N-Alkylated 3,5-bis(arylidene)-4-piperidones. Synthetic Approaches, X-ray Structure and Anticancer Activity

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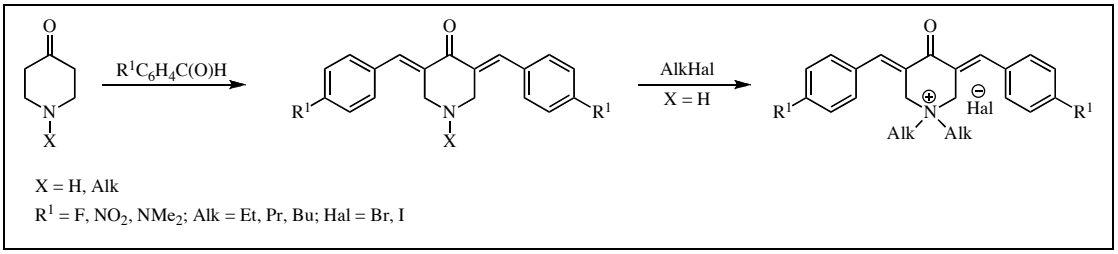

In a search for cytotoxic fluorescent materials a series of N-alkylated and N,N-dialkylated 3,5-bis(arylidene)piperidones was synthesized. Alkylation of 3,5-bis(arylidene)-4-piperidone afforded quaternary salts only while condensation of N-alkyl-4-piperidones with substituted benzaldehydes was a convenient route to the corresponding N-alkylated compounds. Compounds and their pharmaceutically acceptable salts demonstrated high activity against resistant human lung carcinoma cell line A549 with IC_{50} values in the range of 0.3-6.5 μM.

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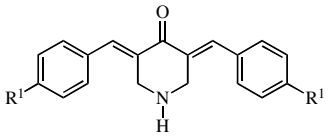
INTRODUCTION

Recently NH-3,5-bis(arylidene)-4-piperidones **1** bearing F, Cl, NO_2 or NMe_2 substituents in the *para*-position of the aromatic ring (Scheme 1) were found to possess cytotoxic and antitumor activity [1-3]. In addition, their hydrochloride salts were well tolerated in mice (up to 240 mg/kg) [3]. The idea of their synthesis and evaluation of antineoplastic properties initially was based on the reasons that such compounds may be considered as Mannich base of a dienone and α,β-unsaturated ketones display anticancer properties *via* the supposed mechanism of action comprising interaction with cellular thiols with little or no affinity for hydroxyl and amino groups in nucleic acids [4]. Among those curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione), a natural component of the rhizome of *curcuma longa* possessing a conjugated structure, proved to be the powerful chemopreventive and anticancer agent [5] having also anti-inflammatory [6a], antibacterial [6b] and antioxidative [6c] properties. Thus, curcumin and other enones were supposed to have multiple targets and interacting macromolecules within the cell [3,5]. It should be mentioned also that a few N-alkylated 3,5-bis-(arylidene)-4-piperidones as curcumin analogues were reported as good free radical scavengers possessing also high cytostatic properties. However, the above mentioned properties were attributed to the presence of hydroxyl groups in the *para*-position of aromatic rings and alkoxy groups at the adjacent aromatic carbon atoms [10].

According to the Dimmock's hypothesis [1] for 3,5-bis(arylidene)-4-piperidones the molecular modification at the nitrogen atom such as N-acylation **2a**, affects the capacity of transportation of these biologically active molecules acting as a thiol acylators *via* the cellular membrane and therefore results in significantly higher level of anticancer activity. Similar approach comprises phosphorylation of such NH-piperidones (compounds **2b,**

Scheme 1



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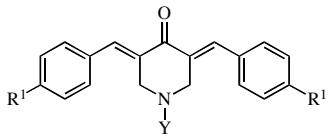
R^1 = Cl, F, NO_2, OMe



2a,b

R^1 = NMe_2
2a: Y = $C(O)CH_3$, C(O)OEt, $C(O)CH=CH_2$
2b: Y = $P(O)(OCH_2CF_3)_2$, $P(O)(OPh)_2$, P(O)Me(OPh)

Scheme 1) [7]. Both acylated and phosphorylated compounds may be considered as pro-drugs giving the parent NH-compound *via* hydrolysis *in vivo* under the action of the suitable enzymes.

Therefore, 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore group observed both in alicyclic unsaturated enones and cyclic piperidone derivatives was speculated as interacting with cellular constituents while the nature of the group on the heterocyclic nitrogen atom in the latter ones could influence the cytotoxic properties of a compound. In other words, it can increase the cytotoxic properties by facilitating of the cytotoxin approach to a specific binding site or reduce them preventing their interaction.

Recent observations demonstrated fluorescent properties of some representatives of 3,5-bis(arylidene)-4-piperidones [7-9]. Natural fluorescence in the case of cytotoxic materials might be very helpful in tracking of their cellular pathway. Structure-activity relationships allow suggesting that cytotoxic activity increases with the increase of electron withdrawing properties of the phenyl ring substituents, which at the same time decrease the fluorescent activity. Combination of anti-tumor activity and two-photon fluorescent properties makes this type of compounds exceedingly prospective as drug-candidates for photodynamic chemotherapy of superficial malignant tumors and will aid in tracing their pathways during chemotherapeutic treatment. Therefore, in order to develop efficient antitumor agents among the bis(arylidene)-4-piperidone derivatives it seems necessary to find the appropriate balance between these two essential features.

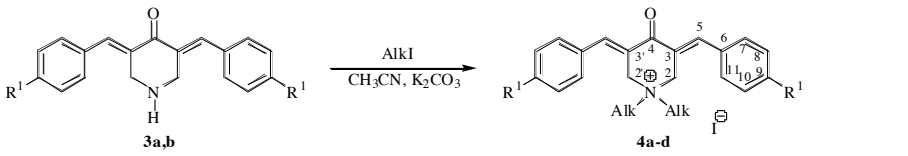
Fluorescent properties of the compounds belonging to the class of 3,5-bis(arylidene)-4-piperidones were reported for the first time for the corresponding N-methyl derivatives [8,9]. Therefore, despite the rupture of N-C(alkyl) bond in such compounds *in vivo* resulting NH-piperidones **1** is less obvious comparing with derivatives **2a,b**, it was of interest to estimate the anticancer activity of such N-alkyl substituted substances. Thus, in this paper we report our results on the synthesis, structural characterization, and assessment of anticancer activity of a series of N-alkylated 3,.5-bis(arylidene)-4-piperidones and their derivatives with quaternized nitrogen atom.

