

REVIEW

Brief insight into the *in silico* properties, structure–activity relationships and biotransformation of fruquintinib, an anticancer drug of a new generation containing a privileged benzofuran scaffold

Stručný pohľad na vlastnosti *in silico*, vzťahy štruktúra–aktivita a biotransformáciu fruquintinibu, protinádorovo účinkujúceho liečiva novej generácie obsahujúceho privilegované benzofuránové zoskupenie

Dominika Nádaská • Lucia Hudecová • Gustáv Kováč • Ivan Malík

Received September 29, 2023 / Accepted October 30, 2023

Summary

Current trends in drug design notably consider so-called privileged scaffolds as the core structural fragments with decisive impact on affinity to properly chosen biological targets, potency, selectivity and toxicological characteristics of drugs and prospective drug candidates. **Fruquintinib (1)** is a novel synthetic selective inhibitor of vascular endothelial growth factor receptor (VEGFR) isoforms, i.e., VEGFR-1, VEGFR-2 and VEGFR-3. The therapeutic agent (1) consists of a flat bicyclic heteroaromatic ring, in which two nitrogens are suitably incorporated, a core bicyclic heteroaromatic

ring – privileged (substituted) benzofuran scaffold, and a pair of hydrogen bond (*H*-bond) donor and acceptor group, i.e., amide functional moiety. **Fruquintinib (1)** was first approved in China for the treatment of metastatic colorectal cancer, a severe malignant disease with a high mortality rate. The review article offered a brief insight into the topic of privileged structures, their drug-like ranges of several parameters, pharmacodynamic characteristics of **fruquintinib (1)** and various *in silico* descriptors characterizing drug's structural and physicochemical properties (molecular weight, number of heavy atoms, number of aromatic heavy atoms, fraction of *sp*³ C-atoms, number of *H*-bond acceptors, number of *H*-bond donors, total polar surface area, molar refractivity, molecular volume as well as parameters of lipophilicity and solubility). Some of these descriptors were related to pharmacokinetics and distribution of **fruquintinib (1)**, and, in addition, might help predict its ability to cross passively the blood–brain barrier (BBB). Moreover, a possible connection between the induction potential on cytochrome P450 isoenzymes (CYP1A2 and CYP3A4) and passive transport of a given drug into the central nervous system *via* BBB was investigated. Current clinical experience and future directions regarding of **fruquintinib (1)** were also briefly outlined.

Key words: privileged scaffold • fruquintinib • *in silico* properties • structure–activity relationships • pharmacokinetics

Súhrn

Súčasný trendy projekcie liečiv významne reflektujú tzv. privilegované zoskupenia ako základné (tzv. jadrové)

D. Nádaská

Department of Pharmaceutical Chemistry

Faculty of Pharmacy, Comenius University Bratislava, Slovak Republic

L. Hudecová • G. Kováč

Institute of Chemistry, Clinical Biochemistry and Laboratory Medicine

Faculty of Medicine, Slovak Medical University in Bratislava, Slovak Republic

Republic

Assoc. Prof. PharmDr. Ivan Malík, PhD. (✉)

¹Department of Pharmaceutical Chemistry

Faculty of Pharmacy, Comenius University Bratislava

Odbojárov 10, 832 32 Bratislava, Slovak Republic

²Institute of Chemistry, Clinical Biochemistry and Laboratory Medicine

Faculty of Medicine, Slovak Medical University in Bratislava

Limbová 12, 833 03 Bratislava, Slovak Republic

e-mail: malik2@uniba.sk

štruktúrne fragmenty s rozhodujúcim vplyvom na afinitu k vhodne zvoleným biologickým cieľom, účinnok, selektivitu aj toxikologické charakteristiky týchto liečiv a perspektívnych kandidátov na liečivá. **Fruquintinib (1)** je nový syntetický selektívny inhibítor izoforiem receptora vaskulárneho endotelového rastového faktora (z *angl.* vascular endothelial growth factor receptor; VEGFR), t. j. VEGFR-1, VEGFR-2 a VEGFR-3. Terapeutikum (1) obsahuje planárne bicycklé heteroaromatické jadro, v ktorom sú vhodne inkorporované dva atómy dusíka, základný (jadrový) bicycklý heteroaromatický kruh – privilegované (substituované) benzofuránové zoskupenie a skupinu pôsobiacu ako donor a akceptor väzby vodíkovým mostíkom (VVM), t. j. amidové funkčné zoskupenie. **Fruquintinib (1)** bol prvýkrát schválený v Číne pre liečbu metastázujúceho kolorektálneho karcinómu, závažného nádorového ochorenia s vysokou mortalitou. Táto prehľadová publikácia ponúkla stručný pohľad na tému privilegovaných štruktúr, ich niekoľkých parametrov, ktorých rozsah približuje tzv. liečivu podobné (drug-like) vlastnosti, farmakodynamické charakteristiky **fruquintinibu (1)** a rôzne *in silico*-deskriptory definujúce štruktúrne a fyzikálno-chemické vlastnosti tohto liečiva (molekulová hmotnosť, počet ťažkých atómov, počet aromatických ťažkých atómov, frakcia C-atómov v sp^3 -hybridizovanom stave, počet akceptorov VVM, počet donorov VVM, celkový polárny povrch, molekulová refrakcia, molekulový objem aj parametre lipofily a rozpustnosti). Niektoré z týchto deskriptorov súviseli s farmakokinetikou aj distribúciou **fruquintinibu (1)** a navyše by mohli pomôcť predikovať jeho schopnosť pasívne prechádzať hematoencefalickou bariérou (HEB). V publikácii sa hodnotila aj eventuálna súvislosť medzi indukčným potenciálom liečiva (1) voči izoenzýmom cytochrómu P450 (CYP1A2 a CYP3A4) a jeho pasívnym transportom do centrálného nervového systému *via* HEB. Stručne boli takisto načrtnuté súčasné klinické skúsenosti s **fruquintinibom (1)** a budúce liečebné možnosti tohto terapeutika.

Kľúčové slová: privilegované zoskupenie • fruquintinib • vlastnosti *in silico* • vzťahy štruktúra–aktivita • farmakokinetika

Introduction

Current definition of the term privileged scaffold¹⁾ as well as its practical interpretation among medicinal chemists might "slightly" differ from the original perception of such scaffold coined by the research of Evans and his co-workers²⁾. The privileged scaffold was first introduced as a single molecular framework capable to provide high-affinity ligands for more than one type of a receptor²⁾. Following the more recent view and opinion of Zhao and Dietrich (2015), a chemical core structure can be regarded as a privileged scaffold if it is more probable that its derivatives can interact with several biological (protein) targets with high affinity and selectivity than other structures¹⁾.

