Journal of Research in Pharmacy

Research Article

www.jrespharm.com

# Absorption, distribution, metabolism and excretion (ADME) of sorafenib and its two analogues of 2-aminoquinolone, in rat animal model, in silico – in vivo interplay

**Nasir M. IDKAIDEK[1]*, Hiba SALEH[2], Noor WADI[2], Ahmad Al-SHEIKH[1], Nidal QINNA[1], Gaida ALDABET[1], Ayman RABAYIA[2], Haya TUFFAHA[1], Laith ALSHOAIBI[1] Ahmad AL-GHAZAWI[2]**

1 University of Petra, College of Pharmacy, Amman, Jordan
2 Triumpharmac LLC, Amman, Jordan

* Corresponding Author. E-mail: nidkaidek@uop.edu.jo, (N.M.I); Tel +962(6)5799555, Fax +962(6)5715570, PO Box 961343, Amman, Jordan.

Received: 7 February 2025 / Revised: 30 March 2025 / Accepted: 1 April 2025

**ABSTRACT**: The aim of this project is to synthesize Sorafenib two derivatives of 2-amino-6-phenoxyquinolone: AH1 & P64, then conduct ADME studies in healthy rats and correlate results with in vitro and in silico results. The absolute bioavailability of sorafenib derivatives were found very low 2.2 & 12 % for AH1 & P64 after in vivo oral and IV studies of the derivatives. Also, the relative bioavailability of sorafenib derivatives were found very low 0.3 & 0.6 % for AH1 & P64 after in vivo studies of sorafenib and its derivatives. In vitro stability tests showed stable derivatives in all degradation tests over the time course of the experiments which suggests stable derivatives in vivo too. However, in vitro diffusion study showed that derivatives permeability values are more than 60 times lower than sorafenib permeability which explains the low bioavailability of the derivatives as compared with sorafenib. Sorafenib derivatives were shown to have more in vitro anti-cancer activity, yet low in vivo bioavailability due to low intestinal permeability.

**KEYWORDS**: ADME; ADMIT Lab; sorafenib; pharmacokinetics

## 1. INTRODUCTION

Derivatives of 2-substituted quinolone represent a promising class of compounds as novel drugs with anticancer activity. These compounds have long been used as effective anticancer drugs against a broad spectrum of cancer. Depending on their chemical structures, 2-substituted quinolone can kill cancer cells by diverse mechanisms. Researchers reported the synthesis and cytotoxic activity of 2-phenylquinolone derivatives that displayed significant antitumor-promoting activity [1, 2]. They disclosed a method of inhibiting tumor cell growth in mammalian subject, administering a therapeutically effective amount of selected 2- phenylquinolone derivatives, disclosed synthesis and anti-cancer activity of novel phosphate derivatives of 2-arylquinolones [3].

The result of preliminary screening revealed that these phosphates showed significant anti-cancer activity. Researchers reported the synthesis of a series of 2-aminoquinoline derivatives. Most derivatives showed potent anti-proliferative activity against human prostatic cancer PC-3 cell line. 5-Chloro-2-(pyridin-2-ylamino)quinolin-8-ol was the most effective compound against human prostatic cancer PC-3 cell line with GI50 values of 1.29 µM. Mechanistic studies revealed that 5-Chloro-2-(pyridin-2-ylamino)quinolin-8-ol was a potential Pim-1 kinase inhibitor [4].

Recently, new derivatives of 2-aminoquinolone have been synthesized and evaluated for their in-vitro anti-cancer activity against 3 human cancer cell lines, Renal cancer cell lines (A498 and Caki-1) and Hepatocellular carcinoma (HepG-2). The results revealed that 2-aminoquinolone derivatives showed anti-

**How to cite this article:** Idakaidek NM, Saleh H, Wadi N, Al-Sheik A, Qinna N, Aldabet G, Rabayia A, Tuffaha H, Alshoaibi L, Al-Ghazawi A. Absorption, distribution, metabolism and excretion (ADME) of sorafenib and its two analogues of 2-aminoquinolone, in rat animal model, in silico – in vivo interplay. J Res Pharm. 2025; 29(3): 1239-1247.

