silico* ADME prediction in o-aminophenol and its byproducts.

Entry	1	2	3	4	5	6	7	8
TPSA * ($Å^2$)	345.26	37.3	20.23	52.6	52.6	93.06	74.6	52.6
Consensus * Log Po/w	-2.01	1.24	4.55	9.03	4.01	4.56	0.84	13.21
Mol wt (g/mol)	755.9	163.39	234.38	502.77	292.37	398.45	166.13	647.07
nRB	6	1	2	26	11	8	2	39
nOHA	14	2	1	4	4	6	4	4
nOHD	4	1	1	0	0	2	2	0
WLOGP	-5.64	1.44	4.6	9.84	3.99	5.53	1.08	13.71
Water solubility	Soluble	Soluble	Moderately soluble	Insoluble	Moderately soluble	Poorly soluble	Soluble	Insoluble
P-gp substrate **	Y	N	N	N	N	N	N	Y
GI absorption **	Low	High	High	Low	High	High	High	Low
BBB permeant **	N	Y	Y	N	Y	N	N	N
CYP3A4 inhibitor **	N	N	N	N	N	N	N	N
CYP1A2 inhibitor **	N	N	N	N	Y	N	N	N
CYP2C19 inhibitor **	N	N	N	N	Y	Y	N	N
CYP2C9 inhibitor **	N	N	N	N	N	Y	N	N
CYP2D6 inhibitor **	N	N	Y	N	N	N	N	N
Lipinski violation	2	0	1	2	0	0	0	2
Log Kp (cm/s)	-12.33	-6.35	-3.73	-1.4	-4.76	-4.68	-6.8	2.9
Bioavailability score ***	0.11	0.85	0.55	0.17	0.55	0.55	0.85	0.17

N: No, Y: Yes, TPSA: topological polar surface area, nRB: no. of rotable bond, nHA: no. of H-bond acceptor, nHD: no. of H-bond donor, GI: gastrointestinal absorption, BBB: blood-brain barrier, WLOGP: water partition coefficient, P-gp: permeability glycoprotein, CYP: cytochrome P_{450}. Entry: 1: o-aminophenol, 2: Trichloroacetic acid, 3: Phenol-2, 4-bis (1,1-dimethylethyl), 4: Didoceyl phthalate, 5: 1,2 Benzenedicarboxylic acid, dinonyl ester 6: 11H dibenzo [b,e] [1,4]dioxepin-11-one, 7: Phthalic acid, 8: Octadecylester

Table 2: Toxicity analysis in o-aminophenol and its byproducts.

Compounds	Hepatotoxicity		Carcinogenicity		Cytotoxicity		Immunotoxicity		Mutagenicity		Predicted LD_{50} (mg/kg)	Toxicity Level
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2		
o-aminophenol	H	0.51	Ncr	0.59	Ncy	0.61	Ni	0.97	Nm	0.61	2497	V
Trichloroacetic acid	Nh	0.81	Cr	0.86	Ncy	0.71	Ni	0.99	Nm	0.87	2820	V
Phenol-2,4-bis (1,1-dimethylethyl)	Nh	0.78	Ncr	0.52	Ncy	0.91	Ni	0.84	Nm	0.99	1420	IV
Didoceyl phthalate	Nh	0.84	Cr	0.75	Ncy	0.89	Ni	0.69	Nm	0.99	1340	IV
1,2-Benzenedicarboxylic acid, dinonyl ester	Nh	0.83	Cr	0.62	Ncy	0.88	Ni	0.69	Nm	0.94	1340	IV
11H dibenzo [b,e] [1,4] dioxepin-11-one	Nh	0.74	Ncr	0.51	Ncy	0.7	I	0.82	Nm	0.72	1310	IV
Phthalic acid	Nh	0.65	Ncr	0.69	Ncy	0.9	Ni	0.99	Nm	0.99	2530	V
Octadecylester	Nh	0.84	Cr	0.71	Ncy	0.87	Ni	0.57	Nm	0.98	8000	VI

P1: Prediction, P2: Probability, H: Hepatotoxic, Nh: Non-hepatotoxic, , Ncy: Non-cytotoxic, I: immunotoxic, Ni: Non-immunotoxic, Nm: non-mutagenic, Cr: Carcinogenic, Ncr: Non-carcinogenic (Toxicity level I: if swallowed, fatal ($LD_{50} \leq 5$), (Toxicity level II: if swallowed, fatal ($5< LD_{50} \leq 50$), (Toxicity level III: if swallowed, toxic ($50< LD_{50} \leq 300$), (Toxicity level IV: if swallowed, harmful ($300< LD_{50} \leq 2000$), (Toxicity level V: if swallowed, possibly harmful ($2000< LD_{50} \leq 5000$), (Toxicity level VI: non-toxic($LD_{50} >5000$)

3.3 *In silico* pass prediction analysis

In Silico Pass prediction for biological activities presented in **table 3**.

