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# Design, synthesis, anti-cancer screening and structure activity relationship studies of biphenyl linked fused imidazoles

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Biphenyl is a privileged scaffold observed in several marketed drugs and is known to predominantly bind to a wide range of proteins with high specificity. Fused imidazole is another privileged structure which is found in several bioactive compounds. The present investigation describes the design and synthesis of a biprivileged compound library comprising biphenyl linked fused imidazoles and their activity against NCI-60 cell line to identify potential 'hits' for further anti-cancer drug discovery. In the preliminary investigation, imidazo[1,2-*a*]pyridine based heterocycles having *tert*-alkyl amine and a biphenyl substituent demonstrated promising results against some of the leukaemia, colon cancer, ovarian cancer as well as breast cancer cell lines. The active compounds were also found to be non-toxic to several other cancer cell lines, warranting further structure activity relationship (SAR) investigation. A systematic structural modifications and bioactivity evaluation against NC-I60 cell line resulted in the identification of 2-aryl-*N*-(2,4,4-trimethylpentan-2-yl)imidazo[1,2-*a*]pyrazin-3-amine scaffold with biphenyl, benzo[*d*][1,3]dioxole and 4-(trifluoromethyl)benzene as substituents at C-2 position showing anticancer activity.

Keywords: NCI-60, privileged scaffold, fused imidazoles, biphenyl, GBB MCR, SAR.

## Introduction

Aromatic heterocyclic scaffolds exist in a wide range of natural products and are being frequently used to probe and understand various biologically significant pathways[1]. In drug discovery process, functionalized aromatic compounds are dominated by their ability to make interactions with hydrophobic residues, polar amide/hydroxyl groups, and charged moieties present in various biomacromolecules. Among such aromatic scaffolds, biphenyl is a privileged structure which comprises 4.3% of the marketed drugs and is present in several bioactive heterocycles[2]. Losartan and valsartan are the top selling drugs which contain the biphenyl scaffold[3]. Biphenyl containing molecules are also known to have potential anti-malarial, anti-microbial, anti-hypertensive as well as anti-atherosclerotic activities[4]. Fesik *et al*. found that the biphenyl containing heterocycles predominantly bind to a wide range of proteins with high specificity[2a]. Very recently, Yang *et al.* designed novel oxime-biphenyl-diarylpyrimidines utilizing a privileged scaffold inspired strategy having potential to treat HIV-1[5]. Due to the flexible nature of the biphenyl scaffold, it can be accommodated into a wide range of enzyme/protein pockets and show favorable interactions with

the receptors. Honokiol (**1**), a natural product comprising a biphenyl scaffold, extracted from the bark of *Magnolia officinalis* exhibits antitumor activity against several cancer cells including colorectal cancer, prostate cancer, leukaemia, and melanoma (Fig. 1)[6]. Biphenyl skeleton is also observed in several other cytotoxic natural products such as eupomatilone-6[7] (**2**), allocolchicine[8] (**3**) and buflavine[9] (**4**). MP5-F9[10] (**5**), the natural product combretastatin A4 (microtubule targeting agent) inspired designed hit also showed anticancer activity via mitotic arrest. Whereas, the nitro-vinyl biphenyl derivative (**6**) showed cytotoxicity in nanomolar range against Hela and MCF-7 cell lines[10].

Hybridization of two different moieties that are biologically active could result in new scaffolds with improved physiochemical as well as pharmacological properties. This approach can easily provide novel scaffolds targeting significant biological pathways. Fused imidazole based scaffold is one of such important privileged structure which was found in several marketed drugs such as Zolpidem (treatment of insomnia), minodronic acid (treatment of osteoporosis), soraprazan, alpedim (treatment of anxiety), zolmidine etc.[11]. Substituted fused imidazole **7** is protein-E (CENP) targeting anticancer agent currently available in the market[12]. Similarly, compounds **8** and **9** are the imidazo[2,1-*b*][1,3,4]thiadiazole analogues of anticancer drug levamisole showing promising results[13]. Based upon the above-mentioned attributes of biphenyl and fused imidazoles, we planned to directly link biphenyl scaffold with imidazole fused pyridine/pyrazine and thiazole skeleton and evaluated their potential against a panel of NCI-60 cancer cell lines.

## Results and discussion

Greobke-Blackburn-Bienaymé (GBB) multicomponent reaction is a one-pot protocol where an amidine, aldehyde and isonitrile reacts together to generate diverse compounds containing imidazole fused heterocycles[14]. Following this strategy we sought to design biphenyl linked fused imidazopyridine, imidazopyrazine, and imidazothiazole based heterocycles. Initially, a set of nine diverse biphenyl linked fused imidazoles were synthesized (Fig. 2) via GBB reaction which was carried out in acetonitrile using HCl as a catalyst to generate the desired compounds in 50–90% yields (Scheme 1). All the products were purified through column chromatography using 230–400 mesh silica gel and characterized by NMR and HRMS analysis. The purity of these compounds was determined by HPLC.