RESULTS AND DISCUSSION

Synthesis of drug candidates. Principally, the target *N*-alkyl-3,5-bis(arylidene)-4-piperidones may be prepared either by direct alkylation of the preformed skeleton of 3,5-bis(arylidene)-4-piperidones or by the condensation of *N*-alkyl-4-piperidones with substituted aromatic aldehydes in the presence of acid or base. However, taking into account commercial availability of the piperidin-4-one hydrochloride salt monohydrate, it seams reasonable to use condensation reaction of piperidin-4-one with appropriate aldehyde followed by introduction of an alkyl group into the corresponding 3,5-bis(arylidene)-4-piperidone by interaction with halogenoalkane.

Assuming that nucleophilicity of the nitrogen atom of an arylidenepiperidone could be diminished by the influence of electron-withdrawing carbonyl group, the reaction would require good alkylating agent, for example, iodoalkane. In order to test these assumption, we carried out the reaction of 3,5-bis[4-(dimethylamino)-

Scheme 2



3a,b

$R^1 = NMe_2$ (**3a**), F (**3b**)

4a-d

$R^1 = NMe_2$; Alk = Et (**4a**), n-Pr (**4b**), n-Bu (**4c**)

R^1 = F; Alk = Et (**4d**)

Table 1

Reaction of bis(arylidene)piperedones **3a,b** with iodoalkanes *via* Scheme 2.

Run	Starting substrate	AlkJ	**3a (3b)**/AlkJ	Temp, ºC	Time, h	Yield of **4**, %[a]
1	**3a**	EtJ	1.3/1	20-25	80	~100(64)
2	**3a**	EtJ	5/1	20-25	80	100(78)
3	**3a**	n-PrJ	5/1	20-25	30	0
4	**3a**	n-PrJ	5/1	80	24	75(37)
5	**3a**	n-BuJ	5/1	80	12	70(44)[b]
6	**3b**	EtJ	5/1	20-25	70	50[c](28)

[a] According to the ^{1}H NMR data, in brackets isolated yields after purification. [b] isolated as complex with KJ. [c] according to the ^{19}F NMR spectrum

benzylidene]piperidin-4-one **3a** with 1.3 equivalents of iodoethane using biphasic system K_2CO_3/CH_3CN (Table 1, run 1). Surprisingly, the reaction mixture comprised mainly the starting compound **3a** and the quaternary salt **4a** according to the NMR data. Pure quaternary salt may be easily obtained by crystallization of reaction product from CH_2Cl_2 – Et_2O. When the alkylation of 3,5-bis[4-(dimethylamino)benzylidene]piperidin-4-one **3a** was carried out with five equivalents of iodoethane, the salt **4a** was the sole reaction product (Scheme 2, Table 1, run 2).

When **3a** was treated with less reactive bromoethane, a mixture of the starting substrate **3a** and the quaternary salt **4a-Br** was obtained as well. However the **4a-Br** salt bearing bromine anion was isolated in a low yield. It is noteworthy, that interaction of piperidin-4-one with bromoethane taken in equimolar amounts, resulted in *N*-ethyl-piperidin-4-one as the only reaction product (see *Experimental part*).

Although the quaternization of **3a** with iodoethane proceeded smoothly at room temperature, it turned out that both *n*-iodopropane and *n*-iodobutane did not alkylate bisbenzylidenepiperidin-4-one **3a** under presented conditions (Table 1, run 3). However, performing the same reactions in refluxing acetonitrile affords the corresponding piperidonium salts **4b**,**c** (Table 1, runs 4, 5). Alkylation of *para*-fluorine substituted derivative **3b** proceeds in similar manner with compound **3a** giving the corresponding salt. Nevertheless, the increase of electron withdrawing properties of the substituent R^1 changes the reaction course. Namely, interaction of 3,5-bis(4-nitrobenzylidene)piperidin-4-one with iodoethane gave N-monoalkylated product (*ca*. 35-45% according ^{1}H NMR) which we failed to separate from other products of non-identified structure.

Therefore, in contrast to piperidin-4-one, the alkylation of its 3,5-arylidene derivatives bearing substituents possessing either donor or moderate acceptor properties in the *para*-position of phenyl rings proceeds according to the quaternization scheme. An interesting feature of some quaternary salts prepared in this study is their ability to form strong complexes with potassium iodide (bromide). For example, recrystallization of crude reaction mixtures resulted in co-crystallizates of **4a-Br, 4c,** and **4d** with potassium bromide or potassium iodide correspondingly rather than the individual salts. In the case of **4d**, the crystallized product also contained iodoethane (this fact was unambiguously confirmed by ^{1}H-NMR spectroscopy). Moreover, repeated crystallization did not remove potassium iodide (bromide) from the above products. Pure salts **4a-Br** and **4d** were obtained only after washing their complexes with water.

The structure of representative quaternary salt **4d** was confirmed by X-ray data. Both olefinic bonds have *E* configuration, which is in agreement with the results

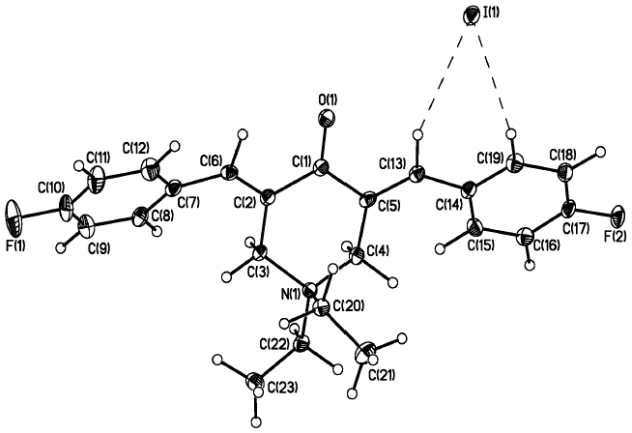


Figure 1. The general view of **4d** in representation of atoms by thermal ellipsoid plots (p=50%). The selected bond lengths (Å): O(1)-C(1) 1.224(3), N(1)-C(3) 1.505(3), N(1)-C(4) 1.508(3), N(1)-C(20) 1.514(3),N(1)-C(22) 1.524(3), C(1)-C(2) 1.485(4), C(1)-C(5) 1.486(4), C(2)-C(6) 1.340(4), C(5)-C(13) 1.349(4); bond angles (º): C(3)-N(1)-C(4) 108.7(2), C(2)-C(1)-C(5) 117.8(2), C(1)-C(2)-C(6) 116.7(2), C(1)-C(5)-C(13), 116.9(2).

