QUESTA E' LA VERSIONE REFERATA DI QUELLA PUBBLICATA IL 5 APRILE 2015;

https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/ejoc.201600210

One-Pot Synthesis of Novel Bi-heterocycles Based on Indole and Azole Scaffolds Using Tryptamines and 1,2-Diaza-1,3-dienes as Building Blocks

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Dedicated to Prof. Achille Umani Ronchi on the occasion of his 80th birthday

Abstract: The rapid and expedient assembly of three new classes of bi-heterocycles of biological interest, viz.: indole-imidazole (**4**), indole-pyrrole (**6**) and indole-triazole (**8**) was accomplished using different combination of tryptamine, 1,2-diaza-1,3-diene, aldehyde and/or alkyne derivatives as readily available building blocks. Twenty-six derivatives were thus prepared in excellent yields (up to 100%) and screened for *in vitro* biological studies. Some of these revealed promising anticancer activity against MCF7 and Caco-2 human tumor cell lines.

Introduction

Since the majority of drugs contain heterocyclic compounds, in particular nitrogen heterocycles,[1,2] a substantial effort has been dedicated in designing new classes of structural entities of medicinal importance. Among them, the indole structure, regarded as *The Lord of the Rings* of heterocyclic compounds, represents the structural core of many natural and non-natural products with a wide range of biological properties.[3] Over the past decades, synthesis and screening of these heterocyclic compounds for drug discovery has been a subject of constant interest in medicinal chemistry. Derivatization of these heterocyclic pharmacophores represents a convenient approach to generate chemical diversity during lead identification and optimization. A careful inspection of a notably array of indole-containing compounds evidences the tryptamine (Tryp) moiety[4] as a common recognizable motif in the whole structure. For example, a number of Tryp derived substances also known as 'triptans' (e.g., sumatriptan, rizatriptan, zolmitriptan) are used for the treatment of migraine headaches.[5] Other drugs belonging to the Tryp class have been reported as efficient agents with high selectivity for the 5-HT_6 serotonin receptor.[6] In addition, tryptamine is a direct precursor to many alkaloid natural products, including approximately 3000 monoterpene indole alkaloids,[7] and is frequently used chemical for many intents and purposes such as the total synthesis of pharmacologically active indole-based alkaloids. Also, the branched tryptamine marine natural alkaloid 2,2-bis(6-bromo-3-indolyl) ethylamine, a member of the large diindole methane family of alkaloids, has generated attention owing to the novel biological activity in leukemia cells (U937)[8] of this class of symmetrical bisindoles. Thus, due to the medicinal and pharmaceutical importance of Tryp derivatives the use of these scaffolds as chemical "navigator"[9] for the synthesis of novel bi-heterocyclic architectures[10] is an active area of research.

On the other hand azoles such as pyrroles,[11] imidazoles,[12] and triazoles[13] constituite important five membered rings of the aromatic heterocycle family due to their presence in a number of biologically active natural products.

Despite many decades of research to efficiently produce mono-azaheterocycles, and participation of tryptamine in many metabolic pathways, methods of generating products with both units, which are ubiquitous elements of biomolecules and pharmacophores, have not been well developed.

Thus, it was thought that an indole core, if coupled to an azole moiety, the resulting compound might produce new entities with unexpected improvements on the interaction with biological targets through a synergistic effect.[14] Recognizing the need for rapid routes to construct hybrid bi-heterocycles that allows efficient preparation of diverse small molecule libraries, newly designed *"indole-azole"* scaffolds such as indole-imidazole (**4**), indole-pyrrole (**6**) and indole-triazole (**8**) have been assembled and evaluated for their *in vitro* antitumoral activities.

The strategies we envisioned utilizes common substrates, specifically tryptamines (Tryps) and 1,2-diaza-1,3-dienes (DDs)[15] to construct indolyl-ethyl azoles through mechanistically different pathways. Three Michael-initiated aza-annulation strategies of general type (Scheme 1) to reach aforementioned targets were considered: a) one-pot sequential addition of tryptamine nucleophiles to DDs followed by thermal assisted cyclization in the presence of aldehydes (route A); b) one-pot sequential addition of Tryps to alkynoates followed by thermal and acid promoted addition/cyclization of DDs (route B); c) one-pot addition reaction of tryptamine derivatives to in situ formed DDs followed by cyclization (route C).

