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Efficient one-pot synthesis of biologically active polysubstituted aromatic amines

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Abstract—An efficient and modular one-pot synthesis of polysubstituted aromatic amines by a mild reductive amination procedure is described and the biological potential of these nitrogen-centered compounds is demonstrated by growth inhibition of murine connective tissue cells and microscopy-based morphological studies.

1. Introduction

Polysubstituted aromatic amines present prevalent constitutional chemotypes and underlying structural motifs in registered drugs and bioactive synthons, such as benzodiazepines (**1**), phenethylamines (**2**), and arylpiperidines (**3**, all shown in Fig. 1a), or tertiary amines of type **4** in general (Fig. 1b),[1,2] which renders the development of efficient procedures for their synthesis an objective of high priority from the perspective of medicinal and bioorganic chemistry. The reductive amination of carbonyls is widely recognized as one of the most important and convergent methods for the preparation of substituted amines and numerous applications have been reported.[3,4] Surprisingly, applications of such methods to a modular and in particular one-pot access to tertiary aromatic amines are much less common and established, due to increased steric hindrance in the second amination step and/or lack of synthetic selectivity.[2,5]

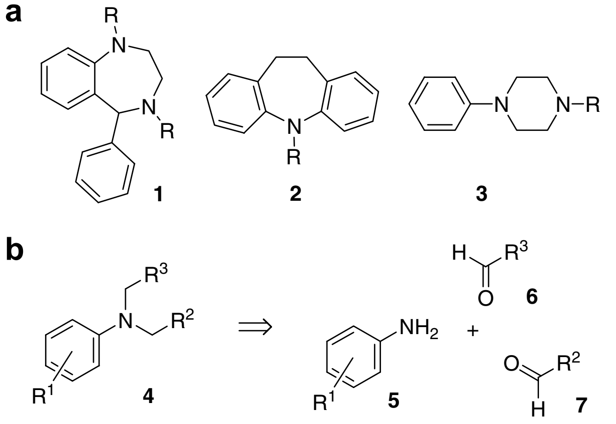

Figure 1. Polysubstituted aromatic amines as bioactive synthons: established motifs (a) and amines of general type **4**, elaborated in this communication (b).

Herein, we report an efficient one-pot procedure for the modular synthesis of sterically hindered aromatic amines of type **4** and demonstrate the biological potential of these compounds in whole cell-based assays and microscopy-based morphological studies. The mild and operationally simple method relies on the convergent assembly of **4** from three building blocks, a starting amine **5** and two different carbonyl components (**6** and **7**) and proceeds by selective reductive amination.

2. Results and discussion

Based on an innovative biosynthetic concept, we have recently developed a direct reductive amination procedure, which uses the Hantzsch ester (HEH, **8**) for transfer hydrogenation and proceeds in the presence of molecular sieves and catalytic amounts of thiourea for

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Keywords: Aromatic amines; Reductive amination; Diversity-oriented synthesis; One-pot synthesis; Cytotoxicity.

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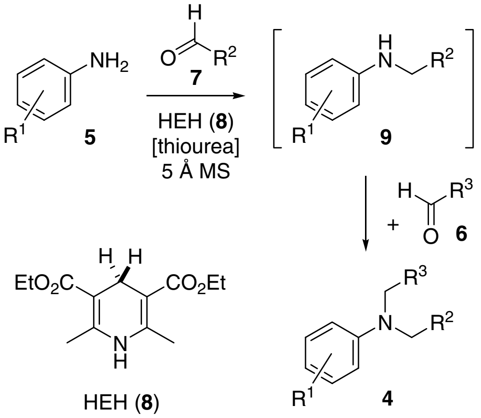


Scheme 1. Modular one-pot synthesis of tertiary aromatic amines.

imine activation.[6,7] In detail, we have successfully developed protocols for the reductive alkylation of primary and secondary aromatic amines to obtain secondary[6a] and tertiary amines,[6c] respectively. The efficiencies of these procedures prompted us to investigate whether this method can be further expanded also to a modular one-pot synthesis of tertiary amines, by stepwise alkylation of a primary amine, yet without isolation of intermediates, in contrast to our previous protocols.[6c] As shown in Scheme 1, this approach would start by reacting amine **5** with a first carbonyl component (**7**) giving initially intermediate **9**, which would then be treated in situ with a second carbonyl (**6**) to give the desired tertiary product **4** in a direct fashion. Notably, the synthetic design is highly convergent and modular and should thus be amenable to combinatorial library synthesis.