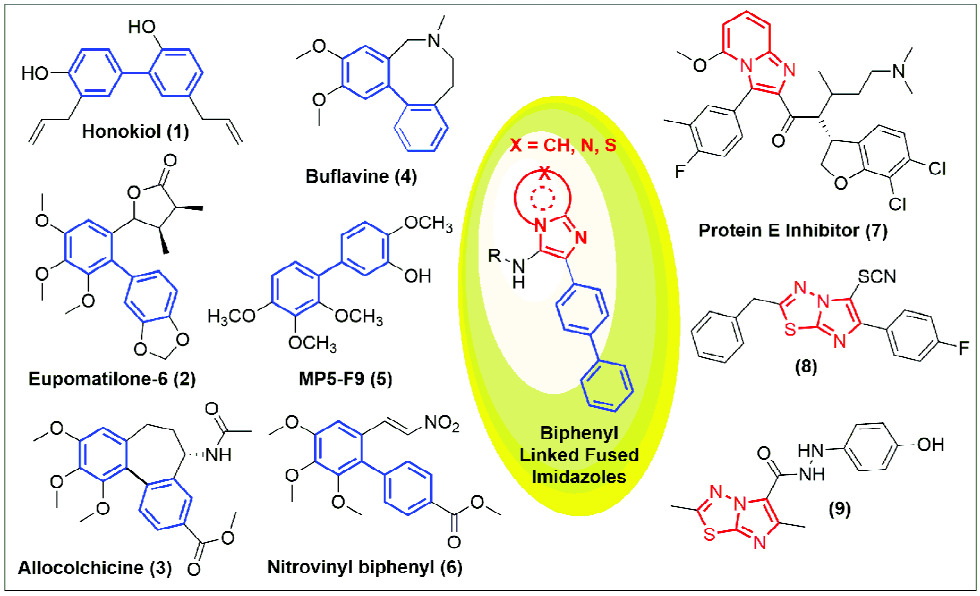



**Fig. 1.** Design of biphenyl linked fused imidazoles.

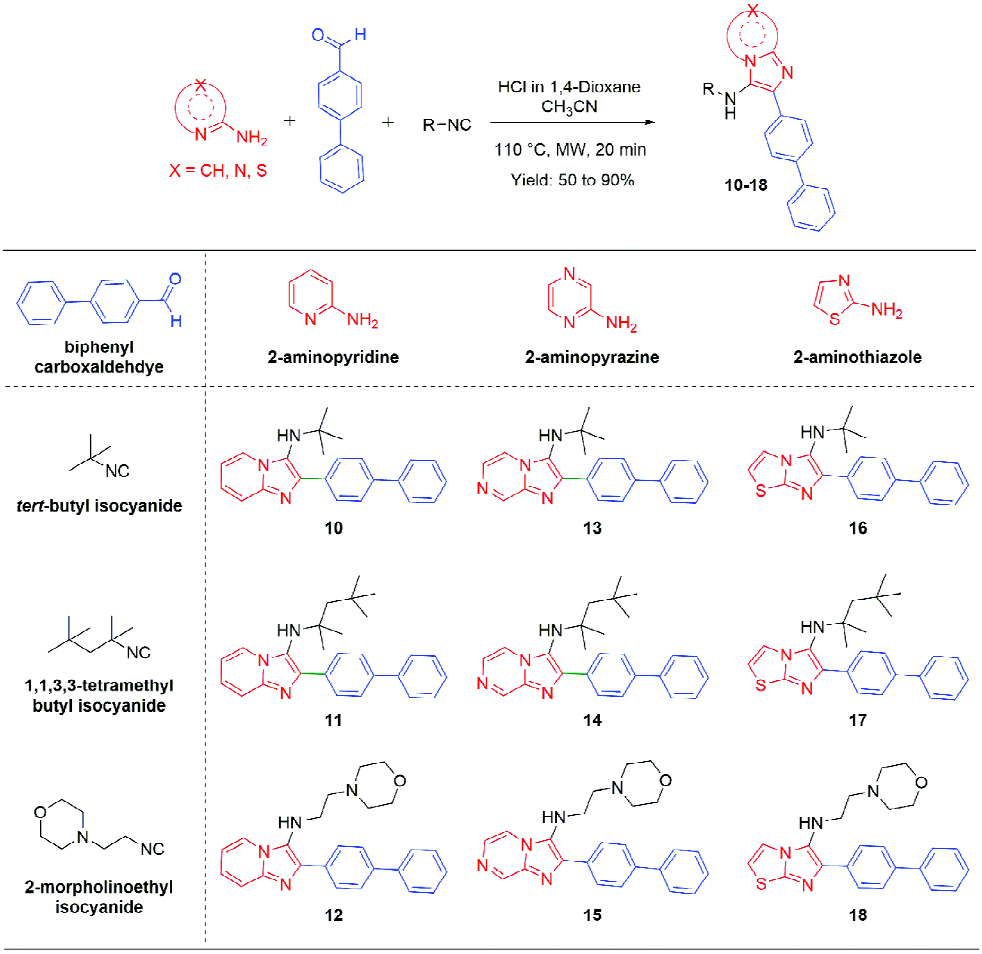
| | Cell Line | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | CCRF-CEM | 33.68 | 15.95 | 40.78 | 18.00 | 27.11 | 22.69 | 18.81 | 4.66 | 68.27 |
| Leukemia | HL-60(TB) | 42.25 | 21.30 | 39.40 | 30.28 | 7.12 | 0.00 | 17.73 | 1.32 | 78.73 |
| Leukemia | K-562 | 71.27 | 44.80 | 44.63 | 43.35 | 37.26 | 11.88 | 57.69 | 16.82 | 15.39 |
| Leukemia | MOLT-4 | 30.71 | 32.75 | 52.61 | 37.53 | 37.47 | 30.14 | 12.69 | 15.83 | 41.65 |
| Leukemia | RPMI-8226 | 42.93 | 29.75 | 39.69 | 42.48 | 31.30 | 36.09 | 21.66 | 7.09 | 55.85 |
| Leukemia | SR | 58.39 | 31.13 | | 42.37 | | | 44.08 | 20.47 | |
| NSCLC | A549/ATCC | 18.90 | 9.66 | 24.29 | 12.69 | 43.57 | 11.55 | 7.21 | 10.35 | 30.41 |
| NSCLC | EKVX | 38.74 | 34.50 | 10.69 | 30.71 | 7.30 | 6.56 | 30.22 | 39.74 | 3.10 |
| NSCLC | HOP-62 | 22.42 | 11.25 | 16.01 | 18.12 | 20.06 | 27.31 | 8.12 | 0.00 | 29.05 |
| NSCLC | HOP-92 | 11.82 | 26.01 | 34.26 | 24.17 | 6.65 | 15.93 | 6.26 | 13.71 | 15.46 |
| NSCLC | NCI-H226 | 22.38 | 11.36 | 7.12 | 16.42 | 12.65 | 10.17 | 30.62 | 3.30 | 14.72 |
| NSCLC | NCI-H23 | 35.22 | 16.49 | 12.03 | 8.84 | 8.49 | 11.17 | 20.92 | 6.52 | 3.18 |
| NSCLC | NCI-H322M | 20.30 | 8.82 | 11.86 | 0.71 | 18.48 | 18.06 | 22.03 | 9.00 | 23.59 |
| NSCLC | NCI-H460 | 20.24 | 12.68 | 18.82 | 10.45 | 19.97 | 1.06 | 11.43 | 10.18 | 12.99 |
| NSCLC | NCI-H522 | 49.59 | 24.42 | 23.14 | 26.59 | 62.52 | 15.78 | 26.23 | 16.84 | 61.89 |
| Colon Cancer | COLO 205 | 26.09 | 6.44 | | 0.00 | | | 0.00 | 0.00 | |
| Colon Cancer | HCC-2998 | 19.34 | 20.71 | 3.99 | 1.70 | 6.35 | 0.00 | 0.00 | 8.42 | 0.68 |
| Colon Cancer | HCT-116 | 46.55 | 21.97 | 33.23 | 35.85 | 40.18 | 16.85 | 19.74 | 11.39 | 24.98 |
| Colon Cancer | HCT-15 | 48.36 | 33.06 | 8.25 | 27.58 | 15.48 | 2.09 | 23.33 | 18.46 | 6.98 |
