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Benzopyrylium salts as new anticancer, antibacterial, and antioxidant agents

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Abstract

Benzopyrylium salts are an unexplored class of compounds and as a first, this study reports them as potential therapeutic agents. In this effort we pursue the synthesis and in vitro anticancer, antibacterial and antioxidant properties of some novel benzopyrylium salts. The benzopyrylium salts were synthesized and further characterized via UV-vis, IR, ¹H-NMR, ¹³C-NMR and mass spectrometry. The benzopyrylium salts were tested in vitro for anticancer activity across NCI 60 cell line panel. **PS-CP-4MO** showed the best activity against the MDA-MB-435 cell line of melanoma cancer in terms of the least GI₅₀ (1.78 μM), TGI (3.47 μM) and LC₅₀ (6.77 μM) values and showed selectivity against melanoma, colon cancer and leukemia. Mechanistic studies indicate that this compound inhibits MCF-7 cancer cells by inducing apoptosis and abrogates colony formation and wound healing in the cancer cells. Antibacterial studies show that some of the benzopyrylium salts are active on *S. aureus* (ATCC 29213) and the best active compound **PS-CP-5Cl** has a MIC of 8 μg/mL. Antioxidant studies indicate that they have good free radical scavenging properties (**PS-CP-5Cl** showed activity 1.48 times ascorbic acid). Fulfillment of the Lipinski's parameters of the benzopyrylium salts in silico showed tremendous drug likeness as potential pharmacophore leads.

Graphical Abstract

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Conflict of interest The authors declare that they have no conflict of interest.

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Computational studies

The Lipinski's parameters of the five benzopyrylium salts were evaluated at: <http://www.scfbioitd.res.in/software/drugdesign/lipinski.jsp> [17, 18].

Keywords

Benzopyrylium salts; Synthesis; Anticancer activity; Antibacterial activity; Antioxidant activity

Introduction

Pyrylium salts are structurally unique as a class of six membered carbon ring system with one carbon replaced by a positively charged oxygen atom, making a positively charged moiety. The study on pyrylium salts has greatly increased over the years. As a result of works done by Balaban in the 1970s [1], the synthesis of pyrylium salts increased rapidly until its decline in the mid-1980s. The decline was observed due to the difficulties in synthesizing these types of compounds. The pyrylium salts ionic character allows the salt to be easily separated from the non-polar starting materials. Pyrylium salts have high reactivity towards nucleophiles, leading to many other products. Pyrylium salts are aromatic and are used to obtain other compounds with strong aromatic character. In general, pyrylium ring acts as a nodal point for many other synthetic routes [2-4].

Pyrylium salts are useful in photographic industry, especially in color photography. Photosensitive layers in film and papers also require the use of pyrylium salts. For photographic rolls, pyrylium salts have been used as markers. The identification of the markers is carried out by the unique fluorescence of the specific pyrylium salt and also under UV light through the respective color. Apart from these, pyrylium salts are used as dyes for fluorescent and as the constituent dye in lasers [5]. In this study, we focus on a specific class of pyrylium salts, i.e., the benzopyrylium salts. The synthesis of some naphthopyrylium salts have already been reported and studied for thermochromic and photochromic properties [6-9]. On the other hand, the benzopyrylium salts were first reported by Borche and Geyer [10] in 1913 and not many studies been carried out for these kind of benzopyrylium salts. Most importantly, the benzopyrylium salts have not been explored for their pharmacological properties.

As part of our endeavors to explore novel pharmacophores, benzopyrylium salts have been selected as the leads due to the lack of their wide applications as therapeutic agents. To evaluate their biological relevance we chose cancer and bacterial infections as our targets as these are some of the most common disease types in terms of causing illness in human beings. As a first, with the encouraging in vitro anticancer results obtained against the

60 cell line panel of NCI, U.S.A., we are reporting the therapeutic applications of the benzopyrylium salts. Further, mechanistic studies have been carried out on MCF-7 breast cancer cell lines. To explore their other therapeutic applications we have also evaluated their antibacterial and antioxidant properties.

Results and discussion

Synthesis and characterization

Five benzopyrylium salts have been synthesized as shown in Scheme 1 (in “Materials and method” section) and characterized using the standard techniques. The compounds synthesized along with their sample codes and yields are shown in Table 1.