2.1. One-pot synthesis of polysubstituted amines

In order to evaluate the viability of such a process, we first analyzed the selectivity of the two amination steps involved. This revealed the first reductive alkylation to be much faster, presumably due to steric reasons, enabling a highly selective monoalkylation. Upon extended reaction times, however, also the second reductive amination may proceed under similar reaction conditions (i.e., temperature and solvent) to give the desired product **4**. After optimizing reaction conditions, an effective protocol was established which involved use of 3 equiv Hantzsch ester, 0.2 equiv of thiourea, 1.05 equiv of the first, and 1.5 equiv of the second carbonyl component and proceeds at 60 °C in toluene.[8]

As shown in Figure 2, this protocol was applied to the synthesis of variously substituted tertiary amines.[9] The desired products were obtained in good (**4a**, **4b**, **4d**, **4g**, **4h**, **4j–l**) to useful (**4c**, **4e**, **4f**, **4i**, **4m**) yields without further adaption of conditions to specific substrates. Aliphatic and aromatic aldehydes are accepted as substrates (e.g., **4b**, **4j**, **4k**) and variations in the electronic and steric properties are tolerated (e.g., **4c**, **4g**, **4m**), which demonstrates the usefulness of this method to the modular synthesis of diverse as well as sterically demanding tertiary amines.

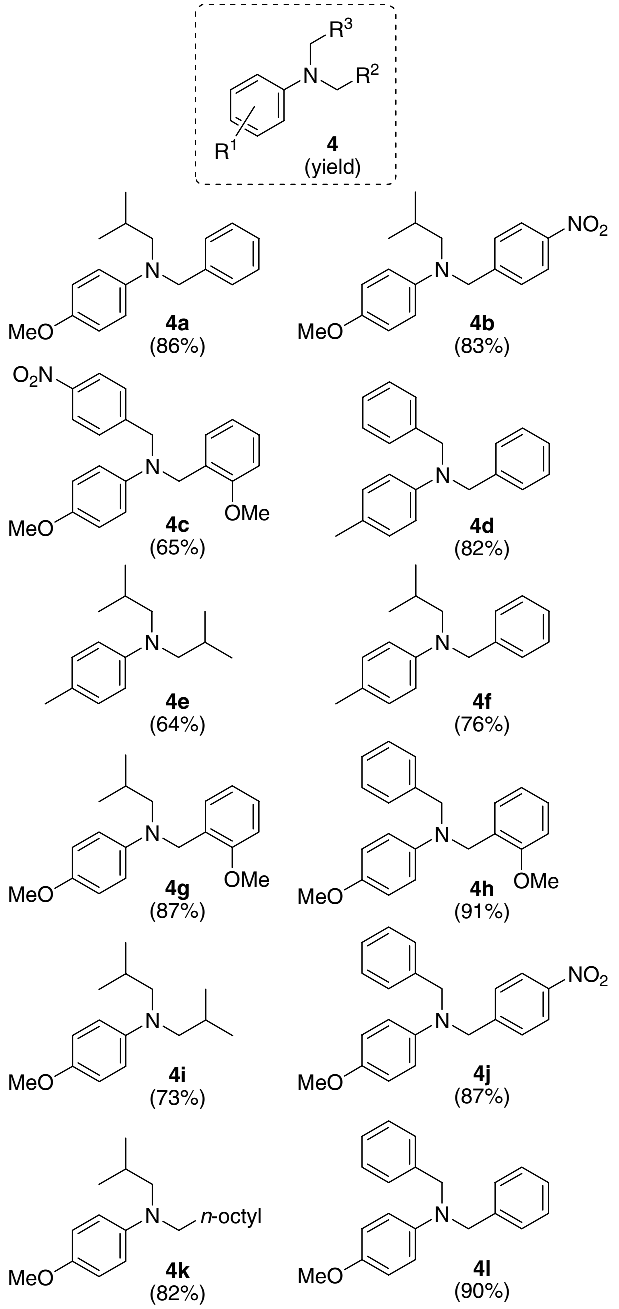


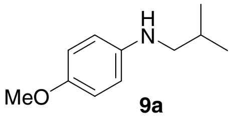
Figure 2. Tertiary amines prepared with the indicated yields by one-pot direct reductive amination (yields correspond to attachment in the order of R^2 and then R^3 according to general structure **4**).