| Colon Cancer | HT29 | 83.31 | 39.39 | 21.10 | 32.49 | 69.46 | 9.57 | 44.48 | 15.05 | 13.76 |
| Colon Cancer | KM12 | 42.07 | 17.68 | 9.95 | 12.61 | 20.10 | 0.00 | 18.30 | 6.93 | 10.38 |
| Colon Cancer | SW-620 | 6.77 | 5.29 | 10.49 | 0.00 | 2.89 | 3.36 | 0.39 | 0.00 | 4.33 |
| CNS Cancer | SF-268 | 10.63 | 4.55 | 14.77 | 0.99 | 18.52 | 3.08 | 3.98 | 1.69 | 28.46 |
| CNS Cancer | SF-295 | 29.22 | 0.00 | 4.53 | 21.16 | 2.43 | 3.97 | 11.23 | 0.00 | 1.10 |
| CNS Cancer | SF-539 | 6.17 | 0.00 | 2.54 | 14.26 | 9.49 | 12.13 | 2.18 | 0.15 | 7.44 |
| CNS Cancer | SNB-19 | 14.04 | 15.78 | 2.34 | 16.30 | 5.68 | 2.56 | 13.28 | 8.95 | 9.36 |
| CNS Cancer | SNB-75 | | 0.00 | 19.33 | 4.69 | 11.30 | 20.26 | | 1.44 | 22.82 |
| CNS Cancer | U251 | 18.79 | 11.06 | 11.16 | 12.15 | 40.33 | 3.34 | 7.13 | 6.51 | 27.59 |
| Melanoma | LOX IMVI | 22.34 | 0.00 | 3.74 | 8.41 | 2.79 | 0.00 | 9.66 | 0.00 | 5.35 |
| Melanoma | MALME-3M | 8.59 | 14.94 | 10.26 | 25.19 | 0.00 | 6.74 | 4.76 | 3.82 | 0.97 |
| Melanoma | M14 | 29.08 | 0.00 | 12.38 | 7.11 | 10.75 | 7.10 | 21.03 | 11.20 | 17.43 |
| Melanoma | MDA-MB-435 | 18.13 | 0.00 | 10.63 | 4.33 | 17.25 | 0.00 | 0.00 | 0.00 | 6.04 |
| Melanoma | SK-MEL-2 | 21.83 | 5.87 | 9.32 | 5.06 | 27.81 | 7.12 | 22.48 | 0.00 | 11.36 |
| Melanoma | SK-MEL-28 | 5.93 | 6.67 | 4.91 | 6.58 | 0.00 | 4.07 | 0.00 | 0.00 | 0.81 |
| Melanoma | SK-MEL-5 | 32.92 | 13.06 | 10.09 | 26.47 | 0.00 | 8.69 | 20.70 | 3.26 | 0.00 |
| Melanoma | UACC-257 | 17.49 | 0.00 | 3.30 | 2.77 | 16.71 | 0.73 | 6.45 | 0.00 | 10.29 |
| Melanoma | UACC-62 | 32.43 | 18.26 | 6.29 | 22.14 | 24.13 | 9.29 | 13.28 | 12.17 | 7.48 |
| Ovarian Cancer | IGROV1 | 14.81 | 36.81 | 7.52 | 17.71 | 33.99 | 9.27 | 8.71 | 32.81 | 8.11 |
| Ovarian Cancer | OVCAR-3 | 26.70 | 23.76 | 17.42 | 12.62 | 9.85 | 0.00 | 7.56 | 8.58 | 23.61 |
| Ovarian Cancer | OVCAR-4 | 41.47 | 32.15 | 18.47 | 31.84 | 100.00 | | 8.39 | 13.38 | 16.84 |
