Antitumour and acute toxicity studies of 4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one against ehrlich ascites carcinoma and sarcoma-180

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ABSTRACT

In an effort to discover an effective and selective antitumour agent, synthesis and anti-cancer potential of 4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1*H*)-one (**SK-25**), which has been reported earlier by us with significant cytotoxicity towards MiaPaCa-2 malignant cells, with an IC₅₀ value of 1.95 μ M and was found to instigate apoptosis. In the present study, the antitumour efficacy of **SK-25** was investigated on Ehrlich ascites tumour (solid), Sarcoma 180 (solid) tumour and Ehrlich ascites carcinoma. The compound was found to inhibit tumour development by 94.71% in Ehrlich ascites carcinoma (EAC), 59.06% in Ehrlich tumour (ET, solid) and 45.68% in Sarcoma-180 (solid) at 30 mg/kg dose. Additionally, **SK-25** was established to be non-toxic at a maximum tolerated dose of 1000 mg/kg in acute oral toxicity in Swiss-albino mice. Computer-based predictions also show that the compounds could have an interesting DMPK profile. The current study provides insight for further investigation of the antitumour potential of the molecule.

keywords: antitumour; cancer; chalcone; DMPK; *in silico*; *in vivo*

1 Introduction

Cancer is the main cause of human deaths in economically advanced countries, having devastating effects. This is irrespective of the significant advancement in therapeutic innovation towards its diagnosis and treatment [1]. Although patients in these countries have access to state of the art chemotherapeutics and major steps taken towards clinical management of the disease, the dilemma of undesirable side effects and emergent resistance of malignant cells to drugs has made numerous regimens ineffective. Thus, major efforts must be dedicated to hunting for innovative therapeutic candidates [2].

New anticancer drug candidates are often either designed on the basis of designing small molecules against specific targets or tumour types or by large-scale drug screening methods, e.g. virtual screening of electronic libraries of high throughput screening of compound collections. Due to the specificity of different cancer types, such drug candidates must show higher efficacy and lesser side effects than known cytotoxic agents. The investigation of new antitumour agents often couples *in silico*, *in vitro* and *in vivo* techniques. *In vitro* screening is one of the prime fundamental steps (sometimes preceded by the *in silico* screening of millions of virtual compounds), aimed at identifying preliminary hits for further drug development. *In silico* and *in vitro* techniques are relatively less costly and less tedious, thus permitting the evaluation of more drug candidates, when compared with *in vivo* methods. For the aforementioned reasons, it is practically impossible to generate *in vivo* data for large datasets of anticancer drug candidates. However, *in vitro* experiments mainly serve to select the preliminary lead compound(s), which could then be further investigated in living organisms (*in vivo*) [3,4]. A step-wise process is often followed from *in vitro* to *in vivo* to reduce the number of potential anticancer agents to be tested to just a few candidates, which could then be further taken to clinical trials. The current study reports *in vivo* studies of one of our previously

reported potent anticancer chalcones on mice. This rodent was selected because their genetic, biological and behavioral characteristics closely related with humans [5].

Chalcones are a class of biaryl propenones, which have demonstrates toxicity against diverse malignant cells *via* the interaction with tubulin at its colchicine fastening position [6-10]. Chalcones, considered as the starting materials of flavonoids and isoflavonoids, have a broad range of pharmacological properties, including antitumour [11-13], anti-inflammatory [14,15], anti-fungal and anti-tubercular properties [16]. Chemically, chalcones (1,3-diaryl-2- propen-1-ones) hold an enone connection among two aromatic rings [17-23]. Both naturally occurring and synthetic chalcones have revealed interesting biological profiles. They have serve as lead compounds for the discovery of new anti-inflammatory, anti-infective, anti-cancer and antioxidant agents [24]. A literature report shows that chalcones are capable of inducing the death of cancer cells through apoptosis, as well as subsiding the mitochondrial membrane potential [25-27].