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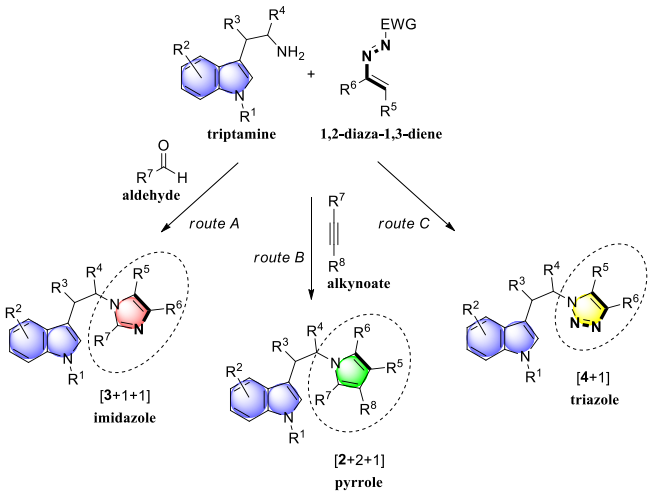
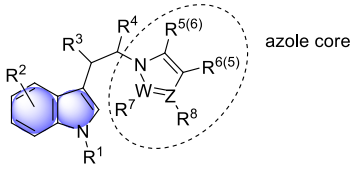


Scheme 1. Outline of Synthetic Strategy towards Substituted Indole-Containing Azoles (1,2-diaza-1,3-diene building block highlighted).

Notably, each of these three bi-heterocyclic scaffolds contains up to eight points of diversity (R1 to R8): two positions at indole core (C5 or C6 and N1 substitution), up to four positions at azole core (C2, C4 and C5 substitution in imidazole; C2, C3, C4 and C5 substitution in pyrrole; C4 and C5 substitution in triazole), and two diversity position at the ethylene spacer between the heterocyclic systems. (Figure 1).



Indole-Imidazole (W=C; Z=N) **4**
Indole-Pyrrole (W,Z=C) **6**
Indole-Triazole (W,Z=N) **8**

Figure 1. General structure of the target bi-heterocycles.

Results and Discussion

The generation of 1,2-diaza-1,3-dienes from hydrazone derivatives by the polarity reversal of carbonyl functional groups represents one valuable umpolung tactic, which provides a useful complementary strategy to access variously functionalized heterocyclic compounds.[15] In our research on the synthetic potential of 1,2-diaza-1,3-dienes in organic synthesis, we have recently developed a convenient one-pot preparation of imidazo-4-carboxylates via 1,5-electrocyclization reaction.[16] The imidazole ring is generated by one-pot sequential reaction between primary amines or aminoesters and DDs followed by heating in the presence of aldehydes. To further expand the scope of this three component reaction (3CR), we herein report a convenient chemo- and regioselective one-pot assembly to a new class of bi-heterocycles *e.g.*, *N*-linked (imidazol-1-yl)triptamine derivatives (**4**) by using Tryp derivatives (**1**) as amine components (Figure 2).

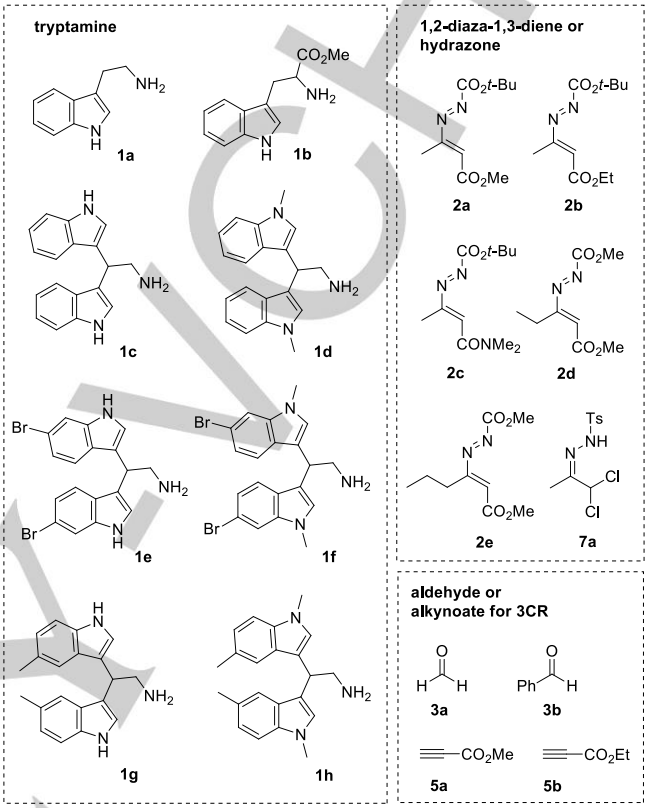
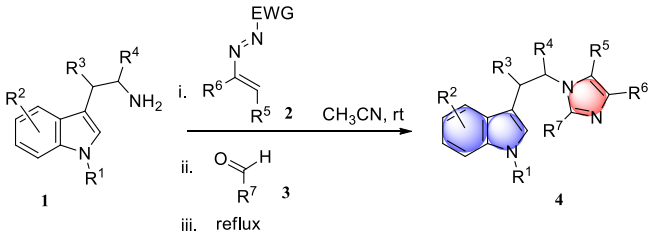