Table 3: Pass prediction for biological activity in o-aminophenol and its byproducts.

Compound	Carcinogenic	Hyperglycemia	Mutagenic	Non mutagenic	Cytotoxic	Hypoglycemia	Inflammation
	Pac>Pin	Pac>Pin	Pac>Pin	Pac>Pin	Pac>Pin	Pac>Pin	Pac>Pin
1	0.67>0.01	0.73>0.02	0.57>0.01	-	0.23>0.08	0.25>0.05	0.52>0.07
3	0.26>0.05	0.47>0.11	0.10>0.05	0.41>0.04	0.19>0.10	0.17>0.13	0.42>0.12
4	0.35>0.03	0.69>0.03	0.13>0.05	0.86>0.05	0.21>0.08	0.38>0.02	0.57>0.05
5	0.3>0.04	0.65>0.04	0.12>0.05	0.85>0.01	0.2>0.1	0.36>0.02	0.56>0.05
6	0.21>0.07	-	-	0.32>0.06	0.23>0.07	0.17>0.13	0.49>0.08
7	0.35>0.03	0.76>0.02	0.2>0.03	0.73>0.01	0.22>0.08	0.37>0.01	0.59>0.04
8	0.48>0.02	0.73>0.02	0.20>0.03	0.71>0.02	0.35>0.05	0.34>0.02	0.54>0.06

Pac= probable activity, Pin= probable inactivity

Alexy et al 2000[19] depicts the compound have Pac>0.7, found the substance is more likely to possess activity in analysis. Pac value lies in between 0.5 to 0.7; the substance is likely to exhibits activity in analysis. Pac<0.5, the substance is not likely to have activity in the analysis. Though the presence of this activity is admitted in analysis depicts new compound might be possible a new existing chemical. This study demonstrated o-aminophenol (Pac<0.7) exhibits likely to have carcinogenic activity. O-aminophenol, Phthalic acid, Octadecylester possess more likely (Pac<0.7) and 1, 2 Benzenedicarboxylic acid, Didoceyl phthalate exhibits likely (0.5<Pac<0.7) to have hyperglycemic potential. O-aminophenol (0.5<Pac<0.7) likely to have mutagenic activity. Didoceyl phthalate 1, 2 Benzenedicarboxylic acid, Phthalic acid, Octadecylester exhibits more likely (Pac<0.7) to have non mutagenic potential. O-aminophenol, Didoceyl phthalate, 1,2 Benzenedicarboxylic acid, Phthalic acid, Octadecylester possess likely (0.5<Pac<0.7) to have inflammation property. All the substance have less likely (Pac<0.5) to have cytotoxic property. Nevertheless further study could be done on the biological efficacy of these compounds.

CONCLUSION

Pollutants are the major risk for the health concern purpose. On the oxidative coupling of o-aminophenol (organic pollutant) some of the side products were also observed. As byproduct compounds are not often to be studied, only focussed on the main compound, present study was done to comparative evaluation of main compound (Substrate o-aminophenol) with byproduct compound efficacy on the basis of pharmacokinetic analysis. ADME/Tox predicts that o-aminophenol slightly exhibits carcinogenic activity while on the contrary Phenol-2,4-bis (1,1-dimethylethyl), 11H dibenzo [b,e] [1,4]dioxepin-11-one and Phthalic acid are non carcinogenic compounds. On the basis of clinical manifestation, pass study predicts almost all compounds are non-hepatotoxic, non- immunotoxic, non-cytotoxic and non-mutagenic in nature. Toxicity class demonstrates these compounds are slightly toxic if swallowed. Octadecylester is non-toxic in nature. On the premise of phartmacokinetic study, these by-products could be effectively used for the experimental biological efficacy for future implementation in pharma industries.

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