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Drug repurposing for Breast Cancer: Preliminary medicinal chemistry investigations and future perspectives

Prashant S. Kharkar*[a,b], Chetan P. Shah[b] and Niteshkumar U. Sahu[b]

[a]Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Matunga (East), Mumbai-400 019, India

[b]Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM's NMIMS, Vile Parle (West), Mumbai-400 056, India

E-mail: ps.kharkar@ictmumbai.edu.in

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Triple-negative breast cancer (TNBC) is one of the leading causes of mortality in women. Despite the availability of varied treatment modalities, including chemotherapeutic and immunotherapeutic drugs, TNBC remains a major health concern worldwide. In the present study, we investigated the drug repurposing for TNBC with the hope to identify safer and efficacious drugs for its treatment. Of the 70 drugs tested against highly metastatic MDA-MB-231 cell line in MTT assay, a total of 11 drugs demonstrated potent cytotoxicity. Further *in vitro* and *in vivo* (animal efficacy) biological investigations are needed to prove the anti-TNBC potential of the identified hits – Domperidone, Candesartan cilexetil, Felodipine, Atorvastatin calcium, Sertraline HCl, Nisoldipine, Lopinavir, Clotrimazole, Desloratadine, Carvedilol phosphate and amlodipine besylate.

Keywords: Breast Cancer, TNBC, drug repurposing, MDA-MB-231, MTT.

Introduction

Triple-negative breast cancer (TNBC) represents a clinical disease with early onset, aggressive progression to higher stages and poor prognosis, compared to its hormone receptor- and HER2-positive counter-parts[1]. Currently, there is no effective treatment option available for patients afflicted with TNBC. Several investigational and recently discovered therapeutics, such as immune checkpoint inhibitors, e.g. anti-programmed death-ligand 1 (anti-PD-L1) antibody atezolizumab (launched in 2016) are being investigated alone or in combination with chemotherapeutic drugs for their efficacy in TNBC treatment[2]. The encouraging results from the IMpassion130 trial assured that there was still hope. In short, there is a dire need for more efficacious anti-TNBC therapeutics, currently an unmet medical need.

Drug repurposing, i.e. finding new uses for approved drugs, is a well-established approach in drug discovery and development[3]. Experimental and computational drug repurposing approaches have contributed signifycantly in times of medical emergency, such as COVID-19[4]. Inspired by the success stories and our own experience in experimental and computational drug repurposing[5], we set out with the sole objective of discovering new therapeutic options for TNBC treatment. The present study is the culmination of our meticulous preliminary investigations in this direction, where we screened the in-house Drugs Library (70 drugs) against a highly aggressive and metastatic breast cancer cell line, MDA-MB-231. To our astonishment, the *in vitro* screening yielded a total of 11 hits (Table 1), belonging to varied therapeutic classes, with relatively potent cytotoxicity. The *in vitro* screening results of all the tested drugs are listed in Table 1S and the calculated/predicted molecular, physicochemical and pharmacokinetic properties of the hits are presented in Table 2S (Supporting Information section). The molecular structures of the hits are given in Fig. 1.

Here, we present the tip of the iceberg, in the form of identified hits, which are supposedly safer approved drugs for varied indications. Further extended biological investigation is warranted to fully validate the anti-TNBC potential of the identified hits.

Table 1. Cytotoxicity of the hits (drugs to be repurposed for breast cancer)

Sr. No.	Drug name	% Inhibition at 10 μM concentration[a] MDA-MD-231[b]
1.	Doxorubicin HCl[c]	97.14±1.03
2.	Domperidone (**1**[d])	97.42±3.33
3.	Candesartan cilexetil (**2**)	96.65±0.31
4.	Felodipine (**3**)	98.23±0.18
5.	Atorvastatin calcium (**4**)	92.94±0.80
6.	Sertraline HCl (**5**)	98.07±0.14
7.	Nisoldipine (**6**)	95.66±1.61
8.	Lopinavir (**7**)	95.24±1.08
9.	Clotrimazole (**8**)	96.38±0.94
10.	Desloratadine (**9**)	96.62±0.54
11.	Carvedilol phosphate (**10**)	97.77±0.52
12.	Amlodipine besylate (**11**)	98.00±0.22

[a]% Inhibition data expressed as mean ± SD (results are average of triplicate analysis); [b]MDA-MB-231 – Breast adenocarcinoma; [c]Positive control; [d]The drug molecular structure is given in Fig. 1.