Figure 2. Components used for bi-heterocycles assembly.

Thus, the reactions between different substituted Tryp substrates (**1a−c,e−h**), DDs (**2a−e**) and aldehydes (**3a,b**) were examined, and the results summarized in Table 1. It was found that the reaction was compatible with various tryptamine derivatives including tryptamine (**1a**), tryptophan (**1b**) and different substituted bis-indolylethanamines (**1c,e−h**). Notably, *N*-H and *N*-Me protected indoles as well as electron-rich (5-methyl), electron-deficient (6-bromo), and neutral groups on the benzo ring were well tolerated affording the corresponding products **4** in good to excellent yields. Also, DDs **2a−e** differently substituted (Table 1, EWG = CO_2Me, CO_2t-Bu; R^5 = CO_2Me, CO_2Et, $CONMe_2$; R^6 = Me, Et, Pr) were compatible with this protocol. In the case of the aldehyde partner both paraformaldehyde and benzaldehyde were used. When benzaldehyde **3b** was used, *N*-linked (imidazol-1-yl)triptamine **4** was still obtained, albeit in lower yield (Table 1, entry 4).

The mechanism for the formation of the indole-imidazole system appears to involve the conjugated addition of tryptamine to 1,2-diaza-1,3-diene followed by treatment with aldehyde to yield iminium ion which undergoes 1,5-electrocyclization, and subsequent aromatization with loss of carbamate to afford the desired imidazole ring.[16]

Table 1. Sequential Three Component Synthesis of Indole-Containing Imidazoles **4**.



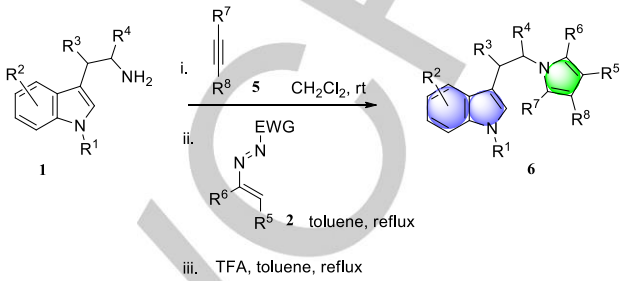
Entry	Tryp **1**	DD **2**	Aldehyde **3**	Indole-Imidazole **4**	Yield (%)[a]
1	**1a**	**2a**	**3a**	**4aaa**	98
2	**1a**	**2c**	**3a**	**4aca**	70
3	**1a**	**2d**	**3a**	**4ada**	76
4	**1a**	**2a**	**3b**	**4aab**	24
5	**1b**	**2a**	**3a**	**4baa**	53
6	**1b**	**2b**	**3a**	**4bba**	67
7	**1b**	**2d**	**3a**	**4bda**	73
8	**1c**	**2a**	**3a**	**4caa**	75
9	**1e**	**2a**	**3a**	**4eaa**	72
10	**1e**	**2d**	**3a**	**4eda**	83
11	**1f**	**2e**	**3a**	**4fea**	55
12	**1g**	**2a**	**3a**	**4gaa**	69
13	**1g**	**2d**	**3a**	**4gda**	85
14	**1h**	**2b**	**3a**	**4hba**	77

[a] Yield of the isolated purified compounds **4** based on the starting Tryps **1**.

