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Synthesis and study of some new *N*-substituted imide derivatives as potential anticancer agents

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Abstract

A new series of *N*-substituted imide derivatives have been synthesized by treating phthalic anhydride, naphthalic anhydride and their substituted derivatives with 2-hydrazino-1-imidazoline hydrobromide, various para-substituted aryl amines, aminoglutethimide and 2,4-dinitrophenyl hydrazine. Compounds **9**, **10**, **12**, **18**, **19**, **23**, **24** and **34–36** have been selected and screened for antineoplastic activity by National Cancer Institute, Bethesda, USA. Some newer aminoglutethimide derivatives **37–39** have also been prepared in order to study the effect of *N*-substitution on its pharmacological profile for the treatment of carcinoma. These compounds **(37–39)** have exhibited weak inhibition of human placental aromatase as compared to aminoglutethimide.

Keywords: Anticancer agents; aminoglutethimide; aromatase; 60-cell line; *N*-imide derivatives

1. Introduction

Intercalation to DNA by small organic molecules has been extensively studied due to medicinal significance and still occupies an important research area in the search for potent antineoplastic agents [1]. DNA intercalating ability and antitumor activity resides in a wide variety of chemical entities such as anthraquinone derivatives [2,3], alkanesulfoanilides [4], acridine [5,6], substituted phenazine-1-carboxamides [7], methylindolo[3,2-*b*]quinolines [8], 6-phenylphenanthridine-4-carboxamides [9], anthrapyrazoles [10], perimidines [11], 1,4-disubstituted anthracene derivatives [12] and *N*-(aminoalkyl)imide derivatives etc. [13–15]. Of all, imide derivatives have been reported as a potential class of antineoplastic agents with compounds like mitonafide **1,** amonafide **2** and azonafide **3** in the market [14–16] (Chart 1). These derivatives have also been explored as potential antifolate thymidylate synthase inhibitors [17].

Another imide derivative, aminoglutethimide **4** (Chart 1) is the pioneer drug of all nonsteroidal, reversible competitive inhibitors of aromatase enzyme [18] and is used in the treatment of mammary carcinoma. A number of modifications of **4** have been carried out with the hope of achieving better antineoplastic agents. Aromatase is a cytochrome P_{450} enzyme, which is responsible for the conversion of androgens to estrogens in the final step of the steroid biosynthetic pathway [19]. On the other hand, drugs that abolish trophic influences of natural oestrogens have also been developed to treat breast cancer. The antiestrogen, tamoxifen **5** is the gold standard adjuvant treatment in primary breast cancer but has also been linked with some adverse effects [20]. The alkylamino side chain of tamoxifen plays an important role in its estrogen modulating activity. Further, several studies have also revealed an increased response of antitumor efficacy of tamoxifen in combination with other cytotoxic chemotherapeutics and/or aromatase inhibitors over tamoxifen alone [21,22]. In continuation with our earlier efforts on anticancer drug design [23,24], it was thought worthwhile to synthesize some newer *N*-substituted imide derivatives by incorporating the essential structural features of the above-mentioned potent cyto-

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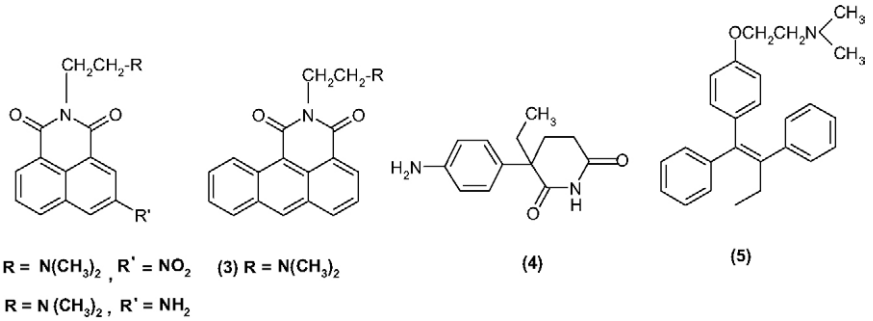


Chart 1. Structures of mitonafide (1), amonafide (2), azonafide (3), aminoglutethimide (4) and tamoxifen (5).

toxic drugs in order to obtain synergistic effects, which we report herein.

2. Experimental Procedures

2.1. Chemistry

Melting points (melting point apparatus MP I, Veego, Mumbai, India) reported are uncorrected. ^{1}H-NMR spectra were recorded on Bruker AC-300F, 300 MHz NMR instrument (Bruker, Fällanden, Switzerland) using tetramethylsilane (TMS) as the internal standard (chemical shifts in δ, ppm). IR spectra were recorded on Perkin-Elmer 882 spectrophotometer model (Perkin-Elmer, Beaconsfield, Buckinghamshire, England) and were obtained with potassium bromide pellets (ν_{max} in cm^{-1}). The purity of the compounds was established by thin layer chromatography (TLC) and by elemental analyses (C, H, N). Elemental analyses were performed on a Perkin-Elmer 2400 (Perkin-Elmer, Norwalk, CT, USA) and agreed with theoretical values to within 0.3%. Anhydrous sodium sulphate was used as drying agent. Plates for TLC were prepared with silica gel G using ethyl acetate. Iodine vapors were used to develop the plates. The complete NMR data of the compounds **18–20,23,24,30–32,35,36–38,39** was in agreement with the proposed structures.

