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Revisiting aryl amidine synthesis using metal amide and/or ammonia gas: Novel molecules and their biological evaluation

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ABSTRACT
Amidines, due to their unique biocompatibility and desirable physical characteristics, have been the functionality of choice as a scaffold for large number of drug synthesis. But still synthesis of amidines in the presence of other active functional groups or pharmacophore, remained a challenge. In this work, a simple and reliable protocol for conversion of nitrile-amide to unsubstituted amidine–amide is developed using metal amide and/or ammonia gas. The scope and efficiency of this synthetic strategy are demonstrated on several substrates which differ in functional groups will be discussed. In this process, 10 novel aryl amidines in good yields (upto 85%) were synthesized. Biological evaluation revealed that compound 4-(aminoiminomethyl)-*N*-(2-furanyl methyl) benzamide ($IC_{50} = 9\ \mu M$) and 4-(aminoiminomethyl)-*N*-(3-pyridinylmethyl) benzamide (73.36% growth inhibition) showed moderate efficacy for cancer cells.

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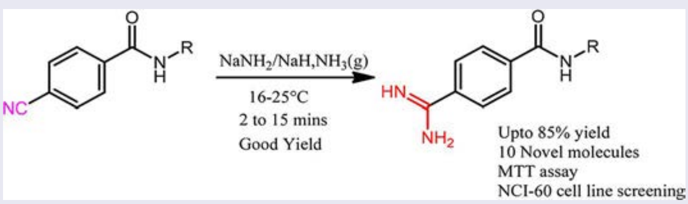
KEYWORDS
Amide; amidine; antiproliferative activity; NCl; single crystal

GRAPHICAL ABSTRACT



Introduction

Amidine functionality serves as synthon for the synthesis of variety of heterocyclic compounds.[1] On the other hand, amidines with their unique structural properties and biocompatibility are present in various active pharmaceuticals ingredients (API) used for antiviral, anti-inflammatory, and anticancer.[2] This latter aspect will open up new scope for amidines as a pharmacophore.

Synthesis of amidines have been explored by many scientists in the past. Out of the various reagents available for the synthesis of amidine, single-step conversion of nitrile to amidine remained choice of synthetic chemists. In this method, the nitrile functionality

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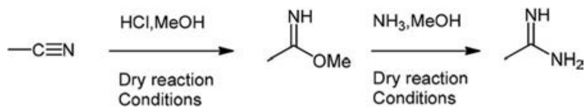
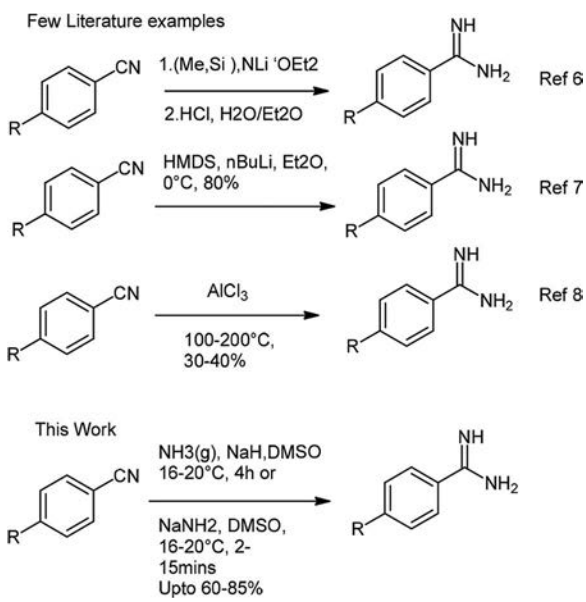


Scheme 1. Pinner reaction condition.

is activated by the electron-withdrawing groups or lewis acids[3,4] to give the desired products.

Pinner reaction, to the best of our knowledge, happens to be the most commonly used technique for the amidine synthesis from the parent nitrile compound.[5] Contemplating the mechanism, first step being the cyanide activation using dry HCl gas in dry methanol, leads to the formation of amidinates as shown in Scheme 1. Ammonia attack is the next step that leads to amidine, the desired product, in good yield. Key step in this process remains the activation of nitrile group by HCl. This may lead to unwanted chemical reactions if additional functional groups are present on substrate. This is what is observed in our case, nitrile-amide system, where latter functional group started reacting before nitrile activation leading to the formation of undesired products. Hence for our substrate, there is a need to find alternative methodology where nitrile group is activated before amide linkage.

Further literature search resulted in few more alternative methods where scientist have used different reagents/reaction conditions for the conversion of nitrile to amidine (Scheme 2).[3,6,7] Cornell et al.[8] reported the synthesis of aliphatic and aromatic amidines from their parent nitriles using liquefied ammonia gas with various metal amides. Criticality of this reaction remained with the difficulty in handling liquefied ammonia gas in standard synthetic laboratory. Newbery et al.[9] used same reagent (metal amides)



Scheme 2. Literature work for amidine synthesis.