With the quest to discover safe and potent prospective novel chalcones as anticancer agents, a novel 4,6-diaryl pyrimidone derivative, *4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one* (**SK-25**) was reported by our research group, which involved the fusion of pyrimidone and chalcone to a rigid chalcone framework (Fig. 1) [28]. The aim of the current study is to investigate the *in vivo* anti-cancer potential of the synthesized compound, which restrains the tumour enlargement in tumour mice models like Ehrlich tumour (ET, solid), Ehrlich ascites carcinoma (EAC), and sarcoma-180 (solid). In addition, acute oral toxicity of SK-25 was also performed as per OECD guidelines No 423. This compound was observed to be most dynamic and potent with IC₅₀ value of 1.95 μ M against MiaPaCa-2 cell lines and findings of the optimized lead compound are also summarized in tabular form in (Table 1).

2 Materials and methods

2.1 Chemicals. All the chemicals and reagents were purchased from Merck, CDH, Sigma Aldrich, Spectro chem., Loba chem., India and utilized without of additional purification.

Biotage Microwave Synthesizer (Model: Initiator) was used for carrying out the reactions operating at 150 °C, with the microwave power maximum level of 400W. ^1H NMR and ^{13}C NMR (75 MHz) spectra were recorded using JEOL (300 MHz) NMR spectrometer. The spectra were determined in DMSO- d_6 relative to TMS (0.00 ppm). Chemical shifts were reported in δ values utilizing tetramethylsilane as an internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd- double doublet) and coupling constants (J) in Hz (Hertz) in ^1H NMR. Melting points were determined in open capillaries and were uncorrected.

2.2. Chemistry

2.2.1. Preparation of tetrafluoroboric acid catalyst ($\text{HBF}_4\text{-SiO}_2$). The tetrafluoroboric acid catalyst system was prepared following the formerly reported strategy [29]. A 40% aq. HBF_4 (3.3 g, 8.25 mL, 15 mmol) and silica gel (26.7 g, 300-400 mesh) were stirred for 3 hours in diethyl ether (75 ml). The above contents of mixture were concentrated and the deposits were dried under the vacuum for 72 h at 100 °C to get $\text{HBF}_4\text{-SiO}_2$ (0.5 mmol HBF_4/g), as a free-flowing powder [30].

2.2.2. General method for the synthesis and characterization of 4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one (SK-25). Substituted acetophenones (1 mmol, 1 eq), benzaldehydes (1 mmol, 1 eq), urea (1 mmol, 1 eq) and 5 mol % of silicated flouroboric acid catalyst were added in 50 mL dried conical flask. The above contents were mixed properly and placed in microwave for 10 min. After the completion of reaction, contents were made dissovded in methanol and adsorbed on to silica (60-120 #). The desired purified product was obtained by passing through column chromatography with increasing proportions of ethyl acetate in hexane as eluting agent.²¹ This was confirmed by spectroscopic techniques. Light brown powder; Yield- 88 %; mp: 275-276 °C; ^1H NMR (DMSO- d_6 , 300 MHz, δ , TMS = 0) : 8.73 (2H, *d*, J = 4 Hz), 8.19 (1H, *d*, J = 4 Hz), 8.00 (2H, *bs*), 7.83 (2H, *m*), 7.23 (1H, *dd*, J = 2 and 4 Hz) ; ^{13}C NMR (CDCl_3 , 75 MHz, δ , TMS = 0): 164.67, 163.33, 152.33, 149.92, 135.52, 134.28, 132.57, 129.67, 128.85, 128.35, 127.56, 124.29, 106.41; Anal. Calcd. for $\text{C}_{13}\text{H}_9\text{N}_3\text{OS}$: C, 61.16; H, 3.55; N, 16.46; S, 12.56 Found: C, 60.97; H, 3.66; N, 16.09; S, 12.44.

2.3. Bioassays

2.3.1. *In vivo* antitumour activity. All animal experiments used in this study were approved by the Institutional Animal Ethical Committee, Guru Nanak Dev University, Amritsar, Punjab, India (approval No 226/CPCSEA). The Swiss albino mice weighed in the range of (18–23 g) were housed and maintained under the controlled conditions at a temperature ($23 \pm 2^\circ\text{C}$), relative humidity (50-60 %). The room was ventilated with 100 % fresh air. Animals were fed with standard pellet diet (M/s Ashirwad Industries, Chandigarh, India) and autoclaved water was provided *ad libitum*. The studies for *in vivo* anti-cancer activities were conducted according to the guidelines issued by National Cancer Institute (NCI) [31].

