Synthesis and Evaluation of Novel *N*-Substituted-6-methoxynaphthalene-2-Carboxamides as Potential Chemosensitizing Agents for Cancer

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Received November 27, 2007; accepted April 15, 2008; published online April 28, 2008

A novel class of molecules with structure *N*-[3-(heteroaryl)propyl]-6-methoxynaphthalene-2-carboxamides 8—13 were synthesized by condensing 6-methoxy-2-naphthoyl chloride 1 with 3-(heteroaryl)propyl amines 2—7. Compounds 8—12 were evaluated *in vitro*, in P388 murine lymphocytic leukemia cell line (P388) using SRB assay for cytotoxicity and in adriamycin resistant P388 murine lymphocytic leukemia cell line (P388/ADR) using MTT assay for resistant reversal activity. Compounds 8—12 were non-toxic at lower dose of 20 μg/ml, and effectively reversed adriamycin resistance. However, at higher doses (40, 80 μg/ml) they showed significant cytotxicity and hence reversal potency was not determined at these concentrations.

Key words 6-methoxynaphthalene-2-carboxamide; multidrug resistance reversal agent; chemosensitizer

Effective cancer chemotherapy can be severely impaired by drug resistance, wherein P-glycoprotein (Pgp), a membrane protein is invovlved in effluxing cytotoxic agents from cell.[1)] The agents used to combat drug resistance are called chemosensitizers or resistant reversal agents. Reversal agents like verapamil, cyclosporine, propafenone and quinidine were non-specific in their action and produced toxicity when used *in vivo*.[2)] Non-toxic chemosensitizing agents were then developed and among them Elacridar (GF-120918) showed good promise.[3)]

In our laboratory a pharmacophore for chemosensitizing activity was developed using Elacridar as a query molecule and based on this pharmacophore, a series of substituted 6-methoxynaphthalene-2-carboxamides were synthesized and they exhibited good Chemosensitizing activity.[4)] These agents contained substituted piperazinopropyl as substituents on amide nitrogen.

Our continued interest in this area led to the development of another novel series of chemosensitizing agents. In the present work we replaced the piperazine ring by other heterocyclic systems in order to study their potential for contributing to chemosensitizing activity on evaluation. The heterocyclic systems chosen were based on their lipophilicity and their ability to act as an hydrogen bond acceptor/donor group. It is proposed to synthesise these novel molecules and evaluate them for cytotoxicity and for chemosensitizing activity in resistant cancer cell lines.

Experimental

Chemistry All melting points were recorded on Thermonik melting point apparatus and are uncorrected; fourier-transform infrared (FT-IR) spectra (KBr discs) were recorded in Jasco FT-IR 5300 instrument. Proton-NMR spectra were recorded on Varian VX-300 NMR spectrophotometer (300 MHz) using $CDCl_3$ and DMSO-d_6 as solvents. The chemical shift values are reported in δ units (ppm) relative to internal standard tetramethylsilane (TMS). Column Chromatography was performed using Silica gel, 60—120 mesh, from Qualigens fine chemicals, India. Elemental analysis values for final compounds were within $\pm$0.4% of theoretical value.

General Method for Preparation of *N*-[3-(Heteroaryl)propyl]-6-methoxynaphthalene-2-carboxamides (8—13) 6-Methoxy-2-naphthoyl chloride **1** (10 mmol) was condensed with different heteroaryl propylamines **2**—**7** (12 mmol) in turn at room temperature in chloroform for 3—4 h to get compounds **8**—**13**. Chloroform was removed under vacuum and the residue obtained was washed with water and then purified by column chromatography using, chloroform : ethyl acetate, 80 : 20 as eluent and then recrystallized from alcohol.

N-[3-(Pyrrolidin-2-one-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (**8**): Yield 74.4%. mp 113—115 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.3—1.6 (2H, m, $-CH_2$), 1.8—2.1 (2H, m, $-CH_2$), 2.3 (2H, t, $-CH_2$, J=7.45 Hz), 2.6—2.8 (4H, m, 2 $-CH_2$), 3.3 (2H, t, $-CH_2$, J=7.95 Hz) 3.6 (3H, s, $-OCH_3$), 6.2 (1H, s, –NH), 7.1—7.2 (2H, m, Ar-H), 7.6 (1H, s, Ar-H), 7.72—7.86 (2H, m, Ar-H), 7.88 (1H, s, Ar-H). IR (KBr) cm^{-1}: 3431, 1641. *Anal.* Calcd for $C_{19}H_{22}N_2O_3$: C, 69.85; H, 6.74; N, 8.57. Found: C, 70.08; H, 6.75; N, 8.75.

