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A greener approach towards double heteroarylation of N, O and S nucleophiles: synthesis of bioactive polynuclear fused *N*-heteroarenes†

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A catalyst, ligand and solvent free method for double heteroarylation of N, O and S nucleophiles has been developed for the first time leading towards the synthesis of compounds containing an indole ring fused with pyrrolo-, furo- and thieno[2,3-*b*]quinoxaline moieties. This general and greener approach afforded novel compounds of medicinal importance.

Polynuclear fused *N*-heteroarenes are attractive templates for the discovery and development of new bioactive molecules and drugs. For example, the indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine scaffold has been used for the identification of potent antitumor agents.[1] Likewise, the indolo[2,3-*b*]quinoxaline[2] framework has been used for the identification of anti-viral (*e.g.* B-220, Fig. 1)[2a] and anti-tumor agents (*e.g.* NCA0424 and NCA0465, Fig. 1).[2b] Additionally, polynuclear fused *N*-heteroarenes can be realized by introducing conformational restriction to their flexible parent molecules as this strategy could provide valuable insights regarding the interaction of the precursory flexible molecule with the putative receptor or enzyme.[2c] All these reports and our continuing interest in quinoxaline derivatives[3] prompted us to explore indolo[3′,2′:4,5]pyrrolo-, indolo[3′,2′:4,5]furo- and indolo[3′,2′:4,5]thieno[2,3-*b*]quinoxaline derivatives represented by **A** (Fig. 2) as potential and novel antiproliferative agents.

While various polynuclear fused *N*-heteroarenes are known in the literature[4] molecules represented by **A** are unknown. It was therefore necessary to develop a suitable and general synthetic route for accessing molecules designed based on **A**. A retrosynthetic analysis of compound **A** revealed that its synthesis can be achieved *via* a 2-(1*H*-indol-3-yl)quinoxaline species **B** which in turn could be accessed from an indole derivative **C** (Fig. 2). While this route appeared to be feasible the major challenging issues however emerged as if conversion of **B** to **A** could be performed (i) with high efficiency (considering unknown reactivity of **B** towards the reactants employed), (ii) under metal catalyst free conditions, (iii) *via* a single-step method that is common and general for X = NR^2, S, O *etc.* (Fig. 1). We envisaged that both the leaving groups of **B** could be displaced by a single nucleophile in a single step under appropriate reaction conditions to afford **A**. Herein we report our preliminary results on a catalyst, ligand and solvent free greener synthesis of **A** or **3** from **1** (Scheme 1).

The key starting material **1** was prepared from indolin-2-one (**4**) that was converted to **5** (Scheme 2).[5] The quinoxaline ring was constructed using the oxo-ester side chain of **5** following a

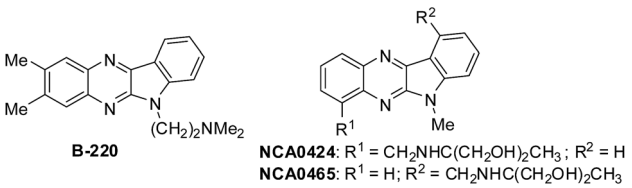

Fig. 1 Examples of reported bioactive indolo[2,3-*b*]quinoxaline derivatives.

Fig. 2 New polynuclear fused *N*-heteroarenes (A) and its retrosynthetic analysis.

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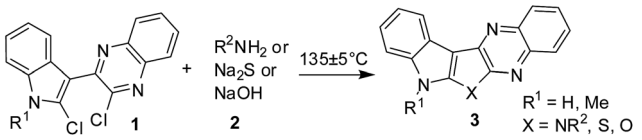
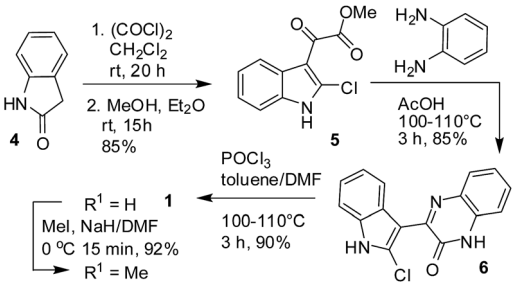
† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, and copies of spectra. See DOI: 10.1039/c5ra16727b



Scheme 1 Catalyst, ligand and solvent free synthesis of compound **3**.

