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DESIGN, SYNTHESIS AND ANTIMYCOTIC ACTIVITY OF (*N*-HETEROARYL)ARYLMETHANAMINES

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The design, synthesis and antimycotic activities of 18 (*N*-heteroaryl)arylmethanamines are reported. The MIC against *Candida* strains of the most active amine, 3-(p-methylbenzylamino)quinoline, is comparable to that of pyrrolnitrin.

INTRODUCTION

We have previously reported the synthesis and the antifungal activity of (1*H*-imidazol-l-ylmethyl-1,2-benzenamine and (1*H*-1,2,4-triazol-1-ylmethyl) benzenamine derivatives[1,2].

Recently derived principal properties (PP) for both substituents[3] and heteroaromatics[4] exhibit the advantages over traditional descriptors of being orthogonal and thus suitable for statistical design and less influenced by measurement errors and system specific variations. PP for heteroaromatics have shown[5,6] their potential as descriptors in the study of the influence of heteroaromatic modifications on biological activities, an effect not yet widely investigated compared with other structural modifications (such as substituent effects) owing to the lack of suitable descriptors. In this paper, we report the design of a series of (*N*-heteroaryl)arylmethanamines with general formula $ArCH_2NH$-Het (Table 1) as potential antimycotic agents. The above series, designed with simultaneous variation of both the *C*-linked aromatic ring (Ar) and the *N*-linked heteroaromatic (Het), could then be studied in relation to the effects of both moieties on biological activity, suggesting strategies for further syntheses. This approach should have the advantage of exploring the maximum range of Ar and Het variability in a stepwise procedure involving first the synthesis of a very limited number of compounds with maximum information and subsequently the synthesis of more active compounds.

RESULTS AND DISCUSSION

Synthesis

The reaction sequence for the synthesis of heteroaromatic secondary amines **1–18** consists in the condensation of aromatic aldehydes with heteroaromatic amines to yield imines which can be easily reduced with $NaBH_4$ to give the corresponding amines.

Imines were synthesized either in ethanol or by azeotropic removal of water in benzene (see Experimental). No significant formation of [bis(heteroarylamino)methyl]arenes[7] was observed in the reactions examined. Consequently, it was not necessary to proceed to reductive amination by the Leuckart–Wallach reaction, in which the formation of polymeric products lowers the yields significantly.[7]

The (*p*-carboxyethyl)benzyl derivative **5**, required by the design (see later), was synthesized by reaction of (*p*-carboxyethyl)benzyl bromide with 2-aminopyrimidine. The main spectroscopic features of the new compounds, characterized by IR, NMR and mass spectrometry, are consistent with those previously

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Table 1. Structures of $ArCH_2NH$-Het (**1–18**)

1	Ph	2-Pyrimidyl
2	4-PhC_6H_4	2-Thiazolyl
3	4-$BuOC_6H_4$	2-Benzothiazolyl
4	4-BrC_6H_4	2-Benzothiazolyl
5	4-$(CO_2Et)C_6H_4$	2-Pyrimidyl
6	Ph	3-Quinolyl
7	2-Thienyl	2-Thiazolyl
8	2-Thienyl	2-Pyrimidyl
9	3-Indolyl	2-Benzothiazolyl
10	2-Furyl	2-Benzothiazolyl
11	2-Quinolyl	3-Quinolyl
12	2-Thienyl	3-Quinolyl
13	3-Indolyl	3-Quinolyl
14	4-MeC_6H_4	3-Quinolyl
15	4-$BuOC_6H_4$	3-Quinolyl
16	4-BrC_6H_4	3-Quinolyl
17	4-$NO_2C_6H_4$	3-Quinolyl
18	4-PhC_6H_4	3-Quinolyl

reported for analogous heterocyclic derivatives[7,8] and with those of *N*-benzylaniline[9–11].