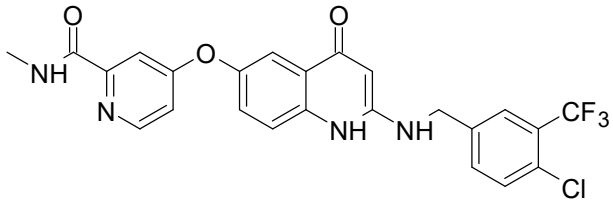

ISSN: 2630-6344

https://dx.doi.org/10.12991/jrespharm.1633820

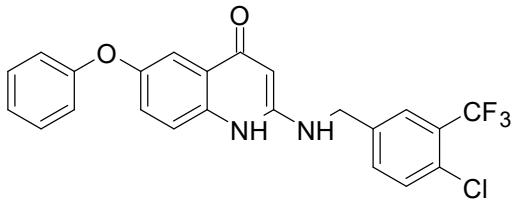
Journal of Research in Pharmacy
Research Article

cancer activity against all cell lines with better or similar potency of known anti-cancer drug Sorafenib. On the other hand, the 2-aminoquinolone derivatives showed less growth inhibitory effect than Sorafenib against fibroblast normal cell line [5].

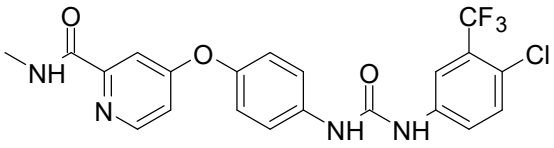
The aim of this project is to synthesize Sorafenib two analogues of 2-aminoquinolone 1(AH1) and 2(P64) (Figure 1), then conduct ADME studies in healthy rats. In vitro stability and permeability tests will also be done using rat homogenates and diffusion cells respectively. Perform in silico simulation of Sorafenib and its analogues as model drugs using ADMIT predictor program.




**1 (AH1)**




**2 (P64)**




**Sorafenib**

**Figure 1.** The chemical structures of 2-aminoquinolone derivatives 1(AH1) and 2(P64) and Sorafenib.

## 2. RESULTS AND DISSCUSSION

### 2.1. *In vivo* results

Sorafenib derivatives showed significantly ($P < 0.05$) lower Cmax, AUC, Kel, CL; higher V and similar Tmax of IV route as compared to oral route. Also, derivatives showed significantly ($P < 0.05$) lower Cmax, AUC; higher V, Kel, CL and similar Tmax after oral route as compared to sorafenib. Some p-values are not significant ($P > 0.05$) due to low sample size and high variability (up to more than 50%). Table 1 shows the statistical analysis of Sorafenib derivatives, while Table 2 shows the pharmacokinetics properties of Sorafenib and its derivatives. Figures 2 and 3 show sorafenib and its derivatives in IV and oral doses.

The absolute bioavailability of sorafenib derivatives were found very low 2.2 & 12 % for AH1 & P64 after in vivo oral and IV studies of the derivatives. Also, the relative bioavailability of sorafenib derivatives were found very low 0.3 & 0.6 % for AH1 & P64 after in vivo studies of sorafenib and its derivatives. This is in agreement with in silico predictions in humans, which validates the rat animal model.

### 2.2. *In vitro* results

To investigate some of the causes of such low bioavailability, in vitro degradation tests were done in liver homogenate, intestinal homogenate, acidic pH, basic pH and blood using the same concentrations used in the in vivo studies. All stability tests showed stable derivatives in all degradation tests over the time course of the experiments (km= 0 - 0.48 h-1) up to 3 hours. This suggests stable derivatives in vivo too as shown in Figures 4 and 5.

Journal of Research in Pharmacy
Research Article

**Table 1.** Statistical analysis of Sorafenib derivatives

| Route | Compound | Kel ($h^{-1}$) | Tmax (h) | Cmax/D (ng/ml) | AUC 0-6/D (ng.h) | Vz (ml) | CL (ml/h) | F |
|---|---|---|---|---|---|---|---|---|
| t-test (derivative PO vs Parent PO) | AH1 | 0.01 | 0.69 | 0.00 | 0.00 | 0.00 | 0.00 | 0.003 |
| t-test (derivative PO vs IV) | AH1 | 0.49 | 0.53 | 0.00 | 0.00 | 0.02 | 0.02 | 0.022 |
| t-test (derivative PO vs Parent PO) | P64 | 0.63 | 0.22 | 0.00 | 0.00 | 0.00 | 0.06 | 0.006 |
| t-test (derivative PO vs IV) | P64 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 |