Two out of the five synthesized compounds are novel. The compounds **PS-CP-SAL** [6, 11], **PS-CP-4MO** [11], and **PS-CP-5Br** [9] have already been reported previously though their biological activities have not been evaluated till date. The UV-vis spectra show three distinctive peaks, foremost in between 250 and 260 nm, followed by the subsequent peak in the range of 290 and 320 nm and the final peak between 400 and 530 nm. IR spectroscopy shows the absence of any C = O stretch in the 1760–1660 cm⁻¹ region confirming the formation of the pyrylium cation and absence of any carbonyl impurities. The C = C double bond stretch was observed in the 1635–1600 cm⁻¹ region. Signals in the between 1550 and 1400 cm⁻¹ pertain to the aromatic skeletal bands and that in 900–650 cm⁻¹ denote C–H out of plane bend. Strong signals indicating the tri substituted benzene rings were observed in between 850 and 800 cm⁻¹. The C–O stretch was seen between 1380 and 1100 cm⁻¹ whereas the C–Cl and the C–Br showed strong bands in the region of 1080–1030 cm⁻¹.

In ¹H-NMR, depending on the degree of shielding, the aromatic protons and the olefinic protons were observed between δ 6.60 and 8.40. Weak signals in the ¹³C-NMR are identified as the quaternary carbons. The carbons in the methoxy substituent are observed between δ 56 and 40. The pyrylium-carbon has a shift between δ 170 and 165, which is on the lower range of carbonyl carbon shifts. Protons H-3 and H-4 are seen as broad singlets at δ 3.49 and δ 3.54 ppm respectively in **PS-CP-SAL**. The splitting is not resolved due to the weak coupling between these two sets of protons. The signal at δ 2.04 is from the acetic acid-d4. We have observed that in the ¹H-NMR of the compounds carried out in 400 MHz instrument these signals come as a multiplet.

The anticipated molecular ion peaks were obtained for all the benzopyrylium salts. The bromo substituted compound had the distinctive [M]⁺ and [M + 2]⁺ peaks with 1:1 ratio whereas the chloro substituted compound had the [M]⁺ and [M + 2]⁺ peaks with 3:1 ratio.

The detailed characterization data of the synthesized benzopyrylium salts have been given in the Supplementary section.

In vitro studies

Anticancer studies—NCI considers the compounds showing at least 32% of growth inhibition in the cell lines as active.

PS-CP-4MO is the only active compound in the entire series of the benzopyrylium salts at 10 μ M concentration. It has been found to have very significant activity (growth) against Leukemia (CCRF-CEM 9.94%; MOLT-4 0.52%; SR -2.10%), Colon Cancer HCT-116 -64.10%; HCT-15 4.44%), Melanoma (LOX IMVI 7.79%; MDA-MB-435 2.85%; Ovarian Cancer (IGROV1 9.32%), Renal Cancer (786-0 -6.35%;), Prostate Cancer (PC-3 8.84%) types. **PS-CP-SAL**, **PS-CP-5MO**, **PS-CP-5Cl-SAL**, and **PS-CP-5Br-SAL** did not exhibit any significant activity. This indicates that the introduction of halogens at the 5th position in the benzopyrylium moiety reduces the anticancer potency. Greater the number of hydrogen bonds formed in the ligand-protein interaction, better is the binding and hence better activity. Hence, **PS-CP-4MO** has the best anticancer activity as compared to **PS-CP-5Cl** and **PS-CP-5Br** as it contains -OCH₃ groups which form more hydrogen bonds with the receptor as opposed to the -Cl and -Br group. **PS-CP-5MO** has no activity at all whereas **PS-CP-4MO** is the lead identified in this study. Though these aforementioned molecules are structural isomers one is active whereas the other is inactive. This brings out the fact that the position of the -OCH₃ substituent matters for potent activity.

Owing to the promising potency showed by **PS-CP-4MO**, it was selected for further five dose study at the NCI, U.S.A. The in vitro anticancer activity results at single dose (10 μ M) and 5 dose against the NCI 60 cell-line panel are given below in Table 2.