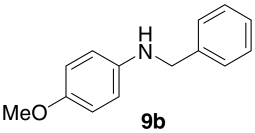
2.2. Biological activity

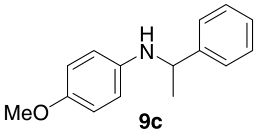
This direct and modular access to substituted tertiary aromatic amines allowed assessment of their bioactivities, relative to representative secondary congeners.[6a] In view of the cytotoxic activity of simple aromatic amines,[10] it appeared rewarding to likewise test these more substituted nitrogen-centered structures in whole cell-based assays. Consequently, the inhibitory effect on the growth of the murine connective tissue cell line L-929 was analyzed. As shown in Table 1, various representatives showed potent cytotoxicity with IC_{50} values in the low micromolar range. Interestingly, the trisubstituted struc-

Table 1. Inhibitory effects of tertiary aromatic amines (**4**) in comparison to secondary amines (**9**) on the growth of mammalian murine connective tissue cell line

Compound[a]	Growth inhibition L-929 IC_{50}, μg/mL (μM)	Compound[a]	Growth inhibition L-929 IC_{50}, μg/mL (μM)
4a	10 (37)	**9b**	30 (141)
4b	>40	**9c**	30 (132)
4c	>40	**9d**	>40
4d	11 (38)	**9e**	12 (48)
4e	13 (59)	**9f**	25 (103)
4f	5 (20)	**9g**	9 (37)
4g	15 (50)	**9h**	>40
4h	>40	**9i**	>40
4i	25 (106)	**9j**	22 (98)
4j	>40	**9k**	35 (190)
4k	20 (66)	**9l**	20 (73)
4l	12 (40)	**9m**	40 (184)
9a	>40	**9n**	30 (129)


9a


9b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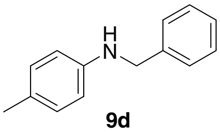
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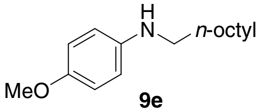

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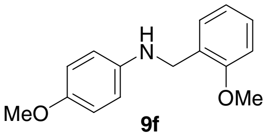
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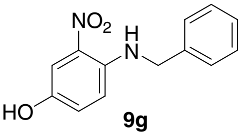
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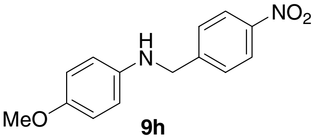
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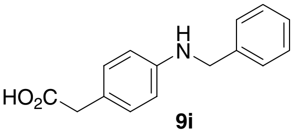
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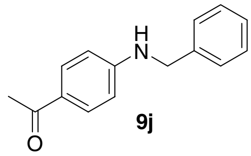
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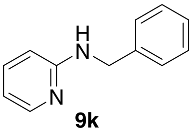
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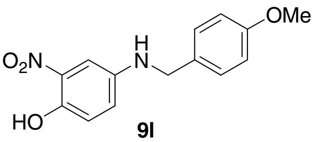
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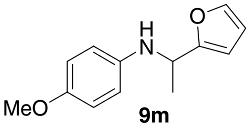
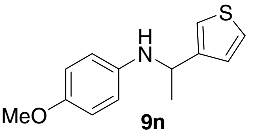

9m


9n

[a] The synthesis of the secondary amines (**9**) by application of our methodology has been previously reported in preliminary form.[6a,b] Full details are given in the Supplementary material.

