Original research article

PREDICTING PHARMACOKINETIC PROFILES OF SUNFLOWER’S (Helianthus annuus L.) ACTIVE COMPOUNDS USING IN SILICO APPROACH

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Abstract

Introduction: Sunflower (Helianthus annuus L.) widely known as medicinal plant for treating several diseases, such as hypertension, allergy, pain, inflammation, and cancer. It contains various bioactive compounds which some of them were hellianuols. Hellianuols are a sesquiterpene lactones which marked by benzene fused 6- to 8-membered cyclic ether ring structure. To make sure that hellianuols were adequate for development as a new chemical entities, we predicted some pharmacokinetic parameters of several hellianuols compounds (A to L) through in silico approach. Methods: We constructed 3 dimensional structures of hellianuol A, B, C, D, E, F, G, H, I, J, K and L then generated the SMILE codes of each compound. These codes then used as main material for running pkCSM online tool to predict absorption, distribution, metabolism, and excretion profile of each compounds. Results: Hellianuols predicted to be well absorbed in intestine (90.793% to 95.384% permeability), skin (Log Kp: -2.662 to -3.570), and high permeability against monolayer Caco-2 cell lines (LogP_app: 1.186 to 1.341 ×10⁻⁶ cm/s). Unfortunately, it had been predicted that hellianuols poorly distributed in the body based on volume of distribution at steady state (VDss: 0.094 to 0.317) value. But its also predicted that most of hellianuols had a capability to pass through blood-brain barriers (LogBBB: up to 0.389) and penetrated into central nervous system as well. Only hellianuol G, H and K predicted to be metabolized by CYP1A2 inhibitor and only hellianuol A, B, D, E and K metabolized by CYP2C19. Also predicted that hellianuols were excreted in around 0.719 to 1.082 mg/kg/day. Conclusion: Hellianuols contained in leaf aqueous extract of sunflower predicted to be a good new pharmaceutical entities candidate based on its pharmacokinetic profiles.
INTRODUCTION

The discovery of new chemical entities originated from plants has attracted much attention in the last two decades. It is becoming more important to implement modern methodologies and innovative strategy for designing an effective, safe, and acceptable new drug (1). Qualified drug must meet some standards of pharmacokinetics aspects, abbreviated with ADME (Absorption, Distribution, Metabolism, Excretion). It takes more time and also costs for conducting in vivo and in vitro data to assure that drug candidates are good in absorption, distribution, metabolism, and excretion. Predicting pharmacokinetic aspects through in silico and computational modelling is important nowadays, especially to cut off time and cost requirement of a new drug discovery (2).

Macias et al. were successfully isolated and elucidated 12 kinds of sesquiterpene compounds from aqueous extract of Helianthus annuus L. cv. SH-222 leaves, namely: Hellianuol A, B, C, D, E, F, G, H, I, J, K, and L (3,4). Hellianuols structure marked with the presence of benzene fused 6- to 8-membered cyclic ether ring so they are included in sesquiterpene compounds (5).

Some hellianuol (B, G, H) have a benzoxepine ring inside their structure. The structure of helianuols A-L represented in Figure 1. According to Kuntala et al., derivatives of benzoxepine showed various pharmacological effects such as: antibacterial and anticancer activity, anti-hypertension agent, estrogen receptor modulator and anti-HIV agent (6–10). Hellianuols itself widely observed as natural herbicide agents due to its herbicidal activities against wide range of monocotyledons and dicotyledons (11).

MATERIALS AND METHODS

The structure of twelve hellianuols were designed using ChemBio2D Draw Ultra v12.0 (CambridgeSoft) and obtained the SMILE string of each compound. Using the SMILE string, we predicted absorption, distribution, metabolism and excretion (ADME) profiles of hellianuol A-L. The prediction of twelve compounds was conducted using pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediciton).

RESULTS AND DISCUSSION

The pharmacokinetic properties of hellianuol A-L according to pkCSM online tool are presented in Table 1.

Absorption

Hellianuol A-L exhibited high value (90.763% to 95.384%) of predicted % human intestinal absorption as their values are above 80% (12). Hellianuol A-L predicted to be skin permeable as their skin permeability constant values (−2.662 to −3.570 logKp) are below -2.5 (13). The ability of hellianuols to transport through intestinal mucosa membrane were predicted by apparent permeabilities of Caco-2 cell line parameter (Papp) (14).