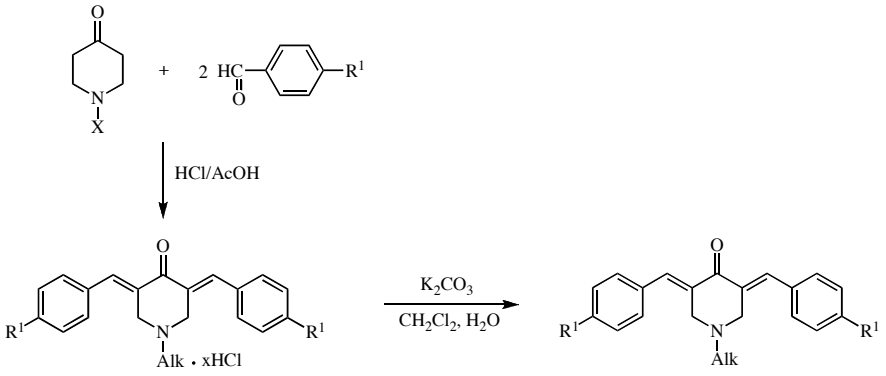
obtained earlier for similar compounds [6,7,8,11-15]. Taking into consideration the general similarity of the spectral data we also assigned the structures of compounds **4a**-**c** to *E,E*-isomers. Principal geometrical parameters of **4d** are in range of standard values with typical lengthening of the N-C bonds from the protonated nitrogen N(1) to values 1.50 Å. The heterocyclic piperidone ring of **4d** has the flattened chair conformation (Figure 1) with the significant deviation of N(1) and slight deviation of C(1) atoms by 0.701(4) and - 0.109(4) Å respectively from the plane formed by C(2), C(3), C(4) and C(5) atoms (rms deviation 0.012 Å) caused by sp^2 character of carbon atom C(1). It should be noted that for similar compounds deviation of C(1) atom from such plane might be positive or negative [11-15] so central core conformation of these molecules might be described as flattened chair or boat; this conformation also can be characterized as a sofa.

To obtain the desired N-monoalkylated bis(arylidene)-piperidones **6a-d,** we have chosen the other possibility that was mentioned above and performed a condensation of pre-alkylated N-alkyl-piperidones with the corresponding aromatic aldehydes followed by transformation of the hydrochloride salts to the free bases (Scheme 3).

Condensation of aromatic aldehydes with *N*-alkyl-4-piperidones may be performed in the presence of either acid or base. The conditions which were reported in literature include the application of aqueous ethanolic potassium hydroxide or hydrogen chloride in absolute ethanol [16].

In some cases, alkylpiperidone is able itself to catalyze this reaction. Indeed, condensation of *N*-methyl-4-

Scheme 3



piperidone with aldehydes proceeds smoothly when carried out in boiling ethanol [17]. Sometimes organic solvent is not present, and a mixture of aldehyde and alkyl-4-piperidone is heated in the presence of concentrated hydrochloric acid [18]. In the present work we have used the procedure described by Dimmock *et al.* [2a], *i.e.* bubbling of gaseous hydrogen chloride through the solution of *N*-alkyl-4-piperidone and aldehyde in glacial acetic acid. Under these conditions the interaction proceeds smoothly to give the hydrochlorides of the desired compounds in 69-90% yield depending on the substituents in the reacting substrates. The free bases were easily obtained on treatment with potassium carbonate in water/CH_2Cl_2 two-phase system. Both salts **5** and free bases **6** were isolated as solids of bright-yellow to dark-orange color.

Cytotoxic activity. The cytotoxic activity of 12 compounds synthesized was tested on human cell line A549 (lung carcinoma). This cell line is known to be rather stable for drugs as possessing ABC-transporters, namely BCRP, LRP, MDR1 (proteins responsible for multiple drug resistance) [9]. The choice of such cell line was connected with our suggestion that good results in this particular case would provide even better activity when applying non-resistant tumor cell lines.

For comparison the activity of parent NH-piperidons **3a**-**c** bearing the same substituents in the phenyl rings was estimated in the same assay. Anticancer agent Melphalan (Sarcolysin) was used as a positive control similar to assays of cytotoxic properties of other 3,5-bis(arylidene)-4-piperidone derivatives described in literature [1-7]. It should be noted that the activity of some analogues of compounds **6** against non-resistant murine tumor cell lines was reported in ref. 6.

In principle, introduction of alkyl groups to the heterocyclic nitrogen atom resulted in decrease of cytotoxic activity excluding compound **4a** being more active that a parent NH-piperidone **3a** and the most active one among the compounds tested. Alkylated piperidones **6c,e** and the corresponding hydrochloride salts **5c,e** bearing NO_2 substituents in the benzene rings possess excellent cytotoxic activity for this cell line (IC_{50}<1.5, Table 2) which was at least twice higher than that for Melphalan. The activity in this series of compounds reduced with decreasing of acceptor properties and appearance of donor properties of the substituents R^1, *i.e.* for compounds **5d, 6d** (R^1=F) and **5a,b, 6a,b** (R^1=$NAlk_2$). In all the cases salts **5** were slightly more active that the corresponding free bases **6**. Such higher activity is apparently attributed to the better solubility of salts **5** in aqueous media in comparison with **6**. On the other hand, for the salts **4**, the above-mentioned compound **4a** with R^1=NMe_2 was more then 10-fold active than its analogue **4d** with the *p*-fluorine atoms. Therefore, we may mention the clear tendency: formation of piperidonium structures

Table 2

In vitro growth inhibition concentrations (IC_{50}) of the testedcompounds against A549 tumor cell line

Cmpd.	R^1	Alk	Alk/H	Hal	IC_{50} (μM)
3a	NMe_2	-	H	-	1.4±0.4
3b	F	-	H	-	0.8±0.07
3c	NO_2		H	-	0.4±0.1
4a	NMe_2	Et	Et	J	0.30±0.05
4d	F	Et	Et	J	4.0±0.2
5a	NMe_2	Me	H	Cl	5.00±0.17
6a	NMe_2	Me	-	-	5.00±0.26
5b	NEt_2	Me	H	Cl	4.00±0.13
6b	NEt_2	Me	-		6.5±0.2
5c	NO_2	Me	H	Cl	0.72±0.01
6c	NO_2	Me	-		1.50±0.05
5d	F	Me	H	Cl	1.50±0.07
6d	F	Me	-		3.0±0.2
5e	NO_2	Et	H	Cl	1.3±0.1
6e	NO_2	Et	-		1.50±0.05
Melphalan					4.0±0.1

result in increase of the biological activity in a series of bis(3,5-arylidene)piperidin-4-ones.