Encouraged by the successful incorporation of the DDs **2** as annulation reaction partners in the 1,5-electrocyclization, we next explored the possibility to assemble the pyrrole nucleus instead of imidazole. Thus, treatment of Tryps **1a,d−f,h** with alkynoates **5a,b** in CH_2Cl_2 followed by addition of DDs **2a,b** in toluene at reflux and upon acid catalysis furnished indole-pyrrole **6** (Table 2)[17]. Commercially available tryptamine (**1a**), easily prepared bis(indolyl)ethanamino derivatives (**1d−f,h**) with either methyl- and ethyl-propiolate (**5a,b**) and DDs (**2a,b**) were compatible with the procedure described above.

The mechanism for the formation of the indole-pyrrole system probably involve the preliminary formation of the tryptamine-derived enamino ester via conjugated addition of Tryp to alkynoate followed by in situ Michael addition with 1,2-diaza-1,3-diene compound and subsequent intramolecular ring closure with loss of carbazate.[17]

Table 2. Sequential Three Component Synthesis of Indole-Containing Pyrroles **6**.



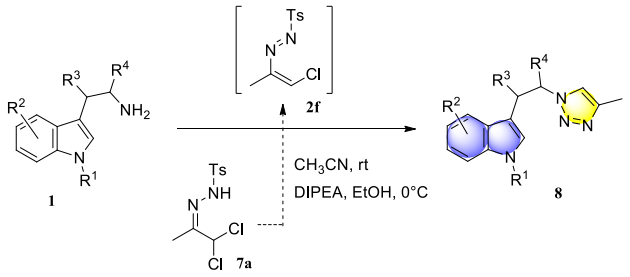
Entry	Tryp **1**	DD **2**	Alkynoate **3**	Indole-Pyrrole **6**	Yield (%)[a]
1	**1a**	**2a**	**5b**	**6aab**	57
2	**1a**	**2b**	**5a**	**6aba**	59
3	**1d**	**2b**	**5a**	**6dba**	37
4	**1e**	**2a**	**5b**	**6eab**	70
5	**1f**	**2b**	**5a**	**6fba**	31
6	**1h**	**2a**	**5b**	**6hab**	40

[a] Yield of the isolated purified compounds **6** based on the starting Tryps **1**.

Finally, inspired by previous Westermann's[18] study on Sakai reaction,[19] we focused on the construction of the indole-triazole structure (**8**), which is a recurrent motif in pharmaceutical compounds. As elegant and mild alternative metal-free strategy to the traditional Huisgen (azide-alkyne) cycloaddition,[20] we employed the Sakai reaction of α,α-dichlorotosylhydrazone **7a** (as DD precursor) and tryptamine derivatives (**1a−d,f,h**) to prepare indole-triazole **8** in a single step. In accordance with this synthetic protocol, in situ generated 1,2-diaza-1,3-diene **2f** from the hydrazone precursor **7a** would serve as a four- atom component (C2N2 building blocks) for [4+1] annulation. The reactions were performed in a solvent mixture of acetonitrile and ethanol (1:1 v/v %) in the presence of six equivalents of DIPEA. All the tryptamine substrates (**1a−d,f,h**) tested were suitable for the synthesis of various indole-containing triazoles **8** and the yields of the isolated products were excellent (Table 3).

The accepted mechanism for the Sakai two component reaction (2CR) involve first the Michael addition of Tryp component on in situ formed chloro-1,2-diaza-1,3-diene **2f** by deprotonation of α,α-dichlorotosylhydrazone **7a**. After deprotonation and elimination of the tosyl group, subsequent cyclization would furnish the triazole ring.[19]

Table 3. Two Component Synthesis of Indole-Containing Triazoles **8**.



Entry	Tryp **1**	Hydrazone **7a**	Alkynoate **3**	Yield (%)[a]
1	**1a**	**7a**	**8aa**	95
2	**1b**	**7a**	**8ba**	quant.
3	**1c**	**7a**	**8ca**	quant.
4	**1d**	**7a**	**8da**	90
5	**1f**	**7a**	**8fa**	89
6	**1h**	**7a**	**8ha**	87

[a] Yield of the isolated purified compounds **8** based on the starting Tryps **1**.