2.3.2. Ehrlich ascites carcinoma (EAC). The *in vivo* anti-cancer assay was performed as earlier reported against Ehrlich ascites carcinoma (EAC) model [31]. EAC (1×10^7) cells were collected from 8-10 days old Swiss albino mice having old ascitic tumour and injected intraperitoneally on day zero (Table 2). On day 1, all animals were randomized and estranged into four groups. **SK-25** was administered i.p. in a dose of 20 mg/kg and 30 mg/kg to Group I and Group II from days 1–9. Similarly, a positive control (Group III) was administered 5- fluorouracil (5-FU, 20 mg/kg i.p) and normal saline (0.2 ml, i.p.). On day 13, all animals were sacrificed and % tumour growth inhibition was calculated.

2.3.3. Ehrlich tumours (Solid) and Sarcoma-180 (Solid). Ehrlich ascites carcinoma (EAC) 1×10^7 cells were collected from the peritoneal cavity of animals having tumour and injected intramuscularly in the right thigh of all animals on day 0. The next day, all animals were randomized and alienated into four groups: Group I and Group II received **SK-25** (20 mg/kg and 30 mg/kg), Group III received positive control (5-FU) and Group IV received (normal saline) for 9 consecutive days. On day 13, all animals were sacrificed, and average tumour weight was calculated.

2.3.4. Hematology and biochemical analysis of EAT-bearing mice. After completing the whole experiment, hematological and biochemical parameters were analyzed. Blood was withdrawn from retro-orbital plexus of the mice and aspirated in an automated hemato analyser (Sysmex

1800, Germany). For assessment of the biochemical analysis, blood samples were centrifuged at 1000g for 10 min at 4 °C. Plasma samples were assayed for the determination of all biochemical parameters with the use of automated biochem analyzer (Erba EM360, Japan) in the process.

2.4. Acute oral toxicity assay. **SK-25** was evaluated for acute oral toxicity in mice [32-34]. Six mice in each group, weighing between 18-23 g, were divided into different groups. Seven days acclimatized mice were used. Mice of group 1 served as control, while the remaining four groups were kept as treated groups.

2.4.1. Administration of test item. The animals were subjected to fasting along with water overnight (16-18 hours) before dosing. Each mouse received test formulations orally by gavage. Animals were fed on a normal diet after 3-4 hours of dosing. The compound **SK-25** was suspended in 0.5 % xanthan gum solution in distilled water and was administered at 5, 50, 300 and 1000 mg/kg to animals of groups 2, 3, 4 and 5, respectively. Animals of normal control were administered only vehicle.

2.5. Observational and gross pathological Study

Clinical signs and mortality. The animals of all groups were checked for mortality and any toxic symptoms at two-hourly intervals for up to 24 hours. A case side clinical examination was noted for another 13 days which included any alteration in mucous membrane, eyes, skin and fur, central nervous system, respiratory patterns, somatomotor activity and behavior pattern and other responses, e.g. lachrymation, etc. Particular observations, e.g. loose bowels, convulsions, tremors, salivation, laziness, sleep and coma were also recorded [32].

2.6. Assessment of adverse effects

2.6.1. Locomotor activity. The impulsive motor activity was evaluated using **SK-25** with actophotometer [35]. Each mouse was placed in a square bugged field arena (30 cm × 30 cm × 30 cm) fitted with six photocells before and after administration of test compounds for 5 min. Scores (locomotor activities) were expressed as total counts of photo beam interruption for 5

min.

2.6.2. Rotarod test. The effect of **SK-25** on motor coordination and grip strength were assessed using rotarod apparatus as previously reported [36]. In brief, before the start of the experiment, animals were subjected to training using rotarod (3.7 cm in diameter, 10 rpm) waiting that they could stay on for 60 s without falling. Rota rod test was performed for 5 min after 30 min of treatment.