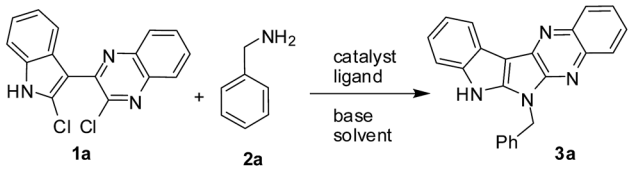
similar procedure reported earlier[6] to give **6**. The chlorination of **6** afforded **1** that was either used directly (or after *N*-methylation) for the next step. The Pd-catalyzed double *N*-arylation of primary amines with 2,2′-dihalobiphenyl has become a powerful method for the construction of central 5-membered pyrrole ring of various carbazole derivatives.[7] The methodology has been used for the elegant synthesis of several carbazole based alkaloids or natural products. We took inspiration from these works and decided to use a similar approach *i.e.* a double heteroarylation strategy for our synthesis. Accordingly, the compound **1a** was reacted with amine **2a** under various conditions (Table 1). Initially, several combinations of a Pd catalyst [*e.g.* $Pd(OAc)_2$, $Pd_2(dba)_3$ and $PdCl_2$] and a ligand (*e.g.* xantphos or BINAP) were used for the coupling of **1a** with **2a** at 80–100 °C depending on the solvent used *e.g.* 1,4-dioxane, DMF, toluene and MeCN (entries 1–8, Table 1). Generally, Cs_2CO_3 was used as a base in most of these cases. While the reaction proceeded in all these cases the yield of desired product **3a** was not satisfactory except in two cases (entries 2 & 3, Table 1) and was poor when Et_3N was used as a base (entry 5, Table 1). We then performed the reaction in the absence of any Pd-catalyst and ligands (entries 6–13, Table 1). To our surprise, the product yield was improved significantly in these cases. Change of base from Cs_2CO_3 to K_2CO_3 or DBU did not affect the yield of **3a** dramatically (entries 14 & 15, Table 1) whereas Et_3N once again found to be an inferior base in the present reaction (entry 16, Table 1). Interestingly, the product yield was continued to increase when the reaction was performed in the absence of any base (entries 17 & 18, Table 1) and finally in the absence of any solvent (entry 19, Table 1). Indeed the reaction was completed within 6 h in these cases affording **3a** in 83–87% yield. Though it required marginally higher reaction temperature, we were delighted particularly with the catalyst, ligand and solvent free method (entry 19, Table 1) as this approach not only avoids the environmental hazard but also reduce the cost. Moreover, the product **3a** was isolated in pure form without performing any chromatographic purification process [*i.e.* after completion of the reaction, the mixture was cooled to room temperature, diluted with cold water, filtered, and the solid obtained was titrated with methyl *t*-butyl ether (MTBE), see ESI†]. Thus the condition of entry 19 of Table 1 was appeared to be optimal and used for further study.

To test the generality and scope of this method the optimized reaction conditions were applied to a variety of substrates *e.g.* **1a**, **b** and **2a–o** (Table 2). Thus amines containing aliphatic and aromatic side chain or functional groups like ether, hydroxyl *etc.* or a chiral center in the side chain were examined. The reaction proceeded well in all these cases affording the desired products in good to excellent yields (entries 1–12, Table 2). The reaction also proceeded well with other N, O and S



Scheme 2 Preparation of starting material **1**.