| Ovarian Cancer | OVCAR-5 | 8.76 | 6.61 | 18.79 | 10.42 | 11.90 | 22.65 | 1.17 | 11.82 | 11.82 |
| Ovarian Cancer | OVCAR-8 | 9.48 | 9.34 | 23.39 | 12.14 | 29.79 | 16.66 | 0.00 | 5.15 | 30.51 |
| Ovarian Cancer | NCI/ADR-RES | 17.74 | 18.26 | 5.31 | 22.91 | 2.31 | 4.74 | 4.82 | 10.51 | 2.61 |
| Ovarian Cancer | SK-OV-3 | 19.11 | 19.90 | 23.47 | 18.35 | 23.69 | 20.23 | 5.42 | 17.33 | 16.63 |
| Renal Cancer | 786-0 | 14.51 | 2.21 | 9.71 | 13.00 | 19.35 | 4.85 | 0.00 | 0.73 | 54.43 |
| Renal Cancer | A498 | 38.47 | 10.15 | 21.32 | 22.28 | 24.09 | 3.30 | 15.54 | 0.00 | 9.00 |
| Renal Cancer | ACHN | 12.66 | 9.30 | 2.00 | 6.62 | 0.00 | 5.85 | 10.05 | 2.83 | 7.20 |
| Renal Cancer | CAKI-1 | 33.12 | 34.59 | 15.60 | 20.22 | 23.89 | 16.70 | 25.76 | 28.72 | 27.59 |
| Renal Cancer | RXF 393 | 23.50 | | 8.34 | | 5.57 | 0.27 | 15.24 | | 9.85 |
| Renal Cancer | SN12C | 23.75 | 16.22 | 9.50 | 22.86 | 1.27 | 3.76 | 19.84 | 6.37 | 16.93 |
| Renal Cancer | TK-10 | 12.07 | 10.72 | 4.71 | 1.78 | 13.37 | 0.00 | 0.00 | 8.73 | 0.00 |
| Renal Cancer | UO-31 | 38.36 | 43.53 | 35.59 | 27.63 | 31.22 | 30.21 | 26.23 | 32.04 | 55.77 |
| Prostate Cancer | PC-3 | 31.87 | 47.08 | 37.31 | 13.06 | 34.62 | 17.49 | 9.72 | 21.92 | 33.92 |
| Prostate Cancer | DU-145 | 18.71 | 2.60 | 19.84 | 7.66 | 5.09 | 4.39 | 9.27 | 0.00 | 7.46 |
| Breast Cancer | MCF7 | 54.43 | 55.66 | 21.34 | 21.09 | 17.68 | 8.05 | 41.07 | 46.96 | 28.20 |
| Breast Cancer | MDA-MB-231 | 9.93 | 10.99 | 21.21 | 15.46 | 9.42 | 18.82 | 9.51 | 10.25 | 16.88 |
| Breast Cancer | HS 578T | 0.00 | 0.00 | 13.72 | 0.00 | 4.49 | 10.05 | 3.31 | 0.00 | 12.39 |
| Breast Cancer | BT-549 | 23.72 | 4.91 | 18.22 | 24.66 | 4.99 | 10.87 | 0.00 | 7.62 | 48.35 |
| Breast Cancer | T-47D | 69.29 | 47.07 | | 51.41 | | | 50.28 | 48.22 | |
| Breast Cancer | MDA-MB-468 | | 12.14 | 21.40 | 23.24 | 0.00 | 0.00 | | 0.00 | 15.40 |