Various structurally "simple" heterocycles containing O-, S- or N-atoms, such as morpholine, thiazole or suitably substituted triazine, incorporated in the structure of biologically active compounds, would be considered the privileged scaffolds³⁻⁵⁾ from a certain point of view. In fact, the given heterocyclic moieties meet the description according to Maclean et al. (2000) – privileged scaffolds are substructural features, which confer desirable (often drug-like) properties on compounds containing those features⁶⁾. Other very well-known examples of privileged scaffolds⁷⁻¹¹⁾ are phenyl-substituted monocycles (biphenyls, N-arylpiperidines, N-arylpiperazines, 1,4-dihydropyridines or dihydropyrimidones), fused [7-6] ring systems (1,4-benzodiazepin-2-ones, 1,5-benzodiazepin-2-ones, 1,4-benzodiazepin-2,5-diones, pyrrolo[2,1-c][1,4]benzodiazepin-5,11-diones, 1,4-benzothiazepin-5-ones or 5,11-dihydrobenzo[e]pyrido[3,2-b][1,4]diazepin-6-ones), fused [6-6] ring systems (benzopyrans, chromones, coumarins and pyranocoumarins or various quinoxalines / quinazolines) or fused [5-6] ring systems (indoles, benzimidazoles, azolo-1,2,4-triazines, azolopyrimidines, benzofurans or benzothiophenes).

Bicyclic privileged structures could be defined by drug-like ranges of several parameters as follows¹²⁾: $260.0 \leq \text{molecular weight (M.W.)} \leq 524.0$, $0.9 \leq \text{lipophilicity descriptor generated in silico (ALogP)} \leq 5.4$, $2 \leq \text{number of heteroatoms (n}_{\text{het}}; \text{O, N, S or P})}$ with one or more lone pairs, excluding atoms with positive formal charges, amide and pyrrole-type N-atoms, and aromatic O- and S-atoms in a bicyclic system ≤ 8 , n_{het} (O, N, S or P) with one or more attached H-atoms ≤ 3 , $21.0 \text{ \AA}^2 \leq \text{polar surface area (calculated parameter using a 2D approximation; PSA)} \leq 128.6 \text{ \AA}^2$, $6.3 \leq \text{ratio of the PSA value divided by a total surface area (TPSA) value} \leq 34.2$, $1 \leq \text{number of rotatable bonds (n}_{\text{rotb}}) \leq 10$, $2 \leq \text{number of rings} \leq 5$, $1 \leq \text{number of chain assemblies} \leq 7$, and atoms marked as EvenAtomStereo, OddAtomStereo or UnknownAtomStereo, and atoms that are internally perceived as having stereo and that are not marked as EvenAtomStereo or OddAtomStereo ≤ 4 .

In addition, both number of hydrogen-bond (H-bond) donors and n_{rotb} mainly modified the absorption of designed compounds (drugs), while lipophilicity, flexibility, degree of branching and existence of some functional groups determined their fate in a metabolic process^{12, 13)}.

In fact, the design and development of biologically active compounds containing one or more privileged scaffolds has to be very precise. For example, a 2-aminothiazole (privileged) moiety is considered an integral part of various synthetic molecules providing notable pharmacodynamic effects. On the other hand, numerous pan-assay interference compounds contain a given chemotype suggesting potential risks of off-target activity if those molecules will be chosen for further development and optimization¹⁴⁾.

Biologically active benzofuran derivatives – brief overview

Heterocyclic systems containing one or more O-atoms take up a central role as core components within a structure of synthetic or natural compounds showing diverse biological activities. These include anticancer, antimicrobial, antimycobacterial, antifungal, anti-inflammatory, analgesic, antioxidant, antiparasitic, antiviral, and antiseizure agents or the therapeutics for neuropsychiatric diseases, as published in various research and review papers^{15–21}.

The structure of a benzofuran core consists of a fused benzene and furan ring. Following the frontier orbital theory, as the frontier electron populations of the parent benzo[*b*]furan are greater, the corresponding C-atoms are more reactive toward electrophiles²². Besides, a 2,3-dihydrobenzo[*b*]furan core is also considered a benzofuran unit. This framework can be found in the structure^{23,24} of natural molecules (**ganodone**, **δ-viniferin**, or **ε-viniferin**) as well as synthetic compounds – clinical drugs (**citalopram** or **ramelteon**).

Synthetic routes providing various (substituted) benzofurans were briefly mentioned in a review article²⁵, for example, and several unconventional synthetic methodologies were comprehensively summarized and published²⁶ as well. The introduction of proper substituents at specified positions of a benzofuran core leads to new derivatives with unique structural characteristics that may possess an excellent therapeutic value^{27, 28}. The molecules containing a privileged benzofuran scaffold effectively interact with various biological targets and are considered powerful anticancer, antibacterial, antimycobacterial, antifungal, antiviral, anti-inflammatory, analgesic, antipyretic, antioxidant, anti-ulcer and anti-hyperlipidemic agents, insecticides, trypanocides as well as the compounds showing a beneficial impact in various phases of progressive neurodegenerative disorders such as Alzheimer's disease^{25, 29–31}.

Inhibition of vascular endothelial-derived growth factors by the activity of fruquintinib (1) as an important therapeutic strategy to suppress tumor growth

Vascular endothelial growth factors (VEGFs), alternatively termed vascular permeability factors, play critical roles in angiogenesis, promoting cell survival as well as the growth and proliferation of endothelial cells. These VEGFs are classified into five isoforms, i.e., VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, and placenta growth factor (abbreviation used: PIGF) as well. The VEGF-A is regarded as the most important factor commonly termed VEGF. Biological effects of given multifunctional peptides are mediated *via* canonical activation of several transmembrane VEGF receptor (VEGFR) subtypes, i.e., VEGFR-1 (also termed Flt-1 or fms-like Ty-

rosine Kinase 1), VEGFR-2 (KDR Kinase Domain Region / flk-1 or Fetal Liver Kinase 1) and VEGFR-3 (Flt-4), and neuropilins^{32–34}. These VEGFRs belong to a family of receptor protein tyrosine kinases, whose upregulation has been observed in various tumors, both benign and malignant³⁵.

Scientific literature survey offers numerous molecules effectively targeting VEGFRs, which are or would be clinically used to treat different types of cancer. The VEGFR inhibitory activity was observed for various compounds of natural origin, including **epigallocatechin gallate**, **resveratrol**, **curcumin**, **wogonin**, **triptolide**, or **farnesiferol C**, for example³³. Many potent synthetic VEGFR inhibitors can potentially inhibit VEGFR-1, VEGFR-2 and VEGFR-3. These molecules are classified as pan-VEGFR inhibitors such as **axitinib**, **cediranib**, **dovitinib**, **motesanib**, **nintedanib**, **lenvatinib**, **pazopanib**, **regorafenib** or **tivozanib**. Synthetic selective-VEGFR inhibitors, e.g., **apatinib**, **brivanib**, **cabozantinib**, **foretinib**, **ponatinib**, **semaxanib**, **sorafenib**, **sunitinib** or **vandetanib**, can suppress one or two of VEGFRs. Chemical structures of the small-molecule pan-VEGFR as well as selective-VEGFR inhibitors (characterized with *M.W.* < 900–1000 Da, among others) for targeted cancer therapy, their mechanism of action, biological targets and several data from their clinical trials can be found in the review papers^{33, 36, 37}.