Preliminary screening to assess the biological activity of newly synthesized compounds was carried out against two human cancer cell lines, MCF7 and Caco-2. The results of the *in vitro* antitumor activities of selected compounds as compared to either reference compounds 2,2-bis(6-bromo-3-indolyl) ethylamine (**1e**) and 3,3'-diindolylmethane (DIM)[21] are reported in Table 4.

Table 4. In vitro Anticancer Activities of Indole-Containing Azoles **4**, **6**, **8**.

Entry	Compound	IC_{50} (µM)[a] MCF7	IC_{50} (µM)[a] Caco-2
1	**4aaa**	–	43.8 ± 8.3
2	**4ada**	–	–
3	**4aab**	–	–
4	**4baa**	–	–
5	**4bda**	~ 50	–
6	**4caa**	~ 40	16.3 ± 6.4
7	**4eaa**	10.0 ± 0.1	11.5 ± 1.9
8	**4eda**	12.1 ± 1.2	9.5 ± 0.4
9	**4fea**	64.1 ± 4.5	32.4 ± 9.1
10	**4gaa**	12.0 ± 0.1	31.8 ± 7.2
11	**4gda**	12.4 ± 0.1	11.2 ± 0.4
12	**6eab**	42.2 ± 5.6	8.5 ± 2.4
13	**8ba**	–	–
14	**8da**	76.9 ± 12.8	16.5 ± 3.1
15	**8fa**	–	23.9 ± 6.2
16	**1e**	4.4 ± 1.9	1.5 ± 0.2
17	**DIM**	49.8 ± 6.4	12.8 ± 2.0

[a] IC_{50} (µM) is 50% inhibitory concentration.

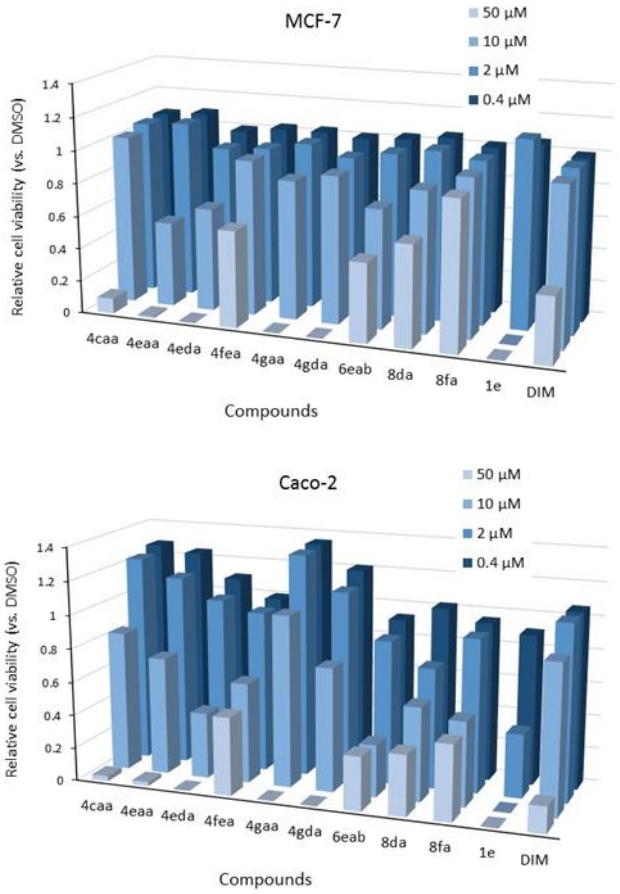


Figure 3. Effect of **4caa, 4eaa, 4eda, 4fea, 4gaa, 4gda, 6eab, 8da, 8fa, 1e** and **DIM** on MCF-7 and Caco-2 cell viability. Cells were treated for 3 days with 0.4, 2, 10 and 50 µM of selected compounds or vehicle only (DMSO). Cell viability was evaluated by MTS assay. Data are expressed as relative cell viability vs. controls (vehicle only).

