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REVIEW ON THE DISCOVERY OF ROHITUKINE-INSPIRED CDK INHIBITORS

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ABSTRACT

Two synthetic flavones, flavopiridol (**2**, Alvocidib; L868275; HMR-1275; NSC 649890 of Sanofi-Aventis + NCI) and P-276-00 (**3**, Piramal) are advanced in clinical trials for the treatment of cancer. Though these flavones are totally synthetic molecules, the basis for their novel structure is a natural product, rohitukine (**1)**, an alkaloid, isolated from plant *Dysoxylum binectariferum* (Meliaceae) which is phylogenetically related to the Ayurvedic plant *D. malabaricum.* Recently a semisynthetic derivative of rohitukine; IIIM-290 (**4**) has been emerged as potential preclinical cyclin-dependent kinase (CDK) inhibitor. CDK has discovered as a key regulator of cell cycle. Therefore, CDK inhibitors or modulators are of great interest to explore as novel therapeutic agents. The present review contains discovery, chemistry, pharmacology and SAR of rohitukine-inspired CDK inhibitors.

KEY WORDS: Cancer, cyclin-dependent kinase, *Dysoxylum binectariferum*, flavopiridol, P-276-00, IIIM-290, rohitukine.

INTRODUCTION

The cyclin-dependent kinase (CDK) has discovered as a key regulator of cell cycle, there are thirteen members of human CDK family, they all are belongs to serine/threonine protein kinases. CDK1, 2, 3, 4, and 6 directly interfere in the cell cycle, according to general function CDKs classified as regulatory or cell cycle CDK (mainly CDK1, 2, 4 and 6) and transcriptional (mainly CDK7 and 9). Any alteration and deregulation of CDK activity have a pathogenic link to the cancer. In multiple studies, it has been shown that many cancers are associated with hyper-activation of CDKs as a result of mutation of the CDK genes or CDK inhibitor genes. Therefore, CDK inhibitors or modulators are of great interest to explore as novel therapeutic agents against cancer (Senderowicz, 2001).

Because CDK inhibitors are ATP competitive ligands hence earlier they were typically described as purine class of compounds for example dimethylaminopurine a first substance to be known as a CDK inhibitor (IC_{50}, CDK1/cyclin B: 120 µM) (Neant and Guerrier, 1988) olomoucine (IC_{50}, CDK1/cyclin B: 7 µM, CDK2/A &

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CDK1/B: 7000 nM, CDK6/D3: 250000 nM, (Vesely et al., 1994, Mariaule and Belmont, 2014) and roscovitine (IC_{50}, CDK1: 2.7 μM, CDK2: 0.1 μM, CDK7: 0.5 μM, CDK9: 0.8 μM) (Mariaule and Belmont, 2014, Meijer et al., 1997).

Later in several studies, it has been revealed that many flavonoids like quercetin, genistein, baicalein, baicalin, fisetin, apigenin, luteolin, kaempferol, and chrysin have shown CDK inhibitory activity as mentioned in Figure 1.