The GI₅₀ values for **PS-CP-4MO** are lesser than 10 μ M in many cases and the highest GI₅₀ was observed as 18.50 μ M. The TGI and the LC₅₀ values for **PS-CP-4MO** are in double digits in many cases.

PS-CP-4MO showed the best activity against the MDA-MB-435 cell line of melanoma cancer in terms of the least GI₅₀ (1.78 μ M), TGI (3.47 μ M) and LC₅₀ (6.77 μ M) values. If the aforementioned three parameters of GI₅₀, TGI and LC₅₀ against all the cell-lines are compared then **PS-CP-4MO** has the best activity in the colon cancer followed by melanoma cancer. The lower values indicate more selectivity in the anticancer activity. The compound's activity against breast cancer, ovarian cancer and renal cancer is also significant.

Among all the breast cancer types, **PS-CP-4MO** showed the best activity in terms of the GI₅₀, TGI and LC₅₀ against the MDA-MB-231/ATCC cell line followed by the MCF-7 cell line. Since a considerable activity was observed, MCF-7 cell line was chosen for further mechanistic studies.

Mechanistic studies—PS-CP-4MO was selected for further mechanistic studies against the breast cancer cell line MCF-7. Wound-healing assay was performed with the MCF-7 cells in absence/presence of the compound (10 μ M) for 24 h. As shown in Fig. 1a it is evident that **PS-CP-4MO** abrogates proliferation ability of the MCF-7 cancer cells. The MCF-7 cells were treated with the compound **PS-CP-4MO** (0.1 μ M) for 24 h and colony formation was observed with methylene blue staining. Figure 1b given clearly shows that **PS-CP-4MO** stops colony formation of the MCF-7 cells.

Further FACS studies were employed to determine the mode of action in MCF-7 upon treating with **PS-CP-4MO**. The results are shown below in Fig. 2.

Flow cytometry with DMSO treatment as a control was carried out. The study showed that after an incubation time of 24 h, apoptotic cells were observed. The proportion of the apoptotic cells grew after 24 h of incubation with **PS-CP-4MO**. The study concludes that the compound **PS-CP-4MO** increases apoptosis in the MCF-7 cells there by exhibiting its potent anticancer property. From the cell cycle analysis it was observed that the cell cycle was arrested mostly in the G₁ phase of the cell cycle. The above studies indicate that these benzopyrylium salts hold promise as anticancer compounds.

Antibacterial studies—The benzopyrylium salts were screened for their antibacterial properties against ESKAPE pathogen panel [12] and found **PS-CP-5Cl** being the most potent compound against *S. aureus* ATCC 29213. The results are tabulated below in Table 3.

The benzopyrylium salts were found to be inactive against *E.coli* (ATCC 25922), *K. pneumoniae* (BAA 1705), *A.baumannii* (BAA 1605), and *P.aeruginosa* (ATCC 27853) strains. However they were found to have some activity against *S. aureus* (ATCC 29213). The best active compound **PS-CP-5Cl** has a MIC of 8 µg/mL. Halogen containing benzopyrylium salts showed better potency than the parent compound **PS-CP-SAL**. Interestingly, **PS-CP-4MO** which showed very good anticancer properties is the second active compound in the antibacterial screening with a MIC of 16 µg/mL. This observation further emphasizes the fact that **PS-CP-4MO** is an active lead from this series of benzopyrylium salts.

Four out of the five benzopyrylium salts showed antibacterial properties. **PS-CP-5MO** was found to possess no anticancer and antibacterial activities indicating that having an –OCH₃ substituent at that particular position is not ideal for biological properties. Overall the study concludes that these benzopyrylium salts possess antibacterial properties though require lead optimizations.

Antioxidant properties—The antioxidant efficacy of the benzopyrylium salts at 10 µM were carried out using the standard DPPH assay. The results are tabulated below in Table 4.

All the benzopyrylium salts were observed to possess antioxidant properties owing to their aromatic structures. The parent molecule **PS-CP-SAL** and the –Br substituted salt **PS-CP-5Br** showed activity lesser than the standard ascorbic acid. The other benzopyrylium salts showed better activity than ascorbic acid with **PS-CP-5Cl** being the best among all. The compound **PS-CP-4MO** which showed the best anticancer activity also exhibited significant antioxidant properties (1.02 times ascorbic acid). The extensive conjugation and the presence of phenolic moiety are the two major factors because of which these benzopyrylium salts have good antioxidant properties.