N-[3-(Benzimidazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (**9**): Yield 72.5%. mp 95—96 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.5—1.7 (2H, m, $-CH_2$), 2.8—2.9 (4H, m, 2-CH_2), 3.98 (3H, s, $-OCH_3$), 6.2 (1H, s, –NH), 7.12—7.22 (2H, m, Ar-H), 7.30—7.38 (2H, m, Ar-H), 7.6 (1H, s, Ar-H), 7.74—7.88 (2H, m, Ar-H), 7.98 (1H, s, Ar-H), 8.26 (2H, d, Ar-H, J=8.19 Hz), 8.52 (1H, s, Ar-H), IR (KBr) cm^{-1}: 3435, 1705. *Anal.* Calcd for $C_{22}H_{21}N_3O_2$: C, 73.45; H, 5.84; N, 11.68. Found: C, 73.55; H, 5.86; N, 11.74.

N-[3-(2-Methylbenzimidazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (**10**): Yield 70.6%. mp 72—74 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.5—1.7 (2H, m, $-CH_2$), 2.24 (3H, s, $-CH_3$), 2.8—2.9 (4H, m, 2-CH_2), 3.98 (3H, s, $-OCH_3$), 6.2 (1H, s, –NH), 7.12—7.20 (2H, m, Ar-H), 7.30—7.50 (2H, m, Ar-H), 7.6 (1H, s, Ar-H), 7.74—7.88 (2H, m, Ar-H), 8.0 (1H, s, Ar-H), 8.2 (2H, d, Ar-H, J=8.08 Hz), IR (KBr) cm^{-1}: 3447, 1684. *Anal.* Calcd for $C_{23}H_{23}N_3O_2$: C, 73.90; H, 6.15; N, 11.24. Found: C, 74.01; H, 6.16; N, 11.34.

N-[3-(Benzotriazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (**11**): Yield 74.5%. mp 188—190 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.5—1.6 (2H, m, $-CH_2$), 2.8—2.9 (4H, m, 2-CH_2), 3.98 (3H, s, $-OCH_3$), 6.2 (1H, s, –NH), 7.18—7.26 (2H, m, Ar-H), 7.42—7.48 (2H, dd, Ar-H, J=6.86 Hz), 7.6 (1H, s, Ar-H), 7.84 (2H, d, Ar-H, J=8.55 Hz), 8.20 (2H, d, Ar-H, J=8.27 Hz), 8.32 (1H, s, Ar-H), IR (KBr) cm^{-1}: 3315, 1620. *Anal.* Calcd for $C_{21}H_{20}N_4O_2$: C, 69.92; H, 5.54; N, 15.53. Found: C, 70.02; H, 5.55; N, 15.62.

N-[3-(1,2,3,4-Tetrahydroisoquinolin-2-yl)propyl]-6-methoxynaphthalene-2-carboxamide (**12**): Yield 75%. mp 148—150 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.4—1.6 (2H, m, $-CH_2$), 2.2—2.4 (4H, m, 2-CH_2), 2.8 (4H, t, 2-CH_2, J=6.30 Hz), 3.4 (2H, d, $-CH_2$) 3.98 (3H, s, $-OCH_3$), 6.2 (1H, s, –NH), 7.14—7.24 (6H, m, Ar-H), 7.6 (1H, s, Ar-H), 7.74—7.82 (2H, dd, Ar-H, J=8.53 Hz), 8.0 (1H, s, Ar-H). IR (KBr) cm^{-1}: 3433, 1618. *Anal.* Calcd for $C_{24}H_{26}N_2O_2$: C, 76.90; H, 6.94; N, 7.47. Found: C, 76.92; H, 6.95; N, 7.54.

N-[3-(4-Amino-3-methylthio-5-phenyl-1,2,4-triazol-4-yl)-propyl]-6-methoxynaphthalene-2-carboxamide (**13**): Yield 76%. mp 194—195 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.4—1.5 (2H, m, $-CH_2$), 2.6 (3H, s,