2.1.1. General procedure for the synthesis of compounds 9, 10, 12

A solution of 2-hydrazino-1-imidazoline hydrobromide **8** (0.25 g, 1.48 mmol) in absolute ethanol (25 ml) was added to a solution of appropriate anhydride **(6**, **7**, **11)** in absolute ethanol. The resultant reaction mixture was stirred at 80 °C for 1 h. For compound **12,** solution was stirred at room temperature for 3 h in the presence of 0.2 ml triethylamine. Solvent was removed under vacuum and residue was dried in desiccator overnight. The dried residue was refluxed in toluene for 3 h. The solid material obtained after removal of solvent was washed with ether, 5% solution of sodium bicarbonate, water, and finally dried and crystallized from methanol to give **9**, **10** and **12.**

2.1.2. General procedure for the synthesis of compounds 17–20, 22–24 and 26

A solution of suitable 3-substituted-1,8-naphthalic anhydride **11** or **21** and appropriate 4-(2-dialkylaminoethoxy)-aniline derivatives **13–16** (0.45 g, 2.50 mmol) in pyridine (40 ml) was refluxed for 6 h. The residue obtained after removal of solvent was washed with distilled water, dried and crystallized from methanol to afford **17–20** and **22–24**, respectively. Condensation of **11** (0.5 g, 2.06 mmol) with 2,4-dinitrophenylhydrazine **25** (0.41 g) afforded compound **26**.

2.1.3. General procedure for the synthesis of compounds 30–32

A solution of required amine in dichloromethane (10 ml) was added drop-wise to a solution of **29** (0.25 g, 0.657 mmol) in dichloromethane (100 ml). The reaction mixture was stirred for 4 h at 5–8 °C. The reaction mixture was treated with sodium carbonate solution, water and solvent removed under reduced pressure to obtain a residue, which was crystallized from solvent ether to afford **30–32**, respectively.

2.1.4. General procedure for the synthesis of compounds 34–36

A solution of **33** (0.6 g, 2.58 mmol) and required anhydride **(6**, **7, 11)** in dry pyridine (10 ml) was refluxed for 1 h. Solvent was removed under vacuum and water was added to the obtained residue. The solid product obtained was filtered, washed with water, dried and crystallized from methanol to afford **34–36**, respectively.

2.1.5. General procedure for the synthesis of hydrochlorides of compounds 37–39

A mixture of aminoglutethimide **33** (0.2 g, 0.86 mmol) and potassium carbonate (2.5 g) in dry acetone (50 ml) was refluxed together for 4 h. Appropriate hydrochlorides of alkylaminoethyl halides, potassium iodide (5 mg) and triethylamine (four drops) were added to the refluxing solution and the progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was filtered and the filtrate was removed under reduced pressure to obtain an oily residue. The traces of moisture in the residue were removed by repeatedly refluxing the residue with dry solvent ether and recovering the solvent. The dried residue was then refluxed with dry ether for about an hour and filtered. The filtrate was dried to give an oily residue, which could not be crystallized. Dry hydrochloride gas was passed through the ethereal solution of the oily product to obtain the hygroscopic hydrochloride salts of **37–39**.

N-(4,5-Dihydro-1*H*-imidazol-2-ylamino)phthalimide (9)

From **6** (0.3 g, 2.025 mmol). Yield: 0.2 g, 43.47%. m.p. > 300 °C. IR (KBr): 3413 (-NH), 1700 (C=O), 1660 (N-C=O) cm^{-1}.

N-(4,5-Dihydro-1*H*-imidazol-2-ylamino)-5,6-dimethoxyphthalimide (10)

From **7** (0.25 g, 1.21 mmol). Yield: 0.2 g, 28.98%. m.p. 254–256 °C. IR (KBr): 3411 (-NH), 1704 (C=O), 1660 (N-C=O) cm^{-1}.

N-(4,5-Dihydro-1*H*-imidazol-2-ylamino)-1,8-naphthalimide (12)

From **11** (0.25 g, 1.028 mmol). Yield: 0.26 g, 78.78%. m.p. > 350 °C. IR (KBr): 3404 (-NH), 1707 (C=O), 1660 (N-C=O) cm^{-1}.