Lipinski's parameters—Evaluation of the Lipinski parameters were carried out by measuring the log P (partition coefficient), molecular mass, number of hydrogen donor(s),

number of hydrogen acceptor (s) and molar refractivity of the benzopyrylium salts in silico. The likeness score of the benzopyrylium salts mentioned above are shown in Table 5.

For **PS-CP-4MO**, all the Lipinski parameters are fulfilled. This reinforces the fact that this particular benzopyrylium salt is a potential anticancer lead. On the other hand, the compound **PS-CP-5Br** has a log P value of 5.91. This value does not satisfy the Lipinski's rule where the log P values should be less than 5. This fact indicates the compound **PS-CP-5Br** is highly lipophilic and hence will not be an ideal lead candidate.

All the benzopyrylium salts have log *P* values greater than 4 thus showing higher lipophilicity. Overall, with structural optimizations to improve log P and other key features, these benzopyrylium salts definitely hold promise as anticancer agents.

Conclusions

As a first, this study reported anticancer activity of a few synthesized and characterized benzopyrylium salts. Two out of the five reported molecules are novel. The anticancer activity across the NCI 60 cancer cell line panel was evaluated in vitro for the benzopyrylium salts. The compound **PS-CP-4MO** exhibited the best potency and hence is identified as the anticancer lead of this study. The five dose studies conclude that **PS-CP-4MO** has selectivity against leukemia, colon cancer and melanoma. Mechanistic studies concluded that **PS-CP-4MO** induces apoptosis in MCF-7 cancer cells. Also it abrogates colony formation and wound healing of the MCF-7 cancer cells. Four out of the five benzopyrylium salts showed antibacterial properties against *S. aureus* (ATCC 29213). Halogen containing benzopyrylium salts **PS-CP-5Cl** and **PS-CP-5Br** showed better potency than the parent compound **PS-CP-SAL** with **PS-CP-5Cl** exhibiting the best antibacterial activity with a MIC of 8 µg/ml. **PS-CP-5MO** showed no anticancer and antibacterial activity indicating that the position of the –OCH₃ substituent is important. Antioxidant studies revealed that all the benzopyrylium salts have good radical scavenging properties with three of them showing better activity than the standard ascorbic acid. And finally, the fulfillment of the Lipinski's parameters confirms that the benzopyrylium salts are potential therapeutic leads especially against cancer.

Materials and methods

General scheme for synthesis of the benzopyrylium salts

The benzopyrylium salts were synthesized following the scheme shown in Scheme 1 [10]. Briefly, 20 ml of glacial acetic acid was saturated with dry HCl gas after which a mixture of the ketone (0.01 mol), the required substituted salicylaldehyde (0.02 mol) was added under nitrogen atmosphere. The reaction mixture was stirred till the contents dissolved completely to give a clear dark colored solution, the stirring was removed and the reaction vessel was left to stand overnight. Formation of crystals in the reaction vessel indicated the completion of the reaction. The crystals were filtered, washed with dry diethyl ether, dried and recrystallized from acetic acid.