**Table 2.** Pharmacokinetics properties of Sorafenib and its derivatives.

| Route | Compound | Kel ($h^{-1}$) | Tmax (h) | Cmax/D (ng/ml) | AUC 0-6/D (ng.h) | Vz (ml) | CL (ml/h) |
|---|---|---|---|---|---|---|---|
| IV dosed at 0.75 | AH1 | 0.29 (0.12) | 3.56 (2.51) | 247.03 (1396.3) | 1729.88 (593.2) | 1855.31 (1018.2) | 414.23 (73.4) |
| Oral dosed at 7.5 mg | AH1 | 0.17 (0.09) | 4 (1.41) | 10.41 (1.79) | 37.53 (0.99) | 88303.1 (16054) | 3140.45 (5472.5) |
| Oral dosed at 0.75 mg | Sorafenib | 0.17 (0.09) | 4 (0.0) | 1984.5 (824.9) | 11117.91 (417.9) | 522.27 (169.2) | 18.68 (2.82) |
| IV dosed at 0.75 | P64 | 0.08 (0.04) | 1.25 (1.64) | 129.5 (40.7) | 447.71 (203.3) | 8727.8 (1542) | 714.3 (337.8) |
| Oral dosed at 7.5 mg | P64 | 0.12 (0.06) | 0.83 (0.24) | 154.27 (42) | 531.64 (57.6) | 50697.02 (1822.1) | 6072.64 (3185.8) |