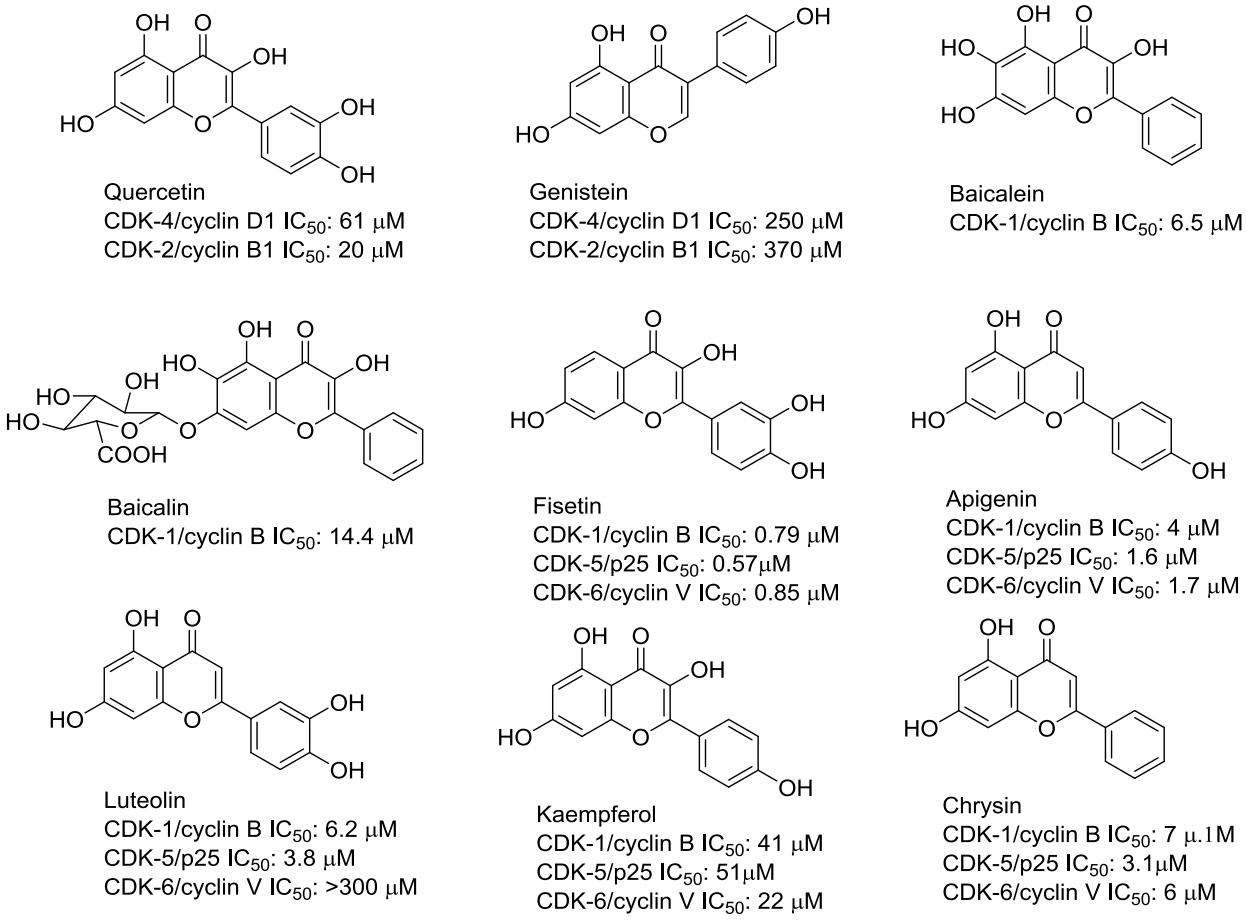

Figure 1. CDK inhibitory profile of naturally occurring flavonoids.

Baicalein and quercetin are also reported to arrest cell growth G1 and G2 by the down-regulation of cyclin D2, cyclin A, CDK1 and CDK2 (Lee et al., 2005, Vijayababu et al., 2005). Recent discoveries from clinical trials data have suggested that with pan-specific inhibitors (like flavopiridol) have more positive outcomes in general (Liu et al., 2012). However, in specific cases, better results have been achieved with specific inhibitors like Palbociclib (specially CDK4/CDK6). Discover and develop a specific inhibitor a challenging task since all CDKs have a very similar active site. Another major issue identification of the specific patient for specific therapy (Malinkova et al., 2015).

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Rohitukine, a naturally occurring alkaloid first isolated from Amoora rohituka (Harmon et al., 1979). Later *Dysoxylum binectariferum* bark and subsequently leaves has been identified as the enriched source of rohitukine (Kumar et al., 2016). Rohitukine has been investigated for anti-inflammatory, anti-implantation, anti-fertility, anti-proliferative and immunomodulatory properties (Safia et al., 2015). Recent investigations identified anti-cancer potential of rohitukine through both CDK and MAPK pathway inhibition (Safia et al., 2015, Bharate et al., 2018).