Instrumentation

The ^1H NMR spectra were obtained on Bruker Ascend 400 MHz, and ^{13}C NMR on Bruker Ascend 100 MHz. TMS was used as the internal standard in a mixture of CD_3OD (0.45 ml) and CD_3COOD (0.05 mL) as the solvent. For **PS-CP-SAL**, the ^1H NMR spectra were obtained on Bruker Avance III 800 MHz, and ^{13}C -NMR on Bruker Avance III 200 MHz at 10 °C using CD_3COOD as the solvent. The mass spectra were obtained on a Shimadzu GC/MS. KBr pellets were used for the FT-IR spectra and were recorded in a Thermo-Nicolet Avatar 370 spectrophotometer between 400 and 4000 cm^{-1} . Shimadzu 2450 spectrophotometer was used for recording the UV-Vis spectra in the wavelength range 200-600 nm using methanol as the solvent. Agilent UPLC 1290 infinity coupled with Agilent 1290 infinity Autosampler and Agilent 6550 Q-TOF LC/MS with dual jet stream ionization source was used as the Analytical instrument. For HPLC, Agilent 1260 Infinity high performance liquid chromatography system, equipped with a quaternary solvent deliver system, inline degasser, autosampler and photo diode array detector, was used. A Waters Nova—Pak C18 (3.9 mm $\times$ 150 mm) connected with a Zorbax C18 guard column (20 mm $\times$ 4 mm, 5 μm) was applied for all analyses. Detection wavelengths were set at the respective λ_{max} for each compound (characterization data). The mobile phase A consisted of methanol and mobile phase B was acetonitrile. A flow rate of 1 ml/min was maintained and the elution was conducted using a linear gradient mode as shown below in Table 6. The separation was carried out at 25 °C with an injection volume of 20 μl and samples were analysed in triplicate. Spectral characteristics of eluted peaks were recorded using diode array detector from 200 to 700 nm.

In vitro anticancer activity

The in vitro anticancer studies were done at the National Cancer Institute (NCI), USA. The NCI single dose study is carried across a panel of 60 cancer cell lines at single dose (10 μM) [13]. (protocol given as Supplementary information). The five dose studies were further carried out against the same panel at five different concentrations.

In vitro anticancer mechanistic studies on the MCF-7 cells

Wound healing assay—A 2-D wound-healing assay was carried out to assess the migration ability into an acellular area of the cancer cells as per reported literature [14]. A denuded area was created at the centre of each well with a rubber policeman when the cells achieved 70–80% confluence. Then the cells were treated with **PS-CP-4MO** followed by 24 h of incubation. Metamorph Imaging Software was used to determine and quantify the rate of migration. All measurements were normalized to the values for the control.

Colony formation assay—At around 100 cells per well, the MCF-7 cells were seeded and were then treated with **PS-CP-4MO**. After 24 h, the contents were washed and replaced with regular DMEM-medium. At least 50 cells were used to define a colony arbitrarily. 4% formaldehyde was used to secure the colonies post 10 days and then crystal violet was used as the staining agent followed by counting [15].

Flow cytometry studies—As described by Ritu Arora et al. 2015, the flow cytometry studies were carried out with a few modifications [16]. First the floating or loose cells

were collected by mild shaking of the culture dishes and followed by transferring the culture medium comprising of the cells into centrifuge tubes. Native MCF-7 cells and the MCF-7 cell with **PS-CP-4MO** treatment were harvested via trypsinization in 0.25% trypsin/EDTA. Both the trypsinized and the loose cells were further mixed and centrifuged. After centrifugation the cells were collected and then stained using the PE Annexin V apoptosis detection kit to identify apoptotic cells as per the manufacturer's instructions. Flow cytometry assay was then carried out to determine the quantity of viable cells, early apoptotic cells and late apoptotic cells. Mod Fit LT software was employed to analyse the percentage of cell population in apoptosis (Verity Software House, Topsham, ME).

In vitro antibiotic susceptibility testing

Broth microdilution assay was utilized for the antibiotic susceptibility testing as per the CLSI guidelines [12]. The stock solutions of test compounds were prepared at a concentration of 10 mg/mL in DMSO. MHBII was used as the inoculating agent for the bacterial cultures. 600 nm wavelength was used for measuring the optical density (OD) of the solution which was followed by dilution. The dilution was carried out till $\sim 10^6$ CFU/mL. The test for the compounds was then conducted in two-fold serial diluted fashion. The concentration range taken was 64–0.5 mg/L and to a 96-well round bottom microtiter plate, 2.5 μ L of each concentration was added. Bacterial suspension was consequently added at a concentration of 97.5 μ L to each well consisting of the control and the compounds of interest. For 18–24 h incubation of the 96-well plate was carried out at 37 °C followed by the determination of MIC. MIC for a compound is the least concentration of the compound required to achieve the absence of any visible bacterial growth. The determinations of MIC for each compound were carried out in triplicates using duplicate samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

All the data has been represented in the Manuscript and the supplementary data.

