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Synthesis, spasmolytic activity and structure–activity relationship study of a series of polypharmacological thiobenzanilides

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ABSTRACT

Recently we presented a series of benzanilide derivatives with a selective spasmolytic effect on terminal ileum preparations of the guinea pig. In this report we demonstrate a further development of these compounds. The exchange of the amide oxygen against a sulfur atom resulted in an up to 325 fold increase of the antispasmodic activity of the thiobenzanilide (IC_{50} of 0.1 μM) compared to its benzanilide derivative. Considering their mode of action the compounds interacted with several molecular targets, suggesting that we identified a chemical identity able to modulate multiple targets simultaneously. Furthermore, based on this data set, we present a structure–activity relationship study supporting the important role of the sulfur atom.

1. Introduction

Benzanilide derivatives showed a selective spasmolytic activity, as displayed in the guinea pig ileum test, which correlates with the N–H angle of the amide bond (Brunhofer et al., 2008). This class of compounds was derived from the stilbenoid scaffold which represents a highly active principle. Resveratrol, gigantol, lusianthridin and batatasin III are stilbenoids with remarkable spasmolytic activity (Estrada et al., 1999; Hernandez-Romero et al., 2004; Handler et al., 2007). The basis of gastrointestinal disorders, like the irritable bowel syndrome (IBS) which is characterized by symptoms such as gastrointestinal hypermotility and abdominal pain, is still not understood. Therefore, the principle of polypharmacology or the modulation of several molecular targets could be a beneficial strategy in the treatment of IBS (Hopkins et al., 2006). The compounds presented are an example for so-called "multi-target-directed ligands", a single chemical entity able to modulate several targets at the same time (Bolognesi et al., 2009). Within this study we modified the benzanilide scaffold and exchanged the amide oxygen against a sulfur atom. The so-obtained thiobenzanilide derivatives exhibit a significantly higher spasmolytic activity compared to their corresponding benzanilide derivatives. The most active compound **18** shows a 325 fold increase in spasmolytic activity compared to its oxygen analogue. Investigations of the mechanism of action reveal that the spasmolytic activity is a result of the modulation of different molecular targets. The following study presents a further development of a new class of compounds with spasmolytic activity that demonstrates the feasibility of accessing a chemical space which intersects several molecular targets thus representing possible treatment options for multifactorial diseases such as IBS.

2. Materials and methods

2.1. Chemistry

2.1.1. General methods

All chemicals obtained from commercial suppliers were used as received and were of analytical grade. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPx200 (200 and 50 MHz). Chemical shifts are reported in (units (ppm) relative to Me_4Si line as internal standard and *J* values are reported in Hertz. Mass spectra were obtained by a Hewlett Packard (MS: 5970) spectrometer. Solutions in organic solvents were dried over anhydrous sodium sulphate. The ^1H, ^{13}C NMR and mass spectra of the new synthesized compounds are in agreement with the assigned structures. C,H,N analysis data were within ±0.4% of the theoretical

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values. Column chromatography was performed using silica gel 60, 70-230 mesh ASTM (Merck).

*2.1.2. General procedure for the synthesis of the benzanilide derivatives **1–10***

The synthesis and analytical data of the benzanilide derivatives **1–10** are described in a previous study (Brunhofer et al., 2008).

*2.1.3. General procedure for the synthesis of the thiobenzanilide derivatives **11–24** and **27–30***

To a solution of 5 mmol of the corresponding benzanilide derivative in 10 ml anhydrous THF 4 mmol (1.6 g) Lawesson's reagent were added and heated to reflux till the reaction was completed (monitored by TLC). The organic solvent was removed *in vacuo* and the so-obtained crude product was purified by column chromatography or recrystallization.

*2.1.4. General procedure for the synthesis of the acetothioamide derivatives **25** and **26***

In a dry three-necked flask 10 mmol of the appropriate phenylacetic acid derivative and 1 ml thionyl chloride were heated to reflux for 2 h. After the removal of the thionyl chloride *in vacuo* 8 ml anhydrous 1,4-dioxane, 10 mmol (1.5 ml) 2,4-dimethoxybenzylamine and 10 mmol (1.0 ml) triethylamine were added. The so-obtained acetoamide was recrystallized in ethanol. The synthesis of the corresponding acetothioamide derivative followed the procedure described in Section 2.1.3.