DISCOVERY

Flavone alkaloid rohitukine (1) was isolated by chemists at Hoechst India Ltd. in the early 1990s from *Dysoxylum binectariferum* Hook. which is related to the Ayurvedic plant, *D. malabaricum* Bedd (Jain et al., 2012a)

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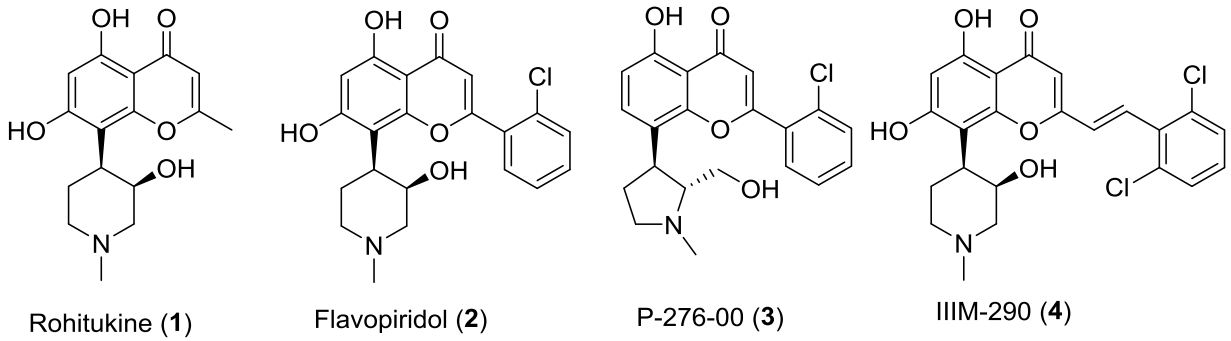

Figure 2. Chemical structure of rohitukine, flavopiridol, and congeners.

This plant is documented in the traditional literature for rheumatoid arthritis. Apart from rohitukine, other potential natural compounds have also been isolated from *Dysoxylum binectariferum* such as dysoline (Jain et al., 2013), camptothecin and methoxy camptothecin (Jain et al., 2014). Rohitukine initially identified as a constituent responsible for the anti-inflammatory and immunomodulatory activity. Medicinal chemistry efforts around this nature-derived flavone alkaloid synthesized hundreds of compound to screen (Kattige et al., 1990). This led the discovery of two promising clinical candidates for the treatment of cancer viz. flavopiridol (2), and P-276-00 (3). A semi-synthetic regime on rohitukine discovers a potent and selective CDK9 inhibitor IIIM-290 (4) under development (Bharate et al., 2018, Jain et al., 2012b).

PHARMACOLOGY

Flavopiridol is the first CDK inhibitor that shown to cause potential effects on the chemotherapy through cell cycle arrest, induces apoptosis, and inhibit angiogenesis (Cimini et al., 2017). Moreover, flavopiridol has shown potent cytotoxicity against different cell lines, (IC_{50} µM): SW620 = 0.2, HOP-92 = 1, NCIH322 = 4, DU145 = 2, LOXIMVI = 4, HCT116 = 1, Panc-1 = 1, NCIH522 = 0.8 similarly flavopiridol has been identified as pan CDK inhibitor, CDK (IC_{50} nM): CDK-1/B1 = 30, CDK-2/E = 170, CDK-5/p25 = 170, CDK-6/D1 = 80, CDK-9/T1 = 20 (6).

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Its preclinical pharmacokinetic studies indicating optimum parameter: $T_{½}$ = 1.1 h, C_{max} = 6.9 ng/ml, T_{max} = 0.5 h, AUC_{0-t} = 13.2 µM*h/ml. The clinical data of flavopiridol (2) has been reviewed by several researchers (Awan et al., 2016). Flavopiridol (2) has been studied in a wide range of indications using a variety of dosing schedules, but promising results have only emerged in the treatment of chronic lymphocytic leukemia (CLL) (Brown, 2005, Christian et al., 2007). These studies indicate that unusually high plasma concentrations of flavopiridol may be required for efficacy due to high plasma protein binding (LaCerte et al., 2017). Recent clinical results, however, indicate that flavopiridol may still find niche utilities as single-agent therapy employing finely tailored dosing schedules for particularly susceptible cancers, such as chronic lymphocytic leukemia (CLL). However, in Europe, flavopiridol has already got the status of an orphan drug for treatment of CLL (Albert et al., 2014).