Fruquintinib (1), chemically 6-(6,7-dimethoxyquinazolin-4-yloxy)-*N*,2-dimethylbenzofuran-3-carboxamide (CAS Registry Number: 1194506-26-7), is a novel, orally available drug containing a privileged benzofuran core. The molecule (**1**), also termed **HMPL-013**, potently and highly selectively inhibits desired biological targets, i.e., VEGFR-1, VEGFR-2, and VEGFR-3, for long term³⁶. High selectivity to those receptors was observed when exploring a panel of more than 250 kinases, in which the synthetic compound (**1**) was found to potently inhibit VEGFR-1, VEGFR-2, and VEGFR-3 and provided weak to no inhibitory effect on all other kinases³⁸.

The drug (**1**) received its first global approval in China for patients with metastatic colorectal cancer (mCRC) who have failed at least two prior systemic antineoplastic therapies, including **fluoropyrimidine**, **oxaliplatin**, and **irinotecan**³⁶. **Fruquintinib (1)** is the first Chinese original orally available small-molecule anticancer agent of a new generation approved by the National Medical Products Administration of China³⁹ in 2018.

A brief view on particular phases of clinical development as well as the list of completed and ongoing clinical trials involving **fruquintinib (1)** as the treatment for mCRC, (advanced) non-squamous non-small cell lung cancer, advanced gastric or gastroesophageal junction adenocarcinoma, and advanced solid tumors were summarized in^{40, 41}. The current review paper focused primarily on *in silico* properties, structure–anti-VEGFR-2 inhibitory activity, and biotransformation pathways of this drug.

Fundamental physicochemical characteristics of fruquintinib (1) and structure–activity relationships in terms of interactions between a given ligand and vascular endothelial–derived growth factor receptor 2

The chemical structure of **fruquintinib (1)** can be "virtually" divided into several parts (Fig. 1) as follows:

- i) flat bicyclic heteroaromatic ring containing two *N*-atoms (fragment **A**),
- ii) core bicyclic heteroaromatic ring – a (substituted) benzofuran scaffold (**B**),
- iii) pair of *H*-bond donor and acceptor groups consisting of an amide functional moiety (**C**).

In addition, the structure of other VEGFR-2 inhibitors also contains a monocyclic/bicyclic (hetero)aromatic ring, which can be unsubstituted (substituted with hydrogen, in fact) or substituted with one or more halogen atoms⁴², interacting with an allosteric hydrophobic pocket *via* numerous hydrophobic interactions.

Several physicochemical properties of the compound (**1**) were calculated by the authors of the current paper using a SwissADME applet⁴³, a free web tool, in which a structure of **fruquintinib (1)** was converted to an appropriate digital notation, i.e., Simplified Molecular Input Line Entry System (SMILES). Based on generated SMILES, the obtained descriptors were as follows: *M.W.* = 393.39 g/mol (or in Da units), number of heavy atoms (n_{ha}) = 29, number of aromatic heavy atoms (n_{ar-ha}) = 19, fraction of sp^3 C-atoms ($Csp3$) = 0.19, n_{rotb} = 6, number of *H*-bond acceptors (n_{ON}) = 7, number of *H*-bond donors (n_{OHNH}) = 1, *TPSA* = 95.7 Å², molar refractivity (*M.R.*) = 106.8 Å² and logarithm of a partition coefficient ($\log P$) for an octan-1-ol/water partition system predicted by a Moriguchi^{44, 45} topological method (MLOGP) = 1.41.

In addition, the lipophilicity was also predicted by a fragment-based computational approach⁴⁶ (CLOGP) = 3.03 with a Perkin Elmer's ChemDraw *ver.*

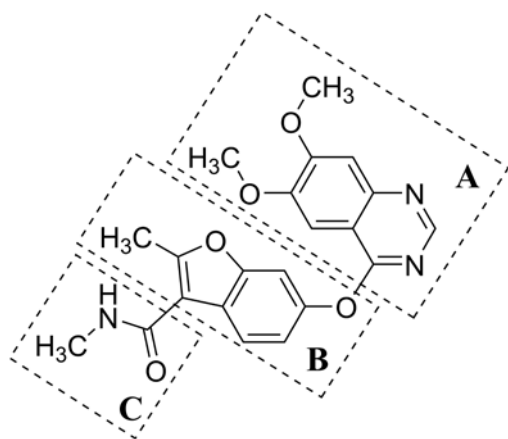


Fig. 1. Chemical structure of **fruquintinib (1)**, an effective VEGFR-1, VEGFR-2 and VEGFR-3 inhibitor, virtually divided into three compartments **A–C**

19.0.0.22 (PerkinElmer Informatics, Waltham, MA, USA) software package⁴⁷.

Molecular volume (*V*, in Å³ units) of **fruquintinib (1)**, i.e., $V = 341.3$ Å³, was also predicted using a Molinspiration Cheminformatics⁴⁸ property engine (Molinspiration Cheminformatics, Slovenský Grob, Slovak Republic), in which the structure of (**1**) was converted to SMILES as in the case of the analysis by the SwissADME predictor.

The compound (**1**) was "moderately soluble" based on a generated $\log S$ value describing solubility (*S*) according to a method of Ali and his co-workers⁴⁹. The $\log S$ parameter was found in a range^{43, 49} of $-6 < \log S < -4$, as the calculation procedure showed. Fragmental method integrated within a Filter-IT program *ver.* 1.0.2 (Silicos-IT, Wijnegem, Antwerpen, Belgium) assigned the category "poorly soluble"^{43, 50}. Thus, the classification group for (**1**) was defined with the $\log S$ value belonging to an interval of $-10 < \log S < -6$.

Some of these parameters were used to provide a more detailed "theoretical" view on the compound's ability of being passively absorbed *per os* as well as its capability to pass the blood–brain barrier (BBB) into the central nervous system (CNS) by a passive mechanism.

The VEGFR-2, as a type of membrane-bound receptor tyrosine kinases, regulates the process of vasculogenesis and angiogenesis⁵¹. The inhibitors of a given 210 to 230 kDa glycoprotein can be roughly classified into three types⁵², i.e., adenosine-5'-triphosphate (ATP) competitive inhibitors, which are able to bind at a pocket reserved for the accommodation of an adenine ring of ATP (type I), inhibitors interacting specifically to the inactive (DFG-out) conformation of VEGFR-2 (type II), and inhibitors, which covalently interact to the active site of this receptor at a hinge region in order to prevent the ATP binding at a catalytic domain (type III).