As evidenced, compounds **4caa, 4eaa, 4eda, 4fea, 4gaa, 4gda, 6eab, 8da, 8fa** exhibited moderate to very good cytotoxicity in DMSO. Detailed dose-responses of the active compounds are reported in figure 3. All these results highlight the importance of

the 2,2-bis(3-indolyl) ethylamino template into the bioactive parent DIM.[21,22]

Conclusions

In summary, rapid and efficient synthetic pathways to build different kinds of ethylene linked bi-azaheterocycles viz., indolyl-imidazoles (**4**), pyrroles (**6**) and triazoles (**8**) has been accomplished employing the azoalkene chemistry. In these reactions, 1,2-diaza-1,3-diene serve as two- (2C), three- (2C+1N), or four (2C+2N) synthons undergoing [2+2+1], [3+1+1], or [4+1] annulations in both 2 and 3CRs, when opportunely coupled with tryptamines **1** (2CRs) or tryptamine **1**, and aldehydes/alkynoates **3**/**5** (3CRs). Furthermore, the use of bis(indolyl)ethylamine derivatives (**1c−h**) allows the possibility of introduce the biologically important DIM (3,3'-diindolylmethane) molecular unit (or two indole units) into the corresponding tricyclic structures **4**, **6**, **8** which be useful for further synthetic elaborations, e.g. for the preparation of indolocarbazole (ICZ)[23] derivatives. On the basis of the breadth of biological activity known for indoles and azoles, the incorporation of these two privileged pharmacophores into novel bi-heterocyclic scaffolds as a new application of versatile 1,2-diaza-1,3-diene building blocks in organic synthesis, could open the way towards new entities of potential usefulness in medicinal chemistry. Besides, each resulting bi-heterocycle having different reactive sites/functional groups could provide further opportunities of derivatization to new highly attractive complex structures.

Experimental Section

General Remarks: All the commercially available reagents and solvents were used without further purification. Indoles (**9a−f**), tryptamine (**1a**), tryptophan methyl ester hydrochloride (**1b**), paraformaldehyde (**3a**), benzaldehyde (**3b**), methyl and ethyl propiolate (**5a** and **5b**) were commercial materials; *N*-trifluoroacetylamino acetal (**10**), bis-indolylethanamines (**1c−h**), and 1,2-diaza-1,3-dienes (DDs) **2a–e** were synthesized according to literature procedure (see Supporting Information). Chromatographic purification of compounds was carried out on silica gel (60–200 μm). TLC analysis was performed on pre-loaded (0.25 mm) glass supported silica gel plates (Kieselgel 60); compounds were visualized by exposure to UV light and by dipping the plates in 1% $Ce(SO_4)\cdot 4H_2O$, 2.5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 10% sulphuric acid followed by heating on a hot plate. All ^1H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using [D_6]DMSO or CD_3OD as solvent. Chemical shift (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent and are sorted in ascending order within each group. The following abbreviations are used to describe peak patterns where appropriate: s = singlet, d = doublet, t = triplet q = quartet, sex = sextet, m = multiplet and br = broad signal. All coupling constants (*J* value) are given in Hertz [Hz]. FT-IR spectra were obtained as Nujol mulls or neat. Mass spectra were recorded in the EI or ESI mode. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were within ± 0.4 of the theoretical values (C, H, N).

General procedure for the Synthesis of Indole-Containing Imidazoles (4): To a stirred solution of tryptamine derivative **1** (0.4 mmol) in acetonitrile (2 mL) DD derivative **2** (0.4 mmol) was added at room temperature. After the disappearance of the reagents, aldehyde **3** (0.8 mmol) was added, and then the resulting mixture was refluxed for 2–4 h (TLC check). The solvent was evaporated under reduced pressure and the crude residue was purified by column chromatography to yield the desired indole-imidazole **4**. In the case of tryptophan methyl ester hydrochloride (**1b**) the following modified procedure was used to a stirred solution of **1b** (0.4 mmol) in $CHCl_3$ (2 mL) DIPEA (0.4 mmol) was added at room temperature. After 30 min., DD **2** (0.4 mmol) was added and stirring was continued for additional 3 h at room temperature until completion of the reaction (TLC check). The reaction mixture was diluted with chloroform (5 mL) and washed with saturated NH_4Cl aq. (3 × 5 mL). After drying over dry Na_2SO_4, the organic solution was concentrated in vacuo and the resulting crude product was subjected to subsequent treatment with aldehyde **3** (0.8 mmol) in acetonitrile (2 mL) at reflux as above described.