N-[4-(2-*N,N*-Dimethylaminoethoxy)phenyl]-3-nitro-1,8-naphthalimide (17)

From **11** (0.5 g, 2.06 mmol) and 4-(2-*N,N*-dimethylaminoethoxy)aniline **13** (0.45 g, 2.50 mmol). Yield:0.3 g, 36.1%. m.p. 226–228 °C. IR (KBr): 3060 (Ar C-H), 1705 (C=O), 1660 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 2.37 (s, 6H, -N(CH_3)$_2$), 2.79 (t, 2H, -CH_2N<, J = 6 Hz), 4.14 (t, 2H, -OCH_2-, J = 6 Hz), 7.10 (d, 2H, Ar-*H* of phenyl ring ortho to the six membered imide functionality, J_o = 7 Hz), 7.20 (d, 2H, Ar-*H* of phenyl ring ortho to the alkoxy functionality, J_o = 7 Hz), 7.98 (t, 1H, 6-C*H*, J_o = 8 Hz), 8.47 (d, 1H, 5-C*H*, J_o = 8 Hz), 8.81 (d, 1H, 7-C*H*, J_o = 7 Hz), 9.18 (d, 1H, 4-C*H*, J_m = 2 Hz), 9.32 (d, 1H, 2-C*H*, J_m = 2 Hz).

N-[4-(2-*N,N*-Diethylaminoethoxy)phenyl]-3-nitro-1,8-naphthalimide (18)

From **11** (0.5 g, 2.06 mmol) and 4-(2-*N,N*-diethylaminoethoxy)aniline **14** (0.60 g, 2.88 mmol). Yield: 0.25 g, 27.7%. m.p.196–198 °C. IR (KBr): 1705 (C=O), 1665 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 1.1 (t, 6H, -N(CH_2CH_3)$_2$, J = 7 Hz), 2.66 (q, 4H, -N(CH_2CH_3)$_2$, J = 7 Hz), 2.93 (t, 2H, CH_2N<, J = 6 Hz), 4.12 (t, 2H, -OCH_2, J = 6 Hz).

N-[4-(2-Pyrrolidinoethoxy)phenyl]-3-nitro-1,8-naphthalimide (19)

From **11** (0.5 g, 2.06 mmol) and 4-(2-pyrrolidinoethoxy)-aniline **15** (0.5 g, 2.42 mmol). Yield: 0.415 g, 51.87%. m.p.198–200 °C. IR (KBr): 1705 (C=O), 1660 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 1.83 (m, 4H, 3-CH_2 and 4-CH_2-pyrrolidine), 2.65 (m, 4H, 2-CH_2 and 5-CH_2-pyrrolidine), 2.95 (t, 2H, -CH_2N<, J = 6 Hz), 4.18 (t, 2H, -OCH_2-, J = 6 Hz).

N-[4-(2-Piperidinoethoxy)phenyl]-3-nitro-1, 8-naphthalimide (20)

From **11** (0.5 g, 2.06 mmol) and 4-(2-(piperidinoethoxy)-aniline **16** (0.6 g, 2.91 mmol). Yield: 0.43 g, 46.41%. m.p. 202–204 °C. IR (KBr): 1710 (C=O), 1660 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 1.48 (m, 2H, 4-CH_2-piperidine), 1.65 (m, 4H, 3-CH_2 and 5-CH_2-piperidine), 2.53 (m, 4H, 2-CH_2 and 6-CH_2-piperidine), 2.82 (t, 2H, -CH_2N<, J = 6 Hz), 4.18 (t, 2H, -OCH_2-, J = 6 Hz).

N-[4-(2-*N,N*-Dimethylaminoethoxy)phenyl]-3-amino-1, 8-naphthalimide (22)

From **21** (0.5 g, 2.345 mmol) and 4-(2-*N,N*-dimethylaminoethoxy)aniline **13** (0.45 g, 2.50 mmol). Yield: 0.46 g, 52.27%. IR (KBr): 3429 (N-H), 1701 (C=O), 1663 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 2.25 (s, 6H, -N(CH_3)$_2$), 2.67 (t, 2H, -CH_2N<, J = 6 Hz), 4.11 (t, 2H, -OCH_2-, J = 6 Hz), 5.98 (s, 2H, -NH_2, disappeared on D_2O exchange), 7.05 (d, 2H, Ar-*H* of phenyl ring ortho to the six membered imide functionality, J_o = 9 Hz), 7.24 (d, 2H, Ar-*H* of phenyl ring ortho to the alkoxy functionality, J_o = 9 Hz), 7.32 (d, 1H, 4-C*H*, J_m = 2 Hz), 7.61 (t, 1H, 6-C*H*, J_o = 8 Hz), 7.98 (d, 1H, 2-C*H*, J = 2 Hz), 8.05 (m, 2H, 5-C*H* and 7-C*H*).

N-[4-(2-Pyrrolidinoethoxy)phenyl]-3-amino -1, 8-naphthalimide (23)

From **21** (0.5 g, 2.345 mmol) and 4-(2-pyrrolidinoethoxy)aniline **15** (0.5 g, 2.42 mmol). Yield: 0.50 g, 53.19%. m.p. 174–176 °C. IR (KBr): 3377 (N-H), 1707 (C=O), 1667 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 1.77 (m, 4H, 3-CH_2 and 4-CH_2 -pyrrolidine), 2.61 (m, 4H, 2-CH_2 and 5-CH_2-pyrrolidine), 2.89 (t, 2H, -CH_2N<, J = 6 Hz), 4.14 (t, 2H, -OCH_2-, J = 6 Hz).