Table 1 Reaction of **1a** with **2a** under various conditions[a]



Entry	Catalyst/ligand	Base	Solvent	%Yield[b]
1	$Pd(OAc)_2$/xantphos	Cs_2CO_3	1,4-Dioxane	40
2	$Pd(OAc)_2$/BINAP	Cs_2CO_3	1,4-Dioxane	62
3	$Pd(OAc)_2$/BINAP	Cs_2CO_3	1,4-Dioxane	60
4	$Pd_2(dba)_3$/BINAP	Cs_2CO_3	1,4-Dioxane	50
5	$Pd(OAc)_2$/BINAP	Et_3N	1,4-Dioxane	5
6	$PdCl_2$/BINAP	Cs_2CO_3	DMF	10
7	$Pd(OAc)_2$/BINAP	Cs_2CO_3	Toluene	30
8	$Pd(OAc)_2$/BINAP	Cs_2CO_3	MeCN	10
9	—	Cs_2CO_3	1,4-Dioxane	50
10	—	Cs_2CO_3	DMF	75
11	—	Cs_2CO_3	DMSO	70
12	—	Cs_2CO_3	*o*-Xylene	65
13	—	Cs_2CO_3	NMP	76
14	—	K_2CO_3	DMF	68
15	—	DBU	Toluene	69
16	—	Et_3N	DMF	23
17	—	—	DMF	83[c]
18	—	—	NMP	82[c]
19	—	—	—	87[c,d]

[a] Reaction conditions: **1a** (1.0 mmol), amine **2a** (1.1 mmol) and a base (3 mmol) at 80–100 °C for 8 h. [b] Isolated yield. [c] The reaction was performed for 6 h. [d] The reaction was performed at 135 ± 5 °C.

nucleophiles when the reaction time was shorter (entries 13–17, Table 2). Thus, indole fused with furo- and thieno[2,3-*b*]quinoxalines (**3m**, **3n**, **3p** and **3q**) were prepared by reacting **1a**, **b** with NaOH and Na_2S separately. All these reactions were performed under open air as the process was not sensitive towards aerial oxygen or moisture. Moreover, NaCl or HCl (that can be neutralized by NaOH to harmless NaCl) being the byproduct in these reactions all the products were isolated in pure form after treating with water followed by titration with MTBE (see ESI†). Notably, the Pd-based strategy for double arylation[7] (leading to carbazoles) was successful only with amines and the use of O- or S- reactants is not common.[8a] Though a different strategy has been reported[8b,c] for the double arylation of S- reactants (leading

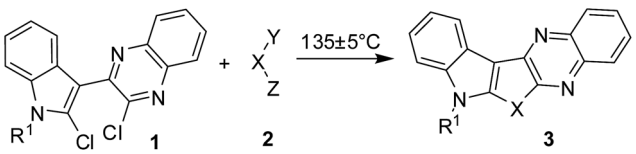
to dibenzo[*b*,*d*]thiophene derivatives) involving the sequential use of *n*-BuLi followed by S_2Cl_2 (as sulfur source) none is known for double arylation of oxygen reactants leading to dibenzo[*b*,*d*] furan derivatives. Moreover, all these reactions required the use of an inert or anhydrous atmosphere. Thus our strategy presented here appeared to be a general one as all N, O and S nucleophiles could be reacted with **1** under a common reaction condition. To test the scale-up potential of this method the reaction of **1a** with **2a** (*cf.* entry 19, Table 1) was performed in g scale [*i.e.* 1.57 g (~5 mmol) of **1a** and 5.1 mmol of **2a**] when **3a** was isolated in 93% yield.