Experimental

Drugs library:

The in-house Drugs Library was a result of our concerted collection efforts over 5–6 years. Various drugs were added to the library following thorough analytical characterization including purity (HPLC, LC-MS/MS) and identity (melting point, and spectroscopic characterization using FT-IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, and thermochemical method such as differential scanning calorimetry). All the samples in the Drugs Library were maintained at –80°C. For the present study, all the library samples were removed from the freezer and thawed before weighing.

Cells and reagents:

The cell line MDA-MB-231 (breast adenocarcinoma) was sourced from National Centre for Cell Science (NCCS), Pune, and processed as previously described[6]. The list of reagents for the cell culture work and their corresponding vendor sources is given in Supporting Information section (Table 3S).

MTT assay:

The assay was performed following a standard protocol as described previously[6]. In brief, MDA-MB-231 cells (in the logarithmic growth phase) at previously determined optimum plating efficiency (10,000 cells/well) seeded in a 96-well plate, were incubated for 24 h (humidified conditions, 5% CO_2) at 37°C and observed under microscope. The drug solutions (10 μM), prepared from 10 mM stock solution in DMSO, after appropriate dilutions, were added to the wells in duplicate along with DMSO as vehicle control. Doxorubicin HCl was used a positive control. Post-drug treatment, the plates were incubated at 37°C under 5% CO_2 humidified conditions. Further, the assay plates were centrifuged twice at 3000 rpm for 3 min, and the resultant supernatant discarded. Subsequently, each well was treated with MTT (100 μL of 5 mg/mL solution) and incubated for 4 h under 37°C and 5% CO_2 humidified conditions. The plate was centrifuged again and the supernatant was removed. To the wells, DMSO (200 μL) was added to solubilize formazan crystals and subsequent absorbance measurement at 540 nm (630 nm for background scan) was carried out using EPOCH 2 Biotek microplate reader. The results were expressed as %inhibition (average of n=2 ± standard deviation).

Results and discussion

The present study was initiated with the objective to discover safer repurposed drugs as potential treatment options for TNBC. The preliminary investigations led to the identification of 11 promising hits (Table 1, Fig. 1) with potent activity against highly aggressive, metastatic, and poorly differentiated TNBC cell line, MDA-MB-231. Intrigued by the exciting outcome, we went on fishing the literature to find out if similar anticancer studies involving the drugs tested were done previously. We were equally curious to understand if their original indication had something to do with the demonstrated anticancer activity, i.e. to find the missing link, if any, between the molecular target(s)/off-target(s) of the hits and the molecular basis of their cytotoxicity against MDA-MB-231 cells. In other words, we were particularly interested in uncovering if the hits were cytotoxic due to activity at their original molecular target/off-target(s).

Initially, the SciFinder search was attempted with keywords such as ‘drug_name and cancer’, ‘drug_name and anticancer’, ‘anticancer activity of drug_name’, etc. Similar searches were carried out by replacing the word ‘cancer’ with ‘breast cancer’. The search results were interesting from many perspectives. Here, we attempt to rationalize the cytotoxicity of the hits and their potential as plausible TNBC therapeutics.