Molecular docking of the VEGFR-2 inhibitors approved by the U.S. Food and Drug Administration indicated the occupation of mainly four regions of the glycoprotein, i.e., hydrophobic region I, hydrophobic region II, *H*-bond rich region (DFG domain), and hinge region. Thus, those ligands are regarded as indirectly competitive inhibitors⁴².

The *N*-atom within an **A** fragment, which was opposite to an *O*-aryl group (Figure 1), interacted at the hinge region of VEGFR-2, forming *H*-bonds with an amino acid (A.A.) residuum (Cys919). The interaction was essentially required for the inhibitory activity of (**1**). The central **B** ring interacted with another A.A. residuum (Lys868) within a hydrophobic region I. In addition, the *H*-bond-rich region was engaged by an amide group within a **C** compartment of **fruquintinib (1)**, forming *H*-bonds with specific AAs⁴², i.e., Glu885 (interaction with an NH-moiety of a drug) and Asp1046 (CO-group).

Several *in silico* predictions connected with pharmacokinetics and distribution of fruquintinib (1)

When the drug molecule was characterized by *M.W.* > 500 Da, CLOGP > 5.00 (or Moriguchi's MLOGP

> 4.15), $n_{\text{ON}} > 10$, $n_{\text{OHNH}} > 5$, $n_{\text{rotb}} > 10$ and $\text{PSA} > 140.0 \text{ \AA}^2$, i.e., if its $(n_{\text{ON}} + n_{\text{OHNH}}) > 12$, it would not be sufficiently absorbed passively from a gastrointestinal tract when being administered *per os*^{44–46, 53, 54}. In fact, the values of these descriptors calculated for **fruquintinib** (**1**) supported its oral administration⁵⁵.

Kelder and his co-workers⁵⁶ found out that non-CNS drugs transported passively and transcellularly needed PSA (TPSA) $\leq 120.0 \text{ \AA}^2$, whereas the drugs can be targeted to the CNS with PSA (TPSA) $< 60.0\text{--}70.0 \text{ \AA}^2$. On the other hand, van de Waterbeemd with his scientific team⁵⁷ suggested a cut-off limit of PSA (TPSA) for CNS penetration to $\leq 90.0 \text{ \AA}^2$ and $M.W. < 450 \text{ Da}$. Levin⁵⁸ proposed the $M.W.$ value cut-off $\leq 400 \text{ Da}$. Hansch and Leo⁵⁹ found out that BBB penetration was optimal when the value of a $\log P$ descriptor for a CNS-active drug was within an interval of 1.5–2.7. In addition, the small-molecule drugs with $V = 740.0\text{--}970.0 \text{ \AA}^3$ could passively permeate *via* BBB⁶⁰. Following the criteria published in^{56, 57, 59, 60}, **fruquintinib** (**1**) would not be passively transported *via* BBB into CNS.

Moreover, the research⁶¹ summarized several essential attributes of successful CNS-active drugs as follows: $M.W. < 450 \text{ Da}$, $\text{CLOGP} < 5.00$ (currently investigated anticancer compound (**1**) passed both criteria), $n_{\text{ON}} < 7$ (did not meet), $n_{\text{OHNH}} < 3$ (passed), $n_{\text{rotb}} < 8$ (passed), $H\text{-bonds} < 8$ (did not meet) and PSA (TPSA) $< 60.0\text{--}70.0 \text{ \AA}^2$ (did not meet), respectively. If the difference $\text{CLOGP} - (\text{number of nitrogens} + \text{number of oxygens}) > 0.00$, then the compound had a high probability of entering the CNS.

The predicted solubility of (**1**) in aqueous environment⁴⁹ was $2.98 \mu\text{g/ml}$; however, the CNS-active agents should be characterized with solubility $> 60.00 \mu\text{g/ml}$ ⁶¹.

In general, the compounds possessing a tertiary N -atom show a higher degree of brain permeation. The predicted value of an acid-base dissociation constant (pK_a) parameter⁶² for the molecule (**1**) was 14.99. The research⁶³ regarded $\text{pK}_a = 4\text{--}10$ as an optimal interval for passive permeation of a drug through BBB; the

study⁶¹ was slightly more rigorous and indicated a neutral or basic molecule with $\text{pK}_a = 7.5\text{--}10.5$ (avoiding acids) as a suitable CNS-active drug.

Considering the values of all given descriptors and the total number of N - and O -atoms^{56, 57, 59–61} within the structure of **fruquintinib** (**1**), it might be concluded that this anticancer drug would not be passively transported *via* BBB into the CNS. In fact, its distributions in adipose, adrenal, and kidney were slightly higher than or almost equivalent to the corresponding plasma levels. The lowest distribution was observed in the brain, testis, and bone marrow when experimental animal models (rats) were used⁵⁵.

Biotransformation of fruquintinib (1)

Regarding **fruquintinib's** (**1**) metabolism, three major oxidative metabolites, i.e., a metabolite **M1** (**2**), **M2** (**3**), and a series of **M3** (**4**) metabolites, were identified in liver microsomes of several animal models (mouse, rat, dog, and monkey) and humans (Figure 2). Oxidation (**M1**) and oxidative O -demethylation (**M2**) within phase I of biotransformation, followed by O -glucuronidation (phase II) are the major *in vivo* metabolic pathways⁵⁵.

Two cytochrome P450 (CYP) enzymes are responsible for oxidation and oxidative O -demethylation *in vitro* of (**1**), leading to **M1** (**2**) and **M2** (**3**), respectively. These enzymes are well-known CYP3A4, a main enzyme biotransforming (**1**), and CYP2D6. The oxidation *in vitro* of a fundamental structural core, as schematically drawn for the metabolites from a series **M3** (**4**) of a parent compound (**1**), was possible *via* the activity of CYP3A4, CYP2D6 as well as CYP2C19 (Fig. 2). It was predicted that **fruquintinib** (**1**) might show favorable human pharmacokinetic properties and low efficacious dose⁵⁵. The compound (**1**) provided no induction potential on CYP1A2 and CYP3A4 in human hepatocytes and probably did not induce some transporters, like P-glycoprotein (P-gp), after multi-dosing⁵⁵.