As mentioned above, the presence of conjugated bonds system results in fluorescent properties of 3,5-bis-(arylidene)piperid-4-one derivatives (two-photon absorption) [7-9]. Fluorescent properties of the compounds obtained allowed one to estimate their distribution in cancer cells and such experiment was performed using compound **6b** as a representative example. The result presented in Fig. 2 indicated that the substance does not enter the cell nuclei and is located in cell cytoplasm only (blank and white spots on the Figure respectively).

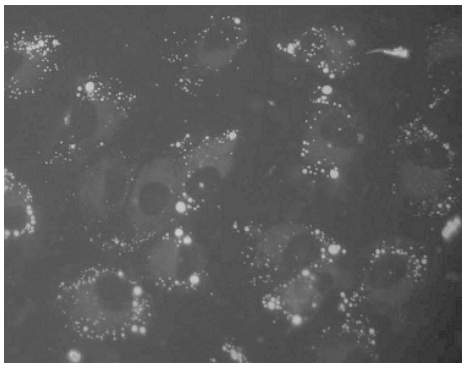


Figure 2. Fluorescence image of the A 549 cells, taken 2 hours after treatment with **6b** compound (1 μM). The nuclei were stained by Hoechst 33258 (black spots).

In conclusion, for the synthesis of N-C_1-C_4-alkylated 3,5-bis(arylidene)-4-piperidones useful as anticancer drug-candidates which activity depends on the lipophilic nature of molecular structure as well as their solubility in body liquids, a condensation of preformed N-alkyl-4-piperidones with the substituted benzaldehydes presents only one convenient route.

EXPERIMENTAL

NMR spectra were recorded with a Bruker AMX-400 spectrometer (^{1}H, 400.13, ^{19}F, 376.3 and ^{13}C, 100.61 MHz) using residual proton signals of deuterated solvent as an internal standard (^{1}H, ^{13}C) and $CFCl_3$ (^{19}F) as an external standard. The ^{13}C NMR spectra were registered using the JMODECHO mode; the signals for the C atom bearing odd and even numbers of protons have opposite polarities. IR spectra were recorded in KBr pellets on a Fourier-spectrometer "Magna-IR750" (Nicolet), resolution 2 cm^{-1}, 128 scans. Melting points were uncorrected. The starting 3,5-bis(arylidene)4-piperidones **3a-c** were obtained by the known procedures including condensation of piperidine-4-one with the corresponding aldehydes in glacial acetic acid in the presence of gaseous hydrogen chloride as reported in ref. [2a] and had the same physical-chemical constants as described in literature [2a]. N-Methyl-piperidone was obtained from Aldrich and used without further purification.

(3*E*,5*E*)-3,5-Bis[4-(dimethylamino)benzylidene]-1,1-diethyl-4-oxopiperidinium iodide (4a). A mixture of 3,5-bis[4-(dimethylamino)benzylidene]piperidin-4-one **3a** (0.36 g, 1 mmol), iodoethane (0.78 g, 5 mmol) and potassium carbonate (0.41 g, 3 mmol) was stirred in CH_3CN (10 mL) for about 80 hours at room temperature. Then CH_2Cl_2 was added to the reaction mixture, and inorganic salts were filtered off. Volatiles were removed in vacuum. The solid was dissolved in CH_2Cl_2, and the solution was filtered. After evaporation of the solvent, the solid (0.54 g) was crystallized from a mixture of CH_3CN/Et_2O to give compound **4a** (0.42 g, 78 %) as red-orange solid. Mp. 235 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ: 1.21 (t, $^3J_{HH}$ = 7.1 Hz, 6H, $N(CH_2CH_3)_2$), 3.05 (s, 12H, 2 $N(CH_3)_2$), 3.70 (q, $^3J_{HH}$ = 7.1 Hz, 4H, $N(CH_2CH_3)$, 5.06 (m, 4H, NCH_2), 6.73 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.36 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 8.10 (s, 2H, CH=). ^{13}C NMR ($CDCl_3$) δ: 7.91 ($N^+CH_2CH_3$), 38.85 (NCH_3), 53.63 ($N^+CH_2CH_3$), 58.59 (N^+CH_2 (cyclic)), 111.79 (C^8, C^{10}), 117.57 (C^6), 120.21 (CH=*C*), 133.15 (C^7,C^{11}), 144.21 (*C*H=C), 151.60 (C^9), 180.00 (C=O). IR (KBr) ν, cm^{-1}: 2972, 2917, 2855, 1655 (C=O), 1614 (C=C), 1574, 1522, 1443, 1371, 1314, 1280, 1231, 1186, 1171, 1127, 984, 941, 818. *Anal.* Calcd. for $C_{27}H_{36}IN_3O$: C, 59.45%; H, 6.65%; N, 7.70%. Found: C, 59.28%; H, 6.74%; N, 7.61%.

When the reactants were used in 1:1.3 molar ratio the salt **4a** was isolated in 64% yield after recrystallization from CH_2Cl_2/Et_2O as red-orange solid.

(3*E*,5*E*)-3,5-Bis[4-(dimethylamino)benzylidene]-1,1-diethyl-4-oxopiperidinium bromide (4a-Br). Using similar procedure and starting from **3a** (0.36 g, 1 mmol) and bromoethane (0.55 g, 5 mmol), complex of compound **4a-Br** with potassium bromide was isolated as red-orange solid (0.18 g, 35 %). *Anal.* Calcd. for $(C_{27}H_{36}BrN_3O)_5$•KBr: C, 62.09%; H, 6.95%; N, 8.05%. Found: C, 62.14%; H, 7.02%; N, 7.75%. Mp. 230 – 233 °C. ^{1}H NMR ($CDCl_3$) δ: 1.21 (t, $^3J_{HH}$ = 7.1 Hz, 6H, $N(CH_2CH_3)_2$), 3.05 (s, 12H, 2 $N(CH_3)_2$), 3.70 (q, $^3J_{HH}$ = 7.1 Hz, 4H, $N(CH_2CH_3)$, 5.06 (m, 4H, NCH_2 (cyclic), 6.73 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.36 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 8.10 (s, 2H, CH=). This complex was treated with a mixture of water (15 mL) and CH_2Cl_2 (15 mL). Organic phase was separated, dried over Na_2SO_4 and evaporated at reduced pressure to afford free compound **4a-Br** as red-orange solid (0.15 g, 30 %) with the same ^{1}H NMR. Mp. 230 – 233 °C (decomp.). *Anal.* Calcd. for $C_{27}H_{36}BrN_3O$: C, 65.05%; H, 7.28%; N, 8.43%. Found: C, 64.73%; H, 7.19%; N, 8.05%.