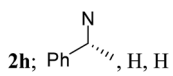
Mechanistically, the reaction may follow either "path a" or "path b" (Scheme 3). The path a involve a nucleophilic attack on the chloro group bearing C-2 of the quinoxaline ring to give **E-1** whereas path b involve a similar attack on the chloro group bearing C-2 of the indole ring leading to **E-2**. Both **E-1** and **E-2** can undergo a second nucleophilic attack on its =C–Cl moiety in an intramolecular fashion to afford the product **3**.[9a] To gain evidence on which path was actually followed we revisited the reaction of **1a** with **2a** under various conditions. This allowed us to isolate an intermediate (**4**) in 53% yield along with **3a** when the reaction was performed at 120 °C and stopped after 3 h (Scheme 4). While the initial spectral/analytical data indicated compound **4** as a monochloro derivative [(HRMS: *m*/*z* [M + 1] calcd for $C_{23}H_{18}N_4Cl$ (M + H): 385.1220; found: 385.1207) that was formed after displacing one of the two chloro groups of **1a** by **2a**] two alternative structures *i.e.* **4A** and **4B** (Fig. 3) were possible in this case. However, the structure of **4** was confirmed as **4A** that showed single NOE interaction between NH and C(7)–H of indole ring (Fig. 3). Notably, two NOE interactions were expected in case of **4B**. Finally, the intermediate **4** afforded **3a** when heated at 130 °C for 3 h indicating its intermediacy in the present reaction. It is to be mentioned that compound **4** did not react with **2a** when treated with **2a** in a separate experiment but afforded **3a** indicating intramolecular cyclization of **4** leading to **3a** was preferred over other reaction.[9b] Thus, all these observations suggested that the reaction followed path a rather than path b.

In view of known anti-tumor/anti-cancer properties of related indolo[2,3-*b*]quinoxaline derivatives,[2] all the synthesized compounds were evaluated for their ability to inhibit the growth of cancer cells. These compounds were tested at 10 μM against three cancer cells *e.g.* A549 (lung), MCF-7 (breast) and TZM-BL (cervical) using the sulphorhodamine B (SRB) assay[10a,b] with gemcitabine[10c] as a reference compound. Among these compounds, **3a–l** showed >90% inhibition against lung cancer cells (comparable to gemcitabine's 90% inhibition), >75% inhibition against breast cancer cells (gemcitabine 49%), and >60% against cervical cancer cells (gemcitabine 90%). Moreover, these compounds did not showed significant effect on normal HEK 293T cells (10–15% inhibition *vs.* gemcitabine's 25%) indicating their selectivity toward cancer cells.

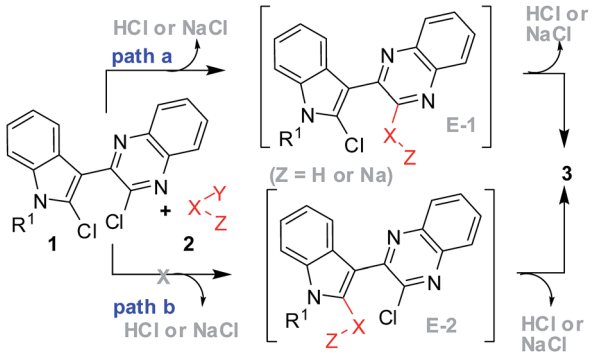
In conclusion, a straightforward yet innovative method has been developed for the double heteroarylation of N, O and S

Table 2 Synthesis of indole fused pyrrolo-, furo- and thieno[2,3-*b*]quinoxalines (**3**)[a]