Methyl 1-(2-(1*H*-indol-3-yl)ethyl)-4-methyl-1*H*-imidazole-5-carboxylate (4aaa): Indole-Imidazole **4aaa** was isolated by column chromatography (ethyl acetate/cyclohexane 50:50) in 98% yield (111.1 mg) (entry 1, Table 1); Pale yellow solid; mp: 181−183 °C; ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 2.33 (s, 3 H, CH_3), 3.05 (t, *J* = 7.6 Hz, 2 H, CH_2CH_2N), 3.79 (s, 3 H, OCH_3), 4.44 (t, *J* = 6.4 Hz, 2 H, CH_2CH_2N), 6.97 (t, *J* = 6.8 Hz, 1 H, Ar-H), 7.03−7.09 (m, 2 H, Ar-H), 7.32 (d, *J* = 8.0 Hz, 1 H, Ar-H), 7.57 (d, *J* = 8.0 Hz, 1 H, Ar-H), 7.63 (s, 1 H, Ar-H), 10.84 (s, 1 H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C): δ = 15.9, 27.3, 40.0, 51.3, 111.3, 111.5, 118.0, 118.3, 119.5, 122.2, 122.5, 126.9, 136.3, 140.6, 148.5, 161.5; IR (nujol): ν_{max} = 3367, 1703 cm^{-1}; MS (EI) *m/z* (%) = 283 (M^+) (69), 154 (77), 130 (100); anal. calcd. for $C_{16}H_{17}N_3O_2$ (283.33): C 67.83, H 6.05, N 14.83; found: C 67.71, H 5.96, N 14.75.

General procedure for the Synthesis of Indole-Containing Pyrroles (6): A mixture of tryptamine derivative **1** (0.4 mmol), and alkynoate **5** (0.44 mmol) was stirred in dichloromethane (1 mL) overnight at room temperature. After the disappearance of the reagents, DD **2** (0.6 mmol) in toluene (4 mL) was added and the reaction was refluxed for 2 h. Catalytic amount of TFA (2 drops) was added once the DD was consumed completely (TLC check) and the reaction was refluxed for additional 2-4 h. After removal of the solvent, the crude mixture was purified by column chromatography on silica gel to afford product **6**.

4-Ethyl 3-methyl 1-(2-(1*H*-indol-3-yl)ethyl)-2-methyl-1*H*-pyrrole-3,4-dicarboxylate (6aab): Indole-Pyrrole **6aab** was isolated by column chromatography (ethyl acetate/cyclohexane 35:65) in 57% yield (80.8 mg) (entry 1, Table 2); Pale yellow oil; ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 1.23 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3), 2.22 (s, 3 H, CH_3), 3.09 (t, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 3.69 (s, 3 H, OCH_3), 4.09−4.19 (m, 4 H, CH_2CH_2N), 7.01 (t, *J* = 7.2 Hz, 1 H, Ar-H) 7.06−7.13 (m, 2 H, Ar-H), 7.35−7.39 (m, 2 H, Ar-H), 7.58 (t, *J* = 8.0 Hz, 1 H, Ar-H), 10.87 (s, 1 H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C): δ = 10.6, 14.7, 26.9, 47.5, 51.4, 59.8, 110.6, 111.9, 112.5, 114.4, 118.7, 118.9, 121.5, 123.7, 126.9, 127.4, 135.0, 136.6, 163.8, 165.7; IR (nujol): ν_{max} = 3386, 1683, 1701 cm^{-1}; MS (EI) *m/z* (%) = 354(M^+) (26), 130 (100); anal. calcd. for $C_{20}H_{22}N_2O_4$ (354.40): C 67.78, H 6.26, N 7.90; found: C 67.91, H 6.15, N 8.01.

General procedure for the Synthesis of Indole-Containing Triazoles (8): To a cooled solution (0 °C) of tryptamine derivative **1** (0.4 mmol) in ethanol (5 mL) was added *N*,*N*-diisopropylethylamine (2.4 mmol, 6 eq.; 2.8 mmol, 7 eq. only when **1b** was used). The solution was stirred for 10 min. after which hydrazone **7a** (0.52 mmol, 1.3 eq.) dissolved in acetonitrile (4 mL) was after to the cooled solution and stirring was continued at room temperature until completion of the reaction (TLC check). After completion of the reaction all volatiles were removed under reduced pressure and the residue was purified by column chromatography to yield **8**.

