Full Paper

The Design and Cytotoxic Evaluation of Some 1-Aryl-3-isopropylamino-1-propanone Hydrochlorides towards Human Huh-7 Hepatoma Cells

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A series of 1-aryl-3-isopropylamino-1-propanone hydrochlorides **1** and a related heterocyclic analog **2** as candidate antineoplastic agents were prepared and the rationale for designing these compounds is presented. A specific objective in this study is the discovery of novel compounds possessing growth-inhibiting properties of hepatoma cells. The compounds in series **1** and **2** were prepared and their structures established unequivocally. X-ray crystallography of two representative compounds **1d** and **1g** were achieved. Over half of the compounds are more potent than 5-fluorouracil which is an established drug used in treating liver cancers. QSAR evaluations and molecular modeling studies were undertaken with a view to detecting some physicochemical parameters which govern cytotoxic potencies. A number of guidelines for amplification of the project have been formulated.

Keywords: Anticancer drug design / Huh-7 cells / Mannich bases / Molecular modeling / QSAR

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Introduction

A major goal in our laboratories is the discovery of novel compounds, which can alleviate the devastating effects of liver cancers. The importance of such goals is axiomatic when one considers, for example, that approximately 20% of cancer-related deaths in the United States are due to primary liver tumors (arising from the hepatobiliary system) or secondary malignancies (tumors of extrahepatic origins, which metastasize in the liver) [1]. The most common primary liver cancers are hepatocellular carcinomas, which are often referred to as hepatomas. Hence, the first step in our long-term strategy is to find some prototypic molecules, which are cytotoxic to a human hepatoma cell line.

The design of the cluster of molecules described in this report, namely series **1** and **2**, was based on the following considerations.

(1) The compounds are structurally divergent from contemporary drugs used in treating hepatomas, such as 5-fluorouracil [2] and doxorubicin [3]. Thus, series **1** and **2** may have different modes of action than the drugs used today, and hence be efficacious in treating drug-resistant tumors.

(2) Series **1** and **2** are acyclic Mannich bases and a number of these compounds have demonstrated cytotoxic and anticancer properties [4–9].

(3) Various cytotoxic Mannich bases exert their bioactivity, at least in part, by interfering with respiration in mitochondria [10, 11]. Recent surveys have emphasized a number of biochemical differences between mitochondria isolated from tumors and normal cells [12-14]. On occasion, greater toxicity

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to neoplasms than the corresponding non-malignant tissues may result.

(4) Solutions of acyclic Mannich bases such as **1** and **2**, which possess a methine group (CH) adjacent to the carbonyl substituent, may undergo deamination to the corresponding α,β-unsaturated ketones [15, 16]. In general, these enones react preferentially or exclusively with thiols rather than amino or hydroxy groups [17, 18]. Since thiols are absent in nucleic acids, α,β-unsaturated ketones may not cause the genotoxic effects associated with a number of the anticancer drugs used today [19].

(5) Cytotoxins should have a balance between hydrophilic and hydrophobic groups, so that adequate quantities of the compounds can reach the sites of action. Hydrophobic groups were incorporated into series **1** and **2**, such as the aryl and heteroaryl rings as well as the isopropylamino group, which should increase the rate and extent of absorption *via* membranes. However, increasing the log*P* values of the molecules reduces aqueous solubility, which in turn lowers the absorption of compounds from body compartments. Hence, the hydrophilic amine hydrochloride group is present in **1** and **2**.

(6) Various substituents were placed in the aryl ring of **1a–i**, which differ in the magnitude of their electronic, hydrophobic, and steric properties. This design was undertaken with the goal of ascertaining whether cytotoxic potencies are correlated with one or more of these physicochemical parameters. Compound **2** was prepared in order to compare its cytotoxicity with that of its bioisostere **1a**.

(7) A secondary amino group is present in series **1** and **2** in order to allow future molecular modifications at this portion of the molecule to occur depending on the initial bioactivity noted with **1a–i** and **2**. For example, *N*-acylation may be deemed appropriate in attempts to improve pharmacodynamic and/or pharmacokinetic properties.