tures were 2–10 times more potent than the respective secondary amines (as shown e.g., by comparing **4a**/**4l** with **9b**, **4g** with **9a**, or **4i** with **9a**) and also more potent as compared to simple primary aromatic amines.[11] In comparison to aliphatic residues, additional aromatic moieties appeared to increase biological activity (e.g., **4a** vs **4i**, **4f** vs **4 e**) which might be related to variations in membrane permeability. Among the aromatics studied, electron donating groups (e.g., OH: **9g** or OMe: **4j**) appeared to be beneficial for activity, while electron-withdrawing groups lead to a slight decrease (e.g., **9k**, **9j**) in this assay. These data demonstrate that the biological function of such polyfunctional amines is quite versatile. No activity below 40 μg/mL was observed for various secondary and tertiary amines, e.g., **9a**, **9d**, **4b**, and **4h**, which further corroborates the modular potential of these polysubstitued amines as flexible bioactive synthons.

To get further hints about the mode of action, the amines **4f** and **9g** as exemplary representatives of this compound class were checked for effects on the morphology of PtK_2 potoroo cells. The cultured cells were stained by labeling the nuclei and a marker protein of the endoplasmatic reticulum, and inspected by fluorescence microscopy. As shown in Figure 3, treated cells showed striking alterations of the inner membrane structure of the cytoplasm. They displayed big vacuoles near the nucleus or a cushion-like pattern, the first being more pronounced in the **9g** treated cells. Compound **4f** seems to be less effective in vacuolization, rather leading to cell enlargement. The effect of **9g** is very similar to the corallidicyals, sesquiterpene hydroquinones from the Caribbean Sponge *Aka coralliphagum*,[12] which suggests that a similar mode of action might be involved. Possibly, redox processes might be associated. The notion that the tertiary amine **4f** is less effective in vacuolization as compared to **9g**, might be related to the fact that the central nitrogen is sterically much more hindered and thus less accessible to oxidation–reduction processes. Significantly, tertiary amines are more potent in the cell culture assays in comparison to primary or secondary amines, which might suggest that also other effects may be involved for these more encumbered nitrogen-containing structures.

3. Conclusions

In conclusion, we have reported polysubstituted aromatic amines as promising and modular bioactive synthons. The synthesis of these compounds was enabled in a versatile, one-pot fashion by reductive amination, which enables a rapid entry into diverse tertiary aromatic amines. These compounds exhibited pronounced inhibitory effects on the growth of the murine connective tissue cell line L-929 in the low micromolar range. Two exemplary representatives were shown to cause alterations of the inner membrane structure of the cytoplasm by microscopy-based studies. Tertiary amines were more active than the corresponding secondary or primary congeners in the cell culture assays, while being less effective in vacuolization of the membrane, which warrants further research into this class of compounds a potentially rewarding task. It is expected, that our procedure will find further applications in medicinal chemistry and chemical biology.