*2.1.5. N-(4-Methylthiophenyl)-3,5-difluorobenzothioamide (**11**) (Erker et al., 2007)*

Yield 0.58 g (39%), mp 142–145 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.92 (s, 1H, NH), 7.71–7.65 (m, 2H, phenyl-H), 7.43–7.20 (m, 4H, phenyl-H), 7.05–6.84 (m, 1H, phenyl-H), 2.50 (s, 3H, SCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 137.9, 135.5, 126.7, 124.0, 110.0 ($J_{C,F}$ = 26.7 Hz), 106.3 (t, $J_{C,F}$ = 26.3 Hz), 15.7. MS: *m/z* 295 (M^+, 46%), 156 (100%).

*2.1.6. N-(2,3-Dimethylphenyl)-4-methoxybenzothioamide (**12**) (Erker et al., 2007)*

Yield 0.92 g (68%), mp 150 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.67 (s, broad, NH), 7.97–7.86 (m, 2H, phenyl-H), 7.27–7.13 (m, 3H, phenyl-H), 6.96–7.84 (m, 2H, phenyl-H), 3.86 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3), 2.19 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 193.0, 138.1, 129.6, 128.7, 126.0, 124.7, 113.6, 55.5, 20.4, 14.2. MS: *m/z* 271 (M^+, 11%), 256 (100%).

*2.1.7. N-(3,4,5-Trimethoxyphenyl)-3,5-difluorobenzothioamide (**13**) (Erker et al., 2007)*

Yield 1.07 g (63%), mp 174–175 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.05 (s, 1H, NH), 7.41–7.25 (m, 2H, phenyl-H), 7.09 (s, 2H, phenyl-H), 7.07–6.85 (m, 1H, phenyl-H), 3.84 (s, 9H, OCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 153.2, 134.4, 109.9 ($J_{C,F}$ = 26.7 Hz), 106.2 (t, $J_{C,F}$ = 26.3 Hz), 100.8, 60.9, 56.1. MS: *m/z* 339 (M^+, 45%), 157 (100%).

*2.1.8. N-(3,5-Difluorophenyl)-4-fluorobenzothioamide (**14**) (Erker et al., 2007)*

Yield 0.84 g (63%), mp 145 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.93 (s, 1H, NH), 7.83–7.76 (m, 2H, phenyl-H), 7.40–7.37 (m, 2H, phenyl-H), 7.10 (t, $J_{H,H}$ = 8.6 Hz, 2H, phenyl-H), 6.97–6.68 (tt, $J_{H,H}$ = 8.6 Hz, $J_{H,F}$ = 2.3 Hz, 1H, phenyl-H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 186.9, 164.7 ($J_{C,F}$ = 253.6 Hz), 163.0 ($J_{C,F}$ = 248.5 Hz), 162.8 ($J_{C,F}$ = 248.5 Hz), 148.8, 140.8, 129.0 ($J_{C,F}$ = 8.8 Hz), 115.8 ($J_{C,F}$ = 21.8 Hz), 106.5 ($J_{C,F}$ = 29.5 Hz), 102.2 (t, $J_{C,F}$ = 25.5 Hz). MS: *m/z* 267 (M^+, 19%), 139 (100%).

*2.1.9. N-(4-Fluorophenyl)-3,5-difluorobenzothioamide (**15**) (Erker et al., 2007)*

Yield 1.15 g (86%), mp 136–139 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.96 (s, 1H, NH), 7.79–7.58 (m, 2H, phenyl-H), 7.48–7.28 (m, 2H, phenyl-H), 7.22–7.05 (m, 2H, phenyl-H), 7.04–6.83 (m, 1H, phenyl-H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 183.5, 162.8 ($J_{C,F}$ = 250.6 Hz), 162.5 ($J_{C,F}$ = 250.9 Hz), 161.0 ($J_{C,F}$ = 248.0 Hz), 134.4 ($J_{C,F}$ = 3.3 Hz), 126.0 ($J_{C,F}$ = 8.4 Hz), 116.0 ($J_{C,F}$ = 23.0 Hz), 110.0 ($J_{C,F}$ = 26.7 Hz), 106.4 (t, $J_{C,F}$ = 25.2 Hz). MS: *m/z* 267 (M^+, 28%), 157 (100%).