In fact, these pharmacokinetic features, if taken "separately", did not provide the light to clearly

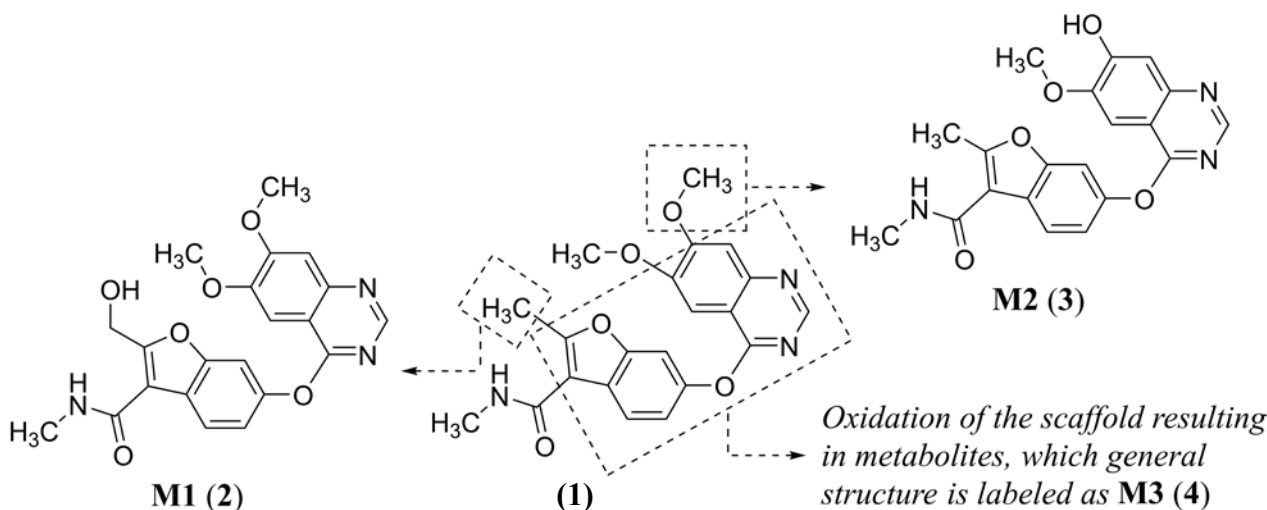


Fig. 2. Proposed biotransformation *in vitro*⁵⁵ of **fruquintinib** (**1**) with indication of main metabolites **M1** (**2**)–**M3** (**4**)

answer the question of whether **fruquintinib (1)** might be passively transported *via* BBB into the CNS or not. The reason was that a successful CNS-active drug must have no significant CYP2D6 metabolism. In the case of **(1)**, the meeting this criterion was quite questionable, as the research⁵⁵⁾ indicated. Furthermore, the CNS-active drug must be a nonpotent CYP3A4 inducer⁶¹⁾ – the compound **(1)** passed⁵⁵⁾ that requirement – and must have no to low affinity *in vivo* to the P-gp transporter^{61,64)}. Focusing on such affinity studies, however, no relevant *in vivo* (using experimental animal models) or clinical data were available.

Current clinical experience and future directions

From a clinical point of view, beneficial therapeutic interventions against some (severe) cancers might be done employing **fruquintinib (1)**, as the conclusions from various clinical trials indicated. For example, the administration of a given compound after local radiotherapy may be an effective option in the treatment of specific populations suffering from mCRC with high microsatellite instability and v-Ki-ras2 Kirsten rat sarcoma viral oncogene (KRAS) exon 2 p. G12D mutation⁶⁵⁾.

Combining **fruquintinib (1)** and a suitable immune checkpoint inhibitor, i.e., programmed cell death protein 1 (programmed cell death receptor-1; PD-1) / PD-ligand 1 (PD-L1) antibody, might improve the efficacy compared to monotherapy when treating various cancers in preclinical and clinical studies. **Sintilimab**, as a fully human IgG4 monoclonal antibody, binds to a programmed cell PD-1 in order to block the interaction between PD-1 and its ligands (PD-L1 and PD-L2) and consequently help to restore the endogenous antitumour T-cell response⁶⁶⁾. The clinical trial studies^{67, 68)} regarding **fruquintinib (1)** plus **sintilimab** and **fruquintinib (1)** plus **toripalimab**, a recombinant, humanized PD-1 monoclonal antibody⁶⁹⁾, respectively, showed promising clinical activity of these combinations in patients with mismatch repair-proficient mCRC. In addition, **fruquintinib (1)** was reported for the first time to have favorable efficacy and a convenient safety profile as an optional treatment modality for patients with advanced bone and soft tissue sarcoma who failed in multi-line therapies⁷⁰⁾.

The drug **(1)** also showed promising efficacy and acceptable toxicity as second or further-line therapy in advanced or metastatic biliary tract cancer⁷¹⁾, a quite rare but aggressive disease.

On the other hand, **fruquintinib (1)** as a relatively new drug brought not only clinical benefits but also several adverse reactions as the first case of **fruquintinib (1)**-associated aortic dissection was recently reported⁷²⁾. The reactions might be suppressed by administering a pH-triggered size-converted nano-drug delivery system to co-deliver **fruquintinib (1)** together with other chemotherapeutic agents, such as **doxorubicin**. The clinical use of such a nanoparticle

system could simultaneously achieve rapid tumor tissue enrichment and high efficiency tumor tissue penetration providing excellent antitumor effects of both anticancer therapeutics as well as effective inhibition of tumor growth and metastasis⁷³⁾.

Conclusions

Colorectal cancer (CRC) belongs to a group of the most predominant malignancies with a high mortality rate globally. Approximately a quarter of CRC patients presented metastatic disease (mCRC) at diagnosis, while almost half of them will develop metastases. The treatment paradigm for CRC/mCRC is nowadays moving towards a tailored approach based on clinical and molecular characteristics. **Fruquintinib (1)**, an orally bioavailable innovative small-molecule tyrosine kinase inhibitor highly selectively targeting VEGFR-1, VEGFR-2, and VEGFR-3, is successfully utilized clinically for the treatment of patients suffering from mCRC. The structural arrangement of the molecule **(1)** favors its interactions with particular VEGFR subtypes. Several *in silico* descriptors listed in this paper supported the "decision" to administer **fruquintinib (1)** *per os*, and, in addition, they could indicate the compound's (in)ability to be passively transported *via* BBB into CNS. Accordingly, CNS side effects of a given molecule **(1)** were expected to be quite low. The antiangiogenic agent **(1)** containing a privileged benzofuran scaffold could significantly improve major efficacy parameters, including response rate, progression-free survival, and overall survival. Moreover, **fruquintinib (1)** might be successfully involved in combination therapy focusing on the treatment of not only rat sarcoma (RAS) oncogene-mutated or chemo-refractory mCRC but also (locally) advanced gastric cancer, non-small cell lung cancer, (locally) advanced rectal cancer, advanced pancreatic cancer or esophageal squamous cell carcinoma in order to prolong survival and improve the quality of patients' life.

Acknowledgments

We would like to thank the Czech and Slovak Pharmacy Journal (*Česká a slovenská farmacie*) for the opportunity to publish this scientific article.

Conflict of interest: none.