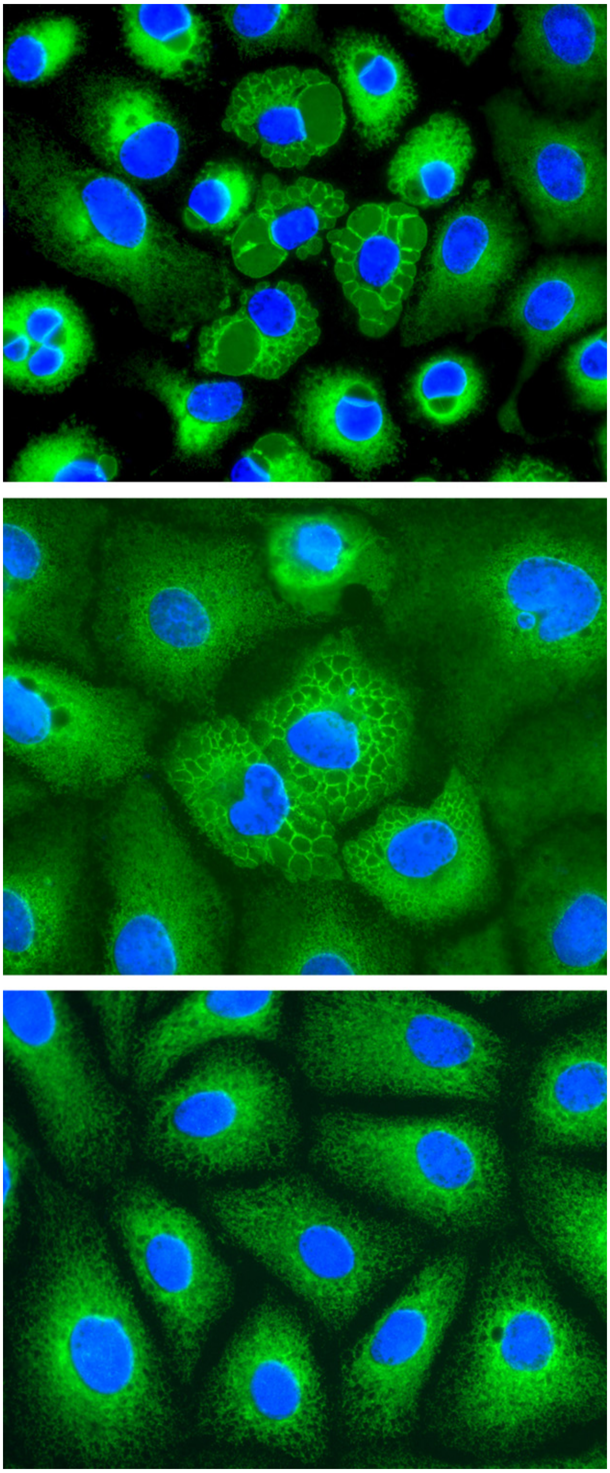


Figure 3. Changes in the morphology of cultivated PtK_2 potoroo cells upon treatment with the aromatic amines **9 g** (top), and **4f** (middle) in comparison to control cells (bottom). Cells were incubated with 50 μg/mL for 18 h and stained for nuclei (blue) and ER structure (green).

4. Experimental

NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 spectrometer. EI and DCI mass spectra (reactant gas ammonia) were obtained on a Finnigan MAT 95 spectrometer, high resolution data were acquired using peak matching (M/DM = 10,000). Analytical TLC (TLC aluminum sheets silica gel Si 60 F_{254} (Merck), solvent: mixtures of ethylacetate/petroleum ether, detection: UV absorption at 254 nm, dark blue spots on staining with cerium(IV)sulfate-phosphomolybdic acid in sulfuric acid followed by charring. Reagents were obtained from commercial suppliers and used without further purification. Dry toluene was obtained from Fluka.

4.1. General procedure for the synthesis of tertiary aromatic amines

A solution of the amine (**5**, 1.00 mmol) and the first carbonyl component (**7**, 1.05 mmol) in toluene (5 mL) was treated with the Hantzsch ester (**8**, 950 mg, 3 mmol), thiourea (15.2 mg, 0.200 mmol), and MS 5 Å (2.0 g) and the mixture was stirred under nitrogen at 60 °C for 24 h, after which the second carbonyl component (**6**, 1.50 mmol) was added and the reaction mixture was stirred at 60 °C until complete conversion (24–72 h). After filtration over Celite, the solvent was evaporated and the residue purified by flash chromatography on silica gel using mixtures of petroleum ether and ethyl acetate as eluents to give the product tertiary amine (**4**) in analytically pure form.

4.1.1. Synthesis of *N*-Benzyl-*N*-isobutyl-4-methoxy-aniline (4a). According to the general procedure, *para*-anisidine was treated with benzaldehyde and *iso*-butyraldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4a** as a yellow precipitate (262 mg, 860 μmol, 86%). Mp 35–37 °C; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.96$ (d, $J = 6.7$ Hz, 6H), 2.11 (tsept, $J = 7.1$, $J = 6.7$ Hz, 1H), 3.14 (d, $J = 7.1$ Hz, 2H), 3.74 (s, 3H), 4.52 (s, 2H), 6.67 (d, $J = 9.0$ Hz, 2H), 6.79 (d, $J = 9.0$ Hz, 2H), 7.14–7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 20.59$, 27.4, 55.8, 56.4, 60.4, 114.7, 126.6, 126.9, 128.4, 139.3, 143.7, 151.4; HRMS (ESI): *m*/*z*: calcd for $C_{18}H_{24}NO$ $[M+H]^+$: 270.1858. Found: 270.1859.