Another compound, P-276-00 (3), was identified as potent CDK inhibitor, which selectively inhibits CDK-4/cyclin D1, CDK-1/cyclin B, and CDK-9/cyclin T1 and shows relevant antitumor activity in a broad panel of cancer-cell lines. The flavone P-276-00 (3) was identified as 40-fold selective toward CDK-4/cylin D1, compared with CDK-2/cyclin E (Joshi et al., 2007a). The preclinical evaluation of P-276-00 (Raje et al., 2009) and the results of Phase I clinical trials (Hirte et al., 2007) revealed that interesting selectivity of the parent compound flavopiridol is maintained, particularly its potency against CDK-9/cyclin T1 (P-276-00: IC_{50} = 20 nM). P276-00 (3) is active in human colon and non-small cell lung cancer xenografts (Joshi et al., 2007b). Results of first-in-human study of P276-00 has been reported (Hirte et al., 2007). Currently, P-276-00 (3) is in phase II clinical studies for advanced refractory neoplasms and multiple myeloma.

Another semi-synthetic 2,6-dichloro-styryl derivative IIIM-290 (4) of rohitukine has recently emerged as a most potent CDK-9/T1 inhibitor (IC_{50} 1.9 nM). The preclinical study of this compound has suggested as a potential lead to be developed for clinical drug discovery. IIIM-290 has shown Molt-4/MIAPaCa-2 cell growth (GI50 < 1.0 µM) and was found to be highly selective for cancer cells over normal fibroblast cells. It inhibited the cell growth of MIAPaCa-2 cells via caspase-dependent apoptosis. It achieved 71% oral bioavailability with *in vivo* efficacy in pancreatic, colon, and leukemia xenografts at 50 mg/kg, p.o. It did not have CYP/efflux pump liability, was not mutagenic/genotoxic or cardiotoxic, and was metabolically stable (Bharate et al., 2018).

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP

First total synthesis of rohitukine (1) (Naik et al., 1988) and flavopiridol (2) (Kattige et al., 1990) was reported by Hoechst researchers in 1990s (Figure 3). Tabaka et al. (Tabaka et al., 1999) provided the improved version of this synthetic strategy for racemic flavopiridol and its salt (Figure 3). Piramal has disclosed a new process (Figure 4) for the preparation of pure P276-00 (3) pyrrolidino-flavone enantiomer based on the optimized resolution of a synthetic intermediate prior to the formation of the pyrone ring (Sivakumar et al., 2008). Further, this intermediate 22 was converted to P-276-00 (3) using a similar procedure as reported for flavopiridol.

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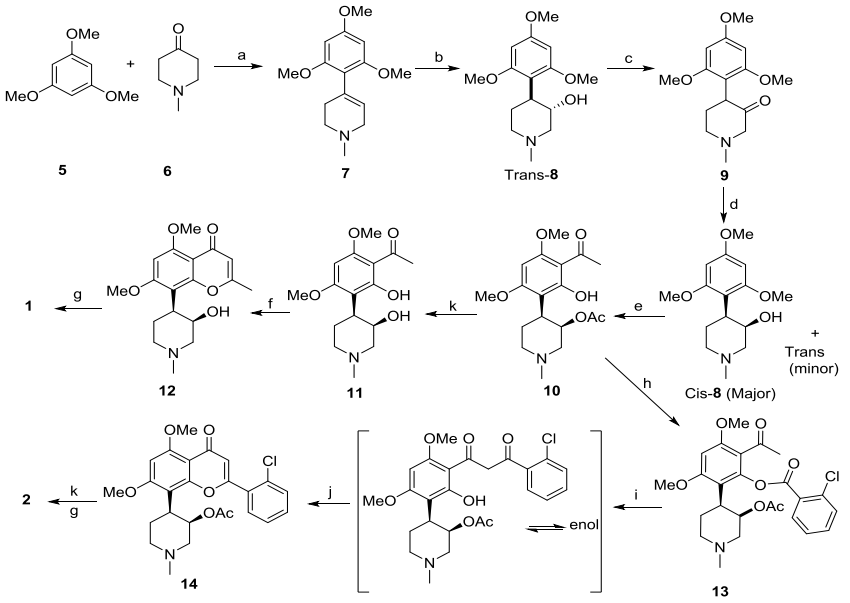