(3*E*,5*E*)-3,5-Bis[4-(dimethylamino)benzylidene]-4-oxo-1,1-dipropylpiperidinium iodide (4b). A mixture of 3,5-bis[4-(dimethylamino)benzylidene]piperidin-4-one **3a** (0.36 g, 1 mmol), iodopropane (0.85 g, 5 mmol) and calcined potassium carbonate (0.61 g, 4.4 mmol) was refluxed with stirring in CH_3CN (10 mL) for about 24 h. Then CH_2Cl_2 was added to the reaction mixture, and inorganic salts were filtered off. Volatiles were removed in vacuum, and the solid (0.5 g) was crystallized twice from a mixture of CH_3CN/Et_2O to give compound **4b** (0.21 g, 37 %) as dark-red solid. Mp. 223 – 226 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, $^3J_{HH}$ = 7.0 Hz, 6H, $N(CH_2CH_2CH_3)_2$), 1.55 (m, 4H, $N(CH_2CH_2CH_3)_2$), 3.05 (s, 12H, 2 $N(CH_3)_2$), 3.55 (m, 4H, $N(CH_2CH_2CH_3)_2$), 5.11 (m, 4H, NCH_2 (cyclic), 6.73 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 7.36 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 8.11 (s, 2H, CH=). ^{13}C NMR ($CDCl_3$) δ: 10.47 ($N^+CH_2CH_2CH_3$), 15.68 ($N^+CH_2CH_2CH_3$), 39.88 (NCH_3), 59.57 (N^+CH_2 (cyclic)), 60.14 ($N^+CH_2CH_2CH_3$), 111.87 (C^8,C^{10}), 117.65 (C^6), 120.28 (CH=*C*), 133.20 (C^7,C^{11}), 144.31 (*C*H=C), 151.66 (C^9), 179.98 (C=O). IR (KBr) ν, cm^{-1}: 2963, 2930, 2873, 1661 (C=O), 1612 (C=C), 1575, 1522, 1436, 1373, 1315, 1278, 1231, 1184, 1167, 1131, 989, 814, 522. *Anal.* Calcd. for

$C_{29}H_{40}IN_3O$: C, 60.73%; H, 7.03%; N, 7.33%. Found: C, 60.89%; H, 7.04%; N, 7.51%.

(3*E*,5*E*)-1,1-Dibutyl-3,5-bis[4-(dimethylamino)benzylidene]-4-oxopiperidinium iodide (4c). Using the similar procedure except the reaction time (see, Table 1) the salt **4c** as dark-red solid complex with KI (0.26 g, 37%) of composition **(4c)$_9$•KI** was obtained from **3a** (0.36 g, 1 mmol) and iodobutane (0.92 g, 5 mmol) after double recrystallization from a mixture of $CHCl_3$/hexane. The mother liquor was evaporated and the remaining compound was subjected to column chromatography on silica gel. Elution with a mixture of acetone/CH_2Cl_2 = 1/1 followed by crystallization afforded an additional amount (0.05 g) of compound **(4c)$_9$•KI**. Total yield of the complex was 0.31 g, 44%. Mp. 162 – 165 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 6H, N($CH_2CH_2CH_2CH_3$)$_2$), 1.36 (sextet, $^3J_{HH}$ = 7.0 Hz, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 1.46 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 3.06 (s, 12H, 2 N(CH_3)$_2$), 3.56 (m, 4H, N($CH_2CH_2CH_3$)$_2$), 5.11 (m, 4H, NCH_2 (cyclic), 6.74 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4), 7.36 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4), 8.11 (s, 2H, CH=). ^{13}C NMR ($CDCl_3$) δ: 13.44 ($N^+CH_2CH_2CH_2CH_3$), 19.25 ($N^+CH_2CH_2CH_2CH_3$), 23.82 ($N^+CH_2CH_2CH_2CH_3$), 39.87 (NCH_3), 58.36 (NCH_2), 59.54 (NCH_2), 111.84 (C^8,C^{10}), 117.77 (C^6), 120.29 (CH=*C*), 133.14 (C^7,C^{11}), 144.30 (*C*H=C), 151.63 (C^9), 180.11 (C=O). *Anal.* Calcd. for $(C_{31}H_{44}IN_3O)_9$•KI: C, 60.05%; H, 7.15%; N, 6.78%, I, 22.74%. Found: C, 60.04%; H, 7.11%; N, 6.65%, I, 22.50%.

(3*E*,5*E*)-1,1-Diethyl-3,5-bis(4-fluorobenzylidene)-4-oxopiperidinium iodide (4d). A mixture of 3,5-bis(4-fluorobenzylidene)piperidin-4-one (0.46 g, 1.5 mmol), iodoethane (1.18 g, 7.5 mmol) and calcined potassium carbonate (0.43 g, 3.0 mmol) was stirred in CH_3CN (10 mL) for 70 h (^{19}F monitoring). Then CH_2Cl_2 was added to the reaction mixture, and inorganic salts were filtered off. Volatiles components were removed in vacuum, and the solid was recrystallized from a mixture of $CHCl_3$/Et_2O to give 0.36 g of the complex formed by **4d**, iodoethane, and potassium iodide. *Anal.* Calcd. for $(C_{23}H_{24}F_2INO)_{10}$•$(C_2H_5I)_4$•3KI: C, 47.05%; H, 4.31%; N, 2.31%. Found: C, 47.08%; H, 4.17%; N, 2.44%. The complex was added into 10 mL of water, then stirred for 15 min and left under the water layer for 48 h. After filtration and drying over phosphorus pentoxide the analytically pure compound **4d** was isolated in 28% (0.21 g) as bright-yellow powder. Mp. 192 - 195 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.19 (t, $^3J_{HH}$ = 7.2 Hz, 6H, N(CH_2CH_3)$_2$), 3.70 (q, $^3J_{HH}$ = 7.2 Hz, 4H, N(CH_2CH_3), 5.29 (m, 4 H, NCH_2 (cyclic)), 7.21 (appeared t, $^3J_{HH}$ = $^3J_{FH}$ = 8.5 Hz, 4H, C_6H_4), 7.48 (dd, $^3J_{HH}$ = 8.5 Hz, $^3J_{FH}$ = 5.4 Hz, 4H, C_6H_4), 8.21 (s, 2H, CH=). ^{19}F-{^{1}H} NMR: -109.91 ppm ($CDCl_3$). *Anal.* Calcd. for $C_{23}H_{24}F_2INO$: C, 55.77%; H, 4.88%; N, 2.83%. Found: C, 55.79%; H, 4.97%; N, 2.84%.