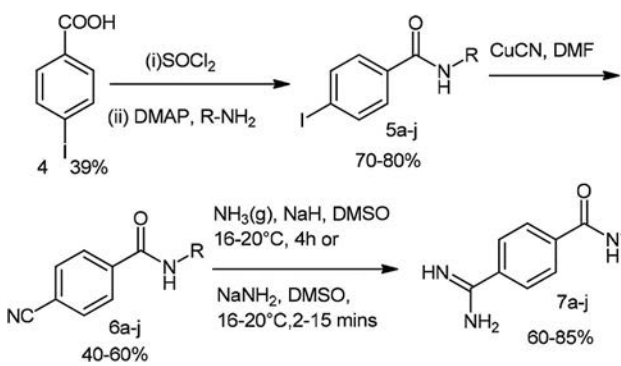
but with a slightly milder conditions, using benzene as solvent at high temperature. The drawback of using this method was the solubility of the parent nitriles in benzene and also the toxicity of the solvent.[10] Interestingly, today's drug design challenge revolves around presence of more than one functional group (pharmacophores) on a single API.[11]

Results and discussion

Keeping above literature survey in mind, our efforts were focused on amidine synthesis in presence of amide (R.CO.NH_2) functionality, our substrates, using amide anion (NH_2^-). This process can be considered as a "direct" amidine formation method, since it requires no prior activation of nitrile functionality. Now, NH_2^- can be generated in situ as well as ex situ means. Therefore we planned both these methods: Method 1- in situ generation: dry NH_3 (g) with sodium hydride in DMSO, and Method 2- ex situ generation: Sodamide in DMSO (Scheme 3). The role of sodium hydride in the Method-1 is to abstract proton from ammonia and form NH_2^- (in situ generation). Both these methods, when performed at room temperature (16–25 °C), gave good yields with substantial reduction in the time of the reaction as compared to the literature. Interestingly bottle-neck for both these modified method remains in the contact time of sodamide and nitrile functionality, which can be controlled by tailoring the reaction conditions. Table 1 shows results of screening different reaction conditions for both these methods on 4-(aminoiminomethyl)-benzamide, a standard substrate with the presence of amide functionality with nitrile. Thus, standardized optimum conditions were used to synthesis of 10 new compounds with amidine–amide linkages.

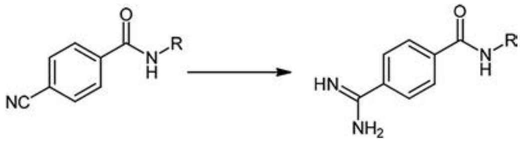
The synthesis of 4-(aminoiminomethyl)-benzamide, one of the standard lead compound, was performed using Scheme 3. To validate these synthetic procedures, different substituents in the form of halogens, heterocycles were introduced near amide functionality. These structures are tabulated in Table 2. While tailoring the structures with different substituent, PAINS (Pan Assay Interference Compounds) were kept in mind.[12] PAINS functionality does not discriminate between target and nontarget moieties leading to plethora of side effects. We observed key step during the synthesis of this series compounds remained in the formation of amidine from parent nitrile functionality.

4-iodo-*N*-(4-methoxyphenyl) benzamide **5a** was obtained from 4-iodobenzoic acid **4** (refer ESI for synthesis) in two stages, treatment with thionyl chloride in first step and DMAP/R-NH_2[13–15] in second step (Scheme 2). For the latter step, we used variety of bases



Scheme 3. Synthesis of target 4-(aminoiminomethyl)-benzamide derivatives.

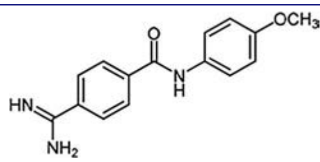
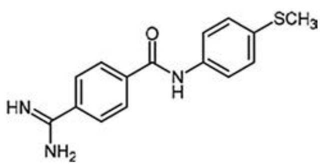
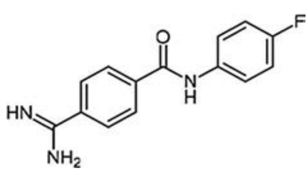
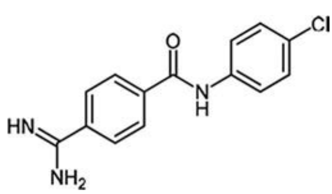
Table 1. Investigation of solvents and reagents on our substrates.



Entry	Reagent[b]	Solvent	Yield[c] %
1	Na, NH_4Cl	MeOH	NR
2	HCl(g), EtOH/NH_3(g)	EtOH	NR
2	NH_2OH.HCl,TEA/NH_3(g)	EtOH	NR
3	NH_4Cl, Si[d]	MeOH	NR
4	EtOH.HCl/NH_4Cl	MeOH	NR
5	NH_3(g), NaH	THF	15
6	NH_3(g), NaH	DMF	35
7	NH_3(g), NaH	Toluene	NR
8	NH_4Cl, NaH	Toluene	NR
9	NH_4Cl, NaH	DMSO	NR
10	NH_3(g), NaH	DMSO	83
11	$NaNH_2$	DMSO	85

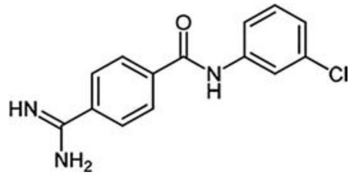
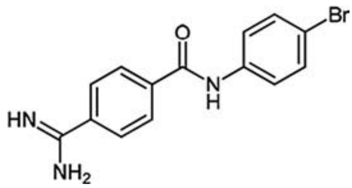
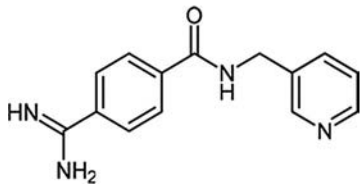
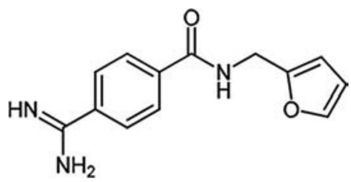
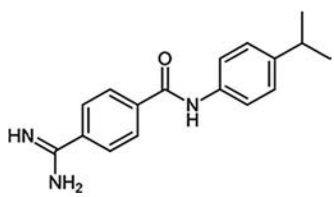
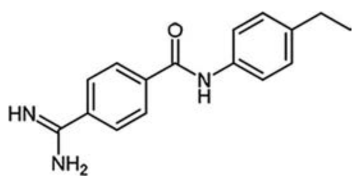
*NR, No reaction, desired product is not obtained.
[b]Reactions were performed based on the literature procedure.
[c]Yield of the isolated product after silica gel chromatography.
[d]Reaction was performed in a sealed tube.