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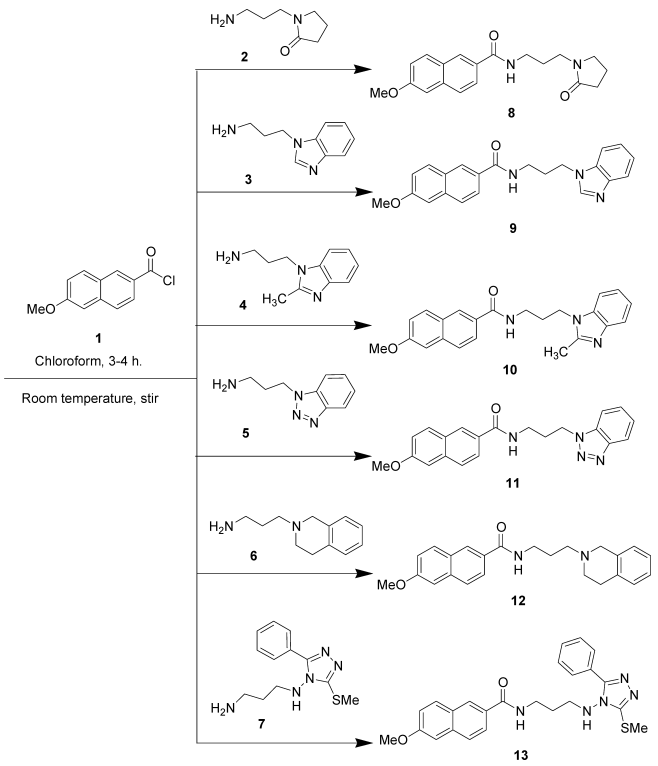

Chart 1. Synthesis of Test Compounds **8**—**13**

–SCH_3), 2.8—3.0 (4H, m, 2-CH_2), 3.9 (3H, s, –OCH_3), 6.2 (1H, s, –NH), 7.28—7.34 (2H, m, Ar-H), 7.46—7.54 (3H, m, Ar-H), 7.76 (1H, s, Ar-H), 8.00—8.10 (2H, m, Ar-H), 8.1—8.2 (2H, m, Ar-H), 8.28 (1H, s, Ar-H), 8.84 (1H, s, –NH). IR (KBr) cm^{-1}: 3412, 1776. *Anal.* Calcd for $C_{24}H_{25}N_5O_2S$: C, 64.34; H, 5.58; N, 15.64. Found: C, 64.44; H, 5.59; N, 15.73.

Pharmacology Test compounds **8**—**12** were evaluated *in vitro*, for cytotoxicity in P388 murine lymphocytic leukemia cell line (P388) using SRB assay[4,5] at 10, 20, 40 and 80 μg/ml concentrations (Table 1) and for resistance reversal activity in adriamycin resistant P388 murine lymphocytic leukemia cell line (P388/ADR) using MTT assay[4,6] at 20 μg/ml concentrations while concentration of adriamycin used was 40 μg/ml (Table 2). Verapamil (VRP) was used as a standard chemo-modulator and was evaluated at the same concentration as that of test compounds. Test compound **13** showed poor solubility and hence was not evaluated. All experiments were carried out in triplicates. At 40 and 80 μg/ml concentrations compounds showed significant cytotxicity and hence reversal potency was not determined at these concentrations (Table 3).

Results and Discussion

A novel series of 6-methoxynaphthalene-2-carboxamides **8**—**13** with various heteroarylpropyl substituents on the amide nitrogen was synthesised by condensing 6-methoxy-2-naphthoyl chloride **1** with heteroaryl propylamines **2**—**7** in chloroform. Small amount of DMF was added to facilitate the reaction. The 6-methoxy-2-naphthoyl chloride **1** was prepared from the corresponding acid[4] by reacting with oxalyl chloride in dichloromethane.

The heteroaryl propylamines **2**—**7** were synthesized by condensing *N*-(3-bromopropyl)phthalimide with heterocycles like, pyrrolidine-2-one, benzimidazole, 2-methyl benzimidazole, benzotriazole, 1,2,3,4-tetrahydroisoquinoline and 4-amino-3-methylthio-5-phenyl-1,2,4-triazole in DMF in presence of base for 4—6 h at 110—120 °C and cleaved by reaction with hydrazine hydrate and acidified with HCl to obtain heterocyclic propylamines. The amines were isolated by extraction in chloroform : ethyl acetate mixture (1 : 2).

Table 1. *In Vitro* Cytotoxic Activity in P388 Murine Lymphocytic Leukemia Cell Line for Test Compounds **8**—**12**

Compound	% inhibition of cell growth			
	10 μg/ml	20 μg/ml	40 μg/ml	80 μg/ml
8	1.3	15.4	31.4	44.4
9	1.8	8.1	12.7	21.9
10	1.9	2.4	7.4	27.5
11	0.0	0.0	5.7	19.0
12	0.0	2.3	11.3	20.5
VRP	1.8	7.1	10.2	15.4
ADR	63.0	65.2	71.5	89.0

Table 2. *In Vitro* MDR Reversal Activity in P388/ADR Cell Line for Test Compounds **8**—**12** at 20 μg/ml