Design

Since biological screening is time consuming and expensive, it is desirable to select by statistical design the compounds to be synthesized for the preliminary screening tests. Recently reported orthogonal PP for substituents[3] and for heteroaromatics[4] provided a suitable non-random criterion for the selection of pairs of aromatic aldehydes and heteroaromatic amines to be reacted for the synthesis of a first set of compounds. With PP now available for 100 substituents[3] and 48 heteroaromatic moieties,[4] in theory 4800 *para*-substituted-(*N*-heteroaryl)benzylamines and 2304 (*N*-heteroaryl)heteroarylmethanamines could be synthesized and tested for antimycotic activity. The criteria adopted in the selection of compounds **1–11** for preliminary screening tests will be briefly outlined below. Each of two sets of *para*-substituted benzaldehydes and heteroaromatic aldehydes has to be reacted with a set of heteroaromatic amines. For the selection of the *para* substituents in the benzaldehyde the first three substituents PP, standardized to have a variation from −1 to +1 similarly to the coded design variables in a factorial design,[12] place each substituent in one of the eight octants of the 'substituents' three-dimensional factorial design cube.[3] Obviously it is impossible to construct a factorial design with substituents in the corners of the cube, as PP exhibit a discrete rather than a continuous variation. Therefore, it would be desirable to select one substituent from each of the eight 'octants' in the design cube, where each 'octant' has one corner in common with the original design cube (in which each variable ranges from −1 to +1) and one corner in the centre (coordinates 0, 0, 0). A fractional factorial design implying only four substituents appropriately located in each of four selected octants was difficult to achieve synthetically. The 'real' design, in fact, has to account also for synthetic difficulties, especially in the preliminary screening, which is expected to explore the maximum three-dimensional PP space with the minimum synthetic effort. In our opinion, a reasonable compromise between statistical design and synthetic availability could be reached in the present case by selecting only five substituents, reported in Table 2, which span five of the eight design cube octants. Most of the corresponding *para*-substituted benzaldehydes, used as starting reagents, are in fact commercially available or easily synthesizable. The *p*-carboxyethyl derivative **5** was synthesized in one step starting from the corresponding benzyl bromide (see also Experimental).

Further efforts at the synthesis of amines with substituents in the missing octants[3] (1 −1 1, −1 1 1 and −1 −1 −1) could be carried out later, when more information is available on the effects of the *N*-linked heteroaromatic moiety. The selection of heteroaromatic amines was restricted to four commercially available compounds whose PP,[4] standardized in order to have a variation from −1 to +1, placed them in four different octants of the 'heteroaromatic' design cube (cf. Table 2).

The combination of five *para*- substituted benzaldehydes with four heteroaromatic amines implies the synthesis of 20 compounds. The number was further restricted with the selection of compounds **1–6** combining the reagents according to the following considerations: (a) ease of synthetic conditions based on previous experience;[7,8] (b) check of the D-efficiency of the design (i.e. the relative number of experiments required to obtain an equally good design with fully orthogonal variables) according to the D-optimal design;[13] and (c) inclusion of two reactions with benzaldehyde, as aromatic descriptors are also available for the phenyl moiety[4] and consequently the relative compounds could also be used in the 'heteroaromatic' set (see below).

Analogous considerations led to the selection of seven compounds (**1**, **6–11**) as the 'heteroaromatic' set spanning five octants of the 'heteroaromatic' design cube for aldehydes (ArCHO) and the former four octants for the amines (Het-NH_2). The corresponding standardized PP_S (t_1, t_2, t_3) are reported in table 2, together with the relevant octants.