4.1.2. Synthesis of *N*-isobutyl-4-methoxy-*N*-(4-nitrobenzyl)aniline (4b). According to the general procedure, *para*-anisidine was treated with 4-nitro-benzaldehyde and *iso*-butyraldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4b** as a yellow oil (260 mg, 830 μmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.95$ (d, $J = 6.6$ Hz, 6H), 2.11 (tsept, $J = 7.1$,$J = 6.6$ Hz, 1H), 3.12 (d, $J = 7.1$ Hz, 2H), 3.72 (s, 3H), 6.63 (d, $J = 9.1$ Hz, 2H), 6.77 (d, $J = 9.1$ Hz, 2H), 7.37 (d, $J = 9.1$ Hz, 2H), 8.13 (d, $J = 9.1$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 20.6$, 27.4, 55.7, 56.4, 61.2, 114.8, 115.4, 123.7, 127.7, 142.9, 147.0, 147.5, 152.1; HRMS (ESI): *m*/*z*: calcd for $C_{18}H_{22}N_2O_3$ $[M]^+$: 314.1630. Found: 314.1634.

4.1.3. Synthesis of *N*-(2-Methoxybenzyl)-4-methoxy-*N*-(4-nitrobenzyl)-aniline (4c). According to the general procedure, *para*-anisidine was treated with 2-methoxybenzaldehyde and 4-nitro-benzaldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4c** as a yellow oil (275 mg, 650 μmol, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.71$ (s, 3H), 3.81 (s, 3H), 4.56 (s, 2H), 4.64

(s, 2H), 6.61 (d, $J = 8.9$ Hz, 2H), 6.75 (d, $J = 8.9$ Hz, 2H), 6.9 (d, $J = 7.9$ Hz, 2H), 7.14 (d, $J = 7.2$ Hz, 2H), 7.41 (d, $J = 8.9$Hz, 2H), 8.15 (d, $J = 8.9$ Hz, 2H); HRMS (ESI): *m*/*z*: calcd for $C_{22}H_{22}N_3O_6$ $[M+H]^+$: 424.1509. Found: 424.1509.

4.1.4. Synthesis of *N*,*N*-dibenzyl-4-methylaniline (4d). In analogy to the general procedure, 4-methylaniline was directly treated with an excess of benzaldehyde (2.55 mmol) to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4d** (235 mg, 820 μmol, 82%) as a white solid. Mp 63–64 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H), 4.61 (s, 4H), 6.66 (d, $J = 8.7$ Hz, 2H), 6.97 (d, $J = 8.7$ Hz, 2H), 7.23 (m, 6H), 7.31 (m, 4H); ^{13}C NMR: δ = (75 MHz, $CDCl_3$): 20.2, 54.5 (C-2), 112.9 (C-2), 126.1, 126.8 (C-6), 128.6 (C-4), 129.8 (C-2), 139.0 (C-2), 147.2; HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{21}N$ $[M]^+$: 287.1674. Found: 287.1674.

4.1.5. Synthesis of *N*,*N*-diisobutyl-4-methylanilin (4e). In analogy to the general procedure, 4-methylaniline was directly treated with an excess of *iso*-butyraldehyde (2.55 mmol) to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4e** as a yellow solid (140 mg, 640 μmol, 64%). Mp 58–60 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.88$ (d, $J = 6.6$ Hz, 12H), 2.05 (tsept, $J = 6.9$ Hz, $J = 6.6$ Hz, 2H), 2.23 (s, 3H), 3.09 (d, $J = 7.2$ Hz, 4H), 6.58 (d, $J = 8.8$ Hz, 2H), 7.00 (d, $J = 8.3$ Hz, 2H); ^{13}C NMR δ (75 MHz, $CDCl_3$): 20.17, 20.47, 26.43, 60.09, 112.90, 124.37, 129.59, 146.38; HRMS (ESI): *m*/*z*: calcd for $C_{15}H_{26}N$ $[M+H]^+$: 220.2065. Found: 220.2065.