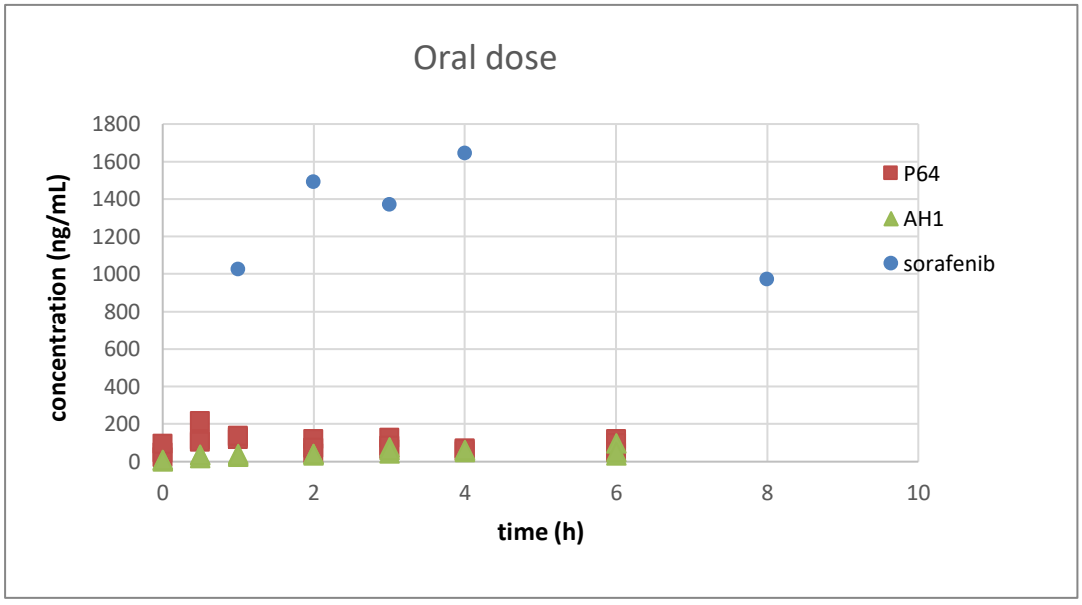



**Figure 2.** Oral dose concentrations for sorafenib and its derivatives

https://dx.doi.org/10.12991/jrespharm.1633820
J Res Pharm 2025; 29(5): 1239-1247

Journal of Research in Pharmacy
Research Article

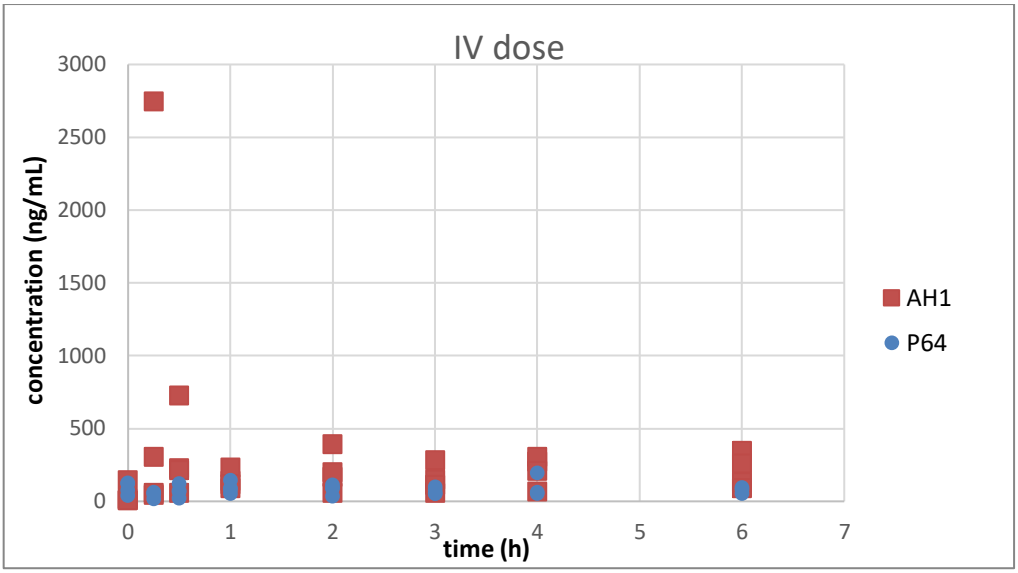



**Figure 3.** Sorafenib derivatives concentration in IV dose

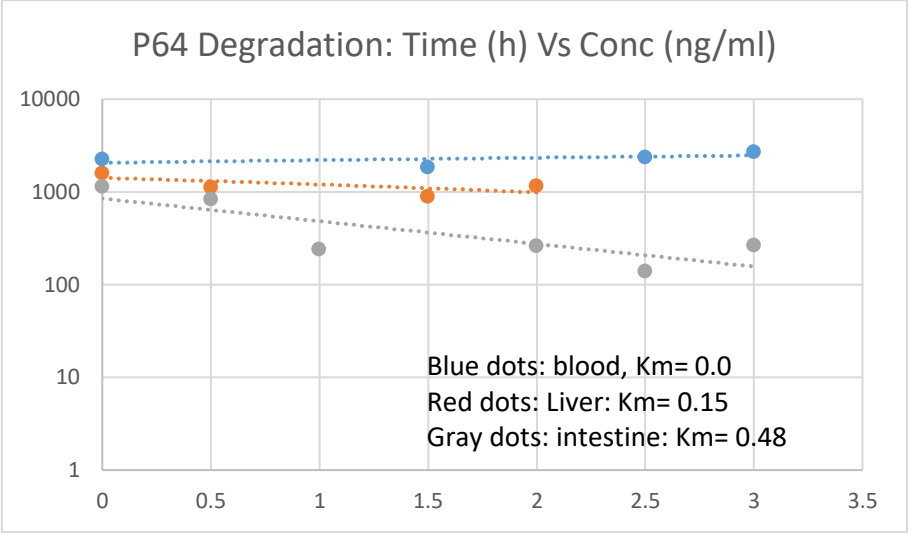



**Figure 4.** P64 degradation profile

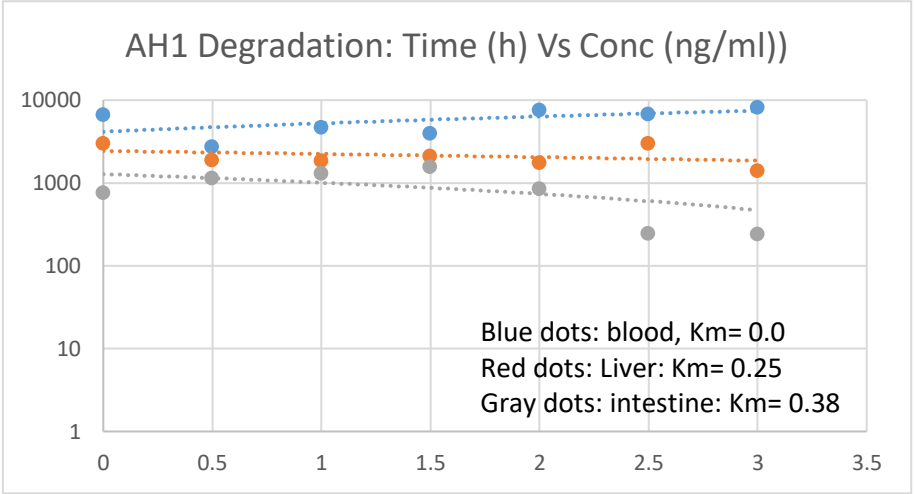



**Figure 5.** AH1 degradation profile

https://dx.doi.org/10.12991/jrespharm.1633820
J Res Pharm 2025; 29(5): 1239-1247

Moreover, in vitro permeation experiment was done using Franz diffusion cells to calculate permeability coefficients from the slope of the line that represents drug flux. Our results showed low derivatives permeability values of 6.65X10-6 and 7.53X10-5 cm/sec for AH1 and P64 respectively. These are more than 60 times lower than sorafenib permeability of 4.07X10-4 cm/sec [6]. This explains the low bioavailability of the derivatives as compared with sorafenib as shown in Figure 6.