References

1. Balaban AT. 2,4,6-Trimethylpyrylium Tetrafluoroborate. *Encycl Reagents Org Synth*. Wiley Online Library; 2001.
2. Dinculescu A, Balaban TS, Popescu C, Toader D, Balaban AT. Synthesis of pyrylium salts with various anions. *Bull des Societes Chim Belges*. 1991;100:665–72.

3. Boiko II, Dergunova Mé, Boiko TN. Reactions of acetophenones and boron trifluoride etherate in the presence of homologs of orthoformic ester. *Chem Heterocycl Compd*. 1987;23:1169–71.
4. Wang J-X, Shi X-N, Zhao L-B. A convenient synthesis of 2-aryl-3, 5-diarylfuran under microwave irradiation. *J Chem Res*. 2003;2003:586–7.
5. Joule JA, Mills K. *Heterocyclic chemistry*. John Wiley & Sons; United Kingdom; 2008.
6. Schiele C, Wilhelm A, Hendriks D, Stepec M, Paal G. Über o-Hydroxyarylvinylpyryliumsalze—I. *Tetrahedron*. 1968;24:5029–36.
7. Heilbron IM, Heslop RN, Irving F. 112. Styrylpyrylium salts. Part XIV. Further study of the colour phenomenon associated with benzonaphtha- and dinaphtha-spiro pyrans. *J Chem Soc*. 1933;430–4. 10.1039/JR9330000430.
8. Sarac AS, Sezer E, Ustamehmetoglu B, Mannschreck A, Stephan B. Ring opening process of some spirochromenes by photo-produced HCl in poly (N-vinyl carbazole). *Polym Adv Technol*. 1997;8:563–7.
9. Schiele C, Wilhelm A, Paal G. Spirobipyrene aus o-Hydroxyarylvinylpyryliumsalzen. *Justus Liebigs Ann Chem*. 1969;722:162–72.
10. Borsche W, Geyer A. Oxonium compounds. 1. Tricyclic benzopyrylium compounds. *Justus Liebigs Ann Chem*. 1913;393:29–60.
11. Kanitz A, Hartmann H, Czerney P. On the formation and reactivity of 2-alkylidene-benzopyrans and their 2-amino-5, 6-benzo-2H-pyran precursors. *J für Prakt Chemie/Chemiker-Zeitung*. 1998;340:34–44.
12. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*; Clin. Lab. Stand. Inst.; Pennsylvania, USA; 2012.
13. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst*. 1991;83:757–66.
14. Jones J, Wang H, Karanam B, Theodore S, Dean-Colomb W, Welch DR, et al. Nuclear localization of Kaiso promotes the poorly differentiated phenotype and EMT in infiltrating ductal carcinomas. *Clin Exp Metastasis*. 2014;31:497–510. [PubMed: 24570268]
15. Kama P, Gundala SR, Gupta MV, Shamsi SA, Pace RD, Yates C, et al. Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells in vitro and in vivo. *Carcinogenesis*. 2011;32:1872–80. [PubMed: 21948980]
16. Arora R, Schmitt D, Karanam B, Tan M, Yates C, Dean-Colomb W. Inhibition of the Warburg effect with a natural compound reveals a novel measurement for determining the metastatic potential of breast cancers. *Oncotarget*. 2015;6:662. [PubMed: 25575825]
17. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol*. 2004;1:337–41. [PubMed: 24981612]
18. Jayaram B, Singh T, Mukherjee G, Mathur A, Shekhar S, Shekhar V. Sanjeevini: a freely accessible web-server for target directed lead molecule discovery. *BMC Bioinf*. 2012;13:S7.

Fig. 1.
a PS-CP-4MO abrogates migration ability. **b** PS-CP-4MO (0.1 μ M) inhibiting colony formation in 24 h

Fig. 2. Annexin/propidium iodide staining to determine the apoptotic rate of MCF-7 cells subsequent to treatment with PS-CP-4MO. **a** Control group (DMSO) **(b)** Cells treated with 10 μ M PS-CP-4MO. Left upper quadrant—necrotic cells, due to mechanical injury; left lower quadrant—normal cells; right upper quadrant—late apoptotic cells; right lower quadrant—early apoptotic cells

Scheme 1.
Benzopyrylium salt formation reaction

Table 1

Structure, IUPAC names, % yield, and melting points of the synthesized benzopyrylium salts