(8) Finally **1a–i** and **2** possess a number of druglike properties. Thus, the rules of Lipinski (molecular weight: <500, log*P* < 5, hydrogen bond donors: <5, hydrogen bond acceptors: <10) [20] and Veber (rotatable bonds: <10, polar surface area: <140 Å^2 or hydrogen bonds: <12) [21] are observed.

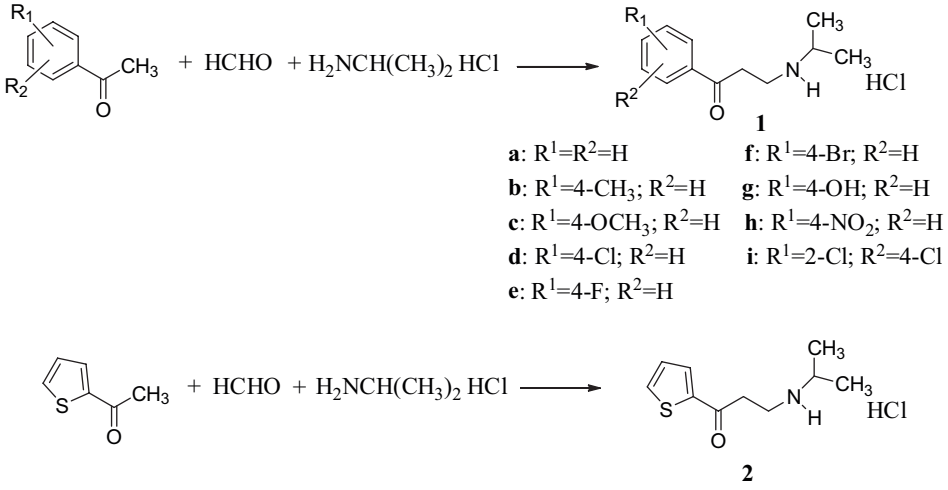
In summary, the aims of the present investigation were to prepare series **1** and **2** and evaluate their cytotoxic potencies towards a human hepatoma cell line, and secondly to undertake analyses of the biodata with a view to establishing guidelines for expansion of the project.

Results

The compounds in series **1** and **2** were prepared by the synthetic chemical route outlined in Scheme 1. X-ray crystallography was undertaken on **1d** and **1g** and ORTEP diagrams of these two compounds are presented in Figs. 1 and 2. All of the compounds were evaluated against human hepatoma Huh-7 cells and the biodata generated is portrayed in Table 1. Quantitative structure-activity relationship (QSAR) and molecular modeling techniques were undertaken using the biodata generated and various physicochemical properties of **1a–i** and **2**.

Discussion

The compounds in series **1** and **2** were prepared by the Mannich reaction. The use of a primary amine means that the initial Mannich base formed could react further with a ketone and formaldehyde [22]. For example, in the case of the attempted synthesis of series **1**, the formation of the corresponding *N*,*N*-bis(3-aryl-3-oxopropyl)isopropylamine hydrochlorides may have occurred. Furthermore, Mannich reactions involving an aryl ethyl ketone, which contain a



Scheme 1. The synthetic chemical route used to prepare the compounds in series **1** and **2**.

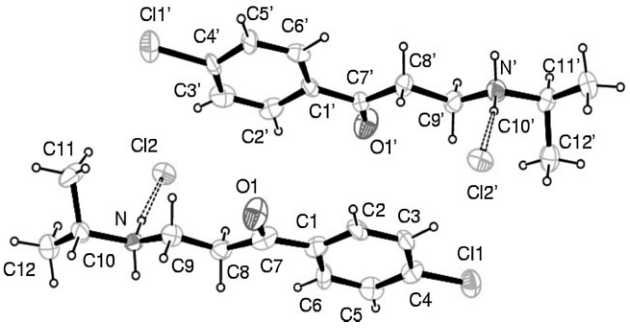


Figure 1. An ORTEP drawing of **1d**. The displacement ellipsoids are drawn at the 40% probability level.