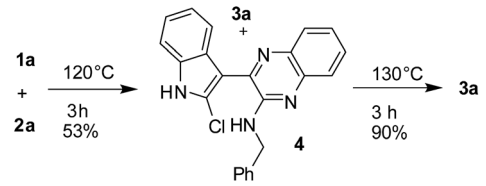


Entry	Compd (**1**), R^1 =	Nucleophile (**2**), X, Y, Z =	*T* (h)	Product (**3**)	Yield[b] (%)
1	**1a**; H	**2a**; $PhCH_2N$, H, H	6	**3a**	87
2	**1a**	**2b**; $Ph(CH_2)_2N$, H, H	5	**3b**	89
3	**1a**	**2c**; (pyridin-2-yl)CH_2N, H, H	6.5	**3c**	85
4	**1a**	**2d**; $EtO(CH_2)_3N$, H, H	5	**3d**	86
5	**1a**	**2e**; *p*-$MeOC_6H_4CH_2N$, H, H	5	**3e**	87
6	**1a**	**2f**; $MeO(CH_2)_3N$, H, H	5	**3f**	87
7	**1a**	**2g**; $Me(CH_2)_5N$, H, H	5	**3g**	85
8	**1a**	**2h**;  , H, H	9	**3h**	78
9	**1a**	**2i**; *n*-BuN, H, H	5	**3i**	80
10	**1a**	**2j**; 3,4-di-$MeOC_6H_3(CH_2)_2N$, H, H	5	**3j**	82
11	**1a**	**2k**; $HO(CH_2)_2N$, H, H	4	**3k**	78
12	**1a**	**2l**; 3,5-di-$MeC_6H_3CH_2N$, H, H	7.5	**3l**	79
13	**1a**	**2m**; S, Na, Na	1	**3m**	91
14	**1a**	**2n**; O, Na, H	2	**3n**	67
15	**1a**	**2o**; NH_2N, H, H	1.5	**3o**	85
16	**1b**; Me	**2n**	1.5	**3p**	70
17	**1b**	**2m**	0.5	**3q**	91

[a] The reaction was carried out using **1** (1.0 mmol), and **2** (1.1 mmol) at 135 ± 5 °C. [b] Isolated yield.



Scheme 3 Proposed reaction mechanism.



Scheme 4 The preparation of intermediate 4 and its conversion to 3a.

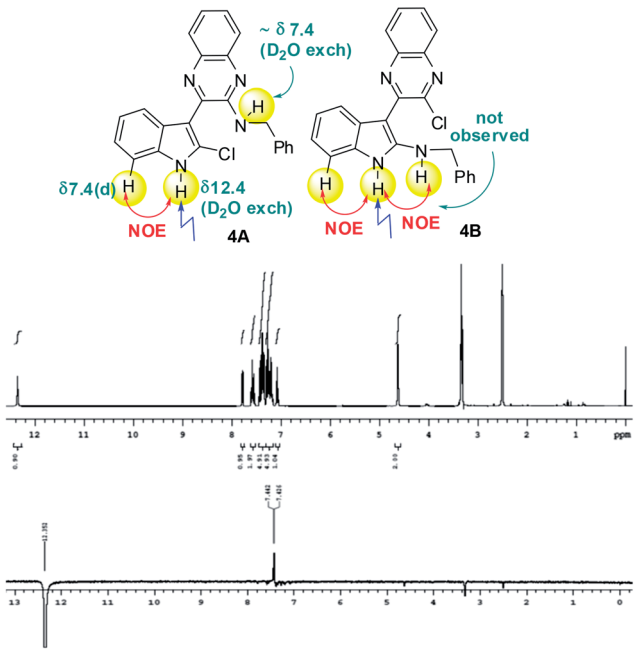


Fig. 3 Alternative structures and NOE spectra of compound 4.

nucleophiles leading towards the synthesis of polynuclear *N*-heteroarenes. This operationally simple, general, greener and cost-effective method can be performed under open air and is amenable for scale-up. The methodology afforded a library of novel compounds containing indole ring fused with pyrrolo-, furo- and thieno[2,3-*b*]quinoxaline moiety without the need of chromatographic purification. Several of these compounds showed promising and selective cytotoxicities against cancer cells. Overall, being unprecedented the present strategy of catalyst/ligand/solvent free double heteroarylation of N, O and S nucleophiles could be useful in accessing a wide range of novel bioactive molecules of medicinal importance.

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9 (*a*) Since products **3a–l** and **3o** were never isolated as a HCl salt after usual work-up, it appeared that the HCl generated during the reaction in most cases formed a weak salt with **3** which however was dissociated during aqueous work-up. This was supported by the acidic nature of aqueous filtrate collected that was later neutralized by dil NaOH solution; (*b*) The reaction of **1a** (1 mmol) with excess of **2a** (upto 10 mmol) also afforded **3a** but no di-benzylamino substituted product [*i.e.* *N*-benzyl-3-(2-(benzylamino)-1*H*-indol-3-yl)quinoxalin-2-amine] supported this observation.

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