Table 2. List of derivatives of amidine–amide conjugates (7).

Entry	Structure (R)	Yield*	Melting point (°C)
7a		82	200–202
7b		85	230–232
7c		74	204–205
7d		78	198–200

(*Continued*)

Table 2. Continued.

Entry	Structure (R)	Yield*	Melting point (°C)
7e		70	135–140
7f		60	220–222
7g		65	156–158
7h		75	144–146
7i		83	170–172
7j		72	180–183

*Yields are reported for the final step f.

triethyl amine, diethyl amine, and (dimethyl amino pyridine) DMAP along with number of solvents such as dry ACN, MDC, $CHCl_3$, and CCl_4. The best yields were obtained with MDC and DMAP. Traditionally, Iodo/nitrile exchange uses NaCN or KCN but in our present strategy we preferred “green” nitrile source in the form of cuprous cyanide (CuCN). Good yield of 4-cyano-*N*-(4-methoxyphenyl) benzamide **(6a–j)** was accessed by reacting 4-iodo-*N*-(4-methoxyphenyl) benzamide (**5a–j)** with the requisite CuCN as the nitrile source in dry DMF (refer ESI for detailed experimental procedure).

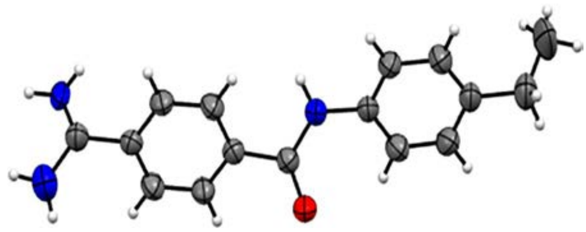


Figure 1. ORTEP diagram of **7j** with 50% probability.

All the new compounds were characterized by FTIR, ^{1}HNMR, ^{13}C NMR, Mass spectrometry, and micro analysis (Refer ESI for spectral details). Spectra analyses were consistent with the assigned structures.

Figure 1 shows ORTEP diagram for single crystal of **7j**. It crystallizes in triclinic crystal system with P1 space group, with two molecules in an asymmetric unit. No prominent hydrogen bonding is observed in spite of having the presence of strong hydrogen bond donor and hydrogen bond acceptor groups. Significant and expected short contact among two neighboring amide bonds through NH···O bonding (3.247 Å and 171.5°) is observed along *a*-axis. Although this interaction is considered to be weak in nature, it is reported that it plays an important role in the protein–drug binding.[16] This short-contact also helps in maintaining planarity of two aromatic groups and hence pharmacophore. (CCDC no. 1432792, detailed structure information can be obtained from supporting information).

Cytotoxicity studies[17] on HeLa cell line was performed for all the new compounds. Cis-platin was used as the reference drug. Percentage cell viability of synthesized compounds on HeLa cell line at various concentrations was checked and then from that IC_{50} was calculated (One-way ANOVA (nonparametric test was performed. P value $= 0.0062$(**)). Table 3 shows results of IC_{50} in micromolar concentrations.

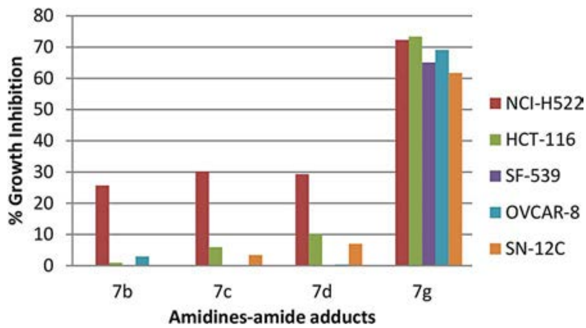
All molecules were initially screened for antiproliferative activity in silico by National Cancer Institute (NCI), USA (Refer ESI for experimental procedure). Out of this, compound **7b–7g** were further selected for actual screening antiproliferative activity at 10 µM concentration. Graph 1 shows comparative study of compound **7b–7g** on selected human-derived cell lines NCI-H522 (Nonsmall cell lung cancer), HCT-116 (Colon cancer), SF-539 (CNS cancer), OVCAR-8 (Ovarian cancer), and SN-12 (Renal cancer). **7g** shows 73.36% growth inhibition in HCT-116 colon cancer cell line (mean growth inhibition) at 10 µM concentration.

Two heterocyclic structure containing derivatives of furan and picolyamine were found to be most potent among all. Both compounds **7g** and **7h** have a heterocycle in conjugation with NH side of amide linkage, but also have flexible –CH_2 bridge. Thus, from anticancer activity perspective we can conclude that 4-(aminoiminomethyl)-*N*-(3-pyridinylmethyl) benzamide **(7g)** and 4-(aminoiminomethyl)-*N*-(2-furanylmethyl)benzamide **(7h)** can be investigated further for the development as new leads.

Table 3. IC_{50} values of the compounds **7a–j** on HeLa cells.

Compound	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	Cisplatin
IC_{50} (µM)[a]	>200	189	>200	153	>200	ND[b]	>200	9	>200	ND[c]	28

[a]IC_{50} values are the mean of four independent determinations.
[b]IC_{50} value exceeded the mM concentration.
[c]Poor solubility.