strongly electron-releasing group in the aryl ring such as a 4-hydroxy substituent, may undergo ring aminomethylation [23]. Hence, the products isolated were examined very carefully using multiple analytical techniques, namely ^{1}H- and ^{13}C-NMR as well as infrared spectroscopy, mass spectrometry, and elemental analyses. In addition, X-ray crystallography of two representative compounds **1d** and **1g** provided unequivocal evidence that the Mannich bases prepared have the structures presented in Scheme 1.

The IC_{50} values of **1a–i** and **2** against human hepatoma Huh-7 cells, which are presented in Table 1, reveal that the objective of finding one or more compounds with growth-inhibiting properties towards a liver cancer has been achieved. In terms of structure-activity relationships (SAR), the following observations were made. First, the potencies of the compounds reveal three groups of molecules, namely **1a–c**, **g**, **i** and **2** having IC_{50} values in the 15–20 μM range, **1h** possessing an IC_{50} figure of 65 μM and **1d–f** displaying IC_{50} values in excess of 100 μM. Second, the six compounds with the lowest IC_{50} figures are 2.1–2.8 times more potent than 5-fluorouracil and even **1h** has 0.7 times the potency of this established drug. Thus, further development will be based on the results obtained for **1a–c**, **g**, **i** and **2**. Third, the bioisosteres **1a** and **2** have comparable potencies. Fourth, the insertion of an *ortho* chloro substituent into **1d** leading to **1i** is accompanied by a 19-fold increase in potency.

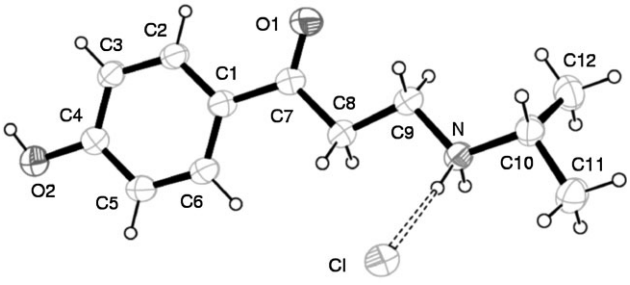
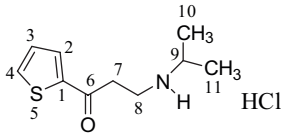


Figure 2. An ORTEP drawing of **1g**. The displacement ellipsoids are drawn at the 40% probability level.

The next phase of the investigation was aimed at detecting correlations between the cytotoxic potencies of **1a–i** and a number of physicochemical parameters of the compounds. The magnitude of the electronic, hydrophobic and steric properties of the aryl substituents may be expressed by the Hammett σ/Taft σ^*, Hansch π and molar refractivity (MR) values, respectively, of these groups. Linear and semilogarithmic plots were constructed between the IC_{50} values of **1a–i** and the σ/σ^*, π and MR figures of the aryl substituents. In addition, logarithmic plots were made between the MR values and the IC_{50} data. However, no correlations ($p > 0.05$) or trends to significance ($p > 0.1$) were found. If the principal mode of action of **1a–i** is the reaction of cellular thiols with the α,β-unsaturated ketones liberated from **1a–i** by deamination, then the electrophilicity of the enones, which is dependent on the σ and σ^* values of the aryl substituents, should correlate negatively with the IC_{50} figures. This lack of correlation noted in the statistical analyses suggests that alternative mechanism(s) of action are primarily responsible for the cytotoxic potencies.

The variation in cytotoxic potencies observed may have been due to differences in the shapes of the molecules. Hence, the decision was made to measure certain torsion angles, interatomic distances and atomic charges, which might govern the magnitude of the biological responses. Molecular models of **1a–i** and **2** were therefore built. The numbers allocated to certain atoms of **1a–i** are displayed in Fig. 2, while Fig. 3 indicates the numbering of **2**.