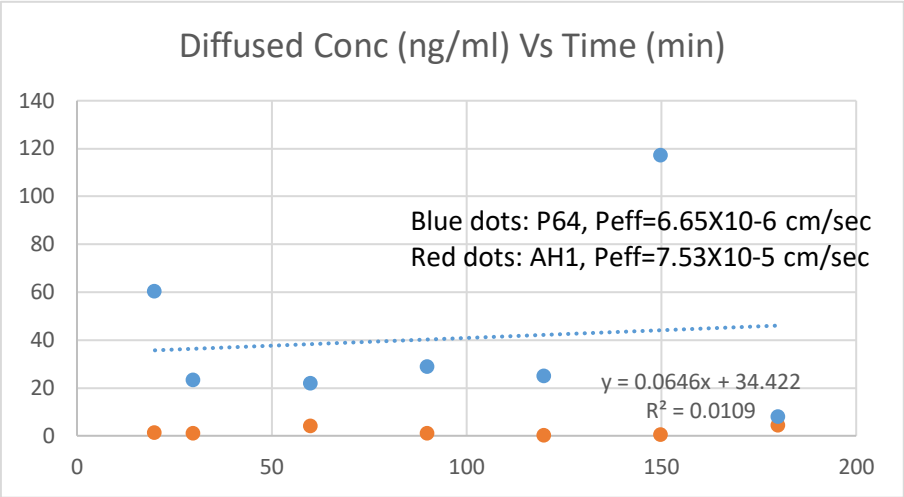



**Figure 6.** P64 & AH1 diffusion profiles

### 2.3. *In silico* results

In silico simulation was done using ADMET Lab V2. It showed MDCK permeability values of 6X10-6 cm/sec, 7X10-6 cm/sec and 1.3X10-5 cm/sec for AH1, P64 and sorafenib respectively. Also, bioavaiability is < 20 % in all compounds. This is in agreement with our in vivo results, which validates in silico prediction.

## 3. CONCLUSION

Sorafenib derivatives were shown to have more in vitro anti-cancer activity, yet low in vivo bioavailability due to low intestinal permeability. Structure improvement by optimization of hydrophilic-liophillic balance is needed to increase derivatives permeability and bioavailability.

## 4. MATERIALS AND METHODS

### 4.1. Synthesis of the 2-aminoquinolone derivatives:

2-(4-chloro-3-(trifluoromethyl)benzylamino)-6-phenoxyquinolin-4(1H)-one 2(P64) was prepared by the reported procedure [5].

1(AH1) were synthesized by nucleophilic displacement of the SMe groups in the prec ound 5-[bis(methylsulfanyl)-methylidene]-2,2-dimethyl-1,3-dioxane-4,6-dione 3, using the appropriate aniline and benzylamine. This was followed by cyclization through heating at 230°C to give the ap SD=22.27 inolone derivatives (Figure 2).

4-(2-(4-chloro-3-(trifluoromethyl)benzylamino)-1,4-dihydro-4-oxoquinolin-6-yloxy)-N-methylpyridine-2-carboxamide 1 (AH1)

A mixture of 5-(bismethylsulfanylmethelene)-2,2-dimethyl-1,3-dioxane-4,6-dione 3 (1eq) and 4-(4-aminophenoxy)-N-methylpyridine-2-carboxamide (1.1eq) in THF was heated at reflux for 1.5h and then (4-chloro-3-(trifluoromethyl)phenyl) methanamine (1.1 eq) was added. The reaction was stirred for an additional 2h. The THF was evaporated and the crude solid was washed with diethyl ether to yield the intermediate 4. The intermediate 4 was heated in diphenylether (3 mL) at 230 °C for 10 minutes. The reaction mixture was cooled to r.t then the diethyl ether (30 mL) was added. The resulting precipitate was filtered off to give 1(AH1) as off-white solid.

1H NMR (400 MHz; DMSO-d6): δ (ppm): 2.79 (s, 3H, CH3), 4.56 (s, 2H, CH2), 5.26(s, 1H, HC=C), 7.15-8.8 (m, 9H, Ar-H), 10.98 (s, 1H, NH). HRMS ESI, ([M + H]+): calculated m/z 503.1097; found, 503.1076.