Figure 3. Scheme for synthesis of rohitukine (1) (Naik et al., 1988) and flavopiridol (2) (Tabaka et al., 1999, Kattige et al., 1990). Reagent and conditions: (a). CH_3COOH, HCl, 100 °C, 3 h, 80%; (b). BF_3-OEt, $NaBH_4$, THF, conc. HCl, then NaOH, H_2O_2, 70%; (c). $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 76%; (d). $NaBH_4$, EtOH, 66%; (e). BF_3-OEt, Ac_2O, CH_2Cl_2, 73%; (f). EtOAc, Na, reflux, 3 h; (g). pyridinium HCl, quinoline, 180 °C, 2 h, 82%; (h). 2-Cl-benzoylchloride, pyridine, 76%; (i). KOH, pyridine, reflux; (j). AcOH/ H_2SO_4; (k). NaOH/H_2O, MeOH.

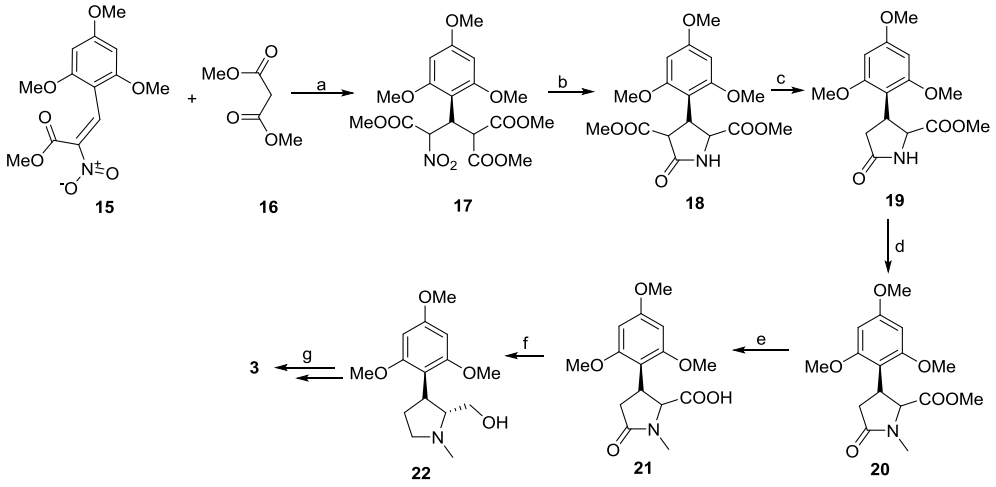

Figure 4. Scheme for synthesis of P-276-00 (**3**) by chemists from Piramal Life Sciences India (Sivakumar et al., 2008). Reagent and conditions: (a). N-methylmorpholine, catalyst complex, $CHCl_3$, H_2O, Mol. sieve,12 h, rt, 69%; (b). $SnCl_2$, ethyl acetate, 55 °C, 2 h, 67%; (c). N-methylpyrrolidone, NaCl, H_2O, 170 °C, 5 h, 44%; (d). NaH, CH_3I, DMF, 0 °C to rt, 1 h, 95%; (e). KOH, MeOH, H_2O, 65 °C, 3 h, 61%; (f). $LiAlH_4$, THF, 50 °C. 1.5 h, 100% ; (g). steps e, h, i, j. g from Figure 3.

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Rohitukine (1) was dissolved in a solution of methanol containing KOH. To this mixture was added 6-dichlorobenzaldehyde, and the resultant mixture was stirred at 100 ºC for 3-4 h. After completion of the reaction, the mixture was cooled and neutralized with 6 N HCl. The precipitate was filtered and washed it was recrystallized using methanol/chloroform.