1-Ethyl-piperidin-4-one. A mixture of piperidone hydrate hydrochloride (1.08 g, 0.007 mol) and calcinated potassium carbonate (1.24 g, 0.009 mol) in CH_3CN (12 mL) was stirred for 2.5 h. Inorganic salts were filtered off and washed with CH_3CN (4 mL). Filtrate and washings were combined and mixed with bromoethane (0.80 g, 0.007 mol) and calcinated potassium carbonate (1.24 g, 0.009 mol). Reaction mixture was stirred for 21 h. Inorganic salts were filtered off and washed with benzene. The solvent was removed in vacuum; the remaining liquid was dissolved in benzene and the solution was filtered. Evaporation of benzene afforded 1-ethyl-piperidin-4-one (0.55 g, 62 %) as light yellow liquid. ^{1}H NMR ($CDCl_3$) δ: 1.00 (t, $^3J_{HH}$ = 7.0 Hz, 3H, NCH_2CH_3), 2.32 (m, 4H), 2.40 (q, $^3J_{HH}$ = 7.0 Hz, 2H, NCH_2CH_3), 2.61 (m, 4H) (*cf.* [19]).

(3*E*,5*E*)-1-Methyl-3,5-bis(4-fluorobenzylidene)-4-oxopiperidinium chloride (5d). *N*-Methyl-piperidin-4-one (1.02 g, 9 mmol) and 4-fluorobenzaldehyde (2.23 g, 18 mmol) were dissolved in glacial acetic acid (15 mL). Hydrogen chloride prepared from ammonium chloride (2.6 g, 48 mmol) was bubbled through the solution. The reaction solution was allowed to stay at room temperature for about 40 hours. During this period, the reaction mixture got crimson color, and the formation of the precipitate was observed. The precipitate was filtered off and washed with acetic acid. The light-yellow precipitate was dried in vacuum at 145 ºC to give pure **5d** (2.08 g, 64%). The mother liquor and the washings were combined, and acetic acid was removed in vacuum. Acetone (5 mL) was added to the residue, and yellow powder (0.19 g) was filtered off. Drying of this powder in vacuum at 145 ºC afforded an additional amount of **5d** (0.16 g). Total yield of **5d** was 2.24 g, 69%. Mp. 248 – 251 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 2.97 (s, 3H, NCH_3), 4.64 (m, 4H, NCH_2 (cyclic)), 7.38 (appeared t, $^3J_{HH}$ = $^3J_{FH}$ = 8.8 Hz, 4H, C_6H_4), 7.64 (dd, $^3J_{HH}$ = 8.5 Hz, $^3J_{FH}$ = 5.5 Hz, 4H, C_6H_4), 7.88 (s, 2H, CH=). ^{19}F-{^{1}H} NMR (DMSO-d_6) δ: -109.5. ^{13}C NMR (DMSO-d_6) δ: 42.36 (NCH_3), 53.12 (NCH_2), 116.29 (d, C^8, $^2J_{CF}$ 21.0 Hz), 127.05 (CH=*C*), 130.36 (d, C^6, $^4J_{CF}$ 2.9 Hz), 133.60 (d, C^7, $^3J_{CF}$ 8.8 Hz), 138.58 (*C*H=C), 163.15 (d, C^9, $^1J_{CF}$ 250 Hz), 181.55 (C=O). *Anal.* Calcd. for $C_{20}H_{18}ClF_2NO$: C, 66.39%; H, 5.01%; N, 3.87%. Found: C, 66.24%; H, 5.01%; N, 3.77%.

Compounds **5a**-**c** were obtained from *N*-methyl-piperidin-4-one (5.1 mL, 0.044 mol) and 0.088 mol of the corresponding substituted aldehyde following the procedure for **5d**.

(3*E*,5*E*)-3,5-Bis[4-(dimethylamino)benzylidene]-1-methyl-4-oxopiperidinium chloride (5a). Yield 90 %. Mp. 245 – 248 °C (decomp.). ^{1}H NMR (DMSO-d_6) δ: 3.01 (s, 3H, NCH_3), 3.04 (s, 12H, 2 N(CH_3)$_2$), 4.62 (m, 4H, NCH_2 (cyclic)), 6.85 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.42 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.78 (s, 2H, CH=), 11.17 (br. s, N^+H).IR (KBr) ν, cm^{-1}: 3012, 2896, 2349, 1674 (C=O), 1608 (C=C), 1561, 1552, 1433, 1367, 1317, 1280, 1235, 1192, 1168, 979, 941, 809, 577, 529. *Anal.* Calcd. for $C_{24}H_{29}N_3O$•2HCl: C, 64.28%; H, 6.97%; Cl, 15.81%; N, 9.37%. Found: C, 63.94%; H, 7.06%; Cl, 15.62%; N, 9.28%.

(3*E*,5*E*)-3,5-Bis[4-(diethylamino)benzylidene]-1-methyl-4-oxopiperidinium chloride (5b). Yield 87%. Mp. 233-234 °C (decomp.). ^{1}H NMR (D_2O) δ: 1.03 (t, $^3J_{HH}$ 7.6 Hz, 12H, N(CH_2CH_3)$_2$), 2.94 (s, 3H, NCH_3), 3.57 (q, $^3J_{HH}$ = 7.6 Hz, 8H, N(CH_2CH_3)$_2$), 4.65 (br, NCH_2 (cyclic) partially overlapped by signal of H_2O in D_2O), 7.50 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.57 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 8.03 (s, 2H, CH=). IR (KBr) ν, cm^{-1}: 2967, 2926, 2511, 2458, 1685, 1615, 1575, 1520, 1472, 1431, 1354, 1314, 1278, 1264, 1177, 1158, 977, 818, 568, 511. *Anal.* Calcd. for $C_{28}H_{37}N_3O$•2.25HCl: C, 65.47%; H, 7.70%; N, 8.18%. Found: C, 65.52%; H, 7.50%; N, 8.20%.