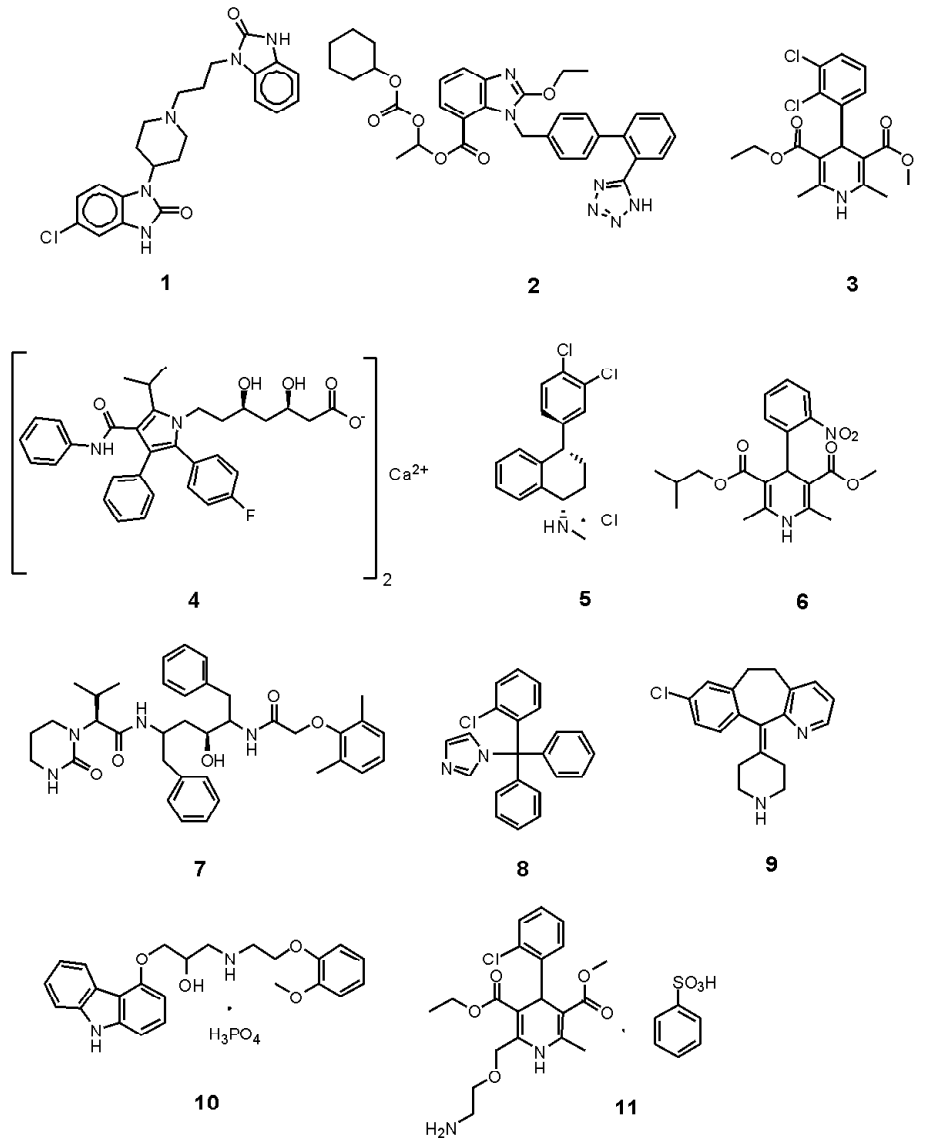

Fig. 1. Molecular structures of hits (drugs to be repurposed for breast cancer).

The first hit, domperidone (**1**), is therapeutically indicated for its antiemetic, gastric prokinetic and galactogogue activities. It is a peripherally selective dopamine D_2 receptor antagonist. SciFinder search led us to a Chinese patent application featuring use/mention of **1** for treating cancer, particularly non-small-cell lung cancer (NSCLC), either alone or in combination with paclitaxel[8]. The treatment of human lung cancer cell line H460 with **1** alone and with paclitaxel induced apoptosis; the combination demonstrated superior synergy. The *in vitro* results were successfully translated in SCID mice (*in vivo* efficacy studies). None of the literature reports mentioned anticancer potential of **1** for any other type of cancer. To the best of our knowledge, the present investigation is the first report featuring the anticancer activity of **1**, with particular relevance to TNBC.

Candesartan cilexetil (**2**) is a prodrug of an antihypertensive medication, candesartan, which acts by selective blockade of angiotensin II receptor (AT_1 subtype)[9]. In a very recent report, **2** was listed as a potential anticancer lead acting via inhibition of Nedd8-activating enzyme (NAE, E1) in ATP-competitive manner[10]. The neddylation pathway, emerged recently as an attractive therapeutic target, is hyperactivated

in a variety of human lung cancers, thereby correlating well with the disease progression. The authors of the neddylation study confirmed the apoptotic induction and tumor suppression using A549 (human lung cancer cell line) cell line *in vitro* and *in vivo*.