Biological assays and selection of amines 12–18

The antimycotic activities against three strains of *Candida albicans* and three strains of other *Candida specie* for compounds **1–11** (the preliminary substituent and heteroaromatic sets) expressed as MIC (minimum inhibition concentrations) at pH 7·2 are recorded in Table 3. The data show clearly that, despite the large descriptor space explored both for the *C*-linked and the

Table 2. Standardized principal properties (PP for X substituents [a] and t for heteroaromatics [b] in the design of $ArCH_2NH$-Het (Ar = *p*-X-C_6H_4 or heteroaromatic; Het = heteroaryl)

X	PP_1	PP_2	PP_3	Octant
H	−1·000	0·612	−0·480	−1, 1, −1
Ph	0·364	0·008	0·170	1, 1, 1
OBu	0·434	0·338	−0·346	1, 1, −1
Br	−0·479	−0·195	0·057	−1, −1, 1
CO_2Et	0·095	−0·534	−0·296	1, −1, −1
NO_2	−0·678	−0·746	−0·177	−1, −1, −1
Me	−0·636	0·519	0·03	−1, 1, 1
Het	t_1	t_2	t_3	
2-Pyrimidyl	0·55	−0·64	0·75	1, −1, 1
2-Thiazolyl	−0·06	−0·57	0·61	−1, −1, 1
2-Benzothiazolyl	−0·37	0·89	0·67	−1, 1, 1
3-Quinolyl	0·41	0·93	−0·36	1, 1, −1
Ar	t_1	t_2	t_3	
Phenyl	0·55	−0·53	−0·53	1, −1, -1
2-Thienyl	−0·31	−0·47	−0·24	−1, −1, −1
3-Indolyl	0·14	0·74	−0·28	1, 1, -1
2-Furyl	−0·90	−0·92	−0·31	−1, -1, 1
2-Quinolyl	0·41	0·93	0·53	1, 1, 1

[a] From Ref. 3
[b] Standardized in the interval—1, 1 from original values in Ref. 4.

Table 3. Antimycotic activity (MIC, μg ml^{-1}) of amines **1–18** against three strains of *Candida albicans*[a] and three other strains of *Candida species*[b] at pH 7·2

Compound	I	II	III	IV	V	VI	M·p(°C)	Ref.
1	>800	>800	>800	>800	>800	>800	79–81	14
2	>800	>800	>800	>800	>800	>800	157–159	8
3	>800	>800	>800	>800	>800	>800	180–181	6
4	>800	>800	>800	>800	>800	>800	197–200	[c]
5	>800	>800	>800	>800	>800	>800	88–90	[c]
6	50	50	50	75	62·5	50	96–98	8,15
7	>800	>800	>800	>800	>800	>800	110	7
8	>800	>800	>800	>800	>800	>800	95	7
9	>800	>800	>800	>800	>800	>800	184–185	7,16
10	>800	>800	>800	>800	>800	>800	119–121	7
11	75	75	50	400	25	200	190–192	7
12	62·5	100	100	62·5	100	37·5	91–93	[c]
13	150	125	250	>800	150	200	125–127	[c]
14	37·5	25	50	50	18·75	50	131–133	8
15	>800	>800	>800	>800	>800	>800	91–92	[c]
16	>800	>800	>800	>800	>800	>800	148–149	[c]
17	>800	500	500	>800	500	200	115–116	[c]
18	>800	>800	>800	>800	>800	>800	193–194	8
Pyrrolnitrin	6·25	25	25	3·12	25	1·56	128–129	—

[a] **I** = *C. albicans* d'Alessandro; **II** = *C. albicans* Porcari; **III** = *C. albicans* BP.
[b] **IV** = *C. tropicalis* SA 204; **V** = *C. glabrata* SA 199; **VI** = *C. guillermondii* SA258.
[c] This work.

N-linked moieties, only derivatives with a 3-quinolyl moiety linked to the amine nitrogen exhibit antimycotic activity. The significance of this result, which could at first appear trivial, lies in the fact that it has been achieved with very little synthetic effort spanning a large number of possible structures. The obvious consequence of the above finding is that further synthesis should be restricted to the reaction of aromatic aldehydes with 3-quinolineamine. 2-Thiophene and 3-indole aldehydes afforded compounds **12** and **13** respectively, while 2-furaldehyde gave polymeric products.