N-[4-(2-Piperidinoethoxy)phenyl]-3-amino-1,8-naphthalimide (24)

From **21** (0.5 g, 2.345 mmol) and 4-(2-piperidinoethoxy)aniline **16** (0.6 g, 2.91 mmol). Yield: 0.48 g, 49.48%. m.p. 92–94 °C. IR (KBr): 3349 (N-H), 1705 (C=O), 1664 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 1.43 (m, 2H, 4-CH_2 -piperidine), 1.56 (m, 4H, 3-CH_2 and 5-CH_2 -piperidine), 2.51 (m, 4H, 2-CH_2 and 6-CH_2 -piperidine), 2.76 (t, 2H, -CH_2N<, J = 6 Hz), 4.14 (t, 2H, -OCH_2, J = 6 Hz).

N-(2,4-Dinitro-1-anilino)-3-nitro-1,8-naphthalimide (26)

Yield: 0.45 g, 51.72%. m.p. 292–294 °C. IR (KBr): 3290 (N-H), 1705 (C=O), 1680 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 7.56 (d, 1H, 6-C*H* -dinitrophenylhydrazine ring, J_o = 9 Hz), 8.14 (t, 1H, 6-C*H*, J_o = 8 Hz), 8.21 (dd, 1H, 5-C*H* -dinitrophenylhydrazine ring, J_o = 9 Hz, J_m = 2 Hz), 8.79 (d, 1H, 5-C*H*, J_o = 8 Hz) 8.90 (d, 1H, 7-C*H*, J_o = 8 Hz), 9.01 (d, 1H, 3-C*H* -dinitrophenylhydrazine ring, J_m = 2 Hz), 9.08 (d, 1H, 4-C*H*, J_m = 2 Hz), 9.6 (d, 1H, 2-C*H*, J_m = 2 Hz), 10.62 (s, 1H, -N*H*, disappeared on D_2O exchange).

4-(3-Nitro-1,8-naphthalimido)benzoic acid (28)

A mixture of **11** (0.25 g, 1.03 mmol), *p*-aminobenzoic acid **27** (0.15 g, 1.09 mmol) and triethylamine (0.2 ml) in aldehyde free alcohol (150 ml) was stirred for 4 h. Excess ethanol was removed and toluene (100 ml) was added to the residue and refluxed for 4 h using Dean-Stark apparatus. Toluene was removed under reduced pressure; distilled water was added and allowed to stand overnight. The slurry was filtered, washed thoroughly with distilled water, dried and crystallized from methanol to afford **28** (0.30 g, 80.50%). Melting point decompose at 280 °C. IR (KBr): 3100 (broad, -OH), 1710 (C=O), 1679 (N-C=O) cm^{-1}. ^{1}H-NMR: δ 7.48 (d, 2H, Ar-*H* of phenyl ring ortho to six membered imide functionality, J_o = 8.4 Hz), 8.07 (t, 1H, 6-C*H*), 8.13 (d, 2H, Ar-*H* of phenyl ring ortho to carboxylic group, J_o = 7.8 Hz), 8.75 (m, 2H, 5-C*H* and 7-C*H*), 9.09 (d, 1H, 4-C*H*, J_m = 2.5 Hz), 9.48 (d, 1H, 2-C*H*, J_m = 2.2 Hz).

4-(3-Nitro-1,8-naphthalimido)benzoyl chloride (29)

Compound **28** (0.25 g, 0.69 mmol) was refluxed in thionyl chloride (3 ml) for 4 h under anhydrous conditions. Thionyl

chloride was removed under reduced pressure to afford a brown colored solid **29** (0.25 g, 93.3%). IR (KBr): 1780 (C=O), 1745 (C=O stretch -aroyl chloride), 1670 (N-C=O) cm^{-1}.

4-(3-Nitro-1,8-naphthalimido)-*N*-(2-dimethylaminoethyl)-benzamide (30)

From 2-(*N,N*-dimethylamino)ethylamine (3 ml). Yield: 0.13 g, 45.8%. Melting point decompose at 290 °C. IR (KBr): 3310 (N-H), 1710 (C=O), 1670 (N-C=O), 1630 (amide C=O) cm^{-1}. ^{1}H-NMR: δ 2.32 (s, 6H, -N(CH_3)$_2$), 2.58 (t, 2H, -CH_2N(CH_3)$_2$, J = 6.2 Hz), 3.54 (q, 2H, -NHCH_2-, J = 6.0 Hz).

4-(3-Nitro-1,8-naphthalimido)-*N*-(t-butyl)benzamide (31)

From *t*-butylamine (3 ml). Yield: 0.15 g, 54.7%. Melting point decompose at 290 °C. IR (KBr): 3390 (N-H), 1710 (C=O), 1670 (N-C=O), 1640 (amide C=O) cm^{-1}. ^{1}H-NMR: δ 1.48 (s, 9H, 3 × CH_3).