In an interesting patent application, the inventors disclosed the utility of **2** as an antitumor agent by targeting cyclin-dependent kinase (CDK4)[11]. The CDK4 IC_{50} was reported to be 5.2 mM. The potential indications of a relatively potent CDK4 inhibitor (e.g. **2**) could be melanoma, mammary gland carcinoma, NSCLC, among others. As seen from Table 1S (Supporting Information section), there was no significant difference between the cytotoxicity of **2** and its active form, i.e. candesartan, potentially ruling out the critical requirement of the -COOH functionality for their cytotoxicity. Similar to **1**, there was no definite report on the putative activity of **2** against breast cancer cell lines.

Felodipine (**3**)[12], a well-known calcium channel blocker used as antihypertensive, exhibited cytotoxicity towards Mz-ChA-1 cells (human malignant cholangiocytes) (IC_{50} = 26 μM) and other cholangiocarcinoma cell lines (KMCH-1, CC-LP-1 and TFK-1) *in vitro*[13]. In nude athymic mice, co-administration of **3** with gem-citabine was efficacious in reducing the growth of Mz-ChA-1 cell xenografts. There were a couple of Chinese patents where cardiovascular drugs were tested for their anticancer activity against a panel of cancer cell lines. But we could not find any mention of anticancer activity of **3**. The literature search did not locate any report featuring the anti-breast cancer activity of **3**.

Atorvastatin calcium (**4**) is a blockbuster statin antihyperlipidemic drug targeting HMG-CoA reductase[14]. Statins are well-known for their anticancer effects (Refer Supporting Information section for additional references). Very recently, the anti-breast cancer activity of **4** was demonstrated[15]; downregulation of the PTEN/AKT pathway by promotion of Ras homolog family member B (RhoB) was the underlying mechanism. It inhibited proliferation, invasion, epithelial-mesenchymal transition (EMT) and induced apoptosis in breast cancer cells. Overall, the investigation successfully unearthed the potential utility of RhoB as a promising breast cancer target.

Next hit, sertraline HCl (**5**)[16], a selective serotonin reuptake inhibitor (SSRI) prescribed for major depression, was reported for its anticancer activity evaluation against human colorectal cancer cell lines – HT29 and multidrug-resistant LS1034, *in vitro* and HT29 xenografted CD1 nude mice[17]. The IC_{50}s for the two cell lines ranged from 8 to 15 μM. The flow cytometry analysis clearly demonstrated the cell cycle arrest by **5** at G_0/G_1 stage with dose-dependent induction of DNA fragmentation and apoptosis. The significant reduction in tumor volume was observed on treatment with **5** in efficacy studies. None of the literature reports indicated the anti-TNBC potential of **5**.

Nisoldipine (**6**)[18], an L-type calcium channel blocker belonging to 1,4-dihydropyridine class, is prescribed as an antihypertensive. In a recently filed patent application, **6** was listed as one of the dihydropyridines used for the treatment of cancer, either alone or in combination with loperamide[19]. The cytotoxicity studies of **6** in A549 cells exhibited relatively higher IC_{50} of 30–40 μM. There were no additional reports linking **6** with breast cancer therapeutic potential.

The next hit, lopinavir (**7**)[20], a well-established HIV-1 protease inhibitor, was reported to be useful in treating and/or preventing skin cancers and premalignant dermal conditions[21]. In yet another interesting study on solid tumors, **7** demonstrated the most potent, specific and dose-dependent cancer stemness inhibitory potential[22]. The outcome of the above study was particularly important for the treatment of solid tumors with poor prognosis.

Previously, an age-old antifungal, clotrimazole (**8**)[23] was identified as a calmodulin antagonist and shown to decrease human breast cancer cell viability in MCF-7 cells (IC_{50} = 88.6±5.3 μM) by altering cytoskeleton-associated glycolytic enzymes[24]. In a related investigation, **8** was found to inhibit proliferation, viability and glycolysis in human breast cancer cells- MCF10A, MCF- 7 and MDA-MB- 231, albeit at bit higher IC_{50} vlaues[25]. The effect was more prominent and selective in invasive MDA-MB-231 cells.