References

1. Zhao H., Dietrich J. Privileged scaffolds in lead generation. *Expert Opin. Drug. Discov.* 2015; 10, 781–790. doi: 10.1517/17460441.2015.1041496
2. Evans B. E., Rittle K. E., Bock M. G., DiPardo R. M., Freidinger R. M., Whitter W. L., Lundell G. F., Veber D. F., Anderson P. S., Chang R. S. L., Lotti V. J., Cerino D. J., Chen T. B., Kling P. J., Kunkel K. A., Springer J. P., Hirshfield J. Methods for drug discovery: development of potent, selective, orally effective cholecystin antago-

- nists. *J. Med. Chem.* 1988; 31, 2235–2246. doi: 10.1021/jm00120a002
3. **Kourounakis A. P., Xanthopoulos D., Tzara A.** Morpholine as a privileged structure: A review on the medicinal chemistry and pharmacological activity of morpholine containing bioactive molecules. *Med. Res. Rev.* 2020; 40, 709–752. doi: 10.1002/med.21634
 4. **Datusalia A. K., Khatik G. L.** Thiazole heterocycle: A privileged scaffold for drug design and discovery. *Curr. Drug Discov. Technol.* 2018; 15, 162. doi: 10.2174/157016381503180620153423
 5. **Gharat R., Prabhu A., Khambete M. P.** Potential of triazines in Alzheimer's disease: A versatile privileged scaffold. *Arch. Pharm. (Weinheim)* 2022; 355, art. no. e2100388 (12 pp.). doi: 10.1002/ardp.202100388
 6. **Maclean D., Baldwin J. J., Ivanov V. T., Kato Y., Shaw A., Schenider P., Gordon E. M.** Glossary of terms used in combinatorial chemistry (technical report). *J. Comb. Chem.* 2000; 2, 562–578. doi: 10.1021/cc000071u
 7. **Horton D. A., Bourne G. T., Smythe M. L.** The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.* 2003; 103, 893–930. doi: 10.1021/cr020033s
 8. **Costantino L., Barlocco D.** Privileged structures as leads in medicinal chemistry. *Curr. Med. Chem.* 2006; 13, 65–85. doi: 10.2174/092986706775197999
 9. **Rusinov V. L., Charushin V. N., Chupakhin O. N.** Biologically active azolo-1,2,4-triazines and azolopyrimidines. *Russ. Chem. Bull.* 2018; 67, 573–599. doi: 10.1007/s11172-018-2113-8
 10. **Voinkov E. K., Drokin R. A., Fedotov V. V., Butorin I. I., Savateev K. V., Lyapustin D. N., Gazizov D. A., Gorbunov E. B., Slepukhin P. A., Gerasimova N. A., Evstigneeva N. P., Zilberberg N. V., Kungurov N. V., Ulomsky E. N., Rusinov V. L.** Azolo[5,1-c][1,2,4]triazines and azoloazapurines: Synthesis, antimicrobial activity and *in silico* studies. *ChemistrySelect* 2022; 7, art. no. e202104253 (8 pp.). doi: 10.1002/slct.202104253
 11. **Savateev K. V., Ulomsky E. N., Butorin I. I., Charushin V. N., Rusinov V. L., Chupakhin O. N.** Azoloazines as A_{2a} receptor antagonists. Structure–activity relationship. *Russ. Chem. Rev.* 2018; 87, 636–669. doi: 10.1070/RCR4792
 12. **Han Ch., Zhang J., Zheng M., Xiao Y., Li Y., Liu G.** An integrated drug-likeness study for bicyclic privileged structures: from physicochemical properties to *in vitro* ADME properties. *Mol. Divers.* 2011; 15, 857–876. doi: 10.1007/s11030-011-9317-2
 13. **Hubatsch I., Ragnarsson E. G. E., Artursson P.** Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat. Protoc.* 2007; 2, 2111–2119. doi: 10.1038/nprot.2007.303
 14. **Jakopin Ž.** 2-Aminothiazoles in drug discovery: Privileged structures or toxicophores? *Chem. Biol. Interact.* 2020; 330, art. no. 109244 (8 pp.). doi: 10.1016/j.cbi.2020.109244
 15. **Atmaram U. A., Roopan S. M.** Biological activity of oxadiazole and thiadiazole derivatives. *Appl. Microbiol. Biotechnol.* 2022; 106, 3489–3505. doi: 10.1007/s00253-022-11969-0
 16. **He M., Fan M., Peng Z., Wang G.** An overview of hydroxypyranone and hydroxypyridinone as privileged scaffolds for novel drug discovery. *Eur. J. Med. Chem.* 2021; 221, art. no. 113546 (29 pp.). doi: 10.1016/j.ejmech.2021.113546
 17. **Rakesh K. P., Shantharam C. S., Sridhara M. B., Manukumar H. M., Qin H.-L.** Benzisoxazole: a privileged scaffold for medicinal chemistry. *Med. Chem. Commun.* 2017; 8, 2023–2039. doi: 10.1039/c7md00449d
 18. **Saroja B., Kumar G., Kumari M., Kaur R., Raghav N., Sharma P. K., Kumar N., Kumar S.** A decennary update on diverse heterocycles and their intermediates as privileged scaffolds for cathepsin B inhibition. *Int. J. Biol. Macromol.* 2022; 222 (Part B), 2270–2308. doi: 10.1016/j.ijbiomac.2022.10.017
 19. **Avula S. K., Das B., Csuk R., Al-Harrasi A.** Naturally occurring *O*-heterocycles as anticancer agents. *Anticancer Agents Med. Chem.* 2022; 22, 3208–3218. doi: 10.2174/1871520621666211108091444
 20. **Pairas G. N., Perperopoulou F., Tsoungas P. G., Varvounis G.** The isoxazole ring and its *N*-oxide: A privileged core structure in neuropsychiatric therapeutics. *ChemMedChem.* 2017; 12, 408–419. doi: 10.1002/cmdc.201700023
 21. **Wang Xi., Wang Xu., Zhao Y., Zhang X.** Two previously undescribed benzofuran derivatives from the flowers of *Callistephus chinensis*. *Phytochem. Lett.* 2022; 51, 145–148. doi: 10.1016/j.phytol.2022.08.012
 22. **Abu-Hashem A. A., Hussein H. A. R., Aly A. S., Gouda M. A.** Reactivity of benzofuran derivatives. *Synth. Commun.* 2014; 44, 2899–2920. doi: 10.1080/00397911.2014.907425
 23. **Abbas A. A., Dawood K. M.** Anticancer therapeutic potential of benzofuran scaffolds. *RSC Adv.* 2023; 13, 11096–11120. doi: 10.1039/d3ra01383a
 24. **Fuloria Sh., Sekar M., Khattulanuar F. S., Gan S. H., Rani N. N. I. M., Ravi S., Subramaniyan V., Jeyabalan S., Begum M. Y., Chidambaram K., Sathasivam K. V., Safi Sh. Z., Wu Y. S., Nordin R., Maziz M. N. H., Kumarasamy V., Lum P. T., Fuloria N. K.** Chemistry, biosynthesis and pharmacology of viniferin: Potential resveratrol-derived molecules for new drug discovery, development and therapy. *Molecules* 2022; 27, art. no. 5072 (33 pp.). doi: 10.3390/molecules27165072
 25. **Khanam H., Shamsuzzaman.** Bioactive benzofuran derivatives: A review. *Eur. J. Med. Chem.* 2015; 97, 483–504. doi: 10.1016/j.ejmech.2014.11.039
 26. **Chiummiento L., D'Orsi R., Funicello M., Lupattelli P.** Last decade of unconventional methodologies for the synthesis of substituted benzofurans. *Molecules* 2020; 25, art. no. 2327 (52 pp.). doi: 10.3390/molecules25102327
 27. **Modell A. E., Blosser S. L., Arora P. S.** Systematic targeting of protein–protein interactions. *Trends Pharmacol. Sci.* 2016; 37, 702–713. doi: 10.1016/j.tips.2016.05.008
 28. **Farhat J., Alzyoud L., Alwahsh M., Al-Omari B.** Structure–activity relationship of benzofuran derivatives with potential anticancer activity. *Cancers (Basel)* 2022; 14, art. no. 2196 (22 pp.). doi: 10.3390/cancers14092196