2.6.3. Assessment of body weight and relative organ weight. In addition to sighting analysis, body weights were also recorded. The animals were sacrificed and were subjected to thorough necropsy inspection of the exterior of the whole body and vital organs [37,38]. Weights of organs like kidney, liver, heart, spleen, brain and lungs were recorded and the relative weight of every organ was determined after 14 days using the given formula:

$$\text{ROW} = (\text{OW}/\text{BW}) \times 100$$

where; ROW = Relative Organ Weight

OW = Organ Weight

BW = Body Weight

2.6.4. Histopathological study. After determining the weight, specific organs like liver, kidney and hearts to be fixed in 10% buffered formalin solution for 24h, dehydrated in different grades of alcohol (70, 90, 95, and 100%). Tissues were entrenched in paraffin wax, sliced into 4–5 μm wide sections, and subjected to hematoxylin-eosin staining for photo microscopic examinations using a phase contrast fluorescent microscope.

2.6.5 Statistical Analysis. Comparisons were made among control and test groups using Student's t-test. Values are represented as mean $\pm$ S.E.M. (n = 10 for control group, n = 7 for test group). ***P < 0.001, **P < 0.01 for each analysis.

2.6.6. Computer-based predictions. The low energy 3D model of **SK-25** was generated using MOE [39]. The compound was further prepared by the LigPrep tool of Maestro [40,41].

Physicochemical properties related to drug metabolism and pharmacokinetics were predicted using QikProp [42].

3 Results

3.1. *In vivo* antitumour efficacy

3.1.1. Effect of SK-25 on Ehrlich ascites carcinoma (EAC) and Ehrlich tumour (solid). SK-25 evaluated for *in vivo* efficacy in EAC and Ehrlich tumour (solid) models were employed [31]. Moreover, no loss of weight was observed in the treated group of animals (Table 2). SK-25 revealed total growth inhibition of 91.56% and 94.71% (Table 3) in EAC and 38.64 % and 59.06% (Table 4) in Ehrlich tumour (solid) at 20 mg/kg/ i.p and 30 mg/ kg/ i.p respectively. SK-25 caused significant inhibition of tumour growth in EAC and solid tumour models of mice (Fig. 3). SK-25 was shown to be non-toxic at all the tested doses and no mortality was observed.

3.1.2. Effect of SK-25 on Sarcoma-180 (Solid) model. Efficacy of SK-25 is determined using the sarcoma-180 (Solid) model. SK-25 produced 32.90 % and 45.68 % inhibition of tumour growth (Table S1, Supplementary data) in sarcoma-180 (Solid) at 20 mg/kg/ i.p and 30 mg/ kg/ i.p, respectively. Images were taken by the digital camera (Fig. 4). Interestingly, it was found that SK-25 did not show any mortality (0/7) at all tested doses.

3.1.3. Hematology and biochemical analysis EAT and Sarcoma-180 (solid) tumour-bearing mice. The outcomes of blood analysis and serum biochemical parameters are in normal ranges which revealed that SK-25 is non-toxic at both the doses 20 mg/kg and 30 mg/kg. No significant changes were observed when compared with positive and normal controls. The results are shown on Tables S2 and S3 (Supplementary data).

3.2 Assessment of adverse effects

3.2.1. Acute oral toxicity assay. The molecule SK-25 was additionally analyzed for *in vivo* acute toxicity [32,33]. Overall, the study showed that compound SK-25 was well tolerated by the Swiss-albino mice for maximum dose level of 1000 mg/kg p.o. A schematic representation of the experimental design of acute toxicity of SK-25 is depicted in Fig. 5.

3.2.2. Observational and gross pathological analysis. All the animals were frequently observed for mortality and any toxic symptoms at two-hourly intervals for up to 24 hours. A case side clinical examination was noted for another 13 days, there was no significant alteration in mucous membrane, eyes, skin and fur, central nervous system, respiratory patterns, somatomotor activity and behaviour pattern, occurrence of tremors, convulsions, abdominal contortions and another responses, e.g. lachrymation, etc [32]. Particular observations like loose bowels, convulsions, tremors, salivation, laziness, sleep and coma were also shown in Table S4 (Supplementary data).

3.2.3. Actophotometer test. No physical sign of toxicity was evidenced by behavioural changes. Locomotor activity was assessed using actophotometer. It was found that there is no significant decrease in locomotor activity with **SK-25** at all the tested doses (Table 5).