4-(3-Nitro-1,8-naphthalimido)-*N*-pentylbenzamide (32)

From *n*-pentylamine (3 ml). Yield: 0.2 g, 70%. Melting point decompose at 290 °C. IR (KBr): 3315 (N-H), 1710 (C=O), 1670 (N-C=O), 1630 (amide C=O) cm^{-1}. ^{1}H-NMR: δ 0.93 (t, 3H, -CH_3), 1.37 (m, 4H, -$CH_2CH_2CH_3$), 1.64 (m, 2H, -NHCH$_2$$CH_2$-), 3.39 (q, 2H, -NH$CH_2$).

3-[4-(Phthalimido)phenyl]-3-ethyl-2,6-piperidinedione (34)

From **6** (0.5 g). Yield: 0.8 g, 85.36%. Melting point 210–212 °C. IR (KBr): 3192 (N-H), 1708 (C=O) cm^{-1}. ^{1}H-NMR: δ 0.90 (t, 3H, -CH_3), 2.0 (m, 2H, -CH_2CH_3), 2.40 (m, 4H, -CH_2-CH_2-piperidine-2,6-dione), 3.2 (s, 1H, N*H*, disappeared on D_2O exchange), 7.56 (s, 4H, Ar), 8.00 (m, 4H, Ar).

3-[4-(4,5-Dimethoxyphthalimido)phenyl]-3-ethyl-2,6-piperidinedione (35)

From **7** (0.44 g). Yield: 0.70 g, 76.96%. Melting point 282–284 °C. IR (KBr): 3234 (N-H), 1710 (C=O) cm^{-1}. ^{1}H-NMR: δ 4.1 (s, 6H, -OCH_3).

3-[4-(3-Nitro-1, 8-naphthalimido)phenyl]-3-ethyl-2,6-piperidinedione (36)

From **11** (0.6 g, 2.46 mmol). Yield: 0.54 g, 48.2%. Melting point 282–284 °C. IR (KBr): 3200 (N-H), 1685 (N-C=O), 1650 (amide C=O) cm^{-1}. ^{1}H-NMR: δ 7.36 (d, 2H, Ar-*H* ortho to piperidinedione ring, J_o = 8 Hz), 7.48 (d, 2H, Ar-*H* ortho to imide functionality, J_o = 8 Hz).

3-(4-Aminophenyl)-3-ethyl-1-(2-morpholin-4-ylethyl)piperidine-2,6-dione (37) hydrochloride

From 4-(2-chloroethyl)morpholine hydrochloride (0.17 g, 0.86 mmol). IR (KBr): 3400, 3240 (N-H), 1710 (C=O), 1680 (N-C=O), 1120 (C-N stretch) cm^{-1}. ^{1}H-NMR: δ 0.82 (t, 3H, -CH_2CH_3), 1.99 (m, 2H, -CH_2CH_3), 2.39 (m, 2H, 4-CH_2-piperidine-2,6-dione), 2.71 (m, 2H, 5-CH_2 piperidine-2,6-dione) 3.16 (s, 2H, -$^+$N(CH_2)-morpholine), 3.34 (d, 2H, N(CH_2)$_2$-morpholine) 3.68 (m, 2H, -OCH_2 -morpholine), 3.97 (m, 2H, -OCH_2-morpholine), 4.15 (m, 4H, -CH_2- attached to nitrogen of piperidine-2,6-dione ring and -CH_2-$^+$N-(CH_2)$_2$-), 7.33 (d, 2H, J_o = 8.34 Hz, Ar-*H* ortho to amino group), 7.53 (d, 2H, J_o = 8.44 Hz, Ar-*H* ortho to piperidine-2,6-dione ring), 11.6 (s, 1H, HCl).

3-(4-Aminophenyl)-1-(dimethylaminoethyl)-3-ethylpiperidine-2,6-dione (38) hydrochloride

From 2-dimethylaminoethyl chloride hydrochloride (0.17 g, 1.18 mmol). IR (KBr): 3400, 3240 (N-H), 1710 (C=O), 1680 (N-C=O), 1120 (C-N stretch) cm^{-1}. ^{1}H-NMR: δ 2.92 (s, 6H, -$^+$N(CH_3)$_2$), 3.41 (m, 4H, -NH_2), 3.33 (s, 4H, 5-CH_2-piperidine-2,6-dione and -CH_2-$^+$N(CH_3)$_2$).

3-(4-Aminophenyl)-3-ethyl-1-(2-pyrrolidin-1-ylethyl)piperidine-2,6-dione (39) hydrochloride

From 4-(2-chloroethyl)pyrrolidine hydrochloride (0.22 g, 1.29 mmol). IR (KBr): 3400, 3240 (N-H), 1710 (C=O), 1680 (N-C=O), 1120 (C-N stretch) cm^{-1}. ^{1}H-NMR: δ 2.14 (m, 4H, -CH_2CH_2- pyrrolidine), 3.21 (d, 4H, -$^+$N(CH_2)$_2$ -pyrrolidine), 3.86 (m, 4H, -CH_2-$^+$N(CH_2)$_2$ -pyrrolidine and -NH_2).