29. **Dawood K. M.** Benzofuran derivatives: a patent review. *Expert Opin. Ther. Pat.* 2013; 23, 1133–1156. doi: 10.1517/13543776.2013.801455
30. **Xu Zh., Zhao Sh., Lv Z., Feng L., Wang Y., Zhang F., Bai L., Deng J.** Benzofuran derivatives and their anti-tubercular, antibacterial activities. *Eur. J. Med. Chem.* 2019; 162, 266–276. doi: 10.1016/j.ejmech.2018.11.025
31. **Nevagi R. J., Dighe S. N., Dighe S. N.** Biological and medicinal significance of benzofuran. *Eur. J. Med. Chem.* 2015; 97, 561–581. doi: 10.1016/j.ejmech.2014.10.085
32. **Ahmad A., Nawaz M. I.** Molecular mechanism of VEGF and its role in pathological angiogenesis. *J. Cell. Biochem.* 2022; 123, 1938–1965. doi: 10.1002/jcb.30344
33. **Malekan M., Ebrahimzadeh M. A.** Vascular endothelial growth factor receptors [VEGFR] as target in breast cancer treatment: Current status in preclinical and clinical studies and future directions. *Curr. Top. Med. Chem.* 2022; 22, 891–920. doi: 10.2174/1568026622666220308161710
34. **Olsson A.-K., Dimberg A., Kreuger J., Claesson-Welsh L.** VEGF receptor signalling – in control of vascular function. *Nat. Rev. Mol. Cell Biol.* 2006; 7, 359–371. doi: 10.1038/nrm1911
35. **Mabeta P., Steenkamp V.** The VEGF/VEGFR axis revisited: Implications for cancer therapy. *Int. J. Mol. Sci.* 2022; 23, art. no. 15585 (14 pp.). doi: 10.3390/ijms232415585
36. **Zhang Y., Zou J.-Y., Wang Zh., Wang Y.** Fruquintinib: a novel antivasular endothelial growth factor receptor tyrosine kinase inhibitor for the treatment of metastatic colorectal cancer. *Cancer Manag. Res.* 2019; 11, 7787–7803. doi: 10.2147/CMAR.S215533
37. **Li X., Zhou J., Wang X., Li Ch., Ma Z., Wan Q., Peng F.** New advances in the research of clinical treatment and novel anticancer agents in tumor angiogenesis. *Biomed. Pharmacother.* 2023; 163, art. no. 114806 (16 pp.). doi: 10.1016/j.biopha.2023.114806
38. **Sun Q., Zhou J., Zhang Zh., Guo M., Liang J., Zhou F., Long J., Zhang W., Yin F., Cai H., Yang H., Zhang W., Gu Y., Ni L., Sai Y., Cui Y., Zhang M., Hong M., Sun J., Yang Zh., Qing W., Su W., Ren Y.** Discovery of fruquintinib, a potent and highly selective small molecule inhibitor of VEGFR 1, 2, 3 tyrosine kinases for cancer therapy. *Cancer Biol. Ther.* 2014; 15, 1635–1645. doi: 10.4161/15384047.2014.964087
39. **Chen Zh., Jiang L.** The clinical application of fruquintinib on colorectal cancer. *Expert Rev. Clin. Pharmacol.* 2019; 12, 713–721. doi: 10.1080/17512433.2019.1630272
40. **Shirley M.** Fruquintinib: First global approval. *Drugs* 2018; 78, 1757–1761. doi: 10.1007/s40265-018-0998-z
41. **Lavacchi D., Roviello G., Guidolin A., Romano S., Venturini J., Caliman E., Vannini A., Giommoni E., Pellegri E., Brugia M., Pillozzi S., Antonuzzo L.** Evaluation of fruquintinib in the continuum of care of patients with colorectal cancer. *Int. J. Mol. Sci.* 2023; 24, art. no. 5840 (12 pp.). doi: 10.3390/ijms24065840
42. **Modi S. J., Kulkarni V. K.** Exploration of structural requirements for the inhibition of VEGFR-2 tyrosine kinase: Binding site analysis of type II, 'DFG-out' inhibitors. *J. Biomol. Struct. Dyn.* 2022; 40, 5712–5727. doi: 10.1080/07391102.2021.1872417
43. **Daina A., Michielin O., Zoete V.** SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* 2017; 7, art. no. 42717 (13 pp.). doi: 10.1038/srep42717
44. **Moriguchi I., Hirono Sh., Liu Q., Nakagome I., Matsushita Y.** Simple method of calculating octanol / water partition coefficient. *Chem. Pharm. Bull.* 1992; 40, 127–130. doi: 10.1248/cpb.40.127
45. **Moriguchi I., Hirono Sh., Nakagome I., Hirano H.** Comparison of reliability of log *P* values for drugs calculated by several methods. *Chem. Pharm. Bull.* 1994; 42, 976–978. doi: 10.1248/cpb.42.976
46. **Lipinski Ch. A., Lombardo F., Dominy D. W., Feeney P. J.** Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 2001; 46, 3–26. doi: 10.1016/s0169-409x(00)00129-0
47. **PerkinElmer.** <https://www.perkinelmer.com/analytical-and-enterprise-solutions.html> (accessed on: September 24, 2023)
48. **Molinspiration Cheminformatics.** <https://www.molinspiration.com/cgi-bin/properties> (accessed on: September 24, 2023)
49. **Ali J., Camilleri P., Brown M. B., Hutt A. J., Kirton S. B.** *In silico* prediction of aqueous solubility using simple QSPR models: the importance of phenol and phenol-like moieties. *J. Chem. Inf. Model.* 2012; 52, 2950–2957. doi: 10.1021/ci300447c
50. **Silicos-IT.** <https://www.silicos-it.be/> (accessed on: September 24, 2023)
51. **Wang X., Bove A. M., Simone G., Ma B.** Molecular bases of VEGFR-2-mediated physiological function and pathological role. *Front. Cell Dev. Biol.* 2020; 8, art. no. 599281 (12 pp.). doi: 10.3389/fcell.2020.599281
52. **Peng F.-W., Liu D.-K., Zhang Q.-W., Xu Y.-G., Shi L.** VEGFR-2 inhibitors and the therapeutic applications thereof: a patent review (2012–2016). *Expert Opin. Ther. Pat.* 2017; 27, 987–1004. doi: 10.1080/13543776.2017.1344215
53. **Lipinski Ch. A.** Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov. Today Technol.* 2004; 1, 337–341. doi: 10.1016/j.ddtec.2004.11.007
54. **Veber D. F., Johnson S. R., Cheng H.-Y., Smith B. R., Ward K. W., Kopple K. D.** Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* 2002; 45, 2615–2623. doi: 10.1021/jm020017n
55. **Gu Y., Wang J., Li K., Zhang L., Ren H., Guo L., Sai Y., Zhang W., Su W.** Preclinical pharmacokinetics and disposition of a novel selective VEGFR inhibitor fruquintinib (HMPL-013) and the prediction of its human pharmacokinetics. *Cancer Chemother. Pharmacol.* 2014; 74, 95–115. doi: 10.1007/s00280-014-2471-3
56. **Kelder J., Grootenhuys P. D. J., Bayada D. M., Delbressine L. P. C., Ploemen J.-P.** Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. *Pharm. Res.* 1999; 16, 1514–1519. doi: 10.1023/A:1015040217741
57. **van de Waterbeemd H., Camenish G., Folkers G., Chretien J. R., Raevsky O. A.** Estimation of blood–brain bar-