CODE	IUPAC NAME	SUBSTITUENT	YIELD (%)
PS-CP-SAL	(E)-3-(2-hydroxybenzylidene)-2,3-dihydro-1H-cyclopenta[b] chromen-4-ium chloride	R ¹ = -H R ² = -H	90
PS-CP-4MO	(E)-3-(2-hydroxy-4-methoxybenzylidene)-6-methoxy-2,3-dihydro-1H-cyclopenta[b]chromen-4-ium chloride	R ¹ = -H R ² = -OCH ₃	92
PS-CP-5MO	(E)-3-(2-hydroxy-5-methoxybenzylidene)-7-methoxy-2,3-dihydro-1H-cyclopenta[b]chromen-4-ium chloride	R ¹ = -OCH ₃ R ² = -H	88
PS-CP-5Cl	(E)-7-chloro-3-(5-chloro-2-hydroxybenzylidene)-2,3-dihydro-1H-cyclopenta[b]chromen-4-ium chloride	R ¹ = -Cl R ² = -H	89
PS-CP-5Br	(E)-7-bromo-3-(5-bromo-2-hydroxybenzylidene)-2,3-dihydro-1H-cyclopenta[b]chromen-4-ium chloride	R ¹ = -Br R ² = -H	87

Table 2The in vitro studies at one dose (10 μ M) and at 5 doses for PS-CP-4MO

PANEL	CELL	Growth % at 10 μ M	GI ₅₀ μ M	TGI μ M	LC ₅₀ μ M
Leukemia	CCRF-CEM	<u>9.94</u>	3.88	>100	>100
	HL-60(TB)	60.84	4.96	61.40	>100
	K-562	<u>39.06</u>	11.40	93.80	>100
	MOLT-4	<u>0.52</u>	3.71	>100	>100
	RPMI-8226	<u>14.05</u>	3.17	12.90	>100
Non-small cell lung cancer	SR	<u>-2.10</u>	3.04	45.50	>100
	A549/ATCC	94.90	18.50	41.60	93.60
	EKVX	75.72	12.50	26.80	57.60
	HOP-62	95.85	13.10	28.00	59.60
	HOP-92	56.05	11.70	29.10	72.20
	NCI-H226	81.46	12.30	25.70	53.60
	NCI-H23	72.67	10.40	30.10	87.50
	NCI-H322M	84.56	14.00	28.00	56.00
	NCI-H460	25.30	2.80	9.74	52.90
	NCI-H522	77.46	12.7	30.50	73.30
Colon cancer	COLO 205	84.09	2.58	10.70	54.90
	HCC-2998	64.33	15.3	32.60	69.40
	HCT-116	<u>-64.10</u>	1.80	3.52	-
	HCT-15	4.44	2.25	6.03	26.50
	HT29	29.56	2.12	4.67	15.40
CNS cancer	KM12	<u>45.41</u>	2.65	7.44	57.30
	SW-620	<u>11.88</u>	2.23	5.09	20.00
	SF-268	82.18	2.80	9.83	72.30
	SF-295	106.26	16.30	38.00	88.40
	SF-539	108.62	15.10	31.80	67.10
	SNB-19	63.08	8.48	25.60	70.10
	SNB-75	90.94	12.90	27.90	60.60
	U251	69.43	2.30	5.57	30.30