3.2.4. Rota rod test. The mice were observed for behavioural changes and mortality within 24 h. The effect of **SK-25** was evaluated on the motor performance of the animals. The compound did not show any change in spontaneous locomotion when compared with vehicle-treated control group (Table 5).

3.2.5. Body weight and Mortality. In adding to sighting study, body weights and mortality were also recorded (Table S5, Supplementary data). However, no alteration in food consumption and no significant reduction of the body weight was observed, when compared with the control group.

3.2.6. Relative organ weight. After the animals were sacrificed and subjected to detailed necropsy assessment of the peripheral surface of the body for any gross pathological changes, all animals were observed for toxic signs and any pre-terminal deaths daily (Table S6, Supplementary data). In all the treated groups, there were no considerable changes in relative organ weight of vital organs like liver, heart, kidney, spleen, brain and lungs were when compared with control group depicted in (Fig. 6) [37].

3.2.7. Histopathological analyses of vital organ. No apparent changes in histological examinations were found in all organs at a dose of 1000 mg/kg as compared with normal control groups (Fig. 7). Findings confirmed no signs of toxicity, such as reduction in body weight, organ weight, and mortality at dose of 1000 mg/kg.

3.2.8. Computer modelling and prediction. The computed DMPK predictions led to 46 computed parameters (Table S7, Supplementary data), all of which fall with the recommended range for 95% of known drugs, including Lipinski's "rule of Five" for drug-like compounds and the "Rule of Three" for Lead-like compounds.

4 Discussion

The search for safe and potent anticancer chemotherapeutic agents with natural product scaffolds, e.g. chalcones, has been a hot topic [43,44]. The compound under investigation was recently synthesized by a microwave assisted multicomponent strategy and proven to be an antiproliferative agent [28, 45, 46]. Equal amounts of aromatic ketone (5.0 mmol, 1 eq), aromatic aldehyde (5.0 mmol, 1 eq), urea (5.0 mmol, 1 eq) and 5 mol % of silicated fluoroboric acids catalyst were mixed and placed in a microwave synthesizer for 10 minutes (Fig. 2, Scheme 1). In our previous study, the cytotoxicity of **SK-25** was evaluated against four different human cancer cell lines, viz. MiaPaCa-2 (pancreatic cancer), PC-3 (prostate cancer), A-549 (lung cancer) and HCT-116 (colon cancer). The compound had exhibited a percentage inhibition of 93 % and IC₅₀ value of 1.95 μ M towards MiaPaca-2 malignant cells. Apoptosis was confirmed by annexin V/PI binding assay, which is a hallmark of cancer [47]. Results demonstrate a dose-dependent boost in the number of apoptotic cells in MiaPaCa-2 malignant cell lines. One of the most remarkable features of the designed compound was the selectivity displayed by the synthesized pyrimidones. The compound was selectively active against MiaPaCa-2 cell line whereas A-549 and PC-3 were resistant against the experience of test molecules. **SK-25** possessing heteroaryl rings at both 4 and 6 locations where the most powerful agent cause percentage inhibition of 93 % and IC₅₀ value 1.95 μ M against MiaPaCa-2 cell lines. This is because of hydrogen bonding capacity of heteroatom. **SK-25** was one of the most effective inhibitors because high aromatic character credited due to the incorporation of heterocyclic rings.

A detailed mechanistic study was further investigated using **SK-25** in MiaPaca-2 cell lines. The molecule induces apoptosis which is revealed by DAPI staining and phase contrast microscopy. Loss of mitochondrial membrane potential has been measured as an indicator of cell death. **SK-25** causes significant reduction of mitochondrial membrane potential, MMP loss is an early apoptotic incident and smashed mitochondria impart numerous signals to downwards that initiate inherent apoptotic death signals in cells and cells treated with compound were displaying cell cycle arrest in G0/G1 phase, which established the apoptotic capability of the molecule. The molecule **SK-25** showed the 30.33 % arrest of apoptotic population in test treated with 20 μ M of **SK-25** [28]. Within the series of previously synthesised and tested chalcones possessing diheteroaryl rings additionally evaluated for cell death mechanism, **SK-25** was found to be the most active. Various experiments such as DAPI staining, Phase contrast microscopy, measurement of MMP loss and cell cycle analysis were performed to gain mechanistic insights. It was observed that **SK-25** causes an apoptotic induction to 30.33 % was observed on treatment at 20 μ M [28].