Graph 1. % Growth **inhibition** on selected cell lines at 10 µM concentration NCI-H522: Nonsmall cell lung cancer cell line; HCT-116: Colon cancer cell line; SF-539: CNS cancer cell line; OVCAR-8: Ovarian cancer cell line; SN-12: Renal cancer cell line.

Experimental

This includes synthesis and characterization of one of the new molecules. Experimental procedures for the intermediates and rest of the derivatives are presented in supporting information.

Materials and methods

All the compounds were purified using column chromatography (2000–400 mesh silica) before characterization. TLC analysis was done using precoated silica on aluminum sheets. Melting points were recorded in Thiele's tube using paraffin oil and are uncorrected. FTIR (KBr pellets) spectra were recorded in the 4000–400 cm^{-1} range using a PerkinElmer FTIR spectrometer. The NMR spectra were obtained on a Bruker AV-III 400 MHz spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), coupling constants (J) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), broad (b) as well as multiplet (m). The microanalysis was performed using a PerkinElmer IA 2400 series elemental analyzer. The mass spectra were recorded on Thermo scientific DSQ-II. All chemicals and solvents were of commercial grade and were used without further purification. Single crystal data were collected with Xcalibur, EoS, Gemini.

General procedure for the synthesis of compound 7

Method 1: Dry DMSO and NaH (60% suspension in oil, 0.100 mg, 0.0025 mol) were stirred at 16–20 °C for 15 min. Compound **6a** (0.100 g, 0.0037 mol) was then added and ammonia gas was purged in it till the completion of the reaction (monitored by TLC, eluent, petroleum ether/ethyl acetate, 1/4, V/V), cooled the reaction. Then the water was added slowly such that temperature of reaction should not exceed 30 °C. Residue was extracted with ethyl acetate (3 × 10 mL) and purified by neutral alumina column chromatography (eluent, petroleum ether/ethyl acetate, 1/4, v/v) to afford compound **7** as off white solid to pale yellow solid. Yield: 60–85%. Melting point: 200–202 °C.

Method 2: Dry DMSO and compound **6a** (0.100 g, 0.0037 mol) were stirred at 25 °C for 5 min, added sodamide in it and stirred for 2–15 min at same temperature. Reaction was monitored by TLC (eluent, petroleum ether/ethyl acetate, 1/4, v/v), the reaction was cooled, and water was added slowly such that temperature of reaction should not exceed 30 °C. Residue was extracted with ethylacetate (3 × 10 mL) and purified by neutral alumina column chromatography (eluent: petroleum ether/ethylacetate, 1/4, v/v) to afford compound **7** as off-white solid to pale yellow solid. Yield: 60–85%.

4-(Aminoiminomethyl)-N-(4-methoxyphenyl) benzamide (7a)

Following the above general procedure title compound was synthesized. Off-white solid. Yield: 82%; M.P: 200–202 °C; IR (KBr) γ: 3336.77 (amidine-NH), 2923 (w), 1681.31 (C=O), 1647.57 (C=N), 1534.10 (NH), 1269.39 (C–N), 1249.80 (C–O), 1031.31 (C–O amide), 824.13 (*para* substitution) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.37 (s, NH), 8.04 (d, 2H, $J = 8$ Hz), 7.99 (d, 2H, $J = 8$ Hz), 7.59 (d, 2H, $J = 8.8$ Hz), 6.92 (d, 2H, $J = 8.8$ Hz), 3.75 (s, 3H), 2.65 (s, 3H, amidine) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 198.9 (C=NH), 165.3 (C=O), 156.4 (C–O), 139.1 (C–C=O), 131.8 (C–C=NH), 128.7 (2C), 128.3 (2C), 122.9 (2C), 114.3 (2C), 55.6 (O–CH_3) ppm; MS (m/z): (M^+) 269.15; Micro Analysis: Anal. Calc. for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60%; Found: C 67.20; H, 5.40; N, 15.82%.

Conclusion

Conversion from nitrile to amidine can be achieved effectively in a single step and in the presence of amide functionality using metal amide and/or ammonia gas. This method is extended for the synthesis of ten new amidines–amide conjugates where strategy works effectively in presence of heterocyclic functionality as well. Apart from nearing room temperature most of the time yield observed in the modified reaction conditions clocks above 70% for nitrile to amidine conversion. Our preliminary results confirmed that present strategy, amidine–amide conjugates can act as antiproliferative active compounds, similar to the ones observed in the literature.[18] In short, this study paves a way to synthesize not only novel amidines but also amidine–amide conjugates, a strategy for future drug design.[19]

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Supplementary data

Supplementary data (experimental procedures and full spectroscopic data for all new compounds) associated with this article can be found, in the online version. CCDC 1432792 (7j) contains the supplementary crystallographic data for this paper. These data can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data request/cif.

Conflict of interest

The authors declare no conflict of interest.

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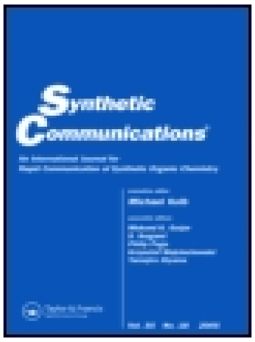

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Synthesis of new 1,2,3-triazole linked benzimidazole molecules as anti-proliferative agents

Ishani I. Sahay & Prasanna S. Ghalsasi

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Synthesis of new 1,2,3-triazole linked benzimidazole molecules as anti-proliferative agents

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ABSTRACT
One pot click chemistry is used to link triazole and benzimidazole pharmacophore to get *N*-((1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)aniline and its derivatives. Flexible linkages in the form of –CH_2–R or –O–R/–N–R were designed during synthesis. All the newly synthesized compounds were characterized by FT-IR and NMR spectroscopy as well as high-resolution mass spectrometry. Selected compounds were screened for *in vitro* anti-proliferative activity using National Cancer Institute (NCI)-60 human tumor cell line screening program. The most potent structure *N*-((1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)-4-chloroaniline **7e** showed 40% growth inhibition in renal cancer cell line (UO-31) at 10 μM concentration.