**Fig. 2.** Heatmap of NCI-60 cell line screening for compounds **10**-**18**.

The anticancer potential of the synthesized analogues (**10**-**18**) was evaluated against NCI-60 cancer cell line panel[15]. The diverse cell line panel provide a unique platform to compare tested compounds by their effect patterns and identify

potential 'hits' for further drug discovery[16]. The synthesized library of compounds were screened against the NCI-60 cell line panel at a single dose (10 μM). The output was processed and reported as percentage (%) inhibition heatmap (Fig. 2).

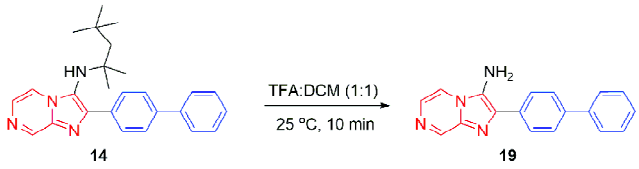
The compounds **10**, **14** and **18**, which are placed diagonally in Scheme 1 showed promising activities. Specifically, the imidazo[1,2-*a*]pyridine-based compound **10** having *tert*-butyl amine and a privileged biphenyl substituent demonstrated substantial inhibition of leukaemia cell lines K-562 (71% inhibition) and SR (58% inhibition). Compound **10** was also found to be active against colon cancer cell line HT29 (83% inhibition) as well as breast cancer cell line T-47D (69% inhibition). The structurally similar analogue having *tert*-octylamine substituent on the imidazo[1,2-*a*]pyrazine heterocycle (**14**) was observed to be selectively toxic to the colon cancer cell line HT29 (69% inhibition) and was also found to be active against non-small cell lung cancer cell line H522 (62% inhibition). It was noteworthy to observe that the compound **14** completely inhibited the growth of the ovarian cancer cell line OVCAR-4 at 10 μM concentration. Among the initial small set of compounds, the biphenyl linked imidazo[2,1-*b*]thiazole **18** having 2-morpholinoethyl amine substituent was another active analogue showing potential anticancer activity against leukemia CCRF-CEM, HL-60 as well as non-small cell lung cancer cell line H522 with more than 60% inhibition at the tested concentration. The three active compounds as well as the other analogues were found to be non-toxic to several other cell lines and also a clear relationship between the substituents and the observed ac-




**Scheme 1.** A diverse set of biphenyl linked fused imidazole.

tivity was observed which demanded further structure activity relationship (SAR) investigation.

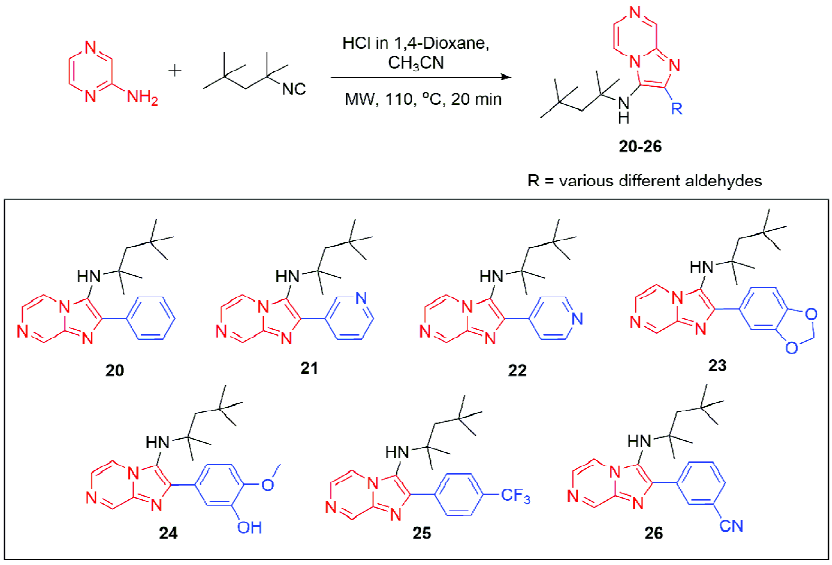
To increase the diversity of the synthesized library and to further understand the SAR, dealkylation of the preliminary 'hit' (compound **14**) with imidazo[1,2-*a*]pyrazine scaffold was carried out to observe the effect of the free amino functionality on the anticancer activity (Scheme 2). The dealkylation reaction was executed in the presence of 1:1 mixture of $CF_3COOH$ and $CH_2Cl_2$ at room temperature to generate the primary amine product **19** in 90% yield.




**Scheme 2.** Dealkylation of HIT compound **14**.

Complete loss of anticancer activity by compound **19** against all the cancer cell lines tested confirmed the importance of the *tert*-alkyl substituent in this scaffold. Further, some more analogues of the compound **14** were synthesized using similar GBB reaction condition (Scheme 3). The imidazo[1,2-*a*]pyrazine with 2-octylamino substituents were kept constant and various aldehydes were selected instead of biphenyl-4-carboxaldehyde so as to understand the appropriate structural requirements at C-2 position of the heterocycle for improved anticancer activity.

In particular, the compound **20** was synthesized to check the importance of the biphenyl moiety in the 'hit' scaffold **14**. Further, the phenyl group was replaced with pyridyl functionality and both 3-pyridinecarboxaldehyde as well as 4-pyridinecarboxaldehyde were selected to synthesize analogues **21** and **22**, respectively. It was important to observe that the analogues **20**, **21** as well as **22** did not show any prominent inhibition of any of the cancer cell line tested (Fig. 3). The compound **23** with benzo[*d*][1,3]dioxole substitution instead of a biphenyl group, inhibited 94% of the colon cancer cell line (HCT-116) at 10 μM whereas the structurally related 3-hydroxy-4-methoxybenzene substituted compound **24** was found to bereft of any anticancer activity. Interestingly, the 4-(trifluoromethyl)benzene substituted compound **25** showed complete inhibition (100% inhibition) of colon cancer cell line (HCT-116), melanoma cell line (M14) as well as renal cancer cell line (786-0). Further modification in this series with 3-benzonitrile substituent resulted in compound **26** with complete loss of the anticancer activity. Overall, the present investigation resulted in the discovery of 2-aryl-*N*-(2,4,4-trimethylpentan-2-yl)imidazo[1,2-*a*]pyrazin-3-amine