On occasions the size of the torsion angle between an aryl ring and an adjacent unsaturated group is correlated with biological potencies [24]. The torsion angles (θ) C6-C1-C7-O in **1a–i** and C2-C1-C6-O in **2** were determined and are presented in Table 1. The θ values of **1a–i** may be influenced by the electronic properties of the aryl substituents. For example, electron-releasing groups may lower θ figures by enhancing the overlap of the π orbitals of the aryl ring with the adjacent carbonyl group. A linear plot between the σ/σ^* values of the aryl substituents in **1a–i** and the θ figures supported this view since a positive trend towards significance ($p = 0.1$) was revealed. Since four of the five compounds in series **1**, which possess IC_{50} values in the 15–20 μM range, have σ figures of zero (**1a**) or are negative (**1b**, **c**, **g**), future studies should include the insertion of one or multiple aryl substituents,



2

Figure 3. The numbering of the atoms in **2** which are used in the molecular modeling studies.

Table 1. Evaluation of **1a–i** and **2** against Huh-7 cells and some physicochemical characteristics of these molecules

Compound	IC_{50}[a]	θ[b]	O-NH[c]	Atomic charges (esu)	
	(μM)	(°)	(Å)	C8	O
1a	19	33.53	2.299	−0.218	−0.297
1b	18	32.31	2.304	−0.218	−0.299
1c	19	29.87	2.308	−0.218	−0.303
1d	286	33.51	2.303	−0.218	−0.293
1e	385	31.89	2.308	−0.218	−0.295
1f	225	34.83	2.301	−0.217	−0.290
1g	19	29.93	2.309	−0.218	−0.320
1h	65	40.46	2.330	−0.208	−0.280
1i	15	99.90	2.341	−0.211	−0.273
2	20	1.06	2.332	—	—
5-Fluorouracil	42.2	—	—	—	—

[a] The IC_{50} value is the average of two independent determinations.[b] The θ figures are the torsion angles between the C6-C1-C7-O atoms in **1a–i** and the C2-C1-C6-O atoms in the case of **2**.[c] The O-NH distance is the span between the carbonyl oxygen atom and the nitrogen atom attached to the isopropyl group in **1a–i** and **2**.

which are strongly electron-releasing such as the dimethylamino group ($\sigma_p = -0.83$) [25]. A second observation of interest is as follows. The θ value of the 4-chloro analog **1d** is 33.51°. This figure was tripled when an *ortho* chloro atom was inserted into the molecule giving rise to **1i**, which was accompanied by a huge rise in potency. Thus, future modifications should explore carefully the importance of placing *ortho* substituents in the aryl ring in terms of potency variation. Third, the C2-C1-C6-O torsion angle in **2** is substantially lower than the value found in **1a–i**. The fact that **2** has favorable cytotoxic properties suggests that replacement of the 2-thienyl group by other heterocycles, which create different θ values, should be undertaken such as the formation of the 2-pyrimidinyl or 6-methyl-2-pyridinyl analogs. Fourth, X-ray crystallography of **1d** and **1g** was carried out, not only to confirm the structures proposed for series **1**, but also to determine if the θ values varied in compounds with aryl substituents having markedly divergent σ_p values. The 4-chloro analog **1d** has two independent conformational isomers in each asymmetric unit and the θ figures are 173.8° and 170.1°. In the case of the 4-hydroxy Mannich base **1g**, the θ value is −5.9°. As noted with the molecular modeling determinations, the θ figure of **1d** is slightly greater than **1g**. The θ figures for **1d** and **1g**, which were generated using X-ray crystallography, are lower than the values obtained by molecular modeling. This observation may be attributed to variations in the physical state of the molecules when the determinations were made.