ARTICLE HISTORY
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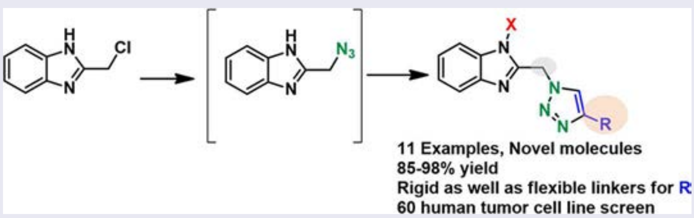
KEYWORDS
Anti-proliferative; benzimidazole; click reaction; 1,2,3-triazole

GRAPHICAL ABSTRACT



Introduction

Pharmacophore-driven synthesis for achieving biological activity is well known in literature. But designing efficient, high yielding regio-specific synthesis with more than one pharmacophore in a single molecule still remained challenge.

The broad and the potent activity of triazoles and benzimidazoles have established them as pharmacologically significant scaffolds[1–4] in an array of drug categories such as anti-microbial, anti-inflammatory, analgesic, anti-peptic, anti-viral, anti-neoplastic, anti-tubercular, anti-Parkinson's, anti-diabetic, and anti-depressant (Fig. 1).

Over the last decade 1,2,3-triazoles[5–9] have received increasing attention in medicinal chemistry[10–14] and this was made possible because of the discovery of the highly useful

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Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/lsyc.
Supplemental data full experimental details and analytical data for new molecules can be accessed on the publisher's website.

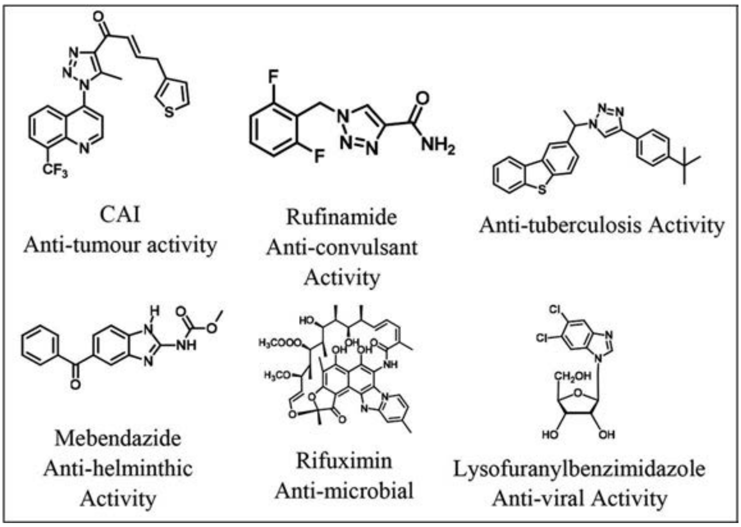


Figure 1. List of Food and Drug Administration (FDA) approved drugs with benzimidazole or triazole in their core structure.

and widely applicable 1,3-dipolar cycloaddition reaction between azide and alkynes (click chemistry)[15] catalyzed by copper salts and ruthenium complexes.[16] The most striking features of considering 1,2,3-triazoles as drug candidate,[17–20] schematically shown in Fig. 2, are (1) formation of hydrogen bonds for improving solubility and ability to interact with biomolecular targets (hydrogen bond acceptor sites, N_2 and N_3 centers; and hydrogen bond donor site, C_5 center); (2) can act as intercalating agent through π–π stacking interactions; (3) can participate in C–H hydrogen bonding interactions; (4) can substitute for an amide linkage without altering the binding pose; and (5) are highly stable to metabolic degradation as compared to other compounds containing three adjacent nitrogen (N) atoms can be metabolically inert (1,2,4-triazoles).[21–23]

On the other hand, benzimidazole, a heterocyclic aromatic organic compound, is an important pharmacophore[24,25] and a privileged structure in medicinal chemistry, especially for anti-cancer activity.[26–30] It is observed that hydrogen bond donor and acceptor site, that is, N_1 and N_3 in the benzimidazole molecule (Fig. 3), which exhibits tautomerism,[31] play a critical role in binding to the biological targets.

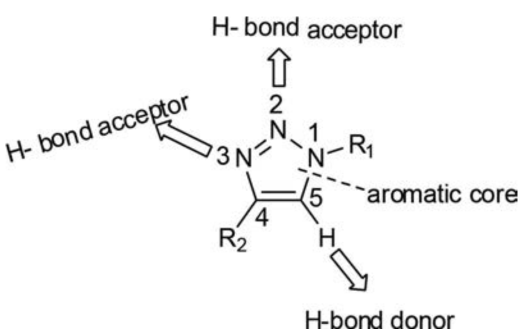


Figure 2. 1,2,3-triazoles as potential binding interactions.

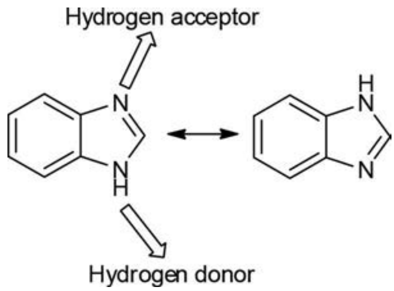


Figure 3. Benzimidazole binding interaction sites.