3-(2-(4-methyl-1*H*-1,2,3-triazol-1-yl)ethyl)-1*H*-indole (8aa): Indole-Triazole **8aa** was isolated by column chromatography (ethyl acetate/cyclohexane 70:30) in 95% yield (86.0 mg) (entry 1, Table 3); Yellow oil; ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 2.21 (d, *J* = 0.8 Hz, 3 H, CH_3), 3.25 (t, *J* = 7.2 Hz, 2 H, CH_2CH_2N), 4.58 (t, *J* = 7.2 Hz, 2 H, CH_2CH_2N), 7.00 (td, J_1 = 8.0 Hz, J_2 = 0.8

Hz, 1 H, Ar-H), 7.06−7.12 (m, 2 H, Ar-H), 7.35 (dt, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1 H, Ar-H), 7.53 (d, J = 8.0 Hz, 1 H, Ar-H), 7.78 (d, J = 0.8 Hz, 1 H, Ar-H), 10.83 (s, 1 H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C): δ = 11.0, 26.4, 50.2, 110.6, 111.9, 118.6, 118.9, 121.5, 122.6, 123.6, 127.4, 136.6, 142.1; IR (nujol): ν_{max} = 3368 cm^{-1}; MS (EI) *m/z* (%) = 226 (M^+) (10), 143 (100),130 (98); anal. calcd. for $C_{13}H_{14}N_4$ (226.28): C 69.00, H 6.24, N 24.76; found: C 68.87, H 6.18, N 24.89.

Biological Tests

Cell Cultures: The human breast cancer MCF-7 and colorectal cancer Caco-2 cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in DMEM supplemented with 10 % fetal bovine serum, 10 mg/ L insulin (MCF-7), 2 mmol/L L-glutamine, 1x MEM Non-essential Amino Acid Solution, 0.1 mg/ml streptomycin and 0.1 U/L penicillin. Cells were maintained in a humidified incubator (5 % CO_2) at 37 °C. All cell culture materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).

MTS cell proliferation assay: Triplicate samples of 5x10^3 MCF-7 and Caco-2 cells in 96-well plates were treated for 3 days with 0.2, 2, 10 and 50 µM of the indole-containing compounds. Cell viabilities were evaluated using a CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) based on the ability of viable cells to convert soluble tetrazolium salt (MTS) into a formazan product (see Supporting Information). The results are expressed as the relative number of viable cells in treated samples relative to controls (DMSO-treated cells).

Acknowledgements

We gratefully acknowledge the financial support from the University of Urbino “Carlo Bo”, and the PhD Programs of Ministry of Education of Italy.

Keywords: bi-heterocycles • indoles • azoles • tryptamines • 1,2-diaza-1,3-dienes

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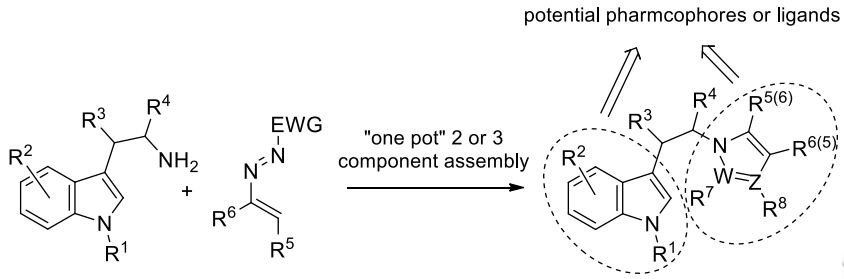

The incorporation of indole and azole pharmacophores into novel bi-heterocyclic scaffolds as potential anticancer agents was realized using different combination of tryptamine, 1.2-diaza-1,3-diene, aldehyde and/or alkyne derivatives as readily available building blocks.

Keywords: **Tryptamines / 1,2-Diaza-1,3-dienes / Indoles / Azoles / Anticancer activity**

Nitrogen Heterocycles

Serena Mantenuto, Simone Lucarini, Mauro De Santi, Giovanni Piersanti, Giorgio Brandi, Gianfranco Favi, and Fabio Mantellini*

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One-Pot Synthesis of Novel Bi-heterocycles Based on Indole and Azole Scaffolds Using Tryptamines and 1,2-Diaza-1,3-dienes as Building Blocks