- rier crossing of drugs using molecular size and shape, and *H*-bonding descriptors. *J. Drug Target.* 1998; 6, 151–156. doi: 10.3109/10611869808997889
58. **Levin V. A.** Relationship of octanol / water partition coefficient and molecular weight to rat brain capillary permeability. *J. Med. Chem.* 1980; 23, 682–684. doi: 10.1021/jm00180a022
59. **Hansch C., Leo A. J.** Substituent constant for correlation analysis in chemistry and biology. New York: Wiley 1979. doi: 10.1002/jps.2600690938
60. **Ghose A. K., Herbertz T., Hudkins R. L., Dorsey B. D., Mallamo J. P.** Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. *ACS Chem. Neurosci.* 2012; 3, 50–68. doi: 10.1021/cn200100h
61. **Pajouhesh H., Lenz G. R.** Medicinal chemical properties of successful central nervous system drugs. *NeuroRx.* 2005; 2, 541–553. doi: 10.1602/neurorx.2.4.541
62. **de Klerk D. J., Honeywell R. J., Jansen G., Peters G. J.** Transporter and lysosomal mediated (multi)drug resistance to tyrosine kinase inhibitors and potential strategies to overcome resistance. *Cancers (Basel)* 2018; 10, art. no. 503 (27 pp.). doi: 10.3390/cancers10120503
63. **Fischer H., Gottschlich R., Seelig A.** Blood–brain barrier permeation: Molecular parameters governing passive diffusion. *J. Membr. Biol.* 1998; 165, 201–211. doi: 10.1007/s002329900434
64. **Raub T. J., Lutzke B. S., Andrus P. K., Sawada G. A., Stanton B. A.** Early preclinical evaluation of brain exposure in support of hit identification and lead optimization. In: Borchardt R. T., Kerns E. H., Hageman M. J., Thakker D. R., Stevens J. L. (eds.) *Optimizing the "Drug-Like" Properties of Leads in Drug Discovery. Biotechnology: Pharmaceutical Aspects, Vol. IV.* New York: Springer 2006; 355–410. doi: 10.1007/978-0-387-44961-6_16
65. **Wang R., Cong D., Bai Y., Zhang W.** Case report: long-term sustained remission in a case of metastatic colon cancer with high microsatellite instability and KRAS exon 2 p.G12D mutation treated with fruquintinib after local radiotherapy: a case report and literature review. *Front. Pharmacol.* 2023; 14, art. no. 1207369 (8 pp.). doi: 10.3389/fphar.2023.1207369
66. **Hoy S. M.** Sintilimab: First global approval. *Drugs* 2019; 79, 341–346. doi: 10.1007/s40265-019-1066-z
67. **Guo Y., Zhang W., Ying J., Zhang Y., Pan Y., Qiu W., Fan Q., Xu Q., Ma Y., Wang G., Guo J., Su W., Fan S., Tan P., Wang Y., Luo Y., Zhou H., Li J.** Phase 1b/2 trial of fruquintinib plus sintilimab in treating advanced solid tumours: The dose-escalation and metastatic colorectal cancer cohort in the dose-expansion phases. *Eur. J. Cancer* 2023; 181, 26–37. doi: 10.1016/j.ejca.2022.12.004
68. **Ma Sh., Chen R., Duan L., Li Ch., Yang T., Wang J., Zhao D.** Efficacy and safety of toripalimab with fruquintinib in the third-line treatment of refractory advanced metastatic colorectal cancer: results of a single-arm, single-center, prospective, phase II clinical study. *J. Gastrointest. Oncol.* 2023; 14, 1052–1063. doi: 10.21037/jgo-23-108
69. **Keam S. J.** Toripalimab: First global approval. *Drugs* 2019; 79, 573–578. doi: 10.1007/s40265-019-01076-2
70. **Ding X., Liu Y., Zhang Y., Liang J., Li Q., Hu H., Zhou Y.** Efficacy and safety of fruquintinib as third- or further-line therapy for patients with advanced bone and soft tissue sarcoma: a multicenter retrospective study. *Anticancer Drugs* 2023; 34, 877–882. doi: 10.1097/CAD.0000000000001482
71. **Zhang P., Yang Y., Gou H., Li Q.** Phase II study of fruquintinib as second- or further-line therapy for patients with advanced biliary tract cancer. *J. Clin. Oncol.* 2023; 41(Suppl), art. no. e16161 (1 pp.). doi: 10.1200/JCO.2023.41.16_suppl.e16161
72. **Deng Y.-Y., Chen Y.-W., Wang M.-X., Zhu P.-F., Pan Sh.-Y., Jiang D.-Y., Chen Zh.-L., Yang L.** Acute aortic dissection caused by fruquintinib for metastatic colorectal cancer—a case report and literature review. *Transl. Cancer Res.* 2023; 12, 177–185. doi: 10.21037/tcr-22-1872
73. **Zhang N., Xin X., Feng N., Wu D., Zhang J., Yu T., Jiang Q., Gao M., Yang H., Zhao S., Tian Q., Zhang Zh.** Combining fruquintinib and doxorubicin in size-converted nano-drug carriers for tumor therapy. *ACS Biomater. Sci. Eng.* 2022; 8, 1907–1920. doi: 10.1021/acsbiomaterials.1c01606