The compounds in series **1** and **2** are flexible molecules permitting rotation about a number of single bonds, such as the C7-C8 and C8-C9 bonds in series **1**. Hence, the question arises as to whether the shapes of the molecules differ in terms of distances between these atoms in series **1** and **2**, which would be predicted to react at a binding site. The spans between the carbonyl oxygen and the basic nitrogen atoms in **1a–i** and **2** were measured and these data are presented in Table 1. The results indicate that there is little variation in the O-NH distances. In order to bring about changes in the O-NH distances, various groups should be placed in carbon atoms 8 and 9 in **1a–i** and 7 and 8 in **2**, which may lead to significant differences in the topography of the molecules including the O-N distances, which could result in variations in potencies.

Finally, the atomic charges on the C8 atoms and the oxygen atoms of the carbonyl groups in **1a–i**, which are presented in Table 1 were determined for the following reasons. The rate of elimination of isopropylamine hydrochloride from **1a–i** will be influenced by the lability of the protons on the C8 methylene group, which in turn will be controlled by the atomic charge on the C8 atoms. The data in Table 1 reveal that the atomic charges on the C8 atoms of **1a–i** are very similar and hence the difference in potencies among these compounds is unlikely to be due to variations in the rates of formation of the corresponding α,β-unsaturated ketones. The size of the atomic charge on the carbonyl oxygen atom is perceived to govern both the ability and extent of the formation of hydrogen bonds at a receptor. Linear plots between these charges and the σ/σ^* constants of the aryl substituents of **1a–i** revealed a positive correlation ($p < 0.01$). Thus, electron-releasing groups in the aryl rings in series **1** increase the size of the negative change on this oxygen atom. However linear, semilogarithmic and logarithmic plots between the IC_{50} values of **1a–i** and the atomic charge on this oxygen atom did not reveal any relationship ($p > 0.1$).

Thus from this initial investigation, various guidelines for analog development may be made. First, **1a–c**, **g**, **i** and **2** are lead molecules and the aryl substituents present in **1b**, **c**, **g**, **i** should be placed in different locations in the aryl ring. In particular, since three of four of these compounds have

electron-releasing aryl groups, priority should be given to incorporating multiple methyl, methoxy and hydroxy groups into the aryl rings such as the formation of the 3,4,5-trimethyl analog. Second, the influence of ortho substituents on IC_{50} figures needs to be developed in considerable detail. Third, changes in the conformation of the molecule by inserting groups on the C8 and C9 atoms in series **1** and C7 and C8 atoms in series **2** should be undertaken.

Conclusions

A number of reasons for preparing series **1** and **2** as candidate antineoplastics have been given and in particular the aspiration of their ability to inhibit the growth of human hepatoma cells. A series of prototypic molecules were prepared and their structures thoroughly established. Not less than 60% of the compounds evaluated have greater potency than 5-fluorouracil against human Huh-7 hepatoma cells. By assessing various physicochemical properties of these molecules, guidelines for the design of further analogs were achieved. Future investigations that should be undertaken include mode of action studies, especially to evaluate whether the compounds interfere with mitochondrial function, stability studies of solutions of the compounds and whether greater toxicity to neoplasms than normal cells is demonstrated.

Experimental

General

Melting points were determined using a Buchi 530 instrument and are uncorrected. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) of **1a–i** and **2** were determined on a Varian Mercury Plus spectrometer. The electron-impact mass spectra were obtained using a Thermo-Finnegan mass analyzer while the infrared spectra were determined as potassium bromide discs on a Mattson1000 FT-IR spectrophotometer. The elemental analyses were generated using a Leco CHNS-932 instrument.

Synthesis of series 1 and 2

A mixture of the appropriate ketone (50 mmol), paraformaldehyde (50 mmol), and isopropylamine hydrochloride (27 mmol) was heated in an oil bath at 130°C. The reaction vessel was then removed from the oil bath and when the temperature of the mixture dropped to 65°C, ethyl acetate (40–80 mL) was added. The mixture was stirred at room temperature for 24 h and the resultant precipitates were then collected and, with the exception of **1f**, were recrystallized from ether/methanol (**1a**, **b**, **d**, **2**) or methanol (**1c**, **e**, **g–i**). The Mannich base **1f** was passed through a column of silica gel 60 (70–230 mesh) using methanol as the eluant and after evaporation of the solvent, the product was recrystallized from methanol.