4.1.6. Synthesis of *N*-Benzyl-*N*-isobutyl-4-methyl-aniline (4f). According to the general procedure, 4-methylaniline was treated with benzaldehyde and *iso*-butyraldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 20:1) amine **4f** as a colorless oil (192 mg, 760 μmol, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.96$ (d, $J = 6.6$ Hz, 6H), 2.14 (tsept, $J = 7.1$, 6.6 Hz, 1H), 2.23 (s, 3H), 3.20 (d, J=7.1 Hz, 2H), 4.57 (s, 2H), 6.62 (d, $J = 8.6$ Hz, 2H), 6.99 (d, $J = 8.6$ Hz, 2H), 7.18 - 7.32 (m, 19H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 20.1$, 129.6, 139.2, 146.8; HRMS (ESI): *m*/*z*: calcd for $C_{18}H_{24}N$ $[M+H]^+$: 254.1909. Found: 254.1908.

4.1.7. Synthesis of *N*-(2-Methoxybenzyl)-*N*-isobutyl-4-methoxy-aniline (4g). According to the general procedure, *para*-anisidine was treated with 2-methoxybenzaldehyde and *iso*-butyraldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4g** as a slightly yellow oil (260 mg, 870 μmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.96$ (d, $J = 6.6$ Hz, 6H), 2.11 (tsept, $J = 7.1$, 6.6 Hz, 1H), 3.17 (d, $J = 7.1$ Hz, 2H), 3.73 (s, 3H), 3.87 (s, 3H), 4.51 (s, 2H), 6.60 (d, $J = 9.1$ Hz, 2H), 6.77 (d, $J = 9.1$ Hz, 2H), 6.83 (t, $J = 7.3$ Hz, 1H) 6.88 (d, $J = 8.1$ Hz, 1H), 7.02 (d, $J = 7.1$ Hz, 1H), 7.19 (t, $J = 8.3$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 20.6$, 27.6, 50.8, 55.2, 55.8, 60.3, 109.9, 113.5, 114.8, 120.3, 126.7, 127.4, 127.5, 143.7, 150.8, 157.3; HRMS (ESI): *m*/*z*: calcd for $C_{19}H_{26}NO_2$ $[M+H]^+$: 300.1964. Found: 300.1962.

4.1.8. Synthesis of *N*-2-Methoxybenzyl)-*N*-benzyl-4-methoxy-aniline (4h). According to the general procedure, *para*-anisidine was treated with 2-methoxybenzaldehyde and benzaldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4h** as a slightly yellow solid (303 mg, 910 μmol, 91%). Mp 53–55 °C. ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 3.72$ (s, 3H), 3.83 (s, 3H), 4.60 (s, 2H), 4.62 (s, 2H), 6.65 (d, $J = 9.1$ Hz, 2H) 6.76 (d, $J = 9.1$ Hz, 2H), 6.90 (t, $J = 8.1$ Hz, 2H), 7.15–7.37 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 50.3$, 55.1, 55.8, 110.0, 113.6, 114.8, 120.4, 126.7, 127.4, 127.7, 128.5, 139.4, 143.8, 151.4, 157.3; HRMS (ESI): *m*/*z*: calcd for $C_{22}H_{24}NO_2$ $[M+H]^+$: 334.1807. Found: 334.1807.

4.1.9. Synthesis of *N*,*N*-diisobutyl-4-methoxy-aniline (4i). In analogy to the general procedure, *para*-anisidine was directly treated with an excess of *iso*-butyraldehyde (2.55 mmol) to give after purification by flash chromatography (petroleum ether/ethyl acetate, 20:1) amine **4i** as a colorless oil (171 mg, 730 μmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.91$ (d, $J = 6.6$ Hz, 12H), 2.00 (tsept, $J = 7.2$ Hz, $J = 6.8$ Hz, 2H), 3.04 (d, $J = 7.2$ Hz, 4H), 3.76 (s, 3H), 6.67 (m, 2H), 6.82 (m, 2H); ^{13}C NMR δ = (75 MHz, $CDCl_3$): 20.5, 26.5, 55.8, 61.4, 114.7, 115.0, 143.7, 151.1; HRMS (ESI): *m*/*z*: calcd for $C_{15}H_{26}NO$ $[M+H]^+$: 236.2014. Found: 236.2013.