(3*E*,5*E*)-1-Methyl-3,5-bis(4-nitrobenzylidene)-4-oxopiperidinium chloride (5c). Yield 74%. Mp. 250-251 °C (decomp.). ^{1}H NMR (DMSO-d_6) δ: 2.99 (s, 3H, NCH_3), 4.70 (s, 4H, NCH_2 (cyclic)), 7.83 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 8.01 (s, 2H, CH=), 8.35 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 11.08 (br. s, N^+H). IR (KBr) ν, cm^{-1}: 3108, 2928, 2450, 1680 (C=O), 1614 (C=C), 1599, 1593 (C=C), 1519 (NO_2, ν_{as}), 1466, 1414, 1348 (NO_2, ν_s), 1317, 1265, 1170, 1109, 852, 756, 686. *Anal.* Calcd. for $C_{20}H_{17}N_3O_5$•HCl: C, 57.77%; H, 4.36%; Cl, 8.53%; N, 10.11%. Found: C, 57.74%; H, 4.38%; Cl, 8.68%; N, 10.01%.

(3*E*,5*E*)-1-Ethyl-3,5-bis(4-nitrobenzylidene)-4-oxopiperidinium chloride (5e). *N*-ethyl-piperidin-4-one (0.70 g, 5.5 mmol) and 4-nitrobenzaldehyde (1.51 g, 10 mmol) were mixed

in glacial acetic acid (25 mL). Hydrogen chloride prepared from ammonium chloride (3 g, 56 mmol) was bubbled through the solution. The reaction solution was allowed to stay at room temperature for approximately 72 hours. The crystals formed were collected, washed with acetone and dried in air to give **5e** as yellow crystals (0.95 g, 44 %). Mp. 250-251 °C (decomp.).^{1}H NMR (DMSO-d_6) δ: 1.24 (t, $^3J_{HH}$ 7.0 Hz, 3H, NCH_2CH_3), 3.40 (br, NCH_2CH_3, partially overlapped by signal of H_2O in DMSO-d_6), 4.64 (m, 4H, NCH_2 (cyclic), 7.86 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 8.35 (d, $^3J_{HH}$ = 8.7 Hz, 4H, C_6H_4), 8.01 (s, 2H, CH=), 11.13 (s, 1H, N-H). IR (KBr) ν, cm^{-1}: 3104, 2928, 2452, 1685 (C=O), 1617, 1601 (C=C), 1593, 1515 (NO_2, ν_{as}), 1414, 1341 (NO_2, ν_s), 1319, 1260, 1195, 1116, 1105, 990, 864, 853, 814, 756, 689. *Anal.* Calcd. for $C_{21}H_{20}ClN_3O_5$: C, 58.68%; H, 4.69%; N, 9.78%. Found: C, 58.61%; H, 4.77%; N, 9.64%.

(3*E*,5*E*)-1-Methyl-3,5-bis(4-fluorobenzylidene)-piperidin-4-one (6d). A suspension of 1-methyl-3,5-bis(4-fluorobenzylidene)-4-oxopiperidinium chloride **5d** (0.51 g, 1.4 mmol) and potassium carbonate (1.54 g, 11 mmol) in a mixture of water (10 mL) and CH_2Cl_2 (15 mL) was stirred for about 3 hours at room temperature. The yellow organic solution was separated and dried over Na_2SO_4. After removal of dichlorometane in vacuum, and the desired compound **6d** (0.45 g, 98%) was obtained as light-yellow powder. Mp. 174 – 177 °C. ^{1}H NMR ($CDCl_3$) δ: 2.47 (s, 3H, NCH_3), 3.76 (m, 4H, NCH_2 (cyclic)), 7.11 (appeared t, $^3J_{HH}$ = $^3J_{FH}$ = 8.6 Hz, 4H, C_6H_4), 7.37 (dd, $^3J_{HH}$ = 8.6 Hz, $^3J_{FH}$ = 5.5 Hz, 4H, C_6H_4), 7.78 (s, 2H, CH=). ^{19}F-{^{1}H} NMR ($CDCl_3$) δ: -110.6 ppm. ^{13}C NMR ($CDCl_3$) δ: 45.70 (NCH_3), 56.77 (NCH_2), 115.58 (d, C^8, C^{10}, $^2J_{CF}$ = 22.0 Hz), 131.17 (d, C^6, $^4J_{CF}$ = 3.7 Hz), 132.17 (d, C^7, C^{11}, $^3J_{CF}$ = 8.8 Hz), 132.49 (CH=*C*), 135.09 (*C*H=C), 162.74 (d, C^9, $^1J_{CF}$ = 251 Hz), 186.37 (C=O). IR (KBr) ν, cm^{-1}: 2850, 2782, 1672 (C=O), 1613, 1601 (C=C), 1580, 1509, 1504, 1458, 1411, 1293, 1273, 1232, 1227, 1175, 1160, 1130, 1107, 1086, 1056, 983, 924, 853, 835, 791. *Anal.* Calcd. for $C_{20}H_{17}F_2NO$: C, 73.83%; H, 5.27%; N, 4.31%. Found: C, 73.81%; H, 5.19%; N, 4.35%.

The free bases **6a-c,e** were obtained according to the similar procedure.

(3*E*,5*E*)-3,5-Bis[4-(dimethylamino)benzylidene]-1-methyl-piperidin-4-one (6a). Yield 86 %. Mp. 229 – 232 °C (lit.: Mp.223 – 225 °C [17]). ^{1}H NMR ($CDCl_3$) δ: 2.48 (s, 3H, NCH_3), 2.99 (s, 12H, 2 $N(CH_3)_2$), 3.78 (s, 4H, NCH_2 (cyclic)), 6.69 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.32 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.76 (s, 2H, CH=). ^{13}C NMR ($CDCl_3$) δ: 39.86 (NCH_3), 45.60 (NCH_3), 57.14 (NCH_2 (cyclic)), 111.48 (C^8, C^{10}), 123.17 (C^6), 128.92 (CH=*C*), 132.33 (C^7, C^{11}), 136.35 (*C*H=C), 150.38 (C^9), 186.26 (C=O). IR (KBr) ν, cm^{-1}: 2941, 1654 (C=O), 1615 (C=C), 1579, 1524 (C=C, aromatic), 1433, 1372, 1310, 1285, 1232, 1163, 1052, 986, 811, 514. *Anal.* Calcd. for $C_{24}H_{29}N_3O$: C, 76.77%; H, 7.78%; N, 11.19%. Found: C, 76.74%; H, 7.82%; N, 11.14%.