The ^{1}H-NMR, ^{13}C-NMR and IR spectroscopy as well as the mass spectra and elemental analyses of **1a–d**, **h**, which have been described previously, are consistent with the proposed structures. The melting points and yields of these compounds are as follows, namely **1a**: 172–174°C (lit. [26] m.p. 174–176°C), 55%; **1b**: 170–171°C (lit. [27] m.p. 171–172°C), 58%; **1c**: 176–178°C (lit. [28] 183°C), 64%; **1d**: 168–170°C (lit. [27] m.p. 167–168°C, 52%; **1h**: 206–207°C (lit. [28] m.p. 207–208°C), 20%.

*1-(4-Fluorophenyl)-3-isopropylamino-1-propanone hydrochloride **1e***

M.p. 189–190°C. Yield: 44%. ^{1}H-NMR ($CDCl_3$) δ 1.51 (d, J = 6.8 Hz, 6H, CH(CH_3)$_2$), 3.36–3.40 (m, 3H, C*H*(CH_3)$_2$ and 2 × H-2), 3.75 (t, J = 7.1 Hz, 2H, 2 × H-3), 7.04–7.08 (m, 2H, H-3'/5'), 7.94–7.96 (m, 2H, H-2'/6'), 9.55 (brs, 2H, NH_2^+); ^{13}C-NMR ($CDCl_3$) δ 19.4 (CH(CH_3)$_2$), 35.1, 40.3, 51.3, 116.2, 131.1, 132.5, 166.4, 195.5 (CO); MS (EI) m/z: 194.1 (M − CH_3)$^+$, 209.1 (M)$^+$; IR (KBr, cm^{-1}): 2446 (NH_2^+), 1681 (CO). Calcd. for $C_{12}H_{17}ClFNO$ (245.72): C, 58.66; H, 6.97; N, 5.70. Found: C, 58.51; H, 6.94; N, 5.74.

*1-(4-Bromophenyl)-3-isopropylamino-1-propanone hydrochloride **1f***

M.p. 174–176°C. Yield: 38%. ^{1}H-NMR ($CDCl_3$) δ 1.49 (d, J = 6.8 Hz, 6H, CH(CH_3)$_2$), 3.34–3.38 (m, 3H, C*H*(CH_3)$_2$ and 2 × H-2), 3.73 (t, J = 7.3 Hz, 2H, 2 × H-3), 7.50 (d, J = 8.4 Hz, 2H, H-3'/5'), 7.76 (d, 2H, J = 8.4 Hz, H-2'/6'), 9.55 (brs, 2H, NH_2^+); ^{13}C-NMR ($CDCl_3$) δ 19.4 (CH(CH_3)$_2$), 35.3, 40.2, 51.3, 129.4, 129.8, 132.3, 134.7, 195.8 (CO); MS (EI) m/z: 254 (M − CH_3)$^+$, 256 (M − CH_3 + 2)$^+$, 270.2 (M + H)$^+$, 272.2 (M + H + 2)$^+$; IR (KBr, cm^{-1}): 2462 (NH_2^+), 1684 (CO). Calcd. for $C_{12}H_{17}BrClNO$ (306.63): C, 47.00; H, 5.59; N, 4.57. Found: C, 46.74; H, 5.52; N, 4.59.