According to US FDA, Papp values <1×10⁻⁶ cm/s indicate poor permeability and $P_{app} >10×10^{-6}$ cm/s indicate high permeability, then hellianuol A-L exhibited 1.186 to 1.341 ×10⁻⁶ cm/s LogPapp (15), it could be predicted that hellianuol A-L were poorly permeable through Caco-2 cell line. Another aspect of absorption were predicted through hellianuols interactions to P-glycoprotein (P-gp), a transporter protein that play several important roles during drug absorption, especially for expelling molecules out of cells (16). Based on their structures, there are eight hellianuols (A, B, C, D, E, G, H, L) predicted to be the substrate of P-gp, thus only hellianuol F, I, J and K could be absorbed without extracted out of cells.

Distribution

Steady state volume of distribution (Vdss) is a pharmacokinetic parameter to estimate drug distribution within tissues. The higher the Vdss value, the more drug concentration is distributed into the tissue.
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Figure 1. Chemical structures and common names of the 12 hellianuol compounds investigated in this study.

than plasma (17). Vdss considered low if its logarithmic form under -0.15 (logVdss < -0.15) and high if above 0.45 (logVdss > 0.45) (18). Hellianuols showed moderately distributed with logVdss values range between 0.094 – 0.317.

Based on pkCSM result, LogBB values of hellianuols vary between -0.616 and 0.389. Hellianuol A, B, D, G, H, and K have such chemical structures that allows them to readily cross the blood-brain barrier (LogBB > 0.3), while hellianuol C, E, F, I, J, and K predicted as moderately distributed to the brain (LogBB < 0.3) (18). It is also predicted that hellianuol A, B, C, and D would shows such good in situ brain perfusions result (logPS > -2), while only hellianuol L showed low prediction value (LogPS = -3.189).

PkCSM could predict how molecules are distributed within the body based on their structure. From the data supplemented in Table 1, we predicted
that hellianuols could be well distributed in the body. It could be assumed as well that some hellianuols would be distributed to the brain. Especially hellianuol A, B, and D which have high values of LogBB and LogPS, so they are predicted to readily cross the blood-brain barrier and penetrated directly into the central nervous system (19,20).

Metabolism

Cytochrome P450s is an important enzyme for xenobiotics metabolism in liver. Two main subtype of cytochrome P450 are CYP2D6 and CYP3A4. Metabolism of hellianuols are predicted based on models for different CYP isoforms (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) (18,21). The results showed that hellianuol G and H were predicted to be CYP1A2 inhibitor, and hellianuol A, B, D, and E were predicted to be CYP2C19 inhibitor. This suggested that hellianuols may not metabolised directly in liver as they were not being the substrate of Cytochrome P450 enzymes family, but hellianuol A, B, D, E, G, H, K may affect another drug metabolisms, as they were predicted could be the Cytochrome P450 inhibitor.

Excretion

Excretion profile of hellianuols were predicted using drug total clearance parameter (CLtot). Total clearance is a combination of hepatic and renal clearance, measured in log ml/min/kg (22). The prediction results show that the total clearance of hellianuol C is the highest followed by hellianuol E, B, D, K, A, G, H, F, L, I and J. Indicating that bioavailability of hellianuol J is the highest. The results also suggested that hellianuols may not be the substrates of Organic Cation transporter 2 (OCT2), a renal uptake transporter that plays an important role in drug elimination through kidney. From the result above, we can considerate that hellianuols excreted through kidney in another mechanism besides OCT2.

CONCLUSION

Based on in silico pharmacokinetic profiles study of hellianuol A-L contained in aqueous extract of Helianthus annuus L. cv. SH-222 leaves using pkCSM online tool, it can be concluded that overall could be well absorbed through oral administration. Hellianuols were quite promising to be developed as a an anticancer drug especially for brain cancer, as they were predicted to be able to penetrated blood-brain barrier into central nervous system. Their properties exhibited such a good pharmacokinetics as well.

This study widely opens opportunities for further research on hellianuols, especially to produce more accurate result prospectively in in vitro and in vivo studies.