PANEL	CELL	Growth % at 10 μ M	GI ₅₀ μ M	TGI μ M	LC ₅₀ μ M
Melanoma	LOX IMVI	<u>7.79</u>	2.12	5.02	>100
	MALME-3M	<u>16.86</u>	3.08	11.70	45.80
	M14	<u>25.99</u>	3.10	14.60	>100
	MDA-MB-435	<u>2.85</u>	<u>1.78</u>	<u>3.47</u>	<u>6.77</u>
	SK-MEL-2	85.62	11.80	25.30	54.30
Ovarian cancer	SK-MEL-28	<u>47.83</u>	2.30	5.62	22.30
	SK-MEL-5	<u>67.83</u>	14.00	41.10	>100
	UACC-257	87.37	15.80	32.00	64.80
	UACC-62	<u>47.55</u>	5.20	19.90	51.00
	IGROV1	<u>9.32</u>	2.71	27.60	>100
	OVCAR-3	<u>49.84</u>	2.48	6.09	22.90
	OVCAR-4	75.95	5.45	20.60	52.50
	OVCAR-5	114.64	17.20	40.30	94.40
	OVCAR-8	<u>42.89</u>	2.27	6.13	37.00
	NCI/ADR-RES	<u>53.84</u>	4.16	50.60	>100
Renal cancer	SK-OV-3	103.04	12.30	25.30	52.10
	786-0	<u>-6.35</u>	3.97	19.80	>100
	A498	94.94	—	—	—
	ACHN	<u>34.26</u>	4.15	15.80	40.20
	CAKI-1	80.97	10.70	22.50	47.50
Prostate cancer	RXF 393	71.39	7.08	24.60	69.40
	SN12C	<u>13.59</u>	2.51	7.93	38.80
	TK-10	135.22	11.70	24.70	52.10
	UO-31	<u>-28.66</u>	2.39	8.04	28.20
	PC-3	<u>8.84</u>	2.74	16.20	>100
Breast cancer	DU-145	76.72	8.15	20.50	45.20
	MCF7	<u>48.15</u>	<u>4.31</u>	<u>22.00</u>	<u>76.40</u>
	MDA-MB-231/ATCC	36.72	3.75	18.80	57.40
	HS 578 T	<u>48.15</u>	4.28	34.50	>100
	BT-549	<u>36.72</u>	12.10	31.70	83.40
	T-47D	<u>67.22</u>	3.04	22.10	99.40

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PANEL	CELL	Growth % at 10 μ M	GI ₅₀ μ M	TGI μ M	LC ₅₀ μ M
	MDA-MB-468	97.12	14.00	34.90	87.30

The bold and italics indicate or rather highlight that the biological activity is quite significant. It is necessary for the readers to understand in which cell-lines the compounds are active

Table 3

MIC data of the benzopyrylium salts for their antibacterial screening

Sample Code	MIC (µg/ml)				
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> BAA 1705	<i>A. baumannii</i> BAA 1605	<i>P. aeruginosa</i> ATCC 27853
PS-CP-SAL	>64	64	>64	>64	>64
PS-CP-5MO	>64	>64	>64	>64	>64
PS-CP-4MO	>64	16	>64	>64	>64
PS-CP-5Cl	>64	8	>64	>64	>64
PS-CP-5Br	>64	32	>64	>64	>64
Levofloxacin	0.0156	0.25	64	8	1

The bold and italics indicate or rather highlight that the biological activity is quite significant. It is necessary for the readers to understand in which cell-lines the compounds are active

Table 4

Antioxidant efficacy of the benzopyrylium salts by DPPH assay

	Absorbance at 517 nm	Activity (at 10 μ M)		
		In mAu	In %	w.r.t. Ascorbic Acid
DPPH	0.795 ± 0.03	—	—	—
Ascorbic Acid	0.495 ± 0.02	0.3771	37.71	1.00
PS-CP-SAL	0.505 ± 0.03	0.3641	36.41	0.97
PS-CP-4MO	0.489 ± 0.01	0.3846	38.46	1.02
PS-CP-5MO	0.373 ± 0.004	0.5306	53.06	1.41
PS-CP-5Cl	0.351 ± 0.02	0.5579	55.79	1.48
PS-CP-5Br	0.544 ± 0.02	0.3154	31.54	0.84

Table 5

Lipinski parameters of the benzopyrylium salts

Benzopyrylium salts	Molecular mass	No. of hydrogen bond donors	No. of hydrogen bond acceptors	Log <i>P</i>	Molar refractivity
PS-CP-SAL	310.50	1	2	4.38	84.81
PS-CP-4MO	370.50	1	4	4.27	98.27
PS-CP-5MO	370.50	1	4	4.27	98.27
PS-CP-5Cl	379.50	1	2	4.51	91.38
PS-CP-5Br	466.50	1	2	5.91	100.21

Table 6

HPLC gradient program

Time (min)	Solvent A (%) (Methanol)	Solvent B (%) (ACN)
0	100	0
15	5	95
17	100	0
20	100	0