Results and discussion

Our efforts were focused on synthesizing new triazole linked benzimidazole molecules and checking its anti-proliferative activity, keeping pan assay interference compounds (PAINS)[32] structure in mind. Most PAINS function as reactive chemicals rather than discriminating drugs. The designing of compounds (Fig. 4) was driven by three basic principles: (1) inserting flexible linker between benzimidazole and triazole pharmacophore: use of methylene bridge; (2) derivatizing triazole at C_4 position with flexible group: CH_2–O/CH_2–N; and (3) increasing solubility and/or bioavailability by derivatizing benzimidazole pharmacophore: N-ethylation. Interestingly, this design strategy forced us to use three different synthetic routes, for synthesizing 11 new compounds, as discussed below.

Strategy 1

2-Chloro benzimidazole, **2**, was synthesized by reacting o-phenylenediamine with two different reagents (1) 2-chloroacetyl chloride and (2) 2-chloroacetic acid[6,33,34] (Scheme 1). Both these reactions gave comparable yield. **2** was transformed into 2-(azidomethyl)-1*H*-benzo[*d*]imidazole, **3**, using sodium azide in dry DMSO.[6] The reaction condition, especially solvent selection, was optimized to get the best yields. In accordance with theory, DMSO, aprotic polar solvent proved choice of solvent, since it favors conversion of **2**–**3**, S_N2 reaction, as well as 1,3-dipolar cycloaddition. Without isolating compound 3, *in situ*, alkyne derivative was added to obtain final compound **4**. Fluorine is known for its pharmacophorical activity; therefore, its addition on triazole nucleus was planned and performed similar to Scheme 1.

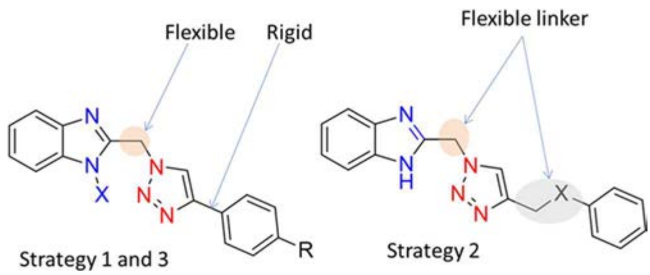
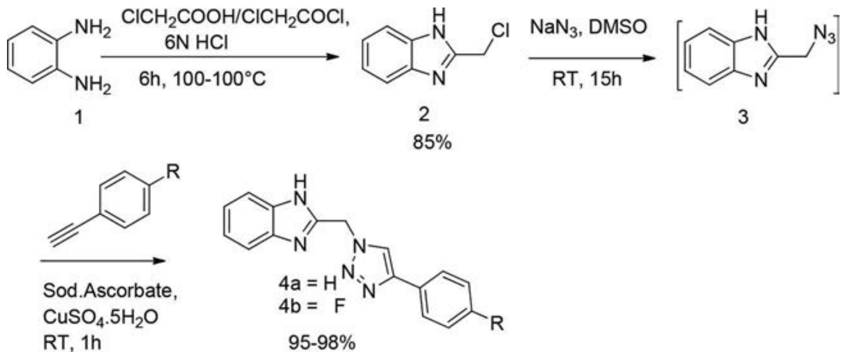


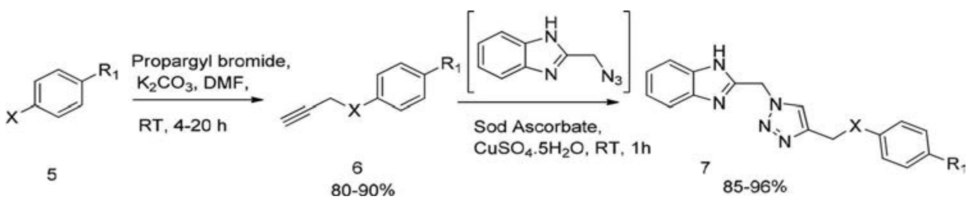
Figure 4. Designing of new triazole linked benzimidazole compounds.



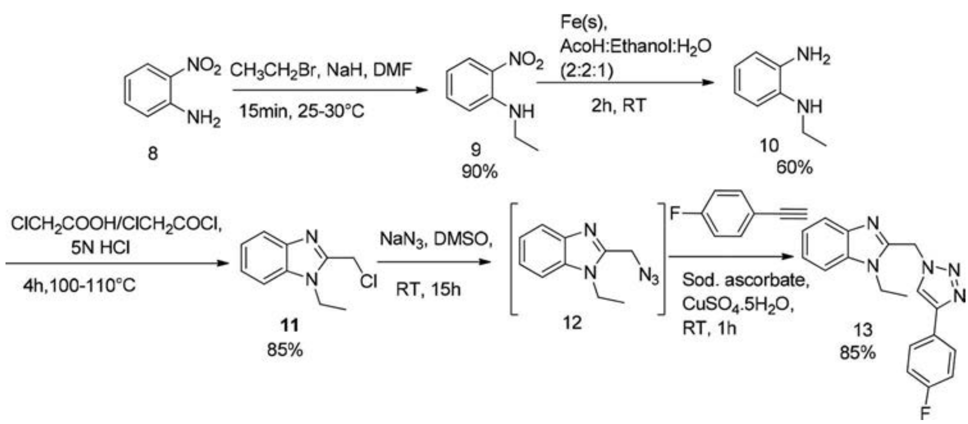
Scheme 1. Synthesis of 2-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1*H*-benzo[*d*]imidazole (**4a** and **4b**).