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### Table 1. Predicted ADME properties of hellianuols

| Properties                                      | Hellianuol |     |     |     |     |     |     |     |     |     |     |     |
|------------------------------------------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Empirical formula                              | C₁₄H₂₀O₃  | C₁₅H₂₀O₃ | C₁₆H₂₂O₃ | C₁₅H₂₂O₃ | C₁₆H₂₂O₃ | C₁₅H₂₀O₄ | C₁₅H₂₀O₃ | C₁₅H₂₀O₃ | C₁₅H₂₀O₄ | C₁₅H₂₀O₃ | C₁₆H₂₂O₃ | C₁₅H₂₀O₄ |
| Molecular weight (g/mol)                        | 236.31     | 248.32  | 262.34  | 250.33  | 262.34  | 264.32  | 248.32  | 248.32  | 264.32  | 264.32  | 264.32  | 248.32  | 266.33  |
| Intestinal absorption (% absorbed)             | 90,793     | 91,239  | 91,473  | 90,763  | 91,369  | 94,255  | 93,800  | 93,800  | 92,542  | 92,542  | 93,593  | 95,384  |
| Skin permeability (log Kp)                     | -3.21      | -3.218  | -3.241  | -3.186  | -3.215  | -3.570  | -2.942  | -2.942  | -3.314  | -3.314  | -2.662  | -3.109  |
| Caco-2 permeability (log \(P_{app}\) in 10⁻⁶ cm/s) | 1.297      | 1.283   | 1.252   | 1.295   | 1.295   | 1.248   | 1.287   | 1.287   | 1.186   | 1.186   | 1.341   | 1.206   |
| P-glycoprotein substrate                       | Yes        | Yes     | Yes     | Yes     | Yes     | No      | Yes     | Yes     | No      | No      | No      | Yes     |
| P-glycoprotein I inhibitor                     | No         | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      |
| P-glycoprotein II inhibitor                    | No         | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      |
| Vdᵣ (log L/kg)                                 | 0.094      | 0.150   | 0.076   | 0.159   | 0.172   | 0.096   | 0.317   | 0.317   | 0.270   | 0.270   | 0.193   | 0.115   |
| BBB permeability (logBB)                       | 0.341      | 0.311   | 0.296   | 0.313   | 0.021   | 0.092   | 0.389   | 0.389   | 0.112   | 0.112   | 0.378   | -0.616  |
| CYP2D6 substrate                               | No         | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      |
| CYP3A4 substrate                               | No         | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      | No      |
| CYP1A2 inhibitor                               | No         | No      | No      | No      | No      | Yes     | Yes     | No      | Yes     | No      | Yes     | No      |
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| CYP2C19 inhibitor | Yes | Yes | No | Yes | Yes | No | No | No | No | Yes | No |
|-------------------|-----|-----|----|-----|-----|----|----|----|----|-----|----|
| **Properties**    |     |     |    |     |     |    |    |    |    |     |    |
| Hellianuol        |     |     |    |     |     |    |    |    |    |     |    |
| **A**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **B**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **C**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **D**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **E**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **F**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **G**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **H**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **I**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **J**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **K**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **L**             | No  | No  | No | No  | No  | No | No | No | No | No  | No |
| **CYP2D6 inhibitor** |     |     |    |     |     |    |    |    |    |     |    |
| **CYP3A4 inhibitor** |     |     |    |     |     |    |    |    |    |     |    |
| **Excretion**     |     |     |    |     |     |    |    |    |    |     |    |
| Total clearance   | 0.947 | 1.011 | 1.082 | 0.948 | 1.078 | 0.895 | 0.905 | 0.905 | 0.719 | 0.719 | 0.974 | 0.824 |
| (log ml/min/kg)   |     |     |    |     |     |    |    |    |    |     |    |
| Renal OCT2        | No  | No  | No | No  | No  | No | No | No | No | No  | No |

ADME, absorption, distribution, metabolism, excretion; $P_{\text{app}}$, apparent permeability coefficient; Kp, skin permeability constant; $V_{\text{dss}}$, volume of distribution at steady state; BBB, blood-brain barrier; BB, blood-brain; CNS, central nervous system; PS, permeability-surface area; OCT2, organic cation transporter-2