2.2. Antineoplastic activity

2.2.1. 60-Cell line assay

The compounds **(9, 10, 12, 18, 19, 23, 24** and **34–36)** selected by National Cancer Institute, Bethesda, USA have been screened for their in vitro antitumor activity against the cell panel consisting of 60-cell lines at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous exposure protocol was used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth [25]. Two standard drugs, meaning that their activities against the cell lines are well documented, are tested against each cell line: NSC 19893 (5-Fluorouracil) and NSC 123127 (Adriamycin).

2.2.2. 3-Cell line assay

The compounds **37–39** have been selected and evaluated by NCI for their antineoplastic activity using in vitro 3-cell lines [MCF-7 (Breast), NCl-H460 (Lung) and SF-268 (CNS)] one dose prescreen. In the current protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added and the culture incubated for 48 h. End-point determinations are made with alamar blue. Results for each test agent are reported as the percent growth of the treated cells when compared to the untreated control cells.

2.3. Inhibition of aromatase in vitro

2.3.1. Preparation of Aromatase

The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri [26]. The isolated microsomes were suspended in the minimum volume of phosphate buffer (0.05 M, pH 7.4) and stored at –30 °C as described [26]. No loss of activity was observed within 4 months.

2.3.2. Inhibition of aromatase

This assay was performed similar to described methods [27,28] monitoring enzyme activity by measuring the ^3H_2O formed from [1β,2β-^{3}H] testosterone during aromatization. Each incubation tube contained 0.225 μCi of (1β,2β-^{3}H) tes-

tosterone, 5 μM unlabeled testosterone, 2 mM NADPH, 20 mM glucose-6-phosphate, 1EU glucose-6-phosphate dehydrogenase, and inhibitor (0–250 μM) in phosphate buffer (0.05 M, pH 7.4). The test compounds had been dissolved in ethanol and diluted with buffer. The final ethanol concentration of control and inhibitor incubation was 2%. Each tube was preincubated for 5 min at 30 °C in a shaking water bath. Microsomal protein (0.5 mg) was added to start the reaction. The total volume for each incubation was 0.5 ml. The reaction was terminated by withdrawing 100 μl aliquots at 0, 7, 14 and 21 min and pipetting them into 200 μl of a cold 1 mM $HgCl_2$ solution. After addition of 200 μl of an aqueous dextran-coated charcoal (DCC) suspension (2%), the vials were shaken for 20 min and centrifuged at 1500 g for 5 min to separate the charcoal-adsorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture in a Beckman liquid scintillation spectrometer (LS 8000).

3. Results

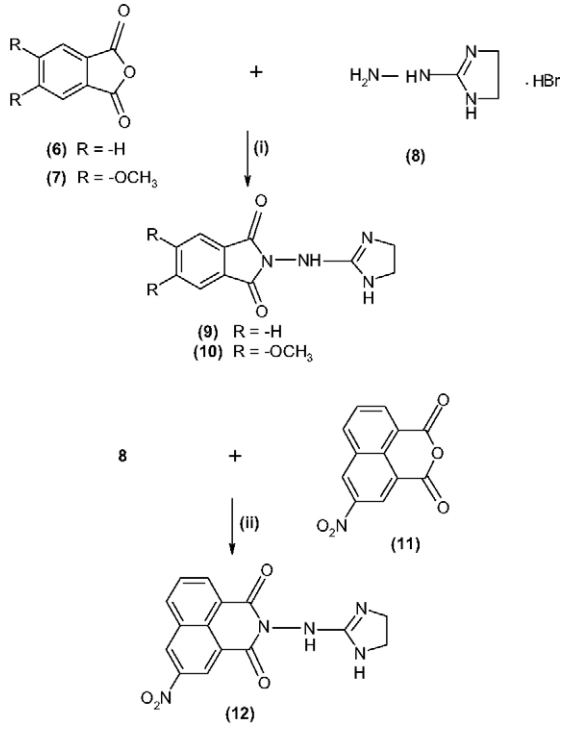
3.1. Chemistry

Respective imidazoline derivatives **9, 10** and **12** were prepared by treating ethanolic solutions of 2-hydrazino-1-imidazoline hydrobromide **8** with phthalic anhydride **(6),** dimethoxyphthalic anhydride **7** and 3-nitro-1,8-naphthalic anhydride **11** and subsequent reflux in toluene (Scheme 1). 3-Nitro-1,8-naphthalic anhydride **11** and 3-amino-1,8-naphthalic anhydride **21** were condensed with appropriate 4-(2-dialkylaminoethoxy)aniline derivatives **13–16** in refluxing pyridine to afford **17–20** and **22–24**, respectively (Scheme 2). Similarly, 2,4-dinitrophenylhydrazone derivative **26** was also prepared by treating **11** with 2,4-dinitrophenylhydrazine **25** (Scheme 2). Spectral studies and elemental data were in agreement with the proposed structures.