The story of desloratadine (**9**)[26], a relatively older histamine H_1-antagonist, was a bit different. A latest (2020) study examined the association between the H_1-antagonist use (six drugs including **9**) and breast cancer-specific and overall mortality in a nation-wide study (#61,627 Swedish women diagnosed with breast cancer during 2006-2013)[27]. The authors clearly observed consistently improved survival in users of **9**, relative to non-users. Based on the out loud conclusion, the authors of the above study recommended to formally investigate the discovered facts with the objective of

uncovering the underlying mechanism. A recent patent application disclosed the utility of **9** in the preparation of anti-liver cancer drugs[28]. In brief, **9** holds a great potential as an anticancer agent.

Carvedilol phosphate (**10**)[29], a β-blocker with multiple indications, was shown to attenuate UV radiation induced skin carcinogenesis in an established epidermal model for studying skin carcinogenesis, i.e. JB6 P^+ cells[30]. Later, the same research group utilized phosphoproteome profiling as a modality to understand **10**-mediated cancer prevention[31], wherein **10** strongly inhibited epidermal growth factor (EGF)-induced neoplastic transformation of JB6 P^+. In A375 melanoma xenografted SCID mice, oral treatment with **10** was highly efficacious in inhibiting the tumor growth.

The last hit, amlodipine besylate (**11**)[32], yet another 1,4-dihydropyridine calcium channel blocker, was devoid of any report linking it to anticancer activity. Overall, the whole investigation turned out to be more exciting and intellectually satisfying that we could ever expect. The outcome of this study is significant from many perspectives such as the cytotoxicity against the TNBC-relevant breast cancer cell line for few hits is reported for the first time. Further studies based on the results of the present investigation are likely to validate the proof-of-concept generated at the conclusion of the present investigation. Some hits were already reported for anti-breast cancer activity, which in a way, corroborated our results.

Conclusions

In the present preliminary study, a total of 11 hits were identified with relatively potent activity at therapeutically relevant concentration (10 μM) against a highly aggressive and invasive breast cancer cell line (MDA-MB-231), potentially representing clinical TNBC disease. Few hits were reported in the literature for their anticancer (other than breast cancer) potential while most of them were never evaluated for cytotoxicity against breast cancer cells. The interesting part of such a study is to understand the association, if any, between the original indication of the drug and its, say anti-TNBC activity. At times, the mechanism of action for its therapeutic indication could be totally different than the one related to its anticancer activity. Further investigations in the direction of repurposing few of these hits for TNBC would be very rewarding in totality, given the lack of promising and efficacious therapeutic options for its treatment. Further research in delineating the mechanism of cytotoxicity against MDA-MB-231 may yield few promising targets for intervening the difficult-to-treat TNBC. We hope our results would motivate the biologists to take up such explorations so that better therapeutic options for TNBC patients be available in record time, which is likely to alleviate sufferings of the millions.

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Supporting Information

The % inhibition data at 10 μM concentration from the MTT assay using MDA-MB-231 cell line for all the tested (#70) drugs (Table 1S), calculated/predicted molecular, physicochemical, and pharmacokinetic properties data for all the 70 drugs (Table 2S) along with list of reagents for cell culture studies (Table 3S), along with are provided for the interested readers. Also, additional references on anticancer activity of atorvastatin are given.