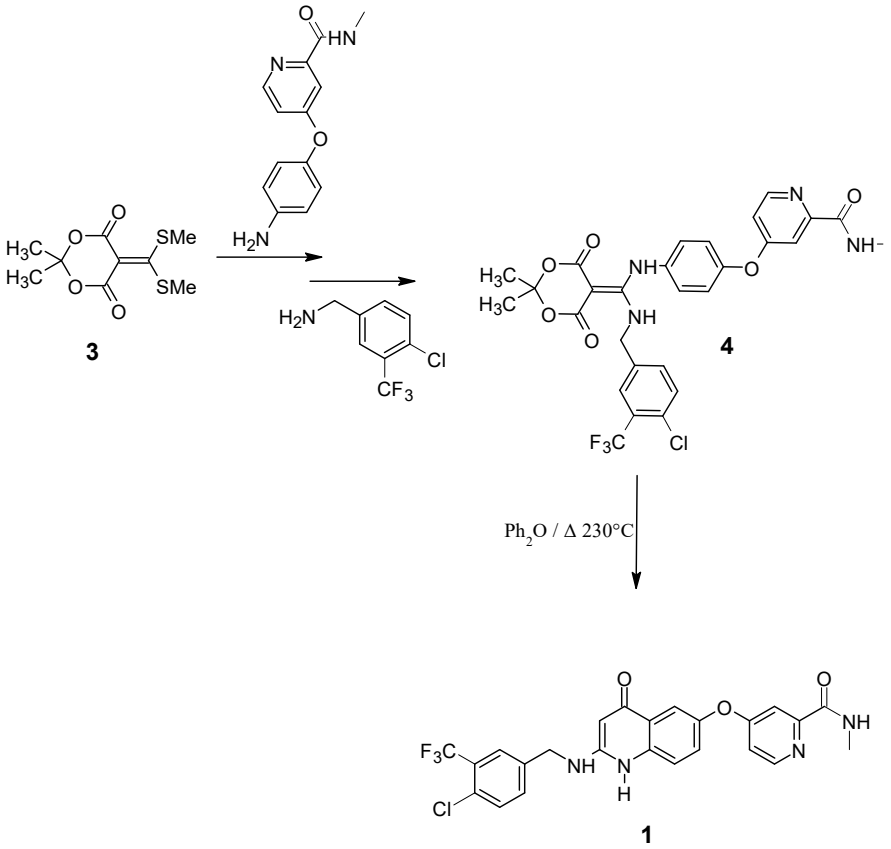



**Figure 7.** Synthesis of 2-aminoquinolone derivative 1(AH1).

### 4.2. Animal Model Setup

Adult male Sprague Dawley (SD) rats with an average weight of 255 ± 12 g were acclimatized 10 days at the Animal House of the University of Petra, Amman, Jordan. Rats were housed under controlled conditions such as temperature (22–24 °C), humidity (55–65%), and photoperiod cycles (12 h light/12 h dark). All experiments were performed in accordance with the University of Petra Institutional Guidelines on Animal Use, which adopts the guidelines of the Federation of European Laboratory Animal Science Association. The protocols for the animal study were revised and approved by the Research Committee at the Faculty of Pharmacy and Medical Sciences, University of Petra (Amman, Jordan) (E/A/8/2/2024).

*4.2.1. Preparation of Solutions*

Drugs were dissolved in 10% Dimtethylysulfoxide (DMSO), using 1% w/v Carboxymethyl cellulose (CMC) as a solvent to prepare the working solution. The final concentration of Sorafenib and its derivatives was 7.5mg/mL.

*4.2.2. In-vivo Experimental Protocol*

Rats were randomly divided into five groups as follows (n=7); group 1 administered 3 mg/kg of Sorafenib as a control, groups 2 and 3 were treated orally with 30 mg/kg of derivative 1 and 2 respectively, groups 4 and 5 injected intravenously (IV) with 3 mg/kg of derivative 1 and 2 respectively. Rats were fasted overnight (for 18 to 22 hours) with water offered ad libitum. Food was provided 4 hours post-drug administration.

*4.2.3. Sample Collection*

Blood samples were collected into heparinized capillary tubes via the retro-orbital sinus at 0, 1, 2, 3, 4, & 6 hours after drug administration. The blood samples were centrifuged 2000×g for 10 min, and the plasma samples obtained were stored at -80 °C for LC-MSMS analysis.

**4.3. Sample assay & validation using LC-MSMS**

Solution Preparation: Sorafenib-Stock Solution: (1mg/ml). Weight equivalent 20.0 mg of Sorafenib standard in 20.0 mL of DMSO, vortex well. Sorafenib -Working Solution: (50µg/ml). Dilute 1.0 mL of stoke solution to 20.0ml with ACN, vortex well. Table 1 shows Sorafenib serial solution. Diluent used is Acetonitrile. Alectinib is used as an Internal standard-Stock solution: (1mg/ml)), then weigh an equivalent amount to 20.0 mg of Alectinib and dissolve in 20.0 ml of ACN, vortex well. The Internal standard (Alectinib)-Working solution: (40µg/ml). Dilute 800.0 µL of Alectinib -Stock solution to 20.0 mL with ACN, vortex well.