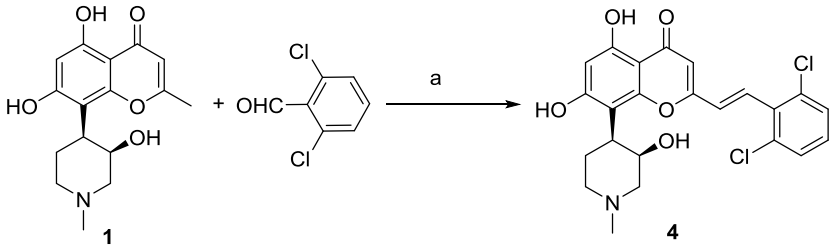

Figure 5. Scheme for synthesis of IIIM-290. Reagent and conditions: (a). KOH (10 eq), MeOH, 110 ºC, 10 h, 65%.

Structure-activity relationship (SAR), results indicated that substitution at C2-methyl with double bond moiety resulted in enhancement of CDK inhibition. The previous report also supports that C2-position is the most tunable position for CDK inhibition activity. The replacement of C2-methyl with 2-chlorophenyl group provided flavopiridol (2, IC_{50}: 20 nM), which possesses 15-fold improvement in CDK-9/T1 compared to rohitukine (1, IC_{50}: 300 nM). The styryl moiety was further substituted with two "Cl" at the ortho position, resulted in tremendous improvement in the CDK-9/T1 inhibition; this 2,6-dichloro derivative IIIM-290 (4, IC_{50} 1.9 nM) possesses ~160-fold improvement in CDK-9/T1. SAR with respect to CDKs inhibition has been summarized in Figure 6.

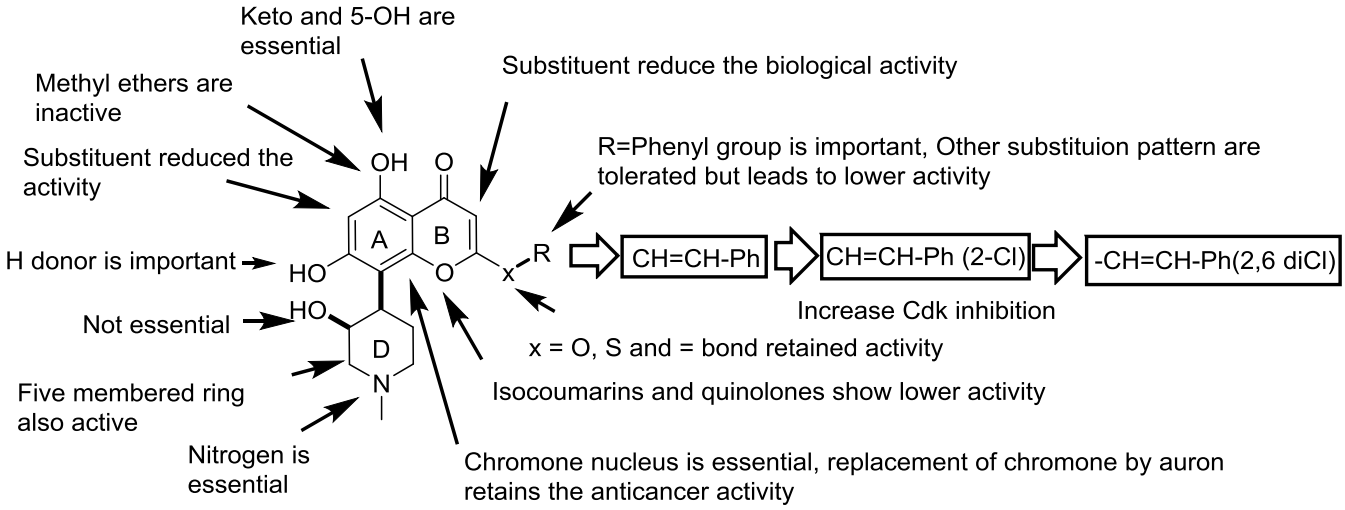

Figure 6. Structure-activity relationship (SAR).

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CONCLUSION

Over more than 60% of the current anticancer drugs have their origin in one way or another from natural sources. Amongst these, flavonoids have a great potential to emerge as therapeutic agents. Naturally, occurring flavone alkaloid rohitukine (1) has already delivered two clinical candidates flavopiridol (2), P-276-00 (3) and IIIM-290 (4) is under preclinical development.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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