A report of extensive investigations on chalcones shows that the presence of two aryl nuclei increases the anti-tumour prospectives [45,46]. Thus, investigations on constrained chalcone analogues remains an area of pharmacological interest, offering immense scope for investigations on this privilege class of tubulin inhibitors. The remarkable anti-cancer potential of pyrimidine/ones, as evidenced by the number of research articles further motivated us to utilise it as a constraint for attaining a rigid arrangement of the two aromatic rings [28]. Keeping in view the significant anti-cancer potential of constrained chalcone analogues, the present study involves *in vivo* antitumour efficacy of **SK-25** on Ehrlich ascites tumour (solid), Sarcoma 180 (solid) tumour and Ehrlich ascites for proving the anticancer potential of the synthesized chalcone *in vivo*.

In recent years, it happens to be more and more evident that the innovation of novel drugs alone is not satisfactory to make sure the evolution in drug development. Exhilarating experimental results obtained *in vitro* is very frequently followed by disappointing results from *in vivo* studies. The foremost reasons for disappointment include the deficient concentration of

drug at the target site, side effects, denied specificity, higher incidence of drug resistance to cancer cells. Consequently, the current research was snooping out the *in vivo* anti-cancer potential of **SK-25** in an experimental tumour model. To investigate the potential of **SK-25** for increased therapeutic benefit against cancer, *in vivo* antitumour activity was performed. The molecule **SK-25** was administered to tumour-bearing mice through intra-peritoneal route and it noticeably inhibits 45-95% tumour growth in all investigational tumour models.

Toxicological scrutiny often helps to drive a decision for a novel chemical entity for its clinical use as safe and effective candidate [48-50]. Moreover, no mortality was observed and the tested animals did not show any stern adverse effects. Moreover, 5-FU has various adverse effects in current therapy and shows lesser effect than **SK-25**. The results revealed that compound **SK-25** had more strapping effects in ascites tumour than solid tumours. Thus, **SK-25** could be used in the treatment of non-solid tumours in future. In this report, **SK-25** has been shown to be non-toxic and inhibit tumour growth in all tumours models. Since there is no mortality throughout the experiment. Moreover, no changes were observed during hematological and biochemical toxicity after treatment with **SK-25** in sarcoma-180 (solid) and EAC (solid) tumour models which make it as a choice of treatment, therapeutically effective and safe. Similarly, no mortality and signs of toxicity like reduction in body weight, food intake, relative organ weight, gross pathology of vital organs during treatment with different doses as compared with control in acute toxicity study. Moreover, no noticeable changes observed in histological examinations of major tissues when compared to normal control. Despite, the promising anti-cancer efficacy, **SK-25** was also found to be safe up to the dose of 1000 mg/kg. Therefore, **SK-25** could be further considered as a potential anti-cancer drug candidate. Nevertheless, sub-acute and chronic toxicity analysis should be done to search for any unfavourable impact on the repetitive administration of compound **SK-25** for its future development.

Conclusions

The well-established cell-killing potential of **SK-25** motivated and tempted us to explore the anti-cancer potential of 4,6-diaryl pyrimidones. **SK-25** displayed noteworthy anti-cancer effects both from *in vitro* and *in vivo* studies. It was accomplished that the cytotoxicity of **SK-25** with

IC₅₀ values 1.95 μ M against human pancreatic cancer cell line was confirmed by *in vivo* experiments. The present study describes the anti-cancer potential of **SK-25** through both solid tumours and does not show any sign of toxicity such as mortality, reduction in body weight, changes in hematological and biochemical toxicity studies in the tested animals. Furthermore, **SK-25** was found to be safe up to the 1000 mg/kg. In addition, the acute oral toxicity of **SK-25** was also performed as per OECD guidelines No 423. Molecular modelling studies and *in silico* predictions also showed that the compounds could have an interested DMPK profile, since 46 all computed parameters fall within the accepted range for approved (marketed) drugs. This compound was observed to be most dynamic and potent with an IC₅₀ value of 1.95 μ M against MiaPaCa-2 cell lines and findings of the optimized lead compound have been provided. It could be concluded that **SK-25** is safe and moderate antitumour lead molecule along with anti-proliferative and apoptosis-inducing properties. Nevertheless, it has potential as a good candidate in future for treatment of cancer.