*1-(4-Hydroxyphenyl)-1-isopropylamino-1-propanone hydrochloride **1g***

M.p 195–196°C. Yield: 10%. ^{1}H-NMR (CD_3OD) δ 1.39 (d, J = 6.4 Hz, 6H, CH(CH_3)$_2$), 3.37–3.46 (m, 5H, C*H*(CH_3)$_2$, 2 × H-2 and 2 × H-3), 4.87 (brs, 1H, OH), 6.87 (d, J = 8.8 Hz, 2H, H-3'/5'), 7.92 (d, J = 8.8 Hz, 2H, H-2'/6'); ^{13}C-NMR (CD_3OD) δ 18.0 (CH(*C*H$_3$)$_2$), 34.0, 40.2, 51.2, 115.3, 128.0, 130.7, 163.2, 195.6 (CO); MS (EI) m/z: 192.4 (M − CH_3)$^+$, 206.3 (M − H)$^+$; IR (KBr, cm^{-1}): 2449 (NH_2^+), 1658 (CO). Calcd. for $C_{12}H_{18}ClNO_2$ (243.73): C, 59.13; H, 7.44, N, 5.75. Found: C, 58.97; H, 7.62; N, 5.76.

*3-(2,4-Dichlorophenyl)-1-isopropylamino-1-propanone hydrochloride **1i***

M.p. 158–159°C. Yield: 37%. ^{1}H-NMR ($CDCl_3$) δ 1.47 (d, J = 6.8 Hz, 6H, CH(CH_3)$_2$), 3.34–3.39 (m, 3H, C*H*(CH_3)$_2$ and 2 × H-2), 3.73 (t, J = 7.1 Hz, 2H, 2 × H-3), 7.24–7.26 (m, 1H, H-5'), 7.37 (s, 1H, H-3'), 7.64 (d, J = 8.4 Hz, 1H, H-6'), 9.52 (brs 2H, NH_2^+); ^{13}C-NMR ($CDCl_3$) δ 19.4 (CH(CH_3)$_2$), 39.1, 39.8, 51.4, 127.7, 130.9, 131.4, 132.7, 135.6, 138.6, 197.6 (CO); MS (EI) m/z: 244.1(M − CH_3)$^+$, 246.1(M − CH_3 + 2)$^+$, 248(M − CH_3 + 4)$^+$, 259.1 (M)$^+$; IR (KBr, cm^{-1}): 2451 (NH_2^+), 1682 (CO). Calcd. for $C_{12}H_{16}Cl_3NO$ (296.62): C, 48.59; H, 5.44; N, 4.72. Found: C, 48.50; H, 5.36; N, 4.77.

*3-Isopropylamino-1-(2-thienyl)-1-propanone hydrochloride **2***

M.p. 165–166°C. Yield: 25%. ^{1}H-NMR ($CDCl_3$) δ 1.51 (d, J = 6.4 Hz, 6H, CH(CH_3)$_2$), 3.37–3.41 (m, 3H, C*H*(CH_3)$_2$ and 2 × H-2), 3.73 (t, J = 7.3 Hz, 2H, 2 × H-3), 7.05 (t, J = 4.4 Hz, 1H, H-4'), 7.63 (d, J = 4.8 Hz, 1H, H-3'), 7.78 (d, J = 3.6 Hz, 1H, H-5'), 9.56 (brs, 2H, NH_2^+); ^{13}C-NMR δ 19.4 (CH(*C*H$_3$)$_2$), 35.6, 40.2, 51.2, 128.6, 133.4, 134.9, 142.9, 189.7 (CO); MS (EI) m/z: 182.1 (M − CH_3)$^+$, 197.0 (M)$^+$;

IR (KBr, cm^{-1}): 2460 (NH_2^+), 1659 (CO). Calcd. for $C_{10}H_{16}ClNOS$ (233.76): C, 51.38; H, 6.90: N, 5.99; S, 13.72. Found: C, 51.37; H, 6.96; N, 6.09; S, 13.92.

Statistical analyses

The σ, π, and MR values were culled from an appropriate reference [29], while the σ^* figure was obtained from the literature [30]. The linear, semilogarithmic and logarithmic plots as well as the multilinear regression analyses were made using a commercial software package [31].

Molecular modeling

Models of the compounds **1a–i** and **2** were built using a Spartan software [32]. The structures were reoptimized using semi-empirical quantum mechanical calculations by the AM1 method.