(3*E*,5*E*)-3,5-Bis[4-(diethylamino)benzylidene]-1-methyl-piperidin-4-one (6b). Yield 92 %. Mp. 195 – 198 °C. ^{1}H NMR ($CDCl_3$) δ: 1.18 (t, $^3J_{HH}$ = 7.0 Hz, 12H, $N(CH_2CH_3)_2$), 2.49 (s, 3H, NCH_3), 3.38 (q, $^3J_{HH}$ = 7.0 Hz, 8H, $N(CH_2CH_3)_2$), 3.79 (s, 4H, NCH_2 (cyclic)), 6.66 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.32 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.75 (s, 2H, CH=). ^{13}C NMR ($CDCl_3$) δ: 12.42 ($N(CH_2CH_3)_2$), 44.18 ($N(CH_2CH_3)_2$), 45.76 (NCH_3), 57.29 (NCH_2 (cyclic)), 110.83 (C^8, C^{10}), 122.37 (C^6), 128.62 (CH=*C*), 132.67 (C^7, C^{11}), 136.19 (*C*H=C), 147.88 (C^9), 186.35 (C=O). IR (KBr) ν, cm^{-1}: 2968, 1660 (C=O), 1587, 1519, 1432, 1355, 1315, 1288, 1268, 1199, 1179, 1172, 1152, 1075, 1012, 987, 820. *Anal.* Calcd. for $C_{28}H_{37}N_3O$: C, 77.92%; H, 8.64%; N, 9.74%. Found: C, 78.01%; H, 8.67%; N, 9.64%.

(3*E*,5*E*)-1-Methyl-3,5-bis(4-nitrobenzylidene)-piperidin-4-one (6c). Yield 89 %. Mp. 222 – 225 °C. (lit.: Mp.229 – 231 °C [17]). ^{1}H NMR ($CDCl_3$) δ: 2.49 (s, 3H, NCH_3), 3.79 (s, 4H, NCH_2 (cyclic)), 7.53 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4), 7.83 (s, 2H, CH=), 8.29 (d, $^3J_{HH}$ = 8.9 Hz, 4H, C_6H_4). IR (KBr) ν, cm^{-1}: 1670 (C=O), 1598, 1589 (C=C, aromatic), 1510 (NO_2, ν_{as}), 1342 (NO_2, ν_s), 1328, 1314, 1251, 1167, 1106, 980, 928, 861, 850, 802, 757, 688. *Anal.* Calcd. for $C_{20}H_{17}N_3O_5$: C, 63.32%; H, 4.52%; N, 11.08%. Found: C, 63.47%; H, 4.51%; N, 11.04%.

(3*E*,5*E*)-1-Ethyl-3,5-bis(4-nitrobenzylidene)piperidin-4-one (6e). After the solvent was removed in vacuum, the crude product was recrystallized from a mixture of CH_2Cl_2/heptane to give a bright yellow solid **6e** in 72 %. Mp. 178 – 183 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ: 1.05 (t, $^3J_{HH}$ = 7.0 Hz, 3H, NCH_2CH_3), 2.61 (q, $^3J_{HH}$ = 7.0 Hz, 2H, NCH_2CH_3), 3.80 (m, 4H, NCH_2 (cyclic)), 7.53 (d, $^3J_{HH}$ = 8.8 Hz, 4H, C_6H_4), 7.81 (s, 2H, CH=), 8.28 (d, $^3J_{HH}$ = 8.8 Hz, 4H, C_6H_4). ^{13}C NMR ($CDCl_3$) δ: 12.16 (NCH_2CH_3), 51.29 (NCH_2CH_3), 54.07 (NCH_2), 123.74 (C^8,C^{10}), 130.71 (C^7,C^{11}) 134.05 (*C*H=C), 135.75 (CH=*C*), 141.26(C^6), 147.48 (C^9), 186.36 (C=O). IR (KBr) ν, cm^{-1}: 1679 (C=O), 1600, 1592 (C=C, aromatic), 1513 (NO_2, ν_{as}), 1346 (NO_2, ν_s), 1310, 1261, 1231, 1172, 1109, 989, 853, 756, 687. *Anal.* Calcd. for $C_{21}H_{19}N_3O_5$: C, 64.12%; H, 4.87%; N, 10.68%. Found: C, 64.07%; H, 4.78% N, 10.69%

X-ray Crystallography. Crystals of **4d** suitable for X-ray diffraction were grown by slow evaporation of CH_3CN/Et_2O solution. Crystallographic data for **4d** ($C_{23}H_{24}F_2NOI$) at 100K: crystals (0.40×0.24×0.20 mm) are monoclinic, space group $P2_1/c$, a= 9.377(2), b = 14.511(3), c= 15.742(3) Å, β=105.560(7)°, V= 2063.5(7) Å^3, Z=4 (Z'=1), M= 495.33, d_{calc}= 1.594 gcm^{-3}, μ(MoKα)=15.83 cm^{-1}, F(000)= 992. Intensities of 15498 reflections were measured with an APEX II CCD diffractometer at 100K (λ(MoKα)=0.71072Å, 2θ<59°) and 5469 independent reflections (R_{int}= 0.0449) were used in the further refinement. The structure was solved by direct method and refined by the full-matrix least-squares technique against F^2 in the anisotropic-isotropic approximation. The refinement converged to wR_2= 0.0901 and GOF=0.967 for all independent reflections (R1=0.0337 was calculated based on F for 4215 observed reflections with I>2σ(I)). All calculations were performed using SHELXTL PLUS 5.0 [20]. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited to the Cambridge Crystallographic Data Centre as supplementary no. CCDC 635654. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ UK (Fax: (internat.) +44-1223/336-033; E-mail: deposit @ccdc.cam.ac.uk).

Biological evaluations. Cell line used for estimation was A549 - human lung carcinoma cell line. Cells were grown in RPMI-1640 medium (Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (FBS, HyClone, USA), 2 mML-glutamine and gentamicin.

Cells were plated at a density of 2x10^5 cells/mL in culture medium with increasing drug concentrations. The compounds were primary dissolved in dimethylsulphoxide (DMSO) and the using solutions were in FBS free culture medium. Control preparations contained similar amounts of DMSO. Plates were incubated for 48 h at 37ºC in a humidified atmosphere containing 5%CO_2. After incubation the percentage survival of

the cells was recorded. The viable cells were determined by trypan blue exclusion. Each compound including parent NH-piperidones **3a**-**c** was examined in triplicate and the IC_{50} values were determined graphically. The concentrations of compounds used were $5x10^{-5}$, 10^{-5}, 10^{-6}, 10^{-7}M. Commercially available Melphalan (Sarcolysin) purchased from Arkelan-Glaxo was used as a positive control in the assay.

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