Declaration of interest

The authors have declared that no competing interests exist.

Consent for publication

All authors gave their consent for submission of the manuscript towards publication.

Author contributions

Designed the experiments (GS, SKJ, GKG and FNK), carried out the experiments (DK, PS, PJS, KN, GM, MJM, AS, DMM and GS), provided experimental material (GS, PS, SKJ, GKG, KN, GM, MJM, AS, DMM and FNK), analysed data (DK, PS, PJS, KN, GM, MJM, AS, SKJ, GKG and FNK), wrote the draft manuscript (DK, PS and FNK), and approved the manuscript for submission (all authors).

Availability of Data and Materials

Not applicable

Acknowledgements

One of the authors (DK) is grateful to University Grants Commission New Delhi, for providing research fellowship under UPE (Focus Area-Health Care, Drug Development and Sports Medicines) Scheme (Sanction No. 25994/ Estt./A-11). FNK is currently a Georg Forster (HERMES) fellow of the Alexander von Humboldt Foundation, Germany. The funders played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Abbreviations

EAC	Ehrlich ascites carcinoma
	Ehrlich ascites tumour/ Ehrlich
EAT/ET	tumour
NCI	National cancer institute
5-FU	5- Fluorouracil
	Inhibit cellular proliferation by 50 %
IC ₅₀	concentration
μM	Micro molar
mg	Milligram
mL	Milliliter
Kg	Kilogram
i.p	Intra-peritoneal
DAPI	DAPI = 4',6-diamidino-2-phenylindole
MMP	Mitochondrial membrane Potential
H&E	Hematoxylin and eosin

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FIGURES

Fig. 1. Design strategy for the synthesis of SK-25

Fig. 2 (Scheme 1). Synthesis of 4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one

Fig. 3. Anticancer activity of SK-25 in Ehrlich tumor solid model

Fig. 4. Evaluation of *in-vivo* anti-cancer activity of SK-25 in Sarcoma-180 (solid) models. (A) Schematic representation of experimental plan, (B) Images of Ehrlich ascites tumor mice following treatment at 13 day

Fig. 5. Schematic representation of the experimental design for *in-vivo* acute oral toxicity of SK-25

Fig. 6. Effect of SK-25 as a single acute oral dose at 5, 50, 300 and 1000 mg/kg on relative organ weight in Swiss-albino mice

Fig. 7. Histology examination of kidney, heart and liver with single acute oral dose 1000 mg/kg of SK-25.

Table 1. Profile of optimized lead compound SK-25

Code	SK-25			
Molecular Formula	C ₁₃ H ₉ N ₃ OS			
IUPAC Name	4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one			
State	Solid, powder			
Color	Light brown			
Structure
Melting Point	275-276 °C			
Yield	88 %			
¹ H NMR	(DMSO-d ₆ , 300 MHz, δ, TMS = 0) : 8.73 (2H, <i>d</i> , <i>J</i> = 4 Hz), 8.19 (1H, <i>d</i> , <i>J</i> = 4 Hz), 8.00 (2H, <i>bs</i>), 7.83 (2H, <i>m</i>), 7.23 (1H, <i>dd</i> , <i>J</i> = 2 and 4 Hz)			
C ₁₃ NMR	164.67, 163.33, 152.33, 149.92, 135.52, 134.28, 132.57, 129.67, 128.85, 128.35, 127.56, 124.29, 106.41			
Percentage growth Inhibition at 50 μM	Pancreatic (MiaPaCa-2)	Prostate (PC-3)	Lung (A-549)	Colon (HCT-116)
	93	13	--	49
Elemental Analysis	C, 61.16; H, 3.55; N, 16.46; S, 12.56 Found: C, 60.97; H, 3.66; N, 16.09; S, 12.44.			
IC ₅₀ Values	1.95 μM			
MMP Loss	51.2 % at 20 μM			
Apoptosis Induction	30.33 % at 20 μM			
Ehrlich ascitic carcinoma (EAC)	% Tumor Cell Growth		% tumor growth inhibition	
	20 mg/kg		8.43 91.56	
	30 mg/kg		5.28 94.71	
Ehrlich tumor (Solid)	Average Tumor Weight		% tumor growth inhibition	
	20 mg/kg		719.85 ± 62.35 38.64	
	30 mg/kg		480.28 ± 72.04 59.06	
Sarcoma-180 (Solid)				
20	816.78 ± 120.89		32.90	
30	661.14 ± 95		45.68	
Toxicity	Acute oral-well tolerated up to 1000 mg/kg single dose			
Observational Parameters	Normal			
Gross pathological Changes	No change			