X-Ray Crystallography

For the crystal structure determination, the single-crystals of the compounds **1d** and **1g** were used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area IP detector). The graphite-monochromatized Mo K_α radiation ($\lambda = 0.71073$ Å) and oscillation scans technique with $\Delta\omega = 5^\circ$ for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2 > 2\ \sigma\ (F^2)$. Integration of the intensities, correction for Lorentz and polarization effects and cell refinement was performed using CrystalClear [33] The structures were solved by direct methods using SHELXS-97 [34] and refined by a full-matrix least-squares procedure using the program SHELXL-97 [34]. The final difference Fourier maps showed no peaks of chemical significance.

Crystal data for **1d**: $C_{12}H_{17}Cl_2NO$, crystal system, space group: orthorhombic, *P*212121; (no:2); unit cell dimensions: $a = 7.2652$ (2) Å, $b = 12.9951$ (3) Å, $c = 29.2708$ (4) Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$; volume: 2763.4(2) $Å^3$; $Z = 8$; calculated density: 1.26 mg/m^3; absorption coefficient: 0.451 mm^{-1}; *F*(000): 1104; θ range for data collection 2.6–26.5°; refinement method: full-matrix least-square on F^2; data/parameters: 2557/293; goodness-of-fit on F^2: 1.019; final *R* indices [$I > 2\ \sigma(I)$]: $R_1 = 0.071$, $w\,R_2 = 0.149$; *R* indices (all data): $R_1 = 0.161$, $w\,R_2 = 0.190$; largest diff. peak and hole: 0.222 and −0.185 e $Å^{-3}$.

Crystal data for **1g**: $C_{12}H_{18}ClNO_2$, crystal system, space group: triclinic, *P*-1; (no:2); unit cell dimensions: $a = 7.7720(2)$ Å, $b = 8.7180(3)$ Å, $c = 10.3490(4)$ Å, $\alpha = 76.70(2)^\circ$, $\beta = 72.33(3)^\circ$, $\gamma = 86.27(2)^\circ$; volume: 650.2(2) $Å^3$; $Z = 2$; calculated density: 1.24 mg/m^3; absorption coefficient: 0.280 mm^{-1}; *F*(000): 260; θ range for data collection 2.1–26.4°; refinement method: full-matrix least-square on F^2; data/parameters: 2661/156; goodness-of-fit on F^2: 1.012; final *R* indices [$I > 2\ \sigma(I)$]: $R_1 = 0.056$, $w\,R_2 = 0.126$; *R* indices (all data): $R_1 = 0.091$, $w\,R_2 = 0.147$; largest diff. peak and hole: 0.173 and −0.214 e $Å^{-3}$.

In the structure **1d**, hydrochlorides are joined by N-H···Cl *H*-bonds [N'···Cl(2) = 3.117(7) Å, N'-H(2B)···Cl(2) = 170°; N···Cl(2') = 3.139(7) Å, N-H(1A)···Cl(2') = 169°]. The compound **1g** crystallizes in the triclinic space group P-1, with two molecules in the unit cell. In this structure, hydrochlorides are joined by N-H···Cl and O-H···Cl *H* bonds [N···Cl = 3.196(3) Å, N-H···Cl = 177°; N···Cl^a = 3.199(3) Å, N-H(1B)···Cl^a = 166°; O(2)···Cl^b = 3.063(3) Å, O(2)-H(2A)···Cl^b = 171°, symmetry code (^{a}); 1-*x*,-*y*,1-*z* (^{b}); 1-*x*, 1-*y*,-*z*], which lead to the formation of double polymeric chain. Crystallographic data for the structures **1d** and **1g** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-767024 and 752376, respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk/data_request/cif).

Cytotoxic evaluation of 1a-i and 2 on Huh-7 cells

The Mannich bases **1a–i** and **2** were evaluated against Huh-7 cells by a reported procedure [35]. The IC_{50} values, which were obtained by a literature method [35] are the average of two independent determinations which differed by less than 10%.

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The authors have declared no conflict of interest.

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