*2.1.10. N-(4-Dimethylaminophenyl)-3,5-difluorobenzothioamide (**16**) (Erker et al., 2007)*

Yield 0.85 g (58%), mp 116–117 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.90 (s, 1H, NH), 7.53 (A-part of AB-system, $J_{A,B}$ = 8.9 Hz, 2H, phenyl-H), 7.40–7.25 (m, 2H, phenyl-H), 7.02–6.84 (m, 1H, phenyl-H), 6.71 (B-part of AB-system, $J_{A,B}$ = 8.9 Hz, 2H, phenyl-H), 2.98 (s, 6H, $N(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 162.7 ($J_{C,F}$ = 250.2 Hz), 149.4, 127.6, 124.9, 111.9, 110.3–109.7 (m), 105.9 (t, $J_{C,F}$ = 25.2 Hz), 40.4. MS: *m/z* 292 (M^+, 75%), 259 (100%).

*2.1.11. N-(4-Methylthiophenyl)-4-fluorobenzothioamide (**17**) (Erker et al., 2007)*

Yield 0.78 g (56%), mp 148–152 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.95 (s, broad, 1H, NH), 7.89–7.76 (m, 2H, phenyl-H), 7.75–7.53 (m, 2H, phenyl-H), 7.36–7.19 (m, 2H, phenyl-H), 7.18–6.96 (m, 2H, phenyl-H), 2.49 (s, 3H, SCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 135.9, 129.0–128.8 (m), 126.7, 124.2, 115.5 ($J_{C,F}$ = 22.0 Hz), 15.7. MS: *m/z* 277 (M^+, 25%), 139 (100%).

*2.1.12. N-(2,4,6-Trifluorophenyl)-3,5-dinitrobenzothioamide (**18**) (Erker et al., 2007)*

Yield 1.23 g (70%), mp 223–225 °C. ^{1}H NMR (d_6-DMSO, 200 MHz): δ 12.25 (s, 1H, NH), 9.16–8.96 (m, 3H, phenyl-H), 7.59–7.33 (m, 2H, phenyl-H). ^{13}C NMR (d_6-DMSO, 50 MHz): δ 195.3, 163.7, 159.8 (q, $^1J_{C,F}$ = 29.1 Hz, $^2J_{C,F}$ = 64.0 Hz), 155.1 (q, $^1J_{C,F}$ = 29.1 Hz, $^2J_{C,F}$ = 64.0 Hz), 147.8, 140.8, 127.6, 120.9, 102.2–101.1 (m). MS: *m/z* 357 (M^+, 28%), 69 (100%).

*2.1.13. N-(3,4,5-Trimethoxyphenyl)-3,5-dinitrobenzothioamide (**19**) (Erker et al., 2007)*

Yield 1.69 g (86%), mp 202–210 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 11.58 (s, broad, 1H, NH), 9.05 (s, 3H, phenyl-H), 7.25 (s, 2H, phenyl-H), 3.87 (s, 9H, OCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 190.8, 152.8, 147.6, 145.3, 135.0, 127.5, 119.3, 101.0, 60.7, 56.0. MS: *m/z* 393 (M^+, 25%), 139 (100%).

*2.1.14. N-(2,6-Dimethylphenyl)-3,5-dimethoxybenzothioamide (**20**) (Erker et al., 2007)*

Yield 1.29 g (86%), mp 114 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.65 (s, 1H, NH), 7.22–7.09 (m, 3H, phenyl-H), 7.04 (d, $J_{H,H}$ = 2.3 Hz, 2H, phenyl-H), 6.62–6.53 (t, 1H, phenyl-H), 3.84 (s, 6H, OCH_3), 2.28 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 199.0, 160.7, 143.6, 136.1, 135.5, 128.4, 128.4, 104.9, 103.1, 55.6, 18.0. MS: *m/z* 301 (M^+, 13%), 286 (100%).

*2.1.15. N-(2,6-Dimethylphenyl)-3,4,5-trimethoxybenzothioamide (**21**) (Erker et al., 2007)*

Yield 1.23 g (74%), mp 209 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.64 (s, 1H, NH), 7.30–7.11 (m, 5H, phenyl-H), 3.94 (s, 6H, OCH_3), 3.89 (s, 3H, OCH_3), 2.31 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.7, 153.0, 137.1, 136.3, 135.5, 128.5, 128.4, 104.4, 60.9, 56.3, 18.1. MS: *m/z* 331 (M^+, 19%), 316 (100%).