4.1.10. Synthesis of *N*-benzyl-4-methoxy-*N*-(4-nitrobenzyl)aniline (4j). According to the general procedure, *para*-anisidine was treated with 4-nitro-benzaldehyde and benzaldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4j** as a red solid (263 mg, 870 μmol, 87%). Mp 93–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 3.74$ (s, 3H), 4.56 (s, 2H), 4.60 (s, 2H), 6.71 (d, $J = 9.1$ Hz, 2H), 6.79 (d, $J = 9.1$ Hz, 2H), 7.23–7.37 (m, 5H), 7.42 (d, $J = 9.1$ Hz, 2H), 8.16 (d, $J = 8.7$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 54.9$, 55.7, 56.1, 114.9, 115.4, 123.8, 127.2, 127.7, 128.6, 138.3, 143.2, 147.1, 152.6; HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{22}NO$ $[M+H]^+$: 304.1701. Found: 304.1701.

4.1.11. Synthesis of *N*-isobutyl-4-methoxy-*N*-nonylaniline (4k). According to the general procedure, *para*-anisidine was treated with nonanal and *iso*-butyraldehyde to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4k** as a slightly yellow oil (250 mg, 820 μmol, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.89$ (m, 3H), 0.92 (d, $J = 6.6$ Hz, 6H), 1.28 (br s, 12H), 1.53 (m, 2H), 1.97 (tsept, $J = 7.4$ Hz, $J = 6.6$ Hz, 1H), 2.96 (d, $J = 7.2$ Hz, 2H), 3.21 (t, $J = 7.5$ Hz, 2H), 3.75 (s, 3H), 6.64 (d, $J = 9.2$ Hz, 2H), 6.81 (d, $J = 9.2$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$, 20.5 (C-2), 22.7, 26.8, 27.1; 27.3; 29.3, 29.6, 29.7, 31.9, 53.0, 55.9, 60.2, 114.5 (C-2), 114.8 (C-2), 143.7, 151.0; HRMS (ESI): *m*/*z*: calcd for $C_{20}H_{36}NO$ $[M+H]^+$: 306.2797. Found: 306.2791.

4.1.12. Synthesis of *N*,*N*-dibenzyl-4-methoxy-aniline (4l). In analogy to the general procedure, *para*-anisidine was directly treated with an excess of benzaldehyde (2.55 mmol) to give after purification by flash chromatography (petroleum ether/ethyl acetate, 15:1) amine **4l**

as a colorless solid (273 mg, 900 μmol, 90%). Mp 85–87 °C; ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 3.72$ (s, 3H), 4.55 (s, 4H), 6.69 (d, $J = 9.0$ Hz, 2H), 6.76 (d, $J = 9.2$ Hz, 2H), 7.28 (m, 10H); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 55.2$ (C-2), 55.8, 114.6 (C-2), 114.8 (C-2), 126.9 (C-2), 127.0 (C-4), 128.6 (C-4), 139.1 (C-2), 143.9, 151.9; HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{21}NO$ [M]$^+$: 303.1623. Found: 303.1626.

4.2. Biological activity: cell culture and growth inhibition assay

L-929 mouse cell line was from the German collection of Microorganisms and Cell Cultures (DSMZ) and cultivated in DME medium (GIBCO BRL) plus 10% newborn calf serum at 37 °C and 10% CO_2 in a moist atmosphere. Growth inhibition was measured on microtiterplates. Aliquots of 120 μL of the suspended cells (50,000 mL^{-1}) were given to 60 μL of a serial dilution of the inhibitor. After 5 days, metabolic activity per well was determined using the MTT assay.[13]

4.3. Biological activity: cell staining

PtK$_2$ cells (ATCC CCL-56) grown on glass coverslips were fixed with cold (−20 °C) MeOH/acetone (1:1) for 10 min, incubated with a primary antibody against GRP-94 (1:1000; Affinity Bioreagents) and then with a secondary Alexa Fluor 488 goat anti-rat IgG antibody (1 μg/mL; Molecular Probes), and mounted in ProLong Antifade Gold (Molecular Probes), which included DAPI to stain the nuclei.

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Supplementary data

Experimental details and spectroscopic data for secondary amines (**9**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.048.

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