Test system	Concentration in μg/ml (μmol)	% inhibition of cell growth	Enhancement in ADR activity (%)	RP[a] at 20 μg/ml (RP[a] at 0.040 μmol)
8	20 (0.061)	2.8		
8+ADR	20+40	20.8	309.09	3.09 (2.02)
9	20 (0.055)	6.1		
9+ADR	20+40	20.3	261.36	2.61 (1.89)
10	20 (0.053)	6.4		
10+ADR	20+40	15.9	115.90	1.15 (0.86)
11	20 (0.055)	5.3		
11+ADR	20+40	18.2	193.18	1.93 (1.40)
12	20 (0.053)	4.7		
12+ADR	20+40	12.2	70.45	0.70 (0.52)
VRP	20 (0.040)	0.0		
VRP+ADR	20+40	40.5	820.4	8.20
ADR	40 (0.068)	4.4		

a) Reversal potency.

Table 3. % Inhibibition of Cell Growth in P388/ADR Cell Line (*in Vitro*) for Test Compounds **8**—**12** at 40 and 80 μg/ml Concentrations

Test system	Concentration in μg/ml (μmol)	% inhibition of cell growth	Concentration in μg/ml (μmol)	% inhibition of cell growth
8	80 (0.245)	22.8	40 (0.122)	11.5
8+ADR	80+80	58.3	40+60	35.3
9	80 (0.222)	24.1	40 (0.111)	13.4
9+ADR	80+80	47.8	40+60	30.4
10	80 (0.214)	23.8	40 (0.107)	10.1
10+ADR	80+80	57.4	40+60	28.1
11	80 (0.220)	29.9	40 (0.110)	14.1
11+ADR	80+80	64.4	40+60	27.1
12	80 (0.212)	31.6	40 (0.106)	15.7
12+ADR	80+80	45.8	40+60	28.9
VRP	80 (0.162)	9.3	40 (0.081)	2.7
VRP+ADR	80+80	54.0	40+60	45.3
ADR	80 (0.137)	31.1	60 (0.103)	13.53

The 4-amino-3-methylthio-5-phenyl-1,2,4-triazole was prepared from benzamide, by reacting it with hydrazine hydrate at 80—90 °C, and the benzohydrazide formed was converted to potassium-3-benzoyl dithiocarbazate salt by stirring with carbon disulfide and potassium hydroxide in absolute alcohol at room temperature. The salt was then cyclised with hydrazine hydrate and then methylated with methyl iodide in 1 N KOH at 0 °C to obtain 4-amino-3-methylthio-5-phenyl-

1,2,4-triazole. Yield 86%. mp 158—159 °C.

Test compounds **8**—**12** were evaluated for cytotoxicity *in vitro* in P388 cell line using SRB assay[4,5] and were found to be nontoxic at lower doses 10 and 20 μg/ml, were slightly toxic at 40 μg/ml and more toxic at 80 μg/ml (Table 1).

Test compounds **8**—**12** were then evaluated *in vitro* for chemosensitizing activity in P388/ADR cell line by MTT assay[4,6] using verapamil as standard and the results are given in Tables 2 and 3.

The percentage enhancement in adriamycin activity was calculated by the following equation:

% enhancement in ADR activity = 100 [% inhibition of (test + ADR) − (% inhibition of ADR + % inhibition of test)]/% inhibition of ADR

The reversal potency was expressed by the ratio of % inhibition of (test + ADR) − (% inhibition of ADR + % inhibition of test) and % inhibition of ADR.

Test compounds **8**—**12** effectively reversed adriamycin resistance at the dose 20 μg/ml (Table 2). The % enhancement in ADR activity at this concentration was in the range 70—309 and the reversal potency was in the range of 0.70—3.09, while verapamil exhibited 820% enhancement in activity with reversal potency of 8.2 at this concentration. As the test compounds **8**—**12** and varapamil have different molecular weights, the reversal potency values were calculated at the same molar concentrations as that of the standard (VRP) and these values are given in parentheses. At higher doses (40, 80 μg/ml) compounds showed significant cytotxicity and hence reversal potency was not determined at these concentrations (Table 3). Finally it is observed that replacing substituted piperazines with other heterocyclic systems has led to compounds which enhanced adriamycin activity (% enhancement 70—309 at 20 μg/ml) and has reversal potency in the range 0.52—2.02 at 0.04 μmol concentration. Thus the present work has led to generation of newer structural leads, which can be further modified to reduce their inherent cytotoxicity and to enhance chemosensitizing activity.

Acknowledgements Authors are thankful to All India Council for Technical Education (AICTE), New Delhi; Indian Pharmaceutical Association-Shri. Ramanbhai B. Patel foundation (IRF), Mumbai and Sir. Ratan Tata Trust, Mumbai for financial support.

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