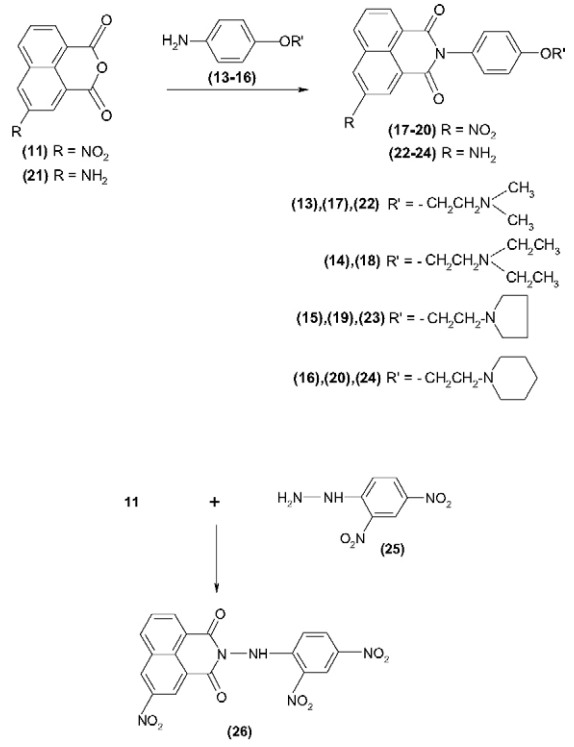
Treatment of **11** with *p*-aminobenzoic acid **(27)** in presence of triethylamine in aldehyde free alcohol yielded **28**, which exhibited ^{1}H-NMR signals at δ 9.09 (d, 4-C*H*) and 9.48 ppm (d, 2-C*H*). Characteristic bands for six membered imides appeared at 1710 and 1679 cm^{-1} in IR spectrum. Refluxing **28** with thionyl chloride yielded **29**, which showed peak at 1745 cm^{-1} in IR spectrum characteristic for aroyl chlorides. Compound **29** was treated with different amines in dichloromethane at 5–8 °C to obtain compounds **30–32**, respectively (Scheme 3).

It was also thought worthwhile to condense anhydrides **6, 7** and **11** with aminoglutethimide **33**, an established aromatase inhibitor, to have synergistic effects. This was achieved by refluxing these anhydrides and **33** in pyridine to yield phthalic anhydride derivatives **34, 35** and naphthalic anhydride derivative **36**, respectively (Scheme 4). A triplet of -CH_3 group appeared at δ 0.90 whereas two protons of -CH_2 of substituted ethyl group coupled separately with -CH_3 and 4-CH_2 of piperidinedione ring (long range coupling) to give two multiplets in close proximity at δ 2.0 (merged) for **34**, 1.96 (merged) for **35** and 1.95 and 2.07 ppm for **36**.

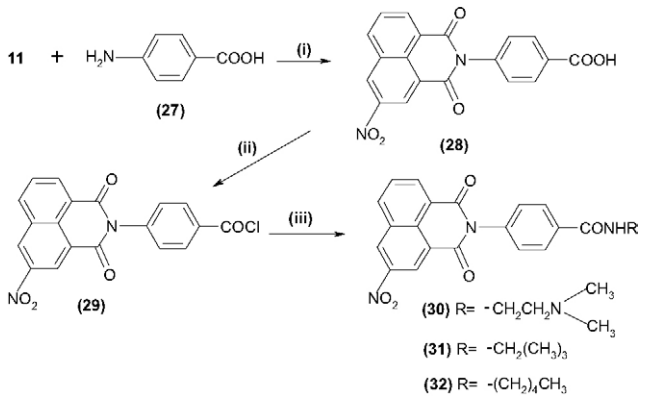
N-Substituted aminoglutethimide derivatives were prepared by treating **33** with various hydrochlorides of alkylami-



Scheme 1. Synthetic procedures of compounds 9, 10 and 12. Reagents and conditions: (i) absolute alcohol, 80 °C, 1 h and (ii) absolute alcohol, triethylamine, room temperature.



Scheme 2. Synthetic procedures of compounds 17–20, 22–24 and 26. Reagents and conditions: (i) pyridine, reflux.



Scheme 3. Synthetic procedures of compounds 27–32. Reagents and conditions: (i) triethylamine, aldehyde free alcohol; (ii) thionyl chloride and (iii) dichloromethane, 5–8 °C, respective amines.

noethyl chlorides in acetone under anhydrous conditions to afford **37–39** as oily residues, respectively (Scheme 4). Since the oily residues could not be crystallized, the compounds were characterized as their hydrochloride salts.