For the mobile phase preparation mix 30% of (10 µm ammonium acetate in water + 0.1% formic acid) with 70% ACN, shake well. For the Seal/Needle Wash solution mix 500 mL of H2O with 500 m ACN, shake well.

For Solvent Extraction:

Extraction solvent: MTBE (methyl tert-butyl ether).

Sample extraction is done as the following: Pipette 80 µL of Blank Plasma /100µL of Spiked Plasma into Pre-labeled glass test tube. Add 20 µL of Serial Solution into blank plasma. Then add 50 µL of (Alectinib (IS)) working solution (40µg/ml). Vortex the samples for about 20 sec. Dispense 4.0 mL of (Extraction Solvent). Vortex the samples for about 2.0 min. Centrifuge the samples at 4000 rpm for about 5.0 min at Room Temperature. Decant the organic layer into another labeled clean test tube. Evaporated the solvent under steam of compressed air in water bath 40C. Reconstituted the samples with 400 µl of (500 mL of H2O with 500 m ACN), then vortex well. Transfer the supernatant into Auto-sampler vials insert, cap the vials and transfer them into the auto sampler racks.

**4.4. Chromatographic Conditions**

The HPLC conditions used were: the flow rate was 0.8 ml/min. the column temperature was 25°C and the auto sampler temperature was 5° C. the injection volume 15 mcL, total runtime was 4.5 minutes. The no. of smooth was 7. The needle wash and seal wash used was Acetonitrile: de-ionized water,1:1, v/v and the analytical column used was Inertsil –ODS-3, C18 5 um, 4.6 x 100 mm. Table 2 shows the standards detection and retention times

**Table 3.** Standards Detection and Retention Times

| Standard | Detection | Retention Time (min) |
|---|---|---|
| **Sorafenib** | Parent 465.200and daughter 252.000 | 2.85 min. |
| **Alectinib** | Parent 483.300 and daughter 396.300 | 3.22 min. |
| **AH1** | Parent 503.100 and daughter 193.100 | 1.54 min. |
| **P64** | Parent 445.100 and daughter 252.000 | 2.11 min. |

**4.5. Calibration Curve**

Alectinib calibration curve in human plasma was prepared by including 8 standard points: 5, 10, 50, 250, 500, 1000, 1500 and 2000, (ng/mL). The calibration curve was generated using the peak area ratios of Sorafenib and the internal standard versus the Sorafenib concentration by least-squares linear regression analysis. According to US FDA guidance for bioanalytical technique validation, the calibration curve must have a coefficient of variation (r2) of > 0.99.

The back-calculated concentrations for the standard points and the quality control samples should be within 15% of the nominal concentration except for the LLOQ level, it should not exceed 20% of the nominal concentration for the LLOQ. A double blank, system suitability sample, zero standard, calibration curve

consisting of eight non-zero samples covering the whole range, and quality control (QC) samples at three concentration levels were included in the analytical method validation run.

### 4.6. Accuracy and Precision

Intra-day precision and accuracy were calculated at LLOQ (10 ng/mL), low quality-control (15 ng/mL), medium quality-control (1200 ng/mL), and high quality-control (1700 ng/mL) with three replicates for each level.

### 4.7. Selectivity and Specificity

To confirm the absence of interfering substances around the retention time of analyst, blank samples were analyzed. Sorafenib and the Alectinib (internal standard) were well separated from the endogenous components. There were no interferences at the retention time of both Sorafenib and the internal standard. The peaks were in good shape, completely resolved from the plasma components. The matrix peak was less than 5 % of the peak area of the internal standard, which is acceptable per the US FDA guidanceTo confirm the absence of interfering substances around the retention time of analyst, blank samples were analyzed. Sorafenib and the Alectinib (internal standard) were well separated from the endogenous components. There were no interferences at the retention time of both Sorafenib and the internal standard. The peaks were in good shape, completely resolved from the plasma components. The matrix peak was less than 5 % of the peak area of the internal standard, which is acceptable per the US FDA guidance.

### 4.8. In vivo and in silico ADME calculations

For the in vivo ADME properties calculations Winnonlin Program V5.2 was used. NCA (non-compartmental analysis) method was used to calculate AUC 0-6, Cmax, Tmax Kel, CL and V. The statistical comparisons of log transformed data were done using t-test in Excel**.**

For the in silico calculations the calculation of molecules physicochemical and ADME properties will be done using ADMIT Lab V2 [7].