The *para*-substituted benzene series was extended with the synthesis of compounds **14–18**, including substituents placed in seven of the eight octants of the substituent design cube (see Table 2). The MIC of the *p*-methyl derivative **14** is comparable to that of pyrrolnitrin for two strains of *Candida albicans* and even better for *Candida glabrata* SA199.

CONCLUSIONS

Although no quantitative effect of heteroaromatic moieties on antimycotic activity was found in the present case, this work indicates the possibility of exploring wide variations in substituents and heteroaromatics with few experiments planned by means of PP_S and statistical design. The information derived from preliminary screening of the above set provided guidelines for the synthesis of more active compounds.

EXPERIMENTAL

Melting points are uncorrected. Pyrrolnitrin was obtained from Schiapparelli. IR spectra (KBr discs) were recorded on a Perkin-Elmer Model 684 spectrophotometer. NMR spectra were recorded at 200 MHz on a Bruker spectrometer (TMS as internal standard) using $CDCl_3$ as solvent, unless stated otherwise. Mass spectra were recorded by direct insertion into a VG-ZAB 2SE double-focusing mass spectrometer under the following conditions: ionization energy, 70 eV; source temperature, 200 °C; trap current, 100 μA; acceleration voltage, 8 kV; sample temperature, 30 °C; and resolution 1500.

Synthesis of imines. Method A: a solution of the aldehyde and of the amine (equimolar amounts, 10 mmol), was refluxed in 10 ml of dry ethanol. The time required varied from a few hours to 19 h, depending on the basicity of the amine. The solvent was evaporated under reduced pressure and the composition of the residue was examined by TLC. The crude imines thus obtained were crystalline and in some cases, owing to their low stabilities, were not purified before reduction. Mixtures of chloroform and light petroleum (b.p. 40–70 °C) were used as the crystallization solvent.

Method B: 50 ml of dry benzene and two drops of glacial acetic acid were added to a solution of 0·007 mol of the appropriate benzaldehyde and 0·0066 mol of heteroarylamine in 100 ml of dry ethanol and the mixture was refluxed for 24 h. Water formed during the reaction was eliminated with a Dean–Stark apparatus. Evaporation of the solvent yielded a residue which was crystallized from dry ethanol.

Reduction of imines.. To a warm solution or suspension of the imine (10 mmol) in methanol (10 ml), sodium borohydride (20 mmol) was added in small portions (20 min) and the mixture was heated on a steam-bath for 2 h with stirring. The reaction mixture was then cooled and treated with water. The product was washed with water and crystallized from ethanol.

2-(4-Bromobenzylideneamino)benzothiazole: yield 30%. Method B. Time 18 h. Yellow microcrystals, m.p. 175–177 °C.

3-(2-Thienylideneamino)quinoline: yield 62%. Method A. Time 8 h. Yellow needles, m.p. 82–84 °C.

3-(3-Indolideneamino)quinoline: yield 56%. Method A. Time 19 h. Yellow microcrystals, m.p. 223–225 °C.

3-(4-Butoxybenzylideneamino)quinoline: yield 77%. Method B. Time 18 h. White prisms, m.p. 56–59 °C.

3-(4-Bromobenzylideneamino) quinoline: yield 59%. Method A. Time 14 h. White needles, m.p. 135–136 °C.

3-(4-Nitrobenzylideneamino)quinoline: yield 71%. Method B. Time 18 h. Yellow prisms, m.p. 160–163 °C.

2-[(4-Bromobenzylamino)]benzothiazole (***4***): yield 50%. White needles, m.p. 197–200 °C. MS, *m*/*z* (%): 318 (M^+, 87), 195 (21), 193 (23), 171 (100), 136 (15). IR (KBr): 3200 cm^{-1} (NH). ^1H NMR, δ: 4·6 (CH_2, 2H, d, $J = 1·5$ Hz), 8·5(N*H*, 1H, b), 6·1–8·1 (8*H*, m).

