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

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Effective cancer chemotherapy can be severely impaired by drug resistance, wherein P-glycoprotein (Pgp), a membrane protein is involved in effluxing cytotoxic agents from cell.1) The agents used to combat drug resistance are called chemosensitizers or resistant reversal agents. Reversal agents contained substituted piperazinopropyl as substituents for their ability to act as an hydrogen bond acceptor/donor and 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 pharmaphore for chemo sensitizing activity was developed using Elacridar as a query molecule and based on this pharmaphore, 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 chemo sensitizing 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 chemo sensitizing activity in resistant cancer cell lines.

Experimental

Chemistry All melting points were recorded on Thermomik 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 CDCl3, and DMSO-d6 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 ±0.4% of theoretical value.

General Method for Preparation of N-[3-(Heteroaryl)propyl]-6-methoxynaphthalene-2-carboxamides (8—13): 6-Methoxy-2-naphthyl chloride 1 (10 mmol) was condensed with different heteroaryl propionylamines 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 (1:3) and then recrystallized from alcohol.

N-[3-(Pyrolidin-2-one-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (8): Yield 74.4%. mp 113—115 °C. 1H-NMR (300 MHz, CDCl3) δ: 1.3—1.6 (2H, m, –CH2), 1.8—2.1 (2H, m, –CH2), 2.5 (2H, t, –CH2, J =7.45 Hz), 2.6—2.8 (4H, m, 2 –CH2), 3.2 (2H, t, –CH2, J =7.95 Hz) 3.6 (3H, s, –OCH3), 6.2 (1H, s, –NH), 7.1—7.2 (2H, m, Ar-H), 7.6 (1H, s, Ar-H), 8.2 (2H, d, Ar-H, J =8.19 Hz), 8.52 (1H, s, Ar-H), 8.88 (1H, s, Ar-H, IR (KBr) cm−1: 3431, 1641. Anal. Caled for C23H23N3O2: C, 73.90; H, 6.15; N, 11.24. Found: C, 74.01; H, 6.16; N, 11.34.

N-[3-(Benzimidazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (9): Yield 72.5%. mp 95—96 °C. 1H-NMR (300 MHz, CDCl3) δ: 1.5—1.7 (2H, m, –CH2), 2.8—2.9 (4H, m, 2-CH2), 3.98 (3H, s, –OCH3), 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. Caled for C23H22N3O2: C, 73.45; H, 5.84; N, 11.68. Found: C, 73.55; H, 5.86; N, 11.74.

N-[3-(Benzotriazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (10): Yield 76.6%. mp 72—74 °C. 1H-NMR (300 MHz, CDCl3) δ: 1.5—1.7 (2H, m, –CH2), 2.24 (3H, s, –CH3), 2.8—2.9 (4H, m, 2-CH2), 3.98 (3H, s, –OCH3), 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 =8.05 Hz), IR (KBr) cm−1: 3447, 1684. Anal. Caled for C22H21N3O2: C, 73.90; H, 6.15; N, 11.24. Found: C, 74.01; H, 6.16; N, 11.34.

N-[3-Benzamidazol-1-yl)propyl]-6-methoxynaphthalene-2-carboxamide (11): Yield 74.5%. mp 188—190 °C. 1H-NMR (300 MHz, DMSO-d6) δ: 1.5—1.6 (2H, m, –CH2), 2.8—2.9 (4H, m, 2-CH2), 3.98 (3H, s, –OCH3), 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. Caled for C23H21N3O2: C, 69.92; H, 5.54; N, 15.53. Found: C, 70.02; H, 5.55; N, 15.62.

N-[3-(Benzotriazol-1-yl)-6-methoxynaphthalene-2-carboxamide (12): Yield 75%. mp 148—150 °C. 1H-NMR (300 MHz, CDCl3) δ: 1.4—1.6 (2H, m, –CH2), 2.2—2.4 (4H, m, 2-CH2), 2.8 (4H, t, 2-CH2, J =6.30 Hz), 3.4 (2H, d, –CH2, J =7.95 Hz), 6.2 (1H, s, –NH), 7.14—7.24 (6H, m, Ar-H), 7.6 (1H, s, Ar-H), 7.74—7.82 (2H, d, Ar-H, J =8.53 Hz), 8.0 (1H, s, Ar-H), IR (KBr) cm−1: 3433, 1618. Anal. Caled for C22H21N3O2: C, 76.90; H, 6.94; N, 7.47. Found: C, 76.92; H, 6.95; N, 7.54.

N-[3-(Amino-3-methylthio-5-phenyl-1,2,4-triazol-4-yl)propyl]-6-methoxynaphthalene-2-carboxamide (13): Yield 76%. mp 194—195 °C. 1H-NMR (300 MHz, DMSO-d6) δ: 1.4—1.5 (2H, m, –CH2), 2.6 (3H, s, 300 MHz) they showed significant cytotoxicity and hence reversal potency was not determined at these concentrations.

Key words 6-methoxynaphthalene-2-carboxamide; multidrug resistance reversal agent; chemo sensitizer
traction in chloroform: ethyl acetate mixture (1:2). The amines were isolated by extraction with hydrazine hydrate and acidified with HCl to obtain condensing 6-methoxy-2-naphthoyl chloride 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).

The heteroaryl propylamines with various heteroarylpropyl substituents on the 4-amino-3-methylthio-5-phenyl-1,2,4-triazole was prepared from the corresponding acid 4) by reacting with oxalyl chloride in dichloromethane. Small amount of DMF was added to facilitate the reaction. The 6-methoxy-2-naphthoyl chloride was prepared from the corresponding acid by reacting with oxalyl chloride in dichloromethane.

## 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-naphthyl chloride 1 with heteroaryl propylamines 2—7 in chloroform. Small amount of DMF was added to facilitate the reaction. The 6-methoxy-2-naphthyl chloride 1 was prepared from the corresponding acid 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, benzimidazol-2-yl, benzimidazole, benzotriazole, 1,2,3,4-tetrahydrosquinozine 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).
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 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 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:

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\text{% enhancement in ADR activity} = 100 \left(\frac{\text{% inhibition of (test + ADR) - (\text{% inhibition of ADR + \text{% inhibition of test})}}{\text{% inhibition of ADR}}\right)
\]

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 verapamil 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 cytotoxicity 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.

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