### 4.9. Assessment of derivatives stability in liver and intestinal homogenates

The drug solution was initially prepared by dissolving the derivatives in 10% DMSO using 1% w/v CMC as a solvent to prepare the working solution in a concentration of 0.75 g/mL. Male Sprague Dawley rats, fasted overnight (18-22 hours), were euthanized by CO2. To prepare liver and intestinal homogenates, the organs were removed through an abdominal incision. A 1g of liver and jejunum were homogenized separately with phosphate-buffered saline (PBS), (pH 6.5), (Euroclone®,Netherlands) in a 1:5 weight ratio, using a hand homogenizer. One milliliter of sorafenib, derivative 1 and derivative 2 stock solutions was added to the homogenates in a centrifuge tube, and then the mixture was incubated at 37°C. Samples of 200 µL were collected every 30 minutes over 3 hours. After each collection, an equivalent volume of PBS was added back to the homogenate. The collected samples were centrifuged at 3000rpm for 3 minutes to separate the drug from the homogenate (8). The supernatant was separated and stored at -80 °C until analysis by LC-MSMS. The experiment was performed in triplicates for each compound. Data analysis was performed by plotting the drug concentrations on a logarithmic scale, and the first-order degradation rate constants and half-lives were calculated using the STATISTICA software. On the other hand, diffusion of derivative molecules through intestinal membrane was done using Franz-diffusion cells. Samples were withdrawn every 30 minutes for 3 hours to calculate intestinal permeability [9, 10]**.**

**Acknowledgements:** This research was supported by Petra University financial grant # 2-4-2023.
**Author contributions:** Concept – NI, AS.; Design – NI, AS.; Supervision – NI, AS.; Resources – AG, NI.; Materials AG, NI, AS; Data Collection and/or Processing NI, NW, HS.; Analysis and/or Interpretation – AR, NI, NW, HS, NQ; Literature Search – NI, NW, HS.; Writing – AS, HT.; Critical Reviews NQ, GA.
**Conflict of interest statement:** The authors declared no conflict of interest

## REFERENCES

[1] Nakamura S, Kozuka M, Bastow KF, Tokuda H, Nishino H, Suzuki M, Tatsuzaki J, Morris Natschke SL, Kuo SC, Lee KH. Cancer preventive agents, Part 2: Synthesis and evaluation of 2-phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives as novel antitumor promoters. Bioorg Med Chem. 2005;13(14):4396-4401. https://doi.org/10.1016/j.bmc.2005.04.078.

[2] Lee K, Kuo S, Wu T, Wang HK, Li L. Preparation of 2-aryl-4-quinolones as antitumor agents. PCT Int Appl. 1996; WO 9610563 A1.

[3] Kuo S, Teng C, Lee K, Huang L, Chou L, Chang C, Sun C, Wu T, Pan S, Way T. Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents and their preparation and use in the treatment of solid cancer PCT Int Appl. 2008; WO 2008070176 A1.
[4] Li K, Li Y, Zhou D, Fan Y, Guo H, Ma T, Wen J, Liu D, Zhao L. Synthesis and biological evaluation of quinoline derivatives as potential anti-prostate cancer agents and Pim-1 kinase inhibitors. Bioorg Med Chem. 2016;24(8):1889-1897. https://doi.org/10.1016/j.bmc.2016.03.016.
[5] Al-Sheikh A, Arafat T, Abuqatusa L, Mallah E. Substituted quinolone compounds, their use in the treatment of cancer, and a method for preparation. PCT Int Appl. 2020; WO 2020136693 A1 20200702.
[6] Wei J, Liu R, Zhang J, Liu S, Yan D, Wen X, Tian X. Baicalin Enhanced Oral Bioavailability of Sorafenib in Rats by Inducing Intestine Absorption. Front Pharmacol. 2021;12:761763. https://doi.org/10.3389/fphar.2021.761763.
[7] https://admetmesh.scbdd.com
[8] Takamatsu N, Welage LS, Idkaidek NM, Liu DY, Lee PI, Hayashi Y, Rhie JK, Lennernäs H, Barnett JL, Shah VP, Lesko L, Amidon GL. Human intestinal permeability of piroxicam, propranolol, phenylalanine, and PEG 400 determined by jejunal perfusion. Pharm Res. 1997;14(9):1127-1132. https://doi.org/10.1023/a:1012134219095.
[9] Andrea N Edginton, Eric I Zimmerman, Aksana Vasilyeva, Sharyn D Baker, John C Panetta. Sorafenib Metabolism, Transport, and Enterohepatic Recycling: Physiologically Based Modeling and Simulation in Mice. Cancer Chemother Pharmacol. 2016, 77(5):1039–1052. https://doi.org/10.1007/s00280-016-3018-6.
[10] Abbiati RA, Craparo EF, Manca D, Cavallaro Gennara. Sorafenib in Mice – A Pharmacokinetic Study. Chem Eng Transact. 2015; 43:283. https://doi.org/10.3303/CET1543048.