2-[(4-Carboethoxybenzylamino)]pyrimidine (***5***): 0·01 mol of *p*-carboethoxybenzyl bromide,[17,18] 0·01 mol of 2-aminopyrimidine and 0·01 mol of Na_2CO_3 in 20 ml of chloroform were refluxed for 24 h. The precipitate was filtered, washed with water, extracted with chloroform and purified twice on an alumina column using chloroform as eluent. Yield 46%. White microcrystals, m.p. 88–90 °C. MS, *m*/*z* (%): 257 (M^+, 100), 256 (19), 258 (16), 228 (16), 212 (14), 184 (29), 178 (24). Ir (KBr): 3240 cm^{-1} (NH). ^1H NMR, δ: 4·6 (CH_2, 2H, d), 5·9 (N*H*, 1H, b), 7·2–8·3 (7*H*, m), 4·3 (2*H*, m), 2·3 (3*H*, t).

3-[(Thiophen-2-ylmethyl)amino]quinoline (***12***): yield 80%. Yellow microcrystals, m.p. 91–93 °C. MS, *m*/*z* (%): 240 (M^+, 49), 97 (100). IR (KBr): 3198 cm^{-1} (NH). ^1H Nmr δ: 4·59 (CH_2, 2H, dd,

$J = 5{\cdot}66$, 1·86 Hz), 3·4 (N***H***, 1H, s). ^{13}C NMR, δ: 43 (***C***H_2).

3-[(Indol-3-ylmethyl)amino]quinoline (*13*): yield 87%. Yellow microcrystals, m.p. 125–127 °C. MS, *m*/*z* (%): 273 (M^+, 15), 144 (100), 130 (91), 129 (32), 128 (9). IR (KBr): 3256 cm^{-1} (NH). ^{1}H NMR, δ: 4·6(C***H***$_2$, 2H, d, $J = 5{\cdot}01$ Hz), 5·80 (N***H***, 1H, b). ^{13}C NMR: 38·38 (***C***H_2).

3-(4-Butoxybenzylamino) quinoline (*15*): yield 61%. White plates, m.p. 91–92 °C. MS, *m*/*z* (%): 306 (M^+, 20), 247 (9), 163 (74), 143 (10), 128 (13), 115 (10), 107 (100). IR (KBr): 3240 cm^{-1} (NH). ^{1}H NMR, δ: 0·98 (C***H***$_3$, 3H, t), 1·44–1·81 (C***H***$_2$, 4H, m), 3·96 (C***H***$_2$, 2H, t), 4·34 (C***H***$_2$N***H***; 3H, b), 6·87–8·47 (Ar, 10 H, m).

3-(4-Bromobenzylamino) quinoline (*16*): yield 91%. White microcrystals, m.p. 115–116 °C. MS, *m*/*z* (%): 314 (88), 312 (M^+, 92·5), 171 (98), 169 (100), 128 (25). Ir (KBr): 3268 cm^{-1} (NH). ^{1}H NMR, δ: 4·39 (C***H***$_2$, 2H, d, $J = 5{\cdot}18$ Hz), 4·43 (N***H***, b). ^{13}C NMR: 47·25 (***C***H_2).

3-(Nitrobenzylamino)quinoline (*17*): yield 42%. Yellow microcrystals, m.p. 148–150 °C. MS, *m*/*z*(%) 279 (M^+, 83), 157 (19), 143 (73), 128 (18), 116 (100), 106 (50), 90 (35), 89 (75), 78 (20). IR (KBr): 3220 cm^{-1} (NH). ^{1}H-NMR (CD_3COCD_3), δ: 4·67 (C***H***$_2$, 2H, d, $J = 3$ Hz), 6·34 (N***H***, b), 6·93–8·70 (Ar, 10 H, m).