**Scheme 3.** Further optimization of HIT compound **14**.

| | Cell Line | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|
| Leukemia | CCRF-CEM | 35.27 | 4.09 | 0.00 | 8.37 | 10.91 | 19.92 | 14.23 | 13.79 |
| Leukemia | HL-60(TB) | 39.12 | 4.08 | 0.00 | 0.00 | 22.16 | 23.69 | 20.64 | 15.31 |
| Leukemia | K-562 | 20.11 | 6.01 | 2.21 | 1.65 | 28.02 | 19.51 | 34.45 | 29.14 |
| Leukemia | MOLT-4 | 15.86 | 9.96 | 3.62 | 5.93 | 30.64 | 30.52 | 31.50 | 27.36 |
| Leukemia | RPMI-8226 | 13.00 | 14.95 | 11.96 | | 30.29 | | 37.10 | |
| Leukemia | SR | 16.58 | 18.54 | 5.43 | | 26.89 | | 40.24 | |
| NSCLC | A549/ATCC | 9.59 | 9.64 | 3.92 | 3.64 | 17.26 | 12.69 | 16.90 | 18.09 |
| NSCLC | EKVX | 7.93 | 8.92 | 9.95 | 6.91 | 19.67 | 6.64 | 20.62 | 18.95 |
| NSCLC | HOP-62 | 4.95 | 2.37 | 0.00 | 0.00 | 2.06 | 0.00 | 7.26 | 4.01 |
| NSCLC | HOP-92 | 0.00 | 26.19 | 5.87 | 12.91 | 29.74 | 7.31 | 17.41 | 33.71 |
| NSCLC | NCI-H226 | 3.70 | 3.34 | 4.21 | 9.75 | 9.91 | 14.53 | 7.49 | 9.59 |
| NSCLC | NCI-H23 | 3.88 | 0.00 | 3.93 | 0.76 | 2.30 | 3.28 | 17.59 | 0.00 |
| NSCLC | NCI-H322M | 3.33 | 1.29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.77 |
| NSCLC | NCI-H460 | 0.00 | 0.00 | 0.00 | 0.00 | 2.80 | 0.91 | 10.51 | 3.46 |
| NSCLC | NCI-H522 | 19.49 | 17.93 | 13.76 | 12.94 | 25.86 | 18.41 | 32.55 | 25.50 |
| Colon Cancer | COLO 205 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.54 | 4.04 | 11.19 |
| Colon Cancer | HCC-2998 | 0.66 | 1.03 | 0.00 | 0.00 | 6.26 | 0.00 | 7.90 | 0.00 |
| Colon Cancer | HCT-116 | 3.56 | 9.59 | 10.06 | 9.88 | 94.64 | 14.44 | 100.00 | 31.35 |
| Colon Cancer | HCT-15 | 4.61 | 4.28 | 1.65 | 2.06 | 15.91 | 11.54 | 26.15 | 15.74 |
| Colon Cancer | HT29 | 5.35 | 8.94 | 5.50 | 6.99 | 20.04 | 0.00 | 42.59 | 10.40 |
| Colon Cancer | KM12 | 2.53 | 0.76 | 0.00 | 1.90 | 3.80 | 0.37 | 16.21 | 5.23 |
| Colon Cancer | SW-620 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.59 |
| CNS Cancer | SF-268 | 8.89 | 11.23 | 4.51 | 1.43 | 15.88 | 18.24 | 11.16 | 7.61 |
| CNS Cancer | SF-295 | 7.23 | 4.09 | 4.13 | 0.00 | 13.76 | 7.90 | 13.73 | 14.83 |
| CNS Cancer | SF-539 | 8.10 | 9.46 | 9.53 | 6.18 | 8.50 | 16.04 | 14.03 | 7.46 |
| CNS Cancer | SNB-19 | 8.67 | 7.94 | 4.60 | 2.70 | 9.15 | 7.77 | 8.21 | 9.97 |
| CNS Cancer | SNB-75 | 0.00 | 13.49 | 5.57 | 19.56 | 32.50 | 49.31 | 18.93 | 32.52 |
| CNS Cancer | U251 | 4.67 | 4.90 | 8.42 | 0.81 | 4.39 | 10.24 | 6.98 | 12.00 |
| Melanoma | LOX IMVI | 0.88 | 0.00 | 6.08 | 1.21 | 5.96 | 1.21 | 2.40 | 6.64 |
| Melanoma | MALME-3M | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 13.28 | 0.00 | 0.00 |
| Melanoma | M14 | 0.00 | 0.00 | 0.00 | 0.00 | 54.09 | 15.25 | 100.00 | 0.00 |
| Melanoma | MDA-MB-435 | 10.02 | 0.00 | 0.00 | 0.80 | 0.91 | 15.57 | 1.25 | 1.67 |
| Melanoma | SK-MEL-2 | 3.51 | 1.93 | 1.56 | 0.00 | 4.44 | 0.00 | 8.97 | 0.00 |
| Melanoma | SK-MEL-28 | 6.58 | 0.00 | 1.46 | 0.00 | 5.66 | 22.07 | 6.11 | 0.00 |
| Melanoma | SK-MEL-5 | 6.62 | 3.73 | 3.29 | 0.63 | 12.28 | 9.10 | 9.97 | 8.55 |
| Melanoma | UACC-257 | 2.49 | 0.53 | 0.42 | 0.00 | 0.00 | 12.08 | 1.35 | 2.54 |
| Melanoma | UACC-62 | 5.79 | 19.86 | 4.72 | 7.09 | 34.02 | 22.21 | 27.14 | 33.53 |
| Ovarian Cancer | IGROV1 | 0.00 | 10.31 | 0.09 | 0.21 | 27.86 | 9.07 | 32.58 | 7.96 |
| Ovarian Cancer | OVCAR-3 | 0.00 | 0.00 | 0.00 | 0.00 | 7.53 | 0.50 | 15.23 | 1.29 |
| Ovarian Cancer | OVCAR-4 | 3.72 | 9.76 | 3.93 | 15.99 | 13.12 | 13.20 | 20.58 | 16.31 |
| Ovarian Cancer | OVCAR-5 | 2.00 | 9.42 | 6.37 | 6.28 | 8.77 | 0.38 | 0.00 | 0.93 |
| Ovarian Cancer | OVCAR-8 | 2.68 | 5.57 | 0.00 | 4.45 | 4.95 | 5.36 | 10.82 | 13.64 |
| Ovarian Cancer | NCI/ADR-RES | 1.36 | 7.30 | 9.36 | 0.46 | 12.32 | 6.99 | 18.11 | 10.84 |
| Ovarian Cancer | SK-OV-3 | 0.00 | 8.82 | 0.00 | 7.19 | 8.04 | 0.00 | 17.34 | 4.58 |
| Renal Cancer | 786-0 | 12.93 | 5.38 | 4.23 | 3.43 | 32.28 | 2.32 | 100.00 | 13.15 |
| Renal Cancer | A498 | 11.39 | 15.05 | 9.67 | 9.11 | 24.40 | 15.09 | 16.25 | 34.38 |
| Renal Cancer | ACHN | 0.79 | 4.80 | 6.89 | 12.35 | 13.51 | 9.97 | 12.98 | 12.67 |
| Renal Cancer | CAKI-1 | 11.96 | 20.00 | 12.66 | 19.41 | 30.43 | 21.77 | 24.63 | 30.04 |
| Renal Cancer | RXF 393 | | | | 6.21 | | 11.95 | | 9.54 |
| Renal Cancer | SN12C | 10.91 | 6.29 | 2.53 | 0.00 | 7.18 | 8.16 | 13.92 | 2.67 |
| Renal Cancer | TK-10 | 0.23 | 0.07 | 0.00 | 0.76 | 1.67 | 0.00 | 0.00 | 3.55 |
| Renal Cancer | UO-31 | 16.26 | 28.96 | 20.27 | 13.28 | 36.86 | 29.20 | 35.85 | 29.11 |
| Prostate Cancer | PC-3 | 2.30 | 21.12 | 3.93 | 2.32 | 32.98 | 10.79 | 31.52 | 16.58 |
| Prostate Cancer | DU-145 | 2.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 7.01 | 4.61 |
| Breast Cancer | MCF7 | 11.31 | 6.50 | 4.36 | 3.45 | 25.91 | 24.20 | 33.54 | 16.67 |
| Breast Cancer | MDA-MB-231 | 0.00 | 4.23 | 13.26 | 5.05 | 16.10 | 10.24 | 13.98 | 16.68 |
| Breast Cancer | HS 578T | 0.00 | 5.26 | 0.00 | 7.80 | 11.46 | 5.56 | 0.00 | 8.84 |
| Breast Cancer | BT-549 | 8.57 | 10.51 | 10.26 | 1.37 | 17.82 | 12.92 | 24.05 | 5.10 |
| Breast Cancer | T-47D | 11.69 | 10.17 | 8.36 | 13.89 | 15.84 | 7.66 | 25.64 | 9.28 |
| Breast Cancer | MDA-MB-468 | 2.30 | 0.00 | 7.95 | 12.50 | 4.78 | 5.78 | 0.00 | 1.14 |