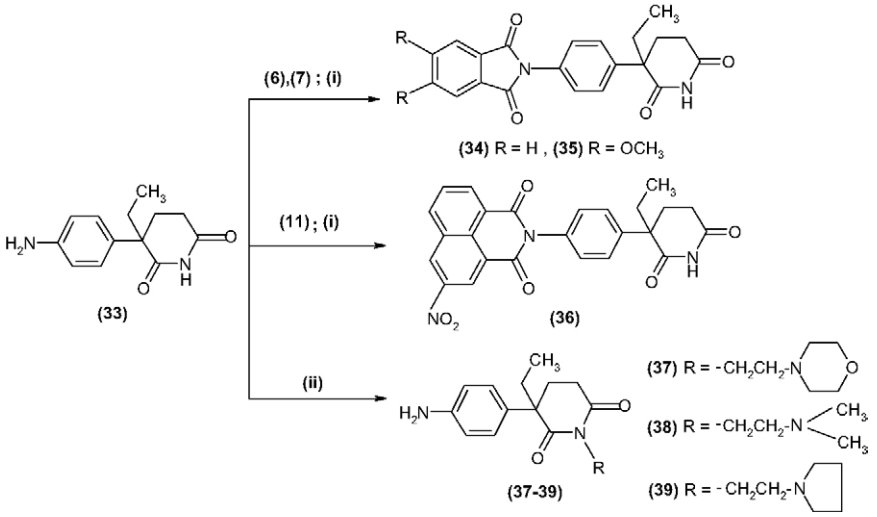
3.2. Antineoplastic activity

The compounds **9, 10, 12, 18, 19, 23, 24** and **34–36** selected by Drug Synthesis and Chemistry Branch, National Cancer Institute, based in general, on the basis of degree of novelty of the structure and computer modeling techniques, were assayed in vitro against a panel consisting of 60 human tumor cell lines, derived from nine cancers types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers), using five concentrations at 10-fold dilutions, the highest being 10^{-4} M. Mean log dose response parameters such as GI50 (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) are summarized in Table 1.

The aminoglutethimide derivatives **37–39** were tested using a one dose (10^{-4} M) primary anticancer in vitro assay against tumor in the three-cell line panel consisting of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS) and the data are shown in Table 2. Compounds, which reduce the growth of any one of the cell lines to approximately 32% or less, are passed on for evaluation in the full panel of 60-cell lines over a 5-log dose range.

3.3. Aromatase inhibitory activity

The aromatase inhibitory activity of aminoglutethimide derivatives **37–39** was determined in vitro using human pla-



Scheme 4. Synthetic procedures of compounds 34–39. Reagents and conditions: (i) pyridine, reflux and (ii) respective hydrochlorides of alkylaminoethyl chlorides, potassium carbonate, potassium iodide, triethylamine, dry acetone.

Table 1
Mean log dose–response parameters such as GI50, TGI and LC50 in the 60-cell line screening

Compound	NSC No.	Mean $\log_{10}$ GI50 (Molar)	Mean $\log_{10}$ TGI (Molar)	Mean $\log_{10}$ LC50 (Molar)
9 (DPJ-231)	664226	–4.00	–4.00	–4.00
10 (DPJ-236)	664227	–4.00	–4.00	–4.00
12 (DPJ-229)	645467	–4.00	–4.00	–4.00
18 (DPJ-519)	679263	–5.12	–4.57	–4.18
19 (DPJ-525)	679264	–4.76	–4.28	–4.05
23 (DPJ-544)	682467	–4.77	–4.44	–4.15
24 (DPJ-545)	682468	–4.80	–4.49	–4.21
34 (DPJ-329)	663902	–5.00	–5.00	–5.00
35 (DPJ-327)	663901	–5.00	–5.00	–5.00
36 (DPJ-534)	679226	–4.80	–4.26	–4.05

Table 2
Growth percentage at 10^{-4} molar concentration in the 3-cell line screening

Compound	NSC No.	Growth percentage		
		Breast	Non-small cell lung	CNS
37 (RG-DPJ-148)	728329	116	146	119
38 (RG-DPJ-149)	728330	79	149	114
39 (RG-DPJ-160)	728333	112	96	107

cental microsomes and [1β, 2β-^{3}H] testosterone. The compounds **37–39** showed weak inhibition of enzyme (15%, 10% and 2% inhibition at 36 μM, respectively) as compared to aminoglutethimide.

4. Discussion

The newly synthesized *N*-substituted imide derivatives (**9,10,12,18,19,23,24,34–36**) have exhibited less cytotoxicity than the original compounds in the 60-cell line assay over a 5-log dose range with mean $\log_{10}$ GI50 more than –5. In general, a compound is selected for in vivo studies if its mean $\log_{10}$ GI50 ≤ –6 in 60-cell line assay. It seems that the introduction of aromatic ring system between imidic nitrogen and dialkylaminoalkyl side chain resulted in the decrease in antitumor activity of the newly synthesized mitonafide and amonafide analogues (**18,19,23,24**). Surprisingly, the *N*-imidazolinoamino derivatives (**9,10,12**) also could not produce good cytotoxic effects.

N-Dialkylaminoalkyl aminoglutethimide derivatives (**37–39**) have exhibited weak aromatase inhibitory activity and insignificant cytotoxic activity. It is revealed that even if dialkylaminoalkyl side chain, the very active structural component of some potent anticancer agents, is introduced at secondary nitrogen of aminoglutethimide, it leads to the loss of activity. Therefore, it may be concluded that the unsubstituted -NH is prerequisite requirement of aminoglutethimide as an aromatase inhibitor. Though the pharmacological results are not very encouraging, the study provides useful information to further design newer anticancer agents.

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