References

1. (a) A. C. Garrido-Castro, N. U. Lin and K. Polyak, *Cancer Discov.,* 2019, **9**, 176; (b) A. Marra, G. Viale and G. Curigliano, *BMC Med.*, 2019, **17**, 90.
2. P. Schmid, S. Adams, H. S. Rugo, *et al., N. Engl. J. Med.*, 2018, **379**, 2108.
3. (a) S. Pushpakom, F. Iorio, P. A. Eyers, *et al., Nat. Rev. Drug Discov.*, 2018, **18**, 41; (b) P. S. Kharkar, P. Ramasami, Y. Siew, R. Lydia and S. Warrier, *RSC Adv.*, 2016, **6**, 26329; (c) N. U. Sahu and P. S. Kharkar, *Curr. Top. Med. Chem.*, 2016, **16**, 2069.
4. (a) R. P. Guy, R. S. DiPaola, F. Romanelli and R. E. Dutch, *Science*, 2020, **368**, 829; (b) J. Wang, *J. Chem. Inf. Model.*, 2020, **60**, 3277.
5. (a) S. Dutta, P. S. Kharkar, N. U. Sahu and A. Khanna, *Life Sci.*, 2017, **185**, 73; (b) P. S. Kharkar, S. Borhade, A. Dangi and S. Warrier, *J. Comput. Sci.*, 2015, **10**, 215.
6. (a) K. Padharia, M. Athavale, S. Srivastava and P. S. Kharkar, *Drug Develop. Res.*, 2020, **81**, 356; (b) K. Padharia, M. Athavale, S. Srivastava and P. S. Kharkar, *Drug Develop. Res.*, 2020 (In Press).
7. Domperidone. DrugBank DB01184 https://www.drugbank.ca/drugs/DB01184 (Accessed on July 9, 2020).
8. Chinese Patent Application CN110507653A (2019).
9. Candesartan cilaxetil. Drug Bank DB00796 https://www.drugbank.ca/drugs/DB00796 (Accessed on July 9, 2020).
10. S. Ni, X. Chen, Q. Yu, *et al., Eur. J. Med. Chem.,* 2020, **185**, 111848.

11. Chinese Patent Application CN108379265A (2018).
12. Felodipine. DrugBank DB01023 https://www.drugbank.ca/drugs/DB01023 (Accessed on July 10, 2020).
13. C. Braconi, E. Swenson, T. Kogure, N. Huang and T. Patel, *PLoS One*, 2010, **5**, e15195.
14. Atorvastatin. Drug Bank DB01076. https://www.drugbank.ca/drugs/DB01076 (Accessed on July 10, 2020).
15. Q. Ma, Y. Gao, P, Xu, *et al., Biomed. Res. Int.,* 2019, **2019**, 3235021.
16. Sertraline. Drug Bank DB01104 https://www.drugbank.ca/drugs/DB01104 (Accessed on July 10, 2020).
17. I. Gil-Ad, A. Zolokov and L. Lomnitski, *et al., Int. J. Oncol.,* 2008, **33**, 277.
18. Nisoldipine. DrugBank DB00401 https://www.drugbank.ca/drugs/DB00401 (Accessed on July 10, 2020).
19. WO2019/008516A2 (2019).
20. Lopinavir. DrugBank DB01601 https://www.drugbank.ca/drugs/DB01601 (Accessed on July 10, 2020).
21. WO2019/224780A1 (2019).
22. C. Y. Darini, P. Martin, S. Azoulay, M. D. Drici, P. Hofman, S. Obba, C. Dani and A. Ladoux, *Cell Death Dis.,* 2013, **4**, e706.
23. Clotrimazole. DrugBank DB00257 https://www.drugbank.ca/drugs/DB00257 (Accessed on July 10, 2020).
24. D. D. Meira, M. M. Marinho-Carval-ho, C. A. Teixeira, *et al.*, *Mol. Genet. Metab.,* 2005, **84**, 354.
25. C. M. Furtado, M. C. Marcondes, M. Sola-Penna, M. L. de Souza and P. Zancan, *PLoS One*, 2012, **7**, e30462.
26. Desloratadine. DrugBank DB00697 https://www.drugbank.ca/drugs/DB00967 (Accessed on July 10, 2020).
27. I. Fritz, P. Wagner, P. Broberg, R. Einefors and H. Olsson, *Acta Oncol.,* 2020, 1.
28. CN110755431A (2020).
29. Carvedilol, DrugBank DB01136 https://www.drugbank.ca/drugs/DB01136 (Accessed on July 10, 2020).
30. K. M. Huang, S. Liang, S. Yeung, *et al., Cancer Prev. Res. (Phila),* 2017, **10**, 598.
31. K. H. Cleveland, S. Yeung, K. M. Huang, S. Liang, B. T. Andresen and Y. Huang, *Mol. Carcinog.,* 2018, **57**, 997.
32. Amlodipine. DrugBank DB00381 https://www.drugbank.ca/drugs/DB00381 (Accessed on July 10, 2020).