Strategy 2

Our aim in this strategy is to derivatize triazole ring at C_4 position using the *N*-(prop-2-yn-1-yl) aniline/phenolic groups. Substituted phenol and aniline derivatives were allowed to react with propargyl bromide in the presence of K_2CO_3 base and DMF as solvent[35,36] (Scheme 2). Terminal alkynes are known to provide an efficient method for the synthesis of triazole.[37] Krim et al.[38] reported (prop-2-ynyloxy) benzene as an intermediate, for the synthesis of triazole through Huisgen dipolar cycloaddition method using click chemistry, which is followed in the present study.

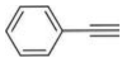
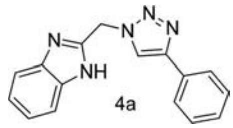
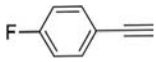
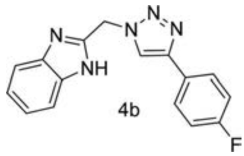
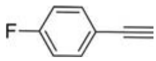
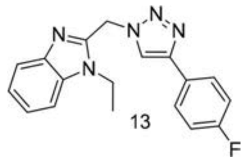
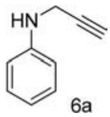
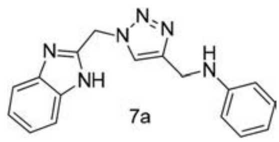
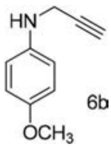
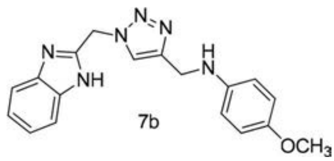
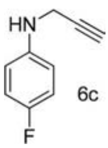
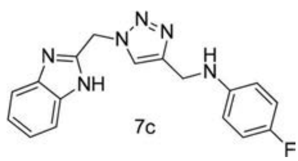
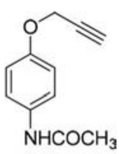
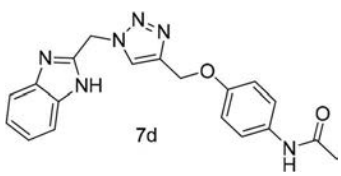
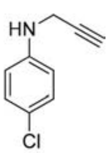
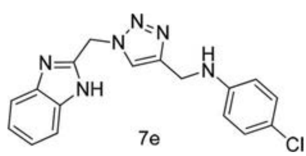
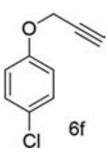
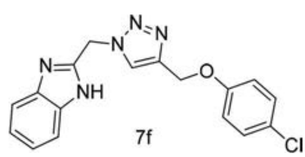


Scheme 2. Synthesis of *N*-((1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)aniline and its derivatives.



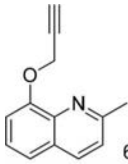
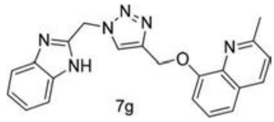
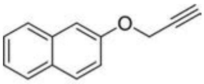
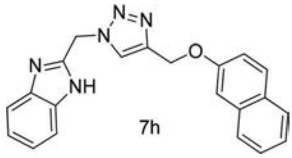
Scheme 3. Synthesis of 1-ethyl-2-((4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-1*H*-benzo[*d*]imidazole.

Table 1. New benzimidazole linked triazole compounds.

No	Terminal alkyne	Product	Yield* (%)	Melting point (°C)
1		 4a	98	185–187
2		 4b	95	135–139
3		 13	85	105–107
4	 6a	 7a	95	135–140
5	 6b	 7b	96	130–135
6	 6c	 7c	94	155–160
7	 6d	 7d	96	210–215
8	 6e	 7e	85	175–180
9	 6f	 7f	92	180–185

(*Continued*)

Table 1. Continued.

No	Terminal alkyne	Product	Yield* (%)	Melting point (°C)
10	 6g		95	105–110
11	 6h		95	175–180

*Yields are given for the final step.

To extend our study, we thought of using paracetamol as an additional pharmacophore. We synthesized, **7d**, where phenolic group of paracetamol was attached at C_4 position of triazole.

Strategy 3

Bioavailability and solubility of benzimidazole can be increased by inserting ethyl or methyl groups at N_3 position. Therefore, we carried out direct methlylation/ethylation of compound **2**. But all the attempts resulted in difficult to characterize polymeric product. Hence, *N*-ethylation is performed initially on *o*-nitro aniline (Scheme 3). *o*-Nitro aniline was converted to *N*-ethyl derivative using ethyl iodide as per the reported procedure.[39] Furthermore, compound **9** was reduced to compound **10** using iron powder.[40] All the further steps were followed according to Scheme 1.

All column purified 11 new molecules (Table 1) with triazole linked benzimidazole pharmacophore were characterized by standard spectroscopic techniques. Literature showed hydrogen bonding ability of C_3 proton in triazole molecule.[21]

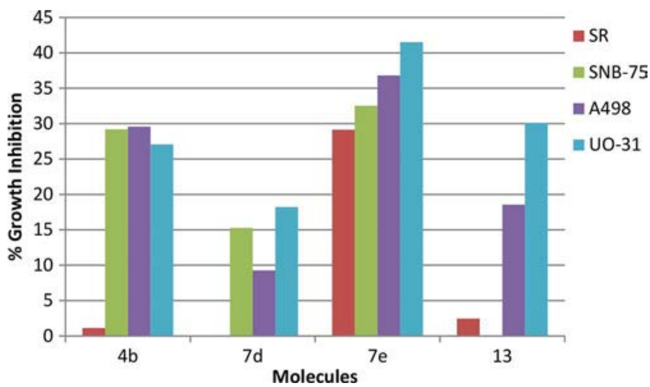


Figure 5. % Growth inhibition on selected cell lines at 10 μM concentration.