Microbiological assays. Compounds **1–18** were tested for their *in vitro* antifungal activity against three strains of *Candida albicans* (*C. albicans* d'Alessandro, *C. albicans* Porcari, *C. albicans* BP) and three other strains of *Candida specie* (*C. tropicalis*, *C. glabrata* SA 199, *C. guillermondii* SA 258). Pyrrolnitrin was used as a positive control. The MIC ($\mu g\ ml^{-1}$) was determined using the method of progressive double dilutions in solid media.[19] Data were recorded after 48 h of incubation at 37 °C. The substances were dissolved in dimethyl sulphoxide (5 mg ml^{-1}); further diluition in the test medium furnished the required concentration, generally in the range 0·1–800 $\mu g\ ml^{-1}$. The cultures were obtained on Sabouraud (BBL) after 18 h of incubation at 37 °C. Tests were carried out using Sabouraud agar (BBL). Inocula were 10^3 cells ml^{-1}.

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REFERENCES

1. M. Scalzo, M. Biava, F. Cerreto, G. C. Porretta, R. Mercantini and C. Fanelli, *Eur. J. Med. Chem.* **24**, 537–540 (1989).
2. S. Panico, A. Villa, N. Simonetti, G. C. Porretta and M. Scalzo, *Drugs Exp. Clin. Res.* **16**, 181–186 (1990).
3. B. Skagerberg, D. Bonelli, S. Clementi, G. Cruciani and C. Ebert, *Quant. Struct.–Act. Relat.* **8**, 32–38 (1989).
4. L. Caruso, G. Musumarra and A. R. Katritzky, *Quant. Struct.–Act. Relat.* **12**, 146–151 (1993).
5. G. Musumarra and M. Stella, *Quant. Struct-Act. Relat.* **12**, 256–260 (1993).
6. M. Biava, R. Fioravanti, G. C. Porretta, L. Caruso, G. Musumarra, N. Simonetti and A Villa, in *Trends in QSAR and Molecular Modelling 92*, edited by C. G. Wermuth, pp. 319–320. ESCOM, Leiden (1993).
7. G. Musumarra and C. Sergi, *Heterocycles* **37**, 1033–1039 (1994).
8. R. Fioravanti, M. Biava, G. C. Porretta, S. Foti, G. Musumarra and R. Saletti, *Heterocycles* **37**, 367–377 (1994).
9. B. Castro and C. Selve, *Bull. Soc. Chim. Fr.*, 4368–4373 (1971).
10. C. Camacho, M. A. Paz-Sandoval and R. Contreras, *Polyhedron* **5**, 1723–1732 (1986).
11. M. A. Paz-Sandoval, C. Camacho and R. Contreras, *Spectrochim. Acta* **43**, 1331–1335 (1987).
12. G. E. P. Box, W. G. Hunter and J. S. Hunter, *Statistics for Experimenters*. Wiley, New York, (1978).
13. S. Clementi, G. Cruciani, M. Baroni and G. Costantino, in: *3D QSAR in Drug Design Theory, Methods and Applications*, edited by H. Kubinyi, pp. 567–582. ESCOM, Leiden (1993).
14. *Fr. Pat.* 1 547 635; *Chem. Abstr.* **71**, 81396z (1969).
15. J. Renault, J. Berlot and J. C. Carton, *Bull. Soc. Chim. Fr.*, 2797–2801 (1969).
16. G. N. Walker and M. A. Moore, *J. Org. Chem.* **26**, 432–439 (1961).
17. J. A. Zderich, M. M. J. Kubitschek and W. A. Bonner, *J. Org. Chem.* **26**, 1635–1637 (1961).
18. D. D. Tanner, J. A. Plambeck, D. W. Reed and T. W. Mojelsky, *J. Org Chem.* **45**, 5177–5183 (1980).
19. S. Shadomy and A. Espinel, in *Manual of Clinical Microbiology*, 3rd ed., pp. 647–651. American Society for Microbiology, Washington, DC (1980).