Histopathological study

No change

Table 2. Effect of SK-25 on body weight of mice in Ehrlich ascites carcinoma assay

Treatment	Dose (mg/Kg/i.p)	Average body weight (g)			
		Day 1st	Day 5th	Day 9th	Day 12th
Control	0.2 ml N.S. i.p	22.16	23.52	24.31	26.42
SK-25	20 mg/kg/ i.p	21.12	22.14	23.26	23.15
SK-25	30 mg/Kg/i.p	21.23	22.16	23.17	23.14
5-FU	20 mg/kg/ i.p	21.18	22.43	22.65	22.12

Table 3. *In vivo* anticancer activity of SK-25 on against EAC assay.

Ehrlich ascites carcinoma (EAC)								
Treatment	Dose Mg/Kg/i.p	Day 12						
		Av. Volume of fluid (ml)	Av. Weight of ascitic fluid (g)	Av. No. of tumor cells ($\times 10^7$)	% Tumor cell growth	% Tumor growth inhibition	Mortality	
Control	0.2 ml N.S.	8.02 $\pm$ 1.42	7.9 $\pm$ 1.4	168.3 $\pm$ 34.5	100	--	0/10	
SK-25	20	1.74 $\pm$ 0.03	1.7 $\pm$ 0.08	14.2 $\pm$ 1.8***	8.43	91.56	0/7	
SK-25	30	0.70 $\pm$ 0.08	0.6 $\pm$ 0.08	8.90 $\pm$ 1.6***	5.28	94.71	0/7	
5-FU	20	0.22 $\pm$ 0.07	0.11 $\pm$ 0.05	3.57 $\pm$ 0.52***	2.13	97.87	0/7	

***P < 0.001, **P < 0.01

Table 4. *In vivo* anticancer activity of SK-25 against Ehrlich tumour (Solid).

Ehrlich tumor (solid)								
Treatment	Dose (mg/kg/i.p.)	Average body weight (g)						
		Day 1st	Day 5th	Day 9th	Day 13th	Av. Tumor weights (mg)	%Tumor Growth Inhibition	Mortality
Control	0.2 ml N.S.	21.86	22.52	23.91	25.42	1173.25 $\pm$ 88.28	-	0/10
SK-25	20	21.31	22.37	23.46	23.59	719.85 $\pm$ 62.35***	38.64	0/7
SK-25	30	21.45	22.62	23.03	22.84	480.28 $\pm$ 72.04***	59.06	0/7
5-FU	22	21.11	22.27	22.41	22.49	597.92 $\pm$ 89.1***	49.03	0/7

***P < 0.001, **P < 0.01

Table 5. Effect of SK-25 on assessment of behavioral effects in acute toxicity assay.

Treatment/dose(mg/Kg)	Acute toxicity assay	
	Locomotor activity Counts/5min	Retention time/latency to fall
Vehicle Control	389.11 ± 21.19	299.12 ± 18.09
SK-25 (5mg/kg)	385.05 ± 19.89	297.75 ± 22.09
SK-25 (50mg/kg)	380.54 ± 22.15	298.50 ± 21.71
SK-25 (300mg/kg)	378.34 ± 23.11	295.22 ± 26.42
SK-25 (1000mg/kg)	378.88 ± 23.32	294.72 ± 28.10