All the molecules were initially screened for anti-proliferative activity *in silico* by National Cancer Institute (NCI), USA (Experimental details are provided in Supporting Information). Compound **4b, 7d, 7e**, and **13** were further selected for actual screening anti-proliferative activity at 10 μM concentration. Figure 5 shows the comparative study of compound **4b, 7d, 7e**, and **13** on selected cell lines SR(Leukemia), SNB-75(CNS), A498(kidney), UO-31(kidney). **7e** shows 40% growth inhibition in UO-31 renal cancer cell line (mean growth inhibition) at 10 μM concentration. Although this activity is moderate, the structure of **7e** can help in developing or designing novel drug candidates.

Experimental

This includes synthesis and characterization of one of the new molecules. Experimental procedures for the intermediates and rest of the derivatives are presented in Supporting Information.

Materials and methods

All the compounds were purified using column chromatography (2000–400 mesh silica) before characterization. Thin layer chromatography (TLC) analysis was done using pre-coated silica on aluminum sheets. Melting points were recorded in Thiele's tube using paraffin oil and are uncorrected. FT-IR (KBr pellets) spectra were recorded in the 4,000–400 cm^{-1} range using a PerkinElmer FT-IR spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV-III 400 MHz spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), coupling constants (J) were expressed in hertz (Hz), and signals were described as singlet (s), doublet (d), triplet (t), broad (b) as well as multiplet (m). High-resolution mass spectrometry was performed on Micromass Q-TOF Micro instrument. All chemicals and solvents were of commercial grade and were used without further purification.

*General method for synthesis of 2-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1*H*-benzo[*d*]imidazole 4a*

Synthesis of compound **4a**[41] was done in single step. A mixture of compound **2** (0.500 g, 3 mmol) and sodium azide (0.195 g, 3 mmol) was charged in DMSO (5 mL) at room temperature for 15–16 h.[22] Reaction completion was monitored using TLC (EtOAc: PET, 7:3). After *in situ* formation of compound **3**, the respective alkyne derivative (0.321 g, 3.14 mmol) was added and mixture of $CuSO_4 \cdot 5H_2O$ (0.112 g, 0.4 mmol) and sodium ascorbate (0.267 g, 1.35 mmol) in water (0.5 mL) was added as shown in Scheme 1.[25,26] The reaction was stirred at room temperature and its completion was monitored by TLC. Compound **4a** was then isolated and further purified by column chromatography using various concentrations of ethyl acetate and petroleum ether to afford the desired compound **4a** as a white solid. FT-IR (KBr) γ: 690.52 (Mono substitution), 738.74 (*o*-substitution), 1089.78 (C–N stretch), 1276.88 (C–N stretch), 1442.75 (N=N), 1587.42 (C=N), 3095.75 (N–H stretch) cm^{-1}. ^{1}H NMR(400 MHz, DMSO-d_6) δ: 5.96 (2H, s), 7.19 (2H, t, $J = 9.6$ Hz), 7.35 (1H, m), 7.45 (2H, t, $J = 8$ Hz), 7.50 (1H, d, $J = 7.6$ Hz), 7.61 (1H, d, $J = 7.6$ Hz), 7.89 (2H, d, $J = 8$ Hz), 8.71 (1H, s), 12.69 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 52.7, 127.4, 130.4, 133.3, 134.2, 135.7, 151.8, 153.5 ppm; HR-MS (ESI+) [$C_{16}H_{13}N_5$+Na]: Calculated, 298.1069; Observed, 298.1064.

*1-Ethyl-2-((4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-1*H*-benzo[*d*]imidazole 13*

Title compound was synthesized following the same procedure as reported for compound **4a** and isolated as white solid. FT-IR (KBr) γ: 734.88 (*o*-substitution), 829.39 (*p*-substitution), 1228.66 (C–N stretch), 1458.18 (N=N), 1496.76 (–C=N of benzimidazole), 2972.31 (–C–H); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (3H, t, $J = 7.2$ Hz), 4.40 (2H, q, $J = 7.2$ Hz), 6.08 (2H, s), 7.22 (1H, d, $J = 7.2$ Hz), 7.28 (3H, t, $J = 8.8$ Hz), 7.62 (2H, t, $J = 6.8$ Hz), 7.93 (2H, t, $J = 5.2$ Hz), 8.705 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.3, 38.8, 46.6, 111, 116.2, 116.4, 119.7, 122.4, 122.6, 123.2, 127.5, 127.5, 127.7, 127.8, 135.2, 142.5, 146.3, 148.3, 161.1, 163.5 ppm; HR-MS (ESI+) [$C_{18}H_{16}FN_5$+Na]: Calculated, 344.1287; Observed, 344.1284.

Conclusion

In conclusion, click reaction can conjoin triazole pharmacophore with benzimidazole in a faster and efficient method. The reaction proceeds without isolation of azide intermediate without compromising in good yields. Commercially available *o*-phenylenediamine was used during the synthesis of series of *N*-((1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)aniline compounds. The *in vitro* anti-cancer activity of selected compounds using NCI-60 human cell line screening program revealed that most of the title compounds showed moderate bioactivity at 10 μM concentration. Flexible structured molecule, **7e**, *N*-((1-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)-4-chloroaniline, showed a 40% growth inhibition in human renal cancer cell line (UO-31) which needs further investigation.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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