**Fig. 3.** Heatmap of NCI-60 cell line screening for compounds **19**-**26**.

scaffold with biphenyl, benzo[*d*][1,3]dioxole and 4-(trifluoromethyl)benzene as prominent aryl substituents at C-2 position showing prominent anticancer activity.

## Conclusions

In this study, a set of compounds based on a privileged molecular scaffold comprising biphenyl linked fused imidazoles were synthesized via GBB-MCR methodology and screened through NCI-60 cancer cell line panel for their anticancer activity. Preliminary screening (at a single dose of 10 μM), furnished compound **10** which showed substantial inhibition of leukaemia cell line (K-562, 71% inhibition; SR, 58% inhibition), colon cancer cell line (HT29, 83% inhibition) as well as breast cancer cell line (T-47D, 69% inhibition). The structurally similar analogue **14** completely inhibited the growth of the ovarian cancer cell line OVCAR-4 at 10 μM concentration. Further SAR investigation, confirmed the important role of C-3 *tert*-alkyl amino as well as biphenyl substituent at the C-2 position on the imidazo[1,2-*a*]pyrazine scaffold. Compound **19** without *tert*-alkyl substituent as well as the analogues synthesized by the replacement of biphenyl group with phenyl (**20**), pyridyl (**21**, **22**) and a few more substituted phenyl analogues (**24**, **26**) were observed to be relatively less active. Interestingly, the compound **23** with benzo[*d*][1,3]dioxole substitution instead of a biphenyl group, inhibited 94% of the colon cancer cell line (HCT-116) at 10 μM whereas the 4-(trifluoromethyl)benzene substituted compound **25** showed complete inhibition of colon cancer cell line (HCT-116), melanoma cell line (M14) as well as renal cancer cell line (786-0).

## Experimental

*General procedure for the synthesis of compounds (**10-18**) and (**20-26**):* To a solution of 2-amidine (1.59 mmol) and aldehyde (3.18 mmol) in anhydrous acetonitrile (5 mL), isonitrile (1.59 mmol) was added followed by the addition of 4 *N* HCl/dioxane (5 μL) at room temperature. The reaction mixture was then allowed to react under microwave irradiation at 110°C for 20 min. After the completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature, solvent was evaporated under reduced pressure and the crude mixture obtained was purified using flash column chromatography to yield the target compounds (**10-18** and **20-26**) in 50–90% yield.

*2-([1,1′-Biphenyl]-4-yl)-N-(2,4,4-trimethylpentan-2-yl)imidazo[1,2-a]pyrazin-3-amine (**14**):* Yield 70%; $^1$H NMR (500 MHz, $CDCl_3$): δ 9.01 (d, *J* 1.3 Hz, 1H), 8.15 (dd, *J* 4.6, 1.4 Hz, 1H), 7.97–7.94 (m, 2H), 7.87 (d, *J* 4.6 Hz, 1H), 7.74–7.70 (m, 2H), 7.69–7.66 (m, 2H), 7.48–7.45 (m, 2H), 7.38–7.35 (m, 1H), 1.61 (s, 2H), 1.05 (s, 9H), 1.00 (s, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$): δ 143.5, 142.4, 140.9, 140.7, 137.5, 133.5, 129.0, 128.9, 128.9, 127.6, 127.3, 127.2, 125.2, 116.6, 61.5, 57.2, 31.9, 31.9, 29.3; HRMS *m/z*: calculated for $C_{26}H_{31}N_4^+$ $[M+H]^+$ 399.25432, found 399.25505.

*2-(4-(Trifluoromethyl)phenyl)-N-(2,4,4-trimethylpentan-2-yl)imidazo[1,2-a]pyrazin-3-amine (**25**):* Yield 80%; $^1$H NMR (598 MHz, DMSO-$d_6$): δ 8.97 (d, *J* 1.3 Hz, 1H), 8.45 (dd, *J* 4.7, 1.3 Hz, 1H), 8.37 (d, *J* 8.2 Hz, 2H), 7.89 (d, *J* 4.7 Hz, 1H), 7.79 (d, *J* 8.3 Hz, 2H), 4.74 (s, 1H), 1.58 (s, 2H), 0.95 (s, 9H), 0.93 (s, 6H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 142.9, 139.1, 138.8, 136.8, 128.6, 128.5, 127.9, 127.6, 126.2, 125.8, 125.2, 124.9, 124.9, 123.4, 117.5, 60.6, 55.8, 31.5, 31.2, 28.6; HRMS *m/z*: calculated for $C_{21}H_{26}F_3N_4^+$ $[M+H]^+$ 391.21041, found 391.21114.

*Procedure for the synthesis of 2-([1,1′-biphenyl]-4-yl)imidazo[1,2-a]pyrazin-3-amine (**19**):* To a solution of compound **14** (100 mg, 0.25 mmol) in anhydrous $CH_2Cl_2$ (1 mL), trifluoroacetic acid (1 mL) was added and the final reaction mixture was stirred at room temperaturefor 20 min. After the completion of reaction, trifluoroacetic acid was neutralized with saturated aqueous solution of $NaHCO_3$ (50 ml) and formed compound was extracted in dichloromethane. The organic layer was collected, dried over anhydrous $Na_2SO_4$ and concentrated to obtain a crude residue, which was purified using column chromatography to obtain product **19** in 90% yield; $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.82 (d, *J* 1.4 Hz, 1H), 8.28 (dd, *J* 4.7, 1.4 Hz, 1H), 8.17–8.08 (m, 2H), 7.75 (ddt, *J* 8.2, 6.3, 1.5 Hz, 5H), 7.52–7.44 (m, 2H), 7.40–7.31 (m, 1H), 5.89 (s, 2H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 142.2, 139.8, 138.3, 134.1, 133.4, 129.0, 128.0, 127.8, 127.4, 126.9, 126.7, 126.5, 115.1; HRMS *m/z*: calculated for $C_{18}H_{15}N_4^+$ $[M+H]^+$ 287.12912, found 287.12808.

*NCI-60 Screening methodology:*

Growth inhibition experiments were performed at the US National Cancer Institute (NCI) according to the method as described by Boyd and Paull[15*a*].

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## Supporting Information

The Supporting Information (analytical data, $^{1}H$ NMR, $^{13}C$ NMR, HRMS, and HPLC spectra of all the synthesized compounds) is available at www.indianchemicalsociety.com.

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